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Chymotrypsin for Ophthalmic Solution

DEFINITION

Chymotrypsin for Ophthalmic Solution is sterile Chymotrypsin. When constituted as directed in the labeling, it yields a solution containing NLT 80.0% and NMT 120.0% of the labeled potency.

IDENTIFICATION

• **A.**

Monobasic potassium phosphate solution: 9.08 mg/mL of monobasic potassium phosphate in water

Dibasic sodium phosphate solution: 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

Phosphate buffer: Mix 38.9 mL of *Monobasic potassium phosphate solution* and 61.1 mL of *Dibasic sodium phosphate solution*. If necessary, adjust by the dropwise addition of *Dibasic sodium phosphate solution* to a pH of 7.0.

Substrate solution: Transfer 237.0 mg of *N*-acetyl-L-tyrosine ethyl ester, suitable for use in assaying chymotrypsin, to a 100-mL volumetric flask, add 2 mL of alcohol, and swirl until solution is effected. Add 20 mL of *Phosphate buffer*, 1 mL of methyl red–methylene blue TS, and dilute with water to volume. If necessary, adjust by the dropwise addition of *Monobasic potassium phosphate solution* to a pH of 7.0.

Sample solution: Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 1 mL of saline TS.

Analysis: Transfer 0.2 mL of the *Sample solution* to a suitable dish, and add 0.2 mL of the *Substrate solution*.

Acceptance criteria: A purple color is produced within 3 min.

[NOTE—This is distinct from trypsin, which produces no purple color within 3 min.]

ASSAY

• **PROCEDURE**

Monobasic potassium phosphate solution: 9.08 mg/mL of monobasic potassium phosphate in water

Dibasic sodium phosphate solution: 9.46 mg/mL of anhydrous dibasic sodium phosphate in water

Phosphate buffer: *Monobasic potassium phosphate solution* and *Dibasic sodium phosphate solution* (38.9: 61.1). If necessary, adjust by the dropwise addition of *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 assaying chymotrypsin, in 50 mL of *Phosphate buffer*, with warming. When the solution is cool, dilute with additional *Phosphate buffer* to 100 mL. [NOTE—*Substrate solution* may be stored in the frozen state and used after thawing, but it is important to freeze it immediately after preparation.]

Sample stock solution: Dissolve the contents of 1 vial of Chymotrypsin for Ophthalmic Solution in 5.0 mL of 0.0012 N hydrochloric acid.

Sample solution: Dilute a volume (V_2, in milliliters) of the *Sample stock solution*, equivalent to 300 USP Chymotrypsin Units, with 0.0012 N hydrochloric acid to 25.0 mL. The dilution is correct if, during the conduct of the *Assay*, there is a change in absorbance of between 0.008 and 0.012 in each 30-s interval.

Blank solution: Mix 0.2 mL of 0.0012 N hydrochloric acid and 3 mL of water.

Analysis

Samples: *Substrate solution*, *Sample stock solution*, *Sample solution*, and *Blank solution*

[NOTE—Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the *Analysis* using USP Chymotrypsin RS in place of the *Sample solution*.]

Conduct the *Assay* in a suitable spectrophotometer equipped to maintain a temperature of 25 ± 1.0° in the cell compartment. Determine the temperature in the reaction cell before and after the absorbance measurement to ensure that the temperature does not change by more than 1.0°. Pipet 3.0 mL of *Blank solution* into a 1-cm cell. Place the cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.00 at 237 nm. Pipet 0.2 mL of *Sample solution* into another 1-cm cell, add 3 mL of *Substrate solution*, and place the cell in the spectrophotometer. [NOTE—Carefully follow this order of addition, and begin timing the reaction from the addition of the *Substrate solution*.] Read the absorbance at 30-s intervals for NLT 5 min. Repeat the procedure on the same dilution at least once. Absolute absorbance values are less important than a constant rate of absorbance change. If the rate of change fails to remain constant for NLT 3 min, repeat the test and, if necessary, use a lower concentration. The duplicate determination at the same dilution matches the first determination in rate of absorbance change.

Determine the average absorbance change per minute, using only the values within the 3-min portion of the curve where the rate of absorbance change is constant. Plot a curve of absorbance against time. One USP Chymotrypsin Unit is the activity causing a change in absorbance of 0.0075/min under the conditions specified in the *Assay*.

Calculate the percentage of the labeled potency of USP Chymotrypsin Units in a vial:

$$\text{Result} = [F_1 \times (V_1/V_2) \times (A_2 - A_1)]/(T \times F_2 \times F_3)$$

F_1 = total USP Chymotrypsin Units in the *Sample solution*, 300

V_1 = volume of the *Sample stock solution*, 5 mL

V_2 = volume as defined in the *Sample solution* (mL)

A_2 = absorbance straight-line initial reading

A_1 = absorbance straight-line final reading

T = time elapsed between the initial and final readings (min)

F_2 = number of USP Chymotrypsin Units in the solution on which the absorbance was determined, 2.4

F_3 = chymotrypsin activity conversion factor, 0.0075/min

Acceptance criteria: 80.0%–120.0% of the labeled potency

PERFORMANCE TESTS

• **Uniformity of Dosage Units ⟨905⟩**

Analysis: Assay 10 individual units as directed in the *Assay*, and calculate the average of the 10 results.

Acceptance criteria: Meets the requirements of the chapter, and the average is 80.0%–120.0% of the labeled potency. The contents of NMT 2 vials deviate by more than 10% from the average content. The contents of none of the vials deviate by more than 15% from the average.

IMPURITIES

Change to read:

• **Limit of Trypsin**

Tris buffer: Dissolve 294 mg of calcium chloride in 40 mL of 0.20 M tris(hydroxymethyl)aminomethane. Adjust with 1 N hydrochloric acid to a pH of 8.1, and dilute with water to 100 mL.

Substrate solution: Transfer 98.5 mg of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride, suitable for use in assaying trypsin, to a 25-mL volumetric flask. Add 5 mL of *Tris buffer*, and swirl until the substrate dissolves. Add 0.25 mL of methyl red–methylene blue TS, and dilute with water to volume.

Sample solution: 10 mg/mL of Chymotrypsin for Ophthalmic Solution

Analysis

[Note—Determine the suitability of the substrate by performing the *Analysis* using the appropriate amount of ▲USP Trypsin Bovine RS▲ (USP 1-Dec-2021) in place of the *Sample solution*.]

By means of a micropipet, transfer 50 µL of the *Sample solution* to a depression on a white spot plate. Add 0.2 mL of the *Substrate solution*.

Acceptance criteria: No purple color develops within 3 min (NMT 1% of trypsin).

SPECIFIC TESTS

• **pH ⟨791⟩:** 4.3–8.7, in the solution constituted as directed in the labeling

• **Sterility Tests ⟨71⟩:** Meets the requirements

• **Completeness of Solution ⟨641⟩:** It dissolves in the solvent and in the concentration recommended in the labeling to yield a clear solution.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in single-dose containers, preferably of Type I glass, and avoid exposure to excessive heat.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Chymotrypsin RS

▲USP Trypsin Bovine RS▲ (USP 1-Dec-2021)

Topic/Question	Contact	Expert Committee
CHYMOTRYPSIN FOR OPHTHALMIC SOLUTION	Julie Zhang Associate Science & Standards Liaison	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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