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Chymotrypsin

Chymotrypsin

CAS RN®: 9004-07-3.

DEFINITION

Chymotrypsin is a proteolytic enzyme crystallized from an extract of the pancreas gland of the ox, *Bos taurus* L. (Fam. Bovidae). It contains NLT 1000 USP Chymotrypsin Units/mg, calculated on the dried basis, and NLT 90.0% and NMT 110.0% of the labeled potency, as determined by the *Assay*.

Add the following:

▲IDENTIFICATION

• **A.** It meets the requirements in the *Assay*.

• **B.**

Solution A: 0.1% Phosphoric acid in water

Solution B: 0.1% Phosphoric acid in acetonitrile

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	75	25
25	55	45
30	10	90
34	10	90
35	75	25
45	75	25

Diluent: 1 mM hydrochloric acid

Standard solution: 1 mg/mL of USP Chymotrypsin RS in *Diluent*

Sample solution: 1 mg/mL of Chymotrypsin in *Diluent*

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm × 25-cm; 5-µm packing L26, pore size 300 Å

Temperatures

Column: 60°

Autosampler: 5°

Flow rate: 1.0 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

[NOTE—The retention time for the major peak of chymotrypsin is 16.65–20.35 min.]

Suitability requirements

Relative standard deviation: NMT 2.0% for the chymotrypsin peak, from triplicate injections

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the major peak of the *Standard solution*.▲ (USP 1-Dec-2021)

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: 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: 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 solution: Dissolve a quantity of Chymotrypsin in 0.0012 N hydrochloric acid to yield a solution containing 12–16 USP Chymotrypsin Units/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 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 of the *Sample solution* matches the first determination, of the same dilution, in rate of absorbance change.

Determine the average absorbance change per min, 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 number of USP Chymotrypsin Units/mg in the portion of Chymotrypsin taken:

$$\text{Result} = (A_2 - A_1)/(T \times W \times F)$$

A_2 = absorbance straight-line initial reading

A_1 = absorbance straight-line final reading

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

W = weight of Chymotrypsin in the volume of solution used in determining the absorbance (mg)

F = Chymotrypsin activity conversion factor, 0.0075/min

Acceptance criteria: NLT 1000 USP Chymotrypsin Units/mg on the dried basis; 90.0%–110.0% of the labeled potency

IMPURITIES

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 2.5%

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 in water

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 *Sample solution* to a depression on a white spot plate. Add 0.2 mL of *Substrate solution*.

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

SPECIFIC TESTS

Change to read:

• **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** ▲Total aerobic microbial count should be NMT 10^2 cfu/g. The total yeasts and molds count should be NMT 10^1 cfu/g.▲ (USP 1-Dec-2021) It meets the requirements of the tests for absence of *Pseudomonas aeruginosa*, *Salmonella* species, ▲*Escherichia coli*,▲ (USP 1-Dec-2021) and *Staphylococcus aureus*.

• **LOSS ON DRYING ⟨731⟩**

Analysis: Dry under vacuum at 60° for 4 h.

Acceptance criteria: NMT 5.0%

ADDITIONAL REQUIREMENTS

• **PACKAGING AND STORAGE:** Preserve in tight containers, and avoid exposure to excessive heat.

Change to read:

• **USP REFERENCE STANDARDS ⟨11⟩**

USP Chymotrypsin RS

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

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

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

Chromatographic Database Information: Chromatographic Database

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