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Change to read:

▲ (Official 1-Dec-2022) Trypsin

▲ (Title for this monograph—not to become official until December 1, 2022)

(Prior to December 1, 2022, the current practice of labeling the article of commerce with the name Crystallized Trypsin may be continued.

Use of the name Trypsin will be permitted as of June 1, 2020; however, the use of this name will not be mandatory until December 1, 2022. The 30-month extension will provide the time needed by manufacturers and users to make necessary changes.)▲ (Official 1-Dec-2022)

Add the following:

▲

```
IVGGYTGAN TVPYQVSLNS GYHFCGGSLI NSQWVSAAH CYKSGIQVRL
GEDNINVVEG NEQFISASKS IVHPSYNSNT LNNDIMLIKL KSAASLNSRV
ASISLPTSCA SAGTQCLISG WGNTKSSGTS YPDVLKCLKA PILSDSSCKS
AYPGQITSNM FCAGYLEGGK DSCQGDSSGP VVCSGKLQGI VSWGSGCAQK
NKPGVYTKVC NYVSWIKQTI ASN
```

```
IVGGYTCAAN SIPYQVSLNS GSHFCGGSLI NSQWVSAAH CYKSRIQVRL
GEHNIDVLEG NEQFINAAKI ITHPNFNGNT LDNDIMLIKL SSPATLNSRV
ATVSLPRSCA AAGTECLISG WGNTKSSGSS YPSLLQCLKA PVLSDSSCKS
SYPGQITGNM ICVGFLEGGK DSCQGDSSGP VVCNGQLQGI VSWGYGCAQK
NKPGVYTKVC NYVNWIQQTI AAN
```

▲C₁₀₁₂H₁₅₈₅N₂₇₉O₃₂₄S₁₄▲ (ERR 1-Dec-2020) 23,293 (for bovine β-Trypsin)

C₁₀₂₀H₁₅₉₇N₂₈₇O₃₂₁S₁₄ 23,463 (for porcine β-Trypsin) CAS RN®: 9002-07-7.▲ (USP 1-Dec-2020)

DEFINITION

Change to read:

▲▲ (USP 1-Dec-2020) Trypsin is a proteolytic enzyme ▲purified▲ (USP 1-Dec-2020) from an extract of the pancreas of healthy bovine or porcine animals. ▲▲ (USP 1-Dec-2020) When assayed as directed herein, it contains NLT ▲85 Trypsin Units/mg.▲ (USP 1-Dec-2020) calculated on the dried basis, and NLT 90.0% and NMT 110.0% of the labeled potency.

▲▲ (USP 1-Dec-2020)

IDENTIFICATION

Add the following:

▲• A. It meets the requirements in the Assay.▲ (USP 1-Dec-2020)

Add the following:

▲• B.

Solution A: 0.1% (v/v) phosphoric acid in water prepared as follows. To 1000 mL of [water](#) add 1 mL of 85% [phosphoric acid](#).

Solution B: 0.1% (v/v) phosphoric acid in acetonitrile prepared as follows. To 1000 mL of [acetonitrile](#) add 1 mL of 85% [phosphoric acid](#).

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: 20 mM calcium chloride and 0.01 N hydrochloric acid, pH 2.0 ± 0.2 prepared as follows. Dissolve 2.9 g of [calcium chloride dihydrate](#) in approximately 700 mL of [water](#), add 2.5 mL of 4 N [hydrochloric acid](#), mix well, and dilute with [water](#) to a final volume of 1000 mL. Adjust, if necessary, with 4 N [hydrochloric acid](#) to a pH of 2.0 ± 0.2 .

Standard solution

For [USP Trypsin Bovine RS](#): Dissolve a weighed quantity of [USP Trypsin Bovine RS](#) in *Diluent* to obtain a concentration of 70 mg/mL. Brief centrifugation at 2° – 8° may be necessary to remove the insoluble particles.

For [USP Trypsin Recombinant Porcine RS](#): The target protein concentration is 70 ± 10 mg/mL. Thaw 100 μ L of [USP Trypsin Recombinant Porcine RS](#) at room temperature for about 1 h and mix.

Sample solution: Dissolve a weighed quantity of Trypsin of the appropriate species in *Diluent* to obtain a concentration of 70 mg/mL. Brief centrifugation at 2° – 8° may be necessary to remove the insoluble portion of the sample.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 280 nm

Column: 4.6-mm $\times$ 25-cm; 3- μ m packing [L1](#), pore size 200 Å

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 1.0 mL/min

Injection volume: 1 μ L

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for α -trypsin and β -trypsin are 0.96 and 1.0, respectively. The retention time for the β -trypsin from bovine trypsin is approximately 11 min and from porcine trypsin is approximately 14 min.]

Suitability requirements

Resolution: NLT 1 between the α -trypsin and β -trypsin peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Acceptance criteria: The retention time of the β -trypsin peak of the *Sample solution* corresponds to that of the appropriate species of the *Standard solution*. ▲ (USP 1-Dec-2020)

ASSAY

Change to read:

• PROCEDURE

▲ **Buffer:** 100 mM Tris and 20 mM calcium chloride, pH 8.0, prepared as follows. Dissolve 1.21 g of [tris\(hydroxymethyl\)aminomethane](#) and 0.29 g of [calcium chloride dihydrate](#) in 100 mL of [water](#), and adjust with 2 N [hydrochloric acid](#) to a pH of 8.0 (at $25 \pm 1^{\circ}$).

Diluent: 20 mM calcium chloride and 0.01 N hydrochloric acid, pH 2.0 ± 0.2 , prepared as follows. Dissolve 2.9 g of [calcium chloride dihydrate](#) in approximately 700 mL of [water](#), add 2.5 mL of 4 N [hydrochloric acid](#), mix well, and dilute with [water](#) to a final volume of 1000 mL. Adjust, if necessary, with 4 N [hydrochloric acid](#) to a pH of 2.0 ± 0.2 .