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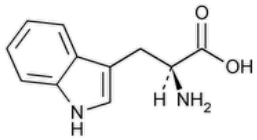
Tryptophan



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

L-Tryptophan CAS RN®: 73-22-3; UNII: 8DUH1N11BX.

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

- **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 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.]

Change to read:

- ▲**Iron ⟨241⟩, *Procedures, Procedure 1*▲** (CN 1-Jun-2023) **:** NMT 30 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

Time (min)	Solution A (%)	Solution B (%)
2	95	5
37	35	65
42	0	100
47	0	100
50	95	5
60	95	5

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *System suitability solution*

Suitability requirement

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of each unspecified impurity in the *Sample solution*

r_S = peak area of tryptophan related compound B in the *Standard solution*

C_S = concentration of USP Tryptophan Related Compound B RS in the *Standard solution* (µg/mL)

C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria

Total impurities 1: NMT 0.01% of the total impurities eluting prior to the tryptophan peak

Total impurities 2: NMT 0.03% of the total impurities eluting after the tryptophan peak. [Note—Exclude the peak for tryptophan related compound B.]

Tryptophan related compound A: If a peak for tryptophan related compound A is observed in the *Sample solution*, then perform the test for *Procedure 2: Limit of Tryptophan Related Compound A*, below.

- **Procedure 2: Limit of Tryptophan Related Compound A**

Solution A: 18 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (9:1)

Solution B: 10 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (1:1)

Solution C: Acetonitrile in water (7:3)

Standard solution: 0.1 mg/L of USP Tryptophan Related Compound A RS in water

Sample solution: 10.0 mg/mL of Tryptophan in water

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)	Solution C (%)
0	100	0	0
30	44	56	0
30.1	0	0	100
45	0	0	100
45.1	100	0	0
60	100	0	0

Chromatographic system

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

Mode: LC

Detector: UV 216 nm

Column: 3.9-mm × 15-cm; 5-µm packing L1

Column temperature: 30°

Flow rate: 1 mL/min

Injection size: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirement

Relative standard deviation: NMT 5.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of tryptophan related compound A in the portion of Tryptophan taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak area of tryptophan related compound A in the *Sample solution*

r_S = peak area of tryptophan related compound A in the *Standard solution*

C_S = concentration of USP Tryptophan Related Compound A RS in the *Standard solution* (µg/mL)

C_U = concentration of Tryptophan in the *Sample solution* (µg/mL)

Acceptance criteria: NMT 10 ppm

SPECIFIC TESTS

- **Optical Rotation, *Specific Rotation*⟨781S⟩:** −29.4° to −32.8°

  Sample solution: 10 mg/mL, in water. [Note—Heat gently to dissolve, if necessary.]
- **pH ⟨791⟩:** 5.5–7.0, in a solution (1 in 100)
- **Loss on Drying ⟨731⟩:** Dry a sample at 105° for 3 h: it loses NMT 0.3% of its weight.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in well-closed containers.
- **USP Reference Standards ⟨11⟩**

USP L-Tryptophan RS

USP Tryptophan Related Compound A RS

(2*S*,2'*S*)-3,3'-[Ethane-1,1-diylbis(1*H*-indole-1,3-diyl)]bis(2-aminopropanoic acid).

$C_{24}H_{26}N_4O_4$ 434.50

USP Tryptophan Related Compound B RS

2-Acetamido-3-(1*H*-indol-3-yl)propanoic acid.

$C_{13}H_{14}N_2O_3$ 246.3

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

Topic/Question	Contact	Expert Committee
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REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	NBDS2020 Non-botanical Dietary Supplements

Chromatographic Database Information: Chromatographic Database

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