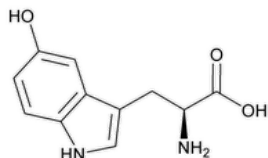


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5-Hydroxy-L-tryptophan



$C_{11}H_{12}N_2O_3$ 220.23

(S)-2-Amino-3-(5-hydroxy-1H-indol-3-yl)propanoic acid CAS RN®: 4350-09-8.

DEFINITION

5-Hydroxy-L-tryptophan contains NLT 98.5% and NMT 101.5% of 5-hydroxy-L-tryptophan ($C_{11}H_{12}N_2O_3$), calculated on the dried basis.

IDENTIFICATION

Change to read:

- A. [▲ SPECTROSCOPIC IDENTIFICATION TESTS \(197\), Infrared Spectroscopy: 197K ▲](#) (CN 1-MAY-2020)

ASSAY

• PROCEDURE

Sample: 200 mg of 5-Hydroxy-L-tryptophan

Blank: Mix 3 mL of formic acid and 50 mL of glacial acetic acid.

Titrimetric system

(See [Titrimetry \(541\)](#).)

Mode: Direct titration

Titrant: 0.1 N perchloric acid VS

Endpoint detection: Potentiometric

Analysis

Samples: *Sample* and *Blank*

Dissolve the *Sample* in a mixture of 3 mL of formic acid and 50 mL of glacial acetic acid, and titrate with the *Titrant*. Perform a *Blank* titration, and make any necessary correction.

Calculate the percentage of 5-hydroxy-L-tryptophan ($C_{11}H_{12}N_2O_3$) in the *Sample* taken:

$$\text{Result} = \{[(V_S - V_B) \times N \times F] / W\} \times 100$$

V_S = *Titrant* volume consumed by the *Sample* (mL)

V_B = *Titrant* volume consumed by the *Blank* (mL)

N = actual normality of the *Titrant* (mEq/mL)

F = equivalency factor, 220.2 mg/mEq

W = *Sample* weight (mg)

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

IMPURITIES

- [RESIDUE ON IGNITION \(281\)](#): NMT 0.2%
- [CHLORIDE AND SULFATE, Chloride \(221\)](#).

Standard solution: 0.50 mL of 0.020 N hydrochloric acid

Sample: 0.73 g of 5-Hydroxy-L-tryptophan

Acceptance criteria: NMT 0.05%

• **CHLORIDE AND SULFATE, *Sulfate* (221).**

Standard solution: 0.10 mL of 0.020 N sulfuric acid

Sample: 0.33 g of 5-Hydroxy-L-tryptophan

Acceptance criteria: NMT 0.03%

• **ORGANIC IMPURITIES**

Solution A: 1 mL/L of trifluoroacetic acid in water

Solution B: 1 mL/L of trifluoroacetic acid in a mixture of acetonitrile and water (80:20)

Mobile phase: See [Table 1](#).

Table 1

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

Standard solution: 1.0 µg/mL of [USP 5-Hydroxy-L-tryptophan RS](#) and 50 µg/mL of [USP L-Tryptophan RS](#) in water

Sample solution: 10.0 mg/mL of 5-Hydroxy-L-tryptophan in water

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 volume: 20 µL

System suitability

Sample: *Standard solution*

[NOTE—The relative retention times for 5-hydroxy-L-tryptophan and L-tryptophan are 1.0 and 1.6, respectively.]

Suitability requirements

Relative standard deviation: NMT 5.0% for the 5-hydroxy-L-tryptophan and L-tryptophan peaks

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each unspecified impurity in the portion of 5-Hydroxy-L-tryptophan taken:

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

r_U = peak response of each unspecified impurity from the *Sample solution*

r_S = peak response of 5-hydroxy-L-tryptophan from the *Standard solution*

C_S = concentration of [USP 5-Hydroxy-L-tryptophan RS](#) in the *Standard solution* (µg/mL)

C_U = concentration of 5-Hydroxy-L-tryptophan in the *Sample solution* (µg/mL)