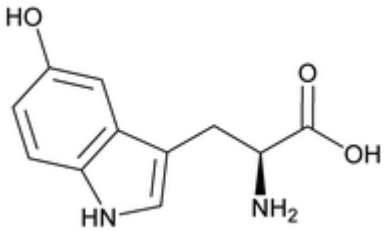
5-Hydroxy- L-tryptophan



$C_{11}H_{12}N_2O_3$ 220.23
(*S*)-2-Amino-3-(5-hydroxy-1*H*-indol-3-yl)propanoic acid [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)

Calculate the percentage of tryptophan 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 tryptophan from the *Sample solution*
r_S = peak response of tryptophan from the *Standard solution*
C_S = concentration of USP L-Tryptophan RS in the *Standard solution* (µg/mL)
C_U = concentration of 5-Hydroxy-L-tryptophan in the *Sample solution* (µg/mL)

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Acceptance criteria
Total impurities 1: NMT 0.01% of the total impurities eluting prior to the 5-hydroxy-L-tryptophan peak
Total impurities 2: NMT 0.03% of the total impurities eluting after the 5-hydroxy-L-tryptophan peak. [NOTE—Exclude the peak for tryptophan.]
Tryptophan: NMT 0.5%

SPECIFIC TESTS

- **OPTICAL ROTATION,** *Specific Rotation* ⟨781⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: –30.0° to –38.0°
- **PH** ⟨791⟩
Sample solution: 10 mg/mL in water
Acceptance criteria: 4.0–6.0
- **LOSS ON DRYING** ⟨731⟩
Analysis: Dry at 105° for 3 h.
Acceptance criteria: NMT 2.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP 5-Hydroxy-L-tryptophan RS
USP L-Tryptophan RS

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