L-Tryptophan[1]

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

L-Tryptophan, when dried, contains not less than 99.0 percent and not more than 101.0 percent of L-Tryptophan ($C_{11}H_{12}N_2O_2$).

Description

White to yellowish white crystals or crystalline powder; slightly bitter taste.
Freely soluble in formic acid, slightly soluble in water, very slightly soluble in ethanol (95).
Dissolves in dilute hydrochloric acid.

Identification

Compare the infrared absorption spectrum of the sample with that of the standard by potassium bromide disc method.

Specifications

Item	Limit	Test
Specific rotation $[\alpha]_D^{20}$	-30.5 to -32.5°	AJI TEST 1 [Dried sample, C=1, H_2O, dissolve by warming][2]
State of solution (Transmittance)	Clear Not less than 95.0%	AJI TEST 2 [0.5g in 20mL of 2mol/L HCl, spectrophotometer, 430nm, 10mm cell thickness]
Chloride (Cl)	Not more than 0.020%	AJI TEST 3 [0.5g, A-1, ref: 0.28mL of 0.01mol/L HCl]
Ammonium (NH_4)	Not more than 0.02%	AJI TEST 4 [D-1]
Sulfate (SO_4)	Not more than 0.020%	AJI TEST 5 [0.85g, (1), ref: 0.35mL of 0.005mol/L H_2SO_4]
Iron (Fe)	Not more than 10ppm	AJI TEST 6 [0.75g, B-2, ref: 0.75mL of Iron Std. (0.01mg/mL)]
Heavy metals (Pb)	Not more than 10ppm	AJI TEST 7 [1.0g, (4), ref: 1.0mL of Pb Std. (0.01mg/mL)]
Arsenic (As_2O_3)	Not more than 1ppm	AJI TEST 8 [1.0g, (1), ref: 1.0mL of As_2O_3 Std.]
Related substances	1) Conforms 2) Any unspecified impurity: Not more than 0.20% Total impurities: Not more than 0.50% 3) EBT [3] not detected[4] Total Impurities 1 Not more than 100ppm Total Impurities 2 Not more than 100ppm	AJI TEST 9 [1mol/L HCl, test sample: 50μg, B-6-a, control; L-Trp 0.25μg][5] AJI TEST 26[6] HPLC [Mayo method][7]

L-Tryptophan[1]

Specifications (cont'd)

Item	Limit	Test
Loss on drying	Not more than 0.20%	AJI TEST 11 [1g, at 105℃ for 3 hours]
Residue on ignition (Sulfated)	Not more than 0.10%	AJI TEST 13 [1g, at 550℃ to 650℃ for 3 hours]
Assay	99.0 to 101.0%	AJI TEST 14 [Dried sample, 200mg, (1), 3mL of formic acid, 50mL of acetic acid (100), 0.1mol/L $HClO_4$ 1mL=20.42mg $C_{11}H_{12}N_2O_2$]
pH	5.5 to 6.4	AJI TEST 33 [1.0g in 100mL of H_2O]

The test for Endotoxin when the material will be used for manufacturing parenteral products is as follows:

Item	Limit	Test
Endotoxin	Less than 6.0EU/g	AJI TEST 34 [C=1, ultrafiltration, kinetic-turbidimetric technique]

[1] This product, in terms of actual quality, conforms to USP, EP, and JP.

[2] Temperature coefficient of $[\alpha]_D^t$: +0.07°

[3] 1,1'-ethylidene-bis-tryptophan (EBT=peakE=DTAA)

[4] Report as "not detected" when the result is less than the detection limit.

[5] Test solution preparation:

Dissolve 0.50g in 2mL of 1mol/L HCl and 25mL of H_2O, fill up to 50mL with water.

[6] Disregard limit: 0.05%

[7] Related substances : Based on the Mayo Method

-Procedure 1

Mobile phase A: Trifluoroacetic acid in water (1mL/L)

Mobile phase B: Trifluoroacetic acid in an acetonitrile and water solution (80:20) (1mL/L trifluoroacetic acid solution)

Standard solution: 1.0mg/L of USP Tryptophan Related Compound A RS (=EBT) in water (For confirmation of retention time)
1.0mg/L of USP Tryptophan Related Compound B (=N-Ac-L-Trp) RS in water
1.0mg/L of IDPT (IDPT Std.) in water (For confirmation of retention time)

Sample solution: 10.0mg/mL of tryptophan in water

System suitability solution: 1.0mg/L of USP Tryptophan Related Compound B RS in water

Mobile phase: See the gradient table below.

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

L-Tryptophan[1]

Analytical condition
Detector: UV220nm
Column: 4.6mm × 25cm; Beckman Ultrasphere ODS 5μm
Column temperature: 30℃
Flow rate: 1mL/min
Injection size: 20μL
System suitability
Sample: System suitability solution
Suitability requirement
Relative standard deviation: NMT 5.0%

Analysis
Inject 20μL each of sample and standard solution, detect by HPLC, and calculate the concentration of each unspecified impurity in the portion of Tryptophan taken:

$$\text{Concentration (ppm)} = (r_u/r_s) \times (c_s/w_u) \times 100 \text{ (mL)}$$

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 (1.0μg/mL)
w_u=Sample(L-tryptophan) amount (g)

Total impurities 1: The total impurities eluting prior to the tryptophan peak
Total impurities 2: The total impurities eluting after the tryptophan peak

Disregard limit:4ppm

If a peak for tryptophan related compound A is detected in the Sample solution, then perform the test for Procedure 2 below.

-Procedure 2
Mobile phase A: 18mM monobasic sodium phosphate, filtered and degassed(pH2.5), and acetonitrile (9:1)
Mobile phase B: 10mM monobasic sodium phosphate, filtered and degassed(pH2.5), and acetonitrile (1:1)
Mobile phase C: Acetonitrile in water (7:3)
Standard solution: 0.1mg/L of USP Tryptophan Related Compound A RS in water
Sample solution: 10.0mg/mL of L-tryptophan in water

Mobile phase: See the gradient table below.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Mobile phase 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

L-Tryptophan[1]

Analytical condition
Detector: UV216nm
Column: 3.9mm × 15cm; Waters Delta-Pak C18 5μm
Column temperature: 30℃
Flow rate: 1mL/min
Injection size: 20μL

System suitability
Sample: System suitability solution
Suitability requirement
Relative standard deviation: NMT 5.0%

Analysis
Inject 20μL each of sample and standard solution, detect by HPLC.

Acceptance criteria: The peak is not detected in the vicinity of the retention time of tryptophan related compound A.

If a peak is detected in the vicinity of the retention time of tryptophan related compound A, then perform the test for Procedure 3: spike test.

-Procedure 3: spike test
The analytical condition is the same as that of Procedure 2.
If the substance suspected to be tryptophan related compound A is detected, calculate the concentration using the following formula:

$$\text{Concentration (ppm)} = (r_u/r_s) \times (c_s/w_u) \times 100 \text{ (mL)}$$

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 (1.0μg/mL)
w_u=Sample (L-tryptophan) amount (g)

1. Measure the sample solution and the sample solution added with USP Tryptophan Related Compound A RS whose concentration is the same as the concentration[8] of the substance suspected to be tryptophan related compound A, at the same time.
2. Evaluate if the peak top of the substance suspected to be tryptophan related compound A is corresponding to the peak top of tryptophan related compound A.
3. If the peak top of the substance suspected to be tryptophan related compound A isn't corresponding to the peak top of tryptophan related compound A, the substance is not tryptophan related compound A.

Remark：Inject a water blank before injecting the sample, and remove the detected impurities.

[8] If the concentration is NMT the detection limit, add USP Tryptophan Related Compound A RS whose concentration is the detection limit.

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