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

1) Compare the infrared absorption spectrum of the sample with that of the standard by potassium bromide disc method.
2) Compare the position and ninhydrin reaction of the test solution with that of the reference solution by chromatographic separation technique.

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 [0.5 g in 20 mL of 2 mol/L HCl, spectrophotometer, 430 nm, 10 mm cell thickness]
Chloride (Cl)	Not more than 0.020%	AJI TEST 3 [0.5 g, A-1, ref: 0.28 mL of 0.01 mol/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 [0.85 g, (1), ref: 0.35 mL of 0.005 mol/L H_2SO_4]
Iron (Fe)	Not more than 10 ppm	AJI TEST 6 [0.75 g, B-2, ref: 0.75 mL of Iron Std. (0.01 mg/mL)]
Heavy metals (Pb)	Not more than 10 ppm	AJI TEST 7 [1.0 g, (4), ref: 1.0 mL of Pb Std. (0.01 mg/mL)]
Arsenic (As_2O_3)	Not more than 1 ppm	AJI TEST 8 [1.0 g, (1), ref: 1.0 mL of As_2O_3 Std.]
Related substances	1) Conforms	AJI TEST 9 [1 mol/L HCl, test sample: 50 μg, B-6-a, control; L-Trp 0.25 μg][3]
	2) Any unspecified impurity: Not more than 0.20% Total impurities: Not more than 0.50%	AJI TEST 26[4]
	3) EBT[5] Not detected[6] Total Impurities 1 Not more than 100 ppm Total Impurities 2 Not more than 100 ppm	HPLC [Mayo method][7]

Specifications (cont'd)

Item	Limit	Test
Loss on drying	Not more than 0.20%	AJI TEST 11 [1 g, at 105°C for 3 hours]
Residue on ignition (Sulfated)	Not more than 0.10%	AJI TEST 13 [1 g, at 550°C to 650°C for 3 hours]
Assay	99.0 to 101.0%	AJI TEST 14 [Dried sample, 200 mg, (1), 3 mL of formic acid, 50 mL of acetic acid (100), 0.1 mol/L $HClO_4$ 1 mL = 20.42 mg $C_{11}H_{12}N_2O_2$]
pH	5.5 to 6.4	AJI TEST 33 [1.0 g in 100 mL 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 6.0 EU/g	AJI TEST 34 [C = 1, ultrafiltration, kinetic-turbidimetric technique]

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

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

[3] Test solution preparation:
Dissolve 0.50 g in 2 mL of 1 mol/L HCl and 25 mL of H_2O, fill up to 50 mL with water.

[4] Disregard limit: 0.05%
The quantitative results are presented numerically to the CoA

[5] 1,1'-ethylidene-bis-tryptophan (EBT = peak E = DTAA)

[6] Report as "Not detected" when the result is less than the detection limit.

[7] Related substances: Based on the Mayo Method

-Procedure 1

[Solution preparation]

Mobile phase A	: Trifluoroacetic acid in water (1 mL/L)
Mobile phase B	: Trifluoroacetic acid in an acetonitrile and water solution (80:20) (1 mL/L trifluoroacetic acid solution)
Standard solution A	: 1.0 mg/L of USP Tryptophan Related Compound A RS (= EBT) in water (For confirmation of retention time)
Standard solution B	: 1.0 mg/L of USP Tryptophan Related Compound B (= N-Ac-L-Trp) RS in water
Standard solution C	: 1.0 mg/L of IDPT (IDPT Std.) in water (For confirmation of retention time)
Sample solution	: 10.0 mg/mL of L-Tryptophan in water
System suitability solution	: Use the Standard solution B

[Gradient condition]

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

[Analytical condition]

Detector : UV 220 nm
Column : 4.6 nm × 25 cm; Hichrom Ultrasphere ODS 5 μm
Column temperature : 30°C
Flow rate : 1 mL/min
Injection size : 20 μL

[System suitability]

Sample : System suitability solution
Suitability requirement : Relative standard deviation Not more than 5.0%

[Analysis]

Inject 20 μL each of the Standard solution (A, B and C) and the Sample solution, and calculate the concentration of each unspecified impurity and IDPT in L-Tryptophan. Also, confirm that EBT is not detected. If EBT is detected, perform **Procedure 2** as “Impurity suspected of EBT”.

Calculation formula:

Impurity (ppm) = $(r_u/r_s) \times (c_s/w_u) \times 100$ (mL)

r_u = peak area of each impurity in the Sample solution
r_s = peak area of Tryptophan Related Compound B in the Standard solution B
c_s = concentration of Tryptophan Related Compound B in the Standard solution B (1.0 μg/mL)
w_u = Sample (L-Tryptophan) amount (g)

Disregard limit: 4 ppm

Impurities:

EBT : An impurity detected in the vicinity of the peak retention time of Tryptophan Related Compound A in the Standard solution A.
IDPT : An impurity detected in the vicinity of the peak retention time of IDPT in the Standard solution C.
Total impurities 1 : The total impurities eluting prior to the tryptophan peak.
Total impurities 2 : The total impurities eluting after the tryptophan peak.

-Procedure 2

Identify “Impurity suspected of EBT” detected in **Procedure 1**.

[Solution preparation]

Mobile phase A : 18 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (9:1)
Mobile phase B : 10 mM monobasic sodium phosphate, filtered and degassed (pH 2.5), and acetonitrile (1:1)
Mobile phase 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 L-Tryptophan in water

[Gradient condition]

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

[Analytical condition]

Detector : UV 216 nm
Column : 3.9 mm × 15 cm; Waters Delta-Pak C18 5 μm
Column temperature : 30℃
Flow rate : 1 mL/min
Injection size : 20 μL

[System suitability]

Sample : Standard solution
Suitability requirement : Relative standard deviation: Not more than 5.0%

[Analysis]

Inject 20 μL each of the Standard solution and the Sample solution.

Criteria:

The peak is not detected in the vicinity of the peak retention time of the Standard solution (EBT is “Not detected”).
If the peak is detected, perform the test for **Procedure 3: spike test** as “Impurity suspected of EBT”.

-Procedure 3: spike test

"Impurity suspected of EBT" detected in **Procedure 2** is further identified by using spike test.

[Solution preparation]

Sample solution : 10.0 mg/mL of L-Tryptophan in water (same as **Procedure 2)**

Sample solution with EBT addition : USP Tryptophan Related Compound A RS (=EBT) in the Sample solution. The amount of addition is equivalent to that of "Impurity suspected of EBT" detected in **Procedure 2** calculated by the following equation. If the amount of addition is not more than the detection limit, the amount corresponding to the detection limit is added.

Impurity (ppm) = $(r_u/r_s) \times (c_s/w_u) \times 100$ (mL)

r_u = peak area of the impurity suspected to be 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 (0.1 μg/mL)

w_u = Sample (L-Tryptophan) amount (g)

[Analytical condition]

The analytical condition of **Procedure 3** is the same as that of **Procedure 2**.

[Analysis]

Inject 20 μL each of water (Blank), the Sample solution and the Sample solution with EBT addition. Considering the condition of peak detection of water (Blank) and the Sample solution, confirm the separation between the peak top of "Tryptophan Related Compound A (=EBT)" and that of "Impurity suspected of EBT" obtained from the Sample solution with EBT addition.

Criteria:

These peak tops don't overlap completely (EBT is "Not detected").

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