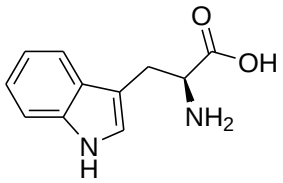
Tryptophan

L-Tryptophan



$C_{11}H_{12}N_2O_2$ Mol. Wt. 204.2

Tryptophan contains not less than 98.5 per cent and not more than 101.5 per cent of $C_{11}H_{12}N_2O_2$, calculated on the dried basis.

Category. Amino acid

Description. A white to slightly yellowish-white crystals.

Identification

Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *L-tryptophan IPRS* or with the reference spectrum of L-tryptophan.

Tests

pH (2.4.24). 5.5 to 7.0, determined in 1 per cent w/v solution.

Specific optical rotation (2.4.22). –32.8° to –29.4°, determined in 1.0 per cent w/v solution in *water*, heat gently to dissolve.

Related substances. Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10.0 ml of *water*.

Reference solution (a). A solution containing 0.0001 per cent w/v, each of, *tryptophan related compound A IPRS* and *tryptophan related compound B IPRS* in *water*.

Reference solution (b). A 0.0001 per cent w/v solution of *tryptophan related compound B IPRS* in *water*.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. 0.1 per cent v/v of *trifluoroacetic acid* in *water*,
  B. 0.1 per cent v/v solution of *trifluoroacetic acid* in a mixture of 80 volumes of *acetonitrile* and 20 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 220 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
2	95	5
37	35	65
42	0	100
47	0	100
50	95	5
60	95	5

Inject reference solution (b). The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the sum of the areas of all the secondary peaks, eluting prior to tryptophan peak is not more than the area of the peak due to tryptophan related compound B in the chromatogram obtained with reference solution (a) (0.01 per cent) and the sum of the areas of all the secondary peaks, eluting after the tryptophan peak other than tryptophan related

compound B peak is not more than 3 times the area of the peak due to tryptophan related compound B in the chromatogram obtained with reference solution (a) (0.03 per cent).

NOTE - If a peak for tryptophan related compound A is observed in the test solution, then perform the test for Tryptophan related compound A.

Tryptophan related compound A. Not more than 10 ppm.

Determine by liquid chromatography (2.4.14).

Test solution. Dissolve 100 mg of the substance under examination in 10.0 ml of *water*.

Reference solution. A 0.001 per cent w/v solution of *tryptophan related compound A IPRS* in *water*. Dilute 1.0 ml of the solution to 100.0 ml with *water*.

Chromatographic system

- a stainless steel column 15 cm x 3.9 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: A. a mixture of 90 volumes of *0.018 M monobasic sodium phosphate*, adjusted to pH 2.5 and 10 volumes of *acetonitrile*,
  B. a mixture of 50 volumes of *0.01 M monobasic sodium phosphate*, adjusted to pH 2.5 and 50 volumes of *acetonitrile*,
  C. a mixture of 70 volumes of *acetonitrile* and 30 volumes of *water*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 216 nm,
- injection volume: 20 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)	Mobile phase C (per cent v/v)
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

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 5.0 per cent.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to tryptophan related compound A is not more than the area of the principal peak in the chromatogram obtained with the reference solution.

Iron (2.3.14). 1.33 g complies with the limit test for iron (30 ppm).

Chlorides (2.3.12). 0.5 g complies with the limit test for chlorides (500 ppm).

Sulphates (2.3.17). 0.5 g complies with the limit test for sulphates (300 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Loss on drying (2.4.19). Not more than 0.3 per cent, determined on 1.0 g by drying in an oven at 105° for 3 hours.

Assay. Dissolve 0.2 g of the substance under examination, in a mixture of 3 ml of *formic acid* and 50 ml of *glacial acetic acid* and titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (2.4.25). Carry out a blank titration.

1 ml of *0.1 M perchloric acid* is equivalent to 0.02042 g of $C_{11}H_{12}N_2O_2$.

Storage. Store protected from moisture.

Solubility. Soluble in hot *ethanol* and in *dilute hydrochloric acid.*