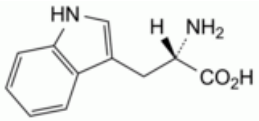
Edition: BP 2025 (Ph. Eur. 11.6 update)

Tryptophan

General Notices

(Ph. Eur. monograph 1272)



$C_{11}H_{12}N_2O_2$ 204.2 *73-22-3*

Ph Eur

DEFINITION

(2*S*)-2-Amino-3-(1*H*-indol-3-yl)propanoic acid.

Product of fermentation or of protein hydrolysis.

Content

98.5 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance

White or almost white, crystalline or amorphous powder.

Solubility

Sparingly soluble in water, slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

First identification: A, B.

Second identification: A, C, D.

A. Specific optical rotation (see Tests).
B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *tryptophan CRS*.

C. Thin-layer chromatography (*2.2.27*).

Solvent mixture *glacial acetic acid R*, *water R* (50:50 *V/V*).

Test solution Dissolve 10 mg of the substance to be examined in the solvent mixture and dilute to 50 mL with the solvent mixture.

Reference solution Dissolve 10 mg of *tryptophan CRS* in the solvent mixture and dilute to 50 mL with the solvent mixture.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (20:20:60 *V/V/V*).

Application 5 µL.

Development Over 2/3 of the plate.

Drying In air.

Detection Spray with *ninhydrin solution R* and heat at 105 °C for 15 min.

Results The principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. Dissolve about 20 mg in 10 mL of *water R*. Add 5 mL of *dimethylaminobenzaldehyde solution R6* and 2 mL of *hydrochloric acid R1*. Heat on a water-bath. A purple-blue colour develops.

TESTS

Appearance of solution

The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Dissolve 0.1 g in *1 M hydrochloric acid* and dilute to 10 mL with the same acid.

Specific optical rotation (*2.2.7*)

-33.0 to -30.0 (dried substance).

Dissolve 0.25 g in *water R*, heating on a water-bath if necessary, and dilute to 25.0 mL with the same solvent.

Ninhydrin-positive substances

Amino acid analysis (*2.2.56*). For analysis, use Method 1.

The concentrations of the test solution and the reference solutions may be adapted according to the sensitivity of the equipment used. The concentrations of all solutions are adjusted so that the system suitability requirements described in general chapter *2.2.46* are fulfilled, keeping the ratios of concentrations between all solutions as described.

Solution A *dilute hydrochloric acid R1* or a sample preparation buffer suitable for the apparatus used.

Test solution Dissolve 30.0 mg of the substance to be examined in solution A and dilute to 50.0 mL with solution A.

Reference solution (a) Dilute 1.0 mL of the test solution to 100.0 mL with solution A. Dilute 2.0 mL of this solution to 10.0 mL with solution A.

Reference solution (b) Dissolve 30.0 mg of *proline R* in solution A and dilute to 100.0 mL with solution A. Dilute 1.0 mL of the solution to 250.0 mL with solution A.

Reference solution (c) Dilute 6.0 mL of *ammonium standard solution (100 ppm NH_4) R* to 50.0 mL with solution A. Dilute 1.0 mL of this solution to 100.0 mL with solution A.

Reference solution (d) Dissolve 30 mg of *isoleucine R* and 30 mg of *leucine R* in solution A and dilute to 50.0 mL with solution A. Dilute 1.0 mL of the solution to 200.0 mL with solution A.

Blank solution Solution A.

Inject suitable, equal amounts of the test, blank and reference solutions into the amino acid analyser. Run a program suitable for the determination of physiological amino acids.

System suitability Reference solution (d):

— *resolution*: minimum 1.5 between the peaks due to isoleucine and leucine.

Calculation of percentage contents:

— for any ninhydrin-positive substance detected at 570 nm, use the concentration of tryptophan in reference solution (a);

— for any ninhydrin-positive substance detected at 440 nm, use the concentration of proline in reference solution (b); if a peak is above the reporting threshold at both wavelengths, use the result obtained at 570 nm for quantification.

Limits:

— *any ninhydrin-positive substance*: for each impurity, maximum 0.2 per cent;

— *total*: maximum 0.5 per cent;

— *reporting threshold*: 0.05 per cent.

The thresholds indicated under Related substances (Table 2034.-1) in the general monograph *Substances for pharmaceutical use (2034)* do not apply.

Impurity A and other related substances

Liquid chromatography (*2.2.29*). *Prepare the standard, test and reference solutions immediately before use.*

Buffer solution pH 2.3 Dissolve 3.90 g of *sodium dihydrogen phosphate R* in 1000 mL of *water R*. Add about 700 mL of a 2.9 g/L solution of *phosphoric acid R* and adjust to pH 2.3 with the same acid solution.

Solvent mixture *acetonitrile R*, *water R* (10:90 *V/V*).

Standard solution Dissolve 10.0 mg of *N-acetyltryptophan R* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 2.0 mL of this solution to 100.0 mL with the solvent mixture.

Test solution (a) Dissolve 0.10 g of the substance to be examined in the solvent mixture and dilute to 10.0 mL with the solvent mixture.

Test solution (b) Dissolve 0.10 g of the substance to be examined in the standard solution and dilute to 10.0 mL with the standard solution.

Reference solution (a) Dissolve the contents of a vial of *1,1'-ethylidenebistryptophan CRS* (impurity A) in 1.0 mL of the solvent mixture.

Reference solution (b) Dissolve the contents of a vial of *1,1'-ethylidenebistryptophan CRS* (impurity A) in 1.0 mL of the standard solution.

Reference solution (c) Dilute 0.5 mL of reference solution (a) to 5.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, *Ø* = 4.6 mm;

— *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);

— *temperature*: 40 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R*, buffer solution pH 2.3 (115:885 *V/V*);

— *mobile phase B*: *acetonitrile R*, buffer solution pH 2.3 (350:650 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	100	0
10 - 45	100 → 0	0 → 100
45 - 65	0	100

Flow rate 0.7 mL/min.

Detection Spectrophotometer at 220 nm.

Injection 20 µL of test solutions (a) and (b) and reference solutions (b) and (c).

Retention time Tryptophan = about 8 min; *N*-acetyltryptophan = about 29 min; impurity A = about 34 min.

System suitability:

— *resolution*: minimum 8.0 between the peaks due to *N*-acetyltryptophan and impurity A in the chromatogram obtained with reference solution (b); if necessary, adjust the time programme for the elution gradient (an increase in the duration of elution with mobile phase A produces longer retention times and a better resolution);

— *signal-to-noise ratio*: minimum 15 for the principal peak in the chromatogram obtained with reference solution (c);

— *symmetry factor*: maximum 3.5 for the peak due to impurity A in the chromatogram obtained with reference solution (b);

— in the chromatogram obtained with test solution (a) there is no peak with the same retention time as *N*-acetyltryptophan (in such case correct the area of the *N*-acetyltryptophan peak).

Limits Test solution (b):

— *impurity A*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (10 ppm);

— *sum of impurities with a retention time less than that of tryptophan*: not more than 0.6 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (100 ppm);

— *sum of impurities with a retention time greater than that of tryptophan and up to 1.8 times the retention time of N-acetyltryptophan*: not more than 1.9 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b) (300 ppm);

— *disregard limit*: 0.02 times the area of the peak due to *N*-acetyltryptophan in the chromatogram obtained with reference solution (b); disregard the peak due to *N*-acetyltryptophan.

Chlorides (*2.4.4*)

Maximum 200 ppm.

Dissolve 0.25 g in 3 mL of *dilute nitric acid R* and dilute to 15 mL with *water R*. The solution, without any further addition of nitric acid, complies with the test.

Sulfates (*2.4.13*)

Maximum 300 ppm.

Dissolve 0.5 g in a mixture of 5 volumes of *dilute hydrochloric acid R* and 25 volumes of *distilled water R*, and dilute to 15 mL with the same mixture of solvents.

Ammonium

Amino acid analysis (*2.2.56*) as described in the test for ninhydrin-positive substances with the following modifications.

Injection Test solution, reference solution (c) and blank solution.

Limit:

— *ammonium at 570 nm*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.02 per cent), taking into account the peak due to ammonium in the chromatogram obtained with the blank solution.

Iron (*2.4.9*)

Maximum 20 ppm.

In a separating funnel, dissolve 0.50 g in 10 mL of *dilute hydrochloric acid R*. Shake with 3 quantities, each of 10 mL, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 mL of *water R* and shake for 3 min. Examine the aqueous layer.

Loss on drying (*2.2.32*)

Maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*)

Maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 3 mL of *anhydrous formic acid R*. Add 50 mL of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

1 mL of *0.1 M perchloric acid* is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

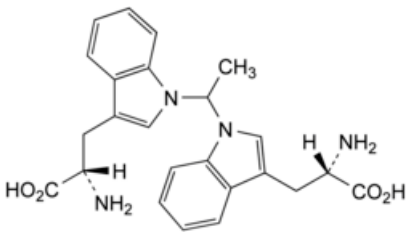
STORAGE

Protected from light.

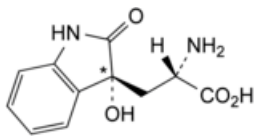
IMPURITIES

Specified impurities A.

Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) B, C, D, E, F, G, H, I, J, K, L.

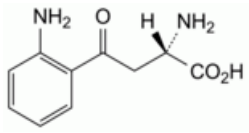


A. 3,3′-[ethane-1,1-diylbis(1*H*-indol-1,3-diyl)]bis[(2*S*)-2-aminopropanoic acid] (ethane-1,1-diylbistryptophan),

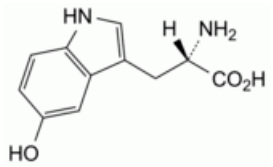


and epimer at C*

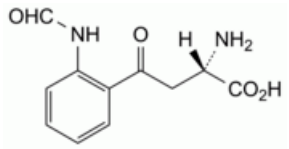
B. (2*S*)-2-amino-3-[(3*RS*)-3-hydroxy-2-oxo-2,3-dihydro-1*H*-indol-3-yl]propanoic acid (dioxyindolylalanine),



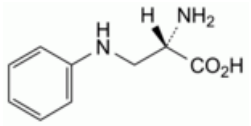
C. (2*S*)-2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (kynurenine),



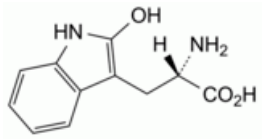
D. (2*S*)-2-amino-3-(5-hydroxy-1*H*-indol-3-yl)propanoic acid (5-hydroxytryptophan),



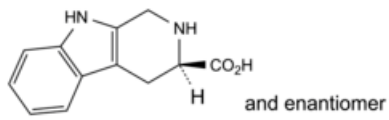
E. (2*S*)-2-amino-4-[2-(formylamino)phenyl]-4-oxobutanoic acid (*N*-formylkynurenine),



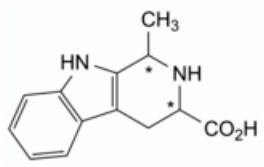
F. (2*S*)-2-amino-3-(phenylamino)propanoic acid (3-phenylaminoalanine),



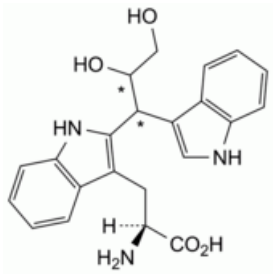
G. (2*S*)-2-amino-3-(2-hydroxy-1*H*-indol-3-yl)propanoic acid (2-hydroxytryptophan),



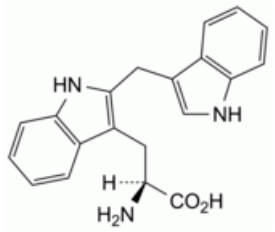
H. (3*RS*)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid,



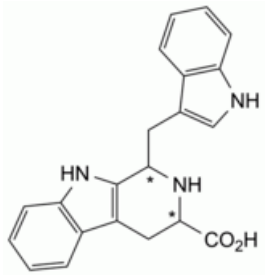
I. 1-methyl-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid,



J. (2*S*)-2-amino-3-[2-[2,3-dihydroxy-1-(1*H*-indol-3-yl)propyl]-1*H*-indol-3-yl]propanoic acid,



K. (2*S*)-2-amino-3-[2-(1*H*-indol-3-ylmethyl)-1*H*-indol-3-yl]propanoic acid,



L. 1-(1*H*-indol-3-ylmethyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole-3-carboxylic acid.

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