Second identification: A, B, D.

A. It complies with the test for specific optical rotation (see Tests).

B. Dissolve 0.50 g in *water R* and dilute to 25 ml with the same solvent. The solution is laevorotatory.

C. Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *leucine CRS*. Examine the substances prepared as discs.

D. Examine the chromatograms obtained in the test for ninhydrin-positive substances. The principal spot in the chromatogram obtained with test solution (b) is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).

TESTS

Appearance of solution. Dissolve 0.5 g in *1 M hydrochloric acid* and dilute to 10 ml with the same acid. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dissolve 1.00 g in *hydrochloric acid R1* and dilute to 25.0 ml with the same acid. The specific optical rotation is + 14.5 to + 16.5, calculated with reference to the dried substance.

Ninhydrin-positive substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel plate R*.

Test solution (a). Dissolve 0.10 g of the substance to be examined in *0.1 M hydrochloric acid* and dilute to 10 ml with the same acid.

Test solution (b). Dilute 1 ml of test solution (a) to 50 ml with *water R*.

Reference solution (a). Dissolve 10 mg of *leucine CRS* in *0.1 M hydrochloric acid* and dilute to 50 ml with the same acid.

Reference solution (b). Dilute 5 ml of test solution (b) to 20 ml with *water R*.

Reference solution (c). Dissolve 10 mg of *leucine CRS* and 10 mg of *valine CRS* in *0.1 M hydrochloric acid* and dilute to 25 ml with the same acid.

Apply separately to the plate 5 µl of each solution. Allow the plate to dry in air. Develop over a path of 15 cm using a mixture of 20 volumes of *glacial acetic acid R*, 20 volumes of *water R* and 60 volumes of *butanol R*. Allow the plate to dry in air, spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 15 min. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). The test is not valid unless the chromatogram obtained with reference solution (c) shows two clearly separated spots.

Chlorides (*2.4.4*). Dissolve 0.25 g in *water R* and dilute to 15 ml with the same solvent. The solution complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*). Dissolve 0.5 g in 3 ml of *dilute hydrochloric acid R* and dilute to 15 ml with *distilled water R*. The solution complies with the limit test for sulphates (300 ppm).

Ammonium (*2.4.1*). 50 mg complies with limit test B for ammonium (200 ppm). Prepare the standard using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*.

Iron (*2.4.9*). In a separating funnel, dissolve 1.0 g in 10 ml of *dilute hydrochloric acid R*. Shake with three 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. The aqueous layer complies with the limit test for iron (10 ppm).

Heavy metals (*2.4.8*). 2.0 g complies with limit test D for heavy metals (10 ppm). Prepare the standard using 2 ml of *lead standard solution (10 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.100 g in 3 ml of *anhydrous formic acid R*. Add 30 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid* using 0.1 ml of *naphtholbenzein solution R* as indicator, until the colour changes from brownish-yellow to green.

1 ml of *0.1 M perchloric acid* is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

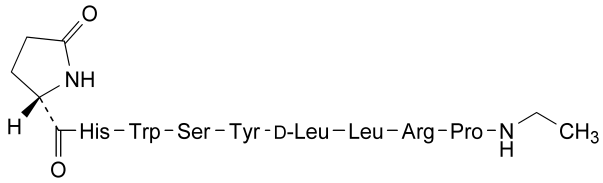
STORAGE

Store protected from light.

01/2008:1442

LEUPRORELIN

Leuprorelinum



$C_{59}H_{84}N_{16}O_{12}$ M_r 1209
[53714-56-0]

DEFINITION

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-*N*-ethyl-L-prolinamide.

Synthetic nonapeptide analogue of the hypothalamic peptide, gonadorelin. It is obtained by chemical synthesis and is available as an acetate.

Content: 97.0 per cent to 103.0 per cent (anhydrous and acetic acid-free substance).

CHARACTERS

Appearance: hygroscopic, white or almost white powder.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs of *potassium bromide R*.

Comparison: *Ph. Eur. reference spectrum of leuprorelin*.

B. Examine the chromatograms obtained in the assay.

Results: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (b).

C. Amino acid analysis (*2.2.56*). For hydrolysis use Method 1 and for analysis use Method 1.

Express the content of each amino acid in moles. Calculate the relative proportions of the amino acids taking one seventh of the sum of the number of moles of histidine, glutamic acid, leucine, proline, tyrosine and arginine as equal to 1. The values fall within the following limits: serine present; glutamic acid = 0.85 to 1.1; proline = 0.85 to 1.1; leucine = 1.8 to 2.2; tyrosine = 0.85 to 1.1; histidine = 0.85 to 1.1 and arginine = 0.85 to 1.1. Not more than traces of other amino acids are present, with the exception of tryptophan.

TESTS

Specific optical rotation (*2.2.7*): – 38.0 to – 42.0 (anhydrous and acetic acid-free substance).

Dissolve the substance to be examined in a 1 per cent *V/V* solution of *glacial acetic acid R* to obtain a concentration of 10.0 mg/ml.

Related substances. Liquid chromatography (*2.2.29*): use the normalisation procedure.

Test solution (a). Dissolve the substance to be examined in the mobile phase to obtain a concentration of 1.0 mg/ml.

Test solution (b). Dilute 0.5 ml of test solution (a) to 10.0 ml with the mobile phase.

Reference solution (a). Dissolve *leuprorelin CRS* in the mobile phase to obtain a concentration of 1.0 mg/ml.

Reference solution (b). Dilute 0.5 ml of reference solution (a) to 10.0 ml with the mobile phase.

Resolution solution. Dilute 5.0 ml of reference solution (a) to 50.0 ml with *water R*. To 5 ml of the solution add 100 µl of *1 M sodium hydroxide* and shake vigorously. Heat in an oven at 100 °C for 60 min, cool immediately and add 50 µl of *dilute phosphoric acid R*. Shake vigorously.

Column:

– *size*: *l* = 0.10 m, Ø = 4.6 mm;

– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase: dissolve about 15.2 g of *triethylamine R* in 800 ml of *water R*, adjust to pH 3.0 with *phosphoric acid R* and dilute to 1000 ml with *water R*. Add 850 ml of this solution to 150 ml of a mixture of 2 volumes of *propanol R* and 3 volumes of *acetonitrile R*.

Flow rate: 1.0-1.5 ml/min.

Detection: spectrophotometer at 220 nm.

Injection: 20 µl of test solution (a) and the resolution solution.

Run time: 90 min.

Relative retention with reference to leuprorelin (retention time = 41-49 min): impurity E = about 0.7; impurity F = about 0.7; impurity H = about 0.78; impurity A = about 0.8; impurity B = about 0.9; impurity I = about 0.94; impurity J = about 1.09; impurity C = about 1.2; impurity G = about 1.3; impurity K = about 1.31; impurity D = about 1.5.

System suitability: resolution solution:

– *resolution*: minimum 1.5 between the peaks due to impurity B and leuprorelin.

Limits:

– *impurity D*: maximum 1.0 per cent;

– *impurities A, B, C*: for each impurity, maximum 0.5 per cent;

– *unspecified impurities*: for each impurity, maximum 0.5 per cent;

– *total*: maximum 2.5 per cent;

– *disregard limit*: 0.1 per cent.

Acetic acid (*2.5.34*): 4.7 per cent to 9.0 per cent.

Test solution. Dissolve 10.0 mg of the substance to be examined in a mixture of 5 volumes of mobile phase B and 95 volumes of mobile phase A and dilute to 10.0 ml with the same mixture of mobile phases.

Water (*2.5.32*): maximum 5.0 per cent.

Sulphated ash (*2.4.14*): maximum 0.3 per cent.

Bacterial endotoxins (*2.6.14, Method D*): less than 16.7 IU/mg, if intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for removal of bacterial endotoxins.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modifications.

Run time: 60 min.

Injection: 20 µl of test solution (b) and reference solution (b).

Calculate the content of leuprorelin ($C_{59}H_{84}N_{16}O_{12}$) using the areas of the peaks and the declared content of $C_{59}H_{84}N_{16}O_{12}$ in *leuprorelin CRS*.

STORAGE

In an airtight container, protected from light, at a temperature not exceeding 30 °C.

If the substance is sterile, store in a sterile, airtight, tamper-proof container.

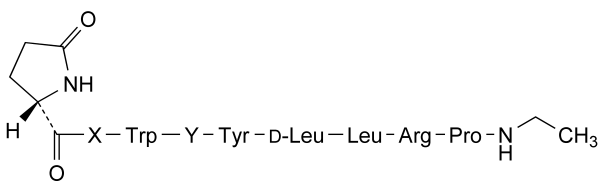
LABELLING

The label states the mass of peptide in the container.

IMPURITIES

Specified impurities: A, B, C, D.

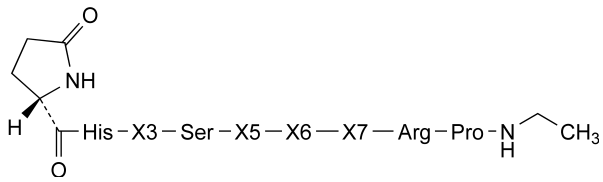
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. 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*): *E, F, G, H, I, J, K.*



A. X = L-His, Y = D-Ser: [4-D-serine]leuprorelin,

B. X = D-His, Y = L-Ser: [2-D-histidine]leuprorelin,

F. X = D-His, Y = D-Ser: [2-D-histidine,4-D-serine]leuprorelin,

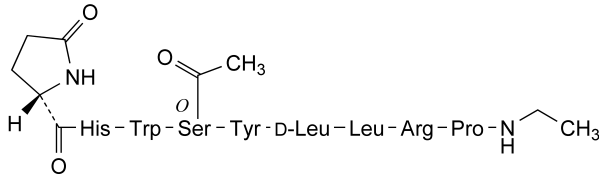


C. X3 = L-Trp, X5 = L-Tyr, X6 = X7 = L-Leu:
[6-L-leucine]leuprorelin,

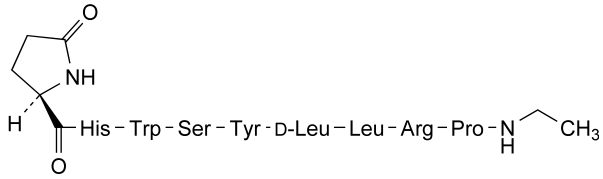
E. X3 = D-Trp, X5 = L-Tyr, X6 = D-Leu, X7 = L-Leu:
[3-D-tryptophane]leuprorelin,

G. X3 = L-Trp, X5 = D-Tyr, X6 = D-Leu, X7 = L-Leu:
[5-D-tyrosine]leuprorelin,

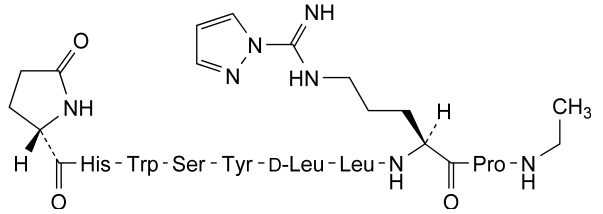
H. X3 = L-Trp, X5 = L-Tyr, X6 = X7 = D-Leu:
[7-D-leucine]leuprorelin,



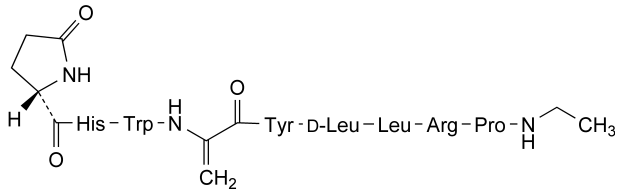
D. [4-(*O*-acetyl-L-serine)]leuprorelin,



I. [1-(5-oxo-D-proline)]leuprorelin,



J. [8-[5-*N*-[imino(1*H*-pyrazol-1-yl)methyl]-L-ornithine]]leuprorelin,

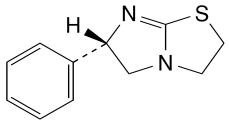


K. [4-dehydroalanine]leuprorelin.

01/2008:1728

LEVAMISOLE FOR VETERINARY USE

Levamisolum ad usum veterinarium



$C_{11}H_{12}N_2S$ M_r 204.3
[14769-73-4]

DEFINITION

(6*S*)-6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole.

Content: 98.5 per cent to 101.5 per cent (anhydrous substance).

CHARACTERS

Appearance: white or almost white powder.

Solubility: slightly soluble in water, freely soluble in alcohol and in methanol.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. It complies with the test for specific optical rotation (see Tests).

B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *Ph. Eur. reference spectrum of levamisole.*

If the spectra show differences, dissolve the substance to be examined in *methylene chloride R*, evaporate to dryness and record a new spectrum using the residue.

TESTS

Solution S. Dissolve 2.50 g in *ethanol R* and dilute to 50.0 ml with the same solvent.

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

Specific optical rotation (*2.2.7*): – 85 to – 89 (anhydrous substance), determined on solution S.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use, protect from light and keep below 25 °C.*

Test solution. Dissolve 0.100 g of the substance to be examined in *methanol R* and dilute to 10.0 ml with the same solvent.

Reference solution (a). Dissolve 50 mg of *levamisole hydrochloride for system suitability CRS* in *methanol R*, add 0.5 ml of *concentrated ammonia R* and dilute to 5.0 ml with *methanol R*.

Reference solution (b). Dilute 1.0 ml of the test solution to 100.0 ml with *methanol R*. Dilute 5.0 ml of the solution to 25.0 ml with *methanol R*.

Column:

– *size*: *l* = 0.10 m, Ø = 4.6 mm,
– *stationary phase*: *base-deactivated octadecylsilyl silica gel for chromatography R* (3 µm).

Mobile phase:

– *mobile phase A*: dissolve 0.5 g of *ammonium dihydrogen phosphate R* in 90 ml of *water R*; adjust to pH 6.5 with a 40 g/l solution of *sodium hydroxide R* and dilute to 100 ml with *water R*,
– *mobile phase B*: *acetonitrile R*.

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 8	90 → 30	10 → 70
8 - 10	30	70
10 - 11	30 → 90	70 → 10

Flow rate: 1.5 ml/min.

Detection: spectrophotometer at 215 nm.

Equilibration: at least 4 min with the mobile phase at the initial composition.

Injection: 10 µl.