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

Leuprorelin

General Notices

(Ph. Eur. Monograph 1442)

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

Action and use

Gonadotropin releasing hormone (gonadorelin) analogue; treatment of prostate cancer.

Preparation

Leuprorelin Injection

Ph Eur

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.

Sulfated 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 preparations 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-evident container.

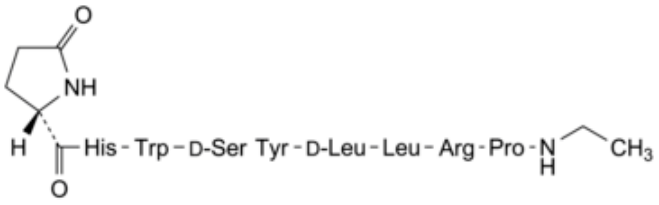
LABELLING

The label states the mass of peptide in the container.

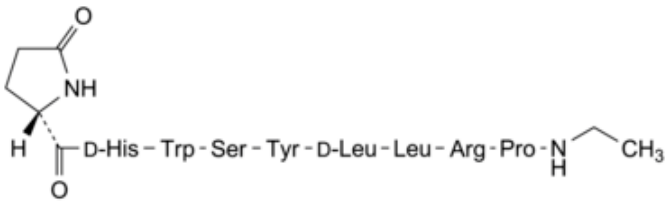
IMPURITIES

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.*

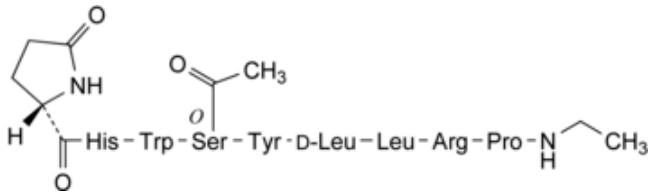


A. [4-D-serine]leuprorelin,



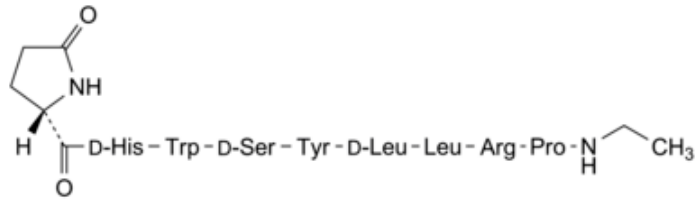
B. [2-D-histidine]leuprorelin,

C. [6-L-leucine]leuprorelin,



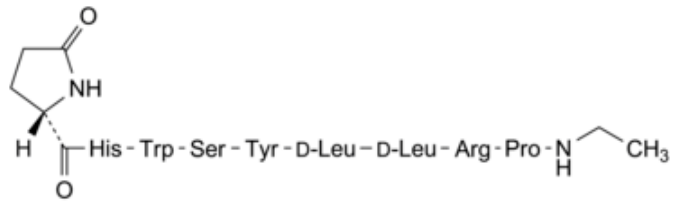
D. [4-(*O*-acetyl-L-serine)]leuprorelin,

E. [3-D-tryptophane]leuprorelin,

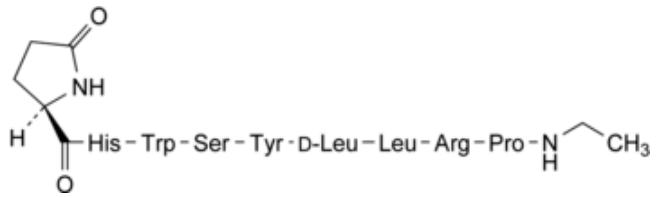


F. [2-D-histidine,4-D-serine]leuprorelin,

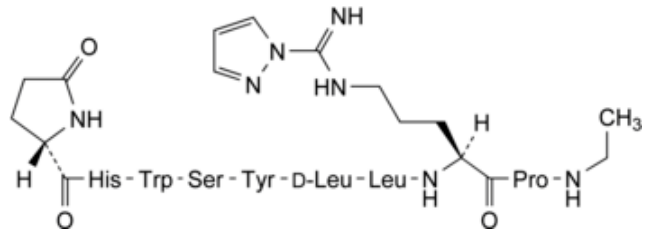
G. [5-D-tyrosine]leuprorelin,



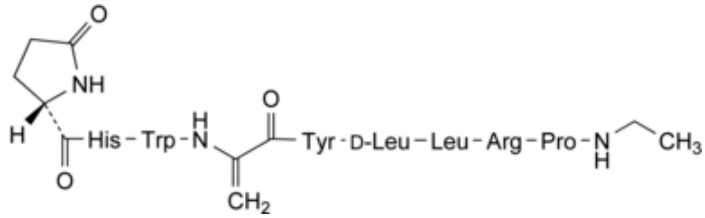
H. [7-D-leucine]leuprorelin,



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.

Ph Eur