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

Leuprorelin Injection

General Notices

Action and use

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

DEFINITION

Leuprorelin Injection is a sterile suspension of Leuprorelin in a suitable liquid. It is prepared by suspending Leuprorelin for Injection in the requisite amount of the liquid stated on the label immediately before use.

The injection complies with the requirements stated under Parenteral Preparations and with the following requirements.

Acidity

pH of the injection when constituted in accordance with the manufacturer's instructions, 5.0 to 7.0, Appendix V L.

STORAGE

Leuprorelin Injection should be used immediately after preparation.

LEUPRORELIN FOR INJECTION

DEFINITION

Leuprorelin for Injection is a sterile powder consisting of Leuprorelin with or without excipients. It is supplied in a sealed container. Leuprorelin for Injection is formulated so that the medicament is released over a period of weeks.

The contents of the sealed container comply with the requirements for Powders for Injections or Infusions stated under Parenteral Preparations and with the following requirements.

Content of leuprorelin, $C_{59}H_{84}N_{16}O_{12}$

95.0 to 105.0% of the stated amount of the peptide.

IDENTIFICATION

A. To a quantity of the powder containing the equivalent of 4.5 mg of the peptide in a separating funnel add 15 mL of *dichloromethane* and 35 mL of a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with *dilute ammonia R1*. Shake for 1 minute and extract the aqueous layer. Dilute the aqueous extract to 50 mL with a

1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 6M *ammonia*. The *light absorption* of the resulting solution, Appendix II B, exhibits a maximum between 277 and 282 nm.
B In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that of the principal peak in the chromatogram obtained with solution (2).

TESTS

Related substances

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions and the *normalisation procedure*.

(1) To a quantity of the powder containing the equivalent of 4.5 mg of the peptide in a separating funnel add 15 mL of *dichloromethane* and 35 mL of a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with *dilute ammonia R1*. Shake for 1 minute and extract the aqueous layer. Dilute the aqueous extract to 50 mL with a 1.15% w/v solution of *ammonium dihydrogen orthophosphate*, previously adjusted to pH 6.0 with 6M *ammonia*.
(2) 0.1% w/v of *leuprorelin EPCRS* in the mobile phase.
(3) Dilute 5 volumes of solution (2) to 50 volumes with *water*. To 5 mL of the solution add 100 µL of 1M *sodium hydroxide* and shake vigorously. Heat in an oven at 100° for 60 minutes, cool immediately and add 50 µL of 2M *orthophosphoric acid*. Shake vigorously (generation of impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (10 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Wakosil-II 3C18 HG is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 1.0 to 1.5 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 220 nm.
(f) Inject 20 µL each of solutions (1) and (3).

MOBILE PHASE

150 volumes of a mixture of 2 volumes of *propan-1-ol* and 3 volumes of *acetonitrile* and 850 volumes of the buffer solution prepared as described below.

To prepare the buffer solution, dissolve about 15.2 g of *triethylamine* in 800 mL of *water*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions the relative retentions with reference to leuprorelin (retention time = 41 to 49 minutes) are: 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

The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution factor* between the peaks corresponding to impurity B and leuprorelin is at least 1.5.

LIMITS

In the chromatogram obtained with solution (1):

the area of any peak corresponding to impurity D is not greater than 1.0%;

the areas of any peaks corresponding to impurities A, B and C are not greater than 0.5%;

the area of any other *secondary peak* is not greater than 0.5%;

the sum of the areas of any *secondary peaks* is not greater than 2.5%.

Disregard any peak with an area less than 0.1%.

Water

Not more than 5.0 % w/w, Appendix IX C, Method III.

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is 11.6 IU per mg of leuprorelin.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions.

(1) Dissolve a quantity of the powder containing the equivalent of 9 mg of the peptide in 100 mL of *dimethyl sulfoxide*.
(2) 0.01% w/v of *leuprorelin EPCRS* dissolved in the mobile phase.
(3) 0.1% w/v of *leuprorelin EPCRS* dissolved in the mobile phase.
(4) Dilute 5 volumes of solution (3) to 50 volumes with *water*. To 5 mL of the solution add 100 µL of 1M *sodium hydroxide* and shake vigorously. Heat in an oven at 100° for 60 minutes, cool immediately and add 50 µL of 2M *orthophosphoric acid*. Shake vigorously (generation of impurity B).

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (5 cm × 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Wakosil-II 3C18 AR is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 0.8 mL per minute.
(d) Use an ambient column temperature.
(e) Use a detection wavelength of 220 nm.
(f) Inject 20 µL of solutions (1), (2) and (4).

MOBILE PHASE

150 volumes of a mixture of 2 volumes of *propan-1-ol* and 3 volumes of *acetonitrile* and 850 volumes of the buffer solution as described below.

To prepare the buffer solution, add 15.2 g of *triethylamine* to 800 mL of *water*, adjust to pH 3.0 with *orthophosphoric acid* and add sufficient *water* to produce 1000 mL.

When the chromatograms are recorded under the prescribed conditions the relative retention time of impurity B with reference to leuprorelin (retention time is approximately 22 minutes) is approximately 0.9.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (4), the *resolution factor* between the peaks corresponding to impurity B and that of leuprorelin is at least 1.2.

DETERMINATION OF CONTENT

Calculate the content of $C_{59}H_{84}N_{16}O_{12}$ using the declared content of $C_{59}H_{84}N_{16}O_{12}$ in *leuprorelin EPCRS*.

STORAGE

The sealed container should be stored at a temperature not exceeding 30°.

LABELLING

The label of the sealed container states the amount of the peptide in milligrams.

IMPURITIES

The impurities limited by the requirements of this monograph include those listed under Leuprorelin.