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

Insulin Lispro Injection

General Notices

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Lispro Injection is a sterile, aqueous solution of Insulin Lispro.

The injection complies with the requirements stated under Injectable Insulin Preparations, with the exception of the test for Insulin in the supernatant, and with the modifications described below.

Content of insulin lispro, $C_{257}H_{383}N_{65}O_{77}S_6$

90.0 to 105.0% of the stated amount.

CHARACTERISTICS

A clear and colourless solution, free from turbidity and foreign matter.

IDENTIFICATION

In the Assay, the retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to that in the chromatogram obtained with solution (2).

TESTS

Impurities with molecular masses greater than that of insulin lispro

The sum of the areas of the peaks in the chromatogram obtained with the test solution with a retention time less than that of the principal peak is not more than 1.5% of the total area of the peaks. Disregard any peak with a retention time greater than that of the peak due to insulin lispro monomer.

Related proteins

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

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to produce a solution containing 0.35% w/v of Insulin Lispro. Add 4 µL of *6M hydrochloric acid* per mL of this solution. Maintain the solution at 2° to 8° and use within 56 hours.
(2) Prepare a 0.35% w/v solution of Insulin Lispro in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido

insulin lispro.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (5 µm) with a pore size of 30 nm (Vydac Protein and Peptide C18 is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 1 mL per minute.
(d) Use a column temperature of 40°.
(e) Use a detection wavelength of 214 nm.
(f) Inject 20 µL for each solution.

MOBILE PHASE

Mobile phase A Mix 82 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid* and 18 volumes of *acetonitrile for chromatography*.

Mobile phase B Mix equal volumes of a 2.84% w/v solution of *anhydrous sodium sulfate*, adjusted to pH 2.3 with *orthophosphoric acid*, and *acetonitrile for chromatography*.

Use the following gradient.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0-60	81	19	Isocratic
60-83	81 → 51	19 → 49	Linear gradient
83-84	51 → 81	49 → 19	Linear gradient
84-94	81	19	Re-equilibration

SYSTEM SUITABILITY

Adjust the mobile phase composition to obtain a retention time of about 41 minutes for insulin lispro; A21 desamido insulin lispro elutes near the start of the gradient elution. The test is not valid unless, in the chromatogram obtained with solution (2), the *resolution* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro) is at least 1.5 and the *symmetry factor* for the peak due to insulin lispro is less than 2.0.

LIMITS

In the chromatogram obtained with solution (1), the amount of A21 desamido insulin lispro is not more than 1.5%. The total amount of related proteins (excluding A21) is not greater than 4.0%.

Total zinc

14 to 35 µg per 100 units of insulin lispro, determined by *atomic absorption spectrometry*, Appendix II D, Method I.

Test solution Shake the preparation gently and dilute a volume containing 200 units of insulin lispro to 25.0 mL with 0.01M *hydrochloric acid*. Dilute if necessary to a suitable concentration of zinc (for example 0.4 µg to 1.6 µg of Zn per mL) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.40 µg, 0.80 µg, 1.00 µg, 1.20 µg and 1.60 µg of Zn per mL, freshly prepared by diluting *zinc standard solution (5 mg per mL Zn)* with 0.01M *hydrochloric acid*.

Measure the absorbance at 213.9 nm using a zinc hollow-cathode lamp as source of radiation and an air-acetylene flame of suitable composition (for example 11 litres of air and 2 litres of acetylene per minute).

Bacterial endotoxins

Carry out the *test for bacterial endotoxins*, Appendix XIV C. The endotoxin limit concentration is less than 80 IU of endotoxin per 100 units of insulin lispro.

ASSAY

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

(1) Dilute, if necessary, the preparation being examined with 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Add 4 µL of *6M hydrochloric acid* per mL of this solution. Maintain the solution at 2 to 8° and use within 48 hours.
(2) Dissolve the contents of a vial of *insulin lispro EPCRS* in 0.01M *hydrochloric acid* to obtain a 0.08% w/v solution. Maintain the solution at 2° to 8° and use within 48 hours.
(3) Prepare a 0.1% w/v solution of the preparation being examined in 0.01M *hydrochloric acid* and add 4 µL of *6M hydrochloric acid* per mL. Allow to stand at room temperature to obtain a solution containing between 0.8% and 11% of A21 desamido insulin lispro. Maintain the solution at 2° to 8° and use within 14 days.

CHROMATOGRAPHIC CONDITIONS

(a) Stainless steel column (10 cm x 4.6 mm) packed with *octadecylsilyl silica gel for chromatography* (3 µm) (Spherisorb ODS 2 is suitable).
(b) Isocratic elution using the mobile phase described below.
(c) Flow rate of 0.8 mL per minute.
(d) Column temperature of 40°.
(e) Detection wavelength of 214 nm.
(f) Injection volume of 20 µL for each solution.

MOBILE PHASE

Mix 255 volumes of *acetonitrile for chromatography* and 745 volumes of a 2.84% w/v solution of *anhydrous sodium sulfate* adjusted to pH 2.3 with *orthophosphoric acid*.

SYSTEM SUITABILITY

When the chromatograms are recorded in the prescribed conditions, the retention time of insulin lispro is approximately 24 minutes. The test is not valid unless, in the chromatogram obtained with solution (3), the *resolution* between the first peak (insulin lispro) and the second peak (A21 desamido insulin lispro), is greater than 1.8, and the maximum relative standard deviation for replicate injections is 1.1% after 3 injections.

DETERMINATION OF CONTENT

Calculate the content of insulin lispro $C_{257}H_{383}N_{65}O_{77}S_6$ from the chromatograms obtained and the declared content of $C_{257}H_{383}N_{65}O_{77}S_6$[1] in *insulin lispro EPCRS*.

LABELLING

The label states the potency in units per mL.

1 100 IU are equivalent to 3.47 mg of insulin lispro.