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Insulin Glargine Injection

General Notices

Action and use

Hormone; treatment of diabetes mellitus.

DEFINITION

Insulin Glargine Injection is a sterile, acidic, aqueous solution of Insulin Glargine.

The injection complies with the requirements stated under Injectable Insulin Preparations with the modifications described below.

Content of insulin glargine, $C_{267}H_{404}N_{72}O_{78}S_6$

95.0 to 105.0% of the stated amount.

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

Acidity

pH, 3.5 to 4.5, Appendix V L.

Impurities with molecular masses greater than that of insulin glargine

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

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.
(2) Dry 1 vial of *insulin glargine EPCRS* in an oven at 100° for 1.5 to 3 hours. Dissolve the contents of the vial in 1.5 mL of 0.01M *hydrochloric acid* and dilute to 10.0 mL with *water*.
(3) Dilute 1 volume of solution (1) to 100 volumes with *water*, dilute 3 volumes of this solution to 20 volumes with *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use two stainless steel columns (30 cm × 8.0 mm) coupled in series and packed with *hydrophilic silica gel for chromatography* (5 µm) with a pore size of 15 nm of a grade suitable for the fractionation of globular proteins in the relative molecular mass range of 2000 to 80,000 (Shodex PROTEIN KW-802.5 is suitable).
(b) Use isocratic elution and the mobile phase described below.
(c) Use a flow rate of 0.5 mL per minute.
(d) Use an ambient column temperature.

(e) Use a detection wavelength of 276 nm.
(f) Inject 100 µL of each solution. If splitting of the principal peak is observed, the injection volume may be decreased according to the provisions given in Appendix III.
(g) Allow the chromatography to proceed for 1.8 times the retention time of insulin glargine.

MOBILE PHASE

Mix 200 volumes of *anhydrous acetic acid*, 300 volumes of *acetonitrile for chromatography* and 400 volumes of *water*, adjust to pH 3.0 with *concentrated ammonia* and dilute to 1000 volumes with *water*.

When the chromatograms are recorded under the prescribed conditions the retention time of insulin glargine is about 35 minutes.

SYSTEM SUITABILITY

The test is not valid unless:

in the chromatogram obtained with solution (2), the *symmetry factor* of the peak due to insulin glargine is not more than 2.0;

in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 2, where H_p = height above the baseline due to high molecular mass proteins and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to insulin glargine;

in the chromatogram obtained with solution (3), the *signal-to-noise ratio* of the principal peak is at least 10.

LIMITS

In the chromatogram obtained with solution (1) the sum of the areas of any *secondary peaks* with a retention time less than that of the peak due to insulin glargine is not greater than 0.3% by normalisation.

Disregard any peaks with a retention time greater than that of the peak due to insulin glargine.

Related proteins

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store the solutions at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.
(2) Dissolve the contents of a vial of *insulin glargine for peak identification EPCRS* (containing 0^A-Arg-insulin glargine) in 0.3 mL of 0.01M *hydrochloric acid* and add 1.7 mL of *water*.

CHROMATOGRAPHIC CONDITIONS

(a) Use a stainless steel column (25 cm × 3.0 mm) packed with *end-capped octadecylsilyl silica gel for chromatography* (4 µm) (Merck Superspher 100-RP-18e is suitable).
(b) Use gradient elution and the mobile phase described below.
(c) Use a flow rate of 0.6 mL per minute.
(d) Use a column temperature of 35°.
(e) Use a detection wavelength of 214 nm.
(f) Inject 5 µL of each solution.

MOBILE PHASE

Mobile phase A Dissolve 18.4 g of *sodium chloride* in 250 mL of buffer solution prepared as described below; add 250 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water*.

To prepare the buffer solution, dissolve 20.7 g of *anhydrous sodium dihydrogen phosphate* in 900 mL of *water*, adjust to pH 2.5 with *orthophosphoric acid* and dilute to 1000 mL with *water*.

Mobile phase B Dissolve 3.2 g of *sodium chloride* in 250 mL of the buffer solution prepared as described above; add 650 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water for chromatography*.

Time (Minutes)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comment
0-20	96→83	4→17	linear gradient
20-30	83→63	17→37	linear gradient
30-40	63→96	37→4	linear gradient
40-48	96	4	re-equilibration

When the chromatograms are recorded under the prescribed conditions the relative retention time of insulin glargine is about 20 minutes.

SYSTEM SUITABILITY

The test is not valid unless, in the chromatogram obtained with solution (2), the *peak-to-valley ratio* is at least 2, where H_p = height above the baseline due to 0^A-Arg-insulin glargine and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to insulin glargine.

LIMITS

In the chromatogram obtained with solution (1):

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

the sum of the areas of all *secondary peaks* is not greater than 2.0% by *normalisation*.

Total zinc

27 to 33 µg per 100 units of insulin glargine, determined by *atomic absorption spectrometry*, Appendix II D, Method I.

Test solution Dilute, if necessary, to a suitable concentration of zinc (for example 0.2 µg to 0.6 µg of Zn per mL) with 0.01M *hydrochloric acid*.

Reference solutions Use solutions containing a suitable range of concentrations, for example 0.20 µg, 0.40 µg and 0.60 µg of Zn per mL, freshly prepared by diluting *zinc standard solution (10 ppm 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 endoxtoxin per 100 units of insulin glargine.

ASSAY

Carry out the method for *liquid chromatography*, Appendix III D, using the following solutions. Store the solutions at a temperature of 2° to 8°.

(1) Dilute the injection, if necessary, with sufficient *water* to produce a solution containing 0.15% w/v of Insulin Glargine.
(2) Dissolve the contents of a vial of *insulin glargine EPCRS* in 1.5 mL of 0.01M *hydrochloric acid* and dilute to 10.0 mL with *water*.

CHROMATOGRAPHIC CONDITIONS

The chromatographic conditions described under Related proteins may be used.

DETERMINATION OF CONTENT

Calculate the content of insulin glargine, $C_{267}H_{404}N_{72}O_{78}S_6$, in the injection from the chromatograms obtained and the declared content of $C_{267}H_{404}N_{72}O_{78}S_6$[1] in *insulin glargine EPCRS*.

LABELLING

The label states the potency in units per mL.

[1] 100 IU are equivalent to 3.64 mg of insulin glargine.