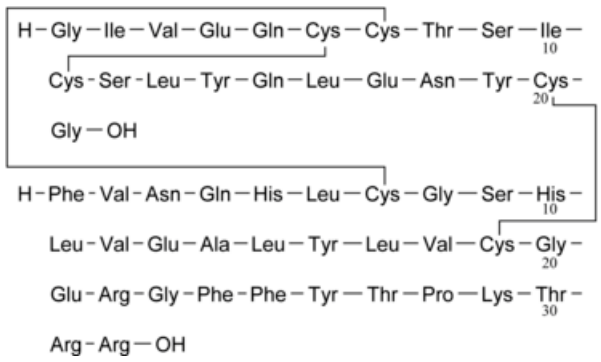
Edition: BP 2025 (Ph. Eur. 11.6 update)

Insulin Glargine

General Notices

(Ph. Eur. monograph 2571)



$C_{267}H_{404}N_{72}O_{78}S_6$ 6063

Action and use

Hormone; treatment of diabetes mellitus.

Preparation

Insulin Glargine Injection

Ph Eur

DEFINITION

21^{A}-Glycine-30^{B}a-L-arginine-30^{B}b-L-arginine-insulin (human).

2-chain peptide containing 53 amino acids. The A-chain is composed of 21 amino acids and the B-chain is composed of 32 amino acids. It is identical in primary structure to human insulin, only differing in amino acid sequence at position 21 in the A-chain and at the C-terminal end of the B-chain where it contains 2 additional amino acids. Human insulin is Asn(A21), whereas insulin glargine is Gly(A21), Arg(B31), Arg(B32). As in human insulin, insulin glargine contains 2 interchain disulfide bonds and 1 intrachain disulfide bond.

Content

94.0 per cent to 105.0 per cent (anhydrous substance).

By convention, for the purpose of labelling insulin glargine preparations, 0.0364 mg of insulin glargine is equivalent to 1 unit.

PRODUCTION

Insulin glargine is produced by a method based on recombinant DNA (rDNA) technology under conditions designed to minimise the degree of microbial contamination.

Prior to release, the following tests are carried out on each batch of insulin glargine, unless exemption has been granted by the competent authority.

Host-cell-derived proteins

The limit is approved by the competent authority.

Single-chain precursor

The limit is approved by the competent authority. Use a suitably sensitive method.

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Practically insoluble in water and in anhydrous ethanol, soluble in dilute mineral acids.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay.

Results The principal peak in the chromatogram obtained with the test solution is similar in retention time to the principal peak in the chromatogram obtained with the reference solution.

B. Peptide mapping (2.2.55).

SELECTIVE CLEAVAGE OF THE PEPTIDE BONDS

Test solution Prepare a 10.0 mg/mL solution of the substance to be examined in a 1 g/L solution of *hydrochloric acid R* and transfer 5 µL of the solution to a clean tube. Add 1.0 mL of *1 M tris-hydrochloride buffer solution pH 7.5 R* and 100 µL of a 20 U/mL solution of *Staphylococcus aureus strain V8 protease, type XVII-B R* in *1 M tris-hydrochloride buffer solution pH 7.5 R*. Mix and incubate at 45 °C for about 2 h. Stop the reaction by adding 2 µL of *phosphoric acid R*.

Reference solution Prepare at the same time and in the same manner as for the test solution but using *insulin glargine CRS* instead of the substance to be examined.

CHROMATOGRAPHIC SEPARATION. Liquid chromatography (2.2.29).

Buffer solution Dissolve 11.6 g of *phosphoric acid R* and 42.1 g of *sodium perchlorate R* in 1600 mL of *water for chromatography R*, adjust to pH 2.3 with *triethylamine R* and dilute to 2000 mL with *water for chromatography R*.

Column:

— *size*: *l* = 0.125 m, Ø = 3.0 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R1*, buffer solution (7:93 *V/V*);

— *mobile phase B*: buffer solution, *acetonitrile R1* (43:57 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 30	90 → 20	10 → 80
30 - 35	20	80

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Equilibration At initial conditions for at least 15 min.

Injection 50 µL.

Retention time Insulin glargine fragment II = about 14 min; insulin glargine fragment III = about 15 min.

System suitability:

— the chromatogram obtained with the reference solution is qualitatively similar to the chromatogram of insulin glargine digest supplied with *insulin glargine CRS*;

— in the chromatogram obtained with the reference solution, identify the peaks due to digest fragments II and III:

symmetry factor Maximum 1.5 for the peaks due to fragments II and III;

resolution Minimum 3.4 between the peaks due to fragments II and III.

Results The profile of the chromatogram obtained with the test solution corresponds to that of the chromatogram obtained with the reference solution.

NOTE: the retention times of fragments I and IV are the same as for human insulin; the retention times of fragments II and III differ from human insulin due to the difference in the sequence at position 21 of the A-chain and to the 2 additional amino acids of the B-chain.

TESTS

Impurities with molecular masses greater than that of insulin glargine

Size-exclusion chromatography (*2.2.30*): use the normalisation procedure.

Test solution Prepare a 4 mg/mL solution of the substance to be examined in a 1 g/L solution of *hydrochloric acid R*.

Resolution solution Dry about 200 mg of the substance to be examined in an oven at 100 °C for 1.5-3 h. Dissolve 15.0 mg of the dried substance in 1.5 mL of a 1 g/L solution of *hydrochloric acid R* and dilute to 10.0 mL with *water R*.

Reference solution Dilute 1.0 mL of the test solution to 100.0 mL with *water R*. Dilute 3.0 mL of this solution to 20.0 mL with *water R*.

Column:

— *size*: *l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *hydrophilic silica gel for chromatography R* (10 µm) with a pore size of 12.5 nm, of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 5000 to 150 000.

Mobile phase Mix 15 volumes of *glacial acetic acid R*, 20 volumes of *acetonitrile R* and 65 volumes of a 1.0 g/L solution of *arginine R*; filter and degas.

Flow rate 0.5 mL/min.

Detection Spectrophotometer at 276 nm.

Injection 100 µL.

Run time About 35 min.

Retention time Insulin glargine = about 18 min.

System suitability:

— *signal-to-noise ratio*: minimum 10 for the principal peak in the chromatogram obtained with the reference solution;

— *symmetry factor*: maximum 2.0 for the peak due to insulin glargine in the chromatogram obtained with the resolution solution;

— *peak-to-valley ratio*: minimum 2.0, where H_p = height above the baseline of the peak 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 the resolution solution.

Limits:

— *total of impurities with a retention time less than that of insulin glargine*: maximum 0.3 per cent of the total area of the peaks; disregard any peak with a retention time greater than that of the peak due to insulin glargine.

Related proteins

Liquid chromatography (2.2.29): use the normalisation procedure. *Maintain the solutions at 2-8 °C.*

Test solution Dissolve 15.0 mg of the substance to be examined in 1.5 mL of a 1 g/L solution of *hydrochloric acid R* and dilute to 10.0 mL with *water R*.

Reference solution Dissolve the contents of a vial of *insulin glargine CRS* in 1.5 mL of a 1 g/L solution of *hydrochloric acid R*, transfer the solution with *water R* to a 10 mL volumetric flask and dilute to 10.0 mL with *water R*.

Resolution solution Dissolve the contents of a vial of *insulin glargine for peak identification CRS* (containing 0^A-Arg-insulin glargine) in 0.3 mL of a 1 g/L solution of *hydrochloric acid R* and add 1.7 mL of *water R*.

Buffer solution Dissolve 20.7 g of *anhydrous sodium dihydrogen phosphate R* in 900 mL of *water for chromatography R*, adjust to pH 2.5 with *phosphoric acid R* and dilute to 1000 mL with *water for chromatography R*.

Column:

— *size*: *l* = 0.25 m, Ø = 3.0 mm;

— *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 35 °C.

Mobile phase:

— *mobile phase A*: dissolve 18.4 g of *sodium chloride R* in 250 mL of the buffer solution, add 250 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water for chromatography R*;

— *mobile phase B*: dissolve 3.2 g of *sodium chloride R* in 250 mL of the buffer solution, add 650 mL of *acetonitrile R1* and mix; dilute to 1000 mL with *water for chromatography R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 20	96 → 83	4 → 17
20 - 30	83 → 63	17 → 37
30 - 33	63 → 96	37 → 4
33 - 40	96	4

Flow rate 0.6 mL/min.

Detection Spectrophotometer at 214 nm.

Injection 5 µL of the test solution and the resolution solution.

Retention time Insulin glargine = about 20 min.

System suitability Resolution solution:

— *peak-to-valley ratio*: minimum 2, where H_p = height above the baseline of the peak 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:

— *any impurity*: for each impurity, maximum 0.4 per cent;

— *total*: maximum 1.0 per cent.

Zinc

Maximum 0.80 per cent.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution Dissolve 45.0 mg of the substance to be examined in a 1 g/L solution of *hydrochloric acid R* and dilute to 50.0 mL with the same solution. Dilute 10.0 mL of the solution to 100.0 mL with a 1 g/L solution of *hydrochloric acid R*.

Reference solutions Prepare reference solutions containing 0.2 µg, 0.4 µg and 0.6 µg of zinc per millilitre by diluting *zinc standard solution (10 ppm Zn) R* with a 1 g/L solution of *hydrochloric acid R*.

Source Zinc hollow-cathode lamp.

Wavelength 213.9 nm.

Atomisation device Air-acetylene flame of suitable composition (for example, 11 L of air and 2 L of acetylene per minute).

Water (*2.5.32*)

Maximum 8.0 per cent, determined on 30.0 mg.

Bacterial endotoxins (*2.6.14, Method D*)

Less than 10 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins.

ASSAY

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

Injection 5 µL of the test solution and the reference solution.

Calculate the content of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) taking into account the assigned content of *insulin glargine CRS*.

STORAGE

In an airtight container, protected from light, at a temperature of -20 ± 5 °C.

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