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Insulin Glargine

$C_{267}H_{404}N_{72}O_{78}S_6$ 6062.96

Insulin (human), 21^{A}-glycine-30^{B}a-L-arginine-30^{B}b-L-arginine CAS RN®: 160337-95-1; UNII: 2ZM8CX04RZ.

DEFINITION

Insulin Glargine is a two-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 to the primary structure of Human Insulin except for position A21 which has Gly rather than Asn as in Human Insulin and two additional amino acids at the C terminal of the B-chain Arg (B31) and Arg (B32). Insulin Glargine is produced by methods based on recombinant DNA technology. Residual host cell protein (HCP) content is determined by a validated method and is NMT 10 ppm (ng HCP per mg of Insulin Glargine). Insulin Glargine contains NLT 94.0% and NMT 105.0% of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$), calculated on the anhydrous basis, or on the dried basis when other volatile solvents in addition to water are present.

[NOTE—One USP Insulin Glargine Unit is equivalent to 0.0364 mg of pure Insulin Glargine.]

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B. PEPTIDE MAPPING**

Phosphate/perchlorate buffer: Dissolve 11.6 g of phosphoric acid and 42.1 g of sodium perchlorate in 1600 mL of water. Adjust with triethylamine to a pH of 2.3, and dilute with water to a final volume of 2000 mL.

Solution A: Prepare a filtered and degassed mixture of acetonitrile and *Phosphate/perchlorate buffer* (7:93).

Solution B: Prepare a filtered and degassed mixture of acetonitrile and *Phosphate/perchlorate buffer* (57:43).

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	90	10
30	20	80
35	20	80
36	90	10

Tris buffer solution: Dissolve 12.11 g of tris(hydroxymethyl)aminomethane in 90 mL of water. Adjust with hydrochloric acid to a pH of 7.5, and dilute with water to a final volume of 100 mL.

Enzyme solution: Prepare a solution of *Staphylococcus aureus* V-8 protease in *Tris buffer solution* having an activity of 20 Units/mL (using Z-Phe-Leu-Glu-4-nitroanilide as the substrate).

Standard solution: Transfer to a vial 35 µL of the *Standard solution* from the *Assay*. To this vial, add 1.0 mL of *Tris buffer solution* and 100 µL of *Enzyme solution*, and incubate at 45° for 2–3 h. Quench the digestion by adding 2 µL of phosphoric acid.

Sample solution: Transfer to a vial 35 µL of the *Sample solution* from the *Assay*. To this vial, add 1.0 mL of *Tris buffer solution* and 100 µL of *Enzyme solution*, and incubate at 45° for 2–3 h. Quench the digestion by adding 2 µL of phosphoric acid.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 12.5-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 0.6 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 3.4 between the peaks indicated as fragments II and III

Tailing factor: NMT 1.5 for the peaks indicated as fragments II and III

Chromatogram similarity: In the chromatogram from the *Standard solution*, identify the peaks due to digest fragments I, II, III, and IV. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with USP Insulin Glargine RS.

Analysis

Samples: *Standard solution* and *Sample solution*

Run a blank, and record the chromatograms.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*. All four fragments–I, II, III, and IV–must be present.

ASSAY

• **Procedure**

Buffer: Dissolve 20.7 g of anhydrous monobasic sodium phosphate in 900 mL of water. Adjust with phosphoric acid to a pH of 2.5, and dilute with water to a final volume of 1000 mL.

Solution A: Dissolve 18.4 g of sodium chloride in 250 mL of *Buffer*, add 250 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Solution B: Dissolve 3.2 g of sodium chloride in 250 mL of *Buffer*, add 650 mL of acetonitrile, and mix. Dilute the solution with water to a final volume of 1000 mL.

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	96	4
20	83	17
30	63	37
33	96	4

[Note—Adjust the *Mobile phase* composition and the gradient by a parallel shift to obtain a retention time of 18–23 min for the insulin glargine main peak.]

System suitability solution: Dissolve the contents of 1 vial of USP Insulin Glargine for Peak Identification RS in 0.3 mL of 0.01 N hydrochloric acid, and add 1.7 mL of water.

Standard solution: Dissolve the contents of 1 vial of USP Insulin Glargine RS in 1.5 mL of 0.01 N hydrochloric acid, transfer the solution to a 10-mL volumetric flask, and dilute with water to volume.

Sample solution: Dissolve 15 mg of Insulin Glargine in 1.5 mL of 0.01 N hydrochloric acid, and dilute with water to a final volume of 10 mL.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: UV 214 nm

Column: 3.0-mm × 25.0-cm; 4-µm packing L1

Column temperature: 35°

Flow rate: 0.6 mL/min

Injection volume: 5 µL

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 for the ratio of the height of the 0^A-Arg-insulin glargine peak to the height of the valley between the 0^A-Arg-insulin glargine peak and the insulin glargine peak, *System suitability solution*

Tailing factor: NMT 1.8 for the insulin glargine peak, *System suitability solution*

Relative standard deviation: NMT 2.0% for six replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the potency, in percent, of insulin glargine ($C_{267}H_{404}N_{72}O_{78}S_6$) in the portion of Insulin Glargine taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of insulin glargine from the *Sample solution*

r_S = peak response of insulin glargine from the *Standard solution*

C_S = concentration of USP Insulin Glargine RS in the *Standard solution* (mg/mL)

C_U = concentration of the *Sample solution* (corrected for the water content or loss on drying) (mg/mL)

Acceptance criteria: 94.0%–105.0% on the anhydrous basis or dried basis

OTHER COMPONENTS

• **Zinc Determination**

Blank: 0.01 N hydrochloric acid

Standard stock solution: 10 µg/mL of zinc in *Blank*, from a commercially available zinc standard solution for atomic absorption

Standard solutions: 0.2, 0.4, and 0.6 µg/mL of zinc from the *Standard stock solution* diluted with *Blank*

Sample solution: Dissolve 45 mg of Insulin Glargine, accurately weighed, in 50 mL of *Blank*. Dilute 10 mL of the solution with *Blank* to a final volume of 100 mL.

Instrumental conditions

(See *Atomic Absorption Spectroscopy (852)*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: Zinc absorption line at 213.9 nm

Flame: Air–acetylene flame of suitable composition (for example, 11 L of air and 2 L of acetylene per min)

Lamp: Suitable radiation source such as zinc hollow-cathode or electrodeless-discharge-lamp (EDL)

System suitability

Samples: *Blank* and *Standard solutions*

Using the *Standard solutions* and *Blank*, construct a calibration curve by plotting the absorbances of the *Standard solutions* versus their concentrations, and draw the straight line best fitting the three plotted points.

Suitability requirements

Correlation coefficient: NLT 0.999

Analysis

Samples: *Blank*, *Standard solutions*, and *Sample solution*

Determine the concentration, *C*, in µg/mL of zinc in the *Sample solution* using the calibration curve.

Calculate the percentage of zinc in the portion of Insulin Glargine taken:

$$\text{Result} = [C \times F_1 \times V \times (F_2/W)] \times 100$$

C = concentration of zinc in the *Sample solution* (µg/mL)

F_1 = conversion factor from µg/mL to mg/mL, 0.001

V = volume of the *Sample solution*, 100 mL

F_2 = sampling factor, 5

W = weight of Insulin Glargine taken (mg)

Acceptance criteria: NMT 0.80%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

• **Product-Related Substances**

Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Analysis

Sample: *Sample solution*

Calculate the percentage of each individual insulin glargine related substance ($\%i_x$) in the portion of Insulin Glargine taken:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of the insulin glargine related substance from the *Sample solution*

r_T = sum of all the peak responses from the *Sample solution*

Calculate the total percentage of insulin glargine related substances in the portion of Insulin Glargine taken:

$$\text{Result} = \Sigma\%i_x$$

$\Sigma\%i_x$ = total percentage of insulin glargine related substances from the *Sample solution*

Acceptance criteria

Any individual insulin glargine related substance: NMT 0.5%

Total insulin glargine related substances: NMT 1.5%

Delete the following:

▲• **Limit of High Molecular Weight Proteins**▲ (USP 1-Dec-2022)

Add the following:

▲• **Physicochemical Analytical Procedures for Insulins** ⟨121.1⟩*, Limit of High Molecular Weight Proteins*: Meets the requirements

Acceptance criteria: NMT 0.3%▲ (USP 1-Dec-2022)

SPECIFIC TESTS

• **Insulin Assays** ⟨121⟩*, Assay, Bioidentity Test*: Meets the requirements

Change to read:

• **Bacterial Endotoxins Test** ⟨85⟩: ▲Meets the requirements▲ (USP 1-Dec-2022)

Change to read:

• **Microbial Enumeration Tests** ⟨61⟩ and **Tests For Specified Microorganisms** ⟨62⟩: The total bacterial count does not exceed ▲3×10^2▲ (USP 1-Dec-2022) cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.

• **Water Determination** ⟨921⟩*, Method I, Method Ic*: NMT 8.0%. [Note—Use this test when the drug substance predominantly contains water.]

• **Loss on Drying** ⟨731⟩: NMT 10.0%. [Note—Use this test when the drug substance contains water and other volatile solvents.]

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, protected from light, and store in a freezer.

Change to read:

• **Labeling:** Label it to indicate that the material is produced by methods based on recombinant DNA technology. Where it is a dried basis, the label so indicates. ▲Where Insulin Glargine must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.▲ (USP 1-Dec-2022)

• **USP Reference Standards** ⟨11⟩

USP Insulin Glargine RS

USP Insulin Glargine for Peak Identification RS

Contains insulin glargine and 0^A-Arg-insulin glargine.

Topic/Question	Contact	Expert Committee
INSULIN GLARGINE	Jennifer Tong Sun Senior Scientist II	BIO2 Biologics Monographs 2 - Proteins

Chromatographic Database Information: Chromatographic Database

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