Insulin Glargine

$C_{267}H_{404}N_{72}O_{78}S_6$ 6062.89

Insulin (human), 21[A]-glycine-30[B]a-L-arginine-30[B]b-L-arginine [160337-95-1].

DEFINITION

Change to read:

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.• (IRA 1-Nov-2016)

[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*.

Change to read:

- **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-nitranilide as the substrate).• (IRA 1-Nov-2016)

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

Change to read:

- **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• (IRA 1-Nov-2016)) (mg/mL)

Acceptance criteria: 94.0%–105.0% on the anhydrous basis •or dried basis• (IRA 1-Nov-2016)

OTHER COMPONENTS

• **ZINC DETERMINATION**
Standard stock solution: 10 µg/mL of zinc in 0.01 N hydrochloric acid, 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 0.01 N hydrochloric acid
Sample solution: Dissolve 45 mg of Insulin Glargine, accurately weighed, in 50 mL of 0.01 N hydrochloric acid. Dilute 10 mL of the solution with 0.01 N hydrochloric acid to a final volume of 100 mL.
Blank: 0.01 N hydrochloric acid
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: *Standard solutions* and *Blank*
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: *Standard solutions*, *Sample solution*, and *Blank*
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

Change to read:

• **•PRODUCT-RELATED SUBSTANCES•** (IRA 1-Nov-2016)
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• (IRA 1-Nov-2016) ($\%i_x$) 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• (IRA 1-Nov-2016) from the *Sample solution*
r_T = sum of the responses for all of the peaks from the *Sample solution*

Calculate the total percentage of insulin glargine related •substances• (IRA 1-Nov-2016) in the portion of Insulin Glargine taken:

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

$\Sigma\%i_x$ = total percentage of insulin glargine related •substances• (IRA 1-Nov-2016) from the *Sample solution*

Acceptance criteria
Any individual insulin glargine related •substance:• (IRA 1-Nov-2016) NMT 0.5%
Total insulin glargine related •substances:• (IRA 1-Nov-2016) NMT 1.5%

• **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**
Mobile phase: Prepare a mixture of acetonitrile, water, and glacial acetic acid (300:400:200). Adjust with concentrated ammonia (25%–30%) to a pH of 3.0, and dilute with water to a final volume of 1000 mL.
System suitability solution: Dissolve 15 mg of Insulin Glargine containing more than 0.4% high molecular

weight proteins in 1.5 mL of 0.01 N hydrochloric acid. Dilute with water to a final volume of 10 mL. [NOTE—Insulin Glargine containing the indicated percentage of high molecular weight proteins may be prepared by incubating Insulin Glargine at 100° for 1.5–3 h.]

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

Chromatographic system
(See *Chromatography* ⟨621⟩, *System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: Two 8.0-mm × 30-cm in series; 5-µm packing L20
Column temperature: Ambient
Flow rate: 0.5 mL/min
Injection volume: 100 µL

System suitability
Sample: *System suitability solution*
[NOTE—The retention time for the insulin monomer is about 35 min, and the high molecular weight proteins elute earlier.]
Suitability requirements
Resolution: The ratio of the height of the high molecular weight proteins peak to the height of the valley between the high molecular weight proteins peak and the insulin glargine peak is NLT 2.
Tailing factor: NMT 2.0 for the insulin glargine peak

Analysis
Sample: *Sample solution*
Measure the areas of the peak responses, disregarding any peaks having retention times greater than that of the insulin monomer.
Calculate the percentage of high molecular weight proteins in the portion of Insulin Glargine taken:

$$\text{Result} = [\Sigma r_H/(\Sigma r_H + r_U)] \times 100$$

Σr_H = sum of the responses for all peaks having retention times less than that of insulin glargine from the *Sample solution*
r_U = peak response of insulin glargine from the *Sample solution*

Acceptance criteria: NMT 0.3%

SPECIFIC TESTS

- **INSULIN ASSAYS ⟨121⟩, *Bioidentity Test*:** Meets the requirements
- **BACTERIAL ENDOTOXINS TEST ⟨85⟩:** NMT 10 USP Endotoxin Units/mg of Insulin Glargine
- **MICROBIAL ENUMERATION TESTS ⟨61⟩** and **TESTS FOR SPECIFIED MICROORGANISMS ⟨62⟩:** The total bacterial count does not exceed 300 cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.

Change to read:

- **WATER DETERMINATION ⟨921⟩, *Method Ic*:** NMT 8.0%. •[NOTE—Use this test when the drug substance predominantly contains water.]• (IRA 1-Nov-2016)

Add the following:

•• **LOSS ON DRYING ⟨731⟩:** NMT 10.0%. [NOTE—Use this test when the drug substance contains water and other volatile solvents.]• (IRA 1-Nov-2016)

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.• (IRA 1-Nov-2016)
- **USP REFERENCE STANDARDS ⟨11⟩**
  USP Endotoxin RS
  USP Insulin Glargine RS
  USP Insulin Glargine for Peak Identification RS
  Contains insulin glargine and 0^A-Arg-insulin glargine.