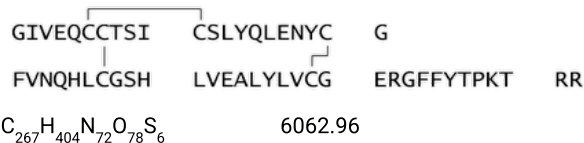


Status: Currently Official on 15-Feb-2025
Official Date: Official as of 01-Dec-2022
Document Type: USP Monographs
DocId: GUID-1FB2BBF9-9095-4966-BB9B-4B549843ED43_5_en-US
DOI: https://doi.org/10.31003/USPNF_M40550_05_01
DOI Ref: 075ua

© 2025 USPC
Do not distribute

Insulin Glargine



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