

Insulin Glargine Injection

DEFINITION

Insulin Glargine Injection is a sterile solution of Insulin Glargine in Water for Injection. It has a potency of NLT 95.0 and NMT 105.0 USP Insulin Glargine Units/mL.

IDENTIFICATION

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

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 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96	4
20	83	17
30	63	37
40	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 1: 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 5-mL volumetric flask, and dilute with water to volume. Dilute 4 mL of this solution with water (IRA 1-Nov-2016) to 10 mL in a volumetric flask.

Standard solution 2: 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.

Standard solution 3: 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 5-mL volumetric flask, and dilute with water to volume. Dilute 3 mL of this solution with water (IRA 1-Nov-2016) to 5 mL in a volumetric flask.

Sample solution: Quantitatively dilute a portion of Injection with water to obtain a solution containing about 40 USP Insulin Glargine Units/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: 0.55 mL/min

Injection volume: 5 μL

System suitability

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

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%, calculated from six response factors from two duplicate injections of each *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

Analysis

Samples: *Standard solutions* and *Sample solution*
Measure the responses of the major peaks. Prepare a calibration curve based on the peak responses from the *Standard solutions* versus the concentrations (USP Insulin Glargine Units/mL) using linear regression. Calculate the potency, in USP Insulin Glargine Units/mL, of the portion of Injection taken:

$$\text{Result} = [(r_u - b)/a] \times D$$

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

b = y -intercept of the calibration curve

a = slope of the calibration curve

D = dilution factor used to prepare the *Sample solution*

Acceptance criteria: 95.0–105.0 USP Insulin Glargine Units/mL

OTHER COMPONENTS

Change to read:

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: Dilute 1 mL of Injection with 0.01 N hydrochloric acid to 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.

2 Insulin

Suitability requirements

Correlation coefficient: NLT 0.999

Analysis

Samples: Standard solutions, Sample solution, and Blank

Determine the concentration, C , in $\mu\text{g/mL}$ of zinc in the Sample solution using the calibration curve. Calculate the quantity of zinc in the portion of Injection taken:

$$\text{Result} = C \times D$$

C = concentration of zinc in the Sample solution ($\mu\text{g/mL}$)

D = dilution factor, 100

Acceptance criteria: 20–40 $\mu\text{g/mL}$ (IRA 1-Nov-2016)

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

- **PRODUCT-RELATED SUBSTANCES** (IRA 1-Nov-2016)
Mobile phase, System suitability solution, Standard solutions, 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 Injection taken:

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

r_i = peak response of the insulin glargine related substance (IRA 1-Nov-2016) from the Sample solution

r_r = sum of all the peak responses from the Sample solution

Calculate the total percentage of insulin glargine related substances (IRA 1-Nov-2016) in the portion of Injection 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 2.0%

Change to read:

- **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: Quantitatively dilute a portion of Injection with water to obtain a solution containing about 40 USP Insulin Glargine Units/mL.

Chromatographic system

(See Chromatography <621>, System Suitability.)

Mode: LC

Detector: UV 276 nm

Column: Two 8.0-mm \times 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 Injection 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 0.5% (IRA 1-Nov-2016)

SPECIFIC TESTS

- **PH <791>:** 3.5–4.5
- **BACTERIAL ENDOTOXINS TEST <85>:** NMT 80 USP Endotoxin Units per 100 USP Insulin Glargine Units
- **STERILITY TESTS <71>:** Test for Sterility of the Product to Be Examined, Membrane Filtration: Meets the requirements
- **PARTICULATE MATTER IN INJECTIONS <788>:** Meets the requirements for small-volume injections
- **INJECTIONS AND IMPLANTED DRUG PRODUCTS <1>:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protected from sunlight, and avoid freezing.
- **LABELING:** States that it has been prepared with Insulin Glargine produced by methods based on recombinant DNA technology. Label it to state that it is to be stored in a refrigerator and that freezing is to be avoided. The label states the potency in USP Insulin Glargine Units/mL.
- **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.