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**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>30</td>
<td>63</td>
<td>37</td>
</tr>
<tr>
<td>40</td>
<td>96</td>
<td>4</td>
</tr>
</tbody>
</table>

[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 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 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°-Arg-insulin glargine peak to the height of the valley between the 0°-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:

\[
\text{Result} = \left( \frac{r_U - b}{a} \right) \times D
\]

- **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.

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Insulin

**PRODUCT-RELATED SUBSTANCES AND IMPURITIES**

**Change to read:**

- **PRODUCT-RELATED SUBSTANCES**
  - Mobile phase, System suitability solution, Standard solutions, Sample solution, Chromatographic system, and System suitability: Proceed as directed in the Assay.
  - Analysis
    - Sample: Sample solution
    - Extract the insulin glargine related *substance* from the Sample solution
    - Calculate the total percentage of insulin glargine related *substances* in the portion of injection taken:
      \[ \text{Result} = \sum \% i x \]
      \[ \% i x = \text{total percentage of insulin glargine related *substances* from the Sample solution} \]
    - Acceptance criteria
      - Any individual insulin glargine related *substance*: NMT 0.5% (IRA 1-Nov-2016)
      - 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°F 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 × 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:
  \[ \text{Result} = \left( \frac{\Sigma r_h / (\Sigma r_h + r_U)}{100} \right) \]
  \[ \Sigma r_h = \text{sum of the responses for all peaks having retention times less than that of insulin glargine from the Sample solution} \]
  \[ r_U = \text{peak response of insulin glargine from the Sample solution} \]
- 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 repack. 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 0A-Arg-insulin glargine.