Isophane Insulin Human Suspension

DEFINITION

Isophane Insulin Human Suspension is a sterile suspension of zinc-insulin human crystals and Protamine Sulfate in buffered Water for Injection, combined in a manner such that the solid phase of the suspension consists of crystals composed of insulin human, protamine, and zinc. The Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* Linné (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, as in the *Assay*, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units in each mL.

IDENTIFICATION

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

ASSAY

- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into this solution, and adjust, if necessary, with ethanolamine to a pH of 2.3.

Mobile phase: Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to a temperature equal to or higher than 20° to avoid precipitation.]

System suitability solution: Dissolve about 1.5 mg of insulin in 1.0 mL of 0.01 N hydrochloric acid. Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Standard solution: 1.5 mg/mL of USP Insulin Human RS in 0.01 N hydrochloric acid

Sample solution A (for Suspension labeled as containing 40 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension to clarify, and mix.

Sample solution B (for Suspension labeled as containing 100 USP Insulin Human Units/mL)**:** Add 2.5 µL of 9.6 N hydrochloric acid per mL of a volume of Suspension. Allow the suspension, if present, to clarify, and mix. [NOTE—Pooling of several package units may be necessary to obtain sufficient volume of the sample.] Pipet 2 mL of this solution into a 5-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix. [NOTE—The *Standard solution* and *Sample solutions* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 214 nm
Column: 4.6-mm × 15-cm; packing L1
Column temperature: 40°
Flow rate: 1 mL/min
Injection volume: 20 µL

System suitability
Samples: *System suitability solution* and *Standard solution*
Suitability requirements
Resolution: NLT 2.0 between the insulin and A-21 desamido insulin peaks, *System suitability solution*
Tailing factor: NMT 1.8 for the insulin peak, *System suitability solution*
Relative standard deviation: NMT 1.6%, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution A* or *Sample solution B*

From the measured chromatographic responses for insulin and A-21 desamido insulin, calculate the potency, in USP Insulin Human Units/mL, of Suspension taken:

$$\text{Result} = (\Sigma r_U/\Sigma r_S) \times (C \times D)$$

Σr_U = sum of the peak areas of insulin and A-21 desamido insulin from the *Sample solution*
Σr_S = sum of the peak areas of insulin and A-21 desamido insulin from the *Standard solution*
C = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
D = dilution factor

Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Human Units in each mL

OTHER COMPONENTS

- **ZINC DETERMINATION ⟨591⟩:** 0.021–0.04 mg for each 100 USP Insulin Human Units

IMPURITIES

- **LIMIT OF HIGH MOLECULAR WEIGHT PROTEINS**

Solution A: 1 mg/mL of L-arginine in water

Mobile phase: Acetonitrile, *Solution A*, and glacial acetic acid (20:65:15)

Resolution solution: Dissolve 4 mg of insulin containing more than 0.4% high molecular weight proteins in 1 mL of 0.01 N hydrochloric acid. Store this solution in a refrigerator, and use within 7 days. [NOTE—Insulin containing the indicated percentage of high molecular weight proteins may be prepared by allowing insulin to stand at room temperature for about 5 days.]

Sample solution: Quantitatively add 4 µL of 6 N hydrochloric acid per mL of a volume of Suspension, and mix.

Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 276 nm
Column: 7.8-mm × 30-cm; packing L20
Flow rate: About 0.5 mL/min
Injection volume: 100 µL

System suitability
Sample: *Resolution solution*
[NOTE—The relative retention times for the polymeric insulin complexes, for the covalent insulin dimer, and for the insulin monomer, with salts eluting after the insulin monomer, are 13–17, about 17.5, and 18–22 min, respectively.]
Suitability requirements
Resolution: NLT 2.0 for the ratio of the height of the covalent insulin dimer peak to the height of the valley between the covalent insulin dimer peak and the insulin monomer peak

Analysis
Sample: *Sample solution*

Inject the *Sample solution*, and measure the peak area responses, disregarding any peaks having retention times greater than the insulin monomer.

Calculate the percentage of high molecular weight proteins in the portion of Suspension taken:

$$\text{Result} = 100\Sigma r_H/(\Sigma r_H + r_M)$$

Σr_H = sum of the peak responses for all peaks having retention times less than that of the insulin monomer

r_M = peak response of the insulin monomer
Acceptance criteria: NMT 3.0% for insulin monomer

SPECIFIC TESTS

- **INSULIN IN THE SUPERNATANT**
  Sample solution: Centrifuge 10 mL of the Suspension at 1500 × *g* for 10 min. Use the supernatant.
  Analysis: Determine the insulin content of the *Sample solution* by a suitable method.
  Acceptance criteria: NMT 1.0 USP Insulin Human Unit/mL
- **pH** ⟨**791**⟩**:** 7.0–7.5, determined potentiometrically
- **BACTERIAL ENDOTOXINS TEST** ⟨**85**⟩**:** It contains NMT 80 USP Endotoxin Units/100 USP Insulin Human Units.

Change to read:

• **STERILITY TESTS** ⟨**71**⟩**:** It meets the requirements, when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*, •and the Suspension being filtered immediately after it has been put into solution using a validated suitable solvent.• (RB 1-Jul-2012)

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in the unopened multiple-dose container provided by the manufacturer. Do not repackage. Store in a refrigerator, protect from sunlight, and avoid freezing.
- **LABELING:** The Suspension container label states that the Suspension is to be shaken carefully before use. The labeling states also that it has been prepared with insulin human of semisynthetic origin (i.e., derived by enzyme modification of pork pancreas insulin) or with insulin human of recombinant DNA origin (i.e., obtained from microbial synthesis), whichever is applicable. 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 Units/mL.
- **USP REFERENCE STANDARDS** ⟨**11**⟩
  USP Endotoxin RS
  USP Insulin (Pork) RS
  USP Insulin Human RS