## 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. Protamine Sulfate is prepared from the sperm or from the mature testes of fish belonging to the genus *Oncorhynchus* Suckley, or *Salmo* L. (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components as determined in the *Assay*, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Human Units/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 with ethanolamine to a pH of 2.3, if necessary.

**Mobile phase:** Acetonitrile and *Solution A* (26:74). [NOTE—The acetonitrile is warmed to NLT 20° to avoid precipitation.]

**System suitability solution:** 1.5 mg/mL of insulin human in 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 human.

**Standard solution:** 1.5 mg/mL of USP Insulin Beef 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 to each milliliter of an accurately measured 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 to each milliliter of an accurately measured volume of Suspension. Allow the suspension 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.

**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 insulin human and A-21 desamido insulin human, *System suitability solution*
**Tailing factor:** NMT 1.8 for the insulin human peak, *System suitability solution*
**Relative standard deviation:** NMT 1.6%, *Standard solution*

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

Calculate the potency, in USP Insulin Human Units/mL, of Suspension taken:

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

$r_U$ = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Sample solution*
$r_S$ = sum of the peak responses of insulin human and A-21 desamido insulin human from the *Standard solution*
$C_S$ = concentration of USP Insulin Human RS in the *Standard solution* (USP Insulin Human Units/mL)
$D$ = dilution factor used to prepare the *Sample solution*

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

### OTHER COMPONENTS

***Change to read:***

▲• **ZINC DETERMINATION** ⟨591⟩**:**▲ (IRA 1-Jan-2019) 0.021–0.04 mg for every 100 USP Insulin Human Units

### PRODUCT-RELATED SUBSTANCES AND IMPURITIES

- **PHYSICOCHEMICAL ANALYTICAL PROCEDURES FOR INSULINS** ⟨121.1⟩**,** *Limit of High Molecular Weight Proteins*

Proceed as directed in the chapter, except for the *Sample solution*. It meets the requirements.

**Sample solution:** Quantitatively add 4 µL of 6 N hydrochloric acid to each milliliter of an accurately measured volume of Suspension, and mix.

**Acceptance criteria:** NMT 3.0%

### SPECIFIC TESTS

- **INSULIN IN THE SUPERNATANT**

**Sample solution:** Centrifuge 10 mL of 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
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Human Units
- **STERILITY TESTS** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed and the Suspension being filtered immediately after it has been put into a solution using a validated suitable solvent.

### 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 that it has been prepared with Insulin Human produced by methods based on recombinant DNA technology or that it is derived by enzymatic modification of insulin from porcine pancreas. 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 Human Units/mL.
- **USP REFERENCE STANDARDS** ⟨11⟩
USP Insulin Human RS