Isophane Insulin Suspension

Type of Posting	Revision Bulletin
Posting Date	29–Mar–2019
Official Date	01–May–2019
Expert Committee	Biologics Monographs 1–Peptides
Reason for Revision	Omission of USP Insulin Beef RS and requirements related to bovine insulin due to the absence of commercial bovine insulin products in the United States

In accordance with the Rules and Procedures of the 2015–2020 Council of Experts, the Biologics Monographs 1–Peptides Expert Committee has revised the Isophane Insulin Suspension monograph. The purpose for the revision is to remove the USP Insulin Beef RS and requirements related to bovine insulin because there are no approved manufacturers of therapeutic bovine insulin in the United States, and suitable reference materials are not available.

Although USP Insulin Beef RS is used as a standard for *Identification* and *Assay* for insulins of bovine origin, the only use for non-bovine products is as an *Identification solution*, where the USP Insulin Pork RS and USP Insulin Beef RS are mixed together and used as a standard for identification based on retention time. The USP Insulin Beef RS and USP Insulin Pork RS are well resolved in the *Assay*, with retention times of approximately 14 and 21 min, respectively, so the USP Insulin Beef RS is not necessary for identification of insulin pork. Therefore, references to bovine insulin and to the USP Insulin Beef RS have been removed from *Identification* and *Assay*, and associated changes have also been made to *Definition*, *Labeling*, and *USP Reference Standards*.

The Isophane Insulin Suspension Revision Bulletin supersedes the currently official monograph.

Should you have any questions or concerns, please contact Diane McCarthy, Senior Manager (301-692-3637 or diane.mccarthy@usp.org).

Isophane Insulin Suspension

DEFINITION

Isophane Insulin Suspension is a sterile suspension of zinc–insulin 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, 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* L. (Fam. Salmonidae). Its potency, based on the sum of its insulin and desamido insulin components, is NLT 95.0% and NMT 105.0% of the potency stated on the label, expressed in USP Insulin Units/mL.

IDENTIFICATION

Change to read:

- **A.** The retention time of the insulin ▲pork▲ (RB 1-May-2019) peak of *Sample solution A* or *Sample solution B* corresponds to that of the ▲main peak▲ (RB 1-May-2019) of the *Identification solution*, as obtained in the *Assay*▲, and no other significant peaks are observed.▲ (RB 1-May-2019) [NOTE—It may be necessary to inject a mixture of *Sample solution* and *Identification solution*.]

ASSAY

Change to read:

- **PROCEDURE**

Solution A: Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water. Pipet 2.7 mL of phosphoric acid into the 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 ▲▲ (RB 1-May-2019) insulin pork in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019) Allow to stand at room temperature for NLT 3 days to obtain a solution containing NLT 5% of A-21 desamido insulin.

Identification solution: 0.6 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. [NOTE—The *Identification solution* may be stored at room temperature for up to 12 h or in a refrigerator for up to 48 h.]

Standard solution: 1.5 mg/mL of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in 0.01 N hydrochloric acid. ▲▲ (RB 1-May-2019)

Sample solution A (for Suspension labeled as containing 40 USP Insulin Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for 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 Units/mL): Add 2.5 µL of 9.6 N hydrochloric acid for each milliliter of an accurately measured volume of Suspension. Allow the suspension to clarify, and mix. [NOTE—Pooling 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 and A-21 desamido insulin, *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: *Identification solution*, *Standard solution*, and either *Sample solution A* or *Sample solution B*
Measure the peak responses for insulin and A-21 desamido insulin ▲▲ (RB 1-May-2019), using the chromatogram of the *Identification solution* to identify the insulin peaks.
▲▲ (RB 1-May-2019) Calculate the potency, in USP Insulin Units/mL, in the portion 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 and A-21 desamido insulin from the *Sample solution*
r_S = sum of the peak responses of insulin and A-21 desamido insulin from the *Standard solution*
C_S = concentration of ▲▲ (RB 1-May-2019) USP Insulin Pork RS in the *Standard solution* (USP Insulin Units/mL)
D = dilution factor used to prepare the *Sample solution*

▲▲ (RB 1-May-2019)
Acceptance criteria: 95.0%–105.0% of the potency stated on the label, expressed in USP Insulin Units/mL

OTHER COMPONENTS

- **ZINC DETERMINATION** ⟨591⟩**:** 10–40 µg for every 100 USP Insulin 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 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 Unit/mL

- **pH** ⟨791⟩**:** 7.0–7.8
- **BACTERIAL ENDOTOXINS TEST** ⟨85⟩**:** NMT 80 USP Endotoxin Units per 100 USP Insulin Units
- **STERILITY TESTS** ⟨71⟩**,** *Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements when tested as directed in the chapter 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.

Change to read:

- **LABELING:** Label it as ▲ ▲ (RB 1-May-2019) porcine ▲ ▲ (RB 1-May-2019). If the Isophane Insulin Suspension is made from insulin that is purified, label it as such. The Suspension container label states that the Suspension is to be shaken carefully before use. 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.

Change to read:

- **USP REFERENCE STANDARDS** ⟨11⟩
▲ ▲ (RB 1-May-2019)
USP Insulin Pork RS