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Iron Sucrose Injection

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-iron-sucrose-inj-20230331.

DEFINITION

Iron Sucrose Injection is a sterile, colloidal solution of ferric hydroxide in complex with Sucrose in Water for Injection. It contains NLT 95.0% and NMT 105.0% of the labeled amount of iron. Sodium Hydroxide may be added to adjust the pH. It contains no antimicrobial agent, chelating agent, dextran, gluconate, or other added substances.

IDENTIFICATION

• **A. Iron**

To 2.5 mL of Injection add 17.5 mL of water and 5 mL of hydrochloric acid. Mix and heat the solution for 5 min in a boiling water bath. Cool, add dropwise 13.5 N ammonium hydroxide until no further precipitation of ferric hydroxide occurs, and filter. Wash the precipitate with water to remove excess ammonium hydroxide, dissolve the precipitate in a minimum volume of 2 N hydrochloric acid, and add sufficient water to make a volume of 20 mL. To 3 mL of the solution add 1 mL of 2 N hydrochloric acid and 1 mL of potassium thiocyanate TS: the resulting solution (Solution 1) is red. To 1 mL of Solution 1 add 5 mL of amyl alcohol or ethyl ether, shake, and allow to stand: the organic layer is pink. To a separate 1-mL aliquot of Solution 1 add 2 mL of mercuric chloride TS: a red color is discharged [iron (III) salts].

• **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay* for *Sucrose*.

• **C. Molecular Weight Determination**

Mobile phase: Dissolve 7.12 g of sodium phosphate, dibasic, dihydrate, 5.52 g of sodium phosphate, monobasic and 0.40 g of sodium azide in 2 L of water.

System suitability solution: Dissolve 200 mg of high molecular weight dextran and 100 mg of glucose in 20 mL of *Mobile phase*.

Standard solutions: Transfer about 20 mg of each polysaccharide molecular weight standard (5,000–400,000 Da) to separate 5-mL volumetric flasks. Add 4 mL of *Mobile phase* to each flask, and allow each aliquot to stand at or below 25° for a minimum of 12 h. After the agglomerate particles of each *Standard solution* have swelled to their fullest extent, gently swirl each *Standard solution* until dissolved. [Note—The chromatograms of freshly prepared *Standard solutions* regularly show a small, unidentified secondary peak following the main peak. Discard the *Standard solutions* if the secondary peak reaches half the height of the main peak.]

Sample solution: Transfer 5.0 mL of Injection to a 10-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: Refractive index, maintained at a constant temperature of 45°

Columns: Two 7.8-mm × 30-cm; packing L39 with pore sizes of 1000 and 120 Å, respectively

Column temperature: 45 ± 2°

Flow rate: 0.5 mL/min

Injection volume: 25 µL

System suitability

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

Suitability requirements

Resolution: NLT 4.0 between dextran and glucose, *System suitability solution*

Correlation coefficient: NLT 0.98 for the calibration curve generated using a suitable program, plotting the retention times of the *Standard solutions* and their molecular weights to generate a third order (cubic) calibration curve

Analysis

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

The molecular weight of the complex is calculated from the calibration curve. The molecular weight distribution curve of the sample is sliced into fractions.

Calculate the weight-average molecular weight (M_W) as follows:

$$\text{Result} = \Sigma(A_T M_T)/\Sigma A_T$$

Calculate the number-average molecular weight (M_N) as follows:

$$\text{Result} = \Sigma(A_T)/\Sigma(A_T/M_T)$$

A_T = area of each fraction of the sample distribution

M_T = corresponding mean molecular weight of each fraction as determined from its retention time on the calibration curve

Acceptance criteria: The molecular weight distribution curve of the Injection conforms to the following parameters.

M_W: 34,000–60,000 Da

M_N: NLT 24,000 Da

M_W/M_N: NMT 1.7

ASSAY

- **Sucrose**

Mobile phase: Acetonitrile and water (79:21)

Standard solutions: Individual solutions of 13, 16, 18, 21, and 23 mg/mL of sucrose from USP Sucrose RS, in water

Sample solution: Transfer about 1.875 g of Injection to a 25-mL flask. Add 1.25 mL of water and mix. Add 1.25 mL of a sodium phosphate, monobasic solution, prepared by dissolving 30 g in 50 mL, and mix. Allow the resulting solution to stand for 10 min to precipitate the colloidal ferric hydroxide. Dilute with water to volume. Centrifuge this solution at 3000 rpm for 15 min. Pass the resulting solution through a filter, discarding the first 2 mL of the filtrate.

Chromatographic system

(See *Chromatography ⟨621⟩, System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4-mm × 25-cm; packing L8

Temperatures

Detector: 20–25° (±2°)

Column: 20–25° (±2°)

Flow rate: 2 mL/min

Injection volume: 20 µL

System suitability

Samples: *Standard solutions*

[Note—The retention time for sucrose is about 8 min.]

Suitability requirements

Correlation coefficient: NLT 0.998 from the linear regression of the *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the peak area for each *Standard solution* versus concentration of sucrose in mg/mL, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration of sucrose, in mg/mL, in the *Sample solution*.

Calculate the quantity of sucrose, in mg, in each mL of Injection taken:

$$\text{Result} = (C_U \times D \times G)/W$$

C_U = concentration of sucrose in the *Sample solution* (mg/mL)

D = dilution volume of the *Sample solution* (mL)

G = density of Injection taken (g/mL)

W = weight of Injection taken (g)

Acceptance criteria: 260–340 mg/mL

- **Iron**

Solution A: Transfer 2.64 g of calcium chloride to a 1000-mL volumetric flask, add 500 mL of water, and swirl to dissolve. Add 5.0 mL of hydrochloric acid, and dilute with water to volume.

Standard stock solution: 50 µg/mL of iron prepared as follows. Transfer about 350 mg of ferrous ammonium sulfate to a 1000-mL volumetric flask. Add water to dissolve, dilute with water to volume, and mix.

Standard solutions: Individual solutions containing 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL of iron in *Solution A* from the *Standard stock solution*

Sample stock solution: Using a "to contain" pipet, transfer 2.0 mL of Injection to a 100-mL volumetric flask. Rinse the pipet several times with *Solution A*. Add 5 mL of hydrochloric acid, and swirl until the solution turns yellow. After the solution has cooled to room temperature, dilute with *Solution A* to volume, and mix.

Sample solution: Nominally 8.0 µg/mL of iron prepared as follows. Pipet 2.0 mL of the *Sample stock solution* to a 100-mL volumetric flask, and dilute with *Solution A* to volume.

Instrumental conditions

(See *Atomic Absorption Spectroscopy ⟨852⟩*.)

Mode: Atomic absorption spectrophotometry

Analytical wavelength: 248.3 nm iron emission line

Lamp: Iron hollow-cathode

Flame: Air–acetylene

Blank: *Solution A*

Analysis

Samples: *Standard solutions* and *Sample solution*

Plot the absorbance of each *Standard solution* versus concentration, in µg/mL, of iron, and draw the straight line best fitting the five plotted points. From the graph, determine the concentration, in µg/mL, of iron in the *Sample solution*.

Calculate the percentage of the labeled amount of iron in each mL of Injection taken:

$$\text{Result} = (C_A/C_U) \times 100$$

C_A = actual concentration of iron in the *Sample solution* determined from the calibration curve (µg/mL)

C_U = nominal concentration of iron in the *Sample solution* (µg/mL)

Acceptance criteria: 95.0%–105.0%

OTHER COMPONENTS

• **Content of Chloride**

Sample: About 12 g of Injection

Analysis: Transfer the *Sample* to a 50-mL beaker. Add 40 mL of water and 0.3 mL of 65% nitric acid, and, while stirring, titrate with 0.01 N silver nitrate VS, determining the endpoint potentiometrically with silver-glass electrodes.

Calculate the content of chloride, in mg, of Injection taken. Each mL of 0.01 N silver nitrate consumed is equal to 0.3545 mg of chloride (Cl).

Acceptance criteria: 0.012%–0.025%

IMPURITIES

• **Limit of Iron [Fe(II)]**

Supplementary electrolyte solution: Dissolve 15.0 g of sodium acetate in 100 mL of water and adjust with 0.1 N acetic acid to a pH of 7.0.

Sample solution: Volume of Injection equivalent to 20–120 µg/mL of elemental iron

Analysis: Transfer a suitable amount of *Supplementary electrolyte solution* to a polarographic cell equipped with a mercury drop electrode. With the electrode submerged in the liquid, bubble nitrogen through the liquid for 5 min. Avoiding any undue exposure to air, immediately transfer the *Sample solution* to the polarographic cell. The sample must be analyzed immediately upon opening the container.

Record the polarogram from 0 mV and −1700 mV. The iron [Fe(III)/Fe(II)] peak is detected at −750 ± 50 mV and the iron [Fe(II)/Fe(0)] peak is detected at −1400 ± 50 mV. Measure the iron [Fe(II)/Fe(III)] peak responses obtained from the polarogram, and perform a blank determination.

Calculate the content of iron [Fe(II)], in % w/v, in the volume of Injection taken:

$$\text{Result} = [1 - (2/R)] \times C_T$$

R = peak response ratio of iron [Fe(II)] to iron [Fe(III)]

C_T = total iron concentration of the Injection (% w/v)

Acceptance criteria: NMT 0.4%

SPECIFIC TESTS

• **pH** ⟨791⟩**:** 10.5–11.1 at 20°

• **Turbidity**

Sample solution: Transfer 0.5 g of Injection to a 150-mL beaker. Add 100 mL of water and, with constant stirring, adjust with 0.1 N hydrochloric acid VS to a pH of 6.0.

Analysis: Remove the pH electrode from the solution. Adjust a light source such that the beam hits the beaker at a parallel angle 2 cm below the surface of the liquid. The light must shine through to the surface, and the solution must not have any turbidity. Measurement must be carried out in a room as dark as possible. Slowly add 0.1 N hydrochloric acid VS, dropwise, until a slight but lasting turbidity develops. Record the pH of the solution as the turbidity point of the Injection.

Acceptance criteria: 4.4–5.3

• **Absence of Low-Molecular Weight Iron [Fe(II) and Fe(III)] Complexes:** In the polarograms obtained in the test for *Limit of Iron [Fe(II)]*, no additional peaks are found.

• **Alkalinity**

Sample solution: 5 mL of Injection

Analysis: Titrate the *Sample solution* with 0.1 N hydrochloric acid VS with constant stirring to a pH of 7.4. Record the volume of 0.1 N hydrochloric acid VS consumed, and calculate the alkalinity of the Injection as the volume of acid, in mL, consumed per mL of Injection.

Acceptance criteria: 0.5–0.8 mL of 0.1 N hydrochloric acid VS is consumed per mL of Injection.

• **Osmolality and Osmolarity** ⟨785⟩

Osmolarity

Sample solution: Dilute Injection in water (1 in 10).

Acceptance criteria: 1150–1350 mOsmol/L

- **Specific Gravity ⟨841⟩:** 1.135–1.165 at 20°
- **Particulate Matter in Injections ⟨788⟩,** *Method 1 Light Obscuration Particle Count Test*

Sample solution: Prepare a solution of Injection (1 in 40) using water that has been passed through a filter having a 1.2-µm or finer pore size.

Acceptance criteria: Meets the requirements for small-volume injections

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 3.7 USP Endotoxin Units/mg of iron contained in Injection
- **Other Requirements:** Meets the requirements in *Injections and Implanted Drug Products ⟨1⟩*

ADDITIONAL REQUIREMENTS

Change to read:

- **Packaging and Storage:** Preserve in single-dose containers▲, preferably▲ (RB 1-Apr-2023) of Type I glass. Store at controlled room temperature. Do not freeze.
- **Labeling:** Label it to indicate that it is for intravenous use only, and that when administered by intravenous infusion, the Injection must be diluted with 0.9% Sodium Chloride Injection to a concentration of 1.0–2.0 mg/mL of elemental iron. Label it also to state the total osmolarity of the solution expressed in mOsmol/L.
- **USP Reference Standards ⟨11⟩**

USP Sucrose RS

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
IRON SUCROSE INJECTION	Documentary Standards Support	SM22020 Small Molecules 2

Chromatographic Database Information: Chromatographic Database

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