Insulin

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 Insulin 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, Assay, and the test for Product-Related Substances 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. Furthermore, Identification B, Peptide Mapping, can easily distinguish between bovine and porcine insulin. Therefore, references to bovine insulin and to the USP Insulin Beef RS have been removed from the Identification, Assay, and the test for Product-Related Substances, and associated changes have also been made to the chemical information, Definition, Labeling, and USP Reference Standards.

The Insulin 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).
Insulin

Change to read:

GIVEQCCTSICSLYQLENYCN
FVNQHLGSHLVEALYLVGERGFFYTPKA

C_{25S}H_{381}N_{86}O_{25}S_8 5777.54
Insulin (pig) [12584-58-6].

▲ ▲ (RB 1-May-2019)

DEFINITION

Change to read:

Insulin is a two-chain peptide hormone consisting of 51 amino acids, and its structure corresponds to native insulin produced in vivo by the beta cells of the pancreas. The A-chain is composed of 21 amino acids, and the B-chain is composed of 30 amino acids. ▲ ▲ (USP 1-May-2019) It is obtained from the pancreas of healthy ▲ ▲ (RB 1-May-2019) porcine animals, ▲ ▲ (RB 1-May-2019) used for food by humans. Its potency is NLT 26.5 USP Insulin Units/mg, calculated on the dried basis; Insulin labeled as purified contains NLT 0.0345 mg of pure Insulin derived from pork.

IDENTIFICATION

Change to read:

- **A.** The retention time of the major peak in the Sample solution corresponds to that ▲ ▲ (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.]

Delete the following:

**B. Peptide Mapping**

Sulfate buffer: 2.0 M ammonium sulfate and 0.5 M sulfuric acid (1:1)

Enzyme solution: 500 units/mL of Staphylococcus aureus V-8 protease activity in water

HEPES buffer: 0.1 M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). Adjust with 5 M sodium hydroxide to a pH of 7.5 before diluting with water to a final volume.

Solution A: Acetonitrile, water, and Sulfate buffer (100:700:200)

Solution B: Acetonitrile, water, and Sulfate buffer (400:400:200)

Mobile phase: See 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>90</td>
<td>10</td>
</tr>
<tr>
<td>60</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>65</td>
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<td>100</td>
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</table>

Table 1 (continued)

<table>
<thead>
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<th>Time (min)</th>
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<th>Solution B (%)</th>
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<tbody>
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<td></td>
<td>100</td>
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<tr>
<td>71</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>86</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Standard digest solution: 2 mg/mL of USP Insulin RS of the appropriate species in 0.01 N hydrochloric acid.

Add the following:

**B. Physicochemical Analytical Procedures for Insulins (121.1), Peptide Mapping** Proceed as directed in the chapter, except for the Mobile phase and System suitability.

Mobile phase: See Table 1.
Change to read:

**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 the solution, and adjust with ethanolamine to a pH of 2.3, if necessary.

Mobile phase: Acetonitrile and Solution A (26:74).

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

Identification solution: 0.6 mg/mL of USP Insulin Pork RS in 0.01 N hydrochloric acid

Standard solution: 1.5 mg/mL of *USP Insulin Pork RS* (92:1-May-2019) in 0.01 N hydrochloric acid

Sample solution: 1.5 mg/mL of Insulin in 0.01 N hydrochloric acid

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

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<tbody>
<tr>
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<td>86</td>
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System suitability

Sample: Standard solution

Suitability requirements

Resolution: NLT 1.9 between digest fragments II and III

Chromatogram similarity: The chromatogram of the Standard solution corresponds to that of the reference chromatogram provided with USP Insulin Pork RS.

Acceptance criteria: Meets the requirements USP 1-May-2019

Add the following:

**PRODUCT-RELATED SUBSTANCES AND IMPURITIES**

**PRODUCT-RELATED SUBSTANCES**

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.

Solution B: Acetonitrile and Solution A (18:82)

Solution C: Acetonitrile and Solution A (50:50)

Mobile phase: See Table 2.

Table 2

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution B (%)</th>
<th>Solution C (%)</th>
</tr>
</thead>
<tbody>
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<td>81</td>
<td>19</td>
</tr>
<tr>
<td>60</td>
<td>81</td>
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<td>91</td>
<td>36</td>
<td>64</td>
</tr>
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<td>92</td>
<td>81</td>
<td>19</td>
</tr>
</tbody>
</table>

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

**OTHER COMPONENTS**

**PRODUCT-RELATED SUBSTANCES**

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.

Solution B: Acetonitrile and Solution A (18:82)

Solution C: Acetonitrile and Solution A (50:50)

Mobile phase: See Table 2.

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 Sample solution

Measure the peak responses for insulin and A-21 desamido insulin, using the chromatogram of the Identification solution to identify the insulin peaks.

Result = \((2r_d / r_s) \times (C_d / C_s)\)

\(r_d\) = 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

\(C_d\) = concentration of *USP Insulin Pork RS* in the Standard solution (USP Insulin Units/mL)

\(C_s\) = concentration of Insulin in the Sample solution (mg/mL)

Acceptance criteria: NLT 26.5 USP Insulin Units/mg on the dried basis; Insulin labeled as purified contains NLT 27.0 USP Insulin Units/mg on the dried basis.
[NOTE—Standard solutions A–C may be stored at room temperature for up to 12 h and in a refrigerator for up to 48 h.]

**Standard solution A:** 3.75 mg/mL of USP Insulin Pork RS (RB 1-May-2019) in 0.01 N hydrochloric acid (RB 1-May-2019)

**Standard solution B:** 0.375 mg/mL of USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of Standard solution A into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Standard solution C:** 0.0375 mg/mL of USP Insulin Pork RS in 0.01 N hydrochloric acid prepared as follows. Pipet 1 mL of Standard solution B into a 10-mL volumetric flask, dilute with 0.01 N hydrochloric acid to volume, and mix.

**Sample solution:** 3.75 mg/mL of Insulin in 0.01 N hydrochloric acid. Prepare the solution in a capped vial, volume, and mix.

**Chromatographic system**

**System suitability**

**Suitability requirements for the Standard solutions**

Sample: System suitability solution, Standard solution A, Standard solution B, and Standard solution C

[Note—Adjust the Mobile phase composition and the duration of the isocratic elution to obtain a retention time of about 31 min for insulin, with the A-21 desamido insulin eluting just prior to the start of the gradient elution phase.]

**Suitability requirements for the System suitability solution**

**Resolution:** NLT 2.0 between insulin and A-21 desamido insulin

**Tailing factor:** NMT 1.8 for the insulin peak

**Suitability requirements for the Standard solutions**

Calculate the factor X₁:

\[ X_1 = \frac{(r_B/r_S) \times D}{r_B} = \text{peak response from Standard solution B} \]
\[ r_S = \text{peak response from Standard solution A} \]
\[ D = \text{dilution factor, 10} \]

**Result:** Between 0.91 and 1.09

Calculate the factor X₂:

\[ X_2 = \frac{(r_C/r_S) \times D}{r_C} = \text{peak response from Standard solution C} \]
\[ r_S = \text{peak response from Standard solution A} \]
\[ D = \text{dilution factor, 100} \]

**Result:** Between 0.7 and 1.3

**Analysis**

Sample: Sample solution

Calculate the percentage of insulin, A-21 desamido insulin, and other insulin-related substances, USP 1-May-2019, in the portion of insulin taken:

Result = \((r_C/r_S) \times 100\)

**Calculate the percentage of A-21 desamido insulin (%D):**

\[ \text{Result} = \left( \frac{r_C}{r_S} \right) \times 100 \]

**Acceptance criteria:** NMT 10.0% of A-21 desamido insulin

**Process-related Impurities**

Add the following:

**Proinsulin Content:** NMT 10 ng/mg, determined by a validated method, USP 1-May-2019

**Specific Tests**

Delete the following:

**Insulin Assays** (121), Assay, Bioidentity Test: Meets the requirements, USP 1-May-2019

**Loss on Drying** (731)

Sample: 200 mg

Analysis: Dry the Sample at 105° for 16 h.

**Acceptance criteria:** NMT 10.0%

Delete the following:

**Zinc Determination** (591), Procedure, Dithizone Method

Sample: 10 mg

**Acceptance criteria:** NMT 1.0% on the dried basis, USP 1-May-2019

**Bacterial Endotoxins Test** (85): NMT 10 USP Endotoxin Units/mg of insulin

**Microbial Enumeration Tests** (61) and Tests for Specified Microorganisms (62): The total bacterial count does not exceed 3 \( \times 10^{2} \) cfu/g, the test being performed on a portion of about 0.2 g, accurately weighed.

**Additional Requirements**

**Packaging and Storage:** Preserve in tight containers. Store, protected from light, in a freezer.

**Change to read:**

**Labeling:** Label it as pork. If the Insulin is purified, label it as such.
Change to read:

- **USP Reference Standards** (11)

(RB 1-May-2019)