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Exenatide Injection

DEFINITION

Exenatide Injection is a sterile solution of exenatide acetate in a suitable diluent. It may contain suitable preservatives. It possesses, in each milliliter, an activity of NLT 90.0% and NMT 110.0% of the labeled amount of exenatide ($C_{184}H_{282}N_{50}O_{60}S$).

IDENTIFICATION

- **A. HPLC**

Diluent, Buffer solution A, Solution A, Buffer solution B, Solution B, Mobile phase, Standard solution, System suitability solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in *Product-Related Substances and Impurities*.

Identity sample solution: 0.25 mg/mL of USP Exenatide RS in *Diluent* mixed with 0.25 mg/mL of Injection

Analysis

Samples: *Standard solution*, *Sample solution*, and *Identity sample solution*

Acceptance criteria: The retention time of the exenatide peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in *Product-Related Substances and Impurities*. In addition, the major peaks of the *Identity sample solution* coelute.

- **B.** The average mass by *Mass Spectrometry ⟨736⟩* is 4186.6 ± 1.0 Da.

ASSAY

- **Procedure**

Diluent: A 2-L aqueous solution containing 3.26 g of sodium acetate trihydrate, 2.98 g of L-methionine, and 2.06 mL of glacial acetic acid, pH 4.5. Pass through a membrane filter of 0.2-µm pore size.

[Note—Alternative suitable diluents may also be used.]

Buffer solution: 0.13 M sodium sulfate solution

Mobile phase: *Buffer solution*, acetonitrile, and trifluoroacetic acid (1500:500:2). Pass through a membrane filter of 0.45-µm pore size.

Standard solution: 0.25 mg/mL of USP Exenatide RS in *Diluent*

Soybean trypsin inhibitor stock solution: 1 mg/mL of soybean trypsin inhibitor in water. Prepare in duplicate.

System suitability solution: Transfer 10–50 µL of *Soybean trypsin inhibitor stock solution* to 1 mL of the *Standard solution* and mix with a vortex mixer for at least 30 s. Determine the soybean trypsin inhibitor peak level before initiating sample analysis. The soybean trypsin inhibitor peak level should be ≥2.0% but ≤5.0% relative to the total peak area. If the peak level is <2.0%, transfer additional *Soybean trypsin inhibitor stock solution* to the *System suitability solution*. If the peak level is >5.0%, discard and prepare a new *System suitability solution*.

Sample solution: Transfer an adequate quantity of each Injection (0.25 mg/mL) from its container directly to an HPLC vial, without contact with any intermediate container or transfer device.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 7.8-mm × 30-cm; 5-µm packing L20

Flow rate: 0.8 mL/min

Injection volume: 20 µL

Temperatures

Autosampler: 2°–8°

Column: 25°

System suitability

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

Suitability requirements

Resolution: NLT 1.3 between the exenatide peak and soybean trypsin inhibitor peak, *System suitability solution*

Tailing factor: NLT 0.8 and NMT 1.4, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of exenatide ($C_{184}H_{282}N_{50}O_{60}S$) in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = mean peak response from the *Standard solution*

C_S = concentration of USP Exenatide RS in the *Standard solution* (mg/mL)

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

Acceptance criteria: 90.0%–110.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

Change to read:

• **Procedure**

Diluent: A 2-L aqueous solution containing 3.26 g of sodium acetate trihydrate, 2.98 g of L-methionine, and 2.06 mL of glacial acetic acid, pH 4.5. Pass through a membrane filter of 0.2-µm pore size.
[Note—Alternative suitable diluents may also be used.]

Buffer solution A: Transfer 27.20 g of anhydrous monobasic potassium phosphate to a 2-L flask or beaker. Add approximately 1900 mL of water and mix well until dissolved. Adjust with phosphoric acid to a pH of 3.30. Transfer the buffer solution to a 2-L volumetric flask, dilute with water to volume, and mix well.

Solution A: Transfer 1670 mL of *Buffer solution A* to a glass container, add 1673 mL of acetonitrile, and mix well. Pass through a membrane filter of 0.45-µm pore size.

Buffer solution B: Transfer 27.20 g of anhydrous monobasic potassium phosphate and 56.20 g of sodium perchlorate monohydrate to a 2-L flask or beaker. Add approximately 1950 mL of water, heat to 65° for 5 min, and mix well until dissolved. Adjust with phosphoric acid to a pH of 3.30. Transfer the buffer solution to a 2-L volumetric flask, dilute with water to volume, and mix well.

Solution B: Transfer 1083 mL of *Buffer solution B* to a glass container, add 1115 mL of acetonitrile, and mix well. Pass through a membrane filter of 0.45-µm pore size.

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5.0	100	0
45.0	40	60
50.0	0	100
52.0	0	100
54.0	100	0
65.0	100	0

0.3% Hydrogen peroxide: Transfer 1 mL of 3% hydrogen peroxide to a 10-mL volumetric flask and dilute with water to volume. Stopper and mix well.

Standard solution: 0.25 mg/mL of USP Exenatide RS in *Diluent*

System suitability solution: Transfer 5 µL of *0.3% Hydrogen peroxide* to 1 mL of *Standard solution* and mix well by mixing with a vortex mixer for at least 30 s. Incubate the tightly capped vial in a boiling water bath for 30 min. Cool the vial rapidly to room temperature and transfer the contents to a glass HPLC vial without any contact with an intermediate transfer device.

Sample solution: Transfer an adequate quantity of each Injection (0.25 mg/mL) from its container directly to an HPLC vial, without contact with any intermediate container or transfer device.

Chromatographic system

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

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 10-cm; 3-µm packing L52▲; two columns in series▲ (ERR 1-Aug-2024)

Flow rate: 1.2 mL/min

Injection volume: 40 µL

Temperatures

Autosampler: 2°–8°

Column: 40°

System suitability

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

Suitability requirements

Resolution: NLT 2.0 between the exenatide peak and the peak at an approximate relative retention time of 1.09, *System suitability solution*

Column efficiency: NLT 15,000 theoretical plates, *Standard solution*

Tailing factor: Between 0.8 and 1.5 for the exenatide peak, *Standard solution*

Relative standard deviation: NMT 2.0% for 5 replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Record the chromatograms and measure the response for each peak. Determine the corrected total peak area by excluding system peaks and peaks below 0.10% of the total peak area.

Calculate the percentage of each impurity in the portion of Injection taken by using the corrected total peak area:

$$\text{Result} = (r_i/r_T) \times 100$$

r_i = peak response of each impurity from the *Sample solution*

r_T = corrected total peak area

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[3-39]-Exenatide[a]	0.32	1.00
Exenatide	1.00	—
[Asu^{9}]-exenatide	1.22	1.00
Unspecified impurities	—	1.00
Total impurities	—	10.0

[a] Peptide fragment peak resulting from degradation.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** NMT 50 USP Endotoxin Units/mL
- **Sterility Tests ⟨71⟩, *Test for Sterility of the Product to Be Examined, Membrane Filtration*:** Meets the requirements
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements for small-volume injections
- **Subvisible Particulate Matter in Therapeutic Protein Injections ⟨787⟩:** NMT 6000 counts/container ≥10 µm, NMT 600 counts/container ≥25 µm
- **pH ⟨791⟩:** 4.2–4.8
- **Injections and Implanted Drug Products ⟨1⟩:** Meets the requirements

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Store at 2°–8°. Do not freeze.
- **Labeling:** Meets the requirements in *Labeling ⟨7⟩*.
- **USP Reference Standards ⟨11⟩**
  USP Exenatide RS

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

Topic/Question	Contact	Expert Committee
EXENATIDE INJECTION	Ying Han Associate Science & Standards Liaison	BIO12020 Biologics Monographs 1 - Peptides

Chromatographic Database Information: Chromatographic Database

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