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Exenatide

HGEGTFTSDL SKQMEEEAVR LFIEWLKNGG PSSGAPPPS—NH_2

$C_{184}H_{282}N_{50}O_{60}S$ 4186.57

L-Histidylglycyl-L-glutamylglycyl-L-threonyl-L-phenylalanyl-L-threonyl-L-seryl-L-aspartyl-L-leucyl-L-seryl-L-lysyl-L-glutaminyl-L-methionyl-L-glutamyl-L-glutamyl-L-glutamyl-L-alanyl-L-valyl-L-arginyl-L-leucyl-L-phenylalanyl-L-isoleucyl-L-glutamyl-L-tryptophyl-L-leucyl-L-lysyl-L-asparaginylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl-L-serinamide CAS RN®: 141758-74-9.

DEFINITION

Exenatide is a 39 amino acid synthetic peptide agonist for glucagon-like peptide-1 (GLP-1) receptor. Exenatide contains NLT 95% and NMT 105% of exenatide ($C_{184}H_{282}N_{50}O_{60}S$), calculated on the anhydrous, acetic acid-free basis. Exenatide is a white to off-white powder.

[NOTE—Exenatide is very hygroscopic. Protect from exposure to moisture.]

IDENTIFICATION

• **A. HPLC**

Solution A, Solution B, Mobile phase, System suitability solution, Standard solution, Sample solution, Chromatographic system, and **System suitability:** Proceed as directed in the *Assay*.

Identity sample solution: Mix equal volumes of the *Standard solution* and the *Sample solution*.

Analysis

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

Examine the chromatograms of the *Standard solution*, *Sample solution*, and *Identity sample solution*.

Acceptance criteria: The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, and the major peak from the *Identity sample solution* elutes as a single peak.

• **B. AMINO ACID ANALYSIS**

For further discussion of the theory and applications, see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩, which may be a helpful, but not mandatory, resource. [NOTE—Use a suitable, validated hydrolysis and separation and calculation procedure including amino acids used in the calculation (see *Biotechnology-Derived Articles—Amino Acid Analysis* ⟨1052⟩.)]

Standardize the instrument with a mixture containing equal molar per volume amounts (except for L-cystine which is half the molar amount) of glycine and the L-form of the following amino acids: alanine, arginine, aspartic acid, cystine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, norleucine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine.

Hydrolysis solution: 6 N hydrochloric acid containing 4% of phenol

Sample solution: Accurately weigh out between 0.4 and 1.0 mg of Exenatide in glass ampuls. Add a minimum of 1.0 mL of *Hydrolysis solution*, freeze the sample ampul, and flame seal under vacuum. Hydrolyze at 110° for about 22 h. After hydrolysis, dry the test sample under vacuum to remove any residual acid. To the ampul add 2 mL of a buffer solution that is suitable for the amino acid analyzer, and pass through a filter of 0.45-µm pore size.

Procedure: Prepare a co-injection of the *Standard solution* and the test sample. Inject a suitable volume into the amino acid analyzer, and record and measure the responses for each amino acid peak in the *Standard solution*. Express the content of each amino acid in nanomoles.

Calculate the mean nanomole of the amino acids:

$$\text{Result} = (\text{nmol found in the } \textit{Analysis} \text{ for Ala, Arg, Asx, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Trp, Val})/30$$

Divide the nanomole of each amino acid by the *Result* to determine the amino acid ratios that must meet the *Acceptance criteria*.

Acceptance criteria: See *Table 1*.

Table 1

Name	Acceptance Criteria (amino acid ratio)
Aspartic acid, threonine, phenylalanine, lysine, alanine	1.5–2.5
Serine	4.2–5.5
Glutamic acid	5.2–6.8
Proline	3.5–4.5
Glycine	4.5–5.5
Valine	0.5–1.5
Methionine, isoleucine, histidine, arginine	0.5–1.5
Leucine	2.5–3.5
Tryptophan	0.5–1.5

• **C.** The average mass by *Mass Spectrometry ⟨736⟩* is 4186.6 ± 1.0 mass units.

ASSAY

• **Procedure**

Solution A: 10 mM ammonium hydrogen carbonate, pH 9.5, 0.0375% ammonia

Solution B: Acetonitrile and *Solution A* (90:10)

Mobile phase: See *Table 2*.

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	74	26
0.5	74	26
33	63	37
35	10	90
36	10	90
36.1	74	26
42	74	26

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL each of USP [Glu^{13}]-Exenatide RS and USP [$Met(O)^{14}$]-Exenatide RS

Standard solutions: 1.0 mg/mL of USP Exenatide RS in water, prepared in duplicate. [Note—The retention time for the exenatide peak is approximately 14–20 min.]

Sample solutions: 1.0 mg/mL of Exenatide in water, prepared in duplicate

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L1

Temperatures

Column: 60°

Autosampler: 10°

Flow rate: 0.8 mL/min

Injection volume: 10 µL

System suitability

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

Suitability requirements

Mean peak area: Maximum difference between the mean peak area of the two *Standard solutions* is ±2%.

Resolution: NLT 1.0 between the [Glu^{13}]-exenatide and [Met(O)^{14}]-exenatide peaks, *System suitability solution*

Relative standard deviation: NMT 2.0% for the exenatide peak area and retention time from three replicate injections of the two *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solutions*

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

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

r_U = peak response of exenatide from the *Sample solutions*

r_S = peak response of exenatide from the *Standard solutions*

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

C_U = concentration of Exenatide in the *Sample solutions* (mg/mL), calculated on the anhydrous, acetic acid-free basis

Acceptance criteria: 95%–105% on the anhydrous, acetic acid-free basis

IMPURITIES

• **Procedure 1: Exenatide Related Substances and Impurities**

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Mobile phase, System suitability solution, Standard solutions, Sample solutions, and **Chromatographic system:** Proceed as directed in the *Assay*.

Acceptance criteria

Individual impurities: See *Table 3*.

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
[Glu^{13}]-exenatide	0.65–0.68	0.50
Sum of [Asp^{28}]-exenatide and [Met(O)^{14}]-exenatide	0.68–0.76	0.50
Exenatide	1.00	—
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 1*

Reporting limit: 0.05%

• **Procedure 2: *N*-Acetyl His^1-Exenatide**

[Note—Manufacturers should determine the suitability of their related substances method for their process-related and degradation impurities. For any impurity peak above the limit for unspecified impurity peaks, identification and appropriate qualification is required.]

Solution A: 0.1 M sodium perchlorate, pH 2.7

Solution B: Acetonitrile

Mobile phase: See *Table 4*.

Table 4

Time (min)	Solution A (%)	Solution B (%)
0	63	37
40	59	41
60	39	61

System suitability solution: 1.0 mg/mL of USP Exenatide RS and 0.005 mg/mL of USP [*N*-Acetyl-His[1]]-Exenatide RS

Standard solution: 1.0 mg/mL of USP Exenatide RS in water, prepared in duplicate

Sample solution: 1.0 mg/mL of Exenatide in water

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 3.0-mm × 15-cm; 3-µm packing L1

Temperatures

Column: 55°

Autosampler: 10°

Flow rate: 0.6 mL/min

Injection volume: 15 µL

System suitability

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

Suitability requirements

Resolution: NLT 1.0 between the main exenatide peak and the [*N*-acetyl-His[1]]-exenatide impurity peak, *System suitability solution*

Retention time variability: NMT ±5% of mean for the exenatide peak, *Standard solution*

Relative standard deviation: NMT 2.0% for the exenatide peak from three replicate injections, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Exenatide taken, disregarding any peak with an area less than 0.05% of the main peak:

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

r_I = peak area of each individual impurity from the *Sample solution*, other than the solvent peak and the main exenatide acetate peak

r_T = sum of the areas of all the peaks from the *Sample solution*, excluding that of the solvent peak

Acceptance criteria

Individual impurities: See *Table 5*.

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Exenatide	1.00	—
[*N*-Acetyl-His[1]]-exenatide	1.10–1.13	1.0

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Unspecified impurities	—	0.50

Total impurities: NMT 3.0% from *Procedure 2*

• **Procedure 3: Limit of D-His[1] Exenatide**

System suitability solution A: 100 mg of L-amino acids and 0.5 mg of corresponding D-enantiomers are dissolved in 500 mL of water. An aliquot of 700 µL is dried in a SpeedVac and derivatized as described in the *Sample solution*.

Sample solution: Hydrolyze 1 mg of the sample in 350 µL of 6 N deuterium chloride (DCl) in heavy water (D_2O) at 110° for 8 h. After removal of excess reagent by a stream of nitrogen, the sample is esterified with 250 µL of 4 M deuterochloric acid-ethyl alcohol at 110° for 20 min. After cooling to about 40°, open the vial and evaporate the reagent with a gentle stream of nitrogen at moderate temperature. Dissolve the residue in 250 µL of trifluoroacetic anhydride in trifluoroacetic acid ethyl ester (1:2). Tightly close the vials and heat to 130° for 10 min. After cooling to room temperature, remove the excess reagent by a stream of nitrogen. Add 50 µL of isobutyl chloroformate to the sample and heat the closed vial to 110° for 10 min. After removal of the excess reagent under a nitrogen stream, dissolve the residue in 250 µL of dichloromethane.

System suitability solution B: About 100 µg of each D- and L-amino acid is derivatized as described in *Sample solution*.

Chromatographic system

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

Mode: GC–MS (see *Mass Spectrometry ⟨736⟩*)

Detector: Mass

Column: 20-m × 0.28-mm; 0.28-µm packing G49

Temperatures

Oven: 155°, 3 min isotherm, 4°/min to 190°, 5 min

Injection port: 220°

Carrier gas: Hydrogen

Flow rate: 26 mL/min

Injection volume: 1.0 µL

System suitability

Samples: *System suitability solution A* and *System suitability solution B*

Suitability requirements

Resolution: NLT 1.0 for enantiomers of the analyte and for any additional peak next to the analyte, *System suitability solution B*

Tailing factor: NMT 2.0 for the representative enantiomer, *System suitability solution B*

Relative standard deviation: NMT ±0.1% from the calculated content of D-Ala, D-Pro, D-Asp, D-Glu, D-Lys, and D-Arg, *System suitability solution A*

Analysis

Sample: *Sample solution*. The mass fragment of 379 Da is used for detection.

Calculate the percentage of D-His[1]-exenatide impurity in the portion of Exenatide taken:

$$\text{Result} = [A_D/(A_D + A_L)] \times 100$$

A_D = peak response of D-His from the *Sample solution*

A_L = peak response of L-His from the *Sample solution*

Acceptance criteria: NMT 1.0%

PROCESS-RELATED IMPURITIES

• **Procedure 4: Limit of Phosphate**

[Note—Limit of phosphate should be performed if phosphate is used in manufacture.]

Solution A: 50 mM potassium hydroxide in water

Solution B: Water

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	2	98
3	2	98
20	50	50
21	100	0

Diluent: 1 mM sodium hydroxide in water

Standard solution: 2 µg/mL of phosphate in *Diluent*

Sample solution: 2 mg/mL of Exenatide in *Diluent*

System suitability solution: *Standard solution* and *Sample solution* (1:1)

Chromatographic system

(See *Ion Chromatography* ⟨1065⟩.)

Mode: IC

Detector: Conductivity

Column: 2-mm × 25-cm; 10.5-µm packing L83

Temperatures

Column: 30°

Sample: 15°

Suppressor: Anion self-regenerating suppressor

Suppressant: Autosuppression

Flow rate: 0.25 mL/min

Injection volume: 25 µL

System suitability

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

Suitability requirements

Tailing factor: NMT 3%, *Standard solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

The peak area for the phosphate ion peak in the chromatograms obtained with the *Standard solution* shows a peak corresponding to phosphate ion at a retention time of 15–21 min. The identity of the phosphate peak in the exenatide sample is confirmed by the *System suitability solution* showing one single peak. The phosphate content in exenatide is >0.1%, if the mean peak area of the *Sample solution* is higher than the mean peak area of the *Standard solution*. The phosphate content in exenatide is ≤0.1%, if the mean peak area of the *Sample solution* is equal to or smaller than the mean peak area for the *Standard solution*.

Acceptance criteria: NMT 0.1%

• **Procedure 5: Trifluoroacetic Acid (TFA) in Peptides** ⟨503.1⟩**:** Exenatide must contain NMT 0.25% trifluoroacetic acid.

OTHER COMPONENTS

• **Acetic Acid in Peptides** ⟨503⟩**:** NMT 5.0%

SPECIFIC TESTS

• **Bacterial Endotoxins Test** ⟨85⟩**:** It contains NMT 10 USP Endotoxin Units/mg of Exenatide.

• **Microbial Enumeration Tests** ⟨61⟩and**Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is less than 200 cfu/g and the total combined yeasts and molds count does not exceed 200 cfu/g.

• **Water Determination** ⟨921⟩, *Method I*, *Method Ic***:** NMT 7.0%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight, light-resistant containers at −20 ± 5°.

• **USP Reference Standards** ⟨11⟩

USP [N-Acetyl-His^{1}]-Exenatide RS

USP Exenatide RS

USP [Glu^{13}]-Exenatide RS

USP [Met(O)^{14}]-Exenatide RS

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

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

Chromatographic Database Information: Chromatographic Database

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