

Teriparatide

Type of Posting	Notice of Intent to Revise
Posting Date	29–May–2020
Targeted Official Date	To Be Determined, Revision Bulletin
Expert Committee	Biologics Monographs 1 – Peptides & Insulins

In accordance with section 7.04 (c) of the 2015–2020 Rules and Procedures of the Council of Experts and the [Pending Monograph Guideline](#), this is to provide notice that the Biologics Monographs 1 – Peptides & Insulins Expert Committee intends to revise the Teriparatide monograph.

Based on the supporting data received from a manufacturer awaiting FDA approval, the Expert Committee proposes to revise the *Definition* and the *Labeling* sections of the monograph to include wording for synthetic.

The proposed revision is contingent on FDA approval of a product that meets the proposed monograph specifications. The proposed revision will be published as a Revision Bulletin and an official date will be assigned to coincide as closely as possible with the FDA approval of the associated product.

An additional revision will be proposed for public comment in the future with additional requirements for products of synthetic origin. This future revision will be processed via an In-Process Revision at a date yet to be determined.

See below for additional information about the proposed text.¹

Should you have any questions, please contact Elena Curti, Associate Science and Standards Liaison (301-998-6803 or eac@usp.org).

¹ This text is not the official version of a *USP–NF* monograph and may not reflect the full and accurate contents of the currently official monograph. Please refer to the current edition of the *USP–NF* for official text.

USP provides this text to indicate changes that we anticipate will be made official once the product subject to this proposed revision under the Pending Monograph Program receives FDA approval. Once FDA approval is granted for the associated revision request, a Revision Bulletin will be posted that will include the changes indicated herein, as well as any changes indicated in the product's final approval, combined with the text of the monograph as effective on the date of approval. Any revisions made to a monograph under the Pending Monograph Program that are posted without prior publication for comment in the *Pharmacopeial Forum* must also meet the requirements outlined in the [USP Guideline on Use of Accelerated Processes for Revisions to the USP–NF](#).

Teriparatide

SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHNF—OH

Click image to enlarge

$C_{181}H_{291}N_{55}O_{51}S_2$ 4117.72 (as free base)

L-Phenylalanine, L-seryl-L-valyl-L-seryl-L- α -glutamyl-L-isoleucyl-L-glutamyl-L-leucyl-L-methionyl-L-histidyl-L-asparaginyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparaginyl-L-seryl-L-methionyl-L- α -glutamyl-L-arginyl-L-valyl-L- α -glutamyl-L-tryptophyl-L-leucyl-L-arginyl-L-lysyl-L-lysyl-L-leucyl-L-glutamyl-L- α -aspartyl-L-valyl-L-histidyl-L-asparaginyl-;

L-Seryl-L-valyl-L-seryl-L- α -glutamyl-L-isoleucyl-L-glutamyl-L-leucyl-L-methionyl-L-histidyl-L-asparaginyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparaginyl-L-seryl-L-methionyl-L- α -glutamyl-L-arginyl-L-valyl-L- α -glutamyl-L-tryptophyl-L-leucyl-L-arginyl-L-lysyl-L-lysyl-L-leucyl-L-glutamyl-L- α -aspartyl-L-valyl-L-histidyl-L-asparaginyl-L-phenylalanine. [52232-67-4]; UNII: 10T9CSU89I.

Change to read:

DEFINITION

Teriparatide, also called rhPTH (1-34), is a single-chain peptide containing 34 amino acids identical to the 34 N-terminal amino acids of human parathyroid hormone. Teriparatide is produced by [▲]either [▲](TBD) a method based on recombinant DNA (rDNA) technology [▲]or chemical synthesis. [▲](TBD) The host cell-derived protein content [▲]of Teriparatide produced from an rDNA process [▲](TBD) is below the limit approved by the competent authority. In addition, the host cell-derived and vector-derived DNA [▲]of Teriparatide produced from an rDNA process [▲](TBD) are below the limit approved by the competent authority. Teriparatide is a white to almost white powder. It contains NLT 95.0% and NMT 105.0% of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$), calculated on the anhydrous, acetic acid-free, and chloride-free basis.

IDENTIFICATION

● **A.** The ratio of the retention time of the teriparatide peak of the *Sample solution* to that of the *Standard solution*, as obtained in the *Assay*, is 1.00 ± 0.03 .

● **B. PEPTIDE MAPPING**

Solution A: 0.1% (v/v) [trifluoroacetic acid](#) in [water](#)

Solution B: [Acetonitrile](#), [trifluoroacetic acid](#), and [water](#) (60:0.1:40)

Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	96	4
6	96	4
20	45	55
25	0	100
25.1	96	4
35	96	4

20 mM sodium phosphate buffer: 2.30 mg/mL of [anhydrous dibasic sodium phosphate](#) and 0.60 mg/mL of [sodium phosphate monobasic monohydrate](#) in [water](#). Adjust with [sodium hydroxide](#) or [phosphoric acid](#) to a pH of 7.8 before final dilution.

Enzyme solution: About 0.25 mg/mL of *Staphylococcus aureus* V8 protease in 20 mM sodium phosphate buffer. Stable for 72 h when stored at 2°–8°.

Standard solution: Prepare a 1.5 ± 0.15 mg/mL solution of [USP Teriparatide RS](#) in 20 mM sodium phosphate buffer. Combine this solution with *Enzyme solution* for a teriparatide to protease ratio of 10:1 (w/w). Mix and incubate at 37° for 18–24 h. Quench the digestion by adding the volume of *Solution A* needed to reach a final digested teriparatide concentration of approximately 0.25 mg/mL. Stable for 72 h when stored at 2°–8°.

Sample solution: Prepare a 1.5 ± 0.15 mg/mL solution of Teriparatide in 20 mM sodium phosphate buffer. Combine this solution with *Enzyme solution* for a Teriparatide to protease ratio of 10:1 (w/w). Mix and incubate at 37° for 18–24 h. Quench the digestion by adding the volume of *Solution A* needed to reach a final digested teriparatide concentration of approximately 0.25 mg/mL. Stable for 72 h when stored at 2°–8°.

Blank: Combine 20 mM sodium phosphate buffer with *Enzyme solution* in the same proportions used for the *Standard solution* and the *Sample solution*. Mix and incubate at 37° for 18–24 h. Quench by adding the same volume of *Solution A* as for the *Standard solution* and the *Sample solution*. Stable for 72 h when stored at 2°–8°.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5- μ m packing [L1](#)

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 μ L

System suitability

Sample: *Standard solution*

[NOTE—The *System suitability* procedure must be performed both before starting sample testing and at the end of each sample run.]

Suitability requirements

Chromatogram similarity: In the chromatogram from the *Standard solution*, identify the peaks due to digest fragments I, II, III, IV, and V. The chromatogram of the *Standard solution* corresponds to that of the typical chromatogram provided with the USP Certificate for [USP Teriparatide RS](#).

Resolution: NLT 1.5 between peaks indicated as fragments III and I

Tailing factor: NMT 2.3 for the peak indicated as fragment IV

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Record the chromatograms. For each of the five fragments, determine the ratio of the fragment retention time from the *Sample solution* to the corresponding fragment retention time from the *Standard solution* obtained from the first *System suitability* injection.

Acceptance criteria: The chromatographic profile of the *Sample solution* corresponds to that of the *Standard solution*. All five fragments, I, II, III, IV, and V, must be present. The fragment retention time ratio is 1.00 ± 0.03 for all five fragments.

● C. BIOIDENTITY

Basic medium: Sterile Dulbecco's modified Eagle's medium (DMEM) containing high glucose, L-glutamine, pyridoxine hydrochloride, and 25mM HEPES¹

Growth medium: 10% [fetal bovine serum](#) (FBS) in *Basic medium* prepared as follows. To 450 mL of *Basic medium*, add 50 mL of heat inactivated irradiated FBS² and mix. Sterilize the solution by filtering the solution using a 0.22- μ m, low-protein-binding, sterile filter unit and store at 2°–8°.

Serum starve medium: 0.1% (w/v) [bovine serum albumin](#) (BSA)-fraction V in *Basic medium* prepared as follows. Add 0.50 g of BSA-fraction V to 500 mL of *Basic medium*, and mix. Sterilize the solution by filtering the solution using a 0.22- μ m, low-protein-binding, sterile filter unit and store at 2°–8°.

Vehicle: 150 mM [sodium chloride](#), 0.1% (w/v) BSA-fraction V, and 0.001 N [hydrochloric acid](#) prepared as follows. Dissolve 1.75 g of [sodium chloride](#) and 0.2 g of BSA-fraction V in 180 mL of [water](#). Add 20.0 mL of 0.01 N [hydrochloric acid](#) to the solution. Sterilize the solution by filtering the solution using a 0.22- μ m, low-protein-binding, sterile filter unit and store at 2°–8°.

600 mM IBMX solution: Add 0.30 g of 3-isobutyl-1-methyl-xanthine (IBMX) to 2.25 mL of [dimethyl sulfoxide](#) (DMSO) and vortex to dissolve. Aliquot and store at –18° to –24°.

Cell culture preparation: Culture UMR-106 rat osteogenic sarcoma cell line³ in *Growth medium* at $37 \pm 2^\circ$ and $10 \pm 2\%$ carbon dioxide (CO₂) atmosphere in a humidified incubator. Cells should be passaged when the cultures are approximately 65%–85% confluent as determined microscopically at an appropriate magnification (such as 200–400 \times). [NOTE—Do not allow the cells to go beyond 85% confluence for cell passage or analysis.] For cell passage, remove the media from the cells. Rinse the cells once with sterile Dulbecco's phosphate buffer saline (DPBS) without calcium and magnesium.⁴ Rinse the cells with approximately 5–10 mL of 0.25% (w/v) trypsin in 1 mM [EDTA](#) solution,⁵ and immediately remove all but approximately 1–2 mL of the 0.25% (w/v) trypsin with 1 mM [EDTA](#) solution from the cells. Allow the 0.25% (w/v) trypsin with 1 mM [EDTA](#) solution to remain on the cells for 1–2 min at a temperature ranging from room temperature to 37° until the cells begin to round and release from the culture surface. Resuspend the cells in an appropriate volume of *Growth medium* and count them. Cells are passaged into a T162 cm² flask with approximately 30 mL of *Growth medium* at $0.75\text{--}6 \times 10^6$ cells per flask.

Preparation of cells for analysis: Use cells that are between passages 4 and 20, 65%–85% confluent, and 2–5 days post-passage. Following the procedure described in *Cell culture preparation*, prepare an appropriate volume of cell solution at 0.75×10^5 viable cells per mL in *Growth medium*. To 96-well flat-bottom plates,⁶ add 200 μ L of cell solution per well. Mix cell solution frequently during dispensing to prevent cells from settling and to ensure consistent density throughout the plate. Incubate plates for 18–26 h at $37 \pm 2^\circ$ and $10 \pm 2\%$ carbon dioxide (CO₂). Following the incubation, remove media from the cells and add 200 μ L of *Serum starve medium* to each well. Incubate plates for 18–26 h at $37 \pm 2^\circ$ and $10 \pm 2\%$ carbon dioxide (CO₂).

Diluent A: 1 mM IBMX in Hanks' balanced salt solution (HBSS) with phenol red⁷ prepared as follows. Very slowly add 500 μ L of warmed (30°–40°) *600 mM IBMX solution* to 300 mL of warmed HBSS with phenol red while continuously mixing on a stir plate. [NOTE—It is acceptable to substitute HBSS containing phenol red with HBSS without phenol red.⁸ HBSS containing phenol red is preferred because it is easier to visualize the wells.]

Assay/lysis solution: 0.55 mM IBMX in assay/lysis buffer from a suitable cAMP immunoassay kit for 96-well plates⁹ prepared as follows. Very slowly add 27.5 μ L of warmed *600 mM IBMX solution* to 30 mL of warmed assay/lysis buffer.

Diluted cAMP-AP conjugate: Dilute the cAMP-alkaline phosphatase (AP) conjugate (1:100) with the conjugate dilution buffer from the same cAMP kit used for the *Assay/lysis solution*.⁹ Prepare 2.5 mL of diluted conjugate per 96-well plate. Use within 4 h.

Standard stock solution: Dissolve the contents of 1 vial of [USP Teriparatide RS](#) in an appropriate volume of *Vehicle* to obtain a 250- μ g/mL solution.

Standard solution: Prepare a 1- μ M solution by mixing 82.4 μ L of the *Standard stock solution* with 4.92 mL of *Vehicle*.

Sample stock solution: Prepare a 1-mg/mL solution of Teriparatide in *Vehicle*.

Sample solution: Prepare a 1- μ M solution by mixing 20 μ L of the *Sample stock solution* with 4.98 mL of *Vehicle*.

[NOTE—Following the preparation of the *Standard solution* and *Sample solution*, the diluting and delivery of the samples to the cells must occur within 45 min. Dilutions must be made in borosilicate glass tubes. Allow all solutions to equilibrate to room temperature prior to use.]

Preparation of diluted standard solutions and sample solutions: Prepare three separate dilution sets from the *Standard solution* and *Sample solution* in borosilicate glass tubes at various concentrations (e.g., 3.0, 1.0, 0.333, 0.167, 0.0833, 0.0417, 0.0208, 0.0069, and 0.0023 nM) using *Diluent A*. [NOTE—Only a single standard and a single sample (three separate dilution sets for each) should be prepared and run for each assay plate. For each assay plate, a freshly prepared standard and sample must be used. Each assay consists of at least three independent runs (or three assay plates).]

Analysis: Following cell serum starvation, wash cells at least twice with 300 μ L per well of HBSS without phenol red⁴ at room temperature. Place 100 μ L per well of each dilution prepared from the *Standard solution* and *Sample solution* into appropriate wells of the plate. [NOTE—See [Design and Development of Biological Assays \(1032\)](#) for helpful information on randomization of samples and plate layout.] Incubate the plates at $25 \pm 2^\circ$ for 20 ± 5 min with gentle shaking. Discard the solutions and wash cells twice with 300 μ L per well of HBSS without phenol red at room temperature. Add 100 μ L per well of *Assay/lysis solution* and incubate the plates at $37 \pm 2^\circ$ for 30 ± 5 min to lyse the cells. Mix cell lysate with a multi-channel pipette prior to transfer. Transfer 60 μ L of cell lysate to the appropriate wells of the 96-well assay plate from the cAMP immunoassay assay kit. Add 30 μ L of *Diluted cAMP-AP conjugate* to each well containing the cell lysate that is derived from the cells treated with the *Diluted standard solutions* or *Diluted sample solutions*, and mix on a plate shaker for approximately 1–2 min. Add 60 μ L of anti-cAMP antibody from the cAMP kit to the wells and incubate at $25 \pm 2^\circ$ for 60 ± 5 min on a plate shaker with gentle shaking. Discard the solutions and wash the plates six times with 300 μ L per well of wash buffer from the cAMP kit, blotting the plate between each wash. Add 100 μ L of substrate/enhancer solution from the cAMP kit to each well. Mix on a plate shaker for 1–2 min. Remove the plates from the shaker and incubate the plates at room temperature (such as 20° – 27°) for 40 ± 10 min. Read the plate in a suitable microtiter plate luminescence reader.

Calculations: Fit a constrained 4-parameter logistic curve to the median relative light units (RLU) at each concentration from the *Diluted standard solutions* and *Diluted sample solutions*. Calculate the relative potency of each teriparatide sample compared to the standards of each run by EC_{50} . Determine the combined weighted percent mean relative potency of the runs following [Design and Analysis of Biological Assays \(111\)](#), [Combination of Independent Assays, Method 2](#).

System suitability

Samples: *Diluted standard solutions* and *Diluted sample solutions*

Suitability requirements

Asymptote ratio: NLT 3.0 for the ratio of the upper asymptote to the lower asymptote of the 4-parameter logistic curve from each run of both the *Diluted standard solutions* and *Diluted sample solutions*

Slope: NLT 1.0 for each run

L term: NMT 0.2000 for each run. [NOTE—L term is determined by subtracting the log of the 95% lower confidence limit from the log of the 95% upper confidence limit of the relative potency.]

Combined assay L term: NMT 0.1500

[NOTE—See [Design and Analysis of Biological Assays \(111\)](#), [Combination of Independent Assays, Method 2](#) for the calculation.]

Acceptance criteria: 60%–120% of the relative potency to [USP Teriparatide RS](#) on the as-is basis

ASSAY

● PROCEDURE

0.2 M sulfate buffer: 28.4 g/L of [anhydrous sodium sulfate](#) in [water](#). Adjust with 85% [phosphoric acid](#) to a pH of 2.3.

Solution A: [Acetonitrile](#) and 0.2 M sulfate buffer (10:90)

Solution B: [Acetonitrile](#) and 0.2 M sulfate buffer (50:50)

Mobile phase: *Solution A* and *Solution B* (63:37). [NOTE—The *Mobile phase* composition may be adjusted to obtain the desired retention time for the teriparatide main peak.]

Diluent: [Acetonitrile](#) and 0.2 M sulfate buffer (25:75)

Standard solutions: Prepare in triplicate 250 µg/mL of [USP Teriparatide RS](#) in *Diluent*. Stable for 72 h when stored at 2°–8° in a sealed container.

Sample solutions: Prepare in duplicate approximately 250 µg/mL of Teriparatide in *Diluent*. Stable for 72 h when stored at 2°–8° in a sealed container. [NOTE—Teriparatide should be equilibrated and weighed in a controlled humidity chamber of 25 ± 5% relative humidity, then dissolved in *Diluent*. Determine the [water](#) content within 24 h of weighing.]

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing [L1](#)

Temperatures

Autosampler: 2°–8°

Column: 40 ± 5°

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Run time: 15 min

System suitability

Samples: *Standard solutions*

[NOTE—The retention time for teriparatide is 7.5–11.7 min.]

Suitability requirements

Tailing factor: NMT 1.5 for the teriparatide peak

Relative standard deviation: NMT 1.25% calculated from the injection of three separate *Standard solutions*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Calculate the concentration of teriparatide (C_S), in µg/mL, in each of the *Standard solutions*:

$$C_S = (L_S/V_S)$$

L_S = content of teriparatide in [USP Teriparatide RS](#) (µg)

V_S = volume of *Diluent* used for the *Standard solutions* (mL)

For each injection of the *Standard solution*, calculate a response factor (F_R):

$$F_R = (r_S/C_S)$$

r_S = peak response of teriparatide from the *Standard solution*

C_S = concentration of [USP Teriparatide RS](#) in the *Standard solution* (µg/mL)

Calculate the mean response factor (F_M) for all three *Standard solutions*.

Calculate the percentage (P_U) of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) in the portion of Teriparatide taken:

$$P_U = (r_U/F_M) \times [V_U/(W \times F)] \times 100$$

r_U = peak response of teriparatide from the *Sample solution*

F_M = mean response factor for all three *Standard solutions*

V_U = volume of *Diluent* used to prepare the *Sample solutions* (mL)

W = weight of Teriparatide taken to prepare the *Sample solutions* (mg)

F = conversion factor from mg to μ g, 1000

Calculate the percentage of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) corrected for water, acetate, and chloride contents:

$$\text{Result} = P_U / [(100 - \% \text{ of water} - \% \text{ of acetate} - \% \text{ of chloride}) / 100]$$

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free, and chloride-free basis

OTHER COMPONENTS

• ACETATE CONTENT

Mobile phase: 0.01 N [sulfuric acid](#) in [water](#)

Standard solution 1: 0.072 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 100 mg of [sodium acetate anhydrous](#), place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Standard solution 2: 0.144 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 200 mg of [sodium acetate anhydrous](#), place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Standard solution 3: 0.216 mg/mL of acetate in *Mobile phase* prepared as follows. Weigh approximately 300 mg of [sodium acetate anhydrous](#), place in a 100-mL volumetric flask, and dilute with *Mobile phase* to volume. Dilute 1 mL of this solution with *Mobile phase* to 10.0 mL. Stable for 72 h when stored at 2°–8°.

Sample solution: Under controlled relative humidity of $25 \pm 5\%$, prepare in duplicate approximately 5 mg/mL of Teriparatide in *Mobile phase*. Stable for 72 h when stored at 2°–8°.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 210 nm

Column: 9.0-mm \times 25-cm; packing [L22](#)

Temperatures

Autosampler: 2°–8°

Column: Ambient

Flow rate: 1.0 mL/min

Injection volume: 100 μ L

Run time: 12–15 min

System suitability

Samples: *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

[NOTE—The retention time of acetate is 9.2–11.6 min.]

Suitability requirements

Tailing factor: NMT 1.5, *Standard solution 2*

Relative standard deviation: NMT 1.25% from triplicate injections, *Standard solution 2*

Percentage of deviation: NMT 2.0 for each concentration from triplicate injections, *Standard solution 1*, *Standard solution 2*, and *Standard solution 3*

$$\text{Result} = \{[(r_S - b)/a]/C_S\} \times 100$$

r_S = peak response of acetate from *Standard solution 1*, *Standard solution 2*, or *Standard solution 3*

b = y -intercept of the calibration curve described in the *Analysis*

a = slope of the calibration curve described in the *Analysis*

C_S = concentration of acetate in *Standard solution 1*, *Standard solution 2*, or *Standard solution 3* (mg/mL)

Analysis

Samples: *Standard solutions* and *Sample solution*

Determine the peak response of acetate.

Calculate the concentration of acetate (C_S), in mg/mL, in the *Standard solutions*:

$$C_S = (W_S/V_S) \times F \times D$$

W_S = weight of anhydrous sodium acetate (corrected for purity) (mg)

V_S = initial volume of *Mobile phase*, 100 mL

F = ratio of molecular weight of acetate (59.04 g/mol) to molecular weight of anhydrous sodium acetate (82.03 g/mol), 0.7197

D = dilution factor from 1 mL to 10 mL, 0.1

Construct a least-squares calibration curve, using the peak area of the *Standard solutions* versus their acetate concentrations (mg/mL). Determine the concentration of acetate (C_U), in mg/mL, in the

Sample solution, using the equation of a line for the calibration curve.

Calculate the percentage of acetate in the portion of Teriparatide taken:

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

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

V_U = volume of the *Sample solution* (mL)

W_U = weight of Teriparatide taken (mg)

Acceptance criteria: NMT 5.0%

● CHLORIDE CONTENT

Mobile phase: 1.7 mM [sodium bicarbonate](#) and 1.8 mM [sodium carbonate](#) in [water](#)

Standard stock solution: Dry a sufficient quantity of [sodium chloride](#) at 105° for approximately 30 min.

Weigh about 165.9 mg of previously dried [sodium chloride](#), and place in a 100-mL volumetric flask. Dilute with [water](#) to volume. Mix until completely dissolved. This solution contains the equivalent of 1000 µg/mL of chloride.

System suitability solution: Weigh approximately 150 mg of [sodium nitrite](#), and place in a 100-mL volumetric flask. Dilute with [water](#) to volume. Transfer 1.0 mL of this solution to a 100-mL volumetric flask. Transfer 2.5 mL of the *Standard stock solution* to the same flask. Dilute with [water](#) to volume, and mix well.

Standard solutions: Transfer 1.0, 2.0, 3.0, and 4.0 mL of *Standard stock solution* to separate 100-mL volumetric flasks. Dilute with [water](#) to volume, and mix well.

Sample solution: Under controlled relative humidity of $25 \pm 5\%$, prepare in duplicate 1.0 mg/mL of Teriparatide in [water](#). Stable for 72 h when stored at ambient temperature.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: Conductivity with anion suppressor current at 100 mA

Columns

Guard: 4.0-mm \times 5.0-cm; packing [L94](#)

Analytical: 4.0-mm \times 25-cm; 13- μ m packing [L94](#)

Column temperature: Ambient

Flow rate: 2 mL/min

Injection volume: 50 μ L

Run time: 10 min

System suitability

Sample: *System suitability solution*

[NOTE—Chloride elutes earlier than nitrite.]

Suitability requirements

Resolution: NLT 1.5 between chloride and nitrite peaks

Tailing factor: <2.0 for chloride and nitrite peaks

Relative standard deviation of standard curve: <3.0%

Relative standard deviation: <2.0% for chloride and nitrite peaks from five replicate injections

Analysis

Samples: *Standard solutions* and *Sample solution*

Inject the *Standard solutions* followed by the *Sample solution*.

Calculate the concentration of chloride (C_S), in μ g/mL, in the *Standard stock solution*:

$$C_S = (W_S/V_S) \times F \times D$$

W_S = weight of sodium chloride (mg)

V_S = volume of water, 100 mL

F = ratio of molecular weight of chloride (35.46 g/mol) to molecular weight of sodium chloride (58.45 g/mol), 0.607

D = conversion factor from mg to μ g, 1000

Determine the concentrations of chloride, in μ g/mL, in the *Standard solutions*.

Construct a least-squares calibration curve, using the peak area of the *Standard solutions* versus their chloride concentrations (μ g/mL). Determine the concentration of chloride (C_U), in μ g/mL, in the

Sample solution, using the equation of a line for the calibration curve.

Calculate the percentage of chloride in the portion of Teriparatide taken:

$$\text{Result} = [(C_U \times V_U \times F)/W_U] \times 100$$

C_U = concentration of chloride in the *Sample solution* (μ g/mL)

V_U = volume of the *Sample solution* (mL)

F = conversion factor from μ g to mg, 0.001

W_U = weight of Teriparatide (mg)

Acceptance criteria: NMT 4.0%

PRODUCT-RELATED SUBSTANCES AND IMPURITIES

● PRODUCT-RELATED IMPURITIES

0.2 M sulfate buffer: 28.4 g/L of [anhydrous sodium sulfate](#) in [water](#). Adjust with 85% phosphoric acid to a pH of 2.3.

Solution A: [Acetonitrile](#) and 0.2 M sulfate buffer (10:90)

Solution B: [Acetonitrile](#) and 0.2 M sulfate buffer (50:50)

[NOTE—If the sodium sulfate precipitates, gentle heating and continuous stirring may be required. The sodium sulfate should not re-precipitate if this procedure is followed.]

Mobile phase: See [Table 2](#). [NOTE—The *Mobile phase* composition may be adjusted to obtain the desired retention time of the teriparatide peak. The *Solution B* percentage at 5 and 35 min should be changed to alter the retention time, but the same gradient slope should be maintained.]

Table 2

Time (min)	Solution A (%)	Solution B (%)
0	100	0
5	65	35
35	60	40
45	0	100
45.1	100	0
55	100	0

System suitability solution: Use an appropriate solution containing approximately 0.8% of the first post-main peak in *Solution A*. [NOTE—Teriparatide containing the first post-main peak may be prepared by dissolving Teriparatide in [water](#) to obtain a concentration of 2 mg/mL. Adjust with hydrochloric acid to a pH of 3.0. Incubate this solution at 50° for 9 days. The solution may be aliquoted and stored frozen. Dilute 1:1 with *Solution A* prior to injection. The first post-main peak is a degradation product resulting from this process and elutes immediately after the teriparatide peak. The relative retention times for teriparatide and this first post-main peak are 1.00 and 1.05, respectively.]

Sample solution: Approximately 0.7 mg/mL of Teriparatide in *Solution A*

Blank: *Solution A*

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 214 nm

Column: 4.6-mm × 15-cm; 3.5-μm packing [L1](#)

Temperatures

Autosampler: 5°

Column: 40°

Flow rate: 1.0 mL/min

Injection volume: 20 μL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time for teriparatide is 19.8–24.8 min.]

Suitability requirements

Peak-to-valley ratio: The ratio of the height of the first post-main peak to the valley between the teriparatide peak and the first post-main peak is NLT 1.5.

Tailing factor: NMT 2.0 for the teriparatide peak

Analysis

Sample: *Sample solution*

Measure the peak responses for all integrated peaks.

Calculate the percentage of methionyl sulfoxides of teriparatide in the portion of Teriparatide taken:

$$\text{Result} = [(r_{\text{Met+O}(8)} + r_{\text{Met+O}(18)} + r_{\text{Met+O}(8,18)})/r_T] \times 100$$

$r_{\text{Met+O}(8)}$ = peak response of *Met+O(8)* teriparatide (oxidized on Met 8)

$r_{\text{Met+O}(18)}$ = peak response of *Met+O(18)* teriparatide (oxidized on Met 18)

$r_{\text{Met+O}(8,18)}$ = peak response of *Met+O(8,18)* teriparatide (oxidized on Met 8 and Met 18)

r_T = sum of all the peak responses

Calculate the percentage of the largest other related impurity of teriparatide in the portion of Teriparatide taken:

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

r_i = peak response of the largest other related impurity of teriparatide

r_T = sum of all the peak responses

Calculate the total percentage of teriparatide related impurities in the portion of Teriparatide taken:

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

r_I = sum of the peak responses of the teriparatide related impurities

r_T = sum of all the peak responses

Acceptance criteria: See [Table 3](#).

Table 3

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
<i>Met+O(8,18)</i> teriparatide	0.38	—
<i>Met+O(8)</i> teriparatide	0.48	—
<i>Met+O(18)</i> teriparatide	0.58	—
Teriparatide	1.0	—
Total of methionyl sulfoxides of teriparatide [consisting of <i>Met+O(8)</i> teriparatide, <i>Met+O(18)</i> teriparatide, and <i>Met+O(8,18)</i>]	—	0.5
Largest other individual related impurities	—	0.5
Total impurities	—	2.5

SPECIFIC TESTS

- **BACTERIAL ENDOTOXINS TEST** [\(85\)](#): NMT 50 USP Endotoxin Units/mg of Teriparatide drug substance
- **MICROBIAL ENUMERATION TESTS** [\(61\)](#) and **TESTS FOR SPECIFIED MICROORGANISMS** [\(62\)](#): The total aerobic microbial count is NMT 100 cfu/g of Teriparatide drug substance.
- **WATER DETERMINATION** [\(921\)](#), *Method I, Method Ic*: NMT 10.0%

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE**: Unless otherwise prescribed, store in an airtight container, protected from light, at a temperature lower than -10° .

Change to read:

- **LABELING**: Label it to indicate that the material has been produced by methods based on recombinant DNA technology [▲]or synthetic processes. [▲](TBD)
- **USP REFERENCE STANDARDS** [\(11\)](#)
[USP Teriparatide RS](#)

¹ ThermoFisher catalog number 12430054 or suitable equivalent.

² GE Healthcare Life Sciences catalog number SH30070.03HI or suitable equivalent.

³ American Type Culture Collection, catalog number CRL-1661.

⁴ ThermoFisher catalog number 14190144 or suitable equivalent.

⁵ ThermoFisher catalog number 25200056 or suitable equivalent.

⁶ Corning Costar catalog number 3595 or suitable equivalent.

⁷ ThermoFisher catalog number 24020117 or suitable equivalent.

⁸ ThermoFisher catalog number 14025092 or suitable equivalent.

⁹ ThermoFisher catalog number 4412182 or 4412183 or suitable equivalent.

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