Teriparatide

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Posting Date	29-Mar-2024
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Expert Committee	Biologics Monographs 1 - Peptides & Oligonucleotides

In accordance with the Rules and Procedures of the Council of Experts, the Biologics Monographs 1 - Peptides & Oligonucleotides Expert Committee has revised the Teriparatide monograph. The purpose of this revision is to revise the following sections to accommodate FDA-approved products with different manufacturing process.

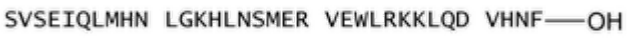
- In the *Definition* section, add "If hydrochloride is not used in a manufacturing process, it contains NLT 95.0% and NMT 105.0% of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$), calculated on the anhydrous and acetic acid-free basis".
- In the *Identification C. Bioidentity* section, change the acceptance criteria to:
  "For teriparatide produced by a recombinant DNA technology: 60%–120% of the relative potency to USP Teriparatide RS on the as-is basis.
  For teriparatide produced by chemical synthesis: 60%–125% of the relative potency to USP Teriparatide RS on the as-is basis".
- In the *Assay* section, add "If hydrochloride is not used in the manufacturing process, calculate the percentage of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) corrected for water and acetate contents: Result = P_U/[(100 − % of water − % of acetate)/100]; change the acceptance criteria to "95.0%–105.0% on the anhydrous, acetic acid-free, and chloride-free basis or 95.0%–105.0% on the anhydrous and acetic acid-free basis if hydrochloride is not used in the manufacturing process".
- In the *Acetate Content* section, change the acceptance criteria to:
  "For teriparatide produced by a recombinant DNA technology: NMT 5.0%
  For teriparatide produced by chemical synthesis: NMT 10%".
- In the *Chloride Content* section, add a note "Perform this test if hydrochloride is used in the manufacturing process".

The Teriparatide Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Julie Zhang, Team Lead/Principal Scientist (301-816-8350 or julie.zhang@usp.org).

Revision Bulletin
Official: April 1, 2024

Teriparatide



Click image to enlarge

$C_{181}H_{291}N_{55}O_{51}S_2$ 4117.77 (as free base)
L-Phenylalanine, L-seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutaminyl-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-triptophyl-L-leucyl-L-arginyl-L-lysyl-L-lysyl-L-leucyl-L-glutaminyl-L-α-aspartyl-L-valyl-L-histidyl-L-asparaginyl-;
L-Seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutaminyl-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-glutaminyl-L-α-aspartyl-L-valyl-L-histidyl-L-asparaginyl-L-phenylalanine. CAS RN®: 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 a method based on recombinant DNA (rDNA) technology or chemical synthesis. The host cell-derived protein content of Teriparatide produced from an rDNA process 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 are below the limit approved by the competent authority. 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. ▲If hydrochloride is not used in a manufacturing process, 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 and acetic acid-free basis.▲ (RB 1-Apr-2024)

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**

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

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*. The solution is 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. The solution is 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. The solution is 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*. The solution is 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-µm packing L1

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 20 µL

System suitability

Sample: *Standard solution*

Suitability requirements

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

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

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.

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.

Change to read:

- **C. Bioidentity**

Basic medium: Sterile Dulbecco's modified Eagle's medium (DMEM) containing high glucose, L-glutamine, pyridoxine hydrochloride, and 25mM HEPES[1]

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 FBS[2] and mix. Sterilize the solution by filtering the solution using a 0.22-µm, 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-µm, 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-µm, 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 line[3] in *Growth medium* at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2) 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×). [Note—Do not allow the cells to go beyond 85% confluence for cell passage or analysis. Higher levels of confluency can reduce the dynamic range of the assay.] 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 cm^2 flask with approximately 30 mL of *Growth medium* from 0.75×10^6 to 6×10^6 cells per flask.

Preparation of cells for analysis: Use cells that are between passages 4 and 10, 65%–85% confluent, and 2–5 days post-passage. [Note—Higher levels of confluency can reduce the dynamic range of the assay.] 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 µL 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 ± 2° and 10 ± 2% carbon dioxide (CO_2). Following the incubation, remove media from the cells and add 200 µL of *Serum starve medium* to each well. Incubate plates for 18–26 h at 37 ± 2° and 10 ± 2% carbon dioxide (CO_2).

Diluent A: 1 mM IBMX in Hanks' balanced salt solution (HBSS) with phenol red[7] prepared as follows. Very slowly add 500 µL 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—Avoid precipitation of IBMX solution during the preparation of *Diluent A*. Discard the solution if precipitated. 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 plates[9] prepared as follows. Very slowly add 27.5 µL of warmed *600 mM IBMX solution* to 30 mL of warmed assay/lysis buffer. [Note—Avoid precipitation of IBMX solution during the preparation of *Diluent A*. Discard the solution if precipitated.]

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-µg/mL solution.

Standard solution: Prepare a 1-µM solution by mixing 82.4 µL 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-µM solution by mixing 20 µL 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 µL per well of HBSS without phenol red[4] at room temperature. Place 100 µL 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 ± 2° for 20 ± 5 min with gentle shaking. Discard the solutions and wash cells twice with 300 µL per well of HBSS without phenol red at room temperature. Add 100 µL per well of *Assay/lysis solution* and incubate the plates at 37 ± 2° for 30 ± 5 min to lyse the cells. Mix cell lysate with a multi-channel pipette prior to transfer. Transfer 60 µL of cell lysate to the appropriate wells of the 96-well assay plate from the cAMP immunoassay assay kit. Add 30 µL 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 µL of anti-cAMP antibody from the cAMP kit to the wells and incubate at 25 ± 2° for 60 ± 5 min on a plate shaker with gentle shaking. Discard the solutions and wash the plates six times with 300 µL per well of wash buffer from the cAMP kit, blotting the plate between each wash. Add 100 µL 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

▲**For teriparatide produced by a recombinant DNA technology:** 60%–120% of the relative potency to USP Teriparatide RS on the as-is basis

For teriparatide produced by chemical synthesis: 60%–125% of the relative potency to USP Teriparatide RS on the as-is basis▲ (RB 1-Apr-2024)

ASSAY

Change to read:

• **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*. The solution is 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*. The solution is 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 (mg/µg), 1000

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

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

▲If hydrochloride is not used in the manufacturing process, calculate the percentage of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$) corrected for water and acetate contents:

$$\text{Result} = P_U/[(100 - \% \text{ of water} - \% \text{ of acetate})/100]$$ ▲ (RB 1-Apr-2024)

Acceptance criteria: 95.0%–105.0% on the anhydrous, acetic acid-free, and chloride-free basis ▲or 95.0%–105.0% on the anhydrous and acetic acid-free basis if hydrochloride is not used in the manufacturing process▲ (RB 1-Apr-2024)

OTHER COMPONENTS

Change to read:

- **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. The solution is 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. The solution is 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. The solution is stable for 72 h when stored at 2°–8°.

Sample solution: Under controlled relative humidity of 25 ± 5%, prepare in duplicate approximately 5 mg/mL of Teriparatide in *Mobile phase*. The solution is 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 × 25-cm; packing L22

Temperatures

Autosampler: 2°–8°

Column: Ambient

Flow rate: 1.0 mL/min

Injection volume: 100 µL

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 sodium acetate, anhydrous, (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 sodium acetate, anhydrous, (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

▲**For teriparatide produced by a recombinant DNA technology:** NMT 5.0%

For teriparatide produced by chemical synthesis: NMT 10%▲ (RB 1-Apr-2024)

Change to read:

- **Chloride Content**

▲[Note—Perform this test if hydrochloride is used in the manufacturing process.]▲ (RB 1-Apr-2024)

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 ± 5%, prepare in duplicate 1.0 mg/mL of Teriparatide in water. The solution is 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 × 5.0-cm; packing L94

Analytical: 4.0-mm × 25-cm; 13-µm packing L94

Column temperature: Ambient

Flow rate: 2 mL/min

Injection volume: 50 µL

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 µg/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 (mg/µg), 1000

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

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

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

F = conversion factor (µg/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 sodium sulfate, anhydrous 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:

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

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

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

$r_{Met+O(8,18)}$ = 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 (%)
Met+O(8,18) teriparatide	0.38	—
Met+O(8) teriparatide	0.48	—
Met+O(18) teriparatide	0.58	—
Teriparatide	1.0	—
Total of methionyl sulfoxides of teriparatide [consisting of *Met+O(8)* teriparatide, *Met+O(18)* teriparatide, and *Met+O(8,18)*]	—	0.5
Largest other individual related impurities	—	0.5
Total impurities	—	2.5

SPECIFIC TESTS

- **Bacterial Endotoxins Test** ⟨85⟩**:** Where the label states that Teriparatide must be subjected to further processing during the preparation of injectable dosage forms, the level of bacterial endotoxins is such that the requirement under the relevant dosage form monograph(s) in which Teriparatide is used can be met.
- **Microbial Enumeration Tests** ⟨61⟩ and **Tests for Specified Microorganisms** ⟨62⟩**:** The total aerobic microbial count is NMT 10^2 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°.
- **Labeling:** Label it to indicate that the material has been produced by methods based on recombinant DNA technology or synthetic processes. Where Teriparatide must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.
- **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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