

Status: Currently Official on 16-Feb-2025
Official Date: Official as of 01-Apr-2024
Document Type: USP Monographs
DocId: GUID-DDD188FC-7C33-43E7-8F63-A4A7084BE61F_6_en-US
DOI: https://doi.org/10.31003/USPNF_M80910_06_01
DOI Ref: hzyf3

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Teriparatide

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SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHN—OH

$C_{181}H_{291}N_{55}O_{51}S_2$ 4117.77 (as free base)

L-Phenylalanine, L-seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutamyl-L-leucyl-L-methionyl-L-histidyl-L-asparagyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparagyl-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-asparagyl-L-seryl-L-valyl-L-seryl-L-α-glutamyl-L-isoleucyl-L-glutamyl-L-leucyl-L-methionyl-L-histidyl-L-asparagyl-L-leucylglycyl-L-lysyl-L-histidyl-L-leucyl-L-asparagyl-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-asparagyl-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	

Time (min)	Solution A (%)	Solution B (%)
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 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-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 line³ in *Growth medium* at $37 \pm 2^{\circ}$ and $10 \pm 2\%$ 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 $\times$). [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 $37 \pm 2^{\circ}$ and $10 \pm 2\%$ 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 $37 \pm 2^{\circ}$ and $10 \pm 2\%$ carbon dioxide (CO_2).

Diluent A: 1 mM IBMX in Hanks' balanced salt solution (HBSS) with phenol red⁷ 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 plates⁹ 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- $\mu\text{g}/\text{mL}$ 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 red⁴ 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 $25 \pm 2^{\circ}$ 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 $37 \pm 2^{\circ}$ 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 $25 \pm 2^{\circ}$ for 60 ± 5 min on a plate shaker with gentle shaking. Discard the solutions and wash the plates six times with 300 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