Teriparatide Injection

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 Injection monograph.

Based on the supporting data received from a manufacturer awaiting FDA approval, the Expert Committee proposes to revise the *Labeling* section 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 Injection

DEFINITION

Teriparatide Injection is a sterile solution of Teriparatide in Water for Injection. It contains NLT 90.0% and NMT 105.0% of the labeled amount of teriparatide ($C_{181}H_{291}N_{55}O_{51}S_2$). The formulation may contain a suitable preservative.

IDENTIFICATION

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

● **B. Bioidentity**

Basic medium: Sterile Dulbecco's modified Eagle's medium (DMEM) containing high glucose, L-glutamine, pyridoxine hydrochloride, and 25 mM 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.] 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 cells in an appropriate volume of *Growth medium* and count. Cells are passaged into a T162 cm^2 flask with approximately 30 mL of *Growth medium* at 0.75–6 × 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 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—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.

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 solution: Prepare a 1-µM solution by diluting the Injection with *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: 75%–125% 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* (61:39). [Note—The *Mobile phase* composition may be adjusted to obtain the retention time of approximately 8 min for the teriparatide main peak.]

Diluent for standard solution: Acetonitrile and *0.2 M sulfate buffer* (25:75)

0.27 M sulfate buffer: 38.8 g/L of anhydrous sodium sulfate in water. Adjust with 85% phosphoric acid to a pH of 2.3.

Diluent for sample solution: Acetonitrile and *0.27 M sulfate buffer* (31:69)

Standard solutions: Prepare in triplicate 100 µg/mL of USP Teriparatide RS in *Diluent for standard solution*. *Standard solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

Sample solutions: Prepare in duplicate approximately 50–100 µg/mL of teriparatide in *Diluent for sample solution*. *Sample solutions* are stable for 48 h when stored at 2°–8° in a sealed container.

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: 0.8 mL/min

Injection volume: 25 µL

Run time: 20 min

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5 for the teriparatide peak

Relative standard deviation: NMT 1.25% calculated from three injections of one *Standard solution*

Analysis

Samples: *Standard solutions* and *Sample solutions*

Measure the peak responses corresponding to teriparatide.

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 for standard solution* used for each *Standard solution* (mL)

Determine the average concentration of teriparatide (C_M) for all three *Standard solutions*.

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

$$F_M = (r_M/C_M)$$

r_M = average peak response of teriparatide from the *Standard solutions*

C_M = average concentration of teriparatide in the *Standard solutions* (µg/mL)

Calculate the concentration of teriparatide (C_U), in µg/mL, in the portion of Injection taken:

$$C_U = (r_U \times F)/F_M$$

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

F = dilution factor used to prepare the *Sample solution*

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

Calculate the percentage of the labeled amount of teriparatide in the portion of Injection taken:

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

C_U = concentration of teriparatide in the portion of Injection taken (µg/mL)

C_L = nominal concentration of teriparatide in the portion of Injection taken (µg/mL)

Acceptance criteria: 90.0%–105.0%

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 1*. [Note—The *Mobile phase* composition may be adjusted to obtain the desired retention time of the teriparatide peak. *Solution B* percentage at 8 min and 68 min may also be changed, if necessary, to obtain the desired retention time, but the same gradient slopes should be maintained. A change of 0.5% of *Solution B* will alter the retention time of the main peak approximately 100 s.]

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	100	0

Time (min)	Solution A (%)	Solution B (%)
2	100	0
8	76	24
68	60	40
75	0	100
80	0	100

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 1:3 with *Solution A* to approximately the same concentration as the Injection portion 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 1.02, respectively.]

Sample solution: Use the solution from an undiluted Injection container.

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: 50 µL

System suitability

Sample: *System suitability solution*

[NOTE—The retention time for teriparatide is 60.83–66.67 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 a related impurity, rhPTH (1–30) (a cleavage product of teriparatide at Asn 30), in the portion of Injection taken:

$$\text{Result} = (r_{rhPTH(1–30)}/r_T) \times 100$$

$r_{rhPTH(1–30)}$ = peak response of rhPTH(1–30)

r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

Calculate the percentage of a related impurity, teriparatide succinimide (30) (formation of succinimide on Asn 30), in the portion of Injection taken:

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

r_{Suc} = peak response of teriparatide succinimide (30)

r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

Calculate the percentage of the largest other related impurity of teriparatide in the portion of Injection 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 excluding peaks due to added preservatives or excipients

Calculate the percentage of total related impurities in the portion of Injection taken:

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

r_T = sum of all the peak responses excluding peaks due to added preservatives or excipients

r_S = peak response of teriparatide

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
rhPTH (1–30)	0.77–0.78	1.2
Teriparatide succinimide (30)	0.98–0.99	1.2
Teriparatide	1.0	—
Largest other individual related impurity	—	1.0
Total impurities	—	7.0

SPECIFIC TESTS

- **pH** ⟨791⟩**:** 3.8–4.5
- **Bacterial Endotoxins Test** ⟨85⟩**:** NMT 100 USP Endotoxin Units/mg of teriparatide drug product
- **Sterility Tests** ⟨71⟩*, Test for Sterility of the Product to Be Examined, Membrane Filtration***:** Meets the requirements
- **Particulate Matter in Injections** ⟨788⟩**:** Meets the requirements for small-volume injections

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Unless otherwise prescribed, store in a sterile, airtight, tamper-proof container, protected from light, at a temperature of 2°–8°. The Injection is not to be frozen.

Change to read:

- **Labeling:** Label it to indicate that the material has been produced by methods based on recombinant DNA technology ▲or chemical synthesis.▲ (TBD)

- **USP Reference Standards** ⟨11⟩
  USP Endotoxin RS
  USP Teriparatide RS

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[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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