Teriparatide Injection

Recombinant Human Parathyroid Hormone (rhPTH\textsuperscript{1-34}) injection

Recombinant Human Parathyroid Hormone (rhPTH\textsuperscript{1-34}) injection, is a sterile solution for injection containing teriparatide (rhPTH\textsuperscript{1-34}) as an active ingredient with suitable buffering and preservative agents.

Teriparatide injection contains not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of teriparatide

**Usual Strength.** Pre-filled cartridge / vial contains 250 µg per ml of teriparatide (rhPTH\textsuperscript{1-34}).

**Description.** Clear, colorless or slightly yellowish solution.

**Identification**

A. It shows the biological activity as described under potency.

B. Determine by Liquid chromatography (2.4.14) as described in assay. In assay, the retention time of the Teriparatide peak in the chromatogram obtained with the test solution corresponds to that of the peak in the chromatogram obtained with the reference solution.

C. Determine by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (2.4.12) under non-reducing conditions.

*Gel dimensions.* 1.5 mm thick 10 cm × 10.5 cm.

*Resolving gel.* 20 per cent acrylamide. Mix the components in the order shown.

<table>
<thead>
<tr>
<th>Solution components</th>
<th>Component volume (ml) per gel volume of 7.5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.20</td>
</tr>
<tr>
<td>50 per cent acrylamide solution*</td>
<td>3.00</td>
</tr>
<tr>
<td>Tris-SDS solution (pH 8.45)</td>
<td>2.49</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.79</td>
</tr>
<tr>
<td>10 per cent APSφ</td>
<td>0.025</td>
</tr>
<tr>
<td>TEMED†</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*50 per cent acrylamide solution: 50 per cent acrylamide/bisacrylamide (50:1) solution

φ10 per cent APS: a 100 g per litre solution of ammonium persulfate. Prepare freshly.

†TEMED: tetramethylethlenediamine

*Stacking gel.* 4 per cent acrylamide. Mix the components in the order shown.
Solution components

<table>
<thead>
<tr>
<th>Component volume (ml) per gel volume of 5.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
</tr>
<tr>
<td>30 per cent acrylamide solution*</td>
</tr>
<tr>
<td>Tris SDS solution (pH 8.45)</td>
</tr>
<tr>
<td>10 per cent APS*</td>
</tr>
<tr>
<td>TEMED†</td>
</tr>
</tbody>
</table>

*30 per cent acrylamide solution: 30 per cent acrylamide / bisacrylamide (29.2:0.8) solution
* 10 per cent APS: a 100 g per L solution of ammonium persulfate. Prepare freshly.
† TEMED: tetramethylthlenediamine

Tris-SDS solution. A mixture of 0.3 per cent sodium dodecyl sulfate in 3.0 M tris-hydrochloride buffer solution, pH 8.45.

Sample buffer. A mixture of 0.2 M tris-hydrochloride buffer solution pH 6.8 containing 48 per cent glycerol, 2 per cent sodium dodecyl sulfate, 0.4 mg per ml coomassie G250.

Test solution. Dilute the preparation under examination in sample buffer to obtain a concentration of 8µg per well.

Reference solution (a). A solution of protein molecular weight markers suitable for calibrating SDS-polyacrylamide gels.

Reference solution (b). Dilute teriparatide RS in water to obtain a concentration of 0.25 mg per ml. To 38.4 µl of this solution add 15 µl of sample buffer. Dilute to 60 µl with water.

Reference solution (c). Dilute the test solution in sample buffer to obtain a concentration of 8µg per well.

Sample treatment. Boil for 2 minutes.

Application:

<table>
<thead>
<tr>
<th>Well</th>
<th>Solution(s)</th>
<th>Volume (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reference solution (a)</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Reference solution (b)</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Test solution</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Reference solution (c)</td>
<td>50</td>
</tr>
</tbody>
</table>

Cathode buffer. 0.1 M tris and 0.1 M tricine and 0.1 per cent sodium dodecyl sulfate. Do not adjust pH.

Anode buffer. 0.2 M tris-hydrochloride buffer solution pH 8.9.
Detection. By silver staining.

The test is not valid unless; a band is seen in the electropherogram obtained with reference solution (c)

The electropherogram obtained with the test solution shows a single broad band corresponding in position and intensity to the single broad band obtained with the reference solution (b).

Tests

pH (2.4.24). 3.8 to 4.5

Related Impurities. Determine by Liquid chromatography (2.4.14) using normalization procedure. Use method A or B.

Method A

Test solution. Dilute the preparation under examination with mobile phase A to obtain a protein concentration of 0.25 mg per ml.

Reference solution. To a volume of the test solution, add a suitable volume of 0.06 per cent hydrogen peroxide and 3 mM acetic acid to obtain a final protein concentration of 0.88 mg per ml. Allow to stand at room temperature for 1 hour. Add approx. 30 mg of L-methionine per ml of solution and mix.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecysilyl silica gel (3 µm) with a pore size of 20 nm,
- mobile phase: A. 0.1 per cent v/v solution of trifluoroacetic acid,
  B. to 800 ml of acetonitrile add 1.0 ml of trifluoroacetic acid and 200 ml of water for chromatography,
- a gradient programme using the conditions given below,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 40 µl.

Equilibrate the column for at least 15 minutes maintaining the temperature of the column at 40°C.

<table>
<thead>
<tr>
<th>Time (in min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>26</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>26.01</td>
<td>66.5</td>
<td>33.5</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>30.01</td>
<td>76</td>
<td>24</td>
</tr>
</tbody>
</table>
Inject the reference solution. The test is not valid unless in the chromatogram obtained with the reference solution three peaks corresponding to oxidized teriparatide appear at retention time of 0.79, 0.66 and 0.46 relative to principal peak.

Inject the test solution. The chromatogram obtained with the test solution corresponds to that obtained with the reference solution. In the chromatogram obtained with the test solution, the sum of the area of any peaks other than the principal peak is not greater than 3.0 per cent of the total area of the peaks. The sum of the area of peaks corresponding to the oxidized form is not greater than 2.0 per cent of the total area of the peaks.

**Method B**

**Test solution.** Dissolve the preparation under examination in mobile phase A to obtain a concentration of approximately 250 µg per ml.

**Reference solution.** Dissolve the content of teriparatide RS in water to obtain a concentration of 2 mg per ml. Adjust the pH to 3.0 with hydrochloric acid. Incubate at 50º for 9 days. The solution may be aliquoted and stored frozen. Dilute 1 volume of this solution with 3 volume of mobile phase A prior to injection. This gives approximately 0.8 per cent solution of first post-main peak. The first post-main peak is a degradation product resulting from this process and elutes immediately after teriparatide peak.

**0.2 M Sulfate buffer.** 28.4 grams per litre of anhydrous sodium sulfate in water. Adjust with 85 per cent phosphoric acid to a pH of 2.3.

**Chromatographic system**

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (3.5 µm),
- autosampler temperature: 5º,
- mobile phase: A. A mixture of 10 volumes of acetonitrile and 90 volumes of 0.2 M sulfate buffer (pH 2.3)
  B. A mixture of 50 volumes of acetonitrile and 50 volumes of 0.2 M sulfate buffer (pH 2.3).

(Note: If the sodium sulfate precipitates, gentle heating and continuous stirring may be required. The sodium sulfate should not re-precipitate if this exercise is followed),

- a linear gradient programme using the conditions given below,
- flow rate: 1.0 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 50 µl.

<table>
<thead>
<tr>
<th>Time (in min)</th>
<th>Mobile phase A (per cent v/v)</th>
<th>Mobile phase B (per cent v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Equilibrate the column for at least 15 minutes maintaining the temperature of the column at 40°C.

Inject the reference solution. The test is not valid unless (i) the ratio of the height of first post main peak to the valley between the teriparatide peak and the first post peak is not less than 1.5 (ii) the tailing factor for the teriparatide peak is not more than 2.0.

Inject the test solution. The relative retention time of rhPTH1-30 is about 0.77 to 0.78 and teriparatide succinimide (30) is about 0.98 to 0.99 with reference to teriparatide.

Calculate the percentage of a related impurity, the cleavage product of teriparatide, rhPTH (1-30), in the portion of injection taken using the following expression:

\[
\left( \frac{r_{\text{rhPTH}(1-30)}}{r_T} \right) \times 100
\]

where,

\[
r_{\text{rhPTH}(1-30)} = \text{peak response of rhPTH (1-30) (a cleavage product of teriparatide at Asn 30)}
\]

\[
r_T = \text{sum of all the peak responses excluding peaks due to added preservatives or excipients}
\]

Calculate the percentage of a related impurity, teriparatide succinimide (30) in the portion of injection taken using the following expression:

\[
\left( \frac{r_{\text{Suc}}}{r_T} \right) \times 100
\]

where,

\[
r_{\text{Suc}} = \text{peak response of teriparatide succinimide (30) (formation of succinimide on Asn 30)}
\]

\[
r_T = \text{sum of all the peak responses excluding peaks due to added preservatives or excipients}
\]

Calculate the percentage of the largest other related impurity of teriparatide in the portion of teriparatide taken using the following expression:

\[
\left( \frac{r_i}{r_T} \right) \times 100
\]

where,

\[
r_i = \text{peak response of the largest other related impurity of teriparatide}
\]

\[
r_T = \text{sum of the responses for all of the peaks excluding peaks due to added preservatives or excipients}
\]
Calculate the total percentage of teriparatide related impurities in the portion of teriparatide taken using the following expression:

\[
\left( \frac{r_l}{r_T} \right) \times 100
\]

where,

- \( r_l \) = sum of peak response of the teriparatide related compound
- \( r_T \) = sum of the responses for all of the peaks excluding peaks due to added preservatives or excipients

The content of (i) rhPTH\(^{1-30}\) is not more than 1.2 per cent; (ii) teriparatide succinimide (30) is not more than 1.2 per cent; (iii) largest other individual related impurity is not more than 1.0 per cent and (iv) the total related impurities is not more than 7.0 per cent.

**Bacterial endotoxins** (2.2.3). Not more than 100 EU per mg of teriparatide drug product.

**Other tests.** Complies with the tests stated under Parenteral Preparations (Injections).

**Assay**

A. **Potency.**

The biological activity of rhPTH\(^{1-34}\) is estimated to be equipotent to that of innovator based on its comparative ability to stimulate the accumulation of cAMP in UMR-106 cells with respect to the innovator's product.

Determination of the biological activity of rhPTH\(^{1-34}\) is based on the stimulation of adenylate cyclase activity in the rat osteosarcoma cell line UMR 106. Activation of the PTH receptor initiates a cascade event which culminates in an intracellular rise in cAMP concentration.

The method uses cAMP end-point measurement by ELISA. Incubate UMR-106 cells in tissue culture plate until an even monolayer is observed. Maintain the cells in serum free media for 18 to 26 hours in order to decrease the levels of endogenous cAMP. Suitably wash the cells and incubate with varying dilutions of test and reference preparations for up to 8 dilutions for the suitable time. Collect the cell lysate containing the cAMP by freeze-thaw cycle. Determine the cAMP accumulation by using a suitable ELISA or detection assay.

Analyze the data by fitting a sigmoidal dose–response curve to the data obtained and by using a suitable statistical method, for example the 4-parameter or parallel line model.

Report the relative potency. The estimated potency is not less than 80 percent and not more than 125 percent of the stated potency.

B. **Determine by liquid chromatography (2.4. 14).**

Store the solution at 2° to 8° and use within 48 hours.
Solution (a). Dissolve 28.4 g of anhydrous sodium sulfate in 900 ml water and adjust to pH 2.3 with 85 per cent phosphoric acid. Dilute to 1000 ml with water to obtain a solution of 0.20 M.

Solution (b). Dissolve 38.8 g of anhydrous sodium sulfate in 900 ml water and adjust to pH 2.3 with 85 per cent phosphoric acid. Dilute to 1000 ml with water to obtain a solution of 0.27 M.

Solution (c). Mix 75 volumes of solution (a) and 25 volumes of acetonitrile.

Solution (d). Mix 69 volumes of solution (b) and 31 volumes of acetonitrile.

Test solution. Prepare the solution in duplicate. Dissolve the preparation under examination in solution (d) to obtain a concentration of approximately 50 to 100 µg per ml. Test solution is stable for not more than 48 hours when stored at refrigerated temperature in sealed container.

Reference solution. Prepare the solution in triplicate. Dissolve the content of teriparatide RS in solution (c) to obtain a concentration of 100 µg per ml. Reference solution is stable for not more than 48 hours when stored at refrigerated temperature in sealed container.

Chromatographic system
- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilyl silica gel (3.5 µm),
- mobile phase: A. a mixture of 90 volumes of solution (a) and 10 volumes of acetonitrile,
  B. a mixture of 50 volumes of solution (a) and 50 volumes of acetonitrile.
- a linear gradient programme using the conditions given below,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 214 nm,
- injection volume: 25 µl,
- Run time: 20 minutes.

Note: The Mobile phase composition may be adjusted to obtain the retention time of approximately 8 min for the teriparatide main peak.

Equilibrate the column for at least 15 minutes maintaining the temperature of the column at 40°.

Inject the reference solution in triplicate. The test is not valid unless (i) The relative standard deviation (RSD) of rh-PTH (1–34) peak area is not more than 1.25 per cent; (ii) the tailing factor of the teriparatide peak is not more than 1.5.

Inject the test solution. The estimated potency is not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of teriparatide.

Storage. Store in sterile airtight, tamper proof container at temperature of 2° to 8°. Do not freeze.

Labelling. The label states (i) the teriparatide content in µg per ml; (ii) the name and the concentration of any other excipients; (iii) indication that the material has been produced by methods based on recombinant DNA technology.