

STABILIZED TERIPARATIDE SOLUTIONS

TECHNICAL FIELD

This invention relates to pharmaceutical compositions containing a
5 parathyroid hormone. More particularly, the invention relates to teriparatide,
PTH(1-34), stabilized solution formulations

BACKGROUND OF THE INVENTION

Parathyroid hormone (PTH) is a secreted, 84 amino acid product of the
10 mammalian parathyroid gland that controls serum calcium levels through its action
on various tissues, including bone. Studies in humans with certain forms of PTH
have demonstrated an anabolic effect on bone, and have prompted significant
interest in its use for the treatment of osteoporosis and related bone disorders.

Using the N-terminal 34 amino acids of the bovine and human hormone for
15 example, which by all published accounts are deemed biologically equivalent to the
full length hormone, it has been demonstrated in humans that parathyroid hormone
enhances bone growth particularly when administered in pulsatile fashion by the
subcutaneous route. A slightly different form of PTH, human PTH(1-38) has shown
similar results.

20 PTH preparations have been reconstituted from fresh or lyophilized
hormone, and incorporate various forms of carrier, excipient and vehicle. Most are
prepared in water-based vehicles such as saline, or water acidified typically with
acetic acid to solubilize the hormone. The majority of reported formulations also
incorporate albumin as a stabilizer (see for example Reeve et al., Br. Med. J., 1980,
25 280:6228; Reeve et al., Lancet, 1976, 1:1035; Reeve et al., Calcif. Tissue Res., 1976,
21:469; Hodsman et al., Bone Miner; 1990, 9(2):137; Tsai et al., J. Clin. Endocrinol
Metab., 1989, 69(5):1024; Isaac et al., Horm. Metab. Res., 1980, 12(9):487; Law et
al., J. Clin Invest. 1983, 72(3):1106; and Hulter, J. Clin Hypertens, 1986, 2(4):360).
Other reported formulations have incorporated an excipient such as mannitol, which
30 is present either with the lyophilized hormone or in the reconstitution vehicle.
Formulations representative of those employed for human studies include a human

PTH(1-34) (SEQ ID NO: 2) preparation consisting, upon reconstitution, of mannitol, heat inactivated human serum albumin, and caproic acid (a protease inhibitor) as absorption enhancer (see Reeve et al., 1976, *Calcif. Tissue Res.*, 21, Suppl., 469-477); a human PTH(1-38) preparation reconstituted into a saline vehicle (see
5 Hodsman et al., 1991, 14(1), 67-83); and a bovine PTH(1-34) preparation in aqueous vehicle pH adjusted with acetic acid and containing albumin. There is also an International Reference preparation which for human PTH (1-84) (SEQ ID NO: 1) consists of 100 ng of hormone ampouled with 250 µg human serum albumin and 1.25 mg lactose (1981), and for bovine PTH (1-84) consists of 10 µg lyophilized
10 hormone in 0.01M acetic acid and 0.1% w/v mannitol (see Martindale, *The Extra Pharmacopoeia*, The Pharmaceutical Press, London, 29th Edition, 1989 at p. 1338).

A recent attempt at improving the stability for the lyophilized preparation of h-PTH(1-34) (SEQ ID NO: 2) is reported in EP 619 119 with a combination of sugar and sodium chloride. Also U.S. Pat. No. 5,496,801 describes a freeze-dried
15 composition for the natural hormone, PTH(1-84), containing mannitol as an excipient and a citrate source as a non-volatile buffering agent.

Commercial exploitation of parathyroid hormone requires the development of a formulation that is acceptable in terms of storage stability and ease of preparation. Because it is a protein and thus far more labile than the traditionally
20 small molecular weight drugs, however, the formulation of parathyroid hormone presents challenges not commonly encountered by the pharmaceutical industry. Furthermore, like other proteins that have been formulated successfully, PTH is particularly sensitive to oxidation, deamidation and hydrolysis, and requires that its N-terminal and C-terminal sequences remain intact in order to preserve bioactivity.
25 It is an object of the present invention to provide a pharmaceutically useful PTH preparation, particularly one comprising, as active ingredient, teriparatide, PTH(1-34) (SEQ ID NO: 2).

SUMMARY OF THE INVENTION

30 The present invention provides a pharmaceutical composition in the form of a stabilized solution containing a parathyroid hormone (PTH) in a therapeutically

effective amount. The solution is storage stable and, in sterile form, may be stored in vials or cartridges ready for parenteral administration in human patients. The advantages of the present solution is the elimination of the need for lyophilization.

Accordingly, the present invention is a parathyroid hormone solution

5 including:

- (a) a therapeutically effective amount of a parathyroid hormone;
- (b) an effective amount of a stabilizing agent;
- (c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
- 10 (d) the balance being water.

This solution may, if desired, undergo lyophilization to form a freeze-dried powder containing not more than 2% water by weight.

Another aspect of the present invention is a parathyroid hormone solution including:

- 15 (a) a therapeutically effective amount of a parathyroid hormone;
- (b) from about 1 to 20 wt-% of a stabilizing agent;
- (c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7 and selected from an acetate or tartrate source;
- 20 (d) from about 0.1 to 2 wt-% of a parenterally acceptable preservative; and
- (e) the balance being water.

Still another aspect of the present invention is a pharmaceutical composition in the form of a freeze-dried powder prior to reconstitution including:

- 25 (a) a therapeutically effective amount of a fragmented parathyroid hormone selected from the group consisting of PTH (1-34), PTH (1-37), PTH (1-38), and PTH (1-41);
- (b) an effective amount of a stabilizing agent;
- (c) a buffering agent in an amount sufficient to maintain the pH
- 30 of the composition within a range of about 3-7; and
- (d) less than 2% water by weight.

DETAILED DESCRIPTION

The invention relates to parathyroid hormone solutions that exhibit storage stability in terms of hormone composition and activity.

5 As active ingredient, the composition or solution may incorporate the full length, 84 amino acid form of parathyroid hormone, particularly the human form, hPTH (1-84) (SEQ ID NO: 1), obtained either recombinantly, by peptide synthesis or by extraction from human fluid. See, for example, U.S. Pat. No. 5,208,041, incorporated herein by reference. The amino acid sequence for hPTH (1-84) is
10 reported by Kimura et al. in Biochem. Biophys. Res. Comm., 114(2):493 (SEQ ID NO: 1).

 The composition or solution may also incorporate as active ingredient fragments or variants of fragments of human PTH or of rat, porcine or bovine PTH that have human PTH activity as determined in the ovariectomized rat model of
15 osteoporosis reported by Kimmel et al., Endocrinology, 1993, 32(4):1577.

 The parathyroid hormone fragments desirably incorporate at least the first 34 N-terminal residues, such as PTH(1-34) (SEQ ID NO: 2), PTH(1-37), PTH(1-38) and PTH(1-41). Alternatives in the form of PTH variants incorporate from 1 to 5 amino acid substitutions that improve PTH stability and half-life, such as the
20 replacement of methionine residues at positions 8 and/or 18 with leucine or other hydrophobic amino acid that improves PTH stability against oxidation and the replacement of amino acids in the 25-27 region with trypsin-insensitive amino acids such as histidine or other amino acid that improves PTH stability against protease. These forms of PTH are embraced by the term "parathyroid hormone" as used
25 generically herein. The preferred hormone is human PTH(1-34) (SEQ ID NO: 2) also known as teriparatide. The hormones may be obtained by known recombinant or synthetic methods, such as described in U.S. Pat. No. 4,086,196, incorporated herein by reference.

 The stabilizing agent incorporated into the solution or composition includes a
30 polyol which includes a saccharide, preferably a monosaccharide or disaccharide, e.g., glucose, trehalose, raffinose, or sucrose; a sugar alcohol such as, for example,

mannitol, sorbitol or inositol, and a polyhydric alcohol such as glycerine or propylene glycol or mixtures thereof. A preferred polyol is mannitol or propylene glycol. The concentration of polyol may range from about 1 to about 20 wt-%, preferably about 3 to 10 wt-% of the total solution.

5 The buffering agent employed in the solution or composition of the present invention may be any acid or salt combination which is pharmaceutically acceptable and capable of maintaining the aqueous solution at a pH range of 3 to 7, preferably 3-6. Useful buffering systems are, for example, acetate, tartrate or citrate sources. Preferred buffer systems are acetate or tartrate sources, most preferred is an acetate
10 source. The concentration of buffer may be in the range of about 2 mM to about 500 mM, preferably about 2 mM to 100 mM.

 The stabilized solution or composition of the present invention may also include a parenterally acceptable preservative. Such preservatives include, for example, cresols, benzyl alcohol, phenol, benzalkonium chloride, benzethonium
15 chloride, chlorobutanol, phenylethyl alcohol, methyl paraben, propyl paraben, thimerosal and phenylmercuric nitrate and acetate. A preferred preservative is m-cresol or benzyl alcohol; most preferred is m-cresol. The amount of preservative employed may range from about 0.1 to about 2 wt-%, preferably about 0.3 to about 1.0 wt-% of the total solution.

20 Thus, the present invention has provided, for example, a stabilized teriparatide solution containing mannitol, acetate and m-cresol with a predicted shelf-life of over 15 months at 5°C.

 The parathyroid hormone compositions of the present invention may, if desired, be provided in a powder form containing not more than 2% water by
25 weight, that results from the freeze-drying of a sterile, aqueous hormone solution prepared by mixing the selected parathyroid hormone, a buffering agent and a stabilizing agent as above described. Especially useful as a buffering agent when preparing lyophilized powders is a tartrate source. Particularly useful stabilizing agents include glycine, mannitol, sucrose, trehalose, raffinose or a mixture thereof.

30 The PTH solution and composition of the present invention incorporate PTH in a medically effective amount, a term used with reference to amounts useful either

therapeutically or in medical diagnosis. The particular amount of parathyroid hormone incorporated in the preparation can be pre-determined based on the type of PTH selected and on the intended end-use of the preparation. In one application, the preparations are exploited for therapeutic purposes, and particularly for the treatment of osteoporosis. Osteoporosis therapy entails administration of the reconstituted preparation by injection, desirably subcutaneous injection, in unit doses that reflect the prescribed treatment regimen but are, by way of example, for human PTH(1-34) (SEQ ID NO: 2), within the range from 25 µg PTH/mL of injected solution to 1000 µg/mL of injected solution per patient, with injection volumes being desirably from 0.02 to 1.3 mL. Accordingly, the purified PTH is desirably incorporated with the buffering agent and excipient to form an aqueous solution containing PTH in a concentration range from 25 µg/mL to 1000 µg/mL, preferably 100 µg/mL to 500 µg/mL, which is then sterile-filtered and filled into a vial or cartridge for use.

Once the preparation is obtained as an aqueous solution containing desired amounts and concentrations of the buffering agent, excipient and PTH, individual vials are filled with the solution to the desired volume. The advantage of the present invention is that the above solution may be prepared with sterile water without the need to undergo a freeze-drying process.

In an alternative embodiment of the invention, the preparations are provided in a form that yields a unit container of 100-500 µg human PTH(1-34) (SEQ ID NO: 2) upon reconstitution into about 1 mL (0.8-1.2 mL) of the reconstitution vehicle, and the vials are accordingly loaded with about 1 mL of the aqueous PTH preparation, for subsequent freeze-drying.

In a preferred alternative embodiment of the invention, the PTH preparation subjected to freeze-drying comprises from 25 to 1000 µg/mL of human PTH(1-34) (SEQ ID NO: 2), from 2 to 8% by weight of mannitol, and a tartrate source in an amount capable of buffering the preparation to within the range from 3.0 to 6.5 upon reconstitution in sterile water. In specific embodiments of the invention, the tartrate buffering agent is incorporated in an amount sufficient to buffer the pH to 3.5 to 5.5.

In addition to their therapeutic use, the present PTH composition can be formulated and administered to aid in medical diagnosis, and particularly to assist in

establishing the diagnosis of hypoparathyroidism and pseudohypoparathyroidism in hypocalcemic patients. Except for the dose of PTH, the composition of the PTH preparation will remain as described herein for therapeutic use. An intravenously infused, single dose of human PTH(1-34) (SEQ ID NO: 2) that is equal to 200

- 5 International Units of PTH activity is appropriate for this diagnostic purpose. Diagnosis is then made by determining the effect of administered PTH or urinary cAMP levels, with cAMP elevation being indicative of the hypoparathyroidism condition, rather than its pseudoform.

The examples which follow are illustrative of the invention and are not
10 intended to be limiting.

EXAMPLES

Example 1

0.1 mg rhPTH (1-34) (SEQ ID NO: 2), 50 mg mannitol, 2.5 mg m-cresol,
15 0.52 mg acetic acid and 0.12 mg sodium acetate were mixed into a solution with 1 ml of distilled water.

Example 2

0.25 mg rhPTH (1-34) (SEQ ID NO: 2), 45.4 mg mannitol, 3 mg m-cresol,
0.41 mg acetic acid and 0.1 mg sodium acetate were mixed into a solution with 1 ml
20 of distilled water.

The formulations of the present invention, Examples 1 and 2 were compared to solutions containing no stabilizer, 0.9% NaCl, 20 mM acetate and 10 mM acetate as primary stabilizer. The stability was measured by determining the amount in % of rhPTH (1-34) (SEQ ID NO: 2) remaining after a certain time. The measurement
25 was made by HPLC. The results are shown in Tables 1 and 2.

Table 1

Effect of Primary Stabilizer on Chemical Stability
of rhPTH (1-34) at 50°C

	Water	0.9% NaCl	20 mM acetate	10 mM acetate
Time, days	% Remaining			
Initial	100	100	100	100
7	74	81	84	80
14	55	58	67	71

5

Table 2

Comparison of Stability of rhPTH (1-34) at 30°C

	20 mM acetate	10 mM acetate	Example 1	Example 2
Time, days	% Remaining			
Initial	100	100	100	100
7	96	94	100	--
14	94	92	96	100
21	90	93	97	--
30	--	81	96	96

Example 3

The following experiment was carried out to show that lyophilized powder
 10 formulations prepared from stabilized solutions of the present invention are more
 stable than a control which was prepared from PTH(1-34) and mannitol alone.

A control solution and solutions for samples A through O were prepared as
 previously described with the ingredients and concentrations shown in Table 3. The
 solutions were then freeze-dried and the resulting lyophilized powder formulations
 15 were stored at 40°C for a one month period. The amount of PTH(1-34) remaining in
 each sample was then measured by HPLC. The results are shown in Table 3.

Table 3

Stability of PTH(1-34) Lyophilized Formulations at 40°C for One Month

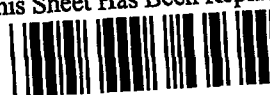
Sample	PTH(1-34) mg/mL	Bulking Agent	Bulking Agent Conc. mg/mL	Buffer	Buffer Conc. mM	% PTH Remaining
Control	0.2	mannitol	40	--	--	78
A	0.5	mannitol	30	acetate	5	90
B	0.5	glycine	30	acetate	5	98
C	0.5	sucrose	30	acetate	5	98
D	0.5	trehalose	30	acetate	5	97
E	0.5	raffinose	30	acetate	5	99
F	0.75	mannitol	30	tartrate	15	95
G	1.5	sucrose & mannitol	5/25	tartrate	5	99
H	0.75	sucrose & mannitol	5/25	tartrate	15	99
I	1.5	mannitol	30	tartrate	5	96
J	1.5	sucrose	30	tartrate	15	100
K	1.5	mannitol	30	tartrate	15	99
L	0.75	sucrose	30	tartrate	15	100
M	0.75	sucrose	30	tartrate	5	100
N	1.5	sucrose & mannitol	5/25	tartrate	15	99
O	1.5	sucrose & mannitol	5/25	acetate	5	91*

* the stability at 2 months was 96%

**WE CLAIM:**

1. A parathyroid hormone solution comprising:
 - (a) a therapeutically effective amount of a parathyroid hormone;
 - (b) an effective amount of a stabilizing agent;
 - 5 (c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and
 - (d) the balance being water.
2. The solution of claim 1, wherein the hormone is a fragmented hormone selected from the group consisting of PTH(1-34), PTH(1-37), PTH(1-38),
10 and PTH(1-41).
3. The solution of claim 1, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).
4. The solution of claim 1, wherein the hormone is human PTH(1-84) (SEQ ID NO: 1).
- 15 5. The solution of claim 1, wherein the stabilizing agent is a polyol.
6. The solution of claim 5, wherein the polyol is mannitol.
7. The solution of claim 5, wherein the polyol is propylene glycol.
8. The solution of claim 1, wherein the buffering agent is an acetate or tartrate source.
- 20 9. The solution of claim 8, wherein the buffering agent is acetate.
10. The solution of claim 1, which further comprises a parenterally acceptable preservative.
11. The solution of claim 10, wherein the preservative is m-cresol or benzyl alcohol.
- 25 12. The solution of claim 11, wherein the preservative is m-cresol.
13. A composition according to claim 1, in the form of a freeze-dried powder containing less than 2% water by weight.
14. A parathyroid hormone solution comprising:
 - (a) a therapeutically effective amount of a parathyroid hormone;
 - 30 (b) from about 1 to 20 wt-% of a stabilizing agent;

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(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7 and selected from an acetate or tartrate source;

5 (d) from about 0.1 to 2 wt-% of a parenterally acceptable preservative; and

(e) the balance being water.

15. The solution of claim 14, wherein the hormone is PTH(1-84).

16. The solution of claim 14, wherein the hormone is selected from the group consisting of PTH(1-34), PTH(1-37), PTH(1-38), and PTH(1-41).

10 17. The solution of claim 16, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).

18. The solution of claim 14, wherein the stabilizing agent is a polyol in an amount of about 3 to about 10 wt-%.

15 19. The solution of claim 14, wherein the preservative is m-cresol or benzylalcohol in an amount of about 0.3 to about 1.0 wt-%.

20. A pharmaceutical composition in the form of a freeze-dried powder comprising:

20 (a) a therapeutically effective amount of a fragmented parathyroid hormone selected from the group consisting of PTH(1-34), PTH(1-37), PTH(1-38), and PTH(1-41);

(b) an effective amount of a stabilizing agent;

(c) a buffering agent in an amount sufficient to maintain the pH of the composition within a range of about 3-7; and

(d) less than 2 wt-% water by weight.

25 21. The composition of claim 20, wherein the hormone is human PTH(1-34) (SEQ ID NO: 2).

22. The composition of claim 20, wherein the stabilizing agent is selected from the group consisting of glycine, mannitol, sucrose, trehalose, raffinose and a mixture thereof.

30 23. The composition of claim 20, wherein the buffering agent is an acetate or tartrate source.

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24. The composition of claim 23, wherein the buffering agent is a tartrate source.

25. The composition of claim 20, wherein the stabilizing agent is in an amount of about 1 to about 20 wt-%.



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