



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(54) Title:</b> A STABILIZED PHARMACEUTICAL FORMULATION COMPRISING GROWTH HORMONE AND ASPARAGINE		
<b>(57) Abstract</b>  A pharmaceutical preparation comprising a growth hormone and asparagine as additive or buffering substance shows a very high stability against deamidation, oxidation and cleavage of peptide bonds. The stability of the product allows for the storing and shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved preparation at ambient temperature.		

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**TITLE**

A stabilized pharmaceutical formulation comprising growth hormone and asparagine.

**FIELD OF THE INVENTION**

The present invention relates to a stabilized pharmaceutical formulation comprising growth hormone, to a method of making such formulation, and the use of asparagine for stabilizing a formulation of growth hormone.

**BACKGROUND OF THE INVENTION**

The growth hormones from man and from the common domestic animals are proteins of approximately 191 amino acids, synthesized and secreted from the anterior lobe of the pituitary gland. Human growth hormone consists of 191 amino acids.

Growth hormone is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids. The major effect of growth hormone is to promote growth.

The organ systems affected by growth hormone include the skeleton, connective tissue, muscles, and viscera such as liver, intestine, and kidneys.

Until the development of the recombinant technology and the cloning of the growth hormone gene now giving rise to production of e.g. human growth hormone (hGH) and Met-hGH in industrial scale, human growth hormone could only be obtained by extraction from the pituitary glands of human cadavers. The very limited supplies of growth hormone restricted the use thereof to longitudinal growth promotion in childhood and puberty for treatment of dwarfism, even though it has been proposed for inter alia treatment of short stature (due to growth hormone deficiency, normal short stature and Turner

syndrom), growth hormone deficiency in adults, infertility, treatment of burns, wound healing, dystrophy, bone knitting, osteoporosis, diffuse gastric bleeding, and pseudoarthrosis.

Furthermore, growth hormone has been proposed for increasing  
5 the rate of growth of domestic animals or for decreasing the proportion of fat in animals to be slaughtered for human consumption.

Pharmaceutical preparations of growth hormone tend to be unstable. Degradation products such as deamidated or sulfoxy-  
10 dated products and dimer or polymer forms are generated - especially in solutions of growth hormone.

The predominant degradation reactions of hGH are 1) deamidation by direct hydrolysis or via a cyclic succinimide intermediate to form various amounts of L-asp-hGH, L-iso-asp-hGH,  
15 D-asp-hGH, and D-iso-asp-hGH (ref 1-3), 2) oxidation of the methionine residues in positions 14 and 125 (ref 4-9), and 3) cleavage of peptide bonds.

Deamidation especially takes place at the Asn in position 149.

20 hGH is rather easily oxidized in positions 14 and 125, especially in solution (4-8).

The oxidation of hGH in solution forming sulfoxides is normally due to the oxygen dissolved in the preparation. The solubility of oxygen in distilled water is about 200  $\mu\text{M}$  (9).  
25 As the concentration of hGH in a preparation comprising 4 IU/ml is 1.3 mg/ml corresponding to 60nM hGH, oxygen will, at normal storing conditions, be present in an excess of about 3000 times the stoichiometric amount for oxidation of hGH. It is not feasible to try to solve the problem by degassing of  
30 buffers before tapping and packing the preparations.

At present, it is not believed that these degradation products should have toxic or altered biological activity or receptor binding properties, but there is indication to the effect that the conformation stability of the sulfoxides is 5 reduced as compared to native hGH.

For the development of a stable, dissolved preparation comprising hGH it is of importance to know the rate of formation of sulfoxides as well as means to control the oxidation.

The kinetics of degradation depend on temperature, pH and 10 various additives or adjuvants in the hGH formulation.

Due to the instability, growth hormone is, at present, lyophilized and stored in the lyophilized form at 4°C until it is reconstituted for use in order to minimize the degradation.

15 The lyophilized pharmaceutical preparations comprising hGH are, at present, reconstituted by the patient and then stored as a solution during the use for a period of up to 14 days at 4°C, during which some degradation will take place.

Furthermore, the process of reconstitution of the lyophilized 20 growth hormone tends to provide difficulties for the patient.

Thus, it is at present preferred to reconstitute the growth hormone as late as possible before use and to store and ship the preparation in a lyophilized state. The chain from the manufacturer to the pharmacy is apt for handling the prepara- 25 tions at a controlled low temperature of e.g. 4°C which allows for a long shelf life of up to two years.

However, the extended use of pen systems for self-medication and the expanded field of use calls for a preparation which is stable for a sufficient long time with the end user under

conditions where "sufficient" cooling is not always available.

Preferably, a preparation should be stable with the end user in a lyophilized state for about one month and additionally  
5 for one month in a reconstituted state in a pen device for the intended period of use of a cartridge.

Thus, there is a need for more stable preparations of growth hormone being stable in a lyophilized state at a relative high temperature for a period and additionally for a period  
10 of use at a relatively high temperature in solution. Such stabilization is of very great importance when moving the administration of the growth hormone from clinics to the homes of the individuals to be treated where optimal storage may not be available as indicated above.

15 Furthermore, the shift in pattern of administration of growth hormone to the use of pen devices calls for a stable dissolved preparation comprising growth hormone in order to facilitate the handling to be performed by the patient. A stable dissolved preparation comprising growth hormone may be pro-  
20 duced ready to use in the form of cartridges fitting into the pen device used by the patient who may then avoid the reconstitution of the preparation and, hence, will not have to be in the possession of a lyophilized preparation, a suitable vehicle for reconstitution as well as the necessary skill and  
25 sterile equipment for sterile reconstitution of the preparation.

For safety reasons it will also be desirable to avoid the reconstitution of a lyophilized preparation just before the use of the preparation.

30 Furthermore, it would also be an advantage to avoid the lyophilization step in the production of growth hormone preparations. Lyophilization is a time consuming and costly process

and is also often a "bottleneck" in the production due to the limited capacity of the freeze drier.

Thus, there is a need to reduce the rate of the degradation processes in order to allow for dissolved hGH preparations being stable during shelf life and during the period of use of up to one month.

Prior attempts to stabilize hGH has not fully succeeded in preventing the formation of dimer. The problems associated with dimer formation is e.g noted in Becker, G.W., Biotechnology and Applied Biochemistry 9, 478 (1987).

International Patent Publication No. WO 89/09614 and Australian patent application No. 30771/89 disclose a stable pharmaceutical formulation containing human growth hormone, glycine, and mannitol. Such a preparation shows improved stability during normal processing and storage in a lyophilized state as well as in the period of use after the reconstitution.

Published European patent application No. 303 746 discloses that animal growth hormone may be stabilized with various stabilizers to give decreased formation of insolubles and preservation of the soluble activity in aqueous environments, such stabilizers including certain polyols, amino acids, polymers of amino acids having a charged side group at physiological pH, and choline salts. Polyols are selected from the group consisting of non-reducing sugars, sugar alcohols, sugar acids, pentaerythritol, lactose, water-soluble dextrans and Ficoll; amino acids are selected from the group consisting of glycine, sarcosine, lysine or salts thereof, serine, arginine or salts thereof, betaine, N,N,-dimethyl-glycine, aspartic acid or salts thereof, glutamic acid or salts thereof; a polymer of an amino acid having a charged side group at physiological pH may be selected from polylysine, polyaspartic acid, polyglutamic acid, polyarginine, polyhistidine, polyor-

nithine and salts thereof; and choline derivatives are selected from the group consisting of choline chloride, choline dihydrogen citrate, choline bitartrate, choline bicarbonate, tricholine citrate, choline ascorbate, choline borate, choline gluconate, choline phosphate, di(choline)sulphate and di-choline mucate.

#### BRIEF DESCRIPTION OF THE INVENTION

It has now surprisingly been found that a preparation of human growth hormone comprising asparagin as additive or buffering substance shows a very high stability against deamidation, oxidation and cleavage of peptide bonds. The stability of the product allows for the storing and shipment thereof in a lyophilized state or in the form of a dissolved or re-dissolved preparation.

Thus, the preparation of the invention may be in the form of a lyophilized powder to be reconstituted later using conventional vehicles such as distilled water or water for injection or in the form of a solution comprising growth hormone. Such vehicles may comprise conventional preservatives such as m-cresol and benzyl alcohol.

A preferred embodiment of the invention is in the form of a pharmaceutical preparation of human growth hormone comprising asparagine in the form of a buffered aqueous solution of growth hormone buffered with asparagine buffer. Such preparation is in a ready-to-use form and may be stored and shipped as an aqueous solution without any considerable degradation.

For stability reasons the pH of a solution is preferably adjusted to a value in the interval from 2-8. Preparations having a pH from 5 to 8 are more preferred and most preferred are preparations having a pH from 6 to 7.5.

In order to obtain the stabilizing effect asparagine is preferably added in an amount of up to 100 mM. The concentration of asparagine is preferably on the interval from 2 to 20 mM, most preferred from 2 to 10 mM

5 The pharmaceutical preparation of the invention may also be in the form of a lyophilized preparation.

The pharmaceutical preparation of the invention may furthermore comprise salts and saccharides in order to facilitate the processing thereof, e.g. lyophilization. Conventionally  
10 mannitol is used as a bulking agent for lyophilization.

Still another aspect of the invention relates to the use of asparagine for the preparation of a stabilized preparation of growth hormone.

In the present context "growth hormone" may be growth hormone  
15 from any origin such as avian, bovine, equine, human, ovine, porcine, salmon, trout or tuna growth hormone, preferably bovine, human or porcine growth hormone, human growth hormone being most preferred. The growth hormone used in accordance  
20 a natural source, e.g. by extracting pituitary glands in a conventional manner, or a growth hormone produced by recombinant techniques, e.g. as described in E.B. Jensen and S. Carlsen in *biotech and Bioeng.* 36, 1-11 (1990). The "growth hormone" may also be a truncated form of growth hormone  
25 wherein one or more amino acid residues has (have) been deleted; an analogue thereof wherein one or more amino acid residues in the native molecule has (have) been substituted by another amino acid residue, preferably a natural amino acid residue, as long as the substitution does not have any  
30 adverse effect such as antigenicity or reduced action; or a derivative thereof, e.g. having an N- or C-terminal extension such as Met-hGH, Met-Glu-Ala-Glu-hGH or Ala-Glu-hGH. The preferred growth hormone is hGH.

In the present context "high stability" is obtained when the preparation is more stable than the conventional formulation comprising phosphate buffer.

The term "salts" used in this specification to designate additional constituents of a pharmaceutical preparation comprises conventional such salts such as alkaline metal, alkaline earth metal or ammonium salts of organic acids such as citric acid, tartaric acid, or acetic acid, e.g. sodium citrate, sodium tartrate or sodium acetate, or of mineral acids such as hydrochloric acid, e.g. sodium chloride.

"Saccharides" may be monosaccharides, disaccharides or sugar alcohols which are conventionally used for pharmaceutical preparations such as lactose and mannitol.

The solvent used in the preparations of the invention may be water, alcohols such as ethyl, n-propyl or isopropyl, butyl alcohol or mixtures thereof. The solvent may comprise a preservative such as m-cresol or benzyl alcohol.

#### DETAILED DESCRIPTION OF THE INVENTION

The invention is explained more in detail in the below Examples which illustrate the invention. They are not to be considered as limiting the scope of the invention being defined by the appended claims.

#### EXPERIMENTAL PART

##### EXAMPLE

25 Reduction of the deamidation.

The rate of deamidation was examined at 25°C for hGH preparations comprising 6 IU hGH at pH 6.5 in the presence of 10 mM Asn as compared to 8 mM phosphate buffer at the same pH and pH 7.3.

5 The hGH preparations were prepared by dissolving 20 mg hGH in 10 ml of 10 mM asparagine solution prepared by dissolving 13.2 mg, of asparagine in 10 ml deionized water containing 0.9% (v/v) of benzyl alcohol and adding 0.1 N hydrochloric acid to the stated pH.

10 The hGH formulations stated in the below Table were stored at 25°C and analyzed for the desamido contents after 14 and 30 days by IE-HPLC. The results appear from the below Table.

## Table.

Contents of desamido hGH as determined by IE-HPLC as a function of the formulation and the time in solution at 25°C:

5	Formulation (*)	Formation of desamido compound at 25°C	
		14 days (')	30 days
	8 mM di-Na-Phosfat pH 6.5	7.8	10.8
10	8 mM di-Na-Phosfat pH 7.3	15.2	20.3
	8 mM di-Na-phosfat pH 6.5, 0.3% m-cresol	9.4	13.2
15	10 mM Asp, pH 6.5	21.7	nd
	10 mM Asn, pH 6.5	6.5	8.3
	10 mM Glu, pH 6.5	14.8	nd

\*: Comprises 0.9% benzyl alcohol except formulation # 3.  
The contents of desamido-hGH in starting material was: 2.1 %

20 From the above Table it appears that the de-amidation of hGH is reduced by approximately 25% by the addition of asparagine as compared with phosphate buffer at pH 6.5.

Addition of Asp or Glu increases the rate of deamidation as compare to phosphate at pH 6.5.

25 The above results show that the rate of de-amidation is reduced by lowering the pH and by adding asparagine in a low con-

centration of up to 100 mM, preferably about 5mM. The rate of de-amidation may be reduced by more than 50% by lowering the pH and substituting the phosphate buffer with asparagine.

The use of m-cresol or benzyl alcohol as preservative seems to have no influence on the rate of de-amidation.

Split-formation (hydrolysis of peptide bonds) is reduced by asparagine at pH 6.5 in comparison with phosphate.

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**CLAIMS**

1. A pharmaceutical preparation comprising a growth hormone and asparagine.
2. A pharmaceutical preparation as claimed in claim 1 in the form of a buffered aqueous solution of growth hormone containing asparagine.
3. A pharmaceutical preparation as claimed in claim 1 or 2 wherein the pH is adjusted to a value in the interval 2-8.
4. A pharmaceutical preparation as claimed in claim any of the preceding claims wherein the concentration of asparagine is up to 100 mM.
5. A pharmaceutical preparation as claimed in claim any of the preceding claims further comprising salts and saccharides.
6. A pharmaceutical preparation as claimed in any of claims 1-6 wherein the growth hormone is hGH.
7. Use of asparagine for the preparation of a stabilized preparation of growth hormone.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 92/00378

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: A61K 37/36

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, WPI, BIOSIS, CLAIMS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A2, 0374120 (MONSANTO COMPANY), 20 June 1990 (20.06.90), whole document, especially claim 16  --	1-7
X	US, A, 4816568 (HAMILTON, JR. ET AL), 28 March 1989 (28.03.89), column 2, line 67 - column 3, line 16; column 4, line 36 - line 44, claims 17,27  --	1-7
X	US, A, 4917685 (VISWANATHAN ET AL), 17 April 1990 (17.04.90)  -- -----	1-7

 Further documents are listed in the continuation of Box C.
  See patent family annex.

\* Special categories of cited documents:

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**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

26/02/93

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0374120	20/06/90	AU-A- 4617689 CA-A- 2005226 JP-A- 2204418	21/06/90 13/06/90 14/08/90
US-A- 4816568	28/03/89	NONE	
US-A- 4917685	17/04/90	NONE	