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The Second International Standard for Somatropin (Recombinant DNA-derived Human Growth Hormone): Preparation and Calibration in an International Collaborative Study

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Abstract. A preparation of somatropin (recombinant DNA-derived human growth hormone) was prepared as lyophilised ampoules according to WHO procedures for international biological standards. The candidate preparation (98/574) was evaluated in an international collaborative study (16 laboratories, nine countries), with the following aims: (i) to determine the suitability of the preparation to serve as the International Standard for somatropin by studying its performance in the current range of physico-chemical and biological assay methods employed for somatropin; (ii) to assign a content in terms of the existing (first) International Standard for somatropin, using the currently recognised assay procedure (Size Exclusion High Performance Liquid Chromatography, SE HPLC); (iii) to confirm the specific biological activity of the candidate preparation; (iv) to confirm the stability of the candidate preparation.

On the basis of the collaborative study WHO agreed that: the preparation in ampoules coded 98/574 is suitable to serve as the next WHO International Standard for somatropin; the preparation in ampoules coded 98/574 should be established as the second International Standard for somatropin, with a defined ampoule content of 1·95 mg total somatropin plus somatropin-related proteins per ampoule; the specific activity of the preparation should be defined as 3·0 IU/mg somatropin.

Introduction

Human growth hormone is a polypeptide growth-promoting hormone of the anterior pituitary gland.[1] Somatropin is the purified, recombinant DNA-derived preparation of the major 191 amino acid residue protein present in preparations of pituitary growth hormone, which, since 1984 has become widely used in the treatment of growth-hormone deficiency in both children and adults.

The World Health Organization has provided international biological standards for growth hormone since 1955,[2] which have supported biological assays for therapeutic preparations of pituitary growth hormone, immunoassays used in clinical measurement and diagnosis, and most recently, physico-chemical assays used in control of the recombinant somatropin.[3] Establishment of the first International Standard for somatropin coincided with an international consensus to replace the bioassays with physico-chemical analytical methods for the routine batch release of somatropin, and an international agreement that the specific activity of pure somatropin monomer is an intrinsic property of the molecule and should be considered to be 3·0 IU/mg.

The first World Health Organization International Standard for somatropin (recombinant DNA growth hormone), 88/624, was therefore established in 1994,[4] with a dual definition of content, in both protein content (mg) and specific activity (3·0 IU/mg), reflecting the international consensus.

The first International Standard for somatropin has become exhausted. The present report describes evaluation and calibration of a candidate replacement preparation, and its formal adoption and

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establishment by WHO as the second International Standard for somatropin.

Materials and methods

The candidate second International Standard for somatropin

Bulk material. Bulk somatropin, prepared by recombinant DNA technology in *Escherchia coli*, was generously donated by Novo Nordisk. The preparation was supplied with a certificate of analysis showing the material to be consistent with current pharmaceutical grades of somatropin.

Ampoules. The candidate second International Standard for somatropin consists of a batch of 10 000 ampoules coded 98/574, containing lyophilised somatropin plus excipients. In order to maintain similarity with the existing International Standard and also with other local and pharmacopoeial standards, the candidate standard was prepared such that each ampoule contains the lyophilized residue of 1 ml of a solution, which contained per ml: 2 mg somatropin, 20 mg glycine; 2 mg mannitol, 2 mg lactose, 2·5 mg sodium bicarbonate (pH 7·3).

Ampoules were prepared according to procedures used for the preparation of WHO International Standards. Briefly, bulk somatropin (20 g) was dissolved in carrier solution to give a 10 × concentrate. This was filtered through a 0·45 μm filter, diluted to a final volume of 10 l with carrier solution, and distributed in 10 000 × 1 ml aliquots into neutral glass ampoules. After lyophilization the ampoules were sealed under nitrogen by heat fusion.

For the fill 98/574, the recorded parameters were: mean solution weight: 1·0088 g/ampoule; relative standard deviation (liquid content): 0·07%, residual moisture: 0·66%; relative standard deviation (moisture content) 6·5%.

International collaborative study. An international collaborative study was organized to determine and confirm: (i) *Potency* [assay against the current international standard (88/624) by methods based on size exclusion HPLC]. (ii) *Purity* (percentage of somatropin-related proteins determined by reverse-phase HPLC, and the percentage of aggregates and dimers determined by SE HPLC). (iii) *Biological activity* (biological assay based either on growth promotion *in vivo* or cell proliferation *in vitro*). (iv) *Identity* (physico-chemical identity tests using the techniques of peptide mapping, iso-electric focusing and capillary zone electrophoresis). (v) *Stability* (physico-chemical stability of the candidate standard by analysis of thermally degraded samples).

Participants in the collaborative study were: Dr Emmanuelle Charton (European Department for the Quality of Medicines, Strasbourg, France); Dr Akihiro Furukawa (Sumitomo Pharmaceuticals Co. Ltd, Osaka, Japan); Masafumi Tatsumi, Mitsuhiro Kawaguchi and Jun-Ichi Kajihara (JCR Pharmaceuticals Co. Ltd, Kobe, Japan), Dr Anne Meunier (Genentech Inc. San Francisco, U.S.A.); Dr Takao Hayakawa (National Institute of Health Sciences, Japan); SJ Facility, Japan; Jeewon Joung (Korea Food and Drugs Administration, Seoul, South Korea); Dr Paula K. Davis (Eli Lilly, Indianapolis); Dr Alain Bayol, P. Jeannin and B. Viard Bouleuc (Sanofi Recherche, Labege, France); Atsuya Ogura (Novo Nordisk Pharma Ltd, Tokyo, Japan); S. Birnbaum (Pharmacia & Uphohn, Sweden); Anne Munk Jespersen (Novo Nordisk, Denmark); D. Smythe, (TGA, Woden, Australia); E. Pithon (Laboratoire Serono, Switzerland); Chikako Yamota (National Institute of Health Sciences (Osaka Branch), Osaka, Japan); Dr M. Girard (Bureau of Biologics and Radiopharmaceuticals Ottawa (Ontario) Canada).

Throughout this report, participating laboratories are referred to by code number. The assignation of code numbers is random, and does not reflect the order of listing above.

Methods

Details of analytical methods are summarized in Table 1.

Full details and raw data for all individual physico-chemical and biological assays were reported but are not reproduced in this report.

Size exclusion (SE) HPLC, reverse-phase (RP) HPLC, gel iso-electric focusing and peptide mapping were either as described in the European Pharmacopoeia monograph for somatropin or were validated modifications.[5] Relevant specific details, where they were supplied, including column specifications, are given in the specific results tables.

Full details of unpublished analytical procedures (ion-exchange and hydrophobic interaction HPLC, capillary zone electrophoresis) were supplied, but are not reproduced in this report as they do not contribute quantitative data to potency estimates.

In vivo biological assays, based either on weight gain or tibial width increase in hypophysectomized

Table 1. Methods contributed to the study

Method	Contributing laboratories	Details
SE HPLC (assay)	1, 3, 4, 5, 6, 7, 9, 8, 10, 11, 12, 13, 14, 15, 16	
SE HPLC (purity test)	1, 2, 3, 4, 5, 6, 7, 9, 8, 10, 11, 12, 13, 14, 15, 16	
RP HPLC	1, 2, 3, 4, 5, 6, 7, 9, 8, 10, 11, 12, 13, 14, 15, 16	
Ion-exchange HPLC	3, 12	
Peptide mapping	1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16	
In vivo bioassay	7	Tibial width
	11, 12, 13	Weight gain
In vitro bioassay	2, 16	Cell proliferation in FDC-P1 cells
Iso-electric focusing	1, 2, 3, 4, 5, 7, 13, 14, 15	
UV spectroscopy	3	
Capillary zone electrophoresis	9, 15	
Hydrophobic interaction HPLC	15	

rats, were as described in the European Pharmacopoeia.[6]

In vitro biological assay based on proliferation of FDC-P1 cells, was as described in the draft United States Pharmacopoeia monograph for somatropin.[7]

Results and discussion

The candidate second IS for somatropin (98/574): determination of content

In most pharmaceutical licensing regions, SE HPLC is now accepted as the assay for somatropin. Assays of the candidate standard, 98/574, in terms of the current international standard, 88/624, are summarized in Table 2.

In seeking to calibrate the candidate standard, 98/574 in terms of the current international standard, 88/624, it should be emphasized that the assignation of content of 88/624 was done on the basis of amino acid analysis. Thus the formally assigned content of 88/624, 2·0 mg/vial, represents the sum of all proteins, i.e. the total of somatropin plus somatropin-related impurities. In defining the standard in this way, it was recognized that the content of somatropin monomer would: (i) depend on the assay method used, and (ii) possibly change with time.

The specific content of somatropin monomer was therefore *not* defined in the first International Standard. It is intended that this approach should be continued with the second standard, and Table 2 therefore shows assays of total somatropin plus somatropin related proteins (all peaks) in 98/574 against the total somatropin plus somatropin related proteins (all peaks) in 88/624, assuming the latter to be 2·0 mg/ampoule.

Estimates were generally in good agreement with each other, even where differing methods of analysis had been employed. The overall mean estimate of 1·93 mg/ampoule (in-house methods excluded) had a relative standard deviation of 2·65%, where 11/16 provided estimates within 2·0% of this value. However when viewing the distribution of the SE HPLC assay results (Fig. 1), a consensus was made on assigning a value of 1·95 mg/ampoule, then 12/16 laboratories provided estimates within 2·0% of this value.

It should also be noted that, whilst the assignment of content is traceable to the amino acid analysis carried out on the first International Standard for somatropin, the present study uses quantitative HPLC rather than amino acid analysis, and calibrates the second IS in terms of the first using the comparative technique.[8] This approach takes advantage of the greater precision offered by the chromatographic procedure, avoiding a possible discontinuity which might arise from attempts to re-calibrate the second IS by amino acid analysis.

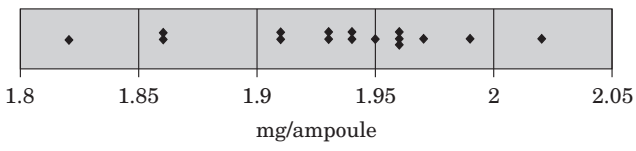

Figure 1. Distribution of SE HPLC assay results.

Table 2. SE HPLC assay of 98/574: estimates of total somatropin plus somatropin related proteins (in terms of 88/624, assuming total somatropin plus somatropin related proteins for 88/624=2·0 mg). Specific method details are given in Tables 3 and 4. Mean of laboratory means 1·932 mg/ampoule; standard deviation 0·051; relative standard deviation 2·65%

Laboratory	98/574 (mg/ampoule)	Laboratory mean	Comments
1	1·84 1·88	1·86	Each assay value based on mean of five determinations of both test and standard.
2	1·93 1·95	1·94	Each assay value based on mean of two determinations of both test and standard.
3a	1·92 1·85	1·91	Each assay value based on mean of two determinations of both test and standard.
3b	1·95 1·88		
4	1·98 1·94	1·96	Each standard=mean of five determinations, each test=mean of two.
5	1·90 1·95	1·93	
6	2·02 2·01	2·02	
7	1·96	1·96	Mean of five determinations (standard deviation=0·0041).
8	1·96 1·96	1·96	
9	1·83 1·80	1·82	
10	1·95 1·93 1·96	1·95	Data obtained by regression analysis using three concentrations each of test and standard.
11	1·88 1·93	1·91	
12	1·93 1·93 1·95	1·94	Each estimate is the mean of three determinations on one separate ampoule.
13a	1·96		EP SEC method.
	2·01	1·99	
13b	1·96 2·08	(2·02)	In-house SEC method.
14	1·94 1·92	1·93	
15	1·95 1·99	1·97	Each estimate is the mean of three determinations on one separate ampule.
16a		1·86	European Pharmacopoeia method.
16b		(1·84)	In-house method (means of duplicate determinations).

The first, and (candidate) second International Standards for somatropin: estimates of purity by SE HPLC

Estimates of purity by SE HPLC are shown in Tables 3 and 4. The candidate standard, 98/574, is of a higher purity than the earlier standard, with estimates of dimer ranging from 0·2–1·9% and estimates of aggregate from 0–2·5%. For the earlier standard these impurities were in the ranges 0·1–4·3% and 0–2·6% respectively. It is interesting that these data do confirm the general observation that estimates of purity are considerably more variable than estimates of total protein derived from the same data set. The results are clearly method-dependent, as shown, for example, by method 3b, which runs in detergent and therefore appears to underestimate the degree of aggregation, presumably by dissociation of the complexes. Similarly,

Table 3. 98/574: SE HPLC estimates of purity

Laboratory	Monomer (%)	Dimer (%)	Aggregate (%)	Comments
1	98·87	0·68	0·45	Each estimate is the mean of six injections on separate
	98·89	0·63	0·48	ampoules. TSK G2000SW, EP 2000 mobile phase.
2	95·57	1·89	2·54	Each estimate is the mean of two injections on separate
	95·85	1·72	2·43	ampoules. Column not specified, mobile phase=25 mM sodium bicarbonate.
3a	99·3	0·7	0	EP 2000 method.
	99·4	0·6	0	
3b	99·7	0·3	0	In-house method (G2000SWXL column, mobile
	99·7	0·3	0	phase=phosphate/SDS/Tween (full details supplied).
4	99·24	0·67	0·09	EP2000 method. Means of duplicate estimates.
	99·20	0·71	0·09	
5	98·15	0·87	0·98	G2000SWXL, 50 mM ammonium bicarbonate.
	98·08	0·84	1·08	
6	98·95	1·05	<0·01	EP2000 method. Means of triplicate estimations.
	98·99	1·01	<0·01	
7	99·37	0·63	Not detected	G3000SWXL, 50 mM ammonium bicarbonate. Mean of five determinations.
8	99·28	0·60	0·18	EP2000 method. Means of duplicate determinations.
	99·28	0·60	0·18	
9	99·26	0·74	(Dimer+agg)	TSKG2000SWXL. EP2000 mobile phase.
	99·26	0·74		
10	96·86	1·24	1·91	Bio-sil SEC-125. 0·395% ammonium bicarbonate as mobile
	98·31	0·84	0·86	phase.
	97·98	0·86	1·17	
11	98·8	0·2	0	EP2000 method.
	99·1	0·5	0·4	
12	98·71	1·29		EP 2000 method.
	98·65	1·35	(Dimer+agg)	
	98·61	1·39		
13a	98·7	0·7	0·5	
	98·8	0·7	0·5	
13b	97·5	1·2	1·2	
	98·0	1·1	0·8	
14	97·76	1·23	1·01	Zorbax G250. EP2000 method.
	98·54	0·96	0·5	
15	99·21	0·79	nd	EP method. TSK 3000 column. Each value is the mean of
	99·23	0·77	nd	three determinations on an independent ampoule
16a	97·03	1·18	1·79	EP method.
	97·29	1·13	1·58	
16b	96·85	1·06	2·13	In-house method.
	97·11	1·10	1·79	

method 2, which runs at a lower ionic strength than other methods, gave apparently higher values for both standards. It is also clear however, that even comparing results from apparently identical procedures, there is a greater spread of results than for total protein. For example, for 98/574, estimates of dimer obtained using the EP method ranged from 0·2% (laboratory 11) to 1·2% (laboratory 14).

In summary estimates of purity by SE HPLC suggest: (i) the candidate standard, 98/574, is significantly purer than the previous standard, with less than half the level of high molecular weight impurities, and, (ii) assignation of content in terms of total somatropin peptides rather than the specific monomer content remains the most appropriate course of action.

Table 4. 88/624: SE HPLC estimates of purity

Laboratory	Monomer (%)	Dimer (%)	Aggregate (%)	Comments
1	95·69	3·1	1·21	Each estimate is the mean of six injections on separate ampoules. TSK G2000SW, EP 2000 mobile phase.
	96·1	2·99	0·91	
2	94·58	3·99	1·43	Each estimate is the mean of two injections on separate ampoules. Column not specified, mobile phase=25 mM sodium bicarbonate.
	94·6	4·26	1·14	
3a	97·0	1·9	1·1	EP 2000 method.
	97·6	1·7	1·6	
3b	99·9	0·1	0	In-house method (G2000SWXL column, mobile phase=phosphate/SDS/Tween (full details supplied)
	99·8	0·2	0	
4	97·15	1·90	0·95	EP2000 method. Means of duplicate estimates.
	97·35	1·85	0·8	
5	96·29	2·22	1·49	G2000SWXL, 50 mM ammonium bicarbonate.
	96·27	2·07	1·66	
6	94·99	2·41	2·60	EP2000 method. Means of triplicate estimations.
	94·96	2·41	2·63	
	94·98	2·41	2·61	
7	97·91	2·01		G3000SWXL, 50 mM ammonium bicarbonate. Dimer+aggregate reported as total HMW forms
8	97·5	2·5		EP2000 method. Dimer+aggregate reported as total HMW forms.
9	97·52	2·48		TSKG2000SWXL. EP2000 mobile phase. Dimer+aggregate reported as total HMW forms.
	97·54	2·46		
10	97·12	1·91	0·96	Bio-sil SEC-125. 0·395% ammonium bicarbonate as mobile phase.
	97·35	1·56	1·10	
	96·39	2·20	1·41	
11	98·8	1·0	0·2	EP2000 method.
	98·3	1·3	0·2	
12	96·26	3·74		
	96·51	3·49		
	96·70	3·3		
13a	Not reported			
14	97·31	1·97	0·73	
	96·33	1·97	1·70	
15	97·38	1·79	0·83	Mean of two or three determinations.
	97·52	1·75	0·73	
16a	95·90	2·08	2·03	EP method.
	95·28	2·27	2·44	
16b	95·51	2·07	2·42	In-house method.
	94·74	2·27	2·99	

Estimates of purity by reverse-phase/ion-exchange/hydrophobic interaction high-performance liquid chromatography/capillary zone electrophoresis. Somatropin-related impurities in the candidate standard, 98/574, determined by RP or ion exchange (IE) HPLC, are shown in Table 5. The pharmacopoeial procedure, procedure based on RP HPLC using a C4 column, with neutral Tris-HCl and propan-1-ol as the organic modifier has largely become the RP method of choice for somatropin. Nonetheless, the method is capable of giving significant variations in performance, with measured levels of impurity ranging from <0·2% (laboratory 6) to some 2% (laboratory 12a). It was also notable that the capability of the method of resolv-ing and quantifying desamido somatropin was rather variable between laboratories. Overall however, the data suggest that 98/574 contains some 1–3% total monomeric impurities, comprising mostly desamido somatropin and the methionine sulphoxide form, of which the latter is present in somewhat higher levels. It may be concluded that the candidate standard 98/574 complies

Table 5. 98/574: determination of related proteins: by RP HPLC, IE HPLC and capillary zone electrophoresis (RP HPLC unless otherwise stated)

Laboratory	Somatropin (%)	Impurities (%)	Comments
1	99·70	0·22	EP 2000 method. Two additional impurities identified but not quantified.
	99·72	0·59 0·22	
2	99·47	0·53	''Neutral pH RP-HPLC''.
	99·39	0·61	
3a	98·7	1·3	RP HPLC method.
	98·8	1·2	
3b	100	0	IEX HPLC (TSK DEAE 3SW, details supplied). Detected
	100	0	Impurity identified as desamido-GH.
4	98·33	1·67	Measured impurity is the sum of six (run 1) or five (run 2)
	98·33	1·67	impurities ranging from 0·9 to 0·002%.
5	99·01	0·99	Vydac 214-TP54. Neutral Tris-propan-l-ol.
	98·40	1·60	
6	>99·8	<0·02	EP 2000 method.
	>99·8	<0·02	
7	98·868	2·132	In-house method similar to EP. Estimates are means of five runs,
	(0·183)	(0·183)	(SDs in parenthesis).
8	98·82	1·18	EP method.
	98·97	1·03	
9	98·35	1·65	Supelco LC-304 column. EP run conditions.
	98·27	1·83	
	98·62	1·38	Capillary zone electrophoresis. Impurity identified as
	98·71	1·29	desamido-somatropin.
10	98·63	0·92	EP method. Impurity peaks identified as sulphoxide and
	98·03	0·66 1·22	desamido, respectively.
11	98·9	1·1	
	99·3	0·8	
12a	98·1	1·9	RP HPLC. Impurity identified as sulphoxide.
	97·9	2·1	
12b	99·0	1·0	IE HPLC. Impurity identified as desamido-GH.
	99·1	0·9	
13	99·9	0·1	EP method.
	98·1	1·9	
	98·5	1·5	In-house method.
	97·5	2·5	
14	98·45	1·55	EP method.
	99·30	0·70	
15	97·43	2·57	Method corresponding to EP, and validated.
	98·70	1·30	
15	100	0	Hydrophobic interaction HPLC. Method identifies 5–6%
	100	0	impurities (trisulphide) in 88/624.
15	99·16	0·84	Capillary zone electrophoresis.
	99·01	0·99	
16	96·45	0·453[1] 2·05[2]	Impurities identified as [1]sulphoxide, [2]desamido.
	96·59	0·40 2·02	

with the current specifications for therapeutic somatropin.

The single laboratory performing hydrophobic interaction HPLC suggested a qualitative difference between the first and candidate second International Standards, with the former containing 5–6% of an impurity identified as the trisulphide, which is entirely absent in the candidate second International Standard.

Estimates of biological activity. Assuming the content of 98/574 to be 1·95 mg/ampoule (minus about

Table 6. 98/574: Biological assay estimates of content in International Units. Unweighted mean of laboratory means: 5·8 IU/ampoule

Laboratory	Assay method	Individual estimates, IU/ampoule (fiducial limits)	Combined estimates, IU/ampoule (fiducial limits)
2	*In vitro* cell proliferation*		104·57 (RSD=8·37%) 102·88 (RSD=3·48%)
7	*In vivo* tibial width	5·87 5·85 6·02	5·91 (SD=0·09)
11	*In vivo* weight gain		5·2 (4·5–6·0)
12	*In vivo* weight gain	5·0 (4·0–6·2) 5·2 (4·5–6·1)	
13	*In vivo* weight gain	7·5 (5·8–9·78) 5·6 (4·4–7·0)	6·4 (5·4–7·5)
16	*In vitro* cell proliferation**	5·62 6·92 5·74	6·09 (RSD=11·6%)

*Results expressed as percentages relative to 88/624, each estimate is the mean of 6 determinations.

**Results presented as IU/mg. Recalculated as IU/ampoule assuming 2 mg/ampoule.

1% aggregates), and the specific activity of somatropin monomer to be 3·0 IU/mg, estimates of biological activity should be in the region of 5·8 IU/ampoule. Six bioassay estimates were contributed to the study, comprising three *in vivo* weight gain assay estimates, one *in vivo* tibial width estimate and two *in vitro* cell proliferation estimates (Table 6). All estimates were consistent with the internationally agreed potency of 3·0 IU/mg, indeed, the overall mean of all estimates (5·8 IU/ampoule) was almost exactly the predicted value.

Physico-chemical identity tests. In addition to the quantitative tests reported above, most participants performed the additional physico-chemical identity tests described in the European Pharmacopoeia; the HPLC tryptic map and gel iso-electric focusing.

All participants reported that the tryptic maps obtained from the candidate standard, 98/574, and the current international standard, 88/624, were essentially identical. One participant (laboratory 7) assigned sequence identity to each of the resolved peptides, further confirming the primary sequence of the test material.

Participants reporting results of iso-electric focusing conformed the candidate standard to behave essentially identically to the current standard, and to comply with the test specifications.

Stability testing. In order to assess the stability of the candidate International Standard, ampoules were stored at temperatures between −20°C and +45°C for a period of 180 days. Analysis of these materials was performed by SE HPLC and by RP HPLC. Results are summarized in Table 7.

Loss of activity after storage at elevated temperatures forms the basis of the accelerated degradation test, which is used to predict the stability of WHO International Standards. In this analytical procedure, the rate of loss of activity is related to temperature using the Arrhenius equation, which can then be used to predict the long-term stability on storage at lower temperatures.[9]

For the candidate standard, 98/574, the relative proportions of monomer to dimer/aggregate in the candidate standard were very stable with respect to accelerated degradation. Only the +45°C storage temperature caused any significant loss of monomer, and the data cannot be used to predict loss of activity at lower temperatures, as there was insufficient loss of activity at temperatures between 4 and 37°C to produce a significant Arrhenius equation slope. It can only be concluded that at temperatures below 37°C the percentage of somatropin monomer in the candidate standard and thereby the biological activity appears to be stable.

Elevated temperature does, however, significantly degrade monomeric somatropin into forms resolvable by RP HPLC, six months at 37 or 45°C causing 36% and 69% loss of purity respectively. Analysis of the loss of RP HPLC main peak with

Table 7. 98/574: HPLC analysis of thermally degraded ampoules

Storage temp.	SE HPLC			RP HPLC	
	Purity (%)		Assay (somatropin monomer relative to frozen baseline)	Main peak (%)	Total impurities (%)
	Monomer	Aggregates			
Frozen baseline*	97·6	2·4	100·6%	95·23	4·77
− 20°C	97·9	2·1	100·8%	96·43	3·57
+4°C	98·3	1·7	100·8%	95·91	4·09
+20°C	98·2	1·8	101·5%	93·51	6·49
+37°C	97·3	2·7	101·0%	63·48	36·52
+45°C	95·0	4·8**	98·2%	30·96	69·04

*The ampoules described as ''frozen baseline'' are stored continuously at − 150°C without lyophilisation.

**Remaining material appears as an increased tail on the monomer peak.

respect to time using the Arrhenius equation produced the predicted losses of purity shown in Table 8.

Whilst the deamidation and sulphoxidation of the somatropin monomer was significant and extensive at 20–45°C found by RP HPLC, there was little or no loss at lower temperatures, producing an Arrhenius plot with a very steep slope, and therefore very low (0·017%/year) predicted degradation on storage at − 20°C, the normal storage conditions for International Standards.

It may be concluded therefore that by chromatographic analysis of thermally degraded samples, the candidate standard, 98/574, is sufficiently stable to serve as an International Standard.

Table 8. 98/574: Predicted loss of purity (%/year, RP HPLC)

Storage temperature	Predicted loss in purity (RP HPLC) (%/year)
− 150°C	0
− 70°C	0
− 20°C	0·017
4°C	0·975
20°C	9·54
37°C	59·79

Loss of somatropin (main peak) measured by RP HPLC (Table 7) was used to estimate the first order rate constant for the rate of degredation of somatropin at the temperatures studied. The values were then used to predict, according to the Arrhenius equation, the rates of degradation (%/year) at the temperatures shown.[8]

Conclusions and proposals

On the basis of the data described in this report, it was proposed to the World Health Organization that: (i) the preparation in ampoules coded 98/574 is suitable to serve as the next WHO International Standard for somatropin; (ii) the preparation in ampoules coded 98/574 should be established as the second International Standard for somatropin, with a defined ampoule content of 1·95 mg total somatropin plus somatropin related proteins per ampoule; and (iii) the specific activity of the preparation should be defined as 3·0 IU/mg somatropin.

Based on these recommendations, the Expert Committee on Biological Standardization of the World Health Organization formally adopted the preparations as the second International Standard for somatropin. The material is available on request from the National Institute for Biological Standards and Control.

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