

Peptide Factors as Pharmaceuticals: Criteria for Application

E. WÜNSCH, *Max-Planck-Institut für Biochemie, Abteilung Peptidchemie, 8033 Martinsried, Federal Republic of Germany*

Synopsis

The increasing interest in the pharmaceutical use of peptide factors in human medicine presents formidable challenges for peptide chemistry. Fully reproducible multistage syntheses with a definite assessment of the degree of purity represent the crucial premise for the introduction of peptide factors as pharmaceuticals. Extensive studies of the stability of peptidic material on storage allows identification of the most suitable form of administration. Nevertheless, the high clearance rate of peptides as pharmaceuticals presents new challenges for improvement by structural modification of resistance to enzyme degradation without creating new problems related to metabolites.

INTRODUCTION

After years of stagnation, peptide factors are regaining great importance as powerful pharmaceutical agents both in diagnosis and in therapy. From structure–function studies of a series of biologically active peptides, it is known that even slight structural changes can provoke drastic modifications both in the potency and in the activity profile. Therefore, the pharmaceutical use of peptides strongly implies an unequivocal control of their state of purity and extensive studies of their stability on storage and of their form of application.

One main difficulty in the control of purity in the case of peptide factors arises from the fact that no reference substance characterized by 100% purity is available. Additionally, depending on the production method, i.e., by isolation from natural sources, chemical synthesis, semisynthesis or gentechnological procedures, the resulting peptide preparations may contain side products or contaminants of a completely different nature. Thus, to qualify such peptide preparations as of high purity—a quality indispensable for their pharmaceutical use—a large spectrum of analytical tests of differentiated specificity has to be performed. Nevertheless, a final judgment will only be possible on the basis of the greatest probability and this only until a new analytical method may demonstrate the opposite. However, standardized procedures, both in the isolation of the natural products and in the different synthesis strategies, may simplify analytical control of peptide preparations once the most efficient and significant assays have been specifically elaborated.

CRITERIA OF PURITY

This analytical process and the related critical aspects are exemplified on synthetic somatostatin-14, a peptide factor¹ that in a short time has become an important pharmaceutical with several different therapeutical applications.²

As already traditional, the material obtained by chemical synthesis³ in the first instance was analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). This was followed by amino acid analyses, both qualitative and quantitative, of the acid and aminopeptidase-M hydrolysates with concomitant determination of the peptide contents and of the recoveries from the enzymatic digests. In this context, it is noteworthy that different conditions of hydrolysis may lead to different results. Thus, the most indicative conditions of hydrolysis have to be experimentally determined by comparative studies for any new peptide preparation. In the case of somatostatin-14, the amino acid analysis of the aminopeptidase-M digest was found to produce more significant values than the analysis of the acid hydrolysate, even if performed under different standardized conditions.⁴ Consequently, for an analytical comparison of different somatostatin-14 samples, the acid hydrolysis was performed in the presence of 2.5% thioglycolic acid⁵ to recover tryptophan and in the absence of this additive for reliable evaluation of the serine (Table I); the values for tryptophan and cystine, however, were more precisely determined by analyses of the enzymatic digests, as shown in Table II. Unfortunately, asparagine is partly hydrolyzed in the incubation media, preventing an exact determination of possibly partially desamidated somatostatin-14. Valuable analytical information results from comparison of the values related to peptide content and to recovery from enzymatic digestion (Table III). For most of the somatostatin-14 samples analyzed these values coincide fairly well within the limits of error of this analytical procedure. The limits of error of the quantitative amino acid analysis are at least 2–3%.

TABLE I
Amino Acid Analyses of Acid Hydrolysates^a of Different Somatostatin-14 Samples

	Diamalt-Serono		CuraMed		Clin Midy		Genentech		UCB		Bachem	
	A	B	A	B	A	B	A	B	A	B	A	B
Asp	1.01	1.01	1.02	1.07	1.00	1.19	0.97	1.08	1.01	1.00	1.01	1.14
Thr	1.98	1.98	2.02	1.99	1.98	1.92	1.93	1.93	1.94	1.93	1.98	1.96
Ser	0.96	0.88	0.97	0.85	0.94	0.85	0.93	0.83	0.97	0.88	0.95	0.87
Gly	1.00	1.00	1.01	1.00	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.01
Ala	1.00	1.00	0.99	1.00	1.00	1.02	1.01	1.00	1.00	1.00	1.00	1.01
Phe	2.92	2.91	2.99	3.10	2.90	2.82	2.92	2.91	2.94	2.94	2.96	2.90
Lys	1.96	1.92	2.06	1.96	1.99	1.90	2.02	1.98	1.91	1.93	2.05	1.94
Trp	—	0.92	—	1.00	—	1.03	—	0.94	—	0.96	—	1.04

^a Acid hydrolysis: A, 6M HCl, 24 h, 110°C; B, 6M HCl, 24 h, 110°C; 2.5% thioglycolic acid.

TABLE II
Amino Acid Analyses of Aminopeptidase-M Digests^a of Different Somatostatin-14 Samples

	Diamalt-Serono	CuraMed	Clin Midy	Genentech	UCB	Bachem
Asn + Asp ^b	0.99	1.04	1.10	0.97	0.90	1.07
Thr	1.99	1.99	2.01	1.99	1.96	1.98
Ser	1.02	1.01	1.03	0.99	0.96	1.02
Gly	0.97	1.00	0.99	1.03	0.99	0.97
Ala	0.96	1.00	1.01	1.03	1.01	1.03
(Cys) ₂	0.98	0.97	0.92	0.96	0.96	1.02
Phe	2.93	3.09	3.09	3.07	2.97	3.09
Lys	2.11	2.01	2.04	1.98	2.00	2.04
Trp	1.02	0.92	1.01	0.97	0.97	1.00

^a Aminopeptidase-M digestion: Sigma AP-M, 37°C; 24 h.

^b Aspartic acid is formed by desamidation in the incubation media; the observed values correspond to those obtained with asparagine.

The peptide content determined by amino acid analysis constitutes basic information that is then used for most of the physicochemical, biological, and immunological assays. Therefore, the results of these analyses, even if the methods applied *a priori* possess a higher degree of precision, are characterized by a limit of error that can not be lower than 2–3%.

Additionally, the various analytical methods exhibit different quantitative specificity versus the possibly accompanying substances, impurities etc. An example in this context is given by the differentiated sensitivity of the HPTLC and HPLC of a series of somatostatin-14 samples (Figs. 1 and 2). The Stilamin ampules contain a large amount of mannitol, which is weakly visualized by means of the chlorine test and hardly detected in HPLC because of the low absorption of this alcohol at 280 nm. On the other side, the high degree of purity of the Genentech material, as judged by HPTLC, is not confirmed by the HPLC as shown in Fig. 2. In fact, several well-separated uv-absorbing contaminants are revealed in the elution profile. A quantitative determination of the contaminants by integrating the peak areas gives only approximate values, since their absorption coefficients are unknown. Thus, for our somatostatin-14 preparation the pooled side fractions presenting visible impurities on HPLC and HPTLC

TABLE III
Peptide Contents and Digestion Rates of Different Somatostatin-14 Samples^a

	Diamalt-Serono	CuraMed	Clin Midy	Genentech	UCB	Bachem
Peptide content (±3%)	77.4	79.9	87.6	59.7	87.5	78.6
Digestion rate (±3%)	77.8	76.4	79.2	61.2	84.7	73.0

^a Determined by quantitative amino acid analysis ($M_r = 1637.94$).

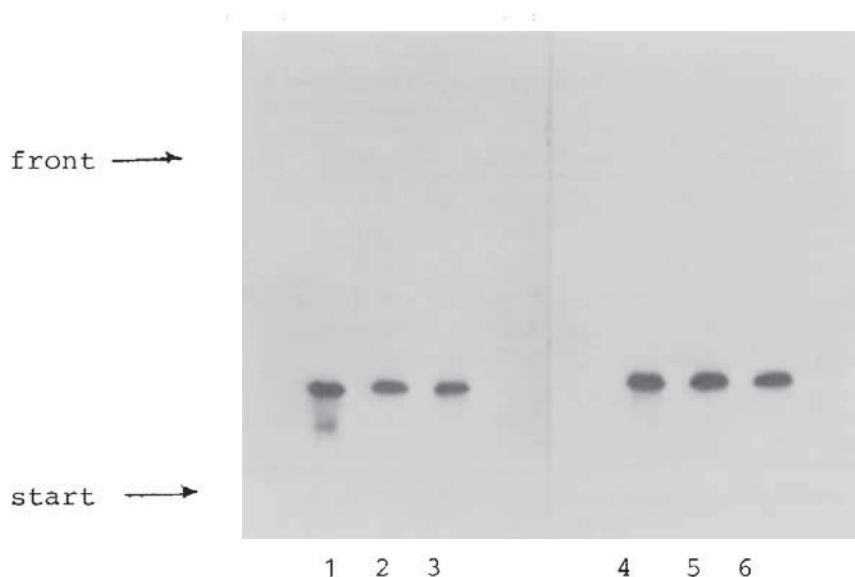


Fig. 1. HPTLC of somatostatin-14 samples on precoated silica gel-60 plates (Merck AG, Darmstadt). Solvent system: 1-butanol/acetic acid/water/pyridine (45:10:20:15); 40 μ g/sample. Columns: 1, Stilamin (Serono); 2, Diamalt; 3, Curamed; 4, UCB; 5, Clin Midy; 6, Genentech.

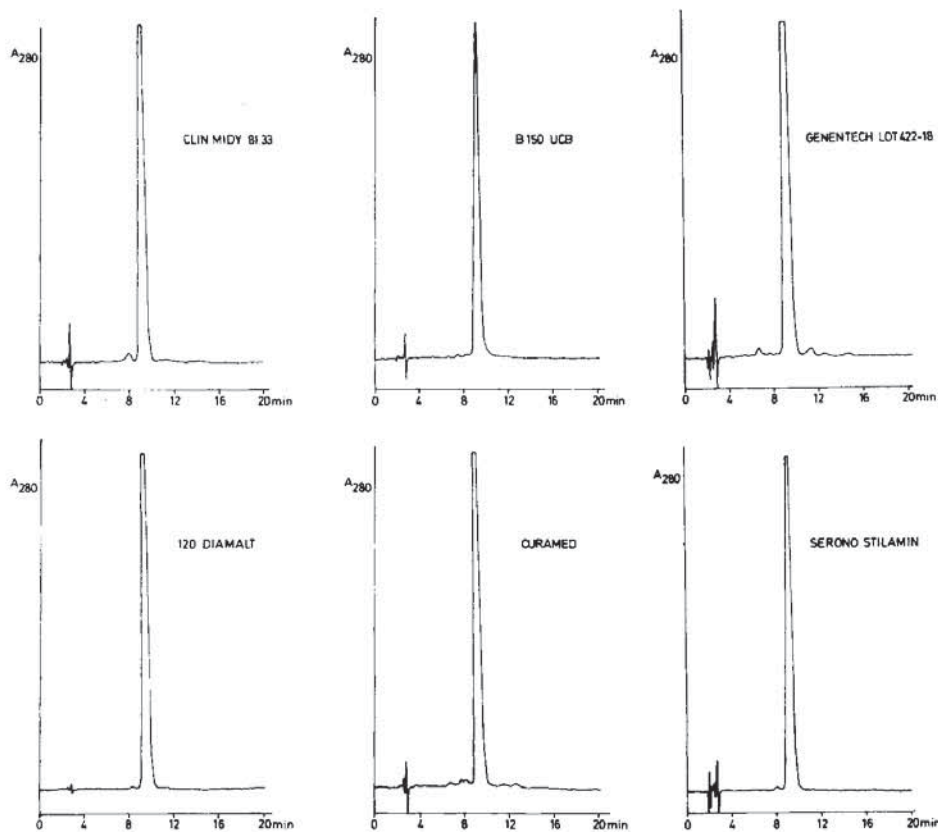


Fig. 2. HPLC of somatostatin-14 samples on μ -Bondapak C18 (30 \times 0.4 cm); eluent: acetonitrile/0.2M ammonium acetate (pH 4.0); 29:71 (v/v); flow rate, 1.3 mL/min.

were chromatographed on a Lobar RP-8 column; the impurities were separated, pooled again, and then found to represent less than 1% on a weight basis.⁶ Consequently, the main somatostatin fraction, wherein these impurities could not be detected by the above-mentioned methods, should exhibit a correspondingly higher degree of purity.

To summarize our experiences in the case of somatostatin-14, prior to its use in human medicine, a whole set of analyses has to be performed as follows:

Chromatographic Methods

1. Amino acid analysis of acid and enzymatic hydrolysates;
2. Gas-chromatographic analysis of the derivatized acid hydrolysate on Chirasil-Val glass capillary columns for postsynthetic chiral analysis (Table IV);
3. TLC on normal and high-performance precoated plates in different solvent systems;
4. HPLC possibly using different reversed-phase supports and eluent systems with qualitative and quantitative runs to confirm the total elution of applied material;
5. Electrophoresis on supports or in the free-flow systems to make additional characterization of the homogeneity of the substance on the basis of its mobility;
6. Micropreparative gel filtration to detect polymeric forms in the case of somatostatin-14.

Spectroscopic Methods

1. Uv measurements;
2. Nmr to detect, via ¹H and ¹³C resonance, possible synthetic side products such as alkylated tryptophans or residual protecting groups, etc.;
3. Ir to detect possible column material resulting from the chromatographic steps (repeated Millipore filtrations on 0.2- μ m filters were found to be useful to avoid such contaminations);
4. Chemical ionization, field desorption and fast-atom bombardment

TABLE IV
Gas-Chromatographic Separation of Amino Acid Enantiomers of the Acid Hydrolysates of Different Somatostatin-14 Samples^a

D-Amino Acid	Diamalt-Serono	CuraMed	UCB	Genentech	Clin Midy	Bachem
Ala	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Lys	1.5	1.8	1.8	1.5	2.1	1.8
Asp	3.3	2.9	3.1	3.0	3.4	3.0
Phe	1.0	1.4	1.7	0.7	1.3	1.4
Trp	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Thr	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Ser	<0.5	2.7	0.6	<0.5	<0.5	<0.5

^a Acid hydrolysis in 6M HCl at 110°C, 20 h. Derivatization to *N*-pentafluoropropionyl amino acid isopropylesters and gas-chromatographic separation on a glass capillary column coated with *N*-propionyl-L-valine *tert*-butylamide polysiloxane according to Ref. 10.

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