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Rapid analysis of somatostatin in pharmaceutical preparations by HPLC with a micropellicular reversed-phase column

VITTORIO BRIZZI*† and DANILO CORRADINI‡

†*Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Banchi di Sotto 55, 53100 Siena, Italy*
‡*Istituto di Cromatografia del C.N.R., Area della Ricerca di Roma, P.O. Box 10, 00016 Monterotondo Stazione, Roma, Italy*

Abstract: A rapid high-performance liquid chromatography method for the analysis of somatostatin in pharmaceutical preparations is described. A commercially available column packed with 2 μm spherical non-porous silica-based reversed-phase sorbent is used, along with a mobile phase consisting of acetonitrile and aqueous phosphoric acid, adjusted to pH 2.8 with sodium hydroxide. The effect of the organic modifier content and column temperature on the retention behaviour of somatostatin is reported. The method is found to be highly selective and specific, as indicated by the baseline separation of a mixture containing somatostatin and two analogue peptides, which differ from the analyte for one and two amino acids, respectively. Down to 10 ng of somatostatin can be detected and the detector response is linear over the concentration range from 4.14 to 20.75 μg ml^{-1}. The application of this method to two commercial pharmaceutical formulations of somatostatin is found to give a mean percentage recovery from each of the two commercial samples, subjected to multiple injection analysis (n = 5), of 100.9% with a RSD of 0.92%, and 102.6% with a RSD of 1.56%, respectively.

Keywords: *HPLC; somatostatin; micropellicular sorbent.*

Introduction

Somatostatin (Fig. 1) is a tetradecapeptide hormone with a 3-14 disulphide bond that was first isolated in ovine hypothalamus [1]. Somatostatin is also present in the duodenum, in the antral mucosa of the stomach and it is widely distributed in the central cord, where it has the function of a neurotransmitter [2]. Other biological activities of somatostatin are suppression of the release of peptide hormones, mainly at the pituitary, pancreatic and gastrointestinal sites. Thus this hormone affects the secretion of growth hormone, insulin, glucagon, gastrin, pepsin, secretin and vasoactive intestinal peptides.

Owing to these various biological activities, somatostatin is employed as a therapeutic agent for the treatment of acute gastrointestinal bleedings and pancreatitis, in the adjuvant therapy of diabetic keto-acidosis, and for the treatment of endocrinous gastrointestinal tumours and of acromegaly. Recently it has been brought to attention for a further therapeutic indication: the intra-articular treatment of arthropathies [3].

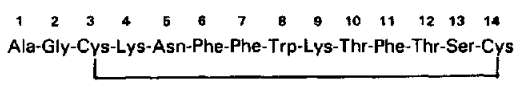

Figure 1
Amino acid sequence of somatostatin.

Reversed-phase HPLC has already been used for analytical and preparative scale separation of somatostatin from several tissues and species [4–6], and for the purification of the synthetic peptide [7, 8]. So far, no HPLC methods have been reported for routine analysis of somatostatin in pharmaceutical preparations.

This paper presents the results of a study of the chromatographic conditions for the rapid and specific analysis of somatostatin in commercial pharmaceutical preparations by RP-HPLC with a micropellicular silica-based column.

Micropellicular sorbents, made of a fluid-impervious microspherical support with a thin retentive layer at the surface, have recently been introduced for rapid separation of pep-

* Author to whom correspondence should be addressed.

tides and proteins by reversed-phase [9–12], ion-exchange [13], hydrophobic interaction [13] and affinity chromatography [14].

A major advantage of micropellicular sorbents is that significant intraparticular diffusion resistances are absent [15]. Furthermore the interaction of eluates with the retentive function of the stationary phase is highly facilitated by their confinement to a thin layer at the surface, which permits complete exposure to the mobile phase stream in the interstitial space of the column packing. As a result, the fast mass transfer in the stationary phase and in the mobile phase due to the small particle size, in the range of 1.0–2.5 μm, makes it possible to obtain high column efficiency even at relatively high flow velocities. At the same time, the absence of pores offers the further advantage of good mass recoveries for proteins and large peptides.

Although the idea of employing pellicular sorbents was introduced by Horvàth and Lipisky for the separation of small molecules in the early stages of HPLC [16], their employment has not come into general use. Only recently, since Unger and co-workers [9] showed that non-porous sorbents of small diameter particles are very useful for rapid separation of proteins, has there been a renewed interest in pellicular stationary phases for analytical applications.

The aim of this study was to examine the potential of reversed-phase high-performance liquid chromatography on a micropellicular silica-based sorbent for the rapid and specific qualitative and quantitative determination of somatostatin in pharmaceutical preparations. The use of this technique resulted in a fast analytical procedure with low detection and quantitation limits, specificity, highly reproducible retention times and high mass recovery.

Materials and Methods

Chemicals and samples

Reagent grade phosphoric acid and sodium hydroxide were obtained from C. Erba (Milan, Italy); HPLC grade acetonitrile was purchased from E. Merck (Darmstadt, Germany). Somatostatin, [Tyr^1]-somatostatin and [D-Tyr^8,D-Cys^{14}]-somatostatin were purchased from Sigma (St Louis, MO, USA). Samples from two different manufacturers of pharmaceutical preparations of somatostatin, namely A and B, were obtained from a commercial source. Sample A contained 3.0 mg of lyophilized somatostatin and 20 mg of mannitole, whereas sample B consisted of 2.0 mg of lyophilized somatostatin without any excipient. Distilled water further purified with a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all solutions and eluents.

Equipment

The experiments were performed with a Gilson (Middleton, WI, USA) Liquid Chromatograph consisting of a Model 305 solvent delivery pump, a Model 805 manometric module, a Model 115 variable wavelength UV detector and a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 20 μl sample loop. Chromatograms were recorded and the peak area measured by a Gilson Model 712 System Controller Software. A Hy-TACH C_{18} silica-based micropellicular octadecyl column (105 × 4.6 mm i.d.) was supplied by Glycotech (Hamdem, CT, USA).

Chromatographic conditions

Chromatographic runs consisted of isocratic elution with various water–acetonitrile mixtures containing phosphoric acid adjusted to the appropriate pH with sodium hydroxide. The eluent flow rate was 0.5 ml min^{-1} in all experiments. All mobile phases were filtered through type HA 0.45 μm membrane filter (Millipore, Bedford, MA, USA), and degassed by sparging with helium before use. The column effluent was monitored at 214 nm.

Calibration graphs

Standard solutions of somatostatin at five different concentrations within the range from 4.15 to 20.75 μg ml^{-1} were prepared in water and injected (20 μl) onto the HPLC column in triplicate. The linearity of calibration graph was checked by peak-area method, linear regression giving the following data: $y = 1.17x + 0.32$, correlation coefficient 0.998.

Sample preparation

The ampoule containing the lyophilized pharmaceutical preparation was weighed, dissolved in 200 ml of water in a volumetric flask and then subjected to HPLC analysis.

Results and Discussion

The object of this investigation was to

demonstrate fast, specific and accurate HPLC analysis of somatostatin by using a short column packed with a silica-based octadecyl micropellicular sorbent. In order to find optimum isocratic conditions, a study of the influence of mobile phase composition and temperature on the chromatographic behaviour of the cyclic tetradecapeptide was undertaken.

Experiments were performed under isocratic conditions using as eluent, various water–acetonitrile mixtures containing phosphoric acid in the concentration range from 5 to 50 mM at pH values ranging from 1.8 to 3.5. Acidic conditions were selected in order to suppress ionization of the residual silanol groups on the stationary phase and to facilitate ion-pairing formation of the phosphate ions with the positively charged somatostatin. Variations of phosphoric acid content in the mobile phase did not greatly affect the retention of somatostatin, due to the low hydrophobicity of the phosphate ion, whereas a pH of 2.8 was found to be the most appropriate in obtaining symmetrical peaks. Therefore, 20 mM phosphoric acid adjusted to pH 2.8 with sodium hydroxide was adopted as the buffer composition in the subsequent chromatographic experiments.

A dramatic change in somatostatin retention (k') with small changes in isocratic concentrations of acetonitrile in the eluent was observed. The range of acetonitrile concentration within which somatostatin was eluted with a practical k' value was only 14–22% (v/v). Under these conditions, plots of the logarithmic retention factor showed linear dependence with acetonitrile concentration in the mobile phase (Fig. 2) in the whole range of temperatures investigated. An increase in temperature from 40 to 70°C resulted in a decrease in column inlet pressure from 2870 to 1710 psi, due to a corresponding decrease in the eluent viscosity. With increasing temperature, chromatographic retention of somatostatin decreased with concomitant decrease of peak width (increasing efficiency), due to increased kinetic mass transfer of the solute between the stationary and the mobile phase.

On the basis of the results obtained, qualitative and quantitative analysis of somatostatin was performed at 40°C with the eluent containing 20% (v/v) acetonitrile in 20 mM phosphate buffer at pH 2.8.

Assay validation involved repetitive in-

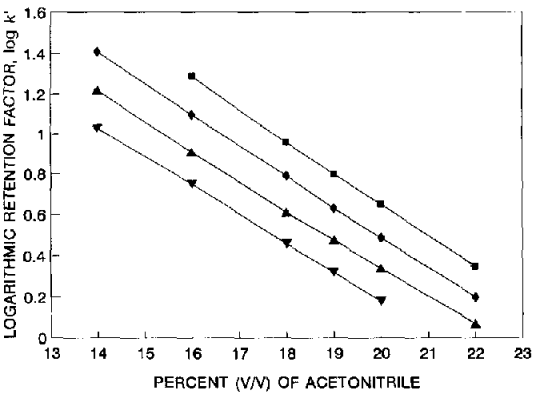

Figure 2
Plot of logarithmic retention factor as a function of acetonitrile content of the mobile phase at various temperatures: (▼) 70°C, (▲) 60°C, (◆) 50°C, (■) 40°C.

jections of a standard solution of somatostatin in water. The mean value, and the relative standard deviation (RSD) of the retention times were calculated by seven repeated injections of a sample solution containing 12 μg ml^{-1} of somatostatin (mean value, 4.86 min; RSD, 0.57%).

Quantification was performed by the method of the external standard. The linearity of the calibration graph, was determined by analysing solutions of standard somatostatin in HPLC-grade water with concentrations between 4.15 and 20.75 μg ml^{-1}. The sample concentration was limited to the above range in order to avoid peak-tailing and retention time shifting which could occur when the sample size approaches the column sample load capacity [17, 18], which for micropellicular sorbents is quite low, due to the low value of the phase ratio. The linear regression analysis provided the equation $y = 1.17x + 0.32$, with a correlation coefficient of 0.998.

The limit of detection was defined as the amount of injected sample which gave a signal-to-noise ratio of 3 and was calculated to be 10 ng.

Furthermore, two analogues of the tetradecapeptide, [Tyr^1]-somatostatin and [D-Tyr^8, D-Cys^{14}]-somatostatin, which differ from somatostatin for one and two amino acids, respectively, were selected to investigate the ability of the method to detect the homogeneity of somatostatin. As it can be seen from the chromatogram in Fig. 3, the two analogues were baseline separated from somatostatin, indicating the high specificity of the method for the analysed peptide.

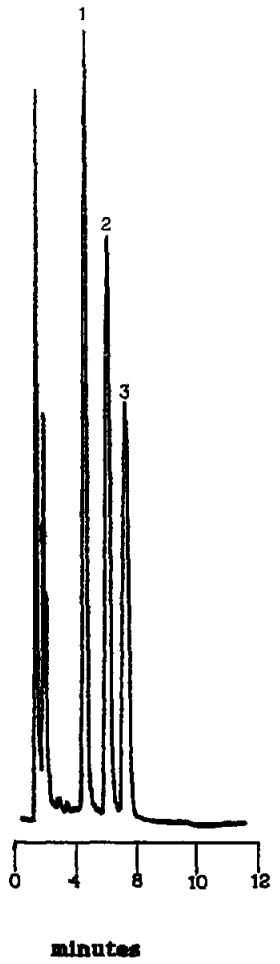

Figure 3
Chromatogram of analogue peptides: (1) [D-Try^{8}, D-Cys^{14}]-somatostatin, (2) somatostatin, (3) [Tyr^{1}]-somatostatin. Column, Hy-TACH C_{18} (105 × 4.6 i.d.); mobile phase 20% acetonitrile in 20 mM phosphate buffer (pH 2.8); flow rate, 0.5 ml min^{-1}; temperature, 40°C; detector sensitivity, 0.016 AUFS at 214 nm.

The method was applied to the analysis of two somatostatin based commercial preparations with and without excipient, as all formulations marketed in Italy [19] contain lyophilized somatostatin alone or with an excipient. No peaks other than somatostatin's were detected by injecting samples from pharmaceutical preparations containing quantities of somatostatin up to a thousand times the detection limit.

The precision and accuracy of the method were determined by spiking the solution of two commercial pharmaceutical preparations containing somatostatin with appropriate quantities of the standard peptide and by analysing the resulting solutions. The average recovery from each of the two commercial samples, subjected to multiple injection analysis (n = 5), was 100.9% (RSD, 0.92%) and 102.6% (RSD, 1.56%), respectively.

In conclusion, RP-HPLC on the micropellicular column was found to be a suitable method for rapid, linear, precise and accurate analysis of somatostatin in pharmaceutical preparations.

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