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CZE determination of somatostatin in pharmaceutical preparations

G.P. Cartoni *, F. Coccioli, R. Jasionowska, M. Masci

Dipartimento di Chimica, Università 'La Sapienza', P. le A. Moro 5, 00185 Roma, Italy

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Abstract

We propose a simple and accurate method for CE quantitative determination of somatostatin in pharmaceutical preparations. The method is specific for somatostatin as indicated by the resolution between the analyte and the analogue peptides which differ from somatostatin by one aminoacid. The linearity range is from 0.02 to 0.35 mg/ml. The recovery of the somatostatin from a pharmaceutical product is about 100.0%. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Somatostatin; Capillary zone electrophoresis

1. Introduction

Somatostatin is a tetradecapeptide hormone with various biological activities. It has inhibitory properties on various endocrine secretions, for ex. growth hormone, insulin, glucagon, gastrin and other peptide hormons. It has recently been suggested that somatosatin can be used in combination with melatonin for the treatment of tumours [1].

There are many substances that are similar to somatostatin but do not have this biological activity. It is therefore very important to find specific reliable methods in order to distinguish somatostatin from other similar substances and to carry out quantitative analysis.

A HPLC method has recently been put forward for checking pharmaceutical products containing somatostatin [2]. Capillary electrophoresis has been used to separate somatostatin analogues [3–5] but no quantitative studies have been carried out. This paper presents a simple and accurate method to analyse somatostatin in pharmaceutical products, by means of capillary electrophoresis.

2. Experimental

2.1. Chemicals

All reagents used were of analytical grade purity. Potassium dihydrogen phosphate, disodium hydrogen phosphate, methanol, benzyl alcohol, tyrosine, somatostatin, Tyr-1–somatostatin, Tyr-

* Corresponding author. Tel.: +39-6-4958251; fax: +39-6-490631.

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11–somatostatin were from Sigma (St Louis, MO). The pharmaceutical product containing somatostatin was obtained from commercial source ('Serono Pharma', Roma, Italy).

2.2. Instrumentation

Capillary electrophoresis was performed on a Spectraphoresis 1000 instrument from Thermo Quest (Palo Alto, CA) with a rapid scan spectrophotometric detection. A weakly hydrophobic coated capillary (ES2) from SGE (Melbourne, Australia), 50 μm I.D., total length 45 cm (38 cm to the detector) was used.

2.3. Buffer and samples preparation

Standard solutions for the calibration curve were prepared by dissolving somatostatin (1 mg/ml) and tyrosine (internal standard) in deionized water. Five solutions at different concentrations within the range from 0.02 to 0.35 mg/ml were prepared and analysed.

The sample of pharmaceutical preparation was obtained by solubilization of the product in deionized water. The content of the vial (somatostatin declared: 3 mg) was dissolved in 1 ml of deionized water. The declared composition of pharmaceutical product was: somatostatin and mannite as excipient. All solutions were stable for 3–4 weeks if maintained at 5°C.

The phosphate buffer solution at pH 7.0 consist of the mixture of potassium dihydrogen phosphate 25 mM and disodium hydrogen phosphate 25 mM.

2.4. Optimization of operating conditions

In order to propose a specific and accurate way of analysing pharmaceutical products containing somatostatin, by using capillary zone electrophoresis, it is essential to find the best experimental conditions in which the analyte can be separated from its analogues. Various operating conditions were explored. Firstly, the choise of capillary type was fundamental. Peptides analysed in an uncoated capillary yield tailed peaks due to the great adsorbtion of these analytes on fused silica capillary walls. This problem is eliminated by using a weakly coated hydrophobic capillary. Using this capillary the electroosmotic flow was lowered but not completely suppressed. The choise of suitable BGE at appropriate pH was very important. The borate buffer, tris buffer and phosphate buffer at various ionic strength were tested for this purpose. Only in the phosphate buffer the separation of the analytes was satisfactory. In order to improve the method selectivity methanol was added to the phosphate buffer in a ratio of 2:1. The optimal experimental conditions were: BGE (background electrolyte) 25 mM phosphate buffer at pH 7 containing methanol (2:1), the operating temperature: 25.0 ± 0.1°C, detection was carried out at 200 nm. The samples were injected on the anodic end of the capillary by hydrodynamic mode for 2 s (about 8 nl). Applied voltage was 10 kV ($i = 10$ μA).

Before analysis the capillary was washed with methanol (5 min), deionized water (3 min) and running buffer (3 min). Then some trial runs were performed to condition the capillary until electroosmotic flow (measured with benzyl alchol) become repeatable. Every five runs the buffer vial was renewed.

3. Results and discussion

In optimal operating conditions we obtained the electropherogram shown in Fig. 1. The somatostatin is separated from its analogues (tyr-1–somatostatin and tyr-11–somatostatin which differ from the analyte for one aminoacid). This allows checking the homogeneity of the pharmaceutical products containing somatostatin.

The internal standard method was used in order to carry out quantitative analysis. Tyrosine was chosen as reference peak. The calibration curve reports R (the ratio of the corrected areas of the two peaks) versus the somatostatin concentration in mg/ml. The graph is linear in the concentration range from 0.02 to 0.35 mg/ml.

The suggested method was applied in checking a pharmaceutical product containing somato-

statin. The analysis was carried out in the experimental conditions described above and yielded an electropherogram with only one peak corresponing to somatostatin migration time.

4. Validation

The response of all the analytes and the internal standard was found to be linear in the range 0.02–0.35 mg/ml. The limit of the detection was 10 μg/ml. The limit of quantitation (LOQ) was 0.02 mg/ml. The equation of the calibration graph given by linear regression method is $y = (7.5554 \pm 0.1726) \times -(0.0789 \pm 0.0313)$ with the correlation coefficient $r^2 = 0.9979$.

The precision of the method was satisfactory: the RSD% of the migration time was 0.4% and of the corrected area 3.0% (the data were calculated for five consecutive runs).

In order to evaluate the accuracy of the method, we analysed solutions containing the commercially available product to which were added known quantities of the somatostatin standard. The average recovery was about 100.0% while the somatostatin concentration in the pharmaceutical product was 3.0 mg/ml, in good agree-

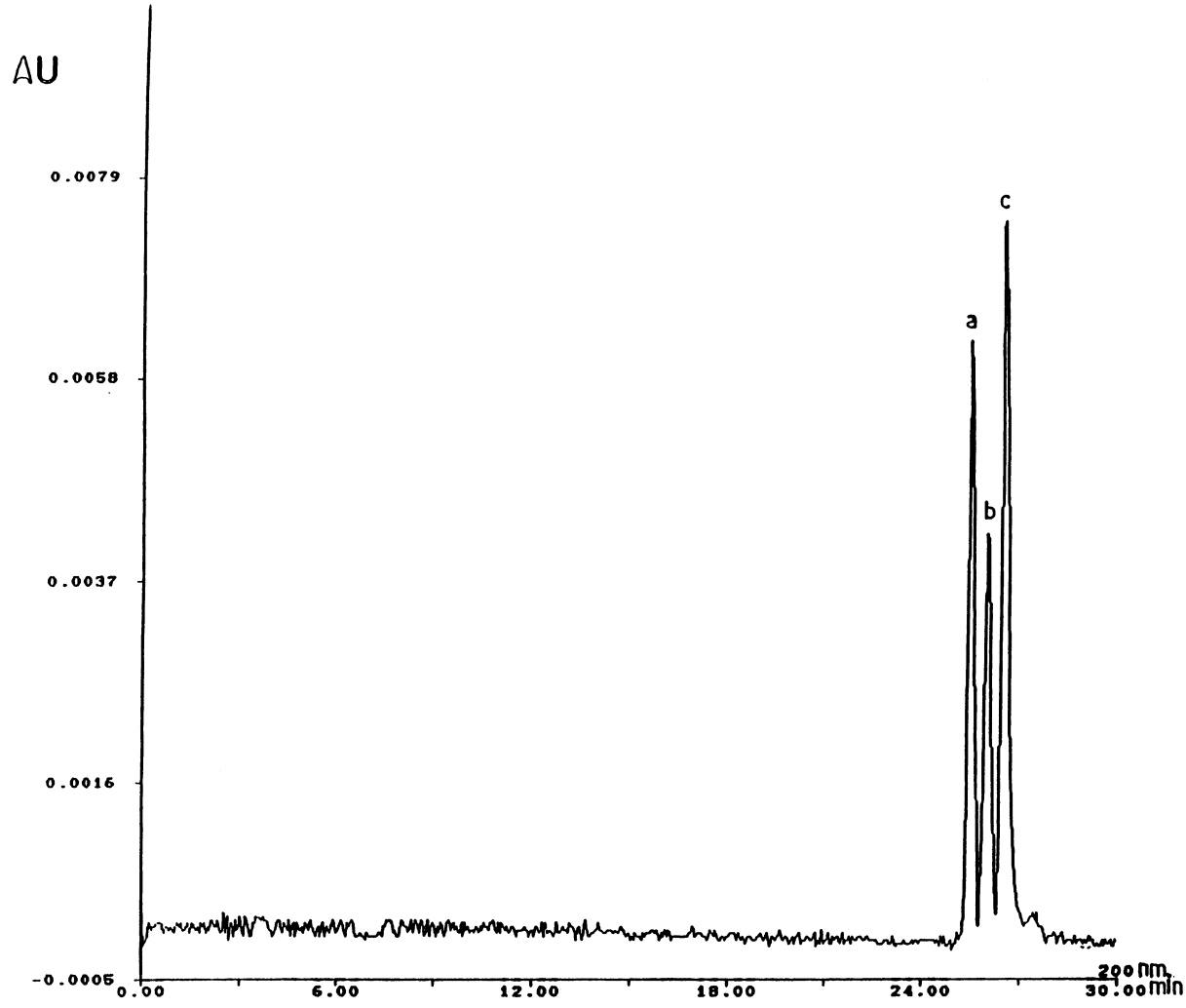

Fig. 1. Specificity of the method: separation of the mixture containing (b) tyr-11–somatostatin and (c) tyr-1–somatostatin.

ment with the value given by the pharmaceutical producing company.

Acknowledgements

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