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Short communication

On-line assay of the *S*-enantiomers of enalapril, ramipril and pentopril using a sequential injection analysis/amperometric biosensor system

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Abstract

A sequential injection analysis/amperometric biosensor system is proposed for the enantioselective analysis of the *S*-enantiomer of enalapril, ramipril and pentopril. The amperometric biosensor used as detector in the sequential injection analysis was designed by immobilization of L-amino acid oxidase in carbon paste. The proposed SIA system can be utilized reliably for the enantioanalysis of the *S*-enantiomer from the raw materials as well as from their pharmaceutical formulations, with a rate of 75 samples per hour and R.S.D. values better than 0.1% ($n = 10$).

Keywords: *S*-enalapril; *S*-ramipril; *S*-pentopril; Amperometric biosensors; Sequential injection analysis

1. Introduction

Enalapril (Fig. 1a), ramipril (Fig. 1b) and pentopril (Fig. 1c) are part of the angiotensin-converting enzyme inhibitors (ACE) class. For angiotensin-converting enzyme inhibitors (ACE) only the *S*-enantiomer is active one [1]. Accordingly, enantioselective analysis became increasingly important in the analysis of raw materials as well as of their pharmaceutical formulations. For this purpose it is necessary to develop highly reliable methods of enantiopurity testing.

Sequential injection analysis (SIA) has proved to be a good alternative to the chromatographic techniques used in the enantioanalysis of the compounds with a chiral moiety, especially when electrochemical sensors are used as detectors [2]. The main advantage over chromatographic techniques are high reliability of the analytical information, high rate of sample analysis, no need for a laborious sample preparation before the introduction of the sample in the SIA system and very low consumption of sample and reagents.

Despite the importance of analyzing only the *S*-enantiomer of enalapril, ramipril and pentopril, not all the methods reported upto now are enantioselective (e.g., CZE [3,4], LC [5,6], spectrometric methods [7,8]). Some of the proposed methods are able to discriminate between the enantiomers (e.g., HPLC [9], electrochemical methods [10–16]). Previously, amperometric biosensors [10,13,17] and enantioselective, potentiometric membrane electrodes (EPMEs) [11,14,15,18] were proposed for the enantioanalysis of different ACE inhibitors, such as captopril, ramipril, enalapril, cilazapril, pentopril, trandolapril and perindopril. Few of these sensors were used as detectors in flow systems (flow injection analysis, (FIA) and sequential injection analysis (SIA)), e.g., amperometric biosensors and EPMEs were used as detectors in FIA or/and SIA systems for the determination of enantiopurity of perindopril and captopril [19–21]. The advantages of using the electrochemical sensors as detectors in flow systems versus the manual method [16] are higher re-

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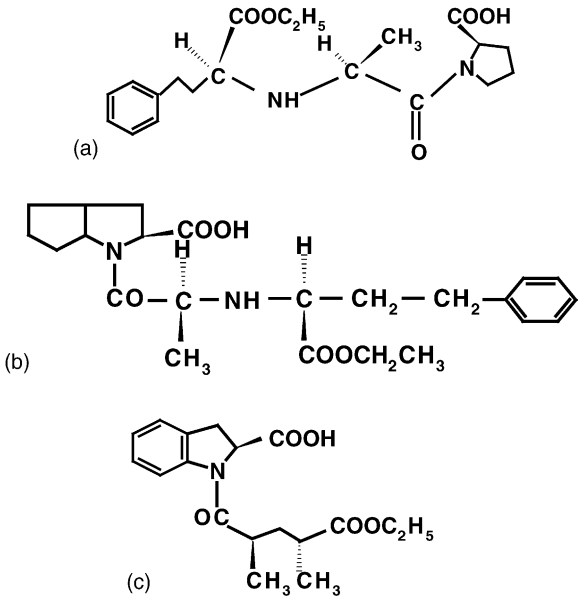

Fig. 1. (a) *S*-enalapril; (b) *S*-ramipril; (c) *S*-pentopril.

liability of the analytical information and higher throughput for on-line enantiopurity tests of ACE inhibitors – raw materials. Accordingly, it is a need of automatization of existing manual electrochemical methods for enantioanalysis of ACE inhibitors.

The emphasis of this paper is on the SIA system utilized for the enantioanalysis of the enantiomers of enalapril, ramipril and pentopril using an amperometric biosensor based on L-amino acid oxidase as detector.

2. Experimental

Reagents and materials. Graphite powder 1–2 μm, synthetic L- and D-proline were purchased from Aldrich (Milwaukee, WI, USA), paraffin oil from Fluka (Buchus, Switzerland) and phosphate buffer (pH = 7.0) from Merck (Darmstadt, Germany). De-ionized water from a Modulab system (continental water systems, San Antonio, TX, USA) was used for all solution preparations. The *S*-enantiomers of enalapril, ramipril and pentopril were obtained from Merck Sharp and Dohme (Great Britain), Hoechst (Germany) and Ciba-Geigy (New Jersey, USA), respectively.

2.1. Amperometric biosensor design

Paraffin oil and graphite powder were mixed in a ratio 1:4 (w/w) to form a graphite paste. The 100 μL L-amino acid oxidase (L-AAOD) (E.C. 1.4.3.2. Type I: Crude Dried Venom from *Crotalus adamanteus*, Sigma) solution (1 mg enzyme/mL in 0.1 mol/L phosphate buffer, pH = 7.0) was added to graphite paste. A plastic tip was filled with the corresponding graphite-paraffin oil paste leaving an empty space of 3–4 mm in the top part filled with carbon paste containing

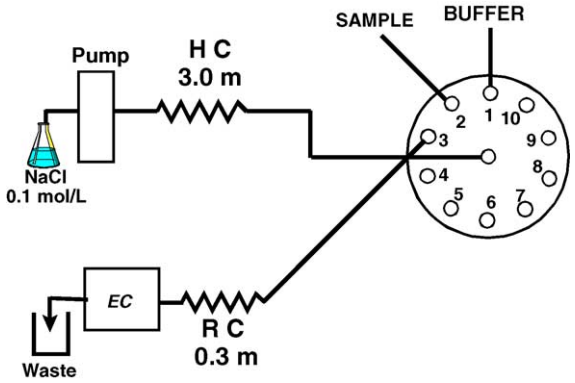

Fig. 2. Schematic flow diagram of SIA system used for the enantioanalysis of the *S*-enantiomers of enalapril, ramipril and pentopril.

the enzyme. The diameter of the sensor was 3 mm. Electrical contact was obtained by inserting a silver wire into the carbon paste. The electrode tip was gently rubbed on fine paper to produce a flat surface. The surface of the electrode was wetted with de-ionized water and then polished with an alumina paper (polished strips 30144-001, Orion) before use (this is also the only regeneration treatment that is performed). The biosensor was stored dry at 4 °C.

2.2. Apparatus

A 663 VA stand (Metrohm, Herisau, Switzerland) in connection with a PGSTAT 20 and software (Eco Chemie Version 4.9) was used for all chronoamperometric measurements. A Pt electrode and an Ag/AgCl electrode served as counter and reference electrodes in the cell.

2.3. Sequential injection system

The biosensors were incorporated into the conduits of the SIA system (Fig. 2) constructed from a Gilson Minipuls peristaltic pump and a 10-port electrically actuated selection valve (Model ECSD10P, Valco Instruments, Houston, TX).

Table 1
Device sequence for one cycle of the SIA system

Time (s)	Pump	Valve	Description
0	Off	Buffer	Pump stops, select buffer stream (valve position 1)
5	Reverse	Buffer	Draw up buffer solution
9.5	Off		Pump stops
10.5		Sample	Select sample stream (valve position 2)
11.5	Reverse	Sample	Draw up sample solution
16	Off		Pump stops
17		Electrochemical cell	Select electrochemical cell line (valve position 3)
18	Forward		Pump stack of zones to electrochemical cell
48	Off		Pump stops, return valve to starting position (valve position 1)

Table 2
Response characteristics for the amperometric biosensors designed for the *S*-enantiomers of enalapril, ramipril and pentopril in SIA system

Analyte	Linear conc. range (μmol/L)	Detection limit	Equation of calibration[a]	Correlation coefficient (*r*)
S-enalapril	0.08–1.50	4.33 nmol/L	$H = 0.03 + 6.3C$	0.9998
S-ramipril	0.12–0.60	0.01 μmol/L	$H = 0.4 + 12.9C$	0.9973
S-pentopril	0.2–6.0	0.1 μmol/L	$H = 2.9 + 20.7C$	0.9997

[a] *H* is the peak height in μA and *C* is the concentration of the *S*-enantiomer in μmol/L.

Tygon tubing (0.76 mm i.d. for the holding coil and 0.89 mm i.d. for the mixing coil) was used to construct the manifold [22]; coils were wound around suitable lengths of glass tubing (15 mm o.d.); 0.1 mol/L NaCl was used as carrier. The capacity of the system is about 75 samples per hour. The device operating sequence is shown in Table 1. The device control was achieved using a PC30-B interface board (Eagle Electric, Cape Town, South Africa). The FlowTEK [23] software package (obtained from MINTEK) for computer-aided flow analysis was used through out for device control.

3. Results and discussion

An optimum flow rate of 3.61 mL/min was used to propel the solutions. In the SIA system, the sample and buffer consumption is only 270 μL each per measurement enantiomer, which is very economical.

3.1. Response characteristics of the amperometric biosensors

The response characteristics of the electrodes were measured at 650 mV versus Ag/AgCl electrode and are shown in Table 2. The working concentration ranges as well as the limits of detection (in the nmol/L magnitude order) demonstrated the suitability of the proposed amperometric biosensor for the on-line monitoring of the *S*-enantiomers. The response of the biosensor revealed good stability and reproducibility for tests performed over one week (R.S.D. < 0.1%, when used daily).

3.2. Selectivity of the amperometric biosensors

The selectivity of the amperometric biosensors was checked using both the mixed and separate solutions methods. Amperometric selectivity coefficients were determined following the method proposed by Wang [24]. In the evaluation, the concentration of the supposed interferent was selected to be 10 times higher than that of the *S*-enantiomer. The enantioselectivity was checked over the *R*-enantiomer as well as over D-proline, because the compounds have an L-proline moiety. L-Proline and D-proline are possible byproducts in the synthesis of these drugs. Polyvinylpyrolidone (PVP) is used for the formulation of these pharmaceutical products and it is the only potential interferent in the determination of the *S*-enantiomer of enalapril, ramipril and pentopril in their pharmaceutical formulations. Its influence on the measurements of the *S*-enantiomer was determined to be negligible? From Table 3, it can be seen that the only interferent in the determination of the *S*-enantiomer is L-proline, the SIA/amperometric biosensor system being able to perform the enantioanalysis of the raw material and pharmaceutical formulation of each of the drugs.

Table 3
Amperometric selectivity coefficients of the amperometric biosensor used as detector in SIA system (all values are the average of 10 determinations)

Analyte	Interfering species, pK_{amp}			
	R-enantiomer	D-Proline	L-Proline	PVP
S-enalapril	>4.00	4.00	2.55	3.09
S-ramipril	>4.00	3.81	1.81	2.86
S-pentopril	>4.00	3.93	1.34	2.51

3.3. Analytical applications

The *S*-enantiomer can be assayed with average recoveries ($n = 10$) of 99.783 ± 0.005% (*S*-enalapril), 99.271 ± 0.004 (*S*-ramipril) and 99.982 ± 0.003% (*S*-pentopril) from the raw material, using the SIA/amperometric biosensor system. Accordingly, the proposed SIA/amperometric biosensor system proved to assure high reliable analytical information for the enantioanalysis of the raw materials of enalapril, ramipril and pentopril.

4. Conclusion

The main advantages of the proposed system are: high precision and accuracy of the analytical information, simplicity of operation and, low cost of analysis. The high precision of the SIA over the manual method [16] is due to the fact that all measurements are done after the same interval of time and the surface of the biosensors are continuously brushed by sodium chloride used as carrier in the sequential injection analysis.

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