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Flow injection biamperometric determination of captopril

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Abstract

An automated method to determine captopril (1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline) is proposed. A flow injection manifold based on the indirect biamperometric detection of the captopril by using Fe(III)/Fe(II) as an indicating redox system and a Z-shaped flow-cell configuration, was developed. The calibration curve is linear over the range 0.03–3.6 μg ml^{-1} of captopril. The relative standard deviation for the determination of 0.76 μg ml^{-1} of captopril is 0.97% ($n = 12$) and the sample throughput is 69 h^{-1}. This method was applied to the determination of captopril to commercially available pharmaceutical preparations.

Keywords: Biamperometry; Flow injection; Captopril

1. Introduction

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline, is an angiotesin converting enzyme inhibitor, which reduces peripheral resistance and lowers blood pressure. It is widely used on hypertensive disease treatment [1] and in the congestive heart failure treatment, as such or in combination with other drugs.

Several analytical methods have been proposed for captopril determinations and other thiol drugs of pharmaceutical interest including: chemiluminometry [2,3], spectrometry [4–9], fluorimetry [10], polarography [11,12] and chromatographic techniques [13,14]. Only four Flow Injection (FI) methods has been described [10,15–17] with spectrophotometric, fluorimetric and chemiluminescence detection.

Flow Injection Analysis (FIA) has advantages that makes it suitable for use in several fields of routine analysis. The application of amperometric detection with two platinum electrodes also called biamperometry, combined with FIA possess desirable analytical features such as simplicity, low cost, high sample throughput and selectivity [18,19]. The aim of this communication is to develop a simple, sensitive and rapid flow injection method for the determination of captopril in pharmaceutical formulation.

This analytical technique is based on measurement of the intensity of current passing through two identical inert electrodes, to which a small potential difference is applied. The electrical cur-

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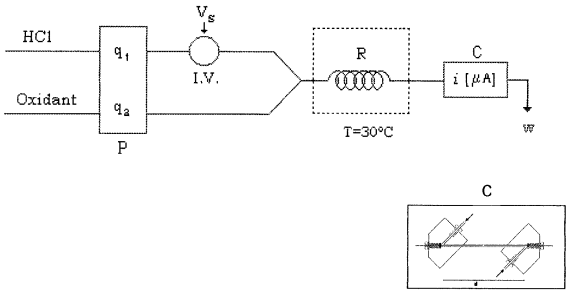

Fig. 1. FIA manifold, P, peristaltic pump; IV, Injection valve; V_s, sample volume; q_1, carrier flow rate; q_2, oxidant flow rate; W, waste; C, Z-shaped cell.

rent flowing into the detection flow cell is only observed when solution contacting the electrodes contains two forms of reversible redox couple. The measured signal of the biamperometric detector is related to the amount of the original analyte. So, the choice of the detector is a critical part for flow injection procedures with this type of electrochemical detectors. A Z-shaped flow cell for the biamperometric detection was used (C in Fig. 1), analytical sensitivity improvement was verified [20].

2. Experimental

2.1. Apparatus

Biamperometric measurements were carried out by means of a PO_4 polariter Radiometer Copenhagen.

The design of the modified home-made flow through cell has been described earlier [20]. Two platinum electrodes (0.5 mm diameter and 10 cm length) were used. The exposed length of wire electrodes to the flowing solution was 8 cm. The electrodes were set into an alkaline phosphate solution (50 g Na_2HPO_4 and 20 g KOH in 1 l of water) at ≈ 80 °C, their surfaces were cleaned electrochemically by alternating the polarisation over the range (-4)–$(+4)$ V every 10 s during 10 min, daily before using on the measurement system. This treatment of the working electrodes allowed us to obtain a maximum sensitivity of detector response.

The propulsion system consisted of a Gilson Minipuls 3 peristaltic pump.

A Rheodyne (5041) injection valve was used. All the reaction coils were made of PTFE tubing (i.d. 0.5 mm).

2.2. Reagents and solutions

Chemicals of analytical reagent grade and distilled water were used to prepare all solutions.

Captopril was obtained from Parafarm (Sweden).

A 30 μg ml^{-1} captopril stock solution was prepared daily.

The Fe(III) solution 0.1 M was prepared by dissolving a weighed portion of $FeCl_3 \cdot 6H_2O$ in 1.5 M HCl medium.

2.3. Sample preparation

Twenty tablets were weighed to calculate the average tablet weight. They were finely powdered and homogenised. Equivalent to about 5.0 mg of captopril of the powder was accurately weighed and dissolved with 30 ml of distilled–deionised water, by stirring for 20 min on a magnetic stirrer. The resulting mixture was filtered and diluted with water in a calibrated 50 ml flask; 100 μl of this solution was diluted with water in a 25 ml flask for further sample analysis.

When the Standard Addition Method was applied the same volume of the solution above described was introduced into the flask and added them standard solution of increased concentrations, then they made up to 25 ml with distilled water.

3. Procedure

A double channel FIA manifold was developed (Fig. 1). The captopril sample was injected into a HCl (10^{-3} M) carrier stream which merges with the oxidant solution ($FeCl_3$ in HCl 1.5 M) into a reactor, where the reaction takes place. The reactor was thermostathised at 30 °C and the applied potential was 100 mV. A current signal was obtained from the produced redox couple.

Table 1
Comparison of different indicating redox systems for the biamperometric determination of captopril

	Linear range (μg ml^{-1})	Equation	r^2	Base line
Fe(III)/Fe(II)	0.06–8.4	$\mu A = 0.9197x - 0.0042$	0.9979	Good
I_2/I^-	0.67–10.1	$\mu A = 0.0126x + 0.0033$	0.9847	Good
$[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$	0.30–8.4	$\mu A = 0.8767x + 0.7676$	0.9837	Bad
VO_3^-/VO_2^+	Low reproducibility	Bad		

4. Results and discussion

4.1. Optimisation of chemical and FIA variables

4.1.1. Studies with different indicating redox systems

Preliminary studies with different indicating reversible redox systems were carried out in a double channel FIA manifold. The sample (300 μl) was injected into a carrier, then it merged and mixed with an oxidant stream inside a reactor (100 cm). The polarising voltage was 100 mV.

Four different oxidant species were studied: Fe(III), hexacyanoferrate(III), Iodine and VO_3^-. Optimisation of chemical parameters were carried out through a calibration graph of captopril, for each of oxidant solutions. Thus, the influence of oxidant concentration and acid concentration added to the oxidant solution, were evaluated over a linear detector response.

4.1.1.1. Fe(III) system. The Fe(III) concentration was tested from 0.05 to 0.5 M. The best slope of the calibration curves was obtained at 0.1 M. The acid concentration in the oxidant solution was studied with sulphuric and hydrochloride acid. The HCl was selected and its concentration was varied from 0.5 to 1.5 M. The optimum value was 1.5 M.

Bidistilled water and solutions of different concentrations of HCl were studied as carrier stream. The suitable carrier stream was HCl 10^{-3} M.

4.1.1.2. $[Fe(CN)_6]^{3-}$ system. The optimum concentration in the reagent stream was 0.05 M in HCl 1 M. Distilled water was used as a carrier stream.

4.1.1.3. I_2 system. The reagent concentration was studied on the range 1×10^{-4}–5×10^{-4} and 1.4×10^{-4} M was selected. Acid and alkaline medium was tested and the signal was not improved.

4.1.1.4. VO_3^- system. The reagent concentration range between 1.3×10^{-4}–8.0×10^{-2} M was studied, but results and base lines had low reproducibility.

The obtained results are summarised in Table 1.

The Fe(III) system was selected for the determination of captopril.

4.1.2. Temperature optimisation

Immersing the reaction coil inside a thermostat bath tested the influence of this parameter on the signal. In the tested temperature range (20–60 °C) the signal increased as the temperature increased but a significative increase of the noise amplitude of the base line was observed. Thus, 30 °C was assumed to be the optimum value for the experimental work.

4.1.3. FIA variables optimisation

FIA variables were optimised by univariant method. This preliminary information was useful to set up the limit values of the FIA variables in the Modified Simplex Method [21,22].

Sample volume, reactor length, oxidant and carrier flow rate streams were the studied variables by using 6 μg ml^{-1} captopril solution. Three different Simplex models were tested as showed in Table 2. The selected variables of Simplex III showed the best reproducibility and sample throughput.

The selected variables are shown in Table 2 (in bold format).

4.1.4. Electrochemical parameter optimisation

Electrochemical parameter, as polarising voltage and exposed length wire electrodes were optimised.

Polarising voltage was studied with a 6 μg ml^{-1} of captopril, the effect of different polarising voltages in the current signal was proved between 50 and 250 mV. The optimum value was 100 mV.

Different exposed length wire electrodes were tested, (4.0, 5.5, 8.0 cm). The study was carried out over calibration curves of captopril, in the concentration range between 0.06 and 1.2 μg ml^{-1}. Sensitivity and linearity of the calibration curve were considered to select the optimum value. Best results were obtained with an exposed length wire of 8.0 cm.

Table 2
Obtained results by application of different simplex models

FIA variable	Simplex I	Simplex II	Simplex III
q_1^* (ml min^{-1})	1.25–4.4	2.3–4.4	1.25–3.35
q_2^* (ml min^{-1})	0.24–1.8	0.9–1.5	0.55–1.2
R^* (cm)	50–200	100–200	100–180
V_s^* (μl)	100–500	300–500	250–450
Optimum value			
q_1 (ml min^{-1})	3.8	2.8	**2.9**
q_2 (ml min^{-1})	1.2	1.0	**1.0**
R (cm)	173.2	121.8	**163.2**
V_s (μl)	428.4	485.1	**408.1**
RSD% (n)	1.34 (11)	2.55 (8)	0.88 (10)
Sample throughput (h^{-1})	63	52	69

Selected variables are in bold format. q_1, carrier flow rate; q_2, oxidant flow rate; V_s, sample volume; R, reactor length.

Table 3
Interfering ions tolerance limits

Ions tested	Tolerance limit (μg ml^{-1})	Error (%)
$Na_2B_4O_7 \cdot H_2O$	1050	1.3
Lactose	5000	4.5
$ZnSO_4 \cdot 7H_2O$	2500	3.5
Glucose	1800	3.8
Sodium citrate	5200	−2.8
HB_3O_3	18 000	−1.8
$NaHCO_3$	1200	3
EDTA	300	3
Starch	Saturated solution	3.3%

4.2. Interferences

The influence of foreign compounds which can be found in typical pharmaceutical samples containing captopril was investigated by using solutions containing 0.84 μg ml^{-1} of the drug and adding various concentrations of the interfering compounds.

The tolerance limits were obtained by comparing the peak height with that obtained by injecting an aqueous solution of pure captopril. It was taken as the measured signal variation $\pm 5\%$. Table 3 summarises the obtained results. This method presents a good tolerance against potential interfering compounds.

4.3. Applications

Under the selected experimental conditions above described, the calibration graph was linear over the range 0.03–3.60 μg ml^{-1} of captopril and the detection limit (LOD) for $S/N = 3$ was 0.012 μg ml^{-1}. The calibration line is $\mu A = (0.024 \pm 0.006) + (1.450 \pm 0.010)X$, with a correlation coefficient of 0.999 (where μA is the current in micro amperes and X the concentration of captopril in μg ml^{-1}). In order to obtain the reproducibility, eight calibration graphs were obtained on different days and with different conditions (standard solution, reagent solution, etc.) and mean of the slope obtained was 1.490_8 with $RSD\% = 3.2$.

The relative standard deviation of the proposed procedure and the sample throughput were obtained by $n = 12$ replicates of samples containing 0.76 μg ml^{-1} captopril injected by duplicate. The results obtained were 0.97% and 69 h^{-1}, respectively.

To validate the proposed method we would have used the official method. Since the chromatographic column, which is needed for that purpose, was not available for us, we decided to do the validation by using two different analytical techniques.

Table 4
Recovery from captopril added to real samples

Sample	Amount (mg per tablet)		Added (mg)	Recovered	Recovery (%)
	Label	Found ± S.D. ($n = 5$)			
Capoten®[a]	25	$25.7 \pm 0.36_7$	2.6	28.1	101.8
			7.9	33.9	103.0
			25	50.5	101.0

[a] Capoten® was purchased from Bristol-Myers Squibb.

One of them was carried out with spiked real samples and the recovery percentages are shown in (Table 4).

The other applied method was standard additions to the real samples. Six different calibration curves were obtained with the technique before described (Section 2.3). Captopril concentrations obtained from calibration curves were shown in Table 5.

The six calibration curves were made on a different way, the mean obtained from the results, 25.7_9 μg ml^{-1}, was compared with 25.7 μg ml^{-1} obtained with the proposed method.

Also, a comparison of two estimated variances, S_1^2 and S_2^2, was performed by F-test. The calculated value (1.001) was smaller than the critical $F_{(0.05;5.4)}$ value (9.36), consequently it was accepted that both variances are equal. As this condition was fulfilled, it was be able to obtain a pooled estimated variance ($S^2 = 0.1355$).

Calculating the statistic then performed the t-test. Since, calculated t value (0.85) was smaller than the critical $t_{(0.05;9)}$ value (2.26), the hypothesis H_0: $\mu_1 = \mu_2$ was accepted. So, the obtained concentration of captopril into the sample by both methodologies is the same.

Through the reproducibility of the obtained results we can proved that the proposed method is robust.

5. Conclusion

The proposed method results a simple, fast and inexpensive analytical technique to determine captopril in commercial pharmaceutical preparations.

From the FIA methods reported in bibliography we can concluded that the proposed method shows lower LOD, a comparable concentration range of the calibration curve and a suitable sample throughput.

Thus, the fluorimetric method [10] needed an oxidation step of captopril before the detection of the quantitative oxidation product of the analyte. The spectrophotometric photometric method [15] used a packed mini-column in the FIA system. So the minimum values obtained for the calibration curves in both methods were higher than the proposed method.

Also, spectrophotometric method [17] which used a rather expensive reagent ($PdCl_2$) presented similar results as above mentioned with a higher LOD than the proposed method.

Moreover, the chemiluminescence method [16] which is a very sensitive and selective technique gave a LOD higher than the proposed method.

Table 5
Obtained results by application Standard Addition Method on real sample

Calibration curve number	Found (mg per tablet)	
1	25.9	
2	26.2	$X = 25.79$ (mg per tablet)
3	25.6_3	
4	25.4	S.D. $= 0.36_8$
5	25.7	
6	26.2	$N = 6$

References

[1] K. Fiorey (Ed.), Analytical Profiles of Drugs Substances, vol. 11, Academic Press, New York, 1982, p. 81.
[2] X.R. Zhang, W.R.G. Baeyens, G. Vanderweken, A.C. Calokerinos, K. Nakashima, Anal. Chim. Acta 303 (1) (1995) 121.
[3] Z. Xinrong, W.R.G. Baeyens, G. Vanderweken, A.C. Calokerinos, K. Nakashima, J. Pharm. Biomed. Anal. 13 (4–5) (1995) 425.
[4] C.S.P. Sastry, K.R. Srinivas, K.M.M.K. Prasad, Anal. Lett. 29 (8) (1996) 1329.
[5] H.F. Askal, Talanta 38 (10) (1991) 1155.
[6] S.M. Elashry, F.A. Ibrahim, Anal. Lett. 25 (9) (1992) 1657.
[7] I. Panderi, M. Parissipoulou, Int. J. Pharm. 86 (2–3) (1992) 99.
[8] K.M. Emara, A.M.I. Mohamed, H.F. Askal, I.A. Darwish, Anal. Lett. 26 (11) (1993) 2385.
[9] C.S.P. Sastry, S.G. Rao, P.Y. Naidu, K.R. Srinivas, Anal. Lett. 31 (2) (1998) 263.
[10] R.S. Guerrero, S.S. Vives, J.M. Calatayud, Microchem. J. 43 (3) (1991) 176.
[11] J.A. Squella, I. Lemus, Y. Borges, I.J. Nuñez Vergara, Bol. Soc. Chilena Quim. 37 (4) (1992) 259.
[12] J.M.G. Fraga, A.I.J. Abizanda, F.J. Moreno, J.J.A. Leon, Talanta 46 (1) (1998) 75.
[13] J. Ouyang, W.R.G. Baeyens, J. Delanghe, G. Van der Waken, W. Van Daele, D. De Keukelaire, A.M. Garcia-Campana, Anal. Chim. Acta 386 (3) (1999) 257.
[14] J. Russell, J.A. McKeown, C. Hensman, W.E. Smith, J. Reglinski, J. Pharm. Biomed. Anal. 15 (11) (1997) 1757.
[15] R. Karlicek, P. Solich, Pharmazie 53 (8) (1998) 549.
[16] Z.D. Zhang, W.R.G. Baejens, X.R. Zhang, G. Van Der Waken, J. Pharm. Biomed. Anal. 14 (8–10) (1996) 939.
[17] M.I. Albero, C. Sanchez petrena, M.S. García, V. Rodenas, J. Pharm. Biomed. Anal. 11 (10) (1993) 887.
[18] T.P. Tougas, J.M. Janetti, W.G. Collier, Anal. Chem. 57 (1985) 1377.
[19] M. Trojanowicz, J. Michalowski, J. Flow Injection Anal. 11 (1) (1994) 34–44.
[20] M. Palomeque, B.S. Fernández Band, paper presented at The Tenth ICFIA 99, Prague, Czech Republic, June 1999.
[21] L.A. Yabroand, S.N. Deming, Anal. Chim. Acta 73 (1973) 391.
[22] S.L. Morgan, S.N. Deming, Anal. Chem. 46 (1974) 1170.