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Voltammetric determination of cilazapril in pharmaceutical formulations

Uğur Tamer [a], Nuran Pekmez Özçiçek [b], Okan Atay [a], Attila Yıldız [b,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey*
[b] *Department of Analytical Chemistry, Hacettepe University, 06532 Beytepe, Ankara, Turkey*

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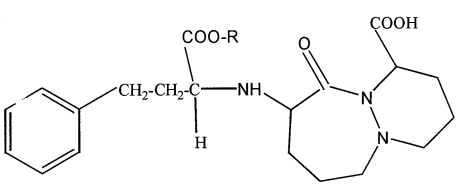
Abstract

A sensitive adsorptive stripping voltammetric method for the measurement of cilazapril in 0.04 M Britton–Robinson buffer (pH 9.0) solution was described. The method was based on the adsorptive accumulation of the drug at a hanging mercury drop electrode (HMDE), followed by differential pulse voltammetry. The response was evaluated with respect to pre-concentration time, pH effect, accumulation potential, accumulation time and scan rate. The peak potential was −0.60 V (vs. Ag/AgCl). The peak current was directly proportional to the concentration of cilazapril with a detection limit of 17.6 ng ml^{-1} at an accumulation time of 10 s. The reduction process was irreversible and the wave showed adsorptive characteristics. The results were compared to those obtained using a HPLC procedure. A reversed-phase C18e column with aqueous phosphate buffer (pH 3.5; 0.125 M)–acetonitrile (67:33, v/v) mobile phase and benazapril as internal standard was used. UV detector was set at 254 nm. Results obtained in HPLC were comparable to those obtained by adsorptive stripping voltammetric method.

Keywords: Cilazapril; Adsorptive stripping voltammetry; Reversed-phase HPLC

1. Introduction

Cilazapril, [1*S*-[1α,9α(R*)]]-9-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]octahydro-10-oxo-6*H*-pyridazino[1.2*a*][1,2]diazepine-1-carboxylicacid monohydrate is a potent and specific angiotensin-converting enzyme (ACE) inhibitor which lowers peripheral vascular resistance without affecting heart rate. It is used in the treatment of hypertension and congestive heart failure [1]. This substance which has the biological activity prevents the transformation of angiotensin I to angiotensin II by inhibiting ACE. It also prevents the reabsorption of sodium and water from renal tubulus and decrease the heart flow-rate [2,3].



R = CH_2CH_3 Cilazapril
R = H Cilazaprilat

* Corresponding author. Tel.: +90-312-297-7964; fax: +90-312-299-2163.
E-mail address: yildiz@hacettepe.edu.tr (A. Yıldız).

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Following oral administration, cilazapril undergoes de-esterification in the liver to its active di-acid form, cilazaprilat. This metobolite is more polar than cilazapril, making it more difficult for the intestine to absorb, but cilazapril, although not active, is well absorbed orally Doses of cilazapril range from 0.5 to 5 mg per day [4,5].

Analytical methods for the direct determination of this drug and its metabolite are very scarce. Analysis of the activity of these compounds were usually carried out indirectly by measuring ACE inhibition angiotensin II or renine levels [5,6]. Direct determination in plasma and serum was carried out by enzyme immunoessay [7]. UV–Vis spectrophotometry was applied to the determination of cilazapril in pharmaceutical formulations [8,9]. High-performance liquid chromatographic method with UV [10,11] and amperometric detections [12] were developed for the quantitation of cilazapril and its active metabolite and degradation product cilazaprilat in urine and tablets. Capillary electrophoresis was also applied to the determination of the drug [13,14]. Other structurally similar ACE inhibitors are usually measured using GC–MS, but this involves two tedious derivatization steps for the amine and the carboxylic acid groups and the carboxylic acid groups ficantly affect the quality of the results [15–17].

Electroanalytical techniques, especially modern pulse techniques such as differential pulse and square wave voltammetry, have been used for the sensitive determination of a wide range of pharmaceuticals with the advantages that there is, in most instances, no need for derivatization, and that these methods are less sensitive to matrix effects than other analytical techniques [18]. Thus, the sensitive determination of compounds, even in complex matrices, is possible without tedious extraction procedures being necessary before the voltammetric measurements. In the literature, no electrochemical data concerning the electrochemical behavior of cilazapril on hanging mercury drop electrode (HMDE). The aim of the present work was the development of a sensitive electroanalytical procedure for the determination of cilazapril in dosage form without an extraction procedure being necessary prior to the voltammetric measurement. The adsorptive behavior of cilazapril on HMDE and in different media was also investigated in order to find the best experimental conditions for the electroanalysis of cilazapril. The utility of the developed method to determine the content of active substances in pharmaceutical preparation was also demonstrated. The results were compared with those obtained using a HPLC method.

2. Experimental

2.1. Voltammetric method

2.1.1. Apparatus

A model 174 A polarographic Analyzer (EG&G Princeton Applied Research Corporation) was employed with a Model 303 static mercury drop electrode. The voltammograms were recorded using a Research Re 0150 Model X–Y recorder. A Hanna Model pH meter was used for pH measurements. The three electrode system consisted of a 10 ml quartz cell. The working electrode was a static mercury drop electrode, a KCl saturated Ag/AgCl electrode was used as a reference electrode. Platinum wire was used as an auxiliary electrode. All experiments were performed at room temperature and the dissolved oxygen was removed by passing pure nitrogen through the solutions.

2.1.2. Reagent and solutions

Cilazapril (generously provided by Fako Drug Inc., Istanbul, Turkey) was used without further purification. Pharmaceutical formulations Inhibace and Inhibace Plus (commercialized by Roche, Istanbul, Turkey) were analyzed throughout the study. The composition of the two pharmaceutical formulations are as follows; Inhibace tablets containing cilazapril, 5 mg and Inhibace Plus tablets containing cilazapril 5 mg; hydrochlorothiazide 12.5 mg). For the preparation of standard cilazapril stock solution (1000 μg ml^{-1}), 100 mg cilazapril was accurately weighed, dissolved in methanol and then adjusted to 100 ml with methanol. Standard solutions were prepared by appropriate dilutions of the stock solution.

Britton–Robinson buffer (Merck) (0.04 M) was prepared and used as supporting electrolyte. The ionic strength was kept constant by adjusting with 0.1 M KCl. All the chemicals were of analytical reagent grade (Merck). Triple distilled water was used throughout.

2.1.3. Procedure

2.1.3.1. Application to cilazapril tablets. Twenty tablets were weighed and powdered in a mortar. An accurately weighed portion of the powder equivalent to about 10 mg of cilazapril was transferred to a 100 ml volumetric flask and 50 ml of methanol were added and shaken for 15 min and then completed to 100 ml. This solution (100 μg ml^{-1}) was used for voltammetry. An aliquots of this concentrated solution were diluted and measured under the optimized conditions. Calibration solutions were made by appropriate dilution in water of the concentrated standard solution.

For the determination of cilazapril in commercial formulation, 1 ml of 1 mol l^{-1} KCl was added to 5 ml of Britton–Robinson buffer (pH 9.0; 0.04 M) with 1 ml methanol and the solution was diluted to 10 ml with tri-distilled water. Deoxygenation with pre-purified nitrogen was applied for 12 min. An accumulation potential of − 0.60 V vs. Ag/AgCl was applied to the working electrode while the solution was stirred continuously. After a 10 s a cathodic differential pulse scan was initiated, and the resulting voltammograms were recorded.

2.2. HPLC

2.2.1. Apparatus

The high-performance liquid chromatographic system consisted of a Hewlett-Packard Co. Ltd. 1050 series delivery pump system equipped with a 1050 UV–Vis detector. The detector was set at 254 nm and peak areas were integrated automatically by a 3396 A multimode integrator.

2.2.2. Reagent and solutions

HPLC grade methanol (Merck) was used. The phosphate buffer was prepared by adding *o*-phosphoric acid to potassium dihydrogen phosphate to obtain a final pH of 3.5. The stock solution of cilazapril (1.0 mg ml^{-1}) and internal standard benazapril (1.0 mg ml^{-1}) were prepared in methanol. These solutions were stable for at least 2 weeks if stored at 4 °C.

2.2.3. Chromatographic condition

Chromatographic separation was carried out on LiChrospher 100 RP-18e column (250×4.6 mm^2 i.d. 5 μm). The mobile phase used in HPLC consisted 0.01 M phosphate buffer (pH 3.5; 0.125 M)–acetonitrile, (67:33, v/v). The mobile phase was prepared daily and filtered through a Alltech 47 mm, 0.45 μm membrane, degassed for 15 min in an ultrasonic bath before use. The flow rate was 0.8 ml min^{-1}. The detector was set at 254 nm. The injection volume was 60 μl. All assay was performed at ambient temperature.

Standard solutions of cilazapril and benazapril contained concentration ranges of 1–5 μg ml^{-1}. Internal standard benazapril concentration was fixed as 1 μg ml^{-1} for every synthetic mixtures. All appropriate dilutions were prepared daily. Sixty microliter volume of each synthetic samples was injected and all application were repeated three times. The peak area ratios of active substances to internal standard were plotted against the corresponding concentration ratios of cilazpril to benazapril.

2.2.4. Application to cilazapril tablets

Twenty tablets (Inhibace and Inhibace Plus) were weighed and powdered. A portion of the powder equivalent to about 5 mg cilazpril was weighed accurately, transferred to a 50 ml volumetric flask and was stirred with 40 ml methanol on a magnetic stirrer for 30 min. The solution was filtered and diluted with methanol. Two milliliter of this solution and 1 ml internal standard were added into 10 ml flask and completed with methanol. Sixty microliter volume of sample solution was injected into a column.

3. Result and discussion

Fig. 1 shows repetitive cyclic voltammograms of 0.6 μg ml^{-1} cilazapril, recorded after pre-concentration at − 1.0 V vs. Ag/AgCl for 10 s.

A large and well defined cathodic peak was observed at the first scan (curve 1) at 1.33 V vs. Ag/AgCl. A substantial decrease in the peak current value was seen in subsequent scans reaching a steady state, indicating that cilazapril shows adsorptive characteristics at a mercury electrode. No peak was observed on the reverse anodic scan, indicating that the reduction of cilazapril at the mercury electrode was irreversible. Adsorptive stripping voltammograms obtained for increasing values of the scan rate showed the existence of a linear dependence of the peak current intensity on the scan rate between 2 and 10 mV s^{-1}. The peak currents were directly proportional to the scan rate indicating that the system was adsorption-controlled.

Well defined stripping peaks were obtained with a peak potential of -1.33 V vs. Ag/AgCl for the concentration range of 0.2–1.2 μg ml^{-1}. Fig. 2a shows the plot of the differential pulse voltammetric peak current vs. accumulation time for 0.4 μg ml^{-1} cilazapril.

As can be seen in Fig. 2a, the peak current value depends strongly on the accumulation time, suggesting an effective adsorption of cilazapril

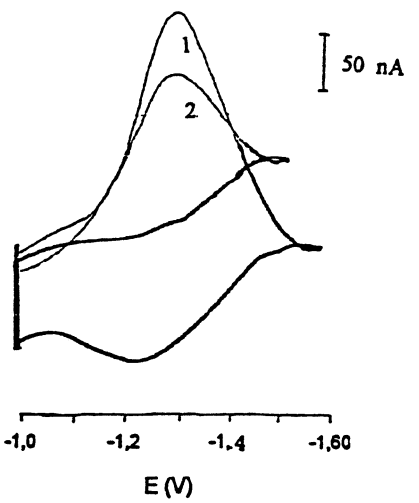

Fig. 1. Cyclic voltammetry of 0.6 μg ml^{-1} cilazapril. Scan 1 was done immediately after extrusion of new mercury drop; scan 2 was a repeat scan on the same drop.

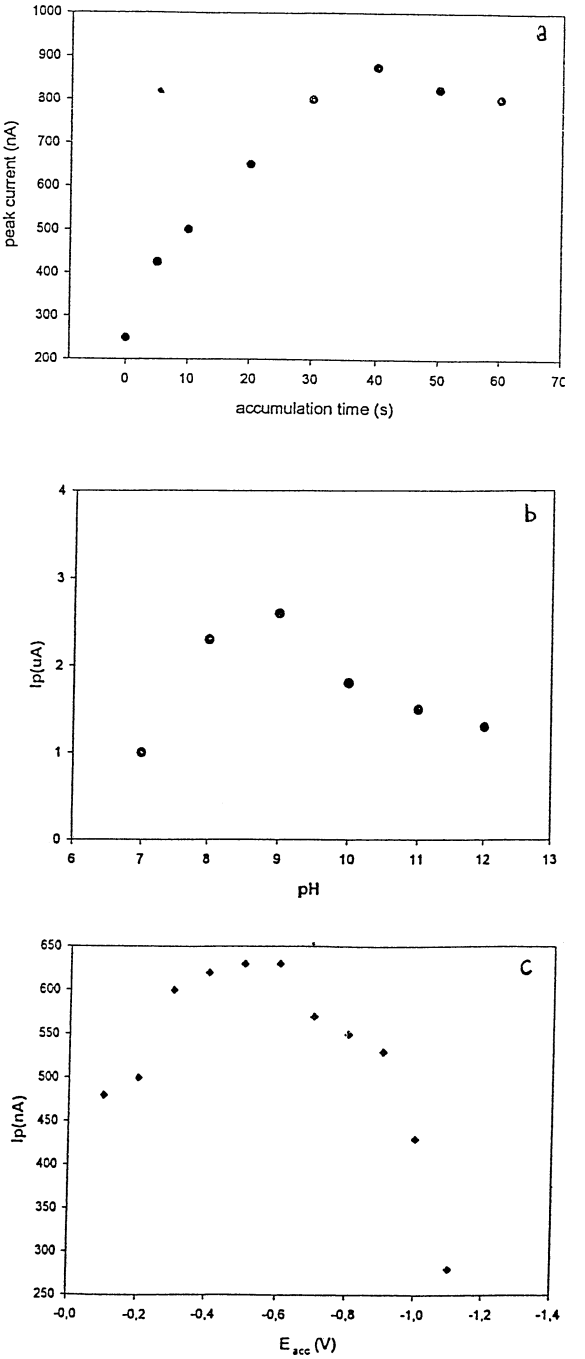

Fig. 2. The effect of varying: (a) the accumulation time on the peak height 0.4 μg ml^{-1} cilazapril; (b) pH on the differential pulse adsorptive stripping response for 1.0 μg ml^{-1} cilazapril. Adsorption potential -0.6 V; accumulation time 10 s; (c) adsorption potential on the differential pulse adsorptive stripping response for 0.6 μg ml^{-1} cilazapril.

complex on the HMDE. The peak current increased linearly with the accumulation time. After a certain accumulation time, the peak current leveled off, illustrating that the adsorptive equilibrium of cilazapril on the mercury electrode surface was finally achieved. An accumulation time of 10 s was selected as an optimum for concentrations of cilazapril lower than 1.2 μg ml^{-1}.

The effect of the solution pH in the range of 2–12 on the peak current values for the cilazapril was investigated by using adsorptive differential pulse stripping voltammetry. It was found that the peak currents were higher in basic media and the cilazapril peak potential was pH independent. At pH values smaller than 7.0, the adsorptive differential-pulse stripping voltammetric peak was not observed (Fig. 2b). Various supporting electrolytes, such as NH_3–NH_4Cl, NaOH and Britton–Robinson buffer solution were tested in adsorptive differential pulse stripping voltammetry. The maximum signal was obtained using Britton–Robinson buffer of pH 9.0. Therefore, 0.04 M Britton–Robinson buffer of pH 9.0 and ionic strength of 0.1 M was selected and employed throughout the study.

The influence of the adsorption potential on the peak height was studied over a wide range of potentials as shown in Fig. 2c. The peak height was almost constant and maximum when the adsorption potential lied between −0.3 and −0.6 V vs. Ag/AgCl. When the potential was made more negative the peak height decreased due to reduction in the amount of the adsorbed cilazapril. Therefore, the deposition potential was fixed at −0.6 V vs. Ag/AgCl for all further experimental measurements.

3.1. Quantitative determination of cilazapril

The electrochemical analysis was carried out at −0.6 V vs. Ag/AgCl accumulation potential, 10 s accumulation time, scan rate 10 mV s^{-1} and pH of 9.0. Regression analysis was carried out on the slope, intercept and the correlation coefficient using the results obtained by electroanalyis and HPLC analysis (Table 1).

The linearity of the calibration graph was validated by the high value of the correlation coefficient of the regression equation and by the value of the intercept on the ordinate. This way a sensitive and rapid determination of cilazapril could be achieved and the results were in good agreement with the labelled amount of cilazapril (Table 2).

Recovery studies on the proposed method were performed by analysing spiking sample of the powdered tablets with appropriate amounts of the stock solution of the cilazapril. The recovery was calculated from five measurements of cilazapril. The mean recovery was 103% and the relative standard deviations was 4.80%. The intra-day precision for cilazapril (calculated from five measurements of 0.60 μg ml^{-1} cilazapril during 1 working day) was 4.80%. The inter-day precision (calculated from five measurements of 0.60 μg ml^{-1} cilazapril over 1-week period) was 4.05%. The results show the accuracy and reproducibility of the assay.

Under the optimum experimental condition selected above two different commercial formulations inhibace and Inhibace Plus were also analyzed. For 10 s accumulation time, detection limit (LOD) was found to be 17.6 ng ml^{-1} according to the 3 s m^{-1} definition where s is the

Table 1
Statistical analysis for the calibration curve of cilazapril

	Linearity range (μg ml^{-1})	Regression equation	Correlation coefficient
Voltammetry	0.20–1.2	$y = 436.6C + 293.9$; SD of slope = 4.7; SD of intercept = 1.8	0.9998
HPLC	0.50–20	$y = 0.29C + 0.0018$; SD of slope = 7.6×10^{-3}; SD of intercept = 25×10^{-3}	0.9911

C is the concentration of the analyte (μg ml^{-1}).

Table 2
Results obtained in the determination of cilazapril in two different commercial tablets using adsorptive stripping voltammetry

Sample no.	Inhibace		Inhibace Plus	
	Amount found (mg per tablet)	Amount labelled (5 mg per tablet)	Amount found (mg per tablet)	Amount labelled (5 mg per tablet)
1	5.32	Mean = 4.99	5.25	Mean = 5.08
2	5.06	SD = 0.303	4.83	SD = 0.292
3	5.22	CV = 6.07	5.08	CV = 5.75
4	4.77	CF = 4.63–5.35	5.48	CL = 4.72–5.44
5	4.60	($P = 0.05$)	4.78	($P = 0.05$)

SD, standard deviation; CV, coefficient of variation; CL, confidence limits.

standard deviation ($n = 10$) of the signal from 0.2 μg ml^{-1}) cilazapril aliquots and m is the slope of the calibration graph [19]. Limit of quantification (LOQ) was calculated to be 176 ng ml^{-1} (10 times the limit of detection).

Quantitative determination of cilazapril in tablets was also conducted by HPLC method for comparison. Figs. 3 and 4 show typical chromatograms obtained from the analysis of standard cilazapril solution and cilazapril containing tablet solution, respectively. Benazapril was used as internal standard (1.0 mg ml^{-1}). As shown in these figures, standard cilazpril and tablet solutions were eluted, forming well shaped, symmetrical single peaks and well separated from the solvent front.

The values obtained with HPLC method was in good agreement with those obtained by adsorptive stripping method (Table 3). The results of both methods were compared by Student's t-test and f (fisher)-test. When precision of the both methods were compared with f-test, there was no significant difference between the standard deviations of the two methods. In addition, as far as Student's t-test results were concerned, there existed no significant difference between the two methods with respect to the mean values (Table 4).

The method can be used for the estimation of cilazapril without prior separation hydrochlorothiazide employed in Inhibace Plus tablets. Since hydrochlorothiazide and other excipients are electroinactive under these condition, they did not interfere with the assay of cilazapril. This observa-

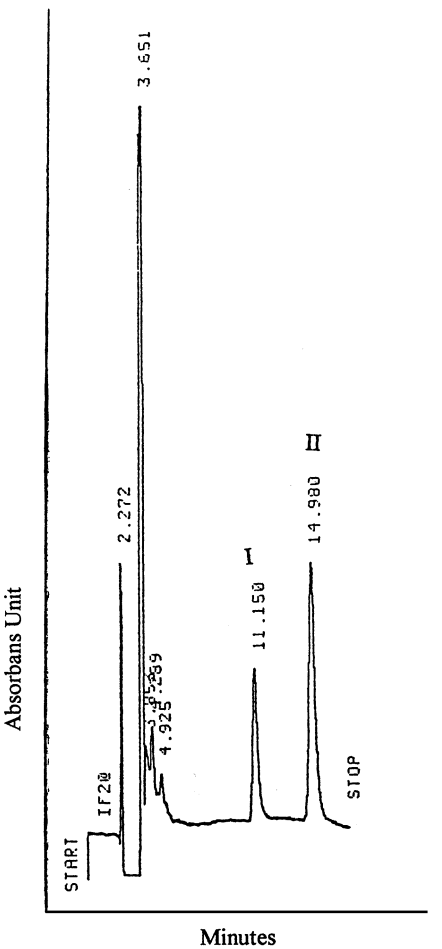

Fig. 3. HPLC chromatogram of cilazapril standard in acetonitrile–phosphate buffer: (I) cilazapril (2 μg ml^{-1}); (II) benezapril (1 μg ml^{-1}).

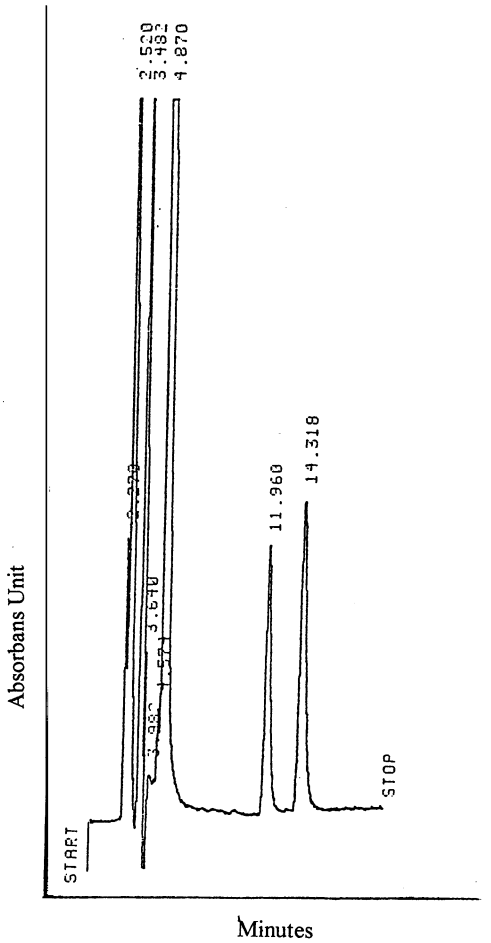

Fig. 4. HPLC chromatogram of commercial tablet (Inhibace Plus) solution in acetonitrile–phosphate buffer.

Table 4
Comparison of two methods

Amount labelled (5 mg per tablet)	Voltammetric method	HPLC method
Amount found (mg per tablet) average values ($n = 5$)	4.99	4.78
CV	6.07	2.82
$t_{calculated} = 1.33$	$t_{theoretical} = 2.31$	$(P = 0.05)$
$f_{calculated} = 5.04$	$f_{theoretical} = 6.39$	$(P = 0.05)$

tion indicate that the method is selective for cilazapril. Also voltammetric method can not be developed as a stability indicating assay method because of the lack of the knowledge of the potential decomposition products.

4. Conclusion

The developed method is suitable technique for application to the determination of cilazapril in pharmaceutical products. In comparison, chromatographic methods for the determination of cilazapril need expensive equipment and materials, also include time-consuming extraction steps to eliminate the additives. In voltammetric methods, high percentage of recovery shows that the compounds are completely extracted from tablet formulations and the results indicate that the developed method can be used to quantify cilazapril in binary combination without interference from other ingredients. Most spectrophotometric methods include complex reactions which cause

Table 3
Results obtained in the determination of cilazpril in tablets using HPLC method

Sample no.	Inhibace		Inhibace Plus	
	Amount found (mg per tablet)	Amount labelled (5 mg per tablet)	Amount found (mg per tablet)	Amount labelled (5 mg per tablet)
1	4.60	Mean = 4.78	4.48	Mean = 4.67
2	4.72	SD = 0.135	4.67	SD = 0.117
3	4.74	CV = 2.82	4.71	CV = 2.25
4	4.88	CL = 4.61–4.95	4.68	CL = 4.52–4.82
5	4.94	$(P = 0.05)$	4.80	$(P = 0.05)$

contamination and loss of substance and all of them have a lower sensitivity than proposed voltammetric method.

It can be concluded that, the proposed voltammetric method has the advantages of being simpler, faster, less tedious and expensive than published procedures for the analysis of cilazapril. The described method is a direct method for the determination of cilazapril and does not include any extraction process.

References

[1] J.R. Rosenthal, U. Osowski, Cardiology 87 (1) (1996) 54–59.
[2] O.W. Foye, Principles of Medicinal Chemistry, vol. 19, 3rd ed., Lea & Jebliger, Philadelphia, 1989, pp. 401–412.
[3] G.A. Gilman, T.W. Rall, A.S. Nies, P. Taylor, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, New York, 1990, pp. 718–712.
[4] F. Deget, R.N. Brogden, Drugs 41 (5) (1991) 799–820.
[5] T. Szucs, Drugs 41 (Suppl. 1) (1991) 18–24.
[6] I.L. Natoff, J.S. Nixon, R.J. Francis, L.R. Klevans, M. Brewster, J. Budd, A.T. Patel, J. Wenger, E. Worth, J. Cardiovasc. Pharmacol. 7 (3) (1985) 569–580.
[7] H. Tanaka, Y. Yoneyama, J. Pharm. Sci. 76 (3) (1987) 224–227.
[8] N. Erk, F. Onur, Anal. Lett. 29 (11) (1996) 1963–1974.
[9] N. Erk, J. Pharm. Biomed. Anal. 20 (1–2) (1999) 155–167.
[10] A. Gumieniczek, L. Pryborowsski, J. Liq. Chromatogr. Rel. Technol. 20 (13) (1997) 2135–2142.
[11] O. Atay, U. Tamer, D. Arikan, Anal. Lett. 34 (7) (2001) 1153–1161.
[12] J.A. Prieto, R.M. Cimenez, R.M. Alonso, J. Chromatogr. B 714 (1998) 285–292.
[13] S. Hillaert, W. Van den Bossche, J. Chromatogr. A 895 (1–2) (2000) 33–42.
[14] J.A. Prieto, U. Akesolo, R.M. Jimenez, R.M. Alonso, J. Chromatogr. A 916 (1–2) (2001) 279–288.
[15] A. Sioufi, F. Pommier, J. Chromatogr. 434 (1988) 239–246.
[16] H.J. Leis, M. Leis, W. Welz, J. Chromatogr. 529 (1990) 299–308.
[17] N. Goto, T. Kamata, K. Ikegami, J. Chromatogr. 578 (1992) 195–201.
[18] P.M. Bersier, J. Bersier, in: M.R. Smyth, J.G. Vos (Eds.), Comprehensive Analytical Chemistry, vol. XXVII, Elsevier, Amsterdam, New York, 1992, pp. 159–465.
[19] K. Hasabe, J. Osteryoung, Anal. Chem. 47 (1975) 2412–2418.