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Square wave voltammetric determination of the angiotensin-converting enzyme inhibitors cilazapril, quinapril and ramipril in pharmaceutical formulations

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Abstract

The angiotensin-converting enzyme inhibitors cilazapril, quinapril and ramipril are reduced at a hanging mercury drop electrode in the pH range 3.5–13 using Britton–Robinson buffers as supporting electrolyte and KCl as ionic medium. Square wave voltammetry has proved to be the most suitable electroanalytical technique for the quantitative voltammetric determination of these antihypertensive drugs. Optimisation of the chemical and instrumental variables was carried out. Analyses were performed in 0.02 M borate buffer at pH 9.5 and 0.5 M KCl as ionic medium, using a pulse amplitude of 50 mV and a frequency of 150 Hz. A linear relationship between peak current and concentration was found in the interval 0.5–8 μg/ml for cilazapril and up to 6 μg/ml for quinapril and ramipril, allowing the direct determination of their pharmaceutical formulations alone or mixed with hydrochlorothiazide. Good accuracy and repeatativity were obtained.

Keywords: Square wave voltammetry; Cilazapril; Quinapril; Ramipril; Pharmaceuticals

1. Introduction

Cilazapril, quinapril and ramipril are potent and specific angiotensin-converting enzyme (ACE) inhibitors that lower peripheral vascular resistance without affecting heart rate. They are used in treatment of hypertension and congestive heart failure [1–3]. The role of these kinds of drugs is to inhibit the last step of the biosynthesis of angiotensin II, a potent vasoconstrictor, and therefore, it causes a general vasodilatation and lowers blood pressure [4,5]. The use of ACE inhibitors is acquiring increasing importance, being nowadays the most prescribed group of antihypertensive drugs. Lever et al. have shown that the long-term use of ACE inhibitors might protect against cancer [6].

Following oral administration, all drugs undergo deesterification in the liver to their active di-acid forms, cilazaprilat, quinaprilat and ramiprilat, respectively. These metabolites are more polar than the corresponding prodrug and thus their intestinal absorption is low. However, the ester prodrugs are well absorbed orally. Little pharmacological differences have been found between all available ACE inhibitors, although ramipril is the preferred choice if the renal function is impaired [7,8]. The structural formulae of the ACE inhibitors studied in this work are shown in Fig. 1.

Analytical methods for the determination of these drugs in their pharmaceutical formulations are scarce. Cilazapril and its mixture with hydrochlorothiazide have been determined by derivative spectrophotometry [9,10]. The determination of ramipril by spectrophotometric methods has been carried out by means of the formation of coloured complexes with different ligands [11], or extraction of ternary compounds in which a metal atom is involved and measured by AAS [12,13]. These drugs have been also determined by liquid chromatography, cilazapril [14–16], quinapril [17] and

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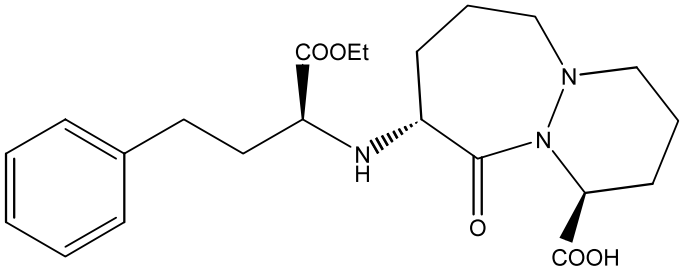
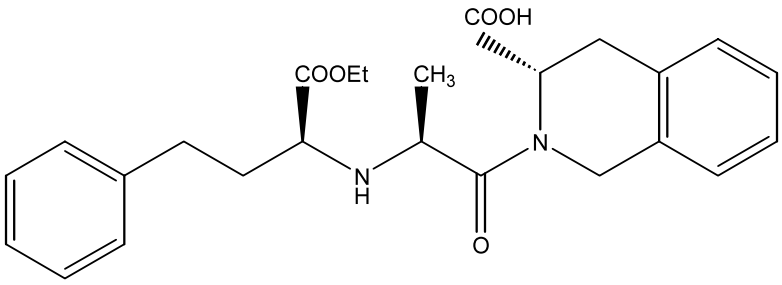
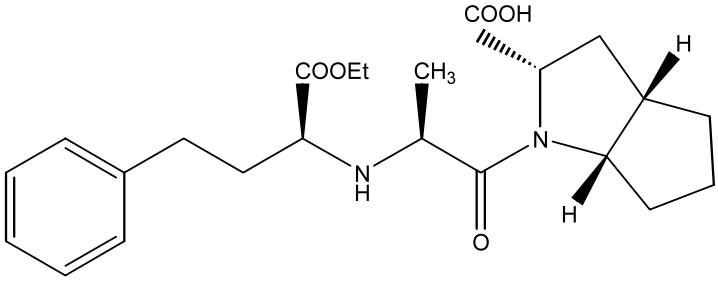


Cilazapril



Quinapril



Ramipril

Fig. 1. Structural formulae of cilazapril, quinapril and ramipril.

ramipril [18,19]. A mixture of several ACE inhibitors has been separated and their formulations determined by HPLC [20] and capillary electrophoresis [21]. Cilazapril [22] and quinapril [23] have also been determined in its pharmaceutical forms by capillary electrophoresis.

Electroanalytical methods have been preferentially applied to captopril, the first commercialised member of this family of antihypertensive drugs, which is a thiol containing drug and, thus easily oxidisable. It is worthwhile to mention the works reported by Aboul-Enein et al. [24] on the construction of potentiometric plastic membrane sensors for the analysis of cilazapril and ramipril among other ACE inhibitors. Stefan et al. [25] have developed amperometric biosensors based on L-amino acid oxidase for the analysis of S-enantiomers of enalapril maleate and ramipril. The same authors have used developed electrodes impregnated with cyclodextrins for the determination of *S*-cilazapril among other ACE inhibitors [26].

Three voltammetric methods are found in bibliography. Cilazapril has been analysed in its dosage forms by adsorptive stripping voltammetry at a hanging mercury drop electrode (HMDE) after accumulation at −0.6 V and subsequent determination by differential pulse voltammetry (DPV) [27]. Ramipril has been determined in its dosage forms, plasma and urine using three different voltammetric techniques: direct current polarography (DC), differential pulse polarography and alternating current polarography (AC). The three mentioned modes were useful for the analysis of tablets, but only AC polarography was applicable to its analysis in plasma and urine [28]. A more recent method reports the determination of ramipril by the same techniques mentioned above after its derivatization with nitrous acid [29].

The lack of suitable chromophores and the low wavelength of absorption of these drugs make difficult their determination by UV–Vis spectrophotometry, thus the development of rapid and simple methods for their direct determination in tablets is required.

The aim of this paper is to study the electrochemical behaviour of cilazapril, quinapril and ramipril in order to establish a method for their determination in pharmaceutical formulations.

2. Experimental

2.1. Reagents and solutions

The studied ACE inhibitors were kindly supplied by the pharmaceutical companies: cilazapril hydrate by Roche Farma (Barcelona, Spain), quinapril hydrochloride by Parke-Davis (Barcelona), and ramipril by Hoechst (Barcelona). Stock solutions containing 200 μg/ml of the drugs were prepared in water and stored in the dark under refrigeration.

Stock solutions of Britton–Robinson (B–R) buffers, which were 0.04 M in acetic acid, boric acid and phosphoric acid, were adjusted to the desired pH by adding the necessary drops of 3 M KOH or concentrated HCl. All the acids used were supplied by Merck (Darmstadt, Germany) and were pro analysis quality.

Salt solutions (KCl, KNO_3 and $NaClO_4$) at a concentration 2.5 M were prepared as ionic media, all them were analytical-reagent grade (Merck). Mercury was Merck and for polarography. Water was obtained from a Waters (Milford, MA) Milli-RO, Milli-Q system.

All solutions were degassed by passing N_2 through for 4 min prior to electrochemical analyses.

2.2. Apparatus

Voltammetric measurements were performed using an Eco Chemie (Utrecht, The Netherlands) μAutolab voltammetric analyser coupled to a Metrohm (Herisau, Switzerland) Model VA 663 three-electrode stand. The auxiliary electrode was a platinum rod, the reference electrode was a saturated calomel electrode and the working electrode was a HMDE (surface area 0.5 mm^2). Data collection and manipulation was performed using the program GPES (Eco Chemie).

The pH value of the solutions was measured using a Radiometer (Copenhagen, Denmark) PHM84 voltmeter with a Crison (Alella, Spain) combined glass/Ag/AgCl(s)/KCl electrode.

2.3. Experimental conditions

Quantitative analyses of cilazapril, quinapril and ramipril were performed on a solution containing 0.02 M borate buffer at pH 9.5 as supporting electrolyte and 0.5 M KCl as ionic medium. Instrumental conditions were: technique square wave voltammetry (SWV), purge time 240 s with N_2, initial potential −0.8 V, final potential −1.5 V, equilibration time 5 s, frequency 150 Hz, pulse amplitude 50 mV and step potential 5 mV. All measurements were performed at room temperature and the peak intensity recorded was the average value of three consecutive measurements.

Cyclic voltammetric analyses were performed on solutions containing 0.02 M B–R buffer and different pH values, and 0.5 M KCl as ionic media. The initial and final potential were variable, depending on the pH value and the cut-off of the electrolyte. Measurements in the range 10–2000 mV/min were carried out.

2.4. Procedure for tablets

Eleven different pharmaceutical formulations were analysed in this work, five of them containing cilazapril: Inhibace (cilazapril 1 and 5 mg) and Inhibace plus (cilazapril 5 mg and hydrochlorothiazide 12.5 mg) commercialised by Andreu-Roche (Barcelona, Spain); Inocar (cilazapril 1 mg) and Inocar plus (cilazapril 5 mg and hydrochlorothiazide 12.5 mg) commercialised by Nezel (Barcelona, Spain). Three contained quinapril as active component: Ectren (quinapril 20 and 40 mg) and Bicetil (quinapril 20 mg and hydrochlorothiazide 12.5 mg), from Menarini (Barcelona). Finally the content of ramipril was determined in Carasel (ramipril 2.5 and 5 mg) commercialised by Hoechst (Barcelona), and Acovil (ramipril 2.5 mg) from Almirall (Barcelona).

Four tablets of each formulation were weighed and then crushed into a fine powder in a mortar. A suitable amount of this powder was accurately weighed, dissolved in desionised water and sonicated for 10 min. The solutions were centrifuged for 5 min at $800 \times g$, and the liquid was transferred to a volumetric flask. The residual solids were washed twice with water in order to extract completely the active components. The clear solutions were added to the volumetric flasks, which were finally made up with water.

Aliquots of these solutions were added to the pH 9.5 borate buffer and the ionic medium, in order to prepare sample solutions within the calibration range (see Section 3.2), degassed and measured under calibration conditions. The process was repeated using two different groups of tablets. Three replicates of each measurement were done.

2.5. Quantitative determination

All measurements are the mean value of three replicates. The repeatability of the method was determined at three concentration levels, using solutions containing 0.5, 3 and 6 μg/ml of each drug. Intra-day precision was performed by calculating the %RSD of 11 determinations at each concentration level. Inter-day repeatativity was calculated as the %RSD of the mean peak intensity produced by each solution in the time spell of 5 days using the ANOVA method [32].

Accuracy was estimated by preparing different solutions containing cilazapril, quinapril or ramipril. Two different solutions were used and three replicates of each were done. The mean value was used to calculate the concentration by interpolation, introducing the peak currents in the calibration curve.

Detection limits were determined as the concentration of cilazapril, quinapril or ramipril that produced a signal-to-noise ratio of 3, and quantitation limits as those that produced a S/N ratio of 10. Instrumental noise was estimated from a blank solution.

3. Results and discussion

The reduction of cilazapril, quinapril and ramipril at a HMDE was studied, using SWV and DPV. These ACE inhibitors are reduced in the pH range 3.5–13 using 0.02 M B–R buffers and 0.5 M KCl as ionic medium. DPV showed to be a much less sensitive technique for quantitative purposes than SWV, and yielded very badly defined peaks, thus DPV was not used for further studies. The oxidation of the drugs was also attempted using DPV and SWV at several pH values on a glassy carbon electrode, but no voltammetric peak was obtained for any of them.

In order to choose the appropriate pH value for the determination of these ACE inhibitors, a study of the influence of pH on the reduction process was carried out. KCl was selected as ionic medium for voltammetric experiments because it permitted the wider range of pH to be tested without interference of the cut-off of the electrolyte. The voltammograms of cilazapril, quinapril and ramipril at several pH values are shown in Fig. 2 along with the peak potential E_p–pH plots. All studied drugs had very similar electrochemical behaviour, three straight lines are obtained for cilazapril and two for quinapril and ramipril. Wide peaks are always obtained, this fact is especially remarkable for the reduction of ramipril at the most acidic pH values, where two peaks are obtained. This can be attributed to the coexistence of two rotamers, due to the slow rotation through the amide bound, which has a partial double bound character and gives rise to two different diasteroisomers [30]. In all cases, the peak potential and intensity remains constant at the most alkaline pH values, pH 9–12 for cilazapril, pH 8–12 for quinapril and pH 7–12 for ramipril. In addition, the highest sensitivity was obtained at these pH values, although no great variations were observed in the pH interval. The intersection of the straight lines obtained for cilazapril, quinapril and ramipril were pH 8.19, 7.04 and 6.61, respectively. These values do not correspond to the pK_a values of their amine groups, which are 6.0 for cilazapril and 5.5 for quinapril and ramipril [31].

Cyclic voltammograms of the three compounds were carried out at three pH values for cilazapril and two for quinapril and ramipril. The selected pH values were located in the middle of the pH intervals delimited by straight lines in Fig. 2. In all cases, only cathodic waves were obtained, and the system was irreversible, also corroborated by the linear relationship between peak potential and the logarithm of scan rate. The nature of the reduction process was adsorption controlled, as peak intensities were proportional to the scan rate, also noticed by Tamer et al. [27]. However, little is known about the electrode reaction, although Al-Majed et al. suggest that the carbonyl group of the amide bound, activated by the neighbouring nitrogen, might undergo reduction to alcohol, taking two electrons and two protons [28].

A pH value of 9.5 was selected for quantitative purposes, since small changes in the pH did not affect either the peak potential or the intensity. Once the pH value was fixed, 0.02 M borate buffer at pH 9.5 was used as supporting electrolyte instead of B–R buffer, since no difference was observed and it avoided the use of acetic and phosphoric acids, which do not participate in the buffer capacity at that pH.

The following task was the optimisation of the chemical variables in order to obtain the best peak shapes and sensitivity. Firstly, a study of the influence of the ionic medium was carried out. Three different compositions were tested: 0.5 M KCl, KNO_3, $NaClO_4$. Although the influence of the ionic medium was negligible, KCl was chosen since slightly more sensitive and narrower peaks were obtained. The influence of the concentration of KCl was also studied in the range 0.05–0.5 M. KCl at a concentration 0.5 M was selected because slightly more sensitive peaks were obtained. The absence of ionic medium produced slightly wider and less sensitive peaks.

The next step was the optimisation of the instrumental variables. The influence of the frequency was studied in the range 25–175 Hz and the pulse amplitude from 25 to 100 mV. In all cases, peak intensities increased with frequency, but at 175 Hz the sweep was so quick that peak definition was lost. A frequency of 150 Hz was selected as optimal. Pulse amplitude was an important variable in order to obtain symmetrical and narrow peaks. A pulse amplitude of 50 mV was selected, since it produced the best peak shape, although the sensitivity is not the maximum and the presence of shoulders could not be completely avoided for ramipril. Fig. 3 shows, as example, the different voltammograms obtained for quinapril during the process of optimisation of the instrumental variables.

3.1. Quantitative determination

A calibration curve was made for each antihypertensive agent, showing the linear dependence between peak current and concentration in the range from 0.5 to 8 μg/ml for cilazapril and from 0.5 to 6 μg/ml for quinapril and ramipril. These relationships were used for the analysis of tablets. The quantitative and statistic parameters obtained for the validation of the methods are collected in Table 1.

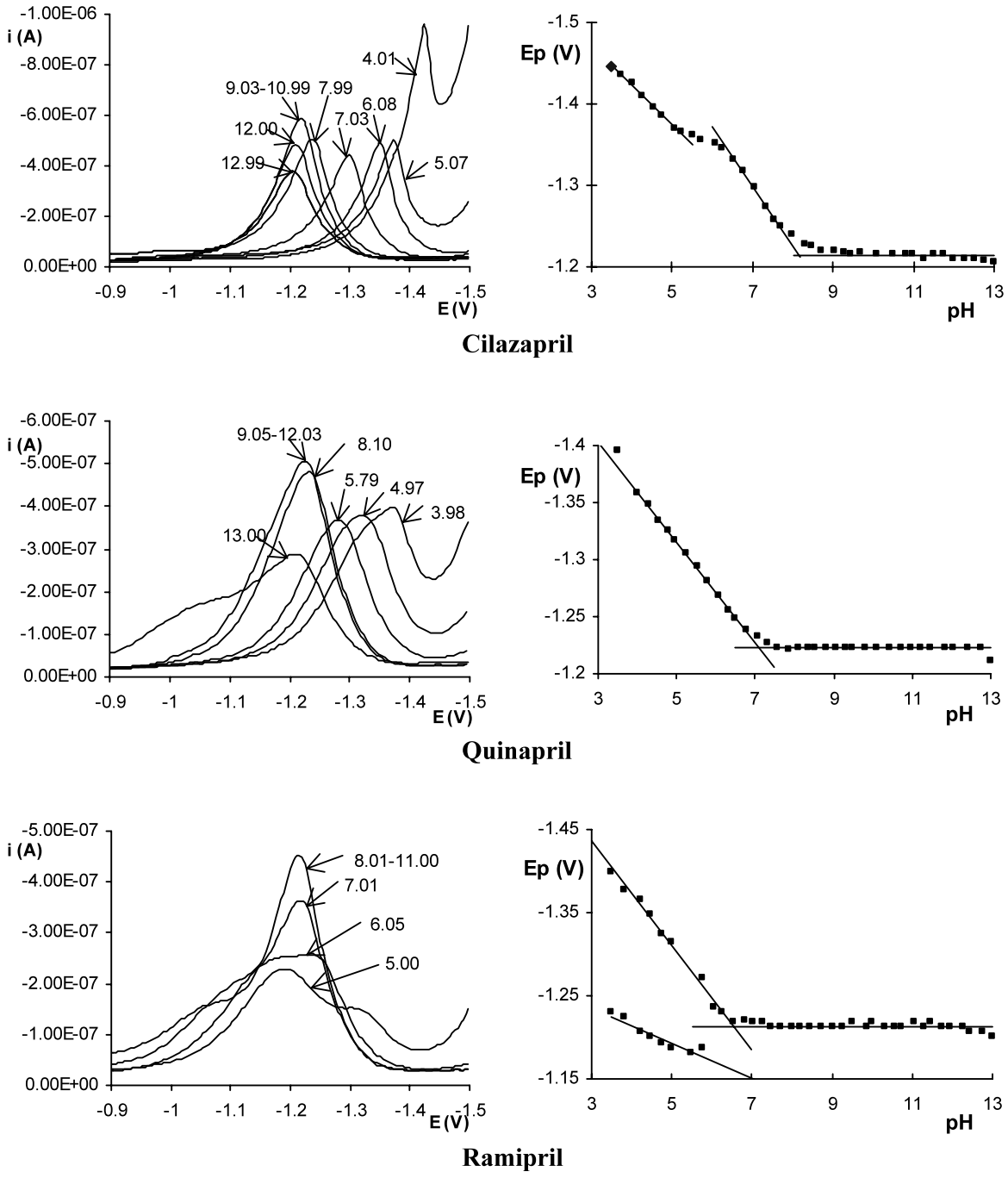


Fig. 2. Square wave voltammograms and peak potential–pH plots of cilazapril, quinapril and ramipril at several pH values. Concentration of the drugs 4 μg/ml, ionic medium 0.5 M KCl, supporting electrolyte 0.02 M B–R buffers, frequency 150 Hz, pulse amplitude 50 mV.

3.2. Analytical applications

Aliquots of the stock tablet solutions were added to the borate buffer and the KCl in order to prepare sample solutions containing ca. 4 μg/ml of cilazapril and 2 μg/ml of quinapril and ramipril, taking into account the calibration interval for each drug. Results for all pharmaceutical formulations containing cilazapril were much lower than the expected taking into account the certified value. Tablets contain a surface active excipient (sodium stearil fumarate) which produced bubbles and suds when the solution is purged with N_2. This compound is probably adsorbed on the mercury surface, inhibiting the reduction of cilazapril. However, this interference could be avoided if sample solutions of lower concentration were prepared. The peak current i_p-concentration relationship was plotted for the five pharmaceutical formulations containing cilazapril. A linear relationship was obtained up to a certain value, where the straight line bent due to the presence of the

Table 1
Quantitative determination of cilazapril, quinapril and ramipril by SWV

Parameter	Cilazapril	Quinapril	Ramipril
Peak potential (V)	−1.21	−1.22	−1.21
Calibration interval (μg/ml)	0.5–8	0.5–6	0.5–6
Slope ± ts, (nA/μg/ml) (95%), $n = 6$	132 ± 8	109 ± 3	94 ± 2
Intercept ± ts, (nA) (95%), $n = 6$	7 ± 21	1 ± 9	2 ± 6
Correlation coefficient r^2	0.998	0.999	0.999
Intra-day repeatativity (11 replicates), (%RSD) [a]	1.0; 1.8; 1.1	1.6; 2.2; 1.5	1.7; 1.5; 0.8
Inter-day repeatativity (5 days), (%RSD) [a]	10.4; 6.4; 5.4	3.0; 6.0; 2.7	3.1; 2.6; 2.6
Accuracy (% error)	6.4; 0.34; −1.9	−0.20; 2.9; 0.3	4.5; −1.6; 0.15
Detection limit (ng/ml), (S/N = 3)	75	90	110
Determination limit (ng/ml), (S/N = 10)	250	300	370

[a] Concentration of the solutions 0.5, 3 and 6 μg/ml, respectively.

interfering excipient (Fig. 4). From this plot, it was deduced that the sample solutions of Inhibace plus and Inocar plus should be prepared to give an approximate final concentration of cilazapril 3 μg/ml, 2 μg/ml for Inhibace 5 mg and 1.5 μg/ml for Inhibace and Inocar 1 mg. If the sample solutions do not exceed these values of concentration, the results obtained are correct. The quantitative determination of the 11 studied pharmaceuticals is presented in Table 2. In the case of Inhibace plus, Inocar plus and Bicetil, which also contain 12.5 mg of hydrochlorothiazide, no interference of the diuretic was found. Relative errors are less than 2% in all cases except in the case of Inhibace 5 mg, which had expired 2 years before. The voltammograms that correspond to different pharmaceuticals of the three antihypertensive agents are shown in Fig. 5.

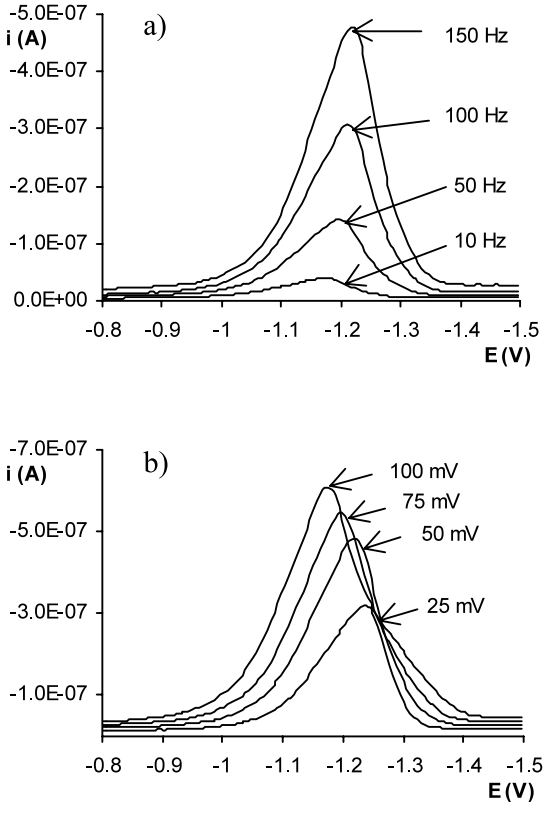


Fig. 3. (a) Influence of the frequency on the square wave voltammetric peaks, pulse amplitude 50 mV, (b) Influence of the pulse amplitude on the square wave voltammograms, frequency 150 Hz. Concentration of quinapril 4 μg/ml, ionic medium 0.5 M KCl, supporting electrolyte 0.02 M borate buffer at pH 9.5.

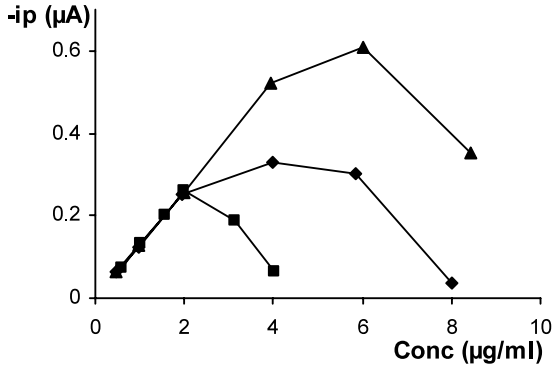


Fig. 4. Dependence of peak intensity (i_p) with the concentration of cilazapril for different tablet samples: (■) Inhibace and Inocar 1 mg, (▲) Inhibace 5 mg and (◆) Inhibace Plus and Inocar Plus. Ionic medium 0.5 M KCl, supporting electrolyte 0.02 M borate buffer at pH 9.5, frequency 150 Hz, modulation amplitude 50 mV.

Table 2
Determination of cilazapril, quinapril and ramipril in its pharmaceutical formulations

Formulation	Certified content (mg)	Found ± SD (mg) [a]
Inocar	1 cilazapril	1.01 ± 0.02; 0.99 ± 0.01
Inocar Plus	5 cilazapril, 12.5 HCT	5.01 ± 0.05; 4.97 ± 0.09
Inhibace	1 cilazapril	1.00 ± 0.02; 0.98 ± 0.01
Inhibace Plus	5 cilazapril, 12.5 HCT	4.98 ± 0.06; 4.99 ± 0.07
Inhibace (expired)	5 cilazapril	4.54 ± 0.02; 4.51 ± 0.03
Ectrén	40 quinapril	40.3 ± 0.6; 40.1 ± 0.5
Ectrén	20 quinapril	20.1 ± 0.6; 20.2 ± 0.3
Bicetil	20 quinapril, 12.5 HCT	20.1 ± 0.4; 20.2 ± 0.2
Carasel	5 ramipril	5.02 ± 0.11; 4.94 ± 0.01
Carasel	2.5 ramipril	2.48 ± 0.02; 2.49 ± 0.05
Acovil	2.5 ramipril	2.45 ± 0.03; 2.47 ± 0.05

[a] Two different batches. Three replicates of each determination.

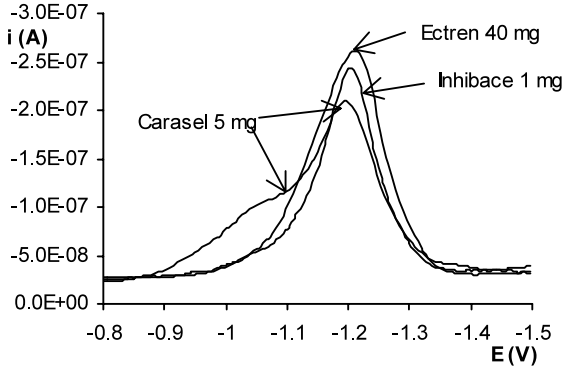


Fig. 5. Square wave voltammograms of the pharmaceutical formulations Inhibace, cilazapril 1 mg; Ectren, quinapril 40 mg, and Carasel, ramipril 5 mg. Concentration of cilazapril in the solution 1.5 μg/ml, concentration of quinapril and ramipril 2 μg/ml, ionic medium 0.5 M KCl, supporting electrolyte 0.02 M borate buffer at pH 9.5, frequency 150 Hz, modulation amplitude 50 mV.

4. Conclusions

From the results obtained, it can be concluded that cilazapril, quinapril and ramipril are reduced at a HMDE in the pH interval 3.5–13. The electrodic processes allow the development of voltammetric methods for the determination of these drugs. The methods are sensitive and reproducible enough to allow their reliable determination.

Direct and rapid determination of the tablets dissolved in water can be carried out, avoiding the lack of sensitivity and the low wavelength of absorption that these kinds of compounds show in UV spectrophotometry. Also the simplicity of the method avoids the use of the expensive solvents used in HPLC.

Acknowledgements

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