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Short communication

A study of the determination of the hypertensive drug captopril by square wave cathodic adsorptive stripping voltammetry

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Abstract

In this work, the determination of captopril (CPL) was studied by square wave cathodic adsorptive stripping voltammetry (SWCAdSV) on a hanging mercury drop electrode (HMDE). CPL was adsorptively preconcentrated on the mercury surface as a sparingly soluble mercury salt under stirring of the solution and then the accumulated species was reduced by a cathodic square wave voltammetric scan. The reduction current was related to the CPL concentration in the sample. The chemical and instrumental parameters affecting the response were investigated and optimized for the CPL determination. The calibration curve was linear from 0.5 to 180 $\mu g\,l^{-1}$ of CPL (depending on the preconcentration time), the limit of detection at a S/N ratio of 3 was 0.5 $\mu g\,l^{-1}$ with 300 s of preconcentration and the relative standard deviation was 3.2% at the 20 $\mu g\ l^{-1}$ level (with 120 s of preconcentration, $n = 8$). The method was applied to the determination of CPL in two pharmaceutical formulations with recoveries of 97.9 and 98.8%. Finally, the potential for applying the proposed method to the determination of CPL in biological media is briefly discussed.

Keywords: Captopril; Square wave cathodic adsorptive stripping voltammetry

1. Introduction

Captoril, 1-[(2S)-3-mercapto-2-methylpropion-yl]-L-proline (CPL) (Fig. 1), is a synthetic dipeptide serving as an orally active inhibitor of the angiontensin-converting enzyme and has been widely used as antihypertensive drug [1] and to moderate heart failure [2]. It is, also, sometimes prescribed for angina pectoris (crushing chest pain), Raynaud's phenomenon (a disorder of the blood vessels that causes the fingers to turn white when exposed to cold) and rheumatoid arthritis [3]. Unfortunately, administering CPL for therapeutic purposes leads to undesirable side-effects. Preliminary research has indicated significant loss of zinc in urine due to the intake of CPL [4]. Although details remain unclear, it now appears that chronic use of CPL may lead to a zinc deficiency [5]. An uncommon yet potentially serious side-effect of CPL treatment (in common with other angiotensin-converting enzyme inhibitors) is increased blood potassium levels [6–8]. CPL is metabolized in the liver (it is oxidized into

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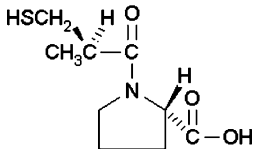

Fig. 1. The structure of CPL.

the corresponding disulfide) and is excreted mainly with the urine with 40–60% of the drug excreted unchanged [9].

Therefore, the determination of CPL is important from a physiological point of view as well as for the purposes of quality control. Various instrumental techniques have been developed to determine CPL including spectrophotometry [10,11], fluorimetry [12], chemiluminescence [13] and chromatography [14–17]. Electroanalytical techniques (such as voltammetry, amperometry and polarography) are characterized by simplicity, sensitivity, cost-effectiveness, precision, accuracy and speed and have been used for the determination of CPL [18–20].

The goal of this work was the development of a new voltammetric method for the direct determination of CPL in pharmaceutical formulations. The proposed method is based on the application of square wave cathodic adsorptive stripping voltammetry (SWCAdSV) [21]. CPL was preconcentrated on a mercury electrode as its sparingly soluble salt with mercury under stirring of the solution and then the accumulated species was reduced by a square wave voltammetric scan. The reduction current was related to the CPL concentration in the sample. The high sensitivity, considerable speed and low background current of the square wave modulation [22] make this method particularly suitable for the determination of CPL at low concentrations.

2. Experimental

2.1. Chemicals

All the chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) unless stated otherwise. Doubly distilled water was used for the preparation of the solutions. CPL was purchased from Fluka (Buchs, Switzerland). A 10 mg l^{-1} CPL solution was prepared daily and kept at 4 °C in the dark. More dilute solutions were prepared by serial dilution. Capoten (Bristol Myers Squibb, 25 mg CPL per tablet) and Pertacilon (Elpen A.E., 25 mg CPL per tablet) were purchased from a local drug store.

2.2. Equipment

Voltammetric measurements were performed with a home-made potentiostat [23] interfaced to a Pentium PC by means of a 6024E PCI multipurpose interface card (National Instruments, Austin, TX). The external potential input of the potentiostat was connected to a DAC of the interface card for potential control. The current output of the potentiostat was connected to an ADC for current sampling. The control and acquisition program was developed in-house using the LABVIEW 5.1 instrumentation software package (National Instruments) [23]. The voltammetric data were saved in ASCII format for further manipulation. Auxiliary programs for background subtraction, for peak height calculation and peak position identification were also written in LABVIEW 5.1.

The voltammetric cell was a standard 50 ml glass cell (Metrohm, Switzerland). The cell was equipped with a hanging mercury drop electrode (HMDE), a Ag/AgCl reference electrode and a Pt wire counter electrode all from Metrohm. A PTFE magnetic stirring bar, agitated by a magnetic stirrer, was placed in the bottom of the cell for stirring during the preconcentration stage. The stirrer was activated by a relay controlled by a transistor through a digital TTL line from the interface card.

An HPLC method, as recommended by the Greek Pharmacopoeia [24], was employed for the validation of this method in pharmaceutical tablets.

2.3. Experimental procedure

For aqueous solutions, 20 ml of supporting electrolyte was introduced into the cell and purged with nitrogen for 5 min. The solution was spiked with a CPL standard to give the required CPL concentration, a new mercury drop was formed and preconcentration was carried out under stirring at +0.20 V. Then, the solution was let to equilibrate at rest for 10 s and the voltammogram was recorded from +0.20 to −0.6 V.

For the determination of CPL in tablets, five tablets were mixed and powdered. A portion of the powder containing approximately 2.5 mg of CPL was accurately weighted and dissolved in 100 ml of water. 20 ml of supporting electrolyte was introduced into the cell and purged with nitrogen for 5 min. 20 μl of the sample were added, a new mercury drop was formed and preconcentration was carried out under stirring at +0.2 V. Then, the solution was let to equilibrate at rest for 10 s and the voltammogram was recorded from +0.2 to −0.6 V. Standard additions were directly made in the cell and all measurements were made in duplicate.

3. Results and discussion

3.1. Accumulation of CPL on the HMDE

CPL, in common with other thiols, forms sparingly soluble salts with Hg(II) [25]. This property can be exploited for the accumulation of the drug on the surface of the HMDE [21]. During the accumulation period, the potential of the mercury electrode is maintained at a positive potential where oxidation of metallic mercury to Hg(II) occurs according to the reaction:

$$Hg \rightleftarrows Hg^{2+} + 2e^{-}$$

The thiol diffusing to the electrode surface under convective conditions (stirring) reacts with Hg(II) and forms an insoluble salt that immediately adsorbs on the surface of the electrode:

$$2RSH_{sol} + Hg^{2+} \rightleftarrows [(RS)_2Hg]_{ad} + 2H^{+}$$

Using this accumulation step, the electrode is "enriched" with the analytically important species leading to an increase in the sensitivity of the determination. Following this preconcentration step, a voltammetric scan to the cathodic direction causes the reduction of Hg(II) in its salt with CPL:

$$[(RS)_2Hg]_{ad} + 2e^{-} + 2H^{+} \rightleftarrows 2RSH_{sol} + Hg$$

The reduction current can then be related to the initial CPL concentration in the sample.

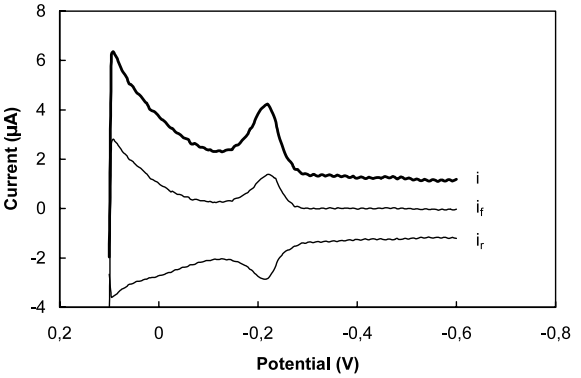

Fig. 2. SW voltammogram of a CPL solution showing the differential current, i, and the constituent forward, i_f, and differential, i_r, currents. CPL concentration: 25 μg l^{-1}; preconcentration time: 120 s; supporting electrolyte: HNO_3 0.1 mol l^{-1}; SW frequency 50 Hz; pulse height 40 mV; scan increment 4 mV; preconcentration potential +0.1 V.

3.2. Study of the chemical and instrumental parameters

A typical SW voltammogram of CPL, after preconcentration, is illustrated in Fig. 2, showing a well defined peak due to the reduction of the adsorbed $[(RS)_2Hg]$. The actual response arises as the difference between the forward and reverse currents [22]. Since the two constituent currents were of opposite polarity, a signal enhancement was achieved leading to a increase in the analytical sensitivity of the voltammetric detection.

The effect of the supporting electrolyte and the pH on the CPL peak height and position were investigated next. It was observed that the CPL peak shifted to the cathodic direction and split into two peaks upon increase of the pH. Double peaks have been observed in earlier voltammetric studies of thiol compounds in alkaline media [25] and in

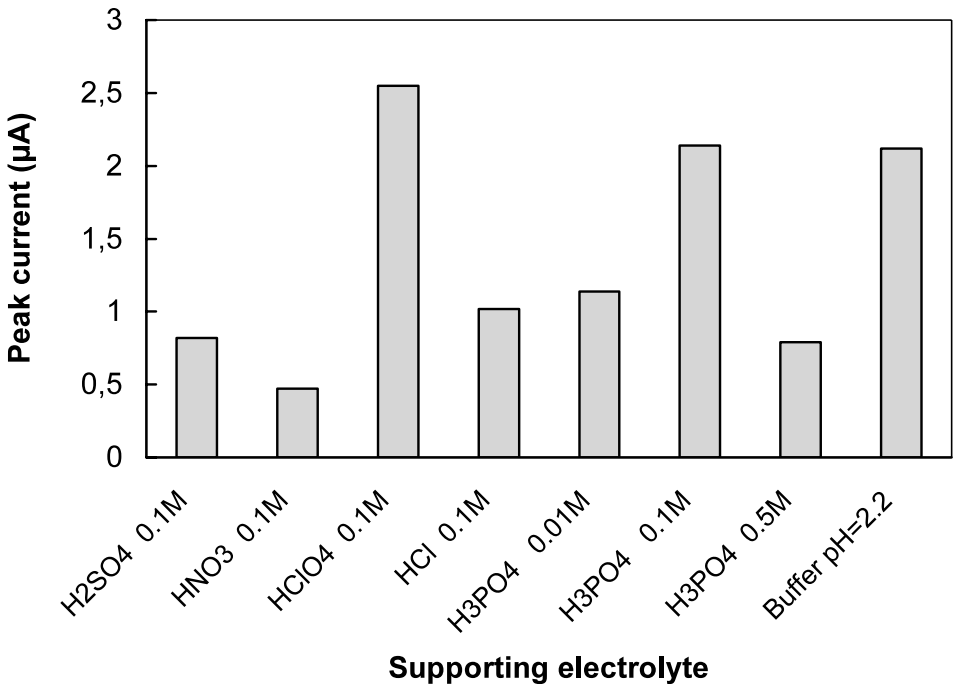

Fig. 3. Histogram showing the effect of different supporting electrolytes in the CPL stripping current. CPL concentration: 50 μg l^{-1}; preconcentration time: 60 s; SW frequency 50 Hz; pulse height 40 mV; scan increment 4 mV; preconcentration potential: +0.2 V.

this case the second peak can be attributed to the reduction of the CPL disulfide that forms and adsorbs on the electrode surface under these conditions. To avoid split peaks, pH < 3 was required. The effect of different acidic supporting electrolytes is illustrated in Fig. 3. Maximum signals were obtained for Britton–Robinson (B–R) buffer at pH 2.2, 0.1 mol l^{-1} $HClO_4$ and 0.1 mol l^{-1} H_3PO_4. In subsequent experiments, the B–R buffer pH 2.2 was used as it provided both a better baseline and minimal degradation of CPL [26].

In stripping voltammetry, the current peak height typically increases linearly with the accumulation time and eventually the rate of increase drops as saturation of the electrode gradually occurs. The plots of the stripping current versus the preconcentration time at different CPL concentrations were rectilinear (Fig. 4(a)), exhibiting an almost linear increase at short deposition times with gradual drop in the rate of increase at longer deposition times. At higher concentrations, the linear part of the plot was narrower as saturation of the electrode was reached at shorter deposition times.

The effect of the preconcentration potential is illustrated in Fig. 4(b). At potentials much more negative than +0.1 V, mercury oxidation was negligible resulting in limited salt formation and weak signals; in other words, at potentials more negative than +0.1 V the response was limited by the availability of Hg(II). As the preconcentration potential became more negative, the stripping signal increased and at potentials more positive than +0.1 V the reduction current stabilized. At this potential region, ample Hg(II) ions were generated but the current was limited by the rate of diffusion of CPL to the electrode surface in order to react with Hg(II). In subsequent experiments, an accumulation potential of +0.2 V was used.

The deleterious effect of oxygen in cathodic voltammetry is well documented as oxygen reduces initially to H_2O_2 and then to H_2O producing broad waves that interfere with the peaks of reducible species. Since the reduction of oxygen

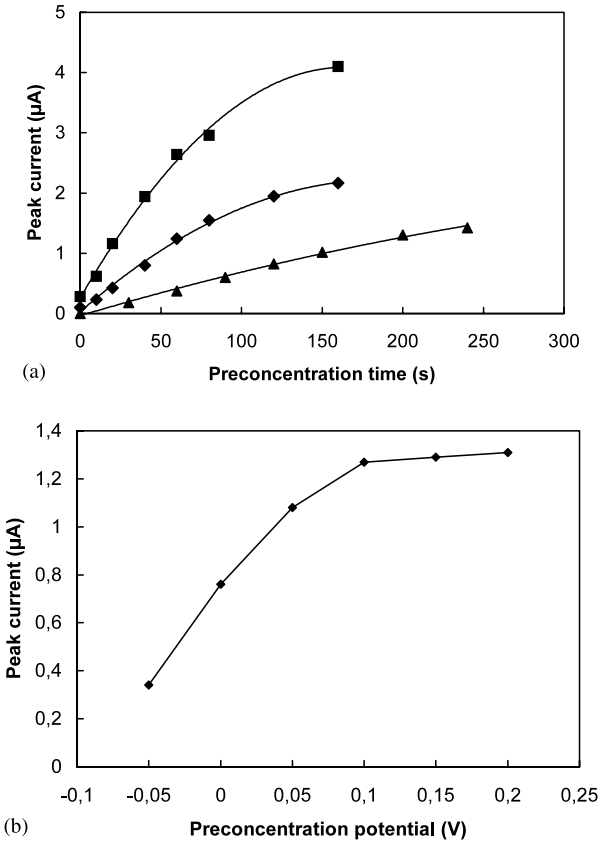

Fig. 4. (a) The effect of the preconcentration time on the CPL stripping current at different CPL concentrations: (▲) 4 μg l^{-1}, (◆) 10 μg l^{-1}, (■) 20 μg l^{-1}. Preconcentration time: 120 s; supporting electrolyte: B–R pH 2.2; SW frequency: 25 Hz; pulse height: 40 mV; scan increment: 4 mV; preconcentration potential: +0.2 V. (b): The effect of the preconcentration potential on the CPL stripping current. CPL concentration: 50 μg l^{-1}; preconcentration time: 30 s; supporting electrolyte B–R pH 2.2; SW frequency 50 Hz; pulse height 40 mV; scan increment 4 mV.

is a non-reversible electrochemical reaction, square wave voltammetry is able to discriminate against the presence of oxygen [22]. However, in this work oxygen was found to interfere with CPL determinations. A comparison between deoxygenated and undeoxygenated CPL solutions revealed that no peak was obtained in the unpurged solution. This was attributed to the oxidation of the accumulated CPL by the hydrogen peroxide formed as a result of oxygen reduction. To alleviate this problem, it was necessary to purge the solution with nitrogen for 5 min.

Selection of the SW parameters is paramount for the successful application of the proposed method. These parameters are the SW frequency, the pulse height and the scan increment. The effective scan rate, v, is related to the frequency, f, and the scan increment, SI, according to the formula: $v = f \cdot SI$. Increasing the SW frequency caused an increase in the net CPL peak height as indicated in Fig. 5(a) due to the increase in the effective scan rate. However, at frequencies higher than 50 Hz there was a deterioration of the peak shape and the background. This was attributed to the increased contribution of the capacitive current as a result of the shorter pulse width at higher SW frequency [27]. Increasing the scan increment also caused an increase in the effective scan rate and the CPL peak height increased (Fig. 5(b)). However, higher scan rates also led to a decrease of the sampled current values and the definition of the voltammograms deteriorated [22,27]. The effect of the pulse height is illustrated in Fig. 5(c). The current increased for pulse heights up to 40 mV while at higher pulse heights the peaks broadened and the background deteriorated. Therefore, the selected parameters were: SW frequency 50 Hz; pulse height 40 mV, and; scan increment 4 mV.

3.3. Calibration and figures of merit

In order to exploit the surface accumulation of CPL on the HMDE for quantitative analysis there must be a linear relationship between the stripping peak current and the CPL concentration in the sample. Calibration linearity was shown to be satisfactory both at low concentrations (0.5–10 μg l^{-1}) and higher concentrations (10–150 mg l^{-1}). The calibration figures of merit are collected in Table 1. Typically for stripping measurements, for longer deposition times the sensitivity and the lower limit of the linear range increased whereas the linear range decreased. Therefore, a compromise between sensitivity and linearity must be made. Loss of linearity at higher deposition times was due to gradual surface saturation. When complete monolayer coverage of the electrode surface was exceeded, the main CPL stripping

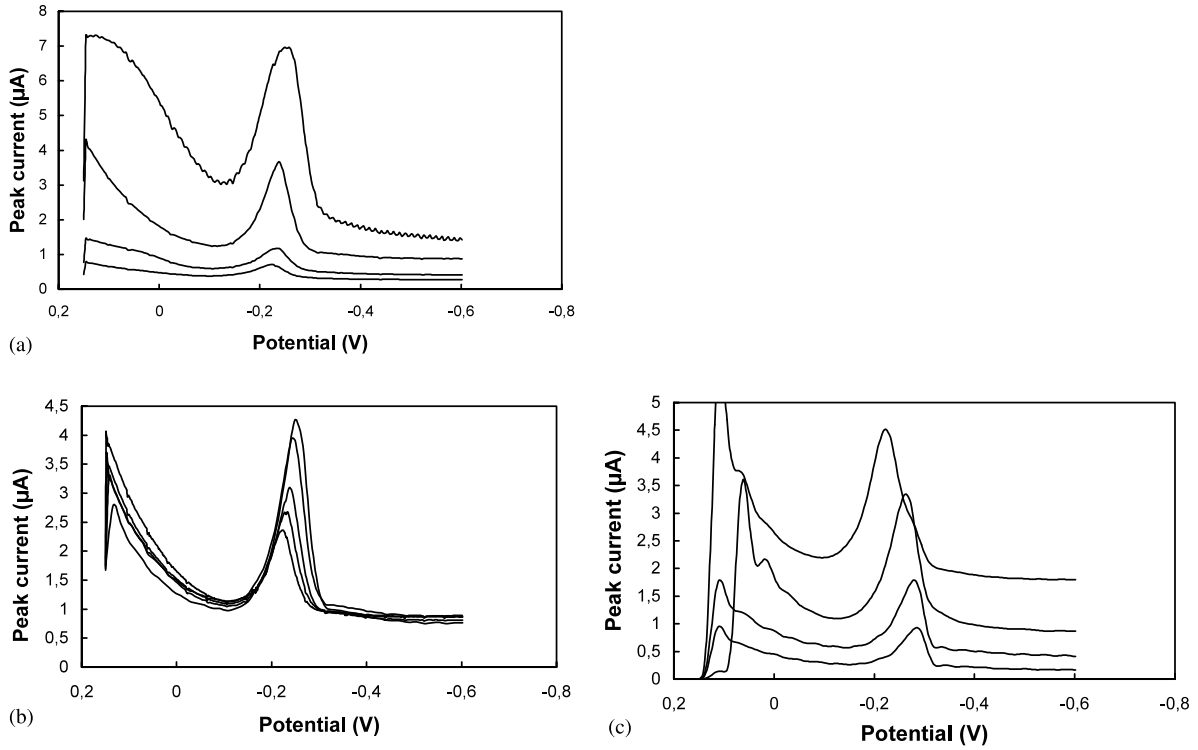

Fig. 5. (a) The effect of the SW frequency on the CPL stripping current (from below SW frequencies: 12,5, 25, 50, and 75 Hz). CPL concentration: 50 μg l^{-1}; preconcentration time: 60 s; supporting electrolyte: B–R pH 2.2; pulse height: 40 mV; scan increment: 4 mV; preconcentration potential: +0.2 V. (b) The effect of the SW scan increment on the CPL stripping current (from below scan increments: 1, 2, 4, 8 and 16 mV). CPL concentration: 50 μg l^{-1}; preconcentration time: 60 s; supporting electrolyte: B–R pH 2.2; pulse height: 40 mV; SW frequency: 50 Hz; preconcentration potential: +0.2 V. (c) The effect of the SW pulse height on the CPL stripping current (from below pulse height: 10, 20, 40 and 80 mV). CPL concentration: 50 μg l^{-1}; preconcentration time: 60 s; supporting electrolyte: B–R pH 2.2; scan increment: 4 mV; SW frequency: 50 Hz; preconcentration potential: +0.2 V.

Table 1
Calibration parameters of the CPL determination by SWCAdSV on the HMDE at different preconcentration times

Preconcentration time (s)	30	60	120
Sensitivity (μA μg^{-1} l)	0.028	0.045	0.071
Linear range (μg l^{-1})	5–180	2–110	1–60
R^2	0.996	0.997	0.996

Supporting electrolyte: B–R pH 2.2; SW frequency: 50 Hz; pulse height 40 mV; scan increment: 4 mV; preconcentration potential: +0.2 V.

peak abruptly increased excessively in height and a pre-peak appeared, in the form of a shoulder in the anodic side of the main peak. This may be indicative of a multi-layer adsorption mechanism, repulsive forces at high electrode coverage, redox centers with different standard potentials or rearrangement of the adsorbed CPL layer [28]. The reproducibility for the same electrode was calculated from eight consecutive measurements in the same solution. The relative standard deviation was calculated as 3.2% for a 20 μg l^{-1} CPL solution with 120 s preconcentration demonstrating a satisfactory level of precision. The limit of detection calculated for a S/N ratio of 3 was 0.5 μg l^{-1} CPL at a deposition time of 300 s. These figures of merit demonstrate that the method could be successfully applied to the analysis of CPL.

3.4. Analytical application

The procedure was applied to the analysis of two brands of pharmaceutical tablets containing 25 mg CPL per tablet. Only dilution of the sample

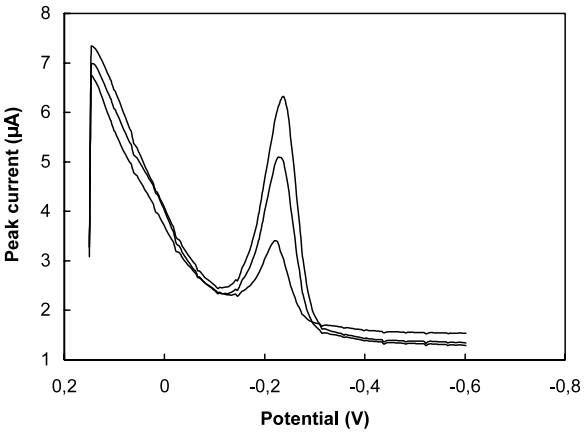

Fig. 6. Voltammograms for the determination of CPL in pharmaceutical tablets (from below: sample and two successive additions of 25 μg l^{-1} CPL). Preconcentration time: 80 s; supporting electrolyte: B–R pH 2.2; SW frequency: 50 Hz; pulse height: 40 mV; scan increment: 4 mV; preconcentration potential: +0.2 V.

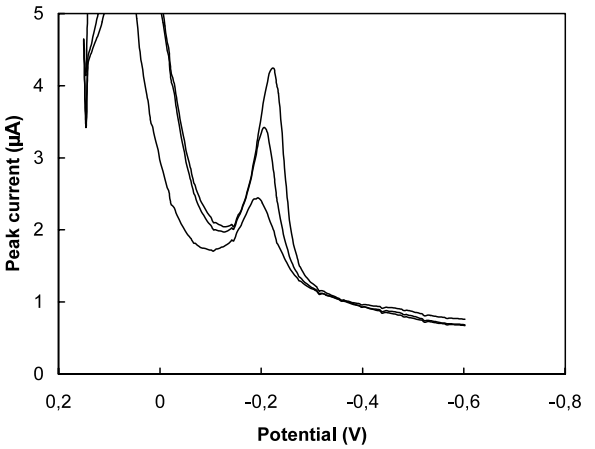

Fig. 7. Voltammograms for the determination of CPL in urine containing 2 mg l^{-1} CPL after its 1:100 (v/v) dilution with B–R buffer pH 2.2 (from below: sample and two successive additions of 20 μg l^{-1} CPL). Preconcentration time: 60 s; SW frequency: 50 Hz; pulse height: 40 mV; scan increment: 4 mV; preconcentration potential: +0.2 V.

Table 2
The results of the CPL determination in pharmaceutical tablets and urine

	Label[a]	Found (HPLC)[b]	Found (SWCAdSV)[b]	Added	Recovered (SWCAdSV)	Recovery (%)[b]
Pertacilon (mg)	25	24.6±1.0	24.4±0.6	25	49.1±1.1	98.8
Capoten (mg)	25	24.8±0.9	24.7±0.5	25	49.2±1.0	97.9

[a] CPL content labeled on the package.
[b] Mean of five determinations.

was necessary before the analysis. Representative voltammograms for capoten tablets are illustrated in Fig. 6. The results of the analysis are shown in Table 2.

Preliminary results indicated that the suggested procedure may be potentially applicable to the analysis of CPL in biological samples such as urine. Urine spiked with CPL at a final concentration of 2 mg l^{-1} was diluted 1:100 (v/v) with B–R buffer pH 2.2 (in which the lowest degradation rate of CPL occurs [26]), thus producing a sample with a CPL concentration of 20 μg l^{-1} CPL in the cell. Given that a daily therapeutic dose of CPL is normally 50–100 mg and 40–60% of the drug is excreted unchanged [9], this level of sensitivity is more than adequate for urinary analysis [26]. The diluted urine sample was subjected to SWCAdSV analysis with the method of standard additions and produced well-defined CPL peaks with a recovery of 93.6% (Fig. 7). The low recoveries of CPL commonly obtained in the analysis of free CPL in urine (typically lower than 90% [29]) are attributed to the oxidation of CPL and the formation of the disulfide or mixed disulfides with other thiols [30]. Further work is needed in this direction in order to confirm the suitability of this method for the determination of CPL in biological media.

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