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ORIGINAL PAPER

Square-wave adsorptive stripping voltammetric determination of candesartan cilexetil in pharmaceutical formulations

İncilay Süslü · Nuran Özaltın · Sacide Altınöz

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Abstract A sensitive, simple and rapid square-wave adsorptive stripping voltammetric method was developed and validated for the determination of candesartan cilexetil in pharmaceutical formulations. The proposed method was based on electrochemical reduction of candesartan cilexetil at a hanging mercury drop electrode in phosphate buffer at pH 5.0. A well-defined reduction peak was observed at −1340 mV with 30 s of accumulation time and −1100 mV of accumulation potential. Under these optimized conditions, the square-wave adsorptive stripping voltammetric peak current showed a linear correlation on drug concentration over the range of 0.25–1.34 μg mL^{-1} with a correlation coefficient of 0.9986 for the proposed method. The detection and quantitation limits for this method were 1×10^{-2} and 2.5×10^{-1} μg mL^{-1}, respectively. The results obtained for intra-day and inter-day precision (as RSD%) were between 1.10 and 3.90%. This method was applied successfully for the determination of candesartan cilexetil in its tablet dosage forms with mean recoveries of 101.13 ± 0.78% with RSD of 2.06% for 8 mg tablet and 99.84 ± 0.89% with RSD of 2.36% for 16 mg tablet. The results obtained from the developed square-wave adsorptive stripping voltammetric method were compared with those obtained by the analytical method reported in the literature.

İ. Süslü (✉) · N. Özaltın · S. Altınöz
Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100 Sıhhıye, Ankara, Turkey
e-mail: isuslu@hacettepe.edu.tr

N. Özaltın
e-mail: nozaltin@hacettepe.edu.tr

S. Altınöz
e-mail: saltinoz@hacettepe.edu.tr

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1 Introduction

Angiotensin-converting enzyme (ACE) inhibitors have a central role in the management of heart failure [1]. Angiotensin II-receptor antagonists (ARA-IIs) are safe and effective agents for the treatment of hypertension and heart failure. They have been proposed as an alternative to the more traditional ACE inhibitors and may offer further benefits compared with ACE inhibitors [1–3]. Candesartan cilexetil (CAN), (±-1-cyclohexyloxycarbonyloxy)ethyl-2-ethoxy-l-{[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]-methyl}-1H-benzimidazole-7-carboxylate (Fig. 1), is a potent, long-acting and selective angiotensin II receptor antagonist that has been shown to be safe and efficacious in the treatment of hypertension [4–7]. In a short-time dose finding study, CAN improved exercise capacity and alleviated symptoms in patients with chronic congestive heart failure [6–8]. Candesartan is orally administered as the ester pro-drug, which is rapidly and completely converted to the active compound during absorption from the gastrointestinal tract [9].

Various analytical methods have been described in the literature for determination of CAN in pharmaceutical formulations and biological fluids. These methods rely on the use of spectrophotometric [10], fluorimetric [11] and chromatographic methods such as high-performance liquid chromatography (HPLC) [5, 12–15] and capillary electrophoresis [2, 16–18]. A voltammetric method based on the oxidation of CAN at the glassy carbon electrode was also reported [19].

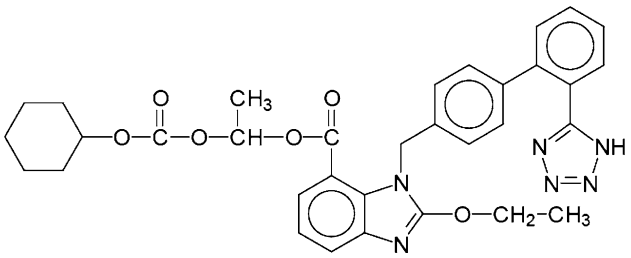

Fig. 1 Chemical structure of CAN

There is no information about the electrochemical redox properties of CAN at a hanging mercury drop electrode (HMDE) and its analytical applications in the literature.

The aim of this study was to develop a new, simple, rapid and sensitive square-wave adsorptive stripping voltammetric (SWAdSV) method for determination of CAN in bulk form and pharmaceutical formulations. The experimental and instrumental parameters effecting the drug response were investigated and optimized for CAN determination. This method was fully validated and applied to the pharmaceutical formulations of CAN. This work was also aimed to investigate the electrochemical behavior of CAN using cyclic voltammetry (CV) and chronoamperometry (CA). The validity of the proposed method was also tested by comparing our results with those of the voltammetric method reported in the literature [19].

2 Experimental

2.1 Apparatus

All experiments were performed using a BAS 100 B/W (Bioanalytical System, USA) electrochemical analyzer. A three electrode system consisted of HMDE as working electrode, an Ag/AgCl with saturated KCl as reference electrode and a platinum wire as counter electrode were used. A magnetic stirrer and stirring bar provided the convective transport during pre-concentration. The peak heights were automatically or manually measured using the "tangent fit" capability of the instrument. All measurements were performed at room temperature. All pH measurements were made with a Mettler Orion Model 420A digital pH meter calibrated with standard buffers.

2.2 Reagents and solutions

CAN and its pharmaceutical dosage forms Atacand® Tablets (containing 8 and 16 mg CAN per tablet) were kindly provided by Sanovel (Istanbul, Turkey) and Astra Zeneca Pharm. Ind., (Istanbul, Turkey), respectively. CAN was used without further purification. Melting point, UV and IR spectra were evaluated to check purity of CAN and no impurities were found. All chemicals used for preparation of buffers and supporting electrolytes were of analytical reagent grade (Merck or Sigma).

The stock solution of CAN (1000 μg mL^{-1}) was prepared in methanol and kept in dark at +4 °C. Standard solutions of CAN were prepared daily by appropriate dilution of the stock solution with methanol:water (1:1, v/v).

Different supporting electrolytes, namely borate, phosphate, acetate and Britton–Robinson buffers (BR) were used. All supporting electrolytes were prepared in Milli-Q water and then the pH was adjusted with 0.1 M HCl or 0.1 M NaOH.

2.3 Procedure

2.3.1 General analytical procedure

3 mL of the phosphate buffer at pH 5.0 as supporting electrolyte was added to the colored electrochemical cell and the solution was purged with pure nitrogen for 12 min. The required accumulation potential ($E_{acc} = -1100$ mV) was applied to the working electrode for a selected accumulation time ($t_{acc} = 30$ s), while the solution was stirred continuously at 400 rpm. The stirring was stopped and after equilibrium time of 10 s, a negative-going potential scan was initiated using the following parameters; frequency (f) = 10 Hz, scan increment (ΔE) = 4 mV and pulse amplitude (E_{SW}) = 25 mV. After recording the voltammogram of the supporting electrolyte, aliquots of CAN standard were added and the square-wave voltammetric cycles were repeated using a new mercury drop. The SWAdSV scan was conducted from −1100 to −1500 mV.

2.3.2 Analysis of Atacand® tablets

Ten tablets of Atacand® were accurately weighed, finely powdered and then mixed. An amount of powder equivalent to the average weight of one tablet was transferred into a 100 mL volumetric flask and 50 mL of methanol was added. The content of the flask was sonicated for 15 min and diluted to volume with methanol. This solution was centrifuged for 15 min at 5000 rpm to separate out the insoluble excipients. Appropriate solutions were prepared by diluting aliquots of clear supernatant with methanol:water (1:1, v/v) to obtain the appropriate concentrations. The procedure was then completed as described under the Sect. 2.3.1.

3 Results and discussion

No voltammetric method concerning the reduction of CAN at the mercury electrode has been reported. Also its

adsorption, electrochemical behavior and reduction mechanism at HMDE have not been investigated.

The high sensitivity, considerable speed and low background current of the square wave modulation make this method particularly suitable for determination of CAN at low concentrations. Thus, SWAdSV method was used for the analytical determination of CAN.

3.1 Optimization of the experimental conditions and instrumental parameters

The voltammetric response of drugs is mainly dependent on the pH of the buffer. Therefore, the electrochemical behavior of CAN was evaluated over a pH range of 2.0–9.0 at HMDE using the SWAdSV method. In preliminary experiments, various buffers such as phosphate, borate, acetate and BR at different pH values were examined. Maximum peak currents and good peak shapes were obtained using phosphate buffer. CAN exhibited a cathodic peak in phosphate buffer over the pH range 3.0–6.0 (Figs. 2, 3). No peak was observed at pH values of 7.0–9.0. Among the investigated supporting electrolytes such as phosphate, citrate, acetate and BR at pH 5.0, the maximum peak current, a well-defined and reproducible cathodic peak, was obtained in phosphate buffer. Therefore, phosphate buffer at pH 5.0 was chosen as the supporting electrolyte for optimization of other variables and for the analysis of CAN (Fig. 4).

Various tetra ammonium salts and potassium chloride were added at different concentrations to increase the ionic strength of the selected supporting electrolyte. As the peak current of CAN decreased with the addition of salts, no salt was added to the phosphate buffer.

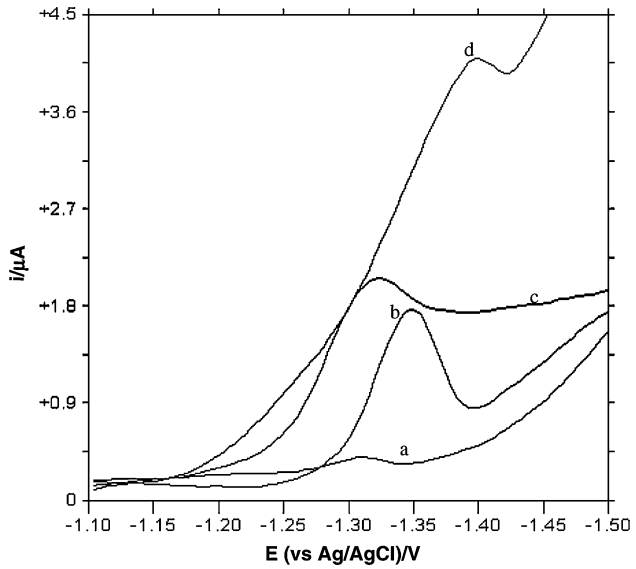

Fig. 2 Effect of pH of phosphate buffer on the SWAdSV peak current of CAN (0.57 μg mL^{-1}) at $E_{acc} = -1100$ mV, $t_{acc} = 30$ s, at $f = 15$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV. (a) pH = 6.0, (b) pH = 5.0, (c) pH = 4.0, (d) pH = 3.0

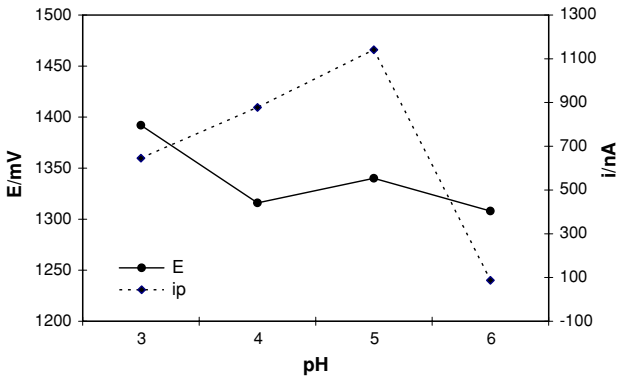

Fig. 3 Effect of pH of phosphate buffer on the SWAdSV peak potential and peak current of CAN (0.57 μg mL^{-1}) at $E_{acc} = -1100$ mV, $t_{acc} = 30$ s, at $f = 15$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV

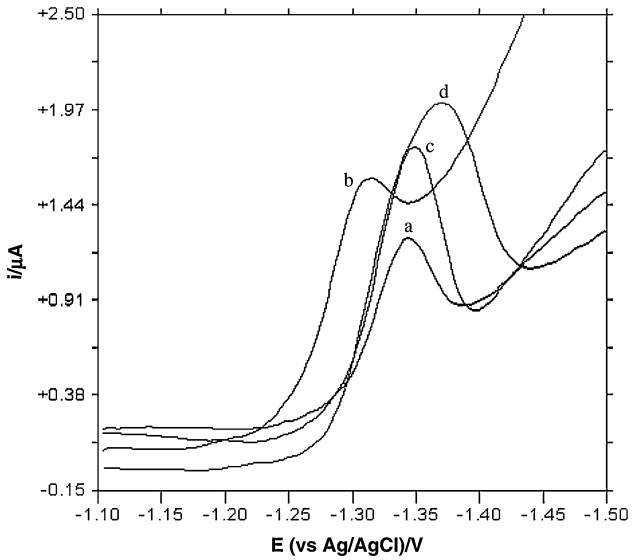

Fig. 4 Effect of different buffers at pH = 5.0 on the SWAdSV peak current of CAN (0.57 μg mL^{-1}) at $E_{acc} = -1100$ mV, $t_{acc} = 30$ s, at $f = 10$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV. (a) Citrate, (b) acetate, (c) phosphate, (d) BR buffers

The adsorptive behavior of CAN at HMDE was essentially dependent on the accumulation time (t_{acc}) and accumulation potential (E_{acc}). Therefore, the effect of t_{acc} on SWAdSV response for 0.83 μg mL^{-1} CAN was investigated from 10 to 50 s at $E_{acc} = -1000$ mV (Fig. 5). When the t_{acc} was increased, a remarkable enhancement was observed for the peak current of CAN up to 30 s: it then decreased sharply due to saturation of the surface of the working electrode. To maximize the sensitivity of the method, $t_{acc} = 30$ s was selected as the optimal value since it provided relatively high peak current with adequate practical time.

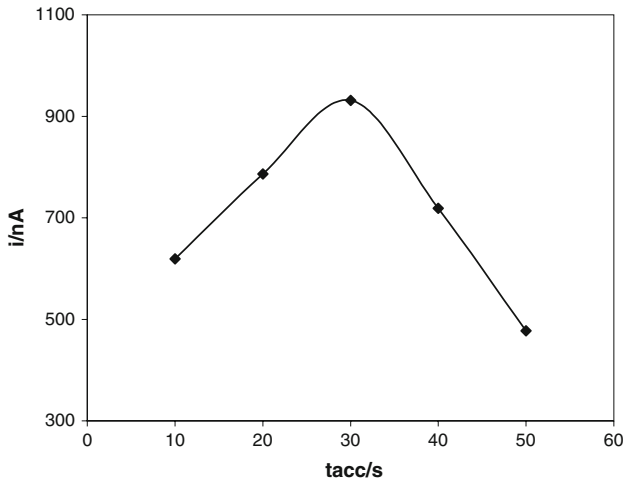

Fig. 5 Effect of the t_{acc} on the SWAdSV peak current response (i_p) for CAN (0.83 μg mL^{-1}) at $E_{acc} = -1000$ mV, $f = 15$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV

The effect of E_{acc} on the stripping voltammetric signal of 0.83 μg mL^{-1} CAN was examined over the range −900 to −1250 mV at a constant $t_{acc} = 30$ s in stirred solution (Fig. 6). The peak current increased steadily and reached its maximum value at −1100 mV and then decreased sharply. Hence, $E_{acc} = -1100$ mV was chosen for optimal analytical sensitivity.

The SWAdSV response of the accumulated drug markedly depends on the instrumental parameters. In order to obtain the maximum peak current and the best peak shape for CAN, frequency (*f*), scan increment (ΔE) and pulse amplitude (E_{SW}) were investigated for 0.83 μg mL^{-1} CAN solution in phosphate buffer at pH 5.0 after accumulation at $E_{acc} = -1100$ mV for 30 s in proposed method.

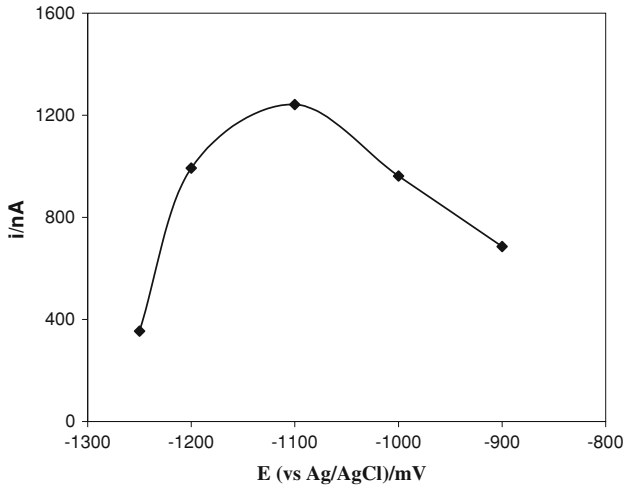

Fig. 6 Effect of the E_{acc} on the SWAdSV peak current response (i_p) for CAN (0.83 μg mL^{-1}) after 30 s preconcentration time at $f = 15$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV

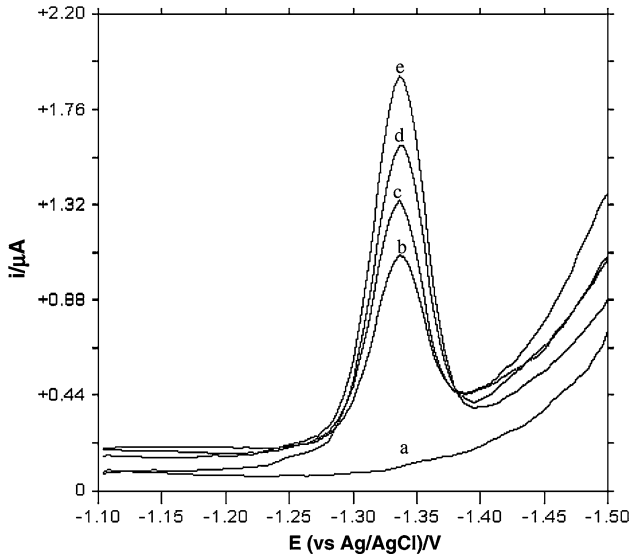

Fig. 7 The SWAdS voltammograms of CAN at different concentrations using the optimum conditions. (a) Supporting electrolyte, (b) 0.49, (c) 0.57, (d) 0.73 and (e) 0.88 μg mL^{-1} of CAN

f was varied from 5 to 50 Hz using 4 mV (ΔE) and 25 mV (E_{SW}). The highest peak current with the best peak definition was found at $f = 10$ Hz, which is used in this study.

At 10 Hz (*f*) and 25 mV (E_{SW}), ΔE was varied from 2 to 6 mV. The peak current increased until 4 mV and then decreased. Hence, $\Delta E = 4$ mV was chosen for the determination of CAN.

At 10 Hz (*f*) and 4 mV (ΔE), E_{SW} varied from 5 to 35 mV. Higher peak currents were obtained by increasing the E_{SW}. When E_{SW} was higher than 25 mV, a broad peak was obtained which was not useful for analytical purpose. Maximum peak current and the narrowest square-wave stripping peak were obtained at 25 mV, and this is an optimal value for analytical determination.

Consequently, the highest peak current values with the best peak definition were found for $f = 10$ Hz, $\Delta E = 4$ mV and $E_{SW} = 25$ mV, and these values were used for further measurements of CAN. In these optimal experimental and instrumental conditions, a well-defined SWAdSV peak was observed at −1340 mV for reduction of CAN in the proposed method (Fig. 7).

3.2 Characterization of the electrode reaction (cyclic voltammetry studies)

The reversibility of the reduction process of CAN was investigated using the CV method. The cyclic voltammogram of 9.90 μg mL^{-1} CAN solution in phosphate buffer at pH 5.0 at HMDE exhibited a single well-defined peak, in the cathodic direction at −1340 mV (Fig. 8). No anodic peak was observed on the reverse scan showed that the

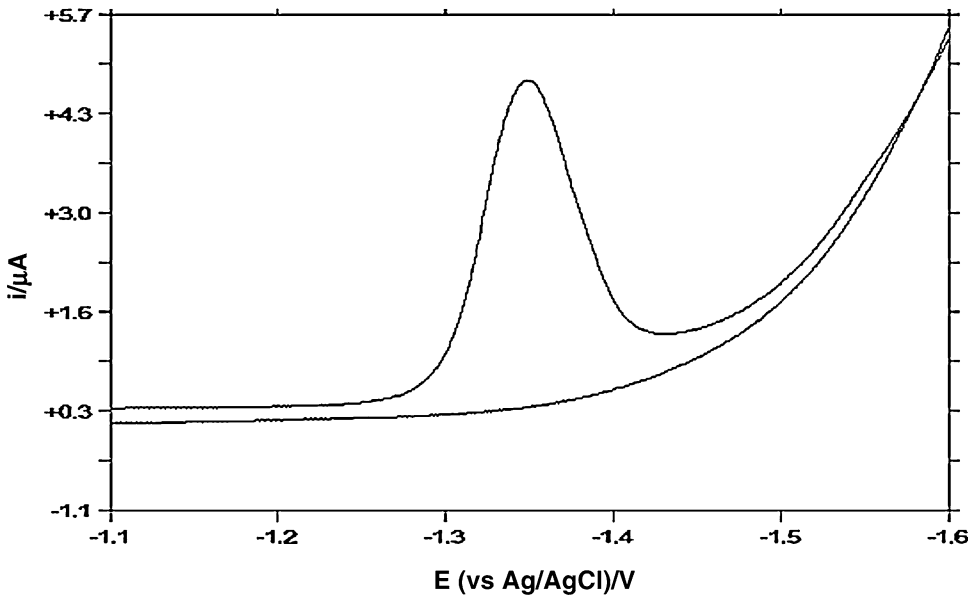

Fig. 8 Cyclic voltammogram of 9.90 μg mL^{-1} CAN solution at HMDE (scan rate = 100 mV s^{-1})

reduction reaction was irreversible. Moreover, the peak potential shifted to a more negative value with increasing scan rate, confirming the irreversible nature of the reduction process [20].

The effect of scan rate (v) on the peak current (i_p) and the peak potential (E_p) was also examined within the range 10–100 mV s^{-1}. About 80 mV negative shifts in the peak potential confirmed the irreversibility of the reduction process. At a scan rate higher than 100 mV s^{-1}, no reduction peak was observed. It is thought that the reduction on the HMDE is very slow and the reduction rate cannot be reached at scan rates higher than 100 mV s^{-1}. This observation also shows the irreversibility of the electrode process.

A linear correlation was obtained between the peak intensity i_p (μA) and the square root of the scan rate $v^{1/2}$ (mV s^{-1}) while the scan rate was varied from 10 to 100 mV s^{-1} in 9.90 μg mL^{-1} CAN solution. The regression equation of this relation demonstrated the diffusional behaviour of current (i_p (μA) = 0.6016 $v^{1/2}$ (mV s^{-1}) −0.6541, $r = 0.9848$, $n = 6$) [20, 21].

Plotting log i_p versus log v gave a straight line in the same scan rate range (10–100 mV s^{-1}) (log i_p (μA) = 0.6154 log v (mV s^{-1}) −0.5002, $r = 0.9784$, $n = 6$). The slope value of 0.6154 is close to the theoretical value of 0.5 for a diffusion controlled process [21].

The number of electrons was also calculated by employing the CV method at HMDE using the following equation [22]:

$$E_p - E_{p/2} = 0.057/n$$

where E_p is the peak potential (mV), $E_{p/2}$ is the peak potential (mV) corresponding to 85.2% of peak current (A), n is the number of electrons transferred per molecule. The number of electron was calculated as 2.88 ± 0.07 ($n = 7$).

It can be seen from the chemical structure of CAN (Fig. 1) that the molecule possesses a carbonyl group, and benzimidazole and tetrazol groups. The carboxyl group is electroinactive because the reduction wave of the carbonyl group (C=O) on carboxyl group can only be observed at −2.0 V in organic solvent as media. The discharge of hydrogen ions in bulk solution overlaps the reduction wave of the carbonyl group, which makes it impossible to observe the latter in aqueous solution. The ability of some organic compounds to bring about catalytic hydrogen evolution seems to be due to the presence of an unshared pair of electrons, to which a proton may be added. The benzimidazole group is not reducible in aqueous solution [23]. The nitrogen atom in the benzimidazole group of the CAN molecule may combine with a proton. Accordingly, it was deduced that the reduction wave of CAN at −1340 mV in the optimal supporting electrolyte should be the result of the reduction of the proton rather than that of the other groups; this produces a catalytic hydrogen wave. Owing to the ability of the nitrogen atom to combine with a hydrogen ion being higher than that of an oxygen atom, the nitrogen atom of CAN combines with a hydrogen ion in the benzimidazole and tetrazol groups, yielding electroactive compounds on the cathode [24, 25].

The experimental Cottrell slope was determined from the chronoamperometric i_p versus $t^{-1/2}$ plot. The diffusion coefficient was calculated from Cottrell Equation [26]. The constant potential applied was slightly more cathodic than the cyclic voltammetric E_p from −1200 to −1500 mV. A HMDE with a surface area of 0.0199 cm^2 was employed.

The diffusion coefficient was calculated as $2.30 \times 10^{-6} \pm 1.14 \times 10^{-7}$ ($n = 7$).

3.3 Validation of the method

The proposed SWAdSV method was fully validated in terms of stability, linearity, sensitivity, precision, accuracy, recovery, selectivity, robustness, and ruggedness [27–33].

3.3.1 Stability

A standard stock solution of CAN was kept in the dark at +4 °C. The stability of CAN standard stock solution (1000 μg mL^{-1}) was tested for one month and it was found to be stable during this period. Under the optimum conditions, the short time stability 25.00 μg mL^{-1} CAN standard solution was evaluated by the proposed method. No changes were observed in the peak potential and peak current over a period of 8 h.

3.3.2 Linearity

The linearity is defined as the ability of the method to obtain test results that are directly proportional to the concentration of the analyte within a given range. The range is the interval between the high and low levels of analyte studied. Under the optimum conditions, SWAdSV voltammograms recorded with increasing amounts of CAN showed that the peak current increased linearly with increasing concentration (Fig. 7). A good linear correlation was obtained between the electrochemical response of CAN and its concentration in the range 0.25–1.34 μg mL^{-1}. The parameters of the concentration-peak current straight line were calculated by the least-squares method. Voltammograms were recorded at least twice using a new mercury drop. The linearity was checked by preparing standard solutions for 10 different concentrations for the proposed SWAdSV method. The regression equation obtained for the calibration curve is given as:

$$i_p = 2582.4396 \pm 4.53\,C - 586.1890 \pm 3.07,$$
$$r = 0.9986\ (n = 6)$$

where i_p is the SWAdSV peak current (nA) and C is the CAN concentration (μg mL^{-1}), r is the correlation coefficient. The analytical characteristics of the proposed method are summarized in Table 1.

3.3.3 Sensitivity

The sensitivity of the developed method was checked in terms of limits of detection (LOD) and quantitation (LOQ) values. The LOD is defined as the lowest concentration of an analyte in a sample which can be detected. LOD may be calculated according the formula:

$$\mathrm{LOD} = 3.3(\mathrm{SD}/\mathrm{S})$$

where SD is the standard deviation of y-intercepts of regression lines and S is the mean slope of the calibration curves. The calculated LOD value for the proposed method was 1×10^{-2} μg mL^{-1}.

The LOQ is defined as the lowest concentration of an analyte in a sample which can be determined quantitatively with an acceptable level of accuracy and precision under the optimum conditions of the method. The LOQ value of this method was found to be 0.25 μg mL^{-1} (RSD = 2.03%) ($n = 6$).

These LOD and LOQ data indicate that the proposed method is sensitive for the analysis of CAN in pharmaceutical formulations.

3.3.4 Precision

Precision is defined as the closeness of agreement between independent test results obtained under optimum conditions and is normally expressed as the percentage relative standard deviation (RSD%). The precision of the proposed method was investigated with respect to repeatability and intermediate precision. In order to measure repeatability of the voltammetric instrument, 10 consecutive measurements

Table 1 Analytical characteristics of developed SWAdSV method ($n = 6$)

	SWAdSV method
Regression equation of calibration curve method[a]	$y = 2582.4396\ x - 586.1890$
Standard error of slope	4.53
Standard error of intercept	3.07
Correlation coefficient (r)	0.9986
Regression equation of Standard addition method[a]	$y = 2585.2454\ x + 1135.6118$
Linearity range (μg mL^{-1})	0.25–1.34
Number of data points	10
Limit of detection (LOD) (μg mL^{-1})	1×10^{-2}
Limit of quantitation (LOQ) (μg mL^{-1})	0.25

[a] $y = bx + a$;
x = concentration (μg mL^{-1}),
y = peak current (nA),
a = intercept, b = slope

Table 2 Precision and accuracy of the proposed method ($n = 6$)

Intra-day				Inter-day		
Added (μg mL^{-1})	Found[a] (μg mL^{-1})	Precision (RSD%)	Accuracy[b] (Bias%)	Found[a] (μg mL^{-1})	Precision (RSD%)	Accuracy[b] (Bias%)
0.33	0.33 ± 0.01	2.89	0	0.32 ± 0.01	1.45	−3.03
0.57	0.58 ± 0.01	3.90	1.75	0.60 ± 0.01	1.93	5.26
1.04	1.02 ± 0.01	1.60	−1.92	1.03 ± 0.01	1.10	−0.96

Found[a] $= \bar{x}$ = Mean ± standard error, *RSD%* relative standard deviation, Accuracy[b] = [(Found − Added)/Added] × 100

were made with the same standard solution 0.57 μg mL^{-1} CAN under the optimum conditions over a short interval of the time. The mean of measured peak potential and peak current were 1339.50 ± 0.89 mV with RSD of 0.21% and 947.05 ± 6.58 nA with RSD of 2.20%, respectively.

Three different concentrations of CAN (0.33, 0.57 and 1.04 μg mL^{-1}, in the linear range) were analyzed in 6 independent series in the same day (intra-day precision) and 6 consecutive days (inter-day precision). Every sample in each series was analyzed three times under the optimum conditions. The RSD values of intra- and inter-day studies were varied from 1.60–3.90% and 1.10–1.93%, respectively indicate that the precision of the proposed method is satisfactory (Table 2).

3.3.5 Accuracy and recovery

Accuracy is defined as the closeness of the test results obtained by the analytical method to the true value. It is determined by calculating the percentage relative error (bias%) between the measured mean concentrations and added concentrations. The intra- and inter-day accuracy were evaluated as described in Sect. 3.3.4. The results obtained from intra- and inter-day accuracy of the developed method were −1.92 to 0% and −3.03 to 5.26%, respectively (Table 2).

The accuracy of the proposed method was also tested by recovery experiments. For this purpose, the recovery test was performed by the standard addition method. For this purpose, appropriate volume of tablet solution was added to supporting electrolyte. After the voltammogram was recorded, three known concentration levels of CAN standard solutions (0.56, 0.63 and 0.71 μg mL^{-1}) were added and voltammograms were recorded after each addition. The peak current measured was plotted against the added concentration of CAN. The concentration was calculated from the extrapolation of the line in the negative zone of the abscissa. The data obtained from this experiment are summarized in Table 3. The recoveries of CAN were 98.94–101.72%. These data showed that there was no interaction of excipients from tablet dosage forms in the analysis of CAN.

3.3.6 Selectivity

Selectivity is defined as the ability of the method to determine the analyte response accurately and specifically in the presence of other components of a sample matrix under the stated conditions of the analysis. Comparison of the recorded voltammograms obtained from pharmaceutical tablet formulation and standard solutions in the same concentration showed that the peak potential and peak current of CAN did not change. The average regression equation for standard addition method was $y = 2585.2454 \pm 8.51\ C + 1135.6118 \pm 5.34$, $r = 0.9928$. i_p is the SWAdSV peak current (nA) and C is the added CAN concentration (μg mL^{-1}), r is the correlation coefficient. There was no difference between the slopes of the two methods (calibration curve and standard addition methods). These values showed that there was no significant excipients interference from tablet dosage forms. Thus, the developed procedure was able to determine CAN in the presence of excipients and the developed method can be considered selective.

3.3.7 Robustness

The robustness of the method shows the reliability of an analytical method with respect to small, but deliberate variations in method performance parameters. These parameters including pH of supporting electrolytes

Table 3 Recovery data of the developed method for the analysis of CAN ($n = 6$)

Added concentration (μg mL^{-1})	Found concentration (μg mL^{-1})	Recovery (%)	RSD of recovery (%)	Bias%
0.56	0.55 ± 5.78 × 10^{-3}	98.94 ± 1.43	2.50	−1.79
0.63	0.63 ± 5.78 × 10^{-3}	100.55 ± 1.09	1.88	0
0.71	0.72 ± 1 × 10^{-2}	101.72 ± 1.57	2.66	1.41

Found $= \bar{x}$ = Mean ± standard error, *RSD%* relative standard deviation

Table 4 The robustness data of SWAdSV method ($n = 3$)

	Found (μg mL^{-1})	RSD%
Standard (0.57 μg mL^{-1})	$0.59 \pm 5.78 \times 10^{-3}$	1.69
pH 4.9	$0.56 \pm 5.78 \times 10^{-3}$	1.79
pH 5.1	$0.56 \pm 5.78 \times 10^{-3}$	1.79
Accumulation potential (−1050 mV)	0.57 ± 0.01	3.51
Accumulation potential (−1150 mV)	$0.58 \pm 5.78 \times 10^{-3}$	1.72
Accumulation time (25 s)	$0.58 \pm 5.78 \times 10^{-3}$	1.72
Accumulation time (35 s)	$0.57 \pm 5.78 \times 10^{-3}$	1.75
Friedman analysis: $p = 0.069 > p = 0.05$		

$\bar{x}$ = Mean ± standard error, *RSD%* relative standard deviation

(4.9–5.1), E_{acc} (1050–1150 mV) and t_{acc} (25–35 s) were investigated for 0.57 μg mL^{-1} of CAN (Table 4). Only one parameter was changed in each experiment. The RSD% value was found to be 1.69–3.51%. The statistical comparison was done with Friedman analysis and no difference was found the results. The results obtained from the various conditions were not different compared to the optimum conditions and none of these important variables significantly affected the assay of CAN. Consequently, this SWAdSV method was reliable for the analysis of CAN and the proposed method could be considered robust.

3.3.8 Ruggedness

The ruggedness of the proposed method was evaluated by applying the developed procedure to assay of 0.57 μg mL^{-1} CAN using the same instrument by two different analysts under the same optimized conditions at different days. The results were compared by means of the *t*- and *F*-tests (Table 5). Since there was no significant difference between the results obtained by the two analysts, the proposed method may be considered rugged.

3.3.9 Analytical application

The developed and validated SWAdSV method was successfully applied to commercially available Atacand® tablets at two different dose strengths (8 and 16 mg). The CAN content of tablets was determined using the calibration curve method. The obtained amount of CAN and statistical analysis are given in Table 6. Mean recoveries were 101.13 ± 0.78% with RSD of 2.06% for 8 mg tablet and 99.84 ± 0.89% with RSD of 2.36% for 16 mg tablet. The results agree with those declared by the manufacturer.

A voltammetric method reported in the literature [19] was used for comparison and also to show the reliability of the proposed method. The statistical analysis of the results obtained from two methods was performed using the *t*- and *F*-tests. At the 95% of the confidence level, the calculated *t*- and *F*-test values were lower than that of theoretical *t*- and *F*-test values, showing that there is no significant differences between the proposed and reference method (Table 6).

Table 5 The ruggedness of the proposed method (added of CAN 0.57 μg mL^{-1}) ($n = 6$)

1. Analyst found (μg mL^{-1})	2. Analyst found (μg mL^{-1})
$\bar{x} = 0.58 \pm 0.01$	$\bar{x} = 0.59 \pm 0.01$
RSD% = 1.72	RSD% = 2.64
$t_c = 0.86$, $t_t = 2.23$, $p > 0.05$	
$F_c = 4.00$, $F_t = 5.05$, $p > 0.05$	

$\bar{x}$ = Mean ± standard error, *RSD%* relative standard deviation

t_c Calculated *t*-value, t_t tabulated *t*-value, F_c calculated *F*-value, F_t tabulated *F*-value

Table 6 The results of commercial pharmaceutical formulations containing CAN ($n = 7$)

	SWAdSV method	Comparison method [19]
Atacand® tablets (8 mg CAN)	$\bar{x} = 8.09 \pm 0.06$	$\bar{x} = 8.30 \pm 0.10$
	RSD% = 2.07	RSD% = 3.10
	$t_c = 1.75$, $t_t = 2.18$, $p > 0.05$	
	$F_c = 2.33$, $F_t = 4.28$, $p > 0.05$	
Atacand® tablets (16 mg CAN)	$\bar{x} = 15.97 \pm 0.14$	$\bar{x} = 16.33 \pm 0.13$
	RSD% = 2.36	RSD% = 2.14
	$t_c = 1.84$, $t_t = 2.18$, $p > 0.05$	
	$F_c = 1.17$, $F_t = 4.28$, $p > 0.05$	

$\bar{x}$ = Mean ± standard error, *RSD%* relative standard deviation

t_c Calculated *t*-value, t_t tabulated *t*-value, F_c calculated *F*-value, F_t tabulated *F*-value

4 Conclusion

A simple, fast, sensitive and precise SWAdSV method was developed for the determination of CAN in pharmaceutical formulations. This method is based on the reduction of CAN at HMDE. The electrochemical behavior of CAN at a mercury electrode was also investigated.

The method is simpler, faster (requiring <2 min to perform) and requires less expensive equipment than chromatographic methods.

The sensitivity of the method significantly enhanced adsorption of the drug on the electrode surface and after careful choice of the operating parameters, extremely low LOD and LOQ values can be reached.

The proposed method is sufficiently accurate and precise and it was successfully applied to assay of CAN in its tablet formulations. The analysis was performed with no

interference from the excipients present in the tablets. This is a rapid one step procedure which only requires a simple sample treatment; so it is an inexpensive and fast procedure which does not need time-consuming separation and extraction steps.

In the compared method [19] based on the oxidation of the drug at a GCE (Glassy carbon electrode), before each measurement the GCE should be polished manually with alumina on a polishing cloth and it has time-consuming procedure and also high running costs. But, the proposed method in our study is rapid and simple to perform and is a low cost quantitative voltammetric method. The LOD and LOQ values were lower than those of the compared voltammetric method. Thus, the developed method is much more sensitive and it can be used as an alternative method to the voltammetric method reported in the literature.

It can be concluded that this SWAdSV method can be successfully and reliably applied to the routine analysis in quality control laboratories for the analysis of CAN in bulk form and pharmaceutical formulations.

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