Sci. Journal of Oct. 6Uni. (SJO6U), *ISSN: 2314-8640:* Vol. 2(1): 40-49, 2014
www.o6u.edu.eg

Determination of Losartan in tablet dosage form and some biological body fluids via copmlexation with Cu (II) using Cathodic Adsorptive Stripping Voltammetry

[*]Ahmed M.Abdel-Megied[1], Ahmed A. Abdel-Gaber[2], Osama H. Abdelmageed[3] and Mahmoud A. Omar[3]

[1*]Pharmaceutical Analytical Chemistry Dept, October 6 University, 6th October, Giza, Egypt
[2]Chemistry Dept, Faculty of Science, Minia University, Minia , Egypt
[3]Analytical Chemistry Dept, Faculty of Pharmacy ,Minia university, Minia , Egypt

Received: 18-11-2013
Accepted: 07-1-2014
Published:01-03-2014

***Corresponding author:** E. mail: dr_ahmed80@hotmail.com

Abstract

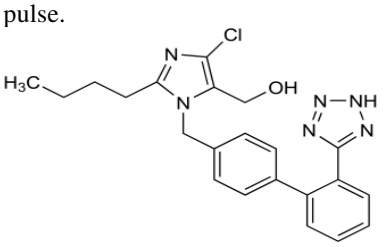
A square-wave voltammetric procedure for electroanalytical determination of Losartan (LOS) in its tablets, human urine and blood serum in Britton-Robinson buffer (pH 3.0, 0.1 mol L^{-1}) as a supporting electrolyte were studied. The electrochemical stripping procedure for trace measurement of LOS was developed based on the adsorption of Cu (II)- LOS complex on a hanging mercury drop electrode and applied to quantification of the drug . Cyclic voltammetry was used to characterize the interfacial and redox behavior of the adsorbed Cu (II)- LOS complex. The solution conditions and instrumental parameters for quantitative determination were optimized and a linear variation ranging from 5×10^{-10} mol L^{-1} to 5×10^{-7} mol L^{-1} with detection limit (LOD) 1.87×10^{-10} mol.L^{-1} in presence of 5×10^{-7} mol.L^{-1} of Cu (II) ions for quantification of LOS. The sharp peak of the adsorbed Cu (II) - LOS complex associated with an effective interfacial accumulation of the drug with good recoveries. The observed data had been subjected to statistical analysis, which revealed high reliability and precision. The degree of interference from potentially present metal ions and organic compounds on (SWAdCSV) signal for Cu (II) - LOS complex was evaluated. The intra-day relative standard deviation (RSDs) ranged from 0.331 to 0.418% at three different concentrations and inter-day RSDs ranged from 0.055 to 0.271%. The procedure was successfully applied to the quantification of the drug in human urine and serum samples. The mean percentage recoveries were 99.6 ± 0.15 and 98.0 ± 0.256, respectively. The determination of the LOS in its tablets was achieved using the standard addition method.

Key Words: Losartan, Cathodic Adsorptive Stripping Voltammetry, Cyclic voltammetry, biological fluids and HMDE

Introduction

Hypertension represents a widespread cardiovascular condition although usually asymptomatic, is a major risk factor for myocardial ischemia, renal failure and stroke. Therefore, it is often called the "silent killer" (1). Losartan, with an IUPAC name chemically it is 2-n-butyl-4-chloro-5-hydroxymethyl-1-((2c-(1H-tetrazol-5yl) (biphenyl-4yl) methyl) imidazole potassium salt, it is a strong, non-peptide, angiotensin II receptor antagonist that affect renin-angiotensin system, used mainly for the treatment of hypertension (2). Several reported methods have been described for the determination of LOS in its tablet. These methods such as spectrophotometry (3-8), Spectrofluorimetry (9), HPLC (10-17), HPTLC (5, 18), capillary electrophoresis (19-22) and electro-chemical methods (23, 24). Square wave

voltammetry is a large-amplitude differential technique in which a waveform composed of a symmetrical square-wave is applied to the working electrode (27, 28). The current is sampled twice during each square wave cycle, once at the end of the forward pulse and once at the end of the reverse pulse.



Losartan

The difference between the two measurements is plotted versus the base staircase potential. The resulting peak current is proportional to the concentration of the analyte. Comparison of square wave and differential pulse voltammetry for reversible and irreversible cases indicated that the square wave current are 4 and 3.3 times higher, respectively, than the analogues differential pulse response (25-27). The major advantage of square wave voltammetry is its great speed. The present work describes a validated square wave adsorptive stripping SWAdCS voltammetric procedure for trace determination of LOS in bulk form, tablets and biological fluids without the need for sample pretreatment or evaporation steps prior to the drug analysis.

2. Materials and Methods

2.1. Apparatus

The voltammetric measurements were done with a complete computerized polarographic trace analyzer AMEL model 433–A (Milano, Italy). A voltammetric cell bottom was fitted with an Ag/AgCl/ KCl reference electrode and a platinum wire as a counter electrode and the mercury working electrode which is used as hanging mercury drop electrode (HMDE). Stirring of the solution was performed using a magnetic stirrer (rotating – field type) and a stirring bar to provide the connective transport during the accumulation step.

2.2. Chemicals and reagents

●LOS (El-Hikma Pharmaceuticals, Amman, Jordan) was kindly provided from the manufacture as a working standard with an overall general estimated purities 99.2 % w/w.

●Cozaar® 50 mg B.N. NHO/9450 (Global Napi Pharmaceuticals- Egypt under license from: Merck & Co. Inc., Whitehouse station, N.J., USA), labeled to contain 50 mg of Losartan potassium per tablet.

●Sodium hydroxide, potassium nitrate, sodium percholrate, potassium dihydrogen phosphate, concentrated hydrochloric acid, concentrated nitric acid, orthophosphoric acid, glacial acetic acid and boric acid (Merck, Darmstadt, Germany).

●Cu (II) nitrate (Merck Co.) was prepared as 1× 10-3 M aqueous solution.

● Double distilled water was used throughout the experimental work.

2.3. Preparation of standard stock Solutions

Stock standard solution (1×10^{-3}M) of LOS was prepared. 0.0423 g of drug was accurately weighed, carefully transferred into 100 mL volumetric flasks and dissolved into B.R buffer then sonicated for about 5 minutes for complete dissolution, and kept at 4ºC. Under these conditions, the solution was stable and hence drug concentration did not change with time. Finally the resultant solution was completed to the volume with bidistilled water. Serial dilutions were prepared to obtain working standards in the range 10^{-3} M to 10^{-7}M.

2.4 Preparation of Voltammetric Cell

A known volume 10.0mL of the supporting electrolyte was added to the cell and de-aerated by passage of nitrogen for 16min. Preliminary experiments were carried out to determine impurities that may be found in supporting electrolyte. The preconcentration potential was adjusted depending upon the analyte to be sought, and it was applied to a new mercury drop for the selected time, while the solution was stirred at 500 rpm. The stirring was then stopped automatically depending on the preconcentration time and after 15 seconds as rest time (equilibrium time), the voltammogram goes to a negative potential scan was recorded. All data were obtained at room temperature (air conditioning 25 ± 1 ºC).

2.5. Sample preparation

2.5. 1. Tablets

Ten Cozaar® tablets were accurately and finely powdered. A portion of the powder, equivalent to 50.0 mg of LOS was transferred to a 100 mL volumetric flask and dissolved in double distilled water. The mixture was sonicated for 30 min and then completed to the mark with the same solvent. The solution was filtered and the desired different sample for the drug was then obtained by accurate dilution with bidistilled water. Suitable aliquot of

the diluted solutions of LOS were transferred to a voltammetric cell in presence of 5×10^{-7} M Cu (II) nitrate.

2.5.2. Urine samples

Fresh urine samples were collected from a healthy person. 5 and 10.0 µL were pipetted directly to the voltammetric cell containing the supporting electrolyte i.e. the dilution factor of the urine sample in the cell was 1: 1000. Then 10 µL spikes of the standard solution were introduced in presence of 5×10^{-7} M Cu (II) nitrate.

2.5.3. Serum samples

Serum samples were obtained from healthy volunteers and stored frozen until the analysis were performed. After gently thawing, an aliquot volume of sample was fortified with LOS dissolved in methanol to achieve appropriate concentration. The solution was centrifuged for 30 min at 3600 rpm to remove the precipitated serum proteins and the supernatant was taken carefully. Appropriate volume of the supernatant liquor was transferred to the voltammetric cell in presence of 5×10^{-7} M Cu (II) nitrate.

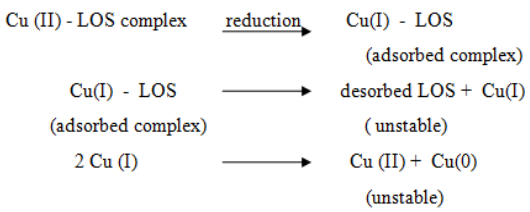
2.6. Calibration and linearity

After optimizing all the reaction parameters, the general procedures were repeated under the optimum conditions using different concentrations of the drug solutions. The peak height in presence of 5×10^{-7} M Cu(II) nitrate was directly proportional to LOS concentration in the linear range (1×10^{-10} - 5×10^{-7}M). Alternatively, the corresponding regression equation was derived as follow

Ip (µA) = a + b C (µM)

3. Results and discussion

In absence of copper, to investigate the adsorptive behavior of LOS at the hanging mercury drop electrode (HMDE), a cyclic voltammogram of 1×10^{-6} M LOS in 0.04 M Britton Robinson buffer pH= 9.0 solution was recorded after 30 sec of stirring at accumulation -0.6 V versus Ag/AgCl electrode. A clearly defined single cathodic peak corresponding to the reduction of the adsorbed drug was observed at -1.2V; Fig. (1). No peak were observed in anodic branch, indicating that LOS reduction is irreversible process and desorption of the adsorbed product inhibits the appearance of the oxidation product. A maximum developed peak current (i_p) was achieved after accumulation of the drug into the electrode surface for 30s, increasing the accumulation time had no effect on the peak current (i_p) . The low currents produced by LOS in the absence of Cu (II) are suggested to be of nonfaradaic nature. The height of the cathodic peak was shown not to be directly proportional to the scan rate and accumulation time ($t_{acc.}$), the drug was not adsorbed; Fig. (2). In presence of Cu (II) , cyclic voltammetry of LOS in the presence of metal ions, which are capable of forming complexes with the investigated drug or depositing at the Hg electrode is interesting. Upon the addition of Cu (II) to LOS solution, the formation of Cu (I) -LOS complex is expected, due to stabilization of Cu (I) by adsorption at the electrode surface. It was found that, in the presence of imidazole, Cu (II)-complex splits into Cu (II) / Cu(I) and Cu (I) / Cu (0) [28, 29]. In analogy, we can suggest that the electrolysis of LOS in presence of copper is due to the copper (I) – LOS complex that is adsorbed on the HMDE. During the subsequent negative potential sweep, this copper (I) complex is reduced, allowing the drug to be determined.



Here, to investigate the adsorptive behavior of LOS in presence of 5×10^{-7} M Cu (II) nitrate at the hanging mercury drop electrode (HMDE), a cyclic voltammogram of 1×10^{-6} M LOS in 0.04 M B.R. buffer pH= 4.0 solution was recorded after 60 sec of stirring at 0.0 V versus Ag /AgCl. As can be seen in; Fig. (3). A new peak at -0.420 mV after addition of copper ions was observed. No peak were observed in anodic branch, indicating that Cu (I)-LOS reduction is irreversible process and the desorption of the adsorbed product inhibits the appearance of the oxidation product. The height of the peak is dependent on both LOS and copper concentrations, pH, accumulation potential and accumulation time. Also, the adsorptive stripping cycles was carried out for increased values of scan rate (ν) gave rise to a reduction peak with intensities that showed a linear increase with scan rate between 40 and 200 mVs^{-1}, followed the relationship

Log ip(µA) = 1.589 + 0.975 Log ν (mVs^{-1})

r= 0.9992. The slope value, 0.975 is close to theoretical value of 1.0 that is expected for an ideal reaction of surface species [30]. Moreover, the peak potential was shifted linearly to more negative values with increase the scan rate that confirmed

the irreversibility nature of electrode reaction as shown in Fig. (4).

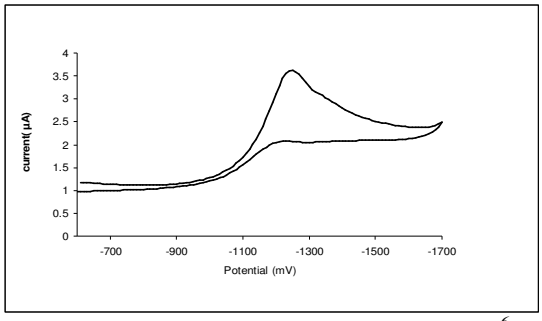


Figure (1): *Cyclic voltammogram for 1× 10^{-6} M **Los** in B.R. buffer pH= 9.0 at scan rate 200 mVs^{-1}, 30 sec preconcentration and equilibrium time = 15 s.*

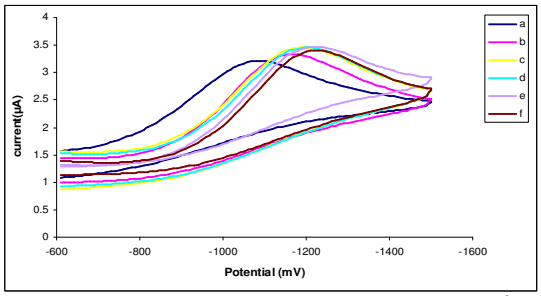


Figure (2): *Cyclic voltammogram for 1× 10^{-6} M **Los** in B.R. buffer pH= 9.0 at scan rate 200 mVs^{-1}, equilibrium time = 15 s*
(a) without accumulation (b) after 30 sec accumulation at -0.6 V
(C) after 60 sec accumulation at -0.6 V
(d) after 90 sec accumulation at -0.6 V
(e) after 120 sec accumulation at -0.6 V
(f) after 180 sec accumulation at -0.6 V

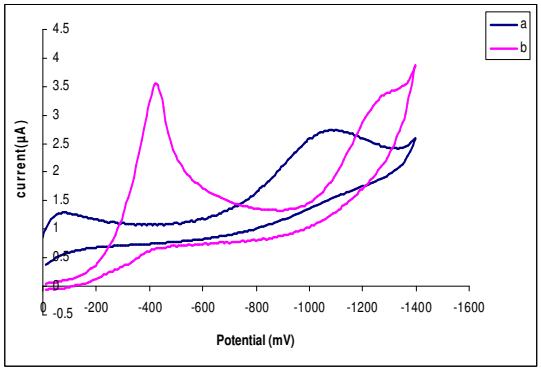


Figure (3): *Cyclic voltammogram for Los-cu complex using 5×10^{-7}M **Los** in presence of 5×10^{-7}M Cu (II) in B.R. buffer pH= 4.0 at scan rate 200 mVs^{-1}, equilibrium time = 15 s*
(a) without accumulation at 0.0 V
(b) after 60 sec accumulation at -0.0 V

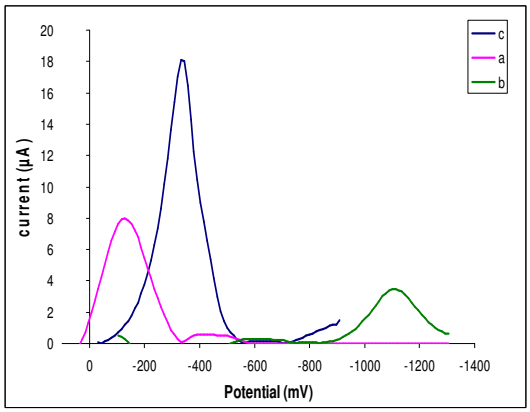


Figure (4): *SWAdCS voltammetry peak current (i_p) in B.R. buffer pH= 4.0, E_{acc}= 0.0 V, t_{acc}= 60 sec, rest time = 15 s, wave increment = 12 mV, wave amplitude E_{sw}=150 mV and wave period = 60ms, sampling time = 10 ms using,*
*(a) 5×10^{-7}M Cu (II) nitrate in absence of **Los***
(b) 5×10^{-7}M Los
*(c) Mixture of 5×10^{-7}M **Los** in presence of 5×10^{-7}M Cu (II)*

3.1. Optimization of the operational parameters

3.1.1. Effect of accumulation potential (E_{acc})

The influence of accumulation potential (E_{acc}) on the monitored electrochemical response of 5×10^{-7} M LOS in presence of 5×10^{-7} M Cu (II) in 0.04 M B.R. buffer pH = 3.0 was examined over the potential + 0.2 to –0.8 V. The peak current increased in the negative direction until it reached to the maximum value at E_{acc} =0.0 V, where it decreased sharply after this potential. Thus, E_{acc} =0.0 V will be adopted as optimum value for this work

3.1.2. Effect of accumulation time (t_{acc})

The dependence of SWAdCS voltammetry peak current on accumulation time was tested at five concentrations 1×10^{-8} M, 5×10^{-8} M, 1×10-^{7} M, 5×10^{-7} M, 1×10^{-6} M of LOS in presence of the 5×10^{-7} M Cu (II) and also 1×10^{-8} M, 5×10-8 M, 1×10^{-7} M, 5×10^{-7} M, 1×10^{-6} M Cu (II) in presence of 5×10^{-7} M LOS in B.R. buffer pH = 3.0, E_{acc}= 0.0 V. These concentrations were examined over the accumulation times range 30 to 180 sec. The longer the accumulation time, the larger peak current and more drug adsorbed, for the concentration higher than 1×10^{-8} M of LOS the SWAdCS voltammetry peak height was increased with increasing the accumulation time and the

break observed at 90 sec means that surface coverage was attained.

3.1.3. Effect of wave amplitude (E_{sw})

The effect of wave amplitude (E_{sw}) on the SWAdCS voltammetry peak current was also studied in B.R. buffer pH = 3.0 showed that, the peak current was enhanced upon increase of wave amplitude (25-200). A wave amplitude of 100 mV gave more sharp and defined peak so preferable in the study.

3.1.4. Effect of wave period

The effect of wave period on the SWAdCS voltammetry peak current was studied in B.R. buffer. pH = 3.0 showed that, the peak current was enhanced upon increase of wave period (50-120 ms). A wave period of 60 ms was preferable in the present study. Moreover, the peak potential shifted linearly to more negative values with increase of wave period that confirmed the irreversibility nature of electrode reaction.

4. Method validation

4.1. Linearity and range

Calibration curves for LOS were attempted under the optimized procedure conditions followed different accumulation time periods at 0.0 V. The regression equation associated with the calibration curves; Table (1) exhibited a good linearity that supported the proposed procedure. The small values of slopes (S_b) and standard deviation of residuals ($S_{y/x}$) indicated the high sensitivity of the proposed method while the small intercept reflected the low interfering background (31, 32).

4.2. Accuracy

The accuracy of the proposed method should be established across the specified range of the analytical procedures on pure forms of studied drugs. Table (2) shows accuracy of the results expressed as bias:

$$\text{Bias } \% = \frac{\text{(measured concentration - concentration taken)}}{\text{concentration taken}} \times 100$$

Then the results were statistically compared with the reported spectrophotometric method for determination of LOS through formation a charge transfer complex with iodine as σ-acceptor (8); using student's *t*-test and the variance ratio *F*-test (31, 32); Table (3). This comparison did not show any significance difference indicating good accuracy of the proposed method.

4.3 Repeatability and intermediate precision

Repeatability and intermediate precision were examined by performing six successive measurements for three concentrations 5×10^{-7} M , 5×10^{-8} M and 5×10^{-9} M of authentic LOS after 90s accumulation demonstrated the reproducibility of the results obtained by the proposed procedure. For intra- assay precision, recoveries were calculated from repeated analysis during one day and for inter-assay precision, recoveries were calculated from repeated analysis for five days over a period of one week. Data of such analysis are summarized in Table (2).

4.4. Specificity and interference study

It was examined by addition of 1×10^{-5} M up to 1×10^{-4} M of (sodium carbonate, magnesium stearate, uric acid, starch and glycine) as a common interference in pharmaceutical preparations, Table (4) show that there is no change in the peak current of the drug was observed, this indicate that there is no significant interference. The addition of 1×10^{-5} M up to 1×10^{-4} M of Pb (II), Cd (II), Ni (II), Zn (II) and Fe (III) result in (10-20%) enhancement of complex peak.

5. Analytical application

5.1. Tablet analysis

The proposed SWAdCS voltammetric method was successfully applied to the determination of LOS in its tablet, the validity was assessed by applying the standard addition method. On plotting of peak height versus concentration of LOS , a straight line is obtained over a range 1×10^{-9} M to 5×10^{-7} M for Cozaar® tablets. The average percentage recovery was 99.0% ± 0.152 and 99.8% ± 0.134, as shown in Table (5a,b).

5.2. Assay of LOS in spiked human urine

LOS was successfully determined in spiked human urine samples by applying the optimized procedure without any prior extraction steps. The displayed voltammograms of six standard additions of LOS in human urine samples: addition affecting drug concentration of 5×10^{-7} M and 1×10^{-8} M , 90 sec accumulation time was employed, the peak current versus drug concentration for samples a and b respectively was presented by a straight line followed by the equations:

ip (μA) = 0.79 (M / 10^{-10}) C + 8.14 r= 0.9997

ip (μA) = 0.91 (M / 10^{-10}) C + 2.43 r= 0.9996

the collected data are illustrated in Tables (6a,b).

5.3. Assay of LOS in spiked human serum

The optimized procedures were successfully applied determination of LOS in protein free spiked human serum samples. No extraction steps other than the centrifugal protein separation were required prior to the assay of drug, the response of successive additions of 0.5×10^{-8} M and 1×10^{-8} M, 90 sec accumulation time was employed. The peak current versus drug concentration for samples a and b respectively was presented by straight line followed by the equation:

ip (µA) = 8.48 C (M / 10^{-8}) + 4.22 r= 0.9949
ip (µA) = 8.56 C (M / 10^{-8}) + 8.28 r= 0.9966
The collected data are illustrated in Table (7a, b).

Table (1): Characteristics of the calibration plots of Los in presence of 5×10^{-7} M Cu(II) at E_{acc}= 0.0 V, rest time = 15 sec, wave increment = 12 mV, t_{acc}= 60s , 90 s and wave amplitude Esw= 100 mV

Parameter	t_{acc}= 60s	t_{acc}= 90s
Linear range (M)	1×10^{-9} -- 5×10^{-7}	1×10^{-10} - 5×10^{-7}
Linear regression Eq.	I_p= 10.23 + 4×10^{7} c	I_p= 12.18 + 1×10^{7} c
Intercept (a)	10.82	11.89
SE of intercept (S_a)	0.154	0.084
Slope (µA/µM)	39	12.39
SE of slope (S_b)	0.603	0.329
Correlation Coefficient (r)	0.9992	0.9997
Determination coeff. (r^2)	0.9982	0.9995
SD of residuals (S_{yx})	0.236	0.131
LOD (M)	1.27×10^{-8}	2.25×10^{-9}
LOQ (M)	3.87×10^{-8}	6.8×10^{-9}

Table (2): Accuracy and precision of the proposed SWAdCS voltammetric method for determination of Los at E_{acc}= 0.0 V, rest time = 15 sec, wave increment = 12 mV, t_{acc}= 90s and wave amplitude E_{sw}= 100 mV

Parameter		Losartan*		
		5×10^{-7} M	5×10^{-8} M	1×10^{-9} M
Intraday	1	100.27	98.91	99.53
	2	100.91	101.08	99.82
	3	99.80	99.45	99.23
	Mean	100.326	99.81	99.53
	S.D	0.557	1.13	0.295
	R.S.D	0.555	1.132	0.296
	Bias %	0.013	-1.08	-0.11
Interday				
	1	101.46	98.38	100.23
	2	100.73	98.91	99.78
	3	100.05	99.45	99.54
	Mean	100.75	98.91	99.85
	S.D	0.705	0.535	0.350
	R.S.D	0.704	0.540	0.351
	Bias %	0.182	-0.541	-0.20

S.D = Standard deviation. R.S.D = relative standard deviation. * Average of three determination

Table (3): Results for the analyzed pharmaceutical preparations at E_{acc}= 0.0 V, rest time = 15 sec, wave increment = 12 mV, t_{acc}= 90s and wave amplitude E_{sw} = 100 Mv

Pharmaceutical samples	% Recovery ± S.D* (n=5)		*t*-value	*F*-value
	Proposed method	Reported method		
Cozaar 50 mg® Tab	99.96 ±0.243	99.85 ±0.544	0.383	4.97

* Average of five determinations
The tabulated values at the 95 % confidence limits are *t* = 2.78 and *F* = 6.39.

Table (4): Effect of interfering species on the SWAdCS voltammetric determination of Los 5×10^{-7} M in presence of 5×10^{-7} M Cu(II)nitrate.

Interfering species	Concentration (M)	(%) R ± SD (n= 3)
Sodium carbonate	1×10^{-4} M 1×10^{-5} M	99.46 ± 0.13 98.91 ± 0.49
Magnesium stearate	1×10^{-4} M 1×10^{-5} M	99.35 ± 0.18 98.41 ± 0.84
Starch	1×10^{-4} M 1×10^{-5} M	100.13 ± 0.47 99.95 ± 0.61
Uric acid	1×10^{-4} M 1×10^{-5} M	100.17 ± 0.37 99.71 ± 0.77
Glycine	1×10^{-4} M 1×10^{-5} M	100.35 ± 0.14 99.51 ± 0.31

Table (5a): Los in different dosage form samples and its statistical parameter by standard addition method at E_{acc}= 0.0 V, t_{acc}=90 sec, rest time = 15 s, wave increment dE = 12 mV, wave amplitude E_{sw}=100 mV and wave period= 60ms

Sample No.	Concentration (M)	Slope i_p/C	Standard addition method	
			Corr. Coeff.	S.E
a	0.5×10^{-8}	13.54	0.9989	0.334
b	5×10^{-8}	12.37	0.9992	0.159

Table (5b): Recovery results in dosage forms (n= 5)

Samples	Added(mol/ l)	Found(mol/ l)	Mean recovery(%)	RSD(%)
Cozaar ® 50mg	0.5×10^{-8}	4.99×10^{-7}	99.8	0.134
Cozaar ® 50mg	5×10^{-8}	4.95×10^{-8}	99.0	0.152

*Average of five determinations

Table (6a): Los in different urine samples and its statistical parameter by standard addition method at E_{acc}= 0.0 V, t_{acc}=90 sec, rest time = 15 s, wave increment dE = 12 mV, wave amplitude E_{sw}=100 mV and wave period= 60ms

Sample No.	Concentration (M)	Slope i_p/C	Standard addition method	
			Corr. Coeff.	S.E
a	0.25×10^{-8}	9.19	0.9997	0.333
b	1×10^{-8}	8.37	0.9996	0.255

Table (6b): Recovery % results in urine samples (n= 5)

Samples	Added(mol/ l)	Found(mol/ l)	Mean recovery(%)	RSD(%)
Urine Samples	0.25×10^{-8}	0.248×10^{-8}	99.6	0.153
	1×10^{-8}	1.02×10^{-8}	102.02	1.06

*Average of five determinations

Table (7a): Los in different serum samples and its statistical parameter by standard addition method at E_{acc}= 0.0 V, t_{acc}=90 sec, rest time = 15 s, wave increment dE = 12 mV, wave amplitude E_{sw}=100 mV and wave period= 60ms

Sample No.	Concentration (nM)	Slope i_p/C	Standard addition method	
			Corr. Coeff.	S.E
a	0.5×10^{-8}	10.06	0.9949	0.49
b	1×10^{-8}	9.85	0.9966	0.40

Table (7b): Recovery % results in serum samples (n= 5)

Samples	Added(mol/ l)	Found(mol/ l)	Mean recovery(%)	RSD (%)
Serum Samples	0.5×10^{-8}	0.49×10^{-8}	98.0	0.256
	1×10^{-8}	1.01×10^{-8}	100.67	0.305

*Average of five determinations

5. Conclusions

The proposed SWAdCS voltammetric method is direct and rapid for the determination of LOS and does not include any extraction process. It is precise, accurate, sensitive, and easy to use while it might be preferred more than other available methods for assay of the drug in clinical and quality control laboratories.

References

1. Sweetman, S., Martindale:"The Complete Drug Reference". 32th ed. 2007, Electronic version, London, U.K: The pharmaceutical press.

2. M.J. Mycek, R.A.H., P.C. Champe, B.D. Fisher, Lippincott's Illustrated Reviews: Pharmacology. 2nd ed. 2000, London, U.K: Lippincott Williams & Wilkins.

3. Lande, N.R., et al., Simultaneous spectrophotometric estimation of losartan potassium and hydrochlorothiazide in tablet dosage form. Indian Drugs, 2000. 37(12): p. 577-81

4. Lastra, O.C., et al., Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets. J Pharm Biomed Anal, 2003. 33(2): p. 175-80.

5. Shah, S.A., et al., Simultaneous determination of losartan and hydrochlorothiazide in combined dosage forms by first-derivative spectroscopy and high-performance thin-layer chromatography. J AOAC Int, 2001. 84(6): p. 1715-23.

6. Ulu, S.T. and S. Saglik, Comparison of UV- and second derivative spectrophotometric and high-performance liquid chromatographic methods for the determination of losartan in tablets. Turkish J Pharm Sci, 2004. 1(3): p. 165-75.

7. Prabhakar, A.H. and R. Giridhar, A rapid colorimetric method for the determination of Losartan potassium in bulk and in synthetic mixture for solid dosage form. J Pharm Biomed Anal, 2002. 27(6): p. 861-6.

8. Darwish, I.A., Analytical study for the charge-transfer complexes of losartan potassium. Anal Chim Acta, 2005. 549(1-2): p. 212-20

9. Bebawy, L.I., et al., Application of first-derivative, ratio derivative spectrophotometry, TLC-densitometry and spectrofluorimetry for the simultaneous determination of telmisartan and hydrochlorothiazide in pharmaceutical dosage forms and plasma. Farmaco, 2005. 60(10): p. 859-67.

10. Furtek, C.I. and M.W. Lo, Simultaneous determination of a novel angiotensin II receptor blocking agent, losartan, and its metabolite in human plasma and urine by high-performance liquid chromatography. J Chromatogr, 1992. 573(2): p. 295-301.

11. Lee, H., et al., Simultaneous determination of losartan and active metabolite EXP3174 in rat plasma by HPLC with column switching. Chromatographia 1996. 42(1-2): p. 39-42

12. Ritter, M.A., C.I. Furtek, and M.W. Lo, An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Pharm Biomed Anal, 1997. 15(7): p. 1021-9.

13. Farthing, D., et al., Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate. J Chromatogr B Biomed Sci Appl, 1997. 704(1-2): p. 374-8.

14. Zhao, Z., et al., Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS. J Pharm Biomed Anal, 1999. 20(1-2): p. 129-36.

15. Kanumula, G.V. and B. Raman, Simultaneous determination of hydrochlorothiazide and losartan potassium in pharmaceutical dosage by reverse phase high performance liquid chromatography. Indian Drugs, 2000. 37 (1): p. 38-41

16. Argekar, A.P. and J.G. Sawant, A gradient reversed-phase high-performance liquid chromatography method for simultaneous determination of hydrochlorothiazide (HCT) and losartan potassium (Los) from tablets. Anal Lett, 2000. 33 (5): p. 869-80

17. Obando, M.A., J.M. Estela, and V. Cerda, Simultaneous determination of hydrochlorothiazide and losartan potassium in tablets by high-performance low-pressure chromatography using a multi-syringe burette coupled to a monolithic column. Anal Bioanal Chem, 2008. 391(6): p. 2349-56.

18. McCarthy, K.E., et al., Determination of losartan and its degradates in COZAAR tablets by reversed-phase high-performance thin-layer chromatography. J Pharm Biomed Anal, 1998. 17(4-5): p. 671-77.

19. Zhang, M., et al., Novel polymer monolith microextraction using a poly(methacrylic acid-ethylene glycol dimethacrylate) monolith and its application to simultaneous analysis of several

angiotensin II receptor antagonists in human urine by capillary zone electrophoresis. J Chromatogr A, 2006. 1102(1-2): p. 294-301.

20. Hillaert, S. and W. Van den Bossche, Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis. J Pharm Biomed Anal, 2003. 31(2): p. 329-39.

21. Quaglia, M.G., et al., Determination of losartan and hydrochlorothiazide in tablets by CE and CEC. J Pharm Biomed Anal, 2002. 29(6): p. 981-7.

22. Williams, R.C., et al., Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar tablets. J Pharm Biomed Anal, 1996. 14(11): p. 1539-46.

23. Habib, I.H., et al., Stripping voltammetric determination of valsartan in bulk and pharmaceutical products. Pharmazie, 2008. 63(5): p. 337-41.

24. Habib, I.H., et al., Cathodic Stripping Voltammetric Determination of Losartan in Bulk and Pharmaceutical Products. Portugaliae Electrochimica Acta, 2008(26/4): p. 315-24.

25. Wang, J., "Stripping analysis; Principals, Instrumentation and Application". 3rd ed. 1985, Florida, USA: Deerfield Beach.

26. Fogg, A.G. and J. Wang, Terminology and Convention for Electrochemical Stripping Analysis. Pure Appl Chem, 1999. 71: p. 891-97.

27. Wang, J., "Analytical Electrochemistry". 3rd ed. 2006, Chap.3, New York, USA: John Wiley & Sons, Inc. 67-94.

28. Glodowski, S., R. Bilewicz, and Z. Kublik, Determination of traces of purine by cathodic stripping voltammetry at the hanging copper amalgam drop electrode. Anal Chim Acta, 1986. 186: p. 39.

29. Shubietah, R.M., A.Z. Abu Zuhri, and A.G. Fogg, Adsorptive Stripping Voltammetric Determination of Dimenhydrinate at a Hanging Mercury Drop Electrode. Mikrochim Acta, 1999. 130: p. 165-71.

30. Laviron, E., A multilayer model for the study of space distributed redox modified electrodes: Part I. Description and discussion of the model. J Electroanalytical Chem, 1980. 112: p. 1-9.

31. ICH Harmonized Tripartie Guidline Q2B (R1) : " Validation of Analytical Proceudures: Text and Methodology". (available at http://www.ich.org): Incorporated in November 2005.

32. ICH Harmonized Tripartie Guidline Q2A: "Text on validation of analytical procedures". (available at http://www.ich.org): Incorporated in November 2005.