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DEVELOPMENT OF VOLTAMMETRIC TECHNIQUES FOR THE DETERMINATION OF SILODOSIN IN PHARMACEUTICAL FORMULATION AT GLASSY CARBON ELECTRODE

Berk TOKER,[a,*] Engin ER[a] and Nevin ERK[a]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University 06100 Ankara, Turkey

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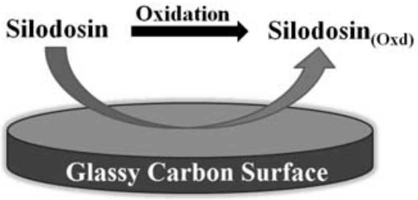
This study related to development of rapid, simple, sensitive and cost effective electrochemical and spectrophotometric methods for quantitative determination of silodosin (SLD) in pharmaceutical formulations. The electrochemical oxidation of SLD on glassy carbon electrode (GCE) was thorougly investigated by cyclic voltammetry and differential pulse voltammetry. The effect of scan rate, pH and concentration on the peak currents were investigated under the optimized experimental conditions for quantitative determination of SLD. The oxidation peak of SLD was observed on GCE at 696.0 mV in pH 2.0 BRT by using differential pulse voltammetry. The oxidation process was observed to be irreversible and diffusion-controlled. The obtained results were statistically compared with those of first derivative UV spectrophotometric method and the differences were found as insignificant.



INTRODUCTION

Silodosin (SLD) is a selective antagonist of alfa-1-adrenoreceptors and a class of medications called alpha-blockers. It is used for the symptomatic treatment benign prostatic hyperplasia. It relieves the symptoms of benign prostatic hyperplasia by relaxing the muscles of the bladder and prostate.[1] SLD has an apparent volume of distribution of 49.5 L and is approximately 97% protein bound. Oral administration of ^{14}C-labeled SLD, the recovery of radioactivity after 10 days was approximately 33.5% in urine and 54.9% in feces. After intravenous administration, the plasma clearance of SLD was approximately 10 L/hour.[1,2]

SLD is chemically known as (1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2,2,2-trifluoroethoxy) phenoxy] ethyl}amino) propyl] indoline -7-carboxamide and the molecular formula is $C_{25}H_{32}F_3N_3O_4$ (Fig. 1). Structural formula is given below:[2]

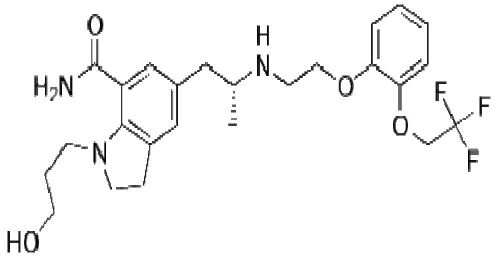


Fig. 1 – Chemical structure of SLD.

SLD is a novel drug. Therefore literature studies show only one analytical method reported for the determination of SLD in human plasma by using liquid chromatography-tandem mass spectrometry.[3] No analytical methods have been reported to date for the determination of novel active material in pharmaceutical formulation.

The electrochemical methods are very popular in the determination of active ingredient in pharmaceutical applications due to their sensitivity, selectivity, rapid response, cost effectiveness, and

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[*] Corresponding author: btoker@ankara.edu.tr

simplicity. Therefore, the voltammetric techniques such as cyclic voltammetry, differential pulse voltammetry have been often used in pharmaceuticals applications.[4-12]

The purpose of this study was to develop and validate a new simple, cost effective, selective, sensitive, short analysis time and reproducible for the routine analysis of commercial pharmaceutical formulations containing SLD. Analytical parameters for these techniques have also been established and compared with those established for the first derivative UV spectrophotometry.

EXPERIMENTAL

1. Reagents and drug

The bulk drug of SLD was supplied from Yeni Recordati Pharm. Co. and Urorec® capsules (8.0 mg SLD per capsule) were purchased local pharmacy of Turkey. All the chemicals were reagent grade.

2. Apparatus

Voltammetric measurements were carried out using BAS 100W (Bio Analytical System, USA) electrochemical analyzers. The three electrode cell used in all experiments contained a Ag/AgCl (BAS, MF-1063) as reference electrode, glassy carbon electrode (GCE) (BAS, MF- 2012) as working electrode and a platinum wire (BAS, MF-1032) as auxiliary electrode. GCE surface was polished with alumina powder, it was rinsed with double-distilled water then dried on a filter paper before each experiment.

The analytical application conditions were used between the range of 0.0 – 1200.0 mV with scan rate of 20.0 mVs^{-1}; pulse amplitude: 50.0 mV; pulse width: 50.0 ms for the differential pulse voltammetry.

The spectrophotometric analysis were performed on a Shimadzu UV-1601 visible double beam spectrophotometer using 10 mm path length quartz cells. Derivative conditions were as follows: scan rate 60.0 nm / min; spectral slit width 2.0 nm and $\Delta\lambda$: 6.0 nm.

3. Solutions preparation

A standard stock solution 10.0 mM of SLD was prepared for voltammetric studies by dissolving an accurate mass of the bulk drug in an appropriate volume of NaCl:methanol (30:70 v/v) mixture, then kept in a refrigerator. SLD working solutions (0.01- 1 mM) were prepared by dilution of the stock solution with supporting electrolyte.

A series of Britton-Robinson buffers (BRT) of pH: 2.0-10.0, used as supporting electrolyte, were prepared in double-distilled deionized water.

In addition, standard stock solutions 1.0 mM of SLD for the first derivative spectrophotometric studies was accurately weighed, dissolved in methanol and then adjusted with the same solvent to give the appropriate concentration.

4. Analysis of pharmaceutical formulation

Urorec® capsules was obtained in a local pharmacy. The average mass of ten hard capsules was determined. The contents of ten capsules were completely removed from shells. An adequate amount of powder, corresponding to a stock solution of concentration 1.0 mM, was weighed, transferred into a 100 mL calibrated flask and completed to the volume with NaCl:methanol (30:70 v/v) mixture. The contents were sonicated for about 15 min to achive complete dissolution. The excipient was separated by filtration. Appropriate solutions were prepared by taking suitable aliquots of the clear filtrate and then diluting with the supporting electrolyte. Each solutions was transferred to the voltammetric cell.

RESULTS AND DISCUSSION

1. Voltammetric studies

Electrochemical behavior of SLD on GCE

The electrochemical oxidation of SLD was investigated in pH 2.0 BRT at GCE by using cyclic voltammetry (Fig. 2). As shown in Fig. 2, a well-defined anodic peak was observed in cyclic voltamogram (CV) of 1.0 mM SLD at +746.0 mV versus Ag/AgCl in pH 2.0 BRT. However, no peak was observed in the cathodic sweep, pointing to the SLD oxidation is an irreversible process. The successive CVs of SLD in Fig. 3 show that the peak current decreases in the second and third sweeps. This behavior may be caused by the fact that the adsorption of SLD or the oxidized product may not be electroactive at the surface of GCE.

The effect of scan rate and pH on the peak current

The effect of scan rate on the peak current was investigated on GCE in Fig. 4. As seen from Fig. 4, the oxidation peak current increased with the increasing square root of scan rate which was the characteristic features of the diffusion controlled mechanism. The linear relation of (I_p (μA) = 0.4835 $v^{1/2}$ (mV s^{-1}) + 0.1966, R^2= 0.9975) between the oxidation peak current (I_p) and the square root of scan rate ($v^{1/2}$) at different scan rates (10-500 mVs^{-1}) shows that the electrode process was totally diffusion controlled. In addition, the anodic peak potential shifted to positive direction with the increasing scan rate which confirms irreversible electrode reactions.[13,14]

The effect of pH on the peak current for 1.0 mM SLD was investigated between pH range of 2.0-10.0 in BRT on GCE. According to the obtained results, the maximum peak current was observed at 696.0 mV in pH 2.0 and the peak potential was shifted towards negative values with incerasing pH as shown in Fig. 5. The relation between the peak potential and pH can be expressed with following equation Ep (mV) = -32.933 pH + 737.16 (R^2=0.9827). According to this equation, the slope is approximately calculated to be 33 mV per pH.

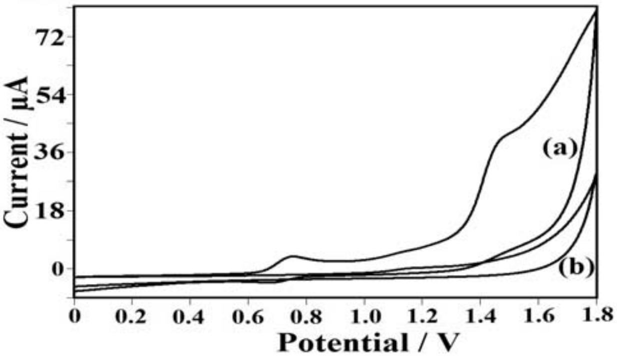

Fig. 2 – CVs of 1.0 mM SLD in pH 2.0 BRT (a), blank solution pH 2.0 BRT (b) on GCE at sweep rate 100 mVs^{-1}.

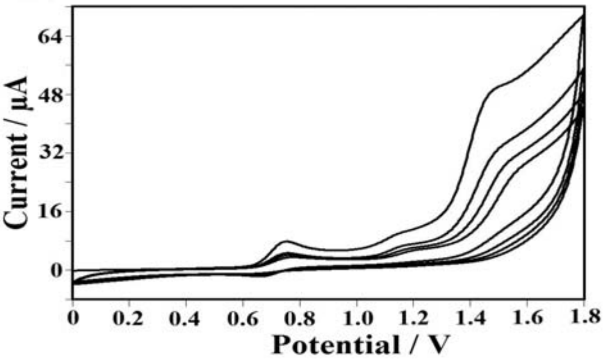

Fig. 3 – Successive CVs of 1.0 mM SLD in pH 2.0 BRT on GCE at sweep rate 100 mVs^{-1}.

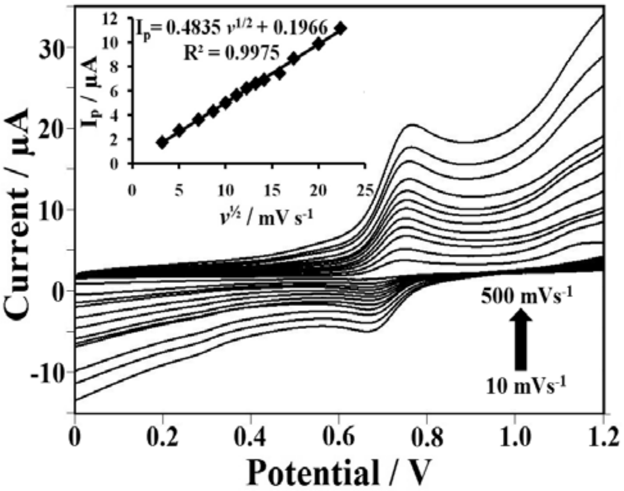

Fig. 4 – CVs of 1.0 mM SLD on GRE at various scan rates (10-500 mVs^{-1}); (Inset graphic: The curve of peak current (I_p) against the square root of scan rates ($v½$)).

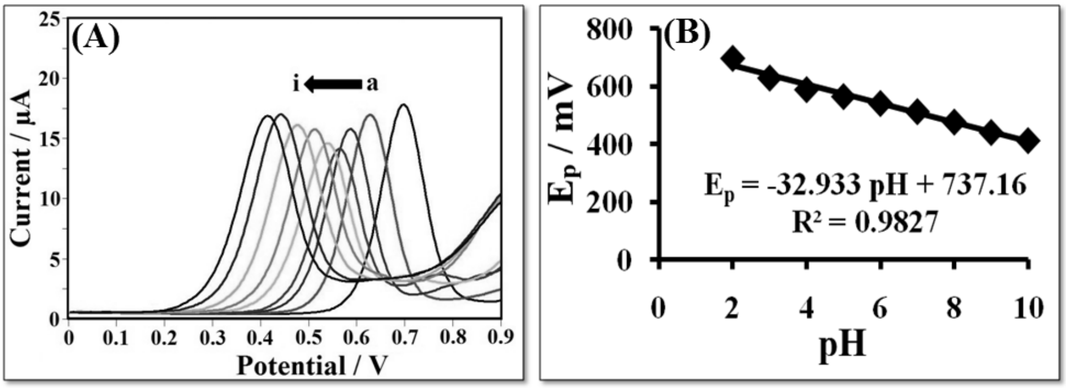

Fig. 5 – (A) DPVs of 1.0 mM SLD on GRE at different pH values (a-i) pH=2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0; (B) The effect of pH upon the oxidation potential.

Determination of SLD in standard solutions

The relation between of SLD concentration and the peak current was investigated under the optimized conditions (pH: 2.0, scan rate: 50 mV/s) by the use of differential pulse voltammetry method (Fig. 6). A linear range concentration and limit of detection were estimated to be 0.001-1.0 mM and 11.6 µM (S/N=3).

4. First derivative spectrophotometric studies

SLD in methanol solution yields a characteristic curve when scanned in the ultraviolet wavelength range between 235.0-400.0 nm in Fig. 7. The first derivative UV spectrums of SLD is shown in Fig. 8. The best results were obtained at amplitude of 260.40 nm the proposed first derivative UV spectrophotometric method. Under the optimum conditions, the linearity range of SLD was calculated to be 18.2-182.0 μM with the detection limit of 6.51 μM.

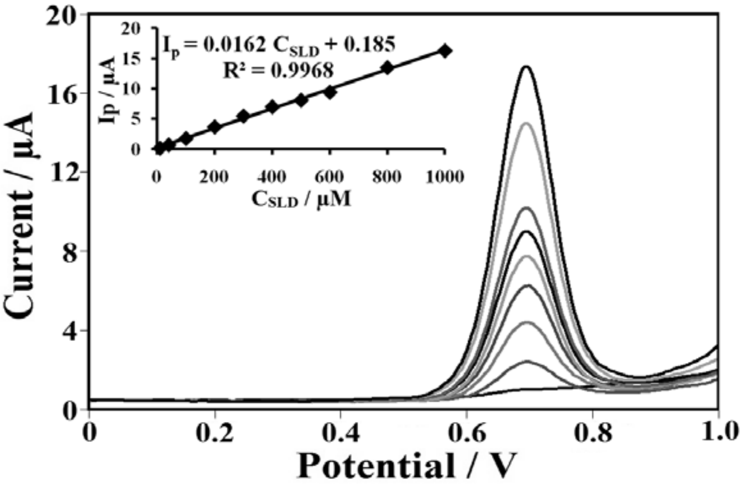

Fig. 6 – DPVs of SLD at different concentrations in pH 2.0 BRT on GRE;
Inset: The relation between the peak current (I_p) and the concentration of SLD (C_{SLD}).

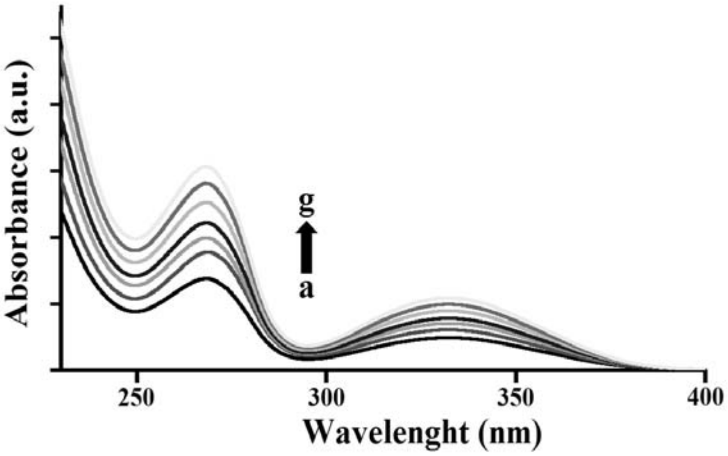

Fig. 7 – The absorption spectra of SLD **(a)** 75.7 μM; **(b)** 90.8 μM;
(c) 106 μM; **(d)** 121 μM; **(e)** 126 μM; **(f)** 151 μM; **(g)** 167 μM in methanol solution.

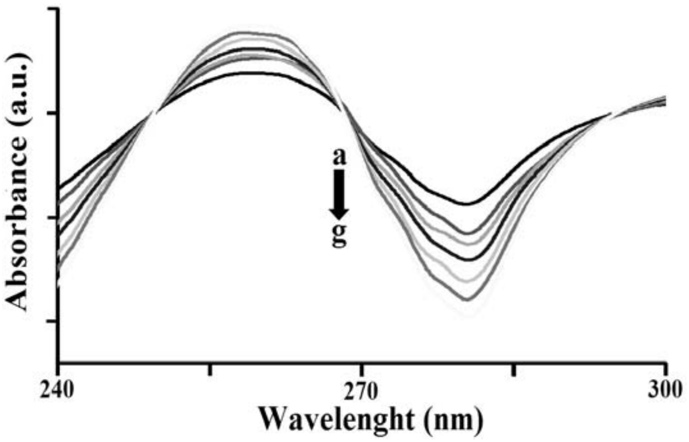

Fig. 8 – The first derivative UV spectra of SLD **(a)** 75.7 μM; **(b)** 90.8 μM; **(c)** 106 μM; **(d)** 121 μM; **(e)** 126 μM;
(f) 151 μM; **(g)** 167 μM in methanol solution (scan rate: 60.0 nm/min; spectral slit width: 2.0 nm and Δλ: 6.0 nm).

Analytical validation of the proposed methods

A calibration curves for voltammetric methods were drawn between peak current and the concentration of SLD. All the calibration curves revealed that the peak current increases linearly with the concentration of SLD under the optimized conditions using described voltammetric methods. Also, the quantitative analysis of SLD was developed using the derivative absorbance value at 260.40 nm as a function of the concentration of SLD for first derivative UV spectrophotometric method. Table 1 represented the characteristics of the calibration curves established, the good linearity of the calibration curves are clearly evident from values of the correlation coefficients and standard deviations. The limits of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations: LOD = 3s/m and LOQ = 10 s/m where, s is the standard deviation of the intercept and m is the slope of the calibration curve.[15-18] The detection limits reported for the proposed methods are listed in Table 1. The repeatability of all proposed methods were examined by performing ten replicate measurements for 800 µM of SLD. The RSD (%) value of anodic peak currents was calculated to be 0.676 %. These RSD (%) and mean recovery obtained values were confirmed repeatability and precision of the all proposed methods. The precision and accuracy of a method is defined as the closeness of agreement between independent test results obtained under prescribed conditions.[15,19,20] Within-day and between-day precision and accuracy of the proposed methods were evaluated by assaying freshly prepared solutions in 200, 800 and 1000µM SLD. Between-day precision and accuracy of the method calculated from the individual recovery data was evaluated by assaying freshly prepared solutions, for three successive days. The RSD (%) ranged from 0.25 to 3.02 for the proposed methods (Table 2), which indicated high precision and accuracy of all the proposed methods. Specificity of the optimized procedures for the determination of SLD was examined in the presence of some common excipients (talc, povidone, starch, magnesium stearete and lactose e.g.). The mean percentage recoveries and relative standard deviations of the drug from this type of common excipients were achieved to be 101.27% and 2.11% for differential pulse voltammetric technique. These results showed no significant interference effect with the excipients for SLD analysis in capsule.

Therefore, the proposed methods can be suitable for quantitative analysis of SLD in pharmaceutical formulations with the sufficient selectivity and sensitivity.

Table 1

Characteristics of the calibration curves of the proposed methods in the determination of SLD

Parameters	Differential Pulse Voltammetry	First Derivate UV Spectrophotometry
Linearity Range, (M)	$1.0\text{x}10^{-3}$ - $1.0\text{x}10^{-6}$	$1.82\text{x}10^{-4}$ - $1.82\ \text{x}10^{-5}$
Slope, (M)	$1.63\text{x}10^{-2}$	$2.46\text{x}10^{2}$
Intercept, (M)	$1.24\text{x}10^{-7}$	$1.18\text{x}10^{-3}$
Standard Deviation of Slope, (M)	$8.62\text{x}10^{-4}$	1.34
Standard Deviation of intercept, (M)	$5.40\text{x}10^{-7}$	$3.04\text{x}10^{-4}$
Limit of Detection (LOD), (M)	$1.16\text{x}10^{-5}$	$6.51\text{x}10^{-6}$
Limit of Quantification (LOQ), (M)	$3.82\text{x}10^{-4}$	$2.15\text{x}10^{-5}$
Regression Coefficients (R^2)	0.9970	0.9889
Repeatability of current /Derivative Absorbance, Recovery (%)	100.30	95.12
Repeatability of current/Derivative Absorbance, RSD (%)	0.676	0.416

Table 2

Precision and accuracy for quantitative determination of SLD by using the proposed voltammetric techniques

	Taken (M)	Found (M)	SD[a]	RSD (%)[b]
Between-day				
Differential pulse voltammetry				
1. day	$8.0\text{x}10^{-4}$	$7.89\text{x}10^{-4}$	$3.93\text{x}10^{-6}$	0.49
2. day	$8.0\text{x}10^{-4}$	$8.04\text{x}10^{-4}$	$5.87\text{x}10^{-6}$	0.73
3. day	$8.0\text{x}10^{-4}$	$8.24\text{x}10^{-4}$	$2.72\text{x}10^{-5}$	3.02

Table 2 (continued)

Within-day				
differential pulse voltammetry	$2.0x10^{-4}$ $8.0x10^{-4}$ $1.0x10^{-3}$	$2.15x10^{-4}$ $7.98x10^{-4}$ $1.00x10^{-4}$	$4.08x10^{-6}$ $3.98x10^{-6}$ $3.32x10^{-5}$	1.90 0.49 3.39

[a] SD: Standard deviation

[b] RSD: Relative Standard deviation

Table 3

The results obtained in determination of SLD in pharmaceutical formulation[a]

Tablet	Differential Pulse Voltammetry	First Derivative UV Spectrophotometry
Mean (mg)[b]	8.29	7.64
SD	0.26	0.36
t-test	1.67	$t_{Theoretical}$(%95):2.78
F-test	1.88	$F_{Theoretical}$(%95):6.39

[a] Urorec® was labeled to contain 8.0 mg SLD, per one dose.

[b] Each value is the mean of five experiments; SD: standard deviation.

SLD assay in capsules

The proposed methods were successfully applied for the determination of SLD in its commercial formulations. As can be seen in Table 3, the obtained results in the analysis capsules by using proposed methods were summarized. In the present, the first derivative UV spectrophotometric method was chosen as the reference method. The statistical comparison of methods were made by t-test and F-test. According to the results of t-test and F-test at the probability level, insignificant differences appeared between the voltammetric techniques regarding their accuracy, precision and recoveries.

CONCLUSIONS

In this study, the voltammetric and first derivative UV spectrophotometric methods were developed for quantitation analysis of SLD in pharmaceutical formulations. These methods proved to be rapid, simple, sensitive, reliable, fast and reproducible. Thus, the proposed methods appear to be a promising alternative for pharmacokinetic studies and routine analysis in pharmaceutical applications.

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