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Adsorptive stripping voltammetric methods for determination of ezetimibe in tablets

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Abstract

In this study, square-wave adsorptive stripping voltammetry (SWAdSV) and differential pulse adsorptive stripping voltammetry (DPAdSV) were used for determination of ezetimibe (EZE) in the presence of 0.1 M K_2HPO_4–$Na_2B_4O_7$ (1:1) supporting electrolyte. The well-defined peaks for SWAdSV were observed at accumulation time of 15 s, accumulation potential of -0.80 V, frequency of 15 Hz, pulse amplitude of 25 mV, potential increment of 4 mV. For DPAdSV, accumulation time of 15 s, accumulation potential of -0.85 mV, scan rate of 20 mV/s and pulse amplitude of 50 mV were found as the best apparatus parameters. EZE gave rise to a single voltammetric peak in the potential interval from -1236 to -1252 mV for SWAdSV and interval from -1190 to -1210 mV for DPAdSV. The developed methods were validated according to the ICH guideline and were found to be linear, sensitive, specific, precise and accurate. The linearity ranges of EZE for SWAdSV and DPAdSV are 33–596 ng/ml and 66–400 ng/ml, respectively. The developed method was applied successfully for the determination of EZE in tablet dosage form.

Keywords: differential pulse adsorptive stripping voltammetry; ezetimibe; pharmaceutical formulation; square-wave adsorptive stripping voltammetry; validation.

Introduction

Ezetimibe, EZE, (3*R*,4*S*)-1-(4-fluorophenyl)-3-[(3*S*)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin-2-one (Figure 1), is the first member of the cholesterol absorption inhibitors, a novel class of lipid modifying, which potently inhibit the absorption of biliary and dietary cholesterol from the small intestine without affecting the absorption of fat-soluble vitamins, triglycerides or bile acids (Mycek et al. 2001). It is indicated for monotherapy or in combination with 3-hydroxy-3-methyglutaryl coenzyme A-reductase inhibitors (statins) in patients with primary hypercholesterolemia, in combination with simvastatin or atorvastatin in patients with homozygous familial hypercholesterolemia and as monotherapy in patients with homozygous familial sitosterolemia (Jeu and Judy 2003).

Several methods have been reported for the determination of EZE alone or in combination with statins in biological fluids and in pharmaceutical dosage forms including chromatography (Rajkondawar 2006, Rao et al. 2006, Basha et al. 2007, Chaudhari et al. 2007, Dhaneshwar et al. 2007, Li et al. 2007, Mahadik and Dhaneshwar 2007, Oliveira Paulo et al. 2007, Ozaltin and Ucakturk 2007, Qutab et al. 2007, Shivshanker et al. 2007, Sonawane et al. 2007, Doshi et al. 2008, Dixit et al. 2008, Neelima et al. 2008, Pawar et al. 2008, Seshachalam and Kothapally 2008, Ucakturk et al. 2009), spectrophotometry (Sankar et al. 2005a,b, 2006, Imran et al. 2006, Shrivastava et al. 2006, Mishra et al. 2007, Anandakumar et al. 2008, Deshmukh et al. 2008, Kothapalli et al. 2008, Palabiyik et al. 2008, Baraka et al. 2008, Dhandapani et al. 2009), and micellar electrokinetic chromatography (Dalmora Sergio et al. 2008). Chromatographic methods for the determination of EZE need expensive equipment and materials and also include time-consuming extraction steps to eliminate the excipients. The described methods are direct methods for the determination of EZE without using any reagents, which cause interferences and contaminations. In addition, these electroanalytical methods were developed in order to contribute to knowledge in the literature. The aim of this study was to develop square-wave adsorptive stripping voltammetry (SWAdSV) and differential pulse adsorptive stripping voltammetry (DPAdSV) methods for determination of EZE in pharmaceutical preparation. The validation parameters of the methods were evaluated. The developed methods were applied to the analysis of a commercial pharmaceutical preparation. The results were compared with those obtained from an UV-spectrophotometric method given in the literature (Shrivastava et al. 2006).

Materials and methods

Reagents

EZE was supplied from the Central Institute of Hygiene in Turkey (Ankara). It was tested for purity by measuring its melting point, UV and IR spectra, and no impurities were found. All other chemicals were of analytical reagent grade [Merck (Darmstadt, Germany) and Sigma (Steinheim, Germany)]. Triple-distilled mercury was used throughout.

EZE stock standard solution (1000 μg/ml) was prepared by dissolving 10 mg of EZE in 10 ml MeOH. Working standard solutions were prepared daily by appropriate dilution of the

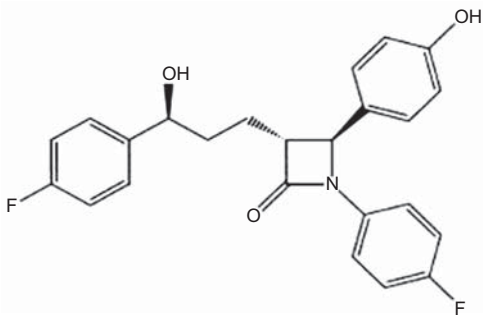

Figure 1 Chemical structure of EZE.

stock standard solution with MeOH. Supporting electrolyte was prepared by mixing the solutions of 0.1 M K_2HPO_4 and 0.1 M $Na_2B_4O_7 \cdot 2H_2O$ (25:25).

Tablet solutions

Ten tablets were weighed and powdered. Equivalent amount to one tablet was accurately weighed and transferred to a 50 ml volumetric flask. MeOH (30 ml) was added and the flask was sonicated for 15 min to complete dissolution and diluted to the mark with MeOH. A 5-ml portion was centrifuged for 10 min. Appropriate solutions were prepared from the supernatant by dilution with MeOH.

Synthetic tablet preparations

Synthetic tablets were prepared by mixing excipients (croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, povidone, sodium lauryl sulfate) and labeled amount (10 mg) of EZE. Then, the mixture was transferred to a 50-ml volumetric flask and appropriate solutions were prepared according to the tablet solution procedures.

Apparatus

A BAS 100 B/W model electrochemical workstation (West Lafayette, IN, USA) was used. The reference electrode was Ag/AgCl and a platinum wire was used as the auxiliary electrode and a hanging mercury drop electrode (HMDE) was used as the working electrode.

An Agilent 8453 single beam UV-visible spectrophotometer (Waldbronn, Germany) was used for spectrophotometric analysis. All pH measurements were made with a Metler Toledo MA 235 instrument (Giesson, Germany).

Procedure

A 3.0-ml volume of supporting electrolyte [K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25) (pH 9.0)] was deoxygenated with prepurified nitrogen for 10 min. After the voltammogram of this solution had been recorded, EZE standard solution was added by micropipette. Nitrogen was passed through the solution for 1 min to mix the solution. The voltammogram was recorded again. This procedure was repeated until the peak height no longer increased.

Results and discussion

Electrochemical behaviors of EZE

To study the nature of the electrode process occurring at the electrode surface, cyclic voltammetry (CV) investigations of EZE were performed. In the CV technique, the effects of scan rate (v) on the peak current (I_p) of EZE were evaluated between 10 and 1000 mV/s (Figure 2). A plot of log I_p vs. log v gave a straight line with a slope 0.45. A slope of <0.50 shows that reduction current of EZE was diffusion controlled. In addition, the peak potential shifted to negative values by increasing concentration of EZE. It was thought that the reduction reaction was irreversible (O'Dea et al. 1993). The reversibility of EZE reduction was also studied by the CV method. The loss of the anodic peak on the reverse scan showed that the reduction reaction was not reversible. The peak potential shifted to negative values when the scan rate was increased (Bond 1980). These results also confirmed that the reduction reaction was irreversible. As a result of CV and square-wave voltammetry (SWV) experiments, it is concluded that the reduction mechanism included two electrons and two protons. The peak potential was shifted to more negative values with increasing pH, as can be seen from Figure 3. This behavior indicates that hydrogen ion is participating in the electrode process (Heyrovsky and Kuta 1966). The following equation which is related to irreversible systems was used to calculate the number of protons:

$$E_p = E^0 - 2.303\ p\ R\ T/\alpha\ n\ F\ pH \qquad [1]$$

The number of protons calculated from Eq. [1] was two protons.

In the CV method, the following equation, expressed as the Randles-Sevcik equation, was used to calculate the diffusion coefficient of EZE (Greef et al. 1990):

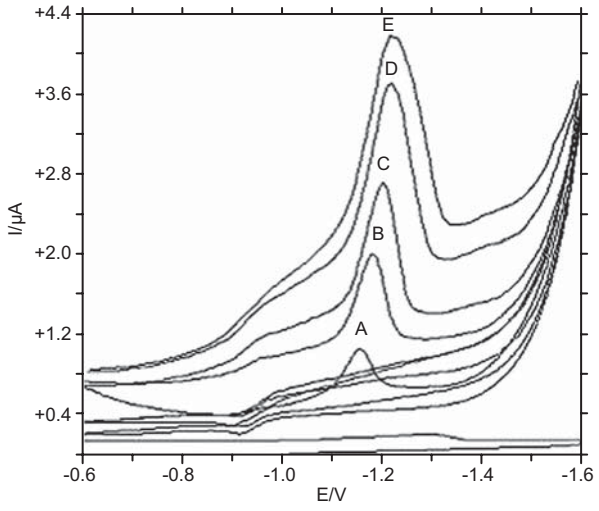

Figure 2 Cyclic voltammogram of EZE on HMDE. Supporting electrolyte: K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25, v/v) (pH 9.0); (A) 25 mV/s; (B) 50 mV/s; (C) 100 mV/s; (D) 250 mV/s; (E) 500 mV/s.

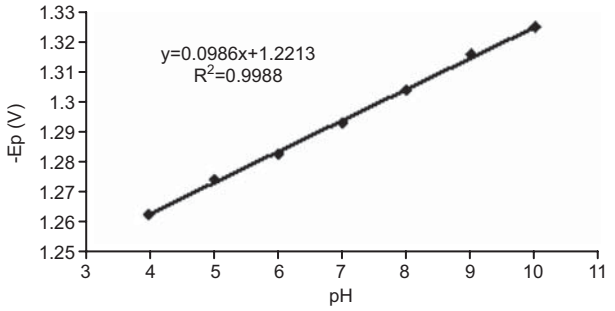

Figure 3 Plot of pH vs. peak potential for 4.76 µg/ml EZE in the presence of K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25, v/v).

$$I_p=(2.99\times10^5)\ n\ (\alpha_C n_\alpha)^{1/2} A\ C_0\ D_0^{1/2}\ v^{1/2} \quad [2]$$

Diffusion coefficient of EZE calculated from these equations was 3.50×10^{-4} cm^2/s.

In addition, it is known that carbonyl compounds are reduced to alcohols in alkaline media at high over-voltage cathodes such as Hg and reduced to pinacols in acidic media (Kolthaoo and Lingane 1952). It is thought that carbonyl (-C=O) belonging to the group of azetidinone found in the structure of EZE is reduced to -CH(OH) at HMDE. We therefore propose Figure 4 as a possible mechanism to explain the electrochemical reduction of EZE. Chemical reaction (C) following electrochemical step (E) can occur as a strong hydrogen bond between flour and hydrogen. Because this product is stable, reoxidation does not occur. Owing to this situation, anodic peak on the reverse scan is not observed. As can be seen from Figures 5 and 6, the peak current value cannot depend strongly on the accumulation time, suggesting a little adsorption of EZE on the HMDE. The peak current decreased with the accumulation time. An accumulation time of 15 s was selected as an optimum condition for both techniques.

Effect of supporting electrolyte and pH

A series of supporting electrolytes [borate, acetate, phosphate and K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25)] were tested in the presence of 1.64 µg/ml EZE. The results showed that EZE in K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25) mixture gave a signal response (Figure 7). The solution condition such as the pH and the concentration of EZE affect the peak potential and peak current significantly. Because SWV is a rapid method, this method was used to investigate effect of supporting electrolyte and pH.

Peak current increases gradually up to pH 9.0. Therefore, pH 9.0 was selected as optimum pH (Figure 8). As shown, as the equilibrium was reached, the concentration of the anion form increases by increasing pH. Increasing of peak current by increasing pH showed that anion form of EZE was reduced at HMDE. Observation of reduction peak only in K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25) mixture

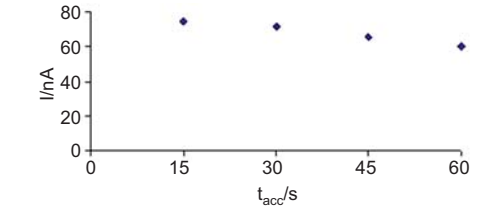

Figure 5 Effect of the accumulation time (t_{acc}) on the square-wave adsorptive peak current response for 300.00 ng/ml EZE in [K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25, v/v) (pH 9.0)] at E_{acc}=-0.80 V.

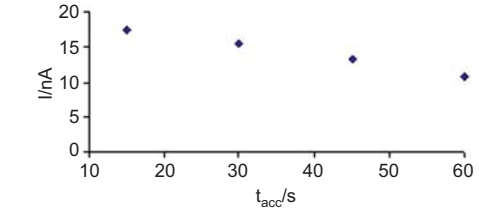

Figure 6 Effect of the accumulation time (t_{acc}) on the differential pulse adsorptive peak current response for 200.00 ng/ml EZE in [K_2HPO_4 (0.1 M)–$Na_2B_4O_7$ (0.1 M) (25:25, v/v) (pH 9.0)] at E_{acc}= -0.85 V.

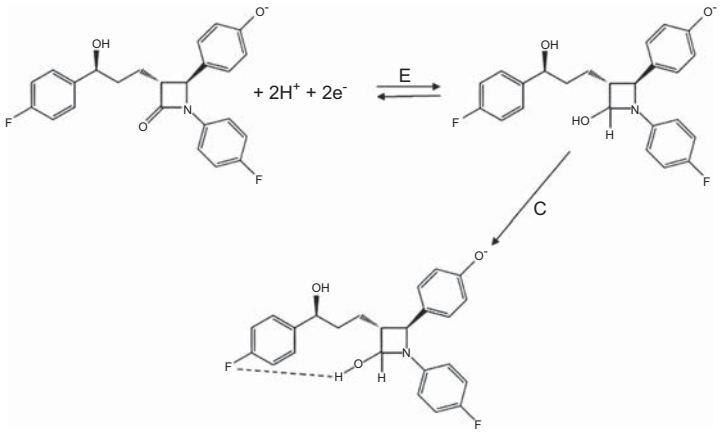

Figure 4 Reduction mechanism of EZE.

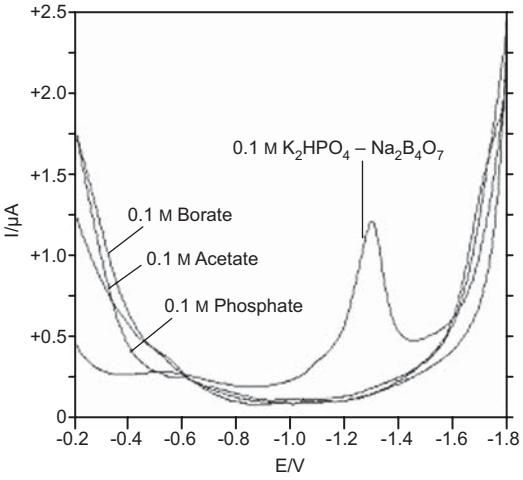

Figure 7 Effect of supporting electrolyte on the peak current of EZE.

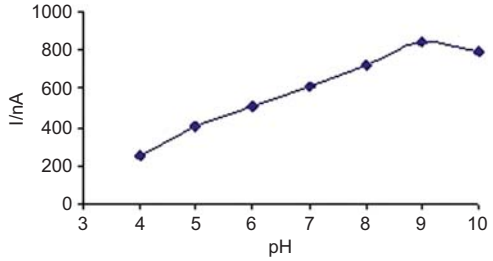

Figure 8 Effect of pH on the peak current of EZE.

means anion form of EZE was stable only in this supporting electrolyte.

Optimization of the experimental conditions

SWAdSV and DPAdSV were applied for the quantitation estimation for EZE at the HMDE. Both techniques require some parameter adjustment. The optimum instrumental conditions for frequency (f), scan increment (Δs), pulse amplitude (E_{sw}), accumulation time (t_{acc}), accumulation potential (E_{acc}) and scan rate (v) were examined.

The influence of the accumulation potential on the peak height was studied over a wide range of potentials. When the potential was made more negative, the peak height decreased due to reduction in the amount of the adsorbed EZE. Therefore, accumulation potential was fixed at -0.80 V vs. Ag/AgCl for SWAdSV, -0.85 V vs. Ag/AgCl for DPAdSV.

For SWAdSV, the frequency varied from 10 to 100 Hz and the scan increment varied from 2 to 5 mV. Although the signal response increased with frequency above 15 Hz the peak shape was deformed. When peak height and the peak shape were taken into consideration, 4 mV of scan increment was chosen. When the pulse amplitude was varied in the range of 20–60 mV for both techniques, the peak current increased with increasing pulse amplitude; however, above 25 mV for SWAdSV and above 50 mV for DPAdSV peak broadening was observed. For DPAdSV, the scan rate varied from 10 mV/s to 40 mV/s. The highest peak current was observed at 20 mV/s.

Hence, the well-defined peaks for SWAdSV were observed at accumulation time of 15 s, accumulation potential of -0.80 V, frequency of 15 Hz, pulse amplitude of 25 mV, and potential increment of 4 mV. For DPAdSV, accumulation

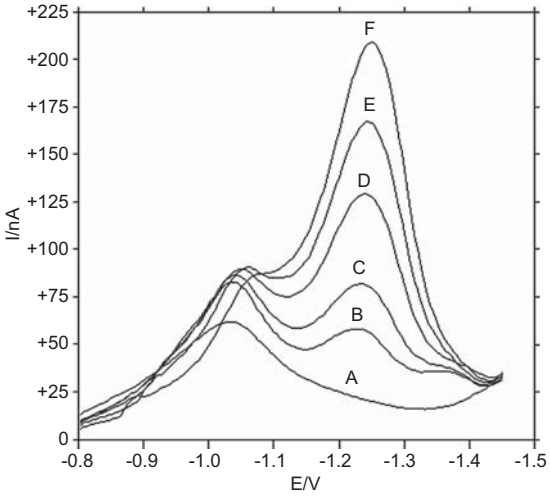

Figure 9 Effect of concentration on the peak current of EZE with the SWAdSV method (A) supporting electrolyte; (B) 66.00, (C) 99.00, (D) 323.00, (E) 446.00, (F) 596.0 ng/ml EZE (accumulation time of 15 s, accumulation potential of -0.80 mV, frequency of 15 Hz, amplitude of 25 mV, potential increment of 4 mV).

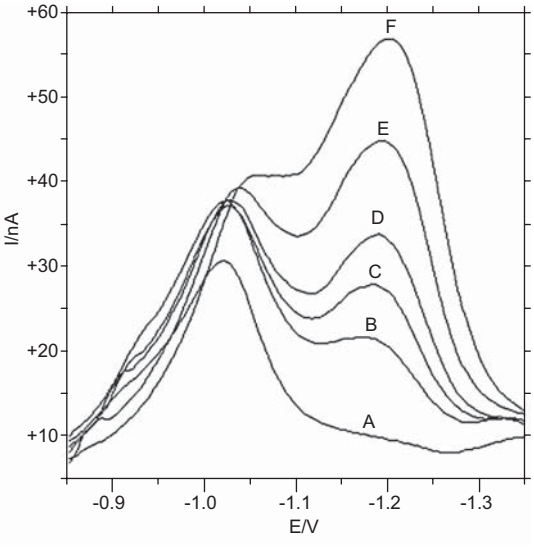

Figure 10 Effect of concentration on the peak current of EZE with the DPAdSV method (A) supporting electrolyte; (B) 66.00, (C) 115.00, (D) 148.00, (E) 212.00, (F) 400.00 ng/ml EZE (accumulation time of 15 s, accumulation potential of -0.85 mV, scan rate of 20 mV/s, amplitude of 50 mV).

Table 1 Data of the calibration curves for the proposed methods (n=7).

	SWAdSV	DPAdSV
Regression equation	y=0.2485x+2.79	y=0.0856x-0.49
Standard error of slope	0.54	0.37
Standard error of intercept	0.63	0.23
Correlation coefficient (r)	0.9987	0.9998
Linearity range (ng/ml)	33–596	66–400
Number of data points	8	8
LOD (ng/ml)	11.53	20.26
LOQ (ng/ml)	31.73	62.46

y=ax+b; y, peak current (nA); x, EZE concentration (ng/ml); a, slope; b, intercept; LOD, limit of detection; LOQ, limit of quantitation.

time of 15 s, accumulation potential of -0.85 mV, scan rate of 20 mV/s, and pulse amplitude of 50 mV were found as the best apparatus parameters.

Validation of the proposed method

Stability The standard stock solutions of EZE were stored in two different conditions at 4°C for 30 days (long-term stability) and at ambient temperature for 12 h (short-term stability). During this period, the solutions were analyzed by the developed method and no significant differences was found in EZE concentrations. It was decided that EZE is highly stable in the mentioned conditions.

Linearity range Voltammograms recorded with increasing amount of EZE (Figures 9 and 10) show that the peak currents increased linearly with increasing concentration. Each point of the calibration graph corresponded to the mean value obtained from seven independent measurements. Data of the calibration curves for the proposed methods are given in Table 1.

Limit of quantification (LOQ) and limit of detection (LOD) Limit of quantification was estimated by the equation: LOQ=10 S/m; limit of detection was estimated by the equation: LOD=3.3 S/m, where S is the standard deviation of the intercept and m is the slope of the regression line (ICH Harmonised Tripartite Guideline 2005). LOD and LOQ were found to be 11.53 ng/ml and 31.73 ng/ml for SWAdSV and 20.26 ng/ml and 62.46 ng/ml for DPAdSV, respectively.

Precision Three different concentrations of EZE (66.00, 228.00, 446.00 ng/ml EZE for SWAdSV and 115.00, 212.00, 360.00 ng/ml EZE for DPAdSV) in the linear range were analyzed in six independent series on the same day (intra-day precision) and six consecutive days (inter-day precision) from six measurements of every sample in each series (Tables 2 and 3). The RSD values varied from 0.62 to 1.43 for intra-day and from 0.63 to 1.72 for inter-day precision in SWAdSV and from 0.60 to 1.05 for intra-day and from 0.67 to 1.35 for inter-day precision in DPAdSV. The low RSD values of intra-day and inter-day indicated that the developed methods have high precision (Green 1996).

Accuracy The accuracy of an analytical method expresses the closeness between the reference value and found value. Accuracy was evaluated as percentage relative error between the measured mean concentrations and added concentrations for EZE (bias%). Both results obtained for intra-day and inter-day accuracy were ≤2.00% (Tables 2 and 3) (Green 1996).

Table 2 Precision and accuracy of the SWAdSV method (n=6).

Added (ng/ml)	Intra-day			Inter-day		
	Found[a] (ng/ml)	Precision[b] (%)	Accuracy[c] (%)	Found[a] (ng/ml)	Precision[b] (%)	Accuracy[c] (%)
66.00	66.34±0.39	1.43	0.89	65.80±0.46	1.72	1.04
228.00	227.28±0.66	0.71	0.61	229.02±0.86	0.92	0.71
446.00	444.80±1.14	0.62	0.42	445.02±1.15	0.63	0.56

[a]Mean±standard error; [b]precision %: relative standard deviation; [c]bias %: [(found-added)/added]×100%.

Table 3 Precision and accuracy of the DPAdSV method (n=6).

Added (ng/ml)	Intra-day			Inter-day		
	Found[a] (ng/ml)	Precision[b] (%)	Accuracy[c] (%)	Found[a] (ng/ml)	Precision[b] (%)	Accuracy[c] (%)
115.00	115.14±0.49	1.05	0.70	114.96±0.63	1.35	0.72
212.00	212.74±0.52	0.60	0.67	212.96±0.93	1.07	0.82
360.00	360.74±0.95	0.65	0.58	360.49±0.98	0.67	0.61

[a]Mean±standard error; [b]precision %: relative standard deviation; [c]bias %: [(found-added)/added]×100%.

Recovery The determination of EZE in a synthetic preparation [the mixture of excipients and labeled amount (10 mg EZE)] were made. The recovery percentage values ranged between 99.10% and 101.30% with RSD <2.00. Closeness of the results to 100.00% showed that recovery of the developed methods were very good (Green 1996).

Selectivity The voltammograms obtained from tablet solution and synthetic preparation were identical with that obtained from standard solution containing an equivalent of EZE (Figures 11 and 12). In addition, the standard addition technique was applied to the same preparations which were analyzed by calibration curves. The regression equations of the standard addition curve for SWAdSV and DPAdSV were found to be y=0.2512x+11.62 and y=0.0904x+2.32, respectively. There was no significant difference between slopes of calibration curves and standard addition curves. These results show that there was no interference from matrix components. Therefore, it can be concluded that the developed method is highly selective.

Robustness and ruggedness

The effect of different analysts (two) on the results for 164.00 ng/ml EZE was evaluated. The obtained results were compared by the Wilcoxon test and there was no significant difference between the results of the two analysts (p>0.05). Robustness testing was performed with deliberate small changes at buffer pH (pH 8.90 and pH 9.10), accumulation time (10 s and 20 s), and accumulation potential (-0.9 V and -0.7 V). Each deliberate small change was analyzed by seven independent series containing 164.00 ng/ml EZE. These results were compared by the Wilcoxon test and there was no significant difference between the results of changed conditions (p>0.05). Thus, it can be concluded that the developed methods are rugged and robust.

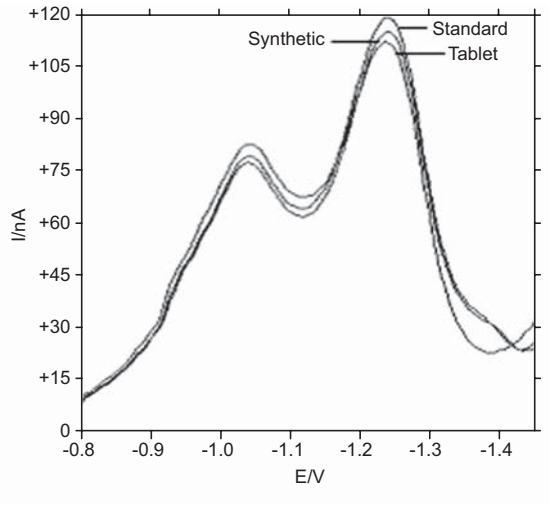

Figure 11 Voltammograms of synthetic preparation, tablet and standard solution for SWAdSV (228.00 ng/ml EZE).

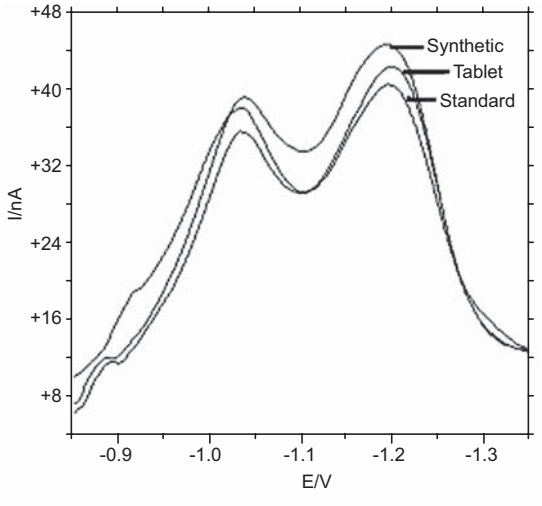

Figure 12 Voltammograms of synthetic preparation, tablet and standard solution for DPAdSV (210.00 ng/ml EZE).

Determination of EZE in a pharmaceutical formulation

To check the applicability of the proposed methods, commercial tablet formulations containing EZE (10 mg per tablet) were analyzed. A spectrophotometric method was employed as a comparison to evaluate the validity of the developed methods. Table 4 gives the results obtained by the three methods for the determination of EZE in pharmaceutical preparations. The results were compared by the Kruskal-Wallis test and there was no significant difference between the methods (p>0.05).

Conclusion

It can be concluded that SWAdSV and DPAdSV are good techniques for determination of EZE in pharmaceutical products. With these methods, a high percentage of recovery

Table 4 Comparison of the results obtained by the SWAdSV, DPAdSV and UV methods for Ezetrol® containing 10 mg EZE (n=7).

Tablet no.	EZE found/mg per tablet		
	SWAdSV	DPAdSV	UV
1	10.09	10.11	10.22
2	9.98	10.02	10.15
3	10.27	9.87	10.02
4	10.08	9.96	10.04
5	10.06	10.12	10.20
6	9.95	9.84	10.06
7	9.89	10.07	10.17
$\overline{X}$	10.05±0.05	10.00±0.04	10.12±0.03
SD	0.12	0.11	0.08
RSD	1.23	1.12	0.79
$KW_{(calculated)}=2.19<KW_{(tabulated)}=7.815$, p>0.05			

$\overline{X}$, mean±standard error; SD, standard deviation; RSD%, relative standard deviation.

shows that the SWAdSV and DPAdSV methods can be used to quantify EZE without interference from the excipients. Chromatographic methods for the determination of EZE need expensive equipment and materials and also include time-consuming extraction steps to eliminate the excipients. The described methods are direct methods for the determination of EZE without using any reagents, which cause interferences and contaminations.

In conclusion, the proposed methods are precise, accurate, sensitive, rapid, cheap, easy to use and might be preferred to published chromatographic and spectrophotometric methods for determination of EZE in pharmaceutical preparations.

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