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Electroanalytical characteristics of antipsychotic drug ziprasidone and its determination in pharmaceuticals and serum samples on solid electrodes

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ABSTRACT

Ziprasidone is a psychotropic agent used for the treatment of schizophrenia. Its oxidation was investigated electrochemically at boron-doped diamond and glassy carbon electrodes using cyclic, differential pulse, and square wave voltammetry. The dependence of the peak current and peak potentials on pH, concentration, nature of the buffer, and scan rate were examined. The process was diffusion and adsorption controlled for boron-doped diamond and glassy carbon electrodes, respectively. The possible mechanism of oxidation was discussed with some model compounds that have indole and piperazine oxidations. A linear response was obtained between 8×10^{-7} and 8×10^{-5} M for the first peak in acetate buffer (pH 5.5) and between 2×10^{-6} and 2×10^{-4} M for the second peak in 0.1 M H_2SO_4 with boron-doped diamond electrode for differential pulse and square wave voltammetric techniques. The reproducibility and accuracy of the proposed methods were found between 0.31 and 1.20, 99.27 and 100.22, respectively. The recovery studies were also achieved to check selectivity and accuracy of the methods. The proposed methods were applied for the determination of ziprasidone from pharmaceutical dosage forms and human serum samples without any time-consuming extraction, separation, evaporation or adsorption steps prior to drug assay except precipitation of the proteins using acetonitrile. The results were statistically compared with those obtained through an established LC–UV technique, no significant differences were been found between the voltammetric and LC methods.

1. Introduction

Schizophrenia is a psychiatric disorder characterized by abnormalities in the perception or expression of reality. It has a high affinity for dopamine, serotonin, and alpha-adrenergic receptors and a moderate affinity for histamine receptors, where it is believed to act as an antagonist [1].

The systemic bioavailability of ZPR administered intramuscularly is 100% or orally is 60%. ZPR absorption is not optimally achieved when administered without food. ZPR is hepatically metabolized by aldehyde oxidase; minor metabolism occurs via cytochrome P450 3A4 (CYP3A4) [1–4]. Mean peak serum concentration of unchanged drug is reported as about 45 ng ml^{-1} (between 30.0 and 62.0 ng ml^{-1}) and the mean AUC of 335.7 ng h ml^{-1} [4]. Major urinary metabolites were reported as oxindole-acetic acid and its glucuronide conjugate, benzisothiazole-3-yl-piperazine and its derivatives.

ZPR has a warning due to increased mortality in elderly patients with dementia-related psychosis. Withdrawal must be done gradually or else cardiac complications may arise, possibly resulting in death [5,6].

ZPR is 5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2*H*-indol-2-one (Scheme 1).

ZPR has been studied and determined by very few studies. Some analytical methods were described for the determination of ZPR including liquid chromatography [7–14], mass spectrometry [11–13], electrophoresis [15], and spectrophotometry [16]. There is no information on the electrochemical behavior of ZPR reported, and no quantitative determination method has been proposed for its analysis in dosage forms or biological media by using any voltammetric techniques.

Boron-doped diamond electrodes (BDD) have a hard, chemically inert, and easy-cleaning surface that proves high stability, optical properties, low voltammetric background currents, high thermal conductivity, and very low capacitance [17–20]. Glassy carbon (GC) electrodes are the most common carbon-based electrodes because of their excellent mechanical and electrical properties, wide potential range, chemically inert nature, and impermeability to gases [20].

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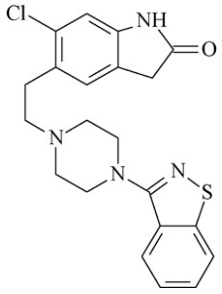


Scheme 1. Structure of ziprasidone.

The aim of this work is to carry out a detailed investigation on the electrochemical behavior and possible oxidation mechanism of ZPR with BDD and GC electrodes by using cyclic (CV), linear sweep (LSV), differential pulse (DPV), and square wave voltammetric (SWV) techniques. Also simple, selective, sensitive, fully validated, rapid, and reliable voltammetric methods were developed for the direct determination. These techniques did not require sample pre-treatment or any time-consuming extraction step prior to drug assay in dosage forms. For the spiked human serum samples, very simple precipitation and centrifugation steps were enough to carry out the voltammetric studies. The proposed methods might be alternatives to the LC techniques in therapeutic drug monitoring or the experimental data might be used for the development LC–EC method.

2. Experimental

2.1. Apparatus

Voltammetric measurements were recorded using BAS 100 W (Bionalytical System, USA) electrochemical Analyzer with a standard three-electrode configuration. The three-electrode system consisted of a boron-doped diamond (BDD, Windsor Scientific Ltd; ϕ: 3 mm, diameter) or glassy carbon (GC, BAS; ϕ: 3 mm, diameter) as working electrode, a platinum wire counter electrode, and an Ag/AgCl saturated KCl reference electrode. BDD and GC electrodes were polished manually with aqueous slurry of alumina powder (ϕ: 0.01 μm) on a damp smooth polishing cloth (BAS velvet polishing pad) just before each experiment.

Operating conditions for DPV were: pulse amplitude, 50 mV; pulse width, 50 ms; scan rate, 20 mV s^{-1} and for SWV were: pulse amplitude, 25 mV; frequency, 15 Hz; potential step, 4 mV.

The pH measurements were made using a model 538, WTW pH-meter (Austria) with a combined electrode (Glass-reference electrodes) with an accuracy of pH ±0.05.

For the comparison study, LC experiments were proposed. A chromatographic system consisted of Shimadzu Model LC 20 AD/T LPGE KIT pump with manual injection (20 μl) and diode array detector system (SPD M 20 A) was used. This equipment has a column oven (CTO 20 AC) and a degasser system (DGU 20 A 5). A Symmetry Shield C-8 (5 μm; 150 mm × 3.9 mm ID) column was used at 40 °C. The data were handled with Shimadzu LC Solution software. Throughout this study, the compounds were separated using isocratic system and the mobile phases assayed were methanol:water (50:50, v/v) with 15 mM o-phosphoric acid. The pH of the mobile phase was adjusted to 2.70 with 1 M NaOH. The mobile phase was prepared daily, filtered, and sonicated before use, delivered at a flow rate of 0.75 ml min^{-1} and the effluent was monitored at 210 nm for ZPR and ramipril (internal standard). The mobile phase mixtures were filtered through a 0.45 μm pore nylon membrane filters (Millipore, Bedford, MA). A total of 20 μl of each solution was injected and chromatograms were recorded.

2.2. Reagents

ZPR and its pharmaceutical dosage form (Zeldox®, including 40 mg of ZPR per capsule) were supplied from Pfizer (Istanbul, Turkey). For the model compound study, sertindole, zuclopenthixol, fluvastatin sodium, and sildenafil citrate, were supplied from different pharmaceutical companies in Turkey and Sigma. Piperazine, indole, and the other chemicals were reagent grade (Merck or Sigma) and used without any purification.

Stock solutions of ZPR (1×10^{-3} M) were prepared in methanol and stored in a dark and cold (+4 °C) environment. Working solutions of ZPR for the voltammetric experiments were prepared by direct dilution of the stock solution with the selected supporting electrolyte. All of the working solutions contained a constant amount of methanol (40% or 20%, v/v). ZPR started to precipitate even at low concentrations, when the amount of methanol was 20% (v/v) at pH ≥ 7.0. Therefore, lower concentrations of ZPR (e.g. 4×10^{-5} M) and 40% (v/v) methanol was used for further studies after pH ≥ 7.0. Since no precipitation was observed at pH < 7.0 in 20% (v/v) methanol, these conditions were selected for higher concentrations of ZPR (e.g. 1×10^{-4} M). As expected, the peak current decreased owing to a lowering of the diffusion coefficient with changing ionic strength and viscosity of the medium. For analytical purposes, best response (with regard to peak current sensitivity, morphology, reproducibility and solubility) was obtained by working with 20% constant amount of methanol in selected supporting electrolyte. For this reason, all pH studies were realized using 40% methanol for the uniformity. The 20% methanol amount was used only the electroanalytical part below pH < 7.0.

Four different types of supporting electrolytes, sulfuric acid solution (0.1 M H_2SO_4), phosphate buffer (0.2 M H_3PO_4; 0.2 M KH_2PO_4.2 H_2O; pH 2.0–8.0), Britton–Robinson buffer (BR; 0.04 M H_3BO_3; 0.04 M H_3PO_4, and 0.04 M CH_3COOH; pH 2.0–12.0), and acetate buffer (0.2 M CH_3COOH; pH 3.5–5.5) were used for electrochemical measurements.

2.3. Validation of the analytical procedure

For the validation of the studied methods, the ruggedness, precision, and accuracy were checked by assaying five replicate samples on the same day (within day) and different days (between days) over a week period for two different concentrations of ZPR [20–23].

To avoid decomposition, all solutions were protected from light and used within 24 h. All measurements were carried out at ambient temperature of the laboratory (23–27 °C). Voltammograms of the sample solutions recorded a week after preparation did not show any appreciable change in assay values. The calibration equations for DPV and SWV techniques with BDD electrode were constructed by plotting the peak current against ZPR concentration. The quantitation studies could not be achieved with GC electrode.

The same validation parameters were calculated for HLPC–UV method.

2.4. Capsule assay procedure

Contents of ten capsules were thoroughly grounded to a fine powder. A sufficient amount of the powder was accurately weighted to prepare a stock solution including 1×10^{-3} M ZPR, transferred to a 50 ml of calibrated flask, and completed to the volume with methanol. The prepared solution was sonicated for 10 min to complete dissolution. The sample taken from the clear supernatant liquor was diluted with the selected supporting electrolyte containing a constant amount of methanol (20%, v/v). This solution was used to receive voltammograms by using the selected techniques for BDD electrode. The nominal content of the capsule amounts were calculated from the corresponding regression

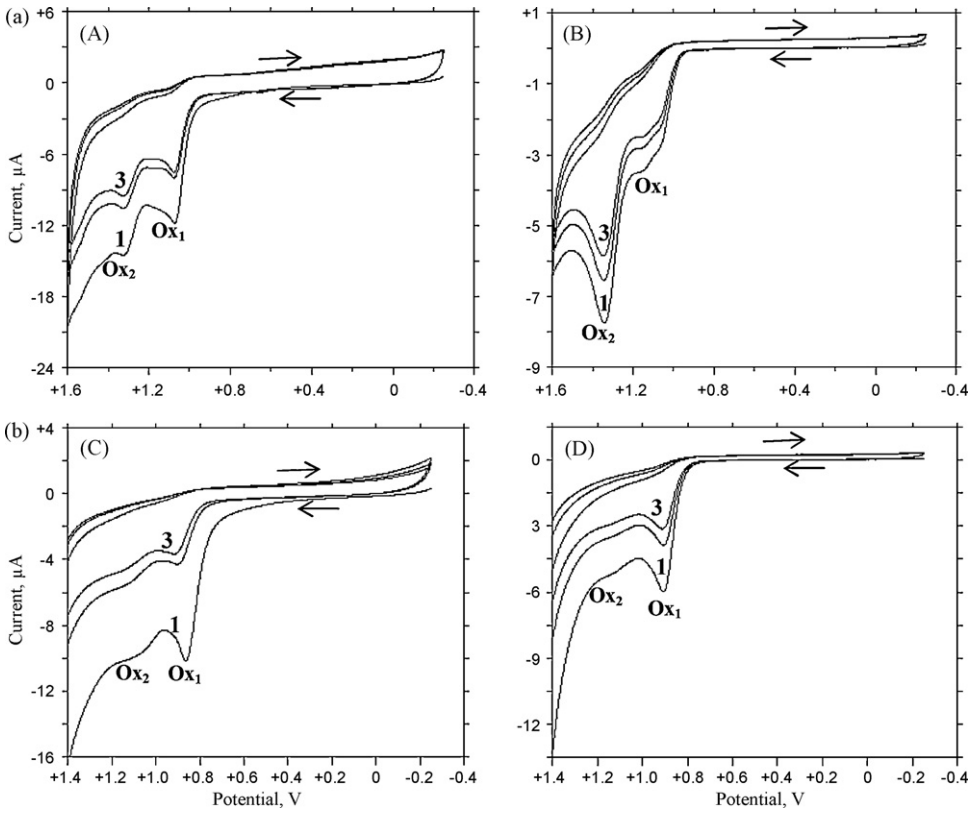


Fig. 1. Multisweep cyclic voltammograms of 1×10^{-4} M ZPR solution (with 20% methanol) in (a) 0.1 M H_2SO_4 and (b) acetate buffer at pH 5.50 for (A)–(C) GC and (B)–(D) BDD electrodes. Curves are between −0.25 V and +1.6 V. Scan rate is 100 mV s^{-1}.

equations of previously plotted calibration curves for only BDD electrode.

2.5. Recovery studies

For clarifying the accuracy, and reproducibility of the methods and check interference from the excipients used in the formulations, recovery experiments were carried [20–23]. For this purpose, a known amount of the pure ZPR was added to the pre-analyzed capsule formulation of ZPR. The recovery results were obtained by using the related calibration equation for both techniques for five repeated measurements.

2.6. Analysis and validation of spiked human serum samples

Drug-free serum samples were obtained from healthy people and stored frozen in the dark until assay. An aliquot volume of the serum samples was fortified with ZPR dissolved in methanol to achieve a final concentration of 1×10^{-3} M which is used as stock serum sample. The solution was treated with acetonitrile as serum denaturing and precipitating agent. The volume was completed to 5 ml with the same serum sample. The appropriate acetonitrile to serum ratio of volumes to eliminate the protein in serum was 1.5:1 (v:v). After vortexing for 30 s, the mixture was centrifuged for 10 min at $5000 \times g$ to eliminate serum protein residues. Required volumes of the supernatant were transferred into the volumetric flask and diluted to the volume with the selected supporting electrolyte. The amount of methanol was 20% (v/v) for all of the working serum solutions. The concentration of ZPR was between 2×10^{-6} and 6×10^{-5} M for DPV and SWV with BDD electrode. Quantifications were performed by means of the calibration curves from the related calibration equations for BDD electrode.

3. Results and discussion

In order to characterize the electrochemical oxidation behavior of ZPR, cyclic (CV) and linear sweep voltammetry (LSV) were carried out in the potential range of −0.25 to 1.6 V. CV experiments were achieved over a range from acidic (0.1 M H_2SO_4) to alkaline (pH 12.00) in acidic solutions and different buffer aqueous media at GC and BDD electrodes. Differential pulse (DPV) and square wave (SWV) voltammetric techniques were developed for the determination of ZPR for only BDD electrode.

CV measurements gave the irreversible nature of the oxidation processes of ZPR. Cyclic voltammograms of 4×10^{-5} M ZPR solution with 40% (v/v) methanol, at a scan rate of 100 mV s^{-1}, were from −0.25 V to 1.6 V in the positive direction. ZPR started to precipitate after pH ≥ 7.0. For BDD electrode, a sharp and well-defined peak and a wave were observed in the CV curves with acetate buffer at pH between 3.5 and 5.5. In 0.1 M H_2SO_4, the first anodic oxidation peak of ZPR did not occur until about +1.05 V and +1.13 V and the second anodic oxidation response of ZPR did not occur until about +1.31 V and +1.33 V on GC and BDD electrodes, respectively (Fig. 1). The sharp oxidation peak was observed at about +0.90 V with BDD electrode in acetate buffer.

By reversing at +1.60 V, no reduction signals corresponding to the anodic response were observed on the cathodic branch for both electrodes. Before pH < 7.0, CV curves were also obtained using 1×10^{-4} M ZPR solutions with 20% (v/v) methanol to see the reduction signals clearly without precipitation. On repetitive cyclic voltammograms the second and successive scans show a substantially smaller peak indicating passivation of the electrode surface by the oxidation product. Voltammograms obtained for ZPR at both electrodes presented an irreversible chemical behavior (Fig. 1).

The effect of pH on the peak potential and intensity were investigated between pH 1.00 and 12.00 using CV, DPV, and SWV

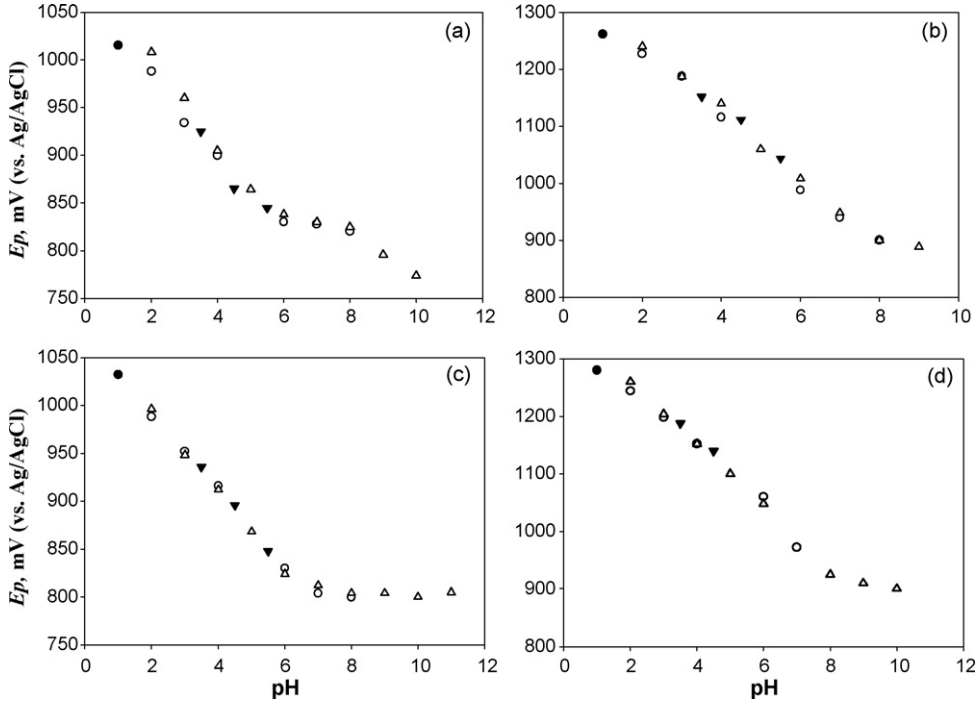


Fig. 2. Effects of pH on ZPR peak potentials (E_p) obtained with (a) Ox_1; (b) Ox_2 for GC electrode and (c) Ox_1; (d) Ox_2 for BDD electrode. ZPR concentration is 4×10^{-5} M with 40% methanol. (●) 0.1 M H_2SO_4; (○) 0.2 M phosphate buffer; (▼) 0.2 M acetate buffer; (△) 0.04 M BR buffer using DPV technique.

techniques for both electrodes. The peak potential of the anodic process moved to less positive potential values and an ill-defined oxidation peak occurred by increasing pH. DPV results were given to show the pH dependence of the oxidation of ZPR. Plots of E_p–pH were given in Fig. 2a and b for GC and Fig. 2c and d for BDD electrodes.

pH dependence of the peak potentials and the intersections in the E_p–pH plots showed that the electroactive group that created the oxidation peak has pK_a values about 6.00 (Ox_1) and 8.00 (Ox_2) for both electrodes. The results for both electrodes are close to the theoretical pK_a values which were reported as 5.44 ± 0.7 and 8.25 ± 0.5 in the literature [24]. For BDD electrode, there was no peak after pH 10.0. Above pH 8.0, the peak potential became nearly pH-independent (Fig. 2a–d). The second peak interaction is located around pH 7.5 which was close to the pK_a of the piperazine moiety [25]. When pH > pK_a, the conjugate base must be formed by a rapid dissociation of the protonated form. At pH < pK_a, the conjugate base predominates in the supporting electrolyte. The pH-independent zones above pH 8.0 and 6.0 mean that there are no proton transfer steps before the electron transfer rate-determining step. According to Fig. 2a–d, the plot of the peak potential (E_p) vs. pH gave one straight line between 1.0 and 6.0 for the first peak and between 1.0 and 8.0 for the second peak for both electrodes, which can be expressed by the following equations in all studied buffer systems:

$$E_p\ (\text{mV}) = 1069 - 40.59\text{pH}; \quad r = 0.981,$$
$$n = 9\ (\text{for GC electrode},\ Ox_1) \tag{1}$$

$$E_p\ (\text{mV}) = 1343 - 54.89\text{pH}; \quad r = 0.994,$$
$$n = 11\ (\text{for GC electrode},\ Ox_2) \tag{2}$$

$$E_p\ (\text{mV}) = 1077 - 41.46\text{pH}; \quad r = 0.998,$$
$$n = 9\ (\text{for BDD electrode},\ Ox_1) \tag{3}$$

$$E_p\ (\text{mV}) = 1361 - 53.42\text{pH}; \quad r = 0.993,$$
$$n = 11\ (\text{for BDD electrode},\ Ox_2) \tag{4}$$

The linearity observed in the pH range of 1.0 and 6.0 for Ox_1 gave a negative slope of 40.59 mV/pH for GC electrode and 41.46 mV/pH for BDD electrode. The similar linearity for Ox_2 between 1.0 and 8.0 for GC and BDD electrodes gave a negative slope of 54.89 mV/pH and 53.42 mV/pH, respectively. These slopes being close to the expected theoretical value of 59 mV/pH indicates that the number of proton and electron involved in the oxidation of ZPR is equal [26–28]. The oxidation steps of ZPR were located on the indole and piperazine moiety, which represented typical redox systems with two electron processes in acidic and basic media. Thus, we may assume that electroactive centers, indole and piperazine, of ZPR may be responsible of these pK_a values.

The relationship between pH and ZPR current (I_p) was also studied for both electrodes. The peak intensity decreased with the raising pH values behaving similar as the peak potential. For GC electrode, first peak gave a single and sharp peak in BR buffer at pH 4.0. For BDD electrode, first peak had a single, sharp and better peak shape in acetate buffer at pH 5.5, whereas second peak had a single, sharp, and better peak shape in 0.1 M H_2SO_4. For this reason, these supporting electrolytes were selected for the determination studies.

Scan rate studies were carried out to understand that the process was diffusion or adsorption controlled. The peak potential shifted to more positive potentials to the anodic direction about 102 mV in BR buffer at pH 4.0 for GC electrode and 70 mV (in acetate buffer at pH 5.5) and 47 mV (in 0.1 M H_2SO_4) for BDD electrode, when the scan rate increased. When the scan rate was varied from 5 to 1000 mV s^{-1} in 4×10^{-5} M ZPR solution, a linear dependence ($r \geq 0.99$) of the peak intensity I_p (μA) upon the square root of the scan rate $\nu^{1/2}$ (mV s^{-1}) was found for both electrodes, demonstrating a diffusional behavior.

The variation of the logarithm of the peak current as a function of the logarithm of the scan rate in the range of 5–1000 mV s^{-1} showed

Table 1
The results of the logarithm of the peak current against the logarithm of ZPR concentration in 0.1 M H_2SO_4 for BDD electrode.

Peak	Peak potential (V)	Method	Linearity range ($\times 10^{-4}$ M)	n	$\log I_p$ (μA) vs. $\log C$ (M) equations	Correlation coefficient (r)
Ox_1	1.024	DPV	0.02–2.00	11	$\log I_p = 0.643 \log C + 2.883$	0.997
Ox_1	1.082	SWV	0.02–2.00	11	$\log I_p = 0.552 \log C + 2.626$	0.993
Ox_2	1.284	DPV	0.04–1.00	9	$\log I_p = 0.590 \log C + 2.837$	0.991
Ox_2	1.316	SWV	0.06–1.00	8	$\log I_p = 0.539 \log C + 2.773$	0.992

that the process was effected adsorption for GC electrode, since the value of the straight line $\log I_p = f(\log \nu)$ was equal to 0.85. The slope is close to the theoretical value of 1.0, which is expected for an ideal reaction of surface species and confirming some adsorption-controlled effects [29]. Therefore, the electrode process may be controlled by diffusion and/or adsorption. The plot of logarithm of peak current vs. logarithm of scan rate gave a straight line with a slope of 0.50 (in acetate buffer at pH 5.5) and 0.55 (in 0.1 M H_2SO_4) for BDD electrode. These values close to the theoretical value of 0.5, an ideal reaction to the diffusion-controlled electrode process for both media [29].

The Tafel plots ($\log I$ vs. E) were obtained for the scan rate of 5 mV s^{-1} beginning a steady-state potential for both electrodes. The α_n value of the anodic reaction from the slope of the linear part of the Tafel plot was 0.37 in BR buffer at pH 4.0 for GC electrode and 0.84 in acetate buffer at pH 5.5 and 0.16 in 0.1 M H_2SO_4 for BDD electrode. The exchange current density (I_o) values obtained from the intercepts of $\log I - f(E)$ plots were 5.25×10^{-12} μA cm^{-2} for GC electrode and 1.32×10^{-18} μA cm^{-2} in acetate buffer at pH 5.5 and 5.62×10^{-10} μA cm^{-2} in 0.1 M H_2SO_4 for BDD electrode. These values together with the absence of the cathodic wave and the positive shift of the peak potential with the scan rate confirmed the irreversibility of the oxidation reaction for both electrodes.

The logarithm of the peak current against the logarithm of ZPR concentration in 0.1 M H_2SO_4 was plotted to find out the reaction order for BDD electrode. Related equations are given in Table 1. The slope values of the equations in Table 1 gave the reaction order, which seemed to be first order.

Voltammetric methods, especially CV, are the most suitable method for investigating the redox behavior of new pharmaceutical compounds which can give insights into their metabolic fates [26–28]. CV curves from the redox properties of active compounds [30,31] and biomolecules [31–34] might have profound effects on the understanding of the redox mechanism related to the activity of the ZPR compound. To identify the group responsible for the main oxidation process, the oxidation of ZPR was also compared with those of the model compounds with piperazine and indole groups. Therefore, several measurements with different electrochemical techniques were performed using various supporting electrolytes (sulfuric acid, acetate, phosphate, and BR buffers) in order to obtain such information.

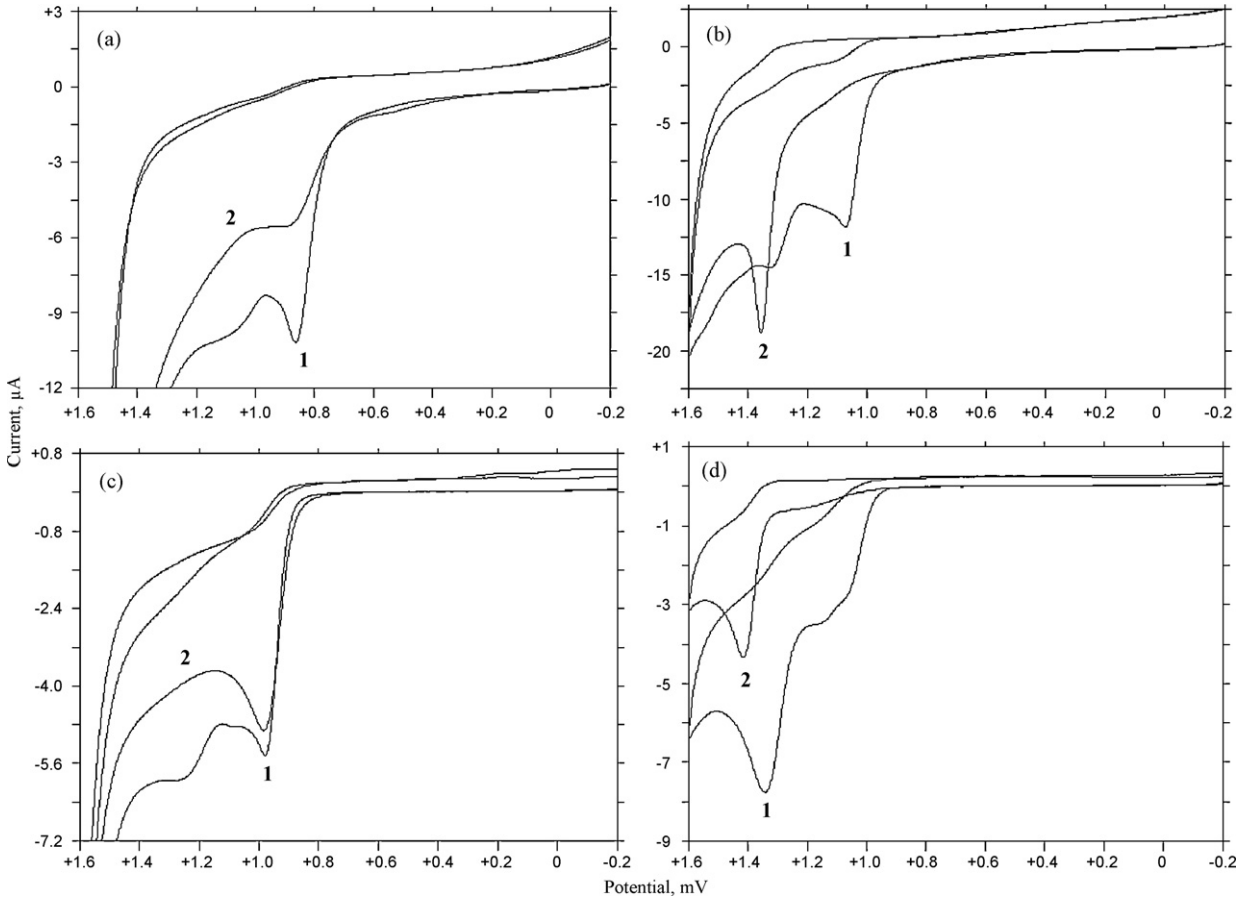


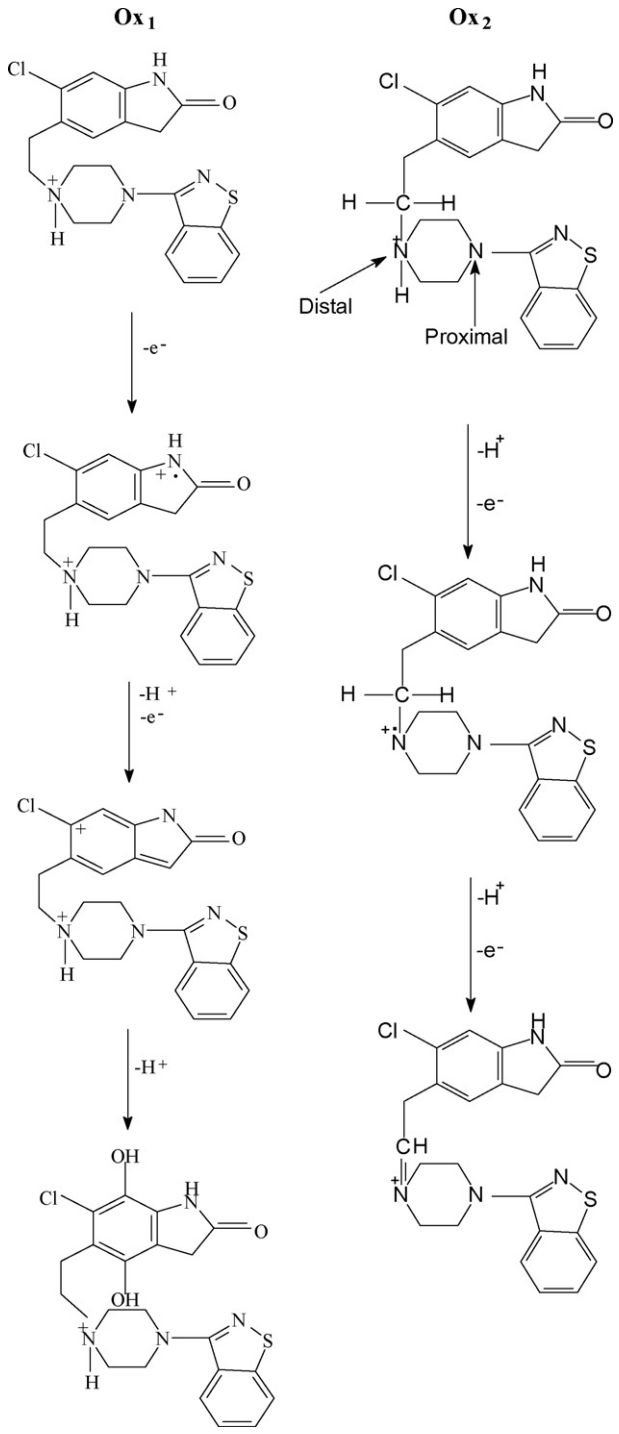
Fig. 3. Cyclic voltammograms of 1×10^{-4} M ZPR (1); indole (2) (in a and b); and sildenafil citrate (2) (in c and d) in acetate buffer at pH 5.5 (a); in 0.1 M H_2SO_4 (b and d) and in Britton-Robinson buffer at pH 4.00 (c) with constant amount methanol (20%). Scan rate, 100 mV s^{-1}. (a and b) GC electrode and (c and d) BDD electrode.

Cyclic voltammetric measurements on the positive potential direction showed an irreversible nature of the oxidation process (Fig. 1). Although the exact oxidation mechanism was not determined, some conclusions about the potentially electroactive centers under working conditions could be reached. Comparative study with model substances was also realized for GC and BDD electrodes. From the CV curves, the main voltammetric behavior of piperazine and indole derivatives, which are structurally related to the mechanism of oxidation of ZPR, may be postulated by the oxidation of these groups [34–42]. Both piperazine and indole may be shown an electrooxidation response on CV. Both redox responses were shown for some selected model compounds in Fig. 3. Our results on model compounds show similar behavior that the electroactive center corresponding to the first and second anodic peak was the nitrogen atom on the indole ring and piperazine moiety, respectively.

It was assumed that the oxidation processes occurred firstly on the nitrogen atom of indole ring and secondly on the nitrogen atom of piperazine ring of the molecule. In acidic pH (pH < 6.0) values, both indole and piperazine responses was obtained clearly and separately. However, these responses were more separated in some supporting electrolyte solutions (Figs. 1 and 3). The anodic oxidative behavior of ZPR was comparable to the indole and piperazine oxidations that were reported in our previous studies and the literature values [34–42]. To support the working hypothesis, the oxidation of piperazine (similar to sildenafil, quetiapine, trazodone, and nefazodone oxidation step) and indole groups (similar oxidation pathway with indol, indol-3-acetic acid, etodolac, fluvastatine, and zuclopenthixol) of ZPR gave two separate steps. The first anodic peak Ox_1 (related with indole moiety) and second anodic peak Ox_2 (related with piperazine moiety) of ZPR were compared to some model compounds for the confirmation of the hypothesis (Scheme 2).

Comparative study on indole, indole-3-acetic acid, etodolac, fluvastatine, and zuclopenthixol were realized by CV at a function of pH in order to identify the oxidation process of ZPR. For all model compounds, the intersection at about pH 6.0 was supposed to correspond to the pK_a value of indole moiety [34]. Considering the above comparison and the break point of E_p vs. pH plot for the first process of ZPR which was obtained at about pH 6.0 and bearing in mind the oxidative process of nitrogen atom in the indole ring leading finally to hydroxylation of the benzene ring [31,32,34–38].

The study on nefazodone, trazodone, sildenafil, and quetiapine was realized by CV at GC and BDD electrodes as a function of pH in order to identify the responsible atom for the second oxidation process. Taking into account that the voltammograms of these substances closely matched the voltammograms of ZPR, we assumed that the second oxidation step of ZPR was located on the piperazine moiety, which represented a typical redox system with two electrons in acidic and basic media [25,31,32,39–44]. For all model substances, the intersection at about pH 7.5 was supposed to correspond to the pK_a value of piperazine moiety [25]. Thus, we might postulate when the aliphatic nitrogen, which is distal to the benzene ring of the molecule, of the piperazine moiety was protonated, oxidation occurred on the proximal nitrogen with the removal of a proton. Above pH 8.0, oxidation occurred exclusively at the most basic piperazine nitrogen (distal) [25,31,32,39–44]. ZPR lost an electron to form a cation radical, which on losing a proton and an electron in subsequent steps form a quaternary Schiff base.



Scheme 2. Possible oxidation pathways of ZPR for Ox_1 [34–38] and for Ox_2 [25,39,40].

3.1. Validation of the analytical procedure

Quantitative evaluation is based on the linear correlation between the peak current and concentration. For analytical purposes, best response was obtained in the selected media consisting of a 20% constant amount of methanol for BDD electrode. The best single peak shapes, peak current sensitivity, and reproducibility for these techniques were obtained in acetate buffer at pH 5.5 for the first peak (Ox_1) and 0.1 M H_2SO_4 for the second peak (Ox_2). The graph of ZPR concentration vs. the peak current gave linear calibration curves in both media using both techniques, indicating a diffusion-controlled process. In acetate buffer at pH 5.5, the plot of the calibration curve for Ox_1 was linear between 8×10^{-7} and 8×10^{-5} M for both voltammetric methods. In 0.1 M H_2SO_4, the linearity of the calibration curve of Ox_2 was obtained in the range of 2×10^{-6} to 2×10^{-4} M for both DPV and SWV techniques. Above these concentration ranges, the loss of linearity was probably due

Table 2
Regression data of the calibration lines for quantitative determination of ZPR in acetate buffer at pH 5.5 and 0.1 M H_2SO_4 by DPV and SWV for BDD electrode.

	Ox_1 in acetate buffer at pH 5.5		Ox_2 in 0.1 M H_2SO_4		LC–UV method
	DPV	SWV	DPV	SWV	
Measured potential (V)/retention time (min)	0.840	0.872	1.280	1.316	3.070
Linearity range ($\times 10^{-4}$ M)	0.008–0.80	0.008–0.80	0.02–2.00	0.02–2.00	0.043–0.22
Slope of the calibration graph ($\mu A\,M^{-1}$)	33,020	38,185	24,019	31,784	38,786
Intercept (μA)	0.0223	0.0207	−0.0187	−0.0935	−0.1384
Correlation coefficient	0.999	0.999	0.999	0.999	0.999
S.E. of slope ($\times 10^2$)	4.67	2.83	1.53	2.65	24.5
S.E. of intercept ($\times 10^{-2}$)	1.56	0.94	1.15	1.99	3.49
LOD ($\times 10^{-7}$ M)	0.75	0.48	3.50	6.60	0.85
LOQ ($\times 10^{-7}$ M)	2.28	1.44	10.60	20.00	2.58
Repeatability of peak current/peak area (R.S.D.%);	0.93	0.79	0.86	0.83	0.22
Repeatability of peak potential/retention time (R.S.D.%)	0.26	0.25	0.15	0.13	0.10
Reproducibility of peak current/peak area (R.S.D.%)	1.20	1.32	1.16	1.05	0.31
Reproducibility of peak potential/retention time (R.S.D.%)	0.48	0.38	0.38	0.30	0.17

to the adsorption of ZPR on the electrode surface. Characteristics of these graphs are given in Table 2. The low values of standard error of the slope and intercept and the greater correlation coefficient than 0.999 confirmed the precision of the proposed methods.

Several approaches are given in the ICH guideline to determine the LOD and LOQ values. Limits of detection (LOD), limits of quantification (LOQ), repeatability (within day), reproducibility (between days), precision, recovery, bias %, and selectivity were evaluated [20–23]. The LOD and LOQ were calculated on the peak current using the equations below:

$$\mathrm{LOD} = 3\frac{s}{m}; \quad \mathrm{LOQ} = 10\frac{s}{m}, \tag{5}$$

where s is the standard deviation of the peak currents (three runs) and m is the slope of the related calibration equation. LOD and LOQ give the sensitivity of the proposed methods [23].

The precision of the methods was calculated by repeating five experiments for the same solutions within the same day (repeatability) and five experiments for six days from different solutions (reproducibility). The ZPR concentrations were selected as 2×10^{-5} and 6×10^{-5} M for the precision experiments. The within day and between day precision, accuracy, and reproducibility were determined as R.S.D.% (Table 2). These results demonstrated good precision, accuracy, and reproducibility [20,21].

Standard sample solutions were stored at +4 °C in the dark and recorded every week. The solutions did not show any appreciable change in assay values even after four-week period. However, all solutions used for the validation experiments were freshly prepared to ensure the stability of analyte in the solutions.

ZPR pharmaceutical dosage forms were also determined with the RP-LC method, which is proposed for the comparison of the proposed voltammetric techniques. As a part of RP-LC validation procedure, system suitability parameters were checked by evaluating different parameters. Tailing and asymmetry factors were 1.25 and 1.20, respectively. Theoretical plate number was 2202. Resolution and selectivity factors for this system were 5.04 and 2.57, respectively. The retention times of ZPR standard sample and capsules were 3.07 and 3.08 min, respectively. The retention times of IS were 5.39 min. The variation of the retention time among five replicate injections of ZPR reference solution was very small (Table 2). The results obtained from system suitability tests are in an agreement with the USP requirements.

The calibration curve for ZPR in mobile phase was drawn by plotting the peak area ratio of ZPR to IS, vs. concentration of ZPR. The developed RP-LC method was validated according to the standard procedures [20–23]. The results are shown in Table 2.

3.2. Capsule assay procedure of ZPR

The proposed DPV and SWV techniques were applied for the determination of ZPR in pharmaceutical dosage forms in acetate buffer at pH 5.5 for the first peak (Ox_1) and 0.1 M H_2SO_4 for the second peak (Ox_2) for BDD electrode (labeled ZPR amount = 40 mg per capsule). The results are given in Table 3. The validity was assessed by applying calibration curves and the standard addition methods. The results showed that the proposed methods could be applied with a great success to ZPR assay in capsule dosage form without any interference (Table 3). The mean results for the determinations of both techniques with both electrodes are very close to the declared value of 40 mg.

On the basis of above results, both DPV and SWV techniques were applied to the direct determination of ZPR in capsule dosage form, using the related calibration straight lines without any sample extraction, evaporation or filtration and after adequate dilutions (Table 3). As far as we know, no official method is described in

Table 3
The results for the determination of ZPR from capsule dosage form and recovery experiments achieved in acetate buffer pH 5.5 and 0.1 M H_2SO_4 for BDD electrode.

Parameters	Ox_1 in acetate buffer at pH 5.5		Ox_2 in 0.1 M H_2SO_4		LC-UV Method
	DPV	SWV	DPV	SWV	
Labeled claim (mg)	40.00	40.00	40.00	40.00	40.00
Amount found[a] (mg)	40.29	40.56	40.48	40.24	40.42
R.S.D.%	0.36	0.55	0.51	0.48	0.28
Bias %	−0.73	−1.40	−1.20	−0.60	−1.05
t_{value}	0.16	0.24	0.55	0.12	$t_{theoretical}$: 2.31
F_{value}	0.67	0.23	0.27	0.34	$F_{theoretical}$: 2.60
Added (mg)	9.00	9.00	9.00	9.00	9.00
Found[a] (mg)	8.95	8.93	9.02	8.98	9.01
Average recovered (%)	99.47	99.27	100.22	99.86	100.02
R.S.D.% of recovery	1.09	0.50	0.44	0.90	0.33
Bias %	−0.55	−0.33	0.22	−0.22	−0.02

[a] Obtained from five experiments.

Table 4
The results obtained for determination of ZPR from spiked serum samples.

	Ox_1 in acetate buffer at pH 5.5		Ox_2 in 0.1 M H_2SO_4	
	DPV	SWV	DPV	SWV
Measured potential (V)	0.972	1.004	1.152	1.192
Linearity range ($\times 10^{-5}$ M)	0.04–1.00	0.04–1.00	0.20–6.00	0.20–6.00
Slope of the calibration graph ($\mu A\ M^{-1}$)	38,290	45,641	19,670	24,722
Intercept (μA)	−0.0056	−0.0167	−0.0323	−0.0605
Correlation coefficient	0.999	0.999	0.998	0.997
S.E. of slope ($\times 10^2$)	3.03	5.38	2.67	5.10
S.E. of intercept ($\times 10^{-2}$)	0.15	0.27	0.72	1.38
LOD ($\times 10^{-7}$ M)	0.28	0.65	2.78	2.98
LOQ ($\times 10^{-7}$ M)	0.92	2.16	9.27	9.93
Repeatability of peak current (R.S.D.%)	0.82	0.96	0.84	0.72
Repeatability of peak potential (R.S.D.%)	0.20	0.10	0.07	0.16
Reproducibility of peak current (R.S.D.%)	1.27	1.24	1.56	1.38
Reproducibility of peak potential (R.S.D.%)	0.39	0.25	0.22	0.23
Added concentration ($\times 10^{-3}$ M)	9.348	9.348	9.348	9.348
Obtained concentration ($\times 10^{-3}$ M)	9.345	9.336	9.428	9.352
Number of experiments	5	5	5	5
Average recovered (%)	99.96	99.88	100.86	100.04
R.S.D.% of recovery	0.79	0.97	0.89	0.63
Bias %	−0.03	−0.13	0.86	0.04

pharmacopoeias related to the ZPR in capsule dosage form. For this reason, ZPR capsules were also determined by RP-LC method, which is proposed for the comparison with the DPV and SWV techniques. Also, the proposed LC method was fully validated (Table 1). Recovery experiments were also performed for LC method (Table 3). All methods showed similar accuracy and precision. Statistical comparisons were performed on data from both LC and voltammetry experiments. Student's *t*- and *F*-tests revealed no statistically significant difference between RP-LC and proposed voltammetric methods with regard to accuracy and precision. However, the proposed method is rapid and selective than LC assay and used without any filtration steps.

In order to detect the interaction between the excipients and active ingredients, recovery studies were carried out after addition of known amounts of the pure drug to various pre-analyzed formulations of ZPR. These results indicated the absence of interference from commonly encountered pharmaceutical excipients used in the capsule formulations. The mean percentage recoveries based on the average of five replicate measurements were showed no significant interference from excipients in the analysis of ZPR (Table 3).

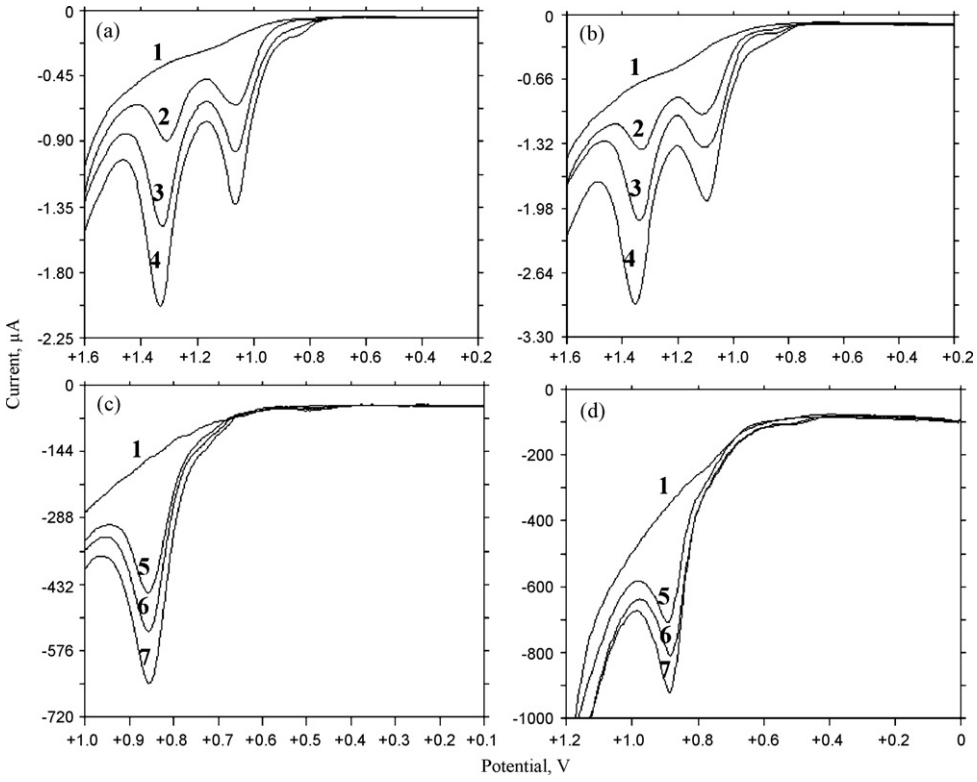


Fig. 4. (a and c) DPV and (b and d) SWV voltammograms obtained for the determination of ZPR from spiked serum in (a and b) 0.1 M H_2SO_4 and (c and d) acetate buffer at pH 5.5, using BDD electrode. (1) Blank; (2) 2×10^{-5} M; (3) 4×10^{-5} M; (4) 6×10^{-5} M; (5) 6×10^{-6} M; (6) 8×10^{-6} M; (7) 1×10^{-5} M.

The precision value around the mean value should not exceed 5% of the R.S.D.% [20–23].

3.3. Analysis and validation of spiked human serum samples

Different amounts of acetonitrile and methanol were used as biological sample precipitating agents. Acetonitrile gave the best results, when 1.5 volume of acetonitrile for 1 volume of serum samples was used. The proposed DPV and SWV techniques were successfully applied for the determination of ZPR from protein-free spiked human serum in acetate buffer at pH 5.5 and 0.1 M H_2SO_4 solution consisting of a 20% constant amount of methanol using BDD electrode. After the experimental steps explained in Section 2, the calibration equations and validation parameters were obtained and given in Table 4. In this study, the proteins and endogenous substances in serum samples are precipitated by the addition of acetonitrile. After the solution was centrifuged at $5000 \times g$, the supernatant was taken, diluted with the supporting electrolyte, and analyzed.

Many people use ascorbic acid regularly as a supplement. In order to evaluate the selectivity of the developed electroanalytical procedure in serum samples, the influence of ascorbic acid was also examined. Ascorbic acid produced no peak in the studied potential range (figure is not shown). It has a peak obtained at about 300 mV less positive potentials than ZPR working peak potentials in selected supporting electrolytes. Hence, we decided ascorbic acid electrooxidation does not affect on ZPR oxidation and determination.

The recovery results of ZPR were calculated from the related linear regression equations (Table 4). Fig. 4 illustrates DP and SW voltammograms obtained from serum spiked at different concentration of ZPR in acetate buffer at pH 5.5 for the first peak (Ox_1) and 0.1 M H_2SO_4 for the second peak (Ox_2) using BDD electrode. As can be seen from Fig. 4, no oxidation compounds and no extra noise peaks presented in the potential range where the analytical peaks appeared.

From the calculation of the LOD value, ZPR concentration was obtained as ng ml^{-1} (about 10^{-8} M) level (between 13 and 139 ng ml^{-1}) depending on the supporting electrolyte and analyzed peak (Table 4). It can be shown that ZPR may be detected using these proposed techniques in the real serum samples. Mean peak serum concentration of unchanged drug is reported in the literature as about 45 ng ml^{-1} [4].

The stability tests were achieved by making five consecutive analyses of the serum samples over a period of approximately 5 h. No significant changes in the peak currents and potentials were observed between the first and the last measurements. The proposed methods gave reproducible results, were easy to perform and sensitive enough for the determination of ZPR in human serum samples.

4. Conclusions

The voltammetric oxidation step of ZPR in different buffer solutions between pH 1.0 and 12.00 has been elucidated with both electrodes. BDD electrode showed perfect results for the electrooxidation and determination of ZPR. However, determination of ZPR could not be possible with GC electrode, since it showed some adsorptive behavior but stripping techniques did not work.

The oxidation of ZPR was compared to some model compounds with both electrodes. CV is used for qualitative diagnosis of chemical reactions during the redox processes [25,31,32,34–44]. The advantages of BDD electrode in comparison to GC electrode for electroanalysis can be explained as wider electrochemical window, relatively low noise-to-signal and background-to-signal ratios, lower adsorption of organic molecules, and response stability.

The analytical procedures were fully validated. ZPR presented two well-defined anodic peaks. For the determination of ZPR using DPV and SWV techniques in pharmaceutical dosage forms and spiked serum samples with BDD electrode, the oxidation step in acetate buffer at pH 5.5 and 0.1 M H_2SO_4 were used as supporting electrolytes for the first peak and the second peak, respectively.

The analytical results obtained from pharmaceutical dosage forms by DPV and SWV using BDD electrode are in good agreement with those obtained by the proposed LC method. Although both voltammetric and LC methods showed similar simplicity, the principal advantage of the proposed voltammetric methods over the LC was the absence of influence of matrix. The proposed methods might be alternatives to the LC techniques in therapeutic drug monitoring or the experimental data might be used for the development LC–EC method.

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