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VALIDATED ELECTROANALYTICAL AND RP-LC ASSAY OF ERTAPENEM IN ITS PHARMACEUTICAL DOSAGE FORM

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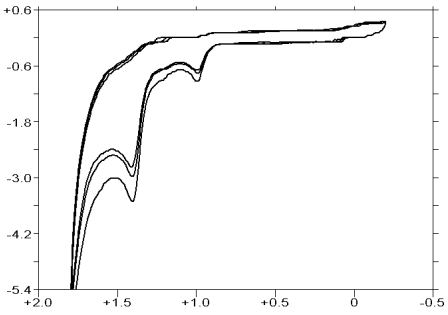
Ertapenem is a member of carbapenem group beta-lactam antibiotics. The electrochemical behavior of ertapenem at the surface on a boron-doped diamond electrode is described. It is investigated and determined using cyclic, linear sweep, differential pulse and square wave voltammetric techniques with this electrode. Two responses of ertapenem are irreversible in nature, and diffusion-controlled. A linear voltammetric response for both peaks was obtained in the concentration range of 1.0 to 12.00 μg.mL^{-1} for differential pulse and square wave voltammetric techniques. Also simple, accurate, precise and fully validated RP-LC assay as a comparison method of this compound in its dosage form has been developed. An X-Select RP-18 column at 25 ^{o}C was used with the mobile phase of water: methanol 55:45 (v/v) adjusted to pH 3.0. The RP-LC method allowed quantitation over the 1.0-15.00 μg.mL^{-1} range for ertapenem. The necessary statistical validation parameters with the system suitability test results revealed that the proposed methods are free from significant systematic errors. Degradation studies were also performed in bulk substance to demonstrate the stability indicating power with using chromatographic technique. The proposed methods successfully applied to the pharmaceutical dosage form.



INTRODUCTION

Ertapenem (ERT), (4R,5S,6S)-3-[(3S,5S)-5-[(3-carboxyphenyl)carbamoyl]Pyrrolidin-3-yl]sulfanyl -6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid, is a member of carbapenem group antibiotics that is structurally related to beta-lactam antibiotics. Carbapenem is most potent with the widest spectrum of antimicrobial activity as a class β-lactams.[1] ERT has used on gram negative and gram positive bacteria.[2] Also, it has clinically useful activity against anaerobic bacteria. ERT is administered as a single agent either via the intravenous or intramuscular route. Unlike imipenem and meropenem, ERT can bound to protein highly, which explains its long half life (4 hours). This specification allows it for once-daily dosing. [3-6]

Several analytical methods have been reported for the determination of ERT based on HPLC-UV detection, [7-12] micellar electrokinetic chromatography,[13] LC-MS[14] and spectrophotometric[15] methods. Many of the reported methods are sensitive but require expensive instruments. The reported methods were influenced by interference of endogenous substances and potential loss of drugs in the re-extraction procedure and involving lengthy, tedious and time-consuming plasma sample preparation and extraction processes.

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The development of a new simple, selective and sensitive method capable of determining drug amount in pharmaceutical dosage forms is very important. Electrochemical methods are well established techniques which when applied to pharmaceutical products, can be considered to be the methods of choice owing to their selectivity, reduced implementation costs and versatility of application. Electroanalytical techniques have already been proved to be useful for sensitive and selective determination in pharmaceutical compounds even in samples containing complex matrix such as syrups, tablets, creams or biological fluids. These methods do not require tedious pre-treatment and involve limited pre-separation, and consequently reduce the cost of analysis.[16-22] Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity.[16-22]

Pharmaceutical compounds have been successfully studied with carbon-based electrodes in their pharmaceutical dosage forms or biological samples. Diamond electrodes can be used at extremely high anodic potentials or aggressive solutions and now commercially polycrystalline boron-doped forms are available. Boron-doped diamond electrodes have more conductive than diamond electrodes. On the other hand, boron-doped diamond electrodes (BDDE) have different excellent properties such as chemical inertness and stability so that they can be used extreme conditions like strong acidic media[17]. BDDE show the superior electrochemical properties such as wide potential window, very low background current, chemical and physical stability. [23-30]

To the best of our knowledge, no scientific literature regarding the electrochemical behavior and voltammetric determination of ERT has been published. Due to the importance of ERT, it is interesting to develop a rapid screening method for its determination in pharmaceutical formulations. Thus, it has been examined the electrochemical behavior of ERT by developing new voltammetric methods to study its content uniformity in the injectable dosage form at BDDE using cyclic, linear sweep, differential pulse, square wave voltammetric techniques. The aim of the present paper is to investigate the electrooxidative behavior of ERT and its quantitative determination by voltammetric methods. As a comparison method, new fully validated stability indicating RP-LC method has also been developed.

RESULTS AND DISCUSSION

Voltammetric studies

There were no electrochemical data available concerning redox behavior of ERT which is an easily oxidizable molecule in the literature. To demonstrate the usefulness of a BDDE for determination of ERT, which may offer advantages for the use of such electrode as sensor, the electrochemical behavior of ERT was investigated as details in this study. In order to characterize the electrochemical oxidation behavior of ERT, cyclic (CV) and linear sweep voltammetry (LSV) were carried out.

CV and LSV experiments were performed over a pH range from acidic (0.1 M H_2SO_4) to alkaline (pH 12.00) in different buffer aqueous media and acidic solutions at BDDE for the characterization of electrochemical oxidation behavior of ERT. The peak currents and peak potentials were determined in different supporting electrolytes using all selected electroanalytical techniques. Differential pulse (DPV) and square wave (SWV) voltammetric techniques were especially used for the quantitative determination of ERT using BDD electrode.

In Fig. 1 a-d, CV curves of ERT represent one or two well-defined anodic peaks at various potential values depending on several aspects such as pH and supporting electrolyte composition. Cyclic voltammetric measurements performed on 10 $\mu g.mL^{-1}$ ERT solutions show an irreversible nature of the oxidation peak in the entire pH range investigated. As pH increased, both peaks shifted to less positive potentials which confirm the irreversibility of the process, with the simultaneous increase in peak current when the scan rate was increased. As shown in Figs. 1 and 2, no cathodic peak was observed. 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 ERT at BDDE presented an irreversible electrochemical behavior for both anodic responses (Fig. 2). Both peaks which are obtained in more acidic media were easily measurable. Hence, all subsequent works were based on the measurements of the magnitude of these steps.

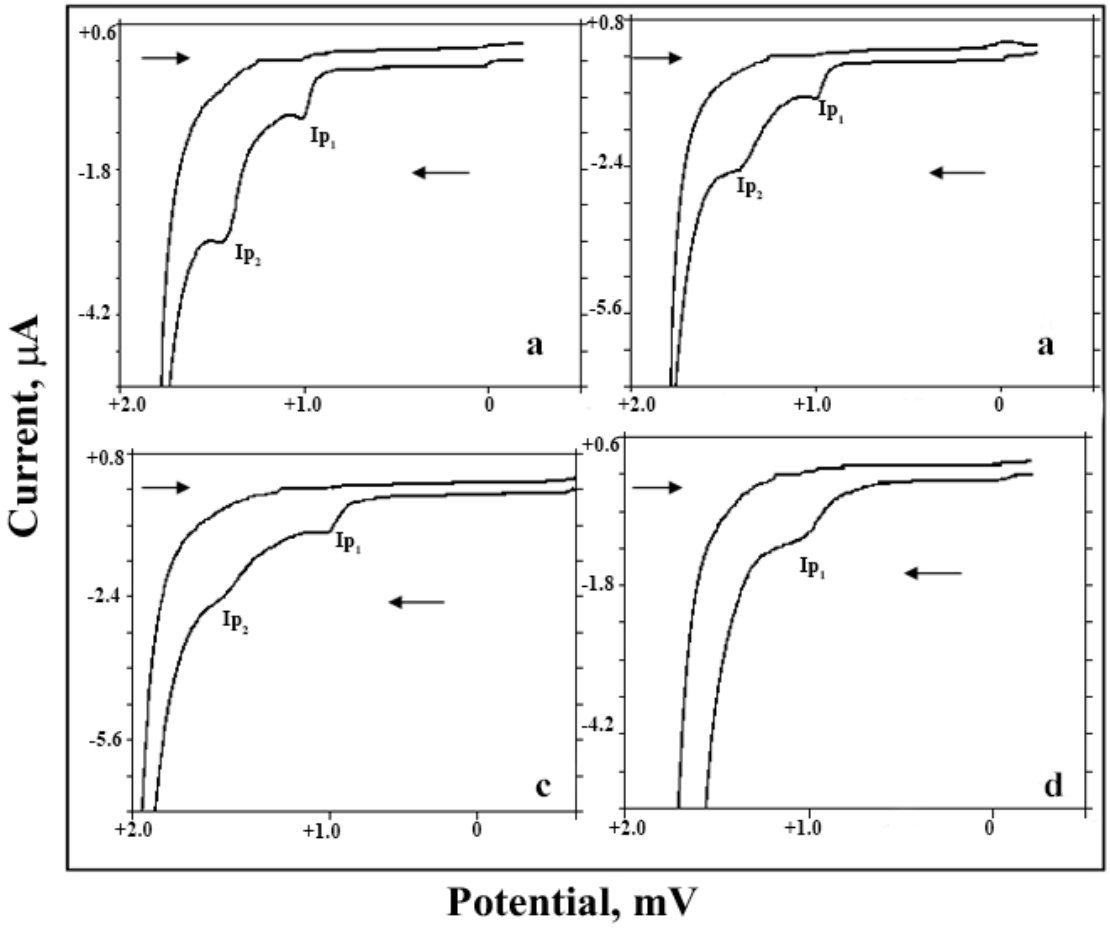

Fig. 1 – Cyclic voltammograms of 10 µg mL^{-1} ERT in pH 3.04 phosphate buffer; (a) Britton-Robinson buffer at pH 4.0; (b) Britton-Robinson buffer at pH 5.0; (c) Britton-Robinson buffer at pH 7.5; (d) Scan rate 100 mV s^{-1}.

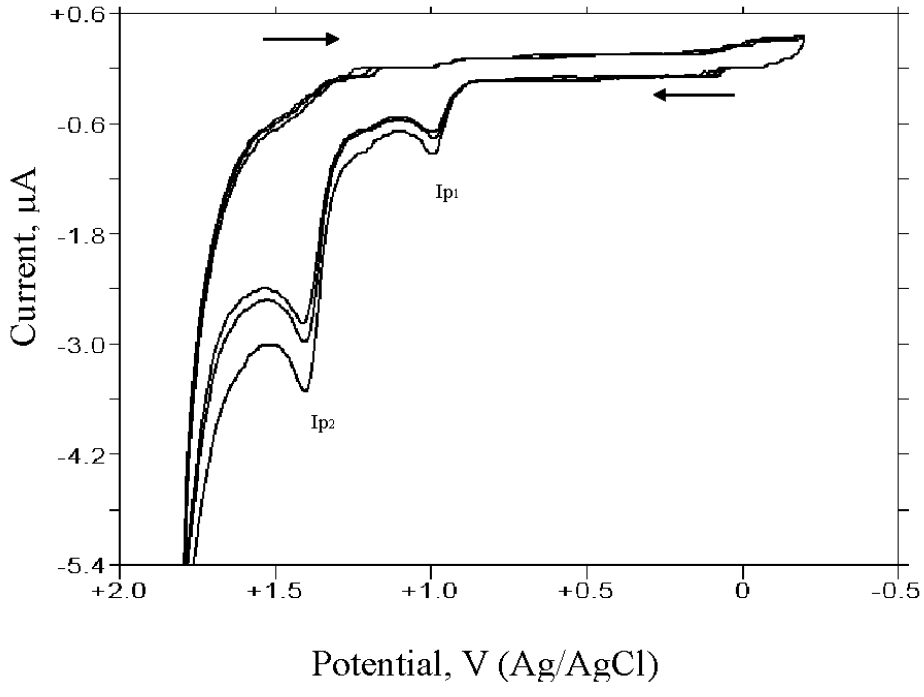

Fig. 2 – Multi sweep cyclic voltammograms of 10 µg mL^{-1} ERT solution in Britton-Robinson buffer at pH 2.0 for BDDE. Curves are between -0.20 V and +1.8 V. Scan rate is 100 mV s^{-1}.

Scan rate studies were then carried out to assess whether the processes on BDDE were under diffusion or adsorption control. The effect of potential scan rate between on the peak current and the peak potential of ERT was evaluated using in Britton-Robinson buffer at pH 2.0 for BDDE. When the scan rate varied from 5 to 1000 mVs^{-1} in 10 $\mu g.mL^{-1}$ ERT solution, a linear dependence of the peak intensity *Ip* (μA) upon the square root of the scan rate $v^{1/2}$ (mV s^{-1}) was found for BDDE, demonstrating a diffusional behavior. The equations are noted below:

I_p (μA) = 0.0218 $v^{½}$ + 0.051; r = 0.999 for the first peak (between 50 and 1000 mVs^{-1})

I_p (μA) = 0.132 $v^{½}$ - 0.121; r = 0.996 for the second peak (between 5 and 1000 mVs^{-1})

As the scan rate increased, the peak potential shifted to more positive potential values for both responses. The 38 mV and 67 mV positive shifts in the peak potential confirmed the irreversibility of the oxidation processes for the first and second peaks, respectively.

$\log I_p$ (μA) = 0.431 log v (mV s^{-1}) - 1.427; r = 0.995 for the first peak (between 50 and 1000 mVs^{-1})

$\log I_p$ (μA) = 0.535 log v (mV s^{-1}) - 0.994; r = 0.999 for the second peak (between 5 and 1000 mVs^{-1})

The relationship between peak potential, Ep and logv can be expressed by the following equation:

Ep (V) = 0.0386 logv (V/s) + 36.61, r = 0.996 (n = 5) in pH 2.0 BR buffer

For a irreversible electrode process, Ep and logv are defined by the following equation:[31]

$$Ep = E^{0'} + (2.303RT/\alpha nF) \log (RTk^0/\alpha nF) + + (2.303RT/ \alpha nF) \log v$$

where $E^{0'}$ is the formal potential, k^0 is the standard heterogeneous rate constant, α is the transfer coefficient of the oxidation of ERT and n is the number of the electron transfer in the rate-determination step. F, R, T symbols have their usual significance. α is generally assumed to be 0.5 in the totally irreversible electrode process.[32] From the slope of the Ep versus logv plot, n was calculated as 3.06 in pH 2.0 BR solution. Therefore, the oxidation of ERT is determined as a three electron transfer process. Up to now, the exact oxidation mechanism has not been determined. According to the literature, the oxidation of ertapenem might be occurred on substituted aniline group of ERT compound. Aniline oxidation in aqueous electrolytes is actually a very complex process, involving several oxidation stages and deprotonation reactions and depends to a large extent on the experimental conditions.[33]

The plot of the logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.43 and 0.54 for the first peak and second peak, respectively. The values of 0.43 and 0.54 were found close to the theoretical value of 0.5. The slope value between 0.50 and 1.0 are expected for ideal reactions of solution and surface species.[34] The oxidation of ERT on BDDE was worked as diffusion controlled for both peaks. Hence, further studies on determination of ERT were realized using DPV and SWV techniques on BDDE.

Various electrolytes, such as sulfuric acid, Britton-Robinson, acetate and phosphate buffers were examined. The best results with respect to signal enhancement were accompanied by sharper response for both responses of ERT (Fig. 2), were obtained with Britton-Robinson buffer at pH 2.0. This supporting electrolyte was chosen for the subsequent experiments. The variation of peak intensity (Fig. 3 a, b) and peak potential (Fig. 3 c, d) with pH for a 10 $\mu g\ mL^{-1}$ ERT solution were studied by cyclic, DPV and SWV techniques between pH 1.8 and 12.00. The peak potential versus pH plots of DPV were similar to that obtained by cyclic and SWV. For this reason, only DPV data were given as Fig. 3. The peak potentials of the first (Ep_1) and second (Ep_2) anodic processes moved to less positive potential values and ill defined oxidation peak and/or wave occurred by increasing pH. DPV results were given to show the pH dependence of the oxidation of ERT. Graph of Ep-pH were given for Ep_1 and Ep_2 in Fig. 3c and 3d, respectively.

The relationship between pH and ERT currents (Ip_1 and Ip_2) was also studied. The peak intensities decreased with the raising pH values behaving similar as the peak potential. Both peaks (Ep_1 and Ep_2) gave a single and sharp peak shapes in BR buffer at pH 2.0. For this reason, this supporting electrolyte was selected for the determination studies (Fig. 3a and b).

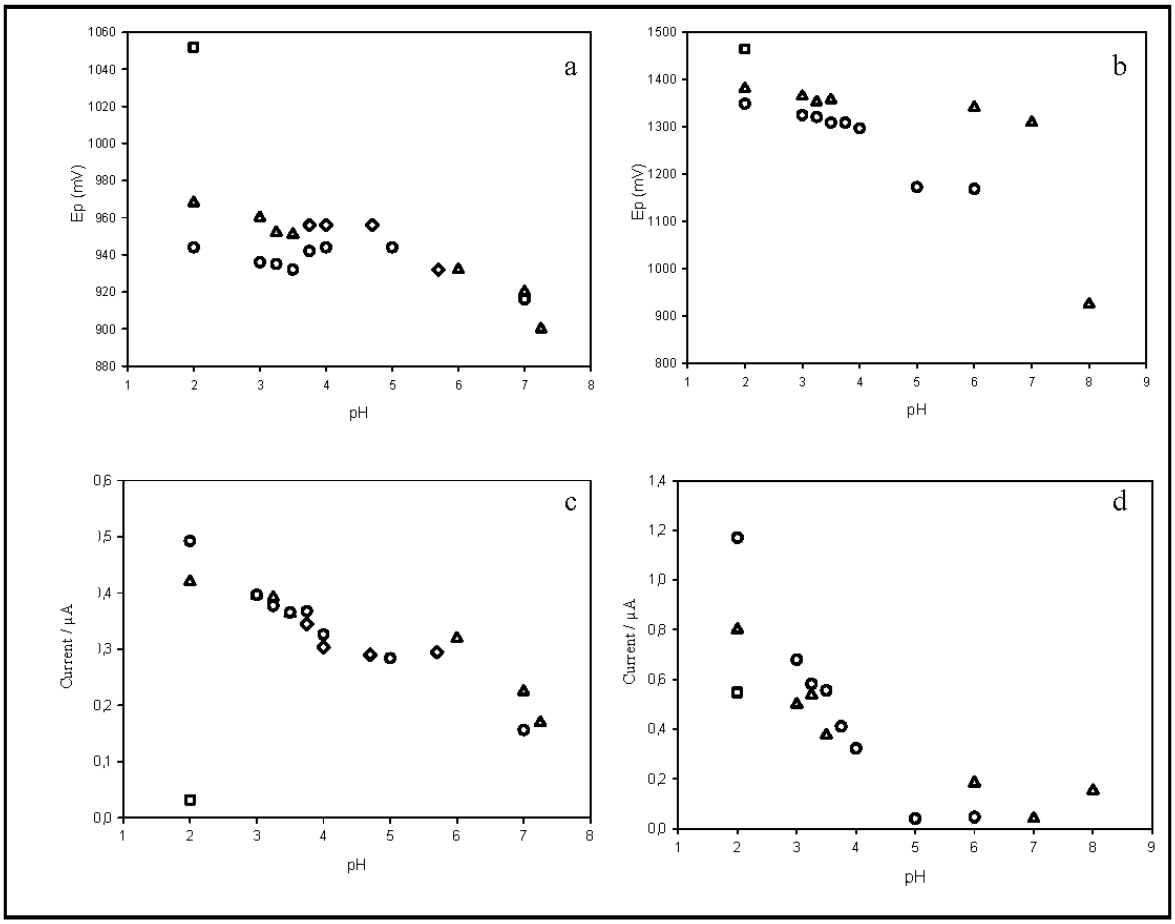

Fig. 3 – Effects of pH on ERT peak potentials obtained with (a) Ep_1; (b) Ep_2 and peak currents obtained with (c) Ip_1; (d) Ip_2. ERT concentration is 10 µg mL^{-1}; (□) 0.1 M H_2SO_4; (Δ) 0.2 M phosphate buffer; (◊) 0.2 M acetate buffer; (○) 0.04 M BR buffer using DPV technique (pulse amplitude, 50mV; pulse width, 50ms; scan rate, 20mVs^{-1}).

Controlled potential coulometry

Controlled potential coulometry was performed to obtain the number of transferred electrons; n values were calculated from the charge consumed at low concentration of ERT. 8.00×10^{-6} M ERT solution was purged with nitrogen. During the electrolysis, the solution was stirred continuously. The total charge consumed was 2.10 mC during the electrolysis of ERT and this value was substituted into Faraday's equation; $Q = nFN$, where Q is the total charge consumed during the electrolysis, N is the number of molar equivalents, and F is the Faraday constant (96,500 C/mol). By using Faraday's equation, the number of transferred electrons was calculated as about 3.

RP-LC studies

According to the obtained results by electrochemical methods, it was possible to apply these techniques for the quantitative analysis of ERT. For showing and proving the accuracy of a proposed method, the results should be compared with other method results using some statistical calculation. That's why a RP-LC technique was proposed and applied to the assay of ERT in its dosage form.

To develop a rugged and suitable RP-LC method for the quantitative determination of ERT, various mobile phase compositions and ratios were employed. Our preliminary trials using different compositions of mobile phases consisting of water, methanol, and acetonitrile, and also different ratios of these solutions, did not give good peak shape. After addition of 15 mM H_3PO_4 (pH 3.00) peak shape of ERT was improved. Finally, by fixing the mobile phase composition consisting of a mixture of methanol:water (45:55; v/v), containing 15 mM phosphoric acid (pH 3.00), ERT and IS were resolved to the baseline and obtained the best peak shape. This mobile phase composition was found to be optimal for good peak shape as well as to

achieve minimal background noise. A reversed-phase Waters X-Select RP-18 (250 x 4.6 mm ID x 5µm) was the most suitable column for RP-LC analysis of ERT. Flow rate of 1.0 mL/min was selected for further studies after several preliminary investigatory chromatographic runs.

Precision and accuracy can often be enhanced by the use of an appropriate internal standard for an LC method, which also serves to correct for fluctuations in the detector response. One of the main reasons for using an internal standard (IS) is for samples requiring significant pretreatment or preparation. When added prior to sample preparation, a properly chosen internal standard can be used to correct for these sample losses. The chemical structure of selected IS (Moxifloxacin (MOX)) is not similar to the ERT structure. However, it was chosen as the internal standard because it showed a shorter retention time with better peak shape and better resolution, compared with other potential internal standards.

Finally, using the selected earlier conditions, a satisfactory chromatographic peak resolution was obtained in a short analysis time, as can be seen in Fig. 5. The chromatographic separation was performed at 25 ^{o}C. Using the optimized operating conditions, the retention times were obtained as 4.28 min for ERT, 4.94 min for IS, being extremely stable among injections. Under the described experimental conditions, the ERT and IS peaks were well defined and free from tailing.

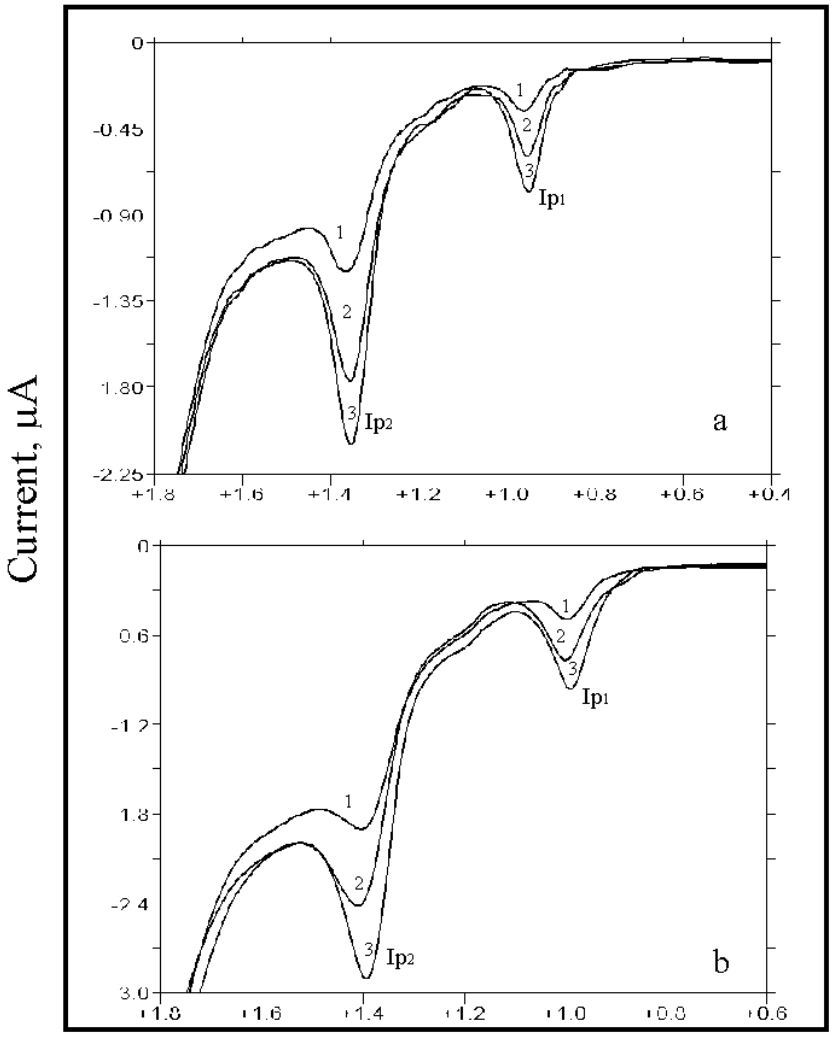

Fig. 4 – (a) DP, (b) SW voltammograms and obtained for the determination of ERT in Britton-Robinson buffer at pH 2.0 using BDD electrode. (1) 5 µg mL^{-1}; (2) 10 µg mL^{-1}; (3) 12 µg mL^{-1} (for DPV technique, pulse amplitude, 50mV; pulse width, 50ms; scan rate, 20mVs^{-1}, for SWV technique, pulse amplitude, 25mV; frequency, 15Hz; potential step, 4mV).

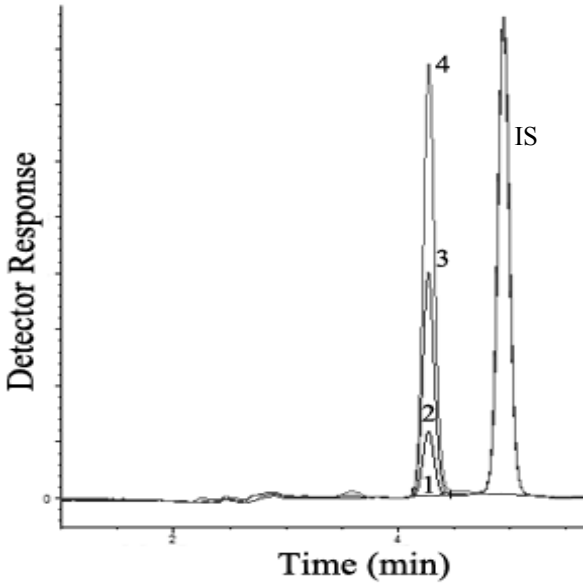

Fig. 5 – RP-LC chromatogram for the determination of ERT;
(1) blank mobile phase; (2) 1 μg mL^{-1}; (3) 4 μg mL^{-1}; (4) 8 μg mL^{-1} and IS 1 μg mL^{-1}.

The proposed RP-LC method provided a simple procedure for the determination of the concentrations of ERT and IS in pharmaceutical preparations by DAD detection at 298 nm. After determining the optimum conditions, a satisfactory resolution was obtained in a short analysis time. Stress testing of the drug substance can help identify the possible degradation products, which can establish the degradation pathways and validate the stability indicating power of the analytical procedures used. The specificity of the developed RP-LC method for ERT was assayed in the presence of its pharmaceutically active compound and degradation products. The chromatograms after being reacted to different degradation conditions were compared with pure active compound solution which is same concentration value. Table 3 shows the results of the forced degradation studies using the proposed method, indicating degradation percentage and purity of ERT signal.

Typical chromatograms obtained from stressed sample with using below conditions are shown in Fig. 6 a-f. Possible degradation products were observed ERT stressed sample that were subjected to acid and alkaline hydrolysis, heat, light and oxidation. ERT was clearly degraded during all applied conditions (Table 3). Peak purity test results derived from DAD with using Chemstation ® software, and the responses were successfully separated from degradation products with the proposed method, as shown in Fig. 6.

Application and Validation of the Proposed Methods

Voltammetric studies

Based on the electrochemical response of ERT, novel methods for its determination are proposed. The greatest advantage of DPV and SWV are increased sensitivity, allowing LOD of various compounds very low level. Due to its high sensitivity, DPV and SWV are particularly useful for trace analysis, *e.g.*, for drug active compounds. [35-37] These techniques prove a good discrimination against background current and low detection limits. [18, 19, 21] They are also suitable for the determination of the drug molecules. SWV has a special property with greater speed of analysis, lower consumption of electroactive species in relation to other electroanalytical techniques and reduced problems with blocking of the electrode surface. SWV showed similar results with DPV technique. In this study, DPV were used as an alternative technique.

For analytical purposes, the DPV and SWV techniques for Ep_1 and Ep_2 in Britton–Robinson buffer at pH 2.0 was selected. Under these conditions, the peak current remains stable and sharp with pH, the signal is well-resolved (Fig. 2 and 3b) and the signal has higher repeatability than signals at other pH values. Further, the signals vary linearly with ERT concentration (Table 1). Typical examples of the differential pulse voltammograms of ERT recorded at the BDDE are presented in

Fig. 4 for Ep_1 and Ep_2 responses using DPV and SWV techniques. The detection (LOD) and quantitation limits (LOQ) of the method were calculated and reported in Table 1. 3.3 s/m and 10 s/m equations were used for the calculation of LOD and LOQ, respectively, [34-41] where s, the noise estimate, is the standard deviation of the peak currents (five runs) of the sample; m is the slope of the calibration curve. All solutions were freshly prepared to ensure stability of analyte in solutions. However, for indicating stability, sample solutions recorded after 4 days did not show any appreciable change in assay values.

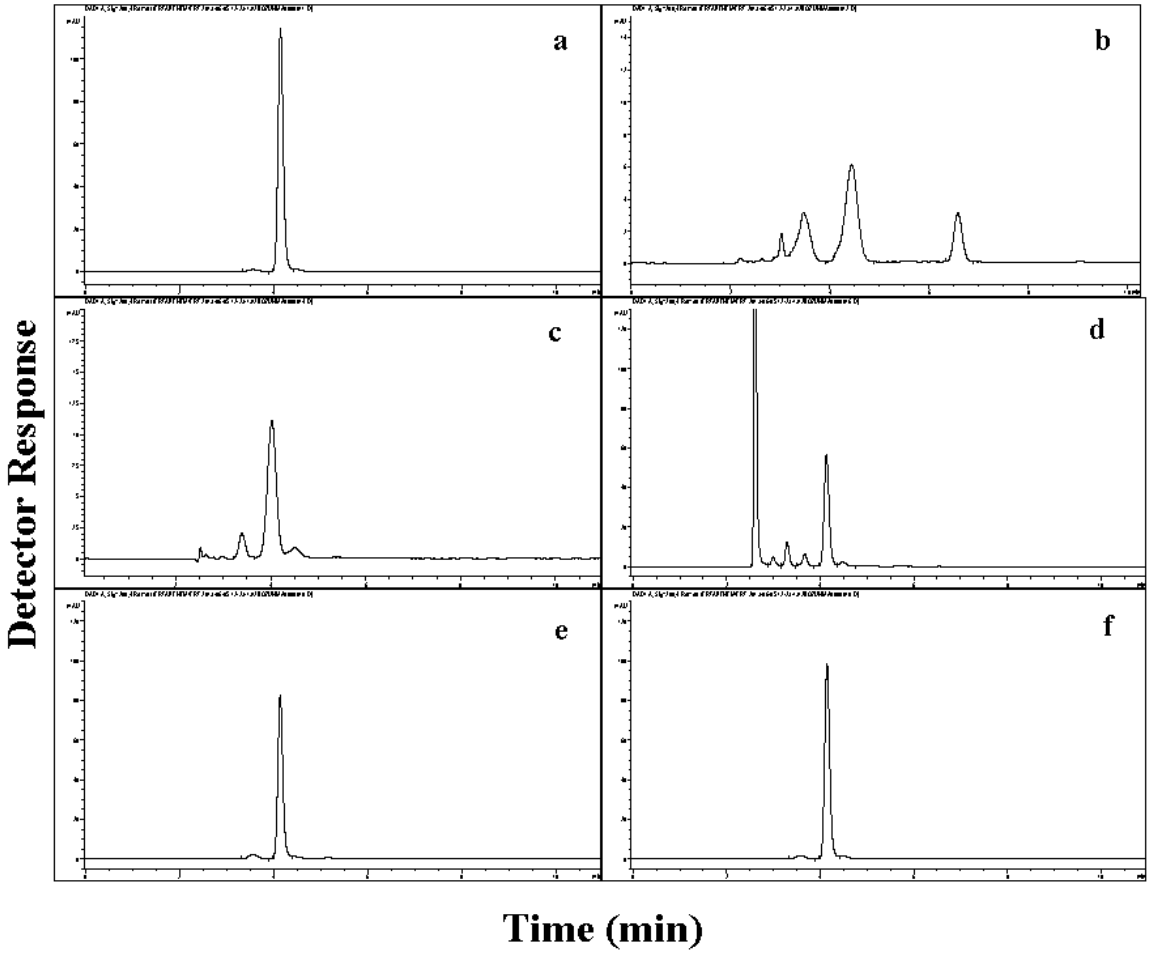

Fig. 6 – Results of degradation studies of 100 μg mL^{-1} ERT. Conditions are
a) bulk form, b) 0.1 M HCl, c) 0.1 M NaOH, d) oxidizing with 3 % H_2O_2, e) UV at 254 nm, f) dry heat at 100 ^oC.

Table 1

Regression data of the calibration lines for quantitative determination of ERT by voltammetric and RP-LC techniques

Technique	Ip_1		Ip_2		HPLC
	DPV	SWV	DPV	SWV	
Potential (mV)/ Retention time(min)	932	988	1344	1388	4.28
Linearity Range (μg mL^{-1})	2.00-12.00	1.00-12.00	1.00-12.00	1.00-12.00	1.00-15.00
Slope	0.041	0.043	0.105	0.124	0.097
Intercept	0.020	0.009	0.060	0.032	0.060
Correlation Coefficient	0.999	0.999	0.999	0.999	0.998

Table 1 (continued)

SE of slope	$6.379x10^{-4}$	$6.218x10^{-4}$	$1.121x10^{-3}$	$1.393x10^{-3}$	2.575x10-3
SE of intercept	$4.893x10^{-3}$	$4.323x10^{-3}$	$7.971x10^{-3}$	$9.905x10^{-3}$	2.321x10-3
Limit of Detection (μg mL^{-1})	0.059	0.072	0.047	0.049	0.184
Limit of Quantification (μg mL^{-1})	0.180	0.218	0.142	0.147	0.612
Within-day Precision[a] (RSD %)	1.048	1.185	0.162	0.338	0.805
Between-day Precision[a] (RSD %)	1.563	1.881	0.570	0.642	1.206

[a] Each value is the mean of five experiments.

Table 2

Assay results and mean recovery studies of ERT in pharmaceutical dosage forms

	Ip_1		Ip_2		HPLC
Technique	**DPV**	**SWV**	**DPV**	**SWV**	
Labeled claim (mg)	1000.00	1000.00	1000.00	1000.00	1000.00
Amount found (mg)[a]	1002.66	1003.44	999.962	1000.19	1004.65
RSD (%)[a]	0.372	0.801	0.065	0.067	0.154
Bias (%)	-0.266	-0.344	0.004	-0.019	-0.465
t_{value}	t_{calc}: 0.36	t_{calc}: 0.43	t_{calc}: 0.14	t_{calc}: 0.26	t_{theo}: 2.31
F_{value}	F_{calc}: 0.22	F_{calc}: 0.27	F_{calc}: 0.27	F_{calc}: 0.77	F_{theo}: 3.79
Added (mg)	10.000	10.000	10.000	10.000	10.000
Found (mg)[a]	10.020	10.033	10.006	10.001	10.007
Recovery (%)	100.207	100.336	100.067	100.016	100.007
RSD% of recovery[a]	1.397	0.409	0.124	0.092	0.114
Bias (%)	-0.207	-0.336	-0.067	-0.016	-0.007

[a] Each value is the mean of five experiments

Table 3

The results of hydrolytic, oxidizing, thermal
and photolytic stressed conditions of ERT by RP-LC

Stress Conditions	**% Degradation of Compound**
0.1 N HCl	87.12
0.1 N NaOH	84.03
3 % H_2O_2	48.00
UV (254 nm)	36.37
Heating (100 ^oC)	24.55

Proposed methods were experienced by replicate analysis of the standard solutions for correctness and reproducibility. Within-day and inter-day reproducibility were deemed adequate with RSD % values lower than 2%. In Table 1, the related analytical parameters are summarized.

The stability of the reference substance and sample solutions were checked by analyzing prepared standard solution of ERT in supporting electrolyte aged at +4 ^{o}C in the dark against the freshly prepared sample. The results demonstrated that the working reference solutions were stable for at least 72 hours.

RP-LC Studies

System suitability tests are an integral part of a liquid chromatographic method. A system suitability test can be defined as a test to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually designed after method development and validation have been completed. The parameters include tailing factor, theoretical plate number, resolution factor, asymmetry factor, selectivity and RSD% of the peak area for repetitive injections. In this study tailing factor is 1.061, theoretical plate number 8255, selectivity 1.40, resolution factor 3.51, symmetry factor 1.085, RSD% of the peak area 0.060%. These tests were carried out on freshly prepared standard stock solutions of ERT. Typically, at least two of these criteria are required to demonstrate system suitability for the proposed method. The linearity was calculated by plotting the peak area ratio of ERT to IS vs. concentration of the compound in the range between 1.0 and 15 μg.mL^{-1}. The developed RP-LC method was also validated according to the standard procedures [29-34] and the results were reported in Table 2 with the electroanalytical results. For the evaluation of stability, several forced degradation procedures were carried out with separately prepared samples via optimized method. Degradation was initiated by dissolving this compound in acidic media (0.1 M HCl), alkaline media (0.1 M NaOH) and for oxidative condition 3 % (w/v) hydrogen peroxide solution and waiting 1 hour. Furthermore, influence of heat was investigated with using solid state of ERT, dry heat in oven at 100 ^{o}C for 12 h. Lastly for light effect, sample of drug substance in the solid state were exposed to 254 nm at room temperature for 12 h. The reactions were carried out at a concentration of 100 μg.mL^{-1}.

Application to analysis of pharmaceuticals

As a final point, the developed DPV, SWV and chromatographic methods were applied to both assays the quantity and the uniformity of ERT in its dosage form. Products in presentation named Invanz® injectable flacon containing 1000.00 mg ERT. On the basis of the above results, optimized methods were applied to the direct determination of ERT in pharmaceutical preparations, using the related calibration equations without sample preparation and after adequate dilutions (Table 2). This is a simple procedure that can be used without any sample extraction, evaporation or filtration. Table 2 shows that these techniques can be effectively applied for the assay of ERT in its dosage forms.

Accuracy, precision and selectivity of the proposed methods and possibility of interference of excipients with the analysis in pharmaceutical dosage forms were evaluated by recovery tests after addition of known amounts of pure drug to various pre-analyzed formulations of ERT. Recovery studies were carried out in pharmaceutical dosage form for both peaks (Ep_1 and Ep_2) and both techniques by addition of a known amount of pure ERT to various pre-analyzed ERT solutions. The precision and accuracy results showed that the used methods could be applied for the determination of ERT in pharmaceuticals without any interference of the inactive ingredients (Table 2).

As far as we know, there is no official method in any pharmacopoeias related to pharmaceutical dosage forms or bulk drugs of ERT. That's why the proposed voltammetric and chromatographic techniques were compared statistically between each other. Both methods showed similar accuracy and precision. Statistical comparisons were performed on data from both chromatographic and voltammetric experiments. Student's t- and F test (Table 2) revealed no statistically significant difference between methods with regard to accuracy and precision. [38-41]

EXPERIMENTAL

Apparatus

Cyclic (CV), linear sweep (LSV), differential pulse (DPV) and square wave voltammetry (SWV) were performed using a BAS 100 W (Bioanalytical System, USA) electrochemical analyzer. A standard one-compartment three-electrode cell of

10 mL capacity, incorporating a BDDE (Windsor Scientific Ltd., Slough, Berkshire, UK; Ø: 3 mm, diameter), was used. A platinum wire and an Ag/AgCl (BAS; 3M KCl) were used as the auxiliary electrode and reference electrode, respectively. The BDDE was polished manually with aqueous slurry of alumina powder (Φ: 0.01µm) on a damp smooth polishing cloth (BAS velvet polishing pad), before each measurement. All measurements were realized at room temperature.

The operating conditions were as follows: For DPV technique, pulse amplitude, 50mV; pulse width, 50ms; scan rate, $20mVs^{-1}$, for SWV technique, pulse amplitude, 25mV; frequency, 15Hz; potential step, 4mV, for CV technique -0.20 V and +1.8V range at 100 $mV.s^{-1}$ scan rate; sample interval 1mV; and quite time 2s; and for LSV technique -0.20 V and +1.8 V range and 100 mV s^{-1} as scan rate were used. Coulometric measurements were performed with large surface area platinum foil as working electrode in an electrochemical cell using AUTOLAB-PGSTAT302 with GPES 4.9. The volume of electrochemical cell was 2.0 mL which coulometric measurements were carried out.

The pH was measured using a pH meter Model 538 (WTW, Weilheim, Germany) using a combined electrode (glass electrode-reference electrode) with an accuracy of ±0.05 pH.

For liquid chromatographic studies; Agilent Technologies HP 1100 series (Wilmington, DE) LC system was used for method development and validation process, equipped with a G1379A degasser, G1311A quaternary pump, 61313A auto sampler, and G1315B diode array detector. The chromatograms were recorded and the peaks were quantified using an automatic integrator. An X-Select (Waters Corp. Milford, MA, USA) RP-18 (250 x 4.6 mm ID x 5µm) column was used as a stationary phase at 25 ^{o}C constant temperature.

Reagents

ERT and its injectable dosage form (Invanz®) were kindly provided by Merck Sharp and Dohme (Istanbul, Turkey). Moxifloxacin (MOX) (IS) was kindly supplied from Abdi Ibrahim (Istanbul, Turkey). Standard stock solution (200 ppm) was prepared by dissolving appropriate weight of ERT standard powder in water while continuous stirring until complete dissolution of the drug. The standard solution was then kept in a refrigerator at 4°C. Four different type of supporting electrolytes were used in this study. 0.1 M H_2SO_4, 0.2 M phosphate buffer at pH 2.14-8.0, 0.04 M Britton-Robinson buffer at pH 2.32-12.0 and 0.2 M acetate buffer at pH 3.7-5.7 were used for supporting electrolyte. Working solutions were freshly prepared just before assay by dilution of the standard stock solution using an appropriate amount of selected supporting electrolytes. For the RP-LC methods, chromatographic grade methanol (Merck, Darmstadt, Germany) were used as organic modifier. Sodium hydroxide (Merck, Darmstadt, Germany) and o-phosphoric acid (Riedel-de Haen, Germany) was used for pH adjustment. Water with conductivity lower than 0.05 µS cm^{-1} for RP-LC studies was obtained for all analysis. The calibration equations were constructed by plotting the peak current against ERT concentrations for voltammetric studies. The calibration curves for RP-LC analysis were constructed by plotting the ratio of the peak area of the drug to that of IS versus drug concentration. The ruggedness and precision were checked in the same day (n=5) and three different days (n=5) over a week period. Precision and ruggedness were expressed as relative standard deviation (RSD %). The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (Bias %). One example of relative error is the accuracy, which describes the deviation from the expected results. [36-39]

All solutions were kept in the dark and were used within 24 h to avoid decomposition. However, voltammograms of the sample solutions recorded a week after preparation did not show any appreciable change in assay values.

Chromatographic procedure

The separation was carried out at ambient temperature, on a reversed-phase Waters X-Select RP-18 column (250 x 4.6 mm ID x 5µm). The chromatographic separation was performed using an isocratic mode. The mobile phase consisted of a mixture of methanol–water (45:55; v/v), containing 15 mM phosphoric acid (pH 3.00). 1.0 mL/min flow rate was used for the separations with a detection of 298 nm. The injection volume was 10 µL. The chromatographic separation was performed at 25 ^{o}C. MOX was chosen as IS. The dead time (t_o) was measured by injecting urasil solution [Sigma, USA, 0.01% (w/v), in water].

Pharmaceutical dosage forms assay procedure

Adequate amount of Invanz® injectable dosage form, claimed to contain 1000.0 mg ERT in the flacon, was dissolved with pure water. The mixture was then homogenized with a vortex to assure the complete dissolution of the drug prior to its dilution to a final volume of 10 mL using the same solvent. An aliquot of this solution was transferred into a 10 mL volumetric flask (for RP-LC studies, adding of the constant amount of IS (1.0 µg mL^{-1})), diluted to the volume with the selected supporting electrolyte and with mobile phase for electroanalytical and RP-LC methods respectively. The nominal content of the injectable dosage form was calculated from the corresponding regression equations of previously plotted calibration plots obtained using BDDE.

Recovery studies

Due to a possible interference of the matrix of the dosage form with the analysis or accurate quantitation of the analyte, potential effects from matrix components must be investigated. Accuracy is the main requirement of electroanalytical methods. It is defined as the closeness of the obtained value to the true value for the sample. Recovery experiments are performed in the presence of the matrix.[36-39] To study the accuracy, reproducibility and to check the interference from the excipients used in the formulations of these techniques, recovery experiments were carried out using the standard addition method. In order to know whether the excipients show any interference with the analysis, known amounts of the pure ERT were added to the pre-analyzed injectable dosage form. The mixtures were analyzed by the both proposed methods. The recovery results obtained after five repeated experiments for voltammetric and RP-LC methods.

CONCLUSIONS

In this study, electrochemical behavior of ERT was investigated for the first time. This work shows that the ERT can be determined by using voltammetric techniques on the basis of its

oxidation processes over the solid electrodes. BDDE was used in different background electrolyte (pH 1.8-12.0) to clarify and understand oxidation process of ERT. ERT exhibits two irreversible anodic responses that are diffusion-controlled at a BDDE. Based on both anodic responses of the drug, DPV and SWV methods for its determination were developed. The developed RP-LC method might serve as a versatile analytical tool suitable for the assay of ERT and interest quality control and therapeutic drug monitoring laboratories. The electroanalytical information and the obtained data might be used for the development of a RP-LC method with an electrochemical detection system. The proposed methods were successfully applied to determine both the quantity and uniformity of ERT in injectable dosage form without interferences. The described procedures allow simple, highly sensitive, accurate, fast response and low cost quantitative method for determination of ERT in the pharmaceutical dosage forms. Preparation of the sample was easy and did not require any previous treatments. All proposed methods require less than 5 min to run a sample. It can be undoubtedly said that studied techniques are a high-quality analytical alternative for determining ERT in pharmaceutical dosage forms. These techniques can be readily adapted to routine in analytical analysis.

REFERENCES

1. P. M. Shah and R. D. Isaacs, *J. Antimicrob. Chemother.*, **2003**, *52*, 538-542.
2. http://en.wikipedia.org/wiki/Ertapenem
3. M. L. Hammond, *J. Antimicrob. Chemother.*, **2004**, *53*, 7-9.
4. H. Teppler, R. M. Gesser, , I. R. Friedland, G. L.Woods, A. Meibohm, G. Herman, G. Mistry and R. Isaacs, *J. Antimicrob. Chemother.*, **2004**, *53*, ii75-ii81.
5. Physicians Desk Reference (PDR), 61st edition, "Medical Economics Company Inc.", Montvale, N.J, 2007.
6. S.C. Sweetman (Ed.). Martindale, "The complete Drug Reference", 36th edition, Pharmaceutical Pres, London, 2009.
7. E.M. Abdel-Moety, N.A. Elragehy, N.Y. Hassan and M.R. Rezk , *J. Chromatogr. Sci.*, **2010**, *48*, 624-630**.**
8. T. Legrand, S. Chhun, E. Rey, B. Blanchet, J.-R. Zahar, F. Lanternier, G. Pons and V. Jullien, *J. Chromatogr. B*, **2008**, *875*, 551-556.
9. M. Zajac, J. Cielecka-Piontek and A. Jelinska, *Chemia Analityczna*, **2006**, *51*, 761-770.
10. J.-B.Gordien, E. Boselli, , C. Fleureau, B. Allaouchiche, G. Janvier, O. Lalaude, M. C. Saux and D. Breilh, *J. Chromatogr. B*, **2006**, *830*, 218-223.
11. C. J. Kitchen, D. G. Musson and A. L. Fisher, *J. Chromatogr. B*, **2004**, *799*, 9-14.
12. D. G. Musson, C. J. Kitchen, J. Y. K. Hsieh and K. L. Birk, *J. Chromatogr.*, *B*, **2002**, *779*, 341-346.
13. K. Michalska, G. Pajchel and S.Tyski, *J. Chromatogr. A*, **2009**, *1216*, 2934-2942.
14. S. Lefeuvre, N.Venisse, S. Marchand, M. Bachelet and W. A. Couet, *J. Chromatogr. B*, **2008**, *862*, 242-245.
15. N. Y. Hassan, E. M. Abdel-Moety, N. A. Elragehy and M. R. Rezk, *Spectrochim. Acta*, **2009**, *72*, 915-921.
16. S. A. Ozkan, B. Uslu and H. Y. Aboul-Enein, *Crit. Rev. Anal. Chem.*, **2003**, *33*, 155-181.
17. B. Uslu and S. A. Ozkan, *Anal. Lett.*, **2007**, *40*, 817-853.
18. B. Uslu and S. A. Ozkan, *Comb. Chem. High Throughput Screen*., **2007**, *10*, 495-513.
19. P.T. Kissinger and W.R. Heineman, "Laboratory Techniques in Electroanalytical Chemistry", 2nd ed., Marcel Dekker, New York, 1996.
20. R. N. Goyal, V. K. Gupta, M. Oyama and N. Bachheti, *Electrochem. Comm.*, **2006**, *8*, 65-70.
21. J. Wang, "Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine", VCH Publishers, New York, 1988.
22. S. Suzen, B.T. Demircigil, E.Büyükbingol and S.A. Ozkan, *New J. Chem.*, **2003**, *27*, 1007-1011.
23. T. N. Rao and A. Fujishima, *Diamond Rel. Mat.*, **2000**, *9*, 384-389.
24. Y.V. Peleskov, *Russ. J. Electrochem.*, **2002**, *38*, 1275-1291.
25. R. Compton, J. Foord and F. Marken, *Electroanalysis*, **2003**, *15*, 1349-1363.
26. N. S. Lawrence, M. Pagels, A. Meredith, T. G. Jones, C. E. J. Hall, C. S. J. Pickles, H. P.Godfried, C. E. Banks, R. G. Compton and L. Jiang, *Talanta*, **2006**, *69*, 829-834.
27. D. Kul, M. Gumustas, B. Uslu and S. A. Ozkan, *Talanta*, **2010**, *82*, 286-295.
28. M. Gumustas and S.A. Ozkan, *Anal. Bioanal. Chem.*, **2010**, *397*, 189-203.
29. B. C. Lourenção, R. A. Medeiros, R. C. Rocha-Filho, L. H. Mazo and O. Fatibello-Filho, *Talanta*, **2009**, *78*, 748-752.
30. P. de Lima-Neto, A. N. Correia, R. R. Portela, M. D. S. Julião, G. F. Linhares-Junior and J. E. S. de Lima, *Talanta*, **2010**, *80*, 1730-1736.
31. Z. Zhang and E. Wang, "Electroanalytical Principles and Methods", Science Press, Beijing, 2000.
32. K. Wu, Y. Sun and S. Hu, *Sensor. Actuat. B-Chem.*, **2003**, *96*, 658-662.
33. M. Mitadera, N. Spataru and A. Fujishima, *J. of App. Electrochem*., **2004**, *34*, 249-254.
34. M. E. Swartz and I. S. Krull, "Analytical Method Development and Validation", Marcell Dekker, New York, 1997.
35. J. Ermer and J. H. Miller, "Method Validation in Pharmaceutical Analysis", Wiley-VCH, Veinheim, 2005.
36. S.A. Ozkan, "Electroanalytical Methods in Pharmaceutical Analysis and Their Validation", HNB Publishing, USA, 2011.
37. I. R. Berry and D. Harpaz, "Validation of Active Pharmaceutical Ingredients", 2nd ed., CRC Press, Washington, 2001.
38. E. Laviron, L. Rouillier and C. Degrand, *J. Electroanal. Chem.*, **1980**, *112*, 11-23.
39. Y. Altun, B. Dogan-Topal, B. Uslu and S. A. Ozkan, *Electrochimica Acta*, **2009**, *54*, 1893-1903.
40. B. Uslu, B. D. Topal and S. A. Ozkan, *Talanta*, **2008**, *74*, 1191-1200.
41. B. Uslu and S. A. Ozkan, *J. Pharm. Biomed. Anal.*, **2003**, *31*, 481-489.