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Voltammetric study of itraconazole an antifungal drug at glassy carbon electrode in acidic medium: A simple plus cost-effective detection method

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In this study, the electro-oxidative behaviour and determination of itraconazole at a glassy carbon electrode have been investigated using cyclic voltammetry, differential pulse anodic stripping voltammetry (DPASV), and square wave anodic stripping voltammetric (SWASV) techniques, under different experimental conditions. The voltammetric peak current for the oxidation of itraconazole has been analyzed at different *p*H, scan rate and concentrations. The voltammograms have exhibited irreversible oxidation of ITRA in B.R. buffer of *p*H 3.0. The oxidation of itraconazole gives a well-defined irreversible peak at glassy carbon electrode *vs.* Ag/AgCl as reference electrode. The oxidation process is adsorption controlled. A linear response has been obtained between 26.7×10^{-6} to 152.8×10^{-6} M in non-aqueous media for all the techniques. The analyzed square wave anodic voltammetric and differential pulse anodic stripping voltammetric methods show limit of detection at 27.28 µM and 65.16 µM respectively. Ultimately, the proposed validated method has been effectively applied for the determination of the antifungal drug which is commercially available in solid form.

Keywords: Itraconazole, antifungal drug, anodic stripping voltammetry, glassy carbon electrode (GCE), BR buffer (*p*H 3.0)

The azoles are the best source of synthetic antifungal agents. Itraconazole is an orally administered triazole antifungal agent. The mechanism of action of this compound is the inhibition of lanosterol demethylase, a cytochrome P-450 enzyme[1,2]. Chemical nomenclature 4-(4-{4-[4-({(2RS,4SR)-2-(2,4-Dichlorophenyl)-2-[(1*H*-1,2,4-triazol-1-yl)methyl]-1,3-dioxolan-4-yl}methoxy) phenyl]piperazin-1-yl}phenyl)-2-[(1RS)-1-methylpropyl] -2,4-dihydro-3H-1,2,4-triazol-3-one[3,4] as shown in Figure 1. Itraconazole is a food and drug administration approved antifungal-drug that has a history of use in clinical trials for the cure of various diseases[5-8]. Also, itraconazole is more effective only at low *p*H and almost insoluble in water.

Various methods are already developed for the investigation of itraconazole drug in bulk form for instance; polarography (DME)[9], spectrofluorimetric[3], spectrophotometric[10-13], and chromatographic (HPLC, LC-MS)[14-27], solid state NMR[28] and voltammetric techniques[29-33] using different electrocatalysts.

In the recent era, voltammetric techniques are used to determine pharmaceutical drug moieties as these methods provide easy handling, smoothness, portability and short examine time. Modern electroanalytical techniques, for instance square wave voltammetry (SWV) and differential pulse voltammetry (DPV) with stripping mode, are utilized for the sensitive and rapid plus effortless investigations of a wide range of drug moieties. Moreover, the stripping voltammetry is an excellent technique and insensitive to matrix effects[4].

In this report, the electrochemical behavior of itraconazole was studied using cyclic voltammetry in acidic BR buffer of *p*H 3 as supporting electrolyte. Also detection of traces amount of itraconazole by differential pulse anodic stripping voltammetry (DPASV) and square wave anodic stripping voltammetry (SWASV) at GCE. The procedures did not require sample pre-treatment/time consuming extraction. To best of our knowledge, the electrochemical determination of itraconazole at glassy carbon electrode is not exactly reported through this procedure and experimental conditions.

Materials and methods

Reagents and instrumentation

Itraconazole was purchased from local pharmacy under the trade name GALITRA-200 mg and was used without purification. Every day, a fresh stock standard solution of bulk itraconazole (2.7 mM

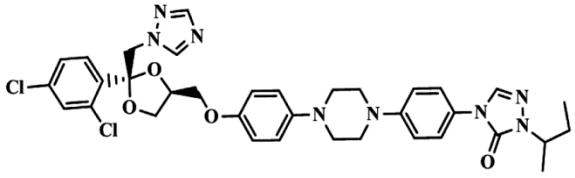


Figure 1 — Chemical structure of itraconazole

concentration) was prepared in dimethylsulfoxide (DMSO) solvent in appropriate ratio. The buffer (Britton Robinson, citrate, and acetate) solutions were prepared and optimized 0.04M BR buffer was used as a supporting electrolyte. Double distilled water (DDW) was used to prepare the solutions and all the reagents used were of analytical grade.

Electrochemical measurements were employed using Model 1230A/SR 400 electrochemical analyzer (CHI Instrument TX, USA), with a totally automated attached computer with proper CHI 100W version 2.3 software for total control of the experiments, data compilation and treatment. A three electrode cell was used with GCE as working electrode for the voltammetric experiments. The calculated active-surface area of GCE is 0.0882 cm^2, as explained in our earlier report[34]. The platinum wire as counter electrode and Ag/AgCl (1M KCl) as reference electrode were used. Moreover, a digital *p*H-meter (CHINO-DB-1011) fitted with a glass-electrode was standardized with buffers of known *p*H and used for measuring the *p*H values of the solutions.

Analytical procedure

Initially, the GC-electrode surface was polished thoroughly with 0.5 µm alumina; additionally it was cleaned in ultrasonic-bath prior to each measurement, and then gently cleaned with a µ-fibered tissue paper. Britton Robinson buffer of *p*H 3.0 with KCl solution and the appropriate concentration of the itraconazole were introduced into the electrochemical cell. And the solution was purged with pure deoxygenated N_2-gas for ~10 min under stirred condition for removal oxygen gas inside the cell, before measurements. Electrochemical pre-treatment was always performed in the same solution in which the measurement was subsequently carried out. After optimization of operational parameters the cyclic and stripping voltammograms were recorded.

Optimization of buffer

Various buffers including BR, citrate, and acetate buffers were prepared and comparatively studied for optimization with 2.7 mM itraconazole using DPASV technique, at an arbitrarily selected *p*H for all buffer solutions. It was found that BR buffer was suitable for electrochemical study of itraconazole as the peak current response is highest, as shown in Figure 2.

Results and Discussion

Electrochemical analysis of 2.7 mM itraconazole were performed by using cyclic voltammograms (CVs), differential pulse anodic stripping voltammograms (DPASVs), and square wave anodic stripping voltammograms (SWASVs). In all electrochemical methods itraconazole gave one well-defined oxidation peak over the potential range 0.2 to 1.0 V. *vs.* Ag/AgCl reference electrode, in BR buffer of *p*H 3.0 at GCE.

Cyclic voltammetric behaviour of itraconazole

Here, itraconazole gave a single well-defined peak in BR buffer of *p*H 3.0 with 1 M KCl at GCE and the cyclic voltammogram is shown in Figure 3. The sharp anodic oxidation peak of itraconazole was observed at 0.769 V peak potential. No reduction peak was observed on the reverse cathodic scan, signifying the irreversible nature of the electrochemical reaction[4,30]. Also, no peak was obtained in blank (without itraconazole) BR buffer of *p*H 3.0.

Effect of scan rate

The cyclic voltammograms of itraconazole in BR buffer exhibits a well-defined oxidation peak in the

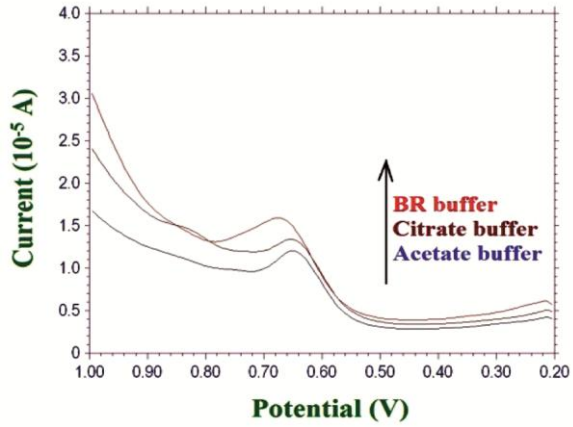


Figure 2 — DPASVs obtained in different buffers

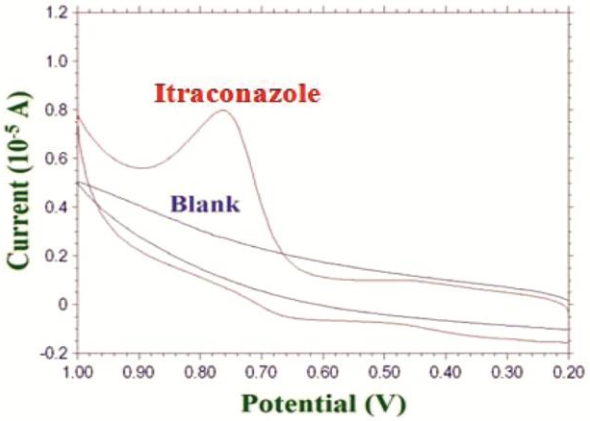


Figure 3 — Cyclic voltammograms recorded at GCE with itraconazole (Red curve) and without itraconazole or blank (Blue curve)

potential range of 0.2 to 1.0 V *vs.* Ag/AgCl reference electrode at various scan rates 60 to 160 mV/s (Figure 4A). The peak potential shifted towards more positive values with increasing scan rate following the Nicholson theory[4,34]. There was no peak was observed in the cathodic direction, suggesting the irreversible nature of the electrode process. This behavior confirms the irreversible character of the oxidation reaction at GCE. Furthermore, linear plots of peak current *vs.* square root of scan rate (Figure 4B) following the $Ip \alpha v^{1/2}$ should be obtained for an adsorption controlled process. A linear plot between *I*p and $v^{1/2}$ indicates about adsorptive nature of electrode process. The linear relationship existing between peak current (*I*p) and square root of the scan rate ($v^{1/2}$) with a slope confirms the adsorptive nature of oxidation of itraconazole; the linear regression equation related to the plot was found as (eq. 1):

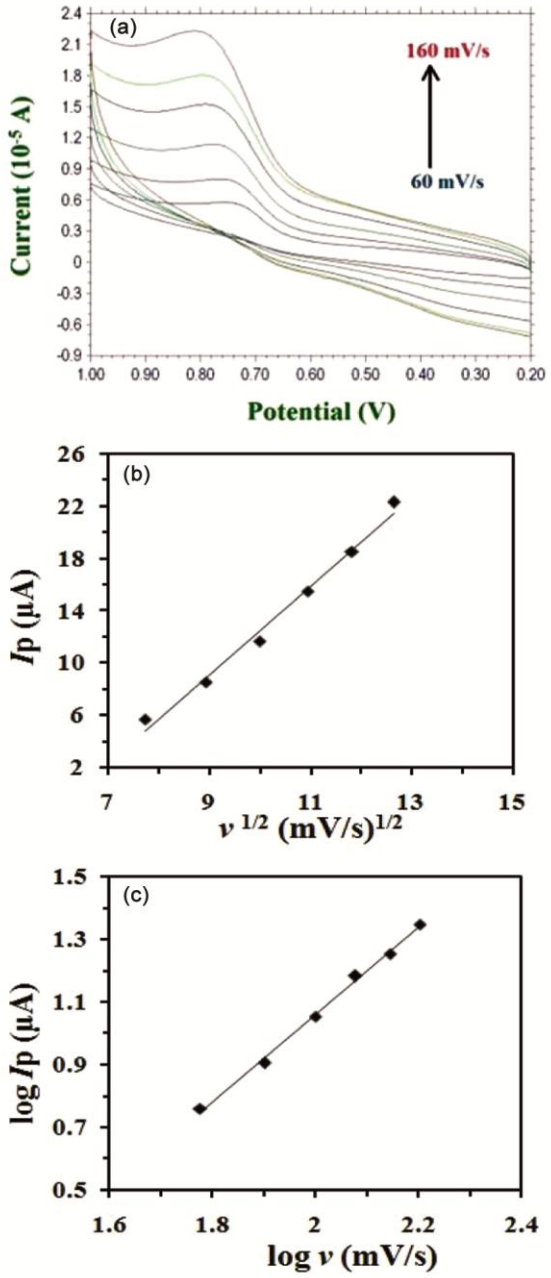


Figure 4 — (A) CVs at different scan rates 60 to 160 mV/s with 2.7 mM itraconazole in BR buffer. (B) Plot of *I*p *vs.* $v^{1/2}$. (C) Plot of log *I*p *vs.* log *v*

$$Ip\ (\mu A) = 3.402\ v^{1/2} - 21.50,\ R^2 = 0.988 \quad (1)$$

Here, Figure 4C illustrates a plot between log *I*p *vs.* log *v* with slope 1.105 log *I*p/log *v* is very near to the theoretical value of one or more than one for a adsorption controlled process[4,34].

Furthermore, the linear regression equation related to the plot of logarithm of peak current *I*p (μA) *vs.* logarithm of scan rate (mV/s) was expressed as (eq. 2):

$$\log Ip = 1.105 \log \nu - 1.736,\ R^2 = 0.996 \quad (2)$$

Influence of *p*H

The influence of the *p*H on the oxidation process was analyzed and a progression of voltammograms was observed. Single voltammetric peak was observed in the whole *p*H range (2-6) studied. The peak current was also dependent on the *p*H, implying the involvement of the protons in the current-limiting electrode process. The maximum peak current value for the voltammetric peak (sharp) was obtained at the *p*H 3.0 (Figure 5A); 2.7 mM itraconazole used. Moreover, the peak height attains maxima at *p*H 3.0 and thereafter decreases (Figure 5B). Therefore, *p*H 3.0 was selected as the optimum *p*H for the study of itraconazole.

The effect of *p*H reveals the involvement of proton in the electrochemical reaction as anodic peak-potential negatively shifted with increasing *p*H of the solution[4,30]. The peak-potential was found to be linearly dependent on *p*H, plotted in Figure 5C, and the corresponding linear regression equation is as followeds (eq. 3):

$$Ep\ (V) = -0.003\ pH + 0.840,\ R^2 = 0.905 \quad (3)$$

The slope of the linear regression equation is -0.003 V/*p*H, which is very far from the theoretical Nernstian value of −0.059 V/*p*H; for the equal-participation of e^- and H^+ in an electrode process[30,31]. Herein, the results of the *p*H study proved that the

number of electrons and protons are not equally participated in the electro-oxidation reaction of itraconazole at GCE.

Concentration effect

To confirm that the peak obtained was solely due to the oxidation of itraconazole moiety, cyclic voltammograms (CVs) were recorded at various concentrations (26.7, 52.9, 78.6, 103.8, 128.6, and 152.8 µM) at scan rate of 100 mV/s. The peak intensity increased with increasing concentration of itraconazole as given in Figure 6A. The peak current linearly increased with increasing concentration as shown in Figure 6B and corresponding linear regression equation was found as (eq. 4):

$$\log Ip = 11975\ C + 26.39,\ R^2 = 0.902 \quad (4)$$

Possible electro-oxidation mechanism of itraconazole

On the basis of anodic voltammetric results, it can be concluded that the observed peak is due to the $2e^-$ and $1H^+$ participation/removal in electrochemical oxidation reaction of itraconazole at GCE as shown in Scheme I, which is analogous with the former studies[4,30,31].

Moreover, the complete mechanism steps are explained in our previous research article[30]. Briefly, the transformation of piperazine heterocyclic ring to tetrahydro-1-pyrazinium heterocyclic ring within itraconazole moiety through single-electron transfer oxidation and deprotonation mechanism steps. The

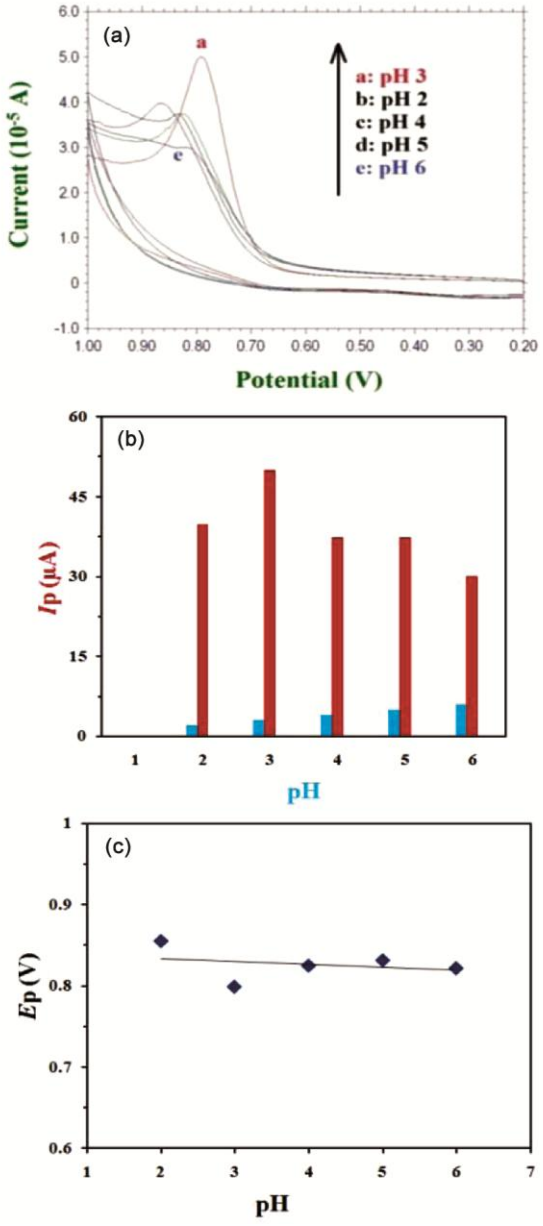


Figure 5 — (A) CVs recorded at different *p*H range of BR buffer at GCE with itraconazole. (B) Plot of *I*p *vs. p*H. (C) Plot of peak *E*p *vs. p*H

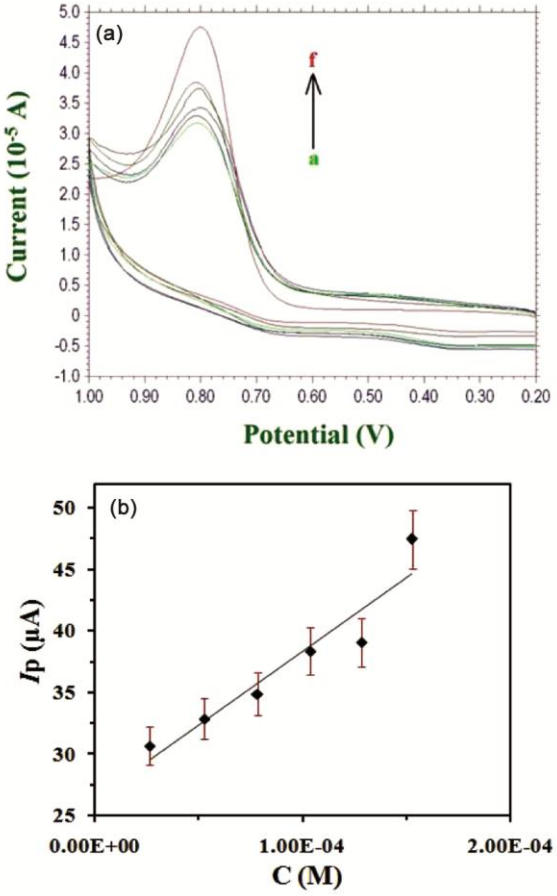


Figure 6 — (A) CVs of itraconazole with increasing concentrations from 26.7 to 152.8 µM in acidic BR buffer, and (B) plot of peak current *vs.* concentration

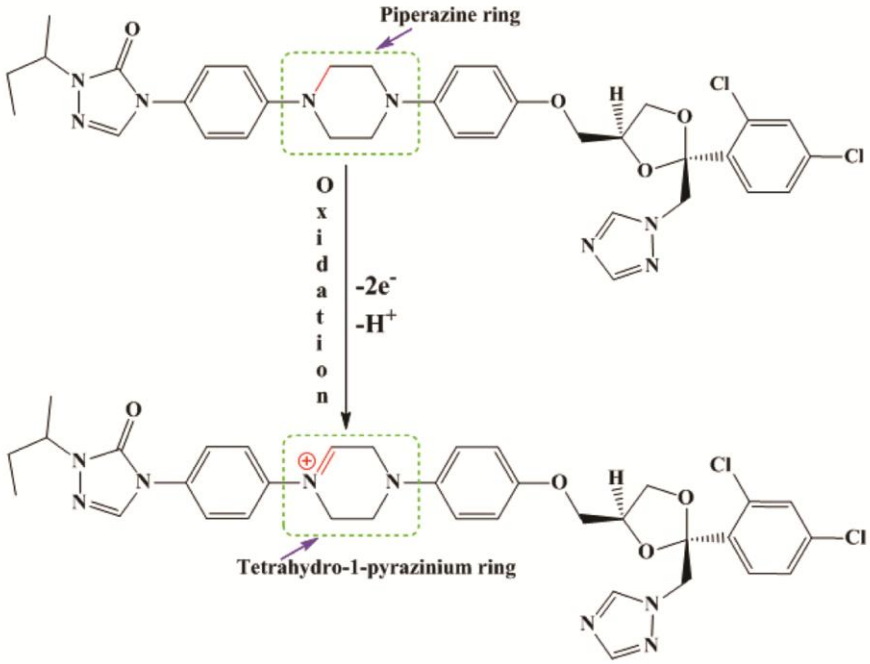
N-radical cation is mainly formed as an intermediate species in single-electron transfer oxidative process[35,36]. Furthermore, the C-based radical produced from C-anion because of the electronegativity (*X*)-factors of N-atom. Eventually, tetrahydro-1-pyrazinium heterocyclic ring is formed due to driving force of stability over radical or radical cation.

Validation of analytical process

DPASV and SWASV modes were selected to develop an electrochemical method for quantitative determination of itraconazole at GCE as it gives improved sensitivity with high speed of current potential curves. According to the obtained results, it was possible to apply this technique to the quantitative analysis of itraconazole in bulk form. The validity of the proposed method was assessed by studying the analytical parameters through DPASVs plus SWASVs.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated, and the results are tabulated in Table I. LOD and LOQ were calculated according to the following equations[4,34]: LOD = 3s/m and LOQ = 10 s/m; Where s = standard deviation, m = slope of linearity.

Under the optimized condition a linear correlation between DPASV and SWASV peak intensity; and the drug concentration was obtained over the range between 26.7 and 152.8 µM, DPASVs and SWASVs were recorded (Figure 7A and Figure 8A). Calibration plot of *Ip vs.* C as graphed in Figure 7B and Figure 8B and their corresponding calibration equations can be written as (eq. 5,6):



Scheme I — Possible mechanism of itraconazole

Table I — The regression parameters obtained from calibration curves for quantitative determination of itraconazole by DPASV and SWASV techniques in acidic BR buffer

Analytical Parameter	DPASV	SWASV
Potential range	0.2-1.0 V	0.2-1.0 V
Linearity range	26.7-103.8 µM	26.7-152.8 µM
Slope	17458 µA/M	14318 µA/M
Correlation coefficient (R^2)	0.965	0.945
RSD	0.32%	9.16%
LOD	27.28 µM	33.82 µM
LOQ	90.93 µM	112.75 µM

Note: Mean values were calculated by three independent repeated measurements.

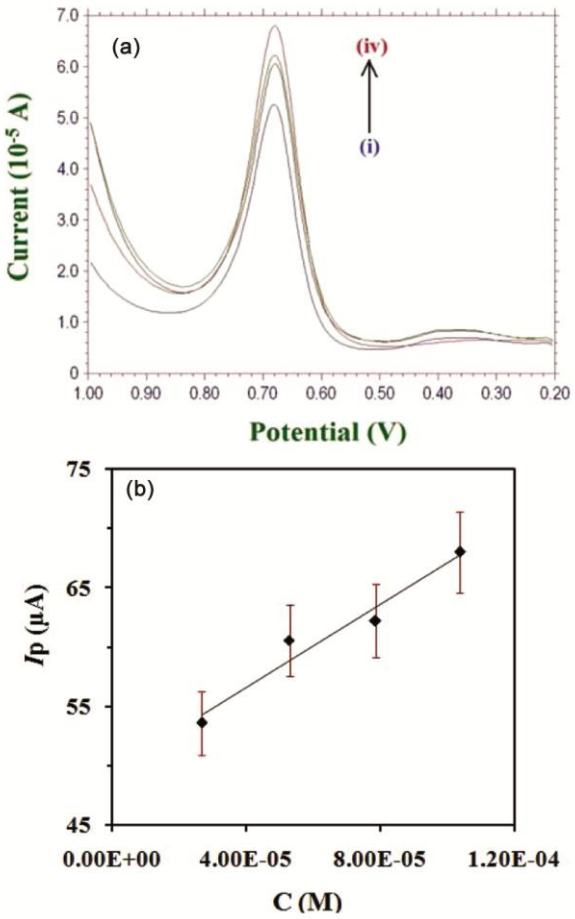


Figure 7 — (A) Calibration graphs: DPASVs of itraconazole with increasing concentrations from 26.7 to 103.8 µM in acidic BR buffer, and (B) plot of peak current *vs.* concentration

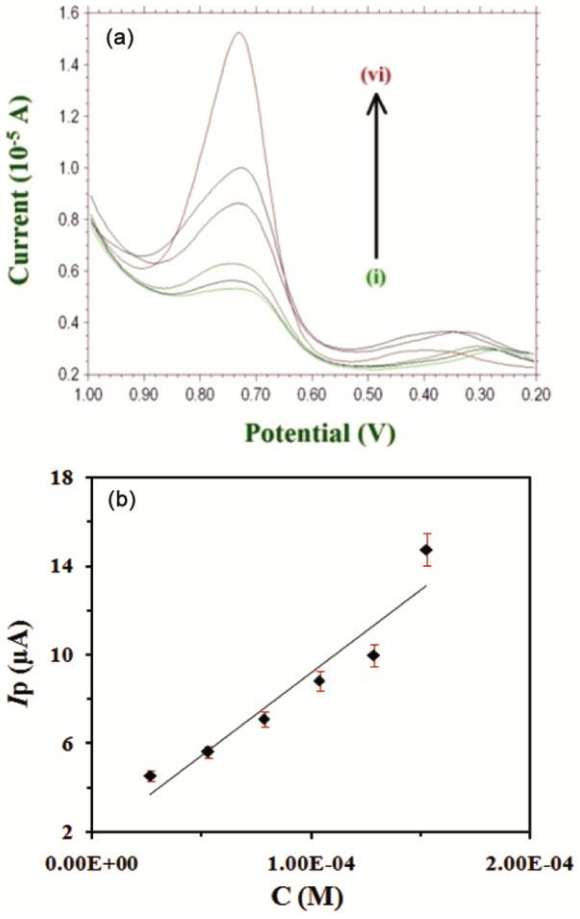


Figure 8 — (A) Calibration graphs: SWASVs of itraconazole with increasing concentrations from 26.7 to 152.8 µM in acidic BR buffer, and (B) plot of peak current *vs.* concentration

DPASV: *I*p (µA) = 17458 C + 49.65, R^2 = 0.965 (5)

SWASV: *I*p (µA) = 14318 C + 1.746, R^2 = 0.945 (6)

Conclusions

In summary, a simple and cost-effective method for itraconazole detection was employed as voltammetric techniques in acidic media at GCE. The electrochemical oxidation of itraconazole under the conditions described is an irreversible process controlled by adsorption. A validated differential pulse and square wave anodic stripping voltammetric path was developed and successfully applied to the quantification of itraconazole in bulk form. Stripping technique is one of the best-known analytical methods. This is justified as the results havebeen acquired from the analysis of moiety with low detection limits. This technique has the advantage of acceptable low detection limits, and suitable for the routine determination of the drug in quality control laboratories.

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