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Adsorptive stripping voltammetric behaviour of azomethine group in pyrimidine-containing drugs

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Abstract

The stripping voltammetric behaviour of buspirone hydrochloride (BUS) and piribedil (PIR), as models of pyrimidine-containing compounds, was studied using a hanging mercury drop electrode (HMDE). A sensitive adsorptive stripping voltammetric method for determination of such drugs is described. The voltammetric peaks were obtained at −1.23 and −1.22 V for BUS and PIR, respectively, which correspond to the reduction of the azomethine group of pyrimidine ring in Britton–Robinson buffer (pH 7). Factors such as pH of supporting electrolyte, accumulation potential and time and instrumental parameters were optimized. Calibration plots and regression data validation, accuracy, precision, limits of detection, limits of quantification, and other aspects of analytical merit are presented. The applicability of the method was evaluated through determination of BUS and PIR in tablet dosage forms. A preliminary study of the analysis of plasma samples, spiked with the investigated drug, after a simple extraction procedure is described.

Keywords: Azomethine group; Pyrimidine-containing drug; Britton–Robinson buffer; Adsorptive stripping voltammetry

1. Introduction

Buspirone hydrochloride (8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4,5]decane-7,9-dione hydrochloride, BUS) is an anxiolytic drug. It has dopaminergic, noradrenergic, and serotonin-modulating properties and its anxiolytic effects appear to be related to its action on serotonin neurotransmission [1].

The USP 24 specifies a HPLC method for the assay of BUS bulk drug and tablets [2]. The drug is not official in BP. Several chromatographic studies concerned with the quantification of BUS or BUS simultaneously with its metabolite, 1-(2-pyrimidinyl)piperazine in human plasma have been reported [3–10]. Spectrophotometric methods based on color reactions have been used for the assay of BUS in tablets [11,12].

Squella et al. [13] investigated the polarographic behaviour of BUS at the dropping mercury electrode and developed a differential pulse polarographic (DPP) method for determining the drug in tablets. The method is based on the reduction of the azomethine

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group of the pyrimidine ring in phosphoric-acetic buffer (pH 6). Later, Chen et al. [14] described a single sweep voltammetric procedure for trace BUS determination. The method deals with the adsorptive voltammetric behaviour at the dropping mercury electrode in presence of Triton X-100. Accordingly, BUS could be measured over the range 0.013–2.1 $\mu g\,ml^{-1}$. The method was adopted for the analysis of BUS in spiked plasma samples. However the interference from different metabolites was not discussed.

Piribedil (2-[4-(3,4-methylenedioxybenzyl)piperazino]pyrimidine or 2-(4-piperonylpiperazin-1-yl)pyrimidine, PIR), is a dopamine D_2-agonist that has been given in the treatment of Parkinsonism and in circulatory disorders [1].

Piribedil is not official in USP or in BP but a few methods for the analysis of PIR and/or its basic metabolites in biological specimens have been reported, including GC [15,16] and HPLC [17]. Spectrophotometric assays through charge-transfer and ion-pair complexation reactions have been used for the analysis of PIR in tablets [18,19]. Recently, differential pulse and square wave voltammetric determination of PIR, based on oxidative mode, has been reported [20].

Buspirone hydrochloride and PIR have a common structure moiety, 1-(2-pyrimidinyl)piperazinyl (Fig. 1), so their polarographic behaviour, based on the C=N bond electroactivity (azomethine group), would be expected to be very similar. The analytical review of PIR revealed that up to the present time, no voltammetric study concerning the reduction at the mercury electrode has been reported. Also, the adsorption behaviour of BUS at hanging mercury drop electrode (HMDE) has not been studied. So it would be of interest to investigate the properties of the adsorption process at HMDE in Britton–Robinson buffer. This work presents a study of the factors that may influence both the accumulation process and the voltammetric response and applications to various sample types.

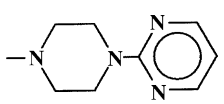

Fig. 1. 1-(2-Pyrimidinyl)piperazinyl moiety.

2. Experimental

2.1. Apparatus

Voltammograms were obtained with a Metrohm 693 VA Processor. A Metrohm 694 VA Stand was used in the HMDE mode. The three-electrode system was completed by means of a Ag/AgCl (3 M KCl) reference electrode and a Pt auxiliary electrode.

2.2. Standard/assay solutions

2.2.1. Preparation of standard buspirone hydrochloride and piribedil solutions

Stock solutions (1.0 $mg\,ml^{-1}$) of BUS and PIR were prepared in methanol and kept in a refrigerator. The solutions were stable for 1 month. From these solutions, intermediate dilution steps were made with methanol in accordance with the concentration ranges used in the analytical technique.

2.2.2. Preparation of tablets assay solutions

A total of 20 tablets (Buspar tablets labeled to contain 10 mg BUS per tablet or Trivastal tablets labeled to contain 20 mg PIR per tablet) were combined and finely powdered. A quantity of the powder, equivalent to 25 mg BUS or PIR, was accurately weighed and mixed with 10 ml of methanol then stirred for 30 min. The solution was filtered into a 25 ml volumetric flask, the residue was washed twice with 5 ml methanol, the washings were added to the filtrate and the volume was completed with methanol. Further dilutions were made to appropriate concentrations (similar to standard working solutions).

2.3. Procedure for voltammetric analysis

Supporting electrolyte (10 ml Britton–Robinson buffer (0.04 M in each of acetic, *o*-phosphoric and boric acids) adjusted to the required pH with 0.2 M sodium hydroxide solution) was placed in the voltammetric cell and an aliquot of standard/assay solution of BUS or PIR was added by micropipette to give a final concentration range of 1–30 $ng\,ml^{-1}$. The stir-

rer was switched on and the solution was purged with nitrogen gas for 5 min. The accumulation potential (E_{acc}) was then applied to a new mercury drop, while stirring the solution. Following the accumulation period (t_{acc}, 200 s for BUS and 140 s for PIR), the stirring was stopped and the system allowed to equilibrate for 10 s. The voltammogram was obtained by applying a negative going potential scan. Unless

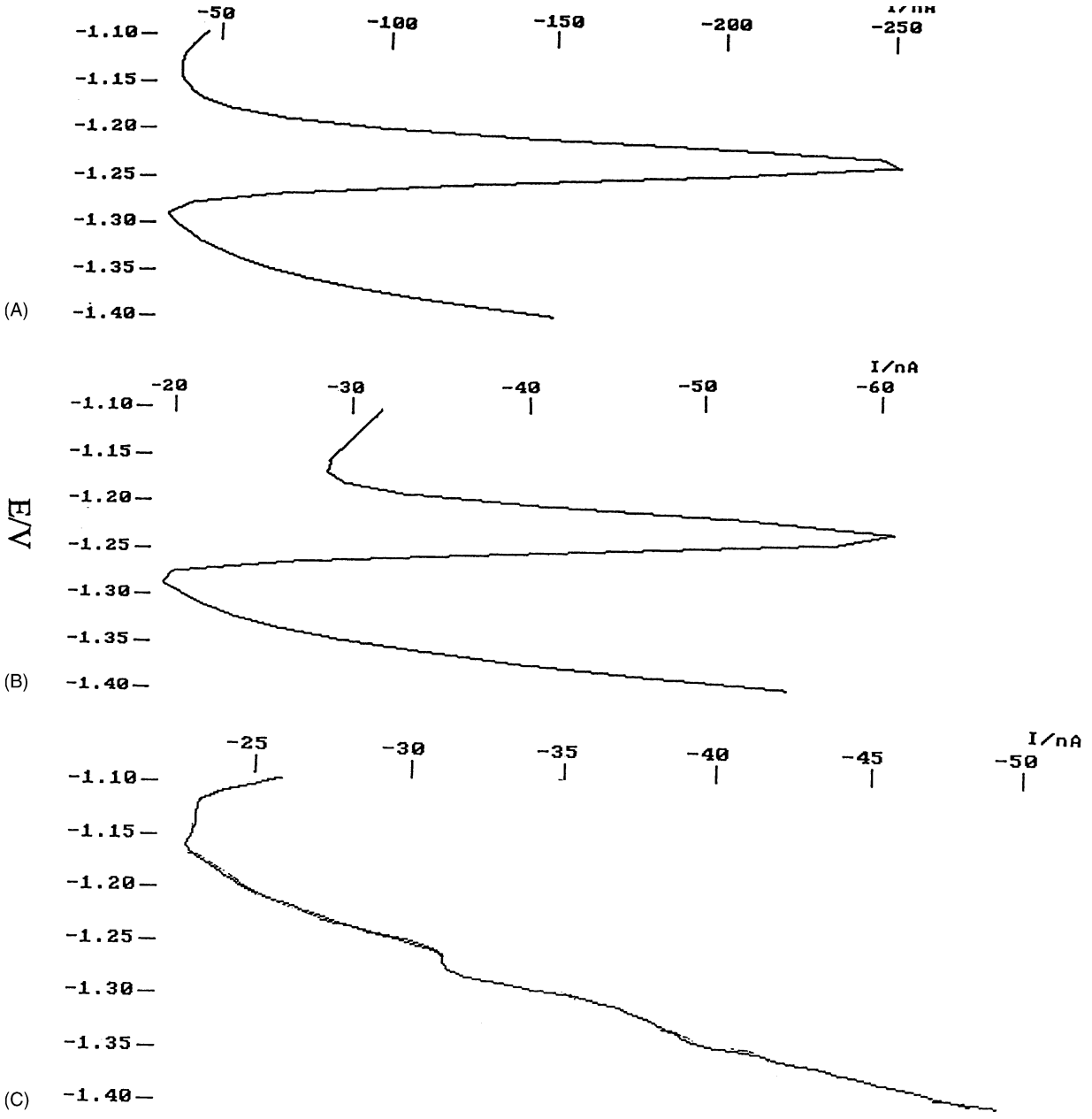

Fig. 2. Voltammograms obtained for BUS (28 ng ml^{-1}) (A) and PIR (4 ng ml^{-1}) (B) in Britton–Robinson buffer at pH 7 (scan rate = 10 mV s^{-1}, pulse amplitude = −100 mV, E_{acc} = −1.100 V, t_{acc} = 200 s (BUS) and 140 s (PIR) and (C), voltammogram of blank, Britton–Robinson buffer at pH 7 at t_{acc} = 140 s.

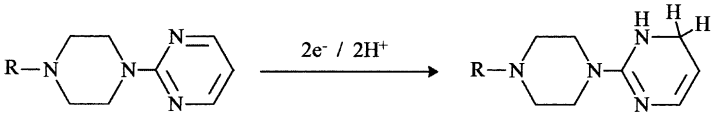

Fig. 3. Electrode reduction process of azomethine bond.

otherwise stated, the following parameters were used; E_{acc} −1.100 V for either BUS or PIR, −100 mV pulse amplitude for differential pulse stripping, scan rate 10 mV s^{-1} and a potential interval 10 mV. The maximum drop size, 9 (ca. 0.6 mm^2 drop area) and constant stirrer speed, 2000 rpm, were used.

3. Results and discussion

3.1. Electrode reaction

Bus and PIR have a common structural moiety, 1-(2-pyrimidinyl)piperazinyl, with an electroactive site, the azomethine group of the pyrimidine ring. The drugs showed similar voltammetric adsorptive stripping characteristics in Britton–Robinson buffer over the pH range 3–9. One well defined adsorptive voltammetric peak at −1.23 V was obtained for BUS (at −1.22 V for PIR) versus Ag/AgCl electrode at pH 7 (Fig. 2). Squella et al. [13] have proposed the electrode reaction to involve two electrons and two protons (Fig. 3) over the pH > 4.

3.2. Influence of pH of the supporting electrolyte

The effect of the pH on the peak current and the reduction potential was studied over the range 3–9. The voltammetric measurements were recorded for a standard solution of the investigated drug of concentration 30 ng ml^{-1} over the pH range examined. Plots of peak potential versus pH and peak current versus pH are given in Figs. 4 and 5 (curves a and b, respectively). The potentials of the adsorptive stripping peaks moved to more negative values with increasing pH, with a change of slope at pH ∼ 6. The slopes of the linear portions from pH 3 to 6 were 96 and 94 mV pH^{-1} for BUS and PIR, respectively, while the slopes from pH 6 to 9 were 64 and 60 mV pH^{-1} for BUS and PIR, respectively. The peak current (of BUS or PIR) has its maximum value at pH ∼ 7.

3.3. Factors influencing the accumulation step

The effect of the accumulation potential on the adsorptive stripping peak current was evaluated over the range from 0 to −1.200 V for BUS (Fig. 6A). Larger peaks were obtained over the range from −0.800 to −1.100 V (curve a), the peak decreased at lower and higher potentials. The plateau region of the curve becomes narrower by increasing the accumulation time (curve b). The study performed on PIR revealed the

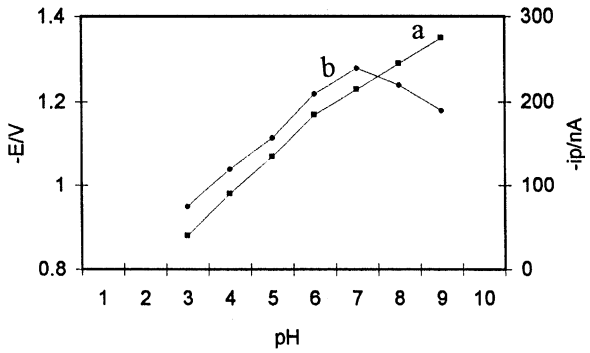

Fig. 4. Influence of pH on the DP adsorptive stripping peak potential (a), and peak current (b), of BUS (30 ng ml^{-1}) in Britton–Robinson buffer at pH 7. Instrumental parameters as in Fig. 2.

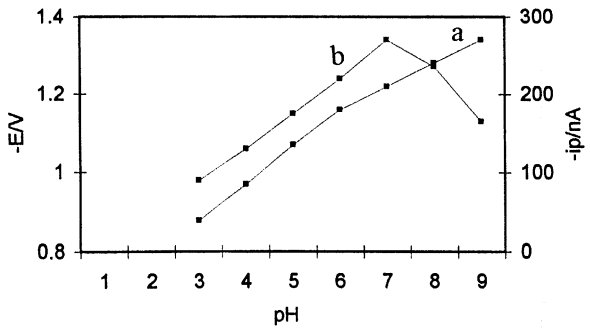

Fig. 5. Influence of pH on the DP adsorptive stripping peak potential (a) and peak current (b), of PIR (30 ng ml^{-1}) in Britton–Robinson buffer at pH 7. Instrumental parameters as in Fig. 2.

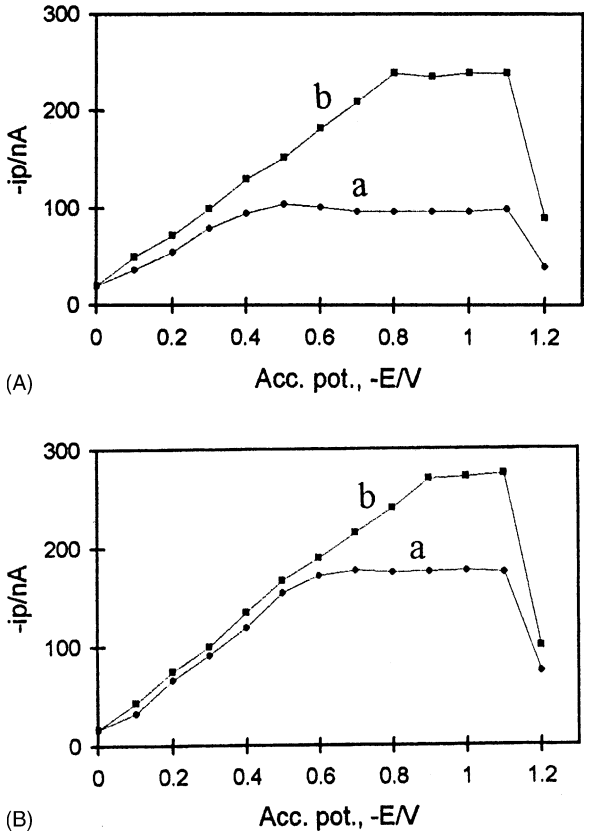

Fig. 6. (A) Effect of accumulation potential on the response to 30 ng ml^{-1} BUS, t_{acc} = 60 s (a), and 200 s (b), in Britton–Robinson buffer at pH 7. Instrumental parameters as in Fig. 2. (B) Analogous study for PIR.

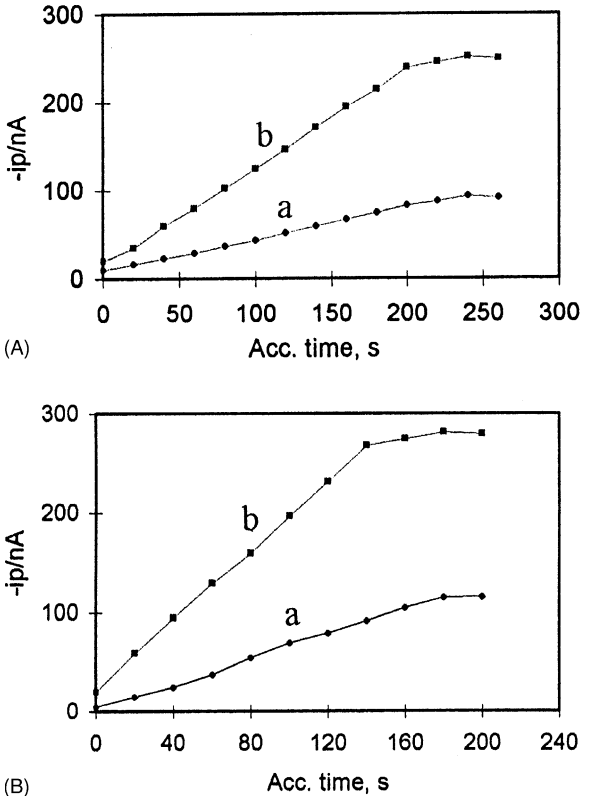

Fig. 7. (A) Effect of accumulation time on the peak current, BUS concentration 10 ng ml^{-1} (a) and 30 ng ml^{-1} (b). Instrumental parameters as in Fig. 2. (B) Analogous study for PIR.

same fashion of adsorption characteristics and showed peak current–accumulation potential plot (Fig. 6B) of similar pattern. For both drugs, an adsorption accumulation potential of −1.100 V was adopted for their analytical determination as it gave the least background (blank, Fig. 2C) current relative to the other accumulation potentials.

Fig. 7A and B display the resulting peak current versus preconcentration time plots for BUS and PIR, respectively. The rapid increase of the current observed at short preconcentration time, was followed by a levelling-off for longer periods. The plots do not pass through the origin possibly because of the adsorption of the analyte at the electrode surface at the equilibrium time which was fixed at 10 s. To maximize the sensitivity, 200 and 140 s accumulation times were used for subsequent quantitative determinations of BUS and PIR, respectively.

3.4. Instrumental parameters

Fig. 8A displays the resulting peak current versus scan rate for BUS and PIR (curves a and b, respectively). Maximum response was obtained at a scan rate 10 mV s^{-1}. Fig. 8B shows the dependence of peak current on the pulse amplitude for BUS and PIR (curves a and b, respectively). The plots are linear up to an amplitude of −100 mV.

3.5. Statistical analysis of results

3.5.1. Concentration ranges and calibration graphs

Under the optimized conditions of E_{acc} of −1.100 V, a scan rate of 10 mV s^{-1} and a pulse amplitude of

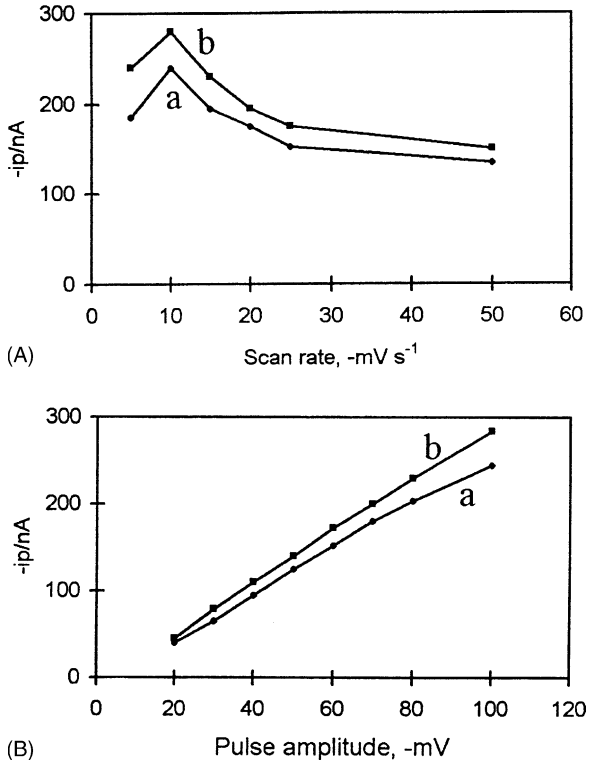

Fig. 8. Dependence of stripping peak current of BUS (30 ng ml^{-1}) (a), and PIR (30 ng ml^{-1}) (b), on the scan rate (A) and pulse amplitude (B). $E_{acc} = -1.100$ V, $t_{acc} = 200$ s (BUS) and 140 s (PIR).

−100 mV, the peak current monitored was found to be proportional to the drug (BUS or PIR) concentration, Table 1 summarizes the characteristics of the calibration plots. The values of standard error of estimate ($S_{y/x}$), standard deviation of intercept (S_a), and standard deviation of slope (S_b) are also recorded in the table.

3.5.2. Detection and quantification limits

In accordance to the official compendial methods [21] and IUPAC [22], the limit of detection, LOD = $3sb^{-1}$, where s is the standard deviation of replicate blank responses (under the same conditions as for sample analysis). Using this formula, the detection limits obtained for the developed voltammetric method are 0.20 and 0.19 ng ml^{-1} for BUS and PIR, respectively. The limits of quantification, LOQ, defined as $10sb^{-1}$, were found to be 0.67 and 0.64 ng ml^{-1} for BUS and PIR, respectively.

3.5.3. Precision and accuracy

In order to assess the precision, as percentage relative standard deviation (R.S.D.%) and the accuracy, as percentage relative error (E_r%) of the proposed method, solutions containing four different concentrations of BUS (or PIR) were prepared and analysed in five replicates. The data obtained from this investigation is summarized in Table 2.

3.6. Analysis of pharmaceutical formulations

The proposed voltammetric method was applied to the determination of BUS and PIR in their tablets (Buspar tablets labeled to contain 10 mg BUS per tablet or Trivastal tablets labeled to contain 20 mg PIR per tablet). The recoveries were calculated with reference to the calibration graphs. As can be seen from the results shown in Table 3, the method gave satisfactory recovery data for both, BUS and PIR. The statistical calculations for the assay results show good precision of the proposed method. For comparisons, BUS and PIR tablets were analysed using the reported DPP [13] and spectrophotometric [19] methods, respectively. The results of the proposed and reference methods were compared in accordance to the Student's

Table 1
Regression and statistical parameters for the DP cathodic stripping voltammetric determination of buspirone hydrochloride and piribedil

Analyte	Linearity range (ng ml^{-1})	Regression data[a]			$S_{y/x}$	S_a	S_b
		a (nA)	b (nA ml ng^{-1})	r			
Buspirone hydrochloride	1–30	0.883	7.97	0.9999	1.034	1.174	0.089
Piribedil	1–30	0.687	9.06	0.9998	0.946	0.990	0.072

a: intercept; b: slope; r: correlation coefficient; $S_{y/x}$: standard error of estimate; S_a: standard deviation of intercept; S_b: standard deviation of slope.

[a] Data number, $n = 5$.

Table 2
Precision and accuracy for the DP cathodic stripping voltammetric determination of buspirone hydrochloride and piribedil

Analyte	Nominal value (ng ml^{-1})	Found ± S.D.[a]	R.S.D. (%)[b]	E_r (%)[c]
Buspirone hydrochloride	5	5.1 ± 0.1	1.2	1.0
	15	15.2 ± 0.1	0.7	1.3
	20	19.9 ± 0.1	0.5	−0.6
	25	25.1 ± 0.1	0.5	0.4
Piribedil	5	4.9 ± 0.1	1.6	−2.0
	15	15.3 ± 0.1	0.9	2.0
	20	19.9 ± 0.2	1.0	−0.5
	25	25.2 ± 0.1	0.4	0.6

[a] Mean ± standard deviation of five determinations.
[b] Percentage relative standard deviation.
[c] Percentage relative error.

t-test and Variance ratio *F*-tests, there were no significant differences between the calculated and theoretical values at $P = 0.05$, demonstrating that the proposed method is as accurate and precise as the respective reference method.

3.7. Preliminary application to plasma samples

The major pathways of hepatic metabolism of BUS and PIR yield different hydroxy derivatives and also 1-(2-pyrimidinyl)piperazine (BUS). Obviously, the presence of azomethine bond-pyrimidine ring, the electroacive site, as a common moiety in the respective parent drug and its metabolites leads to the interference of such metabolites with the selective voltammetric measurement of the drug. It is expected that the parent drug can be carefully extracted into an organic solvent from aqueous alkaline solution (pH ∼ 13) with nil interference from hydroxylated type metabolites. However, under such a condition, the basic metabolite, 1-(2-pyrimidinyl)piperazine, will certainly interfere. A second step of re-extraction, with careful pH adjustment, to be in between the pK_a values, is suggested for separation of BUS and its metabolite, 1-(2-pyrimidinyl)piperazine.

The extraction of the drug (BUS or PIR) from aqueous solutions alkalinized with 2 M NaOH was carried out using chloroform with a recovery ∼98% (confirmed by spectrophotometric measurement) The voltammetric method was applied to the analysis of

Table 3
Assay results for the determination of buspirone hydrochloride and piribedil in tablets

Preparation	Voltammetric method		Reference method
	Declared (ng ml^{-1})	Recovery ± S.D.[a]	Recovery ± S.D.[a]
Buspar tablets[b]	5	99.8 ± 0.7	100.1 ± 0.5[c]
	25	100.1 ± 0.9	
		$t = 0.2$; $F = 2.3$[d]	
Trivastal tablets[e]	5	98.9 ± 0.8	99.4 ± 0.5[f]
	25	99.4 ± 0.7	
		$t = 0.7$; $F = 2.3$[d]	

[a] Mean ± standard deviation of five determinations.
[b] Labeled to contain 10 mg buspirone hydrochloride per tablet. It is manufactured by Bristol-Myers Squibb Co., Cairo, Egypt.
[c] Reference [13].
[d] Tabulated *t*-value for $P = 0.05$ and 13 degree of freedom is 2.16, tabulated *F*-value for $P = 0.05$ and $f_1 = 9$, $f_2 = 4$ is 6.00.
[e] Labeled to contain 20 mg piribedil per tablet. It is manufactured by Servier Egypt Ind. Ltd., 6 October City, Egypt under license of Les Laboratories Servier, France.
[f] Reference [19].

plasma samples, fortified with varying amounts of BUS or PIR (200–30 ng per 1 ml), after chloroform extraction procedure. The recoveries of BUS and PIR were calculated with reference to standard BUS and PIR of the same theoretical concentrations in Britton–Robinson buffer. The recoveries varied between 70 and 80%, i.e. a 20–30% of error in defect. Obviously, interferents which can be co-extracted, result in a decrease of the adsorption capacity for either of the two drugs. Further trials for the analysis of BUS and PIR in plasma samples using standard addition procedure were performed. Recovery ranged from 97 to 99% and R.S.D. ranged from 1 to 3 were obtained.

Accordingly, the preliminary results in spiked plasma samples suggest that this methodology may also have application in the assay of the drug in biological fluids such as plasma.

4. Conclusion

An adsorptive stripping voltammetric technique at HMDE has been described for the measurement of BUS and PIR. The results are adequately accurate and precise and demonstrated a promising sensitivity. More than 10-fold enhancement in sensitivity for voltammetric measurement of BUS was attained compared to the procedure reported by Chen et al. [14]. The method is quick and relatively cheap to operate compared with alternative HPLC [3–6,17] and GC methods [8,9,15,16] currently available. It is suitable for routine analysis in control laboratories, to be applied for the analysis of BUS and PIR in pure form and in tablets. The evaluation of the voltammetric methods towards the analysis of real plasma samples (in vivo study) and establishment of effective extraction procedure to separate different metabolites should be the matter of interest in the future study.

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