Differential pulse, square wave and adsorptive stripping voltammetric quantification of tianeptine in tablets

Azza A. Gazy [*], Hoda Mahgoub, Essam F. Khamis, Rasha M. Youssef, M.A. El-Sayed

Faculty of Pharmacy, Department of Pharmaceutical Analytical Chemistry, University of Alexandria, El-Messalah, Alexandria 21521, Egypt

Abstract

Differential pulse polarographic (DPP) and square wave polarographic (SWP) techniques were applied at hanging mercury drop electrode (HMDE) for quantitative determination of tianeptine (TIA) in tablets. The adsorptive stripping voltammetric (ASV) behavior of TIA was also studied. TIA gave a sensitive reduction peaks at −1256, −1244 and −1072 mV for DPP, SWP and ASV, respectively (versus Ag/AgCl) in Britton–Robinson buffer (B–R buffer) at pH 11. The solution conditions and instrumental parameters were optimized for the determination of TIA in tablets. Calibration plots and regression data validation, accuracy, precision, limit of detection, limit of quantitation and other aspects of analytical merit are presented.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Tianeptine; Differential pulse polarography; Square wave polarography; Adorptive stripping voltammetry; Tablets

1. Introduction

Tianeptine (7-[(3-chloro-6,11-dihydro-6-methyl-dibenzo[c, f] [1,2] thiazepin-11-yl) amino] heptamino acid S, S-dioxide) is antidepressant drug. The pharmacological properties of tianeptine (TIA) are different from those of classical antidepressants. Indeed, it stimulates 5-hydroxy tryptamine (5-HT) uptake in human platelets, increases 5-hydroxyl indoleacetic acid (5-HIAA) concentrations in cerebral tissue and plasma, and reduces serotonergic-induced behavior [1]. As its therapeutic profile appears to be neither stimulating nor sedative, TIA can be placed in a middle position in the bipolar classification of antidepressants. The official method for TIA assay according to British Pharmacopoeia [2] is a potentiometric titration method, where TIA is dissolved in anhydrous acetic acid and titrated with perchloric acid, the end point is determined potentiometrically. Spectrofluorimetric assays have been used for the analysis of TIA in tablets [3,4]. Few chromatographic studies concerned with the quantification of TIA simultaneously with its metabolites in human plasma have been reported [5–7]. The analytical reviews of TIA revealed no voltammetric studies concerning the

reduction at the mercury electrode has been reported. Also its adsorption behavior a hanging mercury drop electrode (HMDE) has not been studied. In the present work, simple and sensitive differential pulse polarographic (DPP), square wave polarographic (SWP) and adsorptive stripping voltammetric (ASV) techniques were developed for the determination of TIA in bulk and tablet forms.

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 Ag/AgCl (3 M KCl) reference electrode and a Pt auxiliary electrode.

2.2. Standard solutions

Stock solution of 100 mg% TIA was prepared in water and stored at 4 °C. Working solutions (10 mg%) were prepared daily by appropriate dilution with Britton–Robinson (B–R) buffer of pH 11 for DPP and SWP procedures. Working solutions

_____
[*] Corresponding author. Tel.: +20 3 4871317; fax: +20 3 4873273.
E-mail address: azzagazy@yahoo.com (A.A. Gazy).

Table 1

Regression and statistical parameters for the determination of TIA by the proposed methods

Parameters	Methods		
	DPP	SWP	ASV
Concentration range (μg/ml)	5–25	2–20	0.04–0.4
Slope	5.08×10^{-3}	1.53×10^{-2}	1.121
Intercept	2.27×10^{-2}	1.28×10^{-2}	-4.70×10^{-4}
Correlation coefficient	0.9999	0.9999	0.9999
S_b	2.40×10^{-5}	9.90×10^{-5}	4.49×10^{-4}
S_a	3.99×10^{-4}	1.03×10^{-3}	2.18×10^{-4}
S_b^2	5.76×10^{-10}	9.80×10^{-9}	2.02×10^{-7}
S_b (%)	0.47	0.65	0.04
LOD	1.00	0.30	0.01
LOQ	4.60	1.50	0.03

S_b is standard deviation of slope, S_a is standard deviation of intercept and S_b^2 is variance around the slope.

(0.1 mg%) were prepared daily by dilution with buffer pH 11 for ASV procedure.

2.3. General analytical procedures

2.3.1. DPP and SWP procedures

Known volumes of working standard solution of TIA were pipetted into 10-ml volumetric flasks within the concentration range stated in Table 1 and then made up to volume with B–R buffer. Then, the solution was introduced into the measuring vessel and deoxygenated with pure nitrogen for 10 min in the first cycle and 30 s for each successive cycle. The determination by DPP or by SWP was carried out with optimum instrumental parameters (Table 2), using HMDE. The potential range scanned was from -700 to -1600 mV. Measurement was carried out at $20 \pm 0.5\,^{\circ}$C and the ionic strength was that provided by the B–R buffer used.

2.3.2. ASV procedure

Different aliquots of working solution of TIA were pipetted into 10 ml volumetric flasks and then within concentration range stated in Table 1 and then made up to volume with B–R buffer. Then the solution was introduced into the measuring vessel. Oxygen free nitrogen was bubbled through the solution for 10 min to deaerate. The accumulative potential (E_{acc}) was then applied to a new mercury drop while stirring the solution. Following the accumulation period (t_{acc} 60 s) the stir-

Table 2

Optimum operational parameters selected for the determination of TIA by DPP and SWP methods

Parameters	Optimum values	
	DPP	SWP
Pulse amplitude (mV)	-100	-50
Scan increment (mV)	12	12
Interval time (s)	0.1	0.3
pH	11	11
Drop size	9	9

ring was stopped and the system was allowed to equilibrate for 10 s. The voltammogram was obtained by applying a negative going potential scan. The following parameters were used: E_{acc} -200 mV, pulse amplitude and scan increment (ΔE_s) were -100 and 10 mV, respectively. A maximum drop size, 9 (ca. 0.6 mm^2 drop area) and a constant stirrer speed of 2000 rpm were used.

2.3.3. Analysis of Stablon® tablets

2.3.3.1. Procedure I. Ten tablets of Stablon® (Servier BN 3H489, labeled to contain 12.5 mg TIA per tablet) were crushed. Adequate amounts of homogeneous powder corresponding to stock solution 100 mg% were individually weighed and transferred into a 25-ml volumetric flask, then dissolved in water by shaking for 20 min. Then the solution was filtered. The filtrate was diluted to obtain the appropriate concentrations (similar to standard working solutions). Then the procedure was completed as mentioned under Section 2.3.

2.3.3.2. Procedure II. Ten tablets of Stablon® were crushed. Adequate amounts of homogeneous powder corresponding to stock solution 100 mg% were individually weighed and transferred into a 25-ml volumetric flask, and then dissolved in water by shaking for 20 min. The solution was directly diluted to obtain the appropriate concentrations (similar to standard working solutions). Then the procedure was completed as mentioned under Section 2.3.

3. Results and discussion

3.1. Development and optimization of the methods

3.1.1. DPP method

TIA exhibited a well defined differential pulse cathodic peak at pH 11.0 (Fig. 1). The voltammetric measurement was recorded for a solution of the investigated drug in B–R buffer as a supporting electrolyte. The effect of pH on the peak current (ip) and the reduction potential (Ep) was investigated over a range between 6 and 12. It was found that a pH value of 11.0 was the optimal for TIA as it gave the highest peak current (ip) at a potential of -1256 mV (Fig. 2a). The potential of the differential pulse peak was shifted to more negative values upon increasing the pH values of the buffer (Fig. 2b).

The pulse amplitude was investigated by using pulse amplitude varying between -10 and -100 mV. It was found that the ip is greatly affected by pulse amplitude and -100 mV was chosen to give the highest peak height (Fig. 3).

The scan increment (ΔE_s) was investigated by using ΔE_s varying between 2 and 12 mV. It was found that ΔE_s of 12 mV gave maximum response for TIA (Fig. 4).

The ip reached a maximum at time interval of 0.1 s, when this variable was changed in the range of 0.1–1 s. The influence of the working electrode area on the peak current was also tested. As expected, an increase in the HMDE area yielded an increase in the peak height, so a large area (0.6 mm^2) was considered suitable.
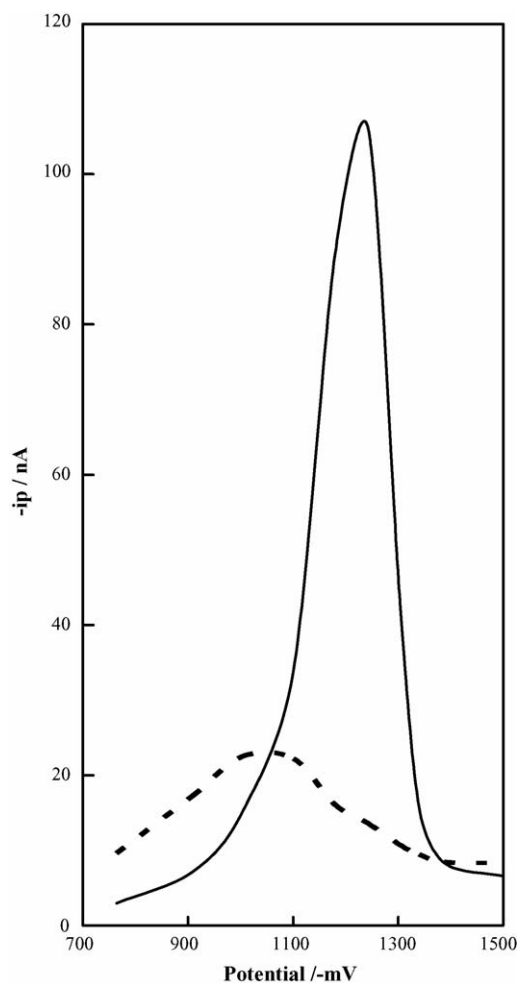
Fig. 1. Differential pulse polarograms obtained for TIA (15 µg/ml) in B–R buffer at pH 11, scan increment 10 mV and pulse amplitude −100 mV (—) and for blank, B–R buffer at pH 11 using the same parameters (- - -).

3.1.2. SWP method

Fig. 5 shows the square wave polarogram of TIA (20 µg/ml solution in B–R buffer pH 11.0). The pulse amplitude was investigated by using pulse amplitude varying between −10 and −50 mV. It was found that the (ip) is greatly affected by pulse amplitude and −100 mV was chosen to give the highest peak height (Fig. 3).

The scan increment (ΔEs) was investigated by using ΔEs varying between 2 and 12 mV. It was found that ΔEs of 12 mV gave maximum response for TIA (Fig. 4).

The differential peak current (Δi) reached was maximum at time interval of 0.3 s, when this variable was changed in the range of 0.3–1.0 s.

3.1.3. ASV method

Fig. 6 shows the adsorptive stripping voltammogram of TIA (0.08 µg/ml solution in B–R buffer pH 11 using optimal operational parameters). The effect of the E_{acc} on the adsorptive stripping peak current was evaluated over the range from 0 to −700 mV. Larger peaks were obtained over the range from −200 to −500 mV but they decreased at lower and higher potentials (Fig. 7). An E_{acc} of −200 mV was adopted for their analytical
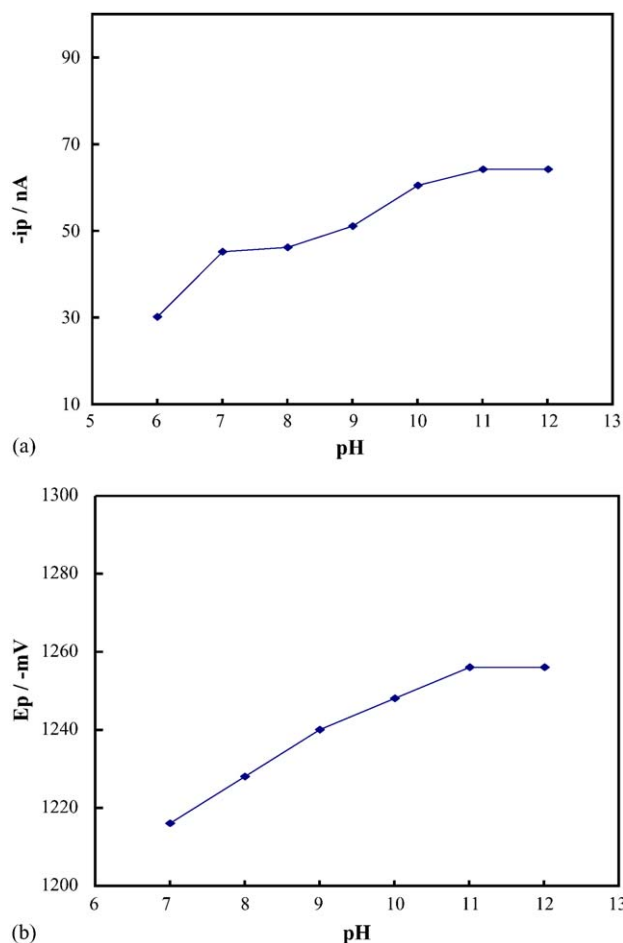

Fig. 2. Influence of pH on the (a) peak current and (b) peak potential of TIA (8 µg/ml) in B–R buffer at pH 11. Instrumental parameters as in Fig. 1.

determination as it gave the least background current relative to the other accumulation potentials. Fig. 8 displays the resulting peak current versus t_{acc} plot for TIA. The rapid increase in the current was observed at short t_{acc} and was followed by a
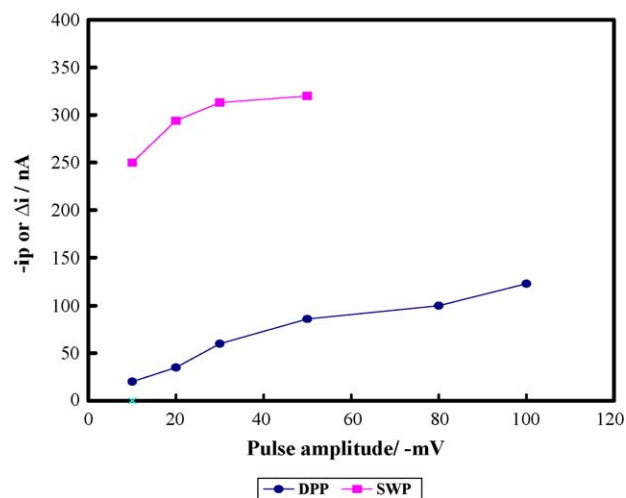

Fig. 3. Effect of pulse amplitude on the peak current of TIA (20 µg/ml) in B–R buffer at pH 11. Instrumental parameters as in Figs. 1 and 5.
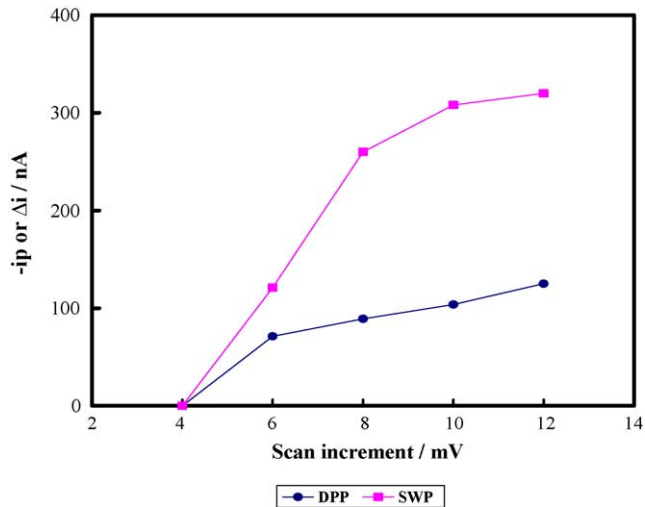
Fig. 4. Effect of scan increment on the peak current of TIA (20 µg/ml) in B–R buffer at pH 11. Instrumental parameters as in Figs. 1 and 5.

leveling off for certain periods. The plot does 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, t_{acc} of 60 s was used for
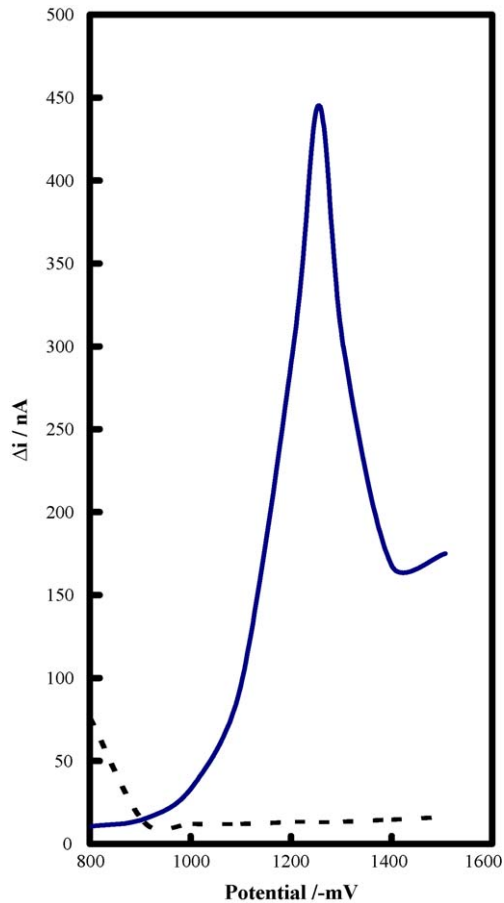

Fig. 6. Adsorptive stripping voltammograms obtained for TIA (0.08 µg/ml) in B–R buffer at pH 11, scan increment 12 mV, time interval 0.1 s, pulse amplitude −100 mV, E_{acc} −200 mV and t_{acc} 60 s (—) and for blank, B–R buffer at pH 11 using the same parameters (- - -).

subsequent quantitative determination of the drug. The peak current increased steadily when the stirring speed increased from 0 to 2000 rpm and the latter was selected as the optimum value. Accordingly, the established optimal operational parameters of

Fig. 5. Square wave polarograms obtained for TIA (20 µg/ml) in B–R buffer at pH 11, scan increment 12 mV, time interval 0.3 s and pulse amplitude −50 mV (—) and for blank, B–R buffer at pH 11 using the same parameters (- - -).
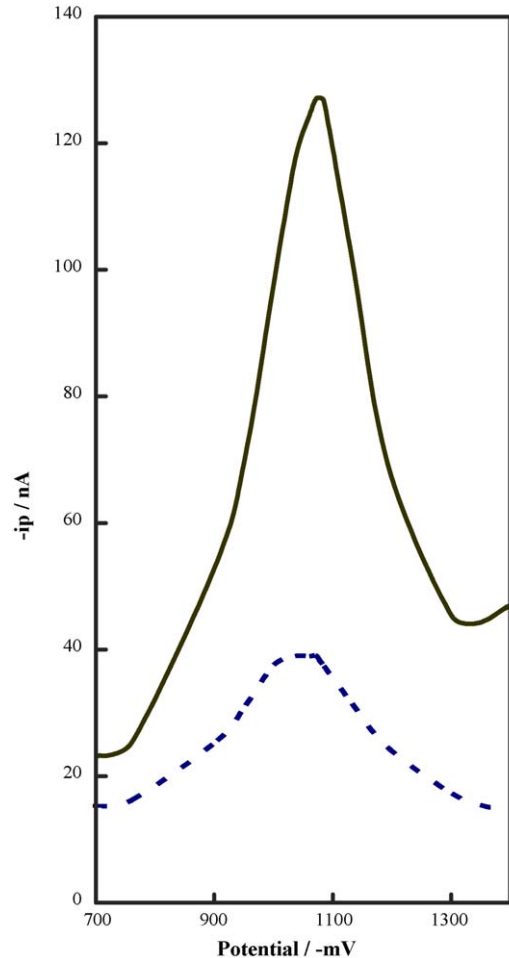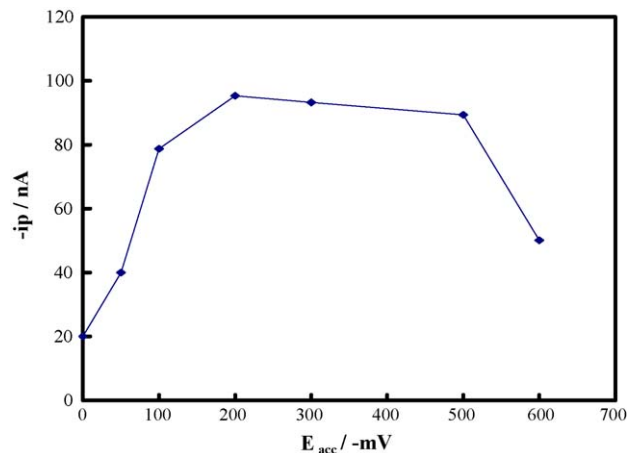

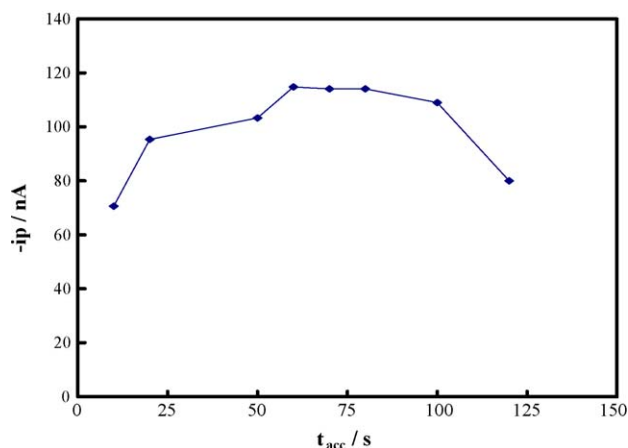Fig. 7. Effect of E_{acc} on the peak current of TIA (0.08 µg/ml) in B–R buffer at pH 11. Instrumental parameters as in Fig. 6.

Fig. 8. Effect of t_{acc} on the peak current of TIA (0.1 μg/ml) in B–R buffer at pH 11. Instrumental parameters as in Fig. 6.

the proposed ASV procedure were: $E_{acc} = -200$ mV, $t_{acc} = 60$ s, potential interval $(\Delta Es) = 12$ mV, pulse amplitude $= -100$ mV, area of the HME $= 0.6$ mm^2, rest period $= 10$ s and a B–R buffer of pH 11.0 as a supporting electrolyte.

3.2. The electrode reaction

TIA shows differential pulse polarographic, square wave polarographic and adsorptive stripping voltammetric peaks at

negative going potential pulse (-50 mV), then a positive pulse ($+50$ mV). It was found that TIA reduction process corresponds to the quasi-reversible criteria:

(1) $Ep^c - Ep^a < |\Delta E|$, where Ep^c is cathodically scanned potential, Ep^a is anodically scanned potential and ΔE is the pulse magnitude.
(2) $ip^a/ip^c < 1$, where ip^a is current measured at anodically scanned potential and ip^c is current measured at cathodically scanned potential.

These values were $Ep^c - Ep^a = 12$ ($\Delta E = 50$ mV) and $ip^a/ip^c = 0.92 (<1)$. The second method studied the effect of pH on Ep, where reduction at HMDE was found to be pH-dependent. The Ep values were shifted to more negative potentials upon increasing the pH which indicated the proton involvement in the electrode process. The relationship shown in Fig. 2b is almost linear with a slope of 10 and a correlation coefficient of 0.9944.

A linear plot of peak current (ip) versus the square root of the scan rate ($v^{1/2}$) was obtained indicating that diffusion is the means of mass transport [9]. Correlation coefficient of the linear plot was 0.9936.

The reduction of chlorobenzenes was reported to take place at pH 11.5 around -1150 mV [10]. Therefore, the reduction of TIA was suggested to be through the reduction of chloride atom at position 3 of dibenzo-thiazepine ring. Based on the above finding, the following scheme is postulated for the electrode reaction of TIA:

-1256, -1244 and -1072 mV, respectively, using HMDE versus Ag/AgCl electrode in B–R buffer (pH 11.0).

Several methods were adopted to test the reversibility of the electrode reaction. The first method used the technique reported by Birke et al. [8]. The DP polarogram was recorded with a
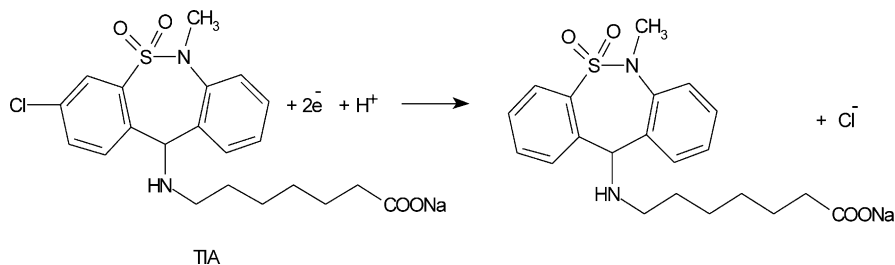
3.3. Validation of the proposed procedures

3.3.1. Concentration ranges and calibration graphs

The determination of TIA was carried out, based on the linear dependence of the peak current (μA) on concentration (μg/ml). For DPP, SWP and ASV procedures linear calibration graphs

Table 3
Statistical comparison between the determination of TIA using the proposed and reference methods in filtered and unfiltered solutions of its tablets

	Mean recoveries ± S.D.[a]						Reference method[b]
	DPP		SWP		ASV		
	Procedure II	Procedure I	Procedure II	Procedure I	Procedure II	Procedure I	
Stablon tablets[c]	99.0 ± 0.90	99.98 ± 0.64	99.1 ± 0.70	100.36 ± 1.18	98.8 ± 0.75	99.96 ± 0.66	99.9 ± 1.56
F[d]	3.0	5.9	4.9	1.7	4.3	5.6	
t[d]	1.11	0.11	1.05	0.53	0.67	0.08	

[a] Standard deviation of five experiments.
[b] Non-aqueous titration method [2].
[c] Labeled to contain 12.5 mg TIA per tablet.
[d] Theoretical values of F and t are 6.390 and 2.306, respectively, at 95% confidence limit.

Table 4
Evaluation of precision and accuracy of the proposed methods for the determination of TIA

Methods	Added[a]	Mean recovery ± R.S.D.%[b]	E_r%[c]
DPP	5	102.0 ± 2.30	2.2
	15	101.4 ± 1.40	1.4
	25	102.0 ± 2.40	2.0
Mean		101.8 ± 2.03	1.9
SWP	2	98.4 ± 1.80	−1.6
	10	99.8 ± 2.30	−0.2
	20	98.8 ± 0.85	−1.2
Mean		99.0 ± 1.65	−1.0
ASV	0.04	102.1 ± 1.20	2.1
	0.10	100.0 ± 2.70	0.0
	0.40	98.8 ± 1.22	−1.2
Mean		100.3 ± 1.70	0.3

[a] Final concentration in µg/ml.
[b] Percentage relative standard deviation of five determinations.
[c] Percentage relative error.

were obtained. Table 1 summarizes the characteristics for calibration plots, standard deviation of intercept (S_a) and slope (S_b).

3.3.2. Limits of detection and quantitation

In accordance to IUPAC [11], the limit of detection, LOD = 3S/b, where S is the standard deviation of replicate blank responses (under the same conditions as for sample analysis). Using this formula, the LOD obtained are 1.0, 0.3 and 0.01 µg/ml for DPP, SWP and ASV methods, respectively. The limits of quantitation, LOQ, defined as 10S/b, were found to be 4.6, 1.8 and 0.03 µg/ml for DPP, SWP and ASV, respectively.

3.3.3. Selectivity

The selectivity of the proposed procedures for the assay of TIA was identified by studying the effect of excipients that are often accompanying TIA in its tablets. An attractive feature of an analytical procedure is its relative freedom from interference by the excipients. The effect of the additives (lactose anhy-

drous, dicalcium phosphate, cornstarch, magnesium stearate, talc, sucrose, sugar granules, chalk and polyvinylpyrolidone) on the assay of TIA was studied by the proposed methods. It was found that these additives had no considerable effect on the accuracy of the drug (Table 3).

3.3.4. Precision and accuracy

In order to assess the precision, as relative standard deviation (R.S.D.%), and the accuracy, as percentage relative error (E_r%) of the proposed methods, solutions containing three different concentrations of TIA were prepared and analyzed in five replicates. The data obtained from this investigation is summarized in Table 4.

3.3.5. Robustness

The robustness of the proposed procedures for analysis of TIA was also examined by evaluating the influence of small variations of some of the most important procedural conditions including pH (10.5–12.0) and t_{acc} (60–80 s) on the recovery of TIA (Table 5). The position of peak potential and its response were not significantly affected. Consequently, the proposed procedures were reliable for assay of TIA and could be considered robust.

3.4. Applications

3.4.1. Analysis of tablets

The proposed techniques were applied to tablets each containing 12.5 mg TIA and the determination was performed in both the filtered and the unfiltered solutions. The determination was carried out on the same batch of tablets and was compared with reference method [2]. Statistical analysis of the results was done using the Student's t-test and variance ratio F-test (Table 3). The calculated values did not exceed the theoretical ones (95% confidence limits for five degrees of freedom), from which one can conclude that there is no significant variation between the proposed methods and the reference method. At the same time, these results confirm the superiority of the polarographic methods in both filtered and unfiltered solutions [12].

Table 5
Effect of slight changes of the pH of the buffer and t_{acc} on the position of the peak potential (Ep) and its response (ip) or (Δi) for the determination of 10 µg/ml TIA by the proposed methods

Procedure conditions	DPP method		SWP method		ASV[a] method	
	Ep (−mV)	ip (−nA)	Ep (−mV)	Δi (nA)	Ep (−mV)	ip (−nA)
pH						
10.5	1256	72	1260	275	1096	115
11.0	1256	74	1260	279	1096	112
11.5	1255	73	1260	280	1090	119
12.0	1255	73	1260	281	1089	116
t_{acc}						
60 s					1096	114
70 s					1096	110
80 s					1096	111

[a] Concentration of TIA in case of ASV method is 0.1 µg/ml.

4. Conclusion

A study of the reduction of TIA in aqueous medium (pH 11.0) has been carried out. Three electro-analytical methods based on DPP, SWP and ASV had been developed. These methods are quick and relatively cheap to operate compared with alternative HPLC methods [5–7]. They are suitable for routine analysis in quality control laboratories, to be applied for the analysis of TIA in pure form and in tablets.

Taking into account the results obtained for the calibration graphs, SWP technique is more sensitive than DPP technique despite having similar coefficients of variation. The LOD value obtained from the SWP technique is lower than that reached using DPP technique. As can be obtained from Figs. 1 and 5 the peak using SWP is four times higher than the one using DPP.

The present results show that ASV at the HMDE is a suitable technique for the determination of TIA in low concentrations even in biological fluids. The sensitivity is significantly enhanced by adsorption of the drug on the electrode surface and, after careful choice of the operating parameters, extremely low LOD can be reached. 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.

References

[1] J. Ortiz, C. Mariscot, E. Alvarez, F. Artigas, J. Affect. Disord. 29 (1993) 227–234.
[2] British Pharmacopoeia, HMSO, London, 2003, pp. 1833–1834.
[3] M. Bulaceanu-Mac-Nair, J.J. Aaron, P. Prognon, G. Mahuzier, Analyst 123 (1998) 2267–2270.
[4] E. Dikici, S.K. Deo, S. Daunert, Anal. Chem. Acta 500 (2003) 237–245.
[5] J.M. Gaulier, P. Marquet, E. Lacassie, R. Desroches, G. Lachatre, J. Chromatogr.-B Biomed. Appl. 748 (2000) 407–414.
[6] G. Nicot, G. Lachatre, C. Gonnet, J. Mallon, E. Mocaer, J. Chromatogr. Biomed. Appl. 54 (1986) 115–126.
[7] G. Nicot, G. Lachatre, C. Gonnet, J.P. Valette, L. Merle, Y. Nouaille, N. Bromet, J. Chromatogr. Biomed. Appl. 31 (1984) 279–290.
[8] R.L. Birke, M.H. Kim, M. Strassfeld, Anal. Chem. 53 (1981) 852–859.
[9] P. Monk, Fundamentals of Electroanalytical Chemistry, John Wiley and Sons, New York, 2001, pp. 162–166.
[10] L. Meites, Polarographic Techniques, 2nd ed., Wiley Interscience, New York, 1965, 683 pp.
[11] J.N. Miller, Analyst 116 (1991) 3–14.
[12] J. Volke, Bioelectrochem. Bioenerg. 10 (1983) 7–10.