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Voltammetric investigations of functional dyspepsia drug acotiamide at pencil graphite electrode: An eco-friendly and cost effective stripping detection method

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First time, the electro-oxidative behaviour and determination of acotiamide at a pencil graphite electrode have been investigated under different experimental conditions using cyclic voltammetry (CV), differential pulse anodic stripping voltammetry (DPASV), and square wave anodic stripping voltammetric (SWASV) techniques. The voltammetric responses of acotiamide have been analyzed at different scan rates, *p*H and concentrations. Oxidation of acotiamide at the surface of pencil graphite electrode gave two well defined irreversible peaks in the voltammograms in BR buffer of *p*H 7.0. The oxidation process is completely diffusion controlled. A linear response of peak current has been obtained between 15.5 to 124 µM in non-aqueous media for all the voltammetric techniques. The proposed DPASV and SWASV techniques show limit of detection at 18.58 and 13.36 µM, respectively.

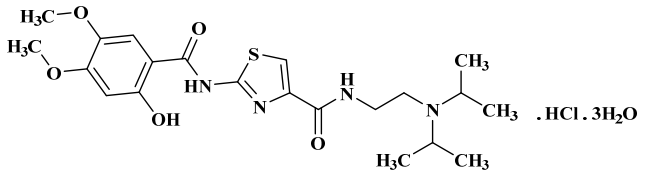
Keyword: Acotiamide, BR buffer, Functional dyspepsia, Pencil graphite electrode, Stripping voltammetry

Chemically acotiamide hydrochloride trihydrate (ACT), is N-[2-[bis (1-methylethyl) amino]ethyl]-2-[(2-hydroxy-4,5-dimethoxybenzoyl) amino]thiazole-4-carboxamide monohydrochloridetrihydrate (Scheme I). It is the first drug approved for the treatment of functional dyspepsia diagnosed by Rome III criteria[1,2]. ACT exerts gastroprokinetic activity via acting as an antagonist on the M1 and M2 muscarinic receptors in the enteric nervous system and inhibiting acetylcholine esterase activity[3-5].

Various analytical methods are already reported for the determination of acotiamide drug moiety, such as UV-Vis absorption spectroscopy[6], HPLC[7-9], Mass spectrometry[10-12], and FT-IR spectroscopy[13].

As a new studying hotspot, it is helpful to develop a low cost, highly sensitive and specific electroanalytical method for the quantification of acotiamide in pharmaceuticals and in biological fluids.Pulse and stripping voltammetric techniques have proved to be highly specific and sensitive for the detection of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids. The advances in pulse and stripping voltammetric methods are due to their eco-friendly nature, low cost, highly sensitivity, simplicity and relatively short analysis time. The use of carbon-based electrodes has increased in recent years, because of their applicability to the determination of drug molecules that undergo either oxidation or reduction. Redox behavior of drugs provides the insights into its metabolic fate and pharmaceutical activity[14-16].

Pencil graphite leads used as working electrodes in electroanalysis are referredas pencil graphite electrodes (PGEs) which are composed of graphite and clay. All the carbon atoms in the graphite are sp^2 hybridized which is responsible for high electrical conductivity. Electron transfer rate on HB pencil leads is similar to other carbon-based electrodes. In order to quantify the different pharmaceuticals in various samples pencil graphite electrodes are used as working electrode and produce well defined and sharp voltammetric



Scheme I — Chemical structure of Acotiamide.

signals. Some common advantages of PGEs over GCE are low cost, easy availability, disposability, lower deposition time, sharper peak, higher sensitivity and direct application in real samples[17,18].

In this paper, the electro-oxidative behavior of acotiamide was reported using cyclic voltammetry in Britton-Robinson buffer of *p*H 7.0 as supporting electrolyte at PGE first time. The trace amount of acotiamide drug were detected by Square Wave Anodic Stripping Voltammetry(SWASV) and Differential Pulse Anodic Striping Voltammetry (DPASV) techniques at PGE as these methods did not require any sample pre-treatment and time-consuming extraction steps.

Experimental Section

Reagents and instrumentation

Acotiamide was purchased from Koye Pharmaceuticals Private Ltd. under the trade name ACOSAT-200 and was used without any further purification. A fresh stock standard solution of acotiamide (3.0 mM concentration) in DMF was prepared every day just before the experiment to avoid any chemical decomposition in the drug. Britton Robinson, citrate, and acetate buffer solutions were prepared and optimized. All the solutions were prepared in the double distilled water (DDW) and all the reagents used were of analytical grade.

All the electrochemical measurements were employed using Model 1230A/SR 400 electrochemical analyzer with a totally automated attached computer with proper CHI 100W version 2.3 software (CHI Instrument TX, USA), for total control of the experiments, data compilation and treatment. A three electrode voltammetric cell (25 mL) was used with PGE as working electrode, the platinum wire as counter electrode and Ag/AgCl (1M KCl) as reference electrode. 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.

Preparation and electrochemical pretreatment of pencil graphite electrode

HP pencil 0.5 mm (length 65 mm) was used to prepare Pencil Graphite electrode (PGE). A 65 mm pencil lead inserted in a plastic tube containing araldite (an epoxy resin 1:1) and a copper wire connected to the one end of the lead to make electrical contact. The working surface of the PGE was polished with emery paper and followed by butter sheet. PGE electrochemically pretreated by scanning positive potential 0.2 to 1.2 V (vs Ag/AgCl electrode) with scan rate 50 mVs^{-1} for 20 cycles in 0.04 M BR buffer.

General analytical procedure

Britton Robinson buffer of *p*H 7.0 with the appropriate concentration of the acotiamide and KCl were taken into the voltammetric cell and the solution was purged with pure deoxygenated N_2 gas for 10 minutes under stirred condition for the removal of oxygen gas inside the cell, before recording of each voltammograms.

Optimization of buffer

BR, acetate and phosphate buffer were comparatively investigated for optimization with 3.0 mM acotiamide solution using cyclic voltammetric technique, at a particular selected *p*H for all buffers. Highest sharp peak observed in BR buffer (as shown in Fig. 1), so it was found suitable for voltammetric study of acotiamide.

Results and Discussion

Voltammograms of 3.0 mM acotiamide were recorded by employing cyclic voltammetry (CV), differential pulse anodic stripping voltammetry (DPASV) and square wave anodic stripping voltammetry (SWASV) techniques. Acotiamide oxidized irreversibly at PGE surface and gave two well defined anodic peaks in the potential range

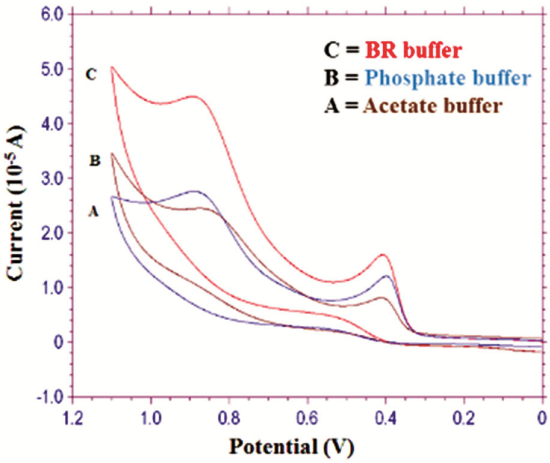


Fig. 1 — Cyclic voltammograms of acotiamide in acetate, phosphate and BR buffers.

0.0 to 1.2 V versus Ag/AgCl reference electrode in BR buffer of *p*H 7.0.

Cyclic voltammetric behaviour of acotiamide

Electro-oxidation of acotiamide gave two well defined peaks in cyclic voltammogram (as shown in Fig. 2) in BR buffer of *p*H 7.0. Two sharp peaks were observed at 0.422V and 0.896 V respectively. No any cathodic peaks were observed during the reverse scan, confirming the irreversible nature of the electrodic reaction.

Influence of scan rates

The cyclic voltammograms of acotiamide were recorded in BR of *p*H 7.0 at various scan rates 10 to 120 mV/s exhibit two well defined anodic peak (Fig. 3a). The peak potential shifted anodically towards more positive value with increasing the scan rate confirming the irreversible nature of electrodic reaction as per Nicholson theory[19]. The slopes of the plots of peak current (Ip) versus square root of scan rate ($v^{1/2}$) are 0.4044 and 0.3116 for both peaks respectively, is less than theoretical value 0.5, confirming the diffusion-controlled nature of electrodic reaction. The linear regression equations related to the plots (Fig. 3b) are

$Ip(\mu A) = 0.4044\ v^{1/2} + 0.1354$; $R^2 = 0.9981$ and
$Ip(\mu A) = 0.3116\ v^{1/2} + 0.3979$; $R^2 = 0.9907$
respectively.

Fig. 3c illustrates plots between log Ip*vs.* log ν with slopes for both peak 0.4728 and 0.4068 respectively is also close to theoretical value 0.5 for a totally diffusion-controlled process. Furthermore, the linear regression equations related to the plots are

$\log Ip = 0.4728 \log v - 0.3261$; $R^2 = 0.9986$and $\log Ip = 0.4068 \log v - 0.2742$; $R^2 = 0.9938$ respectively.

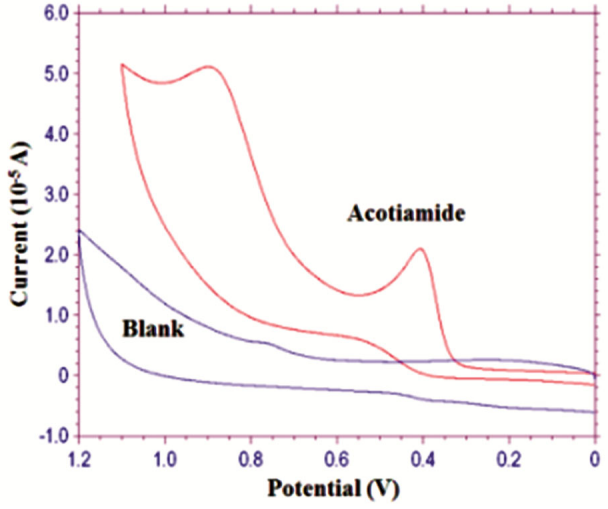


Fig. 2 — Cyclic voltammogram recorded for acotiamide (red line) and without acotiamide or blank (blue line) at PGE.

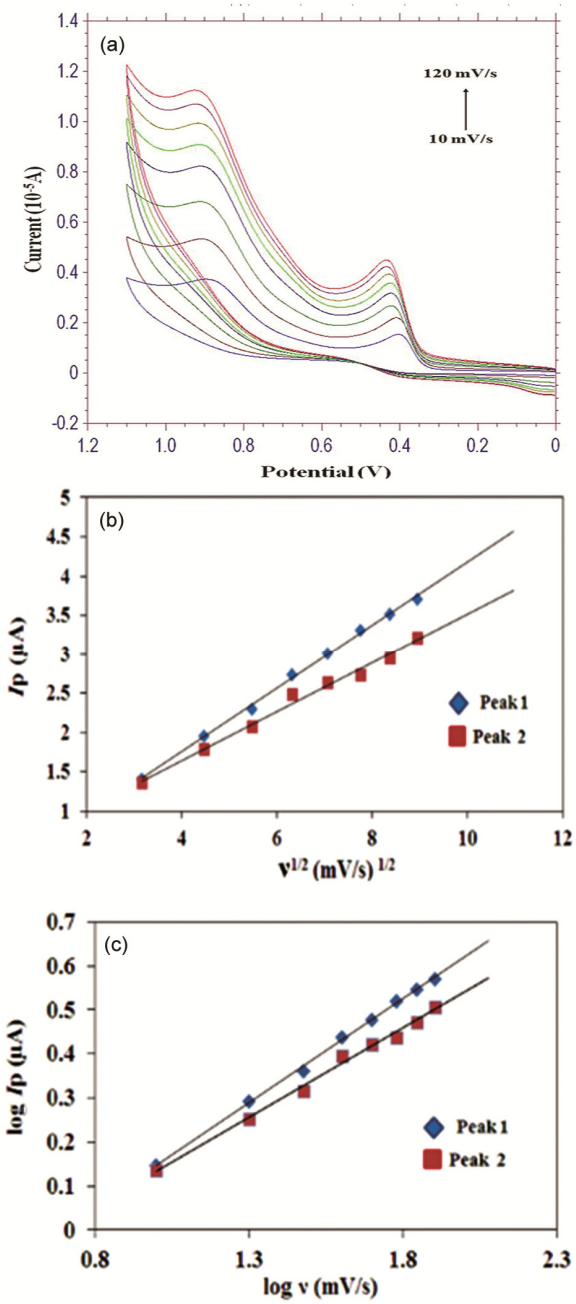


Fig. 3 — (a) Cyclic voltammograms of acotiamide recorded at different scan rates (10-120 mV/s) with 3.0 mM acotiamide in BR buffer solutionof *p*H 7.0 at PGE, (b) Plot of Ip versus $v^{1/2}$ and (c) Plot of log Ip versus log ν.

Influence of pH of buffer solution

The effect of *p*H of Britton Robinson Buffer was investigated on the electro-oxidation of acotiamide. In all *p*H range 3.0-11.0, two irreversible peaks were observed. The maximum peak current with sharp peak is observed at *p*H 7.0 with 3.0 mM acotiamide solution (Fig. 4a). Therefore, *p*H 7.0 is selected for the all voltammetric studies of acotiamide.

For both anodic peaks in cyclic voltammograms the following linear regression equations were found for the plot Ep versus *p*H, (Fig. 4b)

Ep(V) = 0.8485 - 0.0507*p*H; R^2 = 0.9847 (For peak I)

Ep(V)= 0.2957 - 0.0215*p*H; R^2 = 0.9934(For peak II)

The slope of linear regression equation is -0.0507 V/pH, which is very close to theoretical Nernstian value of -0.059 V/pH; as expected for equal number electron and proton participation in the electrodic reaction.So we can conclude that in the first step of the oxidation two electrons and two protons were involved.For the second peak the value of slope is 0.0215 V/pH, which is very far from the theoretical Nernstian value[20] of -0.059 V/pH; suggest that number of electrons and protons participated in second step of oxidation are not equal.

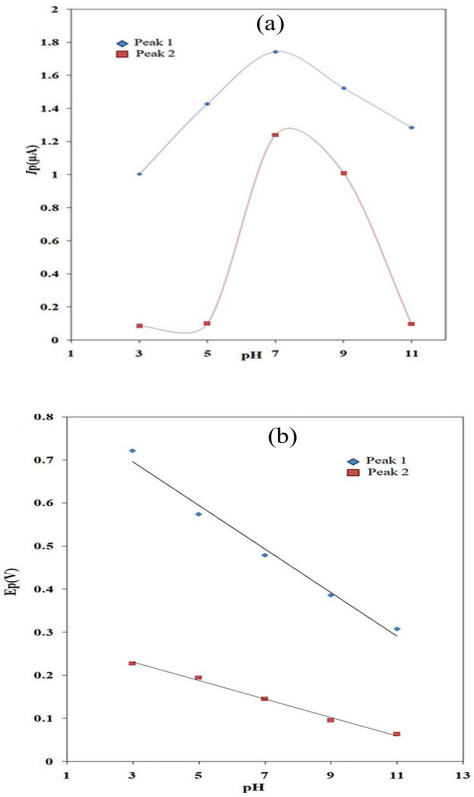


Fig. 4 — (a) Plot of Ip versus *p*H and (b) Plot of Ep versus *p*H.

Concentration effect

At various concentration of acotiamide (15.7, 31.0, 46.5, 62.0, 93.0and 124 µM) cyclic voltammograms were recorded at scan rate 100mV/s in BR buffer of *p*H 7.0. The peak height of both peaks increased with increasing concentration of drug as per Randel Secvick equation[21]. The peak current (Ip) for both the peaks increased linearly with increasing concentration (as shown in Figs 5a & 5b) and linear regression equations found for plot Ip versus concentration are,

Ip(µA) = 2.7771 C + 0.2248, R^2= 0.9961 and
Ip(µA) = 1.6003 C + 0.6677, R^2=0.9809
respectively.

Possible electro-oxidation mechanism of acotiamide

On the basis of the cyclic voltammetric data, it can be concluded that the two electrons and two protons are involved in the first step of electro-oxidation in Scheme 2 which is analogues with the former results[22] and 2-(2-hydroxy-4,5-dimethoxybenzamido)thiazole-4-carboxylic acid formed as a product in step 1.

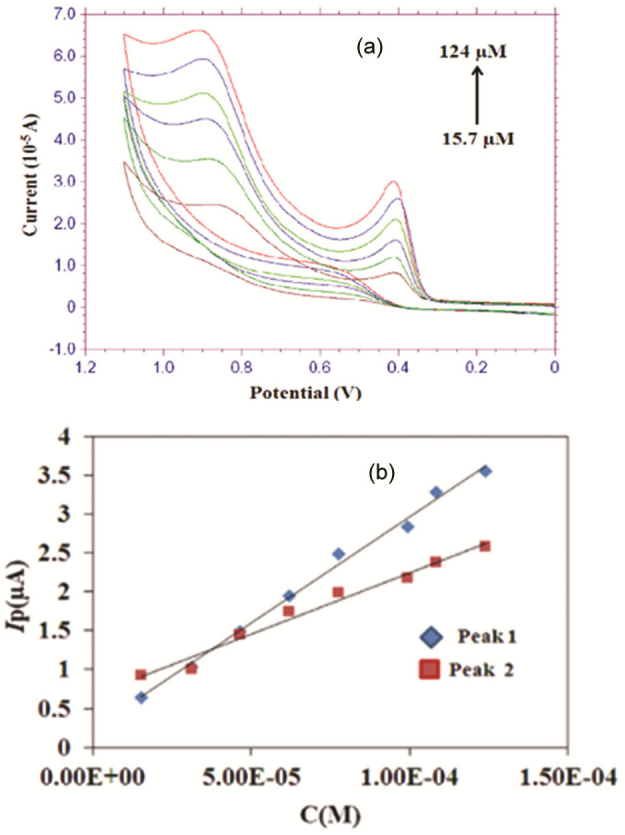
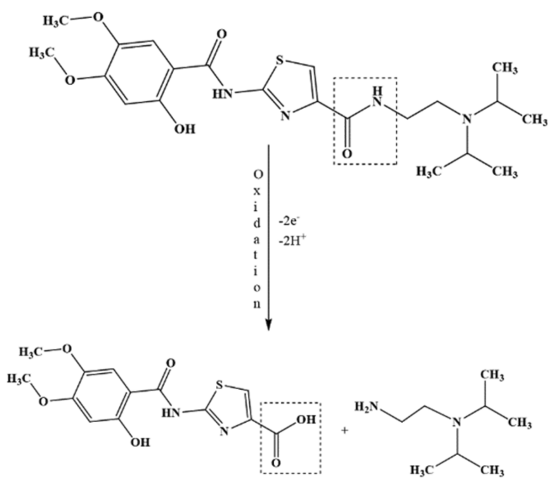


Fig. 5 — (a) Cyclic voltammograms of acotiamide with increasing concentrations from 15.7 to 124 µM in BR bufferof *p*H 7.0. (b) Plot of peak current versus concentration.



Scheme 2 — Probable mechanism of electro-oxidation for peak I.

In the second step two electrons and one proton involved in the oxidation step. In the step 2 the transformation of tertiary amine to quaternary ammonium cation within ACT moiety through de-protonation mechanism steps and electron transfer oxidation[23,24]. TheN-radical cation is produced in single-electron removal process as an intermediate species. Finally, *N*-(2-(2-(2-hydroxy-4,5-dimethoxybenzamido)thiazole-4-carboxamido) ethylidene)-*N*-isopropylpropan -2-aminium is formed (Scheme 3).

Validation of analytical process

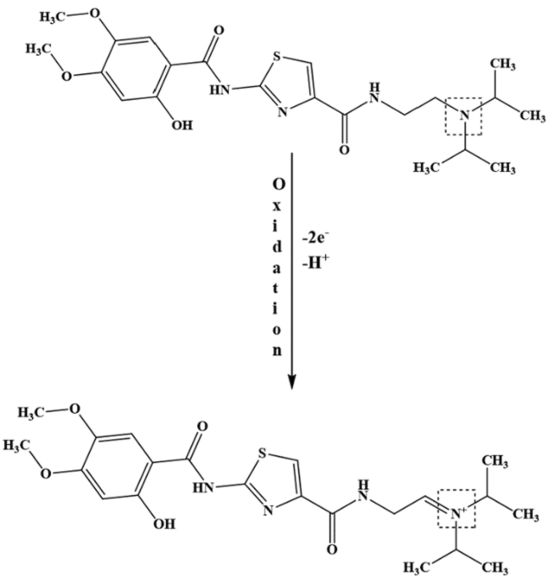
For the quantitative determination of acotiamide DPASV and SWASV methods are selected because these methods give high sensitivity with high speed of current potential relations. These methods can be applied for the quantitative determination of acotiamide in bulk form. Validation of methods was examined via determining stability, linearity range, LOD and LOQ.

Stability

The standard stock solution of acotiamide was stored in two different conditions such as at 4°C during 120 h for long term stability and during 24 h for short term stability. The solutions were analyzed by DPASV method after the end of this period, and no change were found in peak potential and peak current which confirm that acotiamide is highly stable during this period.

Linearity range and calibration Curve

Under the optimized experimental conditions, a linear correlation between DPASV and SWASV peak



Scheme 3 — Probable mechanism of electro-oxidation for peak II.

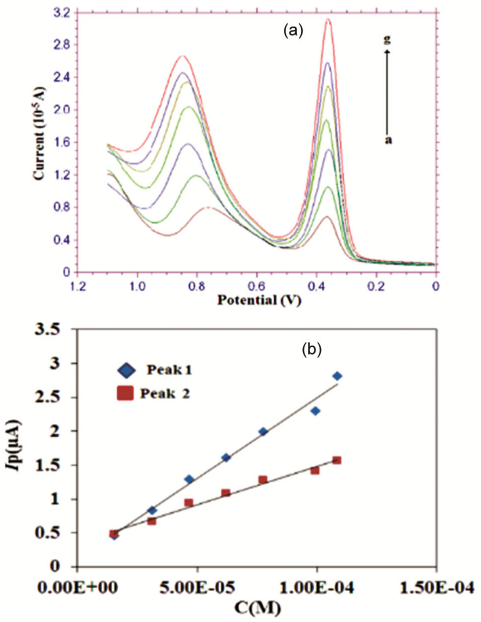


Fig. 6 — (a) DPASV of acotiamide with increasing concentrations from 15.7 to 124 μM in BR buffer of *p*H 7.0 and (b) plot of peak current verses concentration.

current and the acotiamide concentration was obtained over the range concentration between 15.5 to 124μM.

Differential Pulse Anodic stripping voltammograms (DPASVs) and Square Wave Anodic stripping voltammograms (SWASVs) were recordedand corresponding calibration equation found as [Fig. 6(b) and 7(b)].

DPASV:$Ip = 2.4697\ C + 0.0807$; $R^2 = 0.9971$ (Peak I)

$Ip = 1.1759\ C + 0.3327$; $R^2 = 0.9899$ (Peak II)

Table 1 —The regression parameters obtained from calibration curve for quantitative determination of acotiamide by DPASV and SWASV techniques in BR buffer.

Analytical parameter	DPASV		SWASV	
	Peak 1	Peak 2	Peak 1	Peak 2
Potential range (V)	0.0-1.2	0.0-1.2	0.0-1.2	0.0-1.2
Linearity range(µM)	15.5-108.5	15.5-108.5	15.5-108.5	15.5-108.5
Slope (µA/M)	2.4697	1.1759	4.965	2.5371
Correlation coefficient (R^2)	0.9971	0.9899	0.9924	0.993
RSD%	0.68	1.03	0.97	0.73
LOD (µM)	13.36	28.29	19.38	18.58
LOQ (µM)	44.56	94.32	64.61	61.93

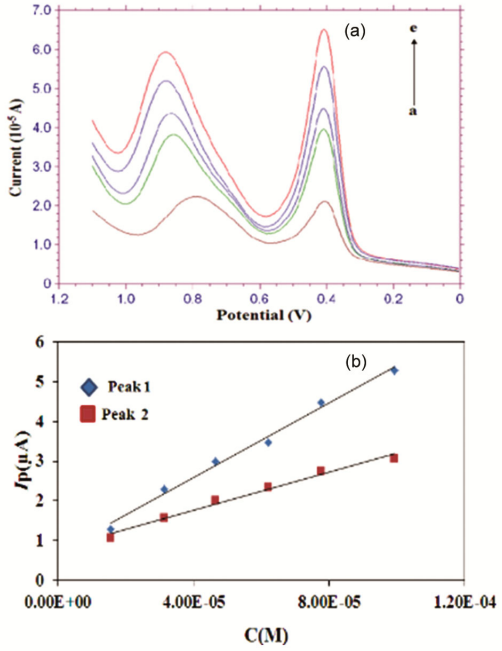


Fig. 7 — (a) SWASV of acotiamide with increasing concentrations from 15.7 to 124 µM in BR buffer of *p*H 7.0 and (b) plot of peak current verses concentration.

SWASV:*I*p = 4.965 C + 0.6063;R^2 = 0.9924 (Peak I)
*I*p = 2.5371 C + 0.7566; R^2= 0.993 (Peak II)

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the following equations:

LOD = 3s/m

AndLOQ = 10 s/m;

Where s = standard deviation and m = slope of linearity of calibration plots. The data are listed in Table 1.

Interference study

The maximum concentration of interference species that cause an error of more than 5% in determination of acotiamide has been defined as interference limit. The effect of some common interfering compounds like glucose, fructose, maltose and NaCl were tested on voltammetric response of acotiamide at pencil graphite electrode (Table 2). The data in the table showed that approx15 fold excess glucose and fructose and 10 fold excess of maltose and NaCl did not have any interference in the voltammetric determination of acotiamide via SWASV method.

Table 2 — Effect of interfering compounds in voltammetric determination of acotiamide.

Tolerance Limit (C_{in}^a/C_{ACT})	Interfering compounds
15.0	Glucose and fructose
10.5	Maltose
10.5	NaCl

C^a_{in} refers to the interferingcompoundconcentration and C_{ACT} refers to the concentration of acotiamide.

Conclusion

In summary, simple eco-friendly and cost-effective voltammetric methodswere employed for the detection of acotiamide in neutral medium at PG electrodefirsttime. The electro-oxidation of acotiamide occurs in two steps and was completely diffusion controlled. Electrodic reaction is completely irreversible in nature. A fully validated differential pulse and square wave anodic stripping voltammetric modes were developed and successfully applied to the quantification of acotiamide in bulk form.These techniqueshave the advantages of high sensitivity, selectivity, low detection limit and suitable for the routine assay of pharmaceuticals in quality control laboratories.

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