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Electrochemical Determination of Ketotifen Antihistamine by Adsorptive Stripping Voltammetric Technique

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Summary. A sensitive and reliable stripping voltammetric method was developed to determine Ketotifen antihistamine drug. This method is based on the adsorptive accumulation of the drug at a hanging mercury drop electrode and then a negative sweep was initiated, which yield a well defined cathodic peak at – 1313 mV versus Ag/AgCl reference electrode. To achieve high sensitivity, various experimental and instrumental variables were investigated such as supporting electrolyte, pH, accumulation time and potential, drug concentration, scan rate, frequency, pulse amplitude, convection rate and working electrode area. The monitored adsorptive current was directly proportional to the concentration of Ketotifen and it shows a linear response in the range from 5 x 10^{-8} to 1 x 10^{-6} mol l^{-1} (correlation coefficient = 0.999) and the detection limit (S/N=3) is 7 x 10^{-10} mol l^{-1} at an accumulation time of 1.5 min. The developed AdSV procedure shows a good reproducibility, the relative standard deviation RSD% (n=10) at a concentration level of 5 x 10^{-7} mol l^{-1} was 1.03%, whereas the method accuracy was indicated via the mean recovery of 99.9%±1.8. Possible interferences by several substances usually present in the pharmaceutical formulations have been also evaluated. The applicability of this approach was illustrated by the determination of Ketotifen in pharmaceutical preparation and biological fluids such as serum and urine.

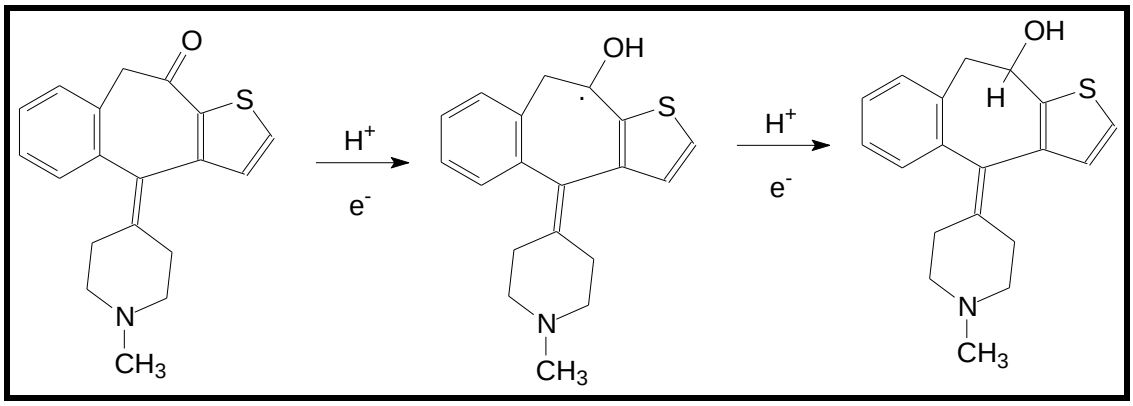
Introduction

Of the most commonly used instrumental techniques, electroanalytical approach is the one of choice, and stripping voltammetric method has provoked particular interest because it is currently the most sensitive and wide used electrochemical technique. Its possibility of applications covers many fields ranging from environment, pharmaceutical and clinical to food and industrial samples. Many of the adsorptive stripping voltammetric (AdSV) approach features such as sensitivity, selectivity, simplicity and versatility attributed to the combination of an effective preconcentartion step based on non-electrolytic adsorptive accumulation process, with an advanced measurement procedures, such as DP or SW[1-5]. Unlike conventional stripping approaches (anodic and cathodic stripping voltammetry), which based on an electrolytic nature of preconcentration step, AdSV approach in contrast is based on adsorptive accumulation of

the analyte on the electrode at open circuit with no charge transferred. Consequently, for a wide range of surface-active organic and inorganic species, which cannot be preconcentrated electrolytically, the adsorption approach allows these analytes to be interfacially accumulated on the electrode and hence analysed. There have been many reviews devoted to emphasize and illustrate the wide spectrum and scope of AdSV applications and potentialities in the analysis of metal ions[6,7] organic analytes[8] and pharmaceutical drugs and biomedical compounds[9,10].

Ketotifen is a broad-spectrum antihistamine, which considered as one of the oldest known antihistamine drugs. Its structure is shown in Scheme 1 and it has been analysed in pharmaceutical formulations and biological samples by various analytical methods such as spectrophotometry[11], HPLC[12], liquid chromatography- mass spectroscopy[13,14], atomic absorption spectroscopy- colorimetric methods[15], polarography and voltammetry[16], and coulometric titration[17].

Although molecular spectroscopic methods were widely used for the analysis of various pharmaceutical drugs, yet most of the applied procedures request a separation and/or pretreatment steps which obviously retain and delay their adequate use for routine clinical analysis. In contrast, based on the wide variety of the practical applications of AdSV in this field, this electrochemical approach was found to be suitable for analyzing pharmaceutical compounds in body fluids either with moderate sample preparation or even with direct analysis of these complex samples when applying the adsorptive-extractive accumulation procedure at carbon paste electrode. Moreover, since most of cited electrochemical studies dedicated for the analysis of Ketotifen were quit old and carried out with relatively old fashion electroanalytical approaches, the aim of present work is to investigate the adsorptive stripping behavior of Ketotifen antihistamine in order to develop a simple, rapid, sensitive and suitable stripping voltammetric procedure for the determination of this drug in biological fluids and pharmaceutical preparations with only limited sample pretreatment. For this purpose, all factors that may influence the AdSV performance were investigated to find out the most sensitive instrumental conditions



Scheme1: the structure and the mechanism of the electrochemical reduction process for ketotifen.

Experimental

Apparatus

All adsorptive stripping measurements were carried out with 797 VA computrace (Metrohm, Switzerland) in connection with Dell computer and controlled by (VA computrace 2.0) control software. Stripping voltammograms were obtained via a hp deskjet 5150 printer. A conventional three electrode system was used in the hanging mercury drop electrode (HMDE) mode. pH values were measured with Metrohm 632 pH meter. Biohit adjustable micropipette (AU), and Brand adjustable micropipette (Germany), were used to measure microliter volumes of the standard solutions.

Reagents

All chemicals used were of analytical reagent grade and were used without further purification. Ketotifen (made in UK, united pharmaceuticals) stock solution of 1×10^{-2} mol l^{-1} was prepared by dissolving the appropriate amount of Ketotifen Hydrogen Fumarate in Methanol in 10 ml volumetric flask. This stock solution was stored in the dark and under refrigeration in order to minimize decomposition. Standard solutions of this antihistamine with lower concentration were prepared daily by diluting the stock solutions with methanol. Britton-Robinson supporting buffer (pH ≈ 2, 0.04M in each constituent) was prepared by dissolving 2.47 g of boric acid (made in UK, winlab) in 500 ml distilled water containing 2.3 ml of glacial acetic acid (made in UK, BDH) and

then adding 2.7 ml of ortho-Phosphoric acid (made in Germany, Riedal-deHaen) and diluting to 1 L with distilled water. In addition, phosphate supporting buffer [0.1 M NaH_2PO_4(made in UK, winlab) and 0.1 M H_3PO_4] was prepared by dissolving 12 g of NaH_2PO_4 and 6.78 g of H_3PO_4 in 1000 ml distilled water. Acetate supporting buffer (0.02 M in each constituent) was prepared by dissolving 1.68 g of sodium acetate(made in UK, winlab) in 500 ml distilled water containing 1.12 ml of acetic acid and diluting to 1 L with distilled water. Finally, carbonate supporting buffer (0.1 M in each constituent) was prepared by dissolving 10.6 g of sodium carbonate(made in UK, BDH) and 8.4 g of sodium hydrogen carbonate(made in UK, winlab) in 1L distilled water.

Procedure

The general procedure adopted for obtaining adsorptive stripping voltammograms was as follows: A 10 ml aliquot of B-R supporting buffer (unless otherwise stated) at desired pH was pipetted in a clean and dry voltammetric cell and the required standard solutions of Ketotifen were added. The test solutions were purged with nitrogen for 5 minutes initially, while the solution was stirred. The accumulation potential of –0.6 V vs. Ag/AgCl was applied to a new mercury drop while the solution was stirred for 90 seconds. Following the preconcentration period, the stripping was stopped and after 20 seconds had elapsed, cathodic scans were carried out over the range 0.0 to –1.6 V. All measurements were made at room temperature.

Results and discussion

Preliminary Observations

When the differential pulse polarographic behavior was investigated for Ketotifen in Britton-Robinson buffer at pH 11, a broad polarographic wave at E_p= -1.313 V was observed. Based on the previous electrochemical studies carried out on Ketotifen, this obtained polarographic wave is probably due to the electrochemical reduction of Carbonyl group (see scheme 1), to alcohol group[18-21]. A proposed mechanism for the electrochemical reduction of this electroactive group is given in Scheme 1. This

mechanism suggesting that the electrochemical reaction is an irreversible process, an assumption which was confirmed by cyclic voltammetric measurement at a scan rate of 50 $mVmin^{-1}$ of Ketotifen in B-R buffer (pH 11). As can be seen from Fig. 1, no anodic peak was observed on the measured cyclic voltammogram, indicating the irreversibility nature of the cathodic reduction process.

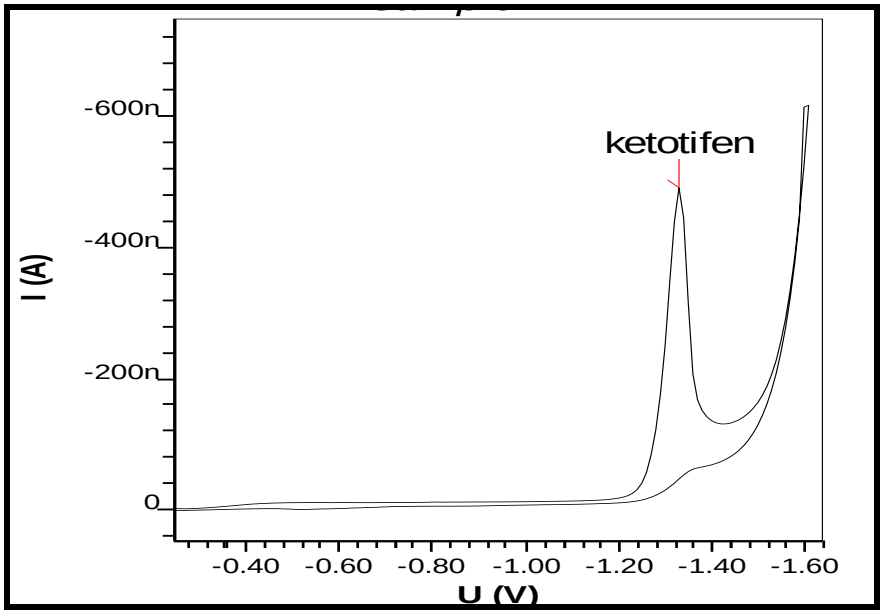


Fig. 1: Cyclic voltammogram of 1 $x10^{-4}$ mol l^{-1} Ketotifen in pH 11 B-R buffer, scan rate 50 mV s^{-1}.

In order to obtain a voltammetric peak with better definition and higher sensitivity, a HMDE was used to study the adsorptive prosperities of Ketotifen. The AdSV behavior of Ketotifen was investigated in various supporting electrolytes at different pH values. This drug yielded a well-developed and defined AdSV peak corresponding to the carbonyl electroactive group at peak potential of –1.3V. A typical adsorptive stripping voltammogram for 5x 10^{-7} mol l^{-1} Ketotifen in B-R buffer is shown in Fig. 2, which illustrates a well observed electrochemical peak indicating a strong and readily adsorption process at the surface of the working electrode.

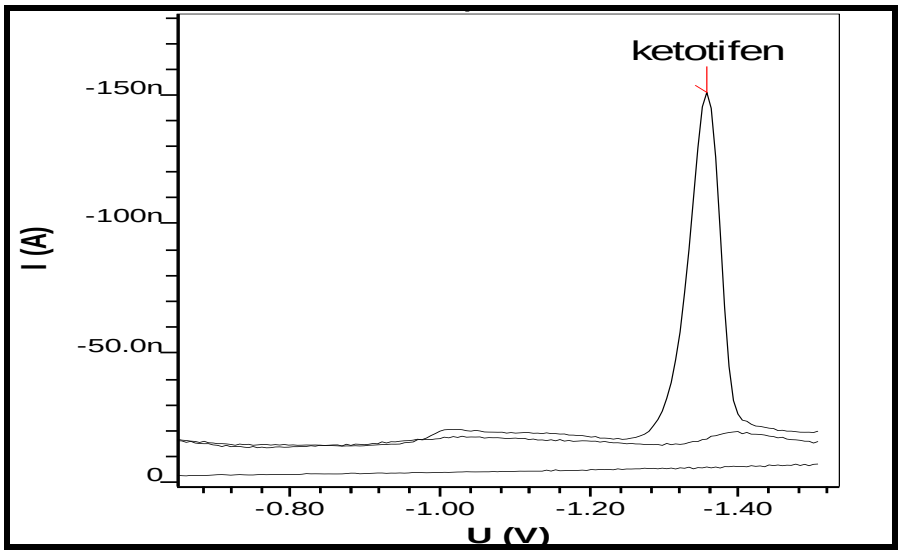


Fig. 2: DP AdSV voltammogram of 5 x10^{-7} mol l^{-1} Ketotifen in pH 11 B-R buffer. Accumulation time 90 sec, accumulation potential –0.6 V and scan rate 1000 mV s^{-1}.

Parametrs Affecting the Adsorptive Stripping Response

Effect of supporting electrolyte and pH

The nature and acidity of the supporting buffer are some of the most important factors which strongly influence the stability of the analyte of and its cathodic reduction and adsorption processes. Among the various investigated buffers (B-R, acetate, carbonate and phosphate) the best voltammetric signal in terms of sensitivity (peak height) and resolution (peak shape) have been secured using B-R buffer. In addition, when the AdSV peak current was measured as a function of pH over 7-12 range, the stripping voltammetric signal increased steadily over the alkaline region and the peak current reached it maximum value at pH 11 which was selected as optimal value for subsequent studies. It is noteworthy that when acidic B-R supporting electrolyte was used, Ketotifen drug was barely detectable and nearly no stripping voltammetric signal was observed. The variation of AdSV peak current with pH, obtained for 5 ×10^{-7} mol l^{-1} ketotifen concentration, accumulated for 1.5 min, is exhibited in Fig. 3. However, the peak potential was shifted gradually to more negative values from – 1220 mV to -1398 mV, when pH increased over the range 7-12, which indicates that E_p was pH dependent

as expected for an electrochemical reduction process consuming proton ions (see the provided mechanism).

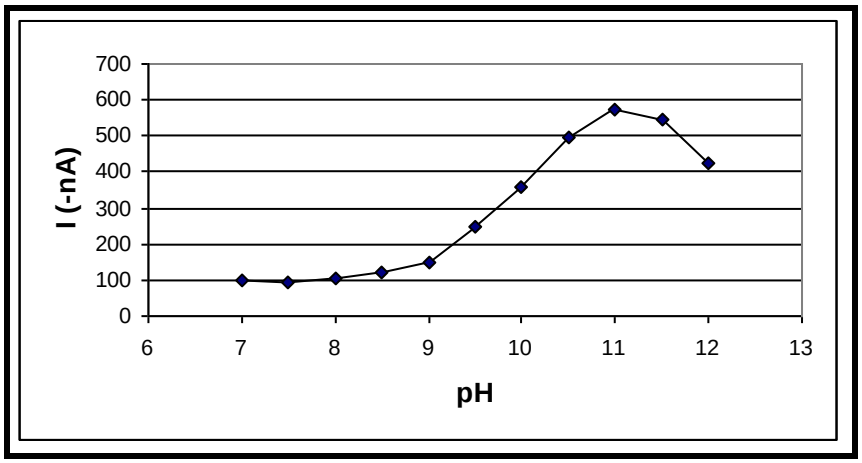


Fig. 3 Fig 3:.Effect of pH on AdSV peak current.

Effect of accumulation time and potential

Preconcentration of the analyzed drug on the surface of HMDE is one of the essential conditions for highly sensitive determinations. Variation of the accumulation time over 0-120 sec period for 5×10^{-7} mol l^{-1} Ketotifen solution at a preconcentration potential of 0.0 V, showed a gradual enhancement for the monitored peak current. The dependence of peak current on accumulation time is presented in

Fig.4. The proportional relationship was nearly observed up to 90 sec and then it becomes virtually curved and leveled off owing to the saturation of the hanging mercury drop by the analyte. For further experiments an accumulation time of 90 sec was selected as optimal because it provided relatively high peak current with adequate practical time. The variation of accumulation time did not produce significant shifts in peak potential value.

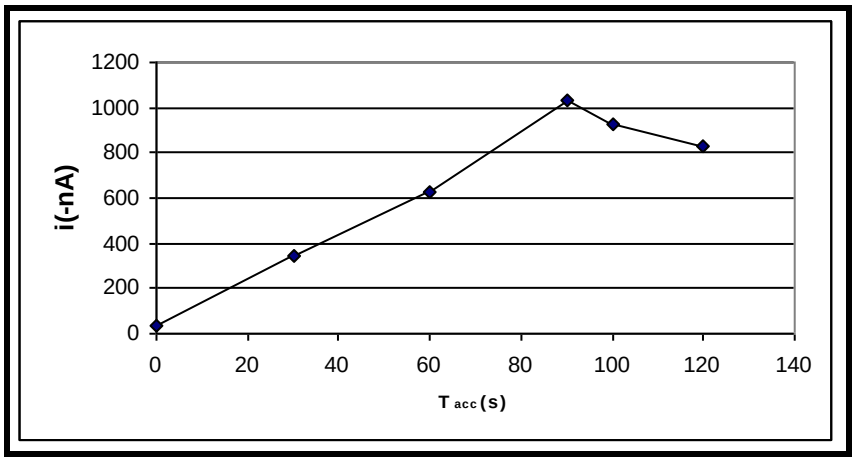


Fig. 4 Effect of time accumulation on AdSV peak current.

In addition, as can be seen from Fig. 5, when the influence of accumulation potential on the monitored electrochemical response was examined over the –0.8 to +0.2 V range at 90 sec preconcentration time, the peak current increased steadily over the positive direction till it reached it maximum value at E_p = -0.6 V where it constant and decreased sharply after potential -0.6V. Thus, E_{acc}= -0.6 V will be adopted as optimum operational value for the following works as it ensured the highest AdSV signal.

Effect of scan rate

The cathodic peak current of Ketotifen was found to be directly proportional to the scan rate, particularly at low scan rate values, a phenomenon characterized for adsorbed materials[22]. When the AdSV peak current of 5×10^{-7} mol l^{-1} Ketotifen in pH 11 B-R buffer was measured over the range 100-1100 mV/s, it was found that peak height was linearly dependent on the scan rate up to1000 mV/s. However, after this maximum value the peak current start to decrease slightly with faster scan rates. Accordingly, 1000 mV/s scan rate value was adopted as optimum condition for further investigations.

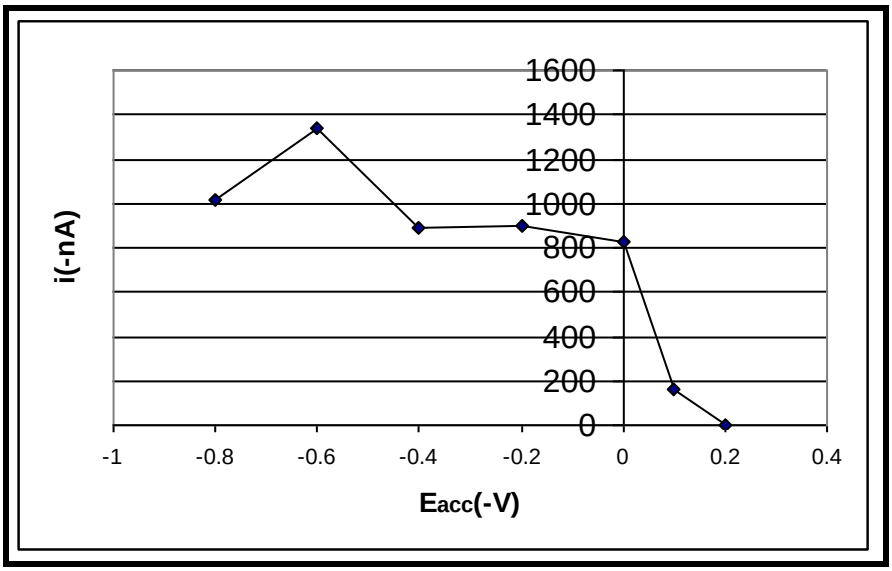


Fig. 5 Effect of accumulation potential on AdSV peak current.

Effect of pulse amplitude and frequency

In addition, the impact of varying the excitation wave pulse amplitude on the voltammetric current intensity was also evaluated. The effect of this operating variable was studied over the rang 10-120 mV (see Fig. 6) and it was concluded that in order to assure maximum peak current, 60 mV pulse amplitude is the ideal choice for this operational parameter. Moreover, varying the value of square wave frequency also plays an important role for the measured signal of SW-AdSV approach. When the AdSV peak current of 5×10^{-7} mol l^{-1} Ketotifen in pH 11 B-R buffer was measured over the range 10-120 Hz (see Fig. 7), it was found that peak height was quasi-linearly dependent on the frequency over the range 10-80 Hz. However, after this maximum value (80 Hz) the peak current start to level off with increasing frequency. Accordingly, for future work 80 Hz SW frequency value was adopted.

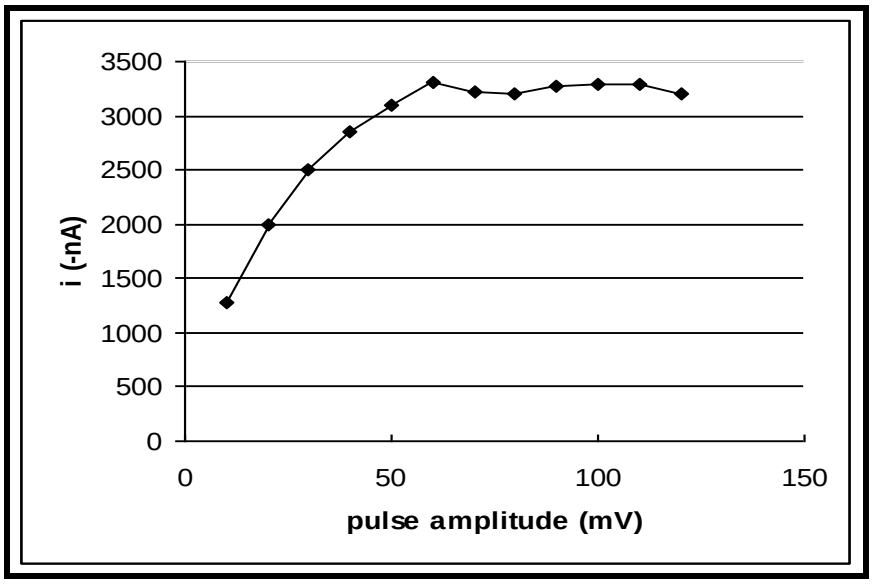


Fig. 6 Effect of pulse amplitude on AdSV peak current.

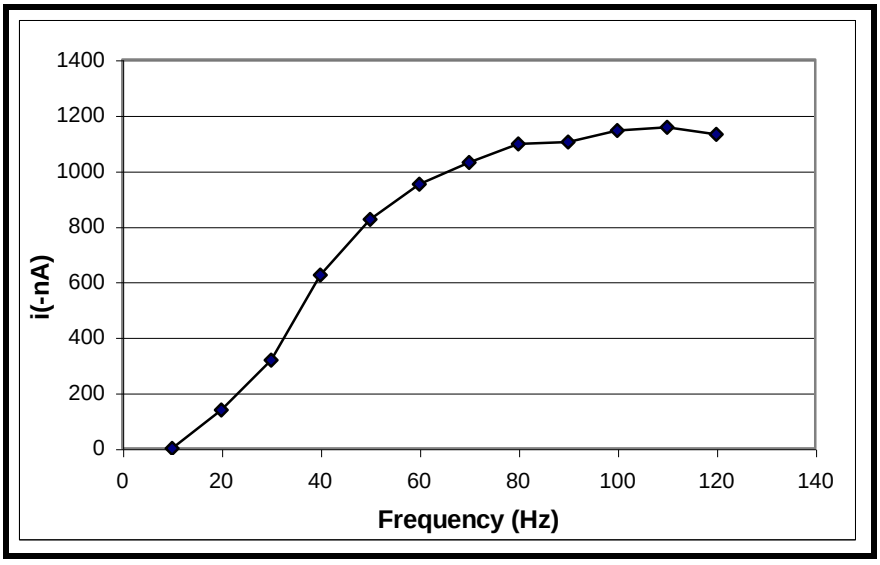


Fig. 7: Effect of Frequency on AdSV peak current.

Effect of instrumental parameters

The monitored AdSV peak height can be further maximized by optimizing other experimental factors that can affect the adsorption process of the analyzed drug. The influence of both the surface size of the mercury drop working electrode and electrode convection rate was also evaluated. An increase in the surface of the working electrode (over 0.15-0.60 mm^2) yielded, as expected, a linear enhancement in the analytical signal

and did not affect the value of the stripping voltammetric potential. In addition,An increase in the stirring rate (raising it from 0.0 to 3000 rpm) yielded, a linear enhancement in the analytical signal from 0.0 to 1200 rpm, after that it is constant and did not affect the value of the stripping voltammetric potential. Thus, for optimal sensitivity, 0.60 mm^2 drop size and 1200 rpm stirring speed were chosen for subsequent practical works.

Analytical Performance (Method Validation)

Once the most ideal and suitable chemical conditions and instrumental parameters for the adsorptive determination were established, a calibration plot for the analyzed drug was recorded to estimate the analytical characteristics of the developed method.

Calibration graph

Under the optimum conditions a very good linear correlation was obtained between the monitored voltammetric peak current and Ketotifen concentration in the range 5×10^{-8}-1×0^{-6} M (see fig. 8). Least-square treatment of the calibration graph yielded the following regression equation:

$$i_p\ (A) = -14.51 + 4.4 \times 10^9\ C\ (mol\ l^{-1}) \qquad r = 0.999, \qquad n = 7.$$

where i_p is the adsorptive stripping peak current, C is the analysed drug concentration and r is the correlation coefficient.

Detection limit

The lowest detectable concentration of this drug was 7×10^{-10} mol l^{-1} (0.22 ppb) , which was estimated based on the signal-to-noise ratio (S/N=3). Such remarkable enhancement for the sensitivity, clearly demonstrates the superiority of this electroanalysis approach over Chemiluminescence (CL) method which only succeeded to achieve 3×10^{-9} M [14] concentration level or voltammetric methods which only succeeded to achieve 2.5×10^{-9} M [16] concentration level.

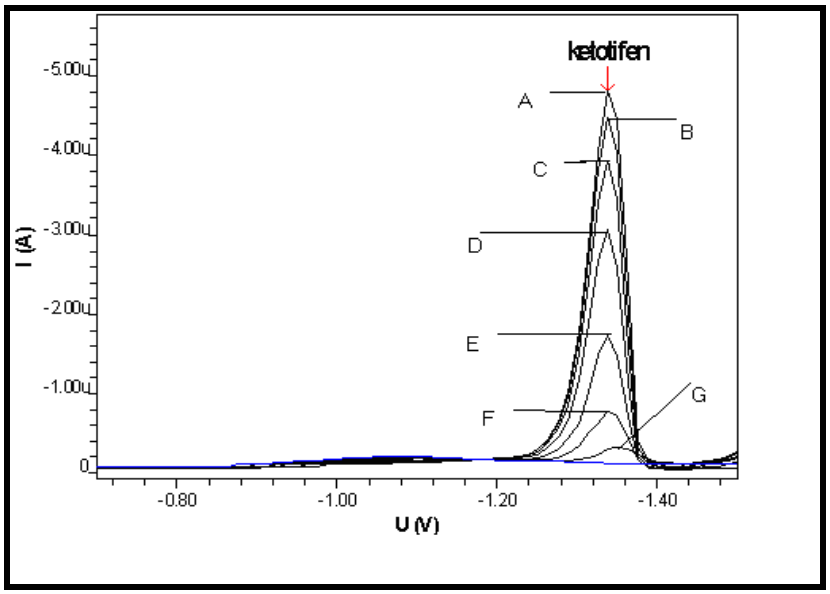


Fig. 8: AdSV voltammogram for Ketotifen in B-R buffer (pH 11), pH=11, T_{acc} =90 sec, E_{acc}= -0.60 V.Drug Conc: (A= $1x10^{-6}$ M, B=$8x10^{-7}$ M, C=$6x10^{-7}$ M, D=$4x10^{-7}$ M, E=$2x10^{-7}$ M, F=$1x10^{-7}$ M, G=$5x10^{-8}$ M).

Reproducibility

The high sensitivity of adsorptive voltammetry is accompanied by very good reproducibility. This analytical performance was evaluated from 10 repeated measurements of electrochemical signal of 5 $\times10^{-7}$ mol l^{-1} Ketotifen solution. The precision of the electrochemical developed method in terms of the relative standard deviation (RSD %) was 1.03%, which was once again preferable than its spectrophotometric counterpart analytical method which yielded 2.0% RSD [11].

Accuracy

The accuracy of the proposed method was checked by calculating the recovery of known amount of Ketotifen (3 $x10^{-7}$ mol l^{-1}) added to B-R buffer solution and analysed via the optimized stripping voltammetric procedure. The value of the recovery obtained by the standard addition method was 99.9%±1.8.

Stability

Under the optimum conditions, the stability of 5 $\times10^{-7}$ mol l^{-1} Ketotifen solution was evaluated by monitoring the changes in the height of AdSV peak over a period of 80 min. The electroanalytical signal was gradually constant and decreased with time. The

basic media (pH 11) of the B-R electrolyte solution probably initiated a slow degradation process for the antihistamine drug.

Interference Studies

In order to evaluate the selectivity of the developed AdSV procedure, the influence of various interferences was examined. Considerable interference can be caused by co-existing surface-active compounds capable of competing with the analyte of interest for the adsorption site on the electrode surface, resulting in decreased peak height. The competitive co-adsorption interference was evaluated in the presence of various substance usually occur in the pharmaceutical tablets and formulations. For these investigations, the interfering species were added at different concentrations (one, 5-fold and 50-fold) higher than the concentration of ketotifen (5×10^{-7} mol l^{-1}). The addition of starch at these concentration levels caused the AdSV peak current to decrease by about 27.5%, 72% and 93%, respectively, of its original peak current. Apparently, this inhibition effect was caused by the working electrode surface blockage due to adsorption of interferences. Also the addition of 50-fold of Cellulose only in the test solution containing 5×10^{-7} mol l^{-1} Ketotifen, caused the AdSV peak current to decrease by about 13%. In contrast, the additions of Lactose, Sucrose, and magnesium setearate at different concentrations (one, 5-fold and 50-fold) in the test solution containing 5×10^{-7} mol l^{-1} Ketotifen, caused no significant effects on the SW-AdSV response of ketotifen.

Practical Applications

The reliability of the proposed AdSV method for the determination of Ketotifen was investigated by assaying this drug in some real samples. Following the developed electroanalytical procedure described above, Ketotifen was analysed in pharmaceutical formulation. The ketotifen content of commercially available tablets (profiler - 1mg ketotifen) was determination directly by the SW-AdSV method after the required dissolving and filtration steps. Five aliquots of the dissolved sample were diluted to the required concentration level and measured via the standard additions approach. For these studies, results obtained gave a recovery mean 99.4% with standard deviation of ±0.55%.

As can be seen from Table 1, these results achieved by the optimized AdSV procedure

were in good agreement with those obtained by HPLC technique for the analysis of the same pharmaceutical tablets (profiler-1mg) manufactured by united pharmaceuticals, Jordon. Based on the statistical evaluation (F-test approach) for these results, there is no significant difference between the results obtained by the developed AdSV procedure and that obtained by the reference method. When comparing the variances of the developed AdSV procedure and the chromatographic reference method (HPLC), the calculated F value is 5.6. Whereas the calculated F-test value (5.6) was less than the critical value (6.94) at the 95% confidence level. There is no statistical evidence that the variance of the proposed method differ significantly from the variance of the reference method.

Table 1: Analysis of Ketotifen in its commercial tablets

	AdSV Method		**Reference Method (HPLC)**	
	Found (mg)	**% Recovery**	**Found (mg)**	**% Recovery**
Labeled Content Ketotifen Tablets 1 mg	0.99	99	1.0	100
	0.99	99	1.01	101
	0.99	99	0.99	99
	1.0	100	1.02	102
	1.0	100		
	Mean	99.4	**Mean**	100.5
	Standard Deviation	±0.55	**Standard Deviation**	±1.3

In addition, the applicability of the AdSV procedure for the analysis of Ketotifen in biological samples was also evaluated by estimating its recovery from spiked human urine and serum samples. A simple and fast pretreatment (clean-up) procedure, which is in fact a slight modification of the sample preparation method develop for the determination of some antagonist drugs [23] was used. By adding a small amount of 5% $ZnSO_4.7H_2O$ solution, NaOH and methanol to the urine or serum samples and centrifuging the mixture, most of the interfering substances (mainly proteins) were simply removed and eliminated by precipitation. As can be extracted from Table 2, this AdSV method (after appropriate dilution) allowed the determination of Ketotifen in

spiked urine and serum samples with mean recoveries 98.4%±0.55 and 97.8%±0.45, respectively.

Table 2 : Analytical result for Ketotifen recovery from biological fluids.

	Spiked Urine	**Spiked serum**
	% Drug Recovery	**% Drug Recovery**
Added Ketotifen 3.0×10^{-7} **mol** l^{-1}	99 99 98 98 98	98 98 98 98 97
Mean	98.4	97.8
Standard Deviation	±0.55	±0.45

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References

1. J. Wang, Analytical Electrochemistry, p 44-54, VCH Publishers, inc., New York (1994).
2. K.H. Brainina, E. Neyman, Electroanalytical Stripping Methods, p 71-120, John Wile, New York (1993).
3. J. Wang, Stripping Analysis: Principles, Instrumentation and applications, VCH Publishers Inc., Florida (1985).
4. R. Kalvoda, Adsorptive Stripping Voltammetry in: Analytical Chemistry II, (J. Zuka, Ed.), Ellis Horwood, New York (1996).
5. J. Wang, Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine, p 17-20, VCH Publishers Inc., New York (1988).

6. P.M. Zaitsev, R.M.F. Salikhdzhanova, N.K. Zaitsev, Indus. Lab Diagno. Mater., **65**, 1-15 (1999).

7. A.Z. Abu Zuhri, W. Voelter, Fresenius J. Anal. Chem., **360**, 1-9 (1998).

8. K.H. Brainina, N.A. Malakhova, N.Y. Stojko, Fresenius J. Anal. Chem., **368**, 307-325 (2000).

9. A. H. Alghamdi, J. Saudi Chem Soc., **6**, 185-198 (2002).

10. J.C. Vire, J.M. Kauffmann, G.J. Patriarche, J. Pharma. Biomed. Anal., **7**, 1323-1335 (1998).

11. W. Szczepaniak, T. Cychowska, T. Przadka, Acta. Pol. Pharm., **49**, 3-5 (1992).

12. M. M. Elsayed, Drug Dev. Ind. Pharm., **32**, 457-461 (2006).

13. F.Q.. Alali, B.M. Tashtoush, N.M. Najib, J. Pharm. Biomed. Anal., **34**, 87-94 (2004).

14. X. Chen, D. Zhonq, D. Liu, Y. Wang, Y. Han, J. Gu, Rapid Com. Mass Spectro., **17**, 2459-2463 (2003).

15. W. Ciesielski, R. Zakrzewski, U. Zlobinska, Pharmazie, **60**, 237-238 (2005)

16. M. B. Pierre, W. S. Maria Ren, Archive der Pharmaize, **325**, 253-259 (2006).

17. N. El-Kousy, L. I. Bebawy, J. Pharm. Biomed. Anal., **20**, 671-679 (1999).

18 W. F. Smyth, Voltammetric Determination of Molecules of Biological Significant, p 65, John Wiley, New York (1992).

19. I. M. Kolthoff and J. J. Lingane, Polarography, 2 th Ed., Vol. **2**, p 683-684, John Wiley, New York (1965).

20. P. Zuman, Topics in Organic Polarography, p 38-67, Plenum Press, New York (1970).

21. Morrison and Boyd, Organic Chemistry, 6 th Ed., p 671-677, Prentice Hall, Inc., New York (1992).

22. C.M.G. Van den Berg, Anal. Chem. Acta, **212**, 31 (1988).

23. G. Stubauer, D. Obendorf, Analyst, **121**, 351-356 (1996).