DETERMINATION OF CLOMIPRAMINE HYDROCHLORIDE FROM ITS COMMERCIAL DRUG FORM BY VOLTAMMETRY

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ARTICLE INFO	ABSTRACT
	In this study, electroanalytical technique was developed for the quantitative analysis of clomipramine hydrochloride from its commercial tablet dosage forms based on its oxidation behavior. The electrochemical determination of clomipramine hydrochloride was easily carried out on glassy carbon electrode (GCE) by two voltammetric techniques. The electrochemical measurements were carried out on GCE surface in different buffer solutions in the pH range from 2.00 to 12.00 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The effect of pH on the anodic peak current and peak potential was investigated. Phosphate buffer (pH 6.50) was selected for analytical purposes. The diffusion-controlled nature of the peak was obtained. A linear calibration plot for DPV analysis was constructed in the clozapine concentration range from 5×10^{-6} mol L^{-1} to 3×10^{-5} mol L^{-1}. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as 2.8×10^{-7} mol L^{-1} and 8.4×10^{-7} mol L^{-1} respectively.

1. INTRODUCTION

As an effective and important antipsychotic drug, clomipramine hydrochloride (Figure 1) is widely used in the treatment of psychiatric disorders. As an effective and important antipsychotic drug, clomipramine hydrochloride (Figure 1) is widely used in the treatment of psychiatric disorders. Many analytical methods have been developed to investigate its characteristics, such as spectrophotometer chemiluminescence, high-performance liquid chromatography, capillary zone electrophoresis, etc. (Huang *et al.,*2008) Nevertheless, there are few reports about clomipramine hydrochloride studied by means of electrochemical methods for its low redox activity under normal conditions. Among the few examples, rotating gold and platinum electrodes were used to study its electrochemical mechanism in sulfuric acid (Bioship and Hussein,1984). The highly boron-doped diamond electrode in HPLC used to determine clomipramine hydrochloride and got satisfactory results, but the preparation of the electrode was complicated and time-consuming (Ivandidi *et al.*,2002). A novel ion-selective

was fabricated electrode based on poly (vinyl chloride) membranes for clomipramine hydrochloride, the electrode had high stability and responded rapidly, while the detection limit of 4×10^{-7} mol L^{-1} seemed somewhat unsatisfactory (Ortuno *et al.*,2006).
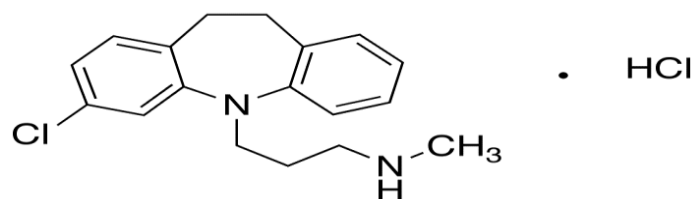

Figure 1. Chemical structure of clomipramine hydrochloride

The purpose of this study is to suggest a rapid and precise voltammetric technique for analyzing the substance in drug dosage forms.

2. MATERIAL AND METHODS

2.1. Apparatus

Electrochemical measurements were carried out using a Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) with a three-electrode system consisting of GCE as working electrode (GCE; φ = 3 mm, Metrohm), a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 M, Metrohm) reference electrode. The GCE was polished with alumina solutions (prepared from φ = 0.01 µm aluminum oxide) on alumina polish pad before each measurement and then, rinsed with ultra-pure deionized water and ethanol. The firstly, the deoxygenation process of the supporting electrolyte solutions were carried out with argon gas for 5 min before all measurement. Firstly, the deoxygenation process of the supporting electrolyte solutions were carried out with argon gas for 5 min before all measurement. Then, the argon gas was passed from the solutions for 60 s after the addition of each sample solution in the measurements. In each new experiment, a new bare electrode surface was used. In each new experiment, a new bare electrode surface was used. Metrohm 744 pH meter (Herisau, Switzerland) was used for pH measurements. All measurements were carried out at ambient temperature of the laboratory (15-20°C). The following parameters were optimized: pulse amplitude 50 mV; pulse time 0.04 s, voltage step 0.009 V, voltage step time 0.04, potential step 10 mV (DPV); the scan rate in the range 10-1000 mVs^{-1} (CV).

2.2. Reagents and materials for analysis

Clomipramine hydrochloride and Anafranil were kindly supplied by (TEOFARMA, Istanbul, Turkey). A stock solution of 1.0×10^{-2} mol L^{-1} of clomipramine hydrochloride was prepared by dissolving an accurate mass of this active material in an appropriate volume of ultra-pure-deionized water and kept in the refrigerator of laboratory. The standard working solutions were prepared by dilution stock solution. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.067 mol L^{-1} phosphate buffer; pH:4.50-8.00 0.04 mol L^{-1} Britton Robinson buffer; pH:2.02-12.00 (acetic acid: Riedel, Seelze, Germany, 100 m/m %; boric acid; Merck, Darmstadt, Germany, and phosphoric acid, Carlo Erba, Rodeno, France, 85 m/m %) were used to the supporting electrolyte solutions. Ultra-pure-deionized (0.055 µS/cm) water obtained from TKA Smart 2 model was used to prepare supporting electrolytes. Other chemicals, all of the analytical-reagent grade (Merck) were used without purification.

2.3. Calibration plot for quantitative analysis of clomipramine hydrochloride

The stock solution of clomipramine hydrochloride was diluted with ultra-pure-- deionized water to obtain various clomipramine hydrochloride concentrations (changed concentrated to dilute). Under the optimum conditions described in the experimental section, a linear calibration plot was constructed in the clomipramine hydrochloride concentration range $5x10^{-6}$-$3x10^{-5}$ mol $L^{-1.}$ The repeatability, accuracy, and precision were determined.

2.4. Analysis of clomipramine hydrochloride from spiked Anafranil tablet dosage forms

Ten tablets were weighed and ground to a fine powder. $1x10^{-2}$ mol L^{-1} clormipramine solution is prepared and centrifuged 20 min at 4000 rpm to complete dissolution and then diluted to volume with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with selected buffer solution as supporting electrolyte. Each solution was transferred to the measurement cell.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Oxidation Behavior of Clomipramine Hydrochloride

The electrochemical determination of clomipramine hydrochloride based on its oxidative behavior at surface of GCE were firstly carried out by CV and DPV techniques. CV measurements performed with clomipramine hydrochloride $1x10^{-4}$ M at various scan rates between $10 - 1000$ mVs^{-1} at surface at GCE in 0.067 mol L^{-1} phosphate buffer (pH 6.50) are given in Figure 2.
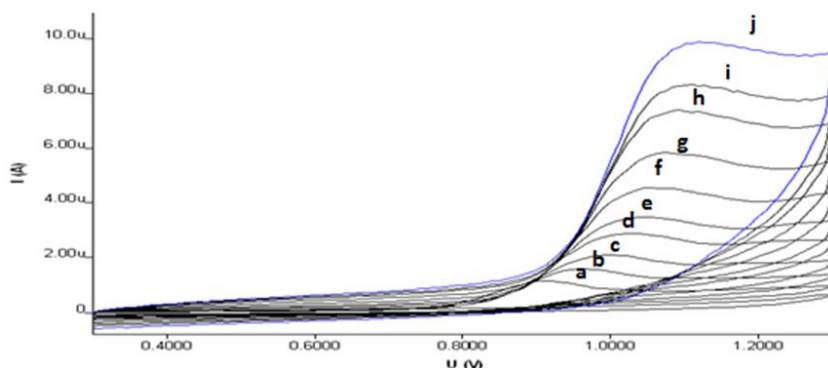

Figure 2. The cyclic voltammograms of $1x10^{-4}$ mol L^{-1} clomipramine hydrochloride in 0.067 M phosphate buffer (pH 6.50) on GCE. Scan rate, mV s^{-1} a) 10, b) 25, c) 50, d) 100, e) 150, f) 250, g) 400, h) 600, i) 750, j) 1000

The best linear relationship existing between peak current and the square root of the scan rate between 10-1000 mV s^{-1} (($Ip(\mu A) = 0.1354v^{1/2} +0.1141$) with correlation coefficient 0.9977 were observed. This result is shown that the oxidation reaction is predominantly diffusion controled. In addition, ideal reaction of solution on surface electrode is determined by the result of correlation coefficient and slope of the peak (logarithm of peak current versus the logarithm of scan rate) 0.9993 and 0.46, respectively (Yılmaz *et al*., 2013; Eker *et al*., 2017).

CV voltammogram of clomipramine hydrochloride gave one anodic peak without reverse scan indicates the irresible properties of electrode reaction. (Figure 2) (Yılmaz *et al*., 2013; Eker *et al*., 2017).

3.2. pH Effect of Peak Current for Clomipramine Hydochloride

The effect of pH on the peak current was investigated in Britton-Robinson, acetate and phosphate buffer solutions. The results showed that the analyses were strongly pH dependent. The DPV peak current of the oxidation was inversely proportional to pH (Fig.3a-c). The convenient pH for the electroanalytical determination of the clomipramine was found as 6.50 in the0.067 mol L^{-1} phosphate buffer.
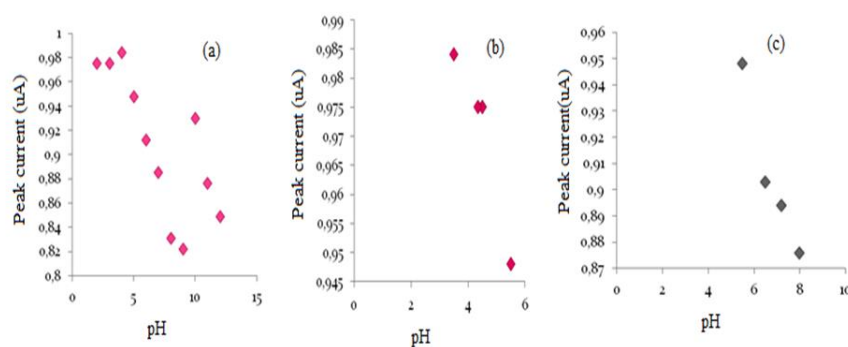
Figure 3. pH changes on the peak current of 5x10^{-5} mol L^{-1} clomipramine hydrochloride in a) 0.04 mol L^{-1} Britton-Robinson Buffer (BRT) , b) 0.2 mol L^{-1} acetate buffer, c) 0.067 mol L^{-1} phosphate buffer as supporting electrolyte by DPV.

3.3. Calibration Plots for The Determination of Clomipramine Hyrochloride

DPV technique was used for quantitate determination of the drug in pharmaceutical formulation. Under the optimized experimental conditions, the linearity value is high in the concentration range of 5x10^{-6}-3x10^{-5} mol L^{-1} Figure 4.
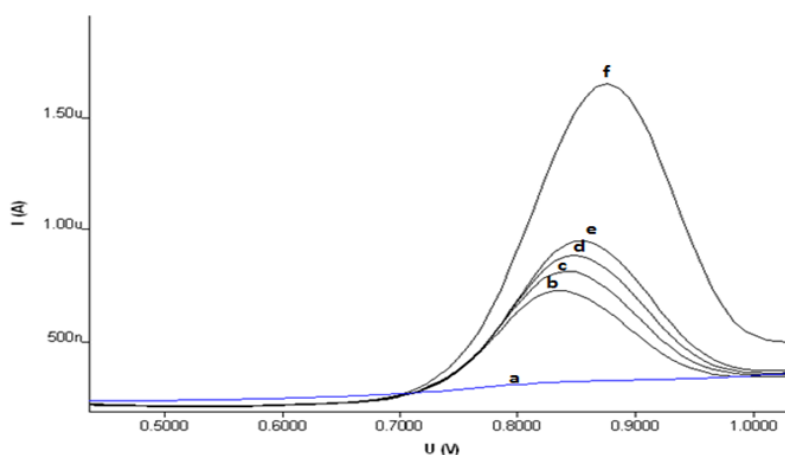
Figure 4. The calibration voltammograms at different concentrations of clomipramine hydrochloride in 0.067 mol L^{-1} phosphate buffer (pH 6.50) on GCE by DPV. a) supporting electrolyte, b) 5x10^{-6} c) 7x10^{-6} d) 9x10^{-6} e) 1x10^{-5} f) 3x10^{-5} (mol L^{-1}).

Applied voltammetric technique was validated for determination of the clozapine with evaluation of the limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and reproducibility) in Table 1, accuracy (bias) and recovery values in Table 2. Table 1 and Table 2 is showed the linearity values. Slope and r of the slope is related with

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lineartiy. It will be better to put linearity knowledge in here (Çıtak *et al.,* 2007; Skrzypek *et al.*, 2005; Yılmaz *et al.*, 2013; Yagmur *et al.*, 2017; Eker *et al.*,2017).

The values of LOD and LOQ were calculated as 2.8×10^{-7} and 8.4×10^{-7} mol L^{-1} respectively. A good repeatability and reproducibility of the anodic peak current and potential were calculated from five independent measurements for 1×10^{-5} mol L^{-1} clomipramine hydrochloride (Skrzypek *et al.*, 2005; Çıtak *et al.,* 2007; Yılmaz *et al.*, 2013; Yagmur *et al.*, 2017; Eker *et al.*, 2017). Repetability peak current and peak potential were found as 1.79 and 0.76 respectively. The RSD values for the reproducibility were found as 0.90 and 0.01 respectively.

The equation of the linear regression plots was Ip(μA)= 3.1996×10^4 C (mol L^{-1}) +0.293 found with correlation coefficient, r=0.999 (n=5 repeat measurements). Standard deviations for intercept and slope of the calibration plot are given in Table 1.

Table 1. Regression analysis of the calibration plot for the analysis of clomipramine hydrochloride. The calibration plots were obtanied in 0.067 mol L^{-1} phosphate buffer (pH 6.50) on surface of GCE by applied DPV technique.

Parameter	Results
Measured potential (V)	0.903
Linear concentration range (mol L^{-1})	5×10^{-6}-3×10^{-5}
Slope (μA mol L^{-1})	3.1996×10^4
SD of slope	1372
Intercept (nA)	0.293
SD of intercept	0.070
Correlation coefficient, r	0.999
Number of measurements, n	5
LOD (mol L^{-1})	2.8×10^{-7}
LOQ (mol L^{-1})	8.4×10^{-6}
Repeatability of peak current (R.S.D %)	1.79 for 1×10^{-5} mol L^{-1}
Repeatability of peak potential (R.S.D %)	0.76 for 1×10^{-5} mol L^{-1}
Reproducibility of peak current (R.S.D %)	0.90 for 1×10^{-5} mol L^{-1}
Reproducibility of peak potential (R.S.D %)	0.01 for 5×10^{-5} mol L^{-1}

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3.4. Analysis of Clomipramine Hydrochloride in Anafranil® Tablets by Voltammeric Techniques

The labeled value of clomipramine hydrochloride in Anafranil commercial tablets was calculated from its calibration values (Table 2). The accuracy of the applied methods were evaluated by recovery tests after the addition of a certain amount of active drug to pre-analyzed formulations of clomipramine hydrochloride to determine whether excipients in the tablets interfered with the analysis, Table 2). The method was validated. The linear range, detection and quantification limits reported for non-electrochemical method and electrochemical methods are given in Table 3.

Table 2. The analysis of clomipramine hydrochloride in Anafranil tablets and mean recoveries on surface of GCE by DPV.

Parameter	Results
Labeled clozapine (mg)	25.00
Amount Found (mg)	24.50
Relative Standard deviation, R.S.D.%	0.96
Bias %	2.00
Added clozapine (mg)	2.50
Found clozapine (mg)	2.25
Number of measurement, n	5
recovery (%)	99.09
Relative standard deviation of recovery, R.S.D. %	0.30
Bias %	0.01

Table 3. Comparison of linear range and detection limits for clomipramine hydrochloride to different known methods.

Linear range	Limit of detection (*LOD*)	Limit of quantification (*LOQ*)	Method	Reference
5×10^{-5} - 1×10^{-6} mol L^{-1}	6×10^{-9} mol L^{-1}	_	voltammetry	Huang *et al.* (2008)
1×10^{-6}-1×10^{7} mol L^{-1}	1×10^{-9} mol L^{-1}	_	UV	Guiying *et al.* (2008)
$4x10^{-6}$-$4x10^{-5}$ mol L^{-1}	$1x10^{-9}$ mol L^{-1}	_	UV	Guiying *et al.* (2008)
$5x10^{-6}$-$3x10^{-5}$ mol L^{-1}	$2.8x10^{-7}$ mol L^{-1}	$8.4x10^{-7}$ mol L^{-1}	voltammetry	**This study**

The important advantage of the applied electrochemical technique over the other techniques such as spectrometry and chromatography is that it can be applied directly to the analysis of pharmaceutical dosage form without the need for extensive sample preparation since there was no interference from the excipients and endogenous substances. Another advantage is that the developed DPV technique is fast, requiring about 5-10 min to run any sample and involves no sample preparing other than dissolving, diluting, precipitating, centrifuging and transferring an aliquot to the supporting electrolyte.

4. CONCLUSIONS

In this study, a simple, low-cost, sensitive and selective DPV technique for the quantitative analysis of clomipramine hydrochloride based on the electrochemical oxidation at surface of GCE was realized. The changing of current with pH, it is understood that electrode reaction process pH dependent. Only oxidation peak was observed. There is no reduction peak indicate that electrode proses is irreversible. Clomipramine hydrochloride was successfully determined in 0.067 mol L^{-1} pH:6.50 phosphate buffer in tablets dosage by DPV technique.

Conflict of Interest Statement

The authors declare no conflict of financial, academic, commercial, political, or personal interests.

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