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Determination of trimetazidine HCl by adsorptive stripping square-wave voltammetry at a glassy carbon electrode

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Abstract

The adsorptive and electrochemical behavior of trimetazidine hydrochloride on a glassy carbon electrode were investigated in acetate buffer solution by using cyclic and square-wave voltammetry. Cyclic voltammetric studies indicated the oxidation of trimetazidine hydrochloride at the electrode surface through a single two-electron irreversible step and fundamentally controlled by adsorption. The solution condition and instrumental parameters were optimized for the determination of the authentic drug using adsorptive square wave stripping voltammetry. Trimetazidine hydrochloride gave a sensitive adsorptive oxidative peak at 0.750 V (vs. Ag/AgCl). The oxidation peak was used to determine authentic trimetazidine hydrochloride concentration in the range 5.0×10^{-8}–5.0×10^{-6} M with a detection limit of 2.0×10^{-8} M. The procedure was successfully applied for assay of trimetazidine hydrochloride in the tablet dosage form (Vastarel). A mean recovery of 94.7% with a relative standard deviation (R.S.D.) of 0.88% was obtained. Applicability to assay the drug in urine samples was illustrated. The peak current was linear with the drug concentration in the range 17–85 μg per ml urine. The detection limit was 1.7 μg ml^{-1} urine.

Keywords: Trimetazidine hydrochloride; Square-wave adsorptive voltammetry; Glassy carbon electrode; Tablet analysis; Human urine determination

1. Introduction

Trimetazidine (I); 1-(2,3,4-Trimethoxbenzyl) piperazine is an antianginal drug [1] that acts as a scavenger of oxygen radicals [2]. It has been used as the dihydrochloride salt in the management of angina pectoris and in ischaemia of neurosensorial tissues as in menier's disease [1]. Whereas its precise mechanism of action remains unknown, antioxidant properties and the ability to preserve high energy phosphate metabolism have been reported [2,3]. Identification and quantitation of trimetazidine and its metabolites in urine and plasma were achieved using modern liquid chromatography-mass spectrophotometric methods [4]. The major drug component observed in urine

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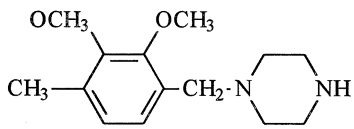
and plasma was unchanged trimetazidine. It is worth-noting that there are no reported methods for the analysis of this antianginal drug in pharmaceutical dosage forms, whereas in biological fluids, only few attempts have been made. These were mainly chromatographic techniques by HPLC method using electrochemical [5] and fluorescence [6] detectors and by gas chromatography-mass spectrophotometry [7]. Recently two stability indicating HPTLC [8] and RPLC [9] assays of the drug in pharmaceutical dosage forms have been reported.



(I)

No electroanalytical methods for the determination of this drug in pharmaceutical formulations or human fluids have been reported in the literature to date.

This work aimed to study of the voltammetric behavior and assay of trimetazidine hydrochloride at a glassy carbon electrode using cyclic and square wave voltammetry. The adsorption nature of the drug at the glassy carbon electrode surface forms the bases for the electroanalytical determination of trimetazidine hydrochloride. An electroanalytical procedure for the trace determination of trimetazidine in its pharmaceutical formulations and human urine was optimized.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using an Electrochemical Trace Analyzer Model 394–PAR (from EG&G). The electrode assembly Model 303A (from EG&G), with a glassy carbon disk electrode (G0197, 7 mm^2 surface area), Ag/AgCl (sat. KCl) reference electrode and a platinum wire auxiliary electrode was used. All measurements were carried out at room temperature.

2.2. Reagents

Trimetazidine hydrochloride and Vastarel® tablets were obtained from the Servier Egypt Industries Limited. A stock solution of trimetazidine hydrochloride (1×10^{-3} M) was prepared. The diluted solutions were prepared daily by accurate dilution with de-ionized water just before use. The trimetazidine hydrochloride solution is stable and its concentration does not change with time. Acetate buffer (0.05 M, pH 5.0) was prepared with sodium acetate and acetic acid. All the chemicals used were of Analar reagent grade and all solutions were prepared with de-ionized water. The de-ionized water was supplied from (PURITE–still plus) de-ionizer connected with a (HAMILTON–aquamatic) double distilled water system.

2.3. Procedure

The glassy carbon electrode (GCE) was polished, at the start of the work, with aqueous slurry of 0.5 μm alumina powder (K0015 from EG&G) on a damp silk cloth until a mirror-like finish was obtained. The accumulation of trimetazidine hydrochloride at the working electrode was carried out for a selected time while the solution was stirred at 1600 rpm. The stirring was then stopped, and after a rest period of 5 s, a cyclic or a square-wave voltammetric stripping, initiated in the anodic direction, was performed. For electrode regeneration, several cyclic scans were carried out in a blank electrolyte solution until a voltammogram corresponding to the residual current was obtained. Before each experiment, a cleaning step for the electrode surface from the accumulated species was applied. This was achieved by means of cycling potential scan for 5 cycles between 0.0 and 1.3 V versus Ag/AgCl at scan rate of 100 mV s^{-1} in acetate buffer solution. The electrode was then ready for use in a next measurement cycle.

2.3.1. Vastarel tablets analysis

Twenty tablets were weighed accurately and finely powdered. A portion of the powder, equivalent to the average weight of one tablet was

transferred to a 100 ml volumetric flask and dissolved in ~50 ml de-ionized water. The mixture was sonicated for few minutes to ensure complete dissolution and then completed to the mark with the same solvent. The solution was then filtered and the first portion of the filtrate was discarded. An accurate measured volume of the filtrate was quantitatively diluted with de-ionized water to yield a sample solution having a final concentration assumed to be 1×10^{-4} M trimetazidine hydrochloride. An aliquot was then transferred to a voltammetric cell containing 10 ml of acetate buffer (pH 5.0) to yield a final concentration of 1×10^{-6} M trimetazidine hydrochloride. The square-wave voltammogram was then recorded without pre-concentration time and after 5 s rest period at open circuit condition. The content of the drug in tablet was determined referring to the regression equation.

Commercially available trimetazidine hydrochloride tablets also contain some additives e.g. starch, sodium bicarbonate, gelatin, lactose and magnesium stearate. For recovery study, a synthetic mixture containing trimetazidine hydrochloride and these additives was prepared in a preparation according to manufacture's batch formulas for 20 mg per tablet. After filtration through a fine milli-pore filter, aliquot of this solution was diluted to produce 1×10^{-6} M trimetazidine hydrochloride solution.

2.3.2. Urine analysis

Urine samples, 1 ml each, were spiked with varying amounts (ranged from 17 to 85 μg) of trimetazidine hydrochloride. The analysis were carried out by adding 0.1 ml of trimetazidine hydrochloride-urine solution to the electrochemical cell containing 9.9 ml of acetate buffer (pH 5.0), The solution was stirred at 1600 rpm at open circuit conditions, and the glassy carbon electrode was immersed for 300 s (pre-concentration step). The electrode was then washed with deionized water, dried, and placed in a measurement cell containing 10 ml of acetate buffer (pH 5.0) and the square-wave voltammogram was recorded following the optimized conditions. Quantification was performed by means of calibration curve method.

3. Results and discussion

3.1. Cyclic voltammetry

The interfacial accumulation of trimetazidine hydrochloride on a glassy carbon electrode is indicated from the cyclic voltammograms of 5×10^{-5} M trimetazidine hydrochloride in 0.05 M acetate buffer (pH 5.0) recorded before and after 180 s accumulation at 0.0 V (Fig. 1). After a short pre-concentration time, a large anodic peak appeared at +0.770 V, which may be attributed to the oxidation dimerization of the –NH center of piperazine ring in the drug molecules through a single two-electron irreversible step. A substantial decrease of the anodic peak is observed in subsequent scans. Such behavior indicates a rapid desorption of the drug from the electrode surface. The fact that no peaks were observed in the reverse scan suggests that the oxidation process is an irreversible one. The effect of scan rate (v) on peak current (i_p) was examined, from 25 to 200 mV s^{-1}, with a plot of log i_p versus log v, giving a straight line which could be expressed by the equation: $\log i_p = 0.95 \log v - 1.12$ ($r = 0.994$, $n =$

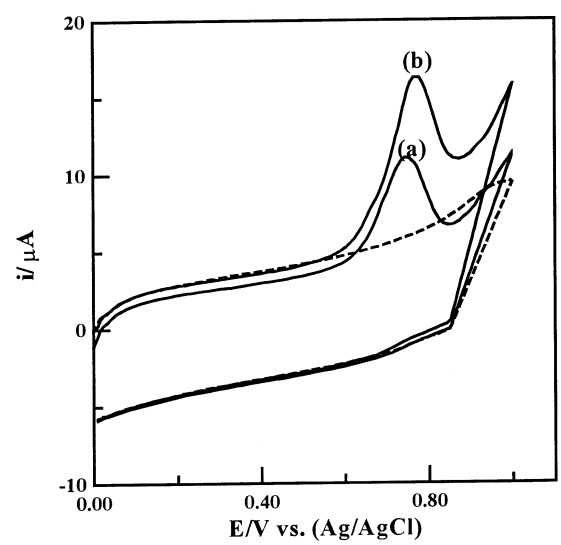


Fig. 1. Cyclic voltammograms for 5.0×10^{-5} M trimetazidine hydrochloride in 0.05 M HOAc-NaOAc buffer solution of pH 5.0 at scan rate 100 mV s^{-1} (a) without accumulation; and (b) after 180 s accumulation at 0.0 V, dashed line represents blank solution.

8). The slope (0.95) is too close to the theoretically expected value (1.0) for an ideal reaction of surface species [10]. The peak potential shifts to less positive value on increasing the scan rate, which confirms the irreversible nature of the oxidation process at the glassy carbon electrode.

3.2. Square-wave adsorptive stripping voltammetry

Square wave voltammetry technique is used to optimize a rapid and sensitive electroanalytical procedure for the determination of authentic trimetazidine hydrochloride and to apply this procedure for the determination of the drug in its pharmaceutical formula and in human urine. The solution conditions affect the enhancement of the peak associated with the preconcentration step. Various electrolytes such as solutions of Britton–Robinson buffer, perchloric acid, borate buffer, ammonia/ammonium chloride, potassium chloride and potassium nitrate were examined and exhibited varying degrees of accumulation. Best results were obtained with 0.05 M acetate buffer (pH 5.0). Thus, this electrolyte was used throughout the present study. The square-wave voltammograms showed improved peak current compared to the linear scan and differential pulse ones, and was used in all subsequent work.

Fig. 2 shows the dependence of the adsorptive peak current on the pre-concentration time at two concentration levels, 5.0×10^{-7} and 1.0×10^{-6} M trimetazidine hydrochloride. The peak current increases with increasing pre-concentration time, indicating the enhancement of trimetazidine hydrochloride concentration at the electrode surface. As the accumulation time increases, the peak current tends to level off, showing that the adsorptive equilibrium is reached. The larger is the concentration, the shorter is the time to reach the equilibrium. For 1.0×10^{-6} M trimetazidine hydrochloride solution following 300 s accumulation, an approximately 3-fold enhancement of the peak current is observed over that attained without accumulation. Thus, a considerable increase in sensitivity can be achieved by application of square wave adsorptive stripping voltammetry (SWAdSV) for determination of trimetazidine hydrochloride.

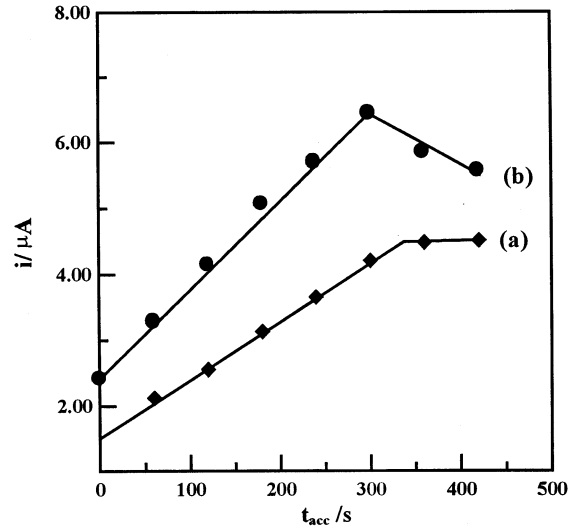


Fig. 2. Effect of the accumulation time on the peak current for (a) 5.0×10^{-7} M and (b) 1.0×10^{-6} M trimetazidine hydrochloride. Square waveform with $f = 20$ Hz, $\Delta s = 10$ and 25 mV amplitude. Other conditions as in Fig. 1.

The effect of accumulation potential on peak intensity was also evaluated for 1.0×10^{-6} M trimetazidine hydrochloride solution following 60 s pre-concentration time over the range -0.3 to $+0.4$ V. Fig. 3 shows that an accumulation potential of 0.0 V gives the highest peak current and, therefore, was chosen for analytical application.

The optimum instrumental conditions were then chosen from a study of the variation of the peak current, of 1.0×10^{-6} M trimetazidine hydrochloride in acetate buffer, with frequency, scan increment and pulse amplitude. With increasing frequency from 20 to 120 Hz, the peak current increases also but the peak becomes less sharp and ill-defined. Higher peak currents were observed by increasing the pulse amplitude from 25 to 100 mV but the background current also increases. The peak current increases linearly with the scan increment up to 10 mV. Thus, the best peak definition was recorded when using 20 Hz frequency, 10 mV scan increment and 25 mV pulse amplitude.

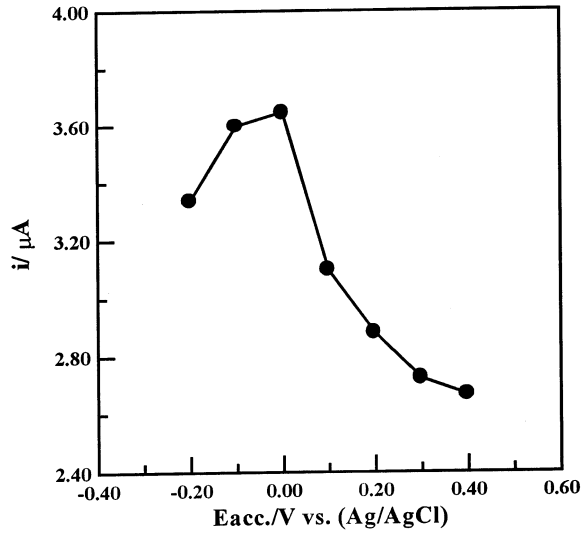


Fig. 3. Effect of pre-concentration potential, on the square-wave stripping signal for 1×10^{-6} M trimetazidine hydrochloride solution, $t_{acc} = 60$ s. Other conditions as in Fig. 2.

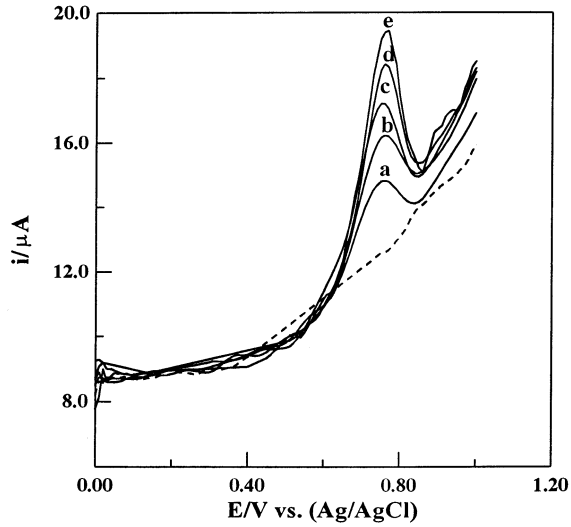


Fig. 4. Adsorptive stripping square wave voltammograms obtained for the determination of trimetazidine hydrochloride in tablets dosage form Vastarel: dashed line represents the blank, lines a–e represent dosage form with increasing concentration of trimetazidine hydrochloride; (a) 1×10^{-6} M; (b) 2×10^{-6} M; (c) 3×10^{-6} M; (d) 4×10^{-6} M; and (e) 5×10^{-6} M. All conditions as in Fig. 2, $t_{acc} = 0$ s.

3.3. Quantitative aspects

Table 1 summarizes the characteristics of the calibration plots established at different accumulation times. It can be seen that the linearity range depends on the accumulation time used. Concentration range from 5.0×10^{-8} up to 5.0×10^{-7} M can, therefore, be determined using the square wave adsorptive stripping voltammetry technique at accumulation time of 300 s. For longer accumulation time, surface saturation is reached. Thus, the ultimate choice of preconcentration time depends on the concentration range studied. The detection limit estimated as 3 s/m [11]; (s, standard deviation of blanks; m, slope of calibration straight line) was 2.0×10^{-8} M, with pre-concentration time of 300 s. The precision of the determination of trimetazidine hydrochloride is tested; at a concentration of 1.0×10^{-7} M and $t_{acc} = 300$ s and the relative standard deviation (R.S.D.) was 1.87% ($n = 7$).

3.3.1. Trimetazidine hydrochloride assay in Vastarel® tablets

Five samples of Vastarel® tablets were analyzed using the proposed stripping voltammetric procedure. Due to the high concentration content of the trimetazidine hydrochloride drug in its dosage form (20 mg per tablet), no accumulation time was needed and thus the assay of tablets was carried out at 0 s accumulation time (Fig. 4). A

Table 1
Characteristics of the calibration plots of trimetazidine hydrochloride

Pre-concentration time (s)	Linearity range (M)	Equation (slope in μA μM^{-1})	Correlation coefficient	Detection limit (M)
0	1×10^{-6}–5×10^{-6}	$y = 1.048x + 0.732$	0.997	8.8×10^{-8}
150	1×10^{-7}–1×10^{-6}	$y = 3.200x + 1.270$	0.996	2.9×10^{-8}
300	5×10^{-8}–5×10^{-7}	$y = 4.600x + 1.900$	0.994	2.0×10^{-8}

mean value of 18.94 mg with R.S.D. of 0.88% was obtained. This compares reasonably well with the stated level of 20 mg per tablet (recovery = 94.7%). The results were favorably compared with those obtained by assay of the same tablet solution with a reported HPTLC method [8] (Table 2). The results indicated that no significant difference between the performance of the two methods regards the accuracy and precision, so, the present method is more simple, fast and less cost tool for trimetazidine hydrochloride analysis.

The effect of the additives on the assay of standard trimetazidine hydrochloride solution was studied by the proposed analysis method. Comparing the assay results in absence and presence of these additives, it was found that these additives had no considerable effect on the accuracy of determination of the drug.

3.3.2. Trimetazidine hydrochloride assay in urine

Direct determination of trimetazidine hydrochloride in urine was not possible due to the huge interfering oxidation peaks of blank urine, and so a medium exchange had to be performed. Medium exchange is efficient in case of determination of trimetazidine hydrochloride because of the completely adsorption nature of the drug at the glassy carbon electrode. However, after medium exchange experiment, a large peak at +0.30 V due to the oxidation of urine constituents was recorded. The height of this peak did not increase with increasing accumulation time and well differentiated from that of trimetazidine hydrochloride. Thus, the peak did not interfere with the determination of trimetazidine hydrochloride. Fig. 5 illustrates the square-wave wave voltammetric response to different concentrations of trimetazidine hydrochloride in urine samples. The response was linearly related to the trimetazidine hydrochloride concentration within the range 17–85 μg per 1.0 ml of urine according to the regression equation: $i_p(\mu A) = 0.048\ C\ (\mu g\ ml^{-1}) + 1.591$, $r = 0.991$. A detection limit of 1.7 μg ml^{-1} was obtained.

The major drug-related component observed in urine was unchanged trimetazidine hydrochloride [4], so the selectivity and sensitivity of the proposed method is sufficient for the determination of the drug in human urine samples.

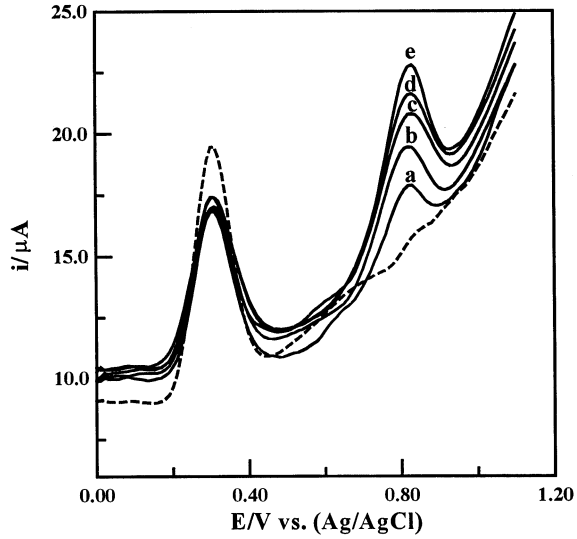


Fig. 5. Adsorptive stripping square wave voltammograms obtained for the determination of trimetazidine hydrochloride in spiked urine sample: dashed line represents the blank, lines a–e represent the spiked sample with increasing concentration of trimetazidine hydrochloride; (a) 17 μg ml^{-1}; (b) 37 μg ml^{-1}; (c) 51 μg ml^{-1}; (d) 68 μg ml^{-1}; and (e) 85 μg ml^{-1} trimetazidine hydrochloride. All conditions as in Fig. 2, t_{acc} = 300 s.

Table 2
Assay of trimetazidine hydrochloride in pharmaceutical formulation solution (Vastarel) using the proposed SWAdS voltammetry procedure and HPTLC reported method [8], based on the average of three separated experiments

Labeled concentration	% Recovery ± S.D.	
	Proposed method	Reported method
20 mg per tablet	94.70 ± 0.88	95.11 ± 0.82

4. Conclusion

The proposed square wave adsorptive stripping voltammetry procedure can be used successfully to determine trimetazidine hydrochloride drug in pharmaceutical formulation and human urine. The method is simple, high sensitive, high accurate, fast, low cost and purging of the trimetazidine hydro-

chloride solutions with nitrogen is not required. The electrochemical renewal of the electrode surface in acetate buffer is efficient and ensures the reproducibility of individual measurements. The detection limit for determination of trimetazidine hydrochloride at a glassy carbon electrode in urine samples after medium exchange is low enough to reach the concentration levels expected in urine after therapeutic doses [4]. The present method could possibly be adopted for the pharmacokinetic studies, as well as quality control laboratories.

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