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Square wave adsorptive stripping voltammetric determination of famotidine in urine

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Abstract

Electrochemical studies of famotidine were carried out using voltammetric techniques: cyclic voltammetry, linear sweep and square wave adsorptive stripping voltammetry. The dependence of the current on pH, buffer concentration, nature of the buffer, and scan rate was investigated. The best results for the determination of famotidine were obtained in MOPS buffer solution at pH 6.7. This electroanalytical procedure enabled to determine famotidine in the concentration range 1×10^{-9}–4×10^{-8} mol L^{-1} by linear sweep adsorptive stripping voltammetry (LS AdSV) and 5×10^{-10}–6×10^{-8} mol L^{-1} by square wave adsorptive stripping voltammetry (SW AdSV). Repeatability, precision and accuracy of the developed methods were checked. The detection and quantification limits were found to be 1.8×10^{-10} and 6.2×10^{-10} mol L^{-1} for LS AdSV and 4.9×10^{-11} and 1.6×10^{-10} mol L^{-1} for SW AdSV, respectively. The method was applied for the determination of famotidine in urine.

Keywords: Famotidine; Square wave adsorptive stripping voltammetry; Linear sweep adsorptive stripping voltammetry; Urine; HPLC

1. Introduction

Famotidine [3-(((2-((aminoiminomethyl)amino)-4-thiazolyl)-methyl)thio)-*N*′-(aminosulfonyl)propanimidamide] (Fig. 1), the third representative of the new generation stress-ulceration inhibitors, is well-known for its excellent histamine H_2 blocking effect. As a specific competitive histamine H_2-receptor antagonist, it inhibits the secretion of histamine — stimulated gastric fluids [1]. The drug is applied both orally and intravenously as an infusion. Therapeutic trials have shown that 20 mg famotidine twice daily or 40 mg at bedtime may be an effective alternative to standard doses of cimetidine in healing duodenal ulcers. The therapeutic level in plasma is 50 μg mL^{-1}. About 15–22% famotidine binds to plasma proteins and between 17 and 30% of the drug appears unchanged in the urine [2].

Methods for the assay of famotidine in phamaceutical dosage forms and biological materials are usually based on high-performance liquid chromatographic (HPLC) determination with ultraviolet detectors [3–7], or tandem mass spectrometry [8,9]. For such applications, however, the operations are time consuming. Other analytical methods have been limited to its spectrophotometric [10], spectrofluorimetric [10], potentiometric [11] and high-performance thin layer chromatographic [12,13] determination. So far, only three papers have been published about the electroanalytical determination of famotidine. Famotidine was determined polarographically from the catalytic proton reduction peak at −1350 mV in Sorensen phosphate buffer (pH 7.8) by Squella et al. [14]. The same authors report that famotidine can be irreversibly oxidized on a glassy-carbon electrode and a method for its differential pulse voltammetric determination in pharmaceutical preparations has been proposed. The peak current shows a linear dependence with famotidine concentration between 8×10^{-6} and 1×10^{-3} mol L^{-1} [2]. Mirceski determined

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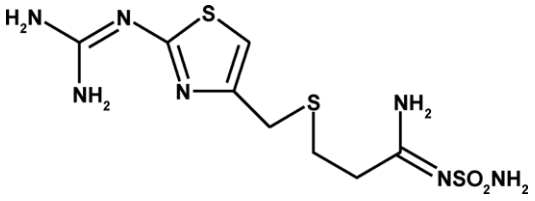

Fig. 1. Chemical structure of famotidine.

famotidine in pure solution in acidic medium (pH 2) by square wave voltammetry [15]. This method allows to determine the compound in the range of 5×10^{-7}–5×10^{-6} mol L^{-1}.

Electrochemical methods, such as cyclic voltammetry, linear sweep voltammetry, differential pulse voltammetry or square wave voltammetry have been widely applied for the determination of compounds of pharmaceutical interest [16–18]. In general, these methods are faster, easier and cheaper than spectrometric and HPLC methods. The sensitivity increases when the stripping voltammetry is employed. The purpose of this work was to develop sensitive, simple, rapid, selective stripping voltammetric methods for the determination of famotidine and to apply it to the pharmaceuticals and biological materials, such as urine or human serum. In the proposed method, there are no sample preparation and time-consuming extraction steps other than centrifugation. In this study, square wave adsorption stripping voltammetry has been proposed as an alternative method to the HPLC techniques in therapeutic drug monitoring.

2. Experimental

2.1. Instrumentation

The experiments were performed on the microAutolab/GPES (General Purpose Electrochemical System, Version 4.7, Eco Chemie) computer-controlled electrochemical system. A controlled growth mercury drop electrode (CGMDE) (Entech s.c, Cracow, Poland) was used. All potentials were referred to the Ag/AgCl (3 mol L^{-1} KCl) reference electrode with a KNO_3 bridge. The counter electrode was a platinum wire. Operating conditions for linear sweep adsorptive stripping voltammetry (LS AdSV) were: scan rate, 100 mV s^{-1}; and step potential, 3 mV, and for square wave adsorptive stripping voltammetry (SW AdSV): pulse amplitude, 25 mV; frequency, 80 Hz; and potential step, 4 mV.

The HPLC system consisted of a Waters model 600 HPLC pump, Rheodyne 7725i Manual and Waters 2487 dual λ absorbance detector, reversed-phase HPLC column preceded by an Alltech guard column packed with Symetry® C_{18} 5 μm 13.9 mm × 150 mm HPLC column. The following equipment was also used: automatic pipettes, a pH-meter type N-517 (Mera-Elwro, Poland), electronics scales-type MC 1 (Sartorius, Germany).

2.2. Reagents and solutions

Fresh stock solution 1×10^{-3} mol L^{-1} of famotidine (Merck) for voltammetric measurements was prepared daily by dissolving a known amount of the compound in 50 mL volumetric flask with addition of 0.1 mL nitric acid (65%) and filling it up with water. This stock solution was then diluted as required. 0.2 mol L^{-1} acetate buffer was prepared by addition of sodium acetate to acetic acid. 0.2 mol L^{-1} ammonium buffer was prepared by addition of nitric acid (V) to ammonium. 0.2 mol L^{-1} MOPS buffer was prepared by addition of sodium hydroxide to 3-(*N*-morpholino)propanesulphonic acid. Sorensen phosphate buffers were prepared using 0.2 mol L^{-1} potassium dihydrogen phosphate anhydrous salt, 0.2 mol L^{-1} disodium hydrogen phosphate dihydrous salt and pH was adjusted by the addition of 0.2 mol L^{-1} sodium hydroxide. All chemicals were analytical grade (POCh SA Gliwice, Poland, or Merck). All solutions were prepared with triply distilled water.

The HPLC determination of famotidine involved preparing stock solution of 0.1 mol L^{-1} famotidine made in methanol (HPLC-grade, Lab-Scan) and stored at 4 °C. The mobile phase used for HPLC analysis consisted of acetonitrile (HPLC-grade, Lab-Scan) and heptanesulfonic acid (Aldrich) (2.5 g L^{-1}) in 0.02 mol L^{-1} sodium acetate buffer (23:77). The mobile phase was adjusted to pH 4.7 with 12 mol L^{-1} HCl and was delivered at the rate of 1.0 mL min^{-1} [3].

2.3. Working voltammetric procedure

The general procedure used to obtain cathodic adsorptive stripping voltammograms was as follows: 10 mL of the supporting electrolyte (a proper amount of buffer mixed up with water) was placed in the voltammetric cell and the solution was purged with argon for 10 min with the stirrer on. When an initial blank was recorded, the required volumes of famotidine were added by means of a micropipette. After forming a new mercury drop, accumulation was effected for the required time at the pre-determined accumulation potential whilst the solution was being stirred. At the end of accumulation period, the stirrer was switched off and after 10 s had elapsed to allow the solution to become quiescent, a negative-going potential scan was initiated. When further volumes of famotidine were added, the solution was de-oxygenated for 20 s before producing further voltammograms. To receive well-shaped peak current of famotidine, the blank was subtracted from the recorded famotidine peak current.

2.4. Working chromatographic procedure

Standard samples of famotidine were made by taking an appropriate volume of stock solution and dissolving in a suitable quantity of the mobile phase. Standard samples of urine were prepared by adding of 2.5 mL urine, a specified amount

of standard solution of famotidine and diluted to 5 mL with the mobile phase. Triplicate (20 μL) injections were made for each solution. Calibration curve was constructed using obtained results.

2.5. Analysis of urine

Urine (morning, mid-stream urine) was obtained daily from a volunteer who took three times a day, every 8 hours "Famogast" tablets containing 40 mg of famotidine was diluted 1:4 with water. Then, 20 μL of the solution was placed in the voltammetric cell according to the general procedure used to record voltammograms.

2.6. Determination of recovery from human serum samples

Serum samples, obtained from healthy volunteers were stored frozen until assay. Two different samples were prepared. The first sample containing 1 mL of serum, 0.5 mL acetonitrile as serum-protein-precipitating agent was spiked with 0.5 mL of 1×10^{-3} mol L^{-1} famotidine solution. The second sample instead of 0.5 mL of famotidine contained 0.5 mL of water (blank). After vortex mixing for 10 min, the mixtures were centrifuged for 10 min at 4000 rpm to separate protein residues. Then 0.5 mL of each supernatant liquor was transferred to two of 50 mL volumetric flasks and diluted with water. After addition of 0.4 mL of the solution to the 9.6 mL of the supporting electrolyte in the voltammetric cell, two voltammograms of the blank and sample with famotidine were recorded.

Serum samples (1 mL) of a volunteer who was treated with "Famogast" were also processed with 0.5 mL acetonitrile as a serum-protein-precipitating agent in the medium of 0.5 mL of water. The next procedure was analogous to the drug-free serum samples.

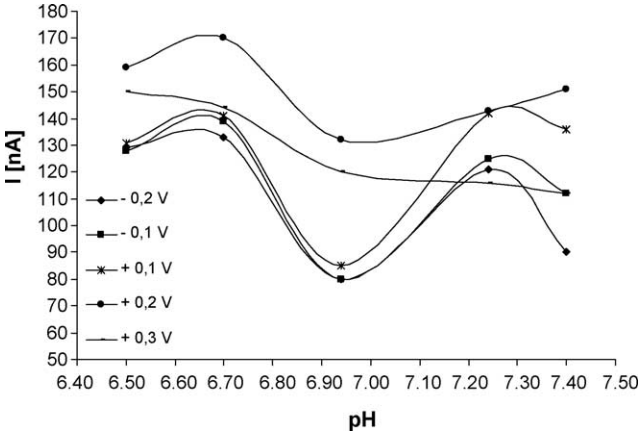

Fig. 2. Dependence of the LS AdSV peak current on pH of the MOPS buffer and potential deposition, $c_{(fam.)} = 1 \times 10^{-7}$ mol L^{-1}, $c_{(buf.)} = 0.05$ mol L^{-1}, $t_d = 120$ s, measuring potential range from 0.3 to −1.7 V.

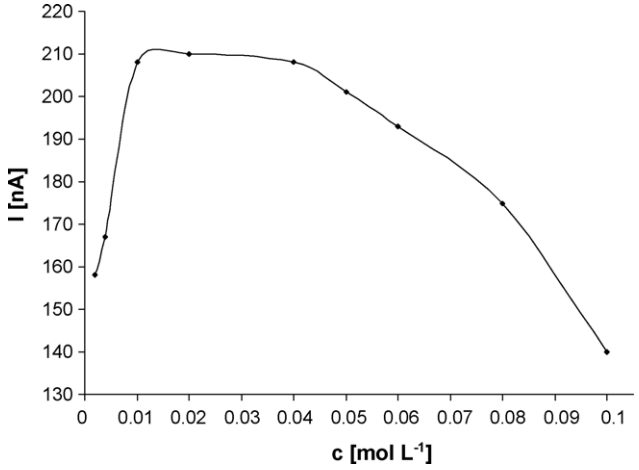

Fig. 3. Dependence of the LS AdSV peak current on the MOPS buffer concentration; pH 6.7, $c_{(fam.)} = 1 \times 10^{-7}$ mol L^{-1}, $t_d = 120$ s, and $E_d = 0.22$ V.

3. Results and discussion

Famotidine exhibits a special behaviour at the dropping mercury electrode. The polarographic response is a catalytic proton reduction process, where famotidine itself is not reduced [14]. As a preliminary study, we investigated the electrochemical behaviour of famotidine over a wide pH range (3.6–8.8) at a HMDE in buffered aqueous media using linear sweep adsorptive stripping voltammetry (LS AdSV). Among the studied electrolytes were Sorensen phosphate, acetate, ammonium and MOPS buffers. For analytical purposes, the best response (with regard to peak current sensitivity and morphology) was obtained with a MOPS buffer.

Cyclic voltammetric measurements performed on 1×10^{-5} mol L^{-1} famotidine showed the irreversible nature of the peak at about −1.4 V in the range of scan rates between 5 and 1000 mV s^{-1}. The cathodic peaks at about 0.2 V are connected with formation of less-soluble complexes of self-oxides mercury with famotidine.

The effect of the potential scan rate between 5 and 1000 mV s^{-1} on the potential and the peak current was evaluated. The linear increase in the reduction peak current with

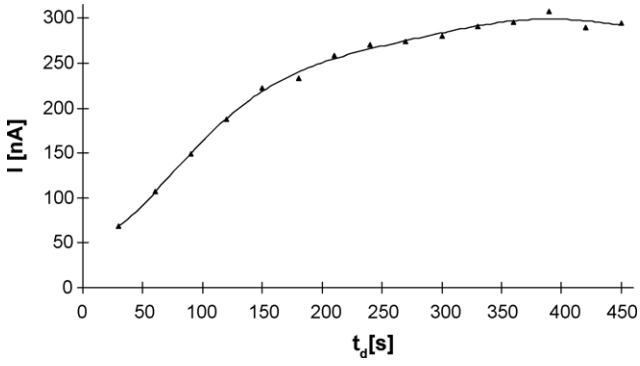

Fig. 4. Dependence of the LS AdSV peak current on deposition time, $c_{(fam.)} = 1 \times 10^{-7}$ mol L^{-1}, $c_{(buf.)} = 0.02$ mol L^{-1} and $E_d = 0.22$ V.

Table 1
Quantitative determination of famotidine in 0.02 mol L^{-1} MOPS buffer pH 6.7 by linear sweep adsorptive stripping voltammetry (LS AdSV) and square wave adsorptive stripping voltammetry (SW AdSV)

	LS AdSV	SW AdSV
Linear concentration range (nM)	1–40	0.5–60
Slope of calibration graph (nA nM^{-1})	3	6
R.S.D. of slope (%)	17	2
Intercept (nA)	0.25	20.72
R.S.D. of intercept (%)	19	9
Correlation coefficient, *r*	0.987	0.997
Number of measurements	3	3
LOD (mol L^{-1})	1.8×10^{-10}	4.9×10^{-11}
LOQ (mol L^{-1})	6.2×10^{-10}	1.6×10^{-10}

The repeatability of the procedure was assessed on the basis of three measurements by LS AdSV and three measurements by SW AdSV at the same famotidine concentration. Repeatability of the catalytic peak current at various famotidine concentrations is shown in Table 2.

the square root of the scan rate (correlation coefficient 0.994) showed the diffusion control process. A 133 mV negative shift in the peak potential was observed, which confirms the irreversibility of the process. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.76 (correlation coefficient 0.999). The values 1.0 and 0.5 are expected for adsorption-controlled and diffusion-controlled reactions, respectively [18]. The received results do not exclude the mechanism postulated by Squella [14]:

$$F + H^+ \rightarrow FH^+$$

$$FH^+ \rightarrow (FH^+)ads$$

$$ads + e \rightarrow (FH)ads \qquad (FH^+)$$

$$2(FH)ads \rightarrow 2F + H_2$$

where F is famotidine and ads indicates adsorption. The CV results prove that the protonated form of famotidine is the electroactive species.

The influences of the deposition potential and pH of MOPS buffer on the famotidine peak current ($c_{fam.} = 1 \times 10^{-7}$ mol L^{-1}) was studied by linear sweep cathodic adsorptive stripping voltammetry. The best results were recorded at pH 6.7, deposition potential $E_d = 0.22$ V (Fig. 2).

The dependence of the peak current on scan rate was also investigated and the scan rate 100 mV s^{-1} was selected for further experiments. The influence of buffer concentration in the range 0.002–0.1 mol L^{-1} at constant pH 6.7 was stated (Fig. 3).

The buffer concentration 0.02 mol L^{-1} was chosen as the optimal. Variation of the deposition time for 1×10^{-7} mol L^{-1} famotidine showed that the peak current increased with the accumulation time and reached plateau after a period longer than 350 s (Fig. 4). The deposition time, 240 s, was chosen for further experiments as it combines good sensitivity and relatively short analysis time.

3.1. Quantitative studies

The applicability of the LS AdSV as an analytical method for the determination of famotidine was tested as a function of its concentration in the range 1×10^{-9}–1×10^{-6} mol L^{-1}. Square wave adsorptive stripping voltammetry method as one of the most selective and sensitive was developed also for quantitative determination. The analytical characteristics of both methods are summarized in Table 1. Detection limits (LOD) and quantification limits (LOQ) of the procedures

Table 2
Repeatability of the famotidine current at various famotidine concentrations

Concentration of famotidine (nM)	LS AdSV		SW AdSV	
	Peak current (average of three) (nA)	R.S.D. of the peak current (%)	Peak current (average of three) (nA)	R.S.D. of the peak current (%)
0.5	–	–	6.2	6
1	2.2	23	29.6	4
4	12.9	16	44.2	3
8	26	2	69.2	0.4
10	29	16	84.9	8
20	72.7	7	140.7	2

Precision and accuracy of the method were investigated by determination of famotidine at three different concentrations in the linear range. Results are presented in Table 3.

Table 3
Accuracy and precision obtained by LS AdSV and SW AdSV

Added nM	Found (nM)		Precision R.S.D. (%)		Accuracy[a] (%)	
	LS AdSV $n = 10$	SW AdSV $n = 10$	LS AdSV	SW AdSV	LS AdSV	SW AdSV
4	4.18 ± 0.49	3.85 ± 0.29[b]	13	8	4.47	−4.00
8	8.20 ± 0.48	8.15 ± 0.18[b]	6	3	2.50	1.75
20	20.26 ± 1.04	20.13 ± 0.62[b]	7	4	1.29	0.65

[a] Accuracy = [(found–added)/added] × 100%.
[b] *t* (*S*/*n* 1/2), *p* = 95%.

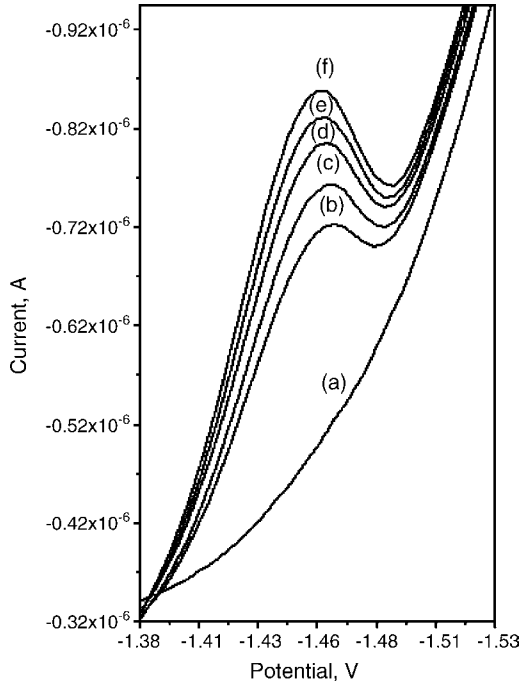

Fig. 5. Square wave adsorptive stripping voltammograms obtained for determination of famotidine in diluted urine samples: (a) blank; (b) sample of urine; (c) as (b) + 1×10^{-8} mol L^{-1}; (d) as (b) + 2×10^{-8} mol L^{-1}; (e) as (b) + 3×10^{-8} mol L^{-1}; (f) as (b) + 4×10^{-8} mol L^{-1}; 0.02 mol L^{-1} MOPS buffer, pH 6.7, E_d = 0.22 V and t_d = 240 s.

were calculated from the peak current by use of the equations LOD = 3 *s*/*m* and LOQ = 10 *s*/*m*, where *s*, the noise estimate, is the standard deviation of the peak currents of the sample and *m* is the slope of the calibration curve (Tables 2 and 3).

3.2. Analytical application

The possibility of applying SW AdSV method to determination of famotidine in urine and human serum was also tested by the method of standard addition. The urine and serum were obtained from the volunteer who was cured with famotidine. Fig. 5b shows typical SW AdSV voltammograms of famotidine in diluted urine. Sometimes voltammetric techniques can pose difficulties in the analysis of biological fluids, which contain reducing substances. As can be seen in Fig. 5, no reduction of compounds present in the urine occurs where the analytical peak appears.

The variation of the peak current versus famotidine concentration (nM) is represented by the straight-line equation I (nA) = 3.7 × c (nM) + 268.3 (R.S.D. of slope 15%, R.S.D. of intercept 2%). The HPLC method [3] was chosen as the analytical reference method in SW AdSV determination of famotidine in urine. The same diluted urine was studied by both methods (Figs. 5 and 6).

The SW AdSV results were statistically estimated by means of the *t*-test of significant. Results (after taking account of dilution) are presented in Table 4.

Table 4
Determination of famotidine in urine by SW AdSV and HPLC methods, n = 3

Method	Concentration (mol L^{-1})	R.S.D. (%)	Student's *t*-test of significance
SW AdSV	1.69×10^{-4}	2	*t*-calculated: 0.386
HPLC	1.35×10^{-4}	2	*t*-theoretical: 2.78

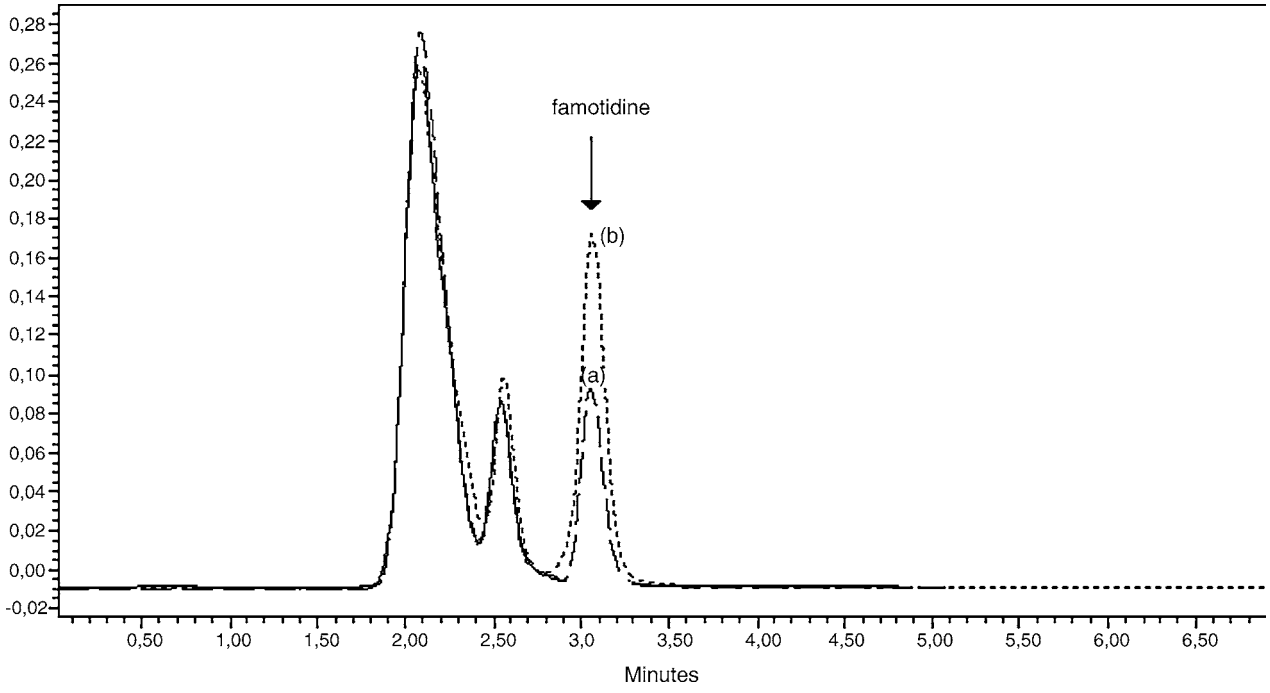

Fig. 6. Chromatogram of famotidine (a) in patient's diluted urine sample, and (b) as (a) + 2.7 nmol of famotidine.

Determination of famotidine by SW AdSV in spiked serum failed. The peak of famotidine was not recorded after subtraction blank serum extract from spiked serum samples. Although only about 22% of famotidine binds to plasma proteins, the signal of the drug was not detected after protein precipitation and centrifugation. Probably, the remaining molecules of famotidine are blocked by other blood components. On the other hand, the peak at -1.4 V was recorded when the stock solution of famotidine (1×10^{-5} mol L^{-1}) was added into voltammetric cell containing 9.6 mL of support electrolyte and 0.4 mL of serum without famotidine. The concentration of famotidine in the cell was at the levels of 4×10^{-8}, 8×10^{-8} and 1.2×10^{-7} mol L^{-1}. The amount of famotidine in human serum was calculated from the appropriate linear regression equations. Good recovery of famotidine was achieved from this type of matrix.

4. Conclusion

Two voltammetric (LS AdSV and SW AdSV) methods have been developed for the determination of famotidine in urine. The principal advantage of the proposed methods over the HPLC method is sensitivity and the separation procedure is not necessary. The proposed voltammetric techniques have the advantages of being simpler, faster, more selective and more cost-effective than HPLC procedure. The LS AdSV and SW AdSV methods are rapid, requiring about 5 min to run sample, and involve no sample preparation other than dissolving, diluting and transferring an aliquot to the supporting electrolyte. The possibility of monitoring of the compound in urine makes the voltammetric methods useful for pharmacokinetic and pharmacodynamic purposes.

Acknowledgments

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