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Original scientific paper

Sensitive voltammetric determination of famotidine in human urine and tablet dosage forms using an ultra trace graphite electrode

SULTAN YAGMUR[1], SELEHATTIN YILMAZ[1]*, GULSEN SAGLIKOGLU[1], BENGI USLU[2], MURAT SADIKOGLU[3] and SIBEL A. OZKAN[2]

[1]*Canakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Analytical, Chemistry, 17020, Canakkale, Turkey,* [2]*Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Ankara, Turkey and* [3]*Gaziosmanpasa University, Faculty of Education, Department of Science Education, 60100, Tokat, Turkey*

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Abstract: In this study, the direct and sensitive determination of famotidine based on its electrochemical oxidation was investigated in spiked human urine and tablet dosage forms. The electrochemical measurements were performed in various buffer solutions in the pH range 0.88–12.08 at an ultra trace graphite electrode (UTGE) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The best results were obtained for the quantitative determination of famotidine by the DPV technique in 0.5 mol L^{-1} H_2SO_4 solution (pH 0.30). In this strong acid medium, one irreversible anodic peak was observed. The effects of pH and scan rate on the peak current and peak potential were investigated. The diffusion-controlled nature of the peak was established. For optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the famotidine concentration range 2×10^{-6}–9×10^{-5} mol L^{-1}. The limit of detection (*LOD*) and limit of quantification (*LOQ*) were 3.73×10^{-7} and 1.24×10^{-6} mol L^{-1} at a UTGE, respectively. The repeatability, precision and accuracy of the developed technique were checked by recovery studies in spiked urine and tablet dosage forms.

Keywords: famotidine; voltammetry; ultra trace graphite electrode; determination; human urine; dosage forms.

INTRODUCTION

Famotidine 3-{[2-(diaminomethyleneamino)thiazol-4-yl]methylthio}-*N*′-sulfamoylpropanimidamide, Scheme 1, is a histamine H_2-receptor antagonist that is used to treat duodenal ulcers and prevent their recurrence. It is also used to treat gastric ulcers and Zollinger–Ellison disease.[1–3] The drug is applied both orally

* Corresponding author. E-mail: seletyilmaz@hotmail.com
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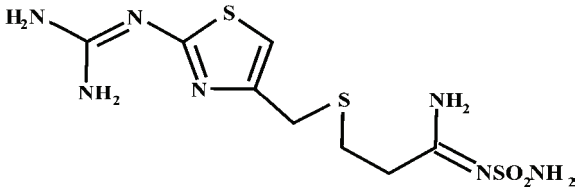
and intravenously as an infusion. Therapeutic trials have shown that 20 mg famotidine twice daily or 40 mg at bedtime may be an effective alternative to standard doses of cimetidine in healing duodenal ulcers. The therapeutic level in plasma is 50 μg mL^{-1}. About 15–22 % of famotidine binds to plasma proteins and between 17–30 % of the drug appears unchanged in the urine.[1–3]



Scheme 1. The structural formula of famotidine.

Methods for the assay of famotidine in pharmaceutical dosage forms and biological materials are usually based on high performance liquid chromatographic (HPLC) techniques with ultraviolet detectors[3–9] or a tandem mass spectrometer.[9–10]

Such applications are, however, time consuming. Other analytical methods have been limited to spectrophotometric and spectrofluorimetric,[11] potentiometric[12] and high performance thin layer chromatographic techniques.[13–14]

Hitherto, only a few papers have been published about the electro-analytical determination of famotidine based on its reduction behavior.[15,16] In this study, famotidine was determined in human urine and tablet dosage forms using an ultra trace graphite electrode by a voltammetric technique.

This technique is particularly well suited for the quantitative determination of redox couples immobilized on an electrode surface.[17–19] This proposed voltammetric technique could be applied directly to the analysis of pharmaceutical dosage forms and biological samples.

EXPERIMENTAL

Apparatus

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) was used for the voltammetric measurements, with a three-electrode system consisting of an ultra trace graphite working electrode (UTGE, surface size φ = 3 mm, Metrohm), a platinum wire auxiliary electrode and an Ag/AgCl (NaCl 3 mol L^{-1}, Metrohm) reference electrode. The UTGE was polished with aluminum oxide on an alumina polish pad. Then its surface was rinsed with deionized water (DI) and ethanol consequently. Oxygen was removed from the supporting electrolyte solution by passing argon gas (analytically pure of 99.99 %) for 5 min before all measurements. The argon gas was passed for 30 s before each measurement. In each new experiment, a new bare electrode surface was used.

All pH measurements were performed with Model Metrohm 744 pH meter (Herisau, Switzerland). All measurements were realized at ambient laboratory temperature (15–20 °C).

For the analytical applications, the following parameters were employed: differential pulse voltammetry (DPV) – pulse amplitude 50 mV; pulse time 0.04 s, voltage step 0.009 V. Potential step 10 mV and scan rate in the range 10–1000 mV s^{-1} (cyclic voltammetry, CV).

Reagents

Famotidine and its Famoser® tablet dosage forms were kindly supplied by Ilsan Inc. (Istanbul, Turkey). A stock solution of 1.0×10^{-2} mol L^{-1} was prepared by dissolving an accurate mass of famotidine in an appropriate volume of ultrapure deionized water (DI) and 20 μL HNO_3. This solution was kept in a refrigerator until the experiments were performed. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution with DI. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.5 mol L^{-1} sulfuric acid (pH 0.30), 0.067 mol L^{-1}; phosphate buffer, pH 4.45–7.39 (Na_2HPO_4 and NaH_2PO_4 as buffer components were purchased from Riedel); 0.2 mol L^{-1} acetate buffer, pH 3.50–5.60 (Riedel); 0.04 mol L^{-1} Britton–Robinson buffer, pH: 2.12–12.00 (acetic acid, Riedel, 100 %); boric acid (Merck) and phosphoric acid (Carlo Erba, 85 m/m %) were used for the supporting electrolytes. The DI water used throughout the experiments with 18.2 MΩ cm^{-1} was obtained from a Sartorius Arium model of ultrapure water system.

Calibration graph for quantitative determination

Famotidine was dissolved in DI to obtain a 1×10^{-2} mol L^{-1} stock solution. This solution was diluted with DI to obtain diluted famotidine concentrations. For the optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the famotidine concentration range of 2×10^{-6}–9×10^{-5} mol L^{-1}. The repeatability, accuracy and precision were checked.

Working voltammetric procedure for spiked human urine

Urine obtained daily from a volunteer was diluted 1:9 with DI. Firstly, 9.4 mL of 0.5 mol L^{-1} H_2SO_4 was put into the voltammetric cell and its voltammogram was taken as the blank. Then 600 μL of the diluted urine solution was added to this solution and its voltammogram was taken as the urine blank. Subsequently, to obtain a 4×10^{-6} mol L^{-1} cell concentration, 40 μL of urine sample (1 mL urine + 8 mL deionized water + 1 mL of 1×10^{-2} mol L^{-1} famotidine stock solution) was added into the voltammetric cell and its voltammogram was recorded. Then, 20 μL of 1×10^{-3} M famotidine standard solution was added four times and the voltammograms of the resulting solutions were individually recorded after each addition. The calibration curve was plotted using obtained results.[17–19]

Working voltammetric procedure for spiked tablet dosage forms

Ten tablets were weighed and ground to a fine powder. From this powder, a 1×10^{-2} mol L^{-1} solution was prepared with deionized water in a 10 mL volumetric flask. The contents of the flask were centrifuged for 20 min at 4000 rpm to affect 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 the selected supporting electrolyte solution. Each solution was transferred into the voltammetric cell. Argon was passed through the solution for 5 min before each measurement. The amount of famotidine was found from the calibration curve.

RESULTS AND DISCUSSION

Electrochemical oxidation of famotidine

In this study, the electrochemical oxidation process and the determination of famotidine were realized by the CV and DPV techniques. The results of the CV measurements performed at a UTGE using 1×10^{-4} mol L^{-1} famotidine solution at a scan rate of 100 mV s^{-1} in various supporting electrolytes and buffers are given in Fig. 1. Cyclic voltammetric measurements showed that an irreversible oxidation process of famotidine occurred.

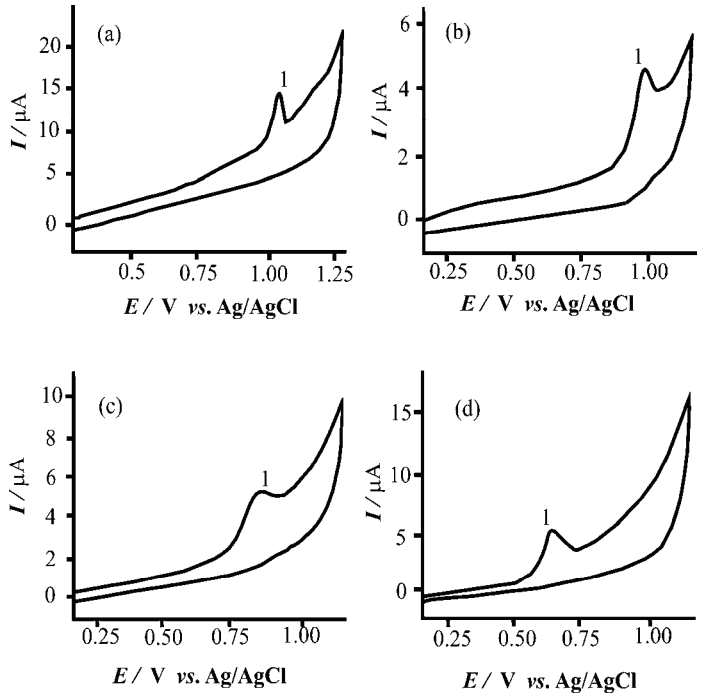
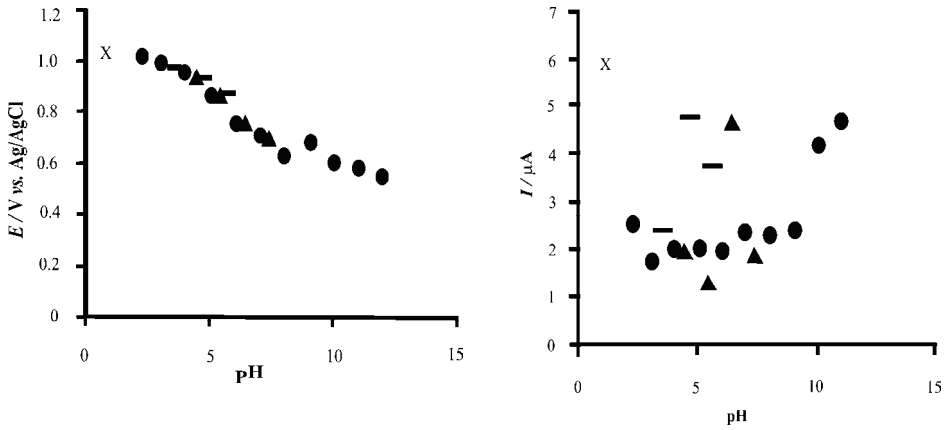


Fig. 1. Cyclic voltammograms of a UTGE electrode in different electrolytes containing 1×10^{-4} mol L^{-1} famotidine; a) 0.5 mol L^{-1} sulfuric acid at pH 0.30, b) 0.2 mol L^{-1} acetate buffer at pH 3.50, c) 0.067 mol L^{-1} phosphate buffer at pH 5.45 and d) 0.04 mol L^{-1} Britton–Robinson buffer at pH 8.02. Scan rate, 100 mV s^{-1}.

The peak potential and the peak current of famotidine were evaluated as in relation to the effects of various scan rates between 10–1000 mV s^{-1}. Scan rate studies were then performed to assess whether the processes at the UTGE were under diffusion or adsorption control.[16–20] Two tests were employed for this procedure. The linear relationship obtained between the peak current and square root of the scan rate between 10–1000 mV s^{-1} was I_p / μA = $0.2489v^{1/2} - 0.6652$ (correlation coefficient, $R = 0.998$). The correlation coefficient was very close to 1.0, showing that the current was diffusion-controlled. A plot of the logarithm of

the peak current *versus* the logarithm of the scan rate gave a straight line with a slope of 0.6571 (very close to 0.5), which is the expected value for a diffusion controlled current.[17–19]

In order to obtain the optimum experimental conditions, some variables affecting the peak current and peak potential, *i.e.*, pH and supporting electrolyte, for a 5×10^{-5} mol L^{-1} famotidine solution were studied at the UTGE using the proposed voltammetric techniques. The voltammetric response was strongly pH dependent. The peak potential shifted to more negative values with increasing pH (Fig. 2a).



(a) (b)

Fig. 2. Effect of pH and on the DPV peak potential (a) and current (b) in 5×10^{-5} mol L^{-1} famotidine; (–) acetate (0.2 mol L^{-1}), (▲) phosphate (0.067 mol L^{-1}) and (•) Britton–Robinson buffers (0.04 mol L^{-1}) and (X) sulfuric acid.

The peak current was maximal at pH 0.30 in 0.5 mol L^{-1} H_2SO_4 solution. Thus, this pH value and supporting electrolyte were chosen for the electro-analytical studies (Fig. 2b).

The DPV technique and 0.5 mol L^{-1} H_2SO_4 solution (pH 0.30) were also selected for further work because they gave not only the highest peak current, but also the best peak shape.

Validation parameters for the quantitative analysis

Based on the electrochemical oxidation of famotidine, DPV techniques were used for the quantitative determination of the pure drug in spiked urine. The optimum experimental conditions were chosen from the studies of the variation of the peak current on pulse amplitude and potential step. Using the optimum conditions described in the experimental section, the voltammograms for various concentrations of famotidine were recorded in 0.5 mol L^{-1} H_2SO_4 solutions pH 0.30 at the UTGE by the applied technique (Fig. 3).

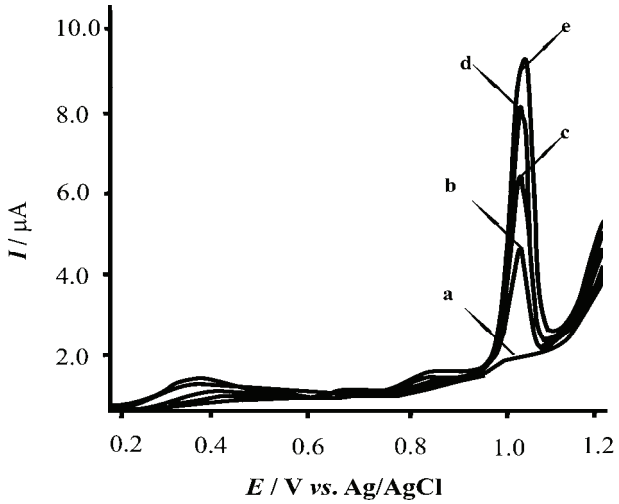


Fig. 3. DPV voltammograms of a UTGE in famotidine solutions. a) Blank, 0.5 mol L^{-1} H_2SO_4 solution (pH 0.30), b) 3.0×10^{-5}, c) 5.0×10^{-5}, d) 7.0×10^{-5} and e) 9.0×10^{-5} mol L^{-1} famotidine.

Quantitative evaluation based on the linear correlation between the oxidation peak current and concentration was performed. Due to this, good correlations were obtained for famotidine in the concentration range 2×10^{-6}–9×10^{-5} mol L^{-1}. The equation of the calibration plot was I_p / μA = 8.0324×10^4(c / mol L^{-1}) – – 0.0398 with a correlation coefficient of r = 0.999 at the UTGE by the DPV technique. Regression data for the calibration plots of famotidine are given in Table I.

TABLE I. Analytical parameters of famotidine obtained in 0.5 mol L^{-1} H_2SO_4 by the DPV technique

Parameter	UTGE
Measured potential, V	1.02
Linear concentration range, mol L^{-1}	2×10^{-6}–9×10^{-5}
Slope, μA M	8.03×10^4
Intercept, μA	–0.0398
Correlation coefficient, *r*	0.999
SE of slope	3.30×10^3
SE of intercept	0.0130
Number of measurements, *N*	10
LOD / mol L^{-1}	3.73×10^{-7}
LOQ / mol L^{-1}	1.24×10^{-6}
Repeatability of peak current, *RSD* / %	1.77
Reproducibility of peak current, *RSD* / %	0.48
Repeatability of peak potential, *RSD* / %	0.69
Reproducibility of peak potential, *RSD* / %	1.26

Validation of the procedure for the quantitative determination of famotidine was examined *via* the evaluation of the limit of detection (*LOD*), limit of quantification (*LOQ*), repeatability, reproducibility, accuracy and precision for the DPV technique (Table I).

The *LOD* and *LOQ* were calculated on the oxidation peak current using the following equations: $LOD = 3s/m$ and $LOQ = 10s/m$ (s is the standard deviation of the peak current for six runs and m is the slope of the calibration curve).[16–19] The achieved limits of detection and quantification were 3.73×10^{-7} and 1.24×10^{-6} mol L^{-1} at the UTGE. The repeatability of the current measurement was calculated for the DPV technique from ten independent runs as 3.27 % *RSD* for 7×10^{-5} mol L^{-1} and 1.77 % *RSD* for 1.0×10^{-5} mol L^{-1} at the UTGE.

The reproducibility of the current measurement was also calculated by the DPV technique from ten independent runs as 0.97 % *RSD* for 7×10^{-5} mol L^{-1} and 0.48 % *RSD* for 1.0×10^{-5} mol L^{-1} famotidine solution at the UTGE, respectively.

Application to human urine samples

The possibility of applying the voltammetric procedure to the quantitative determination of famotidine in spiked human urine was also successfully tested by standard additions of pure drug as described in the experimental section. The voltammograms for famotidine in spiked human urine are given in Fig. 4.

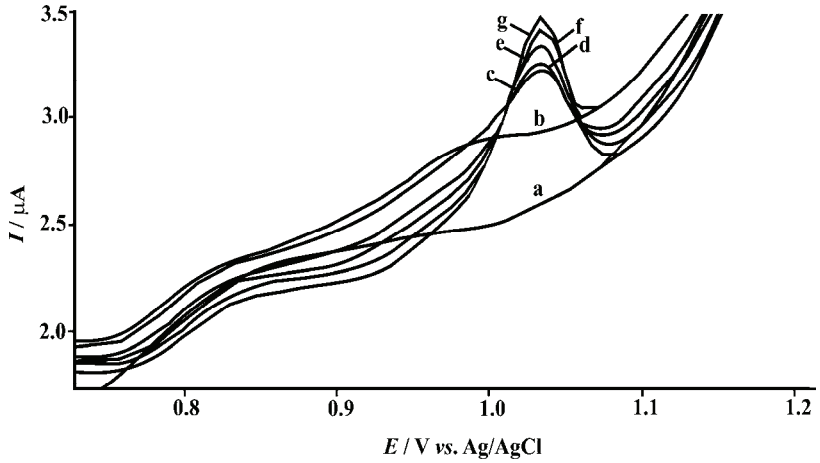


Fig. 4. DPV voltammograms of a UTGE for famotidine in spiked human urine; a) blank (0.5 mol L^{-1} H_2SO_4 solution, pH 0.30); b) a + 600 μL urine (1:9); c) 4.0×10^{-6} mol L^{-1} famotidine with urine; d) 2.0×10^{-6}; e) 4.0×10^{-6}; f) 6.0×10^{-6} and g) 8.0×10^{-6} mol L^{-1} famotidine.

The found amount of famotidine in human urine was calculated from the related linear regression equations. The results of these analyses are summarized in Table II.

TABLE II. Application of the DPV technique at a UTGE to the quantitative determination of famotidine in spiked human urine samples with recovery results

Medium	Human urine
Famotidine spiked, mol L^{-1}	4.00×10^{-6}
Famotidine found, mol L^{-1}	4.08×10^{-6}
Number of measurements, *N*	5
Average recovery, %	102.00
RSD / %	0.42
Bias, %	2.00

As can be seen in Table II, good recovery of famotidine was achieved from this type of matrix at the UTGE. The quantitative assay of spiked urine samples by the proposed technique involved only dilution of spiked urine samples, so it is timesaving and no other procedure steps are required.

Pharmaceutical applications

The amount of famotidine in Famoser® tablets was calculated by reference to the appropriate calibration plots. The results obtained are given in Table III. The proposed technique could be applied with great success to famotidine in tablets without any interference at the UTGE.

TABLE III. The quantitative determination of famotidine in Famoser® tablets by the DPV technique at UTGE

Parameter	Result
Labeled, mg	40.00
Amount found, mg	41.97
RSD / %	2.72
Bias, %	4.92
Famotidine spiked, mg	5.00
Found, mg	5.17
Recovery, %	103.5
Bias, %	3.50
RSD of recovery, %	2.04

The proposed technique was checked by performing recovery tests. To determine whether excipients in the tablets interfered with the analysis, the accuracy of the proposed methods were evaluated by recovery tests after addition of known amounts of pure drug to pre-analyzed formulations of famotidine (Table III).

The results showed the validity of the proposed techniques for the quantitative determination of famotidine in tablets. The proposed DPV technique proved to be sufficiently precise and accurate for reliable electro-analytical analysis of famotidine.

CONCLUSION

In this study, the determination of famotidine based on its electrochemical oxidation at a UTGE was studied by voltammetric techniques. From the CV measurements, it was understood that the electrode reaction process is irreversible and pH dependent. The DPV technique was successfully applied to the quantitative determination of famotidine in 0.5 mol L^{-1} H_2SO_4 solution (pH 0.30) in spiked human urine and commercial drug samples. The analysis was performed with good recoveries without any interference from the excipients in spiked human urine and tablet dosage forms.

The principal advantage of this proposed technique over other techniques is that it may be applied directly to the analysis of pharmaceutical dosage forms and to biological samples without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. Another advantage is that the developed techniques are rapid, requiring about 5 min to run any sample. This paper is not intended to be a study of the pharmacodynamic properties of famotidine, because only healthy volunteers were used for sample collection and results might be of no significance. It only indicated that the possibility of monitoring this compound makes the technique useful for pharmacokinetic and pharmacodynamic purposes.[17–19]

The proposed voltammetric techniques might be a rapid and simple alternative to more complicated chromatographic (HPLC) or spectrometric (UV) techniques for routine analysis of famotidine.

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ИЗВОД

ВОЛТАМЕТРИЈСКО ОДРЕЂИВАЊЕ ФАМОТИДИНА У ХУМАНОМ УРИНУ И ТАБЛЕТНИМ ДОЗИРАНИМ ОБЛИЦИМА ПРИМЕНОМ ЕКСТРЕМНО ОСЕТЉИВЕ ГРАФИТНЕ ЕЛЕКТРОДЕ

SULTAN YAGMUR[1], SELEHATTIN YILMAZ[1], GULSEN SAGLIKOGLU[1], BENGI USLU[2], MURAT SADIKOGLU[3] и SIBEL A. OZKAN[2]

[1]Canakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Analytical, Chemistry, 17020, Canakkale, Turkey, [2]Ankara University, Faculty of Pharmacy, Department of Analytical Chemistry, 06100, Ankara, Turkey и [3]Gaziosmanpasa University, Faculty of Education, Department of Science Education, 60100, Tokat, Turkey

У овом раду је директно и осетљиво одређивање фамотидина, базирано на електрохемијској оксидацији, испитивано на оптерећеним узорцима урина и таблетним дозираним облицима. Електрохемијска мерења су извођена у различитим пуферима pH опсега 0,88–12,08, на екстремно осетљивој електроди (UTGE) цикличном волтаметријом (CV) и диференцијалном пусном волтаметријом (DPV). Најбољи резултати за одређивање фамотидина су добијени DPV техником у раствору H_2SO_4 концентрације 0,5 mol L^{-1}

(pH 0,30). Запажа се један иреверзибилан анодни пик у јако киселој средини. Испитивани су ефекат pH и брзине промене потенцијала на струју и потенцијал пика. Процес регистрован волтаметријским пиком је дифузионо контролисан. Конструисана је линеарна калибрациона крива за оптималне услове, у опсегу концентрација фамотидина 2×10^{-6}–9×10^{-5} mol L^{-1}. Граница детекције (*LOD*) и граница квантификације (*LOQ*) на UTGE износе $3{,}73\times10^{-7}$ и $1{,}24\times10^{-6}$ mol L^{-1}, односно репродуктивност, прецизност и тачност развијене методе су проверени помоћу „*recovery*“ студија на оптерећеним узорцима урина и таблетним дозираним облицима.

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