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Square wave adsorptive stripping voltammetric determination of piromidic acid. Application in urine

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Abstract

A simple procedure for the determination of piromidic acid by square wave adsorptive stripping voltammetry (SW-AdSV) at a hanging mercury drop electrode has been developed. The variables affecting to accumulation process such as concentration of perchloric acid, accumulation potential and accumulation time have been optimised (0.025 mol L^{-1}, −0.25 V and 140 s, respectively) by using response surface methodology. A linear relationship between concentration of piromidic acid and peak intensity has been found in the range 2.22×10^{-9} to 3.33×10^{-8} mol L^{-1}. The detection limit (1.65×10^{-9} mol L^{-1}) has been calculated by the method proposed by Clayton et al. so that protection against both false positive and false negative errors is assured. The procedure was successfully applied to determine piromidic acid in spiked urine samples. The obtained recovery values were in the range 97.3–103.3% at different levels of concentration of piromidic acid.

Keywords: Piromidic acid; Square wave adsorptive stripping voltammetry; Response surface methodology; Central composite design; Urine

1. Introduction

In order to prevent and/or treat a number of diseases in human and animals, antibacterial drugs are often used. The use of these compounds is regulated because of the concerns about their possible effect on human health. Piromidic acid 8-ethyl-5,8-dihydro-5-oxo-2-pyrrolidinopyrido-[2,3-d]pyrimidine-6-carboxylic acid (Scheme 1) belongs to a family called quinolones. It was synthesized for the first time by Shimizu et al. [1].

Quinolones produce a germicide effect. They penetrate in the bacteria through the porines without affecting to the integrity of the cellular wall. Once inside the cell, quinolones act inhibiting an enzyme (DNA-girase, belonging to a group of enzymes called topoisomerases) that prepares DNA for the transcription step. This kind of antibiotics acts against gram-negative bacillus

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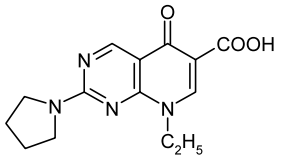


Scheme 1. Structure of piromidic acid.

and they have been preferably used as urinary and intestinal antiseptics, in humans, in animals and in fish farms [2].

Piromidic acid analysis has been performed by spectrophotometry [3], by means of the ternary complex formed with *o*-hydroxyquinolphtaleine–Fe(III); HPLC with several detection systems as ultraviolet [4–11], fluorescence [12,13] or both of them [14,15]; HPLC coupled with MS [16,17]; capillary electrophoresis [18,19] or GC [20,21], in different real samples. About polarographic methods, Corti et al. [22] have proposed the determination of determined piromidic acid and other quinolones, by differential pulse polarography. Recently, Hernández et al. [23] reviewed the analysis of piromidic acid and other quinolones in edible animal products, by HPLC, GC, LC-MS and GC-MS. Most of the determinations of piromidic acid have been performed in fish samples, although there are also determinations in meat samples, urine samples [13,15,22], serum samples [18,22] and pharmaceutical tablets [22]. However, as far as we know, there are not methods based on stripping voltammetric techniques. In this way, a method based on a particularly rapid and sensitive technique (square wave adsorptive stripping voltammetry), at a hanging mercury drop electrode (HMDE), has been developed here. Piromidic acid is adsorbed onto the HMDE electrode, and this phenomenon has been used to develop an adsorptive stripping voltammetric method for the determination of piromidic acid at nanomolar concentration levels.

As a previous step of the method development, response surface methodology (RSM), has been used in order to optimise the principal variables affecting to the accumulative process: concentration of perchloric acid ($HClO_4$), accumulation potential (E_{acc}) and accumulation time (t_{acc}). RSM is a collection of mathematical and statistical techniques aimed for discovering the values of factors (independents variables) that produce an optimum (maximum or minimum) value of a response variable (output variable) which is influenced by them. Although since it intended their employment in analytical chemistry has extended notably, few references exist in the field of voltammetry [24–30]. In RSM the design of experiments [31] is used to select the points where the response will be evaluated. An adequate experimental design allows extracting a lot of information with minimum number of experiments. Central composite design (CCD) [32] was chosen for this work. The aim of this work is to develop a method for the determination of piromidic acid by SW-AdSV on HMDE. Once it has been developed, the method has been applied to the determination of the antibiotic in urine samples. The main advantage of this voltammetric method is that avoids the necessity of a previous extraction step, and it is only necessary a dilution of the sample, due to the high sensitivity of the used technique.

2. Experimental

2.1. Chemicals

HPLC grade dimethylformamide (DMF) (Merck) and MilliQ water (Millipore, Bedford, MA) were used throughout the experiment. Perchloric acid and sodium perchlorate were purchased from Merck. The antibacterial reagent was obtained from Sigma and used without further purification.

Stock solutions of piromidic acid 6.94×10^{-4} mol L^{-1} were prepared in DMF by weighting the appropriate amount of the solid compound. More diluted solutions in water were daily prepared by dilution of stock the solution of piromidic acid.

2.2. Instrumentation

Studies were performed with an Autolab computer-controlled potentiostat (Eco Chemie, Holland) PSTAT 10, equipped with a Methrom (Herisau, Switzerland) 663 VA stand. This system

was connected with a PC 486 microcomputer equipped with the General Purpose Electrochemical System (GPES 3) version 3.2 software package (Eco Chemie). The stand includes a three-electrodes system, an Ag/AgCl–3 M KCl reference electrode, a platinum wire auxiliary electrode, and a mercury drop electrode as working electrode.

All measurements were performed at 25.0 ± 0.1 °C, using a thermostatic cell holder and a Selecta Model Frigiterm thermostatic bath.

2.3. Software

Data analysis was achieved with THE UNSCRAMBLER v. 6.11 (CAMO, Trondheim, Norway). STATGRAPHICS v. 5.0, for experimental design, and QUIMIO (Quimiometría Práctica, Santiago de Compostela, Spain, 1993), for the regression model were also used.

3. Determination of piromidic acid in urine samples

The urine sample was diluted 1:1000 with deionizated water. A suitable volume of this solution was diluted with electrolyte support to 25 ml with 0.025 M perchloric acid and 0.035 M sodium perchlorate. Voltammetric measurements were taken using the following procedure: the solution was purged with nitrogen and stirred for 600 s, then the deposition potential (-0.25 V) was applied for 140 s (accumulation time). The solution was left to rest for an equilibration time of 15 s; then, a cathodic scan from accumulation potential (initial potential) to -1.0 V (final potential) was carried out and the voltammogram was recorded. Other experimental parameters were the following ones: mercury drop size, 0.52 mm^2; stirring rate in the deposition step, 500 rev min^{-1}; square wave amplitude, 50 mV; step potential, 10 mV; frequency, 150 Hz.

4. Results and discussion

Previous studies of aqueous samples of piromidic acid in Britton–Robinson buffer at different pH values in the range 2–12, and in $HClO_4$ media at different concentrations, were carried out by square wave voltammetry, differential pulse polarography and cyclic voltammetry. It could be observed, by square wave voltammetry, that the analyte is electroactive up to pH 8.5 in Britton–Robinson buffer and in $HClO_4$ media. A well-defined reduction peak, corresponding to an irreversible process in acid media was observed (Fig. 1). A linear relationship was obtained between peak intensity and scan rate by cyclic voltammetry. It was observed that the reduction process corresponds to an irreversible adsorptive process (Fig. 1). This phenomenon could also be seen with the temperature coefficients in Sampled DC.

Taking into account the adsorptive nature of the process, a method of adsorptive stripping voltammetry, by using square wave voltammetry, has been developed for the determination of piromidic acid. In Fig. 2, voltammograms of piromidic acid, with and without accumulation process, are shown.

As the obtained response, peak intensity, was notably influenced in the stripping techniques by variables such as perchloric acid concentration, accumulation potential and accumulation time,

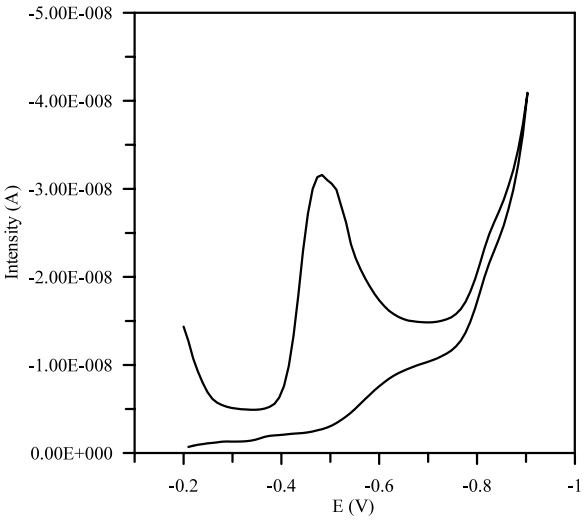


Fig. 1. Cyclic voltammogram of piromidic acid 4.44×10^{-5} mol L^{-1}. Scan rate, 40 mV s^{-1}; $[HClO_4] = [NaClO_4] = 0.05$ mol L^{-1}.

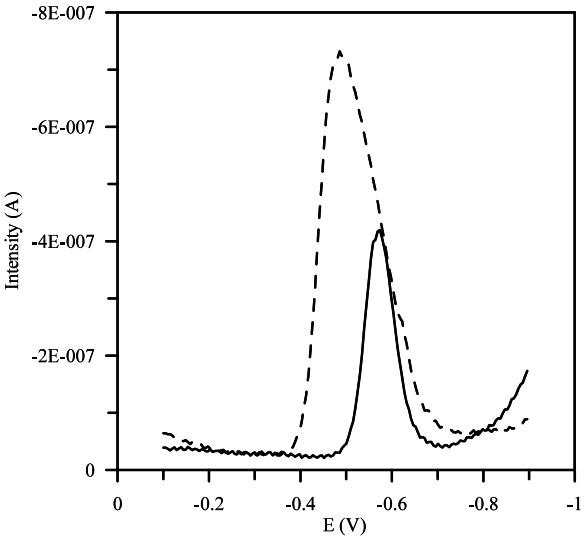


Fig. 2. Square wave adsorptive stripping (---) (potential of accumulation, −0.2 V; accumulation time, 30 s; concentration of perchloric acid, 0.05 mol L^{-1}; concentration of sodium perchlorate, 0.05 mol L^{-1}; step potential, 5 mV; square wave amplitude, 50 mV; frequency, 165 Hz; stirring, 500 rpm) and square wave (—) voltammograms of piromidic acid 1.94×10^{-7} mol L^{-1}.

RSM was used for the optimisation of these variables.

Previous studies about the influence of instrumental variables (square wave amplitude, step potential, frequency, drop size and stirring rate) were also performed, and the values for these variables were selected to get maximum repeatability in the measure of peak intensity. These chosen values were 50 mV for square wave amplitude, 10 mV for step potential, 150 Hz for frequency, 0.52 mm^2 for drop size, and 500 rev min^{-1} for stirring rate.

A CCD was carried out in order to determine the values that lead to the best peak intensity values. A CCD for three factors involves fourteen runs and the central points (six in our case). The individual experiments were performed in a randomised sequence and the operation conditions were constant through all of these experiments. The value of the considered variables, which correspond to the high (+) and low (−) levels and to the central point (0) for each factor, are the following:

Table 1
Results of the CCD for the optimisation of experimental parameters by SW-AdSV ($C_{\text{Piromidic acid}} = 1.94 \times 10^{-7}$ mol L^{-1})

$[HClO_4]$ (mol L^{-1})	E_{acc} (V)	t_{acc} (s)	Peak intensity (nA)
0.010	−0.25	120	133
0.060	−0.25	120	130
0.035	−0.50	120	126
0.035	0.002	120	118
0.035	−0.25	52.7	70.6
0.035	−0.25	187	175
0.020	−0.40	80	93.6
0.050	−0.40	80	91.4
0.020	−0.10	80	97.6
0.050	−0.10	80	95.3
0.020	−0.40	160	162
0.050	−0.40	160	153
0.020	−0.10	160	165
0.050	−0.10	160	158
0.035	−0.25	120	137
0.035	−0.25	120	132
0.035	−0.25	120	148
0.035	−0.25	120	150
0.035	−0.25	120	145
0.035	−0.25	120	151

$[HClO_4](+) = 0.050$ mol L^{-1} $E_{dep}(+) = -0.10$ V $t_{dep}(+) = 160$ s
$[HClO_4](0) = 0.035$ mol L^{-1} $E_{dep}(0) = -0.25$ V $t_{dep}(0) = 120$ s
$[HClO_4](-) = 0.020$ mol L^{-1} $E_{dep}(-) = -0.40$ V $t_{dep}(-) = 80$ s

The results of the analysis are given in Table 1 and the results from the ANOVA is presented in Table 2. As it can be seen only the accumulation time, between the lineal effects, is statistically significant, being affected by a positive sign and also are significant the quadratic effects of perchloric acid, accumulation potential and accumulation time. All the remaining effects are not statistically significant. Fig. 3 shows the predicted values of response at various combinations of two variables, holding constant at its zero level the other factor. This figure allows to visualise the way in which these combinations affect to the peak intensity.

Fig. 3a shows the response surface as a function of perchloric acid concentration and accumulation potential levels, appearing a zone of maximum response.

Table 2
ANOVA with the data of the CCD for the optimisation of experimental parameters by SW-AdSV ($C_{\text{Piromidic acid}} = 1.94 \times 10^{-7}$ mol L^{-1})

	SS	DF	MS	F-ratio	P-value
Variable					
A: $[HClO_4]$	47.783	1	47.783	1.201	0.2988
B: E_{acc}	0.440	1	0.440	0.01105	0.9183
C: t_{acc}	13 890	1	13 890	349.291	0.0000*
AB	0.450	1	0.450	0.01130	0.9174
AC	16.524	1	16.524	0.415	0.5337
BC	0.00337	1	0.003368	0.0000847	0.9928
AA	207.735	1	207.735	5.222	0.0454*
BB	737.937	1	737.937	18.552	0.0015*
CC	679.506	1	679.506	17.083	0.0020*
Model check					
Linear	13 950	3	4649	116.877	
Quadratic	1402	6	233.591	5.872	0.0074*
Error	397.772	10	39.777		
Lack of fit					
Lack of fit	102.939	5	20.588	0.349	0.8636
Pure error	294.833	5	58.967		
Total error	397.772	10	39.667		

SS, sum of squares; DF, degrees of freedom; MS, mean squares; F-ratio, MS_{factor}/MS_{error}; P-value, probability level.
* Significant factors at $\alpha = 0.05$. $R^2 = 0.975$.

Fig. 3b shows the response surface as a function of perchloric acid concentration and accumulation time levels. It is significant the effect of the accumulation time compared to the effect of the acid concentration.

Fig. 3c shows the response surface as a function of accumulation potential and accumulation time levels, being the effect of the accumulation time much more important than the effect of accumulation potential.

Other efficient way to study and locate the optimum can be carried out by using contour plots (representations of the response surfaces, obtained through its projection onto a plane of the factors by means of isoresponse curves). The contour plot for the peak intensity is plotted in Fig. 3d as a function of perchloric acid and accumulation potential level (similar to Fig. 3a). This figure shows that the magnitude of the response is almost constant when the variable ranges are 0.020–0.045 M for acid concentration, and (-0.36) to (-0.16) V for the accumulation potential.

The mathematical analysis leads to an accumulation time of 216 s, out of the experimental domain. Nevertheless, since for values of accumulation time higher than 140 s there is a risk of lack of linearity between the peak intensity and the accumulation time (Fig. 4), 140 s was selected for this variable, which is also decreasing the analysis time.

From all above, the working conditions were selected as follows: accumulation time, 140 s; perchloric acid concentration, 0.025 M; and accumulation potential, -0.25 V.

In order to evaluate the robustness of the proposed method, a fifteen experiments Box-Behnken design, for three variables ($[HClO_4]$, E_{acc} and t_{acc}) was utilised. The variation of the parameters in relation to proposed values (central level) were: $[HClO_4] \pm 0.005$ M; $E_{acc} \pm 0.01$ V, and $t_{acc} \pm 5$ s (these intervals of variation are wider than those ones due to experimental and instrumental errors). A response surface regression analysis for the peak intensity was performed, using a quadratic model. The probability values

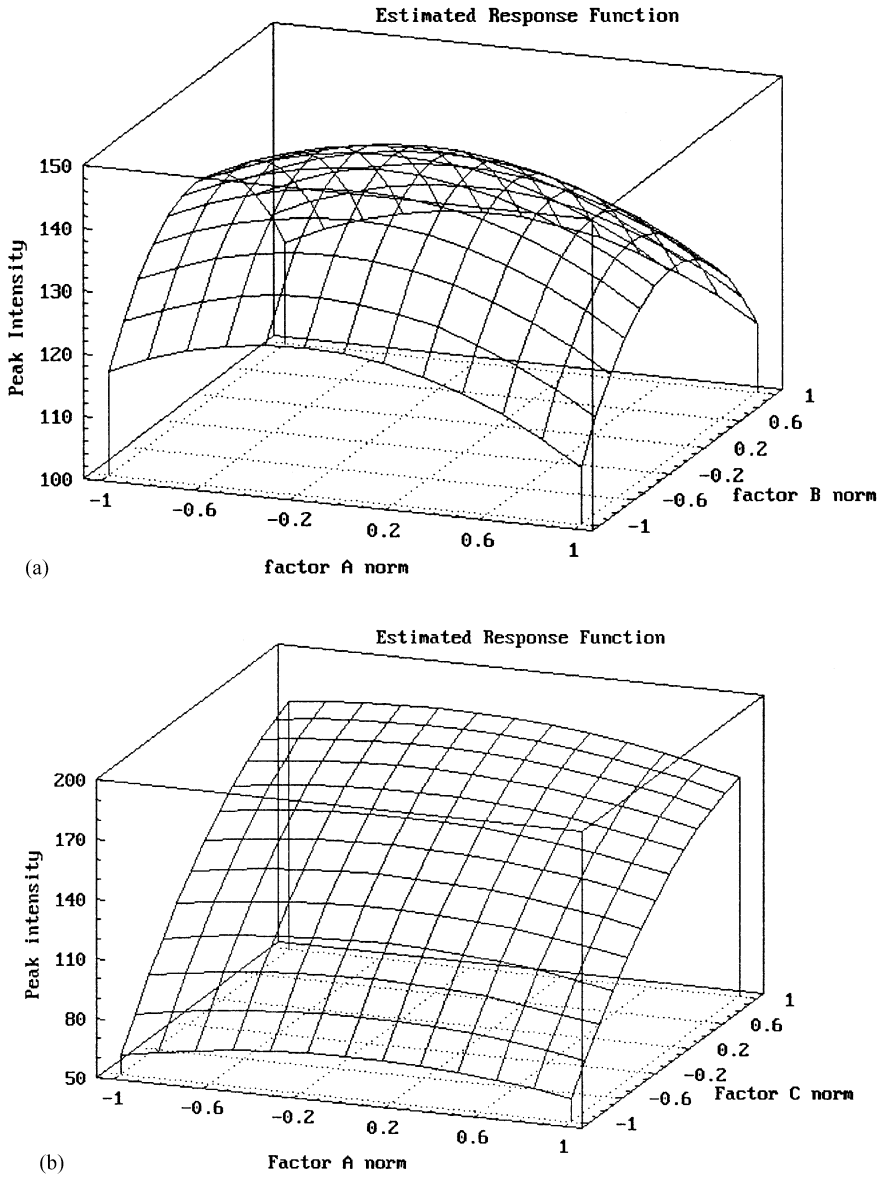
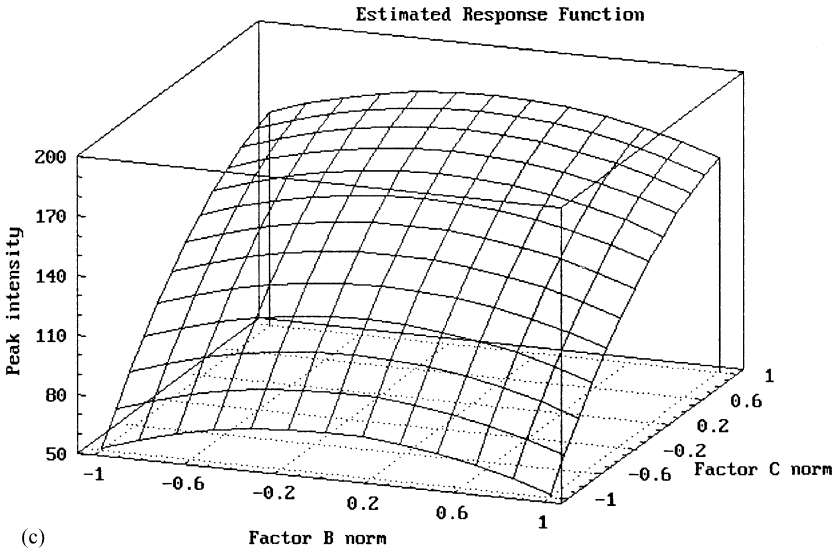


Fig. 3. (a) Response surface obtained plotting [$HClO_4$] (A) against E_{acc} (B) from codified data. (b) Response surface obtained plotting [$HClO_4$] (A) against t_{acc} (C) from codified data. (c) Response surface obtained plotting E_{acc} (B) against t_{acc} (C) from codified data. (d) Isoresponse obtained plotting [$HClO_4$] (A) against E_{acc} (B) from codified data.

(P-values) are shown in Table 3. Since all these P-values are higher than 0.05 it is deduced that, in the intervals above mentioned, there are neither factors nor effects between factors that affect significantly to the response. Therefore the robustness of the method is confirmed with the obtained results.

5. Calibration and detection limit

Once the optimum parameters for the analysis were chosen, a calibration graph, obtained by applying univariate linear regression, was established in the range 2.22×10^{-9} to 3.33×10^{-8} mol L^{-1}, according to the procedure indicated in



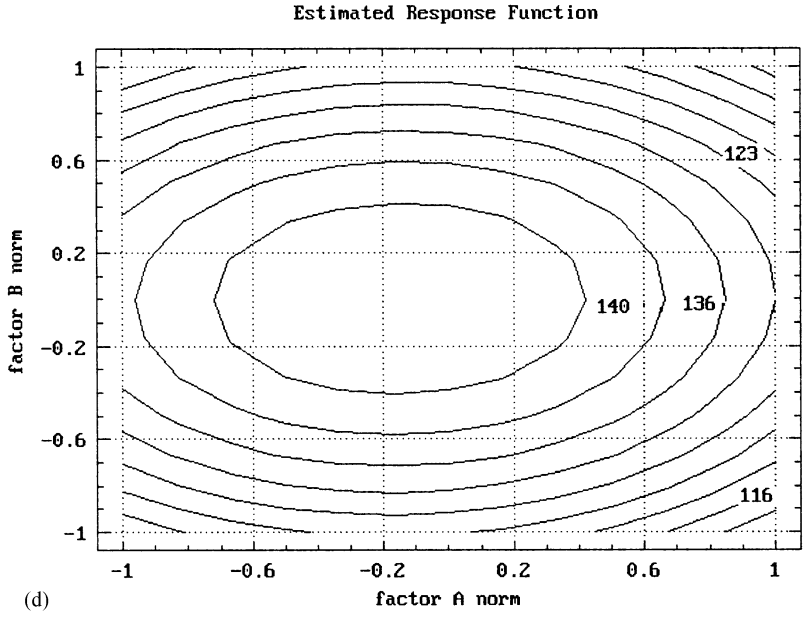


Fig. 3 (*Continued*)

Section 2. Three replicates were used for each concentration. Statistical parameters are given in Table 4. The analysis of the results from the ANOVA was made. It could be seen that a linear relationship is adequate to model the data, because the lack of fit is not significant at the 95% confidence level. The detection limit was calculated by using the method proposed by Clayton et al. [33], with a probability of false positive (α) and false negative (β) errors of 0.05. The obtained value was 1.65×10^{-9} mol L^{-1}. About repeatability ($C_{\text{Piromidic acid}} = 2.08 \times 10^{-8}$ mol L^{-1}),

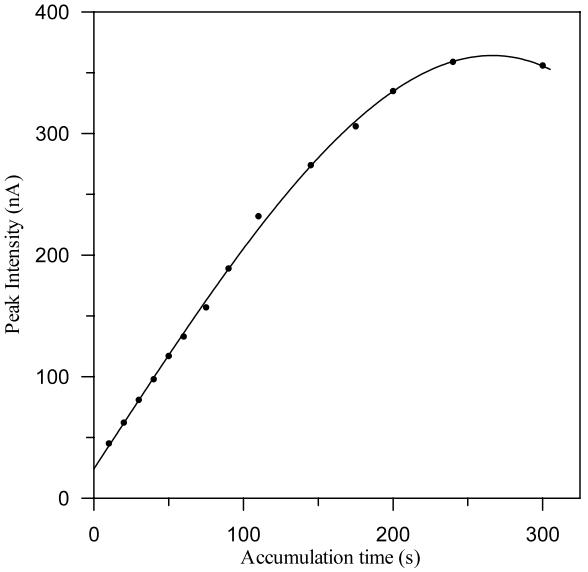


Fig. 4. Influence of accumulation time on peak intensity ($C_{\text{Piromidic acid}} = 1.94 \times 10^{-7}$ mol L^{-1}).

Table 3
Robustness test: P-values

	P-value
Variable	
A: [$HClO_4$]	0.655
B: E_{acc}	0.057
C: t_{acc}	0.472
AB	0.137
AC	0.148
BC	0.817
AA	0.948
BB	0.072
CC	0.910

Table 4
Statistics and performance characteristics of the analytical method from the calibration data set

Number of data[a]	6
Slope (nA mol L^{-1} l)	4.13×10^{9}
Intercept (nA)	1.19
Analytical sensitivity[b] (mol L^{-1})	7×10^{-10}
Coefficient of determination (R^2)	0.9964
Detection limit (mol L^{-1})	1.65×10^{-9}

[a] By triplicate.
[b] See Ref. [34].

when the peak intensity was measured, the obtained value for the error was 4.1% ($n = 11$).

6. Application to the determination of piromidic acid in urine

The concentration of piromidic acid was determined by SW-AdSV, in spiked urine samples, with three known concentrations of analyte (by triplicate) (see Table 5), to evaluate the accuracy of the proposed method. Standard addition and external standard methods were carried out. Both methods provide similar slope values. Good agreement was obtained between the concentrations found and the values of the real concentrations in the spiked samples. SW-AdS voltammograms of spiked and unspiked urine samples are shown in Fig. 5.

7. Conclusions

A square wave adsorptive striping voltammetric procedure for the determination of the antibacterial piromidic acid is presented. The set of values for the variables influencing this stripping technique, perchloric acid concentration, accumulation potential and accumulation time, was optimised using RSM. The proposed method was successfully applied to human urine, at nanomole levels, and proved to be a simple, highly sensitive, highly accurate, fast and low cost method. Other advantage of the developed method is that it is not necessary a pre-treatment of cleaning of the sample, due to the high sensitivity of the used technique that allows a dilution of the urine, and

Table 5
Recovery values of piromidic acid in spiked urine samples

Piromidic acid added in urine (ppm)	Mean piromidic acid found in the urine ($n = 3$)	RSD ($n = 3$)
200.0	206.6	0.6
300.0	291.9	1.5
400.0	390.4	2.3

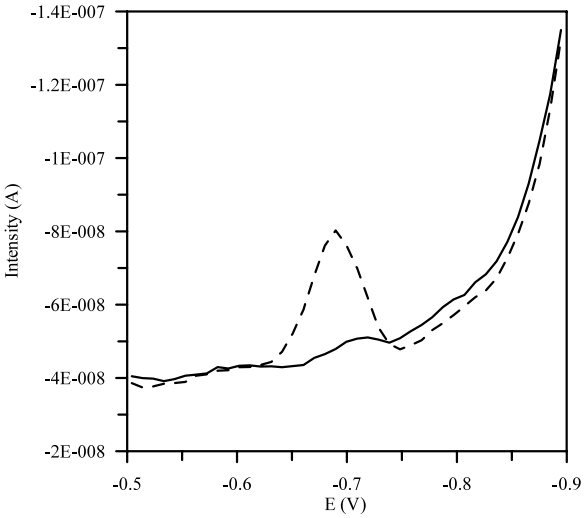


Fig. 5. Square wave adsorptive stripping voltammograms for urine sample (solid line), and for spiked urine sample with piromidic acid (dashed line).

consequently the elimination of the possible interferences, in a great measure.

Acknowledgements

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