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FOOD ANALYSIS

SQUARE-WAVE VOLTAMMETRIC METHOD FOR DETERMINATION OF FUMARIC AND MALEIC ACID—DETERMINATION OF FUMARIC ACID IN WINE

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ABSTRACT

A square-wave voltammetric method for determination of fumaric and maleic acid is developed. Both acids undergo totally irreversible redox reaction in neutral and acid media. The square-wave voltammetric response of the investigated acids is sensitive to pH, concentration of acids, type of the supporting electrolyte, as well as of the exciting signal parameters, such as frequency, amplitude and scan increment. All the experimental conditions as well as the instrumental parameters for quantitative determination of both acids

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were optimized. The detection limits of 116 μg/L for fumaric acid, and 230 μg/L for maleic acid are estimated. The proposed method enables simultaneous determination of fumaric and maleic acid. The method is applied for determination of fumaric acid in 10 kinds of Macedonian wines.

Key Words: Square-wave voltammetry; Fumaric acid; Maleic acid; Wine

INTRODUCTION

The demand for detection of substances of environmental, clinical and forensic significance at a trace levels is growing tremendously. The electrochemical techniques, particularly the pulse voltammetric techniques, have appeared as a powerful and cheap tool in analytical chemistry. Among them, square-wave voltammetry (SWV) is the most advanced electroanalytical technique. It is well suited method for mechanistic study of the electrode reactions, as well as for quantitative determination of the electroactive species (1–6). The fast scan rate together with the large signal amplitude, as well as the ability to discriminate against the capacitate current, are some of the advantages of SWV, which make it a technique of choice developing a particular electroanalytical method (1–9). In our previous work we have applied this technique to develop a method for quantitative determination of aconitic acid (6).

In the present work we have investigated the voltammetric behavior of fumaric and maleic acid in order to develop an analytical method for their quantitative determination. Maleic and fumaric acid as polycarboxilic acids with one double bond are geometric isomers and hence exhibit different physical and physiological properties. Unlike the fumaric acid, which is often used as a foodstuff additive and as a row material for pharmaceutics, plasticizers, synthetic resins etc., the maleic acid is toxic, both to humans and animals, having a harmful effect on the respiratory system and urinary tract (10). It was recently found that fumaric acid has been used with some success to alleviate psoriasis symptoms (11). Several papers have been published on separation and determination procedures of fumaric and maleic acid utilizing the high-performance liquid chromatography (HPLC) in different type of samples such as wine, musts and vinegar (12–13).

Initial reports for the electrochemical properties of both acids at a DME can be found in the works of Elving et al. (14–15). Additional information about polarographic properties of both acids have been collected by

Spirevska et al. (16–17). The presence of a double C=C bond, conjugated with acid carbonyl groups makes the *cis*-*trans* isomers maleic and fumaric acids polarographically active compounds. The scheme for reduction of both acids can be represented as follows:

$$\mathrm{HOOCHC{=}CHCOOH} + 2e^- + 2H^+ \rightarrow \mathrm{HOOCH_2C{-}CH_2COOH}$$

The authors mentioned above, have found that the polarographic responses of both acids are sensitive to pH. Also, they observed one, two or three polarographic waves depending on pH of solutions as well as a shift of the half-wave potential of the acids in negative direction by increasing of the pH.

The analysis of fumaric acid in wines is of considerable importance, because it allows the vivification process to be controlled. Due to the low concentration of fumaric acid in a variety of wine samples (ranges from 0 to 100 mg/L) (12–13) a sensitive and low-cost analytical method for a routine analysis of wine samples is neccessary to be developed. For this purpose an attempt to develop a square-wave voltammetric method for quantitative determination of fumaric acid in wine has been made.

EXPERIMENTAL

All chemicals (fumaric acid, maleic acid, KNO_3, citric acid, NaH_2PO_4, HCl, borax and NaCl, methanol, ethanol, obtained from MERCK) were reagent grade and used as received. The stock solutions of fumaric and maleic as well as all other solutions used for voltammetric measurements were prepared with twice distilled water. The general voltammetric behavior of fumaric acid has been investigated in a various supporting electrolytes with different pH values. The results for the peak potential – E_p, peak current – I_p, as well as half-peak width – $\Delta E_{p/2}$ of the SW responses are listed in the Table 1.

The voltammetric measurements were performed with a Princeton Applied Research Model 384B Polarographic Analyzer. A static mercury drop model 303A with a surface area of 0.0149 cm^2 was used as the working electrode. An Ag/AgCl (sat. KCl) was the reference electrode and Pt wire was the counter electrode.

The solutions were degassed with high-purity nitrogen for 8 min prior to the measurements and for an additional 20 s before each scan. A nitrogen atmosphere was maintained throughout the experiments. Each scan was done on a separate mercury drop at room temperature.

Table 1. The SW Voltammetric Response Parameters as a Function of the Supporting Electrolytes Tested for Fumaric Acid The Conditions Were: c (fumaric acid) $= 1 \times 10^{-4}$ mol/L, $f = 120$ Hz, $E_{sw} = 20$ mV, d$E = 4$ mV

Supporting Electrolyte	I_p/μA	E_p/V	$\Delta E_{p/2}$/mV
0.1 mol/L HCl	1.620	−0.76	110
0.1 mol/L KNO_3 buffered to pH = 2.8	1.840	−0.964	100
0.1 mol/L KNO_3 buffered to pH of 3.5	1.820	−0.996	104
0.1 mol/L KNO_3 buffered to pH of 4.0	1.640	−1.176	120
0.1 mol/L KNO_3 buffered to pH of 6.2	1.800	−1.500	100
0.1 mol/L borax with pH of 8.6	/	/	/

Analytical procedure and sample preparation

A 100 mL volume of each wine sample was subjected to digestion in order to reduce the sample volume to 10 mL. Thereafter, the samples were filtered through a 0.45 μm Milipore filter and passed throughout the C_{18} cartridge. C_{18} cartridge had previously been conditioned by means of successive washes with 10 mL of methanol and 10 mL of water followed by 10 mL of 10% ethanol. The eventual residues of fumaric acid were eluted with 2 mL of 10% ethanol. The fumaric acid was not retarded on the cartridge. This was confirmed by spectrophotometric measurement of fumaric acid in standard solution at 210 nm, passed through the C_{18} cartridge. Final volume of about 10–12 mL was obtained. 100 μL volume of the concentrate were placed in the voltammetric cell which contained 10 mL buffer solution and than SW voltammograms were recorded.

RESULTS AND DISCUSSION

A. Fumaric Acid

The SW voltammetric response of fumaric acid is mainly sensitive to pH, concentration of fumaric acid, as well as of the exciting signal parameters (frequency − f, amplitude − E_{sw} and scan increment − dE).

A few square-wave voltammograms of 1×10^{-4} mol/L fumaric acid solution recorded in supporting electrolyte solutions with different pH values are presented in Figure 1. According to the properties of the voltammetric response, three different pH regions could be recognized. In the first region ($\mathrm{pH} < 3.2$) a single, well defined peak of fumaric acid (peak I in Figure 1) located at $E_\mathrm{p} = -0.880$ V was observed. Within the second region ($3.5 < \mathrm{pH} < 5.5$) the voltammetric response of fumaric acid is consisted of two SW peaks (see peaks I and II in Figure 2). Increasing pH of the medium caused both peaks to shift in negative direction. Within this region, the height of the peak I decreases gradually with increasing pH, while the peak II continuously increases. However, the sum height of these two peaks observed in the second region is equal approximately with the peak height of the response observed at $\mathrm{pH} < 3.2$.

The third pH region is characterized in supporting electrolytes with $\mathrm{pH} > 6.0$, where only a single and well defined peak of fumaric acid was observed (peak III in Figure 1), located at potentials of about $E_\mathrm{p} = -1.60$ V. The height of the peak III is almost equal with the height of the SW peak I,

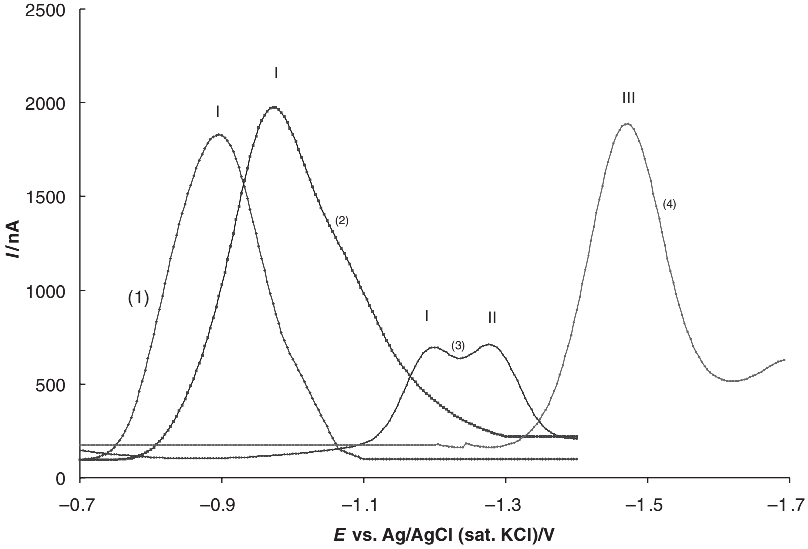

Figure 1. Square-wave voltammograms of fumaric acid recorded in 0.1 mol/L KNO_3 solutions buffered with 0.1 mol/L buffer solution of citric acid and NaH_2PO_4 to pH of 2.8 (curve 1) 3.5 (curve 2), 5.0 (curve 3) and 6.2 (curve 4). The other conditions were: c (fumaric acid) $= 1 \times 10^{-4}$ mol/L, $f = 120$ Hz, $E_\mathrm{sw} = 20$ mV, $\mathrm{d}E = 4$ mV.

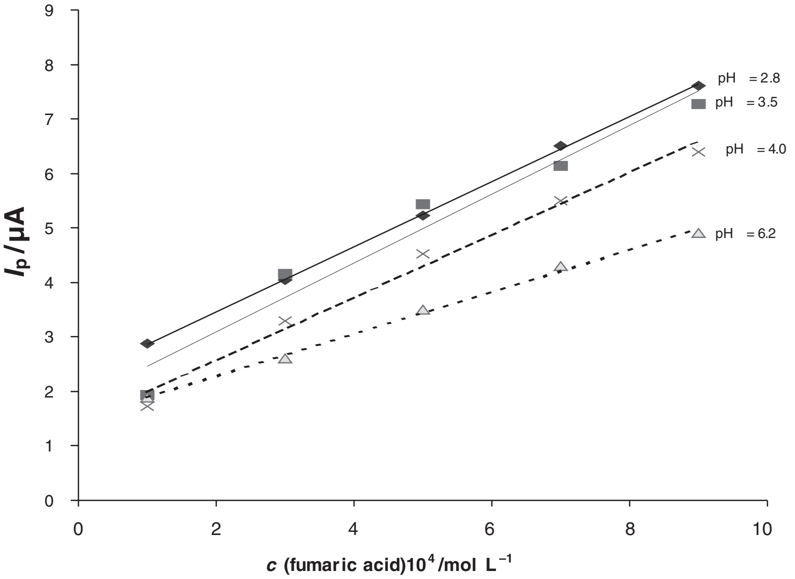

Figure 2. The calibration curves of fumaric acid recorded at different pH of the medium. The experimental conditions are the same as in Figure 1.

observed in the region with $pH < 3.2$. In the solution with $pH > 7$, the SW peak III commences decreasing intensively.

To give reasonable explanation for observed voltammetric behavior of fumaric acid at different pH, one should carefully consider the acid-base equilibrium of this dicarboxylic acid, attributing with two dissociation constants ($pK_1 = 3.45$ and $pK_2 = 4.49$) (18). Hence, depending on pH of the solutions, several different species of fumaric acid and different equilibria could exist. When $pH < pK_1$, only existing electroactive specie form in solution is undissociated form of fumaric acid (HOOCHC=CHCOOH). For this reason, only single SW peak arising from reduction of double C=C bond of undissociated fumaric acid could be expected at $pH < 3.45$ (peak I).

In the region $pK_1 < pH < pK_2$, the following equilibrium in the fumaric acid solutions exists:

$$\mathrm{HOOCHC{=}CHCOOH} \leftrightharpoons \mathrm{HOOCHC{=}CHCOO^-} + \mathrm{H^+}$$

The existence of two electroactive species in the solution causes appearance of two voltammetric SW peaks. The first one (peak I) is attributed from the reduction of undissociated fumaric acid. The second SW peak (peak II) is attributed to the mono-dissociated anionic form of fumaric acid.

Finally, at $\mathrm{pH} > \mathrm{p}K_2$, the dianionic dissociated form of fumaric acid predominantly exists, gaing raise for appearance of a single SW peak located at most negative potentials (peak III).

As shown in Figure 2, the effect of fumaric acid concentration upon the SW peak current was studied in supporting electrolytes with different pH values. The slope of I_p vs. c (fumaric acid) has the highest value in 0.1 mol/L KNO_3 solution buffered with 0.1 mol/L buffer solution of citric acid and NaH_2PO_4 to pH of 2.8, favoring this electrolyte as the most suitable for analytical purposes. So, further optimization of experimental conditions was carried out in this supporting electrolyte.

The SW frequency influences only to the peak current of the SW response of fumaric acid. The I_p is a linear function of the square root of SW frequency, indicating a diffusion-controlled electrode process. The max. ratio $I_p/\Delta E_{p/2}$ was obtained at frequency of 120 Hz.

The SW voltammetric response of fumaric acid was also sensitive to SW amplitude. Changing the SW amplitude from 10 to 50 mV causes increasing of the peak current. At SW amplitude greater than 50 mV, the shape of the SW response significantly disturbed. It should be noted that the peak potential was not affected by the variation of the SW amplitude. An amplitude of 30 mV was chosen as the most suitable for analytical purposes.

As a result of the optimization procedure, the following instrumental conditions were selected as optimal for quantitative determination of fumaric acid: frequency of 120 Hz, amplitude of 30 mV and scan increment of 4 mV. Calibration plots of fumaric acid were constructed over concentration ranges of 10^{-6}, 10^{-5} and 10^{-4} mol/L. The linear dependence between I_p and c(fumaric acid) was observed, which are associated with following regression lines:

$$I_p/\mu\mathrm{A} = 0.1338\ c\ (\mathrm{f.a.}) \times 10^{-6}/\mathrm{mol\ L^{-1}} + 0.308\ (\mathrm{R}^2 = 0.998),$$

$$I_p/\mu\mathrm{A} = 1.007\ c\ (\mathrm{f.a.}) \times 10^{-5}/\mathrm{mol\ L^{-1}} + 0.871\ (\mathrm{R}^2 = 0.992),\ \text{and}$$

$$I_p/\mu\mathrm{A} = 5\ c\ (\mathrm{f.a.}) \times 10^{-4}/\mathrm{mol\ L^{-1}} + 4.88\ (\mathrm{R}^2 = 0.992),$$

for 10^{-6}, 10^{-5} and 10^{-4} mol/L concentration regions, respectively.

The detection limit (three times to noise) of 116 μg/L fumaric acid was estimated. The reproducibility of the method varied from 94.50 to 102.80% (eight measurements at 5×10^{-6} mol/L, 1×10^{-5} mol/L and 5×10^{-5} mol/L fumaric acid concentrations), followed with standard deviation of 0.45 to 3.20%.

B. Maleic Acid

The electrochemical behavior of maleic acid is quite similar to the behavior of fumaric acid. Figure 3 shows the voltammetric responses of 1×10^{-4} mol/L maleic acid solutions recorded in 0.1 mol/L KNO_3 solutions as a supporting electrolyte buffered to different pH values with 0.1 mol/L buffer solution of citric acid and NaH_2PO_4. A single reduction peak at potential of about −0.85 V exists in solutions with pH up to 2.8. Changing pH value from 3.0 to 5.6 two peaks of maleic acid were observed, separated each from other about 200 mV. At pH > 5.6 a single peak of maleic acid was observed again. The origin of these three SW peaks of maleic acid is quite identical as in the case of fumaric acid. The three distinct pH regions characterizing the voltammetric behavior of maleic acid are

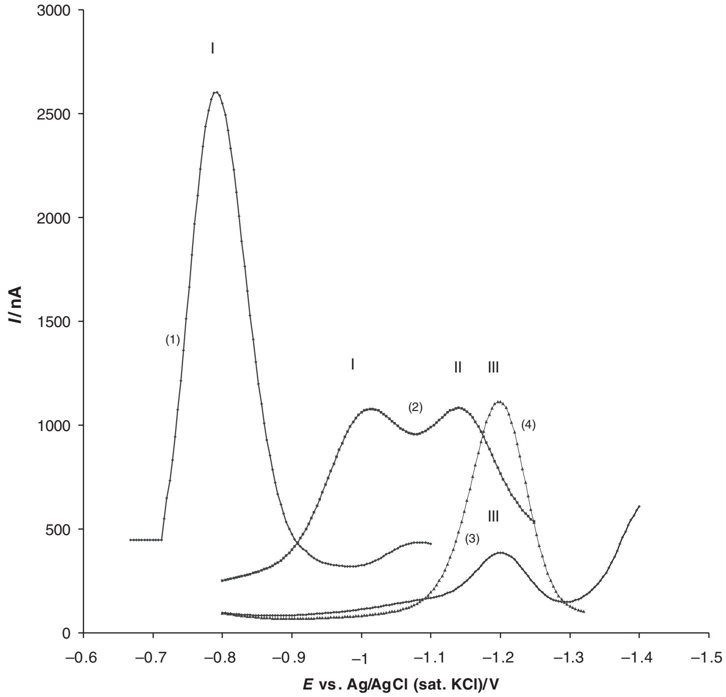

Figure 3. Square-wave voltammograms of maleic acid recorded in 0.1 mol/L KNO_3 solutions buffered to pH of 2.5 (curve 1), 4.0 (curve 2), 5.6 (curve 3) and 6.0 (curve 4). The concentration of maleic acid was $c(\text{maleic acid}) = 1 \times 10^{-4}$ mol/L. The other conditions were: $f = 120$ Hz, $E_{sw} = 20$ m, $dE = 4$ mV.

determined by its dissociation constants which reads $pK_1 = 1.83$ and $pK_2 = 6.07$ (18).

The calibration plot constructed at pH of 1.8 (where single peak was observed) is more useful for quantitative determination of maleic acid as a single compound. Namely, the greatest slope of I_p vs. c (maleic acid) in this case exists.

Generally the SW voltammetric response of maleic acid depends on the exciting signal parameters (f, E_{sw}, dE) almost in same way as for fumaric acid. So, frequency of 120 Hz, SW amplitude of 25 mV and scan increment of 4 mV were chosen as optimal for quantitative determination of maleic acid. The calibration curves were constructed over 10^{-6}, 10^{-5} and 10^{-4} mol/L concentration ranges of maleic acid (R^2 greater than 0.998), with the standard deviation of the results ranged from 0.5% to 4.8%. The detection limit of 230 μg/L maleic acid was found.

C. Mixture of Maleic and Fumaric Acid

It is well known that even though voltammetric measurements may seem to involve the reactivity of individual elements or functional groups (the double C=C bond in the case of fumaric and maleic acid), the effective peak potentials are due to the nature of the molecular environment adjacent to the functional group. Even the molecular environment adjacent to the functioning group is same, their geometric orientation in the space (e.g. *cis* and *trans* isomerism) also produce differences in the peak potentials.

The property of the electrochemical behavior of fumaric and maleic acid enables development of analytical procedure for simultaneous determination of both acids in mixture. Such a method would be of certain interest, since these acids often could be present in various samples. Utilizing the difference in the peak potentials of the SW voltammetric responses in solution with pH of about 3.5, a square-wave voltammetric method for simultaneous quantitative determination of both acids could be developed. Namely, using the derivative square-wave voltammetry is possible to separate both overlaped peaks and a method for simultaneous quantitative determination of both acids could be developed.

D. Determination of Fumaric Acid in Some Wines from Macedonia

The proposed electrochemical method for determination of fumaric acid was successfully applied to several types of Macedonian wines. At first, separation of fumaric acid from the complex matrix sample was neccessary. For this purpose, solid-phase extraction with C_{18} cartridge was applied. This method of separation is very convennient for the wine samples where predominanted compounds are nonpolar because they can be successfully retained on the cartridge. The details of the separation procedure are described in the part ‘‘Analytical procedure and sample preparation’’.

The concentration of fumaric acid in each extracted wine sample was determined by standard addition method in 0.1 mol/L KNO_3 solution buffered to $pH = 2.8$ as a supporting electrolyte. Several voltammetric curves recorded after standard addition of fumaric acid are depicted in the Figure 4. It should be emphasized that four of each wine sample were analyzed. Also, four SW voltammograms were recorded for each probe, and the average peak current was calculated. Subsequently, several standard concentrations of fumaric acid were added in the cell and for each addition four square-wave voltammograms were recorded. The concentration of

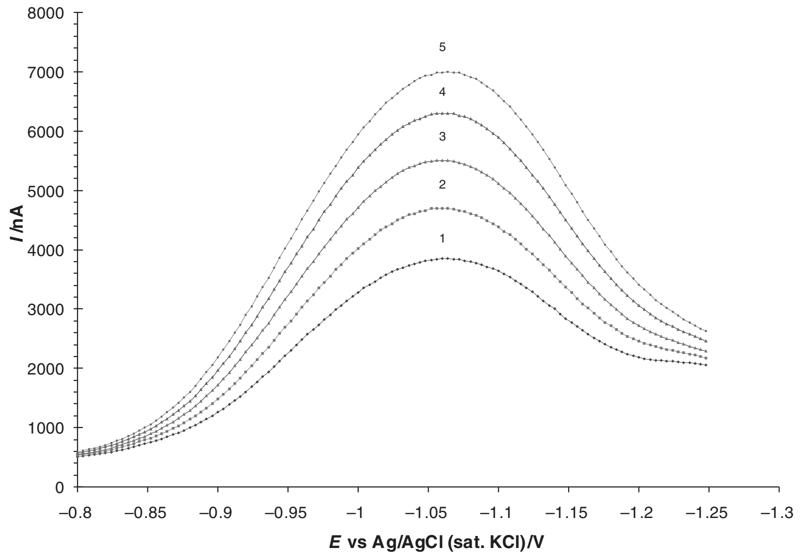

Figure 4. Standard addition SWV method for determination of fumaric acid applied to the ‘‘Smederevka’’ wine. The curve 1 is from sample wine without standard, and the curves 2, 3, 4 and 5 contains 2, 4, 6 and 8×10^{-4} mol/L standard solutions of fumaric acid, respectively. The experimental conditions were $E_{sw} = 30$ mV, $f = 120$ mV, $dE = 4$ mV, Supporitn electrolyte was 0.1 mol/L KNO_3 solution buffered to $pH = 2.8$.

Table 2. Contents of Fumaric Acid Determined by Square-Wave Voltammetry in Some Kind of Wines from Macedonia Supporting Electrolyte 0.1 mol/L KNO_3 Buffered to $pH = 2.8$ Frequency $f = 120\,Hz$, amplitude $E_{sw} = 30\,mV$, Scan Increment $dE = 4\,mV$

Kind of Wine	Y (fum.acid)/mg·L^{-1}	St. deviation/%
Smederevka (from Tikves)	20.05	2.33
Alexandria	22.25	3.45
Belan	17.20	2.45
Temjanika	30.35	1.35
Country White	16.75	0.50
Chardonnay	24.27	3.25
ORO, Grenas Bel	20.20	2.06
Smederevka (from Veles)	15.35	1.85
Belo Velesko	22.85	0.80
Ohridija	18.60	3.60

*each data represents mean of the voltammetric measurements of four samples.

the fumaric acid in each wine sample was estimated according to the formula (19):

$$Y = X + \left|\frac{Y_o - Y_a}{m}\right|,$$

where Y is unknown concentration of fumaric acid in wine sample, X is a concentration of the standard added in the voltammetric cell, Y_o is average peak current obtained from the wine sample, Y_a is average peak current when standard of fumaric acid with concentration X was added in the wine sample and m is a slope of the linear regression line of the dependency $I_p - c$ (standard of fumaric acid), obtained by the least squares method.

The results for concentration of fumaric acid obtained by this method in 10 kinds of wines from Macedonia are listed in Table 2. The concentration of fumaric acid in wine samples ranges from 15 to 30 mg/L. The obtained results are in good agreement with the results presented in the literature (12–13).

CONCLUSION

In summary, square-wave voltammetry is a sensitive, rapid, precise and accurate technique for determination of fumaric and maleic acid, and by extension, of other unsaturated organic acids. This technique is also attractive due to the relatively low cost of the instrumentation and short time required for the analysis. The disadvantages of this approach are addressed to the determination in complex sample types (such a wine). Namely, the existing surface active compounds in the wine samples disable voltammetric determination of fumaric acid instantaneously from the wine. However, these disadvantages could be solved by means of separation techniques. The proposed method for quantitative determination of fumaric acid is a good alternative to other methods based on the HPLC determination (12–13). Proposed method is simple and rapid, enabling easy sample preparation, and hence it could be applied for routine analysis of fumaric acid in wine samples.

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