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Determination of Ascorbic Acid in Tablet Dosage Forms and Some Fruit Juices by DPV

Selehattin Yilmaz[1], *Murat Sadikoglu*[2,*], *Gulsen Saglikoglu*[1], *Sultan Yagmur*[1], *Gokce Askin*[1]

[1] Canakkale Onsekiz Mart University, Faculty of Science and Arts, Department of Chemistry, 17020, Canakkale, Turkey
[2] Gaziosmanpasa University, Faculty of Education, Department of Science Education, 60100, Tokat, Turkey
[*]E-mail: murat.sadikoglu@yahoo.com

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Ascorbic acid, a water-soluble vitamin, is the most common electroactive biological compound found in some fruit species. The electrochemical oxidation of ascorbic acid was investigated at glassy carbon electrode in various aqueous solutions in the pH range of 0.64-10.15 (in Britton-Robinson, acetate, phosphate buffers and 0.5 mol L^{-1} sulphuric acid solutions) by cyclic (CV) and differential pulse (DPV) voltammetry. For the quantitative purposes, a very well-resolved diffusion-controlled voltammetric peak was obtained in 0.2 mol L^{-1} acetate buffer (pH 3.49) at 0.342 V by DPV technique. The linear response was obtained in the concentration range of 6×10^{-6}-8×10^{-4} mol L^{-1} with a detection limit (LOD) of 5.17×10^{-7} and quantitative limit (LOQ) of 1.72×10^{-6} mol L^{-1}. Ascorbic acid in pharmaceutical dosage forms and some fruit juices were determined by DPV. The reliability of the proposed voltammetric technique was established by recovery studies.

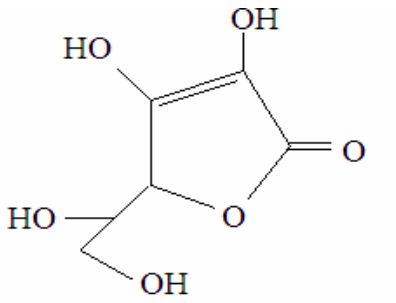
Keywords: Ascorbic acid; Voltammetry; Tablet dosage forms; Fruit juices

1. INTRODUCTION

Ascorbic acid (Scheme 1), a water-soluble vitamin (Vitamin C), is important in forming collagen, a protein that gives structure to bones, muscles and blood vessels. It is one of the most ubiquitous vitamins ever discovered. Besides plays a paramount role as an antioxidant and free radical scavenger, it has been suggested to be an effective antiviral agent [1, 4].

In addition, ascorbic acid has been widely used in the pharmaceutical, chemical, cosmetic and food industry as antioxidant. Therefore, there is a need to find an accurate, reliable, rapid, and easy-to-implement method for measuring the amount of ascorbic acid in a sample. However, there have been difficulties in quantifying ascorbic acid due to its instability in aqueous solution. The instability of

ascorbic acid is due to its oxidation to dehydroascorbic acid, which is a reversible reaction, and subsequently to 2,3-diketo-L-gulonic acid. The later reaction is irreversible [5].



Scheme 1. The chemical structure of ascorbic acid.

Various methods have been employed for the quantitative determination of ascorbic acid in pharmaceutical formulations, fruit juices, urine, plasma etc. These include titration [6, 7], HPLC [7, 10], UV [5,11-13], fluorimetry [14, 15], flow analysis [16, 17], turbidimetry [18] and potentiometry [19]. The electrochemical behavior of ascorbic acid has been examined in detail [20, 23].

The development of a new method to determine of ascorbic acid in pharmaceutical dosage forms is important. Electroanalytical techniques have been used for the determination of a wide range of drug compounds. These techniques are less sensitive toward the matrix effects than the other analytical techniques and there is no need the derivatization of the samples. Furthermore, the application of electrochemistry includes the determination of the redox mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity [1, 24-27].

In this work, DPV technique for the direct quantitative determination of ascorbic acid in tablet dosage form and the some fruit juices was used. Consequently, an electroanalytical method, which is the new, rapid, simple and inexpensive, to determine of ascorbic acid was aimed to be developed. The electrochemical oxidation and determination of ascorbic acid has been carried out at glassy carbon (GC) electrode in various aqueous solutions in the pH range of 0.64-10.15. It is shown that our work describes to be validated of the simple, rapid, sensitive and selective procedures for the oxidation mechanism and quantitative determination of ascorbic acid by CV and DPV techniques on a bare GC surface.

2. EXPERIMENTAL PART

2.1. Apparatus

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) was used for the voltammetric measurements, with a three-electrode system consisting of a glassy carbon working electrode (GCE; 3 mm diameter , Metrohm), a platinum wire auxiliary electrode and Ag/AgCl (NaCl/

3 M, Metrohm) reference electrode. Before each measurements, glassy carbon electrode was polished successfully with polishing alumina (prepared from in 0.01μm aluminum oxide slurries) on alumina polish pad then rinsed with ultra pure deionize water and acetone. All measurements were carried out after solutions deoxidation with argon for 5 min (and for 60 s before each measurement).

All pH measurements were made with Model Metrohm 744 pH meter (Herisau, Switzerland) at ambient temperature of the laboratory (25-30 °C). For the analytical application, the following parameters being employed: pulse amplitude 50 mV; frequency 50 Hz, voltage step 9 mV (DPV); the scan rate in the range 10-1000 mVs^{-1} (CV).

2.2. Reagents

Ascorbic acid tablet dosage form was kindly supplied by Bilim Inc. (Istanbul, Turkey) and powdered form was supplied by Merck. A stock solution of 1.0×10^{-2} mol L^{-1} was prepared by dissolving an accurate mass of the pure ascorbic acid in an appropriate volume of ultrapure-deionize water and freshly used. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution by ultrapure-deionize water. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.5 mol L^{-1} sulphuric acid; pH: 0.64 (Riedel, Germany, 95-97 % m/m), 0.067 mol L^{-1} phosphate buffer (pH 4.5-7.5): natrium hydogen phosphate (Na_2HPO_4, Riedel, Seelze, Germany) and natrium dihydrogen phosphate (NaH_2PO_4, Riedel, Seelze, Germany), 0.2 mol L^{-1} acetate buffer (pH 3.51-5.51): (Acetic acid: Riedel, Seelze, Germany, 100 m/m % and natrium hydroxide: Riedel, Seelze, Germany) and 0.04 mol L^{-1} Britton-Robinson buffer (pH 2.16-11.15): (acetic acid: Riedel, Seelze, Germany, 100 m/m %; boric acid: Merck, Darmstadt, Germany, and phosphoric acide: Carlo Erba, Rodeno, France, 85 m/m %) were used for the supporting electrolytes. Ultra pure-deionize water was obtained from Sartorius Arium model Ultra Pure Water Systems and was used to prepare supporting electrolytes. Other chemicals, all of analytical-reagent grade (Merck) were used. Estervit C® tablets (Bilim, Inc, Istanbul, Turkey) labeled to contain 500 mg ascorbic acid per tablet.

2.3. Calibration graph for voltammetric determination

Ascorbic acid was dissolved in ultra pure-deionize water to obtain 1×10^{-2} mol L^{-1} stock solution. This solution was diluted with ultra pure-deionize water to obtain diluted ascorbic acid concentrations. For optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the ascorbic acid concentration range 8×10^{-6}-8×10^{-4}. The repeatability, accuracy and precision were checked (Table 1).

2.4. Procedure for the analysis of estervit C tablets and recovery studies

Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-2} mol L^{-1} was weighed transferred into a 10

mL calibrated flask and completed to the volume with ultrapure-deionize water. The contents of the flask were centrifuged for 15 min at 4000 rpm to 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 selected supporting electrolyte solution. Each solution was transferred into the voltammetric cell the pure ascorbic acid. The initially, these solutions were deaerated with argon (analytically pure with 99.99 %) for 5 min and than for 60 s before each measurement. The nominal content of the corresponding regression equations of previously was plotted calibration plots (Table 2).

Table 1. Quantitative analyses parameters of ascorbic acid calibration plots in acetate buffer solution (pH 3.50) at GCE by differential pulse technique.

Parameters	Results
Measured potential (mV)	315
Linear concentration range (mol L^{-1})	8×10^{-6}-8×10^{-4}
Slope (mA L mol^{-1})	5285.1
S.D. of slope	11.87
Intercept	0.0305
S.D. of Intercept	7.34
Correlation coefficient, r	0.998
Number of measurements (n)	5
LOD (mol L^{-1})	5.16×10^{-7}
LOQ (mol L^{-1})	1.723×10^{-6}
Repeatability of peak current and Peak potential (R.S.D %)	2.16 for peak current and 1.37 for peak potential

Table 2. The found ascorbic acid values in estervit C and the mean recoveries by differential pulse technique.

Labeled claim (mg)	500
Amount found (mg)	502.25
R.S.D. %	1.57
R.S.D. %	0.45
Added (mg)	15
Found (mg)	14.34
Recovery (%)	95.64
R.S.D. % of recovery	0.64
Bias (%)	4.4

2.5. Working voltammetric procedure of some fruit juices

This study was performed according to conditions mentioned above. Ascorbic acid was determined in fresh orange, grapefruit and lemon. Founded amounts of ascorbic acid in these fruit juices were presented in Table 3.

Table 3. The concentration of ascorbic acid determined in the some fruit juices (n=5)

	Orange	Grapefruit	Lemon
The concentration of ascorbic acid (mg L^{-1}) ±s	26.89 ± 0.49	13.14 ± 0.2	21.86 ± 0.32
R.S.D. %	1.84	1.42	1.47

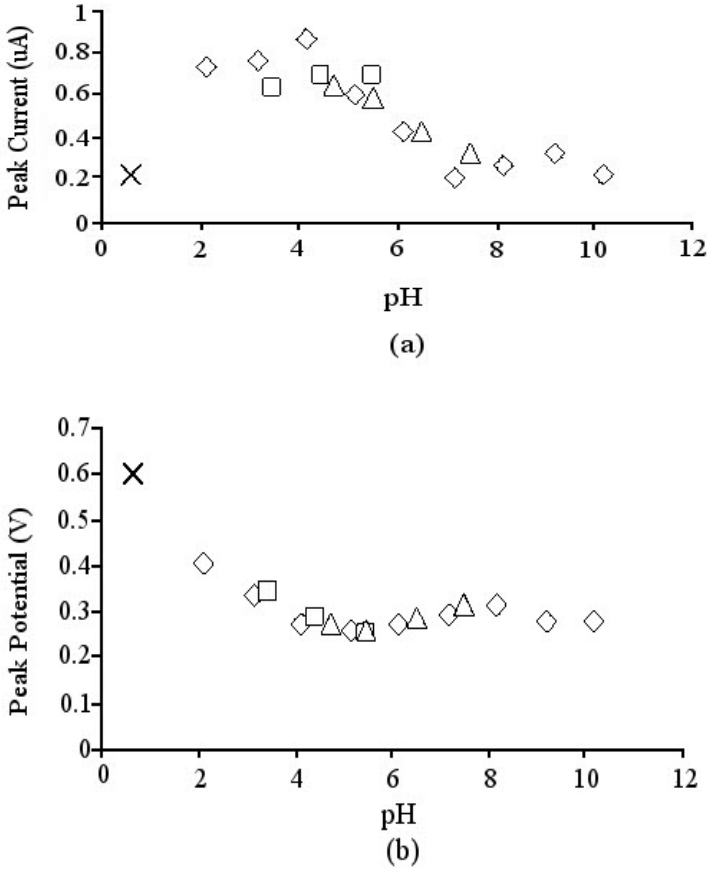


Figure 1. The effect of pH on (a) anodic peak current and (b) peak potential of $1x10^{-4}$ mol L^{-1} ascorbic acid in the different media (×) 0.5 mol L^{-1} H_2SO_4; (Δ) 0.067 mol L^{-1} phosphate buffer; ( ) 0.2 mol L^{-1} acetate buffer ; and (◊) 0.04 mol L^{-1} Britton-Robinson buffer.

3. RESULTS AND DISCUSSION

3.1. Electrochemical oxidation of ascorbic acid

In order to understand the electrochemical oxidation process of ascorbic acid occurring on the GCE, CV and DPV techniques were carried out. Typical cyclic voltammogram of 5×10^{-4} mol L^{-1} ascorbic acid at various scan rates at GCE in 0.2 M acetate buffer (pH 3.50) is given in Fig 1. In addition, the electrochemical oxidation behavior of ascorbic acid was also studied in a wide pH range (between 0.64 and 11.15) at the GCE in diluted H_2SO_4 and buffered aqueous media by DPV technique. The voltammetric response was strongly pH dependent (Fig 2). The peak potential of the anodic peak was shifted positive values with increasing pH (Fig 3).

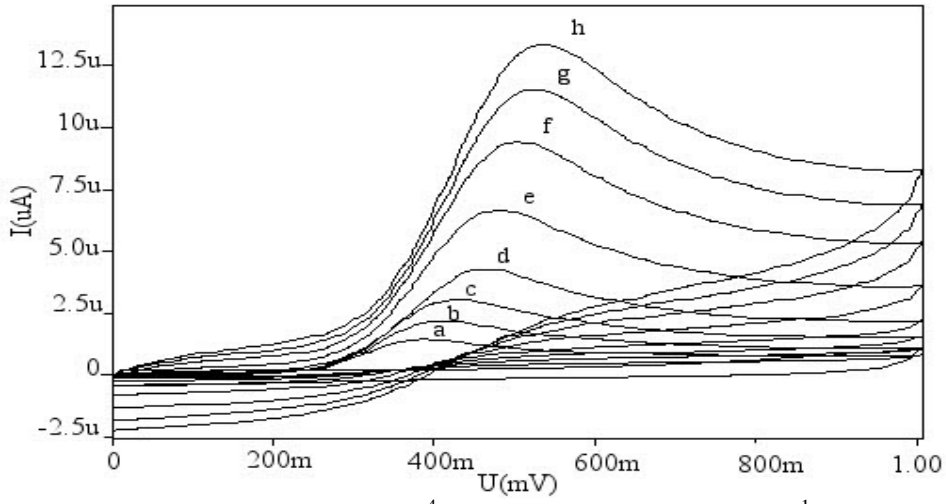


Figure 2. The cyclic voltammograms of 5×10^{-4} M ascorbic acid in 0.2 mol L^{-1} acetate buffer (pH 3.50) at GCE. Scan rates: (a) 10, (b) 25, (c) 50, (d) 100, (e) 250, (f) 500, (g) 750 and (h) 1000 mV s^{-1}.

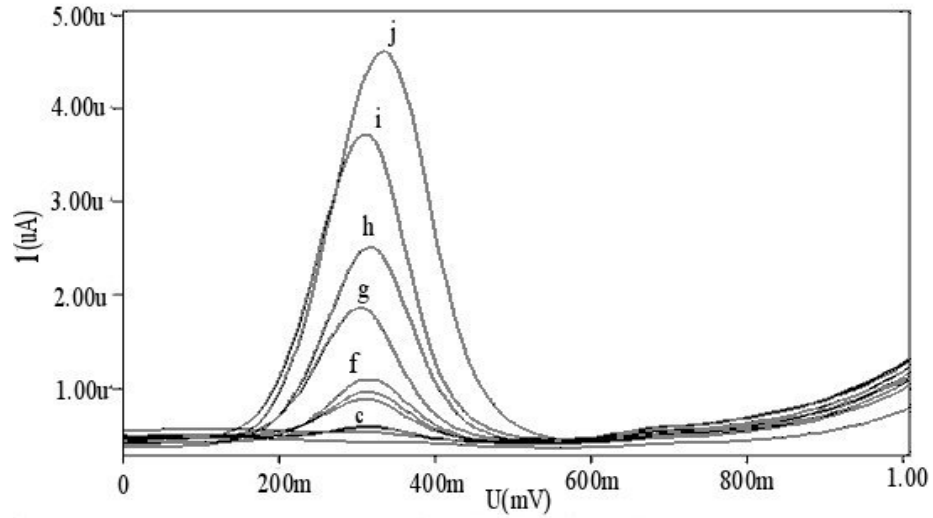
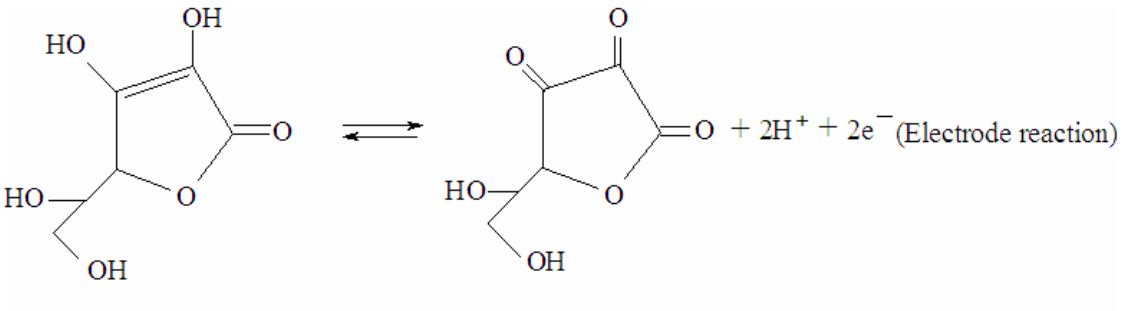


Figure 3. DPV voltammograms obtained from different concentrations of ascorbic acid in 0.2 mol L^{-1} acetate buffer at pH 3.50 (a) supporting electrolyte; (b-j) ascorbic acid : (b)1×10^{-5} mol L^{-1}, (c) 2×10^{-5}, (d) 4×10^{-5}, (e) 6×10^{-5}, (f) 8×10^{-5}, (g)1×10^{-4}, (h) 2×10^{-4}, (i) 4×10^{-4} and (j) 6×10^{-4} mol L^{-1}.

As can be seen from the Fig 2, the maximum peak current was obtained in the acidic media (0.2 M acetate buffer; pH 3.50) that indicates participation of a proton transfer in the electrode process. Also, the experimental results showed that the shape and intensity of the curves were better in acetate buffer pH 3.50. Therefore, the acetate buffer at pH 3.50 was chosen for the analytical determination.

The effect of the potential scan rates between 10-1000 mVs^{-1} on the peak current and potential of ascorbic acid was evaluated. When the potential scan rates was increased from 10 to 1000 mVs^{-1} under the same experiment conditions, a linear relationship was observed between the peak current and square root of the scan rate ($v^{1/2}$) with a correlation coefficient (r= 0.999), demonstrating that the process is diffusion controlled. Also, a plot of the logarithm of peak current versus the logarithm of the scan rate gave a straight line with a slope of 0.44, very close to the theoretical value of 0.5, which is expressed for an ideal reaction of the diffusion-controlled electrode process [1, 28, 29].

The oxidation of ascorbic acid involves two electrons and two protons to produce dehydroascorbic acid, which is followed by an irreversible salvation reaction at pH lower than 4.0. The oxidation mechanism of ascorbic acid given in the following scheme has already been known (Scheme 2).



Scheme 2. The proposed oxidation mechanism of ascorbic acid.

3.2. Analytical applications and validation of the proposed DPV method

In order to develop a voltammetric methodology for determination of the drug, we selected DPV mode, since the peak were sharply and defined well at lower concentration of ascorbic acid. The analytical parameters for the quantitative determination of ascorbic acid were given Table 1. Quantitative evaluation was based on the linear correlation between the peak current and concentration. Good correlation was obtained for ascorbic acid concentration between 6×10^{-6} and 8×10^{-4} mol L^{-1} (Fig. 2). The equation of the calibration plots was obtained as $I_p(\mu A)= 5285.1$ C (mol L^{-1}) – 0.0305 with a correlation coefficient, r=0.998, which was obtained from five measurements. Standard deviations for intercept and slope of the calibration curve were in Table 1. Validation of the procedure for the quantitative determination of the ascorbic acid was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, recovery, accuracy and precision (Table 1).

LOD and LOQ were calculated on the peak current using the following equations:

LOD = 3 s/m, LOQ = 10 s/m

Where s is the standard deviation of the peak currents (n=5) and m is the slope of the calibration curve. LOD and LOQ were obtained as 5.160×10^{-7} and 1.723×10^{-6} mol L^{-1}, respectively.

The precision, accuracy and repeatability were assessed by performing replicate analysis of the standard solutions using DPV technique. The repeatability of the measurement was calculated from five independent runs of ascorbic acid solution. The relative standard deviations (R.S.D. %) were calculated to be 2.16 and 1.37 % for peak current and peak potential, respectively.

To confirm the accuracy and precision of the proposed voltammetric technique, additional recovery experiments were conduct. The recovery procedures were carried out by spiking the already analyzed samples of estervit C tablets with a known amount of standard ascorbic acid. These results were given Table 2. It can be concluded that the proposed method is sufficiently accurate and precise to be applied to pharmaceutical formulations of ascorbic acid. Also, the amounts of ascorbic acid in estervit C tablet forms and fresh fruit juices were determined successfully (Table 2 and Table 3). As a result, the amounts of ascorbic acid in the samples were successfully determined without precipitation, evaporation or extraction steps on GC electrode by DPV technique.

4. CONCLUSIONS

Based on the electrochemical oxidation, the quantitative determination of ascorbic acid in pharmaceutical dosage forms and some fruit juices was developed by the simple, rapid, selective and sensitive DPV technique.

The principle advantages of the electroanalytical techniques are that they may be applied directly to the analysis of pharmaceutical dosage forms and all fruit juices. There is no need for separation, extraction, filtration, or complex sample preparation, since there is no interference from the excipients and other plant substances [1, 29, 30]. In addition, the developed electrochemical technique is less expensive than the other techniques. The oxidation process of ascorbic acid at GCE is irreversible and pH dependent. The developed and proposed DPV technique is rapid, requiring about 5 min to run any sample and involve no sample preparation other than dissolving, diluting, precipitation and centrifugation and transferring an aliquot to the supporting electrolyte. The proposed voltammetric technique is a good alternative to the HPLC, UV and the others drug analysis techniques.

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Ascorbic acid tablet dosage form was kindly supplied by Bilim Inc. (Istanbul, Turkey) and powdered form was supplied by Merck.

References

1. C.S. Erdurak-Kiliç, B. Uslu, B. Dogan, U. Ozgen, S.A. Ozkan, M. Coskun,. *J. Anal. Chem.*, 61 (2006) 1113

2. T., Geetha, A., Garg, K., Chopra, I. P., Kaur, *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 556 (2004) 65
3. F.R. Marín, M.J. Frutos, J.A. Pérez-Alvarez, F.M. Sánchez, J.A. Del Río, *Studies in Natural Product Chem.*, 26 (2002) 741
4. T. Gazivoda, S. Raić-Malić, M. Marjanović, M. Kralj, K. Pavelić, J. Balzarini, E. De Clercq, M., Mintas, *Bioorganic & Medicinal Chem.* 157 (2007) 749
5. W. Zeng, F. Martnuzzi, A. MacGregor, *J. Pharm. and Biomed. Anal.*, 36 (2005)1107
6. N. Lenghor, J. Jakmunee, M. Vilen, R. Sara, G.D. Christian, K. Grudpan, *Talanta*, 58 (2002) 1139
7. H. Rajantie, J. Strutwolf, D. E. Williams, *J. Electroanal. Chem.* 500 (2001) 108
8. L.D. Müller, *J. Pharma. and Biomed. Anal.*, 25 (2001) 985
9. J. Lykkesfeldt, *Anal. Biochem.*, 282 (2000) 89
10. T.N. Shekhovtsova, S.V. Muginova, J.A. Luchinina, A.Z. Galimova, *Anal. Chim. Acta*, 573-574, (2006) 125
11. I.O., Serrano, T.H., Jover, O.M., Belloso, *Food Chemistry*, 105 (2007) 1151
12. A.R., Medina, M.L.F., Córdova, A.M. Díaz, *J. Pharm. and Biomed. Anal.* 20 (1999) 247
13. R. Cantu, O. Evans, F. K. Kawahara, J.A. Shoemaker, A.P. Dufour, *Anal. Chem.* 72(23) (2000) 5820
14. S.P. Arya, M. Mahajan, P. Jain, *Anal. Chim. Acta*, 417 (2000)1
15. I. Tetsuharu, H. Shuuji, Y. Masatoshi, N. Masaru, O. Yosuke, *Chem. & Pharm. Bull.* 33 (1985) 3499
16. A.P.S. Paim, C.M.N.V. Almeida, B.F. Reis, R.A.S. Lapa, E.A.G. Zagotto, J.L.F.C. Lima, *J. Pharm. and Biomed. Anal.* 28 (2002) 1221
17. T. Prez-Ruiz, C. Martinez-Lozano, A. Sanz, A. Guillen, *J. Pharm. and Biomed. Anal.*, 34 (2004) 551
18. M. Spickenreither, S. Braun, G. Bernhardt, S. Dove, A. Buschauer, *Bioorg. & Medicin. Chem. Lett.* 16 (2006) 5313
19. M.K. Amini, S. Shahrokhian, S. Tangestaninejad, V. Mirkhani, *Anal. Biochem*., 290 (2001) 277
20. Q. Sheng, H. Yu, J. Zheng, *J. Electroanal. Chem.* 606 (2007) 39
21. M. Yoshimura, K. Honda, T. Kondo, T.N. Rao, D.A. Tryk, A. Fujishima, *Electrochim. Acta* 47 (2002) 4387
22. M.I. Manzanares, V. Solis, R.H. de Rossi, *J. Electroanal. Chem*. 430 (1997) 163
23. G.-P. Jin, Q.-Z. Chen, Y.-F. Ding, J.-B. He, *Electrochim. Acta* 52 (2007) 2535.
24. L. Bergander, E. Wincent, A. Rannug, M. Foroozesh, W. Alworth, U. Rannug, *Chemico-Biological Interact.* 149 (2004) 151
25. X. Zhao, X. Tao, D. Wei, Q. Song, *Euro. J. Med. Chem.* 41 (2006) 1352
26. B. Uslu, S.A. Ozkan, *Electrochim. Acta*, 49 (2004) 4321
27. S.A., Ozkan, B., Uslu, H.Y., Aboul-Enein, *Critical Reviews in Anal. Chem.* 33 (2003)155
28. S., Skrzpek, W., Ciesielski, A., Sokolowski, S., Yılmaz, D., Kazmierczak, *Talanta* 66, (2005) 1146
29. S., Yılmaz, S., Skrzypek, Y., Dilgin, S., Yagmur, M., Coskun, *Current Anal. Chem*. 3 (2007) 41
30. C.M. Riley, T.W. Rosanske Development and Validation of Analytical Methods, Elsevier Science Ltd.: New York, 1996.