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# Application of Column Solid Phase Extraction of Chromium for Indirect Determination of Ascorbic Acid by Flame Atomic Absorption Spectrometry

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## ABSTRACT

An indirect method for the determination of ascorbic acid in fruit juice and vitamin C tablets by flame atomic absorption spectrometry was described. This method is based on the reduction of chromium (VI) to chromium(III) with the reducing action of ascorbic acid, separation of unreacted Cr(VI) as its 1,5-diphenilcarbazide complex on a column filled with Amberlite XAD-16, elution of the complex by 10 mL of 0.05 mol $L^{-1}$ $H_2SO_4$ in methanol, and determination by flame atomic absorption spectrometry. Amount of the ascorbic acid was calculated from the amount of Cr(VI) reacted with ascorbic acid. The optimum conditions for the determination of ascorbic acid, including pH and volume of sample solution were examined. The effect of interfering species on the recovery of the ascorbic acid was also investigated. The proposed method allows high sensitive and selective determination of ascorbic acid in the range 0.5-20 μg/mL. The proposed method is precise as it provides relative standard deviation of 3.4% during five replicate determinations of 12 μg/mL of ascorbic acid. Accuracy of the procedure was tested by analyzing spiked real samples and applying 967.21 Official Method (AOAC) for the determination of ascorbic acid. The procedure described was successfully applied for the determination of ascorbic acid in pharmaceutical preparation, lemon flavored soft powder drink and fruit juices. Ascorbic acid has been determined in real samples with relative error lower than 8%.

Key words: ascorbic acid, flame atomic absorption spectrometry, Amberlite XAD-16, indirect determination, solid phase extraction

## INTRODUCTION

Ascorbic acid (AsA) is one of the most important water-soluble vitamins, commonly known as Vitamin C that participates in a wide variety of biological events. AsA is also important in the human diet, because it helps forming some tissues and assists the body in assimilating iron and amino acid[1]. It is nutritionally necessary for human diet. Since humans cannot synthesize ascorbate, their main source of the vitamin is dietary fruit and vegetables. Fruits, especially citrus and various tropical fruits, are the best sources of vitamin C. However, vitamin C has limited stability and may be lost from foods during storage, preparation and cooking. Therefore, there are many pharmaceutical preparations that contain ascorbic acid as active ingredient. Vitamin C helps to prevent oxidation, thus is used as food additive with anti-oxidant purpose[1]. Therefore, it is important to detect ascorbic acid selectively and conveniently in routine analyses for pharmaceutical and food industry. An accurate and specific determination of the nutrients content of fruits is extremely important to understand the relationship of dietary intake and human health[2]. Several analytical methods have been reported for the determination of ascorbic acid using titrimetry[3,4], spectrophotometry[1, 5-7], chemiluminescence methods[8], spectrofluorimetry[9], chromatography[10,11], electrochemical[12,13], electrophoresis[14] and enzymatic method[15]. These methods have been reviewed in the articles by Arya *et al.* [16,17].

Recent years, indirect determinations of ascorbic acid by UV-Vis spectrophotometry and atomic absorption spectrometry are widely used[18-21]. Atomic absorption spectrometer is an instrument available in most analytical laboratories. These techniques have been proven as suitable tool for the indirect determination of organic compounds, allowing increases in the range of species accessible with such spectrometers. Yebra-Biurrun proposed an indirect flow injection method for the determination of ascorbic acid in fruit juices based on the reduction of Mn(VII) to Mn(II). The Mn(II) formed was retained online, proportional to the ascorbic acid concentration in the sample, on a poly(aminophosphonic acid) chelating resin, which was selective for only this oxidation state. The non-reduced Mn(VII) was determined

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by flame atomic absorption spectrometry. The proposed method allows the determination of ascorbic acid in the 0.2-34.5 μg/mL range with a relative standard deviation of 2.2%[22]. In another study, iron(III) was reduced to iron(II) by the ascorbic acid. Thus, the iron(II) formed reacts with 1,10-phenanthroline to form a complex that is adsorbed on a non-ionic polymeric adsorbent (Amberlite XAD-4) proportionally to ascorbic acid in the sample. The unadsorbed iron was determined by flame atomic absorption spectrometry. The proposed method allows the determination of ascorbic acid in the range 0.5-25 μg $mL^{-1}$ with a relative standard deviation of 2.9%[23]. In another study, Zhang *et al*. designed a flow injections system in which Cr(VI) was reduced to Cr(III) by ascorbic acid and the product [Cr(III)] was first adsorbed on a cation-exchange resin column then, eluted to nebulizer and measured by FAAS. The analytical signal in absorbance of Cr(III) was proportional to concentration of ascorbic acid[24]. M. Noroozifar and M. Khorasani-Motlagh proposed a method based on the oxidation of ascorbic acid by a known excess amount of potassium chromate followed by the estimation of the unreacted amount of chromate by reactions with sym-diphenylcarbazide. The complex was determined spectrophotometrically without separating Cr(III) ions produced from unreacted Cr(VI)[20]. However, there might be interference effect resulting from other metal ions which form complex compound with sym-diphenylcarbazide.

In this study, an alternative method for the indirect determination of ascorbic acid by flame atomic absorption spectrometry (FAAS) was proposed. It was based on the reduction of a known excess amount of Cr(VI) by ascorbic acid. Afterwards, the non-reduced Cr(VI) was separated from product of Cr(III) by a column solid phase extraction as its diphenylcarbazide (DPC) complex and determination of the non-reduced amount of Cr(VI) in the eluent by FAAS. Separation and speciation of Cr(VI) and Cr(III) by solid phase extraction on a column filled with Amberlite XAD-16 had been optimized in our previous study[25]. In this study, the developed chromium speciation method was adapted for the determination of ascorbic acid and optimum experimental conditions were investigated. The developed method was applied to the determination of ascorbic acid in pharmaceuticals, lemon flavored soft powder drinks and fruit juices.

## MATERIALS AND METHODS

### I. *Apparatus*

A Philips PU 9285 model atomic absorption spectrometer equipped with deuterium lamp background corrector and with an air acetylene burner was used for the analysis under the conditions suggested by the manufacturer. Chromium hollow cathode lamp was used to measure the absorbance of chromium. The operating conditions were as follows: Wavelength, 357.9 nm; lamp current, 12 mA; bandpass, 0.5 nm and fuel flow rate, 1.4 L/min. Deuterium lamp background correction was used. A Unicam UV2-100 double beam UV-Visible spectrometer with 10 mm quartz cell was used for spectrophotometric determination of ascorbic acid. All pH measurements were taken with a Consort digital pH meter and a combined glass electrode.

### II. *Reagents*

All solutions were prepared using doubly distilled deionized water and analytical reagent grade chemicals unless otherwise specified. Standard solutions of ascorbic acid, 1000 μg/mL, was prepared daily by dissolving 0.1000 g of ascorbic acid (Merck) in water and diluted to 100 mL with water in a volumetric flask. 1,5-diphenylcarbazide (DPC) solution (0.01 mol/L) was prepared daily by dissolving appropriate amount of DPC (Merck) in 5 mL of acetone (Merck) and diluting to 25 mL with water. The solution was kept in an amber-glass bottle. A solution of 1000 μg/mL Cr(VI) was prepared by dissolving 0.2829 g of $K_2Cr_2O_7$ (Merck) in water and diluted to 100 mL with water in a volumetric flask. $H_2SO_4$ (95-98%, Merck) and methanol (Merck) were used. Amberlite XAD-16 Resin (Room and Hass; surface area, 800 $m^2/g$; wet mesh size, 20-60 mesh) was used after washing with methanol, 1 mol/L HCl solution and water, respectively and dried for 2 h at 60°C.

### III. *Column Preparation*

A glass column (150 mm length 10 mm i.d) with a glass-wool over its stopcock was used as a mini column. A total of 300 mg of Amberlite XAD-16 resin (~ 1.5 cm bed height) was mixed in water and then placed into the column. A small amount of glass-wool was placed on top to avoid disturbing the adsorbent during sample passage. The column was preconditioned by passing a blank solution of the same pH with the sample solution prior to use. After each use, the resin in the column was washed with dilute HCl and with water before storage in water for the next experiment.

### IV. *Preparation of Samples*

All drug samples tested were fresh and purchased from local pharmacy. Commercially available vitamin C tablets were placed in a mortar and ground to fine powder. 100-150 mg of ascorbic acid powder was weighed and stirred for 2-3 min with 50 mL of water and filtered through Whatman No. 41 filter paper. The insoluble mass was washed with three successive 5-mL portions of water. The filtrate and washings were diluted to volume in a 100 mL calibrated flask. The solution was analyzed immediately by the procedure given below in order to avoid losses of ascorbic acid due to air oxidation during or after dilution.

Fresh orange fruits were purchased from the local market and squeezed mechanically. Then, the sample juices were filtered through Whatman No. 42 filter paper and diluted to an appropriate volume. The solution was analyzed immediately by the procedure given below in order to avoid losses of ascorbic acid due to air oxidation during or after dilution.

An accurately weighed amount of lemon flavored soft powder drink (about 1.0 g) purchased from the local market was transferred into a 50 mL volumetric flask, dissolved with water, and made up to the mark with water. The solution was analyzed immediately by the procedure given below in order to avoid losses of ascorbic acid due to air oxidation during or after dilution.

V. *Procedure*

For the optimization of indirect ascorbic acid determination, 100 mL of spiked sample solutions containing1.2 mL of 100 μg/mL Cr(VI) and ascorbic acid were used. To prepare spiked sample solution, 1 mL of 0.01 mol/L DPC solution and 0.1 mL of 100 mg/L Cr(VI) solution have been added into the 100 mL volumetric flask and the volume is adjusted to 100 mL with water. Then, pH of the sample solution was adjusted to the desired value (pH 1) at which recovery of Cr(VI) was the highest with sulfuric acid. The resulting solution was passed through the column described in Section III at the desired flow rate of 1.5 mL/min. The retained species (Cr-DPC) on the column was eluated with 10 mL of 0.05 mol/L $H_2SO_4$ solution in methanol. Chromium content in the eluate was determined by FAAS. Ascorbic acid content was calculated using the stoichiometric relationship between Cr(VI) and ascorbic acid (1 g of Cr(VI) is equivalent to 5.07 g of ascorbic acid). The reaction scheme is as follows:

$Cr_2O_7^{2-} + 3C_6H_8O_6 + 8H^+ \rightarrow 2Cr^{3+} + 3C_6H_6O_6 + 7H_2O$

Non-reduced $Cr_2O_7^{2-}$+ DPC → Cr-DPC complex

Cr-DPC complex + Amberlite XAD-16 → Adsorbed Cr-DPC onto Amberlite XAD-16

Cr-DPC onto Amberlite XAD-16 + Eluent $H_2SO_4$ → Elution solution containing Cr(VI)

In order to check accuracy of the proposed method, ascorbic acid was also determined in the sample solution by dichloroindophenol titrimetric method of AOAC (AOAC 967.21).

## RESULTS AND DISCUSSION

I. *Optimization of Column Solid Phase Extraction of Chromium Species*

Optimization of the parameters such as pH of the sample solution, eluent type, sample volume, amount of adsorbent, and flow rate of sample solution effecting the column solid phase extraction of chromium species (Cr(III) and Cr(VI)) were investigated and reported in our previous paper[25]. Quantitative recovery (> 95%) was found at the pH 1 with Cr(VI) while recovery of Cr(III) was rather low (< 5%). This could make separation of Cr(VI) from Cr(III)[25] possible. Optimum values for the determination of Cr(VI) are shown in Table 1. Linearity of this system was evaluated for chromium concentration ranging from 1 to 5 mg/L under the experimental condition mentioned above. The calibration graph was found to be linear up to 5 mg/L with regression coefficient above 0.99. The detection limit, as the concentration corresponding to three times the standard deviation of the blank signal (n=12), was 45 μg/L for Cr(VI)[25].

II. *Effect of pH*

The proposed procedure of indirect determination of ascorbic acid was based on its reducing action on Cr(VI) in acidic medium and following the atomic absorption spectrometric determination of the remaining Cr(VI) after separating the Cr(VI) from Cr(III) by a column solid phase extraction of Cr(VI) as its DPC complex onto Amberlite XAD-16. Amberlite XAD-16 resin is selective only for Cr(VI). The dependence of the redox reaction between ascorbic acid and Cr(VI) on the pH of the solution is an important parameter that can have significant influence on the over-all performance of the indirect method. The optimum range of pH for the indirect determination of ascorbic acid was also 1-1.5, adjusted using diluted sulfuric acid.

III. *Precision of the Method*

In order to investigate precision of the method, the above procedure was performed successively as inter-day variability and ascorbic acid was determined in synthetic sample containing 12 μg/mL of ascorbic acid. The mean recovery for five determinations was about 96.7 at the 95% confidence level. Precision of the proposed method was good at relative standard deviation of 3.4% during five replicate determinations of 12 μg/mL of ascorbic acid.

**Table 1.** Optimum conditions for preconcentration/separation of Cr(VI) by the Amberlite XAD-16[25]

| Parameter | Value |
|---|---|
| pH | 1 |
| Eluent (0.05 mol/L $H_2SO_4$ solution in methanol) volume, mL | 10 |
| Amount of adsorbent (mg) | 300 |
| Flow rate of the sample solution (mL/min) | 1.5 |
| Volume of the applicable sample solution (mL) (Containing 10 μg Cr(VI)) | 250 |

IV. *Interference Studies*

To assess validity of the method, a study of interference for ascorbic acid determination was also performed. Cations in the sample would not interfere with the ascorbic acid because they had not adsorbed by Amberlite XAD-16 resin column. The interfering effect of the cations on the separation and determination of Cr(VI) had been investigated in our previous study[25]. Recoveries of Cr(VI) were quantitative when the ratio of interfering ions to chromium (VI) was 10 for Fe(III), 25 for Pb(II) and Al(III), 50 for Cu(II), Ni(II), Mn(II), Co(II) and 100 for Zn(II), Cd(II), Na(I), K(I), Mg(II) and Ca(II)[25] respectively. The interferences caused by foreign species commonly found with ascorbic acid in the analyzed samples (i.e. glucose, fructose citric acid, oxalic acid and tartaric acid) were investigated by adding different amount of other species to a solution containing 12 μg/mL of ascorbic acid. The tolerance limit was defined as the concentration at which the species caused an error <5%. The results are listed in Table 2. According to this work, no interferences were observed for glucose, fructose and citric acid with mass ratio up to 50, for oxalic acid up to 40 and for tartaric acid up to 20.

V. *Effect of Volume of Sample Solution (Ascorbic Acid Concentration)*

The effect of changes in the volume of sample solution on the recovery of ascorbic acid was investigated in order to determine an applicable sample volume or a minimum ascorbic acid concentration. For that purpose, 10, 50, 100 and 250 of sample solutions containing fixed amount of ascorbic acid (120 μg) corresponds 12, 2.4, 1.2 and 0.48 μg/mL, respectively were passed through the column under the optimum conditions determined experimentally. It was found that ascorbic acid could be recovered up to 250 mL of sample solution with a relative error about 8% (Table 3). At higher sample volumes, the recoveries decreased and the relative error increased gradually with increasing volume of sample. It can be concluded that 0.48 μg/mL of ascorbic acid could be determined by this method for 250 mL of sample volume.

VI. *Application*

To investigate applicability to real samples, the proposed method was applied to the determination of ascorbic acid in Vitamin C tablets, lemon flavored soft powder drinks and fruit juices. The results obtained by the proposed method and the AOAC method (AOAC 967.21) are given in Table IV. The t-test assured that results of both methods had no significant difference ($P$ = 0.95). It can be seen that results of the proposed method were in agreement with the values given on packages of the samples.

VII. *Recovery Test*

In order to check accuracy of the proposed method, recovery test was performed using different sample solutions spiked with ascorbic acid. As shown in Table 5, recoveries of ascorbic acid added to orange juice, pharmaceutical samples and lemon flavored soft powder

**Table 2.** The effect of some species on the recovery of ascorbic acid (pH 1; eluent 10 mL of 0.05 mol/L $H_2SO_4$ solution in methanol; sample volume 10 mL; amount of the ascorbic acid 120 μg)

| Species added | Concentration (μg/mL) | % Recovery* |
|---|---|---|
| Oxalic acid | – | 97 ± 2 |
| | 120 | 96 ± 3 |
| | 240 | 100 ± 4 |
| | 360 | 100 ± 3 |
| | 480 | 106 ± 4 |
| | 600 | 119 ± 4 |
| Tartaric acid | – | 97 ± 2 |
| | 120 | 96 ± 2 |
| | 240 | 108 ± 4 |
| | 360 | 117 ± 4 |
| Citric acid | – | 97 ± 2 |
| | 120 | 102 ± 3 |
| | 240 | 100 ± 3 |
| | 600 | 103 ± 3 |
| | 1200 | 118 ± 4 |
| Fructose | – | 97 ± 2 |
| | 120 | 93 ± 2 |
| | 600 | 96 ± 3 |
| | 1200 | 108 ± 4 |
| Glucose | – | 97 ± 2 |
| | 120 | 103 ± 4 |
| | 600 | 107 ± 3 |
| | 1200 | 111 ± 4 |

*Mean ± standard deviation of three determinations.

**Table 3.** Effect of sample volume on the determination of ascorbic acid

| Volume of sample solution (mL) | Added (μg/mL) | Found* (μg/mL) $\bar{x} \pm \frac{ts}{\sqrt{N}}$ | % Recovery |
|---|---|---|---|
| 10 | 12 | 11.9 ± 0.4 | 99 ± 3 |
| 50 | 2.4 | 2.5 ± 0.2 | 104 ± 8 |
| 100 | 1.2 | 1.23 ± 0.04 | 103 ± 3 |
| 250 | 0.48 | 0.44 ± 0.08 | 92 ± 17 |

* Mean of five determinations at 95% confidence level.

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**Table 4.** Determination of ascorbic acid in real samples

| Sample | Concentration found | | Labeled value on the package (Nominal value) |
|---|---|---|---|
| | Proposed method[a] $\bar{x} \pm ts/\sqrt{N}$ | AOAC 967.21[b] $\bar{x} \pm ts/\sqrt{N}$ | |
| Orange juice | 256 ± 9 µg/mL | – | – |
| Redoxon[c] | 1098 ± 80 mg/tablet | 1100 ± 60 mg/tablet | 1000 mg/tablet |
| Calcium Sandoz[d] | 1044 ± 70 mg/tablet | 1070 ± 50 mg/tablet | 1000 mg/tablet |
| Lemon flavored soft powder drink | 8.1 ± 0.4 mg/g | 7.9 ± 0.3 mg/g | 6.0 mg/g |

[a]Mean of five determinations at 95% confidence level.
[b]Mean of four determinations at 95% confidence level.
[c]Each effervescent tablet of Redoxan (from ROCHE, Istanbul, Turkey) contains vitamin C 1000 mg, aspartame and sorbitol.
[d]Each effervescent tablet of Calcium Sandoz (from NOVARTIS, Istanbul, Turkey) contains vitamin C 1000 mg, calcium carbonate 327 mg and calcium lactate gliconate 1000 mg.

**Table 5.** Results of recovery test in the sample solutions

| Sample | Ascorbic acid added (µg/mL) | Ascorbic acid* found (µg/mL) | Recovery % |
|---|---|---|---|
| Orange juice (fresh) | – | 220 ± 10 | – |
| | 100 | 306 ± 6 | 96 ± 4 |
| Redoxon solution | – | 115 ± 8 | – |
| | 50 | 160 ± 10 | 97 ± 8 |
| | 100 | 200 ± 5 | 93 ± 4 |
| Calsium Sandoz solution | – | 75 ± 5 | – |
| | 50 | 116 ± 2 | 93 ± 4 |
| | 100 | 170 ± 4 | 97 ± 4 |
| Lemon flavored soft powder drinks solution | – | 162 ± 8 | – |
| | 100 | 240 ± 20 | 92 ± 8 |

* Mean of five determinations at 95% confidence level.

drinks were all close to 100%. The recovery test results were very good. No interference was found in the presence of a very complex matrix of samples (especially fruit juice) as indicated by the good recovery of ascorbic acid. The proposed method could be applied to the analysis of drugs containing different dosage of ascorbic acid without interference from other constituents and excipients encountered.

## CONCLUSIONS

The proposed method, using the reaction between ascorbic acid and Cr(VI), has realized the off-line preconcentrating/separating by column solid phase extraction and indirect determination of ascorbic acid by flame atomic absorption spectrometry. This method provides interference free determination of ascorbic acid due to prior separation of Cr(VI) from the matrix components. The proposed method could be applied to the analysis of drugs and beverages containing different amounts of ascorbic acid without interference from other constituents and excipients. This method is sensitive, selective and suitable for laboratory rutin control. Repeated use of column is possible. Because of the DPC method is specific for chromium, it can also be concluded that some reducing substances such as iodide and thiosulfate may also be determined indirectly by this method in various matrices. Studies of indirect determinations of iodide and thiosulfate based on the Cr(VI) reduction by FAAS have been continued in our laboratory.

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## REFERENCES

1. Güclü, K., Sözgen, K., Tütem, E., Özyürek, M. and

Apak, R. 2005. Spectrophotometric determination of ascorbic acid using copper(II)-neocuproine reagent in beverages and pharmaceuticals. Talanta 65: 1226-1232.

2. Hernandez, Y., Lobo, M. G. and Gonzalez, M. 2006. Determination of vitamin C in tropical fruits: A comparative evaluation of methods. Food Chem. 96: 654-664.
3. Kabasakalis, V., Siopidou, D. and Moshatou, E. 2000. Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. Food Chem. 70: 325-328.
4. AOAC (1995) Official methods of analysis of AOAC International, 16th edn., vol II, AOAC, Arlington, VA, USA, Chapt. 45, pp 16-17.
5. Özyürek, M., Güçlü, K., Bektaşoğlu, B. and Apak, R. 2007. Spectrophotometric determination of ascorbic acid by the modified CUPRAC method with extractive separation of flavonoids–La(III) complexes. Anal. Chim. Acta 588: 88-95.
6. Shrivas, K., Agrawal, K. and Patel, D. K. 2005. A spectrophotometric determination of ascorbic acid. J. Chinese Chem. Soc. 52: 503-506.
7. Zhang, Z. X., Sun, D. M. and Rong, Z. H. 2004. Catalytic kinetic spectrophotometric determination of trace ascorbic acid. Spectrosc. Spect. Anal. 24: 873-875.
8. Li, B., Wang, D., Xu, C. and Zhang, Z. 2005. Flow-injection simultaneous chemiluminescence determination of ascorbic acid and L-cysteine with partial least squares calibration. Microchim. Acta 149: 205-212.
9. Rezaei, B., Ensafi, A. A. and Nouroozi, S. 2005. Flow-injection determination of ascorbic acid and cysteine simultaneously with spectrofluorometric detection. Anal. Sci. 21: 1067-1071.
10. Silva, F. O. 2005. Total ascorbic acid determination in fresh squeezed orange juice by gas chromatography. Food Control 16: 55-58.
11. Lopes, P., Drinkine, J., Saucier, C., and Glories, Y. 2006. Determination of L-ascorbic acid in wines by direct injection liquid chromatography using a polymeric column Anal. Chim. Acta 555: 242-245.
12. Li, C. X., Zeng, Y. L., Liu, Y. J., et al. 2006. Simultaneous electrochemical determination of uric acid and ascorbic acid on a glassy carbon electrode modified with cobalt(II) tetrakisphenylporphyrin. Anal. Sci. 22: 393-397.
13. Chen, S. M. and Chzo, W. Y. 2006. Simultaneous voltammetric detection of dopamine and ascorbic acid using didodecyldimethylammonium bromide (DDAB) film-modified electrodes. J. Electroanal. Chem. 587: 226-234.
14. Versari, A., Mattioli, A., Parpinello, G. P. and Galassi, S. 2004. Rapid analysis of ascorbic and isoascorbic acids in fruit juice by capillary electrophoresis. Food Control 15: 355-358.
15. Shekhovtsova, T. N., Muginova, S. V., Luchinina, J. A. and Galimova, A. Z. 2006. Enzymatic methods in food analysis: determination of ascorbic acid. Anal. Chim. Acta 573: 125-132.
16. Arya, S. P., Mahajan, M. and Jain, P. 2000. Non-spectrophotometric methods for the determination of vitamin C. Anal. Chim. Acta 417: 1-14.
17. Arya, S. P., Mahajan, M. and Jain, P. 1998. Photometric methods for the determination of vitamin C, Anal. Sci. 14: 889-895.
18. Jiang, Y. C., Zhang, Z. Q. and Zhang, J. 2001. Flow-injection, on-line concentrating and flame atomic absorption spectrometry for indirect determination of ascorbic acid based on reduction of iron(III). Anal. Chim. Acta 435: 351-355.
19. Barrales, P. O., Cordova, M. L. F. and Diaz, A. M. 1998. Indirect determination of ascorbic acid by solid phase spectrophotometry. Anal. Chim. Acta 360: 143-152.
20. Noroozifar, M., Khorasani-Motlagh, M. 2003 Application of potassium chromate diphenylcarbazide in the quantitative determination of ascorbic acid by spectrophotometry. Turk. J. Chem. 27: 717-722.
21. Yebra, M. C., Cespon, R. M. and Moreno-Cid, A. 2001. Automatic determination of ascorbic acid by flame atomic absorption spectrometry. Anal. Chim. Acta 448: 157-164.
22. Yebra-Biurrun, M. C., Cespon-Romero, R. M., and Moreno-Cid, A. 2001. Flow injection flame AAS determination of ascorbic acid based on permanganate reduction. Atom. Spectr. 2: 346-349.
23. Yebra-Biurrun, M. C., Cespo-Romero, R. M. and Bermejo-Barrera, P. 1997. Indirect flow-injection determination of ascorbic acid by flame atomic absorption spectrometry. Microchim. Acta 126: 53-58.
24. Zhang, Z. Q. and Jiang, Y. C. 2000. Flame atomic absorption spectrometry for the automatic indirect determination of ascorbic acid based on flow injection and reduction of chromate. Atom. Spectr. 21: 100-104.
25. Tunceli, A. and Türker, A.R. 2002. Speciation of Cr(III) and Cr(VI) in water after preconcentration of its 1,5-diphenylcarbazone complex on Amberlite XAD-16 resin and determination by FAAS. Talanta 57: 1199-1204.