Research article

Drug Testing and Analysis

Received: 8 February 2011 Revised: 23 March 2011 Accepted: 23 March 2011 Published online in Wiley Online Library: 6 May 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.290

Indirect fibre-optic colorimetric determination of ascorbic acid using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol and cloud point extraction

Hayati Filik* and Derya Giray

A new method has been developed for the indirect determination of ascorbic acid (AA) in commercial syrup preparations based on cloud point extraction (CPE) separation and preconcentration, and determination by molecular absorption spectrometry. The colorimetric method was based on the reduction of Fe(III) to Fe(II) and complexation of Fe(II) with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP), followed by its extraction into Triton X-114. Selectivity of the method was increased with the use of EDTA as a masking agent. The absorbance was measured at 742 nm. Various influencing factors on the separation and preconcentration of AA have been investigated systematically, and the optimized operation conditions were established. The proposed method allows the determination of AA in the range 5–200 μg L^{-1} with a relative standard deviation of 3.0%. The detection limit was found to be 0.9 μg L^{-1} for AA. This method has been applied to the determination of ascorbic acid in commercial pharmaceutical preparations.

Keywords: ascorbic acid; cloud point extraction; spectrophotometry; pharmaceutical analysis.

Introduction

Vitamin C, or L-ascorbic acid, or L-ascorbate is an essential nutrient for humans and certain other animal species, in which it functions as a vitamin.[1] It is added to several pharmaceutical products as an essential ingredient, is a stabilizer for vitamin B complex, and is also used as an antioxidant.[2] However, high levels of ascorbic acid (AA) in the human body could cause adverse effects. In the pharmaceutical and food industries, the determining or monitoring of AA content is particularly important.

Several methods of determining AA have been proposed: polarography, voltammetry, fluorimetry, enzymatic methods, gas chromatography, high performance liquid chromatography, titrimetry, and spectrophotometry.[2–4] Spectrophtometry in visible region is commonly used for the indirect determination of AA by the reagents which produces specific colour reaction. Most of these methods are based on the reducing ability of AA.[2–4] The direct spectrophotometry in UV region is also used, since AA absorbs in this region presenting a maximum absorbance at 243 nm in strongly acid media and at 265 nm in neutral media.[4] To enhance the sensitivity of direct spectrophotometry and make the most of its advantages, solid-phase UV spectrophotometry technique has been developed for the determination of AA by direct measurement of its intrinsic UV absorption after sorption on a solid support.[5–7] Pereira and Fatibello-Filho developed a flow injection spectrophotometric determination of L-ascorbic acid in pharmaceutical formulations with online solid-phase reactor containing copper (II) phosphate immobilized in a polymeric matrix of polyester resin[8]. Solid-phase reactors have been used for a variety of purposes, including preconcentration, sample conversion, immobilized enzymes, and generation of unstable reagents.[9] Basically, the solid-phase reactors are constructed by the incorporation oxidizing or reducing agents with a good catalytic activity into a small column.[9] In recent years, a methodology of separation- and preconcentration-based solid-phase extraction (SPE) methods have been proposed for the indirect determination of AA by UV-Vis spectrophotometry and flame atomic absorption spectrometry (FAAS).[10–16] Atomic absorption spectrometers are analytical instruments available in most analytical laboratories. These techniques have been proved as a suitable tool for the indirect, online determination of organic compounds, making possible an increase in the range of species accessible with such spectrometers.[17] To date, several solvent extraction methods have been reported. In 1945, Robinson and Stotz described a rapid, accurate indophenol-xylene extraction method for the determination of AA in common and highly coloured food products.[18] Arya *et al*. introduced an indirect solvent extraction method for the spectrophotometric determination of AA based on the AA reduction of Fe(III) to Fe(II) and complexation of Fe(II) with 4-(2-pyridylazo)-resorcinol (PAR), followed by its extraction into n-butanol.[19] Gu *et al*.[20] proposed a rapid preconcentration and spectrophotometric method for the determination of traces of AA in water using an organic

* *Correspondence to: Hayati Filik, Istanbul University, Faculty of Engineering, Department of Chemistry, Avcılar, 34320 Istanbul, Turkey.*
E-mail: filik@istanbul.edu.tr

Istanbul University, Faculty of Engineering, Department of Chemistry, Avcılar, 34320 Istanbul, Turkey

solvent-soluble membrane filter. The method was based on the reduction of 1,10-phenanthroline (phen)-Fe(III), which is collected on a nitrocellulose membrane filter as an ion-associate of the cationic complex of tri.phen-iron(II) [ferroin, $Fe(phen)_3^{2+}$] with an anionic surfactant (of dodecyl sulfate). The ion-associate collected is dissolved in a small volume of 2-methoxyethanol together with the filter.[20] The amount of AA has been reported in dog rose samples using conventional extraction method.[21–23]

The aim of this work is to introduce a method for determination of AA in aqueous samples by indirect spectrophotometric determination after preconcentration and separation by the cloud point extraction (CPE) technique. CPE procedure has been widely used for the separation, purification, and preconcentration of a variety of components. As distinct from liquid extraction preconcentration, sorption methods do not require the use of toxic organic solvents; hence, they are more environmentally friendly.[24,25] The proposed method for the indirect determination of AA is based on the reduction of Fe(III) by AA to Fe(II) and complexation of Fe(II) with Br-PADAP, followed by its extraction into a Triton X-114 surfactant-rich phase. Br-PADAP is a very sensitive reagent for the spectrophotometric determination of AA.[23]

Experimental

Instrumentation

Experiments were carried out using a commercially available miniature fibre-optic based spectrometer (HR4000CG-UV-NIR, Ocean Optics Inc., Dunedin, FL, USA) which utilizes a small tungsten halogen lamp (Ocean Optics Inc., Dunedin, FL, USA) as the light source and a charge-coupled device (CCD)-based detector for absorbance measurements. The spectral resolution declared by the manufacturer was 0.02 nm. A thermostated bath maintained at the desired temperature was used for reaction (Hettich, Universal). The pH values of the solutions were measured by a Hanna HI 221 pH-meter using the full range of 0–14. Conical bottom disposable plastic centrifuge tubes (10 mL) made of clear and autoclavable polypropylene were used for phase separations. All glassware was rinsed carefully with 1 : 3 diluted HCl.

Reagents

All reagents used were of analytical grade. Distilled water was used throughout the work. AA stock solution (2.0×10^{-3} mol L^{-1}) was prepared daily by dissolving 35.2 mg of AA in 100 mL of 0.1 mol L^{-1} acetic acid solution. In this work, 0.1 mol L^{-1} acetic acid has been used in preparing the working solutions of AA, because acetic acid works as an effective stabilizer and AA oxidation is slower in it.[24] Further dilutions were made to obtain appropriate concentrations of AA. The Br-PADAP solution was prepared by dissolving an appropriate amount of 5-Br-PADAP in 5 mL ethanol, 1.0 mL concentrated HCl and 2 mL of concentrated TX-114 to give a 1.0×10^{-3} mol L^{-1} solution. The Br-PADAP reagent has a low solubility in water but this problem was turned over by the addition of Triton X-114. The stock solution of 1000 μg L^{-1} Fe(III) was prepared by dissolving $NH_4Fe(SO_4)_2\ 12H_2O$ in 0.1 mol L^{-1} hydrochloric acid solution. Triton X-114 stock solution (5.0% *w*/*v*) was prepared by dissolving 5 mL of concentrated solution (Merck) in distilled water. A 1.0×10^{-3} mol L^{-1} ethylenediaminetetraacetic acid disodium salt (EDTA, purchased from Meck) was used to mask most of the metals. Acetate buffer (pH 5.0) was prepared by dissolving 13.6 g of sodium acetate trihydrate in 80 mL distilled water. Solution pH was adjusted to 4.9 with acetic acid, and the mixture was diluted to 100 mL with water. Pharmaceutical liquid syrups were obtained commercially from different companies, Istanbul, Turkey (Ineldea Co., Bayer Co., Fako Co., Seven Seas Co.).

Sample analysis

Five different liquid syrup samples were analyzed. Commercial liquid syrups were purchased at a local pharmacy (Istanbul, Turkey). Ten millilitres of each pharmaceutical product (*i.e.*, liquid syrups) was measured into a 100-mL volumetric flask and diluted to volume with distilled water. AA was determined after filtration through Whatman No. 42 filter paper. Ten millilitres of the sample was sufficient to determine AA in these samples. The solution was analyzed immediately by the procedure given below in order to avoid losses of AA due to air oxidation during or after dilution.

CPE procedure

For CPE, an aliquot of an AA standard solution (5–200 μg L^{-1}) was transferred to a 10 mL centrifuge tube, 0.5 mL of 1×10^{-4} mol L^{-1} Fe(III) solution, 1.0 mL of 1.0×10^{-4} mol L^{-1} Br-PADAP and 2.0 mL acetate buffer (pH 5.0) were added. After 5 min, 1.0 ml of 2.0×10^{-3} EDTA solution and 0.5 mL of 5% (*v*/*v*) TX-114 solution were added. Next, the solution was taken up to the mark with distilled water and mixed well. The mixture was kept in the thermostatic bath maintained at 50 °C for 5 min. Phase separation was accelerated by centrifuging the tubes at 4000 rpm for 5 min; later 0.3 mL of ethanol was added to the surfactant-rich phase and the solution was transferred to quartz cell for measurement of absorbance at 742 nm.

Results and discussion

Spectral characteristics

The absorption spectrum of the Fe(II)-Br-PADAP complex in surfactant-rich was studied over the range 500–800 nm. The iron(II)-Br-PADAP complex shows absorption maxima at 555 and 742 nm. At 555 nm, the system is more sensitive; however, at 742 nm, the procedure is more selective because only the Fe(II)-Br-PADAP complex absorbs at this wavelength.[26] Therefore, absorbance measurements were made at 742 nm not only because of negligible absorbance of the blank in this region but also due to higher selectivity. Electronic absorption spectra of Fe(II)-Br-PADAP complex in surfactant-rich phase are shown in Figure 1.

Effect of pH

The pH of the sample solution is one of the important factors affecting the formation of complexes and the subsequent extraction. The effect of pH on the CPE of Fe(II)-Br-PADAP complex was studied in the pH range of 3.6 to 8.0. The iron(III) ion reacts with AA according to the following reaction:[26]

$$C_6H_8O_8 + 2Fe^{3+} \rightarrow C_6H_6O_8 + 2H^+ + 2Fe^{2+} \quad (1)$$

$$Fe^{2+} + 2\text{Br-PADAP} \rightarrow Fe^{2+}\ (\text{Br-PADAP})_2 \quad (2)$$

First iron(III) is reduced to iron(II) by AA, the second iron(II) reacts with Br-PADAP in a weak acidic medium to form a coloured

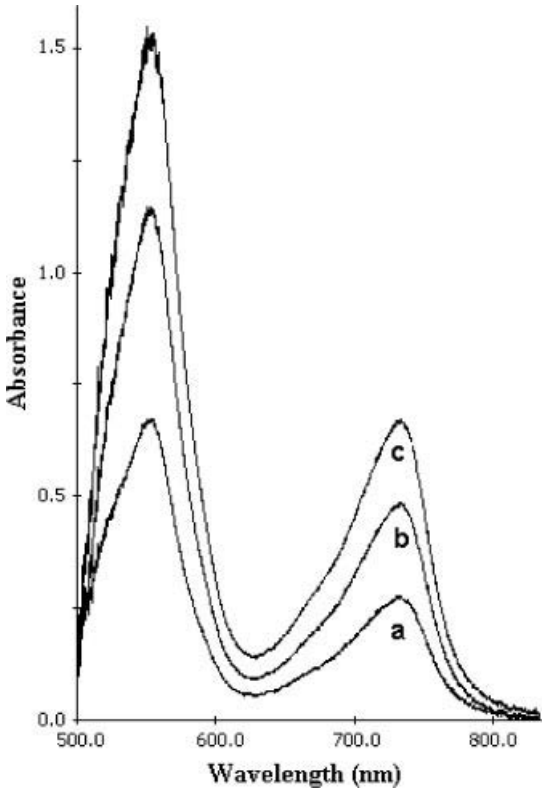

Figure 1. Absorption spectrum of the Fe(II)-Br-PADAP complex. Extraction conditions: 1×10^{-5} mol L^{-1} Br-PADAP, 1.0×10^{-4} mol L^{-1} EDTA, 0.25% (v/v) TX-114, 5.0×10^{-6} mol L^{-1} Fe(III).
a) 35.23 μg L^{-1}, b) 70.45 μg L^{-1}, c) 105.69 μg L^{-1} AA.

complex. The reduction of Fe(III) by AA always requires an acidic medium (Eqn (1)). The reduction rate of Fe(III) by AA decreased drastically as the pH value increased. At pHs lower than 3.7, Fe(III) can be reduced by AA but the Fe(II)-Br-PADAP complex can not be formed (Eqn (2)). Almost constant and maximal absorbance was obtained between pH 5.0 and 6.0. At pH values below and above these, a significant decrease of the absorbance was observed. Graphical representation of the effect of the pH is shown in Figure 2. A buffer solution with a pH of 5.0 was adopted for the determination.

Effect of Br-PADAP

Br-PADAP is widely used for the determination of iron. It reacts with iron(II) and iron(III) cations. For both chelates, the ligand (L) to metal (M) molar ratio was found to be 2 : 1 (L-M).[25] Therefore, Fe(II)-Br-PADAP chelate is electrically neutral (as Fe(II) L_2^0); whereas the Fe(III)-Br-PADAP) chelate is positively charged (as Fe(III) L_2^+) and the Fe(III) chelate is prone to the formation of the ion-pairing species.[27,28] In this experiment, the ligand metal ratio was maintained at 2 : 1. In this work, the ligand concentration was maintained at 1.0×10^{-5} mol L^{-1} and the metal ion concentration was maintained at 5.0×10^{-6} mol L^{-1}. The optimum concentration of Br-PADAP was chosen as 1.0×10^{-5} mol L^{-1}, which is two-fold higher than Fe(III) molar concentration.

Effect of the amount of EDTA

At pH 3.7 – 6.0, Fe(II) reacts with Br-PADAP to form a stable complex and Fe(III) reacts with Br-PADAP to form an unstable complex.

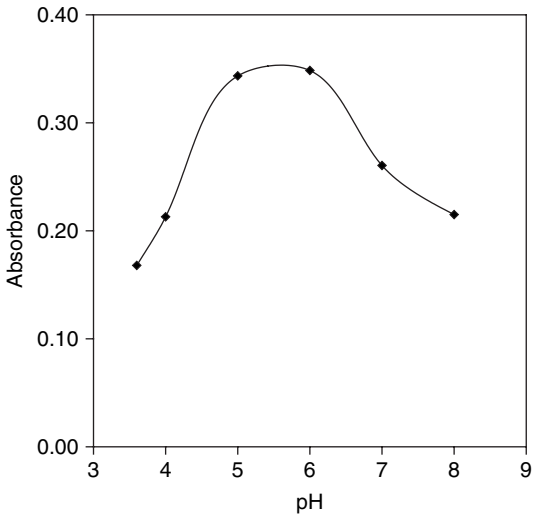

Figure 2. Effect of pH on the CPE of AA. Extraction conditions: 1×10^{-4} mol L^{-1} Br-PADAP, 2.0×10^{-4} mol L^{-1} EDTA, 0.25% (v/v) TX-114, 50.6 μg L^{-1} AA, 5.0×10^{-6} mol L^{-1} Fe(III).

The Fe(III)-Br-PADAP complex is completely decomposed in the presence of EDTA. Thus the Fe(III) ions excess can be eliminated with the EDTA. On the other hand, Br-PADAP is capable of binding a variety of metals (Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Fe(III)), a procedure was needed to eliminate binding of Br-PADAP to metals other than Fe(II). The addition of EDTA allows the stain to bind to Fe(II), but not to other metals. The masking reagent, EDTA is helpful to improve the selectivity of the complexation. The effect of the EDTA concentration on the absorbance of Fe(II)-Br-PADAP complex was studied. The EDTA concentration was varied in the range 0–2.0×10^{-4} mol L^{-1} in order to maximize the absorbance signal. The maximum absorbance was obtained in the concentration range 2.0×10^{-5} and 2.0×10^{-4} mol L^{-1} EDTA. Enough EDTA (2.0×10^{-4} mol L^{-1}) was added to completely mask the excess metal ions but a high concentration of EDTA was avoided. The results are shown in Figure 3.

Influence of order of reagent addition

According to the results obtained, the order of addition of the reagents affects the complex formation. The best order of reagent addition is as follows: Fe(III) solution, AA, chromogenic reagent (Br-PADAP), acetate buffer solution (pH = 5.0) then EDTA and TX-114. EDTA solution must be added after 5 min, when the complex (Fe(II)-Br-PADAP) has already been formed, in order to guarantee the stability and reproducibility of the system and not mask the Fe(II) reaction with Br-PADAP. The absorbance of Fe(II)-Br-PADAP is proportional to the concentration of AA. Other sequences gave lower absorbance values compared with the above sequence. It was observed that the AA behaves as a reductant, thus it must always be added before the buffer addition as the acid medium increases Fe(III) reduction. The iron(III) ions excess was eliminated with the EDTA.

Surfactant concentration

The non-ionic surfactant Triton X-114 was chosen due to its commercial availability in a high purified homogeneous form, low toxicological properties and cost, low cloud point temperature, and high density of surfactant-rich phase, which facilitates

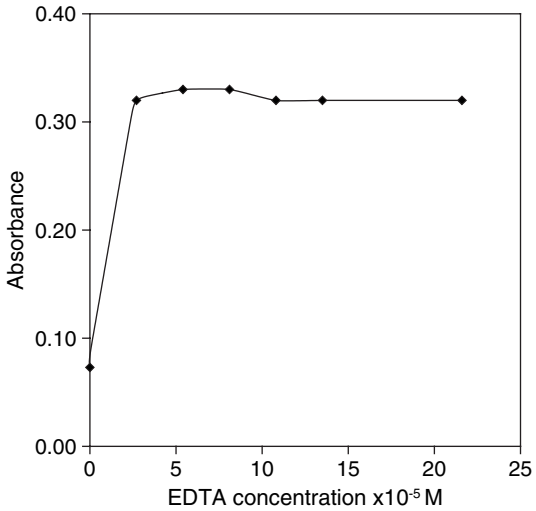

Figure 3. Effect of EDTA on the CPE of AA. Extraction conditions: 1×10^{-5} mol L^{-1} Br-PADAP, 0.25% (*v*/*v*) TX-114, 50.6 μg L^{-1} AA, 5×10^{-6} mol L^{-1} Fe(III).

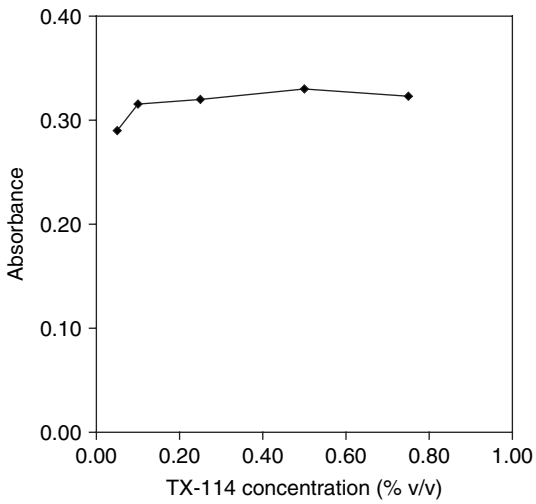

Figure 4. Effect of TX-114 on the CPE of AA. Extraction conditions: 1.0×10^{-5} mol L^{-1} Br-PADAP, 5×10^{-6} mol L^{-1} Fe(III), 1.0×10^{-4} mol L^{-1} EDTA, 50.6 μg L^{-1} AA.

phase separation by centrifugation. The effect of Triton X-114 concentration on the performance of the extraction system was studied. Figure 4 shows the effect of concentration of TX-114 on the analytical signals. The variation of extraction efficiency upon the surfactant concentration was examined within range: C_{TX-114} = 0.05–1.0% (v/v). The maximum signal intensity was observed when the TX-114 concentration was above 0.1% (*v*/*v*). An amount of 0.25% TX-114 was chosen in order to achieve quantitative and thereby the highest possible extraction efficiency.

Effects of equilibration temperature and time

The dependence of extraction efficiency upon equilibration temperature and time was studied with a range of 25–60 °C and 5–10 min, respectively. The results showed that an equilibration temperature of 50 °C and a time of 5 min were adequate to achieve quantitative extraction. A centrifugation time of 5 min at 4000 rpm was selected as optimum, since complete separation occurred for this time and no appreciable improvements were observed for longer times.

Selection of the dilution agent

The surfactant-rich phase was very viscous; ethanol was added to the surfactant-rich phase after CPE to facilitate its transfer into spectrophotometric cell. The amount of 0.3 mL (i.e. final volume 0.5 mL) ethanol was chosen to have an appropriate amount of sample for transffering and measuring the sample absorbance signal. The pH must be in the range of 5.0 to 6.0. Above or below this value a significative decay on the absorbance signal occurs. Because the pH of the final ethanolic solution was already approximately 5, no extra pH-adjustment of the final solution was required.

Analytical characteristics of the method

Performance characteristics of the method were obtained by processing standard solution of AA. Many metals were determination for chelate with Br-PADAP, and the determination wavelength usually is 500–550 nm. The Fe(II)-Br-PADAP system at the same conditions forms a chelate with absorption peaks at 555 and 742 nm. At 742 nm, the system is more selective. Thus all measurements were performed at 742 nm. The calibration graph was linear in the range of 5–200 μg L^{-1} of AA in the initial solution by applying the optimized conditions. The equation for the line was $A = 0.00665\ C + 0.0015$ with regression coefficient (*r*) of 0.9980 ($n = 5$), where *A* is the absorbance and *C* is the AA concentration in solution (μg L^{-1}). The relative standard deviation (R.S.D) for five samples of 20 μg L^{-1} of AA subjected to the complete procedure is 3.0%. The limit of detection, defined as $C_L = 3S_B/m$ (where C_L, S_B, and *m* are the limit of detection, standard deviation of the blank, and slope of the calibration graph, respectively), was 0.9 μg L^{-1} and was therefore more sensitive than the direct method (i.e. without CPE, LOD = 44 μg L^{-1}) reported previously. Phase volume ratio, calculated as the ratio between the volume

Table 1. Influence of other species on the determination of ascorbic acid

Substances	Foreign ion to analyte ratio	Recovery (%)
Acetylsalicylic acid	500	99
Citric acid	500	100
Fructose	500	102
Glucose	500	98
Phosphoric acid	500	100
Saccharin	500	99
Saccharose	500	100
Sodium benzoate	500	101
Sodium chloride	500	98
Tartaric acid	500	98
Cu(II), Ni(II), Co(II), Al(III),	500	97
Mn(II),Zn(II), Ca(II), Mg(II), Cd(II)	500	99
Cr(III), Pb(II),	500	98
PO_4^{3-}, SO_4^{2-}, F^-	1000	99

CPE conditions: 1×10^{-4} mol L^{-1} Br-PADAP, 1.0×10^{-4} mol L^{-1} EDTA, 0.25% (v/v) TX-114, 5×10^{-5} mol L^{-1} Fe(III), 20 μg L^{-1} AA.

of the aqueous phase and the final volume of the surfactant-rich phase, was 20 times.

Interferences

In order to study the selectivity of the proposed method, we tested the effect of various cations and anions on the preconcentration and determination of AA by the proposed method under the optimum conditions. Sample solutions containing 20 μg L^{-1} of AA and different concentration of other ions or compounds were prepared and the developed procedure was applied. The influence of foreign ions on the determination of AA was studied and an error of ±5% in the absorbance reading was considered tolerable. Diverse components of organic and inorganic compounds normally present in fruit, vegetables, beverages, and pharmaceuticals do not interfere. L-cysteine was tolerable in the presence of at least one-fold excess. The results presented in Table 1 show the excellent selectivity of the procedure. It is free from most interfering substances and can be applied for the determination of AA in different samples in the presence of organic and inorganic compounds. The matrix effects with the method were reasonably tolerable (Table 1).

Application

The proposed method was further applied to the determination of AA in several real samples. The results obtained in five individual determinations of AA and their standard deviations are shown in Table 2. The results obtained by the proposed method agreed well with the reference method.[29] The statistical study of precision and accuracy of the proposed method was made from *F* criterion and the *t*-test, respectively. The *t*-test was applied to the results obtained by the proposed and the 1,10-phenanthroline (1,10-PH) method,[29] and it showed that calculated *t* values were lower than the critical *t* value ($t = 2.31$, $P = 0.05$). This suggested that at 95% confidence level, differences between the results obtained by the two methods were statistically not significant. The *F*-test revealed that there is no difference between the precision of the two methods. In every case, the calculated value of *F* was lower than the tabulated value ($F = 6.39$, $P = 0.05$). These results indicated that the methods did not give significantly different values for the mean AA concentration. Ingredients associated with vitamin C and multivitamin products, such as sodium citrate, acetylsalicylic acid, citric acid, saccharine, sodium carbonate, starch, sorbitol, thiourea, sodium benzoate, retinol, chloride, sugars, and vitamin B complex,

Table 2. Ascorbic acid content of commercial syrups (n = 5)

Commercial name of syrup	Supplier	Declared (mg/5 mL)	Extraction method (mg/5 mL)[a]	1,10-PH Method (mg/5 mL)[a]	$F_{calc.}$	$t_{calc.}$
Day & Night	Ineldea	6	5.7 ± 1.82	5.9 ± 1.91	1.09	2.28
Herbazinc	Ineldea	6	6.1 ± 1.52	6.3 ± 2.53	2.77	0.96
Supravit	Bayer	45	46.1 ± 2.48	47.3 ± 2.66	1.15	2.25
Vi-Daylin	Fako	40	39.6 ± 1.67	40.4 ± 1.93	1.33	1.88
Minadex	Seven Seas	17.5	17.0 ± 1.03	16.7 ± 1.14	1.21	2.24

Theoretical value for *F* is 6.39 ($P = 0.05$) and for *t* is 2.31 ($P = 0.05$).
[a] The 95% confidence limits of the mean ($n = 5$).

Table 3. Comparison of the proposed method with some preconcentration methods

Technique	Linear range (μg mL^{-1})	Detection limit (μg mL^{-1})	Reference
[a]SP- UV-spectrophotometry	0.3–5.0	0.05	[5]
SP- UV spectrophotometry	1.76–17.6	0.352	[6]
SP- UV-spectrophotometry	0.2–20	0.02	[7]
SP-reactor	0.88–7.04	0.0528	[8]
SP-Vis-spectrophotometry	0.005–0.090	0.00091	[10]
[b]SP-FAAS	0.1–50	NR	[11]
SP-FAAS	0.4–20	NR	[12]
SP-FAAS	0.5–25	NR	[13]
SP-FAAS	0.5–20	NR	[14]
SP-FAAS	0.2–34.5	NR	[15]
SP-FAAS	0.3–60	NR	[16]
[c]LLE-spectrophotometry	0.02–0.15	NR	[18]
LLE-spectrophotometry	0–5.5	NR	[19]
LLE-spectrophotometry	0–0.010	0.00003	[20]
LLE- (HPLC)	0.5–200	0.20	[23]
Br-PADAP-spectrophotometry	0–2.4	0.044	[24]
Br-PADAP-CPE-spectrophotometry	0.005–0.176	0.0009	This work

[a] Solid phase – UV-spectrophotometry.
[b] Solid phase-flame atomic absorption spectrometry.
[c] Liquid-liquid extraction-spectrophotometry.
NR: not reported.

did not interfere with the determination of AA using the proposed method.

Conclusion

The analytical performance of the presented method is comparable with the other preconcentration methods including SPE [5–16] and liquid-phase extraction (LLE).[18–20,23] Comparative data from some studies on preconcentration of AA by various techniques for the figure of the merits are given in Table 3. As can be seen in the data (Table 3), the proposed CPE and preconcentration method showed a wider linear range,[5–8,11–16,18–20,23] and lower detection limit[5–8,11–16,18,19,23] compared to previous separation preconcentration methods. The method was found to be highly sensitive and selective for the determination of AA in the presence of dehydro-ascorbic acid, all other constituents and excipients possibly present in pharmaceutical preparations. CPE preconcentration and determination of AA in different samples can be utilized as an alternative application.

Acknowledgement

The authors gratefully acknowledge the financial support from the Scientific and Technical Research Council of Turkey (TÜBİTAK Grant No: 109T856).

References

[1] Vitamin C. Available at: http://en.wikipedia.org/wiki/Vitamin_C [8 April 2011].
[2] S. P. Arya. Photometric methods for the determination of vitamin C. *Anal. Sci.* **1998**, *14*, 889.
[3] O. A. Zaporozhets, E. A. Krushinskaya. Determination of ascorbic acid by molecular spectroscopic techniques, *J. Anal. Chem.* **2002**, *57*, 343.
[4] M. C. Yebra-Biurrun. Flow injection determination methods of ascorbic acid. *Talanta* **2000**, *52*, 367.
[5] A. R. Medina, M. L. Fernández de Córdova, A. Molina-Díaz. A rapid and selective solid-phase UV spectrophotometric method for determination of ascorbic acid in pharmaceutical preparations and urine. *J. Pharm. Biomed. Anal.* **1999**, *20*, 247.
[6] J. C. B. Fernandes, G. de Oliveira Neto, L. T. Kubota. Use of column with modified silica for interfering retention in an FIA spectrophotometric method for direct determination of vitamin C in medicine. *Anal. Chim. Acta* **1998**, *366*, 11.
[7] A. Molina-Diaz, A. Ruiz-Medina, M. L. Fernández de Córdova. Determination of ascorbic acid by use of a flow-through solid phase UV spectrophotometric system. *Fresenius J. Anal. Chem.* **1999**, *363*, 92.
[8] A. V. Pereira, O. Fatibello-Filho. Flow injection spectrophotometric determination of L-ascorbic acid in pharmaceutical formulations with on-line solid-phase reactor containing copper (II) phosphate. *Anal. Chim. Acta* **1998**, *366*, 55.
[9] J. Ruzicka, E. H. Hansen. *Flow Injection Analysis*, Wiley: New York, **1988**.
[10] P. O. Barrales, M. L. Fernández de Córdova, A. Molina-Díaz. Indirect determination of ascorbic acid by solid-phase spectrophotometry. *Anal. Chim. Acta* **1998**, *360*, 143.
[11] Y.-C. Jiang, Z.-Q. Zhang, J. Zhang. Flow-injection, on-line concentrating and flame atomic absorption spectrometry for indirect determination of ascorbic acid based on the reduction of iron(III). *Anal. Chim. Acta* **2001**, *435*, 351.
[12] M. C. Yebra, R. M. Cespón, A. Moreno-Cid. Automatic determination of ascorbic acid by flame atomic absorption spectrometry. *Anal. Chim. Acta* **2001**, *448*, 157.
[13] M. C. Yebra-Biurrun, R. M. Cespo-Romero, P. Bermejo-Barrera. Indirect flow-injection determination of ascorbic acid by flame atomic absorption spectrometry. *Microchim. Acta* **1997**, *126*, 53.
[14] A. R. Türker, Ö. Yalçınkaya, A. Tunçeli. Application of column solid phase extraction of chromium for indirect determination of ascorbic acid by flame atomic absorption spectrometry. *J. Food Drug Anal.* **2008**, *16*, 83.
[15] M. C. Yebra-Biurrun, R. M. Cespon-Romero, A. Moreno-Cid. Flow injection flame AAS determination of ascorbic acid based on permanganate reduction. *Atom. Spectrosc.* **2001**, *2*, 346.
[16] Z. Q. Zhang, Y. C. Jiang. Flame atomic absorption spectrometry for the automatic indirect determination of ascorbic acid based on flow injection and reduction of chromate. *Atom. Spectrosc.* **2000**, *21*, 100.
[17] M. C. Yebra. Continuous automatic determinations of organic compounds by flow injection atomic absorption spectrometry. *Trac. Trends Anal. Chem.* **2000**, *19*, 629.
[18] W. B. Robinson, E. Stotz. The indophenol-xylene extraction method for ascorbic acid and modifications for interfering substances. *J. Biol. Chem.* **1945**, *160*, 217.
[19] S. P. Arya, M. Mahajan, P. Jain. Spectrophotometric determination of vitamin C with iron(II)-4-(2-pyridylazo)resorcinol complex. *Anal. Chim. Acta* **2001**, *427*, 245.
[20] X. Gu, C. Chen, T. Zhou. Spectrophotometric method for the determination of ascorbic acid with iron (III)-1,10-phenanthroline after preconcentration on an organic solvent-soluble membrane filter, *Fresenius J. Anal. Chem.* **1996**, *355*, 94.
[21] B. Bozan, B. T. Sagdullaev, M. Kozar, K. H. N. Aripov, K. H. C. Baser. Comparison of ascorbic and citric acid contents in *Rosa canina* L. fruits growing in Central Asian region, *Chem. Nat. Compd.* **1998**, *34*, 687.
[22] J. Halasova, D. Jicinska. Amount of ascorbic acid in the hips of Rosa species. *Folia Geobot.* **1998**, *23*, 181.
[23] S. Nojavan, F. Khalilian, F. M. Kiaie, A. Rahimi, A. Arabanian, S. Chalavi. Extraction and quantitative determination of ascorbic acid during different maturity stages of Rosa canina L. Fruit. *J. Food Compos. Anal.* **2008**, *21*, 300.
[24] S. L. C. Ferreira, M.L.S.F. Bandeira, V. A. Lemos, H. C. dos Santos, A. C. Spinola Costa, D. S. de Jesus. Sensitive spectrophotometric determination of ascorbic acid in fruit juices and pharmaceutical formulations using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP), *Fresenius J. Anal. Chem.* **1997**, *357*, 1174.
[25] E. K. Paleologos, D. L. Giokas, M. I. Karayannis. Micelle-mediated separation and cloud-point extraction. *Trac. Trends Anal. Chem.* **2005**, *24*, 426.
[26] Z. S. Ferrera, C. P. Sanz, C. M. Santana, J. J. S. Rodríguez. The use of micellar systems in the extraction and pre-concentration of organic pollutants in environmental samples, *Trac. Trends Anal. Chem.* **2004**, *23*, 469.
[27] M. Zenki, A. Tanishita, T. Yokoyama. Repetitive determination of ascorbic acid using iron(III)-1.10-phenanthroline–peroxodisulfate system in a circulatory flow injection method, *Talanta* **2004**, *64*, 1273.
[28] S. Oszwaldowski, A. Pikus. Reversed-phase liquid chromatographic simultaneous determination of iron(III) and iron(II) as complexes with 2-(5- bromo-2-pyridylazo)-5-diethylaminophenol Determination of iron(III) and iron(II) in water samples and ultrasound field effect on distribution of iron(III) and iron(II) in micellar solution. *Talanta* **2002**, *58*, 773.
[29] A. Besada. A facile and sensitive spectrophotometric determination of ascorbic acid, *Talanta* **1987**, *34*, 731.