Determination of L-Ascorbic Acid in Pharmaceutical Preparations Using Direct Ultraviolet Spectrophotometry

Mirsad SALKIĆ (✉)
Husejin KERAN
Midhat JAŠIĆ

Summary

A new, selective and accurate direct ultraviolet spectrophotometric method was developed for the determination of L-ascorbic acid in pharmaceuticals. The oxidation of L-ascorbic acid by iodate in an acidic medium was used as a means of correcting for background absorption. The molar absorptivity of the proposed method was found to be 8.71×10^3 dm^3 mol^{-1} cm^{-1} at 250 nm. Beer's law was obeyed in the concentration range of 0.46 – 16.00 μg cm^{-3} for L-ascorbic acid. The relative standard deviation was 0.67 % for a concentration of 8.00 μg cm^{-3} of ascorbic acid (n = 7). The ingredients commonly found in vitamin C and multivitamin products did not interfere. The proposed procedure was successfully applied to assays of L-ascorbic acid in pharmaceutical preparations. The results obtained with the proposed method showed good agreement with those given by the titrimetric method using iodine.

Key words

L-ascorbic acid, iodate, ultraviolet spectrophotometry

University of Tuzla, Faculty of Technology,
Univerzitetska 8, 75000 Tuzla, Bosnia and Herzegovina
✉ e-mail: mirsad.salkic@untz.ba

Received: October 10, 2008 | Accepted: April 3, 2009

Introduction

L-ascorbic acid (2-oxo-L-threo-hexono-1,4-lactone-2,3-enediol) is an essential vitamin which participates in many different biological processes. It occurs naturally in most fruit juices and vegetables. The reversible oxidation of L-ascorbic acid to dehydro-L-ascorbic acid is the basis for its physiological activities and technical applications. L-Ascorbic acid is widely utilized as an additive in juices, soft drinks and foods, and it is also used in pharmaceutical preparations. Sometimes, its addition is carried out during product manufacturing in order to increase the nutritional value or as an antioxidant to lengthen the lifetime of the industrialized products. Many analytical methods have been reported in the literature for the determination of the ascorbic acid contents in different pharmaceutical products, fruits, vegetables and biological fluids. These include titrimetric (Verma et al., 1982), fluorimetric (Wu et al., 2003), electrochemical (Li et al., 2006), high-performance liquid chromatographic (Lykkesfeldt, 2000; Iwase, 2003), spectrophotometric (Fujita et al., 2001; Janghel et al., 2007; Arya et al., 2001) and chemiluminescent (Kato et al., 2005) methods. Of all these methods, spectrophotometric methods are, perhaps, the most commonly used. Direct ultraviolet spectrophotometry can provide a fast, simple and reliable method for the determination of L-ascorbic acid. However, the absorption of UV light by the sample matrix is the major problem with this method. Various background correction techniques such as thermal degradation, UV light decomposition, enzymatic or metal catalytic oxidation and photodestruction of L-ascorbic acid have been proposed to solve this problem. The thermal, UV and metal catalytic decomposition of L-ascorbic acid was too slow to be used practically (Fung et al., 1985). The time required to destroy L-ascorbic acid by photochemical method was 15 minutes (Jung et al., 1995). Some methods based on the Cu(II)-catalyzed oxidation are reported for the assay of pharmaceuticals, soft drinks and fruit juices (Lau et al., 1987; Lau et al., 1986). The presence of Al(III), Mg(II) or Zn(II) gives a negative error due to their catalytic effect on the air oxidation of L-ascorbic acid. Iron(II) interferes more seriously because of its greater catalytic effect. Although the enzymatic methods (bin Saari et al., 1999; Tsumura et al., 1993) are simple, rapid and highly specific for L-ascorbic acid One major obstacle to the wide usage of these methods is the high costs of purified enzymes.

The purpose of this work was to develop a direct and simple ultraviolet spectrophotometric method for the determination of ascorbic acid in pharmaceuticals with background correction based on the oxidation of L-ascorbic acid by iodate. The effects of a number of substances commonly encountered in pharmaceutical preparations on the proposed method were studied. The ascorbic acid contents of a number of pharmaceuticals were determined using the developed method.

Material and methods

Reagents

All reagents used were of analytical-reagent grade.

Buffer solution (pH = 4.00). A mixture of citric acid (0.0073 mol dm^{-3}) and disodium hydrogenphosphate (0.0073 mol dm^{-3}) was prepared by dissolving 1.40 g of citric acid (Fluka) and 1.30 g of $Na_2HPO_4 \cdot 2H_2O$ (Merck) in 1000 cm^3 of distilled water.

Stabilizer solution. A solution of EDTA (2.69×10^{-4} mol dm^{-3}) was prepared by dissolving 0.10 g of ethylenediaminetetraacetic acid disodium salt dihydrate (Fluka) in the buffer solution and making up a volume of 1 dm^3.

L-Ascorbic acid solution (1.13×10^{-3} mol dm^{-3}). Prepared by dissolving 0.05 g of L-ascorbic acid (Riedel-de Haën) in the stabilizer solution and diluting to 250 cm^3 in a volumetric flask.

Potassium iodate solution (0.005 mol dm^{-3}). Prepared by dissolving 0.27 g of potassium iodate (Kemika) in 250 cm^3 of the stabilizer solution.

Iodine solution (0.017 mol dm^{-3}). About 4.5 g of reagent-grade iodine (Sigma) was transferred into a 100 cm^3 beaker containing 20 g of potassium iodide (Merck) dissolved in 25 cm^3 of water. The entire content was stirred and transferred into a glass-stoppered amber liter bottle. The mixture was diluted to 1 dm^3 using distilled water.

Solutions of metal ions, anions, organic acids, vitamins, sugars and amino acids were prepared by dissolving calculated amounts of these substances in the stabilizer solution.

Apparatus

A Cecil 2021 spectrophotometer with 1 cm path length was used for absorbance measurements. The pH measurements were made with a Quatro 220K pH meter.

General procedure

Transfer an aliquot of the sample solution containing 150 – 250 μg of L-ascorbic acid into a 25 cm^3 standard flask. Dilute to the mark with the stabilizer solution and measure the absorbance (A_1) at 250 nm against the stabilizer solution as a blank. Transfer another aliquot of the sample solution into a 25 cm^3 volumetric flask. Add 1.0 cm^3 of the potassium iodate solution and dilute to the mark with the stabilizer solution. Measure the absorbance (A_2) after 10 – 15 minutes at 250 nm against a blank solution prepared by mixing 1.0 cm^3 of the potassium iodate solution with 1.0 cm^3 of the 1.13×10^{-3} mol dm^{-3} ascorbic acid solution in a standard flask and dilute to 25 cm^3 with the stabilizer solution. The value $A_1 - A_2 = A$ is proportional to the ascorbic acid concentration in the sample.

To prepare the calibration graph, transfer a suitable aliquot of the 1.13×10^{-3} mol dm^{-3} ascorbic acid solution into a 25 cm^3 volumetric flask and make up to the mark with the stabilizer solution. Measure the absorbance of the resulting solution at 250 nm against the stabilizer solution as a blank.

Procedure for tablets and granules

Transfer an accurately weighed amount of granules or powder obtained from several tablets into a 100 cm^3 volumetric flask, dissolve and make up to the mark with the stabilizer solution. Filter and dilute a suitable aliquot of the filtrate to 50 cm^3 with the stabilizer solution. Take an aliquot of the

final solution and determine the ascorbic acid content as described under general procedure.

Titrimetric method using iodine

Iodine solution (0.017 mol dm^{-3}) is standardized in the usual way with a primary standard of As_2O_3. Weigh an accurate amount of granules or powdered tablets and pour into a dry volumetric flask of the size chosen. When you are ready to titrate, dissolve granules or powder by adding a volume of distilled water that is equal to about half the volume of the volumetric flask and shake. Fill to the mark. Pipet exactly 25 cm^3 of the vitamin C solution into a 250 cm^3 flask and add 5 cm^3 of starch indicator. Cover the opening of the flask with a piece of cardboard with a small hole for the buret tip. Titrate rapidly to reduce air oxidation of the ascorbic acid, but proceed dropwise near the end point, a deep blue starch-triiodide color.

Statistical methods

The molar absorptivity, regression equation and correlation coefficient were obtained by a linear least-squares treatment of the results. The results obtained by the proposed method were compared with those provided by the titrimetric method using iodine as titrant (Fritz et al., 1987). The *t*-test was used to decide whether there was a significant difference between the results obtained by the two methods. The *F*-test was used in order to see whether the proposed method and the iodine method differ in their precision.

Results and discussion

Oxidation of L-ascorbic acid by iodate

L-Ascorbic acid undergoes the oxidation reaction with iodate in an acidic medium to yield dehydro-L-ascorbic acid. The oxidation of L-ascorbic acid usually takes place in a two-step reaction, in which electrons are transferred. The first step yields a relatively stable ascorbate free radical. In the second one, the L-ascorbic acid free radical donates a second electron, yielding dehydro-L-ascorbic acid (Eitenmiller et al., 1999; Davies et al., 1991; Orlický et al., 2003).

Srividya and Balasubramanian (1996) reported an indirect procedure based on the oxidation of L-ascorbic acid by a known excess of iodate in the presence of acid for the analysis of pharmaceuticals and fresh fruit juices. The unreacted iodate is used for hydroxylamine oxidation to generate nitrite. Sulfanilic acid is diazotized by the nitrite formed and the resulting diazonium ion is coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form an azo dye (λ_{max} = 540 nm). This procedure is a complicated one as it involves many steps. The aim of this paper was the development of a simple method which involves only the oxidation of L-ascorbic acid.

Optimization of conditions

Absorption properties of L-ascorbic acid are dependent upon the ionic species present and, therefore, dependent upon the pH of the aqueous media. Above pH 5.0, L-ascorbic acid exists predominantly as the monoanion and has maximal absorption at 265 nm. Undissociated, at more acid pH levels, maximal absorption occurs around 245 nm (Eitenmiller et al., 1999). Since the position of maximum absorbance is pH-dependent, the citric acid – disodium hydrogenphosphate buffer solution (pH = 4.00) was used throughout this work.

The degradation of L-ascorbic acid in aqueous solutions depends on a number of parameters. Oxygen partial pressure, pH, temperature, light and the presence of heavy metal ions are of great importance. Metal-catalyzed destruction proceeds at a higher rate than noncatalyzed spontaneous autoxidation. Traces of heavy metal ions, particularly Cu^{2+} and Fe^{3+}, result in high losses (Francis, 2000). L-Ascorbic acid can be stabilized with reducing reagents, amino acids, sugars and chelating agents (Francis, 2000; Miyake et al., 1999). In the present work, 2.69×10^{-4} mol dm^{-3} ethylenediaminetetraacetic acid (EDTA) in the buffer solution was used to stabilize L-ascorbic acid in the aqueous media. In the presence of this stabilizer, solutions of L-ascorbic acid remain stable for at least three hours at room temperature.

L-Ascorbic acid is oxidized by iodate in an acidic medium. In our case, this medium was provided by the buffer solution. The effect of the iodate concentration on the oxidation of L-ascorbic acid was studied by adding different amounts of iodate to a solution containing 12.00 μg cm^{-3} of L-ascorbic acid. Although 0.7 – 1.0 cm^3 of the 0.005 mol dm^{-3} potassium iodate solution can be used to oxidize L-ascorbic acid, 1.0 cm^3 of this solution was used for further investigation.

Analytical characteristics

The calibration curve is linear up to an ascorbic acid concentration of 16.00 μg cm^{-3}. A least-square analysis of Beer's plot gave the following linear regression equation (n = 7):

$$A_{250} = 0.0025 + 0.0494\ C,$$

where A_{250} is the absorbance at 250 nm and C is the concentration of L-ascorbic acid in μg cm^{-3}. The detection limit (three times the standard error of the intercept/slope), quantification limit (ten times the standard error of the intercept/slope), molar absorptivity (ε), as well as other analytical characteristics are summarized in Table 1. The precision of the proposed method was checked by calculating the relative standard deviation by seven replicate determination of a standard solution containing 8.00 μg cm^{-3} of L-ascorbic acid (Table 1). The molar absorptivity was calculated from

Table 1. Analytical characteristics of the proposed method

Slope of the calibration graph	0.0494
Intercept	0.0025
Standard error of the slope	0.00022
Standard error of the intercept	0.00227
Correlation coefficient (r)	0.99995
Limit of detection	0.14 μg cm^{-3}
Limit of quantification	0.46 μg cm^{-3}
Linear dynamic range	0.46 – 16.00 μg cm^{-3}
Molar absorptivity (ε)	8.71×10^3 dm^3 mol^{-1} cm^{-1}
Relative standard deviation	0.67%

the slope of the calibration graph to be 8.71×10^3 dm^3 mol^{-1} cm^{-1}. This procedure is more sensitive than other spectrophotometric methods, using 4-chloro-7-nitrobenzofurazane (Abdelmageed et al., 1995) ($\varepsilon = 6.49 \times 10^3$), zinc chloride salt of diazotized 1-aminoanthraquinone (Backheet et al., 1991) ($\varepsilon = 4.07 \times 10^3$), gold(III)ion (Pal et al., 1988) ($\varepsilon = 2.30 \times 10^3$) and *peri*-naphthindan-2,3,4-trione (Hassan et al., 1975) ($\varepsilon = 3.18 \times 10^3$).

Interference studies

To test the selectivity of the proposed method, interferences caused by those foreign species that are commonly found with L-ascorbic acid in the samples analyzed were studied by adding different amounts of other species to a solution containing 8.00 μg cm^{-3} of ascorbic acid. The criterion for the interference was an absorbance varying by 5 % from the expected value. The results are listed in Table 2, where the different substances are classified under such headings as metal ions, anions, vitamins, organic acids, sugars and amino acids.

None of the metal ions investigated interfered with the determination at the levels studied. The results in Table 2 demonstrate that EDTA at a concentration of 2.69×10^{-4} mol dm^{-3} is a suitable stabilizer for L-ascorbic acid in the developed method. This chelating agent forms with metal ions stable complexes which are no longer effective catalysts. EDTA prevents the ascorbic acid-metal ion complex formation and therefore inhibits effectively the oxidation of L-ascorbic acid. Since absorption properties (λ_{max} and ε) of L-ascorbic acid depend on the pH of the aqueous media (Eitenmiller et al., 1999), negative errors caused by magnesium(II) and nicotinic acid may be ascribed to a decrease in the pH of the ascorbic acid solution.

The anions tested except nitrite did not interfere with the determination of ascorbic acid using the proposed method. A serious negative error caused by nitrite may be ascribed to the oxidation of L-ascorbic acid with this oxidant in an acidic medium. The amino acids, sugars and organic acids did not noticeably affect the accuracy of the determination of ascorbic acid, even when these substances were present in large excess amounts compared with that of ascorbic acid. Substances which interfere with other proposed methods for the determination of vitamin C are summarized in Table 3. Obviously the proposed method can be compared favourably with other spectrophotometric methods as regards its selectivity.

Application of the proposed method to real samples

The proposed method was applied to the determination of the ascorbic acid contents in commercial pharmaceutical preparations (granule and tablet). The results obtained are shown in Table 4. In every case, the sample was analyzed by both the proposed and the titrimetric method using iodine as titrant (Fritz et al., 1987). The last one, used as a reference method, is a procedure based on the oxidation of L-ascorbic acid to dehydro-L-ascorbic acid by iodine. The results of applying the proposed method showed good agreement with those provided by the reference method. The results obtained by the developed method also agreed well with the claimed values on the labels in all instances. Ingredients usually associated with vitamin C and multivitamin products, such as acetylsalicylic acid, sodium citrate, starch, saccharine, glycerol, sodium benzoate, magnesium stearate, stearic acid, microcrystalline cellulose, sodium hydrogencarbonate, retinol, sugars and vitamin B complex, did not interfere with the determination of L-ascorbic acid using the proposed method.

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

Table 2. Effect of foreign substances on the determination of L-ascorbic acid

Foreign substance added	Mass ratio (foreign substance:ascorbic acid)	Error, %
Metal ions		
Iron(II)	0.02	-
Copper(II)	0.033	-
Calcium(II)	5	-
Magnesium(II)	2	-1.00
Zinc(II)	1	-
Manganese(II)	1.3	-
Molybdenum(VI)	1	-
Lead(II)	2	-
Nickel(II)	0.4	-
Anions		
Cl^-	10	-
NO_3^-	2.5	-
SO_4^{2-}	10	-
CO_3^{2-}	5	-
HCO_3^-	10	-
$H_2PO_4^-$	14	-
PO_4^{3-}	5	-
NO_2^-	2	-17.04
MoO_4^{2-}	1.7	-
Tartrate	5	-
Acetate	10	-
Oxalate	4	-
Citrate	20	-
Benzoate	2	-
Organic acids		
Citric acid	24	-
Oxalic acid	5	-
Tartaric acid	10	-
Acetic acid	20	-
Lactic acid	20	-
Malic acid	20	-
Vitamins		
B_1 (thiamine hydrochloride)	2	-
B_6 (pyridoxine hydrochloride)	2	-
E (α-tocopheryl acetate)	2	-
Nicotinic acid	2	-1.11
Sugars		
Sucrose	200	-
Glucose	200	-
Fructose	200	-
Lactose	100	-
Maltose	100	-
Amino acids		
L-Proline	10	-
DL-Alanine	10	-
L-Leucine	10	-
L-Arginine	10	-
L(+)-Asparagine	10	-

Table 3. Interferences with other spectrophotometric methods

Reagent	Interference	Reference
Fe(III)-*p*-carboxyphenylfluorone	Nitrite, iodide, oxalate, citrate and hydroxylamine	Fujita et al., 2001
Fe(III)-nitroso-R salt	Oxalic acid, Cu(II) and cysteine	Arya et al., 1997
Fe(III)-2-oximinocyclohexanone thiosemicarbazone	Starch, sucrose, oxalic acid, riboflavin, phenylalanine, niacin, Cu(II), Co(II), Ni(II), Pd(II), IO_3^- and NO_2^-	Salinas et al., 1988
Potassium dichromate	Paracetamol, aspirin, caffeine and reducing substances	Sarkar et al., 1994
Fe(III)-ferrozine	Cu(II), Co(II), Ni(II) and Fe(II)	Jaselskis et al., 1972
Iron(II)-pyridine-picolinic acid	SO_3^{2-}, PO_4^{3-} and $S_2O_3^{2-}$	Arya et al., 1996
Fe(III)-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol	Urea, thiourea, dipirona and paracetamol	Ferreira et al., 1997

tained for the determination of L-ascorbic acid in pharmaceuticals using the developed method were compared with those obtained by the reference method and good agreement was found. Thus, the proposed method can be applied to the determination of vitamin C in commercial pharmaceutical preparations.

References

Abdelmageed O. H., Khashaba P. Y., Askal H. F., Saleh G. A., Refaat I. H. (1995). Selective spectrophotometric determination of ascorbic acid in drugs and foods. Talanta 42: 573-579.

Arya S. P., Mahajan M. (1996). Spectrophotometric determination of ascorbic acid using an iron(II)-pyridine-picolinic acid complex. Anal Sci 12: 941-945.

Arya S. P., Mahajan M. (1997). Nitroso-R salt as a sensitive spectrophotometric reagent for the determination of vitamin C. Anal Lett 30(14): 2541-2553.

Arya S. P., Mahajan M., Jain P. (2001). Spectrophotometric determination of vitamin C with iron(II)-4-(2-pyridylazo) resorcinol complex. Anal Chim Acta 427: 245-251.

Table 4. Determination of L-ascorbic acid in tablets and granules.

Commercial name (Supplier)	Ascorbic acid (mg)[a]		$F_{calc.}$	$t_{calc.}$
	Claimed value	Proposed method[b]	Iodine method[b]		
Vitamin C (PEZ)	60	61.73 ± 0.60	60.95 ± 0.89	2.19	2.02
Aspirin plus C (Bayer Pharma)	240	242.21 ± 1.80	240.06 ± 2.53	1.96	1.92
Andol C (Pliva)	240	240.74 ± 1.89	241.31 ± 2.66	1.95	0.48
Upsavit (Laboratoires UPSA)	1000	1001.51 ± 6.03	992.94 ± 8.51	1.99	2.28
Duovit[c] (Krka)	60	60.05 ± 0.93	58.84 ± 1.42	2.30	1.97
Acerola plus (Natural Wealth Nutrition corp.)	100	99.44 ± 0.75	98.38 ± 1.14	2.25	2.15
Vitamin C (Hansa-pharm Dr. Winter)	120	123.49 ± 1.10	121.50 ± 2.13	3.73	2.29
Vitamin C (Krüger)	180	185.33 ± 1.60	183.28 ± 1.93	1.46	2.26
Bio-C 500 (Pharmamed)	500	492.93 ± 2.47	494.04 ± 4.93	3.96	0.56
Cedevita sport[c,d] (Cedevita)	120	121.81 ± 2.02	119.64 ± 1.79	1.27	2.22

Theoretical value for F is 6.39 ($P = 0.05$) and for t is 2.31 ($P = 0.05$);[a] mg per tablet or mg/100 g; [b] The 95% confidence limits of the mean ($n = 5$); [c] Multivitamin preparation; [d] Granules.

proposed and the iodine method, and it showed that calculated t values were lower than the tabulated 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 critical value ($F = 6.39$, $P = 0.05$). The accuracy of the proposed method was further checked by a thorough replicate analysis of each sample spiked with 2.40 μg cm^{-3} of L-ascorbic acid. The recoveries of ascorbic acid added to the multivitamin and vitamin C products were about 98 – 102 %. This indicates that the proposed method gives accurate results.

Conclusions

The proposed method, which does not require an extraction procedure, is simple, rapid, selective and accurate. The method is quite useful and allows the determination of ascorbic acid in the presence of common excipients usually encountered in pharmaceutical formulations. The results ob-

Backheet E. Y., Emara K. M., Askal H. F., Saleh G. A. (1991). Selective spectrophotometric method for the determination of ascorbic acid in pharmaceutical preparations and fresh fruit juices. Analyst 116: 861-865.

Bin Saari N., Osman A., Selamat J., Fujita S. (1999). Ascorbate oxidase from starfruit (*Averrhoa carambola*): preparation and its application in the determination of ascorbic acid from fruit juices. Food Chem 66: 57-61.

Davies M. B., Austin J., Partridge D.A. (1991). Vitamin C: Its Chemistry and Biochemistry. Royal Society of Chemistry, London.

Eitenmiller R. R., Landen W.O. (1999). Vitamin Analysis for the Health and Food Sciences. CRC Press, USA.

Ferreira S. L. C., Bandeira M. L. S. F., Lemos V. A., dos Santos H. C., Costa A. C. S., de Jesus D. S. (1997). 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 357: 1174-1178.

Francis F.J. (2000). Encyclopedia of Food Science and Technology. John Wiley & Sons, USA.

Fritz J. S., Schenk G.H. (1987). Quantitative Analytical Chemistry. Prentice Hall, New Jersey.

Fujita Y., Mori I., Yamaguchi T., Hoshino M., Shigemura Y., Shimano M. (2001). Spectrophotometric determination of ascorbic acid with iron(III) and *p*-carboxyphenylfluorone in a cationic surfactant micellar medium. Anal Sci 17: 853-857.

Fung Y. S., Luk S.F. (1985). Determination of ascorbic acid in soft drinks and fruit juices. Part 1. Background correction for direct ultraviolet spectrophotometry. Analyst 110: 201-204.

Hassan S. S. M., Abd El Fattah M. M., Zaki M. T. M. (1975). Spectrophotometric determination of vitamin C in citrus fruits using *peri*-naphthindan-2,3,4-trione. Fresenius Z Anal Chem 277: 369-371.

Iwase H. (2003). Routine high-performance liquid chromatographic determination of ascorbic acid in foods using L-methionine for the pre-analysis sample stabilization. Talanta 60: 1011-1021.

Janghel E. K., Gupta V. K., Rai M. K., Rai J. K. (2007). Micro determination of ascorbic acid using methyl viologen. Talanta 72: 1013-1016.

Jaselskis B., Nelapaty S. J. J. (1972). Spectrophotometric determination of microamounts of ascorbic acid in citrus fruits. Anal Chem 44: 379-381.

Jung M. Y., Kim S. K., Kim S. Y. (1995). Riboflavin-sensitized photodynamic UV spectrophotometry for ascorbic acid assay in beverages. J Food Sci 60: 360-364.

Kato T., Ohno O., Nagoshi T., Ichinose Y., Igarashi S. (2005). Determination of small amounts of L-ascorbic acid using the chemiluminescence of an iron-chlorophyllin complex. Anal Sci 21: 579-581.

Lau O. W., Luk S. F., Wong K. S. (1986). Background correction method for the determination of ascorbic acid in soft drinks, fruit juices and cardials using direct ultraviolet spectrophotometry. Analyst 111: 665-670.

Lau O. W., Luk S. F., Wong K. S. (1987). Determination of ascorbic acid in pharmaceuticals using direct ultraviolet spectrophotometry. Analyst 112: 1023-1025.

Li C. X., Zeng Y. L., Liu Y. J., Tang C. R. (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.

Lykkesfeldt J. (2000). Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: Reliable reduction with tris[2-carboxyethyl] phosphine hydrochloride. Anal Biochem 282: 89-93.

Miyake N., Kim M., Kurata T. (1999). Stabilization of L-ascorbic acid by superoxide dismutase and catalase. Biosci Biotechnol Biochem 63: 54-57.

Orlický J., Gmucová K., Thurzo I., Pavlásek J. (2003). Monitoring of oxidation steps of ascorbic acid redox reaction by kinetics-sensitive voltcoulometry in unsupported and supported aqueous solutions and real samples. Anal Sci 19: 505-509.

Pal T., Das P. K. (1988). A comparative study on spectrophotometric, conductometric and potentiometric determination of ascorbic acid. Anal Lett 21: 2333-2343.

Salinas F., Diaz T. G. (1988). Spectrophotometric determination of L-ascorbic acid in pharmaceutical preparations, foods and urine by formation of a 2-oximinocyclohexanone thiosemicarbazone-iron(II) complex. Analyst 113: 1657-1659.

Sarkar M., Sarkar A.R. (1994). Spectrophotometric determination of vitamin C. Analusis 22: 155-157.

Srividya K., Balasubramanian N. (1996). Indirect spectrophotometric determination of ascorbic acid in pharmaceutical samples and fruit juices. Analyst 121: 1653-1655.

Tsumura F., Ohsako Y., Haraguchi Y., Kumagai H., Sakurai H., Ishii K. (1993). Rapid enzymatic assay for ascorbic acid in various foods using peroxidase. J Food Sci 58: 619-622.

Verma K.K. (1982). Determination of ascorbic acid with *o*-iodosobenzoate. Talanta 29: 41-45.

Wu X., Diao Y., Sun C., Yang J., Wang Y., Sun S. (2003). Fluorimetric determination of ascorbic acid with *o*-phenylenediamine. Talanta 59: 95-99.

acs74_46