Direct Spectrophotometric Determination of L-Ascorbic acid in Pharmaceutical Preparations using Sodium Oxalate as a Stabilizer

Amra Selimović*, Mirsad Salkić, Amel Selimović
Department of Analytical Chemistry
Faculty of Technology, University of Tuzla, Bosnia and Herzegovina

***Abstract*--** **A simple and highly sensitive direct spectrophotometric method was developed for the determination of L-ascorbic acid. Sodium oxalate (0,0056 mol/dm^3) was used to stabilize L-ascorbic acid in aqueous medium. The molar absorptivity of the proposed method, which does not require an extraction procedure, was 1.42x10^4 dm^3 mol^{-1} cm^{-1} at 266 nm. Beer's law was obeyed in the concentration range of 0.857 – 12.0 µg ascorbic acid/cm^3. The relative standard deviation was 0.81 % for the determination of 8.0 µg ascorbic acid/cm^3 (n = 7).**

The substances commonly found in vitamin C products do not interfere with the determination of ascorbic acid. Other vitamins, Ca(II) and benzoate interfere. The proposed procedure was successfully applied to the determination of ascorbic acid in pure form and vitamin C preparations.

***Index Term*--** **Spectrophotometry, L-ascorbic acid, sodium oxalate, stabilizer.**

1. INTRODUCTION

Vitamin C has long been recognized as an important nutrient in several food products. The reduced form of the vitamin is referred to as L-ascorbic acid, and the oxidized form is referred to as dehydroascorbic acid. In humans, both forms are biologically active. The total vitamin C activity is the sum of both forms.

Vitamin C is added during the manufacture of juices or soft drinks to improve their nutritional value or to prevent the autoxidation of commercial products. Owing to the wide use of L-ascorbic acid in canned fruits, vegetables and drugs, numerous analytical methods have been proposed for the determination of L-ascorbic acid, including titrimetry[1], fluorimetry[2], spectrophotometry[3-5] and high-performance liquid cromatography (HPLC)[6], each with their advantages and disadvantages.

The use of direct UV for the assay of ascorbic acid has not been easy due to its instability in aqueous solutions. The instability of L-ascorbic acid is due to its oxidation to dehydroascorbic acid which is a reversible reaction and subsequently to 2,3-diketo-L-gulonic acid. This later reaction is irreversible. These reactions can be inhibited by stabilizers.

The purpose of this work was to develop a direct and simple ultraviolet spectrophotometric method for the determination of L-ascorbic acid in vitamin C products with sodium oxalate as a stabilizer for ascorbic acid. The effects of a number of chemical substances commonly found in vitamin C preparations on the proposed method were assessed.

2. EXPERIMENTAL

2.1. Reagents

All reagents used were of analytical-reagent grade.

Buffer solution (pH = 5.4). A mixture of potassium dihydrogenphosphate (0.03 mol/dm^3) and disodium hydrogenphosphate (8.99x10^{-4} mol/dm^3) was prepared by dissolving 4.08 g of KH_2PO_4 (Fluka) and 0.16 g of $Na_2HPO_4 \cdot 2H_2O$ (Merck) in 1000 cm^3 of distilled water.

Sodium oxalate solution (0.0056 mol/dm^3). Prepared by dissolving 0.75 g of sodium oxalate (Sigma) in 1000 cm^3 of the buffer solution.

L-Ascorbic acid solution (1.13x10^{-3} mol/dm^3). A 0.05 g amount of L-ascorbic acid (Riedel-de Haën) was dissolved in 250 cm^3 of the sodium oxalate solution.

Solutions of metal ions, anions, organic acids, amino acids, vitamins and sugars were prepared by dissolving calculated amounts of these substances in the (0.0056 mol/dm^3) sodium oxalate solution.

2.2. Apparatus

All absorbances were determined on Cecil 2021 spectrophotometer using 1 cm path length.

2.3. General procedure

Transfer a portion of the sample solution containing 60 – 300 µg of L-ascorbic acid to a 25 cm^3 standard flask. Dilute to the mark with the (0.0056 mol/dm^3) sodium oxalate solution and measure the absorbance at 266 nm against the sodium oxalate solution as a blank.

2.4. Determination of L-ascorbic acid in tablets

Transfer an accurately weighed amount of powder obtained from several tablets into a 100 cm^3 volumetric flask, dissolve and make up to the mark with the (0.0056 mol/dm^3) sodium oxalate 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.

3. Results and Discussion

3.1. Optimization of conditions

Absorption properties of L-ascorbic acid are 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[7]. Since the position of maximum absorbance is pH-dependent, the potassium dihydrogenphosphate – disodium hydrogenphosphate buffer solution (pH = 5.4) was used throughout this work.

L-Ascorbic acid is readily and reversibly oxidized to dehydrascorbic acid, which is present in aqueous media as a hydrated hemiketal. The biological activity is lost when the dehydroascorbic acid lactone ring is irreversibly opened, giving rise to 2,3-diketogulonic acid. The oxidation of L-ascorbic acid to dehydroascorbic acid and its further degradation products depends on several factors. 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+}, result in high losses. Therefore, a major problem with the analysis of L-ascorbic acid in real samples concerns the prevention of the degradation of the vitamin.

In the present work, sodium oxalate in the buffer solution was used to stabilize L-ascorbic acid in the aqueous media. The effect of sodium oxalate concentration on the stability of L-ascorbic acid was studied in the range of 0.0033 to 0.011 mol/dm^3 in the presence of KH_2PO_4 (0.03 mol/dm^3) and Na_2HPO_4 (8.99x10^{-4} mol/dm^3). When the concentration is within the range of 0.0056 – 0.011 mol/dm^3, solutions of L-ascorbic acid remain stable for at least 30 minutes at room temperature. Therefore, 0.0056 mol/dm^3 sodium oxalate concentration was selected for further investigation.

3.2. Analytical characteristics of the proposed method

By using the proposed method, linear calibration curve was obtained in the range 0.857 – 12.0 μg ascorbic acid/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 I. The precision of the proposed method, expressed as relative standard deviation, for the determination of 8.0 μg/cm^3 ascorbic acid, was 0.81 % (n = 7). The molar absorptivity calculated from the slope of the calibration graph shows that the proposed method is highly sensitive. This procedure is more sensitive than other spectrophotometric methods, using 4-chloro-7-nitrobenzofurazane (ε = 6.49 x 10^3 dm^3 mol^{-1} cm^{-1})[8], zinc chloride salt of diazotized 1-aminoanthraquinone (ε = 4.07 x 10^3 dm^3 mol^{-1} cm^{-1})[9], gold(III) ion (ε = 2.30 x 10^3 dm^3 mol^{-1} cm^{-1})[10], *peri*-naphthindan-2,3,4-trione (ε = 3.18 x 10^3 dm^3 mol^{-1} cm^{-1})[11] and iodate-fluorescein (ε = 8.81 x 10^3 dm^3 mol^{-1} cm^{-1})[12].

Table I
Analytical characteristics of the proposed method

Slope of the calibration line	14241.79
Intercept of the calibration line	0.02755
Standard error of the slope of the calibration line	179.1263
Standard error of the intercept point of the line	0.00693
Correlation coefficient (r)	0.9998
Limit of detection	0.257 μg/cm^3
Limit of quantification	0.857 μg/cm^3
Linear dynamic range	0.857 – 12.0 μg/cm^3
Molar absorptivity (ε)	1.42 x 10^4 dm^3 mol^{-1} cm^{-1}
Relative standard deviation	0.81 %

3.3. Interference studies

To assess 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 II.

The metal ions investigated except Ca(II) did not interfere with the determination at the levels studied. The positive errors (all within 5 %) caused by Fe(II), Cu(II), Mg(II), Mn(II), Mo(VI), Zn(II) and Ni(II) may be ascribed to the absorption of UV light by these substances. Lau et al. reported that the metal ions, such as Fe(II), Mg(II) and Mn(II), caused errors but did not interfere with a direct spectrophotometric method for the determination of ascorbic acid in pharmaceuticals with background correction based on the copper(II)-catalysed oxidation of ascorbic acid.[13] The metal ions investigated did not interfere with other methods described in the literature for the determination of ascorbic acid[14,15] The results in Table II demonstrate that sodium oxalate at a concentration of 0.0056 mol/dm^3 is a suitable stabilizer for L-ascorbic acid in the developed method. This stabilizer forms with metal ions complexes which are no longer effective catalysts. Oxalate prevents the ascorbic acid-metal ion complex formation and therefore inhibits effectively the oxidation of L-ascorbic acid.

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

Foreign substance added	Mass ratio (foreign substance:ascorbic acid)	Error, %
Iron(II)	0.02	3.70
Copper(II)	0.02	2.05
Calcium(II)	5	> 5.00
Magnesium(II)	2	2.30
Manganese(II)	1.3	2.04
Molybdenum(VI)	0.2	2.78
Zinc(II)	2	1.89
Nickel(II)	0.4	1.17
Cl^-	10	0.00
NO_2^-	2	-1.17
Benzoate	2	> 5.00
Acetate	20	0.00
HCO_3^-	10	0.87
Citrate	10	0.00
PO_4^{3-}	5	0.00
Citric acid	5	-1.74
Tartaric acid	5	-0.73
Sucrose	200	0.00
Glucose	200	0.00
Fructose	200	0.00
L-Proline	10	0.00
L-Arginine	10	0.00
L(+)-Asparagine	10	0.00

The anions tested except benzoate did not interfere with the determination of ascorbic acid using the proposed method. Benzoate interfered seriously because of the absorption of UV light. The negative error caused by nitrite may be ascribed to the oxidation of L-ascorbic acid with this oxidant in an acidic medium. Since absorption properties (λ_{max} and ε) of L-ascorbic acid depend on the pH of the aqueous media[7], the positive error caused by hydrogencarbonate may be ascribed to an increase in the pH of the L-ascorbic acid solution. Other workers also reported that anions, such as sulfate, nitrate, chloride, oxalate and acetate ions, did not noticeably affect the accuracy of the determination of ascorbic acid, even when these ions were present in large excess amounts compared with that of ascorbic acid[3,14,16]

The experimental results revealed that a 200-fold excess of sucrose, glucose and fructose and 10-fold proline, asparagine and arginine had no effect on the determination of L-ascorbic acid using the proposed method. The presence of these sugars and amino acids, such as leucine, alanine and arginine, did not interfere with other proposed methods for the determination of vitamin C[13,14,15,17] Negative errors caused by citric and tartaric acids may be ascribed to a decrease in the pH of the ascorbic acid solution after the addition of organic acids. A mixture of thiamine (vitamin B_1), riboflavin (vitamin B_2), nicotinamide (vitamin PP), calcium pantothenate, pyridoxine hydrochloride (vitamin B_6) and cyanocobalamin (vitamin B_{12}) interfered seriously because of the absorption of UV light.

3.4. Application of the proposed method

The proposed method was applied to the determination of the ascorbic acid contents in commercial pharmaceutical preparations (tablets). The results obtained are shown in Table III. In every case, the sample was analyzed by both the proposed and the titrimetric method using iodine as titrant[18]. 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 obtained for the determination of ascorbic acid indicate that many of the ingredients commonly found in vitamin C preparations did not interfere with the proposed method. Other vitamins, such as thiamine, riboflavin and nicotinamide, and large amounts of benzoate do interfere and must be absent.

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 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). Thus, the proposed method can be successfully applied to real samples.

Table III
Determination of L-ascorbic acid in vitamin C preparations

Commercial name (Supplier)	L-Ascorbic acid (mg/tablet)			$F_{exp.}$	$t_{exp.}$
	Claimed value	Proposed method*	Iodine method*		
Vitamin C (Krüger)	180	185.02 ± 1.69	183.28 ± 1.93	1.30	1.88
Plivit C (Pliva)	500	502.09 ± 5.58	498.33 ± 5.12	1.18	1.37
Vitamin C (Galenika)	500	497.77 ± 2.86	498.93 ± 5.38	3.53	0.53

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

4. Conclusions

Sodium oxalate at a concentration of 0.0056 mol/dm^3 is a suitable stabilizer for L-ascorbic acid in a UV method of assay. The proposed method using the stabilizer is simple, sensitive, precise and accurate. Many common ingredients present in pharmaceutical preparations do not interfere. The results of applying the proposed method showed good agreement with those provided by the reference method. The results obtained by the proposed method also agreed well with the claimed values on the labels in all instances. Thus, the proposed method can be applied to the determination of vitamin C in commercial pharmaceutical preparations.

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