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Original Scientific Article

Spectrophotometric Determination of L-Ascorbic Acid in Pharmaceuticals Based on Its Oxidation by Potassium Peroxymonosulfate and Hydrogen Peroxide

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Abstract. Two new, accurate, precise, and sensitive spectrophotometric methods were developed for the assay of L-ascorbic acid in pharmaceutical preparations. The determination of L-ascorbic acid was based on its oxidation by potassium peroxymonosulfate (method A) and hydrogen peroxide in the presence of Cu(II) as a catalyst (method B). The molar absorptivities were found to be 1.48×10^4 and 1.06×10^4 L mol^{-1} cm^{-1} for methods A and B, respectively. Beer's law was obeyed in the concentration range of 0.65–11.20 μg mL^{-1} for method A and 0.51–16.00 μg mL^{-1} for method B. Other compounds commonly found in vitamin C and multivitamin products did not interfere with the determination of L-ascorbic acid.. The proposed methods were successfully applied for the determination of L-ascorbic acid in pharmaceutical formulations. The results obtained with the proposed methods showed good agreement with those given by the titrimetric method using iodine.

Keywords: L-Ascorbic acid, peroxymonosulfate, hydrogen peroxide, spectrophotometry, pharmaceutical preparations

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-used food additive with many functional roles, and is also used in pharmaceutical preparations. Many analytical methods have been reported in the literature for the determination of the ascorbic acid contents in different pharmaceutical products, foods and biological fluids. These include spectrophotometric,[1–4] high-performance liquid chromatographic,[5] electrochemical,[6] fluorimetric[7] and chemiluminescent[8] methods. Of all these methods, spectrophotometric methods are, perhaps, the most commonly used. Direct ultraviolet (UV) spectrophotometry can provide a fast, simple and reliable method for the determination of L-ascorbic acid. However, absorption of UV light by the sample matrix is a major problem with this method. Therefore, several background correction techniques such as thermal degradation, UV light decomposition,[9] enzymatic[10,11] and metal catalytic oxidation[12,13] 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. Some methods based on the Cu(II)-catalyzed oxidation are reported for the assay of pharmaceuticals, soft drinks and fruit juices. The presence of Fe(II), Al(III), Mg(II) or Zn(II) gives a negative error due to their catalytic effect on the air oxidation of L-ascorbic acid. Although the enzymatic methods are simple and highly specific for L-ascorbic acid, a major obstacle to the wide usage of these methods is the high costs of purified enzymes.

The aim of this work was to develop two simple, accurate, and sensitive spectrophotometric methods for the determination of ascorbic acid in pharmaceuticals with background correction based on the oxidation of L-ascorbic acid by peroxymonosulfate or hydrogen peroxide in the presence of Cu(II) catalyst. The effects of a number of substances commonly encountered in pharmaceutical preparations on the proposed methods were studied.

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EXPERIMENTAL

Apparatus

A Cecil 2021 spectrophotometer (Cecil Instruments, Cambridge, UK) with 1 cm path length was used for the absorbance measurements. A Quatro 220K pH meter was used for pH measurements.

Chemicals

All chemicals used were of analytical-reagent grade, and all solutions were prepared fresh daily.

A buffer solution of pH 6.00 was a mixture of glacial acetic acid (0.0087 M) and sodium acetate (0.152 M) in distilled water. A buffer solution of pH 4.50 was prepared in distilled water using glacial acetic acid (0.174 M) and sodium acetate (0.0983 M). A stabilizer solution of ethylenediaminetetraacetic acid (EDTA, 1.34×10^{-3} M) was prepared by dissolving 0.50 g of EDTA disodium salt dihydrate (Fluka) in the buffer solution (pH 6.00) and making up a volume of 1 L. An L-Ascorbic acid solution (1.13×10^{-3} M) was prepared by dissolving 0.05 g of L-ascorbic acid (Riedel-de Haën) in the stabilizer solution (method A) or the buffer solution of pH 4.50 (method B) and diluting to 250 mL in a volumetric flask. A potassium peroxymonosulfate solution (0.021 M) was prepared by dissolving 0.66 g of $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$ (Aldrich) in 100 mL of the stabilizer solution. A hydrogen peroxide solution (0.456 M) was prepared by diluting 4.80 mL of 30 % (w/w) H_2O_2 (Normapur, Prolabo) to 100 mL in a volumetric flask with the buffer solution (pH 4.50). A copper(II) solution (1.13×10^{-4} M) was prepared by dissolving 0.018 g of $CuSO_4$ (Riedel-de Haën) in 1 L of the buffer solution (pH 4.50). Solutions of metal ions, anions, acids, vitamins and sugars were prepared by dissolving calculated amounts of these substances in the stabilizer solution for method A and the buffer solution of pH 4.50 for method B.

General Procedure

Method A

An aliquot of the sample solution containing 50–280 μg of L-ascorbic acid was diluted to 25 mL in a volumetric flask with the stabilizer solution. The absorbance of the resulting solution was measured at 265 nm using the stabilizer solution as a blank and was designated A_1. A volume of 3.0 mL of the 0.021 M potassium peroxymonosulfate solution was added to another aliquot of the sample solution, and volume was completed to 25 mL in a volumetric flask with the stabilizer solution. After 5 min, the absorbance was measured at 265 nm against the stabilizer solution as a blank and was designated A_2. The value $A = A_1 - A_2$ was proportional to the ascorbic acid concentration in the sample.

Method B

An aliquot of the sample solution containing 50–400 μg of L-ascorbic acid was transferred to a 25 mL volumetric flask, and volume was made up to the mark using the buffer solution of pH 4.50. The absorbance (A_1) of the dilute solution was measured at 262 nm using the buffer solution (pH 4.50) as a blank. Another aliquot of the sample solution was transferred into a 25 mL volumetric flask, hydrogen peroxide solution (2.5 mL) and copper(II) solution (3.0 mL) were added, and then the solution was diluted to the mark with the buffer solution of pH 4.50. After 12 min, the absorbance (A_2) was measured at 262 nm against a blank solution prepared by diluting 2.5 mL of the hydrogen peroxide solution and 3.0 mL of the copper(II) solution to 25 mL with the buffer solution (pH 4.50).

Calibration Curve

Into a series of 25 mL volumetric flasks, different aliquots of the 1.13×10^{-3} M ascorbic acid standard solution were transferred and the contents were diluted to the mark with the stabilizer solution (method A) or the buffer solution of pH 4.50 (method B). The absorbance of each solution was measured at 265 and 262 nm for methods A and B, respectively.

Procedure for Tablets

Several tablets were crushed to the powdered form and an accurately weighed amount of the powder was transferred into a 50 mL volumetric flask. The powder was dissolved in the stabilizer solution for method A and the buffer solution of pH 4.50 for method B, and then diluted to the mark. If the powder did not dissolve completely, the solution was filtered through a Whatman No. 42 filter paper, and an aliquot of the filtrate was diluted to 50 mL in a volumetric flask with the stabilizer solution and the buffer solution of pH 4.50 for methods A and B, respectively. The determination of ascorbic acid was completed as described under general procedure.

RESULTS AND DISCUSSION

Oxidation of L-Ascorbic Acid by Peroxymonosulfate

Potassium peroxymonosulfate (also known as potassium monopersulfate) is widely used as an oxidizing agent. It is a component of a triple salt with the formula $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$. The standard electrode potential of $KHSO_5$ is given by the following half cell reaction:

$$HSO_5^- + 2H^+ + 2e^- \rightarrow HSO_4^- + H_2O \quad E° = 1.85 \text{ V}$$

L-Ascorbic acid (H_2A) undergoes the oxidation reaction with peroxymonosulfate in an acidic medium to yield dehydro-L-ascorbic acid (A), insensitive to ultraviolet at 265 nm. The oxidation of L-ascorbic acid usually takes place in a two-step reaction. 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. The redox reaction is:

$$H_2A \rightarrow A + 2H^+ + 2e^-$$

The stoichiometry of the reaction between peroxymonosulfate and L-ascorbic acid is represented by the following:

$$H_2A + HSO_5^- \rightarrow A + HSO_4^- + H_2O$$

Oxidation of L-Ascorbic Acid by Hydrogen Peroxide

The standard electrode potential of H_2O_2 is given by the following reaction:

$$H_2O_2 + 2H^+ + 2e^- \rightleftarrows 2H_2O \quad E° = 1.77 \text{ V}$$

Hydrogen peroxide reacts with L-ascorbic acid yielding dehydro-L-ascorbic acid as the reaction product:

$$H_2A + H_2O_2 \rightleftarrows A + 2H_2O$$

The experimental results in this investigation showed that the rate of reaction between L-ascorbic acid and hydrogen peroxyde in an acidic medium was slow to be used practically for the determination of vitamin C in real samples. It was observed that this reaction was accelerated by the presence of trace amounts of the copper(II) ion. Stabilizers for L-ascorbic acid, such as EDTA and citric acid, were not used in method B. It was found that the copper(II)-catalyzed reaction rate was retarded by the presence of these stabilizers. The inhibition effect of the stabilizers on the catalyzed reaction between L-ascorbic acid and H_2O_2 is attributable to the complex formation of the stabilizers with the copper(II) ion.

Optimization of Conditions

The absorption properties (λ_{max} and ε) of L-ascorbic acid are dependent on the pH of the aqueous media.[14] Because of this pH dependence, the acetic acid–sodium acetate buffer solutions were used throughout this work for both methods.

Method A

Because L-ascorbic acid was not stable at pH > 5.0, 1.34×10^{-3} M EDTA was used in the acetate buffer solution to stabilize L-ascorbic acid in the aqueous medium. In the presence of EDTA, L-ascorbic acid remained stable for at least 2 h at room temperature.

The influence of altering the pH of the acetate buffer (pH 4.00–6.50) on the oxidation of L-ascorbic acid (11.20 μg mL^{-1}) by peroxymonosulfate was investigated in the presence of 0.0026 M $KHSO_5$ and 1.34×10^{-3} M EDTA. The ascorbic acid concentrations were determined by measuring the absorbance values of the pH 5.00, 6.00, and 6.50 solutions at 265 nm, the pH 4.50 solution at 263 nm, and the pH 4.00 solution at 256 nm. The results showed that the time for complete oxidation of ascorbic acid was 5 min at pH 4.00–6.50. The absorption values at 265 nm in the pH 6.00 and 6.50 solutions were about 70 % higher than those at 256 nm in the pH 4.00 solution. The pH 6.00 solution was selected for subsequent analysis of ascorbic acid because of its higher absorption. An oxidation time of 5 min was selected as optimal for the oxidation of L-ascorbic acid.

The influence of the peroxymonosulfate concentration on the oxidation of L-ascorbic acid was examined by adding different volumes of a 0.021 M solution of potassium peroxymonosulfate to a solution containing 11.20 μg mL^{-1} ascorbic acid and 1.34×10^{-3} M EDTA in the acetate buffer (pH 6.00). The final concentration of $KHSO_5$ was varied from 8.59×10^{-4} M to 0.0051 M. Ascorbic acid was oxidized completely after 5 min in the presence of 2.0–6.0 mL of the peroxymonosulfate solution. Although a 2 mL volume of the potassium peroxymonosulfate solution was sufficient to oxidize ascorbic acid, 3.0 mL was selected for subsequent analysis in a total volume of 25 mL (final concentration, 0.0026 M $KHSO_5$).

Method B

Because L-ascorbic acid was unstable at pH > 5.0, the effect of pH on the oxidation of L-ascorbic acid (16.00 μg mL^{-1}) by hydrogen peroxide was investigated over the range 3.00–4.50 in the presence of 0.0456 M H_2O_2. The effect of this variable was investigated by using the acetate buffer. The experimental results showed that the ascorbic acid oxidation rate increased with increasing pH from 3.00 to 4.50 (Figure 1). After 23 min, L-ascorbic acid in solution at pH 4.50 was completely oxidized. Solutions adjusted to pH 4.00 required 33 min and samples at lower pH required longer. The maximum absorbance of L-ascorbic acid was observed at 262 nm at pH 4.50. Therefore, pH 4.50 was selected for further investigation.

In order to investigate the influence of H_2O_2 concentrations on the oxidation of L-ascorbic acid, different aliquots (0.2–4.0 mL) of the 0.456 M H_2O_2

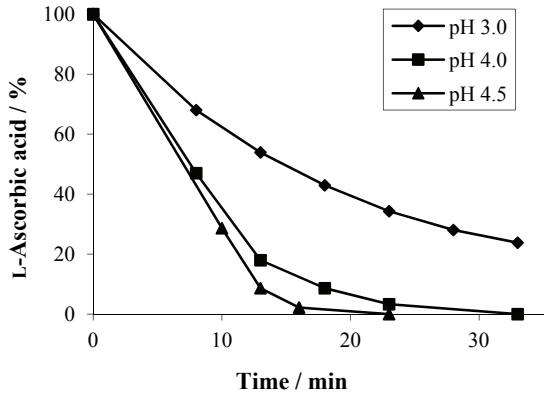

Figure 1. The effect of pH on the oxidation of L-ascorbic acid (16.00 μg mL^{-1}) by hydrogen peroxide (0.0456 M).

solution were added to a solution containing 16.00 μg mL^{-1} ascorbic acid in the acetate buffer (pH 4.50). The final concentration of H_2O_2 was varied from 0.0036 M to 0.073 M. The rate of oxidation of ascorbic acid increased along with an increase in the hydrogen peroxide concentration up to 1.0 mL of the 0.456 M H_2O_2 solution, and remained constant up to 4.0 mL (Figure 2). The time required to oxidize 16.00 μg mL^{-1} ascorbic acid was 23 min in the presence of 1.0–4.0 mL, compared to 33 min in the presence of 0.2 mL of the hydrogen peroxide solution. Therefore, 2.5 mL of the 0.456 M H_2O_2 solution was adopted in a total volume of 25 mL (final concentration, 0.0456 M H_2O_2). The absorbance for H_2O_2 remained constant in the investigated range of the ascorbic acid concentration (2.00–16.00 μg mL^{-1}).

The effect of the concentration of copper(II) on the oxidation of L-ascorbic acid by hydrogen peroxide was studied by adding 1.0–4.0 mL of the 1.13×10^{-4} M copper(II) solution to a solution containing 16.00 μg mL^{-1} ascorbic acid and 0.0456 M H_2O_2 in the

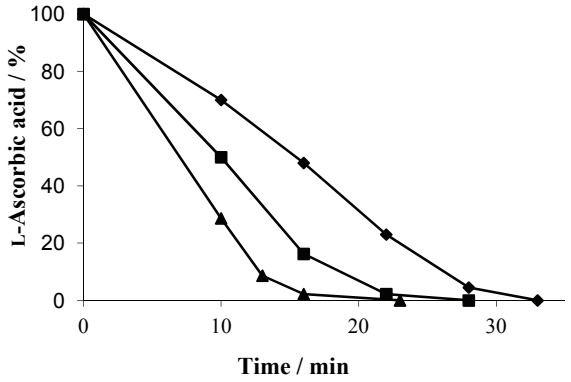

Figure 2. The oxidation of L-ascorbic acid by hydrogen peroxide. Reaction mixtures contained 16.00 μg mL^{-1} ascorbic acid (pH 4.50). The 0.456 M H_2O_2 solution volumes were as follows: (♦) 0.2 mL, (■) 0.5 mL, and (▲) 1.0–4.0 mL.

acetate buffer (pH 4.50). The time for complete oxidation of ascorbic acid was 15 min in the presence of 1.0 mL of the copper(II) solution, and 12 min in the presence of 2.0–4.0 mL. Therefore, subsequent studies were made with 3.0 mL of the 1.13×10^{-4} M Cu(II) solution. An oxidation time of 12 min was selected for the oxidation of ascorbic acid in real samples.

Validation of the Proposed Methods

Linearity, Sensitivity, Limits of Detection and Quantification

The molar absorptivities, correlation coefficients and regression equations were obtained by a linear least-squares treatment of the experimental results (Table 1). Standard calibration curves were constructed by plotting the absorbance against the concentration of L-ascorbic acid in μg mL^{-1}. Beer's law holds over the concentration ranges of 0.65–11.20 and 0.51–16.00 μg mL^{-1} for methods A and B, respectively. The correlation coefficient of the calibration plot was 0.9999 for both methods, confirming good linearity in the working concentration ranges. The limits of detection (LOD) and quantification (LOQ) for the proposed methods were calculated using the following equations:[15]

$$\mathrm{LOD} = \frac{3s}{b}; \quad \mathrm{LOQ} = \frac{10s}{b}$$

where s is the standard error of the intercept and b is the slope of the calibration curve. The LODs and LOQs were obtained from the calibration curves constructed from six data points covering the concentration ranges of 2.00−11.20 and 2.00−16.00 μg mL^{-1} for methods A and B, respectively (each point was obtained from ten measurements). The low values of LOD indicate the

Table 1. Analytical characteristics of the proposed methods

Parameters	Method A	Method B
Linear regression equation[(a)]		
Intercept (a)	0.008	0.002
Slope of the calibration curve (b)	0.0842	0.0603
Standard error of the slope	0.000673	0.000281
Standard error of the intercept	0.005538	0.003093
Correlation coefficient (r)	0.999904	0.999957
Limit of detection / μg mL^{-1}	0.19	0.15
Limit of quantification / μg mL^{-1}	0.65	0.51
Linear dynamic range / μg mL^{-1}	0.65–11.20	0.51–16.00
Molar absorptivity (ε) / L mol^{-1} cm^{-1}	1.48×10^4	1.06×10^4

[(a)] $A = a + bC$, where A is the absorbance and C is the concentration of ascorbic acid in μg mL^{-1}.

Table 2. The intraday and interday precision and accuracy data for L-ascorbic acid using the proposed methods

Method	Taken / μg mL^{-1}	Intraday			Interday		
		Found ± CL[(a)] / μg mL^{-1}	RSD[(c)] / %	E_r[(d)] / %	Found ± CL[(b)] / μg mL^{-1}	RSD[(c)] / %	E_r[(d)] / %
A	4	3.97 ± 0,046	1.25	–0.75	4.04 ± 0.072	1.43	1.00
	8	7.98 ± 0.051	0.70	–0.25	8.03 ± 0.099	0.99	0.38
	11.20	11.22 ± 0.056	0.53	0.18	11.17 ± 0.078	0.56	–0.27
B	4	4.02 ± 0.067	1.80	0.50	4.03 ± 0.107	2.13	0.75
	8	7.98 ± 0.067	0.90	–0.25	8.01 ± 0.076	0.76	0.13
	16	15.98 ± 0.068	0.46	–0.13	16.01 ± 0.103	0.52	0.06

[(a)] The 95 % confidence limits of the mean (n = 7).
[(b)] The 95 % confidence limits of the mean (n = 5).
[(c)] RSD / % - percentage relative standard deviation.
[(d)] E_r / % - percentage relative error.

high sensitivity of the proposed methods (Table 1). The limit of quantification was found to be 0.65 and 0.51 μg mL^{-1} for methods A and B, respectively. The sensitivity of the proposed methods, expressed as the molar absorptivity, was compared with that of other spectrophotometric methods proposed in literature. The present methods are more sensitive than other methods, such as those using iodate-fluorescein[16] ($\varepsilon = 8.81\times10^3$), gold(III) ions[17] ($\varepsilon = 2.30\times10^3$), *peri*-naphthindan-2,3,4-trione[18] ($\varepsilon = 3.18\times10^3$), zinc chloride salt of diazotized 1-aminoanthraquinone[19] ($\varepsilon = 4.07\times10^3$), and 4-chloro-7-nitrobenzofurazane[20] ($\varepsilon = 6.49\times10^3$).

Precision and Accuracy

In order to evaluate the precision and accuracy of the proposed methods, solutions containing three concentrations of pure L-ascorbic acid were analyzed in seven replicates within the day and in five replicates on different days (one measurement per day). The intraday and interday precision and accuracy results are shown in Table 2. The relative standard deviation (RSD / %) values were ≤ 1.80 % (intraday precision) and ≤ 2.13 % (interday precision) indicating high precision of the proposed methods. The accuracy of the proposed methods was determined by calculating relative error (E_r/%), which was varied between –0.75 % and 1.00 %. The percentage relative error was calculated using the following equation:

$$E_r / \% = \frac{(\text{found} - \text{added}) \times 100}{\text{added}}.$$

The analytical results for precision and accuracy in Table 2 show that the proposed methods have good repeatability and reproducibility. The accuracy of the proposed methods was also evaluated by replicate analysis of the pharmaceutical preparation samples after spiking with 2.40 μg mL^{-1} of pure L-ascorbic acid. The recoveries of the added amount were about 97–102.5 % for both methods, which indicates that the proposed methods give accurate results in the presence of common excipients.

Interference Studies

To check the selectivity of the proposed methods, interferences caused by those foreign species that are

Table 3. Effect of foreign substances on the determination of L-ascorbic acid

Foreign substance added	Mass ratio (foreign substance: ascorbic acid)	Error / %	
		Method A	Method B
Iron(II)	0.02	–	–
Copper(II)	0.018	–	–
Calcium(II)	5	–	–
Magnesium(II)	2	–	–
Manganese(II)	0.65	–	–
Molybdenum(VI)	0.2	–	–
Cl^-	10	–	–
NO_3^-	0.04	–	–
SO_4^{2-}	10	–	–
HCO_3^-	10	–	–
NO_2^-	1	–	−3.74
Citrate	20	1.34	–
Benzoate	2	–	–
Citric acid	40	−1.95	–
B_1 (thiamine hydrochloride)	0.5	–	–
B_6 (pyridoxine hydrochloride)	0.5	–	–
Nicotinic acid	1	–	–
Sucrose	200	–	–
Glucose	200	–	–
Fructose	200	–	–

Table 4. Determination of L-ascorbic acid in pharmaceutical preparations using method A

Commercial name (Supplier)	Ascorbic acid (mg/tablet)			$F_{calc.}$	$t_{calc.}$
	Claimed value	Proposed method[(a)]	Iodine method[(a)]		
Aspirin plus C (Bayer)	240	238.82 ± 2.07	240.06 ± 2.53	1.50	1.05
Multivitamin (SchneeKoppe)	80	84.50 ± 0.93	85.09 ± 1.05	1.27	1.16
Andol C (Pliva)	250	252.17 ± 2.19	251.31 ± 2.66	1.47	0.69
Vitamin C (SchneeKoppe)	180	185.19 ± 1.31	183.39 ± 1.89	2.08	2.16
Vitamin C (Sunlife)	180	181.01 ± 1.74	179.04 ± 1.76	1.02	2.21
Multivitamin (dm-drogerie markt)	80	75.57 ± 1.06	75.15 ± 1.22	1.33	0.70
Vitamin C (Biofar)	1000	1005.34 ± 13.43	997.53 ± 10.00	1.80	1.29
Vitamin C Naturelle (Biofar)	120	122.96 ± 1.74	120.92 ± 1.81	1.08	2.24
Vitamin C Depot-Kapseln (dm-drogerie markt)[(b)]	300	296.41 ± 2.27	293.12 ± 3.99	3.07	1.99
Vitamin C (Krüger)	180	184.27 ± 2.46	182.77 ± 1.35	3.33	1.47

Theoretical value for F is 9.60 ($P = 0.05$) and for t is 2.31 ($P = 0.05$).
[(a)] The 95 % confidence limits of the mean ($n = 5$).
[(b)] mg / capsule.

commonly found with L-ascorbic acid in pharmaceutical preparations were studied by adding different amounts of other species to the 8.00 μg mL^{-1} ascorbic acid solution. The criterion for the interference was an absorbance varying by 5 % from the expected value. The results obtained are listed in Table 3.

It was observed that the substances tested did not interfere in the determination of ascorbic acid at the levels studied. Since absorption properties of L-ascorbic acid depend on the pH of the aqueous media,[14] the positive error caused by citrate may be ascribed to an increase in the pH of the L-ascorbic acid solution. The negative error appeared in the presence of nitrite because of its oxidation of L-ascorbic acid in the acidic medium. The negative error caused by large amounts of citric acid may be ascribed to a decrease in the pH of the L-ascorbic acid solution.

Application of the Proposed Methods to Real Samples

The proposed methods were successfully applied for the determination of L-ascorbic acid in vitamin C and multivitamin products. The results are shown in Tables 4 and 5. In every case, the sample was analyzed by the proposed methods and iodine titration as a reference method.[21] The results of applying the proposed methods showed good agreement with those provided by the

Table 5. Determination of L-ascorbic acid in pharmaceutical preparations by method B

Commercial name (Supplier)	Ascorbic acid (mg/tablet)			$F_{calc.}$	$t_{calc.}$
	Claimed value	Proposed method[(a)]	Iodine method[(a)]		
Aspirin plus C (Bayer)	240	237.75 ± 3.08	240.06 ± 2.53	1.48	1.61
Multivitamin (SchneeKoppe)	80	84.69 ± 1.64	85.09 ± 1.05	2.44	0.57
Andol C (Pliva)	250	250.53 ± 3.48	251.31 ± 2.66	1.70	0.45
Vitamin C (SchneeKoppe)	180	185.98 ± 2.54	183.39 ± 1.89	1.79	2.26
Vitamin C (Sunlife)	180	177.81 ± 1.42	177.35 ± 3.15	4.93	0.37
Multivitamin (dm-drogerie markt)	80	76.40 ± 1.02	75.15 ± 1.22	1.43	2.16
Vitamin C (Biofar)	1000	1004.14 ± 4.85	997.53 ± 10.00	4.26	1.65
Vitamin C Naturelle (Biofar)	120	119.89 ± 0.98	120.92 ± 1.81	3.42	1.63
Vitamin C Depot-Kapseln (dm-drogerie markt)[(b)]	300	297.02 ± 2.68	293.12 ± 3.99	2.21	2.25
Vitamin C (Krüger)	180	181.36 ± 1.63	182.77 ± 1.35	1.48	1.85

Theoretical value for F is 9.60 ($P = 0.05$) and for t is 2.31 ($P = 0.05$).
[(a)] The 95 % confidence limits of the mean ($n = 5$).
[(b)] mg / capsule.

reference method. Other ingredients associated with commercial pharmaceutical preparations, such as saccharine, starch, inulin, sodium cyclamate, sorbitol, sodium citrate, citric acid, sodium carbonate, acetylsalicylic acid, sugars and B vitamins, did not interfere with the determination of vitamin C using the proposed methods.

The accuracy and precision of the proposed methods were determined using *t*-test and variance ratio *F*-test,[22] respectively. The calculated *t*-values were lower than the theoretical *t* value ($t = 2.31$, $P = 0.05$), which suggests differences between the results obtained by the proposed methods and reference method were not statistically significant at the 95 % confidence level. The calculated *F*-values did not exceed the critical value ($F = 9.60$, $P = 0.05$) as evident from Tables 4 and 5. Hence, it was concluded that there is no difference between the precision of the proposed and reference methods.

CONCLUSION

The data given in the present work reveal that the proposed methods for the determination of L-ascorbic acid are simple, accurate, precise, selective, and sensitive. L-Ascorbic acid can be analyzed in the presence of ingredients commonly found in vitamin C and multivitamin preparations. The reagents used are cheap, readily available, and the proposed methods do not require any pre-treatment of real samples. Statistical comparison of the results obtained by the proposed methods with those obtained by the reference method indicated no significant difference in precision and accuracy. Thus, the proposed methods can be applied as alternative methods to the reported ones for the determination of L-ascorbic acid in commercial pharmaceutical preparations.

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