Direct Spectrophotometric Determination of L-ascorbic Acid in the Presence of Potassium Cyanide

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Summary

A simple and highly sensitive direct spectrophotometric method was developed for the determination of L-ascorbic acid. Potassium cyanide ($9.21x10^{-5}$ mol dm^{-3}) was used to stabilize ascorbic acid in aqueous medium. The molar absorptivity of the proposed method, which does not require an extraction procedure, was $1.38x10^{4}$ dm^{3} mol^{-1} cm^{-1} at 264 nm. Beer's law was obeyed in the concentration range of 0.26 – 12.0 µg ascorbic acid cm^{-3}. The relative standard deviation was 1.40 % 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 and organic acids interfere. The proposed procedure was successfully applied to the determination of ascorbic acid in pure form and vitamin C preparations.

Key words

spectrophotometry, L-ascorbic acid, potassium cyanide, stabilizer

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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 (Eitenmiller et al., 1999; Fritz et al., 1987), fluorimetry (Wu et al., 2003), spectrophotometry (Fujita et al., 2001; Lau et al., 1987; Jung et al., 1995) and high-performance liquid cromatography (HPLC) (Kacem et al., 1986; Louisi et al., 1998), 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 potassium cyanide 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.

Material and methods

Reagents

All reagents used were of analytical-reagent grade.

Potassium cyanide solution (9.21×10^{-5} mol dm^{-3}). Prepared by dissolving 0.0060 g of potassium cyanide (Riedel-de Haën) in 1000 cm^3 of distilled water.

L-Ascorbic acid solution (1.135×10^{-3} mol dm^{-3}). A 0.05 g amount of L-ascorbic acid (Riedel-de Haën) was dissolved in 250 cm^3 of the potassium cyanide 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, sugars, vitamins and amino acids were prepared by dissolving calculated amounts of these substances in the (9.21×10^{-5} mol dm^{-3}) potassium cyanide solution.

Apparatus

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

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 (9.21×10^{-5} mol dm^{-3}) potassium cyanide solution and measure the absorbance at 264 nm against the potassium cyanide solution as a blank.

Determination of L-ascorbic acid in 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 (9.21×10^{-5} mol dm^{-3}) potassium cyanide 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 Procedure.

Determination of L-ascorbic acid in the presence of common components in pharmaceuticals

Transfer an aliquot of the standard solution (1.135×10^{-3} mol dm^{-3}) containing 200 μg of ascorbic acid to a 25 cm^3 standard flask. Add a suitable aliquot of the co-existing matrix constituent solution. Mix and dilute to the mark with the potassium cyanide solution. Measure the absorbance of the resulting solution at 264 nm against the potassium cyanide solution as a blank. Repeat the procedure using ascorbic acid alone (200 μg) as standard for comparison.

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 vitamin C 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. Pipette 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 burette tip. Titrate rapidly to reduce air oxidation of the ascorbic acid, but precede drop wise near the end point, a deep blue starch-triiodide colour.

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

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+} and Fe^{3+}, 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, potassium cyanide in concentration of 9.21×10^{-5} mol dm^{-3} was used to stabilize L-ascorbic acid in aqueous medium. Potassium cyanide forms with metal ions stable complexes that are no longer effective autoxidation catalysts. This stabilizer prevents the ascorbic acid-metal ion complex formation and therefore inhibits effectively the oxidation of L-ascorbic acid. In the presence of the stabilizer, solutions of L-ascorbic acid remain stable for at least one hour at room temperature. This indicates that potassium cyanide can stabilize ascorbic acid long enough for UV analysis.

By using the proposed method, linear calibration curve was obtained in the range 0.26 – 12.0 μg ascorbic acidcm^{-3}. The molar absorptivity, detection limit, as well as other analytical characteristics are summarized in Table 1. The precision of the proposed method, expressed as relative standard deviation, for the determination of 8.0 μg cm^3 ascorbic acid, was 1.40% (n = 7). The molar absorptivity (ε) 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 (Abdelmageed et al., 1995) ($\varepsilon = 6.49 \times 10^3$ dm^3 mol^{-1} cm^{-1}), zinc chloride salt of diazotized 1-aminoanthraquinone (Backheet et al., 1991) ($\varepsilon = 4.07 \times 10^3$ dm^3 mol^{-1} cm^{-1}), gold(III) ion (Pal et al., 1988) ($\varepsilon = 2.30 \times 10^3$ dm^3 mol^{-1} cm^{-1}) and *peri*-naphthindan-2,3,4-trione (Hassan et al., 1975) ($\varepsilon = 3.18 \times 10^3$ dm^3 mol^{-1} cm^{-1}).

Table 1. Analytical characteristics of the proposed method.

Slope of the calibration line	13801.5
Intercept of the calibration line	0.00253
Standard error of the slope of the calibration line	44.82
Standard error of the intercept point of the line	0.00202
Correlation coefficient (r)	0.99997
Limit of detection	0.077 μg cm^{-3}
Limit of quantification	0.26 μg cm^{-3}
Linear dynamic range	0.26 – 12.0 μg cm^{-3}
Molar absorptivity (ε)	1.38×10^4 dm^3 mol^{-1} cm^{-1}
Standard deviation	6.202×10^{-7} mol dm^{-3}
Relative standard deviation	1.40%

Table 2. Effect of metal cations on the determination of L-ascorbic acid.

Metal cation	Mass ratio (cation : ascorbic acid)	Error (%)
Fe(II)	0.05	0.00
Cu(II)	0.02	0.96
Ca(II)	10	0.00
Mg(II)	2	0.00
Mn(II)	5	1.36
Co(II)	2	1.31

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

Anion	Mass ratio (anion : ascorbic acid)	Error (%)
Cl^-	5	0.00
NO_2^-	1.5	0.00
NO_3^-	1.5	0.00
SO_4^{2-}	10	0.00
CO_3^{2-}	10	0.00
PO_4^{3-}	10	0.00
Oxalate	20	1.74
Acetate	20	1.70

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 8.0 μg cm^{-3} of ascorbic acid. The criterion for interference was an absorbance varying by 5% from the expected value.

None of metal ions investigated interfered with the determination at the levels studied (Table 2). The positive errors (all within 5%) caused by copper(II), manganese(II)

Table 4. Determination of L-ascorbic acid in vitamin C preparations.

Commercial name (Supplier)	L-Ascorbic acid (mg/tablet)			$F_{exp.}$	$t_{exp.}$
	Declared concentration	Proposed method[a]	Iodine method[a]		
Vitamin C (PEZ)	60	59.39 ± 0.74	60.41 ± 0.73	1.03	2.19
Vitamin C (Cedevita)[b]	3600	3647.02 ± 43.59	3588.72 ± 37.68	1.34	2.26
Plivit C (Pliva)	500	503.54 ± 4.93	498.33 ± 4.13	1.42	1.81
Cevitbos (Bosnalijek)	500	484.82 ± 6.16	490.89 ± 3.88	2.52	1.86

[a] Average of five determinations ± standard deviation; [b] mg/100 g; theoretical values for $t = 2.31$ and $F = 6.39$ ($P = 0.05$).

and cobalt(II) may be ascribed to the absorption of UV light by these substances. Lau et al. (1987) 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 (Lau et al., 1987). The metal ions investigated did not interfere with other methods described in the literature for the determination of ascorbic acid (Arya et al., 1997; Ferreira et al., 1997). The results in Table 3 indicate that anions did not cause interferences at the levels studied. Nitrite and oxalate ions caused minor errors due to their UV absorption. Since absorption properties of ascorbic acid depend on the pH of the aqueous media (Eitenmiller et al., 1999), the positive error caused by acetate ions may be ascribed to an increase in pH of the 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 compared with that of ascorbic acid (Fujita et al., 2001; Barrales et al., 1998; Arya et al., 1997).

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. The experimental results revealed that a 200-fold excess of sucrose, glucose, fructose, 100-fold maltose, lactose, 10-fold proline, alanine, asparagine, arginine, leucine 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 (Salinas et al., 1988; Lau et al., 1987; Ferreira et al., 1997; Arya et al., 1997). Two-hundred-fold amounts of citric and malic acids interfered with the proposed method. Errors caused by citric and malic acids may be ascribed to a decrease in pH of the ascorbic acid solution after the addition of organic acids.

The results obtained for the determination of ascorbic acid in the presence of other substances indicate that many of the ingredients commonly found in vitamin C preparations did not interfere with the proposed method. Other vitamins and large amounts of organic acids do interfere and must be absent.

The proposed method was applied to the determination of L-ascorbic acid in commercial pharmaceutical preparations (granule and tablet). The results obtained are shown in Table 4. In every case, the sample was analysed by both the proposed and the titrimetric method using iodine as titrant. The last one was used as a reference method. Ingredients usually associated with granules and tablets, such as sodium bicarbonate, sorbitol, citric acid, sodium citrate, thiourea, starch, cellulose and stearic acid, did not interfere with the determination of ascorbic acid using the proposed method.

The *t*-test was applied to the results obtained by the proposed method 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 proposed method and the reference method 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.

Conclusions

Potassium cyanide at a concentration of 9.21×10^{-5} 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, highly sensitive, precise and accurate. Many common ingredients present in pharmaceuticals do not interfere. The results obtained for the determination of ascorbic acid in pharmaceuticals preparations using the proposed method were compared with those obtained by the iodine method. 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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