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Application of Potassium Chromate-Diphenylcarbazide in the Quantitative Determination of Ascorbic Acid by Spectrophotometry

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A spectrophotometric procedure for the determination of ascorbic acid in pure form and in a number of pharmaceutical preparations and real samples has been developed that offers the advantages of simplicity, accuracy, precision and sensitivity over many other methods. The method is based on the oxidation of ascorbic acid by a known excess amount of potassium chromate followed by the estimation of the unreacted amount of chromate by reactions with sym-diphenylcarbazide. The reacted oxidant corresponds to the ascorbic content. At the maximum absorption of 548 nm, Beer's law is obeyed up to 5 μg/mL of ascorbic acid. Statistical treatment of the experimental results indicates that the procedure is precise and accurate. Excipients used as additives in pharmaceutical formulations did not interfere in the proposed procedure. The reliability of the method was established by parallel determination against the 2,6-dichlorophenolindophenol methods. The procedure described was successfully applied to the determination of bulk drugs, in pharmaceutical formulations and real samples.

Key Words: Ascorbic acid determination, Spectrophotometry, Potassium chromate, Chromium-diphenylcarbazide complex.

Introduction

Ascorbic acid (AsA) is an important vitamin that participates in a wide variety of biological events concerning electron transport reactions, hydroxylation, the oxidative catabolism of aromatic amino acids and so on. Measuring the concentration of a marker chemical commonly assesses food deterioration and product quality; ascorbic acid is one such indicator. It is important to detect it selectively and conveniently in routine analyses. Various methods have been employed for its measurement, such as spectrometry[1−2], thermometric titrimetry[3], HPLC[4], a kinetic method[5], various modified electrodes[6−8], sol-gel[9] and a combination of various other techniques.

Diphenycarbazide (DPC) has been utilized for the selective, sensitive and direct spectrophotometric determination of chromate[10−12]. To the best of our knowledge, however, there is no work in the literature

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about the application of a chromate–diphenycarbazide system for the determination of pharmaceutical products.

The present work aims to demonstrate a simple, sensitive and cheap method suitable for the determination of ascorbic acid using potassium chromate as an oxidant for ascorbic acid and a diphenycarbazide system for measuring unreacted chromate.

Experimental

Reagents

Analytical-reagent grade chemicals and re-distilled water were used throughout this project. All these reagents were purchased from Merck (Darmstadt, Germany). A chromate ion stock solution containing 0.1 g/L of chromium(VI) was prepared by dissolving 0.3735 g of pure potassium chromate in 1 L of water. Ascorbic acid stock solution (1.0 mg/mL) was prepared freshly before measurement by dissolving 0.1000 g of ascorbic acid in 100 mL of water.

The reagent, 4.0×10^{-3}mol/L sym-diphenycarbazide, was prepared daily by dissolving 0.2473 g of Sym-diphenycarbazide (May & Baker Ltd, Dagenham, England) in 250 mL of ethanol.

Apparatus

A Shimadzu UV-VIS spectrophotometer model UV-160, with a 1.0 cm optical path quartz cell was used for spectrophotometric measurements.

Procedure

Five milliliters of standard ascorbic acid or sample solution were pipetted into 25 mL balloons, and 5 mL of 5.2 μg/mL standard chromate ion in 0.8 mol/L HNO_3 solution was added. After mixing, 4 mL of reagent the solution was added and solution was diluted to the mark with distilled water.

Vitamin C tablets

Ten tablets of vitamin C were accurately weighed, ground, powdered and dissolved in doubly distilled water. The content of the flask was shaken for 5 min and then filtered. Titration with 2,6-dichlorophenolindophenol (DCPIP) was employed to validate the response of the method[13]. The samples were diluted to within the working range of the method and assayed.

Real samples, fruit juices and foods

Fresh fruit juice, fruit juice made from concentrate, and flu remedies vitamin C were selected. Foods such as orange, grapefruit, lemon and tomato were squeezed and then filtered. The juice obtained was diluted quantitatively with water for determination.

Results and Discussion

Attempts to oxidize ascorbic acid with a known excess of chromate and to subsequently determine unreacted chromate with diphenycabazide in nitric acid medium as a step in the indirect determination of ascorbic

acid were successful. Hence, we used a chromate-diphenycarbazide system for the determination of ascorbic acid.

The proposed method is based on the oxidation of ascorbic acid by a known excess of chromate in nitric acid medium, and complexing the unreacted chromate with sym-diphenylcarbazide complex, before measuring the absorbance of the latter at 548 nm.

Ascorbic acid, when added in increasing amounts, consumes chromate and decreases chromate-diphenylcardazide complex absorbance. Consequently, there is a concomitant fall in the chromate concentration. The absorbance is found to decrease linearly with increasing concentrations of ascorbic acid, which forms the basis for their determination. The reaction scheme is as follows

$$2CrO_4^{2-} + 3C_6H_8O_6 + 10\ H^+ \longrightarrow 2Cr^{3+} + 3C_6H_6O_6 + 8H_2O$$

$$\text{Unreacted } {CrO_4}^{2-} + \text{ sym-DPC } \xrightarrow{H^+} \text{Cr-DPC complex } (\lambda_{max} = 548 \text{ nm})$$

The chromium(III) produced in the reaction is so small that it does not interfere in the measurement at 548 nm.

Selection of acid concentration

According to the literature, at acid concentrations less than 0.05 mol/L the full color does not develop immediately and at acid concentrations above 0.8 mol/L the complex, chromate-DPC, is less stable[10,12]. Andrade et al. showed that values above 0.5 mol/L produce no changes in the analytical signal, and, consequently, 0.8 mol/L nitric acid was chosen for this purpose[12].

Evaluation of the method

Using the experimental conditions described above, the calibration graph is linear up to 5 μg/mL and is described by the equation A = 0.363-0.057C_{AsA}, r = 0.9989, n = 8, where A is the absorbance, C_{AsA} is the analyte concentration (μg/mL), r is the correlation coefficient and n represents the number of determinations.

The detection limit (DL) was calculated using the equation DL = $3S_{bk}$/m, where S_{bk} is the standard deviation of the blank and m is the slope of the calibration graph. The calculated DL was 0.02 μg/mL.

The precision and accuracy of 10 replicate analyses of a series containing various amounts of ascorbic acid by the variable-time method is shown in Table 1.

Table 1. Precision and accuracy of the method.

[AsA] present (μg/mL)	[AsA] found (μg/mL)	RSD % (n = 10)	Relative error %
7.6	7.8	1.65	2.6
18.9	18.7	1.44	-1.1
74.4	75.8	1.35	1.9
122.3	123.1	1.30	0.65

Recovery tests

Recovery tests using the proposed method were performed using 3 different samples, and the tests for each sample were carried out in triplicate. As shown in Table 2, the recoveries of ascorbic acid added to tomato

juice, lime, orange juice, grapefruit juice and lemon juice were all close to 100%. The results of the recovery tests were very good.

Table 2. Results of recovery test.

Sample	[AsA] added*	[AsA] found* ± S (n = 10)	Recovery
Tomato juice	0.0	15.4 (±1.02)	——
	300.0	316.2 (±2.1)	100.3
Lime	0.0	0.0	——
	300.0	299.3 (±2.9)	99.8
Orange juice	0.0	323.1 (±2.1)	——
	300.0	624.2 (±1.8)	100.2
Grapefruit juice	0.0	16.2 (±0.4)	——
	300.0	317.0 (±1.2)	100.3
Lemon juice	0.0	30.1 (±2.0)	——
	300.0	329.1 (±3.0)	99.7

*mg/100 g

Interference study

Andrade et al. showed the influence of foreign ions on Cr(VI) response by DPC. In all real samples the methods revealed excellent recoveries and no matrix interference[12]. A study of interference for ascorbic acid determination was performed with samples containing 4 μg/mL of ascorbic acid. The tolerance limit was defined as the concentration at which the species caused an error less than ±5%. The results are listed in Table 3 and show no interference caused by the presence of large amounts of compounds such as glucose, sucrose, fructose, lactose, mannitol, saccharose, citric acid, succinic acid, oxalic acid, malic acid, sorbic acid, tartaric acid, fumaric acid, calcium chloride, sodium chloride, maleic acid, benzoic acid, salicylic acid, salicylicamide, acetaminophen, lactic acid and hydroquinone, which are usually present in natural juices, foods and pharmaceutical preparations.

Table 3. Tolerance towards foreign compounds.

Additive type	Tolerance[a] (μg/mL)
Glucose, Sucrose, Fructose, Lactose, Mannitol, Saccharose, Citric acid, Succinic acid, Oxalic acid, Malic acid, Sorbic acid, Tartaric acid, Fumaric acid, Calcium chloride, Sodium chloride	450[b]
Maleic acid, Benzoic acid Salicylic acid, Salicylicamide, Acetaminophen, Lactic acid	200[b]
Hydroquinone	55

[a]Weight ratio of foreign compound to ascorbic acid of 4 μg/mL giving a maximum error of 5%.

[b] Maximum amount tested.

Application to real samples

The proposed method was applied to the determination of ascorbic acid in vitamin C tablets, vitamin C injections, cordials and fresh fruit juices from different locations in Iran. Table 4 lists the results obtained with the proposed method. These results are compared with those obtained with DCPIP method[13], and indicate that the proposed method could be readily implemented using a very simple and stable reagent system.

Table 4. Determination of ascorbic acid in vitamin C tablets, vitamin C injection and fresh fruit juices.

Sample	Proposed method ±S (n = 10)	Standard method (DCPIP) ±S (n = 8)
Vitamin C tablet (1000mg)	994 (±7.3[a])	997.7 (±9.0[a])
Vitamin C tablet (500mg)	494.2 (±4.8[a])	498.1 (±4.7[a])
Vitamin C injection (192 mg/mL)	190.6 (±3.2[b])	191.5 (±1.5[b])
Orange cordial	75.3 (±4.7[c])	74 (±3.5)
	64.5 (±5.1[d]	65.4 (±2.5)
Grapefruit cordial	52.0 (±4.3[c])	53.1 (±3.6)
	46.2 (±3.7[d])	46.7 (±4.0)
Lemon cordial	47.2 (±3.2[c])	46.7 (±3.1)
	54.4 (±2.6[d])	55.1 (±2.7)
Fresh orange juices	552.4 (±3.2[c])	554.1 (±2.4)
	490.3 (±3.3[d])	491.7 (±3.1)
Fresh grapefruit juices	323.1 (±2.2[c])	322.3 (±3.6)
	293.3 (±3.0[d])	294.2 (±4.1)
Fresh tomato juices	77.4 (±4.3[c])	78.0 (±3.4)
	57.8 (±3.7[d])	58.1 (±2.7)

[a] mg/tablet

[b] mg/mL

[c] From the city of Tonekabon in the Iranian province of Mazaderan

[d] From the city of Iranshahr city in the Iranian province of Sistan & Baluchestan

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References

1. N. K. Pandey, **Anal. Chem.**, **54**, 793-796 (1982).
2. O.H. Abdelmageed, P.Y. Khashaba, H.F. Askal, G.A. Saleh and I.H. Refaat, **Talanta, 42**, 573-579 (1995).
3. A.R. Mayers and C.G. Taylor, **Analyst**, **112**, 507-509 (1987).
4. S.P. Sood, L.E. Sartori, D.P. Wittmer and W.G. Haney, **Anal. Chem., 48**, 96-798 (1976).
5. M.I. Karayannis and D.I. Farasoglou, **Analyst**, **112**, 767-770 (1987).
6. G.-D. Liu, Z.-Q. Li, S.-S. Huan, G.-L. Shen and R.-Q. Yu, **Anal. Lett., 33**, 175-192 (2000).

7. X. Han, J. Tang, J. Wang and E. Wang, **Electrochim. Acta**, **46**, 3367-3371 (2001).
8. J. Ren, H. Zhang, Q. Ren, C. Xia and J. Wan, **J. Electroanal. Chem.**, **504**, 59-63 (2001).
9. P. Wang, X. Wang, X. Jing and G. Zhu, **Anal. Chim. Acta**, **424**, 51-56 (2000).
10. J.C. de Andrade, J.C. Rocha, C. Pasquini and N. Baccan, **The Analyst**, **108**, 621-625 (1983).
11. J.E.T. Andersen, **Anal. Chim. Acta**, **361**, 125-131 (1998).
12. F.J. Andrade, M.B. Tudino and O.E. Troccoli, **The Analyst**, **121**, 613-616 (1996).
13. W. Horwitz, "**Official methods of analysis of the Association of Official Analytical Chemists"**, 13 th ed, Association of Official Analytical Chemists, Washington, DC, 476 (1980).