Colorimetric Determination of Ascorbic Acid in Pharmaceuticals and Fruits Using a Chromium(VI)–Diphenylcarbazide Complex

Satya Prakash Arya* **and Meenakshi Mahajan**

Department of Chemistry, Kurukshetra University, Kurukshetra-132119, Haryana State, India

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A simple and rapid method is presented for the determination of ascorbic acid. This method is based on the reducing action of ascorbic acid, which proportionately decreases the color intensity of the Cr(VI)–diphenylcarbazide complex. The absorbance of the complex was measured at 540 nm. A linear relationship between the absorbance and concentration of ascorbic acid was found to hold well up to 3.2 μg ml^{-1}. Most of the common ingredients present in pharmaceutical preparations were found to be tolerated. The method has been successfully applied to the determination of vitamin C in the pure state, dosage forms and citrus fruits.

A daily intake of vitamin C or ascorbic acid is widely acknowledged to be necessary throughout the globe, which has resulted in a variety of food products, tablets, syrups, and canned juices containing the essential vitamins, including vitamin C. Also, in nature it is found in fruits and vegetables, though to a varying degree. Citrus fruits are considered to be the best source of vitamin C. In the absence of such fresh fruits, dependency on fortified foods is increased for the normal intake of ascorbic acid. Keeping in view its importance and its susceptibility to degradation, there has been continuous interest in the development of a determination method for vitamin C.

Although a large number of spectrophotometric methods are available in the literature, they all have their own limitations. Many such methods are time consuming,[1—4] and thus inapplicable for want of quick results. Some of these methods[5—8] lack selectivity, and sometimes require a pretreatment,[9,10] while others are less sensitive.[11,12] To circumvent these problems, a new method is proposed which is fast, facile, sensitive, and selective. This method is based on a corresponding decrease in the color intensity of the chromium(VI)–diphenylcarbazide complex with the addition of ascorbic acid. A detailed account of such studies is presented here.

Experimental

Reagents: All of the chemicals used were of analytical grade, and redistilled water was used for preparing solutions.

Ascorbic Acid (s. d. fine chem): A freshly prepared aqueous solution (100 μg ml^{-1}) was used.

Chromium(VI) Solution: A stock solution of chromium(VI) (1mg ml^{-1}) was prepared by dissolving a calculated amount of potassium dichromate. Suitable dilution of the stock solution gave a lower concentration (20 μg ml^{-1}).

Diphenylcarbazide (DPC) Solution: A 0.2% (w/v) fresh solution was prepared in 50% (v/v) acetone.

Instrument: Shimadzu UV-140-02 (Japan) spectrophotometer with matched 1-cm quartz cells was used.

Procedure: To a 25 ml volumetric flask containing 20 μg of chromium(VI), an aliquot of a standard or sample solution of ascorbic acid was pipetted, followed by the addition of 1.0 ml of DPC solution and 1.5 ml of 1.25 M sulfuric acid (1 M=1 mol dm^{-3}). The contents of the flask were mixed well and diluted to volume with redistilled water. The absorbance of the resulting complex was measured at 540 nm against a reagent blank which had been prepared similarly. The contents of ascorbic acid were determined from the standard calibration curve.

Tablets: An accurately weighed amount of powder, obtained from 10 tablets, equivalent to 20 mg of ascorbic acid, was transferred to a 50 ml volumetric flask; after adding water, the contents of the flask were shaken in order to dissolve the powder. This solution was filtered and further diluted to obtain 100 μg ml^{-1}. Then, an aliquot of this sample solution was analyzed by the recommended procedure.

Ampoule: After a known volume equivalent to 10 mg ascorbic acid was transferred into a 50 ml volumetric flask, the volume was made up to the mark. This solution was further diluted to obtain 100 μg ml^{-1}. A portion of the diluted solution was analyzed in accordance with the general procedure.

Fruit Extracts: Each fruit was squeezed to obtain its juice; the juice was centrifuged to obtain a clear extract. A portion of the clear extract was then immediately analyzed, as described under the procedure.

Results and Discussion

Chromium(VI) is known to form a colored complex with diphenylcarbazide in an acidic solution.[13] It was observed that the color intensity of the chromium(VI)–diphenylcarbazide complex decreases proportionately along with an increase in the ascorbic acid concentration; this observation led to the development of the proposed method. The gradual decrease in the color intensity is likely to be due to an increasing concentration of the chromium(III) formed by the addition of ascorbic acid. Since chromium(III) in an aqueous solution is inert against complex formation, thus not reacting

with DPC, it is responsible for a decrease in the absorbance. Maintenance of the order of adding the reagents, as laid down in the procedure, is desirable; otherwise, no such decrease in the absorbance is observed if the order is reversed.

Spectral Characteristics: Figure 1 represents the absorption spectrum of a reddish-violet colored product as well as that of the reagent blank. The complex exhibits an absorption band at 540 nm, while the reagent blank absorbs strongly at 310 nm, but with a minimum or neglected absorption at 540 nm. Therefore, the region of 540 nm was used for absorbance measurements.

Optimization of Conditions: The parameters which affect the color intensity of the complex were optimized, as presented in Table 1. The intensity of the colored product formed by the reaction of chromium(VI) with diphenylcarbazide is influenced by a variation in the reagent concentration and the acidity of the solution. The optimum conditions obtained by these studies include 1.0—3.0 ml of the reagent solution and 1.5—2.0 ml of 1.25 M sulfuric acid. The complex was found to be stable for 2.5 h under the standard conditions.

Beer's Law and Statistical Parameters: A linear correlation was observed between the absorbance and concentration of the ascorbic acid in the range of 0.4—3.2 μg ml^{-1}. Five replicate determinations with 40 μg of ascorbic acid gave 0.02 as the standard deviation and 3.3% as the coefficient of variation.

Interference Studies: Interference studies of the possible ingredients that may be present in pharmaceutical products were carried out; the basis of their tolerance limits (as shown in Table 2) was a ±2% deviation in the absorbance. No interference was observed from sugars at the levels indicated in Table 2. However, among vitamins only vitamin B_1 and vitamin B_2 were found to interfere. Organic acids other than oxalic acid do not interfere. Other substances (as men-

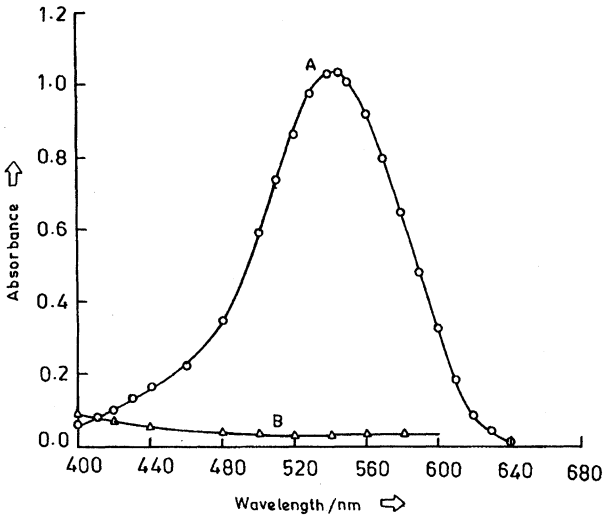

Fig. 1. Absorption spectrum of Cr(VI)–diphenylcarbazide complex. A-Cr(VI) = 1 μg ml^{-1}, B-Reagent blank measured against water.

Table 2. Tolerance Limits of Common Constituents in Vitamin C Formulations

Substance added[a)]	Amount (mg/25 ml)	Error/%
Sugars		
Glucose	100.00	+1.6
Mannose	100.00	—
Sucrose	100.00	+1.6
Fructose	100.00	—
Xylose	100.00	−1.6
Lactose	100.00	+0.8
Maltose	50.00	—
Starch	50.00	—
Vitamins and amino acids		
Glutamic acid	1.0	+0.8
Nicotinic acid	1.0	+0.8
Vit B_6 (Pyrdoxine HCl)	1.0	−1.6
Methionine	0.5	—
Folic acid	0.3	−0.8
Cysteine	0.2	—
Aspartic acid	0.2	+1.6
Cyanocobalamin (Vit B_{12})	0.2	—
Organic acids		
Acetic acid	500.00	+1.6
Benzoic acid	50.00	—
Succinic acid	10.00	+1.6
Tartaric acid	5.00	—
Citric acid	5.00	—
Metal ions		
Calcium(II)	5.0	—
Strontium(II)	5.0	—
Magnesium(II)	5.0	—
Miscellaneous		
Acetone	500.00	—
Glycerol	500.00	—
Methanol	200.00	−1.6
Sodium chloride	100.00	—
Sodium sulfate	100.00	—
Urea	50.00	—
Sodium orthophosphate	5.00	—

a) Substances were added before the addition of ascorbic acid.

Table 1. Effect of Reagent and Sulfuric Acid Concentration on Absorbance of the Complex

Reagent Concentration/ml	0	0.5		1.0—3.0		
Absorbance	0.1	0.77		0.85		
H_2SO_4 acid (1.25 M)/ml	0	0.5	1.0	1.5—2.0	2.5	5.0
Absorbance	0.02	0.75	0.79	0.84	0.77	0.43

Conditions: Chromium(VI) = 20 μg; Volume of reagent (DPC) solution = 1 ml; Sulfuric acid (1.25 M) = 2 ml.

Table 3. Analysis of Pharmaceutical Products

S. No.	Name of product	Manufacturer	Ascorbic acid/μg			Recovery of added ascorbic acid		Recovery %
			Taken	Found				
				Proposed method	I. P. method	Added μg	Found μg	
1.	Ampoule (Calcium+Vit C)	A	50.0	50.7	50.3	20.0	20.1	100.5
2.	Sukcee	B	50.0	50.5	50.8	20.0	20.5	102.5
3.	Celin	C	60.0	59.3	59.2	20.0	20.4	102.0
4.	Calcinova	D	62.3	62.6	62.5	10.0	10.1	101.0
5.	Ostocalcium	E	40.0	39.5	39.8	20.0	20.6	103.0

Table 4. Analysis of Citrus Fruits for Ascorbic Acid Contents

Product	Volume taken ml	Ascorbic acid[a] found/μg	Recovery of added ascorbic acid/ μg	
			Added	Recovered
Moosmi	0.1	70.0	5.0	5.2
Lemon	0.05	31.5	20.0	19.8
	0.1	64.0	10.0	9.9

a) Average of three determinations.

tioned under the heading 'miscellaneous') are also tolerated. Reducing agents interfere with the determination.

Application of the Method to Real Samples: The proposed method was successfully applied to an analysis of ascorbic acid in various pharmaceutical preparations (Table 3). The obtained results were compared with that of I. P. method,[14] and were found to be in good agreement. Recovery tests were performed by adding known amounts of ascorbic acid to pharmaceutical sample solutions; none of these analyses gave less than a 100% recovery. Table 4 gives the ascorbic acid contents of some fresh fruits analyzed by the proposed method. No interference was observed from the very complex matrix of the fruit juices, as indicated by the good recovery of ascorbic acid added to the studied sample.

Conclusions

Most of the reported methods based on the reducing action of ascorbic acid on metal ions invariably make use of an iron(III)–iron(II) redox system,[5,15–17] and a few use copper(II)–copper(I),[4,18] vanadium(V)–vanadium(IV)[19] or molybdenum blue[3,20] formation reactions. The proposed method introduces a new redox system for the determination of ascorbic acid. This method does not require a rigid control of the temperature, as demanded by some of the standard methods used in pathological laboratories.[2] One of the major advantages of the method lies in its rapidity, since no waiting time is required for an absorbance measurement after the addition of the reagents, in contrast to a waiting time of 0.5—1.0 h needed in many of the existing methods.[1–4,21,22] Further, the common additives of pharmaceutical preparations are found to have no adverse effect on the absorbance of the complex. Hence, a method based on the chromium(VI)–DPC complex is proposed, whose characteristics features include simplicity and rapidity, apart from sensitivity and selectivity.

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