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TURBIDIMETRIC DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICALS AND JUICES

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A convenient, rapid, accurate and precise method for the estimation of ascorbic acid in pharmaceutical products and juices has been developed. The method is based on turbidimetric measurement of the fine orange suspension produced by the reaction of ascorbic acid and selenium dioxide. Other reducing agents like, glucose, fructose, metabisulfite, cysteine and cystine do not interfere in the procedure.

INTRODUCTION

Numerous titrimetric, bioassay, polarographic and spectrophotometric methods are available for the determination of ascorbic acid. Titrimetric methods are based on the reducing properties of vitamin C, therefore, it is titrated with iodine, methylene blue, ferricyanide, 2,6-dichlorophenolindophenol and *N*-bromosuccinimide [1–5]. The widely used method for the determination of vitamin C is its oxidation with the dye 2,6-dichlorophenolindophenol. The use of this reagent becomes limited when other reducing substances such as sugars and reductones etc. are present, which generally are found with it in food products. The presence of ferrous and ferric compounds also interfere in the procedure with this dye [6,7]. Iodine and ferricyanide methods are not specific for the determination of vitamin C. The other widely used method for the determination of vitamin C is its titration with *N*-bromosuccinimide, but this gives high results when metabisulfite is present in preserved juices and squashes [8]. However, this was modified [9] by complexing metabisulfite with acetone and then titrating with *N*-bromosuccinimide. Thus the method becomes cumbersome when huge amounts of reducing substances are present alongwith ascorbic acid. The bioassy of vitamin C is quite specific but the method is very expensive and time-consuming.

2,4-Dinitrophenylhydrazine is used for the spectrophotometric determination of ascorbic acid [10] but the method is very tedious and time-consuming. Uranium nitrate [11] has also been used in the spectrophotometric determination of vitamin C, but this method was employed only on test solutions and pharmaceutical preparations.

The present method is also based on the reducing properties of ascorbic acid but in this case reduction is selective. Ascorbic acid reduces selenium dioxide to metallic selenium giving a very fine orange suspension, which is measured turbidimetrically. At low concentrations the suspension does not remain fine, therefore,it settles down. The method is quite accurate, precise, quick and specific for vitamin C. Other substances like glucose, fructose, metabisulfite, cysteine and cystine do not interfere.

EXPERIMENTAL

Reagents

Selenium Dioxide. A 1% w/v solution of this analytical grade reagent was prepared in deionised and distilled water.

Ascorbic Acid. (Merck) A 0.1% w/v solution was prepared in deionised and distilled water and it was standardized [5].

All other reagents used were of analytical grade or comparable purity.

Apparatus

All photometric (turbidimetric) measurements were made by Hilger colorimeter at 430 nm using 1-cm cells.

Procedure

To 0.5 ml solution containing 100 – 1000 μg/10 ml ascorbic acid, was added 5 ml 0.1*M* potassium dihydrogen citrate followed by the addition of 1 ml selenium dioxide solution. The orange turbidity was produced which was diluted to 10 ml after about 2 min. The pH of the resulting solution was between 3–4, which was just right for the procedure. This turbidity was then measured by the Hilger colorimeter at 430 nm with 1-cm glass-cells. The replicate measurements were made and a calibration curve was constructed (Fig. 1). The turbidity obeys Beer's Law.

Procedure for Tablets. Ten tablets were accurately weighed and powdered, then a part of it was taken and weighed which was equivalent to 100 mg of ascorbic acid.

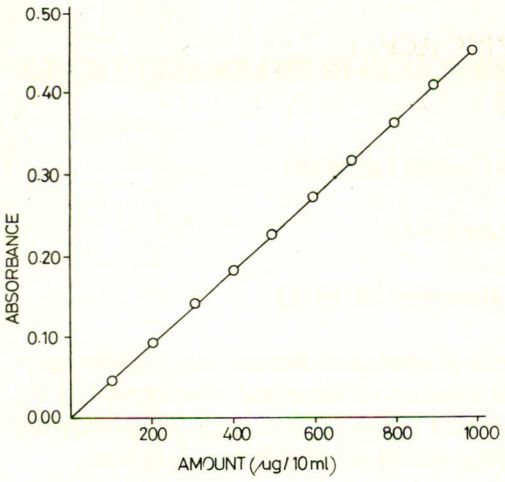


Fig. 1. Calibration curve for ascorbic acid determination.

Then its solution was made by leaching it with 50 ml 0.1*M* metaphosphoric acid and by shaking it vigorously. This was then filtered and transferred quantitatively into a measuring flask and diluted to 100 ml by the addition of water From this solution a suitable aliquot was taken and the procedure was followed as described above.

Procedure for Syrup. This was almost the same as mentioned above, except that in this case a suitable volume of syrup was taken and SeO_2 added and then diluted to the desired volume. The turbidity was measured against syrup blank.

Procedure for Juices. The fruit was peeled off and juice was extracted with electric juicer. The juice was filtered and transferred into a preweighed beaker and weighed again. This gives the weight of the juice. The residue was discarded. A definite weight of the juice was teken and the color developed as described under procedure for pure ascorbic acid, but in this case respective juice was taken as blank. The juices, if desired from other fruits and vegetables can be extracted similarly.

Procedure for Carrots and Tomatoes. The juice was filtered through a filter paper with the help of a vacuum pump. In 10 ml of the juice, was added 10 ml chloroform. The mixture was shaken and allowed to stand till the layers separated. Then 2 ml of the aqueous layer was taken, and 1 ml SeO_2 was added. After about 2 min the volume was made up to 10 ml with distilled water. The turbidimetric

Table 1. Determination of ascorbic acid from pure solution and pharmaceutical preparations and juices.

Sample	Amount presnet (µg)	Amount found* (µg)	Standard deviation (µg)
Ascorbic acid	100	100	0.01
	200	200	0.00
	300	295	0.01

	Amount present[a]	Amount found		Standard deviation	
		with the present method (mg)	by NBS method (mg)	of the present method (mg)	of the NBS method (mg)
Vitamin C tablets†	100.00	99.80	100.00	0.05	0.10
Multivitamin tablets**	100.00	100.00	101.30	0.08	0.30
Multivitamin syrup ‡	75.00	74.50	77.30	0.10	0.80
Orange juice §	–	56.10	57.00	0.20	0.70
Grape fruit juice***	–	26.75	28.10	0.28	0.81
Lemon juice***	–	32.60	33.00	0.25	0.81
Tomatoe juice***	–	12.85	14.00	0.10	0.16
Carrot juice***	–	5.85	6.10	0.19	0.20

* Every result is the average of five determinations.

† Ten tablets were powdered after weighing and an accurately weighed quantity of powder, which was equivalent to 100.00 mg of ascorbic acid, was taken.

** Ten multivitamin tablets were weighed and then powdered. An accurately weighed quantity of powder which was equivalent to 100.00 mg of ascorbic acid was taken.

‡ Each 5 ml of the syrup contained 75.00 mg of ascorbic acid.

§ This was Red Blood orange variety, and amount is mentioned in 100.00 g of juice.

*** The amount is mentioned in 100 g of juice.

[a] The amount of vitamin C was declared by the manufacturing company on its packing.

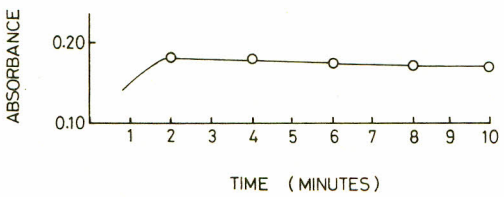


Fig. 2. Effect of time on colour intensity.

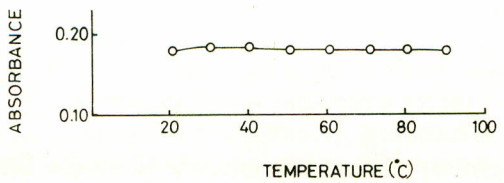


Fig. 3. Effect of temp. on colour intensity.

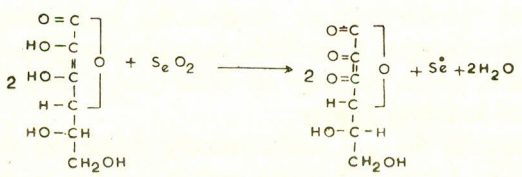
measurements were carried out using 1-cm glass-cells against aqueous extract blank.

RESULTS AND DISCUSSION

For checking the reliability of the present method the pure solution of ascorbic acid was analyzed and it was found that the maximum standard deviation was 0.01 when 100 μg sample was determined. This method was then applied to pharmaceutical preparations and juices. The results are shown in Table 1.

The effect of interfering substances, for example, glucose, fructose, cysteine, cystine and metabisulfite was investigated at pH 3–4 and it was found that they do not interfere even if present up to 200-folds.

The fine orange-colored turbidity is due to the presence of metallic selenium. The oxidizing potential of selenium dioxide is quite low in this system and only ascorbic acid is oxidized when selenium dioxide is added to it. Other reducing substances are not oxidized by this compound. The reaction which takes place between ascorbic acid and selenium dioxide is shown below.



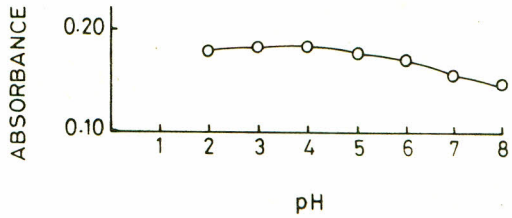


Fig. 4. Effect of pH on colour intensity.

All readings were taken 2 min after the color production, however, there was no appreciable change even after 10 min (Fig. 2).

The effect of temperature and pH on color intensity of the suspensions is shown in Figs. 3 and 4 respectively. Since the color intensity remains constant up to 90° all absorbance measurements were made at room temperature (30°). The color intensity between pH 2.0 – 4.0 remains constant, hence all determinations were carried out between this pH. At pH value more than 7 cysteine, bisulfite and other SH groups start interfering. However, in case of carrots and tomatoes extraction with chloroform is carried out prior to the turbidimetric determination, because their original coloring matter interferes.

From the results shown in Table 1 it can be seen that the present method is reasonable, precise, accurate and offers good scope for the determination of ascorbic acid.

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