

DETERMINATION OF VITAMIN C FROM HUMAN URINE AND BLOOD

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Abstract

Numerous methods have been reported for the determination of vitamin C but most of the methods are tedious., lengthy, time consuming and not free from interference. In the present spectro-photometric method, selenium dioxide reacts with ascorbic acid, produces a fine orange colored suspension. This reaction was utilized for the determination of ascorbic acid in blood and urine. The color was read on UNIGAM SP 600 spectrophotometer at 380 nm. Recoveries of added ascorbic acid were about 98-101%. Reducing agents and many other salts do not interfere in the procedure. There was no change in color intensity for 48 hours. The determination was very steady in the acidic medium (pH 2-6), whereas in basic medium, the absorbance of the color was sufficiently low. It was also observed that 20 to 60°C temperature had no adverse effect on the color intensity. However, there was increase in optical density at higher temperature. The method is quite specific accurate, convenient and less time consuming. (JPMA 30:69 1980).

Introduction

The determination of vitamin C is of great clinical importance for determining the deficiency of this vitamin in various patients. Blood and urine examination is usually taken as an index for checking the adequacy of this vitamin. The methods in vogue are not very many that may be considered satisfactory. A colorimetric method developed by Sarwar et al (Personal Communication) is based on turbidimetric measurement of fine orange colored suspension produced by the reaction of ascorbic acid and selenium dioxide. Since this method had been used only for test solution and pharmaceuticals, the intent of the present investigation was to apply the method to determine ascorbic acid in blood and urine.

Material and Methods

Collection of urine:

Human urine was collected from a normal subject and was kept at room temperature (17-20°C) for the determination of ascorbic acid. To avoid oxidation the urine was preserved by adding 2 ml of glacial acetic acid.

Collection of blood:

Fresh blood was collected from the Lahore slaughter house and was immediately stored in the bottle containing 3 per cent sodium citrate. The blood was just taken at the time of slaughtering the animal, and the sample was then stored at 1-3°C in a refrigerator.

Reagents

Standard Ascorbic Acid:

An accurately weighed amount (80 mg) of A.R. ascorbic acid was dissolved in 100 ml of distilled water containing 2 ml of glacial acetic acid and used as a stock solution.

Selenium Dioxide :

Two per cent solution of selenium dioxide was prepared by adding 2 gm of A.R. selenium dioxide in 100 ml of distilled water.

Carboxy methyl Cetlullose (CMC):

Two per cent solution of carboxy-methyl cellulose was prepared by adding 2 gm of the reagent to the boiling 100 ml distilled water.

Trichloro Acetic Acid (TCA):

Twenty per cent TCA was prepared by dissolving 20 gm of TCA in 100 ml of distilled water.

Depolarization of blood with 20% TCA

Procedure of Barakat et al (1955) was adopted "by adding 5 ml of blood, dropwise with continuous shaking, to 5 ml of 20 per cent trichloroacetic acid in a centrifuge tube and stirred to obtain a fine suspension. The suspension was allowed to stand for 5 minutes, then centrifuged. The clear supernatant solution was filtered.

Procedure for Ascorbic Acid estimation

0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml portions of urine solution were taken in 25 ml measuring flasks separately. 10 ml of 2 per cent CMC and 2 ml of 2 per cent selenium dioxide solution was added in each flask, until an orange colored suspension was developed. The volume in each flask was made up to the mark with distilled water. The maximum absorbance was read at 380 nm in each case.

In another batch of 0.5, 1.0, 1.5, 2.0, 2.5 and 5 ml portions of the urine solutions were taken in six 25 ml measuring flasks separately to check reproducibility. Known amount of ascorbic acid were added in each case. The same procedure as adopted above was followed.

Procedure for Ascorbic Acid in blood

0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml aliquots of deproteinized blood were taken in six separate 25 ml measuring flasks. 10 ml 2 per cent CMC and 2 ml of 1 per cent selenium dioxide were added, until an orange colored suspension was developed. The volume in each flask was made up to the mark. The absorbance was read at 380 nm.

A separate experiment was conducted by adding known amount of ascorbic acid in blood which was not previously deproteinized. The blood containing added ascorbic acid was deproteinized with 20 per cent TCA as previously described. The deproteinized blood was subjected to the chemical reaction as has been described in the above procedure.

Effect of other Environments on the**Stability of Metallic Selenium Suspension**

Three experiments were conducted to observe the effect of temperature, time and pH on the stability of the color intensity of metallic selenium suspension. The details of the procedure are as follows:-

a) Effect of Temperature

2.5 ml portion of the urine containing 2.0 mg of ascorbic acid was taken in 25 ml 2 per cent selenium dioxide and 10 ml CMC solution as has been explained in the aforementioned procedure and the color was read at 380 nm, after heating the reaction mixtures for five minutes at 20, 30, 40, 50, 60, 70, 80, 90 and 100°C. Similar procedure was adopted with blood samples.

b) Effect of Time

2.0 ml portion of the urine containing 1.6 mg of ascorbic acid was taken in 25 ml measuring flask. After the completion of the above mentioned reaction the absorbance of resultant mixture was read at 380 nm after 1, 2, 4, 6, 8, 10, 12, 20, 24, 30, 26, 40 and 48 hours at room temperature (17-20°C). The same procedure was adopted for blood.

c) Effect of pH

Nine flasks of 25 ml capacity were taken and 5 ml portion of urine containing 1.6 mg of ascorbic acid was added in each flask. 10 ml of citrate, phosphate and carbonate - bicarbonate buffer solution (Colowick and Kaplan, 1955) of pH 2-10 were added respectively in the numbered flasks 1, 2, 3, 4, 5, 6, 7, 8, and 9. The rest of the procedure was carried out as previously described. The absorbance of the resultant mixture was read at 380 nm. Similarly experiment was repeated without adding any Buffer solution in the resultant mixture. Same procedure was followed with blood.

Results and Discussion

Numerous titrimetric, colorimetric, polarographic, and spectrophotometric methods for the determination of vitamin C have been developed. The methods generally used either do not take care of possible interference or involve very lengthy and time consuming procedure for the removal of interference making the specificity doubtful. The main purpose of the study, therefore, was to evolve a reasonably good method for the determination of vitamin C in urine and blood. The method should be simple, specific and rapid to give better reproducibility. Present study was undertaken to overcome such difficulties.

The turbidimetric measurement of orange colored suspension produced by the reaction of ascorbic acid and selenium dioxide was taken as the basis of present investigation. The results of determination of added pure ascorbic acid in urine blood have been shown in Table I and II.

Table I

Determination of Ascorbic Acid in Urine

Amount (mg)		Percentage recovery
Taken	Found	
0.25	0.246	98.40
0.45	0.45	100.00
1.86	1.85	99.47
1.90	1.37	98.43
2.03	2.03	100.98
3.00	3.02	100.66

Every result is the average of five determinations.

Table II
Determination of Ascorbic Acid in Blood

Amount (mg)		Percentage Recovery
Taken	Found	
0.230	0.225	97.72
0.4000	0.390	97.50
0.520	0.510	98.08
0.700	0.680	97.50
1.100	1.080	98.20

Every result is the average of five determinations.

It is evident from Table I and II that the recovery of the added compound from urine and blood is about 98-101 per cent. It may be seen that the present method has the advantage that the reducing agents like glucose, fructose, metabisul-phite, cysteine, cystine sulphite and substances like citric acid, tartaric acid, sodium benzoate and metaphosphoric acid do not interfere in the ascorbic acid determination. The present spectrophotometry method is quite specific, quick, accurate and convenient.

Nine sets of determinations each comprising three observations, were done to see the effect of temperature on the stability of results. Same amount of ascorbic acid was tested was raised by heating to 20, 30, 40, 50, 60, 70, 80, 90 and 100°C. The color intensity remained stable between 20-60°C. The corresponding increase in the density was noticed when the temperature exceeded 60°C till 70°C. Beyond 70°C till 100°C again the density readings were stable. However, the density reading was 0.1 more at temperature 70-100°C than 20-60°C. In spite of the fact that the density readings between 70-100°C were stable, the ascorbic acid concentration became over estimated. This suggests that the interfering reducing agents other than ascorbic acid are active beyond 60°C which gave the higher ascorbic acid values than what was actually present.

Time

In most colorimetric analyses the time between production of color and its measurement affects the stability of the results. To study whether the method under trial has much time limitation, known quantity of ascorbic acid was tested at varying time intervals after the production of colors. The results indicated (data not shown) minor variations in color densities at different time intervals after the production of color. The recovery percentage, therefore, was not influenced by time intervals. This suggests that color once produced is fairly stable even upto 48 hours and the absorbance can be measured after the development of the color at any time upto 48 hours. This character provides a fairly good facility to the analyst where large number of samples may not be tested in a short period.

pH:

To see whether pH of the reaction mixtures has any influence on the percentage recovery of ascorbic

acid, it was changed with the help of citrate, phosphate and carbonate/bicarbonate buffers from 2-10. When the pH value increased from 6-10, there was a sharp decline in the absorbance values. The absorbance approached almost to zero at pH 10. This indicated that with the increase in pH above 6, the rate of reaction for color production slowed down consequently giving poor reproducibility. As the absorbance values were stable at pH 2-6 and the recovery percentage was about 100 per cent, the optimum pH of the reaction mixture should be 2-6. Under normal circumstances urine and blood examination by this method provides pR around 3.5. In case pH goes beyond 6, it is advisable to lower it with the help of citrate buffers. This will provide better recovery of ascorbic acid.

References

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