A New Method for the Spectrophotometric Determination of Ascorbic Acid

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Summary:A new spectrophotometric procedure is described for the determination of small quantities of ascorbic acid in different samples. The procedure is based on the reduction of iron(III) to iron(II) which forms a red complex with 1,10-phenanthroline. The absorbance of iron(II)-phenanthroline complex is measured at 515 nm.

Introduction

Because of its significance in human physiology and presence in large variety of matrices ascorbic acid has been determined by a large number of classical and instrumental methods. These include titrimetric [1,2] potentiometric [3], amperiometric [4], fluorimetric [5] and spectrophotometric [6,7,8] methods. Due to reducing properties of ascorbic acid most of the spectrophotometric methods for its determination are based upon oxidation-reduction reactions. In these methods usually either ascorbic acid reduces a metal ion to some lower oxidation state which is then complexed with suitable ligand to produce a coloured complex or reacts directly with some reagent to give a coloured compound. Baker [9] and Guzman [10] reduced Cu(II) ions with ascorbic acid and complexed Cu(I) with neocuproine and cuproine respectively. Al-Zamil [11] and co-workers extracted the Fe(II)-DMG complex in chloroform after reducing Fe(III) with ascorbic acid. 2,4-Dinitrophenyl hydrazine has also been employed to produce an orange-coloured osazone after reacting with oxidized ascorbic acid. The resulted osazone was dissolved in sulphuric acid and absorbance of the red solution was measured [12].

Though a large number of methods have been reported in the literature for the determination of ascorbic acid and most of them need sophisticated instrumentation or rare chemicals or critical control of conditions which make their applications limited. The method described here is based on the reduction of iron(III) to iron(II) by ascorbic acid in the presence of 1,10-phenanthroline. Absorbance of the resulting iron(II)-phenanthroline red complex is measured at 515nm. The method is though not selective, it is quick, simple, and accurate for a number of ascorbic acid-containing drug samples.

Experimental

Equipment and Reagents:

A Pye Unicam SP8-400 double beam UV/Vis spectrophotometer with 10mm glass cells was used for absorption measurements

Iron(II)-Phenanthroline Reagent

Accurately weighed 0.198g (1.0×10^{-3} mol) of AR grade 1,10-phenanthroline monohydrate was

*For correspondence.

mixed with 1 ml of 1N hydrochloric acid in a 100ml flask. Then 0.16g ($3.32x10^{-4}$ mol) of iron(III) ammonium sulphate was added and the contents were dissolved in a few ml of water. The solution was then diluted to the mark with water.

Ascorbic Acid Standard Solution

To prepare 1000μg/ml solution, 0.25g ($1.42x10^{-3}$ mol) of ascorbic acid was dissolved in 250 ml of water. A fresh stock solution was prepared each time and diluted to get 10μg/ml ($5.68x10^{-5}$ M) solution before use.

Calibration

0.2-1ml aliquots of 10μg/ml solution of ascorbic acid was transferred into five 10ml calibrated flasks. 2 ml of iron(III)- phenanthroline reagent was added to each flask.

After 7 minutes the flask contents were diluted to the mark with water. A compensatory blank was prepared by diluting 2 ml of iron(III)-phenanthroline reagents to 10 ml with water. Absorbance of each solution was measured against the blank.

Results and Discussion

The proposed method for the estimation of ascorbic acid is based on the reduction of iron(III) to iron(II). The resulted iron(II) forms a red complex with 1, 10-phenanthroline present in the solution. The absorbance of the solution is measured at 515nm. The principle is very simple and has been used recently for the estimation of adrenaline [13].

Calibration range, Sensitivity and Precision

As shown in Fig.1(a), a linear calibration graph, passing through the origin, was obtained for 0.2-1ppm of ascorbic acid provided the absorbance was measured against a compansatory blank. With water, using as a blank, however calibration with a little intercept along Y-axis, shown in Fig.1(b), was obtained. The calibration started to become slightly curved as the concentration of ascorbic acid exceeded 2.5ppm. Iron(II)- phenanthroline complex showed maximum absorbance at 515nm, hence all the absorbance measurements were made at

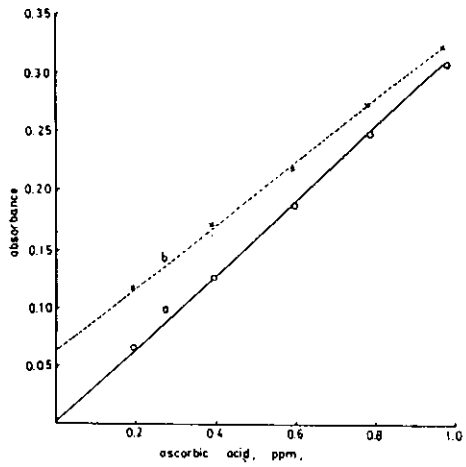

Fig. 1: Calibration curves for ascorbic acid; a- with compensatory blank; b- with water.

515nm. In present work the absorbance is measured in 10mm glass cells; however, the calibration range could be lowered by using long path cells.

To check the precision of the described method relative standard deviation was determined for eight equivolume samples of ascorbic acid. The value obtained for the relative standard deviation was 0.96%

Interferences

To check the possible interferences ascorbic acid samples were determined by the described method in the presence of other ions and compounds. The results obtained are summarized in Table-1. Among the inorganic anions only sulphite and sulphide interfered in the determination severely. Nitrite and oxalate also affected the results but the effect was mild compared to that of sulphite and sulphide. The interferences due to these ions was quite expected because of the fact that the method is based on the reduction of iron(III). Hence any species which can bring about this reduction shall be expected to interfere in the estimation. However ions like chloride, sulphate, phosphate, citrate and tartrate did not affect the results considerably. Besides inorganic ions, effect of a few organic compounds and other vitamins has also been checked. Vitamin 'A' affected the results severely, which can again be attributed to the reduction of iron(III) due to vitamin 'A'. The interference due to vitamin 'A' proved itself a problem

Table 1: Effect of other ions and compounds on ascorbic acid determination

*Interfering ion/ compound	Ascorbic acid found μg/ml	difference	Interfering ion/ compound	Ascorbic acid found μg/ml	difference
None	0.750				
Nitrate	0.890	+0.140	Tartrate	0.73	-0.02
Nitrite	0.780	+0.030	Citrate	0.67	-0.08
Sulphite	5.62	+4.87	Glucose	0.77	+0.02
Sulphide	2.23	+1.48	Fructose	0.76	+0.01
Sulphate	0.76	+0.01	Vitamin A	1.52	+0.77
Chloride	0.71	-0.04	Vitamin E	0.750	0.00
Phosphate	0.75	0.00	Vitamin B12	0.76	+0.01
Oxalate	0.58	-0.17	Niacin	0.750	0.00

*Each interfering ion/compound was added in ten fold excess

during the analyses of the drugs containing vitamin 'A' alongwith ascorbic acid.

pH Effect

To check the effect of hydrogen ion concentration the pH of the solution was changed from 6 to 2 by adding hydrochloric acid. Normally, the reduction of iron(III) was carried out in slightly acidic solutions, at pH 5.5, as a little hydrochloric acid has been added during the preparation if iron(III)-phenanthroline reagent.

As shown in Fig.2, at lower pH values the absorbance of the solution was considerably reduced. This is probably due to the fact that iron(II)-phenanthroline complex is not stable enough in highly acidic solutions.

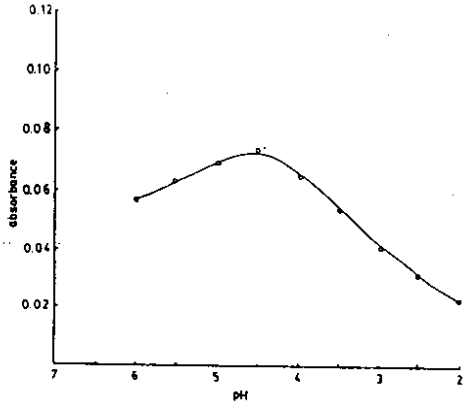

Fig. 2: Effect of pH on absorbance.

Effect of Time

Effect of time was checked by measuring the absorbance of the final solution after different intervals of time. As shown in Fig.3, at least seven minutes are required to develop the colour completely. After seven minutes of mixing the reagents almost constant values of absorbance were obtained which remained unaltered even for hours. This experiment reveals that the reduction of iron(III) to iron(II) and the formation of iron(II)-phenanthroline are relatively slow processes.

Volume of the Reagent

Iron(III)-phenanthroline reagent was a greenish brown solution which on adding ascorbic acid turned red. To avoid the unnecessary volume of the reagent in the flask the volume of the reagent was optimized. It has been noticed that for the range given in the calibration 2 ml of the reagent

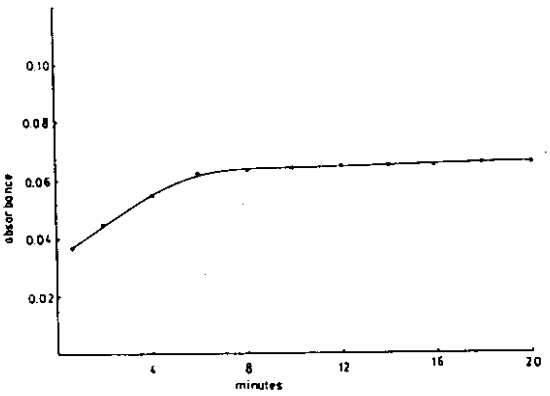

Fig. 3: Effect of time on absorbance.

was enough to complete the reaction. Extra volume of the reagent enhanced the absorbance both of the blank as well as of the sample and hence the intercept of the calibration along the Y-axis increased significantly.

Stability of the Reagents

Stability of the reagents has been checked by measuring the absorbance after mixing the ascorbic acid standards and the iron(III)-phenanthroline reagent, kept for different intervals of time. It has been noticed that iron(III)-phenanthroline remains stable for even more than a week. However to keep the reagent stable for a long time its pH should be less then 5. At high pH values the stability is affected considerably and turbidity has been settled down after a few days.

The standards of ascorbic acid lose their potency with the passage of time. Most probably oxidation due to air and light occurs, hence ascorbic acid standards are either kept in dark air-tight bottles or should be prepared daily.

Samples

To check the accuracy and validity of the described method different pharmaceutical samples have been analysed by the proposed method as well as by a reference method [14]. Solid drug samples were dissolved in water, filtered and the filtrate was used for the analysis of ascorbic acid. Liquid syrups etc. were simply diluted with water to a known volume and analysed.

The results obtained for various samples are summarized in Table- 2. As shown in the table most of the results agree with each other and with those reported for them. However in certain cases non-

Table 2: Comparison of results for analysis of some ascorbic acid containing samples

Sample	Ascorbic acid reported	No. of Detmns. by reference method	Ascorbic acid found (a by described method
Vitera Tab. (Lederle)	150 mg/Tab.	3	142 mg/Tab.136 mg/Tab.
Stress Tab. (Lederle)	600 mg/Tab.	3	593 mg/Tab.584 mg/Tab.
Javeral Cap. (Lederle)	50 mg/Cap.	3	47.5 mg/Cap.46.2 mg/Cap.
Becadexamin Cap. (Glaxo)	30 mg/Cap.	5	30 mg/Cap.27.8 mg/Cap.
Ascorbon Tab. (Pfizer)	500 mg/Tab.	3	489 mg/Tab.492 mg/Tab.
Cebion Tab. (Merck)	500 mg/Tab.	3	495 mg/Tab.497 mg/Tab.
Plubin C. (Hakimsons)	15 mg/ml.	3	13 mg/ml.13.5 mg/ml.
Polybion Forte C. Tab. (Merck)	300 mg/Tab.	5	307 mg/ml.309 mg/ml.

significant differences have been found in the results which may be attributed to the heterogenity of the samples, interfering species and the difference in the techniques used for analysis.

Conclusion

It can be safely concluded from the present work that the proposed procedure for the determination of ascorbic acid especially in drug samples is simple and quick. The reagents used are cheap and easily available. In the absence of certain interferences the described procedure can be used for the estimation of ascorbic acid in various matrices with adequate accuracy.

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