SPECTROPHOTOMETRIC DETERMINATION OF ASCORBIC ACID IN PHARMACEUTICALS BY FLOW INJECTION ANALYSIS USING BROWN MONO 1, 10-PHENANTHROLINE-IRON (III) COMPLEX AS AN OXIDANT

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SUMMARY: A single channel flow injection manifold for spectrophotometric determination of ascorbic acid is described. The method is based on the on-line reduction of mono (1,10-Phenanthroline)-iron (III) complex to tris (1,10-Phenanthroline) - iron (II) complex by reducing action of dienol group of ascorbic acid and the absorbance of the resultant tris chelate was monitored at 510 nm. The effects of reaction coil length, sample volume, flow rate and the reagent concentration on the analytical signal are discussed. A calibration curve for ascorbic acid over the range 0-50 ppm, under optimized conditions is reported. The proposed method was sensitive, rapid (sample rate of 200 s/h) and reproducible (R.S.D 0.5%, n=15). Satisfactory results were obtained in the determination of ascorbic acid in Pharmaceutical preparations.

Key Words: Ascorbic acid, flow injection analysis.

INTRODUCTION

Ascorbic acid is involved in many biological processes and it is an essential compound in the human diet. There is a need for fast, sensitive and automated method for its determination, particularly in routine analysis. In spectrophotometric determination, Fe (III) reduces to Fe (II) by ascorbic acid followed by the determination of Fe (II). Fe (II) can be conveniently measured after its complexation with the chromogenic reagent such as 1, 10-Phenanthroline (1-3), neocuproine (3-6).

In recent years, Flow Injection Analysis (FIA) has been applied to determine ascorbic acid in large number of samples including food, biological and pharmaceuticals (7-10). A recently reported method (10) describes on-line preparation of tris (1,10-Phenanthroline)- iron (III) (ferroin) complex in a two channel manifold followed by injection of sample ascorbic acid and subsequent measurement of absorbance of resultant product tris (1, 10-Phenanthroline)-iron (II) (ferroin) complex.

In this paper a simple, rapid and sensitive flow injection spectrophotometric method for the determination of ascorbic acid in pharmaceutical preparations is developed. In this method, mono (1,10-Phenanthroline) - iron (III)

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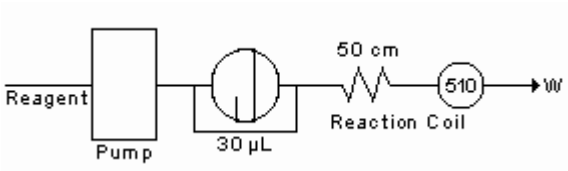
Figure 1: The FIA manifold used in the spectrophotometric determination of ascorbic acid.



was directly reduced to tris (1,10-Phenanthroline) - iron (II) (ferroin) by ascorbic acid and the absorbance of ferroin was monitored at 510 nm. The use of mono (1,10-Phenanthroline) - iron (III) complex as a reagent in place of tris (1,10-Phenanthroline) - iron (III) complex which simplifies the procedure (11). Mono (1,10-Phenanthroline) - iron (III) has also been used for the determination of adrenaline by the classical method (12). A calibration for ascorbic acid over the range 0-50 ppm was developed and method was applied with good results (R.S.D value of 0.5% and a sample rate of 200 s/h) to the routine determination of ascorbic acid in pharmaceutical preparations.

MATERIALS AND METHODS

Reagent

All the reagents used were of Analar grade unless otherwise specified.

Stock solution of mono (1,10-Phenanthroline)- iron (III)

A stock solution of mono (1,10-Phenanthroline) - iron (III) was prepared by dissolving 1.97 g of 1,10-Phenanthroline mono hydrate in 10 ml of 1.0 M HCL, adding 1.67 g of iron (III) ammonium sulfate and diluting to 1.0 L with deionized distilled water. The stock solution was 1:1 ratio with respect to 1,10-Phenanthroline and iron having concentration of 1.0×10^{-3} M and 3.32×10^{-4} M respectively. Working solutions were prepared by suitable dilution with deionized distilled water.

Ascorbic acid standards

A stock (1000 ppm) solution of ascorbic acid (BDH) was prepared by dissolving 0.25 g in deionized distilled water and diluting to 250 ml with the same. A series of standards was prepared by diluting appropriate amounts of stock solution with deionized distilled water.

Real sample solutions

Ten tablets/capsules of each commercial sample were weighed, crushed and powdered. An appropriate amount of powder equivalent to prepare 20-40 ppm solutions with respect to ascorbic acid concentration was dissolved and diluted to 100 ml with distilled deionized water.

Instrumentation and procedure

A single channel manifold (Figure 1) was used in this work. The reagent stream was pumped at the flow rate of 1.1 ml/min (except for the study of flow rate) via a peristaltic pump (Gilson Minipuls 2) equipped with PVC pump tubing (Ana-Chem). The ascorbic acid sample (30 µl except for the study of sample volume) was introduced into the reagent stream via a rotary teflon valve (Rheodyne 5020). Teflon tubing of 0.5 mm was used throughout remainder of the system. The detector was spectrophotometer (Shimadzu 365) equipped with a flow through cell (10 mm, 80 µl) set at 510 nm.

Figure 2: Effect of reagent concentration on the determination of ascorbic acid.

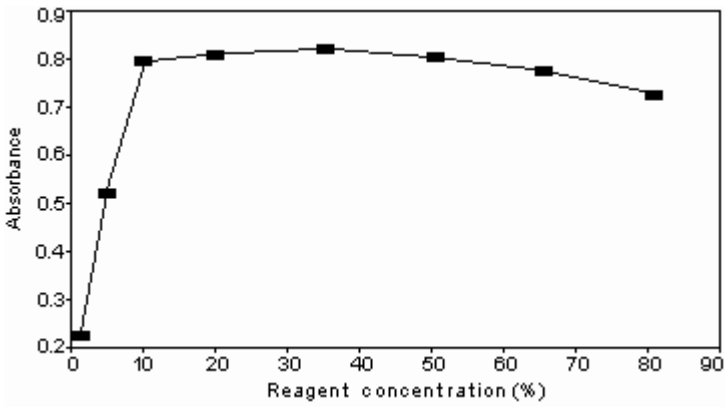
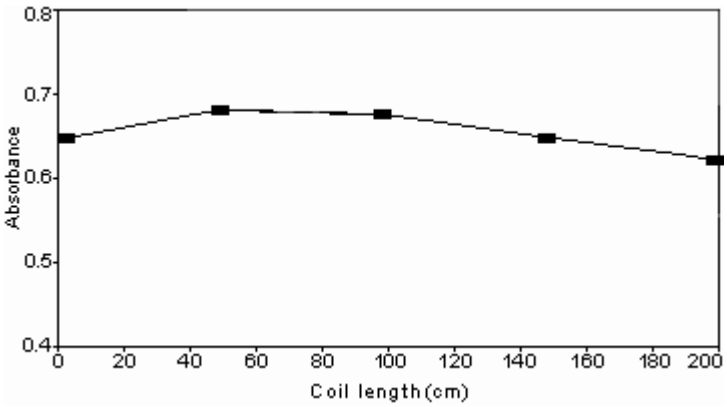


Figure 3: Effect of coil length on the determination of ascorbic acid.



RESULTS AND DISCUSSION

The proposed procedure for the determination of ascorbic acid was based in its reducing reaction on mono (1,10-Phenanthroline) - iron (III) and following the spectrophotometric determination of tris (1,10-Phenanthroline) - iron (II). The absorption spectra measured against reagent blank shows its maximum absorption at 510 nm (11).

Optimization of the FIA system

Flow injection and chemical variables were optimized for the proposed flow injection method. This study was carried out by altering each variable in turn while keeping the others constant.

Reaction coil length

The effect of reaction coil length on analytical signal (Figure 2) was studied in the range of 0 to 200 cm. The maximum signal intensity was observed at 50 cm reaction coil. Hence, an optimized reaction coil length of 50 cm was used in subsequent work.

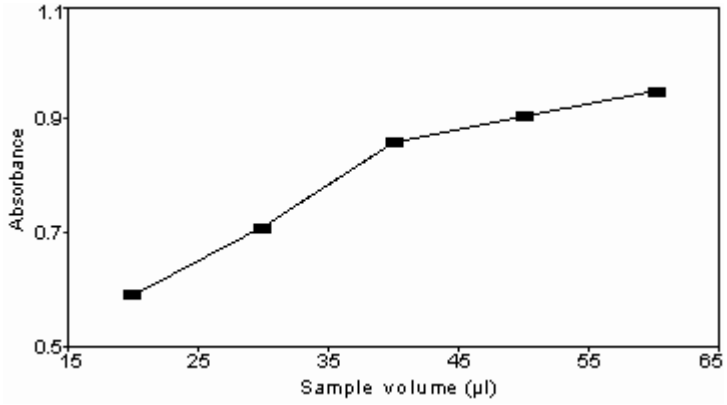
Reagent concentration

The influence of mono (1,10-Phenanthroline) - iron (III) concentration on analytical signal was studied in the range of 1 to 80%. The results obtained are shown in Figure 3 which indicates that maximum signal could be obtained with 35% reagent.

Table 1: Determination of ascorbic acid in commercial samples.

S. No	Composition	Brand name	Manufacturer	Ascorbic acid concentration by present method mg/tablet			DCPIP Method
				Reported	Found	Recovery %	Found
1	Vitamin C	Cecon	Abbot	500	534.3	106.86	542.50
2	Vitamin C	Ascorbon	Dumex	500	509.0	101.80	515.00
3	Multivitamin	Cyanodox forte	Epla	300	315.8	103.27	320.90
4	Multivitamin	Polybion forte C	Merck	300	303.9	101.30	303.30
5	Multivitamin	Stress capsule	Lederle	300	338.0	112.67	342.60
6	Multivitamin with minerals	Vitera	Pfizer	150	153.0	102.00	152.10

Figure 4: Effect of sample volume on the reaction product of ascorbic acid.



Sample volume

The sensitivity of a method can be improved within certain limits by increasing the volume of the injected sample in flow injection analysis. The effect of sample volume in the range of 20-60 µl was studied and results are shown in Figure 4. An increase in absorbance with respect to sample volume over the range used was observed, hence sensitivity was increased two fold with the increase of sample volume.

Flow rate

The effect of flow rate on the absorbance under optimized experimental conditions was examined by changing in the range from 0.6 - 2.7 ml/min (Figure 5). The absorbance remains unchanged throughout the range used. The figure also shows the effect of flow rate on the sample resident time. At maximum signal intensity, sample residence time of 9 seconds does provide sample throughout of 400 s/h. However, considering the practical aspect of the procedure a sample throughout of 200 s/h is recommended.

Calibration graph

With the manifold described above and under the selected experimental conditions, a calibration graph in the range of 0-50 ppm ascorbic acid was obtained (Figure 6). The linear plot has a correlation coefficient of 0.9999 and R.S.D. value (n=15) was 0.5%. Sampling rate was 200 samples per hour.

Figure 5: Effect of sample flow rate on the reaction product of ascorbic acid.

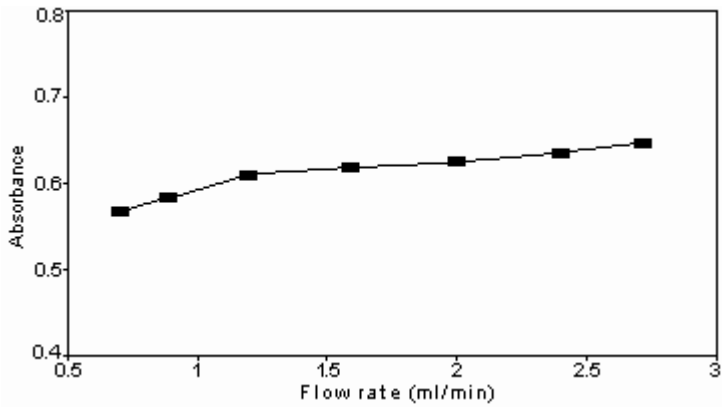
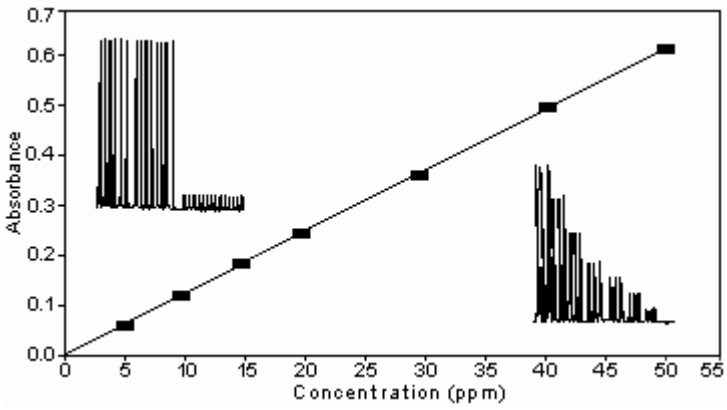


Figure 6 : Calibration curve for ascorbic acid. Calibration traces (lower inset); Reproducibility traces 50 ppm and 5 ppm samples (uper inset).



Application

The procedure was applied successfully for the determination of ascorbic acid in commercial pharmaceutical products. The samples were also analyzed by the reference titrimetric method (13) and results are summarized in Table 1. A good agreement between the results obtained by the two methods was observed.

The available figures were subjected to a statistical evaluation and p value was found within 99% confidence level.

CONCLUSIONS

The proposed method provides a simple, rapid, sensitive and precise flow injection procedure having high sample throughout. Since the time required for sample preparation is short and reagent consumption is low, hence the method is highly economical. The flow injection method using mono (1,10-Phenanthroline) - iron (III) reagent can be used on routine basis for the determination of ascorbic acid in pharmaceutical preparations.

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