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Spectrophotometric determination of vitamin C in pharmaceuticals using Iron (III)-6-Chloro-3-hydroxy-2(4-methoxyphenyl)-4*H*-chromen-4-one complex

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ABSTRACT

A simple, novel, rapid and sensitive procedure is described for the quantitation of vitamin C, which is based on the reducing property of vitamin C. In the proposed method, the iron (III) is found to form a brown colored complex with 6-chloro-3-hydroxy-2(4-methoxyphenyl)-4H-chromen-4-one (CHMC) in acidic medium (pH 3.4-6.8). The absorbance of the brown colored complex formed is measured at 405 nm after extracting it in to dichloromethane. The proposed method obeys Beer's law up to 8.0 µg of vitamin C. The complex formed immediately and the molar absorptivity and Sandell's sensitivity for vitamin C is found to be 9.581×10^3 dm^3 mol^{-1} cm^{-1} and 1.838×10^{-4} µg cm^{-2}. The method has been applied to the analysis of various pharmaceutical samples.

KEY WORDS: Vitamin C, Absorbance, Extraction, Iron (III), Spectrophotometry.

1. INTRODUCTION

Vitamin C is a powerful water soluble antioxidant that is essential for the growth and maintenance of all body tissues. So, a daily intake of vitamin C is necessary for human beings which has resulted a continuous interest for working out rapid, simple and better methods for its determination. A large number of methods based on different instrumental techniques such as fluorometry (Official method of analysis ,1980; Huang, 1995; Park, 2009; Feng, 2005), electrochemical(Craston,1991; Marian, 2000; Teixeira, 2003; Lourencao, 2010; Bunaciu, 2009; Chauhan, 2011), Chemiluminescence (Feng 1995; Pires 2006; Chen 2010; Danet, 2000), spectrometry (Zhang, 2000; Jiang, 2001; Turker, 2008), flow injection methods (Lin, 1999; Rama, 2004; Nobrega, 1996) and enzymic (Esteban, 1997; Casella, 1989; Lee, 1997) methods etc. But the spectrophotometric methods are preferred over these methods for the routine analysis of vitamin C because of their rapidity and simplicity. These methods have been developed based on its ability to couple with diazotized aniline derivatives to yield colored complexes or by using redox property of ascorbic acid. Although many colorimetric methods are reported but these methods are associated with some limitations which require either pre-treatment, or lack of selectivity and sensitivity and many of them are time consuming, thereby restricting their suitability for frequent use.

The purpose of the present work was to develop a rapid sensitive and simple method for the determination of ascorbic acid. The proportionate decrease observed in the color intensity of iron(III)-6-Chloro-3-hydroxy-2(4-methoxyphenyl)-4*H*-chromen-4-one (CHMC) complex with the addition of ascorbic acid and the extraction of complex formed into dichloromethane forms the basis of the proposed method.

2. EXPERIMENTAL METHODS

Instrument: A Systronics spectrophotometer (model 166) and a pair of matched quartz cells was used for measurement of absorbance of complex formed.

Reagents and solutions:

Iron (III) solution: A stock solution (1mg ml^{-1}) of iron (III) was prepared by dissolving accurately weighed amount of Ammonium ferric sulphate in 100 ml of double distilled water and 0.5 ml of concentrated sulphuric acid was added to the solution. Working solutions of the metal ion of required (100 µg mL^{-1}) level are made by proportionate dilutions.

6-Chloro-3-hydroxy-2(4-methoxyphenyl)-4*H*-chromen-4-one (CHMC) solution (Fig.1): A 0.05% (w/v) solution of reagent was prepared by dissolving the reagent in ethanol.

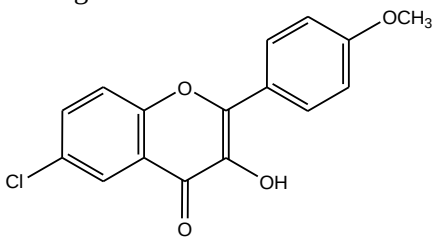


Figure.1. 6-Chloro-3-hydroxy-2(4-methoxyphenyl)-4*H*-chromen-4-one (CHMC)

Vitamin C: The working solution 20 µg ml^{-1} of vitamin C was prepared by dilution of the freshly prepared concentrated solution (1 mg/ml)

Procedure: 50 µg of iron (III) solution was taken in a 100 ml separatory funnel and varying amount of ascorbic acid were added. After mixing the contents, 1 ml of reagent (CHMC) solution was added. The volume was made to 10 ml with deionized water. The brown colored complex was extracted for 1 min into 10 ml of dichloromethane. The extract was then transferred to a 10 ml volumetric flask and its volume was made upto mark with dichloromethane. The absorbance of the brown complex was observed at 405nm against the reagent blank prepared similarly. A standard calibration curve prepared by taking varying amounts of ascorbic acid up to 8.0 µg ml^{-1} and using the conditions of the procedure. From the calibration curve, we can calculate the amount of vitamin C in the various samples.

Determination of Ascorbic acid in pharmaceuticals: A known number of vitamin C tablets or capsules (5-7 items) were ground to the powder form. An accurately weighed amount equivalent to 100 mg of ascorbic acid was dissolved in deionized water. The solution was then filtered and the filtrate was transferred to a volumetric flask of 100ml. The volume was made up to the mark with water. A solution of lower concentration (100 µg ml^{-1}) was prepared by suitable dilution of this solution. The diluted solution was analysed by the recommended procedure.

3. RESULTS AND DISCUSSION

Spectral characteristics: It was observed that Iron (III) forms an extractable colored complex with 6-chloro-3-hydroxy-2 (4-methoxyphenyl)-4*H*-chromen-4-one. With the increase in the amount of ascorbic acid the color intensity of Fe (III)-CHMC complex decreases. The electronic spectrum of Fe (III)-CHMC in dichloromethane was studied along with that of reagent blank over the range 370-600 nm. The electronic spectrum of the brown colored complex reveals the absorption band at 403-407 nm (Fig.2), where the absorption due to reagent blank is almost negligible. Hence, all absorbance were carried out at 405 nm.

Choice of extractant: The extraction behaviour of complex in to different solvents namely dichlorometane, chloroform, carbontetrachloride, benzene, xylene, iso-Amylalcohol and n-hexane as studied (Table.1). The colored complex was extracted in to dichlorometane, carbon tetrachloride, chloroform and benzene. However, Iso-Amyl acetate and n-hexane were found to extract the complex incompletely. Among, these solvents dichloromethane was found to have maximum absorbance value, so chosen for the further studies.

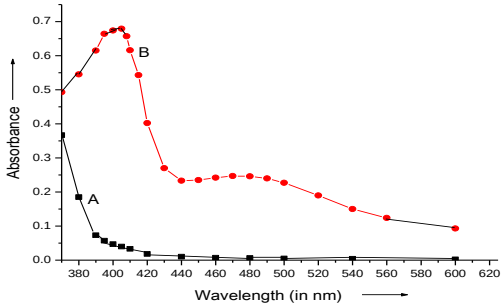


Figure.2. Absorption spectrum of Iron(III)-CHMC complex in Dichloromethane

(Conditions: Fe(III) = 50 µg; CHMC solution = 1 ml)

A – Reagent blank against Dichloromethane

B – Complex against reagent blank

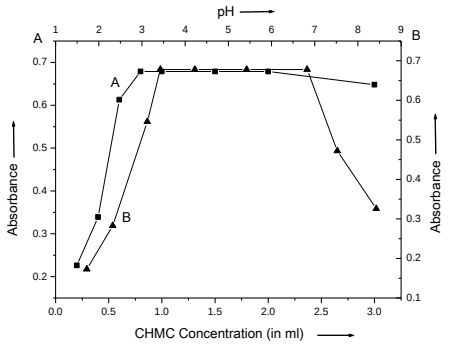
Table.1. Extraction Behaviour of the complex in Different solvents

Solvent	Absorbance*
Dichloromethane	0.679
Chloroform	0.663
Carbon tetrachloride	0.637
Benzene	0.443
Iso-Amyl alcohol	0.174
n-Hexane	0.078

* Measured against respective blank

Optimization of reaction variables: The parameters which affect the absorbance of the brown colored complex were studied as shown in the Table.2. For the study of each reaction variable, 10 ml of the aqueous phase containing 50 µg of Iron (III) was equilibrated with equal amount (10 ml) of dichloromethane. The other conditions used therein are indicated in Table.2.

Effect of the reagent (CHMC) concentration: With the increase in the reagent (CHMC) volume up to 0.8 ml, increases the absorbance of the colored complex, thereafter it remains constant up to 2.0 ml, thereafter a gradual decrease in absorbance above this concentration was observed (Table.2, Fig.3 Curve A). Hence 1.0 ml of the CHMC solution was used for further studies.



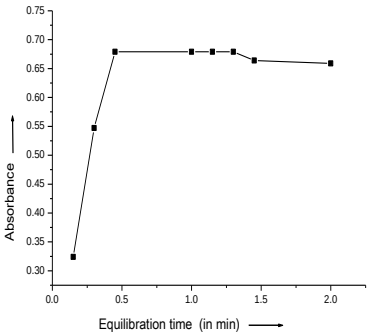


Figure.3. A – Effect of CHMC Concentration B – Effect of pH **Figure.4. Effect of Equilibration Time**

Table.2. Optimization of reaction variables

CHMC Conc. (in ml)	0.2	0.4	0.6	0.8-2.0	2.5	
Absorbance	0.226	0.339	0.613	0.679	0.664	
Equilibration time (in min)	0.15	0.30	0.45-1.30	1.45		
Absorbance	0.324	0.547	0.679	0.662		
pH	1.7	2.3	3.1	3.4-6.8	7.5	8.4
Absorbance	0.174	0.284	0.547	0.679	0.473	0.327

Conditions: Iron (III) = 50 µg; volume of reagent (CHMC) solution = 1.0 ml; volume of dichloromethane = 10 ml; volume of aqueous phase = 10 ml; equilibration time = 1 min λ_{max} = 405 nm.

Effect of pH: The extraction of the Fe (III)-CHMC complex was studied over a pH range 1.7-8.4. It was observed that the complex gives maximum absorbance within pH range of 3.4-6.8 (Table.2, Fig.3 Curve B). However a decrease in absorbance is observed on either sides of this range.

Equilibration time: An increase in the equilibration time between two phases up to 45 sec enhances the extraction of the Fe (III)-CHMC complex. The absorbance of the Fe (III)-CHMC complex remains constant up to 1.30 min (Table.2, Fig.4). Therefore, 1 min equilibration time was chosen for the complete extraction of the complex.

Calibration curve, molar absorptivity and Sandell's sensitivity: Under the optimum conditions, Beer's law obedience and its range was checked at 405 nm by adding different amount of ascorbic acid to Iron (III) solution. A linear relationship between absorbance and concentration of the vitamin C was observed over the range 0.0- 8.0 µg ml^{-1} (Table.3, Fig.5) with Sandell's sensitivity and molar absorptivity of 1.838 µg cm^{-2} and 9.581 x 10^7 l mol^{-1} cm^{-1} respectively.

Table.3. Variation in the Absorbance with Amount of Ascorbic Acid

Amount of ascorbic acid (µg/ 10ml)	**Absorbance**
0	0.679
10	0.595
20	0.526
30	0.446
40	0.359
50	0.272
60	0.177
70	0.105
80	0.032
90	0.018
100	0.014

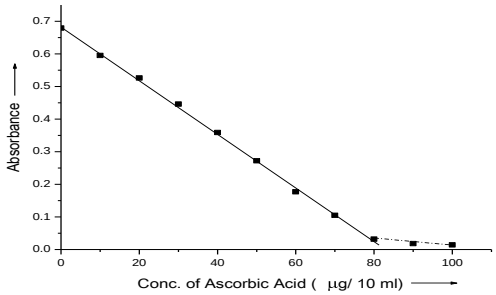


Figure.5. Beer's law curve for varied amount of ascorbic acid

Effect of various substances: Effects of the substances commonly found in pharmaceutical formulations were studied. These includes additives, vitamins and organic acids. The data related to the tolerance of each of these substances for the determination of 50 µg ascorbic acid are as follows (mg amounts given in the parenthesis): glycerol (500); formaldehyde (400); fructose (250); glucose, sucrose (200); maltose (150); lactose , thiourea (100); urea (80); Cl^-, SO_4^{2-} (25); Na(I) and K(I) (20); Ca (II), Mg (II) (15); Al(III) (10); Zn(II), benzoic acid (5); salicylic acid (2.5); Asparatic acid, methionine, succinic acid (2.0); thiamine hydrochloride (1.5); pyridoxine hydrochloride, glutamic acid and nicotinic acid (1.0); riboflavin (0.8); Nicotinamide (0.6); tartaric acid (0.5); cyanocobalamin, cysteine (0.5); folic acid, citric acid (0.03).

Applications: The method can be applied to the analysis of commercial available pharmaceutical preparations. Vitamin C tablets and multivitamin capsules were analysed for ascorbic acid amount by the recommended procedure. The results are mostly in close agreement with the amount given by manufacturer's specification (Table.4); except for the multivitamin formulations where a slight lower amount of the ascorbic acid was found.

Table.4. Analysis of pharmaceutical products

Sr. No.	Preparation	Vitamin C Content per tablet (in mg)	
		Claimed	Found
1	Celin	500	498.7
2	Celin(Chewable)	200	198.6
3	Limcee	100	99.2
4	Supradyn (multivit.)	150	147.8
5	Sym-o-vit (multivit.)	75	72.3

4. CONCLUSION

The proposed method for the determination of ascorbic acid is sufficiently sensitive and selective. The utility of the method was assessed by the analysis of various pharmaceutical formulations containing ascorbic acid as an ingredient.

5. ACKNOWLEDGEMENT

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