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Spectrophotometric Quantitation of Vitamin C Using 3-hydroxy-2-(3-methyl-thiophen-2-yl)-4*H*-chromen-4-one as a Reagent

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ABSTRACT

The proposed method for ascorbic acid (Vitamin C) determination is based on the absorbance of a Fe(III)- 3-hydroxy-2-(3-methyl thiophen-2-yl)-Chromen-4-One (HMTC) as complex in acidic medium, where the absorbance of the formed Fe(III)-HMTC chelate measured at 417 nm after extraction with chloroform gets decreased with the addition of ascorbic acid. This chelates formed immediately and the apparent molar absorptivity and Sandell's sensitivity for vitamin C is found to be 4.413 × 10^4 dm^3 mol^{-1} cm^{-1} and 3.984 × 10^{-7} g cm^{-2}. Beer's law is obeyed up to 1.2 μg ml^{-1} of ascorbic acid. The method has been successfully applied to determination of ascorbic acid in the pure state, dosage forms and multivitamin formulations.

Keywords: Ascorbic acid, Absorption, Extraction, Chloroform

INTRODUCTION

Ascorbic acid commonly known as vitamin C is an important water soluble vitamin. Humans and apes cannot synthesize ascorbic acid due to lack of gulonolactone oxidase enzyme and hence ascorbic acid has to be supplemented from external sources, mainly through vegetables, fruits and pharmaceutical products. Vitamin C is one of the most essential vitamins for both pharmaceutical and food processing industries in view of its nutritional significance, varied use in food products and its daily dose requirement for optimum health. A large number of methods for determination of ascorbic acid include titrimetry [1-4], voltammetry [5-7], amperometry [8-10], potentiometry [11-13], chemilumescence [14-17] and flow injection [18,19] analysis. These methods have been used to increase the analytical sensitivity for ascorbic acid and some of them are automated, but specialized equipments are required for these procedures. Besides, spectrophotometric methods are commonly used for the determination of ascorbic acid. Many reagents such as; fast red [11,20], leucomalachite green [21], rhodamine B [22,23] and methyl viologen [24] etc., to mention a few, are used in the determination of ascorbic acid.

A simple and sensitive analytical method is required for the analysis of a variety of samples. The method using 3-hydroxy-2-(3-methyl thiophen-2-yl)-4*H*-chromen-4-one (HMTC) is described which meets such requisite characteristics of a photometric method. The proposed method is based on the proportionate decrease in the colour intensity of iron (III)-HMTC complex by the addition of ascorbic acid followed by its extraction into chloroform.

EXPERIMENTAL SECTION

Instrument

A Hitachi Schimadzu spectrophotometer (model UV-140-02) with a pair of matched 1 cm quartz cells was used for absorbance measurements.

Reagents

Iron (III) solution

A (1 mg ml^{-1}) iron (III) solution was prepared by dissolving accurately weighed amount of ammonium iron (III) sulphate in 100 ml of deionized water containing 0.5 ml of concentrated sulphuric acid. A lower concentration (10 μg ml^{-1}) was obtained by dilution of the stock solution.

3-hydroxy-2-(3-methyl thiophen-2-yl)-4*H*-Chromen-4-one (HMTC) solution

A 0.05% (w/v) solution was obtained by dissolving the reagent in ethanol (Figure 1).

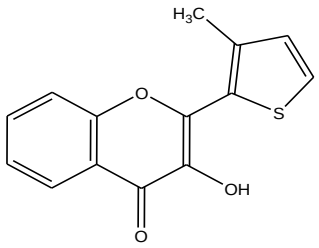


Figure 1: 3-hydroxy-2-(3-methyl-thiophen-2-yl)-4*H*-chromen-4-one

Ascorbic acid

A fresh aqueous solution of ascorbic acid (100 µg/ml) was used. A lower concentration (10 µg/ml^{-1}) was obtained by dilution of the stock solution. Deionized water was used for preparing solutions and all the reagents used were of analytical grade unless otherwise stated.

Procedure

Into a 100 ml separating funnel, 10 µg of iron (III) solution was pipetted followed by addition of an aliquot of ascorbic acid and 0.5 ml of HMTC solution. Enough water was added to make the aqueous phase to 10 ml. The resulting brown complex was extracted for 1 min. with 10 ml of chloroform. The coloured extract was taken into a 10 ml volumetric flask and the volume was made up to the mark with chloroform, if required. The absorbance of the reddish brown complex was measured at 417 nm against the reagent blank prepared similarly. The contents of ascorbic acid were calculated from the standard calibration curve prepared by taking different concentration of ascorbic acid.

Analysis of pharmaceutical products

An accurately weighed amount of the powder, obtained by crushing 5-10 tablets and equivalent to 10 mg ascorbic acid was transferred to a 100 ml volumetric flask, after dissolving the powder, the volume was made up to the mark with deionized water. The working solution (10 µg/ml^{-1}) was obtained by dilution of the stock solution. An aliquot of this solution was analysed for ascorbic acid contents by the recommended procedure.

RESULTS AND DISCUSSION

During preliminary investigation it was observed that the color intensity of iron (III) –HMTC complex diminishes with the increase in the amount of ascorbic acid. This fact was exploited in the development of the proposed method. The extraction behaviour of the complex into different solvents such as chloroform, dichloromethane, carbon tetrachloride, n-butanol, butan-2-one, n-hexane was studied (Table 1). The chloroform was chosen as an extractant since it gives the highest absorbance with more stability of complex. The absorption spectrum of the brown coloured complex along with that of blank was studied. The spectrum shows an absorption band at 414-418 nm (Figure 2), where the reagent blank absorbs very little. Hence, all the absorbance measurements were made at 415 nm.

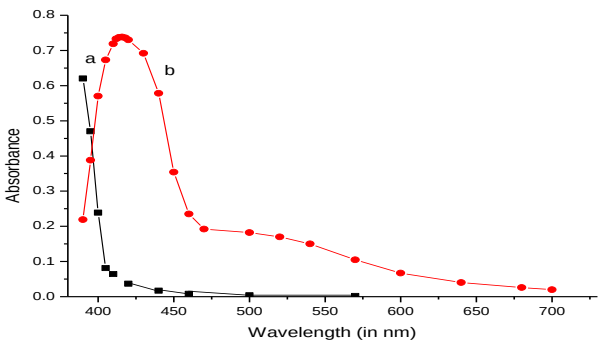
Table 1: Extraction Behaviour of the complex in different solvents

Solvent	Absorbance*
Chloroform	0.79
Dichloromethane	0.75
Carbon tetrachloride	0.72
n-Butanol	0.38
Butan-2-one	0.34
n-Hexane	0.12

*Measured against respective blank

Optimization of reaction variables

The effect of different variables affecting the absorbance and extraction of the complex was studied (Table 2). In the study of these variables, 10 ml of the aqueous phase solution containing 10 µg of iron(III) and varied amounts of the reagent and ascorbic acid was equilibrated for 1 min. with an equal amount of chloroform.



Conditions: Fe(III)=10 µg; HMTC solution=0.5 ml. a: Reagent blank against chloroform; b: Complex against reagent blank

Figure 2: Absorption spectrum of iron(III)-HMTC complex in chloroform

Effect of reagent concentration

The increase in the reagent HMTC concentration through 0.5 ml of HMTC solution leads to increase in the absorbance which remains constant up to 1.0 ml of HMTC solution (Table 2, Figure 3 Curve A). However, further increase in its concentration causes a gradual decrease in the absorbance. Hence, 0.5 ml of the HMTC solution was used for further studies.

Effect of pH

The complex was studied over a pH range 2.5-10.4. It was observed that the complex gives maximum absorbance in the acidic medium between 3.5-6.0 (Table 2 and Figure 3 Curve B). However, a decrease is observed on going either side of this range.

Effect of equilibration time

An increase in the contact time between two phases up to 1 min. enhances the extraction as evidenced by corresponding increase in the absorbance of the complex. It remains constant up to 3 min. of equilibration time (Table 2 and Figure 4). Therefore, equilibration time of 1 min. was chosen.

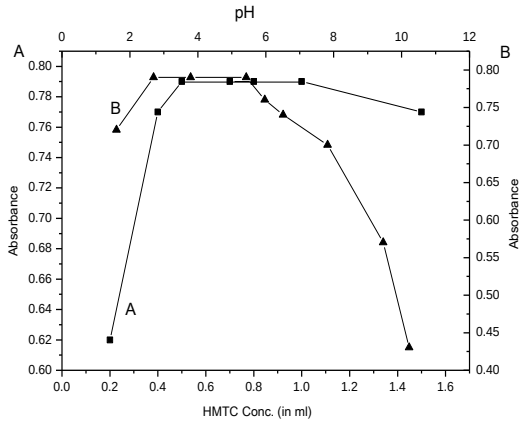


Figure 3: Effect of HMTC concentration

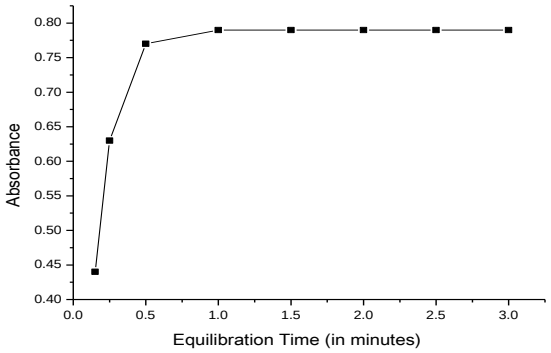


Figure 4: Effect of equilibration time B effect of pH

Table 2: Optimization of reaction variables

HMTC concentration(in ml)	0.2	0.4	0.5-1.0	1.5	2.0		
Absorbance	0.62	0.77	0.79	0.77	0.75		
pH	2.5	3.5-6.0	6.5	7.0	8.2	9.7	10.4
Absorbance	0.72	0.79	0.76	0.74	0.70	0.57	0.43
Equilibration time	0.15	0.25	0.50	1-3			
Absorbance	0.44	0.63	0.77	0.79			
Temperature	20-30	40	50				
Absorbance	0.79	0.72	0.70				

Conditions: iron(III) (10 μg ml^{-1})=1 ml, Volume of reagent (HTMC)=0.5 m, Equilibration time=1 min, Volume of aqueous phase=Volume of chloroform=10 ml, λ_{max} =417 nm

Beer's law and statistical parameter

Under the optimum conditions, a standard calibration curve was constructed at 417 nm by adding different amounts of ascorbic acid to iron(III) solution. A linear relationship between absorbance and the concentration of the analyte was observed over the range 0.0-1.2 μg/ml^{-1} (Table 3 and Figure 5). The calculated value of the molar absorptivity at 417 nm is 4.413×10^4 $dm^3.mol^{-1}.cm^{-1}$.

Table 3: Absorbance value at different concentration of ascorbic acid

Amount of ascorbic acid (µg/10 ml)	Absorbance
0	0.79
1	0.76
2	0.72
4	0.65
6	0.57
8	0.49
10	0.42
12	0.36
13	0.34
14	0.32

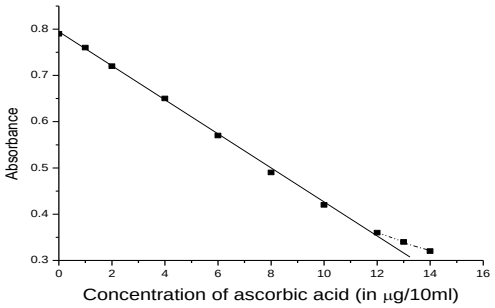


Figure 5: Beer's law curve for ascorbic acid

Interference studies

The effect of the possible ingredients likely to be present in pharmaceutical products was studied. The results of such studies are shown in Table 4. Where the different substances are classified under the headings sugars, vitamins and amino acids, organic acids, cations and anions etc. The tolerance limit for the studies of vitamins and amino acids is comparatively good as compared to cysteine, riboflavin and cyanocobalamin. Organic acids other than citric and salicylic acid do not interfere. Among the tested cations and anions Al(III), Zn(II), Co(II) and Mn(II) are tolerated in traces whereas chloride and sulphate seriously interfere with the determination.

Table 4: Effect of diverse substances

	Substance added[#]	Tolerance limit (mg per 10 ml)
Sugars	Sucrose	150
	Glucose, Fructose	80
	Maltose	40
	Lactose	50
Vitamins and amino acids	Pyridoxine hydrochloride	1
	Thiamine hydrochloride	1
	Methionine	1
	Folic acid	0.25
	Riboflavin, cyanocobalamin	0.15
	Nicotinic acid	0.25
	Glutamic acid	2.5
	Nicotinamide	0.25
	Cysteine	0.15
Organic acids	Aspartic acid	0.03
	Benzoic acid	20
	Succinic acid	8
	Maleic acid	3
	Tartaric acid	1.5
	Salicylic acid	0.4
	Citric acid	0.3
Cations and anions	Ca(II), Mg(II)	5
	Co(II), Mn (II)	0.03
	Zn(II), Al(III)	0.6
	Cl^-, SO_4^{2-}	80
	NO_3^-	2.5
	F^-	4.0
	$S_2O_3^{2-}$	0.8
Miscellaneous	Starch	30
	Formaldehyde	200
	Thiourea, Urea	80
	Glycerol	100

#Substances was added prior to the addition of ascorbic acid.

Applications

The proposed method was successfully applied to the analysis of ascorbic acid in pharmaceutical formulations such as vitamin C tablets and multivitamin capsules as shown in the Table 5.

Table 5: Analysis of pharmaceutical products

S. No.	Preparation	Ascorbic acid content per tablet (in mg)	
		Claimed	Found
1	Celin	500	501.5
2	Limcee	100	100.5
3	Supradyn	150	149.5
4	Celin (Chewable)	200	201.0
5	Sym-o-vit	75	74.7
6	Maxirich	25	26.0

CONCLUSION

The proposed method was naval, rapid, sensitive and highly selective. The method can be used for the routine analysis of vitamin C in various pharmaceutical formulations. The utility of the method was tested by the effect of diverse substances which are common ingredients of various pharmaceutical products.

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