Modified Spectrophotometric Methods for Determination of Iron(III) in Leaves and Pharmaceuticals Using Salicylic Acid

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ABSTRACT

Two simple, accurate, precise, sensitive, and pH-selective visible spectrophotometric methods have been developed and validated for the direct estimation of iron(III) in pure form, leaves, and pharmaceuticals using salicylic acid (SCA) as a chromogenic agent. The methods are based on complexation reaction between iron(III) and SCA at pH 2.26 ($\pm$0.02) (Method A) and 6.1 ($\pm$0.02) (Method B) to yield chromogens with maximum absorption at 520 and 460 nm, respectively. Various factors to yield maximum absorption and sensitivity have been studied and optimized. In both the methods, the absorbance was found to increase linearly with iron(III) concentration as shown by the correlation coefficient (r) of 0.9999 and 0.9988 for Method A and Method B, respectively. The calibration graphs are linear over the concentration ranges of 3.75-37.5 and 2.0-24.0 $\mu g\ mL^{-1}$ in method A and method B, respectively, with apparent molar absorptivity values of 1.71×10^3 and $3.01 \times 10^3\ L\ mol^{-1}\ cm^{-1}$. The stoichiometry between iron(III) and SCA was found as 1:1 and 1:4 in Method A and Method B, respectively. The methods were validated as per the current ICH guidelines. The methods developed were successfully applied to determine iron(III) in curry and drumstick leaves, mustard seeds, and pharmaceuticals. The effect of interferents such as cations, anions, and other organic substances was also studied.

1. INTRODUCTION

Iron is the most wide-spread transition metal found in the living system. It is most commonly found in the form of oxides and hydroxides. To enable the uptake of iron by plants, it is sometimes necessary to mark the iron in an organic complex. In the body of mammals, iron is stored as iron(III) hydroxide particles surrounded by proteinaceous coat, known as ferritin. Iron is transported through the blood stream via transferrins. Iron is a very essential nutrient for human as well as animals. Therefore, consumption of iron-rich food is very pivotal.

Different workers have worked to develop analytical methods to determine iron(III) using several complexing agents, and these include study of kinetics of formation of bidentate monocomplexes of iron(III) with salicylic acid (SCA), sulfosalicylic acid, 8-hydroxyquinoline and salicylaldehyde with possible mechanism [1], bidentate ligands forming six-membered chelate rings with malonic acid n-butylmalonic, benzylmalonic and cyclobutane [2],

study of rates and mechanism of iron(III) monohydroxamato complexes [3]. The rate of iron(III) chelate formation is controlled by the release of first coordinated water molecule or by chelate ring closure as was found in the other reports [4,5] for some bivalent metal chelates containing a six-membered ring. Here, kinetic properties of bidentate hydroxamic acids were compared with those of the bidentate as well as monodentate ligands. Many other workers have also reported [6-8] the kinetics of formation of iron(III) complexes with similar ligands. A spectrophotometric study of the complex formation between iron(III) and SCA was reported by Ogava and Tobe [9]. Several other reagents were used by different workers to determine iron(III) in different samples spectrophotometrically [10,11]. All these reported methods are not describing the procedures to determine iron in sample such as curry leaves and drumstick leaves and pharmaceuticals. Besides, the methods are either kinetic mechanistic reports or simple chelation investigations. Therefore, it is very essential to develop a new rapid and simple validated

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analytical method for assay/determination of iron in these said samples.

Two simple spectrometric methods were developed and validated for the estimation of iron(III) in pure form, leaves, and pharmaceuticals using SCA as a chromogenic agent. The proposed methods were optimized by studying several parameters. Composition and thermodynamic aspects of iron-SCA complex were also examined.

2. EXPERIMENTAL
2.1. Apparatus
All absorbance and pH measurements were made using ultraviolet/visible spectrophotometer (Shimadzu, Double beam) and Elico-pH meter (Ahmadabad, India).

2.2. Reagents
All reagents used in the study were of analytical grade. Bi-distilled water was used wherever necessary. Curry leaves, drumstick leaves, mustard seeds, and Orofer-XT tablets (Emcure Pharmaceuticals Ltd.) of iron(III) were purchased from local market. Ammonium ferric sulfate [Fe(III)AS] (purity 98%), SCA (purity 99.5%), sulfuric acid (H_2SO_4), acetone, sodium hydroxide (NaOH), sodium acetate (NaOAc), ammonia (NH_3), nitric acid (HNO_3), hydrochloric acid (HCl), and other reagents/substances were procured from Merck, Mumbai, India. A standard solution of iron(III) (1000 µg mL^{-1}) was prepared by dissolving the required quantity of Fe(III)AS in water containing few drops of 2 M H_2SO_4. Suitable aliquots were then diluted with water to get 75 and 40 µg mL^{-1} iron(III) for use in Method A and Method B, respectively. SCA (1% w/v) was prepared by dissolving 1 g of substance in 100 ml of acetone. All subsequent dilutions for SCA were made only in acetone. A 2 M NaOAc and all other solutions were prepared in water.

2.3. Procedures
2.3.1. Preparation of calibration curves
2.3.1.1. Method A
Into a series of 10 ml volumetric flasks, different aliquots of 0-5 ml of 75 µg mL^{-1} standard iron(III) solution were placed with the help of a micro burette. To each flask were added 2 ml of 1% SCA. The content was mixed and let to stand for 5 min. Finally, diluted to the mark with distilled water, mixed and the absorbance of each solution was measured at 520 nm against blank.

2.3.1.2. Method B
Varying aliquots of 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml of 40 µg mL^{-1} standard iron(III) solution were placed into a series of 25 ml volumetric flasks. After treating with 2 ml each of 1% SCA and 2 M NaOAc, all flasks were kept aside for 5 min. Then, the flasks were

diluted to the mark with distilled water, mixed and the absorbance measured at 460 nm against reagent blank.

Calibration curves were prepared using absorbance and concentration data for each of the above methods. The concentrations of the unknowns were read using Beer's law relationship or regression data.

2.3.1.3. Determination of iron(III) in curry leaves, drumstick leaves, and mustard seeds
About 5 g of dried curry leaves or drumstick leaves or powdered mustard seeds sample was ignited to ash in a crucible with few drops of concentrated nitric acid. After heating for about 30 min, cooled and dissolved in 25 ml of 1:1 HCl. The solution was boiled for 15 min and filtered. The filtrate was made alkaline with 40% NaOH, boiled for 15 min and the solution was made acidic with 5 ml of concentrated HCl. After diluting the volume to 100 ml, suitable aliquot was used for assay.

2.3.1.4. Determination of iron(III) in pharmaceuticals
Found the weight of 10 iron(III) tablets. The tablet powder equivalent to 100 mg of iron(III) was treated with 30 ml of 1:1 HCl. The solution was boiled for 15 min and filtered. The filtrate was made alkaline with 40% NaOH, and again it was boiled for 15 min. The solution was acidified with 5 ml of concentrated HCl and diluted to 100 ml with water in a standard flask. Stock solution was diluted with water to get 75 and 40 µg mL^{-1} iron(III). Suitable aliquots were then subjected for analysis.

3. RESULTS AND DISCUSSION
3.1. Method Development
From the preliminary experiments, it was found that at pHs 2.26 (±0.02) (Method A) and 6.1 (±0.02) (Method B) iron(III) formed an intense complex with SCA with maximum absorption at 520 and 460 nm,
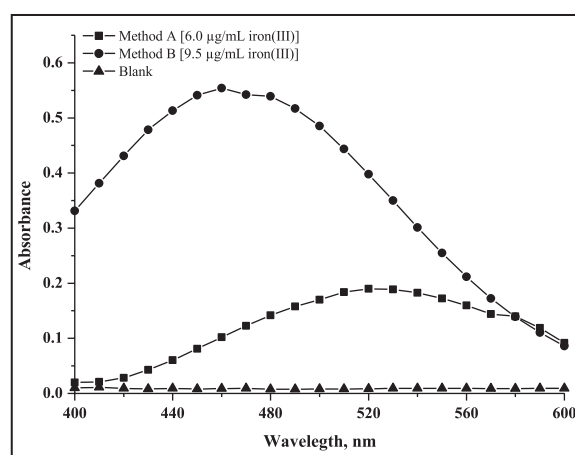

Figure 1: Absorption spectra of iron(III)-salicylic acid complex: (A) At pH 2.26 and (B) at pH 6.1.

respectively (Figure 1). The reaction was completely pH dependent. Various factors which influence the sensitivity of the reaction were studied by measuring the absorbance at either 520 or 460 nm for Method A or Method B, in which one parameter was varied and another was kept as such. The parameters studied were the lower and upper limit of iron(III) and SCA, effect of pH, effect of different acids and bases, effect of standing time, stability of the complex, and composition of the complex.

In Method A, the optimized limits of iron(III) in the complexation reaction were found from 3.75 to 37.5 μg mL^{-1}, whereas in Method B; the same were ranged from 4 to 24 μg mL^{-1}. The maximum absorbance at 520 or 460 nm in Method A or Method B was observed at 2 ml of 1% SCA. In the preliminary investigations for Method B, the reaction showed enhanced sensitivity at pH 6.1. Bases such as NaOH, NH$_3$, hexamine, KOH, and NaOAc were tried in the study. Satisfactory results were obtained with 2 M NaOAc. Hence, 2 ml of 2 M NaOAc was added to bring the pH to 6.1. In both the methods, below and above the optimum limits lesser sensitivity (or low absorbance values) and constant absorbance values were observed, respectively. After the addition of iron(III) it was found that 5 min reaction time was necessary to complete the complexation. The complex obtained was stable for at least 24 h. Hence, these were fixed and used as optimum conditions of the experiments.

3.1.1. Study of the composition of the complex
3.1.1.1. Method A
The composition of the complex was determined by applying Job's continuous variation method [12] at pH 2.25. The composition was established by plotting absorbance in Y-axis and mole ratio of metal in X-axis. The maximum value of absorbance in the curve occurs at a mole ratio of 0.5 (Figure 2) indicating SCA combines with iron(III) in a molar ratio of 1:1.

3.1.1.2. Method B
The limiting logarithmic method [12] was applied to establish the stoichiometry of the iron-SCA complex at pH 6.0-6.2 in Method B. The time dependence of the reaction between iron(III) and SCA was first investigated, and it was found that the reaction proceeds instantaneously. Upon studying the stoichiometric ratio of iron(III) and SCA using a fixed concentration of iron(III) (12 μg mL^{-1}) and SCA (2 ml of 1%) two straight lines with two different slopes (Figure 3) were obtained. The ratio of slopes of two curves was found to be 1:4 and hence the composition between iron(III) and SCA.

3.2. Method Validation
The developed methods were validated for linear dynamic range, precision, accuracy, limit of detection

(LOD) and limit of quantification (LOQ), effect of interferences, application and recovery studies in accordance to the current guidelines to method validation [13].

3.2.1. Linearity
The calibration graphs of Absorbance of complex versus concentration of Fe(III) were found to be linear (Figure 2) for each method, and the related data are presented in Table 1. The calibration graphs followed the linear regression equation:

$$Y = mX + b$$

Where, Y is measured absorbance of 1 cm layer of solution, b and m are intercept and slope of the curve, respectively. For each value of Y, the concentration of Fe(III) in μg mL^{-1}, in each method were calculated. The performance characteristics such as Beer's law limits, molar absorptivity (ε), Sandell's sensitivity, LOD, and LOQ were caclulated and presented in Table 1.

3.2.2. Study of intra-day accuracy and precision
Intra- and inter-day variations were checked by subjecting three different concentrations of Fe(III) for assay 5 times each on the same day and 3 different days, respectively. The amount of Fe(III) obtained, relative error (%), and relative standard deviation (%) values were calculated. The results of these studies are presented in Table 2.
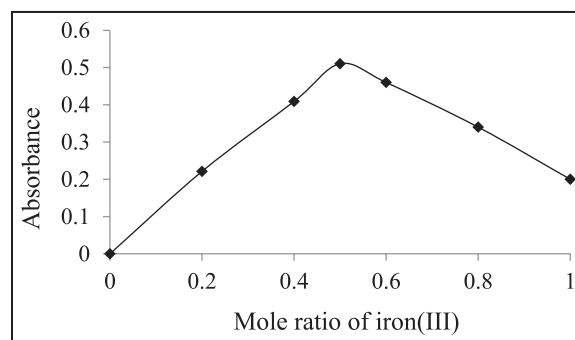

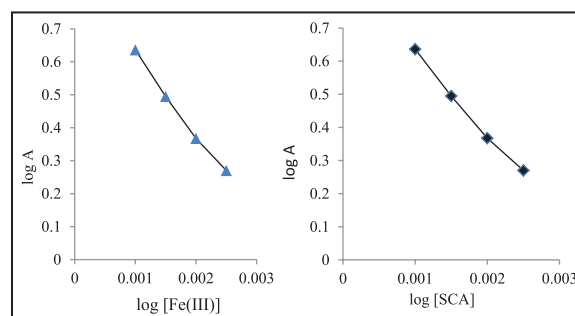**Figure 2:** Job's continuous variations plot for Method A.

Figure 3: Limiting logarithmic plots for Method B.

Table 1: Sensitivity and regression parameters.

Parameter	Method A	Method B
Linear range, $\mu g\ mL^{-1}$	3.75-37.5	2.0-24
Molar absorptivity (ε), $L\ mol^{-1}\ cm^{-1}$	1.71×10^3	3.014×10^3
Limit of detection (LOD), $\mu g\ mL^{-1}$	0.72	0.44
Limit of quantification (LOQ), $\mu g\ mL^{-1}$	2.19	1.34
Intercept (b)	$6.82\times10\text{-}3$	4.99×10^{-2}
Slope (m)	0.0302	0.0256
Regression coefficient	0.9999	0.9988

LOD: Limit of detection, LOQ: Limit of quantification

Table 2: Intra- and inter-day accuracy and precision.

Method	Fe (III) taken, $\mu g\ mL^{-1}$	Inter-day accuracy and precision			Intra-day accuracy and precision		
		Fe(III) found*, $\mu g\ mL^{-1}$	RE, %	RSD, %	Fe(III) found*, $\mu g\ mL^{-1}$	RE, %	RSD, %
A	15.0	14.64	2.27	1.13	15.08	0.53	2.09
	22.5	21.25	5.56	1.34	21.69	3.60	2.00
	30.0	29.30	2.33	0.98	29.70	1.00	1.77
B	8.0	8.20	2.50	2.11	8.05	0.63	1.88
	12.0	12.30	2.50	3.21	12.33	2.75	3.11
	16.0	15.81	1.19	1.98	16.06	0.34	2.88

*Mean value of five measurements. RE: Relative error, RSD: Relative standard deviation

Table 3: Results of assay of iron(III) in pharmaceuticals and statistical comparison with reference method

Tablet brand and nominal amount	Found* (Percent of label claim±SD)		
	Reference method	Method A	Method B
Orofer-XT (100 mg of Fe(III)/tablet)	102.1±1.05	100.6±1.26 t=2.05 F=1.44	101.8±0.88 t=0.49 F=1.42

*Mean value of five determinations. Tabulated t-value at the 95% confidence level and for four degrees of freedom is 2.77. Tabulated F-value at the 95% confidence level and for four degrees of freedom is 6.39. SD: Standard deviation

3.2.3. Study of interferences

The proposed procedures were applied to study various cationic, anionic, and organic interferences. This was done by spiking a known quantity of the above adjuvants into the solution of iron(III)-SCA complex. The adjuvants added to study the other ions interference were Hg(II), Fe(II), Pb(II), Cd(II), Na^+, K^+, Zn^{2+}, Ca^{2+}, Mg^{2+}, Sn^{2+}, NO_3^-, NO_2^-, CO_3^{2-}, SCN^-, oxalate, and citrates. It was revealed by the unchanged absorbance of the Fe(III)-SCA complex at respective absorption maxima that no influence by these foreign ions to the progress of the complexation reaction.

3.2.4. Application of the proposed methods to analysis of iron(III) in curry leaves, drumstick leaves, and mustard seeds

The developed methods were successfully applied to determine the amount of Fe(III) in curry leaves, drumstick leaves, and mustard seeds. The results obtained were satisfactory and comparable with those

of standard values.

3.2.5. Application of the proposed method to analysis of iron(III) in pharmaceuticals

The assay was applied for estimation of iron(III) in Orofer-XT tablets. Obtained results were compared statistically with those of reference method (Sun and Lu, 2006). The accuracy and precision of the proposed methods were checked by performing Student's t-test and variance ratio F-test, respectively. The results in Table 3 revealed that the excipients in tablets did not show interference to the assay.

3.2.6. Recovery study

Recovery experiment was performed by applying the standard addition technique to assess the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed iron(III) tablet solution with pure Fe(III) at three different levels (50%, 100%, and 150% of the content present in the

Table 4: Results of recovery study *via* standard addition method.

Tablets studied	Method A				Method B			
	Fe(III) in tablet, $\mu g\ mL^{-1}$	Pure Fe(III) added, $\mu g\ mL^{-1}$	Total Fe(III) found, $\mu g\ mL^{-1}$	Pure Fe(III) recovered (Percent±SD*)	Fe(III) in tablet, $\mu g\ mL^{-1}$	Pure Fe(III) added, $\mu g\ mL^{-1}$	Total Fe(III) found, $\mu g\ mL^{-1}$	Pure Fe(III) recovered (Percent±SD*)
Orofer-DT	10.0	5.0	14.96	99.2±1.11	8.0	4.0	11.94	98.6±1.56
	10.0	10.0	20.04	100.4±1.35	8.0	8.0	15.97	99.6±1.24
	10.0	15.0	25.29	101.9±1.32	8.0	12.0	19.86	98.8±1.22

*Mean value of three determinations. SD: Standard deviation

tablet powder), and the total was found by the proposed methods. Each test was repeated 3 times. The results of this study are presented in Table 4. The percentage recovery values for Fe(III) in the range from 96.7% to 101% indicates the fairly good accuracy of the methods.

4. CONCLUSION
Two visible spectrophotometric methods are proposed to determine iron(III) using SCA as chromogenic agent. These methods were applied to determine iron(III) in pure form, curry leaves, drumstick leaves, and mustard seeds. The methods have also been extended to application to pharmaceuticals. The proposed methods are free from common interferences such as cations, anions, and organic ions. The methods are very simple to operate and cost-effective. The assay procedures are validated as per the standard guidelines. The accuracy and precision of the methods are excellent when compared with other reported methods with respect to methodology, procedure, reagents, performance characteristic features, etc. Hence, these methods can be used as standard methods for determining iron(III) in its pure form, pharmaceuticals, and in any natural samples.

5. ACKNOWLEDGMENT

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***Bibliographical Sketch**

The corresponding author has graduated from the department of Studies in Chemistry, Manasagangothri, University of Mysore, Mysuru, India, during 2003 to 2005. The author did his Ph.D in the same department during 2008 to 2011. The author has 8 years of post-graduate teaching experience. His current research areas of interest are Analytical Chemistry, Electrochemistry, Bio-analytical Chemistry and Pharmaceutical chemistry. The author has published 62 research articles in various national and international journals of repute. The present H-index of author is found to be 13. The author is a Principal Investigator for research project on chemo sensors which is being funded by UGC, New Delhi, India. The author has guided 52 Masters students for their dissertation/project work.