



## Eugenol [2-Methoxy-4-allylphenol (MAP)] a colorimetric sensing probe for selective determination of Iron(III)

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### Abstract

A simple, precise, sensitive, selective and non-extractive spectrophotometric method is developed for the determination of Fe(III) by using Eugenol [2-Methoxy-4-allylphenol (MAP)] as a chromogenic ligand. The present method is based on instantaneous formation of a green blue color complex of Fe(III) with MAP in a neutral alcoholic medium at room temperature. The MAP forms 1:3 complex with Fe(III) which is stable for 48 hours. The complex exhibits maximum absorption of visible spectrum at 653 nm with molar absorptivity  $0.946 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$  and Sandell's sensitivity  $0.05917 \mu\text{g cm}^{-2}$  of Fe(III). Beers law is found to be valid in the concentration range of  $1 \mu\text{g cm}^{-3}$  to  $60 \mu\text{g cm}^{-3}$ . The standard deviation for ten replicate samples at  $40 \mu\text{g cm}^{-3}$  level of Fe(III) is estimated to be 0.14. The detection limit is  $0.149 \mu\text{g cm}^{-3}$ . MAP shows excellent sensitivity and selectivity for  $\text{Fe}^{+3}$  over other metal ions such as  $\text{Mn}^{+2}$ ,  $\text{Mo}^{+6}$ ,  $\text{Cd}^{+2}$ ,  $\text{Pb}^{+2}$ ,  $\text{Ni}^{+2}$ ,  $\text{Zn}^{+2}$ ,  $\text{Hg}^{+2}$ ,  $\text{Au}^{+3}$  and  $\text{Co}^{+2}$  which permits determination in presence of large number of foreign ions. The present method developed works in neutral and alcoholic conditions and has been employed successfully as an analytical tool for determination of Fe(III) from synthetic mixtures and pharmaceutical preparations.

## 1. Introduction

Iron is one of the supreme trace elements existing in living cells and plays a crucial role in number of biological processes and human health [1-5]. It is involved in large array of key metabolic processes such as oxygen carrier (haemoglobin) [6], oxygen storage (myoglobin) [7], electron carrier (cytochromes) [8], electron transfer (ferredoxins) [9] and deoxyribosenucleic acid (DNA) synthesis. It is also involved in enzymes like catalase, peroxidase, aldehyde oxidase and nitrogenase [10]. The deficiency and excess of Iron than the normal limits leads to physiological disorders and may develop Alzheimer's disease [11], Anemia [12] and hemochromatosis [13].

Hence, rapid, selective and quantitative detection of Iron(III) is of substantial significance in the domain of medical, clinical and environmental concerns. Several analytical techniques such as Atomic absorption spectroscopy (AAS), Fluorometry, Inductively Coupled Plasma Mass Spectrometry (ICPMS) and Voltammetry are utilized in detection of Iron(III) [14-16]. These techniques require sample treatment, availability of sophisticated instrumentation and complexity of operation. However colorimetric method is most simple, sensitive and easily accessible technique. Colorimetric chemosensors, fluorescent chemosensors, chromogenic ligands and molecular probes are available for

detection of Iron(III) [17-21]. But developing a simple, sensitive, selective and readily available chemosensor is challenging, interesting and essential area of research in metal ions detection.

Wide variety of fluorescent chemosensors such as rhodamine b derivative [22], diphenylfluorenes with phosphonic acid [23], squaraine dye [24], nitrogen-doped carbon dots [25] are reported so far for the detection of Iron(III). The fluorescent chemosensors are found to be sensitive and selective but requires sophisticated and expensive instruments. Colorimetric chemosensors proves to be most precise, versatile and simple probes for color change visualized simply by naked eye for metal ion detection. The present work explores the utility of readily and naturally available Eugenol [2-Methoxy-4-allylphenol (MAP)] as highly sensitive and selective colorimetric chemosensor for determination of Iron(III) in alcoholic media under neutral conditions at room temperature. The present work has an edge to use Eugenol [2-Methoxy-4-allylphenol (MAP)] as chemosensor for Iron(III) is that it do not require complex experimental setup and tiresome methods of synthesis as that of other chemosensors in contrast to other known methods [26]. MAP detects Iron(III) in neutral alcoholic media with distinct color change from colorless to green blue (Figure 1). The color response of MAP with different metal ions such as  $Mn^{+2}$ ,  $Mo^{+6}$ ,  $Cd^{+2}$ ,  $Pb^{+2}$ ,  $Ni^{+2}$ ,  $Fe^{+3}$ ,  $Zn^{+2}$ ,  $Hg^{+2}$ ,  $Au^{+3}$  and  $Co^{+2}$  in neutral alcoholic media at room temperature is represented in (Figure 2). Eugenol a natural phenol is extracted from cloves and magnoliae flos and has broad spectrum of applications in medical field [27-31].



**Figure 1.** A) Color Response of MAP with  $Fe^{+3}$  ions in alcoholic medium at ambient temperature. B) Color Response of MAP in absence of  $Fe^{+3}$  ions in alcoholic medium at ambient temperature (Blank).



**Figure 2.** Color Response of MAP with different metal ions in alcoholic medium at ambient temperature , A)  $Mn^{+2}$ , B)  $Mo^{+6}$ , C)  $Cd^{+2}$ , D)  $Pb^{+2}$ , E)  $Ni^{+2}$ , F)  $Fe^{+3}$ , G)  $Zn^{+2}$ , H)  $Hg^{+2}$ , I)  $Au^{+3}$ , J)  $Co^{+2}$ .

## 2. Materials and Methods

### 2.1 Reagents and Solution

In the present method the reagents used were of analytical reagent (AR) grade. The ethanolic standard stock reagent solution of Fe(III) was made available by weighing a 2.904 gm of solid ferric chloride

reagent in 50 to 60 ml ethanol and then finally diluted to 1000 ml in volumetric flask by solvent ethanol. 200  $\mu\text{gcm}^{-3}$  Fe(III) standard working reagent solution was produced from stock reagent solution by utilizing ethanol as solvent. To explore the tolerance limit of various foreign ions in determination of Fe(III), their standard stock reagent solution were made available by weighing and dissolving their salts in ethanol and distilled water mixture or dilute acids. A ferric ion sensor Eugenol [2-Methoxy-4-allylphenol (MAP)] reagent was purchased from Spectrochem PVT. LTD, Mumbai, India and used as such. The boiling point of Eugenol was conducted and found to be in the range of 253-255 $^{\circ}\text{C}$ .

## 2.2 Instruments and Apparatus

For absorbance measurement an Elico make UV-Visible double beam spectrophotometer of Model SL-210 was utilized with matching 1cm quartz cells. Electronic balance Contech make CA123 was employed for weighing purpose. All the glassware's employed in this method were calibrated and thoroughly cleaned. All the glassware's were cleaned by immersing in acidified solution of potassium dichromate and then by washing with liquid soap followed by washing properly with distilled water.

## 2.3 Recommended procedure for determination

An aliquot of Fe(III) solution with concentration 200  $\mu\text{gcm}^{-3}$  was added to graduated 10 ml volumetric flask. To the same flask 2 ml of 0.5M MAP in ethanol was added and final volume of 10 ml was achieved by adding ethanol. A green blue color [Fe(III) - MAP] complex was formed immediately at room temperature conditions without any standing time. The absorbance of was noted at  $\lambda_{\text{max}}$  653 nm on UV-Visible double beam spectrophotometer.

## 3. Results and Discussion

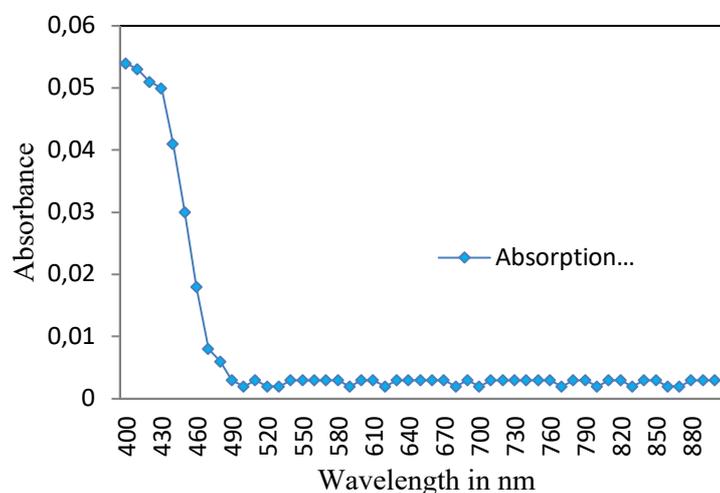
Our current research strategies focus on developing and synthesizing colorimetric chemosensor for efficient, selective and sensitive détermination of métal ions [32-38]. We have introduced Eugénol [2-Methoxy-4-allylphenol (MAP)] as a naked eye colorimetric chemosensor for Fe(III). MAP merits for remarkable selectivity, sensitivity and simple expérimental conditions for détermination of Fe(III). The added advantage of MAP is its readily available nature. The spectrophotometric detection of Fe(III) by MAP was carried out in ethanolic media under neutral conditions at room température. Eugénol [2-Methoxy-4-allylphenol (MAP)] has -OH and -OCH<sub>3</sub>, probable donor sites which may potentially bind with Fe(III) to form green blue complex. Different métal ions were tested with MAP under varying conditions to examine its potential sensitivity and selectivity for colorimetric complex formation. It exhibits remarkable selectivity and sensitivity for Fe(III) against number of different métal ions. The Fe(III) forms a green blue colored 1:3 [Fe(III) - MAP] complex with MAP in neutral ethanolic media. The absorption spectrum of complex shows maximum absorbance at 653 nm and the formed complex is stable for 48 hours. The best conditions have been set up for the détermination of Iron(III) after study of different détermination variables such as standing time, outcome of different acids, MAP concentration and interference study of diverse foreign ions. Stoichiometry of [Fe(III) - MAP] complex is 1:3 as ascertain by Job's method of continuous variation method and Mole ratio method. The spectral, statistical and physico-chemical characteristic figures are reported in **Table 1**.

### 3.1 Absorption Spectra

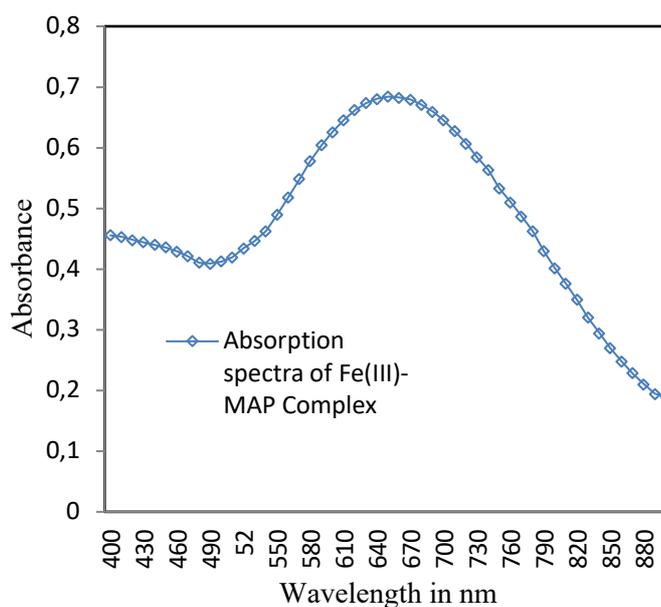
The absorption spectra of MAP in ethanol highlights a negligible absorbance at 653 nm. (**Figure 3**) and the absorption spectra of [Fe(III) - MAP] complex display maximum absorbance at 653 nm (**Figure 4**). Consequently the absorbance measurements were carried out at 653 nm.

**Table 1.** Statistical, Physico-Chemical and Spectral characteristic

Entry	Parameter	Value
1	MAP solvent	Ethanol
2	MAP concentration	0.5M
3	Medium	Alcoholic (Ethanol)
4	$\lambda_{\max}$	653 nm
5	Molar absorptivity	$0.946 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$
6	Sandell's sensitivity	$0.05917 \mu\text{g of Fe(III) cm}^{-2}$
7	Beers law	$1 \mu\text{g cm}^{-3} \text{ to } 60 \mu\text{g cm}^{-3}$
8	Stoichiometry	1:3
9	Limit of detection	$0.149 \mu\text{g cm}^{-3}$
10	Standard deviation	0.14
11	Stability	48 hrs
12	Pearson Correlation coefficient	1.00
13	Regression equation	$y = 0.0172x - 0.0062$



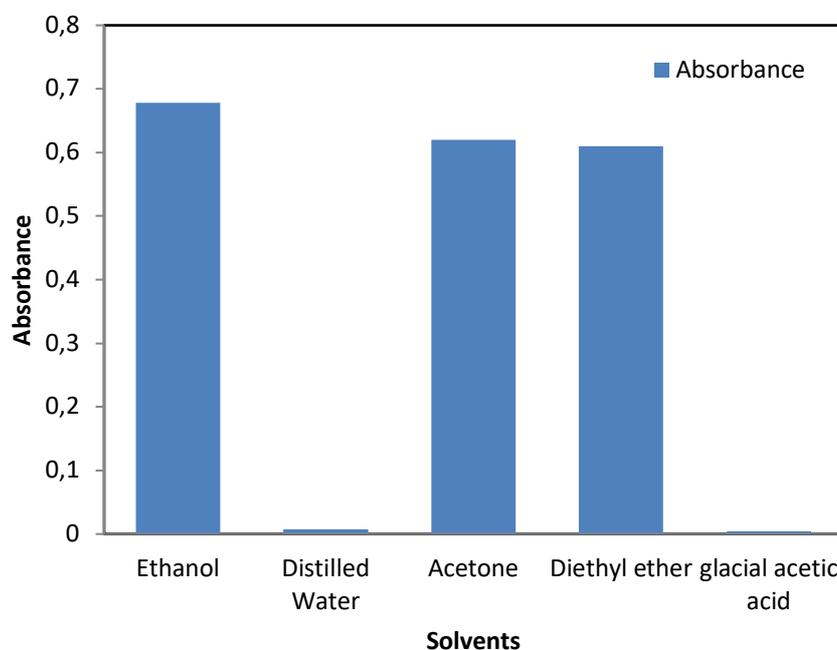
**Figure 3.** Absorption spectra of MAP



**Figure 4.** Absorption spectra of [Fe(III) - MAP] complex for  $50 \mu\text{g cm}^{-3}$  of Fe(III)

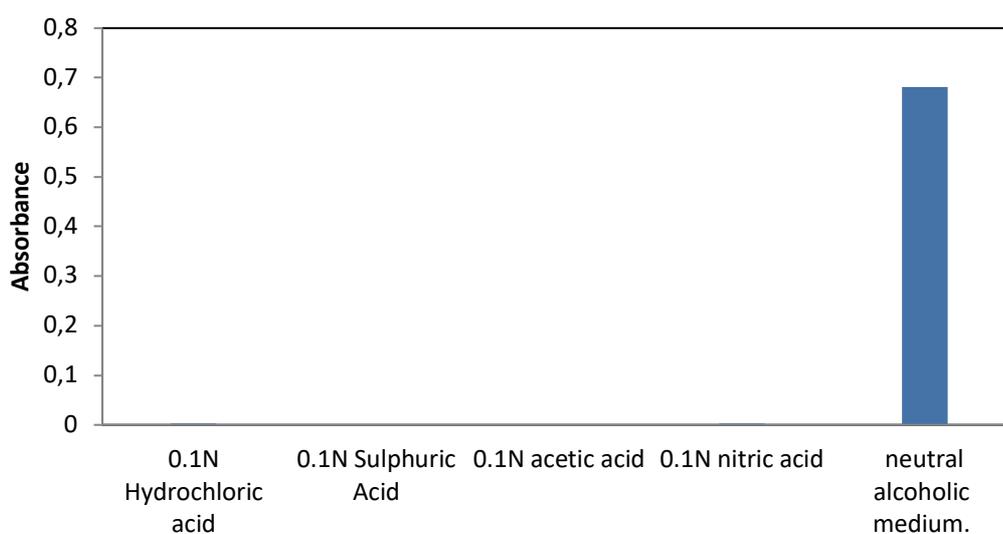
### 3.2 Consequence of solvents and acids

In order to optimize best conditions for [Fe(III) - MAP] complex formation, the outcome of different solvents such as ethanol, acetone, diethyl ether, glacial acetic acid and distilled water was studied. The experimental study reveals that the maximum complex formation takes place in presence of solvent ethanol as compared to acetone and diethyl ether. In glacial acetic acid and distilled water a negligible amount of complex formation take place as outline in **Figure 5**.



**Figure 5.** Effect of different solvents on [Fe(III) - MAP] complex formation

The outcome of different acids was studied for complex formation in present method. It was recognized that negligible amount of complex formation takes place in 0.1N hydrochloric acid, 0.1N sulphuric acid, 0.1N acetic acid, 0.1N nitric acid. The maximum complex formation takes place in non acidic neutral alcoholic medium, **Figure 6**.



**Figure 6.** Effect of different acids in [Fe(III)- MAP] complex formation

### 3.3 Effect of standing period, sequence of addition and stability of complex

When 2 ml solution of 0.5M MAP in ethanol is added to an aliquot of Fe(III) in ethanol a green blue color [Fe(III) - MAP] complex formation takes place instantly with no standing time at room temperature. The complex is stable for 48 hour. The sequence of addition of MAP and Fe(III) solution has no predictable effect on complex formation.

### 3.4 Standard Analytical figures

The acceptable concentration range for Beer's law is  $1 \mu\text{g cm}^{-3}$ -  $60 \mu\text{g cm}^{-3}$  (Figure 7). The estimated values of molar absorptivity and sandells sensitivity of the complex are  $0.946 \times 10^3 \text{ Lmol}^{-1}\text{cm}^{-1}$  and  $0.05917 \mu\text{g cm}^{-2}$  of Fe(III) respectively. The pearson correlation coefficient value with a independent variable as Iron(III) concentration in  $\mu\text{g cm}^{-3}$  and dependent variable as absorbance of [Fe(III) - MAP] complex is found to be 1.0 which stipulate the best linearity between these both variables.

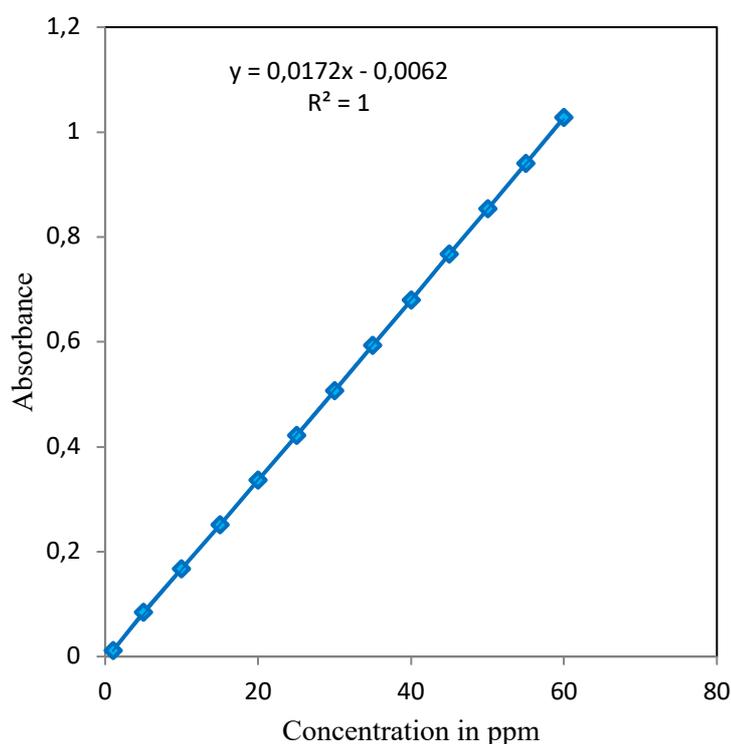


Figure 7. Beer's law plot of [Fe(III)- MAP] complex

### 3.5 Elucidation of Stoichiometry of complex

Job's method of continuous variation and Mole ratio method were employed to elucidate the Stoichiometry of [Fe(III) - MAP] complex. The results of both methods corroborate 1:3 Stoichiometry of [Fe(III) - MAP] complex.

#### 3.5.1 Job's method of continuous variation

In Job's method a set of sample solution containing 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 ml of Iron(III) ( $1 \times 10^{-3} \text{ mol L}^{-1}$ ) in ethanol was prepared in each 10 ml volumetric flask then alcoholic solution of MAP ( $1 \times 10^{-3} \text{ mol L}^{-1}$ ) was added in each volumetric flask as 9, 8.5, 8, 7.5, 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, 1 ml respectively. The absorbance of each solution in flask was measured at 653 nm. The Stoichiometry of [Fe(III) - MAP] complex was found to be 1:3 as indicated by Job's plot Figure 8.

### 3.5.2 Mole ratio method

The Fe(III)  $1 \times 10^{-2} \text{M}$  (1 ml) concentration is maintained constant and the MAP concentration is assorted by maintaining other conditions similar as mentioned in procedure to obtain different mole fractions of Fe(III). The measurement of absorbance is done at 653 nm, the unambiguous break in the graph (Figure 9) analogous to mole ratio 3 indicates, 1:3 stoichiometry of [Fe(III) – MAP] complex.

Fe(III) concentration -  $1 \times 10^{-2} \text{M}$  (1 ml)

MAP concentration -  $1 \times 10^{-2} \text{M}$  (variable)

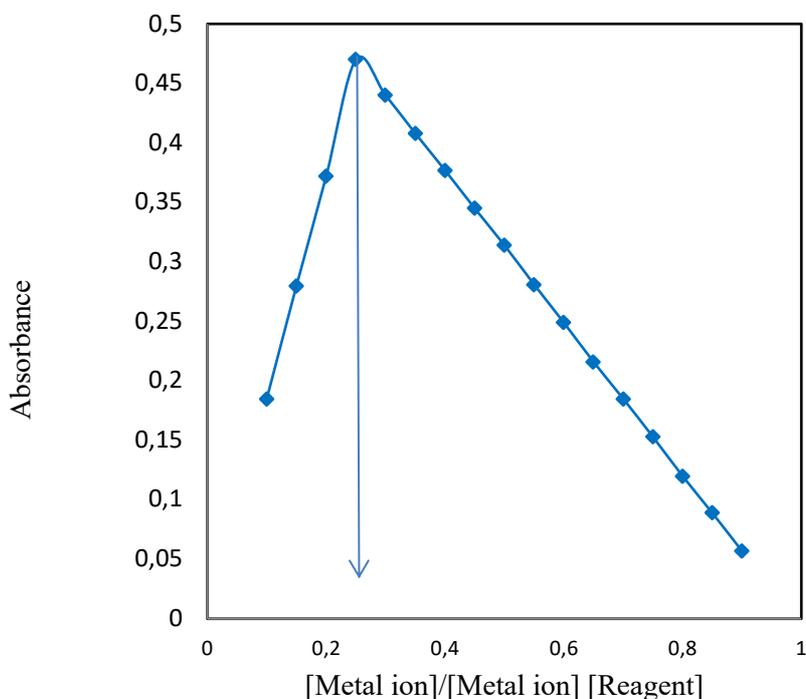


Figure 8. Jobs method of continuous variation for [Fe(III) - MAP] complex

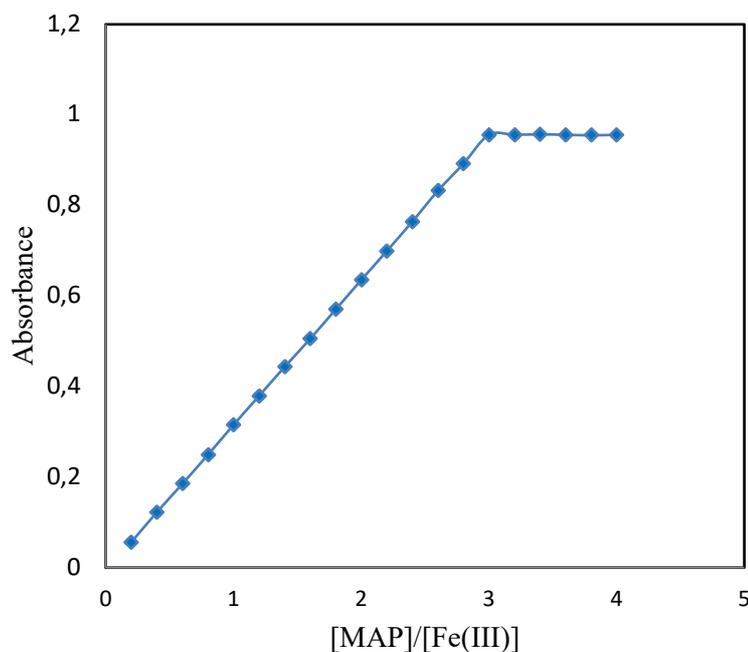


Figure 9. Mole ratio method for [Fe(III) - MAP] complex.

### 3.6 Detection limit and accuracy

To check the precision and accuracy of the results of the present method, absorbance of 10 similar sample solutions containing  $40 \mu\text{g cm}^{-3}$  Iron (III) was noted. Standard deviation value was calculated and found to be 0.14 which reflects good precision and accuracy of the current method. The detection limit of Fe(III) in current method is  $0.149 \mu\text{g cm}^{-3}$ .

### 3.7 Interference Study

For the analytical application of MAP, the effect of foreign was determined by adding known and variable amount of foreign ion in  $40 \mu\text{g cm}^{-3}$  Iron(III). Most of the foreign ions shows higher tolerance limit and do not interfere in determination of Iron(III). The tolerance limit of different foreign ions is presented in **Table 2**.

**Table 2.** Tolerance limit of different foreign ions in determination of  $40 \mu\text{g cm}^{-3}$  Iron(III) with MAP.

Foreign ions	Added as	Tolerance Limit $\mu\text{g/ml}$	Foreign ions	Added as	Tolerance Limit $\mu\text{g/ml}$
Mn(II)	$\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$	350	Al(III)	$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	260
Ba(II)	$\text{BaCl}_2 \cdot 6\text{H}_2\text{O}$	335	Co(II)	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	450
Ti(III)	$\text{Ti}_2(\text{SO}_4)_3$	360	Ni(II)	$\text{Ni}(\text{NO}_3)_2$	330
Au(III)	$\text{HAuClO}_4 \cdot \text{H}_2\text{O}$	380	Fe(II)	$\text{FeSO}_4$	150
Sn(II)	$\text{SnCl}_2 \cdot 6\text{H}_2\text{O}$	400	Citrate	$\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$	850
Hg(II)	$\text{HgCl}_2$	390	Iodide	KI	650
Pb(II)	$\text{Pb}(\text{NO}_3)_2$	390	Bromide	KBr	480
Cd(II)	$\text{CdCl}_2 \cdot 6\text{H}_2\text{O}$	370	Chloride	KCl	560
Cr(III)	$\text{CrCl}_3$	200	Sulphate	$\text{Na}_2\text{SO}_4$	500
Ru(III)	$\text{RuCl}_3 \cdot 6\text{H}_2\text{O}$	320	Nitrate	$\text{KNO}_3$	740
Sb(III)	$\text{Sb}_2\text{O}_3$	320	Acetate	$\text{CH}_3\text{COONa}$	360
Zn(II)	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	320	Urea	Urea	320
Se(IV)	$\text{SeO}_2$	295	Thiourea	Thiourea	300
Mo(VI)	$(\text{NH}_4)_5\text{MO}_7 \cdot 2\text{H}_2\text{O}$	280	Thiosulphate	$\text{Na}_2\text{S}_2\text{O}_3$	240
Mg(II)	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	280	Oxalate	$\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$	270
Ir(III)	$\text{IrCl}_3$	270	EDTA	$\text{Na}_2\text{EDTA}$	90
Rh(III)	$\text{RhCl}_3$	200	Malonate	$\text{CH}_2(\text{COONa})_2$	320

## 4. Applications

To explore the prospectus of current method, Iron(III) was determined from different samples such as pharmaceutical preparations and synthetic mixtures by MAP.

### 4.1 Estimation of Iron(III) from Ferroclix Syrup

A pharmaceutical preparation, Ferroclix Syrup contains Iron (III) hydroxide polymaltose complex (IPC). It is used in prevention and treatment of iron deficiency anemia. The amount of Iron(III) determined by present method is given in **Table-3**.

**Table 3.** Estimation of Iron(III) from Ferroclix Syrup

Composition in mg	Amount of Iron(III) in mg		Standard deviation	Relative standard deviation %
	Certified value	Found value*		
Fe(III) 50	50	49.83	0.19	0.36

#### 4.2 Estimation of Iron(III) from binary synthetic mixtures

A series of binary synthetic mixtures of Iron(III) and different transition metal ions were formulated in the ratio 3:1 (Fe(III) : Metal ion). From these number of synthetic mixtures, amount of Iron(III) was estimated with good effectiveness by present method as shown in **Table 4**.

**Table 4.** Estimation of Iron(III) from binary synthetic mixtures

Composition in µg	Found*	Standard deviation	Relative standard deviation %
Fe(III) 300, Mn(II) 100	299.53	0.53	0.18
Fe(III) 300, Au(III) 100	299.54	0.52	0.17
Fe(III) 300, Zn(II) 100	299.54	0.51	0.16
Fe(III) 300, Ti (III) 100	299.50	0.56	0.19
Fe(III) 300, Pb (II) 100	299.55	0.50	0.17
Fe(III) 300, Hg (II) 100	299.56	0.50	0.17

#### 4.3 Estimation of Iron(III) from ternary synthetic mixtures

Ternary synthetic mixtures of Iron(III) with different transition metal ions were formulated in the ratio 3:1:1 (Fe(III) : Metal ion A : Metal ion B). From these number of synthetic mixtures, amount of Iron(III) was estimated with good effectiveness by present method as shown in **Table 5**.

**Table 5.** Estimation of Iron(III) from ternary synthetic mixtures.

Composition in µg	Found*	Standard deviation	Relative standard deviation %
Fe(III) 300, Pb(II) 100, Sn(II) 100	299.51	0.55	0.18
Fe(III) 300, Mn(II) 100, Cr(II) 100	299.54	0.51	0.16
Fe(III) 300, Hg(II) 100, Mn(II) 100	299.50	0.56	0.19
Fe(III) 300, Zn(II) 100, Au(III) 100	299.52	0.54	0.18
Fe(III) 300, Ba(II) 100, Ti(III) 100	299.53	0.53	0.18

### 5. Conclusion

Eugenol [2-Methoxy-4-allylphenol (MAP)] showed remarkable sensitivity and selectivity for Iron (III) over a number of other metal ions under study. MAP acts as a colorimetric sensing probe for Iron(III) by forming a stable green blue color in neutral and alcoholic media. The present method potentially utilizes a naturally available eugenol (MAP) with advantage of avoiding tedious and time consuming methods of synthesis as compared to other reagents for Iron(III). The method was utilized for determination of Iron(III) from pharmaceutical preparations and synthetic mixtures with variable composition with good efficacy.

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#### Disclosure statement:

##### *Conflict of Interest:*

The authors declare that there are no conflicts of interest in the present research work.

#### *Compliance with Ethical Standards:*

This article does not contain any studies involving human or animal subjects.

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