Spectrophotometric Study and Analytical Applications of Iron (III)-Chelate System with (Mordant Blue-9) Reagent in Aqueous Solution

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Abstract: The reagent 6-(5-chloro-2-hydroxy-3-sulfophenylazo)-5-hydroxy-1-naphthalene sulfonic acid (Mordant blue – 9) is proposed as a spectrophotometric reagent for the determination of trace amounts of iron (III). The proposed chromogenic reagent is sensitive and reacts with iron to form a stable brown 1:2 (Fe (III) – reagent) complex at pH 3.75, Kst = 9.824*109 L^2.mol^{-2}. The apparent molar absorptivity of this complex was 16000 l .mol $^{-1}$ cm^{-1} at 650 nm in aqueous solutions. Beer's law is obeyed with the range of 6 – 75 µg /25 ml i.e 0.24 – 3 ppm. The reagent is relatively selective for iron III. The importance of this work arises from that it allows quantification and determination of Iron without initial expensive and fatigue separation or extraction procedure. Different analytical validations were determined (accuracy, precision, recovery, stability of the complex, study of interferences, etc.) to demonstrate the suitability of this work for the routine quality control labs. The aim of this work was to determine iron III in aqueous solution specrtophotometrically employing mordant blue-9 reagent. The developed method was applied for the determination of Fe III in urine samples. The results agree with AAS with RSD (±0.74 - ±2.64).

Keywords: Iron (III), Spectrophotometry, Mordant blue -9, Analytical Applications

1. Introduction

Iron was probably first used nearly 6000 years ago. It is the fourth most abundant element in the earth’s crust occurring to the extent of 5.1% (Bailar, et al, 1973).The oxidation state encountered in compounds of this transition element ranges from 0 to +6, however the most common compounds are those Fe(II) and Fe(III) (Holzbecher, et al, 1976). many analytical methods are available and have been proposed for the determination of iron, such as spectrophotometry (Jong, et al, 1998), (Hussain, et al, 2006), (Jafarian-Dehk, 2008), (Taies & Jassam, 2013), (Abdelghafar, et al, 2015), (Ghodvinde, et al, 2017) and atomic absorption spectrometry (Sohrin, et al, 1998), and flow injection analysis (Faizullah &Townshend, 1985), (Elsuccary & Salem, 2015), (Barakat, 2017) and chromatography (Schnell, et al, 1998). The present study program involves a research for more reliable, sensitive and selective reagents which can be used for the spectrophotometric determination of iron. Accordingly, preliminary investigations were performed using reagents of different functional groups. One of these reagents examined (Mordant blue-9) was found worthy of further examination, due to its structure. This work describes and explains the use of MB-9 reagent for the spectrophotometric determination of iron-III in aqueous solution with analytical application.

2. Experimental

2.1. Instruments and apparatus. Spectrophotometric measurements were made with Shimadzu model UV-160, using 1cm glass cell. pH measurements at 25°c are made using Philips PW 9421 pH meter, reading to three decimal. Atomic absorption measurements were made using Perkin-Elmer 2280 A.A.S.

2.2. Reagents: All chemicals used were of analytical reagent grade

2.2.1. The stock standard iron solution (1000 ppm). This solution was prepared by dissolving 0.4838 g of $FeCl_3.6H_2O$ (Fluka) in 100ml distilled water.

2.2.2. The working solution (10 ppm), was obtained by diluting the stock Fe (III) solution. This solution is not stable over time, $FeCl_3$ hydrolyzes in water, the hydrolysis was reversed by adding several drops of HCl.

2.2.3. Mordant Blue-9 ($C_{16}H_{11}O_8N_2S_2Cl$), reagent $1*10^{-3}$ M solution. This solution was prepared by dissolving 0.0251 g of this reagent (Hopkins and William, Ltd.) in 50ml distilled water. This solution is stable at least for one week.

2.2.4. pH 3.75 buffer solution. This solution was prepared by mixing 50 ml of 0.1 M 1.0211 g potassium hydrogen phthalate with 4 ml of 0.01 M HCl and diluted the volume to 100 ml with distilled water (Perrin and Dempsey, 1974).

3. Results and Discussions

3.1. Preliminary investigation. This investigation shows that MB-9 reagent gives a brown chelate with iron III at room temperature, which was stable for several hours and absorbs light at 650 nm, while the reagent absorbs light at 515 nm. This colour contrast ($\Delta\lambda$= 135nm) indicates definite reaction in acidic medium between Fe III and MB-9 reagent.

3.2. Optimization of reaction conditions

Note: All the values present in tables and figures are the average value of at least three determination results.

3.2.1. Effect of pH

To an aliquot of solution containing 1 ml of Fe III working solution, 2 ml of 10^{-3} M MB-9 reagent, various amount of HCl were added, (HCl was chosen because the stock iron solution was prepared by dissolving $FeCl_3.6H_2O$ in distilled water and HCl is simple inorganic acid without any side reactions and effects), then the reaction mixture was diluted to 25ml with distilled water. The absorbance against reagent blanks were measured at 650 nm, and the pH of the final reaction mixture was recorded as shown in Fig. (1). The pH 3.5-4.0 is considered optimum because the colour contrast is good and the absorbance is independent of pH. Basic medium is not recommended for the determination of Fe III due to iron precipitation.

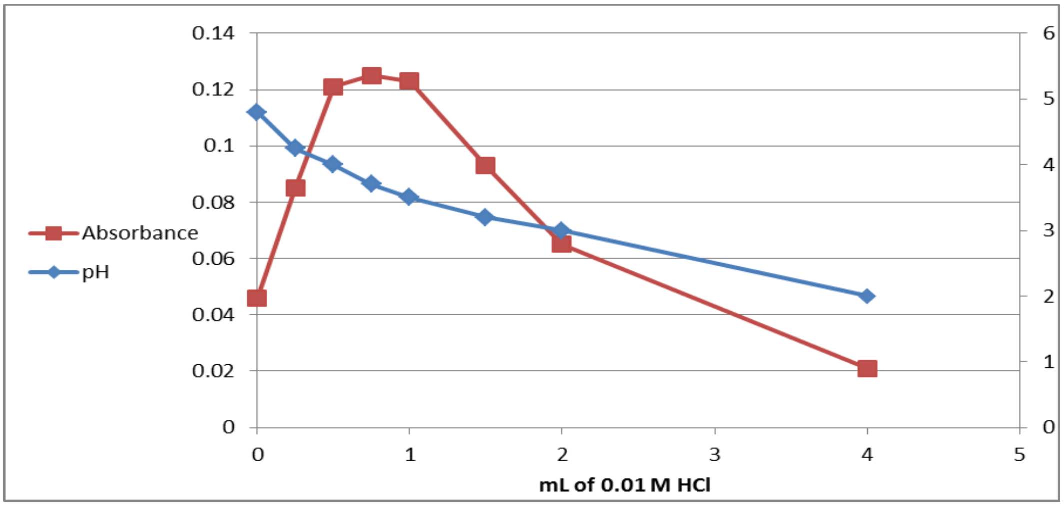

Figure 1: Effect of pH on the absorbance of Fe-MB9

3.2.2. Effect of Buffers. In this respect, buffers of K.H.phthalate 0.1 M, HCl B1,K.H.phosohate 0.1M-HCl B2,KCl 0.2 M-HCl B3,tartaric acid 2 M-NaOH B4,Citric acid NaOH B5,glycine 0.1M-HCl B6,were tried. The effects of these buffers on the absorbance were shown in Fig. (2).

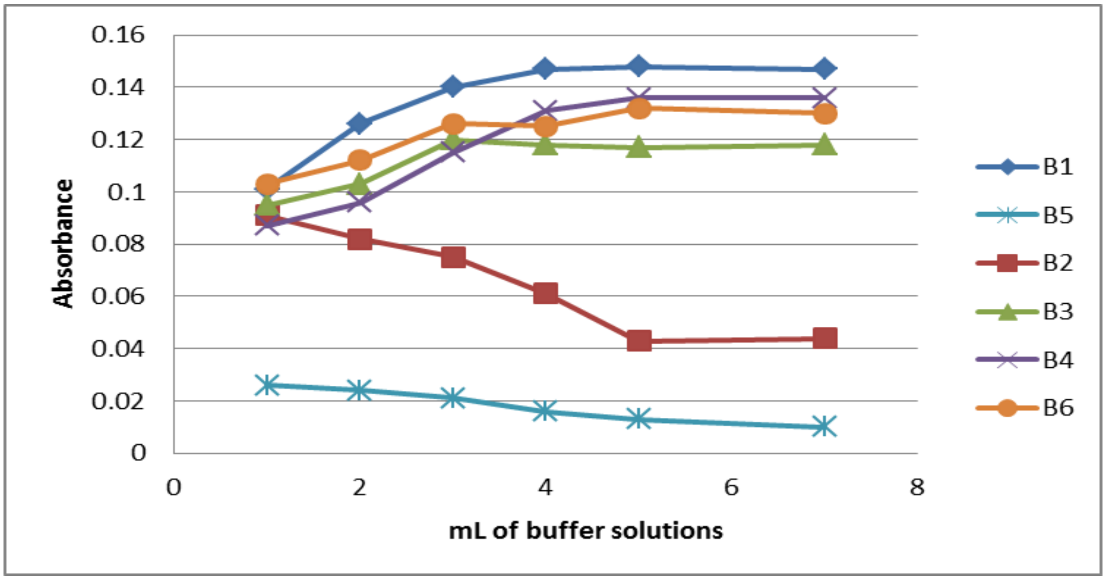

Figure 2: Effects of buffers on the absorbance

A 4 ml of KH- phthalate buffer solution of pH 3.75 was selected for the subsequent experiments because it gives higher capacity and sensitivity than others.

3.2.3. Effect of reagent amount

The influence of the MB-9 reagent amount on the formation of the color is investigated. A 2 ml of the reagent was selected because of the highest sensitivity as shown in Fig. (3).

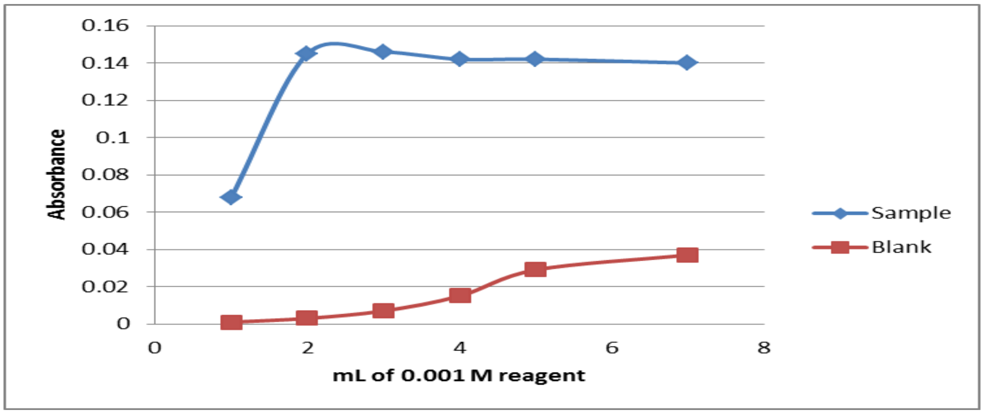

Figure 3: Effect of MB-9 on the absorbance

3.2.4. Effect of time

The reaction between iron III and MB-9 reagent occurs immediately and the absorbance of the complex remains stable. This is good since several determinations can be made at the same time. Fig. (4) shows the results.

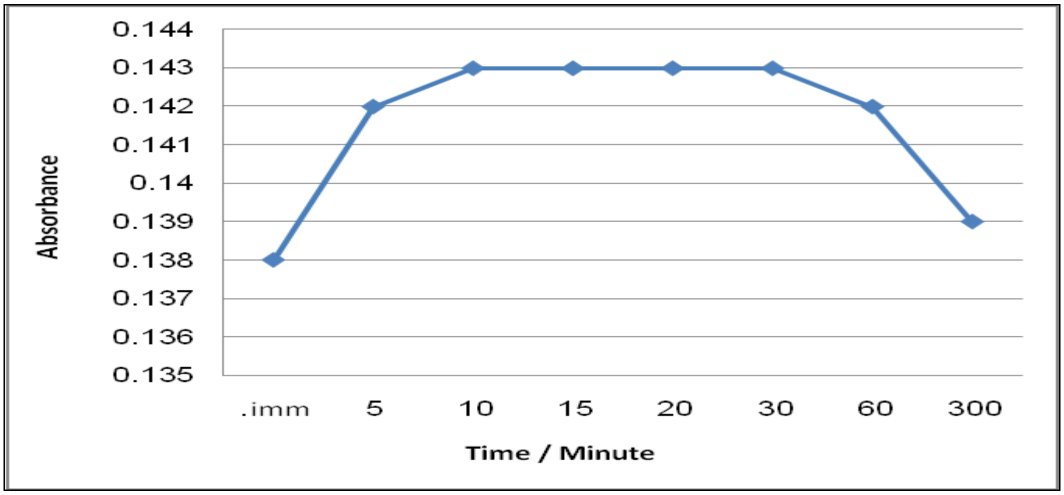

Figure 4: Effect of time

3.2.5. Effect of temperature

The reaction between iron III and reagent was temperature independent as shown in Fig. (5).

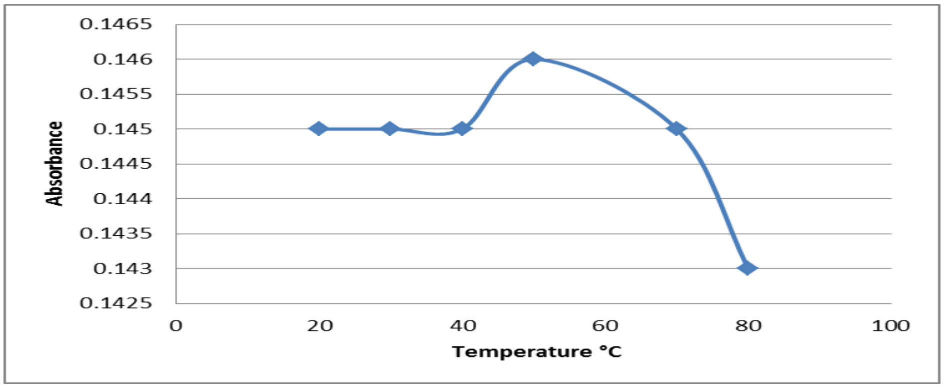

Figure 5: Effect of temperature on absorbance

3.3. Final absorption spectra. When a buffered aqueous solution of iron III ions was mixed with MB-9 reagent at pH 3.75, a characteristic brown colour of binary complex is immediately formed and the absorbance was measured after standing 10 minutes. Fig. (6) shows the absorption spectra of the colored complex with two maxima (This maximum results from the presence of the two interacting hydroxyl groups and coordination of azo group in the chelate molecule) located at 650 nm and 420 nm. The first was applied for the subsequent study treated according to the recommended procedure, but no absorption obtained with the reagent at this wavelength (the reagent absorbs light at λ 515 nm).

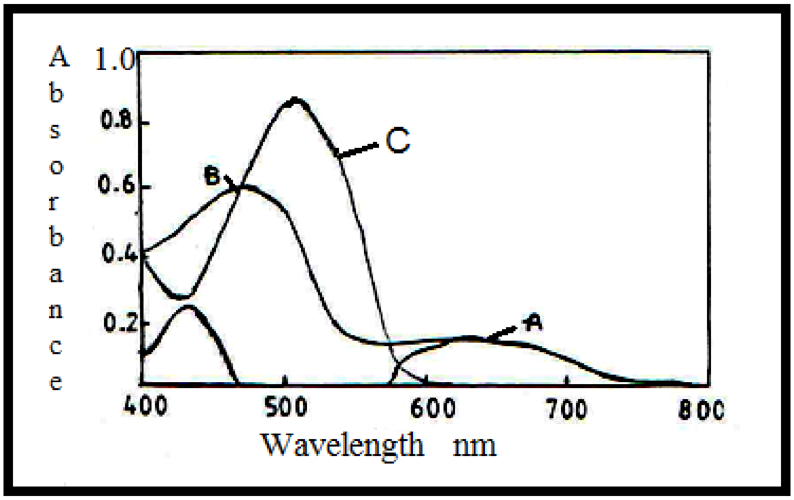

Figure (6): Absorption spectra of the coloured complex in aqueous solution measured

(A) against Blank, (B) against distilled water and (C) blank against distilled water.

3.4. Recommended procedure and calibration graph

To a series of 25 ml volumetric flasks, different amounts of iron 6-100 µg were transferred,4 ml of buffer pH 3.75 solution,2 ml of 10^{-3} M MB-9 reagent were added and the volumes were made to the mark with distilled water. The reaction mixture was mixed and the absorbance was measured at 650 nm after 10 minutes against the reagent blank. A plot of absorbance versus determinant concentrations (Fig. 7) shows that Beer's law was obeyed over the range of 6-75 µg/25 ml, i.e. 0.24-3.0 ppm. The molar absorptivity was calculated to be $1.6*10^{4}$ $l.mol^{-1}.cm^{-1}$, and a Sandell sensitivity index of 0.015 $µg.cm^{-2}$.

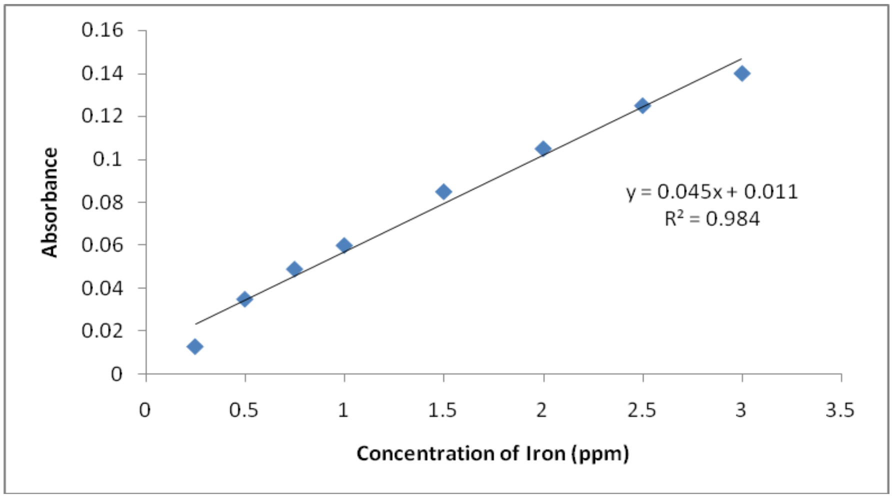

Figure 7: Calibration graph of the complex

3.5. Nature of the complex and stability constant

The composition of the iron (III)-MB-9 chelate had been established by the mole ratio method (Hargis, 1988), Fig. (8).

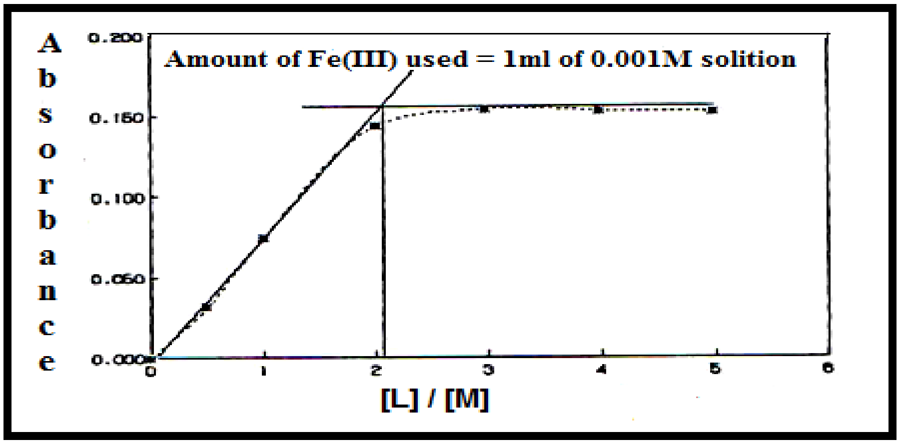

Figure 8: Mole-ratio plot for the Fe-MB-9 chelate

The data reveal that iron III reacts with MB-9 reagent in a 1:2 ratio. The suggested structure of the chelate might be represented as follows Fig(9). The conditional stability constant was estimated using the following formula $K=\frac{1-\alpha}{4C^{2}.\ \alpha^{3}}$ and was found to be $8.824*10^{9}$ $L^{2}.mol^{-2}$ which indicates that the complex is stable.(Hargis.1988).

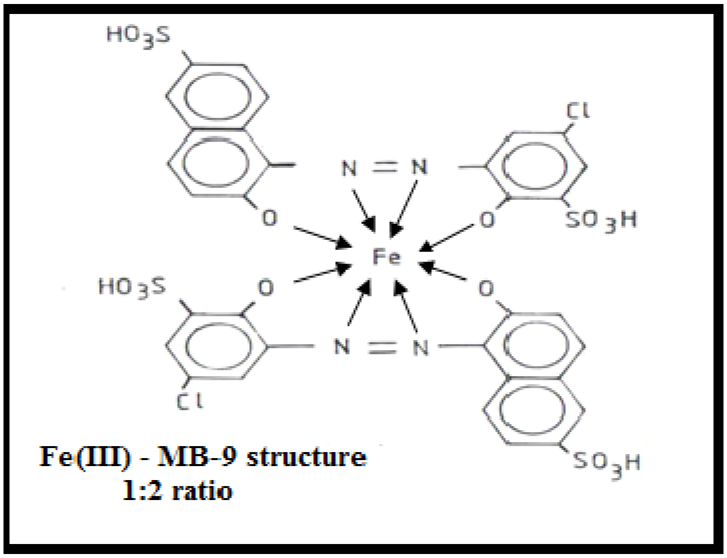

Figure 9: The suggested structure of the complex

3.6. Accuracy and precision of the method

In order to check the accuracy and precision of the method, iron III was determined at four concentrations. The results are shown in Table 1.

Table 1: Accuracy and precision of the method

Amount of Fe(III) taken (µg)	**Recovery* %**	**Relative error* %**	**Relative standard deviation**
10.0	99.2	-0.8	±2.64
25.0	104.8	+4.8	±2.10
35.0	99.43	-0.39	±1.36
50.0	99.6	-0.4	±0.74

*Five determinations.

3.7. Effect of masking agents

In order to enhance the selectivity of the reagent, the effects of several masking agents (**MI solution**) on the colour intensity of iron chelate with MB-9 at pH 3.75 are tested (Wilkinson, 1987). The results are shown in Table 2.

Table 2: Effect of masking agents (M I solution)

Masking agents 0.01M	**Absorbance/mL of masking agents added**		
	0.25	0.5	1.0
1-10-phenanthroline	0.149	0.138	0.125
Ammonium acetate	0.151	0.149	0.148
Ascorbic acid	0.150	0.137	0.127
NTA	0.147	0.134	0.128
Citric acid	0.151	0.149	0.147
Tartaric acid	0.148	0.138	0.130
$NH_2OH.Hcl$	0.149	0.145	0.141
Thiourea	0.150	0.147	0.140
NaF	0.145	0.138	0.129
KI	0.151	0.150	0.148

The results indicated that NTA, 1,10-phenanthroline,ascorbic acid,NaF and tartaric acid caused serious masking action, but acetate and citric acid had little effect on the absorbance, while others had no effect.

3.8. Study of interferences

The effects of various amounts of many foreign ions on the determination of 25µg of iron III per 25 ml solution were examined under the experimental conditions used. Some ions have been found to interfere*. The results are summarized in Table 3.

Table 3: Study of interferences

Foreign ions	**Amount added µg**	**Recovery (%) of 25 µg Fe III ****	
		In absence of M II	**In presence of M II**
Al (III)	200	96	98
Ba (II)	250	98	99
Bi (III)	25	105	101
Ca (II)	200	96	99
Cd (II)	250	95	98
Co (II)	300	95	99
Cr (III)	500	95	98
Cr (VI)	250	96	100
Cu (I)	10	106	101
Cu (II)	25	116	109
Fe (II)	25	108	90
Li (I)	25	95	98
Mg (II)	500	94	98
Mn (II)	500	94	98
Sn (II)	2	95	98
I^-	650	99	98
F^-	25	104	101
$S_2O_3^{-2}$	100	95	96
PO_4^{-3}	100	95	95
Si (IV)	10	96	98
Pb (II)	300	95	98
Ni (II)	250	95	98
Na (I)	250	96	98
SO_4^{-2}	500	99	98
CO_3^{-2}	300	102	100
Zr (IV)	25	105	101

(*) The method suffers serious interferences from Bi III, Cu I, Cu II, Fe,Li, Sn II, Si IV and Zr IV, but the method still stand selective for the determination of iron in various samples as shown later.

(**) M (II) solution : 1 ml of (500 µg 1,10- phenanthroline + 400 µg ascorbic acid + 400 µg F^- + 350 µg tartarate + 500 µg NTA).

3.9. Analytical applications

3.9.1. Determination of iron in urine

20 ml of urine is treated by digestion with 10 ml H_2SO_4 and 10 ml HNO_3. 10 ml $HClO_4$ was then added towards the end of the digestion in order to remove traces of HNO_3, and the solution was heated under reflux with 6N HCl, the volume was completed to 25 ml with distilled water. 1 ml aliquot of the solution was taken for analysis. The results are shown in Table 4.

Table 4: Determination of iron III in urine sample

No.	**Sample**	**Iron content***		**Iron content*** **A.A.S method**
		Present method	**Standard method 1,10 – phenanthroline**	
1.	Human Urine (µg/ml)	3.85	3.4	3.74

* Three determinations

4. Conclusion

Analytical methods based on the measurements of UV or Visible light absorption are considered as one of the most communes used in laboratory practice and commercially available device with low price and easy to operation and control. The developed spectrophotometric method for the determination of Fe III in aqueous solution was described in this work. The method is based on the formation of Fe-MB-9 complex at pH 3.75 and measuring the absorbance at 650 nm. Beer's law was valid between 0.24 – 3.0 ppm Fe. The binary chelate system had a conditional stability constant of $9.824 * 10^9$ $L^2.mol^{-2}$, which indicates that the complex is stable. The present method is considered to be simple because it does not need solvent extraction steps and complications such as hazardous organic solvents and no need for highly sophisticated instrumentation. The proposed method is successfully applied to assay iron in urine samples. The results obtained compared favorably with A.A.S technique.

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