RESEARCH PAPER

Spectrophotometric Determination of Fe(II) and Fe(III) Using 5-(4-Ethylphenyl azo) 2-Hydroxybenzaldehyde Oxime Reagent

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A B S T R A C T

Simple, rapid and sensitive spectrophotometric methods for the determination of trace amounts of iron (II) and iron (III) were developed based on complex formation between 5-(4- Ethylphenyl azo)2-Hydroxybenzaldehyde oxime reagent with iron (II) and iron (III) in acidic medium. The reagent 5-(4-Ethylphenyl azo)2-Hydroxybenzaldehyde oxime has been synthesized and used for iron analysis. The calibration graphs were linear in the concentration range 1.0-6.0 µg/ml and1.0-7.0 µg/ml of iron (II) and iron (III), respectively. The total iron also was determined after reduction of iron (III) to iron (II) employing ascorbic acid solution. The effects of foreign ions and species on the determination of Fe (II) and Fe (III) were investigated to assess the selectivity of the methods. The proposed methods were successfully applied for iron determination in pure form and in pharmaceutical preparation. Good agreement was obtained between the results of the proposed methods and by the reference method.

1.INTRODUCTION

Iron is one of the most abundant metals in our world, it's necessary for most living things, as well as proper human physiology. (Dobrinas et al., 2011, Ensafi et al., 2004, Jha et al., 1996). Many proteins and enzymes that are necessary for optimal health include iron. In human body, iron is an essential component of hemoglobin which is involved in oxygen transport. It is also necessary for the regulation of cell growth and differentiation. Its overload or deficiency may cause health disorders. Iron limits oxygen delivery to cells, resulting in many health problems, but excess amounts of iron can cause toxicity and even death(Derun et al., 2012). In case of clear signs of iron deficiency some medications containing Fe (II) can be given to prevent complications.

There are different kinds of commercially available pharmaceutical products which containing iron, therefore monitoring their qualities are necessary (Balcerzak et al., 2008). It is important to determine trace amounts of iron for public health and environmental protection(Ghosh et al., 2020).

Due to its importance in the context of clinical diagnosis and environmental pollution monitoring many methods for the determination of iron have been reported, such as spectrophotometry(Cheng et al., 2021, Nurchi et al., 2021, Totan et al., 2018, Zannat and Ahmed, 2014), inductively coupled plasma-optical emission spectrometry(Didukh-Shadrina et al., 2019, Proch and Niedzielski, 2021) , atomic absorption spectrometry(Gómez-Nieto et al., 2015, Donnici et al., 2016, Kasa and Bakırdere, 2021) ICP-mass spectrometry(Shariff et al., 2018, Laur et al., 2021) , anodic or cathodic stripping voltammetry(Hassler et al., 2013, Węgiel et al., 2017) chromatography(Frankowski, 2012, Kaasalainen et al., 2016)   cloud point extraction(Durukan et al., 2011, Gondim et al., 2017, Abou El-Reash et al., 2020, Mortada, 2021)

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and flow injection analysis (Xue et al., 2020, Koronkiewicz, 2021) .

Spectrophotometric methods are widely utilized in many laboratories and countries for everyday work due to their simplicity, availability, low cost, and speed thus in the present work, determination of Fe(II) and Fe(III) as well as total iron as azo-oxime complex, in aqueous solution using spectrophotomteric method is reported. The 5-(4-Ethylphenyl azo)2-Hydroxybenzaldehyde oxime is a new ligand was synthesized, characterized and used for Fe(II) and Fe(III) analysis. The method was applied for determination of Fe(II) and Fe(III) in pharmaceutical formulations.

2. MATERIALS AND METODS
All chemicals used were of analytical grade and distilled water was used throughout the experiments.

2.1. Apparatus
A PG instrument T80 + UV-Visible spectrophotometer was used for all spectral and absorbance measurements with 10mm matched quartz cuvettes.

2.2. Reagents
2.2.1. Ferrous and Ferric solutions
A 100 µg/ml of ferrous solution was prepared by dissolving 0.8630 g of $(NH_4)_2$ Fe $(SO_4)_2.12H_2O$ (Fluka) in 0.1 M H_2SO_4 solution in 1000ml volumetric flask. For preparation of ferric solution(1000 µg/ml), a 4.8890 g of $FeCl_3.6H2O$ (Riedel de Haen) was dissolved in 1000ml of 0.1M H_2SO_4 solution. The ferric chloride solution was standardized with EDTA solution. A 100 µg/ml of ferric solution was prepared through dilution of standard solution using 0.1 M H_2SO_4 Solution (Ensafi et al., 2004, Khaled et al., 2007) .

2.2.2. Buffer solutions
The buffer solutions (pH = 4.4 and pH = 5.2) were prepared by mixing an appropriate ratio of 2.0 M acetic acid solution with 2.0 M sodium acetate solution.

2.2.3. Azo oxime ligand solution
This solution was prepared by dissolving 0.1 g of prepared ligand in methanol and adjusting the volume to 100ml.

2.2.4. Ascorbic acid solution
For preparation of 1×10^{-3} M solution of ascorbic acid, 0.0176 g of the ascorbic acid (Riedel de Haen) was dissolved and completed to 100ml with distilled water in a volumetric flask. This solution was used after preparation directly.

2.3. Preparation of the ligand
The synthesis of 5-(4-Ethylphenyl azo)2-Hydroxybenzaldehyde Oxime was carried out according to general procedure described by KARABÖRK et al. (2019) Fifty milliliter of water was mixed with 10 mmol of 4-Ethyl aniline. To the later solution, the concentrated hydrochloric acid solution (2.5 mL) was added then it was filtered. The solution was then chilled on an ice bath (0-5 ºC) and followed by dropwise addition of sodium nitrite solution (13.9 mmol) in water (2 mL). For complete diazonium salt formation the reaction solution was kept for 30 minutes on an ice bath. To the ice-cold solution, salicylaldehyde (10 mmol) in 10% NaOH solution (10 mL) was added drop by drop and the reaction mixture was stirred for about 2.00 hours at room temperature. After addition of salicylaldehyde, yellow precipitate formed which was filtered and washed with water (15 mL) and dried in air.

The azo-aldehyde (1.000 g, 3.9 mmol) was dissolved in ethanol (100 mL). Then sodium acetate (0.8 g) and hydroxylamine hydrochloride (0.68 g) were added to the solutions and refluxed for 2.00 hours. The reaction mixture was then transferred into 250 mL of ice-cold water and the precipitates were filtered and dried in the air.
$C_{15}H_{15}N_3O_2$ yellow solid IR (vmax/cm^{-1}): 3392 (OH), 3052 (C-H) of Ar-H, 2918& 2978 (C-H) of CH_2, 2947& 2999 (C-H) of CH_3, 1649(C=N), 1606 (C=C), 1541 (N=N), 1205 (C-O).
^{1}H NMR (δ, ppm): 1.5 (t, 3H, of CH_2CH_3), 1.5 (q, 2H, of CH_2CH_3), 4.5 (s, 1H, N-OH), 5.5 (s, 1H, Ar-OH),7.00–8.5 (m, 7H, Ar-H), 12.4 (s, 1H, HC=N).

2.4. Analysis of tablets and capsules
Ten tablets or capsules contents, which contains ferrous salts, were weighed accurately, triturated and mixed well then, a weight which equivalent to one tablet or one capsule content was dissolved in 0.1 M H_2SO_4 solution then the solution was filtered with filter paper and the filtrate was collected in 250ml volumetric flask and completed to the mark with 0.1 M H_2SO_4 solution. Further dilution, if necessary, was performed with 0.1 M H_2SO_4 solution.

2.5. Stoichiometric analyses
The mole ratio method was used for the prediction of combination ratio between ligand and ferrous ion or ferric ion, for this purpose a 1.79×10^{-3} M of ferric and ferrous solution was prepared and 1.79×10^{-3} M for the ligand also prepared the

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absorbance of a ten solution is measured in which the volume of metal was held constant (0.5 ml) while that of ligand is varied in 10 ml volumetric flask and fixed volume of buffer solutions were added (Saeidi et al., 2013).

2.6. Recommended procedures
2.6.1. Recommended procedure for determination of Fe (II)

To a series of 10.0 ml volumetric flasks containing Ferrous ion (10.0-70.0 μg),1.0 ml of acetate buffer (2.0 M) solution (pH = 5.2), mixed well then 1.5 ml of 0.1% ligand were added. The solutions were diluted up to the mark with distilled water and the absorbance was measured at 495nm against reagent blank prepared in the same conditions. The measured absorbance was used to make the calibration plot.

2.6.2. Recommended procedure for determination of Fe (III)

To a series of 10.0 ml standard flasks containing Ferric ion (10.0-60.0 μg),1.0 ml of acetate buffer (2.0 M) solution (pH = 4.4), mixed well then 2.5 ml of 0.1% ligand were added. The solutions were diluted to the mark with distilled water and the absorbance was measured at 495 nm against reagent blank prepared in similar conditions. The measured absorbance was used to construct the calibration plot.

2.6.3. Recommended procedure for total iron analysis

To determine total iron the iron (II) must be oxidized to iron (III) or the iron (III) reduced to iron (II), because the optimum pHs for analysis of the two iron oxidation states are different (4.4 and 5.2). Here, the iron (III) was reduced to the iron (II) by ascorbic acid solution. For the total iron estimation, the same procedure which described in section 2.6.1 was used except addition of 0.8 ml of ascorbic acid (10^{-3} M) to the iron solution (20.0 to 60.0 μg) then followed by addition of buffer and ligand reagent in 10 ml volumetric flask..

3.   RESULTS AND DISCUSSION

Ferrous and ferric solutions when reacted with the azo-oxime ligand give a water-soluble complex. The coloured complex showed maximum absorption (λ_{max}) at 495nm for Fe (III) and at

486nm for Fe (II) (Fig1 and 2) while the blank solutions had no significant absorbance at these two wavelengths and therefore, these wavelengths were used for subsequent works. Fe (II) and Fe (III) react with azo-oxime ligand to form an orange-red complex which is suitable for spectrophotometric determination.

Various variables closely related to the iron determination were checked with spectrophotometric method using a fixed iron (III) and iron (II) concentration (5.0 μg/ml) and 2.5 ml and 2.0 ml of azo-oxime ligand for Fe(III) and Fe(II) determination subsequently.
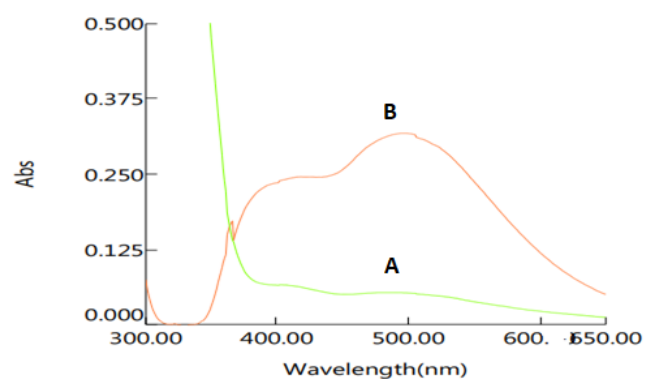

Figure 1. Absorption spectra: (A) Absorption spectra of the azo-oxime ligand against water; (B) Absorption spectra of the azo-oxime ligand -Fe (III) complex against reagent blank.
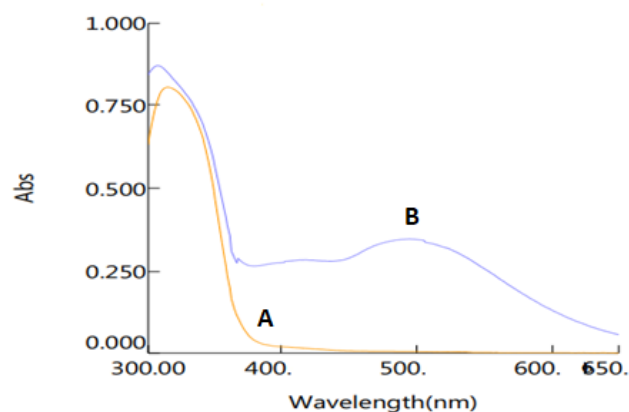

Figure 2. Absorption spectra: (A) Absorption spectra of the azo-oxime ligand against water; (B) Absorption spectra of the azo-oxime ligand -Fe (II) complex against reagent blank.

3.1.1. Effect of pH

The structure of the synthesized ligand contains oxime and hydroxyl groups which may be protonated or deprotonated by manipulation of the pH of the solution (Azzouz and Agha, 2008). Therefore, the effect of pH on colour development

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was studied. The pH of the solution is one of the important parameters for complex formation between metal ions and ligand. The influence of pH on the reaction was studied in the range 3.6-5.6, by using the appropriate amount of acetate buffer solution. The experiments showed that maximum complex formation was achieved at pH 4.4 for Fe (III) and at pH 5.2 for Fe (II) (Fig. 3). Therefore, these pH values were selected as optimum.
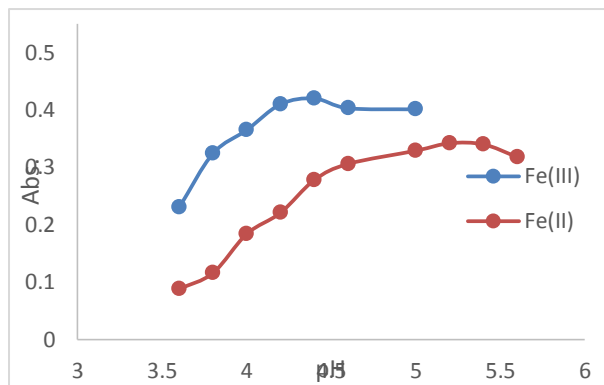

Fig.3: Effect of pH on the formation of Fe (III) and Fe (II) -ligand complexes

3.1.2. Effect of buffer solution concentration:
Under these optimum pH conditions, the effect of the concentration of acetate buffer was studied in the range 0.1–1.0 mol/ L. The colour intensity gave maximum absorbance values in the buffer concentration 0.2 M. Therefore, this concentration was chosen for determination of Fe (III) and Fe (II).
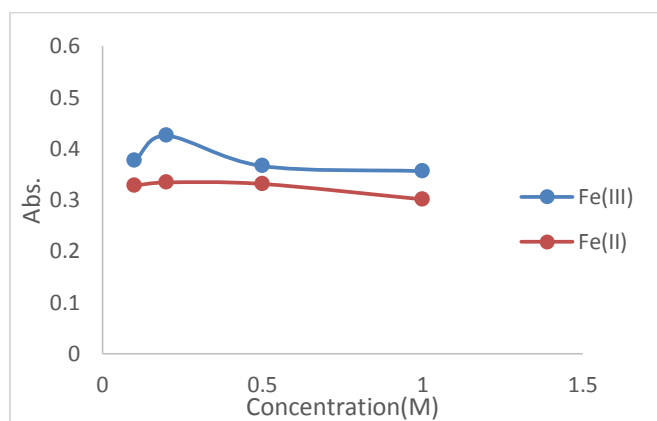

Fig.4: Effect of buffer concentrations on colour development for Fe (III) and Fe (II) determination

3.1.3. Effect of Azo-oxime ligand volumes
The influence of oxime-ligand volumes on the colour development was studied (Fig.5). The absorbance increased by increasing reagent volumes and reaches its maximum value at 2.5 ml for Fe(III) and 1.5 ml for Fe(II) ions and remains constants beyond these values , therefore these volumes were selected for the following studies.
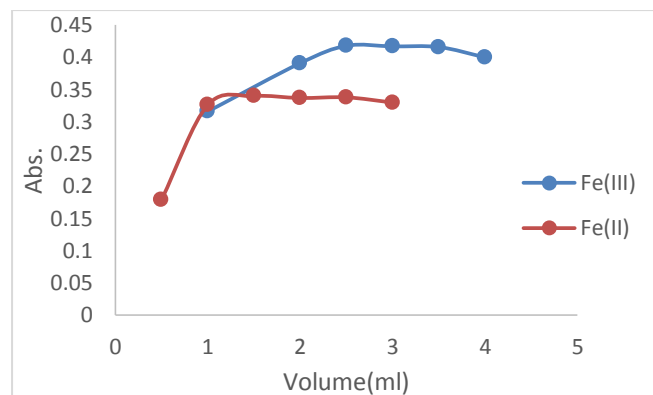

Fig5: Effect of ligand volumes on the colour formation.

3.1.4. Order of addition
Different directions of reagent addition were studied and it was found that the best absorption values were obtain by selecting the following sequence (ferric or ferrous solution+ buffer solution + azo-oxime ligand solution).

3.1.5. Effect of time on colour stability
The results from the optimization showed that the complex formed after mixing of the Fe (III) or Fe (II), the buffer solution and azo-oxime ligand immediately. The colour stability   was examined with Fe (III) and Fe (II) standard solutions and no change in absorbance was observed up to 15minutes.

3.1.6. Final absorption spectra
After obtaining the optimal conditions for Fe (II) and Fe (III) determination and measuring the maximum absorption wavelength, it was found that both oxidation stats have λ_{max} at 495nm.

3.2. Stoichiometric study
The stoichiometry of the reaction between Fe (II) or Fe (III) with the ligand under experimental conditions was investigated using mole ratio method. From Fig. 6 it can be inferred that metal: ligand ratio is 1:3 for ferric and 1:2 for ferrous ions. The suggested reactions between Fe (II) and Fe (III) with azo-oxime ligand are shown in the Scheme 1 and 2. The Fe (II) and Fe (III) prefer the octahedral structure geometry which is agreed

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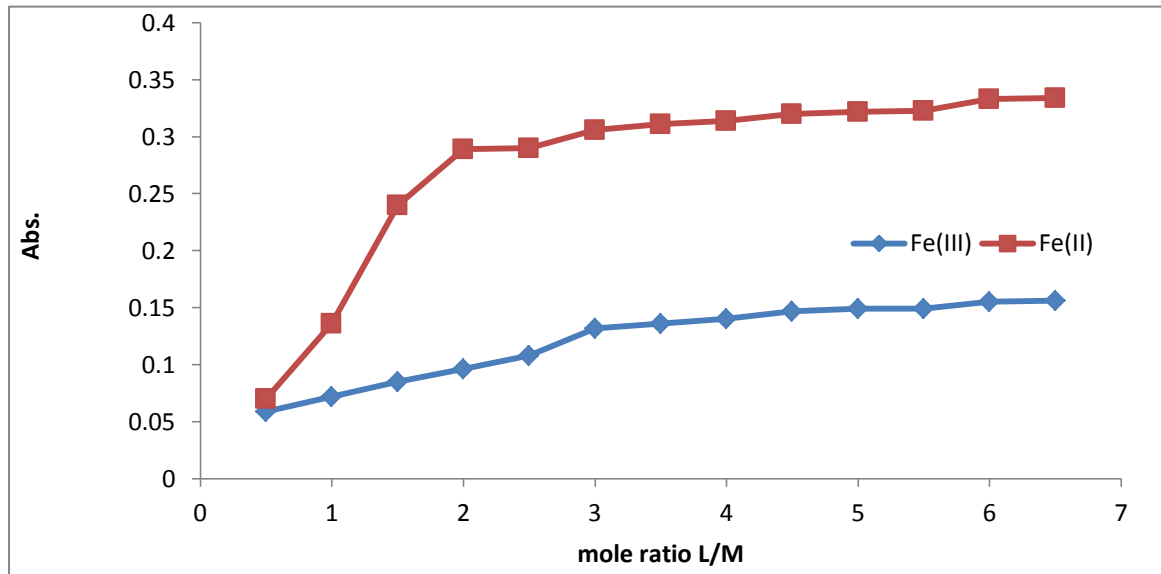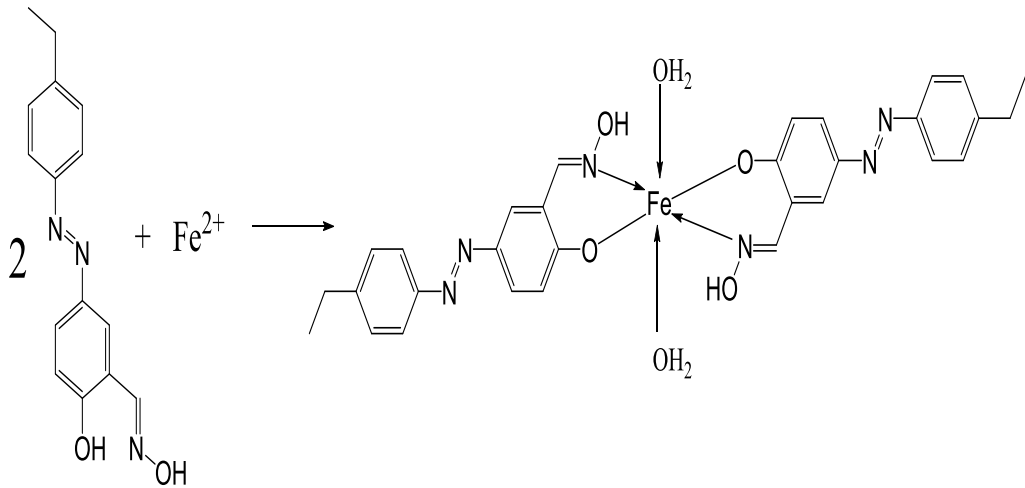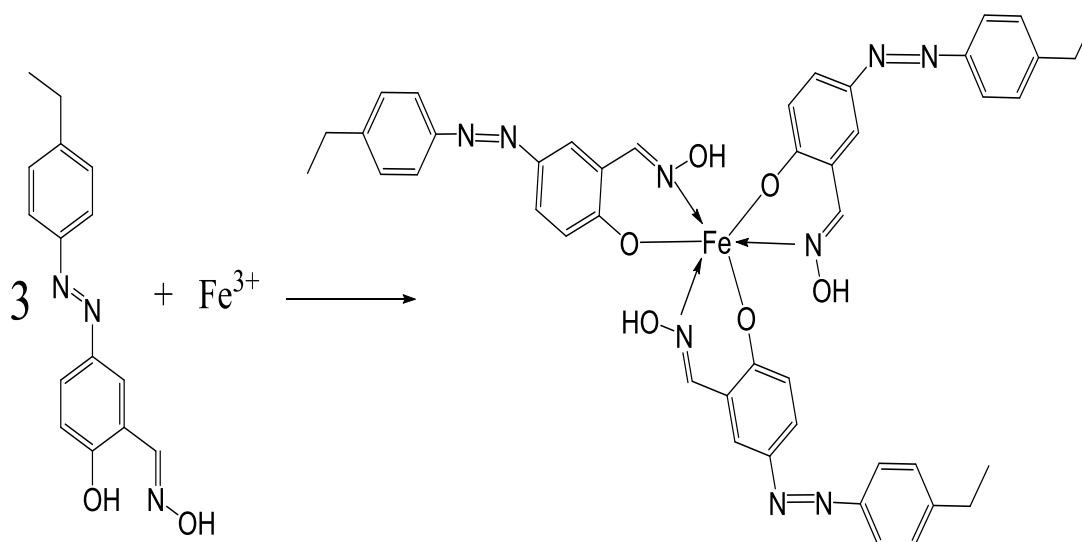with other previous study (Aoun and h Khafagy, 2018).

Fig.6: Determination of the stoichiometry of Fe(III) and Fe(II) with the ligand by the mole ratio method

Schem1: The suggested geometry of Fe (II) Chelate complex

Scheme 2: The suggested geometry of Fe(III) Chelate complex

3.3. Analytical figures of merit

Calibration graph was constructed by plotting absorbance against concentration of Fe(III) or Fe(II). The calibration graph under the optimum conditions is in the linear range between 1.0 to 6.0 µg/ml for Fe(III) and obeys Beers law in the concentration range 1.0 to 7.0 µg/ml (Fig. 7) for Fe(II). The regression equations, coefficient of determinations, Sandell index, molar absorptivity and detection limits are presented in Table 1. The detection limit was calculated according to this relation D. L= 3 S/m here S represent standard deviation of blank and m is the slope of calibration (Harris, 2010, Shrivastava and Gupta, 2011).
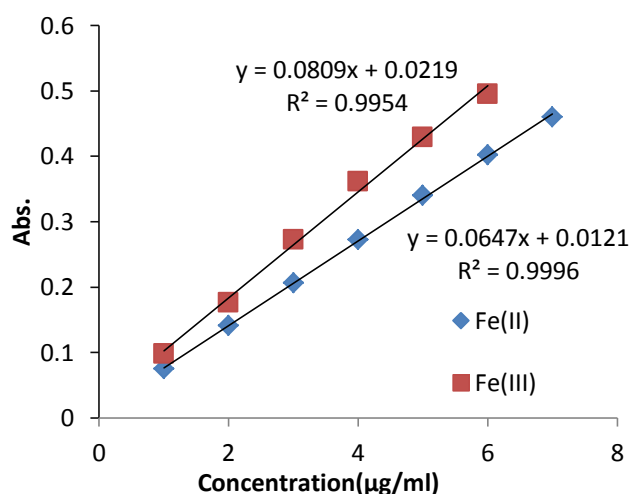

Fig.7: Calibration curves for Fe(III) and Fe(II) determination by proposed methods

Table1: Analytical characteristics of the proposed methods

	Fe (III)	Fe (II)
Linear regression equation	Y=0.0809x+0.0219	Y= 0.0647x+0.012
R^2	0.9954	0.9996
Sandell index	$0.011 \mu g/cm^2$	$0.015 \mu g/cm^2$
Molar absorptivity	4.78×10^3 L/mol.cm	3.64×10^3 L/mol.cm
Detection limit	0.04 µg/ml	0.05 µg/ml

3.4. Accuracy and precision

The accuracy of the method was assessed by analyzing the Fe(II) and Fe(III) at different levels within limits of linearity and the precision was ascertained by calculating the percentage of the relative standard deviation of replicate determinations (n=3) on the same solution containing Fe(III) or Fe(II) at different levels which are presented in Table2 . The %E_r and %RSD indicate the high accuracy and precision for the methods.

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Table 2: accuracy and precision of proposed methods

Concentration (µg/ml)	%Er[Fe(II)]	%Er[Fe(III)]	%RSD[Fe(II)]	%RSD [Fe(III)]
1.0	-2.70	-4.80	0.750	0.700
3.0	-0.10	+3.04	0.057	0.053
6.0	+0.43	-2.53	0.033	0.250

3.5. Effect of interferences

Table 3 shows the tolerable amount of many cations, anions and foreign species on the determination of 4.0 µg/ml of iron (II) or Fe (III).

The tolerance concentration is defined as the foreign species concentration causing error less than ±5.0 % for determining the analyte of interest.

Table 3: Effect of diverse ions and species on the determination of Fe(III) and Fe(II)

Species	Tolerable amount(µg) on Fe (III) determination	Tolerable amount(µg) on Fe (II) determination
Ni $(NO_3)_2$	500.0	300.0
Pb^{2+}	500.0	500.0
Zn^{2+}	2000.0	5000.0
Cu^{2+}	100.0	50.0
Co^{2+}	100.0	5.0
$MgCl_2$	1000.0	1000.0
$CaCl_2$	1000.0	1000.0
$CO_3^{=}$	1000.0	1000.0
HCO_3^{-}	2000.0	1000.0
Cl^{-}	4000.0	2000.0
Folic acid	50.0	200.0
lactose	500.0	500.0

3.6. Total iron analysis

Because various reducing agents, such as ascorbic acid, sodium azide, and hydroxylamine hydrochloride, were employed in earlier researchs (Blain and Treguer, 1995, Khaled et al., 2007, Abdel-Azeem et al., 2013) to reduce Fe (III) to Fe (II), therefore $1x10^{-3}$M ascorbic acid solution was used for total iron measurement. The initial absorbance values of the standard curve generated above for Fe (II) estimation is reduced when

ascorbic acid is used for Fe (III) reduction. As a result, in the presence of this chemical, a new standard curve was created for total iron analysis. A calibration curve is linear in the concentration range 2.0-6.0 µg/ml for total iron analysis. Synthetic samples were prepared for total iron analysis which includes binary mixture of both species. Table 4 shows the total amount of iron added and found out, as well as the % recovery.

Table 4: Determination of total iron in synthetic samples

Added Fe (III) µg/ml	Added Fe (II) µg/ml	Total Iron added µg/ml	Total Iron Found µg/ml	Recovery %
2.0	2.0	4.0	3.98	99.50
4.0	0.0	4.0	3.81	95.25
3.0	3.0	6.0	5.57	92.83

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3.7. Application

The proposed procedures were applied for the determination of iron in different samples. The results are given in Table 5 and compared with those obtained by FAAS. It shows that the results obtained by the proposed methods are in good agreement with the results obtained by FAAS, because the calculated Student's t value (1.344) is lower than the theoretical value (2.353) at the confidence level. 95%.

Table 5: Spectrophotometric determination of iron in some pharmaceutical preparations

Sample	Labeled	Found by present method	Found By FAAS	Error%
Ferrofol	55.430	42.454	41.020	+3.49
Iron gluconate	35.00	30.280	31.503	-3.88
Folic iron	47.00	50.172	48.121	+4.26
Ferrous sulphate	73.520	66.520	64.705	+2.80

4. CONCLUSIONS

This study demonstrated that 5-(4- Ethylphenyl azo)2-Hydroxybenzaldehyde oxime can be utilized as a useful ligand for the spectrophotometric determination of Fe (II) Fe (III) and total iron in different pHs. The methods are free from tedious steps such as heating, extraction step and pH adjustment procedures. The methods offer good analytical characteristics due to their simplicity and rapidity. The methods were succeeded in the determination of iron in different samples with average recoveries agreed with that of the atomic absorption spectrometry method.

Acknowledgements

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