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Full Paper

Determination of Iron Content in Iron Deficiency Drugs by UV-Visible Spectrophotometer

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Abstract: The objective of this work was to validate a simple, precise and accurate spectrophotometric method for the determination of iron in the iron deficiency drugs, namely are Feroglobin B12, Ferose-F and Ferose. The proposed method is based on the reaction of iron with ammonium thiocyanate after the wet digestion of the drugs under study with HNO_3 and H_2O_2. Effects of pH, temperature, standing time and thiocyanate concentration on the determination of iron in drugs containing iron have been investigated. The λ_{max} was 430 nm and the molar absorptivity of 0.0399 L mol^{-1} cm^{-1}. The linear regression was in the range 0.5 - 60 µg/mL for iron content in hemoglobin. The detection limit and the limit of quantification were found to be 0.040 and 0.122 µg mL^{-1} for the iron respectively, and with a linear regression correlation coefficient of 0.998. Recovery measurements ranged from 99.63-100.20%. This method is simple and fast can be used for the determination of iron in the iron deficiency drugs in pharmaceutical laboratories.

Keywords: iron; iron deficiency drugs; thiocyanate; UV spectrophotometer

1. INTRODUCTION

Iron deficiency is very common and affects more than 2 billion world's population [1]. The main function of Iron is to transfer oxygen to the tissue where it is required [2]. It is one of the constituents of hemoglobin present in blood cells in the human body. The red blood cells will not be healthy without iron. So it is an essential micro-nutrient [3]. Iron formulations considered to be a fundamental plan for the prevention and treatment of iron deficiency anemia and can obtain excellent objectives in the achievement of iron deficient people [4]. Iron Deficiency is occurring widely against vulnerable people such as pregnant women, infants and child malnutrition. To avoid these deficiencies, adequate sources of iron are needed. The components of the food will affect iron bio-availability [5]. Iron formulations are the most prescribed medicines. Patients are treated with many drugs and sometimes they use multiple medications [6, 7]. Iron (II) compounds have excellent absorption and they are mainly consumed in oral doses [8]. Most of the oral therapy in use can cause side effects. The drugs industry endeavor to improve the final products by many ways in order to minimize the side effects, but overall, the satisfaction results have not been achieved yet [9, 10]. Iron formulations were found to be effective for treating anemia and iron deficiency [11]. Iron supplementation is one of the most commonly used drugs for the complications arising as a result of iron deficiency in human beings. Iron can be prepared for different drugs form as tablets, capsules, drinking ampules, injectable and syrup. Iron is an essential mineral that is added to multivitamins [12]. Several methods of determination of iron have been employed. These methods include spectrophotometry [13, 14], atomic emission and atomic absorption spectrometer [15, 16], flourimetry [17, 18], flow-injection analysis [19, 20], chemiluminescence [21, 22], voltammetric methods [23-25], capillary electrophoresis [26, 27] and chromatographic techniques [28, 29]. Spectrophotometric method of the determination of iron in multivitamin formulations by the formation of colored complexes of Fe(II) with 2,2'-Bipyridyl was investigated [30]. Iron (III) ions in biological and drug samples were determined by the coupling of cloud-point extraction procedure with molecular spectrophotometry [31]. The determination

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of total iron in drugs and natural water as Fe(II) using a sequential injection system was carried out [32]. The iron in some pharmaceuticals was determined spectrophotometrically by the partial least squares with chromogenic mixed reagents of 1,10-phenanthroline and 5-sulfosalicylic acid in acidic medium [33]. In the present study we validate a simple, cheap, safe, fast, reliable and reproducible spectrophotometric technique for the determination of iron in iron deficiency drugs (Feroglobin B12, Ferose-F and Ferose) using ammonium thiocyanate (NH_4SCN) as a chromogenic reagent

2. MATERIAL AND METHODS

2.1. Chemicals, reagents and samples

Analytical grades of chemicals were used, and deionized water was also used to prepare all solutions. The following available pharmaceutical preparations were analyzed: (i) Feroglobin B12 (15 mg iron as fumarate) capsules, (Thompson and Capper Ltd-United Kingdom) labeled to contain 15 mg iron as fumarate per capsule. (ii) Ferose-F (100 mg of iron) tablets, (SPIMACO, Al-Qassim Pharmaceutical Industries Plant, Saudi Arabia) labeled to contain 100 mg of iron per tablet. (iii) Ferose (100 mg of iron) tablets, (SPIMACO, Al-Qassim Pharmaceutical Industries Plant, Saudi Arabia) labeled to contain 100 mg of iron per tablet.

2.2. Equipment and apparatus

All absorbance measurements were read on a Split beam UV-VIS Spectrophotometer (SP- 3000 Plus model, Optima, Tokyo, Japan) with 1-cm quartz cells. A pH meter (model 3305, Jenway Ltd., United Kingdom) was used. Digital water bath (Model LWB-122D, Daihan Labtech Co. Ltd., Indonesia) was also used.

2.3. Preparation of standard ammonium thiocyanate solution

40.0 g of (NH_4SCN) were dissolved in deionized water, put into a 100 mL standard flask and diluted to the mark with deionized water and mixed well to obtain 40% w/v solution. Then a series of dilution were prepared for the range of (0.5- 40% w/v).

2.4. Preparation of buffer solutions

Buffer solutions of pH ranged from 3.0 - 11.0 were prepared by mixing acetic acid with sodium acetate solution, Na_2CO_3 solution with $NaHCO_3$ solution and NaH_2PO_4 solution with NaOH solution, each buffer solution was prepared and adjusted by a pH meter.

2.5. Preparation of standard solutions of Feroglobin B12 (1000 µg/mL)

The drug was accurately weighed amount of 3.087 g (equivalent to 0.1 g of iron) were digested by transferring them into a 100 mL calibrated beaker, and dissolved in about 12.0 mL of concentrated HNO_3 and 4.0 ml of H_2O_2 and covered by watch glass. The contents of the beakers were heated to 110°C in a hotplate for 30 minutes and then transferred to 100 mL volumetric flasks, completed to volume with deionized water, and mixed well, to give a concentration of 1000 µg mL^{-1} of iron. These stock solutions were diluted to give a series of concentrations in the ranges of 0.5- 120 µg mL^{-1} for the iron. The contents were mixed well and filtered. The prepared solution was used to obtain a suitable concentration for the analysis.

2.6. Preparation of the drug samples

Three tablets of each of the drugs (Feroglobin B12, Ferose-F and Ferose) were powdered in a porcelain mortar and pistol separately. Then they were accurately weighed and transferred into a 100 mL calibrated flask, and digested by the addition of 6.0 mL concentrated HNO_3 and 2 ml of H_2O_2 and covered by watch glass. The contents of the beakers were heated to 110 °C in a hotplate for 30 minutes and transferred to 50 mL volumetric flasks, completed to volume with deionized water, and mixed well, to give a concentration of each tablet. The prepared solutions were diluted quantitatively with deionized water for the three drugs to obtain a suitable concentration for the analysis.

2.7. Spectrophotometric analysis using thiocyanate (SCN^-)

About 2 mL of each of the prepared solutions of Feroglobin B12, Ferose-F and Ferose B12 were transferred into test tube for each drug, 2 mL of pH 3.0 were added for each of the three drugs, followed

by addition of 1 mL of 40.0% (SCN^-) for each drug, the solutions were heated in a thermostat at 30 °C for 5.0 min for the three drugs, the mixtures was diluted with deionized water. The absorbances of the solutions were measured at 430 nm for the three drugs versus blank.

2.8. Job's stoichiometric ratio of the reaction

The Job's method of the account of complexation was applied [34]. Equal concentrations of (10.0 µg mL^{-1}) of aqueous solutions of Feroglobin B12, Ferose-F and Ferose and (SCN^-) were prepared. Series of 10 mL portions of the master solutions of Feroglobin B12, Ferose-F and Ferose and (SCN^-) were made up by the following ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10).

3. RESULTS AND DISCUSSION

3.1. Determination of Absorption spectrum

The absorption spectra of products obtained by the reaction of Feroglobin B12, Ferose-F and Ferose with (NH_4SCN) are shown in Figure 1. The maximum absorption wavelength peaks λ_{max} at 388, 360 and 410 nm, for Feroglobin B12, Ferose-F and Ferose against water respectively. The λ_{max} of (NH_4SCN) has no absorption in the range 400–800 nm, and the λ_{max} for the three drugs with (SCN^-) is 430 nm against the reagent blank.

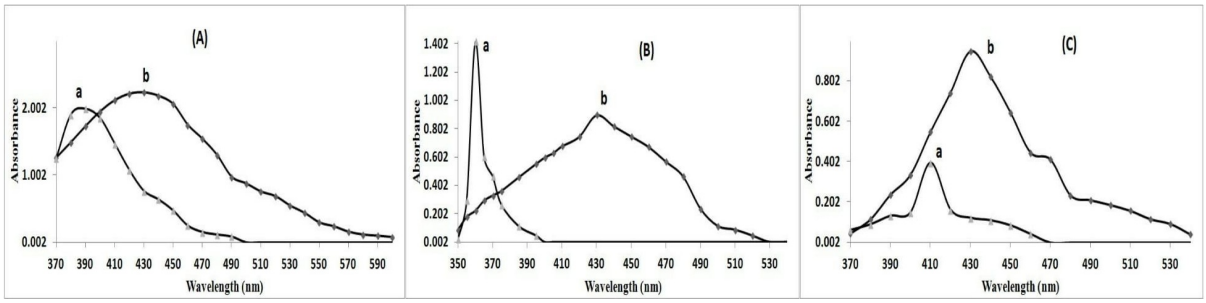

Figure 1. Absorption spectra of (A) Feroglobin B12, (B) Ferose-F, and (C) Ferose (all of the three drug were of 10 µg mL^{-1} : a- absorption spectrum of the drug against water, b- absorption spectrum of reaction of the drug (10 µg mL^{-1}) with (SCN^-) (40.0%).

3.2. Conditioning of the reaction method

The optimum conditions for the reaction in this method were set by changing values of each of the parameters of the pH, volume of buffer, thiocyanate concentrations, temperature and reaction time, and they were found to be 3.0, 2 mL, 40.0 %, 30 °C and 5 minutes respectively, as seen in Figure 2.

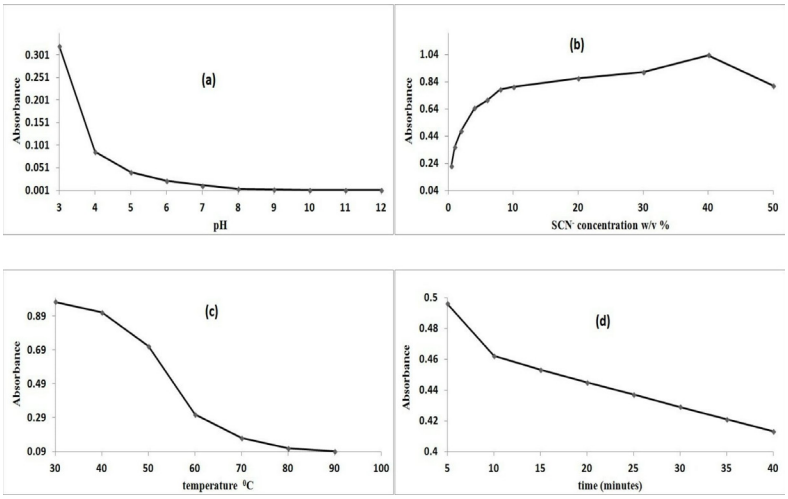

Figure 2. Effect of analytical parameters on the reaction of standard iron solution with (SCN^-): (a) Effect of pH, (b) Effect of (SCN^-) concentrations, (c) Effect of temperature, and (d) Effect of standing time.

3.3. Job's method

The Job's method of continuous variation was employed [34]. Equimolar aqueous solutions of prepared drug samples (Feroglobin B12, Ferose-F and Ferose) and (NH_4SCN) were prepared. Series of 10 mL portions of the solutions of prepared blood sample and (NH_4SCN) were made up by preparing the following ratios of solutions (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10). The shape of Job's graph was obtained, indicated that the ratios of each reaction of (NH_4SCN): studied prepared drug samples were 1:4 as shown in Figure 3.

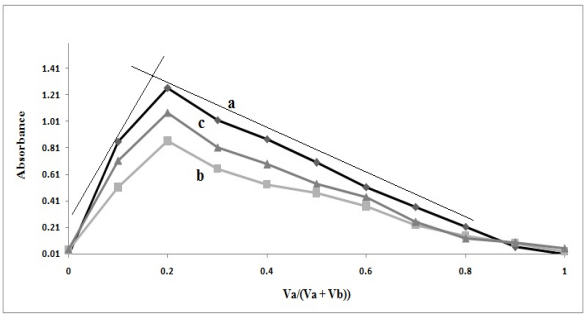

Figure 3. Stoichiometry by Job's method for (SCN) with prepared blood samples: a- Feroglobin B12, b-Ferose and c-Feros-F (Va: SCN^- and Vb iron in drugs).

3.4. Method Validation

Analytical curve for the determination of prepared drug sample (Feroglobin B12) and (NH_4SCN) was constructed by plotting the absorbance as a function of the corresponding concentrations. In the proposed method, linear plot of good correlation coefficient was obtained in 0.5-60 µg/mL as shown in Figure 4.

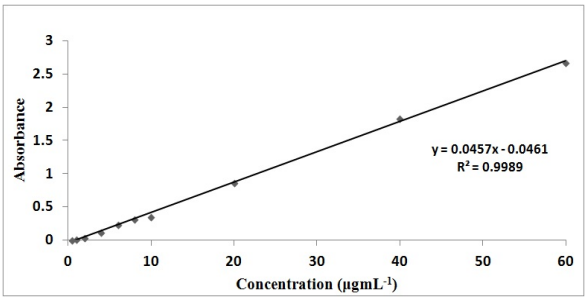

Figure 4. Analytical curve for the determination of iron.

The limit of detection (LOD) was calculated based on the standard deviation of the five replicate determination values of blank and the slope of the analytical curve. The regression equation for the results was Y = 0.045 X - 0.046. The detection and quantification limits were estimated as described by the guidelines of International Conference of Harmonization (ICH) for method validation [35]. The parameters for the analytical performance of the developed methods are listed in Table 1.

Table 1. Summary of the quantitative parameters and statistical data using method.

Parameter	Iron of prepared blood samples and (NH_4SCN)
λ_{max} /nm	430 nm
Beer's law limits (µg mL^{-1})	0.5 - 60
Molar absorptivity, e (L mol^{-1} cm^{-1})	0.0399
Correlation coefficient (R2)	0.998
Regression equation (Y)	Y = 0.045 X - 0.046
Slope	0.045
Intercept	-0.046
LOD (µg mL^{-1})	0.040
LOQ (µg mL^{-1})	0.122

The current method was not affected by the small differences in the studied parameters, which indicates the robustness of the proposed method. The recovery percentage of the changing in the parameters of pH, thiocyanate concentration, temperature and the reaction time was determined. The findings show that small differences in the method parameters did not significantly affect the procedures; recovery values were listed in Table 2.

Table 2. Robustness of the proposed spectrophotometric method of iron in drugs containing iron sample.

Condition	Iron conc. (10.0 µg mL^{-1})	Recovery % ± RSD*
pH	2.80	100.47 ± 1.32
	3.20	99.60 ± 0.10
SCN^- concentration (w/v %)	39.80	100.77 ± 1.93
	40.20	99.60 ± 0.26
Temperature (°C)	28	100.90 ± 1.14
	32	99.60 ± 0.02
Reaction time (min.)	3	101.57 ± 1.66
	7	99.80 ± 0.10

The accuracy and precision of the method were evaluated by performing three replicate analyses on standard iron solutions of the drug samples at three different concentration levels. The relative error (%) was 0.375- 0.738, and intraday precision expressed in relative standard deviation (RSD) (%) was below 2% for the investigated samples, indicating the high accuracy and precision of the method. The results of this study which shown in Table 3 indicating the importance of this method for the analysis of the three investigated drug samples in quality control purposes and also for inter- and intraday accuracy as shown in Table 4.

Table 3. Evaluation of the accuracy and precision of the method**.**

Sample	Taken	Found	Relative error%	SD	RSD%
Feroglobin B12	4.00	3.985	0.375	0.034	0.853
(µg mL^{-1})	8.00	8.059	0.738	0.034	0.422
	10.00	10.067	0.670	0.174	1.728

•SD, standard deviation; RSD, relative standard deviation.

Table 4. Evaluation of Interday and Intraday Accuracy.

Added	Interday (n = 3)				Intraday (n = 3)			
(µg mL^{-1})	Found	Recovery %	± SD	%RSD	Found	Recovery %	± SD	%RSD
6	6.022	100.37	0.044	0.731	6.044	100.73	0.045	0.744
8	7.993	99.91	0.046	0.576	7.985	99.81	0.046	0.576
10	10.010	100.10	0.046	0.460	9.970	99.70	0.034	0.341

It is evident from the above-mentioned results that the proposed method gave satisfactory results of iron in the drug containing iron. Thus, the samples of the three drugs (Feroglobin B12, Ferose-F and Ferose) were subjected to the analysis of their iron contents individually, by the proposed method, as seen in Table 5.

The recovery studies of the method were carried out by taking a fixed amount of the iron in the prepared drug samples (Feroglobin B12), then standard iron was added at three different levels and the total was found by the proposed method. Each test was performed in triplicate. The percent recoveries of the range of 99.63 to 100.20% for the iron in drug sample as shown in Table 6, revealing good accuracy and non-interference.

Table 5. Determination of the studied drugs in their pharmaceutical dosage.

Drug	Pharmaceutical product	Percentage ± SD*
FeroglobinB12	15 mg of iron/tablet	100.33± 0.38
Ferose-F	100 mg of iron/tablet	100.82 ± 0.07
Ferose	100 mg of iron/tablet	99.32 ± 0.03

** Values are mean of three determinations.

Table 6. Recovery of the method.

Sample	Sample iron content (µg mL^{-1})	Standard iron Added (µg mL^{-1})	Found (µg mL^{-1})	Recovery (% ± RSD)*
Feroglobin B12	6.0	2.0	7.970	99.63 ± 0.577
(µg mL^{-1})	6.0	4.0	10.020	100.20 ± 0.559
	6.0	6.0	11.970	99.75 ± 0.384

4. CONCLUSION

This study investigated the use of (Ammonium thiocyanate) reagent in the development of simple, precise and fast spectrophotometric method for the accurate determination of iron in iron containing drugs namely Feroglobin B12, Ferose-F and Ferose. The proposed method is simple. On other hand, all the chemicals used in this method are cheap and

available. The method is practical and importance for its application in laboratories for the determination of each investigated iron deficiency drugs (Feroglobin B12, Ferose-F and Ferose).

5. ACKNOWLEDMENTS

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