ORIGINAL ARTICLE

SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF IRON (III) FROM IRON POLYMALTOSE COMPLEX IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A visible spectrophotometric method has been developed for the quantification of iron (III) from iron polymaltose complex in pure and in pharmaceutical preparations. The method is based on hydrolysis of iron polymaltose complex under acidic conditions and the formation of red colored chromogen with ammonium thiocyanate, which showed absorption peak at 471 nm. This absorption wavelength can be used for the determination of iron (III) from iron polymaltose complex. The limit of detection of iron polymaltose complex at 476 nm was 6.207 ng mL^{-1}. The calibration was linear in the range of 19.8–22.2 μg mL^{-1}. Analytical parameters such as stability, selectivity, accuracy and precision have been established for the method in HAEMOTYL® syrup and evaluated statistically to assess the application of the method. The method was validated under the ICH and USP guidelines and found to comprise the advantages for simplicity, stability, sensitivity, reproducibility and accuracy for using as a method for the routine analysis of the drug in pharmaceutical formulations and in pharmaceutical investigations involving iron polymaltose complex.

Keywords: Spectrophotometry; iron polymaltose complex; pharmaceutical analysis.

INTRODUCTION

Iron is an essential trace element required for survival by practically all-living organisms that has been proposed to participate in biological free radical producing reactions, in particular in the hydroxyl radical producing Fenton's reaction (Tuomainen *et al*., 2003). It can exist in two different physical or redox states and thus catalyses many fundamental biochemical reactions. The most significant of these is oxygen transport by the hemoglobin molecule within red blood cells to metabolizing tissues (Andrews, 2000). Iron deficiency anemia is customarily treated by iron supplementation (Tuomainen *et al*., 1999).

The drug is not official in the United States Pharmacopoeia 2004 or British Pharmacopoeia 2004, thus there is no official method of estimation. No method has been reported yet for the determination of iron (III) from iron polymaltose complex in the literature. Various clinical studies have been reported but there was no information about pharmaceutical analysis of the drug (Erichsen *et al*., 2005; Badhwar, 2001; 2004; Reddy *et al*., 2001; Jacobs *et al*., 2000; Nielsen *et al*., 1994 and Singh & Fong, 2000). Even though a spectrophotometric method (Kiran and Revanasiddappa, 2003) has been suggested as a method of analysis of iron (III) using leuco Xylene cyanol FF but it was not validated from iron polymaltose complex and in pharmaceutical dosage forms. In this study, a simple, visible spectrophotometric method for the determination of iron polymaltose complex by the hydrolysis of the drug has been devised. Analytical parameters for the method have also been established and compared with those established for the official pharmacopeial method.

EXPERIMENTAL

Apparatus

Shimadzu 1601 UV visible spectrophotometer with UVPC ver 3.9 software and quartz cells of 10-mm path length; Analytical balance (AX-200 Shimadzu, Japan); Water bath (China); Whatman 41 filter paper circles of diameter 125 mm (Whatman, Maidstone, England).

Materials

Iron polymaltose complex reference standard (30.781% elemental iron), HAEMOTYL® syrup (50 mg/5mL) were supplied by Noa Hemis Pharmaceuticals, Karachi, Pakistan and was used without any further purification; hydrochloric acid nitric acid and sulfuric acid of analytical grade (Merck, Darmstadt, Germany).

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Stock solution of iron polymaltose complex (335 µg mL^{-1}) was prepared by taking 33.5 mg of iron polymaltose complex accurately weighed in 100 ml volumetric flask with 1 mL nitric acid and 5 mL of hydrochloric acid hydrolyzed over a water bath for 2 minutes, cooled at room temperature and volume was made up to 100 ml with distilled water.

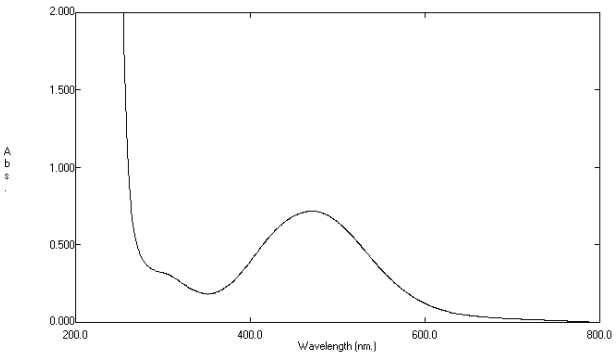

Fig. 1: Wavelength scan for iron polymaltose complex in 0.1N H_2SO_4.

Table 1: Mean absorbance values, regressed values and statistical data of the calibration curve for the estimation of iron polymaltose complex

Concentration (µg mL^{-1})	Mean ABS[a] (±S.E.)	Regressed values[b]
19.8	0.6491 ± 0.00171	0.1818
20.4	0.6685 ± 0.00189	0.5319
21.0	0.6885 ± 0.00213	0.7648
21.6	0.7081 ± 0.00140	0.7692
22.2	0.7278 ± 0.00214	0.1250

[a] Mean of six values. [b] Using regression equation.
Regression equation[++] statistical data.
Intercept (a)= 1.15×10^{-3}; slope (b)= 3.284×10^{-2};
Correlation coefficient = 0.9999; [++], n=42.

Preparation of calibration curve

Suitable weights (33-37 mg) of iron polymaltose complex were weighed accurately in 100 ml volumetric flask with 1 mL of nitric acid and 5 mL of hydrochloric acid for hydrolysis over a water bath for 2 minutes, cooled at room temperature and volume was made up to 100 ml with distilled water. Transferred 3 mL aliquots of the solution and 3 mL of the ammonium thiocyanate (10 %) solution into 50 ml volumetric flasks and the volume was made up to 50 ml with 0.1 N H_2SO_4. The solutions were shaken well for proper mixing and their absorbance measured at 476 nm.
Mean absorbance values (n=6) along with the regressed values (method of least squares) and statistical data for the method are shown in table 1. The optical characteristics for the solution of iron polymaltose complex in 0.1 N H_2SO_4 is given in table 2. Absorptivity scan over the UV wavelength range between 200 and 400 nm for a 20.76 µg mL^{-1} solution of iron polymaltose complex in methanol is shown in fig. 1.

Table 2: Optical characteristics for iron polymaltose complex in reference standard and syrup

Characteristic	Value in reference standard	Value in syrup
Absorption maxima (nm)	471 [a], 304	471 [a]
Beer's law limits [b] (µg mL^{-1})	10-40	10-40
Apparent molar absorptivity [b] (l mol^{-1} cm^{-1})	1836	1836

[a] Analytical wavelength for proposed method.
[b] At analytical wavelength.

Stability

Stability of the solutions of iron polymaltose complex, used for preparing the calibration curves in the method, was ascertained by observing for changes in the absorbance at their respective analytical wavelengths over a period of 24 h.

Precision

In order to determine precision of the method, solutions containing known amounts of pure drug were prepared and analyzed in three replicates and absorbance readings were recorded in six replicates to get the mean. The analytical results obtained from these investigations for the methods are summarized in table 3.

Accuracy and selectivity

The accuracy and selectivity of the method for the estimation of the drug in presence of various syrup excipients such as sugar, sorbitol 70 % solution, liquid glucose, citric acid, methyl paraben, propyl paraben, sodium benzoate, sodium hydroxide, sodium chloride and flavors was investigated.

A placebo comprising 60 % w/v sugar, 20 % w/v sorbitol 70 % solution, 18 % w/v liquid glucose, 0.12 % w/v citric acid, 0.25 % w/v methyl paraben, 0.025 % w/v propyl paraben, 0.1 % w/v sodium benzoate, 0.02 % w/v sodium hydroxide, 0.025 % w/v sodium chloride and 0.32 % w/v flavours was prepared. A 1:1 blend of drug and placebo was prepared. Drug was then hydrolyzed from this blend-using nitric acid and hydrochloric acid. The solution was allowed to cool at room temperature and volume made up with distilled water. Suitable aliquots (8–10 ml) of the stock solution and the 3 mL of the ammonium thiocyanate (10 %) solution were taken into 100 ml volumetric flask and the volume was made up to 100 ml with 0.1 N H_2SO_4. The solutions were shaken well for proper mixing and their absorbance measured at 471 nm. The above procedure was carried out

Table 3: Evaluation of precision of the proposed method in pure drug substance

Amount of drug added (mg)	Individual amounts found (mg) mean (S.D.)[a]	Coefficient of variation (CV)	Relative mean error (RME)	Confidence limits [b]
44.95	44.52	0.445	0.0337	44.31 - 44.60
44.58	44.43			
44.50	44.41			
	44.46 (0.19)			
50.00	49.99	0.766	0.0063	49.97 - 50.03
50.90	50.01			
50.50	50.00			
	50.00 (0.38)			
55.83	55.94	0.475	0.0732	55.62 - 56.25
56.30	56.06			
55.31	55.81			
	55.94 (0.26)			

[a]*n*=6; [b] Confidence limits at *P*=0.95 and two degrees of freedom.

P = 0.05

Table 4: Evaluation of accuracy of the proposed method in drug substance spiked to placebo (HAEMOTYL® syrup)

Amount of drug added (mg)	Individual amounts found (mg) mean (S.D.)[a]	Coefficient of variation (CV)	Relative mean error (RME)	Confidence limits [b]
45.00	44.86	0.353	0.0849	44.33 - 45.06
44.90	44.57			
44.78	44.66			
	44.70 (0.16)			
50.12	50.28	0.569	0.2454	48.89 - 51.00
50.10	50.09			
49.90	49.47			
	49.95 (0.28)			
54.70	54.49	0.359	0.0405	54.39 - 54.74
54.90	54.60			
55.00	54.62			
	54.47 (0.19)			

[a]*n*=6; [b] Confidence limits at *P*=0.95 and two degrees of freedom.

in triplicate and absorbance readings were recorded six times to get the mean. Results of these determinations are included in table 4.

RESULTS AND DISCUSSION

Iron polymaltose complex in 0.1N H_2SO_4 yields a characteristic curve when scanned in the UV-visible wavelength range between 200 and 800 nm. The scan (fig. 1) shows absorption maxima at 471 and 304 nm (table 2) of the red colored chromogen. The absorptivity at 471 nm was found to be 1836 l mol^{-1} cm^{-1} and this wavelength was chosen as the analytical wavelength.

The visible spectra in this case can be attributed mainly to the formation of red colored chromogen of the iron polymaltose complex with ammonium thiocyanate solution. Correlation coefficient was found to be 0.9999, signifying that a linear relation existed between absorbance and concentration of the drug. The limit of detection of iron polymaltose complex at 476 nm was 6.207 ng mL^{-1} calculated by the following formula,

$$y = y_b + 3s_b \quad (1)$$

Where y, y_b and s_b are signal of sample at LOD, blank signal and standard deviation of the blank respectively. According to the equation of line,

$$y = mx + b \quad (2)$$

Where y is the absorbance, m is the slope of the line, x is the concentration of sample and b is the y-intercept of the line. In order to calculate the limit of detection we need to calculate, all the associated values i.e., the values of y, m, b, r (correlation coefficient) and s_b ($s_{y/x}$).
Where,

$$r = \Sigma_i \{(x_i-x_{avg})(y_i-y_{avg})\} / \{[\Sigma_i(x_i-x_{avg})^2][\Sigma_i(y_i-y_{avg})^2]\}^{1/2} \quad (3)$$

$$m = \Sigma_i \{(x_i-x_{avg})(y_i-y_{avg})\} / \Sigma_i(x_i-x_{avg})^2 \quad (4)$$

$$s_{y/x} = \{\Sigma_i(yi-\hat{y})^2/ n-2)\}^{1/2} \quad (5)$$

Beer's law was found to be obeyed between 10 and 40 µg ml^{-1}. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is shown in Table 1. Regression equation was y=0.03284x+0.00115. The variance of the response variable, S_{yx}^2, was calculated to be 4.615×10^{-9} (six degrees of freedom). This low value indicates the closeness of the experimental points to the least squares line. The fact is in concurrence with the low values of the standard error of the mean (S.E.M.) absorbances of the solutions used for preparing the calibration curve. The variance of the methods was compared using `*F*' distribution to determine whether they were significantly different from each other. The calculated `*F*' value was found to be 1.55 for six and five degrees of freedom in the denominator and numerator, respectively. There is no significant difference in the variance and hence no difference in variability exists. This is supported by the narrower range in which Beer's law is obeyed (10–40 µg ml^{-1}) and the higher absorptivity of iron (III) at 471 nm (18360 l mol^{-1} cm^{-1}). The value of Sandell's sensitivity coefficient 5.60×10^{-6} g cm^{-2} per 0.001 abs unit supports the above observation. To examine whether the intercept was significantly different from zero, the intercept was subjected to a `*t*' test. The values of `*t*' was obtained as 3.85 (five degrees of freedom). Thus, acceptance of the null hypothesis indicates that the intercept was not significantly different from zero. Therefore, there is no interference from the solvent used in the method, i.e. methanol.

The stability of iron polymaltose complex in methanol was monitored over a period of 24 h. ANOVA studies of the mean absorbance values of the solutions of different concentrations at preselected time intervals indicated that no significant difference existed between the readings. Thus, iron polymaltose complex is stable over a period of 24 h in methanol.

The precision of the method was carried out using known amounts of pure drug that were subjected to recovery studies in triplicate and evaluated using the S.D. of the results, the coefficient of variation and confidence limits. Table 3 summarizes the results of these investigations. The lower S.D. of the results and the lower coefficient of variation make the method more precise. This is also reflected in the larger confidence limits (table 3) for different levels when the method is used for estimation.

In order to determine the accuracy of method, estimation of iron polymaltose complex was carried out in the presence of various commonly used excipients at the levels they are normally used. From table 4, it can be seen that there is no significant difference between the amount added to the placebo and the amount recovered. Thus, excipients like sugar, sorbitol 70 % solution, liquid glucose, citric acid, methyl paraben, propyl paraben, sodium benzoate, sodium hydroxide, sodium chloride and flavors did not interfere with the estimation. Also, the hydrolysis did not destroy the drug to any extent. Accuracy of method was ascertained by using the `*t*' test at each level. The computed `*t*' values at 45, 50 and 55 mg are 0.248, 0.215 and 0.830 respectively. These lower `*t*' values are indicating that no significant difference between the added and the estimated quantity. To establish the accuracy of method the relative mean error (RME) of method was calculated and is shown in table 4. The relative mean error is less at each level signifying that the method is accurate.

CONCLUSION

Iron polymaltose complex can be estimated using the method at 471 nm. It has the advantages of simplicity, stability, sensitivity, reproducibility and accuracy and is associated with higher sensitivity and precision. The non-interference of syrup excipients makes the method suitable for the estimation of the drug in syrup, hence can be used for routine quality control of iron polymaltose complex formulations of all potencies and in forensic sciences involving the estimation of iron polymaltose complex. It is recommended strongly that a water bath be used when the method is being used for estimation to ensure fast and complete hydrolysis of the drug.

Results of the above study indicate the suitability of the method to estimate iron polymaltose complex in bulk as well as in dosage formulations. The developed method has the novelty of being the only method for the quantitative determination of iron (III) from iron polymaltose complex in pharmaceutical dosage formulations.

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ORIGINAL PAPER

VERIFICATION OF FOLK MEDICINAL POTENTIALITY FOR SOME COMMON PLANTS IN JORDAN

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ABSTRACT:

87 species belonging to 59 genera and 33 plant families were identified and presented in the area of study. The largest 3 families are: Lamiaceae (9 aquatic species), Asteraceae (7 species), and Salicaceae (7 species). The largest genera are Mentha (6 species), Polygonum (5 species), and Salix (5 species). 63 folk medicinal aquatic species (73.3%) have therapeutic similarities with neighbouring countries, while the 24 remaining species (26.7%) haven't such therapeutic similarity. Emerged species (living with close contact with water body) were the most recorded, while amphibious, submerged or floating species were the least.

The folk medicinal importance value of aquatic species recorded was identified according to Friedman parameters. 21 species (24%) have ROP values higher than 50, and therefore; have the highest popularity in folk medicinal potentiality. 26 species (29.9%) have therapeutic effects informed by less than three informants, and therefore, excluded from further consideration. 40 species (46.1%) have ROP values less than 50, and therefore; considered nonpopular medicinal plants.

Keywords: Rank order priority (ROP), relative popular level (RPL), therapeutic effects, ethnobotay, fidelity level (FL), medicinal plants.

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