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Determination Of Iron In The Marketed Dosage Forms And Supplements Using Colorimetric And Titrimetric Methods Of Analysis

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ABSTRACT:
A comparative study of the determination of iron in iron tablets was carried out using colorimetric and titrimetric methods of analysis. The work presented here reports on optimization of phenanthroline method. The maximum absorbance was found to be at 520nm wavelength. A calibration curve was found to be linear up to the concentration range of 0.8 µg/ ml to 3.60 µg/ ml and 10 µg/ ml. total three pharmaceutical samples from different pharmaceutical companies were analyzed and results were compared with the results obtained by standards. The study showed that the total iron concentration in pharmaceutical samples. These values are in good agreement with pharmacopeia range.

Key Words:
Colorimetry, Titrimetry, o-phnenanthroline, ferrous fumarate, ferric ammonium citrate, ferric carboxy maltose, ferrous sulphate, ferrous ammonium sulphate, iron, concentration, absorbance, calibration curve, wavelength.

INTRODUCTION
IRON is a chemical element with the symbol Fe and atomic number 26. Iron makes up 5% of the Earth's crust and is second in abundance to aluminium among the metals and fourth in abundance among the elements. Physiologically, it. exists as an ion in the body. Iron (as Fe2+, ferrous ion) is a necessary trace element used by all known living organisms. Iron-containing enzymes, usually containing Heme prosthetic groups, participate in catalysis of oxidation reactions in biology, and in transport of a number of soluble gases[1]. Iron is an essential constituent of hemoglobin, cytochrome, and other components of respiratory enzyme systems. Its chief functions are in the transport of oxygen to tissue (hemoglobin) and in cellular oxidation mechanisms. Inorganic iron involved in redox reactions is also found in the iron-sulfur clusters of many enzymes, such as nitrogenase (involved in the synthesis of ammonia from nitrogen and hydrogen) and hydrogenase[2]. A class of non-heme iron proteins is responsible for a wide range of functions such as ribonucleotide reductase (reduces ribose to deoxyribose; DNA biosynthesis) and purple acid phosphatase (hydrolysis of phosphate esters). When the body is fighting a bacterial infection, the body sequesters iron inside of cells (mostly stored in the storage molecule ferritin) so that it cannot be used by bacteria. Depletion of iron stores may result in iron-deficiency anemia[3].

Properties of Iron
At room temperature, iron is in the form of ferrite, or α-iron, a body-centered cubic structure. The density of α-iron is 7.86 g/cc. At 910°C it changes to γ-iron, which is face-centered cubic and somewhat softer. At 1535°C iron melts, and boils at 3000°C. For more information on iron structures and the iron-carbon phase diagram, Cobalt melts at 1480°C, nickel at 1455°C[4]. The specific heat of any of the three metals is about 0.107 cal/g-K. The thermal conductivities of Fe, Co and Ni are 3.37, 3.81 and 4.19 cal/s-cm-K. Their electrical resistivities are 9.71, 6.24 and 6.84 µ Ω-cm. These are "worse" than those of copper by factors of only 4 to 6, so the iron metals are very good conductors of electricity and heat. Comparing the numbers shows how similar these metals are in their physical properties. I have not heard whether cobalt and nickel make useful alloys with carbon, as iron does. They are much too expensive to use as structural metals, other than as alloying elements or coatings.

Iron and Blood

Living cells oxidize glucose with atmospheric oxygen, and release carbon dioxide as a result. A cell bathed in water can easily dispose of the carbon dioxide, since it diffuses through the cell wall to the water in which it is very soluble, 1800 cc per litre of water. Oxygen diffuses into the cell from the water where it dissolves to the extent of 50 cc per litre. This is a small, but sufficient concentration. More oxygen would probably be deleterious. A small cell is a colloid, dominated by surface area, not volume. A cell must be small for this strategy to be successful, so most cells and simple organisms are microscopic[6].

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When cells associate in a multicellular organism, the support of respiration by provision of oxygen and carrying-away of carbon dioxide must be arranged. Coelenterata, with an inside and an outside and specialized cells, set up a flow of water through their body that brings in oxygen, and nutrients, with the fresh water, and discharges carbon dioxide and other waste product with the efflux[7].

More complex organisms, such as ourselves, consist of a large assembly of cells that must respire, though out of water and packed tightly against each other. To supply oxygen and eliminate carbon dioxide, a circulatory system is required where a fluid transports the substances between the working cells and organs that communicate with the atmosphere, the lungs. In the atmosphere, the partial pressure of carbon dioxide is very low (0.3 torr), and the partial pressure of oxygen is high, 152 torr[8].

Signs and symptoms

Symptoms of iron deficiency can occur even before the condition has progressed to iron deficiency anemia.

Symptoms of iron deficiency are not unique to iron deficiency (i.e. not pathognomonic)[9,10]. Iron is needed for many enzymes to function normally, so a wide range of symptoms may eventually emerge, either as the secondary result of the anemia, or as other primary results of iron deficiency. Symptoms of iron deficiency include:

- fatigue
- dizziness/lightheadedness
- pallor
- hair loss
- twitches
- irritability
- weakness
- pica
- brittle or grooved nails
- hair thinning

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AIM AND OBJECTIVES

India is one of the countries with a very high prevalence of anemia in the world, affecting almost 75% of the Indian population. Iron supplements are the mainstay of therapy. They are also used for prophylaxis of iron deficiency anemia. There is an increasing concentration over the large- scale availability of various iron supplements as fixed dose combinations with other vitamins/minerals/antioxidants as 'over the counter' agents thereby escalating cost of treatment.

Here our aim is to determine the number, composition, and pharmacological rationale of the various iron supplement formulations available in the Indian market.

Methods:

A novel, safe and sensitive method of spectrophotometric estimation inUV-region has been developed for the assay of iron sucrose injection formulation. The method has been developed and validated for the assay of iron sucrose injection using concentrated HCl 37%, ammonium acetate buffer 32%, hydroxylamine HCl 10%, o-phenanthroline 0.1% and water as diluents. These chemicals do not show any interference in spectrophotometric estimations. Ammonium iron III material used as standard. All the parameters of the analysis were chosen according to ICH guideline and validated statistically.

A Simple, rapid, accurate, economical and reproducible spectrophotometric method has been developed for quantitative estimation of Iron in tablet.

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Procedure:

Method obeys Beers law at concentration range 1-10µg/ml. The iron content was found to be as 99.9. The proposed method was validated statistically and recovery studies. A comparative study of the determination of iron in iron tablets was carried out using spectrophotometric and atomic absorption spectrometric method (AAS). Spectrophotometric method is based on the formation of ferrous tris-o-phenanthroline complex by boiling with hydroxylamine hydrochloride and subsequent addition with 1, 10 - phenanthroline at pH ~ 3±0.2. The work presented here reports on optimization of phenanthroline method. The maximum absorbance was found to be at 510 nm wavelength.

A calibration curve was found to be linear up to the concentration range of 0.2 mg/L to 1.6 mg/L. Total four pharmaceutical samples from different pharmaceutical companies were analyzed and results were compared with the results obtained from atomic absorption spectroscopy. The study showed that the total iron content in pharmaceutical samples ranged from 50 to 54 mg per 60 mg. These values are in good agreement with Pharmacopeial range.

The aim of this investigation was to develop a spectrophotometric method in the visible range for quantitative determination of Fe3+ in a pharmaceutical formulation based on complexation with methylthymol blue. The reaction occurred at a temperature of 30° and the absorbance remains constant after 25-30 min; the blue colored resulting complex had a maximum absorption at 628 nm.

Concentration area of (2-6) µg/ml; the calibration curve is described by the regression line A= 0.1025° (µg/ml)–0.0013 with a correlation coefficient of R2= 0.9997. For the validation of the method the following parameters were studied, linearity, accuracy, limit of detection, limit of quantification and retrieval. Confidence interval of the average retrieval ranged from 98.79 to 101.13 %. The non-interference of excipients in the solution makes the method suitable for routine dosage of iron in polymaltose complexes This visible spectrophotometric method was applied to determine Fe3+ concentration in polymaltose iron complex. The validation parameters confirmed that this method could serve to determine $Fe3^{+}$.

s fumarate, Ferric carboxy maltose dil.H_2SO_4	s sulphate, ferrous fumarate tablet.
d water	mmonium citrate syrup. Starch
mmonium citrate.	carboxy maltose injection.
ylamine hydrochloride.	SO_4, distilled water.
henanthroline, ferrous ammonium sulphate.	potassium permanganate, HCl

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n acetate buffer, glacial acetic acid.	eric ammonium sulphate, ferroin solution.
Cl lium acetate	odium thiosulphate, potassium iodide.

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 thiocyanat concentration on the determination of iron in drugs containing iron have been investigated.

The λmax was 430 nm and the molar absorptivity of 0.0399 L mol-1 cm-1. The linear regression was in the range 0.5 - 60µg/mL for iron content in hemoglobin. The detection limit and the limit of quantification were found to be 0.040 and 0.122µg 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.

TITRIMETRY METHOD-1 (Ceriometry)
Procedure for standard: (standardization of 0.1M CAS)

- Weigh accurately about 0.2g of arsenic trioxide, previously dried at 105^{o}Cfor 1hr, and transfer to a 500ml conical flask.
- Wash down the inner walls of the flask with 25ml of a 8.0% w/v solution of sodium hydroxide, swirl to dissolve, add 100 ml of water and mix.
- Add 30ml of diluted sulfuric acid, 0.15ml of osmic acid solution, 0.1ml of ferroin sulphate solution and sowly titrate with the ceric ammonium solution until the pink colour is changed to a very pale blue, adding the titrant slowly towards the end point.

Each ml of 0.1M ceric ammonium sulphate equivalent to 0.004946g of arsenic trioxide

Procedure for assay:

- Take 10 tablets and weight each one.
- Powder the tablets with the help of motor and pestle.
- Weight 0.667g of ferrous fumarate powder and add 15ml of diluted sulphuric acid (H_2SO_4)
- Heat and cool the solution and add 50ml of distilled water.
- To this solution, add 1/2drops of ferroin, and titrate against 0.1M CAS. We can observe blue colour as end point.

1ml of 0.1M CAS is equivalent to 0.01699g of ferrous fumarate.

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RESULTS AND DISCUSSION

COLORIMETRY:

Determination of λ max:

Colorimetric Method-1:

Determination of λ max using Colorimetric Method 1

length (nm)	rbance

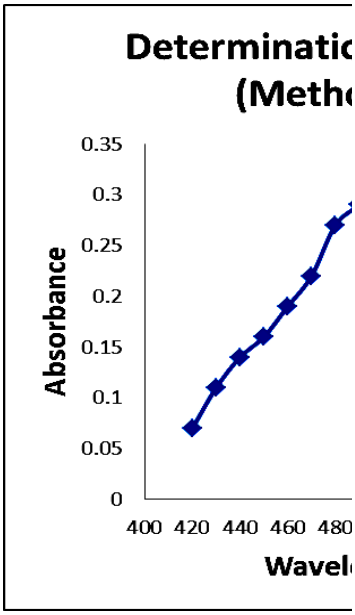

Figure 5.1: Absorption Curve of Colorimetric Method 1

5.1.2 Colorimetric Method-2:

Table 5.2: Determination of λ max using Colorimetric Method 2

length (nm)	rbance

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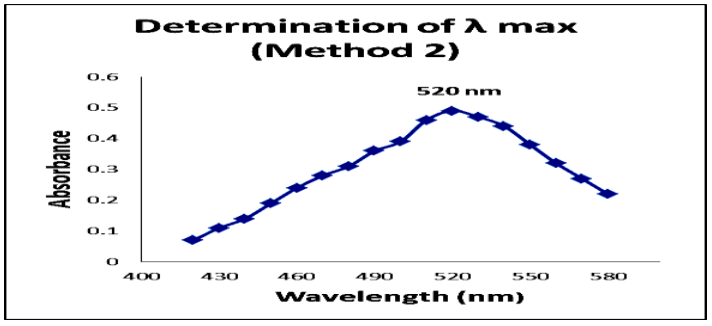

Figure 5.2: Absorption Curve of Colorimetric Method 2

5.1.3 Colorimetric Method-3:

Table 5.3: Determination of λ max using Colorimetric Method 3

length (nm)	rbance

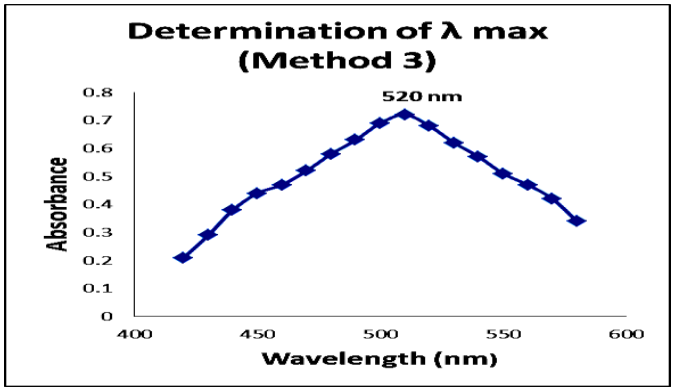

Figure 5.3: Absorption Curve of Colorimetric Method 3

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Colorimetric Method 3:
Absorption determination at 520 nm (3.0µg/ml)

ɾation/ Dosage form	**s**	**on**
ɪrd		
e 1		
e 2		

f dosage form		**d Amount (mg)**	**ɪt**	**Found**	**ay**
	n-Z	tablet			%
	range	15mL			
on	rb	1mL			

ical Analysis

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CONCLUSION:

We were able to determine the concentration of the Unknown sample by finding the absorbance of known standards and plotting them on a standard curve. The unknown concentration Fell within the concentration of the known standards. Once the Standard curve was plotted a line of best fit was determined, the correlation coefficient was high and positive for both methods showing their good applicability for iron estimation. Colorimetric method was found to be more sensitive and also gave more precise and replicate results than the Titrimetric method.

Conflict of Interest: The authors expressed no conflict of interest.

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