Salahaddın Unıversity-Erbil

Spectrophotometric determination of iron in pharmaceutical formulation

Research Project

Submitted to the Department of (Chemistry) in partial fulfillment of the requirements for the degree of BSc. in chemistry

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Abstract

The aim of this study is to determine the iron content using a fast and accurate method for quality control of some imported dietary supplements, based on spectrophotometric measurement of iron after complexation with 1, 10-phenanthroline in an acidic medium. Eight types of vitamin supplementary tablets were randomly collected from the Libyan market and analysed for the iron content. The analysis showed an average value of 61mg Fe/pill for the range of 40.07-112.63 mg Fe/pill. Results showed that 75% of the samples were lower in iron content than that recorded on the dietary products.

Keywords: Dietary Supplement; Spectrophotometer; Iron content; Libyan Market. (Normal Flow and Stopped Flow Injection Spectrophotometric Determination of Quercetin Dihydrate Dietary Supplements, 2017)

1. Introduction

1.1 Physical and chemical properties

Iron is a chemical element with symbol Fe (from Latin: ferrum) and atomic number (26). It is a metal that belongs to the first transition series and group 8 of the periodic table. (Office of Dietary Supplements - Iron, 2022). Iron have a melting point (1538 °C) and boiling point (3000 °C) and oxidation states +2, +3, +4, +6. (Iron - Wikipedia, 2022)

Iron is a lustrous, ductile, malleable, silver-grey metal. It is known to exist in four distinct crystalline forms. Iron rusts in damp air, but not in dry air. It dissolves readily in dilute acids. Iron is chemically active and forms two major series of chemical compounds, the bivalent Iron (II), or ferrous, compounds and the trivalent Iron (III), or ferric, compounds (Iron (Fe) - Chemical properties, Health and Environmental effects, 2022)

Iron, like other metals, conducts heat and electricity, has a lustre, and forms positive ions in its chemical reactions. Pure iron is fairly soft and can easily be shaped and formed when hot. Iron is easily magnetized. When combined with small amounts of carbon, it becomes steel. In the presence of moisture, iron rusts quickly because it combines easily with oxygen in the air. (iron, 2022)

Iron is very active metal; it reacts with other elements to form compounds of iron. Iron can occurs in compounds both ferric compounds and in ferrous compounds. Some of the important iron compounds are: Ferric acetate $Fe(C_2H_3O_2)_3$, used in the dyeing of cloth, Ferric ammonium oxalate $(Fe(NH_4)_3(C_2O_4)_4$, known as blue spirit, Ferric Chloride ($FeCl_3$), used as water purification and sewage treatment system, Ferric Chromate ($Fe_2(CrO_4)_3$, yellow pigment(coloring) for paints and ceramics , Ferric hydroxide $Fe(OH)_3$, is a brown pigment for coloring rubber; water purification system, Ferric phosphate ($FePO_4$) is a Fertilizer; additive for animal and human foods, Ferrous acetate $[Fe(C_2H_30_2)_2]$, is used in dyeing of fabrics and leather; wood preservative, Ferrous gluconate, as a dietary supplement in “iron pills”, Ferrous oxalate (FeC_2O_4)is a yellow pigment for paints, plastic, glass etc., Ferrous sulfate(FeS04), is a water purification and sewage treatment system. (Auwal Balarabe and Zainab Folashade, 2019)

Iron is essential to almost living things, from micro-organisms to humans. (Iron (Fe) - Chemical properties, Health and Environmental effects, 2022)

1.2 Iron in the human body

Iron has several functions in the human body, all contributing to good health and proper functioning. Iron has several functions in the human body, all contributing to good health and proper functioning. Our body needs for the normal function of our immune system, metabolism and oxygen transport. (Iron functions in our body - Active Iron, 2022)

The body of an adult human contains about 4.0 grams (0.005% body weight) of iron, mostly in haemoglobin and myoglobin. These two proteins play essential roles in vertebrate metabolism, respectively oxygen transport by blood and oxygen storage in muscles. To maintain the necessary levels, human iron metabolism requires a minimum of iron in the diet. Iron is also

the metal at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals. (Iron - Wikipedia, 2022)

For almost all living organisms iron is an essential trace element. Iron is not eliminated by any physiological processes, and its uptake, transport and storage is highly regulated. It is so important to living organisms that iron deficiency due to an uncontrolled loss can lead to cell damage, and eventually even death. (Bolm, 2022)

When the amount of iron is too low in our body, a condition known as iron deficiency. People with iron deficiency cannot produce an adequate amount of hemoglobin to meet their body's oxygen-transport needs. Most people are iron deficiency and iron plays such a crucial role in the body, it is important for us to maintain an adequate supply of iron. Our bodies continually lose iron through everyday processes such as urination, defecation, sweating, and sloughing off skin cells. Bleeding contributes to further loss of iron from the body. To compensate for these losses and to maintain an adequate supply of iron, we should consume approximately 18 mg of iron daily. (Auwal Balarabe and Zainab Folashade, 2019)

1.3 Function of iron

Iron is a vital mineral for our body. It helps to:

- Increase energy production.
- Reduce fatigue.
- Improve your cognitive function.
- Keep your immune system strong.
- Transport oxygen in your body.
- Create red blood cells.
- Maintain healthy cell division (Iron functions in our body - Active Iron, 2022)

1.4 Types of iron in our diets.

There are two types of iron found in our diets

1- Haem iron – found in animal tissue such as beef, lamb, kangaroo, chicken and fish. Offal products such as liver and kidney are particularly rich in haem iron (however pregnant women should avoid eating too much offal as it contains large amounts of vitamin A, which can cause birth defects). This form of iron is most easily absorbed by the body.

2- Non-haem iron – found in animal tissue, animal-based products and plant foods such as dried beans and lentils. Good vegetarian sources of non-haem iron include iron-fortified breakfast

cereals, wholegrains and legumes (beans and lentils). If you are vegetarian and have no animal tissue in your diet, you may need almost twice as much dietary iron each day as non-vegetarians. Plant-based sources of iron include: dark green leafy vegetables such as broccoli, raisins, nuts, prunes, dried apricots, seeds, dried beans and peas, and iron-fortified cereals, breads and pastas

Iron absorbed from our diet depends on how much iron stored our body. The healthy body absorbs around 18% of the available iron from a typical western diet (which includes animal foods) and about 10% from a vegetarian diet. However, you may be absorbing much less than that, even if your diet includes iron-rich foods.
The most significant influence on iron absorption is the amount of iron already stored in our body. The body stores iron in various places, including the liver. If our stores are high, our body absorbs less iron from the foods we eat. Conversely, low iron stores increase our ability to absorb iron.
(Iron and iron deficiency - Better Health Channel, 2022)

1.5 Treatment for iron deficiency

There are two way for treatment of iron deficiency depends on our iron status, and the underlying cause (9) and (12):

1- Healthy foods that are high in Iron such as

- Shellfish
- Spinach
- Liver and other organ meats
- Legumes
- Red meat
- Pumpkin seeds
- Quinoa
- Turkey
- Broccoli
- Tofu
- Dark chocolate
- Fish (12 Healthy Foods That Are High in Iron, 2022)

2- Iron supplements

1.6 Spectrophotometer

Spectrophotometry is an experimental technique that is used to measure the concentration of solutes in a specific solution by calculating the amount of light absorbed by those solutes. This technique is powerful because certain compounds will absorb different wavelengths of light at

different intensities. By analysing the light that passes through the solution, you can identify particular dissolved substances in solution and how concentrated those substances are. (2022)

- Advantages of UV Visible Spectroscopy:

- The core advantage is the accuracy of the UV-VIS spectrophotometer
- The UV-VIS spectrometer is easy to handling and use
- Provide robust operation
- UV-VIS spectroscopy is simple to operate
- Cost effective instrument
- Cover the entire of ultraviolet and visible
- It can be utilized in the qualitative and quantitative analysis
- The Derivative graph can be obtained by UV-VIS spectrophotometer
- It can be used in the degradation study of drug
- Only possible for the Analytes which have a chromophore

- Disadvantages of UV Visible Spectroscopy:

- Only those molecules are analysed which have chromophores
- The results of the absorption can be affected by pH, temperature, contaminants, and impurities.
- Only liquid samples are possible to analyse
- It takes time to get ready to use it
- Cuvette handling can affect the reading of the sample. (Spectroscopy and Patil, 2022)

2. Literature Review

Analytical techniques were used for determination of iron in pharmaceutical products.

A direct, rapid and selective Sequential Injection Analysis (SIA) method for determination of iron in pharmaceutical products is proposed. The method is based on monitoring the absorbance at 523 nm due to the complex formed between Fe (II) and 2,2-bipyridyl (α ,α-bipyridyl) in acetate buffer at pH 4.5. Commercial formulations without turbidity were analysed directly after appropriated dilution. The results presented good agreement with the values cited in the manufacturer's label and determined by flame atomic absorption spectrometry (FAAS). The method presented a linear dynamic range from 5.0 to 40.0 mg/ L (r = 0.999) and a detection limit of 0.97 mg/ L, with a sampling frequency of 100 h . 2001 ((2022)

A simple semi-automated spectrophotometric method for determination of Iron (II) is described. The method based on the measurement of absorbance of Iron (II) - 1, 10-Phenathroline complex. The method is sensitive and requires no sample pre-treatment and Iron (II) solution can be analysed

in the range (1.0 - 25) mg/L and a rate of 75 sample/h with R.S.D. % of about 0.06%. The proposed method was allows the determination of Iron (II) in pharmaceutical preparation with satisfactory accuracy with comparing with official BP method. 2003 (

A rapid, sensitive and simple spectrophotometric method was developed for the determination of iron (II). The method was based on the reaction of iron (II) with alizarin red sulphonate reagent to form a brown chelating complex in an aqueous solution. The absorbance of the chelating complex was measured at 566 nm with a molar absorptivity of 7.8×10^3 $L.mol^{-1}.cm^{-1}$. The chelating complex conforms to Beer's law over the range (0.5-5) mg/L. The average recovery % was 100.06% and Precision (RSD) was found to be less than 1.0%. The method was successfully employed for assay of Iron (II) in pharmaceutical formulations (tablets and capsules). The results have been compared with British pharmacopoeia method. 2012 ((2022)

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 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 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 - 60 mg/L for iron content in haemoglobin. The detection limit and the limit of quantification were found to be 0.040 and 0.122 mg/L for the Iron respectively, and with a linear regression correlation coefficient of 0.998. Recovery measurements ranged from 99.63 - 100.20 % . 2017 (2022)

Two different computer vision-based analytical chemistry (CVAC) methods were developed to quantify iron in the commercial pharmaceutical formulations and Ferro sanol. The methods involve using a digital camera or a desktop scanner to capture a digital image of a series of Fe^{2+} standard solutions and the unknown sample upon reaction with *o*-phenanthroline. The images are processed with appropriate software (e.g., the public domain programme ImageJ, from NIH) to obtain a numerical value (analytical signal) based on colour intensity. The fact that such a value is proportional to the analyte concentration allows one to construct a calibration graph from the standards and interpolate the value for the sample in order to determine its concentration. The results thus obtained were compared with those provided by a spectrophotometric method and the US Pharmacopoeia's recommended method. The differences never exceeded 2% . 2018 (Solana-Altabella et al., 2022)

An innovative, flow-through, double-beam, photometric detector with direct injection of the reagents (double-DID) was used for the determination of iron in pharmaceuticals. The total Iron was determined as a Fe (II) with photometric detection using 1, 10-phenanthroline as a complexing agent. The optimum conditions of the propose analytical procedure were established and the method was validated. The calibration graph was linear in the range of 1 - 30 mg/L. The limit of detection (LOD) was 0.5 mg/L. The throughput of the method was 90 samples/hour. The repeatability of the method expressed as the relative standard deviation (R.S.D.) was 2.0 % (n = 10). The method was successfully applied for determination of iron in pharmaceutical products. 2021 (Koronkiewicz, 2021)

3. Spectrophotometric determination of iron in dietary Supplements in Libyan market (2022)

3.1 Materials

All chemicals used in this research were of high purity and deionized water used, 0.2 % of 1,10- phenanthroline (Aldrich 99%), 6 M hydrochloric acid (Aldrich 37%), 10 % sodium acetate (BDH, AnalaR), 10 % hydroxylamine hydrochloride (Chem service inc), ferrous ammonium sulfate hexahydrate (BDH).

3.2 Procedure

Eight types of supplementary tablets (iron or iron-folic acid base) were purchased from the Libyan market; ten tablets of each type were weighed individually to calculate the average weight of one tablet. The tablets were ground manually using an agate Mortar and pestle. Digestion was performed similar to that reported (Atkins 1975). To prepare the stock solution (A), in three replicates 0.10 g of the crushed tablets were placed in 150mL beaker, boiled gently in a fume hood with 25mL of 6M HCl for about 15 minutes while covered with a watch glass. Deionized water was added when necessary to keep a constant volume (15mL). Finally the watch glass was rinsed with water, and the solutions were then filtered directly through Whatman-40 filter paper receiving the filtrates in 250mL volumetric flasks. The remaining precipitate was rinsed thoroughly with deionized water and completed to the mark with deionized water. For the measuring solution (B), to a 100mL volumetric flasks, 2mL of the stock solutions (A) were transferred and the reagents were added in the following amounts: 10.0mL of 10% hydroxylamine hydrochloride, 10.0mL of 0.20% 1, 10-phenanthroline and 8.0mL of 10% sodium acetate.

3.3 Standard solutions

A 10 mg/L Fe standard solution was prepared by accurately dissolving 0.0700g of pure ammonium ferrous sulfate $(NH4)_2Fe(SO_4)_2.6H_2O$ in 1000 mL volumetric flask using deionized water. From the standard solution, 0, 5, 10, 25 and 50mL aliquots were transferred each to 100mL volumetric flasks. The same amounts of the reagents added to the measuring solutions of the samples were added to the standard flasks to give a series of calibration solutions with Fe concentrations of: 0, 0.5, 1.0, 2.5 and 5.0 mg/L respectively. The 5.0 mg/L Fe solution was used to obtain λmax which was found to be 510 nm when fully scanned from 200 to 800nm Using GBC-Cintra 2020 UV-Visible spectrophotometer.

The calibration solutions were then measured quantitatively to gain the absorbance value for each solution. A calibration curve was then established showing a relation between the concentration (mg/L) and the absorbance using the excel program. The samples were measured at

the same conditions and their concentrations (Ci) were calculated from (Equation 1) obtained from the calibration curve (Figure 3.1). Finally, the iron concentration of each sample in mg/pill was then calculated from (Equation 2), which has been designed according to the dilution factor, the total volume and the sample weight.

$Y = 0.2033\ Ci$ (Equation 1)

Where Y = absorbance and Ci is the Fe concentration of each sample solution (mg/L).

$$F(mg/pill) = \frac{Ci \times 100 \times 0.250 \times pill\ weight(g)}{2 \times sample\ weight\ (g)}$$ (Equation 2)

Where Pill weight = average of 10 tables and Sample weight = weight of the crushed portion that was dissolved.

3.4 Results and discussion

3.4.1 Calibration curve

Figure 3.1 shows the calibration curve for iron concentration (mg/L) against the absorbance measured at 510nm. A linear relationship was obtained with a simple equation $Y = 0.2033Ci$, $R2 = 0.9971$ and LOD is 0.21mg/L.

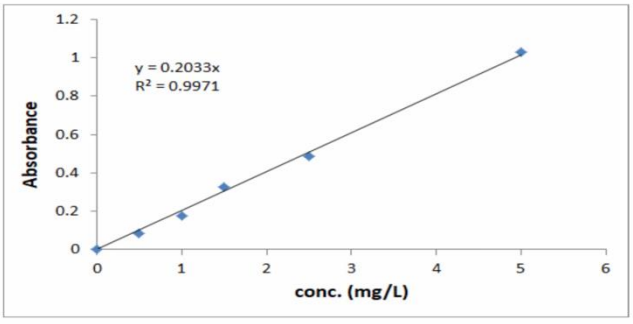

Figure 3.1 UV-Visible Calibration curve for Fe standard solutions

3.4.2 Recovery percent

Method validation was implemented by running standard solutions as samples and the recovery percent was obtained as shown in Table 3.2. From the obtained values one can see that the UV/Vis spectrophotometer is a suitable technique to measure the iron content in medicine tablets.

Table 3.2 method recovery percent

STD soln.	Theoretical value	Measured value	Recovery percent
STD 1	0.50	0.42	84%
STD 3	1.50	1.67	111.3%
STD 4	2.50	2.56	102.4%
STD 5	5.00	4.29	85.8%
Overall average			95.88%

3.4.3 Supplements iron content

The iron content in the analysed dietary supplements differs from one to another. The resulting concentrations ranged from 40.08 mg/pill to 112.63 mg/pill showing an average amount of 60 mg/pill iron, while the median was 50.6 mg/pill. There is a significant difference between the iron content recorded on the supplement pack and the actual measured value in almost all samples, apart from samples S4, S6 and S8 were compatible. A comparison of the obtained result (actual) and those recorded on the tablet container are shown in Figure 3.2.

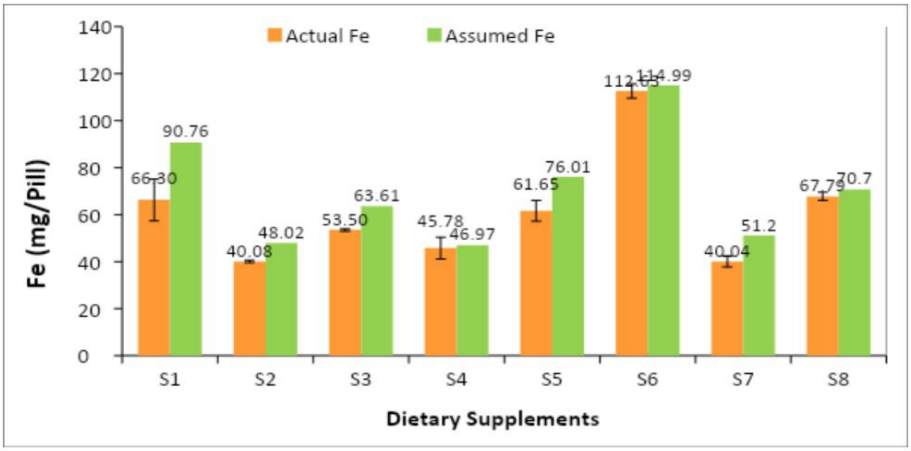

Figure 3.3 Comparison between assumed and actual Fe content (mg/pill)

The samples S1, S2, S3, S4, S5, S6, S7 & S8 showed a % difference of 36.9, 19.8, 18.9, 2.6, 23.3, 2.1, 27.9 and 4.3 respectively.

4.Conclusion

The analysis indicates that the dosage of iron inscribed on the container of the medicine is not equivalent to that measured. This difference may lead to serious health problems especially in anemic pregnant women. According to the WHO guideline report (WHO 2012), pregnant women which are clinically diagnosed as anemic should be initially cured with a higher daily dose of elemental iron (120mg) supplementation until the normal haemoglobin concentration is reached before switching to the standard antenatal dose to prevent repetition of anemia. Therefore, routine analysis of iron in dietary supplements is necessary to ensure that the correct amount of iron is provided.

Accordingly, the authority must take on advance the responsibility for the quality control of any imported medicine before reaching patients. Since there are serious side effects related to the iron content such as anemia, lethargy, heart palpitations and tinnitus low levels of iron while overdosing may be associated with appreciable morbidity and mortality cases. Multi system failure is common in lethal overdoses of iron (Gerald 1994).

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