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Evaluation of iron concentration in selected pharmaceutical ferofolic formulations available in pharmacies in Basra, Iraq: Are the concentrations in the labels accurate

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Abstract

Iron is necessary trace element found in nearly all living organisms, it is important for oxygen transport in the blood, through the hemoglobin. It is also present in some enzymes that catalyze reactions of cellular oxidation such as cytochrome, and catalase Iron deficiency anemia (IDA) is the commonest form of anemia worldwide and maybe due to Blood loss, dietary deficiency, malabsorption and pregnancy. There are many strategies for correcting iron deficiency in populations, Iron supplementation in the form of tablets or capsules is the most common strategy currently used to address iron deficiency in developing countries.

A number of iron preparations are currently marketed in Iraq such as ferrous, ferric, as well as various iron complexes, these products should contain only what is on the label including accurate iron concentration and should not contain any harmful or undesirable substances, such as toxic metals. Atomic absorption-based techniques have gradually assumed prime importance in verifying whether foodstuffs and pharmaceutical products comply with health requirements and/or national or international regulations, therefore AAS was used in this research to investigate whether labeled iron content in different ferofolic pharmaceutical formulations available in pharmacies in Basra, Iraq were accurate. The

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supplements were selected based on their popularity and frequent usage. In which ten commercial preparations (tablets or capsules) of ferofolic supplements from different companies for adult's consumption were analyzed for evaluating their content of iron, in order to preserve brand identity, specific marks of F.F. preparation were identified according to a code assigned in our lab(A,B,C,D,E,F,G,H,I,J). Each selected F.F. formulation was weighed individually and the total of ten tablets or capsules were weighed collectively then 1/10 of total collected weight were ground and crushed thoroughly, 6 sub-samples of each mixture were accurately weighed and used for the subsequent wet digestion method.

The samples were analyzed by Atomic Absorption Spectrophotometer and there were large variations between the measured iron content and the labeled amount found in brands A, B, G, F and J. while brands D, E, H and I have met BP specification and exhibited optimum percent of ferrous content with E being the most remarkable with percent content of 100% .

In general terms, the study evidenced that iron content reported by the manufacturer in labels of D, E, H and I ferofolic supplements were in agreement with the found values according to BP. On the other hand, significant differences in iron concentrations were found among A, B, C, F, G and J ferofolic supplements as compared with accepted BP percent limit.

Introduction

Iron is necessary trace element found in nearly all living organisms, it is important for oxygen transport in the blood, through the hemoglobin. It is also present in some enzymes that catalyze reactions of cellular oxidation such as cytochrome, and catalase [1].The balance of iron metabolism in healthy individuals

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predominantly reflects three variables: nutritional intake,iron loss, and current demand were the main source of iron in humans comes from the destruction of erythrocytes by macrophages of the reticuloendothelial system.[2][3]. Generally Iron deficiency canbe classified as severe iron deffieciencywhen the serum ferritin level isbelow 20–30 µg/L and mild-moderate ID if the serum ferritin level is below 70–100 µg/L.Iron deficiency anemia(IDA) is the commonest form of anemiaworldwide and maybe due to Blood loss, dietary deficiency, malabsorption and pregnancy.IDA may affect visual and auditory functioning and is weakly associated with poorcognitive development in children [4]

During pregnancy, fetalhepcidin controls the placental transfer of iron from maternal plasma to the fetal circulation. When hepcidin concentrations are low, iron enters blood plasma at a high rate. Whenhepcidin concentrations are high, ferroportin is internalized,and iron is trapped in enterocytes, macrophages, andhepatocytes [2] , so more external iron is required to balance increased demandfor iron especially with physiological requirements duringgrowth, pregnancy, and lactation. [6]. Anaemia of pregnancy is generally definedasHb<110 g/L or <115 g/L in some clinical practiceguidelines with a slight variation according to the trimester ofpregnancy [7]. The total iron loss associated with pregnancy andlactation is approximately 1000 mg, Therefore therecommended daily dietary allowance for iron in pregnancy is 27 mg instead of 8 mg in the adult nonpregnantpopulation. Lactation requires a daily dietary allowanceof 10 mg. [6]

There are many strategies for correctingiron deficiency in populations, which can be usedalone or in combination: education combinedwith dietary modification or diversification toimprove iron intake and bioavailability; ironsupplementation; and iron fortification of foods.Iron supplementation in the form oftablets or capsules is the most common strategycurrently used to address iron deficiency in developing

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countries since change of dietary practicesand preferences is difficult and foods that providehighly bioavailable iron (such as meat) areexpensive.[5].

A number of iron preparations are currentlymarketed in Iraq such as ferrous, ferric, as well asvarious iron complexes, which are being used to treat iron deficiency and these products should containonly what is on the label including accurate iron concentration and should not contain any harmful orundesirable substances, such as toxic metals.

The World Health Organization (WHO) estimates that 39% of children younger than 5 years, 48% of children between 5 and 14 years, 42% of all women, and 52% of pregnant women in developing countries are anemic[8].

The analysis of dietary supplement is a challenge because they have a complex matrix and contain many elements in a wide range of concentrations. Atomic absorption-based techniques have gradually assumed prime importance in verifying whether foodstuffs and pharmaceutical products comply with health requirements and/or national or international regulations because they can be applied to all metals measurements and possess high sensitivity and appropriate detection power and it is analytical procedure for the quantitative determination of elemental iron in the products. The present study was designed to investigate whether labeled iron content in different ferofolic pharmaceutical formulations available in pharmacies in Basra, Iraq were accurate. Thesupplements were selected based on their popularity and frequentusage.

Materials and method

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Ten commercial preparations(tablets or capsules) of ferofolic supplements from different company for adult's consumption were analyzed for evaluating content of iron. Such supplements were purchased in 2015 from different pharmacies in Basra, Iraq. To preserve brand identity, specific marks of F.F. preparation were identified according to a code assigned in our lab(A,B,C,D,E,F,G,H,I,J). Table 1 shows a detailed description of the composition of the F.F and their origin as indicated on each Label. Each selected F.F. formulation was weighted individually and the total of ten tablet or capsules were weighed collectively then 1/10 of total collected weight were ground and crushed thoroughly, 6 sub-samples of each mixture were accurately weighed and used for the subsequent wet digestion.

Subsequently, subsamples were digested by wet digestion methodusing acid mixture (3.0 ml HNO_3:1.0 ml HCl+16ml D.W.) then heated at 100°C on a hot plate for 50 min, then allowed to cool and the mixture volume was completed to 800 ml with D.W. and kept in refrigerator for iron analysis

Setting up the atomic absorption spectrophotometer

It is important to ensure that the instrument is properly adjusted to provide the optimum conditions for iron measurement. The instrument was equipped with iron hollow cathodeLamp, the wavelength was adjusted to 248.3nm, the flame was ignited by turning on the regulators for the air and the acetylene fuel. Distilled water were aspirated through the nebulizer for about 10 minutes to allow the instrument to warm up. Then Adjustment of aspiration rateto optimize the flow for the iron determination.

Samples iron analysis

A series of iron standards were prepared from stock solution provided from BUCK company(1000PPM) as shown in figure1. Liquid sampleswere aspirated into a flame via a nebulizer. In the nebulizer, the sample is converted to a mist, and the

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droplets of the mist are easily burned in the flame, which serves as the sample cell. The flame provides a source of neutral atoms or molecules to absorb energy, and acts to desolvate and atomize the sample for successful measurements.

Reading Concentration = Measured concentration *dilution factor

Statistical analysis

Data are presented as Mean ± SD and all the statistical calculations were carried out using one way analysis of variance (ANOVA), supported by Bonferroni's post hoc analysis. Values with $P<0.05$ were considered significantly different. Analysis was performed using GraphPad Prism software for Windows, version 5.0 (GraphPad Software, Inc., San Diego, CA).

Cod	Labeled content(iron conc. in mg/cap)	Origin
A	48.75	India
B	47	Jordan
C	47	Iraq
D	47	Germany
E	47	UAE
F	150	Syria
G	48.75	Egypt
H	27	England
I	20	England
J	48.75	India

Table1Elemental iron content in different ferofolicformulations(tablets or capsules) as found in label description

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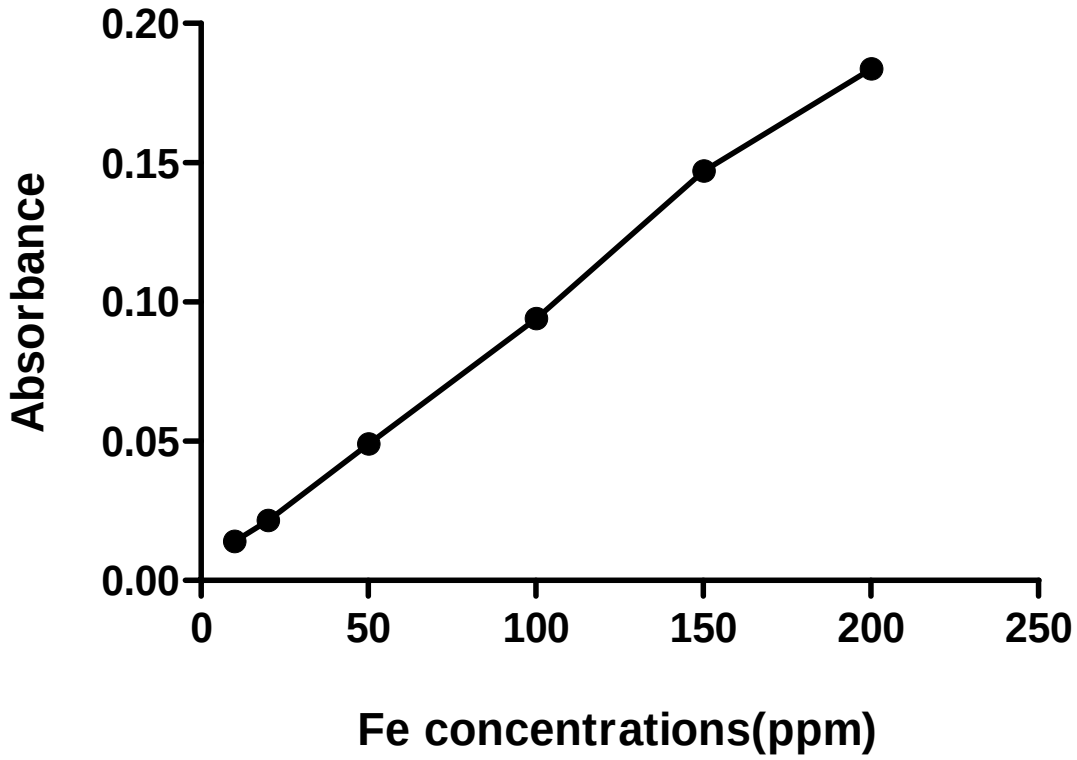

Figure(1):Iron calibration curve as measured by Atomic Absorption Spectrophotometer.

Results

The labeled contents of iron ranged between 20-150 mg/dose elemental iron in the form of various salts. The formulation of the brands that were tested, were found as spansules filled in a capsule. After analysis of the sample preparations, alot of variations in the content of the different types of the formulations were observed.

Formulations of D, H and I have met BP specification and exhibited optimum percent of ferrous content where D , H ,I exhibited percent content of 97.9% , 99.4% and 98.3% respectively. Brands A, B, C, E, F. G and J exhibited significant difference from their labeled amount. The result of C brand indicate that the iron

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content only marginally crossed the specified labeled content and gave a percent content of 107.4%. F, J showed the most difference in their content from their labeled amount with a percent content of 71.6% and 71.8% respectively. these two brands showed the lowest iron content of all the brands that were tested.

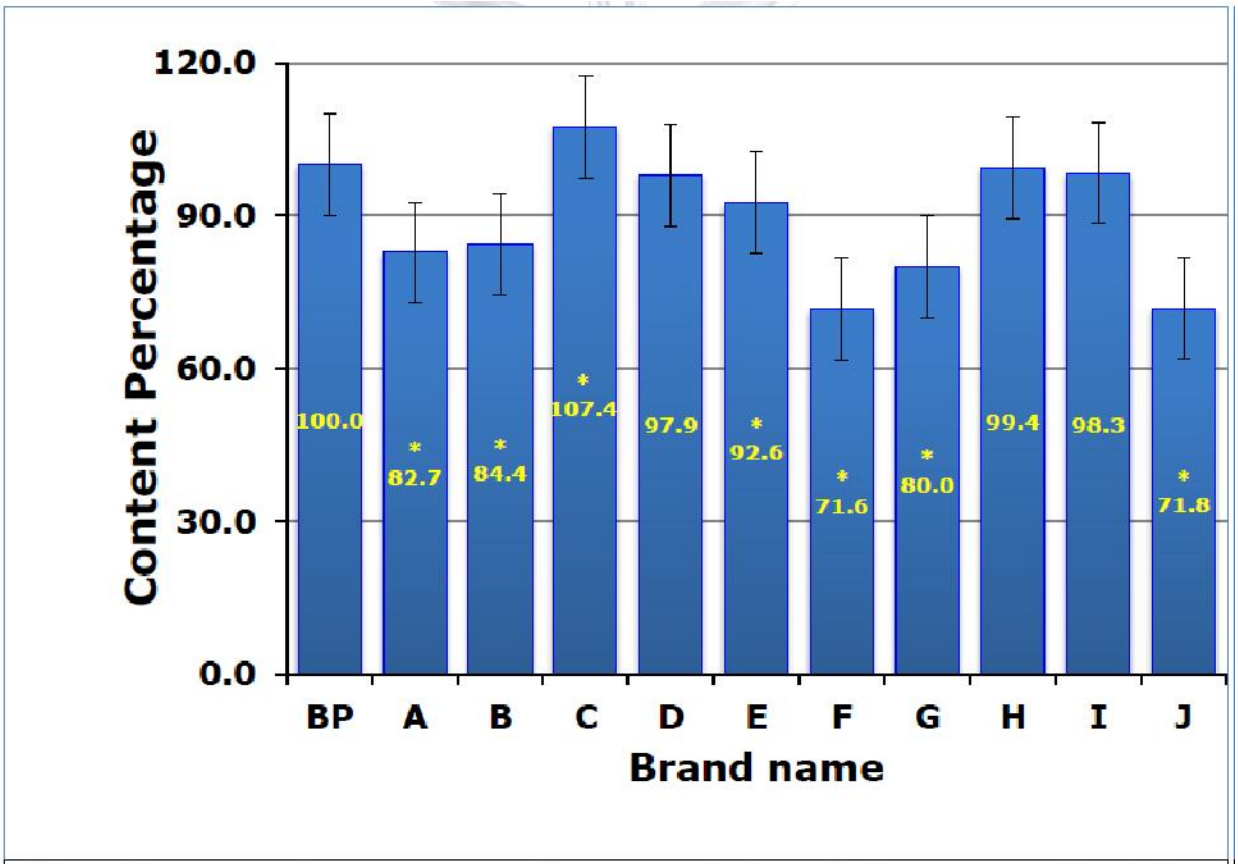

Figure (2): percent of iron content in different ferofolic formulation as compared with BP accepted limit. *Indicate out of range according British pharmacopoeia 2007

Measured and labeled iron concentrations per dosage forms of different ferofolic preparations were clearly summarized in figure (3). The highest elemental iron level observed in sample F while lowest concentration observed in sample I.

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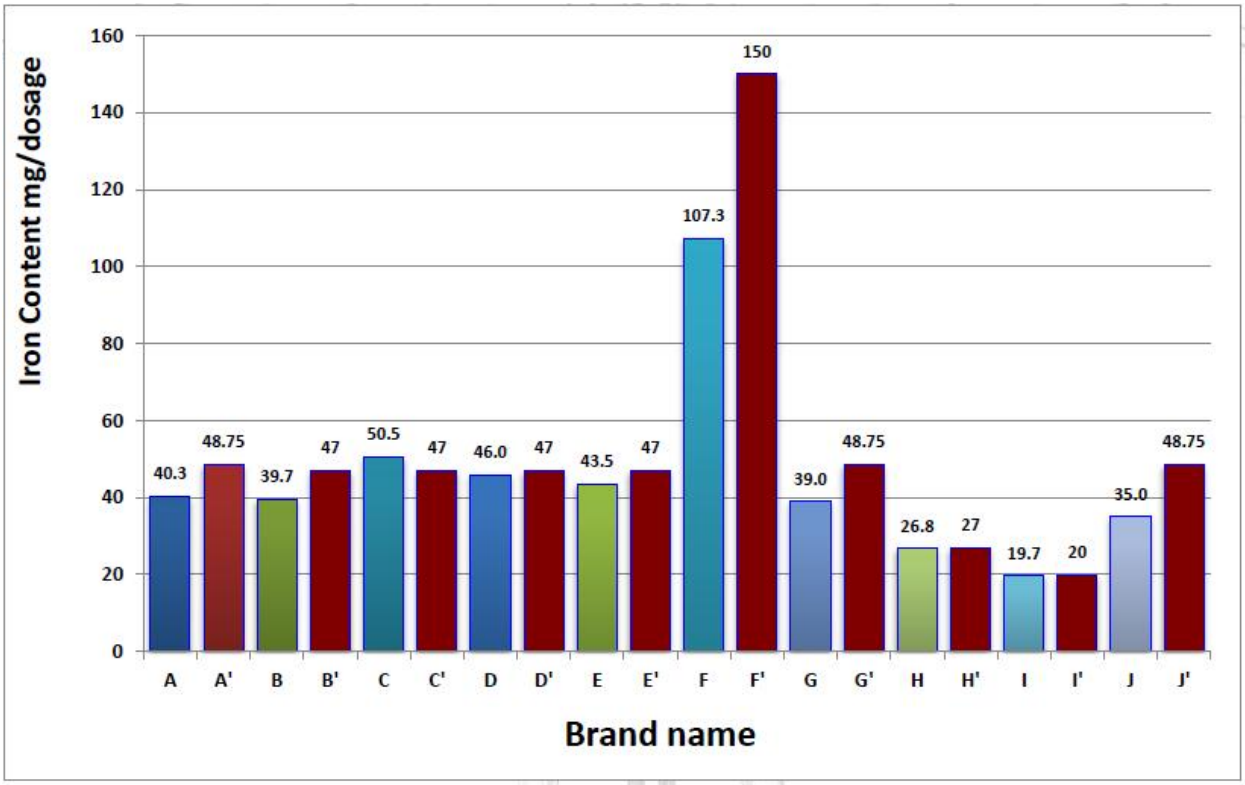

Figure (3) Mean iron concentration in mg of 6 subsamples in various ferofolic formulations available in pharmacies in Basra, Iraq. ' indicate labeled content 2007

Discussion

Trace elements, such as zinc (Zn), Copper (Cu), iron (Fe), selenium (Se) and chromium (Cr) arenecessary for normal growth and normal physiologic function where deficiency or excess of theseelements resulted in variety of disorders [9].The daily and continuous use of these products by adults became usual and its became necessary toensure their quality so the need for the analysis of trace elements in pharmaceuticals is becomingincreasingly more important, both from product quality and patient safety perspectives. The analyticalchallenges associated with sample matrix makes the selection of the method for sample preparationthe

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key to successful analysis. The wide variety of instrumental techniques available, ranging fromflame and graphite furnace AA to newer technologies, such as ICP-MS, makes it possible to monitorall elements at concentrations ranging from sub-ppb's to ppb and ppm. In this study the selection ofacid digestion method came in tune with different analysis study[10].Generally digestions can be performed in a number of ways: open vessel, closed vessel, microwaveassisted (mostly high temperature and high pressure), hot plate. Digestions typically involve the use ofan acid, in which nitric acid being the most commonly used for atomic spectroscopy applications;however, hydrochloric acid, sulfuric acid, and hydrofluoric acid are also used [11].

In the present study, Flame AAS used as an instrument for measurement of iron levels in differentpharmaceutical product that contain iron and folic acid. Despite the availability of more sensitive ormore versatile techniques for elements analysis, atomic absorption based methods are still often usedin the pharmaceutical industry for analysis of different metals [12].In contrast to this idea Marrero J et al, showed that Microwave assisted digestion method followed byanalysis by ICP MS is more accurate and reliable methodology for the determination of metals indietary supplements when compared with flame AAS[13].Valiente et al. reported a comparative study on the determination of Se in tablets of vitamins–minerals–aminoacids, nutritional by electrothermal atomic absorption spectrometry (ETAAS) andhydride generation-atomic absorption spectrometry (HG-AAS). The study evidenced that Se contentreported on the labels was often inaccurate. On the other hand Krawczyk M concluded that themeasured elements contents are in agreement with the certified values according to the t-test for a95% confidence level [14].

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5. Conclusion

In general terms, the study evidenced that iron content reported by the manufacturer in labels of D, Hand I ferofolic supplements were in agreement with the found values according to BP. On the otherhand, significant differences in iron concentrations were found among A, B, C, E, F, G and J ferofolicsupplements as compared with accepted BP percent li

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