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Optimization of Extraction and Measurement Methods in the Determination of Total Iron (Fe) Content in Anti-Anemia Multivitamin Capsule Samples

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ABSTRACT

Iron (Fe) is a transition metal that plays a critical role in human life, particularly as a micronutrient. The objective of this study is to optimize and ascertain the most appropriate method for measuring the total Fe content of anti-anemia multivitamin capsule samples, thereby ensuring the accuracy of the measurement results. The determination of total Fe content can be achieved through two metal extraction methods, namely wet digest and dry ashing methods, and two metal measurement methods, namely UV-Vis spectrophotometer and atomic absorption spectrophotometer (AAS). The findings revealed that the measurement of total Fe content by UV-Vis spectrophotometer through the dry ashing extraction method yielded a lower value (20.92 ± 0.29 mg/g) compared to the wet digest method (67.91 ± 0.83 mg/g). Furthermore, the Fe content determined by AAS analysis exhibited a reduced value in the dry ashing extraction method (1.42 ± 0.02 mg/g), while the wet digest extraction method yielded a substantially higher value (72.91 ± 4.12 mg/g). Statistical tests with the Duncan method revealed that the wet digest extraction method with UV-Vis spectrophotometer measurements is the most effective method for determining the total Fe content, with a significance level equivalent to the theoretical reference value (67.85 ± 0.03 mg/g).

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INTRODUCTION

Iron (Fe) is one of the abundant transition metals in nature, existing in two oxidation states, +2 and +3, and plays a crucial role as a micronutrient in biological aspects. The development of accurate Fe analysis techniques has become an important aspect due to the close relationship of Fe with fields such as environmental science, chemical industry, human physiology, and other domains. Common Fe analysis techniques include atomic absorption spectrophotometry (AAS), ion chromatography (IC), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), controlled-potential techniques, and UV-Vis spectrophotometry. The design and development of

spectrometric techniques for Fe analysis have attracted the attention of researchers due to their high selectivity and sensitivity, relatively low cost, simplicity, and low detection limits [1].

In this study, UV-Vis and AAS spectrophotometry techniques were chosen for the Fe analysis. UV-Vis spectrophotometry for Fe concentration determination utilizes a ligand that can form a colored complex with Fe ions in the sample. The type of ligand used depends on the oxidation state of the Fe ion being analyzed. The +2 oxidation state typically uses ligands such as 1,10-phenanthroline and 2,2'-bipyridine, which form colored complexes with maximum absorption at 512 and 522 nm, respectively. Meanwhile, one of the commonly used ligands for Fe with the +3 oxidation state (Fe^{3+}) is thiocyanate (SCN^-), which forms a stable complex with a maximum absorption at 480 nm, thus representing the total Fe concentration after all Fe in the sample is oxidized to Fe3+ [1]. Therefore, the SCN^- ligand was used in this study. Previous research conducted by [1] successfully developed a method for analyzing Fe(III) concentrations using UV-Vis spectrophotometry with the aid of desferrioxamine B mesylate (DFO) ligand. This ligand was used in determining the Fe concentration in three certified reference materials (CRMs), namely NIST SRM 1643f Natural Water, ClinChek Control Human Urine Level II, and ClinChek Control Blood Serum Level I, with recovery percentages of 102%, 97%, and 103%, respectively. The high recovery percentages make the DFO ligand one of the ligands with high selectivity and sensitivity, with the potential for routine Fe analysis applications in biological and environmental fields.

The Atomic Absorption Spectroscopy (AAS) techniques commonly employed in element analysis are flame AAS (FAAS) and graphite furnace AAS (GFAAS), both of which are capable of measuring at trace levels [2]. Additionally, there are techniques such as hydride generation AAS (HGAAS) and high-resolution continuum source GFAAS (HR-CS GFAAS). Research conducted by Uddin et al. (2016) reported the use of FAAS to measure the Fe content in traditional medicine samples, achieving a 100% recovery rate. Furthermore, HR-CS GFAAS has been successfully utilized to measure Fe as a contaminant in multimineral and multivitamin supplement samples. This technique yields a detection limit of 0.517 µg/g with an accuracy range of 4.3–17% [4].

In practical applications, AAS requires a sample pretreatment process to extract and separate Fe from the sample matrix. Common pretreatment methods in AAS include dry ashing and wet digestion [2]. Dry ashing involves heating the sample in a furnace at 500–600°C, causing the evaporation of water and volatile substances, while organic matter is combusted, forming CO_2 and N_2 oxides. The remaining minerals are then converted into oxides, sulphates, phosphates, chlorides, and silicates. In contrast, wet digestion employs strong acids, oxidizers, or a combination of both to eliminate organic components from the sample. Reagents commonly used in wet digestion include HCl, H_2SO_4, HNO_3, and $HClO_4$. This process effectively removes both organic and inorganic components from the sample matrix, thereby minimizing potential interference [3]. In this study, both pretreatment methods were employed for analysis using a UV-Vis spectrophotometer, forming a colored complex between Fe and SCN^-, allowing a comparison of the measurement accuracy between the two methods. This approach represents an innovative contribution to the measurement of total Fe content in anti-anemia multivitamin samples, as it simultaneously incorporates two distinct metal extraction methods and their corresponding measurements. Subsequently, the combination of these variations was statistically evaluated to identify the optimal treatment based on the accuracy of the measurement results.

Optimization, in essence, is a process aimed at obtaining the most efficient experimental outcomes. In practical work, optimization is essential as it ensures that the experiment is conducted effectively, efficiently, and yields the best possible results based on the available experimental resources. A sound optimization process involves considering the experiment's output parameters, such as purity, analyte concentration, or yield, about input factors like

temperature or time. The optimization procedure begins with screening or identifying the key factors that significantly impact the experimental outcomes, followed by the determination of the optimal levels of these factors to achieve the best possible results. The process concludes with robustness testing to assess the sensitivity of the experimental response to small variations in these factors [5]. This study aims to determine the most effective metal extraction and measurement methods for analyzing total Fe content in anti-anemia multivitamin capsule samples in a practical laboratory setting.

MATERIALS AND METHODS

Materials and Equipment

The materials used are anti-anemia multivitamins (Sangobion), standard Fe^{3+} solution at 1000 ppm (Merck), concentrated H_2SO_4 (Merck), saturated $K_2S_2O_8$ solution, 3 N KSCN, concentrated HCl (Merck), deionized water, and Whatman 42 filter paper. The equipment used includes a UV-Vis spectrophotometer (Shimadzu 1800), 50 mL beakers, 100 mL Erlenmeyer flasks, 30 mL porcelain crucibles, dropper pipettes, 100 mL measuring cylinders, 50 mL volumetric flasks, 100 mL volumetric flasks, 10 mL Mohr pipettes, 10 mL volumetric pipettes, hotplate stirrer (IKA CMAGH-7), AAS (Shimadzu AA-7800), Fe cathode lamp, and 75 mm glass funnels.

Research Procedure

Determination of Sample Weight Data Distribution

Ten anti-anemia multivitamin capsules were prepared, and the capsule shells were opened to weigh each capsule using a 50 mL beaker. The weight of each sample was recorded, and the data were processed to calculate the average value and its standard deviation. This value was used as a reference for determining the theoretical total Fe content for one capsule and per gram of sample.

Wet Digestion Method

The contents of one anti-anemia multivitamin capsule were weighed into a 100 mL Erlenmeyer flask and 5 mL of concentrated H_2SO_4 was added. The mixture was stirred with a hotplate stirrer until homogeneous and then heated gradually to a temperature of 300°C for 1 hour until the mixture became a clear solution. The solution was poured into a volumetric flask containing 50 mL of deionized water, then diluted to 100 mL and homogenized [6].

Dry Ashing Method

The contents of one anti-anemia multivitamin capsule were weighed into a 30 mL porcelain crucible, which was then placed into a furnace. The sample was heated at 500°C for 3 hours until it turned into ash. Afterward, the formed ash was slowly treated with 2 mL of concentrated HCl using a dropper pipette and stirred until dissolved. The mixture was filtered using Whatman 42 filter paper and transferred into a volumetric flask containing 50 mL of deionized water, then diluted to 100 mL and homogenized [7].

Determination of Total Fe Content using UV-Vis Spectrophotometer

The sample solution obtained from the wet digestion method was diluted 100×, and the dry ashing sample was diluted 50× in a 100 mL volumetric flask. A stock solution of Fe^{3+} at 100 ppm was prepared by pipetting 10 mL of the 1000 ppm Fe^{3+} stock solution into a volumetric flask and diluting it to 100 mL and homogenizing. Standard solutions were prepared by pipetting 0.5; 1; 1.5; 2; 2.5; and 3 mL of the 100 ppm Fe^{3+} stock solution into separate volumetric flasks. To each flask, 2 mL of concentrated H_2SO_4 and 2 mL of saturated $K_2S_2O_8$ were added, and the contents

were mixed gently before being diluted to 50 mL and homogenized. Subsequently, 10 mL aliquots of each sample and standard solution were pipetted into 50 mL beakers. Then, 10 mL of water, 1 mL of saturated $K_2S_2O_8$, and 4 mL of 3 N KSCN were added, followed by gentle stirring. The standard solutions have concentrations of 1, 2, 3, 4, 5, and 6 ppm. A blank solution was prepared using deionized water, treated the same as the standards and samples. All solutions were measured using a UV-Vis spectrophotometer at a wavelength of 480 nm [8].

Determination of Total Fe Content using Atomic Absorption Spectrophotometer

The sample solution obtained from the wet digestion method, diluted 100×, and the sample from the dry ashing method, without dilution, were prepared in a 100 mL volumetric flask. A stock solution of Fe^{3+} at a concentration of 100 ppm was prepared by pipetting 10 mL of the 1000 ppm Fe^{3+} stock solution into a volumetric flask and diluting it to 100 mL and homogenizing. Standard solutions were prepared by pipetting 0.5, 1, 1.5, and 2 mL of the 100 ppm Fe^{3+} stock solution into separate 50 mL volumetric flasks and homogenizing. These solutions have concentrations of 1, 2, 3, and 4 ppm. A blank solution was prepared using deionized water and treated the same as the standards and samples. All solutions were measured using AAS with a Fe cathode lamp at a wavelength of 248.3 nm [9]. Calibration curves for both the UV-Vis spectrophotometer and AAS were created from the results of corrected blank measurements, and the sample concentrations were determined by plotting the absorbance values against the standard curve equation. The total Fe content was calculated based on the dilution factor and the sample mass used, and expressed as mg Fe/capsule and mg Fe/gram of anti-anemia multivitamin sample.

Data Analysis

A completely randomized design with one factor was used in this study, followed by evaluation using ANOVA and subsequent Duncan's test at a confidence level (α) of 5% [10]. Statistical analysis was performed using IBM SPSS Statistics (Statistical Package for the Social Sciences) version 25.0. Duncan's test was performed using the following Eq. (1):

$$R_p = r_{\alpha,p,v}\sqrt{MSE/n} \quad (1)$$

MSE stands for mean squared error, r represents repetitions, and α, p, and v are the significant value ranges for Duncan's test. n refers to the degrees of freedom.

RESULTS AND DISCUSSION

Sulfuric acid (H_2SO_4) is used in the wet digestion method for sample preparation in various analytical applications. The role of sulfuric acid is to act as a powerful dehydrating agent, breaking down organic matter by removing water molecules [11]. This helps in converting complex matrices into simpler and more homogeneous solutions. Additionally, sulfuric acid can oxidize organic materials, converting them into carbon dioxide (CO_2), water (H_2O), and stable inorganic residues, which facilitates easier analysis of elemental composition. This is crucial for preparing samples for accurate chemical measurements [12].

Hydrochloric acid (HCl) is used in the dry ashing method to help dissolve ash residues after the sample has been ashed at high temperatures. After the ashing process, the ash residue is dissolved in HCl and then diluted with deionized water [13]. This helps in converting the inorganic residues into a soluble form that can be analyzed for mineral content. The use of HCl during the dissolution step improves the accuracy of the analysis by ensuring that all mineral content is completely dissolved and available for measurement [14]. These functions make sulfuric

acid and HCl essential reagents in the wet digestion and dry ashing methods, particularly for the analysis of total Fe content in samples.

Potassium persulfate ($K_2S_2O_8$) is used in the determination of Fe due to its strong oxidizing properties, which can oxidize iron(II) (Fe^{2+}) to iron(III) (Fe^{3+}), which can then be quantified through UV-Vis spectrophotometric measurement, as described in reaction (Eq. 2) [15]:

$$Fe^{2+}(aq)+S_2O_8^{2-}(aq)\rightarrow Fe^{3+}(aq)+2SO_4^{\bullet-}(aq) \quad (2)$$

Subsequently, the iron(III) form is reacted with KSCN to form the complex compound $[Fe(SCN)]^{2+}$, which exhibits a blood-red color (Figure 1). The formation of this colored complex allows for the measurement of total Fe content in the visible light region (480 nm), where the total Fe concentration is directly proportional to the absorbance of the formed complex, as represented in reaction (Eq. 3) [16]:

$$Fe^{3+}(aq)+SCN^{-}(aq)\rightarrow [Fe(SCN)]^{2+}(aq) \quad (3)$$

Fig. 1. Complex solution $[Fe(SCN)]^{2+}$ with varying Fe concentrations

Table 1. Data distribution of anti-anemia multivitamin sample weights

Test	Sample weight per capsule	Mass of Fe (mg) in 1 capsule	Fe content (mg/g)
1	0.4421	30	67.86
2	0.4425	30	67.80
3	0.4422	30	67.84
4	0.4420	30	67.87
5	0.4421	30	67.86
6	0.4427	30	67.77
7	0.4428	30	67.75
8	0.4426	30	67.78
9	0.4428	30	67.75
10	0.4426	30	67.78
Mean	0.4424	30	67.77
SD	0.003	0.00	0.05
%RSD	0.07	0.00	0.07

Based on the data in Table 1, it is found that each sample capsule of anti-anemia multivitamins contains 250 mg of Fe gluconate, equivalent to 30 mg of elemental Fe per capsule. The average capsule weight tested is 0.4424 ± 0.003 g, meaning the sample has low weight

variability. All capsules contain the same mass of Fe, which is 30 mg, ensuring that the capsules are produced with uniform Fe content. Likewise, the average Fe content also has low variation, with a value of 67.77 ± 0.05 mg/g. The relative standard deviation of the sample weight, Fe mass, and Fe content is less than 2%, indicating that the analytical method meets the acceptance criteria because it is considered to have very high precision [17].

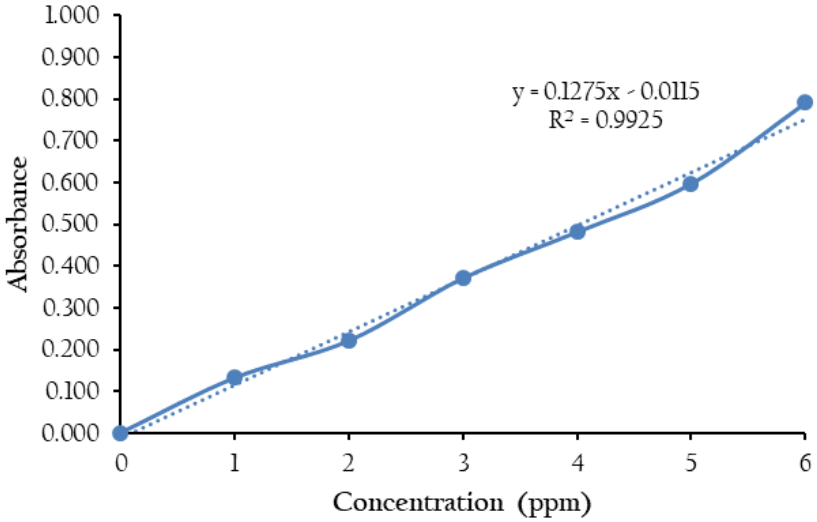

Fig 2. Fe standard curve using UV-Vis spectrophotometer.

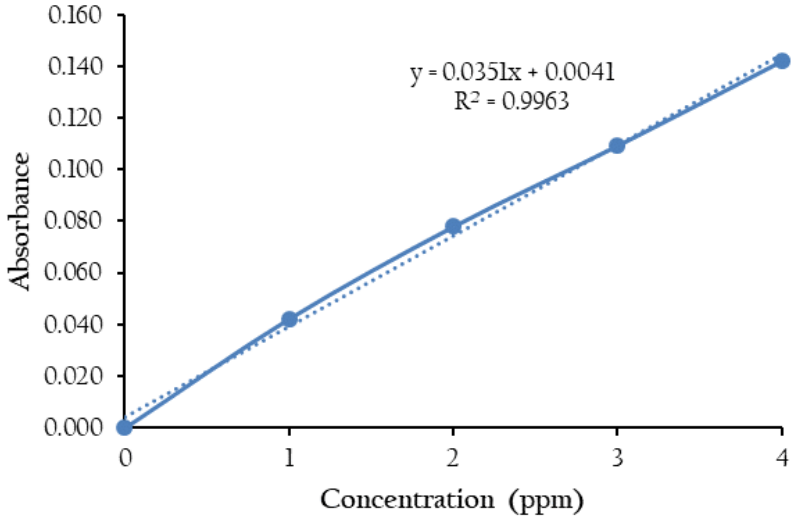

Fig. 3. Fe Standard Curve with AAS

Figure 2 shows a strong linear relationship between the Fe concentration and absorbance on the Fe standard curve using a UV-Vis spectrophotometer. Based on the regression analysis, the equation y = 0.1275x − 0.0115 and the correlation coefficient (R^2) = 0.9925 were obtained. This indicates that for every 1 ppm increase in Fe concentration, the absorbance increases by 0.1275 units. Similarly, the Fe standard curve with AAS shown in Figure 3 also demonstrates a linear relationship. The regression analysis produces the equation y = 0.0351x − 0.0041 and the correlation coefficient (R^2) = 0.9963. This means that for every 1 ppm increase in Fe concentration, the absorbance increases by 0.0351 units. The change in the X-axis value with respect to the Y-axis is consistent with the gradient of the line in the curve [18]. The correlation coefficient value

approaching 0.99 indicates that no multicollinearity occurs, thus the data obtained in this study meet the acceptance criteria [19].

Table 2. Data of total Fe content analysis results with variation of digestion methods and measurement in mg Fe/capsule of multivitamin.

Test	Theoretical Content	Fe Content (mg/capsule)			
		UV-Vis Spectrophotometer		AAS	
		Dry Ashing	Wet Digest	Dry Ashing	Dry Ashing
1	30.00	9.27	30.47	0.63	33.28
2	30.00	9.35	30.08	0.64	35.04
3	30.00	9.16	29.92	0.62	31.03
4	30.00	9.08	29.53	0.62	31.03
5	30.00	9.39	30.31	0.64	31.03
Mean	30.00	9.25	30.06	0.63	32.28
SD	0.00	0.13	0.37	0.01	1.83
%RSD	0.00	1.42	1.22	1.53	5.66

The two extraction methods used in this study were compared to determine the most effective method for analyzing the Fe content in blood booster tablets. These extraction methods are wet digestion and dry ashing. The Fe content measurement was performed using two different spectrophotometric methods, namely UV-Vis and AAS. In one capsule, the sample extracted using the dry ashing method via UV-Vis spectrophotometry yielded an average Fe content of 9.25 ± 0.13 mg/capsule, while the wet digestion method yielded a Fe content of 30.06 ± 0.37 mg/capsule. On AAS, the dry ashing extraction method (0.63 ± 0.01 mg/capsule) also resulted in lower Fe content compared to the wet digestion extraction method (32.28 ± 1.83 mg/capsule). Based on these data, it can be concluded that the wet digestion extraction method results in Fe content closer to the theoretical value (30 mg/capsule), with the highest Fe content obtained from the sample analyzed using AAS. However, the relative standard deviation for the dry ashing and wet digestion extraction methods analyzed by UV-Vis spectrophotometer and the dry ashing extraction method from AAS has a value of ‹2%, proving that the methods used are considered accurate, unlike the wet digestion extraction method on AAS, which shows a value of ›5% or is considered inaccurate [20].

Table 3. Data on the total Fe content analysis results with variations in the digestion method and measurement units in mg Fe/gram of sample.

Test	Theoretical Content	Fe Content (mg/capsule)			
		UV-Vis Spectrophotometer		AAS	
		Dry Ashing	Wet Digest	Dry Ashing	Dry Ashing
1	67.86	20.98	68.83	1.42	75.17
2	67.80	21.14	67.93	1.44	79.14
3	67.84	20.71	67.60	1.40	70.10
4	67.87	20.54	66.69	1.39	70.07
5	67.86	21.24	68.49	1.44	70.10
Mean	67.85	20.92	67.91	1.42	72.91
SD	0.03	0.29	0.83	0.02	4.12
%RSD	0.04	1.40	1.23	1.51	5.65

Through UV-Vis spectrophotometric analysis, each 1 gram of sample yielded a lower Fe content using the dry ashing extraction method (20.92 ± 0.29 mg/g) compared to the wet digestion method (67.91 ± 0.83 mg/g). On the other hand, the Fe content obtained through AAS analysis also showed a lower value for the dry ashing extraction method (1.42 ± 0.02 mg/g), while the wet digestion extraction method yielded a much higher value (72.91 ± 4.12 mg/g). Based on the obtained data, it can be demonstrated that the wet digestion extraction method resulted in Fe content higher than the theoretical value (67.85 ± 0.03 mg/g), especially for the wet digestion sample analyzed using AAS. This is due to the presence of many minerals in the wet digestion extraction method, whereas the dry ashing extraction method results in more minerals being lost due to the high incineration temperature [17]. However, the same level of accuracy was observed for Fe content per 1 gram. The relative standard deviation of less than 2% for the dry ashing and wet digestion extraction methods analyzed by UV-Vis spectrophotometer and the dry ashing extraction method from AAS indicates that the methods used are considered accurate. On the other hand, the wet digestion extraction method on AAS is considered inaccurate due to a relative standard deviation >5%.

Table 4. Post Hoc Duncan Statistical Test Results

Fe Content Unit	Theoretical Content	UV-Vis Spectrophotometer		AAS	
		Dry Ashing	Wet Digest	Dry Ashing	Wet Digest
mg/g	67.85±0.03^{b}	20.92±0.29^{c}	67.91±0.83^{b}	1.42±0.082^{d}	72.91±4.12^{a}
mg/capsule	30.00±0.00^{b}	9.25±0.13^{c}	30.06±0.37^{b}	0.63±0.01^{d}	32.28±1.83^{a}

$^{a-d}$Values followed by different letters are significantly different based on the Duncan test.

Table 4 presents the results of the significant difference test using the Duncan method for the five Fe concentration samples. The Duncan test was selected because it has higher statistical power compared to other multiple comparison tests, such as the Newman-Keuls method. This means that it is more likely to detect real differences among the averages, thereby determining the best combination of methods in a research series [21]. Based on the variance analysis, the use of the wet digest extraction method is highly significant in obtaining Fe concentration from each capsule and per gram of the anti-anemia multivitamin. The Fe concentration obtained through the wet digest extraction method, analyzed using AAS, which is known to give the highest Fe concentration, both per gram and per capsule, showed a significant difference from the dry ashing extraction method measured by UV-Vis spectrophotometry and AAS. The wet digest extraction method analyzed by UV-Vis spectrophotometry did not yield a concentration significantly different from its theoretical value. This indicates that the wet digest extraction method using AAS is more effective in extracting Fe from each capsule and per gram of the sample. Therefore, the dry ashing extraction method is less recommended in this study due to the significantly lower values obtained.

CONCLUSION

The determination of total Fe content in anti-anemia multivitamin samples can be carried out using the wet digest and dry ashing metal extraction methods, with measurements taken using UV-Vis spectrophotometry or AAS. Based on statistical analysis using the Duncan test, it can be concluded that the most accurate method for obtaining total Fe content data is the wet digest metal extraction method with UV-Vis spectrophotometric measurement.

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