How to Cite:
Kadhim, H. H., & Hussein, M. A. (2022). A new method for the determination of iron (II) in a pharmaceutical preparation using the color intensity (RGB) of a smartphone. *International Journal of Health Sciences*, *6*(S9), 261–272. https://doi.org/10.53730/ijhs.v6nS9.12221

A new method for the determination of iron (II) in a pharmaceutical preparation using the color intensity (RGB) of a smartphone

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***Abstract*---**In this research, a new method was used to determine the amount of iron in pharmaceutical, by using the color bio sensor of the smartphone device as bio sensor for the chromatic intensity of the samples images that are examined through a program (color meter) that is downloaded to the phone. An accessory for the mobile device is designed from plastic (black acrylic). In the form of a dark box from the inside equipped with a flow cell and a mirror reflecting the flash light emitted by the mobile device and a green filter complementing the red color, and a micro switch connected to a smartphone device via earphones, and the device is attached to the accessory by the device case. The calibration curve for this method was in the range of mg/L (1-8), the correlation coefficient (R^2) was equal to (0.999), the limit of detection was in the amount of (0.2) mg/L, and the relative standard deviation (RSD%) for the concentration is (4) mg/L, for which the examination was repeated (10) times, and its value was (0.6 %), and the recovery value (Recovery%) was equal to (101.5 %).

***Keywords*---**Ferrous sulfate tablets, sensor, smartphone, (Red, Green, Blue) (RGB), 1.10-Phenanthroline, hydroxylamine hydrochloride.

1. Introduction

Iron is a chemical element with the atomic number 26 and the symbol (Fe). It belongs to the d sub-level elements and is located at the top of the eighth group elements in the periodic table .Iron is the fourth most abundant element on the earth. It is an essential biological trace element for all living organisms. The human body contains an average of 3-4 g of iron [1, 2]. Most of this iron exists in

the complex forms bound to proteins such as hemoglobin and myoglobin. Iron is used in the prevention and treatment of iron-deficiency anemia. Iron (Fe) is a mineral that is available as a dietary supplement. It functions by assisting the body's production of red blood cells. In pharmaceutical industries, iron is used in various forms which are consumed as tablets, capsules, injectable, syrups and drinking ampules. Iron exists in two oxidation states Fe (II) and Fe (III). The balance between these two forms is also important for the metabolism of iron in the biological systems [3]. The oxides of iron are used as inorganic dyes, pigments for cosmetics and food additives [4, 5]. As a necessary component of many bodily activities, including oxidative metabolism, reproduction, cellular growth, wound healing, and numerous metabolic processes, iron is regarded as an important nutrient. Iron's primary function is to transport oxygen to tissues where it is needed [6]. Moreover, it is necessary for the efficient operation of numerous enzymes involved in the production of DNA, energy metabolism, and defense against microorganisms and free radicals [7]. Age, gender, and general health all affect how much iron is required daily. Because their bodies are growing so quickly, infants and toddlers need more iron than most adults do. Additionally, since women lose blood each month during their period, they require more iron [8]. The hemoglobin of red blood cells (RBC) in the human body contains 60 to70 percent of the body's circulating iron [9]. Loss of appetite, stomach pains, fatigue, pale complexion, cold hands and feet, brittle nails, shortness of breath, migraines, etc. are signs of iron deficiency anemia, which reduces the hemoglobin concentration of the blood's ability to carry oxygen. It may consequence from lack of iron, in the diet, insufficient absorption from the gut, or losses, usually through bleeding [10]. More than (2) billion individuals worldwide suffer from iron deficiency anemia, which is one of the main causes of anemia [11]. A large number of analytical methods have been employed for the quantitative determination of iron at trace levels. These methods include spectrophotometry [12, 1 3], fluorimetry [14], voltametric methods [15], atomic emission and absorption spectrometry [16, 17], capillary electrophoresis [18, 19], and chromatographic techniques [20]. In this study, a new method included iron determination in a pharmaceutical by primary color analysis (RGB) of video recorded for samples by smartphone.

2.Experimental

2.1Chemicals

All solutions of chemicals used in water were prepared the degree of analytical reagent. . Ferrous sulfate tablets ($FeSO_4.H_2O$) (Wockhardt) one tablet was taken and dissolved in 1000 mL of distilled water, as 1 mL of the sample was taken, with a concentration of 6.5 mg/L. Iron(II) sulfate heptahydrate solution ($FeSO_4.7H_2O$) (Seelze Hannover*)* was prepared at a concentration of (0.00017 M) by dissolving (0.01 g) in (200 mL) of water .then added 1ml from Concentrated sulfuric acid completes the volume to 200 mL and this solution is its concentration 10 ppm .A solution of 1.10-phenanthroline($C_{12}H_8N_2$. H_2O) (Indiamart) is prepared by taking 0. 1 g and dissolving it in 100 mL distilled water with simple heating to complete the dissolution process. A hydroxylamine hydrochloride solution ($NH_2OH.HCl$) is prepared by taking 5 g and dissolving it in 50 mL distilled water. Sodium acetate (CH_3COONa) solution is prepared by taking 5 g and dissolving it in 50 mL distilled water.

2.2 Equipments

1- UV-Visible Spectrophotometer,EMC-11UV, Emclab, Germany.(single Beam).
2- Electric Balance, BP 301S, Sartorius, Germany.
3- Heater Magnetic Stirrer, EQ-10198, China.
4- Mobile device (Galaxy J 7 Prime 2), China.
5- Micro switch, Singapore.

2.3 Method new that relies on the use of the color sensor on the smartphone device (RGB):

It is a new method that relies on the use of the color sensor on the smartphone device (Galaxy J 7 Prime 2) through a program that is downloaded on the smartphone device, which analyzes the color intensity of the main colors (RGB) of the captured images. This method is based on the formation of a red complex between iron (II) and the compound 1.10-Phenanthroline and this method is sensitive to the determination of iron by the molecular absorbance of this compound $\{(C_{12}H_8N_2)_2Fe\}^{+2}$, The absorption of light by this complex follows the Beer-Lambert law over a wide concentration range. The complex is very stable and the color intensity does not change appreciably over long periods of time. The absorption of light by this complex follows the Beer-Lambert law over a wide concentration range. The color intensity is not affected by the pH of 2-9.
There are several steps that must be taken when conducting tests, which are [21, 22]:

1- The standard solution of iron (II) is prepared as follows:
   0.01 g Iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$) is taken and dissolved in water in 20 mL beaker, then added 1 mL from concentrated sulfuric acid completes the volume to 200 mL and this solution is its concentration 10 ppm.
2- Prepare standard solution of the following concentration: 0, 1, 2, 3 , 4, 6 , and 8.0 ppm.
3- A solution of 1.10-phenanthroline is prepared by taking 0. 1 g and dissolving it in 100 mL distilled water with simple heating to complete the dissolution process.
4- A hydroxylamine hydrochloride solution is prepared by taking 5 g and dissolving it in 50 mL distilled water.
5- Sodium acetate solution is prepared by taking 5 g and dissolving it in 50 mL of distilled water.
6- Measure the absorption spectrum of the red complex formed in each standard flask using the standard solution at wavelength λ_{max}= 508 nm.

2.4 Procedure for the determination of iron (II) in a pharmaceutical (Ferrous Sulfate Tablets)

Pharmaceutical preparation (Ferrous Sulfate Tablets) was measured. Each tablet contains 200 mg of dried ferrous sulfate equivalent to 65 mg of ferrous iron, Fe (II). One tablet was taken and dissolved in 1000 mL of distilled water, and then filtered to get rid of drug additives, as 1 mL of the sample was taken. With a concentration of 6.5 mg/L and we completed it up to 10 mL, where the sample was measured by two methods, the traditional (Spectrophotometer) way, as well as the new way, using a smartphone.

3. Results and Discussion:

3.1 Sample chamber design and RGB measurement method using a smartphone

A rectangular box is designed from plastic (black acrylic) with a length of 6 cm ,a width of 4 cm, and a height of 6 cm, as in Figure 2, and two holes were made in the box, a hole for the entry of light resulting from the flicker the phone (flash) and other for video recording of the models, as well as three holes were made in the cover of the box , the first is through which the solution is injected, and the second is through which the reagent is injected, where each of them is connected to a tube where the distance between the two tubes is at the meeting point of the solution with the detector (2.5 cm), and from it the tube is connected to the entrance to the flow cell, while the third is where the micro switch is installed. It is a key mechanical device, used to cut off or divert current from one path to another within the circuit as in Figure 1 (a), and there is an opening at the bottom of the box for the exit of the solution, through a pipe connected to the other entrance of the flow cell, the size of the injected model is (2ml) for each model, the height of the flow cell is (3 cm), the cell is painted black from the inside and outside except for two ports for the entry and exit of light in the form of a small circle representing the diameter and amount of the cell tubes (1.8 mm) as in Figure 1 (b).

Inside the black box there is a reflective mirror that reflects the flashing light of the phone on one of the light ports of the flow cell near the mirror so that the video is recorded from the other port of the cell, the distance between the light port of the flow cell and the mirror is within (4.5 cm), there is also a small piece of green glass (complementary to the red color) attached to the entry port of the light into the flow cell. As in Figure 2, the phone is fixed to the black box by means of the device case, as in Figure 1 (d).

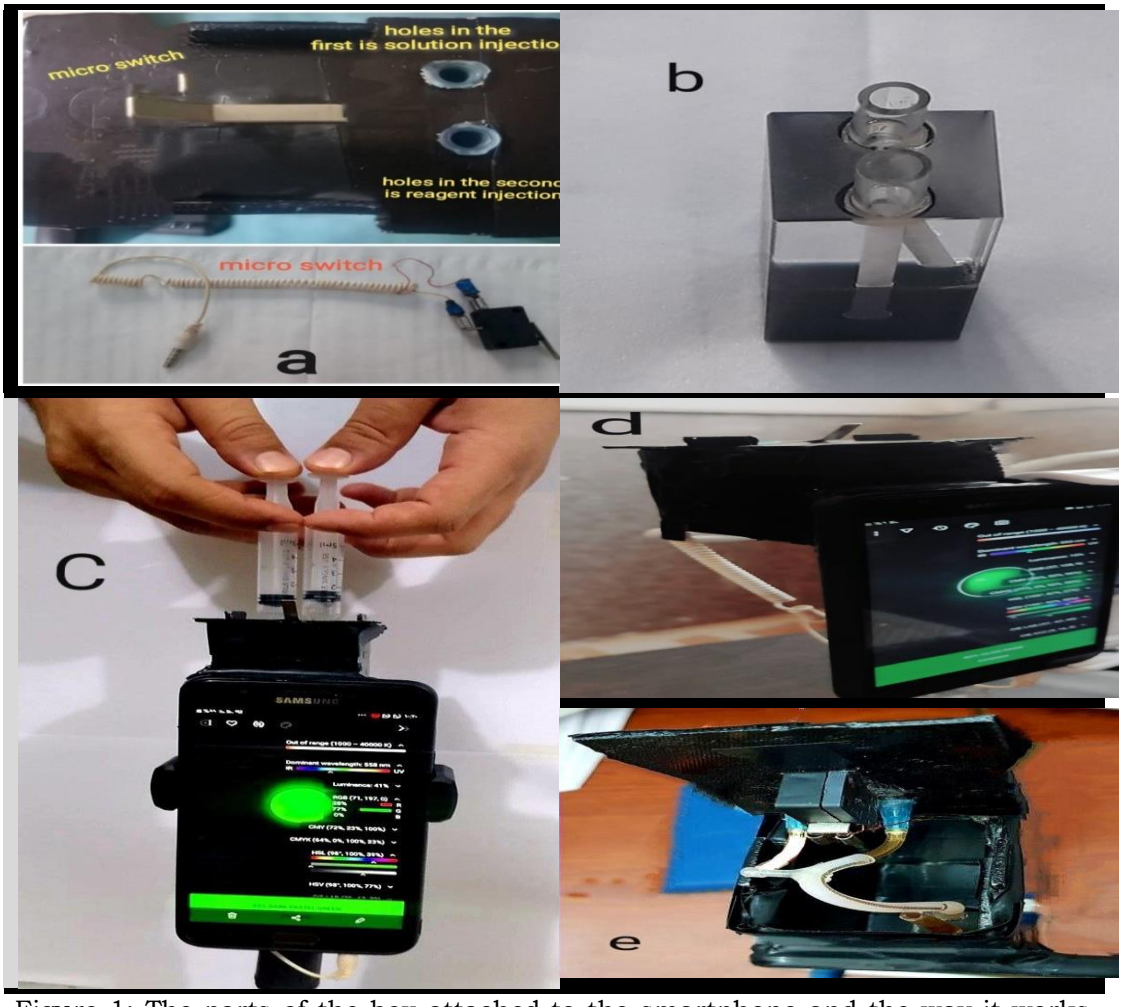

Figure 1: The parts of the box attached to the smartphone and the way it works (a) Box cover and microswitch. (b) Flow cell. (c) Injection of samples into the flow cell. (d) The telephone set is installed on the Black box by device case. (e) The black box from the inside.

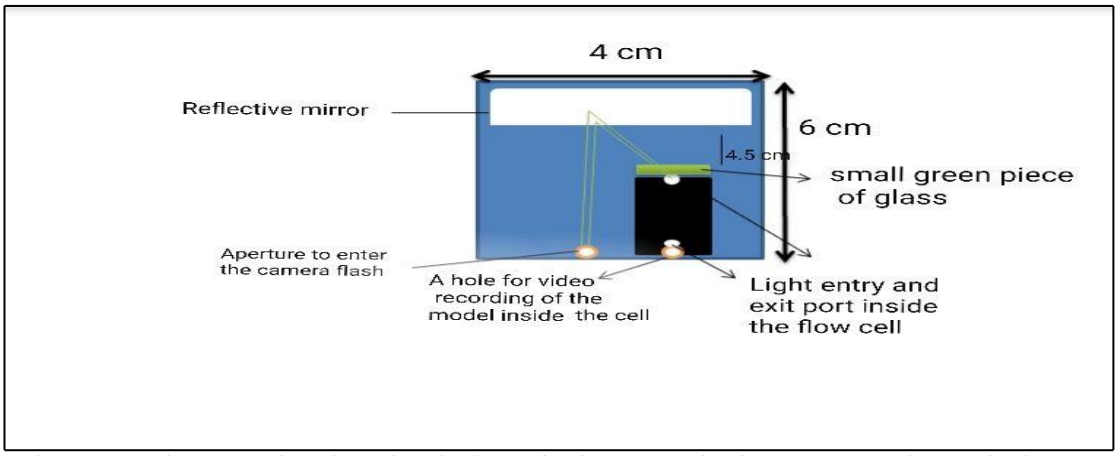

Figure 2: Diagram showing the design of a box attached to a smartphone device.

With draw an amount of the solution and the reagent, and the solutions are manually injected through Hamilton syringes using the sequential method, where both are transferred through the tube to the flow cell installed in the black box as in Figure 1 (c). Install the smartphone device on the front of the box so that the camera hole of the phone applies to the black box slot designated to see the light port of the flow cell, and the flash slot of the device applies to the other slot in the box designated for entering the flash light. During the injection process, press the micro switch which is connected to a smartphone device via earphones, to record the video of the sample inside the flow cell, and after 10 s of time have passed, we press again on the piston to stop the video recording and save it, and a screenshot is taken at the 10 s time of the recorded video, and then analyze the colors of the image using the (Color M1) program to find the (RGB) value for recorded video, which represents the emitting light in Beer-Lambert's law, after completing each inspection process, the flow cell is cleaned with pure water , and the reflective mirror is clean.

We extract the absorbance value of the sample by applying (Beer-Lambert Law) to find the absorbance

$A = - \text{Log} (I / I°)$…… (1)

A = absorbance

I = the transmitted light, and represents the (G) value for recorded video of the model.

I° = the incident light, and it represents the (G) value of green light.

We extract the concentration value of the model through the calibration curve that was prepared in advance and the calibration points were fixed for this purpose, through the linear regression equation, which is expressed by the following equation:

$A = m \times (Conc) \pm b$…… (2)

$Conc = A \pm b / m$ ……. (3)

3.2 Injection time study:

The injection time was studied, by measuring the absorbance of a concentration of 2 ppm of the solution. A change in the absorption of the solution was observed when the time period gradually increased up to 10 s, since the formation of the

complex. It was noted that the compound absorption remained constant for a period of time, so 10 s are the preferred time period.

3.3 Congruence study

To ensure the conformity of the results obtained by color density (RGB) by the smartphone, we studied the congruence of the results of tests for (10) duplicate images taken at a concentration of (4) mg/L, and the (RSD) value of the match was (0.6 %) and the recovery value (Recovery) equals (101.5 %), as in Table (1) and Figure (3) below.

Table 1: The congruence results of the RGB method

Value G		Absorbance	Concentrations measured by chromatic intensity method (mg/L)	RSD%	Recovery%
1	175	0.994	4.078	0.6 %	101.5 %
2	175	0.994	4.078		
3	174	0.101	4.144		
4	175	0.994	4.078		
5	175	0.994	4.078		
6	175	0.994	4.078		
7	175	0.994	4.078		
8	174	0.101	4.144		
9	175	0.994	4.078		
10	175	0.994	4.078		

Figure 3: Images of concordance study of concentration 4 mg/L.

3.4 Studying the detection limit for the (RGB) method

The detection limit represents the least analytical quantity in the substance that can be detected, when measuring the detection limit of the chromatic density (RGB) method, three solutions were prepared with concentrations of 0.2 mg/L, 0.4mg/L, and 0.8mg/L, and the examination of each sample was repeated three times to ensure its accuracy. Results and the 0.2 mg/L concentration were the detection limit value of the RGB method with the smartphone because it is the lowest value that has been detected by this method in practice, as in Figure 4.

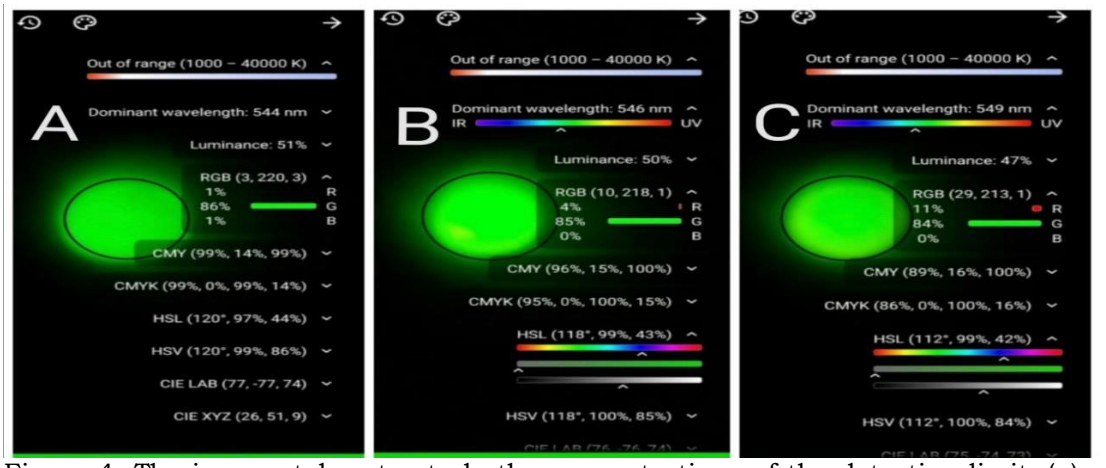

Figure 4: The images taken to study the concentrations of the detection limit, (a) Image of a concentration of (0.2 mg/L). (b) Image of a concentration of (0.4mg/L). (c) Image of a concentration of (0.8mg/L).

3.5 Standard calibration curve of chromatic intensity method (RGB) by smartphone

Under the optimal conditions studied, the calibration curve was obtained for the concentration of iron (II) in the samples. Figure (5) is a graph showing the linearity of the application of (Beer Lambert's Law) within the range (1-8 mg/L) between absorbance and iron II concentrations as in the Table 2, the linear graph has a correlation coefficient (R^2) equal to (0.999), and the value of the relative standard deviation coefficient (RSD%) for the concentration of (4) mg/L for eight repeated assays is (0.6%), and the value of recovery% is equal to (101.5%).

Table 2: The results of the calibration curve for RGB method

NO	Concentration mg/L	G	Absorbance
0	0	220	0
1	1	208	0.0243
2	2	197	0.0479
3	3	185	0.0752
4	4	175	0.0993
5	6	157	0.1465
6	8	141	0.1932

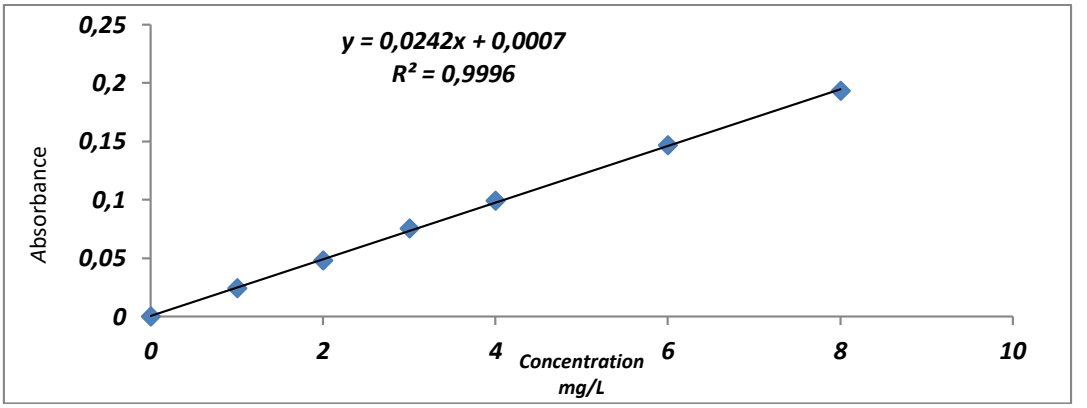

Figure 5: Chart for calibrating the concentrations of iron (II) with absorbance using the RGB method

Table 3 : Optimum conditions for RGB method

Analytical values	Values
Limits of Applicability of the (Beer-Lambert) Law (mg/L)	1-8
(Detection limit) (mg/L)	0.2
(Recovery%) for a concentration of (4 mg/L) for 10 assays	101.5%
(RSD%) for concentration (4 mg/L) for 10 assays	0.6%
Correlation coefficient (R^2)	0.999
(Slope)	0.0242

3.6 Pharmaceutical applications

The pharmaceutical preparation sample was measured (Ferrous Sulfate Tablets), Where a concentration was prepared 6.5 mg/L from the sample, and the samples were measured by the traditional method using the (spectrophotometer) and the chromatic density method (RGB), As in Table 5.

Table 5: The results of examining the pharmaceutical sample in the traditional way and the (RGB) method with a smartphone

pharmaceutical analysis sample mg/L	Traditional method mg/L	RGB Mothed mg/L	RSD% Traditional Method	RSD% (RGB) mothed	Recovery% Traditional method	Recovery % (RGB) mothed
6.5	6.52	6.35	0.62	0.8	97.69%	100.3%

* The average of three assays was extracted for both methods.

3.7 Conclusion

Designing a room in the form of a plastic box (black acrylic), opaque, on which a smartphone case is installed, and inside it a flow cell and a light reflecting mirror,

a green filter complementing the red color, and a microswitch. Iron (II) in pharmaceutical was measured in a new way using the color density (RGB) of a smartphone. Considering the RGB method for measuring iron in pharmaceutical, as a new method compared to traditional measurement methods, and it is characterized by being an easy-to-use and low-cost method, and it can be used in work sites far from the laboratory.

References

1. Bobrowski, Andrzej, and Jerzy Zarębski. "Catalytic systems in adsorptive stripping voltammetry." Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis 12.15 (2000).
2. David, D. J. "Determination of zinc and other elements in plants by atomic-absorption spectroscopy." Analyst 83.993 (1958).
3. De Montalembert, M. "Exploration d'une anémie microcytaire chez l'enfant." Archives de pédiatrie 19.3 (2012).
4. Elgailani, Isam Eldin Hussein, and Hamdan Shoyaib Alamary. "Determination of Iron Content in Iron Deficiency Drugs by UV-Visible Spectrophotometer." Orbital: The Electronic Journal of Chemistry 9.3 (2017).
5. Elgailani, Isam Eldin Hussein, and Hamdan Shoyaib Alamary. "Determination of Iron Content in Iron Deficiency Drugs by UV-Visible Spectrophotometer." Orbital: The Electronic Journal of Chemistry 9.3 (2017).
6. Fuqua, Brie K., Christopher D. Vulpe, and Gregory J. Anderson. "Intestinal iron absorption." Journal of Trace Elements in Medicine and Biology 26.2-3 (2012).
7. Haris, D. C. "Determination of Iron with 1, 10-Phenanthroline." *Quantitative Chemical Analysis,* 6th ed*., WH Freeman & Company, New York* (2003).
8. I Al-Neaimy, Usra, Amal M Saeed, and Thabit S Al-Ghabsha. "Spectrophotometric Assay of Iron (II) in Pharmaceutical Formulation Using Alizarin Red Sulphonate Reagent." JOURNAL OF EDUCATION AND SCIENCE 25.1 (2012).
9. Inoue, Hidenari, and Kuniko Ito. "Determination of trace amounts of iron (II, III) in natural water by reversed-phase high-performance liquid chromatography." Microchemical journal 49.2-3 (1994).
10. Itodo, Adams Udoji, Usman, A., Sulaiman, S. B., & Itodo, H. U. "Color Matching Estimation ofIron Concentrationsin Branded Iron Supplements Marketed in Nigeria." methods 5 (2012).
11. Kassebaum, Nicholas J., Jasrasaria, R., Naghavi, M., Wulf, S. K., Johns, N., Lozano, R., ... & Murray, C. J. "A systematic analysis of global anemia burden from 1990 to 2010." Blood, The Journal of the American Society of Hematology 123.5 (2014).
12. Longo, Dan L., and Clara Camaschella. "Iron-deficiency anemia." N Engl J Med 372.19 (2015).
13. Marczenko, Z., & Balcerzak, M. *Separation, preconcentration and spectrophotometry in inorganic analysis*. Elsevier, (2000).
14. Merli, Daniele, Antonella Profumo, and Carlo Dossi. "An analytical method for Fe (II) and Fe (III) determination in pharmaceutical grade iron sucrose complex and sodium ferric gluconate complex." Journal of Pharmaceutical Analysis 2.6 (2012).

15. Moschonis, George, Papandreou, D., Mavrogianni, C., Giannopoulou, A., Damianidi, L., Malindretos, P., ... & Manios, Y.. "Association of iron depletion with menstruation and dietary intake indices in pubertal girls: the healthy growth study." BioMed Research International 2013 (2013).
16. Nugraha, I. S., & Udi, W. W. (2022). The corelation of pharmaceutical services with the incidence of side effects of phase III COVID vaccination participants in RS Tingkat II Udayana. International Journal of Health & Medical Sciences, 5(4). https://doi.org/10.21744/ijhms.v5n4.1944
17. Oliveira, Fernando, Sara Rocha, and Rúben Fernandes. "Iron metabolism: from health to disease." Journal of clinical laboratory analysis 28.3 (2014).
18. Rajbhandari, Armila, Anjala Aryal, and Sanjeev Das Rajbhandari. "Determination of iron in iron tablets by spectrophotometry and atomic absorption spectroscopy." International Journal of Pharmaceutical and Biological Archives 4.3 (2013).
19. Roldan, Paulo S., Alcântara, I. L., Padilha, C. C., & Padilha, P. M. "Determination of copper, iron, nickel and zinc in gasoline by FAAS after sorption and preconcentration on silica modified with 2-aminotiazole groups." Fuel 84.2-3 (2005).
20. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Get vaccinated when it is your turn and follow the local guidelines. International Journal of Health Sciences, 5(3), x-xv. https://doi.org/10.53730/ijhs.v5n3.2938
21. Swaile, David F., and Michael J. Sepaniak. "Determination of metal ions by capillary zone electrophoresis with on-column chelation using 8-hydroxyquinoline-5-sulfonic acid." Analytical chemistry 63.2 (1991).
22. Tanaka, Y., Ueyama, H., Ogata, M., Daikoku, T., Morimoto, M., Kitagawa, A., ... & Nagata, S. . "Evaluation of nanodispersion of iron oxides using various polymers." Indian Journal of Pharmaceutical Sciences 76.1 (2014).
23. Totan, Maria, Elisabeta Antonescu, and Felicia G. Gligor. "Quantitative spectrophotometric determinations of Fe3+ in Iron Polymaltose solution." Indian Journal of Pharmaceutical Sciences 80.2 (2018).
24. Zeng, Zuotao, and Roger A. Jewsbury. "Fluorimetric determination of iron using 5-(4-methoxyphenylazo)-8-(4-toluenesulfonamido) quinoline." Analyst 125.9 (2000).