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# Study Analytical and Spectrophotometric Methods for Estimation of Ferrous (II) Ion in Pharmaceutical Formulation Using a Paracetamol (PCT) Azo Reagent

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**Abstract** **Original Research Article**

A simple, fast, and the sensitive spectrophotometric method for measuring trace amounts of iron (II) was developed based on the complex formation between ferrous (II) and the paracetamol (PCT) azo reagent in an acidic medium. The paracetamol (PCT) azo reagent has been developed for ferrous ion analysis. The calibration curves were linear throughout the ferrous (II) concentration range of 1–8 g/ml–1µg/ml. The effects of foreign ions and species on the measurement of Fe (II) was investigated in order to assess the methods' selectivity. Both the production of pharmaceuticals and the detection of pure ferrous metals were successfully accomplished using the recommended methods. There was strong agreement between the results of the reference technique and the recommended approaches.

**Keywords:** Paracetamol (PCT) azo, Ferrous sulfate, Pharmaceutical Formulation.



## 1. Introduction

One of the most prevalent metals in the planet, iron is essential to human physiology and the majority of living organisms[1-3]. Iron is one of many proteins and enzymes that are essential for good health. Iron is a necessary component of hemoglobin, which is involved in a transportation of oxygen in the human body. Additionally, it is essential for controlling cell differentiation and development. Health problems may result from either an excess or a shortage of it. Iron restricts the quantity of oxygen that reaches cells, which leads to a number of health issues. However, too much iron can be harmful and even fatal[4]. To avoid difficulties, certain drugs containing Fe (II) may be administered if there are obvious signs of iron deficiency. Iron can be found in a variety of commercially accessible medicinal medicines, Consequently, it is essential to keep an eye on their attributes[5]. Determining trace levels of iron is crucial for protecting the environment and public health[6]. Numerous techniques for determining iron have been documented due to its significance in the context of clinical diagnosis and environmental pollution monitoring, including spectrophotometry[7–10], inductively coupled plasma optical emission spectrometry [11,12],

chromatography[20,21],ICP-mass spectrometry[16–17], atomic absorption spectrometry[13–15], anodic or cathode stripping voltammetry[18–19], cloud point extraction[22–25], and flow injection analysis[26,27]. The determination of Fe(II) as PCT-Azo complex in aqueous solution using the spectrophotometric approach is given in this work because spectrophotometric methods are extensively used in many laboratories and nations for everyday work due to their simplicity, availability, low cost, and speed. A novel reagent called Paracetamol (PCT) azo was created, described, and applied to Ferrous (II) analysis. Ferrous (II) in pharmaceutical formulations was determined using this approach.

## 2. Materials and Methods

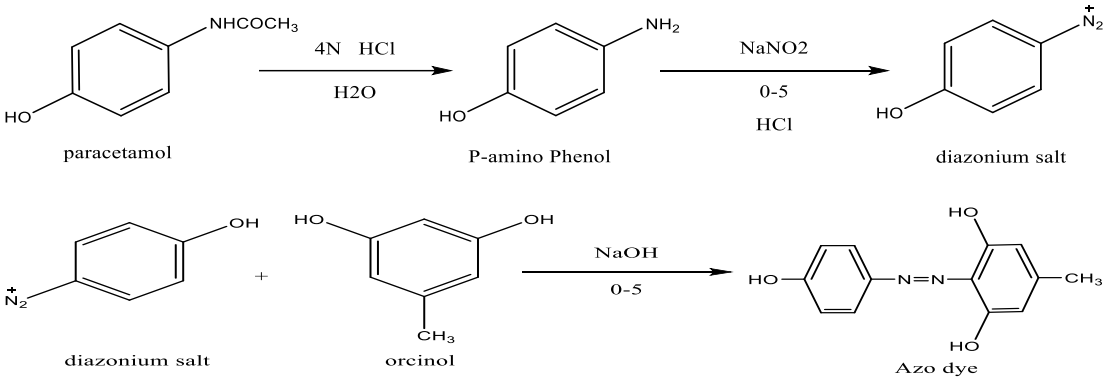
All reagents and chemical compounds used with a highly pure.

### 2.1. Apparatus

All spectrum and the absorbance measurements were performed using a UV-Vis (UV-9200 Pc) spectrophotometer and 10mm matched quartz cuvettes. We utilized a pH meter (type EZDO 6011, China). Additionally, a digital water bath (Model GFL 1083) was employed.

**2.2. Preparation of buffer solutions**: Acetic acid and sodium acetate solution, $Na_2CO_3$ solution and $NaHCO_3$ solution, and $NaH_2PO_4$ solution and NaOH solution were combined to create buffer solutions with pH values ranging from 3.0 to 11.0. A pH meter was used to prepare and adjust each buffer solution.

**2.3. Preparation of reagents solution:** To synthesize the paracetamol(PCT) azo reagent, the amine 4-aminophenol from the obtained paracetamol solution was dissolved in a solution of hydrochloric acid and 100% ethanol, and the combination was stirred for fifteen minutes in an ice bath. After 30 minutes, a drop-by-drop addition of a 10% ice-cold $NaNO_2$ solution was made to the solution. An ice-cooled orcinol solution in alkaline ethanol was added to the solution once it had gone brown, and it was left to sit at 0–5 $^oC$ for the entire night while being stirred continuously. The mixture was neutralized at pH 7 using a solution of ammonia or diluted hydrochloric acid. Following filtering, cold distilled water was used to clean the solid result, and it was then left to dry (Scheme1).




**2.4. Ferrous sulfate solution(1000 µg.ml-1 ):** 0.4964 grams of FeSO4.7H2O were dissolved in 100 milliliters of boiling distilled water to create this solution, which was then titrated using 0.01 N potassium permanganate in an acidic media. Distilled water was used to dilute this solution to create 100 µg.ml$^{-1}$[28].

**2.5. Procedure for calibration:** Transfer increasing volumes of 100 µg to a sequence of 10 ml calibrated

flasks.ml-1 of ferrous(II) solution to cover the concentration range (1-8) µg. $ml^{-1}$, after which 2.5 ml of $1x10^{-4}$ M paracetamol(PCT) azo was added. The solution was then diluted to the appropriate level with distilled water, and after 20 minutes at room temperature, the absorbance at 532 nm was measured against the reagent blank.

**2.6. Analysis of tablets**: Ten precisely weighed tablets (each containing 200 mg of dried ferrous sulfate, or 65 mg of elemental ferrous) were crushed and thoroughly mixed before a weight equal to one tablet was dissolved in 100 ml of distilled water, 0.5 g of charcoal was added, and the mixture was heated for 10 minutes to remove color. The mixture was then filtered, and the filtrate was transferred to a 500 $ml^{-1}$.To quantify iron (II) in tablets, 100 µg.$ml^{-1}$ was generated from this solution by dilution and the calibration process was followed.

**2.7. Analysis of the capsules**: After precisely weighing ten capsules (each containing 150 mg of dried ferrous sulfate, or 47 mg of elemental iron), the weight of one capsule was dissolved in 100 ml of boiled distilled water, 0.5 g of charcoal was added, and the mixture was heated for ten minutes to remove color. The filtrate was then transferred to a 250 ml volumetric flask and finished with distilled water to obtain 188 □g. ml-1. This solution was diluted to create a 100 g/ml-1 solution, which was then calibrated to measure the amount of iron (II) in capsules.

## 3. Results and discussion

### 3.1. The spectra absorption

The reagent (DMPAN) and ferrous(II) complex absorption spectra were scanned against the absolute ethanol ,The results of spectrums was showed in figure 1 and figure 2 , The ligand (PCT-azo) spectra (λmax 514nm) and a ferrous (II) complex spectra (λ max 532nm). So that the increase in the absorbance was explained the coordination between the reagent and ferrous (II) ion.

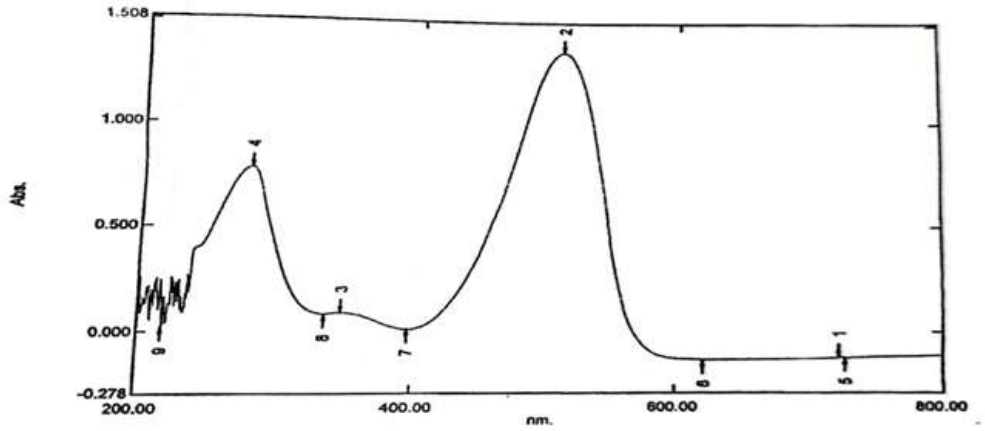



**Figure. 1. Absorption spectra of PCT-Azo.**

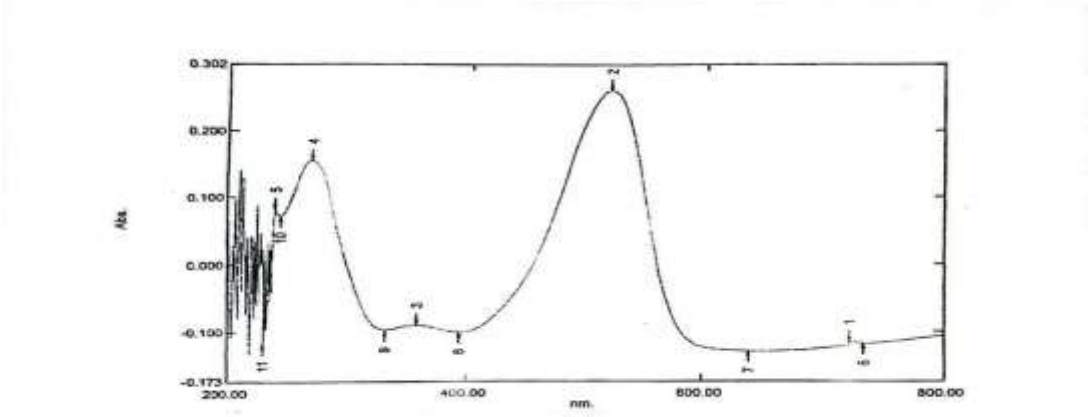



**Figure.2: Absorption spectra of ferrous-PCT azo complex**

### 3.2. Determination of the optimum conditions

**The pH effect**

A Standard concentration series of iron complex solution were prepared and buffered the pH-value range( 2-11) ,The result in figure 3 showed that the highest absorbance was around 6.00 ,Then the absorbance was decreasing , So that the pH=6 was adopted15.

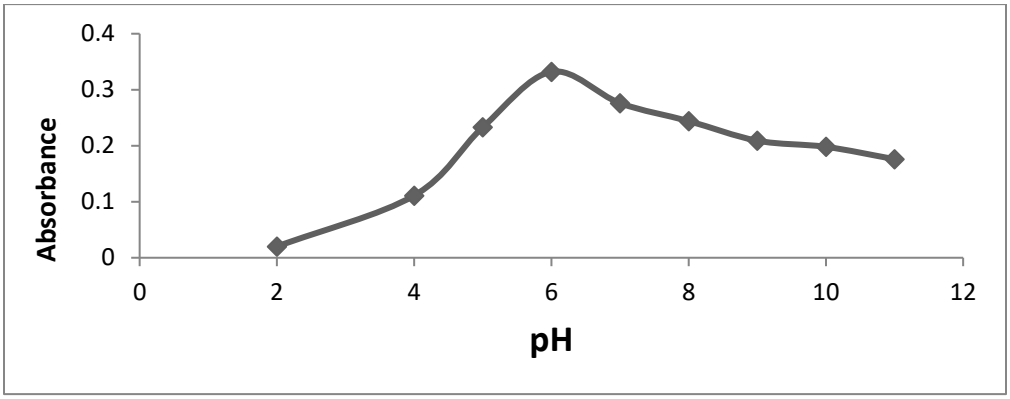



**Figure.3: Effect of pH on absorbance of ferrous(II)-PCT azo complex.**

**Effect of time**

After combining the ingredients, the absorbance stabilizes in the acetone-ethanol solution for at least 200 minutes and reaches its maximum within 15 minutes at room temperature. The ferrous chelates' ethanolic-acetone solutions are stable for a minimum of twenty-four hours. Figure 4 displays the outcomes:

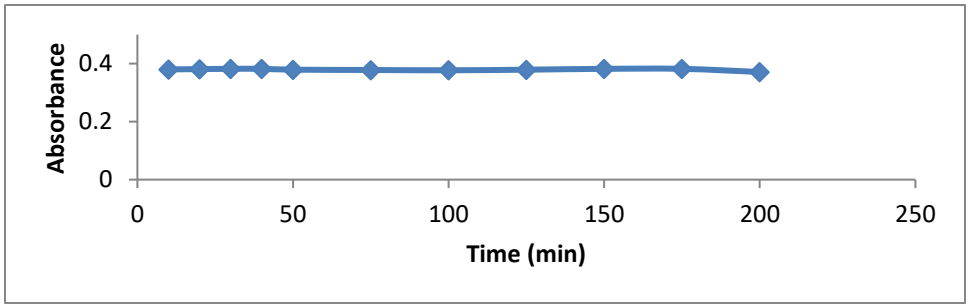



**Figure.4 : Effect of Time on absorbance of ferrous(II)-PCT azo complex.**

**Effect of the temperature.**

The impact of temperature on the ferrous ($I_3$) complex's absorbance was examined. Temperatures between 15°C and 60°C were used for the investigation. At temperatures between 20°C and 30°C, the greatest absorption was achieved. The absorbance steadily drops as the temperature rises over 30°C, which could be explained by the complexes' dissociation. Figure 5 presents the findings.

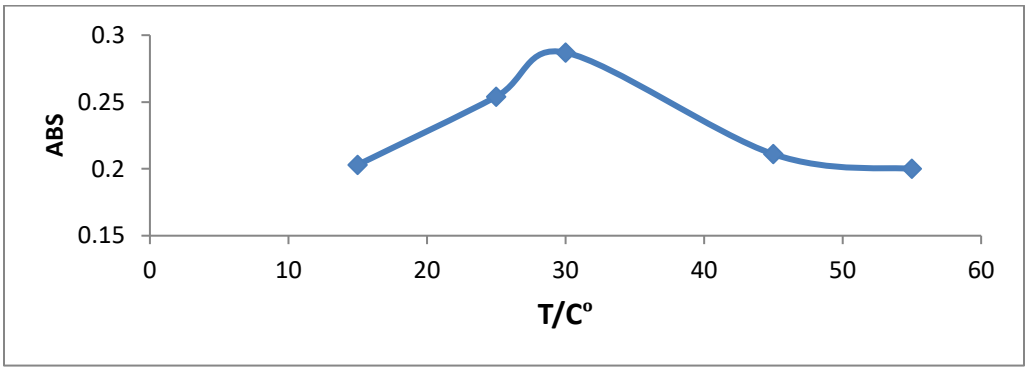



**Figure.5 : Effect of temperature on absorbance of ferrous(II)-PCT azo complex.**

**Effect of PCT-Azo concentration.**

It is clear that the absorbance rises with increasing PCT-Azo concentration and reaches its maximum when 4 mL of 1x10-4 M PCT-Azo is used. The impact of various PCT-Azo concentrations on the absorbance of solution containing 5 g. mLl-1ferrous ($I_3$) was investigated. Consequently, this focus was applied in all ensuing work (figure 6).

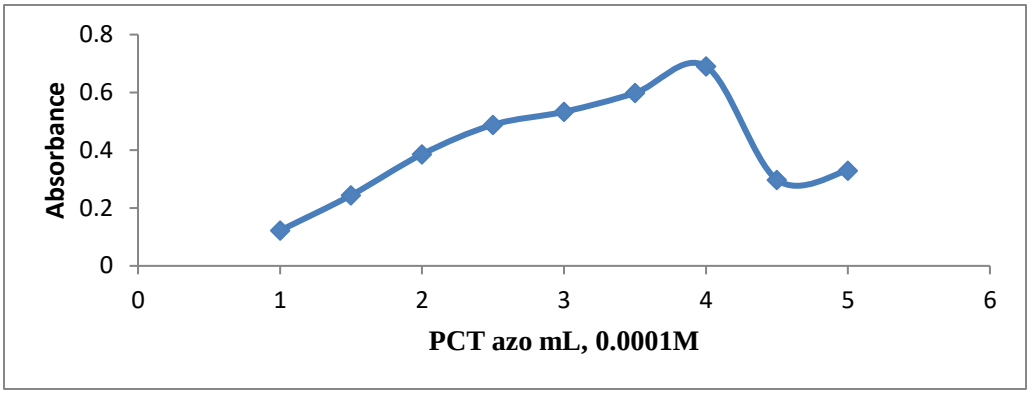



**Figure.6 : Effect of PCT-Azo concentration on absorbance of ferrous(II) complex.**

### 3.3. Calibration graph

Under ideal circumstances, a linear connection with a correlation coefficient of 0.9913 was found between the absorbance and the concentration of ferrous (II), covering the concentration range (1-8) □g. ml-1 (Figure 7). At increasing ferrous (II) concentrations, a negative departure from Beer's law was noted.

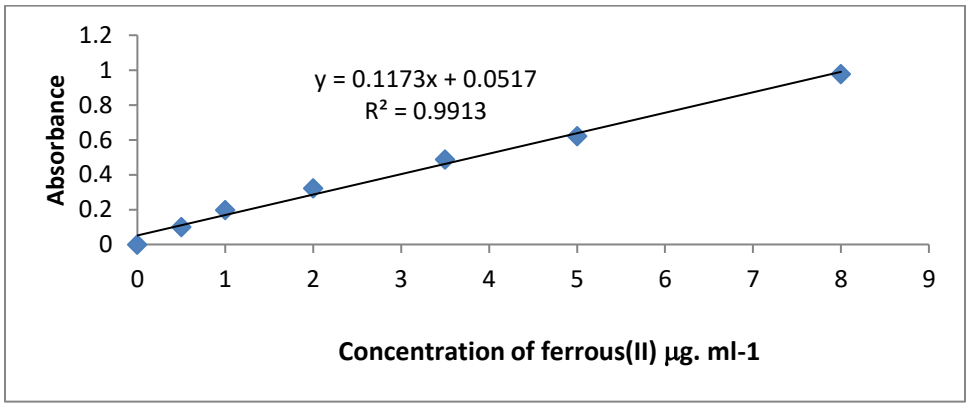



**Figure.7: Calibration curve for the complex of PCT Azo-Ferrous(II).**

### 3.4. Accuracy and precision

Iron (II) was measured at three distinct concentrations to assess the method's accuracy and precision. According to table (1), the precision (RSD) was less than 1% and the accuracy (average recovery%) was 100.024%.

**Table (1): Accuracy and precision of the present proposed method.**

| Amount of ferrous(II) taken μg. $ml^{-1}$ | Recovery*% | Relative standard deviation* (RSD%) |
|---|---|---|
| 1 | 100.23 | 0.87 |
| 2 | 100.13 | 0.77 |
| 3 | 99.56 | 0.98 |
| 4 | 99.78 | 0.67 |
| 5 | 100.42 | 0.65 |

* Average for Ten determinations

### 3.5. Effect of interferences

In the presence of foreign chemicals typically found in pharmaceutical formulations, 3 □g. ml-1 of ferrous (II) was determined using the approved protocol to test the method's selectivity. The results showed that the current approach is selective since there was no interfering effect (Table 2).

**Table (2): Effect of the interferences**

| Foreign compounds | Fold excess | Recovery % |
|---|---|---|
| Folic acid | 5 | 100.03 |
| | 10 | 100.11 |
| | 15 | 99.97 |
| | 20 | 100.07 |
| Starch | 5 | 101.03 |
| | 10 | 101.09 |
| | 15 | 100.5 |
| | 20 | 102.02 |

| | | |
|---|---|---|
| Glucose | 5 | 99.8 |
| | 10 | 99.09 |
| | 15 | 99.78 |
| | 20 | 98.90 |
| EDTA | 5 | 101.19 |
| | 10 | 102.03 |
| | 15 | 102.11 |
| | 20 | 102.09 |
| Sulfate | 5 | 99.98 |
| | 10 | 98.13 |
| | 15 | 98.15 |
| | 20 | 98.23 |
| Phosphate | 5 | 100.98 |
| | 10 | 100.54 |
| | 15 | 100.66 |
| | 20 | 99.89 |
| Oxalate | 5 | 97.33 |
| | 10 | 98.45 |
| | 15 | 97.22 |
| | 20 | 97.87 |

### 3.6. Composition of the complexes

The mole ratio approach was used to determine the complexes' compositions.

### Mole Ratio Method

As seen in Figure 8, this technique demonstrated that the mole ratio of ferrous (II) ions to reagent PCT-Azo was 1:2 (M: L):

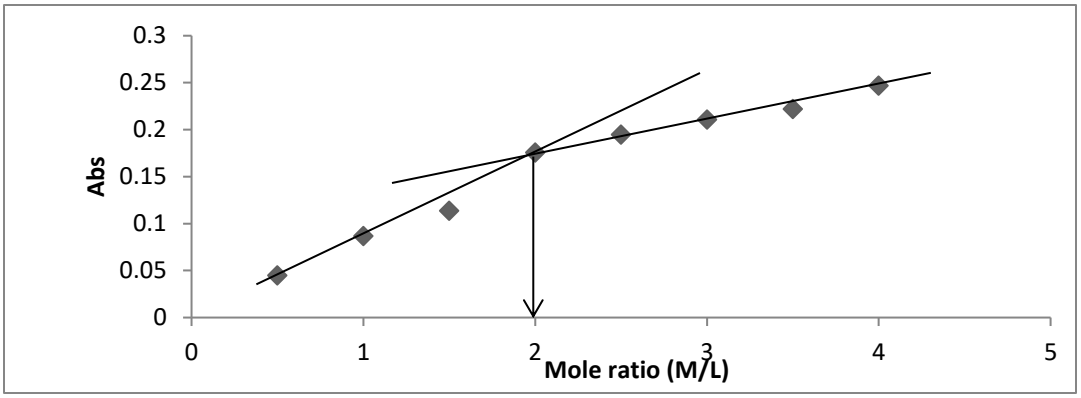



**Figure.8: Mole ratio method for ferrous(II)-complex.**

### 3.7. Analytical applications

Analysis of four different pharmacological formulations containing ferrous (II) revealed good accuracy (Table 3). The suggested approach and the British_ Pharmacopoeia standard method[15] were effectively compared (Table 3), as the T-test at three degrees of freedom and 94.5% confidence limit revealed no significant differences between the two approaches. (t-tabulated = 4.3, t-ex. = 0.42).

**Table (3): Application of the proposed method for determination of Ferrous(II) in pharmaceutical formulations**

| Pharmaceutical preparation of and company | Wt. of tablet (mg) | Certified value of Ferrous(II) (mg) | Amount of Ferrous(II) present (µg.ml$^{-1}$) | Recovery* % | Drug content found (mg) |
|---|---|---|---|---|---|
| Ferrous sulfate/ folic acid tablets Holden medical BV, Lelystad. Netherlands | 200 | 60 | 2<br>4<br>6 | 100.53<br>99.87<br>100.32 | 61.21<br>60.54<br>61.03 |
| Ferrous sulfate folic acid capsules- EIPCO / EGYPT | | | 2 | 101.02 | 45.08 |

| | | | | | |
|---|---|---|---|---|---|
| | 150 | 47 | 4<br>6 | 101.21<br>100.98 | 46.78<br>46.32 |
| Ferrous sulfate tablets Ajanta pharma/ limited, India | 200 | 65 | 2<br>4<br>6 | 100.43<br>100.23<br>99.92 | 63.97<br>64.23<br>65.32 |
| Folicron-Folic acid + iron Julphar, Gulf pharmaceutical industrial, /Ras Al Khaimah,/ U.A.E | 650 | 47 | 2<br>4<br>6 | 99.32<br>98.43<br>98.54 | 46.65<br>46.08<br>46.98 |

**Table (4): Comparing the recommended method with the traditional approach for ferrous (II) in pharmaceutical formulations.**

| Iron (II) pharmaceutical formulation | Present method | | British pharmacopoeia | |
|---|---|---|---|---|
| | Recovery* % | Drug content found mg | Recovery* % | Drug content found mg |
| Ferrous sulfate folic acid tablets | 100.53<br>99.87<br>100.32 | 61.21<br>60.54<br>61.03 | 100.52<br>99.98<br>99.87 | 65.31<br>64.97<br>64.93 |
| Ferrous sulfate folic acid capsules | 101.02<br>101.21<br>100.98 | 45.08<br>46.78<br>46.32 | 100.96<br>99.93<br>99.88 | 47.43<br>46.98<br>46.82 |
| | 100.43 | 63.97 | 100.32 | 63.93 |

| | | | | |
|---|---|---|---|---|
| Ferrous sulfate tablets | 100.23<br>99.92 | 64.23<br>65.32 | 100.53<br>99.87 | 64.24<br>64.03 |
| Folicron | 99.32<br>98.43<br>98.54 | 46.65<br>46.08<br>46.98 | 99.2<br>100.11<br>100.64 | 46.59<br>47.21<br>47.44 |

## 4. Conclusion

This work showed that a good reagent for the spectrophotometric measurement of ferrous (II) at various pHs is paracetamol (PCT) azo. The technique is delicate and easy to use. The disclosed approach does not require buffer solution or solvent extraction, and the product generated is a stable for at least (120 minutes), allowing the quantitative analysis to be carried out with good repeatability. According to the "mole ratio," the metal ligand ratio (M:L) in ethanolic-aceton solutions is 1:2 for ferrous complexes. Four businesses successfully used the reagent to determine the amount of ferrous (II) in various ferrous sulfate drugs.

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