



## A Cost-Effective and Environmentally Friendly Approach in Using Green Tea Extracts for The Determination of Iron Ion with PiCOEXPLORER

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### ABSTRACT

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This research has been developed by using green tea extracts as a green reagent for the determination of iron ion in pharmaceutical products with PiCOEXPLORER as a detector. PiCOEXPLORER is a modern instrument based on the colorimetric system. It is a compact system and a portable device of a new generation. It is a small size colorimeter based on the RGB analysis system which allows displaying RGB intensity and absorbance for each Red (R), Green (G), and Blue (B) mode. The device is controlled by a program using an application on a smartphone with either iOS or Android operating systems. This portable device is convenient for on-site micro-scale analysis. Green tea extracts can be used as an alternative low cost and easily available reagent for the quantification of iron without purifying extraction before use. Parameters affecting the determination of iron content were optimized. Under the optimum conditions developed herein, the results showed good linearity over a Fe(III) concentration range from 2.5 to 30.0 mg·L<sup>-1</sup> gave correlation coefficients of 0.9985 ( $y = 0.0093x - 0.002$ ) with a detection limit (LOD) of 0.5 mg·L<sup>-1</sup> and quantification limit (LOQ) of 1.3 mg·L<sup>-1</sup>. The repeatability of the proposed method was less than 4 %RSD ( $n = 7$ ). The proposed method was successfully applied to pharmaceutical products. The results matched well with those obtained from the standard method as compared at a 95% confidence level with the paired *t*-test. The proposed method is

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common, cost-effective, reduces the sample and reagent consumption, and is an environmentally friendly method. In addition, this device could be used as an alternative tool for spectroscopy study in terms of quantitative analysis instead of using a conventional spectrophotometer.

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## INTRODUCTION

Iron (Fe) is one of the important minerals that help in the formation of red blood cells which is the source of oxygen to feed various parts of the body, development of the brain and regulation of body temperature (1). If the iron content is not enough in the body, it can cause disease as iron-deficiency anemia (2). Thus, iron is produced in the form of iron pills to provide enough iron content where is instead of eating. Therefore, quality control of antianemic preparations and quantification of iron content containing good accuracy and precision, simple, fast, and low-cost systems is interested.

Several methods have been used to quantify iron ion in pharmaceutical samples consist of UV-Visible spectroscopy (3), flame atomic absorption spectroscopy (4, 5), inductively coupled plasma-optical emission spectroscopy (6), electrochemical (7, 8), and high-performance liquid chromatography technique (9, 10). These methods can present a high sensitivity, good accuracy and precision. However, they require skillful analysts, large instruments, high prices and tedious methods. Moreover, these methods use large quantities of a hazardous substance that lead to environmental contamination and health effects. PICOEXPLORER device is a new instrument based on the colorimetric system. This device is a

small instrument that requires a 30  $\mu\text{L}$  of minimum standard/sample volume per time. The analysis of samples was measured in a PCR tube. It can be easily controlled by a mobile phone or tablet and the signal of RGB light absorbance was recorded. Moreover, a calibration graph was constructed and saved in an application on a mobile phone or tablet and also all data will be uploaded to a computer.

Previous studies have reported on the use of green reagents for quantitative analysis of metals ion. For example, the determination of aluminium in pharmaceutical preparations with heartwood extracts (11) and the use of tea leaf extract for copper quantification in tap water samples (12). Both as mentioned above research was used sequential injection system based on spectrophotometric as a detector. The spectrophotometric flow injection system using green reagent extracted from morinda citrifolia root for the determination of aluminium in tea (13).

In this context, we are interested in using green tea extracts as a green reagent for the determination of iron ion due to in the north of Thailand especially in Chaing Rai province have a lot of green tea. Green tea contains the importance of polyphenolic compounds which are catechins, (-)-epigallo-catechin gallate (EGCG), and (-)-epicatechin gallate (ECG). These

compounds can be chelated with iron ion to obtain iron-polyphenolic complexes (14–17). For the determination of iron ion by using green reagent extracts such as the quantification of iron in pharmaceutical samples using flow injection system with green reagent extracts from guava leaf (18) and green tea (19).

The aim of this research is the use of green tea extracts as an alternative green reagent for the determination of iron ion in pharmaceutical products using the PiCO-EXPLORER device as a detector. The proposed method shows several advantages including common, fast analysis, small instrument, cost-effective, environmentally friendly, and reduces the sample and reagent consumption.

## MATERIALS AND METHODS

### *Reagents and chemicals*

A stock solution of  $1000 \text{ mg}\cdot\text{L}^{-1}$  Fe(III) was prepared by dissolving 2.1585 g of ammonium iron(III) sulfate dodecahydrate ( $\text{FeNH}_4(\text{SO}_4)\cdot 12\text{H}_2\text{O}$ , Univar) in deionized (DI) water containing 1 %v/v concentrated sulfuric acid ( $\text{H}_2\text{SO}_4$ , RCI Labscan Limited) and then adjusted with DI water to a volume of 250 mL. For the preparation of acetate buffer solution of pH 5.0 (1.0 M, 1000 mL) was prepared by dissolving 87.83 g of  $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$  in DI water containing 20.30 mL of  $\text{CH}_3\text{COOH}$ .

The study of interferences that affect the analysis of iron content in a real sample was done by adding seven cations into Fe(III) solutions including  $\text{Mg}^{2+}$  ( $\text{Mg}(\text{NO}_3)_2\cdot 6\text{H}_2\text{O}$ , QRëC),

$\text{Ca}^{2+}$  ( $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ , RCI Labscan Limited),  $\text{Ni}^{2+}$  ( $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$ , QRëC),  $\text{Mn}^{2+}$  ( $\text{MnSO}_4\cdot \text{H}_2\text{O}$ , QRëC),  $\text{Cu}^{2+}$  ( $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$ , QRëC),  $\text{Cr}^{2+}$  ( $\text{CrCl}_3\cdot 6\text{H}_2\text{O}$ , QRëC), and  $\text{Co}^{2+}$  ( $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ , QRëC). Green tea was purchased in local stores that are produced in the area of Pasang, Maechan, Chiang Rai, Thailand. Green tea extracts were prepared with 5.00 g of dried green tea in 70.00 mL DI water at  $60^\circ\text{C}$ . The suspension was shaken for 15 min. Then, the suspension was filtered through a piece of No.1 filter paper (Whatman) and finally adjusted with DI water to a volume of 100.00 mL. The extract was prepared daily.

### *Apparatus*

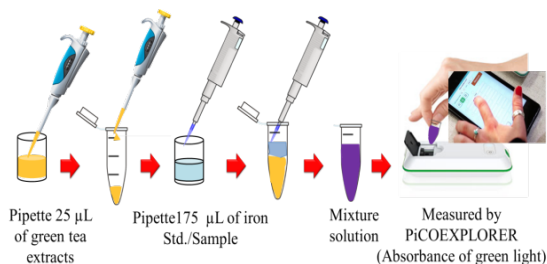
The device used as a detector in this study was the PiCOEXPLORER™ Model PAS-110 photo absorbance sensor from USHIO Inc., Japan as shown in Figure 1. A standard method based on spectrophotometry using 1,10-phenanthroline as reagent was used to compare with the results obtained from the proposed method. A Thermo Scientific Genesys 840-208100 UV/Vis spectrophotometer with quartz cells was used for the detection of the absorbance of the iron-1,10-phenanthroline complex at 510 nm.

### *Sample solutions preparation*

Fifteen tablets were ground to obtain a powder portion. A powder portion which is containing about 40 mg Fe was precisely weighed and mixed with 25.00 mL of DI water and 1.00 mL of concentrated hydrochloric acid (HCL, RCI Labscan Limited). The mixture was

boiled on a hot plate for 30 min, then let it cool down at room temperature, and diluted with DI water to a volume of 500.00 mL. The mixture was filtered through filter paper (Whatman No.1). The filtrate sample solution was diluted (10 times) with DI water and then a 5.00 mL of diluted sample solution was transferred to a 25.00 mL. Standard Fe(III) solution was added to each flask to obtain the final added concentration of 5.0, 7.5, and 10.0  $\text{mg}\cdot\text{L}^{-1}$ . A 0.25 mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ , Merck) was added to make sure the complete oxidization from Fe(II) to Fe(III) before making the final volume to 25.00 mL with DI water.

*Determination of iron ion by using PiCOEXPLORER device as a detector*



**Figure 1** The set-up for determination of iron ion using green tea as a green reagent and recorded the absorbance of green light by PiCOEXPLORER device.

A simple determination of iron ion by PiCOEXPLORER device was set-up as shown in Figure 1. Firstly, 25  $\mu\text{L}$  of green tea extracts and 175  $\mu\text{L}$  of standard or sample solution was added in PCR tube. Then the mixture was reacted for 5 min before the absorbance of green light was detected by PiCOEXPLORER device.

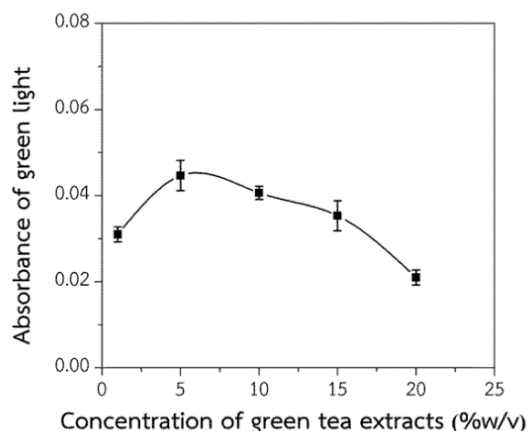
## RESULTS AND DISCUSSION

### *Optimization*

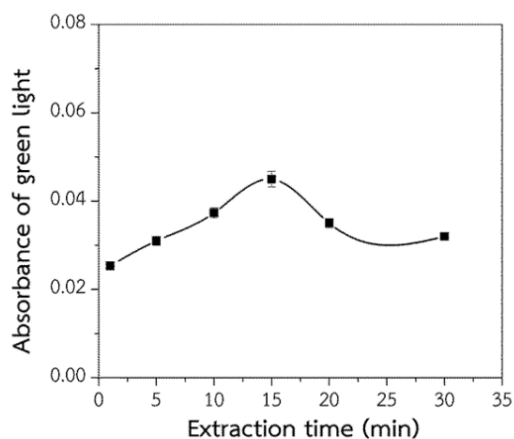
Parameters affecting the analysis of iron contents including the concentration of green tea extracts, extraction time, extraction temperature, buffer solution (pH), reaction time, and volume of green tea extracts were investigated. All results will be discussed below.

### *Effect of the concentration of green tea extracts*

Figure 2 shows the effects of the concentration of green tea extracts (1, 5, 10, 15, and 20 %w/v) that interacted with 10  $\text{mg}\cdot\text{L}^{-1}$  Fe(III). Green tea contains various polyphenolic compounds. The major polyphenolic compounds are catechins, EGCG and ECG. The polyphenolic compound is their capability to reduce and chelate with iron ion and to form Fe-polyphenol complexes. Previous studies have been proposed mechanism about the complex formations that consist of two steps, firstly Fe(III) go through an ion-transferring process when forming the complex with low molecular weight catechin and EGCG, and then Fe(III) is reduced to Fe(II) and the catechin is oxidized to quinone (14, 16). This study showed that the absorbance of green light enhances to 5 %w/v of green tea extracts concentration after that the absorbance decreases due to the concentration of green tea extracts more than 5 %w/v may contain much caffeine. Caffeine cannot interact with Fe(III) making the detection of absorbance of green light decrease. Therefore, the concentration of green tea extracts at 5 %w/v was chosen as the optimum concentration for further studies.



**Figure 2** Absorbance of green light recorded by PiCOEXPLORER device for the study of green tea concentration effect in the range of 1 to 20 %w/v.



**Figure 3** Absorbance of green light recorded by PiCOEXPLORER device for the study of extraction time effect in the range of 1 to 30 min.

#### *Effect of extraction time*

The effect of extraction time was also evaluated since a longer extraction time should allow the more polyphenolic compound to be extracted but will also lead to a longer total analysis time. Extraction time varied from 1 to 30 min (1, 5, 10, 15, 20, and 30 min). The results showed that the absorbance of green light of

extraction time slightly increasing for the first 15 min and then decreased. Extraction time over 15 min making the absorbance of green light decreased due to longer extraction time may cause the amount of caffeine more than polyphenolic compounds which are the major compound in green tea (20). An extraction time of 15 min was chosen because it gave the highest absorbance as shown in Figure 3.

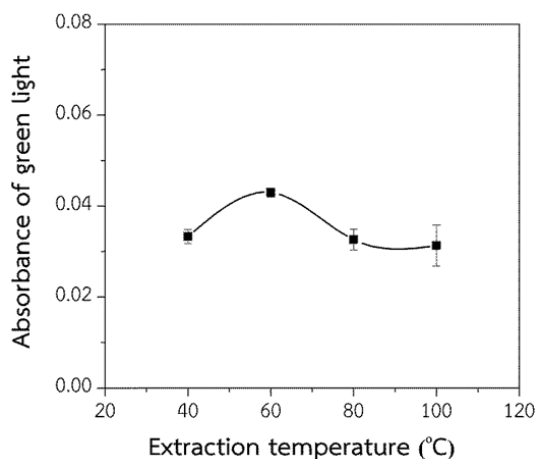
#### *Effect of extraction temperature*

Figure 4 exhibits the effect of the temperature of the solvent for green tea extraction. In this study, an extraction temperature varied from 40 to 100 °C (40, 60, 80, and 100 °C). The result indicating that 60 °C shows the highest absorbance of green light. Therefore extraction temperature at 60 °C was selected.

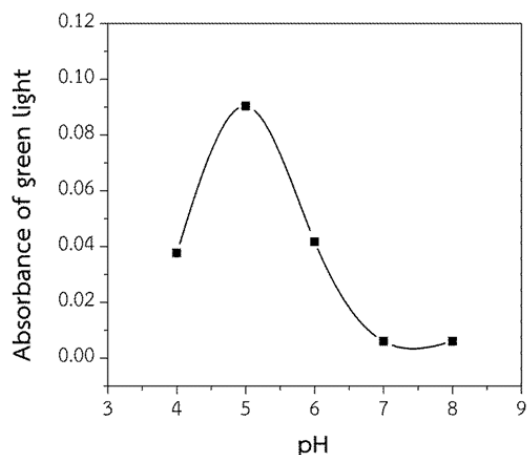
#### *Effect of buffer solution*

This research has been studying the effect of acetate buffer solution (pH 4–6) and a phosphate buffer solution (pH 7–8) on the formation complex of Fe(III) with green tea extracts. The buffer solution was optimized from weak acidic to weak alkaline buffer solution (pH 4, 5, 6, 7, and 8). Figure 5 displays that acidic buffer solution (pH 5) was a suitable condition for the formation of the complex of Fe(III) with green tea extracts because it gave the highest absorbance of green light. At weak acidic, neutral, and weak alkaline buffer solution (pH 6–8) the absorbance is decreased due to this buffer solution making the structure of polyphenolic

compounds change. Therefore an acidic buffer solution (pH 5) was selected.



**Figure 4** Absorbance of green light recorded by PiCOEXPLORER device for the study of extraction temperature effect in the range of 40 to 100 °C.

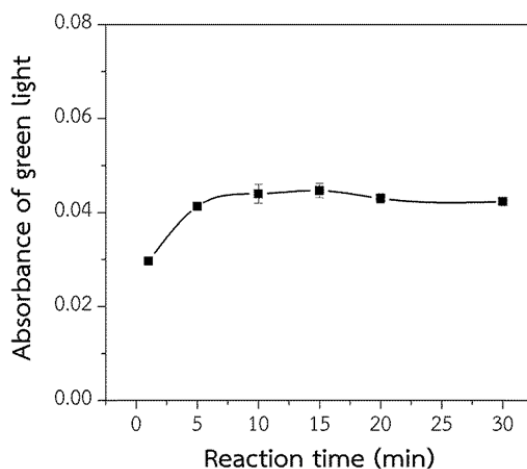


**Figure 5** Absorbance of green light recorded by PiCOEXPLORER device for the study of buffer solution effect in the range of 4 to 8.

#### *Effect of reaction time*

Various reaction times (1, 5, 10, 15, 20, 25, and 30 min) were studied. The variation of reaction time indicated that the absorbance of

green light is proportional to increasing reaction time and constant at long reaction time. As shown in Figure 6 the signal between a reaction time of 5 and 10 min is not different. Thus to save the analysis time the reaction time of 5 min was chosen.

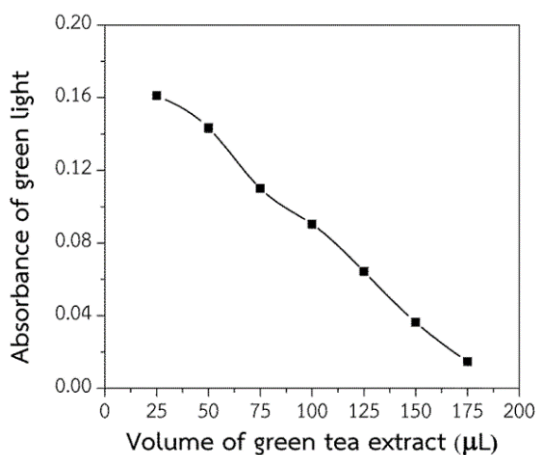


**Figure 6** Absorbance of green light recorded by PiCOEXPLORER device for the study of reaction time effect in the range of 1 to 30 min.

#### *Effect of the ratio between green tea extracts volume and standard/sample solution volume*

Owing to the limitation of PCR tube capacity (PCR tube, polypropylene, 200  $\mu$ L). This research uses the ratio between the natural reagent and standard/sample solution in 200  $\mu$ L as total volume. Figure 7 displays the plot of the volume of green tea extracts (25–175  $\mu$ L) and green light intensity. The results indicated that the volume of green tea extracts to standard/sample solution increase but green light intensity decrease. Therefore, the suitable condition of this study is 25:175  $\mu$ L of the ratio

of green tea extracts and standard/sample solution.



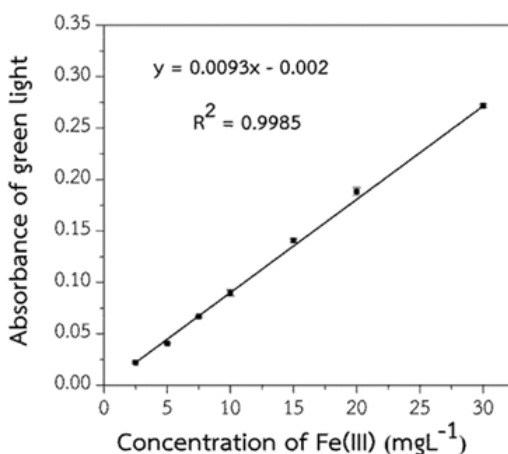
**Figure 7** Absorbance of green light recorded by PiCOEXPLORER device for the study of the ratio of the volume of green tea extracts and standard/sample solution effect in the range of 25 to 175 µL.

From all the above results we can summarize the variable ranges and optimum condition for the determination of iron ion using green tea extracts as a green reagent recorded by the PiCOEXPLORER device which is a newly developed method as shown in Table 1.

#### Study of interference

Interference studies were observed by adding seven cation including  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  into  $\text{Fe(III)}$   $10 \text{ mg}\cdot\text{L}^{-1}$  solutions to obtain the last concentration of 1, 5 and  $10 \text{ mg}\cdot\text{L}^{-1}$  which concentration ratios of  $\text{Fe(III)}$ :interference ion is 10:1, 2:1 and 1:1, respectively. The absorbance of green light obtained from these solutions was compared with the standard  $10 \text{ mg}\cdot\text{L}^{-1}$   $\text{Fe(III)}$ . It was found that absorbance of green light of all

concentration ratio is slightly different with standard  $10 \text{ mg}\cdot\text{L}^{-1}$   $\text{Fe(III)}$  by showing %RSD less than 3%. In this work, we did not study higher ratios of interference:  $\text{Fe(III)}$  concentration because pharmaceutical products usually do not contain these studied interfering ions at a higher quantity.



**Figure 8** The linearity range of  $\text{Fe(III)}$  standard using green tea extracts as a natural reagent recorded by PiCOEXPLORER device

Various parameters were optimized and optimal conditions as shown in Table 2 were applied for the studies of analytical characteristics including linearity range, the limit of detection (LOD), the limit of quantitation (LOQ) and determination of iron in pharmaceutical products. As indicated in Table 2, it was found that the linearity range of standard of  $\text{Fe(III)}$  is  $2.5$  to  $30 \text{ mg}\cdot\text{L}^{-1}$  (Figure 8),  $0.5 \text{ mg}\cdot\text{L}^{-1}$  of LOD and  $1.3 \text{ mg}\cdot\text{L}^{-1}$  of LOQ, respectively. To provide the reproducibility of the developed method, we studied the interaction between green tea extracts with various concentrations of 5, 10, and  $20 \text{ mg}\cdot\text{L}^{-1}$  of

Fe(III) and detected the absorbance of green light by PICOEXPLORER device ( $n = 7$ ). Precision, calculated as the percentage of relative standard deviation (%RSD) of all concentrations are 3.4–4.3, which is an acceptable range.

**Table1** Variable ranges and optimum conditions for the determination of iron using green tea extracts as a natural reagent recorded by PICOEXPLORER device.

Parameter studied	Range studied	Optimum level
Concentration of green tea extracts	1–20 %w/v	5 %w/v
Extraction time	1–30 min	15 min
Extraction temperature	40–100 °C	60 °C
pH	4–8	5
Reaction time	1–30 min	5 min
Volume of green tea extracts	25–175 $\mu$ L	25 $\mu$ L

**Table 2** Study of analytical characteristics obtained from Fe(III) standard solution

Parameter studied	Fe(III) standard solution
Linear regression equation	$y = 0.0093x - 0.002$ ( $R^2 = 0.9985$ )
Linear range (mg/L)	2.5–30
%RSD ( $n = 3$ ) over the linear range	0.0–3.6
LOD ( $3\sigma$ of blank) (mg/L)	0.5
LOQ ( $10\sigma$ of blank) (mg/L)	1.3
Precision (5.0, 10.0 and 20.0mg/L) ( $n = 7$ )	4.3, 3.5, 3.4 %RSD

**Table 3** Determination of iron in pharmaceutical samples by the proposed method and by the standard method based on spectrophotometry system

Sample No.	Form of iron	Amount of Fe(II) (mg/tablet)			%Deviation from standard method
		Label amount	Standard method	Proposed method	
1	Fumarate	100	105 $\pm$ 1	102 $\pm$ 2	-2.9
2	Fumarate	200	197 $\pm$ 6	199 $\pm$ 4	1.0
3	Fumarate	200	234 $\pm$ 10	231 $\pm$ 8	-1.3
4	Sulfate	135	117 $\pm$ 3	116 $\pm$ 3	-0.9



### *Application of green tea extracts for the determination of iron in pharmaceutical products*

The pharmaceutical products were purchased from local drug stores in Chiang Rai, Thailand. The sample preparation procedure was modified from the Association of Official Analytical Chemists (AOAC) method (21). A procedure as mentioned above in the part of experimental, three-drug and vitamin samples were analyzed using green tea extracts as a green reagent with standard addition method. A graph of the standard addition method was established by plotting the absorbance of green light versus the added concentration of iron. The iron contents in samples were evaluated as summarized in Table 3. The standard method based on spectrophotometry system using 1,10-phenanthroline as reagent was used to compare with the results obtained from the proposed method. It was found that the results from the proposed method were in good correlation with the standard method as compared at the 95% confidence level by the paired *t*-test ( $t_{\text{cal}} = 0.4588$ ,  $t_{\text{crit}} = 4.3026$ ).

### CONCLUSIONS

PiCOEXPLORER as a modern instrument based on a colorimetric system was developed for the 1.3 determination of iron in pharmaceutical products using green tea extracts as a green reagent. Green tea extracts can be used as an alternative low cost and easily available reagent for the quantification of iron

without purifying extraction before use. The green extract can be classified as a selective reagent for iron ion assay more than the other studied cation interference at the show on the iron pills products. This proposed method was successful in the determination of iron in pharmaceutical products and provides an alternative to other methods which is common, cost-effective, environmentally friendly, high precision and reduce the sample and reagent consumption. Iron contents were compared with the standard method; the results found both methods were the non-significant difference at 95% confidence level by the paired *t*-test. In addition, the proposed method can be classified as a micro-scale portable device.

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