Research Article

วิธีสเปกโทรโฟโตเมตริกอย่างง่ายสำหรับการวิเคราะห์ปริมาณเหล็ก(III)

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วัตถุประสงค์ของงานนี้ได้พัฒนาวิธีสเปกโทรโฟโตเมตริกอย่างง่ายและประหยัด สำหรับตรวจวัดปริมาณเหล็ก(III)โดยใช้ด๊อกซีไซคลินเป็นรีเอเจนต์ วิธีที่พัฒนาขึ้นนี้ได้นำไปประยุกต์ใช้ในการตรวจวัดปริมาณเหล็ก(III)ในตัวอย่างน้ำผลไม้ ผลการตรวจวิเคราะห์จากวิธีที่นำเสนอนี้ได้มีการเปรียบเทียบผลที่ได้กับวิธีเฟลมอะตอมมิกแอบซอบชั่นสเปกโทรโฟโตเมตริก **วัสดุและวิธีทดลอง:** การศึกษานี้มีพื้นฐานจากการเกิดสารประกอบเชิงซ้อนระหว่างเหล็ก(III) และด๊อกซีไซคลินจากยาด๊อกซีไซคลินแบบแคปซูล ด้วยการวัดค่าการดูดกลืนคลื่นแสงสูงสุดที่ความยาวคลื่น 435 นาโนเมตร สภาวะการทดลองได้หาค่าที่เหมาะสมด้วยวิธียูนิวาริเอตท์ ตัวอย่างน้ำผลไม้จะนำมาเตรียมโดยเทคนิคการย่อยไอออนด้วยความร้อนแบบย้อนกลับ ด้วยสารละลายผสมระหว่างกรดไนตริกและไฮโดเจนเปอร์ออกไซค์เป็นเวลานาน 1.5 ชั่วโมงและนำไปวิเคราะห์ปริมาณเหล็ก(III) **ผลการศึกษา:** กราฟมาตรฐานที่ได้สร้างขึ้นจากสารละลายมาตรฐานเหล็ก(III) มีความเข้มข้นสารละลายเหล็ก(III) ในช่วง 0.3 – 10.0 ไมโครกรัมต่อมิลลิลิตร ในช่วงความเข้มข้นดังกล่าว สมการเส้นตรงซึ่งแกน y แทนค่าดูดกลืนคลื่นแสงของสารละลายเหล็ก(III) แกน x แทนความเข้มข้นของเหล็ก(III) จะได้สมการ $y = 0.0968x + 0.0336$ ($r^2 = 0.9948$, $n=3$) ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ของวิธีที่นำเสนอจากการทดลอง 7 ครั้ง ของเหล็ก(III) ที่ความเข้มข้น 3.0 และ 7.0 ไมโครกรัมต่อมิลลิลิตร มีค่าน้อยกว่า 0.0003 % ของแต่ละความเข้มข้น ค่าเปอร์เซ็นต์การคำนวณย้อนกลับของเหล็ก(III) ที่ความเข้มข้น 3.0 และ 7.0 ไมโครกรัมต่อมิลลิลิตร ($n=7$) มีค่าเท่ากับ 97.86 % และ 103.96 % ตามลำดับ ผลการรบกวนการวิเคราะห์เหล็ก(III) จากไอออนต่างๆ (Na^+, K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, Mn^{2+} และ Fe^{2+}) ได้มีการตรวจสอบด้วยวิธีที่นำเสนอนี้ แคทไอออนส่วนใหญ่รบกวนสัญญาณการวิเคราะห์น้อยกว่า ± 3 % **สรุปผล:** ผลของปริมาณเหล็ก(III)ที่ได้จากวิธีสเปกโทรโฟโตเมตริกที่นำเสนอและวิธีเฟลมอะตอมมิกแอบซอบชั่นสเปกโทรโฟโตเมตริกพบว่าไม่แตกต่างกันโดยใช้สถิติ *t*-test ทดสอบด้วยความเชื่อมั่นที่ระดับ 95% วิธีที่พัฒนาขึ้นนี้ประสบผลสำเร็จเมื่อนำไปใช้ในการวิเคราะห์หาปริมาณเหล็ก(III) ในตัวอย่างน้ำผลไม้ พบว่าเป็นวิธีที่ง่าย ประหยัด ให้ความถูกต้อง และเป็นวิธีทางเลือกอีกวิธีหนึ่งในการวิเคราะห์

คำสำคัญ: เหล็ก(III), ด๊อกซีไซคลิน, รีเอเจนต์จากยา, วิธีสเปกโทรโฟโตเมตริก

Simple Spectrophotometric Method for Determination of Iron(III) Content

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Abstract

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The objective of this work was therefore to develop a simple and cost-effective spectrophotometric method for determination of iron(III) using doxycycline as a reagent. The developed method was applied for analysis of iron(III) content in fruit juice samples. The analysis result from the proposed method was compared to the result from flame atomic absorption spectrophotometric method. **Materials and methods:** This study was based on the complexation of iron(III) and doxycycline reagent from commercial doxycycline capsule characterized by an absorption at maximum wavelength of 435 nm. The experimental conditions were optimized by means of the univariate method. The samples of fruit juice were digested by heating under reflux ion with nitric acid and hydrogen peroxide mixture for 1.5 h and then were measured iron(III) level. **Results:** The linear calibration curve was constructed using iron(III) standard which was contained iron(III) standard solution in range of 0.3–10.0 µg mL^{-1}. Within this concentrations range, linear regression of the absorbance of iron(III) (y) and concentration of iron(III) (x) expressed the equation $y = 0.0968x + 0.0336$ (r^2=0.9948, n=3). The relative standard deviation (RSD) of proposed method calculate from 7 replicate of iron(III) at 3.0 and 7.0 µg mL^{-1} were found to be less than 0.0003 % of each. The percentage recoveries of iron(III) at 3.0 and 7.0 µg mL^{-1} (n=7) were found to be 97.86 % and 103.96 %, respectively. Effect of some possible interfering ions (Na^+, K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, $Mn^{2+}$and Fe^{2+}) on the determination of iron(III) were investigated using present method**.** Most of tested cations were interfered the absorption signal less than ±3 %. **Conclusion:** The iron(III) level in fruit juice samples obtained from the proposed spectrophotometric method and flame atomic absorption spectrophotometric method were in accordance, as compared by the t-test at 95%confidence level. This proposed method was successfully applicable in the determination of iron(III) in fruit juice samples and represented a simple, cost-effective, high precision and alternative to other analytical methods.

Keywords: Iron(III), Doxycycline, Drug reagent, Spectrophotometric method

Introduction

Ideally, reagents are vital to analytical processes, and they are probably the most dynamic area of green analytical chemistry (GAC) research. In this area, GAC has special consideration because of the hazardous nature of the solvents that are often used – reducing contract with a specific hazardous substance, or excluding toxic reagent from the analytical method (Mohamed, 2015). The analytical method would be greener if it reduced consumption of reagent and sample, used safe procedures, avoided the use of hazardous reagents, had short analysis time and used energy-efficient apparatus. In order to implement GAC principle, it is important to know how to green are many step within analytical procedure such as simple sample preparation, using green reagent and save chemical or energy in analytical method (Anastas *et al*., 2010). The objective of this work was therefor to develop a simple and cost-effective spectrophotometric method for determination of iron(III) using doxycycline as a reagent.

Iron is one of the most frequently analysed elements. The iron level is very important to the health of mammals, and iron accumulated in iron deposits of the body as iron-ferritin, where levels below 12 μg mL^{-1} indicate that loss of iron causing anemia (Aggett *et al*., 2002). It is important to determine iron level for public health studies (Whittaker *et al*., 2002).

The IUPAC name of doxycycline is (4S, 4aR,5S,5aR,6R,12aR)-4-(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide which is a semisynthetic tetracycline antibiotic obtained by the modification of oxytetracycline. It is used worldwide as a prophylactic and therapeutic agent in the prevention and treatment of infections caused by gram positive and gram negative bacteria (Lorenzetti *et al*., 2017). Several methods have been reported previously for the determination of iron(III) including ion chromatography (Chen *et al*., 2015), spectrofluorometric (Tümay *et al*., 2018), flow injection analysis (Prasertboonyai *et al*., 2015), potentiometric (Crisponi *et al*., 2008), polarographic (Somer *et al*., 2009) and camera based method (Abbaspour *et al*., 2006). The previous work was reported that tetracycline and derivatives including doxycycline could be formed complexation with iron(III) (Ramesh *et al*., 2011). However, a report of spectrophotometric method for the determination of iron(III) in fruit juice using doxycycline as a reagent has not been yet available in the literature. Thus, it is possible to use the specific spectrophotometric method of the complex to analysis iron(III) content in commercial fruit juice product.

The present work is based on the ability of iron(III) and doxycycline to form metal–drug complex. Palamy *et al*. (2017) was reported the possible mechanism between doxycycline and iron(III) for determination doxycycline in pharmaceutical formulation. Thus, it is possible to use the specific UV-Visible absorption of the complex to determine iron(III) level in interesting samples. The studied was used commercial doxycycline as a reagent which was reacted with iron(III) in sample. The resulting yellow complex is measured at maximum wavelength of 435 nm. The present method was successfully for determination of iron(III) content in fruit juice samples which was compared the resulting with flame atomic absorption spectrophotometric method.

Materials and methods

Chemicals

1. Ammonium ferric sulfate dodecahydrate, Analytical grade, Fluka; UK
2. Nitric acid, Analytical grade, Merck; Germany
3. Perchloric acid, Analytical grade, Merck; Germany
4. Phosphoric acid, Analytical grade, Sigma-Aldrich; Germany
5. Sulfuric acid, Analytical grade, Merck; Germany
6. Hydrochloric acid, Analytical grade, Fluka; UK
7. Hydrogen peroxide, Analytical grade, Sigma-Aldrich; Germany

8. Doxycycline (as HCl), Siadocin® 100 mg capsule, Thailand

Instrumentations

1. UV-Visible spectrophotometer, model 1700, Shimadzu®; Japan

2. Flame atomic absorption spectrophotometer, PinAAcle 900F, PerkinElmer®; USA

3. Deionized water unit, Millipore® Milli-Q; USA

Procedure

Drug reagent preparation

The hydrochloride of doxycycline capsule was purchased from local drug store. The contents of commercial pharmaceutical capsule was 100 mg doxycycline hydrochloride. The powder of twenty capsule of drug was weighted with analytical balance, then ground and mixed well. An amount of drug equivalent to one capsule was accurately weighted and dissolved in deionized water by sonication in a 100 mL volumetric flask and diluting with deionized water to 100 mL which was obtained 1000 µg mL^{-1} stock reagent solution. The doxycycline reagent (250 µg mL^{-1}) was prepared by pipetting 25 mL of stock reagent solution to 100 mL of volumetric flask and made up to the mark with deionized water.

Standard of iron(III) solution preparation

A stock solution of 1000 µg mL^{-1} iron(III) was prepared by dissolving 0.8634 g of ammonium ferric sulfate dodecahydrate in 1 x 10^{-2} mol L^{-1} nitric acid and diluting to 100 mL in a volumetric flask. Solution of working iron(III) standards were obtained by appropriate, accurate dilutions of this solution with 1.0 x 10^{-2} mol L^{-1} nitric acid.

Calibration curve and absorption measuring

The working solution of iron(III) standard for spectrophotometric method with concentrations ranging from 0.3–10.0 µg mL^{-1} were adjusted in 25 mL volumetric flasks by diluting the appropriate amounts of iron(III) stock solution with 1 x 10^{-2} mol L^{-1} nitric acid to graduate mark. The 5 mL assigned iron(III) standard solution was mixed with 10 mL 250 µg mL^{-1} doxycycline reagent solutions in ratio of 1:2 (v/v) which was recorded the absorption signal using UV-Visible spectrophotometer.

Sample preparation

The 100 mL of commercial fruit juice (pomegranate, tangerine, shogun orange, guava, tomato, carrot and red grape) was accurately transferred into 250 mL round bottom flask, and 10 mL of a mixture consisting of nitric acid and hydrogen peroxide (1:9, v/v) were added (Ruengsitagoon, 2008). These sample were digested by heating under reflux for 1.5 h. The cooled samples were transferred into each of 100 mL volumetric flask and made up to the mark with deionized water, mixed well, then subsequently analyzed by the proposed method and flame atomic absorption spectrophotometric method.

Results

Absorption spectra

The UV-visible spectra of complexes prepared from iron(III) and doxycycline from commercial drug. These was used to identify the optimum wavelength for detection of the iron(III) and doxycycline complex. Iron(III) solutions were prepared in 1x10^{-2} mol L^{-1} HNO_3 with the concentration range of 1.0 – 5.0 µg mL^{-1}. The iron(III) solution reacted with doxycycline reagent resulting a soluble yellow complex. The metal ligand ratio was found to be 1:2 using mole ratio and continuous variation method (Palamy *et al*., 2017). The spectra of all mixture were recorded from the wavelength in the range of 200 – 800 nm. It was found that the maximum absorption of the iron(III) – doxycycline complex was at 435 nm (Figure 1). The possible mechanism had shown in Figure 2 which was reacted between iron(III) and doxycycline from commercial drug.

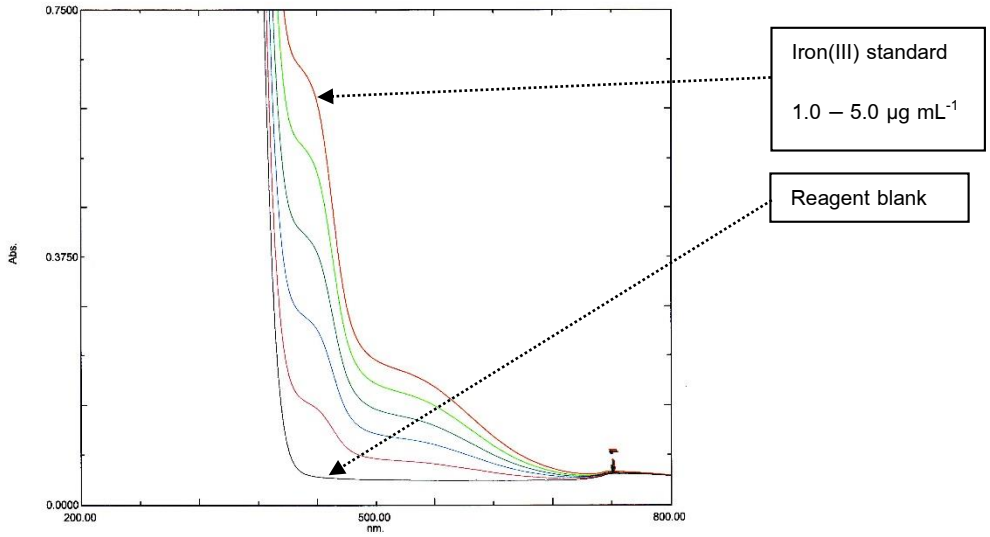

Figure 1. Absorptions spectra of the complex formed between iron(III) 1.0-5.0 µg mL^{-1} and doxycycline from commercial drug.

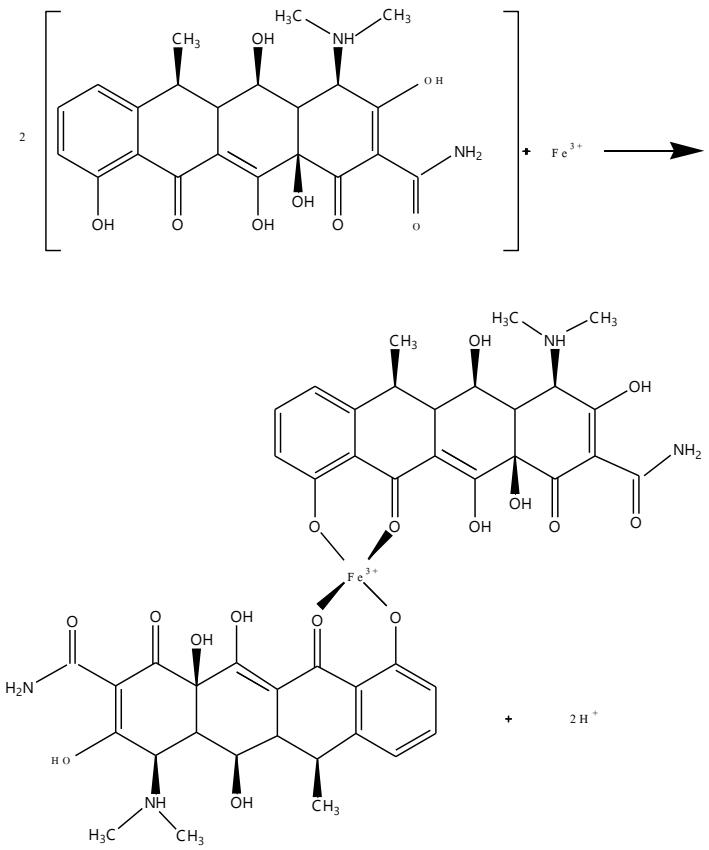

Figure 2. Proposed mechanism of iron(III) and doxycycline.

Validation of the method

The studies were investigated for testing the optimum conditions for using doxycycline as a reagent. The optimization of assay parameters and experimental conditions was carried out by mean of univariate method, in which one variable was modified while maintaining the other variables at constant values (Table 1).

Effect of type of mineral acid and concentration of mineral acid

The effect of type of mineral acid used for preparing iron(III) solution was tested with 1.0 x 10^{-2} mol L^{-1} solutions of nitric acid, phosphoric acid, perchloric acid, sulfuric acid and hydrochloric acid. The relative absorption value of iron(III) – doxycycline complex detected at 435 nm showed that nitric acid gave a higher absorbance than other mineral acid. The effect of various concentrations of nitric acid solution (0.125 x 10^{-2} – 4 x 10^{-2} mol L^{-1}) on the absorption of iron(III) – doxycycline complex was examined. The nitric acid concentration which exhibited the greatest signal was found to be 1.0 x 10^{-2} mol L^{-1} and was therefore chosen as optimum concentration.

Effect of volume ratio of iron(III) and doxycycline

The optimum volume ratio between iron(III) standard solution and doxycycline solution was studied in the ratio of 2:1, 1:1, 1:2, 1:3 and 1:4. The maximum absorbance was obtained with the ratio 1:2 (iron(III) and doxycycline; v:v) and this ratio was selected as the optimum ratio for the subsequent studies.

Effect of the concentration of doxycycline

The effect of varying concentration of doxycycline reagent between 100–750 µg mL^{-1} was investigated. The appropriate absorbance was obtained when the concentration of doxycycline reagent solution was 250 µg mL^{-1} and was therefore chosen as the optimum concentration. However, further increase in doxycycline concentration found the absorption signal to decrease gradually to 750 µg mL^{-1}.

Table 1. The optimum conditions for determination iron(III) using doxycycline as a reagent.

Conditions studied	Range studied	Optimum level
1. Wavelength (nm)	200 – 800	435
2. Type of mineral acid for preparing Iron(III) standard solution	HNO_3, H_3PO_4, $HClO_4$, H_2SO_4, HCl	HNO_3
3. Concentration of HNO_3 (x 10^{-2} mol L^{-1})	0.125, 0.25, 0.50, 1.00, 2.00, 4.00	1.00
4. Ratio of standard or sample and reagent	2:1, 1:1, 1:2, 1:3, 1:4	1:2
5. Concentration of doxycycline as a reagent (µg mL^{-1})	100, 250, 500, 750	250

Analytical characteristics

The linear calibration curve was constructed using iron(III) standard solution which was contained iron(III) standard in rage of 0.3–10.0 μg mL^{-1}. Within this concentrations range, linear regression of the absorbance of iron(III) (*y*) and concentration of iron(III) (*x*) expressed the equation $y = 0.0968x + 0.0336$. The correlation coefficients was shown to be 0.9948 (*n*=3). The limit of detection (LOD) was defined as the concentration of analyte that gave the signal that was different from the blank by an amount equal to three times the standard deviation of the blank signal. It was found to be 0.10 μg mL^{-1} iron(III). The limit of quantitation (LOQ) is defined as the analyte producing a signal that is at least ten times the standard deviation of the blank signal, and was shown to be 0.33 μg mL^{-1} iron(III).

The precision of the proposed method was studied through the repeatability by measuring seven replicated of two standard iron(III) solutions (3.0 and 7.0 μg mL^{-1}). The relative standard deviation (RSD) of proposed method calculate from 7 replicate of iron(III) at 3.0 and 7.0 μg mL^{-1} were found to be less than 0.0003 % of each. Accuracy was tested by means of recovery determination. The percentage recoveries of iron(III) at 3.0 and 7.0 μg mL^{-1} (*n*=7) were found to be 97.86 % and 103.98 %, respectively (Table 2).

Table 2. Analytical characteristics of proposed method for determination of iron(III) content.

Parameters	Optimum value
Linearity of calibration curve	0.3 – 10 μg mL^{-1}
Linear regression equation	$Y = 0.0968X + 0.0336$
Correlation coefficient	0.9948
Limit of detection, LOD	0.10 μg mL^{-1}
Limit of quantitation, LOQ	0.33 μg mL^{-1}
Repeatability (*n*=7); RSD.	
3.0 μg mL^{-1}	0.0003 %
7.0 μg mL^{-1}	0.0003 %
Percentage recoveries (*n*=7)	
3.0 μg mL^{-1}	97.86 %
7.0 μg mL^{-1}	103.98 %

The proposed method was applied to determination iron(III) content in fruit juice samples from 7 commercial products and the results was compared to the flame atomic absorption spectrophotometric method. An acetylene-air flame was used for determination of iron(III). Gas flow rate and burner height were adjusted in order to gave the maximum absorbance signal for analyte. The analytical wavelength of flame atomic absorption spectrophotometric method was set at 248.3 nm (Sadeghi *et al*., 2017). The accuracy was verified by the Student's *t*-test with calculates Student's *t*-test value (0.60) less than the theoretical (2.45) at a confidence level 95% (*P* value of 0.05). Reasonable agreement between the proposed method and flame atomic absorption spectrophotometric methods was found (Table 3).

Table 3. Accuracy of proposed method compared with FAAS method for determination of iron(III) content in fruits juice samples.

No	**Fruit juice**	**Iron(III) found** (μg mL^{-1} ± SD), *n*=5	
		Proposed method	**FAAS method**
1	Pomegranate	0.68 ± 0.04	0.70 ± 0.02
2	Tangerine	0.35 ± 0.02	0.32 ± 0.03
3	Shogun orange	0.34 ± 0.01	0.37 ± 0.01
4	Guava	0.35 ± 0.01	0.30 ± 0.01
5	Tomato	0.48 ± 0.01	0.50 ± 0.01
6	Carrot	1.15 ± 0.01	1.21 ± 0.02
7	Red grape	3.03 ± 0.05	2.83 ± 0.04
t-test at 95% confidence level:			
t-calculation			0.60
t-distribution at (*n*-1)=6,			2.45

Effect of some possible interfering ions (Na^+, K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Al^{3+}, Cu^{2+}, Mn^{2+} and Fe^{2+}) on the determination of iron(III) were investigated with a maximum w/w ratio of interfering ions to iron(III) up to 10:1. Synthetic sample solutions containing 5.0 μg mL^{-1} of iron(III) and different concentrations of some metal ions at 10 and 50 μg mL^{-1} were tested using present method, and absorbance were recorded. Most of tested cations were caused interfered the absorption signal less than ±3 % for determining the analyte of interest. However, the most serious interference from Al(III) ion was observed (10 % and 17 % for 10 and 50 μg mL^{-1} of Al^{3+}). The possible masking reagent for reducing the effect of Al(III) was tartrate (Ahmed *et al.*, 2001).

Discussion and Conclusions

A simple spectrophotometric method was developed for determination of iron(III) content in fruit juice samples. The proposed protocol describes the use of doxycycline as a reagent. This method is based on complexation between iron(III) and doxycycline which iron(III)–doxycycline complex monitored with UV-Visible spectrophotometer at maximum wavelength of 435 nm. The linearity of the calibration graph is in the useful range for quantitation of iron(III) in fruit juice samples. The iron(III) level in fruit juice samples obtained from the proposed spectrophotometric method and flame atomic absorption spectrophotometric method were in accordance, as compared by the *t*-test at 95% confidence level. This proposed method was successfully applicable in the determination of iron(III) in fruit juice samples and represented a simple, cost-effective, high precision, alternative to other analytical methods.

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