Research Article

รีเอเจนต์อย่างง่ายสำหรับการวิเคราะห์หาปริมาณด็อกซีไซคลินและเตตราไซคลินในผลิตภัณฑ์ยาโดยวิธียูวี-วิสิเบิลสเปกโตรโฟโตเมตริก

สุดาวอน พิพัฒน์ผล[1]*, วิรัช เรืองศรีตระกูล[2]

[1] นักศึกษาบัณฑิตศึกษา, คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[2] รองศาสตราจารย์, คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

*** ติดต่อผู้นิพนธ์:** คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002 โทรศัพท์ 0928978970 โทรสาร 043-202379 อีเมล soudavone@kkumail.com

บทคัดย่อ

รีเอเจนต์อย่างง่ายสำหรับการวิเคราะห์หาปริมาณด็อกซีไซคลินและเตตราไซคลินในผลิตภัณฑ์ยาโดยวิธียูวี-วิสิเบิลสเปกโตรโฟโตเมตริก

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วัตถุประสงค์ของงานนี้ได้พัฒนาวิธีการหาปริมาณด็อกซีไซคลินและเตตราไซคลินในผลิตภัณฑ์ยาโดยใช้เฟอร์รัสไอออนในเม็ดวิตามินเป็นรีเอเจนต์ โดยอาศัยหลักการเกิดปฏิกิริยาระหว่างยา-เฟอร์รัสไอออนในกรดไนตริก และสารละลายไฮโดรเจนเปอร์ออกไซด์ที่ช่วยเพิ่มสัญญาณการดูดกลืนแสง **วิธีการศึกษา:** นำด็อกซีไซคลินและเตตราไซคลินในรูปแบบยาแคปซูลและยาเม็ดมาทำปฏิกิริยาเฟอร์รัสไอออนจากเม็ดวิตามินที่มีความเข้มข้น 5.0 × 10^{-2} โมลต่อลิตร ในกรดไนตริก*เข้มข้น* 5.0×10^{-3} โมลต่อลิตรและสารละลายไฮโดรเจนเปอร์ออกไซด์เข้มข้นร้อยละ 2.5 x 10^{-2} (ปริมาตรต่อปริมาตร) ในอัตราส่วน 2.0 : 1.0 : 0.1 (ปริมาตรต่อปริมาตร) ตามลำดับ โดยเกิดสารเชิงซ้อนสีเหลืองที่มีค่าการดูดกลืนแสงที่ความยาวคลื่นสูงสุดที่ 425 นาโนเมตร **ผลการศึกษา:** การวิเคราะห์ด็อกซีไซคลินในช่วงความเข้มข้น 1.0 - 100 ไมโครกรัมต่อมิลลิลิตรและเตตราไซคลินในช่วงความเข้มข้น 1.0 - 200 ไมโครกรัมต่อมิลลิลิตร สามารถแสดงสมการเส้นตรงความสัมพันธ์ระหว่างค่าการดูดกลืนแสง (y) และความเข้มข้นของยาแต่ละชนิด (x) เท่ากับ y = 0.0064x - 0.001 (r^2 = 0.9998) และ y = 0.0069x - 0.0095 (r^2 = 0.9997) สำหรับด็อกซีไซคลินและเตตราไซคลินตามลำดับ ค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (LOD) และ ค่าขีดจำกัดต่ำสุดของการวิเคราะห์เชิงปริมาณ (LOQ) ของยาแต่ละชนิดคือ 0.2 และ 0.6 ไมโครกรัมต่อมิลลิลิตรตามลำดับ ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ (RSD) ของการทำซ้ำสำหรับวิธีการที่นำเสนอมีค่าน้อยกว่า 2.00 % ความถูกต้องแสดงด้วยร้อยละการคืนกลับของยาอยู่ในช่วง 98.62 - 100.56 % สำหรับด็อกซีไซคลิน และ 100.92 - 102.00 % สำหรับเตตราไซคลิน **สรุปผลการศึกษา:** ผลของปริมาณดอกซีไซคลินและเตตราไซคลินที่ได้จากวิธีสเปกโตรโฟโตเมตริกที่นำเสนอและวิธีอ้างอิงให้ผลการวิเคราะห์ไม่แตกต่างกันอย่างมีนัยสำคัญ โดยการทดสอบทางสถิติ (student t-test=0.77, 0.16) ตามลำดับ ที่ t critical = 2.45 ที่ระดับความเชื่อมั่น 95 % ดังนั้นวิธีที่พัฒนาขึ้นเป็นวิธีที่ง่าย ประหยัด ให้ความถูกต้อง และเป็นวิธีทางเลือกอีกวิธีหนึ่งในการวิเคราะห์หาปริมาณด็อกซีไซคลินและเตตราไซคลินในผลิตภัณฑ์ยา

คำสำคัญ: ด็อกซีไซคลิน, เตตราไซคลิน, เฟอร์รัสไอออน, ยาเม็ดวิตามิน, วิธียูวี-วิสิเบิลสเปกโตรโฟโตเมตริก

Simple Reagent for Determination of Doxycycline and Tetracycline in Pharmaceuticals by UV - Visible Spectrophotometric Method

Soudavone Phiphatphon[1]*, Wirat Ruengsitagoon[2]

[1] Graduate student, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
[2] Associate Professor, Faculty of Pharmaceutical Sciences, Khon Kaen 40002, Thailand
***Corresponding author:** Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand
Tel. 0928978970; Fax 043 202379 Email: soudavone@kkumail.com

Abstract

Simple Reagent for Determination of Doxycycline and Tetracycline in Pharmaceuticals by UV - Visible Spectrophotometric Method
Soudavone Phiphatphon[1]*, Wirat Ruengsitagoon[2]
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The objective of this work was developed for the quantification of doxycycline and tetracycline in pharmaceutical products for proposed protocol that describes the use ferrous ion contained in vitamin tablets as reagent. It is based on the reaction between drug-ferrous ion in acidic and dilution of hydrogen peroxide. **Method:** This study was used commercial of doxycycline and tetracycline for capsules and tablets which were reacted with ferrous ion from vitamin tablets were concentration 5.0×10^{-2} mol/L in 5.0×10^{-3} mol/L nitric acid and 2.5×10^{-2} % (v/v) of hydrogen peroxide as reagent in a ratio of 2.0: 1.0: 0.1 (v/v), respectively. The reaction was given a yellow complex with highest absorption was measured at 425 nm. **Results:** The calibration graphs were obtained for doxycycline concentrations in the range of 1.0 - 100 µg/mL and tetracycline 1.0 - 200 µg/mL. Linear regression analysis of the absorbance (y) and concentration of each drug (x) expressed the equation y = 0.0064x - 0.001 (r^2 = 0.9998) and y = 0.0069x - 0.0095 (r^2 = 0.9997) for doxycycline and tetracycline, respectively. The limit of detection (LOD) and limit of quantification (LOQ) for each drug was 0.2 and 0.6 µg/mL, respectively. The percentage relative standard deviation (RSD) of the reproducibility for this proposed method was less than 2.00 %, the percentage recovery was in the range 98.62 - 100.56 % for doxycycline and 100.92 - 102.00 % for tetracycline. **Conclusion:** The quantitative of doxycycline and tetracycline obtained from the proposed spectrophotometric method and reference method were no significant difference by the student *t*-test (0.77, 0.16), respectively by t critical = *2.45* at a confidence level 95%. It is a simple, cost-effective, high precision and alternative to the method of analytical of doxycycline and tetracycline in pharmaceutical products.

Keywords: Doxycycline, Tetracycline, ferrous ion, Vitamin tablets, UV-Visible Spectrophotometric method

Introduction

Doxycycline is a member of the tetracycline group of broad-spectrum antibiotics. It is a group of antibiotics for the treatment of common bacterial infections, widely used in humans and animals, they have also found application in the postharvest preservation of fruits, vegetables and the extermination of insect pasts (Agwuh and MacGowan, 2006; Newton *et al.,* 2005).

Several methods have been used to quantify the tetracycline derivatives in pharmaceutical products and biological fluids including of UV-Visible spectrophotometric method (Ramesh *et al*., 2010; Hasan *et al*., 2016), high performance liquid chromatography (Kogawa *et al*., 2012; Jeyabaskaran *et al*., 2015), flow injection analysis (Al-Abachi *et al*., 2015; Palamy *et al*., 2017), capillary electrophoresis (Castellanos Gil *et al*., 2000; Ghaemi *et al*., 2014) and spectrofluorimetric (Attia *et al*., 2011). The previous studies have reported on the use of green reagents prepared from *Clitoria ternatea, Dendrobium Sania, Beta vulgaris subsp. Vulgris* (Grudpan *et al*., 2011), green tea (Pinyou *et al*., 2010), and guava leaf (Settheeworrarit *et al*., 2005) for assaying pharmaceutical compounds or metal iron. High performance liquid chromatography (HPLC) is recommended in The British Pharmacopoeia (BP) and the United State Pharmacopeia (USP) for the determination of *tetracycline derivatives* (BP, 2015; USP, 2016). Although HPLC provides great selectivity, sensitivity, precision and accuracy for assay of these products, this technique can be unsuitable for use by local pharmaceutical manufacturers and some developing countries due to the expensive instrumentation, the need for specialized skills and the production of hazardous waste from organic solvents.

Nowadays, reagents are important for analytical processes and they are the most dynamic area of green analytical chemistry (GAC) research. As a result, green analytical chemistry features have been increasingly executed in the analysis and it has special consideration due to the dangerous nature of solvents that are often used-reduce contracts with specific hazardous substances, or exclude toxic reagents from the analytical method (Mohamed, 2015). The method should be more environmentally friendly if it reduces the consumption of reagents and samples. Moreover, it is safe, simplified and avoids the use of hazardous reagents by using short time-consuming processes and using energy-efficient instruments (Anastas *et al*., 2010; Galuszka, Migaszewski and Namiesnik, 2013)

In this context, antibiotics in the tetracycline family have the property of forming complexes with metal ions such as iron (III) (Figure 1) has been determined with the continuous variation method as reported (Ramesh *et al*., 2011; Vilayphone *et al*., 2018). In some areas, the acquisition and importation of chemicals are restricted. Other hands, the ferrous ion contained in vitamin tablets and hydrogen peroxide are easily available from the drug store than in the form of the primary standard. Then in the initial standard form based on this condition. It may be related to the development of alternative methods for doxycycline and tetracycline determination under this chemical supply limitation. Thus, it is possible to use the proposed method to describe a simple, rapid, sensitive and inexpensive method for the determination of doxycycline and tetracycline in pharmaceutical formulations based on the spectrophotometric detection of the colored product formed by the reaction between a green reagent containing ferrous ion obtained from vitamin tablets with the drug through hydrogen peroxide (H_2O_2) in the aqueous solution that it is catalyzed by the oxidation of the ferrous ion (Fe^{2+}) to the ferric ion (Fe^{3+}) (Figure 2). The analytical method will be optimized using the univariate method. The developed method can be used satisfactorily with the determination of doxycycline and tetracycline in pharmaceutical products and the results were compared to the reference UV-Visible spectrophotometric method using Folin - Ciocalteu reagent (Ramesh *et al*., 2010).

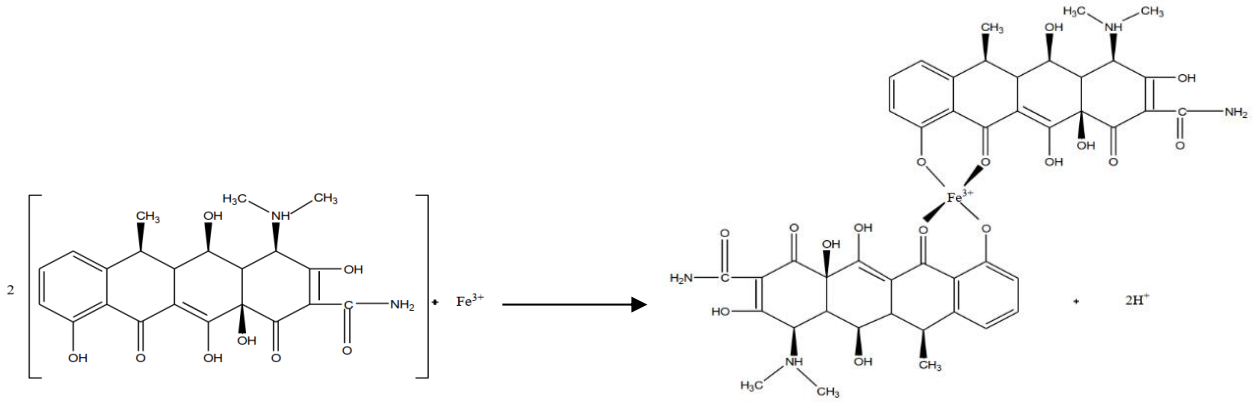

Figure 1. The possible mechanism doxycycline reacts with iron (III)

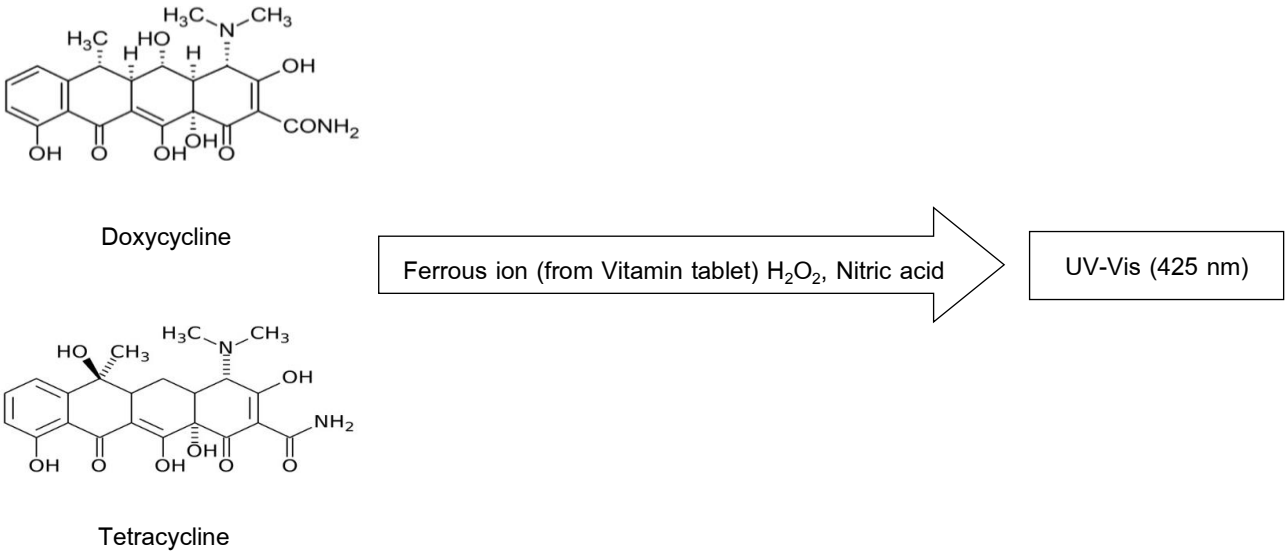

Figure 2. The method to assay doxycycline, tetracycline by complexation using green reagents containing ferrous ion extracted from vitamins tablet

Materials and Methods

Instrumentations

1. UV-Visible spectrophotometer (X-ma 1000)
2. Sonicator (MUJIGAE), Model SD-D300H; Korea
3. Deionized water unit (18.3 mΩ-cm) PURE POWER Korea
4. Analytical balance (OHAUS PA323C), PIONEER; USA

Chemicals

1. Doxycycline HCl standard, Sigma-Aldrich.
2. Tetracycline HCl standard, Sigma-Aldrich.
3. Doxycycline HCl 100 mg capsule and tablet
4. Tetracycline HCl 250 mg and 500 mg capsule
5. Hydrogen peroxide, Merck KGaA, Germany
6. Vitamin tablets (brand A contained 135 mg ferrous sulfate/tablet)
7. Vitamin tablets (brand B contained 10 mg ferrous fumarate/tablet)
8. Vitamin tablets (brand C contained 15 mg ferrous fumarate/tablet)
9. Vitamin tablets (brand D contained 10 mg ferrous fumarate/tablet)
10. Vitamin tablets (brand E contained 14 mg ferrous sulfate/tablet)

11. Nitric acid (HNO_3), Analytical grade, Merck, Germany

12. Hydrochloric acid, Analytical grade, Merck, Germany

13. Sulfuric acid, Analytical grade, Merck, Germany

14. Phosphoric acid, Analytical grade, Merck, Germany

15. Perchloric acid, Analytical grade, Merck, Germany

16. Folin-Ciocalteu's phenol reagent, Merck, Germany

17. Sodium Carbonate anhydrous, Grade AR, Sigma; German)

Procedure

1. Drug Collection

Seven commercial doxycycline and tetracycline in pharmaceutical products (capsules and tablets) and five commercial vitamins of pharmaceutical formulations (tablets) containing ferrous ion from different brands were used as the samples and reagents, respectively. All of the drugs and vitamins were purchased from drug stores in Vientiane Capital, Lao PDR and Khon Kean Province, Thailand.

2. General Analytical Procedure

Different aliquots of the working doxycycline and tetracycline standard solutions in the range of concentration 1.0 - 100 µg/mL and 1.0 - 200 µg/mL, respectively. Pipet the accurate volumes 2.0 mL were transferred into test tubes. To each test tubes added 1.0 mL of 5.0 × 10^{-2} mol/L of ferrous ion in vitamins tablets solution and 0.1 mL of 2.5 × 10^{-2} (v/v) of hydrogen peroxide to mixed well and kept at room temperature. The absorbance of each solution was measured at 425 nm against a reagent blank. The proposed method was compared with the resulting reference method (Ramesh et al., 2010)

3. Reagent Preparation

Solutions of five commercial vitamins tablets from different brands (contained ferrous ion) were *including* brand A (dried ferrous sulfate 135 mg), brand B *(*ferrous fumarate 10 mg), brand C (ferrous fumarate 15 mg), brand D (ferrous fumarate 10 mg) *and* brand E (ferrous sulfate 14 mg) were used by the proposed method.

Twenty vitamin tablets of each commercial brand in pharmaceutical to be studied were weighed exactly. The tablets were grounded to a fine powder and homogenized. A portion of this powder equivalent to the amount of ferrous ion powder from each commercial brand was accurately weighed to be dissolved in 100 mL of nitric acid ($5x10^{-3}$ mol/L) to provide the concentration of 5.0 x 10^{-2} mol/mL and filtered through Whatman No 42-filter before use.

Hydrogen peroxide solution was prepared from the concentrated hydrogen peroxide 30%. Take one milliliter of 30% hydrogen peroxide was diluted with deionized water into 1% (v/v) of hydrogen peroxide in a volumetric flask. Then it was diluted to 2.5 x 10^{-2} % (v/v) hydrogen peroxide with deionized water in a graduated volumetric flask.

4. Standard Preparation

A stock solution of doxycycline and tetracycline standard was prepared by accurately weighing 0.0100 g and 0.0250 g, respectively. The standard drug was dissolved in 5.0 × 10^{-3} mol/L nitric acid and the volume was adjusted to 100 mL of graduate mark to provide a final concentration of 100 µg/mL and 250 µg/mL, respectively.

5. Sample Preparation

Seven commercial samples of pharmaceutical formulations (capsules or tablets) containing doxycycline hyclate equivalent to doxycycline 100 mg and seven commercial samples (capsules) containing tetracycline hydrochloride 250 mg and 500 mg from different brands.

Doxycycline: Twenty capsules or tablets of each commercial brand in pharmaceuticals of doxycycline. The tablets were grounded to a fine powder and homogenized then the amount of powder equivalent to 100 mg of doxycycline was weighed and dissolving in 5.0 x 10^{-3} mol/L nitric acid in 100 mL of volumetric flask and sonicated for 10 min to provide in concentration 100 µg/mL. The solution was filtered through Whatman No 42-filter paper and diluted with 5.0 x 10^{-3} mol/L nitric acid to obtain the appropriate concentrations for analysis.

Tetracycline: Twenty capsules of each commercial brand in pharmaceutical of tetracycline were accurately weighed and an amount equivalent to 250 mg and 500 mg of tetracycline were dissolving in 5.0 x 10^{-3} mol/L nitric acid in 100 mL of a volumetric flask with sonicated for 10 min. the solutions were cooled to room temperature and were filtered through Whatman No 42-filter paper before use.

Results and Discussion

The proposed spectrophotometric method was developed and optimized by a univariate method. The univariate method was applied to select the optimum conditions for the determination of doxycycline which was applied for the determination of tetracycline.

1. Absorption Spectra

The analytical method was based on the reaction between the drug and ferrous ion contained in vitamins tablets in an acidic solution. The presence of hydrogen peroxide enhances the absorption signal. The reaction between standard solutions of doxycycline were preparation in 5.0 x 10^{-3} mol/L nitric acid and reaction with 5.0 x 10^{-2} mol/mL ferrous ion contained in each vitamin tablet and 2.5 x 10^{-2} % (v/v) of hydrogen peroxide in a ratio of 2.0: 1.0: 0.1 (v/v), respectively. The final solution was scanned against the blank to get the UV-Visible absorption spectra in the range of 200 - 700 nm. The objective for studied to select a reagent in the optimum conditions for analysis. The study for relative absorption value of doxycycline - ferrous ion showed that ferrous ion *in* vitamin tablet brand A present the highest absorbance compare with other vitamins tablets due to it is a small tablet and contained ferrous sulfate more than other vitamins tablets. These spectra were used as reference spectra to identify the optimum conditions of complex formation and to select the optimum wavelength for quantitative analysis in further studies. It was found that the maximum absorption of the drug- ferrous ion complex was occurred at 425 nm (Figure 3) and confirmed with the maximum absorption of the iron (III)-doxycycline complex (Palamy *et al*., 2017; Vilayphone *et al*., 2018). Thus, it is possible to use *ferrous ion* contained in vitamin tablets (brand A) as reagent to obtain the green simple, rapid, sensitive and inexpensive method for the determination of doxycycline and tetracycline in pharmaceutical formulations.

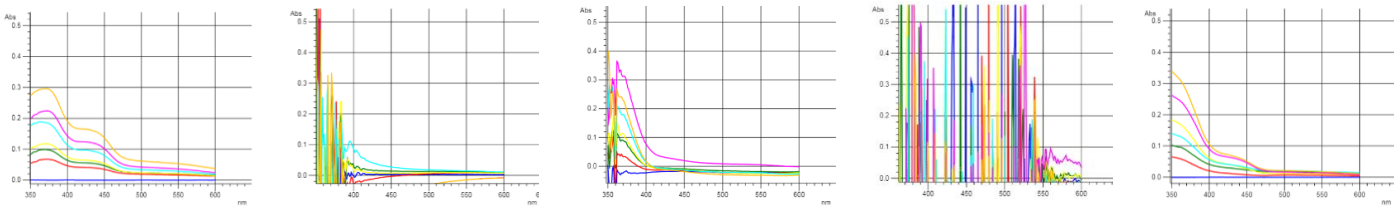

Figure 3. Absorption spectra of the complex formed between doxycycline and ferrous ion contained in vitamin tablets (brand A, B, C, D and E)

2. Effect of Type of Acid and Concentration of The Acid Solution

The solutions of five acids such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4), Perchloric acid ($HClO_4$), nitric acid (HNO_3) and phosphoric acid (H_3PO_4) concentrations were 5.0 x 10^{-3} mol/L for dissolving ferrous ion in vitamin tablets and standard or sample solution was studied. The relative absorption value of the doxycycline - ferrous ion complex detected at 425 nm showed that nitric acid solution provides higher absorbance than other mineral acid solutions. The study on the concentration effect of nitric acid in the range 0.2 x 10^{-3} - 125.0 x 10^{-3} mol/L on the absorption of doxycycline - ferrous ion complex displayed that the concentration of nitric acid gives the greatest signal at 5.0 x

10^{-3} mol/L. Thus, the concentration at 5.0 x 10^{-3} mol/L was therefore selected as the optimum concentration for studies.

3. Effect of Ferrous Ion Contained in Vitamin Tablets *Concentration*

The effect of varying concentrations of ferrous ion in vitamin tablets solution in the range 0.62 x 10^{-2} - 10.0 x 10^{-2} mol/L was shown that ferrous ion in vitamin tablets solution at 10.0 x 10^{-2} mol/L was the highest absorbance but this concentration difficult in preparing the solution which was involved with a precipitate of this solution such as turbid and time-consuming. Thus, the concentration at 5.0 x 10^{-2} mol/L was chosen as the optimum concentration

4. Effect of Hydrogen Peroxide Concentration

The concentration of hydrogen peroxide in the concentration range 1.25 x 10^{-2} - 20.0 x 10^{-2} % (v/v) in deionized water were examined. The maximum absorbance was obtained with 2.5 x 10^{-2} % (v/v). Therefore 2.5 x 10^{-2} % of hydrogen peroxide was selected for the experiment for further study.

Table 1. Variable ranges and optimum conditions for determination of doxycycline and tetracycline in pharmaceutical formulation by UV-Visible spectrophotometric method

Parameter Studied	Range Studied	Optimum Level
Wavelength (nm)	200 - 700	425
Type of acid	HCl, H_2SO_4, $HClO_4$, HNO_3, H_3PO_4	HNO_3
Nitric acid concentration (mol/L)	0.2 x 10^{-3} - 125.0 x 10^{-3}	5 x 10^{-3}
Ferrous sulfate concentration (mol/L)	0.62 x 10^{-2} - 10.0 x 10^{-2}	5 x 10^{-2}
Hydrogen peroxide concentration (%, v/v)	1.25 x 10^{-2} - 20.0 x 10^{-2}	2.5 x 10^{-2}

5. Validation Method

The determination of doxycycline and tetracycline using ferrous ion in vitamin tablets as reagent was optimized and validated according to the current ICH guidelines (ICH guidelines, 2005). Analytical characteristics for the proposed method were determined under the optimum conditions defined (Table 1)

The calibration graphs contained doxycycline and tetracycline standard in the range of 1.0-100 µg/mL and 1.0-200 µg/mL, respectively. Linear regression analysis of the absorbance (y) and concentration of each drug (x) expressed the equation A and B for doxycycline and tetracycline, respectively. (A; y = 0.0064x - 0.001, B; y = 0.0069x - 0.0095). The correlation coefficient was shown to be 0.9998, 0.9997 for doxycycline and tetracycline, respectively. The limit of detection (LOD) for each drug, the concentration of analysis substances that given by calculate the amount equal to three times the standard deviation of the blank signal (s/n=3) was found to be 0.2 µg/mL. The limit of quantitation (LOQ) each drug defined as ten times standard deviation was found to be 0.6 µg/mL for doxycycline and tetracycline.

The precision was studied to followed reproducibility of doxycycline and tetracycline at 5, 10 and 25 ug/mL for twelve replicates shown content 1.51 %, 0.79% and 0.43% (Intra-day), 1.68%, 1.06% and 0.49% (Inter-day) for doxycycline; 1.35 %, 0.47 % and 0.18 % (Intra-day), 1.57%, 0.64% and 0.31% (Inter-day) for tetracycline. The accuracy of the proposed method was determined from seven replicate of each drug sample. After measurement of the absorbance, the recovery of each spiked standard drug was calculated. The percentage recoveries of 3.0, 12.5 and 22.5 µg/mL were found to be 98.62 %, 99.87 %, 100.56 % for doxycycline and 100.92 %, 101.19 % and 102.0 % for tetracycline (*n*=7), respectively (Table 2).

Table 2. Analytical characteristics for determination of doxycycline and tetracycline in pharmaceutical formulation by UV-Visible spectrophotometric method

Analytical Characteristics	Optimum Value			
	Doxycycline		Tetracycline	
Linear of the calibration curve (µg/mL)	1.0 - 100		1.0 - 200	
Linear regression equation (*n*=5)	y = 0.0064x – 0.001		y = 0.0069x-0.0095	
Correlation coefficient (r^2)	0.9998		0.9997	
Limit of detection, LOD	0.2 µg/mL		0.2 µg/mL	
Limit of quantitation, LOQ	0.6 µg/mL		0.6 µg/mL	
Reproducibility (*n*=12); RSD	Intra-day	Inter-day	Intra-day	Inter-day
5.0 µg/mL	1.51%	1.68%	1.35%	1.57%
10.0 µg/mL	0.79%	1.06%	0.47%	0.64%
25.0 µg/mL	0.43%	0.49%	0.18%	0.31%
Percentage recoveries (*n*=7)				
3.0 µg/mL	98.62%		100.92%	
12.5 µg/mL	99.87%		101.19%	
22.5 µg/mL	100.56%		102. 00 %	

6. Application to Pharmaceutical Analysis

The proposed spectrophotometric method for determination of doxycycline (100 mg) in capsules and tablets from seven commercial products and tetracycline (250 mg and 500 mg) in capsules from seven commercial products in pharmaceutical formulation using ferrous ion in vitamin tablets as reagent. The results were compared with those declared on formulation labels and reference method: UV-visible spectrophotometric using Folin - Ciocalteu reagent (Ramesh *et al*., 2010). The drug contain of doxycycline and tetracycline were found to be 101.85 ± 1.77 mg (100 mg/capsule and tablet), 251.15 ± 1.92 mg (250 mg/capsule), 500.21 ± 1.81 mg (500 mg/capsule) (n=7), respectively using proposed method and 101.613 ± 1.34 mg (100 mg/capsule and tablet) and 250.36 ± 1.87 mg (250 mg/capsule), 504.77 ± 1.65 mg (500 mg/capsule) (n=7), respectively using the reference method (Table 3). Statistical analysis of the results from both values using the t-test at 95% confidence level was not significant (Table 3).

Table 3. Accuracy of the proposed method compared with the reference method of doxycycline and tetracycline determination.

No of Products	Doxycycline 100 mg/Capsule, Tablet		Tetracycline 250, 500 mg/Capsule	
	Proposed method[1]	Reference method[2]	Proposed method[1]	Reference method[2]
1	100.02 ± 0.62	101.20 ± 0.30	250.11 ± 1.64	250.02 ± 0.82
2	100.78 ± 0.87	101.09 ± 0.28	249.15 ± 1.90	247.14 ± 1.51
3	103.55 ± 0.91	102.91 ± 0.28	251.28 ± 1.96	250.99 ± 1.76
4	101.14 ± 0.91	101.09 ± 0.23	249.88 ± 1.73	250.00 ± 0.60
5	99.93 ± 0.82	100.03 ± 0.28	252.03 ± 1.91	251.28 ± 1.55
6	103.59 ± 0.90	102.98 ± 0.31	254.45 ± 1.88	252.74 ± 1.06
7	103.91 ± 0.63	102.99 ± 0.32	500.21 ± 1.81*	504.77 ± 1.65*
t-test at 95% confidence level:				
t- calculation		0.77		0.16
t-distribution at (*n*-1) = 6		2.45		2.45

[1]Proposed method: UV-Visible spectrophotometric using Ferrous ion contained in vitamin tablets as reagent
[2] Reference method: UV-Visible spectrophotometric using Folin - Ciocalteu reagent
*Tetracycline 500 mg/Capsule
Student's *t*-test value less than t critical (2.45) at the confidence level 95%

7. Interferences

The effect of some excipient presents in commercial pharmaceutical formulations such as glucose, lactose, starch, magnesium stearate, sucrose, sorbitol, titanium dioxide and cellulose on measurement of the doxycycline and tetracycline were tested. The result was found that glucose, lactose, starch, magnesium stearate, sucrose, sorbitol did not affect doxycycline and tetracycline determination at concentrations up to 5 and 10 times of the drug. The relative percentage of absorption value for this proposed method with some excipients was calculated according to The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines Q2 (R1), 2005. Most ingredients do not interfere in doxycycline and tetracycline determination. However, only titanium dioxide and cellulose have serious effects on the determination of drugs (Table 4).

Table 4. Effect of some excipients on the absorption of doxycycline and tetracycline measured by comparing with 10 µg/mL of each drug

Excipients (mg/mL)		Relative of Absorption (%), *n* = 5	
		Doxycycline	Tetracycline
None of excipients		100.0	100.0
Glucose	(50)	101.4	100.3
Glucose	(100)	101.4	100.7
Lactose	(50)	100.3	100.7
Lactose	(100)	101.0	101.0
Starch	(50)	100.3	101.0
Starch	(100)	100.7	101.0
Mg-stearate	(50)	100.3	100.3
Mg-stearate	(100)	100.3	101.0
Sucrose	(50)	101.3	102.0
Sucrose	(100)	101.3	103.7
Sorbital	(50)	101.4	101.0
Sorbital	(100)	102.4	101.3
Titanium dioxide	(50)	106.2	104.3
Titanium dioxide	(100)	112.5	108.7
Cellulose	(50)	102.0	101.3
Cellulose	(100)	107.5	104.7

Conclusions

A simple spectrophotometric method was developed for the quantification of doxycycline and tetracycline in pharmaceutical products. The proposed protocol describes the use of ferrous ion in vitamin tablets as reagents. The determination of the drug was based on the complexation between drug - ferrous ion in nitric acid and hydrogen peroxide solution by UV-Visible spectrophotometer at a maximum absorption wavelength of 425 nm. This proposed method was successfully applied in the determination of doxycycline and tetracycline in pharmaceutical products and represented a simple, cost-effective, high precision, alternative to other analytical methods.

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