Research Article

การวิเคราะห์หาปริมาณด็อกซีไซคลินโดยใช้เฟอร์รัสซัลเฟตจากเม็ดวิตามินเป็นรีเอเจนต์

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บทคัดย่อ

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วิธีสเปคโทรโฟโทเมตริกอย่างง่ายสำหรับวัดปริมาณด็อกซีไซคลินที่นำเสนอนี้ อาศัยหลักการเกิดปฏิกิริยาระหว่างด็อกซีไซคลินและเฟอร์รัสซัลเฟตจากเม็ดวิตามินในสารละลายกรด โดยใช้ไฮโดรเจนเปอร์ออกไซด์ช่วยเพิ่มสัญญาณการดูดกลืนแสง **วิธีการศึกษา:** วิธีการที่นำเสนอนี้ได้ประยุกต์ในการวิเคราะห์ด็อกซีไซคลินในรูปแบบยาเตรียมต่างๆ โดยใช้วิธีสเปคโทรโฟโทเมตริก วัดค่าการดูดกลืนแสงที่ความยาวคลื่นสูงสุดที่ 425 นาโนเมตรหลังผสมสารละลายต่างๆ เข้าด้วยกัน สารละลายมาตรฐานหรือสารละลายตัวอย่างผสมกับเฟอร์รัสซัลเฟตจากเม็ดวิตามินที่เข้มข้น 5.0 x 10^{-2} โมลต่อลิตรในกรดไนตริกเข้มข้น 5.0 x 10^{-3} โมลต่อลิตรและสารละลายไฮโดรเจนเปอร์ออกไซด์เข้มข้นร้อยละ 2.5 x 10^{-2} (ปริมาตรต่อปริมาตร) ในอัตราส่วน 2.0 : 1.0 : 0.1 (ปริมาตรต่อปริมาตร) ตามลำดับ ตรวจสอบสภาวะที่เหมาะสมสำหรับวิเคราะห์ด็อกซีไซคลินด้วยวิธียูนิวาริเอต **ผลการศึกษา:** ภายใต้สภาวะที่เหมาะสมสำหรับวิเคราะห์ด็อกซีไซคลินสามารถสร้างกราฟมาตรฐานในช่วงความเข้มข้น 1.0 - 50 ไมโครกรัมต่อมิลลิลิตร สมการเส้นตรงแสดงความสัมพันธ์ระหว่างค่าการดูดกลืนแสงของยา (ค่า *y*) และค่าความเข้มข้นของยา (ค่า *x*) เท่ากับ $y = 0.0042x + 0.0017$ มีค่าสหสัมพันธ์ความเป็นเส้นตรงเท่ากับ 0.9994 การตรวจสอบค่าขีดจำกัดต่ำสุดของการวิเคราะห์ (LOD) เมื่อค่าสัญญาณมีค่าเป็นจำนวนสามเท่าของสารละลายเทียบพบค่าเท่ากับ 0.3 ไมโครกรัมต่อมิลลิลิตร และค่าขีดจำกัดต่ำสุดของการวิเคราะห์เชิงปริมาณ (LOQ) เมื่อค่าสัญญาณมีค่าเป็นจำนวนสิบเท่าของสารละลายเทียบพบค่าเท่ากับ 0.9 ไมโครกรัมต่อมิลลิลิตร นอกจากนี้ ส่วนประกอบพื้นฐานที่ใช้เป็นสารช่วยทางเภสัชกรรมในรูปแบบยาเตรียมไม่มีผลรบกวนวิธีการวิเคราะห์นี้ เมื่อเปรียบเทียบผลการวิเคราะห์ด้วยวิธีที่นำเสนอนี้กับวิธีวิเคราะห์อ้างอิง พบว่าไม่มีความแตกต่างกันในเชิงสถิติที่ความเชื่อมั่นร้อยละ 95 (n=7) **สรุปผลการศึกษา:** วิธีสเปคโทรโฟโทรเมตริกที่นำเสนอเป็นวิธีที่ง่ายสำหรับการวิเคราะห์ปริมาณด็อกซีไซคลิน มีข้อดี เช่น สารที่ใช้ในการทำปฏิกิริยาสามารถหาได้ทั่วไป วิธีวิเคราะห์มีความแม่นสูงและการวัดซ้ำให้ผลดี ทำได้รวดเร็ว และสามารถใช้เป็นวิธีวิเคราะห์ทางเลือกหนึ่งสำหรับการควบคุมคุณภาพด็อกซีไซคลินในยาเตรียม

คำสำคัญ: ด็อกซีไซคลิน, วิธีสเปคโทรโฟโทเมตริก, วิตามิน, เฟอร์รัสซัลเฟต

Quantitative Determination of Doxycycline Using Ferrous Sulfate Contained in Vitamin Tablets as Reagent

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Abstract

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A simple spectrophotometric procedure for determining doxycycline is proposed. It is based on the reaction between this drug and ferrous sulfate from vitamin tablets in acidic solution. The presence of hydrogen peroxide enhances the absorption signal. **Methods:** The proposed method was applied successfully to analyze doxycycline in pharmaceutical dosage forms. The highest absorption was measured at 425 nm after mixing the solutions. Either standard or sample solution was mixed with 5.0 x 10^{-2} mol/L of ferrous sulfate from vitamin tablets in 5.0 x 10^{-3} mol/L of nitric acid 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 optimum conditions for determining doxycycline were investigated by the univariate method. **Results:** Using the proposed procedure under optimum conditions, linear calibration graphs were obtained for doxycycline concentrations from 1.0 to 50 µg/mL. Linear regression analysis of the absorbance of drug (y) versus drug concentration (x) yielded the equation $y = 0.0042x + 0.0017$. The correlation coefficient was 0.9994. The detection limit of doxycycline, the concentration of analyte that gave a signal different from the blank by an amount equal to three times the standard deviation of the blank signal (s/n=3), was found to be 0.3 µg/mL. The quantitation limit (defined as ten times the standard deviation of the blank signal) was 0.9 µg/mL. Moreover, the common excipients used as additives in the pharmaceutical dosage forms showed no effect on the proposed method. The results acquired by the proposed method compared favorably with those acquired by the reference method at a 95% confidence level with no significant difference (n=7). **Conclusion:** The proposed spectrophotometric method is a simple method for analysis of doxycycline with many advantages such as simple reagents, high accuracy, high reproducibility, and is therefore a rapid and acceptable alternative method for the routine quality control of doxycycline in drug formulations.

Keywords: Doxycycline, Spectrophotometric method, Vitamin, Ferrous sulfate

Introduction

Doxycycline is a broad-spectrum antibiotic synthetically obtained from oxytetracycline. It is a second-generation tetracycline. Doxycycline has been used worldwide as a preventive and therapeutic agent in the prevention and treatment of infections caused by gram-positive and gram-negative bacteria. It has less toxicity than first generation tetracyclines and has been used to treat a wide range of bacterial infections, depending on the results of antibiotic susceptibility tests. Chemically, doxycycline is called (4S, 4aR, 5S, 5a R-6S, 12aS)-4-Dimethylamino 1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5, -10, 12, 12a-pentahydroxy-6-methyl 1, 11-dioxonaphthacene-2-carboxamide monohydrate. The molecular formula is $C_{22}H_{24}N_2O_8$, HCl and molecular weight is 512.9 g/mol (Sweetman, 2009).

Several methods were reported for analysis of the doxycycline in pharmaceutical products and biological fluids including of fluorimetry (Salinas, Munox and Duran, 1990), *UV-visible spectrophotometric method (Ramesh et al. 2011; Vilayphone et al., 2018)*, thin- layer chromatography (Naidong, Greelen, Roets and Hoogmartens, 1990), liquid chromatography (Bryan and Stewart, 1994; Choma and Pilorz, 2004), *and* flow injection spectrophotometry (Izquierdo et al., 1994). A fast, thin layer chromatography-fluorescence scanning densitometric technique has been developed for doxycycline determination in honey, serum and urine samples (Xie et al., 1997). Doxycycline has also been determined in milk and milk powder by using HPLC (Ding and Mou, 2000). *A mixing solution of copper (II) and hydrogen peroxide (H_2O_2) has been used as a reagent for the determination of doxycycline in pharmaceuticals (Sunaric et al., 2009).*

Nowadays, 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 a toxic reagent from the analytical method (Mohamed, 2015). The analytical method should be greener if it decreases the consumption of reagents and samples, is more safe or simplifies the procedure, avoids the use of hazardous reagents, has no time- consuming processes and uses energy- efficient instruments. To implement GAC principles, it is important to know the appropriate steps within analytical procedures such as simple sample preparation, using green reagents and saving chemicals or energy in the analytical method (Anastas *et al.*, 2010). The objective of this work was therefore to develop a simple and *not sophisticated* spectrophotometric method for the determination of doxycycline using ferrous sulfate from commercial vitamin tablets as a reagent.

The present study describes a spectrophotometric method for the determination of doxycycline in capsules using a simple reagent which reacts with ferrous sulfate from vitamin tablets. In some countries of the Great Maekhong Region i. e. Lao PDR and Myanmar, ferrous sulfate in vitamin tablets can be more easily retrieved from drug stores than in the form of the primary standard. Based on this condition, it may be pertinent to develop an alternative method for doxycycline determination under this chemical supply limitation. The proposed method can measure the absorption of UV- visible light from the complexation between doxycycline and iron in 5.0×10^{-2} mol/L ferrous sulfate from vitamin tablets in 5.0×10^{-3} mol/L using nitric acid and 2.5×10^{-2} % (v/v) and hydrogen peroxide in a ratio of 2.0 : 1.0 : 0.1 (v/v), respectively. The analytical method will be optimized using the univariate method. The developed method has been satisfactorily applied to the determination of doxycycline in pharmaceutical formulations.

Materials and Methods

Apparatus

1. UV-visible spectrophotometer, 1700 model, Shimadzu, Japan.

Reagents

1. Doxycycline standard, Sigma; Germany.
2. Nitric acid, BDH; England.
3. Hydrogen peroxide, Merck; Germany.
4. Commercial vitamin tablets (Haemovit®, contained 135 mg ferrous sulfate/tablet)

Procedure

Recommended Procedure

Accurate volumes (2.0 mL) of doxycycline standard solution over the concentration range 1.0-50 µg/mL were transferred into test tubes and mixed with 1.0 mL of 5.0 x 10^{-2} mol/L ferrous sulfate tablet solution then added to 0.1 mL of 2.5 x 10^{-2} % (v/v) of hydrogen peroxide, and mixed well. The mixing solution was left at room temperature for 1 min before the absorbance measurement. The absorption of the solution was measured at wavelength 425 nm against the corresponding reagent blank using UV-visible spectrophotometer. The proposed method was compared with the reference method (The USP, 2008) for evaluation of the accuracy. The results were compared by the student *t*-test at 95% confidence level.

Sample Preparation

Twenty doxycycline capsules (only powder) were weighed and ground into a fine powder. An amount of powder equivalent to 100 mg of doxycycline was weighed. Then, the sample solution was prepared by dissolving 100 mg of doxycycline powder in 5.0 x10^{-3} mol/L nitric acid and diluting to 100 mL in a volumetric flask. The solution was filtered through Whatman No42 filter paper and diluted with 5.0 x10^{-3} mol/L nitric acid to volume to obtain the appropriate concentration for analysis.

Reagent Preparation

Vitamin tablets purchased from a drug store at Mueang district, Khon Kaen, Thailand were used as the source of ferrous sulfate and 20 vitamin tablets were ground into a fine powder. An amount of ferrous sulfate powder from the vitamin tablets equivalent to 10.0 x 10^{-2} mol/L (0.7596 g) was accurately weighed, transferred to a 100 mL volumetric flask and added with 5.0 x10^{-3} mol/L nitric acid to the graduated mark. Then the solution was mixed well and filtered through Whatman No 42 filter paper before use.

One milliliter of 35% hydrogen peroxide was diluted with distilled deionized water to make 1% (v/v) hydrogen peroxide in 100 mL in a volumetric flask. Then it was diluted to 2.5 x 10^{-2} % (v/v) hydrogen peroxide in a graduated volumetric flask with distilled deionized water.

Results and Discussion

The analytical method of this study was based on the reaction between doxycycline and ferrous sulfate from the vitamin tablets in acidic solution. The presence of hydrogen peroxide enhances the absorption signal. The reaction between sample, 5.0 x 10^{-2} mol/L of ferrous sulfate and 2.5 x 10^{-2} % (v/v) of hydrogen peroxide produces a soluble yellow complex. The volume ratio of sample, ferrous sulfate and hydrogen peroxide solution was 2.0 : 1.0 : 0.1 (v/v). The maximum absorbance was measured at the wavelength of 425 nm. The proposed spectrophotometric method was developed and optimized using the univariate method. The variable method was applied to select the optimum conditions for the determination of doxycycline.

I. UV-visible Spectra

The UV-visible spectra of doxycycline in nitric acid solution were generated using UV-visible spectrophotometer. The spectra were recorded from 200-700 nm. It was found that the maximum absorbance of doxycycline-ferrous sulfate-hydrogen peroxide complex was at 425 nm (Figure 1), which was then used as the optimum value.

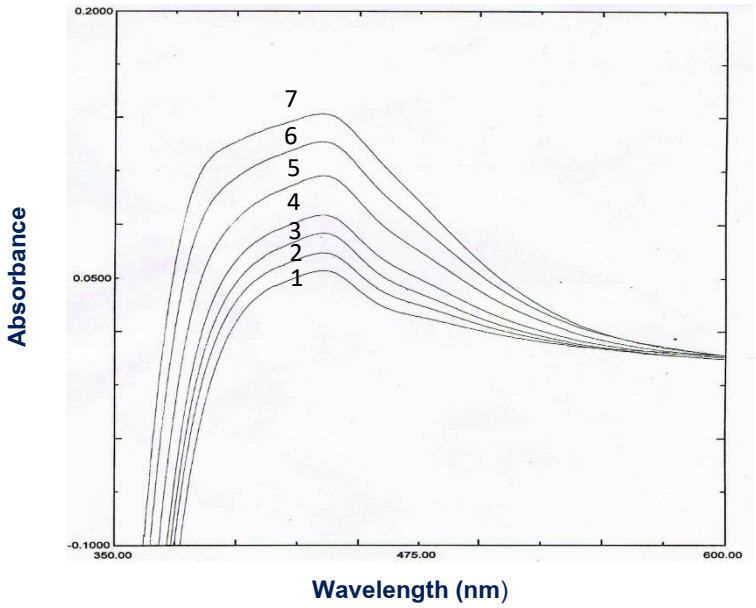

Figure 1 UV-visible spectra of doxycycline (1): Blank, (2): 5, (3): 7.5, (4): 10, (5):15, (6): 20, (7): 25 µg/mL, coupled with ferrous sulfate solution and enhancer (H_2O_2 solution).

II. Type of Acid Solution

Solutions (5.0 x 10^{-3} mol/L) of four mineral acids; nitric acid (HNO_3), sulfuric acid (H_2SO_4), hydrochloric acid (HCl) and phosphoric acid (H_3PO_4) were investigated for dissolving ferrous sulfate from vitamin tablets and standards or samples. The relative absorbances for each of the acids were 100.0%, 87.5%, 87.5% and 17.5%, respectively. The presence of nitric acid solution gave the highest signaling, greater than the other acidic solutions. Thus, nitric acid was selected for subsequent studies (Figure 2).

Various concentrations of nitric acid solution were evaluated in the range 0.2 x 10^{-3} – 125.0 x 10^{-3} mol/L as shown in Figure 3. It was found that the absorbance increased with the increase of nitric acid concentration and reached the maximum analytical signal at 5.0 x 10^{-3} mol/L (Figure 3). The absorbance decreased when nitric acid concentration was higher than 5.0 x 10^{-3} mol/L. Thus, 5.0 x 10^{-3} mol/L nitric acid solution was used as the optimum concentration. Nitric acid gave higher signal than other mineral acids due to its oxidizing power and formation of a stable complex between the drug and iron ion (Kruanetr *et al*., 2007).

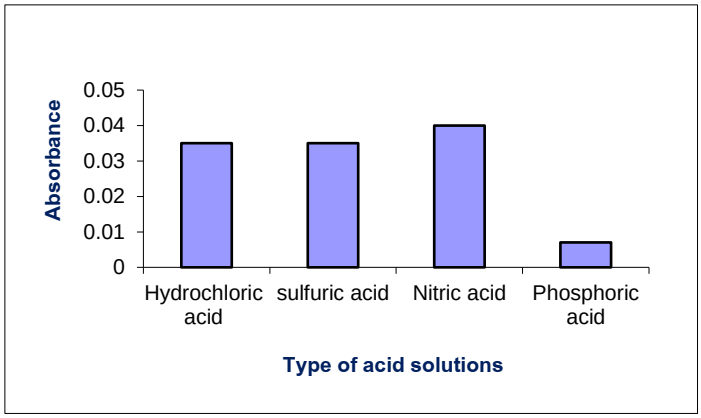

Figure 2 Type of acid solutions on the doxycycline determination.

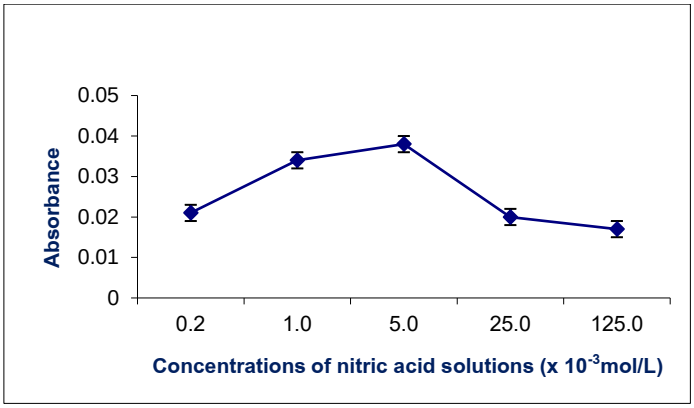

Figure 3 Effect of nitric acid concentration on the doxycycline determination.

III. Effect of Ferrous Sulfate Concentration

The effect of concentration of ferrous sulfate in acidic solution between 0.62 $x10^{-2}$ – 10.0 $x10^{-2}$ mol/L was examined (Figure 4). Although ferrous sulfate solution at 10.0 x 10^{-1} mol/L produced the highest absorbance, it was difficult to prepare, involving the precipitate and was also time-consuming. Thus, the concentration at 5.0 x 10^{-2} mol/L was chosen as the optimum concentration.

IV. Effect of Hydrogen Peroxide Concentration

The effect of varying the concentration of hydrogen peroxide in the range 1.25 $x10^{-2}$ – 20.0 x 10^{-2} % (v/v) was examined. The maximum absorbance was obtained with 2.5 x 10^{-2} % (v/v) of hydrogen peroxide. Therefore 2.5 x 10^{-2} % (v/v) hydrogen peroxide was selected as the optimum concentration for further studies (Figure 5).

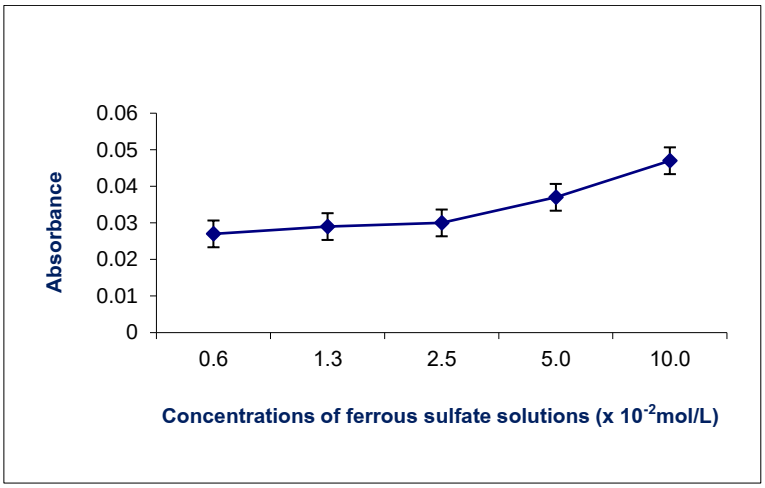

Figure 4 Effect of ferrous sulfate concentrations on the doxycycline determination.

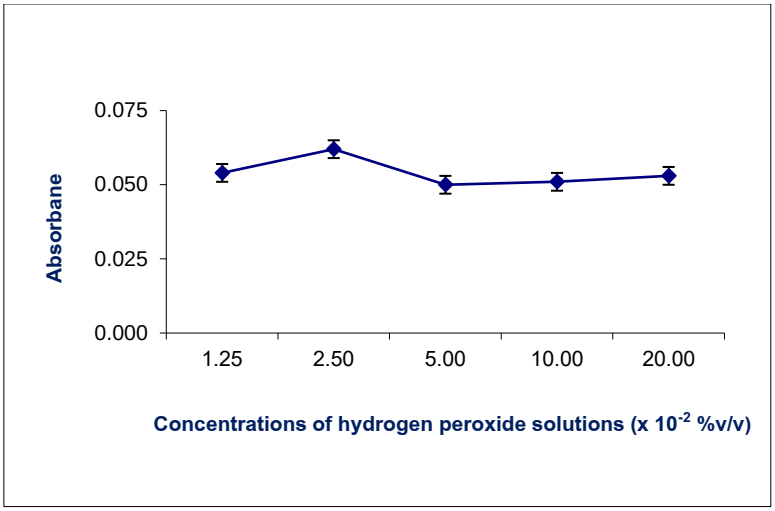

Figure 5 Effect of hydrogen peroxide concentrations on the doxycycline determination.

V. Ferrous Ion, Hydrogen Peroxide and Doxycycline Complex

The proposed mechanism for ferrous ion (Fe^{2+}), hydrogen peroxide (H_2O_2) and doxycycline to form a complex is that ferrous sulfate will react with hydrogen peroxide to give ferric ion (Fe^{3+}). After that, the ferric ion will form a complex with doxycycline at the mole ratio of drug : ferric ion as 2 : 1 (Figure 6).

(a) $Fe^{2+} + H_2O_2$ ---------- > $Fe^{3+} + OH^- + OH^{\cdot}$

(b)

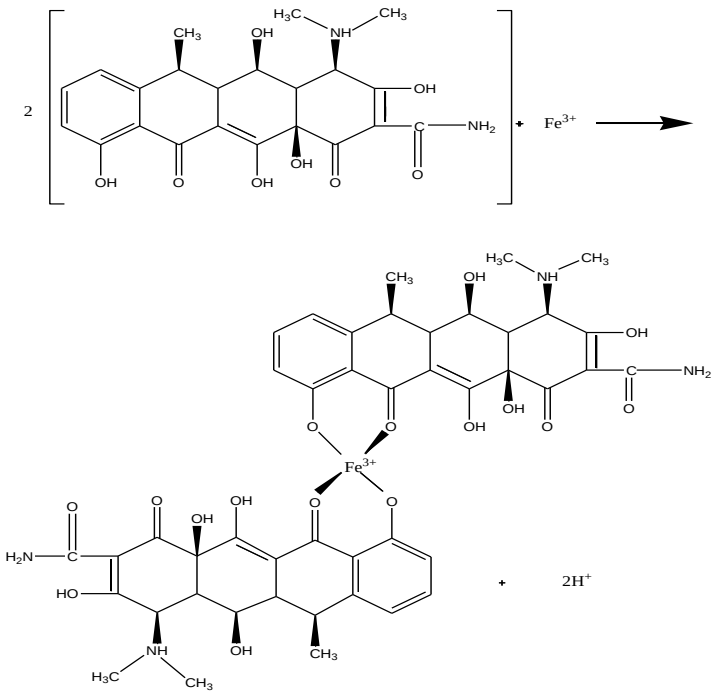

Figure 6 The proposed mechanism of complexation (a) Ferrous sulfate and hydrogen peroxide (b) doxycycline and iron (III) complexation. (Palamy *et al*., 2017)

Table 1. Parameters, range and optimum values of doxycycline determination in this study.

Parameters	Range	Optimum values
Wavelength (nm)	200–700	425
Linear range (µg/mL)	1–100	1–50
Types of acid	HNO_3, H_2SO_4 HCl, H_3PO_4	HNO_3
Nitric acid concentration (mol/L)	0.2×10^{-3}–125.0×10^{-3}	5.0×10^{-3}
Ferrous sulfate concentration (mol/L)	0.62×10^{-2}–10.0×10^{-2}	5.0×10^{-2}
Hydrogen peroxide concentration (%v/v)	1.25×10^{-2}–20.0×10^{-2}	2.5×10^{-2}

V. Analytical Characteristics

Analytical characteristics of doxycycline determination were evaluated under the optimum conditions as shown in Table1.

VI. Calibration Graph

The proposed procedure for doxycycline determination using the optimum conditions was validated according to the current ICH guidelines (ICH guidelines, 2005). Linear calibration graphs were obtained for 1.0-50 µg/mL of the drug (Figure 7). Linear regression analysis of the absorbance (y) versus drug concentration (x) was $y = 0.0042x + 0.0017$. The correlation coefficient was 0.9994. The detection limit of doxycycline, the concentration of analyte that gave a signal different from the blank by an amount equal to three times the standard deviation of the blank signal (s/n=3), was found to be 0.3 µg/mL. The quantitation limit (defined as ten times the standard deviation) was 0.9 µg/mL.

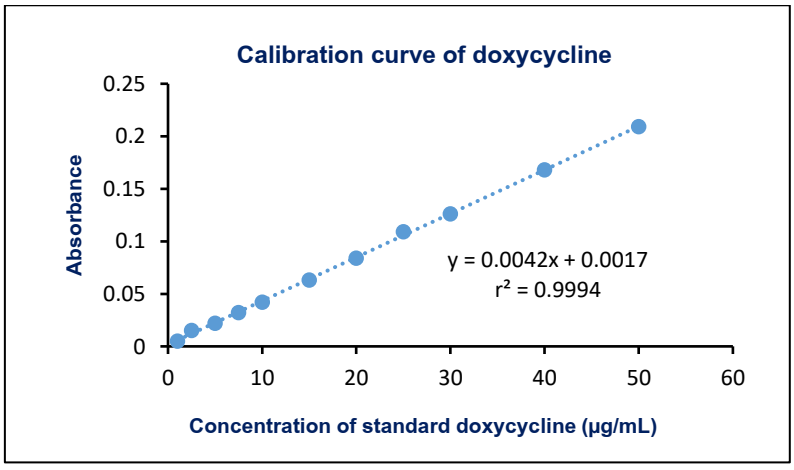

Figure 7 Calibration curve of doxycycline in concentration range of 1.0-50 µg/mL.

VII. Reproducibility and Accuracy

The relative standard deviations (RSD) of the absorbance obtained from the proposed method were calculated from 12 replicates. The RSDs of 5, 10 and 25 µg/mL of doxycycline were found to be 3.15 %, 2.20 %, and 2.36 % (n=12), respectively. The accuracy of the proposed method was determined from 7 replicates of drug samples. After the absorbance measurement, the recovery of each

spiked standard drug was calculated. The percentage recoveries of 3, 12.5 and 22.5 µg/mL of doxycycline were found to be 98.47 %, 98.56% and 100.86%, respectively. The data showed that the proposed method provided accurate results.

VIII. Interferences

The effect of additive ingredients (e.g. glucose, lactose, cellulose, starch, magnesium stearate, sucrose, sorbitol, titanium dioxide and cellulose) were investigated. The sample solutions containing 10 µg/mL doxycycline and various concentrations of some excipients were tested. It was found that glucose, lactose, cellulose, starch, magnesium stearate, sucrose and sorbitol did not affect doxycycline determination at ocncentrations up to 5 and 10 times of the drug. However, titanium dioxide and cellulose were demonstrated to have a serious effect on doxycycline determination (Table 2). In this study, vitamin tablets (Haemovit®) contained aneurine, pyridoxine, cyanocobalamin and vitamin B1-6-12 as excipients. A previously study reported that these excipients did not interfere with doxycycline determination (Campbell and Hasinoff, 1991).

IX. Application

The results obtained from our proposed doxycycline determination method were compared with those declared on the formulation labels and with those obtained using the official method of pharmacopoeia. As per the labelling of doxycycline 100 mg capsules, the doxycycline contents were found to be 103.03 ± 2.08 mg per capsule (n =7) using the proposed method and 101.35 ± 1.79 mg per capsule (n=7) using the official method (Table 3). The results obtained by both methods showed no statistical difference at the 95% confidence level.

Conclusion

The determination of doxycycline was based on the complexation between the drug and ferrous sulfate in a hydrogen peroxide solution. The calibration graph remained linear for the full range of doxycycline content in the commercial formulation. The proposed spectrophotometric method is a simple method for analysis of these drugs and shows many advantages such as the requirement for simple reagents, highly accurate, good reproducibility, and is therefore a rapid and acceptable alternative method for the routine quality control of doxycycline in drug formulations.

Table 2. Effect of some excipients on doxycycline absorption measured by comparing with 10 µg/mL of doxycycline.

Excipients		**Relative of absorption (%); *n*=5**
(µg/mL)		**Doxycycline**
None of excipients		100.0 ± 0.05
Glucose	(50)	102.8 ± 0.15
Glucose	(100)	102.8 ± 0.12
Lactose	(50)	102.1 ± 0.08
Lactose	(100)	104.5 ± 0.19
Starch	(50)	100.0 ± 0.06
Starch	(100)	100.0 ± 0.06
Mg- stearate	(50)	104.2 ± 0.21
Mg- stearate	(100)	104.2 ± 0.22
Sucrose	(50)	100.0 ± 0.06
Sucrose	(100)	100.0 ± 0.07
Sorbital	(50)	100.0 ± 0.09
Sorbital	(100)	103.8 ± 0.13
Titanium dioxide	(50)	110.5 ± 0.28
Titanium dioxide	(100)	115.3 ± 0.35
Cellulose	(50)	105.7 ± 0.11
Cellulose	(100)	108.5 ± 0.15

Table 3. Accuracy of the proposed method compared with the official method of doxycycline determination.

No of Experiment	Doxycycline 100 mg/Capsule	
	Proposed method[1]	HPLC[2]
1	102.46	101.32
2	105.28	107.46
3	101.36	100.28
4	100.61	102.11
5	103.45	101.35
6	102.87	100.28
7	101.55	100.35
t-test at 95% confidence level:		
t-calculation		0.90
t-distribution at (*n*-1) = 6		2.45

[1]Proposed method: UV-visible spectrophotometric method
[2]USP: The United State Pharmacopoeia, USP 31 NF6

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