Research Article

การวิเคราะห์ปริมาณด็อกซีไซคลินในแคปซูลโดยใช้เทคนิคการวัดค่าสีอาร์จีบี

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บทคัดย่อ

การวิเคราะห์ปริมาณด็อกซีไซคลินในแคปซูลโดยใช้เทคนิคการวัดค่าสีอาร์จีบี
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เทคนิคการวัดสีอาร์จีบีได้ถูกพัฒนาขึ้นสำหรับการวิเคราะห์ปริมาณด็อกซีไซคลินในเภสัชภัณฑ์แคปซูล หลักการโดยทั่วไปได้ใช้ภาพถ่ายดิจิทัลของสารประกอบเชิงซ้อนที่ถ่ายโดยโทรศัพท์มือถือที่เป็นระบบแอนดรอยด์ แล้วนำมาอ่านค่าความเข้มของสีอาเรย์อาร์จีบี **วิธีการศึกษา:** ทำการวัดปริมาณด็อกซีไซคลินอย่างง่ายโดยใช้วิธีการอ่านค่าสีแบบอาร์จีบี โดยสารมาตรฐานด็อกซีไซคลินที่ความเข้มข้นต่างๆ (10-100 ไมโครกรัมต่อมิลลิลิตร) ถูกนำมาผสมกับสารละลายเหล็ก(III) เข้มข้น 50 ไมโครกรัมต่อมิลลิลิตร ได้สารประกอบเชิงซ้อนที่มีสีเหลือง ซึ่งความเข้มสีอาร์จีบีมีความสัมพันธ์โดยตรงกับความเข้มข้นของด็อกซีไซคลิน ค่าอัตราส่วนปฏิกิริยาระหว่างด็อกซีไซคลินและเหล็ก(III) มีค่า 2:1 สารละลายมีสีที่เกิดขึ้นถูกบรรจุในหลอดแก้วมาตรฐานที่มีการเรียงลำดับตามความเข้มข้นและถ่ายภาพด้วยโทรศัพท์มือถือ ภาพถ่ายดิจิทัลที่ได้ไม่มีการปรับแต่งภาพก่อนนำไปอ่านค่าอาร์จีบีด้วยโปรแกรมการอ่านค่า (GetPixel) ที่พัฒนาขึ้นมาเอง **ผลการศึกษา:** การหาค่าสภาวะการวิเคราะห์ที่เหมาะสมจะศึกษาด้วยวิธีการวิเคราะห์ตัวแปรเดียว กราฟมาตรฐานถูกสร้างขึ้นระหว่างความเข้มข้นของด็อกซีไซคลิน (ในช่วง 10-100 ไมโครกรัมต่อมิลลิลิตร) กับค่าสีอาร์จีบี ความเป็นเส้นตรงภายในช่วงความเข้มข้นที่เหมาะสมมีสมการแสดงความสัมพันธ์ คือ $y = 0.0671x + 0.1067$ และมีค่าสัมประสิทธิ์ความเป็นเส้นตรง $r^2 = 0.9999$ ค่าขีดจำกัดต่ำสุดของการตรวจวัด (LOD) มีค่าเท่ากับ 1.10 ไมโครกรัมต่อมิลลิลิตร ค่าขีดจำกัดของวิธีตรวจวัด (LOQ) มีค่าเท่ากับ 3.40 ไมโครกรัมต่อมิลลิลิตร ค่าเปอร์เซ็นต์การคำนวณย้อนกลับ (Recovery) มีค่าในช่วง 99.6-103.4 เปอร์เซ็นต์ และค่าการวัดซ้ำๆ มีค่าเบี่ยงเบนมาตรฐานต่ำกว่า 5 เปอร์เซ็นต์ ได้มีการศึกษาผลของสารปรุงแต่งที่ใช้ในทางเภสัชภัณฑ์ พบว่าไม่มีผลใดๆ ต่อการวิเคราะห์ปริมาณด็อกซีไซคลิน ผลของการวิเคราะห์ตัวอย่างด็อกซีไซคลินด้วยวิธีที่นำเสนอเมื่อเปรียบเทียบกับวิธีอ้างอิงแล้ว พบว่าไม่มีความแตกต่างกันในเชิงสถิติด้วยความเชื่อมั่นที่ 95 เปอร์เซ็นต์ (n=10) **สรุปผล:** วิธีการวัดค่าอาร์จีบีที่พัฒนาขึ้นนี้ใช้โทรศัพท์มือถือร่วมกับวิธีการที่ไม่ซับซ้อนและประหยัดเวลาในแต่ละขั้นตอน วิธีการที่นำเสนอได้ให้ผลการวิเคราะห์ที่ดี มีความแม่นยำ ความเที่ยงสูงและการวัดซ้ำได้ผลดี นอกจากนั้นวิธีที่พัฒนาขึ้นนี้สามารถใช้เป็นวิธีทางเลือกหนึ่งสำหรับการวิเคราะห์ปริมาณด็อกซีไซคลิน

คำสำคัญ: วิธีวัดสีอาร์จีบี, ด็อกซีไซคลิน, ภาพดิจิทัล, โทรศัพท์มือถือ

Determination of Doxycycline Capsules Using RGB Colorimetric Method

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Abstract

Determination of Doxycycline Capsules Using RGB Colorimetric Method
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A simple RGB colorimetric method was developed for the quantitative analysis of doxycycline capsules. Principally, digital images of the complex taken by android mobile phone were used for the colorimetric analysis by reading RGB pixels array values. **Method:** Doxycycline content was determined by using the proposed RGB colourimetric method. The various concentrations of standard doxycycline solution (10-100 μg mL^{-1}) were mixed with 50 μg mL^{-1} of iron(III) solution. The yellow colour from complexation were obtained and the RGB pixels array intensity of the solutions was related with doxycycline concentration. The volume ratio of doxycycline and iron(III) was 2:1. The standard glass tubes contained the coloured solutions were arranged standing side by side and digital image was captured by mobile phone. The digital image obtained with no photo effect was detected to get the RGB pixels array values of analyte using the in-house GetPixel program. **Results:** The optimum conditions for the proposed method were studied by univariate analysis. Standard calibration curve was established in concentration ranges 10-100 μg mL^{-1} of doxycycline which was plotted against RGB pixel values. For linearity within the optimum range, regression equation was shown $y = 0.0671x + 0.1067$ and the coefficient of determination (r^2) was 0.9999. The limit of detection (LOD) was 1.10 μg mL^{-1} and the limit of quantification (LOQ) was 3.40 μg mL^{-1}. The percentage recoveries of proposed method had shown in range of 99.6%-103.4% and repeatability was found the standard deviation lesser than 5%. The effect of excipients which are usually added to pharmaceutical formulations was investigated on doxycycline analysis and shown none effected for determination of doxycycline. The difference between the results of proposed and reference methods was figured out statistically and showed no statistical difference at a 95% confidence level (n=10). **Conclusion:** The proposed RGB colorimetric was based on using mobile phone with non-sophisticate processes and less time-consuming step. The presented method was provided good resulting for accuracy, precision and reproducibility. Moreover, this low cost proposed method can be used as an alternative method in quantitative doxycycline analysis.

Keywords: RGB colorimetry, Doxycycline, Digital image, Mobile phone

Introduction

Pharmaceuticals are very important for human health. The purpose of a pharmaceutical development leads to be quality, safety and efficacy. Anyway, pharmaceuticals may be contaminated in every process, which is production of raw materials, storage, transportation, manufacturing process, intermediate product, finished product and finally even product in market. Therefore, analytical methods as a part of quality assurance system play a very important role in pharmaceutical analysis. There are many quantitative analytical methods for pharmaceutical determinations (Toomula N *et al.*, 2011). Gradually, when the negative impact of chemicals on ecosystem has been realized, many advanced green analytical methods are being developed (Samanidon VF, 2014 and Kogawa AC *et al.*, 2016).

Colourimetry is a technique to measure colours by numerical values and it is very useful in analytical chemistry and pharmaceutical analysis. It can be considered as green analytical method because it is complying with principles of green analysis. Colours within visible wavelength ranges can be detected in quantification of pharmaceutical formulation by using visible spectrophotometric and chromatographic methods. In recent years, a new type digital colorimetry which was given by digital devices such as webcams, flatbed scanners, mobile phones, digital cameras have been widely used in qualitative and quantitative determination of food, pharmaceuticals, in clinical laboratories and in environmental monitoring (Schults EV *et al.*, 2019, Monogarova OV *et al.*, 2018, Gummadi S and Kommoju M, 2019, Fernandes GM *et al.*, 2020 and Chaplenko AA *et al.*, 2022).

RGB pixels array model (red, green and blue) can be applied in digital colored images derived from these digital devices. RGB means the combination of three colours. Each colour pixel value is in the range of 0-255 and so the combination of three values produces more than 16,777,216 different colours (Fan Y *et al.*, 2021). There are some examples of research papers in which RGB analysis method was used in quantification of substances including determination of ethanol in beers (Curbani L *et al.*, 2020), sodium in sport drink samples (Labounmi B *et al.*, 2018), analysis of aspirin (Silva RSD and Borges EM, 2019), nitrite in food and water samples (Filgueiras MF *et al.*, 2021) and formaldehyde contamination (Wongniramaikul W *et al.*, 2018).

Nowadays, mobile phones with upgraded software are very useful for many tasks and also high technology in digital camera of the phones produce high resolution photos. The advantages are being able to use easily, not required training to be used, low cost, rapid and no need sophisticated instrumentation. The RGB colour of digital images was captured by mobile phone and it can be measured RGB pixels array values by using colour analysis software. The results (RGB pixels array values) was correlated with the concentrations of analyte which could be extrapolated from calibration curve. The previously report were applied by using mobile phone for related pharmaceutical compounds such as determination of ascorbic acid in fruits (Santos VBD *et al.*, 2019), assay of chlorpromazine tablets (Phadungcharoen N *et al.*, 2020), methamphetamine (Choodum A *et al.*, 2014) and analysis of levocetirizine tablets (Wai MMH *et al.*, 2020).

Doxycycline with IUPAC name is (4*S*,4*aR*,5*S*,5*aR*, 6*R*,12*aR*)-4-(dimethylamino)-1,5,10,11,12*a*-pentahydroxy-6-methyl-3,12-dioxo-4*a*,5,5*a*,6-tetrahydro-4*H*-tetracene-2-carboxamide. This drug is one of tetracycline derivatives which are broad-spectrum antimicrobial activities. It can be used in the treatment of many infections, which was described in a review of doxycycline by Kogawa AC and Salgado HRN, 2012. Doxycycline was found in WHO Model List of Essential Medicine – 22nd (2021). It can be found in three types of dosage forms – oral solid, oral liquid and injection. In treatment of malaria, doxycycline can be used by combination with other antimalarial drugs (Tan KR *et al.*, 2011 and Newton PN *et al.*, 2005). Liquid Chromatographic method was described in British Pharmacopoeia for doxycycline determination (British Pharmacopoeia, 2018). Doxycycline was determined by various methods such as flow injection spectrophotometric method (Palamy S and

Ruengsitagoon W, 2017), high performance thin layer chromatography (HPTLC) (Kumssa L *et al.*, 2021), high performance liquid chromatography (HPLC-UV) method (Kogawa AC and Salgado HRN, 2013), electrochemical impedance spectroscopy method (Dobrova A *et al.*, 2021) and UV-visible spectrophotometric method (Sunaric SM *et al.*, 2009, Ghaemi M and Absalan G, 2015, Awad FH, 2021, Ramesh PJ *et al.*, 2011, Phiphatphon S and Ruengsitagoon W, 2020). Definitely, the instrumental in the previously reported methods are expensive and needed sophisticated processes.

Therefore, the RGB colorimetric method which is low cost technique, simple instrumentation and rapid analysis was developed for quantitative analysis of doxycycline. The yellow color formed by mixing doxycycline and iron(III) in a ratio of 2:1 was captured by mobile phone. Doxycycline analysis was indicated from RGB pixel values from the obtained digital image by using in-house GetPixel Program (Version 2.0). The proposed method was validated using univariate analysis technique.

Materials and Methods

Chemicals and apparatus

All chemicals were of analytical reagent grade and were used without further purifcations. All solutions were prepared with distilled deionized water. The doxycy cline standard, ammonium iron(III) sulfate dodecahydrate and nitric acid were purchased from Sigma-Aldrich, USA; QRëC®; New Zealand and RCI Labscan; Thailand, respectively.

Conventional UV-visible spectrophotometer (Gene sys 180, USA), was used throughout the study. The RGB colorimetric analysis software (GetPixel Version 2.0), using for reading RGB pixel value from digital images

Procedures

Standard and sample preparation

A stock standard 1,000 μg mL^{-1} solution of doxycycline was prepared by accurately weighing 0.1000 g of doxycycline standard in double-distilled deionized water and the volume was adjusted to 100 mL of graduate mark to provide a final concentration of 1,000 μg mL^{-1}.

The sample of doxycycline capsules (100 mg/capsule) were purchased from drug stores in Khon Kaen Province, Thailand. The powder from 20 capsules of doxycycline was collected and an amount equivalent to 100 mg of doxycycline was accurately weighed using an analytical balance. The drug sample was dissolved in 250 mL of double-distilled deionized water and filtered through Whatman No 42 filter paper afterward the sample solution was diluted in double-distilled deionized water to obtain the appropriate concentrations (20 μg mL^{-1} of doxycycline) for analysis.

Iron(III) solution preparation

A solution of 50 μg mL^{-1} iron(III) was prepared by dissolving 4.3170 g of ammonium ferric sulfate dodecahydrate in 5.0×10^{-3} mol L^{-1} nitric acid and diluting to 1,000 ml in a volumetric flask. Solutions of iron(III) at 5, 10, 15 and 50 μg mL^{-1} were obtained by appropriate, accurate dilutions of this solution with 5.0×10^{-3} mol L^{-1} nitric acid.

Preparation for getting digital images

The various concentration of standard doxycycline solution was prepared in range of 0-100 μg mL^{-1} in double-distilled deionized water. The solution of standard doxycycline (2.0 mL) and iron(III) (1.0 mL) were pipetted and mixed well in test tube. Next, the complexation occurred and the yellow colour intensity of the mixtures was found to depend on the drug concentrations (Figure 1). Then, the tube including blank solution were arranged standing side by side. A smartphone (Galaxy A32, Samsung Inc.) was used to acquire images which is consist of 64 MP with f/1.8 as main camera. The digital image of standard or sample solution was taken by using an android smartphone under the ambient temperature in a homemade camera box for protected from the stray light, and stored an image as jpeg format. Finally, the digital image of doxycycline standard or sample was read the RGB pixels array values by using in-house pixels reader program (GetPixel Version 2.0). The region was assigned, (Figure 2), for recorded pixels array values of each digital image.

Calibration curve

The calibration curve was studied in range of 10-100 µg mL^{-1} of doxycycline that reacted with iron(III) reagent. The RGB values of doxycycline and iron(III) complex were recorded from digital image. The calibration curve was constructed when the *x*-axis was represented with doxycycline concentration and *y*-axis was represented with the RGB pixels array values. Afterward the calibration curve was constructed where sample pixels array value were correlated to find out its unknown concentration or amount.

RGB colorimetric software

The GetPixel program (Version 2.0) was utilized in the colorimetric quantitative analysis. This program was developed in-house using Microsoft Visual Basic 6.0. The program was written by Assist. Prof. Dr. Senee Kruanetr, Department of Chemistry, Faculty of Sciences, Mahasarakham University, Thailand. In this program, each colour value (0-255) of red, green, and blue can be detected and also the combination of value of three colours (Figure 2).

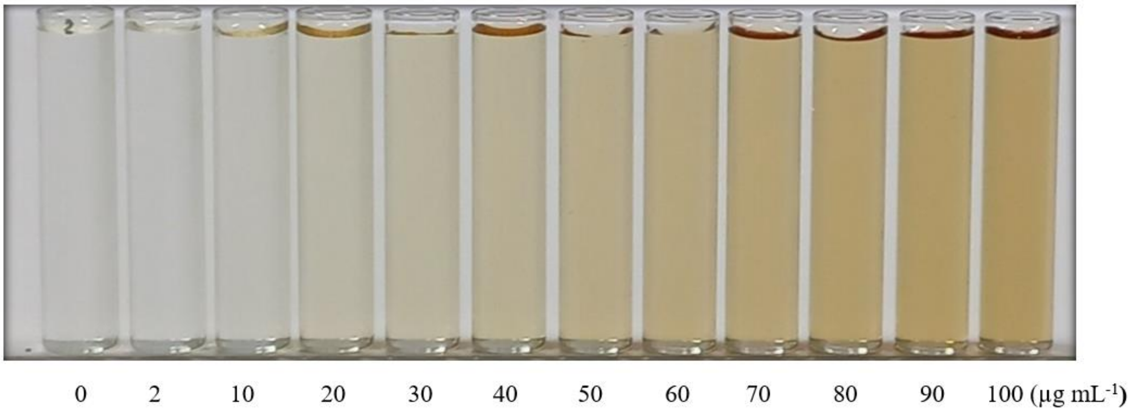

Figure 1. Colors of doxycycline/Fe(III) complexes formed by using different concentration of doxycycline.

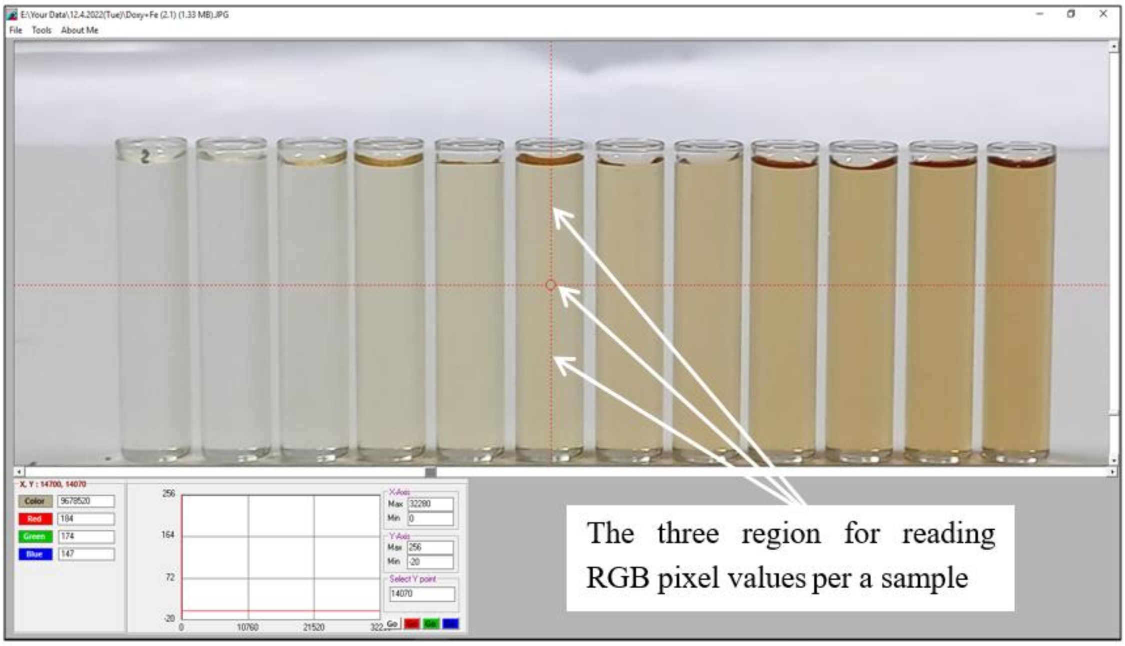

Figure 2. RGB colorimetric analysis program (Version 2.0) where measuring the average RGB values from three region of each standard/sample solution.

Results and Discussion

The optimum conditions for the determination of doxycycline were studied by using univariate analysis technique. This method was developed based on the formation of yellow colour from complexation between doxycycline and iron(III) in nitric acid. The optimum values were selected from the appropriate RGB pixels array values.

Effect of iron(III) concentration

The effect of varying concentration of iron (III) solution between 5-50 µg mL^{-1} was examined. The appropriate RGB pixels array values were obtained when the concentration of iron (III) in acidic solution was 50 µg mL^{-1} and was therefore chosen as the optimum concentration. The result was shown in Figure 3. This was found that the iron(III) concentration at 50 µg mL^{-1} was gave the high sensitivity of present method and precipitate occurred when iron(III) concentration higher than this point.

Effect of acid types and concentration

The effect of acid types such as of nitric (HNO_3), perchloric ($HClO_4$), hydrochloric (HCl) and sulphuric acid (H_2SO_4) in iron(III) solution was studied. The percentage of relative RGB pixels array values were 100.0, 86.0, 83.8, 76.7%, respectively. The presence of HNO_3 gave the highest RGB pixels array values, so it was chosen for subsequent studies (Figure 4).

Once the type of mineral acid was found, the studied the effect of the HNO_3 concentration in the range of 0.005-0.3 mol L^{-1} was investigated and selected the optimum RGB pixels array values. The HNO_3 concentration chosen for further studies was 0.005 mol L^{-1} because this concentration gave a maximum RGB pixels array values (Figure 5) and to reduce the consumption of the reagent. Doxycycline forms a complex with iron(III). Complex formation takes place in acidic medium preferably at lower temperatures (Obaleye JA and Abosede OO, 2019).

The volume ratio of doxycycline and iron(III)

The quantitative ratio of two solutions has an influence on doxycycline determination. The studies of the volume of each solution in mixture are expressed: doxycycline : iron(III); 2:1, 1:1, 1:2 and 1:3, respectively (Figure 6). The volume ration between doxycycline and iron(III) at 2:1 was selected as optimum volume ratio. Previously research findings were exhibited the highest signal that the complete complex formation was occurred at the volume ratio of doxycycline and iron(III) at 2:1 (Ramesh PJ *et al.*, 2011, Palamy S and Ruengsitagoon W, 2017, Phiphatphon S and Ruengsitagoon W, 2020).

Doxycycline formed complex with metallic cations, including iron(III). The proposed structure of the complex formed between doxycycline-iron(III) complex is presented in Figure 7 (Javed J and Zahir E, 2017). The mechanism of chemical reaction is based on metal ion that involves the formation of at least one coordinate covalent bond (Kenner CT and Busch KW, 1979).

Reaction time between two solutions

Doxycycline (50 µg mL^{-1}) reacted with iron(III) (50 µg mL^{-1}) forming a drug-iron(III) complex and the complex solution protected from the light throughout the studied. The yellow colour of complexation was occurred immediately after mixed doxycycline and iron(III) solution. The RGB pixels array values was investigated when mixed both solution from starting reaction until 180 minute. It was found that the RGB pixels array values was not different when leave the complexation solution reached 180 minutes (Figure 8). This studied was selected the reaction times at 15 minute as the optimum times before recorded the digital imaged in order to get the less time of analysis.

Doxycycline, an antibacterial drug, forms a reasonably stable complex with iron(III) and an acidic pH value increases the stability of the drug (Javed J and Zahir E, 2017).

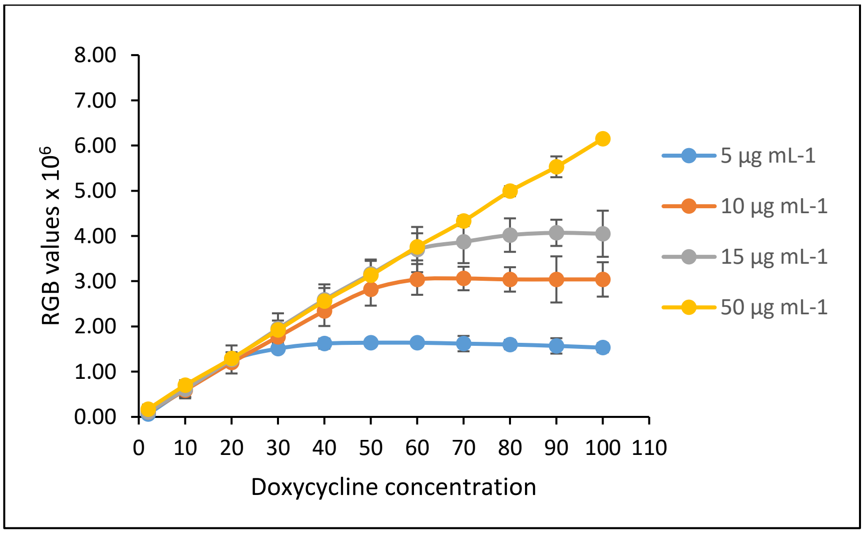

Figure 3. Effect of iron(III) concentrations, 5, 10, 15 and 50 µg mL^{-1} (*n*=3).

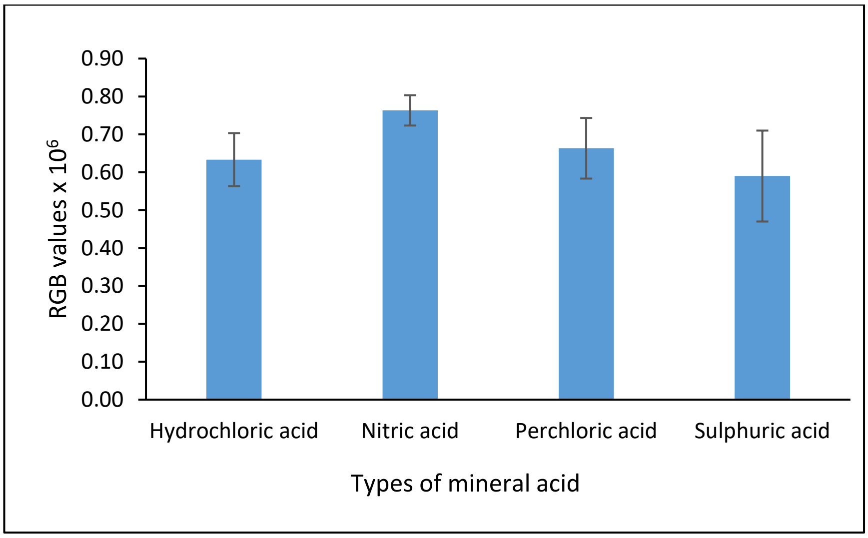

Figure 4. Effect of types of mineral acid (*n*=3).

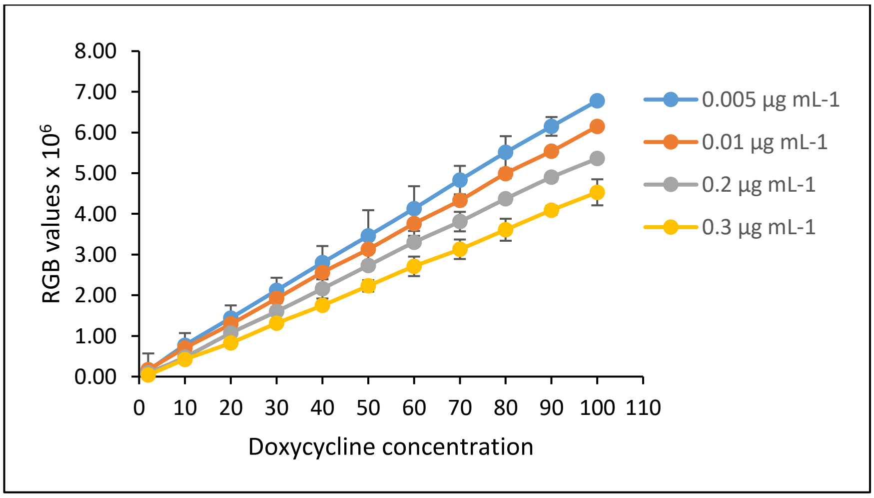

Figure 5. Effect of nitric acid concentrations, 0.005, 0.01, 0.2 and 0.3 mol L^{-1} (*n*=3).

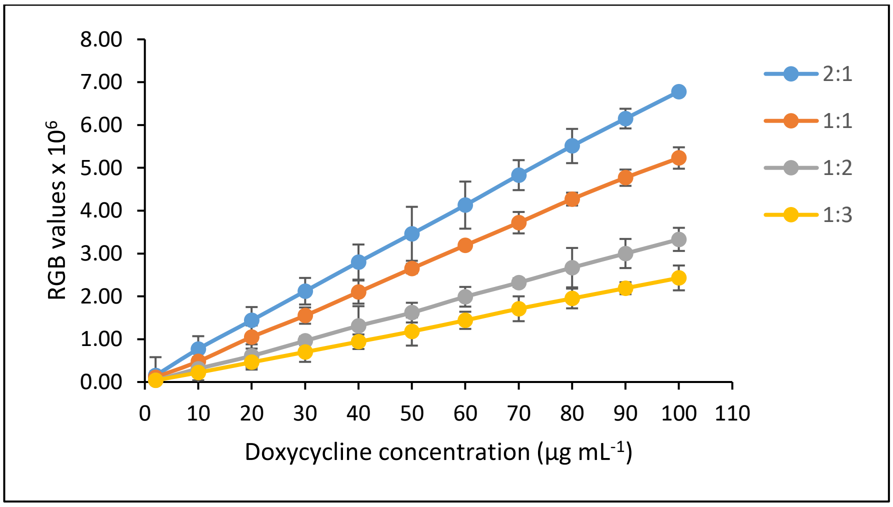

Figure 6. Effect of volume ratio of doxycycline and iron(III), 2:1, 1:1, 1:2 and 1:3 (*n*=3).

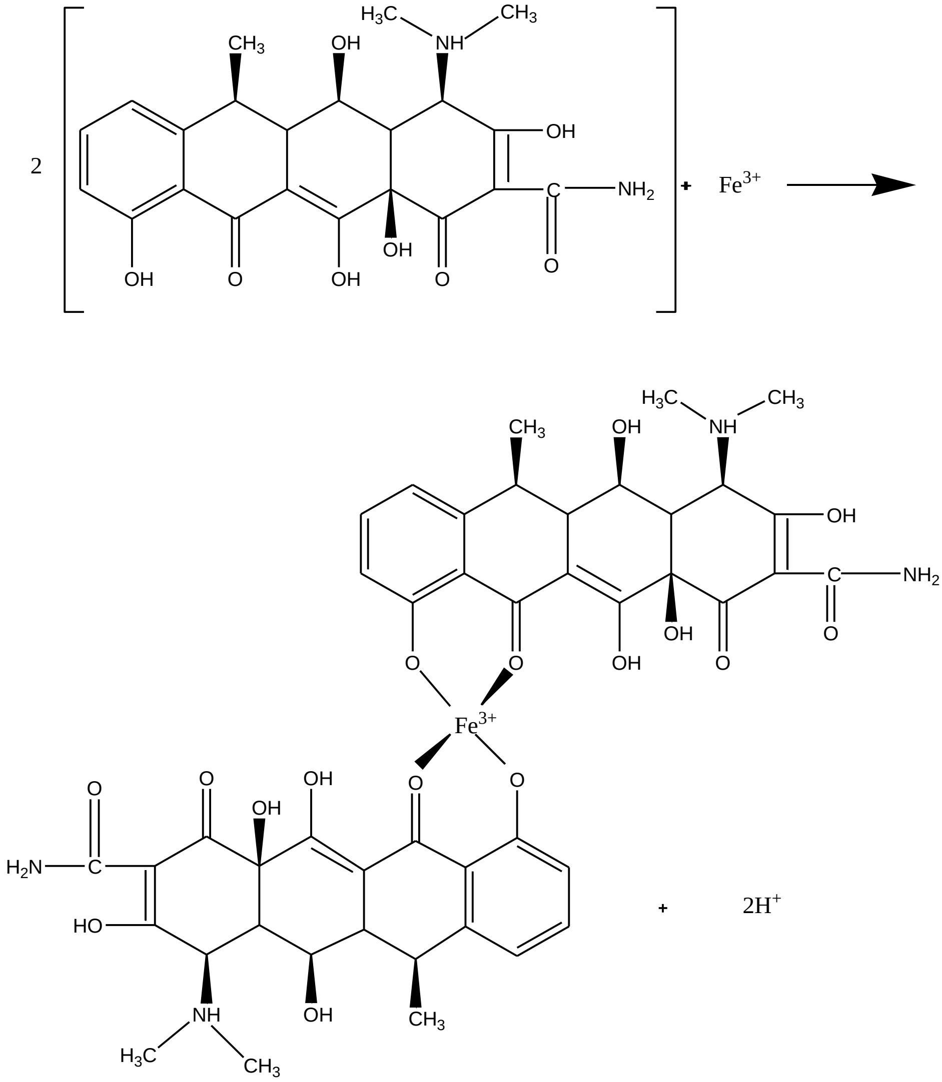

Figure 7. Possible mechanism between doxycycline and iron(III) (Javed J and Zahir E, 2017).

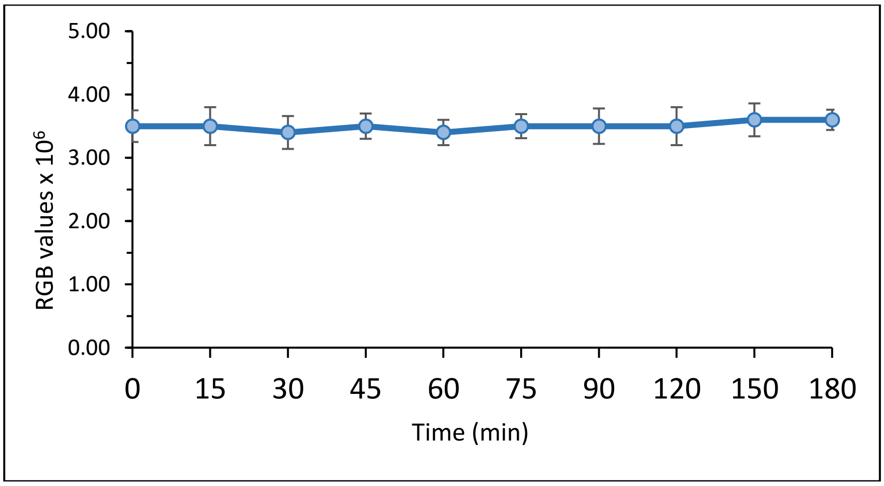

Figure 8. Effect of reaction time between doxycycline and iron(III) (*n*=3).

Table 1. Optimum conditions for doxycycline determination by using RGB colorimetric method.

Conditions	Studied range	Optimum conditions
Iron(III) concentration (ug ml^{-1})	5, 10, 15, 50	50
Nitric acid concentration (M)	0.005, 0.01, 0.2, 0.3	0.005
Types of acid	HCL, HNO_3, $HClO_4$, H_2SO_4	HNO_3
The volume ratio of doxycycline and iron (III)	2:1, 1:1, 1:2, 1:3	2:1
The reaction times (min)	0-180	15

Analytical Characteristics

Calibration curve

Under the optimum conditions (Table 1), linear calibration graphs were obtained for 10.0 - 100.0 μg mL^{-1} of doxycycline. Over the above concentration range, linear regression determination of the RGB pixels array value (*y*) versus drug concentration (*x*) yield equations $y = 0.0671x + 0.1067$. The coefficient of determination of two variables was 0.9999 (*n*=3), (Figure 9).

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined for analysis of doxycycline, based on the criteria that the signal to-noise ratio for LOD and LOQ are 3:1 and 10:1, respectively, for sample to blank. The detection limits for proposed method was 1.10 μg mL^{-1} while the quantitation limits was 3.40 μg mL^{-1}. The coefficient of analysis, detection limits and quantitation limits are in acceptable values.

Precision and accuracy

The relative standard deviation of the proposed method (RGB pixels array value) from 5 repeated of doxycycline 10, 20 and 30 μg mL^{-1} were carried out couple with standard addition method. The percentage of standard deviation of intra-day was 2.40, 1.47 and 1.18 (*n*=5) and inter-day was 3.79, 1.06 and 1.95 (*n*=5), respectively. The recoveries for the proposed method was studied on 10, 20 and 30 μg mL^{-1} and using standard addition method. The results were 103.45%, 99.62% and 100.75% (*n*=5) for intra-day and 100.74%, 101.88% and 100.68% (*n*=5) for inter-day, respectively. The analytical characteristics of this proposed method was summarized in Table 2. The percentage recoveries of proposed method had shown in range of 99.62%-103.45% and repeatability was found the standard deviation lesser than 5% which were proved that this present method was provide accurate and reasonable results.

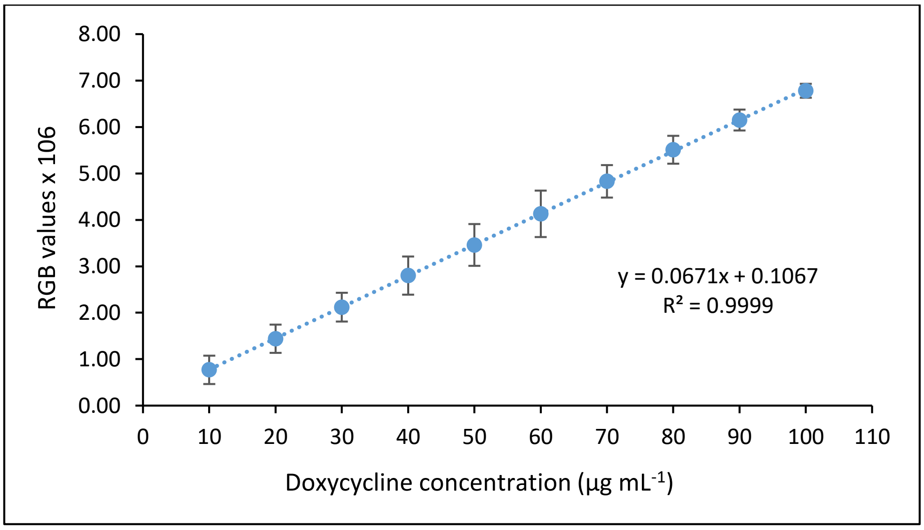

Figure 9. Calibration curve of doxycycline within ranges of 10 – 100 µg mL^{-1} (*n*=3).

Table 2. Analytical characteristics for doxycycline analysis by RGB colorimetric method.

Parameters	**Optimum value**	
Linearity of calibration curve, µg mL^{-1}	10 – 100	
Linear regression equation (*n*=3)	y = 0.0671x + 0.1067	
Correlation coefficient, r^2	0.9999	
Limit of detection (LOD), µg mL^{-1}	1.10	
Limit of quantification (LOQ), µg mL^{-1}	3.40	
Repeatability* (*n*=5); %RSD	Intra-day	Inter-day
10 µgmL^{-1}	2.40 %	3.79 %
20 µgmL^{-1}	1.47 %	1.06 %
30 µgmL^{-1}	1.18 %	1.95 %
Percentage recoveries** (*n*=5)		
10 µgmL^{-1}	103.45 %	100.74 %
20 µgmL^{-1}	99.62 %	101.88 %
30 µgmL^{-1}	100.75 %	100.68 %

* *measuring of desired concentration*

** *using standard addition techniques*

Interference studies

Many excipients are usually added in pharmaceutical formulations. There was an investigation of interference of excipients on doxycycline analysis by the developed method. Excipients were glucose, lactose, starch, magnesium stearate, sucrose, sorbitol and titanium dioxide. Doxycycline (40 µg mL^{-1}) was prepared with the same procedure, in which the concentrations of excipients were added 5 and 10 times greater than the concentration of standard doxycycline. The obtained data was showed a slightly effect from these excipients for the developed method (Table 3).

Application for commercial samples

The proposed method was utilized for the examination of 10 commercial products which contain 100 mg of doxycycline in each capsule. The results of the proposed method were estimated the similarity with that of UV-visible spectrophotometric method (reference method). Doxycycline was determined the absorption at 425 nm with UV-visible spectrophotometer when doxycycline and iron(III) reacted to each other for reference method. The accuracy of proposed method was calculated statistically by using the Student's t-test at a 95% confidence level. It was found that the results obtained by both methods showed no statistical difference at the 95% confidence level. The values are expressed in Table 4.

Table 3. Effects of excipients on doxycycline.

Types of excipients	Amount of excipients	RGB values (*n*=3)	Relative Percentage (%)
Doxycycline* with none excipients		2800219	100.00
Glucose	5 times	2793437	99.75
	10 times	2790266	99.64
Sucrose	5 times	2800560	100.01
	10 times	2787940	99.56
Starch	5 times	2777391	99.18
	10 times	2771768	97.91
Lactose	5 times	2800475	100.01
	10 times	2789406	99.61
Titanium dioxide	5 times	2746871	98.09
	10 times	2637731	94.19
Magnesium stearate	5 times	2771866	98.98
	10 times	2753841	98.34
Sorbitol	5 times	2737473	97.75
	10 times	2710649	96.80

* *40 µg mL^{-1} of doxycycline*

Table 4. Accuracy of proposed method compared with reference method for determination of doxycycline.

Commercial samples	Doxycycline (100 mg/capsule); mean ± sd.	
	Proposed RGB method	Reference method
1	101.01 ± 0.48	99.65 ± 0.65
2	99.99 ± 0.16	100.22 ± 0.87
3	101.31 ± 0.51	100.45 ± 0.92
4	100.47 ± 0.53	99.57 ± 0.68
5	99.87 ± 0.74	99.95 ± 0.68
6	100.54 ± 0.74	98.86 ± 0.66
7	100.41 ± 1.05	100.62 ± 0.99
8	99.94 ± 0.06	100.25 ± 0.27
9	99.89 ± 1.16	100.52 ± 0.65
10	100.17 ± 0.89	98.85 ± 0.27
t-Test at 95% confidence level		
t–calculation		1.75
t-critical value (*n-1*)		2.26

Table 5. Performance comparison of different methods for detecting doxycycline.

Methods	Materials	Linear range (µg mL^{-1})	LOD (µg mL^{-1})	References
UV-Vis spectrophotometry	Microfluidic paper-based	0.5-3.5	0.24	Abdulsattar JO. *et al*.
UV-Vis spectrophotometry	*p*-Bromanil	1.0-50	0.32	Alassaf N and Zankanah FH.
UV-Vis spectrophotometry	Chemometric-assisted	5.0-25	3.30	Eticha T. *et al*.
UV-Vis spectrophotometry	Iron(III)	5.0-250	1.50	Palamy S and Ruengsitagoon W
Fluorimetric	Carbon dot detection	0.4-400	6.40	Su Q. *et al*.
UV-Vis spectrophotometry	Iron(III)	10.0-100	1.28	Ramesh PJ. *et al*.
RGB colorimetric	Iron(III)	10.0-100	1.10	This work

As shown in Table 5, the present method was compared with other methods. The RGB colorimetric method established in this study has reasonable working range and limits of detection value. The proposed method has liner range (10-100 µg mL^{-1}) of calibration curve better than that reported by previously publication (Abdulsattar JO *et al*., 2020, Alassaf N and Zankanah FH., 2019, and Eticha T. *et al*., 2018). The limits of detection of this work was 1.10 µg mL^{-1}. It was found that this better than the studied were presented by some publication (Eticha T. *et al*. 2018, Palamy S and Ruengsitagoon W, 2017, Su Q. *et al*., 2019 and Ramesh PJ. *et al*., 2011).

The advantages of this proposed method may be summed up as follows: (1) Its simplicity compare to the classical UV-Visible spectrophotometric method; (2) This method uses simple instrumentation and program including mobile phone and in-house software (GetPixel, Version 2.0); and (3) This technique may be applying for some scientific units where do not have UV-Visible spectrophotometer in laboratory. The disadvantage of this presented method could be not appropriate for determination of doxycycline which was found quantity lower than the LOQ of the methods and the light conditions could be pay attention when recording the digital image such as using a homemade camera box for protected from the stray light.

Conclusion

The proposed RGB colorimetric method has proven to be simple and sensitive for the determination of doxycycline. The calibration graph remains linear in the useful range for quantitation of this drug in pharmaceutical preparation. This proposed method can be used as an alternative method in quantitative doxycycline analysis.

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