Original Article

Investigating the Spectrophotometric Determination of Doxycycline by Ion-pair Complex Formation With Bromothymol Blue

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ABSTRACT

Background: Doxycycline is a widely used antibiotic, commonly formulated as doxycycline hydrochloride (100 mg). The accurate estimation of doxycycline in pharmaceutical formulations is crucial for quality control and therapeutic efficacy. Spectrophotometry is a preferred analytical technique due to its simplicity, rapidity, accuracy, and cost-effectiveness.

Objectives: To develop a simple, accurate, and cost-effective spectrophotometric method for estimating doxycycline in pharmaceutical formulations by forming an ion-pair complex with bromothymol blue. The study aims to optimize conditions for complex formation, validate the method's linearity, sensitivity, and accuracy, and apply the procedure to pharmaceutical samples.

Methods: The absorbance of doxycycline was measured at 528 nm. The ideal conditions for product formation were systematically studied, including the type and volume of dye, the effects of acid and base, temperature, time, and solvent choice. The method's linearity was evaluated within the range of 5-45 µg/mL, adhering to Beer-Lambert's law. Sensitivity was determined using Sandell's sensitivity; meanwhile, the molar absorptivity (ε) coefficient was calculated. The limits of detection and quantification were also determined. The accuracy and precision of the method were assessed using the percentage recovery and the relative standard deviation. The procedure was applied to the estimation of doxycycline in its pharmaceutical form using direct and standard additive methods.

Results: The developed spectrophotometric method demonstrated an absorbance peak for doxycycline at 528 nm. The linearity of the method was established between 5-45 µg/mL, with Sandell's sensitivity calculated at 0.0625 µg/cm². The molar absorptivity (ε) coefficient was determined at 7.110×10^3 L/mol·cm. The limits of detection and quantification were 0.07950 µg/mL and 0.29573 µg/mL, respectively. The percentage recovery ranged from 95.250% to 99.250%, and the relative standard deviation did not exceed 0.4194. The procedure was successfully applied to estimate doxycycline in its pharmaceutical form by the direct method and the standard additive method, confirming the method's reliability and accuracy.

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Conclusion: A reliable and straightforward spectrophotometric method was successfully developed to estimate doxycycline using bromothymol blue. The method showed good accuracy, precision, and linearity within a 5–45 µg/mL concentration range. It was effectively applied to pharmaceutical formulations, demonstrating high recovery rates and compatibility, confirming its utility for quality control purposes in pharmaceutical analysis.

Keywords: Bromothymol blue, Doxycycline, Ion pair complex, Spectrophotometric, Pharmaceutical formulation

Introduction

The systemic name for doxycycline is 4-(dimethylamino)-S, 4aR, 5S, 5aR, 6R, 12As) -4) 3, 5, 10, 12, 12a-pentahydroxy -6-methyl-, 1,11-dioxo- 1, 4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide, which includes compounds containing four fused rings as well as containing double bonds. Doxycycline belongs to the tetracycline class, its molecular formula is $C_{22}H_{25}ClN_2O_8$ and its molar mass is 480.90 g/mol (Khammas et al., 2016). Doxycycline is an antibiotic that stops the growth of bacteria and is a derivative of oxytetracycline. It is used in the medical treatment of infections caused by various forms of microorganisms, such as rickettsia, mycoplasma, and brucella, for gram-negative and gram-positive bacteria. Its mode of action involves hindering the synthesis of proteins in bacteria. It also treats cancer diseases, including breast cancer (Wikipedia, 2015).

Doxycycline is a treatment option for COVID-19. It has the potential to mitigate the pulmonary consequences of legionella-like pneumonia and mycoplasma pneumonia (Malek et al., 2020; Awad & Taki, 2021). Several analytical methods were used in the quantitative determination of doxycycline, including spectroscopic methods (Ramesh et al., 2010; Khalafa & Othmanb 2022), chromatographic methods (Mashru & Koshti 2021; Kumssa et al., 2021), and electrical methods (Gashu et al., 2022; Xu et al., 2020).

The objective of current research is to enhance a new and simple color spectrophotometric procedure for the estimation of doxycycline by the interaction of the drug with the dye bromothymol blue (BTB) and the creation of a complex, involving an ion pair.

BTB is known in the International Union of Pure and Applied Chemistry as 3,3-Bis[3-bromo-4-hydroxy-2-methyl-5-(propan-2-yl) phenyl]-2,1λ6-benzoxathiole-1,1(3H)-dione (also known as bromothymol sulfone phthalein and BTB) and a pH indicator. Its chemical formula is $C_{27}H_{28}Br_2O_5S$ and its molar mass is 624.38 g/mol. It is mostly used in applications that require measuring substances that would have a relatively neutral pH (near 7). A common use is for measuring the presence of carbonic acid in a liquid. It is typically sold in solid form as the sodium salt of the acid indicator (Peighambari et al., 2022).

BTB has been used in conjunction with phenol red to monitor the fungal asparaginase enzyme activity with phenol red turning pink and BTB turning blue, signaling an increase in pH and enzyme activity (Doriya, 2016).

One of the techniques used to determine compounds in ultraviolet-visible spectrophotometry is the ion-pair complex method. The process of ion pairing in the context of chemistry is formed when a cation and anion, which are present in a solution of an ionizable substance, come together as a discrete chemical species (Burgess & John, 1978).

Accordingly, this study develops a simple, rapid, accurate, and inexpensive spectrophotometric procedure for estimating doxycycline in its pharmaceutical formulation. This study identifies the optimal conditions for product formation and validates the method's reliability and accuracy.

Materials and Methods

Equipment and chemical materials used

Sensitive Balance (Sartorius, Germany), Spectrophotometer, Ultraviolet-Vis Double Beam (Shimadzu-1650-Japan), Ultrasonic Device (LabTech; Korean origin), and substances of a significant degree of purity, which are BTB (Sigma-Aldrich), and indigo carmine (CDH), sodium hydroxide (Fluka-Switzerl), doxycycline (Sigma-Aldrich), phenol red (CDH), amido black

(Sigma-Aldrich) and hydrochloric acid (BDH-U.K) were used in this study.

Preparatimods

Doxycycline standard solution (1000 µg/mL)

The doxycycline standard solution was created by being dissolved in 0.1 g of doxycycline in a 100 mL volumetric flask of distilled water, completing the volume up to the mark with the same dissolvent so that the concentration became 1000 (µg/mL) as a stock solution. Diluted solutions were prepared as needed for the experiment.

Pharmaceutical doxycycline solution (1000 µg/mL)

A total of 10 doxycycline tablets (100 mg) produced by the company, Medochemie LTD in Cyprus, were weighed and grind using a pottery mortar. The mean weight of a single tablet comprising 100 mg of doxycycline was taken. After placing the powder into a 100 mL volumetric flask, a specific quantity of distilled water was subsequently added. Then, the flask was agitated, and the solution underwent filtration using Whatman filter paper No. 42. The volume was supplemented with the same solvent, and then the filtrate that contained 1000 µg/mL of doxycycline was taken and preserved.

Dilute hydrochloric acid solution to an approximate concentration of 0.5 M

to prepare the solution, add 0.83 mL of concentrated acid (12.07 M) gradually to water. Then adjust the volume to the desired level with distilled waterk.

Solution of sodium hydroxide with an approximate concentration of 0.5 M

The sodium hydroxide solution was prepared by weighing 2 g of NaOH, dissolved in distilled water. The volumetric flask was filled to the desired level with distilled water, reaching the mark of 100 mL.

Bromothymol blue dye solution (1000 µg/mL)

The BTB dye solution was prepared by dissolving 0.1 g of BTB dye in a quantity of distilled water, filling the volume to the mark in a volumetric flask (100 mL) with distilled water, so that the concentration became 1000 µg/mL. It was placed in the ultrasonic wave bath for 5 min to complete the dissolution process as a stock solution. Diluted solutions were prepared as needed for the experiment.

Phenol red dye solution (1000 µg/mL)

The phenol red dye solution was prepared by dissolving 0.1 g of phenol red dye in a quantity of distilled water, filling the volume to the mark in a volumetric flask (100 mL) capacity with the same dissolvent, so that the concentration became 1000 µg/mL as a stock solution. Diluted solutions were prepared as needed for the experiment.

Amido black dye solution (1000 µg/mL)

The amido black dye solution was prepared by dissolving 0.1 g of amido black dye in a quantity of distilled water, filling the volume to the mark in a volumetric flask (100 mL) capacity with the same solvent, so that the concentration became (1000 µg/mL) as a stock solution. The diluted solution was prepared as needed for the experiment.

Indigo carmine tincture solution (1000 µg/mL)

The indigo carmine tincture solution was prepared by dissolving 0.1 g of indigo carmine tincture in a quantity of distilled water, filling the volume to the mark in a volumetric flask (100 mL) capacity with distilled water, so that the concentration became (1000 µg/mL) as a stock solution. The diluted solution was prepared as needed for the experiment.

Experimental section

Choosing the type and the volume of the dye

Several dyes were used to choose the best one that gives the best peak and highest absorption. Accordingly, 1 mL of the dyes presented below were added at a concentration of 100 µg/mL to a volumetric flask of 10 mL, containing 1 mL of standard doxycycline at a concentration of 100 µg/mL. As the results show, the dye that gives the highest absorption is BTB, which was used in the following experiments. As for the rest of the dyes, it gave low absorption values. The effect of adding different volumes of BTB dye was studied, and the volume of 3 mL of the dye was the best volume that gives the complex with the highest absorption was selected as the optimal dye volume, as shown in Tables 1 and 2.

The effect of acid and base

A search was conducted to investigate the influence of the hydrochloric acid, the base NaOH, and the base on the formed complex. The highest absorption values occur when the complex is formed, after conducting a

Table 1. Characteristics of the dyes

Dye	λ_{max}	Absorbance
Indigo carmine	540	0.015
Bromothymol blue	528	0.145
Amido black	603	0.01
Phenol red	344	0.109

Table 2. Optimum dye volume for doxycycline ion pair complex

Volume of Bromothymol Blue (mL)	Absorbance
0.1	0.028
0.3	0.042
0.5	0.054
0.8	0.099
1	0.146
1.5	0.161
2	0.192
2.5	0.225
3	0.244
3.5	0.235
4	0.224

Table 3. Influence of acid and base

Substance	Volume (mL)	Absorbance (A)
Hydrochloric acid (0.5 M)	0.1	0.007
	0.2	0.001
	0.3	-0.02
	0.4	-0.047
	0.5	-0.058
Without any addition	...	0.245
NaOH (0.5 M)	0.1	0.006
	0.2	-0.001
	0.3	-0.007
	0.4	-0.033
	0.5	-0.036

Table 4. Influence of temperature

Temperature (°C)	Absorbance (A)
5 (water bath)	0.035
10	0.064
18	0.113
25	0.245
30	0.389
35	0.588
40	0.832
45	0.688
50	0.601
55	0.589
60	0.574

study of the effect of adding hydrochloric acid (0.5 M) and NaOH base (0.5 M) on the reaction of the forming of (ion pair complex) of doxycycline drug. The finding of this study demonstrated that the addition of both the acid and the base resulted in a reduction in the absorption of the colored product. Therefore, their use was avoided, which is shown in Table 3.

Temperature effect

To determine the optimal temperature for achieving maximum absorption of the resulting complex, this study selected the most favorable temperature, a volume of 1 mL of a standard solution of doxycycline drug and 3 mL of a standard solution of BTB was taken in a volumetric flask (10 mL). Completing the volume up to the mark with distilled water, then measurement was performed for the absorption of the resulting complex at the moment of reaction and a temperature range that ranged between (5-60 ˚C). Table 4 presents the consequences of this work, indicating that the highest absorption occurs at a temperature of 40 ˚C. Additionally, a plus in temperature leads to a decrease in the absorption of the colored product formed; therefore, in the subsequent experiments, a temperature of 40 ˚C was employed.

The influence of time

To determine the stability and stability of the complex forms between doxycycline and BTB dye, a study was conducted to identify the optimal time, to maximize the absorption of the formed complex, a volumetric flask was used to combine 1 mL of the drug's standard solution and 3 mL of the dye's standard solution, both having a concentration of 100 µg/mL that have a capacity of 10 mL. Then, the volume was filled up to the mark with distilled water, keeping the temperature and the volume of the reagent at the best conditions. After that, the complex's absorption was assessed at various time points, commencing from the onset of complex preparation and extending up to a maximum time frame of 60 min. The data from Table 5 revealed that the complex exhibited its maximum absorption at 10 min. Subsequently, there was a gradual decrease in absorption until reaching 60 min. Therefore, a time of 10 min was adopted after completing the volume with distilled water, subsequently, the absorption was quantified at 528 nm.

Choosing the appropriate solvent

After adding all the components, a combination of various organic solvents was used to reach the desired volume in a 10 mL volumetric flask, and the absorbance was measured in the range of wavelengths 190-800 nm. To choose the best solvent for the formed complex, the findings are provided in Table 6 and illustrated in Figure 1.

According to Table 6 and Figure 1, the use of methanol gave the highest absorption compared to other solvents, but distilled water was used in the experiment due to its cheapness and availability, it was utilized in subsequent experiments.

Table 5. Influence of time

Time (min)	Absorbance (A)
The start of the reaction	0.833
5	0.981
10	1.02
15	1
20	0.999
25	0.998
30	0.997
35	0.996
40	0.995
45	0.993
50	0.988
55	0.973
60	0.964

Results

The preparation of the ion pair complex was achieved by commixing 1 mL of a 100 µg/mL solution of doxycycline with 1 mL of a 100 µg/mL solution of BTB in a volumetric flask (10 mL), and it was diluted with distilled water until reaching the mark. A scan was conducted for a range of wavelengths ranging from 190-800 nm, where a complex with a purple color was formed. Meanwhile, its absorbance was measured versus the blank solution at 528 nm.

Discussion

The experiments were conducted to study the effect of the experimental condition on the forming of the (ion pair complex), These conditions choose the type of dye and the best volume of the dye, the effect of acid and base, the effect of temperature, the effect of time, and choose the appropriate solvent. A scan was conducted for a range of wavelengths ranging from 190-800 nm, and these experiments were conducted at a wavelength of 528 nm, Beer-Lambert's law was followed in the concentration range 5-45 µg/mL.

The complex correlation ratio

Based on the continuous changes approach of Job's method, research was undertaken to estimate the pro-

Table 6. Influence of the type of solvent

Dissolvent	λ_{max} (nm)	Absorbance
Methanol	560	1.597
Ethanol	548	0.351
Distilled water	528	1.02
Trichloroethylene	---	The separation of two layers
Benzene	----	Slimy

Table 7. Binding ratios of ion-coupling composite of doxycycline with bromothymol blue dye

VD	VR	Absorbance	VD/VD+VR
0.1	0.9	0.022	0.1
0.2	0.8	0.028	0.2
0.3	0.7	0.039	0.3
0.4	0.6	0.041	0.4
0.5	0.5	0.049	0.5
0.6	0.4	0.034	0.6
0.7	0.3	0.029	0.7
0.8	0.2	0.024	0.8
0.9	0.1	0.019	0.9

VD: Volume of drug; VR: Volume of reagent.

portion of the drug to the dye in the ion pair complex. The solutions consisting of the drug and the reagent at equivalent concentrations of 0.00016 M were used. These vials are opposite volumes of a standard 0.9-0.1 mL dye solution. Afterward, the volume was adjusted to the specified mark using distilled water. The absorption values of the complex formed were measured opposite the blank solution at the wavelength 528 nm, as shown in Table 7.

The findings from Job's method indicate that under optimal conditions, a complex is formed consisting of a 1:1 molar proportion between the drug and the dye for both drug and dye, respectively.

The conclusive absorption spectrum

Based on the optimal conditions achieved, the absorption spectrum of the doxycycline complex was measured opposite the blank solution to confirm the formation of the product, revealing the emergence of a new peak in the complex at 528 nm, (λ_{max}) for BTB dye was 426 nm and for doxycycline 276 nm, which is shown in Figure 2.

Calibration curve of the complex

Increasing volumes of a standard solution of doxycycline (100 µg/mL) were added in a chain of volumetric flasks (10 mL), and the volumes ranged from 0.5-4.5

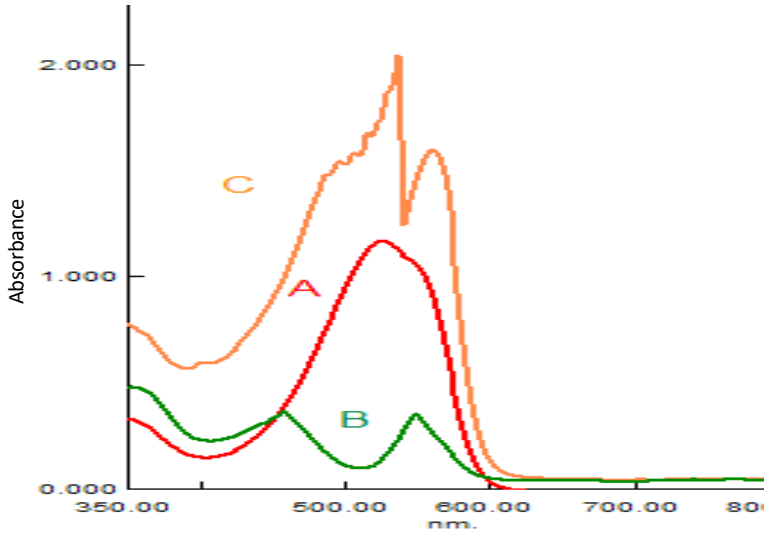

Figure 1. A) Complex absorption spectrum in a water medium, B) Complex absorption spectrum in an ethanol solvent, C) Complex absorption spectrum in methanol

Table 8. Recovery and relative standard deviation values

Concentration of Doxycycline Taken (µg/mL)	Absorbance (A)	Concentraiton of Doxycycline Found (µg/mL)	Percentage Recovery	Relative Standard Deviation Percentage
10	1.01	9.925	99.25	0.4419
25	1.2322	23.8125	95.25	0.0407
35	1.401	34.3625	98.1785	0.4194

Table 9. Detection limit

Concentration of Doxycycline in Pharma Form Taken (µg/mL)	$\overline{X}$	S	Limits of Detection (µg/mL)	Limit of Quantification (µg/mL)
5	0.926	0.005477	0.097592	0.29573

mL, to which a constant volume of 3 mL of a standard dye solution of a concentration of 100 µg/mL was added. Subsequently, it underwent dilution with distilled water until reaching the prescribed marker to obtain concentrations of 5-45 µg/mL of the drug and heated to 40 ˚C, then the absorption of each concentration was measured against the blank solution at 528 nm and under the best pre-established conditions, and a calibration curve was drawn for the drug doxycycline and as the method's linearity spans across a range of 5-45 µg/mL. Sandell's sensitivity was 0.0625 µg/cm^2 and the ϵ coefficient was 7.6944×10^3 L/mol.cm. Accordingly, Figure 3 shows the titration curve of the doxycycline complex.

Precision and accuracy

Five tests were repeatedly taken for 3 different amounts of doxycycline within the range of linearly in the calibration curves. From calculating the (relative standard deviation percentage) and the (percentage recovery percentage), it was found that the method has good precision relative standard deviation percentage) values did not exceed 0.4194 and accuracy values spanned a range of 95.250%-99.250%, as shown in Table 8.

Detection of limit

The detection limit was calculated for the lower concentration (5 µg/mL) in the calibration curve, it was

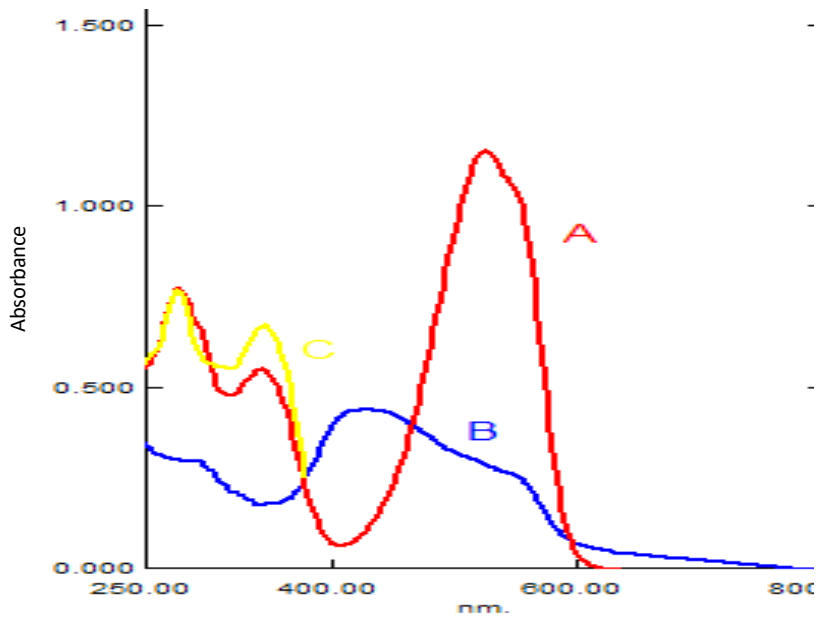

Figure 2. A) Complex's absorption spectrum against the blank solution, B) Dye's absorption spectrum against the dissolvent, C) Drug's absorption spectrum against the dissolvent

Table 10. Results of the determination of doxycycline in pharmaceutical preparation doxycycline (as hydrochloric acid) 100 mg

Concentration of Doxycycline Taken (µg/mL)	Absorbance (A)	Conc. of Doxycycline (µg/mL)	Percentage Recovery	Relative Standard Deviation Percentage
10	1.008	9.8	98	0.0543
25	1.232	23.8	95.2	0.0726
35	1.39	33.675	96.214	0.3951

Table 11. The procedure of single standard additions to the doxycycline complex

Concentration of Doxycycline in Pharma Form Taken (µg/mL)	Concentration of Standard Doxycycline (µg/mL) Added	Absorbance (A)	Concentration of Doxy Found (µg/mL)	Percentage Recovery	Relative Standard Deviation Percentage
10	5	1.08	14.3	95.333	0.413
10	10	1.16	19.3	96.5	0.609
10	30	1.48	39.3	98.25	0.302

equal to 0.097592 µg/mL) and the limit of quantification was 0.29573 µg/mL as shown in Table 9.

Applications

Direct method

The suggested technique was employed to doxycycline as hydrochloric acid 100 mg produced by the company (Medochemie LTD, Cyprus), where different volumes (1, 2.5, 3.5 mL) of a pre-prepared solution (100 µg/mL) were placed in 3 volumetric flasks of 10 mL, and 3 mL of a standard solution of BTB was added to it. Subsequently, the volume was supplemented with distilled water to get various concentrations (10, 25, 35 µg/mL) of the drug, and by making five multiple readings were taken for each measurement, to find out the concentration of the active substance in one pill, the straight line equation of the calibration curves was applied, the results appeared that the procedure is accurate, as the value of percentage recovery is between (95.200%-98.000%) and the value of relative standard deviation percentage does not exceed 0.3951, as shown in Table 10.

Single standard additions method

A total of 1 mL of the previously prepared pharmaceutical solution (100 µg/mL) to three volumetric flasks (10

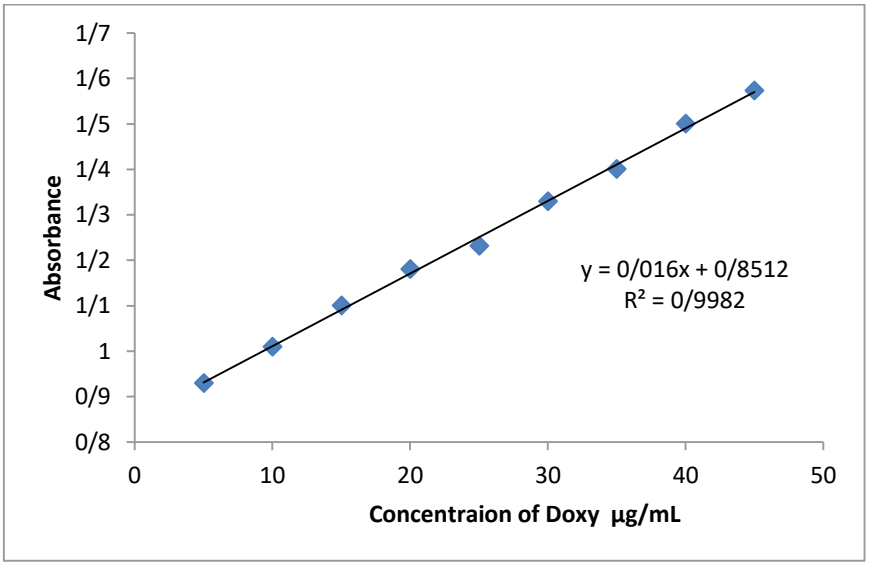

Figure 3. Titration curve of the doxycycline complex

Table 12. Comparison of the suggest procedure for the estimation of doxycycline with several different procedures

Parameter	Reference Procedure (TalibHumeidy, 2018)	Reference Procedure (Khalaf & Othman, 2022)	Present Method
Reagent	4,2-DNPH	4- amino antipyrene	Bromothymol blue
Max (nmλ)	528	515	528
Linearity (µg/mL)	5-60	5-110	5-45
Slope	0.021	0.003	0.016
R^2	0.998	0.995	0. 9982
LOD (µg/mL)	---	0.053	0.097592
LOQ (g/mL)	----	0.176	0.29573
Rec (%)	99.8	-99.06 99.44	99.25-95.25
RSD (%)	0.856-1.043	1.44-1.55	0.4194≥
Molar absorpiliely (L/mol/cm)	10771	1.387×10^3	7.6944×10^3
Sandell's index (µg/cm²)	0.476	0.3334	0.0625

LOD: Limit of detection; LOQ: Limit of quantification.

mL) was added, then increasing volumes of doxycycline standard solution of 100 µg/mL (0.5, 1.30 mL) were added. After that, the standard solution of BTB dye was added in a fixed volume of 3 mL, and distilled water was added until the volume reached the designated mark. The proposed method was applied and the measurement was carried out at 528 nm. The percentage recovery values were between (95.333%-98.250%) and the relative standard deviation percentage values did not exceed 0.609%, which indicates the success of the method in doxycycline estimation as shown in Table 11.

The results are obtained from the application of the single standard addition method to the pharmaceutical product doxycycline capsules 100 mg. The competence and success of the procedure used in the assessment are demonstrated in Table 11.

Method comparison

The suggested procedure for the estimation of mesalamine was compared with several of the procedures used for its estimation, as shown in Table 12.

Conclusion

A good and simple spectrophotometric procedure was developed for the estimation of doxycycline by ion pair complex method. A scan was conducted for a range of wavelengths ranging from 190-800 nm, where a complex with a (purple color) was formed, which gave its greatest absorption at 528 nm, Beer's law was followed in the concentration range 5-45 µg/mL. The procedure was accurate and compatible. The procedure was successfully used to estimate doxycycline within the pharmaceutical form doxycycline 100 mg.

Ethical Considerations

Compliance with ethical guidelines

The research did not involve any ethical considerations.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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