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Spectrophotometric Determination of Doxycycline Hydrochloride in its pure form and its pharmaceutical preparations using reagent 2,4,6 - tribromo-anline by the Azo coupling reaction

A B S T R A C T

This method is aimed at create a simple, fast, sensitive and cost-effective spectrophotometric method for measuring doxycycline hydrochloride in its pure form and in pharmaceutical preparations. The method is based on diazotization and coupling of a 2,4,6-tribromoaniline reagent in an alkaline medium to give an orange-yellow Azo dye and gave the highest absorption at a wavelength of 500 nm. After conducting numerous experiments to obtain optimal conditions, the linear relationship was governed by beer's law within the range of concentrations 0.5-24 (µg/mL),the molar absorptivity was 2.3 x 10^4 ($l.mol^{-1}.cm^{-1}$), the Sandel's sensitivity value was 0.0205 (µg /cm^2), the detection limit (LOD) was 0.0418 (µg/mL), the quantitative limit (LOQ) was 0.287 (µg/mL), and the average recovery percentage was 100.02%, With an accuracy (RSD%) of less than 0.8 %, the method showed that has good accuracy and the method was well applied successfully applied to pharmaceutical preparations as tablets and capsules.

التقدير الطيفي لهيدروكلوريد الدوكسيسيكلين في شكله النقي والمستحضرات الصيدلانية باستخدام كاشف 2، 4، 6 – ثلاثي بروموـأنلين بواسطة تفاعلات الازوتة والاقتران

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الخلاصة:

تهدف هذه الطريقة إلى إنشاء طريقة طيفية بسيطة وسريعة وحساسة وفعالة من حيث التكلفة لقياس هيدروكلوريد الدوكسيسيكلين في شكله النقي وفي المستحضرات الصيدلانية. تعتمد الطريقة على ازوتة واقتران الكاشف 2، 4 ، 6-ثلاثي بروموانيلين في وسط قلوي لتكوين صبغة أزو برتقالية صفراء والتي أعطت أعلى امتصاص عند الطول الموجي 500 نانومتر. بعد إجراء العديد من التجارب لتهيئة الظروف المثلى، كانت العلاقة الخطية تخضع لقانون بير ضمن نطاق التراكيز(0.5-24) مايكروغرام / مللتر، بلغت قيمة حساسية ساندل (0.0205) مايكروغرام / مللتر، وبلغت قيمة معامل الامتصاص المولاري 2.3 x 10^4 (لتر. مول-1.سم-1) ، وكان حد الكشف LOD (0.0418) مايكروغرام / مللتر ، وكان الحد الكمي LOQ (0.287) مايكروغرام / مللتر ، وكان نسبة الاسترجاع المئوية 100.02% . وقيمة الانحراف النسبي أقل من 0.8 %، وتم تطبيق الطريقة بنجاح على المستحضرات الصيدلانية بشكل اقراص وكبسول.

الكلمات المفتاحية: طريقة طيفية، دوكسيسيكلين هايدروكلوريد،2،4،6-ثلاثي بروموانلين، الآزوتة والاقتران

1 INTRODUCTION

Doxycycline hydrochloride belongs to the tetracycline class, doxycycline is an antibiotic derived from oxytetracycline, which includes compounds containing four fused rings as well as double bonds [1], it inhibits germ growth and has been used since 1967 to treat bacterial infections, malaria, and used to treat the resultant infections. for germs such as Rickettsia, Mycoplasma, and Brucella, It discourages bacterial protein synthesis Because of its rapid absorption and long half-life [2,3].

It also addresses malignant illnesses, including as breast cancer, Doxycycline may help alleviate COVID-19 symptoms, including pulmonary complications, Legionella pneumonia-like pneumonia, and inflammation, Mycoplasma in the lungs [4-6]. Doxycycline can be assayed using several methods in the determination of DOX in a pure form a variety of analytical approaches, including spectroscopy. [7-11].and chromatographic methods [12-16],including electrical means [17-22].The Structurer of Doxycycline is ($C_{22}H_{25}ClN_2O_8$), a molecular weight is 480.90 g. mol^{-1} yellow crystalline powder, slightly soluble in alcohol and water, also dissolved in dilute mineral acids and alkaline hydroxides solutions[23,24], Doxycycline has the following structure in Fig. 1.

$C_{22}H_{25}ClN_2O_8$

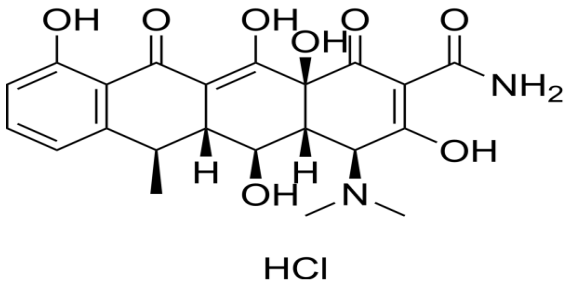


Fig. 1. Structurer of Doxycycline hydrochloride

EXPERIMENTAL

1 Apparatus

Absorbance measurements and absorption spectra were done, using ultraviolet-visible spectroscopy using a device of the Shimadzu UV-Vis 1900 spectrophotometer (Japan) and using glass 1 cm cells, all weighing operations were carried out using a sensitive electronic balance of the AE ADAM type..... In the measurement of weights of chemicals, use a water bath to conduct the heating process of the type (electro. meg).

1.1 Reagents and solutions

All chemicals used were of a high degree of purity.

1.1.1. 2,4,6-Tribromoaniline solution (100 µg/ml)

Prepare the solution by dissolving (0.0100) g of ethanol by adding 20 ml of ethanol, then complete the volume to 100 ml with same solvent in a volumetric flask with a capacity of 100 ml.

1.1.2. Doxycycline solution (100 µg/ml)

This solution was prepared by dissolving 0.0100 g of doxycycline in 100 ml distilled water in a volumetric flask.

1.1.3. Phosphoric acid solution (1 M)

The solution was prepared by diluting (6.1) ml of concentrated phosphoric acid with distilled water up to the mark in a 100 ml volumetric flask.

1.1.4. Sodium hydroxide solution (1 M)

It was prepared by dissolving (4) g of sodium hydroxide with distilled water and complete the volume in a volumetric flask of 100 ml up to the mark.

1.1.5. Urea solution (1%)

Prepare the solution by dissolving (1.0) g of urea with distilled water and then complete the volume in a 100 ml volumetric flask to the mark.

1.1. 6. Sodium nitrite solution (2%)

Prepare the solution by dissolving (2.0) g of sodium nitrite with distilled water and then complete the volume with distilled water in a volumetric flask of 100 ml to the mark.

1.1.7. Pharmaceutical solutions

1-Doxycycline capsule (Doxycycline Capsules 100mg)

dropped the content of eight capsules of (Doxycycline Capsules 100mg) (manufactured by the English pharmaceutical company accord (100mg Dox/capsules), the total weight of the capsules was (1.600)g, After grinding and mixing thoroughly, I took the equivalent of one capsule's content, which includes 1000 mg of doxycycline and dissolved in 5 ml of ethanol, and then filled the container to the mark with distilled water, prepare the sample solution in a 100 ml volumetric flask by diluting the needed volume according to dilution rules, and then add distilled water to the flask.

2. -Doxy-cyclin tablets (Doxy-Denk 100 mg)

Dropped 5 tablets of (Doxy-Denk 100 mg) (manufactured by the German pharmaceutical business Denk Pharma (100mg Dox/tablet), with one tablet weighing roughly 0.2620g and the combined tablets weighing (1.315) g, After grinding and mixing well, take the a equivalent weight of one tablet, which contains 1000 µg of doxycycline was dissolution in 5 mL ethanol and then completes the volume to mark with distilled water, then prepare the sample solution by diluting the required volume using dilution laws and then adding wter that was distilled in a volumetric flask whose volume is 100 ml.

1.2 The method's principle

The essential principle of the proposed method involves two steps, where the first step involves the reaction of 2,4,6 tribromoaniline with an equivalent amount of sodium nitrite in

an acidic medium to form a diazonium salt, second step involves coupling of the formed diazonium salt ortho- anisidine reagent with the reducing doxycycline hydrochloride in alkaline medium to form a colored Azo dye that provides greatest absorption value at 500 nm.

RESULTS AND DISCUSSION

Various experiments have been conducted to study the effect of the reaction components on absorbance and the conditions that give the highest absorbance have been chosen**.**

1. Preliminary Study

The absorption spectrum of the colored product formed by addition of 2 ml of 2,4,6-tri-Bromo-anline reagent was added in a volumetric flask of 10 ml, then 1 ml of the azonized agent sodium nitrite was added to it in an acidic medium by adding 1 ml of Phosphoric acid at a concentration of 1 M. after that, 1 ml of urea was added (to get rid of excess nitrite), then 2 ml of doxycycline solution (100 µg/ml) and after making the medium alkaline by adding 1 ml of sodium hydroxide at of 1 M. it was observed orange gives the highest absorption at 500 nm.

2. Study the optimal conditions

2.1. Study of the type of acid

The effect of various types of strong and weak acids available at a concentration of 1 M was studied, the acid suitable for the diazotization process, which gives the highest absorption, is phosphoric acid, so it was adopted in subsequent studies results obtained in Table 1.

Tab 1. Study of the type of acid

Acid solution used(1M)	HCl	H_2SO_4	**H_3PO_4**	HNO_3	CH_3COOH
Absorbance	0 .423	0 .352	**0 .550**	N.R	0 .010

2.2 Study of the amount of acid used

Increasing amounts have been added of hydrochloric acid and the absorption of solutions versus blank Solutions was measured absorption, and the results obtained in Table (2) show that 1 ml of acid used in previous experiments is the best and was installed in subsequent experiments.

Tab2.Study of the amount of acid used

Vml of H_3PO_4 (1M)	Absorbance/ml of Acid used					
	0.25	0. 5	**1.0**	1.5	2.0	3.0
Absorbance	0.121	0.263	**0.551**	0.511	0.472	0.178

2 .3. Studying of the reagent quantity

This effect was studied by adding different volumes of the reagent 2,4,6-Tri bromo aniline at a concentration of 100 µg/ml in 10 ml volumetric flask and adding the rest of the additives and dilution to the mark and after the completion of the reaction it turned out that 1.5 ml of the reagent is the best volume as shown in table (3), so it was adopted in subsequent studies.

Table 3. Studying of the reagent quantity

Volume of Regent (100 ppm)	0.25	0.5	0.75	1.0	**1.5**	2.0	2.5	3.0
Absorbance	0.071	0.282	0.379	0.423	**0.570**	0.520	0.283	0.240

2.4. Studying of the base type

the effect of different types of bases was studied by adding fixed amounts of 1 ml at (1 M) to each of them individually and it was found that sodium hydroxide gave the highest absorbency as shown in table (4) and thus it was used in subsequent studies.

Table 4. Studying of the base type

Type of base (1M)	KOH	**NaOH**	$NaHCO_3$	Na_2CO_3	NH_4OH
Absorbance	0.226	**0.570**	0.530	0.335	0.171

2.5. Studying of the amount of base

The study was carried out by adding different volumes of sodium hydroxide at a concentration of (1M) to the reaction components, then the absorbency of these solutions was taken against the photo solution and the results showed that the volume of 1.0 ml gives the highest absorbency as shown in Table (5), so it was adopted in subsequent studies.

Table 5. Studying of the amount of base

Volume of base (1M)	250.	0.5	**1.0**	1.5	2.0	2.5	3.0
Absorbance	0.153	0.387	**0.570**	0.462	0.420	0.310	0.280

2.6 Studying the effect of surfactants

This study was carried out using surfactants CTAB, CPC, SDS, Triton X-100, Cetavlon) at 0.1M find out the effect on the color intensity and absorption of the product formed by the interaction of the diazotized tribromoanline reagent with doxycycline hydrochloride in the base medium, the results the inefficiency of surfactants reduce in increasing the absorption formed and improve the sensitivity of the method. therefore, they were not added in subsequent experiments.

2.7 study of the influence of temperature and time on the stability of the pigment formed.

To study the effect of temperature on the stability of the resulting dye, the intensity of absorption of the azo dye formed at different time intervals was tracked and the results showed that the azo dye is formed immediately after the addition of the base and stabilizes after 15

minutes after completion of the additions and dilution and remains stable for 60 minutes at degrees (2 ∓10°C) and this is enough time to make measurements as the results show in the Fig (2).

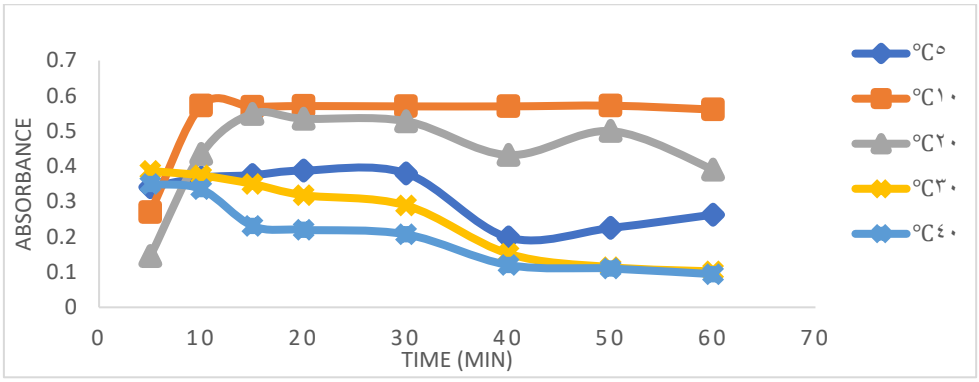


Fig 2. the influence of temperature and time on the stability of the Azo-dye.

3. The final absorption spectrum.

After experimentally installing optimal conditions, a model was prepared in a volumetric flask using 1 ml of doxycycline solution (100 µg/ml) and adding the rest of the components of the proposed method, a yellow-orange dye was formed after waiting 15min , the absorption spectrum of the formed product was plotted with wavelengths from 350 to 800 nm, and it was found that the formed product gives the highest absorption with a wavelength of 500 nm the final absorption of the formed dye versus the blank solution as shown in fig (3).

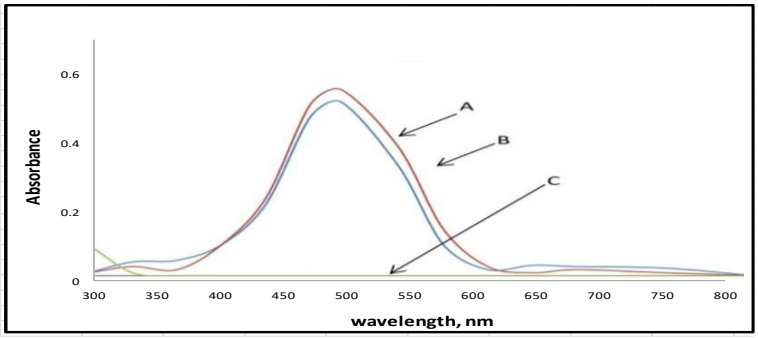


Fig 3. The Absorption spectra of (10 ppm) DOX solution (A)vs blank, B - vs distilled water, C-blank vs distilled water.

4. Procedure and calibration curve

The optimal conditions for estimating doxycycline hydrochloride were applied to draw the standard curve by the working method using a series of 10 ml volumetric flask, where all the necessary additives were added and installed after studying the optimal conditions of the proposed method and completing the volumes to the mark with distilled water and then the absorbency of the resulting colored Solutions was taken after leaving the solutions for 15 minutes versus the blank solution at a wavelength of 500 nm and fig (4) shows that the standard curve indicating that the method follows the beer law for the concentration range of 0.5-24 µg /ml and there is a negative deviation after the upper estimated limits, as indicated by the value of the estimation coefficient (R^2=0.9997) Statistically, the standard curve has excellent linear

characteristics. It was found that the molar absorptivity value of the resulting dye is equal to $2.3x10^4$ l. mol $^{-1}$. cm^{-1} and the value of the Sandel index of sensitivity was 0.0205 µg. cm^{-2}.

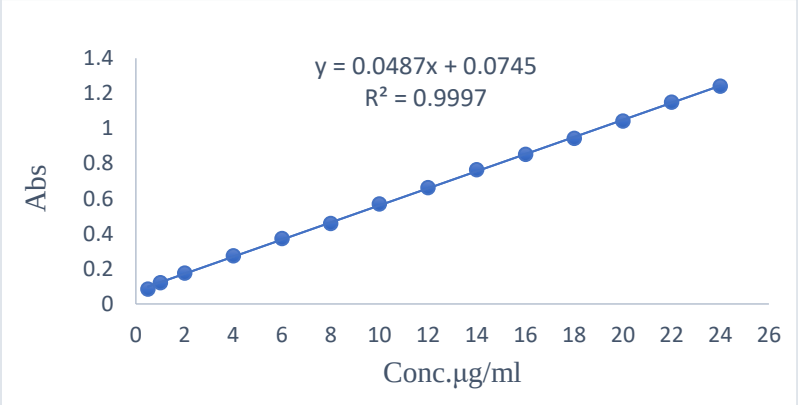


Fig 4. calibration curve for estimating DOX by the proposed method.

5. the linear specification

Table (6) shows the linear specification for the determination of doxycycline hydrochloride with 2,4,6-tribromo-anline.

Beer's law limits (µg/ml)	0.5-24
Limit of detection (LOD) (µg/ml)	0.0418
Limit of quantitation (LOQ) (µg/ml)	0.287
Molar absorptivity ($I.mol^{-1}.cm^{-1}$)	$2.3X10^4$
Sandell's Sensitivity (µg/cm^2)	0.0205
Slope	0.0487
Intercept	0.0745
Correlation Coefficient	0.9997

6. Accuracy and compatibility of the proposed method

The accuracy and compatibility of the method was achieved under optimal conditions for the estimation of doxycycline hydrochloride, the recovery ratio and the relative standard deviation were calculated, where the absorbance of repeats was measured for three different amounts of doxycycline hydrochloride solution and was treated using the approved method and the results were listed in table (7), which indicates that the method has good accuracy and compatibility.

Table 7. Accuracy and compatibility

Amount of Dox (µg/ml) Present	Amount of Dox (µg/ml) found	Recovery %	Average of recovery	R.E %	RSD%
2	2.03	101.5		-1.5	0.78
8	7.83	97.87	100.29	-2.12	0.60
10	10.20	102		2	0.21

6. The nature of the colored azo dye

The method of continuous changes (Job's method) and the method of molar ratios of dilute solutions were applied to find out the ratio of the reaction of doxycycline hydrochloride with the diazotized reagent in a constant amount for all solutions and add the rest of the additives under optimal conditions and complete the volume to 10 ml and then measure the absorption of the product formed against the blank solution at a wavelength of 500. as shown in fig (5), and fig (6), the resulting product is formed in a ratio of 1:1(drug compound : reagent).

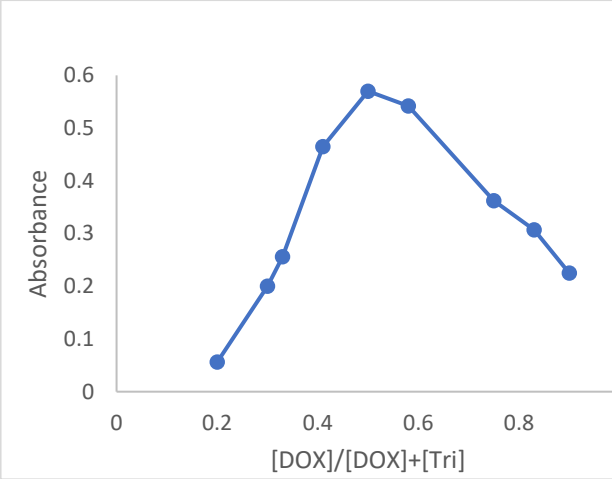


Fig (5) continuous changes (Job's method)

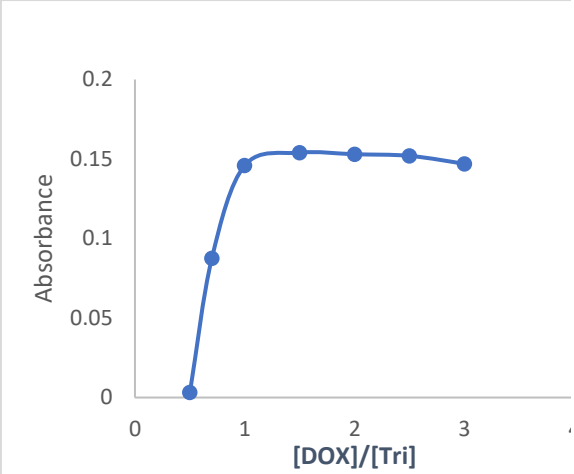
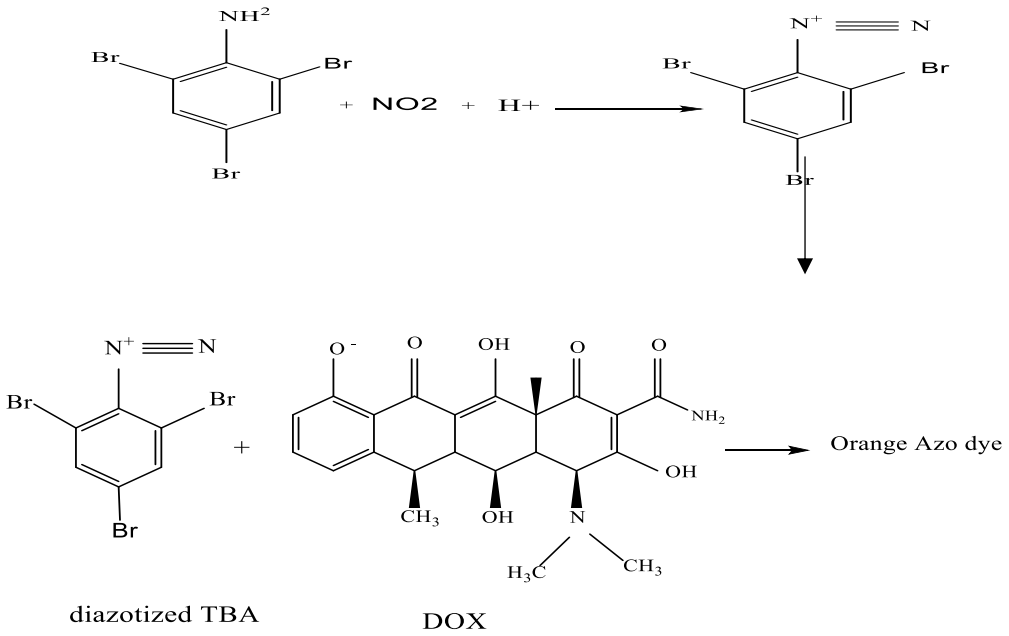


Fig (6) method of molar ratios

7. Proposed chemical formula



Orange-yellow azo dye

Fig (7) Proposed chemical formula

8. Application of the method to a pharmaceutical preparation

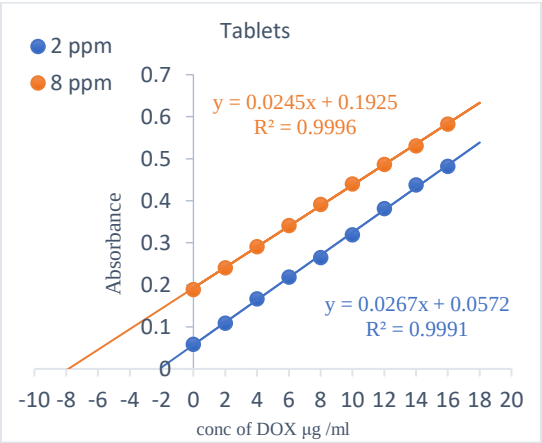
proposed method for determining the amount of doxycycline present in pharmaceutical products such as pharmaceutical tablets and capsules was applied under optimal conditions, and has given the method of action.

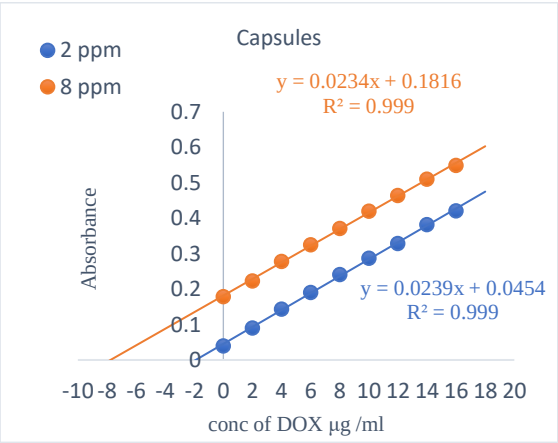
Table 8. Application of the method to a pharmaceutical preparation

Pharmaceutical Preparation	Amount taken (µg/ml)	Amount measured (µg/ml)	Recovery %	Relative error %	RSD%
Doxycycline Capsules 100mg Accord-UK	2	1.89	94.5	-5.5	1.94
	8	8.04	100.5	0.005	0.72
	10	10.07	107	0.7	0.41
Doxy-Denk Tablets 100mg Denk pharma-Germany	2	1.97	98.5	-1.5	1.51
	8	7.79	97.37	-2.62	0.48
	10	9.86	98.6	-1.4	0.455

9. Assessment of the suggested method.

The standard addition method was applied to pharmacological preparations to assess the selectivity of the proposed method for the estimation of doxycycline, the results in scheme (8) and (9) indicate that the results of the standard addition method agree well with the results of the proposed method within an acceptable range of error.





Scheme (8) and (9) Standard addition curve for doxycycline estimation in pharmaceutical preparation.

Pharmaceutical Preparation	DOX Taken (µg/ml)	DOX Measured (µg/ml)	Recovery %
Doxy-Denk Tablets 100mg Denk pharma-Germany	2	1.89	94.5
	8	7.76	97
Doxycycline Capsules 100mg Accord-UK	2	1.97	98.5
	8	7.85	98.13

Conclusion

A simple, fast, sensitive, low-cost spectroscopic method has been developed to estimate doxycycline in its pure form and in its pharmaceutical preparations the method is based on the formation of a yellow - orange Azo dye by coupling doxycycline with the diazotized reagent 6,4,2-tribromoaniline in a basic medium and gave the azo-colored dye the highest absorption at a wavelength of 500 nm, all the information resulting from the study of the optimal conditions for the reaction where the reaction was subject to beer's law within the range of concentrations(0.5-24) micrograms/ml ,the obtained results showed that the method has good accuracy and the method was applied to tablets and capsules and the results were successful.

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