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Determination of Doxycycline as Pure and in Pharmaceutical Preparation (Capsule) Using 4-Aminoantipyrine in Presence of Potassium Periodate

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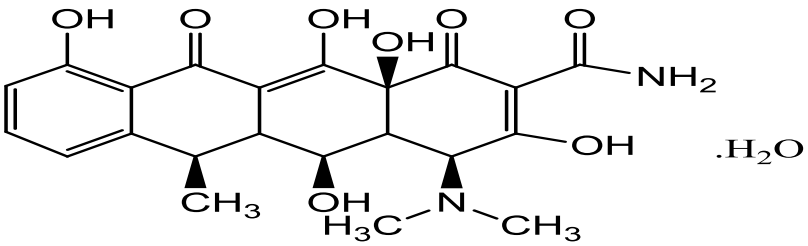
ABSTRACT

A simple spectrophotometric method for the determination of doxycycline in aqueous medium using oxidative coupling reaction has been described. The method was based on the reaction of doxycycline with 4-aminoantipyrene (4-AAP) which gives soluble and stable red colored product that gives the highest absorption at the wavelength 515 nm, and the linearity of Beer's law was in the concentration range from 5 to 110 µg/ml, and the value of the determination coefficient for the standard curve was 0.9995, which statistically indicates that it has excellent linear characteristics. The molar absorptivity was calculated and found to be $1.387x10^3$ $l.mol^{-1}.cm^{-1}$. Sandell's sensitivity value, the Limit of detection (LOD), and limit of quantitation (LOQ) were calculated and equal to 0.3334 $\mu g/cm^2$, 0.114 and 0.38 µg/ml respectively the method was successfully applied for the determination of doxycycline in its pharmaceutical preparation.

Keywords: 4-aminoantipyrene, doxycycline, oxidative-coupling, spectrophotometric method.

INTRODUCTION

Doxycycline is one of the tetracycline-series antibiotics that occupies an important place among the broad-spectrum antibiotics. They overpower the imitation of Gram-negative and positive, and a lot of viruses (Korolkovas and Burckhalter, 1981; Al-Abachi and Al-Nedawi, 2015; Mamani *et al.*, 2006), and is a good-looking handling choice for COVID-19 (Malek *et al*., 2020; Tommy, 2020). Doxycycline monohydrate ($C_{22}H_{24}N_2O_8.H_2O$, M.wt. = 462.5 g/mol) yellow crystalline powder, slightly soluble in alcohol and water, also dissolved in dilute mineral acids and alkaline solutions. Doxycycline has the following structure in scheme 1 (British Pharmacopoeia, 2016).



Scheme (1): The chemical structure of doxycycline.

Various analytical methods have been reported in literature included estimation of Doxycycline in pure and dosage forms, including highperformance liquid chromatography (NP-HPLC and RP-HPLC) (Ghidini *et al*., 2018; Mileva, 2019; Mashru and Koshti, 2021; Dil *et al*., 2020), HPLC-mass (Permana *et al*., 2019), HPTLC (Kumssa *et al*., 2021), potentiometric sensor (Ali *et al*., 2018), ratiometric probe (Tian and Fan, 2021), flow injection spectrophotometry (Tawfeeq and Qassim, 2020), Fluorometric (Sun, 2018) and the spectrophotometry methods using various reagents: Fe(II) with 1,10- phenanthroline (method A) and Fe (II) with 2,2'-bipyridyl (method B) (Awad and Taki, 2021), 4-aminoantipyren in presence of potassium ferriecyanide in alkaline medium. (Al-Kalissy and Mohammed, 2015) diazotized benzocaine (Abbas *et al*., 2020) and UV-spectrometric method at 260 nm (Patil *et al.*, 2020), simultaneous estimation of doxycycline and levofloxacin using wavelengths 273 nm and 287nm in measurements respectively, and iso-absorptive point at 280 nm in phosphate buffer pH 6.8 prepared in Water: Methanol (80:20) (Gholse *et al*., 2022),

Some of the previous methods required heating, and some of them needs expensive apparatus, therefore we developed spectrophotometric method for the estimation of doxycycline as pure and in its dosage form, based on oxidative coupling reaction using 4-aminoantipyrene as a reagent and in the presence of potassium periodate.

EXPERIMENTAL

Apparatus

Spectral measurements and absorbance readings were carried out using a JASCOV-630 spectrometer, and glass cells with a light path of 1 cm were used. The acidity of the solutions was measured using TRANSE BP3001 professional pH meter

Chemicals and prepared solutions

The chemicals used in this research were of high purity and did not require any purification process.

4-aminoantipyrine solution (1.23×10^{-3} M).

This solution was prepared by dissolving 0.0250 g of pure 4-aminoantipyrine in distilled water, then transferred to a 100 ml volumetric flask, and the volume was completed to the mark with distilled water.

Potassium periodate solution (1.5 x 10^{-2}M)

0.3448 g of potassium periodate (Fluka Company) was weighed and dissolved in distilled water, then transferred to a 100 mL volumetric flask and the volume was completed to the mark with distilled water.

Sodium hydroxide solution (4 M)

Sodium hydroxide solution in 4M concentration was prepare by diluting an ampoule of 10 mol/L (100 ml) supplied by Fluka Company with distilled water in a 250 ml volumetric flask, and then kept in a non-glass (plastic) container.

Doxycycline solution (500 µg/ml):

This solution was prepared by dissolving 0.0500 g of pure doxycycline (SDI) in 100 ml of distilled water in a volumetric flask.

Pharmaceutical preparation solutions (500 µg/ml)

5 capsules formulation (Saudi Arabia/ Tabuk), were carefully weighed and mixed well, amount of the powder equivalent to 0.0500 g of pure doxycycline was weighed and dissolved in distilled water then filtered into a volumetric flask of 100 ml and supplemented with distilled water up to the mark.

5 capsules formulation (India / Ajanta pharma limited), were carefully weighed and mixing well, and the same procedure above has been followed.

Procedure and standard curve for the determination of doxycycline

After the optimum conditions for the determination of doxycycline were established, the standard curve was prepared by adding 1.5 ml of 4-aminoantipyrene reagent ($1.25x10^{-3}$ M) to increasing volumes of doxycycline solution at a concentration of 500 µg/ml added firstly to volumetric flasks of 10 ml, then adding 1.25 ml of the oxidizing agent (potassium periodate,$1.5x10^{-2}$M) and finally adding 0.75 mL of sodium hydroxide solution (4 M), then placing the volumetric flasks in the water bath at a temperature of 50 ° C for 15 minutes, leave them for 5 min. after dilution with distilled water to the mark. The absorbance was measured at a wavelength of 515 nm. After drawing the standard curve for the estimation of doxycycline. It was found that the linear range of the concentrations ranged from 5 to 110 µg / mL, and the value of the determination coefficient for the standard curve was 0.9995, which statistically indicates that it has excellent linear specifications Fig. (1). The molar absorbance was calculated and found to be equal to $1.387x10^{3}$ l/mol.cm. Sandell's index value equals to 0.3334 µg/cm^{2}, which indicates the sensitivity of the method. The detection limits and quantitation limits were calculated, and they were 0.114 and 0.38 µg/ml, respectively.

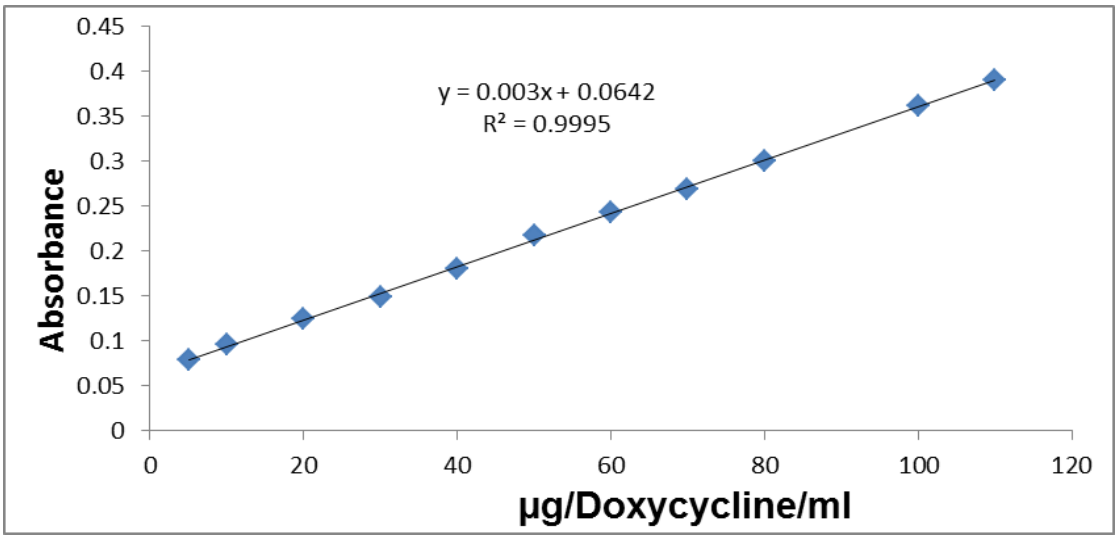


Fig. 1: Standard curve of doxycycline using the proposed method.

RESULTS AND DISCUSSION

All factors affecting absorbance of the formed colored product have been studied:

Preliminary study

The method includes the formation of the colored product via coupling doxycycline with 4-aminoantipyrine in the presence of potassium periodate in alkaline medium (4 M of sodium hydroxide), the reaction needs heating in a water bath at a temperature of 40°C for ten minutes. The colored product gave the highest absorption spectrum at the wavelength of 515 nm, so it was used in the subsequent measurements.

Setting the optimum conditions for the determination of doxycycline:

Subsequent experiments were performed using 1 mL of a 500 µg/mL of pure doxycycline solution in a final volume of 10 mL and the absorbance of the colored product at the wavelength of 515 nm was measured against its blank solution.

Selection of the oxidizing agent

A number of oxidizing agents were tested, such as potassium periodate, sodium periodate, N-bromosuccinimide, and N-chlorosuccinimide, by adding 1 ml of them separately to solutions containing equal concentrations of doxycycline (50 µg/mL) in the presence of fixed amounts of 4-4-aminoantipyrine (1 ml of 1.25 x 10^{-3} M) and a solution of sodium hydroxide 1 mL at a concentration of (4 M) and then heated for 10 minutes at 40° C in a water bath (Table 1).

Table 1: Selection of the oxidizing agent

Oxidizing agent (1.5 x 10^{-2}M)	**Absorbance**	**λ_{max}(nm)**	**Δλ(nm)**
Potassium periodate	0.185	515	125
Sodium periodate	0.021	610	220
N-Bromosucccinimide	0.011	608	218
N-Clorosucccinimide	No color contrast		

The results in (Table 1) indicate that the maximum absorbance was obtained when using potassium periodate, in addition to that the value of the color contrast (Δλ) was good and potassium periodate was recommended to be used in subsequent experiments.

Effect of the amount of potassium periodate solution

The effect of increasing volumes from 0.5 to 2.0 ml of potassium periodae solution (1.5x10^{-2} M) on the absorbance of the colored product from various amount of doxycycline (25-75 µg /mL) was studied, the results shown in (Table 2).

Table 2: Effect of the amount of potassium periodate solution on absorbance.

Oxidizing agent(ml)	**Absorbance /µg Doxycycline/ml**			**R^2**
	25	50	75	
0.5	0.076	0.151	0.199	0.9845
1.0	0.114	0.1754	0.218	0.9892
1.25	0.138	0.203	0.262	0.9992
1.5	0.136	0.201	0.251	0.9960
2.0	0.125	0.188	0.236	0.9928

The results in (Table 2) show that the best volume is 1.25 mL as it gave maximum absorbance and the highest determination coefficient value. Therefore, this volume was used in subsequent experiments.

Effect of the amount of 4-aminoantipyrine reagent solution

Different amounts (1.0 to 2.5 ml) of 4-aminoantipyrine reagent ($(1.25x10^{-3}$ M)) were added to different concentrations of doxycycline ranging from 10 to 100 µg/ml with the addition of 1.25 ml of potassium periodate and heating for 10 minutes in a water bath at 40°C, the results are shown in (Table 3).

Table 3: Effect of the amount of 4-aminoantipyrine (4-AAP) reagent solution on the absorbance of the product

4-AAP (ml)	Absorbance /µg Doxycycline/ml							R^2
	10	20	30	40	50	70	100	
1.0	0.069	0.101	0.134	0.168	0.194	0.233	0.283	0.9725
1.5	0.104	0.124	0.149	0.171	0.198	0.243	0.303	0.9983
2.0	0.094	0.124	0.145	0.170	0.192	0.239	0.294	0.9956
2.5	0.088	0.103	0.128	0.157	0.186	0.226	0.279	0.9909

The results illustrated in (Table 3) show that using 1.5 ml of 4-aminoantipyrine reagent gave the highest absorption and the highest value of the determination factor, so it was confirmed in subsequent experiments.

Base type effect

The effect of different types of bases at a concentration of 4 M and a volume of 1 ml on the absorbance of the formed product was studied by adding 1.5 mL of 4-aminoantipyrine reagent and 1.25 mL of the oxidizing agent KIO_4 to 1 ml of doxycycline solution with a concentration of 500 µg/mL and then heating in the. water bath at 40°C for 10 minutes. The obtained results were illustrated in (Table 4).

Table 4: Selection of the base type

Type of base used (4M)	Absorbance
Without	No color contrast
NaOH	0.200
KOH	0.192
Na_2CO_3	0.181
$NaHCO_3$	0.166

From the results listed in (Table 4), it was found that the reaction needs a strong base medium that is better than the weak, and the base sodium hydroxide gives the highest absorbance, so it has been confirmed in subsequent experiments.

Effect of base quantity

The effect of different volumes of sodium hydroxide on the oxidation and coupling of doxycycline with 4-AAP was studied and the results are shown in (Table 5).

Table 5: Effect of base amount on color product absorbance

Volume of 4M NaOH (ml)	Absorbance	Final pH
0.25	0.168	12.68
0.5	0.187	12.92
0.75	0.205	13.11
1.0	0.199	13.32
1.25	0.191	13.63

It was noted from the results in (Table 5) that the volume of 0.75 mL gave the highest absorbance of the colored product, and therefore it was used in subsequent experiments.

Addition of components sequence effect

In order to choose the best sequence of the reacting components, the sequences shown in (Table 6) were chosen with the addition of the reaction components in the quantities mentioned previously and heating for 10 minutes in a water bath at 40° C.

Table 6: The effect of the sequence of adding reaction components

No.	Reaction components	Absorbance
I*	Dox + 4-AAP + KIO_4 + NaOH	0.207
II	Dox + KIO_4 + 4-AAP + NaOH	0.204
III	KIO_4 + 4-AAP + Dox + NaOH	0.201
IV	NaOH + KIO_4 + Dox + 4-AAP	0.199
V	NaOH + Dox + KIO_4 + 4-AAP	0.199

*Dox = Doxycycline, 4- AAP = 4-Aminoantipyrine, KIO_4 = Potassium periodate, NaOH = Sodium hydroxide

The results of the (Table 6) show that all the addition sequences give close values of absorbance, and the sequence I was chosen to give maximum absorbance.

Effect of temperature

The results listed in (Table 7) show the effect of different temperatures from 25 to 60 °C on the absorbance of the colored product, and it was found that the temperature of 50 °C is optimal for coupling doxycycline with -4-aminoantipyrine in the presence of potassium periodate in the sodium hydroxide medium. Therefore, this degree was fixed in the subsequence experiments.

Table 7: The effect of temperature on absorbance of colored product

Temperature (℃)	25	40	50	60	70
Absorbance	0.121	0.198	0.207	0.195	0.189

Effect of heating time on the formation of the colored product

The time required to complete the coupling process between doxycycline and the reagent 4-aminoantipyrine at 50 °C was studied, and the results in (Table 8) show that 15 minutes gave the highest absorbance of the colored product, and therefore its use was proven in subsequent experiments.

Table 8: Effect of heating time on the formation of the colored product

Time (min.) before dilution	5	10	15	20	25	30	35
Absorbance	0.171	0.197	0.214	0.211	0.201	0.193	0.180

Stability time of the colored product

The effect of time on the stability of the colored product was studied by taking two different concentrations 25 and 50 µg/L and proceed according to the procedure mentioned before, the absorbance was read every 5 or 10 minutes for 60 minutes (Table 9).

Table 9: Study the effect of time on the stability of the formed colored product

µg of Dox.	Absorbance / minute standing time							
	0	5	10	20	30	40	50	60
25	0.101	0.113	0.122	0.122	0.121	0.120	0.119	0.115
50	0.168	0.208	0.214	0.214	0.215	0.214	0.213	0.202

From the results cited in Table 9, the colored product has good stability at least for a period of 55 minutes.

Final absorption spectrum

After creating the optimal conditions shown in (Table 10), the absorption spectrum of the colored product was plotted, which consisted of the reaction of 50 µg/mL of Dox with 1.5 mL of 4-AAP, and according to the optimal conditions, a colored product gave the highest absorption at the wavelength 515 nm whereas the blank has lowest absorbance at this wavelength and the wavelength 515 nm was recommended in the subsequent measurements Fig. (2).

Table 10: The optimal conditions for the proposed method

Variable	Optimality
Reagent 4-aminoantipyrine (ml, M)	1.5, 1.25 x 10^{-3}
Oxidant (ml, M)	Potassium periodate (1.25, 1.5 x 10^{-2})
NaOH (ml, M)	0.75, 4
Temperature (°C)	50
Time of heating, minute	15
Stability (minute)	55

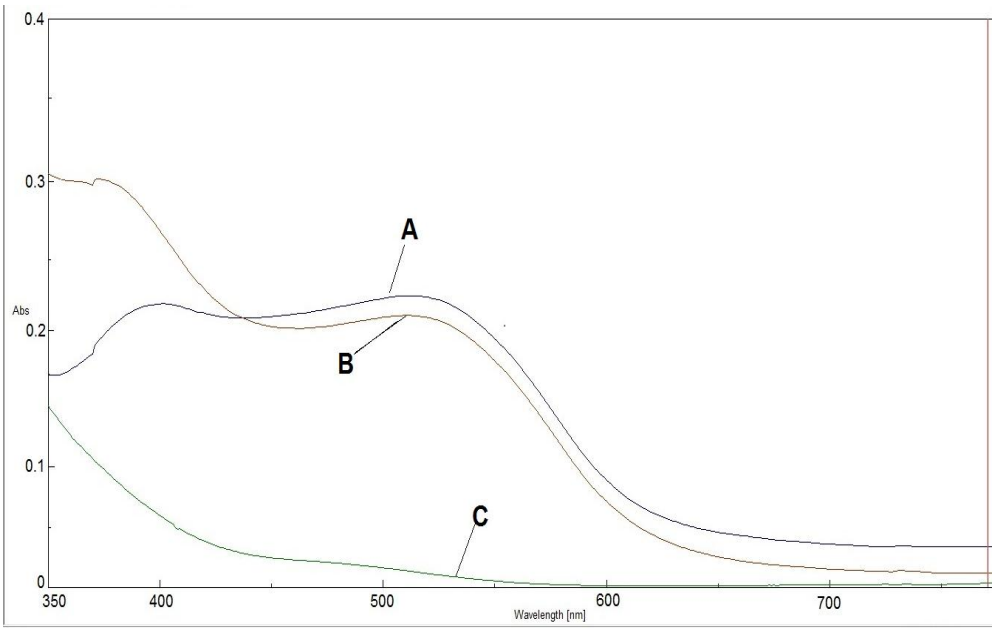


Fig. 2: Absorption spectra A- The colored product of 50 µg/ml Doxycycline treated according to the recommended procedure versus distilled water. B- The colored product versus the blank solution C – blank solution versus distilled water.

Accuracy and precision

Under the optimum conditions shown in the method of work mentioned in (Table 10), the recovery percentage, which expresses accuracy and the relative standard deviation (RSD%), which expresses the precision for two different concentrations of doxycycline 30 and 50 µg / ml were calculated. The results in (Table 11), which indicate that the method has good accuracy and precision.

Table 11: Accuracy and precision of the method

Concentration of Doxycycline (µg/ml)		Recovery* %	RSD%
Present	Found		
30	29.71	99.06	1.55
50	49.72	99.44	1.44

*Average for five determinations.

The nature of the formed colored product

The two methods of continuous variation and mole ratios (Job, 1971) were applied to study the molar structural ratio of the colored product formed between Dox and 4-AAP at a concentration of 1.08×10^{-3} mol/l for each solution, that the final volume of flasks is equal for all solution Fig. (3).

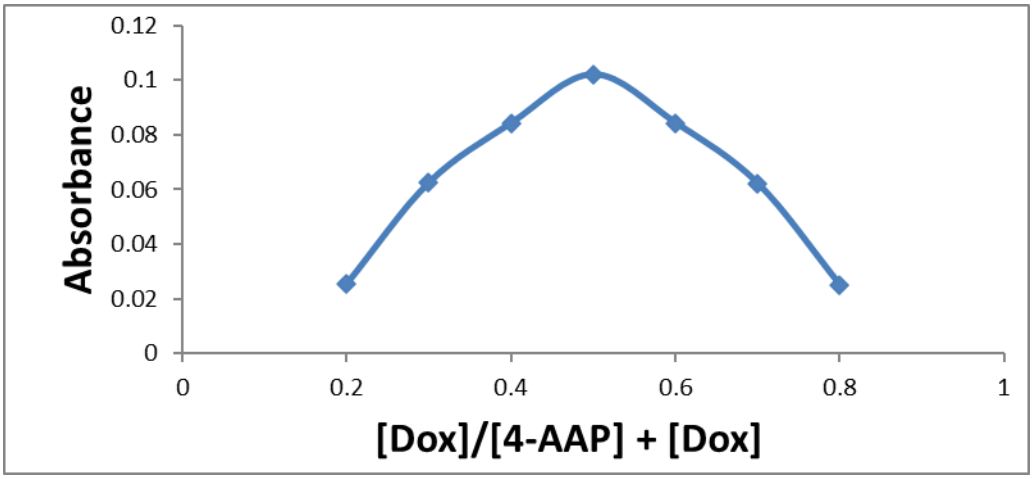


Fig. 3: The plot of continuous variation method for colored product.

From Fig. (3), we infer that the reaction ratio is 1:1, and to verify that this ratio is correct, the method of mole ratio was applied. Fig. (4) shows the mole ratio plot obtained from adding increasing volumes from 0.3 to 4.0 ml of 4-AAP solution at a concentration of 1.08×10^{-3} mol./l to a constant volume of 1 ml of 1.08×10^{-3} mol./l of Dox solution.

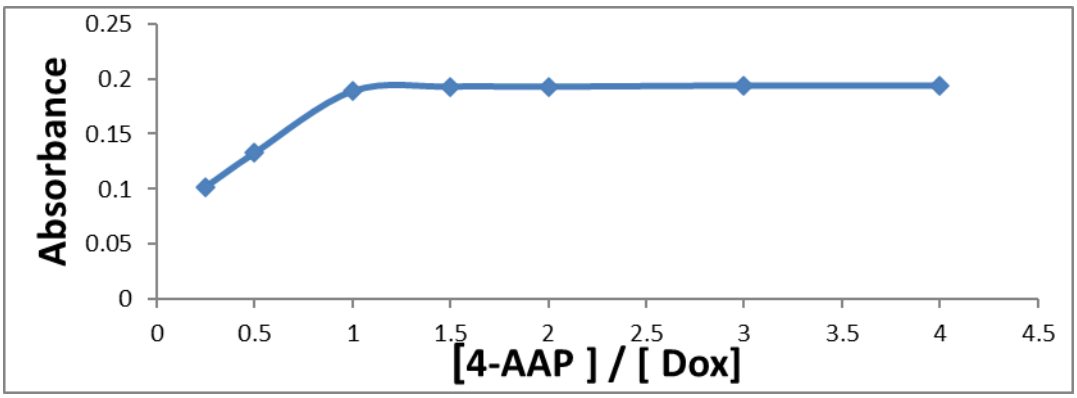
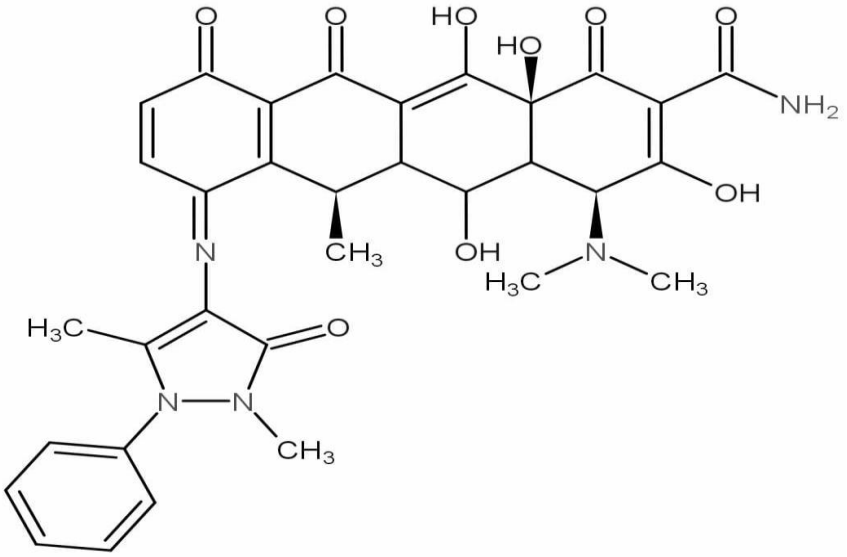


Fig. 4: The plot of the mole ratio method for the colored product.

Based on the results of figures 3 and 4, the proposed chemical structure for the formed colored product was suggested (Scheme 2).



Scheme 2. The proposed chemical structure of the colored product

Application of the method

The suggested method was applied in estimation of Dox in its pharmaceutical preparation's doxycycline capsule. The method was applied by taking different volumes of the stock solution of 500 µg/ml of the pharmaceutical preparation (capsule) to obtain concentrations of 10,30 and 50 µg/mL of pure Dox and treated according to the procedure for pure solution of doxycycline. The obtained results are summarized in (Table 12).

Table 12: Doxycycline capsule analysis according to suggested method

Pharmaceutical preparation	Certified Value (mg)	Amount Present (µg/ml)	Recovery* %	Average %	Drug content found (mg)
Doxycycline capsule / Cyprus / Limassol	100 mg/ capsule	10	99.28	99.75	99.28
		30	100.10		100.10
		50	99.88		99.88
Doxycycline capsule / Saudi Arabia / Tabuk	100 mg/ capsule	10	99.83	100.07	99.83
		30	99.96		99.96
		50	100.42		100.42

*Average for five determinations.

It can be concluded from the results shown in (Table 12) that the recovery% for the analysis of the doxycycline capsule for Tabuk company was 100.07% and for Limassol was 99.75%, which indicates that the method has good efficiency and accuracy in estimation.

Standard addition method

In order to prove that the proposed method is credible and successful in the quantitative determination of doxycycline in pharmaceutical preparations and that it is free from the interferences of standard additives that used in preparation drugs, the standard addition method was applied on concentrations of 10 and 20 µg/mL Dox for the two pharmaceutical preparations, as shown in Fig. (5, 6) and (Table 13).

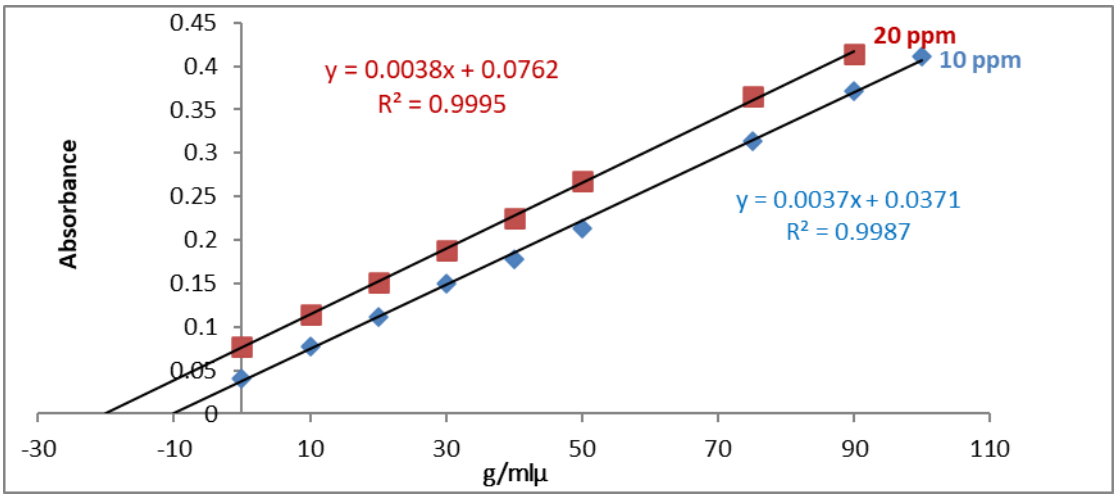


Fig. 5: Standard addition curve for Doxycycline capsule, Limassol Company.

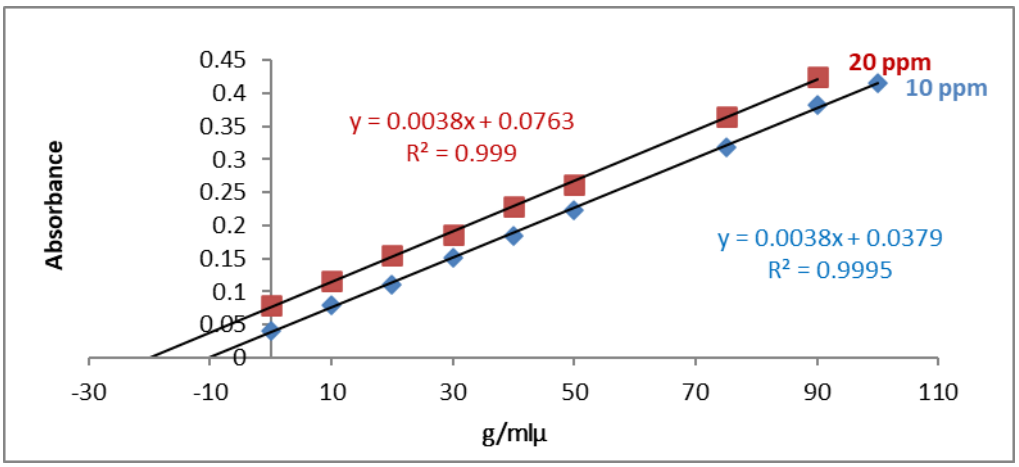


Fig. 6: Standard addition curve for Doxycycline capsule, Tabuk Company

Table 13: Results of the standard addition method for determination of doxycycline

Drug	**Certified Value (mg)**	Amount taken (µg/ml)	Amount measured (µg/ml)	Recovery %	Drug content
Doxycycline capsule / Cyprus / Limassol	100 mg/ capsule	10	10.02	100.20	100.20
		20	20.05	100.25	100.25
Doxycycline capsule / Saudi arabia / Tabuk	100 mg/ capsule	10	9.97	99.70	99.70
		20	20.07	100.35	100.35

From the results shown in (Table 13), we conclude that the developed method has proven its success and credibility in estimating doxycycline in two various types of capsules dosages.

Comparison with other method

A comparison has been made for the most important analytical variables of the current proposed method with its counterparts in other spectroscopic method (Table 14).

Table 14: Comparing some of the important analytical variables of the method with another method

Parameter	**Suggested method**	**Literature Method** (Mahmoud and Abdurahman, 2017)
Type of reaction	Oxidative coupling	Oxidative coupling
Reagent used	4-aminoantipyrine	hydrazine di-hydrochloride
Wavelength (nm)	515	420
Temperature, (℃)	50	Room temperature
RSD%	1.49%	2.27%
LOD(µg/ml)	0.114	0.0631
LOQ(µg/ml)	0.38	0.1912
Beer, slaw µg/ml	5 - 110	3 – 72
ε, l/mol.cm.	$1.387x10^3$	3.0562×10^3

CONCLUSION

A spectrophotometric method was developed for the determination of doxycycline via oxidative coupling reaction. The method was successfully applied in estimating Dox in the pharmaceutical preparation (capsule) from different originators.

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تفاعل الاقتران التأكسدي في تقدير الدوكسي سايكلين كمادة نقية وفي المستحضر الصيدلاني (كبسول) باستخدام 4–أمينو أنتي بايرين بوجود بيريودات البوتاسيوم

الملخص

تم وصف طريقة طيفية بسيطة لتقدير الدوكسي سايكلين في الوسط المائي باستخدام تفاعل الاقتران التأكسدي. تعتمد الطريقة على تفاعل الدوكسي سايكلين مع الكاشف 4–أمينوأنتي بايرين (4-AAP) في وسط قاعدي وباستخدام عامل مؤكسد بيريودات البوتاسيوم، والذي يعطي ناتج أحمر اللون مستقرًا يمتلك أعلى امتصاص عند الطول الموجي515 نانومتر، وكانت حدود قانون بير في نطاق التركيز من 5 إلى 110 مايكروغرام/ مل، وكانت قيمة معامل التقدير للمنحنى القياسي 0.9995، مما يشير إحصائيًا إلى أن له خصائص خطية ممتازة. تم حساب الامتصاصية المولية ووجدت أنها تساوي 1.3 × 10^3 لتر مول$^{-1}$ سم$^{-1}$. تبلغ قيمة حساسية Sandell 0.3333 مايكروغرام/ سم^2، وكانت قيمتي حد الكشف (LOD) وحد التقدير الكمي (LOQ) 0.114 و0.380 مايكروغرام/ مل على التوالي تم تطبيق الطريقة بنجاح لتقدير الدوكسيسيكلين في مستحضره الصيدلاني.

الكلمات الدالة: دوكسي سايكلين، 4–أمينو أنتي بايرين، الاقتران التأكسدي، طريقة طيفية.