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Diazo-coupling reaction in spectrophotometric Determination of doxycycline in pure and its dosage forms

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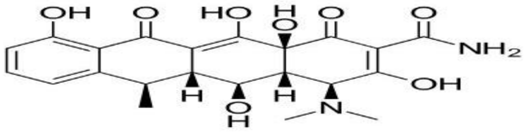
Abstract

A simple and accrued spectrophotometric method was suggested for the determination of doxycycline in pure form and in capsule dosage. The method relied on the formation of an azo dye by coupling of doxycycline with the diazotized reagent 1-amino-4-nitronaphthalein in an alkaline medium using sodium hydroxide as a base and the colored azo dye gave the highest absorbace at the wavelength 466 nm. All parameters affected by the intensity of colored azo dye have been studded and the optimal conditions have been selected. The linearity was from 0.5 to 20 µg/ ml and above the higher concentration of calibration curve gave deviation from Beer's law. The molar absorptivity, Sandell's sensitivity, Limit detection (LOD), and limit quantitation were 7.778 x10^3 l/mol.cm, 0.0595 µg/cm^2, 0.1216 µg/ml, and 0.4046 µg/ml respectively. The method was successfully applied in estimating doxycycline in its dosage form (capsule) from different manufacturers.

Keywords: spectrophotometric;1-amino-4-nitroanaphthalene; doxycycline

1. Introduction

Doxycycline is one of the tetracycline-series antibiotics is occupy an important place among the broad-spectrum antibiotics. They overpower the imitation of Gram-negative and positive, and a lot number of viruses [1-2], and is a good-looking handling choice for COVID-19[4,5]. Doxycycline ($C_{22}H_{24}N_2O_8$, M.Wt = 444.44 g / mol.) yellow crystalline powder, slightly soluble in alcohol and water, also dissolved in dilute mineral acids and alkaline hydroxides solutions. Doxycycline has the following structure in Scheme 1 [6].



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 high-performance liquid chromatography (NP-HPLC and RP-HPLC) [7-12],HPLC-mass[13,14],HPTLC[15], potentiometric sensor [16], ratiometric probe [17], flow injection spectrophotometry[18], Fluorometric [19], spectrophotometric methods using various reagents: Fe(II) with 1,10- phenanthroline in method A and Fe(II) with 2,2'-bipyridyl method B [20] 4-aminoantipyren in presence of potassium ferriecyanide in an alkaline medium. [21] simultaneous estimation of Doxycycline and Levofloxacin at 273 nm and 287 nm respectively, iso-absorptive point at 280 nm in phosphate buffer pH 6.8 [22], UV-spectrometric method at 260 nm [23], 4-aminoantipyrine [24], diazotized benzocaine [25], and Kinetic study of removal doxycycline drug by aluminum oxide surface [26].

The aim of the suggested work is to provide an accurate and precise spectrophotometric method to assay doxycycline in its dosage forms.

2.Experimental

2.1.Apparatus

Spectral measurements and absorbance readings were carried out using a JASCOV-630 spectrometer. Glass cells with a light path of 1 cm were used. The pH of the solutions was measured using a Professional Benchtop pH meter BP3001 (Singapore ;Trans instruments) and using a sensitive balance type Keren ABS-N (Germany ; Keren&Sohn) to perform weighing operations.

2.2.Chemicals used and prepared solutions

All chemicals used were of a high degree of purity.

2.2.1.*Diazotized 1-amino-4-nitronaphthalen nitrated solution(D-1-ANN, 1x10^{-3}M)*

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D-1-ANN solution was prepared by dissolving 0.0188 g of ANN reagent in 5 ml of ethanol and adding 5 ml of concentrated hydrochloric acid (11.8 M) and 80 ml distilled water then cooling with ice bath to 0-5°C and adding 0.0069 g of sodium nitrite (dissolved in cold 5 ml of distilled water). Finally, the volume was completed to the mark of the100-ml volumetric flask with cold distilled water.

2.2.2. Sodium hydroxide solution (1 M)

This solution was prepared by diluting a concentrated solution of ampule (100 ml of 10 M) supplied by Fluke company into a 1-liter volumetric flask with distilled water and stored in a plastic container.

2.2.3 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.

3. Procedure and calibration curve

After the optimum conditions for the determination of doxycycline were established, the standard curve was prepared by adding 1.5 mL of D-1-ANN reagent to an increased volume of doxycycline solution(µg / ml) in a series of 10 ml volumetric flasks and finally add 1.5 ml of sodium hydroxide solution (1M). Then, after dilution with distilled water to mark the absorbance was measured at the wavelength 466 nm. After plotting the standard curve for the determination of doxycycline(Figure 1). The linear range of concentrations is from 0.5 to 20 µg/ml. The value of the determination coefficient for the standard curve was 0.9997, and the molar absorptivity was calculated and found to be 7.786 x 10^3 l./mol.cm. Sandell's sensitivity is 0.0595 µg/cm^2, The low detection limit(LOD) and low quantitate (LOQ) were calculated as equal to 0.2969 µg/ml 0.9880 µg/ml respectively.

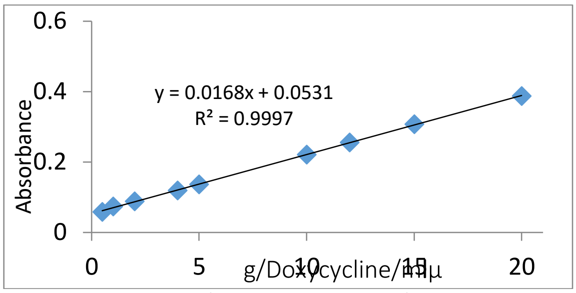


Fig. 1. Standard curve for the determination of doxycycline using the proposed method.

4. 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.

4.1. Preliminary Study

The absorption spectrum of the colored product formed as a result of the coupling of D1-AAN (1 ml of 1x`10^-3 M)with doxycycline (1ml ,100 µg/ml) in presence of 1.25 ml sodium hydroxide (1 M), then completed the volume with distilled water to the mark of 10-ml volumetric flask. The solution left for 5 minutes at room temperature. The spectrum of the resulting yellowish-orange-colored solution against the blank shows the maximum absorption at wavelength 466 nm, which was recommended in the subsequent experiments.

From the results in Table 1, it is clear that 1.5 ml of D-1-ANN reagent gave the highest absorbance and the highest value of determination coefficient, therefore 1.5 ml was confirmed in subsequent experiments.

4.2. Study the type of base

The effect of different types of bases on the absorbance of the formed azo dye was studied by adding 1 ml of various bases (1M) to the components of the reaction and the results obtained in Table 2.

Table 1. Effect of the amount of 1-DANN on the absorbance of azo dye

1-DANN agent (ml of 1x10^{-3}M)	Absorbance/µg Doxycycline/ml					R^2
	5	7	10	12	15	
0.5	0.045	0.078	0.096	0.152	0.187	0.9644
1.0	0.078	0.111	0.137	0.211	0.255	0.9601
1.5	0.129	0.157	0.208	0.256	0.300	0.9970
2.0	0.095	0.116	0.151	0.224	0.262	0.9580
2.5	0.048	0.077	0.112	0.131	0.144	0.9690

Table 2. The effect of various types of base on absorbance of the formed azo dye.

Type of Base used (1M)	NaOH	KOH	Na_2CO_3	$NaHCO_3$
Absorbance	0.202	0.180	0.154	0.135
pH	12.6	12.4	9.87	8.2

The results in Table 2 showed that the reaction takes place in an alkaline medium and sodium hydroxide is the better base used, it gives the highest absorbance of colored azo dye.

4.3. The optimal amount of sodium hydroxide

The optimal amount of sodium hydroxide solution at a concentration of 1 M was studied by adding various volumes from 0.5 to2.0 ml in presence of 1.5ml 1-DANN reagent and 1 ml of doxycycline and the results are listed in Table (3).

Table 3. The optimal amount of sodium hydroxide.

NaOH (1M) , ml	0.5	1.0	1.25	1.5	2.0
Absorbance	0.046	0.138	0.205	0.225	0.179

The results in the above Table show that 1.5 ml of sodium hydroxide gave the highest absorbance and therefore it was used in subsequent experiments.

4.4. Effect of surfactants

The addition of various types of surfactants positive(CPC), negative(SDS), and neutral(Triton-x-100) to the medium of reaction has been studied to increase sensitivity or to give a redshift. The results show that none of the surfactants used in this study gave an improvement in absorbance or spectrum of a formed azo dye, so it was not recommended for their use in the next experiments.

4.5. Solvent effect on the spectrum of formed azo dye

The effect of solvents of different polarities on the absorption spectrum of the formed azo dye under the previously described optimal conditions has been studied. The results cited in Figure (2) and Table (4), included an increase in the absorbance of formed azo dye via using methanol as the solvent of dilution, but because of the economic and advantages of water compared with methanol, so the water was recommended as a medium of dilution.

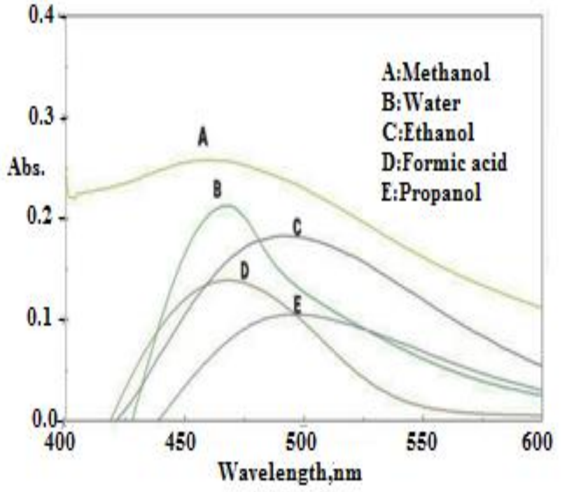


Fig. 2. Spectra of azo dye using different solvents in dilution.

Table 4. Solvent effect on absorbance and molar absorptivity.

Solvent	λmax, nm	Absorbance	$\varepsilon, L/mol.cm$
Ethanol	491	0.182	**8.4×10^3**
Methanol	461	0.257	**1.18×10^4**
Acetone	525	0.054	**1.1×10^3**
Water	466	0.215	**9.9×10^3**
Formic acid	467	0.138	**6.3×10^3**
Propanol	496	0.105	**4.7×10^3**

4.6.The effect of the time on the stability of azo dye

The effect of time on the stability of the formed azo dye was studied by taking two different concentrations 5 and 10 µg/ml of doxycycline and the absorbance was read every of 5 or 10 minutes for a period of 60 minutes. The results are fixed in Table (5).

Table 5. The effect of time on the stability of azo dye.

Absorbance / minute standing time	µg of Doxycycline	
	5	10
Immediately	0.122	0.208
5	0.125	0.212
10	0.129	0.215
15	0.130	0.215
20	0.130	0.216
25	0.131	0.216
30	0.131	0.218
40	0.132	0.218
50	0.131	0.218
60	0.127	0.207

From the results in Table (5), it is clear that the formed azo dye was characterized by high stability for at least 60 minutes

5. Final absorption spectrum

After forming the optimum conditions shown in Table (6), the absorption spectrum of the azo dye was taken, which consisted of the reaction of 10 µg/ml of doxycycline with 1.5 ml of the D-1-ANN, and according to the optimal conditions, an azo-colored dye that gave maximum absorption at wavelength 466 nm against to the blank solution, the blank gives very little absorbance at the maximum measurement wavelength, and 466 nm was fixed in the subsequent experiments. Figure (3).

Table 6. Optimal conditions for the proposed method.

Variable	Optimality
Reagent 1-amino-4-nitronaphthalene	1.5 ml , 1×10^{-3}M
NaOH	1.5ml , 1M
Stability (minute)	60

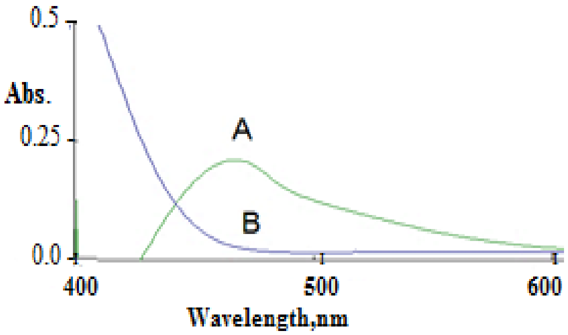


Fig. 3. Absorption spectra A-stained product of doxycycline (10 µg/ml) with 1-amino-4-nitronaphthalein vs. blank solution B - blank solution vs distilled water

6.The nature of the colored azo dye

The continuous variation and molar ratio methods [27] were applied to study the molar structural ratio of azo dye formed from coupling doxycycline [Doxcy] with D-1-amino-4-nitronaphthalene [D-1-ANN]. Figure (4) the continuous variation curve for solutions prepared from the components of the formula of azo dye in different proportions, provided that the final sum is equal, the concentration is for each solution is equal to 2.25 x 10^{-5} M

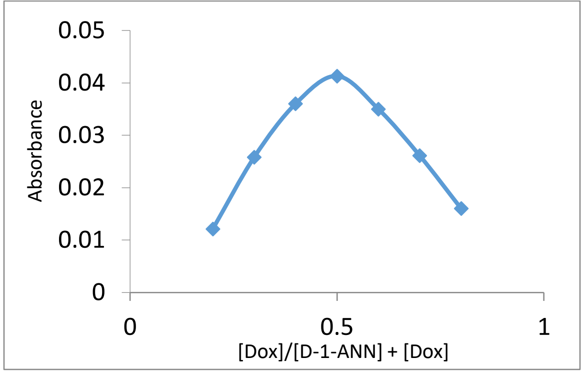


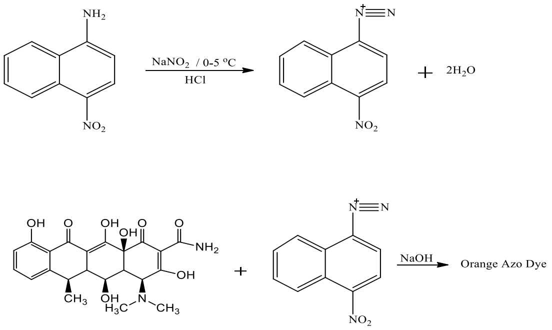
Fig. 4. The plot of the continuous variation method.

Figure (4) proves that the reaction ratio is 1:1, and to verify this ratio, the method of molar ratio was applied. Figure (5) shows the molar ratio curve obtained by adding increasing volumes from 0.25 to 4.0 ml of D-1-ANN solution of 2.25 x 10^{-5} M to a fixed volume of 1 ml of 2.25 x 10^{-5} M of Doxycycline solution**.**

The results in Figures (4 and 5) indicate that the resulting azo dye with a molar ratio of 1:1, the structural formula of the colored azo dye results is proposed (Scheme 2).

7. Effect of additives in pharmaceutical manufacturing

In order to prove the selectivity of the method with the aim of applying it in routine analyzes on different samples, especially pharmaceutical preparations, an increase of additives was added individually to 10 µg / ml of doxycycline and following the optimal conditions for measurement. The results were included in Table 7..



Scheme 2. Chemical structure of the colored azo dye**.**

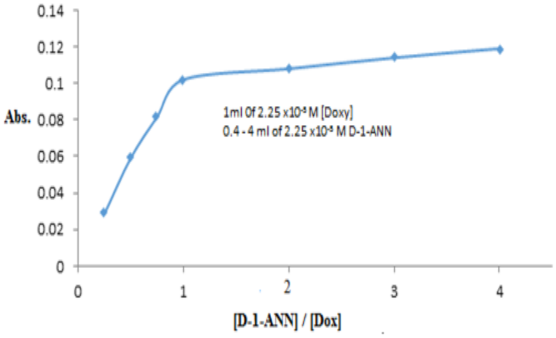


Fig. 5. The plot of the molar ratio method

Table 7. Effect of some excipients on recovery of 10 µg/ml of doxycycline.

Foreign compound	Recovery% of 10 µg Doxycycline in / ml per µg foreign compound added 100	500	1000
Glucose	99.01	97.62	95.55
Starch	98.20	97.92	98.99
Arabic gum	96.86	94.40	98. 59
Sucrose	99.80	99.17	98.82

The results in Table 7 indicated that there is no interferences of glucose, starch, Arabic gum and sucrose in determination of doxycycline via suggested method

8. Application of the method to a pharmaceutical preparation

The method was applied by taking different volumes of the standard solution(100 µg / ml) of the dosage form(capsules) to obtain concentrations of 3 and 10 µg / ml and treated according to the method described before. The obtained results are in Table (8).

It can be concluded from the results in Table (8) that the recovery percentage for the analysis of the doxycycline capsule for Tabuk company was 99.17% and for Ajanta was 98.3%, which indicates that the method has good efficiency and accuracy in estimation of doxycycline capsule form.

9.Standard addition method

In order to prove the success of the proposed method in estimating of doxycycline in its pharmaceutical formulation and its free from additive interference, the standard addition method was applied to estimate doxycycline in capsule dosage form, the results are shown in the following Figures (6) and (7) and Table9.

From the results shown in Table (9), we conclude that the proposed method has proven its success and credibility in estimating doxycycline in capsules form.

10. Comparison with other methods

A comparison was made for the most important analytical variables of the currently proposed method with its counterparts in other spectroscopy methods (Table 10).

Table 8. Doxycycline capsule analysis via proposed method.

Pharmaceutical preparation	d V a l e s		ıc co ver * y	RS D*	% R E	% en t fo ⋮	t_{exp}
Doxycycline capsule / Saudi Arabia / Tabuk	100 mg/ capsule	3	98.2	3.7	-1.8	98.2	1.04
		10	100.14	0.74	0.14	100.14	0.41
Doxycycline capsule / India / Ajanta pharma limited	100 mg/ capsule	3	99.6	4.2	-0.4	99.5	1.63
		10	97.0	0.72	-3.0	97.0	1.3

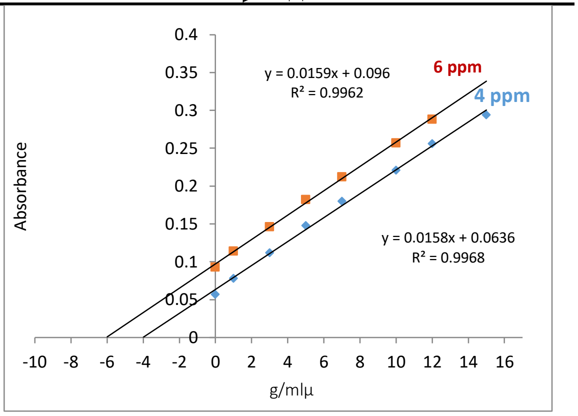


Fig. 6. Standard addition curve for Doxycycline capsule, Tabuk Company.

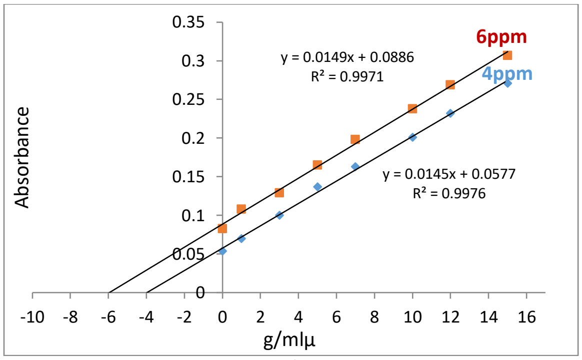


Fig.7. Standard addition curve for Doxycycline capsule, Ajanta

Table 9. The results of the standard addition method.

Drug	Amount taken (µg/ml)	Amount measured (µg/ml)	Recovery (%)	Drug content
Doxycycline capsule Saudi Arabia / Tabuk	4	4.02	100.5	100.5
	6	6.03	100.5	100.5
Doxycycline capsule India / Ajanta pharma limited	4	3.97	99.25	99.25
	6	5.94	99.0	99.0

Table 10. Comparing some of the important analytical variables of the method with other methods.

Parameter	Suggested method	Literature method [20]	Literature method [28]	Literature method [29]
Type of reaction	Diazo-coupling	Redox	Oxidative coupling	**Diazo-coupling**
Reagent used	1-amino-4-nitronaphthalene	1,10-phenanthroline	Hydrazine dihydrochloride	Benzocaine
Wavelength(nm)	466	510	420	480
Temperature (℃)	Room temperature	Room temperature	Room temperature	Room temperature
Medium of reaction	Alkaline	Acidic	Alkaline	Alkaline
Beers law (µg/ml)	0.5 - 20	0.1– 9.0	3 – 72	16 - 34
ε, l/mol.cm.	7.7 X 10^3	8.25x 10^4	3.0562 x 10^3	2.214 x 10^4

From the results shown in Table (10) we conclude that the present method is not more less sensitive than the other methods in comparison.

11. Conclusion

A simple spectrophotometric method was suggested for the assay of doxycycline in pharmaceutical formulation(capsules). By using a diazo-coupling reaction the colored azo dye gave the highest absorption at the wavelength of 466 nm. The method can be applied in estimating doxycycline in its dosage form (capsule) from different manufacturers with satisfactory results**.**

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