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Spectrophotometric Determination of Doxycycline Via Oxidation Reduction Reactions

Firas Hassan Awad*[a], Anmar Ghanim Taki [b]

[a] *Dept. of Dental Industry Techniques, Al-Qalam University College, Kirkuk, Iraq*

[b]*Dept. Of Radiology & Sonar, Al-Noor University College, Mosul, Iraq*

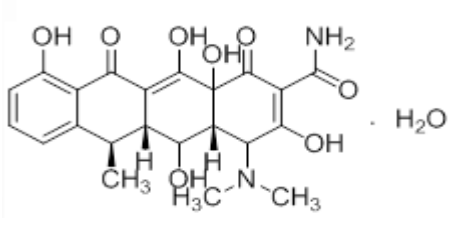
Abstract

Two sensitive methods have been proposed for spectrophotometric determination pure form of Doxycycline (Dox) and pharmaceutical preparation as capsule. The procedures based on the oxidation of the Doxycycline in acidic media by Fe(III). The results Fe(II) reacted with 1,10- phenanthroline in Method A, and the ferroin complex can measure at 510 nm versus the blank of reagent. While method B depends on the reaction of 2,2'-bipyridyl with the resulted Fe(II) to form a stable coloured complex with maximum 522 nm absorption against reagent blank. Beer's law is applicable in the range of 0.1-9.00 µg/ml and 0.15-4.50 µg/ml concentration with molar absorptivity values of 8.25×10^4 and 7.53×10^4 $l.mol^{-1}.cm^{-1}$ for method A and B, respectively. For both processes, the mean percentage of recoveries is 99.9 % and 98.5% respectively. The suggested methods are free from interferences from common excipients. The proposed methods compared favorably with the official and other spectrophotometric methods.

***Keywords*:** Doxycycline; 1,10-Phenanthroline; 2,2'-Bipyridyl; Pharmaceutical analysis; Spectrophotometric

1. Introduction

Doxycycline monohydrate (I) a broad-spectrum antibiotic from a second-generation semi-synthetic tetracycline, which is active versus a variety of microorganisms: include gram-positive, gram-negative bacteria, mycoplasmas, chlamydia, protozoan parasites and rickettsia. These resemble low-cost antibiotics, which are used widely in the treatment of human and animal infections and even at sub-therapeutic levels in animal feed as a growth promoter [1, 2]. Because it is cheap and widely available, Doxycycline has a safe tolerance profile and is an attractive treatment option for COVID-19, potentially alleviating the pulmonary sequela and atypical bacterial pneumonia-like Legionella pneumophilia and Mycoplasma pneumonia [3, 4].



(I) $C_{22}H_{26}N_2O_9$ M.w = 462.45g/mol

Several analytical techniques have been reported for the determination of doxycycline in its pharmaceutical formulations including spectrophotometric methods [5-8], HPLC methods[9-11], Flow injection analysis (FIA)[12], TLC methods[13], Voltammetry[14], electrochemistry methods[15], Kinetic methods [16] and fluorometric methods[17]. Liquid chromatographic methods are the choice of Some Pharmacopoeias for determination of DOX [18, 19].

However, some of these methods have one or other weaknesses, for example, low sensitivity, the use of non-aqueous media, the need for heating, solvent extraction or the use of expensive equipment that require time-consuming, complicated sample preparation and operationally trained staff. Spectrophotometry, on the other hand, always have the advantages of simple instrumental, fast, low cost, ease of transportation, and requires less training for operations.

This paper describes two assay methods for Dox in pure and pharmaceutical formulations. They depend on the Dox oxidation in acidic media associated with ferric salt resulting ferrous ion that makes a complex with a 1,10-phenanthroline reagent in the first method, and with a 2,2'-bipyridyl reagent in the second method.

*Corresponding author e-mail: firas.mlt@alqalam.edu.iq.; (Firas H. Awad).

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2. Experimental

2.1. Apparatus

The measurements of all absorption made on OPTIMA Sp. 300 single beam Spectrophotometer and Phoenix range of UV-210A double-beam spectrophotometer, with cells matching 1.0 cm are used in this work. Philips PW 9420 pH-meter is used for pH measurements; JRAD oven and Diamond MCT 500 balance are used.

2.2. Reagent

The reagents which used were analytical in quality are purchased from Fluka company.

Fe(III) solution(0.03 M):

Was obtained by dissolving 1.212 g $Fe(NO_3)_3.9H_2O$ in distilled water that contains 5ml 0.05 M HNO_3, and diluting the overall solution to 100 ml in a calibrated flask with distilled water.

1,10-Phenanthroline solution (0.025 M):

was obtained by mixing 0.496 g of 1,10-phenanthroline with 5 ml ethanol and diluting to the mark with distilled water in a 100 ml calibrated flask.

2,2'-Bipyridyl solution(0.025 M):

Was obtained by mixing 0.390 g of 2,2'-bipyridyl with 5 ml ethanol then diluting the volume to 100ml with distilled water.

Typical Doxycycline solution (100ppm):

Obtained by dissolving 0.01 g of pure doxycycline (Dox) in distilled water, diluted to the mark in a 100ml calibrated flask with distilled water, and placed in the refrigerator in an amber bottle. As required, the solution was diluted.

2.3. General Procedure

2.3.1. Method A

Increasing volumes (0.01, 0.2, 0.4, 0.6, 0.8 and 0.9 ml) of the standard 100 µg/ml Dox solution did precisely measured and added to a set of calibrated 10 ml flasks. Followed by the addition of 1.0 ml of $Fe(NO_3)_3.9H_2O$ solution and 0.7 ml of 1,10-phenanthroline solution, and the volume was completed with distilled water to 10 ml. The calibrated flasks have been closed, the content was well mixed, with occasional shaking, the flasks let stand for 10 minutes. Then, at 510 nm against the blank reagent, the absorption of each solution was measured.

2.3.2. Method B

Increasing volumes (0.03, 0.1, 0.2, 0.5,0.7,0.8 and 0.9 ml) of Dox standard solution (50 µg/ml) were measured precisely and added to a set of calibrated 10 ml flasks. Followed by the addition of 0.1 ml of $Fe(NO_3)_3.9H_2O$ and 1.5 ml of 2,2'-bipyridyl solution. The volume adjusted to 10 ml with distilled water. The content mixed well, absorption of the solution was measured at 522 nm against blank reagent after 20 minutes.

Standard calibration graphs were prepared for both spectrophotometric methods A and B by plotting the increasing absorption values versus the Doxycycline concentration (µg/ml).

2.3.3. Analysis of dosage forms (Capsule)

The contents of ten capsules of Doxymid (each capsule contains 100 mg Dox) have weighed and finely powdered. A powdered quantity equal to one capsule in water containing a few drops of dilute HCl was dissolved, then filtered. The filtrate made up to 1L with distilled water and the solution was determined by following the prescribed procedure.

3. Results and discussion

The reaction involves Dox oxidation with FeIII salt, the released FeII was reacted in method A with 1,10-phenanthroline and in method B with 2,2'- bipyridyl.

3.1. Principle of the methods

Methods A and B are based on Dox drug oxidation in an acidic medium with Fe(III) and produce Fe(II). The Fe(II) reacts with 1,10- phenanthroline to produce a red coloured complex of tris-1,10-phenanthroline-iron(II) chelate (ferroin) $[Fe(phen)_3]^{2+}$, as shown in scheme1, having the absorption maximum at 510 nm in method A, and reacts with 2,2'-bipyridyl to produce a red coloured complex of tris-2,2'-bipyridyl-iron(II) chelate $[Fe(bipy)_3]^{2+}$, having maximum absorption at 522 nm in method B, as demonstrated in Fig.1.

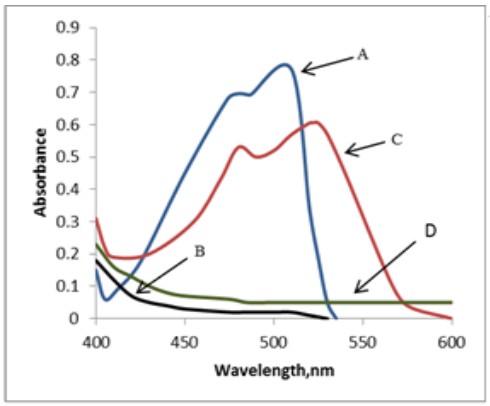
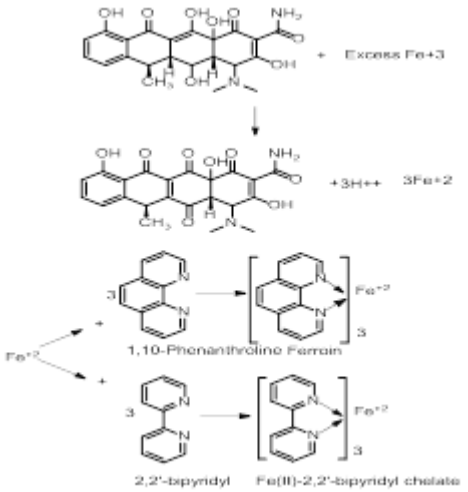


Fig.1. Absorption spectra of (A) (4µg/ml)Dox - $Fe(NO_3)_3.9H_2O$- 1,10-phenanthroline system and its reagent blank (B), (C) Dox (4µg/ml)-$Fe(NO_3)_3.9H_2O$-2,2'-bipyridyl system and its reagent blank(D) in a final



Scheme 1. Proposed Dox assay reaction mechanism

3.2. Optimum reaction conditions

Changing one parameter at a time and maintaining constants for others, the optimal reaction conditions for the determination of Doxycycline quantitatively have been got.

3.2.1. Temperature effect and time of reaction

For each method A and B, the time of reaction is calculated by observing the colour evolution at room temperature and different temperatures in a controlled temperature. The temp. Of water bath. The absorptions quantified against a similarly treated blank reagent at a 5-min interval. At room temperature, or after heating the mixture in a water bath, the absorbance remains the same. Therefore, the reaction performed at room temperature. It found that absorbance reached a maximum after 10 min in method A and 20 min in method B at room temperature, and remain constant for more than 60 min for both methods (Fig. 2).

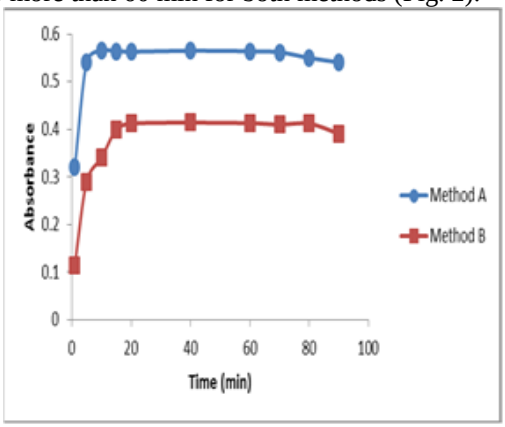
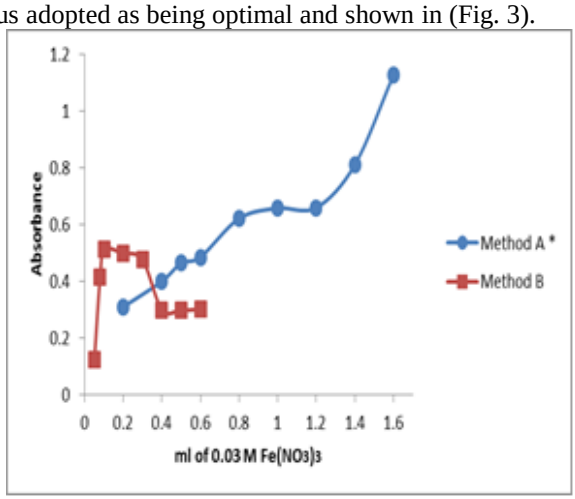


Fig.2. Effect of standing time on the reaction of

(A):1,10-phenanthroline - Dox

3.2.2. Effect of $Fe(NO_3)_3.9H_2O$ concentration

The effect of 1ml of different concentrations of ferric nitrate solution while keeping a fixed concentration of Dox and 1,10- phenanthroline or 2,2'-bipyridyl on the absorbance of a complex in both methods A and B were examined. The 0.03 M concentration of ferric nitrate was found to give maximum absorbance for both methods. The quantity of this concentration has been studied and found that the absorbance has been increased up to 1.0 and 0.1 ml of ferric nitrate in the method A and B respectively, thus adopted as being optimal and shown in (Fig. 3).



* 1.2 and more Turbid

Fig.3. Effect of 0.03M $Fe(NO_3)_3.9H_2O$ concentration on the
(A): Oxidation of 4µg/ml Dox in the presence of 1,10-phenanthroline
(B): Oxidation of 4µg/ml Dox in the presence of 2,2'-bipyridyl

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3.2.3. Effect of 1,10-phenanthroline and 2,2'-bipyridyl reagents concentration

The effect of 1,10-phenanthroline and 2,2'-bipyridyl concentrations on the absorbance of complex in methods A and B respectively investigated. The results indicated that 0.7 ml and 1.5 ml of 0.025 M of 1,10- phenanthroline and 2,2'-bipyridyl respectively gave maximum absorbance, used in the experiments that followed. The absorption was decreased above these concentrations, as shown in (Fig.4).

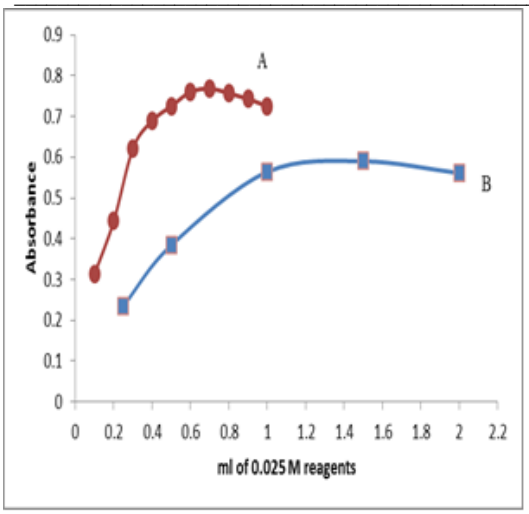


Fig.4. Effect of reagent concentration of
(A):1,10-phenanthroline in the presence of 4µg/ml Dox
(B):2,2'-bipyridyl in the presence of 4µg/ml Dox

3.2.4. Order of Addition

To reach optimum results, the order of reagents addition should follow as indicated in the general procedure, oppositely; a loss of colour intensity has seen.

Therefore, Table 1. Summarizes optimal reaction conditions for the development of colour intensity for the complexes in method A and B.

3.2.5. Quantification

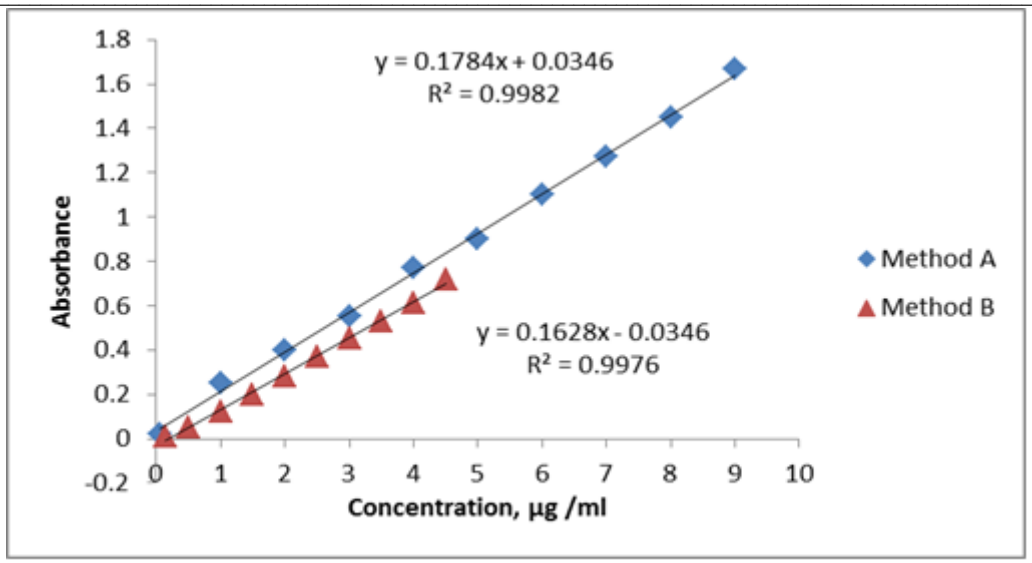
To examine the extent to which coloured complexes comply with Beer's law, after developing the colour, the absorption of the complexes was measured at their selected λmax values by obeying the proposed procedures for a set of solutions with increased quantities of Dox medication. The low limits of Beer's law, molar absorptivity and sensitivity values of Sandell, were estimated and are given in Table 2, which shows that the two methods are sensitive. The regression equation represented the linearity, and the corresponding Dox correlation coefficients determined by the suggested methods represent excellent linearity (Fig.5). For an analysis of six replicates of each of the three different Dox concentrations (1, 4 and 8µg/ml) for method A, and (0.5, 2 and 4.0µg/ml) for method B, the relative standard deviation (RSD) and accuracy (average recovery%) have indicated that the two methods are precise and accurate.

Table1. Optimized conditions for the determination of doxycycline by the suggested methods

Method	λ_{max} (nm)	Temp° (C)	Development time (min)	Stability period (min)	Fe(III) 0.03M (ml)	Reagent 0.025M (ml)
A	510	R.T(28°C)	10	60	1.0	0.7
B	522	R.T(28°C)	20	60	0.1	1.5

Table 2. The proposed methods: Visual properties and statistical data Summary

Parameter	Values of	
	Method A	Method B
Beer's law limits (µg/ml)	0.1-9	0.15-4.5
Molar absorptivity ($l.Mol^{-1}\ cm^{-1}$)	8.25×10^4	7.53×10^4
Sandell's sensitivity (µg cm^{-2})	0.00560	0.00614
Correlation coefficient (r^2)	0.9982	0.9976
Regression equation (*Y*)*		
Slope, *a*	0.1784	0.1628
Intercept, *b*	+0.0346	-0.0346
RSD %	0.9241	1.024
Average recovery %	99.9	98.5
LOD (µg/ml)	0.0531	0.0491
LOQ (µg/ml)	0.2343	0.1279



* $Y = aX + b$, where X is the concentration of doxycycline in µg/ml

Fig.5. Calibration graphs for the Dox determination

3.2.6. Interference

The degree of interferences by some additives accompanying pharmaceutical preparations was investigated by measuring the absorption of solutions containing (2 µg/ml) of Dox and different amounts of various species with a final volume of 10 ml. The additives study which not interfere with the determination of Dox in this dosage shown in Table 3.

4. . Analytical applications

To determine Dox in its pharmaceutical preparations (Doxymid capsule), the current methods successfully used. The outcomes obtained statistically in comparison by using the accuracy *t*-test of the student and the precision *f*-Test variance ratio with the British Pharmacopoeia, at the 95 % confidence level with five degrees - of - freedom, as shown in Table 4. The outcomes revealed that both the *t*-test and the *F*-test were smaller than the theoretical results (t=2.77, F=6.39). So the suggested methods and official method did not have a significant difference.

5. Comparison of current methods with some other spectrophotometric methods

The current method is favourably compared with other spectrophotometric methods published. As shown in Table (5), the suggested methods are more sensitive than many other methods and do not require heating.

Table 3. Influence of additives in doxycycline assay

Additives	The recovery percentage of 2 µg/ml of Dox per µg additives added					
	Method A			Method B		
	100	**500**	**1000**	**200**	**400**	**300**
Lactose	98.2	99.2	101.4	100.1	100.2	98.4
Fructose	99.4	98.6	100.6	97.4	99.8	102.3
Glucose	99.9	100.3	101.0	99.4	97.9	101.1
Starch	100.1	99.2	99.8	98.4	99.9	99.3
Arabic Gum	101.5	99.5	99.1	100.2	98.4	102.0
Sodium Chloride	99.6	99.0	101.0	101.5	99.4	99.8
Urea	102.4	101.9	103.1	100.1	102.4	99.7
Sorbitol	99.8	99.2	101.2	97.4	99.5	101.5
Gum acacia	100.8	101.4	98.7	100.8	97.4	101.9

Table 4. Application of current methods for determination of Dox in capsule and Comparison with the official method

Pharmac. Formula.	Certified value (mg)	Amount present (µg /ml)	Recovery* (%)	Average drug content found (mg)	R.S.D. %	*t-exp*	*F-exp*
**Doxymid Capsule-Phenanthroline	100	1.0	100.2	98.15	1.325	1.02	4.52
		4.0	98.5				
		6.0	96.1				
		8.0	97.8				
**Doxymid Capsule-Bipyridyl	100	1.0	99.6	102.05	1.621	1.98	3.57
		2.0	102.1				
		3.0	102.7				
		4.0	103.8				
British Pharmacopeia	100	0.4	101.54	102.28	0.776	2.77	6.39

*Average of three determinations

** Middle East Pharmaceutical & Chemical Industries -Jordan

Table 5. Current methods compared to published spectrophotometric methods

Analytical parameters	Reagents				
	Current methods		Literature methods		
	1,10-phenanthroline	2,2'-bipyridyl	*DMPD-IO_4 [6]	Chloramine-T [7]	Fe(III) ammonium sulphate [8]
Type of Reaction	Oxidation-Reduction	Oxidation-Reduction	Oxidative coupling	Oxidation-Reduction	Chloroform extraction complex
Colour of dye	Orange-Red	Red	--	--	Yellow
λ_{max}(nm)	510	522	625	525	420
pH	Acidic	Acidic	Neutral	Alkaline	Acidic
Medium	Water	Water	Butanol	Water	Sulfuric acid
Temp.(°C)	RT	RT	RT	65	RT
Development time (min)	10	20	20	5	5
Stability period (min)	60	60	120	60	60
Beer's law (µg/ml)	0.1-9	0.15-4.5	12.2-43.4	8.4-167	10-100
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	8.25×10^4	7.53×10^4	5.46×10^3	3.018×10^3	5.21×10^3
Recovery(%)	99.9	98.5	98.8	103.7	101.57
RSD(%)	< 2.0	< 2.0	1.98	2.14	2.39
Application	Capsules	Capsules	Capsules	Tablets	Tablets
Disadvantages	--	--	Using of organic solvent& Need extraction	Need heating&Less sensitivity	Using H_2SO_4 as solvent&Less sensitivity

6. Conclusion

The methods suggested are simple, sensitive, reasonably precise, and accurate. Sample analysis has shown that the common excipients do not interfere. The advantages of the proposed methods are simple, need no extraction and less time consuming, and the ability to apply in a pharmaceutical preparation with success. The methods applied successfully at room temperature.

7. Conflicts of interest

The authors declare no conflict of interest.

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