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Spectrophotometric Method for the Determination of Tetracycline and Doxycycline by Oxidizing Coupling Reaction with 4-aminoantipyrine

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Received in:13/April/2015 , Accepted in:23/june/2015

Abstract

A simple, sensitive, accurate and low cost effective spectrophotometric method has been developed for the determination of Tetracycline and Doxycycline in pure and pharmaceutical formulations. The method is based on the reaction of methyldopa with 4-aminoantipyren (4-AAP) in presence of potassium ferriecyanide (PFC) in an alkaline medium.

Two optimization methods were applied to determine the optimum conditions of oxidizing coupling reaction variables; univariate and design of experiment (DOE) method. The conditions effecting the reaction; pH, buffer Volume, reagent concentration, oxidant concentration, type of buffer, order of addition, time of reaction and stability were optimized . Under univariate and design of experiment (DOE) method; the Tetracycline colored product having (λmax) at 531nm 535nm. Beer's law was obeyed in concentration range of [two scale (1-18 $\mu g.mL^{-1}$) and (15-70$\mu g.mL^{-1}$)] with correlation coefficient of 0.9997 and 0.9942 respectively and for second method [two scale (1-30 $\mu g.mL^{-1}$) and (40-100$\mu g.mL^{-1}$)] with correlation coefficient of 1 and 0.9913. The assay limits of detection quantification were 0.1732, 0.3123$\mu g.mL^{-1}$ respectively. Under univariate the Doxycycline colored product having (λmax) at 515nm. Beer's law was obeyed in concentration range (10-90 $\mu g.mL^{-1}$) with correlation coefficient of 0.9995. The assay limits of detection quantification were 1.0724$\mu g.mL^{-1}$. The method was successfully applied to the analysis of the drug tablets formulation.

Keywords: Tetracycline, Doxycycline, Spectrophotometric determination, oxidizing coupling reaction, 4-aminoantipyren (4-AAP).

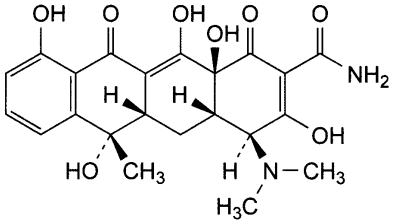
Introduction

Tetracycline chemically{(4S, 4aS, 5aS, 6S, 12aS) - 4 - (Dimethylamino) - 3, 6, 10, 12, 12a-pentahydroxy- 6 - methyl - 1, 11 - dioxo -1, 4, 4a, 5, 5a, 6, 11, 12 a-octahydrotetracene - 2 - carboxamide hydrochloride} chemical structure show down is a class of antibiotics able to inhibit protein synthesis in gram positive and gram negative bacteria by preventing the attachment of aminoacyl-t RNA to the ribosomal acceptor (A) site. [1-3]

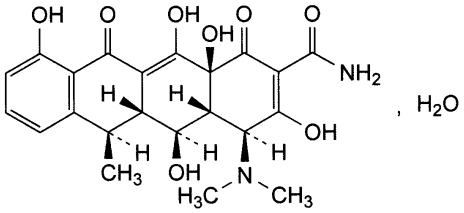
The doxycycline monohydrate chemically {(4S, 4aR, 5S, 5aR, 6R, 12aS) - 4 - (Dimethylamino) - 3, 5, 10, 12, 12a - pentahydroxy - 6 - methyl - 1, 11 - dioxo - 1, 4, 4a, 5, 5a, 6, 11, 12a-octahydrotetracene-2-carboxamide monohydrate} chemical structure show down is a broad-spectrum antibiotic oxytetracycline synthetic derivative used in several countriesis. It is a bacteriostatic inhibiting the bacterial protein synthesis due to the disruption of transfer RNA and messenger RNA at the ribosomal sites1. It is also used for malaria treatment; it was first product by Pfizer Inc. Company in 1960. It has been used to treat infectious diseases and as an additive in animal nutrition to facilitate growth. Doxycycline is more active than tetracycline against many species of bacteria including Streptococcus pyogenes, enterococci, anaerobic, and various Nocardia spp. Cross-resistance is common, although some Staphylococcus aureus resistant to tetracycline respond to doxycycline. Doxycycline is also more active against protozoa, particularly Plasmodium. Doxycycline is useful for the treatment of respiratory tract infections because it provides a hedge against atypical micro-organisms, and since the respiratory pathogens are becoming increasingly resistant to other classes of drugs. Another advantage is that it can also be administered to patients with kidney problems. [3-4]

Several methods have been proposed to quantify Tetracycline and Doxycycline in pharmaceutical formulations, including; high-performance liquid chromatography (HPLC) [5-8], flow injection spectrophotometry, [9-11] voltammetry [12, 13], Extraction [14-16], Electrochemical Methods (biosensor system) [17-19] and spectrophotometry. [20-23]

The aim of the present work is to provide an optimized spectrophotometric method using the univariate and multivariate design of experiment (DOE) method. In the design of experiment method three-interest factors, buffer volume, reagent concentration and oxidant concentration were designated as independent variables and absorbance as response.



Chemical structure of tetracycline



Chemical structure of doxycycline monohydrate

Experimental

Apparatus

All absorption measurements were obtained by using a double beam Shimadzu 1800, Kyoto, Japan UV-Visible Spectrophotometer with 40 mm matched quartz cells and the pH measurements were performed by using i-Trans, BP 3001, Sangapor pH meter.

Reagent

All Chemicals used are of analytical-reagent grade

1. A pure Tetracycline, Doxycycline powder received in pure form (99.99%) was provided as a gift from the State Company for Drug Industries and Medical Appliances Samara-Iraq (SDI). The standard solution of drugs (1000 $\mu g.mL^{-1}$) were prepared by dissolving accurate weighted 100 mg of pure drug in 50 mL of distilled water with mild heating and brought to 100 mL of volumetric flask.

Each working standard solution was freshly prepared by diluting the stock solution with distilled water.
2. 4-aminoantipyrine (4-AAP) stock solution [0.3% (m/v)]: prepared by dissolving 0.3g of 4-AAP in distilled water and diluting to 100 mL in a dark volumetric flask at stored in a refrigerator. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.
3. Potassium ferricyanide (PFC) stock solution [3 % (m/v)]: prepared by dissolving 3 g of PFC in distilled water and diluting to 100 mL in a volumetric flask. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.
4. Borax [0.1 % (m/v)]: prepared by dissolving 0.1 g of Borax in distilled water and diluting to the mark in a 100 mL volumetric flask.
5. Sodium hydroxide [~ 0.5 M]: prepared by dissolving 2g of NaOH in 100 mL of distilled water.
6. Glucose [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of glucose in 10 mL distilled water.
7. Lactose [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of lactose in 10 mL distilled water.
8. Acacia [10000 $\mu g.mL^{-1}$]: prepared by dissolving 0.1 g of acacia in 10 mL distilled water.
9. Soluble Starch [10000 $\mu g.mL^{-1}$]: Triturate 0.1 g of soluble starch with a little cold water into a thin paste, and add boiling water. Boil until a clear solution is obtained (5 minutes) then let it cold and diluting to 10 mL in a volumetric flask. This solution should be freshly prepared as required.
10. Dihydrogen Potassium Phosphate [0.1 %]: prepared by dissolving 0.1 g of KH_2PO4 in 100 mL of distilled water.
11. Hydrochloric acid [~0.5) M]: prepared by taking 4.17 mL of concentrated HCl and diluted to 100 mL with distilled water.
12. Potassium hydroxide [~0.5 M]:prepared by dissolving 2.8 g of KOH in 100 mL of distilled water.

Analysis of Tablets and Capsules

1- Tetracycline

Ten capsules were carefully evacuated; their contents were weighed and finely powdered and average mass per capsule was determined. The amount of powder equivalent to 0.2828 g of India capsule and 0.3332 g of Iraqi capsule, (TET) was dissolved in distilled water with mild heating then diluted to the mark of 100 mL volumetric flask. Each solution was filtered using whatman filter paper No.41 to dispose of any Insoluble materiel before used. Sample solutions were prepared freshly by subsequent dilution with distilled water and analyzed by the recommended procedure.

2- Doxycycline

Ten capsules or tablets were weighed then grinded to a homogenous fine powder, and average mass per capsule was determined. The amount of powder equivalent to 0.1505 g of capsule and 0.1921 g of tablet, (DOX) was dissolved in distilled water with mild heating then diluted to the mark of 100 mL volumetric flask. Each solution was filtered using whatman filter paper No.41 to dispose of any insoluble materiel before used. Sample solutions were prepared freshly by subsequent dilution with distilled water and analyzed by the recommended procedure.

General standard procedures

Two procedures were recommended for the determination of drugs via the proposed methods. The first was carried out following the conditions obtained by univariate optimization, while the second base of those conditions which was obtained by chemometric multivariate design of experiment method of optimization.

I- Univariate Method

1-Tetracycline

0.15mL of the standard solution contains (3-210) µg of TET was directly transferred into quartz cuvette, followed by the addition of 0.6 mL of the borax buffer solution (0.1 % pH=9), 0.45 mL of

2.5% (m/v) potassium ferricyanide solution and 0.45 mL of 0.15% (m/v) 4-AAP solution. After 10 min the mixture diluted with distilled water to make the volume to 3 mL. As soon as the cuvette was covered, the solution was quickly shaken, left to stand for 30 min at room temperate then pleased in the spectrophotometer cell holder, the absorbance of the orange colored compound of TET were measured against the reagent blank at 531nm.

2- Doxycycline

0.45 mL of 2% (m/v) potassium ferricyanide solution was directly transferred into quartz cuvette followed by the addition of 0.75 mL of the borax buffer solution (0.1% pH=9.1) then 0.15mL of the standard solution containing (30-270)µg of Doxycycline, 0.45mL of 0.15%(m/v) 4-AAP solution were added. The mixture was diluted directly with distilled water to make the final volume to 3 mL. As soon as the cuvette was covered, the solution was quickly shaken and pleased in the spectrophotometer cell holder directly, the absorbance of the orang colored compound was measured against the reagent blank at 515nm.

II- Design of Experiment Method

1-Tetracycline

0.15mL of the standard solution contains (15-300) µg of Tetracycline was directly transferred into quartz cuvette, followed by the addition of 0.91 mL of the borax buffer solution (0.1 % pH=9), 0.45 mL of 2.65% (m/v) potassium ferricyanide solution and 0.45 mL of 0.15% (m/v) 4-AAP solution. After 6.75 min the mixture was diluted with distilled water to make the final volume to 3 mL. As soon as the cuvette was covered, the solution was quickly shaken, left to stand for 30 min at room temperate then pleased in the spectrophotometer cell holder. The absorbance of the orange colored compound of TET was measured against the reagent blank at 535nm.

2- Doxycycline

0.45 mL of 2% (m/v) potassium ferricyanide solution was directly transferred into quartz cuvette followed by the addition of 0.75 mL of the borax buffer solution (0.1% pH=9.1) then 0.15mL of the standard solution containing (30-270) µg of Doxycycline and 0.45 mL of 0.15% (m/v) 4-AAP solution were added. The mixture diluted directly with distilled water to make the volume to 3 mL. As soon as the cuvette was covered, the solution was quickly shaken and pleased in the spectrophotometer cell holder directly, the absorbance of the orange colored compound of DOX was measured against the reagent blank at 515 nm.

Results and Discussion

Optimization of reaction variables

A systematic study of the effects of various parameters on development of product color was taken by varying the parameters one at a time and controlling all others fixed.

1-Tetracycline (I-univarite method)

1- Effect of Buffer pH

The influence of buffer pH on the formation of the reaction product was studied over the range of (8.5-10.5) using 0.1% borax buffer solution. Maximum and constant absorption intensities were achieved at pH 9.0, therefore pH 9.0 was chosen as the optimum pH value.

2- Effect of Buffer Volume

Maximum absorbance intensity was achieved using (0.3-1.2) mL of borax buffer solution, so that 0.6 mL of borax buffer solution pH 9.0 was chosen as the optimum buffer volume.

3- Effect of the reagent (4-aminoantipyrene (AAP)) concentration

The influence of concentration of AAP on the absorbance of the color product was examined in the range (0.1-0.2%), the maximum absorbance was obtained with 0.15% 4-AAP. Above this concentration the absorbance value decreased, therefore 0.15% was used in all measurement.

4- Effect of potassium ferricyanide concentration

The studying of PFC concentration revealed that the reaction was depending on PFC as an oxidant agent. The highest absorbance was attained when the concentration of PFC was 2.5%. Above this value a decrease in the absorbance reading occurred.

5- Effect of the type of buffer

Two Buffer solutions (Borax and Dihydrogen Potassium phosphate) were examined to achieve maximum color intensity. Borax buffer proved to be the favorable one due to it yield a high value of absorbance value in addition to instantaneously formation of the color product.

6- Effect of order of addition

The optimum sequence was defined by following the color intensity and maximum absorbance on changing the sequence of addition of drug, reagent, oxidizing agent and buffer. The best condition was {drug solution first – the buffer – the oxidizing agent and the reagent solution} for the maximum absorbance.

7- Effect of reaction time

The reaction time is determined by following the color development at different time intervals when the reaction component were left in dark place at room temperature under the optimum conditions. It was found that maximum absorbance is attained after 10 min further increase of the reaction time result in a gradual decrease in the absorption intensity, therefore 10 min was chosen as the optimum reaction time.

8- Stability of the colored product

Under the aforementioned optimum condition, it was found that the reaction product formed instantaneous and the absorbance reach to a maximum and constant at 30 min, therefore 30 min was chosen as the optimum reaction time.

II- multivariate method: experimental design and statistical analysis

To develop the product color and to find the optimum experimental conditions, Multivariate Method Experimental Design was used. The most three critical variables (buffer volume, reaction time and oxidant concentration) were examined using a central composite design while the other variables obtained from univariate method namely, effect of buffer pH, reagent concentration, effect of the buffer kind, effect of order of addition, effect of reaction time and effect of stability, were kept constant.

To test the curvature of the response three levels of each selected variable were required. The number of experiments needed to investigate the previously noted three parameters at three levels would be 27 (3^3). However, this was reduced to 20 using a central experimental design. Response surface model was applied to study the effect of the three variables, and generate an optimal response surface. [23]

A second order polynomial equation was used to express the absorption as a function of independent variables (buffer volume, reaction time and oxidant concentration).

Absorbance = $\beta_0+\beta_1\times$(buffer volume)$+\beta_2\times$(reaction time)$+\beta_3\times$([PFC)$+\beta_4\times$(buffer volume$\times$reaction time)$+\beta_5\times$ (buffer volume$\times$([PFC])$+\beta_6\times$(reaction time$\times$[PFC])$+\beta_7\times$(buffer volume)$^2+\beta_8\times$(reaction time)$^2+\beta_9\times$ ([PFC])$^{2.}$

The result of optimum conditions according to central composite design and the experimental points used according to the design was listed in Table (1).

The coefficients of the response surface equation were determined by STATISTICA 8.0 software (StatSoft. Inc, release 2007), the results are listed in Table (2).

Optimum conditions that are developed from central composite design for the determination of Tetracycline via oxidation coupling reaction with 4-AAP were calculated mathematically and the results are0.91 mL of buffer volume, 6.75 min for reaction time and 2.65% m/v of oxidant concentration). Figure 3 shows the response surface model if one of the three variables is remained constant.

2-Doxycycline

1- Effect of buffer pH

The influence of buffer pH on the formation of the reaction product was studied over the range of (8.3-9.5) using 0.1% borax buffer solution. Maximum and constant absorption intensities were achieved at pH 9.1, therefore pH 9.1 was chosen as the optimum pH value.

2- Effect of buffer volume

It was found that increasing the volume of borax buffer produces a gradual increase in the absorbance value of the reaction product up to 0.75 mL, after which further increase products a gradual decrease in the absorbance value. Therefore 0.75 mL of borax buffer was chosen as the optimum volume value.

3- Effect of the reagent (4-aminoantipyrene (AAP)) concentration

The influence of concentrations of 4-AAP on the absorbance of the color product was studied using different concentration (0.1-0.2) % (m/v), it was found that the maximum absorbance was obtained with 0.15% 4-AAP. Above this concentration, the absorbance of color product value decreased, therefore 0.15% was used in all measurement.

4- Effect of potassium ferricyanide concentration

The studying of PFC concentration revealed that the reaction was depending on PFC as an oxidant agent. The highest absorbance was attained when the concentration of PFC was 2%, above this value a decrease in the absorbance reading occurred.

5- Effect of the type of buffer

Two Buffer solutions (Borax and Di hydrogen Potassium phosphate) were examined to achieve maximum color intensity. Borax buffer proved to be the most favorable one due to it's highly absorbance value in addition to instantaneously formation of the color product.

6- Effect of order of addition

The optimum sequence was defined by following the color intensity and maximum absorbance on changing the sequence of addition of drug, reagent, oxidizing agent and buffer. The best condition was {oxidizing agent solution first, the buffer solution, the drug solution and the reagent solution} for the maximum absorbance.

7- Effect of reaction time

The reaction time is determined by following the color development at different time intervals when the reaction component were left in dark place at room temperature under the optimum conditions. It was found that maximum absorbance is attained directly (after zero min), that mean the reaction was very fast, and further increase in the reaction time causes a gradual decrease in absorbance intensity a gradual decrease was shown in the absorption intensity, therefore zero min was chosen as the optimum reaction time.

8- Effect of stability

Under the aforementioned optimum condition, it was found that the reaction product was taken place instantaneous, a decrease of the absorbance of reaction product took place. It is recommended to measure the absorbance immediately.

II- Multivariate method: experimental design and statistical analysis

The result of optimum conditions according to central composite design and the experimental points used according to the design was listed in Table 1.

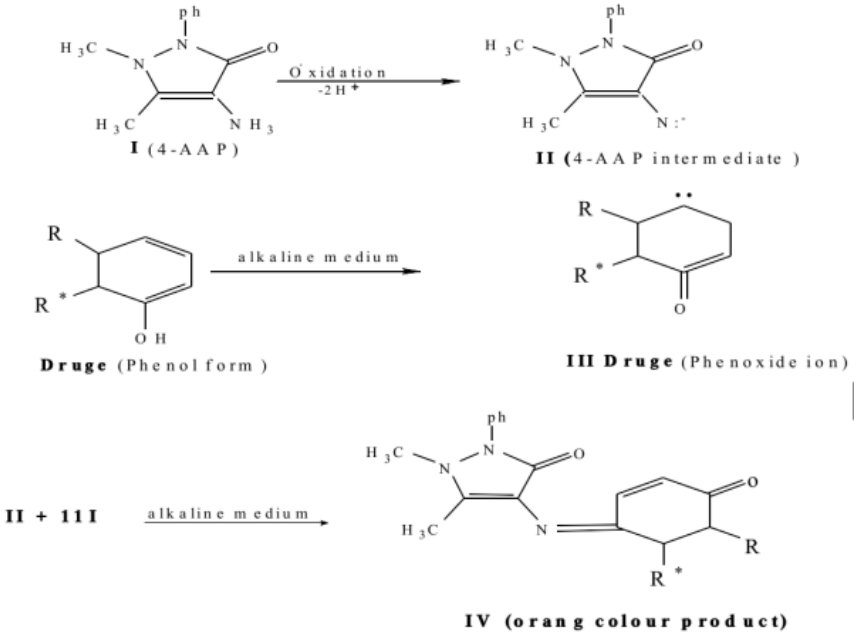
The iniquity results Obtained from Optimum conditions that are developed from central composite design for the determination of Doxycycline via oxidation coupling reaction with 4-AAP, because the reaction very fast and its stability is low, therefore Univariate Method used to determination Doxycycline via oxidation coupling reaction with 4-AAP.

Reaction scheme and absorption spectra

Oxidizing coupling reaction includes reaction of two or more organic materials in the existence of oxidizing agent in suitable conditions, as a result of oxidizing, intermediate compound which reacts with others to produce colored product, that can be measured spectrophotometric.

Under the oxidizing coupling reaction condition, 4-AAP (I) upon oxidation with PFC loses two protons forming a nucleophilic intermediate (II), which has been postulated to be an active coupling species. Drugs (TET or DOX) have a free ortho position to the hydroxyl group, hence the intermediate of 4-AAP (II) undergoes nucleophilic substitution with phenolic moieties (III) of DRUGS in alkaline medium, to form a colored quinonoid type product (IV) [24, 25].

Absorption spectrum for tetracycline was recorded under the optimum conditions (for univariate and Experimental Design method) and showed the maximum absorption (531.0 and 535) nm sequentially for the



dark orange compound against the reagent blank as shown in Figure (1). Absorption spectrum for doxcycycline was recorded under the optimum conditions (for univariate) showed the maximum absorption 515 nm for the dark orange compound against the reagent blank as shown in Fig. (2).

Calibration curves and analytical Data

1-Tetracycline

I- Univariate Method

Employing the experimental condition, two scales of linear calibration curve plotted according to Beer's law (Fig. (1)), The Optical characteristics are listed in Table (3).

II- Design of experiment method

The Optical characteristics are summarized in Table(3).

2- Doxycycline (Univariate method)

Employing the experimental condition, linear calibration curve plotted according to Beer's law and the Optical characteristics are listed in Table 4.

Precision and accuracy

The accuracy and precision of the proposed methods were tested by analyzing three replicate samples of TET or DOX in different concentration levels. The values of the relative error % and precision are summarized in Table 5. These values indicate acceptable accuracy and precision of the method.

Interference study

The extent of interfering by some excipients which often accompanied pharmaceutical preparations were studies by measuring the absorbance of solution containing 40 $\mu g.mL^{-1}$ of TET and 1000 $\mu g.mL^{-1}$. It was found that the studied exhipients do not interfere in the determination of TET or DOX in its dosage forms, Table (6).

Application in pharmaceutical sample

In order to demonstrate the applicability of the proposed method for the determination of TET or DOX, the method was successfully applied to the analysis of TET and DOX in different concentration levels of two type of drug. The results were summarized in Table (7) and Table (8).

Conclusions

Oxidizing coupling reaction of TET or DOX with 4-AAP presence of potassium ferriecyanide (PFC) in alkaline medium was found to be a simple, sensitive and accurate spectrophotometric method for quantitative determination of TET or DOX in pure form and Pharmaceutical. The classical univariate and Design of Experiment method have been used for optimizing the different variable affecting the completion of the reaction. The proposed method offers good linearity and precision. The proposed method can applied in many samples like food samples and biological fluids.

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Table (1): The central composite design with three independent variables (uncoded variables) and their experimental absorption values of 15 µg.mL^{-1} TET, 15 µg.mL^{-1} DOX oxidation coupling product

Exp. no.	TET				DOX			
	Buf. vol. (mL)	Re. time (min)	[Oxi.] (%)	Abs.	Buf. Vol .(mL)	[Reag.] (%)	[Oxi.] (%)	Abs.
1	0.75	10.00	2.00	0.218	0.30	0.20	3.00	0.032
2	0.75	5.00	2.00	0.201	0.75	0.15	3.00	0.153
3	0.75	0.00	2.00	0.206	0.75	0.15	1.50	0.137
4	0.75	5.00	2.00	0.240	0.75	0.15	1.50	0.121
5	0.75	5.00	3.50	0.213	0.75	0.15	1.50	0.119
6	0.75	5.00	0.50	0.124	0.75	0.15	0.30	0.084
7	1.20	10.00	3.50	0.162	0.30	0.10	0.30	0.077
8	0.30	10.00	3.50	0.030	0.75	0.15	1.50	0.113
9	0.75	5.00	2.00	0.229	0.75	0.10	1.50	0.066
10	0.30	10.00	0.50	0.014	0.75	0.15	1.50	0.112
11	1.20	5.00	2.00	0.191	1.20	0.10	0.30	0.066
12	1.20	0.00	3.50	0.215	1.20	0.20	3.00	0.121
13	0.30	5.00	2.00	0.001	0.30	0.10	3.00	0.212
14	0.30	0.00	0.50	0.000	1.20	0.10	3.00	0.062
15	0.30	0.00	3.50	0.011	1.20	0.20	0.30	0.074
16	0.75	5.00	2.00	0.221	0.75	0.15	1.50	0.111
17	1.20	0.00	0.50	0.114	0.75	0.20	1.50	0.132
18	1.20	10.00	0.50	0.120	0.30	0.20	0.30	0.05
19	0.75	5.00	2.00	0.213	1.20	0.15	1.50	0.062
20	0.75	5.00	2.00	0.194	0.30	0.15	1.50	0.08

Table (2): Regression coefficients, P or probability for abs. of TET oxidation coupling product

Variable	Regression coefficient	St. E of coefficient	t-value	P
Constant	-0.330957	0.044284	-7.47351	0.000021
Buffer volume	1.077106	0.118692	9.07484	0.000004
(Buffer volume.)2	-0.605836	0.075081	-8.06906	0.000011
Reaction time	0.001388	0.007349	0.18883	0.854002
(Reaction time)2	0.000453	0.000608	0.74442	0.473752
[PFC]	0.075612	0.029871	2.53132	0.029803
([PFC])2	-0.017192	0.006757	-2.54419	0.029153
Buffer vol.* Reaction time	-0.005167	0.003962	-1.30411	0.221414
Buffer vol.* [PFC]	0.024630	0.013206	1.86502	0.091756
Reaction time * [PFC]	-0.001050	0.001189	-0.88343	0.397745

Table (3): Optical characteristics and statistical data for the determination of Tetracycline by (A) univariate method, (B) Design of Experiment Method. (*(L. mol $^{-1}$.cm $^{-1}$), **(µg.cm^{-2})

Parameter	Value (A)		Value (B)	
	(Scale 1)	(Scale 2)	(Scale 1)	(Scale 2)
λmax (nm), Color,	531 nm, Dark Orang		535 nm, Dark Orange	
Linearity range *	1-18	15-70	1-30	40-100
Regression equation	y=00110[TET.*]-0.0007	y=0.002 [TET.*]+0.1403	y=0.0122[TET*]+0.0165	y=0.0015[TET.*]+0.3105
Calibration sensitivity*	0.0110	0.0020	0.0122	0.0015
Correlation coefficient (r)	0.9997	0.9942	1.0000	0.9913
Correlation of linearity(r^2)	0.9994	0.9884	1.0000	0.9965
Molar absorptivity*	ε = 4.884×10+3	ε = 0.888×10+3	ε = 5.422×10+3	ε = 6.66×10+2
Sandell's sensitivity**	0.0909	0.5000	0.0719	0.1508
Detection limit*	0.173205		0.3123	
Quantification limit*	0.5249	0.9462	0.9465	7.6980

Table (4): Optical characteristics and statistical data and Calibration curve for the determination of Doxycycline by univariate method.(*(L. mol $^{-1}$.cm $^{-1}$), ** (µg.cm^{-2})

Parameter	Value
λmax (nm), Color	515 nm, Dark Orange
Linearity range*	10 -90
Regression equation	Y=0.0068 [DOX.*] +0.1286
Calibration sensitivity*	0.0068
Correlation coefficient (r)	0.9995
Correlation of linearity (r^2)	0.9991
Molar absorptivity*	$3.14473*10^{+3}$
Sandell's sensitivity**	0.0147
Detection limit*	1.0724
Quantification limit*	3.1540

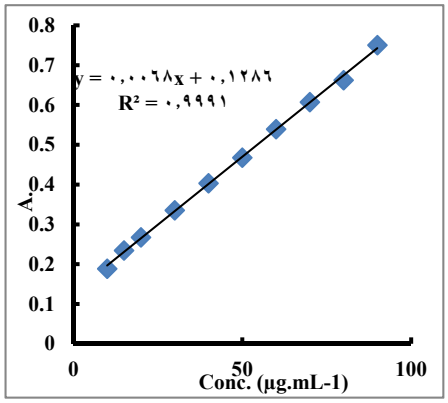


Table (5): Evaluation of accuracy and precision for the determination of TET by proposed methods.(*Average of three determinations).

Conc. of TET (µg.mL^{-1})	Taken	Found*	R. E.%	R. S. D.%
(TET)For univariate	5	4.9936	-0.1280	3.8765
	15	15.0183	0.1220	3.2768
	30	29.2703	-2.4324	4.7749
	60	59.4991	-0.8348	2.7024
(TET)For DOE	5	4.9745	-0.5100	3.8720
	20	19.6455	-1.7725	1.5887
	40	39.6333	-0.9167	1.6981
	60	59.8682	-0.2197	1.7975
(DOX)For univariate	15	15.1514	1.0096	2.7471
	30	29.8226	-0.5913	5.2330
	70	70.1404	0.2005	0.1970

Table (6): Percent recovery for 40 µg.mL^{-1} of TET in the presence of 1000 µg.mL^{-1} of excipients. (*(µg.mL^{-1}), DOX conc. taken (30 µg.mL^{-1}), DOX conc. taken (30 µg.mL^{-1})).

(1000)* Excipients	TET		DOX	
	Conc. found	Recovery%	Conc. found	Recovery%
Acacia	40.70	101.73	30.5166	101.72
Glucose	40.75	101.87	31.2005	104.00
Lactose	41.39	103.48	29.6046	98.68
Starch	42.09	105.22	30.5166	101.72

Table (7) Application of the DOE method to the TET concentration measurements in pharmaceutical sample. (*Average of three determinations, ** (µg.mL^{-1})).

Sample	Weight*found (250mg/100mL)	Conc. Taken**	Conc.* found**	Recovery* %	R.S.D*%
250mg of TET India capsule	242.00	5	4.84	96.89	4.01
	243.63	20	19.49	97.47	4.82
	242.15	40	38.75	96.87	2.86
250mg of TET Iraqi capsule	262.50	5	5.25	104.91	2.68
	261.00	20	20.88	104.40	1.20
	258.31	40	41.33	103.32	5.55

Table (8) Application of the Univariate method to the DOX concentration measurements in pharmaceutical sample. (*Average of three determinations, ** (µg.mL^{-1}))

Sample	Weight* found (100mg/100mL)	Conc. Taken**	Conc.* found**	Recovery* %	R.S.D* %
100mg of DOX Iraqi capsule	98.46	10	9.85	98.46	5.74
	98.66	30	29.60	98.65	5.44
	98.28	60	58.96	98.27	3.02
100mg of DOX India tablet	98.39	10	9.839	98.39	4.5
	97.39	30	29.22	97.41	0.95
	97.67	60	58.59	97.66	1.25

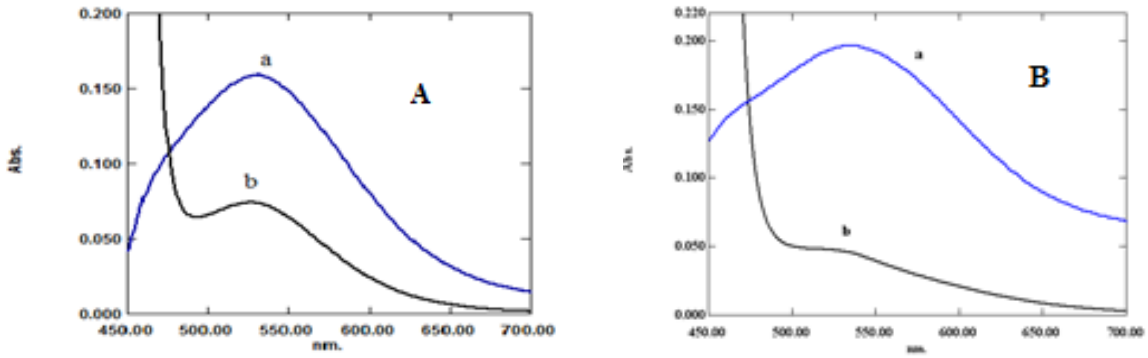


Fig. (1): Absorption spectrum of color product: (a) 15 µg.mL^{-1}Tetracycline against reagent blank, (b) blank solution against distilled water (A) under the univariate method, (B) under the Experimental Design Method conditions.

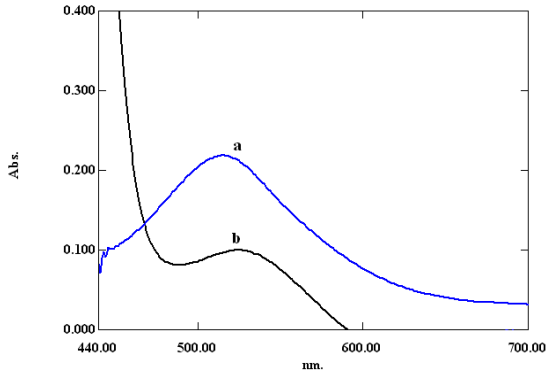
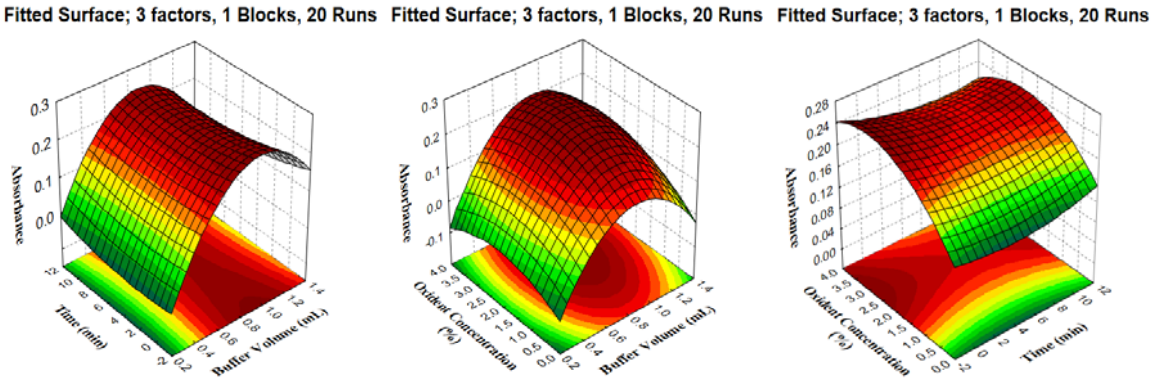


Fig. (2): Absorption spectrum of color product: (a) 15µg.mL^{-1} Doxycycline against reagent blank, (b) blank solution against distilled water under the optimum conditions.



A B C

Fig. (3): A- The response surface for the abs. of TET oxidation coupling product as a function of buffer vol. and reaction time (at cons. optimum value of oxi. conc.:2.65% m/v), B-The response surface for the abs. of TET oxidation coupling product as a function of buffer vol. and oxi. Conc. (at cons. optimum value of reaction time: 6.75 min), C-The response surface for the abs. of TET oxidation coupling product as a function of reaction time and oxi. Conc. (at cons. optimum value of buffer vol.:0.91 mL).

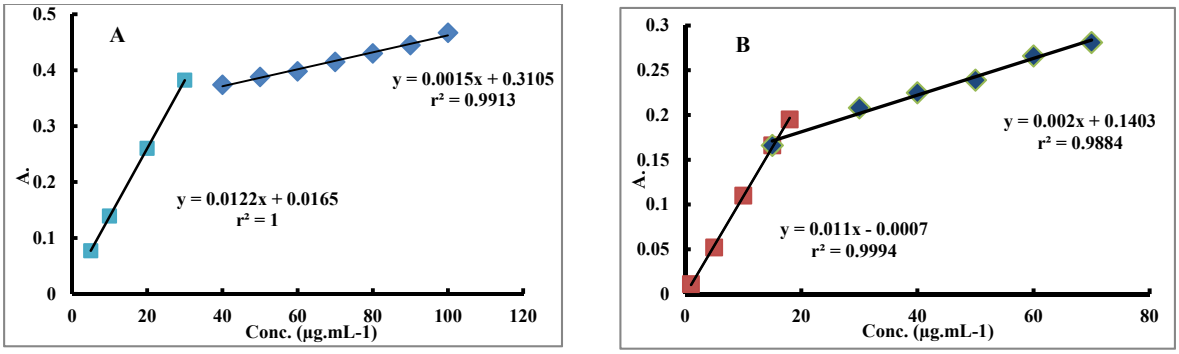


Fig. (4) Calibration curve for the determination of Tetracycline (A) by DOE optimal condition, (B) by univariate optimal condition.

تقدير التتراسايكلين والدوكسي سايكلين طيفيا بتفاعل الازدواج التاكسدي مع 4-امينو انتي بايرين

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استلم البحث في: 13/نيسان/ 2015، قبل البحث في:23/حزيران/ 2015

الخلاصة

طورت طريقة طيفية بسيطة وحساسة ودقيقة وواطئة الكلفة للتقدير الكمي للتتراسايكلين (TET) و الدوكسيسايكلين (DOX) بشكله النقي وفي الشكل الصيدلاني. تعتمد الطريقة على تفاعل للتتراسايكلين (TET) و الدوكسيسايكلين (DOX) مع 4- امينو انتي بايرين بوجود بوتاسيوم فريسيانيد (PFC) في وسط قلوي. وقد تمت دراسة العوامل التي تؤثرفي إتمام تفاعل الازدواج التاكسدي بعناية للحصول على الظروف الفضلى بطريقتين. وذلك باتباع نمط المتغير الواحد وبالاعتماد على وطريقة المتغيرات المتعددة (تصميم التراكم المركزي لثلاثة متغيرات). المتغيرات التي تؤثر على التفاعل هي pH ، تركيز الكاشف، تركيز العامل المؤكسد، تسلسل الاضافه، زمن التفاعل والاستقراريه. الامتصاص الأعظم (λmax) لمركب التتراسايكلين الملون الناتج حسب الطريقتين 531، 535 نانومتر بالتسلسل، وجد أن قانون بير ينطبق على مدى من التراكيز للطريقه الاولى يتراوح بمديين بين $\mu g.mL^{-1}$ (1-18) و $\mu g.mL^{-1}$ (15-70) و بمعامل ارتباط مساوي لـ 0.9942 و0.9997 . وللطريقه الثانيه بمديين بين ($\mu g.mL^{-1}$-1-30) و ($\mu g.mL^{-1}$-40-100) و بمعامل ارتباط مساوي لـ 1 و0.9913 . وكان حد الكشف يساوي0.1732 $\mu g.mL^{-1}$ و0.3123 $\mu g.mL^{-1}$ لكلا الطريقتين بالتتابع . والامتصاص الأعظم (λmax) لمركب الدوكسيسايكلين الملون الناتج حسب طريقة المتغير الواحد515 نانومتر، وجد أن قانون بير ينطبق على مدى من التراكيز يتراوح بمديين بين $\mu g.mL^{-1}$ (10-90) و بمعامل ارتباط مساوي لـ 0.9995 . وكان حد الكشف يساوي$\mu g.mL^{-1}$1.0724. وطبقت الطريقة المقترحة بنجاح لتقدير التتراسايكلين والدوكسيسايكلين في بعض النماذج دوائيه.

الكلمات المفتاحية: تتراسايكلين، دوكسيسايكلين ، التقدير الطيفي، تفاعل الأزدواج التاكسدي،4-امينو انتي بايرين.