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Research Article

Kinetic - Spectrophotometric Estimation of Tetracycline in Bulk and Pharmaceutical Forms

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ABSTRACT
Simple and sensitive spectrophotometric method is described based on the coupling reaction of tetracycline hydrochloride (TC. HCl) with diazotized 4-aminopyridine in bulk and pharmaceutical forms. Colored azo dye formed during this reaction is measured at 433 nm as a function of time. Factors affecting the reaction yield were studied and the conditions were optimized. The kinetic study involves initial rate and fixed time (10 minutes) procedures for constructing the calibration graphs to determine the concentration of (TC. HCl). The graphs were linear for both methods in concentration range of 10.0 to 100.0 µg.mL^{-1}. The recommended procedure was applied successfully in the determination of (TC. HCl) in its commercial formulations.

Keywords: Tetracycline, coupling reaction, azo dye

INTRODUCTION

Tetracycline (TC.HCl), has the IUPAC name[1] (4*S*,4a*S*,5a*S*,6*S*,12a*S*)-4-(Dimethylamino)-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a, 6 , 11,12a-octahydrotetracene-2-carboxamide Figure (1).

Tetracyclines are a group of broad-spectrum antibiotics that have been used for more than 50 years for the treatment of bacterial infections in both humans and animals. The main applications of TC.HCl in animal husbandry are for preventative treatment of bacterial infections and to increase growth rates[2,3].

For the importance of TC.HCl compound, several researches have been conducted to deal with the estimation of that compound. In this respect, different analytical methods have been used for the determination of TC.HCl based on HPLC[4,5], Flow Injection Analysis[6], Voltammetric[7], and spectrophotometric[8-10], have been developed for determination of TC.HCl in pharmaceutical preparations. According to a relative small number of kinetic methods for quantitative determination of TC.HCl have been reported in the literature, the work done to development a new sensitive, selective, and free of interference kinetic-spectrophotometric procedure by coupling TC.HCl with the diazotized 4-Aminopyridine Figure (2).

EXPERIMENTAL

Apparatus

All spectrophotometric measurements were performed using Shimadzu 1800 UV-Visible, with 1 cm silica cells. A Sartorius BL 210S balance, water bath (Memmert W-200 RING- Germany) and hot plate with magnetic stirrer (Germany).

Reagents and chemicals

TC.HCl standard powder was obtained, as a gift, from the State Company for Drug Industries and Medical Appliances, Samara-Iraq (SDI). Charge transfer complexing agent such as *p*-Br (Merck) was supplied from china.

Antibiotic Standard Solutions

A stock solution of tetracycline hydrochloride (TC. HCl) (1000 μg/mL) was prepared by dissolving 0.100 g of TC. HCl standard powder in 100 mL distilled deionized water (DW).

Diazotized 4-Aminopyridine solution (3x10^{-2})[11]

0.25 g of 4-Aminopyridine was dissolved in 30 mL distilled water, with continuous stirring and heating. Then 2 mL of 11.8M HCl is added with continuous stirring, and the mixture is transferred into a brown 100 mL volumetric flask, cooled to 0 - 5 ^{o}C in ice–bath. After then 0.1833g of $NaNO_2$ is added and the mixture is stirred vigorously. Five minutes later, the solution is made up to final volume 100 mL with cold water. The solution is stable for at least one week when is stored in a refrigerator.

Solution for the analysis of TC.HCl in formula preparation

The content of ten capsules were grinded and finely powdered. A quantity of powder containing 100mg of the drug (TC.HCl) was dissolved in 25 mL of DW and left to stand for 5 minutes. The total volume of the formed solution was made to 100 mL in a volumetric flask with DW to obtain 1000µg.mL^{-1} TC.HCl solution. The undissolved materials were filtered-off via Whatman filter paper No.41 before use.

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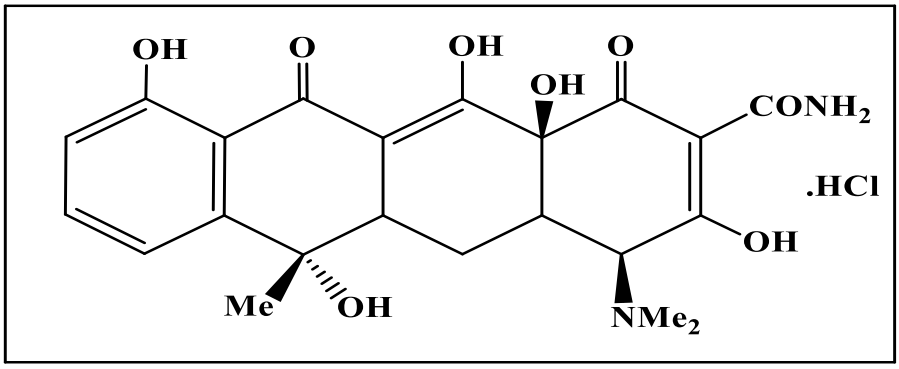

Figure 1: Chemical structure of TC.HCl.

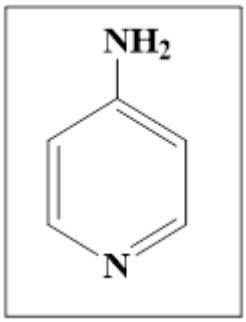

Figure 2: Structure of 4-Aminopyridine.

Table 1: Values of k' for different rates of variable con. of TC.HCl.

[TC.HCl] M	k' (S^{-1})
2.2501×10^{-05}	-6.6534×10^{-04}
6.7502×10^{-05}	-6.9620×10^{-04}
1.8001×10^{-04}	-7.2982×10^{-04} $\times 10^{-3}$

Table 2: Values of reciprocal of time taken at fixed absorbance for different rates of various con. of TC. HCl.

[TC. HCL] M	1/*t* (sec^{-1})
6.7502×10^{-5}	6.0423×10^{-4}
1.1250×10^{-4}	1.9802×10^{-3}
1.3500×10^{-4}	4.3478×10^{-3}
1.8001×10^{-4}	6.8966×10^{-3}
2.2501×10^{-04}	1.6667×10^{-2}

RESULTS AND DISCUSSION

Absorption spectra

Absorption spectrum for the azo dye product formed upon the reaction between the diazotized 4-aminopyridine and TC. HCL was recorded, against the reagent blank. Figure (3) shows that the maximum absorption of the yellow dye under primary conditions is located at 433.0 nm at which the reagent blank shows a negligible absorption.

General procedure for Kinetics of the reaction

Aliquot of 1 mL of the standard drug solution containing various amount (30.0-100.0 µg) of TC. HCL was added conical flask. The flask was left to stand for 1 minute at 70 °C, then after transferred into 4 mL cuvette. 2mL of $3 \times 10^{-2} M$ hot diazonium salt was then added and the absorbance of this solution was immediately measured at 433 nm. The value of absorbance was recorded at 1 s intervals for 30 minutes (i.e. 0 and 1800s).

Kinetics of the reaction

Verification of reaction order

The rate of the reaction was studied to determine its order according to following equation [12, 13]:

$$\text{Rate} = k'[\text{drug}]^{n}$$

This was accomplished by using different concentrations (10.0-100.0) µg.mL^{-1} of TC. HCl solution and a constant concentration of diazonium salt. It was found that the reaction rate is [TC.HCl] dependent. Graphs on Figure (4) shows that reaction rate is increased with the increasing [TC.HCl] concentration.

Measurements were carried out by variable time method, which could be used for rate (in terms of $\Delta A/\Delta t$) estimation, (Figure 4).

The logarithmic form of the above equation could be written as follows:

$$\log \text{rate} = \log \frac{\Delta A}{\Delta t} = \log k' + n \log[\text{Drug}]$$

Where: k' and n represent the rate constant, and the order of the reaction.

A is the absorbance, and t is the measuring time.

Regression least square plot of log [TC. HCl] versus $\log \Delta A/\Delta t$ for the studied drug with the yielded the calibration equation are shown in Figure (5).

This was accomplished by using different concentrations (10.0-100.0 µg.mL^{-1}) of TC. HCL solution and a constant concentration (1.00×10^{-2} M) of diazonium salt. It was found that the reaction rate is [TC. HCL] dependent. Graphs on Figure (5) shown that reaction rate is increased with the increasing [TC. HCL] concentration. Measurements carried out by variable time method, could be used for rate (in terms of $\Delta A/\Delta t$) estimation. The rate equation could also be expressed in logarithmic form as:

$$\log \text{rate} = \log \frac{\Delta A}{\Delta t} = \log k' + n \log[\text{TC. HCL}]$$

Where: A is the absorbance, and t is the measuring time.

Regression least square plot of log [TC. HCL] versus log (rate) is shown in Figure (5). The regression equation is:

$\log(\text{rate}) = 0.9812 \log[\text{TC. HCL}] + 0.1571,$ with correlation coefficient (r) = 0.9925. Accordingly, the value of k'= 1.4358 s^{-1} and the reaction is pseudo-first order since the value of n equals to 0.9812 (≈1).

Quantitation methods

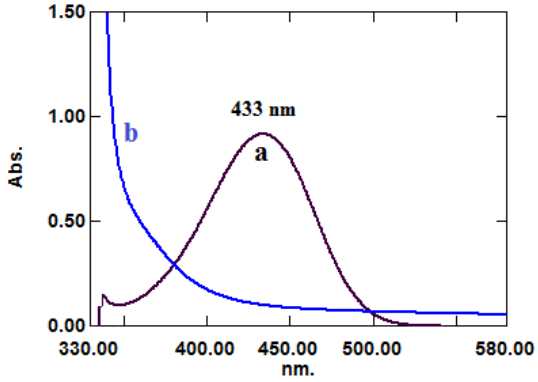

Figure 3: Absorption spectra of: (a) Reaction product of TC.HCl vs reagent blank (b)Blank solution against distilled water.

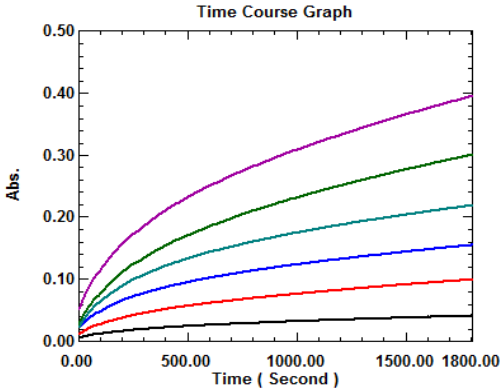

Figure 4: Absorbance versus time for the reaction of different con. of TC.HCl

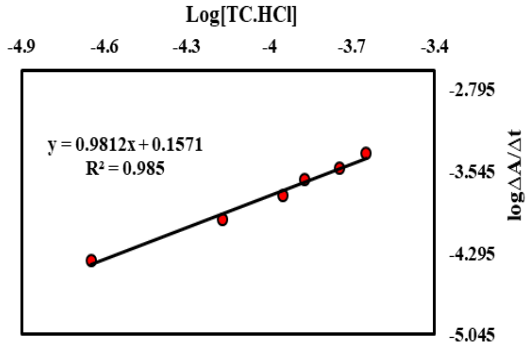

Figure 5: Calibration plot of logarithm rate of the reaction versus molar conc. of TC.HCl.

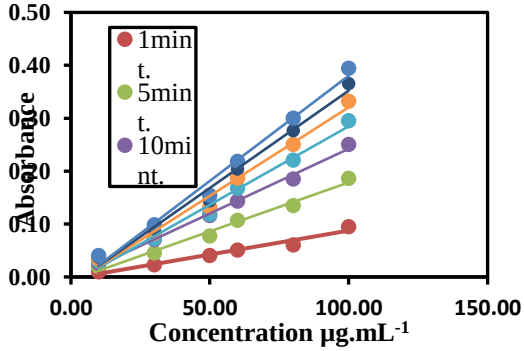

Figure 6: Calibration plot of absorbance versus the concentration of TC. HCL at preselected fixed time.

Table 3: Regression equations at fixed time method for the determination of TC. HCL.

Time (min)	Regression equation	Correlation coefficient (r)	*SD $_{\Delta y}$	**LOD (µg. mL^{-1})	***LOQ (µg.mL^{-1})
1	Y= 0.0009C - 0.0029	0.9832	0.0055	0.2107	0.7023
5	Y= 0.0019C - 0.0065	0.9932	0.0073	0.2785	0.9283
10	Y= 0.0024C - 0.0024	0.9975	0.0059	0.2257	0.7522
15	Y= 0.0030C - 0.0121	0.9934	0.0113	0.4306	1.4353
20	Y= 0.0033C - 0.0140	0.9987	0.0129	0.4964	1.6546
25	Y= 0.0037C - 0.0158	0.9928	0.0145	0.5556	1.8520
30	Y= 0.0040C - 0.0174	0.9925	0.01606	0.6144	2.0480

*SD = for the difference absorbance, **LOD = 3.3 σ /S and ***LOQ = 10 σ/S.

The determination of drugs is based on above equation using the rate data. Different techniques were carried out (i.e. the initial rate, fixed concentration rate constant and fixed time)[14,15] for this purpose. Appropriate methods for analysis were selected according to their applicability, sensitivity, and the values of correlation coefficient (r) and intercept of the regression equations.

Rate constant method

The best way to obtain a rate constant (k') value for the reaction is to plot the logarithm of absorbance versus time for TC.HCl concentrations in the range (2.2501×10^{-05} – 2.2501×10^{-04} M). Pseudo first order rate constant (k') corresponding to different concentrations for each drug was calculated from the slopes multiplied by -2.303 and are presented in Table (1) with regression equation of concentration versus k':

The regressed relation between C vs the values of k' is given by:

$k' = -0.3882 \times -7 \times 10^{-4}$ (r = 0.9764)

The correlation coefficient value indicates poor linearity; thus, this method was abandoned.

Fixed absorbance method

The absorbance of TC. HCL reaction product for different concentrations of drug (6.7502×10^{-5} – 2.2501×10^{-04} M) were recorded Figure (4). The time in seconds at a selected value of absorbance (0.0954), was measured. A plot of the reciprocal value of measured time against initial [TC. HCL], Table (2), was constructed with the following regression equation:

$$\frac{1}{t} = 98.123\ C - 0.008\ (r = 0.9358)$$

The value of correlation coefficient (r = 0.9358) for calibration graph indicated poor, which is considered a

Table 4: Validation of regression and assay for the determination of TC. HCL by the proposed method.

Parameter	Fixed time method (10 mint.)	Initial rate method
λmax (nm)	433.0	
Regression equation	Y=0.0024C - 0.0024	Y=0.9812C + 0.1571
Linearity (μg mL^{-1})	10.0-100.0	
Slope ± SD	0.00244 ± 8.7E-05	0.981167 ± 0.06051
Intercept ± SD	-0.00244 ± 0.00545	0.15707 ± 0.24317
Correlation of linearity (R^2)	0.9864	0.9850
Correlation coefficient (r)	0.9932	0.9925
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	ε =1066.632	ε = 436074.7
Sandell's sensitivity($\mu g.cm^{-2}$)	0.4167	0.00102
Limit of detection ($\mu g.mL^{-1}$)	0.2257	0.17421
Limit of quantification ($\mu g.mL^{-1}$)	0.7522	0.5807

Table 5: Evaluation of the accuracy and precision of the initial rate and fixed time methods for determination of TC. HCL.

Method	Con. of TC. HCL ($\mu g.mL^{-1}$)		RE%	RSD%
	Taken	Found*		
Initial rate	30	31.1816	3.9386	1.9721
	50	50.7757	1.5514	1.7122
	80	79.8561	-0.1799	1.7123
Fixed time	30	31.1458	3.8194	1.8729
	50	49.6412	-0.7177	1.6462
	80	79.4430	-0.6963	1.6590

*Average of three measurements.

Table 6: Analysis of TC. HCL by the initial rate and fixed time methods.

Pharmaceutical	Method	Assay (mg/Capsule)	Con. ($\mu g.mL^{-1}$)		*Recovery	*SD	*RSD%
		Spiked	Found	Taken	*Found	%		
TC.HCl (Iraq) 250mg/Capsule	Initial rate	100.0	109.9720	20	21.9944	109.9719	0.9124	4.1487
			104.0778	50	52.0389	104.0778	1.2502	2.4024
TC.HCl (India) 250mg/Capsule			99.2060	40	39.6824	99.2060	0.3748	0.9445
			97.9218	80	78.3374	97.9217	1.5185	1.9384
TC.HCl (Iraq) 250mg/Capsule	Fixed time	100.0	112.0180	20	22.4036	112.0181	0.8713	3.8891
			101.6562	50	50.8281	101.6563	1.1746	2.3108
TC.HCl (India) 250mg/Capsule			97.9773	40	39.1909	97.9771	0.3539	0.9031
			99.3598	80	79.4878	99.35976	2.6372	3.3177

*Average of three measurements.

disadvantage. Table (2) summarize the values of (1/t) for different TC. HCL concentration.

Initial rate method

In this method, the reaction in the initial rate with respect to the time (0-600 sec.) versus the log [TC. HCL] plots were rectilinear within the range of 10.0 – 100.0 $\mu g.mL^{-1}$ (Figure 5). The reaction was first order by according to the value of slope 0.9812 (~1) and the value of correlation coefficient was (0.9925). The value of detection limit was calculated and found to be 0.17421 $\mu g.mL^{-1}$ while limit quantification was 0.5807 $\mu g.mL^{-1}$. These values indicated the high sensitivity of the proposed method to determine low amounts of TC. HCL.

Fixed time method

In the fixed time method, varying amounts of TC. HCL were used for determination of reaction rate at a preselected fixed time (1, 5, 10, 15, 20, 25 and 30 minute) and the absorbance values were measured and plotted against [TC. HCL].

The low values of standard deviation (SD), limit of detection (LOD) and limit of quantification (LOQ) are given in Table (3). A fixed time of 10 minute indicates that this method could successfully applied for determination of TC. HCL in its pure form and in pharmaceutical preparations, Figure (6).

Validation of the proposed methods

Accuracy and precision

The accuracy and precision of the proposed kinetic spectrophotometric methods were determined at different concentration levels of each drug by analyzing three replicate samples of each concentration by both the initial rate and fixed time methods. The relative standard deviation (RSD %) and the relative error percent of the methods for the results are shown in Table (5). The calculate values of the mentioned parameters prove the high reproducibility and accuracy of the results. These good levels of precision are suitable for quality control analysis of each drug in its pharmaceutical tablet.

Application of the proposed methods

The results obtained for the two suggested kinetic spectrophotometric methods to analyses TC. HCL were satisfactory due to their good agreement with the labeled amounts.

The results shown in Table (6) are for the analysis of TC. HCL in commercial tablet by both methods (initial rate and fixed time). The mean recoveries and RSD% values were 102.7944± 2.3585% and 102.7528± 2.6052039% respectively.

CONCLUSION

In the present study, determination of TC. HCL in its pure form and in its pharmaceutical dosage was investigated by two new kinetic methods (initial rate and fixed time). It was found that the proposed methods are precise, accurate, and sufficiently sensitive to be applied for determination of small amounts of TC. HCL. Therefore, the proposed methods could be recommended for the analysis of TC. HCL in quality control laboratories.

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