Determination of Tetracycline Hydrochloride by Using Dapsone as a Reagent in Developed Spectrophotometric Analysis

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Abstract: Inexpensive spectrophotometric method has been developed to estimate its pure concentration. This method is based on the azo coupling reaction using Diaz onium dapsone, which is formed in a solution containing hydrochloric acid and sodium nitrate. The resulting salt reacts with tetracycline hydrochloride to form a Tetracycline hydrochloride is considered an antibiotic, and a sensitive, easy, fast, and orange azo dye at a wavelength of 380 nanometres. After optimizing the reaction conditions, a recovery rate of 100% was achieved, with a Beer-Lambert limit between (2 - 26) µg /mL, and a Sandal sensitivity of 0.0238 µg.cm². The method also demonstrated high sensitivity without any interference from the excipients present in the formulation, with a sensitivity value of 20160 $L \cdot mol^{-1} \cdot cm^{-1}$ and a correlation coefficient of 0.9992. The limit of detection (LOD) was found to be 0.094 and the limit of quantification (LOQ) was found to be 0.28. The reaction was stable for a sufficient period, making the method suitable for estimating tetracycline in its pure form and in pharmaceutical preparations, such as capsules, without any interference.

1 INTRODUCTION

Tetracycline in IUPAC nomenclature (4S,4aS,5aS,6S,12aR)-4- (dimethyl amino)-1,6,10,11,12a- pentahydroxy-6-methy l-3, dioxo-4,4a,5,5,5a -tetrahydrotetracene-2- carboxamide; hydrochloride 12- (Fig. 1).

They are yellow crystals [1], soluble in water, resulting in a clear yellow solution that can become cloudy if left for a long time. It acts as an antibiotic to eliminate bacterial growth such as acne and urinary tract infections, as it is active against a variety of gram-positive and gram-negative bacteria. It can be used as an anti-ulcer medication in the treatment of acute bronchitis [3], [4], it is economical [5], and in the treatment of certain types of diseases, including cholera, trachoma, rickettsia [6], and others. Due to the importance of this drug, there are many analytical methods to estimate the purity of tetracycline hydrochloride, the most important of which are spectroscopic methods [4]-[9], HPLC [10], flow injection techniques [11]-[14], and TLC [15]. Multispectral determination [16], effective absorption and photo degradation using visible light [17], fluorescence spectrum [18], as well as nanotechnology methods [19], [20].

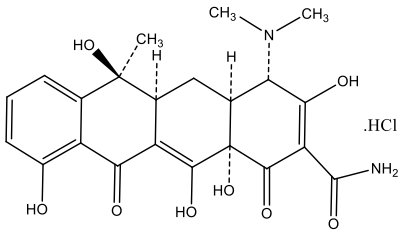

Figure 1: Structure tetracycline hydrochloride [1], [2].

2 EXPERIMENTAL

2.1 Preparation of Solutions

A solution of tetracycline hydrochloride with a concentration of 1000 µg/ml.It was prepared with a weight of 0.1020 grams of the pure substance, which was later dissolved in distilled water. The solution was then transferred to a 100 ml flask, where the volume was completed with the solvent, water, until reaching the mark on the flask. This is the stock solution, and a solution with a

concentration of 250µg /ml is prepared from the stock solution.

2.2 Preparation of a 4× 10-3 M Dapsone Solution

0.1 g of the substance were weighed and dissolved in 25 ml of ethanol in a 100 ml glass flask. Then, the volume of the flask was completed with distilled water to the marked line of the flask.

2.3 Sodium Nitrate 0.01 M

0.06901 grams of the solid were weighed and then dissolved in distilled water. After that, the volume of the flask was filled to the specified mark of 100 ml.

2.4 Preparation of a 1N Hydrochloric Acid Solution

The dilution law was used for the concentrated solution with a concentration of 11.9 M, and from this solution, using the same law, a 0.05 N solution with a volume of 100 ml was prepared. The solvent was distilled water.

2.5 Preparation 1N Sodium Hydroxide Solution

A weight of 4.001 grams of the solid was taken and dissolved in distilled water. The volume was completed until the mark on the 100 ml bottle was reached.

3 SETTING THE OPTIMAL CONDITIONS

A study was conducted to determine the optimal amount of hydrochloric acid solution, which gave the highest absorption of the colored product. 1 ml of the reagent and 1 ml of sodium nitrite were added, along with increasing volumes of acid ranging from 0.5 to 2.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml NaOH. The results indicated that 1.5 ml of acid is the most suitable volume, and this volume was used for subsequent experiments. Table 1.

3.1 Study of the Effect of Sodium Nitrite

A study was conducted to determine the optimal amount of sodium nitrite solution, which gave the highest absorption of the colored product. 1 ml of the reagent and increasing volumes of sodium nitrate from (0.5-2.5) ml, and 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml NaOH. The results indicated that 1 ml of sodium nitrite is the most suitable volume, and this volume was used in subsequent experiments. Table 2.

Table 1: Results in the table No. (1) Quantitative study of acid.

Volume ml (HCl) 0.05 N	0.5	1	1.5	2	2.5
ABS	0.453	0.468	0.489	0.485	0.487

Table 2: Study of the effect of sodium nitrite.

NaNO2(ml)	0.5	1	1.5	2	2.5
0.01 M ABS	0.461	0.479	0.471	0.472	0.470

3.2 Study of the Effect of Sodium Hydrochcide

A study was conducted to determine the optimal amount of sodium hydroxide solution that gave the highest absorption of the colored product. 1 ml of the reagent, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, and 1 ml of the drug were added, along with increasing volumes of sodium hydroxide (2.5-0.5 ml). The results indicated that 1 ml of sodium hydroxide is the most suitable volume, and this volume was used in subsequent experiments. Table 3.

Table 3: Study of the effect of Sodium hydrochcide.

NaOH (1N): ml	0.5	1	1.5	2	2.5
ABS nm	0.468	0.488	0.485	0.487	0.484

3.3 Study the Effect of Dapsone (Reagent) con. 4× 10-3 M

A study was conducted to determine the optimal amount of reagent solution, which gave the highest absorption of the colored product. Increasing volumes of the reagent (0.3 - 2) ml, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml sodium hydroxide were added. The results indicated that 1 ml of the reagent is the most suitable volume, and this volume was used in subsequent experiments. Table 4.

Table 4: To study the effect of dapsone (Reagent) Reaction sequence.

Reagent 4×10-3M	0.3	0.6	1	1.5	2
1000µg/ml ABS	0.366	0.393	0.483	0.480	0.482

3.4 Study of the Effect of the Formed Interaction Time

The effect of time was studied to find the necessary time to reach the stable product in the equilibrium state and completion of the reaction under optimal conditions (1 ml of the reagent, 1 ml of sodium nitrite, 1.5 ml of hydrochloric acid, 1 ml of the drug, and 1 ml of sodium hydroxide). It was observed that under these conditions, the colored product is formed as the absorption increases over time, and the color stabilizes after at least 10 minutes and remains stable for 40 minutes due to the stability and permanence of the product formed over time. Table 5.

4 FINAL ABSORPTION SPECTRUM

When the drug is coupled with the reagent under experimental conditions, the resulting product has an orange color. The result is measured after 10 minutes, and the highest absorption was measured at a wavelength of 380 nanometers compared to the reference solution. Figure 2 illustrates this, showing the resulting product against the reference solution and the absorption spectrum of the reference solution against distilled water and the reference solution against distilled water.

Table 5: Study of the effect of the formed interaction time.

Time minute	0	10	20	30	40	50	60
Abs/ nm	0.454	0.485	0.483	0.438	0.444	0.434	0.399

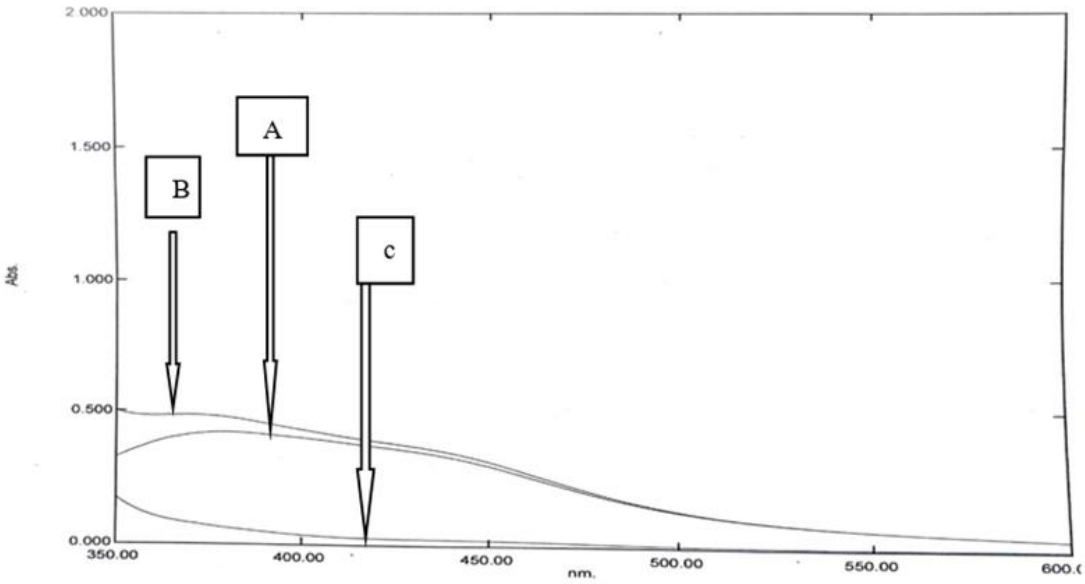

Figure 2: The final (A) absorption spectrum of tetracycline hydrochloride under optimal conditions against the blank solution, (B) absorption spectrum of tetracycline hydrochloride against distilled water, and (C) the blank solution against distilled water.

5 CALIBRATION CURVE

After establishing the optimal conditions, a series of 25 ml glass vials were taken, and 1 ml of the reagent, 1 ml of sodium nitrite, and 1.5 ml of hydrochloric acid were added, along with increasing volumes of the drug 0.2 – 26 ml and 1 ml of 1 M sodium hydroxide. The volume was then completed to the marked line with distilled water. The samples were left for a period of time, and then the absorbance was measured at a wavelength of 380 nm against the blank solution, as shown in Figure 3, which demonstrates Beer's Law for the drug tetracycline hydrochloride at concentrations of 2 - 26 µg/ml in a final volume of 25 ml. The molar absorptivity was calculated from the (1) - (7):

$$Ɛ = a \times 1000 \times M = 20160, \quad (1)$$

$$S = M/Ɛ = 0.0238, \quad (2)$$

$$L.O.D = 0.094, \quad (3)$$

$$Ɛ = L.mol\text{-}1.cm\text{-}1, \quad (4)$$

$$a = ml.µg\text{-}1.cm\text{-}1, \quad (5)$$

$$M = g.mol\text{-}1, \quad (6)$$

$$S = µg.cm\text{-}2. \quad (7)$$

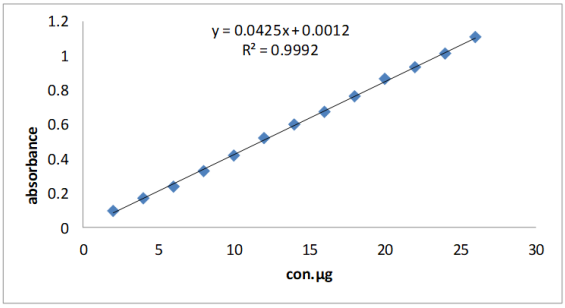

Figure 3: Calibration curve for tetracycline hydrochloride.

6 PRECISION AND COMPATIBILITY OF PURE MATERIAL

Accuracy is the extent to which the measured result is close to the true or accepted value.
Repeatability refers to the degree of repeatability and consistency in the results when repeated measurements are made on the same sample The analysis is considered good if the results are close to each other Table 6.

Table 6: Precision and compatibility of pure material.

RSD%	Average recovery%	Recovery%	Average RE%	Average Abs nm	Con. µg/ml
1.52	-	100	0.011	329	8
2.62	-	100	0.016	609	15

7 THE NATURE OF THE RESULTING OUTPUT

7.1 Job Method

The continuous variable method was used to determine the interaction percentage of the drug tetracycline hydrochloride with the reagent. This method relies on the changes that occur in the solutions with the drug and the reagent. This method was carried out by following the procedure outlined below. Figure 4. Different solutions were prepared containing increasing volumes of the drug from 1-9 ml, and the reagent from 1-9 ml, both at a concentration of 250µg/ml (5.4×10-4 M) in a final volume of 25 ml. The remaining solutions were added under optimal working conditions, and the reaction output was measured against the blank solution at a wavelength of 380 nanometers, indicating a reaction ratio of 1:1.

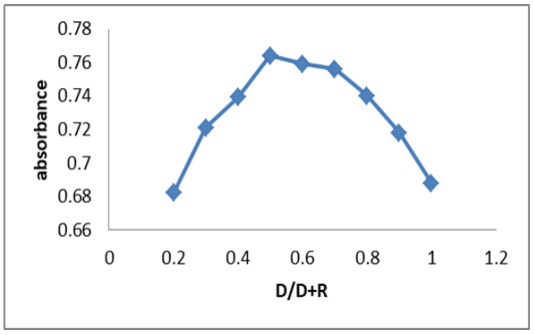

Figure 4: The curve of continuous variables (Job's method of the result formed from the interaction of the solvent and the reagent) between the drug and the reagent-(Dapson)/(tetracycline hydrochloride) + (Dapson).

7.2 Mole Ratio Method

A series of 25 ml volumetric flasks were prepared, and varying volumes of the reagent were added. The remaining variables obtained under optimal conditions were added, followed by the addition of 1 ml of the drug to each flask at the same drug concentration. The absorbance was then measured against the blank solution at a wavelength of 380 nanometers. Figure 5.

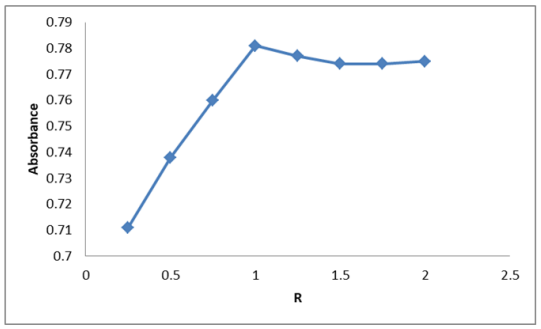

Figure 5: The molar ratio curve of the product formed by the nitration reaction and the coupling, between the drug and the reagent. () tetracycline hydrochloride /(Dapson).

8 REACTION EQUATION

The azo coupling reaction between tetracycline hydrochloride and Dapsone is depicted in Figure 6.

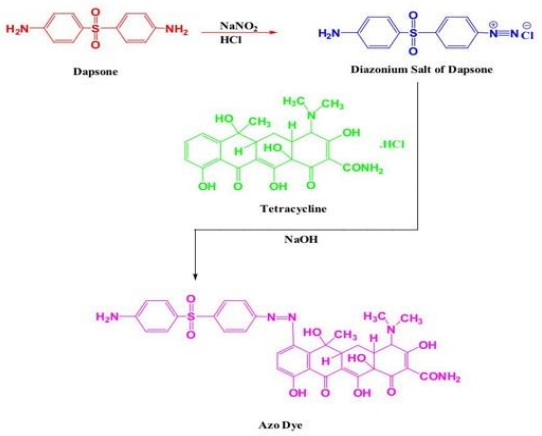

Figure 6: Reaction equation.

9 APPLICATIONS

After the developed method was successful, it was applied to the preparation and different concentrations (30, 15, 8) micrograms/ml were taken. The same working steps followed when preparing the standard curve were applied, and the efficiency of the method was demonstrated as shown in the following Table 7.

$$A1 / A2 = C1 / C2 \quad (8)$$

$$C2 = A2\ C1 / A1 \quad (9)$$

Table 7: Determination for six.

	Amount taken µg/ml	Amount found µg/ml	Recovery,* %	Average Recovery	RSD,* %
Tetracycline Hydrochloride, Company: SDI	8	8.2	102	101.3 %	2.2
	15	15.1	100		2.1
	30	30.8	102		4.0

10 THE STANDARD ADDITIONS METHOD

The experiment was conducted using standard additives to prove that the developed method is reliable, achieves high efficiency, and is not affected by interferences, as shown in the following Figure 7.

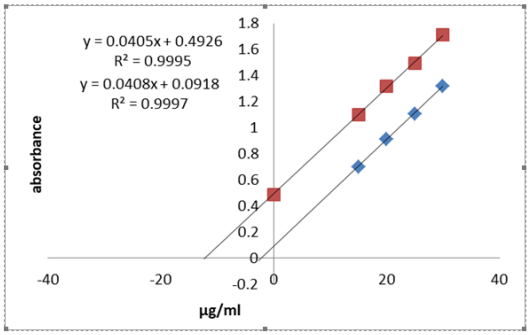

Figure 7: The standard additions method.

From the graph of the standard addition method, the following results emerged Tables 8 and 9.

Table 8: Standard additions method.

Tetracycline hydrochloride	Amount taken	Amount found	recovery	company
	10 ppm	9.95	99.5%	SDI

Table 9: Optical characteristics of the method.

Optical characteristics of the method	
λmax	380 nm
The limits of linearity µg/ml	2-26
Molar absorptivity L.mol-1.cm-1	20160
Color of the dye	orange
Recovery	100%
Sandal allergy µg.cm-2	0.0238
Slop	0.0425
Intercept	0.0012
LOD µg/ml	0.09
LOQ µg/ml	0.2

11 CONCLUSIONS

A simple and accurate spectrophotometric method was developed for the determination of tetracycline hydrochloride, based on the azo coupling reaction between tetracycline hydrochloride and a suitable chemical reagent, resulting in the formation of a colored complex with easily measurable absorbance. Experimental results demonstrated that this method exhibits high reproducibility and excellent accuracy, along with resistance to potential interferences from other components in pharmaceutical samples. The method's linear range was evaluated, showing a proportional response across a wide concentration range, thereby enhancing the reliability of the results. Moreover, this technique is characterized by its simplicity and cost-effectiveness, making it suitable for routine application in quality control laboratories and pharmaceutical product monitoring. Based on these attributes, this method can be considered a reliable and efficient analytical tool for the determination of tetracycline hydrochloride in various dosage forms, contributing to the assurance of pharmaceutical product quality and safety.

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