Spectrophotometric Determination of tetracycline hydrochloride Using 2,4–dinitrophenyl hydrazine as Coupling Reagent

Roaa M. Khaleel¹ and Dawooh H. Mohammed²
¹,²Chemistry Department / /Education College for Girls/ Mosul University
* e-mail: Roaa.mohanad.1989@gmail.com¹ , Dr.alhaboo@uomosul.edu.iq²

ABSTRACT
Simple, highly sensitive and accurate spectrophotometric method has been developed to determination of tetracycline hydrochloride in aqueous solution. The proposed method is based on the coupling of tetracycline hydrochloride with 2,4-dinitrophenylhydrazine (2,4-DNPH) in the presence of potassium periodate to form an intense orange color dye at 360nm. Beer's law is obeyed in the concentration range 0.1-9 μg/ml with the molar absorptivity of 1.262 ×10⁴ liter.mol⁻¹.cm⁻¹, with limit of detection (LOD) of 0.0123μg/ml and limit of quantification (LOQ) of 0.0412μg/ml while the RSD value of 0.184 – 0.467 % depending on the concentration. The proposed method was performed successfully to the determination and analysis of tetracycline hydrochloride in pharmaceutical formulations with average recovery of 100.23 %.

Key words: Spectrophotometric, Tetracycline hydrochloride, 2,4-DNPH reagent.

1. Introduction

Tetracycline hydrochloride is considered a member of the family of tetracycline hydrochloride’s, which offers biomass interpreters on a wide spectrum. It has been frequently used in treating negative Gram and positive Gram and bacteria. It is also considered as one of the least toxic drugs and the best family of medicines. The molecular form of the Tetracycline hydrochloride is C₂₂H₂₄N₂O₈.HCl, while the molecular weight is 480.93 g/mol [1]. Fig.(1) depicts the structure formula of Tetracycline:
tetracycline hydrochloride is mainly effective for bacteriostatic treatment and is used in the field of human and veterinary medicine by linking it with the ribosome 30S. It prevents the arrival of Amino acyl tRNA to future sites in the mRNA complex [2] and exerts its effect against microbes. The practical mechanism is to inhibit the protein industry in bacteria cells through blocking the access of amino acids to the ribosome. The main working area of tetracycline hydrochloride is in the protein industry. It has been found that tetracycline hydrochloride can be disseminated through the outer cell membrane of Gram-negative bacteria [3]. The misuse or overuse of tetracycline hydrochloride yields the formation of bacteria resistant, which reduces its usage [4]. Tetracycline hydrochloride is taken orally and absorbed in the duodenum and rapidly spread in body fluids and tissues. It is concentrated in the bile and excreted through the kidneys and its absorption can be further reduced by milk alkaline materials and products, aluminum salts, magnesium and calcium. It is occasionally available in ointments to treat skin infections and acne [5]. The determination methods of tetracycline hydrochloride are; spectrophotometric methods [6-10], Chromatographic methods [11-17] and Electrical methods [18-20].

2. Experimental

2.1 Apparatus

- Spectrophotometric measurements were performed using a dual-beam spectrophotometer: UV-VIS 1800 spectrophotometer, Japan Shimadzu with quartz type cells 1 cm in all absorption measurements.
- The weighing was performed using the balance type of: artorius BL 201 S

2.2 Reagents

All the used chemicals and analytical reagents were of a high grade of purity and available from commercial sources in the local market and are used directly. Distilled water was used for all dilutions of reagents and samples.

2.3 Material solutions

- Standard tetracycline hydrochloride solution (100 mg / ml): This solution is dissolved 0.01 g of the pure material in distilled water, solution is transferred into a 100ml volumetric flask.
- Potassium periodate (5×10^{-3}M): This solution is dissolved 0.1150 g of pure material in 3 ml of dilute sulfuric acid and complete the volume using distilled water in a 100 ml volumetric flask.
- 2,4 –dinitrophenyl hydrazine solution (2×10^{-3}M): This solution is prepared by dissolving 0.1981 g of material in 5 ml of dilute sulfuric acid and completes the volume with distilled water in a 100 ml volumetric flask.
- Surfactant: the SDS and CTAB surfaces were papered at 0.1% concentration is dissolved 0.1g of it in 100ml of distilled water. Triton x-100 solution is prepared at a concentration 1% is dissolved one gram of it in 100ml of distilled water

3. Procedure for pharmaceutical preparations
10 capsules were grinded well and a certain portion of the final powder was accurately weighted to give an equivalent to about 250 mg of tetracycline hydrochloride was dissolved distilled water. The prepared solution transferred to 100 ml volumetric flask and made up to the mark with distilled water forming a solution of 100μg ml⁻¹ concentration. The solution was filtered by using a filter paper to avoid any suspended particles.

4. Results and discussion

1 ml of 2,4-dinitrophenyl hydrazine solution (2×10⁻³M) were added into a series of 25ml calibrated flask and 2 ml of potassium periodate (5×10⁻³M) followed by the addition of increasing volumes of (100μgml⁻¹) tetracycline hydrochloride and followed by 1 ml of (0.1%) Triton X-100, and wait for three minutes for the purpose of completing the oxidation, then absorbance of all solutions was measured at a wavelength of 360 nm versus their blank solutions.

4.1 Study of the Optimum Reaction Conditions:

The spectrophotometric properties of the colored product as well as the different experimental parameters that affect the color development and its stability were carefully studied and optimized by using 1 ml of tetracycline hydrochloride at a concentration of 100 mg / ml in a final volume of 25 ml (with a final concentration of 4 mg / ml).

4.2 Effect of amount the reagent 2,4-dinitrophenyl hydrazine

The concentrations and volume of different reagents that affect the 2,4-dinitrophenyl hydrazine reactions were also carefully studied and optimized by increasing volumes 0.25-2 ml from 2,4-di-nitrophenyl hydrazine 2x10⁻³ molar to a volume of 25 ml containers, which contains 1 ml of tetracycline hydrochloride solution (100 mg / ml) and 1 ml from a potassium periodate solution(5×10⁻³ molar) and diluted to the mark with distilled water. The experimental results are presented in Table 1.

| ml of 2,4 Dinitrophenyl hydrazine 2×10⁻³M | 1     | 3     | 5     | 7     | 10    |
|-----------------------------------------|-------|-------|-------|-------|-------|
| 0.25                                    | 1.249 | 1.247 | 1.248 | 1.248 | 1.247 |
| 0.5                                     | 1.258 | 1.257 | 1.257 | 1.256 | 1.255 |
| 1                                       | 1.266 | 1.266 | 1.265 | 1.266 | 1.266 |
| 1.5                                     | 1.227 | 1.228 | 1.228 | 1.226 | 1.225 |
| 2                                       | 1.198 | 1.196 | 1.193 | 1.193 | 1.188 |

4.3 Effect of amount of the oxidization agent (potassium periodate) :

The reaction of oxidization amount to reagent was studied. The absorbent was measured at different periods of time. The results are shown in Table 2.

| ml of potassium periodate 5 × 10⁻³ molar | 1     | 3     | 5     | 7     | 10    |
|-----------------------------------------|-------|-------|-------|-------|-------|
| 0.25                                    | 1.249 | 1.247 | 1.248 | 1.248 | 1.247 |
| 0.5                                     | 1.258 | 1.257 | 1.257 | 1.256 | 1.255 |
| 1                                       | 1.266 | 1.266 | 1.265 | 1.266 | 1.266 |
| 1.5                                     | 1.227 | 1.228 | 1.228 | 1.226 | 1.225 |
| 2                                       | 1.198 | 1.196 | 1.193 | 1.193 | 1.188 |

Table 1: Effect of amount 2,4-dinitrophenyl hydrazine

Table 2: Effect of oxidization amount
Effect of surfactants:

The use of different surface tension, cationic, anionic and neutral materials as a result of the instability of the dye formed with a view to show the effect on the intensity and stability of the colored product. The results are shown in Table 3.

| Surfactant       | Absorbance / ml of surfactant used |
|------------------|-----------------------------------|
|                  | 0.5     | 1.0     | 1.5     | 2       | 2.5     |
| SDS (0.1%)       | 1.225   | 1.227   | 1.228   | 1.229   | 1.226   |
| Triton X-100 (1%)| 1.275   | 1.277   | 1.272   | 1.270   | 1.268   |
| CTAB (0.1%)      | 1.249   | 1.250   | 1.253   | 1.258   | 1.256   |

Effect of oxidation time:

The time required to complete the oxidation was studied by taking a set of 25 ml volumetric containers containing 1 ml of tetracycline hydrochloride solution at a concentration of 100 mg/ml, 1 ml of reagent solution 2,4 dinitrophenyl hydrazine 2 x 10^-3 molar, 2ml of potassium periodate 5 x 10^-3 molar and added 1 ml of Triton-X100 at a concentration of 2%. Finally, discard the solutions for different time periods, then dilute it with distilled water up to the mark and then measure the absorption of the solutions at a wavelength of 360 nm against their blank solution. The results are shown in Table 4.

| Tetracycline hydrochloride | Time (Min) | Directly | 3 | 5 | 10 | 15 | 20 |
|---------------------------|------------|----------|---|---|----|----|----|
| Absorbance                | 1.275      | 1.277    | 1.274 | 1.270 | 1.266 | 1.261 |

Order of addition

In order to choose the best addition sequence for the reactants, a number of different laboratory experiments were conducted to find out the effect of the addition sequence of solutions. The results shown in Table 5, it explains that the best addition sequence is (I), to form the colored product and it was adopted in subsequent experiments accordingly.
Table 5: Effect of order of addition on absorption

| Order of addition                  | Order number | Absorbance |
|-----------------------------------|--------------|------------|
| D+R+O+Triton-100                  | I            | 1.277      |
| O+R+Triton-100+D                  | II           | 1.217      |
| D+O+R+Triton-100                  | III          | 1.266      |
| R+D+O+Triton-100                  | IV           | 1.168      |
| D+R+Triton-100+O                  | V            | 1.123      |
| R+O+Triton-100+D                  | VI           | 1.197      |

R: 2,4-dinitrophenyl hydrazine (2×10⁻³M)
O: potassium periodate (5×10⁻³M)
D: Tetracycline hydrochloride (100 mg/ml)

4.7 Final absorption spectra:

The final absorption spectrum for the formed product was drawn up and according to the optimal conditions, where the absorption spectra graph showed the maximum intensity of absorption at a wavelength of 360 nm while the blank solution gained a weak absorption at this region as shown in Fig.(2).

![Absorption spectra of 4 μg/ml tetracycline hydrochloride measured](image)

Fig.(2): Absorption spectra of 4 μg/ml tetracycline hydrochloride measured
[A:sample and blank, B:sample and water, C:blank and water]

4.8 Quantification

Under the optimum conditions, a linear calibration curve is constructed by plotting absorbance versus concentration. Beer's law is obeyed over the range (0.1-9)μg/ml of the solution Fig.(3).
4.9 Accuracy and precision

The RSD ratio was calculated for three concentrations 2, 3.6, 6 mg / ml of tetracycline hydrochloride. By computing the average of six iterations for each and treating according to the optimal conditions, the results were summarized in Table 6. These results tell us that the proposed method provided an efficient accuracy recovery rate (100.23%), and high precision, while the value of RSD did not exceed 0.467. The results are depicted in Table 6.

### Table 6: Accuracy and precision

| Concentration of Tetracycline.HCl taken (µg/ml) | Concentration of Tetracycline.HCl found (µg/ml) | Recovery % | Average Recovery % | Relative Error % | RSD % |
|-----------------------------------------------|-----------------------------------------------|------------|--------------------|------------------|------|
| 2                                             | 1.99                                          | 99.5       | 100.23             | -0.5             | ± 0.467 |
| 3.6                                           | 3.604                                         | 100.11     | 100.23             | 0.11             | ± 0.259 |
| 6                                             | 6.065                                         | 101.08     |                    | 1.08             | ± 0.184 |

4.10 Nature of the product:

Continuous changes methods (Job method) [21] and molar ratio were applied to study the structural ratio of the colored product consisting of the coupling of reagent 2,4-di-nitrophenylhydrazine with tetracycline hydrochloride in the aqueous solution. The concentration of the reagent and tetracycline hydrochloride are $2 \times 10^{-4}$ molar. The number of solutions was prepared by taking volumes from the pharmaceutical compound and the reagent. Therefore, the total volume of the two solutions was fixed by 3 milliliters. The rest of the additions were completed according to the optimal conditions and dissolving with distilled water up to the mark. Absorbance of the solutions was measured wavelength 360 nm versus its blank solutions, Fig.(4) shows that the ratio was 1:1.
In order to know the nature of the resulting dye, the molar ratios method was applied, which included the addition of increased volumes of reagent solution 2,4-di-nitrophenyl hydrazine at a concentration of $2 \times 10^{-4}$ mol to a fixed volume 1 ml of tetracycline hydrochloride solution. The results are shown in Fig. (5). In fact, these results are in agreement with the job’s method result. The proposed dye for the colored product is shown in Fig. (6).

$$\text{[S]} = \text{Tetracycline hydrochloride conc.}, \quad \text{[R]} = \text{Reagent conc.}$$

**Fig.(4): Continuous Change Method for Tetracycline hydrochloride Solution with reagent 2,4-Diphenyl Hydrazine**

**Fig.(5): plotting the molar ratio of the tetracycline reaction with 4,2-di-nitrophenylhydrazine.**
4.11 Proposed chemical reaction:

The Fig.(7) explains the proposed chemical reaction:

![Chemical Reaction Diagram]

4.12 Interference effect:

In order to test the efficiency and selectivity of the proposed method, the effect of the presence of some common pharmaceutical additive such as Sucrose, Fructose, Starch, Sodium chloride and Glucose that are usually present in dosage forms was studied by adding different amounts of foreign substances to 4 mg of tetracycline hydrochloride. The result in Table 7, indicates that there were no significant interference produced by the foreign substances on the proposed procedure.

| compounds        | Recovery% of 4 μgml⁻¹of Tetracycline hydrochloride | Recovery% of foreign compounds added | 100 μgml⁻¹ | 300 μgml⁻¹ | 500 μgml⁻¹ |
|------------------|---------------------------------------------------|-----------------------------------|----------|----------|----------|
| Sucrose          |                                                   |                                   | 100.70   | 100.01   | 101.33   |
| Fructose         |                                                   |                                   | 97.96    | 98.35    | 98.66    |
| Starch           |                                                   |                                   | 100.62   | 100.23   | 100.93   |
| Sodium chloride  |                                                   |                                   | 97.24    | 97.49    | 97.80    |
| Glucose          |                                                   |                                   | 98.66    | 99.06    | 99.45    |
4.13 Standard addition method:

To prove the efficacy of the proposed method and its accuracy in determining that no significant interference, the standard addition was applied to the pharmaceutical preparation. The method included adding 0.5 and 0.75 ml of the pharmaceutical preparation previously prepared. Fig.(8) and Table 8. reflect standard addition of tetracycline hydrochloride.

![Standard addition of tetracycline hydrochloride](image)

**Fig.(8): Standard addition of tetracycline hydrochloride**

**Table 8: Determination of tetracycline hydrochloride in pharmaceutical preparations using the standard addition method**

| Type of drug     | Tetracycline HCL Present mg/ml | Tetracycline HCL Measured µg/ml | Recovery (%) |
|------------------|--------------------------------|---------------------------------|--------------|
| Tetracycline HCL | 2                              | 1.99                            | 99.5         |
| Capsule (250 mg) | 3                              | 3.01                            | 100.33       |

5. Conclusion

In this work could be concluded the proposed method for determination Tetracycline hydrochloride assay was simple, precise, accurate, and sensitive. The proposed method can be satisfactorily applied to the analysis of tetracycline hydrochloride in bulk and pharmaceutical formulations.

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