

Spectrophotometric Determination of Tetracycline Hydrochloride Through Coupling with Sulphanilic Acid

Rasul J Ali (MSc)¹, Hawar J Sadiq Hawezy (MSc)² and Mohammad S Abdullah (PhD)³

Abstract

Background: Tetracycline is a broad-spectrum antibiotic that is used to treat diseases caused by different types of bacteria.

Objective: To estimate tetracycline hydrochloride in pharmaceuticals through coupling with a new reagent, sulfanilic acid.

Patients and Methods: The proposed method for determination of tetracycline hydrochloride was applied to four different types of pharmaceutical drug companies and the results were compared with a standard method (HPLC method) to determine their compatibility and the results were satisfactory.

Results: The method is based on azo coupling reaction between sulfanilic acid and tetracycline in alkaline medium. The colored compound produced by the reaction has a higher absorbance at a wavelength of 403nm. The Beer's law was applied in the concentration range (8.0 - 95.0 µg / ml) and the detection limit, molar absorptivity and correlation coefficient were 0.2 µg / ml, 6.536x10³ L / mol.cm and 0.9997 respectively. The method was successfully applied to the evaluation of tetracycline hydrochloride in pharmaceuticals.

Conclusion: The proposed method for determination of tetracycline hydrochloride is a simple and sensitive method, where the tetracycline hydrochloride can be estimated at low concentration and accurately. This method needs a simple device and the method can be used in quality control centers. The recommended method has been successfully applied to estimate tetracycline hydrochloride in pharmaceuticals.

Key words: Spectrophotometry, Tetracycline, Azo-coupling.

Corresponding Author: mohammad.abdullah@su.edu.krd

Received: 16th April 2018

Accepted: 24th June 2018

<https://doi.org/10.26505/DJM>

¹ Clinical Biochemistry Department -Health Science College- Hawler Medical University-Erbil-Iraq.

² Chemistry Department- Faculty of Science and Health- Koya University- Erbil-Iraq.

³ Chemistry Department- Education College- Salahaddin-Erbil University- Erbil-Iraq.

Introduction

Tetracyclines represent a class of antibacterial compounds with broad spectrum antibiotics for their high activity against nearly all gram-positive and gram-negative bacteria[1]. The tetracyclines are a well-known and widely used family of antibiotics. These compounds are particularly useful in several types of both common and rare infections

including rickettsial infection, chlamydial infections, a typical pneumonias, ehrlichiosis and Lyme disease[2]. Various methods for the determination of tetracycline and its derivative have been used, including spectrophotometry[3-6] HPLC(7-9) , flow injection analysis technique(10-12) voltammetry[13] fluorometry(14,15) and LC-

mass[16].Some of the used methods require expensive instruments and good operation skill. The main purpose of the present study was to develop a simple, fast and new method for determination of tetracycline in pharmaceutical formulation through using new reagent which is sulphanilic acid.

Patients and Methods

Apparatus

Spectral and absorbance measurements were carried out using Cary-100 uv-visible spectrophotometer and 1-cm quartz cuvette was used.

Reagents and materials

All solutions were prepared from analytical-reagent grad chemicals with distilled water. Tetracycline Hydrochloride (obtained from Awamedica co.) Stock solution (1000ppm) was prepared by dissolving 0.10g of Tetracycline Hydrochloride in little amount of distilled water and completed to 100ml in volumetric flask with distilled water. A 2.0% w/v of sodium nitrite solution was prepared by dissolving of 2.0g of NaNO₂(obtained from SD Fine Chem Limited 98%) in distilled water and diluting to 100ml with distilled water. Concentrated Hydrochloric acid solution (purchased from SD Fine Chem Limited 98% , sp.gr 1.83) was used for preparation of 0.5M solution through suitable dilution of the concentrated solution. Sulphanilic acid, obtained from Fluka co., a 0.1% solution was prepared by dissolving 0.1g of sulphanilic acid in distilled water and completing the volume to 100ml with distilled water. A 2.0% potassium hydroxide solution was prepared by

dissolving 2.0g of KOH (Global Chemical Company) in distilled water and diluting to 100ml with distilled water.

Sample preparations

Tetracycline Hydrochloride capsules from different brands were purchased in local drugstore and analyzed by proposed method. The content of ten capsules of each commercial brand were weighed and mixed in a mortar. Accurately equivalent amount of one content of a capsule was weighed and transferred to a beaker. The Tetracycline Hydrochloride was dissolved in distilled water then transferred to 500ml volumetric flask and completed to the mark with distilled water.

Recommended procedure for the determination of Tetracycline Hydrochloride

To a series of 25.0ml volumetric flask a 0.6ml of 2.0% sodium nitrite solution, 0.8ml of 0.5M hydrochloric acid solution and 0.8ml of 0.1% sulphanilic acid were added. The solution mixed well then aliquots of Tetracycline Hydrochloride containing 200.0-2375.0 µg and 2.8ml of 2.0% potassium hydroxide solution were added. The solution was completed to the mark with pure ethanol. The absorbance was measured at 403nm against reagent blank prepared in similar conditions.

Statistical analysis

The data were analyzed by paired t-test at 95% level of confidence [17].

Results

Preliminary investigations: Preliminary tests revealed that the reaction of Tetracycline

Hydrochloride with coupling reagent (diazonium salt of sulphanilic acid) in basic medium give a yellow coloured compound which show maximum absorption at 383nm. To a 25ml volumetric flask 1ml of 2.0% sodium nitrite solution, 0.5ml of 0.5M hydrochloride acid solution and 2.0ml of 0.1% sulphanilic acid solution were added and mixed well. To the same solution a certain volume which contains 500µg of Tetracycline Hydrochloride and 2.0ml of 2.0% sodium hydroxide solution were added. The solution was mixed and diluted to the mark with distilled water. The absorbance was measured immediately after dilution at 383nm against reagent blank.

Effect of sodium nitrite

Table (1) shows the effect of sodium nitrite volumes on the absorbance. It was found that 0.6ml of 2.0% sodium nitrite solution gave best results among studied volumes. Therefore, this volume was selected as a suitable volume for further studies.

Effect of the acids

Different types of acids like hydrochloric acid, acetic acid, nitric acid and sulphuric acid have been tested for maximum absorption achievement. The hydrochloric acid gave the best results in contrast with other acids. The effect of hydrochloric acid

volumes also was investigated as shown in Table(2). The absorbance increased with increasing hydrochloric acid volumes up to 0.8ml, after this volume the colour start to decrease. Therefore, 0.8ml of hydrochloric acid solution was used for subsequent work.

Effect of sulphanilic acid

In order to achieve the optimum condition for the determination of Tetracycline Hydrochloride, different volumes of sulphanilic acid were checked Figure(1). The colour intensity increased with increasing the volume of added sulphanilic acid till 0.8ml after that the absorbance reduced. Therefore, this volume was adopted for the determination.

Effect of the base

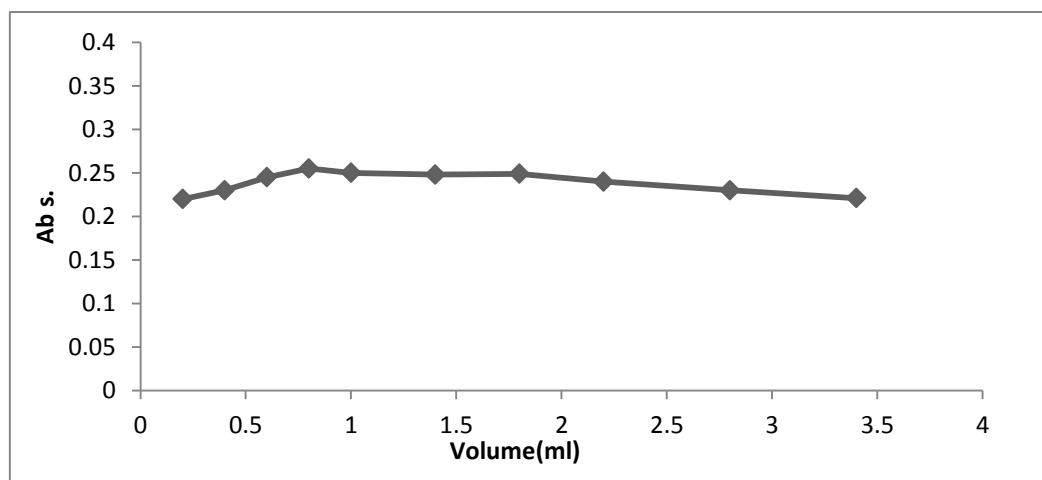
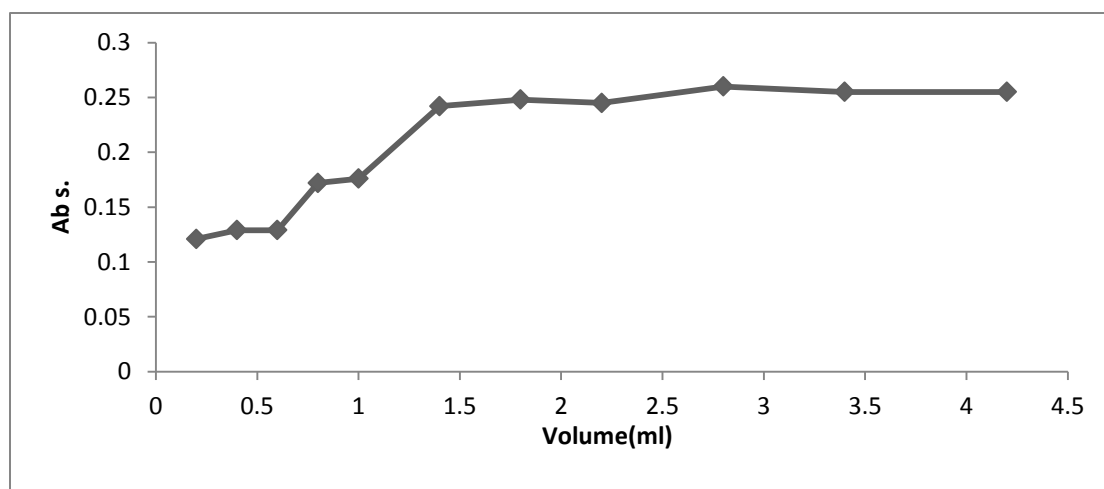
The coupling reaction between diazonium ion and Tetracycline Hydrochloride gives colour in basic medium only as preliminary investigations indicated that. Therefore, different bases like KOH, NaOH and Na₂CO₃ with the same concentration have been used for this purpose. It was found that potassium hydroxide (2%w/v) shows best results. The effect of KOH volumes on color intensity also has been tested and it was found that 2.8ml of KOH solution shows maximum absorbance Figure(2). Therefore, this volume was selected for further work.

Table (1): Effect of Effect of sodium nitrite volumes on colour intensity.

Absorbance	Volume(ml)
0.159	0.2
0.230	0.4
0.240	0.6
0.235	0.8
0.230	1.0
0.220	2.0

Table (2): Effect of hydrochloric acid volumes on colour intensity.

Absorbance	Volume(ml)
0.230	0.2
0.237	0.4
0.244	0.6
0.245	0.8
0.231	1.0
0.196	2.0
0.160	3.0

**Figure(1):**Effect of sulphanic acid volumes on colour intensity.**Figure(2):** Effect of potassium hydroxide volumes on colour intensity.

Effect of temperature and time on colour intensity

It is well-known that aryl and alkyl diazonium ions stability affected by temperature (18). Therefore, the reaction was studied in ice and in room temperature. It has been found that the colour intensity was slightly affected by temperature when the reaction was done in the ice, but the effect was not significant. Therefore the procedure for the determination was done at room temperature which is easier for conducting. The absorbance was not stable when the solution was diluted with distilled water to the final volume. This feature leads to use other solvents for dilution in order to get stable colour, for this purpose different solvents were used for dilution. Among them absolute ethanol produced a stable colour for 75minutes, therefore ethanol was used for the dilution of the reaction mixture. Under the optimum conditions, the final absorption spectra were checked, and it was found that the λ_{max} of the coloured compound shifted to 403nm.

Linearity

After obtaining the optimum conditions for determination of Tetracycline Hydrochloride, a calibration curve was obtained as shown in Fig.3. It was found that the calibration curve was linear in the concentration range within 8.0 - 95.0 $\mu\text{g/ml}$, and the coefficient of determination was 0.9995.

Accuracy and precision

The accuracy of the proposed method was determined for different concentration

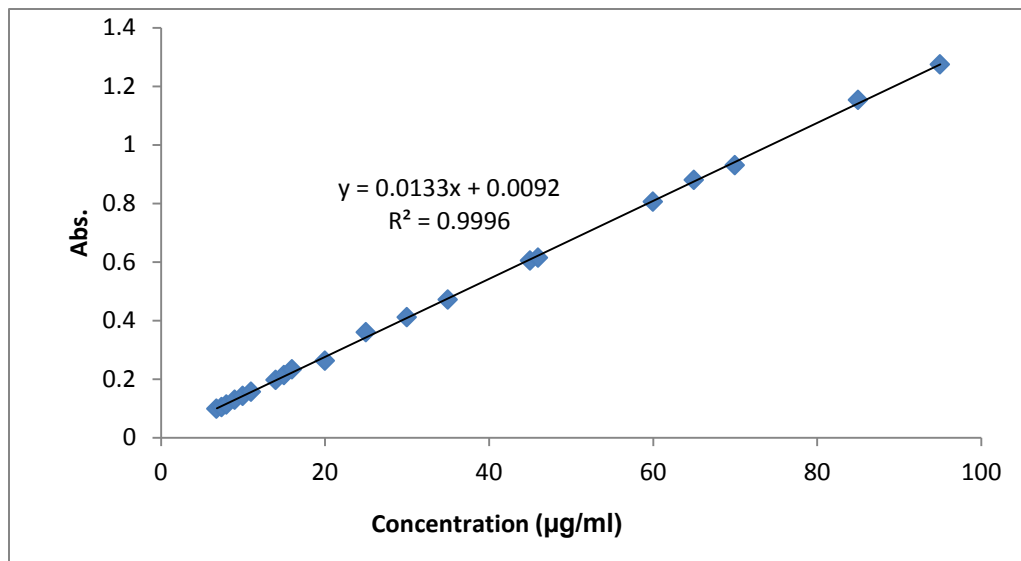
through calculating relative error percentage (Er%) and the precision study was achieved by measuring three replicate ($n=3$) for different concentration and calculating relative standard deviation percentage (RSD %). The results indicating that the proposed method is accurate and precise as shown in Table (3).

Interferences study

The usefulness of the proposed study for assay of commercial Tetracycline Hydrochloride capsules was assessed by studying the effect of some common excipients used in pharmaceutical preparations. The influences of glucose, lactose, maltose, sucrose, KCl and NaCl on the determination of Tetracycline Hydrochloride were tested and the results are shown in Table 4. The proposed method showed good tolerance for interfering species.

Application of the method

The proposed method was applied for the determination of Tetracycline Hydrochloride in commercial pharmaceutical formulations. The results were compared with standard method (HPLC method) [19]. Table 5 shows good agreement between results obtained by both methods. The statistical analysis for the results was done using t-test. The results of the test shows no significant difference at 95% confidence level ($\alpha = 0.05$) between results obtained by present method and that obtained by HPLC method. The calculated t value = 0.54 which is less than tabulated value 2.35.



Figure(3): Calibration curve of spectrophotometric determination of Tetracycline Hydrochloride.

Table (3): Precision and accuracy data of Tetracycline Hydrochloride determination.

Concentration (µg/ml)	S.D	RSD%	Er%
8.0	0.0002	0.390	-3.70
45.0	0.0035	0.601	-0.006
95.0	0.0475	4.070	0.030

Table (4): Effect of foreign species on determination of Tetracycline Hydrochloride.

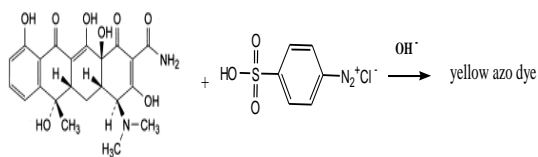
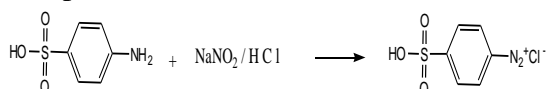
Foreign Species	Conc. (µg/ml)	Er %
Glucose	1600	-2.1
Lactose	1200	1.2
Maltose	1000	3.2
Sucrose	1400	-4.4
Potassium Chloride	1000	2.5
Sodium Chloride	1000	4.4

Table (5): Determination of tetracycline Hydrochloride by proposed method.

Trade name	Company name	Quantity(mg)	Found by present method(mg)	Found by Standard method(mg)	Er%
Tetracycline	Kontam,China	500	491.42	475.27	3.4%
Tetralab	Laborate,India	500	491.59	495.06	-0.7%
Tetramac	Macdols,India	500	471.79	499.78	-5.6%
Apcycline	Ajanta,India	250	242.46	252.70	- 4.05%

Discussion

The tetracycline and their derivatives are widely used as bacteriostatic drugs. Different methods for their assay have been reviewed[3]. The present method based on diazotization of sulphanilic acid by nitrous acid. The probably formed diazonium ion coupled with Tetracycline Hydrochloride in alkaline medium to give yellow coloured compound as follows(6,18):



The method showed that the compound formed has a good stability period for 75 minutes where tetracycline hydrochloride can be estimated at this time without a change in the absorption value. The value of molar absorptivity and the detection unit show that the method is sensitive to low concentration of tetracycline hydrochloride. The Beer law was applied in a wide linear range and has a low detection limit where the calibration curve can be prepared in this range. The method demonstrated the tolerability to many of the interfering substances, which showing selective method for determination of tetracycline hydrochloride. The proposed method was applied to several samples of pharmaceutical preparations as well as compared with the results obtained by standard method. Where the percent of errors

was low and were compatible with each other. It is recommended to determine tetracycline by proposed method in biological fluids in patients.

References

- [1] José LR, Patrícia LW, Helena RP, Leonardo P. Flow-injection spectrophotometric determination of tetracycline and doxycycline in pharmaceutical formulations using chloramine-T as oxidizing agent. *Quim Nova*. 2009;7(32):1764-1769.
- [2] Zeina S, Singh G. Doxycycline and other tetracyclines in treatment of bone metastasis. *Anti-Cancer Drugs*. 2003; 10(14):773-778.
- [3] Salah MS, Ibrahim ZA, Nawal AA. Complexometric-spectrophotometric assay of tetracycline in drug formulations. *Talanta*. 1988; 5(35):375-378.
- [4] Kaml HA, Zainab TY, Basm AA. Spectrophotometric determination of tetracycline in some pharmaceutical preparations. *J. Basrah Researches(Sciences)*. 2006;32:43-48.
- [5] José LR, Flávio CB, Mayara S R, Helena R P, Leonardo P. A simple spectrophotometric method for the determination of tetracycline and doxycycline in pharmaceutical formulations using chloramine-T. *Ecl. Quim Sao Paulo*. 2010;4(35):139-146.
- [6] Nabeel SO, Rasha JA. Spectrophotometric determination of tetracycline by coupling with diazotized 4-aminoantipyrine in presence of cetylpyridinium chloride. *Raf. J. Sci*. 2012; 2(23) :72-84.

- [7] Kazuo I, Norio O, Mitsuru Y. Determination of tetracycline antibiotics by reversed phase high-performance liquid chromatography with fluorescence detection. *J. Chromatography A*. 1992; 623:153-158.
- [8] Johnathan WF, Yugang Z. Simultaneous determination of tetracycline, oxytetracycline and 4-epitetracycline in milk by high performance liquid chromatography. *Food Chem.* 2007; 103: 1297-1313.
- [9] Shalaby AR, Nadia AS, Abou-Raya SH, Wafaa HE, Mehaya FM, Validation HPLC method for determination of tetracycline residue in chicken meat and liver. *Food chem.*, 2011;124 :1660-1666.
- [10] Angelina P, Leonidas P P, Celeste M L, Irene S, Antony CC. Determination of tetracycline and its major degradation products by chemiluminescence. *Analytica Chimica Acta*. 2000 ;405:51-56.
- [11] Xingwang Z, Yang M, Zhjun Z. Flow-injection chemiluminescence determination of tetracyclines with in situ electrogenerated bromine as the oxidant. *Analytica Chimica Acta*. 2001; 440:143149.
- [12] Alan T, Wirat R, Chalernporn T, Saisunee L. Flow injection chemiluminescence determination of tetracycline. *Analytica Chimica Acta*. 2005; 541: 105-111.
- [13] Ricardo TK, Marcelo RS, Antonio DA, Marcos FS. Construction of an electrochemical sensing platform based on platinum nanoparticles supported on carbon for tetracycline determination. *Sensors and Actuators B*. 2016; 228: 207–213.
- [14] Ken-ichi N, Yoshio K, Nobuyasu T. Fluorometric determination of tetracyclines in Honey by High Performance Liquid Chromatography. *Food Hygiene and Safety Science*. 1991; 1(32) :43-47.
- [15] Wen-Bao C, Yi-Bing Z, Yun-Xiang C, Lin-Yun Hu., Spectrofluorimetric determination of tetracycline and anhydrotetracycline in serum and urine. *The Analyst*. 1992; 117(8):1377-1378.
- [16] İbrahim K, Şeyda K, Mansur H. Development of a rapid method for the determination of antibiotic residues in honey using UPLC-ESI-MS/MS. *Food Sci. Technol.* 2016;36(1):90-96.
- [17] David H. *Modern Analytical Chemistry*. 1st ed. McGraw-Hill Higher Education. 2000.
- [18] Robert TM, Robert NB. *Organic chemistry*. 5th ed. Prentice-Hall India; 1989.
- [19] British Pharmacopoeia Commission, *British pharmacopoeia*. The Stationery Office, London. 2009.