ASSAY OF TETRACYCLINE IN PHARMACEUTICAL PREPARATIONS, SPIKED INDUSTRIAL WASTEWATER AND CHICKEN MEAT SAMPLES USING VISIBLE SPECROPHOTOMETER TECHNIQUE

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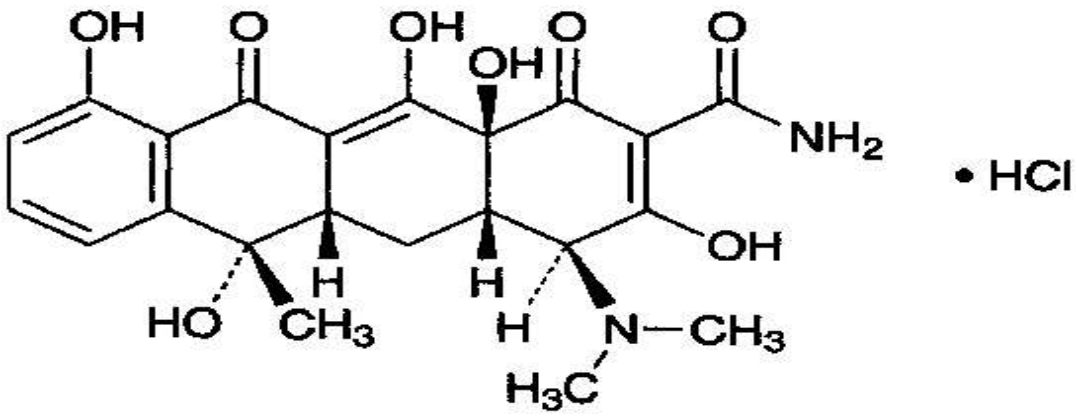
ABSTRACT

A simple, accurate, and rapid visible spectrophotometric method has been developed for the determination of tetracycline hydrochloride in pure, pharmaceutical preparations and environmental water samples. The method is based on the oxidation of tetracycline hydrochloride by sodium hypochlorite in alkaline medium to form a red colored product with an absorption maximum at 400 nm. Beer's Law was obeyed in the range of 2-24 µg/ml with molar absorptivity of 1.346×10^4 $L.mol.^{-1}.cm^{-1}$. The relative standard deviation of the method was less than 2% and accuracy (average recovery) was 100 ± 0.85 % .The optimum conditions for all color development are described and the proposed method has been successfully applied for the determination of tetracycline hydrochloride in pharmaceutical preparations, chicken meat and waste water samples.

INTRODUCTION

Tetracycline hydrochloride, figure 1, is 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, 12a-octahydrotetracene-2- carboxamide monohydrochloride (1). Tetracycline (TC) is

a widely used antibiotic and it has high activity against nearly all Gram–positive and Gram–negative bacteria (2**).** Various methods have been developed for the



$C_{22}H_{24}N_2O_8$, HCl 480.9

Figure 1: **Chemical structure of tetracycline hydrochloride**

determination of tetracycline hydrochloride in pharmaceutical preparations and biological samples including fluorimetry (3), electro chemical method (4,5), atomic Absorption spectrophotometry (6), liquid chromatography (7-11), capillary electrophoresis (12) and chemiluminescence (13-15). UV-Visible spectrophotometry is still considered to be a convenient and low cost method for the analytical determination of tetracycline in Pharmaceuticals formulations. A number of spectrophotometric and colorimetric procedures for the determination of tetracycline in bulk material and dosage forms are reported in the literature (16-20). Recently, attention has been focused on the development of methods for the determination of tetracycline antibiotics in environmental samples and food products and. The low cost of tetracycline antibiotics, high antimicrobial activity and relatively are responsible for their wide use not only in medicine but also in animal.(21) The aim of this work is to develop a very simple, accurate and sensitive spectrophotometric method for the determination of tetracycline hydrochloride**.**

MATERIAL AND METHODS

Apparatus

A spectra scan 50 UV-visible spectrophotometer with 1.0 cm quartz cells and Centrifuge (Labofuge 400, Heraeus Instruments GmbH, Hanau, Germany) was used,

Reagents

All chemicals used were of analytical grade and the tetracycline hydrochloride standard material was provided by state company of drug industries and medical appliance (NDI) Nineveh – Iraq.

Prepared Tetracycline hydrochloride stock solution (1000 ppm) was done by dissolving 0.1g of tetracycline hydrochloride in 100ml distilled water in a volumetric flask.

Tetracycline hydrochloride standard solution (100 ppm) was prepared by diluting10 ml of stock solution to 100 ml of distilled water in a volumetric flask.

Sodium hypochlorite solution (0.1%) was prepared by diluting 2.5 ml of 4% sodium hypochlorite to 100 ml of distilled water; this solution was standardized every 4-5 days and stored in a dark bottle (22, 23).

Sodium hydroxide solution (1N) was prepared by dissolving 4g of NaOH in 100 ml distilled water in a volumetric flask.

Recommended procedure

Aliquots of standard solution of tetracycline hydrochloride (50-600µg) were transferred into a series of 25 ml calibrated flasks, 1 ml of 1 N sodium hydroxide and 1 ml of 0.1% sodium hypochlorite solution; dilute the solution to the mark with distilled water. The absorbance of the red-colored product was measured at 400 nm against a reagent blank.

Assay procedure for pharmaceutical preparations:-

Capsule

An amount of finely ground capsule powder equivalent to 100 mg of tetracycline hydrochloride was accurately weighed into a 100ml calibrated flask, 60ml of distilled water added and shaken for 20 min, the volume was then made up to the mark with distilled water, mixed well, filter into 100 ml calibrated flask, dilute to 100-ml with distilled water mixed well and aliquot of this solution was treated as described under recommended procedure.

Ointment

Preparation a composite sample was done by mixing the contents of five containers and accurately weighs a sample equivalent to 10 mg of tetracycline hydrochloride into a 100-ml beaker. Then 80- ml of hot distilled water was added and heated on a water-bath for 20 min, cooled in freezer, filtered into 100 ml calibrated flask, diluted to 100-ml with distilled water, mixed well and aliquot of this solution was treated as described under recommended procedure.

Procedure for spiked chicken meat

Chicken meat samples were obtained from local shops. Cut and weighed 10 g into a 500-ml beaker. Three blank chicken samples were spiked with a combined working tetracycline stock solution 10ml of a 1000ppm. The contents were homogenized in cutting blender for5 minutes, and transferred back into the beaker plus washings. Samples were then transferred into labeled 15-ml centrifuge tubes. Each sample was then centrifuged at 3500 rpm for 10 minutes The supernatant was poured into a second beaker, filtered into 100 ml calibrated flask, dilute to 100-ml with distilled water and mixed well. Aliquot of this solution was treated as described under recommended procedure.

Procedure for spiked industrial wastewater

To demonstrate the practical applicability of the proposed method, industrial waste water sample from the state company for drug industries and medical appliances, Mosul-Iraq, were analyzed, since industrial waste water sample was found to be free from tetracycline hydrochloride. Synthetic samples were prepared by spiked known amount of tetracycline hydrochloride with the concentrations ranging from 4-16 $\mu g.ml^{-1}$ and aliquot of this solution was treated as described above for recommended procedure.

RESULTS AND DISCUSSION

Tetracycline hydrochloride is oxidized in alkaline medium by sodium hypochlorite solution forming a red-colored product which absorbs maximally at 400nm is shown in

Figure [2]. The colorless reagent blank has practically negligible absorbance at this wavelength and this wavelength was recommended for determination

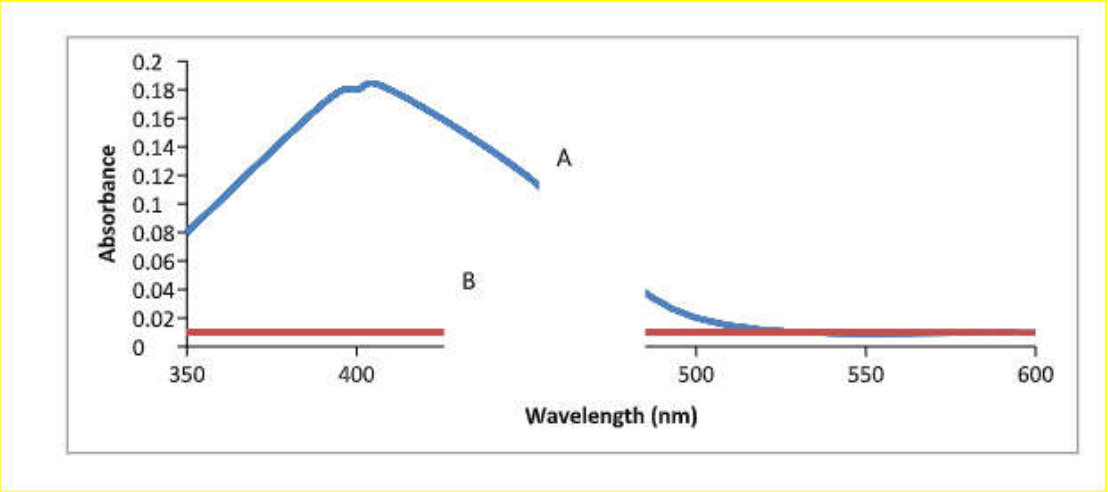
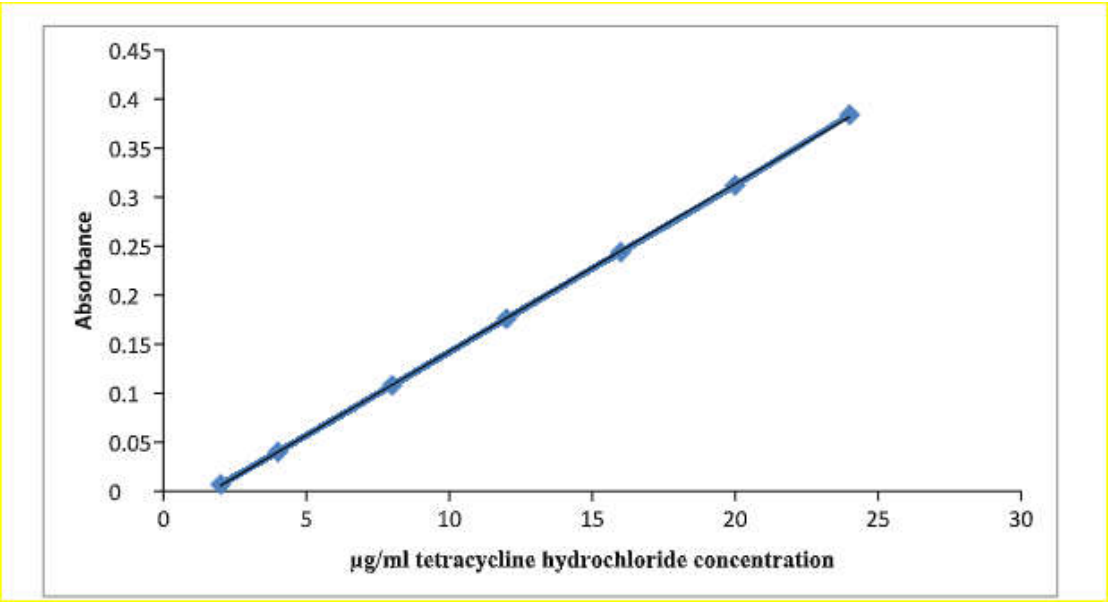


Figure 2: Absorption spectra of A- tetracycline .HCl (12µg/ml) product against blank. B- Blank against distilled water.

The reaction variables were optimized by varying each variable while keeping others constant for obtaining maximum absorbance. The oxidation reaction was found to be quantitative in the sodium hydroxide medium. It was found that 1 ml of 1 N sodium hydroxide solution gives high sensitivity and this amount has been used for subsequent experiments. The effect of the amount of sodium hypochlorite on the absorbance was investigated. A maximum and constant absorbance was founded with 1 to 2 ml of 0.1% sodium hypochlorite solution and 1.0 ml has been used for subsequent experiments. The color reaction occurred at room temperature immediately and remained stable for at least 24h and a reaction time of 5 min was

selected for reproducible results. Under the experimental conditions described, Beer's law is obeyed over the concentration range 2- 24 µg /ml (Figure 3).



. **Figure 3: Calibration graph of tetracycline hydrochloride.**

A regression analysis of Beer's law plot at 400 nm revealed a good correlation (r=0.9999, n=7) .The graph of the absorbance versus the concentration of tetracycline

Hydrochloride showed a low intercept (-0.017) and slope (0.028) and described by a regression equation Y = ax + b (where x is the concentration of tetracycline hydrochloride in µg /ml, Y is the absorbance, a is the slope and b is the intercept. The apparent molar absorptivity was 1.346×10^4 l. mol^{-1} .cm^{-1}. The optical characteristics are given in Table [1].

Table 1: Optical characteristics and statistical data for regression equation of the proposed method

Parameters	Value
λ max (nm)	**400**
Beer´s law limits (µg .ml^{-1})	**2 – 24**
Molar absorpitivity (1.mol^{-1}.cm^{-1})	**1.346×10^4**
Determination coefficient (r^2)	**0.999**
Regression equation (Y= a × + b)	
Slope (a)	**0.028**
Intercept (b)	**-0.017**

Accuracy and precision

The accuracy and precision of the method was established by analyzing the pure drug solution at three different levels. The average recovery which is a measure of accuracy is 100 ± 0.85 revealing high accuracy of the method. The relative standard deviation (RSD) , which is an indicator of precision is better than ±2% . The results are complied in Table [2].

Table 2:Accuracy and precision of the proposed method.

Tetracycline taken µg/ml	Tetracycline found µg/ml	Er (%)[a]	RSD (%)
5	4.96	-0.8	1.3
10	10.085	0.85	1.8
20	20.15	0.75	1.5

a: Mean of six determinations

The effect of foreign compounds or excipients in assay of tetracycline

The interfering effect of foreign species often accompanied with tetracycline hydrochloride in the pharmaceutical preparations were studied by adding different amounts of foreign species to 300µg/25ml of tetracycline hydrochloride in solution and the recommended procedure for the determination of tetracycline hydrochloride was followed . The species are considered to interfere seriously if the cause aching of more than 2% in the absorbance obtained for tetracycline hydrochloride alone (24). The results of the recovery analysis are presented in Table [3]. Excipients at the concentration show in Table [3] do not interfere with the assay .In addition; recoveries in most cases were around 100%.

Table 3: Determination of tetracycline hydrochloride in presence of excipients

Excipients	Amount taken, (µg/ml)	Average recovery, * %
Talc	**700**	**99.95**
	1000	**100.08**
Mannitol	**800**	**100.09**
	1000	**99.95**
Mg – stearate	**600**	**100.05**
	1000	**100.09**
Starch	**500**	**100.08**
	1000	**100.03**
Microcrystalline cellulose	**500**	**99.98**
	1000	**99.94**

* Average of five replicate determinations.

Application of the proposed method

The proposed method was successfully applied to the analysis of tetracycline hydrochloride in capsules, ointment, spiked industrial wastewater and chicken meat samples. The result of analysis for pharmaceutical formulations revels that there is close agreement between the results obtained by the proposed method and the label claim Table [4], and the results of water samples and chicken meat samples. Table [5,6] showed that the recovery values obtained were close to100%.

Table 4:Assay of tetracycline.HCL in pharmaceutical formulations.

Pharmaceutical formulation supplied by NDI	Amount of tetracycline hydrochloride * Proposed method	Label claim	%Recovery
Capsules 250mg	248.8 mg	250 mg	99.52
Ointment 3%	2,98%	3%	99.33

*Mean of ten determinations.

Table 5:Determination of tetracycline .HCL in spiked industrial wastewater sample.

Water samples	Tetracycline.HCL(µg/ml)* Taken	Found	%Recovery
Industrial wastewater	4	4	100
	10	9.95	99.5
	16	15.9	99.37

*Mean of ten determinations.

Table 6:Determination of tetracycline.HCL in spiked chicken meat samples.

samples	Tetracycline.HCL(µg/ml)* Taken	Found	%Recovery
spiked chicken meat	4	4	100
	12	12.15	101.25
	20	20.2	101

*Mean of ten determinations for three samples of chicken meat.

CONCLUSION

In the current study, a simple, rapid, precise and accurate spectrophotometric method was developed and validated for the determination of tetracycline hydrochloride in pharmaceutical preparations and industrial waste water samples .The method free from such experimental variables as heating or solvent extraction step. The method rely on the use of simple and cheap chemicals and techniques and can be used for rapid routine determination and quality control of tetracycline hydrochloride

in pure form, bulk sample ,pharmaceutical preparations, spiked chicken meat and real industrial waste water sample.

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تقدير التتراسايكلين طيفيا في المستحضرات الصيدلانية ولحم الدجاج والمياه الصناعية المطروحة

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الخلاصة

تم اختبار طريقة طيفية جديدة مباشرة لتقدير التتراسايكلين في حالته النقية وفي بعض مستحضراته الدوائية وفي لحم الدجاج وفي المياه الصناعية المطروحة،تتميز الطريقة بالبساطة والانتقائية والحساسية والدقة وتعتمد الطريقة على أكسدة التتراسايكلين بواسطة الهايبوكلورات في الوسط القاعدي لتعطي ناتج احمر اللون له أقصى امتصاص عند الطول ألموجي400 نانوميتر وأمكن تقدير الكميات التي تتراوح بين2-24 مايكروغرام\مل وان معامل الامتصاص المولاري للطريقة المقترحة هو 1.346×10^{4}لتر .مول$^{-1}$.سم$^{-1}$.ان الانحراف القياسي النسبي للطريقة أقل من2 % وبدقة (معدل استرجاعية) 100± 0.85 % . وتم دراسة الظروف المثلى للتفاعل .وطبقت الطريقة بنجاح لتقدير هيدروكلوريد التتراسايكلين في حالته النقية وفي مستحضراته الصيدلانية وفي لحم الدجاج وفي المياه الصناعية المطروحة

REFERENCES

1- The Japanese Pharmacopoeia, (2016). 17th edn, English Version, The Ministry of Health, Labor and Welfare, ,p1670

2-Martindale "The Extra Pharmacopeia(2009)-The Complete Drug Reference" edited by Sean.C,sweetman , ,Pharmaceutical press, London, UK, 36th ed. P.162

3- Shou-Miao Zhao, Su-Qin Han (2010) ;Thioglycolic Acid Capped CdS Quantum Dots as Fluorescence Probe for Ultrasensitive Determination of

Tetracycline and Oxytetracycline, Journal of the Chinese Chemical Society, , 57(6), 1353–1360,

4- Mohammad Bagher Gholivand, and Hamzeh Khani (2013). Determination of Tetracycline at a UV-Irradiated DNA Film Modified Glassy Carbon Electrode, Electroanalysis , , 25(2), 461–467

5- Flow injection analysis of tetracycline in pharmaceutical formulation with pulsed amperometric detection.Anal. Chim. Acta, , 499, 191-197.

6-Ahlam Jameel Abdulghani, Hadi Hassan Jasim, and Abbas Shebeeb Hassan, (2013) ; Determination of Tetracycline in Pharmaceutical Preparation by Molecular and Atomic Absorption Spectrophotometry and High Performance Liquid Chromatography via Complex Formation with Au(III) and Hg(II) Ions in Solutions, International Journal of Analytical Chemistry,. Volume, 1- 11.

7- British pharmacopoeia, 2013, volume 2, p.2186.

8- R. E. Gavil´an, C. Nebot, J. M. Miranda (2016) "Analysis of tetracyclines in medicated feed for food animal production by HPLC-MS/MS," *Antibiotics*, 5(1), 1–10,

9- **United States Pharmacopeia and National Formulary, 2018**, USP 41, NF 36 .P 4013-4017.

10- Zhenzhen Li, Kailin Xu, Bing Liang and Yanfang Li, (2013); Determination of trace tetracyclines in surface water by aluminum hydroxide co precipitation coupled with high-performance liquid chromatography , Anal. Methods**,** 5, 3516-3522.

11- Ewelina Patyra, Ewelina Kowalczyk, Krzysztof Kwiatek,(2013) Development and validation method for the determination of selected tetracyclines in animal medicated feeding stuffs with the use of micellar liquid chromatography, Analytical and Bioanalytical Chemistry, 405(21), 6799-6806

12- Nozal, L., Arce, L., Simonet, B. M., Ríos, A. and Vacárcel, M.;(2004) Rapid determination of trace levels of tetracyclines in surface water using a continuous flow manifold coupled to a capillary electrophoresis system. Anal. Chim. Acta, 517, 89-94.

13- Pena, A., Palilis, L. P., Lino, C. M., Silveira, M. I. and Calokerinos, A. C; (2004) Determination of tetracycline and its major degradation products by chemiluminescence.Anal. Chim. Acta, 405, 51-56.

14- Lau, C., Lu, J. and Kai, M. (2004) Chemiluminescence determination of tetracycline based on radical production in a basic acetonitrile-hydrogen peroxide reaction. Anal.Chim. Acta, 503, 235-239.

15- Townshend, A., Ruengsitagoon, W., Thongpoon, C. and Liawruangrath, S;(2005) Flow injection chemiluminescence determination of tetracycline. Anal . Chim. Acta, ,541, 105-111.

16- Amjadi M,Manzoori J, and Pakpoor F,(2016) "Photometric determination of tetracycline based on surface plasmon resonance of silver nanoparticles," Journal of Analytical Chemistry, ,71(3), 253–258.

17- Yongnian Ni, Na Deng and Serge (2010) A simple kinetic spectrophotometric method for simultaneous determination of tetracyclines by use of chemometrics, Anal. Methods, ,2, 1302-1309.

18- Hind Hadi and Ghadah Fadhil (2014) Sensitive Spectrophotometric Determination of Tetracycline Hydrochloride Indosage forms using Sodium Nitroprusside and Hydroxylamine Hydrochloride Journal of Al-Nahrain University 17 (3), 53-58 .

19-Wish.T,Senee.K,Richard.DBoonsom.L,and Saisunee.L,(2011). Sequential injection spectrophotometric determination of tetracycline antibiotics in pharmaceutical preparations and their residuesin honey and milk samples using yttrium(III) and cationic surfactant,Talanta, 84(5), 1401-1409.

20-Lamia Faaq Mari1 Mohsin Hamza Bakir2 Mohammed Salim Abdul Aziz, (2016).'Spectrophotometric Determination of Tetracycline, in pharmaceutical formulations by oxidative coupling, Kirkuk University Journal /Scientific Studies, , 11(4),159-183.

21- Udalova A, Dmitrienko S, and Apyari V (2015),'Methods for the Separation, Preconcentration, and Determination of Tetracycline Antibiotics, Journal of Analytical Chemistry, , 70(6), 661–676

22-. Nief Rahman Ahmad,(2013). A sensitive spectrophotometric determination of tadalafil in pharmaceutical preparations and industrial wastewater samples, J. Baghdad for Sci.,10(3),1005-1013.

23- Nief Rahman ahmad (2011). An indirect spectrophotometric determination of mesna in pharmaceuticals and environmental samples ;Iraqi National Journal of Chemistry, , 44,492- 500

24- Nief Rahman Ahmad(2012). Facial visible spectrophotometric determination of metformin hydrochloride in glucosam tablets and industrial waste water: Application to content uniformity testing, Iraq J Pharm, , 12(1),75-86.