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Development and Validation of Spectrophotometric method of Tetracycline Hydrochloride in Bulk and Pharmaceutical Preparation

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ABSTRACT

Sensitive spectrophotometric method is described for the determination of tetracycline HCl in bulk and in pharmaceutical formulations. The method is based on the coupling of tetracycline HCl with diazotized 8-hydroxy quinoline reagent in basic medium at room temperature to form a reddish to brown mono azo dye soluble in water with maximum absorption at 395 nm. The reaction was followed up spectrophotometrically by measuring the increase in absorbance at 395 nm. The analytical performance of the method, in terms of accuracy and precision, was statistically validated; the results were satisfactory. The calibration graph is linear in the concentration range 1-20 µg ml^{-1} ,with 0.005 µg ml^{-1} detection limit and 0.324 µg ml^{-1} , 0.980 µg ml^{-1} Limit of detection(LOD) and Limit of quantification(LOQ) respectively. The method has been successfully applied to the determination of the studied drugs in commercial pharmaceutical formulations.

Keywords: Spectrophotometric method, Validation ,Tetracycline HCl, Coupling reaction.

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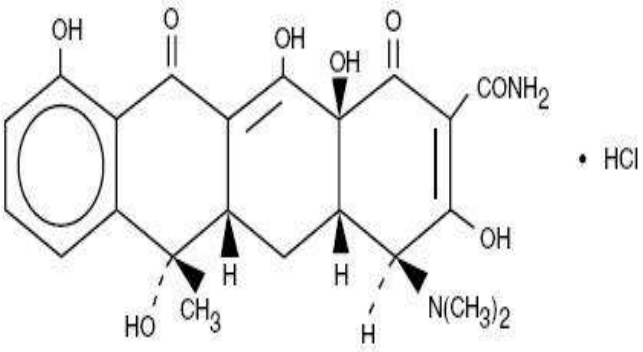
INTRODUCTION

Tetracycline is most important broad spectrum antibiotic, tetracycline hydrochloride is bright yellow, crystalline salt that is stable in air but darkens on exposure to strong sunlight(1,2). Tetracycline is inhibits cell growth by inhibiting translation. It binds to 16 S part of the 30 S ribosomal subunit & prevents the aminoacyl t-RNA from binding to the site of the ribosome(3). Tetracycline have been used in ruminants as both prophylactic and therapeutic agents(4) having a good activity against acute disease caused by gram-positive and gram-negative bacteria, including the species Spirochete, A ctinomyces, Ricketsia and Mycoplasma(5).The chemical name for tetracycline hydrochloride is 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12aoctahydro-3,6,10,12,-12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecar-boxamide monohydrochloride. Its structural formula is as follows:



Schema 1: Tetracycline HCl structure

Molecular weight(480.90)and $C_{22}H_{24}N_20_8$•HCI formula

Several methods have been reported for the determination of tetracycline in dosage formsincluding: Microbiological assay, non-Aqueous volumetric titration, TLC densitometry with fluorescence(6,7).Kinetic spectrophotometric methods(8,9)High Performance Liquid Chromatography (HPLC)(10)chemiluminescence(11) and electrochemical methods(12). In this research a new and simple pectrophotometric method for determination of tetracycline was applied by used coupling reaction with dazotised 8-hydroxy quinoline solution to form azo dye with maximum absorptive at 395nm.

MATERIALS AND METHOD

Apparatus

All spectral and absorbance measurements were carried out A Jena Model 1100, UV-Visible spectrophotometer(Germany) in the technique laboratory of pharmaceutical chemistry department, college of pharmacy, Basra university, Iraq.. The instrument was equipped with a quartz cell with a

1.0cm path length. E. Meter electrical balance is used for weighting the sample. The pH measurements are performed using Philips PW 9421 pH meter.

Reagents

All chemicals used were of analytical reagent grade purity.Pure drugs and pharmaceutical capsules: Pharmaceutical grade tetracycline hydrochloride (Fluka) used as working standards. Tetracycline hydrochloride capsules containing 250 mg: AL-Naser (Egypt) , ZMC pharmaceutical Co., Ltd., China and S.D.I –Iraq were obtained from commercial sources in the local market. Potassium permanganate (Merck, Germany) aqueous solution was freshly prepared. Sodium hydroxide (BDH, England).

Solutions

Tetracycline stock solution (100 µg.ml^{-1}):

A 0.01076 g amount of tetracycline hydrochloride is dissolved in distilled water and the volume is completed to 100 ml in a volumetric flask. This solution is kept in a brown bottle. Tetracycline working standard solution containing (1 – 20) µg ml^{-1} was prepared by suitable dilution with distilled water.

Diazotised 8-hydroxy quinoline solution(5 Mm):

A 0.1452 g of 8-hydroxy quinoline (Fluka) is dissolved in about 50ml of distilled water. Then 1.35ml of concentrated HCl is added and the solution is heated. The mixture is transferred to a 200-ml volumetric flask and is cooled to ≈ 5 C^0. A 5 ml of 1% NaNO2 is added and the mixture is stirred occasionally for 5 min and the volume is completed to 200 ml with additional cooled distilled water(5C^o).This solution is stored in darkness over ice and used after 15 min. This reagent solution when kept in the refrigerator(≈5 **C**^{0}).

Sodium hydroxide solution(5 M):

This solution is prepared by dissolving 20 g of sodium hydroxide in distilled water. Then completing the volume to 100 ml in a volumetric flask with distilled water and transferring it to a plastic bottle.

Procedure for Calibration Graph

Transfer aliquot volumes of tetracycline standard solution covering the working concentration range from 1 to 20.0 µg mL^{-1} in to 25 ml volumetric flasks .About 2.5 ml of 5 mM diazotised 8-hydroxy quinoline reagents are then added . .Allow the reaction mixture to stand for 2min,Then 1 ml of 5 M sodium hydroxide solution is added. Then the volume is completed to the mark with distilled water. Measure the absorbance of the resulting solution at 395 nm against a reagent blank

prepared simultaneously. Plot the values of the absorbance against the final concentration in µg mL^{-1} to get the calibration curve.

Procedure for Tetracycline capsules

Empty the contents of 10 capsules and mix well. Transfer a weighed quantity of the powdered capsules equivalent to 10 mg of tetracycline into 100 ml volumetric flask and made up to the mark with water. The content of the flask was stirred magnetically for 10 minutes, then proceed as described under "Recommended Procedure".

Optimization of reaction conditions

The influence of the various parameters on the colour development of the azo dye were studied and the reaction conditions are optimized (stability of the dye resulting from the reaction of tetracycline with diazotised 8- hydroxyl quinoline in basic medium, Effect of Sodium Nitrite Concentration, Effect of Reagent Concentration, Effect of Temperature , Effect of different solvents , Effect of diazotisation acid, Effect of NaOH Concentration, Effect of variation in reaction time) Such variables were changed individually while the others were kept constant.

Effect of Sodium Nitrite Concentration

The effect of sodium nitrite concentration was tested by using different amounts (1-8 mL) of 1% M NaNO2 solution. It was found that the addition of 5 mL of NaNO2 solution was required with 5minute reaction time to obtain a maximum absorbance

Effect of Reagent Concentration:

The effect of reagent(diazotized 8-hydroxy quinoline) concentration was tested by using different volumes (0.5–4 mL) of 5mM diazotized 8-hydroxy quinoline solution(fig.1). The results showed that 2.5 mL of reagent is sufficient for production of maximum and reproducible color intensity.

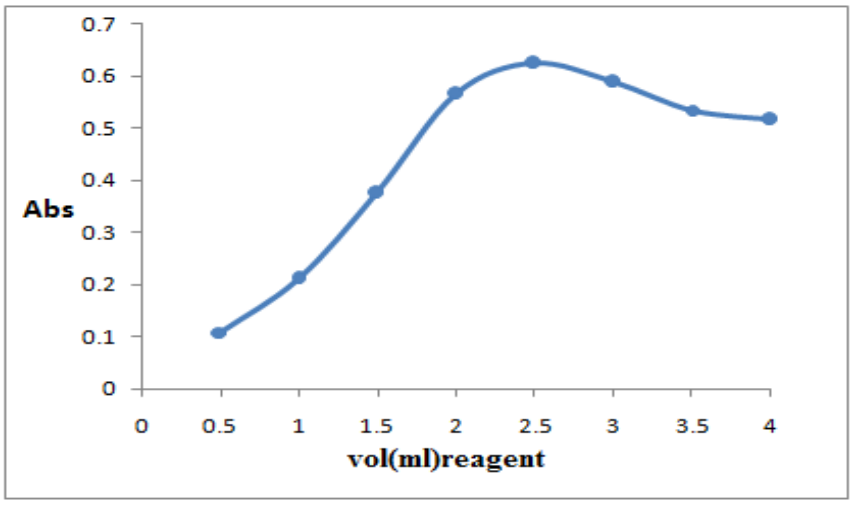


Figure 1: Effect of reagent (diazotized 8-hydroxy quinoline)concentration on absorptivity

Effect of Temperature:

The effect of temperature on the absorption was investigated at different temperatures (5 – 80 C^0). The results revealed that the absorbance relatively stable in the temperature range (5–35 C^0)(fig.2). At higher temperatures, the absorbance value decreased, which was probably due to the dissociation of azo-dye.

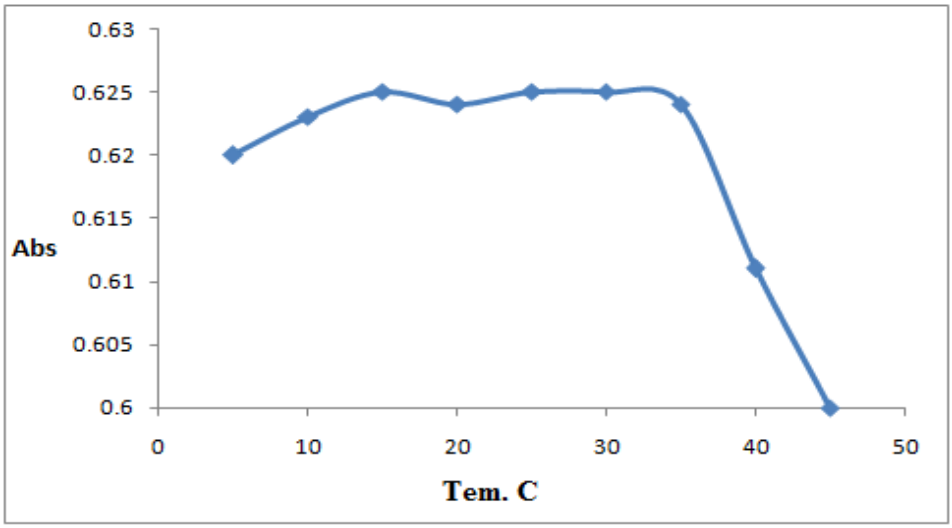


Figure 2: Effect of the temperature on absorptive

Effect of different solvents

Different solvents with different polarities was also studied (13) such as : water, ethanol, methanol, isopropanol and acetone. Results revealed that water was the optimum diluting solvent as it gave the maximum absorbance with the investigated drug. Dilution with water is advantageous as it is the most cheap and environmentally safe solvent.

Effect of diazotisation acid

The diazotisation of 8-hydroxy quinoline is carried out in various acids (weak and strong) to establish the suitable acid for the reaction. Experimental data show that acetic acid, phosphoric acid, and formic acid give turbid solution of diazonium salt and after 10min a precipitate is formed. Hydrochloric acid gives higher results than others. Therefore, it is regarded as the most suitable of the acids tested.

Effect of NaOH Concentration

The influence of NaOH volume on the absorbance of the reaction product was also studied(0.1-2.2ml). It was found that increasing the volume of 5M NaOH resulted in a corresponding increase in the absorbance of the reaction product up to 1 ml. Thus 1 ml of 5 M NaOH was established as the most suitable volume for this study (Figure 3).

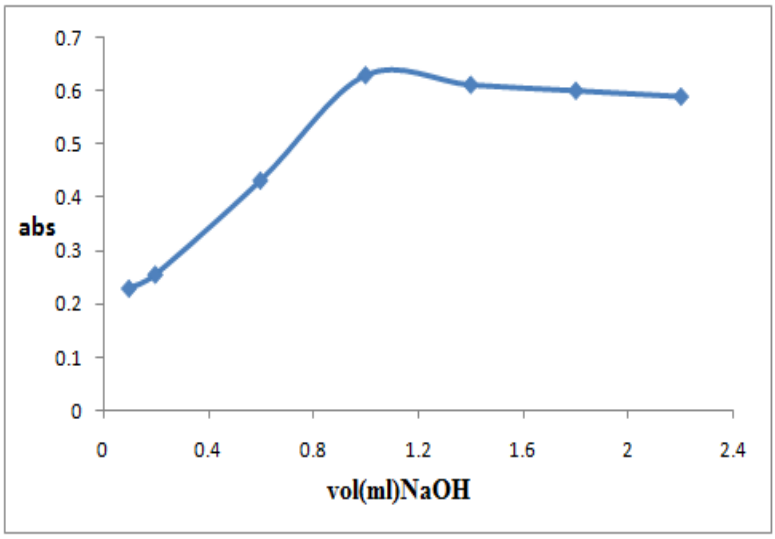


Figure 3: Effect of sodium hydroxide on absorptivity

Effect of variation in reaction time

The reaction was carried out for different periods of time (2 –40 min), and was found to be time dependant. Maximum absorption intensity was obtained after 5 min(fig.4). It was found that 5 minutes time was sufficient for complete colour development and the colour was stable for 24 hours.

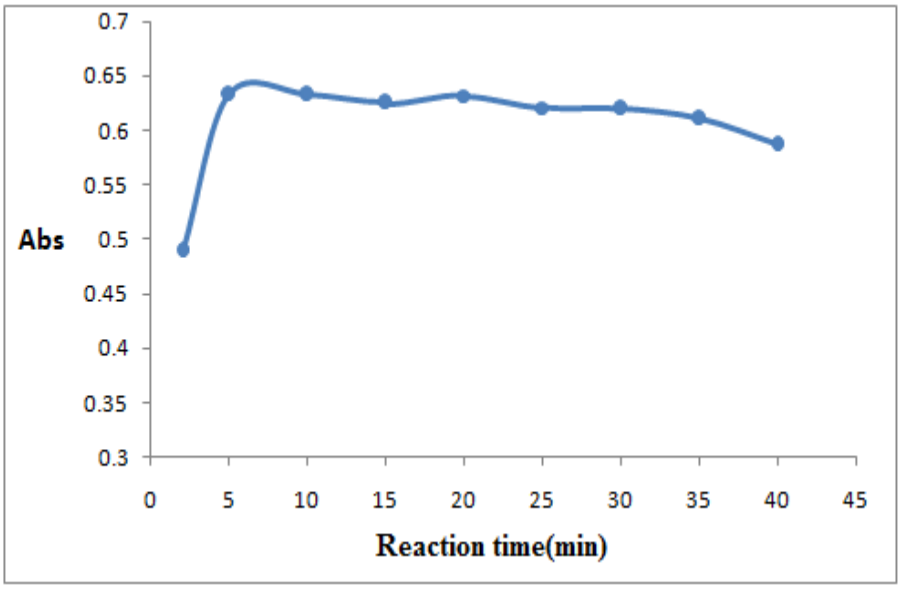


Figure 4: Effect of reaction time on absorptivity

Order of addition of reagents

The order of addition of reagents is crucial. Addition of reagents in the order tetracycline , diazotised 8-hydroxy quinoline reagent, sodium hydroxide and then complete with water gave

constant and maximum absorbance. If sodium hydroxide is added before addition of reagent, sensitivity was affected.

Absorption Spectra

The absorption spectra of the coloured azo dye and the corresponding reagent blank shows in fig.(5). A reddish brown coloured azo dye is formed immediately, when a solution of tetracycline is mixed with diazotised 8-hydroxy quinoline in a highly basic medium .The wavelength of maximum absorption at395 nm has been adopted in all subsequent work

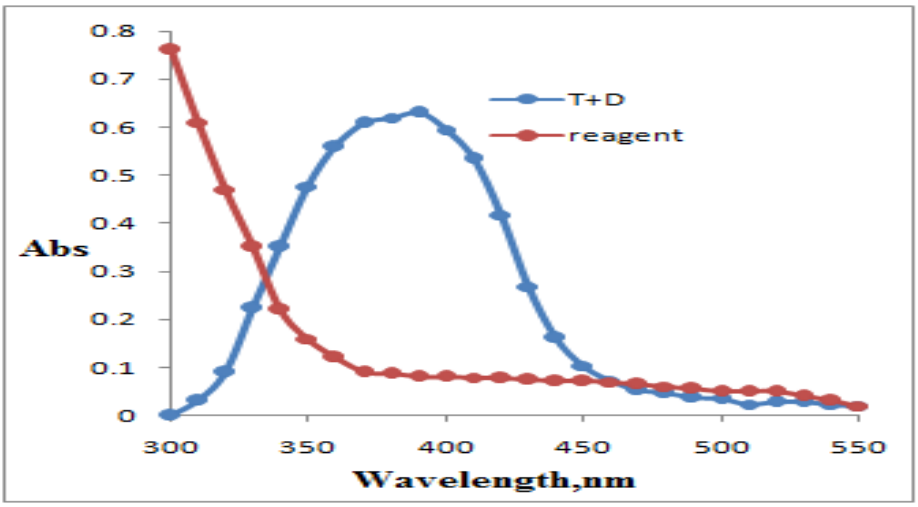


Figure 5: Absorption spectra of 12µg ml^{-1} tetracycline, treated according to the recommended procedure and measured against blank (T+D), reagent measured against distilled water(reagent).

Calibration Graph

Under optimum conditions studied above, standard calibration curves for tetracycline and diazotised 8-hyaroxy quinoline were constructed Fig.6. To a series of 25-ml volumetric flasks are added aliquots of solution containing 1-20 µg ml^{-1} tetracycline.2.5 ml of 5 mM diazotised 8-hyaroxy quinoline reagentsis are added , then the mixtures are shaken well. Then 1 ml of 5 M sodium hydroxide solutions is added and the volume is made to the mark with distilled water. The absorbance are measured at 395 nm against the corresponding reagent blank using 1 cm bath cells.

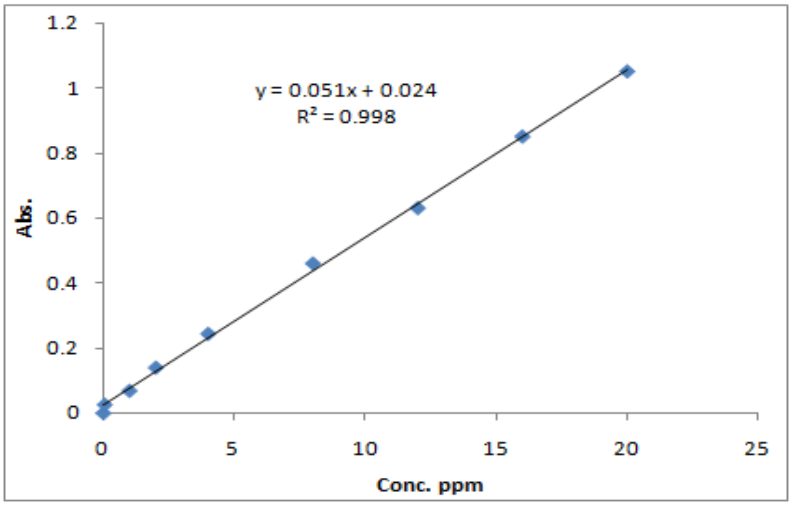


Figure 6: Calibration curve for tetracycline.

Beer's law (14)has been obeyed in the concentration range 1- 20 µg ml^{-1} of tetracycline in a final volume, 25ml.The different parameters of the analytical performance of the proposed method are summarized in Table(1). Limit of Detection LOD and Limit of Quantitation LOQ were calculated by the formula.

LOD= 3.3(SD/a)

LOQ= 10(SD/a)

Where **SD** is standard deviation of the 3.3 or 10 spectrophotometric readings of blank and **a** is a slope of calibration curves obtained in the linearity study.

Table 1: Parameters for the performance of the proposed method.

Parameters	Value
Measurement wavelength(nm)	395
Linear range(µg ml^{-1})	1-20
Intercept	0.024
Slope	0.051
Standard deviation	0.005
Correlation coefficient(R2)	0.9989
Limit of detection ,LOD(µg mL-1)	0.324
Limit of quantification, LOQ (µg mL-1)	0.980
Molar absorptivity, ε (L. mol-1 cm-1)	$0.24x10^4$

Method validation

The proposed methods was validated according to the continuous variation method(15) of equivalent mole method was used to determine the composition of Product. The result is shown in Figure 5. As can be seen, the mole ratio of tetracycline and reagent of Product is 1:1. Based on the observation molar ratio

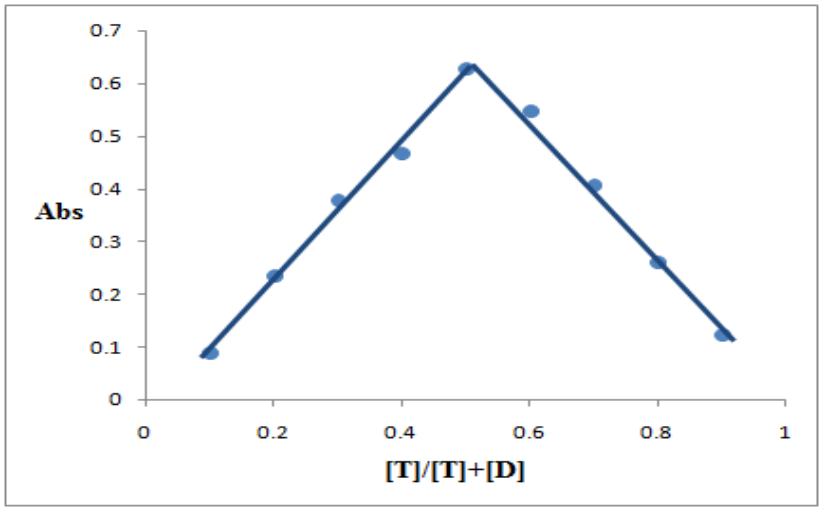


Figure 5: The mole ratio of the reaction between T and T-D

(T=tetracycline, D= diazotised 8-hyaroxy quinoline reagent).

Accuracy and precision of the methods

To evaluate the accuracy and precision of the proposed method, pure drug at foure different levels was determined, each measurement being repeated five times. The results obtained from this study are summarized in Table 2. The spectrophotometric method are fairly accurate and precise as revealed by the relative error (< 2 %) and the relative standard deviation (< 3 %). The reproducibility on a day-to-day basis, was estimated by analyzing the standard drug solution at three levels on five consecutive days.

The recovery of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 99.87-100.79% Intra-day and 99.7-100.31 Inter-day(Table3).

Table 2: Accuracy of the proposed method for the determination of tetracycline-HCl

Amount Taken ($\mu g\ ml^{-1}$)	**Amount Found ($\mu g\ ml^{-1}$)**	**% Accuracy ***	**RSD %**	**Mean ± SD**
4	4.06	101.50	0.921	100.20
8	7.95	99.38		±0.923
12	12.02	100.17		
16	15.96	99.75		

*each value is the average of five separated determinations.

Table 3: Precision of the proposed method for the determination of tetracycline-HCl

Precision			
Intra-day		**Inter-day**	
Recovery ± **SD*%**	RSD*	Recovery ± **SD*%**	RSD*
99.87±0.976	0.977	100.08±0.457	0.459

100.07±0.477	0.478	100.31±0.986	0.987
99.97±0.886	0.887	99.77±0.776	0.778
100.76±0.995	0.996	100.05±1.235	1.236

*****n=5 , SD=Standard deviation , RSD=Relative standard deviation

Recovery studies were also carried out by standard addition method. Good recoveries (96.3 to 102.8%)table(4)indicated good recovery and there is no serious interference in the determination of above drugs in such samples .

Table 4: Results of recovery study by standard addition method

Amount of drug taken (µg ml^{-1})	Amount added (µg ml^{-1})	% Recovery± SD *	%RSD
50	30	100.50±0.855	0.864
50	40	99.50±0.495	0.499
50	50	99.88±0.765	0.767

*each value is the average of five separated determinations

The results obtained by the proposed method were compared with british pharmacopoeia (BP)(16)method, by appalling the F-test and T-test at 95% confidence level(. The theoretical value of t –test and F-test are 2.36 and 8.941 respectively .The calculation values for F and t tests for proposed method did not exceed the theoretical values. These confirming that there are no signification differences between the proposed method with BP method.

Application to tablet analysis

The proposed method was applied successfully for determination of the tetracycline HCl in their pharmaceutical dosage forms. three brands of commercially available tablets were analysed for the active ingredient by the proposed methods. The results of the assay summarized in Table(5) reveal good agreement between the declared content and percent found. The results obtained by the proposed methods were compared with those obtained by the reference method.

Table 2: Application of the proposed method to the determination Tetracycline-HCl in some dosage form

*each value is the average of five separated determinations.

Preparation	Amount Taken (µg ml^{-1})	Amount Found (µg ml^{-1})	%Recovery*	%RSD	Proposed method Mean ± SD	Ref. method Mean ± SD
Tetracycline HCl capsule (ZMC pharma. Com.Ltd.)	4 8 12 16	4.05 8.03 11.95 16.06	101.25 100.38 99.58 100.38	0.462	100.395±0.464	98.50 ± 1.40

Tetracycline HCl capsule (Al-Naser ,Egypt)	4 8 12 16	3.98 7.97 12.05 16.07	99.50 99.63 100.42 100.44	0.278	99.995 ±0.279	98.90 ± 2.10
Tetracycline HCl capsule (S.D.I.-Iraq)	4 8 12 16	4.07 7.96 11.95 16.06	101.00 99.50 99.58 100.38	0.648	100.115±0.469	100.95 ± 1.45

CONCLUSION

In this research, the method is simple and accurate. It is easily applied to determination of the tetracycline in the pure form and in pharmaceutical preparations. The proposed methods was validated with continuous variation method and the result shown that the mole ratio of tetracycline and reagent of Product is 1:1. Beer's law has been obeyed in the concentration range 1-20 µg ml^{-1} of tetracycline .the statistic parameter and the recovery data indicated the high accuracy and reproducibility of the proposed method.

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