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Spectrophotometric Determination of Tetracycline by Coupling with Diazotised 4-Aminoantipyrine in Presence of Cetylpyridinium Chloride

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ABSTRACT

A spectrophotometric method for the determination of microgram amounts of tetracycline -HCl(TCH) has been proposed. The method is based on coupling of tetracycline-HCl with diazotized 4-aminoantipyrine in presence of cetylpyridinium chloride in alkaline medium. The molar absorptivity of the formed dye reached to 1.92×10^4 $l.mol^{-1}.cm^{-1}$ at 537 nm and Beer's law is obeyed within the range of 10-300 µg of tetracycline-HCl /10 ml (i.e.1.0 – 30 ppm). The colour reaction was highly stable, a relative error ranged from - 2.5 to + 0.21 % and a relative standard deviation of ± 0.37 to ± 0.73 %, depending on concentration level of tetracycline-HCl. The proposed method has been applied successfully to the determination of tetracycline-HCl in pharmaceutical preparations.

Keywords: Tetracycline-HCl; 4-aminoantipyrine; diazo-coupling; spectrophotometry.

التقدير الطيفي للترأسايكلين بالاقتران مع العامل المؤزوت 4-امينو انتي بايرين بوجود سيتيل بريدنيوم كلوريد

الملخص

تم اقتراح طريقة طيفيه لتقدير كميات متناهية في الصغر من هيدروكلوريد التتراسايكلين. تعتمد الطريقة على اقتران التتراساكلين مع العامل المؤزوت 4- امينوانتي بايرين بوجود سيتيل بريدنيوم كلوريد في الوسط القاعدي. بلغت قيمة معامل الامتصاص المولاري للصبغة المتكونة 1.92×10^4 لتر.مول$^{-1}$.سم$^{-1}$ عند الطول الموجي الاعظم 537 نانوميتر. وتتبع الطريقه قانون بير في مدى التراكيز من 10 الى 300 مايكروغرام/ 10 مل (أي من1-30 جزء/مليون). يمتاز التفاعل اللوني باستقرارية جيدة وبخطأ نسبي يتراوح بين 2.5 - و 0.21 % وانحراف قياسي نسبي بين ±0.37 و ±0.73 % اعتمادا على مستوى التركيز. تم تطبيق الطريقة بنجاح في تقدير هيدروكلوريد التتراسايكلين في المستحضرات الصيدلانية.

INTRODUCTION

Tetracycline antibiotics are potent, broad spectrum antibacterial agents effective against a host of Gram positive and Gram negative aerobic and anaerobic bacteria. As a result, the tetracyclines are drugs of choice, or well accepted alternatives for a variety of infectious diseases (Albert, 1953). Assay of tetracycline has previously been achieved by several analytical techniques such as high performance liquid chromatography (Shalaby *et al.*, 2011), RPHPLC (Johnathan and Yugang, 2007 ; Kazuo *et al*., 1992), LC (Innocenzo and Fabio, 2009) LC-Mass (Pena *et al*., 2007), flourimetry (Regos *et al*., 1978), adsorptive voltammetry (Parviz *et al*., 2007), flow injection (Saisunee *et al*., 2006; Jose *et al.,* 2009 ; Prinya et al 2008). However, many of these methods require expensive equipments and skilled operation. The most widely methods used for the determination of tetracycline are spectrophotometric methods such as: complexation with iron (Ronald, 2007), oxidative coupling (Ayad *et al*., 1986), diazo- coupling (Al-Abbasi, 2009), charge transfer complex (Khairi, 2008), kinetic spectrophotometric method (Yongnian *et al*., 2010) However, some of these methods suffer form one or more disadvantage such as insufficient sensitivity , poor selectivity or using of solvent extraction procedures. Therefore, a simple and accurate method for assaying of tetracycline in different pharmaceutical preparations is necessary for routine analysis.

EXPERIMENTAL

Apparatus

All absorption measurements were obtained by using a double beam CECIL CE7200 UV-Visible Recording Spectrophotometer with 1.0 cm matched quartz cells and the pH measurements were performed by using HANNA Instrument pH 211microprocesser pH meter.

Reagent

All Chemicals used are of analytical-reagent grade .

A pure tetracycline - HCl (TCH) was obtained from the State Company for Drug Industries and Medical Applicances (SDI), Sammara, Iraq .A solution of 100 μg ml^{-1} was prepared by dissolving 0.01g of tetracycline -HCl in 100-ml distilled water. Sodium carbonate (1M) and different interferences solution (1000 μg ml^{-1}) were prepared by dissolving the proper amounts in distilled water. The diazotised 4-aminoantipyrine (2.5 mM) solution was prepared by dissolving 0.05 g of 4-aminoantipyrine (Fluka) in 60 ml distilled water then 3ml of concentrated hydrochloric acid was added followed by heating. Finally the mixture was transferred to a 100-ml volumetric flask and cooled at 0-5 ^{O}C in an ice-bath. A 0.017 g sodium nitrite was added and the mixture was stirred vigorously After 5 minutes, the solution was made up to 100 ml with cold distilled water and was kept in brown bottle in a refrigerator for 1 hour before using, and it is stable for at least five days.

General procedure and calibration graph

The aqueous solution (0.1 – 3ml) containing tetracycline-HCl (100 μg $.ml^{-1}$) was transferred into a series of 10-ml calibrated flasks. To each flask 1 ml of diazotised 4-aminoantipyrine solution (2.5 mM), 2 ml of sodium carbonate solution (1M) and 4 ml of CPC (1×10^{-3}M) were added then the volume was completed to the mark with distilled

water. The absorbance was measured at 537 nm against a reagent blank which was prepared in a similar way but without the addition of tetracycline-HCl. The calibration graph as shown in Fig. (1) was linear over the range of 10-300 µg of tetracycline-HCl /10 ml (i.e 1– 30 ppm).The apparent molar absorptivity referred to tetracycline – HCl has been found to be 1.92×10^4 $l.mol^{-1}.cm^{-1}$.

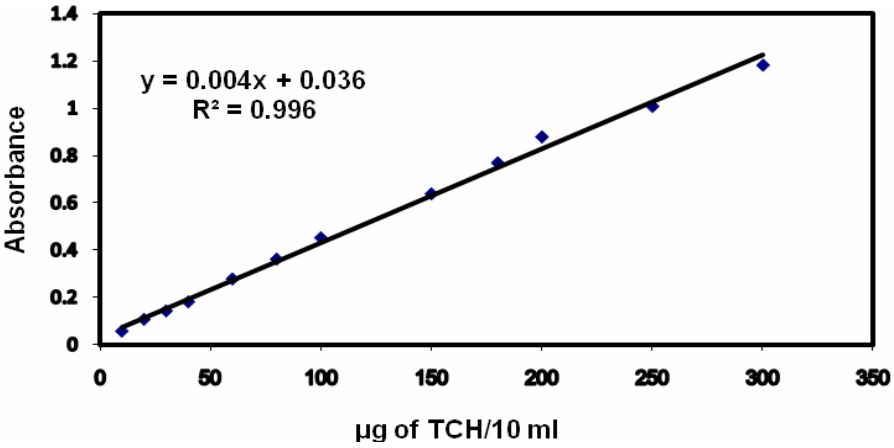


Fig. 1: Calibration graph for tetracycline – HCl determination using the proposed method.

Determination of tetracycline in capsule

Weight and mix the contents of five capsule (each one contains 250 mg tetracycline - HCl), an accurately weighed amount of powder equivalent to 0.01g tetracycline - HCl was dissolved in 100 ml distilled water in a volumetric flask. A suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug .

Determination of tetracycline in ointment

Mixed well the contents of four containers of tetracycline ointment , an accurately weighed amount equivalent to 0.01g tetracycline - HCl was dissolved in a mixture of 3 ml ethanol with 50 ml distilled water, then the mixture was warmed and filtered into a 100- ml volumetric flask and the volume was completed to the mark with distilled water. A suitable aliquot of solution was taken and the recommended procedure was followed for analysis of the drug.

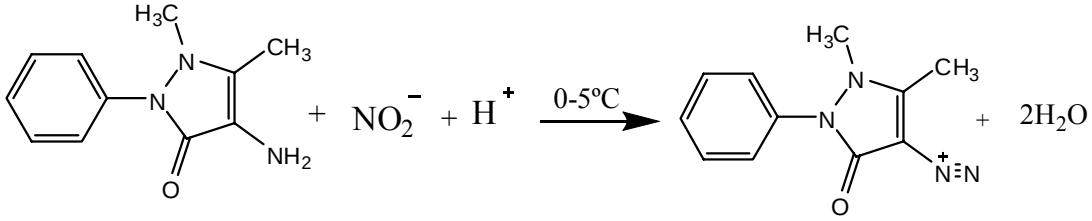
RESULTS AND DISCUSSION

The effect of various variables on the colour development was tested to establish the optimum conditions for the determination of TCH by coupling with diazotized 4-aminoantipyrine reagent.

Principle of the method

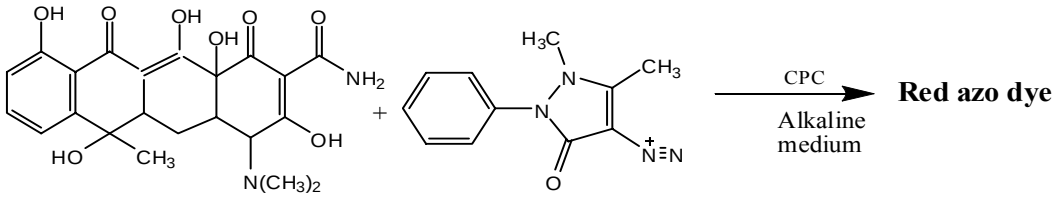
The method included the following steps:

- Preparation of diazotised 4-aminoantipyrine



4-aminoantipyrine **Diazotised 4-aminoantipyrine**

- Coupling of tetracycline with diazotised 4-aminoantipyrine



Tetracycline **Diazotised 4-aminoantipyrine**

Choice of diazotised agent and amount

Several aromatic diazotised agents have been tested for optimum conditions. The results in Table 1 show that p-phenyldiamine give the highest intensity but with low value of colour contrast, while 4-aminoantipyrine gives good sensitive reaction ($\varepsilon = 1.85\times10^4$ $l.mol^{-1}.cm^{-1}$) with high value of colour contrast(165 nm) in alkaline medium. Therefore, it has been selected for the subsequent experiments.

Table 1: The selection of diazotised agent.

Reagent(2.5 mM)	Absorbance	λ_{max}(nm)	$\Delta\lambda_{max}$(nm)	ε ($l.mol^{-1}.cm^{-1}$)
m-Nitroaniline	0.261	352	99	1.26×10^4
p-Phenyldiamine	0.404	352	42	1.95×10^4
Sulphanilic acid	0.114	352.5	102.5	0.55×10^4
4-aminoantipyrine	0.384	537	165	1.85×10^4

*$\Delta\lambda_{max}$ = Colour contrast = $\lambda_{max}S - \lambda_{max}B$ where S=The dye, B=Blank.

The effect of different amounts(0.5 – 2 ml) of diazotised 4-aminoantipyrine reagent on the absorbance of solutions containing different amounts of tetracycline-HCl (50-250 µg/10ml) have been studied. The obtained results indicated that the absorbance increases

with increasing the reagent concentration (diazotised 4-aminoantipyrine) and reached a maximum on using a volume of 1.0 ml of 2.5 mM diazotised 4-aminantipyrine, which also gives the highest value of determination coefficient (r^2 =0.9938). Therefore, 1.0 ml reagent was recommended for the subsequent experiments.

Effect of base

Preliminary experiments have been shown that TCH gives coloured dye with diazotised 4-aminoantipyrine only in basic medium. Various bases (strong and weak) have been used and their results in Table 2 indicate that 2ml of Na_2CO_3 (1M) give the highest intensity of the coloured dye, therefore this volume has been recommended for the subsequent experiments.

Table 2: The effect of base on absorbance **.**

Amount of base used (ml)	**Absorbance/base used (1M)**			
	NaOH	**KOH**	**Na_2CO_3**	**$NaHCO_3$**
0.25	0.015	0.018	0.242	0.009
0.5	0.103	0.019	0.379	0.194
1.0	0.061	0.053	0.386	0.350
2.0	0.057	0.045	0.413	0.354
3.0	0.050	0.032	0.413	0.388
pH range	(2.17- 13.16)	(1.98-13.20)	(6.68-10.73)	(3.75-9.73)
*$\Delta\ \lambda_{max}$	135	137	165	165

*$\Delta\ \lambda_{max}$ = Colour contrast λ_{max}S- λ_{max}B
where S=The dye, B=Blank

Effect of surfactant

The effect of several types of surfactants on colour intensity of the dye has been investigated. (Table 3 and Fig. 2).

Table 3: The effect of surfactant on dye absorbance.

*** Order**	**Absorbance / ml of CPC (1×10^{-3}M)**			**Absorbance/ml of SDS (1×10^{-3} M)**			**Absorbance/ml of Triton-X100 (1%)**		
	1 ml	**3 ml**	**4 ml**	**1 ml**	**3 ml**	**4 ml**	**1 ml**	**3 ml**	**4 ml**
I	0.282	0.263	0.242	0.409	0.407	0.380	0.409	0.407	0.392
II	0.283	0.275	0.244	0.405	0.398	0.386	0.389	0.405	0.420
III	0.429	0.445	0.458	0.401	0.386	0.374	0.397	0.401	0.415

Note Absorbance without surfactant = 0.412
I* =Sample (S) + Surfactant(Sur.) + diazotised (R) + Base (B)
II = S+R+Sur.+B
II = S+R+B +Sur.

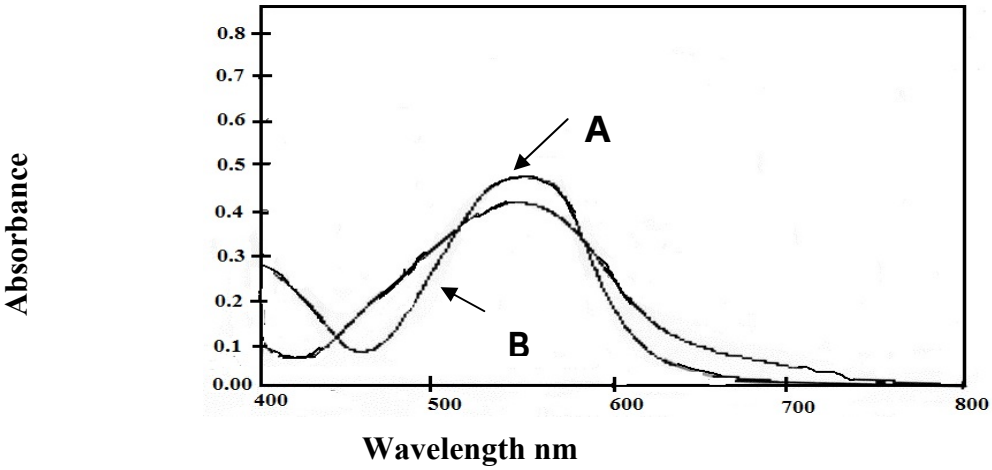


Fig. 2: The effect of CPC on absorbance (order III)
A- Sample with CPC B- Sample without CPC

The results indicate that the addition of CPC in the order (III) increases the intensity of the formed dye, therefore it has been recommended to use 4 ml of CPC (1×10^{-3} M) in this order in subsequent experiments.

Effect of time and amount of TCH on absorbance

The effect of time on the development and stability period of the formed coloured dye was investigated under optimum experimental conditions described before. The formation of coloured dye being complete after mixing the components of reaction and the absorbance of the coloured species remained constant for at least 60 minutes. (Table 4).

Table 4: The effect of time on absorbance.

Time/min.	Absorbance/µg of tetracycline-HCl		
	100	**200**	**250**
0	0.454	0.776	1.006
5	0.458	0.778	1.004
10	0.456	0.774	0.998
20	0.457	0.775	0.995
30	0.454	0.776	0.993
40	0. 456	0.774	0.990
50	0. 455	0.775	0.989
60	0. 450	0.774	0.987

Final Absorption Spectra

An absorption spectra of the formed coloured dye by coupling of TCH with diazotised 4-aminoantipyrine in the presence of CPC and in basic medium, against its corresponding reagent blank shows a maximum absorbance at 537 nm in contrast to the reagent blank which shows maximum absorbance at 425 nm (Fig 3).

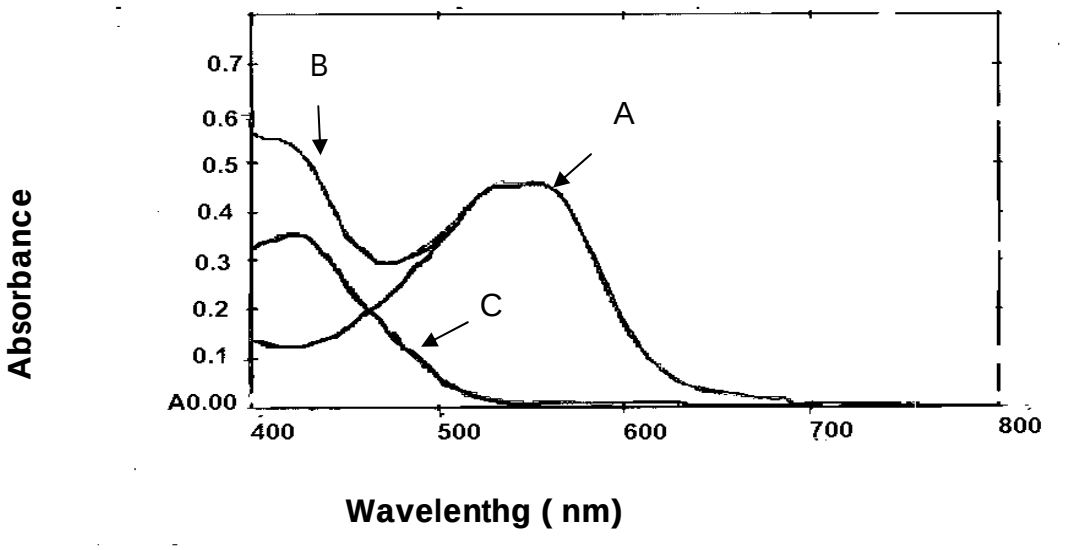


Fig. 3: Absorption spectra of 100µg TCH treated according to the recommended procedure and measured against (A) blank (B) distilled water and (C) blank measured against distilled water

INTERFERENCE

The criterion of interference was an error of not more than ± 5.0% in the absorbance. To test the efficiency and selectivity of the proposed analytical method, a systematic study of excipients (e.g. , glucose , lactose , gum Arabic and starch) at various levels, that usually present in dosage forms. Experimental results showed that there was no interference from additives or excipients up to 1500 µg in the present method as shown in Table 5.

Table 5: Effect of foreign compounds for assay of tetracycline-HCl.

Foreign compound	**Recovery (%) of 100 µg tetracycline-HCl per µg foreign compound added**		
	500	1000	1500
Glucose	99.3	100.0	98.9
Gum Arabic	100.0	99.7	99.3
Lactose	100.2	100.6	100.4
Starch	100.2	99.5	100.6

Accuracy and precision

To check the accuracy and precision of the calibration curve, TCH was determined at two different concentrations. The results illustrated in Table (6) indicate that the method is satisfactory.

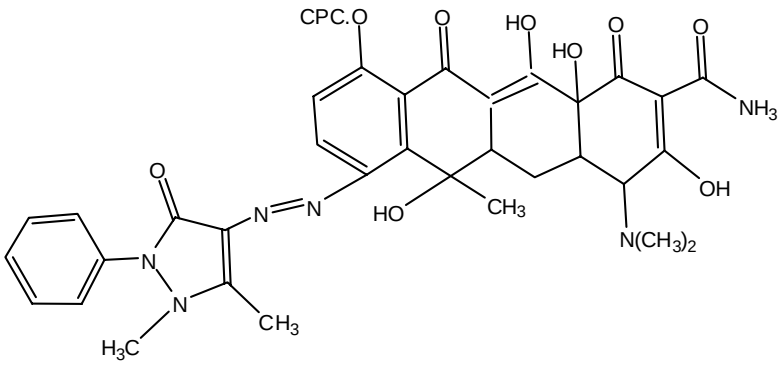
Table 6: Accuracy and precision of the calibration curv.

Pharmaceutical preparation	Amount taken mg	Relative error,%*	Relative standard deviation,%*
Samacycline 250mg /Capsules (S.D.I Iraq)	50	-2.1	±0.73
	100	0.21	±0.37
Apecycline 250mg/Capsoules (India)	50	-2.5	±0.73
	100	-1.09	±0.48

*Average of four determinations

Nature of the azo dye

Job's and mole – ratio methods (Hargis, 1988) indicate that the azo dye has a
Also the Job's method composition of 1:1 [TCH] to diazotised 4-aminoantipyrine. reagent. indicated that the composition of CPC with tetracycline was equal to the ratio 1:1
There for ,the suggested structure was as shown below:



Red azo dye

The average stability constant of the dye in aqueous solution ,under the established experimental condition, is 0.123 x10^{6} Molar $^{-1}$

The effect of organic solvents

Different organic solvents have been examined to evalute their effects on the spectrum of the resulting azo dye, the results are given in Table 7 and Fig. 4

Table 7: The effect of organic solvents compared with water.

Solvent	λmax, nm	Absorbance	ε l.mol^{-1}.cm^{-1}
Ethanol	554	0.396	1.91×10^4
Formic acid	561	0.233	1.12×10^4
Methanol	606	0.396	1.91×10^4
Acetic acid	612	0.051	0.25×10^4
Water	537	0.451	2.178×10^4

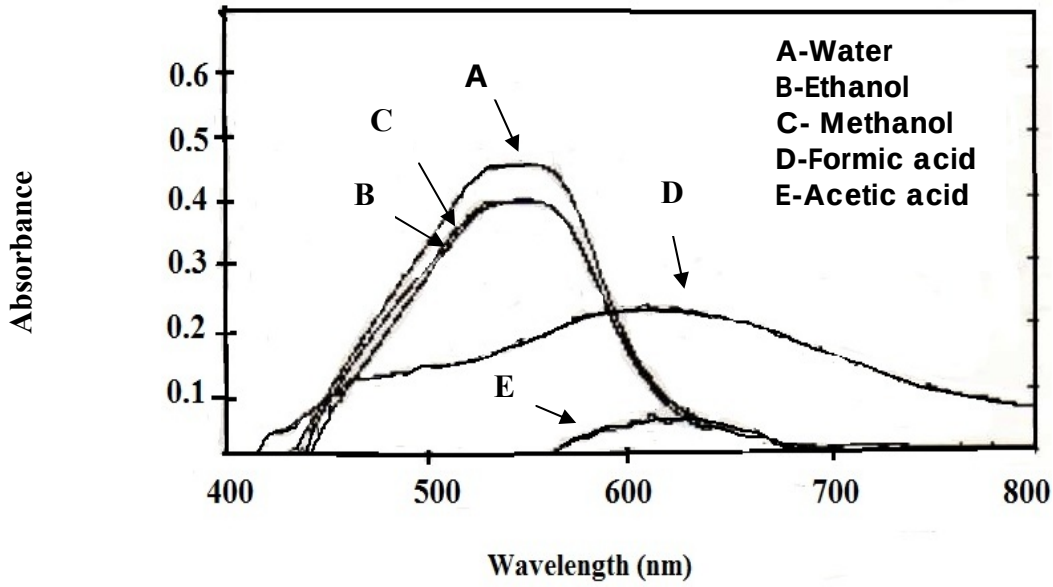


Fig. 4: The effect of solvents

Although formic acid and acetic acid caused red shift, water still used in the subsequent experiments according to the highest sensitivity.

Application of the method

The proposed method was applied to determine tetracycline- HCl in its pharmaceutical preparations(capsules and skin ointment). The results which are shown in Table 8 indicate that a good recovery was obtained.

Table 8:Analytical applications of the proposed method.

Pharmaceutical preparation	Amount taken (µg)	Amount measured	Recovery
Samacycline 250mg /Capsules (S.D.I Iraq)	50	49.7	99.4
	100	100.2	100.2
Apecycline 250mg/Capsules (PVT.SECTOR India)	50	49.7	99.4
	100	99.9	99.9
Samacycline 3% Skin ointment (S.D.I Iraq)	50	49.7	99.4
	100	100	100

Average of four determinations

The results obtained are in the agreement with certified values compared with standard addition method (Fig. 5 and Table 9).

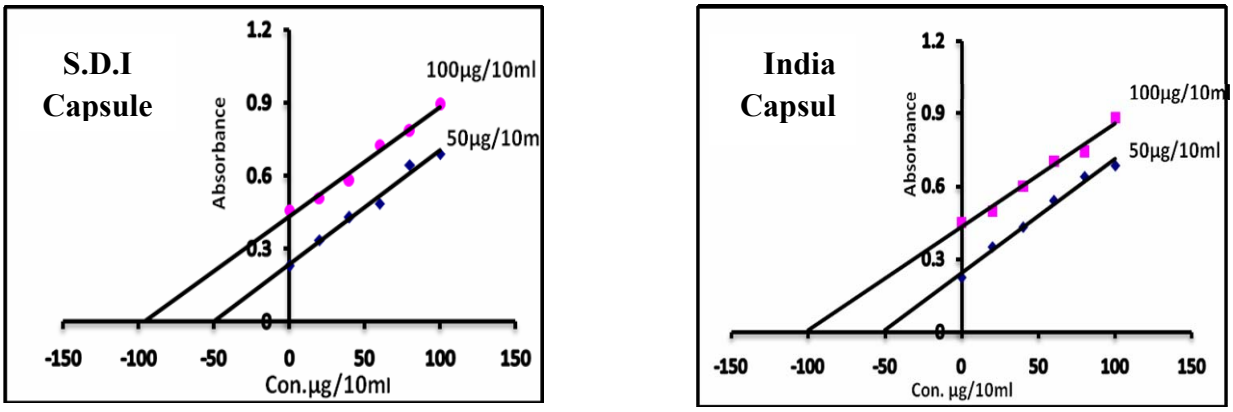


Fig. 5 : Standard addition plot for the recovery of 50 and 100 mg of TCH in S.D.I Iraq capsule and PVT.SECTOR India capsule.

Table 9: The results of standard addition method.

Pharmaceutical preparation	Amount taken (mg)	Amount measured(mg)	Recovery, %
Samacycline 250mg /Capsules S.D.I Iraq	50	50	100
	100	98.5	98.5
Apecycline 250mg/Capsules PVT. SECTOR. India	50	50	100
	100	101	101

The performance of the proposed method for determination of tetracycline in ointment was assessed by calculation of the t-test compared with the standard method (British Pharmacopeia, 2000) (spectrophotometric method) for 95% confidence level with eight degrees of freedom. The results (Table 10) showed that the t-value was less than the critical value, indicating no significant difference between the proposed and standard method for the determination of tetracycline in ointment.

Table 10: Analytical applications of the proposed method and t-values experimental.

Drug	Recovery, % *		t. exp
	Present method	British pharmacopeia method[14]	
Samacycline Skin ointment S.D.I (Iraq)	99.70	99.55	0.459

*Average of five determinations of 100 µg TCH.

Comparison of the methods

Table(11) shows the comparison between some of analytical variables for the present method with that of another literature spectrophotometric methods.

Table 11: Comparison of the methods.

Technique	Reaction	Reagent	λ_{max} (nm)	Berr's law	ε (l.mol^{-1}.cm^{-1})	Reference
FI**/Spec.*	Complexation	Aluminum chloride	376		.....	Saisunee, *et al*., 2006
FI/Spec.	Oxidation	Chloramine -T	535	6.62×10^{-5}-$.72\times10^{-4}$	.....	Jose *et al.*, 2009
Spec.	Oxidative coupling	4-Aminoantipyrine	520	0.04-12 mg/ml	.....	Ayad *et al*., 1986
Spec.	Complexation	Fe^{+3}	435	2.81×10^{-3}-$.50\times10^{-4}$	.....	Ronalad, 2007
Spec.	Charge transfer complexation	Chloranilic acid	540	2.5-30.0 mg/ml	.....	Khairi, 2008
Spec.	Diazo coupling	Diazotised p-nitro aniline	569	2-400 µg/25ml	7.8×10^{4}	Al-Abbasi, 2009
FI/ Spec.		Uranyl acetate	410	1.0-3.0 µg/ml	.....	Prinya *et al.*, 2008
Spec.	Diazo coupling	Diazotised 4-aminoantipyrine	537	1.0-3.0 µg/ml	1.923×10^{4}	Present method

***Spec.= Spectrophtometric method** ** FI= Flow injection method

The results indicate that the proposed method has a good sensitivity and has a wide application part in determination of drug under investigation in its pharmaceutical preparations.

CONCLUSION

The proposed method for the determination of tetracycline -HCl is simple and has a good sensitivity. The proposed method has advantageous over some of the reported visible spectrophotometric methods with respect to reproducibility, precision, accuracy and stability of the coloured species. The proposed method is suitable for the determination of tetracycline -HCl in pure form and in its pharmaceutical preparations.

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