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Improved a Spectrophotometric Estimation of Tetracycline Hydrochloride in Pharmaceutical Preparations through Oxidative Coupling Reaction

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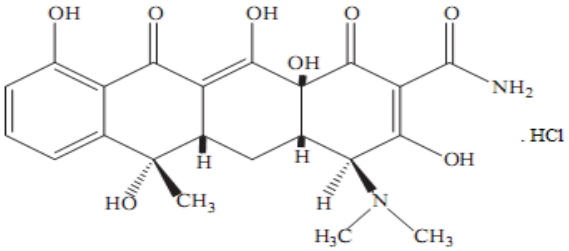
ABSTRACT

This paper comprises the creation of a sensitive, accurate, and reasonably priced spectrophotometric method to analyze tetracycline hydrochloride in its pharmaceutical formsthrough oxidative coupling reaction with p-aminoantipyrine reagent in presence of N-bromosuccinimide as oxidant in the alkaline medium of NaOH to produce a yellow product, which is soluble in water and exhibited maximum absorption peak at 387 nm.The yellow color of the resulting product was stable for more than 1hr and obayed Beer's law in the concentration range of0.5-18.75 µg/mlwith an excellent determination coefficient (R^2= 0.9994)and molar absorptivity1.063x10^4 l/mol.cm.The values of the detection limit (DL) and quantitation limit (QL) were determined and found to be 0.0442 and 0.1475 µg/ml,respectively. Theprecision(RSD) was calculated to be better than 0.66%, whereasthe values of recovery percent was in the range of 99.84% to 101.53%.The nature of the resulting yellow product has been studied between tetracycline hydrochloride andp-aminoantipyrine reagent and it was equal to 1:1.The proposed approach was applied to assay tetracycline hydrochloride in ointment and capsules and no interferences were observed from the common additives found in the drugs.

Keywords: Tetracycline hydrochloride; p-Aminoantipyrine ; Oxidative coupling ; Spectrophotometry

INTRODUCTION

Among broad-spectrum antibiotics, the tetracycline-series antibiotics occupy a leading place . They suppress Rickettsia, acid-resistant bacilli, the reproduction of gram-positive and gram-negative microorganisms and many infections(Mamani *etal*.,2006). Tetracycline hydrochloride (TCH) (Fig.1) is a synthetic agent that has been used in human and veterinary therapy about hundreds of years.It isa yellow powder and chemically acknowledged as [(4S, 4aS, 5aS, 6S, 12aS)-4-dimethylamino-3,6,10,12,12a-pentahydroxy-6-methyl-1 ,11- dioxo-1,4-,4a-,5,5a,6,1,12a-octahydro-tetracene-2-carboxamide monohydrochloride].The chemical formula of TCH is $C_{22}H_{24}N_2O_8$.HCl and its molecular weight 480.94 g/mol (The Japanese Pharmacopoeia, 2016).



The study of the literature found that several different techniques have been described for the direct and indirect valuation of the TCH, they included: cyclic voltammetry using the reduced graphene-oxide (rGO) modified electrode associated with flow-injection analysis(Faria*et al*.,2019),high performance liquid chromatography (HPLC)using iron-tannic nanoparticles as a new adsorbentfor dispersive solid

phase extraction synergized with cloud point extraction (Phomai *et al.*, 2023), HPLC–MS/MS (Pang*etal.*,2021), sulfur quantum dot probes (Lu *etal*., 2021), isotope dilution-liquid chromatography/tandem mass spectrometry(Gab-Allah*et al*.,2023), fluorometric and electrochemical dual-mode nanoprobe for tetracycline (Hu *etal.*, 2020), potentiometric sensor coupled to HPLC (Gil *etal.*,2022) and kinetic spectrophotometry (Alassaf *etal.*, 2019).However, most of these procedures need an expensive equipment and a skilled operation.

UV-Visible spectrophotometry technique is still regarded as a cost-effective and feasible approach in analytical measurements. It is widely used for the analyses of TCH in pharmaceutical formulations.Therefore,different spectrophotometric methods have been employed for the estimation of TCH in the bulk and in the pharmacological preparations. Some of these methodexcluded,a diazotization and coupling reaction which based on the coupling of TCH with diazotizedp-aminoantipyrine in presence of cetylpyridinium chloride (Othman and Al-Ashow, 2012),anthranilic acid (Abd *etal.*,2017), p-nitroaniline (Al-Abbasi, 2009) and sulphanilic acid (Ali *etal.*,2018).Other methods based on the oxidative coupling reaction of TCH with 2,4-dinitrophenylhydrazine (2,4-DNPH) and potassium periodate in a basic medium (Hameedi, 2021), N,N-dimethyl-p-phenylenediamine reagent and sodium meta periodate (Tella *etal.*, 2011) and N,N-diethyl-p-phenylenediamine in presence of oxidizing agent of N-bromosuccinimide in an alkaline medium (Mari *etal.*, 2016). Other spectrophotometric methods have been developed for estimating TCH based on thecomplex formation reactions with cerium(IV) (Al-Sowdani *et al*, 2006), yttrium (III) in presence of cationic surfactant (Thanasarakhana *et al.*, 2011) and zirconium (IV) (Saenjum *etal*., 2022).In addition, the oxidation of TCH with sodium hypochlorite (Ahmed *et al*, 2018) and a charge transfer complex formation of TCH with chloranilic acid (Fahelelbom, 2008) have also been employed for determining TCH spectrophotometrically.

The recent investigation involves a development spectrophotometric method to estimate TCH as pure form and inits drugs (ointment and capsules) through an oxidative coupling reaction using p-aminoantipyrine (p-AAP) reagent in presence of N-bromosuccinimide as an oxidant.

EXPERIMENTAL

Instrumentation

A Jasco V-630 digital double beam UV-Vis. spectrophotometer equipped with 1.0-cm matched fused silica cells and Bp3001 professional bench top pH meter instruments were used for all recording absorption spectra and pH measurements, respectively.

Chemical reagents and standard solutions

All reagents and chemicals used were of a high degree of purity andthe standard material of TCH was attained from drug industries (SDI), Samarra- Iraq.

Standard solution of TCH(100 µg.ml^{-1}).A weight of0.01 g of TCHwas dissolved in a 100 ml of distilled water (dw) using standard flask. The prepared solution was saved in a dark bottle.

p-AAP solution(0.1%w/v). A 0.10 g of p-AAP reagent (Fluka) was dissolved in a little of dw and the volume was then completed to 100 ml with the same solvent and placed in a dark container.

N-Bromosuccinimide solution (0.2%w/v). A weight of0.20 g of N-bromosuccinimide (N-BS) was dissolved in a 100 ml of dw. This solution was placed in brown bottle.

Solution of 0.1M sodium hydroxide was also prepared.

Analysis method and calibration graph

An aliquot of the standard solution of TCH cover the concentration range of 0.5-20 µg/ml of TCH was transferred into a sequence of 20 ml standard flasks.To each flask, a volume of 1.25 ml of p-AAP reagent (0.1%) was added. After 3 minutes 0.75 ml of N-BS solution (0.2%) was then added. The solutions were mixed thoroughly for 2 minutes and followed by adding one ml of 0.1M sodium hydroxide solution. The contents were shaken well and made up to the mark with dw.The absorbance of each solution was then recordedat 387 nm against the blank solution which was prepared similarly but without drug. A linear standard calibration graph was obtained in the concentration range of 0.5-18.75 µg/ml TCH (Fig.2) with a molar absorptivity coefficient of 1.063×10^4 l/mol.cm.

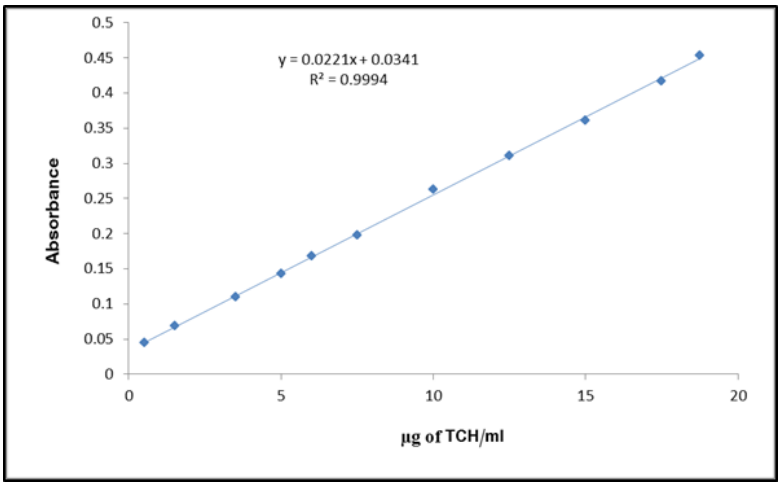


Fig.2: Standard calibration graph of TCH estimation using the recommended procedure

Analysis of TCH in the drugs

For TCH ointment

To obtain (100 µg/ml) of TCH drug, three containers of TCH ointment were mixed thoroughly and a precise amount equivalent to 0.01g TCH was weighed and dissolved in a mixture consisting 3 ml of ethanol and 50 ml of dw. The mixture was warmed and filtered into a 100 ml standard flask and the volume was then diluted up to the mark with dw (Othman and Al-Ashow, 2012).

For TCH capsule

The contents of five capsules[each capsule is containing500 mg of TCH(for tetrasiklin capsule) and 250 mgof TCH(for other capsule drugs)] were weighed and mixed well. An accurate quantity of powder equivalent to 0.01g TCH was taken and dissolved in 100 ml dw using standard flask to obtain a solution of TCH its concentration was equal to 100µg/ml and an aliquot of the drug solution was analysed by following the suggested method.

RESULTS AND DISCUSSION

Absorption spectra

On treatment a solution containing microamount of TCH with p-AAP reagent in presence of N-BS as an oxidant a yellow colure product was produced. The reaction was carried out in a basic solution of sodium hydroxide (0.1M) and in a final volume of 20 ml. The resulting yellow product exhibits a maximum absorption peak at the wavelength of 387 nm against the blank solution (Fig.3).The intensity of the colour is directly proportional to the amount of TCH that originally exist in the sample solution.

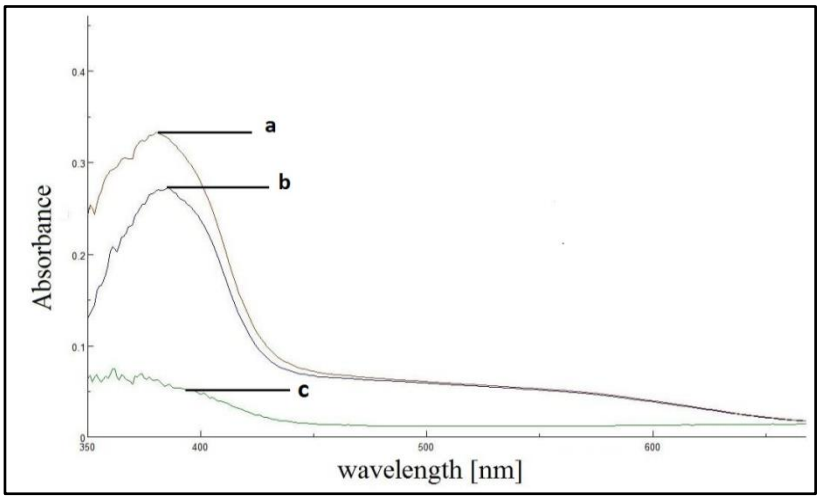


Fig.3: Absorption spectra of the colored product of10µg/ml TCH treated according to the suggested procedure and measured against dw (a),blank solution (b) and the blank solution against dw (c)

Setting the optimum conditions

Subsequent experiments were performed using 200 µg/ml of pure TCH solution in a final volume of 20 mlof standard flask and the absorbance of the colored product was recorded at the wavelength of 387 nm against its blank solution.

Selection of the appropriate oxidizing agent

The influence of various oxidizing agents (0.2%) such as, potassium periodate (KIO_4), N-BS, potassium iodate (KIO_3) and N-chlorosuccinimide (N-CS) on the absorbance of 10µg/ml TCH was investigated and the results are listed in Table 1.

Table 1: Effect of the oxidizing agents on absorbance*

Oxidizing agent(0.2%)	Absorbance	λmax(nm)	Δλmax(nm)
KIO_3	No colour contrast	-	-
KIO_4	0.1544	381	98
NBS	0.2245	387	134
NCS	0.1266	378	120

*[1m of 0.1M NaOH and1ml of p-AAP (0.1%) were used in the procedure]

The results in Table 1 show that the N-BS reagent is suitable for the oxidation of TCH in an alkaline medium of NaOH solution,and a highest absorbance was obtained whena 1.0 ml of N-BS solution was used in the reaction. Therefore,1.0 ml of it was recommended for the subsequent experiments.

Influence of N-BS amount

The influence of different quantities 0.25-1.25 ml of N-BS solution with an increasing quantities100, 200 and300 µg/mlof TCH on the absorbance of the resulting colored product was studied. The results are summarized in Table 2indicate that a 0.75ml of N-BSsolution was found to be the optimum because, it provides a good value of (R^2).Therefore,0.75 ml of N-BSsolution has been established for the nextinvestigations.

Table 2: Effect of the amount of N-BS agent on absorbance.

ml of oxidizing agent (0.2%)	Absorbance /µg TCH added			
	100	200	300	R^2
0.25	0.1329	0.2464	0.3361	0.9954
0.5	0.1363	0.2361	0.3515	0.9983
0.75	0.1394	0.2421	0.3579	0.9988
1.0	0.1322	0.2239	0.3428	0.9945

1.25	0.1257	0.2023	0.3126	0.9893

The effect of oxidation reaction time of N-BS on absorbance was also studied. The results in Table 3 illustrate that the oxidation of TCH with N-BS solution needsat least 2-3 minutes to be completed and thus,2 minuteswasfixed in the subsequent experiments.

Table 3.:Effect of the time of oxidation reaction on absorbance

Time /min	Immediately	1	2	3	5	7	10
Absorbance	0.2422	0.2541	0.2588	0.2587	0.2563	0.2536	0.2436

Effect of p-AAP reagent amount

The effect of diverse quantities 0.5 to 2.0 ml of p-AAP reagent in presence of N-BS reagent as an oxidant and an increasing amounts of TCH on the sensitivity of the method was carried out. The results are explained in Table 4.

Table 4. The effect of p-AAP reagent amount on absorbance.

ml of (p-AAP) solution (0.1%)	Absorbance / µg of TCH added						
	50	70	100	150	200	300	R^2
0.5	0.0512	0.0992	0.1355	0.1754	0.2511	0.3453	0.9862
0.75	0.0618	0.1007	0.1375	0.1822	0.2574	0.3521	0.9924
1.0	0.0881	0.1048	0.1412	0.1871	0.2590	0.3599	0.9971
1.25	0.0887	0.1095	0.1430	0.2026	0.2645	0.3642	0.9984
1.5	0.0796	0.1073	0.1410	0.1905	0.2590	0.3567	0.9975
2.0	0.0763	0.1014	0.1386	0.1839	0.2484	0.3375	0.9961

The results in Table 4 reveal that the volume of 1.25 ml of p-AAP reagent is the optimum because, it shows the greatest intensity of absorption and a good value of the determination coefficient (R^2=0.9984).Therefore, a 1.25 ml of p-AAP was verified in this work.

Effect of the base type and its amount

The effect of different quantities from 0.5 to 2.0 ml of varied types of weak and strong bases (1M) on the absorbance of the product formed was studied by adding 1.25 mL of p-AAP reagent and 0.75 ml of the oxidizing agent N-BS to 10 µg/ml of TCH. The obtained results are illustrated in Fig.4.

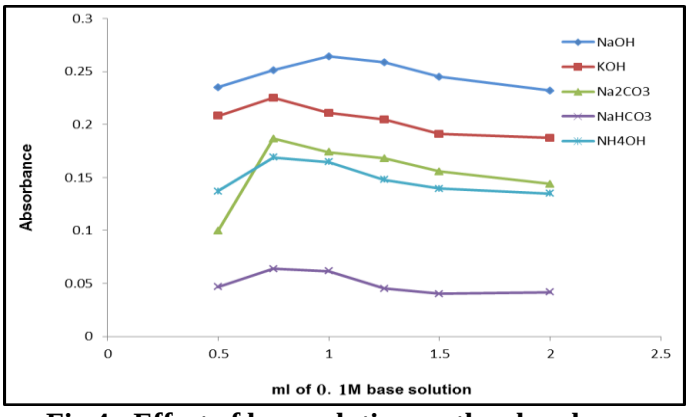


Fig.4 : Effect of base solution on the absorbance

From the results in Fig.4, it was found that a 1.0 ml of sodium hydroxide is still the better quantity to be used as a basic medium for the reactionof TCH with p-AAP in presence of N-BS.

Temperature and reaction time effect

According to the suggested procedure, the influence of the reaction time of p-AAP reagent on absorbance was investigated by following the colour development of the product at room and different

temperatures up to 60°C using a water bath with thermostatic control. The results in (Fig.5) illustrate that the reaction of TCH with p-AAP in presence of N-BS was found to be completed within 3 min at laboratory temperature (25±2).

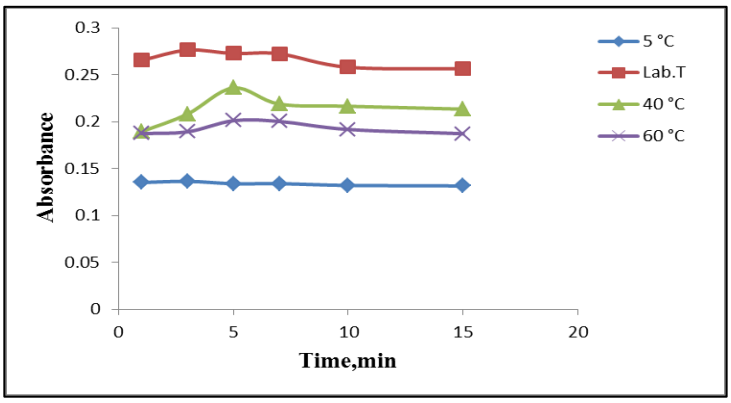


Fig 5: Effect of temperature and reaction time of p-AAP on absorbance

The influence of the addition order of the reacting compounds on the colour development was also examined. Maximum absorbance (0.2824) was achieved by performing the following order of addition (TCH + p-AAP+ N-BS + NaOH).

The effect of time on the colour development of the resulting product was carried out by measuring the absorbance of the final solution at different periods of time. The experimental results in Fig.6 revealed that the colour of the resulting product was formed immediately and no noticeable change was appeared on the absorbance for about 60.0 minutes at laboratory temperature.

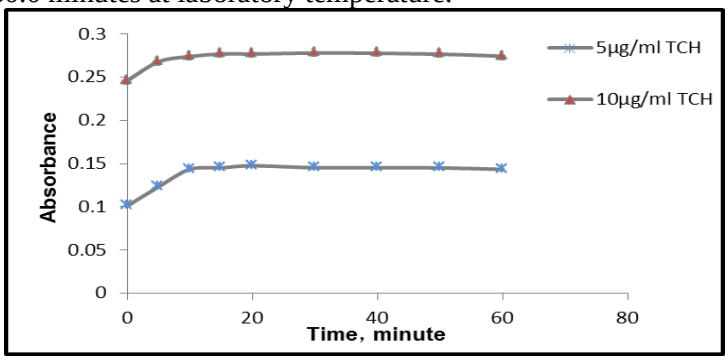


Fig.6: Effect of time on the colour development of the product

Quantification

The limits of Beer's law, molar absorptivities, Sandell's sensitivities, accuracy (recovery %), and precisions (RSD) of the proposed procedurewere evaluated. The linearity of the methods was also described by the equation of regression, as well as the corresponding determination coefficient (R^2) for TCHwas calculated by the recommended method and represented an excellent linearity. The detection limit (DL) and quantitation limit (QL) were found according to the rules guidelines (Shrivastava and Gupta, 2011) andthe results are summarized in Table 5.

Table 5: Analytical data and optical characteristics of the proposed method

Parameters	Value

Beer's law range (µg/ml)	0.5-18.75
Molar absorptivity (l/mol.cm)	$1.063x10^4$
DL (µg/ml)	0.0442
QL(µg/ml)	0.1475
Relative error percent range (%)	-0.68% to1.90%
Recovery percent range (%)	99.84% to 101.53%
RSD (%) (N=5)	better than 0.66%
Determination coefficient (R^2)	0.9994
Slop (a)#	0.0221
Intercept (b)#	0.0341
Range of t-value	0.63-2.20
Range of F-value	1.57-6.35

Regression equation (X = b + ac) , where c is [TCH] in µg/ml.

Composition of the resulting product

The stoichiometry of the coloured product which formed between the reaction of TCH and p-AAP reagent was studied by applying the continuous variation and mole ratio methods (De levic, 1997). In continuous variation method, volumes of 0.5 to 2.5ml of $2.079\times10^{-4}$of p-AAP were coupled according to the recommended procedure with the corresponding complementary volume of 2.079×10^{-4} M TCHsolution to give a total volume of 3 ml and diluted to 20 ml with dw. While in mole ratio method, an increasing volumes from 0.2 to 5.0 ml of 2.079×10^{-4} Mp-AAP solution were added to a 1.0 ml of 2.079×10^{-4} of TCH and the absorbance was recorded at 387nm after dilution to the mark with dw. The obtained results are illustrated in Fig.7 reveal that the resulting product was formed by a 1:1 combining ratio of TCH to p-AAPreagent.

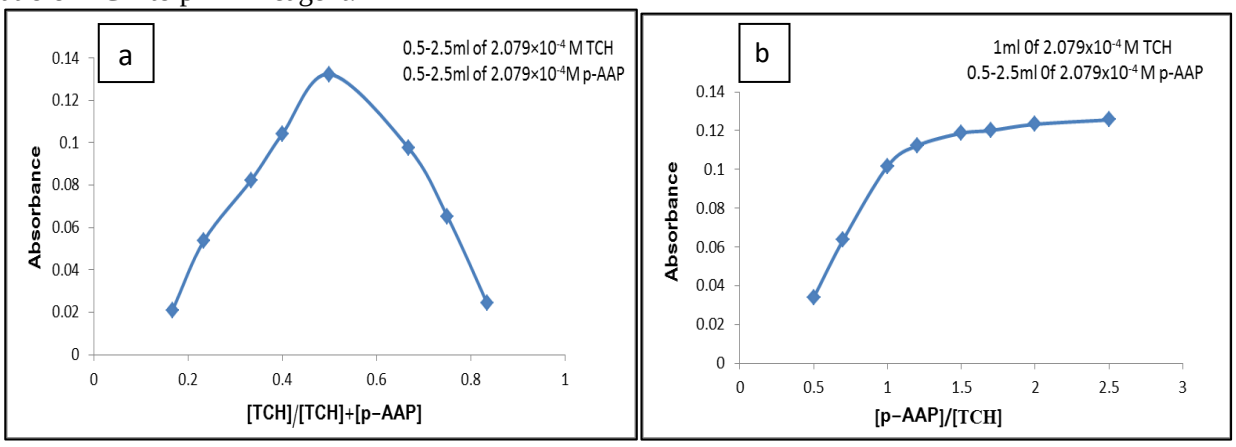
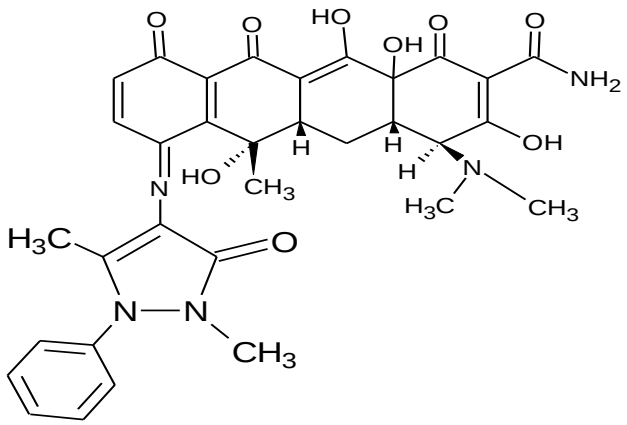


Fig.7: Plots of the (a) mole ratio and (b) continuous variation methods for the colored product

According to the results of stoichiometry study the suggested chemical structure of the colouredproduct formed through the oxidative coupling reactionof TCH with p-AAP can be clarified in the scheme 1.



Scheme 1: The proposed chemical structure of the product

Application

The suggested method has been applied for the analysis of TCH in its pharmaceutical formulations (capsules and skin ointment) from different origins and at three different quantities (100, 200, 300 µg). The results are listed in Table 6 indicate that the development method is a suitable for assaying TCH with an acceptable results.

Table 6: Estimation of TCH in the pharmaceutical formulations

Drug	Certified value	TCH Found(µg)*	R.E. (%)*	Mean recovery (%)	RSD(%) (N=5)	Measured value
Apcycline (India)	250 mg / Capsule	99.47 200.89 298.83	-0.53 0.45 -0.37	99.84	0.47 0.15 0.09	249.60
Tetracycline (Iran)		100.48 198.64 299.32	0.48 -0.68 -0.23	99.86	0.45 0.37 0.25	249.64
Samacycline (SDI-Iraq)		100.15 200.15 301.16	0.15 0.08 0.38	100.20	0.31 0.66 0.08	250.50
Tetrasiklin (Turkey)	500 mg / Capsule	100.29 201.60 301.56	0.29 0.82 0.52	100.54	0.56 0.02 0.53	502.70
Samacycline skin ointment (SDI-Iraq)	3%	101.17 203.80 304.56	1.17 1.90 1.52	101.53	0.58 0.06 0.02	3.046%

*Average of five estimations ,

Evaluation of the method

To evaluate the results of the development method, the statistic factors t-test and F-test values have been investigated and the results in Table 7 reveal that the experimental t and F values are less than the tabulated values at the 95% confidence level (Christian*et al.*, 2014). These results indicated that the difference between the proposed and literature methods(Sultan *et al.*, 1988) was statistically not significant, which confirms the success of applying the proposed method to estimate TCH in its pharmaceutical formulations.

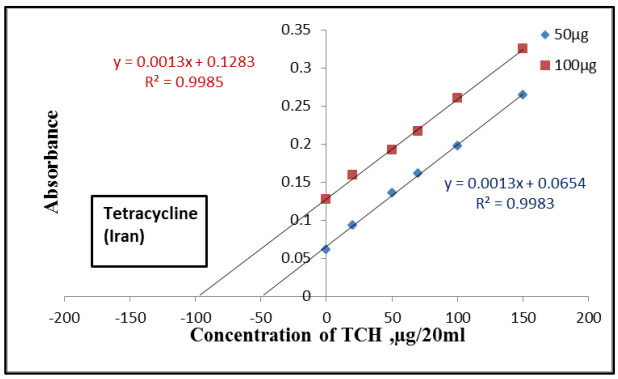
Table 7: Evaluation of the suggested method for the estimation of 100 µgTCH in the drug

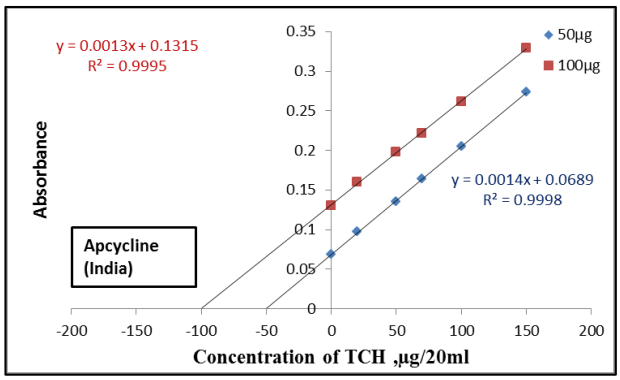
Drug	Certified value	TCH Found (µg)		Recovery ± RSD (%)*		t# value	F# value
		Present method *	Literature method *	Present method	Literature Method		
Apcycline (India)	250 mg / capsule	99.47	99.66	99.47±0.47	99.66±0.21	2.20	2.14
Tetracycline (Iran)		100.48	100.73	100.48±0.45	100.73±0.26	1.84	1.57
macycline (SDI-Iraq)		100.15	100.05	100.15±0.31	100.05±0.20	1.20	2.30
Tetrasiklin (Turkey)	500 mg / capsule	100.28	100.42	100.28±0.56	100.42±0.46	0.63	1.59
Samacycline skin ointment (SDI-Iraq)	3%	101.17	101.47	100.17±0.58	101.47±0.25	2.12	6.35

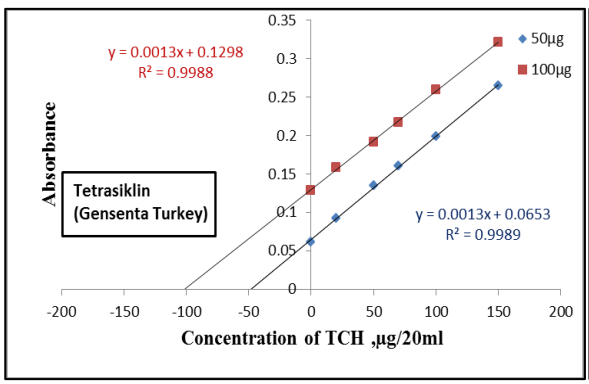
*Average of five estimations , # [$F=S_1^2/S_2^2$ where $S_1^2 > S_2^2$, $\pm t = \frac{x1-x2}{Sp}\sqrt{N1N2/N1+N2}$, N=10]#Tabulated t and F values at 95% confidence level of eight degree of freedom are 2.31 and 6.39

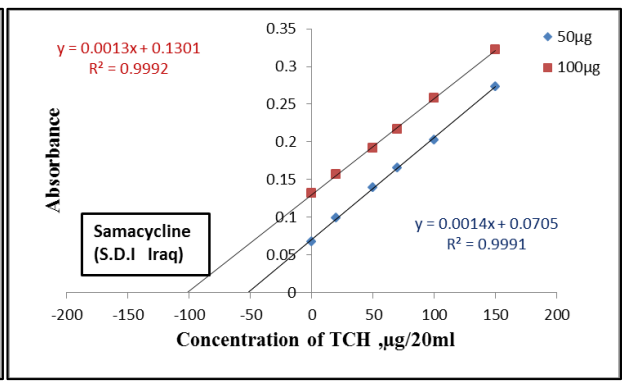
Standard addition method

To prove the efficiency and credibility of the proposed method in the estimation of PPH and to ensure that it was free from the interference of additives, a standard addition method was used. The results are listed in Table 8 and illustrated in Fig.8 indicate that there is a high agreement between the standard addition method and the proposed methodfor the estimation of TCH in its pharmaceutical forms.









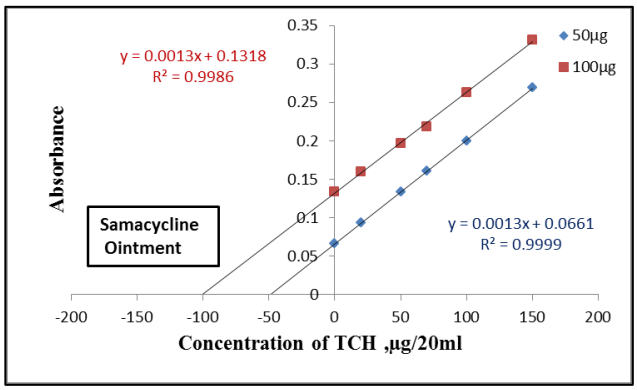


Fig.8:Calibration graphs of standard addition methods for the analysis of TCH in the pharmaceutical formulations

Table 7.: The results of standard addition method for analysis 50 and 100 µg TCH

tracycine Drug	Certified value	TCH found (µg)	Mean recovery (%)	Measured value
Apcycline (India)	250 mg / Capsule	49.21 101.15	99.79	249.48 mg
Tetracycline (Iran)		50.30 98.69	99.65	249.13 mg
Samacycline (SDI- Iraq)		50.36 100.08	100.40	251.00 mg
Tetrasiklin (Turkey)	500 mg / Capsule	50.23 99.85	100.16	500.80 mg
Samacycline skin ointment (SDI-Iraq)	3%	50.36 101.38	101.54	3.0462 %

CONCLUSION

The suggested spectrophotometric methodperformed, based on the reaction of p-AAP reagent with TCH in presence of N-BS as oxidant through oxidative coupling reaction. The proposed method has the advantages of being sensitive, low-cost, accurate and precise enough to replace the current spectrophotometric method. and straightforward because, it does not involveany critical steps such as, temperature control and a solvent extraction process. The suggested approach is also accurate and precise enough to replace the current spectrophotometric method; hence it can be used routinely for the estimation of TCH in capsules and ointment with accepted recoveries.

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