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Colourimetric Method for Assaying Tetracycline Hydrochloride in Pharmaceutical Formulations via Zirconium Complex Formation

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Abstract

This paper includes development of a sensitive, accurate and precise direct spectrophotometric approach to estimate tetracycline hydrochloride in the bulk solution and in its pharmaceutical preparations. The method was based on the reaction of tetracycline hydrochloride with zirconium (IV) in presence of acetate buffer solution at pH3.6 to form a yellow complex with a highest absorption peak at 398 nm. The resulting complex conforms Beer's law to cover the concentration in the range of 0.5 to 12.5 µg/ml with a good determination coefficient (r^2=0.9989), molar absorptivity of 2.7798×10^4 l/mol.cm and Sandell's sensitivity of 0.0173 µg/cm^2. The Limits of detection (LOD) and quantitation (LOQ) were equal to 0.019 and 0.063µg/ml, respectively. A relative standard deviation (precision) was evaluated to be better than 0.91% depending on the concentration levels, whereas the value of recovery percentage were in the range of 97.20% to 103.82%. The approach was useful to the analysis of tetracycline hydrochloride in capsules and skin ointment. The statistic factors t-test and F-test values have also been investigated and revealed that the experimental "t" and "F" results were less than the theoretical results at confidence level of 95%.

Keywords: Tetracycline Hydrochloride, Zirconium (IV), Complexation, Spectrophotometry

Introduction

Tetracycline hydrochloride (TCH) is one of the most important antibiotics which widely use in the treating human and animal infections, it is named tetracyclines due to the four (tetra) hydrocarbon rings (Figure 1). TC-series medicines hold a prominent position among broad-spectrum antibiotics. They inhibit the growth of gram-negative, gram-positive microbes and different contaminations, including Rickettsia and acid-resistant microbes [1]. TCH is chemically identified as ((4S, 4aS, 5aS, 6S, 12aS)-4-dim-ethylamino-3,6,10, 12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4-,4a-,5,5a,6,11-,12a-octahydrotetracene-2-carboxamide monohydrochloride) (M.Wt.=480.935 g/mol) [2].

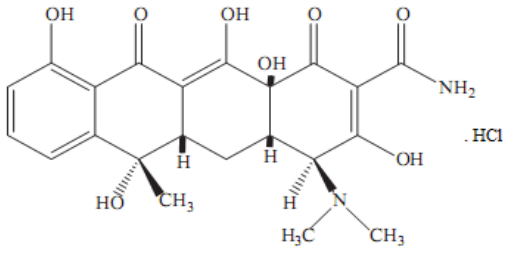


Figure 1: The chemical structure of tetracycline hydrochloride.

TCH is a synthetic agent used in human and veterinary therapy for hundreds of years. Several different methods have been described in the literature for direct and indirect valuation of the TCH in its drug forms, they comprised : HPLC technique with diode array detection [3], sulfur quantum dot probes [4], HPLC-MS/MS [5], a potentiometric sensor coupled to HPLC [6], cyclic voltammetry

associated with the flow-injection analysis [7], fluorometry associated with a nanoprobe for tetracycline prepared from carbon nitride quantum dots and silver nanoparticles [8], and kinetic method [9]. However, several of these procedures need a skilled operation and expensive equipment.

For the analytical measurement of TCH in pharmaceutical formulations, UV-Visible spectrophotometry is still regarded as a cost-effective and feasible approach. TCH has also been assayed in the bulk sample and in its pharmacological formulations by using a variety of spectrophotometric techniques. Some of these approaches comprised diazotization and coupling reactions in which TCH was coupled with diazotized of p-nitroaniline [10], 4-aminoantipyrine (4-AAP) and a cationic surfactant like cetylpyridinium chloride [11], anthranilic acid [12], sulphanilic acid [13]. Other approaches depended on the oxidative and coupling reaction of TCH with N,N-diethyl-p-phenylenediamine and $NaIO_4$ [14], 2,4-dinitrophenylhydrazine (2,4-DNPH) in presence of KIO_4 [15], N,N-diethyl-p-phenylenediamine reagent in existence of N-bromosuccinimide as an oxidant using a basic medium [16] and 4-AAP in incidence of N-bromosuccinimide [17]. TCH has also been estimated through the complex formation with yttrium ion (Y^{3+}) in existence of cationic surfactant [18]. TCH was also determined by spectrophotometric methods via bleaching reaction using indigo carmine dye and N-bromosuccinimide as oxidant [19], charge transfer complex formation using a reagent of chloranilic acid [20] and oxidation reaction with sodium hypochlorite (NaOCl) [21]. However, majority of these methods suffer from various difficulties, for instance, low range of estimation, poor selectivity, moderate sensitivity and low stability of the resulting product and time consuming.

The focal aim of this approach is to create an economic, rapid, simple and inexpensive visible spectrophotometric method. The approach was created on the establishment of a yellow complex by the reaction of TCH with zirconium (IV), which has been used to assay TCH in different capsules and ointment.

Experimental Instrumentation

A devices of double-beam UV-visible spectrophotometer (JASCOV-630) using matched fused silica cells with 1.0-cm light width were used for all measurements of absorbance and absorption spectra. While the pH measurements were recorded by using a professional Benchtop pH meter Model (BP3001).

Chemical Reagents and Materials

All chemicals used were of the highest purity available.
TCH was brought from the state company for drug industries (Sammara-Iraq (SDI)).

TCH working Solution, 100 µg/ml: A 0.010 g quantity of pure TCH was dissolved in little amount of dw (distilled water) and then completed to a final volume of 100 ml using a calibrated flask.

Zirconium (IV) Stock Solution, 1000 µg/ml: A 0.3137 g quantity of zirconyl chloride ZrOCl2.8H2O (Fluka) was dissolved in a 100-ml dw using a calibrated flask.

Zirconium (IV) Working Solution, 250 µg/ml (2.741×10-3 M): This solution was made by diluting 25 ml of the zirconium (IV) stock solution with dw in a 100-ml calibrated flask.

Acetate Buffer solution (pH3.6). It was prepared by mixing 46.3 ml of acetic acid solution (0.2M) with 3.7 ml of sodium acetate solution (0.2M), and the volume was completed to 100 ml with dw using a calibrated flask [22].

Essential Procedure

To sequence of 20 ml calibrated flasks an increasing quantities 10-250 µg of TCH were added followed by 2 ml of acetate buffer solution (pH3.6). To each flask, 1.75 ml of zirconium (IV) working solution (2.741×10-3 M) was added. The contents of the flasks were shaken thoroughly and allowed to stand for 3-5 min. and the final volume was made up to the mark with dw. After that, the absorbance the resulting complex was measured at 398 nm against corresponding reagent blank which contain all materials except TCH.

Pretreatment Procedure for Assaying TCH in the Capsules and Ointment

TCH Capsule Solution (100 µg/ml)

The solution of this medicine was prepared by mixing the contents of five capsules (every capsule containing 250 of TCH and 500 mg (for tetrasiklin capsule only)) were mixed well and weighed. An exact quantity of medicine powder equal to 0.0100 g TCH was dissolved in dw in a 100-ml.calibrated flask and a suitable volume of the TCH capsule solution was pipetted and analyzed according the established procedure.

For TCH Ointment Solution (100 µg/ml)

The contents of three TCH ointment containers were carefully mixed and homogenized well. A precise quantity of the homogenized ointment equal to 0.0100 g pure TCH was dissolved a mixture of 3 and 50 ml ethanol and 50 dw, correspondingly. The final solution was warmed and filtered into a 100-ml calibrated and the volume was made to the mark with dw. A convenient volume of the TCH ointment solution was taken and estimated by following the development procedure [11].

Optimization of the Reaction Parameters

pH Influence and the Type of Buffer Solutions

The pH influence on the absorbance of TCH-Zr complex was studied carefully and the results are illustrated in Figure 2 show that the TCH-Zr complex was produced and exhibited maximum absorbance at optimal pH of 3.6 and λmax=398 nm. Therefore, the value of pH3.6 was selected for the next experiments.

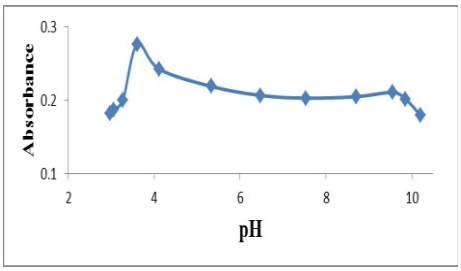


Figure 2: Effect of pH on absorbance of TCH-Zr complex.

Therefore, various buffer solutions of pH 3.6 were prepared and their effect and efficiencies were investigated on the absorbance of the TCH-Zr complex formed. The experimental data are précised in Table 1 and indicated that a 1ml of acetate buffer solution (B3) of pH 3.6 was the optimum and selected for the subsequent measurements.

Type of buffer solution*	Absorbance/ml of buffer solution added							Final pH range
	0.3	0.5	0.7	1.0	1.5	2.0	2.5	
B1	0.2376	0.2406	0.2471	0.2580	0.2601	**0.2620**	0.2473	3.58-3.60
B2				Turbid				
B3	0.1041	0.1190	0.1114	0.0985	0.0952	0.0949	0.0928	3.36-3.54
B4	0.2421	0.2589	0.2577	0.2504	0.2459	0.2404	0.2363	3.58-3.62

*B1: 0.2M acetic acid + 0.2M sodium acetate; B2: 0.1M potassium hydrogen phthalate + 0.1MHCl; B3: 2.0M citric acid + 2.0M sodium hydroxide; B4: 2M acetic acid + 2M sodium acetate.

Table 1: Influence of different buffers on absorbance.

Effect of Zirconium Amount

The effect of different portions 0.5-2.5 ml of zirconium ion on absorbance of the resulting TCH-Zr complex was studied and Table 2 shows the results.

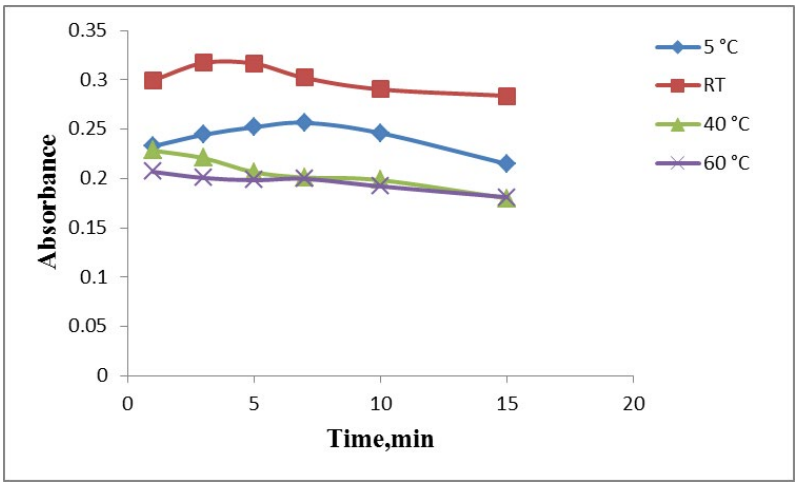
ml of Zr(IV) solution (2.741×10^{-3} M)	Absorbance/ml of TCH solution						r^2
	25	50	75	100	150	200	
0.5	0.0924	0.1161	0.1850	0.2265	0.3437	0.4498	0.9945
1.0	0.0845	0.1295	0.1782	0.2844	0.3733	0.4909	0.9896
1.5	0.0806	0.1535	0.1979	0.2717	0.3912	0.5018	0.9981
1.75	0.0922	0.1612	0.2266	0.2993	0.4231	0.5415	0.9990
2.0	0.0936	0.1598	0.2123	0.2747	0.4188	0.5290	0.9981
2.25	0.0898	0.1418	0.2014	0.2531	0.4021	0.4945	0.9952
2.5	0.0815	0.1315	0.1873	0.2479	0.3875	0.4767	0.9955

Table 2: Zirconium amount effect on absorbance.

The results in the above Table show that the addition of 1.75 ml of zirconium solution gave the highest absorbance of the resulting complex and good determination coefficient (r^2=0.9990), so it was adopted for the next study.

Temperature and Reaction Time Effect

In order to investigate the temperature and time effect on the formation and stability of the TCH-Zr complex, the reaction was performed at different temperatures (5, RT, 40, and 60°C) with different times using a water bath with a thermostatic regulator. The results are illustrated in Figure 3.



RT=Room temperature (25±2 C°)

Figure 3: Effect of temperature and reaction time on absorbance.

The results in Figure 3 show that the reaction of TCH with zirconium ion needs about 3-5 minutes to be completed and to appear the yellow colure of the complex at room temperature.

Time Effect on the Colour Development

The effect of time on the stability of the colour of the resulting complex TCH-Zr was studied at increasing time at the wavelength of 398 nm. Figure 4 reveals that the resulting yellow colour of the complex is appeared directly and remains stable for at least 60 minutes, which was a sufficient period for conducting measurements.

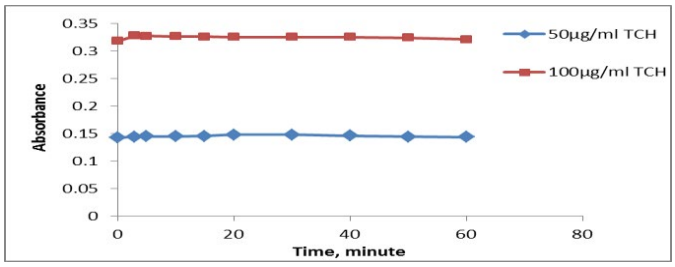


Figure 4: Time effect on the colour development.

Absorption Spectrum

The final absorption spectrum was drawn by adding 1 ml of TCH solution (100 µg/ml), 2 ml of buffer solution (pH3.6), and 1.75 ml of working zirconium solution in a 20 ml calibrated flask. The solution was left for 3-5 minutes and complete to the mark with DW. The resulting complex of TCH-Zr exhibits a maximum absorption peak at 398 nm versus blank solution (Figure 5).

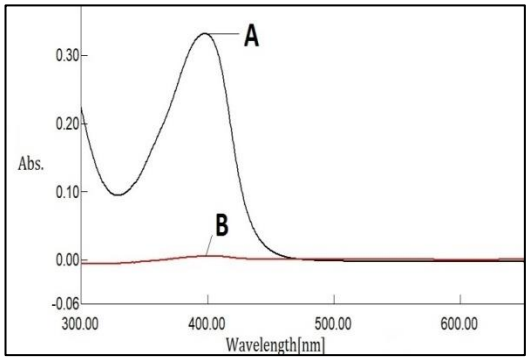


Figure 5: Final absorption spectra of 5 µg/ml of TCH recorded (A) Vs. blank and (B) blank Vs dw.

Calibration Curve, Quantification and Detection limits

A linear standard calibration curve was obtained adheres to Beer's law in the concentration range from 0.5 to 12.5 µg/ml TCH with an excellent determination coefficient (r^2) equal to 0.9989 (Figure 6). The results of molar absorptivity, Sandell's sensitivity, LOD and LOQ were valued and set up to be 2.7798 x 10^4 l/mol.cm, 0.0173 µg/cm^2, 0.019 and 0.063 µg/ml, correspondingly [23], which indicated that the approach is very sensitive.

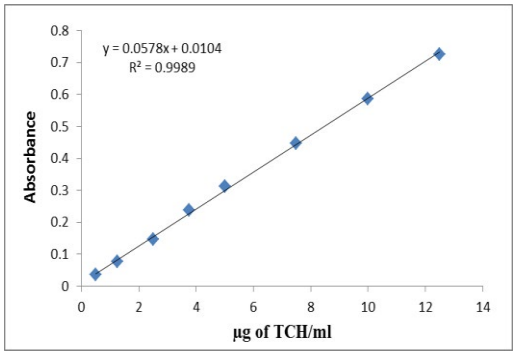


Figure 6: Standard plot for determining TCH according to the proposed method.

Nature of the Resulting Complex

Under the optimized conditions of the essential procedure, continuous variation and slope ratio methods [24] were used to clarify the correlation ratio of TCH with Zr (IV) Both methods were carried out using the same concentrations of TCH and Zr (IV) solutions (2.079×10^{-4}M). The results of both methods are explained in (Figure 7) and (Figure 8) revealed that the complex was created by the reaction of TCH and Zr (IV) at pH3.6 in a ratio of 1:1.

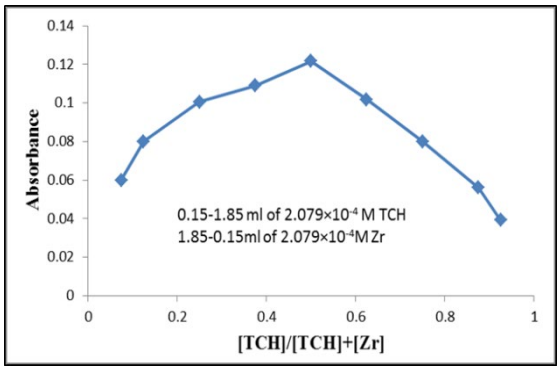


Figure 7: Plot of the continuous variation method.

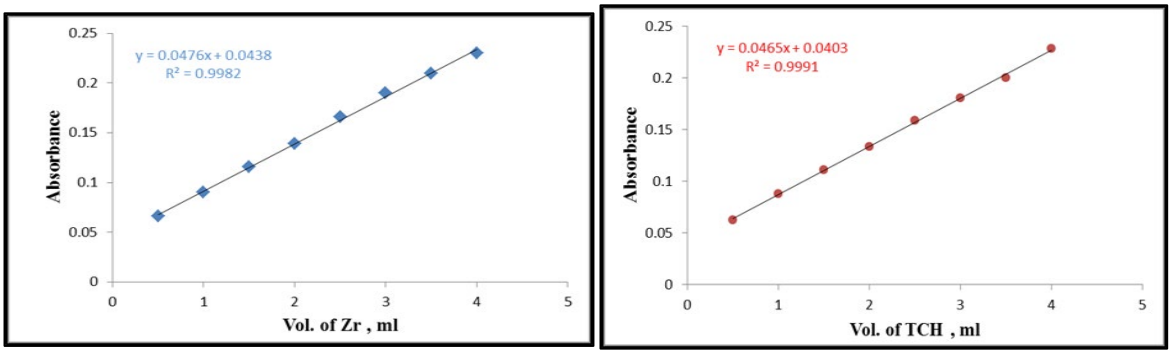


Figure 8: The plots of the slope ratio method of the resulting complex.

Application

The recommended approach has been used to analyze TCH in its pharmaceutical forms (capsules and skin ointment) at three different quantities 50, 100, and 150 µg TCH and from various origins. The results are listed in Table 3 and indicated that the development method is suitable for assaying TCH with an acceptable results.

Pharmaceutical preparation	TCH Found (µg)*	R.E. (%)*	Recovery (%)*	Recovery Average	RSD (%)*	Measured value
Apcycline 250mg/capsule (India)	49.15 100.68 150.09	-1.70 0.68 0.06	98.30 100.68 100.06	99.68	0.48 0.16 0.09	249.20 mg
Tetracycline 250mg/capsule (Iran)	48.60 98.09 149.86	-2.80 -1.90 -0.09	97.20 98.10 99.90	98.40	0.42 0.20 0.07	246.00 mg
Samacycline 250mg/capsule (SDI-Iraq)	50.22 99.96 150.10	0.44 -0.04 0.07	100.44 99.96 100.07	100.16	0.23 0.13 0.91	250.40 mg
Tetrasiklin 500mg/capsule (Gensenta Turkey)	50.49 100.59 149.81	0.98 0.59 -0.13	100.98 100.59 99.87	100.48	0.30 0.23 0.12	502.40 mg
Samacycline Ointment (3%) (SDI- Iraq)	51.91 101.02 151.00	3.82 1.02 0.67	103.82 101.02 100.67	101.84	0.59 0.20 0.11	3.055 %

*Average of five estimations

Table 3: Analysis of TCH in the pharmaceutical formulations.

Evaluation of the Method

The statistic factors t-test and F-test values have been investigated to evaluate the results of the development method and the fallouts in Table 4 reveal that the experimental t and F values are better than the theoretical t and F values at the confidence level of 95% [25]. These results indicated that the difference between the proposed and the literature method was statistically not important, showing the possibility of using the proposed method to assay TCH in its pharmaceutical formulations.

Drug	Recovery(%) ±RSD*		F-value#	t- value#
	Present method	literature method**		
Apcycline 250mg/Capsules (India)	150.092±0.091	150.114±0.087	1.11	0.71
Tetracycline 250mg/Capsules (Iran)	149.86±0.074	149.914±0.040	3.42	2.12
Samacycline 250mg/Capsules (SDI-Iraq)	150.098±0.091	150.066±0.072	1.68	1.00
Tetrasiklin 500mg/Capsules (Gensenta Turkey)	149.804±0.123	149.71±0.122	1.00	1.55
Samacycline 3% Skin ointment (SDI-Iraq)	150.996±0.114	151.076±0.059	3.75	1.84

*Average of five estimations; ** [26]. # ($F=S_1^2/S_2^2$ where $S_1^2>S_2^2$, $\pm t=\frac{\bar{x}1-\bar{x}2}{Sp}\sqrt{N1N2/N1+N2}$, N=10);

#Theoretical t and F values for eight and four degrees of freedom at 95% confidence level are 2.306 and 6.39, respectively.

Table 4: Evaluation of the suggested method for determining 150 µg TCH in the drugs.

Selectivity of the Development Approach

To prove the productivity and credibility of the development approach for the estimation of TCH and to ensure that the method was unrestricted from the undesired additives, a standard addition method was used. The fallouts are illustrated in Figure 9 and itemized in Table 5 indicated that there is a high covenant between the results of the proposed and standard addition methods for the analysis of TCH in its drug forms.

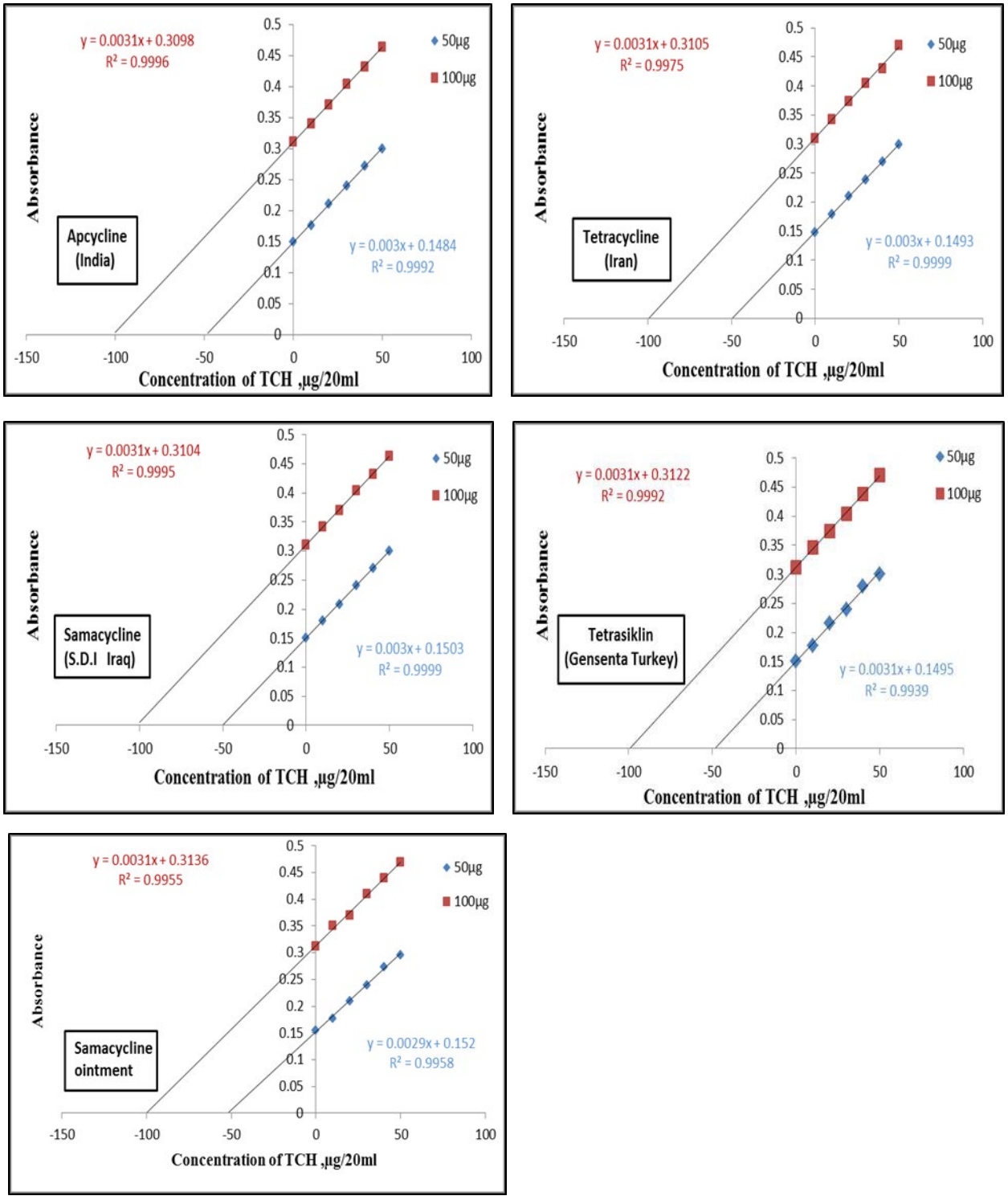


Figure 9: Standard addition plots for determining TCH in capsules and ointment.

Pharmaceutical preparation	TCH Taken, µg	TCH Found, µg	Recov., %	Measured value, mg
Apcycline 250 mg/capsules (India)	50	49.47	98.84	247.10
	100	99.94	99.94	249.85
Tetracycline 250 mg/capsules (Iran)	50	49.77	99.54	248.85
	100	100.16	100.16	250.40
Samacycline 250 mg/capsules (SDI-Iraq)	50	50.17	100.34	240.85
	100	100.13	100.13	250.33
Tetrasiklin 500 mg/capsules (GensentaTurkey)	50	48.23	96.46	482.30
	100	100.71	100.71	503.55
Samacycline (3%) skin ointment (SDI-Iraq)	50	52.41	104.82	3.1446#
	100	101.16	101.16	3.0348#

Conc. in (%)

Table 5: The results of standard addition method for analysis 50 and 100 µg TCH.

Conclusion

The approach defines a simple and easy spectrophotometric procedure for assaying TCH through complex formation reaction. The approach was based on the reaction of TCH with Zr (IV) ion at pH3.6 to give a yellow water soluble complex. It was obeyed Beer's law in the range concentration from 0.5 to 12.5 µg/ml. The suggested approach has the welfares of being sensitive, inexpensive, and accurate enough to replace the recent spectrophotometric method. The development approach does not comprise temperature control nor extraction steps. The development approach has a good selectivity, so it may be frequently utilized for the analysis of TCH in capsules and ointments with recognized recoveries.

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