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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF TETRACYCLINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS USING RHODIUM (II) AS A MEDIATOR METAL

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ABSTRACT

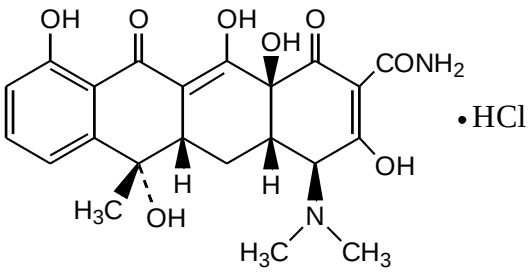
The study includes new developed indirect (FAAS) as well as molecular spectrophotometry for determination tetracycline hydrochloride (TCH) as chelate complex in 2-heptanon as solvent for extraction the complex. Absorption spectrum of yellowish green drug complex (TCH) with Rh(II) at maximum wavelength 430 nm. Linear dynamic range (5-160)µg.ml^{-1}, (D.L. = 0.163 µg.mL^{-1}), (r = 0.9996), (%$E_{rel.}$ = 0.296), (%Recovery = 99.7 ± 0.114), (%RSD = 1.30), (molar absorptivity = 2.891×10^3 L.mol^{-1}.cm^{-1}). The optimum condition: (pH = 1.9), T = 90°C, concentration of (Rh(II) = 60 µg.ml^{-1}), reaction time = 135 second), aqueous to organic phase ration (5:3), shacking complex = 2.5 min.), metal to ligand (1:1) with UV-Vis. method. When using Indirect (FAAS): concentration of (Rh(II) = 20 µg.ml^{-1} one extraction process with 2-heptanon gave (97.60%), linear dynamic range (2-50 µg.ml^{-1}), (r= 0.9995), (%RSD = 0.897), (D.L= 0.140 µg.ml^{-1}), %$E_{rel.}$ = 0.567), Recovery = (100.567 ± 0.0016). Additives did not interfere in this method.

INTRODUCTION

Tetracycline is a group of antibiotics produced of genus streptomyces, it is effective against wide range of gram positive and gram negative bacteria interferiny with protein synthesis in these microorganisms. Tetracycline may cause permanent discoloration of developing teeth and it is not given to the pregnant, lactating women and growing children because of the development of strains of microorganisms resistant to the tetracycline.

Tetracycline hydrochloride (TCH) useful because of broad antimicrobial action, it is chiefly used in treating infections caused by streptococci, staphylococci, gram-negative bacilli, riclkcettsiae and viruses.

The structure formula of TCH[1,2] as shown blow, and have molecular formaula: $C_{22}H_{24}N_2O_8$.HCl



The pH of TCH is (2.0-2.5), crystalline (yellow) powder soluble in water, slightly soluble in alcohol, practically insoluble in acetone and ether, it dissolves in solutions of alkali hydroxide and carbonate. The solution of TCH in water have the maximum wavelengths (213, 271, 344 and 363 nm)[3].

Analytical methods used for determination (TCH), spectrophotometric[4,5], flow injection[6,7], chromatographic[8] fluorometric[9] and titometric[10] methods.

EXPERIMENTAL

(A) Apparatus

1- Shimadzu, UV-Vis spectrophotometer UV-160A.
2- Shimadzu Flame, Atomic absorption spectrophotometer AA-670.
3- pH meter Philips, PW 9420.

(B) REAGENTS

1- Stock solution of Rhodium ion (1000 µg.ml^{-1}) prepared by dissolving (0.1073 gm) of Rhodium acetate $Rh(CH_3COO)_2$ in 5 ml distilled water and some drops of concentrated HCl were added the volume completed to 50 ml with distilled water.
2- Stock solution of TCH (1000 µg.ml^{-1}) prepared by dissolving (0.1000 gm) of TCH in distilled water then completed to 100 ml.
3- Complex solutions: (0.1-2) ml from stock solution TCH (1000 µg.ml^{-1}) were transferred to 5 ml volumetric flasks then 2.5 ml of (100 µg.ml^{-1}) Rh(II), the optimum conditions were fixed and the formed complexes were extracted with 2-heptanone and the absorption spectra were measured versus organic solvents as blank solutions.
4- The solution of pharmaceutical preparation (Apcycline): a solution of (1000 µg.ml^{-1}) from Apcycline was prepared by taking twenty capsules of weighted pharmaceutical preparation and the average of each capsule was (0.2861 gm), the (0.1144 gm) of Apcycline powder was dissolved in distilled water and filtered, the filtrate was diluted to 100 ml and a solution of (400 µg.ml^{-1}) was prepared from the last solution.

RESULTS AND DISCUSSION

(A) Spectrophotometric Studies

1- The drug spectrum (TCH)

The spectrum of the drug TCH (50 µg.ml^{-1}) in the ultraviolet visible region Figure (1) shows the maximum absorption of TCH at different λ_{max} (213, 271, 344 and 363)nm versus water as blank solution.

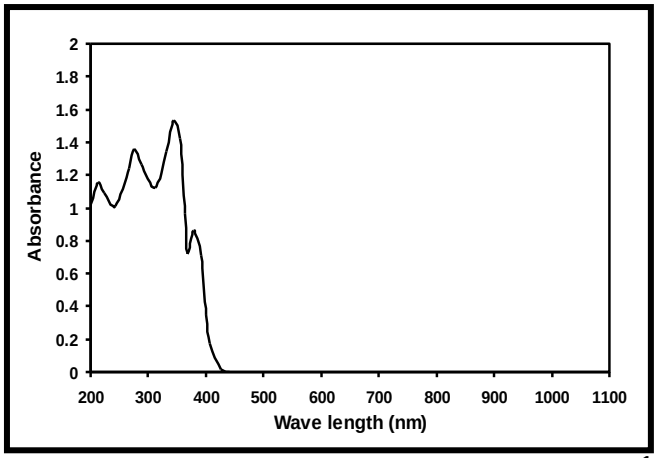


Fig. 1: Molecular absorption spectrum of (50 µg.ml^{-1}) TCH versus water as ablank solution

2- The spectrum of Rhodium(II) ion

Figure (2) shows absorption spectrum of Rhodium(II) (50 µg.ml^{-1}) at (λ_{max} = 215 nm), the measurement was done versus water as a blank solution.

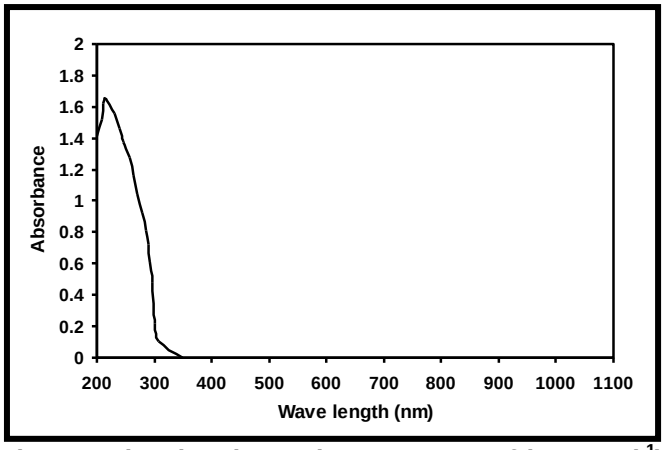


Fig. 2: Molecular absorption spectrum of (50 μg.ml^{-1}) Rhodium ion Rh(II) versus water as a blank solution

3- The spectrum of [TCH-Rh(II)] complex

Figure (3) shows absorption spectrum of yellow green drug complex TCH (100 μg.ml^{-1}) with Rh(II) (50 μg.ml^{-1}) at (λ_{max} = 430 nm), the optimum conditions were fixed and the formed complex was extracted with 2-heptanone and the absorption spectra was measured versus organic solvent as blank solution.

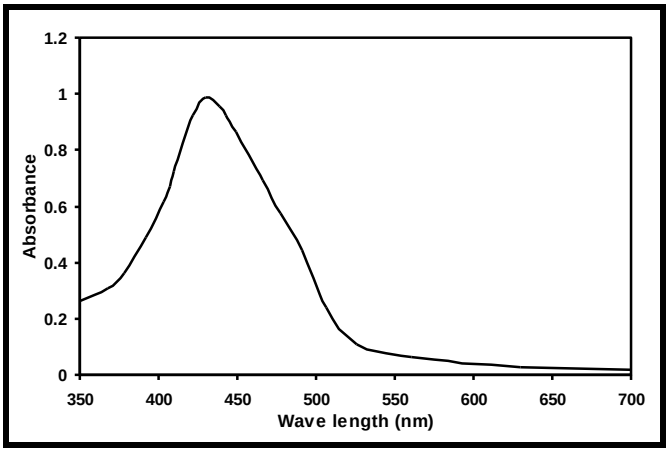


Fig. 3: Molecular absorption spectrum for complex [TCH-Rh(II)]TCH (100 μg.ml^{-1}) + Rh(II) (50 μg.ml^{-1}) and V_o = 3 ml of 2-heptanone

(B) Determination of TCH with Rh(II) using molecular absorption spectroscopy

Optimum Conditions

1- Temperature effect

The reaction between TCH and Rh(II) was very slowly in room temperature therefore the temperature was raised (Figure 4) shows the effect of temperature on the complex formation and the results shown that (90°C) was appropriate to give the highest absorbance intensity, after this temperature the absorbance was decreased because of the partially decomposition of complex.

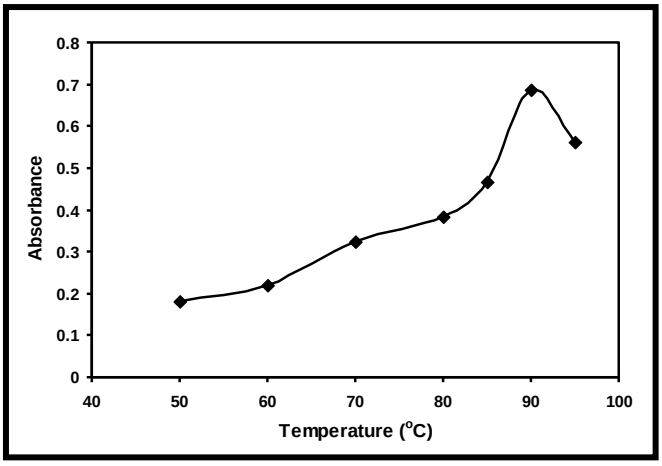


Fig. 4: Effect of temperature on the absorbance for complex [TCH-Rh(II)]

2- pH Effect

The best value of pH was (1.9) which recorded the highest absorbance intensity for the complex [TCH-Rh(II)] against the effect of pH (Figure 5).

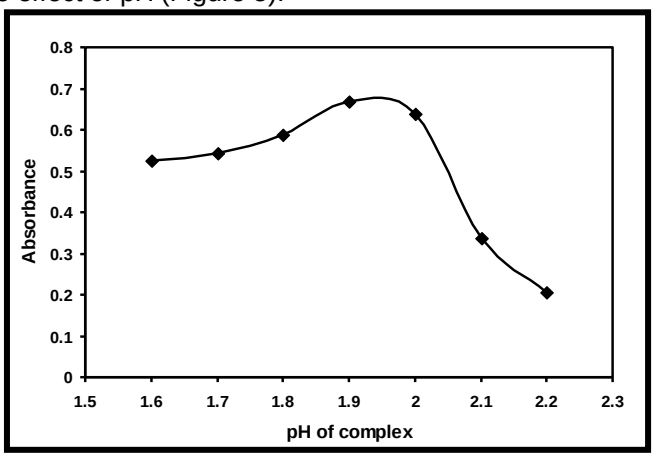


Fig. 5: Effect of pH of the solution on the absorbance for complex [TCH-Rh(II)]

3- Rhodium Ion Concentartion

Figure (6) shows the effect of rhodium ion concentration upon the absorbance intensity of the extracted complex, its formed from reaction (100 µg.ml^{-1}) TCH with Rh(II) ion the best concentration (60 µg.ml^{-1}).

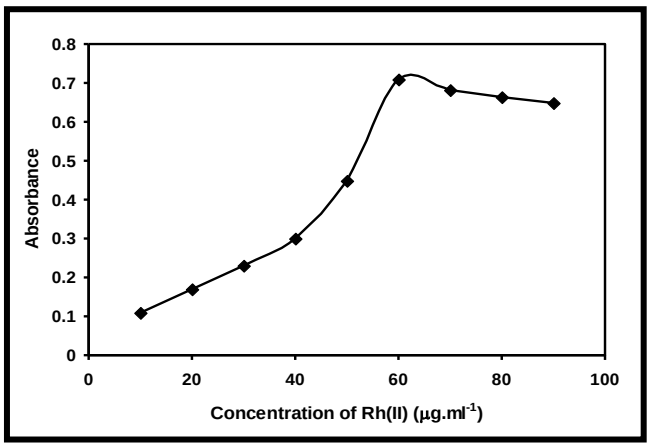


Fig. 6: Effect of metal ion concentration on the absorbance for complex [TCH-Rh(II)]

4- Phase Ratio

Figure (7) shows that the volumes (5 ml) from aqueous layer and (3 ml) from organic layer were sufficient for obtaining the highest absorbance intensity for complex formation.

The absorbance value for extracted complex was decreased with increasing the volume of organic layer after (3 ml), this indicates that the extraction method influences by increasing the volume of organic layer.

The percentage of extraction was calculated depending upon the absorbance value in (Table-1) and according to equations below.

$$\%E = \frac{Intial\ concn.(org.) - Final\ concn.(aq.)}{Intitial\ concn.(org.)} \times 100$$

$$\%E = \frac{100D}{D + \frac{V_{aq.}}{V_o}}$$

%E = 97.94 and distribution ratio D = 79.24.

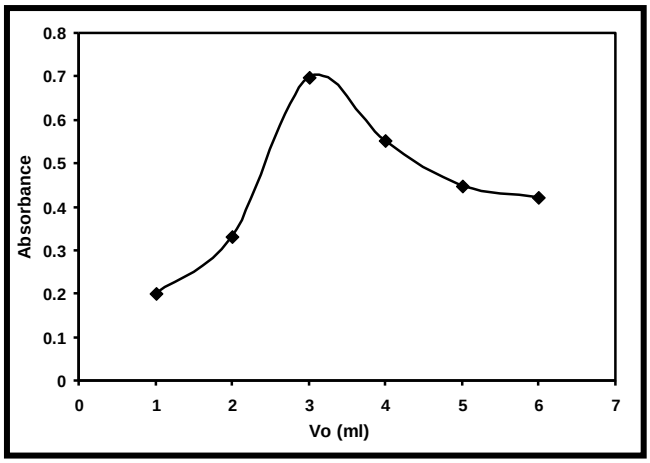


Fig. 7: Effect of phase ratio on the absorbance for complex [TCH-Rh(II)]

5- The Extraction Efficiency

(Table-1) shows the absorbance values for extracted complex from the first extraction method and second extraction for the remaining aqueous layer and compared it with a blank solution the extraction method for once gave suitable efficiency for extraction, the reason belongs to the highest percentage of extraction and distribution ratio.

Table 1: The absorbance values of complex [TCH-Rh(II)] after first and second extraction

TCH ($\mu g.ml^{-1}$)	Rh(II) ($\mu g.ml^{-1}$)	pH	A_1 (Ex.No.1)	A_2 (Ex.No.2)	A_0 blank	%E
100	60	1.9	0.698	0.023	0.003	97.94

6- Reaction Time

Figure (8) shows the effect of reaction time on the complex formation before extraction, through the absorbance which was read for the extracted complex at (135) second the absorbance gave the highest value and increasing the time gave deviation for absorption intensity.

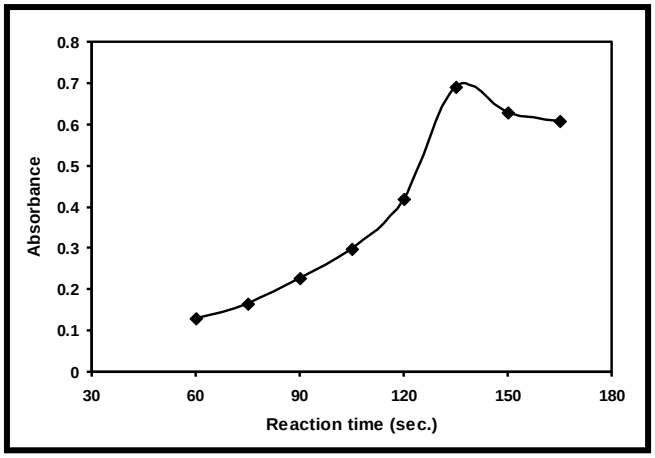


Fig. 8: Effect of reaction time on the absorbance for complex [TCH-Rh(II)]

7- Shaking Time

Figure (9) shows that the absorbance with increasing the time of shaking and (2.5 min.) gave the best absorbance intensity.

The formed complex was partially decomposed if it remains in organic layer more time.

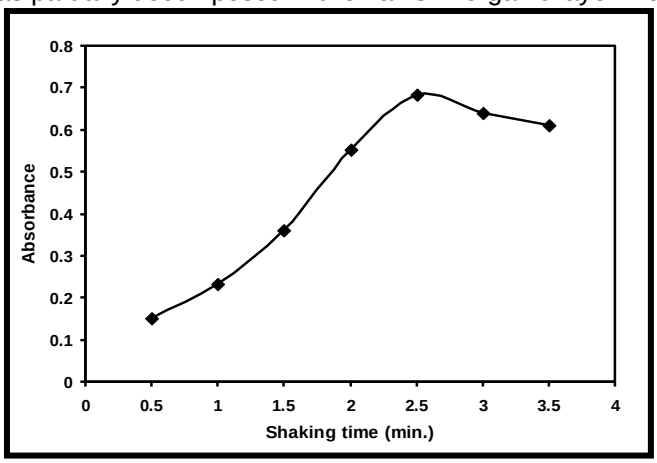


Fig. 9: Effect of shaking time on the absorbance for complex [TCH-Rh(II)]

8- Organic Solvent

Many of organic solvent were used for extraction the complex example: benzyl alcohol, octane, toluene, o-xylene, cyclohexane, benzene, diethyl ether, acetyl aceton and 2-heptanone.

The appropriate solvent for analytical purpose which can extract the complex without extracting the residue of metal or drug was 2-heptanone.

(C) The calibration Curve for Determination TCH as [TCH-Rh(II)] Complex Using Spectrophotometric Method

A calibration curve was prepared from a series of standard solutions of TCH in the range (5-160) $\mu g.ml^{-1}$, by using the optimum conditions for the reaction between TCH and Rh(II) ion and measuring the absorbance at (λ_{max} = 430 nm), the curve was described in (Figure 10).

The curve was deviated negatively concentration (160 $\mu g.ml^{-1}$) toward the concentration axis, the absorbance was decreased because the interactions between complex molecules or with solvent or instrumental factor or formation some polymers when concentration of drug increased.

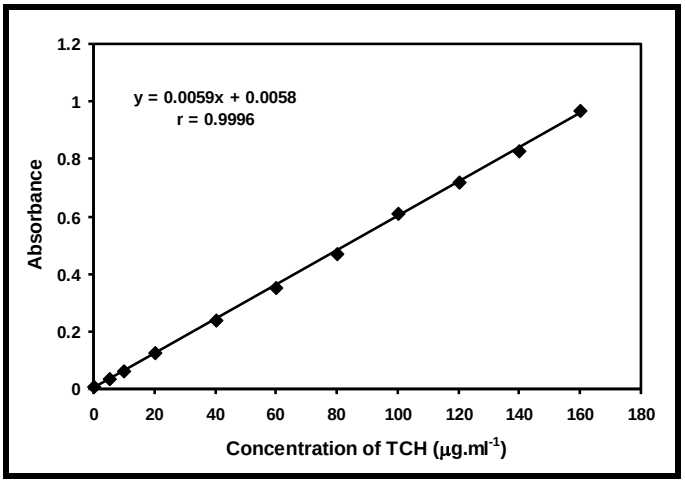


Fig. 10: Calibration curve for determination TCH with Rh(II) as [TCH-Rh(II)] complex [Temp. 90°C, pH=1.9, Rh(II) (60μg.ml^{-1}), V_O = 3 ml, reaction time 135 sec. and shaking time 2.5 min.]

1- Determination of Ligand to Metal Ratio in Complex [TCH-Rh(II)] by mole-Ratio Method

Figure (11) shows the mole ratio between TCH and Rh(II) ion its (1:1). Figure (12) shows the suggested structure for complex [TCH-Rh(II)]. The suggested for the complex [TCH-Rh(II)] produced from reaction TCH with Rh(II).

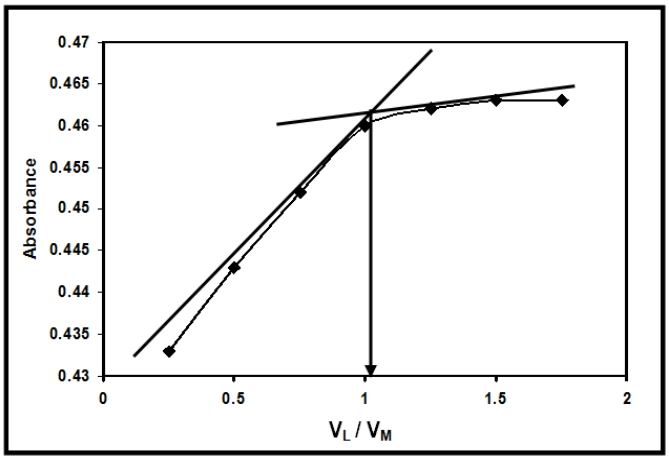


Fig. 11: Mole ratio plot for the complex [TCH-Rh(II)]

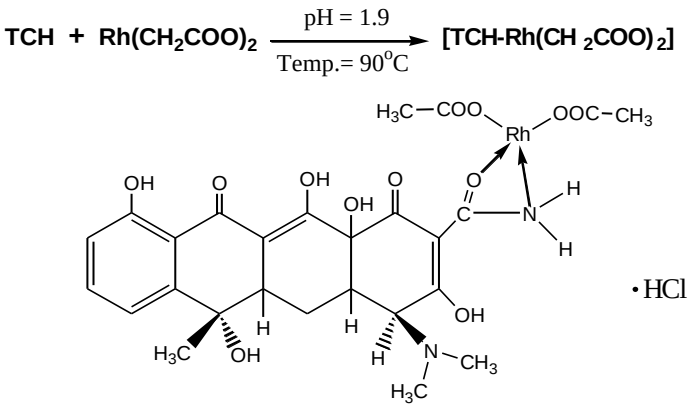


Fig. 12: The suggested structure for complex [TCH-Rh(II)]

2- Calculating the Formation Constant for Complex [TCH-Rh(II)]

The formation constant for complex [TCH-Rh(II)] was (3×10^6 molar^{-1}) which can be calculated depending upon the Figure (11) as this equation[11]

$$k_f = \frac{(A_1 - A_3)(A_2 - A_3)}{(A_2 - A_1)^2 C}$$

k : formation constant
A_1 : absorbance which represents two tangents intercept.
A_2 : absorbance which represents the point of fixing absorbance.
A_3 : absorbance which represents first point.
C : molar concentration against A_1.

Table 2: Absorbance values for mole ratio plot to calculate formation constant

A_1	A_2	A_3	C (Molar)
0.462	0.463	0.433	2.9×10^{-4}

3- Statistical Analytical Data

Through the direct clibration curve, (Figure 10) shows the connection between absorbance and used concentrations and Table (3), (4), (5) show that data treatment results by modern statistical treatment[12].

Table 3: Determination of concentration ranges, detection limits, molar absorbtivity coefficient, Sandell's sensitivity and confidence limits for concentration (80 μg.ml^{-1}) and absorbance to determine TCH as [TCH-Rh(II)] complex at λ_{max}=430 nm using direct calibration curve

λ_{max} (nm)	Linearity (μg.ml^{-1})	D.L* (μg.ml^{-1}) (n=10)	D.L.T** (μg.ml^{-1})	S (μg.cm^{-2})	Conf. Limit Conc. (μg.ml^{-1}) 95% C.L.	Conf. Limit Abs. 95% C.L.	ε (L.mol^{-1}.cm^{-1})
430	5-160	0.163	0.932	0.1664	80.11±0.2348	0.4821±0.0032	2.891×10^{3}

*Experimental , ** Theory

Table 4: Linear regression equation, correlation coefficient (r), two tailed t-test and confidence limits for the slope and intercept at 95% confidence limits

Regre.Eq. y=bx+a	Corr. Coef. (r)	t-test Statistic	Tabulated t-test two tailed (n-2) 95% C.L.	Conf.Limit For the slope b ± tS_b	Conf.Limit For the intercept a ± tS_a
y=0.0059x+0.0058	0.9996	99.97	2.262	0.00595± 0.0000931	0.0058± 0.0085

From comparison between t-statistic and t-tabulated that t-statistic is more than t-tabulated which indicated that there is a linear relationship between concentration and absorbance.

Table 5: Relative standard deviation %RSD, percentage relative error %$E_{rel.}$ and recovery for complex [TCH-Rh(II)]

concentration of TCH taken (μg.ml^{-1})	concentration of TCH found (μg.ml^{-1})	%Rec.	%$E_{rel.}$	%RSD (n=5)	Mean For %Rec. ± S.D	Mean %$E_{rel.}$
40	39.70	99.25	-0.75	0.62	99.7 ± 0.114	-0.296
80	79.75	99.70	-0.30	1.30		
120	120.20	100.16	0.16	0.83		

4- Determination of TCH in the pharmaceutical preparation (Apcycline)

Two methods for the determination of TCH were used the first was direct method which included measuring the absorbance for extracted complex for several concentrations and determining the concentration from direct calibration curve.
The second method included determination of the drug by using standard addition method at maximum wavelength, Figure (13).

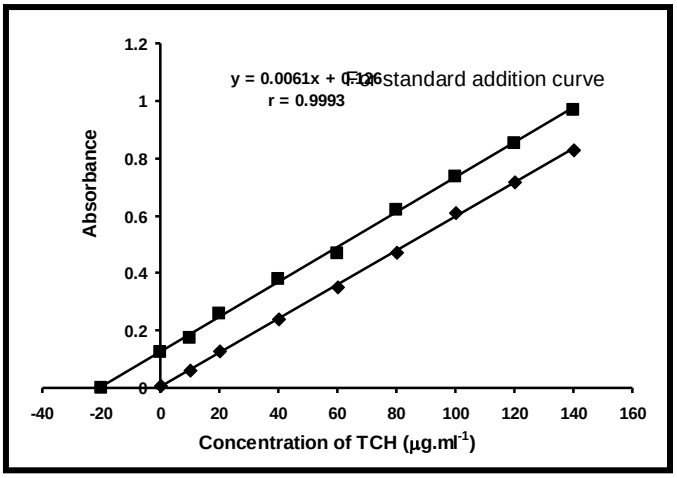


Fig. 13: Determination of TCH in pharmaceutical preparation (Apcycline) by using standard addition method [Temp. 90°C, pH=1.9, Rh(II)(60μg.ml^{-1}), V_O=3ml, reaction time=135 sec. and shaking time=2.5 min.]

Tables (6), (7), (8) show that the data treatment results by modern statistical treatment.

Table 6: Determination of TCH using Rh(II) by standard addition method and direct calibration curve

Name of pharmaceutical	Type of preparation	Stated concentration (μg.ml^{-1})	Found (direct calb.) (μg.ml^{-1})	%$E_{rel.}$	Found (Std. add. calb.) (μg.ml^{-1})	%E_{rel}
Apcycline	Capsules	20	20.08	0.40	19.75	-1.25

Table 7: Linear regression equation for standard addition curve, correlation coefficient, two tailed t-test and concentration value by standard addition method at 95% confidence limits for complex [TCH-Rh(II)]

Regre. Eq. y=bx+a	Corr. Coef. (r)	t-test statistic	Tabulated t-test two tailed (n-2) 95% C.L.	Conf. Limit For X-value XE±t_sXE	%Rec.	%E_{rel}
y=0.0061x+0.126	0.9993	70.67	2.306	19.75±0.2687	98.75	-1.25

The slope of standard addition curve was paralleled to slope of direct calibration curve which means that the connection of Rh(II) ion with standard TCH has the same shape to connect it with Apcycline.

Table 8: Relative standard deviation %RSD, percentage relative error %$E_{rel.}$ and recovery to determine TCH in pharmaceutical preparation by using direct calibration method

Concentration of TCH taken (μg.ml^{-1})	Concentration of TCH found (μg.ml^{-1})	%Rec.	%$E_{rel.}$	%RSD (n=5)	Mean For %Rec. ± S.D	Mean %$E_{rel.}$
40	40.16	100.40	0.40	0.553	100.27±0.098	0.27
80	80.22	100.27	0.27	0.921		
120	120.18	100.15	0.15	1.657		

D) Using Indirect Flame Atomic Absorption Spectroscopy

1- The Optimum Conditions

The optimum conditions were similar to results in using molecular absorption spectroscopy, except Rh(II) ion concentration which was (20μg.ml^{-1}) Figure (14).

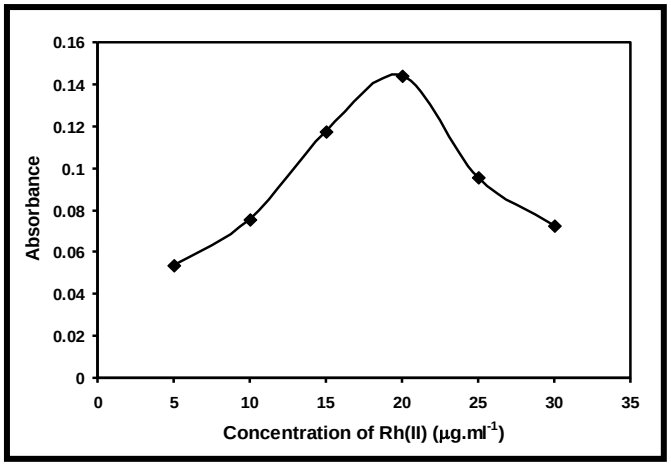


Fig. 14: Effect of Rhodium ion concentration on the absorbance for complex [TCH-Rh(II)] by FAAS

The percentage of extraction was calculated depending upon the absorbance values in Table (9), %E=97.60 and D=67.78.

Table 9: Outline the absorbance values for complex [TCH-Rh(II)], after first and second extraction

TCH (µg.ml^{-1})	Rh(II) (µg.ml^{-1})	pH	A_1 (Ex.No.1)	A_2 (Ex.No.2)	A_0 Blank	%E
50	20	1.9	0.172	0.009	0.002	97.60

2- Calibration Curve for Determination TCH as [TCH-Rh(II)] Complex

Figure (15) shows direct calibration curve for complex [TCH-Rh(II)], the curve plot between the atomic absorbance of Rhodium in the complex against concentration of TCH.

From the curve it found that (50µg.ml^{-1}) was the highest concentration of TCH obeyed Beer's law and after that the curve was deviated toward concentration axis because of the decreasing in concentration of free rhodium atoms.

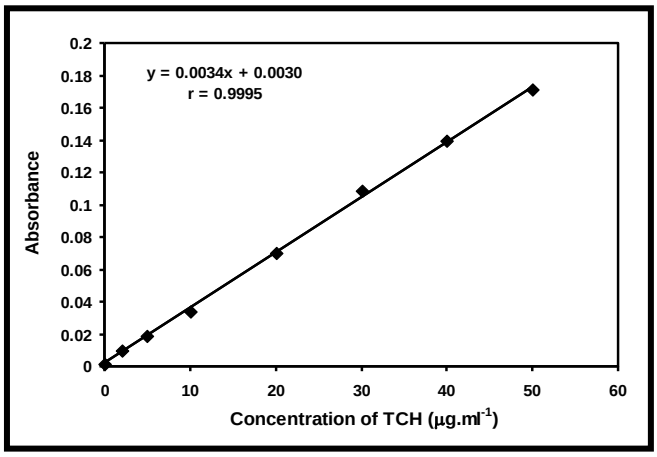


Fig. 15: Calibration curve for determination TCH as [TCH-Rh(II)] complex by using FAAS [Temp. 90°C, pH=1.9, Rh(II) (20µg.ml^{-1}), V_O=3ml, reaction time=135 sec. and shaking time=2.5min.]

Tables (10), (11), (12) show that the data treatment results by modern statistical treatment.

Table 10: The results of concentration ranges, detection limits and confidence limits for concentration and absorbance at 95% confidence limits by using FAAS

Drug	Linearity ($\mu g.ml^{-1}$)	D.L ($\mu g.ml^{-1}$) (n=10)	D.L.T ($\mu g.ml^{-1}$)	Conf. Limit Conc. ($\mu g.ml^{-1}$) 95% C.L.	Conf. Limit Abs. 95% C.L.
TCH	(2-50)	0.140	0.179	25.16±0.041180	0.08688±0.000133

Table 11: Linear regression equation, correlation coefficient (r), two tailed t-test and confidence limits for the slope and intercept at 95% confidence limits

Regre. Eq. y=bx+a	Corr. Coef. (r)	t-test statistic	Tabulated t-test two tailed (n-2) 95% C.L.	Conf. Limit For the slope $b \pm tS_b$	Conf. Limit For the intercept $a \pm tS_a$
y=0.0034x+0.0030	0.9995	70.68	2.447	0.00341±0.000116	0.0030±0.003250

From a comparison between t-statistic and t-tabulated that t-statistic is more than t-tabulated which indicates that there is a linear relationship between concentration and absorbance.

Table 12: Relative standard deviation, percentage relative error and recovery

Concentration of TCH taken ($\mu g.ml^{-1}$)	Concentration of TCH found ($\mu g.ml^{-1}$)	%Rec.	$\%E_{rel.}$	%RSD (n=5)	Mean For %Rec. ± S.D	Mean $\%E_{rel.}$
10	10.205	102.05	2.05	1.54	100.567±0.00164	0.567
20	19.820	99.10	-0.90	0.80		
40	40.220	100.55	0.55	0.35		

3- Determination of TCH in the Pharmaceutical Preparation (Apcycline) Using FAAS by Standard Addition Method

TCH in the pharmaceutical preparation was determined by atomizing the extracted solution for the formation complex which was produced from the reaction between TCH in the pharmaceutical preparation and rhodium ion at the optimum conditions, two methods were followed for the determination direct and standard addition method, Figure (16).

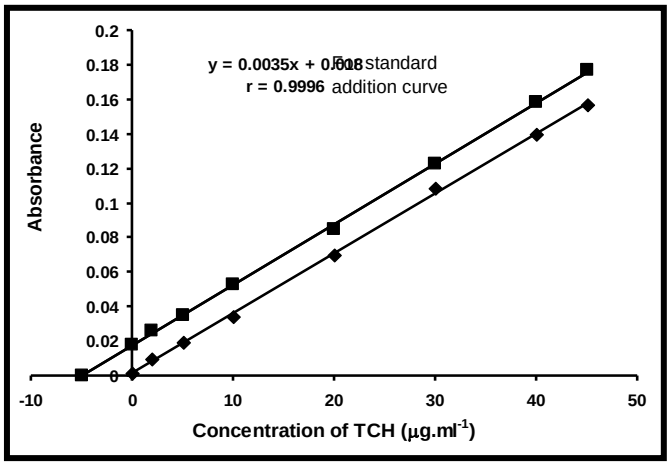


Fig. 16: Determination of TCH in the pharmaceutical preparation (Apcycline) by using standard addition method [Temp. 90°C, pH=1.9, Rh(II)(20$\mu g.ml^{-1}$), V_O=3ml, reaction time 135 sec. and shaking time 2.5 min.]

Table 13: Determination of TCH in the pharmaceutical preparation (Apcycline) by using indirect FAAS by standard addition method and direct calibration curve method

Name of pharmaceutical	Type of preparation	Stated concentration ($\mu g.ml^{-1}$)	Found (direct calb.) ($\mu g.ml^{-1}$)	$\%E_{rel.}$	Found (Std. add. calb.) ($\mu g.ml^{-1}$)	$\%E_{rel}$
Apcycline	Capsules	5	4.88	-2.4	4.85	-3

Table 14: Linear regression equation for standard addition curve, correlation coefficient, two tailed t-test and concentration value by standard addition method at 95% confidence limits

Regre. Eq. y=bx+a	Corr. Coef. (r)	t-test statistic	Tabulated t-test two tailed (n-2) 95% C.L.	Conf. Limit For X-value $XE \pm t_s XE$	%Rec.	$\%E_{rel.}$
y=0.0035x+0.018	0.9996	86.58	2.365	4.85±0.2869	97	-3

Table 15: Percentage relative standard deviation, percentage relative error $\%E_{rel.}$ and recovery for determination TCH in the pharmaceutical preparation by using direct calibration method

Concentration of TCH taken ($\mu g.ml^{-1}$)	Concentration of TCH found ($\mu g.ml^{-1}$)	%Rec.	$\%E_{rel.}$	%RSD (n=5)	Mean For %Rec. ± S.D	Mean $\%E_{rel.}$
10	10.15	101.50	1.50	0.288	101.05±0.0035	1.05
20	20.23	101.15	1.15	1.900		
40	40.20	100.50	0.50	1.110		

Comparison the Results for Determination TCH by Using Spectrophotometric and FAAS Method

Table 16: Comparison the results of spectrophotometric method with the results of FAAS to determine TCH

Method	Linearity ($\mu g.ml^{-1}$)	D.L ($\mu g.ml^{-1}$)	%RSD	Corr. Coef. (r)	Calculated F-test	Tabulated F-test
UV-Method	(5-160)	0.163	0.917	0.9996	1.004	19
FAAS-Method	(2-50)	0.140	0.897	0.9995		

The value of (F) calculated less than value of (F) tabulated at 95% confidence limit and degree of freedom (n-1) that referred the two methods were shown approach in accuracy.

CONCLUSION

1. New chelate complex for tetracycline hydrochloride by reaction with Rhodium ion was prepared at first in this study, the literature survey had not referred to formation of this complex.
2. The results of comparison between FAAS and UV-Vis. methods for new chelate complex were shown approach in accuracy but FAAS high sensitivity, low detection limits and linear range.
3. The results of analysis for Apcycline showed approach with the results labeled on the drug bottle.

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