Determination of Tetracycline Hydrochloride in Pharmaceutical Preparations by Molecular Absorption and Indirect Flame Atomic Absorption Spectrophotometry Using Platinum (IV) as Complexing Metal

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Abstract: *A simple, rapid and sensitive spectrophotometric methods for determination of trace amount of (TCH) as [TCH-Pt(IV)] complex in hexanol as solvent for extraction the complex. The light-violet soble product give maximm absorption at 392 nm, Beer's law is obeyed over the concentration range of (5-120) $\mu g.ml^{-1}$ with molar absorptivity = 8.544×10^3 $L.mol^{-1}.cm^{-1}$), (r = 0.9995), (D.L. = 0.2607 $\mu g.mL^{-1}$), (%RSD = 0.918), with UV-Vis. method. When using Indirect FAAS a linear range of (5-60 $\mu g.ml^{-1}$), (r= 0.9995), (D.L= 0.1571 $\mu g.ml^{-1}$), (%RSD = 0.554). The optimum condition for colour development are described. The proposed methods have been successfully applied for determination (TCH) in the pharmaceutical preparation (Apcycline) using direct and standard addition methods, the common excipients and additives did not interfere in this method.*

Keywords: Tetracycline Hydrochloride, Pharmaceutical Preparations, Flame Atomic Absorption Spectrophotometer

1. 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: $C_{22}H_{24}N_2O_8.HCl$
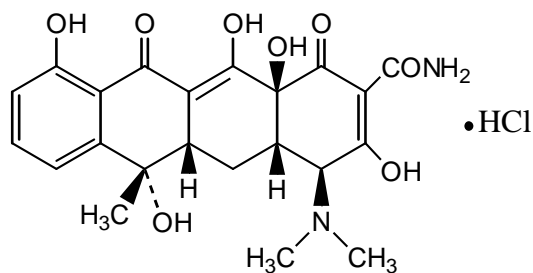
The pH of TCH is (2.0-2.5), crystalline (yellow) powder soluble in water, slightly soluble in alcohol, practically insoluble in acetone and ether, it dissolves in solutions of alkali hydroxide and carbonate.

The solution of TCH in water have the maximum wavelengths (213, 271, 344 and 363 nm)[3].

Analytical methods used for determination (TCH), spectrophotometric[4-6], flow injection[7-9], chromatographic[10] fluorometric[11] and titrometric[12] methods.

2. 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) Standard tetracycline hydrochloride solution (SDI Samara Iraq): stock solution (1000 $\mu g.ml^{-1}$) was prepared by dissolving 0.1000 gm of pure compound (TCH) in distilled water then the volume was completed to 100 ml with distilled water.
2) Stock solution of Platinum ion (1000 $\mu g.ml^{-1}$) prepared by dissolving (0.2492 gm) of Potassium hexachloro palatinate (Fluka) (K_2PtCl_6) in distilled water then the volume was completed to 100 ml with distilled water.
3) Complex solutions: (0.1-2) ml from stock solution (TCH) (1000 $\mu g.ml^{-1}$) were transferred to 5 ml volumetric flasks then 2.5 ml of (100 $\mu g.ml^{-1}$) Pt(IV) was added, the optimum conditions were fixed and the formed complexes were extracted with 1-hexanol and the absorption spectra was measured versus organic solvents as blank solutions.
4) The solution of pharmaceutical preparation (Apcycline) (India 250 mg): a solution of (Apcycline) (1000 $\mu g.ml^{-1}$) was prepared by taking twenty capsules of weighted pharmaceutical preparation and the average of each capsule was (0.2861 gm), then (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 $\mu g.ml^{-1}$) was prepared from the last solution.

3. Results and Discussion

(A) Spectrophotometric Studies

1- The drug spectrum (TCH):
The spectrum of the drug (TCH) (50 $\mu g.ml^{-1}$) 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.
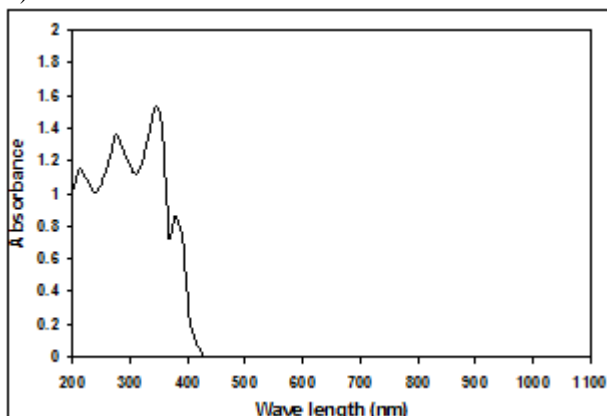

Figure 1: Molecular absorption spectrum of (50 $\mu g.ml^{-1}$) TCH versus water as a blank solution

2- The spectrum of Platinum ion
Figure (2) shows absorption spectrum of Platinum ion (50 $\mu g.ml^{-1}$) which has two maximum wavelength at 215 and 239 nm, the measurement was done versus water as a blank solution.

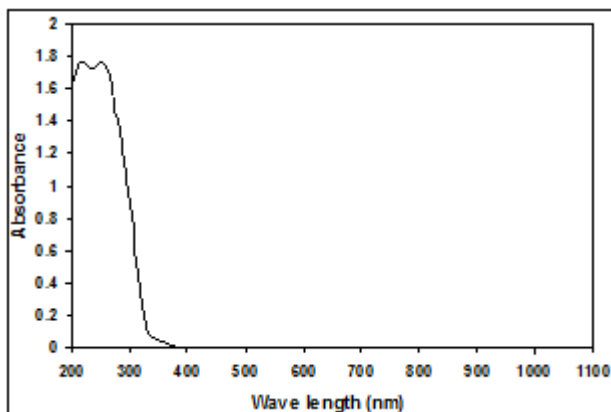**Figure 2:** Molecular absorption spectrum (50 $\mu g.ml^{-1}$) of platinum ion Pt(IV) versus water as a blank solution

3- The spectrum of [TCH-Pt(IV)] complex
Figure (3) shows absorption spectrum of light violet drug complex [TCH (100 $\mu g.ml^{-1}$) with [Pt(IV) (50 $\mu g.ml^{-1}$)] at maximum wavelength 392 nm, the optimum conditions were fixed and the formed complex was extracted with 1-hexanol and the absorption spectra was measured versus organic solvent as blank solution.
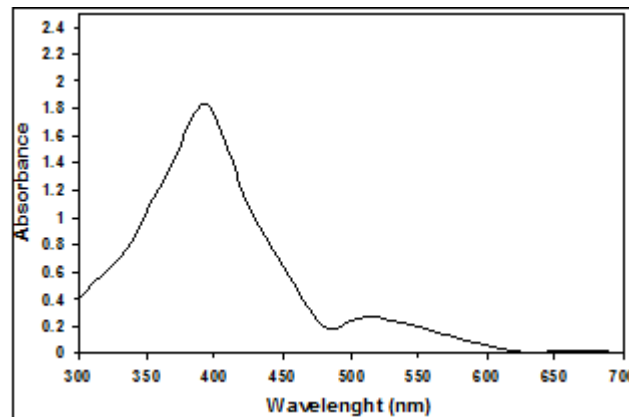

Figure 3: Molecular absorption spectrum for complex [TCH- Pt(IV)]
[TCH (100$\mu g.ml^{-1}$) + Pt(IV) (50$\mu g.ml^{-1}$), V_O=1 ml of 1-hexanol]

(B) Determination of TCH with Pt(IV) using molecular absorption spectroscopy

Choosing Optimum Conditions
1- Temperature effect
The reaction between TCH and Pt(IV) 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 (85°C) was appropriate to give the highest absorbance intensity, after this temperature the absorbance was decreased because of the partially decomposition of complex with increasing the temperature of the solution.
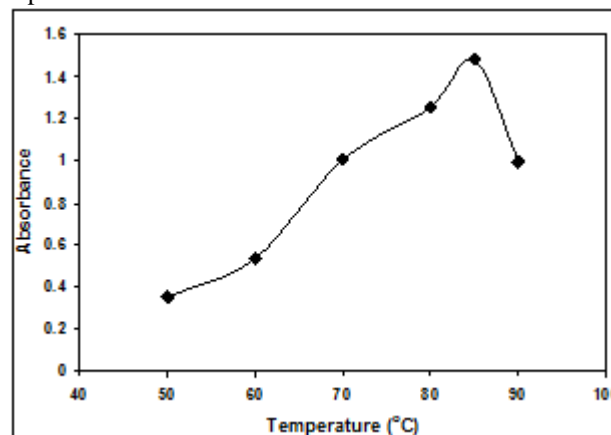

Figure 4: Effect of temperature on the absorbance for complex [TCH-Pt(IV)]

2- pH Effect
The best value of pH was (12.5) which recorded the highest absorbance intensity for the complex [TCH-Pt(IV)] against the effect of pH (Figure 5).
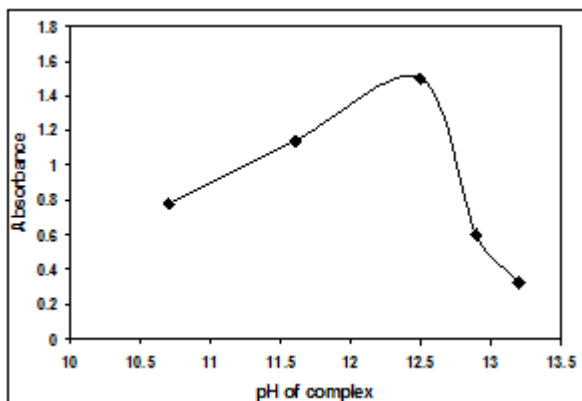
Figure 5: Effect of pH of the solution on the absorbance for complex [TCH-Pt(IV)]

3- Platinum Ion Concentration

Figure (6) shows the effect of Platinum ion concentration upon the absorbance intensity of the extracted complex, its formed from reaction (80 µg.ml^{-1}) TCH with Pt(IV) ion the best concentration of Pt(IV) ion which gave the highest at (50 µg.ml^{-1}).
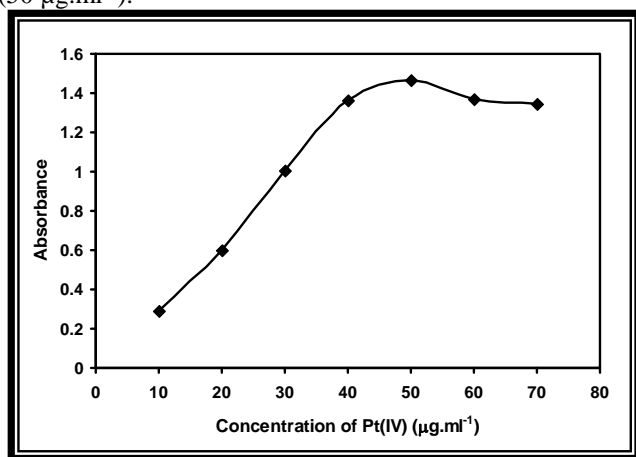

Figure 6: Effect of metal ion concentration on the absorbance for complex [TCH-Pt(IV)]

4- Phase Ratio

Figure (7) shows that the volumes (5 ml) from aqueous layer and (1 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 (1 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), (%E = 98.1) and distribution ratio D = 258.1 according to equations below.

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

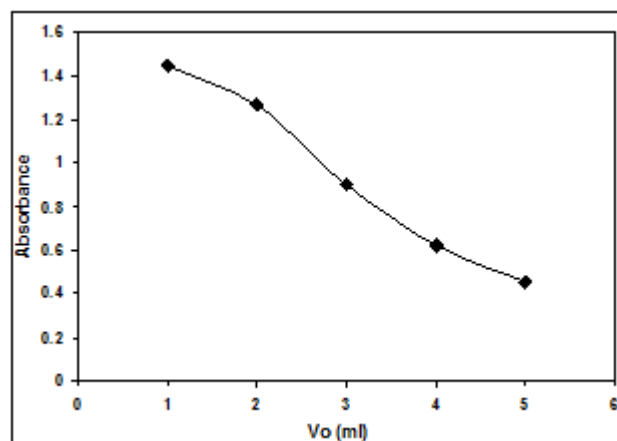$$\%E = \frac{100D}{D + \dfrac{V_{aq.}}{V_o}}$$

Figure 7: Effect of phase ratio on the absorbance for complex [TCH-Pt(IV)]

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 (µg.ml^{-1})	Pt(IV) (µg.ml^{-1})	pH	A$_1$ (Ex.No.1)	A$_2$ (Ex.No.2)	A$_0$ blank	%E
80	50	12.5	1.447	0.081	0.006	98.1

6- Reaction Time

Figure (8) shows that (2.5) min. was enough to complete the formation of complex [TCH-Pt(IV)], when increasing the time gave deviation for absorbance intensity.
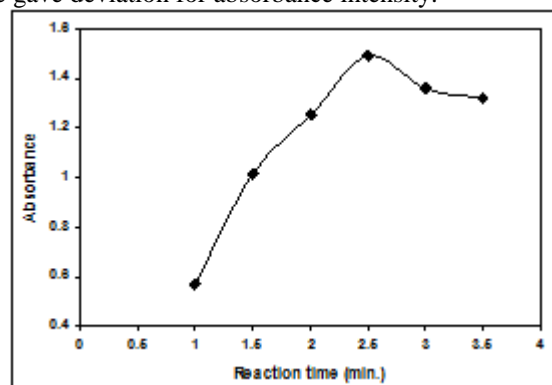

Figure 8: Effect of reaction time on the absorbance for complex [TCH-Pt(IV)]

7- Shaking Time

Figure (9) shows that the chelate complex was needed only (2 min.) to get the highest absorbance intensity.
The formed complex was partially decomposed of it remains in organic layer more time.
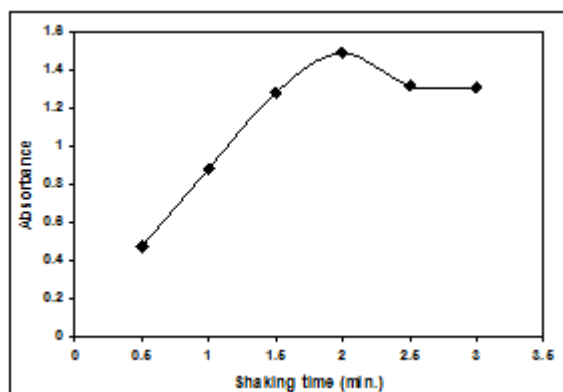
Figure 9: Effect of extraction time on the absorbance for complex [TCH-Pt(IV)]

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 1-hexanol.

The appropriate solvent for analytical purpose which can extract the complex without extracting the residue of metal or drug was 1-hexanol.

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

Figure (10) show the direct calibration curve for determination TCH as complex [TCH-Pt(IV)] in the range (5-120) $\mu g.ml^{-1}$, by using the optimum conditions for the reaction between TCH and Pt(IV) ion and measuring the absorbance at (λ_{max} = 392 nm).

The curve was deviated negatively concentration (120 $\mu g.ml^{-1}$) 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.
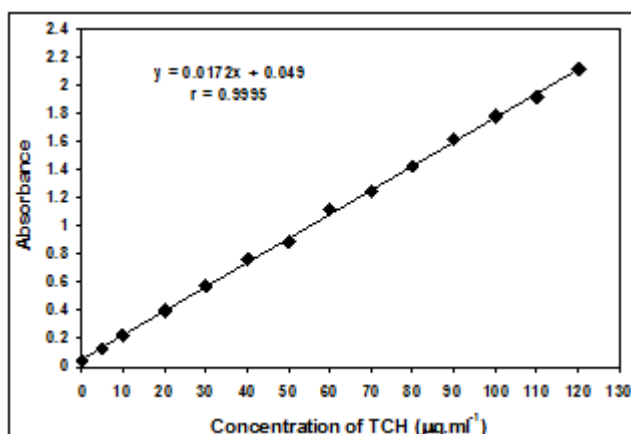

Figure 10: Calibration curve for determination TCH as [TCH-Pt(IV)] complex [Temp. 85°C, pH=12.5, Pt(IV) (50$\mu g.ml^{-1}$) V_O=1ml, reaction time 2.5 min. and shaking time 2 min.]

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

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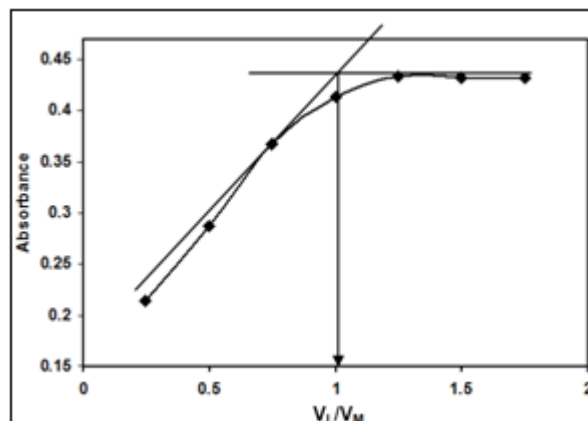

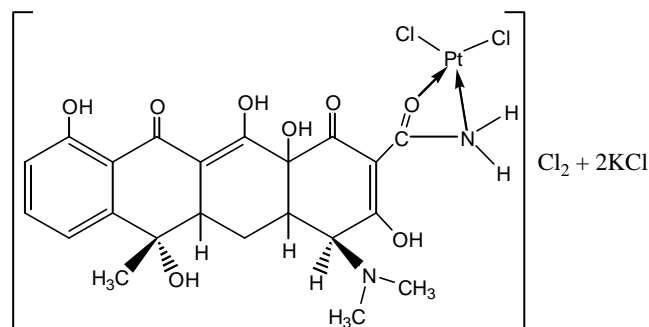**Figure 11:** Mole ratio plot for the complex [TCH-Pt(IV)]

$$TCH \ + \ K_2PtCl_6 \ \xrightarrow[Temp.= 85^oC]{pH = 12.5} \ [TCH-PtCl_2]Cl_2 + {}_2KCl$$
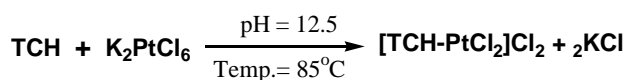
Figure 12: The suggested structure for complex [TCH-Pt(IV)]

2- Calculating the Formation Constant for Complex [TCH-Pt(IV)]

The formation constant for complex [TCH-Pt(IV)] was (6×10^7 molar^{-1}) which can be calculated depending upon the Figure (11) as this equation[13]

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

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)
0.425	0.433	0.214	1.2×10^{-4}

3-Statistical Analytical Data[14]

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

Table 3: Determination of concentration ranges, detection limits, molar absorbtivity coefficient, Sandell's sensitivity and confidence limits for concentration (60 µg.ml⁻¹) and absorbance to determine TCH as [TCH-Pt(IV)] complex at λ_{max}=392 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})
392	(5-120)	0.2607	0.3451	0.0562	59.91±0.2287	1.0734±0.00399	8.544×10^3

*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 using spectrophotometric method

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=0.0172x+0.049	0.9995	104.84	2.179	0.01711±0.000169	0.048±0.01196

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-Pt(IV)]

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$_{rel.}$
30	29.80	99.33	-0.67	1.045		
60	59.87	99.78	-0.22	0.918	99.76 ± 0.0185	-0.24
90	90.16	100.17	0.17	0.592		

4- Determination of TCH in the pharmaceutical preparation (Apcycline) by Using Spectrophotometric Method as Complex [TCH-Pt(IV)]

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).
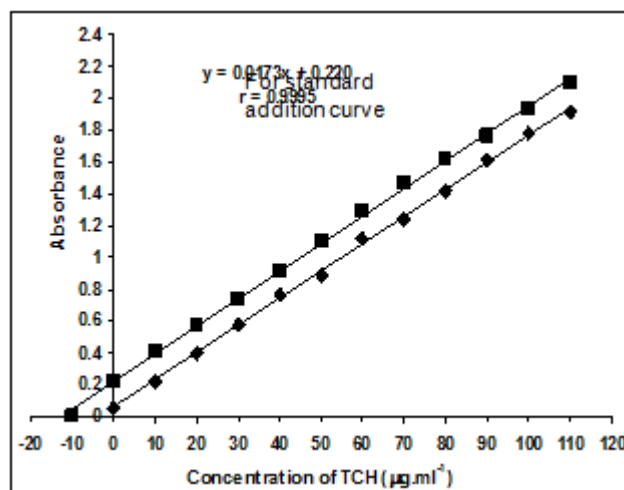

Figure 13: Determination of TCH in the pharmaceutical preparation (Apcycline) using standard addition method [Temp. 85°C, pH=12.5, Pt(IV) (50µg.ml⁻¹) V$_O$=1ml, reaction time 2.5 min. and shaking time 2 min.]

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

Table (6): Determination of TCH using platinum ion 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**	10	10.12	1.2	9.94	-0.6

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

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$_s$XE	%Rec.	%E$_{rel.}$
y=0.0173x+0.220	0.9995	99.96	2.201	9.94±0.1643	99.4	-0.6

The slope of standard addition curve was paralleled to slope of direct calibration curve which means that the connection of Pt(IV) ion with standard TCH has the same shape to connect it with Apcycline, that there is no interference in region.

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 %$E_{rel.}$
30	30.17	100.56	0.56	1.420		
60	59.88	99.80	-0.20	0.870	100.07±0.0345	0.08
90	89.88	99.87	-0.13	0.642		

D) Using Indirect Flame Atomic Absorption Spectroscopy

1- The Optimum Conditions

The optimum conditions were studied such as pH effect, temperature, reaction time, shaking time and the number of extraction methods which were similar to the results in using molecular absorption spectroscopy except platinum ion concentration which was 25 μg.ml^{-1}.Figure (14).
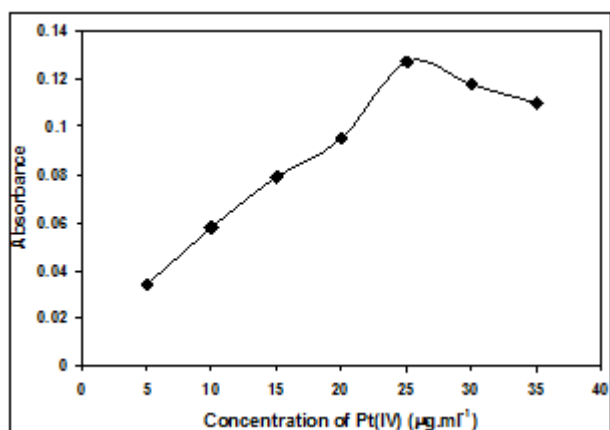

Figure 14: Effect of platinum ion concentration on the absorbance for complex [TCH-Pt(IV)] by FAAS

The percentage of extraction was calculated depending upon the absorbance values in Table (9), %E=98.63 and D=359.96.

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

TCH (μg.ml^{-1})	Pt(IV) (μg.ml^{-1})	pH	A_1 (Ex.No.1)	A_2 (Ex.No.2)	A_0 Blank	%E
60	25	12.5	0.159	0.007	0.003	98.63

2- Calibration Curve for Determination TCH as [TCH-Pt(IV)] Complex

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

From the curve it found that (60 μg.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 Platinum atoms.
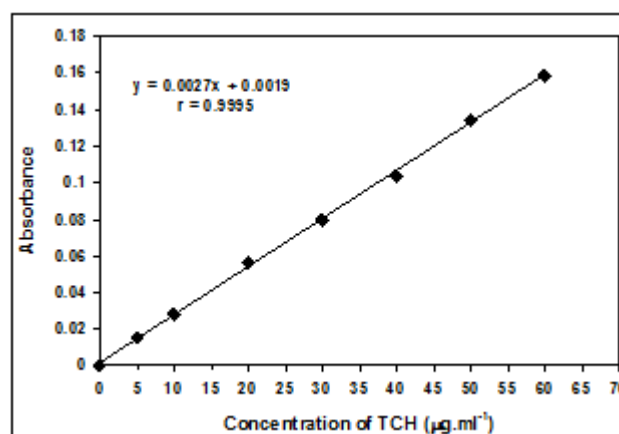

Figure 15: Calibration curve for determination TCH as [TCH-Pt(IV)] complex using FAAS [Temp. 85°C, pH=12.5, Pt(IV) (25μg.ml^{-1}) V_O=1ml, reaction time 2.5 min. and shaking time 2 min.]

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 (μg.ml^{-1})	D.L (μg.ml^{-1}) (n=10)	D.L.T (μg.ml^{-1})	Conf. Limit Conc. (μg.ml^{-1}) 95% C.L.	Conf. Limit Abs. 95% C.L.
TCH	(5-60)	0.1571	0.1760	30.18±0.2039	0.0816±0.000679

Table 11: Linear regression equation, correlation coefficient, 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
0.0027x+0.0019	0.9995	70.68	2.447	0.00264±0.0000422	0.0019±0.001524

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	10.16	101.6	1.60	0.872		
30	30.00	100.0	0.00	0.471	100.42±0.00202	0.63
50	49.83	99.66	-0.34	0.319		

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 platinum ion at the optimum conditions, two methods were followed for the determination direct and standard addition method, Figure (16).
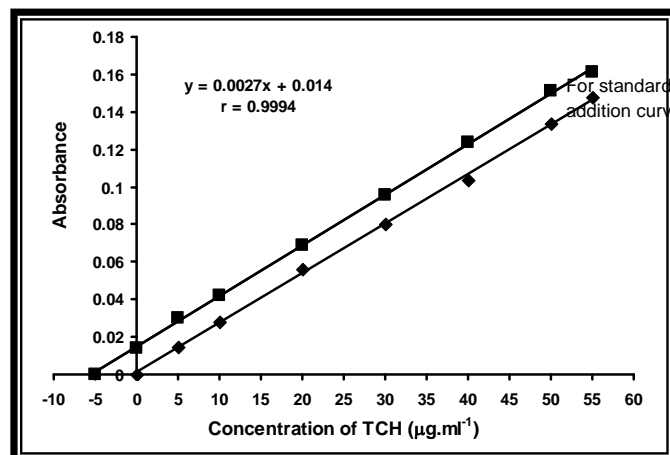

Figure 16: Determination of TCH in the pharmaceutical preparation (Apcycline) using standard addition method [Temp. 85°C, pH=12.5, Pt(IV) ($25\mu g.ml^{-1}$) V_O=1ml, reaction time 2.5 min. and shaking time 2 min.]

Table 13: Determination of TCH in the pharmaceutical preparation using standard addition method and direct calibration curve using FAAS

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	5.12	2.4	5.18	3.6

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 $XE±t_sXE$	%Rec.	%$E_{rel.}$
0.0027X+0.014	0.9994	70.68	2.365	5.18±0.2071	103.6	3.6

Table 15: Relative standard deviation (%RSD), 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	10.13	101.3	1.3	1.474		
30	30.09	100.3	0.3	0.653	100.53±0.00263	0.8
50	50.00	100.0	0.0	0.942		

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	(5-120)	0.2607	0.852	0.9995		
FAAS-Method	(5-60)	0.1571	0.554	0.9995	1.049	5.05

The value of F-statistic was lower than F-tabulated at 95% confidence limits and freedom degrees (n-1), that referred the two methods were shown approach in accuracy.

4. Conclusion

1) New chelate complex for tetracycline hydrochloride by reaction with Platinum 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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