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Analytical Study of Al(III)-Tetracycline Complex for Drug Formulations

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ABSTRACT

An extensive analytical study of aluminum(III)-tetracycline complex formation in drug formulation was performed. The optimum experimental conditions for the analysis were established as follows: Concentration of Al (III) ion was: 0.0001 M; pH within the range 4.5-6.0; absorbance reach maximum stability within 2.0 minutes; and stoichiometric ratio of ligand to metal ratio is 2:1. This study revealed the possibility for using the complex formation for analytical purpose for tetracycline determination; hence it can be simple selective, very reliable, and time saving. The capabilities of the method for the analysis of real samples were evaluated by determination of tetracycline in some pharmaceutical formulations. The analytical data obtained from this procedure were as follow: Linear dynamic range was: (4×10^{-7} - 2×10^{-4} M) for tetracycline; RSD % 0.28; limit of detection: 3×10^{-7} mol L^{-1}, Sandell's sensitivity 0.625, and 0.797 gm.Cm^{-2}, $E_{rel.}$ % 0.03 and Recovery % 100.03.

Keywords: Tetracycline, aluminum(III)-tetracycline complex, pharmaceutical formulations, linear dynamic range, limit of detection, Sandell's sensitivity.

INTRODUCTION

Tetracycline (1,4,4a,5,5a,6,11,12a-octahydronaphthacene, figure-1a, are broad-spectrum antibiotics, exhibiting activity against a wide range of gram-positive and gram-negative bacteria, a typical organisms such as *chlamydiae*, *mycoplasmas*, and *rickettsiae*, and *protozoan* parasites. The favorable antimicrobial properties of these agents and the absence of major adverse side effects has led to their extensive use in the therapy of human and animal infections **[1-3]**.

Tetracycline and its derivatives are strong chelating agents and it appears to be linked to the ability of the molecule to form complexes with a large variety of metal ions **[4-6]**. Many possibilities of the sites of complexation have been proposed, with the corresponding effect of the ions on model compounds, 11,12-*β*-diketone group are active sites for chelation **[6-7]** , as shown in figure 1b.

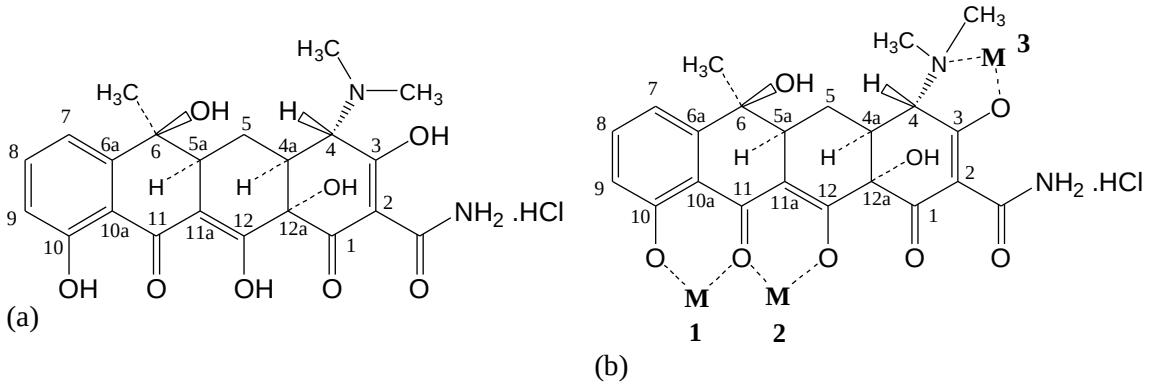

Figure-1: a. Tetracycline structure and b. possible the chelation sites.

Most procedures for the determination of tetracycline use high performance liquid chromatography (HPLC) with UV–Visible fluorescence detectors **[8-11**]. Also flow injection analysis with fluorescence detector **[12]**, and fluorescence spectrophotometry **[13]**, are employed.

UV-Visible spectrophotometry is still considered to be a convenient and low cost method for the analytical determination of tetracyclines in pharmaceuticals formulations. A number of spectrophotometric and colorimetric procedures for the determination of tetracyclines in bulk material and dosage forms are reported in the literature. However, few methods have been developed so far for determination of tetracycline using ultraviolet–visible spectroscopy **[14-17]**.

In the method presented here, tetracycline is bounded to aluminum(III) and the absorbance of the complex formed is measured. The present methods report simple and accurate the determination of tetracycline.

MATERIALS AND METHODS

Chemicals and instruments: All the chemicals were of analytical-reagent grade: Aluminum nitrate nonahydrate, and acetic acid were from Fluka, Germany. Sodium acetate trihydrate and hydrochloric acid (ANALAR) from BDH (UK), and Sodium hydroxide (PURUM) from Merck, Germany. Tetracycline Hydrochloride, B.P from Himedia, India, and Tetracycline Hydrochloride Secondary Reference Standard from Julphar, UAE. All the solutions were prepared in deionized water. UV-Visible 1650 Shimadzu spectrophotometer was used for recording all UV-vis spectra acquisition, controlled by computer operational UV-probe software. The spectrophotometer was equipped with a 1-cm path length quartz cell was and spectra were acquired between 190 and 1100 nm.

Spectrophotometric measurements: Stock standard solution of tetracycline hydrochloride B.P (Himedia) and secondary standard solution (Julphar), were prepared by dissolving the compound in water. This solution was stored in the dark and was found to be stable for at least three weeks changing in the its spectral profile. All the solutions were prepared in distilled water. Stock solution of aluminum (prepared by dissolving aluminum nitrate nonahydrate $Al(NO_3)_3.9H_2O$ powder in distilled water.

General Procedure: Known amounts of tetracycline hydrochloride standard solutions, aluminum nitrate, were placed in a 25 mL volumetric flask and completed to the final volume with distilled water. The absorption spectra of the above solution were measured in the range 190-1100 nm against distilled water as blank. The spectrum is presented in figure-1(a, b, and c).

RESULTS AND DISCUSSION

The absorption spectrum of Aluminum stock solution (4.0×10^{-5} M) shows strong absorption of U.V light below 250 nm wavelength. This strong absorption is related to the presence of nitrate ions in considerable amount as shown in figure-2a. The spectrum of tetracycline hydrochloride secondary standard (8.0×10^{-5} M), obtained from Julphar, UAE, were obtained and presented in Figure-2b. It showed almost continuous absorption along the wave length range 190-600 nm, with distinctive peaks at λ_{max} 358 nm, (ε 7312.5 $L.mol^{-1}.cm^{-1}$), and at λ_{max} 275 nm, (ε 7412.5 $L.mol^{-1}.cm^{-1}$). This electronic spectrum is arise from the n-π* and π-π* allowed transition, definitely effected by conjugations through the fused cyclic and aromatic rings. The sample of tetracycline hydrochloride B.P obtained from Himedia, India behaves in the same way. Figure-2c showed the absorption spectrum of tetracycline hydrochloride secondary standard in the presence of aluminum nitrate. There is a clear change from that of tetracyclines alone; the second peak at 358 nm was shifted towards longer wave length to 381 nm with slight increase in the value of the absorptivity ε 7625 $L.mol^{-1}.cm^{-1}$. However, an important change in the band shape took place, viz. it is well resolved absorption band with much smaller value of the band pass width.

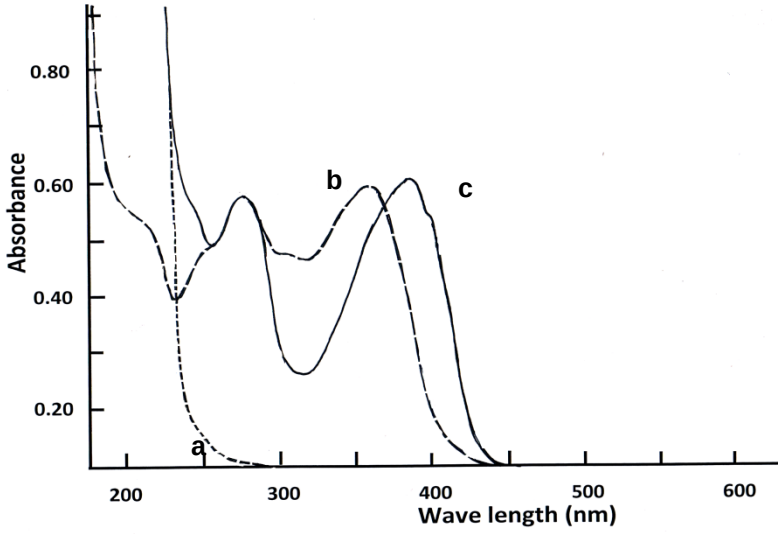

Figure 2: Absorption spectra of: a. Aluminum nitrate solution (4.0×10^{-5} M), (b) tetracycline hydrochloride secondary standard solution (8.0×10^{-5} M), (c) Aluminum (III)-tetracycline complex.

The appearance of the well resolved absorption peak is definitely due to the interaction of the tetracycline molecule, which possesses a variety of functional groups that can form complexes with aluminum metal ion in aqueous solution **[26]**. Although, it is known that tetracycline can be coupled very well to many metals, such as Iron (III) **[27]**, copper (II) **[28]**, magnesium (II) **[29]** etc, but none of them was used for analytical purpose.

Effect of variable concentrations of aluminum ion: The effect of various concentrations of Al (III) solutions was examined on the absorption of the aluminum (III)-tetracycline complex as a peak height (were prepared by the procedure mentioned in section 2-3-3). The absorption was measured at two wave length: 381 and 275 nm increase upon the increase of the concentration of aluminum ion. The absorption at λ_{max} 275 nm remains constant, an indication that it has no connection with complex ion formation. It is a criterion of the UV-Visible absorption part of tetracycline molecule only. The aluminum(III) concentration that exhibited the greatest peak height was found to be 0.0001 M, and was therefore chosen as the optimum concentration as shown in figure-3.

Effect of pH: The effect of varying the pH value of the solutions by using acetate buffer (pH 3-6) on the absorption of the aluminum(III)-tetracycline complex was studied. The result is presented in figure-4, which revealed that the absorption at λ_{max} 381 nm was increased on increasing the pH in the range (3.0-5.0), while at λ_{max} 275 nm, the absorption decreased slowly in the same range. It is possible to conclude that the effect of pH is small at the range (4.5 - 6.0).

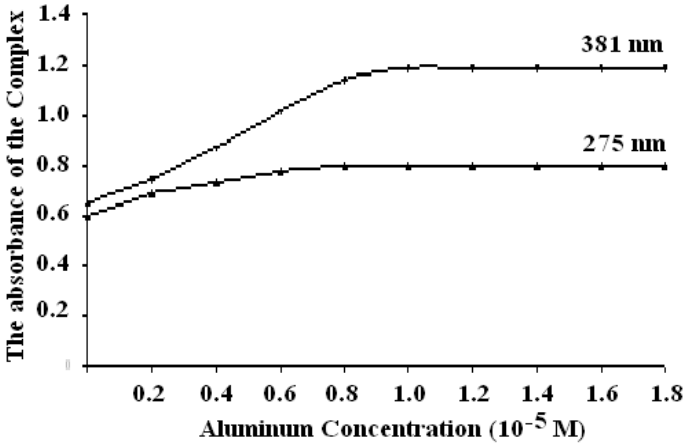

Figure 3. The effect of Al (III) concentration on the absorption of the aluminum(III)-tetracycline complex.

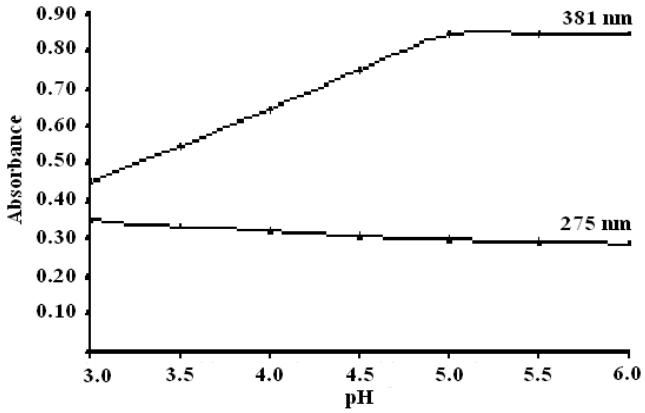

Figure 4. The effect of pH on the mean peak height of the Al(III)-tetracycline complex.

The effect of acid and base concentrations: The effect of extreme pH values on the complex stability was studied at both λ_{max} 381 and 275 nm. The results are shown in figures 5, showing that on increasing the amount of HCl, the absorption at λ_{max} 381 nm reduced while other absorption maxima at λ_{max} 435 nm start to appear until it reached a constant value at 1.5 M. This may be due to the decomposition of the complex aluminum(III)-tetracycline and the appearance of a new absorbing species. It is well known that tetracycline can undergoes an internal molecular rearrangement, as shown in figure 6. The new compound is responsible for the appearance of this new peak, and it seems to have low affinity to complex with aluminum. At higher values of pH, following the addition of NaOH, the absorption at 381 nm continues to increase, passing through a plateau of constant value, it is reduced suddenly to its tenth value with increasing the amount of NaOH. At this point we notice the formation of gelatinous precipitate of $Al(OH)_3$ as a mark of the destruction of Al(III)-tetracycline complex.

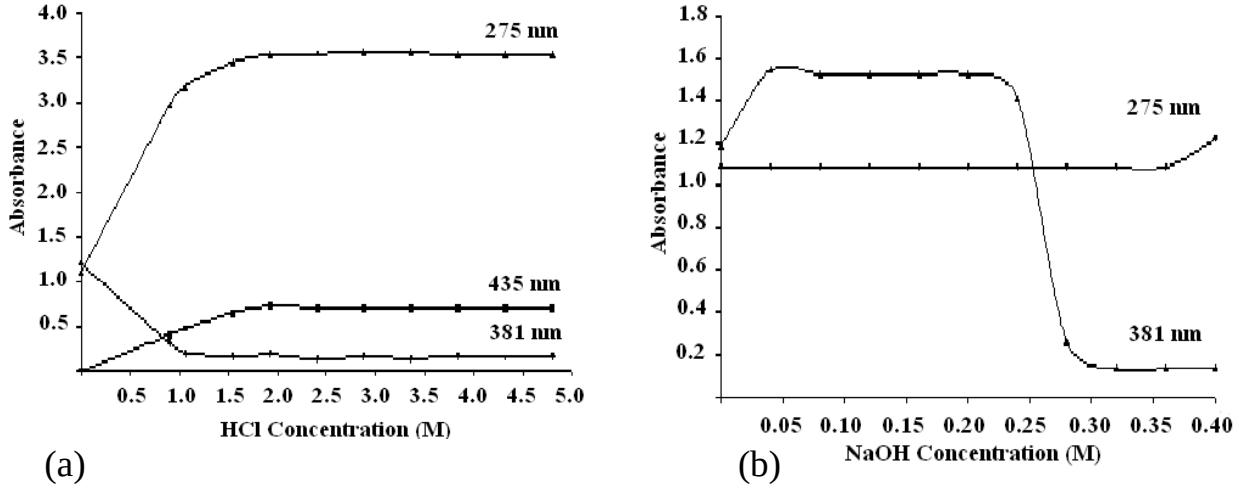

Figure 5: The effect of HCl (a) and NaOH (b) concentrations on the mean peak height of the Al(III)-tetracycline complex.

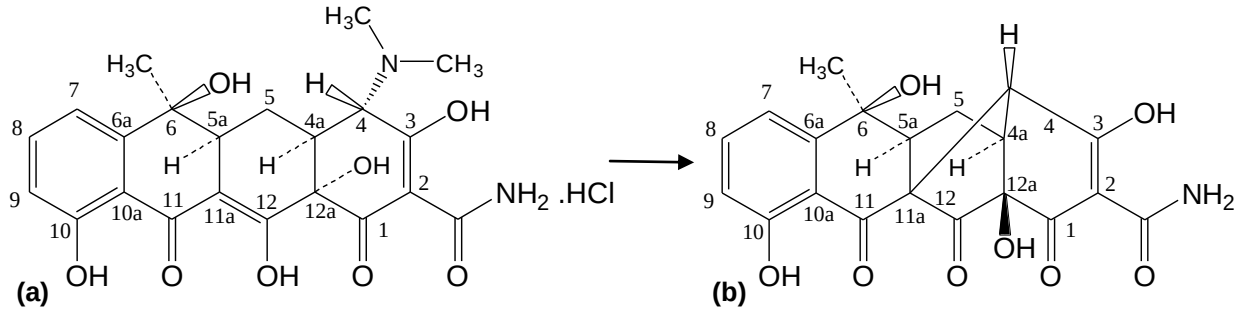

Figure 6: The internal rearrangement of tetracycline.

On the other hand we have noticed that the absorption at 275 nm increase on increasing the acid concentration until it reached a constant value of twice the absorbance to that at the start, while the addition of NaOH cause no significant effect and a plateau type curve occurs at basic medium. However this peak arises from the tetracycline part, which does not participates in the coordination with aluminum.

The stability of Complex with time: The effect of reaction time on the absorption of the aluminum (III)-tetracycline complex at different times was studied. Through the absorption of the complex, it was observed that 2 minutes was enough to obtain maximum absorption of the complex, after that interval the curve becomes constant as shown in figure 7, even four hours.

The effect of the presence of other metal ions: The effect of the presence of various metal ions on the shape and intensity of absorption curves of aluminum(III)- tetracycline complex was studied. Equimolar amount of the metal ion was used in this experimental, and the results were presented in table 1. It was found that the presence of these metal ions contribute no significant effect to the shape and in intensity of aluminum(III)-tetracycline complex.

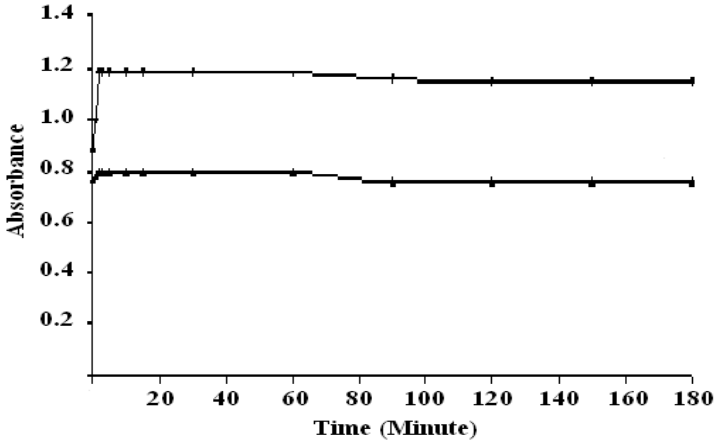

Figure 7. The effect of reaction time on the absorption of the Al (III)-tetracycline complex

Table 1 .The effect of the presence of metal ions on the aluminum(III)-tetracycline complex

No	Metal ion	Error % at λ_{max} 381	Error % at λ_{max} 275
1	Co(II)	<1.0	<1.0
2	Ni(II)	<1.0	<1.0
3	Fe(III)	<1.0	<1.0
4	Cu(II)	<1.5	<1.3
5	Pd(II)	<2.0	<1.6
6	V(IV)	<1.7	<1.4
7	Zr(IV)	<1.0	<1.0
8	Cr(III)	<1.0	<1.0
9	Ca(II)	<1.0	<1.0
10	Mg(II)	<1.0	<1.0

Effect of other interfering antibiotics: The contribution of other interfering antibiotics: (Amoxicillin, Doxycycline, Cefadroxil, Cephalexin, and Penicillin) were studied on the aluminum (III)-tetracycline complex. The result of their contributions reveals that there was no significant effect on the shape and intensity of the aluminum (III)-tetracycline absorption band.

Aluminum (III)-tetracycline complex stoichiometry By the mole ratio method: On applying the experimental procedure, and measuring the absorption of aluminum(III)-tetracycline complex at varying proportion of tetracycline (1×10^{-3}) where volumes: (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5 mL) to a fixed concentration of aluminum (2.5 ml of 1×10^{-3}) were pipetted in 25 mL volumetric flask, the plot in figure 8b, was obtained. It seemed that a single aluminum ion can coordinate to two tetracycline molecule.

Table 2 Effect of other interfering antibiotics on the stability of aluminum(III)-tetracycline complex.

No	Antibiotic*	Error % at λ_{max} 381	Error % at λ_{max} 275
1	Amoxicillin	<1.0	<1.0
2	Doxycycline	<1.0	<1.0
3	Cefadroxil	<2.0	<1.4
4	Cephalexin	<1.5	<1.3
5	Penicillin	<1.0	<1.0

* Himedia, India.

By the continuous variation method (Job method): A set of solutions were prepared from tetracycline hydrochloride B.P and aluminum stock by mixing different volume of 0.001 M of each solution in 25 mL volumetric flask and the results were presented in Figure 8a. The tetracycline structure contains many sites at which have the ability to chelate with metal ions, the most important of these being in the parts of the molecule which contains the two enolized 1,3-diketone groups. Such enol groups readily form six membered-ring with metal ion, with the two oxygen atoms as donors. Job's method showed that the ratio of aluminum(III) to tetracycline is 1: 2. This ratio, accordingly, make it possible to suggest that aluminum(lII)-tetracycline complex is more likely to possess the configuration shown in figure 9 **[19]**.

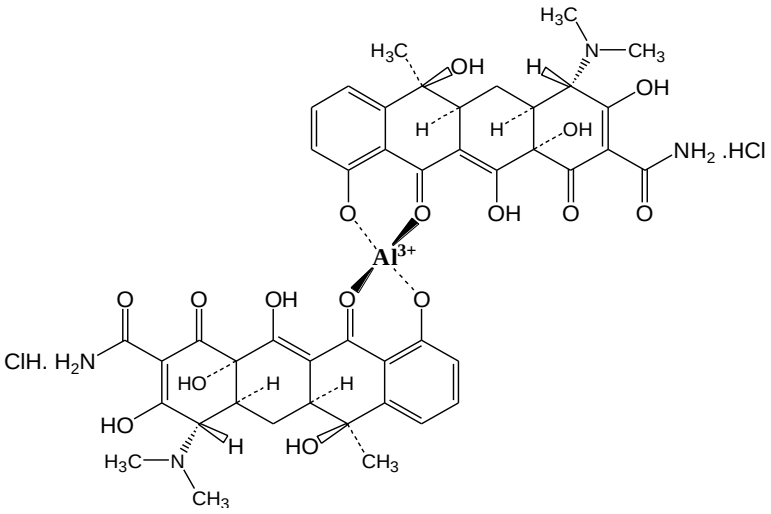

Figure 9. The suggested configuration of aluminum(III) tetracycline complex on following the continuous variation and the molar ratio methods, in which both methods showed ligand to metal stoichiometric ratio of 2:1.

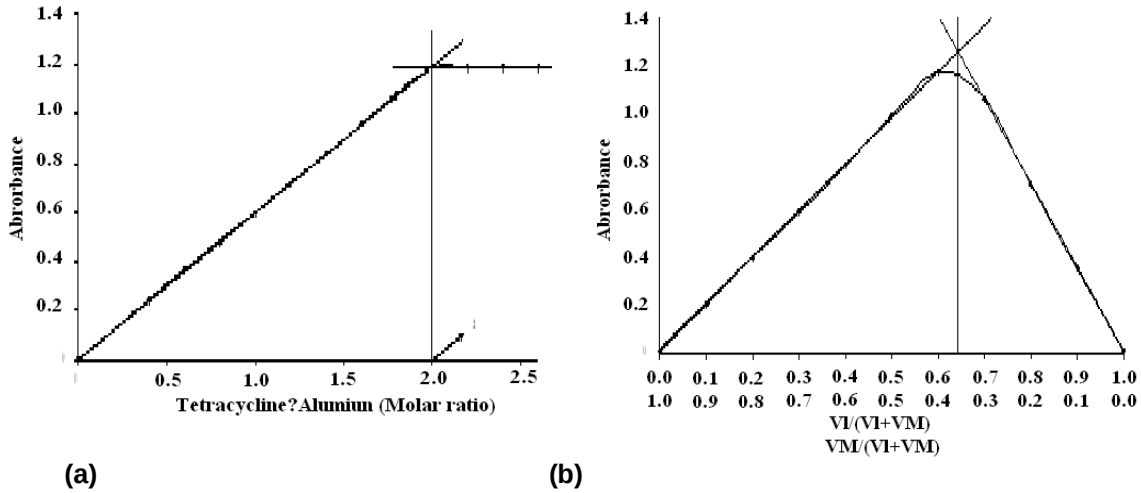

Figure 8. The ligand to metal ratio of the Al(III)-tetracycline complex by a. the continuous variation (Job method) b. mole ratio method at λ_{max} 381 nm

The calibration curve: The calibration curve for the determination of tetracycline under the As it can be seen from Figures 10, using the UV-Visible spectrophotometer under optimum conditions for tetracycline secondary standard, B.P determination using Al(III) ion, a linear calibration graph over the range of (4×10^{-7} - 2×10^{-4} M) tetracycline was established. A 2×10^{-4} mol/l obeyed Beers law and considered to be the optimum concentration.

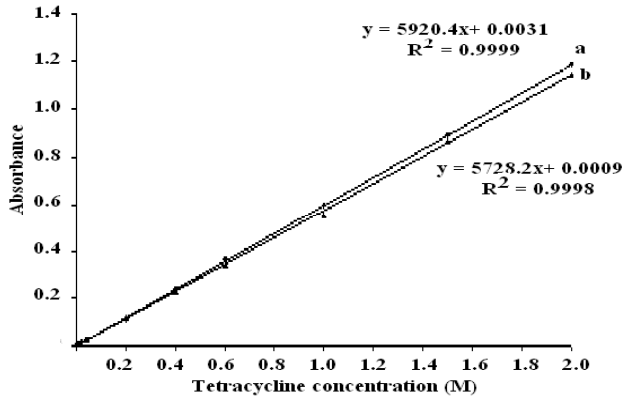

Figure10. The calibration curve for the determination of tetracycline: (a) secondary standard, (b) B.P solutions as an aluminum(III)-tetracycline complex at λ_{max} 381 nm.

Statistical Analysis : The wave length (λ_{max} 381 nm) of the maximum absorption for chelating complex for both tetracyclines (Secondary standard, and B.P) with aluminum was established, a linear calibration graph over the range of (4×10^{-7} –2×10^{-4}M), which obey Beer's law and detection of limits D.L (mol/l) for the proposed method were presented in Table-3. The relative standard deviation (RSD), apparent molar absorptivity ε ($L.mol^{-1}.cm^{-1}$), Sandell's sensitivity (S), recovery percentage and relative error percentage RSD % (n=11), were calculated and presented in table-3. The linear range at (4×10^{-7} - 2×10^{-4} mol/l) indicated that the proposed method has a wide linear range. The detection limit, of tetracycline was determined, and indicated that the method has high sensitivity by molar absorptivity **ε** value for the proposed method, Sandell's sensitivity (g cm^{-2}), the relative standard deviation RSD for tetracycline secondary standard and B.P are equal to (0.28 %). The method was also very accurate with the mean recovery of the tetracycline (Secondary standard, B.P) are being (100.03 %), with standard errors of (0.03 %). The regression equation of calibration graph, the slope at the confidence limit (95 %), correlation coefficient **(r)**, F-test statistics were mentioned**.**

Table 3. The wave length of maximum absorption, detection limits, some statistical analyses obtained from the calibration graph for the aluminum(III)-tetracycline complex.

	(Secondary standard)	**(B.P)**
λ_{max} (nm)	381	381
Conc. range (mol/l)	(2×10^{-4} - 4×10^{-7} M)	(2×10^{-4} - 4×10^{-7} M)
D.L (mol/l)	3×10^{-7}	3×10^{-7}
RSD % (n=11)	0.28	0.28
Recovery %	100.03	100.03
$E_{rel.}$ %	0.03	0.03
ε ($L.mol^{-1}.cm^{-1}$)	0.7697×10^{3}	0.6031×10^{3}
S ($gm.cm^{-2}$)	0.625	0.797
Reg. Eq. Y= a + bx	Y = 5920.4x + 0.0031	Y = 5728.2 x + 0.0009
Corr. Coef. (r)	0.9999	0.9998
F-test tabulated	2.98	2.98
F-test statistics	1.02	1.02

Tetracycline in pharmaceutical preparations: Results for some locally available formulations were investigated by the proposed method for analysis of tetracycline using aluminum(III)-tetracycline complex, and the results of the analysis were presented . Table 4, shown that is a higher value than statistics, so that it is found at confidence limit of (95 %), the standard method is not different from the B.P method, and the standard deviation of each methods is a product are within statistical errors.

Table 4. Accuracy of proposed complexometric method with tetracycline from different origins.

No	Company Name	Country of origin	Indicated dose (mg/cap.)	Measured at λ_{max} 381 nm
1	Ajanta pharma limited	India	250 mg	245 mg
2	Holland medicines Company	Holland	250 mg	252 mg
3	Pharm-Inter SPRL	Belgium	250 mg	243 mg
4	S.D.I	Iraq	250 mg	247 mg
5	Julphar	UAE	250 mg	251 mg

APPLICATIONS

This method offered an analytical procedure for the determination of tetracycline in pharmaceutical preparations such as: capsules, syrups, injections, etc. Due to the noticed stability of this complex in aqueous solutions, there is a good possibility to use it in pharmaceutical preparations.

CONCLUSIONS

The optimum experimental conditions for the analysis of tetracycline as its aluminum complex were established as follows: Concentration of Al (III) ion: 0.0001 M; pH: at the range (4.5 - 6.0); stability with time: 2 min; metal to ligand ratio: (2:1). The analytical figures obtained for the first procedure: linear dynamic range were:(4×10^{-7}– 2×10^{-4}M); RSD %: 0.28; limit of detection: 3×10^{-7} mol L^{-1}; Sandell's sensitivity: 0.625, 0.797g cm^{-2}; E rel. %: 0.03; Rec. %: 100.03. This work can be extended to investigate the possibility to follow similar method to the study the behavior of other types of tetracyclines with aluminum metal. Investigation of the possibility of using spectrofluorometry to the determination of tetracycline as aluminum (III) complex.

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