

Direct Determination of Tetracycline Hydrochloride and the Interaction with Bovine Serum Albumin by UV Spectra in Water

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ABSTRACT

The UV fourth order derivative spectra were selected as the more reliable technique for the direct quantification of pure Tetracycline Hydrochloride in water, after the measurement of the zero, first, second, third and fourth order spectra, within the concentration range at (0.2885-144.27) $\mu\text{g/ml}$ with $R^2=0.9998$ and $\text{RSD}=0.215\%$.

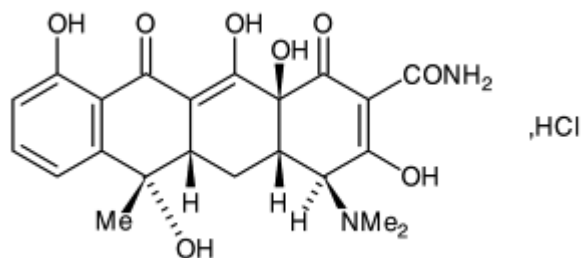
Bovine serum albumin was directly quantified by the fourth order UV derivative technique within the concentration range (6.7-2680) $\mu\text{g/ml}$ with $R^2 = 0.9998$ and $\text{RSD}=0.324\%$. Appreciable interaction between Tetracycline hydrochloride and Bovine serum Albumin was found in accordance with the fraction coefficient and the apparent binding constant.

The UV-fourth order derivative technique appears to be in a good accuracy and precise method for the direct quantification of Tetracycline Hydrochloride and Bovine Serum Albumin (BSA) in water.

Keywords: Tetracycline hydrochloride, Bovine Serum Albumin, Direct quantification.

INTRODUCTION

Tetracycline hydrochloride is a yellow crystalline powder, soluble in water and alcohol, its molecular formula is $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8 \cdot \text{HCl}$ and its structural formula is:



Tetracycline Hydrochloride
M.WT=480.9

It is one of the tetracyclines antibiotics which treats numerous types of gram positive and gram negative bacteria (Navigation, 2005), (Katzung *et al.*, 2009).

Various methods were used for the quantitative determination of tetracycline hydrochloride, spectroscopical method (Masawa *et al.*, 2008), (Abdel Khalek *et al.*, 1983), (Morelli and Peluso, 1985), (Sastry *et al.*, 1996), (Al-Ramadani, 2007), HPLC methods (Ruengsitagoon, 2008), (Bhattacharya, 2003), (Reybroeck *et al.*, 2007), (Fritz and Zuo, 2007), (Moats and Khan, 1996), (Bonta, 2007) and by mass spectrometry (Sczesny *et al.*, 2003), (Jeon and Paeng, 2009).

This work aimed to show the direct determination of pure tetracycline hydrochloride in water, and to study their interactions with albumin by recording the fourth order derivative UV spectra in water at $\lambda=282$ nm and 310°K with a good accuracy and precision and the results were compared with the standard pharmaceutical method.

EXPERIMENTAL

Material

Tetracycline hydrochloride is supplied by the state company for drug industries and medical appliances in Nineveh, Iraq (N.D.I), and was used without any further purification. Bovine serum albumin is supplied by Fluka company.

Preparation of stock solution:

A stock solution of (0.01M) of pure tetracycline hydrochloride was prepared by dissolving 0.1202g in 25ml distilled water. Other concentrations were prepared from this solution by proper dilution.

A stock solution of (0.0001M) of pure bovine serum albumin was prepared by dissolving (0.1675)g in 25ml distilled water. Other solutions were prepared from this solution by proper dilution with distilled water.

Instrumentation

The main spectra measurements were performed by a double beam Shimadzu UV-1800 (UV-Visible) spectrophotometer connected to a computer with a Pentium 4 processor. The system is supplied with a temperature controller type Shimadzu TCC-240 A and TCC-240 CE, which is equipped with insulated constant temperature cell houses. The temperature deviation varied within the limits of ± 0.1 °C. The optimized conditions for spectrophotometric measurement were derivative modes at scan speed fast, slit width 2nm. Derivative UV spectra were recorded over wavelength range (1100-190) nm using teflon stopper quartz cuvette with dimensions of (1×1×4cm). Other used apparatus are mettler sartor us 2004 MP6 electronic semi-micro balance with five digits and Electrothermal melting point apparatus IA 9300.

RESULTS AND DISCUSSION

The zero order UV spectrum of pure tetracycline hydrochloride solution in distilled water was measured against distilled water as a blank. The spectrum showed two bands at 276 nm with $\epsilon_{\max} = 15425 \text{ lit.mol}^{-1}.\text{cm}^{-1}$ and another band at 356nm with $\epsilon_{\max} = 14925 \text{ lit.mol}^{-1}.\text{cm}^{-1}$ Fig. (1).

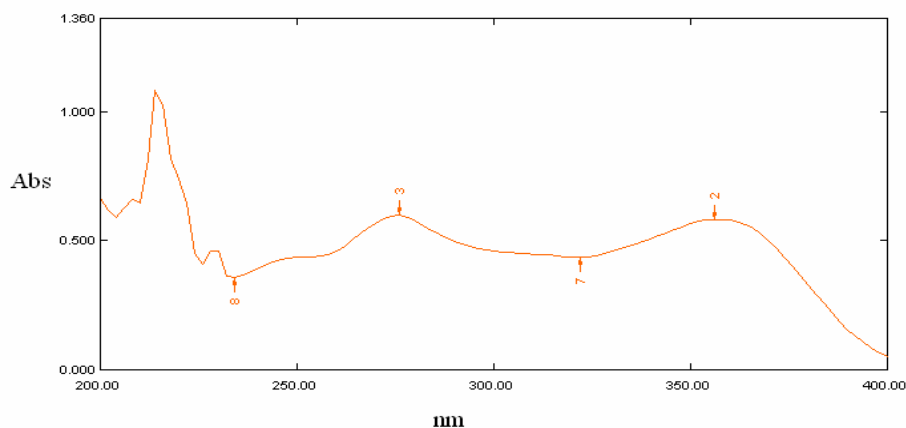


Fig. 1: UV-absorption of zero order spectra of (19.236 μg /ml) of pure tetracycline hydrochloride in water at 298.16°K.

The effect of temperature and time on these spectra showed that ,there is no effect of temperature on the absorbance of pure tetracycline spectra in water within the temperature range (298.16-313.16 °K) as shown in (Table 1).

Table 1: Temperature effect on the absorbance of pure tetracycline hydrochloride (19.236 μg /ml) in water at λ_{\max} 356nm and 298.16 K.

Temperature °K	298.16	303.16	308.16	310.16	313.16
Abs	0.606	0.605	0.605	0.602	0.601

The effect of time on the zero-order spectrum of tetracycline hydrochloride solution in water shows that the solution is stable up to more than 24 hours, as shown in (Table 2).

Table 2: Effect of time on the absorbance of pure tetracycline hydrochloride (19.236 μg /ml) in water at λ_{\max} 356 nm and 298.16°K.

Time (min)	5	15	25	35	50	60	90	120	24h
Abs	0.606	0.606	0.604	0.605	0.604	0.605	0.606	0.604	0.605

The quantification of pure tetracycline hydrochloride in water was accomplished according to the absorbance at $\lambda_{\max} = 356 \text{ nm}$ and at 298.16°K, for this purpose, the spectra of a series of different concentration solutions were recorded and the absorbance at $\lambda_{\max} = 356 \text{ nm}$ plotted versus the molar concentrations. The results showed a linear relationship obeying Beer's-Lambert law within the concentration range (0.385-48.090) $\mu\text{g}/\text{ml}$, $R^2 = 0.9993$ and $\text{RSD} = 0.125$ Fig. (2), (Table 3).

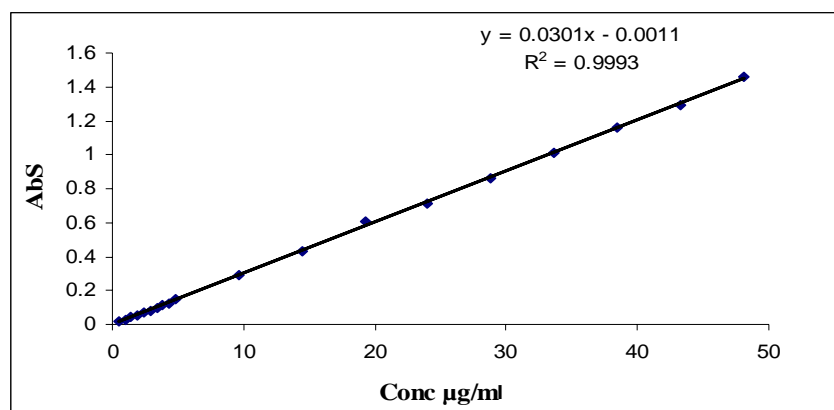


Fig. 2: Calibration curve of the zero order spectra of pure tetracycline hydrochloride solutions in water at 298.16°K and 356nm.

Table 3: Absorbance of the zero order spectra at 356nm and 298.16 °K for different concentration solutions of pure tetracycline hydrochloride in water.

Conc. µg/ml	Abs
0.3847	0.012
0.4809	0.015
0.9618	0.027
1.4427	0.041
1.9236	0.056
2.4045	0.069
2.8854	0.083
3.3663	0.098
3.8472	0.112
4.3281	0.127
4.8090	0.150
9.6180	0.292
14.4270	0.436
19.2360	0.582
24.0450	0.710
28.8540	0.861
33.6630	1.008
38.4720	1.159
43.2810	1.297
48.0900	1.458

The first order derivatives UVspectra of tetracycline hydrochloride water solution were recorded for a series of solution of different concentrations (0.385-144.270) µg/ml. The spectra showed a positive peak at $\lambda=(238-276)$ nm crossing the zero axis at 276 nm and a negative peak at $\lambda=(276-314)$ nm, and another positive peak at $\lambda=(314-356)$ nm crossing the zero axis at 356nm and a negative peak at $\lambda=(356-429)$ nm Fig. (3). The positive peak at $\lambda=(314-356)$ nm was chosen for the direct quantification of tetracycline by measuring the peak height of this peak for each solution and their plot versus the solution concentration. The results showed a straight line obeying Beer's –Lambert law within the range (0.385-48.090) µg/ml with $R^2= 0.9999$ and $RSD=0.381$, (Table 4), Fig. (4).

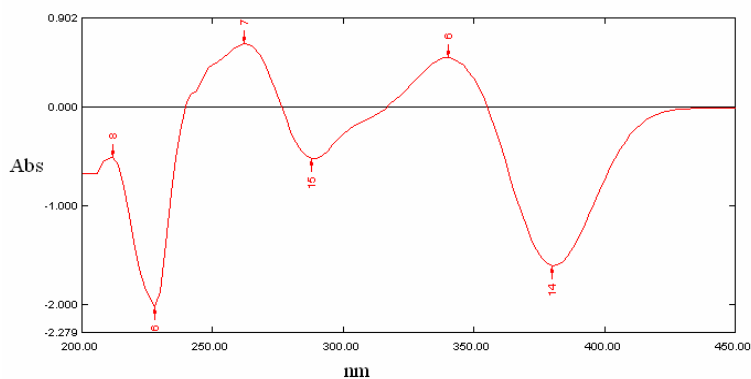


Fig. 3: UV –absorption of first order spectra of (19.236 $\mu\text{g}/\text{ml}$) of pure tetracycline hydrochloride in water at 298.16°K.

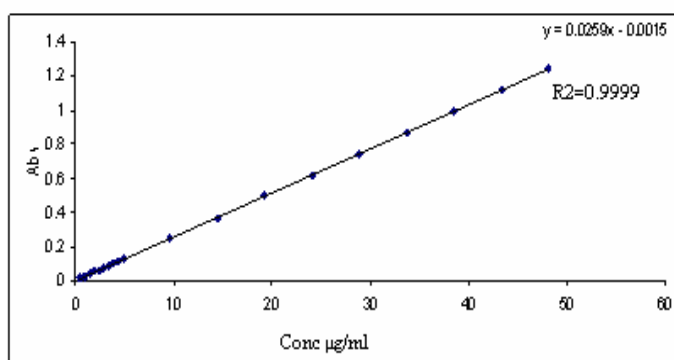


Fig. 4: Calibration curve of the first order derivative spectra of pure tetracycline hydrochloride for the positive peak at $\lambda = (340)\text{nm}$.

Table 4: Absorbance measurement of the first order derivative spectra for the positive peak at $\lambda = (340)\text{nm}$ for different concentration solutions of pure tetracycline hydrochloride.

Conc $\mu\text{g}/\text{ml}$	Abs
0.3847	0.012
0.4809	0.013
0.9618	0.022
1.4427	0.037
1.9236	0.050
2.4045	0.060
2.8854	0.071
3.3663	0.083
3.8472	0.097
4.3281	0.112
4.8090	0.128
9.6180	0.250
14.4270	0.364
19.2360	0.501
24.0450	0.611
28.8540	0.741
33.6630	0.869
38.4720	0.995
43.2810	1.118
48.0900	1.250

The second order derivative of tetracycline hydrochloride in water was recorded for a series of solutions of different concentrations ranging from (0.192-96.180) $\mu\text{g/ml}$. The spectrum shows two main negative peaks ($\lambda_1 = 274\text{nm}$) and ($\lambda_2 = 364\text{nm}$), with two satellites one at the both sides of each peak Fig. (5). The quantification was achieved according to the peak height at ($\lambda = 364\text{nm}$) which was estimated for each concentration of tetracycline solution in water. The plot of these absorbances versus the molar concentrations result in a straight line relation obeying Beer's-Lambert law within the concentration range (0.385-96.180) $\mu\text{g/ml}$ with $R^2=0.9998$ and $\text{RSD}=0.593$ (Table 5), Fig. (6).

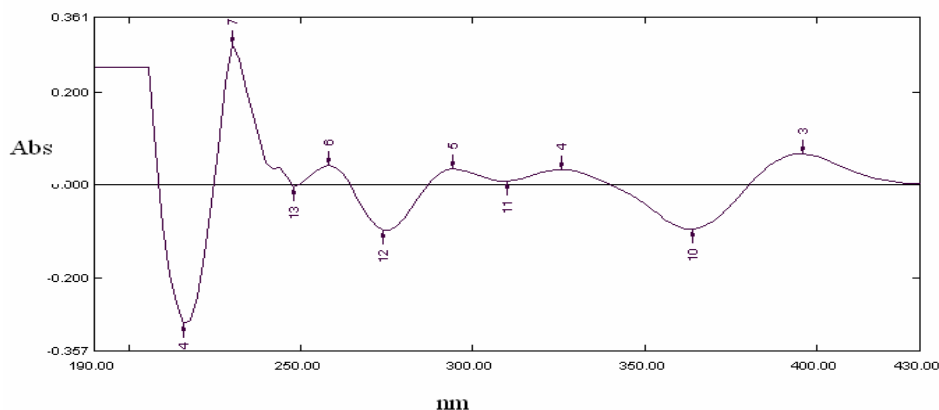


Fig. 5: UV –absorption of second order spectra of (19.236) μg /ml of pure tetracycline hydrochloride in water at 298.16°K.

Table 5: Absorbance measurement of the negative peak of the second order derivative spectra at ($\lambda_{\text{max}} = 364 \text{ nm}$) of pure tetracyclin hydrochloride.

Conc $\mu\text{g/ml}$	Abs
0.3847	0.002
0.4809	0.002
0.9618	0.004
1.4427	0.007
1.9236	0.009
2.4045	0.011
2.8854	0.014
3.3663	0.016
3.8472	0.018
4.3281	0.021
4.8090	0.024
9.6180	0.047
14.4270	0.071
19.2360	0.095
24.0450	0.116
28.8540	0.140
33.6630	0.164
38.4720	0.190
43.2810	0.211
48.0900	0.235
96.1800	0.509

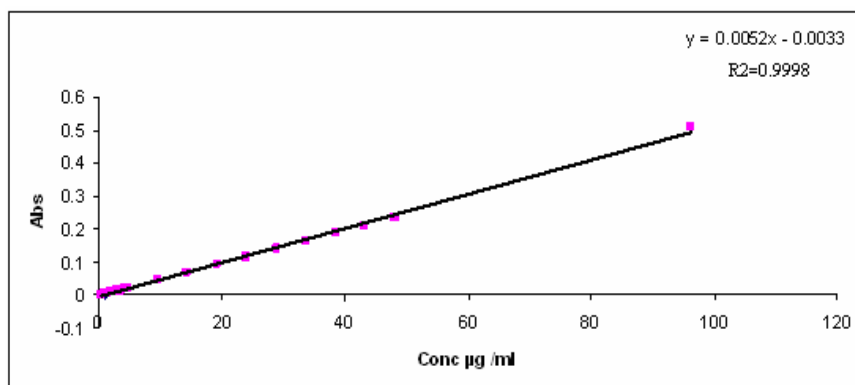


Fig. 6: Calibration curve of the second order derivative spectra of pure tetracycline hydrochloride solutions in water at 298.16°K and 364nm.

The third order derivatives UV spectra were recorded for a series of different concentrations of tetracycline hydrochloride in water. The spectrum consists of a negative peak in the region (258-274) nm crossing the zero axis at 274 nm and a positive peak at (274-292) nm, and a second negative peak at (338-364) nm crossing the zero axis at $\lambda=364$ nm and a positive peak at $\lambda=(364-393)$ nm Fig. (7). The quantification of tetracycline was accomplished according to the positive peak at $\lambda=(364-393)$ nm through the estimation of their peak height and their plot against the concentration which results in a straight line relation obeying Beer's-Lambert law within the range (0.385-48.090) $\mu\text{g/ml}$ with $R^2=0.9995$ and $\text{RSD}=0.612$, Table (6), Fig. (8).

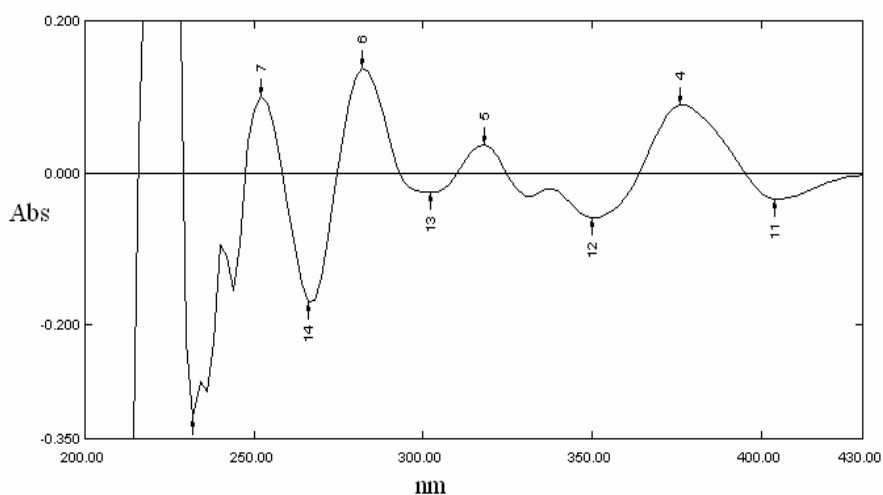


Fig. 7: UV –absorption of the third order spectra of (19.236) $\mu\text{g/ml}$ of pure tetracycline hydrochloride in water at 298.16°K.

Table 6: Absorbance of the positive peak of the third order derivative spectra at $\lambda = 377$ nm of pure tetracycline hydrochloride in water at (298.16 °K).

Conc (ppm) $\mu\text{g/ml}$	Abs
0.3847	0.002
0.4809	0.002
0.9618	0.004
1.4427	0.007
1.9236	0.009
2.4045	0.010
2.8854	0.013
3.3663	0.015
3.8472	0.017
4.3281	0.019
4.8090	0.023
9.6180	0.045
14.4270	0.067
19.2360	0.088
24.0450	0.108
28.8540	0.129
33.6630	0.153
38.4720	0.174
43.2810	0.190
48.0900	0.213

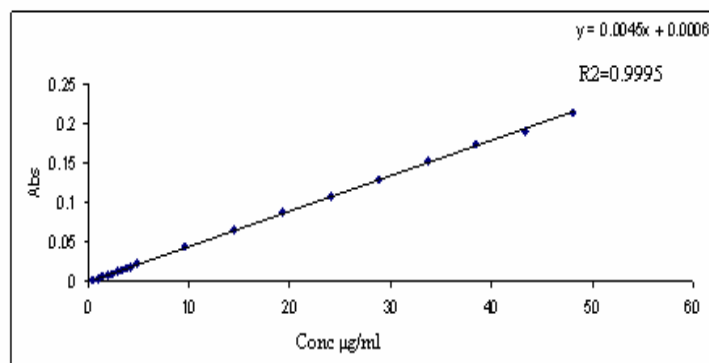


Fig. 8: Calibration curve of the third order derivative spectra of pure tetracycline hydrochloride solutions at λ_{max} 377 nm in water and 298.16°K.

The fourth order derivatives UV spectra of pure tetracycline hydrochloride in water were recorded for a series of different concentrations. The spectrum showed two main positive peaks at $\lambda=274\text{nm}$ and $\lambda=368\text{nm}$ with two satellites at both sides of each peak Fig. (9). The quantification was accomplished according to the peak height at $\lambda=368\text{nm}$ which was plotted versus the concentrations and resulted in a straight line relation obeying Beer's-Lambert law within the concentration range of (0.289 -144.270) $\mu\text{g} /\text{ml}$ with $R^2=0.9998$ and $RSD=0.215$, (Table 7), Fig. (10).

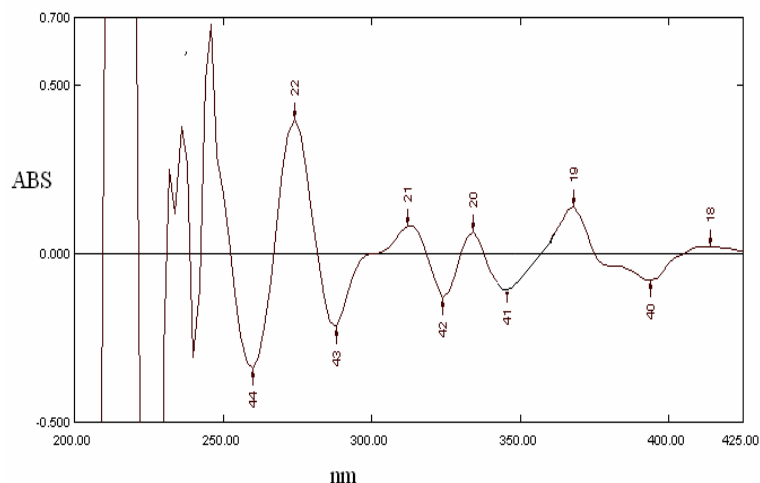


Fig. 9: UV –absorption of fourth order derivative spectra of (19.236) $\mu\text{g/ml}$ of pure tetracycline hydrochloride in water at 298.16°K.

Table 7: Absorbance measurement of the positive peak of the fourth order derivative spectra at λ 368nm of pure tetracycline hydrochloride.

Conc $\mu\text{g/ml}$	AbS
0.2885	0.004
0.3847	0.005
0.4809	0.006
0.9618	0.011
1.4427	0.013
1.9236	0.016
2.4045	0.017
2.8854	0.022
3.3663	0.024
3.8472	0.029
4.3281	0.032
4.8090	0.038
9.6180	0.092
14.4270	0.141
19.2360	0.162
24.0450	0.190
28.8540	0.250
33.6630	0.300
38.4720	0.345
43.2810	0.385
48.0900	0.440
96.1800	0.880
144.2700	1.331

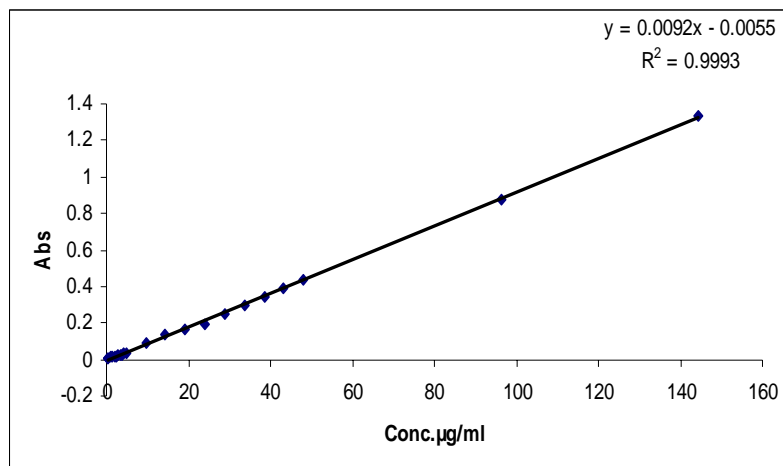


Fig. 10: UV –absorption of fourth order spectra of (19.236) µg /ml of tetracycline hydrochloride in water at 298.16°K at 368 nm in water.

According to the results obtained, it is obvious that the fourth order derivative UV technique is the more reliable technique for the direct quantification of pure tetracycline hydrochloride in water, due to their measurable larger range of concentration.

For the examination of the accuracy and precision of the fourth order derivative technique for the quantification of pure tetracycline hydrochloride in water, the absorbance of three samples at three different concentrations were measured at $\lambda = 368\text{nm}$ and the results reveal a good accuracy and precision as show in Table (8).

Table 8: Accuracy and precision of the fourth order derivative spectra (at 368nm) for the determination of pure tetracycline hydrochloride in water.

Concentration of tetracycline hydrochloride µg /ml		Recovery %	Error %
Taken	Found		
2.40	2.44	101.6	+1.6
38.47	38.09	99.0	-1.0
96.18	96.25	100	0.0

Bovine Serum Albumin (BSA):

The zero order UV spectrum of BSA in distilled water showed a peak at $\lambda_{\text{max}} = 278\text{ nm}$ with $\epsilon_{\text{max}} = 43325\text{ lit.mol}^{-1}.\text{cm}^{-1}$. The absorbance values measured at this wavelength remain unchanged for more than 2hours within a temperatures range 298.16-313.16°K Tables (9 and 10).

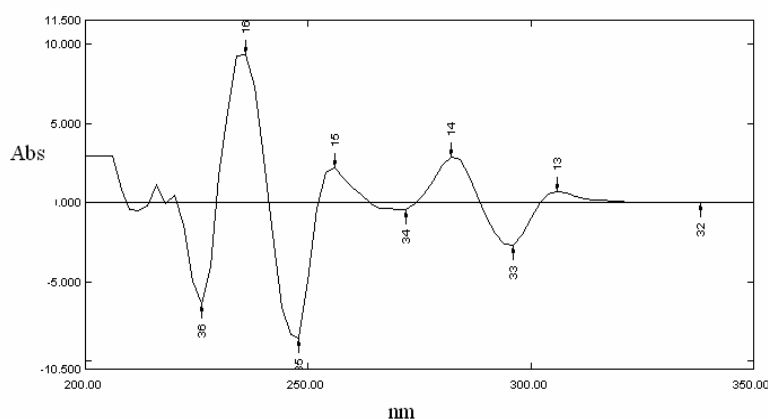
Table 9: Effect of time on the absorbance of (BSA)(2680)µg/ml at $\lambda = 278\text{ nm}$ In water

Time (min)	5	10	15	25	35	50	60	90	120
Abs	1.72	1.72	1.716	1.718	1.714	1.713	1.715	1.716	1.716

Table 10: Effect of temperature on the absorbance of bovine serum albumin solution in water (2680)µg/ml at 278nm.

Temperature. K°	298.16	303.16	308.16	310.16	313.16
Abs	1.751	1.751	1.745	1.744	1.746

The fourth order derivative spectrum of BSA in water showed a main positive peak at $\lambda=282\text{nm}$ with two satellites at each side of the peak. The peak height of the band at $\lambda=282\text{nm}$ was measured for a series of different concentration solutions of pure BSA in water and plotted versus the concentration. The result is linear relation obeying Beer's –Lambert law within a concentration range (6.7-2680) µg/ml with $R^2=0.9998$ and $RSD= 0.0836 \%$ (Table 11), Fig. (11,12).

**Fig. 11: UV –absorption of fourth order spectra of (2680) µg /ml of pure (BSA)in water at 298.16°K.****Table 11: Absorbance measurement of the positive peak of the fourth order derivative spectra at λ_{max} 282nm of pure (BSA).**

Conc µg/ml	AbS
6.7	0.0077
13.4	0.0130
20.1	0.0204
26.8	0.0271
33.5	0.0356
40.2	0.0407
53.6	0.0560
60.3	0.0608
67.0	0.0711
134.0	0.1395
201.0	0.2099
268.0	0.281
335.0	0.3491
402.0	0.4179
469.0	0.4939
536.0	0.5653
603.0	0.6351
670.0	0.7474
1340.0	1.4363
2010.0	2.1657
2680.0	2.8506

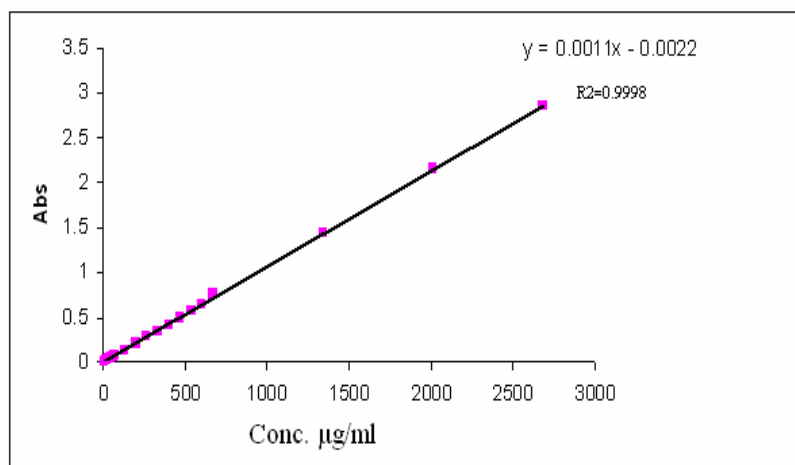


Fig. 12: Calibration curve of the fourth order derivative spectra of pure (BSA) solutions in water.

The accuracy and precision of the quantification of BSA in water was accomplished by the measurement of the peak height at $\lambda = 282\text{nm}$ of three samples at different concentrations of accurately prepared solutions, which indicate a good accuracy and precision for this method, (Table 12).

Table 12: Accuracy and precision of the fourth order derivative spectra at $\lambda = 282\text{nm}$ of the determination of pure (BSA).

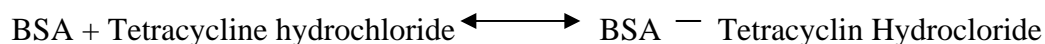
Concentration of bovin serum albumin $\mu\text{g/ml}$		Recovery %	Error %
Taken	Found		
26.80	26.63	99.38	- 0.62
67.00	66.63	99.45	- 0.55
670.00	681.45	101.70	+1.30

The interaction of pure tetracycline hydrochloride with BSA :

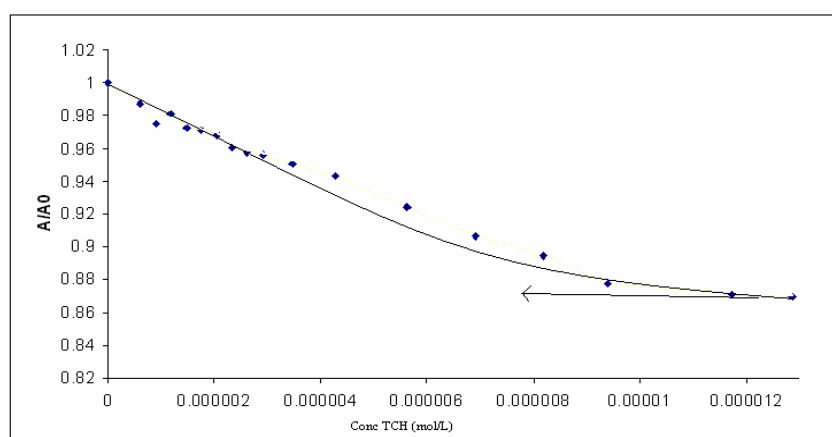
The addition of successive amount of pure tetracycline hydrochloride in water of concentration (9×10^{-5}) M to a solution of (BSA)(1×10^{-5} M) in water, causes a gradual decrease in the measured absorbance at $\lambda = 282\text{nm}$ Table (13).

Table 13: Absorbance after the addition of Tetracyclin Hydrochloride $9 \times 10^{-5} \text{M}$ to (BSA) $1 \times 10^{-5} \text{M}$ in water at $T=310.16^\circ \text{K}$ and $\lambda = 282 \text{nm}$

Conc (TCH) mol/L	Abs $\lambda = 282 \text{ nm}$	A/A_0	$\log 1/[TCH]$	$\log A/(A_0-A)$
0	0.7280	1		
5.96026E-07	0.7190	0.987637		
8.91089E-07	0.7100	0.975275		
1.18421E-06	0.7140	0.980769	5.926571083	1.707
1.47541E-06	0.7080	0.972527	5.831087326	1.549
1.76471E-06	0.7070	0.971154	5.753327667	1.527
2.05212E-06	0.7050	0.968407	5.687797826	1.486
2.33766E-06	0.6990	0.960165	5.63121822	1.382
2.62136E-06	0.6970	0.957418	5.581473461	1.351
2.90323E-06	0.6960	0.956044	5.537119184	1.337
3.46154E-06	0.6920	0.950549	5.460730839	1.283
4.28571E-06	0.6870	0.943681	5.367976785	1.224
0.000005625	0.6726	0.923901	5.249877473	1.084
6.92308E-06	0.6598	0.906319	5.159700843	0.985
8.18182E-06	0.6512	0.894505	5.087150176	0.928
9.40299E-06	0.6390	0.877747	5.026734253	0.856
1.17391E-05	0.6340	0.870879	4.930364072	0.828
1.28571E-05	0.6330	0.869505	4.890855531	0.823
Fractional coefficient(k)	0.88			
binding constant (K)	$9.772 \times 10^3 \text{ M}^{-1}$			



In order to highlight the nature of interaction between the Tetracycline Hydrochloride and BSA in water, The fraction coefficient k was estimated by the plot of $\frac{A}{A_0}$ versus the concentration of the added Tetracycline Hydrochloride ($9 \times 10^{-5} \text{M}$). To BSA of concentration ($1 \times 10^{-5} \text{M}$) Fig. (13).

**Fig. 13: Dependence on A/A_0 ratio with addition of tetracycline chloride at fixed BSA($1 \times 10^{-5} \text{M}$) at 310 K.**

From which the fraction coefficient $k = \frac{A}{A_0}$ was estimated. The equation for voltammetric determination can be deduced according to absorbance equation as described below:

$$\text{Log}\left(\frac{1}{\text{Conc}[TCH](M)}\right) = \text{Log}K + \text{Log}\left(\frac{A}{A_0 - A}\right)$$

Where K is the apparent binding constant, A_0 is the absorbance at $\lambda = 282\text{nm}$ before the addition of tetracycline hydrochloride, A the absorbance after each addition under the assumption of reversible complex of (BSA — Tetracycline hydrochloride) is 1:1 association complex. Then the plot of $\text{Log}(1/\text{conc}[TCH])$ versus $\text{Log}(A/A_0 - A)$ result in a linear relation with the intercept of $\log K$, Fig. (14), from which the K value $= 9.772 \times 10^3$ was calculated indicated that an appreciable interaction between BSA and tetracycline hydrochloride do exist.

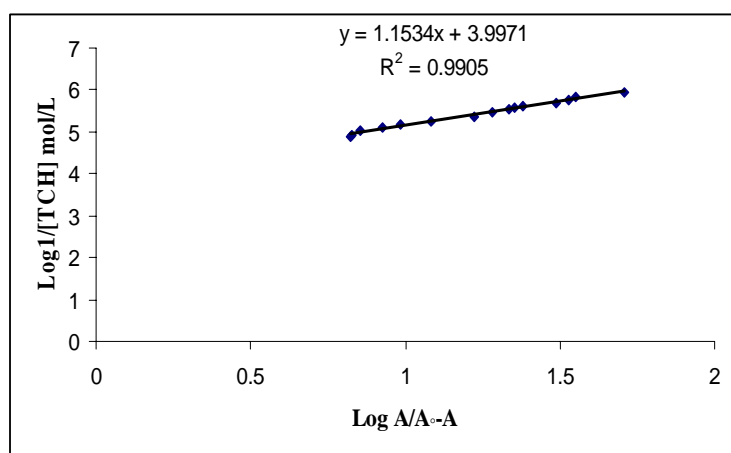


Fig. 14: Plot of $\text{Log } 1/[\text{TCH}]$ versus $\text{Log}A/(A_0 - A)$ at fixed BSA 0.00001M at 310°K.

Determination of tetracycline hydrochloride by standard method (HPLC): (B.P 2009)

The determination of tetracycline hydrochloride by HPLC method at their optimum experimental conditions accomplished at the state company of drug industries and medical appliance in Nineveh, Iraq (NDI), and comparison with our present method (fourth order derivative) indicates that the fourth order derivative method is of a very good recovery percent as shown in (Table 14).

Table 14: Comparison between the standard HPLC method and the present method for the determination of tetracycline hydrochloride in pharmaceutical formulation.

pharmaceutical preparation	Recovery %	
	Present method	Standard method
Tetracycline-HCl 250mg /capsule S.D.I Iraq	100.2	101.8

*Average of three determinations

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