Spectrophotometric Determination Tetracycline.HCl in Pharmacutical Preparation by oxidative coupling

التقدير الطيفي للتتراسايكلين هيدروكلورايد في المستحضرات الصيدلانية بالاقتران التأكسدي

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ABSTRACT

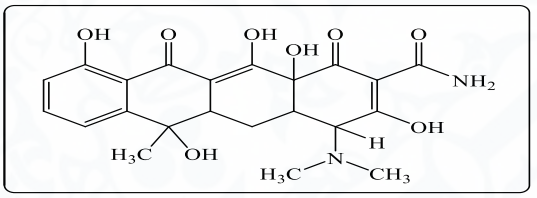
The Development of a spectral method for the estimation of micrograms of tetracycline hydrochloride in oxidative coupling with 4.2-di nitro phenylhydrazine with the presence of $NaIO_4$ as an oxidative agent in the basal medium to form a reddish yield in ethanol and give the highest absorption at wavelength 528 nm. The method obeyed the berr's Law in a range of concentration ranging from 2 to $\mu g.ml^{-1}$, and the molar absorption value was 10^{-4} x 1.8851 $L.mol^{-1}$. cm^{-1}, the Sandal index value is 0.0255 $\mu g. cm^{-2}$, the detection unit 0.0749 $\mu g. ml^{-1}$, the correlation coefficient value 0.9892, and the relative standard deviation of the method does not exceed 1.20%. The method was applied successfully to the pharmaceuticals and in the direct and standard methods, and the reaction ranged between 99-102%.

Key words: tetracycline hydrochloride, ultraviolet and optical spectroscopy.

1- Introduction

Tetracyclinehydrochloride

It is a member of the family of tetracyclate. A non-odorless yellow crystalline powder is easy to dissolve in water and alcohol. It breaks down with bases and strong acid is kept in a dark vial and its molecular formula is $C_{22}H_{24}N_2O_8.HCl$ [1,2] with a molecular weight of 444.4 g. Mol^{-1} and owns the formula:



Spectroscopic [3.4], chromatographic [5.6] and other methods of estimating tetracycline [7,8] are used.

2.Practical Part

2.1 Equipment used: Use the Shimadzu UV-1800 spectroscopy device.

2.2 Reagents and chemicals used: All chemicals and analytical reagents were of high purity.

3-2 Solutions used

- Standard tetracycline solution 1000 $\mu g.ml^{-1}$ (2.25 x 10^{-3}molar): (Prepare this solution by dissolving 0.1 g of tetracycline powder with distilled water in a 100 mL volume vial and complete to the mark with distilled water.
- Tetracycline solution 500 $\mu g.ml^{-1}$ (1.12x10^{-3} molar) :(This solution was prepared by diluting 50 mL of the standard solution 1000 ml.
- Detector solution 2,4 -Di Nitro phenylhydrazine (1 x 10^{-3} molar): (Prepare this solution by dissolving 0.1980 g of powdered material in a

quantity of distilled water after adding 3 ml of concentrated sulfuric acid and complete the volume with distilled distilled water to the mark with distilled water in a 100 mL volume vial.

- sodium hydroxide solution 1 molar Approximate:

Prepare this solution by dissolving 4 g of it and dissolve it with distilled water and then complete the size to the mark with distilled water in a 100 mL volume vial.

- Oxidative Working Solution Metha Sodium Periodate 1 $x10^{-2}$ molar:

Prepare this solution by dissolving 0.2139 g of it in distilled water, and then complete the size to the mark in a 100 ml volume vial.

- Pharmaceuticals solutions 500 $\mu g.ml^{-1}$:

The tetracycline-containing pharmaceuticals are capsules:

Tetra Lab Tetracycline BP (500µg):The drug was prepared at a concentration of 500 $\mu g.ml^{-1}$ with 5 capsules and mixed well and weighed 0.05 g of tetracycline powder and dissolved in 100 ml of distilled water.

3-Preliminary Study

When adding 1.5 ml of sodium periodate solution to 2 ml of tetracycline with a concentration of 500 $\mu g.ml^{-1}$ With 2 ml of the 2,4-dihydrozine phenylhydrazine solution at a concentration of 1x10-3 molar, then 2 ml of concentrated sodium hydroxide The solution was measured in red color with the addition of the base solution. After dilution of the solution with distilled water in a 25 ml volume bottle, the absorbance spectra of the colored product was measured against the solution. It was found to give the highest absorption at 528 nm wavelength. this area.

4- Study of optimum conditions

Subsequent experiments were performed using 2 mL of tetracycline solution with a concentration of 500 $\mu g.ml^{-1}$ in a final volume of 25 ml. The absorption of solutions at 528 nm wavelength was measured against the solution.

1-4 Effect of Oxidation Factor Quantity

The effect of the oxidizing agent was studied with a solution of $1x10^{-2}$ molar sodium methyl periodate by adding volumes from 0.5-3 ml to 25 ml volume bottles containing 2 ml of tetracycline 500 $\mu g.ml^{-1}$ solution. 2 ml 2,4-dienitrophenylhydrazine with concentration $1x10^{-3}$ molar and then add 2 ml of 1 molar sodium hydroxide and complete the volume to the mark mark with distilled water. The results are recorded in Table (1), which found that the best volume gives the highest absorption of the stained product is within the range of 1-2.5, for this size was selected 2 ml and was adopted in subsequent experiments.

Table (1) Effect of oxidative Agent Quantity

ml of $NaIO_4$ 0.005 M	*Abs.*	*ml of $NaIO_4$ 0.005 M*	*Abs*
0.5	0.390	2	0.544
1	0.412	2.5	0.521
1.5	0.576	3	0.591

4.2 Effect of Acid Function

The acidic effect was studied with addition of 0.5-3 ml of sodium hydroxide. The best acidic function was found in 12.6-12.7. Acid function 12.65 was used with 2 mL sodium hydroxide in subsequent experiments and as indicated in the results recorded in Table (2).

Table (2) Effect of acidic Function

ml of NaOH 1 M	*Abs.*	*PH*	*ml of NaOH 0.1 M*	*Abs.*	*PH*
0.5	0.324	11.87	2	0.635	12.65
1	0.479	12.39	2.5	0.701	12.71
1.5	0.528	12.60	3	0.611	12.69

4.3Effect of Reagent Quantity

The effect of the quantity of the coupling detector was investigated by taking different volumes of 2,4-dinitro phenylhydrazine solution with a concentration of $1x10^{-3}$ molar ranging from 0.7-4 ml with different sizes 0.5-4 ml tetracycline 500 $\mu g.ml^{-1}$ solution with 1.5 ml from$NaIO_4$ concentration $1x10^{-2}$ molar and 2 ml of 1 molar of sodium hydroxide at a final volume of 25 mL. The results are shown in Table (3). The results below show that the volume of 2 mL of the 2,4-di nitro phenylhydrazine concentration 1x10-^{3} molar gave the highest value of 0.1196, the highest sensitivity and high value of the correlation coefficient 0.9828, so it was adopted in experiments Subsequent.

Effect of Reagent Quantity) 3 (Table

ml of Reagent $\times10^{-3}$ M	*Conc of Tetracycline .HCL 500µg/ml*					*R^2*	*Slope*
	0.5	*1*	*2*	*3*	*4*		
	0.099	0.135	0.237	0.300	0.348	0.9952	0.0102
0.5	0.086	0.155	0.273	0.374	0.440	0.9858	0.128
0.7	0.089	0.166	0.278	0.385	0.455	0.9872	0.0131
1	0.075	0.272	0.272	0.444	0.484	0.9891	0.0140
1.5	0.065	0.146	0.265	0.430	0.512	0.9976	0.0151
2	0.069	0.145	0.263	0.428	0.533	0.9957	0.0168
2.5	0.055	0.135	0.264	0.415	0.510	0.9944	0.0165
3	0.052	0.122	0.261	0.405	0.490	0.9879	0.0158

4-4 Sequence of entries

The effect of changing the sequence of addition of the reaction materials on the absorption of the colored product was studied by conducting a number of experiments. It was found that the sequence of addition (I) achieved the highest absorption of the colored product. Therefore, it was adopted in subsequent experiments. The results are shown in Table 4. (D), the solution of the detector 2,4-dinitro phenylhydrazine (R), the oxidizing agent solution (O), and the sodium hydroxide solution (B).

Table (4) Sequence of Additions

Order Number	***Order of addition***	***Absorbance***	
		BW	***SB***
I	D+O+R+B	0.008	0.601
II	D+R+O+B	0.021	0.122
III	D+B+O+R	0.005	0.008
IV	D+O+B+R	0.021	0.134

5-4 Temperature Effect

The effect of temperature on the absorption of the colored product produced using temperatures of 15-50 ° C was studied as shown in the results in Table (5).

Table (5)Effect of temperature

Temp.	15	20	25	30	35	40	50	60
Abs.	0.313	0.313	0.313	0.313	0.313	0.313	0.313	0.313

We observe from the above table that the optimum temperature is 15-25 ° C. The absorption value of the red color product is stable at this temperature. At 30, 40 and 50 ° C, the color of the product changes and is less absorbed. Respectively, so the temperature was adopted 15-25 ° C in subsequent experiments.

6-4 The stability of the Reaction Product

This study was carried out by taking three different volumes (1,2,3 ml) of tetracycline concentration with 500 $\mu g.ml^{-1}$ Concentrates (20, 40, 60) $\mu g.ml^{-1}$ Add 2 ml of $NaIO_4 1x10^{-2}$ molar and then add 2 ml of the 2,4-di nitro phenylhydrazine solution with a concentration of 1 $x10^{-3}$ molar and then add 2 ml of 1 molar sodium hydroxide and complete the volume in a 25 mL volume vial and complete the volume with distilled water, The absorption of colored output remains stable for at least 60 minutes and is suitable for completion of many measurements and results recorded in Table (6).

Table (6) Stability of the Output of the Reaction

µg of TCN	Absorbance/min. Standing time								
	5	10	15	20	25	30	40	50	60
20	**0.620**	0.624	0.628	0.618	0.615	0.613	**0.612**	0.611	0.610
40	**0.658**	0.650	0.652	0.651	0.646	0.645	**0.643**	0.642	0.640
60	**0.713**	0.712	0.711	0.710	0.710	0.705	**0.704**	0.704	0.702

Solvent Effect4.6

The effect of some solvents was studied on the reaction of the colored reaction, which is the reaction of tetracycline with the sodium $NaIO_4$, and the addition of 2,4-di nitro phenylhydrazine with the presence of sodium hydroxide in the basal medium. The volume of 25 ml bottles was used after completion of the additives under optimum conditions, To the extent of the mark with different organic solvents and taking the absorbance spectra for each solution versus its photolysis and the results as written in Table (6) and shown in Figure (1) .

Table (6) Solvent Effect

Solvent	Absorbance	Solvent	Absorbance
DMSO	0.850	Acetone	1.128
Ethanol	0.543	Water	0.625

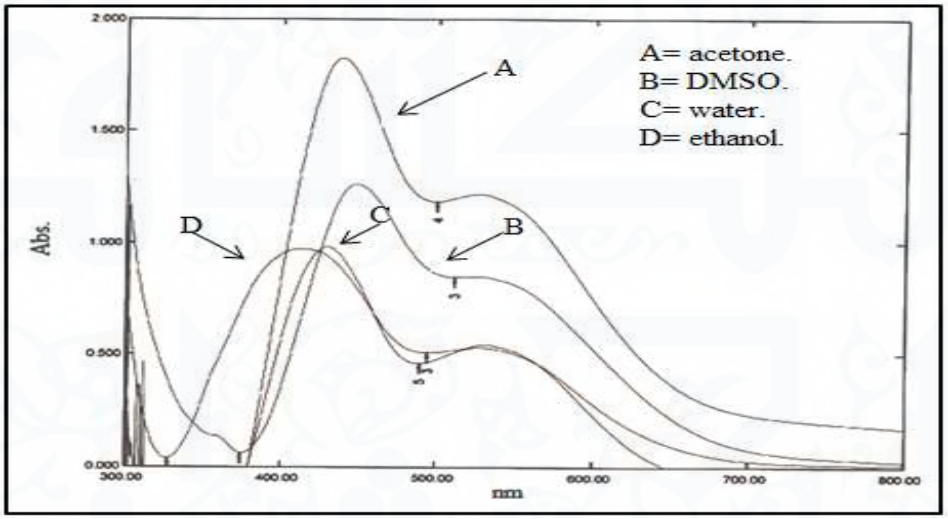


Figure (1) Solvent Effect

5.The Final Absorption Spectra

The final absorbance spectrometry was measured after optimum conditions were shown in Table (7). Then, the absorption of the red-color product was measured against its photolysis, which was found to give the highest absorption at 528 nm while its photolysis did not give any absorption in this area. The SW represents the absorption spectrum of the tetracycline solution versus the distilled water. SB represents the absorption spectrum of the tetracycline acid solution versus the photo solution, and Bw represents the absorption spectrum of the solution compared to the distilled water.

Table (7) Optimal Conditions for Interaction

2ml	500 µg.ml^{-1}	tetracycline
2ml	1x10^{-3} molar	2,4-di nitro phenylhydrazine
2ml	1x10^{-2} molar	$NaIO_4$
2ml	1 molar	NaOH
-----	528nm	Wave lenght
-----	5 min	Time Oxidation

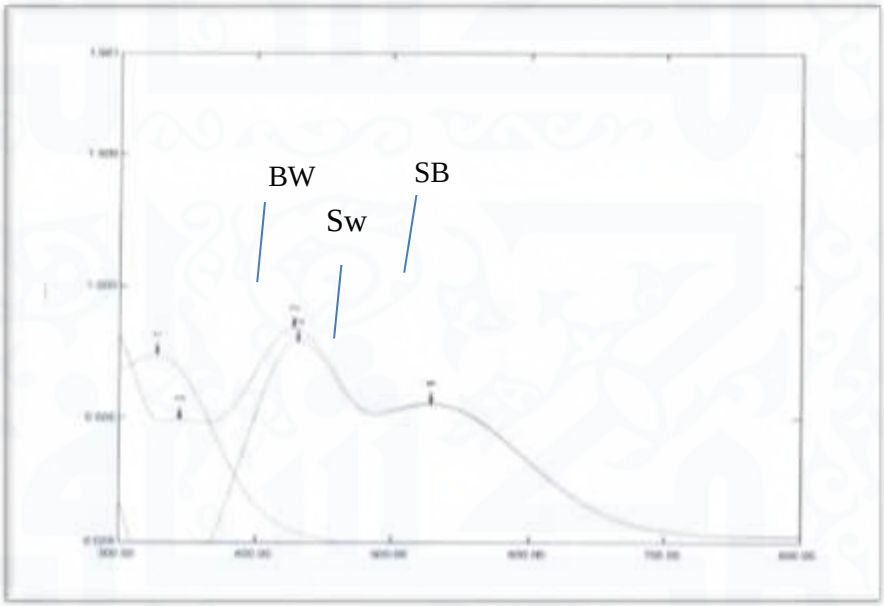
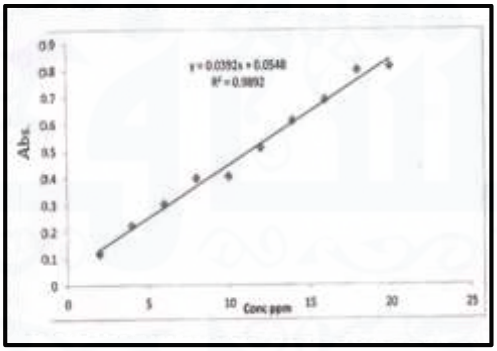


Figure (2):Final Absorption Spectrum

6. Approved Method of Work and Calibrating Factor

After the optimal conditions were established for the determination of tetracycline shown in Table (7), a series of 25 ml volume bottles containing increasing concentrations of 2-20 µg.ml^{-1} were taken from tetracycline solution (0.5 - 5.5 ml) (2-20 µg.ml^{-1}) of the tetracycline solution and makes the deviation from the beer ،s law Act at the time of the Concentrations of more than 20 µg. ml^{-1} , gave a correlation coefficient of 0.9892 and then calculated the value of molar absorption and With a value of 1.8851 L.mol^{-1}.cm^{-1} With a value of sandel index 0.0255 µg. ml^{-2} .



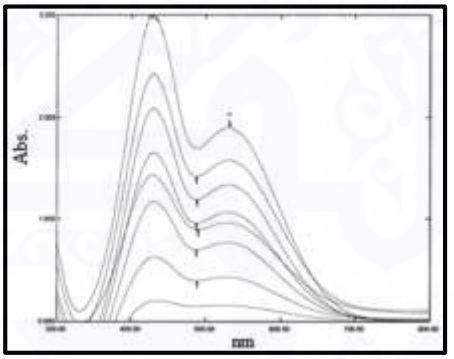


Figure 4: Absorption spectra for concentrations of 20-20 µg / ml Figure (3):Titration curve for the determination of tetracycline of tetracycline

7. Accuracy and Compatibility

The optimum conditions were used in the method of work to select the accuracy of the calibration curve and its compatibility. Six readings of three different amounts of tetracycline solution were used within the limits of the BER law in the calibration curve and the RSD% calculation [9] and the method was found to be of high accuracy (99.51% Average of Recovery) and high correlation (relative standard deviation less than 1.20%), according to the table below.

Table (8) shows Accuracy and compatibility.

Conc. Of TCN µg/ml	*Recovery%*	*Average of Recovery%*	*RSD%*
4	99.71	99.51	1.20
10	99.84		0.540
18	99		0.316

8. Detection Limit

The detection limit [10] was calculated by measuring the absorption of solutions to a minimum concentration of 2 $\mu g.ml^{-1}$ in the calibration curve within the limits of the Birr

law and under the same conditions (optimal conditions). The detection limit was 0.0749 µg.ml^{-1} As in Table 9.

Table (9) Detection Limit

Concentration µg/ml	$\overline{X}$	***S***	***D.L µg/ml***
2	0.1178	0.0014	0.0749

9. The Nature of the Resulting Product

To determine the nature of the red product and the ratio of the bond with the reagent, the two methods of continuous changes (the Job method) and the molar ratio method [1] were applied. In both methods, the concentration of tetracycline solution and the detector solution 2,4-di nitro phenylhydrazine was in the same concentration In the Job method, a series of 25 ml volume bottles were placed in different sizes of the drug solution ranging from 1-9 ml. The supplements were added to 10 ml of the reagent solution and 1 ml of $NaIO_4$ solution Concentrate $1x10^{-2}$ molar and 2 ml of basal hydroxide solution, dilute To the extent of the mark with distilled water and then measure the absorption of these solutions at the wavelength 528 nm and Figure (5) shows the ratio (1: 1).

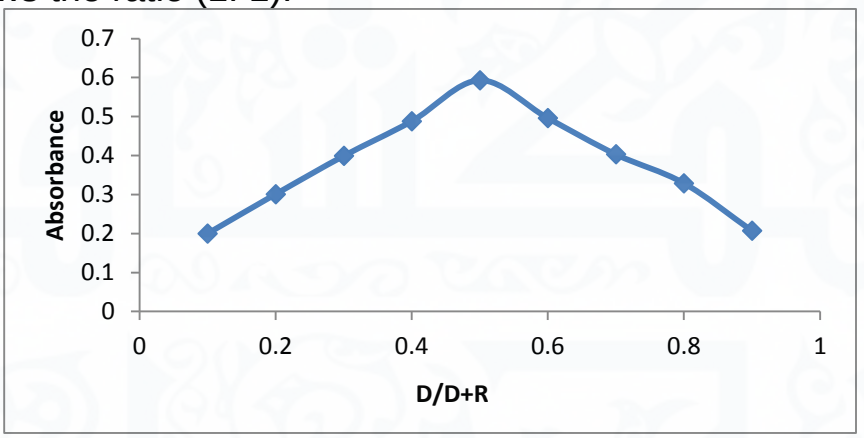


Figure (5) Method of determination of tetracycline with detector 2,4-dinitro phenylhydrazineboog $NaIO_4$

In the method of temperature approximation, 2 ml of the drug solution was placed in a 25 mL volume bottle and the reagent solution was added in sizes ranging from 0.5-5 ml and then added 1 ml of oxidation agent $NaIO_4$ $1x10^{-2}$ molar and 2 ml The basal solution sodium hydroxide was concentrated 1 molar in the 25 distilled water and then measured the absorption of these solutions at the wavelength of 528 nm versus the solution of each solution. It was found that the molar ratio is consistent with the method of continuous changes and the ratio (1:1) In figure (6).

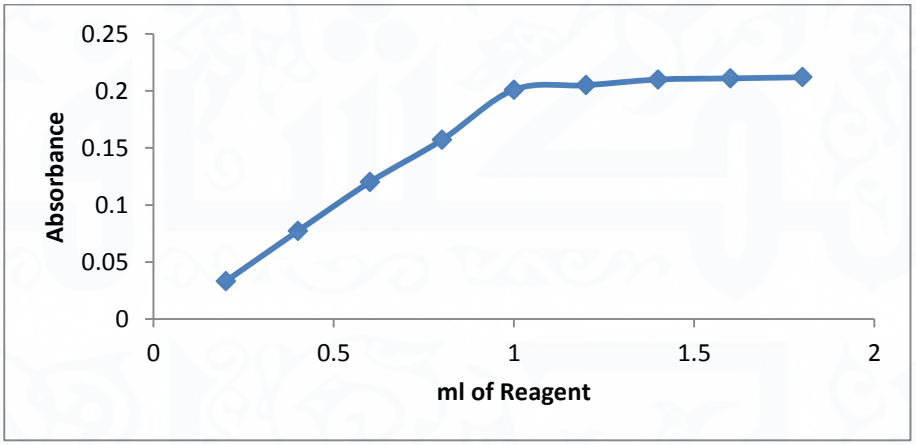
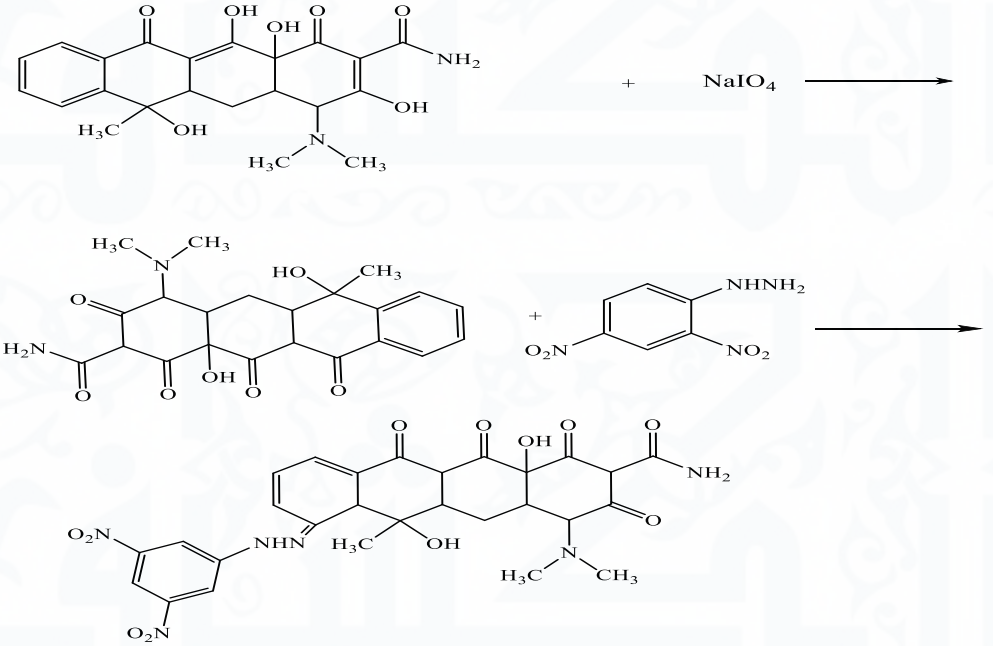


Figure (6):method of temperature approximation for determination of tetracycline with 2,4-dinitro phenyl hydrazine boog $NaIO_4$

There fore, the suggested interaction equation is as follows:



10.Applications

The method was applied to pharmaceuticals containing tetracycline, the pharmaceutical product: Teblet-Tetracycline Capsule BP (500µg).

10.1 Direct Method

Three different concentrations of each solution were taken (4, 8, 16 micrograms, µg. ml^{-1}). The solutions were treated with the same steps as the calibration curve and then measured at 528 nm wavelength compared to the solution. Concentration in addition to the recalculation calculation as in the results listed in Table (10).

Table (10) Direct Method

Conc. of TCN (µg/ml)	***Abs. of Pure TCN***	***Teralab***	
		Abs.	***Recovery%***
4	0.162	0.158	103.21
8	0.621	0.615	100
18	0.697	0.685	100

The results of the above table show the success of the suggested method of estimating tetracycline in the pharmaceuticals containing it. The return rate was 100.97% in Teralab.

10.2: Standard Addition Method

This method was carried out for the purpose of demonstrating the efficiency and accuracy of the proposed method and proving that the method was free from interference. The standard method of addition was used in estimating tetracycline in the Teralab pharmaceutical product.

The method included the addition of a fixed amount of 0.5 ml of the prepared pharmaceutical solution at a concentration of 500 µg. -1 in a series of 25 ml volume bottles, then adding increasing volumes (0, 1, 2, 3, 4 and 5 ml) of the standard tetracycline solution With a concentration of 500 µg. M-1. The above solutions were treated in the same way as the calibration curve. The absorption of all solutions compared to the solution was measured at 528 nm and the results are shown in Table 11 and Figure 7.

Table (١١)Standard Method of Addition.

Type of Drug	***Tetracycline Present µg/ml***	***Tetracycline measured µg/ml***	***Recovery%***
Capsule Tetracycline (500 µg).	4	4.51	103.33

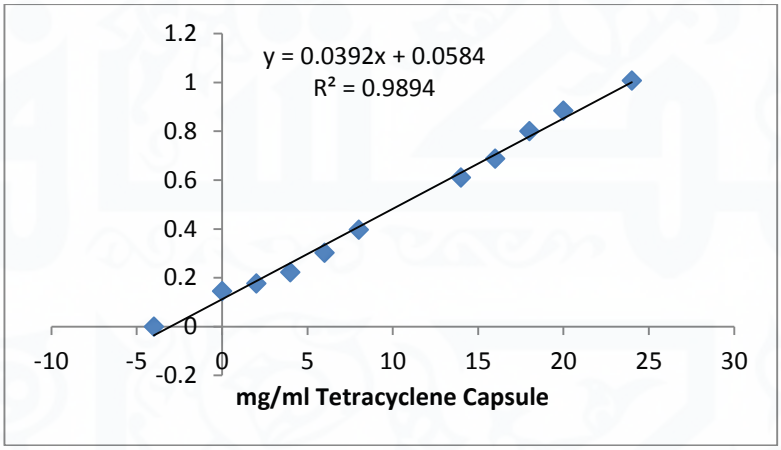


Figure (7) Standard method of addition of tetracycline using Capsule- 4mg

11.Conclusion

A spectral method was developed to estimate tetracycline through the oxidative coupling reaction. The method is based on the oxidation of the drug using sodium periodate in the basal medium and the addition of the 2,4-di nitro phenylhydrazine reagent solution. After completion of the additives, the final product of the red color reaction is more stable Of 50 min, long enough to hold many measurements, gave the highest absorption at 528 nm wavelength and followed the beer's law in the range of concentrations (2-20 µg.ml^{-1}), and the molar absorption value was1.8851 x10^{-1}. L.mol^{-1}.cm $^{-1}$ and Sandal index 0.0255 µg. cm^{-2}, the method was also the degree of accuracy and compatibility and return value 99-102% and the value of the relative deviation 1.20 ± The method was successfully applied to tetracycline in some pharmaceutical preparations.

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