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Determination of Doxycycline Hyclate in pharmaceutical samples via Oxidative Coupling Reaction using Spectrophotometric method

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ABSTRACT

This study involves the development of spectrophotometric method for determination of microgram a suggested mounts of doxycycline Hyclate (DCH) via oxidative coupling with 2,4,dinitrophenylhydrazine reagent (2,4-DNPH) in presence of potassium periodate(KIO_4) in the alkaline medium (pH 12.6) to form a red colored dye which soluble in water and gives the highest intensity of absorption at λmax 528 nm. The method has been obeyed Beer's law in the concentration range 5-60 $\mu g.ml^{-1}$with the molar absorptivity of 10771 L. mol^{-1}. cm^{-1}, Sandel Index value 0.0476 $\mu g.cm^{-2}$ with the relative standard deviation of the method does not exceed its value 1.043 % and the correlation coefficient 0.998. The proposed method was applied successfully for the determination of doxycycline hyclate in its Pharmaceutical samples (Capsule).

Introduction

Doxycycline Hyclate (DCH), is a yellow, crystalline powder, hygroscopic, molar mass is 512.9 gm/mol, chemically is Hydrochloride hemiethanol hemihydrate of (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide[1] Scheme1.

Doxycycline hyclate is a broad spectrum antibody derived from oxytetracycline, which produces its effect by inhibition of protein synthesis. Effective against Gram-positive and Gram-negative bacteria. Doxycycline is a tetracycline antibiotic. It works by slowing the growth of bacteria in the body[2].

Doxycycline is used to treat many different bacterial infections, such as urinary tract infections, acne, gonorrhea, chlamydia, periodontitis (gum disease) and others. Doxycycline is also used to treat blemishes, bumps, and acne-like lesions caused by rosacea. It will not treat facial redness caused by rosacea. Doxycycline may be used in combination with other medicines to treat certain amoeba infections[3].

Doxycycline hyclate was determined in various analytical methods including spectrophotometric methods[5-9],flow-injection[10-12],ion selective electrodes[13] ,potentiometric method [14] and HPLC[15-20], . this study describe development of spectrophotometric method determination of the microgram amounts of doxycycline hyclate (DCH).

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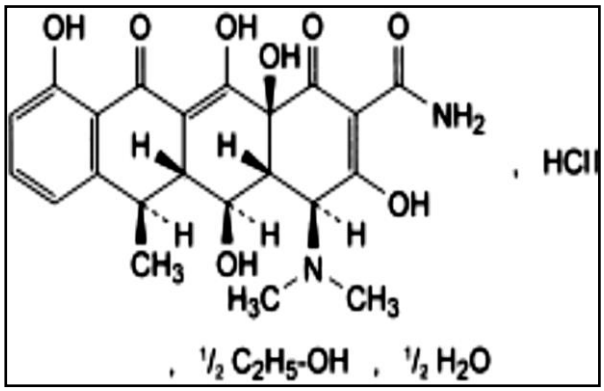


Scheme1: Chemical structure for doxycycline hyclate

Material and Methods

Apparatus

All spectral measurements have been carried out by using shimadzu UV-Visible spectrophotometer UV-160 with 1 cm quartz cells.

Chemicals

All chemicals used are equipped by companies SDI, BDH, Fluka.

Solution of the materials

1. Stock solution of DCH,1000 µg.ml^{-1}

An exactly 0.1g of DCH powdered was dissolved in 100 ml distilled water.

2. Standard solution of DCH, 500 µg.ml^{-1} (9.748 ×10^{-4} M)

To get a solution with a concentration of 500 µg.ml^{-1}, 50 ml of the standard solution of DCH (1000 µg.ml^{-1}) was diluted to 100 ml with distilled water. This solution was attended to use it during a period not exceeding one month

3. 2,4-dinitrophenylhydrazine solution (5×10^{-3}M)

The solution was attended by dissolving 0.0991g of 2,4-dinitro phenyl hydrazine in 3 ml of concentrated sulphuric acid and the volume was completed to 100 ml in a volumetric flask with distilled water.

4. Potassium periodate (KIO_4) solution (1×10^{-2}M)

A 0.2301 g of potassium periodate (KIO_4) was dissolved in a quantity of distilled water and the volume was completed to 100 ml in a volumetric flask with distilled water.

5. Sodium hydroxide solution (approximate, 1.0 M)

The solution was attended by dissolving 4.0000 g of NaOH in100 ml of distilled water.

6. Pharmaceutical preparations of DCH (500 µg.ml^{-1})

Pharmaceutical preparations of doxycycline (monodoks) present in capsules have been obtained from commercial source. Doxycycline hyclate 14-capsules (DEVA HOLDING A. S.), each capsule contain 100 mg of doxycycline**.**

The General Principle of the Method

A 1.5 ml of 500 µg/ml of DCH have been transferred into 25 ml volumetric flask, 1.0 ml of 1×10^{-2}M of potassium periodate solution (KIO_4) were added in the presence of 1.0 ml of 5 ×10^{-3}M of 2,4-dinitro phenyl hydrazine in alkline medium using 1.0 ml of 1.0 M NaOH shows the red color when adding the base solution and then completes the volume with distilled water in a volumetric flask, the resulting solution were measured at 528 nm versus blank reagent treated similarly. For the optimization of conditions and in all subsequent experiments were carried out on 30 µg ml^{-1} of DCH.

Results and Discussion

A typical absorption spectra were measured after reaching optimal conditions, then
measure the absorption of a red color product of DCH - 2,4-DNPH system against the blank reagent which was found to give the highest absorption at 528 nm while the blank reagent did not give any absorption in this region, as shown in Fig. (1), which shows the final absorption of a red colored product measured against blank reagent (A), distilled water (B) and blank reagent against distilled water (C)

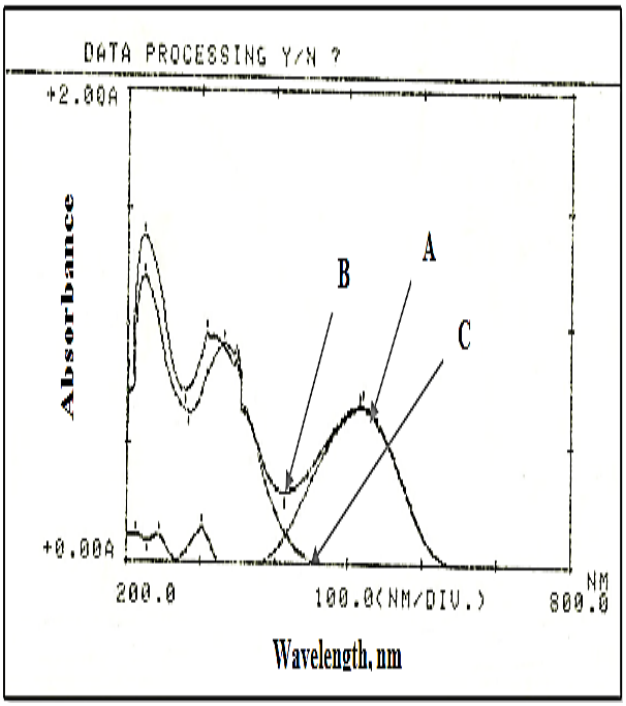


Fig.(1)Absorption spectrum of the colored product.

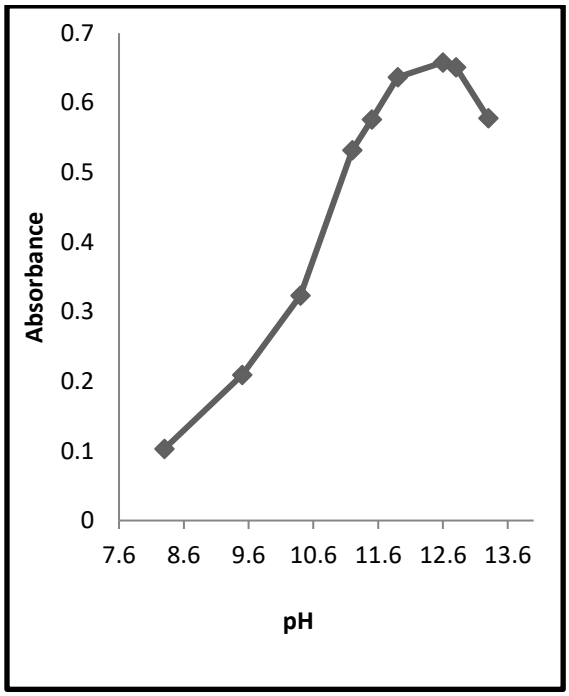


Fig.(2) Effect of pH

Study of The Optimization Experimental Conditions

To establish the optimum conditions, The various factors affecting absorption such as volumes of 2,4-DNPH, KIO_4 and alkaline medium, oxidation time and the stability of a red colored product have been studied at room temperature by using 1.5 ml of doxycycline Hyclate (500 µg/ml), 1.0 ml of 2,4-dinitro phenyl hydrazine (5×10^{-3}M) and 1.0 ml of potassium periodate (KIO_4) (1×10^{-2}M) in alkaline medium. Absorption spectra of a red colored product versus the blank reagent was shown maximum absorption at 528nm.

Effect of pH

After sodium hydroxide has been installed as the best base, The effect of pH was studied and found that the best of pH is in the range 11.5 - 12.9, so the pH of 12.6 was adopted in subsequent studies because it has given the highest absorption, the results as shown in Fig. (2).

Effect of Potassium Periodate (KIO_4) Amount

Increasing volumes (0.3-4.0 ml) of (KIO_4) (1×10^{-2}M) were studied. It was found that the volume of 2.0 ml of (KIO_4) is the optimum value that gives maximum absorption therefore it is considered in subsequent studies Fig.(3).

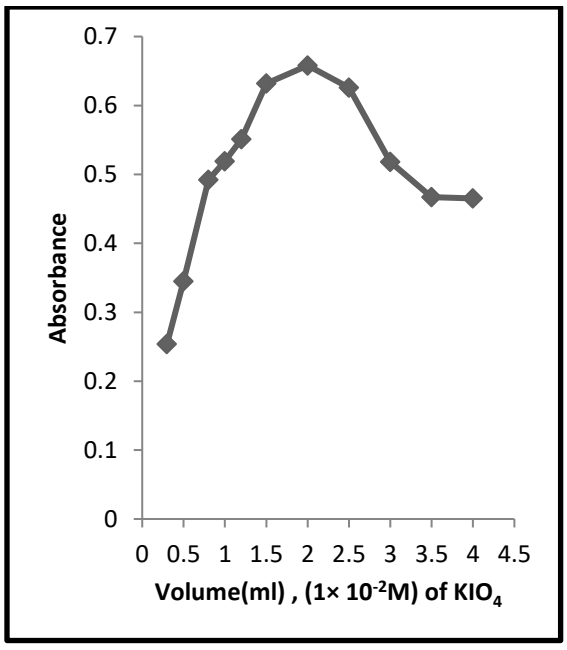


Fig.(3) Effect of KIO_4 amount on the color product absorption.

Effect of 2,4-DNPH Reagent Amount

Various volumes (0.3-4.0ml) of 2,4-dinitrophenylhydrazine (2,4-DNPH) reagent (5×10^{-3}M) were tested. The results shown in the Fig.(4) indicate that using 2.0 ml of coupling reagent gave the highest absorption of colored product at 528 nm therefore it is **chosen for further studies.**

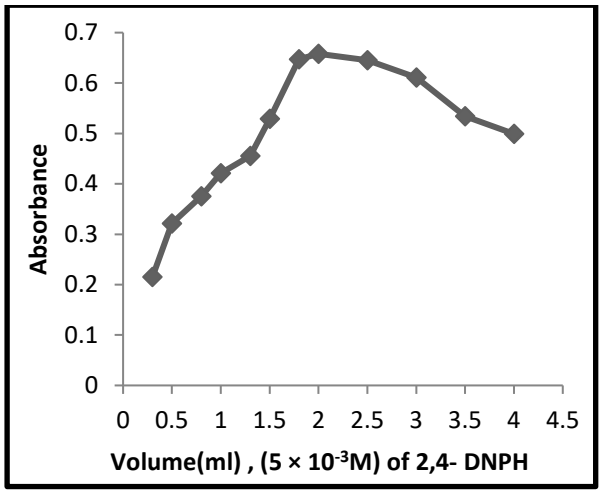


Fig.(4) Effect of 2,4-DNPH reagent amount on the color product absorption.

Effect of Reaction Time on the Absorbance

The effect of reaction time on absorption of color product was tested. The absorption value of the colored product reached its maximum and became was stable after 15 minutes for 90 minutes, therefore (15) minutes were chosen as optimum in the subsequent studies Fig.(5)

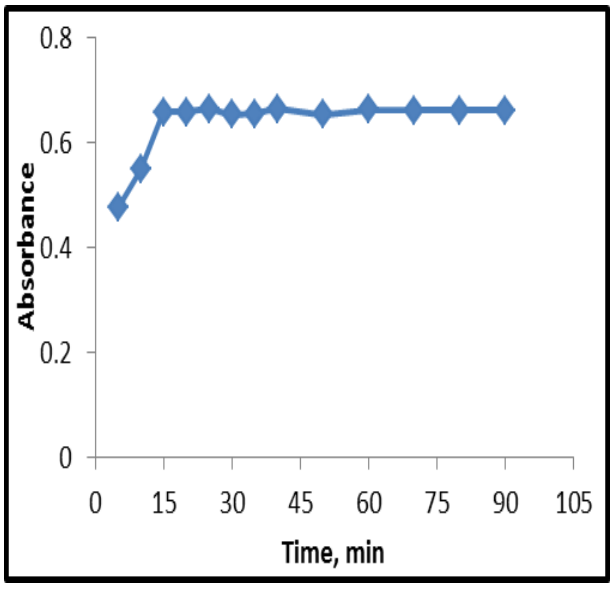


Fig. (5) Effect of reaction time on the absorbance.

Effect of Temperature

The effect of temperature (10-50°C) on the absorption of the resulting product was tested. It was found that the optimum temperature is (25°C) because the absorption value of the red product is stable at this temperature while higher temperatures show change color product and its absorbance becomes less respectively. Therefore, a temperature of 25°C was adopted in subsequent studies, Fig.(6).

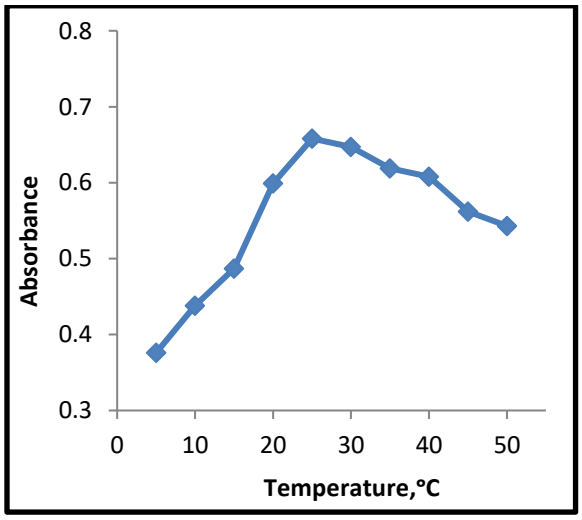


Fig.(6) Effect of temperature on the absorbance.

Effect of the type of solvents used

In this study many organic solvents such as acetone, methanol, ethanol, DMSO and water were used as a medium for reaction. The results showed that water was a good solvent for the reaction and gave an optimal absorption value at 528 nm as well as its availability , so it was used in subsequent studies Table (1).

Table (1) Effect of the organic solvents.

Solvent	Absorbance	λ_{max} ,nm
Water	0.658	528
Methanol	0.578	525
Ethanol	0.563	522
DMSO	0.468	523
Acetone	0.517	519

Analytical Construction Method for Calibration Curve

Through the results of previous experiments and after establish the optimal conditions to determine doxycycline Hyclate, standard calibration curve was constructed as follows:-

increasing volumes (0.25-3.0 ml of 500 µg/ml) of doxycycline Hyclate solution were added to a series of volumetric flasks (25 ml) containing 2.0 ml of 2,4-dinitro phenyl hydrazine(5×10^{-3} M) and 2.0 ml of potassium periodate (KIO_4) (1×10^{-2} M) in basic medium (pH 12.6) at 25°C then the solutions have been left for 15 minutes to complete the oxidation reaction. After completing the oxidation, the volumes are completed to the mark with distilled water . The absorbance of each colored product solution was measured at 528 nm versus the blank reagent. Fig.7 represents the standard calibration curve which follows the Bear law. The linear calibration curve is over the concentration range of 5-60 µg/ml, the molar absorptivity value is10771 liter / mol.cm and the Sandell'ssensitivity index 0.0476 µg.cm^{-2}.

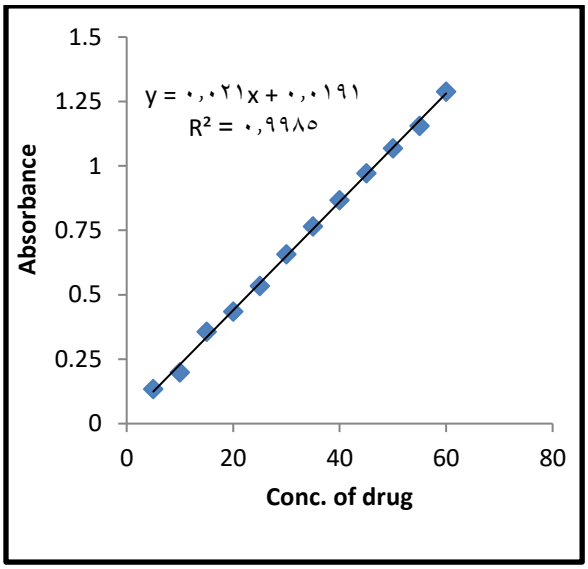


Fig.(7) Calibration curve for DCH determination

Accuracy and Precision of the Suggested Method

The accuracy and precision of the suggested method was calculated under the adopted optimum conditions by taking two different concentrations of doxycycline hyclate within the limits Beer's law. Of the recovery rate (99.80 %) and the relative standard deviation (0.856 - 1.043%) showed that the method was high accuracy and satisfactory accuracy, as shown in Table (2).

Table (2) Results of accuracy and its precision.

Conc. Of DCH (µg/ml)	RSD%	Average recovery%	Recovery* %
10	1.043	99.80	99.87
15	0.856		99.73

* Average of five determinations

Nature of the resulting product

To determine the nature of the red product and to find the relationship between the doxycycline Hyclate and 2,4-dinitro phenyl hydrazine , the Job's and mole ratio methods were applied and in both methods, the concentration of each solution(drug and reagent) is equal to 5×10^{-3}M. (Fig.8) illustrates that the ratio of DCH:2,4-DNPH is 1:2.

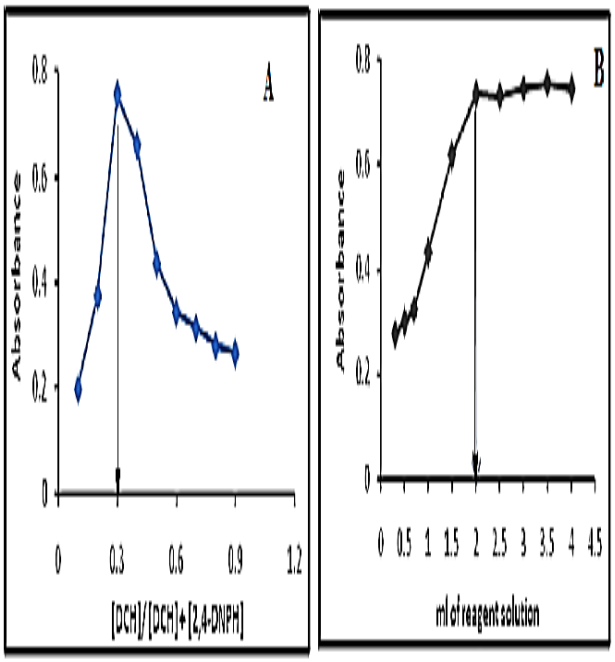
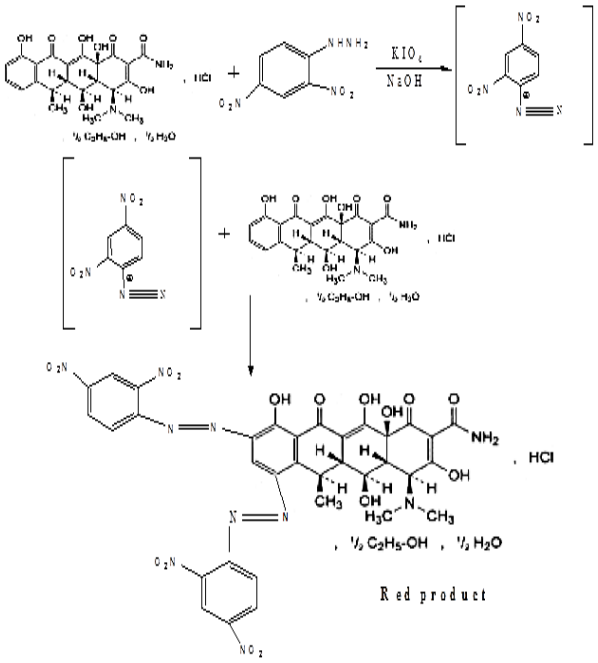


Fig. (8) Job's method(A) and mole ratio method(B) of the resulting product DCH- 2,4-DNPH.

The suggested equation between DCH and 2,4-DNPH is represented in Scheme 2**.**



Scheme 2: the proposed structure of the formed product

Applied Part:

Determination of dch in Its Pharmaceutical Samples by Standard Additions Method

This method was performed for the purpose of establish the efficiency and accuracy of the proposed method and proving that the method is free from interference, the standard addition method was applied in the determination of doxycycline Hyclate (DCH) in monodoks tablets.

The method involved adding different amounts (0.25,0.5) ml of pharmaceutical solution (500 µg /ml) , to a series of volumetric flasks (25ml) and growing volumes (0.25- 2.25 ml) of the standard doxycycline Hyclate solution (500 µg /ml) were while leaving the seventh flask in each series without adding. The above solutions were treated in the same way as the calibration curve. The absorbances of all solutions was measured at 528 nm. Results are shown in Table (3) and Fig.(9

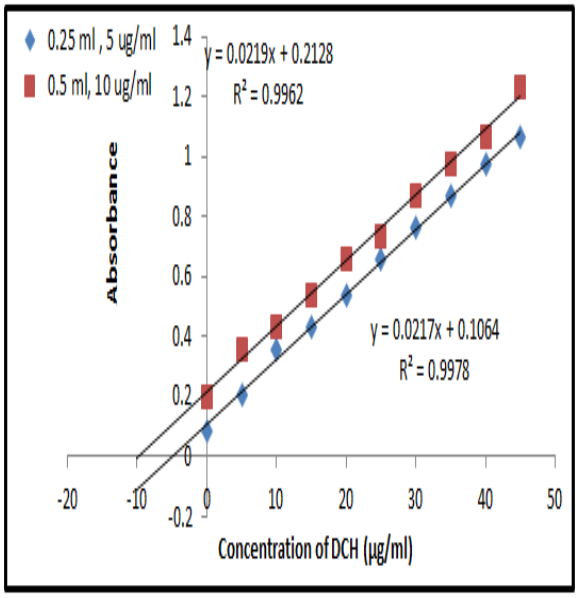


Fig. (9) diagram of standard additions method of the determination of DCH in Monodoks tablets.

Table (3) Results of the analysis of pharmaceutical tablets using standard additions method.

pharmaceutical	DCH present µg/ml	DCH measured µg/ml	Recovery*, %
monodoks tablets	5.000	5.048	100.96
	10.000	10.095	100.95

The results of Table (3) above explaine that the method of standard additions is completely consistent with the proposed method within the

acceptable error range, indicating that the method is satisfactory, does not contain interferences.

Conclusions

Analysis results showed that the proposed method is easy, sensitive and repeatable to determine DCH. This method does not require control of temperature, nor the use of different organic solvents, or extraction and can be successfully applied to determine DCH in pharmaceutical.

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تقدير الدوكسي سايكلين هيكلات في العينات الصيدلانية عن طريق تفاعل الاقتران التاكسدي باستخدام طريقة طيفية

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الخلاصة:

هذه الدراسة تتضمن تطوير طريقة طيفية لتقدير كميات مايكروغرامية من عقار الدوكسي سايكلين هيكلات بالاقتران التأكسدي مع كاشف 2،4- ثنائي نيتروفنيل هيدرازين بوجود بيريودات البوتاسيوم في الوسط قاعدي ذي الدالة الحامضية 12.6 لتكوين صبغة حمراء اللون ذائبة في الماء وتعطي أعلى امتصاص عند الطول الموجي 528 نانوميتر . وتتبع الطريقة قانون بير في مدى التراكيز تراوح بين 5 – 60 مايكروغرام.مل$^{-1}$ من الدوكسي سايكلين هيكلات وبلغت قيمة الامتصاصية المولارية 10771 لتر . مول$^{-1}$.سم$^{-1}$، ودلالة ساندل 0.0476 مايكروغرام. سم$^{-2}$ مع انحراف قياسي نسبي لاتتجاوز قيمته 1.043 % وبمعامل ارتباط 0.998 . الطريقة المقترحة تمّ تطبيقها بنجاح على بعض النماذج الصيدلانية الحاوية على الدوكسي سايكلين هيكلات (كبسول).

الكلمات المفتاحية: الطريقة الطيفية، الدوكسي سايكلين هيكلات ، 2،4 – ثنائي نيتروفنيل هيدرازين