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RESEARCH ARTICLE

SPECTROPHOTOMETRIC DETERMINATION OF DOXYCYCLINE HYCLATE USING OXIDATIVE COUPLING REACTION

***,[1]Kamal M. Mahmoud and [2]Maadh T. Abdurahman**

[1]Department of Chemistry Erbil, College of Science, Salahaddin University- Erbil
[2]Department of Chemistry, College of Science, Tikrit University

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ABSTRACT

A simple spectrophotometric technique has been developed for the determination Doxycycline hyclate (DCH) in pure and pharmaceutical formulations. The proposed method is based on the oxidative coupling reaction using sodium periodate and hydrazine dihydrochloride (HZD) producing an orange colored complex with maximum absorption at 420 nm. The Beer's law is obeyed over the concentration range of (3.0 –72.0) µg ml^{-1}, while the detection limit and quantification limit are 0.0631 and 0.1912 µg ml^{-1} respectively, with a correlation coefficient (r) of 0.9999 and a molar absorptivity of 3.0562×10^3 L $mol^{-1}cm^{-1}$ with Sandell's sensitivity index of 0.1678 µg cm^{-2}. The precision and accuracy of the method are checked by calculating relative standard deviation (RSD) (≤ 2.27%) and average recovery (98.913 %). The value of the stability constant has been calculated and found to be 1.89×10^8 L mol^{-1}. Different experimental parameters affecting the development and stability of the formed color product are carefully studied and optimized. Possible interferences that related to the determination of (DCH) in pharmaceutical capsules are studied and the results showed that the method is successfully applied for determination of DCH in pharmaceutical formulation.

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INTRODUCTION

Chemically, doxycycline hyclate (Fig.1) $C_{22}H_{24}N_2O_8$.HCl, ½C_2H_5OH, ½H2O with molecular mass 512.94 g/mol, is the hydrochloride hemiethanol hemihydrate of doxycycline (Dhal *et al*., 2015). The drug has a yellow color hygroscopic powder in physical appearance and must be stored in an airtight container protected from light (British Pharmacopeia, 2013). Doxycycline was discovered in 1967 and has undergone extensive investigation, both for its antimicrobial properties as well as the effects on the physiology of higher organisms (Dennis *et al*., 2001). Doxycycline is a broad-spectrum tetracycline antibiotic. Although it shares many properties with other tetracyclines, it has great efficacy and more advantageous pharmacokinetics. It is the tetracycline of choice if a tetracycline is required for a patient with renal failure (Rinaldi, 2014) which has a good activity against Gram-positive and Gram-negative bacteria (Sunarić *et al*., 2013). It has been used for the treatment of infectious diseases caused by rickettsiae, mycoplasmas and chlamydiae (Harrison *et al*., 1975; Milingos *et al*., 1983; Smelov *et al*., 2004; Clad and Krause, 2007; Cunha, 2012), and used in triple therapy along with tinidazole and proton pump inhibitor in the treatment of peptic ulcer (Drugs, 2002). There have been several analytical methods reported for their determination, both in pharmaceutical formulations and biological samples (fluids and tissues) including spectrophotometry (Al-Momani and Kanan, 2008; Ramesh *et al*., 2011; Pourmoslemi *et al*., 2016; Saber and Amin, 2010; Ramesh *et al*., 2010; Rokayia and Alaa, 2015; Lotfy *et al*., 2016), spectrofluorimetry (Attia *et al*., 2011), electrochemical methods (Issa *et al*., 2013; Issa *et al*., 2016; Ryasenskii *et al*., 2016), TLC (Selvadurai *et al*., 2012), HPLC (Bie *et al*., 2012; Injac *et al*., 2007), capillary electrophoresis (Gil *et al*., 2000; Mamani *et al*., 2006), and FIA methods (Zhang *et al*., 1998; Townshend *et al*., 2005; Wangfuengkanagul *et al*., 2004; Palaharn *et al*., 2003; Liawruangrath *et al*., 2006; Rufino *et al*., 2009). The main goal in the present work and due to the importance of DCH and it's a broad uses, a simple and sensitive spectrophotometric determination for pure and pharmaceutical capsules is involved, which based on the oxidation reaction of the drug with sodium periodate and then coupling with HZD in basic medium to produce an orange color complex at a λ_{max} = 420 nm.

****Corresponding author: Kamal M. Mahmoud,***
Department of Chemistry Erbil, College of Science, Salahaddin University- Erbil.

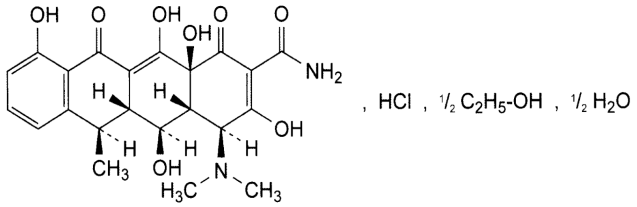

Fig.1. Molecular structure of DCH

MATERIALS AND METHODS

Apparatus

Spectral measurements were carried out on a (CECIL CE 3021- England) UV-Vis digital spectrophotometer, while other measurements were carried out with a (JENWAY 7305 -UK) UV-Vis spectrophotometer equipped with quartz cell of 1.0 cm path length. A digital analytical balance (METTLER TOLEDO AB204 – S), pH meter (PHILIPS pw9421), magnetic stirrer hotplate (Gallenkamp – 400), and water bath (Labacon LWB-104) are used through this investigation.

Reagents and solutions

All chemicals and reagents are used of analytical grade. DCH is provided by Samara Drug Industry (SDI) - Iraq. Distilled water is used in all preparations.

DCH solution (1000 µg ml^{-1})

0.1000 g of DCH is dissolved in amount of distilled water and then made up to 100 ml in a volumetric flask. The working solution of (300 µg ml^{-1}) is prepared by simple dilution of stock solution and stored in an amber glass bottle in a refrigerator.

HZD reagent solution (1.0×10^{-2} M)

0.1562 g of HZD is dissolved in a small volume of distilled water, and then diluted to 100 ml in volumetric flask.

Sodium periodate solution (0.2 M)

This solution is prepared by dissolving 4.2778 g of sodium periodate in distilled water then completed to 100 ml with distilled water.

Sodium hydroxide solution (1.0 M)

4.0 g of sodium hydroxide has been weighted and dissolved in distilled water then completed to 100 ml volumetric flask.

Sample solution of capsules contain DCH (300 µg ml^{-1})

Sample from three different companies are taken and an accurate weight (20 powdered capsules of 100 mg DCH), equivalent to 30 mg DCH of the pure drug is dissolved in distilled water and transferred into a 100 ml volumetric flask (to prepare 300 µg ml^{-1} of drug) and completed to the mark with distilled water. The flask with its contents is shacked well and filtered by filter paper (589/4 S&S Rund filter 150 mm), and stored in an amber glass in a refrigerator.

RESULTS AND DISCUSSION

Preliminary investigations

To a 25 ml volumetric flask containing 2.0 ml standard DCH (300 µg ml^{-1}) added 1.0 ml of (0.2 M) sodium periodate followed by adding 1.0 ml (1.0×10^{-2} M) of hydrazine dihydrochloride in alkaline medium using 0.5 ml (1.0 M) of sodium hydroxide, then diluted to the mark with distilled water. The blank solution is prepared in the same way except DCH. The absorption spectrum of the colored complex (orange) against blank solution shows maximum absorption at 420 nm.

Selection of the oxidizing agent and its concentration

Effect of different types of oxidizing agent1.0 ml of (2.0×10^{-1} M) (sodium periodate, ammonium ceric sulphate, potassium periodate, potassium bromate, ammonium persulphate and sodium selenite anhydrous) in volumetric flask (25 ml) are tested. Results indicate that sodium periodate gives the best absorption maxima, therefore it is used in subsequent experiments. The effect of sodium periodate concentration in the range of (0.06 - 0.7 M) on the sensitivity of the reaction has been studied. It is found that 0.4 M of sodium periodate gives the optimum absorbance.

Selection of the coupling reagent and its concentration

Different coupling reagents are tested depending on the highest value of $\Delta\lambda_{max}$ (λ_{max} of the complex - λ_{max} of the blank). It found that (HZD) is the best coupling reagent due to gives the highest $\Delta\lambda_{max}$ =146 nm (λ_{max} of sample against blank = 420 nm and λ_{max} of blank against water = 274 nm). The effect of HZD concentration in the range of (0.001- 0.06 M) solution has been studied to get a constant amount of DCH (2.0 ml, 300 µg ml^{-1}). It is observed that the maximum color intensity is obtained with 0.01 M, after further increase in concentration, results nearly shows a constant absorbance.

Effect of sodium hydroxide concentration

Different volumes (0.25 - 2.0 ml) of sodium hydroxide (1.0 M) has been added to a series of volumetric flasks (25 ml) containing 2.0 ml of DCH solution (300 µg ml^{-1}) and 2.0 ml of sodium periodate solution (0.2 M), then added 0.5 ml of HZD solution (1.0×10^{-2} M) and completed the volume to mark with distilled water. The best amount of sodium hydroxide is 0.5 ml with the highest absorbance and the pH = 10.75.

Order of addition

The effect of order of addition on the sensitivity of the method has been investigated. The results obtained that the order of addition (Drug sample (DCH) + sodium periodate + (HZD) + sodium hydroxide) give the best sensitivity and more intense color than other probabilities, therefore this order is selected in all subsequent experiments.

Effect of oxidation time and temperature

The oxidation of the drug (DCH) with sodium periodate is tested during different periods (1:00 - 20:00 min) then adding HZD as coupling reagent in basic medium with sodium hydroxide. It has seen that the best complexation occurs

between (5:00 - 10:00 min). Therefore 5:00 min will select for the subsequent experiments because at this time a good stability in the absorbance will occur. It contrast, the effect of temperature is also studied between (5 – 50 °C). It found that the highest absorbance can be recorded in the range of (15 - 30 °C), so it considered 25°C (room temperature) for the subsequent experiments.

Stability of the colored product

The stability of the formed product is tested through different time (0 -70 min). It is found that a high absorbance will be obtained at 5:00 min, and the complex remains stable up to 65:00 min.

Recommended procedure

To a 25.0 ml calibrated flask which contain 2.0 ml of 300 µg ml^{-1} of the working standard solution of DCH (equivalent to 24 µg ml^{-1}) added 2.0 ml of sodium periodate solution (0.2 M) and 0.5 ml of HZD (1.0×10^{-2} M), then after 5:00 min added 0.5 ml sodium hydroxide and complete with distilled water. The blank solution is prepared in the same manner in the absence of DCH. The absorbance is measured against a reagent blank after 5:00 min, at 420 nm. The unknown concentration derived from the calibration graph or computed from the regression equation derived using Beer's law.

Calibration Graph & Statistical Data

Under the optimum experimental conditions and applying the recommended procedure, a calibration graph is obtained by taking different volume of DCH (0.1 - 10.0 ml, 300 µg ml^{-1}). Fig. (2) shows a straight line of the calibration graph which obeyed the Beer's law in the concentration range of (3.0 - 72.0 µg ml^{-1}) of DCH with a correlation coefficient of (0.9999) and detection limit of 0.0631 µg ml^{-1}. The molar absorptivity and Sandell index are found to be 3.0562×10^{3} L $mol^{-1}cm^{-1}$ and 0.1678 µg cm^{-2} respectively.

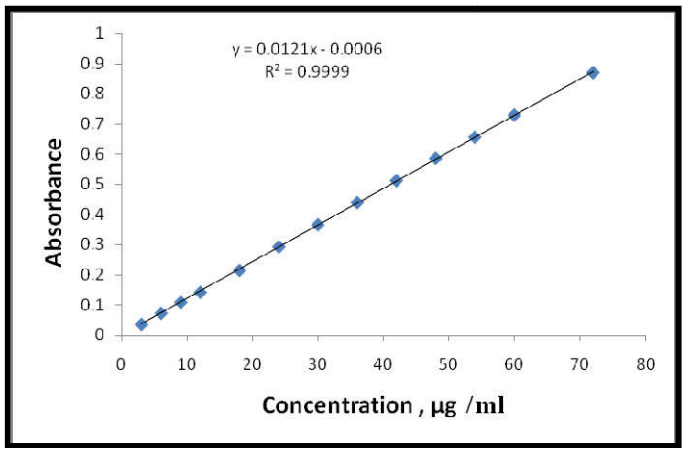

Fig.2. Calibration curve for determination of DCH by oxidative coupling with HZD reagent

Accuracy and Precision

In this study the absorbencies for three different concentrations (12, 30 & 60 µg ml^{-1}) of DCH are measured for ten replicated times (n = 10). The results are shown in Table (1), which indicates a good accuracy and precision, because the average recovery approaches 98.913 % and RSD% ≤ 2.27%.

Table 1. Results of accuracy and precision

Concentrations of DCH (µg ml^{-1})	Recovery % *	Average Recovery %	RSD %
12	98.32	98.913	2.27
30	99.45		1.36
60	98.97		1.31

* Average of ten determinations (n=10)

The nature of the colored product and its mechanism

The stoichiometric relation between oxidized DCH and HZD has been studied employing the molar ratio method. The results which shown in Fig. (3) indicate that the reaction between the oxidized drug and the reagent.

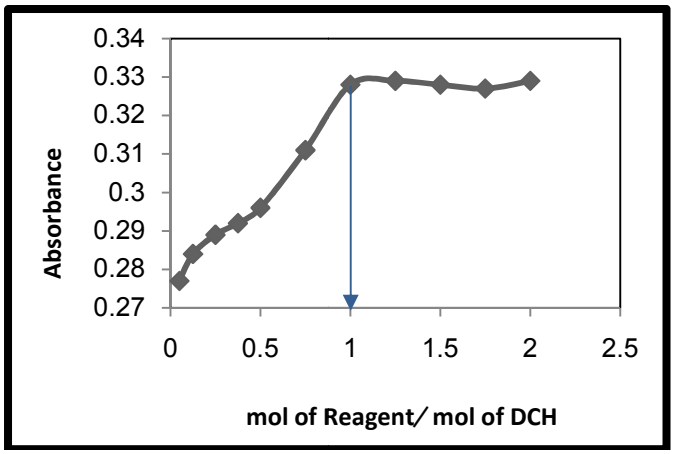

Fig.3. Molar ratio for the product formed by oxidative Coupling of DCH with HZD reagent

The proposed mechanism for the reaction can be written as shown in Fig.(4). The stability constant of complex has been calculated (Maadh T. Abdurhman and Kamal M. Mahmoud, (2016) and found as 1.89×10^{8} L mol^{-1}, which shows high stability.

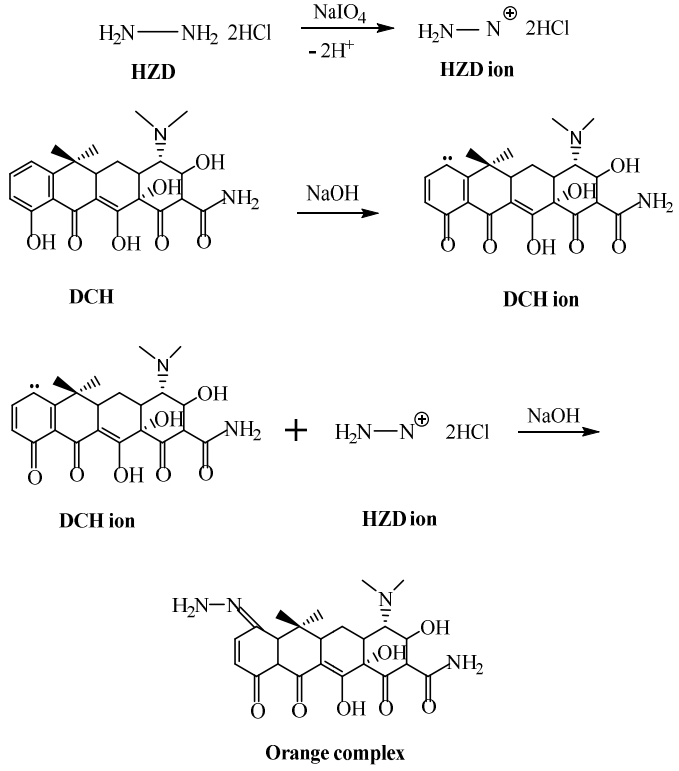

Fig.4. The proposed mechanism for the reaction of DCH & HZD

Table 2. Effect of interference

Foreign Compound	Recovery (%) of 24 μg ml^{-1} of DCH per Foreign compound added (μg ml^{-1})		
	120	**240**	360
starch	99.05	100.22	100.08
fructose	101.29	99.19	99.62
maltose	99.45	100.23	99.03
glucose	99.91	100.87	101.09

Table 3. Direct method for determination of DCH Capsules in pharmaceutical formulations

Pharmaceutical formulations capsules	DCH present (μg ml^{-1})	DCH measured (μg ml^{-1})	Recovery%	RSD%
Doxycycline 100 mg (actavis) UK	9.0	8.944	99.816	0.409
	33.0	33.138	100.098	0.135
	69.0	68.944	99.905	0.232
Monodoks 100 mg (DEVA) TYRKEY	9.0	8.977	100.364	0.499
	33.0	32.954	99.653	0.589
	69.0	69.185	100.059	0.106
Doxycycline Atb 100 mg (Antibiotice) CYPRUS	9.0	8.862	99.815	0.413
	33.0	33.090	100.495	0.974
	69.0	68.702	99.238	0.155

Table 4. Comparison of the proposed method with literature methods

Analytical parameter	Literature (Al-Abachi and Al-Nedawi, 2015) method	Literature (Al-Abachi and Al-Nedawi, 2015) method	Present method
Reagent	metchlopramide	o-nitroanline	Hydrazine dihydrochloride
Beer's law range (μg ml^{-1})	1.0 – 52	0.4-52	3.0- 72
L.O.D. (μg ml^{-1})	0.2352	0.284	0.0631
L.O.Q. (μg ml^{-1})	----------	----------	0.1912
Molar absorptivity (L mol^{-1}cm^{-1})	14.427 ×10^3	14.515×10^3	3.0562× 10^3
Sandel Index (μg cm^{-2})	0.0234	0.035	0.1678
pH	--------	--------	10.75
Temperature °C	25	25	15 – 30
λ_{max} (nm)	436	448	420
Recovery (%)	102.452	99.613	98.913
RSD (%)	1.876	1.528	≤ 2.27
Solvent	Water	Water	Water
stability constant (L mol^{-1})	3.014×10^5	-----	1.89×10^8
Color of the dye	yellow–orange	yellow–orange	orange
Nature of the dye	1 : 1	1 : 2	**1 : 1**
Pharmaceutical preparation	capsules	capsules	capsules

Effect of interferences

To improve the efficiency and selectivity of the proposed method for determination of DCH, the effect of some foreign substances (starch, fructose, maltose and glucose) have been studied, which normally are present in the dosage forms of pharmaceutical preparation. This study is performed by comparing the absorbance obtained when DCH (24 μg ml^{-1}) present alone and in the presence of different concentration of interferences (120 -360 μg ml^{-1}) reach to (5 -15) times of the amount of DCH. The results found that a substance is considered not to interfere if the variation in the absorbance of pure DCH & DCH with interferences equal or less than ± 5.0 % of the recoveries. Table (2) illustrated the recoveries after addition of additives by (5 - 15) fold excess the amount of DCH.

Applications

The present method has been applied for the determination of DCH in different pharmaceutical formulations (capsules) from different companies, which converted to solutions as mentioned in section (2.2.5). It was transferred different volumes (0.75, 2.75, 5.75) ml of a pharmaceutical formulations solution (300 μg ml^{-1}) to volumetric flasks (25ml) then the concentrations become (9.0, 33.0, 69.0) μg ml^{-1}. The optimum conditions have been applied and treated as in the concentration of calibration curve. The absorbance has been measured at 420 nm for five times (n=5). Then it was calculated the recovery and RSD as show in Table (3).

Comparison of the proposed method with literature methods

Table (4) shows the comparison between some of the analytical variables for the present method with that of other spectrophotometric methods in the literature. It is found that the linear range of the Beer′s law for the present method is more longer with lower detection limit, in addition the produce complex has higher stability in a wide range of temperature (15 – 30 °C) which makes the applications is suitable in different temperatures and seasons.

Conclusion

This method is considered to be simple, rapid, sensitive, inexpensive and accurate for the determination of DCH in pure form and pharmaceutical formulations. The method does not require the removal of excipients, any chemical sample pretreatment, solvent extraction step, and expensive reagents and solvents.

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