Spectrophotometric Determination of Doxycycline Hyclate in Pure and Capsule using Diazotization Reaction

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Abstract

A sensitive spectrophotometric method has been developed for the determination of Doxycycline hyclate (DCH) as pure and capsule forms. This method is based on diazotization of primary amine group of benzocaine with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then reacted with DCH in sodium hydroxide medium, to form yellow – orange Azo dye. Beer's law is obeyed in the concentration rang 16-34mg. ml^{-1} at 480 nm with detection limit LOD and molar absorptivity Є were found to be 0.418 $mg.ml^{-1}$ and $2.214\times 10^{+4}$ $L.mol^{-1}.cm^{-1}$, respectively.

Keywords: Doxycycline hyclate, benzocaine, spectrophotometric, Diazotization.

الخلاصة

استخدمت طريقة طيفية بسيطة وحساسة لتقدير الدوكسي سايكلين هايكلات في الصيغة النقية للدواء والكبسول. وتعتمد هذه الطريقة على ازدواج كاشف الامين الاولي في البنزوكائين مع نتريت الصوديوم في وسط حامضي لتكوين ملح الديزانيوم، هذا الملح يتفاعل بعد ذالك مع الدوكسي سايكلين بوجود هيدروكسيد الصوديوم كوسط قاعدي لانتاج صبغة (صفراء– برتقالية) اللون. وكان خطي لقانون لامبرت بير (34-16) مايكروغرام. مل$^{-1}$ مع حدود الكشف ومعامل الامتصاص المولاري0.418 مايكروغرام. مل $^{-1}$و$2.214\times10^{+4}$ لتر. مول$^{-1}$.سم $^{-1}$ على التوالي. واظهرت الفعالية البايولوجية استجابة عالية للبكتريا الموجبة تجاه الصبغة المتكونة.

INTRODUCTION

Doxycycline hyclate (DCH), ($C_{22}H_{24}N_2O_8Hcl.$ ½ $C_2H_5OH.$ ½H_2O), molecular mass 512.94g/mol [1], it is a tetracycline derivative which is Bacterio static with a broad spectrum of antimicrobial efficiency including many aerobic and anaerobic gram- positive and negative pathogenic bacteria and some Protozoa [2].

Some analytical methods have been used for the assessment of doxycyclinehyclate in biological and pharmaceutical samples, these include High performance liquid chromatography [3] [4], spectrophotometric [5] [6] electrochemiluminescent, [7] voltammetric [8], electro capillary chromatography [9] and titrimetric methods [10].

In existing study Doxycycline hyclate was assessment spectrophotometry in pharmaceutical formulations based on the diazotization of Benzocaine by sodium nitrite in acidic medium at (0-5) c^0 followed by coupling with Doxycycline hyclate in alkali medium.

MATERIALS AND METHODS

Equipment

All spectral and absorbance measurements were carried out on Shimadzu UV- visible digital double beam (VARIAN UV-visible) with 1cm path quartz cells.

Reagents

Analytical reagents grade chemicals and distilled water were applied thoroughly:-

1- Doxycycline hyclate (DCH) stock standard solution ($1000\mu g.ml^{-1}$) ($1.95\times 10^{-4} mol.L^{-1}$) was prepared by dissolving (0.1gm) of (DCH) in distilled water, and completed to 100ml distilled water.

2- Hydrochloric acid (BDH- England) (1M):- was prepared by (8.1ml) of (11.64M) of

concentrated HCl with distilled water in 100ml volumetric DCH (1000µg.ml^{-1}) and then completed to 100ml in distilled water.

3-DCH (100ppm)(1.95×10^{-5} mol. L^{-1}): was prepared by take (10ml) from DCH (1000µg. ml^{-1}) and then completed to 100ml in distilled water.

4- diazonium salt reagent (1.5×10^{-2}M): was prepared by dissolving (0.125gm) Benzocaine(BDH –England) in 5ml 1M HCl, then in another beaker dissolving (0.05175gm) $NaNO_2$ in 5ml distilled water, and placed both beakers in ice-bath for 10 min then transfer $NaNO_2$ solution by dropper to Benzocaine solution and shake well, after 5min, the volume complete volume to 50 ml with distilled water, this diazanium salt solution must still in ice bath and prepared daily, and concentration of (1.5 x 10^{-2}M) $NaNO_2$ in equimolar solution(1.5x10^{-2}M) Benzocaine, so we do not need to addition of sulfamic acid in this method to get rid of excess $NaNO_2$,

5- DCH (1.5 X10^{-2}M): Was prepared by dissolving 0.769 gm DCH in 100ml distilled water

6-Sodium hydroxide (0.1M): Was prepared by dissolving 0.4gm NaOH in 100ml distilled water

The Procedure

Calibration curve:-

An aliquot of sample solution containing 4-8.5 ml of (100µg.ml^{-1}) DCH is transferred into a series of 25 ml calibration flask. To each flask, 3 ml of 0.1 M NaOH solution and 4 ml of diazanium salt reagent are added. Then diluted the contents to the mark with distilled water. After 10 min. the absorbance of the azo dye was measured at 480 nm against reagent blank.

Method for the assay of pharmaceutical preparations:-

An accurately weight amount of 20 powder DCH capsule (100 mg of DCH pre each capsule) equivalent to 100 mg of pure drug was transfer into 100 ml volumetric flask then completed to the mark with distilled water to obtain 1000 mg.ml^{-1} of DCH.

RESULTS AND DISCUSSION

Absorption spectra

An orange–yellow colored chromophore was formed by coupling of diazotized (DCH) with Benzocaine in alkaline medium. This Azo(orange-yellow) dye was gained with a maximum absorbance at (480nm) against reagent blank as shown in Figure 4.

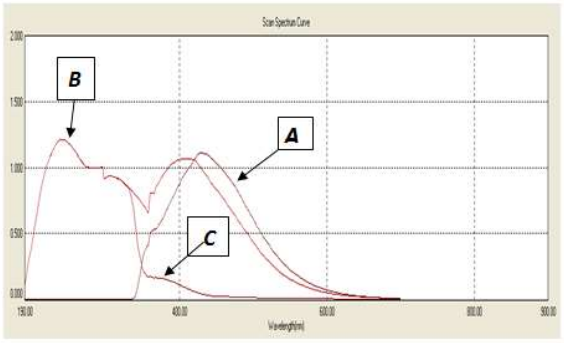


Figure 4:Absorbance spectra of azo dye colored product measured against blank.,B= Azo dye product against distilled water. C= The blank against distilled water.

Study of the optimum reaction conditions

Figure 5 shows the effect of various parameters on the absorbance intensity was studied of the Azo dye formed.

Effect of hydrochloric acid

Different amount of (0.5-2ml) of 1M HCL solution has been studied. A 0.2 ml of 1M HCL were enough to obtain the maximum absorbance.

Effect of diazanium salt reagent

The effect of diazanium salt reagent (1.5 x 10^{-2} mol.L^{-1}), volume (1-6 ml) on the intensity of the absorbance was study and 4ml was found to be optimum.

Effect of NaOH

The effect of NaOH was measure and was found (3ml) 0.1M NaOH is the optimum amount due to the maximum absorbance.

Effect of reaction time

In Figure 6 show the final color of the sample, the color intensity reached a maximum after 10 min and the azo dye was sTable for 2hr.

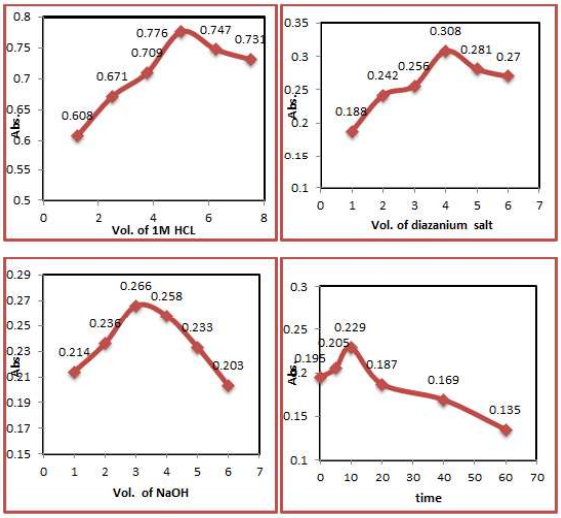


Figure 5:Optimum condition for determination of DCH.

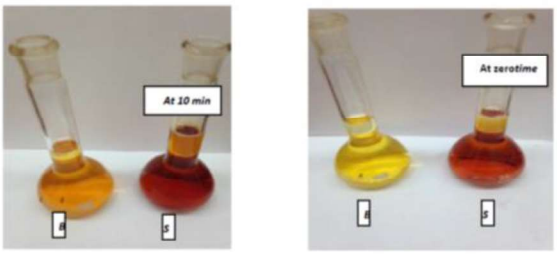


Figure 6:final color of the sample(S) and blank(B) at zero and 10 min.

Study of the dye

The structure of the created complex had been established using the Job method which applied by placing 1 to 5ml of solution of (1.5 x 10-2M) DCH drug into series of 25ml volumetric flask, this was followed by placing 3ml (0.1M) NaOH into each volumetric flask and 5 to 1 ml of (1.5 x 10-2M) diazanium salt reagent, the solutions were diluted to the mark with distilled water, let to stand 10 min, the results in Figure 7 indicate that the azo dye were formed in the ratio 2:1 (benzocaine{R}:DCH{D}).

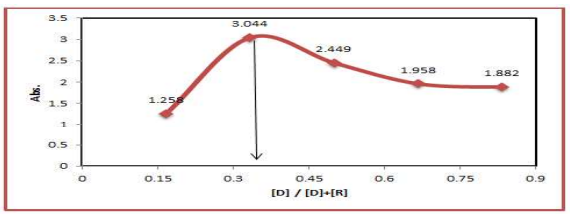
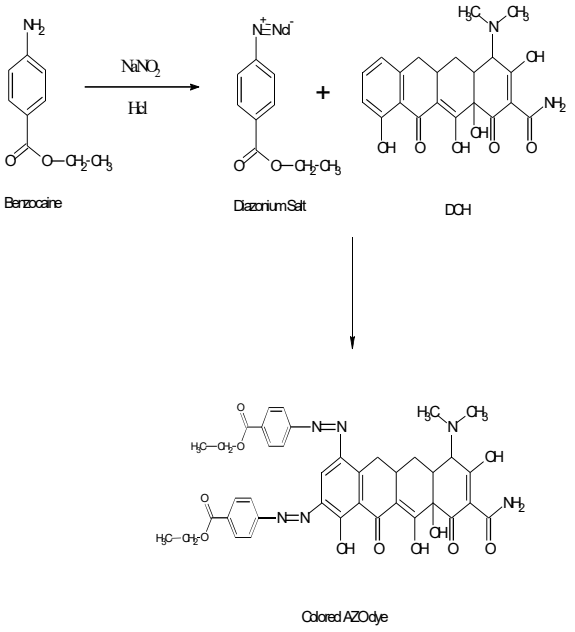


Figure 7:Job's plot for diazotized benzocaine coupled with DCH.

The possible reaction path might be written as follow:



Apparent stability constant was calculated by using Vargas method [11]with the following Equation k=a-(ΔA/ϵ) /nn (ΔA/ϵ)n+1, a = DCH drug conc. (1.5 × 10-2 mol. L-1) in stoichiometric, ΔA=DCH drug absorbance in reagent excess minus the DCH drugstoichiometric, ϵ=2.214×10+4 (L. mol-1. cm-1)stoichiometric method (2).

The stability constant is found to be 2.33×10-4 (L. mol-1), indicating that the product is sTable.

Calibration curve and sensitivity

Under the optimized experiment condition, the calibration graph was gained by the series of standard solution for DCH were constructed Figure 8 , the linearity, regression Equation (y), correlation of determination (r2), slope (b), intercept (a) and different parameters of the analytical achievement of the proposed method are brief in

Table 2.

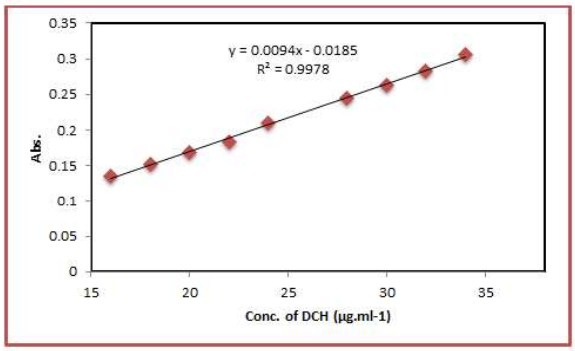


Figure 8:Calibration graph for estimation of DCH

Table 2:The analytical values of parameters and statistical treatment for the calibration curve.

Parameter	Value
Linearity µg.ml-1	16 - 34
Regression Equation (y)	Y= 0.009 × - 0.018
Correlation of determination (r2)	0.997
Slope (b) ml. µg-1	9 × 10-3
Intercept (a)	18 × 10-3
Conf. limit for slope b± tsb	0.009 ± 4.149
Conf. limit for Intercept a ± tsa	0.018 ± 54.67
Molar absorptivity Ɛ(L. mol-1. Cm-1)	2.214×10+4
Sandell's sensitivity ʃ (µg. cm-1)	23.16 × 10-3
Limit of detection LOD (µg. ml-1)	0.418
Limit of quantification LOQ(µg. ml-1)	1.266

Accuracy and precision

At three different concentration of DCH was determined, the accuracy and precision of the calibration curve (see Table 3) indicated a satisfactory of precision and accuracy.

Table 3: Accuracy and precision for present method

A mount of DCH (µg. ml-1) Taken	Found	×100 Erel.%=$\frac{x-xo}{xo}$	Rec* % (100 +E%)
20	20.221	-1.105	101.105
25	24.870	+0.52	99.480
30	30.686	-0.686	102.286

Average of five determination, x= measured value (found), xo= true value (taken).

Application of the method

The suggested method had been applied to the determination of DCH in capsule (Modomycin), and obtain a good recovery and relative error show in Table 4.

Table 4: Application of the suggest for determination of DCH in pharmaceutical capsule

Pharmaceutical capsule	Conc. of DCH µg. ml-1		E*rel %	Rec.* %
Modomycin (DCH) capsule 100mg Limassol kyprus	taken	found		
	20	20.433	+ 2.165	102.165
	25	25.624	+ 2.496	102.496
	30	29.815	- 0.6166	99.383

*Average of five determination

Analysis correlation

The results obtained were compare with those obtained by literature method [12] the same pure and capsule for DCH were analyzed and the result getting by two different methods see Table 5 were statistically compared, using variance ratio f- test and t- test at 95% confidence level, the f and t-test value did not overtake the theoretical values, which indicate that there is no difference between each methods in accuracy and precision in estimation of DCH in pharmaceutical pure and capsule.

Table 5: The comparison of the suggest method with literature method using t and f- test.

Drug form	Suggest method		Literature method	
	Rec. % (xi)1	(xi-$\bar{x}$)12	Rec. % (xi)2	(xi-$\bar{x}$)22
DCH pure	100.957	0.0382	101.583	0.123
	101.348	0.0382	102.286	0.123
Modomycin capsule	Σx1=101.1525	Σ=0.0764 S21=0.0764	Σx2=101.9345	Σ=0.246 S22=0.246
S*		0.401		

F (theor.)	0.310 (19.4)
T (theor.)	1.95 (4.303)

S21=variation $=\frac{\Sigma(xi-\bar{x})_1^2}{n1-1}$,

S22$=\frac{\Sigma(xi-\bar{x})_2^2}{n2-1}$,

t$=\frac{|\bar{x}1-\bar{x}2|}{s\sqrt{\left(\frac{1}{n1}+\frac{1}{n2}\right)}}$,

S*=pooled-standard

deviation$=\sqrt{\frac{(n1-1)s_1^2+(n2-1)s_2^2}{n1+n2-2}}$

REFERENCES

[1] Ramesh P. J., Basavaiah K., Tharpa K.,, *J. of pre – clinical and clinical research,* vol. 4, no. 2, pp. 101-107, 2010,.

[2] Baraka M. M., Elsadek. M. M., Abdelaziz L. M,, *International J. of current pharmaceutical research,* vol. 6, no. 3, 2014.

[3] Patyra E., Kowalczyk E., Bullvet Int. Pulwy, vol. 56, pp. 329-333, 2012.

[4] Gajda A., Posniak A., Zmudzki J., J. chromatogr. B, *anlyt technol. Biomed life sci.,* vol. 928, pp. 113-120, 2013.

[5] Sreeran V., Nagendrakumar A.V.D, Madhu M., *chemical science transactions,* vol. 4, no. 1, pp. 69-74, 2015.

[6] Lofty H. M., Hegha M. A., Mohamed E. H., *spectrochimica Acta part A: molecular and bio molecular spectroscopy,* vol. 153, pp. 321-332, 2016.

[7] Li S., Li J., Lin Q., Wei X.,, *Analyst J,* vol. 140, pp. 4702-4707, 2015.

[8] Gùrler B., Özkorucklu S., Kir E., *J. of pharmaceutical and biomedical analysis,* vol. 84, pp. 263-268, 2013.

[9] Injac R., Kac J., Strukyelj B., *Analytical and Bio analytical chemistry,* vol. 387, no. 2, pp. .695-701, 2007.

[10] Pavagada J., Ramesh K., Divya N., *Association of chemical engineers AchE,* vol. 16, no. 2, pp. 139-148, 2010.

[11] Ahmed N. R., Hassan W. E, *J. Raf. Sci.,* vol. 20, no. 3, pp. 66-73, 2009.

[12] Al-Abachi M. Q., Al-Nedawi Z. A., *J. of Al Nahrain university,* vol. 18, no. 3, pp. 24-32, 2015.