Batch and Flow Injection Spectrophotometric Determination of Doxycycline Hyclate in Pharmaceutical Preparations

Mouayed Q. Al-Abachi and Zaid A. Al-Nedawi

Department of Chemistry, College of Science, University of Baghdad, Baghdad-Iraq.

E-mail: zaid.abdulsatar@yahoo.com.

Abstract

New, simple and sensitive batch and flow injection spectrophotometric methods for the determination of doxycycline hyclate (DCH) in pharmaceutical preparations were developed. These methods were based on diazotization-coupling reaction between diazotized o-nitroaniline (DONA) and DCH in sodium carbonate medium to form yellow-orange colored azo dye product has a maximum absorbance at 448 nm. Calibration graphs of absorbance versus concentration show that Beer’s law were obeyed over the concentration range 0.4-52, 5-200 µg mL\(^{-1}\) of DCH, with limit of detection of 0.284, 1.117 µg mL\(^{-1}\) of DCH for batch and flow injection analysis (FIA) procedure respectively, and sample throughput was 171 h\(^{-1}\) for FIA method. All chemical and physical conditions affected on the developed colored product were carefully studied and the proposed methods were successfully applied for the determination of doxycycline in its pharmaceutical preparations.

Keyword: Diazotized o-nitroaniline, Flow injection analysis, Doxycycline hyclate.

1- Introduction

Doxycycline hyclate (DCH) chemically named as (4S,4aR,5S,5aR,6R,12aS)-4-dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,6,11,12a-octahydotetracene-2-carboxamide hydrochloride hemiethanol hemihydrates and molecular weight 512.9 g mol\(^{-1}\) Fig. (1). DCH more soluble than doxycycline monohydrate, this is one of the main reasons for it’s more frequent use in pharmaceutical samples. DCH is one of the tetracycline derivatives has broad spectrum antibacterial agents effective against a host of Gram positive and Gram negative aerobic and anaerobic bacteria which obtained from oxytetracycline or metacycline. Doxycycline is preferred to other tetracyclines in the treatment of specific infections because of its fairly reliable absorption and its long half-time, it is used to treat chronic prostatic, Syphilis and pelvic inflammatory disease [1-2].

Several methods have been developed for determination DCH in pharmaceutical preparations such as spectrophotometric [3-6], high performance liquid chromatography HPLC [7,8], RP-HPLC [9], potentiometry[10], Chemliumincence [11] and thin layer Chromatography [12], however, some of these methods are time consuming and/or require expensive equipment.

The present paper describe development of batch and FIA methods which based and diazotization-coupling reaction between DCH and diazotized o-nitroaniline (DONA) reagent in sodium carbonate medium, yellow-orange azo dye was produced and spectrophotometrically measured at 448 nm. Both batch and FIA procedures have applied for the determination DCH in its pharmaceutical preparations.

2- Materials and methods

2-1: Apparatus

All spectral and absorbance measurements were carried out by using a shimadzu UV-visible–260 digital double beam recording spectrophotometer (Tokyo–Japan), and using 1 cm quarts cells. A quartz flow cell with 50 µL internal volume and 1 cm bath length used for the absorbance measurements. A two channel manifold Fig. (2) was employed for the FIA spectrophotometer determinations of DCH. A peristaltic pump (Ismatec Lobortechnik –
Analytic, CH–8512, Glatbragg–Zurich, Switzerland, Sixchannels) was used to transport the reagents solutions. Injection valve (Rheodyne, Altex 210, supeko use) was employed to provide appropriate injection volumes of standard solutions and samples, flexible vinyl tubing of 0.5 mm internal diameter was used for the peristaltic pump. Reaction coil (RC) was of Teflon with internal diameter of 0.5 mm. The diazotized o-nitroaniline (DONA) (A) stream was combined Fig. (2) with injected sample (DCH) and they merged with sodium carbonate (B) stream at T – link then mixed in reaction coil (RC) with length (50 cm), injection loop (200 µL), total flow rate 2.5 mLmin⁻¹, the absorbance was measured at 448 nm at temperature 25 °C.

Fig.(2) A schematic diagram of FIA manifold where: (A) & (B), solutions of diazotized o-nitroaniline and sodium carbonate respectively; P = peristaltic pump; S= injection sample of DCH; IV = injection valve; RC=reaction coil; FC = flow cell; D=detector; W= waste.

2-2: Reagents and materials
Analytical reagents grade chemicals and distilled water were used thoroughly.

-Doxycycline hyclate stock solution (500 µg mL⁻¹), (9.748 ×10⁻⁴ M): 0.05 g amount of pure DCH (SDI- Iraq) was dissolved in distilled water, and then completed to 100 mL in a volumetric flask with the same solvent. More dilute solutions were prepared by suitable dilution of the stock solution with distilled water.

-Hydrochloric acid (BDH-England)(1M): Was prepared by diluting 21.5 mL of 11.64 M of concentrated hydrochloric acid with distilled water in 250 mL volumetric flask, and standardized against Na₂CO₃.

-Diazotized o-nitroaniline (DONA) (10×10⁻³ M): Was prepared daily by dissolving 0.138 g of o-nitroaniline (BDH-England) in 5 mL ethanol and add 20 mL of distilled water, then 6mL of HCl (1M) was added. The mixture was transferred to 100 mL volumetric flask and placed in an ice-bath for 5 min, then 0.069 g of sodium nitrite was added to the mixture and shacked well. After 5 min, the volume was made up to the mark with distilled water. More dilute solutions were prepared by appropriate dilutions from stock solution with distilled water.

-Sodium carbonate (BDH-England) (0.3M): 7.948 g of sodium carbonate Na₂CO₃ was dissolved in a 250 mL volumetric flask with distilled water. 0.1 M of sodium carbonate was prepared by dilution with distilled water.

2-3: Pharmaceutical preparations of doxycycline
Pharmaceutical preparations were obtained from commercial sources.
1- Doxycycline hyclate 8-capsules (Actavis-U.K.), each capsule contain 100 mg of doxycycline.
2- Medomycin 10-capsules (Medochemie Ltd.-cyprus), each capsule contain 100 mg of doxycycline. HCl.
3- Tabocine 10-capsules (Tabook- K.S.A.), each capsule contain 100 mg of doxycycline hyclate.

2-4: General procedure
2-4-1: General batch procedure
Into a series of 25 mL volumetric flasks, an increasing volumes of DCH working solution (100 µg mL⁻¹) were transferred to cover the range 0.4-52 µg mL⁻¹ of calibration graph, 1mL of DONA (5×10⁻³M) reagent was added, and followed by adding 1 mL of Na₂CO₃ (0.1 M) for all flasks. The solutions were completed to the mark with distilled water, mixed well and left for 10 min at room temperature (25 °C). The absorbance was measured at 448 nm versus the reagent blank was prepared in same way but without drug. A calibration graph was drawn and the statistical calculation was done for the analytical features obtained as shown in Table (1). For the optimization of conditions and in all subsequent experiments were carried out on 20 µg mL⁻¹ of DCH.
2-4-2: General FIA procedure

Working solution of DCH in the range 5-200 µg mL\(^{-1}\) was prepared from stock solution by appropriate dilution with distilled water. A 200 µL portion of DCH was injected into the stream of DONA (5×10\(^{-3}\)M) reagent and was then combined with a stream of 0.1 M sodium carbonate with a total flow rate 2.5 mL min\(^{-1}\). A calibration graph was prepared and regression equation with other feature was calculated as shown in Table (1). Optimization of conditions was carried out on 40 µg mL\(^{-1}\) of DCH.

### Table (1)

Analytical characteristics of the procedures for the determinations of DCH.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Batch method</th>
<th>FIA method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>Y = 0.0283X+0.0134</td>
<td>Y=0.0066x-0.0064</td>
</tr>
<tr>
<td>Linear range (µg mL(^{-1}))</td>
<td>0.4-52</td>
<td>5-200</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9993</td>
<td>0.9992</td>
</tr>
<tr>
<td>Limit of detection (µg mL(^{-1}))</td>
<td>0.284</td>
<td>1.117</td>
</tr>
<tr>
<td>Average of recovery, %</td>
<td>99.613</td>
<td>100.689</td>
</tr>
<tr>
<td>Relative standard deviation (RSD), %</td>
<td>1.528</td>
<td>0.739</td>
</tr>
<tr>
<td>Sandell’s Sensitivity (µg cm(^{-2}))</td>
<td>0.035</td>
<td>0.151</td>
</tr>
<tr>
<td>Through-put (hr(^{-1}))</td>
<td>6</td>
<td>171</td>
</tr>
<tr>
<td>Molar absorptivity (L mol(^{-1})cm(^{-1}))</td>
<td>14.515×10(^3)</td>
<td>3.385×10(^3)</td>
</tr>
</tbody>
</table>

2-5: Pharmaceutical preparation analysis

An accurately weight amount of 10 powder capsules (100 mg of DCH pre each capsule). Equivalent to 50 mg of the pure drug was transferred into 100 mL volumetric flask and completed to the mark with distilled water. The flask with its contents was shacked well and filtered to produce 500 µg mL\(^{-1}\) solution of DCH capsule. Deferent diluted concentrations were prepared by simple dilution with distilled water in 25 mL flasks final volume and used for analysis; the measurements were carried out as described earlier under general procedure.

3: Results and Discussion

3-1: Batch spectrophotometric determination

The factors affecting on the sensitivity of the colored product resulting from diazotization-coupling reaction between DCH and DONA reagent in basic medium were carefully studied. A typical spectrum for the yellow-orange azo dye formed was measured versus reagent blank which has absorbance at 448 nm Fig.(3).

![Fig.(3) Absorption spectra of the azo dye (20 µg mL\(^{-1}\)) of doxycycline hyclate (A) against reagent Blank (B) and blank against distilled water.](image)

The experimental conditions for the determination of DCH were established. The diazotization process occurred in an acidic medium and hydrochloric acid in concentration (1 M) was selected, the effect of
different volumes of HCl were studied and 3 mL volume seem to be optimum for intense azo dye color produced after coupling with DCH as shown in Fig.(4), effect of the volume of DONA (5×10^{-3}M) reagent was studied in the range (1-5 mL) and 1 mL was found to be optimum Fig.(4). The diazotization-coupling reaction occurred in an alkaline medium, thus, the effect of different alkaline solutions (0.1 M) on the colored product was studied such as sodium hydroxide, ammonium hydroxide, sodium acetate and sodium carbonate. Maximum sensitivity and stability was obtained only when the reaction is carried out in the presence of Na_{2}CO_{3}. The effect of different volumes of sodium carbonate was studied in the range (1-10 mL), A volume of 1mL was found enough to obtain a maximum absorbance Fig.(4), the order of addition of the reagents was studied and found that the order

![Graph](Fig.(4) Effect of different parameters on batch method.)

The stoichiometry of the reaction between DCH and DONA was investigated using continuous variation and mole ratio methods [13]. Equal concentration (4.875×10^{-4} M) were prepared for DCH and DONA reagent. In mole ratio an increasing volumes of DONA reagent were added to a 2 mL solution of DCH drug in a series of 25 mL volumetric flasks, followed by 1 mL of 0.1 M Na_{2}CO_{3}, the volumes were made up to the mark with distilled water, allowed to stand for 10 min; and absorbance was measured at 448nm.

The Job method was applied by placing 0.5 to 8.5 mL of solution of DCH drug into a series of 25 mL volumetric flasks, this was followed by placing 8.5 to 0.5 mL of DONA reagent and 1 mL of (0.1 M) of Na_{2}CO_{3}. The solutions were diluted to the mark with distilled water, allowed to stand for 10 min; and absorbance was measured and results for both stoichiometric methods plotted as shown in Fig.(5) and show that a (1:2) azo dye was formed between DCH and DONA as shown in proposed reaction sequence in Fig.(6). The product was soluble in water and the apparent stability constant was calculated by comparing stoichiometric amount of DCH (4.875 ×10^{-4}M) and DONA (4.875 ×10^{-4}M) (A_0) with that of
solution containing a ten-fold excess of DONA (A_m) and the stability constant was calculated according to the following equations:

\[ k = \frac{1-\alpha}{\alpha^3} \]  

and

\[ \alpha = \frac{A_m - A_s}{A_m} \]  [14]

The analytical features of the procedure a summarized in Table (1). It also summarized the main performance of the flow procedure developed for DCH determination in order to make an effective comparison between the two approaches.

**Fig.(5) Stoichiometric plots for colored dye products: mole ratio plot and continues variation plo.**

**Fig.(6) Reaction sequence.**
3-2: Flow-Injection spectrophotometric determination

The batch method for the determination of DCH was adopted as a basis to develop FIA procedure. The manifolds used for the determination of DCH was so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of DCH with DONA in sodium carbonate medium. Maximum absorbance intensity was obtained when the sample was injected into a stream of DONA and then with a stream of sodium carbonate (0.1 M) as show above in Fig.(2). The influence of different chemical and physical FIA parameters on the sensitivity of the colored product was optimized as follows:

3-2-1: Optimization of chemical parameters

The effect of various concentrations of DONA (1×10^{-3}-10×10^{-3} M) was investigated. A concentration of (5×10^{-3} M) DONA gave the highest absorbance and was chosen for further experiments as shown in Fig.(6). It was observed that the reaction between DONA and DCH depends on alkaline medium, thus, the effect of different concentrations (0.05-0.3 M) of Na_2CO_3 was studied and 0.1 M was found to be the optimum as shown in Fig.(7).

3-2-2: Optimization of physical parameters

The effect of total flow rate on the absorbance intensity of the colored product was investigated in the range of (0.5-4.0 mL min^{-1}). The results obtained show that a total flow rate of 2.5 mL min^{-1} gave the highest absorbance as shown in Fig.(8) and was used in all subsequent experiments.

The reaction coil length is an essential parameter that effects on the sensitivity of the colored reaction product and was investigated in the range of 25-250 cm, the result obtained show that a reaction coil length of 50 cm gave the highest absorbance as shown in Fig.(9), and was used in all subsequent experiments.

The effect of the volume of injected sample was investigated by varying it between 100 and 250 µL using different lengths of sample loop. The result show that injected sample of 200 portion gave the best absorbance with good reproducibility Fig.(10). The reaction time is also an important parameter that affected on the sample throughput and was investigated by calculating the interval time between the sample injection and the appearance of the maximum value of the signal. The reaction time of each sample was 21 sec; therefore, the sample throughput was 171 samples per hour.
Fig.(10) Effect of injected sample volume.

A standard calibration line, obtained for a series of DCH standard and the main analytical feature of the developed method are indicated in Table (1). The accuracy and the precision Table (2) of the proposed method were studied by determinations of three different concentrations and equations results from calibration graphs Table (1) was applied for absorbance to find concentrations, and compared with result obtained from the standard method [2].

Table (2)
The accuracy and precision of the proposed methods (Batch and FI).

| Concentration µg.mL⁻¹ | Batch method | | | Flow injection method |
|----------------------|--------------|------------------|------------------|
|                      | Present (%)  | Found (%)         | RSD %*           | Present (%)  | Found (%)         | RSD %* |
| 8                    | 7.816        | -2.296           | 97.903           | 2.436       | 50                | 50.242  | 0.484           | 100.484  | 0.963 |
| 16                   | 16.212       | 1.325            | 101.325          | 1.228       | 100               | 102.939 | 2.393           | 102.393  | 0.897 |
| 20                   | 19.922       | -0.388           | 99.611           | 0.920       | 150               | 148.787 | -0.808          | 99.191   | 0.357 |

* For five determinations.

3-3: Analytical applications:
The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing DCH. The results are summarized in Table (3) in accordance with those obtained by the official standard method. Finally, the statistical analysis [15] using F- and t- tests reveals that there is no significant difference in precision and accuracy between the proposed methods and the official methods.

Table (3)
Applications of the proposed and official methods to the determinations of some TCH and DCH in pharmaceutical forms.

<table>
<thead>
<tr>
<th>Pharmaceuticals</th>
<th>Concentration µg.mL⁻¹</th>
<th>Found µg.mL⁻¹</th>
<th>Rec. %*</th>
<th>RSD %*</th>
<th>Concentration µg.mL⁻¹</th>
<th>Found µg.mL⁻¹</th>
<th>Rec. %*</th>
<th>RSD %*</th>
<th>Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medomycin (DCH)</td>
<td>8.000</td>
<td>7.808</td>
<td>97.600</td>
<td>3.844</td>
<td>50</td>
<td>51.260</td>
<td>100.257</td>
<td>0.695</td>
<td>101.583</td>
</tr>
<tr>
<td>Capsule 100mg (Kyprus)</td>
<td>16.000</td>
<td>16.057</td>
<td>100.357</td>
<td>1.016</td>
<td>100</td>
<td>100.257</td>
<td>100.257</td>
<td>0.751</td>
<td>102.286</td>
</tr>
<tr>
<td></td>
<td>20.000</td>
<td>19.750</td>
<td>98.750</td>
<td>1.498</td>
<td>150</td>
<td>151.390</td>
<td>100.926</td>
<td>1.522</td>
<td>99.627</td>
</tr>
<tr>
<td>Doxycycline (DCH)</td>
<td>8.000</td>
<td>7.952</td>
<td>99.401</td>
<td>1.982</td>
<td>50</td>
<td>49.393</td>
<td>98.787</td>
<td>0.894</td>
<td>99.191</td>
</tr>
<tr>
<td>Capsule 100mg (actvas, UK)</td>
<td>16.000</td>
<td>15.654</td>
<td>97.841</td>
<td>1.000</td>
<td>100</td>
<td>96.533</td>
<td>96.533</td>
<td>0.640</td>
<td>147.116</td>
</tr>
<tr>
<td></td>
<td>20.000</td>
<td>19.919</td>
<td>99.595</td>
<td>1.505</td>
<td>150</td>
<td>147.116</td>
<td>98.077</td>
<td>1.515</td>
<td>150.748</td>
</tr>
<tr>
<td>Tabocine (DCH) capsul</td>
<td>8.000</td>
<td>8.003</td>
<td>100.043</td>
<td>1.872</td>
<td>50</td>
<td>51.239</td>
<td>102.479</td>
<td>0.850</td>
<td>98.640</td>
</tr>
<tr>
<td>e100mg (Tabok, K.S.A)</td>
<td>16.000</td>
<td>16.130</td>
<td>100.815</td>
<td>1.702</td>
<td>100</td>
<td>98.640</td>
<td>98.640</td>
<td>0.658</td>
<td>100.499</td>
</tr>
<tr>
<td></td>
<td>20.000</td>
<td>20.164</td>
<td>100.820</td>
<td>1.468</td>
<td>150</td>
<td>150.748</td>
<td>100.499</td>
<td>1.118</td>
<td>100.926</td>
</tr>
<tr>
<td>t - test</td>
<td>0.928</td>
<td></td>
<td></td>
<td></td>
<td>0.451</td>
<td></td>
<td></td>
<td></td>
<td>(Thio.)</td>
</tr>
<tr>
<td>F – test</td>
<td>3.605</td>
<td></td>
<td></td>
<td></td>
<td>1.105</td>
<td></td>
<td></td>
<td></td>
<td>(Thio.)</td>
</tr>
</tbody>
</table>

* For five determinations.
4: Conclusion
The developed methods are very adequate for determination of DCH in pharmaceutical preparations at trace level concentration μg mL⁻¹ and without requiring a temperature or pH control. In comparison of the batch with FIA procedure, the letter is more convenient then the former method because of its speed (sample throughput of 171 injection h⁻¹) and wider linear range of the calibration graph.

The proposed method is thus, simple, rapid, and inexpensive and hence can be used in routine analysis of DCH in pharmaceutical preparations.

Acknowledgements
We would like to thank University of Baghdad, Collage of science, and the stuff of chemistry department for their providing the necessary facilities during the study.

References
الخلاصة

يتضمن البحث تطوير طرق طيفية جديدة وحساسة للتقدير المايكروغرامي للدوكسي ساكلين هيكلات في المستحضرات الصيدلانية باستخدام طريقتي الدفعة والحقن الجرياني. تعتمد هذه الطرق على تفاعل الازوتة-ايزوديج بين الأثونينتروانيين الموزوت والدوكسي ساكلين في وسط قاعدي ليتكون لون اصفر-برتقالي من صبغة الأزو الذائبة في الماء التي أعطت أعلى امتصاص عند 448 نانومتر.

تشير منحنى الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن المدى 0.4 - 52 و 5 - 100 مايكروغرام من الدوكسي ساكلين هيكلات وبحدود كشف 0.284 و 1.117 مايكروغرام مل. لكل من طريقتي الدفعة والحقن الجرياني على التوالي. وبمعدل نموذج لطريقة الحقن الجرياني 171 نموذج في الساعة، بعد دراسة كافة المتغيرات الكيميائية والفيزيائية بدقة واختيار الظروف المثلى طبقت الطرقتين بنجاح في تقدير الدوكسي ساكلين في المستحضرات الصيدلانية.