Determination of Doxycycline Hyclate by Batch and Reverse Flow Injection Analysis Based on the Oxidative Coupling Reaction with 3-Methyl-2-benzothiazolinone Hydrazone hydrochloride (MBTH)

Sadeem Subhi Abed                            Omar T. Hussein

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq
E-mail: Omar12th@yahoo.com

Received 22/ 9/2015
Accepted 20 /12 /2015

Abstract

New, simple and sensitive batch and reverse FIA spectrophotometric methods for the determination of doxycycline hyclate in pure form and in pharmaceutical preparations were proposed. These methods based on oxidative coupling reaction between doxycycline hyclate and 3-methylbenzothiazolinone-2-hydrazone hydrochloride (MBTH) in the presence ammonium ceric sulfate in acidic medium, to form green water-soluble dye that is stable and has a maximum absorbance at 626 nm. A calibration graph shows that a Beer's law is obeyed over the concentration range of 1-80 and 0.5-110 μg.mL⁻¹ of DCH for the batch and rFIA respectively with detection limit of 0.325 μg.mL⁻¹ of DCH for r-FIA methods. All different chemicals and physical experimental parameters affecting the development and stability of the colored product were carefully studied. The proposed methods were successfully applied for the determination of DCH in pharmaceutical preparations.

Key words: Reverse FIA, Spectrophotometric, doxycycline hyclate, oxidative coupling, 3-methyl-2-benzthiazolinone hydrazone hydrochloride (MBTH).

Introduction:

Doxycycline hyclate (DCH) is one of the tetracycline derivatives, which has a wide range of antibacterial activities molecular weight 512.95g/mol and structure (Figure 1) given below [1]. It is a broad spectrum antibiotic, with activity against a wide range of gram-positive and gram-negative bacteria. It has been used for the treatment of infectious diseases caused by rickettsiae, mycoplasmas and chlamydiae [2]. The drug is official in the United States Pharmacopoeia (U.S.P) [3] and the British Pharmacopoeia (B.P) [4] which describes the HPLC method for the determination of DCH either in raw material or in pharmaceutical formulations. The literature reveals to several methods for the determination of DCH in pharmaceutical dosage forms, including liquid chromatography [5], FIA-spectrophotometry with copper
carbonate [6], sequential injection chromatography [7], capillary electrophoresis [8], sodium cobalt nitrite [9], uranyl acetate [10] and also 4-aminophenazone/potassium hexacyanoferrate (III) [11]. Chromatographic techniques are the most widely used one. Although these procedures are specific, most of the described methods are time consuming and require multistage extraction procedures [12-14].

**Fig.(1):** Doxycycline Hyclate (DCH)

This work describes spectrophotometric methods (batch and Reverse Flow Injection) for the determination of DCH in pharmaceuticals drugs. The methods were successfully applied to the determination of DCH in three different brands of tablets and capsules with good accuracy, precision. And without detectable interference by standard-addition procedure and the methods were to be simple, accurate and easy to apply to routine analysis.

**Materials and Methods:**

**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 was used for the absorbance measurements. The two channel manifolds were employed for the FIA spectrophotometer determination of VHC. A peristaltic pump (Ismatec Lobortechnik–Analytic, CH–8512, Glatbragg–Zurich, Switzerland, sixchannels) was used to transport the reagent's solutions. Injection valve (Rheodyne,Altex 210, supeko use) was employed to provide appropriate injection volumes of standard and sample solutions. Flexible vinyl tubing of 0.5mm internal diameter was used for the peristaltic pump. Reaction coil (RC) was of Teflon with internal diameter of 0.5 mm. and with length (150 cm), injection loop (100 µL), and total flow rate 2.5 mLmin⁻¹, the absorbance was measured at 626 nm at temperature 25 °C.

**Chemicals:**

**Doxycycline stock solutions (500µg.mL⁻¹)**

When dissolved 0.05 g amount of pure DCH (SDI-Iraq) in distilled water then completed to a 100 mL in a volumetric flask with the same solvent the dilute a solution were prepared by suitable dilution of the stock standard solution with distilled water.

**3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) 0.2 % (w/v)**

Accurately weighed 0.2g of MBTH reagent was transferred into a 100 ml calibrated volumetric flask, dissolved in distilled water, and made up the volume to mark to obtain a solution of 0.2% (w/v).

**Ammonium ceric sulfate (Am.C.S.) 1%**

Prepared the oxidant by dissolving 1.00g of (Am.C.S.) in 100 mL of 0.1M H₂SO₄.

**General Batch procedure:**

Into a series of 10 mL volumetric flasks, an increasing volume of doxycycline working solutions (500 µg.mL⁻¹) were transferred to cover the
range of the calibration graphs (1 – 80 μg mL⁻¹), and then add of 0.8 mL of MBTH (1%) and 1 mL of (NH₄)₄Ce(SO₄)₄·2 H₂O (1%) dissolved in 0.1M H₂SO₄. The solutions were diluted to develop the color to mark with distilled water, mixed well and left for 15 min at room temperature 25 ºC. The absorbance was measured at 626nm versus the reagent blanks prepared in the same way containing no doxycycline drug.

A calibration graph was constructed and regression equations were calculated.

General FIA procedure

Working solution of DCH in the range (0.5-110 μg mL⁻¹) was prepared from stock solution. A 100μL portion of the reagent of MBTH (0.1%) was injected into the stream of the 40 μg mL⁻¹ of DCH and was then combined with a stream of oxidant(Am.C.S. 0.25% in 0.01M H₂SO₄) with a total flow rate of 2.5 mL min⁻¹ and the reaction coil of 150cm. The resulting absorbance of the colored dye was measured at maximum wave length. A calibration graphs was prepared over the range cited in (Table 1).

Sample preparation

Stock solution (500μg.mL⁻¹) was prepared daily by dissolving 0.05 g of the pure DCH in 100 mL of distilled water and serial dilutions with distilled water were made.

Results and Discussion:

The effect factors on the sensitivity and stability of the colored products resulting from the oxidative-coupling reaction of 3-Methyl-2-Benzthiazolinone hydrochloride (MBTH) with DCH in an acidic medium were carefully studied. A typical spectrum for the 40 μg mL⁻¹ of the dye formed was measured versus reagent blank which has negligible absorbance at 626 nm (Figure 2).

Fig.(2): Absorption spectra of (40 μg.mL⁻¹) DCH treated as described under procedure and measured against reagent blank (MBTH and Am.C.S.) and the reagent blank measured against distilled water.

Batch spectrophotometry determinations

After fixing the optimum reaction conditions a calibration graph of DCH was prepared according to the following procedure: Into 10 mL standard flasks, an increasing volumes of DCH working solution (500 μg mL⁻¹) was transferred to cover the range of the calibration graph (1 – 80 μg mL⁻¹). A volume of 0.8 mL of MBTH (1%), 1 mL of (NH₄)₄Ce(SO₄)₄·2 H₂O (1%)dissolved in 0.1M H₂SO₄. The contents of the flasks were diluted to mark with distilled water, mixed well and left for 15 min at room temperature 25 ºC. The absorbance was measured at 626 nm against reagent blank containing all materials except DCH. The calibration graph was then prepared by plotting the absorbance versus concentration of DCH and the regression equation was calculated. The analytical values of statistical treatments for the calibration graph are summarized in (Table 1).
Table (1): Analytical values of statistical treatments for the calibration graph

| Parameter                  | Batch method | rFIA method |
|----------------------------|--------------|-------------|
| Regression equation        | y = 0.005x + 0.012 | y = 0.007x + 0.028 |
| Correlation coefficient, r | 0.999        | 0.999       |
| Linearity percentage, r²%  | 99.8         | 99.8        |
| Linear range (μg mL⁻¹)     | 1 – 80       | 0.5 – 110   |
| Molar absorptivity, ε (L mol⁻¹ cm⁻¹) | 2.5645 × 10⁻⁴ | 3.5903 × 10⁻⁴ |
| Slope, b (mL μg⁻¹)         | 5 × 10⁻⁴     | 7 × 10⁻⁴    |
| Intercept, a               | 12 × 10⁻³    | 2.80 × 10⁻³ |
| Limit of Detection (LOD)   | 0.537        | 0.325       |
| Limit of Quantitation (LOQ)| 1.792        | 1.083       |
| Sandell’s sensitivity, S   | 0.02         | 0.0143      |

The stoichiometry of the formed product was studied by continuous variation (Job’s method) and mole ratio methods. The job’s method was applied by adding decreasing volumes (5 to 0.0 mL) of 3.365 × 10⁻⁴ M of MBTH solution into a series of 10 mL volumetric flasks, followed by adding increasing volumes (0.0 to 5 mL) of DCH of same concentration, 1mL of 1% [(NH₄)₄Ce(SO₄)₄]·2 H₂O dissolved in 0.1% of H₂SO₄. The solutions were diluted to the mark with distilled water, allowed to stand for 15 min, and the absorbance was measured versus reagent blank.

In mole ratio method, an increased volumes (0.25 – 2.5 mL) of 3.365 × 10⁻⁴ M of MBTH solution were added to a series of 10 mL volumetric flasks, followed by 1 mL of DCH of same concentration, 1mL of 1% [(NH₄)₄Ce(SO₄)₄]·2 H₂O dissolved in 0.1% of H₂SO₄. The volumes were made up to the mark with distilled water and allowed to stand for 15 min. The absorbance was measured at 626 nm versus the reagent blank. The results obtained of both methods were plotted and are shown in (Figure 3) and (Figure 4) which indicated the existence of 1:1 (MBTH: DCH) and the reaction equation according to scheme-1.

**FIA determination:**

The batch method for the determination of DCH was adopted as a
basis to develop rFIA 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 DCH drugs with MBTH in presence of (Am.C.S.) was dissolve in sulfuric acid and also medium. Maximum absorbance intensity was obtained when the reagent was injected into a stream of DCH drug and was combined with a stream of oxidant (Am.C.S.) (0.25% in 0.01M H$_2$SO$_4$). The influence of different chemical and physical FIA parameters on the absorbance intensity of the colored product was optimized as follows

**Chemical variables**

**The effect of concentration of MBTH and Ammonium ceric sulfate**

The effect of various concentrations of MBTH in the range (0.05 - 1 %) and (NH$_4$)$_4$Ce(SO$_4$)$_4$.2H$_2$O in the range (0.1 - 1.2 %) in the mixture of MBTH and ammonium ceric sulfate solution were investigated for the rFIA method, while keeping other conditions constant. A concentration of 0.1 % of MBTH and 0.25 % of (NH$_4$)$_4$Ce(SO$_4$)$_4$.2H$_2$O dissolved in 0.01M H$_2$SO$_4$ in mixture gave the highest absorbance for rFIA (Figure 5) and (Figure 6), and was chosen for further use.

**Physical variables**

**The effect of total flow rate**

The effect of total flow rate was investigated in the range of 0.5 to 4.5 mL min$^{-1}$ using equal flow rates of the two channels for FIA manifold. The results showed that a total flow rate of 2.5 mL min$^{-1}$ gave the highest absorbance (Figure 7) and was used in all subsequent experiments. After this rate, the absorbance of colored product decreased by increasing a total flow rate because the residence time is not enough for the reaction to be completed. A total flow rate of 2.5 mL min$^{-1}$ was selected as a compromise between sample throughput rate and sensitivity.
The effect of reaction coil length

The effect of the length of reaction coil was investigated in a range of 25 - 250 cm, at a length of 150cm the absorbance reached to maximum for rFIA, (Figure 8), then it decreased as the reaction coil was increase because of the increase in dispersion. Therefore the optimal length for rFIA procedure was 150 cm, and was used in all subsequent experiment.

Accuracy and precision

Under the optimum conditions the accuracy and precision for the determination of DCH using reverse FIA methods was studied using three different concentrations of standard DCH. (Table 2) shows E%, Rec. %, and RSD% of five determinations of each DCH for three different concentrations (10, 40, 80 \( \mu g.mL^{-1} \)) and satisfactory results were obtained.

| Conc., \( \mu g.mL^{-1} \) | Present | Found | E% | Rec.% | RSD% |
|-----------------------------|---------|-------|----|-------|------|
| 10                          | 10.28   | 2.85  | 102.85 | 1.335 |
| 40                          | 40.28   | 0.714 | 100.714 | 1.196 |
| 80                          | 78.85   | -1.43  | 98.56 | 1.828 |

Pharmaceutical applications

The proposed method was applied for the determination of DCH in capsules by the analysis of three concentrations for each sample using the recommended procedure. The results obtained are summarized in (Table 3) and can be considered to be satisfactory.

Analytical application

In order to evaluate the competence of the proposed methods, the results obtained were compared with those obtained with standard method. The results obtained by the two different methods were statistically compared, using the Student \( t \)-test and variance ratio F-test at 95% confidence level. In all cases, the calculated \( t \)- and \( F \)-values (Table 4) did not exceed the theoretical values, which indicate that there is no significant difference between either methods in accuracy and precision in the determination of DCH pharmaceutical preparations.
Table (3): Application of the proposed method for determination of DCH in pharmaceutical preparations

| Drug form                  | Conc., μg.mL−1 | E%  | Rec. % | RSD% |
|---------------------------|----------------|-----|--------|------|
| Present Found             |                |     |        |      |
| Medochemie LTD (capsule 100 mg) cyprus | 10 | 10.14 | 1.428 | 101.428 | 3.41 |
|                           | 40             | 40.71 | 1.78 | 101.78 | 0.782 |
|                           | 80             | 80.85 | 1.071 | 101.071 | 0.326 |
| Doxycycline (capsule 100 mg) Actavise | 10 | 9.95 | -0.42 | 99.58 | 2.44 |
|                           | 40             | 38.71 | -3.21 | 96.79 | 0.44 |
|                           | 80             | 80.42 | 0.53 | 100.53 | 0.439 |
| Doxycycline (capsule 100mg ) ajanta | 10 | 10.28 | 2.85 | 102.85 | 1.322 |
|                           | 40             | 39.14 | -2.14 | 97.86 | 0.641 |
|                           | 80             | 81.85 | 2.321 | 102.321 | 0.362 |

Table (4): Comparison of the proposed method with standard method using t- and F-statistical tests

| Drug form                  | Proposed method | Standard method |
|---------------------------|-----------------|-----------------|
|                           | Rec. % (X) i     | (X i - X) 2       | Rec. % (X) j     | (X j - X) 2 |
| DOC pure                  | 100.714         | 0.034            | 100.029          | 0.180 |
| Medochemie LTD (capsule 100 mg) cyprus | 101.426 | 0.804 | 101.395 | 0.885 |
| Doxycycline (capsule 100 mg) Actavise | 98.966 | 2.442 | 99.548 | 0.820 |
| Doxycycline (capsule 100mg ) ajanta | 101.010 | 0.231 | 100.845 | 0.152 |
|                           | (X i) 1 =100.529 | (X i - X) 2 =3.511 | (X j) 2 =100.454 | (X j - X) 2 =2.037 |
|                           | S 1 =1.170      | S 2 = 0.679      | S = 0.961       |
| t (2.776)                 | 0.110           | 1.723           |
| F (19.000)                |                 |                 |

Conclusion:
The application of oxidative–coupling reaction of MBTH in (Am.C.S.) in the spectrophotometric determinations of the doxycycline hyclate in pharmaceutical preparations was described by batch and rFIA systems. The rFIA system has several advantages over the batch system simplicity, reproducibility time saving, low reagent consumption need of small sample volume, large dynamic range and high sample throughput (72 sample h−1 for DCH) are important features of the rFIA system.

The proposed methods offer a good linearity and precision and simple and inexpensive since it requires simple instrumentation.

References:
[1] Craig, C. and Stitzel, R. 1990. Modern Pharmacology, 3rd edn., Little Brown and Co., Boston.
[2] Goodman, k. and Gilman’s, B. 2001. The Pharmacological Basis of
Therapeutics, J. G. Hardman, L. E. Limbird, A. G. Gilman, editors, McGraw Hill, New York 10th ed, p. 2045.

[3] U.S. Pharmacopoeia, XXIII. 1995. The United States Pharmacopoeia Convention Inc., Rockville, MD pp. 557–559.

[4] British Pharmacopoeia, 1999. Vol. II, Her Majesty’s Stationary Office, London, p. 1805.

[5] Mitic, S. S.; Miletic, G. Z., Kostc, D. A., Naskovic-Djokic, D. C., Arsic, B. B. and . Rasic I. D. 2008. A Rapid and reliable determination of doxycycline hyclate by HPLC with UV detection in pharmaceutical samples, J. Serb. Chem. Soc. 73(3): 665–671.

[6] Lopez, Paz J. L. and Calatayud, J. M. 1993. Copper carbonate as a solid-bed reactor for spectrophotometric determination of doxycycline and oxytetracycline in an unsegmented continuous flow assembly, J. Pharm. Biomed. Anal. 11(11-12): 1093–1098.

[7] Satinsky, D.; Dos Santos, L. M. L.; Sklenáøová, H.; Solich, P.; Montenegro, M. C. B. S. M. and Araùjo A. N. 2005. Sequential injection chromatographic determination of ambroxol hydrochloride and doxycycline in pharmaceutical preparations, Talanta 68(2): 214–218.

[8] Van Schepdael, A.; Kibaya R.; Roets, E. and Hoogmartens, J. 1995. Analysis of doxycycline by capillary electrophoresis, Chromatographia 41(5): 367–369.

[9] Mahrous, M.S. and Abdel-Khalek, M. M. 1984. Spectrophotomtric determination of phenothiazines, tetracyclines and chloramphenicol with sodium cobaltinitrite, Talanta 31(5): 289-291.

[10] Saha, U., Sen; A. K., Das; T. K. and Bhowal, S. K. 1990. Spectrophotometric determination of tetracyclines in pharmaceutical preparations, with uranyl acetate, Talanta 37(12): 1193-1196.

[11] Karlicek, R. and Solich, P. 1994. Flow-injection spectrophotometric determination of tetracycline antibiotics, Anal. Chim. Acta. 285(1-2): 9-12.

[12] Chandra, Y. S.; Rao, V. S.; Murthy, P. S. R.; Siva Chandra, Y. and Suryanarayana Rao, V. 1996. Determination of hostacycline and doxycycline using thorium(IV) as spectrophotometric reagent, Indian J. Pharm. Sci. 58(11): 157–159.

[13] Sunaric, S. M.; Mitic, S. S.; Miletic, G. Z.; Pavlovic, A. N. and Naskovic-Djokic, D. 2009. Determination of doxycycline in pharmaceuticals based on its degradation by Cu(II)/H₂O₂ reagent in aqueous solution, J. Anal Chem. 64(3): 231–237.

[14] Espinosa-Mansilla, A. F.; Salinas, F. and De Orbe Paya I. 1995. Simultaneous determination of sulfadiazine, doxycycline, furaladone and trimethoprim by partial least squares multivariate calibration, Anal. Chim. Acta. 313(1-2): 103–112.
تقدير الدوكسي سايكلين هايكلات بطريقة الدفعة والحقن الجرياني العكوس
اعتمادًا على تفاعل الأكسدة والازدواج مع كاشف 3-ميثيل-2-بينزوثيازولينون
هايبرازون هايدروكلورايد

绥يم صبري عبد عمر حسن
قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة:

يتضمن البحث تطوير طرائق طيفية جديدة وبسيطة للتقدير الكمي لمقادير ضئيلة من الدوكسي سايكلين هايكلات في المحاليل المائية والمستحضرات الصيدلانية باستخدام طريقة الدفعة والحقن الجرياني العكوس. تعتمد الطريقة على تفاعل الأكسدة والازدواج للدوكي سايكلين هايكلات مع كاشف 3-ميثيل-2-بينزوثيازولينون هايبرازون هايدروكلورايد في وسط حامضي حيث تتكون صبغة خضراء مستقرة وذائبة في الماء والتي أعطت أعلى امتصاص عند طول موجي 424 نانومتر. تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التركيز 0.5-110 مايكروغرام/ملي. تمبريق الدفعة والحقن الجرياني العكوس على التوالي وحد كشف 0.325 مايكروغرام/ملي من الدوكسي سايكلين هايكلات وبمعدل مئوية 72 نموذج في الساعة بطريقة الحقن الجرياني العكوس، تم دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة، طبقت الطريقة بنجاح على المستحضرات الصيدلانية الحالية على الدوكي سايكلين هايكلات.

الكلمات المفتاحية: الحقن الجرياني العكوس، الدوكسي سايكلين هايكلات، 3-ميثيل-2-بينزوثيازولينون
هايبرازون هايدروكلورايد