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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)

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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 $\mu\text{g.mL}^{-1}$ of DCH for the batch and rFIA respectively with detection limit of 0.325 $\mu\text{g.mL}^{-1}$ 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 cobaltnitrite [9], uranyl acetate [10] and also 4-aminophenazone /potassiumhexacyanoferrate (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].

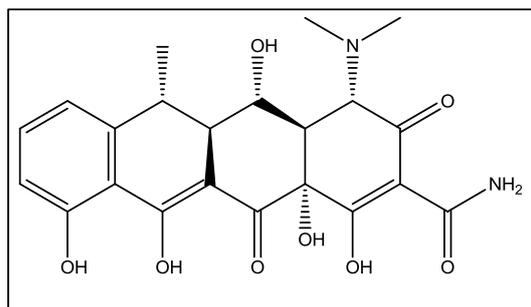


Fig.(1): Doxycycline Hyclate (DCH)

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 quartz 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 Loborteknik–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 $^{\circ}\text{C}$.

Chemicals:

Doxycycline stock solutions ($500\mu\text{g.mL}^{-1}$)

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 H_2SO_4 .

General Batch procedure:

Into a series of 10 mL volumetric flasks , an increasing volume of doxycycline working solutions ($500\mu\text{g.mL}^{-1}$) were transferred to cover the

range of the calibration graphs (1 – 80 $\mu\text{g mL}^{-1}$), and then add of 0.8 mL of MBTH (1%) and 1 mL of $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2 \text{H}_2\text{O}$ (1%) dissolved in 0.1M H_2SO_4 . The solutions were diluted to develop the color to mark with distilled water, mixed well and left for 15 min at room temperature 25 °C. 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 $\mu\text{g mL}^{-1}$) was prepared from stock solution. A 100 μL portion of the reagent of MBTH (0.1%) was injected into the stream of the 40 $\mu\text{g mL}^{-1}$ of DCH and was then combined with a stream of oxidant (Am.C.S. 0.25% in 0.01M H_2SO_4) 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 (500 $\mu\text{g mL}^{-1}$) 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 $\mu\text{g mL}^{-1}$ of the dye formed was measured versus reagent blank which has negligible absorbance at 626 nm (Figure 2).

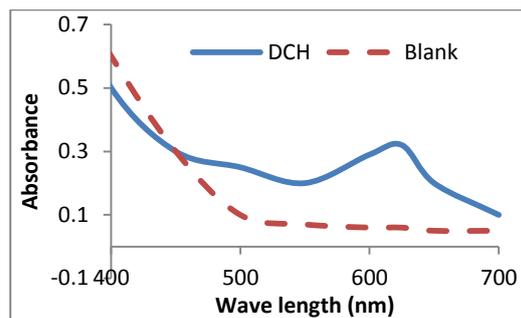


Fig.(2): Absorption spectra of (40 $\mu\text{g mL}^{-1}$) DCH treated as described under procedure and measured against reagent blank (MBTH and Am.C.S.) and the reagent blank measured against distilled water.

Batch spectrophotometry determinations

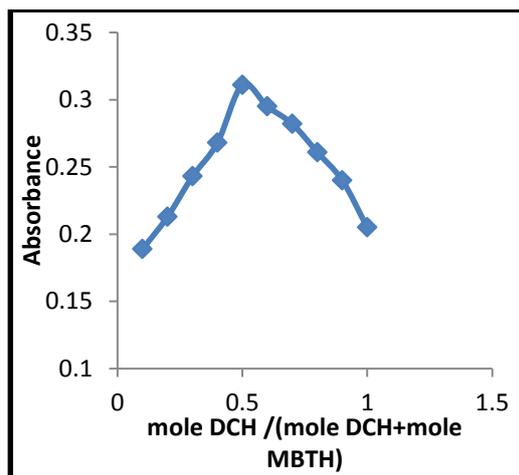
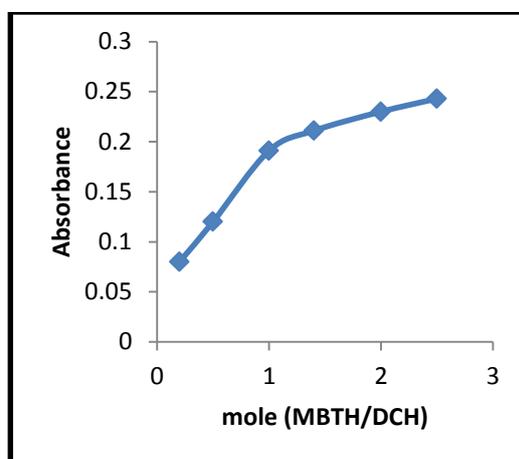
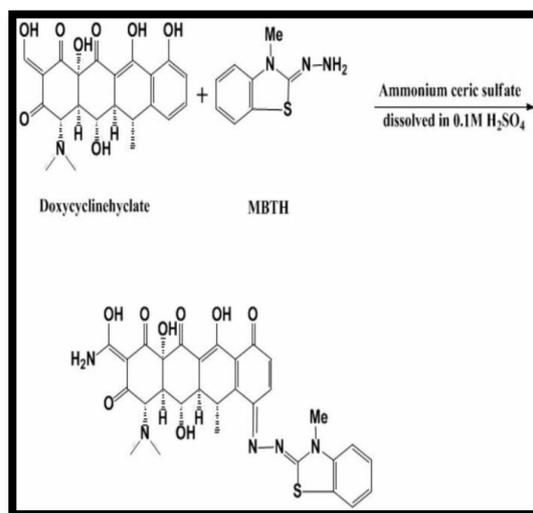
After fixing the optimum reaction conditions a calibration graph of DCH was prepared according to the following procedure: Into 10 mL standard flasks, an increasing volumes of DCH working solution (500 $\mu\text{g mL}^{-1}$) was transferred to cover the range of the calibration graph (1 – 80 $\mu\text{g mL}^{-1}$). A volume of 0.8 mL of MBTH (1%), 1 mL of $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2 \text{H}_2\text{O}$ (1%) dissolved in 0.1M H_2SO_4 . The contents of the flasks were diluted to mark with distilled water, mixed well and left for 15 min at room temperature 25 °C. 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, r ² %	99.8	99.8
Linear range ($\mu\text{g.mL}^{-1}$)	1 – 80	0.5 – 110
Molar absorptivity, ϵ ($\text{L mol}^{-1}\text{cm}^{-1}$)	2.5645×10^4	3.5903×10^4
Slope, b ($\text{mL.}\mu\text{g}^{-1}$)	5×10^{-3}	7×10^{-3}
Intercept, a	12×10^{-3}	2.80×10^{-2}
Limit of Detection (LOD) ($\mu\text{g.mL}^{-1}$)	0.537	0.325
Limit of Quantitation (LOQ) ($\mu\text{g.mL}^{-1}$)	1.792	1.083
Sandell's sensitivity, S ($\mu\text{g.cm}^{-2}$)	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% $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2 \text{H}_2\text{O}$ dissolved in 0.1% of H_2SO_4 . 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% $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2 \text{H}_2\text{O}$ dissolved in 0.1% of H_2SO_4 . 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.

**Fig.(3):** Continuous variation plot**Fig.(4):** Mole ratio plot**Scheme-1:** Reaction between DCH and MBTH.**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 H₂SO₄). 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 (NH₄)₄Ce(SO₄)₄.2H₂O 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 (NH₄)₄Ce(SO₄)₄.2H₂O dissolved in 0.01M H₂SO₄ in mixture gave the highest absorbance for rFIA (Figure 5) and (Figure 6), and was chosen for further use.

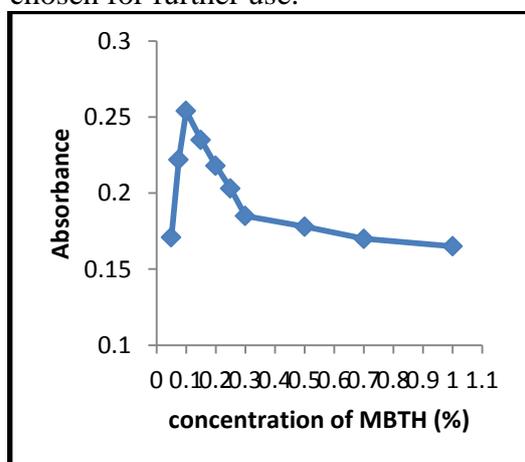


Fig.(5): The effect of MBTH concentration

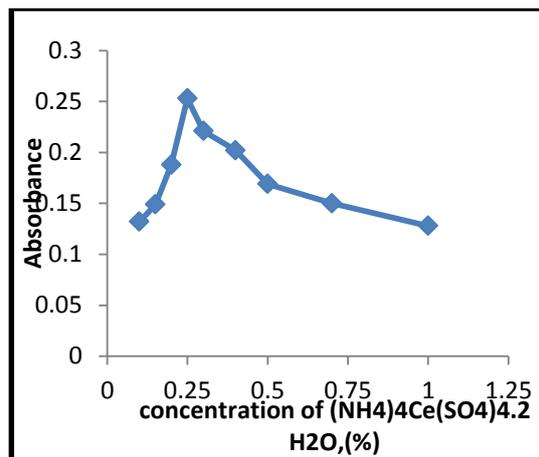


Fig.(6): The effect of (NH₄)₄Ce(SO₄)₄.2H₂O concentration

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.

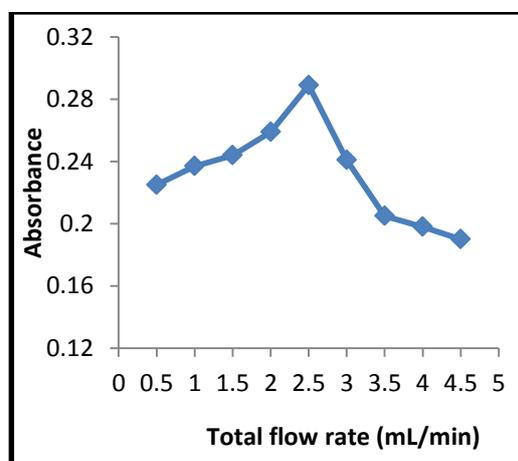


Fig.(7): The effect of total flow rate

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.

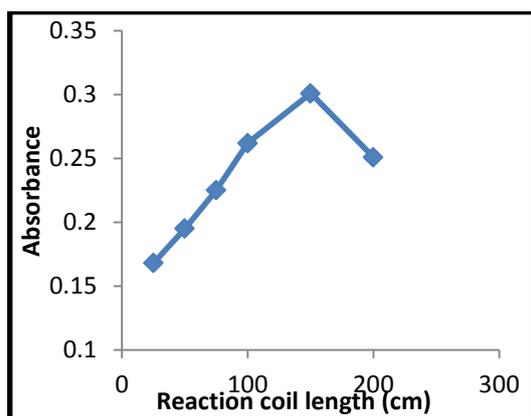


Fig. (8): The effect of reaction coil length

The effect of injected sample volume

The volume of the injected sample was varied between 100 and 250 μL using different lengths of sample loop. The results obtained showed that an injected sample of 100 μL gave the best absorbance for rFIA method (Figure 9). The volume of 100 μL was chosen as optimal for subsequent experiments.

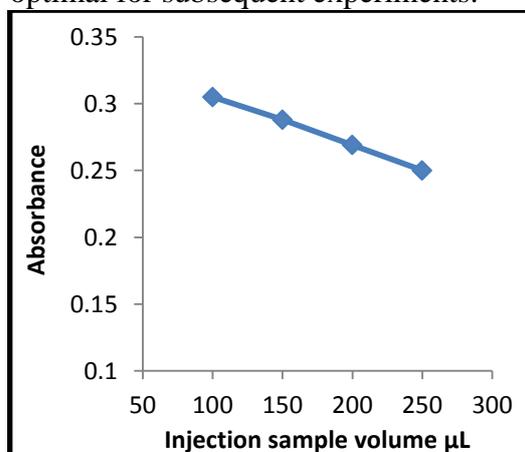


Fig. (9): The effect of injected sample volume

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 $\mu\text{g}\cdot\text{mL}^{-1}$) and satisfactory results were obtained.

Table (2): Accuracy and precision of the proposed method

Conc., $\mu\text{g mL}^{-1}$		E%	Rec.%	RSD%
Present	Found			
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

<i>Drug form</i>	<i>Conc., $\mu\text{g.mL}^{-1}$</i>		<i>E%</i>	<i>Rec. %</i>	<i>RSD%</i>
	<i>Present</i>	<i>Found</i>			
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

<i>Drug form</i>	<i>Proposed method</i>		<i>Standard method</i>	
	Rec.% (X_i) ₁	$(X_i - \bar{X})_1^2$	Rec.% (X_i) ₂	$(X_i - \bar{X})_2^2$
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
	$(\bar{X}_i)_1$ =100.529	$\sum(X_i - \bar{X})_1^2$ =3.511	$(\bar{X}_i)_2$ =100.454	$\sum(X_i - \bar{X})_2^2$ =2.037
S	$S_1^2 = 1.170$		$S_2^2 = 0.679$	
t (2.776)	0.110			S = 0.961
F (19.000)	1.723			

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.

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تقدير الدوكسي سايكلين هايكلات بطريقتي الدفعة والحقن الجرياني العكوس
اعتماداً على تفاعل الاكسدة والازدواج مع كاشف 3-مثيل-2-بينزو ثايازولينون
هايدرازون هايدروكلورايد

عمر طه حسين

سديم صبحي عبد

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 الاكسدة والازدواج للدوكسي سايكلين هايكلات مع كاشف 3-مثيل-2-بينزو ثايازولينون هايدرازون هايدروكلورايد في وسط حامضي حيث تتكون صبغة خضراء مستقرة وذائبة في الماء والتي اعطت اعلى امتصاص عند طول موجي 626 نانوميتر. تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التراكيز 1-80 و110-0.5 مايكروغرام. مل⁻¹ للدفعة والحقن الجرياني العكوس على التوالي وبتحديد كاشف 0.325 مايكروغرام. مل⁻¹ من الدوكسي سايكلين هايكلات وبمعدل نمذجة 72 نموذج في الساعة بطريقة الحقن الجرياني العكوس، تم دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة، طبقت الطريقة بنجاح على المستحضرات الصيدلانية الحاوية على الدوكسي سايكلين هايكلات.

الكلمات المفتاحية: الحقن الجرياني العكوس، الدوكسي سايكلين هايكلات، 3-مثيل-2-بينزو ثايازولينون هايدرازون هايدروكلورايد