Baghdad Science Journal

Volume 22 | Issue 8 Article 6

8-15-2025

Normal and reverse flow injection- spectrophotometric determination of doxycycline hyclate in bulk and pharmaceutical samples

Tabarak Mohammed
Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.,
tabarkchemist@gmail.com

Hind Hadi
Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.,
hind.h@sc.uobaghdad.edu.iq

How to Cite this Article

Mohammed, Tabarak and Hadi, Hind (2025) "Normal and reverse flow injection- spectrophotometric determination of doxycycline hyclate in bulk and pharmaceutical samples," *Baghdad Science Journal*: Vol. 22: Iss. 8, Article 6.
DOI: https://doi.org/10.21123/2411-7986.5016

BAGHDAD SCIENCE JOURNAL 2025;22(8):2539–2549

RESEARCH ARTICLE

Normal and Reverse Flow Injection-Spectrophotometric Determination of Doxycycline Hyclate in Bulk and Pharmaceutical Samples

Tabarak Mohammed *, **Hind Hadi**

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

ABSTRACT

The present paper reports the development and validation of an analytical method for doxycycline hyclate (DOX) quantification in pure and dosage forms by normal and reverse flow injection analysis (FIA) techniques. Two types of FIA approaches were employed to automate a diazotization coupling reaction of DOX with diazotized benzidine dihydrochloride. Red azo dye was formed rapidly and measured at maximum wavelength of 488 nm. Calibration curves were prepared over different DOX concentrations with linearity ranges of 10–250 and 1–180 μg/mL for normal and reverse FIA systems respectively with the limits of detection of 2.95 and 0.46 μg/mL and limits of quantitation of 9.83 and 1.55 μg/mL, respectively. The developed approaches were then used to determine DOX in pharmaceutical dosage forms with high percentage recoveries and low %RSD. In concordance with the standard method, the proposed techniques demonstrated rapid detection and applicability in pharmaceutical analysis.

Keywords: Benzidine dihydrochloride, Doxycycline hyclate, Diazotization, Flow injection analysis, Spectrophotometry

Introduction

Doxycycline hyclate (DOX), chemically named, hydrochloride hemiethanol hemihydrate of (4*S*,4a*R*,5*S*,5a*R*,6*R*,12a*S*)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octa-hydrotetracene-2-Carboxamide, is an antibiotic derived from the tetracycline family and has broad-spectrum bacteriostatic action against prokaryotes and protozoan parasites.[1,2] DOX, is effective against a variety of gram-positive and gram-negative bacteria in a broad spectrum.[3–5] Due to its high absorption and extended half-life, DOX is preferred over other tetracyclines in some illnesses. It is commonly used to treat pelvic inflammatory disease, syphilis, sinusitis, chlamydia, and chronic prostatitis.[6] A review of the literature revealed that numerous analytical techniques were used for the determination DOX in biological and pharmaceutical samples including micellar electrokinetic capillary chromatography,[7] UV-visible spectrophotometry,[8–10] high-performance liquid chromatography,[11–13] flow injection analysis (FIA)[14–17] and liquid chromatography-tandem mass spectrometry.[18] The FIA technique combined with spectrophotometry has received a lot of attention among other analytical techniques. This technique is widely used because of its excellent reproducibility, ease of use, and low cost of apparatus.[19–21] The two most commonly used forms of flow injection analysis techniques are normal FIA, which includes injecting a small quantity of sample into a stream of reagent that travels through a small hole tube to a spectrophotometer for measuring the analyte.[22–24] While in reverse flow mode, the reagent is injected into the sample stream.[25–27] The main advantages of using the reverse mode are reduced reagent consumption (especially expensive or hazardous

Received 4 January 2024; revised 3 June 2024; accepted 5 June 2024.
Available online 15 August 2025

* Corresponding author.
E-mail addresses: tabarkchemist@gmail.com (T. Mohammed), hind.h@sc.uobaghdad.edu.iq (H. Hadi).

https://doi.org/10.21123/2411-7986.5016

reagents),[28,29] improved sample sensitivity, and the determination of many analytes on the same sample by injecting various reagents.[30–32]

In this study, Flow injection methods were used to automate a sensitive diazotization and coupling reaction between DOX and diazotized benzidine dihydrochloride (DBZ). A red azo dye with a maximum wavelength of 488 nm was created and quantified using spectrophotometry.

The aim of the present work estimation of doxycycline hyclate in bulk and pharmaceutical forms using conventional and reverse FIA and comparative study was established between the two forms of FIA after they had been optimized.

Materials and methods

Experimental

Instruments and manifold design

The absorbance was measured using a single-beam UV-Vis spectrophotometer (Shimadzu 1240) equipped with a cell made of quartz (50 μL and 1-cm path length). FIA manifold contained peristaltic pump (Ismatec, Switzerland) utilized for pumping solutions, and an injection valve (Rheodyne, USA) utilized to supply precise amounts of sample solution with different injection volumes. The components of the manifold were connected by transport lines made of polytetrafluoroethylene tubes with an inner diameter of 0.8 mm, while the reaction coil (RC) was constructed using Teflon tubes with a 0.5 mm inner diameter. Two types of double channels FIA manifolds (normal and reverse) were used for the investigation of DOX in its pure and pharmaceutical forms, Fig. 1.

The normal FIA manifold involved injecting 100 μL of DOX solution into the sodium hydroxide solution stream via the injection valve, then combined with the reagent stream (DBZ) at the Y-link, and mixed inside the reaction coil. On the other side, in the reverse FIA manifold, 100 μL of DBZ solution was injected into the DOX solution stream through the injection valve, then combined with the base stream and mixed inside the reaction coil. For the nFIA and rFIA methods, the solutions were pumped into FIA manifolds at a rate of 5.8 and 4 mL/min respectively, using a peristaltic pump. Finally, the red azo dye produced from the coupling reaction of DOX and DBZ, was measured spectrophotometrically at 488 nm.

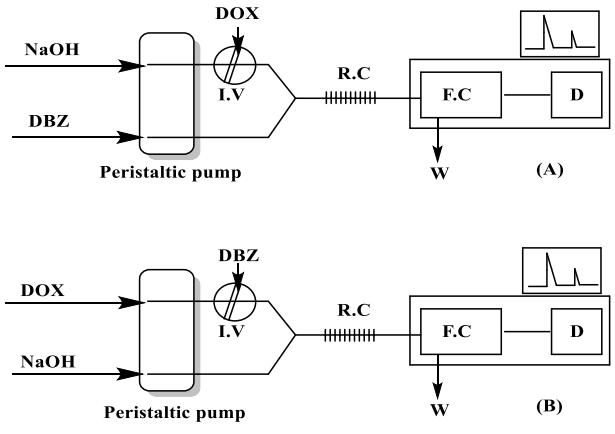


Fig. 1. FIA manifolds: normal (A) and reverse (B) for determination of DOX. (DBZ: diazotized benzidine; W; waste; D; detector; F.C; flow cell; I.V; injection valve; R.C; reaction coil).

Reagents and solutions

All reagents used were of analytical grade, and all solutions were prepared using distilled water. Doxycycline hyclate standard (99.9% w/w) was supplied by the general pharmaceutical manufacturing company (Samarra/Iraq). Benzidine dihydrochloride and hydrochloric acid (36 % w/w) were obtained from Merck (Germany). Sodium hydroxide and sodium nitrite were obtained from DBZ (UK). Pharmaceutical products of doxycycline purchased from local pharmacies included: VIBRAMYCIN®/pfizer-USA, capsules-100 mg, and Accord®/UK, capsules-100 mg of doxycycline monohydrate. Stock standard solution of DOX (500 μg/mL) was prepared by dissolving 0.05 g of standard drug in 100 mL distilled water. Working standard solutions of DOX were prepared by simple dilution of stock solution with distilled water. Diazotized benzidine dihydrochloride (DBZ, 1 mM) solution was prepared by weighing 0.0257 g reagent and dissolving it in 5 mL of distilled water before transferring it to a 100 mL volumetric flask. Next, 3 mL of HCl (1M) was added, and the mixture was placed in an ice bath. Finally, 0.0069 g of $NaNO_2$ was added, and the flask was completed to the mark with distilled water. A stock solution of 0.1 M sodium hydroxide was prepared by dissolving an accurate weight of the base in distilled water.

Preparation of the solution for pharmaceutical applications

Twenty commercial pharmaceutical forms (tablets and/or capsules) with an active ingredient of 100 mg of doxycycline monohydrate were accurately weighed and powdered. An accurately weighted amount equivalent to 50 mg of DOX was dissolved in distilled water and transferred to a 100 mL volumetric flask, then diluted with distilled water and filtered by filter paper. The filtrate was diluted with distilled water to obtain the needed diluted solutions. Lastly, the DOX assay was completed according to the suggested FIA methods.

Procedure for normal FIA and reverse FIA

The general procedure for nFIA was performed as follows: A 100 μL amount of standard solution of DOX range from 10–250 μg/mL was injected by injection valve to the stream of 0.2 M sodium hydroxide solution, then combined with a stream of 1 mM of DBZ at the Y-link. Later, the solutions of two streams were mixed inside the reaction coil (75 cm) with a total flow rate of 5.8 mL/min. In contrast, the reverse FIA includes injecting 100 μL of 4 mM of DBZ into a stream of DOX solution ranging from 1–180 μg/mL then combined with the stream of 0.1 M sodium hydroxide and mixed at 4 mL/min flow rate in a 50 cm reaction coil. For both methods, the red azo dye product was measured spectrophotometrically at 488 nm. All chemical and physical variables were optimized using 75 μg/mL of DOX.

Results and discussion

Batch investigations demonstrated that when DOX was reacted and combined with the diazotized reagent in an alkaline medium, a red azo dye was developed. The reaction product formed in less than five seconds and remained stable for at least two hours. The aromatic amino group in the benzidine dihydrochloride molecule is diazotized in the presence of nitrous acid, resulting in the formation of diazonium ions. The coupling reaction between doxycycline and diazonium salt occurs at the para position of the phenolic group in doxycycline. The absorption spectrum of the colored product against the reagent blank displayed a distinctive wavelength at 488 nm as shown in Fig. 2.

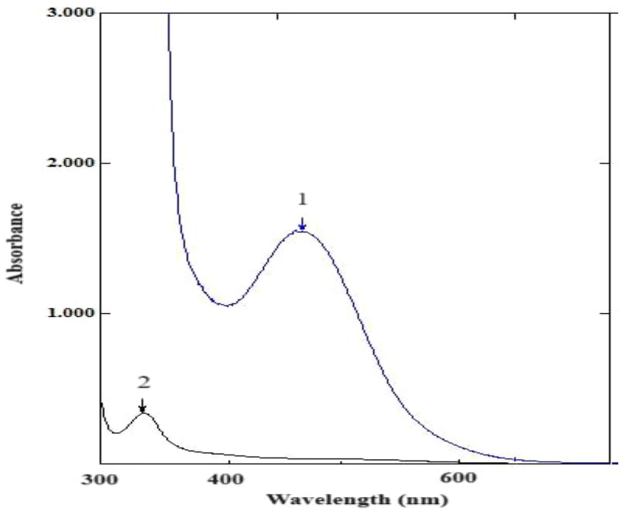


Fig. 2. Absorption spectra of (1) the red dye formed by reacting 50 μg/mL of DOX with DBZ measured versus the blank, and (2) the blank versus distilled water.

Optimization of flow injection parameters

A thorough investigation was conducted on the chemical and physical parameters that were most affecting the red dye product's development and the stability of the analytical signals for both FIA systems.

Selection of the manifold design

The reaction medium and reagent are the two main components of the reaction used for the DOX analysis. As a result, different double-channel manifold designs were examined for both normal and reverse FIA techniques to carry out different reaction pathways. For nFIA and rFIA, manifold B in Fig. 3 offered the highest absorption intensity and good precision, and it was selected to continue utilization.

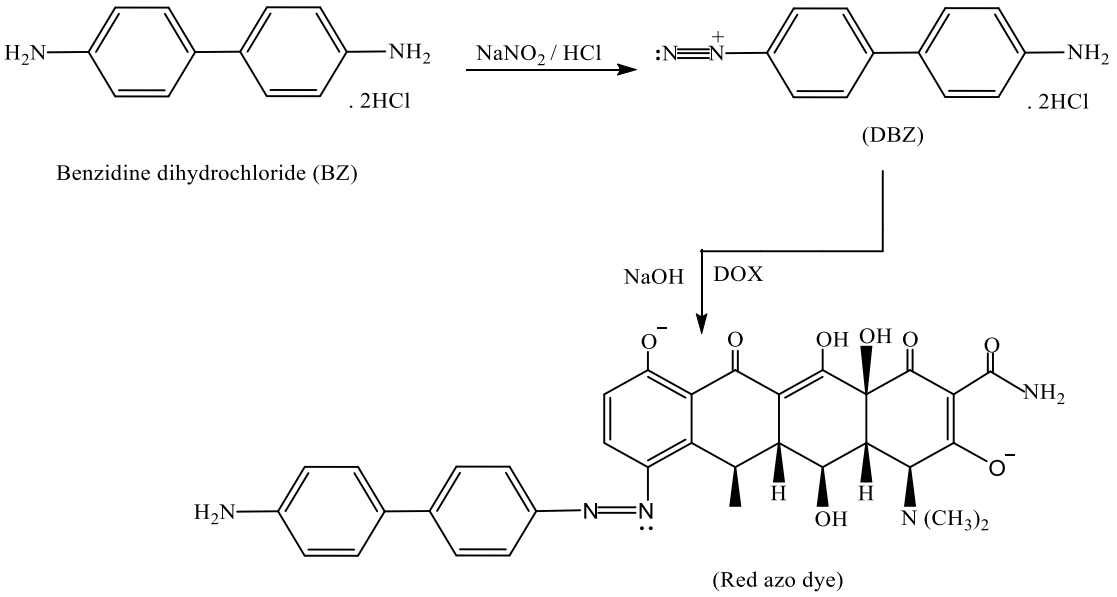
Study of the chemical parameters

The effect of different concentrations of the reagent (DBZ) on the sensitivity of the colored product in normal and reverse FIA systems was examined within ranges of 0.1–6.0 and 1.0–7.0 mM for normal and reverse FIA systems, respectively. Maximum analytical signals were obtained for the concentrations 1 and 4 mM for the normal and reverse systems, respectively, Fig. 4A, and were used for further work. DOX molecule has several phenolic groups. Previous research showed that the presence of an alkaline medium is required for the coupling reaction to convert the Ar-OH group of BZ to a reactive phenoxide group.[33] As a result, the influence of various bases was examined. According to the results in Fig. 4B, the greatest analytical signal was achieved when sodium hydroxide was utilized in both FIA systems. Additionally, for nFIA and rFIA methods, the concentrations of NaOH were examined between 0.01–1 and 0.02–1 M, respectively. The highest absorbance intensities were obtained at 0.2 M for normal and 0.1 M for reverse FIA systems, Fig. 4C.

Study of the physical parameters

Total flow rate

Flow rate is a significant physical factor since it affects the time necessary for the production of the colored product and, as a result, the sampling rate. Therefore, the effect of the total flow rate for the normal and reverse flow systems was investigated in the range of 1.77–10.73 mL/min. As shown in Fig. 5A, the analytical signals were increased as the flow rate increased to 5.83 and 4.0 mL/min for nFIA and rFIA systems, respectively, and then gradually decreased. The dispersion effect and shortened residence time may be responsible for the reduced analytical signal



Scheme 1. Proposed reaction pathway.

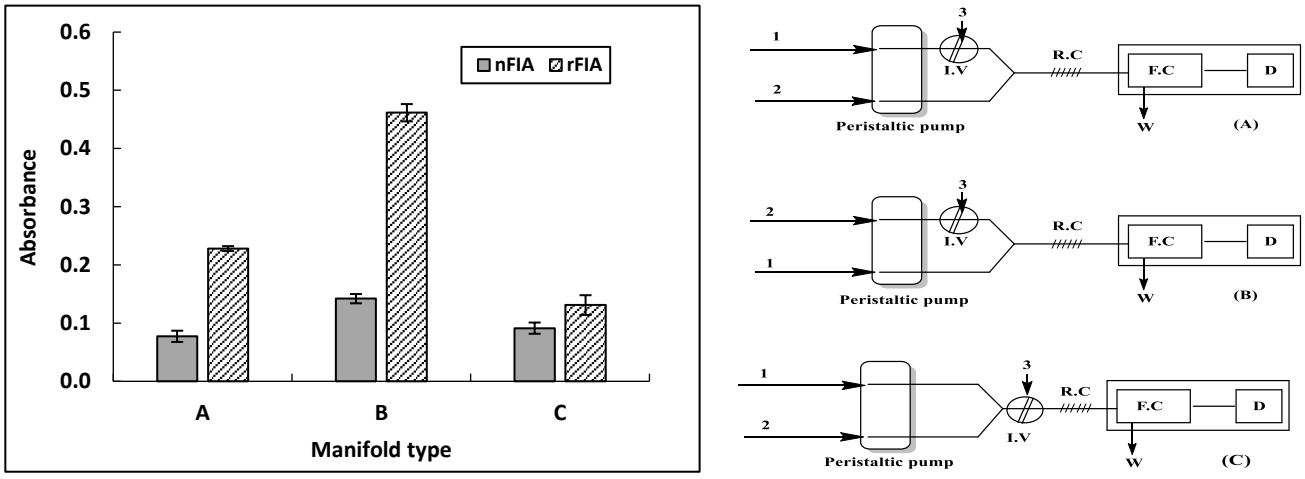


Fig. 3. Effect of manifold design (left) and the manifold designs (right). [For nFIA (1) DBZ, (2) NaOH, and (3) DOX, for rFIA (1) NaOH, (2) DOX, and (3) DBZ].

Table 1. Selected FIA factors for the assay of DOX using normal and reverse FIA methods.

	Studied range		Optimum value	
FIA factors	nFIA	rFIA	nFIA	rFIA
Chemical factors				
Conc. of DBZ (mM)	0.1–6.0	1.0–7.0	1	4
Type of base	NaOH, KOH, NH_4OH, Na_2CO_3		NaOH	NaOH
Conc. of NaOH (M)	0.02–1		0.2	0.1
Physical factors				
Total flow rate (mL/min)	1.77–10.73		5.83	4.0
Length of reaction coil (cm)	0–250		75	50
Injected volume (μL)	50–250		100	100

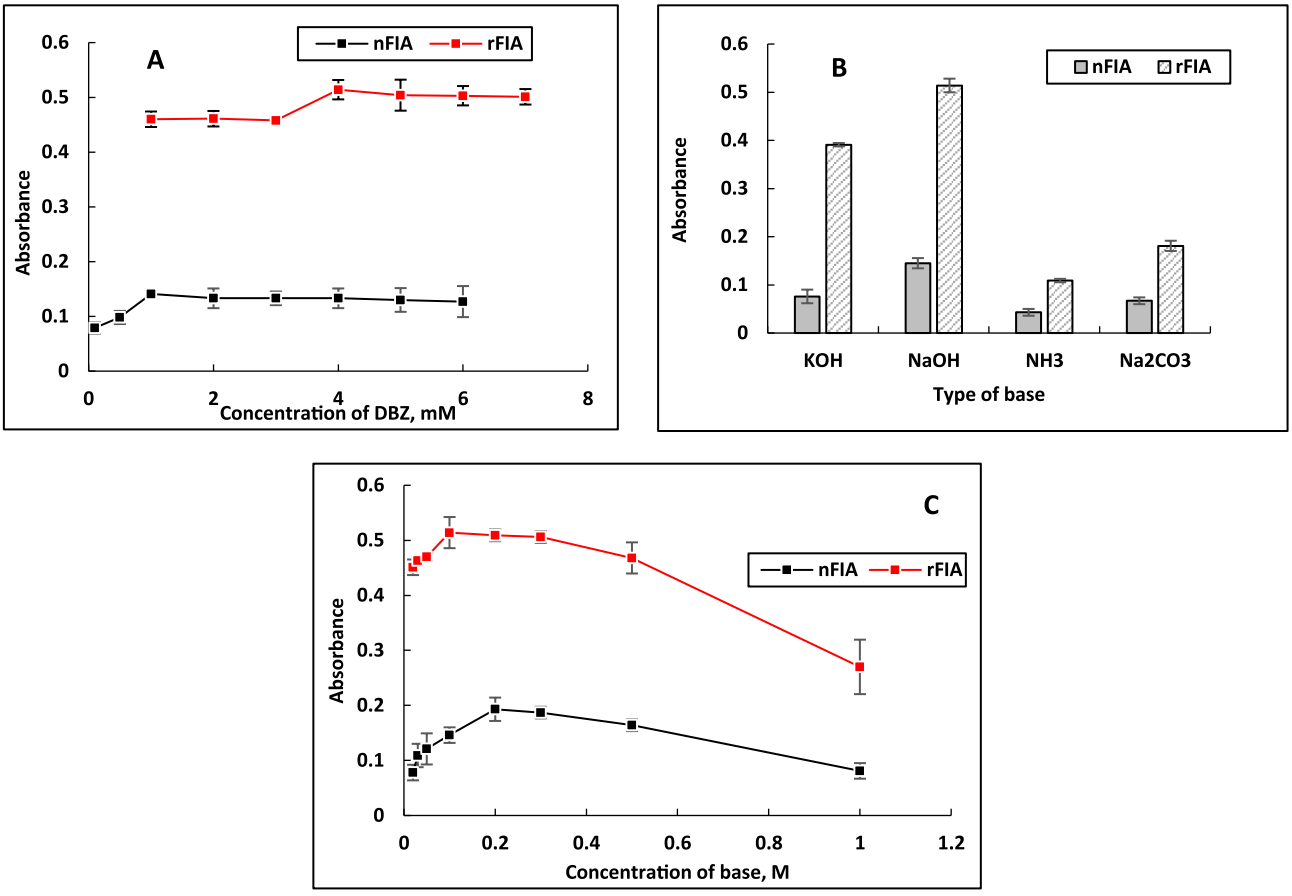


Fig. 4. Study of (A) conc. of DBZ, (B) type of base, (C) conc. of NaOH.

Table 2. Analytical characteristics of the suggested methods.

Parameter	Value	
	nFIA	rFIA
Regression equation	y = 0.0025x + 0.0314	y = 0.0055x + 0.1149
Linear range (μg/mL)	10–250	1–180
Correlation coefficient, r	0.9995	0.9992
Detection limit (S/N = 3) (μg/mL)	2.95	0.46
Limit of quantification (μg/mL)	9.83	1.55
Molar absorptivity, ε (L/mol cm)	1.28×10^3	2.82×10^3
Sandell's sensitivity, S (μg/cm^2)	0.40	0.18
Reproducibility, %	<1.70	<1.12
Recovery, %	99.11–99.73	98.15–99.90
Slope, b (mL/μg)	0.0025	0.0055
Intercept, a	0.0314	0.1149
Standard deviation of residuals, $S_{y/x}$	9.16×10^{-3}	1.50×10^{-2}
Standard deviation of slope, S_b	3.39×10^{-5}	7.64×10^{-5}
Standard deviation of intercept, S_a	4.84×10^{-3}	7.78×10^{-3}

at high flow rates. As a result, it was determined that 5.83 and 4.0 mL/min, respectively, were the optimal rates for nFIA and rFIA systems.

Reaction coil and injected sample volume

To investigate the effect of reaction coil length, several lengths of reaction coil ranging from 0–200 cm were used. For normal and reverse FIA methods, the reaction response reached the highest value at 75 and 50 cm, respectively. Due to the rise in dispersion, the response of both FIA systems began to steadily diminish as the length of the reaction coil increased by more than 75 and 50 cm, for both FIA systems respectively, Fig. 5B. As a result, 75 and 50 cm coil lengths

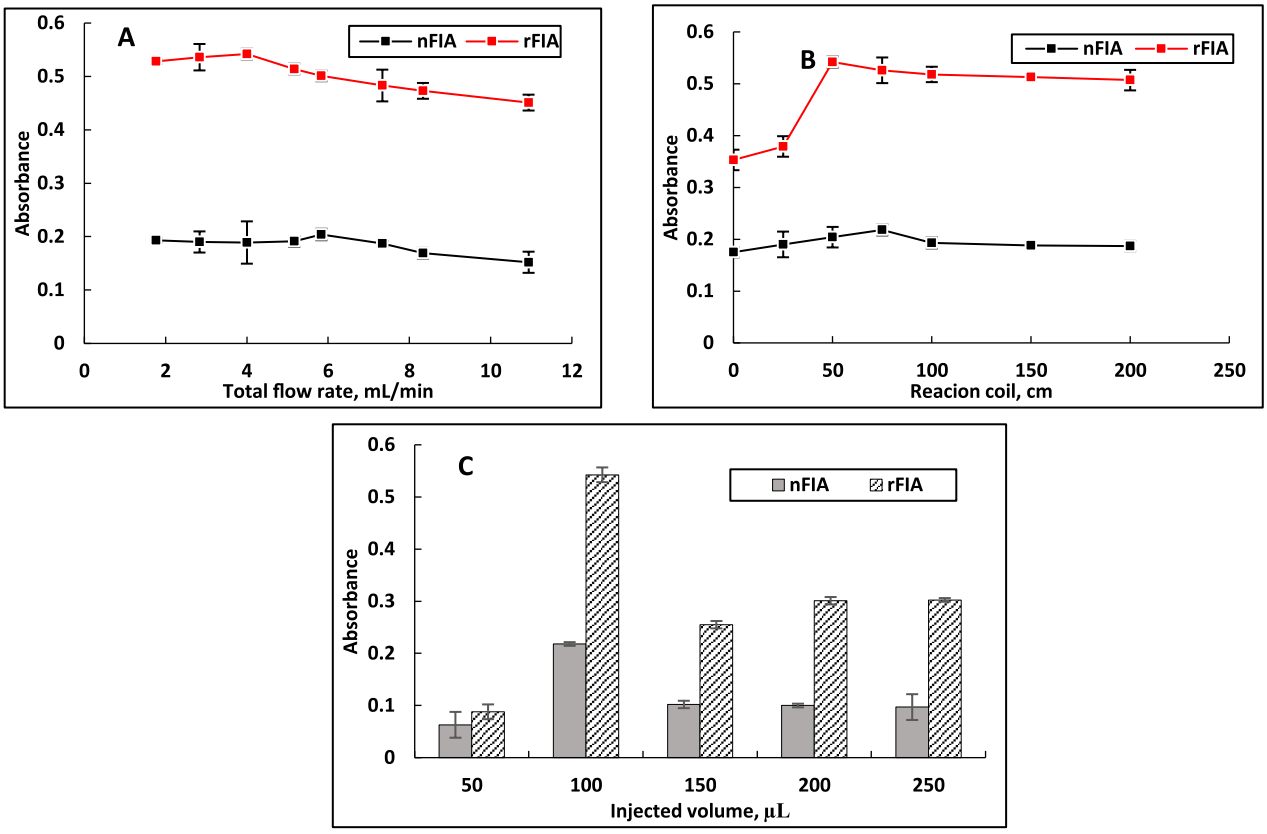


Fig. 5. Influence of (A) flow rate; (B) reaction coil length; and (C) injected volume.

Table 3. Intra and inter-day accuracy and precision for determination of DOX using FIA systems.

		Intra-day (n = 5)				Inter-day (n = 15)			
Method	Taken conc. (μg/mL)	Found conc. (μg/mL)	Relative error (%)	Recovery (%)	RSD (%)	Taken conc. (μg/mL)	Relative error (%)	Recovery (%)	RSD (%)
nFIA	60	59.84	–0.27	99.73	1.70	59.68	–0.53	99.47	1.85
	90	89.20	–0.89	99.11	0.81	89.76	–0.27	99.73	1.26
	150	149.44	–0.37	99.63	0.67	150.05	0.03	100.03	1.43
rFIA	60	60.56	0.93	100.93	0.79	61.21	2.02	102.02	0.78
	90	91.07	1.19	101.19	0.54	90.99	1.10	101.10	0.48
	150	149.80	–0.13	99.87	0.40	149.99	–0.01	99.99	0.36

were selected as optimum lengths. The optimal volume of analyte or reagent injected for both the normal and reverse FIA manifolds was investigated. In this study, several lengths of loop connected to the injection valve with volumes ranging from 50–250 μL were used. The results in Fig. 5C showed that, for both methods, 100 μL of injected volume produced the maximum absorbance with good precision. Due to the

Table 4. Analysis of DOX in the presence of common excipients using nFIA.

Excipients (1000 μg/mL)	Amount of DOX (μg/mL)		(Rec.± SD) % n = 5)
	Added	Found	
Glucose		75.84	101.12 ± 0.42
Polyvinylpyrrolidine		74.52	99.36 ± 0.31
Talc	75	74.31	99.08 ± 0.16
Mg stearate		76.13	101.51 ± 0.38
Starch		74.93	99.91 ± 0.26
All additives		74.38	99.17 ± 0.53

Table 5. Estimation of DOX in tablets using nFIA and rFIA methods.

	Proposed methods															
	nFIA method						rFIA method				UV method					
Dosage form	Taken conc. (μg/mL)	Spiked conc. (μg/mL)	Found conc. (μg/mL)	Rec. (%)[a]	Mean Rec. ± SD (%)	RSD (%)[a]	Found conc. (μg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]	Taken conc. (μg/mL)	Spiked conc. (μg/mL)	Found conc. (μg/mL)	Rec. (%)[a]	Mean Rec. (%)	RSD (%)[a]
VIBRAM-YCIN®	30	30	58.96	98.27	99.82 ± 1.10	2.19	60.72	101.20	99.98 ± 0.87	0.75	20	20	39.91	99.78	99.19 ± 0.74	1.42
		40	69.39	99.13		0.87	70.03	100.04		0.21		30	49.15	98.30		1.88
		50	78.53	98.16		1.46	79.14	98.93		0.56		40	60.18	100.30		1.36
	40	30	70.34	100.49		1.18	70.65	100.93		0.42	30	20	49.30	98.60		1.34
		40	80.72	100.90		1.22	79.30	99.13		0.53		30	59.48	99.13		0.95
		50	89.88	99.87		0.94	90.41	100.46		0.61		40	69.91	99.87		1.57
	50	30	80.71	100.88		0.76	79.64	99.55		0.64	40	20	59.65	99.42		1.42
		40	90.88	100.98		1.35	90.56	100.62		0.50		30	69.46	99.23		1.99
		50	99.73	99.73		1.31	98.97	98.97		0.54		40	78.48	98.10		1.89
Accord®	30	30	59.17	98.62	99.70 ± 1.01	1.12	59.84	99.73	99.87 ± 0.60	1.34	20	20	39.46	98.65	99.46 ± 0.84	0.87
		40	69.18	98.83		1.34	70.05	100.07		0.61		30	49.41	98.82		2.05
		50	79.93	99.91		1.36	80.39	100.49		0.78		40	59.91	99.85		1.92
	40	30	70.14	100.20		2.28	69.97	99.96		0.66	30	20	50.26	100.52		1.93
		40	78.52	98.15		1.79	79.39	99.24		0.78		30	60.36	100.60		1.24
		50	90.73	100.81		1.07	90.50	100.56		1.05		40	69.02	98.60		1.85
	50	30	80.86	101.08		1.52	79.03	98.79		0.89	40	20	59.12	98.53		1.66
		40	89.49	99.43		1.92	89.59	99.54		0.84		30	70.09	100.13		1.54
		50	100.29	100.29		0.69	100.42	100.42		0.61		40	79.52	99.40		1.29
Pure DOX					99.49 ± 0.33				100.66 ± 0.69						99.73 ± 0.25	
t (2.78)[b]					1.14				2.42							
F (19.00)[b]					2.61				2.51							

[a], (n = 5); [b], Theoretical value; RSD, relative standard deviation; Conc., concentration.

high sample-to-reagent volume ratio and increased dispersion, absorbance drops over 100 μL. Table 1 contains an overview of the optimal values for the investigated FIA variables.

Validation of the suggested methods

After examining all physical and chemical variables of both FIA systems, the calibration curves were produced under optimal conditions. Several standard DOX solutions were injected and/or pumped to determine the calibration graphs' linearity for normal and reverse FIA systems, respectively. Several statistical values are included in Table 2, together with the regression equations, slope, correlation coefficient, and molar absorptivity values. The results indicated sufficient accuracy, good linearity, and high sensitivity for DOX estimation.[34] The linearity of the calibration curve ranges from 10–250 μg/mL (LOD 2.95 μg/mL, % RSD $<$ 1.70, n $=$ 5) for the nFIA method and 1–180 μg/mL (LOD 0.46 μg/mL, %RSD $<$ 1.12, n $=$ 5) for rFIA. The small values of the standard deviation of the residuals, slope, and intercept revealed the precision of the proposed approaches as well as the minimal scattering of calibration curve points.

Reproducibility and accuracy of methods

The accuracy and precision of both normal and reverse FIA systems were tested by assaying three different concentrations of DOX solutions five replicates on the same day and over six consecutive days (intra and inter-day variation, respectively). For both methods, the results in Table 3 showed good precision (low values of RSD 0.67–1.70 and 0.40–0.79%, respectively) and a high accuracy (recovery values within the range of 99.11–99.73 and 99.87–101.19%, respectively).

Interferences effect

To evaluate the suitability of the proposed FIA methods, 75 μg/mL of doxycycline was spiked with an excess concentration of various excipients (1000 μg/mL), which frequently accompany the medication in its dosage forms.[35] A good percentage of recoveries were obtained indicating no interference was observed from any of these excipients, indicating a high selectivity for determining the DOX in its dosage forms Table 4.

Sampling rate

The sampling frequency or sampling rate can be computed theoretically by observing the time interval between the injection of the sample and the appearance of the maximum absorbance. This duration was equivalent to 42 and 49 seconds for nFIA and rFIA, respectively, and hence a sample rate of 85 and 73 samples per hour could be achieved for both normal and reverse FIA, respectively.

Assay of DOX in pharmaceutical forms

Two different types of commercial pharmaceutical forms (tablets and capsules) containing doxycycline monohydrate as active ingredient were analyzed to determine the applicability of the recommended FI methods. By spiking pharmaceutical applications at three concentration levels with varying amounts of DOX and analyzing them using the two FIA systems,[36] recovery tests proved the reliability of the presented procedures. Table 5 shows that the obtained spiking recoveries (calculated as the mean value of five determinations) ranged from 99.70–99.82 % and 99.19–99.49 % for normal and reverse FIA respectively, demonstrating the high accuracy of the methods and independence from the effect of the matrix. A statistical comparison was additionally conducted between the proposed FIA methods and UV method (measured at wavelength 278 nm)[37] using student's t and F tests,[38] and the results indicated a non-significant difference in accuracy and precision between the two methods.

Conclusion

The current study developed two types of FIA methods (normal and reverse) for sensitive determination of DOX in bulk and dosage forms without involving temperature control or a separation step. FIA methods provided a high sampling frequency (85 and 73 samples sample/h for nFIA and rFIA, respectively) as well as a wide range of linearity (10–250 and 1–180 μg/mL for both systems respectively) when compared to the conventional batch methods. In addition, FIA procedures are regarded as economical and inexpensive when compared to other techniques such as GC, HPLC, and CE. In addition, when compared to other FIA-spectrophotometric methods, the reverse flow injection approach provided great sensitivity (LOD was less than 0.5 μg/mL) and suitability when utilizing toxic or costly chemicals. Acceptable recoveries (99.70–99.82% and 99.19–99.49% for nFIA and rFIA, respectively) and relative standard deviation values (RSD less than 2% for both methods) obtained from DOX analysis in various applications confirmed the accuracy and precision of both FIA methods. The suggested procedures were used successfully in the assessment of various pharmaceutical forms containing DOX.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' contribution statement

H.H designed the study, performed the interpretation, drafting the MS, revision and proofreading. T.M. performed the experiments and analyzed the data, wrote the paper with input from all authors.

References

1. Chopra I, Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Mircrobiol Mol Rev. 2001;65(2):232–60. https://doi.org/10.1128/mmbr.65.2.232-260.2001
2. Wormser GP. Doxycycline for prevention of spirochetal infections: status report. Clin Infect Dis. 2020;71(8):2014–7. https://doi.org/10.1093/cid/ciaa240
3. Joshi N, Miller DQ. Doxycycline revisited. Arch Intern Med. 1997;157(13):1421–8. https://doi.org/10.1001/archinte.1997.00440340035003
4. Lees KA, Orlandi RR, Oakley G, Alt JA. The role of macrolides and doxycycline in chronic rhinosinusitis. Immunol Allergy Clin North Am. 2020;40(2):303–15. https://doi.org/10.1016/j.iac.2019.12.005
5. Miow QH, Vallejo AF, Wang Y, Hong JM, Bai C, Teo FS, *et al.* Doxycycline host-directed therapy in human pulmonary tuberculosis. J Clin Invest. 2021;131(15):e141895. https://doi.org/10.1172/JCI141895
6. Wüst RC, Coolen BF, Held NM, Daal MR, Alizadeh Tazehkandi V, Baks-te Bulte L, *et al.* The antibiotic doxycycline impairs cardiac mitochondrial and contractile function. Int J Mol Sci. 2021;22(8):4100. https://doi.org/10.3390/ijms22084100
7. Injac R, Kac J, Kreft S, Strukelj B. Determination of doxycycline in pharmaceuticals and human urine by micellar electrokinetic capillary chromatography. Anal Bioanal Chem. 2007;387:695–701. https://doi.org/10.1007/s00216-006-0900-9
8. Abdulghani RH, Hassan RF. Development and Validation of Spectrophotometric Methods for the Quantitative Determination of Doxycycline Hyclate in Pure Form and Pharmaceutical Formulations Using Flow-Injection and Batch procedures: A Comparative Study. 2023;8(13):e202300183. https://doi.org/10.1002/slct.202300183
9. Sahoo PK, Sahoo P, Mohapatra J, Panigrahi D, Patra AK, Mishra A. Development and Validation of Stability Indicating Assay Method of Doxycycline Hyclate by using UV-Spectrophotometer. J Drug Deliv Ther. 2023;13(6):89–94. https://doi.org/10.22270/jddt.v13i6.6087
10. Abdulsattar JO, Richardson S, Iles A, Pamme N. Detection of doxycycline hyclate and oxymetazoline hydrochloride in pharmaceutical preparations via spectrophotometry and microfluidic paper-based analytical device (μPADs). Anal Chim Acta. 2020;1136:196–204. https://doi.org/10.1016/j.aca.2020.09.045
11. Becze A, Resz M-A, Ilea A, Cadar O. A Validated HPLC Multichannel DAD Method for the Simultaneous Determination of Amoxicillin and Doxycycline in Pharmaceutical Formulations and Wastewater Samples. Appl Sci. 2022;12(19):9789. https://doi.org/10.3390/app12199789
12. Pippalla S, Nekkalapudi AR, Jillellamudi SB, Reddy MP, Kumar CV. A stability-indicating, reversed-phase HPLC method for quantification of assay and organic impurities in doxycycline hyclate bulk and parenteral dosage forms. Chromatogr A. 2023;37(6):e5626. https://doi.org/10.1002/bmc.5626
13. Comparative Bioequivalence Studies of doxysam and doxycycline (Pfizer) by reversed phase HPLC. Baghdad Sci J 2010;7(1):601–6. https://doi.org/10.21123/bsj.2010.7.1.601-606
14. Al-Abachi MQ, Al-Nedawi ZA. Batch and flow injection spectrophotometric determination of tetracycline hydrochloride and doxycycline hyclate in pharmaceutical preparations. Iraqi J Sci. 2015;56(2A):909–20.
15. Tawfeeq AH, Qassim BB. A green method for assay of doxycycline hyclate using continuous flow injection/merging zones technique via coupling with azo metol in aqueous medium. Vopr. Khimii Khimicheskoi Tekhnologii. 2020;4:31–7. https://doi.org/10.32434/0321-4095-2020-131-4-31-37
16. Tawfeeq AH, Qassim BB. A novel method of CFIA/Merging zones technique for assay of Doxycycline in bulk and pharmaceutical preparation depending on azo dye formation. Res J Pharm Technol. 2021;14(1):67–74. https://doi.org/10.5958/0974-360X.2021.00013.5
17. Abed SS, Hussein OT. 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). Baghdad Sci J. 2016;13(2.2 NCC):0489. http://dx.doi.org/10.21123/bsj.2016.13.2.2NCC.0489
18. Gajda A, Posyniak A, Zmudzki J, Tomczyk G. Determination of doxycycline in chicken fat by liquid chromatography with UV detection and liquid chromatography–tandem mass spectrometry. J Chromatogr B. 2013;928:113–20. https://doi.org/10.1016/j.jchromb.2013.03.011
19. Hadi H, Mouayed M. Determination of nitrofurantoin in pharmaceutical preparations using flow injection-spectrophotometry. J Assoc Arab Univ Basic Appl Sci. 2017;24:74–80. https://doi.org/10.1016/j.jaubas.2017.03.005
20. Turkey N, N Jeber J. Flow Injection Analysis with Turbidity Detection for the Quantitative Determination of Mebeverine Hydrochloride in Pharmaceutical Formulations. Baghdad Sci J. 2022;19:141–54. http://dx.doi.org/10.21123/bsj.2022.19.1.0141
21. Sadiq HA, Hammood MK. A Continuous Flow Injection/Indirect Photometric Method for the Detection of Fosetyl Aluminum in Commercial Pesticide Formulations. ChemistrySelect. 2022; (15):e202104605. https://doi.org/10.1002/slct.202104605
22. Raju CV, Rani GM, Haribabu J, Kumar SS. Flow injection analysis-based electrochemiluminescence: an overview of experimental design and its biosensing applications. ECS

Sensors Plus. 2022;1(3):031604. https://doi.org/10.1149/2754-2726/ac8d70
23. Wang Y, Rink S, Baeumner AJ, Seidel M. Microfluidic flow-injection aptamer-based chemiluminescence platform for sulfadimethoxine detection. Mikrochim Acta. 2022;189(3):117. https://doi.org/10.1007/s00604-022-05216-6
24. Mohammed FF, Abed SSAS. Flow Injection Spectrophotometric Technique for Determining of Genistein in Pure and Supplements Formulations Through Diazotization Coupling Reaction. Iraqi J Pharm Sci. 2022;31(1):278–84. https://doi.org/10.1002/slct.202300183
25. Abed SS, Hussein OT. Spectrophotometric determination of vancomycin hydrochloride (batch and flow-injection) using O-nitroaniline as diazotized chromogenic reagent. Iraqi J Sci. 2015;56(4B):3025–35. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/9341
26. Hassan RF. New mode semi-automated turbidimetric determination of mefenamic acid by Ayah 6SX1-ST-2D solar cell-CFI analyser. Res J Pharm Technol. 2019;12(12):5773–80. https://doi.org/10.5958/0974-360X.2019.00999.5
27. Turkey N, Hameed S. A Pioneered Homemade NAG-4SX3-3D Analyzer Coupled with Continuous Flow Injection Analysis New Approach for The On- Line Turbidimetric Measurements of Metronidazole in Pure and Pharmaceutical Formulations. Chem Methodol. 2023;7:53–66. https://doi.org/10.22034/CHEMM.2022.356013.1592
28. Mohammed FF, Abed SSAS. Flow Injection Spectrophotometric Technique for Determining of Genistein in Pure and Supplements Formulations Through Diazotization Coupling Reaction. Iraqi J Pharm Sci. 2022;31(1): 78–84. https://doi.org/10.31351/vol31iss1pp278-284
29. Hadi H. Normal and Reverse Flow Injection Analysis Methods for Estimation of Mesalazine in Pharmaceutical Dosage Forms. J Appl Sci. 2023;36(4):232–45. https://doi.org/10.30526/36.4.3143
30. Jasim AN, Turkey NS, Abd-Alrazack HF. Design a continuous microfluidic flow cell for turbidimetric-flow injection technology: A new approach for routine analysis of active pharmaceutical formulations. J Environ Anal Chem. 2022;5(02):90–104. https://doi.org/10.24200/amecj.v5.i02.184
31. Abed SS. Spectrophotometric and reverse flow injection method determination of nitrazepam in pharmaceuticals using O-coumaric acid as a new chromogenic reagent. Baghdad Sci J. 2020;17(1 (Suppl.)):0265. https://dx.doi.org/10.21123/bsj.2020.17.1(Suppl.).0265
32. Mohammed FF, Abed SSAS. Flow Injection Spectrophotometric Technique for Determining of Genistein in Pure and Supplements Formulations Through Diazotization Coupling Reaction. Iraqi J Pharm Sci. 2022;31(1):278–84. https://doi.org/10.31351/vol31iss1pp278-284
33. Hadi H, Mouayed M. Determination of clonazepam in pharmaceutical preparations using simple high-throughput flow injection system. J Anal Chem. 2017;72:226–33. https://doi.org/10.1134/S1061934817020058
34. Hassan B, Hadi H. Development of continuous flow injection analysis method for determination of oxymetazoline and vancomycin hydrochloride in pharmaceutical preparations. Bull Chem Soc Ethiop. 2022;36(2):303–13. https://doi.org/10.4314/bcse.v36i2.5
35. Al-Fatlawi FKK, Alkhafaji AMM, Al-Rufaie MMM. Simple spectrophotometric assay for doxycycline drug in their pure form by using Enzymatic approaches. Cardiometry. 2023;(26):475–80. https://doi.org/10.18137/cardiometry.2023.26.475480
36. Pippalla S, Nekkalapudi AR, Jillellamudi SB, Reddy MP, Kumar CV. A stability-indicating, reversed-phase HPLC method for quantification of assay and organic impurities in doxycycline hyclate bulk and parenteral dosage forms. J Chromatogr B. 2023;37(6):e5626. https://doi.org/10.1002/bmc.5626
37. Gholse YN, Chaple DR, Kasliwal RH. Development and validation of novel analytical simultaneous estimation based UV spectrophotometric method for doxycycline and levofloxacin determination. Biointerface Res App Sci. 2022;12(4):5458–78. https://doi.org/10.33263/BRIACI124.54585478
38. Miller J, Miller JC. Statistics and chemometrics for analytical chemistry: Pearson education. 2018. https://www.africanfoodsafetynetwork.org/wp-content/uploads/2021/09/Statistics-and-Chemometrics-2010.pdf

التقدير الطيفي بطريقة الحقن الجرياني العادي والعكوس للدوكسي سايكلين هايكليت في صيغته النقية والمستحضرات الصيدلانية

تبارك محمد، هند هادي

قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد، العراق.

المستخلص

يتضمن هذا البحث تطوير وتقييم طريقة تحليلية لتقدير الدوكسيسيكلين هيكليت (DOX) في شكله النقي والادوية الصيدلانية عن طريق تقنيات التحليل الحقن الجرياني العادي(nFIA) و العكوس(rFIA). تم استخدام نوعين من طرق الحقن الجرياني عن طريق إتمام تفاعل الازوتة والازدواج للدوكسيسيكلين(DOX) مع ثنائي هيدروكلوريد البنزيدين المؤزوت حيث تتكون صبغة حمراء حساسة يتم قياسها عند الطول الموجي488 نانومتر .تم اعداد منحنيات المعايرة لتراكيز مختلفة للدوكسيسيكلين (DOX) ضمن مدى خطي يتراوح بين 10-250 و1-180 ميكروغرام/مل لكلا النظامين العادي والعكوس على التوالي. مع حدود الكشف 2.95 و0.46 ميكروغرام/مل وحدود التقدير 9.83 و 1.5 ميكروغرام/مل . لكلا النظامين على التوالي. تم استخدام الطرق المطورة لتحديد الدوكسيسيكلين في اشكال الجرعات الصيدلانية مع نسبة استرداد عالية (Rec) ونسبة انحراف قياسي نسبي (RSD)منخفضة بالمقارنة مع الطريقة الطيفية. أظهرت التقنيات المقترحة كشفا سريعاً وقابلية للتطبيق في تحليل المستحضرات الصيدلانية.

الكلمات المفتاحية: ثنائي هيدروكلوريد البنزدينو الدوكسيسيكلين هيكليت، أزوتة، تحليل الحقن الجرياني، القياس الطيفي.