Flow injection spectrophotometric determination of Tetracycline hydrochloride in pharmaceutical samples

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Abstract

Tetracycline hydrochloride (TCH) was determined spectrophoto-metrically in the pure form and in the pharmaceutical formulations using flow injection analysis (FIA). The automated method is based on the reaction between TCH, sodium nitroprusside and hydroxylamine hydrochloride in alkaline medium. The product absorbed maximally at 529 nm. The calibration graph of TCH is linear from 25-1000 μg mL^{-1} with detection limit (S/N=3) of 7.589 μg mL^{-1} and sample through put of 69 h^{-1}. The various chemical and physical variables were optimized and the proposed procedure was applied successfully for estimation of drug in different commercial forms.

Key words: Tetracycline hydrochloride, hydroxylamine hydrochloride, spectrophotometry.

الخلاصة

تم تقدير التتراسايكلين هيدروكلورايد بشكله النقي وفي المستحضرات الصيدلانية طيفيا باستخدام تقنية التحليل بالحقن الجرياني.تعتمد الطريقة المؤتمتة على التفاعل بين التتراسايكلين وهيدروكسيل امين هيدروكلورايد في الوسط القاعدي.الناتج اعطى اعلى امتصاص عند 529 نانومترا وبلغ مدى الخطية من 25–1000 مايكروغرام من التتراسايكلين هيدروكلورايد لكل مللتر من المحلول وبحد كشف 7.589 مايكروغرام $مل^{-1}$ وبمعدل نمذجة 69 نموذج بالساعة.تمت دراسة جميع التغيرات الفيزيائية والكيميائية وتم تطبيق الطريقة وبنجاح لتقدير الدواء بمختلف اشكاله التجارية.

مفتاح الكلمات: التتراسايكلين هيدروكلورايد ، هيدروكسيل امين هيدروكلورايد ، التقدير الطيفي.

Introduction

The tetracycline antibiotics are active against a wide range of Gram-positive and Gram-negative bacteria, being widely used in human and veterinary medicines as well as feed additives[1].Chemically, TCH is:4s-(48, 1α, 4aα, 5aα, 6β,12aα))-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3 ,6 ,10,12,12a-pentahydroxy-6- methyl-1,11-dioxo-2-naphthacene carboxamide mono hydrochloride.Several analytical methods have been reported for the determination of TCH in raw material and dosage forms including spectrophotometry [2-4], voltametry [5, 6], HPLC methods [7] and flow injection analysis(FIA) methods [8,9] ,however, some of these methods are not only expensive and time consuming but also are poor in terms of sensitivity and specificity.FIA technique has found recently wide applications mainly due to reduction of the analysis time and reagent consumption compared with conventional manual procedures. In this paper, FI method using spectrophotometric detection at 529 nm is described for the determination of TCH. The batch method [10] was adopted as a basis to develop a FIA method FIA-spectrophotometric method based on reaction between TCH and hydroxylamine hydrochloride (HAH) in the presence of sodium nitroprusside (SNP) in alkaline medium to form an intense dark red color product which shows an absorption maximum at 529 nm.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV–vis 260 digital double beam recording spectrophotometer. A flow cell with 50 µL internal volume and 1 cm bath length was used for the absorbance measurements. A three-channel manifold (Fig.1) was employed for the FIA spectrophotometric determination of TCH drug. A peristaltic pump (Ismatec, Labortechnik –Analytik, CH–8152, Glatbrugg – Zurich – Switzerland) was used to transport the carries solutions. (Rheodyne, Altex 210,Supelco–USA) injection valve 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. Channel A was used to transport SNP, channel B to transport sodium hydroxide and

channel C to transport HAH solution. The sample was injected into the stream of the mixture of SNP with sodium hydroxide solution, through the injection valve. Solutions were propelled by peristaltic pump with individual flow rate of 0.6 mL min^{-1}. The absorbance was measured at 529 nm.

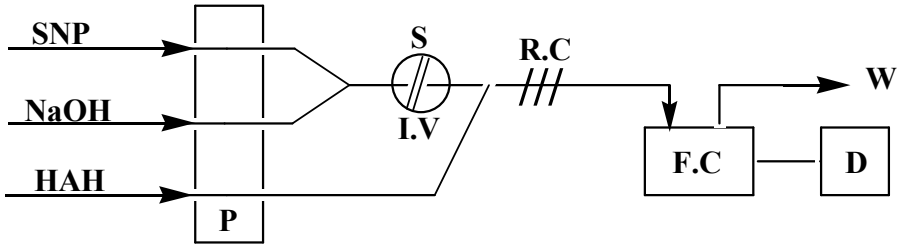


Fig. 1: Manifold employed for FIA-Spectrophotometric determination of TCH with SNP,HAH solution in alkaline medium where IV, Injection valve ; R.C,Reaction Coil ;S, Sample(TCH) ; P, Peristaltic pump ; F.C ,Flow cell ; D , Detector ; W, Waste.

Reagents

- ***Standard tetracycline hydrochloride solution*:** stock solution (1000µg mL^{-1}) was prepared by dissolving 0.1000 g of the pure compound (kindly provided from state company for Drug Industries and Medical Appliance, SDI, Samara. Iraq) in a sufficient amount of distilled water and diluted to 100 mL in a volumetric flask with the same solvent. Working solution (200 µg mL^{-1}) was prepared by a simple dilution with the same solvent.
- ***Sodium nitroprusside solution (BDH, UK):***10 mM was prepared by dissolving 0.2980 g of SNP in 100 mL of distilled water.
- ***Hydroxylamine hydrochloride solution (Merck, Darmstadt, Germany):*** 20mM was prepared by dissolved 0.1389 g of HAH in 100 mL of distilled water.
- ***Sodium hydroxide (BDH, UK):***100 mM was prepared by dissolving 0.4000 g of the base in a 100 mL of distilled water.

General FIA procedure

Working solutions of TCH in the range cited in Table 1 were prepared from stock solutions. A 150 µL portion of TCH was injected into the stream of the mixture of 10 mM SNP and 100 mM sodium hydroxide solution and was then combined with a stream of 20mM HAH with a flow rate of 0.6 mL min^{-1} in each channel (Fig. 1). The resulting absorbance of the red dye

was measured at 529 nm and a calibration graph was prepared over the range cited in Table 1. Optimization of conditions was carried out on 200 μgmL^{-1} of TCH.

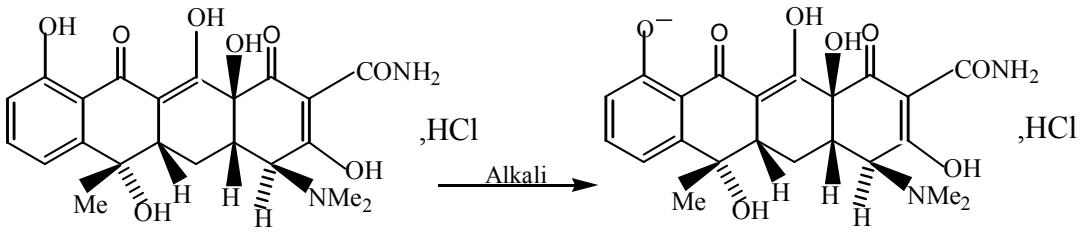
Procedure for capsules and ointment

Capsules samples: An accurately weight amount of 10 powdered capsules equivalent to 50 mg of the pure drug was dissolved in 10 mL distilled water and transferred into 50 mL volumetric flask and completed to the mark with the same solvent .The flask with its contents was shaked well and filtered to obtain 1000 ppm. The measurements were carried out at 529 nm as described earlier under general procedures.

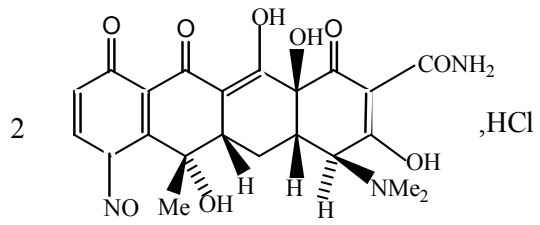
Ointment samples: The contents of five tubes of ointment were mixed. The accurately weighed amount of ointment equivalent to 100 mg of TCH was dissolved in 20 mL diethyl ether and extracted with three 20 mL of 0.01M HCl . The solution was filtered into a 100 mL volumetric flask, the residue was washed with 0.01M HCl and diluted to volume with the same solvent to obtain 1000 µg mL^{-1} of TCH.

Results and Discussion

Tetracycline hydrochloride forms a dark red colored product (λ_{max} at 529 nm) with SNP and HAH in alkaline medium. The batch method [10] for the determination of TCH was adopted as a basis to develop FIA procedure. According to the previous batch methods suggested by Hadi et.al, the mechanism of the reaction was postulated in scheme 1.



$$2\,[Fe(CN)_5NO]^{2-} + 2\,(I) + 6NH_2OH + 6\,OH^- \rightleftharpoons [Fe_2(CN)_{10}]^{10-} + 3\,N_2 + 12H_2O$$



Scheme 1. Proposed mechanism of the reaction between TCH and SNP

The manifold used for the determination of TCH was so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of TCH drug with SNP and HAH in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample was injected into a stream of mixed SNP with sodium hydroxide and was then combined with the stream of HAH (Fig.1). The influence of different chemical and physical FIA parameters on the absorbance intensity of the colored product was optimized as follows.

Optimization of reagents concentration

Effect of SNP concentration

The effects of various concentrations of SNP were investigated. A concentration of 10 mM gave the highest absorbance and was chosen for further use. The results are shown in Fig. 2.

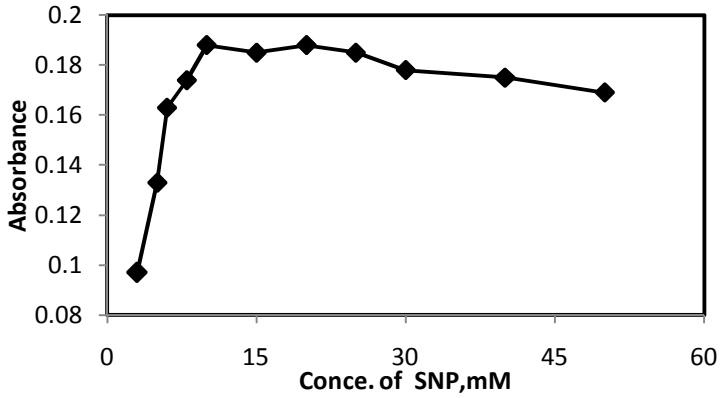


Fig.2: Effect of the concentration of SNP (mM)

The effects of various concentrations of HAH in the range of 3 to 100 mM were investigated. A concentration of 20 mM gave the highest absorbance and was chosen for further use. The results are shown in Fig.3.

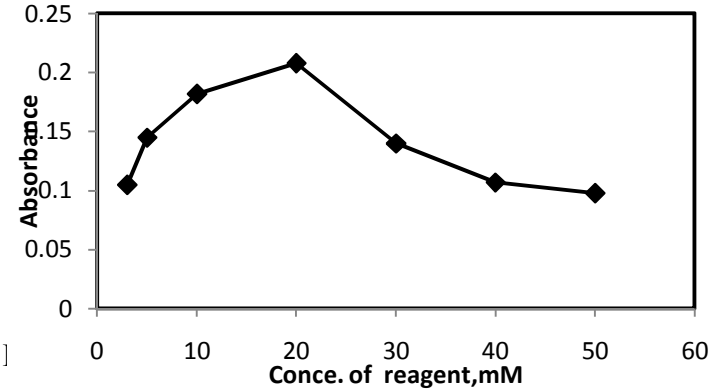


Fig.3: Effect of the concentration of HAH (mM)

Effect of alkaline medium

The sensitive red product was formed only in alkaline medium therefore, the effect of different alkaline solutions were studied such as sodium acetate, sodium carbonate, sodium hydroxide and ammonium hydroxide. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution. The effect of sodium hydroxide concentration on the color reaction was studied in the range of 20-400 mM. A concentration of 100 mM gave the highest absorbance and was chosen for further use (Fig.4).

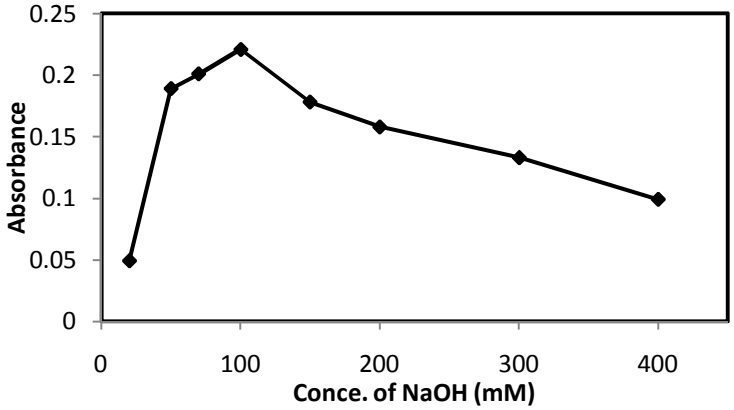


Fig.4: Effect of the concentration of NaOH (mM)

Optimization of manifold parameters

The variables studied under the optimized reagent concentrations were the flow rate, the injected sample volume and the reaction coil length.

The effect of flow rate on the sensitivity of the colored reaction product was investigated in the range of 0.75–3.25 mL min^{-1}. The results obtained showed that a total flow rate of 1.7 mL min^{-1} (0.6 mL min^{-1} in each line) gave the highest absorbance as shown in Fig.5 and was used in all subsequent experiments. The volume of the injected sample was varied between 50 and 250 µL using different length of sample loop. The results obtained showed that injected sample of 150 µL gave the best absorbance (Fig.5).

Coil length is an essential parameter that affected the sensitivity of the colored reaction product and was investigated in the range of 25–250 cm.The result obtained showed that a coil length of 100 cm gave the highest absorbance as shown in Fig. 5 and was used in all subsequent experiments.

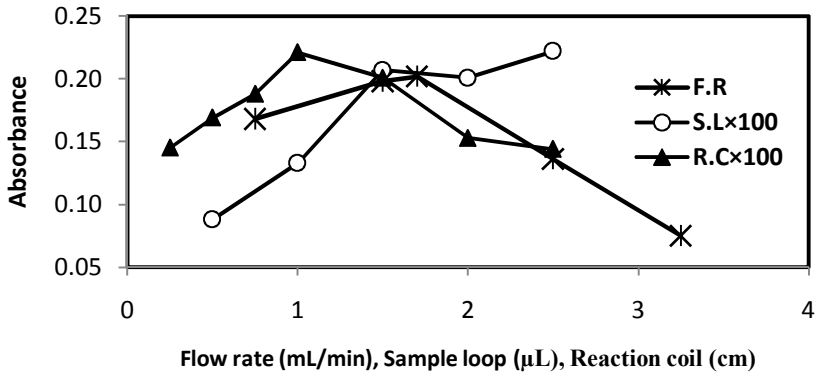


Fig.5: Effect of physical parameters

Optical characteristics

In order to test whether the red product formed obeys Beer's law or not, a series of solutions containing varying amounts of TCH were pumped, each as four replicates and the absorbances were recorded against the reagent blank at 529 nm. The Beer's law limits, the regression equation, correlation coefficient and limit of detection are summarized in Table 1.

The precision and accuracy of the proposed method were studied by analyzing solutions containing known amounts of TCH within the Beer's law limits, and the results indicate that a high precision and accuracy of the method (Table 1) were obtained. Finally, the proposed method was fast and simple through put of 69 injection h^{-1}.

Table 1:Analytical features of the procedure developed for the determination of TCH

Parameter	*Value*
λ_{max} (nm)	529
Regression equation	Y=0.001X+0.0096
Linear range (µg mL^{-1})	25-1000
Correlation coefficient (R^2)	0.9990
Limit of Detection (µg.mL^{-1})	7.589
Limit of Quantitation (µg.mL^{-1})	25.297
Reproducibility %	<2.041
Recovery%	>99.700
Sample through-put (hr^{-1})	69
Molar ratio of the product (TCH:SNP)	1:1

Analytical application

The proposed methods were applied successfully to the analysis of some pharmaceutical forms containing TCH. The results in Table 2 are in accordance with those obtained by the official spectrophotometric method used. Finally, statistical analysis [11], F- and T-test, reveals that there is no significant difference in precision and accuracy between the proposed method and the official spectrophotometric method [12].

Table2: Application of the proposed method for the determination of TCH in pharmaceutical preparations

Pharmaceutical form	*TCH (µg mL^{-1})* *Taken*	*Found*	*RSD (%)**	*Recovery (%)**	*Average Recovery*	*Official method recovery*
Tetracycline HCl capsules 250mg (MEHECO/China)	200.00	200.29	1.84	100.15	99.81	99.00
	400.00	397.31	0.91	99.33		
	600.00	599.73	0.33	99.96		
Ninacycline HCl capsules 250mg (NDI/Iraq)	200.00	201.97	0.895	100.99	100.40	99.50
	400.00	400.89	0.44	100.22		
	600.00	599.95	0.45	99.99		
Tetracycline HCl ointment 3% (Awamedica/Iraq)	200.00	196.72	1.77	98.36	98.48	98.02
	400.00	392.03	1.21	98.01		
	600.00	594.39	0.94	99.07		
		*(t=0.912)***				
		*(F=1.029)***				

** Average of four determinations*

*** Theoretical values at confidence level 95%, t=2.571 and F=9.28*

Conclusion

The developed methodology is very adequate for the determination of TCH in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (ppm) and without requiring any previous separation step, a temperature or a pH control. Moreover, the proposed procedures are very economical when compared to other methods such as those based on the use of LC. In comparison of the batch with FIA procedure, the latter is more convenient than the former method because of its speed (sample through-put of 69 injection h^{-1}) and wider linear range of the calibration graph and also a good recovery was obtained (Table 1).

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