BATCH AND FLOW-INJECTION SPECTROPHOTOMETRIC DETERMINATION OF SODIUM CEFOTAXIME IN PHARMACEUTICAL PREPARATIONS

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Abstract

Simple and sensitive batch and Flow-injecton spectrophotometric methods (FIA) for the determination of Sodium Cefotaxime in pure form and in injections were proposed. These methods were based on a diazotization and coupling reaction between diazotized Sodium Cefotaxime and thymol in alkaline medium to form an intense red water-soluble dye that is stable and has a maximum absorption at 522 nm. A graphs of absorbance versus concentration show that Beer's law is obeyed over the concentration range of 8-160 and 50-2500 $\mu g.ml^{-1}$ of Sodium Cefotaxime, with detection limits of 1.56 and 1.32 $\mu g.ml^{-1}$ of Sodium Cefotaxime for batch and FIA methods respectively. The FIA procedure sample throughput was 65 h^{-1}. All different chemical and physical experimental parameters affecting on the development and stability of the colored product studied and the proposed methods were applied successfully on the determination of Sodium Cefotaxime injections preparations.

Key words: Sodium Cefotaxime, Spectrophotometric determination, Thymol, Diazotization and coupling, Flow injection.

طريقة للتقدير الطيفي لسيفوتاكسيم الصوديوم في المستحضرات الصيدلانية بوساطة طريقتي الدفعة والحقن الجرياني

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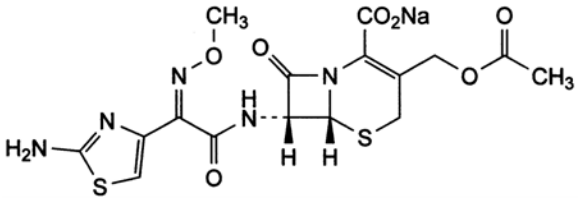
الخلاصة

يتضمن البحث تطوير طرائق طيفية جديدة وبسيطة للتقدير الكمي لمقادير ضئيلة من سيفوتاكسيم الصوديوم في المحاليل المائية والحقن باستخدام طريقة الدفعة التقليدية وتقنية الحقن الجرياني. تعتمد الطريقتين على تفاعل الازوتة والازدواج للدواء المؤزوت مع كاشف الثايمول في وسط قاعدي حيث تتكون صبغة حمراء اللون مستقرة وذائبة في الماء اعطت اعلى قمة امتصاص عند طول موجي ٥٢٢ نانوميتر. تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التراكيز بين ٨-١٦٠ و ٥٠-٢٥٠٠ مايكروغرام. مل$^{-1}$ من سيفوتاكسيم الصوديوم وبحد كشف 1.577 و 1.318 مايكروغرام.مل$^{-1}$ من سيفوتاكسيم الصوديوم لطريقتي الدفعة والحقن الجرياني على التوالي وبمعدل نمذجة 65 نموذج في الساعة لطريقة الحقن الجرياني، تم دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة، طبقت الطريقتين بنجاح على الحقن الحاوية على سيفوتاكسيم الصوديوم.

الكلمات المفتاحية: سيفوتاكسيم الصوديوم، التقدير الطيفي، ثايمول، الازوتة والازدواج، الحقن الجرياني.

Introduction

Sodium Cefotaxime is a Sodium (6*R*,7*R*)-3-[(acetyloxy)methyl]-7-[[(2*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate, $C_{16}H_{16}N_5NaO_7S_2$, its molecular weight is 477.4, it is white or slightly yellow powder, freely soluble in water, sparingly soluble in methanol and its chemical structure is [1]:



Sodium Cefotaxime

Cefotaxime is considered to be broad-spectrum antibiotics, primarily used to treat bacterial infections of the skin, soft tissues and the urinary tract. It belongs to an important class of antibiotics, the cephalosporins. It is referred to the-lactam antibiotics, which is among the oldest and the most valuable clinical antimicrobial agents [2].

A number of analytical methods have been reported for the determination of Sodium Cefotaxime, these include high pressure liquid chromatography [3-8], electrochemical techniques [9], chemiluminescence [10, 11], spectrophtometric – kinetic and flow injection analysis [12-19].

The reaction between Sodium Cefotaxime and $NaNO_2$ in acidic medium give a purple color measured at 500 nm with low sensitivity [20]. The object of the present work describes the development of bacth and FIA methods based on diazotiazation and coupling reaction between diazotized Sodium Cefotaxime and thymol reagent in alkaline medium. The red product was spectrophotometrically measured at 522 nm with high sensitivity than the diazonium salt alone. The analytical procedure is simple, fast, and accurate, it has been satisfactorily applied for the determination of Sodium Cefotaxime in pure and injections preparations, the reaction can be carried out in batch and FIA and the two approaches were compared.

Experimental

Apparatus

-All spectral and absorbance measurements were carried out on a Shimadzu UV-Visble-260 digital double-beam recording spectrophotometer (Tokyo-Japan). A 1-cm quartz flow cell with 50 µl internal volume and 1 cm bath length was used for the absorbance measurements. A two channel manifold was employed for the FIA spectrophotometer determination of Sodium Cefotaxime. A peristaltic pump (Ismatec, Labortechnik-Analytic, CH-8152, Glatbrugg-Zurich, Switzerland, six channels) was used to transport the reagents solutions, Injection valve (Rheodyne, Altex 210, Supelco-USA) 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. In (Figure 3) the hydrochloric acid (R1) stream were combined with injected sample (Sodium Cefotaxime and sodium nitrite) and they merged with thymol and NaOH (R2) streams at T-link then mixed in reaction coil (RC) with length of 100 cm. The injection loop was (150 µl), total flow rate of 1.2 ml/min, the absorbance was measured at 522 nm. and at room temperature (10-15 C°).

Reagents and materials

Analytical reagents grade chemicals and distilled water were used thoroughly.

a-For batch method

Sodium Cefotaxime stock solution (1000 µg. ml^{-1} = 2.09 x 10^{-3}M): a 0.10000 gm amount of pure Sodium Cefotaxime (BDH) was dissolved in amount of distilled water then complete to 100 ml in a volumetric flask with distilled water. Sodium Cefotaxime working solution (100 $\mu g.ml^{-1}$), was prepared by dilution of 10 ml of the stock solution in 100 ml volumetric flask with distilled water.

Sodium nitrite 2.09 x 10^{-3}M: A 0.01442 gm amount of $NaNO_2$ was dissolved in a 100 ml volumetric flask with distilled water.

Thymol (0.1 %) reagent solution: prepared by dissolving 0.1 gm of pure thymol (BDH) in 5 ml ethanol and completed the volume to 100 ml volumetric flask with distilled water.

Sodium hydroxide (1M).

b-For flow-injection method

Sodium Cefotaxime stock solution (1000 µg.ml^{-1} = 2.09 x 10^{-3}M):a 0.10000 gm amount of pure Sodium Cefotaxime (BDH) was dissolved in amount of distilled water then 0.01442 gm of sodium nitrite was added and completed to 100 ml in a volumetric flask with distilled water.

Thymol (0.1 %) reagent solution: prepared by dissolving 0.1 gm of pure thymol (BDH) in 100 ml of (0.7M) sodium hydroxide solution.

Hydrochloric acid (BDH) (0.8M).

More dilute solutions were prepared daily by suitable dilution with distilled water.

Procedure for Injections:

Five types of injections from different companies were analyzed by the developed methods, these include:-

1-Mirocef (250 mg of Sodium Cefotaxime)- Mission Vivacare- India.

2-Cefatac (250 mg of Sodium Cefotaxime)- Sanjiu Enterprise Group- China.

3-Cefosam (500 mg of Sodium Cefotaxime)- SDI –Iraq.

4-Cefatak (1 g of Sodium Cefotaxime)- Laboratorios Torlan- Espain.

5-Cefatak (1 g of Sodium Cefotaxime)- Zentiva Saglik Urunleri San. Ve Tic.- Turkey

For all types of injection, an accurately weighed portion from mixed three vials powder , equivalent to about 0.1 gm of Sodium Cefotaxime, was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with distilled water to obtained 1000 µg.ml^{-1} . Aliquot samples were treated exactly as in the procedure cited for the batch method.

For FIA method, an accurately weighed portion from mixed three vials powder equivalent to about 0.1 gm of Sodium Cefotaxime, 0.01442 gm of sodium nitrite were dissolved in distilled water, shacked well and completed to 100 ml in a volumetric flask with distilled water to obtained 1000 µg.ml^{-1} (2.09 x 10^{-3}M).

More dilute solutions were prepared by simple dilution for the stock solution with distilled water.

Procedures:

General batch procedure

An increasing volume (0.05-4 ml) of 1000 µg.ml^{-1}(2.09 x 10^{-3}M) Sodium Cefotaxime was transferred into a series of 25 ml standard flask. Added (0.05-4 ml) of sodium nitrite (2.09 x10^{-3}M) and 1 ml of 0.8M Hydrochloric acid, shake well then add 2 ml of (0.1% w/v) thymol and 3 ml of sodium hydroxide (1 M). The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 20 min at room temperature (10-15 C^{o}), the absorbance of the red dye formed was measured at 522 nm against a reagent blank containing all materials except Sodium Cefotaxime. A calibration graph was drawn and the regression equation was calculated, for the optimization of conditions and in all subsequent experiments, a 1 ml of (1000 µg.ml^{-1} = 2.09x10^{-3}M) Sodium Cefotaxime was used in a final volume of 25 ml.

General FIA procedure

Mixtures of Sodium Cefotaxime and $NaNO_2$ in the range of 100-2500 µg.ml^{-1} were prepared from the working solution of 1000 µg.ml^{-1} (2.09x10^{-3}M) for Sodium Cefotaxime and stoichiometric amount of $NaNO_2$. A 150 µl portion of Sodium Cefotaxime was injected into the stream of the hydrochloric acid (0.8M) then the diazotized drug combine with a mixture of (0.1% thymol in (0.7 M) NaOH) at T-link with a flow rate of o.6 ml min^{-1} of each channel. The resulting absorbance of the red product was measured at 522 nm and a calibration graph was constructed. Optimizations of conditions were carried out on 500 µg.ml^{-1} of Sodium Cefotaxime.

Results and discussion

The factors affecting on the sensitivity and stability of the colored product resulting from the reaction of the diazotized Sodium Cefotaxime and thymol in alkaline medium were carefully studied. A typical spectrum for the azo dye formed was measured versus reagent blank which has negligible absorbance at λmax 522 nm is shown in (Figure 1).

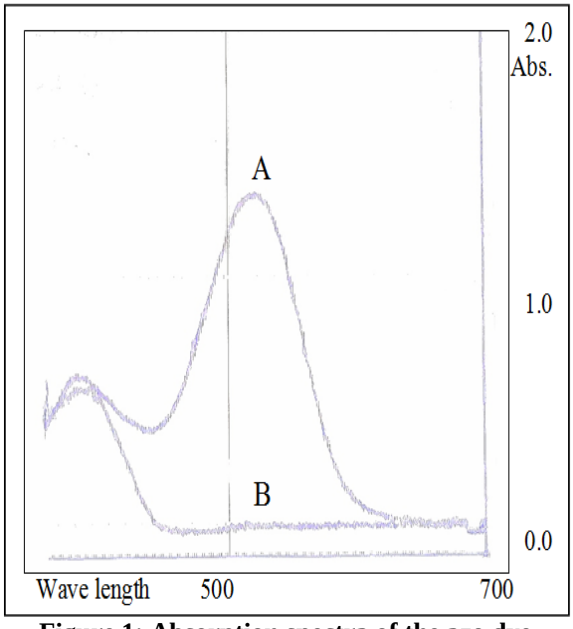


Figure 1: Absorption spectra of the azo dye formed against reagent blank (A) and blank against distilled water (B).

Batch spectrophotometric determination

The experimental conditions for the determination of Sodium Cefotaxime on the basis of sensitivity and stability of the azo dye formed were established. The diazonium reaction occurred in an acidic medium [21] and a hydrochloric acid of concentration of 0.8M was selected [22], the effect of different volumes of 0.8 M of HCl was studied and 1 ml volume seems to be optimum. A 1:1 mole ratio of Sodium Cefotaxime to sodium nitrite of ($2.09x10^{-3}$M) was used in order to prevent the effect of excess of sodium nitrite. The effect of coupling reagent (0.1% w/v) thymol reagent volumes (0.1-5 ml) on the intensity of the dye, were studied and 2 ml was found to be optimum. The absorbance of the dye formed became more intense and stable in alkaline medium, therefore, the effect of different alkaline solutions on the colored product were studied such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, sodium acetate and sodium carbonate. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution. The effect of different concentrations of NaOH were studied, (0.1-4 M) and 1 M seems to be optimum. The effect of (1 M) NaOH volumes were also studied from 0.1 to 5 ml and 3 ml was found optimum.

Experimental results revealed that the colour intensity reach a maximum after Sodium Cefotaxime solution had been reacted with thymol in alkaline medium for 20 min, therefore, a 20 min development time was suggested as the optimum reaction time and remain stable for 120 min. The order of addition of the reagents is an essential part of the experiment, it was found that the order of addition of the reagent cited under general procedure gave maximum color intensity and the minimum absorbance of the blank and was used in all subsequent experiments. The effect of temperature on the colour intensity of the dye was studied. In practice, a higher absorbance was obtained when the reaction was developed at room temperature (10-15 C^o) and when the calibrated flasks were placed in an ice bath (0-5 C^o) or in water bath (60 C^o) a decreased in absorbance were observed.

The stoicheiometry of the reaction between Sodium Cefotaxime and thymol was investigated using a molar ratio method [23]. In this method, an increased volumes of 0.1-3 ml of ($2.09x10^{-3}$M) thymol (V_R) were added to a 1 ml of ($2.09x\ 10^{-3}$M) Sodium Cefotaxime (V_D) which was diazotized using 1 ml of sodium nitrite ($2.09x10^{-3}$M) and 1ml of 0.8 M hydrochloric acid. A 3 ml of 1M NaOH was added and the solution was diluted to 25 ml with distilled water, The result obtained (Figure 2) shows that a (1:1) azo dye was formed between Sodium Cefotaxime and thymol.

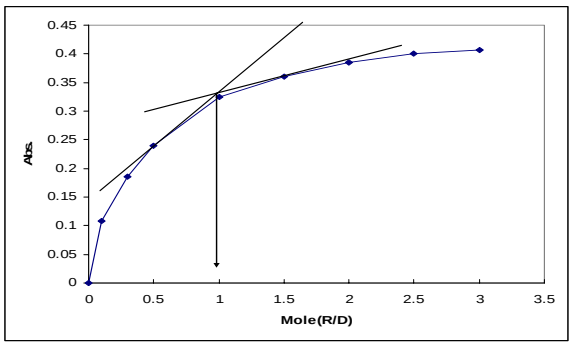
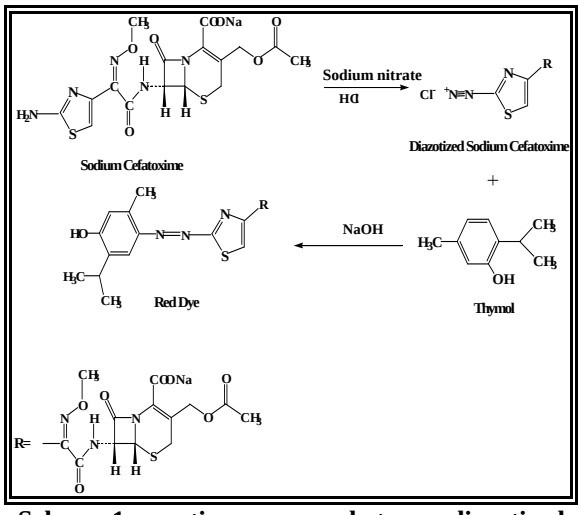


Figure 2: Mole ratio plot of the reaction of the diazotized Sodium Cefotaxime with thymol.

A reaction subsequent based on the above results is shown in Scheme (1).



Scheme 1: reaction sequence between diazotized Sodium Cefotaxime and thymol

The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stiochiometric amount of diazotized Sodium Cefotaxime ($2.09x10^{-3}$M) and thymol (A_S) with that of a solution containing a five – fold excess of thymol reagent (A_m) and according to analytical procedure. The average stability constant (K)= $6.172x10^4$ $L.mol^{-1}$, where is [$K=(1-\alpha)/\ \alpha^2C$] and $\alpha=A_m-A_s/A_m$ [23].

The regression equation obtained, and the analytical features of the procedure are summarized in (Table 1). It also summarized the main performance of the flow procedure developed for Sodium Cefotaxime

determination in order to make an effective comparison between the two approach.

Table 1: Analytical characteristics of the procedure developed for the determination of Sodium Cefotaxime

Parameters	Batch procedure	FIA procedure
Regression equation	Y=0.0075x+ 0.0001	Y=0.0007x+ 0.0032
Linear range (µg ml^{-1})	8-160	50-2500
Correlation coefficient, r^2	0.9975	0.9980
Limit of detection (µg ml^{-1})	5.60	34.28
Relative standard deviation (RSD)%	1.55	1.32
Average of recovery %	100.106	99.983
Molar bsorptivity L $mol^{-1}cm^{-1}$)	$3.522x10^{3}$	$3.191x10^{3}$
Sandell's sensitivity (µg cm^{-2})	$135.547x10^{-3}$	$149.608x10^{-3}$
Sample through-put (hr^{-1})	3	65

FIA-spectrophotometric determination

The batch method for the determination of Sodium Cefotaxime was adopted as a basis to develop FIA procedure. The manifold used for the determination of Sodium Cefotaxime was designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of Sodium Cefotaxime with thymol in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample (Cefotaxime sodume and sodium nitrite) was injected into a stream of hydrochloric acid and then mixed with mixture of thymol reagent and sodium hydroxide as given in (Figure 3). The influence of different chemical and physical FIA parameters on the absorbance intensity of the colored product were optimized as follows:

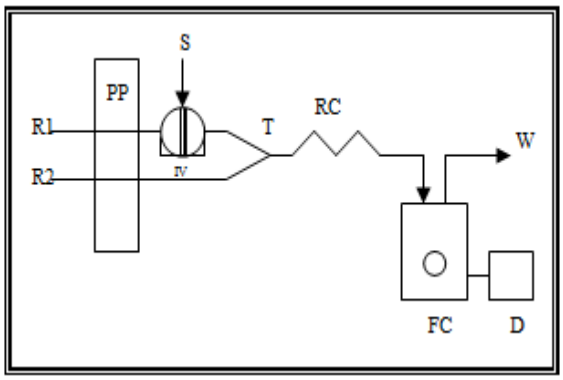


Figure 3: A schematic diagram of FIA manifold Where: R1 and R2, solutions of hydrochloric acid and mixture of (thymol and sodium hydroxide) respectively; PP =peristaltic pump; S= injection sample (Sodium Cefotaxime and sodium nitrite); IV= injection valve; T= T-link; RC= reaction coil; FC= flow cell; D= detector; W= waste.

Optimization of chemical parameters

The effect of various concentrations of hydrochloric acid (0.05-2M) was studied for the formation of diazotized Sodium Cefotaxime in the presence of sodium nitrite, and 0.8 M seems to be optimum as shown in (Figure 4).

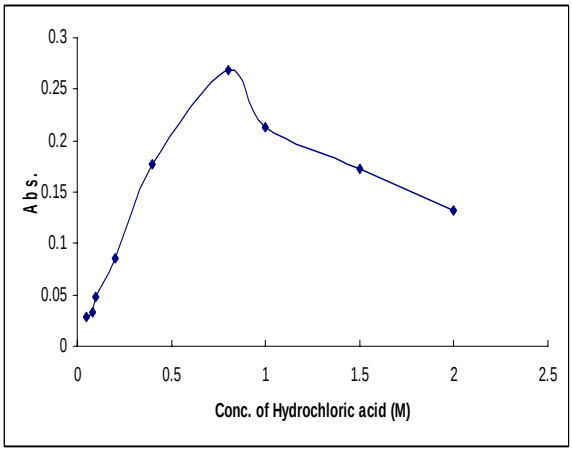


Figure 4: Effect of the concentration of hydrochloric acid in (M)

The effects of various concentrations of thymol was investigated. A concentration of 0.1M thymol, gave the highest absorbance and was chosen for further experiments as shown in (Figure 5). It was observed that the reaction between diazotized Sodium Cefotaxime and thymol depends on alkaline medium. In order to reduce the number of channels of the manifold to get a simple design. The thymol was prepared in sodium hydroxide, therefore the effect of different concentrations of sodium hydroxide was studied and 0.7 M was found to be the optimum for the reaction.

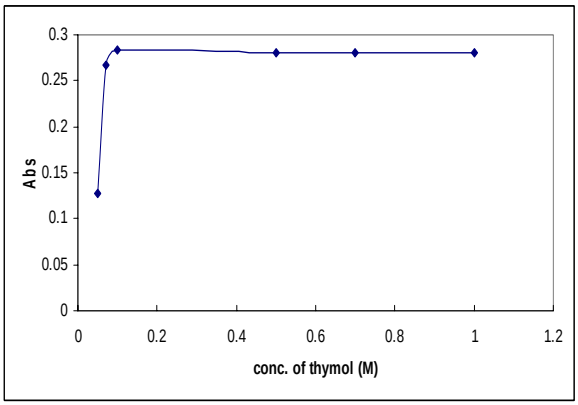


Figure 5: Effect of the concentration of thymol reagent in (0.7M) of NaOH

Optimization of manifold parameters

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

The effect of total flow rate on the sensitivity of the colored reaction product was investigated in the range of 0.6-2.6 ml min^{-1}. The result obtained showed that a total flow rate of 1.2 ml min^{-1}, (0.6 ml min^{-1} in each line) gave the highest absorbance as shown in (Figure 5), and was used in all subsequent experiments.

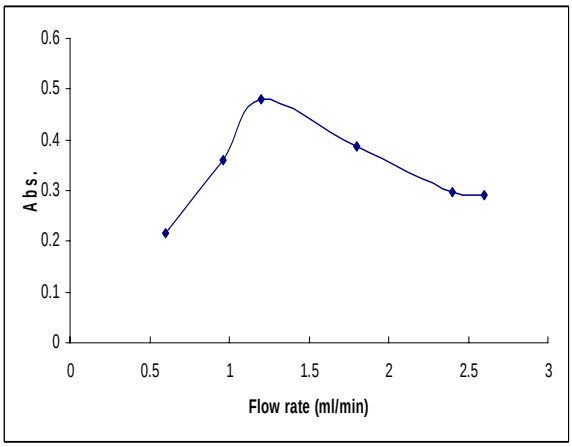


Figure 6: Effect of the total flow rate (ml/min)

The volume of the injection sample was varied between 50-250 µl using different length of sample loop. The results (Figure 7) obtained showed that injected sample of 150 µl gave the best absorbance.

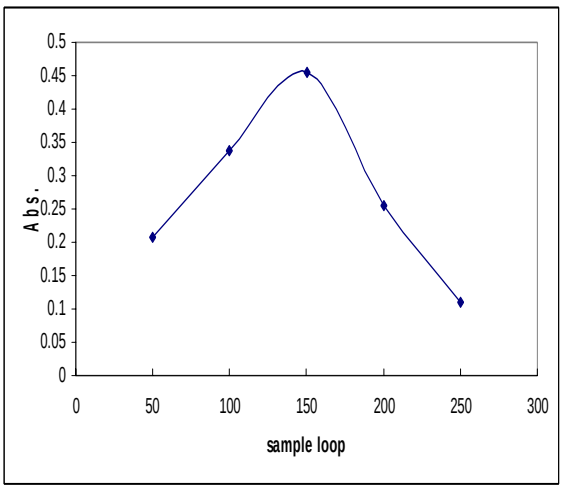


Figure 7: Effect of the injection loop (µl)

The coil length is an essential parameter that affects on the sensitivity of the colored reaction product and was investigated in the range of 25-250 cm. the results obtained showed that a coil length of 100 cm gave the highest absorbance as shown in (Figure 8) and was used in all subsequent experiments.

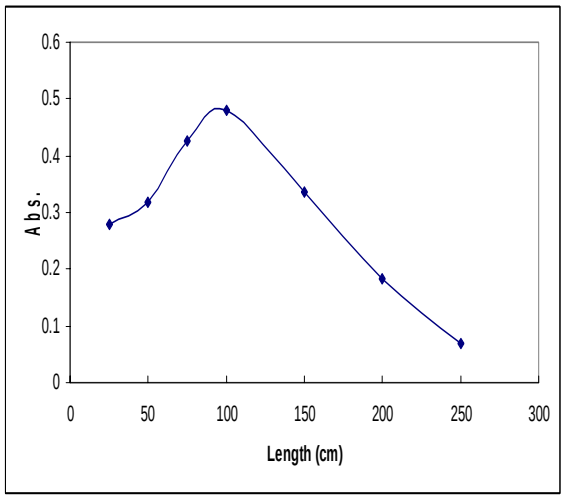


Figure 8: Effect of the length of the reaction coil in (cm)

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 end of the signal. The reaction time of each sample was 55 sec, therefore the sample through put was 65 samples per hour.

Analytical characteristics

Analytical characteristics such as linear range, detection range, correlation coefficient and relative standard deviation (RSD) of each method were determined under the optimized conditions as shown in (Table 1). In comparison

of the batch with the FIA procedure, the later is more convenient than the former method because of its speed (sample through-put of 65 injection h^{-1}), wider linear range of calibration graph, and good recovery were obtained.

Analysis of injection samples

The suggested methods were applied for the quantitative determination of Sodium Cefotaxime in injections. Five types (different origins) of injections preparations containing Sodium Cefotaxime were analyzed and they gave a good accuracy and precision as shown in (Table 2). The proposed method was compared successfully with the official method (liquid chromatography) [1], since F-test and T-test (Table 3) showed that there was no significant difference between the proposed method and the official method.

Table 2: Application of the proposed and official methods for the determination of mouth wash containing Sodium Cefotaxime

Injection samples	Proposed method				Official method Rec.%
	Batch method (80 µg/ml)		FIA method (750 µg/ml)		
	Rec.%*	RSD %*	Rec%*	RSD %*	
Mirocef (0.25g of Sodium Cefotaxime)-India	101.26	1.16	97.18	0.390	100.9
Cefatac (0.25g of Sodium Cefotaxime) Chaina	100.76	0.971	99.87	0.960	103.5
Cefosam (0.5g of Sodium Cefotaxime)-Sammara-Iraq	99.26	1.150	98.66	1.254	99.2
Cefatak (1g of Sodium Cefotaxime)-Espain	100.26	1.790	99.98	1.346	101.3
Cefata (1g of Sodium Cefotaxime)-Turky	100.93	0.912	100.99	0.841	98.7

*** for four determinations**

Table 3: The comparison of the proposed batch method with standard method using T- and F-statistical tests

The pharmaceutical preparations for 80 µg.ml^{-1}	The proposed method		The official method	
	Rec.%	$(Xi\text{-}\bar{Xi})^2_1$	Rec.%	$(Xi\text{-}\bar{Xi})^2_2$
Mirocef -Indian	101.26	0.592	100.9	0.032
Cefatac -Chaina	100.76	0.072	103.5	7.728
Cefosam-Sammara-Iraq	99.26	1.512	99.2	2.310
Cefatak)-Espain	100.26	0.052	101.3	0.336
Cefata -Turky	100.93	0.1936	98.7	4.080
	$(\bar{Xi})_1$= 100.49	$\sum(Xi\text{-}\bar{Xi})^2_1$= 2.421	$(\bar{Xi})_2$= 100.72	$\sum(Xi\text{-}\bar{Xi})^2_2$= 14.486

$F_{calculated} = S_1^2 / S_2^2 = 0.777/1.903 = 0.408$
$F_{theoretical} = 6.39$, $F_{theoretical} > F_{calculated}$ at 95% confidence level,
$T_{calculated} = 0.314$, $T_{theoretical} = 2.776$
$T_{theoretical} > T_{calculated}$; at 95% confidence level.

Conclusion

A batch and FIA methods were described for the determination of Sodium Cefotaxime. Although very few methods are available for the determination of Sodium Cefotaxime by spectrophotometric analysis. The suggested methods, are simple, rapid and offers the advantages of sensitivity more than all reported spectrophotometric methods, which needed a high temperature [17,18] expensive materials and method that obeyed Beer' law gave a good application for the pharmaceutical preparation than the other methods which have low linear range [16] . In addition, the wide applicability of the new method for routine quality control is well established by analyzing the assay of Sodium Cefotaxime at concentration of trace level (ppm) in injectios formulations.

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