FLOW INJECTION- SPECTROPHOTOMETRIC DETERMINATION OF AMOXICILLIN BASED ON ITS OXIDATIVE CONDENSATION WITH 4-AMINOANTIPYRINE

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Abstract

Simple methods were proposed for the spectrophotometric determination of amoxicillin (AMX) in pharmaceutical formulations . The methods are based on the reaction of AMX with 4-aminoantipyrine (4AAP) in the presence of potassium persulphate in alkaline medium .The reaction water soluble red colour product is measured at λ_{max} 510 nm using both batch and flow injection analysis (FIA) approach .The effect of chemical and physical parameters were investigated by univariate method. Under the optimum conditions ,calibration graphs were observed linear from 1-60 and 1-120 μg mL^{-1} AMX with detection limits of 0.173 and 0.395 μg mL^{-1} AMX by batch and FIA procedure respectively. The relative standard deviations of the proposed methods were less than 0.67 and 0.59 by batch and FIA procedure respectively. The FIA sample throughput was 40 h^{-1}. The proposed methods were successfully applied to the determination of AMX in injections and capsules

التقدير الطيفي–الحقن الجرياني الاموكسيسيلين بالاعتماد على تكاثفه التاكسدي مع 4–امينو انتي بايرين

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الخلاصة

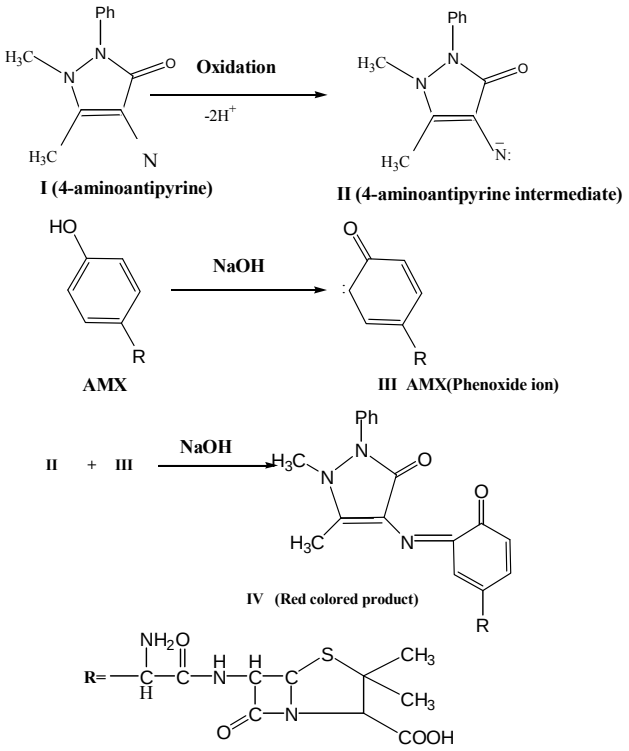
يتضمن البحث تطوير طريقة طيفية جديدة وبسيطة للتقدير الكمي للمقادير الضئيلة من الاموكسيسيلين في المحاليل المائية والمستحضرات الصيدلانية باستخدام المطياف الضوئي–الحقن الجرياني .تعتمد الطريقة على تفاعل الازدواج التاكسدي بين الاموكسيسيلين مع كاشف 4–امينوانتي بايرين في وسط قاعدي حيث يتكون ناتج احمر مستقر وذائب في الماء يعطي اعلى قمة امتصاص عند طول موجي 510 نانوميتر .تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التركيز 1–60 و1–120 مايكروغرام.مل$^{-1}$ من الاموكسيسيلين وبحد كشف 0.173 و0.395 مايكروغرام.مل$^{-1}$ من الاموكسيسيلين لطريقتي الدفعة والحقن الجرياني على التوالي وبمعدل نمذجة 40 نموذج بالساعة.تمت دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة وطبقت الطريقتين بنجاح على المستحضرات الصيدلانية الحاوية على الاموكسيسيلين.

1. Introduction

Amoxicilline (AMX) is an α-amino–substituted β-lactam antibiotic of broad spectrum and clinically widely used [1]. It is possesses some significant advantage over ampicillin,include more complete gastrointestinal absorption, and little or no effect on absorption of food [2].The B.p. was recommended a liquid chromatography (LC) and spectrophotometric (using imidazol-mercury reagent) methods for the determination of AMX in raw material and dosage forms respectively [3].A number of analytical methods in addition to microbiological assay have been reported for the determination of AMX, included spectrophotometric [4,5], polarographic [6,7], flourimetric [8,9],FIA [10] and HPLC methods [11,12]. Recently a flow injection method was reported for the determination of AMX with N,N-dimethyl-p-phenylenediamine and potassium hexacyanoferrate (III) in alkaline medium [13]. Among various methods available for trace analysis, FIA-spectrophotometry continues to be one of the most popular because it is simple and cost effective. There are few FIA-spectrophotometric methods available for the determination of AMX and these methods were not completely satisfactory as they either require temperature or pH control [14].The literature reported only a few FIA-spectrophotometric methods based on oxidative coupling reactions for the determination of AMX using 1-nitroso-2-naphthol as a chromogenic reagent [15]. The present study describes the development of a FIA method based on oxidative coupling reaction between AMX and 4AAP in the presence of potassium persulphate in alkaline medium. The red colored product was spectrophotometrically measured at 510 nm. The analytical procedure is simple, fast and accurate. It has been satisfactorily applied for determination of AMX in pure and dosage forms. The reaction can be carried out in batch and FIA and in this paper the two approaches are compared.

1.1 . Reaction mechanism of the method

Under the reaction conditions , 4AAP (I) upon oxidation with potassium persulphate loses two proton forming a nucleophilic intermediate (II), which has been postulated to be an active coupling species. AMX has a free ortho position to the hydroxyl group, hence the intermediate of 4AAP (II) undergoes nucleophilic substitution with phenolic moieties(III) of AMX in alkaline medium, to form a coloured quinonoid type product (IV) [16] according to scheme 1 [17].



Scheme 1: Proposed mechanism of the reaction between AMX and 4AAP.

Based on the observed molar reactivity of the reaction between AMX and 4AAP in the presence of potassium persulphate in alkaline medium to yield a red-coloured product (λ_{max} of 510 nm with a molar absorption coefficient of 10980 $L.mole^{-1}.cm^{-1}$),the reaction given in scheme 1 was postulated.

2. Experimental

2.1 Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visible 260 digital double beam recording spectrophotometer. A flow cell with 50 µL internal volume and 1cm bath length was used for the absorbance measurements. A two-channel manifold (Figure 1) was employed for the FIA spectrophotometric determination of AMX 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 4AAP ,channel B to transport oxidant solution (mixture of potassium persulphate and sodium hydroxide solutions). The sample solution was injected into the stream of the mixture of 4AAP with oxidant solution, through the injection valve. Solutions were propelled by peristaltic pump with total flow rate of 0.5 mL min^{-1}. The absorbance was measured at 510 nm.

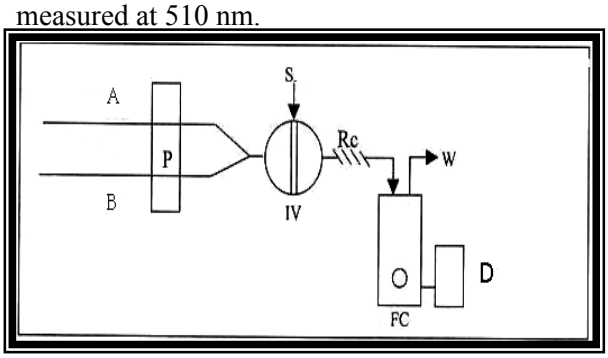


Figure 1:Manifold employed for FIA-Spectrophotometric determination of AMX with 4AAP and potassium persulphate in alkaline medium where: A , 50 mM of 4AAP solution ; B , mixture of 35 mM of potassium persulphate and 60 mM of sodium hydroxide solutions ; IV, Injection valve ; Rc,.Reaction Coil ; S, Sample ; P, Peristaltic pump ; FC, Flow cell ; D , Detector ; W, Waste.

2.2 Reagent and materials

Analytical reagent grade chemicals and distilled water were used throughout. Pure amoxicillin drug sample was kindly provided from state company for Drug Industries and Medical Appliance, SDI, Samara. Iraq.

Dosage forms were obtained from commercial sources. 4AAP (Fluka), 100 mM aqueous solution was prepared daily.Potassium persulphate (BDH), 100 mM solution. Sodium hydroxide (BDH), 200 mM solution.

Oxidant solution, prepared by mixing 35 mL of 100 mM of potassium persulphate with 30 mL of 200 mM sodium hydroxide and the solution was diluted to 100 mL with distilled water .

A 1000 µg mL^{-1} stock solution of AMX was prepared by dissolving 0.1000 g in 10 mL of ethanol and completed to 100 mL with distilled water. Serial dilutions with distilled water were made to cover the working range (Table 1).

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

2.3.Procedures

2.3.1 General batch procedure

An aliquot of sample containing 25-1500 µg of AMX was transferred into a series of 25 mL standard flasks. A volume of 1.0 mL of 50 mM sodium hydroxide solution, 3.0 mL of 10 mM of potassium persulphate and 1.5 mL of 30 mM 4AAP solution were added. The contents of the flasks were diluted to the mark with distilled water, mixed well and left for 30 min.The absorbance was measured at 510 nm (at room temperature 25°C) against reagent blank containing all materials except AMX.

A calibration graph was drawn and the regression equation calculated. For the optimization of conditions and in all subsequent experiments, a solution of 500 µg was used in a final volume of 25 mL .

2.3.2. General FIA procedure

Working solutions of AMX in the range cited in Table 1 were prepared from stock solutions. A 150 µL portion of AMX was injected into the stream of the mixture of 50 mM 4AAP and oxidant solution , with a flow rate of 0.25 mL min^{-1} in each channel (Figure 1).The resulting absorbance of the red product was measured at 510 nm and a calibration graph was concentrated in Table 1. Optimization of conditions were carried out on 100 µg mL^{-1} of AMX.

2.3.3. Procedure for capsules and injections

An accurately weighed amount of 10 powdered capsules or mixed content of 10 vials equivalent to 100 mg of the pure drug, was transferred into a 100 mL calibrated flask and was dissolved in 10 mL ethanol and completed to the mark with distilled water. The flask with its contents was shacked well and filtered. A sample of 500 µg of AMX in a final volume of 25 mL was taken and the measurement was carried out as described earlier under general procedure.

3.Results and discussion

The factors affecting on the sensitivity and stability of the coloured product resulting from the oxidative of AMX with 4AAP and potassium presulphate in alkaline medium were carefully studied. A typical spectrum for AMX

reaction product against reagent blank is shown in Figure 2.

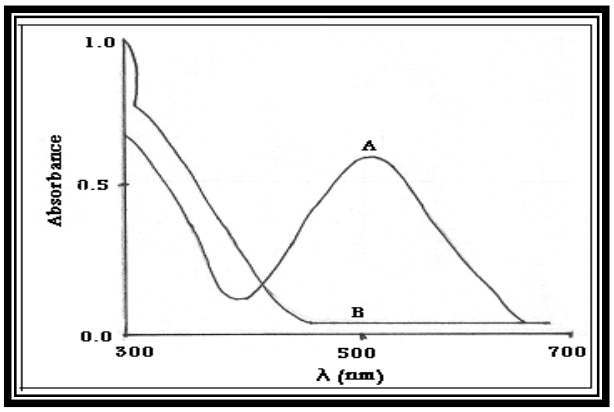


Figure 2: Absorption spectra of A (20 µg mL^{-1}) of AMX treated as described under procedure and measured against blank and B the reagent blank measured against distilled water.

The red dye product was only formed in alkaline medium because the phenolic part of AMX is converted into its salt (phenoxid ion) in the presence of aqueous sodium hydroxide, since phenoxid ion is more stable than phenol (due to its resonance) .The attack on phenoxid ion yield an even more stable -and rapidly formed- colored product with 4AAP [18], therefore, the effect of different alkaline solutions on the colored product were studied such as sodium hydroxide, ammonium hydroxide, sodium carbonate and sodium acetate. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution.

3.1. Batch spectrophotometric determination

The best experimental conditions for the determination of AMX were established for 4AAP 30 mM(from 0.3 to 4 mL), $K_2S_2O_8$ 10mM(from 1 to 4.5 mL) and sodium hydroxide 50 mM(from 0.7 to 3 mL) by adding various volumes of their solutions to a fixed concentration of AMX and measuring the absorbance at 510 nm. The results obtained (Figure 3) showed that 1.5 mL of 30 mM 4AAP, 3 mL of 10 mM potassium persulphate and 1 mL of 50 mM sodium hydroxide gave the maximum color intensity and led to the maximum color stability of the dye product for 500 µg of AMX in a final volume of 25 mL.

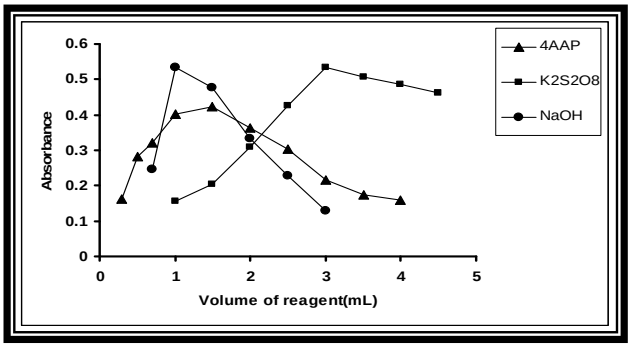


Figure 3: Study of optimum reagent concentrations

Experimental results revealed that the colour intensity reach a maximum after the drug solution had been reacted with 4AAP and potassium persulphate in alkaline medium for 25 min, therefore, a 30 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 a maximum color intensity and a 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 colour was developed at room temperature (25°C) than when the calibrated flasks were placed in an ice-bath at (0°C) or in a water bath at (60 °C).

The stoichiometry of the reaction was studied using equimolar concentrations of the drug and 4AAP at constant potassium persulphate and sodium hydroxide concentrations, adopting Jops method of continuous variation [19], a molar ratio of 1:1 drug to 4AAP was obtained by the applied method as shown in Figure 4. The stability constant of the dye product was calculated [20] by comparing the absorbance of a solution containing stoichiometric amount of AMX and 4AAP with that of solution containing five-fold excess of 4AAP reagent . The stability constant of the dye product in water under the described experimental conditions was 1.86×10^4 L moL^{-1}.

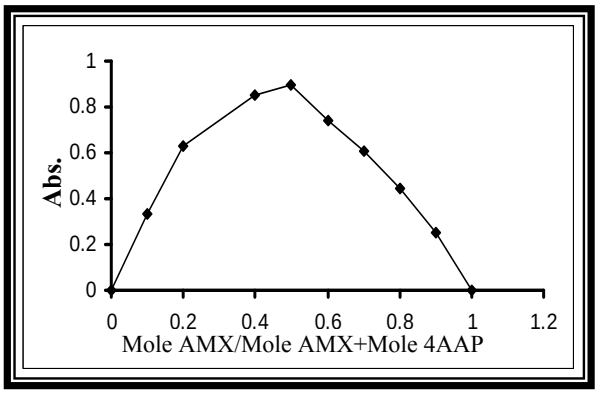


Figure 4: Study of the mole ratio of the reaction between AMX and 4AAP.

In order to assess the possible analytical applications of the proposed methods. The effect of some common excipients frequently found with AMX drugs in pharmaceutical formulations, such as sucrose, glucose, fructose, lactose, starch, talc and magnesium stearate was studied by analyzing synthetic sample solutions containing 20 µg mL^{-1} of AMX and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously. The regression equation obtained, from a series of AMX standards, and the analytical features of this procedure are summarized in Table 1 in which are also summarized the main performance of the flow procedure developed for AMX determination in order to make an effective comparison between the two approach

Table 1: Analytical characteristics of the procedures developed for the determination of AMX

Parameter	Batch procedure	FIA procedure
Regression equation	Y=0.0262 X+0.0125	Y=0.0057X +0.0042
Linear range (µg mL^{-1})	1- 60	1- 120
Correlation coefficient	0.9998	0.9998
Limit of detection (s/n=3) µg mL^{-1}	0.173	0. 395
Reproducibility %	<0.67	<0.59
Average of recovery,%	100.35	99.72
Sandell's Sensitivity (µg cm^{-2})	0.038	0.175
Through-put (hr^{-1})	2	40

3.2 FIA-spectrophotometric determination

The batch method for the determination of AMX was adopted as a basis to develop FIA procedure. The manifold used for the determination of AMX was so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of AMX drug with 4AAP and potassium persulphate in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample was injected into a stream of mixed 4AAP with oxidant solution (mixture of potassium persulphate and sodium hydroxide) as given in Figure 1.The influence of different chemical and physical FIA parameters on the absorbance intensity of the colored product were optimized as follows:

3.2.1 Optimization of chemical parameters

The effects of various concentrations of 4AAP were investigated. A concentration of 50 mM gave the highest absorbance and was chosen for further use. The results are shown in Figure 5.

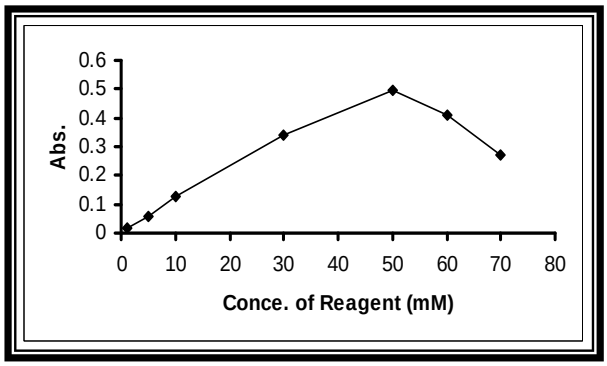


Figure 5: Effect of the concentration of 4AAP in (mM).

It was observed that the reaction between AMX and 4AAP depends on the oxidation process with potassium persulphate in alkaline medium. To simplify the manifold used , a mixture of potassium persulphate and sodium hydroxide was allowed to flow in one stream. The optimum concentrations of the two materials in the mixture were investigated by altering one of the concentrations and keeping the other constant. A mixture solution of concentration of 35 mM potassium persulphate and 60 mM sodium hydroxide gave the best results and minimum blank value as shown in Figure 6 and was considered as optimum value.

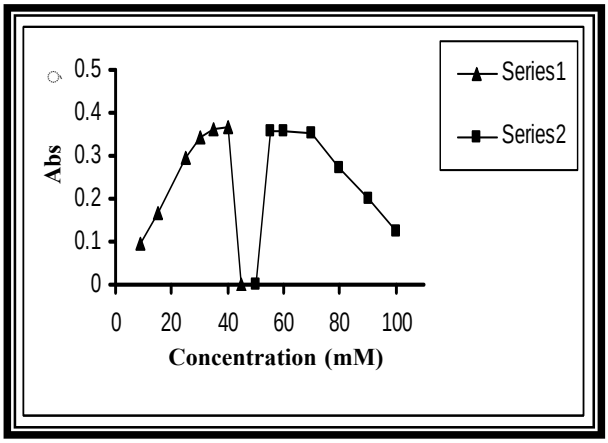


Figure 6: Effect of the concentration of potassium persulphate (series 1) and sodium hydroxide (series 2) in (mM)

3.2.2 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 total flow rate on the sensitivity of the coloured reaction product was investigated in the range of 0.25- 2.5 mL min^{-1}. The results obtained showed that a total flow rate of 0.5 mL min^{-1}. .(0.25 mL min^{-1} in each line) gave the highest absorbance as shown in (Figure 7) and was used in all subsequent experiments

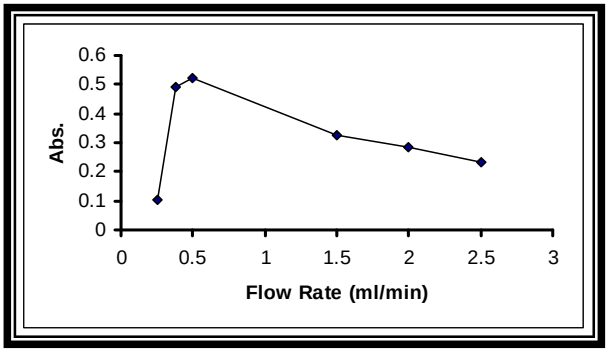


Figure 7: Effect of the total flow rate (mL min^{-1}).

The volume of the injected sample was varied between 50-250 µl using different length of sample loop. The results (Figure 8) obtained showed that injected sample of 150 µL gave the best absorbance.

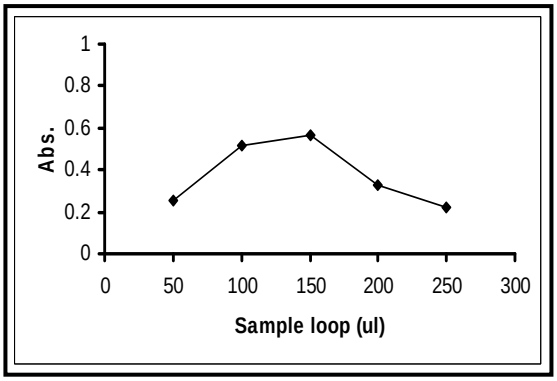


Figure 8: Effect of the injection volume in (µL).

Coil length is an essential parameter that affects the sensitivity of the coloured reaction product and was investigated in the range of 25-150 cm. The result obtained showed that a coil length of 75 cm gave the highest absorbance as shown in Figure 9 and was used in all subsequent experiments.

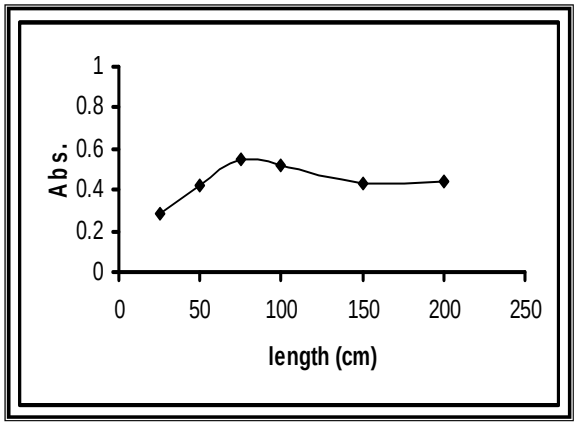


Figure 9: Effect of the length of the reaction coil in (cm)

The reaction time is also an important parameter that affected the sample throughput and was investigated by calculating the interval time between the sample injection and appearance the end of signal. The reaction time for each sample was 90 sec, therefore, the sample through put was 40 samples h^{-1}.

Table 2: Application of the proposed and official methods to the determination of some AMX drug in dosage form

Drug form	Proposed methods				Official Method Recovery %
	Batch method		FIA method		
	Rec.%*	RSD%*	Rec.%*	RSD %*	
Amoline (injection 500 mg) Oubari-pharma-Syria	102.16	0.74	100.87	0.14	102.50
Amoxicillin(injection 500 mg) Pan pharma-France	101.50	0.69	102.43	0.14	101.00
Acamoxil (capsule 250 mg) ACAI-Iraq	97.76	1.89	97.57	0.58	102.00
Amoxicillin (capsule 250 mg) Pharm-Inter-Belgica	99.28	0.94	98.26	0.56	99.00
Amoxicillin (capsule 500 mg) Ajanta-pharm-Limited-India	99.67	1.10	101.87	0.26	98.00
Amoxicillin (capsule 250 mg) SDI-Iraq	98.74	0.76	99.48	0.33	99.00

*For five determinations

Analytical characteristics

Analytical characteristics such as sampling rate, detection range, correlation coefficient and relative standard deviation (RSD) of each method were determined for the above optimized conditions as shown in Table 1. In comparison of the batch with FIA procedure, the later is more convenient than the former method because of its speed (sample through put of 40 injection h^{-1}) and wider linear range of calibration graph . In addition the precision of the methods was evaluated by analyzing pure sample of AMX and a good recovery was obtained.

Analysis of Pharmaceutical samples

The suggested method was applied to the quantitative determination of AMX in pharmaceutical formulations. Four types of capsules and tow types of injections containing AMX have been analyzed and they gave a good accuracy and precision as shown in Table 2.

The proposed method was compared successfully with the British pharmacopeia's standard method, since F-test and T-test showed that there was no significant differences between the proposed and official method using imidazole-mercury reagent [3].

Conclusion

Although very few methods are available for the determination of AMX by FIA-spectrophotometer based on oxidative coupling reaction , the suggested method, which is simple, rapid, offers the advantages of sensitivity more than the reported methods [13,21] , and a wide range of determination without the need for extraction or heating [22]. The wide applicability of the new method for routine quality control is well established by the assay of AMX at concentration level of trace (p.p.m) in pharmaceutical formulations.

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