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SPECTROPHOTOMETRIC DETERMINATION OF AMOXICILLIN IN PHARMACEUTICAL FORMULATIONS USING NORMAL AND REVERSE FLOW INJECTION ANALYSIS SYSTEMS: A COMPARISON STUDY

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ABSTRACT. The combination of flow injection technique and spectrophotometric detection can be employed for the rapid and low-cost methods for antibiotic assay. In this work, two flow injection analysis (FIA) systems, normal and reverse (nFIA and rFIA), combined with UV-Vis spectrophotometric technique were used for amoxicillin determination in its pure and pharmaceutical preparations. A colorimetric coupling reaction between amoxicillin and diazotized p-toluidine produced a bright yellow azo dye and quantified at a maximum wavelength of 426 nm. For nFIA and rFIA procedures, the amoxicillin calibration graphs had an RSD of less than 2% and were linear in the ranges of 5-200 and 1-140 µg/mL, respectively. The limits of detection and quantification were 1.41 and 4.71 µg/mL of amoxicillin for nFIA and 0.39 and 1.31 µg/mL for rFIA, respectively. The physical and chemical factors that could influence color sensitivity were investigated. Various commercial formulations containing different amounts of amoxicillin were successfully tested using the developed approaches, which demonstrated to be easy, rapid, reproducible, and interference-free.

KEY WORDS: Amoxicillin, Normal FIA, Reverse FIA, p-Toluidine, Diazotization reaction

INTRODUCTION

Amoxicillin sodium (AMX), sodium (2*S*,5*R*, 6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl) acetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylate, is a commonly β-lactam antibiotic used for inhibits or kills bacteria that cause infections in humans and animals [1, 2]. It is given for several bacterial infections, including meningitis, respiratory tract infections, skin infections, and salmonella infections [3]. Despite these advantages, concerns regarding AMX residues in food and the possible environmental contamination risk associated with this antibiotic have been raised due to its side effects on human health [4]. The literature covered a variety of analytical techniques [5] for the extraction and determination of AMX, including spectrofluorometry [6, 7], electrochemistry [8-10], liquid chromatography–mass spectroscopy [11-13], high-performance thin layer chromatography (HPTLC) [14], high pressurized liquid chromatography (HPLC) [15], FIA [16-20], magnetic molecularly imprinted polymer [21], and spectrophotometry [22-24]. Flow-injection analysis (FIA) techniques are frequently utilized and have generated great interest because of their reproducibility, simplicity, and low apparatus costs [25-29]. The FIA technique is widely used to analyze a broad range of both inorganic and organic substances [30-32]. Among the several FIA types, the normal and reverse modes received high attention [33, 34]. The normal mode required injecting a small volume of sample into the reagent carrier stream, which was then transported through a thin bore tube to a spectrophotometer, where the derivative was measured. In a reverse flow injection analysis (rFIA), a small amount of reagent solution is injected into both the carrier and sample streams [35].

Utilizing diazotized p-toluidine (DPT) as a chromogenic reagent, the current study employed two fast and easy normal and reverse flow injection techniques to estimate AMX in pharmaceutical forms. To lower the detection limit and enhance the sensitivity assay of the AMX, the reverse mode of the FIA technique was applied and compromised with the normal mode. As soon as the drug and reagent were combined at room temperature, highly sensitive yellow dye

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was generated. The maximum wavelength of the measuring dye was identified at 426 nm. Without any pre-treatment, both approaches were successfully used in the determination of AMX in pharmaceutical preparations.

EXPERIMENTAL

Instruments and FIA manifolds

A single beam UV-Vis spectrophotometer (Shimadzu 1240) supplied with a quartz flow cell (50 µL) was used for measuring absorbance and combined with a flow injection system. The flow injection system is equipped with a six-channel peristaltic pump (Ismatec, Switzerland) that is utilized for pumping reagents and carrier solutions, and a six-port injection valve (Rheodyne, USA) that is employed to introduce sample solution or reagent solution for nFIA and rFIA systems, respectively. Reaction coils (RC) of varying lengths were constructed using Teflon tubes (0.5 mm i.d.), while transport lines and component connections within the FIA manifolds were made with Polytetrafluoroethylene tubes (0.8 mm i.d.). For the analysis of AMX in its bulk and pharmaceutical formulations, two types of double channels FIA manifolds (normal and reverse) were employed (Figure 1).

Manifold design of normal and reverse FIA

The normal FIA setup involved injecting 100 µL of AMX solution via the injection valve into the stream of sodium hydroxide solution. Later, this stream was combined with the stream of diazotized reagent (DPT) at the Y-link and then mixed inside the reaction coil. In contrast, the reverse FIA setup involved injecting 100 µL of DPT solution through the injection valve into the stream of AMX solution. Subsequently, this stream was combined with the sodium hydroxide stream at Y-link and mixed within the reaction coil. The solutions were pumped through a peristaltic pump into FIA manifolds at a rate of 7.5 mL/min for the nFIA and 5.3 mL/min for the rFIA method. At the end of the manifold, the yellow product's absorbance was measured at its maximum wavelength of 426 nm.

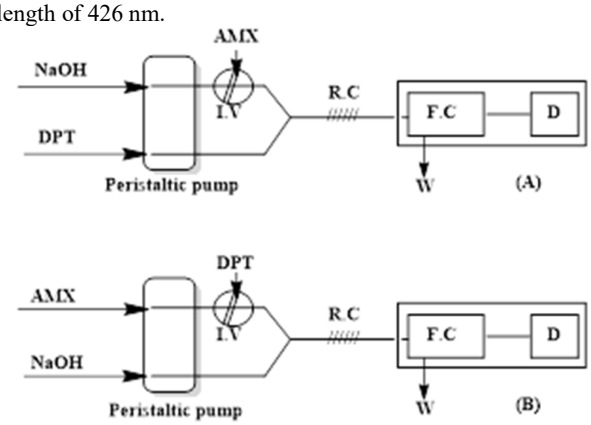


Figure 1. Normal (A) and reverse (B) double channels-FIA manifolds for determination of AMX. (DPT: diazotized p-toluidine; W: waste; D: detector; F.C: flow cell; I.V: injection valve; R.C: reaction coil).

Reagents and solutions

The reagents used were of the analytical reagent grade, and all of the solutions were made using distilled water. AMX standard (99.9% w/w) was provided by the state Company for Drugs

Industry and Medical Appliances (SDI) (Iraq). *p*-Toluidine was purchased from Fluka (Switzerland) and sodium hydroxide and sodium nitrate were purchased from BDH (Dorset, UK). Hydrochloric acid (36% w/w) was obtained from Merck (Darmstadt, Germany). For amoxicillin applications, the active ingredients in amoxicillin injections and tablets were amoxicillin sodium and amoxicillin trihydrate, respectively. The pharmaceutical formulations involved Glomox®/UAE, capsules 500 mg, Pulmoxyl®/India, capsules 500 mg, amoxicillin injection/Germany, 500 mg, and amoxicillin injection/India, 500 mg, were purchased from a local pharmacy in Iraq.

A 500 µg/mL of stock standard solution of AMX was prepared by dissolving 50 mg of AMX in 100 mL distilled water. Working standard solutions of the drug were prepared by serial dilution with distilled water. Diazotized solution of *p*-toluidine (DPT, 3 mM) was prepared as follows: In a 100 mL volumetric flask, 0.0322 g of *p*-toluidine (PT) was dissolved in a minimal amount of ethanol. After the flask was placed in an ice bath, 3 mL of hydrochloric acid (1 M) and 0.0207 g of sodium nitrite were added to the PT solution. After shaking the mixture for five minutes, the flask was complete to the mark with distilled water. The same process was used to prepare 7 mM of DPT; however, 0.0750 g of *p*-toluidine (PT) and 0.0483 g of sodium nitrite were used. To make a stock solution of 1 M sodium hydroxide, an accurate weight of the base was dissolved in 250 mL distilled water and then standardized.

Solution of pharmaceutical formulations

An accurately weighed amount of 20 powdered capsules equivalent to 500 mg of the pure drug, was transferred into a 100 mL calibrated flask and agitated for five minutes with 25 mL of ethanol. The flask with its contents was diluted to the mark with distilled water, shaken well, and filtered. For injection application, an exactly weighed amount of mixed content of 10 vials equivalent to 500 mg of the pure drug, was transferred into a 100 mL calibrated flask, dissolved and diluted with distilled water, and then filtered. Further working solutions of pharmaceutical preparations for FIA procedures were made by simple dilution using distilled water. The AMX assay was performed in compliance with the suggested FIA procedures.

Procedure of normal and reverse FIA

A range of 5–200 µg/mL of standard solutions of AMX were prepared using the drug's stock standard solution. A 100 µL volume of AMX solution was injected into the stream of the 0.1 M sodium hydroxide solution through the injection valve of the nFIA manifold. Later the resultant solution was mixed with a stream of 7 mM DPT inside a 75-cm long reaction coil at a flow rate of 7.5 mL/min. The reverse FIA, on the other hand, involved injecting 100 µL of a solution containing 3 mM of DPT into a stream of a series of AMX solutions ranging from 1 to 140 µg/mL. The solution was then combined with the stream of 0.2 M sodium hydroxide solution and mixed in a 50 cm reaction coil at a flow rate of 5.3 mL/min. The yellow azo dye produced from both systems was measured spectrophotometrically at 426 nm using a UV-Vis spectrophotometer attached to the end of the FIA manifold. All FIA system variables were optimized using 50 µg/mL of AMX.

RESULTS AND DISCUSSION

The outcome of the preliminary investigation indicated that the AMX molecule reacted quickly with diazotized *p*-toluidine, creating a highly sensitive yellow azo dye product. The yellow azo dye product was formed almost immediately after the addition of DPT and NaOH to the AMX solution, and it remained stable for at least two hours. These findings are significant as they meet the requirements for fully automated and sensitive normal and reverse FIA methods for estimating AMX. The absorption spectrum of the colored product was recorded between 350 and 800 nm

(Figure 2), with the maximum intensity observed at a wavelength of 426 nm. According to the suggested reaction pathway, p-toluidine was diazotized with nitrous acid in a basic solution to form diazonium ions. The diazonium ions are then coupled with another aromatic molecule in an alkaline environment (Scheme 1). Various research suggests that the formation of azo-dye compounds through diazotization reactions is a common method for estimating various compounds [36-38]. The 1:1 mole ratio was obtained by employing Job's method for continuous variations to assess the stoichiometry of the AMX to DPT using equimolar quantities of both drug and reagent.

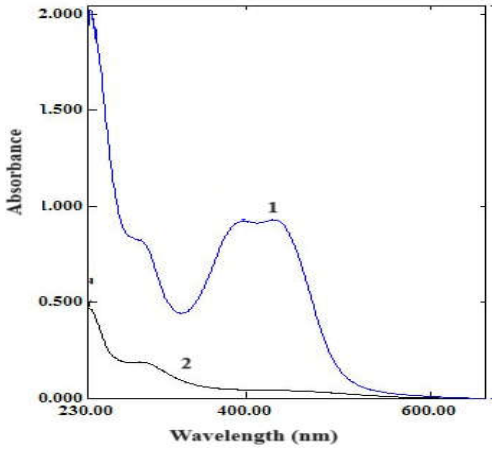
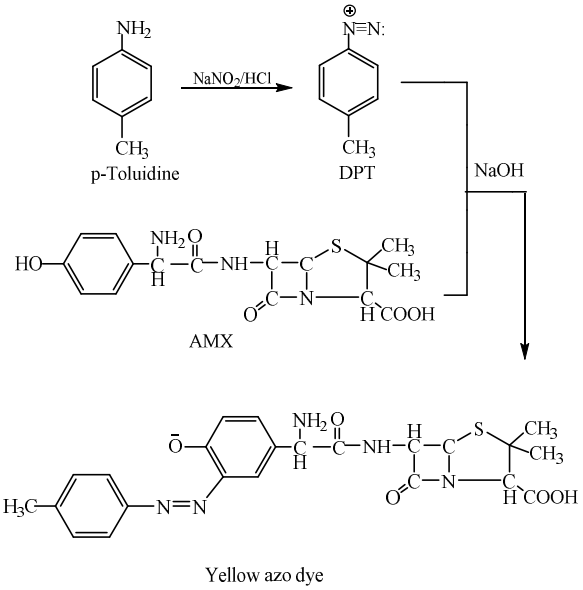


Figure 2. Absorption spectra of the yellow dye formed by reacting 50 µg/mL of AMX with DPT measured versus the blank (1), and the blank versus distilled water (2).



Scheme 1. Proposed reaction pathway.

Study of FIA variables

The chemical and physical variables that were most affecting the formation of the azo dye product and the stability of analytical signals for both FIA systems were carefully examined by changing one variable at a time while maintaining the rest constant.

Selection of the manifold design

Different double-channel manifold designs were explored to carry out different reaction paths using both normal and reverse FIA procedures. According to the findings, manifolds 1 and 2, which are displayed in Figure 1, were chosen for further use because they offered the highest absorbance intensity and good precision for nFIA and rFIA, respectively. The proposed reaction pathway, which involved converting AMX molecule to phenoxide (more active spices) in a basic medium and then coupling with the diazotized reagent, was agreed with the manifold configuration of nFIA.

Study of the chemical variables

The effect of different concentrations of diazotized reagent (DPT) was examined in the range of 1–10 mM, for normal and reverse systems. The findings demonstrated that the optimal concentrations were determined to be 7 and 3 mM, respectively, since these values gave the maximum analytical signal for the nFIA and rFIA systems (Figure 3A). The analytical signal either stayed constant for nFIA or gradually decreased for rFIA beyond these concentrations. This may result from differences in the mechanism for dispersion (dispersing the analyte in the reagent stream or vice versa) between both systems, as well as an increase in the analytical signal of the blank with increasing reagent concentrations [39]. According to preliminary investigations, the sensitive yellow product could only form in an alkaline medium. This medium is essential for converting AMX into the more reactive ionized form (phenoxide) and in facilitating the coupling process with a diazotized reagent [40, 41]. Therefore the effect of the type and concentration of various alkaline solutions on the sensitivity of colored products were investigated. Based on the results, sodium hydroxide was chosen for additional use since it provided the best analytical signal and high precision for both FIA systems (Figure 3B). Moreover, different concentrations of NaOH were examined for nFIA and rFIA procedures between 0.02-0.5 M. The results revealed to increase in the absorbance with increasing the concentration of base up to 0.1 M for normal and 0.2 M for reverse FIA systems, and then gradually decreased (Figure 3C). The decrease in analytical signals could be caused by the highly concentrated base having an impact on the stability of the diazotized reagent (produced in an acidic medium) and the formation of azo dyes [42]. Therefore, 0.1 and 0.2 M of sodium hydroxide were selected as optimum concentrations for normal and reverse FIA systems, respectively.

Effect of total flow rate

Flow rate is an important parameter that most impact the sensitivity of the product, along with sample frequency [43]. A range of total flow rates between 2.9-11.3 mL/min was used to study the effect of flow rate for both FIA systems and under optimum chemical conditions. The analytical signal was developed with increasing flow rate to 7.5 and 5.3 mL/min for nFIA and rFIA systems, respectively (Figure 4A), after which it gradually decreased. The dispersion effect and the shortened residence time, which was required to raise the colored product to its maximum values, could be the reason for the diminished analytical signal at the high value of the flow rate. To achieve both acceptable precision and reduced reagent use, the rates of 7.5 and 5.3 mL/min were selected for the following study.

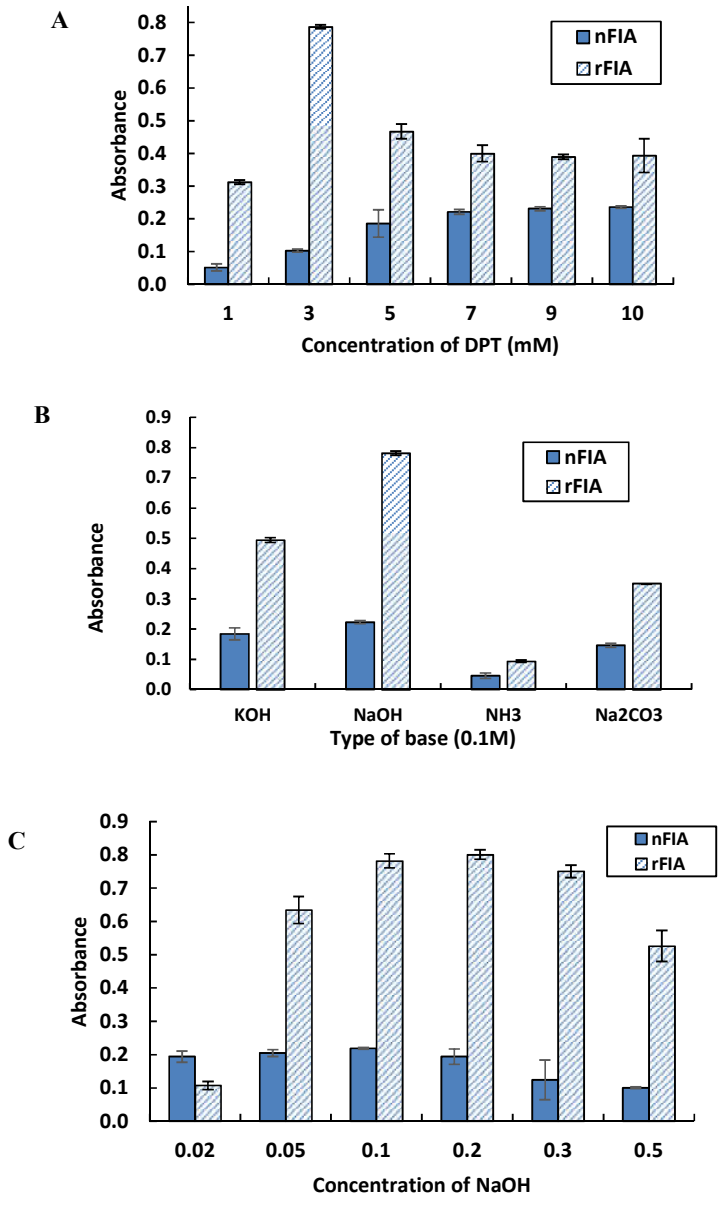


Figure 3. Study of (A) conc. of DPT, (B) type of base and (C) conc. of NaOH.

Effect of reaction coil length

The influence of reaction coil length was analyzed by examining various lengths within the range of 0-200 cm. The maximum value of the analytical signal was achieved at 75 and 50 cm, for both

systems, respectively. Beyond these lengths, the signal steadily declined with increasing coil length due to increased dispersion [44, 45], hence the ideal lengths for subsequent work were 75 and 50 cm for both systems, as shown in Figure 4B.

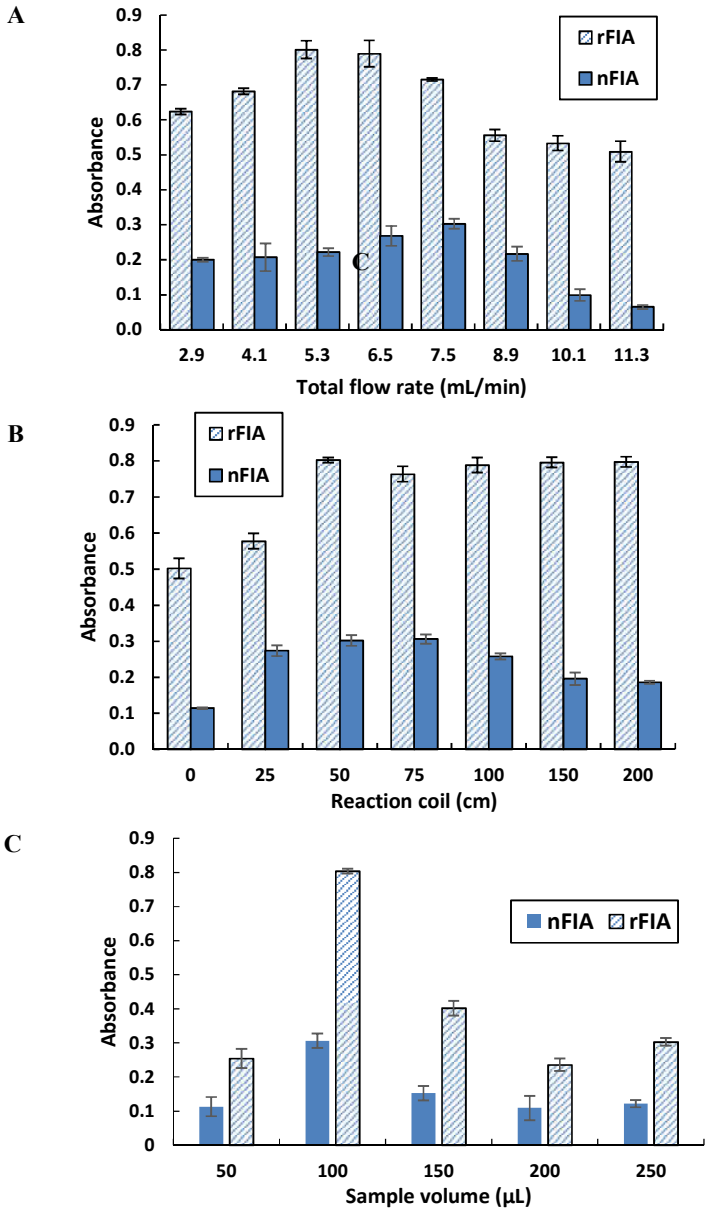


Figure 4. Effect of (A) total flow rate; (B) reaction coil length; and (C) injected volume.

Table 1. Studied range and optimum values of chemical and physical variables for AMX analysis via both FIA systems.

Flow injection variable	Studied range	Selected value	
		nFIA	rFIA
Chemical variable			
Conc. of DPT (mM)	1–10	7	3
Type of base	NaOH, KOH, NH_4OH, Na_2CO_3	NaOH	NaOH
Conc. of NaOH (M)	0.02–0.50	0.1	0.2
Physical variable			
Total flow rate (mL/min)	2.9–11.3	7.5	5.3
Reaction coil length (cm)	0–250	75	50
Sample volume (µL)	50–250	100	100

Effect of injected sample volume

For both normal and reverse FIA systems, the volume of sample or reagent solution injected through the injection valve into the carrier streams was studied. For this investigation, sample loops with varying lengths that offered quantities between 50 and 250 µL and were attached to the injection valve were used. The results (Figure 4C) showed that, for both procedures, an injected volume of 100 µL produced the maximum absorbance with good precision; as a result, it was selected for additional usage. The usage of sample loops greater than 100 µL was found to result in a decreased analytical signal, which was attributed to either the dispersion effect or a high sample-to-reagent ratio [46]. The optimal values for the investigated FIA variables are summarized in Table 1.

Validation of the FIA methods

Using a series of AMX solutions ranged 5-200 µg/mL, a linear range of the calibration graph for the nFIA system was established. A 100 µL volume of AMX solution was injected into the alkaline solution stream (0.1 M NaOH and 7.5 mL/min total flow rate), then combined with the stream of diazotized reagent (7 mM), and mixed through the reaction coil. On the other hand, the linearity of the calibration graph for the rFIA system was achieved through the pumping of several AMX solutions of different concentrations ranging from 1 to 140 µg/mL. A portion of DPT (100 µL of 3 mM) was injected into the drug solution stream, then combined with the basic solution (0.1 M NaOH and 3.5 mL/min total flow) and mixed inside the reaction coil. Along with several statistical values, Table 2 includes the regression equations, slope, correlation coefficient, and molar absorptivity values. Based on the following equation [47], the limits of detection (LOD) and quantification (LOQ) were determined: LOD = 3SD/b, LOQ = 10SD/b, where SD: standard deviation of replicate determination (n = 10) results obtained under identical conditions to sample analysis, but without the analyte (i.e., blank); b is the calibration curve's slope. The linearity of the proposed methods was in the ranges of 5–200 µg/mL (LOD 1.41 µg/mL, %RSD < 0.92, n = 5) for the nFIA method and 1-140 µg/mL (LOD 0.39 µg/mL, %RSD < 1.92, n = 5) for rFIA. The analytical results showed that the proposed methods had sufficient precision, good linearity, and high sensitivity.

Accuracy and precision

The accuracy and repeatability of both normal and reverse FIA procedures were examined. Five replicates of each of the four concentrations of amoxicillin solutions were assayed on the same day and over six consecutive days (intra and inter-day variation, respectively). The results presented in Table 3 for normal and reverse FIA methods showed high accuracy (recovery values within the range of 98.5-99.4% and 98.3-101.8, respectively) and excellent precision (low values of RSD < 2%).

Table 2. Analytical characteristics of the suggested methods.

Parameter	Value	
	nFIA	rFIA
Regression equation	y = 0.0057x + 0.0404	y = 0.0125x + 0.1246
Linear range (µg/mL)	5-200	1-140
Correlation coefficient, r	0.9994	0.9989
Slope, b (mL/µg)	0.0057	0.0125
Intercept, a	0.0404	0.1246
Limit of detection (S/N =3) (µg/mL)	1.41	0.39
Limit of quantification (µg/mL)	4.71	1.31
Molar absorptivity, ε (L/mol cm)	2.21×10^{3}	4.84×10^{3}
Sandell's sensitivity, S (µg/cm^2)	0.18	0.08
Reproducibility, %	0.27-0.92	0.21-1.92
Recovery, %	98.50-101.20	98.30-100.70
$S_{y/x}$	1.40×10^{-2}	2.75×10^{-2}
S_b	6.24×10^{-5}	1.54×10^{-4}
S_a	7.07×10^{-3}	1.23×10^{-2}
Sampling rate (sample/h)	124	92

Table 3. Intra and inter-day variations for determination of AMX via nFIA and rFIA methods.

Method	Taken conc. (µg/mL)	Intra-day (n = 5)				Inter-day (n = 15)			
		Found conc. (µg/mL)	RE* (%)	Recovery (%)	RSD (%)	Found conc. (µg/mL)	RE (%)	Recovery (%)	RSD (%)
nFIA	40	39.75	-0.63	99.37	0.92	40.49	1.23	101.23	0.89
	80	78.81	-1.49	98.51	0.34	78.88	-1.40	98.60	0.49
	100	98.74	-1.26	98.74	0.27	99.03	-0.97	99.03	0.50
	140	139.46	-0.39	99.61	0.31	139.85	-0.11	99.89	0.28
rFIA	10	10.18	1.80	101.80	0.95	10.07	0.70	100.70	1.23
	30	29.73	-0.90	99.10	0.50	30.17	0.57	100.57	1.92
	70	69.26	-1.06	98.94	0.26	68.89	-1.59	98.41	0.34
	130	127.79	-1.70	98.30	0.21	127.70	-1.77	98.23	0.13

*RE; relative error, conc.; concentration, RSD; relative standard deviation, n; number of measurements.

Sampling rate and dispersion value

The sample frequency for each of the two approaches must be determined. The time computed from the beginning of injecting the sample (for nFIA) or reagent (for rFIA) to the appearance of the maximum absorbance can be used to theoretically predict sampling frequency (sampling rate). This interval was equivalent to 29 and 39 seconds for nFIA and rFIA, respectively, under the current methodologies; as a result, the sampling rate was 124 and 92 samples per hour.

Effect of the excipients in pharmaceutical forms

Investigations were conducted into the effects of some possible interfering compounds (excipients), which are typically combined with the active ingredient in tablets and capsules. To conduct the study, 50 µg/mL of amoxicillin was spiked with twenty times the quantity of several excipients [48], including starch, glucose, talc, polyvinylpyrrolidone (PVP), and magnesium stearate. Acceptable recovery values were obtained, as shown in Table 4, indicating negligible interference with current techniques.

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

Excipients (1000 µg/mL)	Amount of AMX (µg/mL)		(Rec. ± SD)% (n = 5)
	Added	Found	
Glucose	50	49.38	98.76 ± 0.39
Talc		49.25	98.50 ± 0.26
PVP		49.38	98.76 ± 0.33
Starch		49.80	99.60 ± 0.22
Mg stearate		49.90	99.80 ± 0.25
All excipients		49.22	98.44 ± 0.75

Table 5. Application of the proposed methods for determination of AMX in pharmaceutical preparations.

Dosage form	Proposed methods										UV method					
		nFIA method					rFIA method									
	Taken (µg/mL)	Spiked (µg/mL)	Found (µg/mL)	Rec. (%)	Mean Rec.± SD (%)	RSD (%)	Found (µg/mL)	Rec. (%)	Mean Rec. (%)	RSD (%)	Taken (µg/mL)	Spiked (µg/mL)	Found (µg/mL)	Rec. (%)	Mean Rec. (%)	RSD (%)
Glomox® Capsules	25	25	50.19	100.38	99.46 ± 0.79	0.89	49.89	99.78	99.32 ± 0.78	0.46	20	20	39.41	98.53	99.56 ± 1.34	1.63
		50	74.63	99.51		0.49	73.37	97.83		0.39		30	49.79	98.58		1.97
		75	99.14	99.14		0.72	99.78	99.78		0.29		40	58.89	98.15		2.10
	50	25	73.73	98.31		0.40	74.38	99.17		0.11	40	20	60.57	100.95		2.95
		50	99.11	99.11		0.25	99.40	99.40		0.27		30	70.89	101.27		2.11
		75	125.38	100.30		0.15	124.94	99.95		0.34		40	79.88	99.85		2.93
Pulmoxy® Capsules	25	25	49.46	98.92	99.57 ± 0.41	0.83	49.47	98.94	98.88 ± 0.72	0.38	20	20	38.54	96.35	98.83 ± 1.39	2.53
		50	75.15	100.20		0.59	73.56	98.08		0.73		30	49.60	99.20		2.27
		75	99.67	99.67		0.45	99.18	99.18		0.40		40	60.33	100.55		2.92
	50	25	74.66	99.55		0.81	73.46	97.95		0.22	40	20	59.28	98.80		1.66
		50	99.50	99.50		0.66	99.33	99.33		0.45		30	69.66	99.51		1.68
		75	124.47	99.58		0.77	124.72	99.78		0.07		40	78.84	98.55		2.33
Trogehamburger® Injection	25	25	49.16	98.32	98.95 ± 0.36	1.66	49.33	98.66	98.89 ± 0.39	1.38	20	20	39.06	97.65	99.3 8± 1.72	2.09
		50	74.42	99.23		1.53	74.20	98.93		0.50		30	50.30	100.60		2.08
		75	98.99	98.99		2.10	99.52	99.52		0.46		40	61.32	102.20		2.58
	50	25	74.19	98.92		1.11	74.22	98.96		0.55	40	20	58.85	98.08		1.53
		50	98.89	98.89		1.02	98.94	98.94		0.66		30	69.43	99.19		2.02
		75	124.19	99.35		0.89	122.93	98.34		2.12		40	78.83	98.54		2.85
Pyxus® Injection	25	25	50.17	100.34	98.90 ± 1.18	1.08	49.59	99.18	99.22 ± 0.36	0.60	20	20	41.04	102.60	99.8 8± 2.04	2.67
		50	73.29	97.72		1.76	74.06	98.75		0.56		30	49.50	99.00		1.56
		75	98.84	98.84		0.95	99.78	99.78		0.43		40	58.36	97.27		2.13
	50	25	73.23	97.64		1.12	74.57	99.43		0.57	40	20	61.18	101.97		2.15
		50	98.61	98.61		1.13	99.22	99.22		0.37		30	69.11	98.73		1.85
		75	125.30	100.24		0.66	123.68	98.94		1.01		40	79.77	99.71		2.66
Pure AMX					99.06 ± 0.52				99.54 ± 1.55						100.67 ± 0.69	
t (2.306)					1.43				1.45		$(n_1 + n_2 - 2) = 8$					
F (9.605)					4.91				5.69		$(n_1 - 1) = 4$, $(n_2 - 1) = 4$					

Assay of AMX in pharmaceutical forms using FIA methods

The applicability of the suggested FIA techniques was assessed by spiking four types of pharmaceutical applications (two types of capsules and two types of injections) at two concentration levels with different amounts of standard AMX and analyzed using the proposed procedures. The acquired results demonstrated a high degree of reliability with a low percentage error between the taken and founded amounts. The recovery values resulting from the two FI techniques were compared to those obtained using the UV method [49]. Using the F and t-tests, the suggested and reference processes were statistically compared [50]. The computed values were less than the theoretical ones, indicating that there was no significant difference in accuracy and precision between the two techniques (Table 5).

CONCLUSION

The current study provided two flow injection spectrophotometric methods (normal and reverse) for determining the concentration of amoxicillin in its bulk and pharmaceutical forms. Its benefits included fast estimation, minimal sample consumption (100 microliters), reduced waste generation, and a high sampling rate. A simple diazotization reaction was adopted for colorimetric analysis of the analyte combined with the FIA technique without any interference. There was no need for pre-extraction or heating, and both procedures were precise and economical. The findings showed that, in comparison to the nFIA method, the reverse FIA technique could significantly increase the sensitivity and precision of AMX assessment. On the other hand, the conventional FIA offers a high sampling rate by reducing the analysis time. The methods were effectively used to determine the amount of AMX in injections and capsules.

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