Spectrophotometric Determination of Amoxicillin Using New Organic Reagent *via* Different Analytical Methods

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Abstract: *New and simple spectrophotometric method was applied for amoxicillin determination by oxidative coupling with an organic reagent 1-(4-aminophenyl)-3-(5-(4-nitrophenyl)-furan-2-yl)-yl)-prop-2-en-1-one (H) to form an orange colored dye with λ_{max} of 490 nm. The molecular structure of the new compound H was characterized using spectral analysis including ^{1}H-NMR, FTIR, Mass spectroscopy, and UV-visible. The concentration range of oxidative coupling obeyed Beer's law was 2–50 µg/mL, the correlation coefficient was 0.9995, molar absorptivity was 0.63 × 10^4 L/mol cm, and the detection limit was 0.189 µg/mL. The concentration range of flow injection obeyed Beer's law was 1–150 µg/mL, the correlation coefficient was 0.9994, molar absorptivity was 0.295 × 10^4 L/mol cm, and the detection limit was 0.407 µg/mL. The proposed method was successfully applied in pharmaceutical formulation for amoxicillin determination. The results showed that amoxicillin could be reacted with a new compound H in the alkaline medium in the presence of oxidative agent $NaIO_4$ and automated by flow injection analysis. The proposed methods have the advantage of simple, fast, very sensitive, good precision and accuracy. The suggested technique was effectively used to estimate amoxicillin in both its pure form and pharmaceutical formulations.*

Keywords: *amoxicillin; chalcone; spectrophotometric; flow injection; oxidative coupling*

■ INTRODUCTION

Amoxicillin (6-[(*R*)-(-)-2-amino-2-(*p*-hydroxyl phenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.]heptane-2-carboxylicacid trihydrate, AMX, (Fig. 1), is one of the mostly given semisynthetic penicillins for acute bacterial sinusitis and community-acquired pneumonia [1-2]. Several methods for AMX determination in pharmaceutical formulations and biological fluids have been reported including HPLC [3-5], chemiluminescence [6-8], spectrofluorimetric [9], and flow-injection analysis [10-14].

Chalcone has a unique structure that consists of two aromatic rings connected by a three-carbon unsaturated carbonyl system with a wide range of functional groups [15-17]. Chalcone and its derivatives have shown a wide spectrum of biological activities [18] such as anti-fungal, anti-microbial, anti-inflammatory, anti-malarial, antiviral, anti-tumor, antioxidant, anti-leishmanial, and anti-cancer [19-20]. These activities were originated from the presence of a reactive keto-ethylenic moiety (–CO–CH=CH–) in their structure. The objective of this study is an alternative method that is more effective and inexpensive because it utilizes agriculture used or by-products such as grain, soybean husk, straw, cottonseed, bark, used newspaper, and others. Paper, such as newspaper, is a material that contains cellulose (50.1%), hemicellulose, and lignin (18.1%); thus, it can be used as

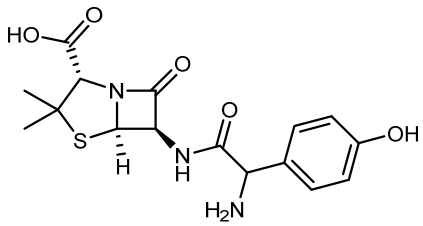


Fig 1. Structure of amoxicillin

an adsorbent [1]. In this work, a simple spectrophotometric method was established for AMX determination by oxidative coupling with a new chalcone reagent.

■ EXPERIMENTAL SECTION

Materials

The chemicals and solvents were obtained from Sigma Aldrich and BDH companies and were used without further purification to create the compounds in this study. 4-amino acetophenone, absolute ethanol, hydrochloric acid, 5-(4-nitrophenyl)furan-2-carbaldehyde and ethanol were obtained from Sigma-Aldrich company. Sodium hydroxide, sulfuric acid, sodium periodate, and nitric acid were obtained from BDH company. AMX was obtained by SDI, a general corporation for the production of pharmaceuticals and medical supplies, in Samara, Iraq.

Instrumentation

Using an open capillary tube in a Gallen-Kamp MFB-600 melting point apparatus, the melting point of produced chalcone was measured. A Shimadzu FTIR-8400S was used to register infrared spectra in the region of 4000–600 cm^{-1}. A mass spectroscopic was captured with Shimadzu QP 2010 Plus. With tetramethylsilane as an internal standard, the model ultra-shield ^{1}H-NMR spectra (DMSO-d_6 solvent) were registered at 300 MHz. The absorbance measurements were done in a spectrophotometric single-beam UV-visible 295 (Lasany-India) with 1.0 cm and 0.5 cm quartz cells. For the flow injection analysis (FIA), the configuration with the three channels (Fig. 2) was used. The peristaltic pump and reaction coil were both powered by a poly(vinyl chloride) tube (0.8 mm internal diameter) peristaltic pump (ALITEA, C4, produced in Sweden).

Procedure

The general procedure of preparation of compound H

Compound H was prepared using the method given in reference [20] with some changes. A 4-aminoacetophenone (0.005 mol, 0.67 g) was dissolved and stirred in a mixture of 30 mL of absolute ethanol and 4 mL of 10% NaOH solution at room temperature. Then, 5-(4-nitrophenyl)furan-2-carbaldehyde (0.005 mol, 1.08 g) was added and stirred for 48 h. After the disappearance of the initial materials, as monitored by TLC, hydrochloric acid was added drop-wise with stirring to the reaction mixture until the formation of a precipitate which was collected after filtration. The product was washed with cold distilled water, dried, and purified by ethanol to afford the orange crystals.

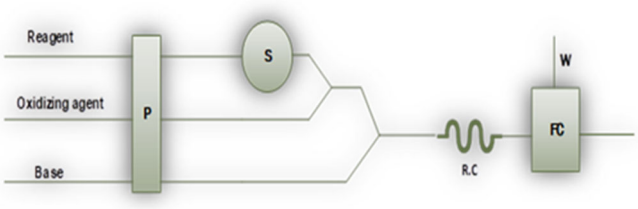


Fig 2. FIA manifolds, peristaltic pump (P), sample injection (S), reaction coil (R.C), flow cell (FC), detector (D), waste (W)

Analytical methods

Preparation of stock solution and reagents solution. To prepare an AMX stock solution (1000 mg/mL), 0.1 g of AMX was dissolved in 100 mL of distilled water. By combining 0.5 mL of sulfuric acid and 0.1 g of the organic reagent in distilled water and stirring, a stock solution of H 1000 g/mL was obtained. NaOH 2 M was obtained by dissolving 8 g of NaOH in 100 mL of distilled water. A 2.13 g of $NaIO_4$ and 1 mL of HNO_3 were dissolved, stirred, and added to 100 mL of distilled water to create 0.1 M $NaIO_4$.

The general procedure of oxidative coupling reaction 1000 µg/mL. The oxidative coupling reaction was prepared for AMX in 20 mL volumetric flask by the addition of 2 mL AMX (1000 µg/mL), 2 mL 1000 µg/mL from organic reagent H with 1 mL of $NaIO_4$ 0.1 M in present of NaOH 1 M that produced orange colored dye at λ_{max} 490 nm. The blank solution was prepared in the same way without the addition of AMX.

The procedure of flow injection

AMX was injected into a carrier stream made of three channels that were mixed together. The first channel carried 0.2 M organic reagent H, and the second channel carried 0.1 M $NaIO_4$ in a T-shape. The reaction is carried out by thoroughly mixing the ingredients in a 50 cm reaction coil, allowing the mixture to flow

through an injector, and then reacting the end product with a stream of 1 M NaOH. The absorbance of the resulting orange dye was then measured at 490 nm.

The Procedure for AMX in capsules

The AMX capsules 500 mg pharmaceutical, equivalent to 100 mg of pure AMX, were transferred into 100 mL volumetric flask to prepare 1000 µg/mL and completed to 100 mL distilled water. Afterward, the solution was filtered to remove any interference. The diluted solution was prepared from the pharmaceutical and then used in the measurement of oxidative coupling and FIA.

■ RESULTS AND DISCUSSION

Compound H was prepared by the reaction of equivalents moles of 4-aminoacetophenone with 5-(4-nitrophenyl)furan-2-carbaldehyde in an appropriate solvent through the Claisen-Schmidt condensation using NaOH as a catalyst. Compound H showed a band at 3483 and 3388 cm^{-1} related to NH_2, bands at 3230 and 3089 cm^{-1} regions due to CH=CH and aromatic C–H bonds, respectively. The C=O absorption band appeared at 1639 cm^{-1}, while the C=C stretching frequency of compound H appeared at 1585 cm^{-1}. Bands at 1502 and 1332 cm^{-1} were related to the NO_2 absorption. The ^{1}H-NMR of compound H revealed a singlet band at 5.4 ppm due to NH_2, the doublet appears at 7.03 ppm related to two Ar–H, while the multiple signals at 8.05–8.73 ppm due to other aromatic hydrogens and two hydrogen of the CH=CH of chalcone.

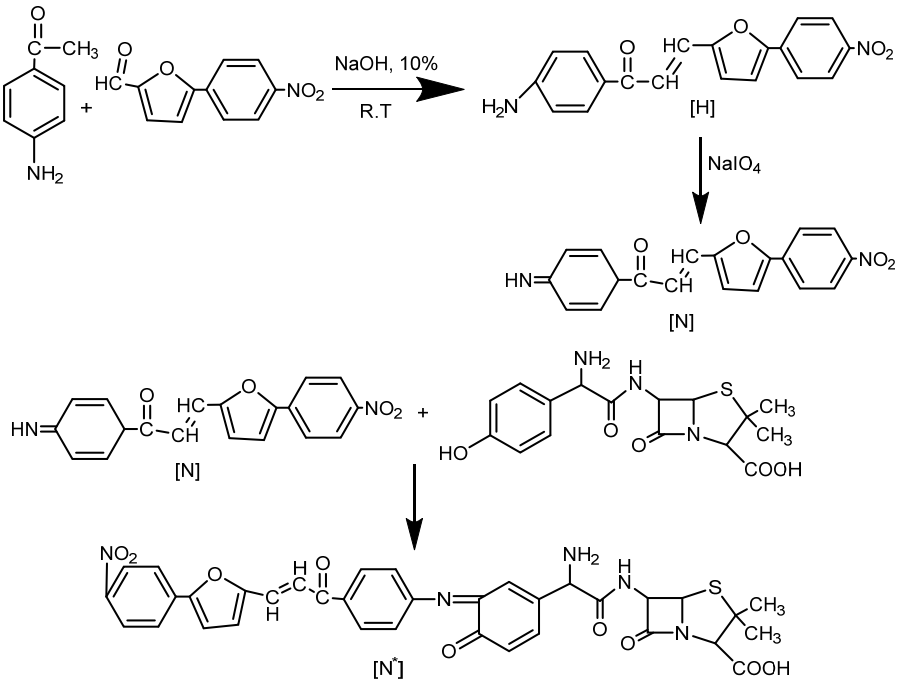
Scheme 1 shows the proposed reaction pathway between the prepared reagent and drug to produce the colored dye derivative. From the mole ratio method shown, the ratio between the drugs and reagents was 1:1, and the proposed formula for the resulting dye produced are therefore as follows [21-22] shown in Scheme 1.

Spectrophotometric Study

Oxidative coupling reaction preliminary investigation showed that the reaction of AMX with organic reagent H in the presences oxidizing of agent and in alkaline media was used to produce the orange-colored dye that has λ_{max} 490 nm, where the absorption spectra of the orange dye were measured against blank.

Effect of Experimental Conditions of Oxidative Coupling

The factors that effect on the stability and sensitivity of the colored product from the oxidative coupling of



Scheme 1. Synthesis of dye derivative

AMX with the organic reagent and oxidative reagent $NaIO_4$ present in an alkaline medium were studied. Different parameters that affected the colored intensity of the oxidative dye were studied, such as the addition of order and medium. The optimal amount of alkaline was 1.5 mL, as shown in Fig. 3(a), when several volumes of NaOH (0.25–2.50 mL) were investigated. Different volumes (0.5–3.0 mL) of 0.2 M organic reagent H were used; the results are shown in Fig. 3(b). The optimal volume of a reagent to produce the most intensely colored product was 2 mL. The amount of oxidizing agent 0.1 M $NaIO_4$ was studied by adding various amounts (0.25–2.50 mL) of an oxidizing agent into a volumetric flask of 20 mL. The greatest absorbance was achieved when 1 mL was employed, and the findings are shown in Fig. 3(c). Effects of temperature, reagent concentration, oxidizing agent quantity, NaOH concentration, and period of oxidation. It depended on experiments that affected the nature of the medium for the oxidative coupling reaction; diverse medium (alkaline, acidity, and neutral) was tested, and the sequence addition (R+D+O+B) for AMX offered the larger intensity of colored. To increase the effectiveness of the oxidative coupling process, as shown in Fig. 3(d), oxidation reactions at different temperatures (0, 25, and 50 °C) were employed to explore the effects of temperature while maintaining the same circumstances. The time required to reach the output has been found to be stable, and it is composition is complete using optimal conditions of interaction. Under these conditions, the product is formed and stabilized after a period of 10 min, the absorption remains stable for not less than 2 h. The result was 0.852 for AMX.

Stoichiometric Ratio Determination

The stoichiometry of the oxidative reaction between AMX and chromogenic reagent H was investigated using the mole ratio method. Fig. 4 shows that the orange dye is formed in the ratio 1:1 chromogenic reagent H:AMX.

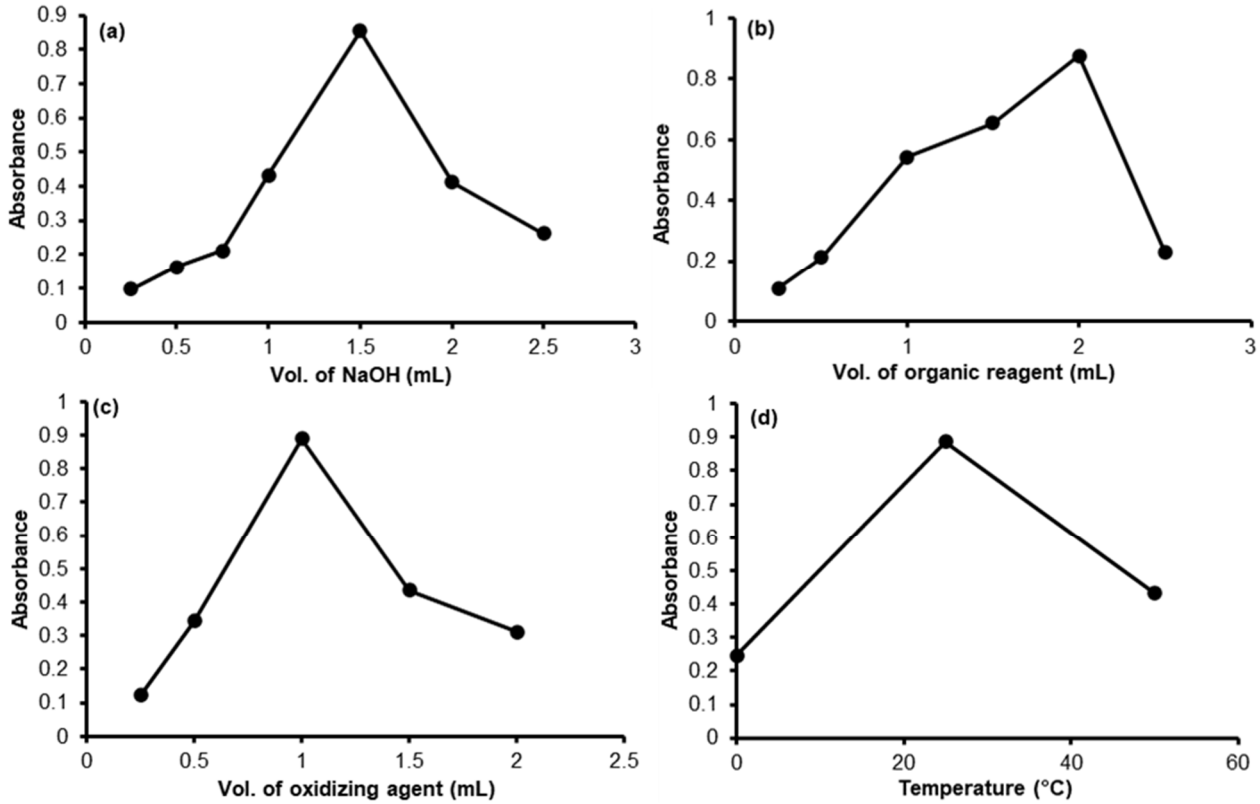


Fig 3. Effect of experimental conditions of oxidative coupling (a) Base, (b) reagent, (c) $NaIO_4$, and (d) temperature

Study the Optimum Reaction Conditions for AMX Determination Using Flow Injection

The concentration of the reagent, the concentration of the oxidizing agent, and the concentration of NaOH were all studied as the chemical parameters' to get ideal conditions for this method. The highest absorbance intensity colored was into 0.2 M when several concentrations (0.025–0.300 M) organic reagent H for the AMX were examined; the findings are shown in Fig. 5(a). The best oxidizing agent concentrate, as shown in Fig. 5(b), was $NaIO_4$ 0.1 M at various concentrations (1×10^{-3}–3×10^{-1} M). NaOH is used as the best alkaline in the flow injection for the AMX to accumulate into. When several NaOH concentrates (0.2–2 M) were examined, the greatest absorbance was at 0.8 M, as shown in Fig. 5(c).

Different physical parameters, such as reaction coil, were also investigated. The reaction coil's length ranged from 25 to 230 cm, and the best reaction coil was 50 cm because it gave the highest absorbance and was most sensitive to low dispersion. The results are shown in Fig. 6(a). The largest absorption occurred when the total flow rate was 2 mL/min because it took a long time for the reaction to complete and result in significant absorption, as shown in Fig. 6(b). Various injection sample volumes between (50–200 µL) were investigated. A volume of 100 µL was chosen as the optimal volume to yield the greatest absorbance and was used in all future trials. The findings are shown in Fig. 6(c).

The calibration graph for the AMX was established by plotting various concentrates of (2–50) and (1–150) µg/mL, respectively. From the calibration graph,

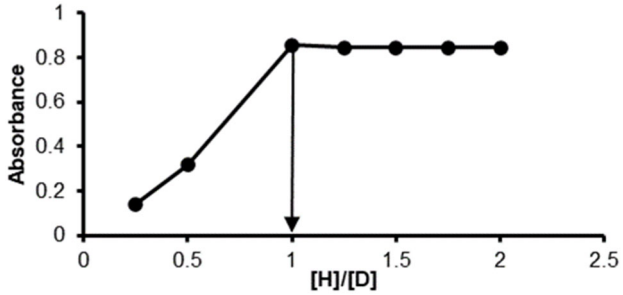


Fig 4. Mole ratio for oxidative AMOX coupled with chromogenic reagent H

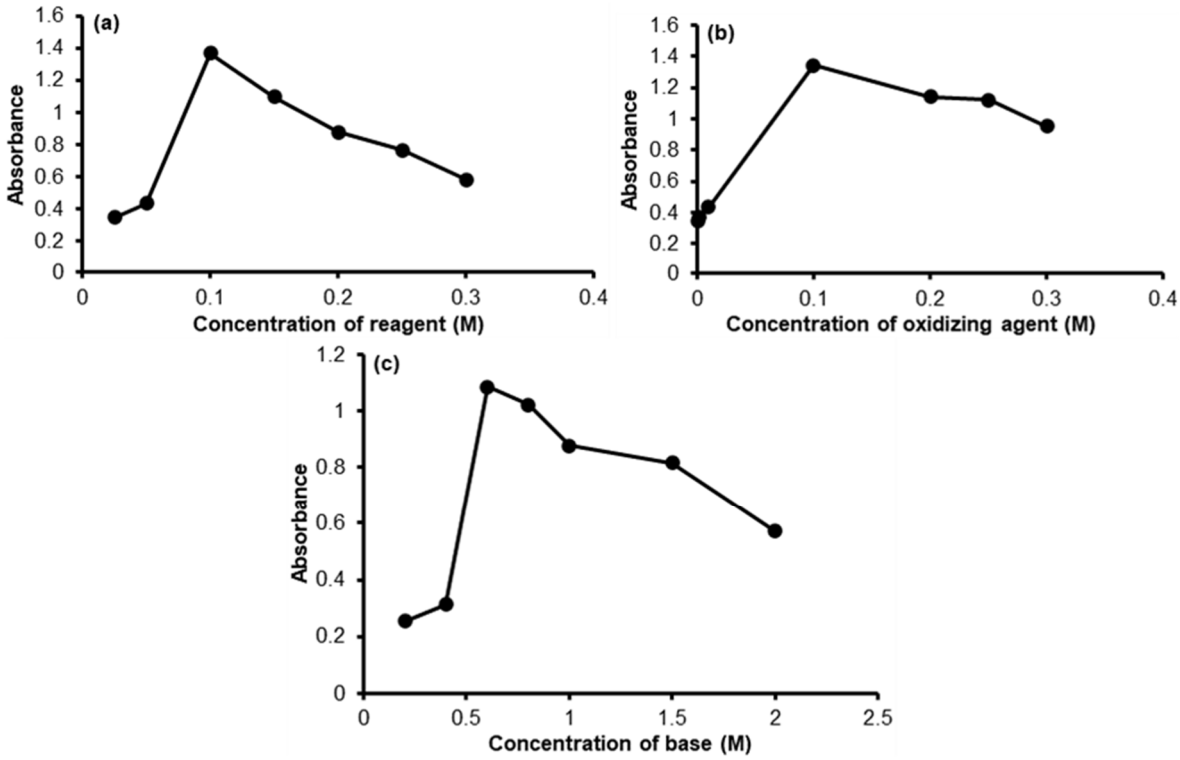


Fig 5. Effect of experimental conditions of flow injection chemical parameters, (a) Concentration of reagent, (b) concentration of oxidative agent, (c) concentration of NaOH

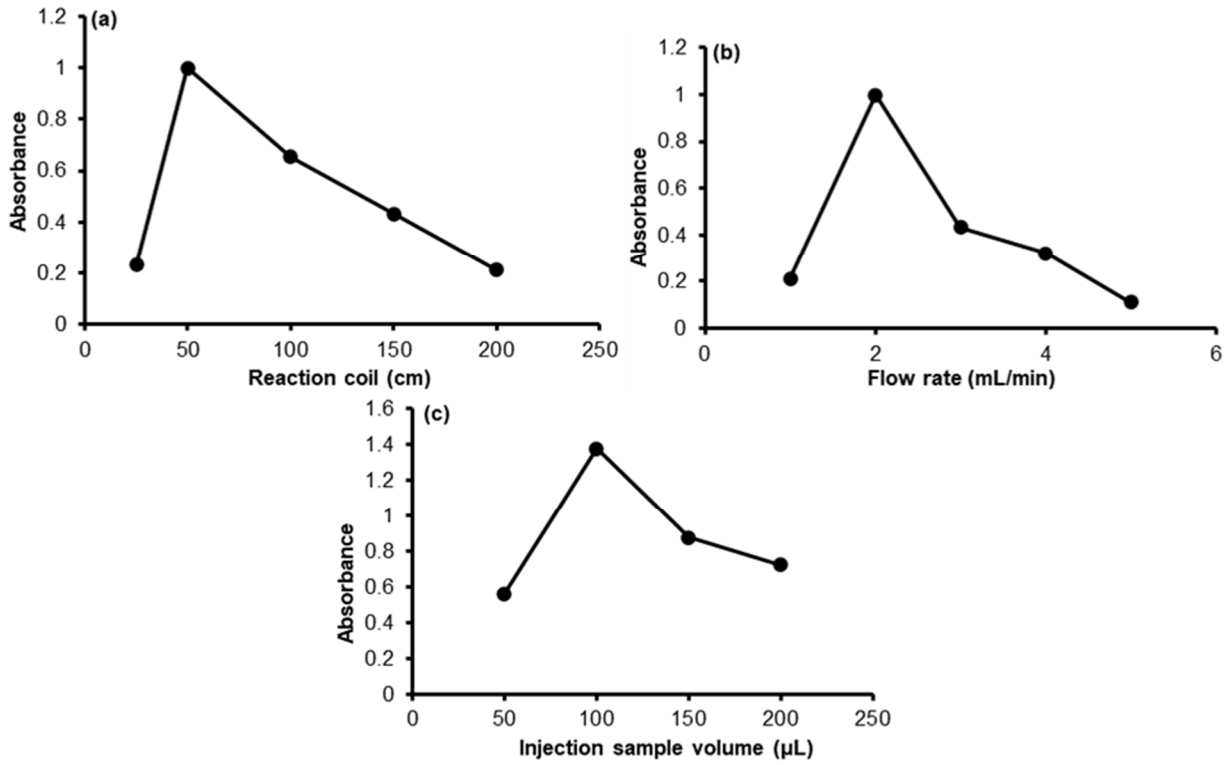


Fig 6. Effect of experimental conditions of flow injection physical parameters, (a) reaction coil, (b) flow rate, and (c) injection sample volume

the linear regression equation, correlation of coefficient (r), slop (a), and intercept (b), and optimal experimental conditions of oxidative coupling and flow injection were obtained. Fig. 7(a, b), and Table 1 display analytical results for the regression equation of the suggested oxidative coupling and FIA methods.

Studies on accuracy and precision were conducted for the suggested methods of flow injection and oxidative coupling under ideal conditions and utilizing a range of concentrations, measuring absorbance at least five times for each concentration. As stated in Tables 2 and 3, relative error (%RE), recovery (%R), and relative standard deviation (%RSD) were used to calculate precision and accuracy.

Table 1. Characteristic parameter for the proposed oxidative coupling and flow injection regression equation

Parameters	Oxidative coupling	Flow injection
λ_{max} (nm)	490	490
Color	Orange	Orange
Regression equation	Y=0.0174x–0.0053	Y=0.0086x+0.1618
Linearity range (µg/mL)	2–50	1–150
Correlation Coefficient (r)	0.9995	0.9995
ε (L/mol.cm)	0.630×10^4	0.295×10^4
Sandal' sensitivity (µg/cm^2)	0.0580	0.1200
Slope (b)	0.0174	0.0081
Intercept (a)	–0.0082	–0.0066
Limit of detection (µg/mL)	0.1890	0.4070
Limit quantification (µg/mL)	0.5710	1.2300

LOD = 3.3×SDb/S, SDb = the standard deviation of intercepts of regression lines [22-23]

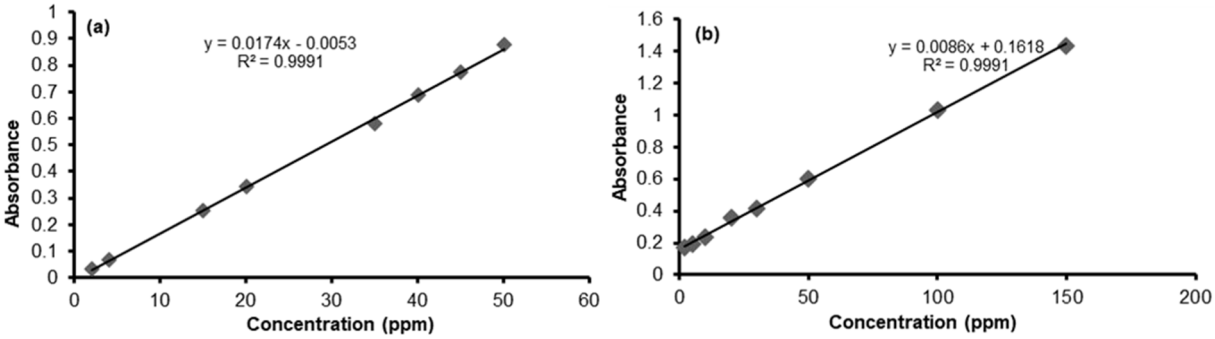


Fig 7. Calibration curve of AMX: (a) Oxidative coupling and (b) flow injection

Table 2. The precision and accuracy of suggested techniques for estimating pure samples

Drug	Amount of drugs (μg/mL)		%RE	%R	Average %R	%RSD (n=5)
	Taken	Found				
			Oxidative method			
AMX	10	9.97	−0.30	99.70		0.13
	20	19.99	−0.05	99.95	99.94	0.42
	30	30.05	0.16	100.16		0.22
			Flow injection method			
AMX	10	9.89	−1.10	98.90		0.26
	30	29.94	−0.40	99.60	99.72	0.59
	50	50.33	0.66	100.66		0.43

Table 3. The proposed method's accuracy and precision for identifying commercial medications

Type of drugs	Amount of drugs (mg)		%RE	%R	Average %R	%RSD (n=5)
	Taken	Found				
			Oxidative method			
Amoxicillin trihydrate 500 mg	10	10.04	0.40	100.40		0.31
Capsules (Global, UAE)	20	19.97	−0.15	99.85	100.26	0.43
	30	29.95	−0.16	99.83		0.51
Amoxicillin 500 mg	10	9.98	−0.20	99.80		0.76
Capsules (Ajanta, India)	20	19.98	−0.10	99.90	99.97	0.21
	30	30.07	0.23	100.23		0.32
			Flow injection method			
Amoxicillin trihydrate 500 mg	10	9.98	−0.20	99.80		0.65
Capsules (Global, UAE)	30	29.99	−0.03	99.98	99.96	0.22
	50	50.05	0.10	100.10		0.12
Amoxicillin 500 mg	10	10.01	0.10	100.10		0.44
Capsules (Ajanta, India)	50	49.97	−0.16	99.83	99.95	0.54
	30	29.95	−0.06	99.94		0.65

Average of five repeats, %E = relative error (Found-taken/taken×100), %R, and %RSD%

■ CONCLUSION

In this paper, amoxicillin reacts with a new chromogenic reagent, (*E*)-1-(4-aminophenyl)-3-(5-(4-nitrophenyl)furan-2-yl)prop-2-en-1-one (compound H), in the alkaline medium in the presence of oxidative agent $NaIO_4$ and automate by flow injection analysis. The ideal chemical parameters, such as the reagent concentration, oxidizing agent concentration, and sodium hydroxide concentration, were studied. These methods have the advantage of being simple, rapid, very sensitive, and having good precision and accuracy.

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