KINETIC SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMOXICILLIN IN PHARMACEUTICAL PREPARATION

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Abstract

Two simple, sensitive and economical kinetic spectrophotometric methods have been developed for the estimation of Amoxicillin in pure form and pharmaceutical preparations.The methods based on the oxidation of the studied drug by a known amount of potassium permanganate in alkaline medium and subsequent determination of manganate ion(Method A)and unconsumed potassium permanganate(Method B),at a suitable λ_{max}=602 and 524 nm,respectively.The reacted oxidant was found to be corresponding to the drug content. The initial rate and fixed time (at 30 minutes) methods are utilized for construction of calibration graphs to determine the concentration of the studied drugs. The calibration graphs are linear in the concentration ranges of (0.5–9.0) $\mu g.ml^{-1}$ and (1-11) $\mu g.ml^{-1}$ using the initial rate and fixed time methods, respectively. The results are validated statistically and checked through recovery studies. The two methods have been successfully applied for the determination of the studied Amoxicillin in commercial dosage form.

طرق طيفية حركية لتقدير الاموكسسلين بصورته النقية وفي مستحضره الصيدلاني

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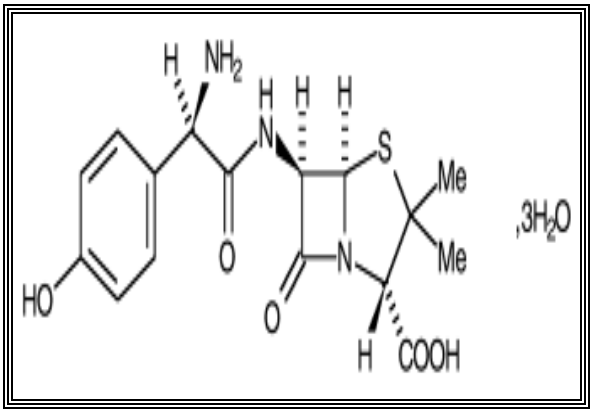
الخلاصة

طورت طريقتين طيفيتين حركيتين بسيطتين، حساستين واقتصاديتين لتقدير دواء الاموكسسلين بصورته النقية وفي المستحضرات الصيدلانيه. تعتمد الطريقتين على تفاعل الاكسدة للدواء بوجود بوتاسيوم فوق المنغنات كعامل مؤكسد في وسط قاعدي ثم تقدير ايون المنغنات(الطريقة الاولى)وبوتاسيوم فوق المنغنات الغير مستهلك(الطريقة الثانية) عند الطوليين الموجيين الاعظميين (602 و524 نانومتر) تتابعيا وقد وجد ان العامل المؤكسد يتناسب مع محتوى الدواء.وتم دراسة حركية التفاعل بواسطة طريقتي معدل السرعة الابتدائية والزمن الثابت عند 30 دقيقة ثم تثبيت الظروف الفضلى للتفاعل لكلا الطريقتين. وكان معدل الخطية لتقدير الدواء بأستخدام الطريقة الاولى (0.5−9.0 مايكروغرام.مل$^{-1}$)وبأستخدام الطريقة الثانية(1−11 مايكروغرام.مل$^{-1}$)،وطبقت الطريقتين بنجاح في تقدير الدواء في المستحضرات الصيدلانية.

1-Introduction

Amoxicillin(AMOX) is (2S,5R,6R)- 6-{[(2R)-2-amino- 2-(4-hydroxyphenyl)-acetyl]amino}- 3,3-dimethyl- 7-oxo- 4-thia- 1-azabicyclo[3.2.0]heptane- 2-carboxylic acid and

has a molecular formula $C_{16}H_{19}N_3O_5S$,whereas its chemicals structure is [1]:



Amoxicillin is a penicillin antibiotic which is used to treat many different types of infections caused by bacteria, such as ear infections, bladder infections, pneumonia, gonorrhea, and E. coli or salmonella infection.

Amoxicillin is also sometimes used together with another antibiotic called clarithromycin (Biaxin) to treat stomach ulcers caused by Helicobacter pylori infection [2].Several methods have been reported for determining this drug in pharmaceutical preparations,including Spectroscopy[3-8] SIA (Sequential Injection Analysis)[9], Voltametric [10],Flow Injection-Chemiluminescence [11,12],FI Flow Injection)[13],UPLC-MS/MS(Ultra Performance Liquid Chromatography-Tandem Mass Spectroscopy)[14],SPE-LC/ESI-MS-MS(Solid Phase Extraction-Liquid Chromatography-Electrospray-Tandem Mass Spectroscopy)[15] and Chromotographic[16,17] methods. The United states Pharmacopeia (USP) involved a high-performance liquid chromatography (HPLC) method for the determination of AMOX. in pharmaceutical preparation but this method requires large amount of high purity organic solvents and long system stabilization time [18]. Hence, it was planned to develop a sensitive and economically viable technique, like kinetic procedures. The literature is still poor in analytical procedures based on kinetics, especially for pharmaceuticals or biological fluids. However, some specific advantages in the application of kinetic methods can be expected such as, selectivity due to the measurement of the evolution of the absorbance with the time of the reaction instead of the measurement of a concrete absorbance value. Potassium permanganate has been frequently utilized in the field of pharmaceutical analysis. Many pharmaceutical compounds have been determined kinetically through this approach such as Telithromycin [19],cephalosporins[20,21],Tetracycline[22] Ramipri[23] . The aim of the present work was to study the reaction between Amoxicillin and alkaline potassium permanganate kinetically in an attempt to evaluate the drug in pharmaceutical preparation. The initial-rate and fixed-time methods were adopted after a full investigation

2-Expermental

2.1 Apparatus

All spectra and absorbance measurements were carried out on a UV/VISIBLE spectrophotometer (VARIAN UV-Visible) digital double-beam recording spectrophotometer using 1-cm quartz cell.

2.2 Reagents

All reagents used were of analytical grade. Amoxicillin was kindly supplied by State company for Drug Industries and Medical Appliance,SDI,Samara,Iraq.Capsule (Amoxicillin 250 mg) were purchased from local market.Potasium permanganate($KMnO_4$) (0.1M) and Sodium hydroxide (NaOH) were from BDH. Distilled water was used for the preparation of all aqueous solution.

2.3 Solutions

1-Amoxicillin (AMOX.) stock solution ($1000\mu g.ml^{-1}$):

This was prepared by dissolving 0.0500 g of AMOX. in distilled water in 50 ml volumetric flask. Working standard solutions were freshly prepared by diluting the stock solution with distilled water to obtain the appropriate concentration.

2-Potassium permanganate solutions (0.05 and 0.025 M):

These were freshly prepared by appropriate dilution of the concentrated volumetric solution (0.1M) with distilled water in volumetric flasks and transferred to brown bottles.

3-Sodium hydroxide solutions (0.5 and 1M):

These were prepared by appropriate dilution of the standard concentrated volumetric solution (5M) with distilled water in volumetric flasks.

2.4 pharmaceutical preparations:

Ten capsules of AMOX.(250 mg) were weighed and finally powdered using a morter.A weighed amount of the powder equivalent to 0.05 mg of AMOX. was dissolved in hot water, cooled and made up to 50 ml with distilled water. The resulting solution was filtered and

treated as described under recommended procedure.

2.5. Recommended Procedure

2.5.1 Initial –rate methods

In the first procedure ,the initial rates of the reactions were determined by measuring the slopes of the initials tangents the absorbance time curves for the first 5 min .A calibration graph was constructed by plotting the logarithm of the initial rate of the reaction versus the logarithm of the molar concentration of AMOX.

2.5.2 Fixed-time methods

In to a series of 20 ml calibrated flask, transfer increasing volume of stock solution (20µg.ml^{-1}) of AMOX. to cover the range of the calibration graph (0.5-9.0 ppm) for method A and (1-11ppm) for method B,add 1.5 ml of (1M) of sodium hydroxide solution, followed by 1.75 ml(0.05M) potassium permanganate for method A, while adding 1.25 ml of (0.5M) NaOH solution, followed by 1.5 ml (0.025M)$KMnO_4$ solution for method B shake well and then dilute the solution to the mark with distilled water. Allow the reaction mixture to stand for 30 min at room temperature, measure the absorbance at 602 nm (method A) and 524 nm (method B) against a reagent blank prepared in the same way but containing no AMOX. The color of the product formed was stable for more than 60 min**.**

3. Result and discussion

3.1 Method A(Determination of Amoxicillin (AMOX) via measuring of the manganate ion):

Permanganate ion oxidizes AMOX. in an alkaline medium, resulting in the formation of manganate ion with a maximum absorbance at 602 nm (Fig 1). The absorbance of the colored products measured versus reagent blank increases with time and then remains stable for at least 60 min.This was used as a basis for a useful kinetic method for the determination of AMOX in pharmaceutical preparations. Initial studies were directed towards the optimization of the experimental conditions in order to establish the optimum conditions necessary for quantitative formation of the product with maximum sensitivity. A 100 µg.ml^{-1} of AMOX was taken In a 20 ml final volume and the absorbance was measured at room temperature (25° C) for series of solutions by varying one and fixing the other parameters at 602 nm versus reagents blank after 30 min from the beginning of the reaction.

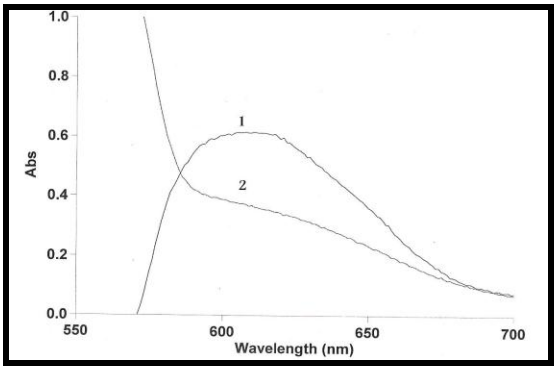


Fig 1: Absorption spectra of the product obtained by the reaction of potassium permanganate with 5 µg.ml^{-1} of 1-AMOX. in alkaline medium versus reagent blank, 2-reagent blank versus distilled water.

The effect of 0.05 M potassium permanganate oxidant (0.5-2.0ml) in alkaline solution was studied. The results obtained indicted that the absorbance increased with the increasing volume of the potassium permanganate solution up to 1.75 ml, further increase in volume resulted in a very slight decrease in the absorbance of the reaction product, thus a 1.75 ml of 0.05M potassium permanganate was found to be the most suitable concentration and was used in the subsequent experiments.

Permanganate is a powerful oxidant in alkaline medium[24]- accordingly oxidation of AMOX was carried out in NaOH solution. Trials were made to determine the AMOX. through its oxidation with potassium permanganate in neutral and acidic media, but very little oxidation of AMOX. was observed. Other alkalies,such as KOH,NH_4OH and Na_2CO_3 were also examined to determine the best alkaline medium .However, their effect on the color development was less than that of NaOH;therefore the latter was used throughout the study.

The effect of different volumes (0.5 to 2.25 ml) of 1M NaOH solution on the formation of MnO_4^{-2} was studied. It was found that increasing the absorbance of the reaction product up to 1.5 ml,further increase in volume resulted in a decrease in the absorbance of the reaction product, thus 1.5 ml of 1M NaOH was adequate for the maximum absorbance.

To obtain optimum results, the order of the addition of reagents should be followed according to the order cited in the procedure, otherwise a loss in color intensity was observed.

3.2. Stoichiometry of the reaction

The stoichiometric ratio between potassium permanganate and AMOX measured at 602 nm was determined by Mole-ratio method and was

found to be ≈1:1(Figure 2) AMOX/$KMnO_4$

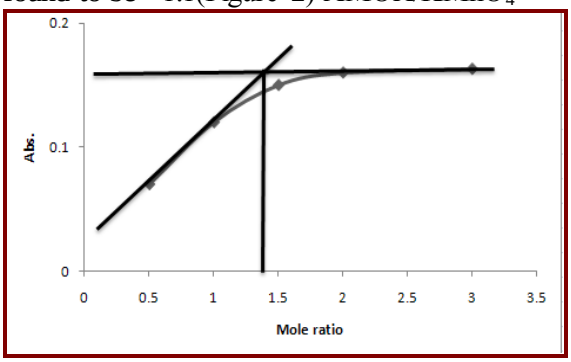


Fig 2:Mole ratio plot of AMOX/$KMnO_4$,Conc. Of $KMnO_4$=6.4×10^{-6} M

Based on the obtained molar reactivity, and depending on the phenolic nature of AMOX., the reaction pathway is proposed to proceed as shown in(Fig 3).

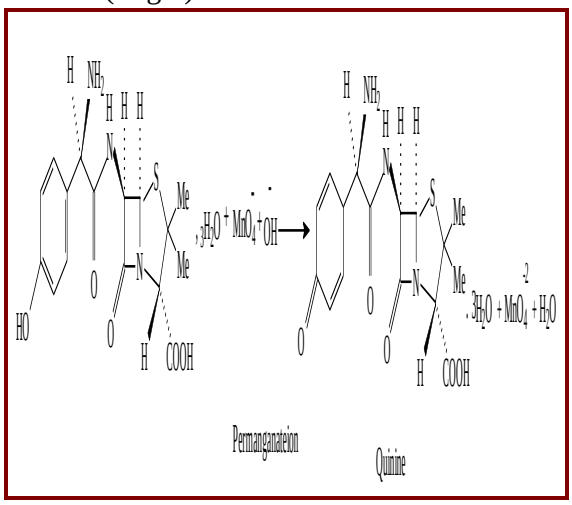


Fig 3:Reaction scheme

3.3Method (B) Determination of Amoxicillin via measuring of the unconsumed potassium permanganate:

An alternative spectrophotometric method for the determination of AMOX. based upon measuring the decrease in the absorbance of potassium permanganate at 524 nm was developed. The absorbance of the reagent blank measured versus colored products (Fig 4) increased with time.

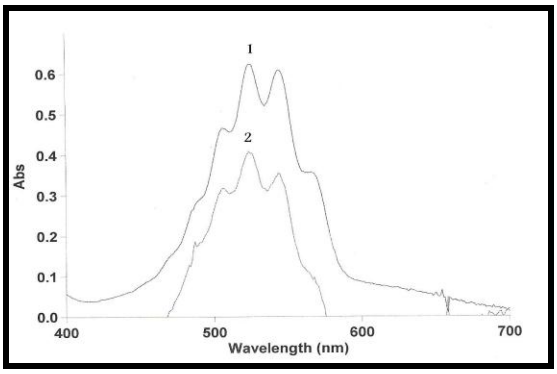


Fig 4:Absorption spectra of blank ($KMnO_4$ +NaOH) versus 1-distilled water, 2-AMOX(20 µg.ml^{-1}) +$KMnO_4$ +NaOH

Because the absorbance of the unconsumed permanganate ion color increased with time, a kinetically based method was elaborated for the determination of AMOX in pharmaceutical preparations. Initial studies were directed towards the optimization of the experimental conditions, in order to establish the optimum conditions that are necessary for a quantitative formation of the product with a maximum sensitivity.

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of AMOX by measuring of the unconsumed potassium permanganate at 524 nm after 30 min from the beginning of the reaction.

The effect of the 0.5M sodium hydroxide concentration (0.25-2 ml) was studied .The results indicated that the absorbance increased with the increasing volume of the sodium hydroxide solution up to 1.25 ml, further increase in volume resulted in a slight decrease in the absorbance.Thus, 1.25 ml of o.5 M NaOH was found to be the most suitable concentration for the maximum absorbance.

The effect of the different volumes (0.25 to 1.5 ml) of 0.025 M potassium permanganate solution on the absorbance was studied. It was found that the absorbance was increased as the volume was increased up to 1.5 ml.Thus; 1.5 ml of (0.025M) potassium permanganate was adequate as the suitable concentration

To obtain optimum results, the order of the addition of reagents should be followed according to the cited procedure, otherwise a loss in color intensity was observed. After obtaining the optimum conditions for the formation of the product, the absorption spectra of the reagent blank versus distilled water and the reagent blank versus product solution were recorded in the range of 400 to 700 nm

(Fig.4).The maximum absorption of the reagent blank was found at 524nm, which was used in all subsequent experiments.

3.4 Calibration graphs

Initial rate method

The initial rate of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance time curve for method A and B.A calibration graph was constructed by plotting the logarithm of the initial rate of the reaction versus the logarithm of the molar concentration of AMOX.(Figs,5,6, 7 and8)

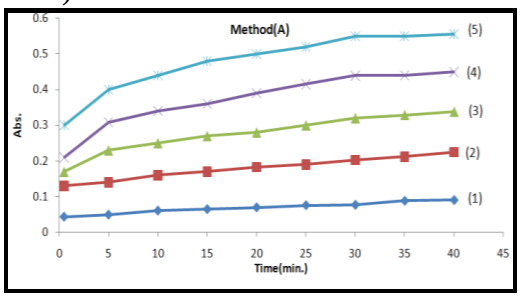


Fig 5:Absorbance versus time for method A,showing the dependence of the reaction rate on AMOX. concentrations:
(1)2.38×10^{-6}M;(2)7.15×10^{-5}M;(3)1.19×10^{-5}M;(4)1.66×10^{-5}M;(5)2.14×10^{-5}M.

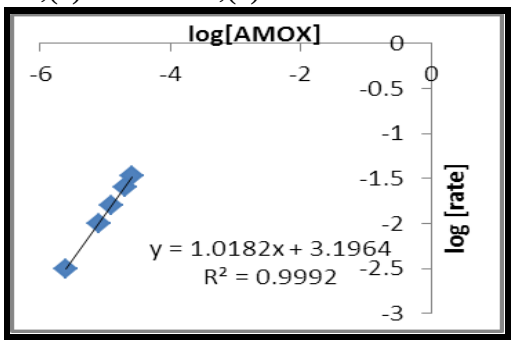


Fig 6:log(rate)versus log(AMOX.)graph for method A.

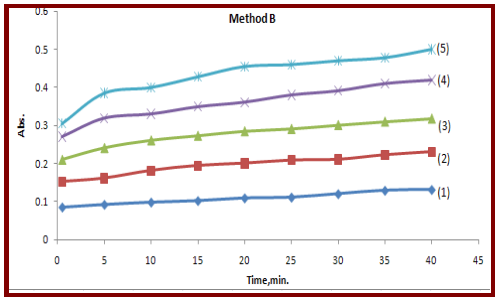


Fig.(7):absorbance versus time for method B,showing the dependence of the reaction rate on AMOX.concentrations:
(1)4.76×10^{-6}M;(2)9.53×10^{-6}M;(3)1.4×10^{-5}M;(4)1.90×10^{-5}M;(5)2.38×10^{-5}M.

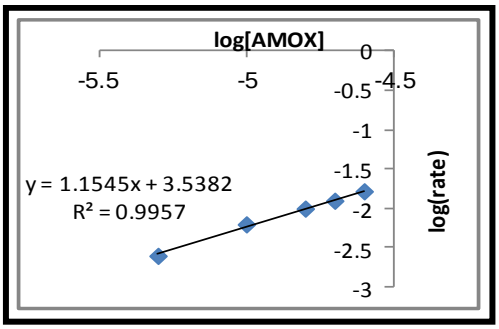


Fig 8:log(rate) versus log(AMOX.) graph for method B.

Reaction rates were determined for different concentrations of AMOX. at a pre-selected fixed time, which was accurately determined the absorbance was measured. Calibration graphs of absorbance versus initial concentration of AMOX. were established at fixed time of 5,10,15,20,25,30,30 and 40 min. with regression equations assembled in (Table 1).

Table 1: Regression equations for AMOX. at different fixed time over range 2.38×10^{-6} to $2.14\times10^{-5}$and range4.76×10^{-6} to 2.38×10^{-5} for method A and B respectively at room temperature

Time min	Method A		Method B	
	Regression equation	Correlation coefficient	Regression equation	Correlation coefficient
5	0.0086+ 0.0434C	0.9994	0.0162+ 0.0372C	0.9990
10	0.0175+ 0.0467C	0.9991	0.0279+ 0.0377C	0.9984
15	0.0138+ 0.0513C	0.9992	0.0269+ 0.0404C	0.9988
20	0.0163+ 0.0539C	0.9993	0.0259+ 0.0427C	0.9991
25	0.024+ 0.0556C	0.9992	0.0288+ 0.0435C	0.9990
30	0.0219+ 0.0593C	0.9995	0.0388+ 0.0435C	0.9992
35	0.0354+ 0.0577C	0.9994	0.0447+ 0.0439C	0.9990
40	0.0499+ 0.0567C	0.9991	0.0409+ 0.0465C	0.9990

Table 2: Analytical values of statistical treatments for the calibration graphs

Parameters	Value	
	Method A	Method B
Correlation coefficient, r	0.9995	0.9993
Linearity percentage r^2 %	0.9990002	0.9984006
Test for a significant correlation t*	89.406975	74.954241
Regression equation	y=0.0593x+ 0.0219	y= 0.0435x + 0.0382
Slope, b(ml. μg^{-1})	5.93×10^{-2}	4.35×10^{-2}
Intercept, a	2.19×10^{-2}	3.82×10^{-2}
Conf. limit for slope b±tsb	0.0593± 0.0011507	0.0435± 0.000680
Conf. Limit for intercept a±t_{sa}	0.0219± 0.0163755	0.0382± 0.013859
Standard deviation of the residuals, $S_{y/x}$	4.3127×10^{-3}	3.16227×10^{-3}
Standard deviation of the slop, S_b	4.816×10^{-4}	2.94467×10^{-4}
Standard deviation of the intercept, S_a	6.9388×10^{-3}	5.99999×10^{-3}
Conf. limit conc. $\mu g.ml^{-1}$ 95% C.I.	8.987±0.1952055	9.95±0.0450000
Conf. limit Abs. 95% C.I.	0.557±0.0052769	0.476±0.0055391
Linearity range ($\mu g.ml^{-1}$)	0.5 -9.0	1-11
Molar absorptivity, ε ($l.mol^{-1}. cm^{-1}$)	3.8161×10^{4}	3.3557×10^{4}
Sandell's sensitivity, S ($\mu g. ml^{-1}$)	1.3157×10^{-2}	1.2499×10^{-2}
Limit of detection, LOD ($\mu g. ml^{-1}$)	2.181804×10^{-1}	2.09302×10^{-1}
Limit of quantification, LOQ ($\mu g. ml^{-1}$)	7.272681×10^{-1}	6.89655×10^{-1}

t*-tabulate=2.31 at confidence level 95% and (n-2)=9 degrees of freedom.

t*-tabulate=2.36 at confidence level 95% and (n-2)=8 degrees of freedom.

It is clear that the slop increases with time and the most acceptable values of the correlation coefficient (r) and the intercept were obtained for a fixed time of 30 min, which was therefore, chosen as the most suitable time interval for measurement.

Fixed time method

In the second procedures, the absorbencies measured at a fixed time of 30 min. were plotted against the final concentrations of AMOX.(Fig.7)and analytical values of statistical treatments for the calibration graphs are summarized in (Table 2).

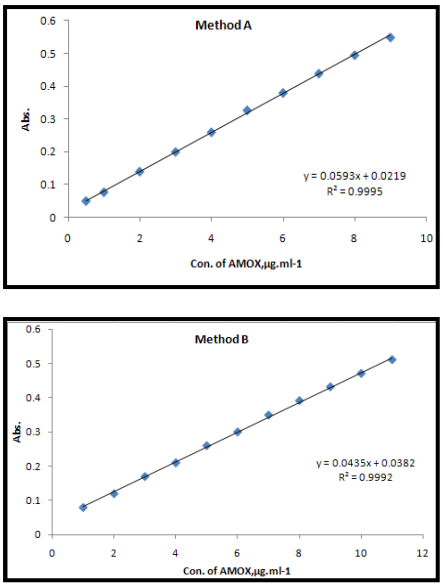


Fig 7:Calibration graph of method A and B at fixed time 30 min.

3.5 Evaluation of the kinetic methods

The rate of reaction was found to be dependent on AMOX. concentration. The rates were followed at room temperature (25° C) with various concentration of AMOX. in the range of (0.5-9.0) $\mu g.ml^{-1}$ and (1-11) $\mu g.ml^{-1}$ for A and B methods respectively keeping $KMnO_4$ and NaOH concentrations constant. The reaction rate was found to obey the following equation:

Rate=K' $[AMOX]^{n}$…… (1)

Where K' is the pseudo-order rate constant and n is the order of the reaction. The rate of the reaction may be estimated by the variable-time method [25] (differential initial rate method)[26] as $\Delta A/\Delta t$, where A is the absorbance and t is the time in minutes.Taking logarithms of rates and concentration, Eq. (1) is transformed into:

Log (rate) =log $\Delta A /\Delta t$ = log K' +n log [AMOX]…….(2)

Regression of log(rate) versus log[AMOX] gave the regression equation:

Log(rate)= 3.196 +1.018Log C(r=0.0.9992) for method A

Hence K=1500 min^{-1}=26 sec^{-1} and reaction is first order (n=1.018), with respect to AMOX concentration.

Log (rate) =3.538+1.154log C (r=0.9957) for method B

Hence K=3471min^{-1}=57 sec^{-1} and the reaction is first order (n=1.154).

3.6. Accuracy and precision of proposed methods

The accuracy and precision of the determination of AMOX. were studied. For five replicates of three different concentration of AMOX., the results in (Table 3)and(Table 4) show a good accuracy and precision.

Table 3: Accuracy and precision of the initial-rate method

Method	Conc.of AMOX,µg.ml^{-1}		E %	Rec.%	RSD%
	Present	Found			
A	3.000	2.987	-0.433	99.566	1.024
	5.000	4.997	-0.200	99.950	0.787
	9.000	9.050	+0.583	100.583	0.994
B	3.000	2.960	-1.333	98.666	0.790
	6.000	6.037	+0.625	100.625	0.826
	9.000	8.932	-0.750	99.250	0.573

Table 4: Accuracy and precision of the fixed-time method

Method	Conc.of AMOX,µg.ml^{-1}		E %	Rec.%	RSD%
	Present	Found			
A	3.000	3.017	+0.566	100.566	0.899
	5.000	5.007	+0.150	100.150	0.619
	9.000	8.987	-0.138	99.861	0.717
B	3.000	3.025	+0.666	100.666	0.318
	6.000	5.975	-0.833	99.583	0.416
	9.000	8.955	-0.500	99.500	0.354

3.7 Pharmaceutical applications

The validity of the proposed methods was tested by adding a fixed amount of pure drug to pre-analyzed pharmaceutical preparation. The results obtained from the investigations are summarized in Table 5 and Table 6, which indicated no interfere with the determination and the proposed methods have good selectivity.

Table 5: Determination of AMOX in pharmaceutical preparations by initial method using the standard addition technique.

Method	Pharmaceutical preparation	Conc. Of AMOX,µg.ml^{-1}			E %	Rec. %
		Added	Present	Found		
A	AMOXYCILLIN(250mg)	3.000	2.000	5.032	+0.645	100.645
		7.000	2.000	9.018	+0.204	100.204
B	AMOXYCILLIN(250mg)	3.000	3.000	5.989	-0.179	99.820
		6.000	3.000	8.978	-0.238	99.761

Table 6: Determination of AMOX in pharmaceutical preparations by the fixed-time method using the standard addition technique.

Method	Pharmaceutical preparation	Conc. Of AMOX,µg.ml^{-1}			E %	Rec. %
		Added	Present	Found		
A	AMOXYCILLIN (250 mg)	3.000	2.000	5.026	+0.539	100.539
		7.000	2.000	9.040	+0.449	100.442
B	AMOXYCILLIN (250 mg)	3.000	3.000	5.972	-0.459	99.540
		6.000	3.000	8.983	-0.222	99.879

The initial-rate and fixed-time methods were applied to determination of AMOX. in pharmaceutical preparation by the analysis two different concentrations of pharmaceutical preparations using the proposed procedures .The results obtained are given in Table 7 and Table 8

Table 7: Application of the proposed methods for determination of AMOX. in pharmaceutical preparations by the initial-rate method.

Method	Pharmaceutical preparation	Con.of AMOX,µg.ml^{-1}		E%	Rec.%
		Present	Found		
A	AMOXYCILLIN (250 mg)	3.000	3.020	+0.674	100.674
		5.000	5.023	+0.460	100.460
B	AMOXYCILLIN (250 mg)	3.000	2.978	-0.716	99.283
		6.000	5.962	-0.627	99.372

Table 8: Application of the proposed methods for determination of AMOX in pharmaceutical preparations by the fixed-time method.

Method	Pharmaceutical preparation	Con.of AMOX,µg.ml^{-1}		E%	Rec.%
		Present	Found		
A	AMOXYCILLIN (250 mg)	3.000	3.018	+0.333	100.333
		5.000	5.042	+0.843	100.843
B	AMOXYCILLIN (250 mg)	3.000	2.983	-0.536	99.463
		6.000	5.972	-0.459	99.540

3.7. Conclusions

The proposed methods are superior to other reported methods by showing good sensitivity, and low detection limit. In addition to competitive precision and sensitivity, the proposed procedures show relevant selectivity allowing analysis without separation steps, and providing suitable alternative to many chromatographic procedures. The proposed methods are advantageous when they are compared with colorimetric methods in having higher sensitivity. The data given above reveal that proposed methods are accurate and sensitive (Method A> Method B) with good precision and accuracy. With these methods, one can do the analysis with speed at low cost without losing accuracy. The most important limitation of the proposed methods is their poor selectivity, especially for compounds of similar structure; this shortcoming does not affect the usefulness in routine analysis and content uniformity determination of AMOX. as it is singly prescribed. The proposed methods can be used as alternative methods to reported ones for the routine determination of AMOX. in the pure form and in pharmaceutical preparations depending upon the availability of chemicals and equipment.

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