Spectrophotometric Determination of Amoxicillin Trihydrate in Pure and Pharmaceutical Dosage Forms

Hemn A. Qader
Dept. of Pharmaceutical Chemistry/College of Pharmacy/Hawler Medical University/ Erbil
Nabil A. Fakhre
Dept. of Chemistry/ College of Education Salahaddin University/ Erbil

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Abstract

A rapid and sensitive method for the determination of amoxicillin trihydrate (AMXT) based on the diazo-coupling reaction was studied. Sulphanilic acid diazotizes with nitrite ion in acidic medium to produce a water soluble, colorless diazonium ion, which subsequently coupled with AMXT to form a colored azo dye in the alkaline medium, having maximum absorption at 455 nm. The calibration graph showed that Beer's law is obeyed over the concentration range of 0.3 – 30.0 µg/mL of AMXT, with the detection limit of 0.15 µg/mL and molar absorptivity was 2.3×10^4 L/mol.cm. The accuracy and the precision were acceptable depending upon the values of error percentage and relative standard deviation. The influence of common interferences was studied and the method was applied with good recovery for the determination of AMXT in pure form and different pharmaceutical formulations, which commercially available in Erbil market.

Keywords: spectrophotometry, amoxicillin trihydrate, diazotization-coupling reaction.

Introduction

Amoxicillin, or hydroxyl ampicillin (scheme 1), is a phenolic β-lactam antibiotic with significant activity against both Gram-positive and Gram-negative bacteria. This antibiotic is widely used to treat infectious diseases in humans and animals, and to enhance both growth and yield in agriculture [(1)]. Amoxicillin kills bacteria by interfering with the synthesis of the bacterial cell wall. As a result, the bacterial cell wall is weakened, the cell swells and then ruptures. Amoxicillin is readily hydrolyzed by the staphylococcal penicillinase[(2)]. In 1972, amoxicillin was introduced for the first time, which maintained broad spectrum activity of ampicillin [(3)], but possesses some significant advantages over ampicillin [(4)], include more complete gastrointestinal absorption and little or no effect on absorption of food, and other advantages over some antibiotics such as high rate of absorption and its stability under acid conditions [(5)]. Various hydrated forms of amoxicillin, including monohydrate, dihydrate, and trihydrate, have been reported, among which, the trihydrate is the most stable hydrated form [(5)].

The therapeutic importance of amoxicillin requires the development of a sensitive and rapid method for industrial quality control and clinical monitoring [(1)]. From this point of view, several analytical procedures established and reported in the literature for the determination of amoxicillin in pure and pharmaceutical dosage forms, including spectrophotometric methods[(6–17)], derivative spectrophotometric methods [(5,18,19)], HPLC methods [(20-22)], and chemiluminescence methods [(23-25)].

In the present work, a spectrophotometric method is established for determination of amoxicillin trihydrate in the bulk and dosage forms, with the aid of diazo-coupling reaction. Sulphanilic acid diazotizes with nitrite ion in acidic medium to produce a water soluble, colorless diazonium ion, which subsequently coupled with amoxicillin to form a colored azo dye in the alkaline medium (Scheme 2).

Experimental

Apparatus

The spectral and absorbance measurements were carried out using 1-cm quartz cells, on a UV/Visible digital single-beam spectrophotometer of BIO-TEK Instruments (BIO-TEK Instruments manufactured in the UK for Bio-Tek instruments, model: KP-99-90283, Milan, ITALY).

Reagents and solutions

All chemicals were used of analytical reagent grade and all of the solutions must be prepared freshly.

Amoxicillin trihydrate (AMXT) (100 µg/mL) (Fluka):

prepared by dissolving 0.01 g of AMXT, in an amount of deionized water, with using ultra sonic devise for dissolving the compound and diluting to 100 mL in a volumetric flask [(5)]. Each working standard solution was freshly prepared by suitable dilution of the stock solution with distilled water.

Sulphanilic acid solution (0.5% w/v) (Hopkin and Williams, England):

0.50 g of this compound was dissolved and diluted to 100 mL with D.W.

Sodium nitrite solution (0.2% w/v) (Scharlau):

0.20 g of the compound was dissolved and diluted to 100 mL with distilled water.

Hydrochloric acid, **HCl (~ 0.01 N) (S D Fine-Chem Limited, Mumbai).**

Sodium carbonate solution (0.5 N) (Scharlau): 2.65 g of sodium carbonate, Na_2CO_3 was dissolved, then heated in order to dissolve and diluted to 100 mL with distilled water in a volumetric flask.

Recommended Procedure

Spectrophotometric determination of amoxicillin trihydrate

In a series of 25 mL volumetric flasks, each one containing 0.5 mL HCl (0.01 N), 1.0 mL $NaNO_2$ (0.2%), and 1.0 mL of sulphanilic acid (0.5%) to produce a water soluble, colorless diazonium ion. The formed diazonium ion subsequently coupled with (7.5 – 75) µg of AMXT to form an orange azo compound after the addition of 1.0 mL of Na_2CO_3 (0.5 N). The volume in each flask was made to the mark with distilled water, and the colored product is monitored spectrophotometrically against a reagent blank at 455 nm. The reagent blank is prepared in the same manner but without AMXT.

Results and Discussions

Absorption spectra

When AMXT was treated according to the recommended procedure, the absorption spectra of the formed azo compound showed a maximum absorption at 455 nm. While, the blank has no significant absorbance in this region, as it is shown in the (Figure-1).

Optimization of reaction conditions

The effect of the type and concentration of acid with concentration of nitrite ion solution to form nitrous acid were studied. The results showed that use of 0.01 M hydrochloric acid with 1.0 mL of 0.2% $NaNO_2$ were found to give better results (Figures 2a, 3 and 4a). The effect of volume of 0.5% sulphanilic acid solutions as diazotizing reagent was examined, 1.0 mL of this solution gave the maximum intensity (Figure 4b). The main factors for controlling azo coupling are pH value and temperature, which are to be arranged to favor coupling rather than diazo decomposition [26]. Therefore, the type and the volume of different alkali solutions were examined. The results indicated that with using of 1.0 mL of 0.5 N of Na_2CO_3 solution, optimum condition can be obtained (Figure 2b and 4c). The order of addition of the reactants should be followed, as mentioned in the recommended procedure.

The effect of using ice bath (low temperature) on the formation of the diazonium and the azo dye was examined, the results showed there was no significant difference in the absorbance measurements of the azo dye, with or without using ice, therefore, the experiments have been carried out at room temperature.

Under the optimized conditions, colour stability of the azo dye was studied, the results indicated that the colour developed instantaneously and remains stable for about 15.0 minutes.

Calibration graph and the statistical data

Under the chosen optimum conditions, a calibration curve was constructed (Figure 5). The graph showed that the colour system is obeyed Beer's law in the concentration range of 7.5 – 750 µg of AMXT in a 25 mL of final volume (i.e. 0.3 – 30 µg/mL of AMXT). (Table-1) shows the statistical data of the calibration curve of spectrophotometric determination of AMXT.

Accuracy and precision

The accuracy and the precision of the method were tested by determining five replicate of standard AMXT solution at three concentration levels. The values of the percentage of

relative error (Error %) and the percentage of the relative standard deviation (RSD %) for these replicate measurements of AMXT were calculated. The results are shown in (Table-2).

Interferences Study

The effects of different additives and excipients (starch, fructose, lactose, glucose, sucrose, mannitol and Mg-stearate on the determination of 15 µg/mL of AMXT via the proposed method were studied. A species considered to be interfere, when its presence caused a relative error percentage greater than ± 5.0% in the absorbance of the sample. Results, presented in Table 3, indicated that the commonly encountered excipients did not interfere in the examined method, even when they present more than 100-folds of cited drug except Mg-stearate which starts to cause interference when its concentration exceed 10-folds that of the analyte.

Application of the Method

The proposed method was applied successfully to the determination of AMXT in different pharmaceutical formulations, which commercially available in Erbil market.

An accurately weighed amount of 10 powdered capsules or mixed content 100 mg from each one of the items under study were dissolved in minimum amount of D. W. with sonication, and transferred into a 100 mL volumetric flask then completed to the mark with distilled water. The flask with its contents was shacked well and filtered. A sample of 375 µg of AMX in a final volume of 25 mL from each sample was taken and the measurement was carried out as described earlier under the described recommended procedure.

In addition, recovery experiments were performed by adding a known amount from the standard AMXT to the pre-determined dosage forms. Then total amount of AMXT, was determined with the proposed method. (Table 4) summarized composition, company, the trade name, determination of amoxicillin contained in pharmaceutical formulations with the proposed method and the recovery of the method.

Conclusions

The method was applied successfully for the determination of amoxicillin and amoxicillin trihydrate in the bulk and different pharmaceutical formulation samples. The proposed method offers some advantages such as; more sensitive than some of the reported methods [1, 4, 6, 9, 11, 13 – 17], rapid colour development, reproducibility, and wide applicable range compare with other published methods [14 – 16, 27, 28]. In addition, the method requires neither extraction process nor heating. The application seems to be inexpensive and yielding results in good recovery (Table 5).

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Table (1): Statistical data of the calibration curve for the determination of AMXT spectrophotometrically

Parameter	Value
λ_{max} (nm)	455
Color	Brownish yellow
Linear range (µg/mL)	0.3-30
Regression equation	A = 0.0602 [AMXT] + 0.0173
Slop (L/mg.cm)	0.0602
Intercept	0.0173
Molar absorptivity (L/mol.cm)	2.3×10^4
Correlation coefficient	0.9999
Detection Limit (µg/mL)	0.15
Sandell's sensitivity (µg/cm^2)	0.01823

Table (2): Accuracy and precision of the proposed spectrophotometric method

AMXT concentration (µg/mL)	*Error %	*RSD %
0.3	– 3.12	3.77
15	– 2.32	0.43
30	– 1.75	0.67

*Results for five replicates.

Table (3): The effect of the presence of the most common excipients on the determination of (15 µg/mL) of AMXT.

Additive and excipients	Added amount (µg)	Error %
Starch	2500	- 3.76
Fructose	1500	- 3.98
Lactose	1750	- 4.16
Glucose	1500	- 3.28
Sucrose	1500	- 4.13
Mannitol	1500	- 4.24
Mg-stearate	150	+ 3.26

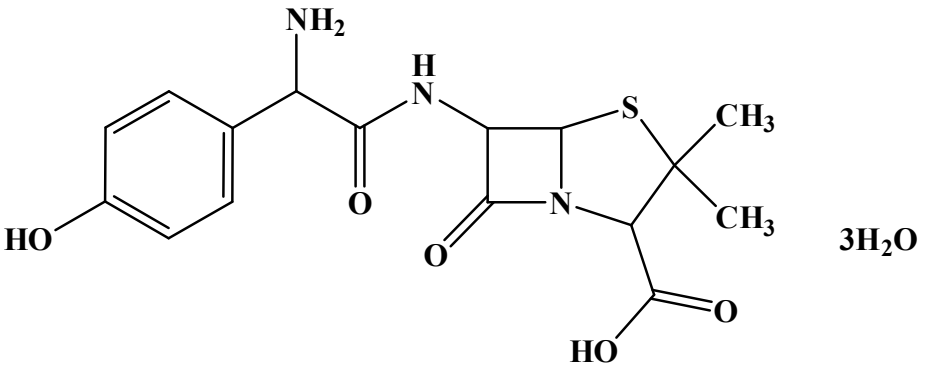
Table (4): Determination of amoxicillin in commercial pharmaceutical formulations with the recovery result of the method.

Item type	Company	Trade name	Found amount (mg)	Recovery %
Capsule, 500 mg as trihydrate	Hikma, Jordan	Penamox	498.172	99.19
Capsule, 500 mg	Global pharma, UAE	Glomax 500	493.19	103.06
Capsule, 250 mg	GSK, USA	Amoxil™	242.44	102.51
Capsule, 250 mg as trihydrate	S.D.I, Iraq	Amoxysam-250	256.28	96.98
Vial, 500 mg	Panpharma S.A., France	Panamoxicilline	515.34	98.64
Oral suspension, 250 mg/5 mL, as trihydrate	Global pharma, UAE	Glomax ®	247.98	99.63

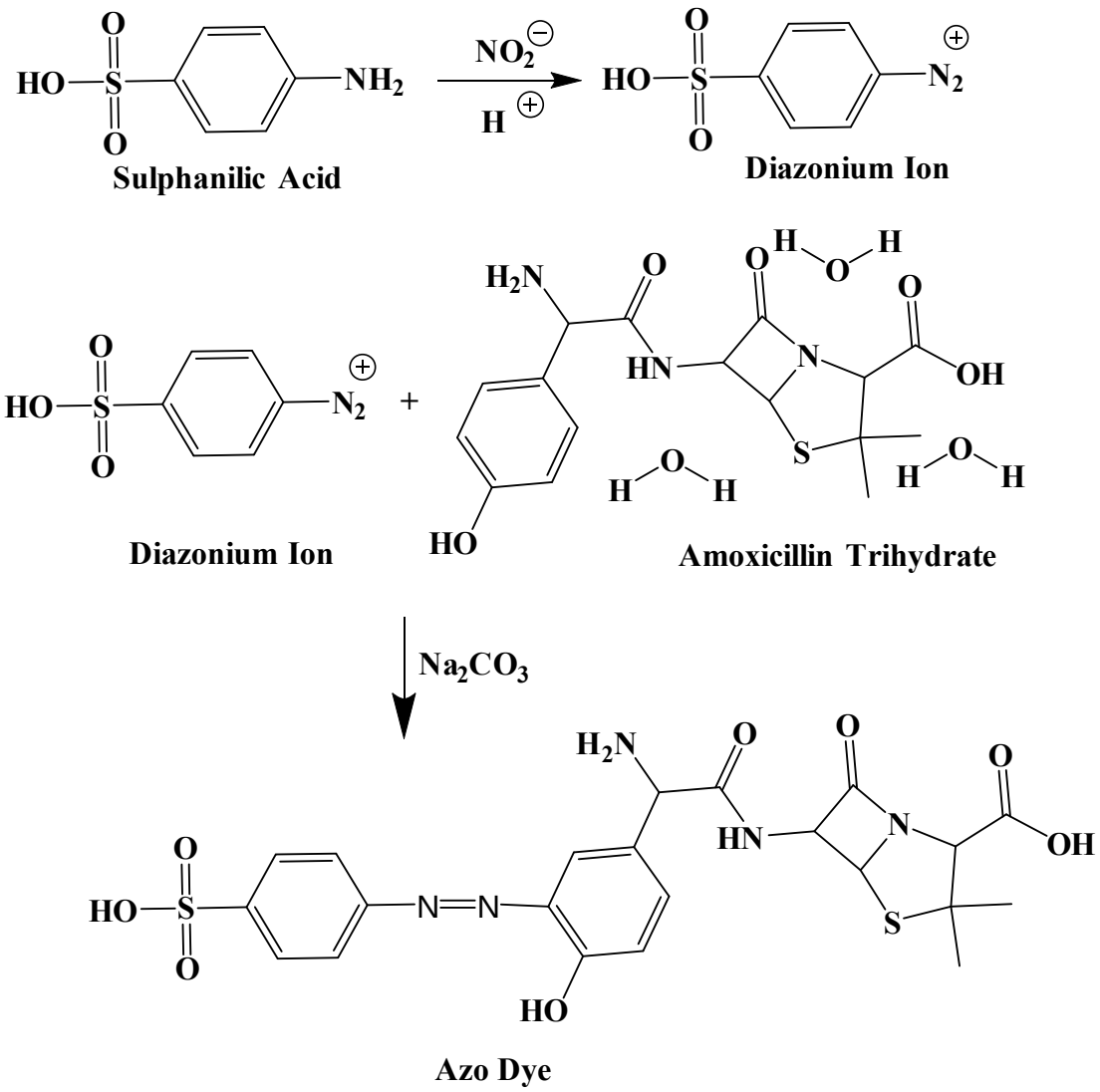
Table (5): Comparison of the present method for the spectrophotometric determination of AMXT with some published methods.

Reagents	λ_{max} nm	Determination limit (µg/ml)	Detection limit (µg/ml)	Ref.
Diazotized *o*-nitroaniline	435	25 – 400	5.1	1
Diazotized *p*-nitroaniline	478	0.5 – 100	0.104	4
Metol (N-methyl-p-hydroxy aniline)	620	5 – 60	1.494	6
4-aminoantipyrine	510	1 – 60	0.173	9
Ninhydrin	578	10 – 80	2.41	11
I_3^-	351	2 – 40		13
Diazotized *p*-amino benzoic acid & Diazotized procaine	435	0.4 – 10	0.1877	14
$FeCl_3$ + 1,10-Phenanthroline	450	0.4 – 14	0.1916	14
2,4-dinitrophenylhydrazine	510	2 – 20		16
Fe^{3+} and hexacyanoferrate (III)	515	1 – 40	0.230	17
Diazotized sulphanilic acid	700	5 – 13.5	0.5628	27
	455	0.3 – 30	0.15	p. w*

* p.w.= present work



Scheme (1): The structure of amoxicillin trihydrate (AMXT).



Scheme (2): The reaction of AMXT with diazotized sulphanilic acid to produce azo dye

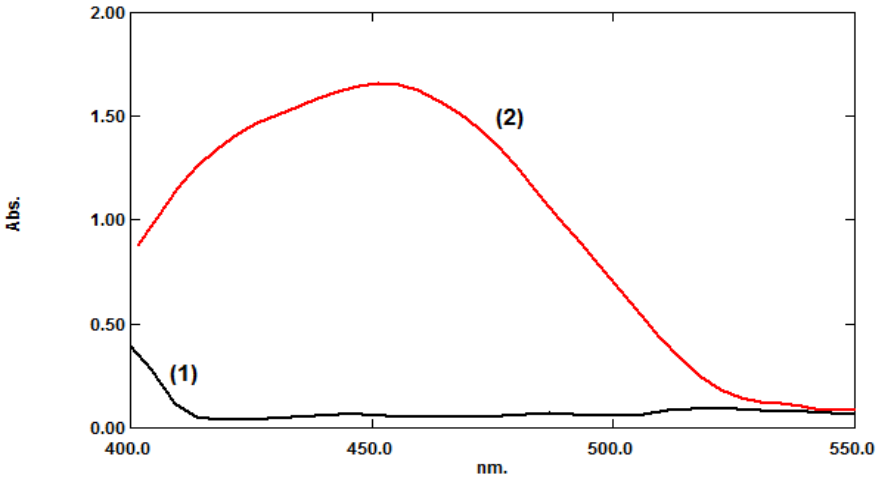


Fig. (1): Absorption spectra of blank against distilled water (1) and azo dye against reagent blank (2) treated according to the recommended procedure.

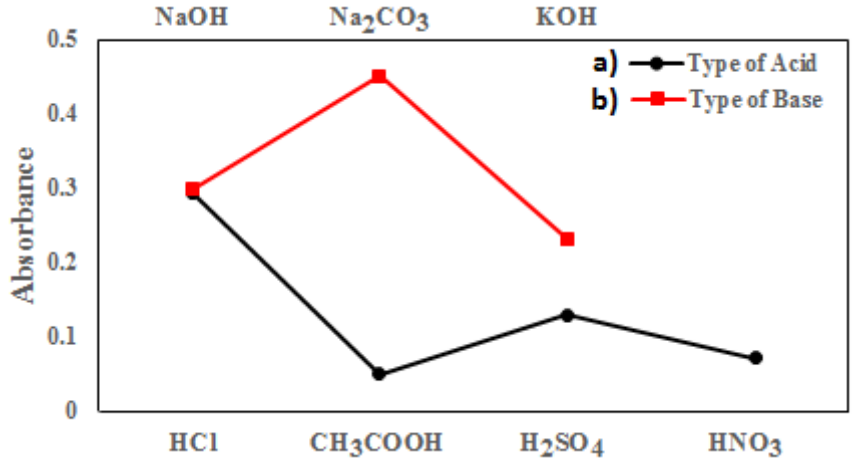


Fig. (2): Effect of (a) type of acid, (b) type of base on the formation of nitrous acid.

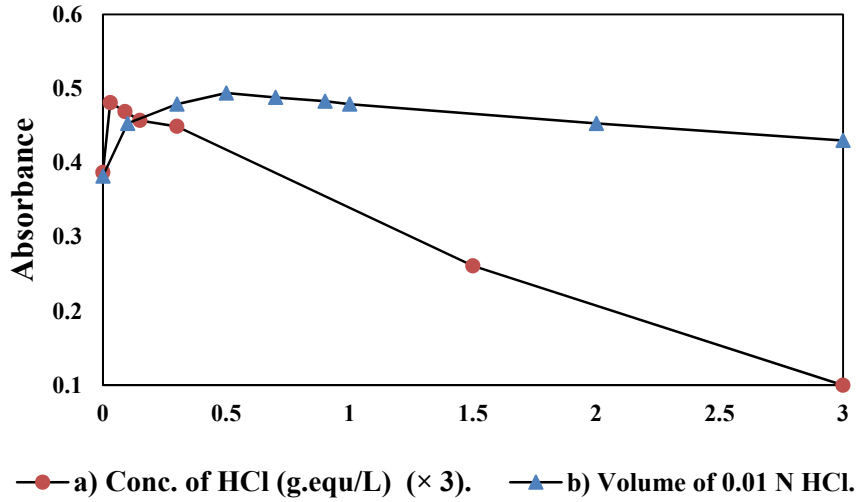


Fig. (3): Effect of concentration and volume of HCl on the reaction product.

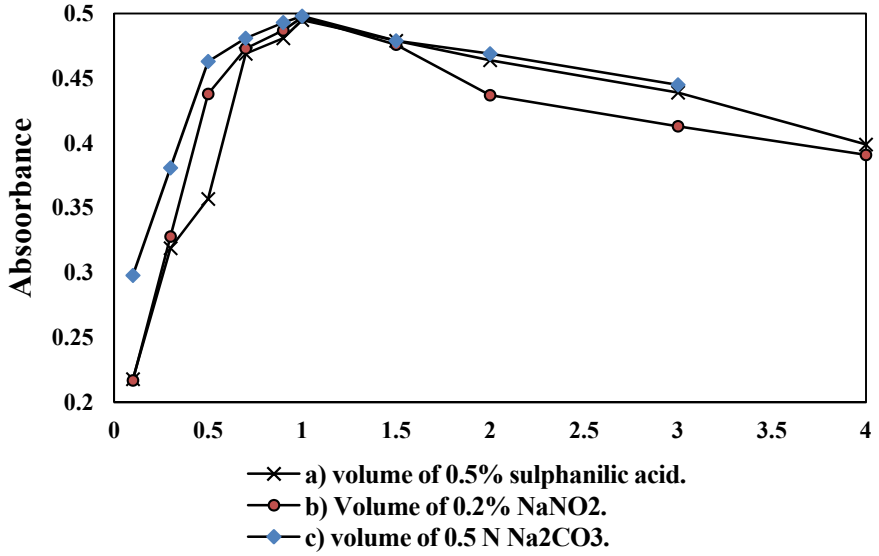


Fig. (4): Effect of volumes of sodium nitrite, sulphanilic acid, and sodium carbonate solution on the reaction product.

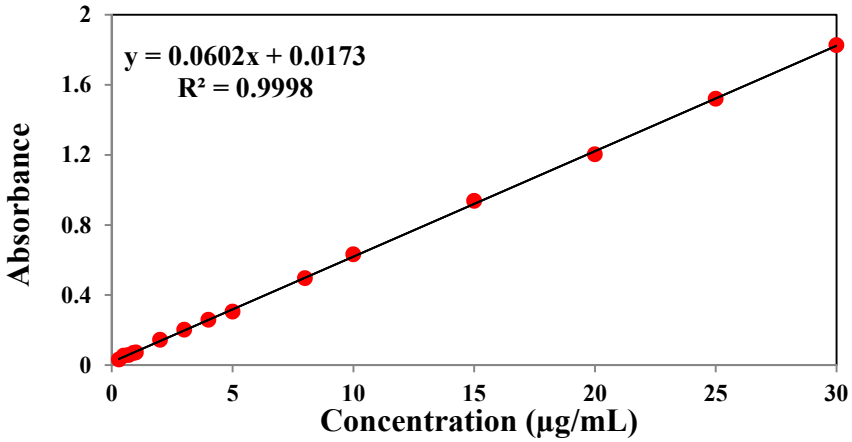


Fig. (5): Calibration curve constructed under optimum conditions.

التقدير الطيفي للأموكسيلين ثلاثي الماء في صورته النقية والمستحضر الصيدلاني

هيمن عبدول قادر
قسم كيمياء الصيدلية/ كلية الصيدلة/ جامعة هولير الطبية/ أربيل
نبيل عادل فخري
قسم الكيمياء/ كلية التربية/ جامعة صلاح الدين

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الخلاصة

تم اجراء دراسة طريقة طيفية سريعة وحساسة لتقدير الأموكسيلين ثلاثي الماء بوساطة تفاعل الأزوتة والازدواج. تمت ازوتة حامض السلفانيليك عن طريق تفاعله مع أيون النتريت في محيط حامضي، لتكوين أيون ديازونيوم ذائب في الماء وغير ملون، ثم أجريت عملية ازدواج لأيون الديازونيوم المتكون مع الأموكسيلين ثلاثي الماء لتكوين صبغة ازو ملونة في محيط قاعدي التي أظهرت اعلى امتصاص عند الطول الموجي 455 نانومترا. يشير الرسم البياني للامتصاص مقابل التركيز بأن قانون بير ينطبق ضمن المدى (0.3 – 30) ميكروغرام/مل وبحد كشف 15.0 ميكروغرام/مل مع امتصاص مولي مقداره 3.2 $\times 10^4$ لتر/مول.سم، وكانت قيم الدقة والتوافق للطريقة المقترحة والمحسوبة اعتمادا على قيم الخطأ النسبي والانحراف القياسي النسبي مقبولة. تم دراسة تأثير المتداخلات الشائعة وطبقت الطريقة المقترحة لغرض تقدير الأموكسيلين ثلاثي الماء في صورته النقية وفي بعض المستحضرات الصيدلانية المختلفة المتوفرة في أسواق أربيل.

الكلمات المفتاحية: المطباقية الضوئية، اموكسولين ثلاثي الماء، تفاعل ازونة، اقتران.