A DEVELOPED SPECTROPHOTOMETRIC DETERMINATION OF AMOXICILLIN FORMS VIA CHARGE- TRANSFER REACTION WITH METOL

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Abstract

A spectrophotometric method for determination of amoxicillin (AMX) in pure and pharmaceutical formulations is described. The proposed method involves the use of metol (N-methyl-p-hydroxy aniline) as a chromogenic reagent.The drug produce a bluish-green coloured soluble charge- transfer complex with metol in the presence of potassium persulphate in alkaline medium. The coloured product is measured at λ_{max} 620 nm . Under the optimum conditions calibration graph was observed a linear from 5-60 μg ml^{-1} AMX and with correlation coefficient not less than 0.9994 which was suitable for the quantitative determination of the drug.The molar absorbitivity was 2.726×10^3 $L.mole^{-1}.cm^{-1}$,and sandell sensitivity of 0.1538 μg cm^{-2} . The relative standard deviation of the proposed method was 0.25-2.44% depending on the concentration of AMX. The proposed method was successfully applied to the determination of AMX in injections and capsules.

Keywords: Spectrophotometric ; Amoxicillin ; metol ; charge-transfer reaction.

Introduction:

Amoxicillin (AMX) is an α-amino–substituted β-Lactam antibiotic of broad spectrum and it is clinically widely used (1). It is possesses some significant advantage over ampicillin, include more complete gastroin-estinal absorption, and little or no effect on absorption of food (2). The B.p. recommended a liquid chromatography (LC) and spectro-photometric (using imidazol-mercury reagent) methods for the determination of AMX in raw material and dosage forms respectively(3).

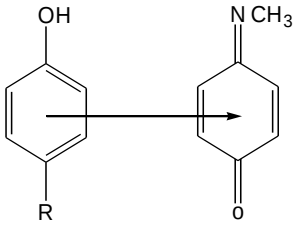
A number of analytical methods in addition to microbiological assay have been reported for the determination of AMX, included spectrophotometric[4,5], polarographic [6,7],flourimetric [8, 9] , flow injection analysis [10,11]and HPLC methods [12,13].

The present study describes the develop-ment of spectrophotometric method based on charge-transfer complex between AMX and metol in the presence of potassium persul-phate in alkaline medium. The analytical procedure is simple, reproducible and accurate. It has been satisfactorily applied for the determination of AMX in pure and dosage forms. The reaction product has been spectrophotometrically measured at 620 nm.

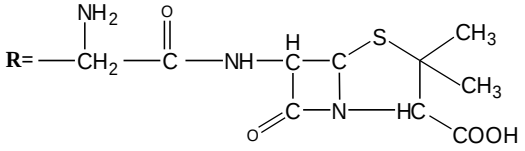
Reaction mechanism of the method:

The reaction between AMX and metol in the presence of potassium persulphate in alkaline medium yield a bluish-green product (λ_{max} of 620 nm with a molar absorption coefficient of $2.726\times10^3 L.mole^{-1}.cm^{-1}$), the reaction given in scheme 1 was postulated. The absorption spectrum of the coloured product is given in Fig.1.

Under the reaction conditions, the complex may be regarded as a charge transfer complex type. The charge transfer may be presumed to be taking place involving electron transfer from the highest occupied (π)molecular orbital of phenolic compound (AMX)to the lowest empty molecular orbital (π*) of metol. Similar overlapping of π- π* orbitals were reported in the complex formed between chloranil and N, N, N^1, N^1 tetra methyl-p-phenylene diamine[14].



AMX metol



Scheme (1) Proposed mechanism of the reaction between AMX and metol.

The apparent stability constant was calculated by comparing the absorbance of solution containing stoichiometric amount of AMX and metol with that of a solution containing a five-fold excess of metol reagent. The stability constant of the product in water under the described experimental conditions was 1.172×10^{4} L.mol^{-1}.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV-visible 260 digital double beam recording spectrophotometer using 1-cm silica cells .

Reagents

All chemicals used were of analytical reagent grade and pure amoxicillin (AMX) drug sample was provided from state company for Drug Industries and Medical Appliance, SDI, Samara. Iraq. Dosage forms were obtained from commercial sources.

Amoxicillin stock and working solution (1000 µg ml^{-1})

The stock solution of AMX was prepared by dissolving 0.1000 gm 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.

Metol reagent solution (1×10^{-2}M)

Prepared by dissolving 0.1230 gm of reagent in distilled water and made up to 100 ml in volumetric flask with the same solvent, and stored in dark bottle.

Potassium persulphate $K_2S_2O_8$ (1×10^{-2}M)

Prepared by dissolving 0.2703 gm in distilled water and made up to 100 ml with distilled water.

Sodium hydroxide (0.1M)

Prepared by dissolving 0.8000 gm of the base in distilled water and made up to 200 ml with distilled water.

Procedure of pure drug

Into a series of 25 ml calibrated flask, transfer an increased volumes of AMX of 500 ppm to cover the range of calibration graph 125-1500 µg of AMX in final volume 25 ml ,followed by 1 ml of NaOH (0.1M) and 2 ml of potassium persulphate (0.01M) and2 ml of metol (0.01M).

The solutions are diluted to the mark with distilled water .The colour reach its maximum intensity and stability on standing for 50 min at room temperature then the absorbance is measured at 620 nm against the reagent blank prepared in the same way but containing no AMX.The colour of the formed product is stable for 120 min.

For the optimization conditions and in all subsequent experiments a 1000 µg in final volume 25 ml was used (i.e. 40 ppm).

Procedure of pharmaceutical forms (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 dissolved in 10 ml ethanol and transferred into 100 ml calibrated flask and completed to the mark with distilled water. The flask with its contents was shaken well and filtered to obtain 1000 ppm, dilute 50 ml of this solution to 100 ml by distilled water to prepared 500 ppm of AMX. The measurement was carried out as described earlier under general procedure using suitable volume of last solution.

Results and Discussion:

The bluish-green colour which is obtained when dilute aqueous solution of AMX is mixed with solution of metol in the presence of oxidizing agent (potassium persulphate) in alkaline medium reach its maximum intensity and stability on standing for 50 min at room temperature. The colour have a maximum

absorption at 620 nm, which is used in all subsequent experiments.

The spectra of the bluish-green product formed and of reagent blank were show in Fig.(1).

Study of the optimum reaction conditions

The factors affecting on the sensitivity and stability of the coloured product resulting from the charge-transfer reaction of AMX with metol and potassium persulphate in alkaline medium were carefully studied. All experiments using 1000 µg of AMX in final volume 25 ml (i.e. 40 ppm).

Effect of Reagent concentration

When various concentrations of metol were added to a fixed amount of AMX solution,2 ml of 1×10^{-2} M solution was found enough to develop the colour to its full intensity and give a minimum blank value , and was considered to be optimum for the concentration range of 125-1500 µg/25 ml of AMX.

Effect of oxidant concentration

When different volumes of potassium persulphate ($K_2S_2O_8$) solution (0.3-5.0 ml) were added to AMX solution, it was found that 2 ml of 1×10^{-2} is enough to give a maximum absorbance and full intensity.

Effect of Base

The bluish–green dye product was only formed in alkaline medium, therefore, the effect of different alkaline solutions were studied such as sodium carbonate, sodium bicarbonate, potassium hydroxide, ammonium hydroxide. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of 1 ml (0.1M) sodium hydroxide solution.

Effect of order of addition

Different orders of addition of reagents were experimented and it was found that the order of addition of reagents cited under general procedure was used in all subsequent experiments.

Effect of temperature

The effect of temperature on the colour intensity of the dye was studied. In practice, high absorbance was obtained when the colour was developed at room temperature (25°C) than when the calibration flasks were placed in an ice-bath at (0°C) or in water bath at (60°C) .

Structure of the product

The stoichiometry of the formed charge-transfer complex between each AMX with metol was investigated under the recommended optimum conditions by applying the molar ratio method. The results obtained from Fig.(2) show that a 1:1 (drug to reagent) charge-transfer complex formed between AMX and metol reagent at 620 nm , therefore, the formation of product probably occurs as illustrated in Scheme 1.

Analytical data

Employing the conditions described under procedure ,a linear calibration graph (Fig.3) for AMX and metol was obtained .The optical characteristics, such as Beer's law, molar absorbativity, and correlation coefficient and other analytical data are summarized in Table (1).

Accuracy and precision

The accuracy and precision of the method was determined at three different concentrations. The results shown in Table (2) indicate that satisfactory precision and accuracy could be obtained with the proposed method.

Analytical Applications

The suggested method was applied to the quantitative determination of AMX in pharmaceutical formulations. Four types of capsules and two types of injections containing AMX have been analyzed and they gave a good accuracy and precision as shown in Table (3).

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 methods[3] Table(4).

In conclusion, the proposed method, which is simple and selective, offers the advantage of a wide range of determination without the need for extraction or heating, or removal of excipients. The precision of the method evaluated by analyzing pure sample of AMX and a good recovery was obtained (Table

(4)).In addition the proposed method was applied successfully to the analysis of some capsules and injections containing AMX ,and finally statistical analysis F-test and T-test, reveals that no significant difference in accuracy and precision between the proposed and official methods.

Table (1)
Analytical data obtained from proposed method.

PARAMETER	VALUE
Beer's Low limits($\mu g.ml^{-1}$)	5-60
Molar absorbativity($lit.mole^{-1}.c$	2.72×10^{3}
Sandell's sensitivity($\mu g.cm^{-2}$)	0.1538
Slope(b)	0.0068
Intercept(a)	0.0041
Correlation coefficient (R^2)	0.9994
λ_{max} (nm)	620
R.S.D (%)	<1.19
Accuracy(mean± SD)	100.44±0.157
Limit of detection ($\mu g.ml^{-1}$)	1.494

Table (2)
Accuracy and precision of the proposed method for the determination of AMX.

Amount of AMX ($\mu g.ml^{-1}$)		Recovery%*	R.S.D %*
Present	Found		
20.00	20.28	101.39	1.91
30.00	30.28	100.93	0.48
60.00	59.39	98.98	0.25

**** Average of four determinations.***

Table (3)
Application of the proposed method for the determination of AMX in pharmaceutical forms.

Drug Sample	Wt of AMX (mg)	AMX($\mu g.ml^{-1}$)		R.S.D %*	Rec. %*
		Taken	Found		
Amoxicillin capsule (ACAI)	250	40	39.38	1.44	98.45
Amoxicillin capsule (Ajanta)	500	40	39.89	0.64	99.73
Amoxicillin capsule (SDI)	250	40	40.09	0.89	100.23
Amoxicillin capsule(Belgica)	250	40	39.69	0.78	99.23
Amoline(Injection) Oubari pharm.syria.	500	40	40.66	1.03	101.65
Amoxicillin (Injection) Pan-pharma France	500	40	40.33	1.04	100.83

**** Average of four determinations***

Table (4)
Comparison of the proposed method with standard method to determination of AMX in pharmaceutical forms.

Amoxicillin preparations	Recovery%*	
	Proposed method	Standard method **
Pure AMX	100.26	100.5
Amoxicillin capsule (ACAI)	98.45	102.00
Amoxicillin capsule (Ajanta)	99.73	98.00
Amoxicillin capsule (SDI)	100.23	99.00
Amoxicillin capsule (Belgica)	99.23	99.00
Amoline(Injection) Oubari pharm.syria.	101.65	102.50
Amoxicillin (Injection) Pan-pharma France	100.83	101.50

**** Average of four determinations.***
***** British Pharmacopoeia standard method.***

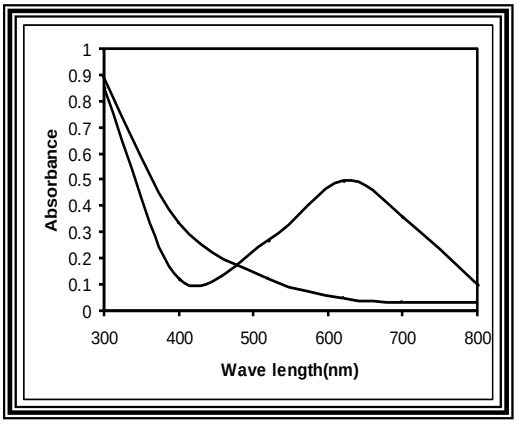


Fig.(1) : Absorption spectra of A (60 µg ml^{-1}) of AMX treated as described under procedure and measured against blank and B the reagent blank measured against distilled water.

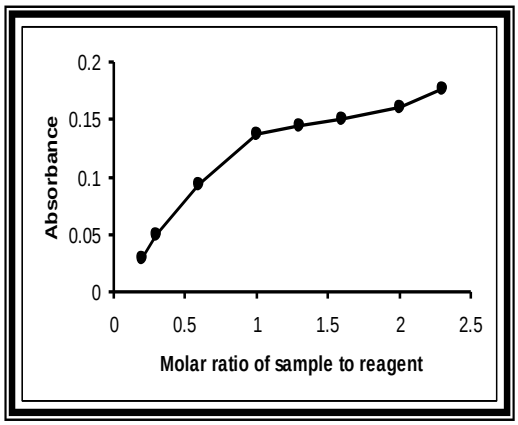


Fig. (2) : Molar ratio of sample to reagent for the product formed. The concentration of both the sample and reagent are 2.38×10^{-3}M.

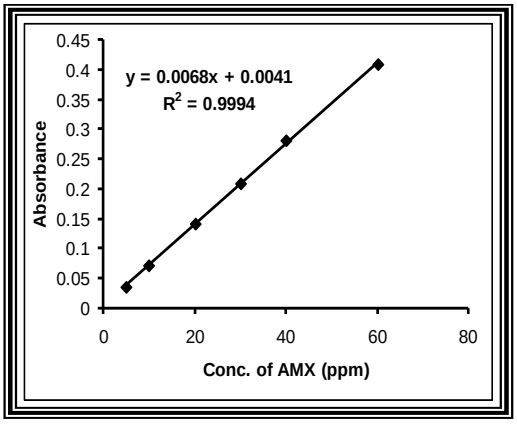


Fig. (3) : Calibration graph of AMX.

References

[1] A. Goodman, T. Rall, A. Nier and P. Taylor , The pharmacology bases of therepeutics , McGraw – Hill , New York, 1996 .

[2] J.N.Delgado, W.r.Remers (Eds.), Wilson and Gisvolds"Text book of Organic Medicinal and pharmaceutical chemistry" Lippincott, Philadilphia, PA,10 th.Ed., 1995, chapter 7.

[3] British Pharmacopaeia, H. M. Stationary Office, London, 1993 .

[4] F Belal ,,M.M.El- kerdawy, S.M. EL-Ashry ,D.R.El-Waseef, IL ARMACO, Vol.55, 2000, pp 680-686.

[5] B. S. Nagaralli, J. Seetharamappa, M. B .Melwanki, J.Pharm. Biom. Anal .Vol. 29, 2002, pp 859-864.

[6] B. Uslu, I. Biryol, J. Pharm. Biom .Anal. Vol. 20, 1999, pp591-596.

[7] I. Biryol, B. Uslu, Z. Kucukyavuz, STP, Pharm. Sci.Vol.8, 1998, pp 383.

[8] V. Kapetanovic, D. Veselinovic, Arch-pharm. Vol. 321, 1998, pp 559.

[9] P. G. Navarro, A. El-Bekkouri, E. R. Reinoso, A. Bult, PharmBiom. Anal, Vol.123, 1990, 2263-2269.

[10] M. A. Vanopstal, R. Wolters, J. S. Blauw, R. C. Vankrimper, W. P. Vankrimper, A. Bult, J. Pharm. Biom. Anal. Vol. 8, No.1, 1990, pp 49.

[11] M. Q. Al-Abachi, H. Hadi, A. M. Q.Al-bachi, Anal.chimi. acta. 554, 2005, 184-189.

[12] J. O. Boison, L. J. Y. Keng, J. AOAC, 81, 1998, 1113.

[13] J. Vanzijveld, E. J. VanHoogdalem , J. Chromatography.726, 1999, 169.

[14] R. Foster , T. J. Thampson, Trans. Faraday soc., 58 1962, 860.

الخلاصة

يتضمن البحث ايجاد طريقة طيفية بسيطة للتقدير الكمي للمقادير الضئيلة من عقار الاموكسيسيلين في المحاليل المائية بأستخدام المطياف الضوئي .تعتمد الطريقة على تفاعل انتقال الشحنة للاموكسيسيلين مع كاشف الميتول بوجود بيرسلفات البوتاسيوم كعامل مؤكسد وفي وس ط قاعدي حيث يتكون ناتج اخضر–مزرق مستقر وذائب في الماء اعطى اعلى امتصاص عند طول موجي 620

نانوميتر. يشير الرسم البياني الخطي للامتصاص مقابل التركيز بأن قانون بير ينطبق ضمن مدى التركيز 125– 1500 مايكروغرام من الاموكسيسيلين في حجم نهائي 25 مل اي ما يكافئ 5– 60 جزء بالمليون وكانت قيمة الامتصاصية المولارية مساوية الى 2.726×10^3 لتر.مول $^{-1}$.سم $^{-1}$ وقيمة حساسية ساندل 0.1538 مايكروغرام .سم$^{-2}$ مع قيمة انحراف قياسي نسبي 0.25–2.44 % أعتمادا على مستوى التركيز المراد تحديده. تمت دراسة الظروف المثلى للتفاعل وتطبيق الطريقة على الحقن والكبسولات الحاوية على الاموكسيسيلين.