Spectrophotometric Determination of Amoxicillin-Application to Capsules

Jwan A. Abdul-Sattar, Abdul-Jabar K. Atia, Sahar A. Kadhom, and Lubna F. Mohammed-Ali.
Department of Chemistry, College of Science, University of Al-Musansiriya.

الخلاصه

خلال هذه الدراسه تم استحداث طريقه طيفيه لتقدير الاموكسسلين في عيناته الصيدلانيه.والطريقه تعتمد على تحضير 6-[α-(4-hydroxy phenyl)-α-(4-Bromo benzelidiene)]acetamindo-pencillic acid. من تفاعل 6-[α-(4-hydroxy phenyl)-amino]acetamido pencillinic acid مع 4- bromobenzaldehyde كعامل ملون في الايثانول النقي.العلاقه الخطيه كانت في مدى (9-350)µg.ml⁻¹ في حدود كشف (0.14)µg.ml⁻¹ ووجد ان معامل الارتباط الخطي (0.9997). وكان متوسط الانحراف القياسي النسبي (RSD=0.49%) مما يعزز جدوى هذه الطريقه.وكان الاختلاف بين نتائج هذه الطريقه والطرق المرجعيه المعتمده صغيرا بحيث يمكن اهماله.وامكن تطبيق هذه الطريقه بنجاح لتقدير العقار في جرعاته.

ABSTRACT

In this study, a novel spectrophotometric method is given for the determination of amoxicillin in its pharmaceutical dosage form. The method is based on the preparation of 6-[α-(4-hydroxy phenyl)-α-(4-Bromo benzelidiene)]acetamido-pencillinic acid from the reaction of 6-[α-(4-hydroxy phenyl)-amino]acetamido pencillic acid (amoxicillin) with (4-bromobenzaldehyde)as a chromogenic reagent in absolute ethanol. Linearity was in the range (9-350) µg.ml⁻¹ with a limit of detection (0.14) µg.ml⁻¹for the drug. Linear response was observed over the tested range of the drug with correlation coefficient (0.9997).The mean relative standard deviation percentage (RSD=0.49%) which confirmed the reproducibility of the assay technique. The method showed insignificant difference with those of reference methods. The method was applied successfully to the determination of amoxicillin in its dosage form.

INTRODUCTION

Amoxicillin belongs to a class of antibiotics called penicillins. Other members of this class include ampicillin (Unasyn), piperacillin (Pipracil), ticarcillin (Ticar) and several others. These antibiotics all have a similar mechanism of action. They do not kill bacteria, but they stop bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together. Bacteria cannot survive without a cell wall. Amoxicillin is effective against many different bacteria including H. influenza, N. gonorrhoea, E. coli, Pneumococci, Streptococci, and certain strains of Staphylococci(1).

Thiazole fused to another heterocyclic ring has attracted wide spread attention due to their diverse applications as anti-bacterial,anti-depressant,anti-viral,anti-tumorial and anti-inflammatory agents,pestici-des,herbicides dyes and analytical reagents(2).Schiff's

bases systems are reported to pass divers' types of biological activities, including antibacterial and cause inhibition to the enzyme activity like Ch.E.(3).Amoxicillin is determined by several spectrophotometric methods based on oxidation(4,5), nucleophilic substitution(6), derivation (7),nitration(8) and ion-pair complexion(9).Compared with other techniques such as NIR (Near-infrared spectroscopy)(10),DRIFTS (Diffuse reflectance infrared Fourier Transform(11),SIA(Sequential Injection Analysis)(12),Chemiluminescence's(13) and Isothermal microcalorimetry(14).Spectrophotometer is very simple,rapid and less expesive.In addition, spectrophotometers are commonly available in all laboratories.

The purpose of this study was to evaluate a spectrophotometric method for the determination of amoxicillin in its pharmaceutical preparation,based on the reaction of amoxicillin with a chromogenic reagent (4-bromobenzaldehyde) to yield a colored product .This method is free from disadvantage like expensive equipments ((12),stringent conditions(13) and narrow range(14),etc.,which other above methods suffer from.

MATERIALS AND METHODS

Apparatus

Spectrophotometric measurements were carried out on a UV/VISIBLE spectrophotometer (VARIAN UV-Visible).

Materials and solutions:
Pure and pharmaceutical dosage forms:

All materials and reagents used were of analytical grade. Amoxicillin was donated by Samara Drug Industry, Iraq.(Amoxicillin)capsule, Iraq, labeled to contain 500 mg of amoxicillin per capsule, as commercial pharmaceutical preparation was obtained from the local pharmacy and subjected to analysis by the proposed method.

Synthesis of 6-[α-(4-hydroxy phenyl)-α-(4-Bromo bezelidene)] acetamido penicillanic acid(15):

To a stirred solution of amoxicillin (0.01 moles) in absolute ethanol (20ml), 4-bromobenzaldehyde was added. The mixture was refluxed for (5 min.)and cooled, and the precipitate thus formed, was filtered,m.p.218-220C°,69% yield.I.R.(KBr)(cm^{-1})2978-2820(C-H alph),3450-2500(OH) of carboxylic acid,3196(OH) of carboxy phenyl1737(C=O) of carboxy acid,1700(C=O)cyclic ring 760(C-Br);U.V.(EtOH),λmax=(300,211)nm.

Preparation of stock and working solutions:

Stock solution of amoxicillin was prepared by dissolving an accurately weighed amount (50) mg of the derivative drug in a (50) ml volumetric flask. The solution is then made up to the volume with distilled water. Suitable aliquot of the stock solution was completed quantitatively with the same solvent to obtain the suitable working standard solution (400) μg.ml^{-1}.

Preparation of dosage form samples(capsules):

The contents of twenty capsules were mixed and finally powdered. An accurately weighed amount equivalent to (500) mg of the drug was derivate,transferred quantitatively to 50 ml volumetric flask and complete to the mark.A suitable aliquot of the stock solution was then diluted quantitatively with the used solvent to obtain the suitable working sample solution for the measurements at the specified range.

Procedures
Calibration curve:

In order to obtain the calibration curve for applying quantitative analysis seven solutions of each of the pure derivative drug were prepared with concentrations in the calibration range (9-350)μg.ml^{-1}. These ranges were previously verified to obey Beer's law for the studied drug.

Procedures for pharmaceutical preparation(Capsules):

Portion of dosage form working solution (0.625) ml was quantitatively transferred to six volumetric flasks, then serial portions of amoxicillin working solutions (0.625-3.75) ml were added to each flask and the solution was completed with the used solvent and measured at the specified wavelength.

RESULT AND DISCUSSION

Amoxicillin derivative Spectra:

A new amoxicillin derivative containing bromo moiety was prepared following the reaction sequence in scheme (1).The starting material for the synthesis of the targeted compound is amoxicillin(15).

The IR spectrum showed the (OH of carboxylic group) stretching absorption at 3450-2500 cm^{-1}(bromo band), in addition to the band at 3196 cm^{-1} for the (OH) of hydroxyl phenyl interference with (OH) of carboxyl group. Finally, the structure of the targeted compound was confirmed by the presence of (Br) stretching vibration at 760 cm^{-1}. U.V. Spectra showed two intense absorption maxima at 300nm and 211nm

which can be attributed to n→π* and π→π*electronic transition, respectively as shown in Fig (1,2).
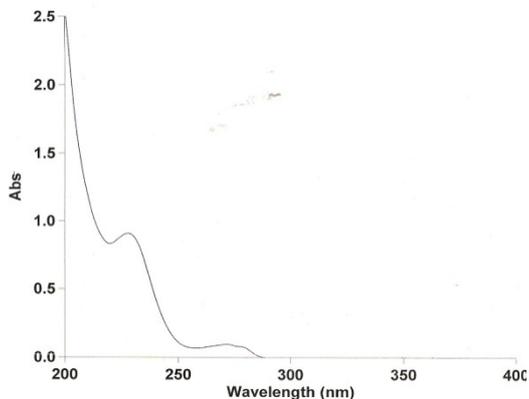

Fig-1: UV-VIsible of amoxicillin.

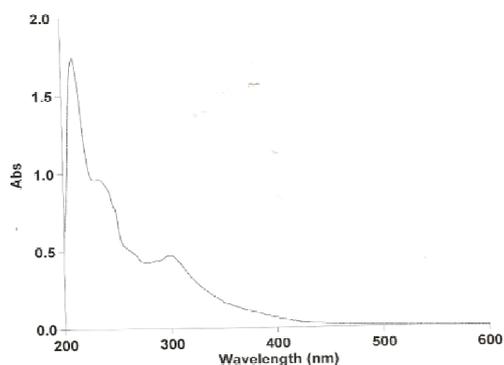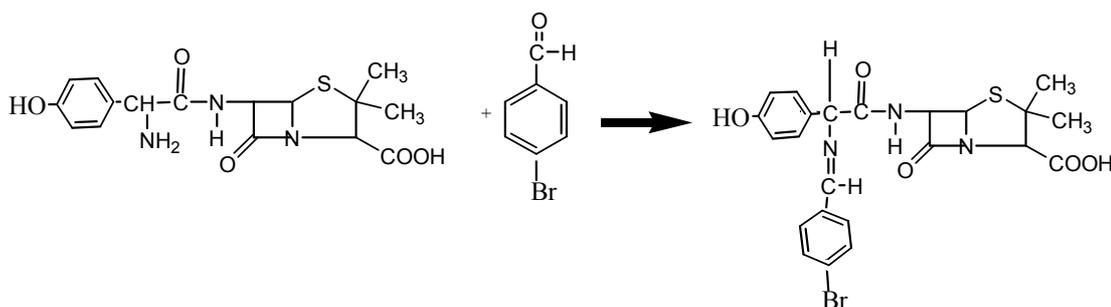

Fig-2:UV-Visible of derivative.

Scheme(1)

Optimization of experimental conditions:

The effects of optimum conditions were studied by measuring the absorbance values at λ=300 nm in order to give the maximum stability and sensitivity.

1-Effect of concentration:

The effect of different concentrations of the derivative drug was investigated. A concentration of (400)µg.ml^{-1} gave the highest absorbance and thus was chosen for further use. Fig(3).

2-Effect of pH:

pH effect was studied in the rang (1-11).It was found that maximum absorbance was observed at pH=2,therefore it was adopted for all experiments within the concentration range of the calibration curve. Fig. (4).

3-Effect of diluting solvents:

It was found that distilled water was the best solvent for dilution. Other solvents were not preferred either because they are poison such as methanol or they are not available like benzyl alcohol, etc.

4-Effect of Temperature on stability:

The absorbance of the colored compound was found to be stable at room temperature for more than (24 hours).
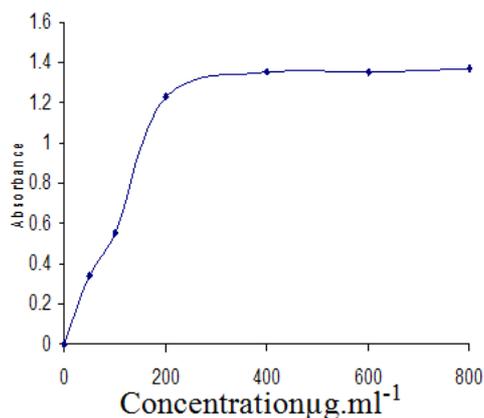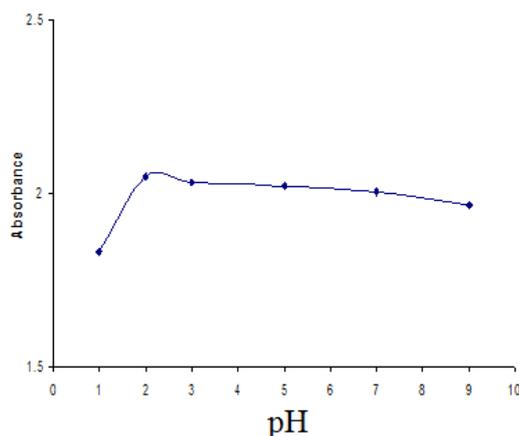

Fig-3:concentration effect   Fig-4:pH effect

Analytical Performances of the method:

Beer's law is obeyed over the amoxicillin concentration range of (9-350) $\mu g.ml^{-1}$ for this method as shown in Fig(3). The proposed method procedure is validated by determining various optical parameters, which are listed in Table(1).The linearity, slope and the intercept have been calculated using the regression equation $Y=aX+b$,where 'y' represents absorbance 'x' the concentration of drug in $\mu g.ml^{-1}$ and   'a'   and   'b'   represent   slope   and intercept,respectively.Precision and accuracy of the proposed method was tested by carrying out determinations of seven replicates of pure and commercial samples of amoxicillin, whose concentration lie within Beer's law range. The values of standard deviation (S.D.), relative standard deviation (R.S.D.), Erel. at 95% confidence level were calculated.

Table -1: Optical characteristics and precision data

Parameters	Values
Beer's Law range(μg.ml^{-1})	9-350
Regression equation:	Y=aX+b
Slope(a)	0.0085
Intercept(b)	0.0058
Conf. limit for slope $b\pm t_{sb}$	0.0058$\pm$0.00024
Conf. Limit for intercept $a\pm t_{sa}$	0.0085$\pm$0.405
Correlation coefficient(r)	0.9997
Limit of detection(μg.ml^{-1})	0.14
Molar absorptivity (L.mol^{-1}.cm^{-1})	6.0*10^3
Sandell's sensitivity(μg.cm^{-2})	0.1
S.D.	0.0086
R.S.D.(%)	0.49 %
E rel.%	0.13 %

Pharmaceutical application:

The analysis of the pharmaceutical formulation (amoxicillin) containing antibiotic was applied to confirm the validity of the analytical method (15), and the results are compared statistically with the reference method as cited in Table (2).

Recovery studies were realized for the capsules regarding accuracy and precision of the proposed method. The results in Table (2) concluded that the proposed method is sufficiently accurate and precise in order to be applied for pharmaceutical dosage form. High percentage recovery data show that this method is free from interference of excipients used in the formulation such as talc, glucose, starch, and lactose and magnesium stearate.Standard addition calibration curve was used for the analysis as shown in Fig (4).
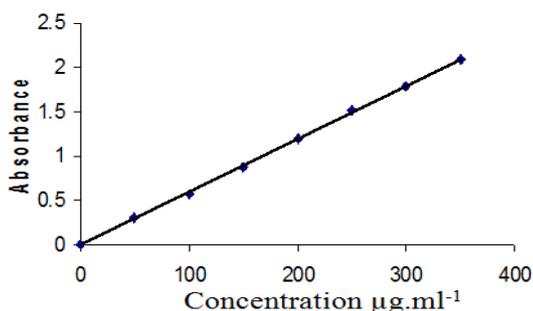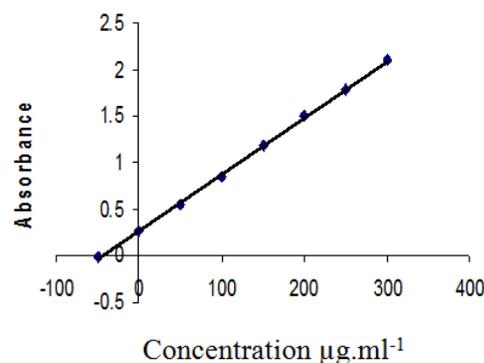

Fig -3: Calibration curve

Fig -4:The standard addition method

Table-2: Determination of antibiotic in pharmaceutical preparation

Pharmaceutical	Type	Manufactures	Proposed method		Reference method	
			Recovery %	RSD %	Recovery %	RSD %
AMOXICILLIN	Capsules	SDI/Iraq	100.5	0.56	98.9	0.9

Conclusion

In this work, the nucleophilic substitution of a β-lactam antibiotic (amoxicillin) by 4-bromobenzaldehyde, has been studied to establish the optical characteristic, precision and accuracy of the proposed method for the determination of (amoxicilin).This method is rapid and does not require any sophisticated apparatus if compared with chromatographic methods. So, the proposed method was completely validated and suitable for quality control laboratories, where economy and time are essential.

REFERENCES

1. British Medical Association and Royal Pharmaceutical Society of Great Britain..British Medical J. Publishing Group and Pharmaceutical Press,United Kingdom,pp.45(2008).
2. Tijen O.,Bilge C. and Fathi S.,Turk.J.Chem,".Synthesis and Antinociceptive activity of 2-[(2-oxo benzothiazolin-3-yl)methyl]-5-amino alkyl/aryl-1,3,4-thiadiazole.",28,461-468(2004).
3. Khalaf A.J.,Kadem.S.A. and Bayati.R.I.,J.college education,"Synthesis of New Nicotinic acid Derivatives.",1:107-112(2008).
4. Revanasiddappa H.D. and Veena M.A.,E.J.Chem,"A Sensitive Spectrophotometric Determination of Ritodrine, Pentazocine, Isoxsuprine Hydrochlorides and Amoxicillin in Pure and Pharmaceutical Samples.",5 (1):100-106(2007).
5. Farhadi K. and Ghadamghi S.,J.Chinese Chem. Soc."Spectrophotometric Determination of Selected Antibiotics Using Prussian blue Reaction",49:993-997(2002).
6. Quanmin L.I. and Zhanjun Y.,Anal.Lett,"Study of spectrophotometric determination of amoxicillin using sodium 1, 2-naphthoquinone-4-sulfonate as the chemical derivative chromogenic reagent.",39 (4-6):763-775(2006).
7. Mohamed A. and Salem H., Thai J.Pharm.Sci.,"Chemometrics-assisted spectrophotometric determination of certain β-lactam antibiotic combinations.",31:9-27(2007).

8. Hesham S.,Anal. Chim. Acta,"Selective spectrophotometric determination of phenolic β-lactam antibiotic in pure forms and in their pharmaceutical formulations.",515(2):333-341(2004).

9. Mohamed G.G.,J. Pharm. and Biomed. Anal.,"Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin and amoxicillin antibiotic drug:ion-pair formation with molybdenum and thiocyanate.",24 (2):561-567(2001).

10. Nan Q. and Mingchao Z., Spectrochimica Acta Part A:Mole. and Biomol. Spectro.,"Nondestructive determination of compound amoxicillin powder by NIR spectroscopy with the aid of chemometrics.", 70(5):1146-1151(2007).

11. Parisotto G. and Flores M.,Brazilian J. Pharm.Sci.,"Determination of amoxicillin content in powdered pharmaceutical formulations using DRIFTS and PLS",43(1):89-96(2007).

12. Pasamontes A. and Callao M.,Anal. Sci.,"Seqential Injection Analysis for the Simultaneous Determination of Clavulanic acid and Amoxicillin in Pharmaceutical Using Second-order Calibration",22(1):131-135(2006).

13. Xie X. and Song Z.,Spectroscopy,"Ultrasensitive determination of amoxicillin using chemiluminescence with flow injection analysis.",20 (1):37-43(2006).

14. Chadha R. and Kashid N.,J.Pharm. Pharmacology,"Kientic studies of the degration of an aminopenicillin antibiotic (amoxicillin trihydrate) in aqueous solution using heat conduction microcalorimety.",55(11):1495-1503(2003).

15. Khalaf A.,J. Dyala,"Synthesis of New Benzidine Derivatives",17:10-24(2003).