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DEVELOPMENT OF A REVESED-PHASE HPLC METHOD FOR DETERMINATION OF AMOXICILLIN IN ORAL DOSAGE FORMS

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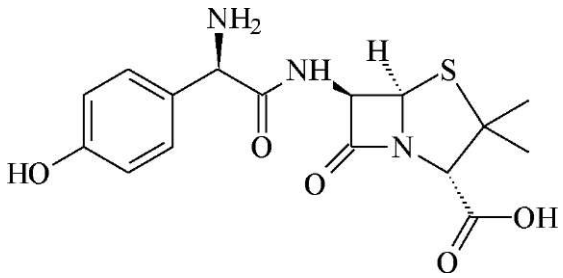
RP HPLC method has been proposed for amoxicillin assay in some drugs. Step bu step development of the chromatographic procedure is considered. The method proposed involvs Reprosil-Pur C18-AQ stationary phase and phosphoric buffer (pH=5) with 5% of acetonitrile addition as a mobile phase with UV-detection (228 nm). The method has been applied for amoxicilline assay in four trademarks of amoxicillin drugs.

Key words: RP HPLC, method of determination, amoxicillin

Introduction

Amoxicillin ((2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)-acetyl]amino}-3,3-dimethyl-7-oxo-4-thia-1azabicyclo[3.2.0]heptane-2-carboxylic acid) belongs to a class of penicillin antibiotics that include also ampicillin, piperacillin, ticarcillin and some others.



Amoxicillin

Amoxicillin is used for a treatment of bacterial infections by gram-positive as well as by gram-negative bacteria being the drug of choice within the penicillin antibiotics because of the better absorption after oral administration than other P-lactam antibiotics. The bioavilability of amoxicillin is not affected by the concomitant food ingestion. It is one of the most common antibiotics prescribed for children [1].

Amoxicillin is a semi-synthetic penicillin with sales of US $2200 million as a bulk formulated drug in 1994 [2]. So it not surprising that there is a special Internet site devoted to amoxicillin - http://www.amoxicillin.com/. It is marketed under many trade names including: *Actimoxi®, Amoxibiotic®, Amoxicilina®, Dispermox®, Isimoxin®, Lamoxy®, Ospamox®, Pamoxicillin®, Polymox®, Trimox®, Tolodina®, Wymox®, Zerrsox®* and *Zimox®*.

Amoxicillin is presently the most commonly used antibiotic. Thus reliable quantitative methods have been developed to quantify the antibiotic by HPLC with direct UV detection at low wavelengths ^=225-330 nm) [1, 3-9]. The methods proposed are of reversed-phase mode because of amoxicillin high polarity: chromatographic separation is carried out on the C18 as well as C8-phases.

The aim of the present investigation was development of a revesed-phase HPLC method for determination of amoxicillin and assay of amoxicillin in some commercial oral dosage forms.

Materials and methods

Materials

In the present work were used amoxicillin (Sigma), acetonitrile (HPLC-gradient grade, Panreac, Spain); distilled water, phosphoric acid (Vecton, RF) and NaOH (REACHIM, RF) of a reagent grade.

The amoxicillin brands "Acamoxil 500" (Iraq), "Glomox 500" (U.A.E.) and "Cimoxil®-500" (CHAPHAR, China) were purchased in local pharmacies in Iraq while "Амоксициллин" (РФ) was purchased in Belgorod.

Instrumentation

HPLC method of amoxicillin determination was performed and amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatographic system that included high pressure pump Beckman 110 B, Rheodyne model 7125 injector with 20 pl sample loop, 250x4 mm Reprosil-Pur C18-AQ or 250 x 4 mm Reprosil-Pur C8 columns, Nicolet LC/9563 Variable UV Detector. Detector signal was processed by MultiChrom 1.5 data management program.

UV-vis spectra were recorded in quartz cuvettes by spectrophotometer SPh-26 (RF). The acidity of the mobile phase was controlled by pH-meter "pH-150" with a combination electrode.

Preparation of the standard solution of amoxicillin

Precise mass sample of the amoxicillin standard powder (in region of 9 ^ 11 mg) was quantitatively transferred into 10 ml volumetric flask; then 4 ^ 6 ml of mobile phase were added and after the sample dissolution the flask volume was made up by the mobile phase. The solution was filtered through syringe filters (0.45 pm). The solution was diluted to get the calibration solutions with desired concentration (0.025 ^ 0.10 mg/ml).

Sample preparation of drug amoxicillin

Some 5-8 tablets of the drug were finely dispersed in a porcelain mortar. Then a precise mass sample of the powder was quantitatively transferred into 10ml volumetric flask; 4 ^ 6 ml of mobile phase were added and after the sample dissolution the flask volume was made up by the mobile phase. Before injection the solution was filtered through syringe filters (0.45 pm).

HPLC procedure

For mobile phase preparation mix 12.5 ml of acetonitrile, 5 ml 0.5 M sodium phosphate buffer (pH=5) and some 100-150 ml of distiller water in the 250 cm^3 volumetric flask and make up the volume with distilled water. Then equilibrate the chromatographic system with 250 x 4 mm Reprosil-Pur C18-AQ with a guard column (effluent monitoring at 228 nm) before sample injection.

The result of amoxicillin determination calculate as mean value of two sample preparations for each of them taking the mean value for two injection, using a calibration curve for amoxicillin concentration calculation.

Results and discussion

Stationary phase choice

Amoxicilline is a highly polar compound thus the reversed-phase mode of HPLC seems to be the most suitable for the chromatographic determination. Meanwhile there are some types of reversed-phase stationary phases available differing by the length of the alkyl radical chemically bound to the silica surface. The most popular are series of trademarks of octadecyl (ODS ore C18) phases, proposed for amoxicillin determination [1, 3-5, 7,8]. Some less popular are octyl (C8) phases, though just a Zorbax SB-C8 column was found to be more suitable (than Zorbax SB-C18) for quantitative determination of amoxicillin in complex mixture with possible degradation products [5].

The background of a stationary phase choice may be proposed by the following way. The solute retention in HPLC belongs upon some parameters (eq.1).

' U o n^(i)/N(mp) N(mp) Xᵥ(i) ' "л ' " W

where k - a capacity factor of the solute *i;*

nSp(i) and *nmp(i)* - quantity of solute *i* in stationary and mobile phases respectively;

N - number of moles of the substances in the phases involved;

Kth(i) - thermodynamic constant of solute *i* distribution;

П - column phase ratio.

Any two columns at a given mobile phase composition may differ by thermodynamic constant as well as by column phase ratio. If the columns are chemically equal it means that they have equal thermodynamic constant of solute distribution between the stationary and mobile phases. Excluding this constant form two equations for two different columns (A and B) we can get the equations (2) - (5).

к Л о _ П(.А)

k_B{i) 4(B)' C²)

$$k_A\{i) = k_B\{i)\text{-}^{r}\text{^-} = a\text{-}k_B\{i). \quad (3)$$

$$= я_0 + a\,r\,\Gamma * \partial(0\,в \quad (4)$$

$$lg *,(/') = lg я + lg *_{„}(/'). \quad (5)$$

So, the retention times of the solute *i* for column A *vs* that for column B should be a linear function (eq. 4) for some mobile phase compositions of any solvent system. The linearity of the dependence for logarithms of capacity factors with slope closed to one is more convenient for the analysis.

If stationary phases under investigation are not chemically equal no linearity will be found between retention times or equation (5) should be replaced by equation (6) with a slope deviating from 1.

$$lg\ k_A(/) = a_o + a_l\text{-}1g k_B(i). \quad (6)$$

Experimentally for columns with C8 and C18 stationary phases of Reprosil trademark a linear relationship for the retention times of amoxicillin were found with intercept closed to zero. This finding indicates that both phases are chemically equivalent - it means that mechanism of retention of amoxicillin is hydrophobic repulsion of the solute onto sorbent surface. Indeed in this case the length of alkyl radical of chemically modified silica could not influence upon solute retention.

Chromatographic columns 250x4 mm Reprosil-Pure C8-AQ and Reprosil-Pur C18.

Mobile phases 2.0 ^5.0 vol.% of CH3CN in water phosphoric buffer (0.01 M NaH2PO4), 1 ml/min.

As the consequence of the fact - C18 stationary phases may be readily replaced by C8 or even C4-phases for the given mobile phase composition. By the way for the two reversed-phase stationary phases built upon the same silica matrix dead times may be closed then the intercept of the equation (4) must also be close to zero.

In spite of the findings column with C18 stationary phases has been explored for the further method development.

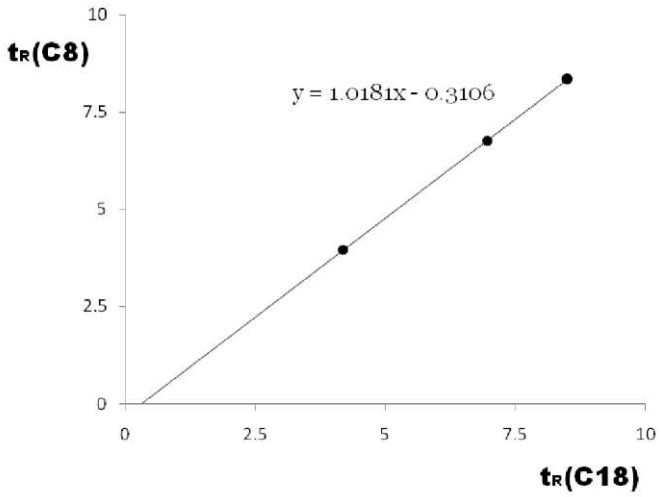


Fig.1. Comparison of retention times of amoxicillin for two column

Mobile phase choice

The choice of mobile phase composition depends upon some factors.

1. As a highly polar compound amoxicillin (LogPexp = 0.87, for phenol LogPexp = 1.46, for aniline LogPexp = 0.90) needs only small additions of organic modifier into water based mobile phase- methanol or acetonitrile (0 - 10 vol.%), fig.2.

The relationship between logarithm of capacity factor of amoxicillin and volume percentage of acetonitrile in a mobile phase is rather linear, so the composition of a mobile phase with the desired amoxicillin retention may be calculated (eq.7).

$$\lg fc(i) = 0.678 - 0.122\cdot\phi \qquad (7)$$

where ϕ - volume percentage of acetonitrile in a mobile phase.

A composition, containing 5 vol.% of CH3CN has been chosen for amoxicillin determination.

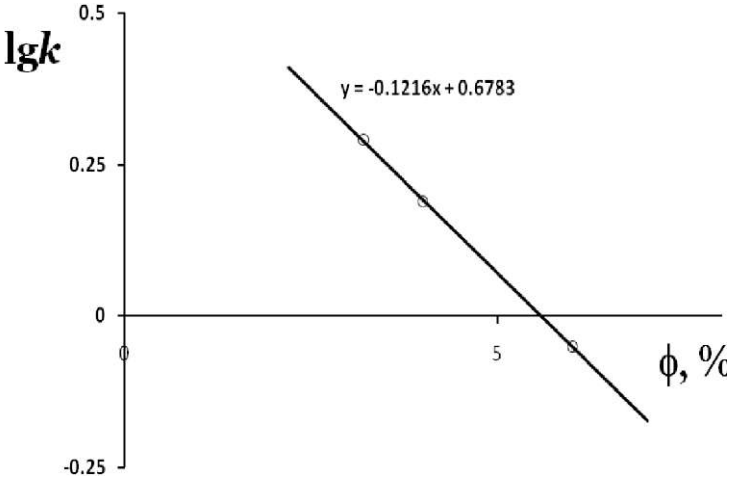


Fig.2. Retention of amoxicillin as a function of vol % of CH3CN in mobile phase

Column: 250 x 4 Reprosil-Pur C18-AQ; mobile phase 0.01 M phosphate buffer pH=5

2. Low organic modifier concentration in the mobile phase may course phase collapse [9] for the ordinary reversed phases. Thus phases sustained in aqueous solutions are preferable. Stationary phases of Reprosil trademark agree the requirement.

3. Chromatographic determination of amoxicillin as ionisable compound must be performed in buffered mobile phases. The choice of buffering system is determined by UV-spectrum on the amoxicillin solution enabling a simple and sensitive detection. The UV-spectrum is presented on fig.3.

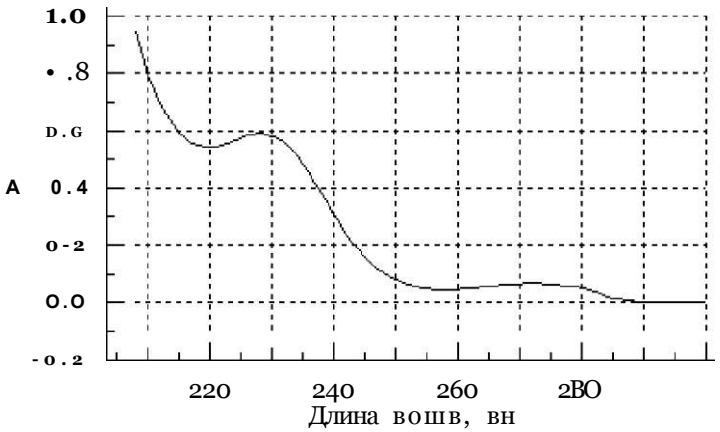
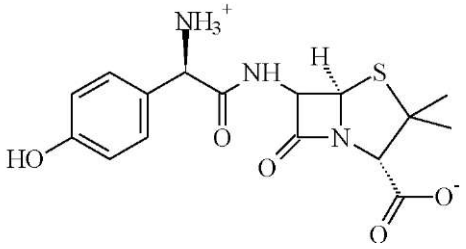


Fig.3. UV sperctrum of amoxicillin solution in water (pH=5)

In consequence of a shot wavelength of analytically convenient absorption maxima (Xmax = 228 nm) the buffering system must be transparent at 228 nm. The most suitable buffering system is the phosphate one. But it should be noticed that at a high pH due to carboxylic group dissociation the molecule will have a negative charge, while at the low pH a charge turns for positive as a consequence of amino-group protonation. The both cases need ion-pair reagent edition into mobile phase, while zwitterionic form will be the better case for reversed-phase retention. Hence pH must be of a moderate value (4 ÷ 5), fig.4.



Zwitterionic form of amoxicillin

Fig.4. Chromatogram of amoxicillin standard solution

Column 250x4 mm, Reprosil-Pur C18-AQ,; mobile phase 5 vol. % in 0.010 M phosphate buffer (pH=5), 1 ml/min. Detection 228 nm.

Method validation

For the developed method of amoxicillin determination taking a result as a mean value for two replicate injection for two replicate sample preparations the overall relative confidence interval (P = 0.95) is composed of relative confidence intervals (CI) of method repeatability (R), sample preparation (SP), and detector calibration (DC), eq.8.

$$CI_Z = CI_R + CI^2_{SP} + CI^2_{DC} = 2.57\% \quad (8)$$

Data for three series of six replicates of amoxicillin solution injections indicates a high repeatability -is no more than 0.93%. CI_{SR} for three replicate sample preparations was found to be 1.71; CI_{DC} (1.68) was estimated for the calibration mode utilizing three different concentrations with two replicate injections for each of them. The calibration dependence proved to be linear with not statistically significant intercept, fig.5.

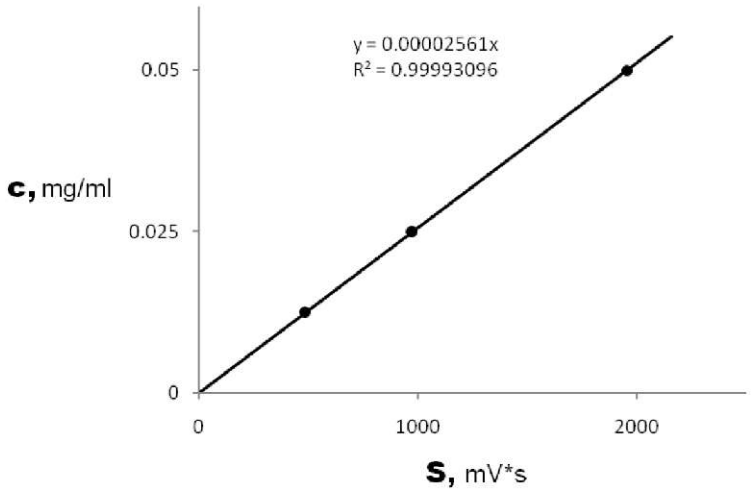


Fig.5. Calibration dependence for amoxicillin determination

By the way, an ordinary presentation of a calibrating curve as "Peak area" vs "Solute concentration" is rather strange because namely the reciprocal dependence must be used for quantitative solute determination.

Drug analysis

The method has been applied for assay of amoxicillin in four trademarks of real drugs.

Table

Results of amoxicillin assay in corresponding trademark drugs

№	Drug trademark	Sample mass, mg	Mass of amoxicillin found, mg	Conformity with stated content, %
1	"Acamoxil 500" (Iraq),	10.3	10.24	99.5±3.7
2	"Glomox 500" (U.A.E.)	10.2	10.56	103.6±3.8
3	"Амоксициллин" (РФ)	10.5	8.66	99.5±3.0
4	"Cimoxil®-500" (CHAPHAR, China)	9.6	9.55	99.6±3.7

The results obtained by the developed method proved the validity of the method and good quality of the drugs under investigation.

Conclussions

For the amoxicillin assay in drugs the RP HPLC method may be explored. The stationary phase must be collapse phase resistant while the length of the alkyl-radical of modified silica is not critical. The mobile phase for isocratic HLPC assay consists of 5 vol.% of acetonitrile in (0.01 M) water phosphoric buffer (pH=5); the rate of mobile phase 1 ml/min. Peaks are registered at 228 nm.

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РАЗРАБОТКА СПОСОБА ОПРЕДЕЛЕНИЯ АМОКСИЦИЛЛИНА В ГОТОВЫХ ФОРМАХ ДЛЯ ОРАЛЬНОГО УПОТРЕБЛЕНИЯ МЕТОДОМ ОБРАЩЕННО-ФАЗОВОЙ ВЭЖХ

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Обращенно-фазовая ВЭЖХ была использована для разработки метода определения амоксициллина в готовых формах для орального употребления. В работе приводится пошаговое объяснение и оптимизация условий определения. Предложенный метод предполагает использование стационарной фазы Reprosil-Pur C18-AQ и водного фосфатного буфера (pH=5) с добавлением 5% ацетонитрила при УФ-детектировании (228 nm). Приведены результаты применения метода для определения амоксициллина в четырех готовых формах.

Ключевые слова: обращенно-фазовая ВЭЖХ, метод определения, амокситциллин.