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Development and Validation RP-HPLC Method for Determination of Amoxicillin in Pure and commercial Preparations

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Abstract:

Amoxicillin was determined using a simple, accurate, and repeatable reverse-phase high-performance liquid chromatographic (RP-HPLC) method on a C-18 column (Hyper ODS2 100-5, 250 x 4.6 mm). Acetonitrile and water (70:30) were used as a mobile phase, with a flow rate of 0.9 ml/min. The detection wavelength was 254 nm. The retention time of AMO was 1.685 min. The developed method was validated via accuracy, precision, linearity range, limit of detection (LOD) (1.834 µg mL^{-1}), and limit of quantification (LOQ) (5.927 µg mL^{-1}) in this technique.

Keywords: Amoxicillin; high-performance liquid chromatography method (HPLC); method development, validation .

طريقة تطوير والتحقق من الصحة الاموكسلين بواسطة كروموتوغرافيا السائلة عالية الاداء العكوس مع مستحضراته القياسية و التجارية

أحمد تركي فرج الحلبوسي ، سرحان علي سلمان

مستخلص:

تـم تقديـر الأموكسيسـيلين (AMO) باسـتخدام طريقـة كروماتوغرافيـا السـائل عاليـة الأداء–الطـور المعكـوس (RP-HPLC) بسـيطة ودقيقـة وقابلـة للتكـرار عـلى عمـود الكربـون-18 (Hyper ODS2 100-5، 250 × 4.6 مـم). اسـتُخدم الأسـيتونتريل والمـاء (70:30) كطـور متحـرك، بمعـدل تدفـق 0.9 مل/ دقيقـة. بلـغ طـول موجـة الكشـف 254 نانومـتر. وكان زمـن احتجـاز الأموكسيسـيلين AMO (1.685) دقيقـة. تـم التحقـق مـن صحـة الطريقـة المطـورة مـن خـلال الدقـة، الضبـط، ومـدى الخطيـة، وحـد الكشـف (LOD) (1.834 ميكروغرام/ مـل$^{-1}$)، وحـد التقديـر الكمـي (LOQ) (5.927 ميكروغرام/ مـل$^{-1}$) في هـذه التقنيـة.

الكلـمات المفتاحيـة : الأموكسيسـيلين ، كروموتوغرافيـا السـائلة عاليـة الاداء ، الطـور المعكـوس (RP-HPLC)، تطويـر و تقييـم .

1. INTRODUCTION

Chromatography is considered one of the analytical methods used to separate two or more fractions using two phases. The first phase is called the mobile phase, and the second phase is called the stationary phase. Chromatography can be defined as an analytical method used to separate the substance to be analyzed. It is considered a physical method for analysis and separation, and the stationary phase is usually placed in a column or on a glass or metal surface. Metallic, while the mobile phase contains the pre-prepared solvent, and the substance to be examined (analyzed) is distributed between the two phases. Between the mobile and stationary phases or by the difference in the solubility of the substance in both phases, depending on the adsorption of the substance in The stationary adsorbed phase and the moving phase, which can be liquid, gas, or supercritical. Generally, the naming Chromatography is classified according to the type of mobile phase followed by the type of stationary phase, and during the separation process, each component exits the stationary phase at a different time than the other component.

The HPLC device consists of six main units, which are:

1 - Bottles for storing the mobile phase.

2 - The pump to move the moving phase and the model.

3 - The injector unit allows the model to be introduced through it.

4 - The separation unit (column) to separate the components of the sample.

5. Detector unit to sense the separated compounds.

6 - Control unit.

7 - Data collection machine to help interpret and store the results.

Advantages of HPLC analysis

1. High-sensitivity technology.

2. Highly efficient.

3. Faster analysis and an increase in the number of samples analyzed.

4. Used in quantitative and qualitative analysis.

5. Used in sample analysis and material diagnosis.

6. Models can be analyzed in small quantities.

The reasons that made us choose

HPLC technology over other techniques are

The HPLC technique (high-performance liquid chromatography) stands out from other chemical analysis techniques due to several characteristics that make it preferred in many applications, especially in the pharmaceutical, chemical, and environmental industries:

Advantages of HPLC over others:

1. High precision and sensitivity: It can detect very small amounts of substance (nanograms or picograms). It provides accurate and reproducible results.

2. Excellent separation of complex components: Compounds are separated even in complex mixtures (such as plant extracts or components of multi-drug formulations). Compounds that are very similar in chemical properties can be separated.

3. High flexibility in use: It can be used to analyze polar and non-polar compounds. It operates at room temperature, unlike GC (gas chromatography), which requires the compounds to be volatile.

4. Performance speed: the analysis usually takes a few minutes to half an hour. The time can be controlled according to the type of mobile and stationary phase.

5. The possibility of connecting to multiple detectors, such as ultraviolet (UV) detectors, mass spectrometry (MS), flame ionization (FID), and others. This allows for precise analysis and confirmation of the chemical identity of the compound.

6. Analysis of non-volatile or heat-sensitive compounds: Unlike GC, which requires the compound to be volatile, HPLC does not need that. Ideal for proteins, vitamins, nucleic acids, and more.

7. The possibility of use in drug analysis and quality monitoring: it is widely used in drug analysis (API), impurity estimation, and stability studies (stability studies).

Aquick comparison with other techniques: technique, advantages, and disadvantages TLC (Thin Layer Chromatography) Simple and inexpensive, Less accurate, qualitative, and does not provide quantitative amounts GC (gas chromatography), High accuracy for volatile com-

pounds Not suitable for non-volatile or heat-sensitive compounds UV-Vis Spectrophotometry, Fast and inexpensive analysis Not suitable for mixtures, does not separate compounds NMR. Provides precise structural information. Expensive, complex, and usually not used for quantitative analysis.

Amoxicillin is a member of the penicillin antibiotic class. A moderate-spectrum beta-lactam antibiotic, amoxicillin is used to treat infections brought on by certain gram-negative bacteria as well as penicillin-sensitive gram-positive bacteria]1 [. The chemical name is (2S, 5R, 6R) -6-(R) -2-amino -2-(4-hydroxyphenyl) acetamido) -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo] 3.2.0 [heptane-2-carboxylic acid, chemical formula: $C_{16}H_{19}N_3O_5S$, and molecular weight 365.40 g/mole. According to reports in the literature, the most popular chromatographic method for determining amoxicillin is HPLC[ ]5–2. Among the many techniques developed for amoxicillin determination was potentiometry]6 [, spectrofluorometry]7[, flow injection analysis methods] 9 ,8 [thin-layer chromatography]10 [, voltammetry]11 [, LC-MS[ ]12, capillary electrophoresis]14 ,13 [, and atomic absorption spectroscopy]15[. Figure (1) depicts the chemical structure of amoxicillin.

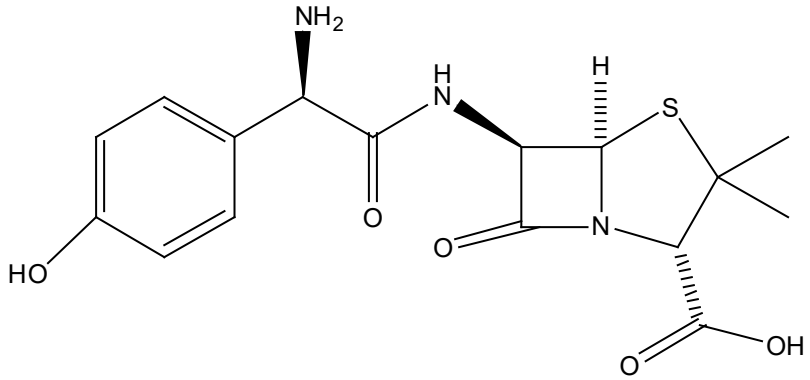

Figure 1: The Chemical structure of amoxicillin

2. Experimental

2.1. The devices and tools used

HPLC device, RP C-18 column (Hyper ODS2, 250 mm x 4.6 mm x 10 MPa), syringe filter, balance, and glassware such as beakers, filter funnels, and volumetric flasks.

2.2. Materials and reagents

Every reagent used in the experiment was of HPLC grade. Iraq General Company for Pharmaceutical Industries, Samarra Pharmaceutical Factory, generously provided AMO. Iraq. Merck provided the HPLC-grade acetonitrile and water. Merck's Whatman polytetrafluoroethylene (PTFE) membrane filter (0.2 μm) was used to filter all sample solutions during the operation.

2.3. Pharmaceutical dosage form

Glomox tablets were labeled to contain 500 mg AMO per tablet manufactured by Globalpharma UAE.

2.4. Preparation of standard drug solutions

2.4.1 Stock solutions

A precisely weighted 0.1 gm of AMO It would help to explicitly describe the dilution process—for instance, mention that after sonication, the volume was adjusted to the 100 mL mark with water, diluted with approximately 80 mL water, and sonicated for 3 min to create the stock standard solution (1000 μg mL^{-1}). The flask was then diluted to 100 mL with water to create a working solution within the concentration range. Indicate that the stock solution was freshly diluted just before use to prepare working solutions.

3. Results and discussion

3.1. Chromatographic conditions

HPLC analysis was carried out under the following guidelines: The analytical column used for the HPLC separation and quantification was an RP C-18 column (Hyper ODS2, 250 mm x 4.6 mm x 10 MPa). Acetonitrile and water (70:30) clarify whether the mobile phase was run isocratically.

The flow rate was 0.9 ml min^{-1}. Every determination was made at a temperature of 25°C. The volume of the injection was 10 μl. The 254 nm wavelength was selected for the detector. Calibration curves explicitly state that they were constructed by plotting peak area versus drug concentration, which would enhance clarity and readability.

3.2. Validation of the methods

The characteristics and the procedures used for validation were those described in USP 24,]17 [and the International Conference of Harmonization (ICH) Guidelines [19 ,18] and *other literature* [21 ,20 ,16].

3.2.1. Linearity and range

By analyzing a series of standard solutions of AMO with varying concentrations ranging from 5 μg mL^{-1} to 75 μg mL^{-1}, Concentrations higher than 75 were present, and by applying the method, we concluded that the highest concentration that adheres to the linear relationship is 75, and the rest were disregarded due to their deviation from linearity. The linearity of the proposed HPLC method was assessed. The calibration curve of the drug under study was obtained by plotting the drug's concentration against each corresponding peak area, specifying that each concentration was analyzed in triplicate for precision. Several analytical parameters were computed, and linear regression analysis was used to analyze them using linear regression (Table 1). Given that the correlation coefficient (r) was 0.9996, the linearity was excellent. Comparing our method with another method Validation of HPLC-UV method for determination of amoxicillin trihydrate in capsule The mobile phase used in the method we are comparing with is potassium monophosphate with methanol at a ratio of 5:95, a flow rate of 1.5 ml/min, and a retention time of 3.5 min. Values LOD = 1.579 μg/mL and LOQ = 4.785 μg/mL, with excellent linearity (R^2 = 0.9998). Precision: Very low RSD (≈ 1.173%), and recovery = 100.06 ± 1.03% within the confidence interval of 95.45–104.5[22].

3.2.2. Limit of detection (LOD) and limit of quantitation (LOQ)

The International Conference on Harmonization (ICH) principles were followed in establishing LOD and LOQ. The standard deviation of the response and the slope of the calibration curve were used to calculate the LOQ and LOD using the formulas LOD = 3.3 SD/Slope and LOQ = 10 SD/slope, where SD is the standard deviation of the intercept and S is the slope of the calibration curve. Table 1 summarizes the findings. 5.927 μg/mL was the limit of quantitation (LOQ), and 1.834 μg/mL was the limit of detection (LOD). This implies that the recommended HPLC analytical approach has a high sensitivity when contrasted with other published analytical procedures.

Table 1: The analytical parameters for determination of AMO by suggested HPLC analytical method

NO.	Parameter	AMO
1	concentration range μgml^{-1}	5-75 μgml^{-1}
2	correlation coefficient (r)	0.9997
3	Determination coefficient (r^2)	0.9995
4	Intercept	–0.241
5	Slope	0.019
6	LOD(μgml^{-1})	1.834
7	LOQ(μgml^{-1})	5.927
8	t-test	4.336
9	F-test	0.023

3.3.3. Accuracy and precision

By examining a solution with a known concentration (the working standard solution) and contrasting the measured and known values, the method's accuracy was ascertained. AMO's mean recovery is 99.323% (n = 5), demonstrating the method's high accuracy (Table 2).

Table 2. Determination of accuracy in samples of known concentration

Sample number	Taken[a]	Found[b]	% recovery
1	15	14.611	97.406
2	30	29.903	99.676
3	45	45.102	100.227
4	60	59.901	99.835
5	75	74.604	99.472

(a) Theoretical concentration expressed in μgml^{-1}
(b) Experimental concentration expressed in μgml^{-1}

Precision

Precision can be measured as repeatability, reproducibility, and intermediate precision. In this work, only repeatability and intermediate precision were studied.

Repeatability

Repeatability tests were performed to determine intra-day variation in peak areas, retention, and migration times. Standard solutions with concentrations of 20, 40, and 60 μgml^{-1}were analyzed

($n = 6$). RSD values for migration times (between 0.254 and 0.983%), retention times (between 0.297 and 0.462%), and peak areas (between 0.595 and 1.663% for HPLC) indicate that the repeatability of this method is acceptable.

Intermediate Precision

Intermediate precision was evaluated over three days (inter-day repeatability) with working solutions of concentrations 10–50 $\mu g ml^{-1}$. These solutions were injected on each of the three days under the same conditions, and the results were used for the repeatability study. The solutions were stored at room temperature (25±225±2ºC during the experimental period) in sunlight, which caused a decrease of recovery values of a maximum of 2.3% for all drugs in methanol. When stored refrigerated in the dark, the recovery decreased by a maximum of 0.7% in three days for all drugs. RSD values for HPLC: 0.491−0.812% for AMO indicate acceptable peak area, migration, and retention time intermediate precision for the HPLC method.

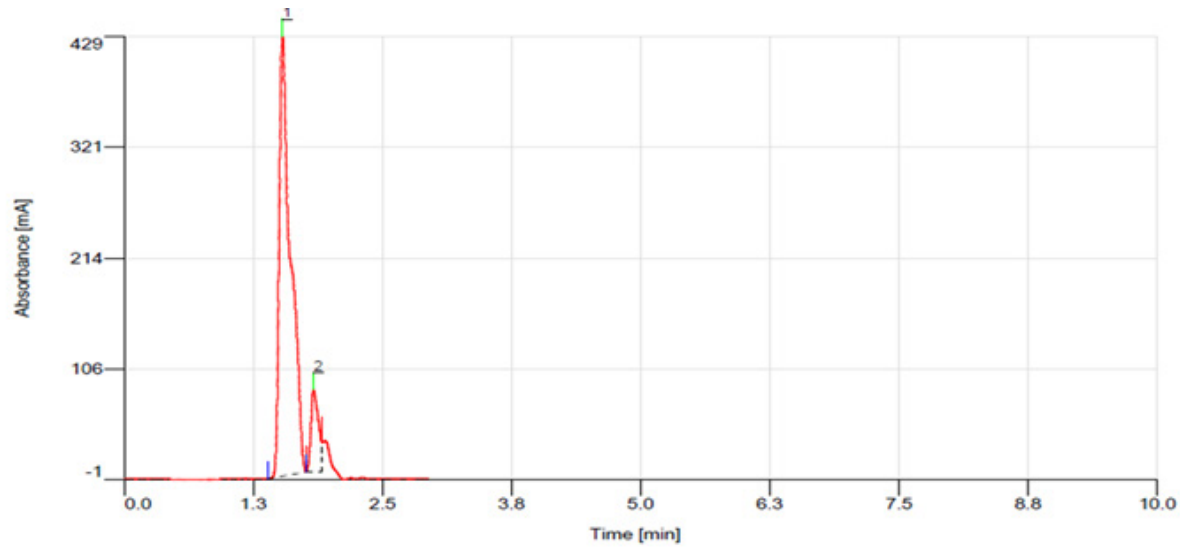

Fig.2: Chromatogram of Standard Amoxicillin (75 μg/mL)

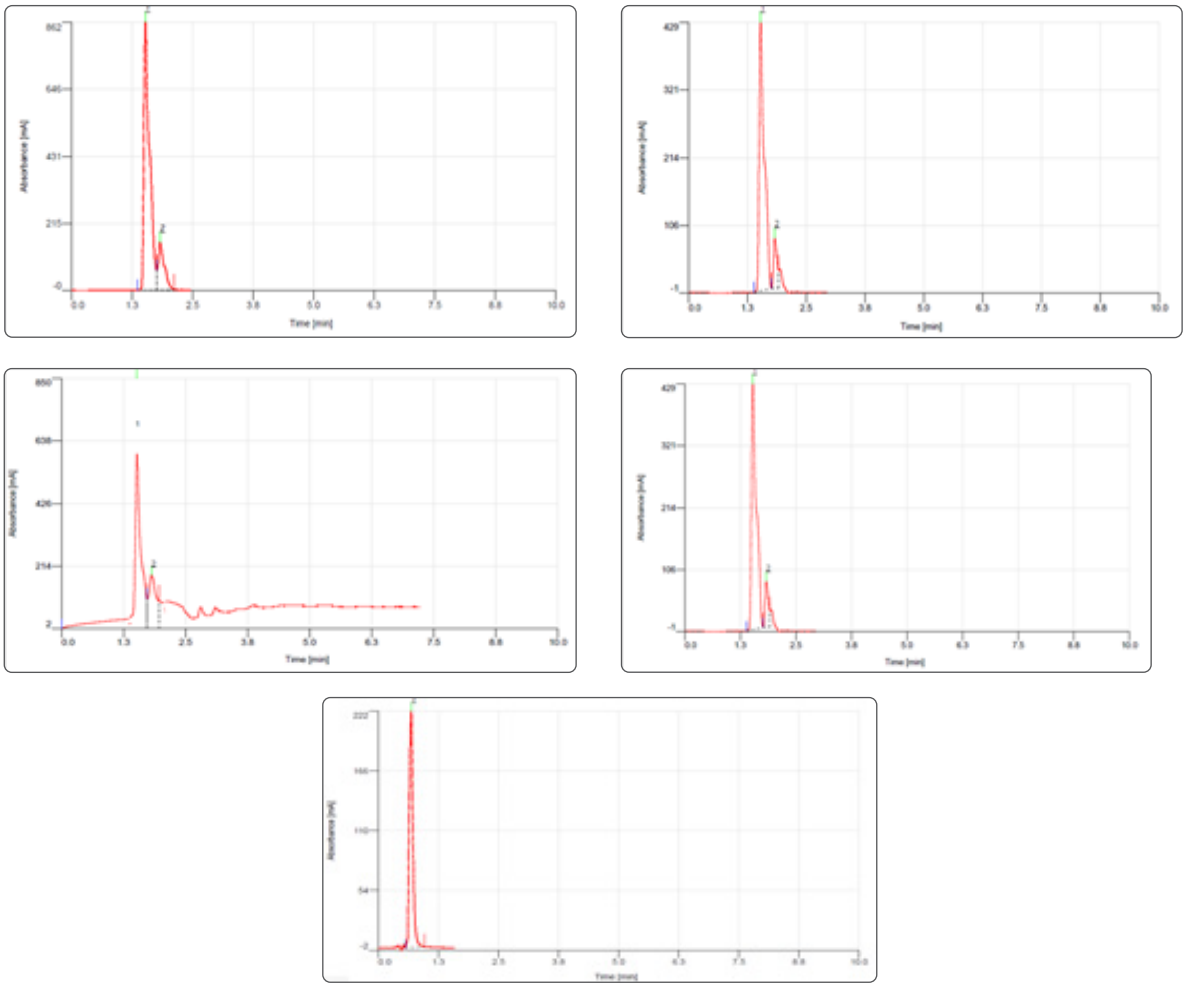

Fig.3: Chromatograms of Standards Amoxicillin (5-75 µg/mL)

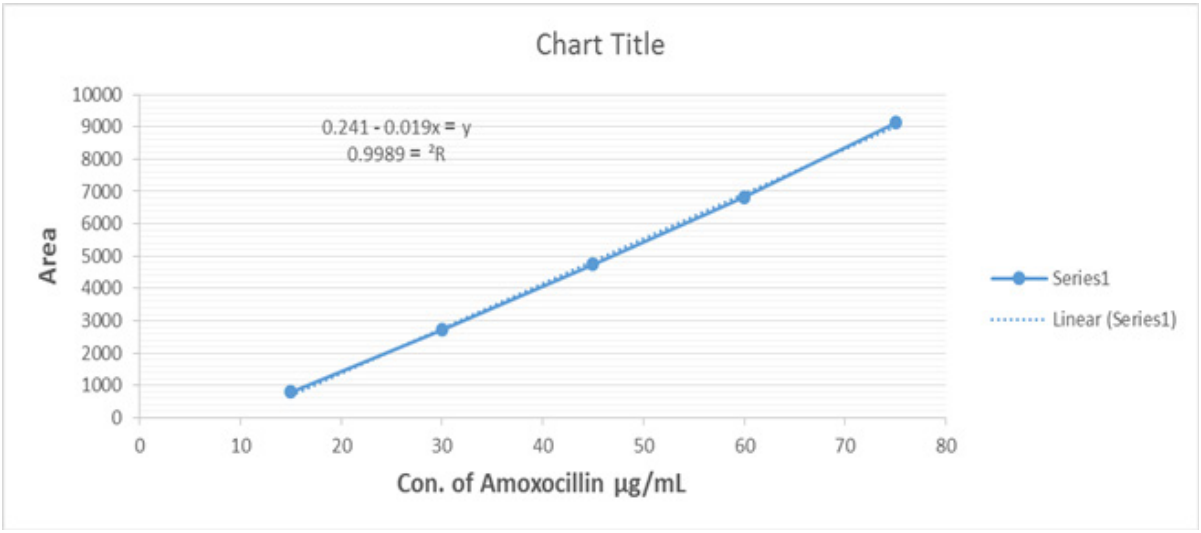

Fig. 4: Linearity of Amoxicillin

4. Conclusion

The chromatographic separation of amoxicillin in our study was characterized with good accuracy and precision. It is characterized by being simple, rapid, sensitive, economical, and reproducible. The credibility of the proposed method has been established by validation as per ICH guidelines. The method employed more common instrumentation with UV detection, a simpler extraction procedure, and a faster run time with no compromise on assay sensitivity. In comparison with the previously developed methods, the present method offers an undoubted advantage in terms of overall analytical performance.

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