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Original article

Development and Validation of a Simple UV-Vis Spectrophotometric Method for the Quantitative Determination of Amoxicillin in Pharmaceutical Dosage Forms

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Abstract
A simple, sensitive, and inexpensive UV/visible spectrophotometric approach was developed and validated for the quantitative measurement of amoxicillin (AMX) in pharmaceutical dosage forms. The approach demonstrated excellent linearity (r^2 = 0.9994) across the concentration range of 1.0-15.0 µg mL^{-1} by measuring absorbance at 228 nm. The determined limits of detection and quantification were established at 0.56 and 1.72 µg mL^{-1} for AMX, respectively. Values of relative standard deviation (RSD) below 2% indicated remarkable repeatability and consistency; intra-day and inter-day studies confirmed accuracy. Recovery tests conducted at three concentration levels yielded results within the acceptable range of 94.15% to 108%, demonstrating the accuracy of the method. The validated method was successfully used to analyze various commercial amoxicillin products in tablet and capsule forms because all the tested samples met the required quality standards. These findings demonstrate that the proposed spectrophotometric method is consistent and adequate for routine quality monitoring of amoxicillin in pharmaceutical formulations.
Keywords: Amoxicillin, UV/Visible Spectrophotometric, Method Validation, Capsules, Tablets.

Introduction

Amoxicillin is a common beta-lactam antibiotic commonly used to treat gram-negative bacterial infections [1, 2]. Similar to other antibiotics of its class, this medicine suppresses cell wall synthesis, resulting in osmotic lysis. Antibiotics are among the most used pharmaceuticals globally and are extensively utilized in both human and veterinary medicine, not only for the treatment or prevention of infections in humans and animals but also for boosting animal growth [3, 4]. AMX serves as a primary antibiotic for addressing minor respiratory infections and various other prevalent infections. Chemically, It is a semi-synthetic antibiotic of the penicillin class, characterized by attaching a thiazolidine ring to a *β*-lactam ring [5, 6]. The chemical structure of amoxicillin (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (Fig. 1) [7]. It is an acid-stable and highly hygroscopic white or slightly off-white powder [8], and is typically administered orally [9]. Due to its extensive bactericidal efficacy and consequent prevalent application in pharmaceuticals, numerous formulations of this drug, including capsules, tablets, oral suspension powder, injections, and combinations with other components [10]. However, its use may also be associated with potential side effects include nausea, vomiting, rashes, and antibiotic-associated colitis [11]. A minor change in the composition or purity of the active pharmaceutical ingredient (API) can influence the therapeutic results and potentially lead to adverse effects of medications. Consequently, it is essential to create enhanced analytical techniques for the pharmaceutical evaluation of medications.

Several analytical methods have been developed to determine AMX, including thin-layer chromatography (TLC) [1], high-performance thin-layer chromatography (HPTLC) [12, 13], high-performance liquid chromatography (HPLC) [14-16], voltammetry[17, 18], electrochemical methods [19, 20], electrophoresis [21], and spectrophotometric methods [22-25]. Among these, the spectrophotometric technique is the most widely used method because of its simplicity and ease of use and because it does not require expensive equipment. This study intends to develop a validated approach that is straightforward, efficient, and economical for the quantitative assessment of amoxicillin in pharmaceutical tablet formulations via UV-Vis spectrophotometric analysis.

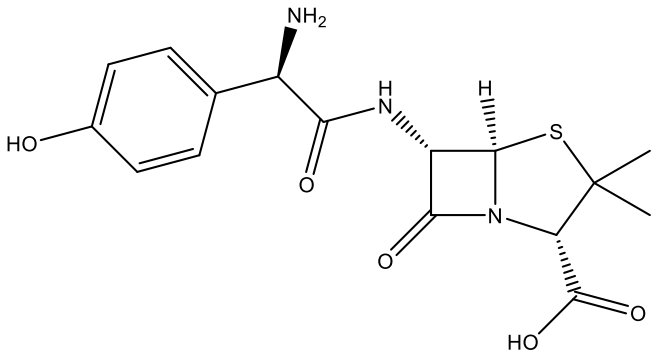

Figure 1. The chemical structure of amoxicillin.

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Materials and methods

Equipment and materials

All spectral scans and measurements were done using a JENWAY 6305 spectrophotometer. We used analytical reagent-grade chemicals throughout this study. Reference standard of pure AMX was purchased from Sigma Aldrich. Two brands of AMX (Amoxicillin Tablets USP, Kwality, India and Ronakamoxi Capsules, Fugen, India) were purchased from a local pharmacy store. Each of them contained 500 mg per the label claim.

Standard preparation

Amoxicillin Solution Preparation

Standard amoxicillin stock solution (1000 µg mL^{-1}) was prepared by dissolving 100 mg in 100 mL (20:30) (V/V MeOH:water) and was stored at 4°C until the analysis.

Test preparation

Ten powdered capsules, each containing 500 mg of AMX, or ten tablets of amoxicillin, also 500 mg each, from each product were weighed individually to determine the average weight of a tablet and subsequently ground into a fine powder using a mortar. A specific amount of powder equivalent to 10 mg of amoxicillin was accurately measured, transferred into a 100 mL volumetric flask, dissolved in a diluted solution, and subjected to sonication for 10 minutes to improve solubility. The solution was subsequently diluted to a final volume of 100 mL using the same diluted solution. The sample underwent filtration through Whatman filter paper no. 41 to remove solid particulates, resulting in a stock solution with a concentration of 100 µg mL^{-1}. A solution of 10 µg mL^{-1} was subsequently prepared and subjected to UV analysis. Each medicinal substance was subjected to three replicates.

Results and Discussion

Validation of Analytical Method

Linearity, limit of detection (LOD), and limit of quantification (LOQ)

Constructing a calibration curve using a total of eight standard solutions allowed the assessment of the linearity of the suggested method. The linearity of the calibration plots was evaluated using standard AMX to cover the concentration range of 1.0-15.0 µg mL^{-1}. The calibration curve was established by plotting the absorption (y) axis versus the AMX concentration (x) axis. The obtained calibration curve was linear over the studied concentration range. The data analysis revealed a regression equation of y = 0.0274x - 0.0035 with a correlation coefficient of r^2 = 0.9994, indicating excellent linearity due to its high r^2 value (Fig. 2). The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations: "LOD = 3.3S/K" and "LOQ = 10S/K", where S is the standard deviation of the intercept and K is the slope of the calibration curve. The LOD and LOQ were determined to be 0.56 µg mL^{-1} and 1.72 µg mL^{-1}, respectively. These results turned out to be an improvement over the previously reported limits of detection using various methods like standard UV-Visible spectrophotometry [22]. HPLC coupled with UV detection [26], and even the more advanced ratio-first derivative zero-crossing UV-Vis technique [27].

Table 1. Analytical parameters obtained from the calibration graph of amoxicillin determined by the proposed spectrophotometric method.

Parameter	Value
λmax	228
Linear range (µg mL^{-1})	1-15
LOD (µg mL^{-1})	0.56
LOQ (µg mL^{-1})	1.72
Slope	0.0274
Intercept	0.0035
Correlation coefficient (r^2)	0.9994

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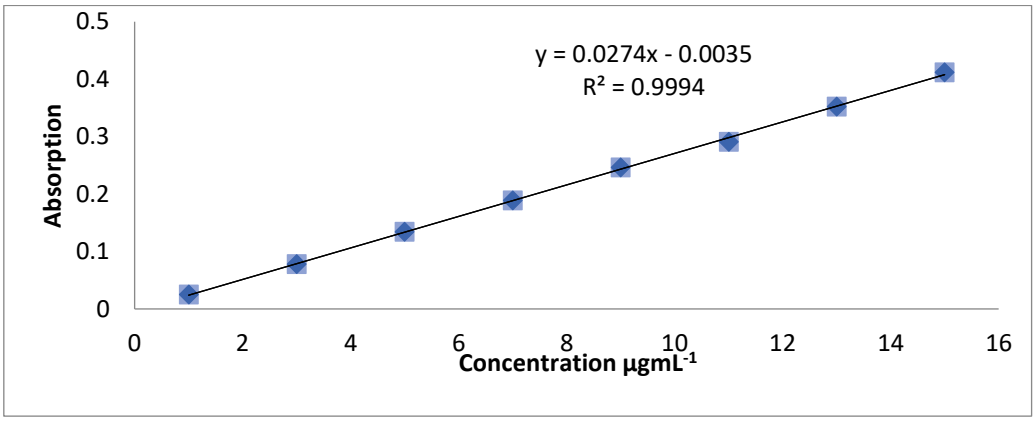

Figure 2. Calibration curve of amoxicillin.

Precision

The term "precision" denotes the extent to which measured values align with reference values. Precision is generally contingent upon the analyte concentration; thus, it is essential to ascertain this concentration while operating within the relevant range of interest. Repeatability (within-day precision) and intermediate precision (between-day precision) were assessed over three consecutive days to evaluate overall precision [28]. Three standard solution preparations were used to check the precision within a single day at three different concentration levels (3, 7, and 9 µg mL^{-1}) with a total of nine measurements. Each concentration was measured three times, and the inter-day precision was evaluated over three consecutive days (n = 27). Table 2 shows that the calculated coefficient of variation for repeatability, intra-day, and inter-day results stayed within the acceptable range, with RSD% not going over 2% [29]. Interestingly, the results were quite similar to those obtained with previously reported methods, like flow injection paired with UV-Vis [30], or standard UV-Vis spectroscopy [31]. The outcomes show that the procedure is very repeatable. A high degree of precision was shown by sufficiently low coefficients of variation. Therefore, the established approach may be used reliably for determining AMX in pharmaceutical formulations.

Table 2. Amoxicillin standard solution's intra-day and inter-day precision (%RSD).

AMX concentration (µg mL^{-1})	**RSD (%)**		
Intra-day precision (n=9)	**0hr**	**1hr**	**2hr**
5	0.71	1.38	1.12
7	0.88	0.93	1.02
9	0.95	1.14	1.29
Inter-day precision (n=27)	**1st day**	**2nd day**	**3rd day**
5	1.94	1.87	1.40
7	1.98	1.22	0.88
9	1.34	1.04	1.57

Recovery

An analytical method's accuracy can be described as the degree to which the result of the value found is close to the reference value [28]. This is a relatively close relationship between the two values. To ascertain the precision of the proposed method, recovery experiments were carried out at three distinct concentrations, namely 3, 7, and 9 µg mL^{-1}. The recoveries were carried out by including determined quantities of AMX into the formulations that had been pretreated. A triplicate of each concentration level was generated, and it was then subjected to UV/Visible three times. Table 3 summarizes the collected results. This indicates that this method has the potential to determine the AMX in tablet dosage formulations, as the outcome demonstrated that the approach was accurate. At each additional concentration, the spiked drug yielded the best recoveries, ranging from 94.15 to 108 percent. This falls within the recommended recovery percentage range of 80 to 110% [32].

Table 3. Recovery of the proposed method.

Sample (µg mL^{-1})	**Added (µg mL^{-1})**	**Found (µg mL^{-1})**	**Recovery ± RSD(%)***
7	3	10.26	108±0.44
7	7	13.59	94.15±0.36
7	9	16.9	97.11±0.044

Analysis of pharmaceutical formulation

The validated method was successfully employed for the testing of two commercial items of different types (tablet and capsule) containing 500 mg of AMX. The findings are derived from the average of three specified

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values presented in Table 4. The result indicates that there were no major differences between the amoxicillin amounts listed by the manufacturer and those measured by the new method, which needed to be between 90% and 110% of the labeled amount [33]. Consequently, all brands examined adhered to USP pharmacopeial standards.

Table 4. Assay results of amoxicillin in different pharmaceutical formulation.

Brand	Amount of drug labelled (mg)	Amount of drug estimated (mg)	% Labelled claim±RSD
Amoxicillin Tablets USP (tablet)	500	513.0	102.6±0.442
Ronakamoxi (capsules)	500	553.5	110.7±0.598

Conclusion

The validation of an analytical method based on UV/visible spectroscopy was applied successfully for the determination of amoxicillin in two pharmaceutical dosage forms, including tablets and capsules. The proposed method was found to be linear within the concentration range of 1.0–15.0 µg mL^{-1} with a correlation coefficient (r^2 = 0.9994). The method demonstrated good sensitivity, evidenced by the low LOD and LOQ values. Precision studies have validated that the method demonstrates exceptional repeatability and reproducibility, with %RSD values consistently remaining under 2%. Accuracy was supported by recovery rates ranging from 94.15% to 108%, which fall within acceptable limits. Furthermore, analysis of commercial amoxicillin products indicated that all tested brands met pharmacopeial standards. The good recoveries and low coefficients of variation that are obtained make the suggested method suitable for the routine quality control analysis of amoxicillin in pharmaceutical dosage formulations.

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