

## TESTING OF AMOXICILLIN LEVELS IN RESISTANCE CASES IN AN EFFORT TO MONITOR ANTIBIOTIC DRUG QUALITY AT THE TASIKMALAYA CITY HEALTH CENTER, INDONESIA

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### ABSTRACT

**Objective:** Acute respiratory infection (ARIs) is an acute inflammation of the upper and lower respiratory tract caused by microorganisms, bacteria, viruses, and infectious rickets. Evaluation of the quality of antibiotics is done to determine the effectiveness of the desired therapy. This study aims to determine the levels of amoxicillin antibiotics used in ARIs treatment. **Methods:** Drug samples were obtained from health centers in the City of Tasikmalaya. Testing the levels of amoxicillin antibiotics was carried out using HPLC instruments, using validation parameters linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). **Results:** The test results showed that the levels of levofloxacin antibiotics that are used to meet the requirements that exist in the United State Pharmacopeia grading 104.95%, and the validation parameters that

meet the requirements, correlation coefficient of 0.9993, recovery percent of 100.1%, precision 0.055%, LOD 0.082 µg/mL and LOQ 0.251 µg/mL. **Conclusions:** The test results showed that the levels of amoxicillin antibiotics that are used to meet the requirements that exist in the United State Pharmacopeia (USP).

**KEYWORDS:** Acute respiratory infections, amoxicillin, resistance of antibiotics, validation parameters.

## INTRODUCTION

Acute Respiratory Infections (ARIs) in Indonesia are still a serious problem and cause 28% mortality in children. Inappropriate use of antibiotics will cause many bacteria to become resistant to antibiotics.<sup>[1]</sup>

The main bacteria that cause ARI include haemolyticus, Streptococcus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae. The main bacteria that cause ARI include haemolyticus, Streptococcus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae.<sup>[2]</sup> Treatment of respiratory diseases is usually administered with amoxicillin antibiotics, but cases of resistance have been reported to Streptococcus haemolyticus, Staphylococcus, and Corynebacterium diphtheriae.<sup>[3]</sup>

This study aims to evaluate the quality of the antibiotic amoxicillin used in all health centers in the city of Tasikmalaya, whether it is in accordance with the requirements listed in the Indonesian Pharmacopoeia.

## MATERIALS AND METHODS

Materials tested were Amoxicillin used in community health center in Tasikmalaya, potassium dihydrogen phosphate, acetonitrile, HCL p.a (PT. Merck Indonesia), aqua bidestilation (Ikapharmindo Putramas).

The tools used in this study is HPLC (Dionex Ultimate 3000) with Accalim Polar Advantage II column, DAD detector, ultrasonic bath (NEY-1510), and glass tools commonly used in the Laboratory Analysis.

### Method

Chromatographic conditions The selected and optimized mobile phase composed of Acetonitrile: Potassium dihydrogen phosphate buffer (pH 5) (1:99v/v) and conditions optimized were with flow rate of 1 ml/minute, wavelength at 254 nm and Run time of 20 min. Here the peaks were separated and showed better resolution, appreciable theoretical plate counts and good peak symmetry.<sup>[4]</sup>

### Preparation of mobile phase

Mobile phase was prepared by taking Acetonitrile: 0.2 M Potassium di hydrogen phosphate buffer (pH 5) (1:99 v/v). Mobile phase was filtered through 0.45 µm membrane filter and

degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1 ml/min.<sup>[4]</sup>

### Preparation of standard solutions

Dissolve 30 mg of amoxicillin working standard in mobile phase and dilute to 50 ml with the same mobile phase. One ml was diluted to 20ml with mobile. Diluted to 1ml of this solution to 50ml with mobile phase. Finally this gave 0.6 ppm solution, then the solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system.<sup>[4]</sup>

### Assay of Amoxicillin

Twenty tablets (Amoxicillin 500 mg) was weighed accurately, average weight was calculated. Then powder equivalent to 50 mg was taken in a 50 ml volumetric flask. Then 20ml of mobile phase was added and kept for 15mins with occasional shaking. Then 10 ml of Glacial acetic acid was added. Then volume was made to 50ml followed by sonication for 15mins. The solution was filtered with 0.45µ filter paper. From the filtrate 10 ml was taken and made to 100ml with the mobile phase. Finally 1ml of the above solution was taken in 10ml volumetric flask and made up to volume with mobile phase and injected into RP-HPLC system. Then Assay was carried out for the amount of amoxicillin content.<sup>[4]</sup>

### Method Validation

The method was validated in accordance with ICH guidelines. The parameters assessed were linearity, accuracy, and precision, reproducibility, robustness and system suitability.<sup>[5]</sup>

### Accuracy

Accuracy was best determined by the standard addition method. Previously analyzed samples of Amoxicillin API were added with standard drug solutions and are analyzed by the proposed method. Recovery (%), RSD (%) and correlation coefficient, limit of detection (LOD), limit of quantification (LOQ) were calculated for each concentration. Accuracy is reported as percentage bias, which is calculated from the expression.<sup>[6]</sup>

$$\% \text{ Bias} = \frac{(\text{measured value} - \text{true value})}{\text{true value}} \times 100$$

**Precision**

System precision: Standard solution prepared as per test method and injected six times and the % RSD value was calculated. Method precision: Six preparations individually using single batch of Amoxicillin drug substance were prepared as per test method and injected each solution induplicate on the same day in to HPLC. % RSD value was calculated to determine intra-day precision.

**Limit of Detection (LOD)**

The Limit of Detection (LOD) of an analytical method may be defined as the concentration, which gives rise to an instrument signal that is significantly different from the blank. For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept ( $S_a$ ), which may be related to LOD and the slope of the calibration curve.

$$\text{LOD} = 3 S_a / b$$

**Limit of Quantitation (LOQ)**

The LOQ is the concentration that can be quantitated reliably with a specified level of accuracy and precision. The LOQ represent the concentration of analyte that would yield a signal-to-noise ratio of 10.

$$\text{LOQ} = 10 S_a / b$$

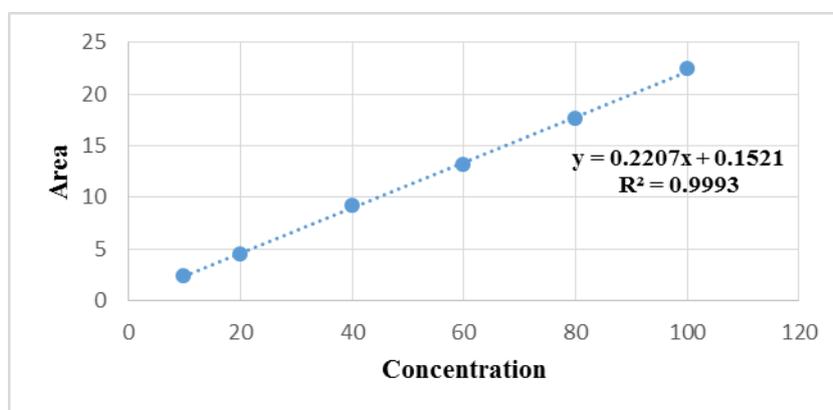
Where,  $S_a$  is the standard deviation of the peak area ratio of analyte to IS (6 injections) of the drugs and  $b$  is slope of the corresponding calibration curve.

**RESULTS AND DISCUSSIONS****Linearity Test**

Linearity test is done with a series of standard solutions which consist of at least four different concentrations in the range of 50-150% of the content of the analyte in the sample. (Riyanto, 2014). The concentration used in the assay was 10 ppm; 20 ppm; 40 ppm; 60 ppm; 80 ppm; and 100 ppm.

The calibration curve showed good linearity in the range of 0.6 - 3.4  $\mu\text{g/ml}$ , for Amoxicillin (API) with correlation coefficient ( $r^2$ ) of 0.9993. The slope and intercept of the calibration graph was calculated by using linear regression analysis. The regression equation of the calibration curve was:  $y = 0.2207x + 0.1521$ ;  $r^2 = 0.9993$ . A correlation coefficient suggests

that the developed HPLC method had an excellent linearity over the investigated range. Correlation coefficient meets the requirements is greater than 0.99.<sup>[5]</sup> The results for linearity are shown in Figure 1.



**Figure 1: Calibration Curve for Amoxicillin.**

### Accuracy Test

Accuracy indicates the degree of closeness of the results of a series of measurements obtained from a homogeneous sample under specified conditions.<sup>[5]</sup> Accuracy expressed as a percent recovery (recovery) the analyte is added. Testing is done by six different of concentration are 10 ppm; 20 ppm; 40 ppm; 60 ppm; 80 ppm; and 100 ppm. The average value of recovery% is 100.1%. This result is acceptable because it is still within the required range 80 - 110%.<sup>[8]</sup>

### Precision Test

Precision is a measurement repeatability of analytical methods derived from multiple measurements on the same sample. Precision is measured as the standard deviation or relative standard deviation (coefficient of variation).<sup>[5]</sup> Precision test criteria can be distinguished as follows:

**Table 1: Criteria of precision test.**

% RSD	Criteria
< 1	very precise
1 – 2	Precise
2 – 5	Midle
> 5	Not pricise

System precision Acceptance criteria: RSD for area should not be more than 1%. The intra & inter day variation of the method was carried out and the high values of mean assay and low

values of standard deviation and % RSD (% RSD < 2%). The RSD percentage of 0.55% indicates that this method has a high degree of accuracy for sample testing.<sup>[5]</sup>

### **LOD and LOQ Test**

The limit of detection is the smallest amount of analyte in a sample that can be detected which still provides significant response compared to the blank and the test parameters limits. Values obtained detection limit is 0.082 µg/mL. Quantification limit is a parameter on the analysis of trace and is defined as the smallest quantity of analyte in the sample were still able to meet the criteria of a careful and thorough. Values obtained quantification limit was 0.251 µg/mL.

### **Assays Amoxicillin**

Determination of amoxicillin antibiotic sample level was done by HPLC method. Levels of antibiotic amoxicillin samples obtained from the calculation of 104.95%. The results of amoxicillin level measurement meet the requirements listed in USP that is 90% -110%.<sup>[5]</sup>

### **CONCLUSION**

Levels of antibiotic amoxicillin used in Tasikmalaya City Health Center is 104.95%. Results are still within the range required by the USP 97% -120%.<sup>[5]</sup>

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