WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 140-146 Research Article ISSN 2278 – 4357

ANTIBIOTIC QUALITY MONITORING: DETERMINATION OF COTRIMOXAZOLE USED IN TREATMENT IN ANTIBIOTIC RESISTANCE CASES AT TASIKMALAYA CITY HEALTH CENTER, INDONESIA

Danni Ramdhani*, Wiwiek Indriyati and Muhammad Aiman

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia, 45363.

Article Received on
09 Dec. 2018,

Revised on 29 Dec. 2018,
Accepted on 19 Jan. 2019

DOI: 10.20959/wjpps20192-13135

***Corresponding Author**
Danni Ramdhani
Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia 45363.

ABSTRACT

Objective: Cotrimoxazole is a broad spectrum anti-microbial used in the management of Acute Respiratory Infection (ARIs) disease. The good quality of antibiotics greatly determines the effectiveness and success in therapy. The level of an antibiotic is one of the quality parameters that must meet the requirements stated in the United State Pharmacopeia (USP). ***Methods:*** The cotrimoxazole antibiotic used in the treatment of ARIs was obtained from the health center of the City of Tasikmalaya. The determination of cotrimoxazole 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 trimethoprim and sulphamethoxazole that were used to meet the requirements that exist in the USP grading 90.88 % and 102.30 %, and the validation parameters that meet the requirements, correlation coefficient of 0.9982, recovery percent of 99.01 %, precision 0.125 %, LOD 0.8359 µg/mL and LOQ 2.533 µg/mL. ***Conclusions:*** The test results showed that the levels of cotrimoxazole antibiotics that are used to meet the requirements that exist in the USP.

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

INTRODUCTION

Co-trimoxazole [trimethoprim (TM)- sulphamethoxazole (SM)] is a broad spectrum anti-microbial agent composed of a fixed combination of a diamino-pyrimidine and a sulphon-amide.[1] Co-trimoxazole has been shown to be effective in acute and persistent or recurrent urinary tract infections, (treatment and prophylaxis), ear, nose, throat infections, acute exacerbation of the chronic bronchitis and enteric fever.[2]

The main bacteria that cause ARI include haemolyticus, Streptococcus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae.[3] The main bacteria that cause ARI include haemolyticus, Streptococcus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae.[4]

Sulfamethoxazole is chemically 4-amino –N (5-Methel-1,2-oxazol-3-yl)benzene-1-sulfonamide. The mechanism of sulfamethoxazole is inhibit conversion of pteridine and p-aminobenzoic acid to dihydropteroic acid by competing with PABA for binding to dihydrofolate synthetase an intermediate of tetrahydrofolic acid synthesis (THF). THF is required for the synthesis of purines and dTMP and inhibition of bacterial growth.[5]

Trimethoprim is chemically 5(3,4,5 trimethoxy phenyl) methyl phrimidine-2,4-di amine. The mechanism of trimethoprim is, it binds to dihydrofolate reductase and inhibits dihydrofolic acid to tetrahydrofolic acid. THF is an essential precursor in the thymidine synthesis pathway and interference with this pathway inhibits bacterial DNA synthesis.[5]

This study aims to evaluate the quality of the antibiotic cotrimoxazole used in all health centers in the city of Tasikmalaya, whether it is in accordance with the requirements listed in the United State Pharmacopeia (USP).

MATERIALS AND METHODS

Materials tested were cotrimoxazole used in community health center in Tasikmalaya. Phosphoric acid, acetonitrile, and ethanol HPLC grade solvents were obtained from PT. Merck Indonesia, aqua bidestilation (Ikapharmindo Putramas). The tools used in this study is HPLC (Dionex Ultimate 3000) with Accalim Polar Advantage II column, UV detector, ultrasonic bath (NEY-1510), and glass tools commonly used in the Laboratory Analysis.

Method

Chromatographic Condition: The mobile phase containing Buffer: Acetonitrile (30:70) was found to resolve Sulfamethoxazole and Trimethoprim. Ortho phosphoric acid was used for pH adjustment of buffer to 4.0. The mobile phase was filtered through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/min. The drug shows good absorbance at 260 nm, which was selected as wavelength for further analysis.[6]

Buffer Preparation: Accurately weighed and transferred 1.143grams of ortho phosphoric acid into 1000 ml of distilled water and adjust pH with triethylamine to 4.0. Filter the solution through 0.45µm nylon filter.[6]

Preparation of Mobile Phase: Prepare, filtered and degassed mixture of buffer and Acetonitrile in the ratio of 30:70 v/v.[6]

Preparation of Standard solution: Accurately weighed and transferred about 50mg of sufamethoxazole and 10mg of trimethoprim working standard into a 10ml volumetric flask add 2ml of mobile phase, sonicated for 15 min and make up to the mark with mobile phase.[6]

Preparation of Sample solution: Crush 20 tablets and transferred accurately weighed powder equivalent to 50 mg of Sulfamethoxazole and 10mg of Trimethoprim into 10ml volumetric flask add 7ml of mobile phase sonicate for 20min to dissolve and make up to the volume with mobile phase. Filter the solution through 0.45µm nylon filter. Transfer 0.1ml of above solution into 10ml volumetric flask and make up to the volume with mobile phase (50 ppm of sulphamethoxazole and 10 ppm of trimethoprim).[6]

Validation procedure: Present study was conducted to obtain an innovative, simple, rapid and affordable method for the determination of cotrimoxazole. The HPLC method development and validation was performed according to the official specifications of Centre of Drug Evaluation and Research (CDER-1994), International Conference on Harmonization and United State Pharmacopeias. The method validation parameters included system suitability, linearity, specificity, accuracy, limit of detection, limit of quantification, precision.[7]

Accuracy: Accuracy was best determined by the standard addition method. Previously analyzed samples of cotrimoxazole 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.[7]

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

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 cotrimoxazole 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.[7]

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 (Sa), which may be related to LOD and the slope of the calibration curve.[7]

$$LOD = 3\ Sa / b$$

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.

$$LOQ = 10\ Sa / b$$

Where, Sa 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.[8]

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. 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 µg/ml, for Cotrimoxazole (API) with correlation coefficient (r2) of 0.9982. The slope and intercept of the calibration graph was calculated by using linear regression analysis. The regression

equation of the calibration curve was: y = 0.4964x - 0.4354. 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 (5). The results for linearity are shown in Figure 1.

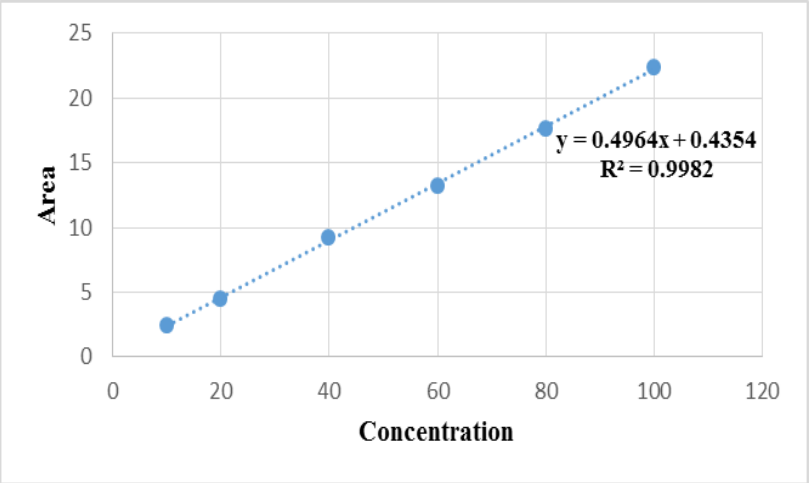

Figure. 1. Calibration Curve for Cotrimoxazole.

Accuracy Test: Accuracy indicates the degree of closeness of the results of a series of measurements obtained from a homogeneous sample under specified conditions (5). 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 99.01s %. This result is acceptable because it is still within the required range 80 - 110%.[11]

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).[9] 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 Aceptance 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 0.125 % indicates that this method has a high degree of accuracy for sample testing.[10]

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 0.8359 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 2.533 µg/mL.[8]

Assays Cotrimoxazole

Determination of cotrimoxazole antibiotic sample level was done by HPLC method. Levels of antibiotic cotrimoxazole samples obtained from the calculation of 104.95 %. The results of cotrimoxazole level measurement meet the requirements listed in USP that is 90% -110%.[7]

CONCLUTION

Levels of antibiotic cotrimoxazole used in Tasikmalaya City Health Center are used to meet the requirements that exist in the United State Pharmacopeia grading trimethoprim 90.88 % and sulphamethoxazole 102.30 % 107.58 %. Results are still within the range required by the USP 97% -120%.[11]

ACKNOWLEDGMENTS

The authors are deeply grateful to the subjects participating in this study. The author would like to thank Tasikmalaya City Health Office. The author also thanked Muhammad Aiman for its cooperation in this study.

REFERENCES

1. Wormser, G.P., Keusch, G.T. and Heel, R. Co-trimoxazole (trimethoprim-sulfamethoxazole): An updated review of its antibacterial activity and clinical efficacy. Drugs, 1982; 24: 459-518.
2. Kumar, A.S. Treatment of anaphylaxis following oral Co-trimoxazole. Indian Pediatr, 1996; 33(3): 249-250.
3. Ministry of Health RI. 2002. Guidelines for the Control of Acute Respiratory Disease. Jakarta: Ministry of Health RI.

4. Southwick, S.F. Infectious Diseases In 30 Days. USA: Mc Graw-Hill Companies Inc. 2003.
5. Richards, R.M., Taylor, R.B. and Zhu, Z.Y. Mechanism for synergism between sulphonamides and trimethoprim clarified. J. Pharm. Pharmacol, 1996; 48(9): 981-984.
6. Behzadian Nejad, G., Rezaee, A. and Kebriaeezadeh, A. High-performance liquid chromatographic determination of trimethoprim in mouse liver. Pharm. Pharmacol. Commun, 1998; 4: 439-441.
7. ICH, Validation of analytical procedures. Methodology harmonized tripartiate guideline prepared within the international conference on harmonization of technical requirements for the registration of pharmaceuticals for human use. ICH-Q2B: Geneva, 1996.
8. Ermer, J. 2005. Method Validation in Pharmaceutical Analysis. Weinheim: Wiley-VCH Verlag GmbH & Co. KGaA.
9. McMaster, M.C, 2007. HPLC A Practical User's Guide, Edisi ke II, New Jersey: John Wiley and Sons Inc.
10. Harmita Implementation Guidance Validation Method and Method Calculation. Pharmaceutical Science Magazine, December, 2004; I(3): 117-135.
11. The United State Pharmacopeial Convention. The United States Pharmacopoeia (USP). 37th Edition. United States: US Pharmacopeial Convention Inc, 2014; 79-82.