EVALUATION OF TETRACYCLINE ANTIBIOTIC RESISTANCE: RELATION OF ANTIBIOTIC QUALITY PARAMETERS TO CASES OF RESISTANCE OCCURRING IN ARIS DISEASE IN TASIKMALAYA CITY HEALTH CENTER

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ABSTRACT

Objective. Tetracycline is an antibiotic that usually used in treatment of acute respiratory infections (ARIs). One of the factors that support the occurrence of cases of antibiotic resistance is the antibiotic quality parameters of the potential and levels of antibiotics used. This study aims to determine the relationship between quality parameters of tetracycline antibiotics used in public health centers in Tasikmalaya city with cases of tetracycline resistance occurring in the area. **Methods.** This research method includes the determination of antibiotics potential with 3 + 3 pattern and the comparative value of the activity using agar diffusion method perforation techniques and antibiotic assay with UV-Visible spectrophotometry method with validation parameters: linearity, precision, accuracy, limit of detection (LOD) and limit of Quantification (LOQ). **Results**. The research shows the antibiotic potential of tetracycline is 101,71%. The validation method demonstrate that the method used fulfill the requirements of standard curve with concentrations in the range of 7.5-20 μg/mL with a correlation coefficient of 0.9978, reproducibility 0.08%, average recovery percent of 99,97%, LOD 0,9961 μg/mL, and LOQ 3.3203 μg/mL. The Results of antibiotic assay is 102,76 %.

KEYWORDS: ARIs, antibiotics, tetracycline, potency, antibiotic assay, validation method.

INTRODUCTION

In Indonesia, the disease Acute Respiratory Infections (ARIs) is still a major cause of morbidity and mortality especially among children under five is 28%. It was reported that one of the respiratory disease is pneumonia is the leading cause of infant mortality second after diarrhea with 15.5% of whom are children under five (Riskesdas, 2007).

In addition, pneumonia is always on the list of 10 biggest disease each year in health facilities. According to the basic medical research, the prevalence of ARI in Indonesia was 25.5% (range: 17.5% - 41.4%) with 16 provinces have prevalence above the national average. Based on the results of basic medical research in West Java province, the prevalence of ARI in the town of Tasikmalaya provides real figures on the number of national prevalence (Winarni, 2010).

The bacteria that cause ARIs are haemoliticus, Streptococcus, Staphylococcus, Pneumococcus, Haemophilus influenzae, Bordetella pertussis, Corynebacterium diphtheriae (Achmadi, 2004). One

treatment of respiratory diseases is done by administering the antibiotic tetracycline. But the resistance was reported to have occurred haemoliticus Streptococcus, Staphylococcus, and Corynebacterium diphtheriae to the antibiotic tetracycline (Chopra, 2001).

In previous studies, it has acquired resistance data of clinical isolates from the oral swab with ARIs to some antibiotics used to treat ISPA in Puskesmas Tasikmalaya. The resistance data are resistant to cefadroxil 70.25%; 68.03% were resistant to amoxicillin, and 43.03% were resistant to ciprofloxacin (Alfaeira et al, 2016).

MATERIALS AND METHODS

Tools
The tools used in this study is an autoclave (Hirayama), micropipette mL volume 5-1000 (Eppendrof), tip micropipette, uv-visble spektrotometer (SPECORD 200-1510), glass vials, ultrasonic bath (NEY-1510), a glass vial of 10 mL, 100 mL brown bottle and glass tools commonly used in the Laboratory Analysis.

Test Materials
Materials tested were Tetracycline from PT. AFI Farma used in community health center in Tasikmalaya, West Java, Indonesia. HCL p.a (Merck), aqua bidestilation (Ikapharmindo Putramas).

Method
A. Preparation of Tetracycline HCl Standard Solution
Weighed approximately 25 mg of tetracycline HCl Baku, put in a 50 mL volumetric flask. Thereafter, 0.1N HCl is added until reaching half the volume of the flask, then sonicated for 5 minutes. Once dissolved, add it back 0.1N HCl to the mark, shaken homogeneous, in order to obtain a standard solution tetrasilklin HCl with a concentration of 500 μg / mL (LS I). From this solution, as many as 5 mL pipette, put in a 50 mL volumetric flask, then diluted with 0.1N HCl to mark boundaries in order to obtain a concentration of 50 μg / mL (LS II) (Nofita et al, 2016).

B. Determination of Wavelength Maximum
Pipette 5 mL LS II, put in a 25 mL volumetric flask. Then add 0.1N HCl to mark boundaries, shaken homogeneous, in order to obtain a solution with a concentration of 10 mg / mL. Thereafter, absorbance was measured at a wavelength of 200-400 nm (Nofita et al, 2016).

C. Determination of a Standard Curve
Solution of tetracycline hydrochloride (50 mg / mL) was taken and put into a 25 mL volumetric flask with -- volume variations as follows:

Table 1: Variations in The Concentration on Standard Curve.

No.	Volume taken (mL)	Solution Concentration (μg/mL)
1.	1	2
2.	2,5	5
3.	5	10
4.	6,25	12,5
5.	7,5	15

- In each flask, add 0.1N HCl to the mark.
- The absorbance was measured at a wavelength of 271 nm.

D. Method Validation
1. Linearity Test
From the standard curve which has been obtained, the calculated value of the correlation coefficient (r2) where the correlation coefficient showing linearity value. Linearity parameters stated in the correlation coefficient value is more than 0.99 at a minimum of five points concentration variations (ICH, 1994).

2. Precision Test
Made standard solution with 5 variations of concentration is 2 mg / mL, 5 mg / mL, 10 mg / mL, 12.5 mg / mL, and 15 mg / mL. Furthermore, each of these concentrations measured absorbance at 271 nm wavelength. 0.1N HCl is used as a blank. Precision expressed as relative standard deviation or coefficient of variation. Parameters acceptable precision of <2% (Harmita, 2004).

3. Accuracy Test
Made standard solution with 5 variations of concentration is 2 mg / mL, 5 mg / mL, 10 mg / mL, 12.5 mg / mL, and 15 mg / mL. Furthermore, each of these concentrations measured absorbance at 271 nm wavelength. Accuracy is obtained through the% recovery (% recovery) by the equation:
% Recovery = (levels of analysis results) / (actual content) x 100%

Parameters required accuracy is 80-110% (Harmita, 2004).

4. Determining LOD and LOQ
From the standard curve which has been obtained, calculated the amount of analyte smallest detectable (LOD) by the equation:

$$LOD = \frac{3\left(\frac{Sx}{y}\right)}{slope}$$

Besides LOD, also calculated the smallest quantity of analyte can still be detected (LOQ) by the equation:

$$LOQ = \frac{10\left(\frac{Sx}{y}\right)}{slope}$$

E. Assays Tetracycline HCl Capsules.
Weighed and mixed the contents of 6 capsules. After that, the samples were weighed tetracycline HCl equivalent to 25 mg tetrasikin raw HCl. Tetracycline HCl was weighed and then added to 50 mL volumetric flask. Thereafter, 0.1N HCl is added until reaching half the volume of the flask, then sonicated for 5 minutes until completely dissolved. Thereafter, absorbance was measured at a wavelength of 271 nm.

RESULT AND DISCUSSION

A. Determination of Wavelength Maximum
Tetracycline standard solution 10 mg / mL was observed absorbance at the wavelength of 200-400 nm with a UV-Vis spectrophotometer. The spectrum measurement results of the maximum wavelength of raw tetracycline with UV-Vis spectrophotometer can be seen in Figure.1.
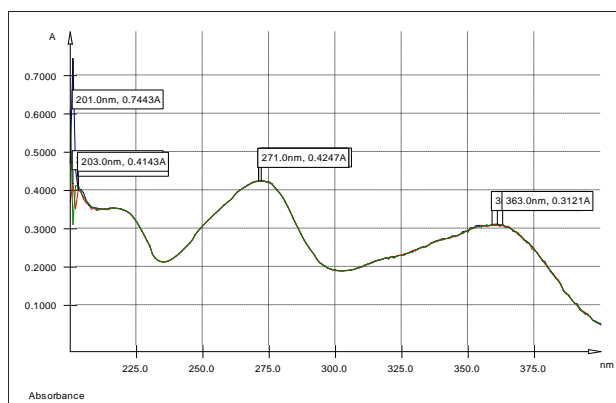
Figure 1: Determination of the Maximum spectrum Wavelength.

Based on the spectrum of the above, it can be seen that there are three peaks, namely at a wavelength of 203 nm, 271 nm, and 363 nm. From the top of the highest peak occurs at a wavelength of 271 nm with the absorbance value of 0.4247. This result is almost the same as the wavelength of tetracyclines according to the literature. Tetracycline has a maximum absorption in an acid solution at a wavelength of 270 nm (Moffat, 2004).

B. Determination of a Standard Curve
Preparation of standard curve aims to determine the relationship between the concentration of the solution with absorbance values so that concentration of the sample can be determined and the sample concentration can be calculated. Standard curve was made by plotting the concentration of standard solution as the x-axis and absorbance as the y-axis. Preparation of standard curve using five variations in the concentration is 2 mg / mL, 5

mg / mL, 10 mg / mL, 12.5 mg / mL, and 15 mg / mL. Tetracycline standard curve is shown in Figure. 2.
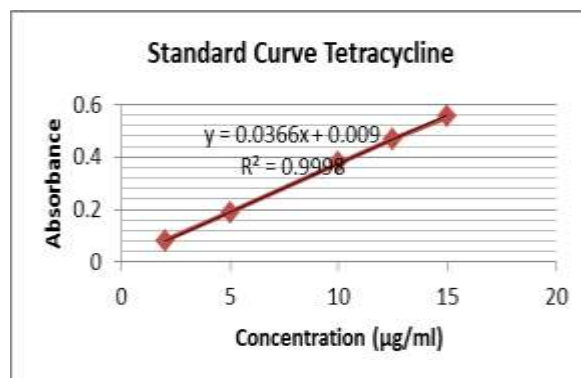

Figure 2. Standard Curve Tetracycline.

The standard curve obtained by a linear regression equation y = 0,0366x + 0.009.

C. Method Validation
1. Linearity Test
The linear regression equation obtained from the standard curve is y = 0,0366x + 0.009 with R^2 = 0.9998. Correlation coefficient meets the requirements is greater than 0.99 (ICH, 1994). Correlation coefficient very close to 1 indicates a highly linear relationship between the concentration of the samples by absorbance, which means the line equation can be used for sample measurements (Harmita, 2004).

2. Precision Test
Precision calculation results can be seen in Table 1.

Table 1: Precision Test Calculation Results.

Concentration (µg/mL)	Absorbance	Absorbance Mean	SD	SBR (%)
2	0,0871 0,0819 0,0789	0,0826	0,00415	0,20606
5	0,1892 0,1951 0,1841	0,1895	0,00552	0,11199
10	0,3747 0,3806 0,3770	0,3775	0,00296	0,02936
12,5	0,4654 0,4683 0,4734	0,4690	0,00404	0,03213
15	0,5535 0,5616 0,5492	0,5548	0,00631	0,04232
Mean				0,08437

3. Accuracy Test
Accuracy is expressed as a percent (%) recovery. The concentration used to test the accuracy of which is 2 mg /

mL, 5 mg / mL, 10 mg / mL, 12.5 mg / mL, and 15 mg / mL. The accuracy of calculation results can be seen in Table 2.

Table 2: Results Calculation Accuracy.

Concentration (µg/mL)	Absorbance Mean	Concentration Results (µg/mL)	Concentration Mean (µg/mL)	Recovery (%)
2	0,0871 0,0819 0,0789	2,169 2,026 1,942	2,012	100,607
5	0,1892 0,1951 0,1841	5,006 5,169 4,863	4,930	98,610
10	0,3747 0,3806 0,3770	10,159 10,322 10,223	10,067	100,671
12,5	0,4654 0,4683 0,4734	12,679 12,757 12,9	12,569	100,554
15	0,5535 0,5616 0,5492	15,126 15,351 15,006	14,912	99,415
Mean				99,971

In this study, the average % recovery of 99.971%. These results meet the required criteria, ie 80-110% (Harmita, 2004).

4. Determining LOD and LOQ.
In this study, the LOD value of 0.9961 mg / mL and the LOQ values of 3.3203 mg / mL. Table calculation of LOD and LOQ can be seen in the table 3.

Table 3: Calculation Result LOD and LOQ.

Concentration (µg/mL)	Absorbance	Absorbance Mean (Y)	Yi	Y - Yi	$(Y-Yi)^2$
2	0,0871 0,0819 0,0789	0,0826	0,0732	0,0094	0,00009
5	0,1892 0,1951 0,1841	0,1895	0,183	0,0065	0,00004
10	0,3747 0,3806 0,3770	0,3775	0,366	0,0115	0,00013
12,5	0,4654 0,4683 0,4734	0,4690	0,4575	0,0115	0,000013
15	0,5535 0,5616 0,5492	0,5548	0,549	0,0058	0,00003
Jumlah					0,00043
SY					0,01195
LOD (µg/mL)					0,9961
LOQ (µg/mL)					3,3203

D. Assays Tetracycline HCl Capsules.
Tetracycline assay results of samples obtained from the calculation is 102, 76%. These levels meet the levels required by the Indonesian Pharmacopoeia IV that is 90-125% (DepKes, 1995).

CONCLUTION

Levels of antibiotic Tetracycline samples used in Tasikmalaya City Health Center is 102.7621%.

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