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LEVOFLOXACIN ANTIBIOTIC TEST USED IN TASIKMALAYA CITY HEALTH CENTER AS QUALITY EVALUATION ON ANTIBIOTIC RESISTANCE CASE

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ABSTRACT

Objective: Acute respiratory infections (ARIs) is an acute inflammation of the upper and lower respiratory tract caused by infection of microorganisms or bacteria, viruses, without or with inflammation of the lung parenchyma. The use of antibiotics is one way to overcome the disease ARIs. However, over time many occur resistant to the antibiotic. The purpose of this study was to determine the effectiveness of levofloxacin antibiotics to treat respiratory syndrome chemically that is by testing the levels of antibiotic drugs as a quality requirement of a drug. **Methods:** Drug sample obtained from health center of tasikmalaya city. The sample is a levofloxacin antibiotic used to treat the ARIs case. Tests carried out using UV-Visible spectrophotometer instrument, with validation parameters: linearity, precision, accuracy, Limit of Detection (LOD) and Limit of Quantification (LOQ). **Result:** The test results showed that the levels of levofloxacin antibiotics that are used to meet the requirements that exist in the Indonesian Pharmacopoeia grading 100,6%, and the validation parameters that meet the requirements, koefesisen correlation of 0.9969, accuracy 97.64%, precision 0.071%, LOD 15.88 and LOQ 52.95

KEYWORDS: Levofloxacin, UV-Visible Spectrophotometer, Validation Parameters, Indonesia Pharmacopoeia.

INTRODUCTION

Acute Respiratory Infection (ARIs) is an acute upper or lower respiratory inflammation caused by microorganism infection or bacteria, virus, without or with lung parenchymal inflammation (Alsagaf, 2009). ISPA is one of the main causes of death in children under 5 years but diagnosis is difficult to enforce (Depkes RI, 2002). Based on data from the WHO and the Ministry of Health of the Republic of Indonesia in 2008, one of ARIs is the main cause of death in children under five are pneumonia (Nasution et al., 2009).

ARIs can be caused by bacteria, viruses and rickets such as Streptococcus genus, Staphylococcus, Pneumococcus, Hemophilus, Bordetella, and Corynebacterium. Virus causes include 9 groups Mexovirus, Adenovirus, Coronavirus, Pikornavirus, Mikoplasma, Herpesvirus, and others (Depkes RI, 2000).

Based on previous research, there has been resistance of some antibiotics used to treat ARIs in Tasikmalaya City health center. The resistance data is 70.25% sefadroksil; 68.03% amoxicillin, and 43.03% resistant to ciprofloxacin (Alfaeira et al, 2016). In this study will be tested the accuracy of Levofloxacin levels which is also an important data to determine the quality of antibiotics used in the health center of Tasikmalaya City. The requirements of antibiotic levels should be in accordance with government regulations contained in Pharmacopoeia Indonesia.

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), ultrasonic bath (NEY-1510).

Materials

Materials tested were Levofloxacine from PT. Sanbe Pharmaceutical Company used in Community Health Center in Tasikmalaya, West Java, Indonesia. HCL p.a (Merck), aqua bidestilation (Ikapharmindo Putramas).

Method

A. Preparation of Levofloxacin Standard Solution

100 µg / mL standard levofloxacin stock solution: 10 mg levofloxacin was dissolved in 100 mL of aquabidestilate (Nur, 2010).

B. Determination of Wavelength Maximum

Determination of the maximum wavelength done by measuring the standard solution of levofloxacin 10 µg/mL were prepared from stock solutions of levofloxacin 60 µg/mL. Solution of maximum absorbance is observed using a UV-Vis spectrophotometer.

C. Method Validation

Validation of the analytical methods used consisted of a test of linearity, limit of detection (LOD), Limit of Quantification (LOQ), precision, and accuracy.

1. Linearity Test

Linearity test is done by calculating the correlation coefficient (r2) with a linearity parameter whose value > 0.99 at least four different concentrations in the range of 50-150% of the content of the analyte in the sample (Harmita, 2004).

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:

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

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

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

D. Assays Levofloxacin

Samples are prepared and weighed as much as 10 mg and dissolved in aquabidestilata up to 100 mL. The solution is taken 1 mL and added with 0.5 mL plasma, then stirred with vortex for 3-5 seconds. The solution was added TCA 10% of 5 mL and centrifuged at 2500 rpm for 15 min. Clear filtrate is separated for absorbance measured at λ max.

RESULT AND DISCUSSION

A. Determination of Wavelength Maximum

Determination of the maximum wavelength needs to be done before determination of antibiotics. It is intended to look at the value of absortivitas which provides the highest measurement sensitivity. Determination the maximum wavelength is done by making the stock standard solution 100 ppm. Standard solution with a concentration of 20 ppm and then measured the absorbance maximum in the wavelength range 200-400 nm. Spectrum results the maximum wavelength measurements with the standard solution of levofloxacin.

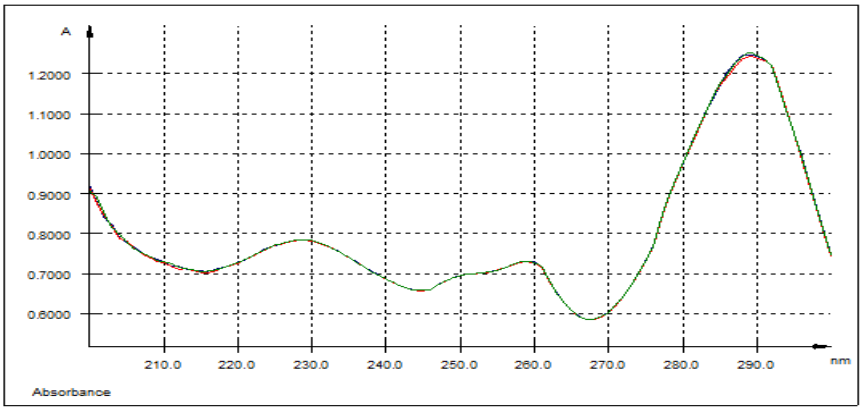

Figure 1: The maximum wavelength spectrum of the standard levofloxacin solution.

The measurement results show that the maximum wavelength of levofloxacin is 289 nm. Wavelengths obtained in accordance with the literature (USP, 2014).

B. Determination of a Standard Curve

The standard curve aims to obtain the equation of standard solution in determining the sample rate. This curve is the relationship between absorbance and concentration, that is by plotting the concentration of standard solution as x axis and absorbance as the y axis. The concentration used is 5, 7.5, 10, 15 and 20 ppm and measured the abortance at 289 nm wavelength. The standard curve of levofloxacin can be seen in Figure 2.

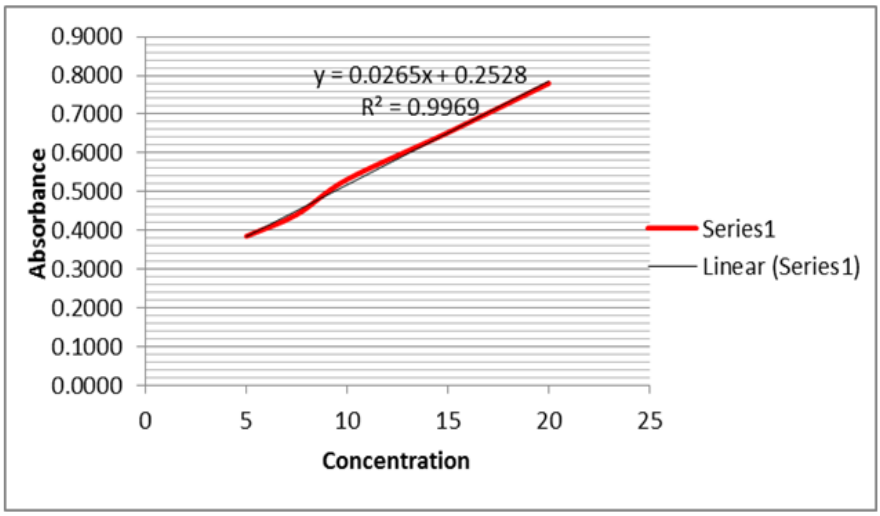

Figure 2: Standard curve lefofloxacin.

The obtained line equation is y = 0.0265 x + 0.2528 with R2 = 0.9969. The above standard curve corresponds to Lambert-Beer's law stating that the intensity passed by the sample solution would be directly proportional to the concentration of the solution and inversely proportional to the transmittance (Day 2002).

C. Method Validation

Validation of analytical methods performed to prove that certain parameters still meet the requirements for its use. The parameters tested were linearity, accuracy, precision and LOD and LOQ.

1. 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 5 ppm; 7.5 ppm; 10 ppm; 12.5 ppm; and 15 ppm. The linearity of the method can describe the precision of the analysis of a method shown by the coefficient of determination of > 0.99 (Chan, 2004). The equation of a straight line obtained standard curve is y = 0.0265 x + 0.2528 with R2 = 0.9969. Correlation coefficient obtained meets the requirements (Chan, 2004). R value close to 1 proved that the regression equation is linear and small standard deviation indicates a fairly high accuracy (Andayani, et al, 2008).

2. Accuracy Test

Accuracy indicates the degree of closeness of the results of a series of measurements obtained from a homogeneous sample under specified conditions (ICH, 1995). Accuracy expressed as a percent recovery (recovery) the analyte is added. Testing is done by five different of concentration is 5 ppm; 7.5 ppm; 10 ppm; 12.5 ppm and 15 ppm. Calculation% recovery can be seen in Table 1.

Table 1: Precision test calculation results.

Concentration	Average Absorption	Concentration Results	Average	% Recovery
5	0.3858	10.296	10.274	105.4888
	0.3794	10.119		
	0.3899	10.408		
7.5	0.4386	11.739	11.801	127.3527
	0.4383	11.730		
	0.4459	11.936		
10	0.5277	14.173	14.282	122.8172
	0.5386	14.469		
	0.5288	14.203		
15	0.6490	17.485	17.587	117.2475
	0.6501	17.516		
	0.659	17.760		
20	0.7783	21.019	21.062	105.3096
	0.7895	21.324		
	0.7718	20.842		
	Average			97.6431

The average value of recovery% is 97.6431%. This result is acceptable because it is still within the required range 80 - 110% (Harmita, 2004).

3. Precision Test

Precision is a measurement repeatability of analytical methods derived from multiple measurements on the same sample (Gandjar and Rohman, 2007). Precision is measured as the standard deviation or relative standard deviation (coefficient of variation) (Harmita, 2004). According to Sherri (2008) precision test criteria can be distinguished as follows:

Table 2: Criteria of precision test.

% SBR	Kriteria
<1	very precise
1-2	precise
2-5	midle
>5	not precise

The concentration of the test used is 5 ppm; 7.5 ppm; 10 ppm; 12.5 ppm and 15 ppm. The test results are shown in Table 3.

Table 3: Results of accuracy test.

Concentration	Average Absorption	Yi	SD	Concentrattion Results	RSD (%)
5	0.3858	0.3850	0.0053	5.894	0.0904
	0.3794				
	0.3899				
7.5	0.4386	0.4409	0.0043	6.774	0.0631
	0.4383				
	0.4459				
10	0.5277	0.5317	0.0060	8.247	0.0723
	0.5386				
	0.5288				
15	0.6490	0.6526	0.0055	9.732	0.0565
	0.6501				
	0.659				
20	0.7783	0.7799	0.0089	12.258	0.0728
	0.7895				
	0.7718				
Average					0.0710

The RSD percentage of 0.0710% indicates that this method has a high degree of accuracy for sample testing.

4. 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 15.88 mg / 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 (Riyanto, 2014). Values obtained quantification limit was 52.95 mg / mL. LOD and LOQ calculation results can be seen in Table 4.

Table 4: Calculation Result LOD and LOQ.

Concentration	Absorbance	Average Absorption	Yi	Y-Yi	$(Y-Yi)^2$
5	0.3858	0.3850	0.1830	0.2020	0.0408
	0.3794				
	0.3899				
7.5	0.4386	0.4409	0.2745	0.1664	0.0277
	0.4383				
	0.4459				
10	0.5277	0.5317	0.3660	0.1657	0.0275
	0.5386				
	0.5288				
15	0.6490	0.6527	0.5490	0.1037	0.0108
	0.6501				
	0.659				
20	0.7783	0.7799	0.7320	0.0479	0.0023
	0.7895				
	0.7718				
Total					0.0218
SY					0.1906
LOD µg/mL					15.8862
LOQ µg/mL					52.9540

D. Assays Levofloxacin

Determination of levofloxacin antibiotic sample level was done by UV spectrophotometric method. The average absorbance obtained from the measurement of 10 µg / mL levofloxacin antibiotic was 0.5204. The absorbance value is inserted into the equation y = 0.0265x + 0.2528 and obtained sample concentration 10,060 µg / mL. Levels of antibiotic levofloxacin samples obtained from the calculation of 100.60%. The results of levofloxacin level measurement meet the requirements listed in USP that is 90% -110% (USP, 2014).

CONCLUTION

Levels of antibiotic levofloxacin used in Tasikmalaya City Health Center is 100.60%. Results are still within the range required by the USP 97% -120% (USP, 2014).

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