Validation Of Analytical Method For Determining Ciprofloxacin's Level In Blood Using High Performace Liquid Chromatography (HPLC).

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ABSTRACT

Ciprofloxacin is a broad-spectrum antibiotic, often used empirically for infection. Ciprofloxacin is an antibiotic whose activity depends on levels in blood (concentration depends), potential effect of ciprofloxacin will be optimal when ciprofloxacin's levels in blood is high compared with its MIC. To measure the level of ciprofloxacin in the patient's blood required a simple and accurate method of analysis. This study aims to validation of analysis method for determining level of ciprofloxacin. This study use of mobile phase 2% acetic acid and acetonitrile (84: 16). HPLC conditions include HPLC devices, YMC C18 column (4,6 x 150 mm: 5 μm), UV detector 280 nm and flow rate is 1,0 mL / min. Validation parameter in this study are selectivity, accuracy, linearity, LOD and LOQ. The results of analysis method for measure levels of ciprofloxacin is selective (α > 1,9177) at time 3,845 minute. The HPLC method of ciprofloxacin meets the acceptability criteria of %recovery value is 96,9%. Eligible precision test % RSD is <3,5%. The value of r^2 on the linearity is 0.9989. LOD value is 1,1 μg/mL and LOQ value is 3,6 μg/mL.

Keywords: ciprofloxacin, HPLC, validation

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INTRODUCTION

Validation in the process to proof that any procedures, processes, equipment, materials or systems used consistently deliver results as expected. Validation of analytical methods is very important for pharmaceutical industry therefore it is important to validate the ciprofloxacin's analytical method. This study aimed to determine the validity of an analytical method. Ciprofloxacin is an antibiotic that is classified as fluoroquinolones, ciprofloxacin works by inhibiting reproduction and repair if genetic material or DNA bacteria (Ali et al., 2011, Katzung et al., 2012). Ciprofloxacin is one antibiotic that has post antibiotic effect (PAE), because they were able to effect even though the levels in the blood is below MIC for 6-8 hours. Therefore, these antibiotics are given in large doses but rarely. Ciprofloxacin is optimal if levels in the blood higher than its MIC value, generally the ratio of Cmax/MIC = 5-10 times. If the ratio is lower than that value then it is possible bacteria will develop into resistant. Analytical of ciprofloxacin's level using HPLC instrument (Mukti et al., 2011, Hakim, 2012)

MATERIALS AND METHOD

Material

Equipment and materials

HPLC instrumentation, YMC C18 column (4,6 x 150 mm : 5 µm), 280 nm UV detector, ohaus's digital analytical balance sheet, ultrasonic bath, pH meter, filtration unit for HPLC, and glassware. The materials used in this study include comparative standards of ciprofloxacin, methanol for HPLC (Merck), sterile water (PT. otsuka), acetonitrile for HPLC (Merck), and KH2PO4

Methods

Determination of retention time and selectivity of ciprofloxacin

Standard solution of Ciprofloxacin 100 ppm was prepared in acetic acid and acetonitrile solvent (84 : 16). Standard solution of Ciprofloxacin 100 ppm is read for retention time with HPLC. Determination of retention time was performed by looked at chromatogram of ciprofloxacin solution 100 ppm.

Selectivity of method is measured by coefficient of selectivity, which is obtained from retention time of ciprofloxacin comparison with retention time of nearest endogenous compound owned by ciprofloxacin in one chromatogram, that is resulted from analysis of blood sample. Retention time of ciprofloxacin is used based on the results of previous determination.

Determination of criteria for accuracy

Blank plasma prepared 3 samples, samples were dispersed with standard solution of ciprofloxacin, so that concentration of ciprofloxacin in plasma to be 5; 10; and 25 µg/mL, replicated for each concentration by 6 times. Acetonitrile added in order to precipitate plasma protein, 250 µL plasma plus internal standard solution and add 150 µL of acetonitrile. Mixture is homogenizedby vortex and centrifuge 15 minutes at 12.000 rpm, 200 µL taken and then transferred to bottle for HPLC analysis, inject 10 µL into HPLC.

Determination of precision criteria

Determination of precision criteria using the same solution is used in determining the criteria of accuracy (Synder et al., 2010). Precision is expressed as SD or relative standard deviation (RSD) of the data series (EMA, 2011).

Relative standard deviation is a measure of relative accuracy and is commonly expressed as a percent. The smaller RSD value of a series of measurement, the method used increasingly appropriate. Test with HPLC typically for RSD values range from 1-2% is required for large amounts of active compounds, while for compound with a small amount, RSD range from 5-15% (Gandjar & Rohman, 2010).

Determination of sensitivity criteria

Sensitivity of an analytical method is known by calculating the smallest range of limits that can be determined for a quantitative analysis known as Limit of Detection (LOD). LOD is a parameter for determination of a sample with smallest concentration but still gives different detector response to the comparison (without sample). Limit of Quantification (LOQ) is the smallest level of sample that can be analyzed which shows adequate accuracy. Lower Limit of Quantification (LLOQ) is the smallest level of samples that can be quantified with precision and accuracy (EMA, 2011). The limits of detection and quantification obtained through equation of linear regression that obtained from determination of standard curve.

Determination of linearity criteria

Blank plasma prepared 7 samples. Sample were dispersed with standard solution of ciprofloxacin concentration to obtain concentration 5; 10; 12; 16; 25; 40,6; 50; and 64,8 µg/mL. Spiking results were prepared and injected into HPLC, recorded the area of ciprofloxacin on each sample's chromatogram. The Calibration curve is made between concentration with the average area of each concentration, then determine the linearity along with the regression equation of the concentration with the area.

<div align="center">

RESULTS AND DISCUSSION

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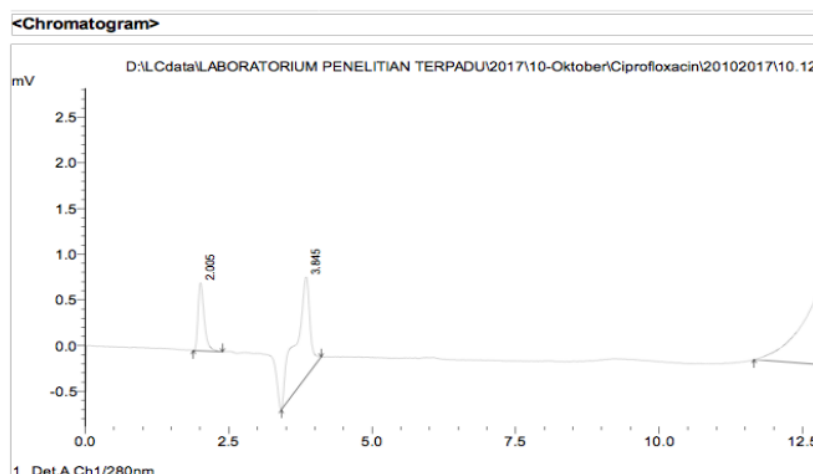**Retention time and selectivity of ciprofloxacin**

Figure 1: Chromatogram of standard ciprofloxacin

The analytical method is expressed selectively as indicated by a single peak on chromatogram, there is no other peak around the retention time of ciprofloxacin. Retention time is 3,845 minutes.
Selectivity test was conducted in this study to know that the analytical method used could detect ciprofloxacin selectively. Based on calculation, obtained value α 1,9177, the results indicate that analytical method used has met the criteria selectivity ($\alpha > 1$).

Accuracy test

Accuracy test is done by calculating % recovery value. Based on calculation results, accuracy value is 96,9%. The validation results show that recovery value is more than 90% and systematic error is less than 10%. The results indicate that method used in this study is considered to be accurate (Hakim, 2011).

Precision test

Based on calculation results, obtained RSD value is not more than 15% that is equal 3,1%. The results indicate that method used is considered to provide eligible precision criteria.

Linearity test

Subsequently made calibration curve in order to obtain the linear equation y = bx + a and calculated the correlation coefficient value (r). Based on linearity test results obtained linear equation y = 12545x – 10071 with r value of 0,9989. Linearity test result qualified acceptance linearity correlation coefficient 0,999 (Mukti et al., 2016).
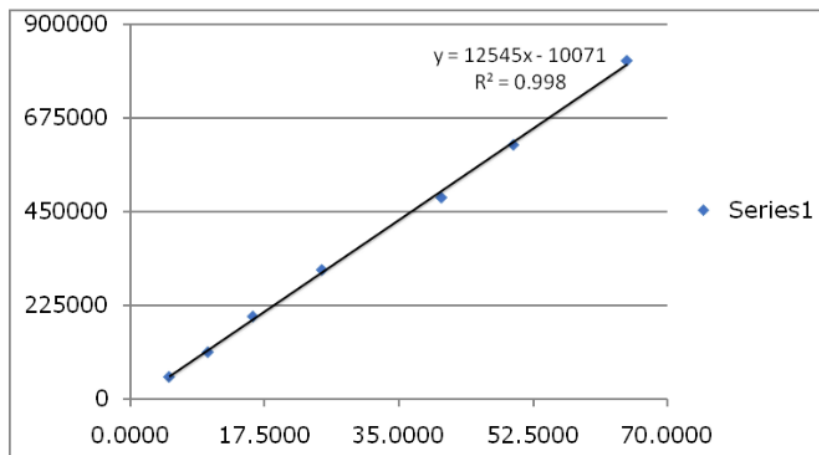

Figure 2: Linearity test curve of ciprofloxacin

LOD and LOQ test

LOD is the lowest concentration of a sample that can still be detected by analytical method, but not necessarily quantified as the right value. In the study obtained LOD value 1,1 µg/mL. LOQ is the lowest sample concentration that can still be detected quantitatively with accurate and acceptable precision. In the study obtained LOQ value 3,6 µg/mL.

CONCLUSION

The results of %recovery value is 96,9%; % RSD of 3,1%. The linear equation y = 12545x – 10071 with r value 0,9989. LOD value is 1,1 µg/mL and LOQ value is 3,6 µg/mL. The analytical method for determination pharmacokinetics profile of ciprofloxacin by HPLC qualified validation parameters of selectivity, accuracy, repeatability precision, linearity, LOD and LOQ.

ACKNOWLEDGEMENTS

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