DEVELOPMENT AND VALIDATION METHOD FOR QUANTITATIVE DETERMINATION OF CIPROFLOXACIN IN HUMAN PLASMA AND ITS APPLICATION IN BIOEQUIVALENCE TEST

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ABSTRACT

Objective: This study aimed to develop and validate an high-performance liquid chromatography method coupled with an ultraviolet detection for determination of ciprofloxacin in spiked human plasma and subsequently used for bioequivalence (BE) study.

Methods: The chromatographic separation was achieved on an RP-C18 column (Lachrom Hitachi, 250×4.6 mm, 5 μm), utilizing a mobile phase of phosphate buffer/acetonitrile (87:13, v/v, pH 3.0±0.1) at a flow rate of 1.5 ml/minutes. Detection is carried out at 276.6 nm using a spectrophotometer. The developed method is statistically validated for the linearity, accuracy, limit of detection (LOD), limit of quantitation, precision, and specificity. The specificity of the method is ascertained by comparing chromatograms of blank plasma and plasma containing ciprofloxacin.

Results: Plasma matrix and ciprofloxacin can be separated very well using the validated method. The LOD and limit of quantification (LOQ) were 0.07 μg/ml and 0.26 μg/ml, respectively. The regression curve of the standard was linear (R>0.999) over a range concentration of 0.01-3.00 μg/ml. The mean recovery of the method ranged between 95.27% and 104.45%. Both intra- and inter-day precision data showed reproducibility (relative standard deviation ≤8.0, n=9). The validated method is successfully applied in pharmacokinetic and BE study of ciprofloxacin tablet in a small number of subjects. The mean values of C_{max}, T_{max}, area under curve $(AUC)_{(0-12)}$, and $AUC_{(0-\infty)}$ for the test (generic) formulation of ciprofloxacin were 2.25±0.29 μg/ml, 1.0 hr, 8.60±0.69 μg/ml/h, and 9.52±0.70 μg/ml/h, respectively. Meanwhile, for the reference formulation (ciproxin tablet), the values were 2.30±0.27 μg/ml, 1.0 hr, 8.54±0.85 μg/ml/h, and 9.38±0.89 μg/ml/h, respectively. From BE study, the 90% confidence intervals for the test formulation/reference formulation ratio for the logarithmic transformations of C_{max}, $AUC_{(0-12)}$, and $AUC_{(0-\infty)}$ were within the BE limit of 80-125% (96.08%, 101.00%, and 102.02%, respectively).

Conclusion: According to the validation result, the method was rapid, simple, and reliable. It can be used for routine analysis of ciprofloxacin in human plasma and BE study. The test formulation (tablet "X") met the regulatory criteria for assuming BE in healthy subjects.

Keywords: Validation, High-performance liquid chromatography, Ciprofloxacin, Plasma, Bioequivalence.

INTRODUCTION

Ciprofloxacin (Fig. 1a) is one of a fluoroquinolone class of antibiotics that are widely used in the treatment of several bacterial infections [1,2]. Ciprofloxacin exhibits a broad spectrum antimicrobial activity against Gram-negative and Gram-positive bacteria [3]. Ciprofloxacin has low toxicity and potential for used as oral therapy in urinary tract as well as skin and soft infections [4].

Ciprofloxacin is rapidly dissolved in the gastrointestinal tract and well absorbed in duodenum and jejunum. Like other fluoroquinolone, it is both metabolized and excreted unchanged in the urine [5,6]. Oral bioavailability is approximately 70%, and a maximum concentration ranging from 1.5 to 2.5 μg/ml is achieved 1-2 hrs after a dose of 500 mg by oral. Area under the serum concentration curve (AUC) range from 6.8 to 12.7 μg/h/ml with an elimination half-life ranging from approximately 3-5 hrs [7].

Various methods have been developed for the determination of ciprofloxacin individually using spectrophotometer [8], spectrofluorometer [9,10], flow injection analysis [11], or high-performance liquid chromatography (HPLC) method that can be found in British Pharmacopeia [12], United States Pharmacopeia [13], and Indonesian Pharmacopeia [14]. In these pharmacopeias, HPLC methods were officially method for the determination of ciprofloxacin as alone. Many applications of HPLC method have been reported in the literature for determination ciprofloxacin in pharmaceuticals preparations [15],

human plasma [6,16], and other biological fluids such as simultaneous analysis of ciprofloxacin and phenazopyridine in solid dosage form using isocratic RP-HPLC that reported Pola and Sankar [17]. In addition, Sachan *et al.* (2010) have reported simultaneous estimation of ciprofloxacin hydrochloride and ofloxacin by reverse phase-HPLC (RP-HPLC) [18]. Some of HPLC methods that involved the use ion-pair reagent to get better peak shape have been also reported in the literature [19,20].

The current study was devised to develop and validate the HPLC method for ciprofloxacin determination in human plasma. The developed method is expected tobe simpler, faster, and more reliable than previously published methods. Validation will be conducted in accordance with ICH harmonization for validation of analytical procedure ICH Q2 (R1) [21]. The validated method is then applied to quantify ciprofloxacin in healthy volunteers human plasma for pharmacokinetic purposes as well as for bioequivalence studies of a generic tablet found in Indonesia. Bioequivalence (BE) study should be done to prove equality between copy or generic drug to the innovator drug and considered to be inter changeable. This is important to improve the protection of the public from drug products that risk to the health and ensure the quality, efficacy, and safety drugs in the market, specially ciprofloxacin drug [22]. The BE study can be a test of pharmacokinetic, pharmacodynamics test, or comparative clinical trials depend on whether the drugs work systemically or locally. National Agency of Drugs and Foods Controls in Indonesia or Badan Pengawasan Obat dan Makanan Indonesia has released guideline for the conduct of
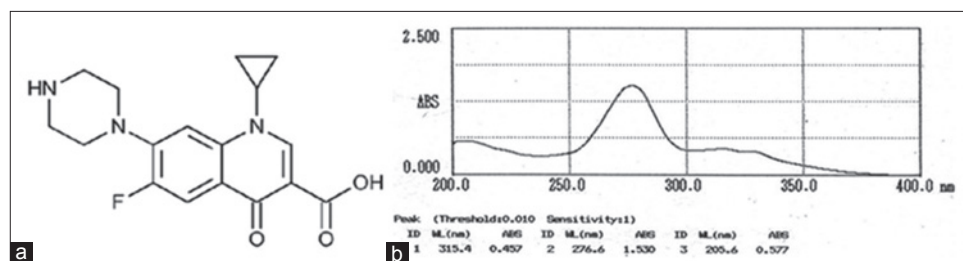
Fig. 1: Structure of ciprofloxacin (a); Ultraviolet spectra of ciprofloxacin (b)

BE study to provide instruction for conducting BE study in accordance with established international standards such as those published by the US Food and Drug Administration (FDA) [23,24] and the European Medicines Agency (EMA) [25,26] as well as ICH harmonization [21]. These guidelines are very important not only for researcher and BE center but also for the evaluators and the pharmaceutical industry [22].

METHODS

Chemical and materials

Ciprofloxacin standard (≥99.0% from Sigma-Aldrich (Dorset, UK), ciprofloxacin generic tablet "X" from Indonesia, ciproxin tablet 500 mg from Bayer. Triethanolamine (TEA), phosphoric acid, and glacial acetic acid are obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile HPLC grade were purchased from Merck (Darmstadt, Germany). Human plasma was from Indonesian Red Cross. HPLC was equipped with ultraviolet (UV)-Vis L-2420, pump L-2130, and Lachrom C18 column (250 mm×4.6 mm; 5 µm) from Hitachi (Japan). Refrigerated centrifuge (Eppendorf), vortex thermolyne 16700 mixer (USA).

Validation of ciprofloxacin analysis in spiked human plasma

Preparation of spiked human plasma

Human plasma 300 µl, spiked with 200 µl of the ciprofloxacin solution (10 µg/ml) and 1000 µl acetonitrile. The mixture is centrifuged with the speed of 10.000 rpm for 10 minutes in 4°C. Supernatant is collected and placed in vial. Plasma is rinsed with 1 ml of acetonitrile and centrifuged again; supernatant is collected and added to the vial which contains the result of the first centrifugation. Supernatant is dried until completely dry. A number of 1.0 ml of solvent is added to the dry extract, then vortexed for 30 seconds and filtered using membrane filter which has pore size 0.2 µm. The filtrate is ready to be injected into HPLC instrument.

Determination of the maximum wavelength of ciprofloxacin

Ciprofloxacin solution in concentration of 20 µg/ml is scanned using the UV spectrophotometer at a wavelength range of 200-400 nm. The maximum wavelength is chosen from the spectrum and used for next analysis.

Optimization of flow rate and mobile phase composition

At the beginning of HPLC system optimization, ciprofloxacin is injected into HPLC and eluted using mobile phase that consisted of phosphate buffer solution 20 mM, pH 3.0±0.1 adjusted with TEA:acetonitrile in ratio (15:85 v/v). The flow rate is controlled in 1.0 ml/minute. Mobile phase composition and flow rate are modified until producing a good performance chromatogram. Detection is conducted using UV-detector at the maximum wavelength. All parameters included retention time (Rt), resolution factor (Rs), tailing factor (Tf), the peak area under curve (AUC), the peak height, height equivalent of theoretical plate, and number of theoretical plates (N) are evaluated to decide which HPLC system can be used for routine analysis.

System suitability tests

Ciprofloxacin solution in concentration of 5 µg/ml is injected into HPLC and eluted using the optimal condition. It was replicated 6 times and calculated relative standard deviation (RSD) of Rt, Rs, AUC, Tf, and N. The parameters evaluated to decide whatever the method is suitable for routine analysis.

Validation of ciprofloxacin determination

Validation method conducted by calculating parameters of validation: Selectivity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), and ruggedness.

The selectivity performed by calculating Rs. The requirement of selectivity parameter is Rs value >2 [27].

Linearity conducted by preparing series concentration of ciprofloxacin standard at the concentration of 0.01, 0.02, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00, and 3.00 (µg/ml). The peak area of each concentration is plotted by concentration to form a linear regression curve. Correlation coefficient (R) and coefficient of determination (R^2) as well as % y-intercept are calculated and evaluated according to acceptance criteria including R≥0.999, R^2≥0.997, and % y-intercept value ≤2.0% [28,29]. The SD of y-intercept is used to measure LOD and LOQ parameters.

Precision performed in three different measurement times by injecting ciprofloxacin that extracted from human plasma for 6 times replication. Then, % RSD was calculated to determine repeatability and reproducibility system. According to AOAC PVM Program, % RSD value for analyte at concentration of 5 µg/ml should be less than 9.36% [30,31].

Accuracy expressed as percentage recovery and determined by standard addition method using three concentration levels which are specific ranges of 80%, 100%, and 120% of the target analyte concentration. Each concentration is injected into HPLC in three times replication. The percentage recovery calculated as the measured value/theoretical value×100%. The used concentrations of ciprofloxacin in human plasma were 1, 2, and 3 µg/ml. Therefore, % recovery value should be in the range 80-110%, whereas for active substance should be 98-102% [30,31].

Ruggedness performed by preparing human plasma containing ciprofloxacin concentration of 1 µg/ml. Plasma filtrate is then injected into difference HPLC instrument from difference laboratories. The data then analyzed by statistical measurement (t-test) to evaluate the ruggedness of the method.

Application in pharmacokinetic and BE test

Study design

The study designed using an open-label, randomized two-way cross over (2 treatments, 2 periods, and 2 sequences) with 7-days washout period between the 2 study arms. Subjects are randomized into two groups. Each subject is assigned a subject number; treatment sequence randomized, and all subjects received the test and reference formulations. The test formulation was generic tablet "X" containing 500 mg ciprofloxacin produced by Indonesian Pharmaceutical Industry. The reference formulation was ciproxin 500 mg (produced by PT. Bayer Indonesia Tbk).

Ethical Clearance (Ref: KE/FK/124/EC; 24 Dec 2013) obtained from Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University. This study is conducted in compliance with the principles of the Declaration of Helsinki. Informed consent signed by all of the subjects after the presentation of verbal and written explanations of the study and before initiation of screening procedures.

Subjects

Healthy volunteers are recruited and assessed for inclusion in the study. A medical history is taken including the recording allergies, illnesses, consumption of alcohol, drugs, and also tobacco. Physical examination is conducted before treatment, including measurement of weight, blood pressure, pulse, respiratory, abdominal. Blood samples (10 ml) collected for full blood count, urea and electrolyte, liver function by determining of protein, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic oxoacetic, and bilirubin. Renal function is also tested by determining of ureum, creatinine, and electrolyte. Urine samples are collected for urine-formed elements with microscopic examination analysis, and urinary pregnancy test is conducted for all the female subjects. All of blood and urine analysis are carried out by Pramitha Clinical Laboratory that already received accreditation by the International Organization for Standardization (Standard No. 15189 and 9001).

Inclusion and exclusion criteria

Subjects were healthy volunteers between the ages of 18 and 55 years that have a body mass index between 18 and 25 kg/m^2. They had passed all screening parameters included physical examination, liver function tests, hemogram, hepatitis B, HIV, and urine analysis. Subjects have no history of allergic responses to ciprofloxacin, a history of drugs alcohol abuse, significant diseases or disorders of liver, cardiovascular, respiratory and kidney or gastrointestinal. Female subjects were not under pregnancy and menstruation during the study. Women who were breastfeeding were also ineligible. All subjects have to sign an informed consent before treatment during the research.

Admission and procedures

Subjects are admitted to the "Rumah Sakit Akademik" Hospital of Gadjah Mada University 1 day before the study. The explanations about the nature and risk of the study are given, and subjects then signed informed consent forms for participation in the study. No other medications or outside foods are permitted. No foods are allowed at least 12 hrs before dosing.

Formulations are given to subjects after 12 hrs fasting and then given 200 ml of water. A light breakfast and lunch are provided after 2 and 6 hrs, respectively. According to the schedule of administration, half subjects received test formulation (tablet "X") and half others subjects received reference formulation (ciproxin tablet). After a washout period of 7 days, the subjects received formulations that different from at first period.

Collection of blood samples

Venous blood samples (3-5 ml) collected in the heparinized tube using wing needle intravenous at 0 hr (shortly before consumption of drug) 0.25, 0.5, 1, 2, 3, 5, 7, 10, and 12 hrs after digestion drugs. After collection, blood samples are immediately centrifuged at 8.000 rpm for 10 minutes. Plasma is separated and placed in vials in duplicate and stored at −80°C until analyzed using HPLC.

Bioanalytical work and statistical analysis

Ciprofloxacin concentration in plasma measured using optimum preparation and HPLC method resulted from validation procedure.

All pharmacokinetic parameters determined by non-compartmental analysis using Pharmacokinetic Functions for Microsoft Excel software. C_{max} and T_{max} are directly read from the concentration-time plots. Elimination rate constant calculated from the linear regression line of the elimination phase.

BE assessment for C_{max}, AUC_{0-12}, and AUC0-∞ are calculated by parametric analysis using paired t-test as well as two-way ANOVA by two-one-sided test at 90% confidence interval (CI). The test formulation (tablet "X") is considered bioequivalent to the standard formulation (ciproxin tablet) if it passes the requirement of 80-125% at CI of 90% for both statistical tests.

RESULTS AND DISCUSSION

From optimization of procedure analysis, the optimum wavelength of ciprofloxacin was 276.6 nm as displayed in Fig. 1b, whereas Table 1 showed the start conditioning of HPLC for ciprofloxacin analysis. Maximum wavelength found in this study was very close to the maximum wavelength in previously publication [32]. Tables 2 and 3 showed the optimization data for flow rate and composition of mobile phase, respectively.

Based on the optimization data (Table 3), the chromatographic condition option 5 is chosen for ciprofloxacin assay in plasma samples for BE study. The optimum condition of HPLC for ciprofloxacin analysis can be summarized as follows in Table 4, while the chromatogram can be found in Fig. 2.

Fig. 2 showed that the Rt of ciprofloxacin was 7.18 minutes, whereas for levofloxacin (as internal standard) was 6.17 minutes. Rt of ciprofloxacin was less than 10 minutes and shorter than previously publications from Vybiralova *et al.* (2005) and Locatelly *et al.* (2015). They stated that the Rts were 14 minutes [33] and 9.19 minutes [34]. Chromatogram Fig. 2 displayed that ciprofloxacin can be perfectly separated from plasma endogenous matrix and internal standard. Plate number and Tf were, respectively, N≥2000 and Tf≤2 that were in agreement with acceptance criteria from Snyder *et al.* [27].

Table 5 displayed parameters of suitability test that meets with validation requirements. Therefore, the optimum HPLC system can be used as routine analysis method for quantification ciprofloxacin in human plasma.

The selectivity parameter (Rs) of ciprofloxacin was 3.4. Based on the Fig. 2, both peak ciprofloxacin and levofloxacin showed that the peaks were symmetric, homogeneous, and there were no co-eluting peaks. It was indicating that the method has high selectivity and can be applied for routine analysis. In addition, the method has a good linearity in the range of 0.01 to 3.00 µg/ml with coefficient of correlation (R) 0.9997, coefficient of determination (R^2) 0.9995, and regression equation Y=101675X−1377.2. The linear regression of calibration curve can be seen in Fig. 3.

Repeatability test is conducted to describe the precision parameter. The precision of method evaluated by performing six replicate analyses of the samples through the complete analytical procedure from sample preparation to the final step. In this case, ciprofloxacin 2 µg/ml (in human plasma) extracted using acetonitrile and subsequently injected into HPLC. Measurement is repeated on three different days and calculated the % RSD. Intermediate precision evaluated by comparing the assays on three different days using different analyst. Acceptance criteria according to AOAC PVM Program was %RSD≤10.59% [30,31]. All of RSD values are in agreement with

Table 1: Starting HPLC condition for ciprofloxacin analysis

Stationary phase	Lachrom Hitachi C18, 250 mm×4.6 mm, 5 µm
Mobile phase	Phosphate buffer solution 20 mM, pH 3.0±0.1 adjusted with TEA: Acetonitrile in ratio (15:85 v/v)
Flow rate of mobile phase	1.0 ml/minute
Volume of injection	20 µL
Detector	UV at 276.6 nm

HPLC: High-performance liquid chromatography

Table 2: Result of the flow rate optimization using mixture of phosphate buffer at pH 3±0.1: Acetonitrile (87:13, v/v)

Flow rate (ml/menit)	Retention time (menit)	Theoretical plate number (N)	Tailing factor (Tf)	Height of equivalent of theoretical plate/HETP (mm)	Peak area (miliarea unit)	Note
1	12.58	11460	1.34	0.002182	6273454	
1.5	7.19	12979	1.31	0.001926	6404905	Optimum
2	2.48	4453	2.54	0.005614	3416720	

HETP: Height equivalent of theoretical plate

Table 3: Result of optimization of mobile phase composition (phosphate buffer at pH 3±0.1 and acetonitrile) with a flow rate of 1.5 ml/minute

Option	Mobile phase composition (phosphate buffer: acetonitrile)	Retention time (menit)	Theoretical plate number (N)	Tf	HETP (mm)	Peak area (miliarea unit)
1	80:20	2.89	4546	1.95	0.0055	5192543
2	83:17	3.57	6632	1.79	0.0037	5868978
3	85:15	4.92	8847	1.63	0.0028	5835432
4	86:14	5.55	9864	1.52	0.0025	5781028
5	87:13	7.19	11460	1.31	0.0022	5915811

Note: Option 5 was the optimum condition. Tf: Tailing factor, HETP: Height of theoretical plate

Table 4: Optimum condition of HPLC for ciprofloxacin in human plasma analysis

Stationary phase	Lachrom Hitachi C18, 250 mm×4.6 mm, 5 µm
Mobile phase	Phosphate buffer solution 20 mM, pH 3.0±0.1 adjusted with TEA: Acetonitrile in ratio (13:87 v/v)
Flow rate of mobile phase	1.5 ml/minute
Volume of injection	20 µL
Detector	UV at 276.6 nm

HPLC: High-performance liquid chromatography

Table 5: Parameters of suitability test of ciprofloxacin analysis using HPLC

Parameters	Acceptance criteria	Replication						Mean	RSD (%)
		1	2	3	4	5	6		
Retention time (menit)	RSD≤1	7.23	7.29	7.24	7.28	7.27	7.29	7.27	0.23
Peak area	RSD≤2	191256	176754	196320	189431	195635	188152	189591	1.95
Tailing factor	≤2	1.22	1.22	1.15	1.16	1.20	1.18	1.19	2.52
Theoretical plate number (N)	>6000	10824	11520	10737	11535	10461	11358	11071	4.11

HPLC: High-performance liquid chromatography, RSD: Relative standard deviation

Table 6: Repeatability parameter of ciprofloxacin analysis using HPLC

Day-1		Day-2		Day-3		Note
µg/ml	RSD (%)	µg/ml	RSD (%)	µg/ml	RSD (%)	
1.92	1.14	1.91	1.28	1.85	0.79	Repeatability
Mean=1.89±0.05						Reproducibility (inter-day precision)
RSD=2.04%						

HPLC: High-performance liquid chromatography, RSD: Relative standard deviation

validation requirement, so this method can be concluded has a good repeatability (Table 6).

To ensure the accuracy and reliability of the method, the recovery study is carried out by adding a known quantity of standard into the sample and then analyzed by the proposed method. Accuracy was measured at three different concentrations. Recovery of analysis calculated by dividing the found concentration with the known concentration that added into the sample. According to the result displayed in Table 7, HPLC method has good accuracy that in line with the requirement of AOAC PVM Program (% recovery with analyte concentration of 1-10 µg/ml should be in 80-110%).

LOD and LOQ measured based on the SD of intercept value (A) and the slope (B) of the calibration curve, using the formulae LOD=3.3

Table 7: Accuracy parameter of ciprofloxacin in human plasma analysis

Concentration (µg/ml)	% recovery	RSD (%)
1	104.45±5.77	5.52
2	96.06±0.36	0.37
3	95.27±1.56	1.63

RSD: Relative standard deviation

SD/B and LOQ=10 SD/B. The results from this research were 0.07 and 0.26 µg/ml for LOD and LOQ, respectively. LOD value obtained in this study is smaller than 1.44 µg/ml the value from Pola *et al.* (2015) [17]. Therefore, the validated method was more sensitive than the previously method.

Table 8: Result of ruggedness test of ciprofloxacin method by using HPLC

Result (µg/ml) from		Significance (P)	Conclusion
UGM Laboratory	BPOM Laboratory		
0.9875	0.9235	0,1776	There are no significant differences between result
1.0128	0.9491		from UGM Laboratory and BPOM Laboratory
1.0154	0.9199		
0.9234	0.9544		
0.9848±0.0428	0.9367±0.0175		

UGM Laboratory is Laboratory in Gadjah Mada University. BPOM Laboratory is Laboratory in National Agency of Drugs and Food Controls in Indonesia. BPOM: Badan Pengawasan Obat dan Makanan, HPLC: High-performance liquid chromatography

Table 9: Pharmacokinetic parameters for ciprofloxacin after administration of test formulation (tablet "X") and reference formulation to healthy subjects

Parameters	Mean±SEM		
	Test formulation	Reference formulation	From literature [35]
T_{max} (hr)	1±0	1±0	1±0.01
C_{max} (µg/ml)	2.25±0.29	2.30±0.27	2.2±0.5
$AUCt_{(0-12)}$ (µg/ml/h)	8.60±0.69	8.54±0.85	
$AUCt_{(0-\infty)}$ (µg/ml/h)	9.52±0.70	9.38±0.89	9.5±1.8
$T_{1/2}$ (h)	3.39±0.11	3.37±0.09	4.4±0.9
Volume of distribution (ml)	146183.53±18344.25	137927.03±18290.99	
Mean residence time (h)	2.59±0.16	2.45±0.13	5.3±1.0
Clearance (l/h)	53900.98±3731.00	55565.96±4766.84	

SEM: Standard error of mean

Table 10: 90% CIs for the mean ratio of the test formulation to reference formulations for C_{max}, AUC_{0-12}, and $AUC_{0-\infty}$ for ciprofloxacin

Parameters	90% CI	Test/reference ratio (%)
$Log10\ C_{max}$	74.08-124.60	96.08
$Log10\ AUC_{(0-12)}$	83.53-122.14	101.00
$Log10\ AUC_{(0-\infty)}$	86.07-120.92	102.02

CI: Confidence interval, Log_{10}: Logarithmic transformation, test formulation: Tablet "X" containing ciprofloxacin 500 mg, reference formulation: Trademark ciproxin tablet 500 mg
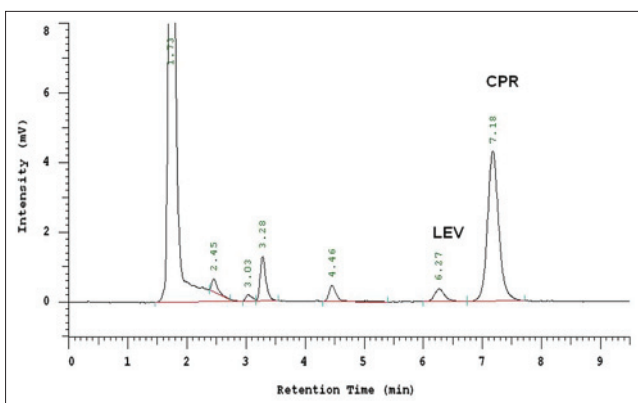

Fig. 2: Chromatogram of ciprofloxacin in human plasma matrix detected using high-performance liquid chromatography with the optimum condition as mentioned in Table 4.

The result of ruggedness test (Table 8) showed that this method was rugged. When the method is applied for ciprofloxacin analysis in two difference laboratories with difference time, person, and equipment, there are no statistically significant differences in results from both laboratories.
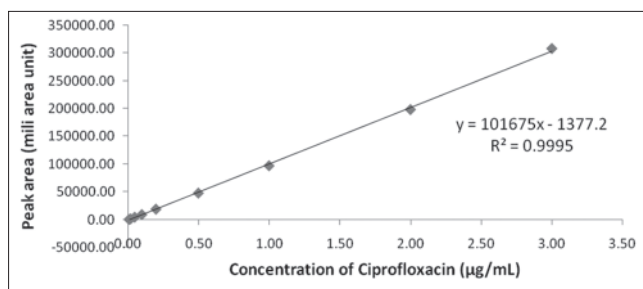
Using validated method from this study, plasma samples from all healthy volunteers are measured to know the pharmacokinetic profile both of tablet "X" (as test formulation) and tablet ciproxin (as reference formulation). Mean concentration-time profile of ciprofloxacin is presented in Fig. 4. The pharmacokinetic parameters of ciprofloxacin, such as AUC, C_{max}, and T_{max} obtained from all subjects, are given in Table 9. As evident from Fig. 4 and Table 9, ciprofloxacin is rapidly absorbed with T_{max} of 1 hr. Other pharmacokinetic parameters of tablet "X" were similar with the parameters of reference tablet. In this study, we also compared these parameters with the results published by Wagenlehner *et al.* (2006) [35]. From the comparison, we can conclude that there are no difference parameters between the result of this study and Wagenlehner's publication.

Table 10 showed the 90% CIs and the mean ratio of the test-to-reference formulation for log_{10} C_{max}, AUC_{0-12}, and $AUC_{0-\infty}$. In this study, the 90% CI for the test formulation/reference Formulation ratio for both log_{10} C_{max} and log_{10} $AUC_{0-\infty}$ were within the BE limit of 80% to 125% (96.08% and 102.02%, respectively). Therefore, the test and reference formulations met the regulatory of FDA, EMA, and Nation Agency for Drug and Food Controls in Indonesia. The test and reference formulations appeared to be bioequivalent.

CONCLUSION

1. HPLC condition for ciprofloxacin analysis consisted of Lachrom Hitachi C18 (250 mm×4.6 mm, 5 µm) as the stationary phase, while as mobile phase was a mixture of phosphate buffer solution 20 mM, pH of 3.0±0.1 that adjusted with TEA:acetonitrile in ratio (13:87 v/v), flow rate of 1.5 ml/minutes, and detected using UV detector at wavelength of 276.6 nm.
2. The validated method successfully applied in pharmacokinetic and BE study of ciprofloxacin tablet in small number of subjects. The mean values of C_{max}, T_{max}, $AUC_{(0-12)}$, and $AUC_{(0-\infty)}$ for the test formulation of ciprofloxacin were 2.25±0.29 µg/ml, 1.0 hr, 8.60±0.69 µg/ml/h, and 9.52±0.70 µg/ml/h, respectively. Meanwhile, for the reference formulation, the values were 2.30±0.27 µg/ml, 1.0 hr, 8.54±0.85 µg/ml/h, and 9.38±0.89 µg/ml/h, respectively. From BE

Fig. 3: Standard curve of ciprofloxacin in human plasma matrix detected by high-performance liquid chromatography
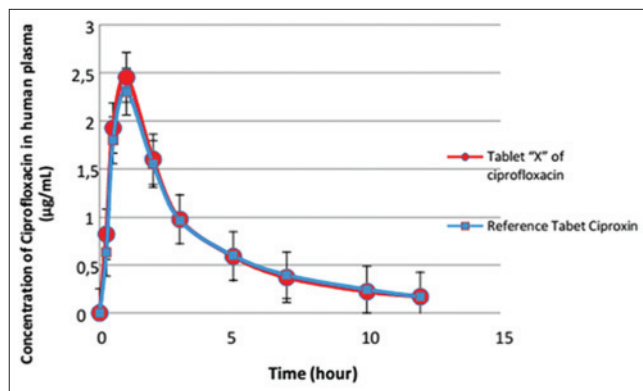

Fig. 4: Mean±standard deviation concentration of ciprofloxacin in human plasma versus time after consuming a single oral dose of ciprofloxacin 500 mg. Tablet "X" was a test formulation and ciproxin 500 mg used as reference tablet

study, the 90% CIs for the test formulation/reference formulation ratio for the logarithmic transformations of C_{max}, $AUC_{(0-12)}$, and $AUC_{(0-\infty)}$ were 75.08-124.60%, 83.53-122.14%, and 86.07-120.92%, respectively. According to the BE test guidelines of the European Commission-EMA, FDA and National Agency for Drug and Food Controls in Indonesia, it can be concluded that the test formulation (tablet "X") met the regulatory criteria for assuming BE in these healthy subjects.

ACKNOWLEDGMENT

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