

BIOEQUIVALENCE AND PHARMACOKINETIC EVALUATION OF ETHAMBUTOL 400 MG FILM-COATED TABLET: A SINGLE-DOSE, OPEN-LABEL, RANDOMIZED, TWO-WAY CROSSOVER DESIGN STUDY

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Abstract

The objective of this study was to determine the bioequivalence of Ethambutol 400 mg Film-Coated Tablet produced by PT Kimia Farma Tbk compared to the WHO-recommended comparator product, Myambutol 400 mg Film-Coated Tablet produced by Pantheon Inc, Ontario, Canada for STI Pharma LLC, in healthy subjects. The study design used was a randomised two-way crossover design, single dose, open label, under fasting conditions. The number of subjects who completed the study was 29 of 32. Blood samples were collected 18 times. Ethambutol concentrations were determined by LC-MS/MS method. Bioequivalence was determined by value of 90% confidence interval (CI) with $\alpha = 5.00\%$ within the range of 80.00–125.00% for AUC and C_{max} . The results of the statistical analysis for the bioequivalence study comparing the test drug to the comparator drug showed a geometric mean ratio (90% CI) of 103.67% (97.23-110.52%) for the area under the concentration-time curve (AUC_{0-t}) and 91.53% (84.80-98.79%) for the maximum concentration (C_{max}). Based on the result, the test drug is bioequivalent to the comparator drug.

KEYWORDS

Ethambutol, AUC, Bioequivalence, Comparator, and Crossover

1. INTRODUCTION

Ethambutol is a bacteriostatic drug, but it exhibits bactericidal properties at higher doses. Ethambutol works by inhibiting RNA synthesis, leading to disruptions in metabolism and cellular multiplication. Ethambutol is effective against organisms such as *M. bovis*, *M. tuberculosis*, *M. marinum*, and some strains of *M. kansasii*, *M. fortuitum*, *M. avium*, and *M. intracellulare* [1]. Ethambutol inhibits arabinosyl transferase (embA, embB, and embC), preventing the formation of arabinogalactan and lipoarabinomannan components in the cell wall and hindering cell division [2]. Ethambutol does not affect other bacteria. This drug is suppressive to the growth of most tuberculosis bacilli resistant to isoniazid and streptomycin. Ethambutol is a specific

bacteriostatic agent against tuberculosis bacilli that are resistant to other antimycobacterial agents [3].

The absorption of Ethambutol in the body ranges from 75%-80%, with peak levels reached within 2-4 hours through the digestive tract. Absorption of the drug may be increased if administered with a high-fat meal [4]. The plasma protein binding of ethambutol ranges from 8-22%. The distribution of ethambutol in the body, including body fluids, is very extensive. It will be contained in high concentrations (red blood cells, kidneys, lungs, and saliva) or in low concentrations (ascitic fluid, pleural fluid, brain, and cerebrospinal fluid). Ethambutol undergoes oxidation in the liver to form an aldehyde, followed by conversion into a dicarboxylic acid [4]. Approximately 50% of ethambutol elimination occurs through urine, with about 8-15% as unchanged drug and 20-22% as metabolites. The remaining elimination occurs through feces, where about 20-22% is eliminated in its original form. The half-life after a single oral dose administration range from approximately 3.3 to 9.3 hours [4-6]. The use of ethambutol can cause side effects include ocular effects (blurred vision, scotoma, color blindness, visual impairment), joint pain, gastrointestinal effects (anorexia, nausea, vomiting, abdominal pain), fever, fatigue, headache, and confusion or lack of focus [4].

Before the drug is marketed, to ensure compliance with BPOM requirements, including effectiveness, safety, and quality, this ethambutol drug needs to undergo clinical testing first at an accredited laboratory. This study was performed to investigate Ethambutol 400 mg Tablets produced by PT Kimia Farma Tbk compared to the comparator product approved by WHO, Myambutol 400 mg Film-Coated Tablets produced by Pantheon Inc, Ontario, Canada, for STI Pharma LLC [7], in healthy subjects by means of bioequivalence studies and pharmacokinetic evaluation.

2. METHODS

2.1. Design Study

The bioequivalence study is utilized by a randomized, single-dose, open-label, two-way crossover design with 7 (seven) days washed-out period between each period, under fasting state at least 8 hours before drug administration [5, 6, 8]. The subjects were randomly administered the investigation drugs in sequence RT or TR.

2.2. Study Protocol

The Faculty of Medicine Ethics Committee, Indonesia University and the Indonesian FDA (Jakarta, Indonesia) has approved the study protocol. The protocol described all term of the study, including bioequivalence design study, procedures of recruitment, screening, and drug administration, procedure of sample handling, procedure of analysis method, procedure of pharmacokinetic and statistical data analysis, procedure of documentation control (including informed consent from subject, case report form (CRF), etc), and all evaluation or deviation of the study which documented on final report.

2.3. Subject Recruitment and Health Screening

To prevent the study from being influenced by the subject's body metabolism, the subject who has been recruited will undergo the health examination including physical examination and laboratory testing so the subject will fulfil the inclusion and exclusion criteria based on the study protocol. The requirement of the inclusion and exclusion criteria is stated on Bioequivalence

Study Guidelines [3]. This health examination is conducted within 7 days prior to the investigation drug administration at Equitrust Lab, Central Jakarta, Indonesia.

2.4. Investigational Drugs Administration

This bioequivalence study for Ethambutol drug consists of two periods, with each period conducted for 36 hours. Subjects will be quarantine at Equitrust Lab at least 12 hours before drug administration. The subjects will be asked by the investigator regarding sickness and the history of medications taken during the last week (for 1st period) or last visit (for 2nd period) and undergo a medical assessment by the responsible physician, including the examination the pressure of blood, temperature of body, respiratory rate, oxygen saturation assessments (SpO₂), and pulse rate, also pregnancy test for female subject. During the quarantine and sampling period, subject is forbidden to smoking, consuming the alcohol, as well as intake of coffee, soda water, fruit juice, bread, chocolate, and tea. The subject also will be fasting at least 8 hours before drug administration.

On the first day of sampling, subjects were instructed to consume 1 tablet of the test drug with 220 mL of water at 07:00 AM, in a seated position, according to the predetermined randomization sequence. The test drug used in this study was Ethambutol 400 mg tablet manufactured by PT Kimia Farma, while the comparator product was Myambutol 400 mg tablet manufactured by Pantheon Inc, Ontario, Canada, and distributed by STI Pharma LLC. Throughout the sampling period, subjects were provided with a standardized meal, and their activities were closely monitored by the researchers to ensure compliance with the study protocol.

2.5. Blood Samples Collection

In this bioequivalence study of ethambutol, blood samples are conducted for 36 hours with 18-time sampling points (0 hours (prior to drug administration), 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, and 36 hours). In each time sampling point, 5 mL of blood will be taken. The deviation of blood sample collection will be recorded.

Blood samples were collected by a qualified phlebotomist and then transported to the laboratory for plasma separation and storage at -20 degrees. Approximately 195 mL of total blood volume was collected from each study subject, including 15 mL of blood for medical check-up.

2.6. Assay Methodology

An assay of Ethambutol in plasma sample was analyzed using LCMS/MS with LLOQ 20.00 ng/mL. The precipitation method is used to samples extractions [9-11]. This analytical approach has undergone rigorous validation in accordance with the European Medicines Agency's (EMA) [12]011 guidelines for bioanalytical method validation [12]. To ensure the accuracy and reliability of bioanalytical results, a range of quality control measures are implemented, including system suitability testing, calibration curve verification, and the analysis of quality control samples at low, medium, and high concentrations [12].

2.7. Pharmacokinetics and Statistical Analysis

The parameters of pharmacokinetics for this study (C_{max} , AUC_{0-t} , AUC_{0-inf} , t_{max} , and $t_{1/2}$ value) were evaluated using Microsoft Excel. Assessment of bioequivalence results using two main pharmacokinetic parameters, namely area under the curve (AUC) and maximal concentration

(C_{max}). Data from the two parameters were compared using Two-way analysis of variance (ANOVA) in the R statistical programme with an acceptance range of 90% confidence interval of log or ln transformed data of 80%-125%. [13].

2.8. Data Quality Assurance

The study was conducted following protocol requirements which has obtained approval from the ethics committee and clinical trial approval from BPOM, and requirement of GCP and GLP (Good Clinical Practice - Good Laboratory Practice). Regular audits by the Quality Assurance Department of Equitrust Lab and Sponsor were conducted during the study in each period. During the bioanalysis, various quality control measures were implemented in accordance with EMEA guidelines [12].

3. RESULT AND DISCUSSION

Throughout the study, all participants maintained good health, free from any underlying medical conditions before and after the study, and did not take any concomitant medications that could potentially influence pharmacokinetic parameters or bioequivalence outcomes. After receiving the medication, some adverse effects were observed, including abdominal pain, headache, and nausea, which were consistent with the product labeling for Ethambutol and have been widely reported in the literature [4]. During the short study duration, both the test and reference preparations were well-tolerated by healthy subjects after two doses.

This study has included a total of 32 subjects consisting of 16 male and 16 female healthy adult subjects. There are three subjects who withdrawal from the study because of sickness and personal reasons. The range of age subject, body weight, body height, and BMI from 32 subject respectively are 18-53 years, 46-74 kg, 148-177 m, and 18.34 – 24.92 kg/m².

The bioanalytical samples were stored in a freezer at a maximum temperature of -20°C until analysis. The analysis started on Monday, June 19th, 2023, and was completed on Monday, July 10th, 2023. The sample bioanalysis method used has been validated. The bioanalysis result of system suitability test, calibration curve linearity, and quality control samples met the requirements.

The assessment of bioavailability and bioequivalence of drugs was based on the pharmacokinetic parameter values (AUC_{0-t} , AUC_{0-inf} , C_{max} , $t_{1/2}$, and t_{max}) of both drugs (test drug and comparator drug) which were then calculated and compared statistically. 90% confidence intervals were calculated with $\alpha = 5\%$ for the individual geometric means and ratios of AUC_{0-inf} and AUC_{0-t} and C_{max} for test drug (BN: J20113NX) and comparator drug Myambutol (BN: CFKFD) were all within 80% - 125% interval.

Data on pharmacokinetic parameter values are shown in Table 1. Statistical calculations of AUC_{0-t} and C_{max} parameters based on the 29 subjects who completed the study are shown in Table 2. The graph of mean plasma concentration vs time after oral administration is shown in Figure 1.

Table 1. The Summary of pharmacokinetic parameters

Parameter	Test Drug (Ethambutol)		Comparator drug (Myambutol)	
	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
AUC_{0-36} (ng.h.mL ⁻¹)	8475.12	2522.33	7998.71	1703.69
AUC_{0-inf} (ng.h.mL ⁻¹)	8882.33	2562.22	8388.72	1740.33
C_{max} (ng.mL ⁻¹)	1375.21	481.52	1507.47	525.70
$t_{1/2}$ (h)	6.72	0.83	6.66	0.92
t_{max} (h)*	3.48 (2.00-5.00)		3.16 (0.67-5.00)	

*mean (range)

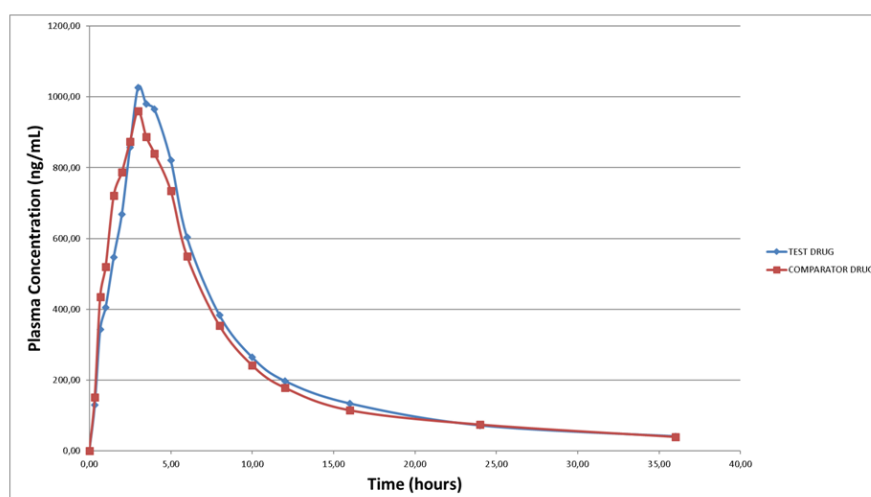


Figure 1. Pharmacokinetic Profile in Graphical Form of Geometric Mean Plasma Concentration vs Time After Administration of Test Drug: Ethambutol and Comparator Drug (R): Myambutol.

Table 2. Bioequivalence Statistical Data for AUC_{0-t} and C_{max} Parameters Of Ethambutol After Administration of Test Drug: Ethambutol and Comparator Drug (R): Myambutol

Parameter	% Ratio of Geometric Means (T/R)	90% Confidence Interval (T/R)		% CV	Power (%)
		Lower Limit	Upper Limit		
AUC_{0-36}	103.67	97.23	110.52	14.39	99.93
C_{max}	91.53	84.80	98.79	17.17	99.99

*) Bioequivalence criteria (90%CI): 80 – 125 %

The t_{max} values of the two drugs (test and comparator drugs) were compared using Wilcoxon signed-rank test on the original data and the result was not significantly different. The half-life values of the test and the comparator drugs were compared using student paired t-test and the result was not significantly different, demonstrating a comparable rate of drug elimination from

the body. In the present study, the intra-subject coefficient of variance (%CV) obtained from the ANOVA for Ethambutol AUC_{0-36h} was 14.39% and 17.17% for C_{max} . Hence, the number of subjects in this study (29 subjects) was adequate to ensure that this study has an adequate 80% power to confirm a statistical conclusion.

4. CONCLUSION

The bioequivalence study of Ethambutol which aims to determine the bioequivalence the ethambutol product produced by PT Kimia Farma Tbk compared to its recommended comparator product by WHO, Myambutol 400 mg Film-Coated Tablet produced by Pantheon Inc, Ontario, Canada for STI Pharma LLC, in healthy subjects already conducted by design study which accordance to the protocol study (randomized, single-dose, open-label, two-way crossover design). The criteria in this study were the ratio of geometric mean values $(AUC)T/(AUC)R=1.00$ with 90% CI=(80-125)% (α : 0.05) and $(C_{max})T/(C_{max})R=1.00$ with 90% CI is (80-125)% (α : 0.05) for both parameter AUC and C_{max} .

From this study, obtained the geometric mean ratios (90% CI) with the result 103.67% (97.23-110.52%) for AUC_{0-t} and 91.53 % (84.80-98.79%) for C_{max} . Based on the AUC_{0-36} and C_{max} values, Ethambutol 400 mg Film-Coated Tablet produced by PT Kimia Farma Tbk is bioequivalent to Myambutol 400 mg Film-Coated Tablet produced by Pantheon Inc, Ontario, Canada for STI Pharma LLC. The intra-subject coefficient of variation (%CV) was 14.39% for AUC_{0-36} and 17.17% for C_{max} .

CONFLICT OF INTEREST

The authors have no conflicts of interest in this research.

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Dyah Juliana Pudjiati and Yosi Reflinda is a representative from PT Kimia Farma Tbk, Jakarta, Indonesia, who in this case as the research sponsor and carries out development and ensures the quality of the drugs being studied.