

PHARMACOKINETIC EVALUATION OF AMLODIPINE TABLET COMPARED WITH NORVASK® TABLET IN HEALTHY INDONESIAN ADULTS

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ABSTRACT

This study objective to evaluate the pharmacokinetics of Amlodipine 10 mg Produced by PT. Tropica Mas Pharmaceuticals compared to Norvask® 10 mg Tablet Produced by PT. Pfizer Indonesia, in healthy Indonesian Adults through bioequivalence study. This study is utilized by randomized, single-dose, open-label, two-way cross-over design with a washout period 14 days and fasting. This study involved 18 subjects, with only 17 subjects included in the statistical analysis, because 1 subject withdrew due to diarrhoea in the 2nd period. Plasma samples were collected 17 times for 72-hour per period. Amlodipine concentrations were measured using LCMS/MS. 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 geometric mean ratios (90% CI) of the test drug vs. the innovator drug were 101.67% (97.16 – 106.39) for AUC_{0-t} and 100.46% (95.00 – 106.23) for C_{max} . Based on the result, the test drug is bioequivalent to the comparator drug.

KEYWORDS

Pharmacokinetic, Bioequivalence, Amlodipine, AUC, and Two-Way Crossover

1. INTRODUCTION

Indonesian Drug and Food Regulatory Agency as evaluator of drugs before they are marketed, grants marketing authorization, and subsequently monitors the drugs after they are marketed to ensure that they meet the required standards for efficacy, safety, and quality. In the assessment of generic drugs, their equivalence to the innovator drug needs to be demonstrated through comparative dissolution testing or bioequivalence and bioavailability testing [1].

Amlodipine is group of calcium channel blockers. It is a derivative of dihydropyridine with a long duration of action. Amlodipine will decreasing the peripheral vascular resistance and blood pressure by relaxing the smooth muscle cells in coronary blood vessels and promoting coronary vasodilation [2]. Amlodipine slowly absorbed in body when administered orally. Peak plasma concentration is reached within 6-12 hours. Amlodipine has a bioavailability approximately 64-90%, and it is not affected by food intake. It binds to plasma proteins to about 93%. The time required for the plasma drug concentration to decrease by half during the elimination phase is approximately 30 to 50 hours, and steady-state drug concentration in the blood plasma can be achieved after continuous administration for 7 to 8 days. Approximately 90% of the metabolic

process occurs in the liver, where it is converted into inactive metabolites, 10% remaining in its basic form and excreted through urine as much 60% in the metabolites form [3, 4]. Amlodipine has side effects of headache and oedema [3]. It also has side effects of dizziness, drowsiness, feeling tired, abdominal pain, nausea, flushing, or skin redness [5].

This study objective to characterize the pharmacokinetics of Amlodipine 10 mg Tablet Produced by PT. Tropica Mas Pharmaceuticals compared to Norvask® 10 mg Tablet Produced by PT. Pfizer Indonesia, in healthy Indonesian Adults through bioequivalence study. Pharmacokinetic parameters were derived by analysing the Amlodipine plasma concentration over time.

2. METHODS

2.1. Design Study

The design study for bioequivalence is utilized by a randomized, of single-dose, an open-label, a two-way cross-over design with a washout period until 14 days, under fasting state at least 8 hours before drug administration, both test drug and the innovator drug [6-9]. Pharmacokinetics of Amlodipine from several references are shown in Table 1.

Table 1. Reference of Amlodipine Pharmacokinetics

Parameter	Pharmacokinetics of Amlodipine			
	Liu Y et al, 2009	Carvalho M et al, 2001	Setiawati Effi et al, 2007	Hu Chaoying et al, 2018
Strength	10 mg	5 mg	10 mg	5 mg
C _{max} (ng/mL)	5.46-5.88	3.8 - 3.9	8.08 - 8.22	3 - 4
t _{max} (h)	7.70 - 9.20	6.0	8.0 - 8.5	6.00
t _{1/2} (h)	38.52 - 38.75	33.9 - 37.0	43.81 - 48.04	51.8
AUC _{0-t} (ng·h/ml)	284.56 - 311.34	147.4 - 151.7	353.15 - 359.99	212
%CV C _{max}	8.95%	20.81%	23.09%	12.20%
%CV AUC _{0-t}	9.34%	17.44%	19.02%	10.80%

The calculated coefficient of variation (%CV) for Amlodipine from previous studies is approximately 8.95% - 23.09%. According to the regulations of the Indonesian Drug and Food Regulatory Agency, drugs with %CV in this range require a minimum of 12 - 24 subjects. So, in this Amlodipine study, 18 subjects have been determined (including allowance for subject dropout) [1].

The washout period is determined based on the minimum washout time requirement, which is 5.5 times the half-life (t_{1/2}). According to earlier kinetic studies, the maximum t_{1/2} is 51.8 hours, suggesting that a minimum washout period of approximately 284.9 hours is required [7]. However, in this Amlodipine study, the washout period lasts for 14 days. After 14 days, the drug is administered again according to the randomization results in the same manner as the previous administration [10].

2.2. Study Protocol

The Faculty of Medicine Ethics Committee, Indonesia University (Jakarta, Indonesia) and the Indonesian FDA (BPOM) (Jakarta, Indonesia) has approved the research protocol. This study followed the ethical principles listed in the Declaration of Helsinki for biomedical research with human subjects, as well as GCP and GLP standards. Before the study began, all participants were provided with comprehensive information regarding all aspects of the study, in accordance with the Indonesian Bioequivalence study guidelines. They also signed a written informed consent.

2.3. Subject Recruitment and Screening

Healthy Indonesian adults were recruited and fulfilled the inclusion and exclusion criteria based on the study protocol with reference to the Declaration of Helsinki and the current Good Clinical Practice. Written informed consent was obtained before the screening was conducted. To ensure the subject was in good health, a medical history, vital signs, physical examination, blood sample laboratory tests and cardiac health (ECG) were conducted.

The inclusion and exclusion criteria established for this study were based on the Bioequivalence Study Guidelines as per BPOM Regulation No. 11 of 2022. The inclusion criteria is including: willing to sign an informed consent; Healthy based on clinical laboratory tests (routine hematology, liver function, kidney function, blood glucose, urinalysis, hepatitis B (HBsAg), hepatitis C (Anti-HCV) and HIV (Anti-HIV), medical history, and physical examination); Male and female subjects (if female, consider the risks for women of childbearing age and perform pregnancy tests); Age between 18-55 years; Normal weight range according to Body Mass Index (BMI) 18-25 kg/m²; Vital signs within the following ranges: systolic blood pressure 110-129 mmHg, diastolic blood pressure 70-84 mmHg, normal pulse rate 60-90 bpm, oxygen saturation (SpO₂) in the normal range of 95-100%, and normal respiratory rate of 12-20/min.

The exclusion criteria for this study including: Smoking more than 10 cigarettes per day; Pregnant or breastfeeding women (Pregnancy tests was performed during screening and prior to the administration of the investigational or comparator drug); History of kidney or liver disease, or history of allergy, hypersensitivity or contraindication to the investigational bioequivalence drug; Clinically significant haematological abnormalities; Abnormal electrocardiogram (ECG); Difficulty accessing veins in the left or right arm; History of significant ongoing clinically or medically significant chronic or acute illness; History of drug or alcohol abuse within the past 12 months (1 year) prior to screening for this study; Positive serology test results for Hepatitis B (HBsAg), Hepatitis C (anti-HCV), HIV (anti-HIV). Positive rapid antigen test results for SARS-CoV-2 (if the BE study is conducted during a pandemic); Have history or condition that can affect drug kinetics; Use of drugs or dietary supplements no more than 7 days since the start of the study; participated in previous clinical trials no more than 3 months from the start of the study; and Blood donation or blood loss of more than 300 ml within 3 months from the start of the study.

2.4. Study Drugs and Administration

The investigational drug used in this study is a 10 mg tablet of Amlodipine produced by PT. Tropica Mas Pharmaceuticals, while the innovator product Norvask® 10 mg tablet is manufactured by PT. Pfizer Indonesia. Subjects are directed to abstain from any medication for a minimum of one week prior to the conduction of the study. In the event of an urgent situation requiring medication or supplements, subjects must promptly notify the investigator, specifying the type and dosage. Smoking, alcohol consumption, as well as intake of coffee, soda water, fruit juice, bread, chocolate, and tea are not allowed for 24 hours prior to the start of the

bioequivalence study. All subjects were conditioned to be in a fasting state for at least 8 hours prior to the administration of both the test drug and the comparison drug. One hour before drug administration, a medical assessment is conducted by the responsible physician to evaluate the subjects' health, including pressure of blood, temperature of body, respiratory rate, oxygen saturation assessments (SpO₂), and pulse rate. The results of the examination were documented in the case report form document and additional pregnancy tests for female subjects.

Drug administration started at 07:00 a.m on the first day. Each subject received 1 tablet of Amlodipine 10 mg (as test drug) or 1 tablet of Norvask® 10 mg (as comparison drug) with 220 mL water volume. The sitting upright position was conditioned until 1 hour after drug administration.

2.5. Blood Samples Collection

The samples (blood from each subject) taken were determined to reflect the absorption, distribution, and elimination phases of the drug. The number of samples was 12-18 collection points consisting of one sample before taking the drug, before peak concentration 2-3, around peak concentration 4-6, and after peak concentration 5-8 points [1].

The time of sampling in this Amlodipine study was conducted for 72 hours with the following sampling points: before drug administration (zero hours), hour one, two, three, four, five, six, seven, eight, nine, ten, twelve, sixteen, twenty-four, thirty-six, forty-eight, and seventy-two hours after taking the test drug or comparator (seventeen points). After that, blood samples were stored and collected into vacutainer tubes filled with anticoagulant. Plasma was separated from blood sample using the centrifuged at certain speed and time. The plasmas were then stored in the freezer until Amlodipine bioanalysis was completed. The total volume of blood collected from each subject was approximately 185 mL (this included the 15 mL blood sample taken at the medical checkup). All deviations that were not in accordance with the study protocol were documented in the CRF (Case Report Form).

Clinical data and vital signs of subjects during the study period were examined by a responsible physician. The examination was monitored before drug administration, and after drug administration at 2 hours; 4; 6; and 12 hours, as well as additional time according to the consideration of the responsible physician in charge. All observation results are documented in the CRF.

2.6. Bioanalytical Method

The bioanalytical method used have been validated accordance to EMEA, 2011, Guideline on Bioanalytical Method Validation, meeting the validation requirements of all parameters [15]. The LLOQ in validation of this method was 0.1 ng/mL.

Amlodipine plasma concentrations were analyzed by LCMS/MS at the Equitrust Laboratory (Jakarta, Indonesia). Chromatographic separation of samples using Acquity HSS T3 column. The solution used for mobile phase is Acetonitrile and Ammonium Acetate solution with a gradient system. Extracted samples were prepared using the precipitation method [11-13].

2.7. Pharmacokinetics and Statistical Evaluation

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. The main parameters to assess bioequivalence were pharmacokinetic parameters area under the curve (AUC) and maximal concentration (C_{max}) with

90% Confidence Interval (90% CI) of log or ln-transformed data. Two-way analysis of variance (ANOVA) of the log or ln-transformed $C_{\max}$ and AUC_{0-t} values for both drugs using the R statistics programme, with 90% confidence interval values of 80.00 - 125.00% for both primary parameters [14].

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 [15].

3. RESULT AND DISCUSSION

3.1. Result

Eighteen adult subjects participated in the study, consist of 15 males and 3 females (subject number SN001 - SN018). Subject number SN012 did not complete the study in the second period due to diarrhoea, so the total subjects who completed the study and were subjected to pharmacokinetic and statistical analyses is 17 subjects. Subjects who participated in the study had met the inclusion (including age and BMI parameters) and exclusion criteria. Subject demographic data are presented in Table 2.

Table 2. Demographic Data of 18 subject

	MIN	MAX
Age (Year)	20	46
BMI (kg/m ²)	18.07	24.61
Body Height (m)	153	176
Body Weight (kg)	48	67

There were adverse events during this bioequivalence study, which were heart palpitations, headache, nausea, and diarrhoea. All adverse events during the study were documented in the case report form and deviations from the study protocol were described in the full report.

Based on the bioanalysis data, pharmacokinetic parameters including AUC_{0-t} , AUC_{0-inf} , $C_{\max}$, $t_{1/2}$, and $t_{\max}$ were calculated. The 90% confidence intervals (CI) with $\alpha = 5.00\%$ for the geometric mean ratios of AUC_{0-inf} , AUC_{0-t} , and $C_{\max}$ between the test drug Amlodipine and the innovator drug were in the range of 80.00% - 125.00%.

The pharmacokinetic parameter results are summarized in Table 3. Furthermore, Table 4 outlines the primary statistical analyses for AUC_{0-t} and $C_{\max}$ of Amlodipine based on data collected from 17 subjects who received both the test and innovator drugs orally. Figures 1 illustrate the plasma concentration-time profiles after administering a single oral dose of the test drug.

Table 3. The summarize of pharmacokinetic parameter results

Parameters	Test Drug (Amlodipine)		Innovator drug (Norvask®)	
	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
AUC_{0-72} (ng.h.mL ⁻¹)	205.60	47.32	201.82	42.44
AUC_{0-inf} (ng.h.mL ⁻¹)	268.57	70.91	262.22	59.88
C_{max} (ng.mL ⁻¹)	5.87	5.84	5.82	1.20
$t_{1/2}$ (h)	31.91	8.88	31.39	4.09
t_{max} (h)*	7.47 (5.00 - 10.00)		7.12 (4.00 – 10.00)	

*mean (range)

Table 4. Statistical analyses were performed on the AUC_{0-72} and C_{max} parameters of Amlodipine after administering a single oral dose of both the test and innovator drugs.

Parameter	% Ratio of Geometric Means (T/R)	90% Confidence Interval (T/R)		% CV	Power (%)
		Lower Limit	Upper Limit		
AUC_{0-72}	101.67	97.16	106.39	7.54	>99
C_{max}	100.46	95.00	106.23	9.30	>99

*)Bioequivalence criteria (90% confidence interval): 80.00 – 125.00%.

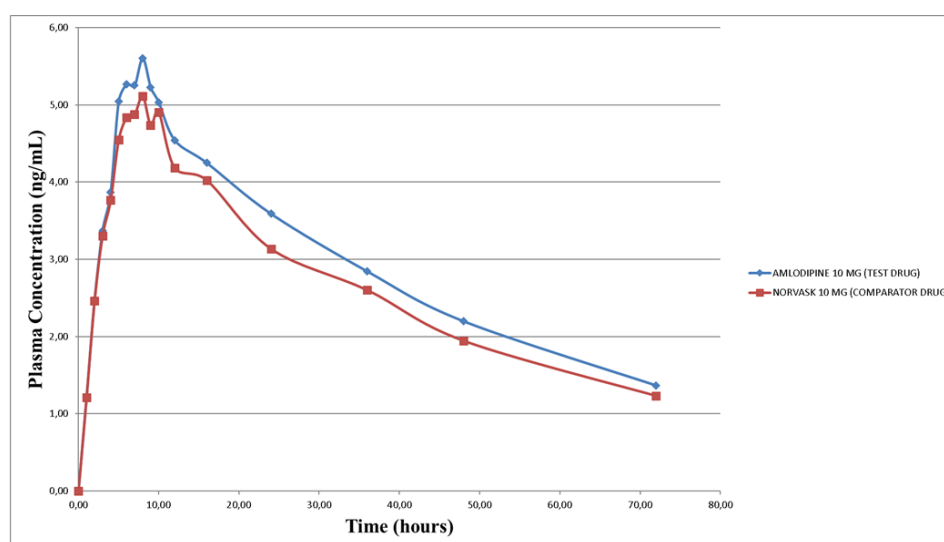


Figure 1. Geometric Means of Plasma Concentration-Time Profiles Following Administration of the Test Drug (T) and Innovator Drug (R).

Statistical comparison of t_{max} between the test drug and the innovator drug was conducted with Wilcoxon signed-rank test. The t_{max} value was not significantly different since p-value was > 0.05. The data is shown in Table 5.

Table 5. Statistical Analysis of $t_{\max}$ Comparison Between the Test Drug and the Innovator Drug Using the Wilcoxon Matched-Pairs Signed-Rank Test.

	Mean Rank	Sum of Ranks
Negative Ranks	5.50	16.50
Positive Ranks	5.50	38.50
Z	-1.165	
p-value	0.244	

However, the $t_{1/2}$ comparison between the test drug and the innovator drug was assessed using a student paired t-test (tested with Kolmogorov-Smirnov Test). Plasma half-life ($t_{1/2}$) of test and innovator drug was not significantly different because the p-value > 0.05. The result is shown in Table 6.

Table 6. Statistical comparison of $t_{1/2}$ between the test drug and the innovator drug based on Student paired t-test.

Normality test (p)	0.888
Distribution	Normal
Paired t	0.448
p-value	0.661

3.2. Discussion

This study objective to characterize the pharmacokinetics of Amlodipine 10 mg Produced by PT. Tropica Mas Pharmaceuticals compared to Norvask® 10 mg Tablet Produced by PT. Pfizer Indonesia, in healthy Indonesian Adults through bioequivalence study. The bioequivalence study is utilized by a randomized, of single-dose, an open-label, a two-way cross-over design with a washout period until 14 days, under fasting state at least 8 hours before drug administration (test drug or the innovator drug). There were 17 from 18 subjects completed the study and analyzed for statistical calculation of Amlodipine. Even though there are 1 (one) subject that are excluded in the study, the power study is meets the criteria (>80.00%). Both the power study of AUC_{0-72} and $C_{\max}$ parameter are >99%.

The key parameters used to assess similarity between the test and innovator formulations of Amlodipine are AUC_{0-72} and $C_{\max}$. Bioequivalence was determined based on a 90% confidence interval criterion, where the geometric mean ratios (AUC)T/(AUC)R = 1.00 with a 90% CI = 80-125% (α : 0.05) and ($C_{\max}$)T/($C_{\max}$)R = 1.00 with a 90% CI = 80-125% (α : 0.05).

The geometric mean ratios (with 90% confidence intervals) for AUC_{0-72} and $C_{\max}$ of the test drug relative to the innovator drug were 101.67% (97.16 – 106.39) and 100.46% (95.00 – 106.23), respectively. Based on these values, Amlodipine (BN: PF017R) demonstrated comparable absorption rates and extents compared to the innovator drug Norvask (BN: G7322). The intra-subject coefficient of variation (%CV) from ANOVA was 7.54% for AUC_{0-72} and 9.30% for $C_{\max}$ in this study.

The mean of range time to reach peak plasma concentration of Amlodipine ($t_{\max}$) for the test drug was 7.47 (5.00-10.00) hours, while for the innovator drug, it was 7.12 (4.00-10.00) hours. Comparison of $t_{\max}$ values between the two drugs (test and innovator) was conducted using the

Wilcoxon signed-rank test on the raw data, was no significant difference as the p-value was greater than 0.05.

The mean or SD value of elimination half-lives ($t_{1/2}$) of Amlodipine for the test drug was 31.91 (5.84) h and the innovator drug was 31.39 (4.09) h. The compared of half-life values of the test and innovator drugs is conducted using a paired Student's t-test, revealing no statistically significant difference. This indicates a similar rate of drug elimination from the body for both formulations.

Meanwhile, the adverse event that occur during this bioequivalence study (heart palpitations, headaches, nausea, and diarrhoea) is related to the drug, but these are not clinically significant and subjects who experience adverse event is continued the study.

4. CONCLUSION

Based on pharmacokinetics and Statistical analysis for AUC_{0-72} and C_{max} values of Amlodipine 10 mg Tablet Produced by PT. Tropica Mas Pharmaceuticals is bioequivalent to Norvask® 10 mg Tablet Produced by PT. Pfizer Indonesia. The geometric mean ratio for AUC_{0-72} and C_{max} of the test drug relative to the innovator drug were 101.67% (97.16 – 106.39) and 100.46% (95.00 – 106.23), respectively. Meanwhile, the intra-subject coefficient of variation (%CV) from ANOVA was 7.54% for AUC_{0-72} and 9.30% for C_{max} in this study. Both the power study of AUC_{0-72} and C_{max} parameter are >99%, so that the study results can be recognized.

CONFLICT OF INTEREST

The authors have no conflicts of interest in this research.

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REFERENCES

- [1] Badan Pengawas Obat dan Makanan. (2022). Tata Laksana Uji Bioekivalensi. Jakarta: BPOM
- [2] MIMS. (2023, February 1st). Amlodipine Dosage and Drug Information. Accessed on: <https://www.mims.com/indonesia/drug/info/amlodipine?mtype=generic>
- [3] American Society of Health-System Pharmacist. (2011). AHFS Drug Information. USA: American Society of Health-System Pharmacist
- [4] Prescribers' Digital Reference. (2023, February 1st). Amlodipine. Accessed on: <https://pdr.net/drug-summary/Norvasc-amlodipine-besylate-1853>
- [5] Drugs.com. (2023, February 2nd). Amlodipine. Accessed on: <https://www.drugs.com/amlodipine.html>
- [6] Carvalho M, et al. (2001). Amlodipine bioequivalence study: Quantification by liquid chromatography coupled to tandem mass spectrometry. *Biopharmaceutics and Drug Disposition*, 22(9), 383–390.
- [7] Hu C, et al. (2018). Bioequivalence and Pharmacokinetics of Bisoprolol–Amlodipine 5 mg/5 mg Combination Tablet versus Bisoprolol 5 mg Tablet and Amlodipine 5 mg Tablet: An Open-Label, Randomized, Two-Sequence Crossover Study in Healthy Chinese Subjects. *Clinical Drug Investigation*. 38(12), 1145–1154.
- [8] Liu Y, et al. (2009). Pharmacokinetics and bioequivalence evaluation of two formulations of 10-mg amlodipine besylate: An open-label, single-dose, randomized, two-way crossover study in healthy chinese male volunteers. *Clinical Therapeutics*. 31(4), 777–783.

- [9] Setiawati E, et al. (2007). Comparative Bioavailability of Two Amlodipine Formulations in Healthy Volunteers. *Arzneimittel-Forschung (Drug Research)*. 57(7):467–471.
- [10] World Health Organization (WHO). (2020, August 25th). Bioequivalence - General Considerations. Accessed on: https://extranet.who.int/prequal/sites/default/files/documents/2-4_Bioequivalence.pdf
- [11] Danafar H, et al. (2016). Method Validation of Amlodipine and atorvastatin by liquid chromatography-mass spectrometry (LC-MS) method in human plasma. *Cogent Medicine*. 3. page 1-11
- [12] Chan-Mei Lv, et al. (2013). Determination of Amlodipine in human plasma by LC-MS/MS and Its Bioequivalence Study in Healthy Chinese Subjects. *Pharmacology&Pharmacy*. 4. page 191-200
- [13] Yacoub, Mahmoud, et al. (2013). Simultaneous determination of amlodipine and atorvastatin with its metabolites; ortho and para hydroxyl atorvastatin; in human plasma by LC-MS/MS. *Journal of Chromatography B*. 917-918. page 36-47
- [14] Badan Pengawas Obat dan Makanan. (2015). *Tata Laksana Persetujuan Uji Klinik*. Jakarta: BPOM
- [15] European Medicines Agency (EMA). (2011). *Guideline on Bioanalytical Method Validation*

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