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Research Article

Bioequivalence Study of Modified Release Formulations of Trimetazidine 35 mg MR Tablets in Healthy Thai Subjects: Single Dose Under Fasting Condition, Single Dose Under Fed Condition and Multiple Dose Under Fed Condition

Chuleegone Sornsuvit[1,2*], Wannakamol Sonsingh[1,2], Jutamas Jiaranaikulwanitch[1,3], Darunee Hongwiset[1,3], Songwut Yotsawimonwat[1,3], Peerapong Srifun[1], Manatchaya Toonkum[1], Parichat Salee[4], Kanokkarn Pinyopornpanish[4]

[1]Pharmacy Service Center, Chiang Mai University, Chiang Mai, 50200, Thailand; [2]Department of Pharmaceutical Care, Chiang Mai University, Chiang Mai, 50200, Thailand; [3]Department of Pharmaceutical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand; [4]Department of Internal Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

ABSTRACT

Objective: This study aimed to determine the bioequivalence of trimetazidine dihydrochloride 35 mg modified-release tablets between a trimetazidine test product and a reference product in healthy subjects.

Significance: Trimetazidine, available as a modified-release dosage form, is approved as an add-on therapy for the treatment of stable angina pectoris patients. Conducting multiple-dose Bioequivalence (BE) studies may be necessary for extended-release dosage forms, in addition to the standard single-dose fasting and non-fasting BE studies.

Methods: The bioequivalence assessment was conducted under three conditions: Single-dose under fasting condition (Study A), single dose under fed condition (Study B), and multiple-doses under fed condition (Study C). An open-label, randomized, two-treatment, two-period, two-sequence, crossover design was employed, with 24 healthy volunteers recruited for each condition. The concentrations of trimetazidine in plasma were measured using validated LC-MS/MS, and pharmacokinetic parameters were calculated to assess bioequivalence.

Results: For fasting and fed condition single-dose testing, the 90% CI for ratios of geometric means for Cmax ranged from 91.89% to 100.12% and 101.65% to 109.65%, respectively. The corresponding values for AUC0-t were 96.17% to 101.25% and 99.76% to 105.41%. In the multiple-dose fed condition, the 90% CI for $C_{max,ss}$, $C_{\tau,ss}$, and $AUC_{(0-\tau),ss}$ were 100.11% to 108.68%, 96.43% to 102.26%, and 93.06% to 101.46%, respectively. All these values fell within the equivalent criteria of 80.00% to 125.00%.

Conclusion: Based on these results, it can be concluded that the two formulations of trimetazidine 35 mg modified-release tablets were found to be bioequivalent.

Keywords: Trimetazidine; Modified-release tablets; Bioequivalence; Multiple doses; Single dose

INTRODUCTION

Trimetazidine is approved as an add-on therapy for the treatment of stable angina pectoris patients who have inadequate control with first-line antianginal therapy. It has also shown beneficial therapeutic effects in the treatment of heart failure when used in addition to conventional treatments. [1] It is chemically described as 1-((2,3,4-trimethoxyphenyl) methyl) piperazine dihydrochloride, with a chemical formula of $C_{14}H_{24}C_{12}N_2O_3$ and a molecular weight of 339.257 g/mol. [2] The structural formula is shown in Figure 1.

Modifying myocardial cell metabolism is the main mechanism of action of Trimetazidine. It blocks mitochondrial long-chain 3-ketoacyl-CoA thiolase in the β-oxidation process, leading to a shift in the source of energy metabolism from free fatty acids to glucose oxidation. This shift requires less oxygen consumption,

Correspondence to: Chuleegone Sornsuvit, Pharmacy Service Center, Chiang Mai University, Chiang Mai, 50200, Thailand, E-mail: chuleegone.sornsuvit@cmu.ac.th; chujang@hotmail.com

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thereby optimizing energy metabolism in ischemic cells. It helps preserve intracellular levels of phosphocreatine and ATP, corrects transmembrane ion exchanges, reduces intracellular acidosis, calcium overload, and free-radical-induced damage. [1-4]

Following the oral administration of a single dose of trimetazidine dihydrochloride 35 mg modified-release tablet, the mean peak plasma concentration is reached approximately 5 hours. The tablet can be taken with or without meals. Trimetazidine exhibits slight binding to human plasma proteins, accounting for approximately 16%. The apparent distribution volume is 4.81/kg. Metabolism of trimetazidine is negligible, with the majority being eliminated unchanged through renal excretion. In young healthy volunteers, the elimination half-life of trimetazidine after administration of the 35 mg modified-release tablet is approximately 7 hours. However, in individuals over the age of 65, the elimination of half-life is extended to around 12 hours. [5-8]

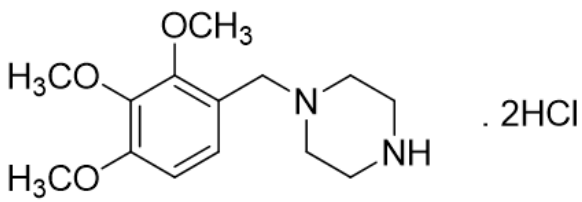

Figure 1: The chemical structure of Trimetazidine (TMT) dihydrochloride.

This study aimed to assess the bioequivalence of trimetazidine dihydrochloride 35 mg modified-release tablets between a test product and a reference product in healthy subjects. The study encompassed three conditions: Single dose under fasting condition, single dose under fed condition, and multiple doses under fed condition. The guidelines provided by the USFDA in 21 CFR 320.27 outline the requirements for designing a multiple-dose *in vivo* Bioequivalence (BE) study. According to these regulations, conducting multiple-dose BE studies may be necessary for extended-release dosage forms, in addition to the standard single-dose fasting and non-fasting BE studies. This ensures comprehensive evaluation of the drug product's bioequivalence. [9] By establishing bioequivalence between the generic and original tablets, a broader range of patients can benefit from this effective additional therapy for angina pectoris and heart failure while maintaining affordability.

MATERIALS AND METHODS

Subjects and study design

The bioequivalence study comprised of 3 separate studies, namely study A, study B and study C. The study design of each study was as follows:

Study A: An open-label, randomized, single dose, two-treatment, two-period, two-sequence, crossover design under fasting condition with one-week washout period between drug administration.

Study B: An open-label, randomized, single dose, two-treatment, two-period, two-sequence, crossover design under fed condition with one-week washout period between drug administration.

Study C: An open-label, randomized, multiple doses, two-treatment, two-period, two-sequence, crossover design under fed condition with two days' washout period between drug administration.

For each study, a total of twenty-four Thai male and female subjects, aged between 18 and 45 years with a Body Mass Index (BMI) in the range of 18.5-25.0 kg/m^2 were recruited as participants.

The study had specific inclusion and exclusion criteria for selecting subjects. Inclusion criteria involved healthy individuals with a clean medical history, passing a physical examination, and normal clinical laboratory test results. Non-smoking status and confirmation of non-pregnancy for female subjects were also required. Exclusion criteria included a known allergy to trimetazidine, a history or currently of alcohol addiction or drug abuse, recent medication or supplement use, consumption of citrus fruits or caffeine within specific timeframes, significant blood donation or loss, and participation in other clinical trials within 90 days prior. Termination from the study could occur if subjects experienced adverse events or allergic reactions, encountered medical problems, demonstrated non-compliance, had a positive pregnancy test, or voluntarily withdrew. Informed consent was obtained from all eligible subjects. For study C, only subjects reaching steady-state plasma concentrations of trimetazidine were included in the bioequivalence evaluation and statistical analysis, excluding those who did not reach steady state.

The clinical study was conducted at the Clinical Trial Unit (CTU) located at the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The study adhered to the Good Clinical Practice (GCP) guidelines established by the International Conference on Harmonization (ICH). The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The approval for the study's protocol was granted on June 20, 2019. Additionally, the study protocol was prospectively registered with the Thai Clinical Trials Registry (TCTR). The registration ID for the study is TCTR20190703003. By complying with GCP guidelines, obtaining ethical approval, and prospectively registering the study, the researchers demonstrated their commitment to conducting the study in an ethical and transparent manner.

Investigational product

The investigational product used was trimetazidine dihydrochloride 35 mg Modified Release (MR) tablets. The test formulation of the investigational product was manufactured by Medreich Limited, India, and imported by Berlin Pharmaceutical Industry Co., Ltd. located in Bangkok, Thailand. The reference product used in the study was Vastarel® MR manufactured by Les Laboratoires Servier Industrie, France, and imported by Servier (Thailand) Ltd., based in Bangkok, Thailand.

Drug product administration

In the single-dose bioequivalence studies (Studies A and B), all subjects were required to fast for at least 10 hours before the administration of the investigation product. They stayed at the clinical trial unit the night before the administration. Each volunteer received a single oral dose of Trimetazidine dihydrochloride 35 mg MR tablets with 220 ml of drinking water. Subjects were allowed to drink water freely, except during the restricted period during the 1 hour before or after taking the investigational product. In the fed condition study (Study B and C) high fat, high calorie breakfast was served 30 minutes before the dose of the investigation product. A standardized meal was provided for lunch and dinner. Lunch

was served 4 hours after the dose of the investigation product, and dinner was served 10 hours after the dose.

For study C, the administration of the investigational product consisted of two periods: The steady state build-up period and the bioequivalence study period. During the steady state build-up period, the subjects were administered one tablet of trimetazidine dihydrochloride 35 mg MR formulation with 220 ml of drinking water in the morning and evening after meal for four consecutive days. The purpose of this period was to reach a steady state of plasma drug levels. Once the steady state of plasma drug levels was achieved, the administration of the investigational product for the bioequivalence study period was performed following the same procedure as detailed above for a single dose study.

The study protocol ensured that the subjects followed a standardized fasting period, received the investigational product with a specified amount of water, and had standardized meals at predetermined intervals. This protocol helps maintain consistency in the study conditions and ensures accurate assessment of bioequivalence between the test and reference formulations.

Blood sample collection

In bioequivalence studies A and B, blood samples were collected to analyze the plasma concentrations of trimetazidine at specific time points: pre-dose (0.0 h) and 1.0 to 48.0 h. In study C, blood samples were collected to analyze at steady-state buildup and at time points 0.0 to 12.0 h as shown in Table 1. In each time-point, a total of 7 mL of blood sample were collected into blood collection tubes coated with K2EDTA, which is an anticoagulant. The plasma samples were separated immediately from the collected blood samples and stored at a temperature of -70°C ± 10 °C at the Pharmacy Service Center, Pharmacy Faculty at Chiang Mai University, until further analysis.

Table 1: Blood sampling time points in bioequivalence study A, B and C.

Period of study		Study A	Study B	Study C
Build-up period	Day 1			Pre-dose 1
	Day 2			
	Day 3			Pre-dose 5, 6
	Day 4			Pre-dose 7, 8
Study period	Period 1	Pre-dose (0.0 h), 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 10.0, 12.0, 24.0, 36.0, 48.0 h		Pre-dose (0.0 h), 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 10.0, 12.0 h
	Period 2			

Bioanalysis of trimetazidine in plasma

The bioanalysis part of the study was conducted following the standard operating procedures of the Pharmacy Service Center, which adhered to the OECD Good Laboratory Practice (GLP) guidelines. Throughout the bioanalysis process, quality assurance personnel closely monitored and reviewed all procedures to ensure adherence to the established protocols, maintaining the highest standards of quality. An analytical method using liquid chromatography tandem mass spectrometry was developed and validated for the determination of Trimetazidine (TMT) in human plasma. The method validation process included assessments of precision, accuracy, selectivity, linearity, recovery, matrix effect, and stability, in accordance with guidance from the European Medicines Agency (EMA) [10], and the U.S. Food and Drug Administration (FDA) [11]. A concise overview of the analytical method is as follows:

Sample Preparation: Ten microliters of 3.9 µg/mL working standard solution of Trimethoprim (TMP), internal standard (IS) was added to 250 µl of plasma used for calibration standards, QC samples and test samples. Then, 20 µl of 25% ammonia solution and 2 mL of 20% dichloromethane in diethyl ether were added to the mixture. The resulting solution was mixed using a Benchmark BV1010 shaker at 2500 rpm for 20 minutes at room temperature, followed by centrifugation at 3000 rpm for 5 minutes at 4°C. A 1 mL portion of the organic phase was collected, transferred to a new test tube, and evaporated to dryness under N2 gas at a bath temperature of 25°C and 0.5 psi for 15 minutes. The dried residue was reconstituted with 100 µL of a reagent consisting of 40% methanol in 0.005% v/v ammonium hydroxide in 2 mM ammonium acetate. The reconstitution was performed by shaking the mixture using a Benchmark BV1010 shaker at 2000 rpm for 10 minutes at room temperature. Finally, an aliquot of 3 µL was injected into the analytical column for further analysis.

The LC-MS/MS analysis was conducted using an Agilent 1260 HPLC system, consisting of a solvent delivery unit, online degasser, auto injector, and column oven, in combination with an API 3200 Mass spectrometer from AB Sciex. Chromatographic separation was achieved using a Supelco Discovery® C18 column with controlled temperature at 30°C and a flow rate of 0.6 mL/min. The elution solvent consisted of a mixture of methanol and 0.1% acetic acid in 2 mM ammonium acetate (40:60 v/v), which was filtered before use. A 3 µL injection volume was used for analysis. The mass spectrometer operated in Multiple Reaction Monitoring (MRM) mode with Electrospray Ionization (ESI) as the ionization source. Quantification was performed using MS/MS detection in positive ion mode. The transitions m/z 267.110 → 166.023 and m/z 291.012 → 123.054 were monitored for the analyte (TMT) and Internal Standard (TMP), respectively. The ion spray voltage was set at 5000 V, and the nebulizer gas, auxiliary gas, curtain gas, and collision gas were set at 60, 50, 35, and 8 psi, respectively. The declustering potential, entrance potential, collision cell entrance potential, collision energy, and collision cell exit potential were optimized at specific voltages for TMT and TMP.

Safety monitoring and evaluation

Vital signs of the subjects were assessed at various time points including pre-dose (0.0), 4.0, 8.0, 12.0, 24.0, 36.0, and 48.0 hours after drug administration and also every time points of dose in steady state build-up period of study C. Physical examinations were done by the physician during screening, on the night before drug product administration on Day 0 and Day 7, and at the end of the study. Continuous monitoring of Adverse Events (AEs) was performed throughout the entire duration of the study, including the confinement periods. Safety-related data analysis focused on AEs that occurred after the initiation of study treatment. The physician evaluated the causal relationship between adverse events and the study drug and reported their findings accordingly.

Pharmacokinetic parameter determination

The pharmacokinetic parameters analyzed in the study included C_{max} (Maximum plasma concentration), t_{max} (Time to reach C_{max}), AUC_{0-t} (Area under the plasma concentration-time curve from time

zero to the last measurable concentration), $AUC_{0\text{-}inf}$ (Area under the plasma concentration-time curve extrapolated to infinity), k_e (elimination rate constant), and $t_{1/2}$ (elimination half-life). C and t_{max} were obtained directly from the observed data. AUC0-t was calculated using the trapezoidal rule with linear up and logarithmic down extrapolation. $AUC_{0\text{-}inf}$ was estimated using $AUC_{0\text{-}t}$, the last measured plasma concentration (Clast), and the elimination rate constant (ke). The ke was determined by applying the slope of the linear regression equation for the log-transformed plasma drug concentrations during the terminal log-linear phase (-2.303 times the slope). The elimination half-life ($t_{1/2}$) was calculated as 0.693 divided by k_e.

In study C, the primary pharmacokinetic parameters analyzed were $AUC_{(0\text{-}\tau)ss}$ (Area under the plasma concentration-time curve during a dosing interval at steady state), $C_{max,ss}$ (maximum plasma concentration at steady state), and Cτ,ss (plasma concentration at the end of the dosing interval at steady state). The secondary parameters analyzed were tmax,ss (time until Cmax,ss is reached), Cmin,ss (minimum plasma concentration at steady state), Cav,ss (average concentration during a dosing interval at steady state, calculated as AUC(0-τ),ss divided by the dosing interval), and fluctuation (calculated as the ratio of the difference between Cmax,ss and Cmin,ss to Cav,ss).

The pharmacokinetic parameter determination was performed using the Phoenix™ WinNonlin® 8.0 computer software.

Statistical analysis and equivalence criteria

The statistical analysis for comparison of bioequivalence involved using the logarithmically transformed data. The ANOVA (Analysis of Variance) method was applied, and the 90% confidence interval was obtained using the Phoenix™ WinNonlin® 8.0 computer software. All pharmacokinetic parameter data from the subjects who completed both periods of the clinical study were included for the statistical analysis.

The criteria for determining bioequivalence were based on the guidelines for the conduct of bioavailability and bioequivalence studies [9,10,12]. According to the guideline for a single dose study (studies A and B), the 90% confidence interval of the ratio of logarithmically transformed C_{max}, and $AUC_{0\text{-}t}$ between the test and reference products should fall within the range of 80.00 to 125.00%. For study C, the bioequivalence of the test and reference products would be concluded if the 90% confidence interval of the ratio of logarithmically transformed $C_{max,ss}$, $C_{\tau,ss}$, and $AUC_{0\text{-}\tau,ss}$ between the test and reference products falls within the range of 80.00 to 125.00.

RESULTS

Out of the original 24 healthy Thai subjects recruited for each study, one subject discontinued participation in study B due to a safety issue related to an adverse event. Additionally, two subjects in study C did not reach steady state build-up and were therefore excluded from the statistical analysis. As a result, the statistical analysis of pharmacokinetic parameters included data from 24 subjects in the fasting study, 23 subjects in the fed study, and 22 subjects in the multiple fed study. This information is depicted in Figure 2. The demographic data, including age, weight, height, and BMI, were summarized in Table 2. It is noteworthy that the physical examination and laboratory data of all subjects fell within an acceptable range, indicating their good health throughout the study.

Quantification of trimetazidine in human plasma was performed with a robust and reliable LC-MS/MS method. The method demonstrated simplicity, sensitivity, and specificity during validation and bioanalysis. The calibration curve constructed for the analyte exhibited linearity over the range of 4-2000 ng/mL, with a coefficient of determination (R^2) greater than 0.99. The Lower Limit of Quantification (LLOQ) for trimetazidine at 4 ng/mL was sensitive enough for this study. The accuracy and precision of the method were assessed throughout the study with the quality control samples, which fell within the acceptable ranges. No matrix and no concomitance medication affected the analysis result. Moreover, the stability data showed that trimetazidine demonstrated good stability in plasma, with the analyte remaining stable for at least 5 hours at room temperature and up to 45 days when stored at -70°C ± 10 °C.

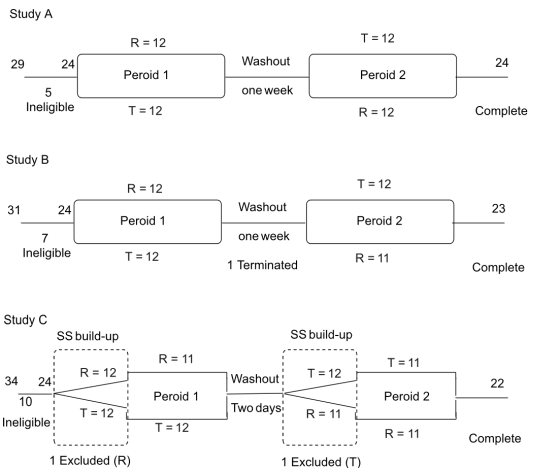

Figure 2: Bioequivalence study diagram of trimetazidine. **Note:** R=Reference product; T=Test product; SS build-up=Steady state build-up.

Table 2: Summary of demographic data including gender, age, weight, height, and BMI.

Study	Number of volunteers		Age (year)	Weight (kg)	Height (cm)	BMI (kg/m2)
A	24 M=12 F=12	Mean	27.7	58.3	164.6	21.5
		SD	5.5	7.3	7.9	1.6
		Range	20-40	45.0-75.0	150-176	18.7-24.2
B	23 M=12 F=12	Mean	25.3	57.5	164.3	21.2
		SD	3.9	8.7	8.4	1.9
		Range	21-35	45.5-72.4	150-181	18.6-25.0
C	22 M=12 F=10	Mean	26.5	59.3	166.8	21.2
		SD	5.8	8.6	8.7	1.6
		Range	19-41	45.3-78.0	150-185	18.8-24.4

Illustrating the plasma trimetazidine concentration-time curve, at various sampling times of all subjects after taking 35 mg trimetazidine diHCl MR tablet single dose under fasting condition, single dose under fed condition, and multiple doses under fed condition (Figure 3).

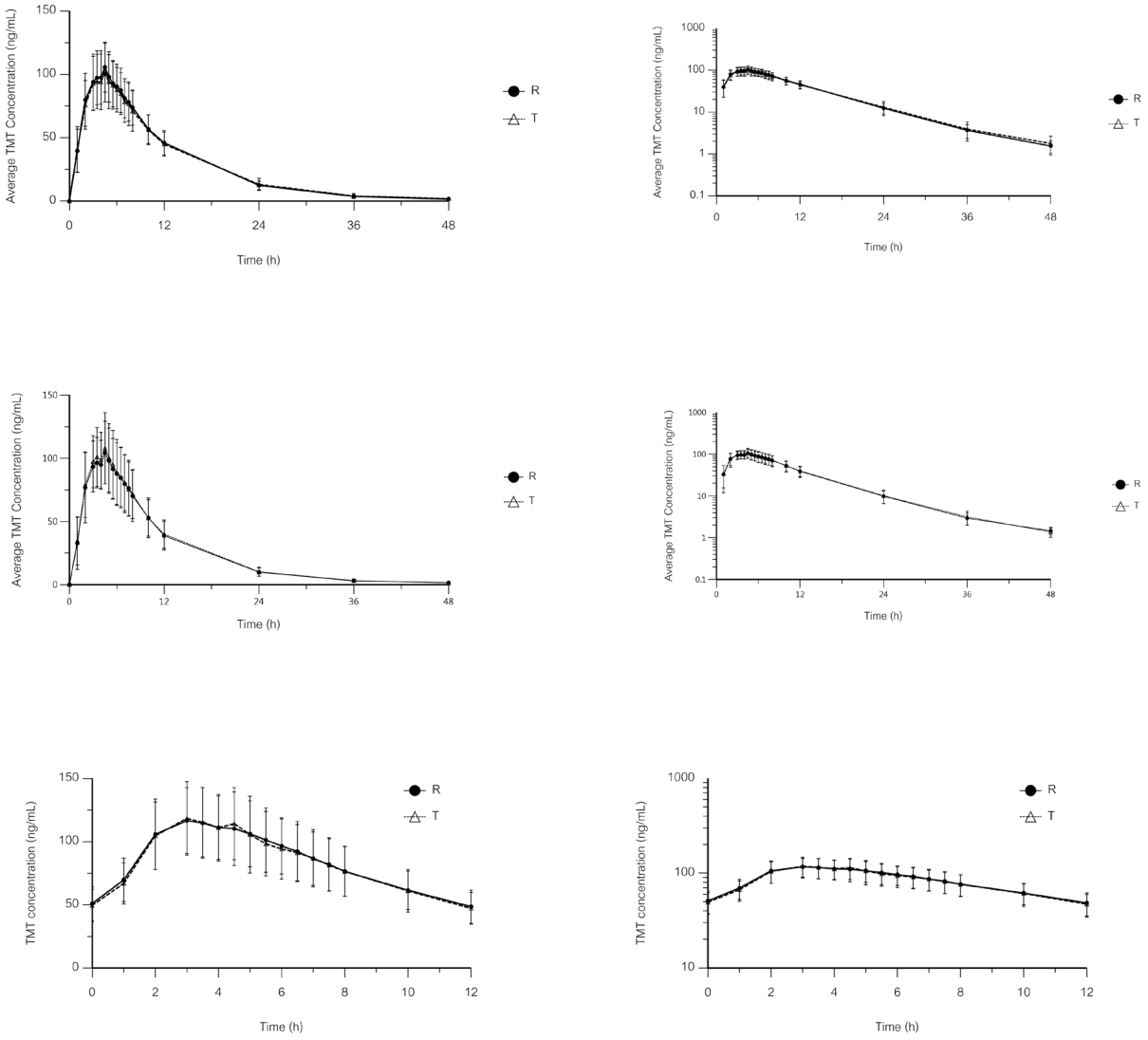

Figure 3: Average plasma trimetazidine concentrations at various sampling times of all subjects after taking 35 mg trimetazidine diHCl MR tablet single dose under fasting condition, n=24. **Note:** A: Single dose under fed condition, n=23; B: Multiple dose under fed condition, n=22; C: Reference formulation, Vastarel® MR (-◆-) and test formulation, trimetazidine diHCl MR tablet 35 mg test product (-△-); (Left) =normal scale (Right) =semi-log scale.

The pharmacokinetic parameters, including C_{max}, $C_{max,ss}$, $C_{\tau,ss}$, $C_{min,ss}$, $C_{av,ss}$, % fluctuation, t_{max}, $t_{max,ss}$, AUC_{0-t}, AUC_{0-inf}, $AUC_{(0-\tau),ss}$, k_e, and $t_{1/2}$, were determined using a non-compartmental model with the assistance of Phoenix™ WinNonlin® 8.0 software. The results of these calculations are summarized in (Tables 3 and 4) for study A, B, and C.

(Table 5) Presents the ratio of least square means and the 90% Confidence Interval (CI) for the ratios of geometric means (test/reference) for various pharmacokinetic parameters. For fasting and fed condition single dose testing, the 90% CI for ratios of geometric means for C_{max} ranged from 91.89% to 100.12% and 101.65% to 109.65%, respectively. The corresponding values for AUC0-t were 96.17% to 101.25% and 99.76% to 105.41%. The 90% CI for AUC0-inf in fasting and fed conditions were 96.30% to 101.41% and 99.73% to 105.24%, respectively. In the multiple dose fed condition, the 90% CI for $C_{max,ss}$, $C_{\tau,ss}$, and $AUC_{(0-\tau),ss}$ were 100.11% to 108.68%, 96.43% to 102.26% ng/mL, and 93.06% to 101.46% ng.h/mL, respectively. All these values fell within the equivalent criteria of 80.00% to 125.00% [9,10,12]. The power of the test for all these parameters' ratios was 100%. Additionally, the intra-subject coefficients of variation were low, ranging from 5.19% to 8.67%.

During the study, Adverse Events (AEs) were closely monitored and recorded based on interviews and physical examinations. Overall, both trimetazidine investigation formulations were well tolerated at the administered dose. Out of the 72 subjects, a total of 21 AEs were reported by 12 individuals (4 subjects from study B and 8 subjects from study C). Among these events, 11 occurred with the test product and 10 with the reference product as shown in Table 6. Most AEs were nonserious and mild in severity, resolving completely without the need for medications. However, there was one serious adverse event involving a subject who experienced abrasion wounds on their left hand and right knee due to a motorcycle accident. This event was determined to be unrelated to the study drug, but the subject was terminated from the study for safety reasons.

Table 3: Pharmacokinetic parameters of trimetazidine for each treatment after a single oral dose of trimetazidine diHCl MR tablet 35 mg under fasting and fed condition (study A and B).

Parameters	Single dose under fasting condition (n=24)		Single dose under fed condition (n=23)	
	Trimetazidine diHCl MR tablet 35 mg test product	VASTAREL® MR	Trimetazidine diHCl MR tablet 35 mg test product	VASTAREL® MR
	Arithmetic mean ± SD			
Cmax (ng/mL)	105.56 ± 22.52	109.42 ± 20.93	116.15 ± 22.29	110.29 ± 21.33
AUC0-t (ng.h/mL)	1257.45 ± 262.93	1268.59 ± 237.94	1183.53 ± 282.13	1152.26 ± 268.22
AUC0-inf (ng.h/mL)	1277.25 ± 268.20	1286.45 ± 240.68	1198.77 ± 283.63	1167.91 ± 269.56
tmax (h)	4.5 (2.0-5.0)	4.5 (2.0-6.5)	4.5 (2.0-5.5)	4.5 (2.0-6.5)
median (min-max)				
t1/2 (h)	7.03 ± 1.06	6.91 ± 1.02	6.69 ± 1.04	6.59 ± 0.96
Ke (h-1)	0.1006 ± 0.0139	0.1022 ± 0.0142	0.1063 ± 0.0190	0.1075 ± 0.0165

Note: C_{max}, maximum observed plasma concentration; AUC_{0-t}, area under the plasma concentration *versus* time curve up to the last; AUC_{0-inf}, area under the plasma concentration *versus* time curve with the concentration extrapolated based on the elimination rate constant; t_{max}, time to C_{max}; $t_{1/2}$, elimination half-life; K_e, elimination rate constant.

Table 4: Arithmetic mean of pharmacokinetic parameters of trimetazidine for each treatment in 22 healthy subjects after multiple oral doses of trimetazidine diHCl MR tablet 35 mg under fed condition (study C).

Parameters	Trimetazidine diHCl MR tablet 35 mg test product	VASTAREL® MR
	arithmetic mean ± SD	
Cmax,ss (ng/mL)	128.33 ± 27.79	123.06 ± 28.38
C□,ss (ng/mL)	47.12 ± 12.57	48.49 ± 13.14
AUC(0-□)ss (ng.h/mL)	996.79 ± 211.32	1004.05 ± 226.60
tmax,ss (h) median (min-max)	3.0 (2.0-5.0)	3.0 (2.0-5.5)
Cmin,ss (ng/mL)	46.24 ± 12.23	46.85 ± 12.28
Cav,ss (ng/mL)	83.07 ± 17.61	83.67 ± 18.88
Fluctuation (%)	99.54 ± 18.50	91.54 ± 14.53

Note: $C_{max,ss}$, maximum plasma concentration at steady state; $C_{\tau,ss}$, plasma concentration at the end of the dosing interval at steady state; $AUC_{(0-\tau),ss}$, area under the plasma concentration-time curve during a dosing interval at steady state; $t_{max,ss}$, time until $C_{max,ss}$ is reached; $C_{min,ss}$, minimum plasma concentration at steady state; Cav,ss, average concentration during a dosing interval at steady state.

Table 5: Ratio of log-transformed least square mean and 90% confidence interval of pharmacokinetic parameters used in bioequivalence evaluation of trimetazidine, intra-subject CV, and powers of test.

Study	Parameter	Cmax (ng/ml) or Cmax, ss* (ng/ml)	Cτ, ss (ng/ml)	AUC0-t (ng.hr/ml) or AUC(0-τ)ss* (ng.hr/ml)	AUC0-inf (ng.hr/ml)
A	Ratio of LSM (%)	95.91		98.68	98.82
	90% CI	91.89-100.12		96.17-101.25	96.30-101.41
	Intra-subject CV (%)	8.67		5.19	5.22
	Powers of test (%)	100		100	100
B	Ratio of LSM (%)	105.57		102.54	102.44
	90% CI	101.65-109.65		99.76-105.41	99.73-105.24
	Intra-subject CV (%)	7.47		5.43	5.3
	Powers of test (%)	100		100	100
C	Ratio of LSM (%)	104.31	99.3	97.17	
	90% CI	100.11-108.68	96.43-102.26	93.06-101.46	
	Intra-subject CV (%)	7.88	5.62	8.29	
		100	100	100	

Note: Value of Study C, C_{max}, maximum observed plasma concentration; AUC_{0-t}, area under the plasma concentration *versus* time curve up to the last; AUC_{0-inf}, area under the plasma concentration *versus* time curve with the concentration extrapolated based on the elimination rate constant; $C_{max,ss}$, maximum plasma concentration at steady state; $C_{\tau,ss}$, plasma concentration at the end of the dosing interval at steady state; $AUC_{(0-\tau),ss}$, area under the plasma concentration-time curve during a dosing interval at steady state.

Table 6: Adverse events experienced during study.

Causal relationship	Adverse events	Test formulation	Reference formulation	Total
Related	Costochondritis	7 events, 1 subject*	1 event, 1 subject*	8 events
	Dizziness	3 events, 3 subjects	1 event, 1 subject	4 events
	Atypical chest pain	0	1 event, 1 subject	1 event
	Acute dermatitis	0	1 event, 1 subject	1 event
	Palpitation	0	2 events, 1 subject	2 events
	Fatigue	0	2 events, 1 subject	2 events
	Chest discomfort	0	1 event, 1 subject	1 event
Unrelated	Allergic rhinitis	1 event, 1 subject	0	1 event
	Abrasion wound at left hand and right knee	0	1 event, 1 subject	1 event
Total		11 events	10 events	11 events

Note: Experienced in the same subject in both test and reference formulation.

DISCUSSION

In the fasting conditions, C_{max} exhibited a slightly lower level compared to the single-fed conditions, while AUC showed a trend towards higher values. However, t_{max}, $t_{1/2}$, and k_e demonstrated similar ranges of values. The influence of food on the absorption of the drug appeared to be minimal, indicating that it can be taken without regard to meals [6]. In the plasma trimetazidine concentration-time curve demonstrates that the maximum concentration of the drug in the single and multiple dosing studies exhibited only a negligible difference. Typically, lower levels of maximum concentration are observed in single dosing compared to multiple dosing, which can be attributed to the accumulation of the drug in the bloodstream following repeated administration.

The ratio of least square means and the 90% Confidence Interval (CI) for the ratios of geometric means (test/reference) for various pharmacokinetic parameters in fasting and fed condition single dose testing and the multiple doses fed condition fell within the equivalent criteria of 80.00% to 125.00% [9,10,12]. Additionally, the power of the test for all these parameters' ratios shows that the sample size of 24 subjects was abundant to indicate bioequivalence with the power of 100%. Furthermore, the intra-subject coefficients of variation were low, less 9%, which is consistent with previously reported [5-7,13].

Dizziness was the most reported AE, observed in 4 subjects, which is consistent with previous reports [1,6-7]. Another subject experienced costochondritis after administration of both investigation formulations. Costochondritis was not reported previously in whom administrated trimetazidine but was reported in similar symptom as gastric or esophageal burning and muscular cramp [1].

CONCLUSION

Based on the analysis of pharmacokinetic parameters and statistical evaluation, it can be concluded that the trimetazidine diHCl MR tablet 35 mg test product is bioequivalent to the reference product Vastarel® MR. The bioequivalence assessment indicates that both products exhibit comparable rates and extents of absorption in the body. This finding suggests that the test product can be considered a suitable substitute for the reference product in terms of therapeutic effectiveness and safety.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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REFERENCES

1. Dézsi CA. Trimetazidine in practice: Review of the clinical and experimental evidence. Am. J. Ther. 2016;23(3):871.
2. Onay-Besikci A, Özkan SA. Trimetazidine revisited: A comprehensive review of the pharmacological effects and analytical techniques for the determination of trimetazidine. Cardiovasc. Ther. 2008;26(2):147-165.
3. Uuetoa T. Clinical overview of trimetazidine (Vastarel MR) in patients with heart failure. Metab. Clin. 2010;(47):23-26.
4. Fragasso G, Alberti L, Lauretta L. Beneficial effects of trimetazidine (Vastarel® MR) in patients with chronic heart failure. Metab. Clin. 2011;53:25-28.
5. Abdelbary A, El-Gazayerly ON, El-Gendy NA, Ali AA. Floating tablet of trimetazidine dihydrochloride: An approach for extended release with zero-order kinetics. Aaps Pharmscitech. 2010;11:1058-1067.
6. Chandranipapongse W, Chatsiricharoenkul S, Ruangnapa T, Ngokpol S, Sathirakul K. Bioequivalence study of two formulations of 35mg trimetazidine modified release tablets in healthy thai volunteers under fasting and fed conditions. J Bioequiv Availab. 2011;3:52-55.
7. Harahap Y, Budi Prasaja MM, Lusthom W, Hardiyanti AF. Bioequivalence of trimetazidine modified release tablet formulations assessed in Indonesian subjects. J Bioequiv Availab. 2013;5:117-120.

8. Barré J, Ledudal P, Oosterhuis B, Brakenhoff JP, Wilkens G, Sollie FA, et al. Pharmacokinetic profile of a modified release formulation of trimetazidine (TMZ MR 35 mg) in the elderly and patients with renal failure. Biopharm Drug Dispos. 2003;24(4):159-164.

9. Bioavailability and bioequivalence studies submitted in NDAs or Inds-general considerations. 2014.

10. Guideline EM. European Medicines Agency, Committee for medicinal products for human use. Guideline on bioanalytical method validation. 2011.

11. Bioanalytical method validation guidance for industry. 2018.

12. Guideline for the conduct of bioavailability and bioequivalence studies. 2009.

13. Shedage A, Khanna A, Gole M, Purandare S, Malhotra G. Comparative steady state cross-over bioequivalence study of 35mg trimetazidine extended-release tablets. J Bioequiv Availab. 2014;20(6):192.