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Pilot study: bioequivalence of dihydroartemisinin in dihydroartemisinin-piperaquine tablet generic formulation in healthy Indonesian volunteers

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ABSTRACT

Bioequivalence test should be carried out for copy medicine, including dihydroartemisinin-piperaquine (DHP), which is used to treat critical diseases requiring medication. To predict the bioequivalence of film coated DHP generic tablets compared to the reference, a randomized controlled trial, single blind, single dose cross over design, two sequences, 2 periods, and wash-out period 7 days was conducted on 8 healthy adults. Blood samples were taken at certain times; plasma levels of dihydroartemisinin (DHA) were determined and analyzed for pharmacokinetics profile using UPLC MS MS system. The mean ±SD of $AUC_{0\text{-}24}$, C_{max}, T_{max}, and T½ of the test drug (T) in the following order were 220.07 ± 64.48 ng.mL-1.hour; 119.00 ± 37.66 ng.mL-1.hour; 1.16 ± 0.30 hour; and 1.06 ± 0.31 hour. The mean ±SD of $AUC_{0\text{-}24}$, C_{max}, T_{max}, and T½ of the reference drug (R) were 301.91 ± 161.30 ng.mL-1.hour; 203.60 ± 91.04 ng.mL-1.hour; 0.94 ± 0.35 hour; and 0.80 ± 0.21 hour. Based on statistical analysis, the geometrics mean ratio (T/R) for the C_{max} and $AUC_{0\text{-}t}$ were 0.6083 with 90% CI (0.4853–0.7624) and 0.7769 with 90% CI (0.6493–0.9295) respectively. Kinetic profiles between the two products were the same, however the test drug is relatively inferior compared to the reference drug.

Keywords: dihydroartemisinin-piperaquine (DHP), dihydroartemisinin (DHA), bioequivalence

Abbreviations:
BE: bioequivalence
CDT: comparative dissolution test
CV: coefficient of variation
DHA: dihydroartemisinin
DHP: dihydroartemisinin - piperaquine
F2: factor 2, similarity factor (factor indicated the similarity of dissolution profile of tablet)

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INTRODUCTION

Malaria disease is still a problem in Indonesia. In 2018, from 514 regencies in Indonesia, there are 285 regencies with malaria-free status, and 229 regencies especially in eastern Indonesia are not yet gained malaria-free status. Based on blood examination in Indonesia Basic Health Survey (Riskesdas) 2018, region with highest malaria is Papua 12.07% and West Papua 8.64% and East Nusa Tenggara (NTT) 1.99%.[1] Therefore malaria medicine is highly needed. Malaria treatment with artemisinin combine based therapy (Artemisinin-based Combination Therapy, ACT) is the best therapy for malaria that is recommended by WHO until now.[2,3] One of ACT that is widely used in Indonesia as first line malaria treatment and prospective in terms of safety and efficacy is dihydroartemisinin-piperaquine (DHP) fixed-combination. This DHP is well tolerated with high effectiveness to cure *falciparum* malaria with cure rate more than 95%, 3 days treatment with follow up to 42 days.[4-7] DHP tablet is imported product as well as all medicine ingredients in Indonesia, estimated 95% is imported. To fulfill demand in Indonesia by producing local medicine, quality test in vitro and in vivo are done to local DHP tablet.

In order to have good quality product and fulfill requirement as generic product, a series of product quality test is needed that is comprised of physical test (tablet description, tablet weight uniformity, tablet thickness, tablet hardness, tablet crispness) and chemical test (rate determination, dissolution, dissolution test profile compared to comparator).[8] Generic medicine has also run through clinical test, like innovator medicine before widely used. Implementing clinical test to generic medicine needs high budget because it needs big sample to decide equivalent. As alternative, bioequivalence (BE) test which has accurate endpoint (medicine rate in plasma) is implemented. This test is used due to low variability, and few samples needed. If the two bioequivalent medicines give the same effect, in terms of efficacy and safety, they can be act as substitute and can be distributed in Indonesia.[8]

Thus, DHP tablet consist of 2 different chemical active substances, one of which is water insoluble dihydroartemisinin (DHA) and the other piperaquine which is a water soluble substance. The BE test has previously been carried out on the first formula using a parallel design. The result is inequivalent.[9] Therefore, in the BE test in this study, a pilot study of the active substance DHA was carried out through reformulation of DHP tablet because this substance is a chemical substance that is difficult to dissolve and difficult to absorb.

MATERIALS AND METHODS

Study design, subjects and ethical consideration

This research is a pilot study of the bioequivalence of a combination of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP) membrane-coated generic tablets produced by the Pharmaceutical Industry as a test tablet compared to a reference drug, namely DHP-Frimal. The study was conducted in a Randomized Controlled Trial, single-blind, single dose, with a cross-over design, using two sequences and 2 periods, was performed on 8 healthy adult Indonesian subjects. Wash-out period 7 days (at least 5.5 × elimination half-life of dihydroartemisinin). Screening was carried out by conducting a health examination to get subjects that meet the inclusion criteria, exclusion criteria and restrictions.[10] The inclusion criteria were as follows: male and female aged 18–55 years; Body mass index (BMI) between 18–25 kg/m^2; healthy subjects based on routine hematology blood chemistry results; and negative serological results (HBsAg, Anti-HCV, Anti-HIV); urinalysis within normal limits; systolic blood pressure in the range of 100–120 mmHg, diastolic 60–80 mmHg and heart rate 60–90 bpm; normal ECG examination; subjects who do

not smoke, or are light smokers (less than 10 cigarettes per day); no history of alcohol or drug dependence; did not participate in another study in the 3 months prior to the study; was able to communicate well; and expressed consent to participate as a subject by signing an informed consent. Subjects were excluded from the study if they had a history of allergy (personal/family) or hypersensitivity or contra-indication to dihydroartemisinin and/or piperaquine or a combination of these drugs; had a medical history that may affect the pharmacokinetics of the test drug, such as chronic gastrointestinal disease (eg, gastric ulcer, duodenal ulcer), diarrhea, gastric surgery, hyperglycemia, renal failure, liver dysfunction, cardiovascular disease and had a history of arrhythmias and QT prolongation drug effect; had a history of bleeding or blood clotting disorders; a history of difficulty taking blood or having difficulty finding a vein in the left or right arm for blood collection; had donated or lost 300 ml (or more) of blood in the 3 months prior to the study; were taking medicines, supplements, herbal medicines within 14 days before administering the test drug. All subjects were prohibited from engaging in strenuous physical activity during sampling times and were prohibited from consuming food and beverages containing xanthin and fruit juice 24 hours before and during the days of sampling. Alcohol-based products were also prohibited from being consumed 24 hours before and during the day of sampling. This research has been approved by the Ethics Committee of the Research and Development Agency through Number LB.02.01/2/KE.309/2017 and because it is a pilot study, the National Agency for Drug and Food Control of Indonesia (BPOM) does not require registration for this clinical trial. This research uses an independent laboratory facilitiy PT. Farmalab Indoutama Integrated Laboratory, Faculty of Pharmacy and Science UHAMKA University which has been accredited in collaboration with the nearest hospital (Islamic Klender Hospital) to provide inpatient and outpatient facilities for drug clinical trials, as well as emergency treatment facilities to anticipate possible emergency conditions for the subject.

Drug selection, randomization and blinding

Each subject was given a single oral dose of the test drug, one generic film-coated tablet of a combination of dihydroartemisinin 40 mg/piperaquine 320 mg produced by an Indonesian drug manufacturer, or a reference drug of one film-coated tablet of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP-Frimal). Each subject will randomly receive a test drug or a reference drug so that the sequence of period-1 and period-2 will consist of TR or RT depending on the random allocation. Random allocation was performed using a block 4 randomization system[11] to determine subjects who received the test drug and subjects who received the reference drug in period-1, and for period-2 received the drug otherwise. The randomization code will be unlocked after the study ends and data analysis has been completed. The blinding procedure was carried out by means of the test drug and the reference drug were administered in a sealed envelope that had been prepared by an independent pharmacist (excluding members of the research team). In the single-blind procedure used in this study, the subjects did not know whether they were taking the test drug (DHP film-coated generic tablet combination of dihydroartemisinin 40 mg/piperaquine 320 mg produced by the Indonesian Pharmaceutical Industry) or the reference drug DHP-Frimal.

Sampling procedure

Subjects received an explanation of the research method, signed the consent form. and were asked to fast for 8 hours before administering the test drug or reference drug. Blood samples were taken at the following times: before drug administration (0 hours), 10 minute; 20; 30; 45 and 1st, 1, 2, 2½, 3, 4, 6, 8, 10, 12 and 24 hours after drug administration. Period-2 blood sampling was carried out using the standard procedure and the same way as period-1 blood

sampling. Blood samples from all subjects collected at each time point were centrifuged at 1538 g (rotor radius = 86 mm) for 15 min to separate the plasma. Plasma is transferred into a clean tube. All plasma samples containing DHA were stored in a freezer at −40°C ± 5°C at the clinical site until the time of DHA level analysis.

Dihydroartemisinin level measurement

Plasma containing DHA was added to the internal standard. Extraction of DHA from plasma through a liquid-liquid extraction process using tert butyl methyl ether. The clear solution extracted / supernatant was put into a tube and evaporated to dryness using nitrogen gas in a water bath at a certain temperature. The resulting residue was then dissolved with a mixture of methanol and distilled water. The dissolving results are then injected automatically into the UPLC MS MS system. The mobile phase used was ammonium formate and acetonitrile with an isocratic system. The column used is Waters Acquity BEH C18 (2.1 × 50 mm, 1.7 m) and the detector is MS MS, m/z dihydroartemisinin 302.00/163.00 and lansoprazole 370.10/252.10. The method was validated before being used to determine the levels of DHA in human plasma. Validation of DHA is shown as the standard calibration curve of DHA, which ranges from 0.51 to 202.69 ng/mL. A linear relationship between concentration and signal intensity was obtained (r = 0.9935 day 1, r = 0.9953 day 2, r = 0.9949 day 3) and the lower limit of quantification (LLOQ) was 0.51 ng/mL.[12]

Pharmacokinetic and statistical analysis

The bioequivalence between the two formulas was assessed based on the geometric mean ratio C_{max}, and $AUC_{0\text{-}24}$ with a 90% confidence interval (CI 90%) from log data or ln data. The log/ln values of $AUC_{0\text{-}24}$ and C_{max} of the 2 products were compared using a 2-way analysis of variance (ANOVA) for a 2-way crossover design that took into account the following sources of variation: drug products compared (Test and Reference), drug administration period (I and II), subject, and sequence (TR and RT). The difference in the average value of $AUC_{0\text{-}24}$ between the test product (test = T) and the comparison product (reference = R) according to the bioequivalence criteria, namely the geometric mean ratio (AUC)T/(AUC)R = 1.00 to 90 % CI = 80.00–125.00% and (C_{max})T/(C_{max})R = 1.00 with 90% CI = 80.00–125.00%.[8,13]

Comparative dissolution test

Prior to the in vivo test, the DHP tablets after being formulated were tested for quality in vitro, such as determining the levels of DHA and the comparative dissolution test (CDT) to obtain an overview of the test and reference tablets that have the same profile and concentration at the in vitro level. The tablet similarity value is indicated by factor 2, similarity factor (factor indicated the similarity of dissolution profile of tablet) (F2) which must have a value above 50 (F2>50). The similarity of profiles is expected to be a determinant of the success of drug absorption in vivo. CDT was carried out on the test and comparison tablets using the paddle-type method on 3 different pH media, namely pH 0.1, 4.5 and 6.8. The number of samples for each test and comparison tablet were 12 tablets.[8,14]

RESULTS

The results of the drug quality test as indicated by the levels of DHA and the CDT showed that the DHA content in the DHP test tablet (with the active subtance dihydroartemisinin 40 mg and piperaquine phosphate 320 mg) was 103.6%; this value is still within the required range

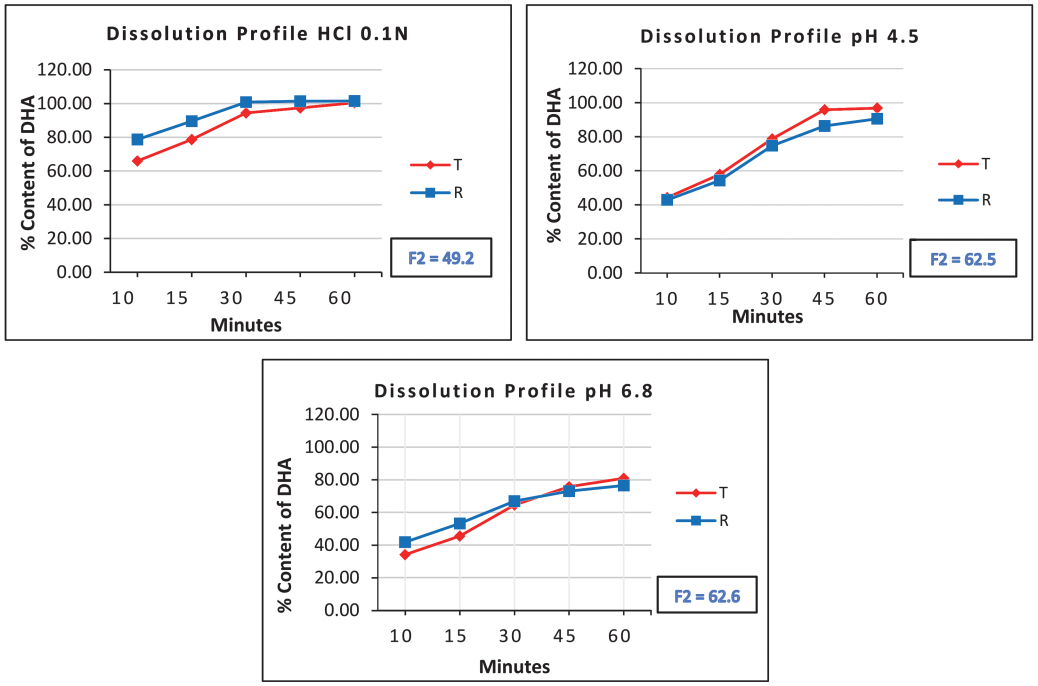

Fig. 1 CDT results of DHA of DHP test tablets compared to DHP-Frimal tablets
CDT: comparative dissolution test
DHA: dihydroartemisinin
DHP: dihydroartemisinin - piperaquine
F2: factor 2, similarity factor (factor indicated the similarity of dissolution profile of tablet)
T: Test drug
R: Reference drug

of 90.0%–110.0% for DHA, and piperaquine phosphate is 97.0%, also still within the required value range of 93.0%–107.0%.

The results of the CDT of the active substances dihydroartemisinin in the DHP trial batch, namely In/17/11/BI 12P0101PUT2142, compared to the reference drug DHP-Frimal with batch 161204 can be seen in Figure 1.

The results of the CDT of the active substance DHA obtained an F2 value which states the similarity of the profiles of the test tablet and reference tablet (DHP-Frimal). CDT was carried out with three pH media, namely pH 0.1; pH 4.5 and pH 6.8 each having an F2 value of 49.2; 62.5 and 62.6, which means that the two tablets have a great similarity, because based on the requirements it is stated similar if the F2 value > 50. Meanwhile for the profile with a pH of 0.1 the F2 value does not reach 50. However, this is the optimal tablet formula that will be followed by a pilot scale bioequivalence test.

Subject screening was carried out on 21 people and 9 people were included in the study. One of the subjects withdrew, so that 8 subjects participated in the study, including 4 male and 4 female, aged between 19–28 years, body weight between 47–65 kg, height between 153–169 cm, and BMI between 19.31–22.89 kg/m^2. Laboratory examination conditions and history were within normal limits. Plasma from 8 subjects obtained as many as 256 samples (8 people × 16 points × 2 periods). Plasma sample analysis was performed simultaneously for period one

and period two for each subject. The results of the analysis of the active substance DHA after administration of DHP tablet are as follow:

Table 1 Pharmacokinetic parameters of DHA after oral administration of single dose generic-coated tablet combination of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP), test drug and reference drug

Parameter	Test Drug		Reference Drug	
	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
AUC_{0-t} (ng.hr.mL^{-1})	220.07	64.48	301.91	161.30
AUC_{0-inf} (ng.hr.mL^{-1})	258.07	65.69	323.28	158.88
C_{max} (ng.mL^{-1})	119.00	37.66	203.60	91.04
$t_{½}$ (hr)	1.06	0.31	0.80	0.21
t_{max} (hr)	1.16	0.30	0.94	0.35

DHA: dihydroartemisinin
DHP: dihydroartemisinin - piperaquine
AUC : area under the curve
C_{max}: maximum concentration
$t_{1/2}$: half-life, time required for plasma concentration of a drug to decrease by 50%
t_{max}: time to maximum plasma concentration

Table 2 Statistical calculations for AUC_{0-t}, and C_{max} of DHA after oral administration of single dose of generic coated tablet combination of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP), T and R

Parameter	% Geometric Mean Ratio (T/R)	90% Confidence Interval (T/R)*)		% CV	Conclusion
		Lower Limit	Upper Limit		
AUC_{0-t} (ng.hr.mL^{-1})	0.7769	0.6493	0.9295	18.47	Bio-inequivalent
C_{max} (ng.mL^{-1})	0.6083	0.4853	0.7624	23.26	

*) Criteria for bioequivalence, T/R (90% CI): 80.00–125.00%
DHA: dihydroartemisinin
DHP: dihydroartemisinin - piperaquine
AUC : area under the curve
C_{max}: maximum concentration
CV: coefficient of variation
T: test drug
R: reference drug

Based on the results of parameter testing and statistical analysis with Equivtest 2, the geometric mean value (T/R) for C_{max} was 0.6083 with 90% CI (0.4853–0.7624) and % coefficient of variation (CV) intrasubject 23.26%. The geometric mean (T/R) AUC_{0-t} was 0.7769 with 90% CI (0.6493–0.9295) and an intrasubject % CV of 18.47%. The kinetic profiles of the two products were relatively the same, with the T_{max} of the test drug (1.16 ± 0.30) hours and the reference drug (0.94 ± 0.35) hours. The test drug is relatively inferior to the reference drug.

The profile of the average DHA levels in the plasma of each subject can be seen in Figure 2. From a total of 16 data, it is known that the higher average DHA levels were obtained from

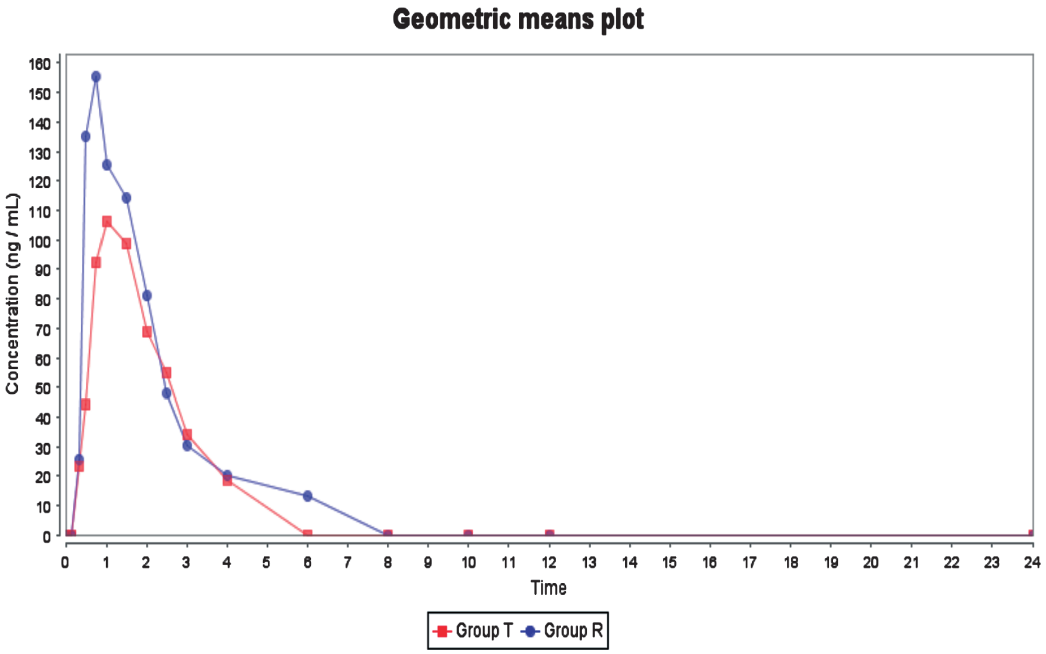

Fig. 2 Profile of average levels of DHA in plasma after oral administration of DHP product (test drug) and DHP-Frimal (reference drug)

DHA: dihydroartemisinin
DHP: dihydroartemisinin - piperaquine
Group T: test drug
Group R: reference drug

6 subjects in the reference group, while in the test drug group only 2 subjects showed high levels of DHA. Overall, the reference drug has a higher mean of DHA levels than the test drug.

DISCUSSION

The results showed that the maximum time of elimination (T_{max}) of DHA in the test drugs ranged from 1.16 ± 0.30 hours. This is in accordance with the literature which states that DHA is absorbed very quickly, with a T_{max} of 1–2 hours after administration of single and repeated doses of DHP tablets. In healthy volunteer subjects, DHA levels will increase by 43% when given together with high-fat/calorie foods. Therefore, in this study the drug was given with mineral water, on an empty stomach and was carried out according to standard procedures in the protocol.

There was evident in this study where the mean percentage of CV obtained for DHA for $AUC_{0\text{-}t}$ ($ng.hr.mL^{-1}$) is 18.47%, and the mean percentage CV obtained for C_{max} ($ng.mL^{-1}$) is 23.26%. Judging from the % CV which is below 30%, it shows that the test tablet formula is not classified as a highly variable drug product. In several DHA studies it is found that % CVs is depending on the design of the BE test method. One of this can be seen in our previous BE test on DHP tablets with the same comparison drug (DHP Frimal) but with a parallel design involving 48 subjects, where it was found that the coefficient of variation of AUC_{inf} was 46.7% and 43.56% for C_{max} of DHA,[9] which indicated a highly variable drug product. The results of

the present study is similar with a study which used crossover design in 24 healthy Vietnamese subjects, the two tested drugs containing dihydroartemisinin and piperaquine were found to be bio-inequivalent.[15]

However, in the present study the results of the BE test (in vivo pharmacokinetics) imply that the DHP test drug is bio-inequivalent to the comparison (reference) drug DHP-Frimal. The 90% confidence intervals of the test/reference ratios for AUC and C_{max} of dihydroartemisinin were outside the acceptable range for bioequivalence. In the BE study, the in vitro-in vivo simulation (IVIVS) approach successfully predicted the BE outcome of drugs. In the development of generics tablets, the IVIVS approach can save time and expenses.[16]

There are several possible reasons why these two DHP products in this study are bio-inequivalent. This possibility is related to the physical properties of one the components of the drug's active ingredients, namely the solubility and the permeability of the substance to the plasma membrane. DHA is a compound that is practically insoluble in water, but soluble in non-polar solvents, such as chloroform and acetone. Biopharmaceutical test (in vitro) showed that the dissolution of the DHA component of the test drug (generic tablet) was not comparable to that of the reference drug at gastric pH (acid), indicated by the slightly lower concentration of DHA of the test drug.

Based on bioequivalence test (in vivo) and biopharmaceutical test (in vitro), according to the Biopharmaceutical Classification System (BCS), the formulation of the test drug (generic tablet) is categorized as class IV, ie, formulation with low solubility and permeability. With such drug characteristics, proper drug formulation is the key to increase solubility and intestinal permeability, which is expected to increase absorption in order to obtain optimal bioavailability. Drugs classified as BCS IV often show inter/intrasubject variability in the bioavailability/bioequivalence (BA/BE) test.[17]

Bioequivalence studies of highly variable drugs generally use more subjects than studies of drugs with lower variability.[18] However, since 2004, the FDA has been looking for alternative solutions to drugs with high variability. The FDA eventually suggested a replicate crossover design for BE tests for highly variable drugs/products. This provides a good approach for evaluating the BE test of a highly variable drug/drug product, thereby effectively reducing sample size without increasing patient risk.[19]

Although the formula in our present study has been improved based on the results of the first (previous) BE test, the tablets are still bio-inequivalent, so they still need to improve the formula to increase the permeability and solubility. The tablet needs to be reformulated by changing substances to accelerate or adding new substances to increase permeability and solubility. However, one of the factors to determine the success of a BE test is the formulation and manufacturing method of the drug.[20,21] This is in line with the survey results where it is known that about 60% of drugs vary widely due to the characteristics of their active substances, while about 20% of those drugs that are highly variable, it is the performance of the formulation that contributes to the high variability.[22]

CONCLUSION

The difference in the mean value of $AUC_{0\text{-}24}$ between the test product (test = T) and the comparison product (reference = R) with a 90% confidence interval (CI 90%), indicates that the DHP tablet tested is inferior, but based on the T_{max} value, both have the same profile.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this study.

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