

Formulation of Dihydroartemisinin-Piperaquine (DHP) Generic Tablet as Antimalarials Drug

Formulasi Tablet Dihidroartemisinin-Piperakuin (DHP) Generik Sebagai Obat Antimalaria

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Abstract

The incidence of malaria in Indonesia is about two million cases annually. Dihydroartemisinin-piperaquine (DHP) is the first line therapy recommended for uncomplicated malaria treatment, whereas DHP is still fully imported. The generic DHP tablet formulation has the potential to become the first of DHP drug which is locally produced. This study is aimed to formulate generic DHP film coated tablets for antimalaria drug. Tablets were compressed with the combination of wet granulation for piperaquine phosphate (PQP) and direct compression method for DHA and coated with a moisture barrier coating material. The parameters to evaluate the quality of DHP tablets are physical properties, assay, and dissolution test. DHA and PQP assay were performed by HPLC method. The dissolution testing was conducted by in house method using HCl 0.1 N medium. The result shows physical properties of film-coated tablets meet the requirement, i.e. uniform weight, 7.0-8.5 kp hardness, 0.02% friability and 3 minute 22 seconds disintegration. The assay to determine DHA in tablet was 95.17% and PQP was 97.05%. The result of dissolution testing shows the content of DHA and PQP in the tablet were 113.51% and 96.55%, respectively. The formulation which is developed meets the general requirement of API in tablet 90–110% and dissolution requirement >75%.

Keywords: Dihydroartemisinin; Piperaquine; Tablets; Film coated

Abstrak

Kejadian malaria di Indonesia masih sangat tinggi, yaitu sekitar dua juta kasus per tahun. Dihidroartemisinin-Piperakuin (DHP) merupakan terapi lini pertama untuk malaria, namun hingga saat ini sediaan yang ada masih merupakan obat impor. Formulasi tablet DHP generik ini berpotensi menjadi obat DHP pertama yang merupakan produksi dalam negeri. Penelitian ini bertujuan untuk menghasilkan formula tablet DHP generik salut selaput untuk obat antimalaria. Tablet dicetak dengan kombinasi dua metode, yaitu granulasi basah untuk piperakuin fosfat (PQP), dan cetak langsung untuk DHA, kemudian disalut untuk melindungi dari pengaruh kelembaban. Kualitas tablet diukur meliputi pemeriksaan fisik, penetapan kadar, dan uji disolusi. Penetapan kadar DHA dan PQP dilakukan menggunakan sistem Kromatografi Cair Kinerja Tinggi (KCKT). Uji disolusi dilakukan dengan metode in house menggunakan medium HCl 0.1 N. Hasil analisis menunjukkan bahwa tablet hasil formulasi memenuhi seluruh persyaratan fisik, yang terdiri dari memiliki keseragaman bobot, kekerasan 7.0-8.5 kP, keregakan 0.02%, dan waktu hancur 3 menit 22 detik. Penetapan kadar menunjukkan bahwa kandungan zat aktif dalam tablet hasil formulasi adalah 95.17% untuk DHA dan 97.05% untuk PQP. Sementara itu, dari hasil uji disolusi diperoleh kandungan DHA dan PQP dalam tablet masing-masing adalah 113.51% dan 96.55%, yang berarti memenuhi persyaratan umum kandungan zat aktif dalam tablet 90–110% dan persyaratan disolusi >75%.

Kata kunci: Dihidroartemisinin; Piperakuin; Tablet; Salut selaput

INTRODUCTION

Malaria is the most important parasite-infection in human that can be treated by antimalarial drugs. Malarial cases are spread through WHO member countries. Eighty eight percent of global malarial cases was found in African region countries, followed by 10% in South East Asian region. Globally, 214 million people in 2015 were at risk of malarial infection, 438 thousand resulted in death. Global commitment in eliminating malaria successfully reduce malarial incidence and death by 37 and 60 percent, respectively, in year 2000 through 2015.¹

In Indonesia, malaria is still one of the diseases with the highest incidence. The biggest burden of the disease is in eastern provinces of Indonesia, where malaria is endemic. Indonesian climate is perfect for mosquito breeding. The decline in malaria cases and deaths were also reported in Indonesia. Indonesian national malaria prevalence decreases from 10.6% in 2010 to 6% in 2013.^{2,3} Between the years 2000 to 2013, Indonesia reported a decrease in malarial incidence of 5.34% per year and a reduction in malaria deaths of 5.19% per year.⁴ Although malaria cases and deaths in Indonesia has been declining, there are still problems encountered in achieving malaria elimination in 2030. Java and Bali has achieved the status of pre-elimination and elimination, while other areas is still considered as malarial endemic areas. Indonesia has 497 districts, and 54% of it is still categorized as an endemic districts.⁵ Malaria cases occurred in all age groups and the highest is reported in the age group of ≥ 15 years. Thirty three percents of malaria

patients were treated by Artemisinin-based Combination Therapy (ACT), and only 14.46% received ACT within the first 24 hours of fever and took the medication for 3 (three) days.³

Artemisinin-based Combination Therapy (ACT) currently is the best antimalarial medication.⁶ Fixed-dose combinations (FDC) of 40 mg dihydroartemisinin (DHA) and 320 mg of piperaquine phosphate (PQP) is recommended by the program bearer since 2008 and is widely used in Indonesia since 2011.^{7, 8} DHP clinical trials against cases of falciparum and vivax malaria without complications, compared to artesunate-amodiaquine (AS-AQ) and artemether-lumefantrine (AL), showed that DHP has better response (recovery $>95\%$) and mild side effects.^{9,10} In addition, DHP combination is safe, more effective, and cheaper than other ACT.^{11,12} Therefore, DHP tablet is currently listed as antimalarial drug by the national drug formulary.¹³ The high incidence of malaria in Indonesia resulted in high demand of antimalarial drug. However, the real problem is the availability of the drug, whereas DHP is still fully imported. This is the main reason for Indonesian government to formulate and produced DHP locally.

Dihydroartemisinin (DHA) is an unstable active ingredient, easily oxidized and hygroscopic. Film-coating is used to protect the active substances contained in tablets from moisture. This study aims to formulate generic DHP film coated tablet as antimalarial drugs to be produced locally in Indonesia.

METHOD

Design of this study is a laboratory-based experimental research. The study was conducted in March to December 2015 in Pharmacy laboratory in Center for Biomedical and Basic Technology of Health and Research and Development laboratory in PT Indofarma, Tbk. Ethical clearance for this research has been granted from Health Research Ethic Committee, NIHRD, No: LB.02.01/5.2/KE.286/2015. Activities performed in this research include core tablet compression, tablet coating, tablet physical analysis, tablet assay, and dissolution study.

Core tablet

Core DHP tablet was constructed using wet granulation method. Kollidon solution was prepared by dissolving Kollidon K30 in aquadest. Piperaquine starch 1500, LHCP LH 21 were added into the super mixer, followed by Kollidon solution and mixed thoroughly, and then sieved using mesh 20 sieve. Drying was performed using oven. Dried granule, together with DHA, talc, and magnesium stearate were added into cone mixer to be homogenized. This granule mass was compressed using tablet compression machine, weighing 494 mg, using round convex punches, with 12 mm diameter.

Tablet coating

Core tablets were placed inside a coating pan. Warm air were blown through a blower, followed by coating material suspension. Opadry AMB suspension in water was used as a coating material with weight gain targeted at 5%.

Tablet physical analysis

The physical analysis of the tablet were conducted on core and film-coated tablet. Quality testing performed included tablet appearance, weight uniformity, hardness, thickness, friability, and disintegration time.¹⁴

Assay

DHP tablet assay was performed by High Performance Liquid Chromatography (HPLC) instrument (Waters). Twenty DHP tablets were weighed, crushed, and mixed. For active pharmaceutical ingredient (API) assay, sufficient amount of samples equal to 25 mg DHA and 10 mg PQP were taken. Assays for DHA and PQP were conducted separately. DHA sample was put in volumetric flask and then diluted with 60% acetonitrile (ACN) to 25 mL. HPLC method applied for DHA analysis was as follow: Trifluoroacetic acid 0.1%-ACN (40-60) adjust pH to 3.0 by adding diluted ammonium; 1.0 mL/min flow; 20 µL injection volume, and UV detection at 216 nm. PQP samples were diluted with 40% ACN in 50 mL volumetric flask. This sample was analysed using HPLC with phosphar buffer pH 7.0-CAN (60:40) as the eluent; flow rate 1.0 mL/min; 20 µL injection volume; and UV detection at 320 nm.

API content was calculated using the formula as follows:

$$\text{Content (\%)} = \frac{Ru \times Ws \times f}{Rs \times W \times g} \times 100\%$$

Ru : main peak area of test substance
 Rs : main peak area of standard reference
 Ws : weight of API in standard solution
 W : weight of API in sample solution
 f : standard equivalence factor
 g : API content in each tablet

Comparative dissolution testing

DHP tablet dissolution testing was performed using type II dissolution tester (Hanson Vision G2 and AutoPlus®) at 100 rpm of speed, using 900 mL HCl 0.1 N as the medium. Dissolution filtrates were taken at minutes 10, 15, and 30, and it must be at 1 cm from tube wall. Dissolution medium was immediately added at the same volume with filtrate taken to maintain the sink condition. The filtrate was then analyzed using spectrophotometer. Each amount gained was plotted against time when the filtrate taken in order to see the dissolution profile. Furthermore the dissolution profile of sample DHP tablets was compared to DHP innovator tablet.

DHA content in dissolution sample was done by measuring standard reference and test substance absorbance at (λ) 238 nm. The ingredient was calculated by following formula:

$$\text{Content (\%)} = \frac{\text{Au} \times \text{Ws} \times \text{f} \times 0,5}{\text{As} \times 40} \times 100 \%$$

Au: absorbance of test substance
 As: absorbance of standard reference
 Ws: weight of DHA in standard solution, mg
 f : standard equivalence factor

PQP content in dissolution sample was done by measuring standard reference and test substance absorbance at (λ) 345 nm. The

content was calculated utilizing following formula:

$$\text{Content (\%)} = \frac{\text{Au} \times \text{Ws} \times \text{f} \times 5}{\text{As} \times 320} \times 100 \%$$

Au : absorbance of test substance
 As : absorbance of standard reference
 Ws : weight of PQP in standard solution, mg
 f : standard equivalence factor

RESULT AND DISCUSSION

Physical quality, API content, and dissolution profile of DHP tablet samples were performed to evaluate the quality of the tablets. Several parameters were done for physical quality, such as physical appearance, weight uniformity, thickness, hardness, friability, and disintegration time.

Film-coated DHP tablet was made through two steps. Wet granulation for PQP was made as the first step. which aimed to improve the granules' flowability. The second step was to add DHA and directly compress the mixture since DHA is unstable to temperature and humidity.¹⁵ Core tablet was then coated with a blue-coloured moisture barrier coating material to protect it from humidity. Tablet physical testing result is reported in Table 1.

Tablet physical appearances comply with all the requirements. Round shape and blue colour was chosen to match the innovator product. Weight uniformity is a very important parameter in determining tablet quality, because it will affect the API content. For tablets weighing more than 200 mg, no more than 2 tablets are allowed to deviate more than 5% from its mean weight. The result shows that the tablets meet the requirement. TIn addition, this formula have a good flow ability which will produce tablets with uniform weight.

Table 1. Tablet physical testing result

Parameter	Requirement	Test result
Appearance	Round shaped, convex, diameter 12 mm, blue coloured	Round shaped, convex, diameter 12 mm, blue coloured
Core tablet weight (mg)	494 ± 15	483 - 504
Film-coated tablet weight (mg)	510 ± 15	508 – 520
Thickness (mm)	4,5 – 4,9	4,6 – 4,7
Hardness (Kp)	7 – 10	7,0 – 8,5
Friability (%)	< 0,3	0,02
Disintegration time (minutes)	< 30	2'12" – 3'22"

The results of friability tablet test is 0.02%. This is an excellent result since coating process require hard tablet. As stated in United State Pharmacopoeia, a good tablet must have friability not exceed than 1%. Less friability may minimize the risk of tablet damage because during coating process tablets should stand against friction and shock, either between tablet and coating pan or between the tablets itself.

In addition, the friability test is also useful to predict the resistance of a tablet when subjected to mechanical stress or abrasion during the manufacturing process, packaging and transport. Friability parameter is important. When a tablet has high friability, larger amount of tablet will be lost during such process and the amount of the core substance would not be in accordance with the predetermined formula and would compromised the API dose when consumed. The low value of friability is due to the use of appropriate binder in the formulation so that the tablet mass became more compact and harder.

Tablet hardness test was performed to illustrate the tablet resistance to pressure, shock, and erosion during the production process, packaging, transport or distribution. The tablets' hardness value was 7.0 to 8.5 Kp which is qualified as a good tablet. An ideal tablet should be hard enough but also should be arranged (not too hard) in order to comply with tablet disintegration and dissolution requirements. If the tablet is too hard, it will took longer time to be dissolved. Tablet that is too soft can not retain its shape in the next process such as coating, packaging, and delivery.^{16,17}

Tablet disintegration time describes the time required by the tablet to disintegrate in body fluids. The process of tablet disintegration is preceded by the absorption of water so that the tablet can be broken into parts. Film coated tablet was found to have disintegration time ranging from 2 minutes 12 seconds to 3 minutes 22 seconds and meet the requirements of less than 30 minutes for coated tablets. Disintegration time is a prerequisite for dissolution.

Table 2. Core and film-coated DHP tablet assay result

Active ingredients	% assay		
	Requirement	Core tablet	Film-coated tablet
DHA	90-110	98,11	95,17
PQP	90-110	99,73	97,05

The tablet will first destroyed, and then the active ingredients will be separated, dissolved, absorbed and distributed into the target site. Longer disintegration time will result in longer dissolution time so the onset of the drug will be delayed. Good tablet disintegration time is due to the excellent ability of tablet filler in absorbing water and expands. This was also enhanced with the addition of disintegrant that will counter the particle bond resulting in faster tablet disintegration time.^{18, 19}

Assay of the active substance in the tablet DHP was performed using in-house developed methods based on research by D'Acquarica since there is no official assay method for this tablet in reference books such as the Indonesian Pharmacopoeia, USP, and the British Pharmacopoeia. The assay results of core and film coated DHP tablet can be seen in Table 2.

Based on the examination of active ingredient, the formulated DHP tablet formulation complied with the common requirements of active ingredient in the drug. This illustrates that the tablet formula and the granulation method is good enough to be developed into a commercial product because it produces products with eligible level of active ingredients.

DHP tablet dissolution testing is conducted using in-house developed methods because there is no official method yet in reference books. The test was done on

each sample with sampling times at minutes 10, 15 and 30. The minimum level to be expected in minute 30 was 75%. Comparative dissolution test was conducted to determine the quality of drugs based on drug bioavailability predictions compared to the innovator drug. The dissolution properties of a drug directly related to its pharmacological activity since it is a prerequisite for the absorption of the drug and clinical response. The comparative dissolution test of generic DHP tablet compared with the results of DHP innovator tablet is shown in Figure 1.

As reported in Figure 1, the dissolution profile of formulated DHP tablet was similar to the innovator tablet. Comparative dissolution test showed that in generic and innovator DHP tablet, both active ingredients has been dissolved more than 70% in 10 minutes. At the end of testing time (30 minutes), it can be seen that the levels of the active substance released from the generic and innovator tablets DHP has met the requirements, which was more than 75%. Tablet formulation, tablet mixing and compressing method, as well as excipients plays important role in creating tablet with good dissolution profile.^{20,21} The final properties of preparation, such as bioavailability and stability is very dependent on the selected excipient, the amount of excipient used, and its interaction with the active substance or other excipients.

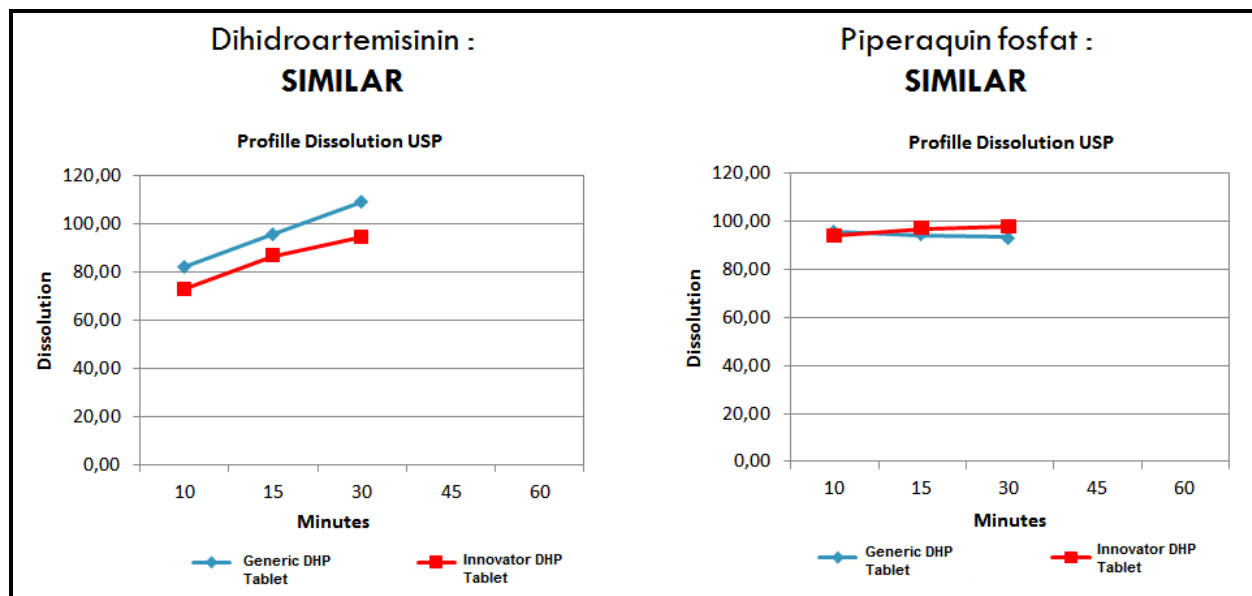


Figure 1. Comparative dissolution test result between generic and innovator DHP tablet

CONCLUSION

Generic film-coated DHP tablet meets all the requirements, e.g physical quality, content uniformity, and dissolution. This formulated generic tablet is very prospective to be up scaled and developed into the first generic DHP tablet produced locally in Indonesia.

SUGGESTION

Further stability testing and bioequivalence study need to be performed on the generic DHP tablet.

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