# Notes on the Design of Bioequivalence Study: Artemether / Lumefantrine

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Unit – Medicines Assessment Team (PQT/MED) are issued to aid manufacturers with the development of their product dossier. Deviations from the approach suggested below can be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines of submission of documentation for WHO prequalification. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-seventh Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2024. WHO Technical Report Series, No. 1052, Annex 8.

Below, additional specific guidance is provided on the invited fixed-dose combination products, containing artemether and lumefantrine.

## Pharmacokinetics of artemether

After oral administration, artemether peak plasma concentrations are reached after about two hours. Concomitant intake of a high fat meal enhances the absorption of artemether, resulting in an increase in the relative bioavailability by more than two-fold.

Artemether is rapidly and extensively metabolized (substantial first pass metabolism) into its main active metabolite, dihydroartemisinin. This metabolite is further converted to inactive metabolites. Artemether and dihydroartemisinin are rapidly cleared from plasma with an elimination half-life of about two hours.

## Pharmacokinetics of lumefantrine

After oral administration of lumefantrine, peak plasma concentrations are observed after about 6–8 hours. Concomitant intake of a high fat meal enhances the absorption of lumefantrine, resulting in an increase in the relative bioavailability by more than 16-fold. In patients with malaria, this increase was only two-fold, probably due to the lower fat content of the food ingested by acutely ill patients. Patients should therefore be encouraged to take the medication with a normal diet as soon as food can be tolerated.

Lumefantrine is metabolized into the active desbutyl-lumefantrine, however, the systemic exposure to this metabolite is low. Lumefantrine is eliminated very slowly with a terminal half-life of 2–3 days in healthy volunteers and 4–6 days in patients with falciparum malaria.

## Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of artemether and lumefantrine, the following guidance with regard to the study design should be taken into account:

**Design:** A single-dose, crossover design is recommended.

**Dose:** The EoI includes 20/mg120 mg, 40mg/240 mg, 60mg/360 mg and 80mg/480 mg tablets as well as 2.5mg/30 mg, 5/mg60 mg and 20mg/120 mg dispersible tablets for children. Each dosage form should be compared with its corresponding comparator product. In the case of the tablets, the highest strength to be developed should be tested, e.g., a single oral dose of the test 80mg/480 mg tablet *versus* four tablets of the comparator 20mg/120 mg tablet. This dose is in line with the recommended dose in adult patients.

In the case of paediatric dispersible tablets, the API amounts are not proportional. Therefore, bioequivalence should be demonstrated in vivo with their corresponding comparators. In the case of the 5mg/60 mg (and 2.5mg/30 mg) dispersible tablets, it is recommended to conduct the bioequivalence study with 1 tablet of the 5/60 mg dispersible tablet (or 2 tablets of the 2.5/30 mg dispersible tablets) vs. 2 tablets of the 2.5mg/30 mg comparator dispersible tablet. In the case of the 20/120 mg dispersible tablet, it is recommended to compare it with 1 tablet of the 20mg/120 mg tablet of the comparator product to administer the same dose of each drug.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. It is considered incorrect to standardise the volume of liquid in all these cases (e.g. administering a glass of water after the intake of a dispersible tablet or rinsing the container where a dispersible tablet has been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions. The total volume of water employed during administration of a paediatric dispersible tablet should not exceed 50 ml.

**Fasted/fed:** As lumefantrine exhibits limited solubility and the manufacturing technology and formulation might affect the bioavailability in fasted and fed state differently, bioequivalence should be demonstrated in principle in both fasted state and fed state, although artemether/lumefantrine tablets are taken only with food (see ICH M13A guideline). The fasted state is recommended to represent the extreme case of a low-fat meal and the fed state study should be conducted with high-fat high-calorie meal. **However, the fasted state study could be waived if the generic and comparator products have similar manufacturing technology and formulation. (Consultation with PQT/MED is suggested to confirm the possibility of a waiver.) In this case, where the fasted state bioequivalence study is waived, the fed state bioequivalence study can be conducted either with a high-fat, high-calorie meal or a low-fat, low-calorie meal.**

**Subjects:** Healthy adult subjects should be used. It is not necessary to include patients in the bioequivalence study.

**Parent or metabolite data for assessment of bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of artemether and lumefantrine.

**Sample size:** Information on artemether and lumefantrine currently available to the PQT/MED indicates that the intra-subject variability for artemether and lumefantrine is around 20-35%. These data will facilitate the calculation of sufficient power for the bioequivalence study.

**Washout:** Taking into account the elimination half-life of lumefantrine in healthy volunteers (2-3 days), a washout period of two or three weeks is considered sufficient to prevent carry over.

**Blood sampling:** As artemether is absorbed rapidly and has a short half-life, blood sampling should be intensive in the first hours after administration to cover the peak of artemether. As lumefantrine is absorbed slowly and has a long elimination half-life, blood sampling should cover 72 hours after administration, e.g. pre-dose and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.33, 3.67, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 8.50, 9.00, 10.00, 12.00, 16.00, 24.00, 36.00, 48.00 and 72.00 hours post dose. It is not necessary to take blood samples over a longer time period, as this will only substantiate the elimination phase lumefantrine.

**Analytical method:** Information currently available to the PQT/MED indicates that it is possible to measure simultaneously artemether, dihydroartemisinin and lumefantrine in human plasma using LC-MS/MS analytical

methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the Cmax in most profiles of each formulation (test or comparator). The bioanalytical method for each analyte should be validated in the presence of the other analyte (see Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information on bioanalytical recommendations).

**Statistical considerations**: The data for artemether and lumefantrine should meet the following bioequivalence standards in a single dose cross-over design study:

- The 90% confidence interval of the relative mean $AUC_{0-t}$ of the test to reference product should be within 80-125%.

- The 90% confidence interval of the relative mean $C_{max}$ of the test to reference product should be within 80-125%.

Information currently available to PQT/MED suggests that the comparator product is a highly variable drug product for both $AUC_{0-t}$ and $C_{max}$ in the fed state. Therefore, if the applicant suspects that the variability of $C_{max}$ or $AUC_{0-t}$ is high (CV>30%), the applicant may prefer to employ a full replicate, crossover study in order to widen the acceptance range of $C_{max}$ and/or $AUC_{0-t}$. For more information on replicate study designs and widening the acceptance limits of average bioequivalence based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 8, TRS 1052 and PQT/MED guidance document "Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED".