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. In particular, 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 20/120 mg, 40/240 mg, 60/360 mg and 80/480 mg tablets as well as 20/120 mg tablets (preferably dispersible) for children. The highest strength to be developed should be tested, e.g. a single oral dose of the test 80/480 mg tablet *versus* four tablets of the reference 20/120 mg tablet. This dose is in line with the recommended dose in adult patients. In the case of paediatric dispersible tablets, the highest strength under development should be compared to the dispersible comparator product.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. In those cases where the test and the comparator product are different dosage forms with different methods of administration (e.g. a tablet that should be taken with a glass of water vs. a dispersible tablet to be dispersed in 10 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. 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 pediatric dispersible tablet should not exceed 50 mL.

Fasting/fed: As it is recommended to take the originator tablets with food, and the absorption is more variable if the tablets are taken in the fasted state, we recommend administration of the tablets with a standard breakfast, not a high-fat, high-calorie meal, as a standard breakfast is considered to be closest to real-life conditions in malaria patients.

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

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 ICH Harmonised Guideline M10 for more information).

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound(s) should be used to assess bioequivalence. Although the results of the parent compound should be used for the decision on bioequivalence, if data are available for the metabolite dihydroartemisinin, pharmacokinetic and statistical results for this metabolite should also be provided as it will help the WHO to understand the relationship between parent and metabolite, and will provide scientific knowledge in the area of decision-making.

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. Widening of the acceptance range for AUC_{0-t} for artemether and/or lumefantrine will be accepted by PQT/MED. Therefore, the applicant may design a replicate cross-over study to estimate variability more accurately and to widen the acceptance range for C_{max} and AUC_{0-t}. For more information on replicate study designs and widening of the acceptance range based on the intra-subject variability of the comparator product, refer to Section 7.9.3 of Annex 8, TRS 1052. If widening of the acceptance range is planned for the AUC_{0-t} parameter, the principles described for C_{max} in Section 7.9.3 will apply and a four period, full replicate design study should be conducted to demonstrate bioequivalence, in order to assess the variability associated with each product.