Notes on the design of bioequivalence study:
Artesunate/Mefloquine

Notes on the design of bioequivalence studies with products invited to be submitted to the WHO Prequalification Team – Medicines (PQTM) 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.


Below, additional specific guidance is provided on the invited immediate release products containing artesunate and mefloquine.

Pharmacokinetics of artesunate and mefloquine
Artesunate is rapidly absorbed after oral administration. Most of the artesunate is promptly biotransformed, mainly through plasma esterases, into the active metabolite dihydroartemisinin (DHA). The median (range) artesunate Tmax value is 0.25 hours (0.25-1.33 h).

Artesunate has a plasma half-life of 3-29 minutes.

When artesunate was taken with a high fat meal in healthy volunteers, the Cmax and AUC0-t of artesunate decreased 66 and 13%, respectively, compared to that observed under fasting conditions. The Cmax and AUC0-t of the active metabolite dihydroartemisinin (DHA) decreased 48 and 5%, respectively, with a high-fat meal, compared to that observed under fasting conditions. Artesunate should not be taken with a high-fat, high-calorie meal.

The maximum plasma concentration of mefloquine is reached within 6 to 24 hours after a single oral dose. The presence of food significantly enhances the rate and extent of absorption. The tablets should be swallowed whole preferably after a meal with plenty of liquid. The RBC concentration of mefloquine is almost twice as high as the plasma level.

The average half-life of mefloquine in Europeans is 21 days. Pharmacokinetic differences have been observed between various ethnic populations.

Guidance for the design of bioequivalence studies:
Taking into account the pharmacokinetic properties of artesunate and mefloquine the following guidance with regard to the study design should be taken into account:

Design: A parallel design is recommended due to the long half-life of mefloquine. A cross-over design would require a wash out period of at least 100 days in principle.

Dose: As the EoI includes artesunate / mefloquine 100 / 200 mg and 25 / 50 mg tablets, the highest strength (100/200 mg) should be tested. As the reference product of mefloquine contains 250 mg and the reference product of artesunate contains 50 mg, the results obtained for mefloquine need to be dose normalized to correct for the difference in administered dose, and two tablets of the reference product for artesunate need to be administered, i.e. 1 x 100/ 200 mg Test product vs. 2 x 50 mg artesunate comparator + 1 x 250 mg mefloquine comparator.
**Fasting/fed:** The bioequivalence study should be conducted in the fed state with standard fat content (500-600 Kcal). A high fat, high calorie meal should not be employed.

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

**Sample size:** Limited information is available on mefloquine variability. Mefloquine values for inter-subject variability seem to range between 35 to 44%. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study. However, a pilot study is recommended to confirm the inter-subject variability, to explore the optimal sampling times, and to explore the feasibility of a cross-over design by investigating the intra-subject variability and the necessary wash-out for a cross-over design.

Artesunate $C_{\text{max}}$ may exhibit high intra-subject variability (47%). Inter-subject variability in a parallel design is expected to be higher. A pilot study is recommended to estimate it.

**Washout:** Not applicable in case of a parallel design and, in principle, at least 100 days in case of a cross-over design. However, a shorter wash-out period may be feasible in the case of multiphasic elimination with most of the being drug eliminated in the initial phases of elimination and plasma levels below 5% of $C_{\text{max}}$ in the final phases of elimination. This can be investigated in the pilot study recommended above.

**Blood sampling:** The blood sampling should be intensive around the first hour for artesunate and around 5.5 hours for mefloquine. It is not necessary to take samples after 72 hours. For example, blood samples might be taken at pre-dose and at 00.17, 00.25, 00.33, 00.50, 00.67, 00.83, 01.00, 01.25, 01.50, 01.75, 02.00, 02.50, 03.00, 03.50, 04.00, 05.00, 06.00, 07.00, 08.00, 10.00, 12.00, 24.00, 48.00 and 72.00 h after drug administration.

**Analytical considerations:** Information currently available indicates that it is possible to measure artesunate and mefloquine in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

**Parent or metabolite data for assessment of bioequivalence:** The parent drugs are considered to best reflect the biopharmaceutical quality of the product. The data for the parent compounds should be used to assess bioequivalence of artesunate and mefloquine.

**Statistical considerations:** The data for artesunate and mefloquine should meet the following bioequivalence standards in a single-dose parallel or cross-over design study:

- The 90% confidence interval of the relative mean $\text{AUC}_{0-t}$ for artesunate and $\text{AUC}_{0-72h}$ for mefloquine of the test to reference product should be within 80-125%.

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