Notes on the design of bioequivalence study: Amodiaquine/Artesunate

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 amodiaquine/artesunate.

Pharmacokinetics of amodiaquine and artesunate

Amodiaquine is quickly absorbed and biotransformed into its main active form, desethylamodiaquine. The median amodiaquine $T_{max}$ value is 0.91 h.

Amodiaquine is eliminated principally through biotransformation with only around 2% excreted unchanged in urine. Its half-life is 24-28 hours.

When amodiaquine was taken with a high fat meal in healthy volunteers, the $C_{max}$ and $AUC_{0-t}$ of amodiaquine increased 23% and 58%, respectively, compared to that observed under fasting conditions. The $C_{max}$ and $AUC_{0-t}$ of the active metabolite desethylamodiaquine increased 18% and 12%, respectively, following a high-fat meal, compared to that observed under fasting conditions.

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 $T_{max}$ 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 $C_{max}$ and $AUC_{0-t}$ of artesunate decreased 66% and 13%, respectively, compared to that observed under fasting conditions. The $C_{max}$ and $AUC_{0-t}$ of the active metabolite dihydroartemisinin (DHA) decreased 48% and 5%, respectively, with a high-fat meal, compared to that observed under fasting conditions.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of amodiaquine and artesunate the following guidance with regard to the study design should be taken into account:

**Design:** A cross-over design is recommended.

**Dose:** As the EoI includes Artesunate/Amodiaquine tablet 25 mg / 67.5 mg, 50 mg / 135 mg, 100 mg / 270 mg, the highest strength 100/270 mg should be tested.
**Fasting/fed:** The bioequivalence study should be conducted in the fasted state since the amodiaquine/artesunate fixed combination should not be taken with a high-fat meal and, although it is generally taken after meals, this seems to be related to tolerability.

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

**Sample size:** Amodiaquine and Artesunate $C_{\text{max}}$ may exhibit high variability at 37 and 47%, respectively, whereas their AUCs do not exhibit high variability at 30 and 22%, respectively. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** Taking into account the elimination half-life of amodiaquine and artesunate at 24 - 28 h and 0.6 h, respectively, a wash out period of 2 weeks is recommended.

**Blood sampling:** The blood sampling should be intensive in the first 2 hours since the median $T_{\text{max}}$ occurs before 1 hour for both drugs. It is not necessary to take samples after 72 hours for amodiaquine. 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 hours after drug administration.

**Analytical considerations:** Information currently available indicates that it is possible to measure amodiaquine and artesunate 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 amodiaquine and artesunate.

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

- The 90% confidence interval of the relative mean $AUC_{0-72h}$ for amodiaquine and $AUC_{0-t}$ for artesunate 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 for both drugs should be within 80-125%.

Information currently available to PQTm suggests that the comparator product is a highly variable drug product for both $C_{\text{max}}$ in the fasted state. Widening of the acceptance range for $AUC_{0-t}$ is not acceptable, but the applicant may design a replicate cross-over study to estimate variability more accurately and to widen the acceptance range for $C_{\text{max}}$. For more information on replicate study designs and average scaled bioequivalence, refer to Section 7.9.3 of Annex 7, TRS 992.