Notes on the design of bioequivalence study: 
Amodiaquine/Artesunate

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.


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

Pharmacokinetics of amodiaquine and artesunate

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

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

When amodiaquine was taken with a high fat meal in healthy volunteers, the $C_{\text{max}}$ and $AUC_{0-t}$ of amodiaquine increased 23% and 58%, respectively, compared to that observed under fasted conditions. The $C_{\text{max}}$ 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 converted, mainly through plasma esterases, into the active metabolite dihydroartemisinin (DHA). The median (range) artesunate $T_{\text{max}}$ value is 0.25 hours (0.25-1.33 h).

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

When artesunate was taken with a high fat meal in healthy volunteers, the $C_{\text{max}}$ and $AUC_{0-t}$ of artesunate decreased 66% and 13%, respectively, compared to that observed under fasting conditions. The $C_{\text{max}}$ 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 single-dose cross-over design is recommended.

Dose: As the EoI includes artesunate/amodiaquine tablet 25 mg / 67.5 mg (preferably dispersible), 50 mg / 135 mg, and 100 mg / 270 mg, the highest strength 100/270 mg should be tested.
**Fasted/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 recruited. It is not necessary to include patients in the bioequivalence study.

**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.

**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 of 24 – 28 h and 0.6 h, respectively, a washout period of 2 weeks is considered sufficient to prevent carry-over.

**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). The bioanalytical method of amodiaquine should be validated in the presence of artesunate and vice versa.

**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 $AUC_{0-1}$ for artesunate of the test to reference product should be within 80.00–125.00%.

- The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product for both drugs should be within 80.00–125.00%.

Information currently available to PQT/MED indicates that the comparator product is a highly variable drug product. Therefore, if the Applicant suspects that the variability of $C_{\text{max}}$ or $AUC_{0-72h}$ / $AUC_{0-1}$ is high (CV>30%), the applicant may prefer to employ a full replicate design study in order to widen the acceptance range of $C_{\text{max}}$ and/or $AUC_{0-72h}$ / $AUC_{0-1}$. For more information on replicate study designs and widening the limits of average bioequivalence, refer to Section 7.9.3 of Annex 6, TRS 1003 and PQT/MED guidance document “Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED”.

---

Notes on the design of bioequivalence study: amodiaquine and artesunate