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.

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 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_{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_{max} and AUC_{0-t} of amodiaquine increased 23% and 58%, respectively, compared to that observed under fasted 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 converted, 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 elimination 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 single-dose crossover design is recommended.

Dose: As the EoI includes artesunate/amodiaquine 25 mg / 67.5 mg and 50 mg / 135 mg dispersible tablets, as well as 50 mg / 135 mg and 100 mg / 270 mg tablets, the highest strength of the applied series should be tested.

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.

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_{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_{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_{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 (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 amodiaquine and artesunate should meet the following bioequivalence standards in a single-dose, crossover design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72h}$ for amodiaquine and $AUC_{0\text{-}t}$ for artesunate of the test to reference product should be within 80.00–125.00%
- The 90% confidence interval of the relative mean C_{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 for both AUC and/or C_{max}. Therefore, if the Applicant suspects that the variability of C_{max} or AUC_{0-72h} / AUC_{0-t} is high (CV>30%), the applicant may prefer to employ a full replicate design study in order to widen the acceptance range of C_{max} and/or AUC_{0-72h} / AUC_{0-t}. For more information on replicate study designs and widening the 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".