Notes on the Design of Bioequivalence Study: Artesunate / Pyronaridine

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 artesunate and pyronaridine.

Pharmacokinetics of artesunate and pyronaridine

Peak plasma concentrations are generally reached between 0.5 and 1.0 hours post-dose for artesunate, and between 2 and 8 hours post-dose for pyronaridine. Exposure to artesunate and pyronaridine was increased by 34% and 20% respectively when artesunate / pyronaridine was administered with a high fat meal, however, these effects were not judged clinically significant, and patients can take artesunate / pyronaridine tablets without regard to meals.

Pyronaridine is eliminated slowly from blood, with an elimination half-life in adults of between 14 - 18 days for the parent compound. In contrast, artesunate has a plasma half-life of 3 – 29 minutes.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of artesunate and pyronaridine 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 Artesunate/Pyronaridine 20 mg/60 mg granules for oral suspension and 60 mg/180 mg tablets. The comparator product is marketed as 20 mg/60 mg granules and 60 mg/180 mg tablets. As these two dosage forms of the comparator product are not bioequivalent, each strength of the test product should be compared with their corresponding comparator strength and dosage form.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use (e.g., disperse the granules with 10 ml of water in a small cup and rinse with additional 10 ml of water until no granules remain in the cup). It is considered incorrect to standardise the volume of liquid in all these cases (e.g., administering a glass of water to disperse the granules or rinsing the container where the granules have been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions.

Fasted/fed: The bioequivalence study should be conducted under fasted conditions since the artesunate/pyronaridine fixed combination can be taken with or without meals.

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 artesunate and pyronaridine.

Sample size: Artesunate C_{max} may exhibit high intra-variability (43-56%), whereas its AUC does not exhibit high intra-subject variability (28%). The intra-subject variability of pyronaridine seems to be moderate (28% for C_{max} and 12% for AUC). These data may facilitate the calculation of a sufficient sample size for a single-dose cross-over bioequivalence study.

Washout: A wash-out period of 6-8 weeks seems to be sufficient to reduce plasma levels below 5% of C_{max}, although half-life of pyronaridine is of 14-18 days, because the elimination of pyronaridine is multiphasic and the half-life appears to be only 6-8 days initially.

Blood sampling: The blood sampling should be intensive in the first hour since median Tmax of artesunate occurs before 1 hour after drug administration. In contrast, median Tmax of pyronaridine occurs at 4 hours post-dose. It is not necessary to take samples after 72 hours for pyronaridine. For example, blood samples might be taken at pre-dose and at 0.083, 0.17, 0.25, 0.33, 0.50, 0.67, 0.83, 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. However, it is not necessary to measure both drugs in all samples since artesunate is not measurable in the last sampling times and frequent sampling in the first hour after drug administration is not necessary for pyronaridine.

Analytical considerations: Information currently available indicates that it is possible to measure artesunate in human plasma and pyronaridine in plasma or whole blood using LC-MS/MS analytical methodology with lower limits of quantification of at least 1.2 ng/ml and 1.47 ng/ml, respectively. 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), which is possible for pyronaridine in all profiles, but it may be difficult in the case of artesunate if a single tablet is administered. The bioanalytical method for each analyte should be validated in the presence of the other analyte (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 artesunate and pyronaridine 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 pyronaridine and $AUC_{0\text{-}t}$ for artesunate of the test to comparator product should be within 80.00 – 125.00%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product for both drugs should be within 80.00 – 125.00%.

Information currently available to PQT/MED suggests that the artesunate C_{max} in the comparator product is highly variable. Therefore, if the Applicant suspects that the variability of C_{max} or $AUC_{0\text{-}t}$ 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 $AUC_{0\text{-}t}$. For more information on replicate study designs and widening of the acceptance limits 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". The long washout period required for this study may make such a design impractical. However, it is also possible to conduct separate bioequivalence studies with different design for each component of the combination if prespecified in the study protocols.