Notes on the Design of Bioequivalence Study: Dihydroartemisinin / Piperaquine Tetraphosphate

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 fixed dose combination products containing dihydroartemisinin and piperaquine phosphate.

Pharmacokinetics of dihydroartemisinin

After oral administration, dihydroartemisinin peak plasma concentrations are reached after approximately 1 – 2 hours. Inter-subject variability was observed to be approximately 47% in C_{max} and 45% in AUC. Concomitant intake of a high fat meal enhances the absorption of dihydroartemisinin, resulting in an increase in the relative bioavailability by about 44%.

Dihydroartemisinin is rapidly cleared from plasma with an elimination half-life of about 1 – 2 hours. The AUC of dihydroartemisinin seems to increase more than proportionally with increasing dose.

Pharmacokinetics of piperaquine

After oral administration of piperaquine tetraphosphate, peak plasma concentrations of piperaquine are observed after approximately five hours. Inter-subject variability was observed to be approximately 62% in C_{max} and 47% in AUC. Concomitant intake of a high fat meal enhances the absorption of piperaquine, resulting in an increase in the relative bioavailability by approximately 2.7 to 3.2-fold.

Piperaquine is eliminated very slowly with a terminal half-life of about 22 days in healthy volunteers. The AUC of piperaquine seems to increase proportionally with dose.

The tablets of dihydroartemisinin and piperaquine tetraphosphate should be taken under fasting conditions.

Guidance for the design of bioequivalence studies:

Considering the pharmacokinetic properties of dihydroartemisinin and piperaquine, the following guidance with regard to the study design should be taken into account:

Design: A single-dose, crossover design is recommended despite the long half-life of piperaquine. However, a parallel study design might also be acceptable. It may also be possible to conduct a separate study for each drug, e.g., a replicate crossover design study for dihydroartemisinin, since it is a highly variable drug with short half-life,

to widen the acceptance range for AUC and C_{max} based on the intrasubject variability of this active substance in the comparator product, and also a two-period two-sequence design study to show bioequivalence for piperaquine. In this latter case, both studies should be conducted with the same batches of the test and the comparator products.

Dose: The EoI includes dihydroartemisinin and piperaquine phosphate 60 mg/480 mg and 80mg/640 mg tablets as well as the paediatrc strengths, 20 mg/160 mg, 30 mg/240 mg and 40 mg/320 mg tablets (preferably dispersible) In those cases where several strengths are developed, one bioequivalence study is necessary for the highest strength (e.g., 80 mg/640 mg), and the other strengths (e.g., 60 mg/480 mg, 40 mg/320 mg, 30 mg/240 mg and 20 mg/160 mg) could be biowaived if the conditions for the additional strengths biowaivers are fulfilled. As the bioequivalence study should be conducted with the highest strength applied and that strength is not available in the reference product, several units may be administered to reach the same dose (e.g. 1 x 80 mg/640 mg test tablet *vs.* 2 x 40 mg/320 mg comparator tablet if all the strengths are in one series or, two studies, one with 80 mg/640 mg for the adult tablets and another with 40 mg/320 mg for the paediatric dispersible tablets (e.g. 1 x 40 mg/320 mg dispersible test tablet *vs.* 1 x 40 mg/320 mg comparator tablet), if the adult and paediatric strengths are separate series).

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 30 – 50 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardize 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 standardization does not occur in real life conditions. The total volume of water employed during administration of a paediatric dispersible tablet should not exceed 50 ml.

Fasted/fed: As dihydroartemisinin and piperaquine tetraphosphate tablets should be taken under fasting conditions, the study should be carried out under fasting conditions.

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 drug is considered to best reflect the biopharmaceutical quality of the proposed product. Therefore, bioequivalence should be based on the determination of dihydroartemisinin and piperaquine.

Sample size: Information on dihydroartemisinin and piperaquine currently available to PQT/MED indicates that the intra-subject variability of dihydroartemisinin ranges from 21.4% to 29.9% for AUC and from 30.3 to 41.1% for C_{max}. Intra-subject variability of piperaquine ranges from 22.2% to 25.8% for AUC and 29.2% to 38.7% for C_{max}. Inter-subject variability for dihydroartemisinin for C_{max} is around 50%, while for piperaquine it is around 60%. These data will facilitate the calculation of sufficient power for the bioequivalence study.

Washout: If a crossover design is employed, taking into account the long elimination half-life of piperaquine, a washout period of at least 100 days may be necessary to prevent a significant carry over (i.e. after 100 days, pre-dose concentrations are observed, but they are lower 5% of Cmax, except in exceptional cases). If bioequivalence for dihydroartemisinin is investigated in a different study, the wash out period of this study only needs to be 3 – 7 days since dihydroartemisinin half-life is short.

Blood sampling: As dihydroartemisinin has a short half-life, blood sampling should be intensive in the first 8 – 10 hours after administration to cover the rate and extent of absorption of dihydroartemisinin. As piperaquine has a long elimination half-life, blood sampling should cover 72 hours after administration. It is not necessary to take blood samples over a longer time period, as this will only substantiate the elimination phase of piperaquine.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure dihydroartemisinin and piperaquine in human plasma using LC-MS/MS analytical methodology (LLOQ of 1 ng/ml

for dihydroartemisinin and 0.3 ng/ml for piperaquine). The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the Cmax in most profiles of each formulation (test or comparator). 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 dihydroartemisinin and piperaquine should meet the following bioequivalence standards in a single dose, crossover or parallel design study:

Dihydroartemisinin:

- The 90% confidence interval of the relative mean AUC_{0-t} 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 should be within 80.00 – 125.00%.

Piperaquine:

- The 90% confidence interval of the relative mean AUC_{0-72h} 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 should be within 80.00 – 125.00%.

Information currently available to PQT/MED suggests that the comparator product is a highly variable drug product for C_{max} of didroartemisinin and piperaquine in the fasted state. Therefore, if the applicant suspects that the intra-subject variability of C_{max} or AUC_{0-t} is high (CV>30%) for any of the active compounds, the applicant may prefer to employ a full replicate, crossover study in order to widen the acceptance range of C_{max} and/or AUC_{0-t}. For more information on replicate study designs and widening the acceptance 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".