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. In particular, 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 60/480 mg and 80/640 mg tablets, as well as dihydroartemisinin and piperaquine phosphate 20/160 mg, 30/240 mg and 40/320 mg paediatric formulations, preferably dispersible.

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 slightly 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.

According to the SmPC of Eurartesim, the tablets should be taken under fasting conditions. Dosing should be based on body weight, i.e. patients weighing 5 to < 7 kg should receive one half of the 20/160 mg tablet, patients weighing 7 to < 13 kg should receive one 20/160 mg tablet, patients weighing 13 to < 24 kg should receive one 40/320 mg tablet, patients weighing 24 to < 36 kg should receive two 40/320 mg tablets, patients weighing 36 to < 75 kg should receive three 40/320 mg tablets and patients weighing 75 – 100 kg should receive four 40/320 mg tablets.

Guidance for the design of bioequivalence studies:

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

Design: A two-period, randomized crossover study 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. A replicate design study for dihydroartemisinin, since it is a highly variable drug with short half-life, to widen the acceptance range for 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: In those cases where several strengths are developed, one bioequivalence study is planned for one of the strengths (e.g., 80/640 mg), and the other strengths (e.g., 60/480 mg, 40/320 mg, 30/240 mg and 20/160 mg) are designed to be biowaived as additional strengths, the bioequivalence study should be conducted with the highest strength (e.g. 1 x 80/640 mg test tablet *vs.* 2 x 40/320 mg comparator tablet if all the strengths are in one series or, two studies, one with 80/640 mg for the adult tablets and another with 40/320 mg for the paediatric dispersible tablets (e.g. 1 x 40/320 mg dispersible test tablet *vs.* 1 x 40/320 mg comparator tablet), if the adult and paediatric strengths are separate series). Multiple tablets can be used if necessary (but equivalent doses for the two treatments in a study should be maintained).

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 maximum volume employed should not exceed 50 ml.

Fasted/fed: As it is recommended to take the originator tablets 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%.

Washout: If a crossover design is employed, taking into account the long elimination half-life of piperaquine (as indicated in the European Medicine's Agency Public Assessment Report for Eurartesim), 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 reach a LLOQ of 1 ng/ml for dihydroartemisinin and 0.3 ng/ml for piperaquine. After administration of one tablet of 40/320 mg to subjects of 60-62 kg on average, dihydroartemisinin C_{max} values of 90 – 156 ng/ml were reached (ranging from 17 to 734 ng/ml) and C_{max} values of piperaquine of 23 – 26 ng/ml were observed (ranging from 4.9 to 97 ng/ml). After administration of one tablet of 80/640 mg to subjects of 65 kg on average, dihydroartemisinin C_{max} values of 275 – 287 ng/ml were reached (ranging from 64 to 1025 ng/ml) and C_{max} values of piperaquine of 91 – 94 ng/ml were observed (ranging from 16 to 274 ng/ml). After administration of four tablets of 40/320 mg to subjects weighing > 75 kg, C_{max} values of about 250 and 200 ng/ml may be expected for dihydroartemisinin and piperaquine, respectively. Considering the dose/exposure relationship of both analytes (see EMA EPAR Eurartesim), comparable values can be expected in case of administration of three tablets to subjects weighing less than 75 kg. For dihydroartemisinin, the analytical method should be sufficiently sensitive to evaluate plasma concentrations over approximately 3–4 half-lives, and to fulfil the requirement of AUC_t/AUC_{inf} >80%. For piperaquine, only in the case of a crossover study, the analytical method should be sufficiently sensitive to analyse plasma concentrations up to 5% of C_{max} to prevent carry-over. In case of a parallel designed study, the analytical method should be sufficiently sensitive to analyse plasma concentrations up to 72 hours after administration. The bioanalytical method for each analyte should be validated in the presence of the other analyte (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: In the case of a single-dose two-period crossover study or a single-dose parallel study, the data should meet the following bioequivalence standards:

Dihydroartemisinin:

- The 90% confidence interval of the relative mean AUC_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 $AUC_{0\text{-}72}$ 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%.

In the case of a single dose, replicate, crossover design study, the C_{max} acceptance range can be widened based on the intra-subject variability observed for the comparator product since 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_{max} is high (CV > 30%), the applicant may prefer to employ a replicate design study in order to scale the acceptance range of C_{max}. For more information on replicate study designs and average scaled bioequivalence, refer to Section 7.9.3 of Annex 8, TRS 1003.