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 Team: medicines (PQTm) 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 fixed dose combination products, containing 20 mg dihydroartemisinin + 160 mg piperaquine tetraphosphate and 40 mg dihydroartemisinin + 320 mg piperaquine tetraphosphate.

**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 $C_{\text{max}}$ 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.

**Pharmacokinetics of piperaquine tetraphosphate**

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

Piperaquine tetraphosphate is eliminated very slowly with a terminal half-life of about 22 days in healthy volunteers.

According to the SmPC of Eurartesim, the tablets should be taken under fasting conditions. Dosing should be based upon body weight, i.e. 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 tetraphosphate, the following guidance with regard to the study design should be taken into account:

**Study design:** A two-period, randomized crossover study can be undertaken. However, considering the long elimination half-life of piperaquine tetraphosphate, a parallel study design is also acceptable.

**Dose:** A single oral dose of three tablets of dihydroartemisinin + piperaquine tetraphosphate 40/320 mg should be employed in the bioequivalence study. This dose advice takes into account the recommended dose in adult patients and the need to obtain sufficiently high plasma concentrations for dihydroartemisinin and piperaquine tetraphosphate for pharmacokinetic analysis.

**Fasting/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 utilized. It is not necessary to include patients in the bioequivalence study.

**Power:** Information on dihydroartemisinin and piperaquine tetraphosphate currently available to PQTm indicates that the inter-subject variability for dihydroartemisinin for C<sub>max</sub> is around 50%, while for piperaquine tetraphosphate it is around 60%. Intra-subject variability is currently not known. The inter-subject variability data may facilitate the calculation of sufficient power for the bioequivalence study, however, it will be likely an overestimation of the power, as the intra-subject variability is normally lower.

**Washout:** If a crossover design is to be employed, taking into account the long elimination half-life of piperaquine tetraphosphate (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 carry over. However, other literature, e.g. Chinh et al.,<sup>1</sup> suggests low piperaquine tetraphosphate plasma levels during the elimination phase may permit the washout period to be shorter.

**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 tetraphosphate 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 tetraphosphate.

**Analytical method:** Information currently available to PQTm indicates that after administration of four tablets 40/320 mg to subjects weighing > 75 kg, C<sub>max</sub> values of about 250 and 200 ng/ml may be expected for dihydroartemisinin and piperaquine tetraphosphate, 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 AUCt/AUCinf >80%. For piperaquine tetraphosphate, only in the case of a crossover study, the analytical method should be sufficiently sensitive to analyse plasma concentrations up to 5% of C<sub>max</sub> 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.

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**Parent or metabolite data for assessment of bioequivalence**: The parent drug is considered to best reflect the biopharmaceutical quality of the proposed product.

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

**Dihydroartemisinin**:

- The 90% confidence interval of the relative mean $AUC_T$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{max}$ of the test to reference product should be within 80–125%.

**Piperaquine tetraphosphate**:

- The 90% confidence interval of the relative mean $AUC_{0-72}$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{max}$ of the test to reference product should be within 80–125%.

In case of a single dose, replicate crossover designed study, the data should meet the following bioequivalence standards:

Information currently available to PQTm 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 for at least the comparator product 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 7, TRS 992.