Notes on the design of bioequivalence study:
Artesunate/Pyronaridine

Notes on the design of bioequivalence studies with products invited to be submitted 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 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 Pyramax was administered with a high fat meal, however, these effects were not judged clinically significant and patients can take Pyramax 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 cross-over design is recommended.

**Dose:** As the EoI includes Artesunate/Pyronaridine tablet 60/180 mg only, this strength should be tested. If necessary, four tablets can be administered simultaneously in order to obtain measurable plasma levels.

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

**Sample size:** Artesunate Cmax 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 Cmax and 12% for AUC). These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.
**Washout:** A wash-out period of 6-8 weeks seems to be sufficient to reduce plasma levels below 5% of Cmax, 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 artemisinin occurs before 1-hour post-dose. 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 artemisinin is not measurable in the last sampling times and frequent sampling in the first hour post-dose is not necessary for pyronaridine.

**Analytical considerations:** Information currently available indicates that it is possible to measure artemisinin in human plasma and pyronaridine in plasma or whole blood using LC-MS/MS analytical methodology. 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).

**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 artemisinin and pyronaridine.

**Statistical considerations:** The data for artemisinin and pyronaridine should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean AUC_{0-72h} for pyronaridine and AUC_{0-t} for artemisinin 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 for both drugs should be within 80-125%.

Given the suspected high intra-subject variability of artemisinin Cmax, an applicant may wish to consider to design a replicate design study for at least the comparator product in order to scale the acceptance range of artemisinin Cmax, however, the long washout period required for this study may make such a design impractical. Refer to Section 7.9.3 of Annex 7, TRS 992 for more information.