Notes on the design of bioequivalence study: Pyrimethamine

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 pyrimethamine.

Pharmacokinetics of pyrimethamine

Pyrimethamine is well absorbed with peak levels occurring between 2 to 6 hours following administration. It is eliminated slowly and has a plasma half-life of approximately 96 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose parallel design is recommended.

Dose: As the EoI includes only pyrimethamine 25 mg tablets, this dose should be tested.

Fasted/fed: The bioequivalence study can be conducted in the fasted state since it is taken with food to avoid vomiting, and a clinically different exposure when taken with food has not been reported.

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 product. Therefore, bioequivalence should be based on the determination of pyrimethamine.

Sample size: Pyrimethamine inter-subject variability values is low (11-14%) for both C_{max} and AUC. These data may facilitate the calculation of a sufficient sample size for the parallel bioequivalence study.

Washout: N/A. Taking into account the long elimination half-life of the pyrimethamine a parallel design is recommended.

Blood sampling: The blood sampling should be intensive between 2 and 7 hours. It is not necessary to take samples after 72 hours. For example, blood samples might be taken at pre-dose, 0.50, 1.00, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 9.00, 10.00, 12.00, 24.00, 48.00 and 72.00 hours after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure pyrimethamine in human plasma using LC-MS/MS analytical methodology. 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). The bioanalytical method should be validated according to the WHO Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6.

Statistical considerations: The data for pyrimethamine should meet the following bioequivalence standards in a single-dose parallel design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72h}$ of the test to reference product should be within 80.00 – 125.00%

- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80.00 – 125.00%.