Notes on the design of bioequivalence study: Pyrimethamine / Sulfadoxine

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 and sulfadoxine.

Pharmacokinetics of pyrimethamine and sulfadoxine

After oral administration of a single tablet containing 500 mg of sulfadoxine and 25 mg of pyrimethamine the following pharmacokinetic parameters were reported:

Peak plasma levels of sulfadoxine are reached in approximately 4 – 5.5 hours (range 1.5 to 8 hours). Sulfadoxine has an elimination half-life of approximately 200 hours (range 100 – 250 hours).

Peak plasma levels of pyrimethamine are achieved within 2.1 – 7.7 hours (median 5.5 h) and it has an elimination half-life of approximately 100 hours (range 54 to 148 hours).

The tablets should be swallowed whole with plenty of fluid after a meal.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of pyrimethamine and sulfadoxine, 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 pyrimethamine and sulfadoxine 12.5/250 mg tablets (preferably dispersible for paediatric use) and 25/500 mg tablets (scored, or scored and dispersible), the highest strength (25/500 mg) should be tested.

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 10 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardise 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 standardisation does not occur in real life conditions. The total volume of water employed during administration of a pediatric dispersible tablet should not exceed 50 mL.

Fasted/fed: The bioequivalence study can be conducted in the fasted state since, although the combination is taken after meals, this seems to be related to tolerability, 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 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 pyrimethamine and sulfadoxine.

Sample size: Pyrimethamine and sulfadoxine inter-subject variability values are low (11-14% for pyrimethamine and 10-11% for sulfadoxine) 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 and sulfadoxine a parallel design is recommended.

Blood sampling: The blood sampling should be intensive between 3 and 7 hours since median T_{max} occurs at 5.5 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, 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 and sulfadoxine 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 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).

Statistical considerations: The data for pyrimethamine and sulfadoxine 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%.