

Notes on the design of bioequivalence study: Sulfamethoxazole/Trimethoprim

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 immediate release products containing sulfamethoxazole and trimethoprim.

Pharmacokinetics of sulfamethoxazole and trimethoprim

After oral administration trimethoprim and sulfamethoxazole are rapidly and nearly completely absorbed. The presence of food does not appear to delay absorption. Peak levels in the blood occur between one and four hours after ingestion and the level attained is dose related.

The elimination half-life of trimethoprim in man is in the range 8.6 to 17 hours in the presence of normal renal function.

The elimination half-life of sulfamethoxazole in man is approximately 9 to 11 hours in the presence of normal renal function.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of sulfamethoxazole and trimethoprim, the following guidance with regard to the study design should be considered:

Design: A single-dose cross-over design is recommended.

Dose: As the EoI includes Sulfamethoxazole /Trimethoprim tablets at the strengths of 400 mg/80 mg and 800 mg/160 mg, as well as in combination with Isoniazid /Pyridoxine at these same strengths, the highest strength of 800mg/160 mg is recommended for the bioequivalence study.

Fasted/fed: The bioequivalence study should be conducted in the fasted state.

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. The data for the parent compound should be used to assess bioequivalence of sulfamethoxazole and trimethoprim.

Sample size: The intra-subject variability of $C_{\max}$ of sulfamethoxazole and trimethoprim is approximately 18% and 14%, respectively. This data may facilitate the calculation of a sufficient sample size for the cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of sulfamethoxazole (up to 11 h) and of trimethoprim (up to 17 h), a washout period of at least 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive during the first four hours after administration to properly characterize the $C_{\max}$ of sulfamethoxazole and trimethoprim. Considering the elimination half-lives, it is sufficient to take blood samples up to 72 hours after administration for the characterization of sulfamethoxazole and trimethoprim pharmacokinetics. For example, samples may be taken at pre-dose and at 0.25, 0.50, 1.00, 1.50, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.50, 5.00, 6.00, 8.00, 12.00, 18.00, 24.00, 30.00, 36.00, 48.00, and 72.00 hours after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure sulfamethoxazole and trimethoprim 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). As per ICH M10, the bioanalytical method of sulfamethoxazole should be validated in the presence of trimethoprim and vice versa.

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

- The 90% confidence interval of the relative mean AUC_{0-t} 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%.