Notes on the Design of Bioequivalence Study: Sulfamethoxazole/Trimethoprim/Isoniazid/Pyridoxine

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, trimethoprim, isoniazid and pyridoxine.

Pharmacokinetics of sulfamethoxazole, trimethoprim, isoniazid and pyridoxine

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.

After oral administration isoniazid produces peak blood levels within 1 to 2 hours. Ingestion of isoniazid with food may reduce its absorption. Isoniazid should be administered preferably on an empty stomach at least 30 minutes before a meal or 2 hours after a meal. Isoniazid is metabolised primarily by acetylation and dehydrazination. The rate of acetylation is genetically determined. The elimination half-life in fast acetylators is 0.5 - 1.6 h and in slow acetylators is 2 – 5 h approximately.

Pyridoxine peak absorption is observed after 1.3 h and its half-life is very short (0.75 h), which may be responsible for a large intra-subject variability. Pyridoxine is considered as a highly soluble and highly permeable drug substance.

Guidance for the design of bioequivalence studies

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

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

Dose: As the EoI includes sulfamethoxazole / trimethoprim / isoniazid / pyridoxine scored tablet containing 800 mg / 160 mg / 300 mg / 25 mg and 400 mg / 80 mg /150 mg /12.5 mg, the bioequivalence study should be conducted with the highest strength with the corresponding fixed combination of sulfamethoxazole and trimethoprim, and the individual comparator of isoniazid.

Fasted/fed: The bioequivalence study should be conducted in 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, trimethoprim and isoniazid.

Pyridoxine component may exceptionally be compared with its comparator based on in vitro dissolution testing under the conditions of BCS biowaivers for BCS class I drugs.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for C_{max} of isoniazid is the most variable parameter of this drugs since it ranges from 15 to 29%. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of sulfamethoxazole (up to 11 h), trimethoprim (up to 17 h) and isoniazid (up to 5 h), a washout period of at least 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first four hours after administration to properly characterize the C_{max} of sulfamethoxazole, trimethoprim, and isoniazid. However, for isoniazid a more frequent sampling is required compared to sulfamethoxazole and trimethoprim. Similarly, although it is necessary to take blood samples up to 72 hours after administration for the characterization of sulfamethoxazole and trimethoprim pharmacokinetics considering it long half-life, it is not necessary to take blood samples beyond 12 hours for the characterization of isoniazid plasma concentration – time profile. For example, blood samples can be taken pre-dose and at 0.17, 0.33, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 5.00, 6.00, 8.00, 12.00 hours for sulfamethoxazole, trimethoprim and isoniazid (some of which can be skipped for sulfamethoxazole and trimethoprim) and 18.00, 24.00, 30.00, 36.00, 48.00, and 72.00 h for sulfamethoxazole and trimethoprim.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure sulfamethoxazole, trimethoprim and isoniazid 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 each of these drugs should be validated in the presence of the other drugs.

Statistical considerations: The data for sulfamethoxazole, trimethoprim, and isoniazid 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%.