Notes on the Design of Bioequivalence Study: Isoniazid / Pyrazinamide / Rifampicin

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (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 isoniazid, pyrazinamide and rifampicin.

Pharmacokinetics of Isoniazid, Pyrazinamide, and Rifampicin

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. Elimination half-life in fast acetylators is 0.5 - 1.6 h and in slow acetylators is 2 – 5 h approximately.

Pyrazinamide is readily absorbed from the gastrointestinal tract. Peak concentrations occur about 2 hours after an oral dose. Plasma elimination half-life is about 9-10 hours.

Rifampicin is readily absorbed and T_{max} occurs about 2 - 4 hours after administration on an empty stomach. Absorption of rifampicin is reduced when the drug is ingested with food. In normal subjects the elimination half-life of rifampicin in serum averages about 3 hours after a 600mg dose and increases to 5.1 hours after a 900mg dose. With repeated administration, the elimination half-life decreases and reaches average values of approximately 2-3 hours.

Guidance for the design of bioequivalence studies:

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

Study design: A single-dose crossover design is recommended.

Dose: As the EoI includes the fixed combination Isoniazid / Pyrazinamide / Rifampicin 50mg / 150mg / 75 mg dispersible tablets, the bioequivalence study should be conducted with the mono-component comparator, i.e., 4 x 50 mg/150 mg/75 mg dispersible test tablet *vs.* 2 x 100 mg isoniazid comparator + 1 x 500 mg pyrazinamide comparator (with dose correction) + 1 x 300 mg rifampicin comparator.

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 30 – 50 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardize 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 standardization 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 should be conducted in the fasting 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 drugs are considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound Therefore, bioequivalence should be based on the determination of isoniazid, pyrazinamide and rifampicin.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for isoniazid, pyrazinamide and rifampicin is around 20–25%, although C_{max} intra-subject variability values around 30% have also been observed. These data may facilitate the calculation of a sufficient sample size for a single-dose crossover bioequivalence study.

Washout: Taking into account the elimination half-life of these drugs (9-10 h for pyrazinamide and up to 5 h for isoniazid and rifampicin), a washout period of 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first three hours after administration to properly characterize the C_{max} of isoniazid, pyrazinamide and rifampicin. Sampling times after 24 – 48 hours are necessary for the quantification of pyrazinamide only. For example, blood samples might 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, 24.00, 48.00 and 60 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure isoniazid, pyrazinamide and rifampicin 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 analytes (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for isoniazid, pyrazinamide, and rifampicin should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to comparator product should be within 80.00 – 125.00%

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