Notes on the design of bioequivalence study: Isoniazid / 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-first Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations,* Geneva. World Health Organization, 2017. WHO Technical Report Series, No. 1003, Annex 6.

Below, additional specific guidance is provided on the invited immediate release products containing isoniazid and rifampicin.

Pharmacokinetics of isoniazid

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.

Pharmacokinetics of rifampicin

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 half-life of rifampicin in serum averages about 3 hours after a 600 mg dose and increases to 5.1 hours after a 900mg dose. With repeated administration, the 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 and rifampicin, the following guidance with regard to the study design should be taken into account:

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

Dose: As the EoI includes 50/75 mg as dispersible tablets as well as 75/150 mg, and 150/300 mg strengths as tablets or capsules, and the FDC comparator is marketed as 150/300 mg strength, the 150/300 mg strengths of test and comparator should be compared in the bioequivalence study. The 75/150 mg strength could be waived if the requirements for an additional strength biowaiver are met. Otherwise, a bioequivalence study would be required between 2 x 75/150 mg of the test product *vs.* 1 x 150/300 mg of the comparator product.

The 50/75 mg strength should be compared with mono-component comparator products (2 x 50/75 mg vs. 1 x 100 mg of isoniazid comparator and 1 x 150 mg of rifampicin comparator) or the 1 x 100/150 mg of the comparator FDC.

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.

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

Subjects: Healthy volunteers 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. Therefore, bioequivalence should be based on the determination of isoniazid and rifampicin.

Sample size: Based on information available to the PQT/MED, intra-subject variability in the rifampicin pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasting state ranges from 9.5 to 27%, but it is generally around 15-20%, so it seems to possess low to moderate variability. Isoniazid seems to exhibit a slightly higher variability (20-30%), although it ranges from 9% to 35%. These data may facilitate the calculation of a sufficient sample size for the single-dose cross-over bioequivalence study.

Washout: Taking into account the elimination half-lives of both drugs, a wash out period of 7 days is considered sufficient to prevent carryover.

Blood sampling: The blood sampling should be intensive for the first 4 hours after administration to properly characterize the C_{max} of isoniazid and rifampicin. It is not necessary to take blood samples beyond 24 hours for the characterization of isoniazid and rifampicin pharmacokinetics. For example, samples can be taken pre-dose and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00, 12.00, 16.00, and 24.00 h after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure isoniazid 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).

Statistical considerations: The data for isoniazid and rifampicin 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 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%.