Notes on the design of bioequivalence study: Isoniazid/Pyrazinamide/Rifampicin

Notes on the design of bioequivalence studies with products invited to be submitted to the WHO Prequalification Team – Medicines (PQTm) 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.


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. 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 half-life of about 9-10 hours.

Rifampicin is readily absorbed and Tmax occur 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 600mg 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, pyrazinamide, and rifampicin the following guidance with regard to the study design should be taken into account:

Design: A cross-over design is recommended.

Dose: As the EoI includes the fixed combinations:

- Isoniazid / Pyrazinamide / Rifampicin, coated tablet/capsule 150mg / 500mg / 150mg
- Isoniazid / Pyrazinamide / Rifampicin, coated tablet/capsule 75mg / 400mg / 150mg
- Isoniazid / Pyrazinamide / Rifampicin 50mg / 150mg / 75 mg (preferably dispersible or crushed tablets)

As none of these strengths are proportional in the quantitative composition of the APIs, bioequivalence should be demonstrated in vivo for each strength. In the case of the pediatric dispersible / crushed tablets, the test product should be administered according to the proposed method of administration i.e., dispersed in a small volume of water (e.g., 50 mL) or crushed, and the comparator products should be administered with a glass of water.
The proposed products should be tested against combinations of the individual monocomponent comparator products:

Isoniazid / Pyrazinamide / Rifampicin 150/500/150 mg: 2 x 150/500/150 mg vs. 1 x 300 mg isoniazid + 2 x 500 pyrazinamide + 1 x 300 mg rifampicin

Isoniazid / Pyrazinamide / Rifampicin 75/400/150 mg: 4 x 75/400/150 mg vs. 1 x 300 isoniazid + 3 x 500 pyrazinamide (with dose correction) + 2 x 300 mg rifampicin

Isoniazid / Pyrazinamide / Rifampicin 50/150/75 mg: 4 x 50/150/75 mg vs. 2 x 100 mg isoniazid + 1 x 500 mg pyrazinamide (with dose correction) + 1 x 300 mg rifampicin.

**Fasting/fed:** The bioequivalence study should be conducted in the fasting state.

**Subjects:** Healthy adult subjects should be included in the bioequivalence study(ies). It is not necessary to include patients.

**Sample size:** Information currently available to PQTm indicates that the intra-subject variability for isoniazid, pyrazinamide, and rifampicin is around 20–25%, although Cmax intra-subject variability values around 30% have also been observed. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

**Washout:** Taking into account the short elimination half-life of the drugs, 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 Cmax 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 at predose, 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 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 Cmax in most profiles of each formulation (test or comparator).

**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 isoniazid, pyrazinamide, and rifampicin.
**Statistical considerations:** The data for isoniazid, pyrazinamide, 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 reference product should be within 80-125%
- The 90% confidence interval of the relative mean $C_{max}$ of the test to reference product should be within 80-125%.

Information currently available to PQTm indicates that the comparator products are not highly variable drug products, although in certain cases $C_{max}$ intra-subject variability around 30% has been observed.