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

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. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, 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 ethambutol, isoniazid, pyrazinamide, and rifampicin.

## Pharmacokinetics of Ethambutol, Isoniazid, Pyrazinamide and Rifampicin

Ethambutol is readily absorbed after oral administration and this absorption is not significantly impaired by food. After a single dose, median $T_{max}$ occurs at 3 hours. Ethambutol elimination half-life is approximately 3 - 5 h.

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.

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

Rifampicin is readily absorbed and $T_{max}$ 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 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 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 ethambutol, isoniazid, pyrazinamide, and rifampicin, the following guidance with regard to the study design should be considered:

**Design:** A single-dose crossover design is recommended.

**Dose:** As the EoI includes only the fixed combination of Ethambutol hydrochloride / Isoniazid / Pyrazinamide / Rifampicin, coated tablet 275 mg / 75 mg / 400 mg / 150 mg, this strength should be tested versus the fixed combination comparator of the same APIs and strengths.

**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 ethambutol, isoniazid, pyrazinamide, and rifampicin.

**Sample size:** Information currently available to PQT/MED indicates that the intra-subject variability for ethambutol, 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 the cross-over bioequivalence study.

**Washout:** Taking into account the short elimination half-life of all these 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 $C_{max}$ of ethambutol, 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 pre-dose, 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 60.00 h after drug administration.

**Analytical considerations:** Information currently available to PQT/MED indicates that it is possible to measure ethambutol, 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 analyte (see Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information).

**Statistical considerations:** The data for ethambutol, 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 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%.