Notes on the design of bioequivalence study:

Ethambutol/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 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 Tmax occurs at 3 hours. Ethambutol 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. 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 ethambutol, 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 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 comparators of the individual monocomponents (or the FDC of isoniazid / rifampicin 75/150 mg or 150/300 mg): e.g., 2 x 275/75/400/150 mg vs. 1 x 150/300 mg isoniazid/rifampicin FDC + 1 x 400 mg ethambutol + 2 x 500 pyrazinamide (with dose correction for the different dose of ethambutol and pyrazinamide). It is also acceptable to compare one test tablet (1 x 275/75/400/150 mg) versus 1 unit of the individual comparators (1 x 400 mg ethambutol + 1 x 100 mg isoniazid + 1 x 500 mg pyrazinamide + 1 x 150 mg rifampicin) and correct by the difference in administered dose. To avoid large differences in the administered doses of ethambutol and
pyrazinamide, it is also acceptable to compare 4 x 275/75/400/150 mg vs. 2 x 400 mg ethambutol + 1 x 300 mg isoniazid + 3 x 500 mg ethambutol + 4 x 150 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. It is not necessary to include patients.

**Sample size:** Information currently available to PQTm indicates that the intra-subject variability for ethambutol, 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 all 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 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 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 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 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 ethambutol, isoniazid, pyrazinamide, and rifampicin.

**Statistical considerations:** The data for ethambutol, 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 AUC0-t of the test to reference product should be within 80-125%

- The 90% confidence interval of the relative mean Cmax 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 Cmax intra-subject variability around 30% has been observed.