Notes on the design of bioequivalence study: Ethambutol/Isoniazid

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.

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: *Forty-ninth report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations,* Geneva: World Health Organization. WHO Technical Report Series, No. 992, 2015, Annex 7.

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

Pharmacokinetics of Ethambutol and Isoniazid

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.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of ethambutol and isoniazid 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, coated tablet /capsule 400 mg / 150 mg, this strength should be tested versus the comparators of the individual monocomponents: e.g., 1 x 400/150 mg vs. 400 mg Ethambutol and 100 mg Isoniazid (with dose correction for the difference in isoniazid dose), or 2 x 400/150 mg vs. 2 x 400 mg ethambutol + 1 x 300 mg isoniazid or 3 x 100 mg of isoniazid.

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 and isoniazid is around 20–25%. 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 both 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 and isoniazid. 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, 16.00 and 24.00 h after drug administration.

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

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 and isoniazid.

Statistical considerations: The data for ethambutol and isoniazid 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 Cmax intra-subject variability around 30% has been observed.