Notes on the Design of Bioequivalence Study: Ethambutol

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. In particular, 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.

Pharmacokinetics of Ethambutol

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.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of ethambutol, 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 ethambutol hydrochloride 400 mg film-coated tablet (scored) and ethambutol hydrochloride 100 mg tablet (scored and dispersible), these strengths should be tested versus the comparators of 400 mg and 100 mg, respectively.

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. The total volume of water employed during administration of a pediatric dispersible tablet should not exceed 50 mL.

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

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for ethambutol, 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 elimination half-life of ethambutol of 3 - 5 h, a washout period of 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first four hours after administration to properly characterize the C_{max} of ethambutol. For example, blood samples might be taken at pre-dose, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 6.00, 8.00, 12.00, 16.00 and 24.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure ethambutol 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 ethambutol 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%.