Notes on the Design of Bioequivalence Study: Pyrazinamide

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 pyrazinamide.

Pharmacokinetics of Pyrazinamide

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

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of pyrazinamide the following guidance with regard to the study design should be taken into account:

Design: A single-dose cross-over design is recommended.

Dose: As the EoI includes pyrazinamide 400 mg tablet (scored) or 500 mg (scored) and pyrazinamide 150 mg tablet (dispersible), these strengths should be tested versus the comparator of 500 mg, which requires adjustment of the results based on the different doses administered (e.g. 1 x 400 mg vs. 1 x 500 mg or 3 x 150 mg vs. 1 x 500 mg).

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 pyrazinamide.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for pyrazinamide 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 pyrazinamide of 9 – 10 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 three hours after administration to properly characterize the C_{max} of pyrazinamide. 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, 4.00, 6.00, 8.00, 12.00, 16.00 and 24.00 and 48.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure pyrazinamide 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). 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 on bioanalytical recommendations.

Statistical considerations: The data for pyrazinamide 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 80.00–125.00%
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80.00–125.00%.

Biowaiver: If the applicant can demonstrate high solubility of pyrazinamide according to the BCS based on the highest single therapeutic dose, a BCS biowaiver would be feasible provided the additional requirements for granting a BCS-based biowaiver are met as outlined in the WHO Guideline on "Biopharmaceutics Classification System-based biowaivers" (TRS1052, Annex 7, 2024) and the PQT/MED guidance "PQT/MED-specific Annotations for the WHO Guideline on Biopharmaceutics Classification System (BCS)-based biowaivers" (2025).