Notes on the Design of Bioequivalence Study: Fluconazole

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 fluconazole.

Pharmacokinetics of fluconazole

After oral administration fluconazole is well absorbed, and plasma levels (and systemic bioavailability) are over 90% of the levels achieved after intravenous administration. Oral absorption is not affected by concomitant food intake. Peak plasma concentrations in the fasting state occur between 0.5 and 6 hours post-dose. Plasma concentrations are proportional to dose. Plasma elimination half-life for fluconazole is approximately 30 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended.

Dose: As the EoI includes fluconazole capsules of 50 and 200 mg, the bioequivalence study should be conducted with the highest strength.

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

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for fluconazole is around 9%. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of Fluconazole of 30 h, a washout period of 14 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first two hours after administration to properly characterize the C_{max} of fluconazole. As fluconazole has a long elimination half-life, blood sampling should cover 72 hours after administration. For example, blood samples should 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, 5.00, 6.00, 8.00, 12.00, 24.00, 48.00 and 72.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure fluconazole 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 fluconazole should meet the following bioequivalence standards in a single-dose crossover design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72h}$ 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: A BCS-based biowaiver for fluconazole is considered a possible alternative to a bioequivalence study, provided the requirements for granting a BCS-based biowaiver are met as outlined in the WHO Guideline "Biopharmaceutics Classification System-Based Biowaivers" (TRS 1052, Annex 7, 2024) and the PQT/MED guidance "PQT/MED-specific Annotations for the WHO Guideline for Biopharmaceutics Classification System (BCS)-based Biowaiver Applications" (2024).