Notes on the Design of Bioequivalence Study:

Tenofovir Disoproxil Fumarate

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 suggestedbelow 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 1052, Annex 8.

Below, additional specific guidance is provided on the invited immediate release products containing tenofovir disoproxil fumarate.

Pharmacokinetics of tenofovir

Following oral administration of tenofovir disoproxil fumarate, tenofovir peak plasma concentrations are typically reached within one hour post-dose in the fasted state and within two hours post-following a standardized high fat meal. Relative to fasting conditions, the administration of a single dose of tenofovir with a standardized high-fat meal increases the tenofovir C_{max} by 14% and the AUC by 40%. For this reason, tenofovir is can be administered without regard to food (according to the FDA label) or with a meal (according to the EU SmPC). Tenofovir displays linear pharmacokinetics. Tenofovir is excreted in urine predominantly unchanged. The mean terminal half-life of tenofovir is 12−18 hours.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of tenofovir, 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 tenofovir disoproxil fumarate tablets 300 mg, the bioequivalence study should be conducted with this strength.

Fasted/fed: The bioequivalence study should be conducted in the fasted state because it can be taken without regard to food in some stringent regulatory authorities.

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: Tenofovir disoproxil fumarate is the water soluble diester prodrug of the active ingredient tenofovir. Following absorption, the prodrug is rapidly converted to tenofovir. Therefore, bioequivalence should be based on the determination of tenofovir.

Sample size: Tenofovir is not a highly variable drug based on information available to PQT/MED. Values for intra-subject CV are generally 15 – 20% for C_{max} and 10 – 15% for AUC. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study. Sample sizes are generally between 24 and 32.

Washout: Taking into account the elimination half-life of tenofovir in the fasted state of 12−18 hours, a washout period of seven days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive during the first 1 hour after administration to properly characterize the C_{max} of tenofovir. Considering the elimination half-life, it is sufficient to take blood samples up to 72 hours after administration for the characterization of tenofovir pharmacokinetics.

Analytical considerations: Tenofovir should be the basis for the bioequivalence assessment. Assay sensitivity for tenofovir (limit of quantitation) is currently 5−10 ng/ml. 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).

Statistical considerations: The data for tenofovir 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 comparator product should be within80.00 − 125.00%.
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within80.00 − 125.00%.

Biowaiver: The European Medicines Agency indicates that tenofovir disoproxil fumarate is a high solubility and low permeability compound (BCS Class III), therefore a BCS based biowaiver could be feasible. Refer to the requirements for granting a BCS-based biowaiver 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).