Notes on the Design of Bioequivalence Study:
Lamivudine/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 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 lamivudine and tenofovir.

Pharmacokinetics of lamivdudine and tenofovir disoproxil fumarate

Maximum lamivudine and tenofovir concentrations are observed in serum within 0.5 to 3.0 hours of dosing in the fasted state (median T_{max} of 1 hour). The elimination half-life of lamivudine is 5–7 hours, whereas the half-life of tenofovir is 10 hours.

Co-administration of lamivudine with food results in a delay of Tmax and a lower C_{max} (decreased by 47%). However, the extent (based on the AUC) of lamivudine absorbed is not influenced. Therefore, lamivudine can be taken with or without food. Administration of tenofovir disoproxil fumarate (TDF) with food increases AUC and C_{max} approximately 35% and 15%, respectively, when administered with a high fat or light meal, compared to administration in the fasted state. In order to optimize the absorption of tenofovir, it is recommended that TDF should preferably be taken with food in the European Union, but with or without food in the United States.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of lamivudine and TDF, 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 lamivudine/tenofovir disoproxil fumarate 300 mg/300 mg tablets, the bioequivalence study should be conducted with this strength versus the same strength of the fixed combination comparator product.

Fasted/fed: The bioequivalence study should be conducted in the fasted state, as tenofovir can be taken with or without meals according to the US-FDA labelling and lamivudine is recommended to be taken with or without food. In addition, the fixed combination comparator product may be administered with or without food according to the US FDA.

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 lamivudine. In contrast, for tenofovir, tablets contain tenofovir disoproxil fumarate, which 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: Information currently available to PQT/MED indicates that the intra-subject variability for lamivudine and for tenofovir is around 20%. These data may facilitate the calculation of the sample size for the cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of lamivudine (5 – 7 hours) and tenofovir (10 hours) in healthy volunteers, a washout period of seven 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 lamivudine and tenofovir. 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, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00, 24.00 and 36 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure lamivudine and tenofovir 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). The bioanalytical method for each analyte should be validated in the presence of the other analyte (see ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for lamivudine and 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 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%.