

Notes on the Design of Bioequivalence Study: Lamivudine / Tenofovir Disoproxil Fumarate / Efavirenz

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 lamivudine/tenofovir/efavirenz.

Pharmacokinetics of lamivudine, tenofovir and efavirenz

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). Efavirenz peak plasma concentrations are typically reached within 5 h post-dose.

The elimination half-life of lamivudine is 5–7 hours and for tenofovir it is 10 hours, whereas the mean terminal half-life of efavirenz is 52–76 h following single doses.

Co-administration of lamivudine with food results in a delay of T_{max} and a lower C_{max} (decreased by 47%). However, the extent (based on the AUC) of lamivudine absorption is not influenced. Therefore, lamivudine can be taken with or without food.

Administration of tenofovir 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 tenofovir should preferably be taken with food in the European Union, but with or without food in the United States.

Relative to fasting conditions, the administration of a single dose of efavirenz with a standardized high-fat meal increases the rate and extent of absorption of efavirenz. For this reason, efavirenz is to be administered on an empty stomach, preferably at bedtime. Therefore, the fixed combination of lamivudine/tenofovir/efavirenz should be administered in fasted state.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of lamivudine, tenofovir and efavirenz, 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 per ICH M13A, for APIs with non-linear pharmacokinetics within the range of strengths due to limited solubility of the API and resulting in less than proportional increases in AUC with increasing dose, bioequivalence studies should be conducted on at least the lowest strength (or a strength in the linear range) and the highest strength of a series of strengths.

As the EoI includes the fixed combinations of lamivudine/tenofovir disoproxil fumarate/efavirenz 300mg/300mg/400mg and 300/300/600 mg tablets for adults, both strengths need to be tested in an in vivo bioequivalence study due to the non-linearity of efavirenz after the administration of a single dose. Both strengths should be tested versus the corresponding mono-components at the same dose level since there is no comparator product containing these three APIs as a fixed combination.

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

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence study(ies).

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 and efavirenz. In contrast, 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 is around 30–35%, and around 20–25% for tenofovir and efavirenz. These data may facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: Taking into account the elimination half-life of efavirenz in the fasted state of 52–76 h, a washout period of 28–35 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, and during the first 5 hours after administration to properly characterize the C_{max} of efavirenz. Blood samples for the characterization of efavirenz pharmacokinetics should be taken for 72 h post-dose in order to determine truncated AUC values (AUC_{0-72h}). 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, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 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 lamivudine, tenofovir and efavirenz 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 two analytes (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).

Statistical considerations: The data for lamivudine, tenofovir and efavirenz 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} for lamivudine and tenofovir, and AUC_{0-72h} for efavirenz 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%.