Notes on the design of bioequivalence study: Emtricitabine/Tenofovir Alafenamide/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. 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 emtricitabine/tenofovir alafenamide/efavirenz.

Pharmacokinetics of Emtricitabine, Tenofovir Alafenamide, and Efavirenz

Maximum emtricitabine are observed in plasma within 0.5 to 3.0 hours of dosing in the fasted state. Tenofovir alafenamide (TAF) peak plasma concentrations are observed after about 1 hour after dosing in fed state. Efavirenz peak plasma concentrations are typically reached within 5 h post-dose.

The elimination half-lives of emtricitabine, TAF, and efavirenz are 10, 0.51, and 52-76 hours, respectively, following single doses.

Administration of emtricitabine with a high-fat meal does not affect systemic exposure ($AUC_{0\text{-}inf}$) of emtricitabine; therefore, emtricitabine may be administered with or without food.

Administration of TAF with a high-fat meal increased the systemic exposure of TAF by 65%; therefore, TAF should be administered with food.

Relative to fasted 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.

Given these conditions, the fixed combination of emtricitabine/tenofovir alafenamide/efavirenz should be administered in the fed state following a meal with standard fat content (500-600 Kcal).

Efavirenz appears to demonstrate non-linear pharmacokinetics with less than proportional increases in AUC observed with increasing dose, due to very limited solubility.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of emtricitabine, TAF, 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 Section 7.4.2 of Annex 8, TRS 1052, 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 such as efavirenz, 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.

However, as the EoI includes only the fixed combination of emtricitabine/tenofovir alafenamide/efavirenz 200mg/25mg/400mg, this dose (strength) needs to be tested in an in vivo bioequivalence study. The 200mg/25mg/400mg strength should be tested versus the corresponding monocomponent products at the same dose level.

Fasted/fed: The bioequivalence study should be administered in the fed state following a meal with standard fat content (500-600 Kcal), not a high-fat, high-calorie meal.

Subjects: Healthy adult subjects should be recruited. It is not necessary to include patients in the bioequivalence studies.

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 emtricitabine, TAF, and efavirenz.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for emtricitabine and efavirenz is around 20-25%, while the intra-subject variability for TAF C_{max} is reported to be approximately 36%. 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 emtricitabine and TAF, and during the first five 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 ($AUC_{0\text{-}72h}$). For example, blood samples might be taken at pre-dose, 0.083, 0.17, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 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 emtricitabine, tenofovir alafenamide, 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 ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for emtricitabine, TAF, and efavirenz should meet the following bioequivalence standards in a single-dose cross-over design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}t}$ for emtricitabine and TAF, and $AUC_{0\text{-}72h}$ 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%.