Notes on the Design of Bioequivalence Study:

Emtricitabine/Tenofovir Disoproxil Fumarate /Dolutegravir

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 may be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines on 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 emtricitabine, tenofovir disoproxil fumarate (TDF), and dolutegravir.

Pharmacokinetics of emtricitabine, tenofovir disoproxil fumarate, and dolutegravir

Maximum emtricitabine and tenofovir concentrations are observed within 0.5 to 3.0 hours of dosing in the fasted state. Dolutegravir T_{max} is observed at 2 to 3 hours post-dose.

The elimination half-life of emtricitabine and tenofovir is 10 hours, whereas dolutegravir has a terminal half-life of approximately 14 hours.

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 TDF with food increases tenofovir 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 disoproxil, it is recommended that TDF should preferably be taken with food in the European Union, but with or without food in the United States.

Food increased the extent and slowed the rate of absorption of dolutegravir. The bioavailability of dolutegravir depends on meal content: low-, moderate-, and high-fat meals increased dolutegravir $AUC_{0\text{-}inf}$ by 33%, 41%, and 66%, C_{max} by 46%, 52%, and 67%, and prolonged T_{max} to 3, 4, and 5 hours, respectively, from 2 hours under fasted conditions. These increases may be clinically relevant in the presence of certain integrase class resistance. Therefore, dolutegravir is recommended to be taken with food by patients infected with HIV with integrase class resistance. Otherwise, dolutegravir can be taken with or without food.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of emtricitabine, tenofovir disoproxil, and dolutegravir, the following guidance with regard to the study design should be taken into account:

Design: A single-dose cross-over study is recommended.

Dose: A single oral dose of one tablet of emtricitabine/tenofovir disoproxil fumarate/dolutegravir 200/300/50 mg should be feasible.

Fasted/fed: The bioequivalence study should be conducted in the fasted state, as TDF can be taken with or without meals according to the US-FDA labelling, and emtricitabine and dolutegravir are recommended to be taken with or without food.

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 emtricitabine and dolutegravir. In contrast, for tenofovir disoproxil fumarate, which is the water soluble diester prodrug of the active ingredient tenofovir and which following absorption rapidly convert into tenofovir, bioequivalence should be based on the determination of tenofovir.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for emtricitabine, tenofovir disoproxil, and dolutegravir is around 20-25%. 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 emtricitabine (10 hours), tenofovir (10 hours), and dolutegravir (14 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 four hours after administration to properly characterize the C_{max} of emtricitabine, tenofovir, and dolutegravir. It is not necessary to take blood samples beyond 72 hours. For example, blood samples might be taken at pre-dose, 0.17, 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 emtricitabine, tenofovir and dolutegravir 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 on bioanalytical recommendations).

Statistical considerations: The data for emtricitabine, tenofovir, and dolutegravir should meet the following bioequivalence standards in a single-dose crossover 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%.