Notes on the Design of Bioequivalence Study: Emtricitabine/Tenofovir/Dolutegravir

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team: medicines (PQTm) 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.


Below, additional specific guidance is provided on the invited immediate release products, containing emtricitabine, tenofovir and dolutegravir.

**Pharmacokinetics of emtricitabine, tenofovir and dolutegravir**

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

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 tenofovir with food increases AUC and $C_{\text{max}}$ 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.

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%, increased $C_{\text{max}}$ by 46%, 52%, and 67%, and prolonged $T_{\text{max}}$ to 3, 4, and 5 hours from 2 hours under fasted conditions, respectively. 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 dolutegravir, the following guidance with regard to the study design should be taken into account:
**Dose:** A single oral dose of one tablet of emtricitabine/tenofovir/dolutegravir 200/300/50 mg should be feasible. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

**Fasting/fed:** The bioequivalence study should be conducted in the fasting state, as tenofovir 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 used. It is not necessary to include patients in the bioequivalence study.

**Sample size:** Information currently available to PQTm indicates that the intra-subject variability for emtricitabine, tenofovir and dolutegravir is around 20%. These data will facilitate the calculation of the sample size for the 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 three hours after administration to properly characterize the $C_{\text{max}}$ of emtricitabine, tenofovir and dolutegravir. It is not necessary to take blood samples beyond 72 hours.

**Analytical considerations:** Information currently available to PQTm 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 $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

**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, 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.

**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$_T$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product should be within 80–125%.
Information currently available to PQTm indicates that the comparator products are not highly variable drug products.