Notes on the Design of Bioequivalence Study: Emtricitabine/Tenofovir disoproxil fumarate/ Levonorgestrel/Ethinyl estradiol

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. 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 tablet containing emtricitabine, tenofovir disoproxil fumarate, levonorgestrel and ethinylestradiol.

Pharmacokinetics of emtricitabine, tenofovir disoproxil fumarate, levonorgestrel and ethinylestradiol.

Maximum emtricitabine plasma concentrations are observed within 0.5 to 3.0 hours of dosing in the fasted state. 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. The elimination half-life of emtricitabine is 10 hours.

After administration of tenofovir disoproxil fumarate maximum tenofovir concentrations are observed within 0.5 to 3.0 hours of dosing in the fasted state. Administration of tenofovir disoproxil fumarate 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 fumarate, it is recommended that tenofovir disoproxil fumarate should preferably be taken with food in the European Union, but with or without food in the United States. The elimination half-life of tenofovir is 10 hours.

Orally administered ethinyl estradiol is rapidly and completely absorbed. Peak serum concentrations are reached within 1.0 – 2.0 hours. Ethinyl estradiol serum levels decrease in two phases, the terminal disposition phase is characterized by a half-life of approximately 15 - 24 hours.

Levonorgestrel is rapidly and completely absorbed. Maximum plasma concentrations are reached just 1.0 hour after ingestion. The elimination has two phases with half-lives of 0.5 and 20 – 60 h.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of emtricitabine, tenofovir disoproxil fumarate, levonorgestrel and ethinyl estradiol, the following guidance with regard to the study design should be taken into account for the 200mg /300 mg/0.15 mg/0.03 mg tablet:

Design: A single-dose crossover study is recommended.

Dose: As the 200mg /300 mg/0.15 mg/0.03 mg tablet is the only invited strength, this strength should be employed.

Fasted/fed: In clinical practice, tenofovir disoproxil fumarate can be taken with or without meals according to the US-FDA labelling. In addition, emtricitabine, levonorgestrel/ethinyl estradiol are recommended to be taken with or without food. The single-dose, crossover design bioequivalence study should be conducted under fasting conditions.

Subjects: Healthy, adult, female 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, levonorgestrel and ethinylestradiol. In contrast, for tenofovir disoproxil fumarate, which is the water soluble diester prodrug of the active ingredient tenofovir and which following absorption rapidly converts 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, levonorgestrel and ethinyl estradiol is around 20 - 25% for C_{max} and AUC. These data may facilitate the calculation of the sample size for the crossover bioequivalence study.

Washout: Taking into account the elimination half-life of emtricitabine (10 hours), tenofovir (10 hours), levonorgestrel (20 – 60 hours) and ethinyl estradiol (15 – 24 pours) in healthy volunteers, a washout period of 21 – 28 days is considered sufficient to prevent carryover.

Blood sampling: The blood sampling should be intensive for the first 4 hours after administration to properly characterize the C_{max} of emtricitabine, tenofovir, levonorgestrel and ethinyl estradiol. 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, 36.00, 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, levonorgestrel and ethinyl estradiol in human plasma using LC-MS/MS analytical methodology with a LLOQ of 20 ng/ml, 3 ng/ml, 25 pg/ml and 1 pg/ml, respectively. 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 emtricitabine, tenofovir, levonorgestrel and ethinyl estradiol should meet the following bioequivalence standards in the single-dose crossover design study conducted under fasting conditions:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}t}$ (or $AUC_{0\text{-}72h}$, where applicable) of the test to comparator product should be within 80.00–125.00%
- The 90% confidence interval of the relative mean C_{max} of the test to comparator product should be within 80.00–125.00%.