Notes on the design of bioequivalence study:
Lamivudine/Abacavir/Efavirenz

Notes on the design of bioequivalence studies with products invited to be submitted 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 can be considered acceptable if justified by sound scientific evidence.


Below, additional specific guidance is provided on the invited immediate release products, containing lamivudine/abacavir/efavirenz.

**Pharmacokinetics of Lamivudine, Abacavir, and Efavirenz**

Maximum lamivudine and abacavir concentrations are observed in serum within 0.5 to 3.0 hours of dosing in the fasted state (median $T_{\text{max}}$ of 1 - 1.5 hour). Efavirenz peak plasma concentrations are typically reached within 5 h post-dose.

The half-life of lamivudine is 5-7 hours and for abacavir it is 1.5 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 $T_{\text{max}}$ and a lower $C_{\text{max}}$ (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.

Food delayed absorption and decreased $C_{\text{max}}$ of abacavir but did not affect overall plasma concentrations (AUC). Therefore, abacavir can be taken with or without food.

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/abacavir/efavirenz should be administered in the fasted state.

**Guidance for the design of bioequivalence studies:**

Taking into account the pharmacokinetic properties of lamivudine, abacavir, and efavirenz the following guidance with regard to the study design should be taken into account:

**Design:** A cross-over design is recommended.

**Dose:** As the EoI includes only the fixed combination of Lamivudine/Abacavir/Efavirenz, tablet 75 mg/150 mg/150 mg scored and dispersible for paediatric patients, that strength should be tested in vivo versus the corresponding monocomponents at the same dose level (4 x 75/150/150 mg vs. Epivir 300 mg tablet + Ziagen 600 mg tablet + Sustiva/Stocrin 600 mg tablet), since there is no comparator product containing these three APIs as a fixed combination.
In the bioequivalence study for Lamivudine/Abacavir/Efavirenz 75 mg/150 mg/150 mg dispersible tablet, the test product should be administered as indicated in the proposed dosing instructions i.e., dispersed in a small volume of water (e.g., 50 mL), and the comparator should be taken whole (without crushing or chewing) with a glass of water.

**Fasting/fed:** The bioequivalence study should be conducted in the fasting state.

**Subjects:** Healthy adult subjects should be included in the bioequivalence study. It is not necessary to include patients.

**Sample size:** Information currently available to PQTm indicates that the intra-subject variability for lamivudine is around 30‒35%, and around 20-25% for abacavir 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 $C_{\text{max}}$ of lamivudine and abacavir, and during the first 5 hours after administration to properly characterize the $C_{\text{max}}$ 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 might be taken at predose, 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 indicates that it is possible to measure lamivudine, abacavir, 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 $C_{\text{max}}$ 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 lamivudine, abacavir, and efavirenz.

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

- The 90% confidence interval of the relative mean $AUC_{0-4}$ for lamivudine and abacavir, and $AUC_{0-72h}$ for efavirenz 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, although the variability of lamivudine $C_{\text{max}}$ may be slightly higher than 30%.