Notes on the Design of Bioequivalence Study: Abacavir / Lamivudine / Lopinavir / Ritonavir

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 abacavir, lamivudine, lopinavir and ritonavir.

Pharmacokinetics of abacavir

Maximum abacavir concentrations are observed in plasma within 0.5 to 3.0 hours of dosing in the fasted state (median T_{max} of 1 - 1.5 hour). The elimination half-life of abacavir is 1.5 hours. Food delayed absorption and decreased C_{max} of abacavir, but did not affect overall plasma concentrations (AUC). Therefore, abacavir can be taken with or without food.

Pharmacokinetics of lamivudine

Maximum lamivudine concentrations are observed in plasma within 0.5 to 3.0 hours of dosing in the fasted state (median T_{max} of 1 - 1.5 hour). The elimination half-life of lamivudine is 5-7 hours. Co-administration of lamivudine with food results in a delay of T_{max} and a lower C_{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.

Pharmacokinetics of lopinavir / ritonavir

Ritonavir inhibits the metabolism of lopinavir, thereby increasing the plasma levels of lopinavir.

Multiple dosing with 400/100 mg of lopinavir / ritonavir twice daily for 2 weeks and without meal restriction produced C_{max} occurring approximately 4 hours after administration. After a single dose, T_{max} was observed between 3 and 4 hours.

Administration of a single 400/100 mg dose of lopinavir / ritonavir tablets under fed conditions (high fat, 872 kcal, 56% from fat) compared to fasted state was associated with no significant changes in C_{max} and AUC_{inf}. Therefore, lopinavir/ritonavir tablets may be taken with or without food.

The effective (peak to trough) half-life of lopinavir over a 12-hour dosing interval averaged 5−6 hours. After a single dose administration, the elimination half-life was of 4−6 hours approximately.

Pharmacokinetics of ritonavir

After oral administration, ritonavir peak plasma concentrations are observed after approximately 3−4 hours.

Ritonavir is metabolized by the hepatic cytochrome P450 system, primarily by the CYP3A isozyme family and to a lesser extent by the CYP2D6 isoform. Low doses of ritonavir have shown profound effects on the pharmacokinetics of other protease inhibitors (and other products metabolized by CYP3A4) and other protease inhibitors may influence the pharmacokinetics of ritonavir. Ritonavir demonstrates nonlinear pharmacokinetics with greater than dose-proportional increases in AUC. Ritonavir elimination half-life when administered with lopinavir has been reported to be 5 – 6 hours.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of abacavir, lamivudine, lopinavir, and ritonavir 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 the EoI includes Lamivudine / Abacavir, granules / minitablets / pellets 15 mg / 30 mg co-mixed with Lopinavir / Ritonavir, granules / minitablets / pellets (heat stable) 40 mg / 10 mg, this product strength should be compared with the simultaneous administration of the comparator for abacavir / lamivudine and the comparator for lopinavir / ritonavir (i.e., 10 units of abacavir / lamivudine / lopinavir / ritonavir 30/15/40/10 mg *vs.* 1 unit of the abacavir 300 mg tablet comparator, 1 unit of the lamivudine 150 mg tablet comparator and 2 units of the lopinavir / ritonavir 200/50 mg.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. In those cases where the test and the comparator product are different dosage forms with different methods of administration (e.g., a tablet that should be taken with a glass of water *vs.* granules, pellets or minitablets to be dispersed in a small volume (e.g., 30 - 50 mL) of water or to be sprinkled in soft food) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardise the volume of liquid in all these cases (e.g., administering a glass of water after the intake of a granules, pellets or minitablets or rinsing the container where granules, pellets or minitablets have been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions.

Fasted/fed: As abacavir, lamivudine and the fixed combination tablet of lopinavir / ritonavir can be taken with or without food, a fasted state study is recommended.

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 drugs are considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of the parent compounds abacavir, lamivudine, lopinavir and ritonavir.

Sample size: Abacavir and lamivudine C_{max} seem to be moderately variable (15 – 25% approx.). Lopinavir and ritonavir pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasted state seem to possess moderate variability (25 – 30%), although high variability (>30%) has been observed in some studies. These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of these drugs, with the highest value for lamivudine (5- 7 hours in healthy volunteers), a wash-out 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 abacavir, lamivudine, lopinavir, and ritonavir. It is not necessary to take blood samples beyond 12 hours for the characterization of abacavir pharmacokinetics and 24 hours for lamivudine, lopinavir and ritonavir. For example, samples can be taken pre-dose and at 0.16, 0.33, 0.50, 0.67, 0.83, 1.00, 1.16, 1.33, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00 and 24.00 hours.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure abacavir, lamivudine, lopinavir, and ritonavir 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).

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

- The 90% confidence interval of the relative mean AUC_{0-t} 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%.