Notes on the design of bioequivalence study:
Lopinavir / Ritonavir

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 lopinavir and ritonavir.

Pharmacokinetics of lopinavir

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 Cmax occurring approximately 4 hours after administration. After a single dose Tmax 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 Cmax and AUCinf. 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 half-life was reported to be approximately 4 - 6 hours.

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 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 lopinavir and ritonavir, 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 200/50 mg and 100/25 mg tablets and 40/10 mg granules/minitablets/pellets. The highest strength 200/50 mg strength is recommended for the bioequivalence study if the conditions are fulfilled to waive the bioequivalence requirements for the additional 100/25 mg strength.

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

Subjects: Healthy volunteers

Sample size: Lopinavir and ritonavir pharmacokinetic parameters, Cmax and AUC_{0-t}, in the fasting state seem to possess moderate variability (25-30%), although high variability (>30%) has been observed in some studies. These data will facilitate the calculation of a sufficient sample size for the bioequivalence study.

Washout: At least seven (7) days.

Blood sampling: Predose, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 8.00, 9.00, 10.00, 12.00, 16.00, and 24.00 h after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure 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).

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.

Statistical considerations: The data for 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 reference product should be within 80-125%

- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80-125%.