Notes on the Design of Bioequivalence Study 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. For guidance on issues related to bioequivalence (BE) studies for immediate-release, solid oral dosage forms, see the ICH Harmonised Guideline M13A Bioequivalence for Immediate-Release Solid Oral Dosage Forms (2024). For BE issues outside the scope of the ICH M13A guideline, e.g., for additional strength biowaivers, 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 lopinavir and ritonavir.

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 two 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. However, the oral solution should be taken with food since administration of the oral solution with a moderate fat meal (500–682 Kcal, 23 to 25% calories from fat) increased lopinavir AUC and C_{max} by 80 and 54%, respectively, and administration of the oral solution with a high fat meal (872 Kcal, 56% from fat) increased lopinavir AUC and C_{max} by 130% and 56%, respectively.

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 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 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, provided the conditions are fulfilled to waive the additional 100/25 mgstrength.

Fasted/fed:

The comparator product employs a complex manufacturing process in order to enhance the dissolution and absorption of these drugs. Due to the low solubility of the APIs and the complexity of the manufacturing process employed, there is an increased risk that the in vivo performance of this type of product will be impacted differently by varying gastrointestinal (GI) conditions between fasted and fed conditions. Therefore, for such drug products, bioavailablity differences (i.e., test/comparator ratios) due to differences in formulation and/or manufacturing process may not be detected with a single BE study, i.e., results from a fed study may not be extrapolated to predict the fasted BE outcome or vice versa, thus both fasting and fed studies should be conducted, unless otherwise justified (e.g., by demonstrating the use of a formulation with the same or similar excipient composition and the same manufacturing process as in the comparator product).

On this scientific basis, for lopinavir/ritonavir products, two bioequivalence studies are required, *i.e.*, single-dose, crossover bioequivalence studies should be conducted under both fasting and high-fat, high-calorie fed conditions.

For the 40/10 mg granules/minitablets/pellet product, the test product should be dispersed in a small amount of water for the fasted state study. Therefore, the test product granules/minitablets/pellets should be dispersed in a small amount of water (e.g., 20 – 40 mL) and the reference product (i.e., tablet) should be taken in the fasted state with a glass of water (e.g., 240 mL). For the fed study, the test product granules/minitablets/pellets should be sprinkled on a paediatric-age-appropriate meal (e.g., apple sauce) or high-fat high-calorie purée and the reference product (e.g., oral solution or tablet) should be taken 30 minutes after starting the intake of the same type of meal. Consequently, the dosing instructions for a proposed 40/10 mg granules/minitablets/pellet would be that the product can be taken sprinkled on food or dispersed in a small amount of water, identifying the volume employed in the bioequivalence study.

Subjects: Healthy volunteers 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 thebiopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of lopinavir and ritonavir.

Sample size: Lopinavir and ritonavir pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasting state seem to possess moderate intra-subject variability (25–30%), although high variability (>30%) has been observed in some study. In fed state the variability is expected to be lower (<20%). These data may facilitate the calculation of a sufficient sample size for the cross-over bioequivalence studies.

Washout: Taking into account the elimination half-life of lopinavir and ritonavir in the fasted state of up to 6 h, a washout period of 7 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive in the first 5 hours, taking into account that T_{max} is expected around 3 h after dosing in fasted state and 4 - 5 h in fed state. It is not necessary to take samples after 24 hours. For example, blood samples might be taken at pre-dose, 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 to PQT/MED 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). The bioanalytical method for each analyte should be validated in the presence of the other analyte (see Guideline on bioanalytical method validation and study sample analysis. In: WHO Technical Report Series, No. 1060, Annex 6, or the ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for lopinavir and ritonavir should meet the following bioequivalence standards in each 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 80.00–125.00%.
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be within 80.00–125.00%.