Notes on the design of bioequivalence study: Ritonavir

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (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 ritonavir.

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 has been reported to be 5 – 6 hours.

Food slightly decreases the bioavailability of the tablet. Administration of a single 100 mg dose of ritonavir tablet with a moderate fat meal (857 kcal, 31% calories from fat) or a high fat meal (907 kcal, 52% calories from fat) was associated with a mean decrease of 20-23% in ritonavir AUC and Cmax.

Guidance for the design of bioequivalence studies:

Taking into account the pharmacokinetic properties of ritonavir, the following guidance with regard to the study design should be taken into account:

Study design: A single-dose crossover design is recommended.

Dose: The EoI includes tablets of 25 and 100 mg and pellets of 25 mg. The bioequivalence study should be conducted with the highest strength of the tablets if both strengths are developed and the conditions for an additional strength biowaiver are fulfilled, and with the 25 mg strength if pellets are developed.

Fasted/fed:

In clinical practice, ritonavir is to be taken with food. However, the Comparator product employs a complex manufacturing process in order to enhance the dissolution and absorption of ritonavir being released from the product. Due to the low solubility of the API 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 although this product should be taken only in fed state because the fasted state represents the worst-case scenario of a light meal.

On this scientific basis, for 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.

In the case of ritonavir pellets, the type of food employed in the fed study should be discussed with PQT/MED prior to undertaking the 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 drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of the parent compound ritonavir.

Sample size: Ritonavir pharmacokinetic parameters, C_{max} and AUC_{0-t}, demonstrate intra-subject variabilities of around 43% for C_{max} and 33% for AUC_{0-t}.. These data may facilitate the calculation of a sufficient sample size for the single-dose crossover bioequivalence studies.

Washout: Taking into account the elimination half-life of ritonavir of 6 – 8 h, a washout period of seven days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive the first five hours after administration to describe adequately the peak exposure of ritonavir, taking into account that T_{max} is expected around 3 h after dosing in the fasted state and 4 - 5 h in the fed state. It is not necessary to take blood samples beyond 36 hours for the characterization of ritonavir pharmacokinetics. For example, samples can be taken pre-dose and at 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.25, 3.50, 3.75, 4.00, 4,25, 4.50, 4.75, 5.00, 5.25, 5.50, 5.75, 6.00, 8.00, 10.00, 12.00, 24.00 and 36.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure 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 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 80.00 – 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%.