Notes on the Design of Bioequivalence Study: Atazanavir/Ritonavir

Notes on the design of bioequivalence studies with products invited to be submitted 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 fixed dose combination products containing atazanavir and ritonavir.

Pharmacokinetics of atazanavir

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

After oral administration, atazanavir peak plasma concentrations are reached after approximately 2–3 hours. Concomitant administration of atazanavir sulfate with a light meal or a high fat meal enhances the relative bioavailability of atazanavir by approximately 70% and 35%, respectively. Co-administration of food significantly also reduces the observed pharmacokinetic variability of atazanavir. Consequently, atazanavir is to be taken with food.

Atazanavir is extensively metabolized, primarily through monooxygenation and dioxygenation pathways. Atazanavir demonstrates nonlinear pharmacokinetics with greater than dose-proportional increases in the AUC (area under the curve) and Cmax values over the dose range of 200−800 mg once daily.

After a single dose of atazanavir/ritonavir, the half-life of atazanavir was approximately 10 – 11 hours.

Pharmacokinetics of ritonavir

After oral administration, ritonavir peak plasma concentrations are observed after approximately 4 hours. Concomitant administration of ritonavir with food improves absorption relative to the fasted state. Ritonavir should preferably be administered with food.

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. After a single dose of atazanavir/ritonavir, the half-life of ritonavir was approximately 5 hours.

Guidance for the design of bioequivalence studies

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

Design: A crossover design is recommended.

Dose: As the EoI includes 300/100 mg and 150/50 mg tablets, the highest recommended therapeutic dose for the combination product should be employed in the bioequivalence study due to the non-linearity of the pharmacokinetics of the active ingredients, if the conditions are fulfilled to waive the additional 150/50 mg strength.

Fasted/fed: In clinical practice, it is recommended to take the originator mono-component tablets with food. However, the comparator product for ritonavir 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 APIs and the complexity of the manufacturing process employed in the comparator product, 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, 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.

Subjects: Healthy adult subjects should be utilized. 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 atazanavir and ritonavir.

Sample size: Atazanavir and ritonavir pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasting state seem to possess moderate variability (25%). 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, 3.33, 3.67, 4.00, 4.33, 4.67, 5.00, 5.33, 5.67, 6.00, 8.00, 10.00, 12.00, 16.00, 24.00, 36.00 and 48.00 h after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure atazanavir 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 on bioanalytical recommendations).

Statistical considerations: The data for atazanavir and ritonavir should meet the following bioequivalence standards in the single-dose, crossover design studies:

- 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%.