Notes on the Design of Bioequivalence Study: Darunavir / 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 darunavir and ritonavir.

Pharmacokinetics of darunavir and ritonavir

Darunavir is primarily metabolized by CYP3A and ritonavir inhibits CYP3A, thereby increasing the plasma concentrations of darunavir considerably. The overall pharmacokinetic enhancement effect by ritonavir is an approximate 14-fold increase in the systemic exposure of darunavir when a single dose of 600 mg darunavir is given orally in combination with ritonavir at 100 mg twice daily.

Darunavir, co-administered with 100 mg ritonavir twice daily, is absorbed following oral administration with a T_{max} of approximately 2.5 – 4 hours. The absolute oral bioavailability of a single 600 mg dose of darunavir alone and after co-administration with 100 mg ritonavir twice daily is 37% and 82%, respectively.

When administered with food, the C_{max} and AUC of darunavir, co-administered with ritonavir, is approximately 30% higher relative to the fasting state. Therefore, darunavir, co-administered with ritonavir, should always be taken with food. Within the range of meals studied, darunavir exposure is similar. The total caloric content of the various meals evaluated ranged from 240 Kcal (12 g fat) to 928 Kcal (56 g fat).

The terminal elimination half-life of darunavir is approximately 15 hours when combined with ritonavir. In adults, linear pharmacokinetics are observed after single dose administration over the 300–1200 mg darunavir dose range with 100 mg ritonavir.

Ritonavir peak plasma concentrations occur in about 2 to 4 hours. Absorption is enhanced when ritonavir is taken with food. The elimination half-life of ritonavir is 3–5 hours. The ritonavir pharmacokinetic parameters C_{max} and AUC display a greater than proportional increase with dose. A 5-fold increase in dose from 20 mg to 100 mg results in approximately 50-fold increase in AUC.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended.

Dose: The darunavir and ritonavir combination tablets in the EoI are 800/100 mg, 600/100 mg, 300/50 mg, 400/50 mg, and 120 mg/20 mg. Bioequivalence should be demonstrated for all strengths unless a waiver for additional strengths can be applied. It appears feasible to manufacture proportional formulations for the 800/100 mg and 400/50 mg strengths, and further also proportional formulations for the 600/100 mg, 300/50 mg and 120/20 mg strengths. In such a case, studies with the highest strengths, 800/100 mg and 600/100 mg, would suffice for the whole range of strengths.

As the amount of darunavir is expected to represent more than 5% of the tablet core weight, the formulation strategy of keeping the amount of excipients constant is not considered suitable to waive the 600/100 mg strength based on the 800/100 mg strength, and the 300/50 mg strength based on the 400/50 mg strength.

The development of bilayer tablets might allow the waiver of additional strengths with proportional composition. In such cases, bioequivalence studies should be performed with the highest strength 800/100 mg due to the low solubility of both drugs and the non-linear (more than proportional) kinetics of ritonavir.

Fasted/fed: Darunavir is recommended to be taken with food because of the increased bioavailability, irrespective of the total caloric contents of the meal. 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 darunavir/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.

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 drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of darunavir and ritonavir.

Sample size: Information on darunavir and ritonavir currently available to the PQT/MED indicates that the intra-subject variability for darunavir is around 20%, but the intra-subject variability of ritonavir is around 43% for C_{max} and 33% for AUC_{0-t}. These data may facilitate the calculation of the sample size of the crossover bioequivalence studies.

Washout: Taking into account the elimination half-life of darunavir in healthy volunteers (about 15 hours), 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 darunavir and ritonavir, taking into account that T_{max} is expected around 3 h after dosing in fasted state and 4 - 5 h in fed state. The sampling times after 4 hours should be selected to characterize adequately the extent of exposure of both drugs since they have a different elimination half-life (i.e. 3–5 hours for ritonavir and 15 hours for darunavir). It is not necessary to take blood samples beyond 72 hours to characterize completely the extent of exposure of darunavir, as this will only characterise the elimination phase of darunavir.

Analytical considerations: Information currently available indicates that it is possible to measure darunavir 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 darunavir and ritonavir should meet the following bioequivalence standards in each single-dose crossover 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%.

Information currently available to PQT/MED indicates that the comparator product is a highly variable drug product. Therefore, if the Applicant suspects that the variability of C_{max} or AUC_{0-t} is high (CV>30%), the applicant may prefer to employ a full replicate design study in order to widen the acceptance range of C_{max} and/or AUC_T. For more information on replicate study designs and widening the limits of average bioequivalence, refer to Section 7.9.3 of Annex 8, TRS 1052 and PQT/MED guidance document “Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED”.