Notes on the Design of Bioequivalence Study: Nirmatrelvir + 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 nirmatrelvir and ritonavir.

Pharmacokinetics of nirmatrelvir

Ritonavir is administered with nirmatrelvir as a pharmacokinetic enhancer or booster resulting in higher systemic concentrations of nirmatrelvir. In healthy volunteers in the fasted state, the mean elimination half-life of a single dose of 150 mg nirmatrelvir administered alone was approximately 2 hours compared to 6-7 hours after administration of a single dose of 250 mg / 100 mg nirmatrelvir / ritonavir.

Less than dose proportional increases in nirmatrelvir exposures were observed after single oral doses of nirmatrelvir ranging from 250 mg to 750 mg (with and without ritonavir 100 mg). Tmax ranged from 2 to 4 h when boosted with ritonavir and from 0.5 to 2 h without ritonavir.

A high fat meal modestly increased the exposure of nirmatrelvir (approximately 15% increase in mean C_{max} and 1.6% increase in mean AUC_{0-t}) relative to the fasted state. Consequently, the comparator product can be administered with or without food.

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.

Guidance for the design of bioequivalence studies:

Please note that the study design recommendations described below are applicable for applications to PQT/MED in which both the nirmatrelvir and ritonavir components of the proposed co-pack product will be subject to the PQT/MED full assessment pathway. If the proposed co-pack product includes an SRA-

approved ritonavir product that is prequalified or seeking prequalification through the abridged assessment route (see PQT/MED website for more information), please note the following:

The study recommendations below remain unchanged including the requirement to co-administer the proposed ritonavir product with the proposed nirmatrelvir product, however, it is not necessary to measure ritonavir concentrations in the collected plasma samples since bioequivalence will not need to be demonstrated for the ritonavir component.

The recommendations noted above also apply if the proposed co-pack includes a ritonavir product that is already on the current list of prequalified products through the full assessment route.

Taking into account the pharmacokinetic properties of nirmatrelvir and 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 150 mg tablets of nirmatrelvir and 100 mg tablets of ritonavir. The bioequivalence study should be conducted preferably with the therapeutic dose 2 x 150 mg nirmatrelvir + 1 x 100 mg ritonavir taking into account the less than dose proportional pharmacokinetics of nirmatrelvir.

Fasted/fed: As the comparator nirmatrelvir / ritonavir product can be taken with or without food, a fasted state study is recommended if nirmatrelvir is the only drug investigated (i.e., ritonavir product has already been prequalified through the full assessment route, is prequalified by the SRA route or seeking prequalification through the abridged assessment route).

However, if bioequivalence has to be demonstrated also for ritonavir, as the comparator product of ritonavir employs a complex manufacturing process in order to enhance the dissolution and absorption of ritonavir being released from the product, both fasted and fed studies should be conducted. 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, bioavailability 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.

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 the biopharmaceutical quality of the product. The data for the parent compounds should be used to assess bioequivalence of nirmatrelvir and ritonavir.

Sample size: Nirmatrelvir pharmacokinetic parameters, C_{max} and AUC_{0-t}, seem to possess moderately high intra-subject variability (22% approximately for AUC_{0-t} and 36% for C_{max}), although available information is limited for 2x2 crossover designs. The intra-subject variability obtained in replicate designs for C_{max} has ranged between 17 and 22%. Ritonavir pharmacokinetic parameters, C_{max} and AUC_{0-t}, in the fasted state seem to possess moderate variability (25 – 30%), although high variability (>30%) has been observed in some studies. This data may facilitate the calculation of a sufficient sample size for a single-dose cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of nirmatrelvir and 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 for the first 4 hours after administration to properly characterize the C_{max} of nirmatrelvir. It is not necessary to take blood samples beyond 36 hours for the characterization of nirmatrelvir pharmacokinetics. For example, samples can be taken pre-dose and at 0.25, 0.50,

0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 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 nirmatrelvir 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 nirmatrelvir and ritonavir should meet the following bioequivalence standards in the single-dose cross-over design study(ies):

- 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 suggests that the comparator product might be a highly variable drug product for both C_{max} and AUC_{0-t}. 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 to estimate intra-subject variability more accurately and to widen the acceptance range for C_{max} and/or AUC_{0-t}. For more information on replicate study designs and widening the acceptance limits of average bioequivalence based on the intra-subject variability of the comparator product, 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".