

Notes on the design of bioequivalence study: Molnupiravir

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. In particular, please consult the "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability" in: *Fifty-first Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. Geneva, World Health Organization, 2017. WHO Technical Report Series, No. 1003, Annex 6.

Below, additional specific guidance is provided on the invited immediate release products containing molnupiravir.

Pharmacokinetics of Molnupiravir

Molnupiravir is a 5'-isobutyrate prodrug that is metabolised to the ribonucleoside analogue N-hydroxycytidine (NHC), which distributes into cells where it is phosphorylated to form the pharmacologically active ribonucleoside triphosphate (NHC-TP). Molnupiravir is hydrolysed to N-hydroxycytidine (NHC) prior to reaching systemic circulation by carboxylesterases present in the intestine and liver during absorption/hepatic first pass, delivering the nucleoside metabolite NHC into systemic circulation following oral administration of molnupiravir.

Following twice daily oral administration of 800 mg molnupiravir, the median time to peak plasma NHC concentrations (T_{max}) was 1.5 hours. In healthy subjects, the administration of a single 200 mg dose of molnupiravir with a high-fat meal resulted in a 35% reduction in NHC peak concentrations (C_{max}), AUC was not significantly affected. Molnupiravir can be taken with or without food. The effective half-life of NHC is approximately 3.3 hours. NHC C_{max} and AUC increases dose-proportionally.

Guidance for the design of bioequivalence studies:

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

Study design: A single-dose cross-over design is recommended.

Dose: The EoI includes 200 mg capsules. The bioequivalence can be conducted with a dose of 1 x 200 mg.

Fasted/fed: As the comparator molnupiravir product can be taken with or without food, a fasted state study is recommended.

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: Molnupiravir is the 5'-isobutyrate prodrug of NHC. Following absorption, the prodrug is rapidly converted to NHC. Therefore, bioequivalence should be based on the determination of NHC, since the parent molnupiravir is not measurable in the systemic circulation.

Sample size: Molnupiravir pharmacokinetic parameters, C_{\max} and AUC_{0-t} , seem to possess moderate intra-subject variability (approximately 22%), although information available is limited and confounding factors might have inflated the intra-subject variability estimation. 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 molnupiravir of 3.3 hours, a washout period of seven days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first 3 hours after administration to properly characterize the C_{\max} of molnupiravir. It is not necessary to take blood samples beyond 12 hours for the characterization of molnupiravir 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, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00, and 12.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure NHC in human plasma using LC-MS/MS analytical methodology with a LLOQ of 1 ng/ml. 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 NHC 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%.

Biowaiver: A BCS-based biowaiver for molnupiravir is considered a possible alternative to a bioequivalence study, provided the requirements for granting a BCS-based biowaiver are met as outlined in the ICH Guideline "Biopharmaceutics Classification System-Based Biowaivers" M9 (2019) and the PQT/MED guidance "*PQT/MED-specific Annotations for the ICH M9 Guideline for Biopharmaceutics Classification System (BCS)-based Biowaiver Applications*" (2021) because the parent drug molnupiravir is metabolized in the enterocytes and hepatocytes during the first-pass effect after drug absorption.