Notes on the Design of Bioequivalence Study: Baloxavir marboxil

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 may be considered acceptable if justified by sound scientific evidence.

The current notes should be read and followed in line with the general guidelines on submission of documentation for WHO prequalification. In particular, 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 baloxavir marboxil.

Pharmacokinetics of baloxavir

After administration of the pro-drug baloxavir marboxil, the pro-drug is rapidly converted by arylacetamide de-acetylase in the intestinal epithelium and liver into the active drug baloxavir. Plasma concentrations of baloxavir marboxil were not detectable (limit of detection 0.1 ng/ml) and therefore, pharmacokinetics are based on baloxavir plasma concentrations.

Maximum baloxavir plasma concentrations are observed around 4h after dosing in the fasting state. AUC and C_{max} increases dose proportionally over the dose range of 6 – 80 mg. The elimination half-life of baloxavir is about 79h.

Administration of baloxavir marboxil with food increased AUC and C_{max} by 36 and 48%, respectively. T_{max} was not affected. These changes were considered not clinically significant, and therefore, baloxavir marboxil may be administered with or without food.

The granules for oral suspension were bioequivalent to the tablets.

Guidance for the design of bioequivalence studies

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

Design: A single-dose, crossover study is recommended.

Dose: As baloxavir marboxil is a low solubility drug and the pharmacokinetics are linear over the 6 - 80 mg dose range, the highest strength, i.e., the 80 mg tablet for the 40 and 80 mg tablet strengths or a 80 mg dose of the oral suspension, should be employed.

Fasted/fed: The bioequivalence study should be conducted in the fasted state, as baloxavir marboxil can be taken with or without food.

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: Baloxavir marboxil is a pro-drug and is rapidly converted into the active drug baloxavir. Plasma concentrations of baloxavir marboxil are almost not detectable and therefore, pharmacokinetics are based upon baloxavir plasma concentrations. The data for baloxavir should be used to assess bioequivalence of baloxavir marboxil.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for baloxavir is around 22% for C_{max} and 15% for AUC. These data may facilitate the calculation of the sample size for the crossover bioequivalence study.

Washout: Taking into account the elimination half-life of baloxavir (79 hours), a washout period of 28 days is considered sufficient to prevent carry-over.

Blood sampling: The blood sampling should be intensive for the first 6 hours after administration to properly characterize the C_{max} of baloxavir. It is not necessary to take blood samples beyond 72 hours. For example, blood samples might be taken at pre-dose, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 10.00, 12.00, 24.00, 48.00, and 72.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure baloxavir in human plasma using LC-MS/MS analytical methodology with a LLOQ of 0.1 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 baloxavir should meet the following bioequivalence standards in a single-dose, crossover design study:

- The 90% confidence interval of the relative mean $AUC_{0\text{-}72h}$ 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%.