Notes on the Design of Bioequivalence Study: Lamivudine/Zidovudine

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. 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 lamivudine and zidovudine.

Pharmacokinetics of lamivudine and zidovudine

Maximum lamivudine concentrations are observed in plasma within 0.5 to 3.0 hours of dosing in the fasted state (median T_{max} of 1–1.5 hour). The elimination half-life of lamivudine is 5–7 hours. Co-administration of lamivudine with food results in a delay of T_{max} and a lower C_{max} (decreased by 47%). However, the extent (based on the AUC) of lamivudine absorption is not influenced. Therefore, lamivudine can be taken with or without food.

Zidovudine is well absorbed with peak serum concentrations occurring within 0.5–1.5 hours. Zidovudine may be administered with or without food. The extent of zidovudine absorption (AUC) was similar when a single dose of zidovudine was administered with food. The mean terminal plasma elimination half-life was 1.1 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended.

Dose: The EoI includes lamivudine/zidovudine 30/60 mg tablet (scored and dispersible), as well as 150/300 and 150/250 mg tablets. Bioequivalence requirements for the dispersible tablet cannot be waived because the same dosage form is not available for the comparator product, and it is not intended to be administered with a full glass of water. The 150/300mg tablet could be waived based on a Biopharmaceutics Classification System (BCS) biowaiver, if the corresponding requirements for BCS class III drugs are fulfilled, since lamivudine is considered to be a BCS class III drug and zidovudine is considered to be a BCS class I drug. A biowaiver is not applicable for the 150/250 mg strength since the same strength combination is not available for the comparator product.

In the case where a biowaiver is not possible, the bioequivalence study should be conducted with the 150/300 mg tablet if the 30/60 and the 150/300 strengths are developed and the conditions for an additional strength biowaiver are fulfilled. If the formulation for the 30/60 mg tablet differs from that of the 150/300mg product then a study will be required for the 30/60 mg tablet. For the 150/250 mg tablet, this strength should be tested in a bioequivalence study against the combination of monocomponent comparator products.

When conducting bioequivalence studies, it is essential to administer the test and the comparator product according to their corresponding instructions for use. In those cases where the test and the comparator product are different dosage forms with different methods of administration (e.g. a tablet that should be taken with a glass of water vs. a

dispersible tablet to be dispersed in 10 mL of water) the bioequivalence study should be conducted employing the intended method of administration of each product. It is considered incorrect to standardize the volume of liquid in all these cases (e.g. administering a glass of water after the intake of a dispersible tablet or rinsing the container where a dispersible tablet has been suspended with the remaining liquid of a glass of water) because this standardization does not occur in real-life conditions.

Fasted/fed: As lamivudine and zidovudine can be taken with or without food, a fasted state study is recommended.

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 drugs are considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of lamivudine and zidovudine.

Sample size: Zidovudine C_{max} seems to be highly variable (31–36% approx.). These 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 lamivudine in healthy volunteers of 5–7 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 hours after administration to properly characterize the C_{max} of lamivudine and zidovudine. It is not necessary to take blood samples beyond 24 hours for the characterization of lamivudine pharmacokinetics and 8 hours for zidovudine. For example, samples can be taken pre-dose and at 0.16, 0.33, 0.50, 0.67, 0.83, 1.00, 1.16, 1.33, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 12.00, 16.00 and 24.00 hours after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure lamivudine andzidovudine 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 ICH Harmonised Guideline M10 for more information).

Statistical considerations: The data for lamivudine and zidovudine should meet the following bioequivalence standards in a 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 suggests that the comparator product might be a highly variable drug product for zidovudine C_{max}, but not for 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 in order to widen the acceptance range of C_{max} and/or AUC_{0-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 WHO guidance document "*Application of reference-scaled criteria for AUC in bioequivalence studies conducted for submission to PQT/MED*".

Biowaiver: A BCS-based biowaiver for lamivudine and zidovudine 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).