Notes on the Design of Bioequivalence Study: 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. 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 zidovudine.

Pharmacokinetics of zidovudine

Zidovudine is well absorbed with peak serum concentrations occurring within 0.5 to 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 zidovudine, the following guidance with regard to the study design should be taken into account:

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

Dose: The EoI includes zidovudine oral solution (50 mg/5ml), 300 mg tablets, and 250 mg capsules.

For the oral solution, a single dose of 250 or 300 mg should be administered. The bioequivalence study of the oral solution can be waived if the qualitative and quantitative composition of the excipients is similar to that of the comparator, i.e. maltitol (6.4 g / 10 ml). Preservatives (i.e. sodium benzoate), buffer agents (i.e. citric acid), and flavours (i.e. strawberry flavour and white sugar flavour) may differ.

The bioequivalence study for the tablets and capsules should be conducted with the corresponding strengths. The bioequivalence study for the tablet and the capsule could be waived according to the requirements for Biopharmaceutics Classification System (BCS) biowaivers since zidovudine is classified as BCS Class I drug (PQT/MED-specific Annotations for the WHO guideline on Biopharmaceutics Classification System-based biowaivers (2024)).

Fasted/fed: As zidovudine may 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 drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of zidovudine.

Sample size: Zidovudine C_{max} seems to exhibit high variability, with approximately 31 – 36% intra-subject variability. 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 zidovudine in healthy volunteers of 1.1 hours, a washout period of 7 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 zidovudine. It is not necessary to take blood samples beyond 8 hours for the characterization of zidovudine pharmacokinetics. For example, samples can be taken pre-dose and at 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00 and 8.00 hours after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure zidovudine 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). 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 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 the PQT/MED suggests that the comparator product might be a highly variable drug product for 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 biowaiver for 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 WHO Guideline "*Biopharmaceutics Classification System-Based Biowaivers*" (TRS1052, Annex 7, 2024) and the PQT/MED guidance "*PQT/MED-specific Annotations for the WHO guideline on Biopharmaceutics Classification System (BCS)-based Biowaiver*" (2024).