Notes on the Design of Bioequivalence Study
Linezolid

Notes on the design of bioequivalence studies with products invited for submission to 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 linezolid.

Pharmacokinetics of linezolid

Linezolid is rapidly and extensively absorbed following oral dosing. Maximum plasma concentrations are reached within 2 hours of dosing. Absolute oral bioavailability of linezolid (oral and intravenous dosing in a crossover study) is complete (approximately 100%). Absorption is not significantly affected by food and absorption from the oral suspension is similar to that achieved with the film-coated tablets. Linezolid may be taken with or without food. The elimination half-life of linezolid averages at about 5–7 hours. A small degree of non-linearity in clearance is observed with increasing doses of linezolid. This appears to be due to lower renal and non-renal clearance at higher linezolid concentrations, however, the difference in clearance is small and is not reflected in the apparent elimination half-life.

Guidance for the design of bioequivalence studies

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

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

Dose: As the EoI includes linezolid 150 mg dispersible tablets, linezolid 600 mg tablet, and linezolid 20 mg/ml powder for oral suspension, the bioequivalencestudy should be conducted with these strengths at the 600 mg dose level.

The bioequivalence study for the tablet and the powder for oral suspension could be waived according to the requirements for Biopharmaceutics Classification System (BCS) biowaivers since linezolid is classified as BCS class I drug. However, for the dispersible tablet the BCS biowaiver is not possible since the comparator product is a tablet.

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, and a dispersible tablet 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 standardise the volume of liquidin all these cases (e.g. administering a glass of water after the intake of a dispersible tablet or rinsing the containerwhere

a dispersible tablet has been suspended with the remaining liquid of a glass of water) because this standardisation does not occur in real life conditions.

Fasted/fed: The bioequivalence study should be conducted in the fasted state.

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. The data for the parent compound should be used to assess bioequivalence.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for linezolid C_{max} is around 12%. 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 linezolid in healthy volunteers of 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 linezolid. Sampling times beyond 36 hours are not necessary for the quantification of linezolid. For example, blood samples might be taken at pre-dose, 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, 6.00, 8.00, 12.00, 16.00, 24.00 and 36.00 hours after drug administration.

Analytical considerations: Information currently available indicates that it is possible to measure linezolid 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).

Statistical considerations: The data for linezolid 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%.

A BCS- based biowaiver for linezolid tablets and powder for oral suspension 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 on *Biopharmaceutics Classification System-Based Biowaivers* 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 7 and the PQT/MED guidance "*PQT/MED-specific Annotations for the WHO Guideline for Biopharmaceutics Classification System-based Biowaiver Applications*" (2024).