Notes on the Design of Bioequivalence Study: Levofloxacin

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 levofloxacin.

Pharmacokinetics of levofloxacin

Orally administered levofloxacin is rapidly and almost completely absorbed with peak plasma concentrations being obtained within 1–2 h. The absolute bioavailability is 99–100%. Food has little effect on the absorption of levofloxacin. The tablets may be taken during meals or between meals. Levofloxacin is eliminated relatively slowly from the plasma (t½: 6–8 hours). Levofloxacin exhibits linear pharmacokinetics over a range of 50–1000 mg.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of levofloxacin 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 levofloxacin 100 mg dispersible tablets and levofloxacin 250 mg tablet or capsule, 500 mg tablet, and 750 mg tablet, the bioequivalencestudy should be conducted preferably with the highest strength of the product series, e.g. 100 mg for the dispersible tablet and 750 mg for the tablet, or 250 mg for the capsule, if all strengths are developed and the requirements for an additional strength biowaiver are fulfilled.

The bioequivalence study for the tablet could be waived according to the requirements for Biopharmaceutics Classification System (BCS) biowaivers since levofloxacin is classified as a BCS class I drug. However, for the capsule and 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 a small volume 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: The bioequivalence study should be conducted in the fasted state since levofloxacin can be taken with or without meals.

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, the determination of bioequivalence should be based on the parent compound.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for levofloxacin $C_{max}$is around 15–18%. These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of levofloxacin in healthy volunteers of 8 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 levofloxacin. Sampling times beyond 24 hours are not necessary for the quantification of levofloxacin. 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 and 24.00 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure levofloxacin in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitiveto detect concentrations that are 5% of the C_{max} in most profiles of each formulation (test or comparator).

Statistical considerations: The data for levofloxacin 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 levofloxacin tablets 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 on Biopharmaceutics Classification System-based Biowaiver Applications*" (2024).