

Notes on the Design of Bioequivalence Study: Cyclophosphamide

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 cyclophosphamide.

Pharmacokinetics of cyclophosphamide

Cyclophosphamide is well absorbed following an oral dose with a mean half-life of 4-8 hours for both oral and parenteral administration.

Guidance for the design of bioequivalence studies

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

Design: A multiple-dose cross-over design is recommended. Period I should begin on the first day of a treatment cycle. On the day following Day 5 of Period I, patients should be switched to receive the other treatment in Period II. Pharmacokinetic sampling should take place on Day 5 of each period. Blood sampling should occur at the same time on Day 5 for both periods to assess the concentration-time curve at pre-dose (0 hour) and at appropriate post-dose sampling times.

Dose: As the EoI includes 5 mg orodispersible minitables (in unit-dose packages) as well as 5 and 10 mg dispersible tablets, the bioequivalence study should be conducted with the highest strength if the waiver of the lowest strength applies. As the comparator is marketed at different strengths, i.e., the 25 or 50 mg strengths, the same dose of both products should be administered in the study periods.

The dose to be administered to the patients should be calculated on the basis of body surface area. Cyclophosphamide should be administered at the current dose of cyclophosphamide: the patient should receive his/her own recommended dose during the study and not be switched to a different dose or regimen for purposes of study enrolment. Each patient should receive his/her calculated dose with no change in dose between study periods, and concomitant medications should be the same for both periods of the study. If it becomes necessary to adjust a patient's dosing regimen, then that patient must be dropped from the bioequivalence study.

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 30 – 50 mL of water or an orodispersible tablet to be taken with a reduced

volume of water or without 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 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 standardisation does not occur in real life conditions. The maximum volume of water employed for a dispersible tablet for paediatric use should not exceed 50 ml.

Fasted/fed: As the comparator product can be taken with or without food, a fasted state study is recommended.

Subjects: Adult patients already receiving oral cyclophosphamide as monotherapy or as part of combination therapy. Furthermore, each patient should have comparable health status between the two periods of the 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 of cyclophosphamide.

Sample size: No information is currently available to PQT/MED on the intra-subject variability of cyclophosphamide in fasted state at steady state after multiple doses.

Washout: As the study is conducted in patients, the washout period of 5 days is active, i.e., patients are switched to the treatment of period 2 at the end of period 1 (on day 6) to continue being treated.

Blood sampling: The blood sampling should be intensive for the first hours after administration to properly characterize the C_{max} of cyclophosphamide. Blood samples for the characterization of cyclophosphamide pharmacokinetics should cover one dosing interval, i.e., 24 h. In addition, pre-dose blood samples (at -48 h and -24 h) should be collected also on the two previous days (day 3 and 4) of dosing in each period to ensure that steady-state blood plasma levels are achieved.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure cyclophosphamide in human plasma using LC-MS/MS analytical methodology. 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 cyclophosphamide should meet the following bioequivalence standards in a multiple-dose, cross-over design study:

- The 90% confidence interval of the relative mean $AUC_{0-t,SS}$ of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean $C_{max,SS}$ of the test to comparator product should be within 80.00 – 125.00%.

If patients were receiving different dosing regimens, dose should be included in the statistical model. Correction for differing dosing regimens by dose-normalization is not recommended.

Biowaiver: A BCS-based biowaiver for cyclophosphamide might be 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"](#) (2024) and the PQT/MED guidance ["PQT/MED-specific Annotations for the WHO Guideline for Biopharmaceutics Classification System \(BCS\)-based Biowaiver Applications"](#) (2025). However, as these products have different strength and dosage form compared to the comparator product, a BCS-based biowaiver is not acceptable in principle, unless otherwise justified (e.g., if bioequivalence between a tablet and an oral solution has been described in the literature). If a BCS-based type biowaiver is being considered, prior consultation with PQT/MED is **strongly advised**.