

Notes on the Design of Bioequivalence Study: Temozolomide

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 temozolomide.

Pharmacokinetics of temozolomide

After oral administration to adult patients, temozolomide is absorbed rapidly, with peak concentrations reached as early as 20 minutes post-administration (mean time between 0.5 and 1.5 hours). The elimination half-life ($t_{1/2}$) in plasma is approximately 1.8 hours.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended with dosing on the first 2 days of a treatment cycle (i.e., Period I and Period II of the study are Day 1 and Day 2, respectively, of the treatment cycle).

Dose: As the EoI includes 10 and 20 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 5 and 20 mg strengths, the 20 mg strengths should be used (e.g., 10 units of test and comparator of 20 mg, see doi: 10.1007/s00280-020-04175-0).

The dose to be administered to the patients should be calculated on the basis of body surface area. Temozolomide should be administered at the current dose of temozolomide: 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 capsule that should be taken with a glass of water vs. a dispersible tablet to be dispersed in 30 – 50 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 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 paediatric dispersible tablet should not exceed 50 ml.

Fasted/fed: As the comparator product should be taken in the fasting state, a fasted state study is recommended.

Subjects: Adult cancer patients who are already receiving or are about to start receiving temozolomide once daily as their calculated individualized dose (e.g., based upon factors such as tumour type, body surface area, cycle number and toxicity).

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 temozolomide.

Sample size: Temozolomide C_{max} seems to be moderately variable (21% approx.). These data may facilitate the calculation of a sufficient sample size for a cross-over bioequivalence study.

Washout: Due to the short half-life of temozolomide, the test and comparator products can be studied on consecutive days with no additional washout period needed.

Blood sampling: The blood sampling should be intensive for the first hours after administration to properly characterize the C_{max} of temozolomide. Blood samples for the characterization of temozolomide pharmacokinetics does not need to cover more than 10 h. For example, samples can be taken pre-dose and at 0.17, 0.33, 0.50, 0.67, 0.83, 1.0, 1.17, 1.33, 1.5, 1.67, 1.83, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 6.0, 8.0 and 10.0 hours after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure temozolomide 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 temozolomide should meet the following bioequivalence standards in a single-dose, cross-over design study:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to comparator product should be within 80.00 – 125.00%.
- The 90% confidence interval of the relative mean C_{max} 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 temozolomide 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 dosage form (and strength) compared to the comparator product, a BCS-based biowaiver is not acceptable in principle, unless otherwise justified (e.g. if bioequivalence between a capsule and an oral solution has been described in the literature). If a BCS-based type biowaiver is planned, consultation with PQT/MED is **strongly advised**.