

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 methotrexate.

Pharmacokinetics of methotrexate

When given in low doses, methotrexate is rapidly absorbed from the gastrointestinal tract giving plasma concentrations equivalent to those achieved after intravenous administration. Peak concentrations in serum are reached within 1 – 2 hours. Generally, a dose of methotrexate of 30 mg/m² or less is absorbed rapidly and completely. The bioavailability of orally administered methotrexate is high (80 – 100%) at doses of 30 mg/m² or less. Saturation of the absorption starts at doses above 30 mg/m² and absorption at doses exceeding 80 mg/m² is incomplete. The elimination follows a triphasic pattern. The half-life is approximately 3 – 10 hours following low dose treatment and 8 – 15 hours following high dose treatment.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of methotrexate, 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 methotrexate 2.5 mg orodispersible minitables and 2.5 mg dispersible tablet, the bioequivalence study should be conducted with this strength and dose.

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) 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, or an orodispersible minitab to be taken with a reduced volume of water or without water) because this standardisation does not occur in real life conditions. The maximum volume of water employed for an orodispersible minitab to be used for paediatric use should not exceed 50 ml.

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

Subjects: Healthy adult subjects can 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 of methotrexate.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for methotrexate in fasted state is around 11-14% (e.g., doi: 10.1007/s00210-022-02337-w). These data may facilitate the calculation of a sufficient sample size for the bioequivalence studies.

Washout: Taking into account the elimination half-life of methotrexate in the fasted state of 10 h, 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 methotrexate. Blood samples for the characterization of methotrexate pharmacokinetics does not need to be taken after 24 h post-dose in order to determine AUC_{0-t} . For example, blood samples might be taken at pre-dose, 0.17, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, and 24.0 h after drug administration.

Analytical considerations: Information currently available to PQT/MED indicates that it is possible to measure methotrexate 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.

Statistical considerations: The data for methotrexate 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%.