

Notes on the Design of Bioequivalence Study: Mercaptopurine

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 mercaptopurine.

Pharmacokinetics of mercaptopurine

After oral administration of mercaptopurine monohydrate $t_{\max}$ was 2.2 hours with a range of 0.5 - 4 hours. In a study with 22 adult patients the mean elimination half-life of mercaptopurine after IV infusion was 0.9 hours.

Guidance for the design of bioequivalence studies

Taking into account the pharmacokinetic properties of mercaptopurine, 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 10 mg scored dispersible tablets or 10 mg dispersible tablets, the bioequivalence study should be conducted with this strength. As the selected comparator is the 50 mg tablet, the dose of 50 mg should be administered for both products (i.e., 5 units of the test product versus 1 unit of the comparator).

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) because this standardisation does not occur in real life conditions. The maximum volume of water employed 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 or patients receiving mercaptopurine in a stable regimen using the same dose. Mercaptopurine therapy often includes daily oral mercaptopurine and weekly oral methotrexate. Since the pharmacological effect of methotrexate may potentially affect the pharmacokinetics of mercaptopurine differently on different days following the methotrexate administration, the study periods of the bioequivalence study of

mercaptopurine should be conducted at weekly intervals (within the daily dosing regimen for mercaptopurine), using the same time interval between methotrexate and study drug administration for each period.

Subjects with inherited deficiency of the enzyme thiopurine methyl transferase should be excluded from these studies.

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 mercaptopurine.

Sample size: Information currently available to PQT/MED indicates that the intra-subject variability for mercaptopurine in fasted state is around 30% (e.g., doi: 10.1002/pbc.30813), although values of 19% have also been reported (doi: 10.1002/pbc.30813). These data may facilitate the calculation of a sufficient sample size for the bioequivalence studies.

Washout: Taking into account the elimination half-life of mercaptopurine in the fasted state of 0.9 h, a washout period of 7 days (or shorter) 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 mercaptopurine. Blood samples for the characterization of mercaptopurine pharmacokinetics does not need to be taken after 12 h post-dose in order to determine AUC_{0-t} . For example, blood samples might be taken at pre-dose, 0.17, 0.33, 0.5, 0.67, 0.83, 1.0, 1.25, 1.5, 1.75, 2.0, 2.33, 2.67, 3.0, 3.33, 3.67, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0 and 12.0 h after drug administration.

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