# Notes on the Design of Bioequivalence Study: Hydroxyurea

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 dosage forms containing hydroxyurea.

## Pharmacokinetics of hydroxyurea

After oral administration, hydroxyurea (or hydroxycarbamide) is readily absorbed from the gastrointestinal tract. Peak plasma concentrations are reached at 0.75 and 1.2 h in children and adult patients, respectively. The oral bioavailability of hydroxycarbamide is almost complete.

In patients with sickle cell syndrome hydroxycarbamide was eliminated with a half-life of approximately six to seven hours, which is longer than reported in other indications.

## Guidance for the design of bioequivalence studies

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

**Design:** A single-dose, crossover design is recommended.

**Dose:** As the EoI includes 500 mg capsules and 500 and 100 mg single-scored soluble or dispersible tablets, the highest strength of each dosage form should be tested. The additional strength can be waived if the conditions for the additional strength biowaiver are met.

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 dispersible tablet to be dispersed in 10 mL of water vs. an oral solution for which water should be taken after each dose to assist accurate and consistent dose delivery to the stomach), 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 because this standardisation does not occur in real life conditions. The total volume of water employed during administration of a paediatric dispersible tablet should not exceed 50 mL.

**However, a Biopharmaceutics Classification System (BCS)-based biowaiver might be feasible for the 500 mg capsules with respect to the comparator 500 mg capsules (see below for information on BCS-based biowaivers). Further, a waiver from the requirement to conduct a BE study for the 500 and 100 mg single-scored soluble or dispersible tablets may also be possible if it is demonstrated that the API is completely dissolved at the time of administration, i.e., it is essentially an oral solution in a small volume of water at the time of administration. To satisfy the data requirements for this waiver, API data satisfying the requirements outlined in the "WHO Guideline on Biopharmaceutics Classification System-based biowaivers" (2024), a comparison of excipients between the proposed product and the oral solution comparator product, and evidence that the API is in solution in a small volume of water (recommended in the posology for the product for administration of the product) at the time of administration should be provided in a biowaiver request.**

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

**Subjects:** Adult male and non-pregnant and non-lactating female patients with sickle cell anaemia with recurrent painful crises who are on stable regimens of hydroxyurea. Blood counts at baseline and throughout the study should be monitored.

**Parent or metabolite data for assessment of bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the product. Therefore, bioequivalence should be based on the determination of hydroxyurea.

**Sample size:** Information currently available to the PQT/MED indicates that the intra-subject variability for hydroxyurea $C_{max}$ is around 21% and 8% for AUC. These data will facilitate the calculation of sufficient sample size for the cross-over bioequivalence study.

**Washout:** Taking into account the elimination half-life of hydroxyurea in patients of 6 – 7 hours, a washout period of one week is considered sufficient to prevent carry over.

**Blood sampling:** As hydroxyurea $t_{max}$ median can range between 0.75 h and 1.2 h, blood sampling should be intensive the first hours after administration to cover the peak of hydroxyurea, e.g., pre-dose and at 0.17, 0,33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 4.50, 6.00, 8.00, 12.00, 16.00 and 24.00 hours post dose.

**Analytical method:** Information currently available to the PQT/MED indicates that it is possible to measure hydroxyurea in human plasma using LC-MS/MS analytical methodology (LLOQ of 0.1 μg/ml). 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 hydroxyurea 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 reference product should be within 80-125%.
- The 90% confidence interval of the relative mean $C_{max}$ of the test to reference product should be within 80-125%.

**Biowaiver**: A BCS-based biowaiver for hydroxyurea 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" (2024) and the PQT/MED guidance "PQT/MED-specific

Annotations for the WHO Guideline for Biopharmaceutics Classification System (BCS)-based Biowaiver Applications" (2025).