

Notes on the Design of Bioequivalence Study: Flucytosine

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team – Medicines (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. In particular, 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 flucytosine.

Pharmacokinetics of flucytosine

Flucytosine is a low molecular weight, water soluble compound. Flucytosine is rapidly and virtually completely absorbed following oral administration. Bioavailability was estimated by comparing the area under the curve of serum concentrations after oral and intravenous administration showed 78% to 89% absorption of the oral dose. Flucytosine is not metabolized significantly when given orally. Peak serum concentrations were reached within 1 - 2 hours of administration. The half-life in healthy subjects ranged between 2.4 and 6 hours, whereas in another study mean elimination half-life was 5.5 h (range 4.2 – 6.9 h). Flucytosine is excreted via the kidneys by means of glomerular filtration without significant tubular reabsorption. More than 90% of the total radioactivity after oral administration was recovered in the urine as intact drug. Flucytosine is deaminated (probably by gut bacteria) to 5-fluorouracil. The area under the curve (AUC) ratio of 5-fluorouracil to flucytosine is 4%. Food decreases the absorption rate, but the total extent absorbed is not affected significantly.

Guidance for the design of bioequivalence studies

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

Design: A single-dose crossover design is recommended. However, a Biopharmaceutics Classification System (BCS) – based biowaiver might be possible if it is shown that the maximum single dose is highly soluble according to the BCS, and the corresponding requirements are met.

Dose: The EoI includes flucytosine 250 mg capsules and 500 mg (scored) tablets. The bioequivalence study should be conducted with one unit of the proposed product versus one unit of the equivalent comparator product.

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

Subjects: Healthy adult subjects should 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. Therefore, bioequivalence should be based on the determination of flucytosine.

Sample size: Information currently available to PQT/MED indicates that the residual / intra-subject variability of flucytosine is around 14 – 19%, when the products were administered in fasting state. These data may facilitate the calculation of sufficient sample size for a single-dose cross-over bioequivalence study.

Washout: Taking into account the elimination half-life of flucytosine in healthy volunteers of 3 – 6 hours, a washout period of seven days is considered sufficient to prevent carry over.

Blood sampling: The blood sampling should be intensive for the first two hours after administration to properly characterize the C_{max} of flucytosine. It is not necessary to take blood samples beyond 24 hours for the characterization of flucytosine. For example, samples can be taken pre-dose and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 6.00, 8.00, 12.00, 18.00 and 24.00 hours.

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

Statistical considerations: The data for flucytosine should meet the following bioequivalence standards in a single-dose crossover design study:

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

Biowaiver: A BCS biowaiver for flucytosine 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 "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" (2024).