Notes on the Design of Bioequivalence Study:
Flucytosine

Notes on the design of bioequivalence studies with products invited to be submitted 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.


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. 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 cross-over design is recommended. However, a Biopharmaceutics Classification System (BCS) – based biowaiver might be possible if it 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 tablets. The bioequivalence study should be conducted with one unit of the proposed product versus one unit of the equivalent comparator product.

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

Subjects: Healthy adult subjects should be utilized. It is not necessary to include patients in the bioequivalence study.
Sample size: Information currently available to PQT/MED indicates that the residual / intra-subject variability of flucytosine is around 19%, when the products were administered in fasting state. These data will facilitate the calculation of sufficient power for the 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 $C_{\text{max}}$ of flucytosine. It is not necessary to take blood samples beyond 18 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, 4.50, 5.00, 8.00, 12.00, 14.00 and 18.00 hours.

Analytical considerations: Information currently available 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 $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

Parent or metabolite data for assessment of bioequivalence: The parent drug is considered to best reflect the biopharmaceutical quality of the product. The disposition of flucytosine should be characterized and the determination of bioequivalence will be based on the parent compound.

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 80–125%
- The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product should be within 80–125%.