Notes on the Design of Bioequivalence Study:
Amphotericin B (liposomal)

Notes on the design of bioequivalence studies with products invited for submission to the WHO Prequalification Team: medicines (PQTm) 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 products containing amphotericin B 50 mg (liposomal) that, in comparison to the comparator product, have the same qualitative composition and very similar quantitative composition in excipients as well as equivalent liposome characteristics, including liposome morphology, liposome size distribution (D10, D50, D90 and span), number of lamellar, electrical surface potential or charge, lipid bilayer phase transition, and in vitro leakage rates. The in vitro liposome characterization tests should be conducted on at least three batches of the test and the comparator product and at least one test product batch should be produced by the commercial scale process and used in the in vivo study/ies.

**Pharmacokinetics of liposomal amphotericin B**

Following the first dose of liposomal amphotericin B, amphotericin B pharmacokinetics appear non-linear such that amphotericin B concentrations are greater than proportional with increasing dose. This non-proportional dose response is believed to be due to saturation of reticuloendothelial liposomal amphotericin B clearance. There was no significant drug accumulation in the plasma following repeated administration of 1 to 7.5 mg/kg/day. The volume of distribution on day 1 and at steady state suggest that there is extensive tissue distribution of amphotericin B.

After repeated administration of liposomal amphotericin B, the terminal elimination half-life (t½β) of amphotericin B was approximately 7 hours. The excretion of liposomal amphotericin B has not been studied. The metabolic pathways of amphotericin B and liposomal amphotericin B are not known. Due to the size of the liposomes, there is no glomerular filtration and renal elimination of liposomal amphotericin B, thus avoiding interaction of amphotericin B with the cells of the distal tubuli and reducing the potential for nephrotoxicity seen with conventional amphotericin B formulations.

**Guidance for the design of bioequivalence studies:**

Taking into account the pharmacokinetic and pharmacodynamic properties of liposomal amphotericin B, the following guidance with regard to the study design should be taken into account:

**Subjects:** Patients who need amphotericin B treatment. The study should not be conducted using healthy subjects because amphotericin B is cytotoxic. The patients should receive their own established amphotericin B dosing regimen during the study. Concomitant medications should remain exactly the same during the study.

**Study design:** A two-period, randomized crossover study at steady-state is recommended. However, a parallel study design might also be acceptable.
**Dose:** Each patient should receive the same dose of amphotericin B Liposomal injection at fixed 24-hour intervals. Patients with dose adjustment during the BE study should be excluded from the study. For crossover studies, patients may receive either the test or the comparator product for 5 days (Day 1-5), and then switch to the other treatment for 5 days (Day 6-10). No washout period is necessary between the two treatments. The study should be conducted with a test product produced by the proposed commercial scale manufacturing process.

**Fasting/fed:** It is essential to avoid concomitant therapy with intravenous fat emulsions, such as total parental nutrition (TPN), since that may change the pharmacokinetic profile of amphotericin B liposomes. A standard non-high-fat diet can be given during the study provided there is no interference with patient care. Alternatively, the treatment can be administered two hours after a standard (non-high-fat) breakfast every day during the study.

**Sample size:** Information on unencapsulated and encapsulated amphotericin B variability is not currently available to PQTm. Therefore, a pilot study is recommended to estimate inter-subject variability in case of parallel designs or intra-subject variability in case of cross-over designs. These variabilities should then be used for the sample size calculation of the pivotal study. In addition, the pilot study may help with the development of a sufficiently sensitive bioanalytical method, selection of sampling times, and confirmation of the time required to reach steady state.

**Washout:** If a crossover design is employed, patients may receive either the test or the comparator product for 5 days (Day 1-5), and then switch to the other treatment for 5 days (Day 6-10). No washout period is necessary between the two treatments.

**Blood sampling:** For each period, two or three trough concentrations are recommended to be measured before the full sampling day to ensure steady-state is reached. On the day of blood sample collection, a series of blood samples should be collected to assess the concentration – time curve.

**Analytical method:** Unencapsulated amphotericin B and encapsulated amphotericin B should be measured in plasma.

**Parent or metabolite data for assessment of bioequivalence:** The parent amphotericin B should be measured since the parent drug is considered to best reflect the biopharmaceutical quality of the proposed product.

**Statistical considerations:** In the case of a two period crossover study or a parallel designed study, the data should meet the following bioequivalence standards:

**Unencapsulated amphotericin B:**
- The 90% confidence interval of the relative mean AUC$_{0-24,ss}$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{\text{max,ss}}$ of the test to reference product should be within 80–125%.

**Encapsulated amphotericin B:**
- The 90% confidence interval of the relative mean AUC$_{0-24,ss}$ of the test to reference product should be within 80–125%
- The 90% confidence interval of the relative mean $C_{\text{max,ss}}$ of the test to reference product should be within 80–125%.
If the proposed product does not contain the same excipients in very similar amounts in comparison to the comparator product, the approach described above is not applicable and a complete comparability exercise is required. This includes comparison of the quality attributes, non-clinical pharmacodynamic and pharmacokinetic comparisons (e.g. distribution studies), pharmacokinetic / bioequivalence comparison, and a clinical comparison in the most sensitive indication to detect differences between formulations.