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 (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 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 (D_{10}, D_{50}, D_{90} 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.

The terminal half-life of encapsulated amphotericin B after a single dose has been shown to range between 7 and 45 h with a mean of 27 h. For non-encapsulated drug, the terminal elimination half-life ranged from 170 to 1215 h with a mean value of 475 h.

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:

Design: A single-dose crossover design is recommended.

Dose: A greater than proportional increase in AUC with increasing dose has been demonstrated for liposomal amphotericin B. Therefore, the highest dose in the non-linear part of the AUC vs. dose curve is considered the most sensitive to detect the differences that may exist between products. Therefore, a dose of 3 mg/kg over 60 (or 120) minutes is recommended.

The study should be conducted with a test product produced by the proposed commercial scale manufacturing process.

Fasted/fed: Study subjects can be given a standard non-high-fat breakfast during the study.

Subjects: Healthy adult subjects can be recruited. It is not necessary to include patients in the bioequivalence study. It is recommended that subjects be pre-treated with acetaminophen (650 mg) and diphenhydramine HCl (50 mg) to reduce infusion-related reactions.

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

Sample size: Information on unencapsulated and encapsulated amphotericin B residual variability currently available to PQT/MED shows 31% CV for unencapsulated and 14% for encapsulated amphotericin B. However, other studies have been able to show lower variability (<20%), which seems to indicate that the intra-subject variability is notably affected by the sample handling and the bioanalytical method. These variabilities could be considered for the sample size calculation.

Washout: A washout period of 28 - 36 days is considered sufficient to prevent carry-over. Shorter periods (e.g. 21 days) may be suitable, but a few subjects would be excluded due to pre-dose levels higher than 5% of the corresponding C_{max}.

Blood sampling: For an infusion time of 60 min, a sampling schedule such as the following could be employed: pre-dose, 0.50, 1.00 (end of the infusion), 1.08, 1.16, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 9.00, 12.00, 24.00, 36.00, 48.00, 72.00, 96.00, 360.00, 600.00, 840.00, 1080.00 and 1320.00 h after the start of the infusion. For encapsulated amphotericin it is sufficient to sample up to 96 h.

Analytical method: Unencapsulated amphotericin B and encapsulated amphotericin B should be measured in plasma. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the Cmax in most profiles of each formulation (test or comparator).

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

Encapsulated amphotericin B:

- 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 $AUC_{0-\infty}$ 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%.

Unencapsulated amphotericin B:

- The 90% confidence interval of the relative mean AUC_{0-t} of the test to reference product should be submitted as supportive information
- The 90% confidence interval of the relative mean $AUC_{0-\infty}$ of the test to reference product should be submitted as supportive information
- The 90% confidence interval of the relative mean C_{max} of the test to reference product should be submitted as supportive information.

The 90% confidence intervals of partial AUCs for encapsulated (e.g., AUC_{0-10h} and AUC_{10h-t}) and unencapsulated (e.g. AUC_{0-24h} and AUC_{24h-t}) amphotericin B should also be submitted as supportive information.

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.