Contains Nonbinding Recommendations

Draft Guidance on Amphotericin B

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA, or the Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the Office of Generic Drugs.

Active Ingredient: Amphotericin B

Dosage Form; Route: Injectable, liposomal; injection

Strength: 50 mg/vial

Recommended Studies: Two studies: in vivo and in vitro

To be eligible for the bioequivalence studies recommended in this guidance, the Test product should meet the following criteria:

- Qualitatively (Q1)[1] and quantitatively (Q2)[2] the same as the Reference Listed Drug (RLD)
- At least one batch of the Test product should be produced by the commercial scale process and be used in the in vivo bioequivalence study
- Have equivalent liposome characteristics, including liposome size distribution, number of lamellar, electrical surface potential or charge, lipid bilayer phase transition, and in vitro leakage rates comparable to the Reference Standard (RS).

---

1. In Vivo Study:

Type of study: Bioequivalence study with pharmacokinetic endpoints
Design: Single-dose, two-treatment, two-period crossover
Strength: 50 mg/vial
Dose: Lowest feasible dose (≤ 3 mg/kg) administered over 120 minutes
Subjects: Healthy males and non-pregnant, non-lactating females
Additional Comments: See comments below

a. Submission of a Bio Investigational New Drug Application (Bio-IND) is required prior to conducting a bioequivalence study for a cytotoxic drug product such as amphotericin B (see 21CFR § 320.31).

b. The following patients should be excluded from the study:
   - Less than 18 years of age
   - Pregnant or lactating women
   - History of hypersensitivity reactions to any components of conventional or

[1] Q1 (Qualitative sameness) means that the test product uses the same inactive ingredient(s) as the RLD product.
[2] Q2 (Quantitative sameness) means that concentrations of the inactive ingredient(s) used in the test product are within ±5% of those used in the RLD product.

liposomal amphotericin B formulations
- Serum creatinine concentration, AST, ALT greater than the upper limit of normal (ULN)

c. The following safety monitoring measures are recommended at baseline and during the course of the study:
   - Vital signs
   - Laboratory evaluation of renal, hepatic and hematopoietic function
   - Serum electrolytes, including magnesium and potassium

d. All study subjects can be given a standard non-high-fat diet during the study.

Analytes to measure: Free amphotericin B (liposome-unbound) and liposome-encapsulated amphotericin B in plasma

Bioequivalence based on (90% CI): Liposome encapsulated amphotericin B.
Submit AUC and C_{max} of free amphotericin B as supportive data.

---

2. In Vitro Study:

Type of study: Liposome size distribution
Design: In vitro bioequivalence study on at least three lots of both Test and RS products

Equivalence based on (95% CI): Population bioequivalence (PBE) based on D_{50} and SPAN [i.e. $(D_{90}-D_{10})/D_{50}$] or alternatively on the harmonic intensity-weighted average particle diameter (z-average) and polydispersity index derived from cumulant analysis of the size intensity distribution. Refer to the product-specific *Guidance on Budesonide* inhalation suspension for additional information regarding PBE.

Additional information:

Same drug product composition
Per 21 CFR § 314.94 (a) (9) (iii), as a parenteral drug product, a generic amphotericin B liposome injection must be Q1 and Q2 the same as the RLD, except differences in buffers, preservatives and antioxidants provided that the applicant identifies and characterizes these differences and demonstrates that the differences do not impact the safety/efficacy profile of the drug product. Currently, FDA has no recommendations for the type of studies that would be needed to demonstrate that differences in buffers, preservatives, and antioxidants do not affect the safety/efficacy profile of the drug product.

Lipid excipients are critical in the liposome formulation. ANDA applicants should obtain lipids from the same category of synthesis route (natural or synthetic) as found in the RLD. Information concerning the chemistry, manufacturing, and control of the lipid components should be provided at the same level of detail expected for a drug substance as suggested in

the liposome drug products draft guidance. ANDA applicants should have specification on lipid excipients that are similar to those used to produce the RLD. Provide additional comparative characterization (beyond meeting specifications) of lipid excipients including the distribution of the molecular species.

Equivalent liposome characteristics
Additional in vitro characterizations are recommended to demonstrate the sameness between the Test and Reference products in terms of liposome composition, liposome morphology and number of lamellar, lipid bilayer phase transitions, liposome size distribution, electrical surface potential or charge, in vitro leakage rates, in vitro red blood cell potassium release assay (i.e. drug concentrations inducing half-maximum potassium release) [3] and state of association of amphotericin B and the lipid bilayer[4,5].The in vitro liposome characterization tests should be conducted on at least three batches of the Test and Reference products. At least one Test batch should be produced by the commercial scale process and used in the in vivo bioequivalence study.

Waiver request of in vivo testing: Not applicable.

Dissolution test method and sampling times: The dissolution information for this drug product can be found on the FDA-Recommended Dissolution Methods Web site, available to the public at the following location: http://www.accessdata.fda.gov/scripts/cder/dissolution/. Conduct comparative dissolution testing on 12 dosage units each of the test and reference products. Specifications will be determined upon review of the abbreviated new drug application (ANDA).

[3] Rivnay et al., Critical process parameters in manufacturing of liposomal formulations of amphotericin B, Int J Pharm, 2019, 565(30): 447-457
[4] Adler et al., AmBisome: liposomal formulation, structure, mechanism of acting and pre-clinical experience, Chemother. 2002, 49, Suppl. S1, 21-30
[5] Fujii et al, The formation of amphotericin B ion channel in lipid bilayers, Biochem, 1997 Apr 22; 36(16): 4959-4968. doi: 10.1021/bi962894z.