

*Contains Nonbinding Recommendations*  
**Draft Guidance on Daunorubicin Citrate**

This draft guidance, once finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the Office of Generic Drugs.

**Active ingredient:** Daunorubicin citrate

**Form/Route:** Liposome injection/Intravenous

**Recommended studies:** 2 Studies

When the test and reference liposome products

- have the same drug product composition (qualitatively (Q1) and quantitatively (Q2)) and
- are manufactured by an active liposome loading process with a pH gradient and
- have equivalent liposome characteristics including liposome composition, internal environment of liposome, liposome size distribution, number of lamellar, electrical surface potential or charge, and in vitro drug release rates.

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We recommend the following in vivo and in vitro studies to demonstrate bioequivalence:

**In Vivo Bioequivalence Study:**

1. Type of study: Fasting\*  
Design: Single-dose, two-way crossover in vivo  
Strength: EQ 2 mg base/mL (available as 50 mg/vial)  
Dose: 40 mg/m<sup>2</sup>  
Subjects: Advanced HIV-associated Kaposi's sarcoma patients

\* If the health conditions of patients prevent fasting, the sponsor can provide a non-high-fat diet during the proposed study. Alternatively, the treatment can be initiated 2 hours after a standard (non-high-fat) breakfast.

**Additional comments:**

1. Daunorubicin citrate liposome injection is a cytotoxic drug. Therefore, a Bio-IND is required for a bioequivalence study of a doxorubicin liposome injection to ensure the safety of human test subjects.
2. Conduct the two arms of the crossover study on two of the days when the patients are scheduled to receive their usual therapy so that the treatment regimen is not altered or delayed.
3. Do not alter the standard of care treatment regimen except to randomize the patients to the test or reference therapy on the specified dosing days.
4. Given that the dosage is every 2 weeks, use two consecutive treatment cycles for the two treatment periods.

5. Any concomitant medications must be the same in both periods of the study.
6. Evaluate cardiac function by means of history and physical examination at Screening Visit and before each dose of study treatment.
7. Determination of left ventricular ejection fraction (LVEF) should be performed at total cumulative doses of daunorubicin citrate liposomal injection 320 mg/m<sup>2</sup> and every 160 mg/m<sup>2</sup> thereafter.
8. Patients who have received prior therapy with anthracyclines (doxorubicin > 300 mg/m<sup>2</sup> or equivalent), have pre-existing cardiac disease, or have received previous radiotherapy encompassing the heart may be less "cardiac" tolerant to treatment with daunorubicin citrate liposomal injection. Therefore, monitoring of LVEF at cumulative daunorubicin citrate liposomal injection doses should occur in these patients prior to therapy and every 160 mg/m<sup>2</sup> of daunorubicin citrate liposomal injection.
9. Obtain Complete Blood Count prior to each dose and withhold dosing if absolute granulocyte count is less than 750 cells/mm<sup>3</sup>.
10. Any patient whose weight changes during the study requiring a  $\pm 5\%$  dose adjustment must be discontinued from the study and excluded from the analysis.
11. Study treatment should be administered only under the supervision of a physician who is experienced in the use of cancer chemotherapeutic agents.
12. Inclusion Criteria (the sponsor may add additional criteria):
  - a) Male or female aged  $\geq 18$  years  $\leq 75$  years.
  - b) Advanced HIV-associated Kaposi's sarcoma.
  - c) Cardiac ejection fraction > 45% by Echo at Screening visit.
  - d) Granulocyte count  $\geq 1500$ /ul or WBC  $\geq 3500$  /ul at Screening visit.
  - e) Platelet count  $\geq 75,000$  and Hgb  $\geq 10$  g/dl at Screening visit.
  - f) Liver and renal function testing with no clinically significant abnormality(ies) at Screening visit
13. Exclusion Criteria (the sponsor may add additional criteria):
  - a) Female who is pregnant, breast feeding, or planning a pregnancy.
  - b) Female of childbearing potential who does not agree to utilize an adequate form of contraception throughout the study.
  - c) Clinically significant or unstable cardiac, liver or kidney disease.
  - d) Total cumulative daunorubicin dose approaching about 550 mg/m<sup>2</sup>.
  - e) Patient receiving other myelotoxic drugs.
  - f) Known allergy or hypersensitivity reaction to daunorubicin, daunorubicin citrate, any reference listed drug excipient or any study treatment excipient.

**Analytes to measure (in appropriate biological fluid):** Free daunorubicin and liposome encapsulated daunorubicin.

**Bioequivalence based on (90% CI):** AUC and C<sub>max</sub> for free daunorubicin and liposome encapsulated daunorubicin.

Note: as daunorubicin is a cytotoxic drug, a Bio-IND is required for bioequivalence studies of daunorubicin liposome injection to ensure that proposed generic products are safe for use in human test subjects and do not expose them to undue risk.

## **In Vitro Study:**

2. Type of study: Liposome Size Distribution  
Design: in vitro bioequivalence study on at least three lots of both test and reference products

**Parameters to measure:**  $D_{10}$ ,  $D_{50}$ ,  $D_{90}$

**Bioequivalence based on (95% CI):** Population bioequivalence based on  $D_{50}$  and SPAN ( $(D_{90}-D_{10})/D_{50}$ ) or polydispersity index.

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**Waiver request of in vivo testing:** Not applicable

Please note that a **Dissolution Methods Database** is available to the public at the OGD website at <http://www.accessdata.fda.gov/scripts/cder/dissolution/>. Please find the dissolution information for this product at this website. Please conduct comparative dissolution testing on 12 dosage units each of all strengths of the test and reference products. Specifications will be determined upon review of the application.

## **Additional information:**

### ***Same drug product composition***

Being a parenteral drug product, a generic daunorubicin liposome injection must be qualitatively and quantitatively the same as the reference listed drug product (RLD), except differences in buffers 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 impact the safety/efficacy profile of the drug product.

Lipid excipients are critical in the liposome formulation. ANDA sponsors should obtain lipids from the same category of synthesis route (natural or synthetic) as found in the RLD. Provide information concerning the chemistry, manufacturing and control of the lipid components at the same level of detail expected for a drug substance as suggested in the liposome drug products draft guidance<sup>1</sup>. ANDA sponsors should have specifications 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.

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<sup>1</sup> Draft guidance for industry: Liposome drug products chemistry, manufacturing, and controls; human pharmacokinetics and bioavailability; and labeling documentation, FDA (2002), <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070570.pdf>

### ***Active liposome loading process with a pH gradient***

In order to meet the compositional equivalence and other equivalence tests, an ANDA sponsor would be expected to use an active loading process with a pH gradient. The major steps include 1) formation of liposomes containing citric acid and sucrose, 2) liposome size reduction, 3) creation of a pH gradient, and 4) active drug loading. The size reduction and drug loading should be conducted at a temperature over the phase transition temperature of lipids. An active loading process uses a pH gradient between the liposome interior and the exterior environment to drive the diffusion of daunorubicin into liposomes.

Sponsors should use a Quality by Design approach to identify critical material attributes and critical process parameters, and guide process optimization. It is recommended to identify the critical process parameters and critical material attributes by evaluating the sensitivity of liposome characteristics to changes in process parameters and attributes. The optimal values of critical process parameters should be selected based on comparison of the resulting liposome characteristics to those of the RLD.

### ***Equivalent liposome characteristics***

In vitro liposome characterization should be conducted on at least three batches of the ANDA and RLD products (at least one ANDA batch should be produced by commercial scale process and used in the in vivo bioequivalence study). Attributes that should be characterized are:

- Liposome composition

Liposome composition including lipid content, free and encapsulated drug, internal and total citric acid concentration, and sucrose concentration should be measured. The drug-to-lipid ratio and the percentage of drug encapsulation can be calculated from liposome composition values.

- Internal environment

The internal environment of daunorubicin liposome includes internal volume, pH, citrate and sucrose concentration, and the status of daunorubicin citrate. Different from doxorubicin salt in Doxil, daunorubicin is unlikely to form precipitates in liposomes<sup>2</sup>. Hence, the measurements of internal volume, pH, citrate and sucrose concentrations are recommended to demonstrate equivalent liposome internal environment between test and reference products.

- Liposome morphology and number of lamellae

Liposome morphology and lamellarity should be determined as drug loading, drug retention, and the rate of drug release from the liposomes are likely influenced by lamellarity.

- Lipid bilayer phase transitions

Equivalence in lipid bilayer phase transitions will contribute to demonstrating equivalence in bilayer fluidity and uniformity. The phase transition profiles of the raw lipid excipients and liposomes should be comparable to those of RLD.

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<sup>2</sup> Dicko A, Kwak S, Frazier A, Mayer L, Liboiron B, Biophysical characterization of a liposomal formulation of cytarabine and daunorubicin. International Journal of Pharmaceutics 2010, 391: 248-259

- Liposome size distribution

Liposome size distribution is critical to ensuring equivalent passive targeting. The ANDA sponsor should select the most appropriate particle size analysis method to determine the particle size distributions of both test and reference product. The number of liposome product vials to be studied should not be fewer than 30 for each of the test and reference products (i.e., no fewer than 10 from each of three batches). See recommended study 2 (above) for details of the recommended statistical equivalence tests.

- Electrical surface potential or charge

Surface charge on liposomes can affect clearance, tissue distribution, and cellular uptake. Liposome surface charge measurement is recommended.

- In vitro leakage under multiple conditions

In vitro drug leakage testing to characterize the physical state of the lipid bilayer and encapsulated daunorubicin should be conducted to support a lack of uncontrolled leakage under a range of physiological conditions and equivalent drug delivery to the tumor cells. Below are some examples of proposed conditions.

**Table 1. Examples of in vitro leakage conditions of daunorubicin liposomes**

<b>In Vitro Drug Leakage Condition</b>	<b>Purpose</b>	<b>Rationale</b>
At 37°C in 50% human plasma for 24 hours	Evaluate liposome stability in blood circulation.	Plasma mostly mimics blood conditions.
At 37°C with pH values 5.5, 6.5, and 7.5 for 24 hours in buffer	Mimic drug release in normal tissues, around cancer cells, or inside cancer cells	Normal tissues: pH 7.3 Cancer tissues: pH 6.6 Inside cancer cells (endosomes and lysosomes ): pH 5-6.
At a range of temperatures (43°C, 47°C, 53°C, 60°C) in pH 6.5 buffer for up to 12 hours or until complete release	Evaluate the lipid bilayer integrity	The phase transition temperature ( $T_m$ ) of lipids is determined by lipid bilayer properties such as rigidity, stiffness and chemical composition. Differences in release as a function of temperature (below or above $T_m$ ) will reflect small differences in lipid properties
At 37°C under low-frequency (20 kHz) ultrasound for 2 hours or until complete release.	Evaluate the state of encapsulated drug in the liposome.	Low-frequency ultrasound (20 kHz) disrupts the lipid bilayer via a transient introduction of pore-like defects and will render the release of daunorubicin controlled by the dissolution of the gel inside the liposome.

***Equivalent in vivo plasma pharmacokinetics of free and encapsulated drug***

A Bio-IND is required to conduct bioequivalence studies of daunorubicin liposome injection in humans since daunorubicin is a cytotoxic drug. We recommend single dose fasting two-way crossover bioequivalence studies in patients diagnosed with advanced HIV-associated Kaposi's sarcoma at 40 mg/m<sup>2</sup> dose. Sponsors should measure both liposome-encapsulated and free daunorubicin to demonstrate the same in vivo stability of generic liposome formulation and RLD. Conduct the studies under either fasted or standard diet conditions depending on patient needs. See recommended study 1 (above) for details of the recommended statistical equivalence tests.