

Draft Guidance on Doxorubicin Hydrochloride

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Active Ingredient: Doxorubicin hydrochloride

Dosage Form; Route: Liposome injection; intravenous

Recommended Studies: Two studies

When the test and reference pegylated liposome products

- have the same drug product composition and
- are manufactured by an active liposome loading process with an ammonium sulfate gradient and
- have equivalent liposome characteristics including liposome composition, state of encapsulated drug, internal environment of liposome, liposome size distribution, number of lamellar, grafted PEG at the liposome surface, electrical surface potential or charge, and in vitro leakage rates.

The following clinical and in vitro studies are recommended to demonstrate bioequivalence:

In Vivo Bioequivalence Study:

1. Type of study: Fasting*

Design: Single-dose, two-way crossover in vivo

Strength: 50 mg/vial or 20 mg/vial

Dose: 50 mg/m²

Subjects: Ovarian cancer patients whose disease has progressed or recurred after platinum-based chemotherapy and who are already receiving or scheduled to start therapy with the reference listed drug (RLD) or the reference standard product.

Additional comments:

- Doxorubicin is a cytotoxic drug. Therefore, a Bio-IND is required for bioequivalence studies of a doxorubicin liposome injection to ensure the safety of human test subjects.
- The two arms of the crossover study are to be conducted 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.
- The standard of care treatment regimen should not be altered except to randomize the patients to the test or reference therapy on the specified dosing days.
- Given that the dosage is every 4 weeks, two consecutive treatment cycles should be used for the two treatment periods.

- Any concomitant medications must be exactly the same in both periods of the study.
- Due to concerns about cardiac toxicity, cardiac status should be documented at baseline.
- 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.

Exclusion Criteria:

- Prior doxorubicin exposure that would result in a total lifetime exposure of 550 mg/m² or more after four cycles of treatment.
- The protocol must exclude patients with significantly impaired hepatic function in their exclusion criteria.
- Patients who have a history of hypersensitivity reactions to a conventional formulation of doxorubicin HCl or the components of the RLD or reference standard should not be entered into the study.
- Females should not be pregnant or lactating.
- Patient is < 18 years of age or > 75 years of age.
- Active opportunistic infection with mycobacteria, cytomegalovirus, toxoplasma, P. carinii or other microorganism if under treatment with myelotoxic drugs.
- Clinically significant cardiac, liver or kidney disease.

* 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.

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

Bioequivalence based on (90% CI): AUC and Cmax for free doxorubicin and liposome encapsulated doxorubicin.

Note: the pivotal bioequivalence study should be conducted using test product produced by the proposed commercial scale manufacturing process.

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: D10, D50, D90

Bioequivalence based on (95% upper confidence bound): D50 and SPAN [(i.e. D90-D10)/D50] or polydispersity index using the population bioequivalence approach.

Dissolution test method and sampling times: The dissolution information for this drug product can be found on the FDA-Recommended Dissolution Methods website 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 all strengths of the test and reference products. Specifications will be determined upon review of the abbreviated new drug application (ANDA).

Additional information:

Same drug product composition

Being a parenteral drug product, a generic doxorubicin HCl liposome injection must be qualitatively and quantitatively the same as the RLD or reference standard, 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 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 or reference standard. 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 guidance¹. ANDA sponsors should have specification on lipid excipients that are similar to those used to produce the RLD or reference standard. Additional comparative characterization (beyond meeting specifications) of lipid excipients including the distribution of the molecular species should be provided.

Active liposome loading process with an ammonium sulfate 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 an ammonium sulfate gradient. The major steps include 1) formation of liposomes containing ammonium sulfate, 2) liposome size reduction, 3) creation of ammonium sulfate gradient, and 4) active drug loading. An active loading process uses an ammonium sulfate concentration gradient between the liposome interior and the exterior environment to drive the diffusion of doxorubicin into the liposomes^{2,3}.

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

¹ 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>

² A. Gabizon, H. Sheemda, Y. Barenholz. Pharmacokinetics of pegylated liposome doxorubicin: review of animal and human studies. Clin Pharmacokinet 42(5): 419-436 (2003)

³ F. Martin. Product evolution and influence of formulation on pharmaceutical properties and pharmacology, Advisory Committee for Pharmaceutical Science Presentation (Jul 2001), http://www.fda.gov/ohrms/dockets/AC/01/slides/3763s2_08_martin.ppt.

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 resulting liposome characteristics to those of the RLD or reference standard.

Equivalent liposome characteristics

As with other locally acting products with complex bioequivalence requirements (such as nasal sprays and inhalation products), in vitro liposome characterization should be conducted on at least three batches of the ANDA and the RLD or reference standard products (at least one ANDA batch should be produced by the commercial scale process and used in the in vivo bioequivalence study). Attributes that should be included in the characterization of ANDAs claiming equivalence to the RLD or reference standard are:

- **Liposome composition:** Liposome composition including lipid content, free and encapsulated drug, internal and total sulfate and ammonium concentration, histidine 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.
- **State of encapsulated drug:** The doxorubicin in the RLD or reference standard is largely in the form of a doxorubicin sulfate precipitate inside the liposome. The generic doxorubicin HCl liposome must contain an equivalent doxorubicin precipitate inside the liposome.
- **Internal environment (volume, pH, sulfate and ammonium ion concentration):** The internal environment of the liposome, including its volume, pH, sulfate and ammonium concentration, maintains the precipitated doxorubicin. The measurements of total and free concentrations of components (including sulfate ions) described in liposome composition section allow the inference of the internal concentration inside the liposome.
- **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 the degree of 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 the RLD or reference standard.
- **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.

- Grafted PEG at the liposome surface: The surface-bound methoxypolyethylene glycol (MPEG) polymer coating protects liposomes from clearance by the mononuclear phagocyte system (MPS) and increases blood circulation time. The PEG layer thickness is known to be thermodynamically limited and estimated to be in the order of several nanometers. The PEG layer thickness should be determined.
- Electrical surface potential or charge: Surface charge on liposomes can affect the clearance, tissue distribution, and cellular uptake. Liposome surface charge should be measured.
- In vitro leakage under multiple conditions: In vitro drug leakage testing to characterize the physical state of the lipid bilayer and encapsulated doxorubicin should be investigated 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 doxorubicin liposomes

In Vitro Drug Leakage Condition	Purpose	Rationale
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 Insider cancer cells (endosomes and lysosomes): pH 5-6 (Endosome and lysosomes of cancer cells may be involved in liposome uptake and induce drug release).
At a range of temperatures (43°C, 47°C, 52°C, 57°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 doxorubicin 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 doxorubicin liposome injection in humans since doxorubicin is a cytotoxic drug. We recommend single dose fasting two-way crossover bioequivalence studies in ovarian cancer patients at 50 mg/m² dose. Sponsors should measure both liposome-encapsulated and free doxorubicin to demonstrate the same in vivo

stability of generic liposome formulation and the RLD or reference standard. The studies may be conducted under either fasted or standard diet conditions depending on patient needs. See recommended study 1 (above) for details of the recommended statistical equivalence tests.