Amphotericin B Liposome: Revisions of the Product Specific Guidance

SBIA 2023—Advancing Generic Drug Development: Translating Science to Approval

Day 1, Session 4: Noteworthy Complex Generic Drug Approvals: Multiphase Systems

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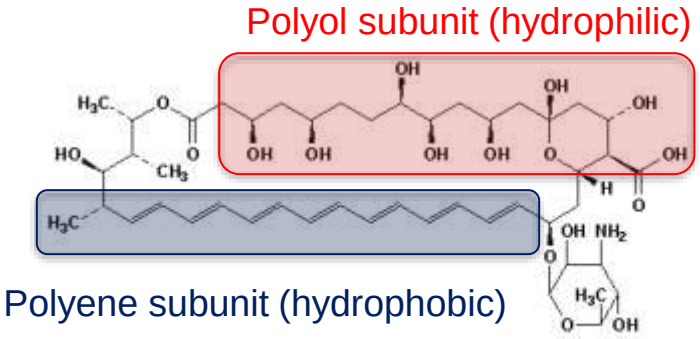
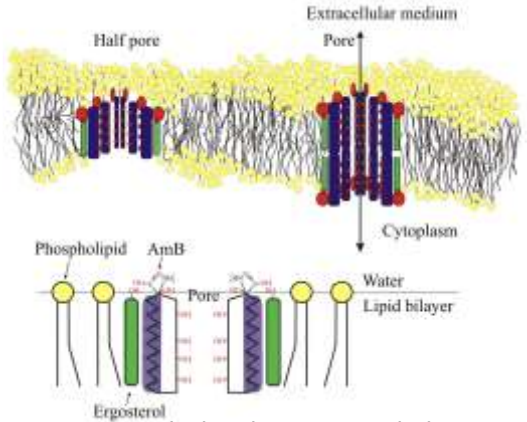
Learning Objectives

- Describe approved amphotericin B drug products and discuss challenges in developing generic amphotericin B liposome.
- Explain FDA's latest revision to the product-specific guidance (PSG) on amphotericin B liposomal injection.
- Illustrate GDUFA research on amphotericin B liposome: understand the link between aggregation status of amphotericin B in the liposomal bilayer and product toxicity.

Amphotericin B

- Amphotericin B is a heptaene macrolide antibiotic active against fungi and yeast
- Forms pores or channels in biological membranes
- Binds to ergosterol of cell membrane of susceptible fungi
- Binds to the cholesterol component of the mammalian cell leading to toxicity
- Amphiphilic feature
  - Poorly soluble in water, self-association in aqueous media
  - Present as monomer, soluble and non-soluble aggregates
  - Heat induced “super-aggregation” reduce in vitro toxicity (Gaboriau *et al*., 1997)





European Biophyscis J, 2014; 43(10): 453-467

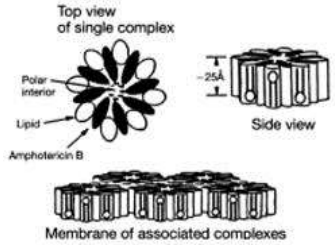
Amphotericin B Formulations

Mar 01,1966 — Nov 20,1995 — Nov 22,1996 — Aug 11,1997

Fungizone

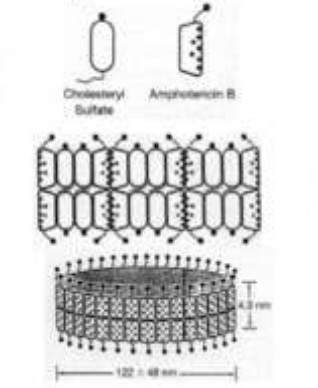
ANDA 060517
Powder of sodium deoxycholate and amphotericin B

Abelcet® ABLC



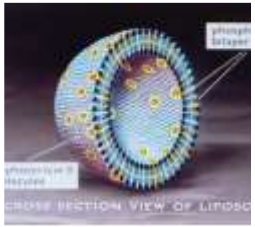
NDA 050724
Ribbon-like particles
Carrier lipids: DMPG, DMPC
Particle size(μm): 1.6-11

Amphotec® ABCD



NDA 050729
Disc-like particles
Carrier lipids: cholesteryl sulfate
Particle size(μm): 0.12-0.14
(Discontinued)

AmBisome



NDA 050740
True unilamellar liposomes
Carrier lipids: DSPG, HSPC, cholesterol
Particle size (μm): 0.08

DMPG: 1-α-dimyristoylphosphatidylglycerol; DMPC: 1-α-dimyristoylphosphatidylcholine
DSPG: distearoyl phosphatidylglycerol; HSPC: hydrogenated soy phosphatidylcholine

Min et al., *Chem. Rev.* 2015 (with modification)

AmBisome

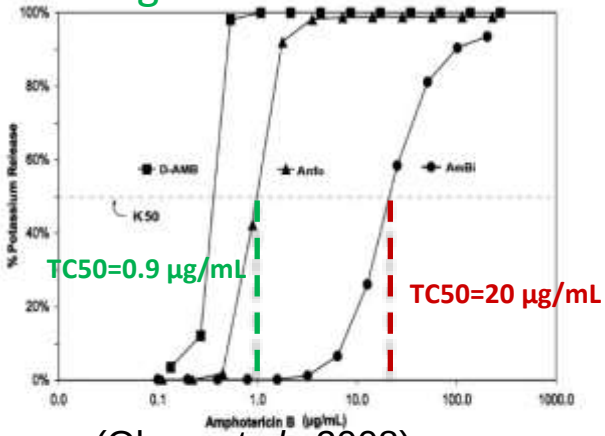
- A liposomal formulation of amphotericin B indicated for the treatment of fungal infection
- Included in WHO List of Essential Medicines; difficult to access in many countries (Gaspani et al. 2013)
- Sales: $540 million globally and $39 millions in the U.S. (2021)
- U.S. patents expired in 2016

Challenges in Developing Generic Amphotericin B Liposome

- Demonstrating bioequivalence
- Technical difficulties in manufacturing
- Special consideration: toxicity
  - Manufacturing process has impact on aggregation status of the amphotericin B drug substance in the liposome bilayer, which could result in different product toxicity.

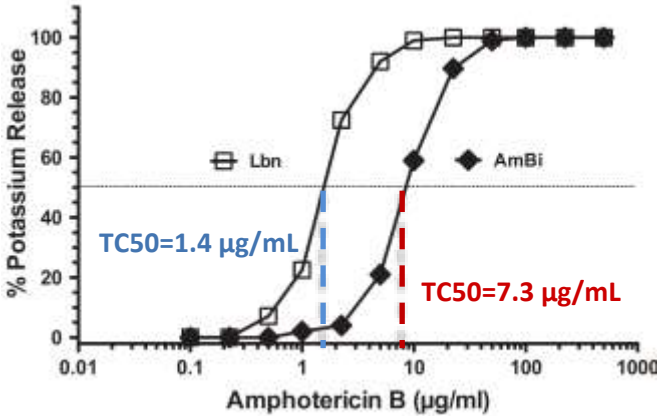
Liposomal 'Follow-on' Products Approved Outside the U.S.

Anfogen vs. AmBisome



(Olson *et al.*, 2008)

Lambin vs. AmBisome

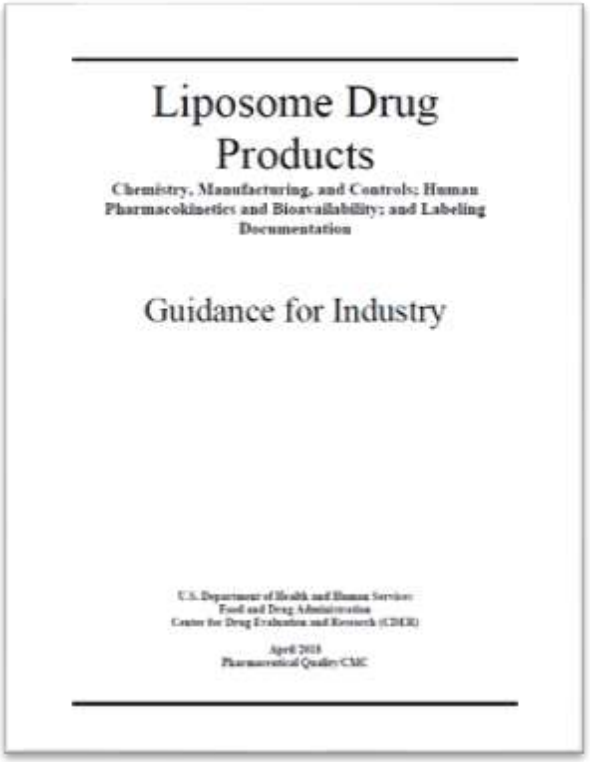


(Olson *et al.*, 2014)

TC_{50}: Drug concentrations inducing half-maximum potassium release

- Anfogen (previously licensed in Argentina) and Lambin (marketed in India) were reported to have the same chemical composition as Ambisome but were manufactured differently.
- Anfogen was withdrawn due to toxicity concerns (Adler-Moore *et al.*, 2016)

FDA's Guidances



Liposome Drug Products

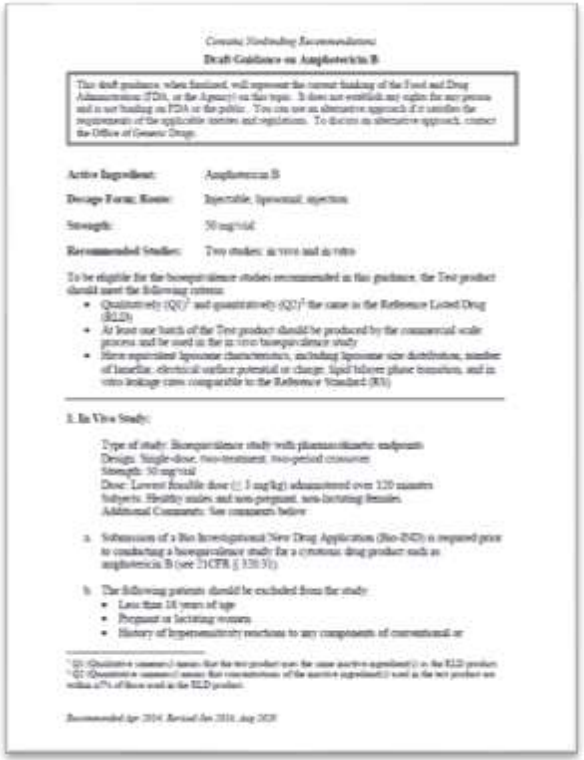
Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

April 2018
Pharmaceutical Quality/CMC

Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation | FDA



Ceramic 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: an *in vivo* and an *in vitro*

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

- Quantitatively (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 lamellae, electrical surface potential or charge, lipid bilayer phase transition, and *in vitro* leakage rates comparable to the Reference Standard (RS)

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

Recommended Apr 2018, Revised Jan 2019, Aug 2020

Product-Specific Guidances for Generic Drug Development (fda.gov)

PSG on Amphotericin B Liposome

- Recommends using in vivo and in vitro studies to demonstrate BE.
- Two changes were made in August 2020 revision:
  - **In vivo study: Changing multi-dose steady-state study in patients to single-dose study in healthy subjects**
  - **In vitro study: adding in vitro red blood cell potassium release assay and state of association of amphotericin B and the lipid bilayer**
- The change in in vivo study design was based on new healthy subject information that was provided by generic drug industry through controlled correspondences and pre-ANDA meetings.
- The addition of in vitro studies was based on finding from relevant GDUFA research projects.

GDUFA Research Projects on Amphotericin B Liposome

- Grant U01FD005249-01: Evaluation of in vitro release methods for liposomal amphotericin B, ZoneOne Pharma, Inc. and University of Michigan
- FDA Internal research: Evaluation of size-based distribution of drug and excipient in amphotericin B liposomal formulation, NCTR
- Contract HHSF223201610093C: Critical process parameters for the preparation of amphotericin B liposomes, Neo-Advent Technologies LLC
- Contract 75F40120C00055: Evaluation of critical process parameters for the preparation of amphotericin B that influence toxicity, Landrau Scientific Innovations

Manufacturing Steps for Amphotericin B Liposome

- A four-step process was used based on the method described in U.S. Patent 5,965,156:

Step 1

Mixing lipids and drug in organic solvents

Step 2

Spay drying

Step 3

Hydration and microfluidization

Step 4

Lyophilization

Design of Experiment (DOE) and Quality by Design (QbD) Screening

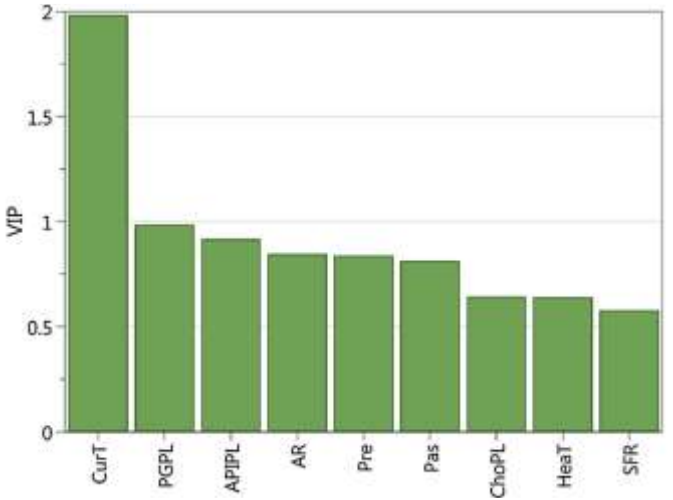
- Nine CPPs and five critical quality attributes (CQAs) were used for DoE and QbD screening analysis.

Experimental design using the fractional factorial design (Resolution III: $2^{(9-5)} + 3$) and experimentation result (See Table 2 for CPPs and CQAs abbreviations).

Exp No.	Organic Mixing			Spraying Drying		Microfluidization			Lyophilization	Critical Drug Product Quality						
	PGPL	ChoPL	APIPL	AR	SFR	CurT	Pre	Pas	HeaT	AMP	APR	PS.M	TC.M	CaiQ	PS.R	TC.R
1	0.237	0.3	0.166	7	0.75	58	70	4	20	331	0.277	141.5	0.313	3.9	236	0.176
2	0.321	0.41	0.122	7	0.75	58	100	4	25	332	0.2946	181.7	0.374	5	1560	0.556
3	0.237	0.41	0.122	12	0.75	58	100	10	20	329	0.3037	118.1	0.532	4	303	0.292
4	0.321	0.3	0.166	12	0.75	58	70	10	25	331	0.2987	173.2	0.798	3.6	192.3	0.845
5	0.237	0.3	0.122	7	1.4	58	100	10	25	322	0.281	173.7	0.187	3.25	299.5	0.555
6	0.321	0.41	0.166	7	1.4	58	70	10	20	331	0.298	234.3	0.499	3.5	158.2	—
7	0.237	0.41	0.166	12	1.4	58	70	4	25	326	0.2866	193.3	0.477	3	155	0.341
8	0.321	0.3	0.122	12	1.4	58	100	4	20	338	0.3178	115	0.29	3.5	—	—
9	0.237	0.41	0.122	7	0.75	72	70	10	25	321	0.2573	212.6	2.462	3.2	756.8	2.114
10	0.321	0.3	0.166	7	0.75	72	100	10	20	321	0.235	387	0.777	1.5	265.9	1.079
11	0.237	0.3	0.166	12	0.75	72	100	4	25	321	0.1788	144.6	1.141	4.45	279	1.818
12	0.321	0.41	0.122	12	0.75	72	70	4	20	321	0.2247	256.5	2.298	4	454	2.453
13	0.237	0.41	0.166	7	1.4	72	100	4	20	321	0.247	386	1.866	1.5	618.5	—
14	0.321	0.3	0.122	7	1.4	72	70	4	25	320	0.272	232	0.67	4.3	139.7	—
15	0.237	0.3	0.122	12	1.4	72	70	10	20	321	0.2735	137.8	1.669	5	254.9	—
16	0.321	0.41	0.166	12	1.4	72	100	10	25	320	0.3173	482.9	1.671	5.4	232	—
17	0.279	0.355	0.144	9	1.075	65	85	7	22.5	323	0.2896	188.3	1.068	3	177.3	0.419
18	0.279	0.355	0.144	9	1.075	65	85	7	22.5	321	0.2381	178.4	0.566	4	1259	0.436
19	0.279	0.355	0.144	9	1.075	65	85	7	22.5	323	0.258	229	0.94	4	172	0.94

—: data not available.

CPPs ranking

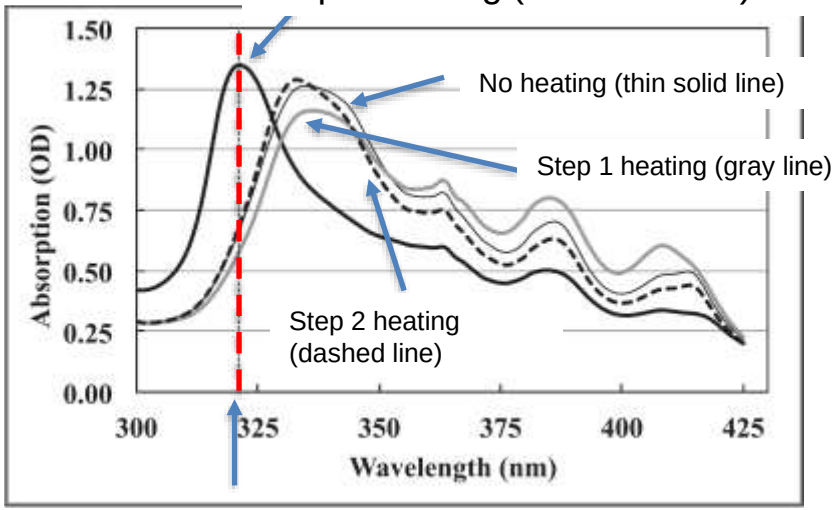


- Curing temperature (CurT) had the greatest effect on CQAs, followed by Q2 formulation differences and lastly by the liposomal processing CPPs

Contract HHSF223201610093C

Liu et al., *Int. J. Pharm.* 2020, 585:119473

Impact of a “Curing” Step



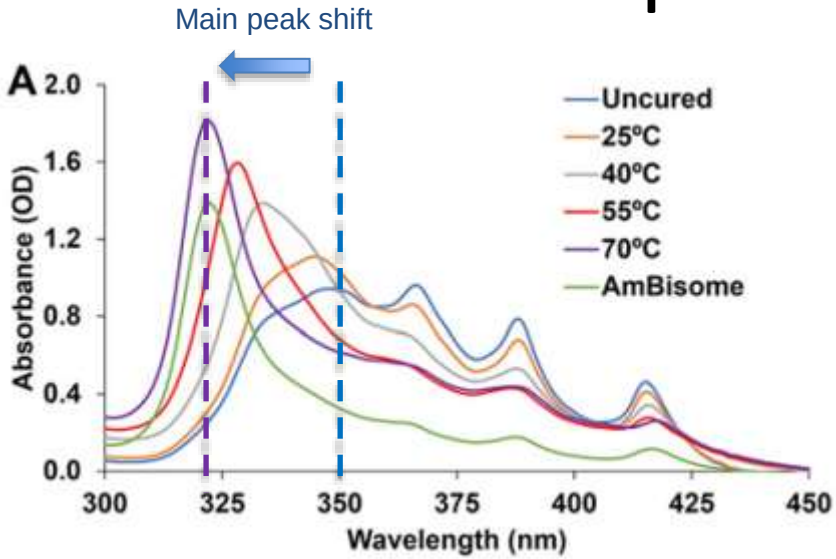
Main peak position of AmBisome

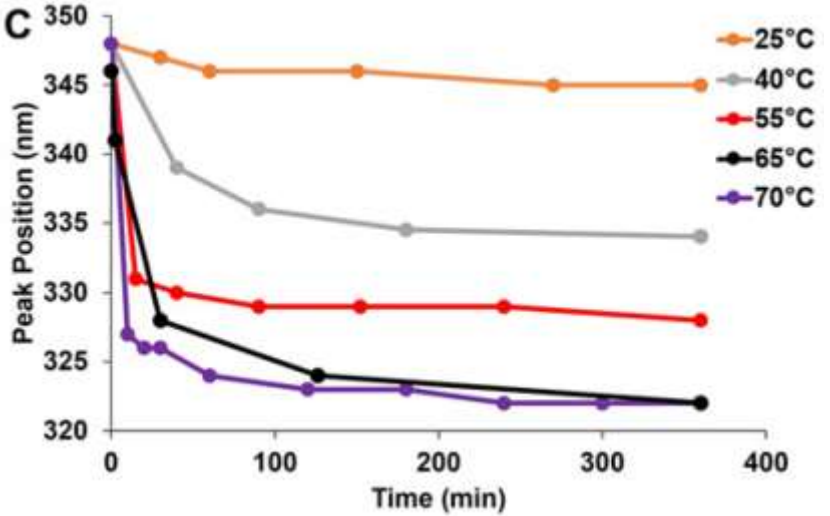
	TC_{50} (µg/mL)
No heating	0.410
Step 1 heating	0.160
Step 2 heating	0.600
Step 3 heating	5.75
AmBisome	1.697

Contract HHSF223201610093C

Rivnay et al., *Int. J. Pharm.* 2019 565:447-457

Impact of Curing at Different Temperatures





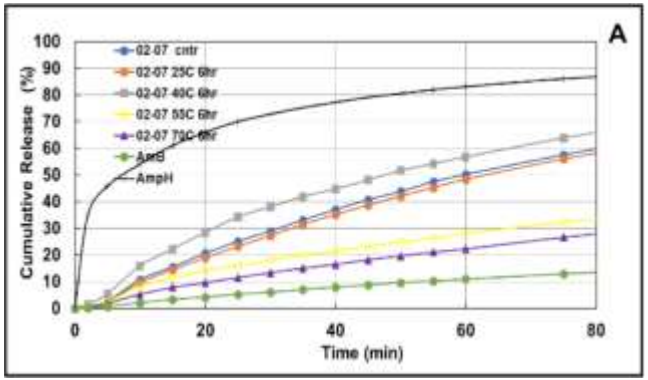
- As curing temperature increased, main peak underwent a blue shift
- Similar trends were seen in main peak ratio (OD_{346nm}/OD_{322nm})

Contract 75F40120C00055
Asian Journal of Pharmaceutical Sciences 17 (2022) 544-556

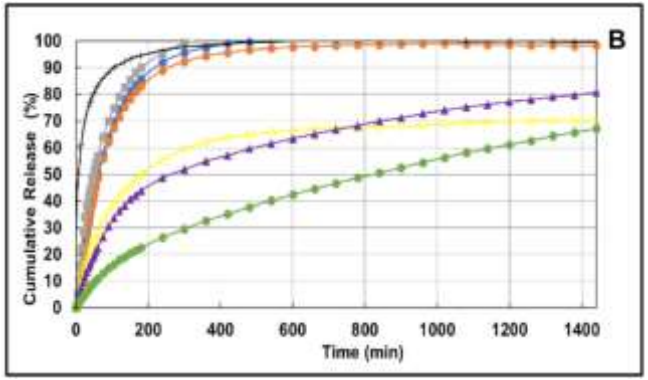
In Vitro Drug Release Test (IVRT)

IVRT Method was adopted from *Eur. J. Pharm. and Biopharm*, 2019, 134:107-116 (funded by **Grant U01FD005249-01**)

- USP-4 apparatus
- Release medium: 5% sucrose, 10 mM HEPES, and 0.01% NaN3 (pH=7.4), γ-cyclodextrin 5% w/v
- 1.5 mL sample were placed in a Float-A-Lyzer membrane compartment (300 kDa Mw cut-off) and inserted into USP 4 flow through cells
- Close loop setting at 16 ml/min
- Temperature: 55°C



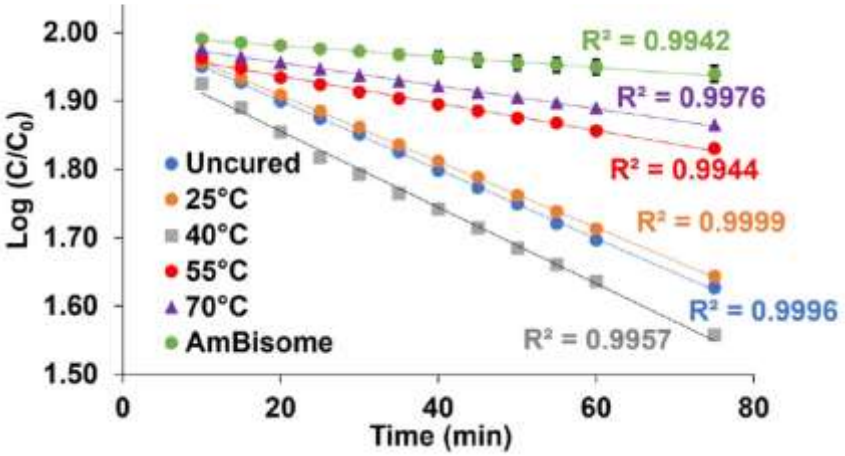
Cumulative release% over 80 min

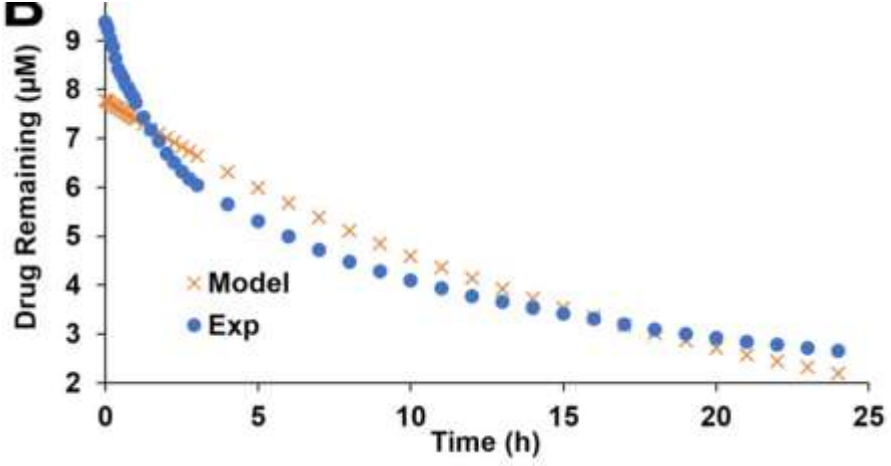


Cumulative release% over 24 hours

First Order One-pool Release Model

$$\log C = \log C_o - Kt/2.303$$





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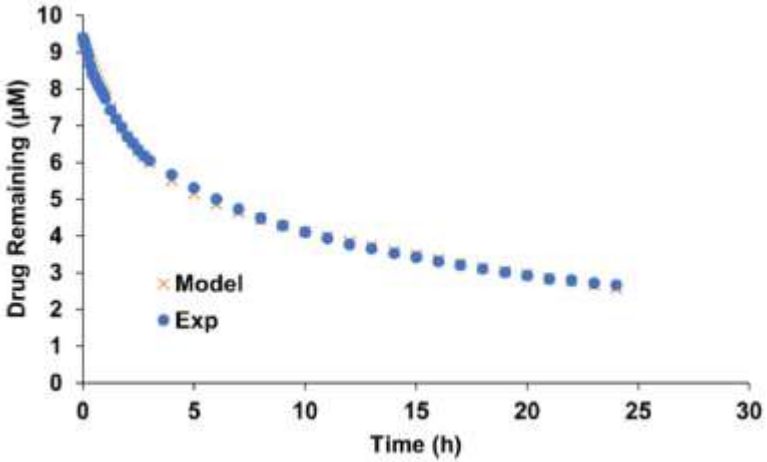
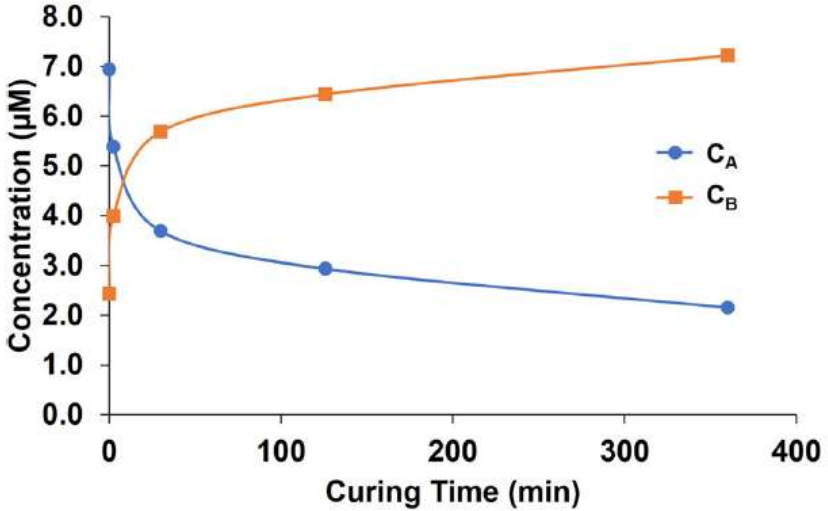
First Order Two-pool Release Model

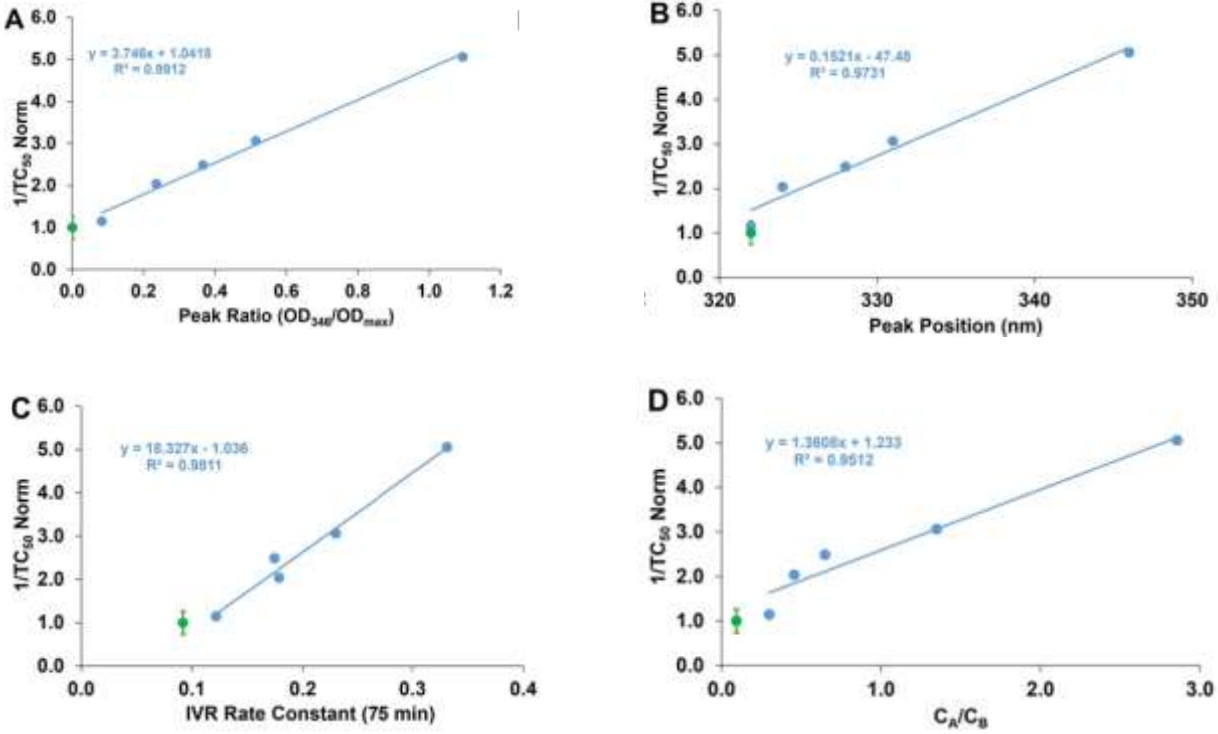
$$C = C_A e^{-K_A t} + C_B e^{-K_B t}$$





- Two pool model fits better than one pool model.
- This model suggested amphotericin B may exist in two different aggregate forms (loose and tight) as deducted from UV/Vis analysis

Correlation of Normalized In Vitro Toxicity with Spectral Analysis and IVRT



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Summary of GDUFA research

- A thermal treatment process (“curing”) was found to be critical for reducing the toxicity of amphotericin B liposome formulations.
- As “curing” progresses, amphotericin B shifted from loose aggregate to tight aggregate, as evidenced by the blue shift in spectral method and release rate changes in IVRT.
- The two physicochemical analytical methods (spectral method and IVRT) correlated well to in vitro toxicity measured by in vitro potassium release assay.

First Generic Amphotericin B Liposome Drugs

The first U.S. generic Amphotericin B liposome products were approved in 2021 and 2022

GENERICS BULLETIN
ONLINE COMMERCIAL

21 Dec 2021 | **News**

Sun Gets CGT Nod For AmBisome Rival

Awarded 180 Days Exclusivity For Amphotericin B Liposome Injection

by Akriti Seth

Sun Pharma is looking to target a market worth $136m after receiving US FDA approval for amphotericin B liposome with a Competitive Generic Therapy designation, bringing the promise of 180 days of CGT exclusivity for the product.

11/22/2022

Eugia Pharma Specialities gets FDA OK for generic AmBisome Liposome for Injection

Amphotericin B liposome for injection, 50 mg /vial is a partnership product from TTY Biopharm.

Sandra Levy
Senior Editor

Aurobindo Pharma's subsidiary, Eugia Pharma Specialities, has obtained the Food and Drug Administration's clearance for amphotericin B liposome for injection, 50 mg /vial, which is a generic of Astellas' AmBisome Liposome for Injection.

This is a partnership product from TTY Biopharm and will be manufactured at their Taiwan facility

Summary

- GDUFA research and information submitted by the generic drug industry gave rise to revised thinking and recommendations in the PSG on amphotericin B liposome.
- Manufacturing process has impact on the aggregation status of amphotericin B in the liposome bilayer, which could result in different product toxicity.
- The product toxicity can be informed via physicochemical characterization (spectral method), IVRT, and in vitro potassium release assay.

Challenge Question #1

Is this statement correct? The in vivo bioequivalence study should not be conducted in healthy volunteers as amphotericin B liposome is toxic.

A. True

B. False

Challenge Question #2

Which of the following supportive comparative physicochemical characterization is NOT relevant to bioequivalence of amphotericin B liposome?

A. Liposome size distribution.

B. In vitro red blood cell potassium release assay (drug concentrations inducing half-maximum potassium release).

C. State of associated drug and the lipid bilayer.

D. Internal environment (volume, pH, sulfate, and ammonium ion concentration.

U.S. FOOD & DRUG ADMINISTRATION

Questions?

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