

Advanced Analytical Methods to Characterize Liposome Drug Products

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Ispra, Italy (via webex)

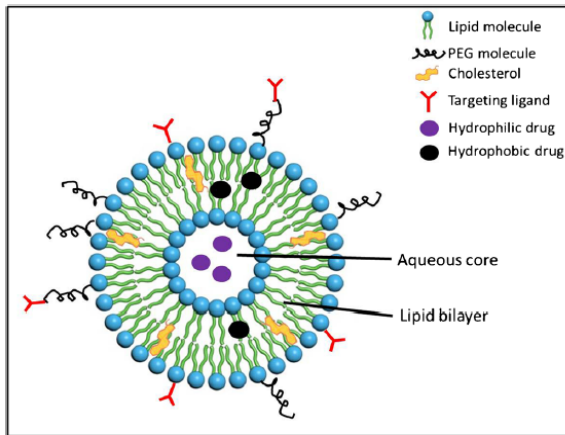
Joint European Commission Joint Research Center (JRC)-National Institute of Standards and Technology (NIST)
Workshop on Characterization Methods and Standards for Nanoparticles in Medical Products

Disclaimer



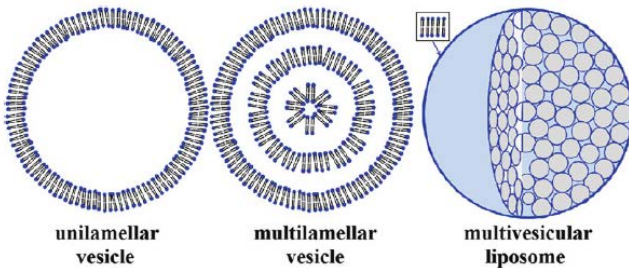
- The views expressed in this presentation are those of the speaker and not necessarily those of the Food and Drug Administration (FDA).
- Drug product names are mentioned in the presentation for clarification not endorsement.

Liposome and Liposome Drug Products



Liposome

- Microvesicle composed of a bilayer and/or a concentric series of multiple bilayers separated by aqueous compartments formed by amphipathic molecules such as phospholipids that enclose a central aqueous compartment



Liposome Drug Product

- A drug product in which the active pharmaceutical ingredient (API) is contained in liposomes

www.fda.gov

FDA Approved Liposome New Drug Applications (NDAs)

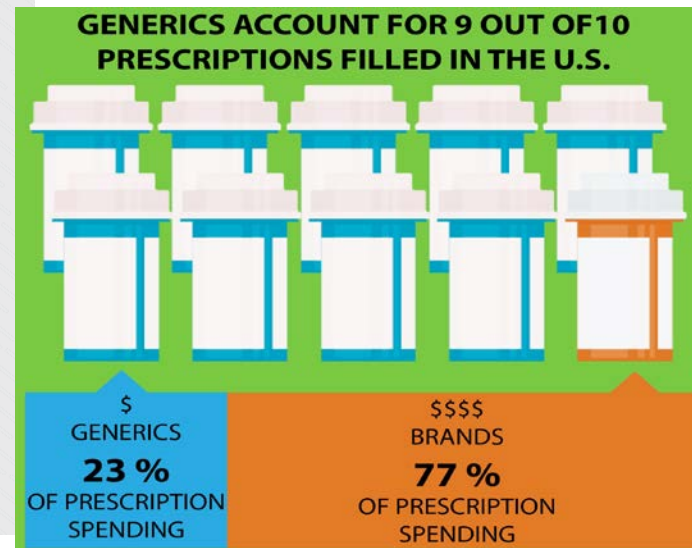
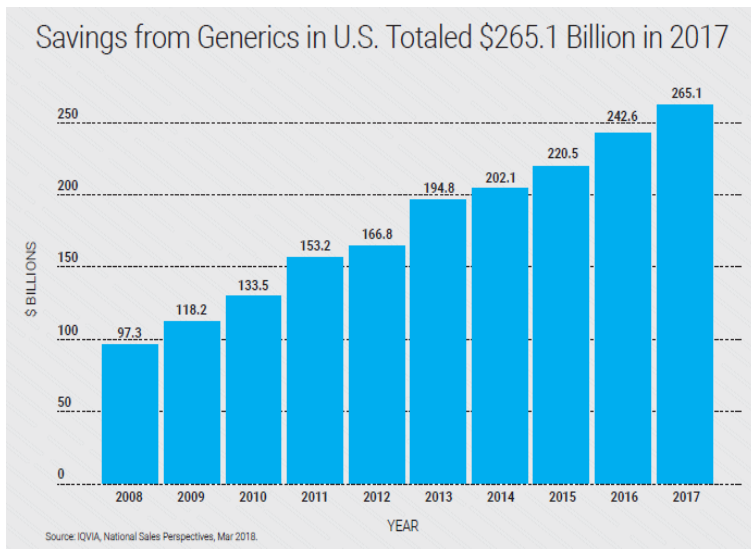


Trade name	Active Ingredient	Indication and Usage	Route	Initial Approval Date	Market Status Available
DOXIL	Doxorubicin HCl	Ovarian cancer, AIDS-related Kaposi's sarcoma, multiple myeloma	Intravenous	11/17/1995	Yes
DAUNOXOME	Daunorubicin Citrate	Advanced HIV-related Kaposi's sarcoma (relapse)	Intravenous	4/8/1996	Yes
AMBISOME	Amphotericin B	Certain fungal infections	Intravenous	08/11/1997	Yes
DEPOCYT	Cytarabine	Lymphomatous meningitis	Intrathecal	04/01/1999	Discontinued
VISUDYNE	Verteporfin	Photosensitizer for treatment of certain patients	Intravenous	04/12/2000	Yes
DEPODUR	Morphine Sulfate	Opioid local analgesic	Epidural	05/18/2004	Discontinued
EXPAREL	Bupivacaine	Postsurgical analgesia	infiltration into the surgical site	10/28/2011	Yes
MARQIBO	Vincristine Sulfate	Acute lymphoblastic leukemia	Intravenous	08/09/2012	Yes
ONIVYDE	Irinotecan HCl	Metastatic pancreatic cancer	Intravenous	10/22/2015	Yes
VYXEOS	Daunorubicin and Cytarabine	Therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC)	Intravenous	08/03/2017	Yes
ARIKAYCE KIT	Amikacin sulfate	Mycobacterium avium complex (MAC) lung disease	Oral inhalation	09/28/2018	

<https://www.accessdata.fda.gov/scripts/cder/daf/>

Generic Drugs

- Generic drugs are “copies” of their respective reference listed drugs (RLDs)
- Generally, this means same active ingredient, dosage form, strength, routes of administration, quality, performance characteristics, safety, efficacy, and intended use.



New Drug Application (NDA) vs. Abbreviated New Drug Application (ANDA)



NDA

1. Chemistry
2. Manufacturing
3. Testing
4. Labeling
5. Inspection
6. Animal Studies
7. Clinical Studies
8. Bioavailability

ANDA

1. Chemistry
2. Manufacturing
3. Testing
4. Labeling
5. Inspection
6. Bioequivalence

Generic Liposome Drug Products



Doxorubicin HCl (liposomal)	ANDA	Manufacturer	Approval Date
	203263	Sun Pharma Global	Feb 4, 2013
	208657	Dr Reddy's Labs LTD	May 15, 2017



Guidance on Liposome Drug Products

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

Physico-chemical Characterization

Liposome components

- Lipids
- Unencapsulated drug vs liposome associated drug

...

Liposome higher order structure

- Particle size
- Morphology
- Lamellarity
- Surface characteristics of the liposomes
- Liposome phase transition temperature

Liposome performance

- In vitro release
- Liposome integrity changes

Product-specific Guidance for Liposome Drug Products

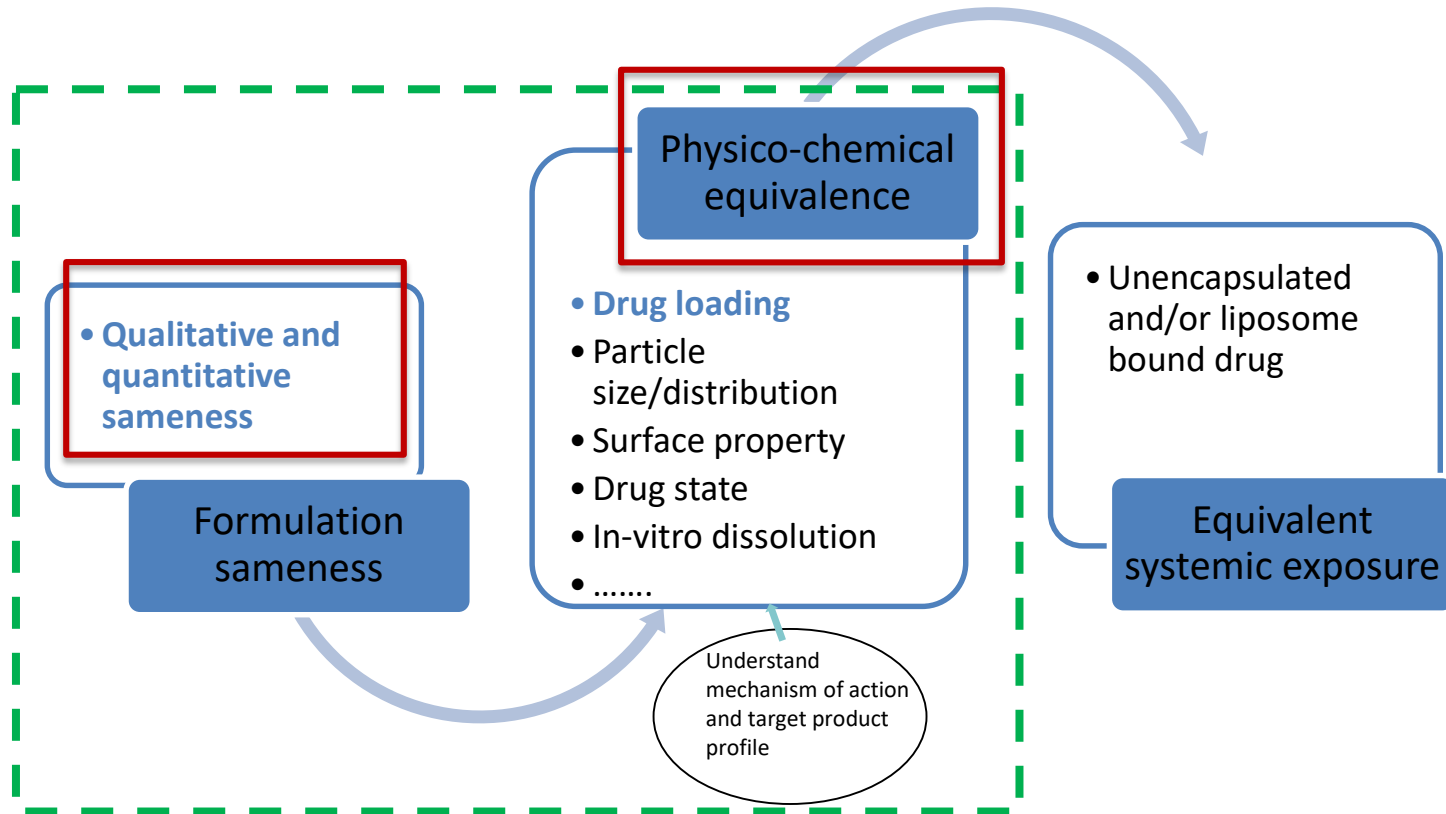


Trade name	Initial Approval Date	Product-Specific Guidance Available
DOXIL	11/17/1995	Yes
DAUNOXOME	4/8/1996	Yes
AMBiSOME	08/11/1997	Yes
DEPOCYT*	04/01/1999	No
VISUDYNE	04/12/2000	Yes
DEPODUR*	05/18/2004	No
EXPAREL	10/28/2011	Yes
MARQIBO	08/09/2012	No
ONIVYDE	10/22/2015	No
VYXEOS	08/03/2017	No
ARIKAYCE KIT	09/28/2018	No

<https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075207.htm>

* Product discontinued

General Paradigm for Therapeutic Equivalence Recommendation of Liposome Drug Products



Unencapsulated drug: drug not associated with liposomes which is either present in the formulation or released from the liposomes

Zheng N, Sun DD, Zou P, Jiang W. Scientific and Regulatory Considerations for Generic Complex Drug Products Containing Nanomaterials. *AAPS J.* 2017 May;19(3):619-631.

Advanced Analytical Methods to Characterize Liposome Compositions



- Unencapsulated and liposome associated drug
- Liposome excipients and active pharmaceutical ingredient (API)
- Liposome excipient degradation products

Direct Quantification of Unencapsulated Drug in Liposome Drug Product

Model Drug: Doxorubicin HCl Liposomes

Proprietary Name: DOXIL

Generic Name:

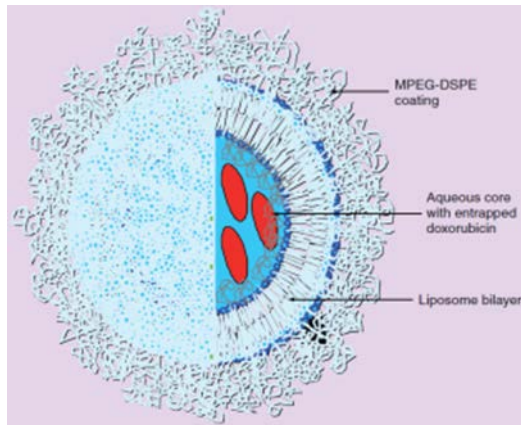
Doxorubicin HCl liposome injection

Indication and Regimen:

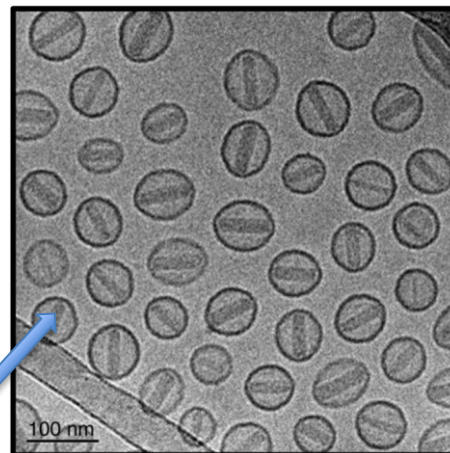
Aids-related Kaposi's Sarcoma

Ovarian cancer

Multiple myeloma in combination with bortezomib



Liposome
associated Dox



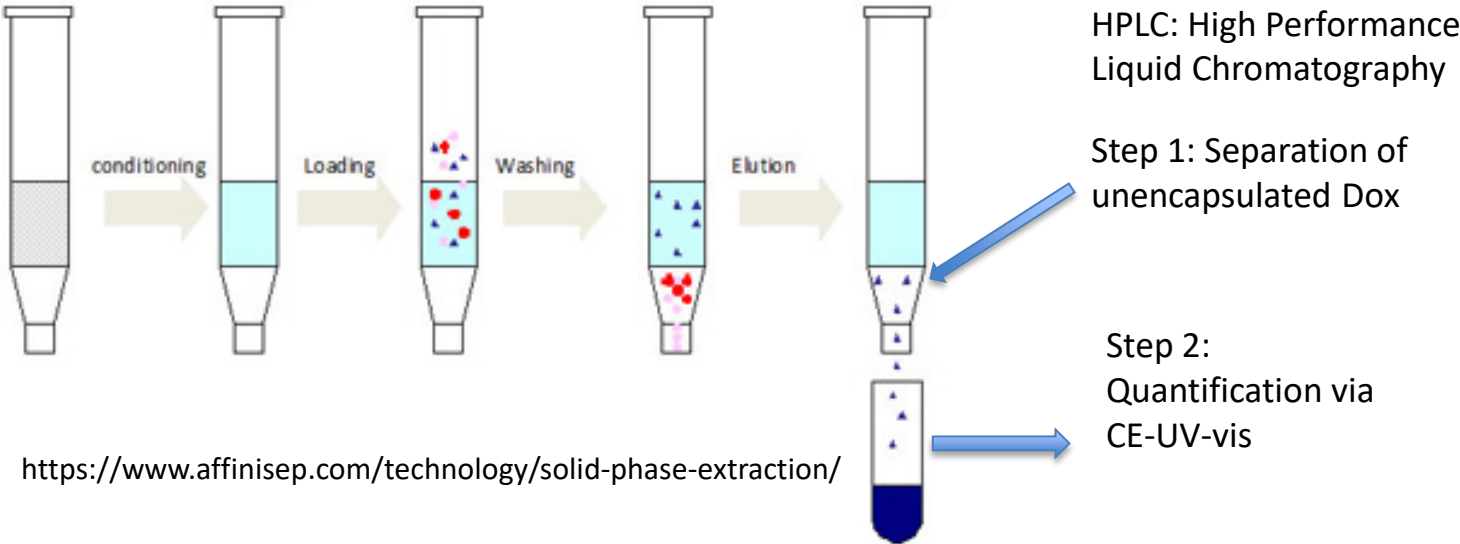
Mechanism of Action

- Passively targets tumor sites due to its small size and persistence in the circulation (Enhanced Permeation and Retention (EPR) effect)
- Free doxorubicin (Dox) HCl becomes available at the tumor cell and binds DNA and inhibits nucleic acid synthesis. The exact mechanism of release is not understood.

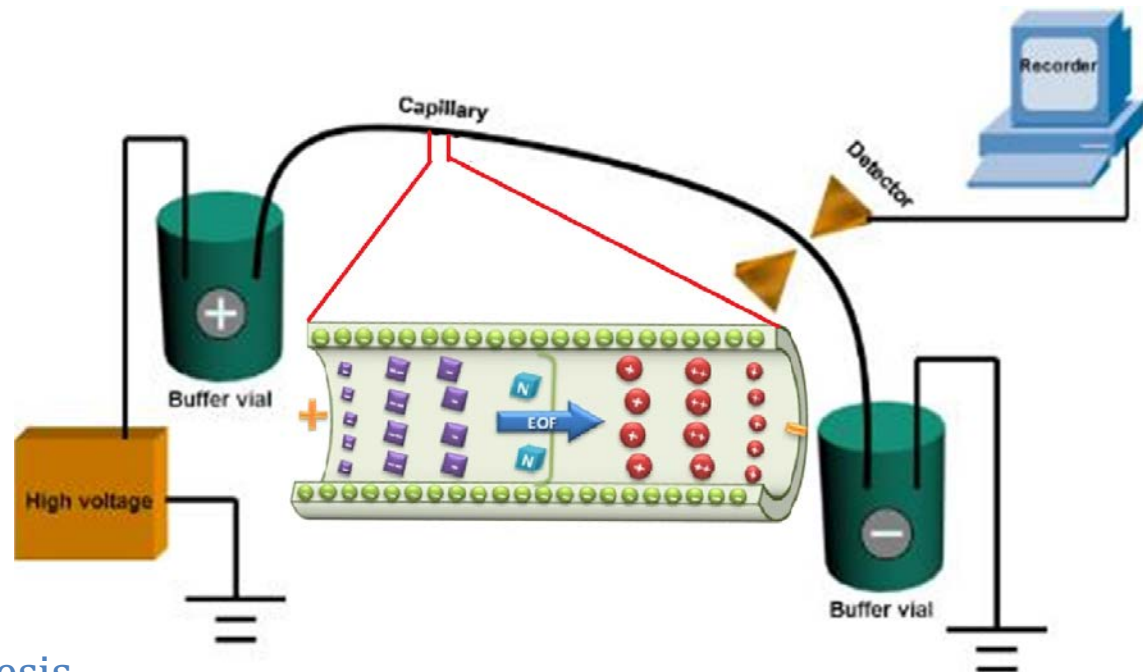
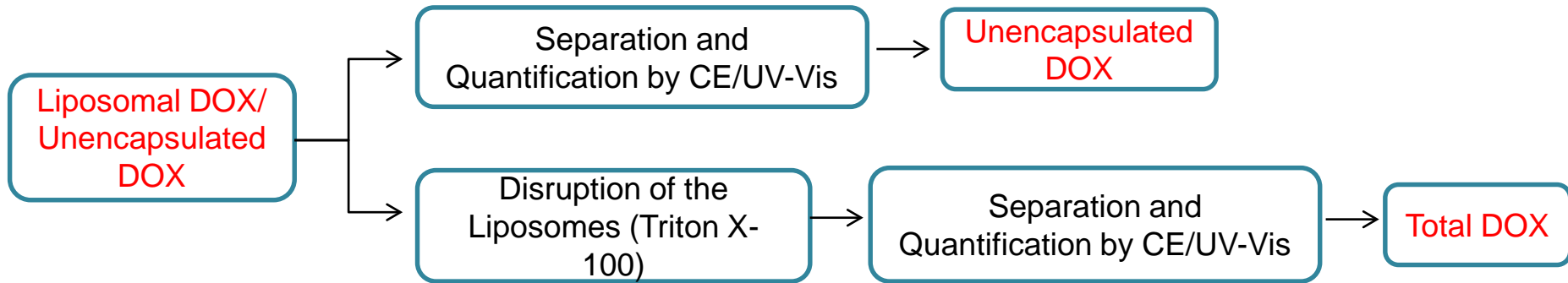
Unencapsulated Dox is known to induce cardiac toxicity

Unencapsulated DOX Determined via Solid Phase Extraction and CE-UV-Vis Method

Liposomal Formulation	Percentage of Unencapsulated DOX (n=3, mean ± SD)
DOXIL	2.4 ± 1.5
Generic	2.9 ± 1.7

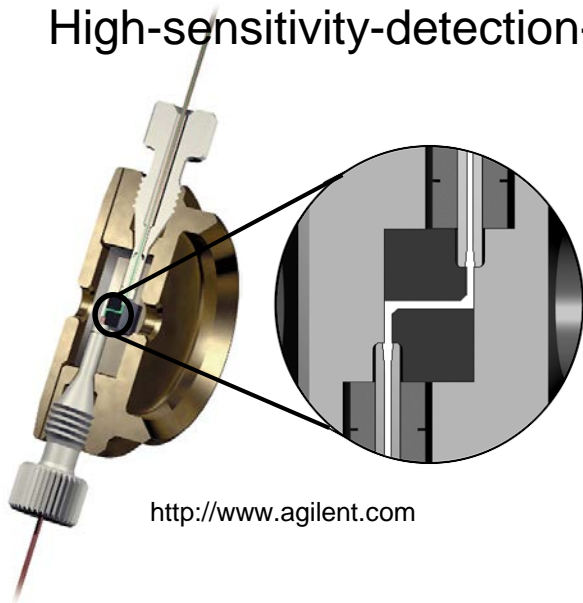


Determination of Unencapsulated Doxorubicin by (CE-UV-Vis)



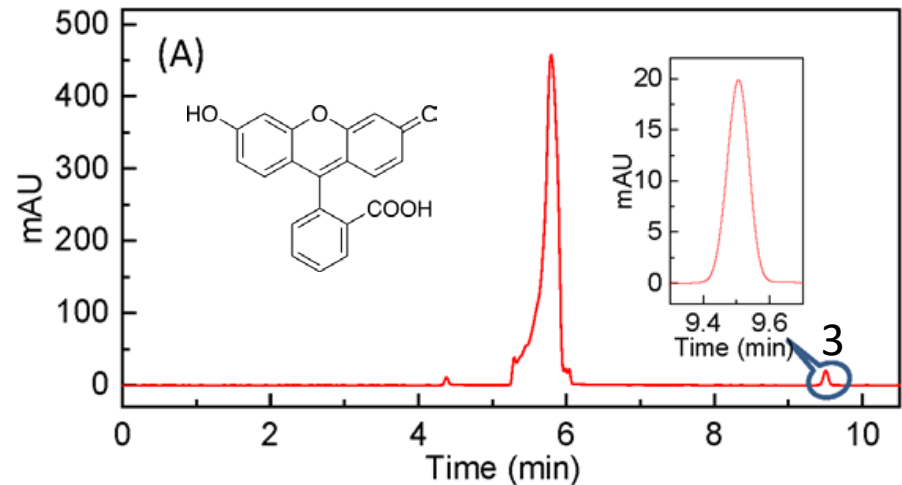
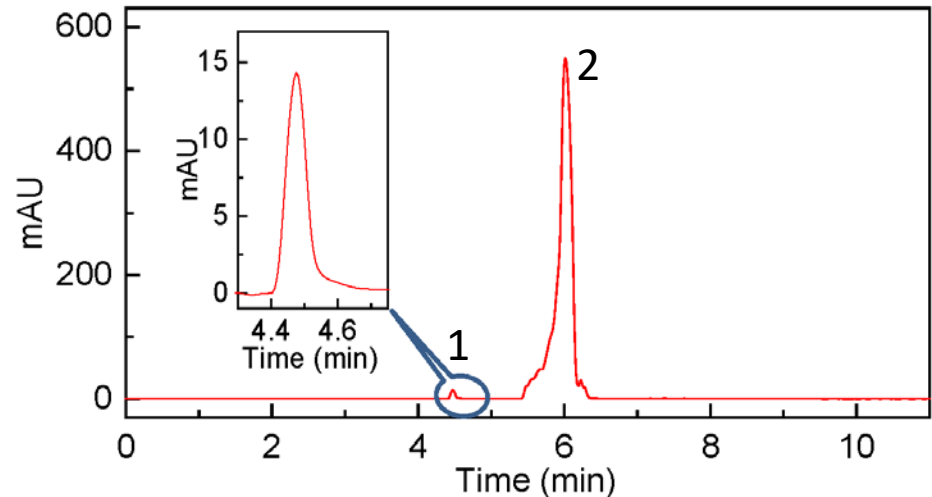
Electropherogram of DOX

High-sensitivity-detection-cell



<http://www.agilent.com>

- 10-min run
- Good separation of unencapsulated and liposome associated Dox
- Sensitivity enhanced by 10-fold by increasing the detection path length



1. Unencapsulated Dox
2. Liposome associated Dox
3. Fluorescein

Quantification Results of Unencapsulated DOX in FDA Approved Liposomal DOX Formulations via CE-UV-Vis



Percentage of Unencapsulated Dox (N=3, mean \pm SD)

Dilution factor	Dilution with 5% Dextrose		Dilution with serum	
	Doxil	Generic	Doxil	Generic
1	0.51 \pm 0.06	0.45 \pm 0.05	0.51 \pm 0.06	0.45 \pm 0.05
2	0.46 \pm 0.04	0.42 \pm 0.03	0.47 \pm 0.08	0.38 \pm 0.11
4	0.48 \pm 0.04	0.39 \pm 0.06	0.50 \pm 0.06	0.43 \pm 0.07
8	0.48 \pm 0.05	0.38 \pm 0.06	0.48 \pm 0.06	0.38 \pm 0.05
16	0.49 \pm 0.08	0.41 \pm 0.05	0.48 \pm 0.04	0.40 \pm 0.05

Generic: Doxorubicin HCl liposomes manufactured by Sun Pharma

Siyam M. Ansar, Wenlei Jiang, Andrew Fong, and Thilak Mudalige, Direct Quantification of Free Drug from Liposome-Associated Drug by Capillary Electrophoresis with UV-Vis Detection. International Journal of Pharmaceutics, 549:109-114, 2018

Method Improvements

Conventional SPE-CE-UV-Vis Method

- Two-step process
- Lengthy separation
- Potential inaccurate quantification due to disruption to liposomes

Newly Developed CE-UV-Vis Method

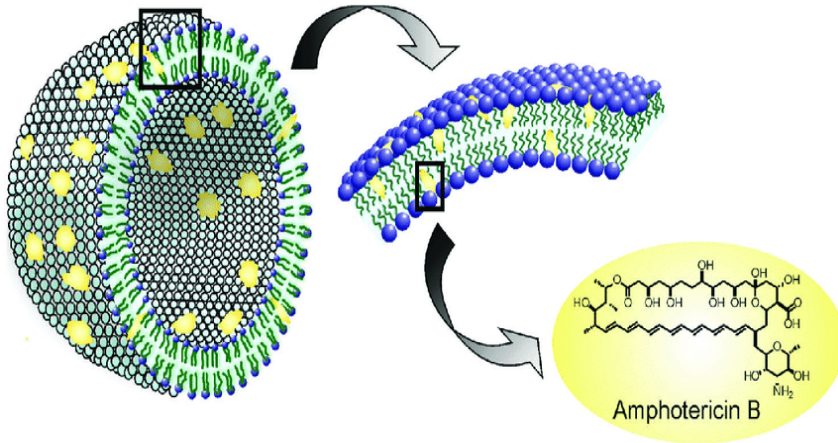
- Direct quantification of unencapsulated drug in liposome formulation
- Fast separation (10 min)
- Minimizing the drug leakage
- Enhanced detection sensitivity

Quantification of Lipid Excipients and Active Pharmaceutical Ingredients (API) in Liposomes

Model Drug: Amphotericin B Liposomes

Mechanism of Action

- AMBISOME has been shown to penetrate the cell wall of both extracellular and intracellular forms of susceptible fungi
- Amphotericin B binds to the sterol component, ergosterol, of the cell membrane of susceptible fungi. It forms transmembrane channels leading to alterations in cell permeability through which monovalent ions (Na^+ , K^+ , H^+ , and Cl^-) leak out of the cell, resulting in cell death.
- While amphotericin B has a higher affinity for the ergosterol component of the fungal cell membrane, it can also bind to the cholesterol component of the mammalian cell, leading to cytotoxicity.



<https://pubs.rsc.org/services/images/RSCpubs.ePlatform.Service.FreeContent.ImageService.svc/ImageService/ArticleImage/2016/CS/c5cs00674k/c5cs00674k-f3.gif>

Proprietary name: AMBISOME

Generic name:

Amphotericin B Liposomal Injection, 50 mg/vial

Indications:

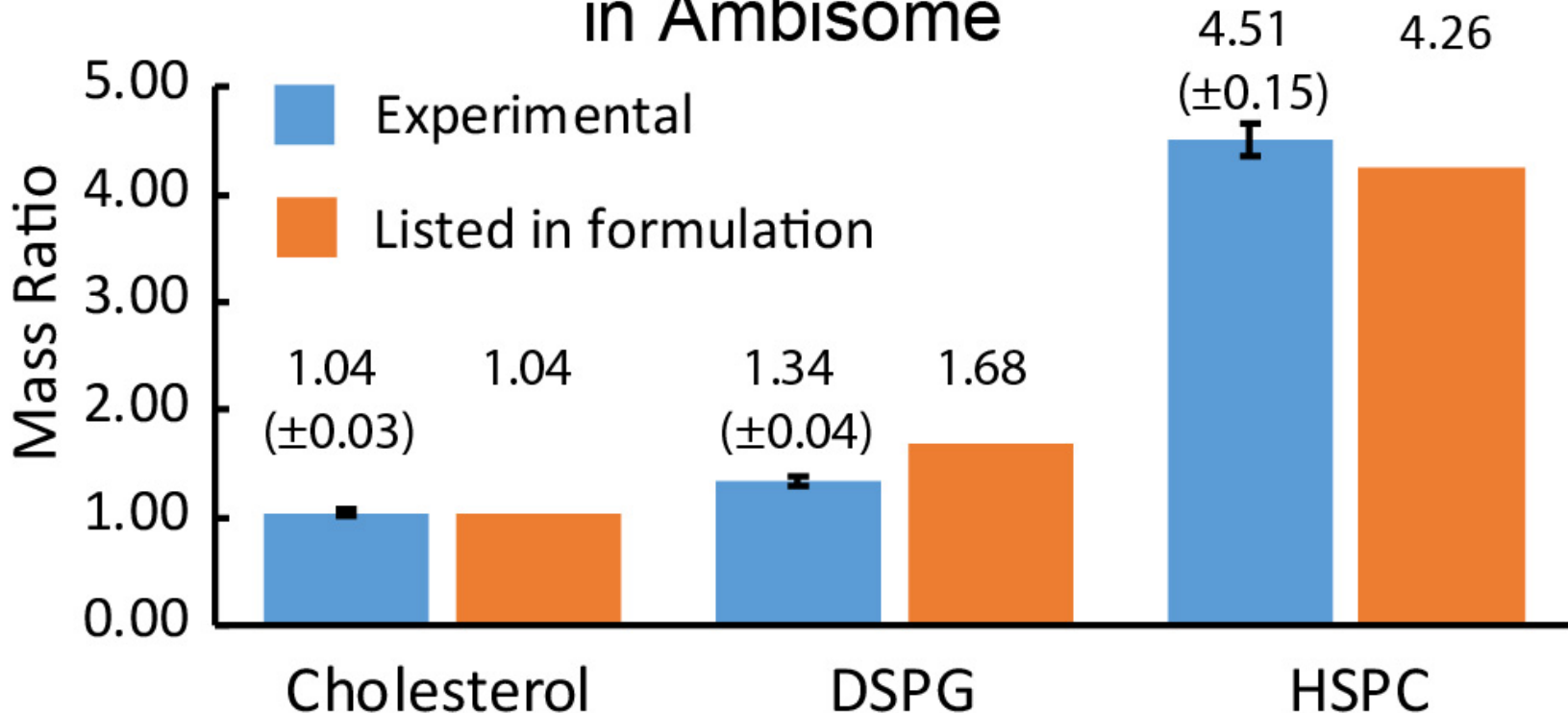
Fungal infection, cryptococcal meningitis, visceral leishmaniasis, and others

https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/050740s021lbl.pdf

Quantification of Lipid Contents in Bulk Liposomes



Mass Ratio of Components to Amphotericin B in Ambisome

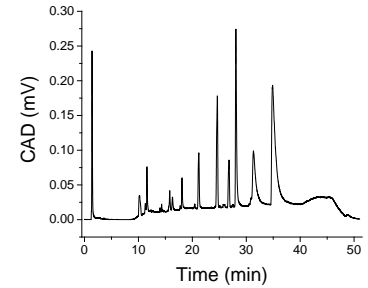
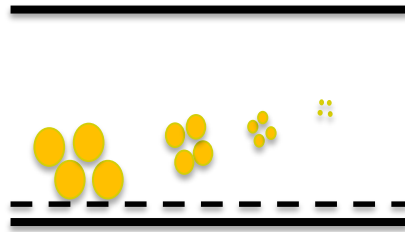
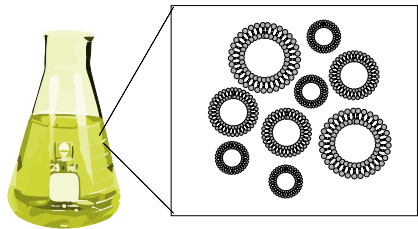
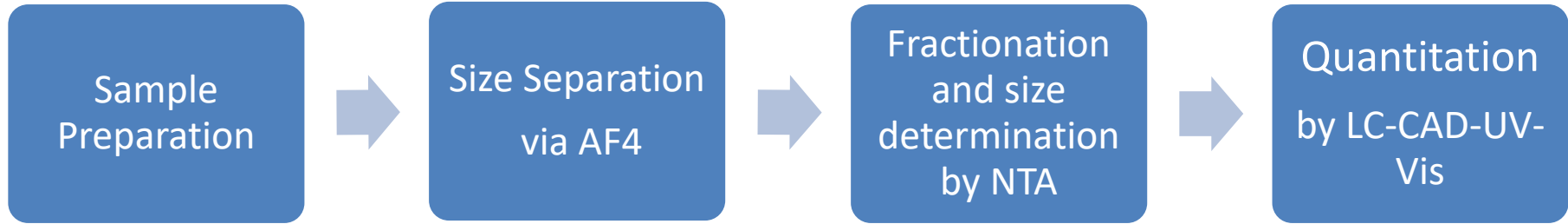


HSPC: Hydrogenated soy phosphatidylcholine

N=3, mean ± SD

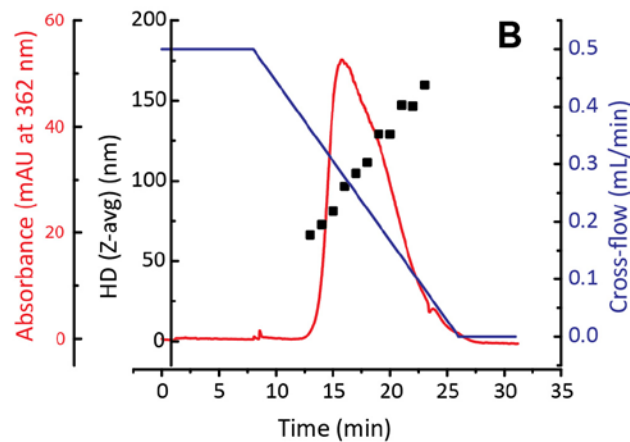
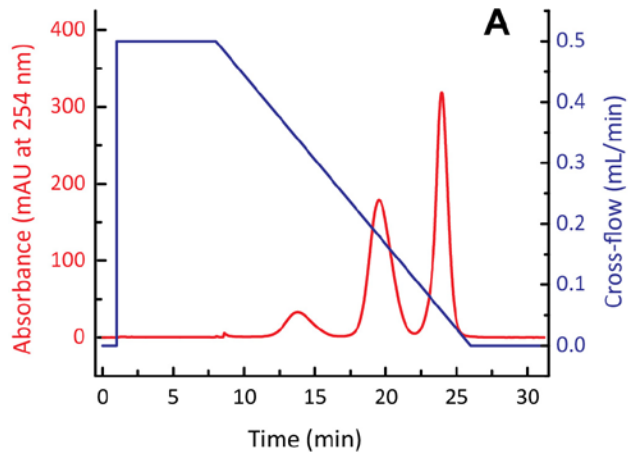
DSPG: Distearoylphosphatidylglycerol

Quantification of Lipid and API via AF4-LC-UV/CAD

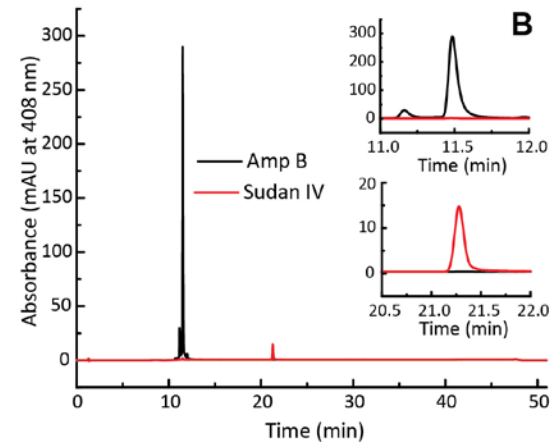
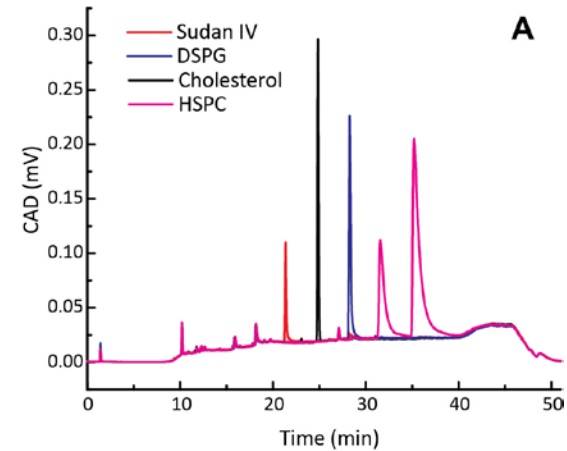


AFFFF (AF4): asymmetric flow - field flow fractionation
NTA: nanoparticle tracking analysis
CAD: charged aerosol detection

Size Separation and Quantification

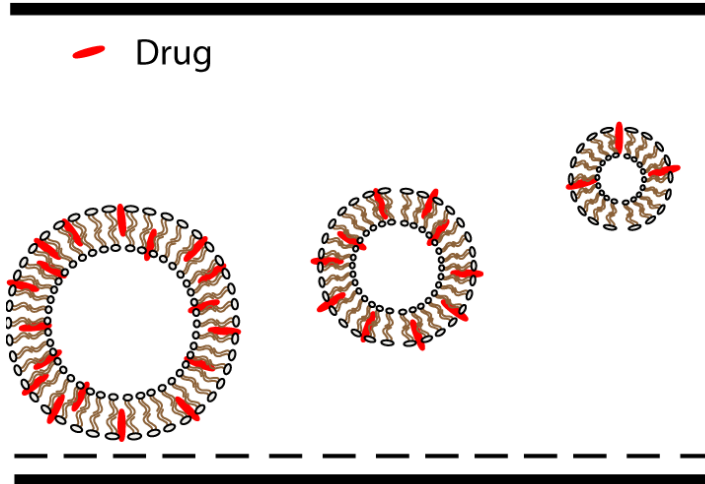


(A) Fractogram of 40 nm, 100 nm, and 200 nm Polystyrene nanoparticle standards mixture and (B) fractogram of Amphotericin B liposomal formulation

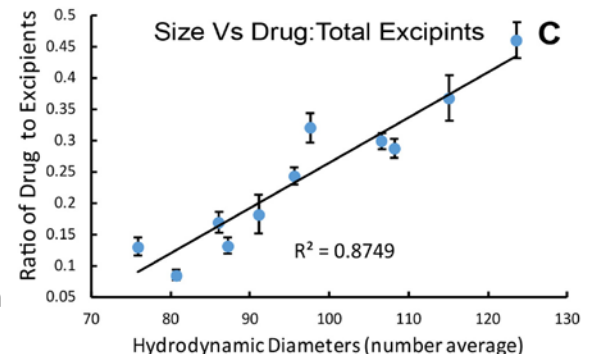
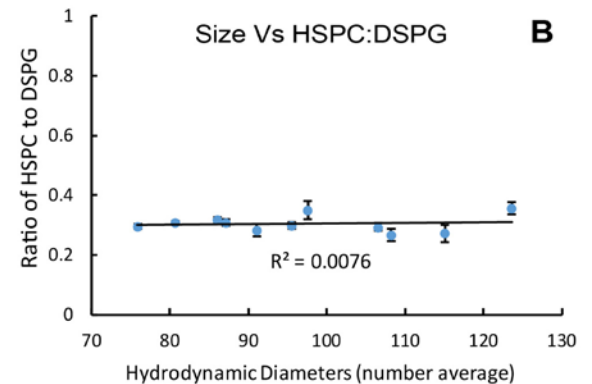
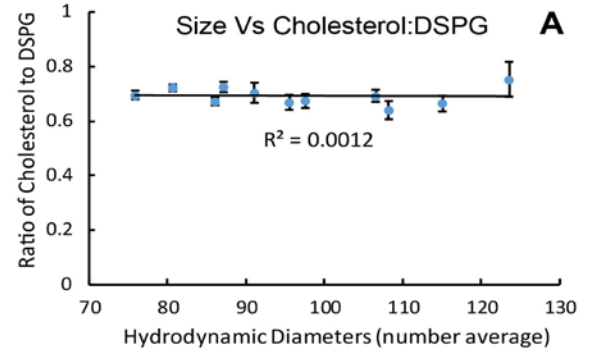


(A) Chromatograms of individual standard solutions collected with CAD and (B) Chromatograms of Amphotericin B solutions collected with UV-Vis detector

Quantification of Lipids and API in Liposomes with Different Sizes (nm)



Fraction Time	Nanoparticle Tracking analysis	Dynamic light scattering (Dh(z))
11 – 12	75.9 ± 1.3	62.2 ± 0.99
12 – 13	80.7 ± 1.8	65.46 ± 0.50
13 – 14	87.2 ± 2.3	72.22 ± 0.76
14 – 15	86.1 ± 1.4	78.58 ± 1.08
15 – 16	91.1 ± 0.3	85.14 ± 1.09
16 – 17	95.6 ± 2.0	91.2 ± 1.44
17 – 18	97.6 ± 0.6	96.9 ± 1.88
18 – 19	106.6 ± 0.4	101.46 ± 1.35
19 – 20	108.2 ± 0.7	108.98 ± 7.07
20 – 21	115.1 ± 1.7	113.34 ± 2.14
21 – 22	123.6 ± 1.3	119.18 ± 2.09



Method Improvements

Conventional Method

- The distribution of active pharmaceutical ingredient (API) and excipients in liposomes with different sizes was removed due to the breakdown of bulk liposomes
- The API and excipients are often quantified by two separate analytical methods.

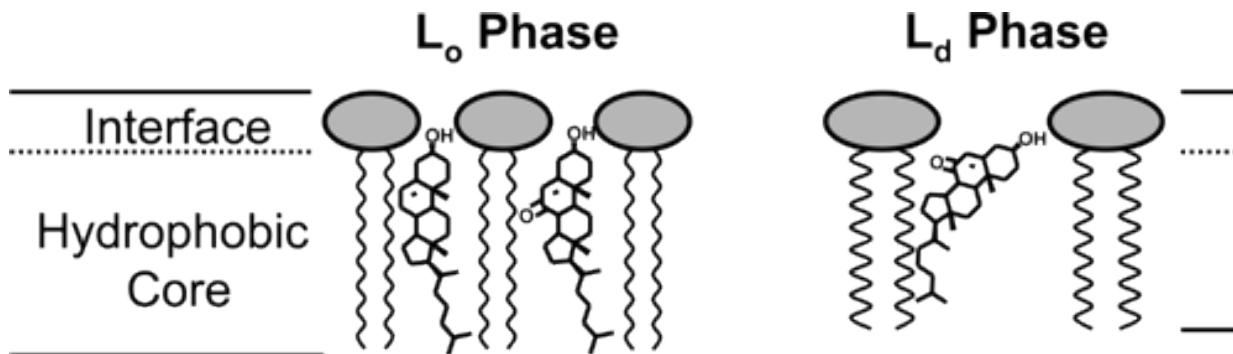
Newly Developed AF4-LC-UV/CAD Method

- Simultaneously obtain the information of the API and excipient distribution in liposomes of different sizes.

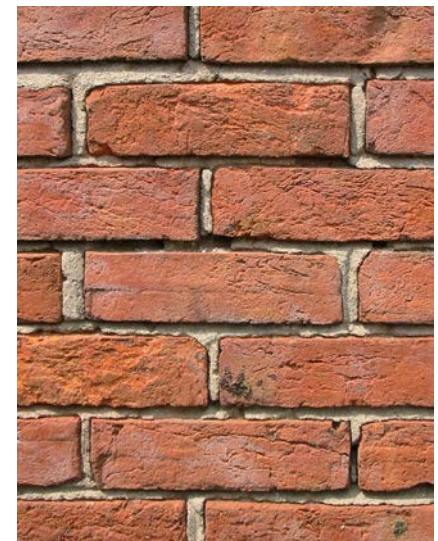
Investigation of Cholesterol Oxidation Products in Raw Materials and Liposome Drug Products

Cholesterol and Its Oxidation Products(COPs)

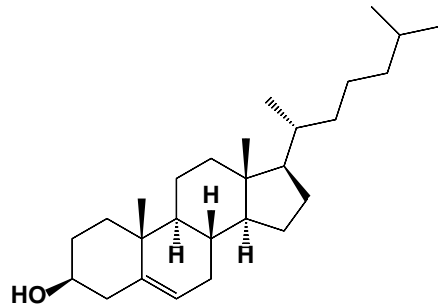
- Cholesterol is a critical excipient in liposome formulations
- Cholesterol oxidation products (COPs) which are oxygenated derivatives of cholesterol may be formed during liposome manufacturing process and liposome storage
- These COPs may affect liposome bilayer integrity



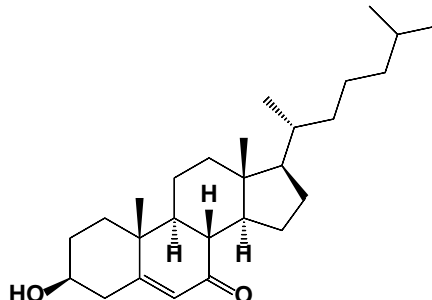
7-ketocholesterol



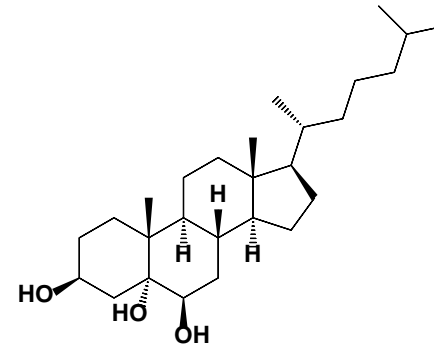
Representative Structures of COPs



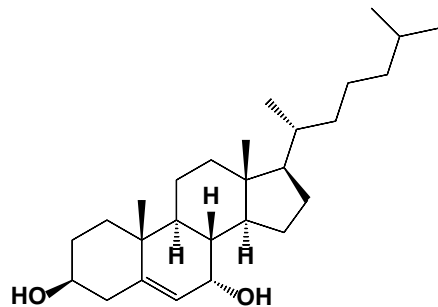
cholesterol
Exact Mass: 386.35487



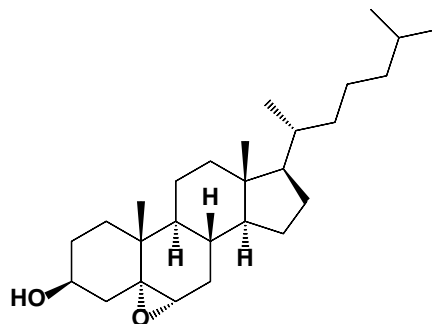
7-ketocholesterol
Exact Mass: 400.33413



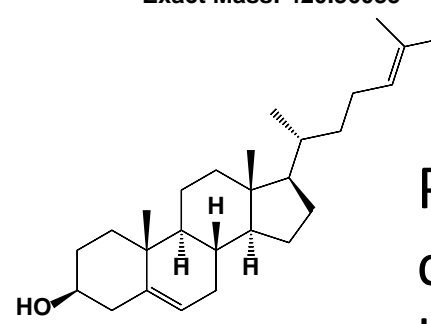
3b, 5a, 6b-Triol-cholesterol
Exact Mass: 420.36035



7a-hydroxycholesterol
Exact Mass: 402.34978

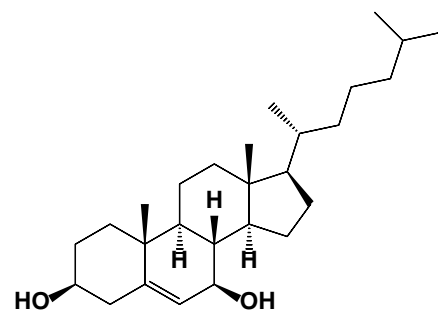


5,6a-epoxycholesterol
Exact Mass: 402.34978

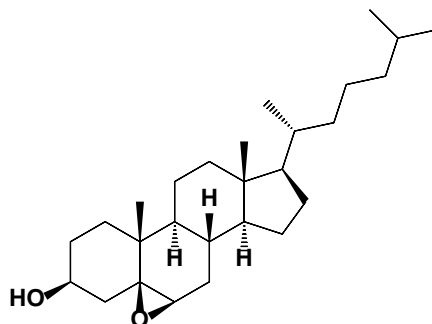


Desmosterol
Exact Mass: 384.33922

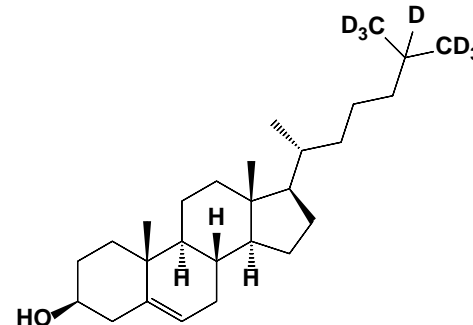
Precursor of
cholesterol
biosynthesis



7b-hydroxycholesterol
Exact Mass: 402.34978

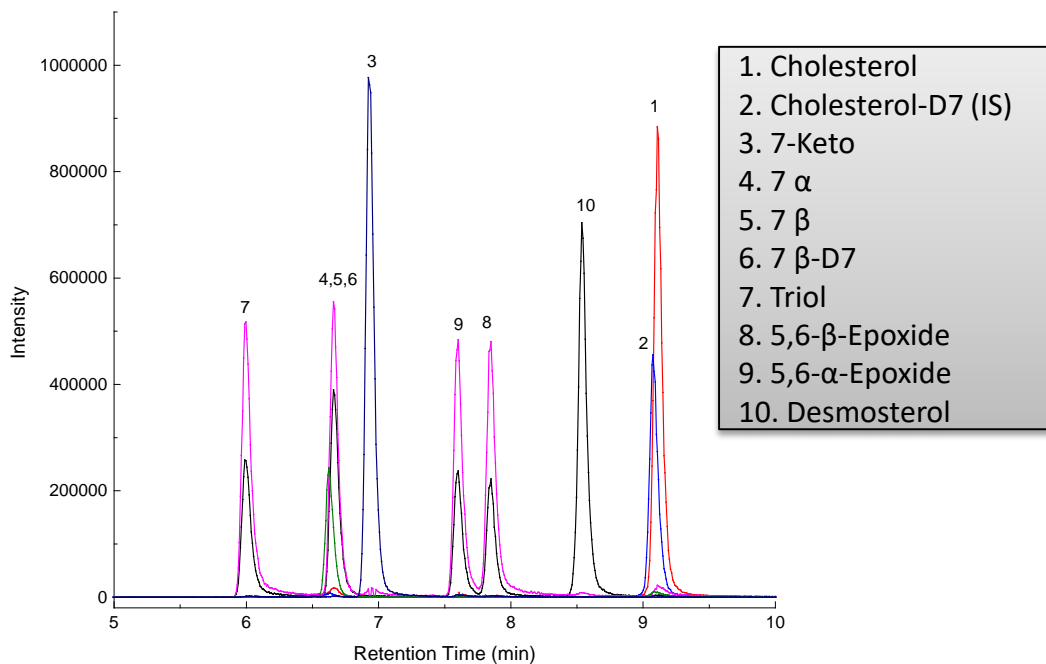


5,6b-epoxycholesterol
Exact Mass: 402.34978

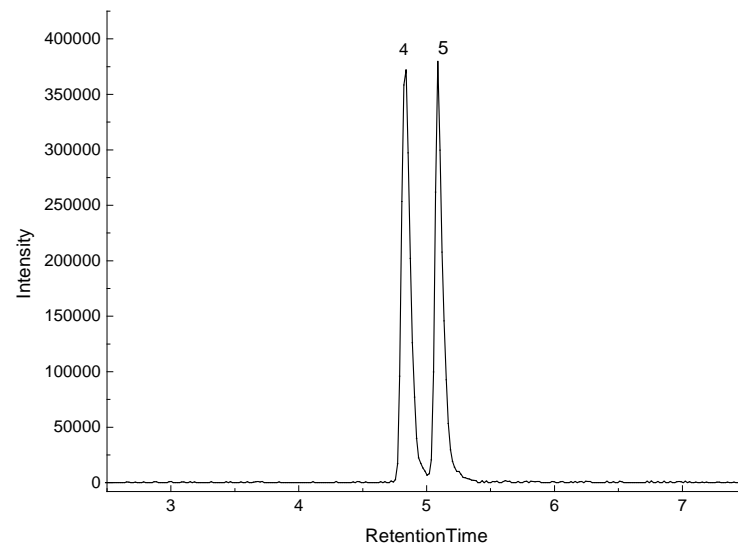


cholesterol -D7 (Internal standards)
Exact Mass: 393.39880

UPLC-QTOF of COPs



RP-HPLC Chromatogram of Standard compounds mixture on C18 column



NP-HPLC Chromatogram of 7 α , 7 β mixture on cyano column.

7 α (4), 7 β (5) are share EIC m/z 385.3471

UPLC: Ultra Performance Liquid Chromatography

QTOF: Quadrupole Time of Flight

Desmosterol Impurities in Raw Cholesterol Materials



Vendor	Biological Source	Manufacturing	Storage	Stabilizer	Detected	vs CHOL	RSD (n=3)
					ng/mL	%	%
Vendor1	Ovine wool	NA	-20°C	NA	321.49	1.47	0.05
Vendor2	Wool Grease	GMP	RT	a-Tocopherol	499.16	2.54	0.03
Vendor3	Synthetic (Plant Derived)	GMP	-20°C	NA	0.00	0.00	0.00
Vendor4	NA	NA	4°C	NA	108.35	0.49	0.10
Vendor5*	NA	NA	-20°C	NA	749.40	3.64	0.01
Vendor6	Sheep wool grease	GMP	RT	a-Tocopherol	551.19	2.66	0.01

- No COPs detected in cholesterol USP raw materials.
- UPS Acceptance Criteria for desmosterol in bulk cholesterol : NMT 3%

* Non USP-NF excipient

RT: room temperature NA: Not available

COPs in Liposomal Drug Products



	7 α			7 β			7-Keto			Desmo			5,6 α			5,6 β			Triol		
DP1	412	±	49	93	±	14	116	±	14	121	±	29	0	±	0	0	±	0	36	±	5
DP2	336	±	100	372	±	43	249	±	14	2062	±	186	0	±	0	271	±	20	77	±	4

All commercial drugs were tested within shelf-life.

DP1: Lot: 008986 Exp: 10/2020

DP2: Lot: HKZSV00 Exp: 06/2019

Changuang Wang, Dumindika A. Siriwardane, Wenlei Jiang, Thilak Mudalige, Quantitative analysis of cholesterol oxidation products and desmosterol in parenteral liposomal drug products, FDA study report

Conclusions

- Complex liposome formulations call for advanced analytical methods for detailed characterization
- A CE-UV-Vis method can provide a fast and direct quantification of unencapsulated doxorubicin in doxorubicin HCl liposomes while minimizing drug leakage
- AF4 in combination with LC-UV-Vis-CAD can simultaneously obtain the information of the API and excipient distribution in liposomes of different sizes.
- UPLC-QTOF method was developed for quantitation of trace amount of COPs in liposome drug products.

Acknowledgements



Office of Regulatory Affairs

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Siyam Ansar

Desiree Van Haute

Changguang Wang

Dumindika Siriwardane

ORS/OGD/CDER

Lei Zhang

Robert Lionberger

Thank you!

Any question? Please contact Wenlei Jiang
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