A Standard Test Method for Lipid Quantitation in Liposomal Formulations Using Ultra-High-Performance Liquid Chromatography with Triple Quadrupole Mass Spectrometry (UHPLC-TQMS)

Sanghamitra Majumdar, Udaya B. Nasini, and Anil K. Patri

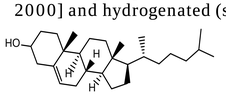
Nanotechnology Core Facility, Office of Scientific Coordination, National Center for Toxicological Research (NCTR), FDA, AR 72079

Abstract

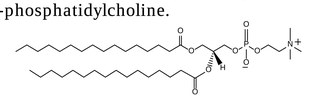
There has been a steady increase in submissions to FDA of products containing nanomaterials for drug delivery, gene delivery, and medical devices. Liposomes are vesicles of nanoscale dimensions, composed of lipid bilayers, used as carriers for small molecules and constitute a third of the drug products submission utilizing nanotechnology. There is a lack of documentary and reference material standards to assess the critical quality attributes that facilitate regulatory review and market entry of safe and effective products. Lipid composition and concentration are key attributes in determining the quality and efficacy of a liposomal drug product. FDA Nanotechnology Task Force Standards sub-committee has identified the development of a standard test method to quantify lipid components in liposomal formulations as a high priority need for collaborative consensus standard development through ASTM E56-08 committee on Nanotechnology. This test method utilizes ultra-high-performance liquid chromatography with triple quadrupole mass spectrometry (UHPLC-TQMS) standard for the identification and quantitation of lipids in liposomal formulations. This test method is specific for liposomal formulations consisting of cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] and hydrogenated (soy) L-α-phosphatidylcholine. This test method is currently undergoing ASTM International E56-08 committee balloting after significant testing in the laboratory, and review by stakeholders. This test method standard will eventually benefit industry in ascertaining quality assessment of liposomal formulations and in monitoring batch-to-batch consistency. The development of this standard is highly relevant for quality control of liposomal formulation and supports FDA's mission to protect and promote public health.

Introduction

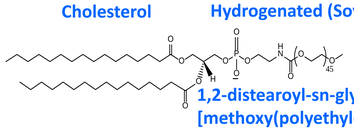
- Liposomes account for a third of the drug products utilizing nanotechnology that have been submitted to FDA for approval.
- Liposomes are vesicles composed of lipid bilayers and used as carriers for small molecules. Hydrophilic drugs are encapsulated in the aqueous core and hydrophobic drugs can be incorporated in the lipid bilayer.
- Lipid bilayers are primarily composed of phospholipids. Cholesterol is incorporated in the phospholipid bilayer to modulate its fluidity by interacting with the lipid components.
- Lipid composition and concentration are key attributes in determining the quality and efficacy of a liposomal drug product, as they influence the stability of liposomes, drug incorporation, release, and pharmacokinetic properties.
- In this test method we develop and validate method for quantitation of lipids in liposomes primarily consisting of cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] and hydrogenated (soy) L-α-phosphatidylcholine.



Cholesterol



Hydrogenated (Soy) L-α-phosphatidylcholine (HSPC)



1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG 2000)

Methods

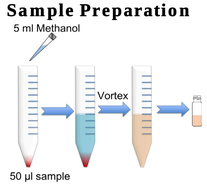
Calibration standards

Calibration curves are established for DSPE-PEG 2000 and HSPC in the range of 2 to 400 ng/g. Due to the low ionization efficiency, the range of the calibration curve for cholesterol (8 to 1600 ng/g) was expanded to about four times higher than the calibration range for DSPE-PEG 2000 and HSPC. For analyte quantitation, known amounts of internal standards (ISTD) including cholesterol-d7, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-550] (DSPE-PEG 500), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) were added to the calibration levels.

Method validation

A full method validation was performed which includes specificity, linearity, accuracy and precision, matrix effect, sensitivity and carry-over effects following FDA Bioanalytical Method Validation Guidance for Industry

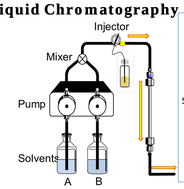
Sample Preparation



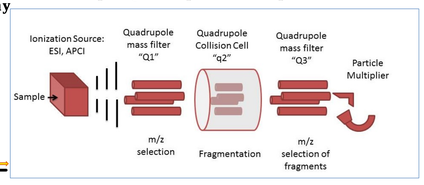
Cholesterol, DSPE-PEG 2000 and HSPC in a liposomal formulation are solubilized in methanol at 1:100 dilution by volume followed by vortex mixing.

The solubilized sample is diluted to appropriate concentrations to fit the calibration range and amended ISTDs. The diluted sample is subjected to quantitative analysis using UHPLC-TQMS.

Ultra-high-performance Liquid Chromatography



Triple Quadrupole Mass Spectrometer



A. Acetonitrile/Water (90/10 v/v) with 0.1% Formic acid + 5 mM Ammonium formate
B. Methanol with 0.1% Formic acid + 5 mM Ammonium formate

Table 1. Retention Times, MRM Ions, and Analyte-specific MS parameters

Analyte	Retention time (min)	MRM transition (Parent > Product)	Cone Voltage (V)	Collision Energy (eV)
DMPC	2.56	678.6 > 184	38	25
Cholesterol-d7	2.68	376.4 > 147.1	38	22
		376.4 > 160.9	38	20
		376.4 > 95.2	38	22
Cholesterol	2.70	369.5 > 161.1	36	24
		369.5 > 95.1	36	26
		369.5 > 147.2	36	22
DSPE-PEG 2000	3.82	607.5 > 95.2	60	25
		607.5 > 109.0	60	
HSPC-1	4.62	762.6 > 184.1	38	25
DSPE-PEG 500	4.66	607.4 > 94.9	60	25
		607.4 > 71.1	60	
HSPC-2	5.80	790.6 > 184.1	38	25

Results and Discussion

UHPLC-TQMS offers higher selectivity, sensitivity, wide linear range of quantitation, high signal-to-noise ratio (S/N), and accuracy, compared to other contemporary techniques utilizing universal detectors, thereby allowing robust and reproducible quantitation of the analytes.

The method detection limits (MDL) for cholesterol, DSPE-PEG 2000, and HSPC are 1.8, 0.39, and 0.8 ng/g, respectively. The measurement range for cholesterol is 8-2000 ng/g. and for DSPE-PEG 2000 and HSPC is 2 to 400 ng/g.

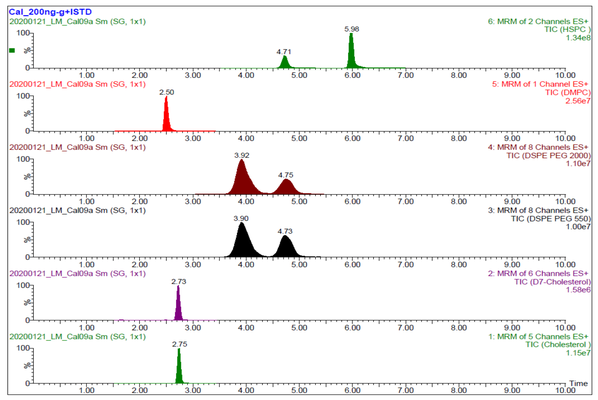


Figure 1. Extracted ion chromatogram of cholesterol and lipid standards using TQMS

Table 2. Chemical composition of a liposomal formulation: total amount of cholesterol DSPE-PEG 2000, and HSPC from three independent measurements

Measurement	Analyte concentration (mg/g)		
	Cholesterol	DSPE-PEG 2000	HSPC
1	3.82	2.56	10.02
2	3.90	2.68	9.74
3	3.89	2.45	9.88
Avg	3.87	2.56	9.88

Table 3. Chemical composition of a liposomal formulation: total amount of cholesterol DSPE-PEG 2000 and HSPC (Mean ± Standard deviation), and the ratio of those components

	Concentration (mg/g)	Component ratio (DSPE-PEG 2000:HSPC:Cholesterol)
DSPE-PEG 2000	2.55 ± 0.09	1.0:3.9:1.5
HSPC	9.85 ± 0.18	
Cholesterol	3.88 ± 0.21	
Total lipid content	16.19 ± 0.27	

Method Validation parameters for this test method

Linearity

Analyte	Replicate	r^2 value
Cholesterol	1	0.998
	2	0.997
	3	0.995
DSPE-PEG 2000	1	0.998
	2	0.997
	3	0.998
HSPC	1	0.996
	2	0.993
	3	0.997

Limit of detection and quantitation

Analyte	LOD (ng/g)	LOQ (ng/g)
Cholesterol	1.76	5.84
DSPE-PEG 2000	0.39	1.29
HSPC	0.8	2.00

Accuracy and recovery (%Rec) for low, medium, and high concentrations

	LOW			MEDIUM			HIGH		
n = 6	Avg. Conc.	%Rec	%RSD	Avg. Conc.	%Rec	%RSD	Avg. Conc.	%Rec	%RSD
Cholesterol	23.23 ± 2.63	92.93	11.31	43.52 ± 3.24	87.03	7.43	83.28 ± 2.98	83.27	3.58
DSPE-PEG 2000	6.92 ± 0.33	110.77	4.72	13.26 ± 0.46	106.08	3.47	25.94 ± 1.05	103.76	4.07
HSPC	5.73 ± 0.30	91.68	5.18	10.99 ± 0.26	87.9	2.35	22.19 ± 1.24	88.76	5.58

Repeatability results for low, medium, and high concentrations

	LOW		MEDIUM		HIGH	
n = 6	Mean Conc.	%RSD	Mean Conc.	%RSD	Mean Conc.	%RSD
Cholesterol	26.90 ± 1.47	5.48	86.89 ± 3.47	4.00	432.29 ± 30.00	6.94
DSPE-PEG 2000	5.42 ± 0.17	3.17	23.44 ± 0.80	3.43	98.15 ± 2.51	2.56
HSPC	5.47 ± 0.40	7.31	26.75 ± 1.35	5.06	102.94 ± 0.58	0.56

Conclusion

- This analytical test method standard has been identified as a high priority need for collaborative consensus standard development through ASTM E56-08 on Nanotechnology and is currently undergoing ballot process.
- This standard test method utilize readily available instrumentation in pharmaceutical labs.
- Consensus standards facilitate regulatory review and helps industry with expedient review of submissions.

Acknowledgement

This work was supported by an Interagency Agreement (IAG) between the U.S. Food And Drug Administration/NCTR and the National Institute of Environmental Health Sciences/NTP (FDA IAG No. 224-18-0502R/NIEHS IAG No. AES12013)

Disclaimer

The interpretation of the data and conclusions are of the presenter, and should not be considered as the official position or policy of U.S. FDA, or the Department of Health and Human Services