Quantification of Lysophosphatidylcholines (LPCs) and Free Fatty Acids (FFAs) in Parenteral Liposomal Formulations by Liquid Chromatography – Mass Spectrometry (LC-MS)

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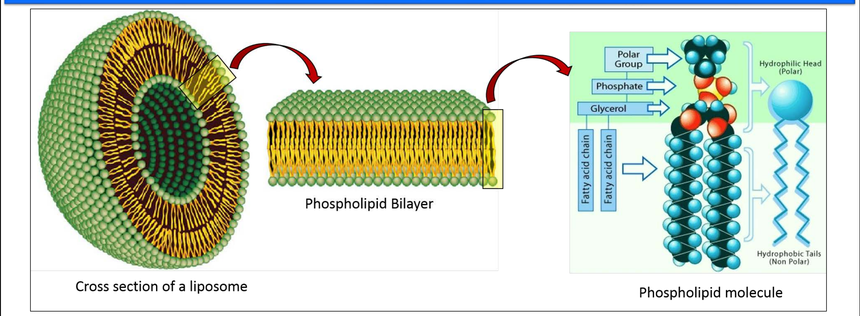
Introduction

Liposomes have been used for decades as carriers of active pharmaceutical ingredients (API) resulting in enhanced drug efficacy and reduced toxicity. Phospholipids are the principal excipients in the liposomal formulations and are susceptible to hydrolysis, generating lipid degradation products such as lysophospholipids and free fatty acids. The lipid hydrolysis may alter the physical and chemical properties of the lipid bilayer, potentially affecting the drug release profiles of liposomal formulations.

Objective

Available GC-FID and HPLC-based methods provide relatively poor selectivity and sensitivity. Therefore, we developed a rapid, sensitive and reproducible LC-QTOF based method to quantitate the phospholipid hydrolysis products, primarily lysophosphatidylcholines (LysoPCs), lysophosphatidylglycerol (LPGs) and free fatty acids (FFA) in liposomal formulations.

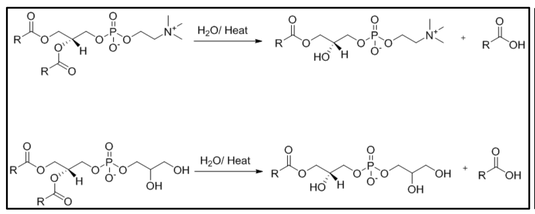
Lipid Hydrolysis



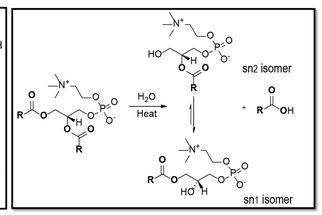
*Adapted from Bitounis, D., Fanciullino, R., Iliadis, A., and Ciccolini, J. (2012) Optimizing Druggability through Liposomal Formulations: New Approaches to an Old Concept, *ISRN pharmaceutics 2012*, 738432, http://syromonoed.com/lecithin-common-phospholipid-human-bodies.

Figure 01. Schematic representation of presence of phospholipids in liposome

Scheme 01. Hydrolysis of Phosphotidylcholines (PCs) and Phosphotidylglycerols (PGs)



Scheme 02. Schematic representation of acyl migration reaction of lysophosphatidylcholine



Spontaneous intramolecular acyl migration reaction under physiological condition can take place by yielding *sn1* and *sn2* regioisomers in a 9:1 ratio. The process of the formation of such regioisomer is called intra-molecular acyl migration.[1]

Table 01. Different Types of Phospholipids Present in Liposomal Formulations[2]

Liposomal Formulation	Active Ingridient	Phospholipid in Formulation
Doxil® (1995)	Doxorubicin	HSPC, PEG 2000-DSPE
DaunoXome® (1996)	Daunorubicin	DSPC
Depocyt® (1999)	Cytarabine/Ara-C	DOPC, DPPG
Myocet® (2000)	Doxorubicin	EPC
Mepact® (2004)	Mifamurtide	DOPS, POPC
Marqibo® (2012)	Vincristine	SM
Onivyde™ (2015)	Irinotecan	DSPC, MPEG-2000:DSPE
Abelcet® (1995)	Amphotericin B	DMPC, DMPG
Ambisome® (1997)	Amphotericin B	HSPC, DSPG
Visudyne® (2000)	Verteporphin	DMPC, EPG
DepoDur™ (2004)	Epidural Morphine sulfate	DOPC, DPPG
Exparel® (2011)	Bupivacaine	DEPC, DPPG

***HSPC** (hydrogenated soy phosphatidylcholine); **PEG** (polyethylene glycol); **DSPE** (distearoyl-sn-glycero-phosphoethanolamine); **DSPC** (distearoylphosphatidylcholine); **DOPC** (dioleoylphosphatidylcholine); **DPPG** (dipalmitoylphosphatidylglycerol); **EPC** (egg phosphatidylcholine); **DOPS** (dioleoylphosphatidylserine); **POPC** (palmitoyloleoylphosphatidylcholine); **SM** (sphingomyelin); **MPEG** (methoxy polyethylene glycol); **DMPC** (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); **DSPG** (distearoylphosphatidylglycerol); **DEPC** (dierucoylphosphatidylcholine); **DOPE** (dioleoly-sn-glycero-phophoethanolamine)

Method Workflow and Optimization

Table 02. Method Information for FFA, LPC, and LPG

Analyte	Column Type	Specifications	Mobile Phase A	Mobile Phase B
FFA	ACQUITY UPLC® CSH™ C8 column	100 X 2.1 mm; particle size 1.7 µm, Waters	100% H_2O 1 mM Ammonium Acetate	ACN:MEOH 8:2 1 mM Ammonium Acetate
LPC	ACQUITY UPLC® CSH™ C18 column	100 X 2.1 mm; particle size 1.8 µm, Waters	100% H_2O + 0.1 FA + 10 mM Ammonium Formate	MEOH + 0.1 % FA+10 mM Ammonium Formate
LPG	ACQUITY UPLC® CSH™ C18 column	100 X 2.1 mm; particle size 1.8 µm, Waters	100% H_2O 1 mM Ammonium Formate	MEOH 1 mM Ammonium Formate

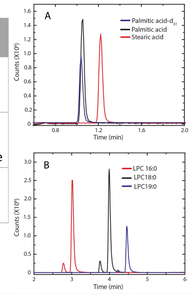


Figure 02. Chromatogram for FFA (A) and LPCs (B)

Analysis of Phospholipid's Degradation Products

Determination of FFAs

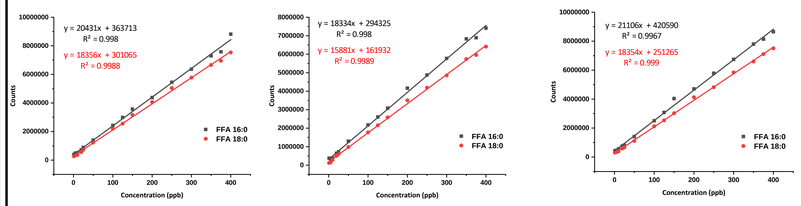


Figure 04. Calibration plots for FFA for three consecutive days

Table 03. Method validation statistics for FFAs

	Inter-day Measured Values [m]					Intra-day Measured Values [n]			
	QC sample concentration (ng/mL)	Mean Concentration (ng/mL)	SD ±	Accuracy (%)	Precision (%)	Mean ng/mL	SD ±	Accuracy (%)	Precision (%)
FFA 16:0	20	20.2	1.8	105	5.4	21.1	0.6	105.7	2.9
	125	126.7	7.5	112	3.6	121.3	2.2	97.1	1.8
	375	356.5	7.6	108	5.7	335.5	3.9	89.5	1.2
FFA 18:0	20	19.8	2.5	98.6	12.7	22.3	0.5	111.4	2.4
	125	124.0	11.1	102	2.0	122.1	1.1	97.7	0.9
	375	366.2	8.1	98	5.2	353.0	6.6	94.1	1.9

Determination of LPCs

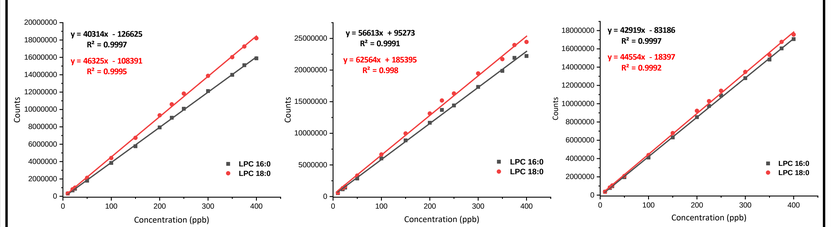


Figure 05. Calibration plots for LPC for three consecutive days

Table 04. Method validation statistics for LPCs

	Inter-day Measured Values [m]					Intra-day Measured Values [n]			
	QC sample concentration (ng/mL)	Mean Concentration (ng/mL)	SD ±	Accuracy (%)	Precision (%)	Mean (ng/mL)	SD ±	Accuracy (%)	Precision (%)
LPC 16:0	20	19.6	0.8	98.2	4.1	19.3	0.2	96.3	1.1
	225	225.0	1.4	100.0	0.6	231.8	2.1	103.0	0.9
	375	374.1	3.1	99.8	0.8	375.4	4.2	100.1	1.1
LPC 18:0	20	19.4	0.3	96.0	1.6	19.6	0.2	97.9	1.3
	225	226.1	1.7	100.5	0.8	235.0	1.9	104.5	0.8
	375	370.8	3.4	98.9	0.9	371.8	3.5	99.1	0.9

Determination of LPGs

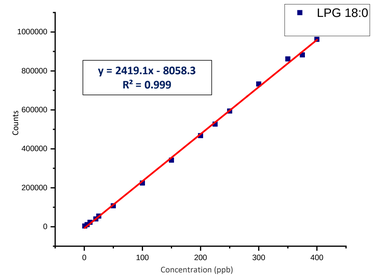


Figure 06. Calibration plot for LPG

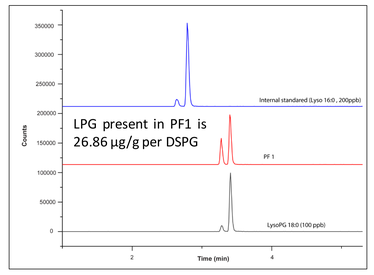


Figure 07. Chromatograms for LPG

Analysis of Bulk Formulation

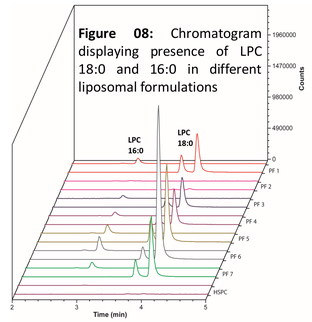


Figure 08: Chromatogram displaying presence of LPC 18:0 and 16:0 in different liposomal formulations

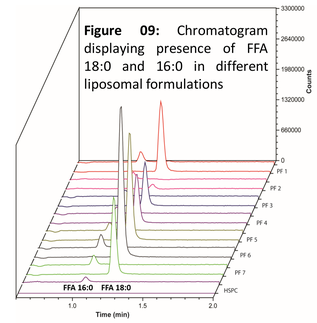


Figure 09: Chromatogram displaying presence of FFA 18:0 and 16:0 in different liposomal formulations

Table 05. Detected LPCs and FFAs in different liposomal formulations as mass percentage

Liposomal Formulation	LPC 16:0 (%)*	LPC 18:0 (%)*	FFA 16:0 (%)*	FFA 18:0 (%)*
HSPC	0.00	0.00	0.00	0.00
PF1	0.14	0.59**	0.13	1.14**
PF2	0.04	0.05	0.15	0.00
PF3	0.10	0.85	0.07	1.16
PF4	0.13	1.02	0.08	1.35
PF5	0.27	2.16	0.01	2.87
PF6	0.44	4.20	0.02	4.70
PF7	0.19	1.51	0.16	2.22

*with respect to HSPC, **with respect to total content of HSPC and DSPG

Summary and Conclusion

- LC-MS methods were developed and validated for the quantitation of lipid degradation products including FFAs, LPCs, and LPGs in liposomal pharmaceutical formulations (PFs).
- The limit of detection (LODs) for FFA 16:0, FFA 18:0, LPC 16:0 and LPC 18:0 are 1.70 ng/mL, 1.03 ng/mL, 2.0 ng/mL, and 2.1 ng/mL, respectively.
- The limit of quantification (LOQs) for FFA 16:0, FFA 18:0, LPC 16:0, and LPC 18:0 are 5 ng/mL, 5 ng/mL, 6.5 ng/mL, and 7.0 ng/mL, respectively.
- The LOD and LOQ for LPG are 2.1 ng/mL and 7.1 ng/mL, respectively.
- Corresponding lipid degradation products (FFA and lyso-lipids) of lipid excipients were detected for all the liposomal formulations.

References

[1] Plueckthun, A., and Dennis, E. A. (1982) Acyl and phosphoryl migration in lysophospholipids: importance in phospholipid synthesis and phospholipase specificity, *Biochemistry 21*, 1743-1750.

[2] Bulbake, U., Doppalapudi, S., Kommineni, N., and Khan, W. (2017) Liposomal Formulations in Clinical Use: An Updated Review, *Pharmaceutics 9*.

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