Qualitative and Quantitative Analysis of Lipids in Exparel® Injectable Liposomal Drug Formulation

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Prabhath L. Gamage[a], Wenlei Jiang[b], Thilak Mudalige[a]

a. Arkansas Laboratory, Office of Regulatory Science, Office of Regulatory Affairs (ORA), U.S. Food and Drug Administration, Jefferson, Arkansas, 72079

b. Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, U.S. Food, and Drug Administration, Silver Spring, Maryland, 20993

E-mail: Wenlei.Jiang@fda.hhs.gov, Tel.: +1 240 402 7963, Thilak.Mudalige@fda.hhs.gov, Tel.: +1-870-543-4665

PURPOSE

Exparel® is a multivesicular liposomal formulation (MVL) of bupivacaine, which provides sustained release at the site of injection resulting in prolong anesthetic capabilities. Since the lipid composition leads to the unique structure and performance of liposome formulation, the quantitation of major lipid constituents and identification of minor lipids, lipid degradation products, and cholesterol oxidation products would help a comprehensive evaluation of the formulation. Lipids are a complex class of components with many structurally similar compounds; hence their identification is difficult when using a simple MS technique such as triple quadrupole MS. A QTOF (quadrupole time of flight) MS technique is advantageous in lipid analysis because it can identify lipid molecules with same nominal mass through scanning in a vast range of parent and fragment ions.

OBJECTIVE(S)

1) To identify all the lipid components and API related impurities using high resolution mass spectrometry, SimLipid® high throughput lipid identification software and currently available literature.
2) To develop UHPLC QTOF method which can simultaneously separate and determine the API, major lipids, cholesterols their oxidation products, and minor lipids.

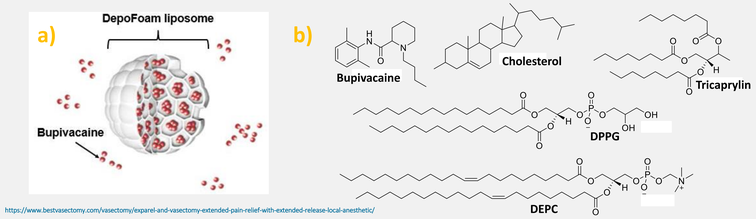

Figure 1. a) Liposomal Exparel® MVL formulation and b) major constituents of liposomal Exparel® formulation

METHOD(S)

The Exparel® was dissolved in methanol or isopropanol. An ultra high-pressure liquid chromatography (UHPLC) QTOF method was used to separate different components in the liposome formulation.

- ✓ Chromatography: Waters C8 Column (2.1mmx100mm, particle size 1.7 µm) except for bupivacaine enantiomer separation where a 250 x 4.6 mm *S*-tert-leucine and *R*-1-(alpha-naphthyl) ethylamine chiral stationary phase column (Chirex® 3020, Phenomenex, Torrance, CA, USA) was used.
- ✓ Mobile phase for reverse phase was methanol and water with 0.1% formic acid and 10 mM ammonium formate and except for the chiral separation where hexane and acetonitrile/IPA mixture was used.
- ✓ Electron spray ionization (ESI) source was used for all the analysis except for the cholesterols where Atmospheric pressure chemical ionization (APCI) was used.
- ✓ Qtof conditions: Positive mode and negative mode analysis with nitrogen sheath gas temperature of 350 °C, at 11 L/min; nebulizer pressure at 60 psi; the capillary voltage at 3500 V; the fragmentor voltage at 175 V.
- ✓ Data Analysis: All the data were analyzed using Premier Biosoft SimLipid® software (Premier Biosoft International, San Francisco, CA USA) and Agilent MassHunter software for qualitative and quantitative analysis, respectively.

RESULT(S)

Table 2. Classes of minor lipids identified in Exparel®

Phosphatidic Acid (PA)	Phospho-glycerols (PG)	Phospho-cholines (PC)	Phospho-serines (PS)	Phospho-ethylamines (PE)	Triacylglycerols (TAG)
(14:0_12:0)	(14:0_14:0)	(16:0_20:4)	(21:0_22:6)	(12:0_18:4)	(12:0_22:6_22:6)
(12:0_17:0)	(16:1_18:1)	(16:0_18:2)	(18:0_18:1)	(18:0_18:1)	(15:0_17:1_15:0)
(14:0_14:0)	(18:1_22:1)	(16:0_18:1)	(17:0_21:0)		
(20:0_22:2)	(18:2_22:2)	(18:0_18:1)			
	(18:0_22:4)	(16:0_22:4)			
	(19:0_22:4)	(16:0_22:6)			
		(17:0_22:4)			

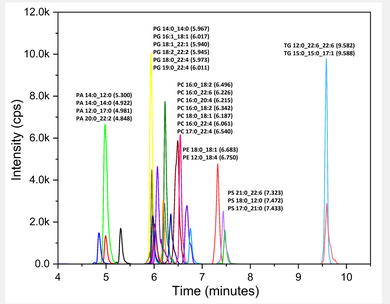

Figure 2. Ion chromatogram of minor lipids found in Exparel®

Table 3. Quantitation of minor lipids and % minor lipids compared to total amount of lipids in Exparel® Mean ± SD N=6

Lipid	Sample 1, mg/L (mM)	Percentage of total amount of lipids	Sample 2, mg/L (mM)	Percentage of total amount of lipids
PC (16:0_20:4)	18.340 ± 1.013 (0.023 ± 0.001)	0.086	20.671 ±0.989 (0.026±0.001)	0.095
PC (16:0_18:2)	5.848 ± 0.772 (0.008 ± 0.001)	0.028	8.174 ±0.532 (0.010±0.001)	0.039
PC (16:0_18:1)	22.873 ± 1.171 (0.030 ± 0.002)	0.11	20.193 ±1.578 (0.026±0.002)	0.096
PC (16:0_22:4)	138.215 ± 3.296 (0.171 ± 0.004)	0.626	124.375 ±3.089 (0.154±0.004)	0.553
PC (18:0_18:1)	35.510 ± 1.735 (0.045 ± 0.002)	0.165	30.037 ±2.045 (0.038±0.003)	0.137
PC (16:0_22:6)	75.225 ±3.688 (0.093±0.005)	0.342	50.414 ±2.830 (0.063±0.004)	0.225
PC (17:0_22:4)	20.797 ±2.965 (0.025±0.004)	0.093	18.184 ±0.933 (0.022±0.001)	0.079
PG (14:0_14:0)	53.400 ±1.611 (0.080±0.003)	0.294	50.783 ±4.234 (0.076±0.006)	0.274
PG (16:1_18:1)	23.850 ±1.978 (0.032±0.003)	0.117	27.721 ±1.403 (0.037±0.002)	0.134
PG (18:1_22:1)	32.293 ±2.024 (0.039±0.002)	0.143	24.334 ±1.469 (0.029±0.002)	0.105
PG (18:2_22:2)	29.820 ±1.597 (0.036±0.002)	0.132	28.768 ±2.223 (0.035±0.003)	0.125
PG (18:0_22:4)	17.488 ±1.268 (0.021±0.002)	0.078	24.511 ±2.058 (0.030±0.002)	0.107
PG (19:0_22:4)	18.392 ±1.426 (0.022±0.002)	0.08	15.271 ±1.166 (0.018±0.001)	0.065
PE (18:0_18:1)	38.509 ±3.982 (0.052±0.005)	0.189	40.226 ±1.362 (0.054±0.002)	0.194
PE (12:0_18:4)	24.217 ±1.197 (0.031±0.002)	0.115	17.885 ±0.910 (0.023±0.001)	0.083
TG (12:0_22:6_22:6)	128.683 ±5.363 (0.144±0.006)	0.527	126.543 ±3.460 (0.141±0.004)	0.509
TG (15:0_15:0_17:1)	34.665 ±1.537 (0.044±0.002)	0.161	30.813 ±0.992 (0.039±0.001)	0.14
PA 14:0_12:0	14.623 ±0.945 (0.026±0.002)	0.095	9.782 ±0.639 (0.017±0.001)	0.062
PA 14:0_14:0	35.259 ±1.944 (0.057±0.003)	0.21	25.711 ±1.883 (0.042±0.003)	0.151
PA 12:0_17:0	19.438 ±1.074 (0.032±0.002)	0.117	22.328 ±0.597 (0.037±0.001)	0.132
PA 20:0_22:2	28.275 ±1.184 (0.036±0.002)	0.131	23.564 ±0.528 (0.030±0.001)	0.107
PS 21:0_22:6	32.667 ±1.324 (0.037±0.002)	0.136	22.690 ±1.101 (0.026±0.001)	0.093
PS 18:0_18:1	27.616 ±1.890 (0.034±0.002)	0.125	18.195 ±0.617 (0.022±0.001)	0.081
PS 17:0_21:0	36.232 ±0.667 (0.044±0.001)	0.162	25.564 ±1.489 (0.031±0.002)	0.112
Total	912.265	4.264	806.734	3.699

Table 4. Quantitation of cholesterol oxidation products in Exparel® Mean ± SD, N=6

Batch	7-oxo-cholestenone, µg/ mL (mM)	Mole-% Compared to total cholesterol	7α-hydroxy-cholesterol µg/mL (mM)	Mole-% Compared to total cholesterol
1	33.16 ± 2.317 (0.083 ± 0.006)	0.711 ± 0.050	18.86 ± 1.663 (0.047 ± 0.004)	0.400 ± 0.035
2	25.83 ± 2.003 (0.065 ± 0.005)	0.534± 0.041	9.841 ± 0.788 (0.024 ± 0.002)	0.202 ± 0.016

Batch	Lot No:	DPPG, mg/L (mM)	DEPC, mg/L (mM)	Tricaprylin, mg/L (mM)	Cholesterol, mg/L (mM)
1	20-4012	0.887 ± 0.042 (1.190 ± 0.056)	7.968 ±0.336 (8.894 ± 0.176)	2.031 ± 0.084 (4.316 ± 0.177)	4.525 ± 0.217 (11.701 ± 0.560)
2	21-6005	0.906 ± 0.045 (1.216 ± 0.060)	8.121 ± 0.183 (9.040 ± 0.203)	2.060 ± 0.077 (4.376 ± 0.163)	4.689 ± 0.264 (12.126 ± 0.683)

Table 5. Comparison of major constituents in Exparel® Mean ± SD, N=6

Table 6. API Bupivacaine quantitation, Mean ± SD N=6

Sample	Lot No:	Racemic-Bupivacaine, mg mL^{-1}	*S*-levobupivacaine, mg mL^{-1}	*R*-dextrobupivacaine/mg mL^{-1}	Ratio R:S
Batch 1	20-4012	12.92 ± 0.16	6.800 ± 0.130	6.698 ± 0.180	1:1.01
Batch 2	21-6005	13.18 ± 0.24	6.902 ± 0.130	6.782 ± 0.100	1:1.01

Table 7. Comparison of major constituents in Exparel® Mean ± SD, N=6

Constituents	Bupivacaine mg/L	DPPG mg/L	DEPC mg/L	Tricaprylin mg/L	Cholesterol mg/L
Labelled data	13.3	0.9	8.2	2.0	4.7
Experimental data	13.055±0.633	0.896±0.021	8.044±0.351	2.036±0.065	4.606±0.194

CONCLUSION(S)

- A UHPLC Qtof methods was developed for the separation, identification, and quantification of the API (bupivacaine), its enantiomers, major and minor lipids, cholesterols, and cholesterol oxidation products in the Exparel® multivesicular liposomal drug formulation.
- All the lipids were quantitatively or semi-quantitatively analyzed and a total of 24 different minor lipids which belong to six different lipid classes were identified in the samples.
- Out of the minor lipids identified, 22 were phospholipids and the others were triacylglycerols.
- It was found that 7-keto cholestenone and 7α-hydroxycholesterol were the major cholesterol oxidation products (COPs) in Exparel® samples.

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