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

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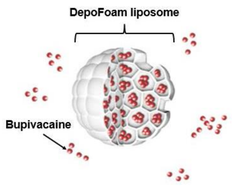
Abstract

Exparel® is a multivesicular liposomal formulation (MVL) of bupivacaine, which provides sustained release at the site of injection resulting in prolonged anesthetic effect. We developed a LC-MS based analytical method to identify and quantify active pharmaceutical ingredients, lipids, minor lipids, and cholesterol oxidation products in two batches of Exparel® injectable liposomal emulsion.

Introduction

Exparel® (bupivacaine) MVLs possess a unique structural assembly of multiple polyhedral chambers separated by lipid septal, providing a prolonged release with the erosion of lipid membranes.



https://www.bestvasectomy.com/vasectomy/exparel-and-vasectomy-extended-pain-relief-with-extended-release-local-anesthetic/

Figure 1. Liposomal Exparel® MVL formulation

The structure and functionality of these liposomes are dictated by their lipid composition, thus providing a broad platform for the drug delivery applications. Given the importance of liposomal lipid composition and their effect on drug incorporation, drug stability, and release properties, the quantification of lipid components is an essential aspect for a complete evaluation of the formulation.

In this study, we utilized a novel UHPLC-ESI-QTOF method and SimLipid® high throughput lipid identification software to identify and quantify all the major lipids, cholesterol, active pharmaceutical ingredients (API) and its enantiomers in two batches of commercially available Exparel® injectable liposomal drug formulation.

The same method was utilized to identify and quantify 24 minor lipids and two cholesterol oxidation products where the two batches of Exparel® were found to contain 4.3% and 3.7% of minor lipids content compared to the total amounts of lipids.

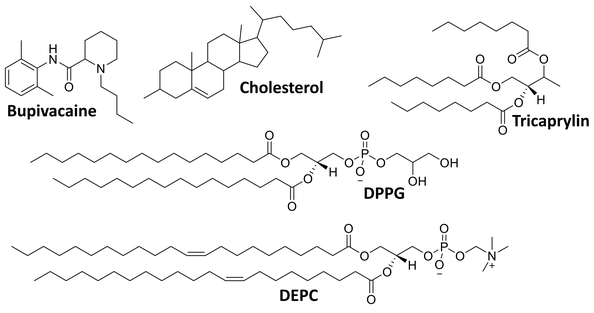


Figure 2. Major Constituents of Exparel® liposomal formulation

Materials and Methods

The Exparel® was dissolved in methanol and the bupivacaine and lipid analyses were performed on a Waters C8 column, (2.1mm×100 mm, particle size 1.7 µm) with a mobile phase flow rate of 400 µL/min with aqueous 0.1% formic acid (FA),10 mM ammonium formate (AF) as the mobile phase A, and 0.1% FA, 10 mM AF in methanol as mobile phase B. The 10 mM AF was used in both mobile phases to reduce peak broadening through ion-pair formation.

Table 1. Binary pump method for API and lipid quantification

Total time	Flow Rate/ µL min^{-1}	A%	B%
0.00	400	75.0	25.0
2.00	400	75.0	25.0
8.00	400	0.00	100.0
12.00	400	0.00	100.0
12.10	400	75.0	25.0
15.00	400	75.0	25.0

ESI ionization was used for the analysis, except for the cholesterols, and the data were acquired in positive mode 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. APCI ionization source was used for the cholesterols ionization. All the major lipids were quantitatively analyzed, while the minor lipids were analyzed either quantitatively or semi-quantitatively depending on the availability of standards.

Results and Discussion

Table 2. API Bupivacaine quantification

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

- **Data presented as mean ± standard deviation, N=6**

Table 3. Quantification of major constituents in Exparel®

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)

- **Data presented as mean ± standard deviation, N=6**

Table 4. Comparison of labelled value and experiment results of Exparel® constituents

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

- **Data presented as mean ± standard deviation, N=6**

Table 5. Quantification of cholesterol oxidation products in Exparel®

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

- **Data presented as mean ± standard deviation, N=6**

Table 6. 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)			

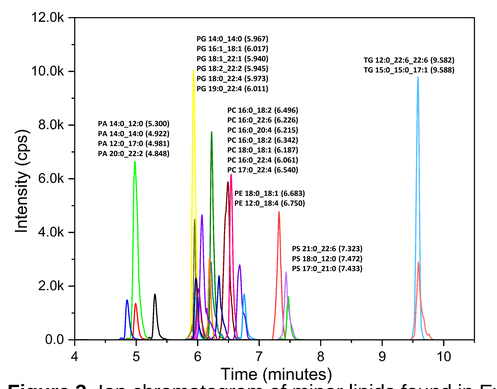


Figure 3. Ion chromatogram of minor lipids found in Exparel®

Table 7. Quantification of minor constituents in Exparel®

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**

- **Data presented as mean ± standard deviation, N=6**

Conclusion

- A LCMS-HRMS 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 were identified in the samples which belong to six different lipid classes.
- Out of the minor lipids identified, 22 were phospholipids and the others were triacylglycerols.
- 2 cholesterol oxidation products (CODs; 7-keto cholestenone and 7α-hydroxycholesterol) were identified and quantified in Exparel® sample.

Acknowledgement and Disclaimer

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