Comprehensive Composition Analysis of Doxorubicin HCl Liposomes

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PURPOSE

Liposomal technologies have advanced over decades, serving as versatile carriers that have transformed cancer therapy with formulations like doxorubicin HCl liposome injection. These formulations deliver the active pharmaceutical ingredient (API) with enhanced efficacy and altered biodistribution, resulting in reduced toxicity. However, lipids are prone to degradation and oxidation. The objective of this study is to develop an analytical method to obtain comprehensive composition and degradation product profiles of doxorubicin HCl liposome formulations.

METHOD(S)

In this investigation, we explored high resolution mass spectrometry methods aimed at identifying and quantifying lipid degradation products, oxidation products, and lipid impurities in doxorubicin HCl liposomal formulations. Various reversed-phase UHPLC techniques were used for lipid component separation. Lipids and their derivatives (Phosphatidylcholines (PCs), Lysophosphatidylcholines (LPCs), Lyso-phosphatidyl-ethanolamines (LPEs), Phosphatidylethanolamines (Pes)) were analyzed in positive ionization mode using an ACQUITY UPLC® CSH™ C18 column with an Electrospray ionization (ESI) ion source. Free fatty acids (FFAs) were separated in negative ionization mode with an ACQUITY UPLC® BEH™ C8 column. Cholesterols and their derivatives were analyzed in positive mode with an ACQUITY UPLC® CSH™ C18 column using an Atmospheric pressure chemical ionization (APCI) ion source. Identification of these compounds was facilitated by Premier Biosoft SimLipid® software, cross-verifying with standards where available. The composition of these identified lipids was determined, employing either quantitative or semi-quantitative methods with the aid of commercially available lipid standards.

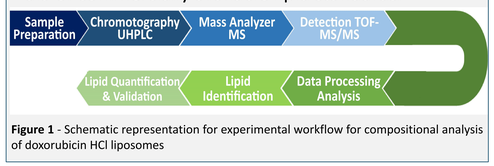

Figure 1 - Schematic representation for experimental workflow for compositional analysis of doxorubicin HCl liposomes

RESULT(S) – LIPID IDENTIFICATION

Phospholipids and Lyso-phospholipids

- Ionized by ESI source in the positive mode.
- **Column:** ACQUITY UPLC CSH™ C18.
- Four lysophospholipids, five phospholipids including three major formulation lipids (PC 18:0-18:0, PC 16:0-18:0, and mPEG2000-DSPE) were identified

Free fatty acids (FFA)

- Ionized by ESI source in the negative mode.
- **Column:** ACQUITY UPLC® BEH™ C8
- 6 free fatty acids were identified

Cholesterol

- Ionized by APCI source in the positive mode.
- Column: ACQUITY UPLC CSH™ C18.®
- 2 cholesterol derivatives were identified

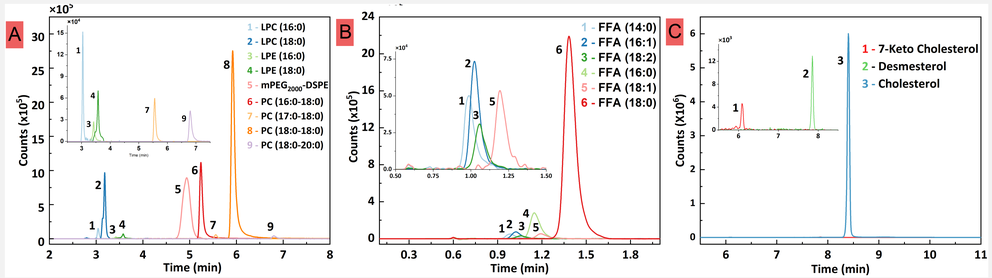

Figure 2 – (A) Chromatogram of lipids, and lipid degradation products in doxorubicin HCl liposomal formulations (in positive ionization mode). The list of compounds in elution order as follows: (1) LPC 16:0, (2) LPC 18:0, (3) LPE 16:0, (4) LPE 18:0, (5) $mPEG_{2000}$-DSPE, (6) PC (16:0-18:0), (7) PC (17:0-18:0), (8) PC (18:0-18:0), and (9) PC (18:0-20:0). Inset shows the magnification of (1) LPC 16:0, (3) LPE 16:0, (4) LPE 18:0, (7) PC (17:0-18:0), (9) PC (18:0-20:0). (B) Chromatogram of FFAs in doxorubicin HCl liposomal formulations (in negative ionization mode); (1) FFA 14:0, (2) FFA 16:1, (3) FFA 18:2, (4) FFA 16:0, (5) FFA 18:1, and (10) FFA 18:0. Inset shows the magnification of (1) FFA 14:0, (2) FFA 16:1, (3) FFA 18:2, (5) FFA (18:1). And (C) Chromatogram of Cholesterol and cholesterol derivative products in HCl liposomal formulations in The list of compounds in elution order as follows: (1) 7-keto cholesterol, (2) Desmosterol, and (3) Cholesterol.

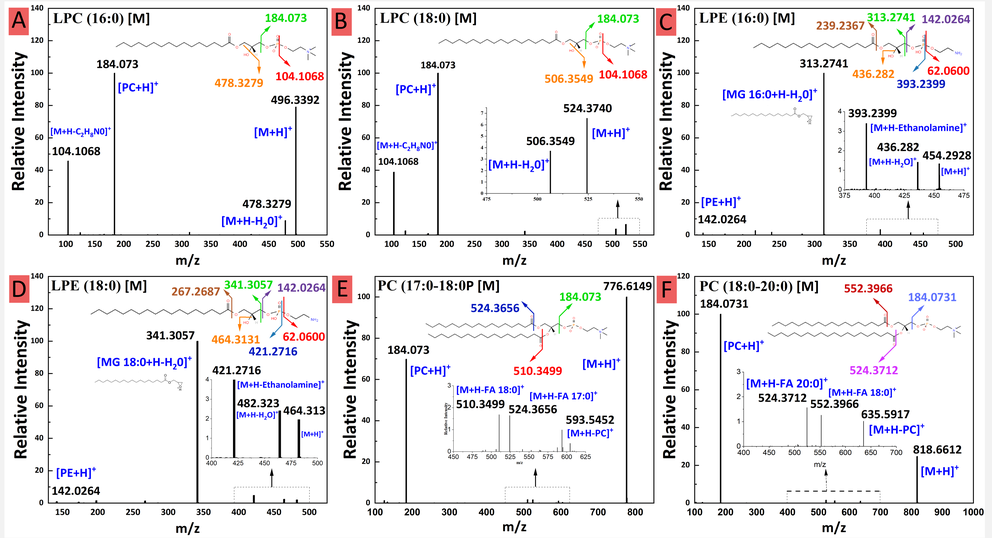

Figure 4 - MS/MS Fragmentation patterns and relevant chemical structures of protonated [M+H]+ (A) LPC (16:0), (B) LPC (18:0), (C) LPE,(16:0), inset image represents magnification of the section between m/z 375 to m./z 47 5, (D) LPE (18:0), inset image represents magnification of m/z 400 to m/z 500, (E) PC (17:0-18:0),), inset image represents magnification of m/z 450 to m/z 625 and (F) PC (18:0-20:0), inset image represents magnification of m/z 400 to m/z 700.

RESULT(S) - LIPID QUANTITATION

Table 1. Quantitative results of lipid components, lipid impurities, and cholesterol across five different liposomal formulations. The table provides a comprehensive overview of lipid composition, aiding in the comparison and analysis of the formulations.

Compound Name	Vendor 1 (µmol/ml)	Vendor 2 (µmol/ml)	Vendor 3 (µmol/ml)	Vendor 4 (µmol/ml)	Vendor 5 (µmol/ml)
PC (16:0/18:0)	3018.24	2972.67	3229.89	3027.24	3225.77
PC (18:0/18:0)	9236.78	9224.20	9046.59	9218.00	9039.35
LPC (16:0)	23.02	33.19	46.14	41.91	27.36
LPC (18:0)	208.77	279.81	378.46	354.51	205.53
PC(17:0/18:0)*	192.48	184.38	197.64	177.98	197.29
PC(18:0/20:0)*	188.65	186.58	205.58	177.41	189.92
Choleterol	8162.32	8123.02	8305.35	8118.96	8341.43
Desmeterol	21.89	29.27	21.37	20.51	22.28
7-Keto-Choleterol	5.12	14.23	74.68	9.88	5.69
FFA 14:0	42.04	53.33	40.94	65.73	52.41
FFA 16:0	37.79	87.67	67.31	61.15	90.12
FFA 16:2*	4.99	ND	ND	ND	7.43
FFA 18:2	9.16	12.19	9.95	3.64	6.31
FFA 18:1	8.43	8.67	8.50	BLD	BLD
FFA 18:0	461.12	492.06	556.03	513.64	259.74
mPEG-DSPE	1156.44	1128.78	1165.47	1169.42	1136.92
LPE 16:0	ND	BLD	ND	BLD	BLD
LPE 18:0	2.24	2.45	BLD	4.03	4.17

*Semi-quantitative analysis using different standards; used 18:0 PC-d70 standard for PC (17:0/18:0) and PC (18:0/20:0), and FFA 18:0-d35 for FFA 16:2 quantification. ND represents not detected in the formulation, BLD represents below limit of detection (LOD).

Figure 5 - Quantitative analysis of phospholipids and lysophospholipids in doxorubicin HCl liposomal formulations. The panel of bar charts (A-I) representing the concentration of various lysophospholipids and phospholipids across five different liposomal formulations. (A) LPC (16:0), (B) LPC (18:0), (C) LPE (16:0), (D) LPE (18:0), (E) $mPEG_{2000}$-DSPE, (F) PC (16:0-18:0), (G) PC (17:0-18:0), (H) PC (18:0-18:0), and (I) PC (18:0-20:0). ND represents not detected in the sample and BLD represent below limit of detection (LOD). Compd Name represents compound name.

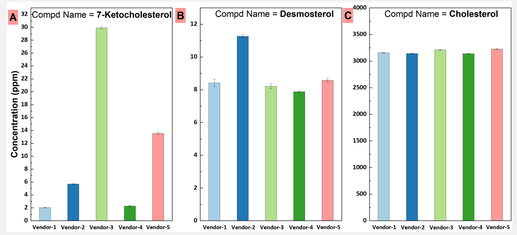

Figure 7 – Quantitative Analysis of cholesterol and derivatives in doxorubicin HCl liposomal formulations. The panel of bar charts represents the composition of cholesterol-related compounds present across all five formulations. (A) 7-Ketocholesterol, (B) Desmosterol, and (C) Cholesterol.

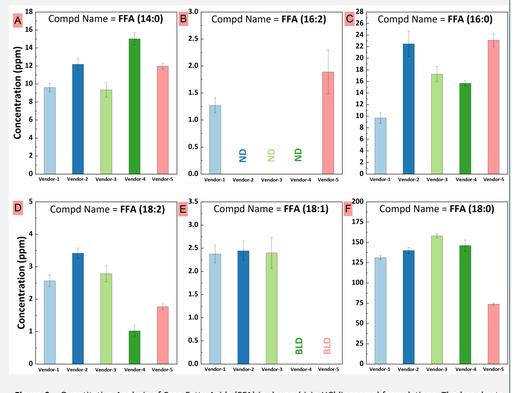

Figure 6 – Quantitative Analysis of Free Fatty Acids (FFA) in doxorubicin HCl liposomal formulations. The bar charts illustrate the concentration of six FFAs across five different liposomal formulations. (A) FFA 14:0, (B) FFA 16:2, (C) FFA 16:0, (D) FFA 18:2, (E) FFA 18:1, and (F) FFA 18:0. The charts highlight the variability in FFAs in each formulation. ND represents Not detected and BLD represent below limit of detection (LOD).

CONCLUSION(S)

- **Phospholipid impurities:** PC (17:0-18:0) and PC (18:0-20:0), are present in concentrations of ~178 to 198 µmol/ml and ~177 to 205 µmol/ml respectively across all formulations, making up less than 2.4 mole% of the total lipids in the formulations.
- **Lysophospholipids (LPCs and LPEs)** were detected in all formulations, with LPCs higher in Vendor 2, Vendor 3, and Vendor 4, and total lysophospholipids were below ~1.25 mole%, with Vendor 1 and Vendor 5 showing even lower levels.
- **Free fatty acids (FFAs),** including FFA 16:0 and FFA 18:0, are present in all formulations with FFA 18:0 being most abundant, total FFAs constitute less than 2.5% mole-% of the total phospholipids.
- **Cholesterol derivative products:** Desmosterol levels ranged from 7.89 ng/ml to 11.26 ng/ml, and 7-keto-cholesterol levels varied from 5 µmol/ml to 74 µmol/ml, with cholesterol derivative products comprising less than ~0.3% mole-% of total cholesterol.

ACKNOWLEDGEMENT AND DISCLAIMER

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