4D Analysis of Lipid Nanoparticles (LNP) using Elute-timsTOF Pro 2 with VIP-HESI source

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Introduction

Lipid nanoparticles (LNPs) have emerged from the recent COVID-19 pandemic as effective delivery vehicles for mRNA vaccines,[1,2] and pharmaceutical formulations in nucleic acid-based drugs. LNPs are typically composed of four critical lipid species components:

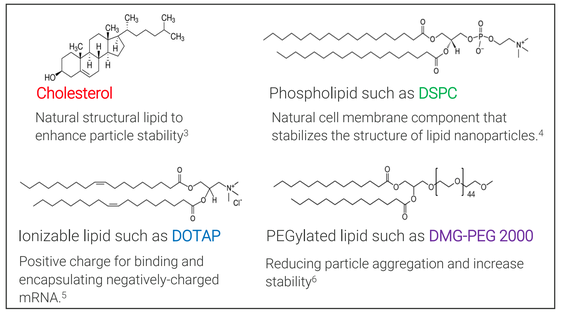

Figure 1. Structure of four components of lipid nanoparticles (LNP).

As LNPs become more commonly considered for use by drug and vaccine manufacturers, the research interest in their composition, stability, and biological interactions is driving a need for bioanalytical solutions capable of in-depth sample characterization. Bruker's 4D-Lipidomics™ solution leverages powerful multidimensional separation by combining ultra-high performance liquid chromatography (UHPLC) with trapped ion mobility spectrometry (TIMS) and high resolution mass spectrometry (HRMS) in a single hyphenated system capable of separating and describing sample components by their retention time, collision cross section (CCS), and mass-to-charge (*m/z*) measurements, respectively. Here, four components of LNPs are analyzed using the Elute–timsTOF Pro 2 system with CCS-capability via a VIP-HESI source. Analysis of LNP lipid species in a complex biological matrix is also investigated.

Methods

Working solution chemical standards of DSPC, DOTAP, DMG-PEG-2000 (Avanti Polar Lipids) and cholesterol (Sigma-Aldrich) with concentrations ranging from 1 ppb – 100 ppm of the resulting LNP component mixture were analyzed using Elute-timsTOF Pro 2 with both ESI and VIP-HESI sources.

Lipids were extracted from NIST SRM 1950 plasma (Sigma-Aldrich) by MTBE. One aliquot was analyzed as is and the other aliquot was spiked with LNP component mixture (5 ppm cholesterol, 1 ppm DMG-PEG 2000, and 50 ppb DOTAP/DSPC).

LC System	Elute		
Column	YMC-Triart C18, 100x2.1 mm, 1.9 µm, P/N TA12SP9-10Q1PT		
Mobile Phase A	600/390/10 MeCN/Water/1M ammonium formate in 0.1% formic acid		
Mobile Phase B	900/90/10 IPA/MeCN/1M ammonium formate in 0.1% formic acid		
Gradient	Time	%A	%B
	0.0	50	50
	0.5	47	53
	4.0	45	55
	7.0	35	65
	7.5	20	80
	10.0	1	99
	13.0	1	99
	13.1	50	50
Flow Rate	0.4 mL/min		
Column Temp.	55 °C		
Sample Temp.	4 °C		
Injection Volume	2 µL		

MS System	timsTOF Pro2	
Method	default 4D-Lipidomics_pos	
VIP-HESI Source Parameter	Parameter	Value
	End Plate Offset	500 V
	Capillary Voltage	4500 V
	Nebulizer	2.0 Bar
	Dry Gas	8.0 L/min
	Dry Temp	230 °C
	Prob Gas Temp	400 °C
	Probe Gas	4.0 L/min
ESI Source Parameter	Parameter	Value
	End Plate Offset	500 V
	Capillary Voltage	4000 V
	Nebulizer	2.2 Bar
	Dry Gas	10.0 L/min
	Dry Temp	220 °C

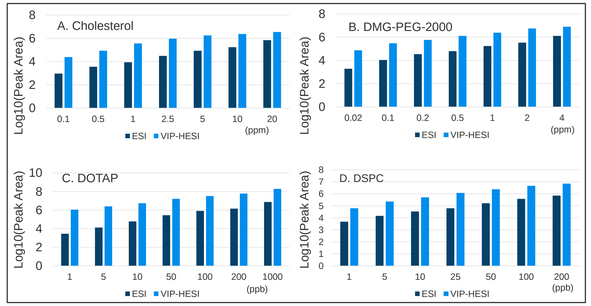

Figure 2. Log10 of peak area of representative EIC peaks from the four lipid species comparing standard ESI source vs. VIP-HESI source for cholesterol at *m/z* 369.3516, DMG-PEG-2000 at *m/z* 854.2496, DOTAP at *m/z* 662.6087, and DSPC at *m/z* 790.6320.

Results

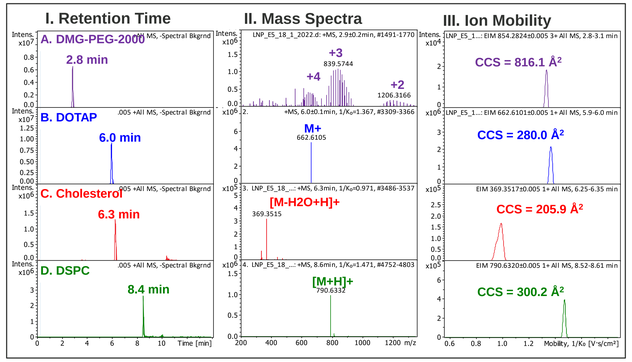

Figure 3. Results of (I) extracted ion chromatogram, (II) extracted mass spectra, and (III) extracted ion mobilograms for the analysis of a mixture of four LNP components: (A) DMG-PEG-2000 (*m/z* 854.2496 for +3 of 44 repeating PEG unit), (B) DOTAP for the cation (*m/z* 662.6087), (C) Cholesterol with water loss (*m/z* 369.3516), and (D) DSPC (*m/z* 790.6320)

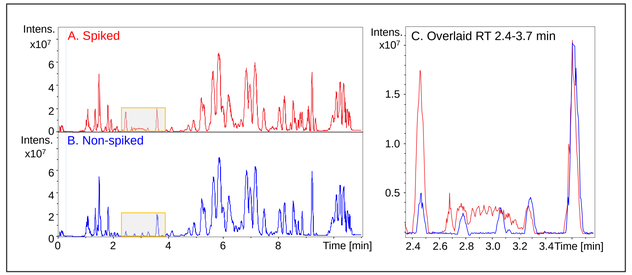

Figure 4. Base peak chromatogram of SRM 1950 lipid extract (A) with and (B) without LNP component mixture spike, (C) Overlaid chromatogram of RT 2.4 – 3.7 region.

References

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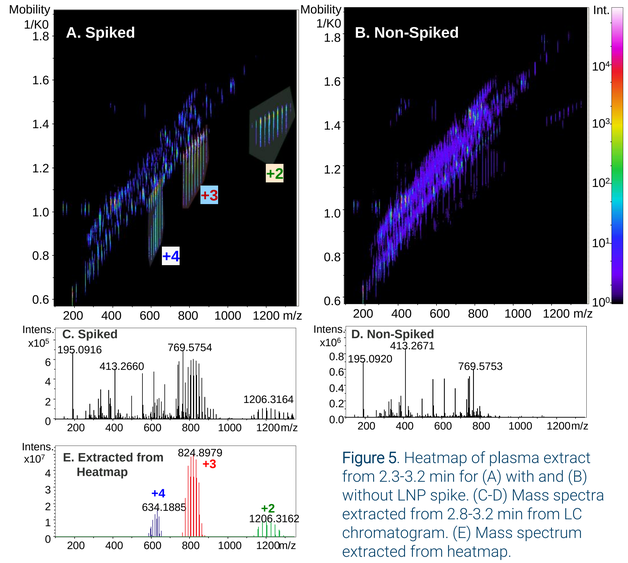

Figure 5. Heatmap of plasma extract from 2.3-3.2 min for (A) with and (B) without LNP spike. (C-D) Mass spectra extracted from 2.8-3.2 min from LC chromatogram. (E) Mass spectrum extracted from heatmap.

Summary

- LNP components are readily separated and detected by reverse phase UHPLC-MS. VIP-HESI source improved ion signals by 5-25 folds (Fig. 2-3).
- CCS values of all components are measured by TIMS which provides additional dimension of separation for characterization (Fig. 3).
- PEGylated lipids are co-eluting with endogenous species when LNP mixture is spiked into plasma extract. They can be well separated in the TIMS dimension (Fig.4-5).

Conclusion

- Rapid analysis of LNP components is provided by UHPLC-TIMS-HRMS. The VIP-HESI source demonstrates significant sensitivity enhancement for ionizing LNP materials.
- The additional dimension of ion mobility spectrometry provides CCS information for LNP compound identification and can separate PEGylated lipids from complex plasma samples.

4D-Lipidomics Elute-timsTOF Pro 2