Simultaneous separations of lipid nanoparticle components using nearly monodisperse, mixed-mode stationary phases

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Introduction

- Lipid nanoparticles (LNPs) enable drug delivery for mRNA therapeutics, as demonstrated by the FDA-approved COVID-19 vaccines.
- LNP component separation is important for quantitation and optimization of lipid composition for desired physical properties, such as surface charge and particle size.
- mRNA-LNPs are typically analyzed over multiple methods due to the opposing physicochemical properties of their components.
- Two stationary phase chemistries, C12 and C18/Ar, were used to separate empty LNPs composed of components used in FDA approved LNP formulations within a single method.
- Mac-Mod EvoSphere columns with nearly monodispersed particle packings were selected to enhance peak shape and efficiency by minimizing eddy diffusion.

Methods

Empty LNPs were composed of SM-102 (ionizable lipid), DSPC (phospholipid), cholesterol, and DMG-PEG(2000) (PEG-lipid) in a 50:10:38.5:1.5 molar ratio. The solution was diluted to 1:10 in phosphate buffer.

	C12 Separation	MAX C18/Ar Separation
Column	Evosphere C12 (2.1 mm i.d. x 100 mm, 3µm)	Evosphere MAX C18/Ar (2.1 mm i.d. x 100 mm, 3µm)
Mobile Phase	A: 0.01% TFA B: IPA, 0.01% TFA	A: 0.1% FA B: IPA, 0.1% FA
Oven Temperature	50˚C	50˚C
Time Program	Gradient, 70-95 B% (5 min); hold 3	Gradient, 70-95 B% (5 min); hold 3
Flow Rate:	0.20 mL/min	0.20 mL/min
Detection:	1Q MS, SCAN (+), SIM (+): m/z 369, 711, 791	1Q MS, SCAN (+), SIM (+): m/z 369, 711, 791

Results

C12 Separation of LNP Components

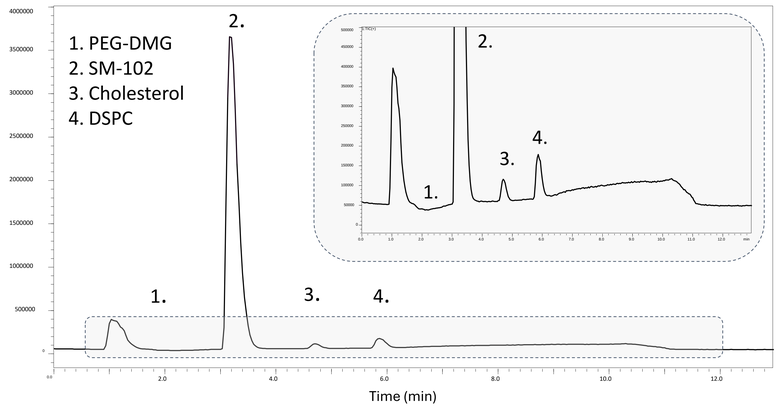

Figure 1 - Chromatogram of LNP components resolved using the C12 column.

MAX C18/Ar Separation of LNP Components

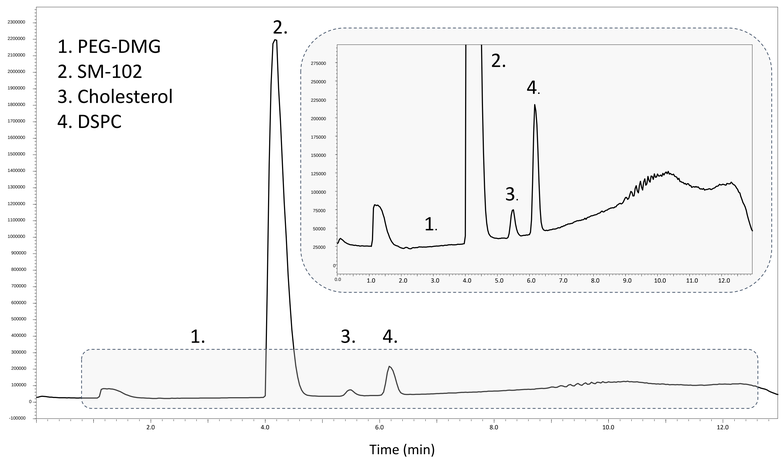

Figure 2 - Chromatogram of LNP components resolved using the C18/Ar column.

TFA Optimization Study

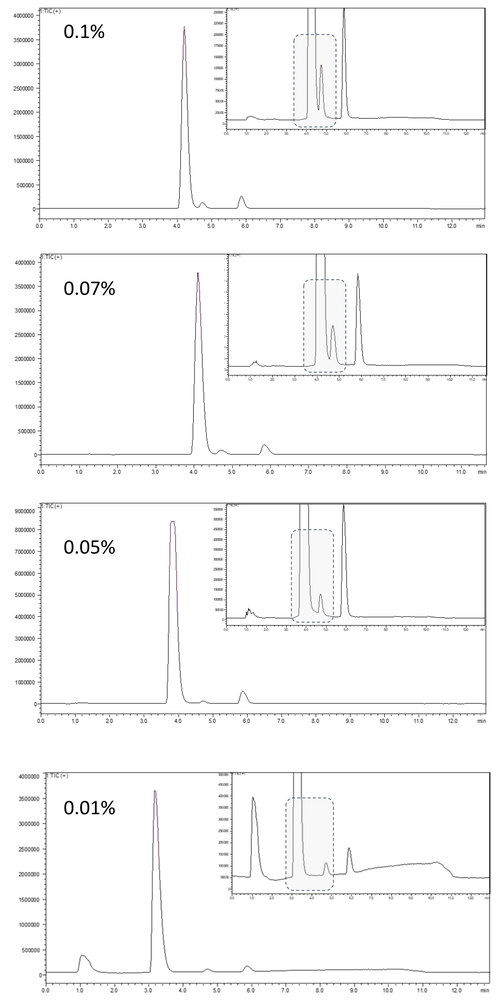

Figure 3 - TFA percentage of mobile phase was systematically optimized to 0.01%. Reducing the TFA concentration improved the resolution of SM-102 and Cholesterol.

PEG-DMG Mass Spectra

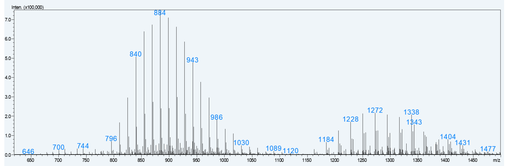

Figure 4 – PEG-DMG mass spectra from the chromatogram in Figure 1 as proof of detection. The diluted PEG-lipid did not present a peak in the chromatogram.

Discussion

C12 separation

- The ion pairing agent, Trifluoroacetic Acid (TFA), improved resolution by increasing the retention time of charged species.
- Initially, SM-102 and cholesterol partially co-eluded. A reduction in TFA further improved separation, thus the TFA percentage of the mobile phase was systematically optimized from 0.1% to 0.01%.
- The low concentration and high polydispersity of DMG-PEG led to weak signal response, but was still detectable (Figure 4), eluting before the other components.

MAX C18/Ar separation

- The C18/Ar column provided a comparable lipid separation without the need for a TFA ion pairing agent.
- The aromatic groups provide “ring stacking” interactions with cholesterol, thus retaining longer than SM-102.
- Ion pairing agents can cause column and MS contamination. These reagents are also environmentally toxic.
- This method will be used for lipid quantitation and subsequently optimized for RNA and lipid separations.

Sources

1. Li Li, Joe P. Foley, Roy Helmy. Simultaneous separation of small interfering RNA and lipids using ion-pair reversed-phase liquid chromatography. Journal of Chromatography A, Volume 1601, 2019, Pages 145-154. ISSN 0021-9673.
2. Sylwia Studzińska, Szymon Bocian, Luca Rivoira, Ed Faden, Geoff Faden. Separation and identification of oligonucleotides impurities and degradation products by reversed phase ultra-high performance liquid chromatography using phenyl-bonded stationary phases without ion pairs - A step towards sustainability. Journal of Chromatography A, Volume 1736, 2024, 465380, ISSN 0021-9673.

Acknowledgements

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