Rapid Analysis of Lipid Nanoparticle Components Using BioAccord™ LC-MS System

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Introduction

- The recent success of mRNA vaccines in SARS-CoV-2 clinical trials is in part due to the development of lipid nanoparticle (LNP) delivery systems.
- Incorporating the mRNA into LNP protects the mRNA from enzymatic attack and enhances cell uptake and expression (1).
- The LNP used in delivery contain four lipid components: cholesterol, a phospholipid, an ionizable lipid and PEGylated lipid (Figure1).
- A simple, rapid, and routine LC-MS method was developed for the characterization and analysis of LNP components using an ACQUITY™ Premier CSH C18 Column and the BioAccord System.

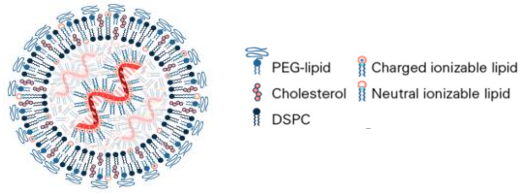

Figure 1. Cartoon of mRNA encapsulated in LNP (1).

Experimental

LC method: Mobile phase A was 600/390/10 (ACN/Water/1M aqueous ammonium formate) in 0.1% formic acid and B was 900/90/10 (IPA/ACN/1 M aqueous ammonium formate). An ACQUITY Premier CSH C18 Column (100 x 2.1mm) was used.

MS method: Data was acquired in positive mode from m/z 50-2000 with a cone voltage of 30 V and fragmentation cone voltage ramp 120-200 V.

Results

Single Lipid Analysis

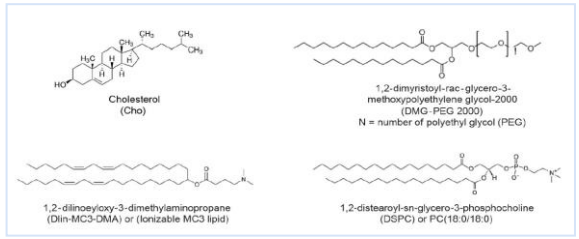

Figure 2. Four lipid nanoparticle components investigated.

The MS detector and reversed-phase chromatography enabled both detection of these spectroscopically silent species and separation of similar lipids within a common class.

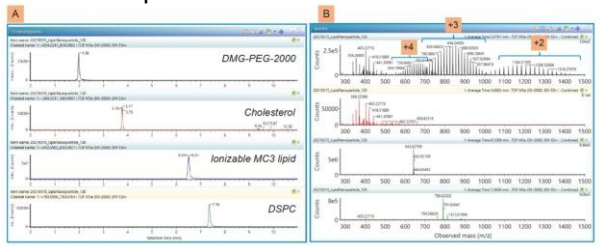

Figure 3. (A) Extracted Ion chromatograms and (B) corresponding spectra of the four lipid components.

Serial dilution and LOD

Lipid nanoparticle	5pg/µL	50pg/µL	100pg/µL	250pg/µL	500pg/µL
PC 18:0_18:0	25pg*				
Cationic Lipid MC3	25pg*				
Cholesterol				1.25ng*	
DG(14:0/14:0)-PEG 2000	25pg*				

*LOD on column

Results

Complex Lipid Analysis

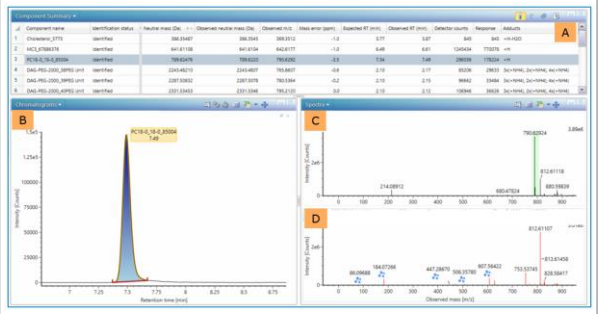

Figure 4. Component summary plot showing (A) the identified lipid nanoparticles of cholesterol, cationic lipid MC3, DSPC and 14 different DMG-PEG-2000 (B) Example extracted ion chromatogram of DSPC (C) Low energy exact mass of DSPC and (D) High energy fragment ion spectrum of DSPC. The blue icon in panel D indicates matched predicted in silico and experimental fragment ions.

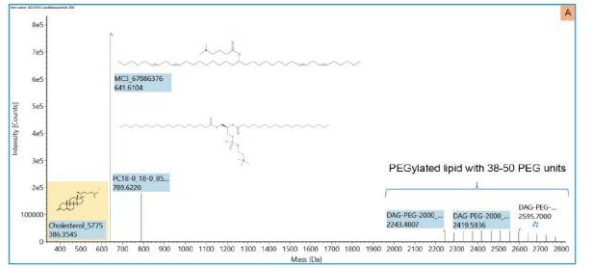

Figure 5. Component plot of the four classes of lipids commonly used in lipid nanoparticle formulations. The PEGylated lipid had the most complex spectra with multiple charges states (+2, +3, +4) under ESI positive ion mode and has variable chain lengths from 38 to 50 PEG repeat units.

References:
1. Vaccines 2021, *9*(1), 65; https://doi.org/10.3390/vaccines9010065

Results

New Peak Detection and Binary comparison

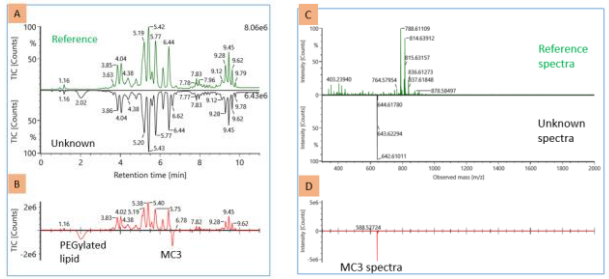

Figure 6: (A) Chromatogram binary comparison of liver lipid extract (reference) compared to a sample with additional lipids spiked into the sample (unknown). New peaks detected identified with arrows. (B) Difference plot of reference and unknown chromatograms.

(C) Combined spectra binary comparison of ionizable MC3 lipid (RT 6.6 min). (D) Spectra difference plot between the reference and unknown from figure C.

Conclusions

- A simple, rapid, and routine RP LC-MS method was developed for the analysis of LNP composition.
- The BioAccord System is useful for the single components ID and degradation, process and raw material impurities and process development and quality control analyses.
- For more information, please refer to the Waters application note: "Rapid Analysis of Lipid Nanoparticle Components Using BioAccord LC-MS System," 2021.

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