Rapid Analysis and Characterization of Lipid Nanoparticle Components for mRNA Delivery

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PURPOSE

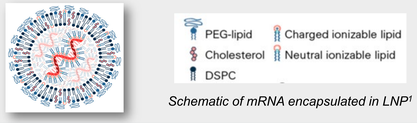
Success of mRNA vaccines for SARS-CoV-2 highlight the potential of mRNA-based therapeutics and the importance of lipid nanoparticle (LNP) delivery systems.

The success of mRNA vaccines in SARS-CoV-2 clinical trials is in part due to the development of lipid nanoparticle (LNP) delivery systems.

LNP delivery system with four lipids (cholesterol, a phospholipid, an ionizable lipid and PEGylated lipid) require careful compositional and associated impurities analysis for efficacy and safety.

Incorporating the mRNA into LNP protects the mRNA from enzymatic attack and enhances cell uptake and expression.[1]

A systematic approach for characterization of LNP and potentially associated impurities and degradation products is needed.



Schematic of mRNA encapsulated in LNP[1]

OBJECTIVE(S)

Development of simple, rapid, and routine LC-MS methods were for the characterization and analysis of LNP components using an ACQUITY™ Premier CSH C18 Column and the BioAccord System.

METHOD(S)

LC method:

ACQUITY™ Premier and ACQUITY Premier CSH C18 Column (1.7 µm,100 x 2.1mm) was used for separation. Mobile phase A: 600/390/10 (ACN/Water/1M aqueous ammonium formate) in 0.1% formic acid; Mobile phase B was 900/90/10 (IPA/ACN/1 M aqueous ammonium formate).

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.

Data Management:

UNIFI Scientific Information System under waters_connect was used for data acquisition and processing.

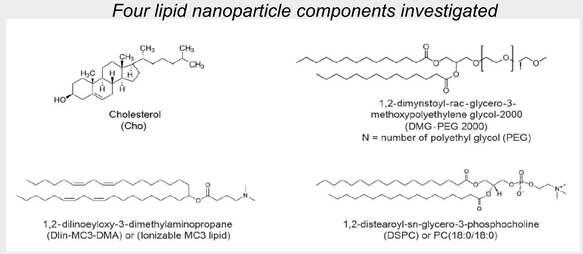
Instrumentation:

ACQUITY Premier BioAccord LC-MS System

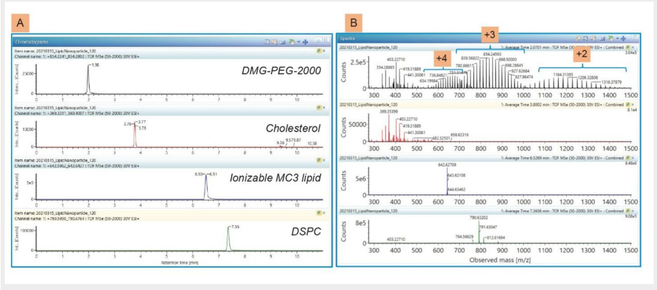
RESULT(S)

Single Lipid Analysis

Four lipid nanoparticle components investigated

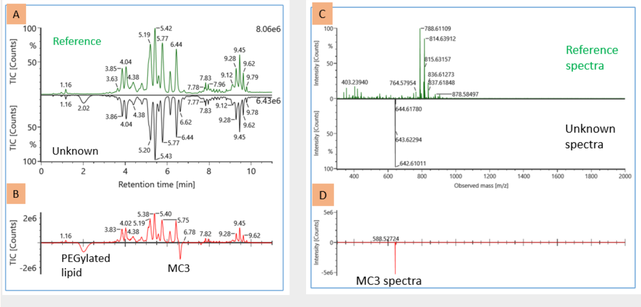


The MS detector and reversed-phase chromatography enabled both detection of these spectroscopically silent species and separation of similar lipids within a common class.



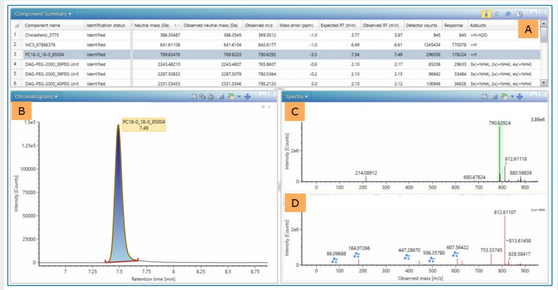
(A) Extracted Ion chromatograms and (B) corresponding spectra of the four lipid components

New Peak Detection and Binary Comparison

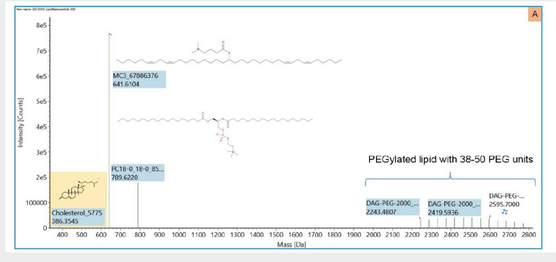


(A) Chromatogram binary comparison of liver lipid extract (reference) compared to a sample with additional lipids spiked into the sample (unknown). New peaks detected identified with arrows. (B) Difference plot of reference and unknown chromatograms. (C) Combined spectra binary comparison of ionizable MC3 lipid (RT 6.6 min). (D) Spectra difference plot between the reference and unknown from figure C.

Complex Lipid Analysis



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.



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.

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

CONCLUSION(S)

- A simple, rapid, and routine RP LC-MS method was developed for the analysis of Lipid Nanoparticle composition.
- The built-in library feature on waters_connect/UNIFI informatics system allows for quick screening and quantification of LNP components in a complex mixture.
- Single components identification of new species, introduced in manufacturing or result of degradation, can achieved using built in structural interrogation tools.
- Newly identified species can be added to the library for quick screening in the future analysis.
- Excellent sensitivity and dynamics range for potential use in both formulation process development and raw material/impurities characterization and quality control.
- For more information, please refer to the Waters application note: “Rapid Analysis of Lipid Nanoparticle Components Using BioAccord LC-MS System,” 2021.

REFERENCE

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

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