LIPID NANOPARTICLES—GET TO KNOW THEM ANALYTICALLY

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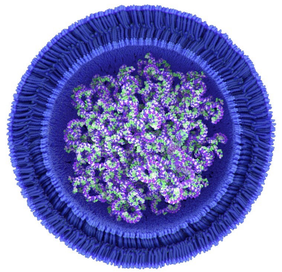
INTRODUCTION

Lipid Nanoparticles (LNPs)

The recent success of mRNA vaccines is largely due to their ability to use human ribosomes to produce the correct protein. This was discovered multiple years prior to their Emergency Use Authorization as a vaccine. They transitioned from "promising technology" to "breakthrough science" after successful delivery, LNPs, were developed.

The LNP used in delivery contain four lipid components: cholesterol, a phospholipid, an ionizable lipid and the PEGylated lipids. Due to high demand companies are sourcing **raw materials** from multiple manufacturers and **quality and analytical impurity analysis** is paramount to demonstrate similarity.

. Liquid chromatography (LC) can separate complex lipid mixtures and mass spectrometry (MS) can provide detailed information regarding the identity and purity of a lipid.



The BioAccord System, an ACQUITY UPLC I-Class LC coupled with ACQUITY RDa Detector was used to characterize LNP. This easy-to-operate LC-HRMS is the ideal pairing with LNP characterization as many pivot to this new class of therapies.

Figure 1. Cartoon of mRNA encapsulated in a LNP.

METHODS

Samples

Lipid standard of cholesterol, 1,2 –distearoyl-sn-glycero-3-phosophocholine (DSPC), and 1,2-dilinoeyloxy-3-dimethylaminopropane (MC3) were purchased from Sigma and 1,2,-dimyristolyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG-2000) was purchased from Avanti Polar Lipids. Stock solutions of 1mg/mL were prepared for each lipid dissolved in either methanol (cholesterol and DMG-PEG-2000) or a 1:1 solution of methanol/chloroform (DSPC and MC3)

Instrument methods

LC method: Mobile phase A was 600/390/10 (ACN/Water/1M aqueous ammonium formate) in 0.1% formic acid and Mobile phase B was 900/90/10 (IPA/ACN/1 M aqueous ammonium formate). An ACQUITY Premier CSH C18 column with 1.7 μM particle size was used.

MS method: The data acquired in positive mode from *m/z* 50-2000 with a cone voltage of 30 V and fragmentation voltage 4-6x greater

Informatics software

Data processing: Data were processed using the UNIFI Scientific Information system. A custom database/library was created within the UNIFI Scientific library. It was run under waters_connect with accurate mass screening workflow.

RESULTS

Single Lipid Analysis

Lipid Components

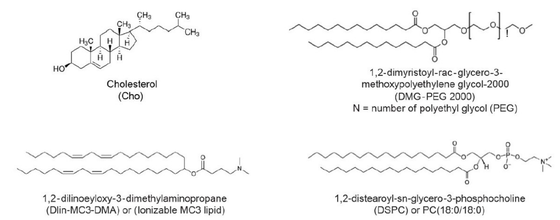
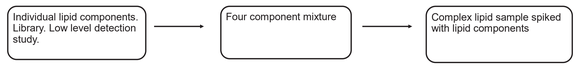


Figure 2: Four Lipid nanoparticle components investigated.

In addition to detection of these four classes of lipids another important aspect is any lot-to-lot variation, supplier variation, or degradant detections. Common degradation pathways for lipids include: oxidation, hydrolysis, hydrogenation, aggregation and oligomer formation.

Workflow



BioAccord Results for Single Lipids

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

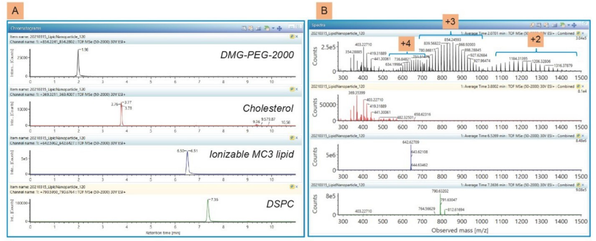


Figure 3. Extracted Ion chromatograms (A) and corresponding spectra (B).

Lipid Complex Analysis

Low Level Concentrations

The four lipid samples were diluted to 1 pg/uL, 5 pg/uL, 50 pg/mL, 100 pg/mL, 250 pg/uL and 500 pg/uL to estimate the lower limit of detection under the conditions of this study. Results of this dilution experiment showed that the instrument had a similar threshold for three of the lipids with the limit of detection for cholesterol being significantly higher.

Lipid	Lowest concentration studied
MC3	5 pg/µL
DSPC	5 pg/µL
DMG-PEG-2000	5 pg/µL
cholesterol	250 pg/µL

Table 1: Low level detection study. 5 pg/µL (25 pg on column) and 250 pg/µL (1.25 ng on column).

Lipid Complex Analysis

The PEGylated lipid had the most complex spectra with multiple charges states (+2, +3, +4) under ESI positive mode and has variable chain lengths from 38 to 50 units derived MS fragments.

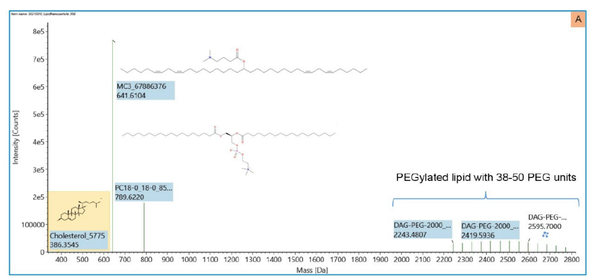


Figure 4: Component plot of the four classes of lipids commonly used in lipid nanoparticle formulations. Individual lipids were added to a custom library.

Unknown Impurities

New Peaks Detected

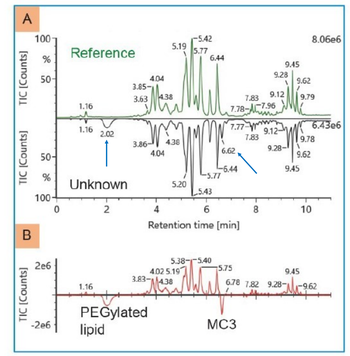


Figure 5: A. Chromatogram binary comparison of liver lipid extract (reference) compared to a sample with additional lipids spiked into the sample (unknown). New peaks detected identified with arrows. B. Difference plot of reference and unknown chromatograms.

Binary Comparison

Figure 6C and E is the combined spectra at RT 2.02 min and RT 6.62 min from Figure 5. The profile of the difference plots in D and F are consistent with the single lipid spectra of PEGylated lipid, DMG-PEG200 (D) and MC3 lipid (F) .

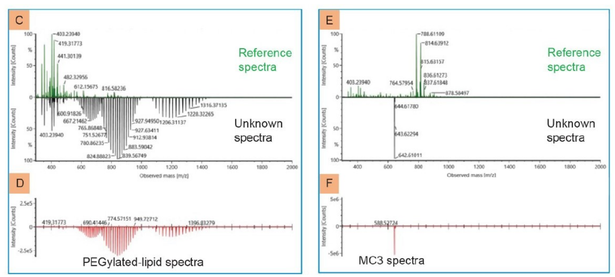


Figure 6: C. Combined spectra binary comparison of RT 2.02 min peak. D. Spectra difference between reference and unknown in C. E. Combined spectra binary comparison of RT 6.62 min peak.

CONCLUSION

- The BioAccord LC-MS system can be quickly adopted into the following workflows for LNPs:
  - Simultaneous single components ID and degradation
  - Process and raw material impurities
  - Process development and quality control
- For more information on details of this study please refer to the Waters application note: "Rapid Analysis of Lipid Nanoparticle Components Using BioAccord LC-MS System," 2021.

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