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Identification and Characterization of Impurities in Lipid Nanoparticle Components Using TOF-MS with Predictive In-silico Fragmentation and Automated Data Processing

Kellen DeLaney, Duanduan Han, Robert E. Birdsall, Ying Qing Yu, Ann Gray

Waters Corporation, Milford, MA

aaps National Biotechnology CONFERENCE

PURPOSE

The development of lipid nanoparticles (LNPs) as delivery vehicles has enabled the success of mRNA-based vaccines and other nucleic-acid based therapeutics. These lipid shells are comprised of four components that spontaneously self-assemble around genetic material when mixed at the correct ratios. Before an LNP formulation can be released into the market, impurities must be screened for and appropriately characterized to ensure both safety and efficacy of the released product. Regardless of whether these LNP components are synthesized in-house or procured from third-party vendors, rigorous analysis and screening of impurities are critical to ensuring products are of an acceptable quality and purity. Here, we show how the BioAccord™ System can be used with the integrated waters_connect™ Platform to facilitate the identification of impurities in LNPs.

OBJECTIVES

- Develop a streamlined workflow for the identification and routine monitoring of lipid impurities
- Identify and localize sites of modifications in impurity peaks

METHODS

All lipids in this study were used exclusively for research and demonstration purposes. Five lipid nanoparticle components were analyzed in this study, including cholesterol (CHO), distearoylphosphatidylcholine (DSPC), PEGylated lipid DMG-PEG 2000, and ionizable lipids SM-102 and Dlin-MC3-DMA. Stocks of each lipid were prepared in methanol at 1 mg/mL and diluted to the appropriate concentration at 90/10 methanol/water (v/v). Samples were separated with UPLC™ using an ACQUITY™ Premier CSH™ Phenyl-Hexyl column (1.7 µm, 2.1 mm × 50 mm) at 50°C over a 12-min gradient. MS acquisition was performed on a BioAccord System using Data Independent Acquisition (MS^E), using alternate high low collision energy switching. Data acquisition and analysis were performed in the waters_connect Platform with the integrated UNIFI™ Application.

RESULTS

This study used the waters_connect Platform to facilitate interpretation of MS data collected on the BioAccord System. In-silico predicted fragment ion matching was used to confirm the identification of known components (Figure 1) and impurity peaks (Figure 2), as well as enable localization of modifications on impurities (Figure 3). The platform was shown to be capable of analysis of low-abundance impurities down to 0.1% base peak (Figure 4).

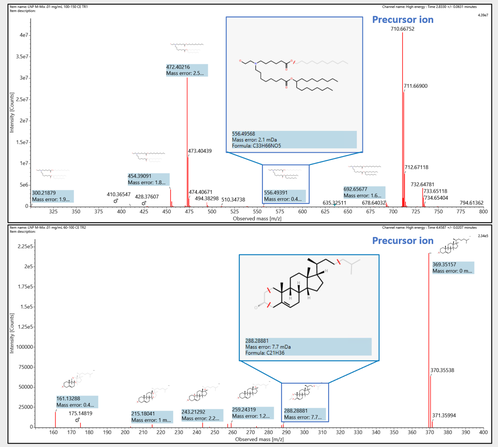

Figure 1. In-silico predicted structures were generated for fragment ions in the UNIFI App within the waters_connect Platform. By loading .mol structure files of each LNP component into the scientific library database, experimentally obtained fragment ions were able to be matched to theoretical structures to improve identification confidence, as shown for (top) SM-102 and (bottom) cholesterol.

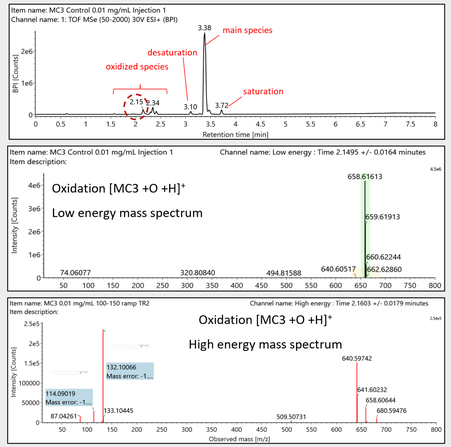

Figure 2. Accurate mass screening for possible modifications was followed by in-silico predicted fragment ion matching to identify impurity peaks in ionizable lipid samples, such as is shown here for Dlin-MC3-DMA. The top base peak ion chromatogram shows impurity peaks, and the middle and bottom spectra show the precursor and fragment ion MS spectra, respectively, for an oxidation peak.

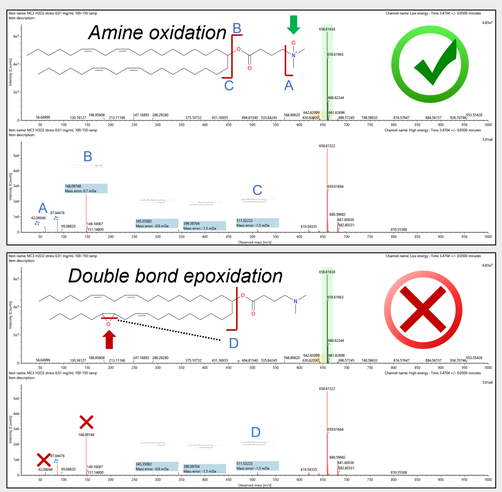

Figure 3. Oxidation can occur at multiple sites on the Dlin-MC3-DMA molecule. Here, two potential oxidation sites are compared using the in-silico predicted fragment ion matching. In the top structure, oxidation of the amine group, nearly all observed fragment ions can be matched to a predicted structure, including ions A, B, and C, which are indicative of the location of oxidation. In the bottom structure, double bond epoxidation, two of the most intense fragment ions cannot be matched to a predicted structure, and fragment ion D matches an unlikely structure. These results indicate that oxidation occurs on the amine group for this peak.

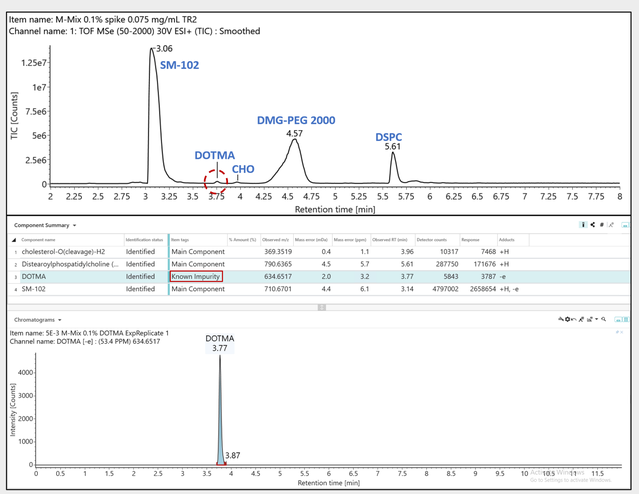

Figure 4. To evaluate the platform's capability for detected low-level impurities in an LNP component mixture, a mixture of 4 LNP components, SM-102, cholesterol, DMG-PEG 2000, and distearoylphosphatidylcholine (DSPC), were analyzed using the BioAccord System with another ionizable lipid, DOTMA, spiked in at 0.1% relative to SM-102. The top total ion chromatogram (TIC) shows the resulting peaks. As expected, the DOTMA peak's intensity is very low. However, it can still be reliably detected, as shown in the component summary (middle panel) where it has been labeled as a "known impurity" (red box), as well as in the extracted ion chromatogram (bottom panel). These results demonstrate that the platform can reliably detect low abundance impurities.

CONCLUSIONS

Rigorous analysis of impurities is necessary throughout the development and manufacturing processes to ensure the safety and efficacy of released LNP products. Accurate mass data of both precursor ions and fragment ions offers both sensitive detection and confident identification of unknown peaks in an LNP profile. This analysis workflow facilitates both accurate mass screening and interpretation of fragmentation data by taking advantage of instrument and informatics tools.

REFERENCES

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