Comprehensive characterization of the lipid nanoparticle (LNP), ALC-0315, and its impurities using electron-activated dissociation (EAD)

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ABSTRACT

Lipid nanoparticle (LNP) vehicles have been crucial to the success of the COVID-19 vaccine. It was shown that ionizable lipids influence mRNA stability through counter ion production that stimulates stable complex formation between the LNP and the mRNA. To ensure product quality, detailed and sensitive structural characterization of the ionizable lipid and its related impurities is necessary but also challenging with current liquid chromatography-mass spectrometry (LC-MS)-based methodologies using collision induced dissociation (CID). Here, the applicability of EAD for the characterization of the ionizable lipid used for LNP formulation, ALC-0315, was tested. Within a single experiment, low-abundant impurities, including oxidative species in which the location of oxygen incorporation (5.9%) and the transformation from hydroxyl to carboxyl group (0.2%), were identified in a stressed ALC-0315 sample.

INTRODUCTION

The use of LNPs as drug delivery devices has dramatically increased since the advent of the COVID-19 vaccine and the introduction of recent gene therapy therapeutics. Lipid impurities in the LNP can attenuate the effectiveness of the active pharmaceutical ingredient (API). For example, N-oxidation of ionizable lipids might lead to covalent modification of ribonucleotides and a loss of mRNA potency.[1] It is, therefore, necessary to ensure detailed and sensitive characterization of the ionizable lipid and its related impurities. However, the detailed characterization of LNPs is challenging using current CID-based LC-MS methodologies. Only EAD can be used to provide structurally diagnostic fragment ions to elucidate the distinctive structures of complex lipids.[2]

Here, [(4-hydroxybutyl)azanediyl]di(hexane-6,1-diyl) bis(2-hexyldecanoate), commonly known as ALC-0315, and its impurities at relative abundances as low as 0.19% were structurally characterized using EAD fragmentation on the ZenoTOF 7600 system. This lipid plays a crucial role in the function of LNPs by stabilizing the negatively charged mRNA, and its impurities might affect the therapeutic effectiveness of the LNP formulation. CID did not generate sufficient fragments to fully characterize ALC-0315 and impurities. In contrast, EAD provided abundant diagnostic fragments to allow near-complete structural elucidation of singly charged compounds.

MATERIALS AND METHODS

Chemicals and materials: ALC-0315 was provided by Acuitas Therapeutics Inc. Methanol (MeOH) was purchased from LiChrosolv (PN: 1.06035.2500). Ammonium acetate was from Fluka Analytical (PN: 73594-25G). Acetonitrile (ACN) was from Sigma-Aldrich (PN: 34851-4L).

Sample preparation: ALC-0315 standard suspended in PBS with 300mM sucrose was aliquoted into 5 mg per vial and subjected to 60ºC incubation for 3 days or 5 days for forced degradation.

The intact and degraded ALC-0315 stock solutions were diluted 100-fold with a solution of 60:40 (v/v), ACN/MeOH with 10mM ammonium acetate (mobile phase B). The resulting solution was further diluted 100-fold with a solution containing 15% water, 30% MeOH, and 55% ACN with 10mM ammonium acetate (mobile phase A). The solution was then analyzed directly by LC-MS/MS.

LC-MS/MS analysis: Samples were analyzed by an LC-MS/MS system equipped with a Waters H-class UPLC and a SCIEX ZenoTOF 7600 system. The samples were separated using a UHPLC Peptide BEH C18 column (2.1 × 150 mm, 1.7 µm, 130 Å, Waters, PN: 186003687). The gradient conditions are in reference 3. The flow rate was set to 0.3 mL/min, and the column temperature was set to 70ºC. The injection volume was set to 2 µL.

LC-MS/MS data were acquired using data-dependent acquisition (DDA) scan mode. The parameters used in these experiments can be found in reference 3.

Data processing: Structural elucidation was performed, and relative quantitation was determined using the Explorer module of SCIEX OS software.

RESULTS

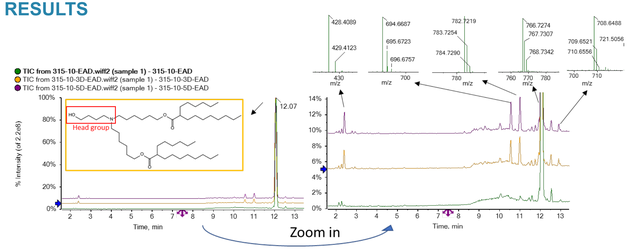

Figure 1. Total Ion Chromatogram (TIC) of ALC-0315 exposed to forced degradation. The structure of ALC-0315 is shown in the yellow inset square. Forced degradation of ALC-0315 was achieved by incubating the compound at 60° C for 3 or 5 days. The m/z values for 4 impurities are shown above the TIC. These degradation products included the loss of an acyl chain (m/z = 428.4087), the loss of the head group (m/z = 694.6690), oxidation (m/z = 782.7216) and the loss of an alkyl chain (m/z = 708.6495).

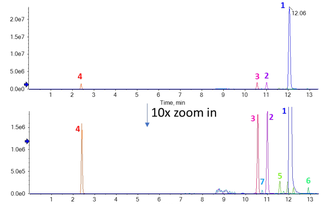

Figure 2. Extracted ion chromatogram (XIC) of intact ALC-0315 and 6 low-abundant impurities after 5 days of forced degradation. The top panel shows the normalized XIC of all compounds, in which ALC-0315 (peak 1) is the base peak of the chromatogram. The bottom panel shows the same chromatogram with a 10x zoom of the y-axis to better visualize the low-abundant impurities (peaks 2-7). Impurities were identified at relative intensities as low as 0.2%.

Table 1. The compounds identified by EAD in ALC-0315-5Day corresponds to the peaks in Figure 2.

Compound	Formula	Relative intensity%	Measured m/z	m/z error (ppm)
1. ALC-0315	$C_{48}H_{95}NO_5$	*100*	*766.7274*	*-1.83*
2. Oxidation at N	$C_{48}H_{95}NO_6$	*5.88*	*782.7217*	*-2.64*
3. Loss of head group	$C_{44}H_{87}NO_4$	*5.54*	*694.6692*	*-3.02*
4. Loss of acyl chain	$C_{28}H_{53}NO_3$	*3.87*	*428.4089*	*-3.41*
5. Loss of CH_2	$C_{47}H_{93}NO_5$	*0.75*	*752.7108*	*-3.19*
6. Loss of C_4H_8 and 2 H	$C_{44}H_{85}NO_5$	*0.34*	*708.6495*	*-2.39*
7. Hydroxyl to carboxyl group	$C_{48}H_{93}NO_6$	*0.19*	*780.7061*	*-2.58*

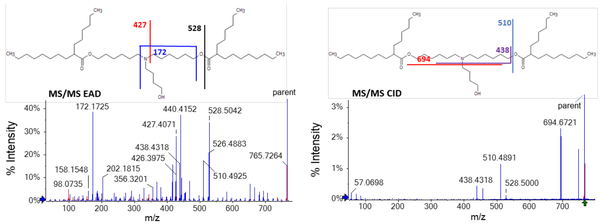

Figure 3. MS/MS spectra acquired for ALC-0315 (m/z = 766.7) using EAD (left) and CID (right). Blue peaks were assigned to a fragment of the structure. Red peaks were not assigned. More than 70% of the EAD-generated peaks were assigned to fragments. EAD generated many more assigned fragments than CID, including an important fragment from the head group of the molecule (m/z = 172) that was used for structural elucidation.

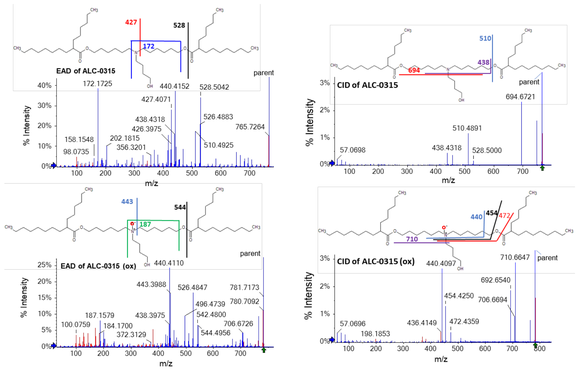

Figure 4. MS/MS spectra acquired for ALC-0315 (m/z = 766.7) and its oxidized impurity (m/z = 782.7) using EAD and CID. Blue peaks were assigned to a fragment of the structure. Red peaks were not assigned. More than 70% of the EAD-based fragment peaks were assigned for both spectra. Peaks at 544, 443 and 187 from EAD-derived MS/MS of oxidized ALC-0315 indicate N-oxidation compared to the peaks at 528, 427 and 172 from EAD-derived MS/MS of unoxidized ALC-0315. The peaks from CID are not sufficient to pinpoint the location of oxidation (right panels).

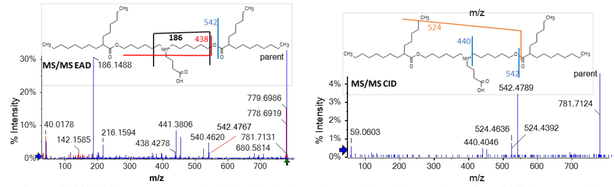

Figure 5. MS/MS spectra acquired for the hydroxyl-to-carboxyl impurity using EAD (top panel) and CID (bottom panel) (m/z = 780.7). Blue peaks were assigned to a fragment of the structure. Red peaks were not assigned. The peak of m/z = 438 indicates N is not oxidized. EAD generated a fragment containing the head group (peak of m/z = 186), indicating the oxidation of the hydroxyl moiety to a carboxylic acid functional group. CID was unable to distinguish the case of hydroxyl to carboxyl transformation or case of oxidation and desaturation.

CONCLUSIONS

- Untargeted analysis by mass spectrometry using EAD provided an in-depth structural characterization of singly charged, ionizable lipids and related impurities with a single LC-MS/MS run
- The ZenoTOF 7600 system provides improved risk assessment of formulated LNPs through explicit structural elucidation and localization of O incorporation into impurities derived from cationic lipids, such as ALC-0315
- The interscan dynamic range and quantitative sensitivity of the ZenoTOF 7600 system can decrease the risk of missing critical low-abundant impurities

REFERENCES

1. Jones JW, et al. Electron-induced dissociation (EID) for structure characterization of glycerophosphatidylcholine: determination of double-bond positions and localization of acyl chains. J Mass Spectrom. 2015 Dec;50(12):1327-39. PMID: 26634966

2. Baba T, et al. Structural identification of triacylglycerol isomers using electron impact excitation of ions from organics (EIEIO). J Lipid Res. 2016 Nov;57(11):2015-2027. PMID: 27457033

3. Structural characterization of the cationic lipid nanoparticle component, ALC-0315, and its impurities using electron-activated dissociation (EAD)-based MS/MS fragmentation. SCIEX technical note. MKT-26966-A.

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