Lipid Nanoparticle Impurity Monitoring Using Single Quadrupole Mass Detection for Regulated Environments

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Introduction

Lipid nanoparticles (LNPs) represent a novel solution to deliver gene-based therapeutics that is challenging to execute. LNPs are a multi-component biologic comprised of lipid species with varying properties impacting chromatography and detector response. New approaches are needed for characterization and monitoring to reduce the associated risks and ensure active pharmaceutical ingredients are delivered in a safe and efficacious manner. We present an LC-based optical/MS dual-detector platform with improved sensitivity/diagnostic power for LNP workflows. A single quadrupole mass detector provides complementary mass data for raw material impurity screening, MS spectral library-based lipid confirmation, and stability monitoring. Supported by compliant-ready informatics, this workflow is easy to deploy in both non-regulated and regulated environments alike for efficient method development and migration.

Lipid Nanoparticles

Composition

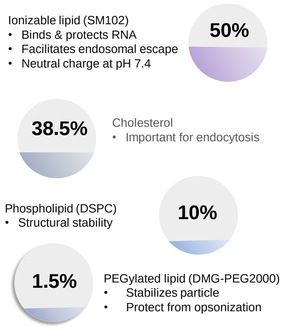

Figure 1. Four lipid components of LNP. Cholesterol, DSPC and DMG-PEG2000 provide structural stability and are typically outsourced. The ionizable lipids are often proprietary and produced in-house to protect intellectual property.

Detection

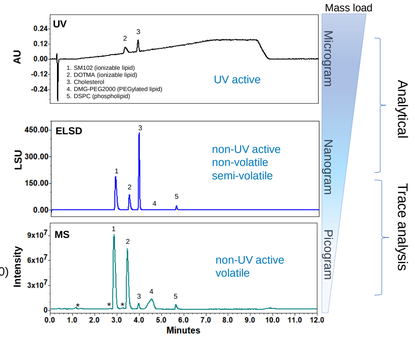

Figure 2. Detector type and response. Detector response is shown across 3 detector types for a panel of lipids representative of components used in the production of lipid nanoparticles. Peaks 1-5 are SM102, DOTMA, cholesterol, DMG-PEG2000, and DSPC. SM102, cholesterol, DMG-PEG200, and DSPC were prepared in a molar ratio of 50:38.5:1.5:10 representative of a formulated LNP sample. DOTMA was spiked-in at half the concentration relative to SM102.

Methods

LC: ACQUITY™ UPLC™ Premier System
Sample(s):
- Cholesterol (CHO)
- Distearoylphosphatidylcholine (DSPC)
- PEGylated lipid DMG-PEG 2000*
- SM-102 (ionizable lipid)
- Dlin-MC3-DMA (ionizable lipid)

0.1 mg/mL in 10:90 water:methanol (v/v)
*0.01 mg/mL for DMG-PEG2000

Injection volume: 3 µL
Sample temp.: ambient
Column: ACQUITY Premier CSH™ Phenyl-Hexyl Column 1.7 µm, 2.1 mm X 50 mm (p/n:186009474)
Column temp.: 50 ˚C
MS: ACQUITY QDa™ Mass Detector
Scan range: 150-840 m/z @ 5Hz
Capillary voltage: 1.5 kV, cone voltage: 15 V, probe temp.: 600 ˚C

MP A: H_2O, 0.4% formic acid v/v (MS-grade)
MP B: MeCN, 0.6% formic acid v/v (MS-grade)

Gradient:

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.4	40	60	Initial
6.0	0.4	10	90	6
8.0	0.4	10	90	6
8.5	0.4	40	60	6
12.0	0.4	40	60	6

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Results

Enhanced analysis capabilities with complementary mass data

Increased sensitivity

Concentration (mg/mL)	Mass load	Area ELSD	Area QDa Detector XIC	Area QDa Detector SIR
0.4	1200 ng	1987600		
0.3	900 ng	1297792		
0.2	600 ng	688005		
0.1	300 ng	249497		
0.08	240 ng	176789		
0.06	180 ng	113036		
0.05	150 ng	78060	562333317	846393213
0.04	120 ng	57763	472562227	747932620
0.03	90 ng	36662	410236965	658197917
0.02	60 ng	15683	318308125	549289939
0.01	30 ng	5772	210593141	370460055
0.005	15 ng	1556	116377502	205457099
0.001	3 ng		34254753	52498772
0.0005	1.5 ng		16775156	26897524
0.0001	300 pg		4290533	6276022
0.00005	150 pg		1852079	3090689
0.00001	30 pg		383648	599480
0.000005	15 pg		159956	266054
0.0000001	3 pg		39766	55138
0.0000005	1.5 pg			24379
Linear response		R²=0.998	R²=0.999	R²=0.999

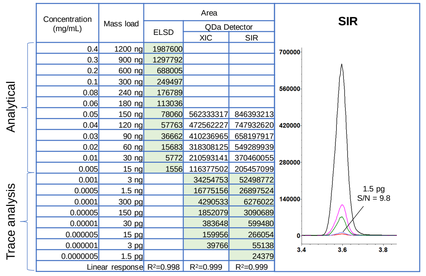


Figure 3. ELSD meets the analytical needs of labs, detecting lipid mass loads as low as 15 ng. Selected ion recording (SIR)drives down LOD to 1.5 pg and extends LOD by 4 orders which is suitable to assess trace impurities. Green shaded concentrations represent the dynamic range. Sample: ionizable lipid DOTMA.

Drug formulation and stability

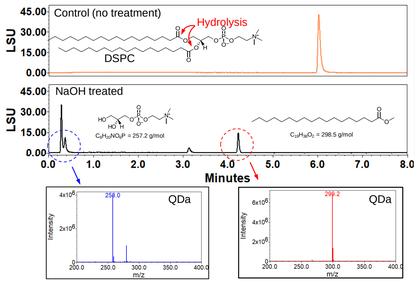
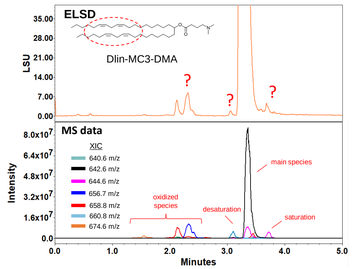


Figure 4. Labile bonds such as esters are subject to degradation. An example of which is shown here for the hydrolysis of DSPC under high pH. MS provides mass data for tentative assignment of degradant peaks as the methylated fatty acid methyl stearate (299.2 m/z) and the polar head group (258.0 m/z).

Product / process related impurities



Compound	m/z	Proposed cause
[MC3 -2H +H]⁺	640.6	Desaturation
[MC3 +H]⁺	642.6	Main peak
[MC3 +2H +H]⁺	644.6	Saturation
[MC3 -2H +O +H]⁺	656.7	Desaturation/Oxidation
[MC3 +O +H]⁺	658.8	Oxidation
[MC3 +2H +O +H]⁺	660.8	Saturation/Oxidation
[MC3 +2O +H]⁺	674.6	Saturation/Double oxidation

Figure 5. Process/product related impurities. For more complex profiles, ELSD alone may not be as easily interpreted as impurities may represent an individual or set of peaks, as well as the quality of the raw material. Using extracted ion chromatograms from the MS data we can putatively assign which peaks are associated with oxidation events versus desaturation or saturation events related to the MC3 ionizable lipid. Confirmation of which can be made from fragmentation patterns using HRMS platforms.

Product consistency

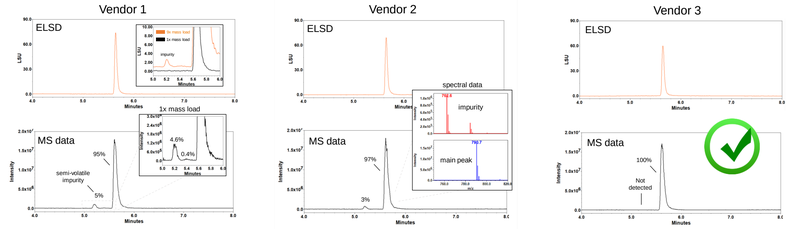
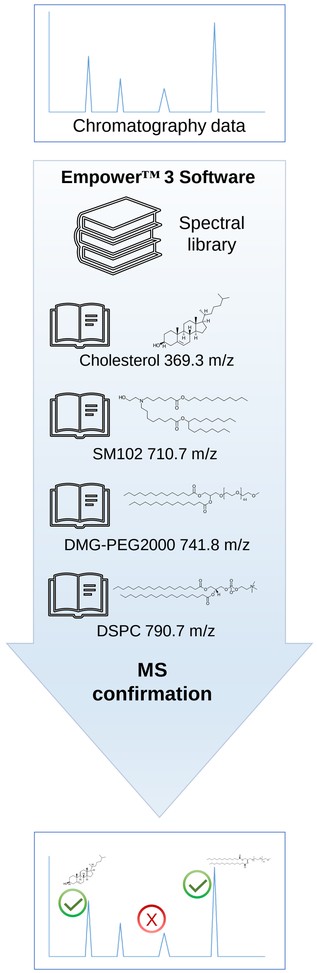


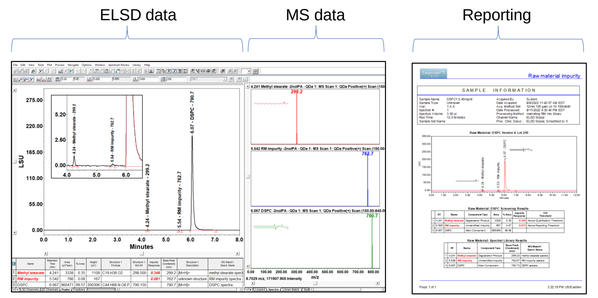
Figure 6. MS data reveal volatile impurities (up to 5% peak area) which otherwise would have gone undetected in the ELSD, requiring 9 times the mass load to be detected. Sample: DSPC, typically outsourced by LNP manufacturers.

Leverage MS to reduce risks in process development and manufacturing workflows

a) ELSD/MS workflow



b) Raw material screening



c) Compositional analysis

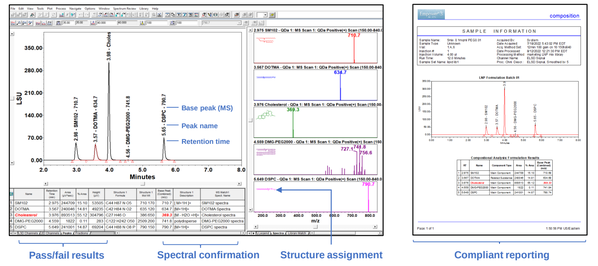


Figure 7. Complementary mass data reduces error in process development and manufacturing workflows through spectral confirmation. a) Spectral libraries based on retention time and mass can be utilized to confirm peak identity in raw material screening and compositional analysis. b) To demonstrate this, ELSD and mass data were acquired in the same acquisition run for the DSPC lipid spiked with methyl stearate to act as a low-level impurity present in a raw material shipment. In this example, we can view both the ELSD and QDa information from the results window with pertinent peak information. The table shows best match (and structural information if available) found in the library for the spectra associated with each peak seen in the ELSD chromatogram. c) This workflow is also applicable in a compositional analysis setting that may occur in formulation or release assays as part of QA/QC testing.

Conclusion

- Multi-detector array for efficient development and manufacturing
- MS-support for increased sensitivity and complementary mass data
- Spectral library support for identification and structure assignment
- Compliant-ready software solution to facilitate easy method migration