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Application Note

Lipid Nanoparticle Analysis: Leveraging MS to Reduce Risk

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Abstract

Lipid nanoparticles (LNPs) represent an elegant solution in the delivery of gene-based therapeutics that can be challenging to execute. As a multi-component biologic comprised of lipid species with varying properties that impact chromatography and detector response, new approaches are needed in their characterization and monitoring. This application note proposes a liquid chromatography (LC)-based optical/mass-spectrometry (MS) dual-detector platform with improved sensitivity and diagnostic power for lipid nanoparticle workflows. In this workflow, the ACQUITY™ QDa™ Mass Detector provides complementary mass data for raw material impurity screening, MS spectral library-based lipid component confirmation, and stability monitoring. As an Empower™ Software-based method, the proposed workflow is easy to deploy in both non-regulated and regulated environments alike for efficient development and migration of lipid nanoparticle workflows.

Benefits

- MS offers lower limits of detection and increased diagnostic power for impurity analyses
- Complementary mass data offers orthogonal detection and confirmation of lipid species
- Empower Software-based workflows ensure compliance and straightforward method migration

Introduction

Gene or nucleic acid-based therapeutics and vaccines represent the fastest growing segment of the pharmaceutical industry. As new modalities, they have proven to be highly effective in the treatment of disease and, more recently, as a vaccine for COVID-19.[1] A unique aspect of these modalities is that they need to be delivered into targeted cells via a "delivery vehicle" in order to work. LNPs represent one of the approaches for targeted delivery of the nucleic acid payload, which is the Active Pharmaceutical Ingredient (API).[2]

The concept of an LNP as a "delivery vehicle" is simple in theory; however, their execution is rather complex. As a multi-component delivery vehicle, the LNP relies on four lipid components, each with a specific function and requiring a specific ratio to deliver the API payload effectively and safely. Three of these four components account for 50% of the LNP composition. These include cholesterol (~38.5%) for particle fluidity, a helper lipid (~10%), typically a phospholipid, for particle structure, and a pegylated lipid (~1.5%) that adds stability and hydrophilicity to the nanoparticle. These components, which are common and serve a structure and stability role, are often outsourced as a raw material from third-party vendors. The other half of the LNP is comprised of cationic lipids or ionizable lipids. In addition to stability and structure, these lipids are the primary driver of potency and help bind and protect the API payload. Due to their role and specificity towards the disease being treated, the ionizable lipids are often proprietary and produced in-house to protect intellectual property. In either case, whether the components are outsourced or manufactured in-house, analytical techniques are needed to ensure these drug products are safe and efficacious.[3]

LC-based methods are frequently used as the principal platform in the analysis of LNPs. MS-based methods tend to be deployed in upstream activity for characterization and identification purposes, whereas optical-based methods using detectors such as evaporative light scattering detectors (ELSD) are deployed in late-stage development or manufacturing environments as part of monitoring or screening assays. However, as evident from the data shown in Figure 1, even the detector of choice is nuanced as the components used in lipid nanoparticles do not respond equally across detector types. This can present challenges as the limit of detection (LOD) can impede the quantitation of low-abundance species and/or impurities. The purpose of this study is to demonstrate how MS, when configured in a dual-detector workflow, can be used to improve the analysis of LNPs to mitigate risk associated with the production and manufacturing of these new modalities. The workflow will leverage the Empower Chromatography Data System (CDS) and ACQUITY QDa Mass Detector to facilitate easier deployment in downstream labs or manufacturing environments.

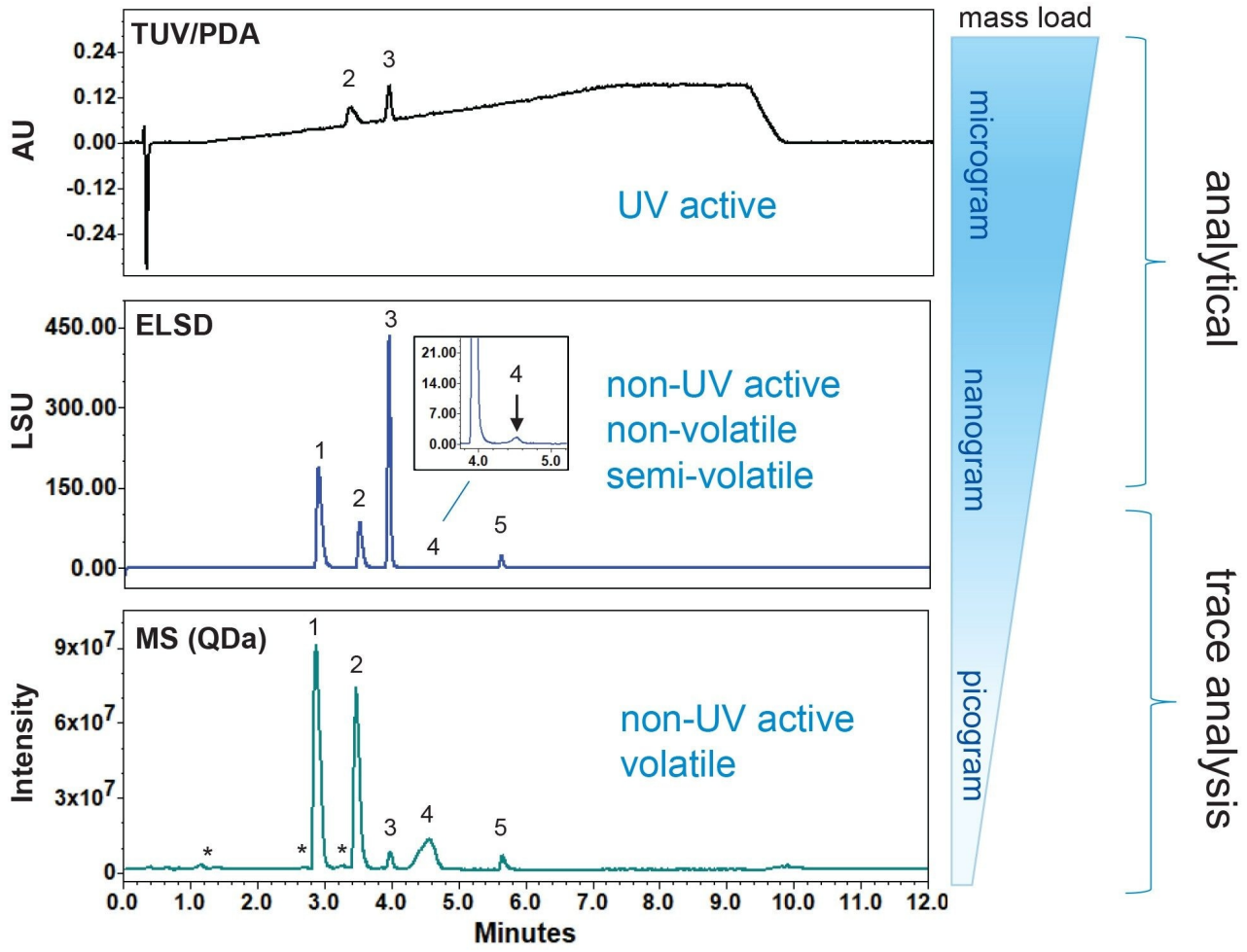


Figure 1. Detector Type and Response. Detector response is shown across 3 detector types for a panel of lipids representative of components used in the production of lipid nanoparticles. Peaks 1-5 are SM102, DOTMA, cholesterol, DMG-PEG2000, and DSPC. SM102, cholesterol, DMG-PEG200, and DSPC were prepared 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.

Experimental

Cholesterol, Dlin-MC3-DMA, PEGDMG-2000, SM-102, DSPC, and DOTMA were purchased from commercially available sources. Comparative studies were conducted using samples from different vendors. All lipids purchased were used for research and demonstration purposes only.

Lipid stocks were prepared in methanol at 1 mg/mL. Working samples were diluted to different concentrations using water and methanol. The final composition was 10:90 water:methanol. PDA, ELSD, and QDa were serviced prior to the start of experiments to ensure detectors were operating within manufacturer specification.

LC System Conditions

LC system:	ACQUITY™ Premier™ System (BSM-variant)
Detection:	ACQUITY™ PDA, FC=Ti 5 mm, λ range=190–400 nm
Vials:	TruView™ Max Recovery Vials, (p/n: 186005662CV)
Vial caps:	Polyethylene Septumless Screw Cap, (p/n: 186004169)
Column(s):	ACQUITY Premier CSH™ Phenyl-Hexyl Column, 1.7 µm, 2.1 mm X 50 mm, (p/n: 186009474)
Column temperature:	50 °C
Sample temperature:	Ambient
Injection volume:	3 µL
Flow rate:	0.400 mL/min
Mobile phase A:	H_2O, 0.4% FA v/v (MS-grade)

Mobile phase B:	25:75 IPA:MeCN, 0.6% FA v/v (MS-grade)

ELSD Settings

Gain:	100
Sample rate:	5 Hz
Nebulizer mode:	Heating
Power level:	80%
Drift tube temperature:	48.0 °C
Gas pressure:	20.0 psi (1.64 slpm)

QDa Settings

Ionization mode:	ESI+
Acquisition mode:	Full Scan
Acquisition range:	High (150–840 *m/z*)
Scan rate:	5 Hz
Capillary voltage:	1.5 kV
Cone voltage:	15 V
Probe temperature:	600 °C

Chromatography software:	Empower 3, FR4

Gradient Table

Time	Flow (mL/min)	%A	%B	Curve
Initial	0.400	40.0	60.0	Initial
6.00	0.400	10.0	90.0	6
8.00	0.400	10.0	90.0	6
8.50	0.400	40.0	60.0	6
12.00	0.400	40.0	60.0	6

Results and Discussion

One of the benefits of adding MS to lipid nanoparticle assays is the increased sensitivity and specificity afforded by MS detection. While the ELSD is well suited in the detection of non-volatile/semi-volatile analytes and is capable of meeting most analytical needs with a modest dynamic range and ability to detect low nanogram amounts of sample, the ACQUITY QDa Mass Detector increases detection limits by multiple orders of magnitude, providing users with the ability to detect significantly lower amounts of sample. In addition, users have access to orthogonal spectral data as well that can be utilized to discern differences in samples and profiles. Some key examples of how complementary mass data can be utilized in LNP workflows are discussed below.

Raw Material Screening

As shown in Figure 2, having access to mass data can make a critical difference when assessing raw material in the production and manufacturing of LNPs. In this example, one of the common component lipids (DSPC) was outsourced from three different commercial vendors and analyzed with both ELS and mass detection using the same method. In this example, the ELSD chromatograms were observed to be relatively free of extraneous peaks, yet the same samples showed varying levels of a semi-volatile impurity preceding the main peak when using the

ACQUITY QDa Mass Detector. This would have otherwise gone undetected in the ELSD-only workflow, which required a 9-fold increase in mass load (0.3 µg vs. 2.7 µg) to detect the main impurity (Figure 2A, top inset). In addition to detecting the low-abundance species, the ACQUITY QDa-enabled workflow provided spectral information that was utilized to assess the presence of multiple impurities. As shown in the bottom inset of Figure 2A, three impurities were discovered representing a total peak area of 6.4%. In this case, the number of impurities present was only discernable due the ability to utilize extracted ion chromatograms (XICs) within the mass data to reveal a partial co-elution at 5.4 minutes. Samples from vendors 2 and 3 were assessed in a similar fashion where vendor 3 was found to have no detectable impurities in both the ELSD and mass data. This example demonstrates how complementary mass data can be utilized to identify suitable raw material to use in the production and manufacturing of LNPs.

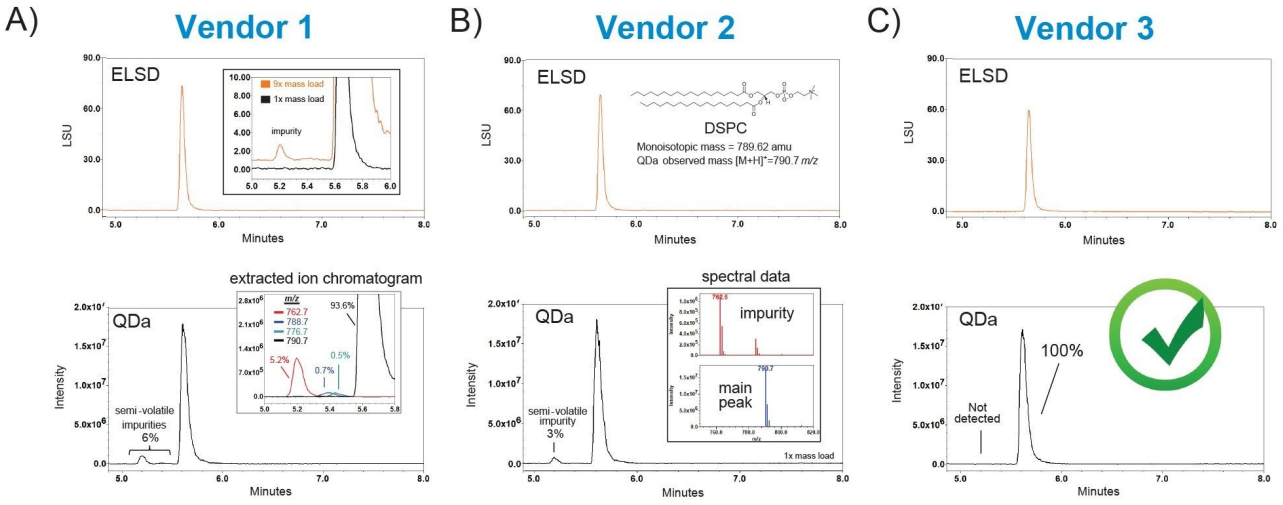


Figure 2. Raw Material Screening. Analysis of the lipid nanoparticle component DSPC using ELS and MS detection is shown from 3 different vendors. Semi-volatile Impurities were assessed using extracted ion chromatograms from MS data with A) 6%, B) 3%, and C) 0% total impurities observed for vendors 1–3 respectively.

Process Development

The ability to interrogate samples at a spectral level provides users the means to understand and adapt development and manufacturing processes more efficiently. An example is shown in Figure 3 for a research-grade sample of Dlin-MC3-DMA which is the ionizable lipid used in patisiran, a therapeutic used in the treatment

of hereditary transthyretin-mediated amyloidosis. Lipids that contain amines and/or unsaturated fatty acids can be subject to oxidation as well as desaturation/saturation events as part of process/product related impurities. In these instances, the optical-based chromatogram may not be as easily interpreted, as each event can generate a unique peak or set of peaks depending on the impurities present (Figure 3A). Using extracted ion chromatograms from the MS data, we can tentatively assign possible pathways associated with the impurity profile (Figure 3B) to guide impurity identification using high resolution MS platforms. Considering many of the ionizable lipids are manufactured in-house, this example demonstrates how complementary mass information can be utilized to improve process consistency from batch to batch.

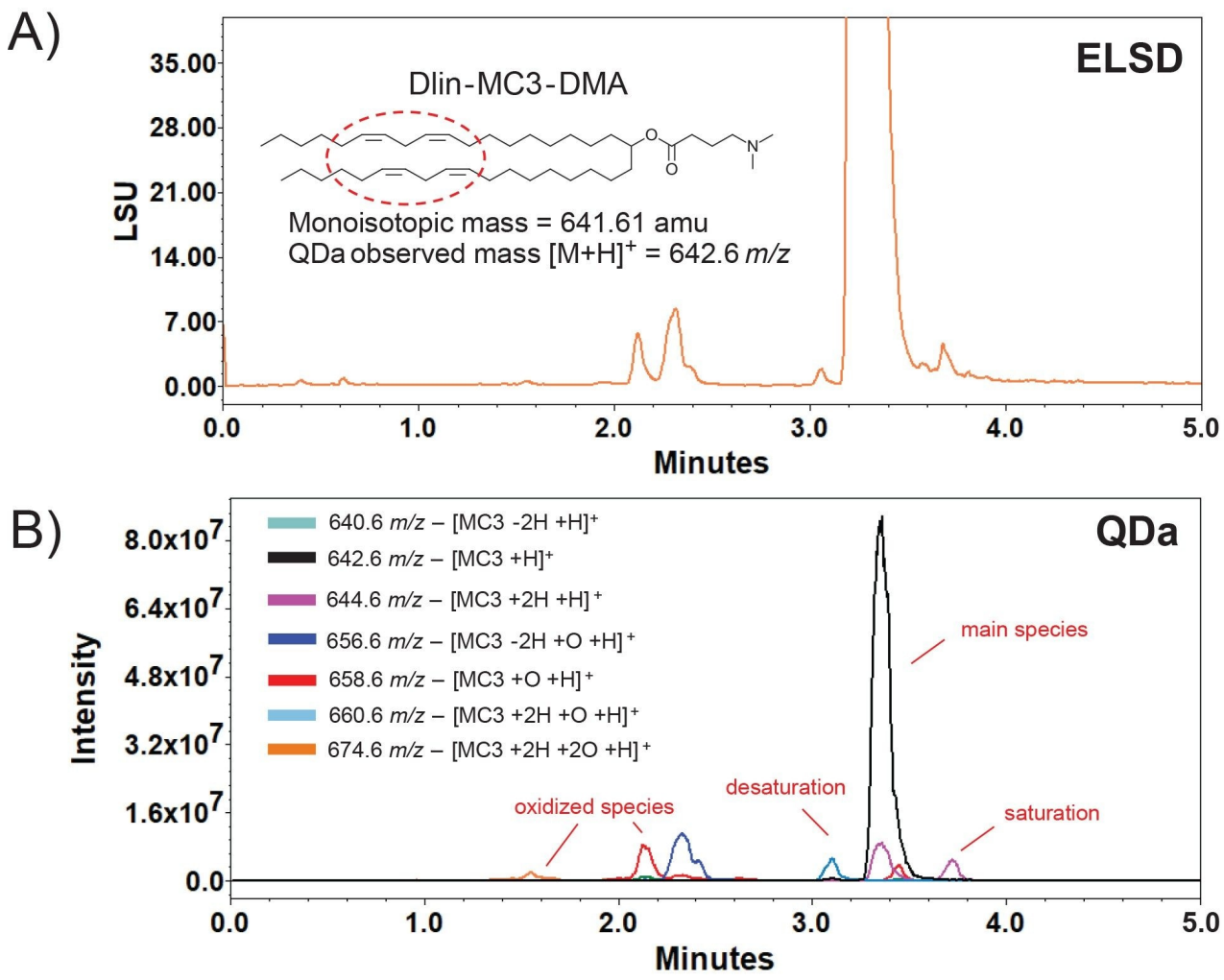


Figure 3. Process Development. Analysis of the ionizable lipid Dlin-MC3-DMA using A) ELS and B) MS detection is shown. Calculated mass based on saturation, desaturation, and oxidation events of the main component were used for extracted ion chromatograms to identify peaks of interest. Note: Mobile phase B was prepared as MeCN, 0.4% FA v/v to enhance spectral response.

Formulation and Stability studies

In another aspect, complementary mass data was explored as part of formulation and stability studies. In addition to oxidation and saturation/desaturation, components of the lipid nanoparticle can be subject to hydrolysis events which can impact the stability and safety of lipid nanoparticle therapies. To demonstrate this, a forced degradation study of the phospholipid DSPC was performed. As shown in the ELSD chromatograms of

Figure 4, when exposed to a 0.01N solution of NaOH, the main DSPC peak degrades into multiple peaks. From this data alone, it can be concluded that DSPC is sensitive to pH. However, when looking at the accompanying mass spectrum acquired with the ACQUITY QDa Mass Detector, we can tentatively assign the peaks at 0.3 minutes and 4.2 minutes as the polar head group (258.0 *m/z*) and methylated fatty acid methyl stearate (299.2 *m/z*), respectively. This information tentatively confirms that the ester linkage of DSPC is susceptible to hydrolysis which can provide more guidance in determining stable matrices for formulated products.

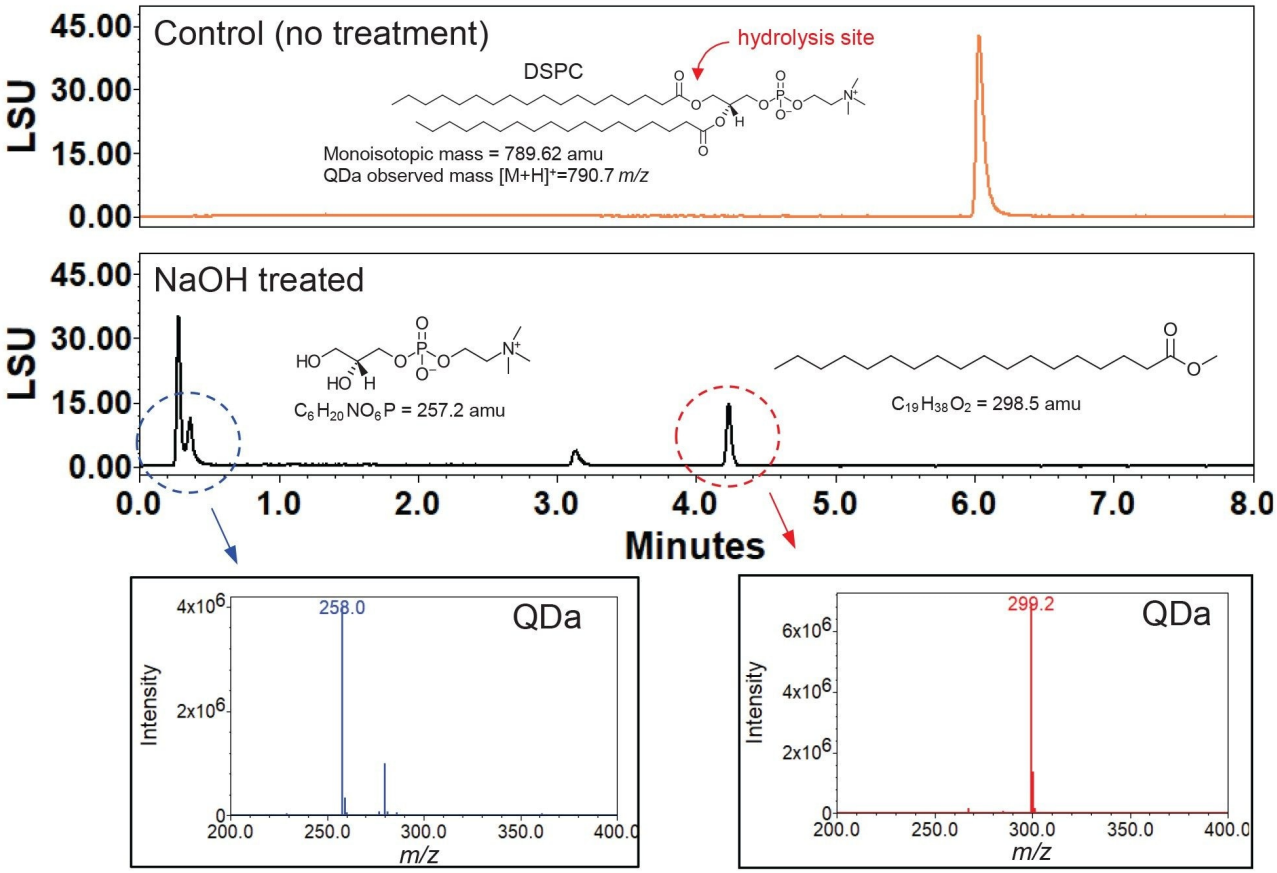


Figure 4. Formulation and Stability Studies. Forced degradation study of the lipid nanoparticle component DSPC using a 0.01N solution of NaOH. ELSD data and corresponding MS spectral information acquired with the QDa is shown for peaks of interest representing putative degradation species. Note: Mobile phase B was prepared as MeCN, 0.4% FA v/v to enhance spectral response.

Managing MS Data

With the value of mass data as an investigative tool to help better understand LNP components and their associated impurities established, the question remains as to how to incorporate mass information in a practical way with existing workflows to help mitigate risk in the development and manufacturing of lipid nanoparticles. As part of its design, the ACQUITY QDa Mass Detector is fully integrated with Empower 2 (FR5) and Empower 3 CDS Software. This integration allows for easy migration of methods to downstream labs for broader deployment, as well as allowing users to take advantage of MS-based features within the Empower CDS. These features include access to spectral libraries to manage and catalog spectral information as well as the "Impurity" and "Limits" processing workflows, which enable users to define acceptance thresholds for results based on regulatory guidance and/or validation studies.

Spectral Library Support

An example of the spectral library functionality within Empower is shown in Figure 5. In this example, the ELSD and ACQUITY QDa Mass Detector were configured in a dual-detector workflow where the eluent from the column was split between the detectors. When acquired in this manner, spectral information is automatically linked to the optical data, allowing users access to spectral data as a means for orthogonal confirmation of LNP species or identification of impurities. In this case, a spectral library was created as part of a raw material screening library for the phospholipid DSPC. When selected within the processing method (Figure 5A), spectral information associated with the ELSD results are cross-referenced against the library for matching. The best "spectral match" for each peak is shown in the table results for the processed data (Figure 5B). Tabular results that exceed defined impurity thresholds based on regulatory guidance (e.g. NMT 0.1% by Area%) are automatically color-coded red to notify users of results that are outside specification. In this way, the proposed Empower-based dual detector workflow allows users to quickly assess impurities from LNP samples using the integrated impurity processing workflow while taking advantage of spectral information to confirm peak identity as well to potentially identify new impurities based on spectra stored in the spectral library.

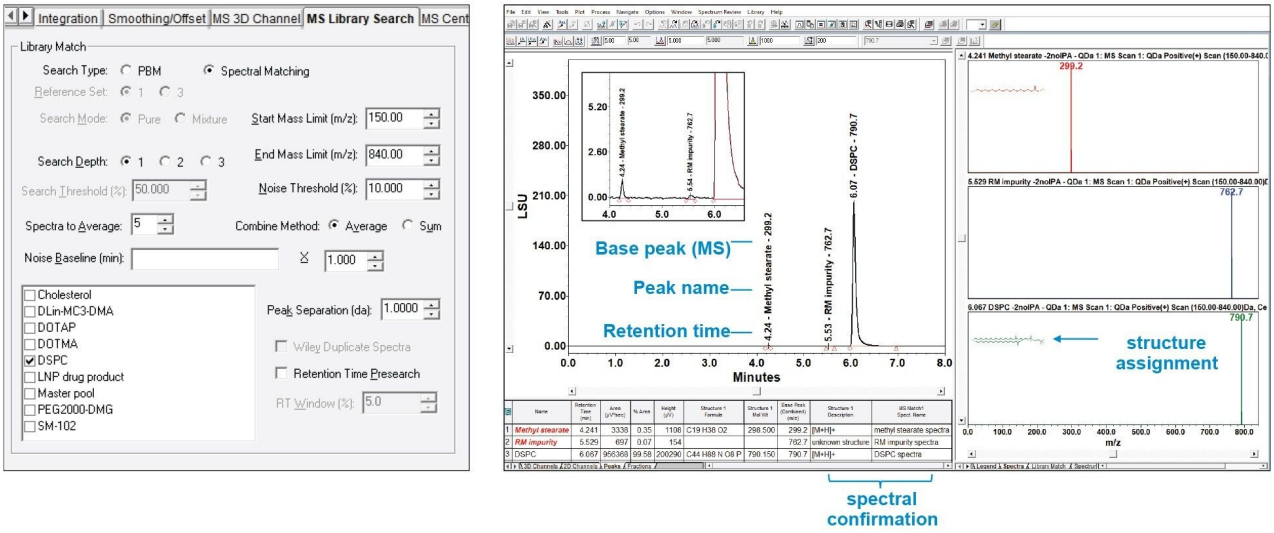


Figure 5. Spectral Library Support. A) An example of the Spectral Library Search feature of an Empower processing method and how to define parameters to perform a spectral search of MS data. B) ELSD and corresponding spectral results for an analysis of raw material impurities present in the lipid nanoparticle component DSPC. The "best" spectral match is displayed in the right two columns of the results table. Results in red indicate impurities that exceeded ICH impurity thresholds (Reporting: 0.05, Identification: 0.10, Qualification: 0.15) for % Area as per criteria set in the impurities tab of the Empower processing method.

Release Assays

As methods migrate downstream, users may need to access mass data to define acceptance thresholds as part of method validation. In addition to the Impurity processing workflow, Empower offers users the ability to define thresholds for different field types. An example of this is shown in Figure 6 for a panel of lipids representing a formulated drug substance. As shown in Figure 6A, an acceptance criteria of ±1 *m/z* was used as a mass tolerance threshold for each lipid based on their $[M+1H]^+$ *m/z* value. In addition to this criterion, the results were processed with a "master pool" spectral library with "Retention Time Presearch" enabled for increased specificity. As shown in the processed results of Figure 6B, all components of the LNP mixture were identified correctly based on RT as well as spectra. However, cholesterol was color-coded red, indicating the associated mass information did not match the acceptance criteria defined within the "Limits" processing parameters (369.3 *m/z* vs. 387.6±1 *m/z*). In this case the difference (Δ 18.3 *m/z*) is within 0.3 Da of a loss of water which could have occurred during the ionization process. Similar to previous examples, this example demonstrates how mass

information can be used in a dual detector workflow to expedite data review and increase confidence in results as methods migrate to downstream labs. Furthermore, as shown in Figure 7, users can take advantage of the comprehensive reporting capability within Empower to customize and format results using both optical and spectral data for efficient development and migration of methods as part of lipid nanoparticle workflows.

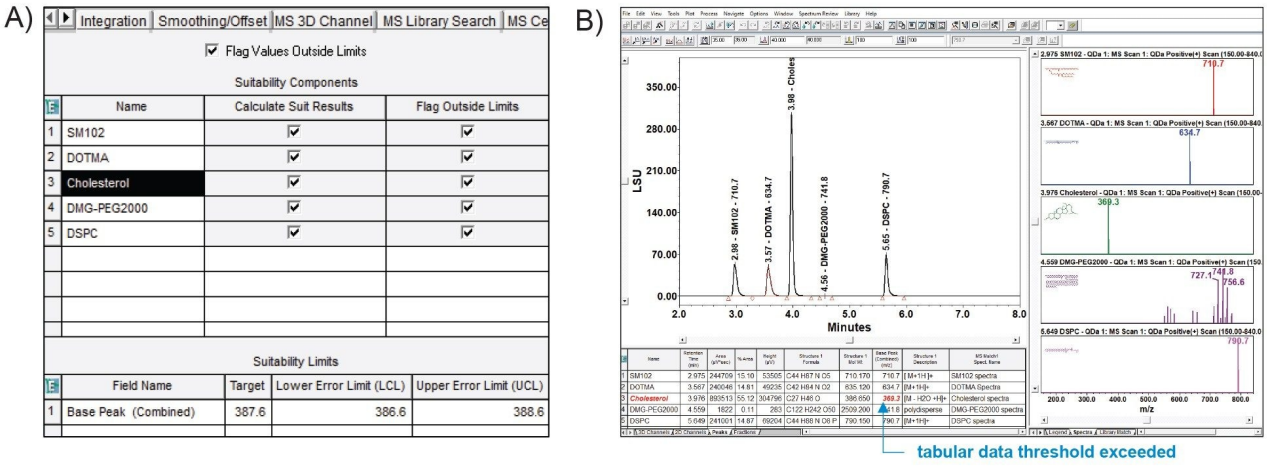


Figure 6. Release Assays. A) An example setting thresholds within an Empower processing method and how to define a lower and upper error limit for a specified field. B) ELS data and corresponding spectral results for an analysis using a sample comprised of a panel of lipids commonly used in the production of lipid nanoparticles. Results in red indicate components that exceeded the mass tolerance (±1 m/z) defined in the processing method "Limits" window. Note: System suitability license required to access "Limits" as a processing option.

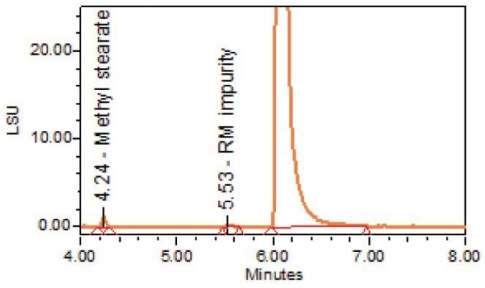
Empower 3

Leveraging MS to reduce risk

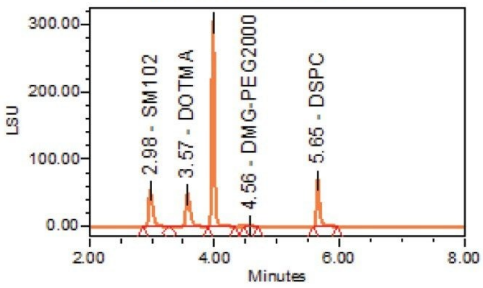
SAMPLE INFORMATION

Sample Name:	DSPC1 0.45mgml	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	7/18/2022 5:43:02 PM EDT,
Vial:	1:A, 6	Acq. Method Set:	12min 100 gain cn 10 150to840
Injection #:	1	Date Processed:	8/11/2022 4:30:40 PM EDT,
Injection Volume:	4.00, 3.00 ul	Processing Method	marketing RM mix library,
Run Time:	12.0 Minutes	Channel Name:	ELSD Signal
Sample Set Name:	lipid lib1	Proc. Chnl. Descr.:	ELSD Signal, Smoothed by 5

raw material screening



compositional analysis



LNP:Raw Material Screening Results

	RT	Name	Component Type	Area	% Area	Impurity Response	ICH Threshold
1	4.241	*Methyl stearate*	Degradation Product	3338	0.35	*0.348*	Above Qualification Threshold
2	5.529	*RM impurity*	Unidentified Impurity	697	0.07	*0.073*	Above Reporting Threshold
3	6.067	DSPC	Main Component	956368	99.58		

LNP: Composition Analysis Results

	RT	Component Type	Name	Area (µV*sec)	% Area	Base Peak (Combined) (m/z)
1	2.975	Main Component	SM102	244709	15.10	710.68
2	3.567	Related Substance	DOTMA	240046	14.81	634.68
3	3.976	Main Component	*Cholesterol*	893513	55.12	*369.33*
4	4.559	Main Component	DMG-PEG2000	1822	0.11	741.84
5	5.649	Main Component	DSPC	241001	14.87	790.74

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Figure 7. Reporting. An example of an Empower report is shown for an analysis of lipid nanoparticles using dual

ELS and mass detection. Tabular results that exceeded thresholds or limits defined in the processing method are automatically annotated in red.

Conclusion

Gene-based therapies represent an exciting new chapter in pharmaceutical development and hold great promise for the treatment and prevention of disease. Lipid nanoparticles as "delivery vehicles" are part of the final drug product and reflect the added complexity and analytical challenges associated with these new modalities. This study demonstrated how complementary mass data acquired with the ACQUITY QDa Mass Detector can be leveraged in a dual-detector configuration to increase the analytical sensitivity and diagnostic power of LNP workflows. As an Empower-based method, the proposed workflow is easy to deploy in both non-regulated and regulated environments alike, facilitating efficient method development and migration of methods in the development and manufacturing of lipid nanoparticle therapies and vaccines.

References

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