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Optimization of Lipid Nanoparticle Analysis Across Universal Detectors

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Introduction

Lipid nanoparticles (LNPs) are a drug delivery system for nucleic acids which target cancers and other diseases. Most recently, LNPs have become a critical component in mRNA vaccines. LNPs consist of four components that encapsulate a payload for delivery into target cells: phospholipid (allows the LNP to fuse to the target cell), ionizable lipid (encapsulates the nucleic acids), PEG-lipid and cholesterol (contributes to the overall stability of the LNP). These components must be present in specific ratios to properly control potency and efficacy of an LNP.

Traditional UV-based methods of quantitation are not suitable for LNPs, because the lipid components lack necessary chromophores. For this reason, universal detection methods are commonly used instead, such as evaporative light scattering detection (ELSD) and charged aerosol detection (CAD). In this study, a representative LNP formulation based on typical mRNA vaccines consisting of cholesterol, DSPC, DSPE-PEG 2000, and SM-102 was examined. An LC method was developed and optimized using ELSD and CAD detectors with an ACQUITY™ Premier UPLC™ System. Differences in sensitivity (LOD/LOQ), linearity, and accuracy are assessed.

Method

Standards were prepared at nine levels from 5 to 500 µg/g. They were composed of cholesterol, DSPC, DSPE-PEG 2000, and SM-102 in equal concentrations. Representative samples at the typical concentration ratio of 50: 38.5: 10: 1.5 SM-102: cholesterol: DSPC: DSPE-PEG 2000 were created at LOQ levels for DSPE-PEG 2000 on the two detectors. The final concentrations for the sample on the CAD system were 167 µg/g SM-102, 128 µg/g cholesterol, 33 µg/g DSPC, and 5 µg/g DSPE-PEG 2000. The concentrations on the ELSD sample were 500 µg/g SM-102, 385 µg/g cholesterol, 100 µg/g DSPC, and 15 µg/g DSPE-PEG 2000.

Method Conditions	
System	ACQUITY Premier UPLC System
Detection	ELSD and CAD
Column	CORTECS® Phenyl Column, 90Å, 1.6 µm, 2.1 x 100 mm (p/n 186008381)
Mobile Phase A	10 mM Ammonium Acetate in 90/10 Methanol/Water
Mobile Phase B	10 mM Ammonium Acetate in 90/10 Acetonitrile/Water
Needle Wash	50/50 Water/Acetonitrile
Flow Rate	0.400 mL/min
Inj. Volume	5 µL
Sample Temp.	12 °C
Column Temp.	30 °C

Time	%A	%B	Curve
Initial	100.0	0.0	Initial
3	85.0	15.0	6
5	85.0	15.0	6
5.01	40.0	60.0	6
7	40.0	60.0	6
7.01	0.0	100.0	6
9	0.0	100.0	6
9.01	100.0	0.0	6
12	100.0	0.0	6

CAD Detection	
Power Function Value	1
Evaporator Temp.	40 °C
Data Rate	2 Hz

ELS Detection	
Nebulizer Mode	Cooling
Drift Tube Temp.	50 °C
Gas Pressure	40 psi
Gain	125
Data Rate	2 Hz

Results and Discussion

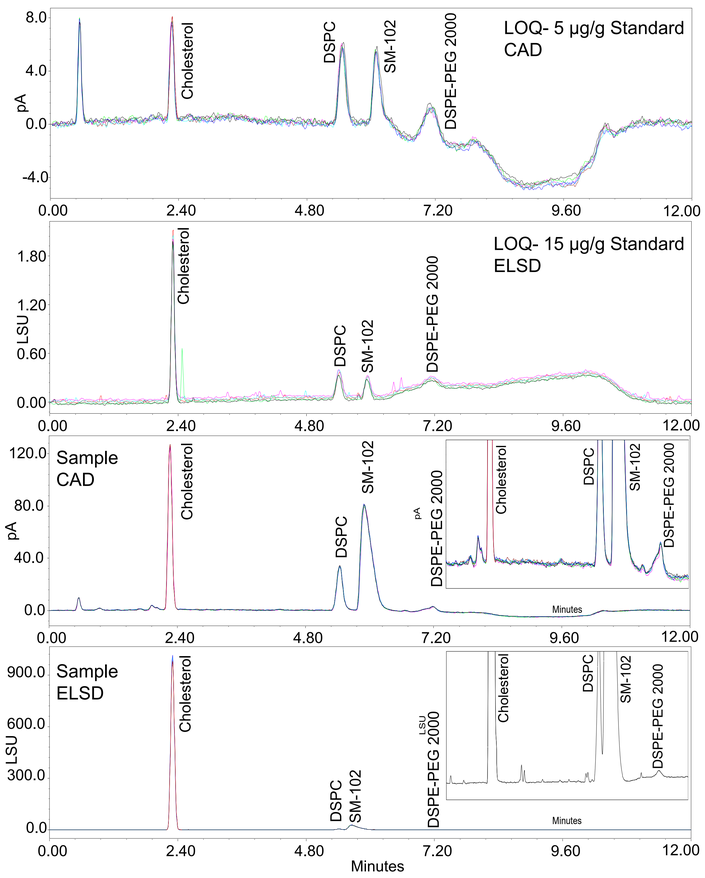

Figure 1. Chromatogram overlays (n=6) of LOQ standards and LNP samples on CAD and ELSD detectors. Sample chromatograms contain inlays of lower responding peaks for visibility.

Chromatographic results are shown in Figure 1. The CAD LOQ standard is shown at 5 µg/g, while the ELSD LOQ standard is shown at 15 µg/g (Figure 1). The criteria for individual LOQ values was a signal to noise ratio of greater than or equal to 10 and an area percent RSD less than or equal to 15% across six injections at each concentration. The individual component LOQ values across each detector are summarized in Figure 2, and the standard shown is based on the highest individual component LOQ. Figure 1 also shows representative chromatograms for the sample formulation on both detectors. The second sample at the higher concentrations was necessary due to the higher LOQ results for DSPC and DSPE-PEG 2000 on the ELSD. Due to the large differences in individual component concentrations within each sample, an inlay is shown for the lower responding peaks. The separation between the DSPC and SM-102 can also be seen in the zoomed in chromatogram, where it is shown that there is more resolution on the critical pair on the CAD compared to the ELSD. This resolution difference can be expected due to the increased concentration of the ELSD sample compared to the CAD sample.

LOQ Results (µg/g)

Detection	Cholesterol	DSPC	SM-102	DSPE-PEG 2000
Waters CAD	5.0	5.0	5.0	5.0
Waters ELSD	5.0	10.0	5.0	15.0

Calibration Curve R^2 Results (LOQ to 500 µg/g)

Detection	Cholesterol	DSPC	SM-102	DSPE-PEG 2000
Waters CAD	0.999	0.998	0.999	0.999
Waters ELSD	0.994	0.996	0.998	0.996

Figure 2. LOQ results for each peak and R-squared values of log-log linear calibration curves from LOQ to 500 µg/g using up to nine standards. LOQ criteria: (signal-to-noise ≥ 10 and area %RSD ≤ 15%.)

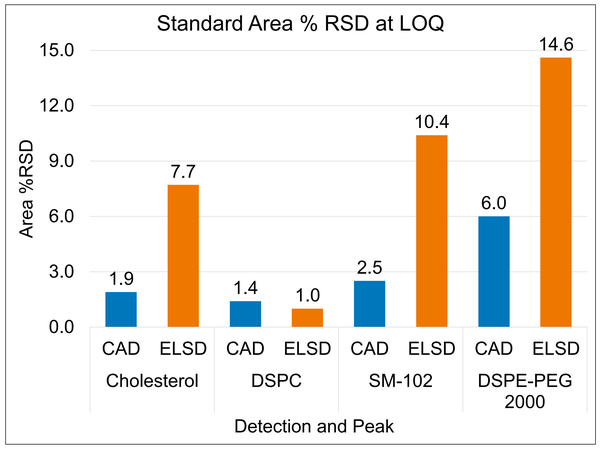

Figure 3. Area % RSD results for LOQ standards across detectors. LOQ concentrations shown in Figure 2.

Sample Recovery (%)	Cholesterol	DSPC	SM-102	DSPE-PEG 2000
Waters CAD	105.8	93.8	103.9	102.9
Waters ELSD	84.5	79.8	137.2	98.6

Figure 4. Calculated average sample recoveries across detectors using the established linearity curves (n=6).

The LOQ results summarized in Figure 2 show that every component LOQ was equal or lower on CAD compared to ELSD. Due to the differing mechanisms of detection, there was greater sensitivity observed on the CAD. DSPC peaks could be observed in the 5 µg/g standard on ELSD, but the repeatability was insufficient to be used as LOQ. The DSPE-PEG 2000 peak was not discernable from the baseline at concentrations below 15 µg/g. The linearity results of the log-log calibration curves for each component are also shown (Figure 2). These were determined from the LOQ standard to the 500 µg/g standard for each individual component, and thus each curve consisted of seven to nine data points depending on the LOQ level, with the ELSD curves of DSPC and DSPE-PEG 2000 being comprised of fewer points. All r-squared values show very precise data overall for both detectors, and the calculated values for each component were higher on the CAD than the ELSD across a wider range.

Area % RSD results were calculated for the LOQ standards (Figure 3). Results are shown for each individual component LOQ. For the two peaks where LOQ was at equal concentration (cholesterol and SM-102), there was better repeatability on the CAD compared to the ELSD. For DSPC, results were very similar across the two detectors, while for DSPE-PEG 2000, there was significantly lower RSD on CAD despite being at a lower concentration. This indicates better overall repeatability using CAD at low concentrations.

Percent recoveries for samples using the established calibration curves are shown in Figure 4. There is a smaller range of recoveries on the CAD, indicating better performance precision across all compounds. Additionally, all compounds recover close to 100%, showing the CAD is an ideal detector for sample accuracy when running LNP applications.

Conclusions

- CAD achieved uniformly lower LOQ values (5 µg/g for all components) compared to ELSD, which required 10–15 µg/g for DSPC and DSPE-PEG 2000
- CAD demonstrated superior repeatability at low concentrations, with area %RSD values approximately half those observed on ELSD for cholesterol, SM-102, and DSPE-PEG 2000
- Both detectors yielded strong log-log calibration linearity ($R^2 \geq 0.994$), though CAD consistently produced higher R^2 values across all four lipid components over a wider range
- CAD is the recommended universal detection method for LNP quantitation, offering greater sensitivity, precision, and chromatographic resolution on the critical DSPC/SM-102 pair

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