Optimization of Universal Detection Methods for Lipid Nanoparticles

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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 (ELS) and charged aerosol detection (CAD). In this study, two spiked sample solutions based on typical mRNA vaccines, consisting of DSPC, DSPE-PEG2000, cholesterol, and SM-102, was examined as a representative LNP. An LC method was developed and optimized using ELS and CAD detectors with an ACQUITY™ Premier UPLC™ System. Differences in sensitivity (LOD/LOQ), accuracy, and linearity are assessed.

Discussion and Method(s)

Method Conditions	
System	ACQUITY Premier UPLC System
Detection	ELS and CAD
Column	CORTECS™ Phenyl Column, 90Å, 1.6 µm, 2.1 x 50 mm (p/n 186008379)
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

Gradient Table

Time	%A	%B	Curve
Initial	100.0	0.0	Initial
7.00	0.0	100.0	6
9.00	0.0	100.0	6
9.01	100.0	0.0	6
14.00	100.0	0.0	6

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

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

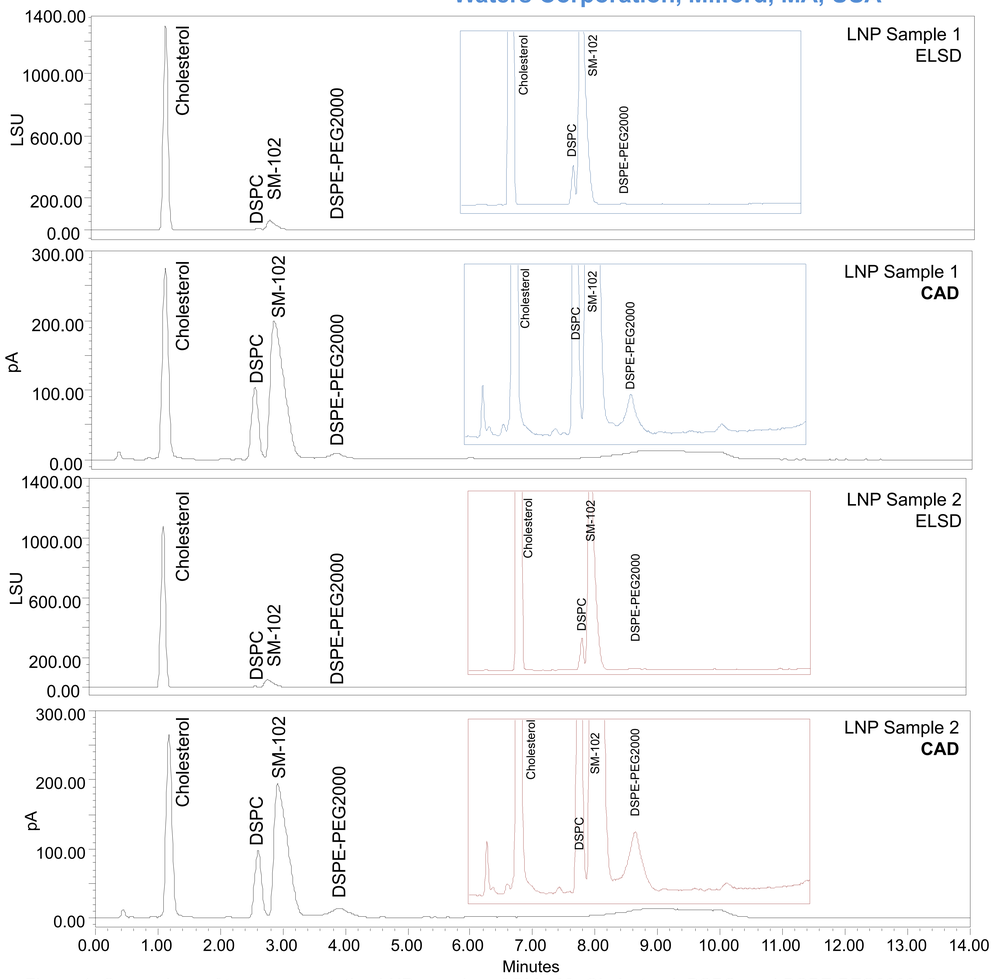

Figure 1. Comparison of two representative LNP samples of SM-102, Cholesterol, DSPC, and DSPE-PEG2000. Sample 1 ratio is 50:38.5:10.1.5 and Sample 2 ratio is 49.5:38:9.5:3 (total 1000 µg/g).

An optimized gradient method for LNPs was developed for use with ELS and CAD detectors on an ACQUITY Premier UPLC System. To compare the two detection methods, the same standards and samples were evaluated using equivalent parameters and data rates where possible. To assess linearity and establish LOD/LOQ for ELSD and CAD detection methods, up to eight standard levels consisting of cholesterol, DSPC, and DSPE-PEG2000 were assessed ranging from LOQ to 300 µg/g. Two representative sample solutions were spiked with known amounts of cholesterol, DSPC, DSPE-PEG2000, and SM-102 and assessed by ELS and CAD detection methods.

LOQ (µg/g) Results

Detection	Cholesterol	DSPC	DSPE-PEG2000
ELSD	10.0	10.0	15.0
CAD	5.0	5.0	10.0

Calibration Curve R² Results

Detection	Cholesterol	DSPC	DSPE-PEG2000
ELSD	0.993	0.986	0.991
CAD	0.999	0.998	0.995

Figure 2. LOQ results for each peak and R-square values of log-log linear calibration curves from LOQ to 300 µg/g using up to eight standards. LOQ criteria: (signal-to-noise ≥ 10 and area %RSD ≤ 15%.)

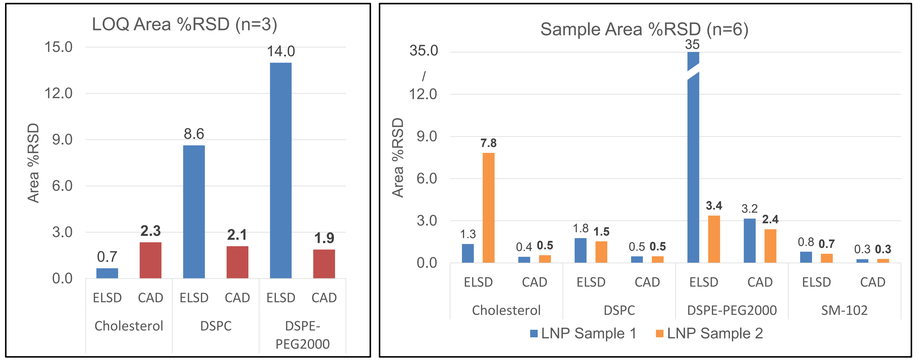

Figure 3. Area % RSD results for LOQ and Sample Formulation 1 and 2.

In the standards, the cholesterol peak produced the largest response. Sensitivity for DSPC and DSPE-PEG2000 was overall greater on CAD than ELSD. In the sample formulations, sensitivity for DSPC, DSPE-PEG2000, and SM-102 was greater on CAD than ELSD (Figure 1).

LOQ values were determined (Figure 2). LOQ levels established for ELSD were 10 µg/g for cholesterol and DSPC, and 15 µg/g for DSPE-PEG2000. LOQ levels on CAD were lower due to greater sensitivity, 5 µg/g for cholesterol and DSPC, and 10 µg/g DSPE-PEG2000.

Curves were created for each peak from LOQ to 300 µg/g, consisting of either six or eight standards. The curve fits use log-log linear response, the best practice for ELSD and CAD as they have limited linear dynamic ranges. Cholesterol had similar R^2 values between detectors, but DSPC and DSPE-PEG2000 had lower R^2 on ELSD than on CAD. At low levels, the charge to particle size response of CAD appears to be more consistent than light scattering efficiency to particle size response of ELS.

The relationship between detection and response consistency is shown in Figure 3. Area %RSD of each peak is shown for LOQs and the two sample solutions. In the LOQ standards, DSPC and DSPE-PEG2000 both showed lower %RSD on CAD than on ELSD, with no peak exceeding 2.3%. In sample solutions, %RSD on CAD were overall lower, indicating better repeatability on CAD than on ELSD.

Conclusions

- LNP analysis, which has become a critical application for many labs, requires universal detection
- For LNP analysis, CAD detection provides improved precision and sensitivity when compared to ELSD
- CAD is the preferred method for quantitation, especially at lower concentrations

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