# Optimization of Universal Detection Methods for Lipid Nanoparticles

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## Introduction

Lipid nanoparticles (LNPs) are a drug delivery mechanism that has gained popularity in recent years due to use in mRNA vaccines. LNPs are comprised of four components that encapsulate RNA for delivery into cells. These components must be present in specific ratios to properly control potency and efficacy. Traditional UV based methods of quantitation are not suitable for LNPs because many of the lipid components lack the necessary chromophores for UV detection, so universal detection is commonly used for this analysis. The two primary forms of universal detectors for LNP analysis are evaporative light scattering detection (ELSD) and charged aerosol detection (CAD). Due to the differing mechanisms of detection, CAD is typically more sensitive at low analyte concentration and has tools to improve linearity.

In this study, a formulation of cholesterol, DSPE-PEG 2000, and HSPC is used as a representative LNP. An LC method was developed and optimized for specific detectors (CAD and ELS) across various UHPLC platforms. The results are compared across detectors to examine the differences in sensitivity (limit of quantitation), accuracy, and linearity.

## Discussion and Method(s)

### Method Parameters

| Condition | Parameter |
|---|---|
| System | A) ACQUITY™ UPLC™ H-Class PLUS Quaternary System with Charged Aerosol Detector<br>B) ACQUITY Premier System with ACQUITY ELS Detector<br>C) UHPLC System with ELS Detection |
| 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 |
| Injection Volume | 5 µL |
| Sample Temp | 12 °C |
| Column Temp | 30 °C |

### Detector Parameters - CAD

| Condition (System A) | Parameter |
|---|---|
| Power Function Value | 1 |
| Evaporator Temperature | 40 °C |
| Ion Trap Voltage | 20 V |
| Data Rate | 10 Hz |

### Detector Parameters - ELS

| Condition (System B) | Parameter |
|---|---|
| Nebulizer Mode | Cooling |
| Drift Tube Temperature | 50 °C |
| Gas Pressure | 40 psi |
| Gain | 150 |
| Data Rate | 10 Hz |

### Gradient Table

| Time | %A | %B | Curve |
|---|---|---|---|
| Initial | 100.0 | 0.0 | Initial |
| 3.00 | 0.0 | 100.0 | 6 |
| 5.00 | 0.0 | 100.0 | 6 |
| 5.01 | 100.0 | 0.0 | 6 |
| 10.00 | 100.0 | 0.0 | 6 |

Standards were prepared ranging from 5 to 300 µg/g of cholesterol, DSPE-PEG, and HSPC in methanol. All glassware was rinsed in triplicate using appropriate solvent prior to use to minimize any potential impurities and interference peaks. Mobile phases were freshly prepared every two days.

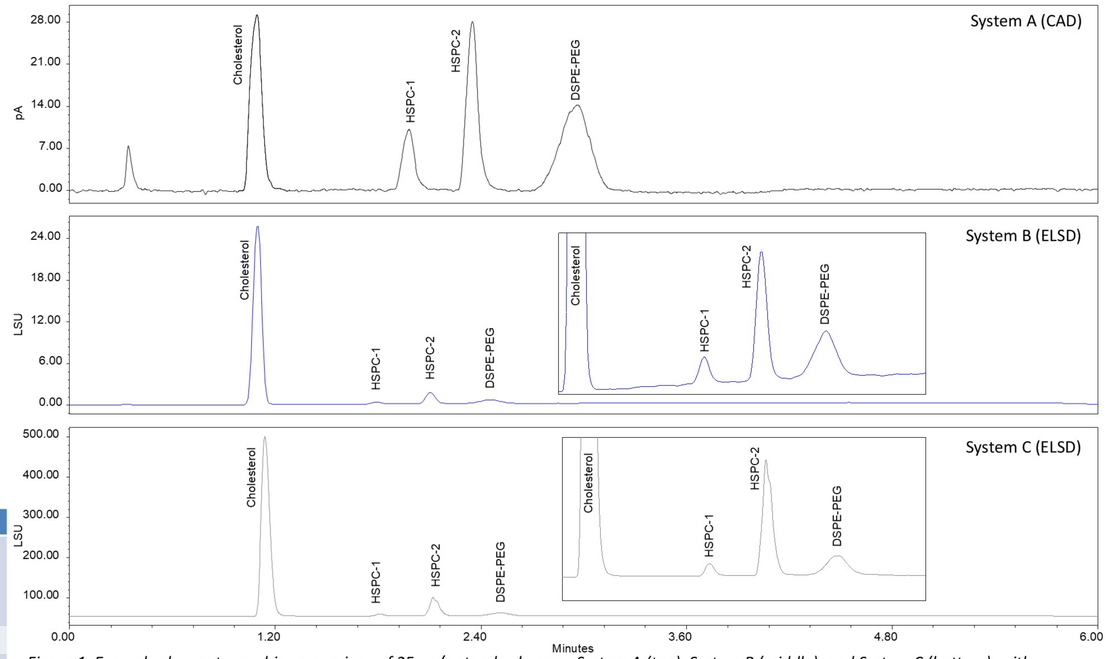


*Figure 1. Example chromatographic comparison of 25 µg/g standard across System A (top), System B (middle), and System C (bottom), with enlarged views of lower responding peaks on Systems B and C.*

Method optimization for all parameters was performed for each detector. The optimization process was completed more efficiently on CAD than either of the ELS systems. The main parameter that needed to be optimized was the evaporator temperature, with other parameters making less of an impact. On the other hand, the ELS had more parameters that made large impacts in results. Following optimization, the sensitivity remained better on System A with CAD.

Chromatographic results of the 25 µg/g standard are shown in Figure 1 above. The 25 µg/g standard is the minimum concentration required to achieve at least LOQ levels of signal to noise (S:N > 10.0) for all peaks on all systems. As can be seen in the chromatograms, the cholesterol peak has the largest height on all systems. However, HSPC and DSPE-PEG show more comparable signal response to the cholesterol peak on System A with CAD, with the area counts of all three components being very similar. On the ELS systems, the non-cholesterol peaks have a significantly lower response than the cholesterol peak.

A full table of the determined LOQ values are shown in Figure 2. The lowest level standard tested in this study was 5 µg/g. For the highest responding peak, cholesterol, this was the LOQ on all systems. The other peaks had varying concentrations where LOQ was established. System A with CAD detection was determined to have LOQs at least equal to the best performing ELS System. Additionally, the S:N values for these peaks at the LOQ levels were the highest with System A, suggesting that an intermediate standard concentration could achieve lower LOQ values on the system than 25 µg/g. The r-squared values of the calibration curves are also shown in Figure 2. Curves were constructed using all standard concentration levels from LOQ to 300 µg/g, meaning that curves could be results of five, six or seven standards. The curve fits used log-log linear response, which is the general best practice for both CAD and ELS as they have limited linear dynamic ranges. The two HSPC peaks and the DSPE-PEG peak had similar results across all systems, with the lowest result being 0.994. However, the cholesterol peak had an r-squared value of 0.999 on the CAD system and values of 0.986 and 0.980 on the two ELS systems, showing a difference in relationship between concentration and response between the two types of detectors. Especially at low levels, the charge to particle size response of CAD is much more consistent than the light scattering efficiency to particle size response of ELS.

### LOQ (µg/g) Results

| System | Cholesterol | HSPC-1 | HSPC-2 | DSPE-PEG |
|---|---|---|---|---|
| A | 5.0 | 25.0 | 5.0 | 25.0 |
| B | 5.0 | 25.0 | 5.0 | 25.0 |
| C | 5.0 | 25.0 | 10.0 | 25.0 |

### Calibration Curve $R^2$ Results

| System | Cholesterol | HSPC-1 | HSPC-2 | DSPE-PEG |
|---|---|---|---|---|
| A | 0.999 | 0.999 | 0.998 | 0.996 |
| B | 0.986 | 0.999 | 0.999 | 0.999 |
| C | 0.980 | 0.999 | 0.994 | 0.999 |

*Figure 2. LOQ results for each peak across all systems and R-squared values of log-log linear calibration curves from LOQ to 300 µg/g using a minimum of five standards.*

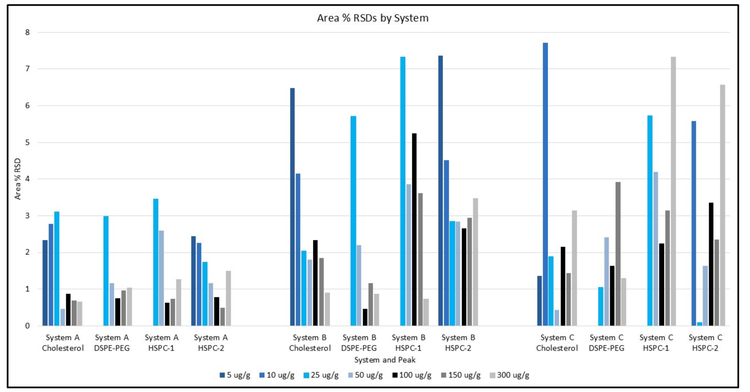


*Figure 3. Area % RSD results (n=3 at all concentrations) for all peaks across the three systems used throughout the study.*

The relationship between detection methods and response consistency is also shown in Figure 3. The area % RSD of each peak at all standard levels of quantitation is shown for the three systems. While there are fluctuations at individual levels, there are a few general trends that can be observed. The results on System A with CAD have a much smaller range of variability, with the highest RSD being 3.4% compared to 7.4% and 7.7% for Systems B and C using ELSD, respectively. This is particularly evident at the LOQ levels of each peak on each system, where each ELS system had at least two peaks where the RSD is a minimum of 5.6%. For some peaks, the higher standard concentrations have comparable results across systems, while for others the repeatability remains more precise when CAD is used with System A.

## Conclusions

In this study, a comparison of universal detectors, specifically CAD and ELSD, was performed for the purposes of LNP analysis. LOQ results were determined to be equal or lower on System A with CAD, and the calibration curves also showed r-squared values that were similar for some peaks and significantly higher for cholesterol. The area % RSD responses were generally lower with CAD detection and were also much more consistent across concentration levels, demonstrating improved precision for the detector.

LNP analysis is quickly becoming a critical application for many labs due to their emerging popularity. They require the use of universal detection, and CAD detection is the preferred method for quantitation, particularly at lower concentrations.

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