Method development for improving lipid nanoparticle quantification on charge aerosol detector

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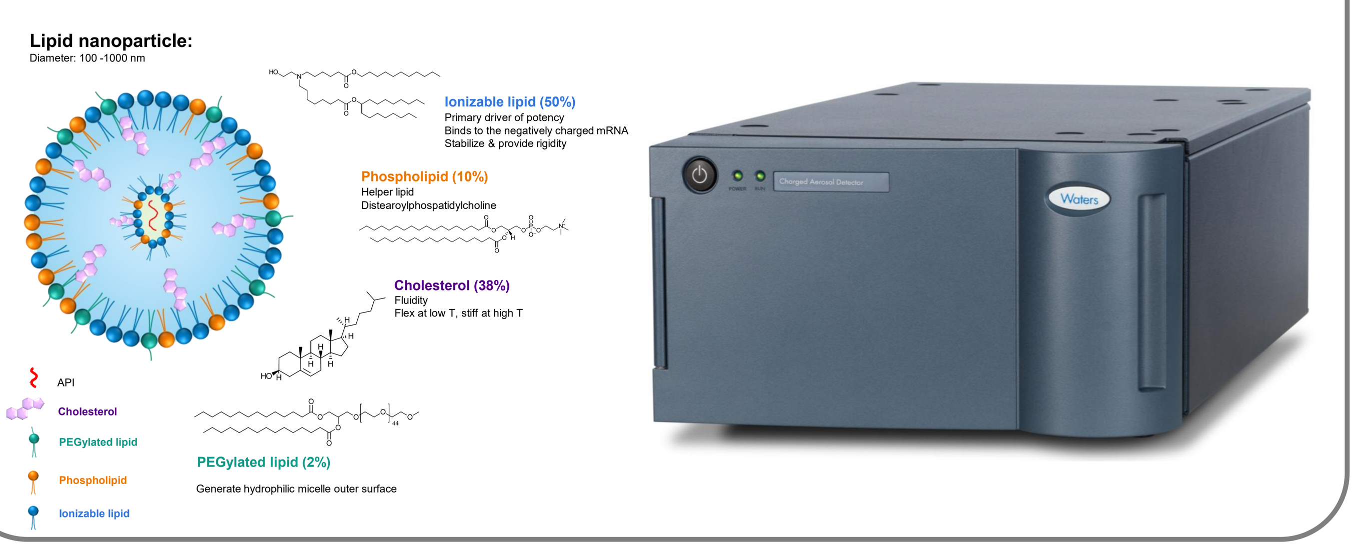
Overview

Lipid nanoparticles (LNPs) played a pivotal role in enabling mRNA vaccine delivery during the COVID-19 pandemic, and their success has driven continued interest in LNPs as a versatile platform for delivering a broad range of therapeutics. As with any excipient used in regulated biopharmaceutical manufacturing, robust analytical control strategies are essential to ensure accurate quantification and process consistency.[1,2] Traditional optical detectors such as TUVs and PDAs are commonly used in analytical laboratories but are limited by the lack of UV chromophores in several LNP components. This limitation has increased the adoption of universal detectors, including the charge aerosol detector (CAD), for LNP analysis.

To evaluate the suitability of CAD for detection and quantification, we assessed a panel of lipid standards across dilution series. Through optimization of separation selectivity and implementation of an innovative particle-selection approach, we achieved a significant improvement in the limit of detection (LOD) for DMG-PEG, one of the more challenging components to quantify. The resulting method is compatible with compliance-ready analytical software and is therefore well suited for deployment in manufacturing environments that require high throughput, ease of use, and automation to maintain productivity.



Experimental

All lipids in this study were used for research and demonstration purposes and were purchased from the following vendors: cholesterol and DSPC from Sigma-Aldrich; DMG-PEG 2000 and SM-102 from Cayman Chemical. Stocks of each lipid were prepared in methanol at 5 mg/mL and diluted to the appropriate concentration at 90/10 water/methanol (v/v).

LC system:	ACQUITY™ Premier System (BSM)	
Detection:	TUV, λ = 200 / 280 nm FC = 5mm Ti, 1Hz, time constant = normal	
Chromatography software:	Empower™ 3.9.0 CDS	
CAD settings:		
Sampling rate:	10 Hz	
Time constant:	Normal	
Ion trap:	20 V	
Evaporation temperature:	35 °C	
Column:	ACQUITY™ Premier™ CSH Phenyl-Hexyl 1.7µm, 130Å, 2.1 × 50 mm, (p/n: 186009474)	Waters GTxResolve™ Lipid Phenyl-Hexyl+ RP Column, MaxPeak™ Premier Technology, SPP, 1.6µm, 230Å, 2.1 x 50 mm (p/n: 186011698)
Column temperature:	50 °C	50 °C
Sample temperature:	Ambient	Ambient
Injection volume:	3 µL	3 µL
Flow rate:	0.400 mL/min	0.400 mL/min
Mobile phase:	A: 0.1% formic acid in water B: 0.1% formic acid in ACN	A: 0.1% formic acid in water B: 0.1% formic acid in 50:50 MeOH:ACN

Gradient Table:

Time (min)	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

Time (min)	Flow (mL/min)	% A	% B	Curve
Initial	0.400	50.0	50.0	Initial
6.00	0.400	10.0	90.0	6
8.00	0.400	10.0	90.0	6
8.50	0.400	50.0	50.0	6
12.00	0.400	50.0	50.0	6

Results & Discussion

ELSD VS CAD Response Behavior

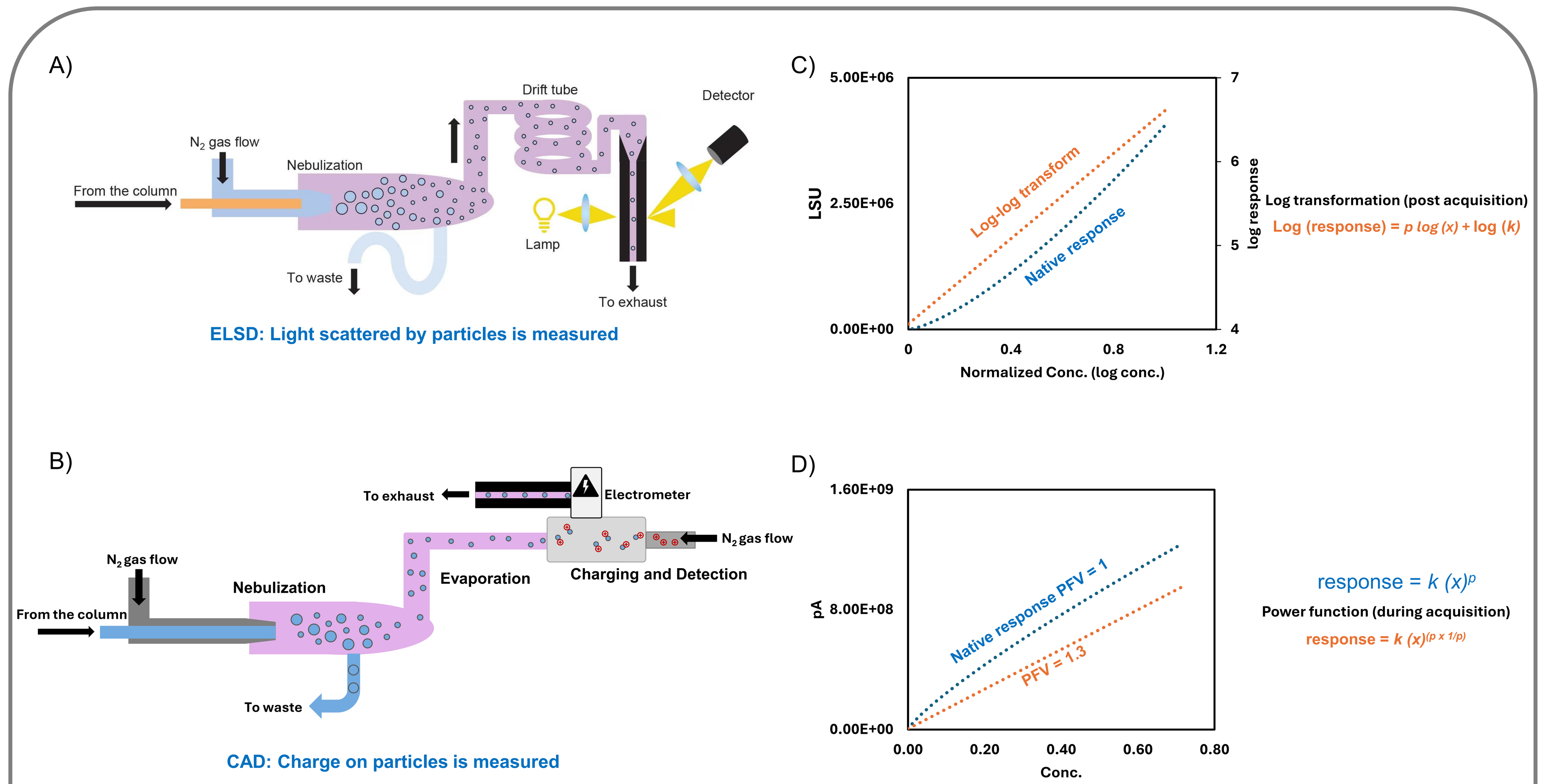


Figure 1. A) ELSD measures the light scattered by analyte particles. B) CAD measures the charge accumulated on particles; both detectors exhibit non-linear response to analytes' concentration. C) ELSD data are typically linearized post-acquisition using a log–log transformation, yielding a linear relationship between detector response and concentration. D) CAD linearizes the response during acquisition through transformation of the exponential "p-term" in the power law relationship.

LNP analysis[3]

N=3	SM102		Cholesterol		DMG-PEG		DSPC	
Conc. (mg/mL)	Area	RSD (%)	Area	RSD (%)	Area	RSD (%)	Area	RSD (%)
0.75	1.11E+09	6.55	1.05E+09	0.04	1.51E+09	4.74	1.41E+09	6.46
0.50	7.40E+08	4.14	7.36E+08	2.86	1.04E+09	2.91	9.82E+08	5.18
0.25	3.65E+08	0.86	3.72E+08	2.46	5.53E+08	0.51	5.15E+08	1.00
0.100	1.44E+08	0.04	1.45E+08	0.07	2.21E+08	0.90	2.28E+08	1.27
0.075	1.17E+08	1.17	1.11E+08	0.78	1.76E+08	0.12	1.86E+08	0.42
0.050	7.68E+07	0.95	6.71E+07	0.28	1.12E+08	1.00	1.30E+08	2.11
0.025	3.83E+07	0.55	3.36E+07	0.74	5.67E+07	0.47	6.62E+07	0.56
0.0100	1.46E+07	1.77	1.24E+07	0.32	2.04E+07	1.47	2.55E+07	0.15
0.0075	1.17E+07	0.75	9.01E+06	0.70	1.62E+07	1.32	2.01E+07	0.90
0.0050	7.28E+06	1.01	5.29E+06	3.10	9.55E+06	1.94	1.27E+07	1.38
0.0025	3.49E+06	2.18	2.54E+06	3.36	4.62E+06	2.57	6.18E+06	0.49
0.00100	1.24E+06	1.83	1.11E+06	2.81	1.93E+06	3.67	2.23E+06	2.79
0.00075	1.11E+06	1.99	8.24E+05	2.26	1.50E+06	0.54	1.92E+06	2.18
0.00050	6.33E+05	0.59	4.75E+05	2.79	~	~	1.18E+06	2.58
0.00025	2.95E+05	0.52	2.21E+05	2.79	~	~	6.93E+05	1.10
0.00010	1.52E+05	1.98			~	~	3.33E+05	1.30
0.000075	~	~			~	~	1.86E+05	3.20

Table 1. Calibration data table for individual LNP components collected on ACQUITY Premier CSH Phenyl-Hexyl column. Samples covering four orders of magnitude in concentration were evaluated, highlighting the wide dynamic range of the charged aerosol detector.

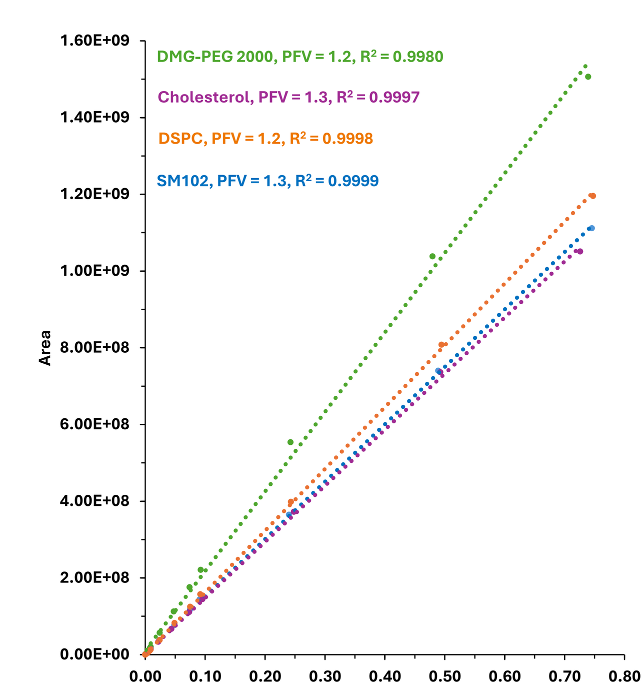
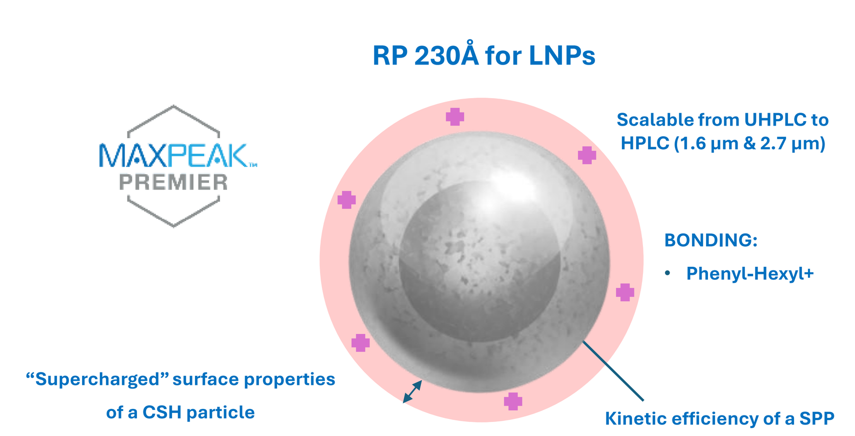


Figure 2. Application of PFV to individual LNP components enabled linear fitting across a wide dynamic range.

GTxResolve™ Lipid Phenyl-Hexyl+ RP Column



***Supercharged** lipid separations with a tailored particle that provides a **unique selectivity**, higher resolution for critical pairs, and outstanding reproducibility on U(H)PLC and HPLC systems.*

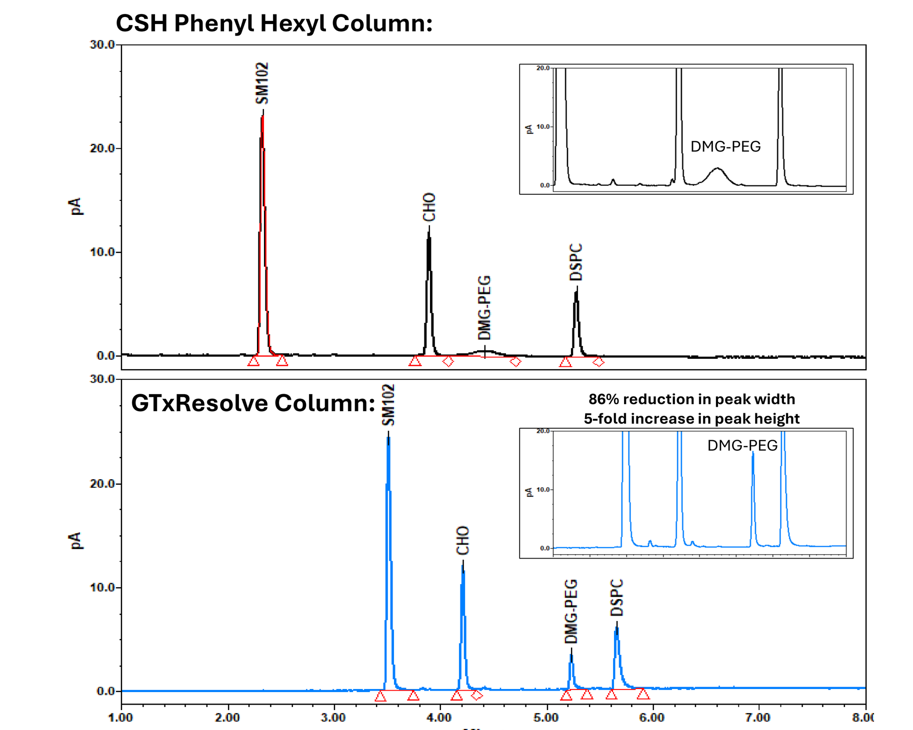
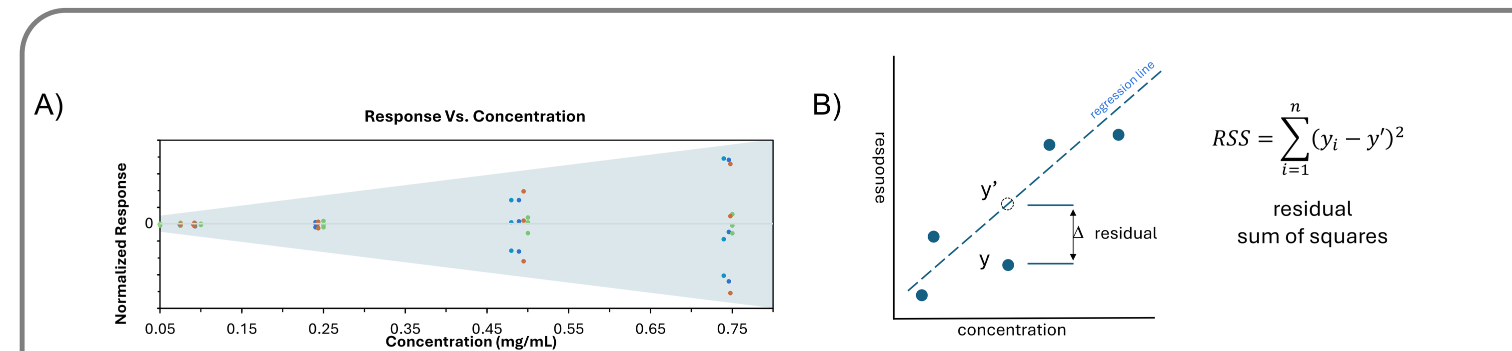
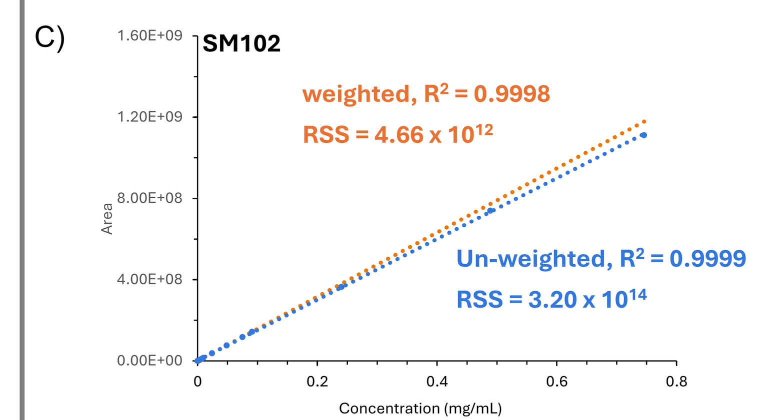


Figure 3. Comparison of the LNP panel at 0.1 mg/mL SM102 with a concentration ratio of SM102:cholesterol:DSPC:DMG-PEG= 1:0.42:0.22:0.11. Using the GTxResolve Lipid Phenyl-Hexyl+ RP column significantly improved DMG-PEG peak performance, resulting in an 86% reduction in peak width and a 5-fold increase in peak height.

Empower CDS Software CAD-based Workflow



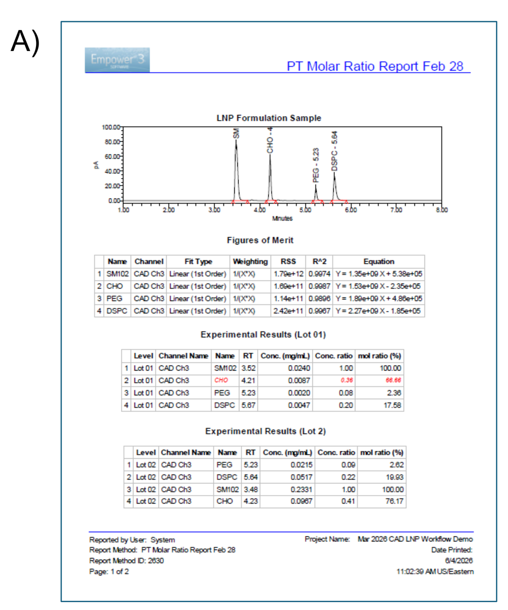


D) SM102, PFV = 1.3

Conc. (mg/mL)	Area	No weighting Error (%)		1/x² weighting Error (%)	
0.75	1.11E+09	0.58	Acceptable	5.69	Acceptable
0.50	7.40E+08	-0.87		4.23	
0.25	3.65E+08	-0.96		3.89	
0.100	1.44E+08	-4.29		-0.10	
0.075	1.17E+08	-3.07		0.79	
0.050	7.68E+07	-2.99		0.00	
0.025	3.83E+07	-1.18		-0.71	
0.0100	1.46E+07	6.92		0.15	
0.0075	1.17E+07	11.74	Unacceptable	2.98	
0.0050	7.28E+06	19.97		5.06	
0.0025	3.49E+06	35.76		8.12	
0.00100	1.33E+06	58.12		5.95	
0.00075	1.11E+06	62.09		4.02	
0.00050	6.33E+05	74.58		8.68	
0.00025	2.95E+05	86.09		3.83	

Figure 4. A) The variability in the LNP response grows with concentration, pointing to heteroscedastic behavior. B) Residual sum of squares (RSS) as a metric for fit quality. C) Example of applying weighting to SM102 fitting, resulting in reduced RSS thus better fitting. D) Weighted data reduces residuals effectively, yielding a distribution more consistent with homoscedastic data.

A)



B) Figures of Merit

	Name	Channel	Fit Type	Weighting	RSS	R^2	Equation
1	SM102	CAD Ch3	Linear (1st Order)	1/(X*X)	1.79e+12	0.9974	Y = 1.35e+09 X + 5.38e+05
2	CHO	CAD Ch3	Linear (1st Order)	1/(X*X)	1.69e+11	0.9987	Y = 1.53e+09 X - 2.35e+05
3	PEG	CAD Ch3	Linear (1st Order)	1/(X*X)	1.14e+11	0.9896	Y = 1.89e+09 X + 4.86e+05
4	DSPC	CAD Ch3	Linear (1st Order)	1/(X*X)	2.42e+11	0.9967	Y = 2.27e+09 X - 1.85e+05

C)

Concentration ratio

$$\frac{Component\ Amount\ (\frac{mg}{mL})}{SM102\ Amount\ (\frac{mg}{mL})}$$

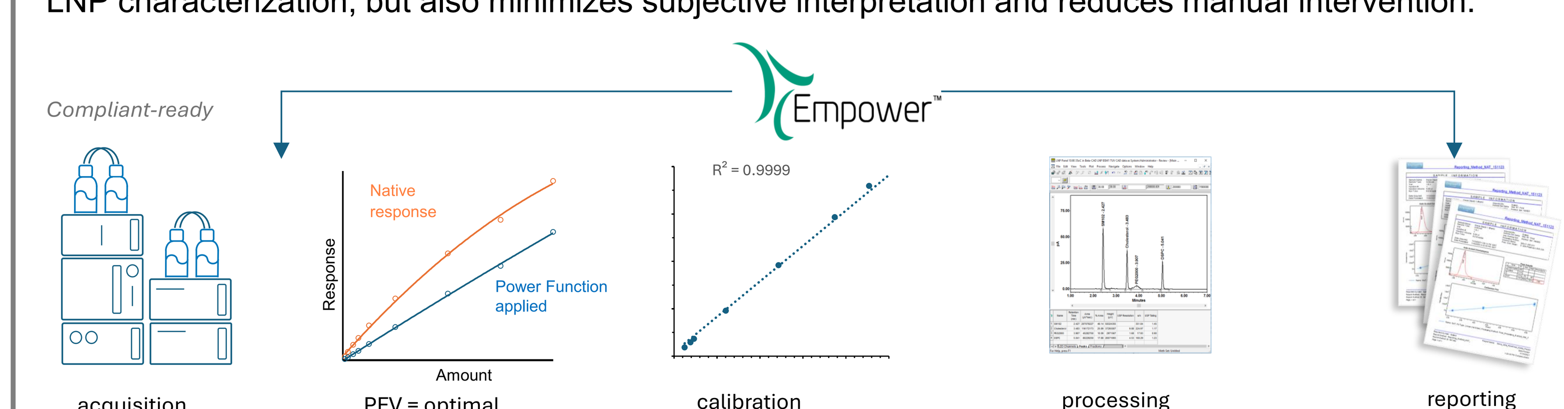
Molar ratio

$$\left(\frac{Component\ Amount\ (mg/mL)}{Molecular\ weight\ (g/mol)} \Big/ \frac{SM102(mg/mL)}{Molecular\ weight\ (g/mol)}\right) \times inj\ vol\ (L) \times 100$$

Figure 5. A) Empower report of LNP panel sample analysis results, including chromatography, Figures of Merit, and component analysis using custom field. B) Zoomed-in view of the Figures of Merit table. C) Illustration of how concentration ratio and molar ratio is considered in Empower custom fields.

Conclusion

The capability to evaluate and linearize response curves for multiple LNP components using CAD within Empower CDS provides key advantages for method development, including streamlined calibration approaches, improved quantitative reliability, and enhanced readiness for regulated environments. This approach not only accelerates the development of reliable quantitative methods for LNP characterization, but also minimizes subjective interpretation and reduces manual intervention.



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1. Fekete S, Doneanu C, Addepalli B, et al. Challenges and emerging trends in liquid chromatography-based analyses of mRNA pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis. 2023 Feb;224:115174. DOI: 10.1016/j.jpba.2022.115174.
2. Han, D., Birdsall, R., Simeone, J., Fogwill, M., Yu, Y. Comparing ELSD and CAD Performance on Polysorbate Quantification in Infliximab Drug Products. (2022), Waters Application Note 720007501.
3. Birdsall R., Du, X., Bigos, P., Han, D., Bhiwankar, N. Automating Charged Aerosol Detection (CAD) Analysis with Empower™ CDS Using a Single-Vendor Integrated LC Platform (2026), Waters Application Note 720009297.

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