ADVANCES IN CHARGED AEROSOL DETECTION FOR QUANTITATIVE ANALYSIS OF BIOPHARMACEUTICAL EXCIPIENTS

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Poster number: P 070

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INTRODUCTION

Charged aerosol detection (CAD) has emerged as a versatile analytical technology for the quantification of non-volatile analytes in complex biopharmaceutical samples. Unlike optical or mass-based detectors that rely on chromophores or ionizable functional groups, CAD provides a near-universal response to non-volatile compounds, enabling broad applicability across diverse therapeutic modalities and formulation components. In biopharmaceutical development, CAD is increasingly utilized for the analysis of excipients and delivery system components including polysorbates and lipids used in lipid nanoparticle (LNP) formulations.

This poster will highlight key CAD operating parameters that can impact quantitative performance, including ion trap voltage and power function values, and their optimization for different analyte classes. Examples will include the accurate quantification of polysorbates and lipids in lipid nanoparticle (LNP) formulations, demonstrating how CAD settings can improve sensitivity, precision, and quantitative reliability.

METHODS

Polysorbate Analysis[1]

LC System Settings:

LC system:	ACQUITY™ Premier System
Column:	Oasis™ MAX Column, 80Å, 30 µm, 2.1 x 20 mm (p/n: 186002052)
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	30 µL
Mobile phase A:	2.0% formic acid in water
Mobile phase B:	2.0% formic acid in isopropanol

CAD Settings:

Sampling rate:	5 Hz
Time constant:	Normal
Ion trap voltage:	600 V
Evaporator temp.:	40 °C

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.000	90	10	Initial
1.0	1.000	80	20	6
3.0	1.000	80	20	6
3.1	1.000	0	100	6
5.1	1.000	0	100	6
5.2	1.000	90	10	6
8.0	1.000	90	10	6

LNP Analysis[2,3]

LC System Settings:

LC system:	ACQUITY Premier System
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 temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	3 µL
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in ACN for the CSH Phenyl-Hexyl Column; 0.1% formic acid in 50:50 MeOH:ACN for GTxResolve Lipid Phenyl-Hexyl+RP Column

CAD Settings:

Sampling rate:	10 Hz
Time constant:	Normal
Ion trap voltage:	20 V
Evaporator temp.:	35 °C

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.400	50	50	Initial
6.0	0.400	10	90	6
8.0	0.400	10	90	6
8.5	0.400	50	50	6
12.00	0.400	50	50	6

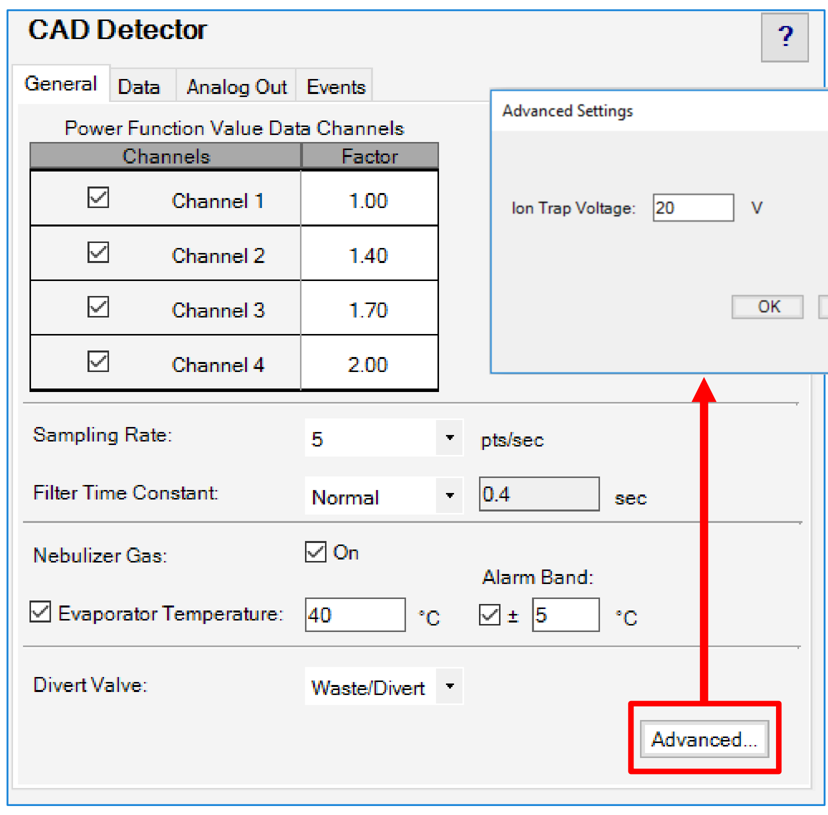
CONCLUSIONS

- **Optimization of the ion-trap voltage enhances method performance by extending the calibration range and reducing baseline noise.**
- **Spike–recovery experiments confirmed accurate quantification of PS80 and PS20, with measured concentrations closely aligning with expected values and relative standard deviations below 1%.**
- **The GTxResolve Lipid Phenyl-Hexyl+ RP Column improved DMG-PEG peak performance, expanding the assay's dynamic range and lowering the LOD, while monitoring multiple Power Function values in a single analysis further enhanced CAD sensitivity for LNP component detection.**

References

1. Bigos P, Birdsall RE, Du X, Han D, Bhiwankar N. Quantification of Polysorbates Using the Waters Charged Aerosol Detector. Waters Application Note. July 2026. 720009502EN.
2. Birdsall RE, Du X, Bigos P, Han D, Bhiwankar N. Automating Charged Aerosol Detection (CAD) Analysis with Empower CDS Using a Single-Vendor Integrated LC Platform. Waters Application Note. April 2026. 720009297EN.
3. Birdsall RE, Du X, Bigos P, Han D, Simeone J, Bhiwankar N. Predicting Linear Operating Range for Charged Aerosol Detection Using an Inverse Power Function Framework. Waters Application Note. June 2026. 720009440EN.

RESULTS AND DISCUSSION



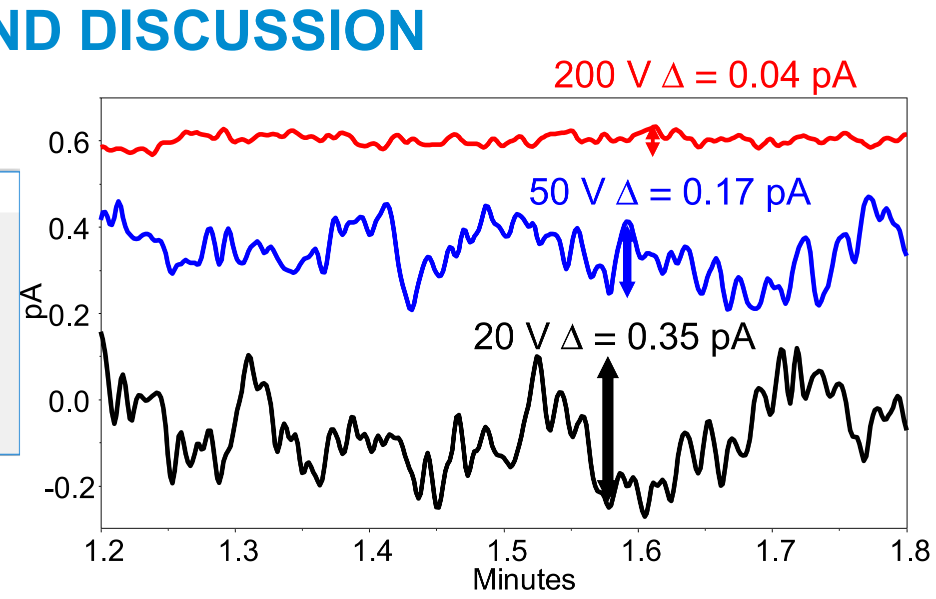


Figure 1: Ion trap voltage can be configured within the advanced settings of the Empower™ instrument method editor over a range of 20 – 600 V. This parameter controls the strength of the electric field applied within the ion trap region of the CAD, located before the electrometer. This electric field removes excess aerosol ions generated from the corona charger while allowing the larger charged analyte particles to pass to the detector. By filtering excess ions prior to detection, the ion trap helps reduce background signal and improve baseline stability.

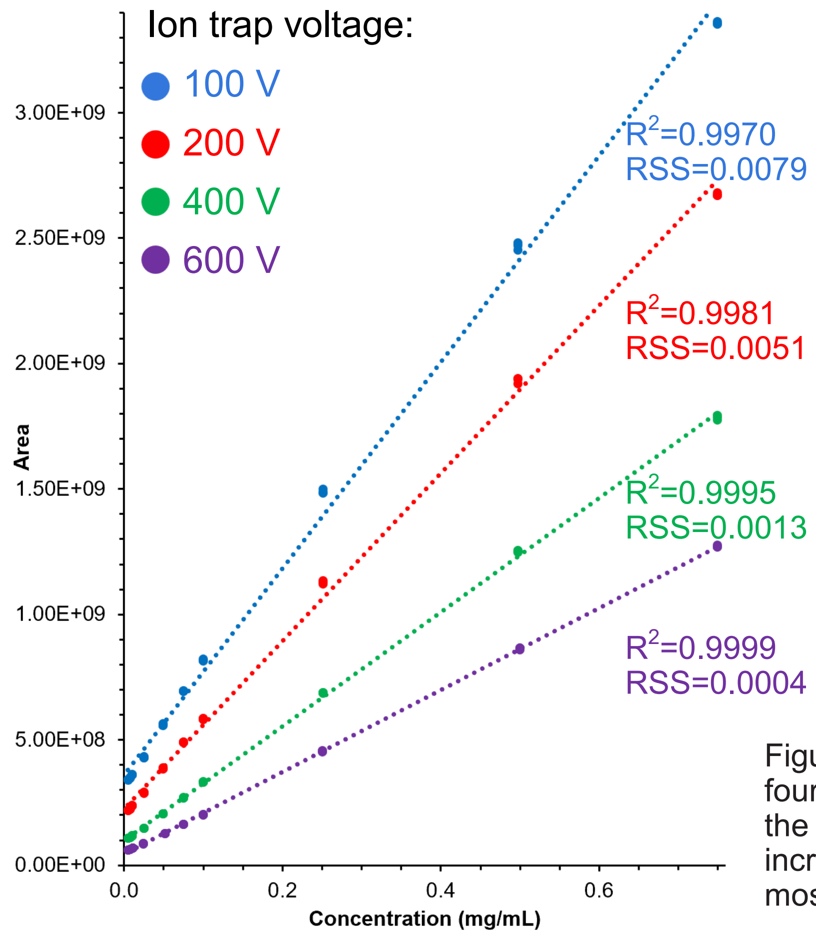


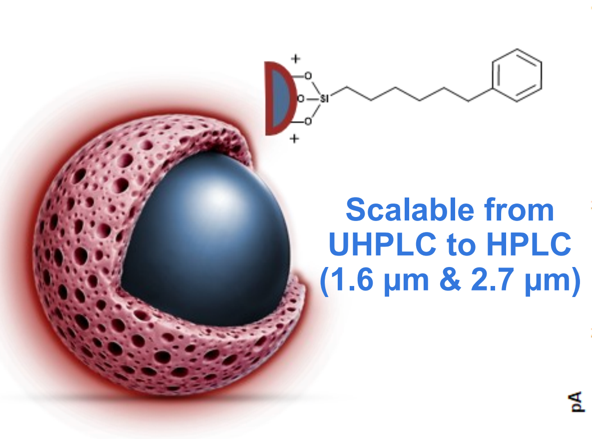
Figure 2: Calibration curves for PS20 (0.005—0.75 mg/mL) were generated at four ion trap voltages using a fixed power function value (PFV) of 1.0 to evaluate the impact of ion-trap voltage on detector response. As ion trap voltage is increased, calibration linearity improves, with the 600 V condition yielding the most linear response and the lowest residual sum of squares (RSS).

N=6	Polysorbate spike-recovery results					
Spiked amount (mg/mL)	Polysorbate 80			Polysorbate 20		
	Exp. (mg/mL)	% Dev.	% RSD	Exp. (mg/mL)	% Dev.	% RSD
~0.100	0.102	1.64	0.23	0.102	2.18	0.45
~0.300	0.296	1.74	0.94	0.296	1.38	0.69
~0.500	0.492	2.80	0.80	0.512	1.65	0.64

Figure 3: Spike-recovery experiments were performed using a well-characterized mAb (NISTmAb) spiked with known concentrations of PS20 and PS80. Three concentration levels were evaluated and each level was injected 6 times. The table shows excellent accuracy and precision across the tested concentration ranges for both PS80 and PS20.

GTxResolve Lipid Phenyl-Hexyl+ RP Column

230Å for LNPs



Scalable from UHPLC to HPLC (1.6 µm & 2.7 µm)

"Supercharged" surface properties of a CSH particle

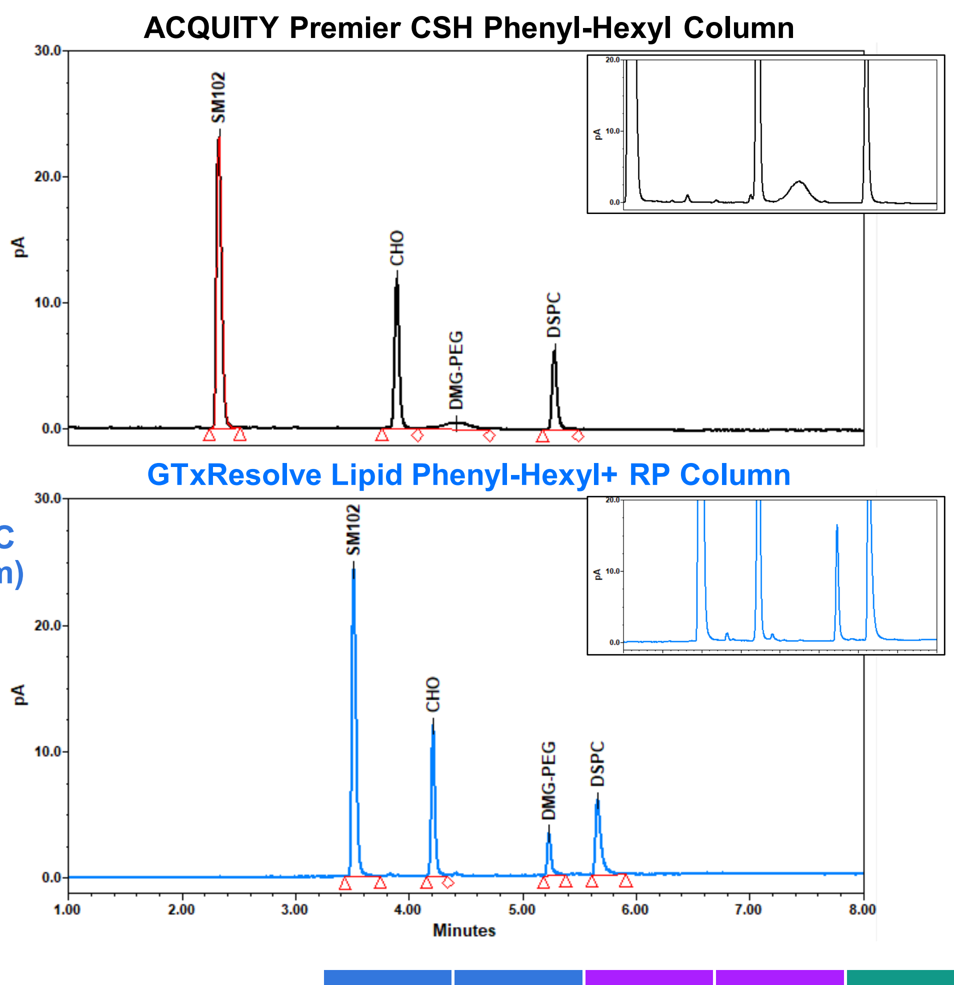
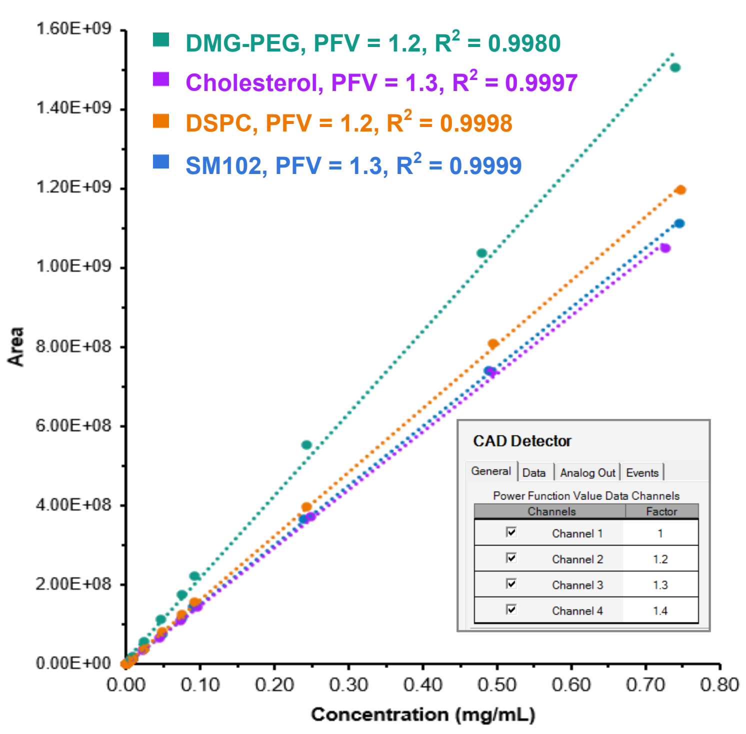


Figure 4: Comparison of the LNP panel containing 0.1 mg/mL SM102 with a concentration ratio of SM102 / cholesterol /DSPC / DMG-PEG = 1 / 0.42 / 0.22 / 0.11. The GTxResolve Lipid Phenyl-Hexyl+ RP Column, which utilizes "supercharged" superficially porous particles significantly improves the chromatographic performance of DMG-PEG peak when compared to the ACQUITY Premier CSH Phenyl-Hexyl Column. The improved performance resulted in an 86% reduction in peak width and a 5-fold increase in peak height.



Conc. (mg/mL)	Area	Error (%)	Area	Error (%)	Area	Error (%)	Area	Error (%)
0.75	9.59E+08	1.35	~	~	~	~	~	~
0.50	6.65E+08	0.07	~	~	~	~	~	~
0.25	3.49E+08	-4.45	5.35E+08	2.61	~	~	~	~
0.100	1.47E+08	-7.92	3.63E+08	3.14	~	~	~	~
0.075	1.05E+08	-7.30	1.87E+08	0.64	1.98E+08	-14.70	~	~
0.050	6.49E+07	-6.08	7.76E+07	-2.38	1.32E+08	-11.51	~	~
0.025	3.21E+07	-3.84	5.50E+07	-1.28	6.33E+07	-7.39	3.42E+08	3.02
0.0100	1.25E+07	3.28	3.37E+07	-0.87	2.39E+07	-1.12	2.41E+08	-0.07
0.0075	9.34E+06	5.74	1.62E+07	0.40	1.66E+07	2.20	1.25E+08	-4.20
0.0050	5.70E+06	12.81	6.01E+06	2.00	9.67E+06	6.51	5.02E+07	-4.33
0.0025	~	~	4.46E+06	-0.76	4.40E+06	10.91	3.44E+07	-0.26
0.00100	~	~	2.68E+06	-6.44	1.63E+06	6.62	2.05E+07	1.91
0.00075	~	~	~	~	1.13E+06	5.97	9.39E+06	4.50
0.00050	~	~	~	~	6.03E+05	-1.07	3.29E+06	0.56
0.00025	~	~	~	~	~	~	2.39E+06	-8.18
0.00010	~	~	~	~	~	~	1.46E+06	-48.83
0.00007	~			~	~	~	~	~
0.00005	~			~	~	~	~	~

New LOD (s/n=3)

Figure 5: The improved sensitivity achieved with the GTxResolve Lipid Phenyl-Hexyl+ RP Column enabled accurate detection of three additional concentration levels, supported by the 5-fold increase in DMG-PEG peak height. These improvements effectively expand the dynamic range and lower the LOD of the composition assay, while the ability to monitor different Power Function values within a single analysis further enhances CAD sensitivity for the detection of LNP components, as shown in the table and calibration plot.

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