QUANTIFICATION OF POLYSORBATE IN MONOCLONAL ANTIBODY FORMULATIONS USING CHARGED AEROSOL DETECTION

Pawel Bigos, Duanduan Han, Robert Birdsall, Nikhil Bhiwankar

Waters Corporation

INTRODUCTION

Polysorbate 80 (PS80) and polysorbate 20 (PS20) are non-ionic surfactants widely used in pharmaceutical and biopharmaceutical formulations. In protein therapeutics, polysorbates act as stabilizing excipients that reduce surface adsorption and protect proteins from stress-induced aggregation caused by agitation, shear, or interfacial interactions.[1] From a quality control perspective, accurate determination of polysorbate concentration in final drug products is important, however, its structural heterogeneity presents analytical challenges. Polysorbates are composed of a complex mixture of closely related species that lack strong UV chromophores and typically produce broad, unresolved peaks under gradient chromatographic conditions, rendering conventional UV detection unsuitable for accurate quantification. Consequently, a trap-and-elute reversed-phase liquid chromatography (RPLC) method coupled with charged aerosol detection (CAD) was employed for polysorbate analysis.[2]

METHODS

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
Evaporation temp.:	40 °C

Software:

LC CDS: Empower™ Software, 3.9.0

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

CONCLUSIONS

- **The double-valve column manager configuration removes unswept volume within the valve system, thereby minimizing residual protein matrix coelution.**
- **Optimization of the ion-trap voltage enhances method performance by extending the calibration range and reducing baseline noise.**
- **The CAD exhibited excellent linearity over approximately two orders of magnitude for both PS80 and PS20.**
- **Spike–recovery experiments confirmed accurate quantification of PS80 and PS20, with measured concentrations closely aligning with expected values and relative standard deviations below 1%.**

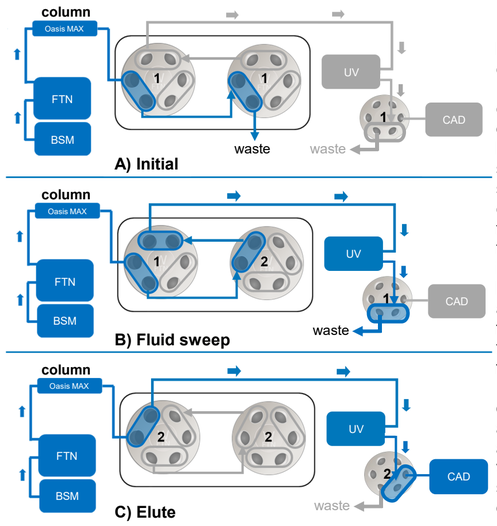
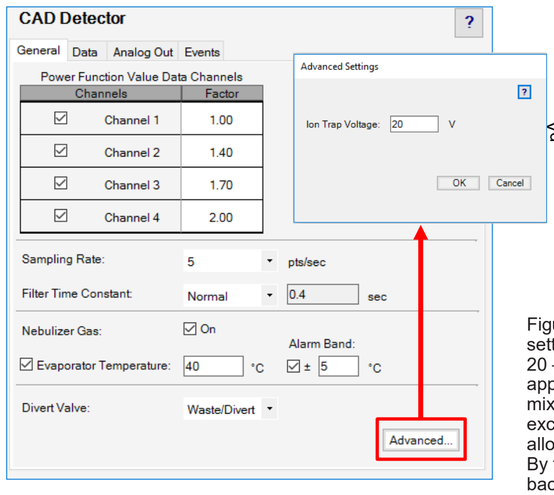


Figure 1: The double-valve configuration in the column manager provided the most consistent performance, delivering the lowest % RSD for both calibration standards and spiked samples. The valve configuration consists of three stages: (A) initial trapping, where the sample is loaded onto the Oasis MAX column and proteins are directed to waste; (B) a fluid sweep step, in which the second valve redirects flow to actively flush previously unswept regions of the first valve and associated tubing to waste; and (C) elution, where all valves are positioned to send polysorbate to the detector.

RESULTS AND DISCUSSION



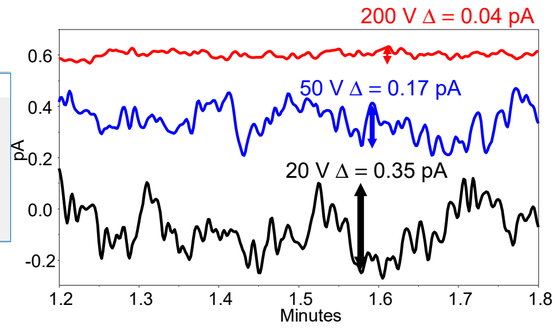
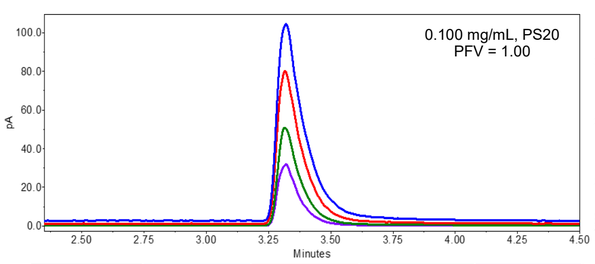


Figure 2: The ion-trap voltage can be configured within the advanced settings of the Empower instrument method editor over a range of 20 – 600 V. This parameter controls the strength of the electric field applied within the ion-trap region of the CAD, located between the mixing chamber and the electrometer. 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.



Statistics	100 V	200 V	400 V	600 V
R^2	0.9970	0.9981	0.9995	0.9999
RSS	0.0079	0.0051	0.0013	0.0004

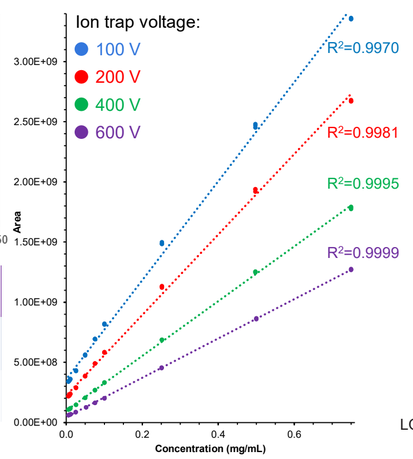
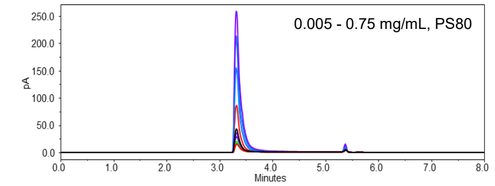


Figure 3: Calibration curves for PS20 (0.005—0.75 mg/mL) were generated at four ion-trap voltages using a fixed power function value (PFV) of 1.0 to evaluate the impact of ion-trap voltage on detector response. At lower ion-trap voltages (100-200 V), the response exhibits noticeable nonlinearity, reflected by lower R^2 values and higher residual sum of squares (RSS). As the ion-trap voltage is increased, calibration linearity improves, with the 600 V condition yielding the most linear response and the lowest RSS, indicating minimal deviation from the regression model.



Conc. (mg/mL)	Area	Error (%)	%RSD	Area	Error (%)	%RSD
0.7500	1.65E09	-0.39	0.20	1.40E09	-0.25	0.18
0.5000	1.16E09	0.86	0.64	9.89E08	-0.89	0.10
0.2500	6.52E08	0.73	0.44	5.57E08	-0.64	0.08
0.1000	3.42E08	-2.33	0.81	3.03E08	-2.17	0.22
0.0750	2.96E08	-2.66	0.30	2.62E08	-2.40	0.24
0.0500	2.45E08	-3.21	0.60	2.21E08	-3.09	0.46
0.0250	1.97E08	0.94	0.37	1.79E08	-2.42	0.14
0.0100	1.70E08	10.19	0.06	1.56E08	8.76	0.17
0.0075	1.63E08	9.84	0.36	1.52E08	15.01	0.32
0.0050	1.58E08	14.12	0.26	1.51E08	62.85	0.24

LOQ

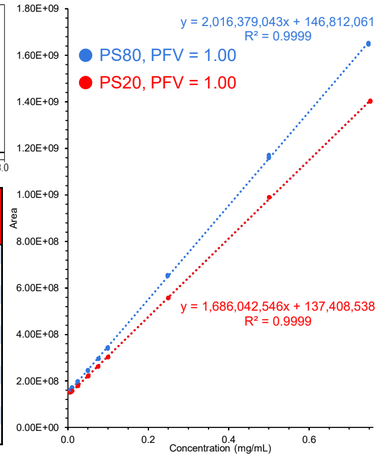


Figure 4: Calibration performance for PS80 and PS20 was assessed (0.005 to 0.75 mg/mL) to define the linear range of the detector. PS80 and PS20 demonstrate strong linear behavior across this range, with R^2 values of 0.9999. The tabular results show low % RSD values across all calibration levels. Percent error values remain within acceptable limits for PS80 at all concentration levels, although slightly higher deviations were observed for the lowest calibration level for PS20.

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 5: 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 to evaluate repeatability and quantitative performance. The table shows excellent accuracy and precision across the tested concentration ranges for both PS80 and PS20. Measured concentrations closely matched the expected spike levels, with minimal percent deviation, demonstrating accurate recovery of both surfactants from the NISTmAb.

References

1. Martos A, et al. Trends on Analytical Characterization of Polysorbates and Their Degradation Products in Biopharmaceutical Formulations. J Pharm Sci. 2017;106 (7):1722–1735. doi:10.1016/j.xphs.2017.03.001.
2. Hewitt D, et al. Quantitation of Polysorbate 20 in Protein Solutions Using Mixed-Mode Chromatography and Evaporative Light Scattering Detection. J Chromatogr A. 2008;1215(1–2):156–160. doi:10.1016/j.chroma.2008.11.017.

Learn More

720009464EN

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS