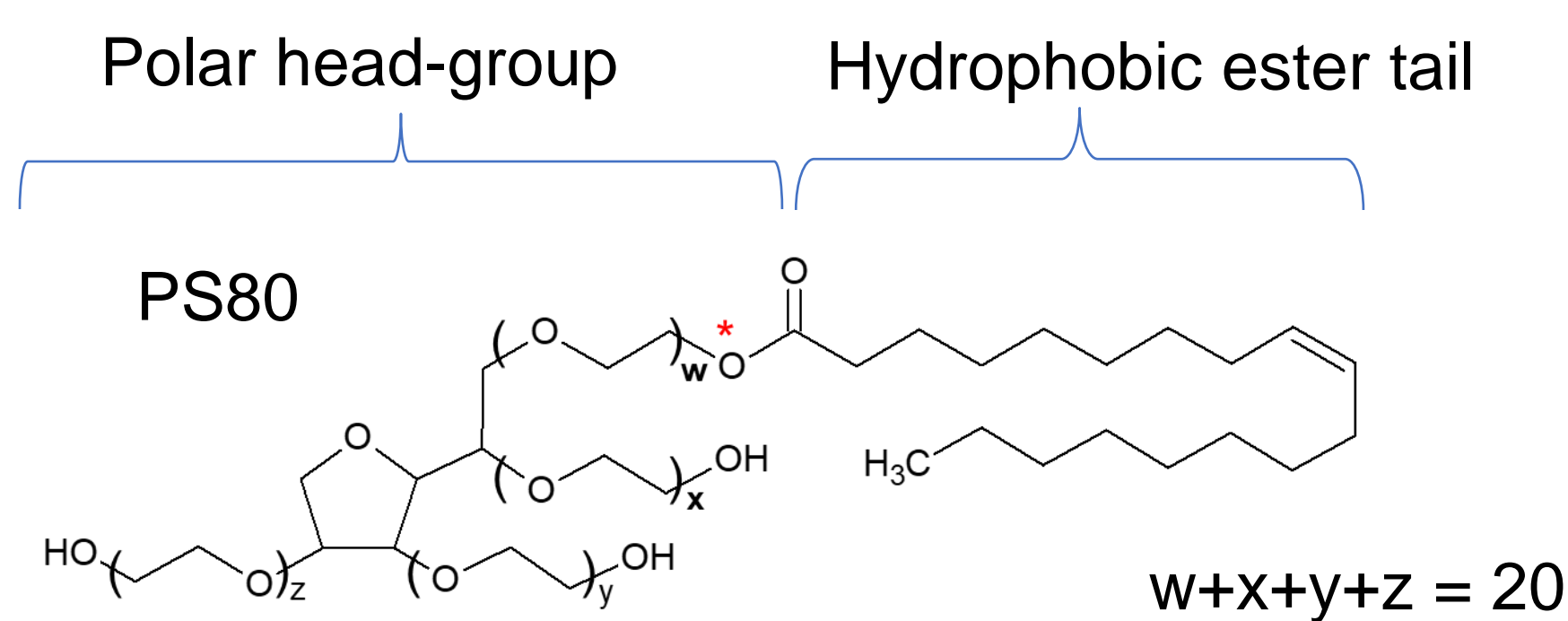# ELSD Performance on Polysorbate Quantification in Infliximab Drug Products
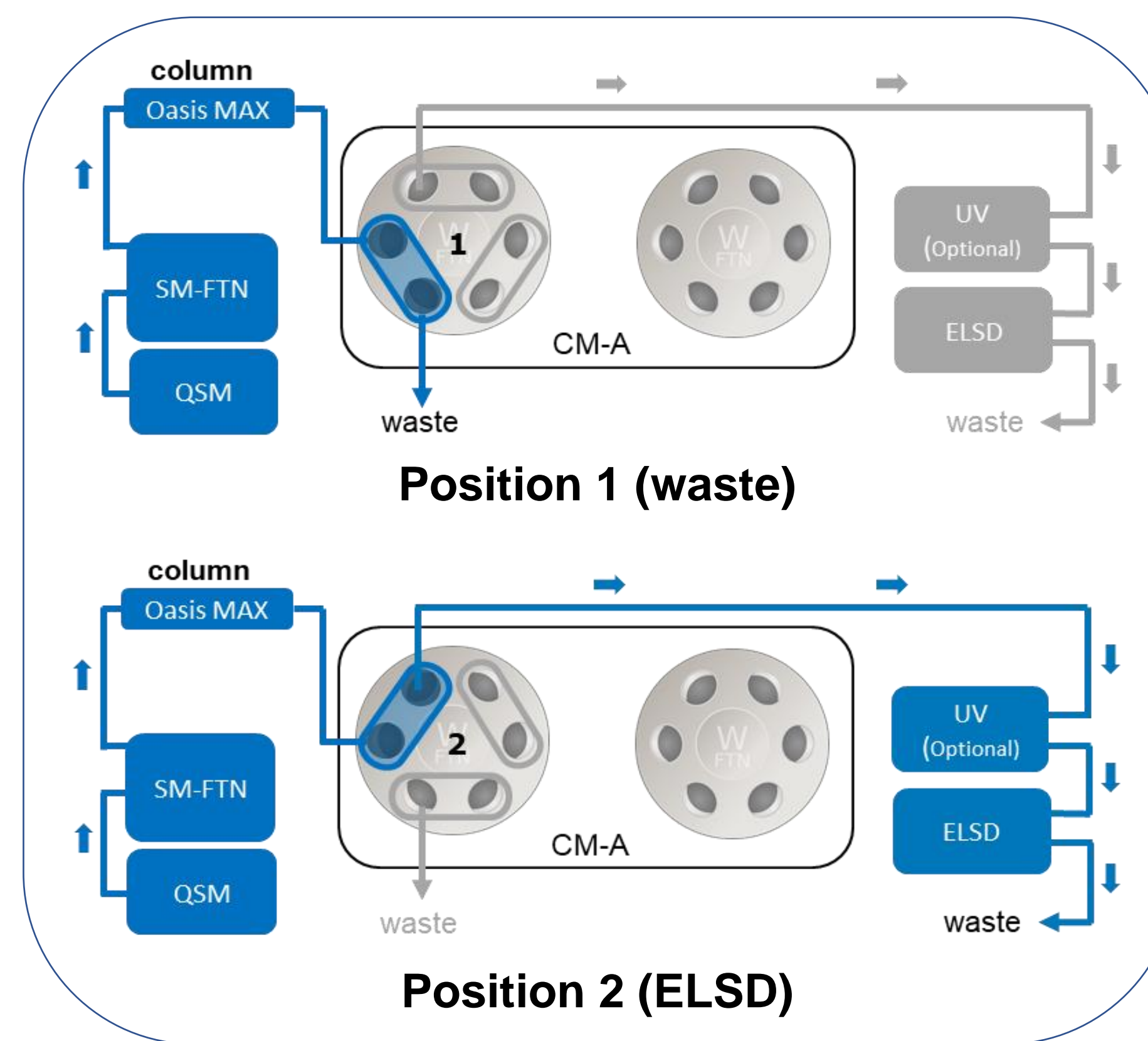
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Waters Corporation, Milford, MA

INTRODUCTION

Polar head-group   Hydrophobic ester tail

PS80

$w+x+y+z = 20$

Figure 1. Polyoxyethylene sorbitan monooleate.

Due to its biocompatibility and low toxicity, polysorbate (PS) is a common stabilizing excipient for drug products. To ensure PS-containing drug products are safe to administer and efficacious as designed, accurate quantification of PS is desired. However, as a non-UV active excipient (Figure 1) present at ppm levels in a biological matrix, novel approaches are required in the isolation, detection, and quantitation of polysorbate.

METHODS

Principles of evaporative light scattering

Step 3: Detection

Step 1: Nebulization

Step 2: Evaporation and Desolvation

column
Oasis MAX
SM-FTN
QSM
CM-A
UV (Optional)
ELSD
waste
Position 1 (waste)

column
Oasis MAX
SM-FTN
QSM
CM-A
UV (Optional)
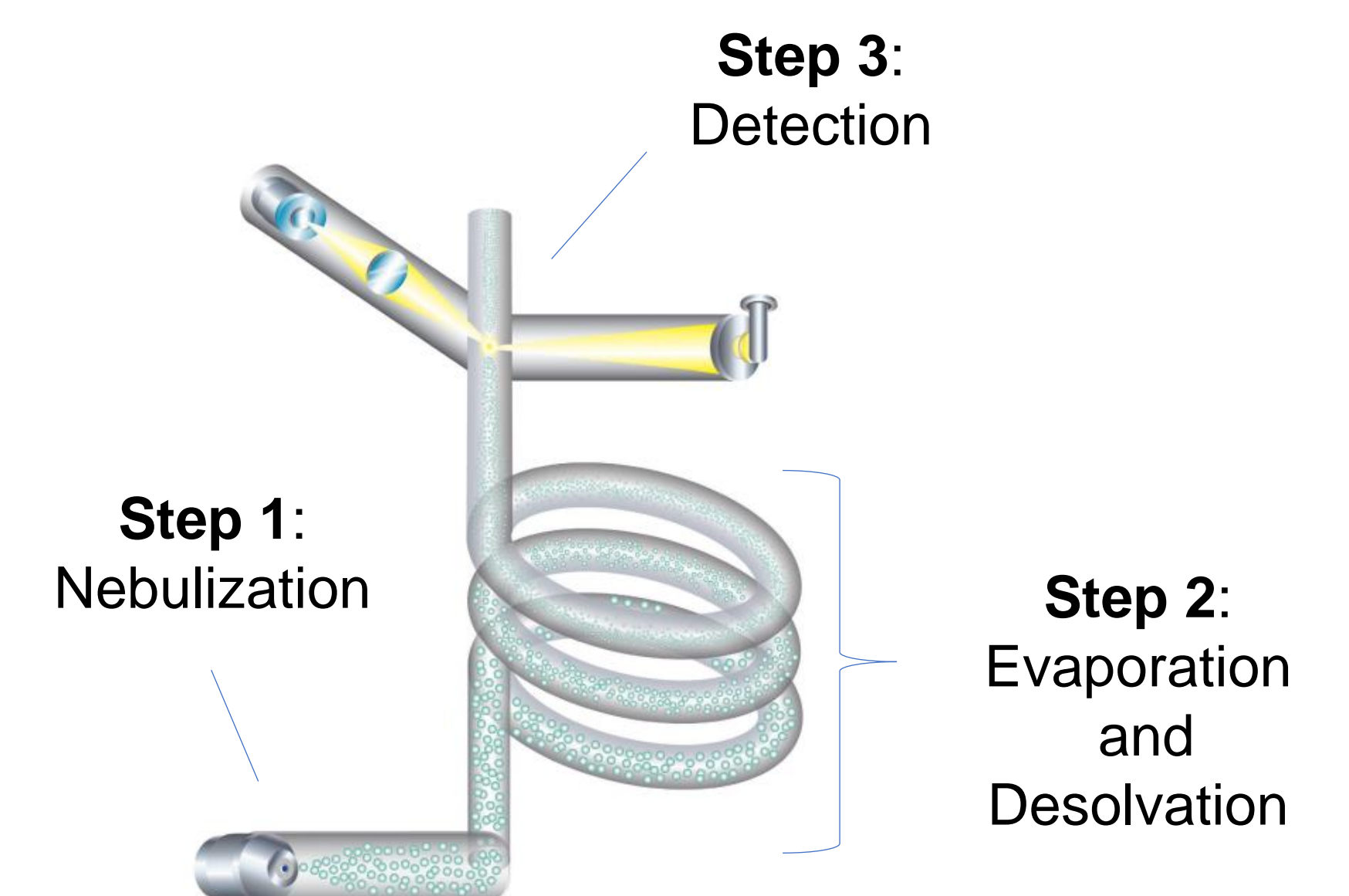ELSD
waste
Position 2 (ELSD)

Figure 2. A mixed-mode chromatographic column (Oasis™ Max, 30 µm, 2.1 × 20 mm column) was employed in a trap-and-elute configuration using Waters advance column manager (CM-A) to control a 2-position 6-port valve. Under acidic conditions (H_2O, 2% formic acid) the positively charged protein is passed to waste (valve position 1), while the PS is retained on the column. The valve is then switched to position 2, and the PS is eluted to the ELSD (Gain: 100, Power: 75%, Temp.: 80℃, Gas: 20 psi) using organic solvent (IPA, 2% formic acid).

RESULTS

Water blank

Step gradient   ELSD response

0.1 µg mass load

Polysorbate   Without baseline subtraction

10 µg mass load

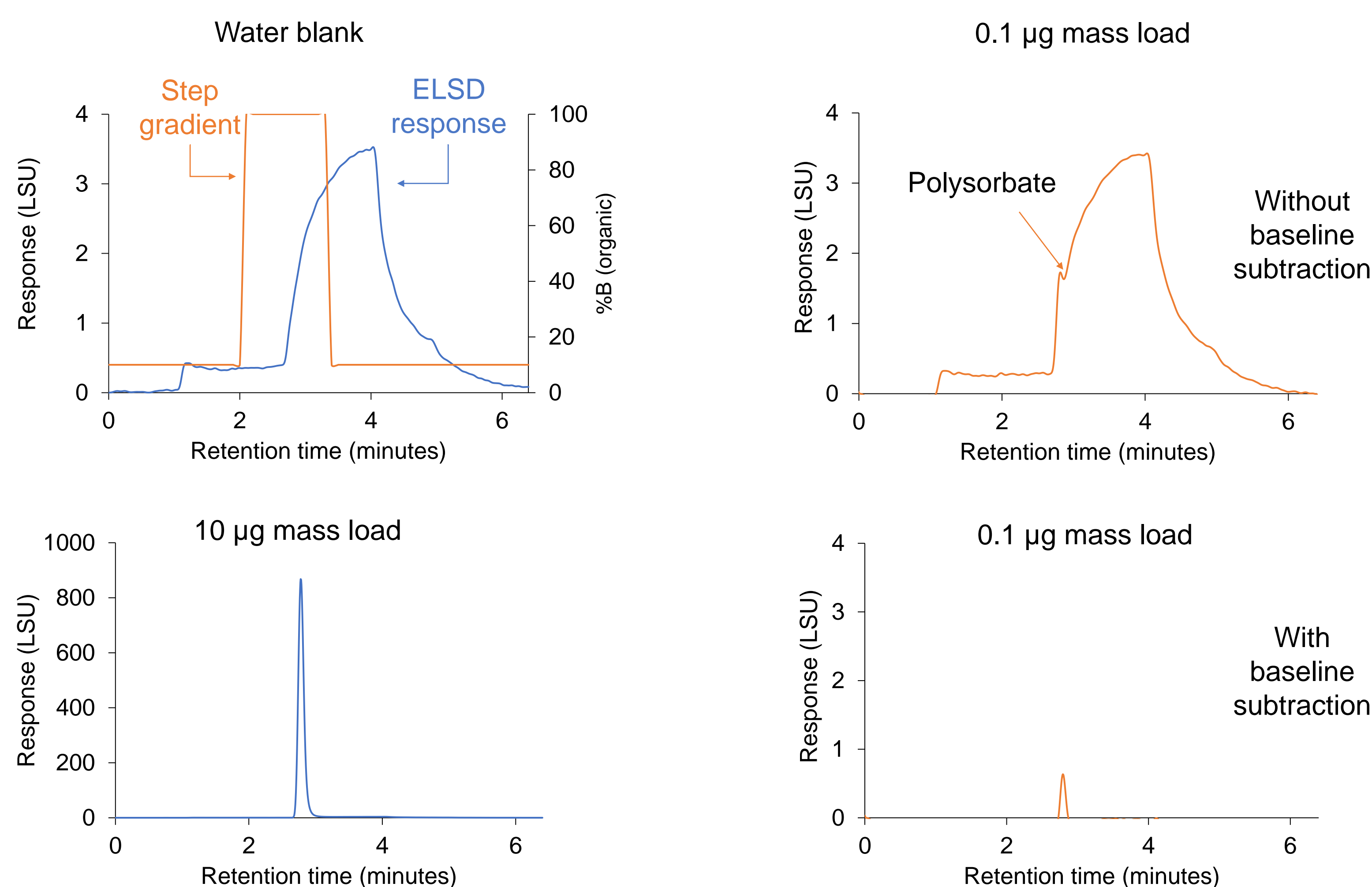0.1 µg mass load   With baseline subtraction

Figure 3. Baseline rise in step gradients can interfere with peak detection at low mass loads requiring manual curation of data which adds time to data processing and is prone to error as a subjective process. With baseline subtraction, the polysorbate peak is not affected by the basline rise and can be reliably detected and integrated in an automated fashion using a processing method. LC system: ACQUITY™ UPLC™ H-Class PLUS Bio System. Detector: 2424 evaporative light scattering detector.

Polysorbate 80 Results (baseline subtracted)					
Sample	Expected (mg/mL)	ELSD		CAD	
		Experimental (mg/mL)	RSD	Experimental (mg/mL)	RSD
Remicade™ sample	0.05	0.045	1.44%	0.029	3.63%
Avsola™ sample	0.05	0.045	2.48%	0.040	6.64%
Inflectra™ sample	0.05	0.044	1.04%	0.047	5.13%
Renflexis™ sample	0.05	0.042	1.48%	0.038	10.87%

PS80
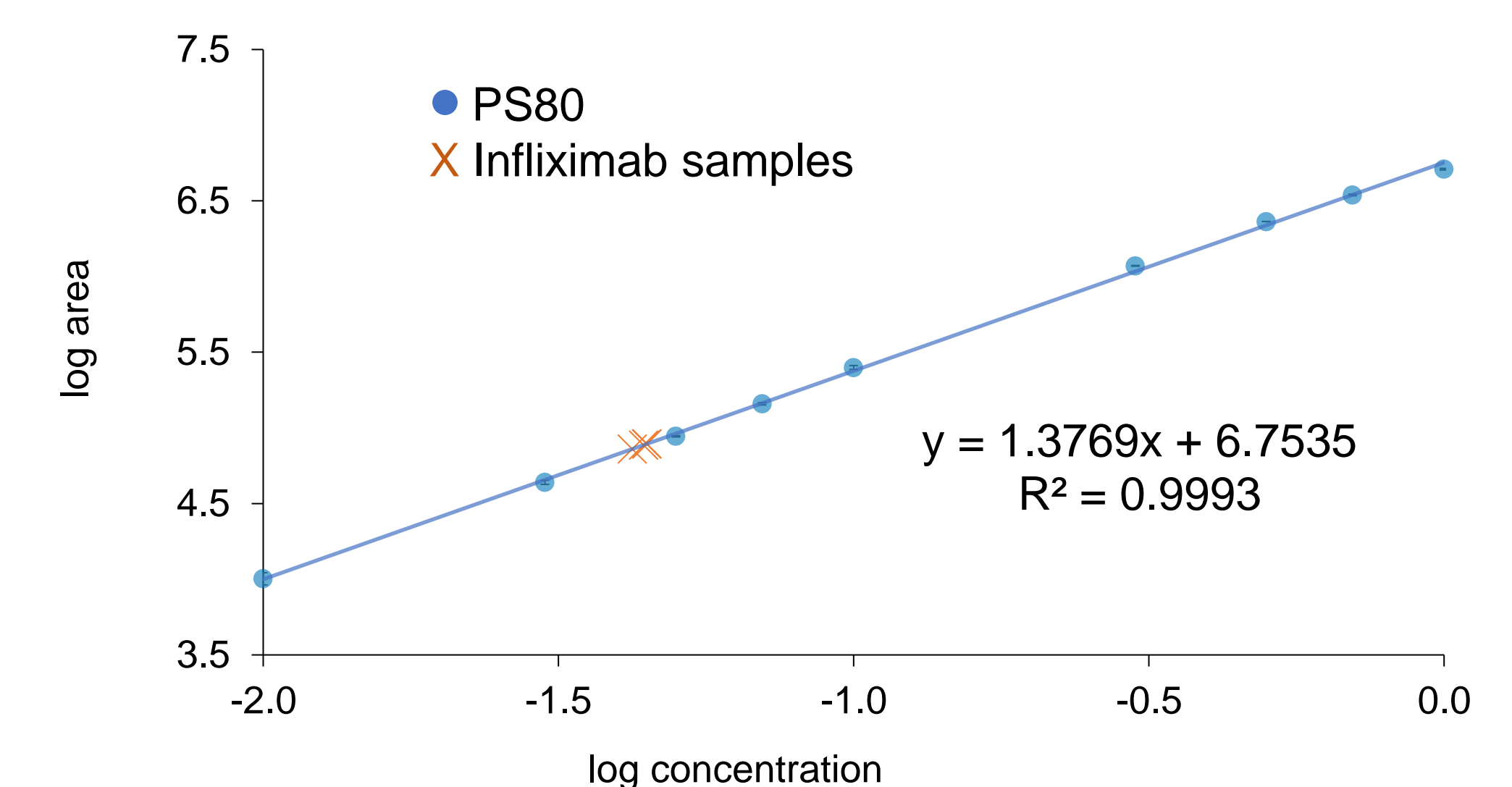X Infliximab samples

$y = 1.3769x + 6.7535$
$R^2 = 0.9993$

Figure 5. Using the baseline subtraction method, concentration of PS was determined in 4 Infliximab drug products. The proposed method was demonstrated to have better accuracy and reproducibility when compared to detectors such as charged aerosol detector (CAD).

Polysorbate 0.01-1 mg/mL

with subtraction $R^2 = 0.9722$
without subtraction
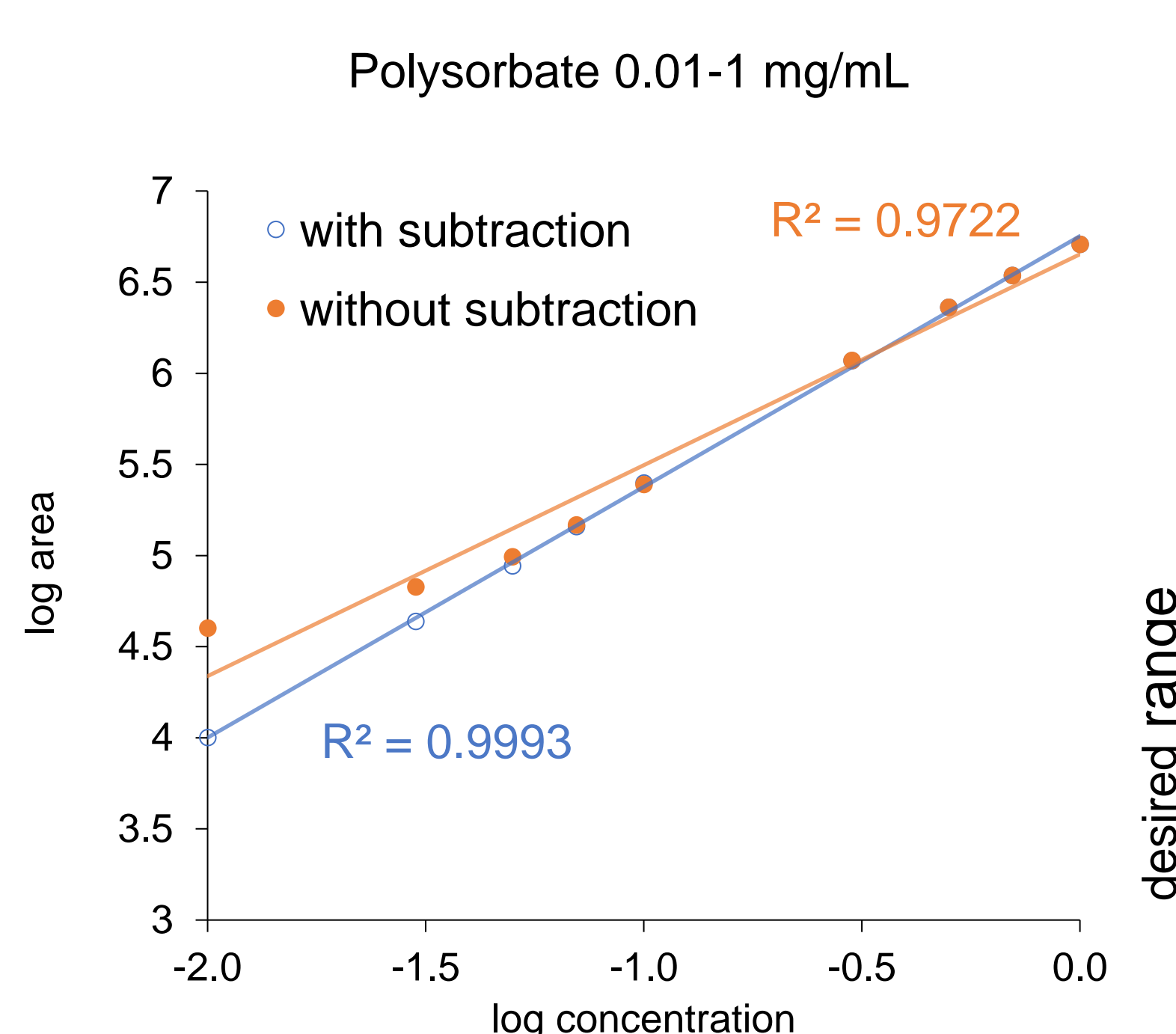
$R^2 = 0.9993$

Polysorbate (mg/mL)	% weight	Without baseline subtraction		With baseline subtraction	
		Response (R^2=0.9722)	RSD	Response (R^2=0.9993)	RSD
1.0	0.100	6.71	0.07%	6.71	0.08%
0.7	0.070	6.54	0.09%	6.54	0.09%
0.5	0.050	6.36	0.02%	6.36	0.02%
0.3	0.030	6.07	0.05%	6.07	0.04%
0.1	0.010	5.39	0.34%	5.40	0.23%
0.07	0.007	5.17	0.19%	4.16	0.14%
0.05	0.005	4.99	0.09%	4.94	0.07%
0.03	0.003	4.83	0.13%	4.64	0.29%
0.01	0.001	4.60	0.59%	4.00	1.02%

desired range

* Highlighted data required manual curation

Figure 4. When using baseline subtraction, the dynamic range is extended from one order (0.1-1 mg/mL) to two orders (0.01-1 mg/mL) allowing for detection and accurate quantitation of PS using a pre-defined processing method within the Empower™ 3 Software.

CONCLUSION

- ELSD can quantify intact polysorbate at formulation levels for biopharmaceuticals
- Baseline subtraction improves LOD for intact polysorbate analysis with ELSD
- Empower 3 Software provides an easy-to-use compliant-ready data processing solution for ELSD
- ELSD provides comparable performance to CAD in polysorbate analysis

References
- Han, D. et al. Comparing ELSD and CAD Performance on Polysorbate Quantification in Infliximab Drug Products, Waters Application Note, 2022. 720007501EN
- McCarthy, S. Quantification of Polysorbate Using the ACQUITY UPLC H-Class Bio System with ELS Detection, Waters Application Note, 2015. 720005323EN

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