Application note | 003506

Pharma/Biopharma

Characterization of polysorbate 80 in (bio)pharmaceuticals using HPLC-CAD

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Keywords

Polysorbate 80 (PS80), Charged Aerosol Detector (CAD), Vanquish Inverse Gradient LC System, (bio)pharmaceuticals, power value (PV)

Application benefits

- Characterize polysorbate 80 formulation through group-based quantitation applying the power value (PV) concept
- Identify the perfect linear response quickly using simultaneous acquisition of multiple channels with different PVs
- Facilitate automated peak area integration by eliminating unretained compounds using the diverter valve in the Thermo Scientific™ Vanquish™ Charged Aerosol Detector P series (CAD)

Goal

Investigating the use of the diverter valve in the CAD and the PV concept for detector response optimization in the profiling of polysorbate 80 formulations

Introduction

Polysorbate (PS) is a non-ionic surfactant commonly used in (bio)pharmaceutical products. It is a complex mixture comprising hundreds of molecules, a complexity that arises from the heterogeneous nature of the raw materials used in its synthesis and the synthetic processes involved in producing the final product. PS profiling is often accomplished using reversed-phase high-performance liquid chromatography (RP-HPLC). Because PS lacks relevant chromophores, the CAD is the preferred detection choice. A previous study developed an HPLC-CAD method to monitor the characteristics of polysorbate 80 (PS80) samples.[1]

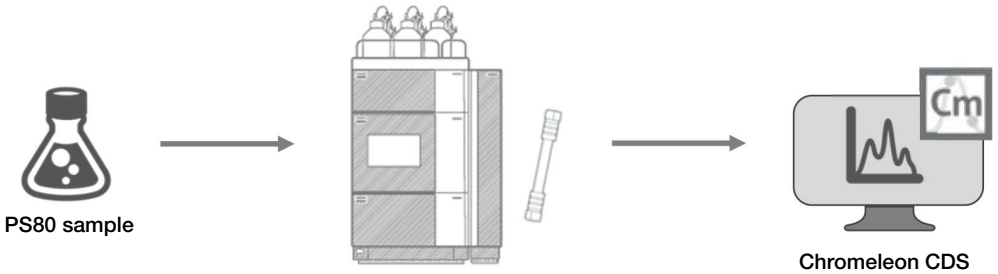

Figure 1. Schematic workflow of PS80 profiling

The purpose of this work is two-fold. First, to evaluate the use of the diverter valve to eliminate possible contamination from PS80 sample formulation ingredients. Second, to investigate the use of the PV, a parameter used to linearize response within a specific concentration range. Linearization is particularly important when evaluating relative peak areas, such as in PS80 profiling.

The workflow uses a Thermo Scientific™ Vanquish™ Flex Inverse Gradient LC system with Thermo Scientific™ Vanquish™ Charged Aerosol Detector HP and Thermo Scientific™ Accucore™ C18 column (Figure 1). Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) is used for data acquisition and processing.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification (P/N 50136149)
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955)
- Fisher Scientific™ Isopropanol, Optima™ LC/MS grade (P/N A461)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (P/N A117)
- Fisher Scientific™ Ammonium formate, Optima™ LC/MS grade (P/N A115)
- PS 80, histidine and sucrose were purchased from a reputable vendor.

Sample handling

- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes: 100–1000 µL (P/N 4641100N)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes: 10–100 µL (P/N 4641070N)
- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes: 1–10 µL (P/N 4641030N)
- Fisherbrand™ Mini Vortexer (P/N 14-955-152)
- Thermo Scientific™ Orion 3 Star™ pH Benchtop meter (P/N 13-644-928)
- Thermo Scientific™ SureSTART™ 2 mL Amber Glass Short Thread Screw Top Vials, 100/pack, Level 2 (P/N 6ASV9-2P)
- Thermo Scientific™ SureSTART™ Blue Polypropylene 9 mm AVCS™ Screw Caps with Soft Blue Silicone/Clear PTFE Septa, 100/pack, Level 3 (P/N 6PSC9ST101)

The Fisher Scientific product codes can be unique to different countries, the codes given above should be compatible across the EU and USA.

Instrumentation

Vanquish Flex Inverse Gradient LC system consisting of:

- Vanquish System Base Horizon/Flex (P/N VF-S01-A-02)
- Vanquish Dual Pump F (P/N VF-P32-A-01)
- Vanquish Split Sampler FT (P/N VF-A10-A-02)
- Vanquish Column Compartment H (P/N VH-C10-A-03)
- Vanquish Charged Aerosol Detector HP (P/N VH-D21-A-01)
- Workflow Kit, Vanquish Inverse Gradient LC systems (P/N 6036.2010)

Sample preparation

A stock solution of PS80 was prepared by dissolving 25 mg of PS80 in a 10 mL volumetric flask, and then filling it to the mark with ultrapure water. This stock solution was subsequently diluted with ultrapure water to prepare calibration standards at concentrations of 2.0 mg/mL, 1.5 mg/mL, 1.0 mg/mL, and 0.5 mg/mL.

A representative PS80 formulation was prepared by adding 4 mg histidine and 55 mg sucrose to 1 mL of 1 mg/mL PS80 stock solution.

Mobile phase preparation

Solvent A was prepared by dissolving ammonium formate in ultrapure water at a concentration of 5 mM. The pH was adjusted to pH 4.8 by adding formic acid to the solvent.

Tip: To prevent contamination by the pH electrode, avoid measuring the pH directly in the solvent bottle. Instead, test the pH using a small aliquot of the prepared solvent, and discard it after the measurement.

Chromatographic conditions

Table 1. Chromatographic conditions

Parameter	Value			
Column	Accucore C18 150 × 2.1 mm; 2.6 µm, P/N 17126-152130			
Solvent A	5 mM ammonium formate, pH 4.8			
Solvent B	50/50 isopropanol/acetonitrile (v/v)			
Gradient	Analytical		Inverse*	
	Time (min)	% B	Time (min)	% B
	0	9	0	100
	3	9	1.147	100
	10	22	4.147	100
	10	57	11.147	87
	21	69	11.147	52
	21	84	22.147	40
	26	85	22.147	25
	35	100	27.147	24
	45	100	36.147	9
	46	9	46.147	9
	56	9	47.147	100
			56	100
Flow rate	0.4 mL/min			
Column temperature	50 °C with active pre-heater at 50 °C, forced air mode, fan speed 5			
Autosampler temperature	10 °C			
Needle wash solution	10/90 water/isopropanol (v/v)			
Needle wash mode	Both (before and after draw)			
Injection volume	10 µL			
CAD settings	• Data collection rate: 20 Hz • Filter setting: 3.6 s, PV 1.5, PV 1.8, PV 2.25, PV 2.4 • Evaporation temperature: 50 °C • Diverter valve position: 0–1.3 min to waste, 1.3–56 min to nebulizer or 0–56 min to nebulizer			

* The inverse gradient was automatically calculated using the wizard in the Chromatography Data System (CDS) with calculation mode *"Keep solvent composition"*.

Chromatography Data System

Chromeleon CDS 7.3.2 was used for data acquisition and processing.

Results and discussion

The inverse gradient UHPLC-CAD method, as detailed in previous work,[1,2] was employed for profiling a PS80 formulation sample. When using an inverse gradient, the overall organic composition entering the detector in gradient mode remains the same, ensuring an accurate mass balance between species with different degrees of esterification. The target sample in this study was a model formulation that included PS80, histidine, and sucrose, but excluded the protein component. For an example of protein removal, refer to the work by Carnes *et al.,*[3] among many others. Histidine and sucrose, common formulation ingredients, are not retained on the Accucore C18 column. As illustrated in Figure 2, both compounds elute within the first minute of the chromatogram. Given that these compounds are not of interest, the diverter valve in the CAD can redirect this portion to waste, thereby minimizing the risk of contamination of the instrument. Figure 2 also depicts the PS80 profile in this sample, where group 1 includes free polyoxyethylene (POE), sorbitan-POE, and isosorbide POE. Groups 2, 3, and 4 represent compounds with an increasing degree of esterification. The identification of these groups has been previously published in the application note *"Polysorbate 80 profiling by HPLC with mass and charged aerosol detection."*[1]

Although it is not necessary to quantify every individual compound in PS80 formulations, group-based quantitation allows for effective monitoring of changes of PS80 in a formulation over time or evaluation of PS80 batch quality prior to use in a final product. Problematic degradative mechanisms observed in PS80 include oxidative or hydrolytic processes. The purity of the PS80 sample is evaluated by summing the peak area across all groups in the sample. Thus, the entire chromatogram, from group 1 through group 4, is integrated and the peak area assessed as a single value.

While the CAD is known as a non-linear detector, the signal can be "linearized" in a certain concentration range by applying a specific PV. The ideal PV for a given concentration range is found by acquiring data with four PVs simultaneously using four discrete channels in the instrument method (Figure 3). Four different PVs can be set to four different channels in the instrument method and acquired simultaneously (Figure 3). In this work, channels for PV of 1.5 (lowest possible value), 1.8 (default value), 2.25, and 2.4 (highest possible value) were acquired all together in the same run. Calibration ranged from 0.5 mg/mL up to 2.5 mg/mL PS80 in water, and each calibration point was measured in triplicate. Table 2 presents the coefficient of determination (R^2) for the four measured PVs. Although the variations in R^2 for a linear curve fit type are not significant for this particular application, the residual error (relative amount deviation) indicates that the lower end of the calibration curve achieves the best linearity at a PV of 1.8, with a residual error of less than 4%.

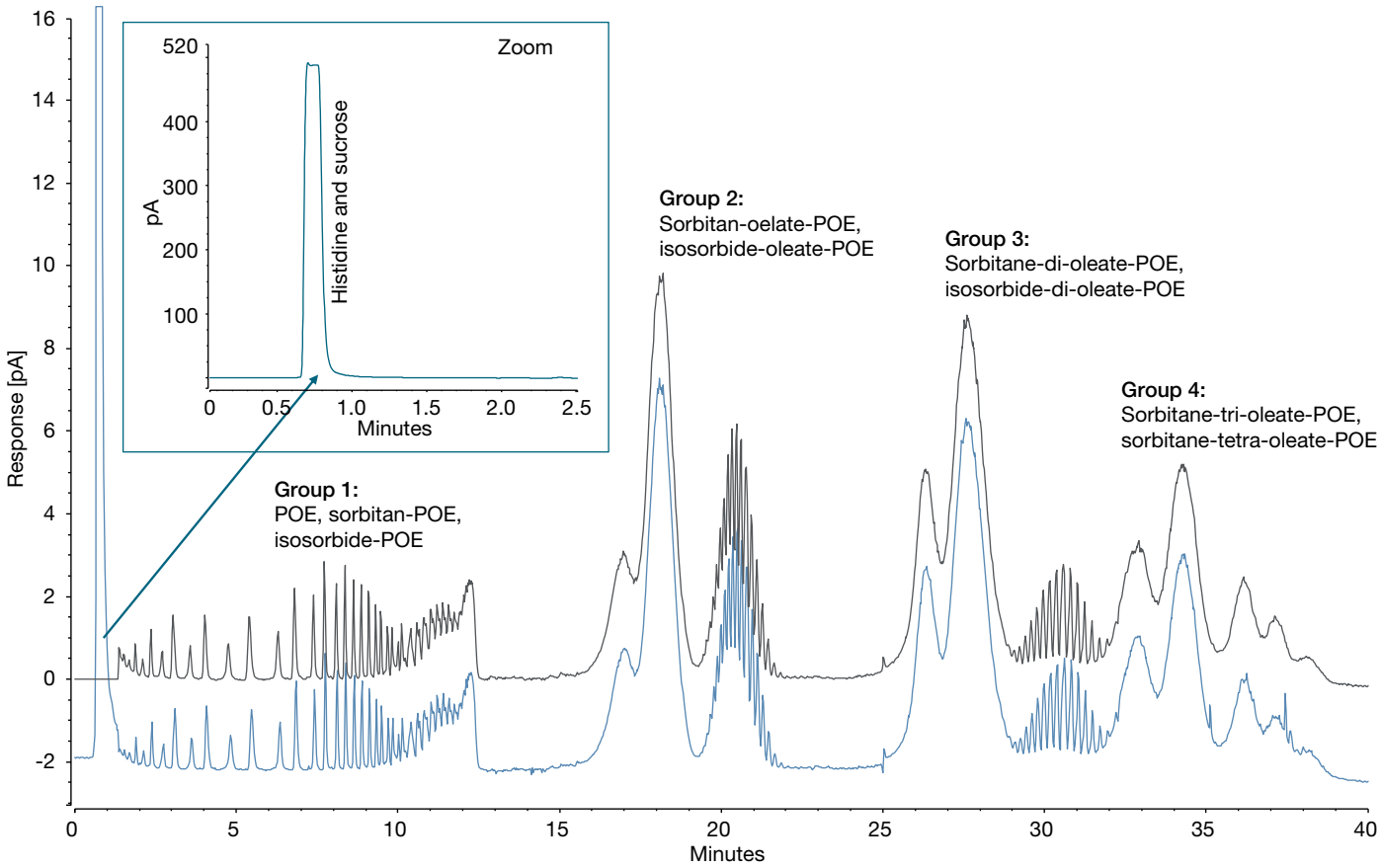

Figure 2. Overlaid chromatograms (with signal offset 10%) of the model formulation sample (1 mg/mL PS80 + 4 mg/mL histidine + 55 mg/mL sucrose) run by the method outlined in Table 1. Black trace: Diverter valve to waste from 0 to 1.3 min and to nebulizer from 1.3 to 56 min; blue trace: diverter valve to nebulizer 0–56 min. The peak in the first minute of the blue chromatogram refers to histidine and sucrose. PS80 profiling group assignments are based on reference 1.

Channel settings | Timetable

Channel start settings:

No	Channel	Power Value	Acquisition on [min]	Acquisition off [min]
1	☑ CAD_1	1.80	Start Run	Stop Run
2	☑ CAD_2	2.25	Start Run	Stop Run
3	☑ CAD_3	1.50	Start Run	Stop Run
4	☑ CAD_4	2.40	Start Run	Stop Run

Figure 3. CAD channel settings in instrument method. Four power values (PVs) can be measured simultaneously in one single run.

Table 2. Coefficient of determination at different PVs. Value was obtained for total peak area of all four groups and a 5-point calibration curve (n=3) with curve fit type: linear.

Coefficient of determination (R^2)			
PV 1.5	PV 1.8	PV 2.25	PV 2.40
0.9976	0.9997	0.9987	0.9973

Figure 4 illustrates the assessment of the residual error in concentration-error plots. If the residual error is “0”, represented by the horizontal target line (pink), then the measured calibration point is in ideal alignment with the calibration curve. The further a point deviates from this target line, the greater the residual error and the poorer the linear fit. As a result, the calibration curve with PV 1.8 was used to determine that the purity of PS80 in the sample was 96.2%.

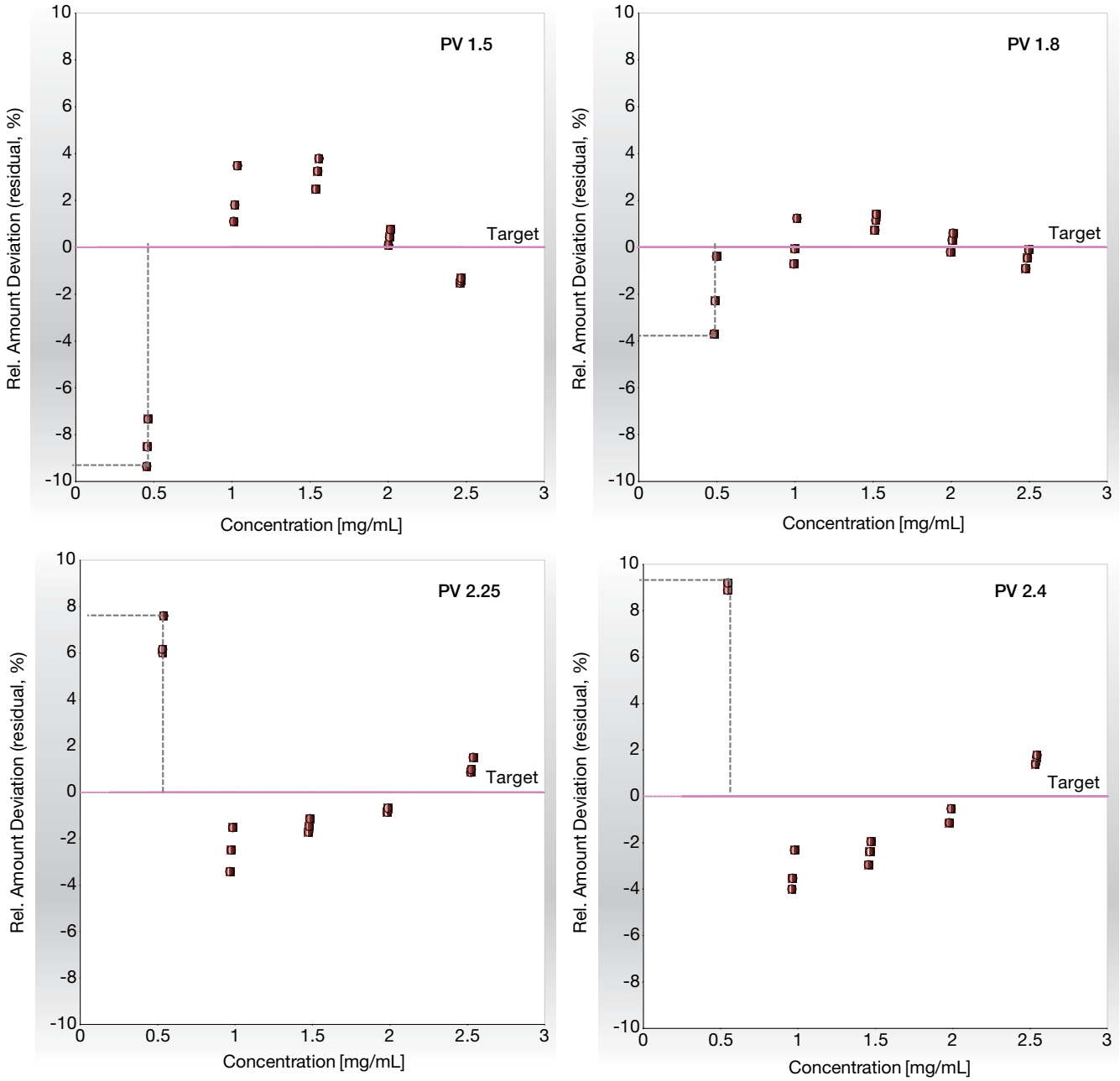

Figure 4. Residual error plots of the four measured PVs. The smaller the residual error, the closer the measured value is compared to the value estimated by the calibration curve. In addition to R^2, this also provides information about the linearity in a certain area of the calibration range. For further details on creating residual error plots in Chromeleon CDS, refer to Appendix B of the technical note in reference 4.

The PS80 formulation is characterized by determining relative peak areas found in the elution profile. This approach is used for lot-to-lot comparisons to identify any variation in PS80 raw material in formulation development or quality control laboratories.[1,2] The inverse gradient allows for a uniform detector response, resulting in a real mass balance among species with different levels of esterification. An optimized linearization of response curve is mandatory for this determination to avoid problems with accuracy at the lower concentration range, as would be the case with a quadratic fit. Table 3 shows the result of peak groups along with their respective relative peak areas and relative standard deviation (RSD) in the sample. Diversion of the histidine and sucrose peak to waste using the integrated valve in the CAD facilitated the automated integration of the first group by allowing for more precise identification of the integration starting point. In contrast, when the histidine and sucrose peak were allowed to enter the detector, the integration of the first group was only manually possible but inconsistent due to baseline variation caused by these compounds. Consequently, the first group showed a 3-fold improvement in RSD peak area when the diverter valve was used, as compared to runs where the histidine and sucrose peak entered the detector (data not shown).

Table 3. Relative peak areas and RSD of the peak groups in PS80 formulation. The data is the average of three injections.

	Relative peak area [%]	RSD rel. area [%]
Group 1	12.48	0.79
Group 2	34.53	1.56
Group 3	30.67	1.02
Group 4	22.32	2.81

Conclusion

The Vanquish Flex Inverse Gradient LC system, equipped with a Vanquish Charged Aerosol Detector P series and its capabilities, proved to be an optimal choice for profiling PS80 formulations.

- The diversion of the matrix peaks to waste not only improves robustness, by avoiding contamination of the detector, but also increases productivity, by facilitating automated peak area integration while eliminating interference from early eluting analytes.
- Four different PVs (1.5, 1.8, 2.25, and 2.4) were measured simultaneously as separate channels in the Chromeleon CDS to easily identify the optimum linearity with PV 1.8 and with a residual error below 4% for all calibration points. The PV of 1.8 is the default setting for the Vanquish Charged Aerosol Detector P series.
- The purity of PS80 samples was assessed by summing the peak areas across all four groups and was determined to be 96.2%.
- The PS80 formulation was characterized by determining the relative peak area of the four separated peak groups.

References

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2. De Pra, M.; Ispan, D.A.; Meding, S.; Müllner, T.; Lovejoy, K.S.; Grosse, S.; Cook, K.; Carillo, S.; Steiner, F.; Bones, J. Degradation of polysorbate investigated by a high-performance liquid chromatography multi-detector system with charged aerosol and mass detection, *Journal of Chromatography A* **2023,** *1710,* 464405. https://www.sciencedirect.com/science/article/pii/S0021967323006301
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4. Thermo Fisher Scientific Technical Note 73299: Charged Aerosol Detection – Use of the power function and robust calibration practices to achieve the best quantitative results, **2019,** Appendix B.

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