Pharma and biopharma

Characterization of polysorbate 80 in (bio)pharmaceuticals using HPLC with charged aerosol detection

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Abstract

Purpose: This work demonstrates the characterization of a polysorbate 80 (PS80) formulation based on the relative peak areas of the peak groups in the sample. The key objectives are:

1. Achieve uniform detector response, ensuring real mass balance among species with varying esterification levels
2. Enable optimized linearization for calibration curves in a single run, reducing time and solvent consumption by using advanced detector settings
3. Divert matrix ingredients (e.g., histidine, sucrose) to waste, enhancing robustness and productivity by preventing detector contamination and facilitating automated accurate peak area integration

Methods: 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.

Results: The Vanquish Flex Inverse Gradient LC system with the Vanquish Charged Aerosol Detector P series enabled effective profiling of polysorbate 80 formulations. Matrix peak diversion to waste improved robustness and productivity by eliminating early eluting analytes to allow for more precise automated integration. Optimal linearity was achieved at power value (PV) 1.8 with residual error below 4%, and PS80 purity was determined to be 96.2%. The relative peak area of four separated groups provided comprehensive formulation characterization, essential for lot-to-lot comparisons to identify any variations in PS80 raw material.

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. Considering PS80, the product is obtained by esterification of oleic acid with sorbitan polyoxyethylene (POE). The oleic acid originates from natural sources and contains other fatty acid impurities including but not limited to palmitic, linoleic, and stearic acids. These impurities will participate in the esterification reactions, thereby increasing complexity. The additional presence of the precursor and side product of sorbitan, respectively sorbitol and isosorbide, along with different degrees of ethoxylation of main and by-products, results in a mixture of hundreds of components. PS profiling is often accomplished using reversed-phase high-performance liquid chromatography (RP-HPLC). While quantification of every component of a PS formulation is not required, a simple analytical technique capable of profiling the main features of the PS sample and enabling lot-to-lot comparison is highly desirable.

Because PS and its impurities lack relevant chromophores, the charged aerosol detector (CAD) is the preferred detection choice. A previous study has shown that an HPLC-CAD method with an inverse gradient is capable of resolving PS80 components based on the degree of esterification. In combination with a single quadrupole mass spectrometer, the identity of the main components was confirmed.[1]

This study characterizes a PS80 formulation through group-based quantitation using RP-HPLC-CAD.[2] By investigating the use of the diverter valve in the CAD and the power value (PV) concept[3] for detector response optimization, this research shows improved accuracy and efficiency, ultimately contributing to better quality control and formulation development in pharmaceutical and biochemical applications.

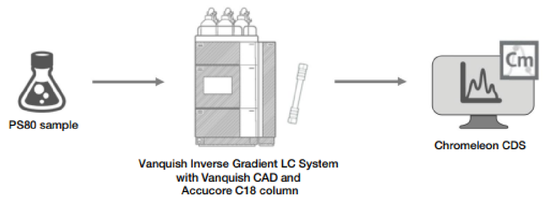

Figure 1. Schematic workflow of PS80 profiling.

Materials and methods

Sample Preparation

A stock solution of PS80 was prepared by dissolving 25 mg of PS80 in a 10 mL volumetric flask and 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.

Test Method(s)

Table 1 shows the chromatographic conditions of the RP-HPLC-CAD method for PS80 analysis.

Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.3.2 was used for data acquisition and processing.

Table 1. Chromatographic conditions.

Parameter	Value			
Column	Accucore C18 150 x 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 preheater 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)			
Injection volume	10 µL			
CAD settings	Data collection rate: 20 Hz			
	Filter setting. 3.6 s			
	Power values: 1.5, 1.8, 2.25, 2.4			
	Evaporation temperature: 50° C			
	Diverter valve position: 0 – 1.3 min to waste, 1.3 – 56 min to nebulizer			

* The inverse gradient was automatically calculated using the wizard in the CDS with calculation mode “Keep solvent composition”

Results

Eliminating Matrix Peaks

The target sample in this study was a model formulation that included PS80, histidine, and sucrose, but excluded the protein component. 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.

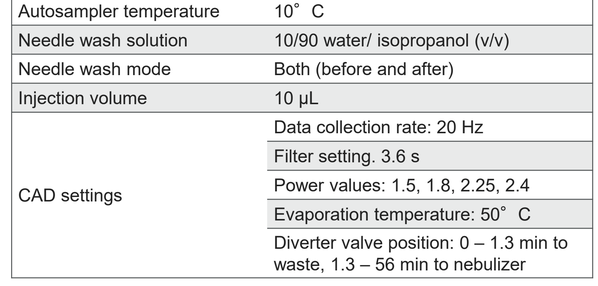

Figure 2. Left: integrated diverter valve in the Vanquish CAD P used to switch between waste and nebulizer and right: Overlaid chromatograms (with signal offset 10%) of the model formulation sample (1 mg/mL PS80 + 4 mg/mL histidine + 55 mg/mL sucrose) run by the method outlined in Table 1. Black trace: 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.

Optimizing linearization for calibration

Calibration ranged from 0.5 mg/mL up to 2.5 mg/mL PS80 in water, and each calibration point was measured in triplicate. 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. Four different PVs can be acquired at once as four different channels in the instrument method of the Vanquish CAD P Series (Figure 3). Technical details on the PV concept can be found in Reference 3.

Figure 3. CAD channel settings in instrument method editor. Four PVs can be measured simultaneously in one single run.

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 4).

Linearization is particularly important when evaluating relative peak areas, such as in PS80 profiling.

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

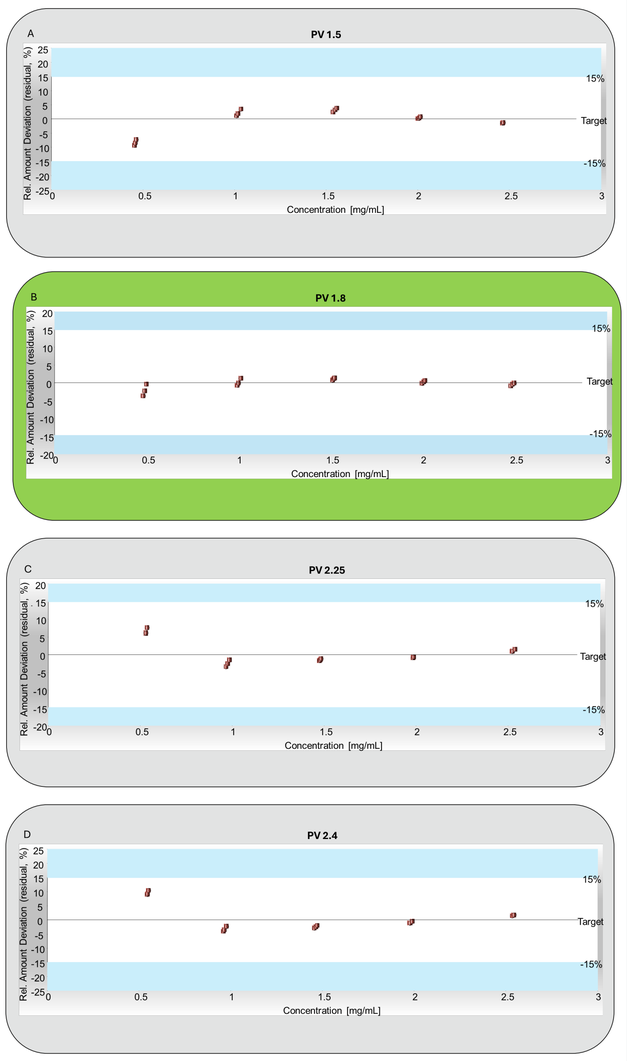

Figure 4. Residual error plots of the four measured PVs. The smaller the residual error, the closer the measured value is compared to the value estimated by the calibration curve. In addition to R^2, this also provides information about the linearity in a certain area of the calibration range. For further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

Mass balance and sample purity assessment

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.[4,5] The inverse gradient allows for a uniform detector response, resulting in a real mass balance among species with different levels of esterification. Table 3 shows the result of peak groups along with their respective relative peak areas and relative standard deviation (RSD) in the sample.

The assessment of PS80 sample purity is done by summing the peak areas across all four groups. The purity of PS80 in the sample was found to be 96.2%.[2]

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

Conclusions

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 to waste not only improves robustness, by avoiding contamination of the detector, but also increases productivity, by facilitating reliable 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. Thermo Fisher Scientific Application Note 003506: Characterization of polysorbate 80 in (bio)pharmaceuticals using HPLC-CAD, **2025**
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