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Biopharma

Simultaneous quantification of poloxamer 188 and polysorbate 80 in biopharmaceutical formulations using charged aerosol detection

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Keywords

Vanquish Core HPLC, charged aerosol detector (CAD), surfactant, poloxamer 188, polysorbate 80, Acclaim Surfactant Plus column, Chromeleon CDS, recombinant protein

Application benefits

- A single, simple, accurate, and reproducible method for quantification of poloxamer 188 and polysorbate 80 in biopharmaceutical formulations
- Thermo Scientific™ Acclaim™ Surfactant Plus column provides good separation and peak shape for poloxamer 188 and polysorbate 80
- Thermo Scientific™ Chromeleon™ CDS provides a simple approach to automatically subtract the chromatogram baseline
- Linear range was expanded by optimizing the power function value of CAD

Goal

Development and validation of an accurate, sensitive, and reproducible HPLC-CAD method for the simultaneous determination of poloxamer 188 and polysorbate 80 in biopharmaceutical formulations

Introduction

Surfactants play a key role in stabilizing protein-based formulations through manufacturing, storage, and transportation. As of 2018, more than 75% of the European Medicines Agency-approved liquid protein formulations contained surfactants. Almost 50% of these liquid products are formulated with polysorbate 80 (PS80), 40% with polysorbate 20 (PS20), and approximately 10% with poloxamer 188 (P188).[1] Polysorbates (PS) are synthetic nonionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan, which dominate the group of surfactants in protein-based formulations due to their excellent stabilizing properties for proteins. However, ester bonds as well as unsaturated moieties in polysorbates make them susceptible to degradation by hydrolysis and oxidation in liquid formulations. The concerns regarding the stability and degradation products of PS have risen rapidly in recent years. [2,3] Although not as widely used as PS, P188 has emerged as an alternative solubilizing agent and surfactant used in biopharmaceutical products, regarded as more stable and safer in formulations.[4] To reduce the potential risk caused by a single surfactant, the use of a mixture of polysorbate and poloxamer 188 in biopharmaceutical formulations has also been reported.[5,6]

To ensure the safety and efficacious quality control of surfactants containing drug products and meet the regulatory requirements to specify the composition and content of drug products, the accurate and sensitive quantification of these surfactants is particularly important. However, it can be challenging to develop methods for the quantification of PS and P188. There are no chromophores in their structures, which is why common UV/VIS detection is not possible. Additionally, both commercially available P188 and PS are complex mixtures of different chemical variations of the parent structures. To improve the sensitivity, it is preferred to elute them as single peaks respectively in the method.

Here, we demonstrate a HPLC-CAD method for the simultaneous quantification of P188 and PS80 in biopharmaceutical formulations. CAD is a universal detection technique that can be used to detect non-volatile and some semi-volatile compounds with or without a UV chromophore, which makes it ideal for surfactants analysis. An Acclaim Surfactant Plus column, which is specifically designed for this type of analysis, was used in this method. By using this column, the main compounds of PS80 and P188 were eluted as single peaks with excellent peak shape and outstanding selectivity. The method linear range, limit of detection (LOD), limit of quantification (LOQ), accuracy, and reproducibility were further assessed during method development and validation.

Experimental

Instrumentation

- Thermo Scientific™ Vanquish™ Core HPLC system consisting of:
  - Vanquish System Base Core (P/N VC-S01-A)
  - Vanquish Quaternary Pump CN (P/N VC-P21-A)
  - Vanquish Split Sampler CT (P/N VC-A12-A)
  - Vanquish Column Compartment C (P/N VC-C10-A)
  - Vanquish Charged Aerosol Detector F (P/N VF-D20-A)
- Vanquish 6-position, 7-port Switching Valve (P/N 6036.2530)
- Thermo Scientific™ Sorvall™ Legend™ Micro 21R centrifuge (P/N 75002447)

Software

- Chromeleon Chromatography Data System (CDS), Version 7.3

Reagents and consumables

- The water used was purified by a Thermo Scientific™ Barnstead™ GenPure™ Pro water purification system with a resistivity 18.2 MΩ·cm or higher.
- Isopropanol (IPA), Optima™ LC/MS grade, Fisher Chemical™ (P/N A461-4)
- Formic acid, Optima™ LC/MS grade, Fisher Chemical™ (P/N A117-50)
- Gibco™ Pluronic™ F-68 (P188) 10% solution, (P/N 24040032)
- Thermo Scientific™ SureSTART™ 2 mL glass vials (amber), (P/N 6ASV9-2P)
- Thermo Scientific™ SureSTART™ 9 mm vial caps with septum (P/N 6ASC9ST1)
- PS80 and recombinant protein samples were provided by National Institutes for Food and Drug Control (NIFDC), China.

Sample preparation

Use glass pipettes, inserts, vials, and bottles to transfer, prepare, and store PS80 and P188 solutions/samples, as PS80 can adsorb to plastic pipettes and vials.

Stock solutions were prepared in a 5 mL brown glass bottle to a final concentration of 5.0 mg/mL by diluting the PS80 and P188 with deionized water.

Standard solutions were prepared by diluting the stock solution with deionized water. The concentrations of the standard solutions were 5.0 µg/mL, 10.0 µg/mL, 25.0 µg/mL, 50.0 µg/mL, 80.0 µg/mL, 100.0 µg/mL, 250.0 µg/mL, 400.0 µg/mL, 500.0 µg/mL.

Sample solutions were prepared by diluting protein samples with deionized water to an appropriate concentration. The solution was then centrifuged at 4,000 rpm for 10 minutes, and the supernatant was used for injection.

Table 1. Chromatographic conditions

Parameter	Value		
Column	Acclaim Surfactant Plus, 3 µm, 4.6 × 150 mm (P/N 078950)		
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in isopropanol		
Gradient	Time (min)	%A	%B
	0	80	20
	1.8	80	20
	2.0	67	33
	3.0	67	33
	3.5	0	100
	8.5	0	100
	9.0	80	20
	17.0	80	20
Flow rate	0.6 mL/min		
Column temperature	25 °C		
Autosampler temperature	4 °C		
Injection volume	10 µL		
Needle wash solvent	100% water		
Detection	Evaporation temperature: 50 °C Power function value: 1.25 Data collection rate: 10 Hz		

Results and discussion

P188 are block copolymers that consist of a hydrophobic chain of poly (propylene oxide) flanked by two hydrophilic blocks of poly (ethylene oxide). On average, the number of poly (propylene oxide) ranges from 25 to 30, and the poly (ethylene oxide) block is composed of 75 to 85 ethylene oxide units.[4] The typical manufacturing process for PS80 begins with anhydrization of sorbitol and is followed by esterification with oleic acid and ethoxylation with ethylene oxide (EO) in excess molar ratio for each mole of sorbitol or sorbitol anhydride. Each step generates a complex mixture of products that contribute to the heterogeneity of PS80.[6] The chemical structures of the main compounds of P188 and PS80 are shown in Figure 1.

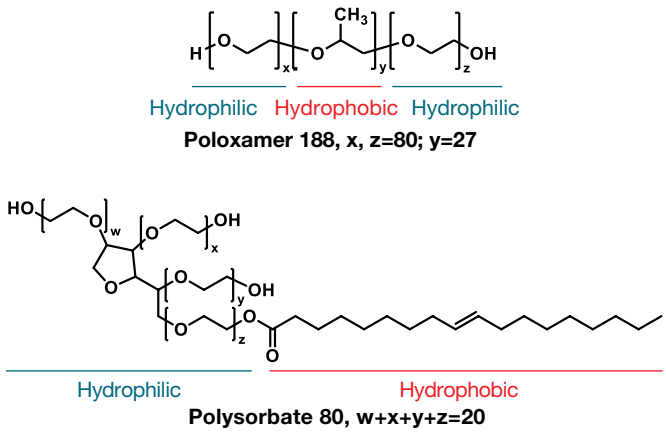

Figure 1. Chemical structures of the main compounds of poloxamer 188 and polysorbate 80

To elute PS80 and P188 as single and sharp peaks, isopropanol, which has a strong elution strength, was used. The chromatographic conditions and CAD settings are shown in Table 1. The gradient starts with 20% isopropanol, which is used to remove the unretained positively charged protein, and then increases to 33% and held for 1 minute to elute other commonly used excipients such as sucrose, lactose, and mannitol. The hold time is kept to 1 minute to avoid peak splitting and broadening for PS80 with longer hold times. Then, for the consecutive elution of P188 and PS80, solvent B (0.1% formic acid in isopropanol) is increased to 100% rapidly in 0.5 minutes and held for 5 minutes. The gradient change also causes the fluctuation of the baseline, as shown in Figure 2A. The rise of baseline at 7.5 minutes results in a split peak for the low concentration of PS80, making it less practical to use the automatic integration process. This issue could be resolved using the automated baseline subtraction feature in Chromeleon CDS. The chromatogram after baseline subtraction is shown in Figure 2B. As can be seen, the effects of baseline rise are fully eliminated.

The peak eluting at 2.78 min in PS80 standard solution was identified as the mixture of unesterified polyoxyethylene (POE), polyoxyethylene sorbitan(sorbitan-POE), and polyoxyethylene isosorbide (isosorbide-POE) by single quadrupole mass detection (data not shown). These compounds lack the hydrophobic group and amphiphilic properties and are regarded as by-products due to the incomplete esterification or ester interchange reactions in manufacturing process. The ratio of POE, sorbitan-POE, and isosorbide-POE varies among different batches of PS80, so it is necessary to use the same batch of PS80 for quantitative analysis.

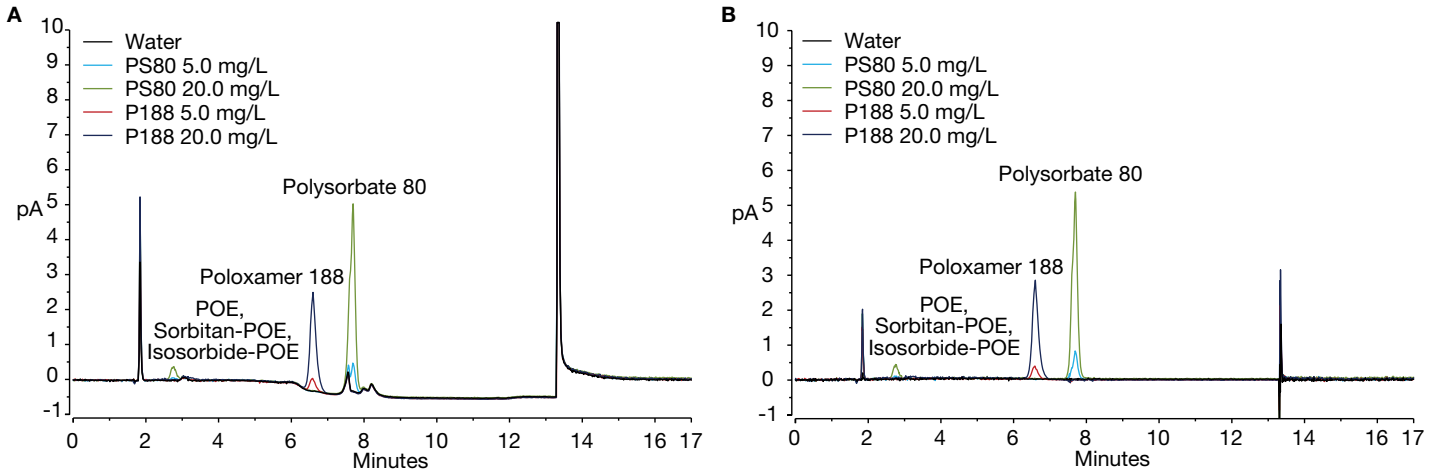

Figure 2. Effects of baseline subtraction. (A) Baseline rise affects the peak shape and automatic integration process for low concentration of polysorbate 80. (B) After baseline subtraction in Chromeleon CDS, the effects of baseline rise were eliminated.

The response of CAD over a wide range of analyte concentrations is non-linear, while for a narrow range (1.5 to 2 orders), CAD response can be treated as linear. For a given method, it is possible to linearize the response for the analyte concentration range of interest by optimizing the power function value (PFV). Chromeleon CDS provides a power law processing functionality that can apply a new PFV to an existing channel with the data points recalculated and simulated and the results being reported in a new channel. Therefore, analysts do not need to inject samples with every PFV they want to optimize. The linearity and R^2 value of P188 and PS80 ranging from 5 mg/L to 100 mg/L with different PFV are shown in Figures 3A and 3B. For PFV 1.2 and 1.3, it was observed that P188 and PS80 have the highest degree of linearity (R^2>0.999) between CAD response and concentration. As a second optimization step, standard solutions were injected using PFV 1.20, 1.25, and 1.30 to find the optimal value. Finally, PFV 1.25 was chosen as the best condition. The linear regressions for P188 and PS80 ranging from 5 mg/L to 100 mg/L using PFV 1.25 are shown in Figures 3C and 3D. R^2 for the regression of P188 and PS80 were 0.9997 and 1.000, respectively.

This linearity range covers the concentration as low as 5.0 mg/L, about 0.0005% in formulations. For high concentration samples (greater than 100 mg/L), only dilution was needed or the use of a nonlinear fit (quadratic fit). The results for a nonlinear fit are shown in Figures 3E and 3F, which can be used to quantitively analyze for P188 and PS80 up to 500 mg/L. This range is suitable for almost all biopharmaceutical formulations in the market.

Limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting diluted solutions with known concentration and calculating the signal-to-noise ratio (S/N). Minimum levels with the S/N above 3 and 10 were defined as LOD and LOQ, respectively. LOD and LOQ for P188 in this method were 2.0 mg/L (S/N = 8.7) and 5.0 mg/L (S/N=18.6), and for PS80 were 1.0 mg/L (S/N=5.6) and 2.0 mg/L (S/N=17.1).

Accuracy was assessed by preparing triplicate protein samples spiked with P188 and PS80 at three levels. Recovery was calculated and used to evaluate the accuracy. Results are shown in Table 2. Recovery values for P188 ranged from 96.8% to 110.0% with all relative standard deviation (RSD) lower than 5.0% at three levels, and the recovery for PS80 ranged from 95.7% to 99.01% with all RSD>5.0% at three different levels.

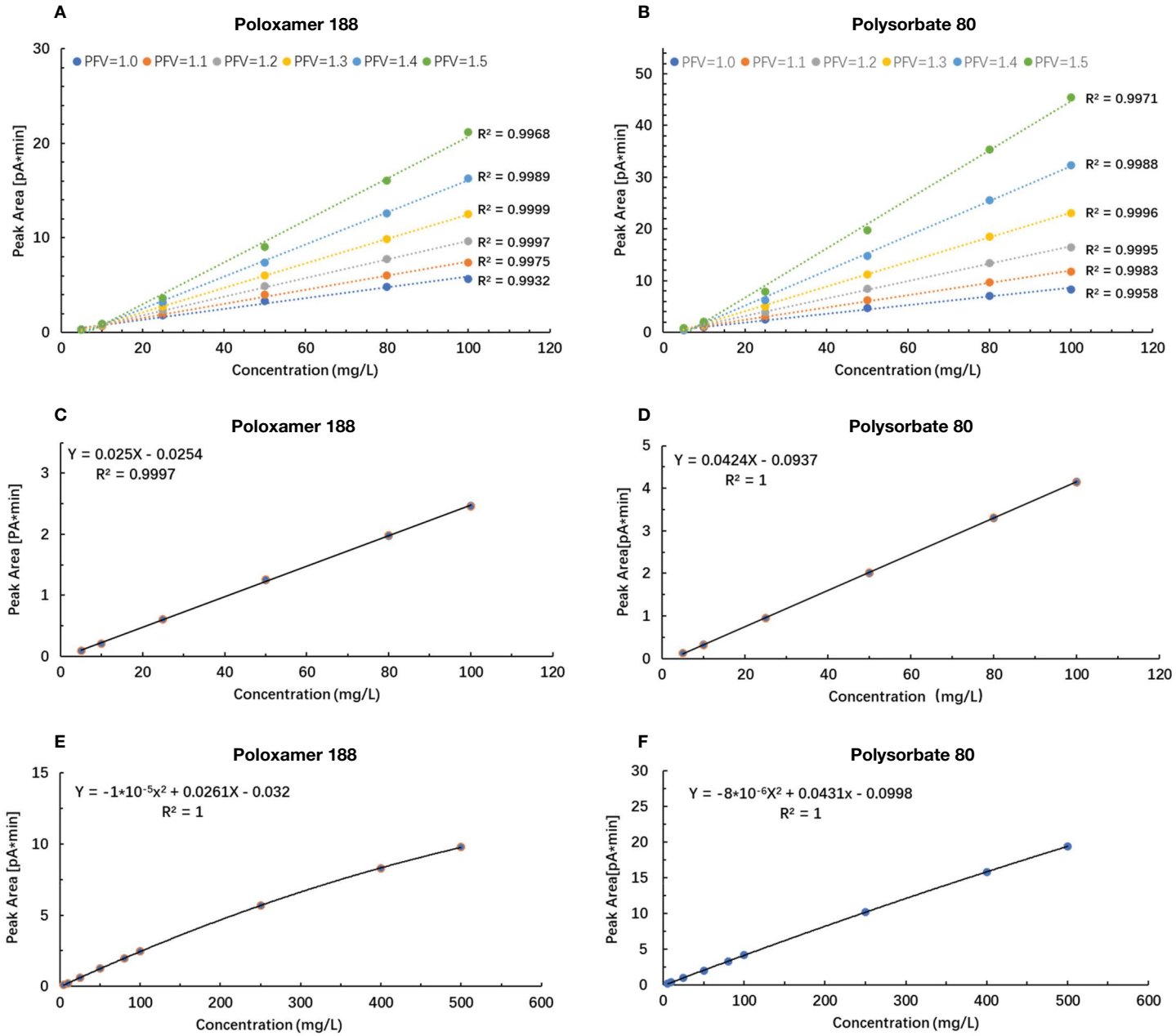

Figure 3. Calibration curves for P188 and PS80. The linearity regression for P188 (A) and PS80 (B) ranged from 5 mg/L to 100 mg/L by using different power function values. The linearity regression for P188 (C) and PS80 (D) ranged from 5 mg/L to 100 mg/L with PFV 1.25. The nonlinear fit (quadratic fit) for P188 (E) and PS80 (F) ranged from 5 mg/L to 500 mg/L with PFV 1.25.

Table 2. Recovery results of poloxamer 188 and polysorbate 80 (n=3)

Level	Added amount (mg/L)	Poloxamer 188			Polysorbate 80		
		Detected amount (mg/L)	Recovery (%)	RSD of recovery (%)	Detected amount (mg/L)	Recovery (%)	RSD of recovery (%)
Low concentration (10 mg/L)	10	11.0	110.0	2.16	9.57	95.7	3.14
Middle concentration (40 mg/L)	40	38.7	96.8	1.58	39.60	99.01	0.90
High concentration (80 mg/L)	80	82.5	103.1	0.45	79.14	98.92	0.66

Repeatability was assessed by preparing three spiked protein samples at three levels on three different days and injecting into two different HPLC systems. The RSD of peak area was used to evaluate the repeatability. As shown in Table 3, the RSD ranged from 1.95% to 3.56% for P188 at three different levels, and from 1.82% to 4.15% for PS80 at three levels, which demonstrates the excellent reproducibility of the Acclaim Surfactant Plus column and Vanquish Core HPLC system.

Table 3. Repeatability results of poloxamer 188 and polysorbate 80 (n=9)

Level	Poloxamer 188 RSD of peak area (%)	Polysorbate 80 RSD of peak area (%)
Low concentration (10 mg/L)	3.56	4.15
Middle concentration (40 mg/L)	1.95	1.82
High concentration (80 mg/L)	2.06	2.17

To further evaluate the applicability of the method for the detection and quantification of P188 and PS80 in biological formulations, two batches of recombinant protein samples containing PS80 were analyzed. The data demonstrates that test results are consistent with the expected value with an RSD between 1.09% and 2.54%, which indicates that this method is well suited for PS80 analysis in biopharmaceutical products (Table 4).

The chromatogram of the recombinant protein sample is shown in Figure 4A. The protein and excipients are eluted before 4.0 minutes. To avoid the high content of protein and excipients contaminating the CAD, a column switching valve that switches the eluent before 4.0 minutes to waste is recommended. Figure 4B shows the results after applying the switching valve approach.

Table 4. Polysorbate 80 test results in recombinant protein sample (n=6)

Sample	Dilution ratio	Protein amount (mg/g)	Expected polysorbate 80 amount (mg/L)	Detected polysorbate 80 amount (mg/L)	RSD of detected amount (%)
Recombinant protein 1	1	0.42	20–50	33.2	1.82
Recombinant protein 2	1	0.42	20–50	32.5	2.54
Recombinant protein 1	2	0.21	10–25	16.5	1.09
Recombinant protein 2	2	0.21	10–25	16.1	1.59

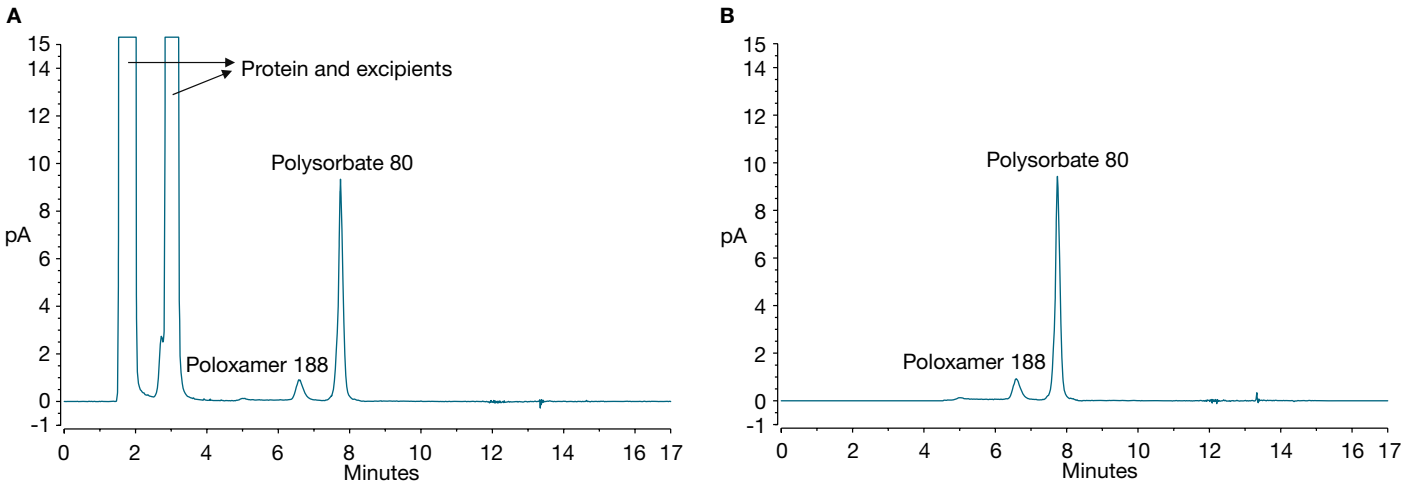

Figure 4. Chromatogram of recombinant protein sample. Poloxamer 188 was added to this sample. (A) Without switching valve. (B) With switching valve to transfer the eluent before 4.0 min to waste.

Conclusion

- Using CAD combined with an Acclaim Surfactant Plus column, a single HPLC method was developed for the quantification of P188 and esters in PS80 simultaneously.
- This method was demonstrated with a wider linearity range, lower LOQ and LOD, and good accuracy and reproducibility.
- The validation results indicate that this method is well suited for the analysis of P188 and PS80 in biopharmaceutical products.

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