Application Note

Biopharma/Pharma

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Profiling Poloxamer Oxidation in Adeno-Associated Virus Formulations

Using an Agilent InfinityLab Pro iQ Series single quadrupole mass detector

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Abstract

Poloxamers, particularly poloxamer 188, are emerging as an alternate option of nonionic surfactants in biopharmaceutical formulations, offering protein stabilization and aggregation prevention. Unlike polysorbates, these polymers are not susceptible to hydrolysis. However, oxidative degradation remains a significant risk, potentially compromising product quality and safety. This application note demonstrates a streamlined approach for screening poloxamer oxidation and related impurities using an Agilent AdvanceBio Surfactant Profiling HPLC column coupled to an Agilent InfinityLab Pro iQ Series single quadrupole mass detector. The method offers rapid analysis and simplified data interpretation, enabling proactive monitoring of critical quality attributes (CQAs) in therapeutic protein and adeno-associated virus (AAV) formulations.

Introduction

Poloxamers are used as excipients in biopharmaceutical formulations due to their ability to stabilize proteins and other biologics against interfacial stress, aggregation, and particle formation. Among the available grades, poloxamer 188 is commonly employed as a nonionic surfactant to protect drug substances and drug products during manufacturing, storage, and administration. By preferentially adsorbing to hydrophobic interfaces such as air-liquid, liquid-solid, and liquid-liquid boundaries, poloxamers reduce protein unfolding and surface induced aggregation, contributing to improved product stability and robustness.

Structurally, poloxamers are amphiphilic triblock copolymers composed of a central hydrophobic poly(propylene oxide) (PPO) block flanked by two hydrophilic poly(ethylene oxide) (PEO) chains. This PEO-PPO-PEO architecture imparts favorable biocompatibility and surfactant behavior while minimizing direct chemical interactions with active pharmaceutical ingredients. Poloxamers are polydisperse by nature, with molecular weights and block ratios tailored to achieve specific surface activity and formulation performance, making them promising alternatives to polysorbates in certain biopharmaceutical applications.

Despite their favorable stability profile, poloxamers are not immune to chemical degradation. Oxidative stress, elevated temperature, light exposure, and formulation components such as buffers and trace metal impurities can initiate polymer chain cleavage, particularly within the PPO block. Degradation of poloxamers may lead to the formation of low molecular weight acids and aldehydes, changes in molecular weight distribution, and loss of surfactant functionality. These changes can compromise the protective effect of the surfactant and potentially impact product quality attributes such as particle levels, pH stability, and long term shelf life.

Given the critical role of poloxamers in stabilizing sensitive biopharmaceutical products, robust analytical strategies are required to monitor poloxamer integrity and detect early signs of degradation. Understanding both the structural features that enable poloxamer performance and the conditions that promote its degradation is essential for formulation development and risk assessment of modern biologic drug products. This application note highlights the Agilent AdvanceBio Surfactant Profiling HPLC column coupled to an Agilent Pro iQ Series single quadrupole mass detector to monitor poloxamer degradation.

Experimental

Materials

All chemicals and reagents were obtained from Sigma-Aldrich, unless otherwise specified. Poloxamer 188 solution (P5556–100 mL), iron(III) chloride hexahydrate (236489-5G), L-histidine (H8125-256), and hydrogen peroxide (95321–100 mL) were used as received. An adeno-associated virus serotype 8 (AAV8) formulation (RS-AAV8-FL, 7.05×10^{11} GC/mL) was acquired from Charles River Laboratories. Ultrapure water was obtained from a Milli-Q water purification system. A bench-top incubator/heat block was used to maintain samples at 25 °C during stability analysis.

Chromatographic analyses were performed using an AdvanceBio Surfactant Profiling HPLC column (2.1 × 100 mm, 3.5 µm particle size) in conjunction with a matching AdvanceBio Surfactant Profiling guard column (2.1 × 5 mm).

Instrumentation

An Agilent 1290 Infinity III LC system was coupled to an Agilent InfinityLab Pro iQ Plus mass detector.

- Agilent 1290 Infinity III Bio High-Speed Pump (G7132A)
- Agilent 1290 Infinity III Bio Multisampler (G7137A)
- Agilent 1290 Infinity III Multicolumn Thermostat (G7116B)
- Agilent 1290 Infinity II Diode Array Detector (DAD) (G7117B)
- Agilent InfinityLab Pro iQ Plus mass detector (G6170A)

Software

The Pro iQ Plus and the 1290 Infinity III LC were operated with the Agilent OpenLab CDS software (version 2.8). The sequence override function within the OpenLab CDS 2.8 was employed to systematically vary ESI source parameters, enabling the identification of conditions that maximized the signal intensity of poloxamer 188. Data analysis was conducted with the Open Lab CDS software (version 2.8).

Method conditions

Table 1. LC method parameters.

Agilent 1290 Infinity III LC System	
Column	Agilent AdvanceBio Surfactant Profiling HPLC column 2.1 × 100 mm, 3.5 µm (p/n 861775-907) with guard (p/n 821126-927)
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	0.1% formic acid in acetonitrile
Flow Rate	0.6 mL/min
Column Temperature	50 °C
LC Gradient	5–95% MP B in 4 minutes
Needle Wash	1:1 methanol: water

Table 2. MS parameters.

Agilent InfinityLab Pro iQ Series Single Quadrupole Mass Detector	
Ion Source	ESI
Polarity	Positive
Gas Temperature	200 °C
Nebulizer	30 psi
Gas Flow	12 L/min
Capillary Voltage	3000 V
Fragmentor	150 V
Scan Type	Scan
Scan Time	1512 ms
MS Spectrum Range	*m/z* 100–2,000

Sample preparation

Samples of AAV8 formulation and poloxamer 188 were subjected to oxidative stress via the Fenton reaction at 25 °C, with aliquots collected at T0, 1 day, and 10 days. Following incubation, samples were transferred into 2 mL HPLC vials containing glass inserts and directly injected into the LC/MS system for analysis.

Results and discussion

Method development and signal optimization for P188

Initial method development prioritized LC conditions that produced sharp, well-resolved poloxamer 188 peaks while maintaining mobile-phase compatibility with mass spectrometric detection. Multiple mobile phase systems and gradients were evaluated with the AdvanceBio Surfactant Profiling HPLC column to understand the impact of organic modifier, mobile-phase additives, and gradient profile on retention behavior and peak shape.

Using ammonium acetate in water with methanol as the organic modifier resulted in elution of the main poloxamer peak during the high organic wash step for both short and long gradients, indicating strong retention under these conditions. Although the peak was detected, late elution limited flexibility for resolving potential pre-peaks or degradation products. Replacing methanol with acetonitrile significantly reduced retention, allowing both the main peak and an associated pre-peak to elute closer to the center of the gradient. This shift improved chromatographic efficiency and increased the analytical window for resolving structurally related species, although some peak broadening was observed under both short- and long-gradient conditions (Figure 1).

Acidic mobile phases containing formic acid in water and acetonitrile were also evaluated. The results were similar to ammonium acetate in water and acetonitrile. Additional experiments incorporating a lower *m/z* acquisition range, alternative stationary phases, and negative-ion polarity did not yield measurable improvements in chromatographic resolution or ion detection for intact poloxamer species. Collectively, these results indicated that acidic-to-neutral, volatile-buffered mobile phases combined with acetonitrile provided the most effective balance between retention control, peak shape, and MS compatibility for poloxamer 188 analysis. Ultimately, water and acetonitrile with 0.1% formic acid was selected as the mobile phase of choice for performance and ease of preparation.

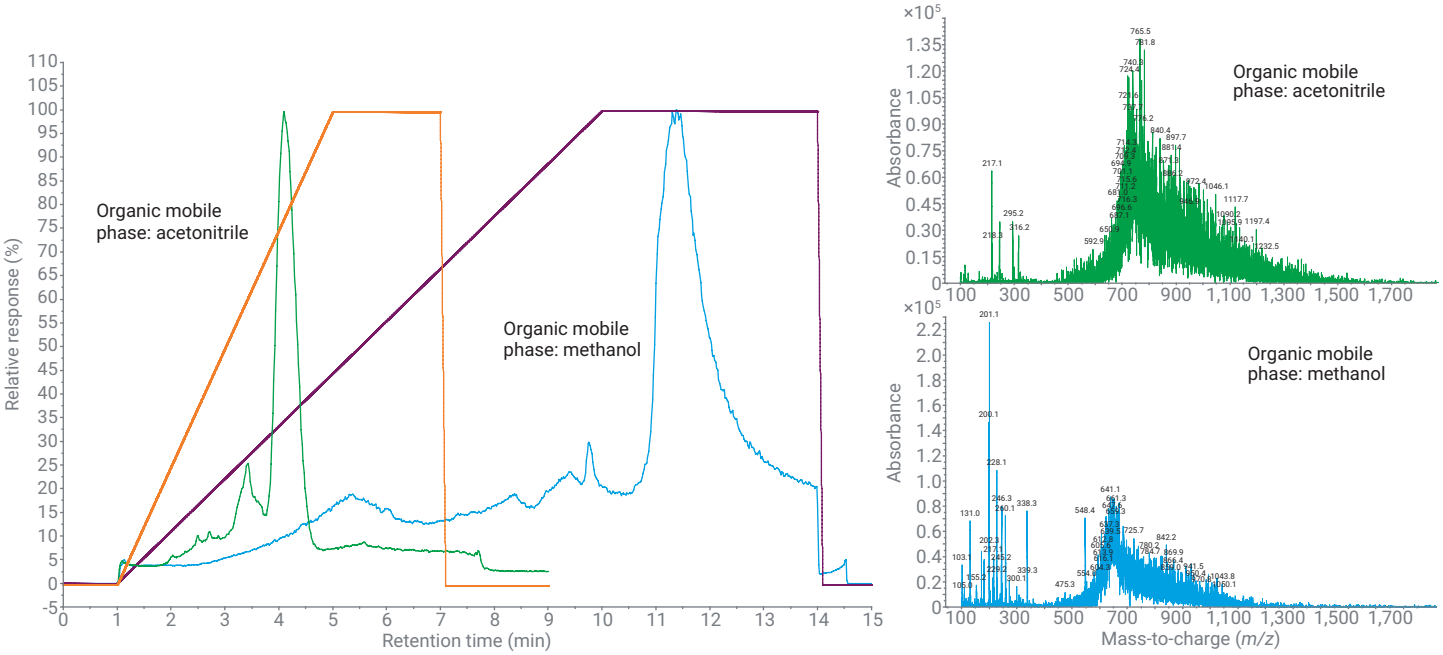

Figure 1. Acetonitrile shows improved signal over methanol as the organic mobile phase. Additionally, poloxamer elutes in the middle of the acetonitrile gradient, allowing for increased flexibility in gradient conditions.

Optimization of MS source parameters to maximize signal

Following the selection of LC conditions that enabled consistent retention of poloxamer 188, MS source parameters were systematically optimized to maximize the total ion current (TIC) signal and improve spectral quality. Fragmentor voltage and capillary voltage were identified as critical parameters influencing ion transmission and signal intensity for this nonionic surfactant.

A series of experiments demonstrated that moderate fragmentor voltages enhanced ion transmission without inducing in-source fragmentation, while higher capillary voltages improved overall ionization efficiency. Optimal performance was achieved at a fragmentor voltage of 150 V and a capillary voltage of 3000 V, which together produced the highest TIC signal for poloxamer 188 under the developed LC conditions. These gentler ionization settings did not measurably alter the overall mass spectral distribution, indicating that increased sensitivity was achieved without compromising spectral integrity (Figure 2).

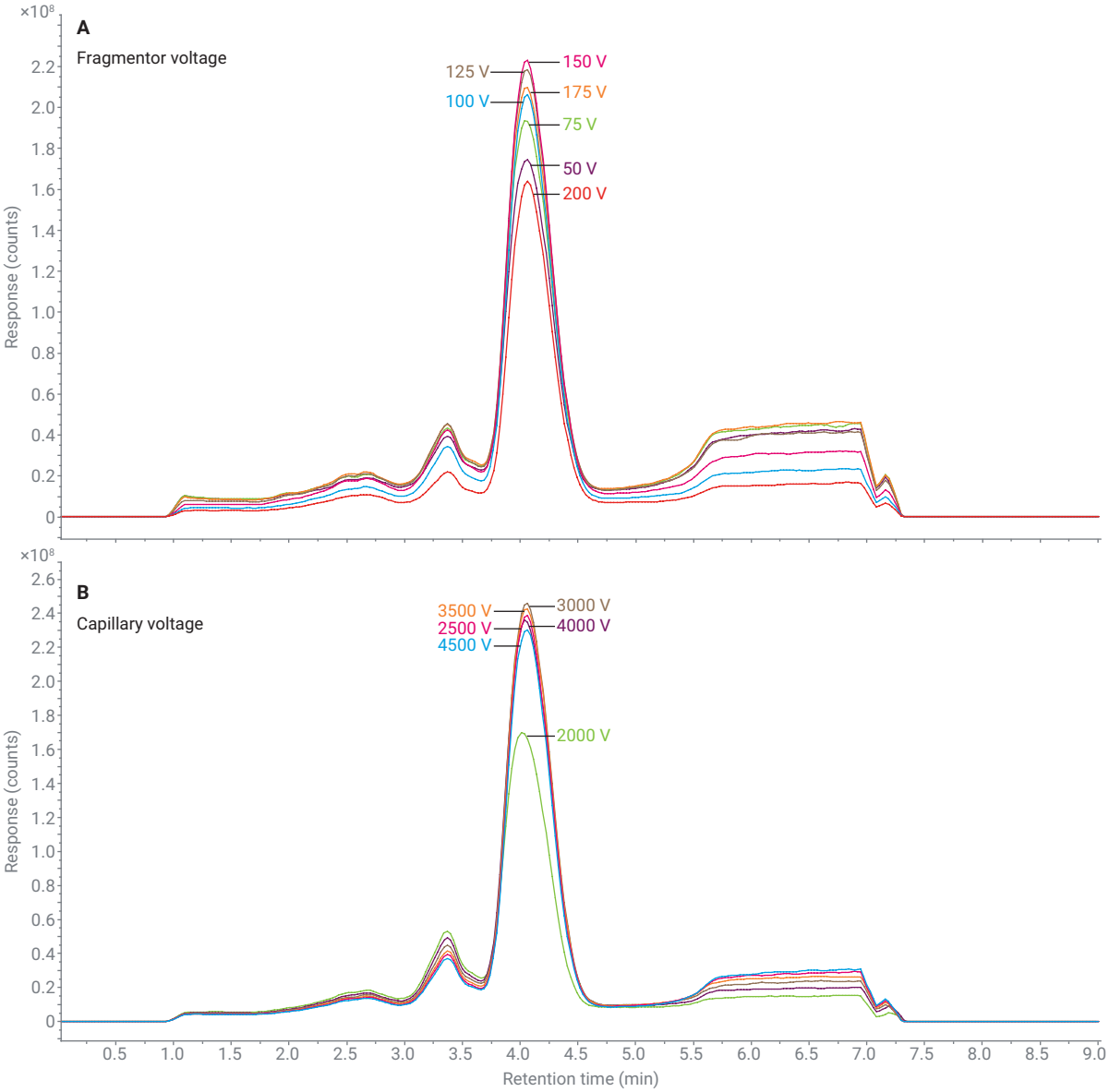

Figure 2. Mass spectrometry source conditions were optimized for poloxamer signal while ensuring minimal in-source fragmentation.

Neat poloxamer degradation under oxidative stress

Following method development, optimized LC/MS conditions were applied to evaluate induced degradation of neat poloxamer 188, using the Fenton reaction alongside hydrogen peroxide incubation, iron chloride incubation, and unstressed controls. TICs collected after one day of incubation revealed a clear chromatographic change unique to the Fenton reaction. The appearance of a nearly resolved shoulder peak preceding the main poloxamer peak was observed, while control, hydrogen peroxide, and iron-only samples remained chromatographically unchanged. The mass spectral data reveals a lower mass shift in the overall charged envelope, when comparing the shoulder peak to the main peak, with the apex of the peak shifting from *m/z* 716 to *m/z* 643 (Figure 3).

Furthermore, this emerging shoulder peak increased in abundance with extended incubation, accompanied by a substantial loss in main peak intensity after one week post Fenton reaction. In addition to reduced peak height, the degraded samples exhibited a shift toward earlier retention times (Figure 4).

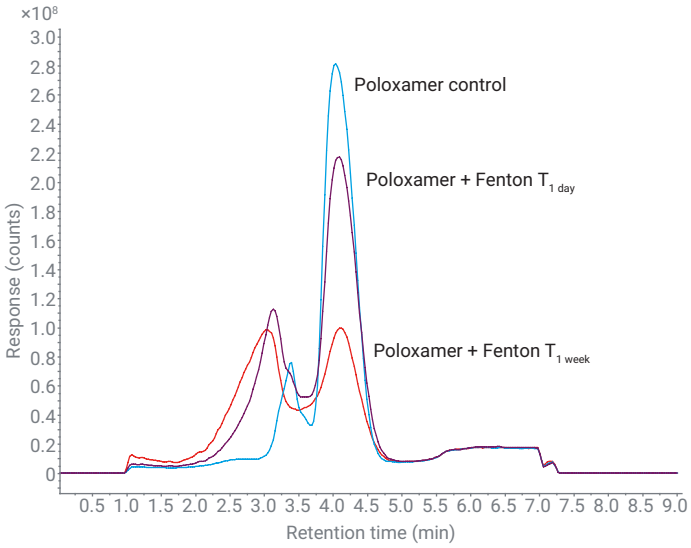

Figure 4. Poloxamer control versus Fenton reaction after one day and one week. Over time, the intensity of the main poloxamer peak decreases, while a shoulder peak associated with degradation becomes more pronounced.

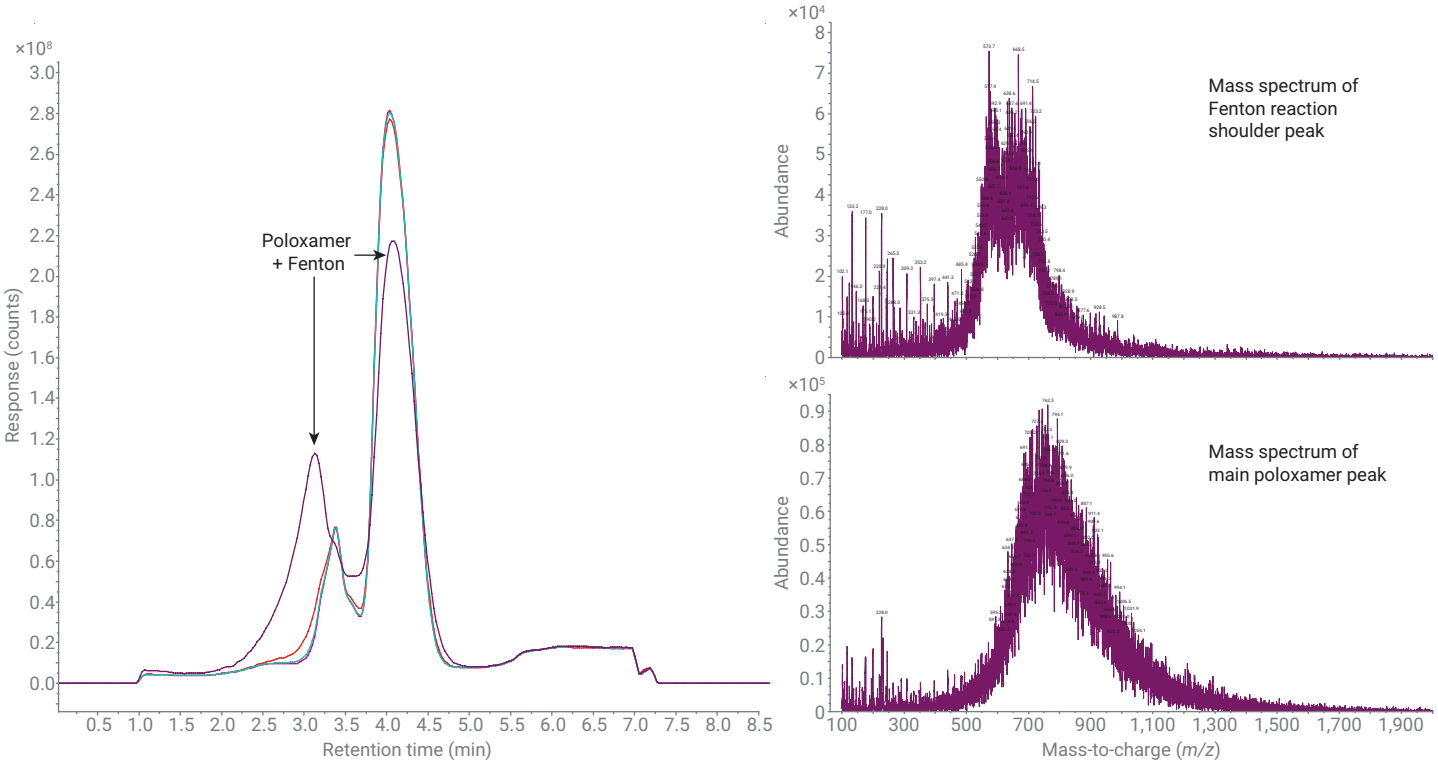

Figure 3. The appearance of a chromatographic shoulder peak and a reduced *m/z* shift in the accompanying shoulder peak after the Fenton reaction indicate poloxamer degradation.

Mass spectral changes associated with degradation

Mass spectral analysis provided further qualitative evidence of poloxamer degradation. Spectra extracted from the shoulder peak under Fenton conditions showed a noticeable shift toward lower *m/z* values compared to spectra from the intact main peak. In addition, the spectra extracted from the main peak reveal a complex structure with a Gaussian charge distribution, limiting direct structural interpretation (Figure 5). When observing the mass spectra in the earlier eluting shoulder peak, they are more resolved, although the spectra are still quite complex (Figure 6). However, the increased resolution indicates poloxamer degradation (Figure 7).

Multiple losses of 44 Da and 58 Da indicate loss of PEO and PPO groups respectively, consistent with cleavage of the polymer backbone.

Collectively, the chromatographic and mass spectral data demonstrate that oxidative stress under Fenton conditions induces measurable degradation of poloxamer 188, observable as reduced main peak intensity, formation of a shoulder peak, earlier elution, and a shift toward lower *m/z* ion populations. Significantly, the overall evidence of degraded samples remained broadly similar to previously reported poloxamer degradation patterns in the literature, lending confidence to the qualitative trends observed.

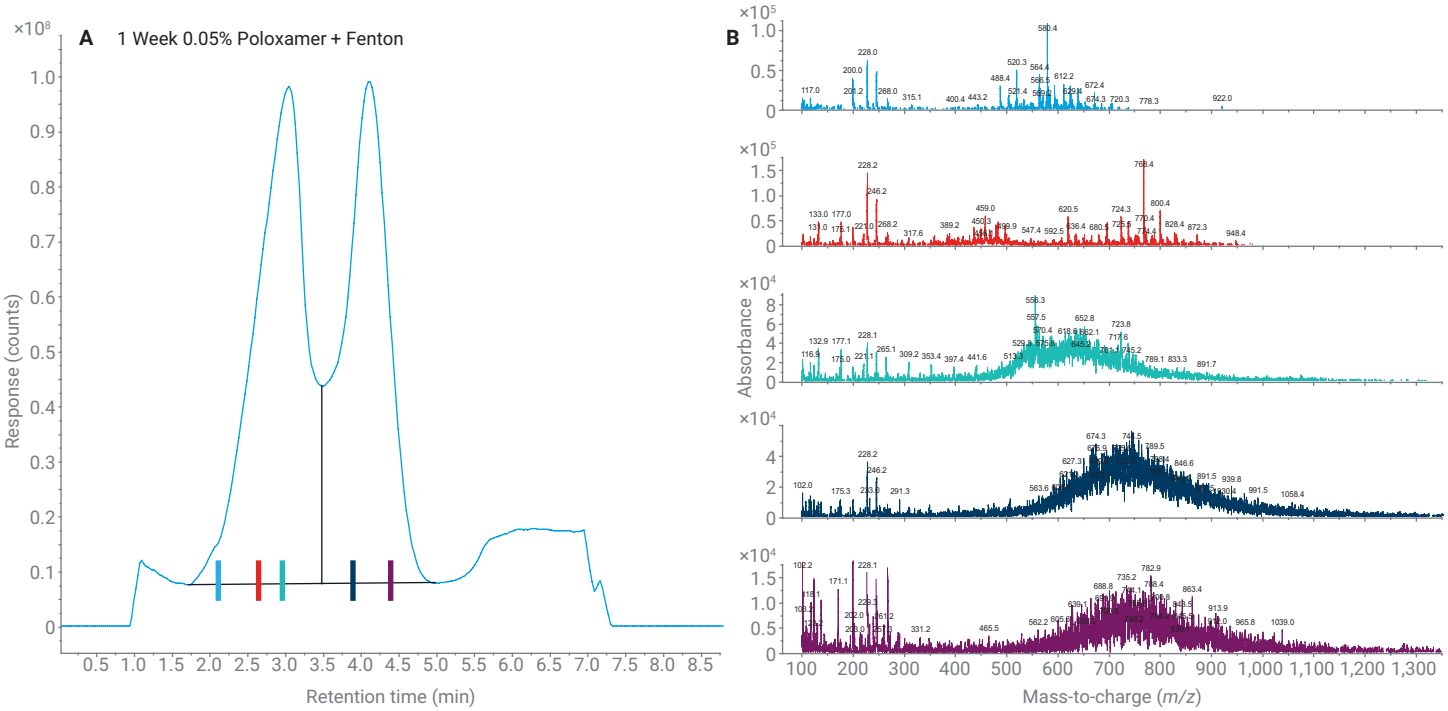

Figure 5. The colored bars on the bottom of the chromatogram (A) correspond to the same color of the mass spectra (B), indicating where the mass spectra were extracted. The main peak (dark blue and purple distributions) indicates a complex structure with overlapping charges.

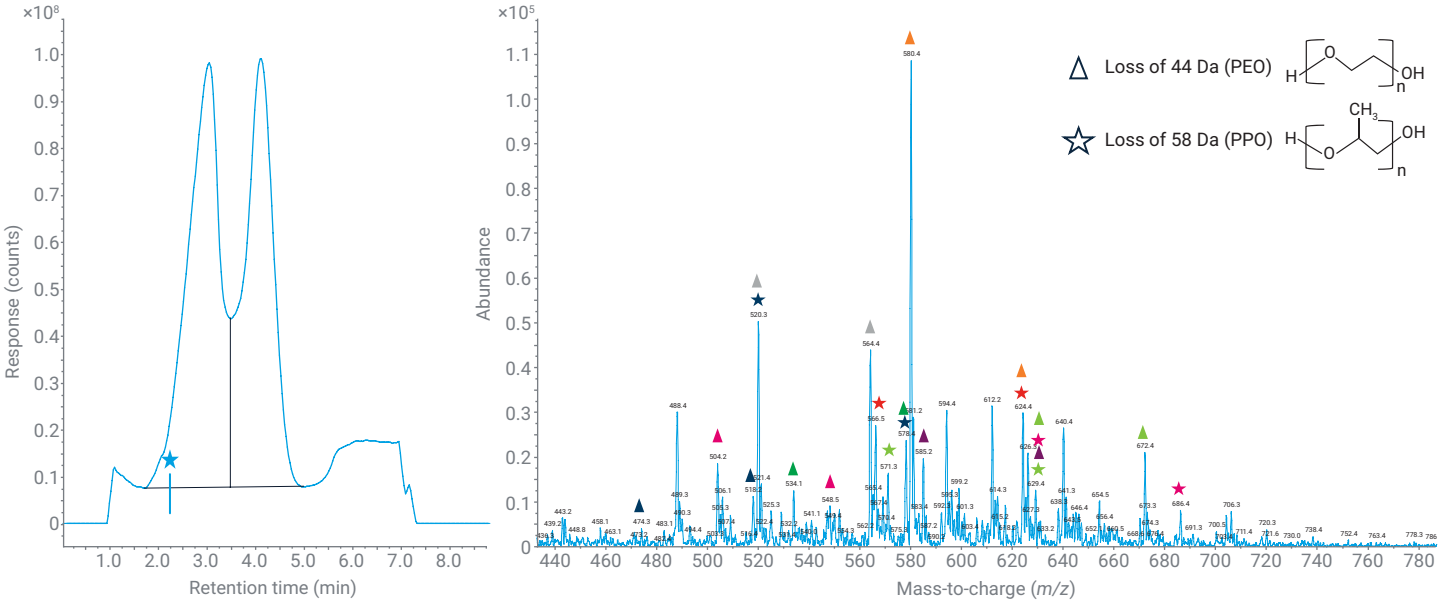

Figure 6. The mass spectrum extracted from an early part of the shoulder peak that emerged post Fenton reaction reveals a complex mixture. Instead of an unresolved Gaussian distribution, most of the peaks are baseline resolved and reveal numerous PEO and PPO losses, 44 Da and 58 Da, respectively.

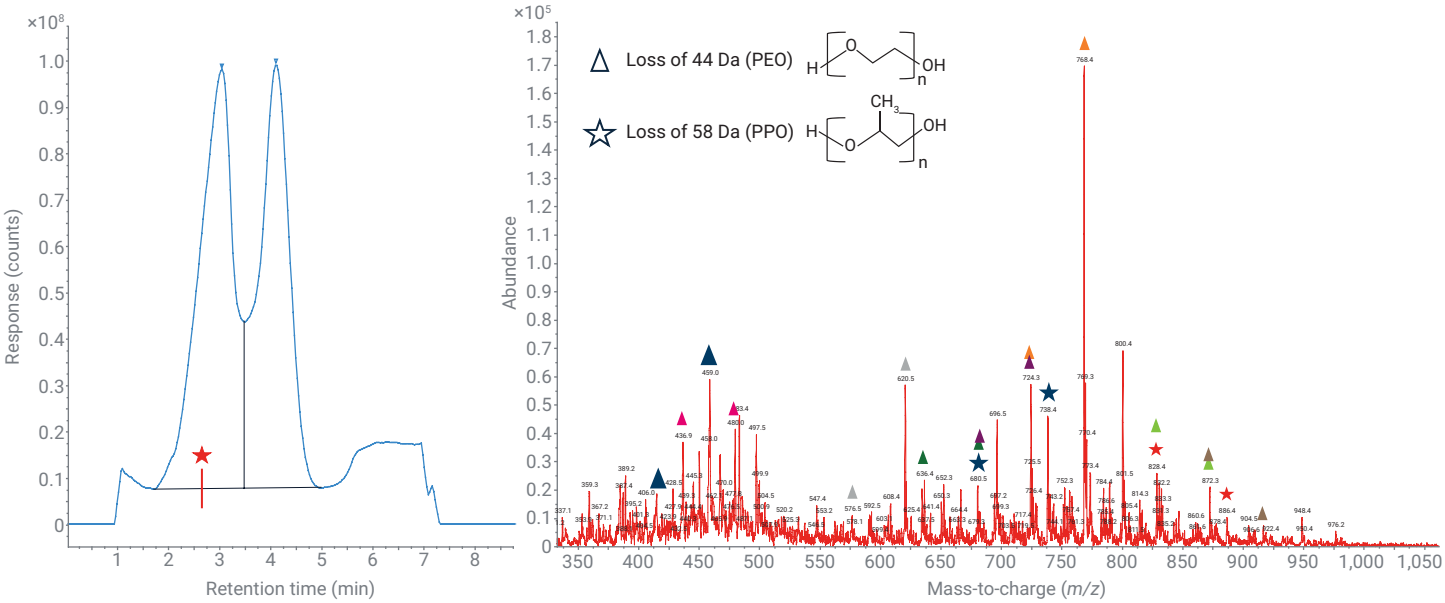

Figure 7. The mass spectrum extracted from the center part of the shoulder peak that emerged post Fenton reaction reveals a complex mixture. Instead of an unresolved Gaussian distribution, most of the peaks are baseline resolved and reveal numerous PEO and PPO losses, 44 Da and 58 Da, respectively.

Poloxamer degradation in formulation

Poloxamer 188 degradation was evaluated in an AAV formulation. The overall degradation response under Fenton conditions after 1 (T1) and 10 days (T2) was reduced relative to the neat poloxamer stress study, consistent with a less efficient oxidation pathway with reduced histidine buffer. The main poloxamer peak showed a consistent decrease in intensity, accompanied by the appearance of the expected shoulder peak eluting ahead of the main poloxamer peak.

Even with this lower reactivity, the chromatographic profiles remained reproducible across stressed formulation samples and retained the same qualitative signature of degradation with shoulder peak height percent CVs of 3.51% and 4.12%, for T1 and T2, respectively. Although the magnitude of these changes was smaller than that observed for neat poloxamer, the combined loss of the main peak and formation of the earlier eluting shoulder peak indicate that oxidative degradation still occurred in formulation. The AAV presumably elutes in the void volume, and its concentration is sufficiently low to allow injection onto the column without the risk of clogging (Figure 8).

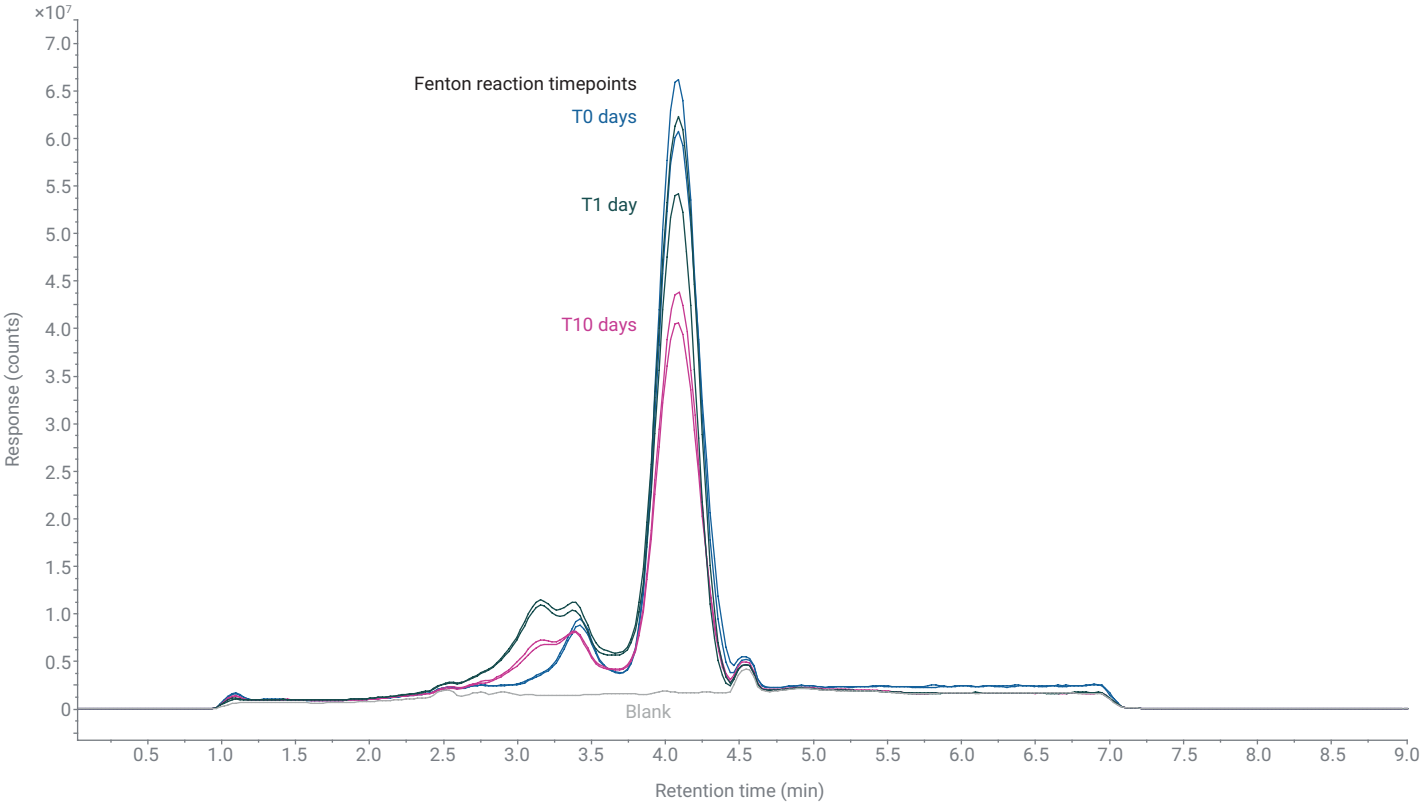

Figure 8. In an AAV formulation, the Fenton reaction produces a reproducible degradation response after 1 and 10 days, characterized by decreased intensity of the main poloxamer peak and the appearance of an earlier eluting peak consistent with degradation. The extracted ion chromatograms range from *m/z* 300–2,000 to omit noise, allowing for a smoother and clearer chromatogram.

Conclusion

This application note demonstrates that the Agilent AdvanceBio Surfactant Profiling HPLC column coupled with the Agilent InfinityLab Pro iQ Series single quadrupole mass detector provides a practical and effective approach for monitoring the degradation of poloxamer 188. Optimized LC and MS conditions enabled reproducible detection of intact poloxamer and degradation-related changes, including reduced main peak intensity, and the formation of an earlier eluting degradation-associated peak under Fenton stress. Mass spectral analysis further supported these observations through a shift toward lower *m/z* species and the appearance of resolved ion populations consistent with polymer backbone cleavage. Together, these results show that the method can sensitively track qualitative degradation trends in both neat poloxamer and AAV formulation matrices, supporting its use as a streamlined screening tool for formulation development, stress testing, and proactive monitoring of surfactant-related critical quality attributes.

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