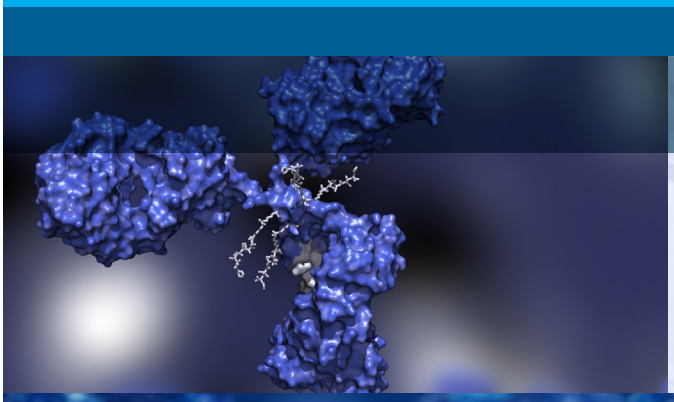
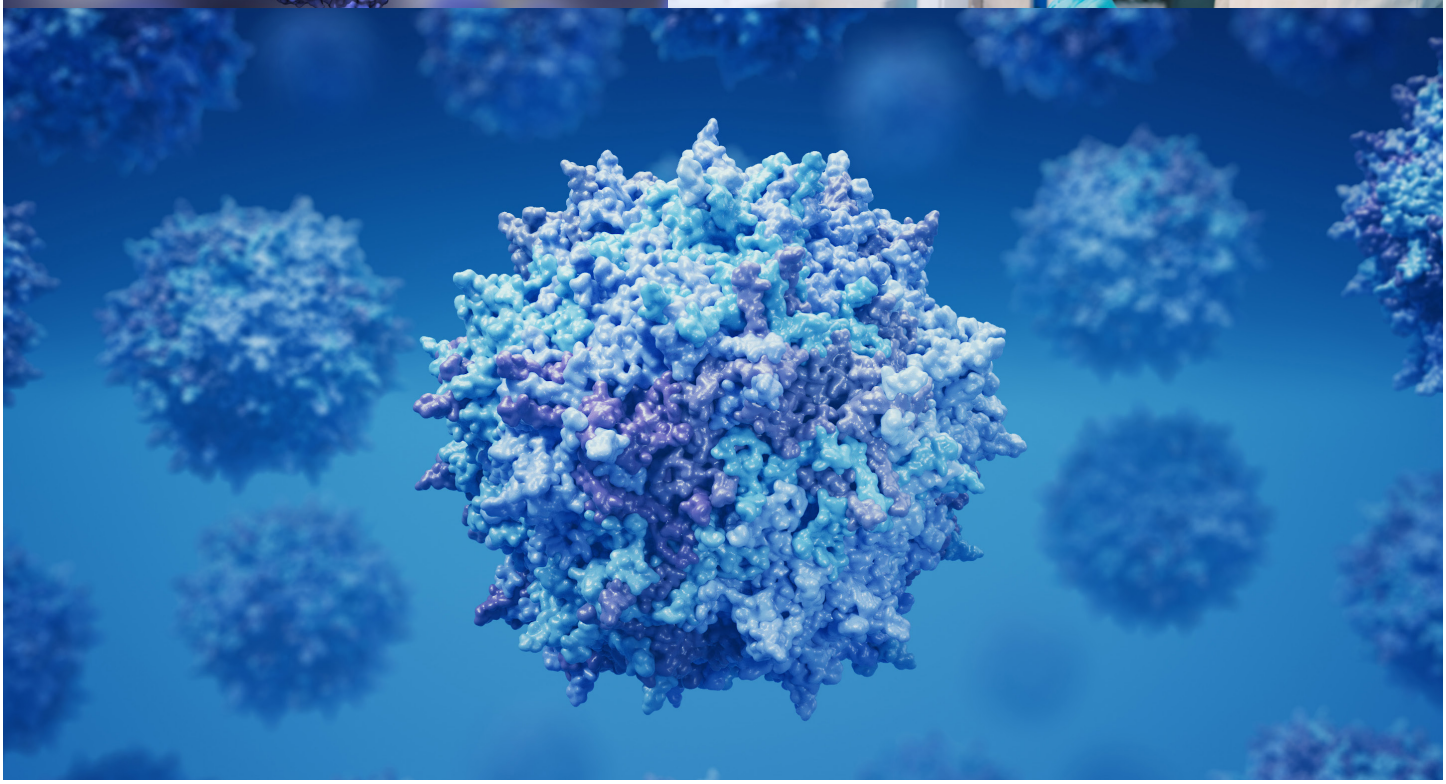
Agilent

Trusted Answers

Agilent AdvanceBio Surfactant Profiling HPLC columns

In-depth characterization of surfactant and surfactant degradation in biotherapeutic applications





Surfactants: Balancing the Benefits and Risks

Surfactants are a vital component of biologic formulation buffers, preventing biologic aggregation and adsorption at the air-liquid interface. Formulations are carefully optimized to ensure the correct amount of each component, including surfactants, is present and functioning effectively. Unfortunately, surfactants are prone to degradation, leading to lower-than-expected levels in the formulation and other adverse effects.

Polysorbate hydrolysis, mainly mediated by host cell protein enzymes, can produce proteinaceous or free fatty acid particles (Figure 1). Polysorbate or poloxamer oxidation may cause oxidation of the biologic itself or other formulation components (Figure 2). Poor product quality can impact regulatory approval and biologic shelf life. Therefore, surfactants, including polysorbate, should be monitored for degradation so that any issues can be addressed early in the development process.

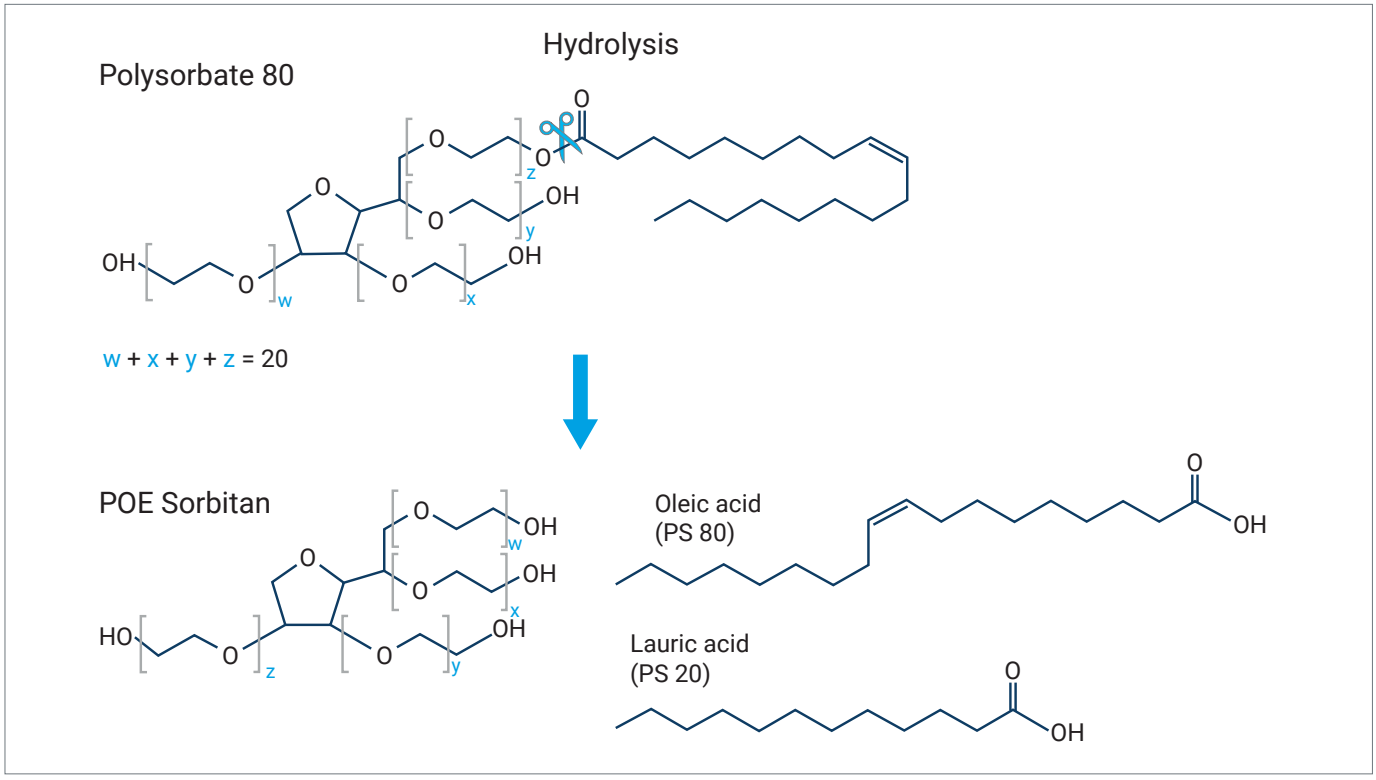
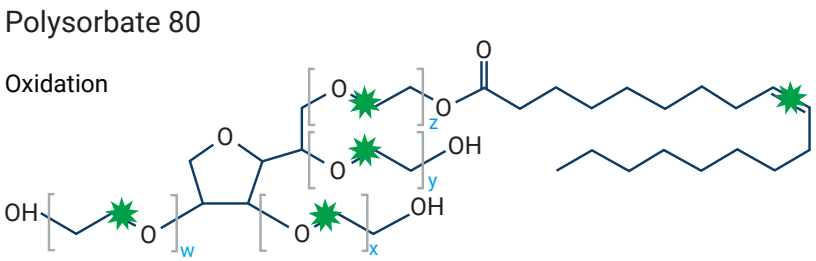


Figure 1. Polysorbate hydrolysis cleaves the fatty acid, which may cause formation of proteinaceous or free fatty acid particles.



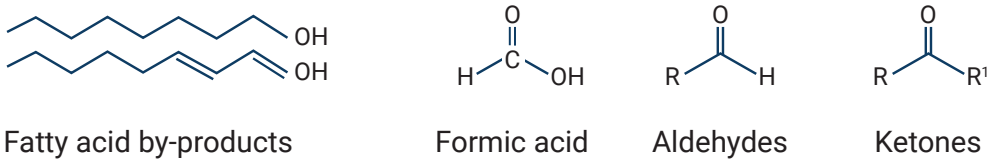
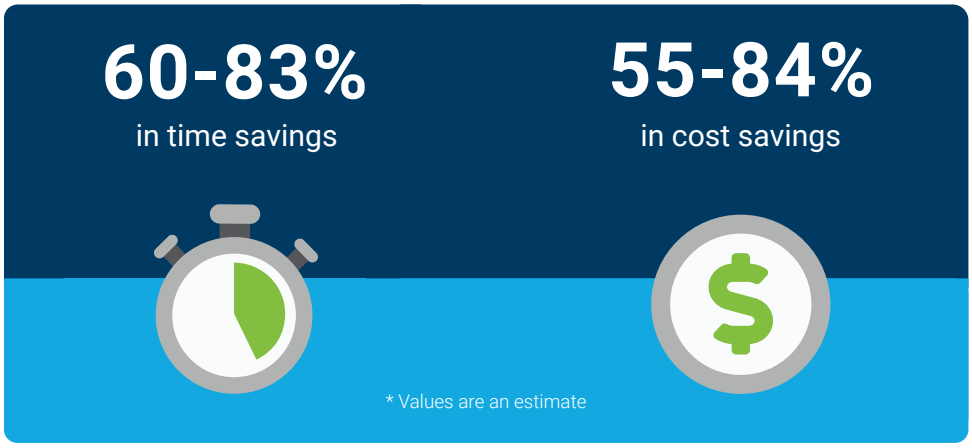


Figure 2. Oxidation can occur at multiple sites on the polysorbate structure. Many by-products can form, and oxidation may also affect the biologic.

Switching from a traditional reversed-phase column to the AdvanceBio Surfactant Profiling column saves both time and money



Beyond Surfactant Quantitation

Current regulations support surfactant quantitation, a rapid method that generally requires less than 10 minutes to complete and yields only a single peak, making analysis easy. Surfactant quantitation is usually performed with a mixed-mode column, and in the case of polysorbate, the mono-, di-, tri-, and tetraesters co-elute. This is an ideal method for reporting the expected versus actual percentage of surfactant in the formulation buffer.

However, surfactant quantitation alone is insufficient for surfactant characterization. If the measured amount is lower than expected, further characterization is necessary because degradation products that elute in the void are not detected with mixed-mode columns (Figure 3). With increasing awareness and regulatory scrutiny, conducting a more thorough characterization assay in the early stages of the development process is important. Ideally, this additional assay should be easy to implement, provide easily interpretable data, offer comprehensive characterization, and be as high throughput as possible. The Agilent AdvanceBio Surfactant Profiling HPLC column was designed with these characteristics in mind.

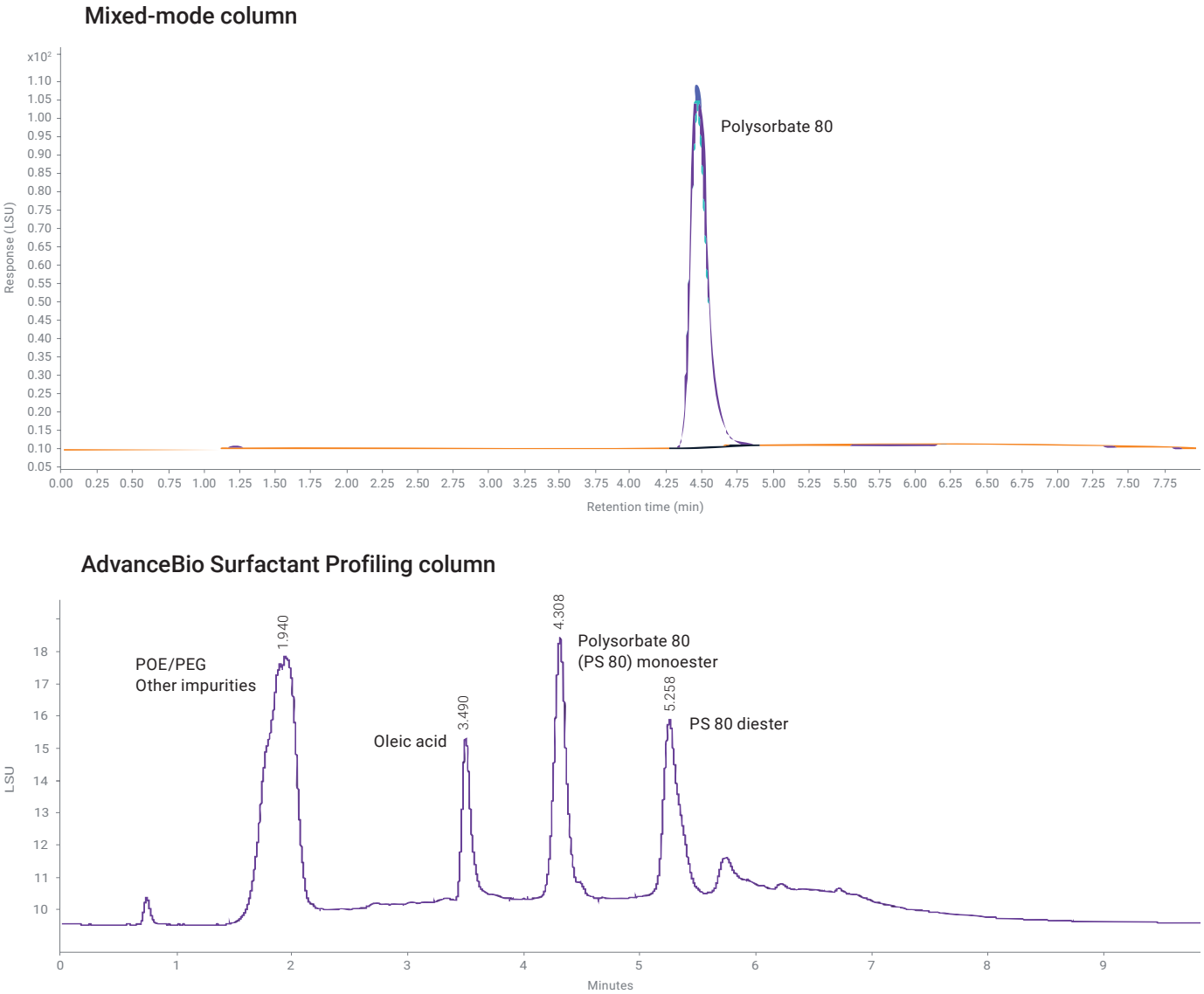


Figure 3. While a mixed-mode column is sufficient for surfactant quantitation, it is unable to detect polysorbate degradation products such as polyoxyethylene moieties or free fatty acids. The Agilent AdvanceBio Surfactant Profiling column can detect surfactant degradation products with a short run time.

Why the AdvanceBio Surfactant Profiling HPLC Column?

Many current surfactant degradation characterization methods use longer columns and extended methods to ensure separation between surfactant components (Figure 4). The unique chemistry of the AdvanceBio Surfactant Profiling HPLC column offers alternate selectivity, enabling improved separations with shorter columns in less time (Figure 5). Shorter methods save solvent, time, and money. The AdvanceBio Surfactant Profiling column certificate of performance includes an application-specific QC test using oleic acid and polysorbate 80 to demonstrate the column's capabilities.

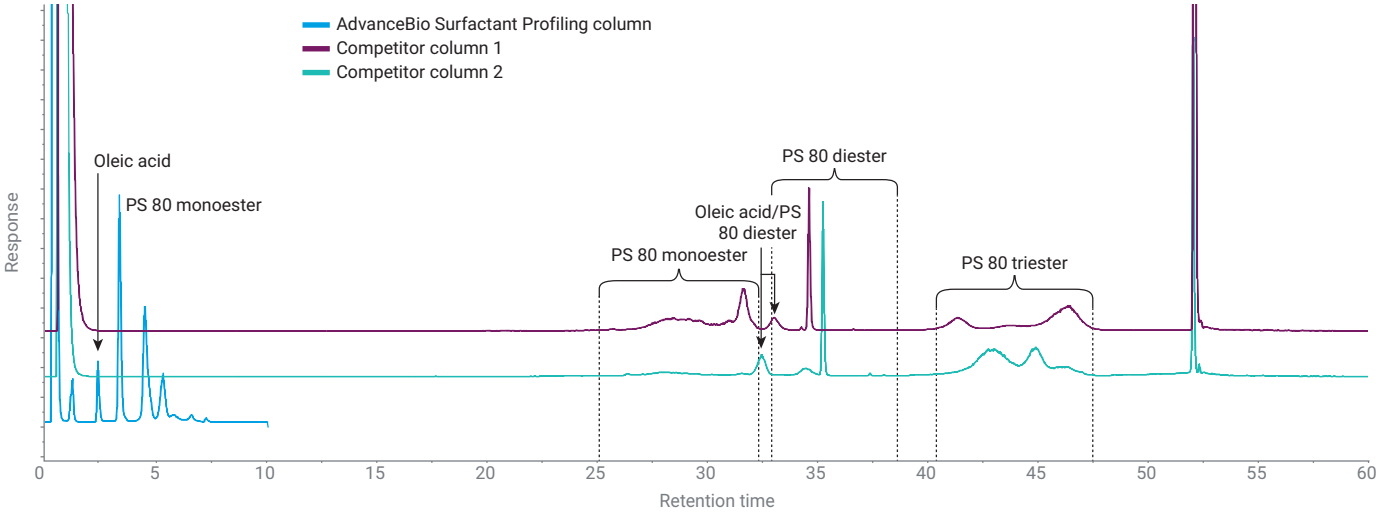


Figure 4. The Agilent AdvanceBio Surfactant Profiling column easily resolves the free fatty acids from the polysorbate monoester peak in under 10 minutes. The competitive columns typically used for this analysis have lower resolution in 60-minute run times.

Although it may seem daunting to adopt a new assay or switch methods, the benefits provided, along with the long-term cost and time savings, are worthwhile. Benefits include more detailed information about the integrity of the polysorbate and the identification of triggers that prompt investigation into sources of oxidation or the presence of host cell proteins.

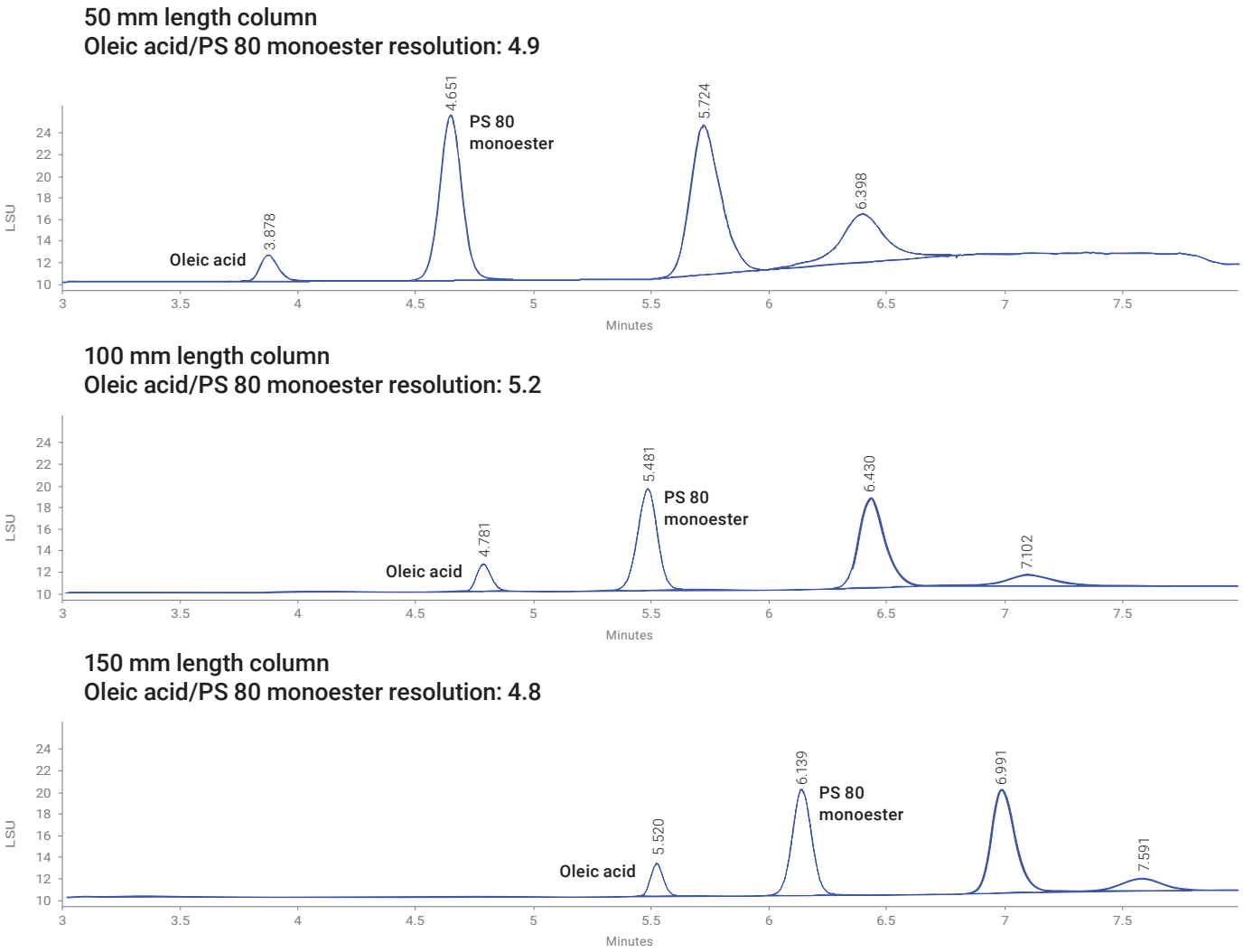


Figure 5. While increasing the length of the column is known to increase resolution, it offers negligible improvement for fatty acid/monoester separation. A 50 mm length column is sufficient when monitoring hydrolysis. Increased lengths may be helpful for screening oxidation, as there are more degradation by-products.

Cost savings with improved column lifetime

To maximize cost savings, the AdvanceBio Surfactant Profiling columns offer guards, which help protect and increase the lifetime of the analytical column (Figure 6). Guards are particularly important with this application, as the formulation matrix can be complex. Maximizing column lifetime saves money. Measures such as proper sample preparation and avoiding the column's temperature and pressure limits promote longer column life. Guards are another way to protect the column, helping to maximize lab budget.

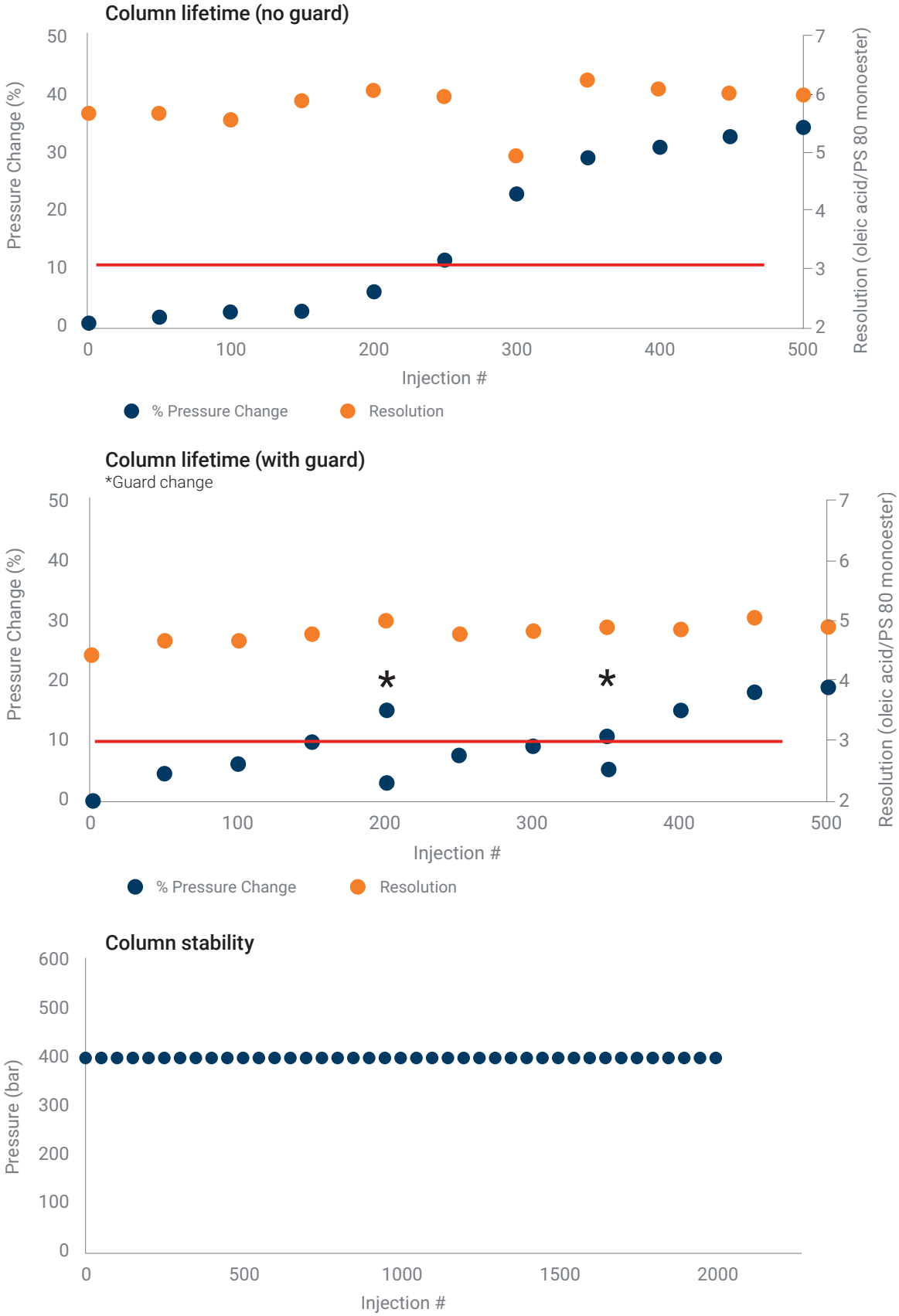


Figure 6. Lifetime testing was performed with samples in a heavy salt buffer to mimic formulation conditions. Guards were replaced every 150-200 injections. While column pressures eventually increased over 10%, the resolution between oleic acid and polysorbate 80 monoester was maintained. Additionally, column lifetime testing at the column pressure limit was performed with a uracil probe, showing no pressure change over 2000 injections and demonstrating mechanical stability. Red lines indicate a 10% increase in pressure.

Ordering Information

Description	Part Number
AdvanceBio Surfactant Profiling, 2.1 x 50 mm	865750-907
AdvanceBio Surfactant Profiling, 2.1 x 100 mm	861775-907
AdvanceBio Surfactant Profiling, 2.1 x 150 mm	863750-907
AdvanceBio Surfactant Profiling, 2.1 mm, fast guard	821126-927
AdvanceBio Surfactant Profiling, 4.6 x 50 mm	865973-907
AdvanceBio Surfactant Profiling, 4.6 mm, fast guard	820951-927

To learn more visit:
www.agilent.com/chem/advancebio-surfactant-profiling

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