

Organic Solvent in Size Exclusion Chromatography of an Antibody Drug Conjugate Surrogate

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Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, some analytes may present some challenges. For example, Antibody Drug Conjugates (ADCs), because of their hydrophobic nature, may have a propensity to interact with the stationary phase.

In this application note, we demonstrate the effect of organic solvent on SEC analysis of an ADC “mimic.” This sample is a monoclonal antibody that has been conjugated to a dansyl group and functions as a surrogate for the typically cytotoxic agent commonly used for most ADC modalities.

Figure 1 demonstrates even with 5 % isopropanol (IPA) added to the mobile phase (black trace), hydrophobic adsorption is still observed for the ADC mimic, leading to poor peak shape and recovery. Overlay of 10 % IPA improves peak shape and recoveries.

Figure 2 shows full recovery of aggregate, and good peak shape for monomer. Percent aggregate by peak area was measured at 2 %. Additionally, post-peak, putative fragments which are common with cysteine-based ADCs, are partially separated.

In summary, ideal size exclusion chromatography separations should minimize secondary interactions. Although column selection is a consideration, some analytes such as ADCs may be too hydrophobic to run with phosphate containing mobile phases. Consequently, the addition of isopropanol may improve peak shape, thus allowing for the separation of monomer and aggregate for the calculation of purity by percent peak area.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: [00H-4787-E0](#)
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2 + IPA
Flow Rate: 0.35 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: ADC Mimic



Figure 1. SEC chromatographic overlays for ADC mimic, demonstrating the effect of organic solvent to peak shape and recovery.

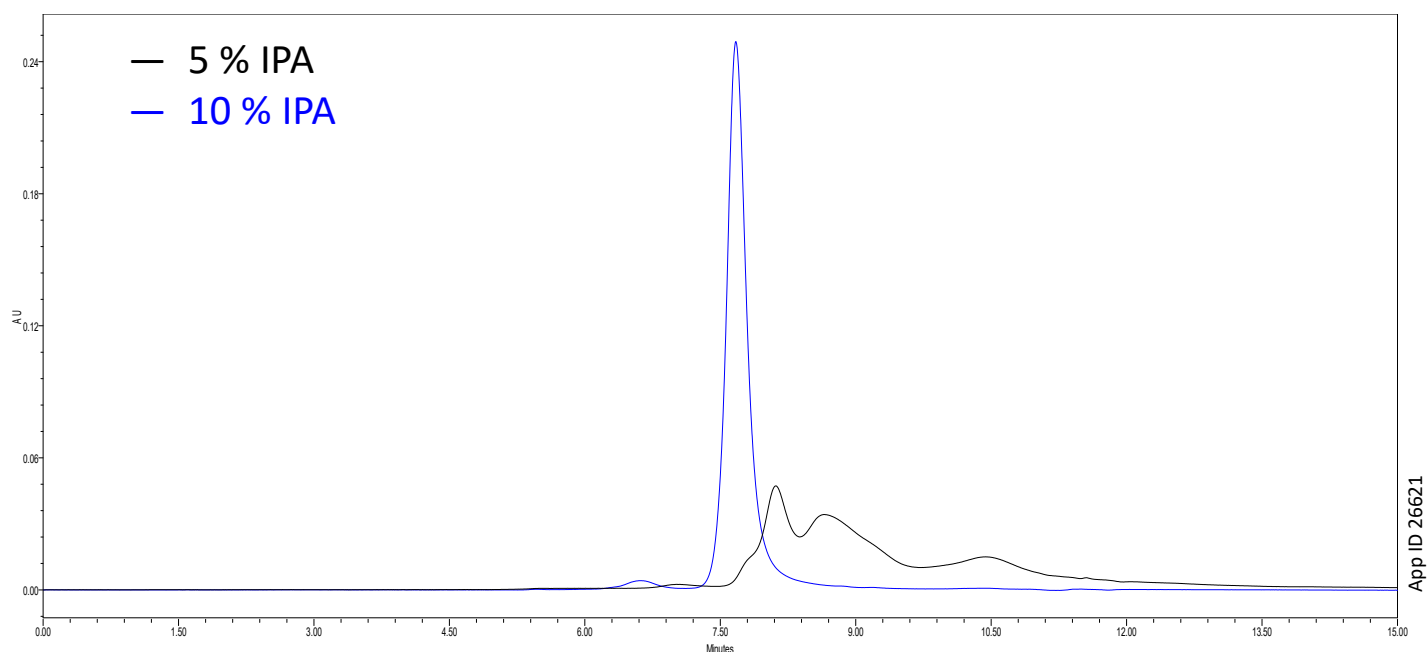
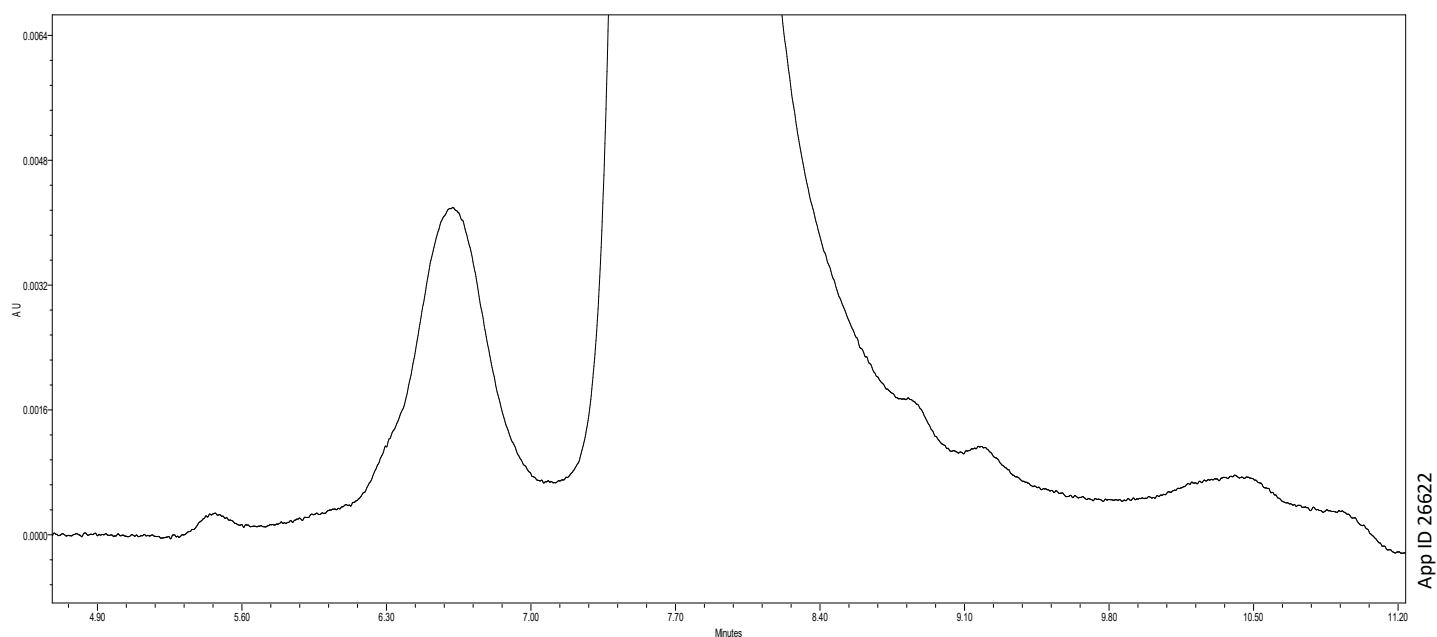


Figure 2. SEC chromatogram for 10% IPA. Sufficient separation of aggregate and monomer allows for integration to determine percent purity by peak area.



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