

Size Exclusion Chromatography Analysis of a Monoclonal Antibody and Antibody Drug Conjugate

Using an Agilent AdvanceBio SEC 200 Å 1.9 µm
column

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Abstract

Size exclusion chromatography (SEC) is a common technique used to characterize size variants from biotherapeutic proteins. This Application Note compares SEC analysis of monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) with 2 µm and sub-2 µm SEC columns from different vendors. The Agilent AdvanceBio SEC 200 Å 1.9 µm column provides a unique advantage for high-resolution separation of mAb and ADC.

Introduction

High molecular weight (HMW) aggregates and low molecular weight (LMW) fragments of biotherapeutic proteins induced by various stress conditions can be formed during the process of drug development, storage, shipment, or delivery. These size variants are critical quality attributes (CQAs) that need to be well characterized. Immunogenic responses and differences in pharmacokinetics or potency could arise due to the existence of these species in the drug product^{1,2}. SEC is a standard technique to analyze these size variants from mAbs and ADCs. SEC analysis of ADCs may be challenging due to the presence of hydrophobic payloads. These features can cause some undesirable secondary interactions with the column stationary phase. This Application Note demonstrates the SEC analysis of an mAb and ADC with an AdvanceBio SEC 200 Å 1.9 µm column and two leading alternative SEC columns from other vendors, both of which are columns with 2 µm or sub-2 µm particles. The unique chemistry of the AdvanceBio SEC 200 Å 1.9 µm column offers superior performance compared to other vendor columns for the analysis of mAbs and ADCs. The column provides excellent peak shape, resolution, and reduced secondary interactions.

Experimental

Reagents, samples, and materials

SILu Lite SigmaMAb universal antibody standard and SigmaMAb ADC mimic were purchased from MilliporeSigma, and reconstituted with water to 1 mg/mL. Monobasic and dibasic sodium hydrogen phosphate and sodium chloride were purchased from MilliporeSigma. All chemicals used were ≥99.5 % pure. Water was purified from a Milli-Q A10 water purification system (Millipore). Solutions were prepared fresh daily, and filtered through a 0.22 µm membrane filter prior to use.

Instrumentation

LC system

An Agilent 1260 Infinity LC with the following configuration was used:

- Agilent 1260 Infinity II bio-inert quaternary pump (G5654A)
- Agilent 1260 Infinity II bio-inert multisampler (G5668A) with sample cooler (option #100)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A) with bio-inert heat exchanger (option #019)
- Agilent 1260 Infinity II variable wavelength detector (G7114A)

Columns

- Agilent AdvanceBio SEC 200 Å 1.9 µm (4.6 × 300 mm, 1.9 µm, 200 Å)
- Vendor A (4.6 × 300 mm, 1.7 µm, 200 Å)
- Vendor B (4.6 × 300 mm, 2.0 µm, 250 Å)

Software

Agilent OpenLab 2.2 CDS.

Parameter	1260 Infinity II LC
Column Temperature	25 °C
Mobile Phase	50 mM sodium phosphate, 200 mM NaCl, pH 7.0
Flow Rate	0.35 mL/min
Injection Volume	1 to 2 µL
Detection	UV at 220 nm

Results and discussion

Figure 1 shows the comparative SEC chromatograms of the separation of an IgG1 mAb (SigmaMAb) sample (mixed with its F(ab')₂ and Fc fragments). As shown in Table 1, the AdvanceBio SEC 200 Å 1.9 µm column gives sharper peaks for monomer and low molecular weight species compared to both column A and column B. Dimer/monomer and monomer/LMW1 are both better resolved on the AdvanceBio SEC 200 Å 1.9 µm versus the other two columns. Among the three columns, the AdvanceBio SEC 200 Å 1.9 µm column offers the best performance for the separation of HMW and LMW species from mAb monomer. This column provides the narrowest peaks, best resolution, and a backpressure less than column A. Although column B has the lowest backpressure due to its slightly larger particle size, its performance has been compromised.

Figure 2 compares the SEC chromatograms of an ADC mimic (SigmaMAb ADC) using three SEC columns of the same dimensions (4.6 × 300 mm) from different vendors. For column A, the peak is wide, and splits into at least two or more peaks, indicating secondary interactions of hydrophobic payloads with column stationary phase. The AdvanceBio SEC 200 Å 1.9 µm column results in a sharper peak than column B. Additionally, the AdvanceBio SEC 200 Å 1.9 µm column can well resolve (Rs = 2.55) the LMW fragment peak (circled in red). By comparison, with column A, the peak cannot be separated at all, and column B can only partially resolve it. These results demonstrate the superior performance of the AdvanceBio SEC 200 Å 1.9 µm column in analyzing ADCs.

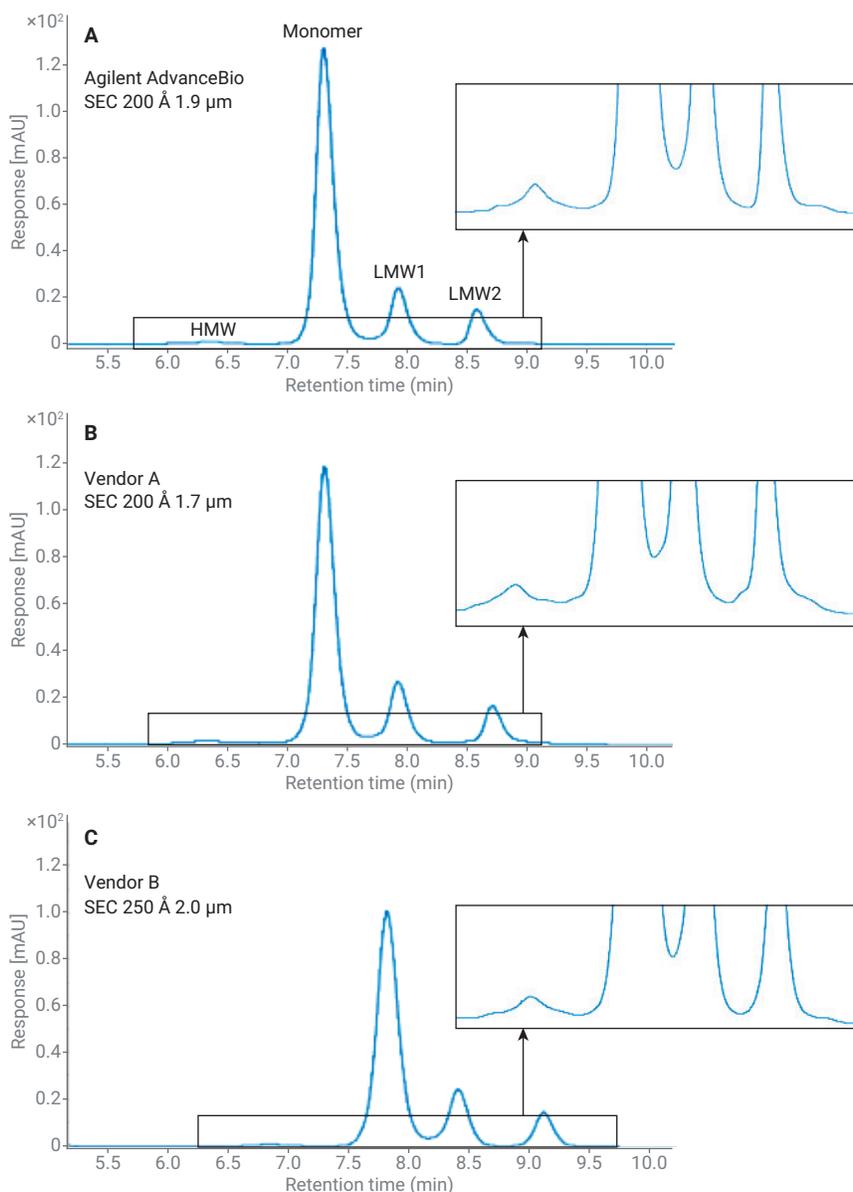


Figure 1. Size exclusion chromatograms of SigmaMAb (mixed with its F(ab')₂ and Fc fragments) using 4.6 × 300 mm SEC columns running with 50 mM sodium phosphate, 200 mM NaCl, pH 7.0 at 0.35 mL/min.

Table 1. Peak width, resolution, and backpressure comparison of the three SEC columns.

Column	Peak Width at Half Height			Resolution		Backpressure (bar)
	Monomer	LMW1	LMW2	Dimer/Monomer	Monomer/LMW1	
Agilent AdvanceBio SEC 200 Å 1.9 µm	0.159	0.154	0.148	2.79	2.28	340
Vendor A SEC 200 Å 1.7 µm	0.172	0.166	0.160	2.46	2.09	354
Vendor B SEC 250 Å 2.0 µm	0.194	0.182	0.169	2.49	1.83	260

Conclusions

This study compares the performance of three columns, including the AdvanceBio SEC 200 Å 1.9 µm column and other vendors' 2 µm and sub-2 µm columns, for the analysis of an mAb and ADC in terms of peak shape and separation of HMW and LMW size variants. The AdvanceBio SEC 200 Å 1.9 µm column shows better peak shape and resolution for HWM and LMW species when analyzing the mAb compared to the other vendor columns. For more hydrophobic samples such as ADCs, the AdvanceBio SEC 200 Å 1.9 µm column provides clear benefits. Undesirable secondary interactions are greatly reduced, resulting in a sharp, symmetrical peak and a well resolved LMW fragment peak. By contrast, the two other vendor columns suffer from severe nonspecific binding, with split or broader peaks, and are unable to resolve the LMW peak.

References

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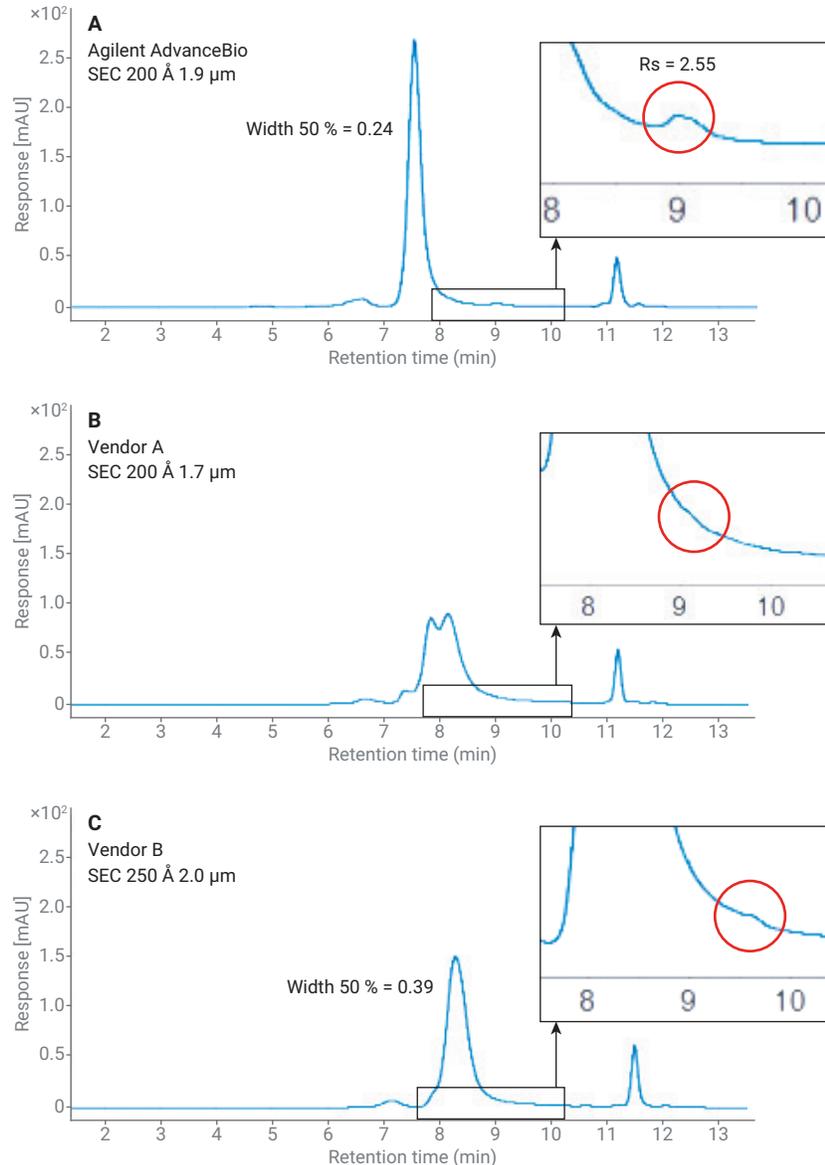


Figure 2. Size exclusion chromatograms of SigmaMAb ADC using 4.6 × 300 mm SEC columns running with 50 mM sodium phosphate, 200 mM NaCl, pH 7.0 at 0.35 mL/min.

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