Size Exclusion Chromatography Analysis of Antibody Drug Conjugates

Using the Agilent 1260 Infinity II Bio-inert LC System

Application Note

Biopharma

Authors

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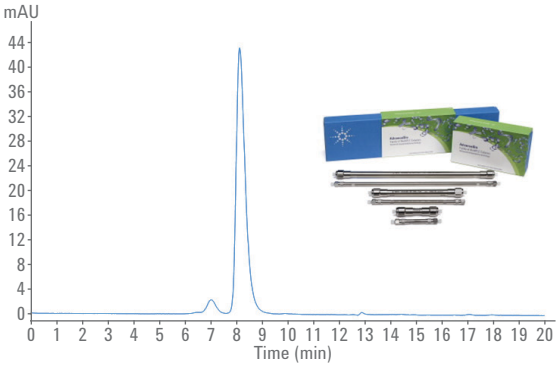
Abstract

The Agilent 1260 Infinity II Bio-inert LC with the Agilent AdvanceBio SEC column proved an ideal solution for the aggregate analysis of antibodies and Antibody Drug Conjugates (ADCs). This investigation compared the performance of the AdvanceBio SEC column with the Agilent Bio SEC-3 column for the analysis of monoclonal antibodies and two ADCs. The benefit of using the AdvanceBio SEC columns for the analysis of ADCs was clearly demonstrated by the sharp peaks observed with no peak tailing, indicative of size-based separations with no nonspecific interactions. Therefore, baseline separation of the aggregates was achieved, presenting the option to perform quantitation of the aggregates.



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Introduction

The efficacy of monoclonal antibodies (mAbs) is connected to the correct primary, secondary, tertiary, and quaternary structure. Production processes as well as storage and transportation conditions can stimulate aggregation. The presence of any type of aggregates can lead to activity loss, decreased solubility, and enhanced immunogenicity. Therefore, it is essential to monitor product stability during every step of the development and production process.

Antibody drug conjugates (ADCs) represent a special class of mAbs, which are challenging to analyze due to their complex and heterogeneous structure[1]. ADCs are immunoconjugates composed of a monoclonal antibody chemically linked to a potent small molecule cytotoxin. The small molecules attached to the mAb are often relatively hydrophobic. This hydrophobicity can be problematic during manufacturing and storage in terms of aggregate formation as well as in analytical characterization of the latter[1].

Size exclusion chromatography (SEC) is the most commonly used liquid chromatography (LC) technique to determine the amount of aggregates for mAbs, and this technique can be applied for ADCs as well. Agilent AdvanceBio SEC columns have been designed to provide maximum efficiency without risk of shear degradation of samples, or clogging between particles. The unique method of manufacture of AdvanceBio SEC, controlling pore size, structure, and volume, then applying a hydrophilic polymeric coating, ensures that protein peaks are sharp and well resolved. This method also ensures that there are minimal nonspecific interactions even with hydrophobic molecules[2].

The Agilent 1260 Infinity II Bio-inert LC system is the next generation of Agilent Bio-inert LC, specially designed for conditions used in biochromatography, that is, high salt concentrations (2 M NaCl, up to 8 M urea), and high and low pH solvents (0.5 M NaOH, 0.5 M HCL), by working with a completely inert sample flowpath. All capillaries and fittings throughout the multisampler, multicolumn thermostat, and detectors are completely metal-free so that biomolecules come in contact only with ceramics or PEEK[3]. Especially, when working with high salt buffers, as often found in intact protein analysis methods such as hydrophobic interaction chromatography (HIC), ion exchange chromatography (IEX), and SEC, the inert flowpath can prevent many of the typical issues of stainless steel systems, such as corrosive effects.

This Application Note shows the aggregation analysis of trastuzumab and two ADCs with the AdvanceBio SEC column. The chromatographic performance is also compared to an older generation Bio SEC column that has a different surface chemistry, which is more likely to exhibit nonspecific interactions.

Experimental and Instrumentation

The Agilent 1260 Infinity II Bio-inert LC system used consisted of:

- Agilent 1260 Infinity II Bio-inert 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 Diode Array Detector WR (G7115A) with bio-inert flow cell (option #028)

Columns

- Agilent AdvanceBio SEC 300 Å, 7.8 × 300 mm, 2.7 µm (p/n PL1180-5301)
- Agilent Bio SEC-3, 300 Å, 7.8 × 300 mm, 3 µm (p/n 5190-2511)

Software

Agilent OpenLAB CDS Version 2.1

Samples

Trastuzumab emtansine (T-DM1, Kadcyla), Trastuzumab (Herceptin), and Brentuximab Vedotin (Adcetris)

Table 1. Chromatographic conditions.

Parameter	Value
Mobile phase	PBS (pH 7.4)
Flow rate	0.8 mL/min
Stop time	20 minutes
Needle wash mode	Standard Wash
Injection volume	10 µL
Column temperature	30 °C
DAD	280 nm/4 nm, Ref. Off >0.05 minutes (1 second response time) (5 Hz)

Chemicals

Sodium phosphate monobasic and dibasic as well as phosphate-buffered saline tablets were purchased from Sigma-Aldrich, St. Louis, Missouri, US. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22 µm membrane point-of-use cartridge (Millipak).

Results and Discussion

This Application Note compared two columns, the Agilent AdvanceBio SEC column and an older generation Agilent Bio SEC-3 of similar pore size. Both had the same dimensions, but different surface chemistry. The AdvanceBio SEC column had a smaller particle size (2.7 µm in the AdvanceBio SEC compared to 3 µm for the Bio SEC-3 column). Figure 1 shows the chromatograms for the analysis of trastuzumab on both columns. No major differences can be observed on both columns for the analysis of a simple mAb. Both columns deliver sharp symmetrical peaks indicative of a size separation with no nonspecific interactions between the column and the sample.

The situation is different when analyzing ADCs. Due to the hydrophobic contribution of the conjugated small molecule, the ADC is too hydrophobic to achieve a size-based separation with the Bio SEC-3 column, using an aqueous mobile phase. This leads to immense peak tailing, as shown in Figure 2. In this figure, the analysis of trastuzumab emtansine is shown on both columns. Note that the ADC shows peak tailing over more than 5 minutes when analyzed on a Bio SEC-3 column. Conversely, a sharp peak can be achieved on the AdvanceBio SEC column, which has been designed with a unique surface chemistry to minimize nonspecific interactions for hydrophobic samples, such as the ADC. In addition, the aggregates and monomer are only baseline-separated using the AdvanceBio SEC column.

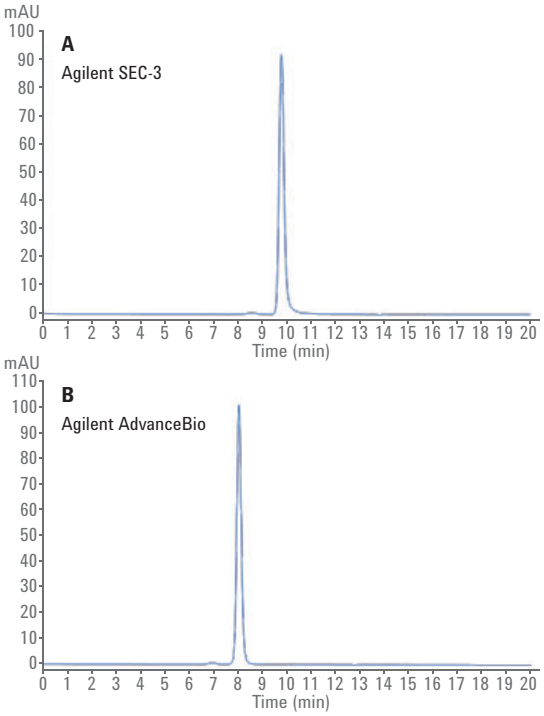

Figure 1. Analysis of trastuzumab on the Agillent Bio SEC-3 (A) and on the Agilent AdvanceBio SEC column (B). No major difference can be observed for the analysis.

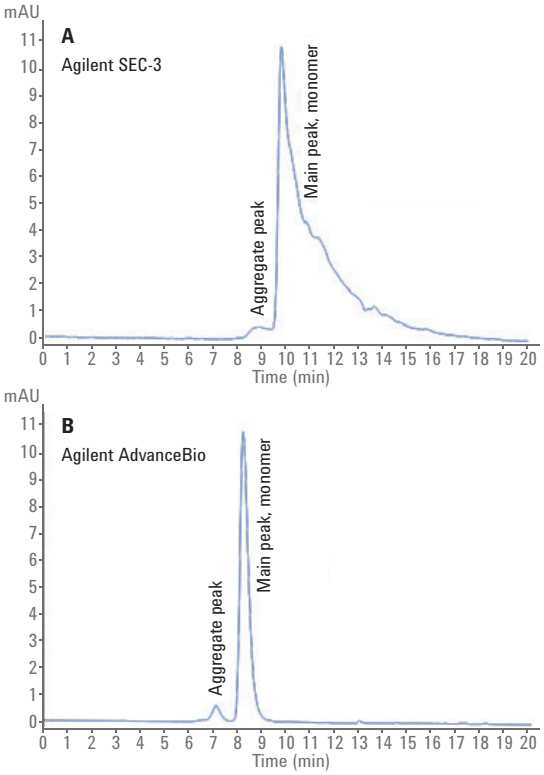

Figure 2. Separation of trastuzumab emtansine on an Agilent Bio SEC-3 column (A) and an Agilent AdvanceBio SEC column (B).

The narrow and highly symmetrical peak shape for ADCs on the AdvanceBio SEC column is a clear indicator of the benefits of the highly shielded particle surface of the packing material. The ideal SEC separation is a pure sieving mechanism based on the size of the compounds analyzed. Retardation or adsorption effects are not desired, and result in poor peak shapes. The AdvanceBio SEC silica particles are hydrophilically bonded, and allow a pure SEC separation mechanism, even for hydrophobic ADCs and modified mAbs. This results in symmetrical peak shapes as well as high resolution.

Figure 3 shows that a similar effect was observed in the chromatograms of the analysis of brentuximab vedotin. The effect of peak tailing was again observed with the Bio SEC-3 column. The peak also resulted in at least two or more peaks distributed over several minutes. Conversely, using the AdvanceBio SEC column resulted in a sharp peak and baseline separation of the aggregates. Under the conditions reported here, only with the AdvanceBio SEC column would it be possible to quantify the level of aggregation in the ADC samples.

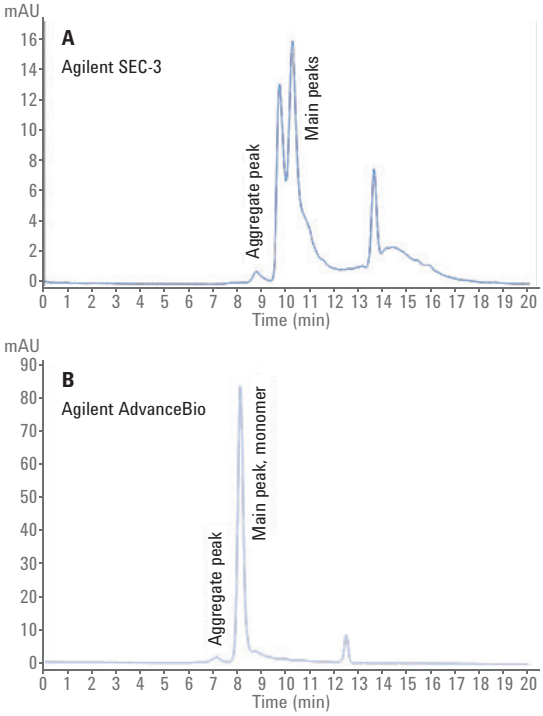

Figure 3. Separation of brentuximab vedotin on the Agilent Bio SEC-3 column (A) and the Agilent AdvanceBio SEC column (B).

Conclusion

The performance of two columns was compared for the analysis of mAbs and ADCs in terms of peak shape and baseline separation of aggregates. The Agilent AdvanceBio SEC column showed clear advantages for the analysis of ADCs. Both ADC samples evaluated (trastuzumab emtansine and brentuximab vedotin) showed size exclusion chromatography with a sharp peak shape and baseline separation of the aggregates on the AdvanceBio SEC column. The peak shape of both ADCs produced using the older technology, the Agilent Bio SEC-3, was not satisfactory, and it was not possible to quantify the level of aggregation in the ADC. For less hydrophobic mAbs such as trastuzumab, acceptable peak shape was seen with both columns. However, other mAbs, especially modified or conjugated antibodies could be affected. This Application Note demonstrates the AdvanceBio SEC column together with the Agilent 1260 Infinity II Bio-inert System as an ideal combination for the aggregate analysis of ADCs.

References

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