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應用手冊

Size Exclusion Chromatography Method Transfer of a Monoclonal Antibody Across UHPLC Systems

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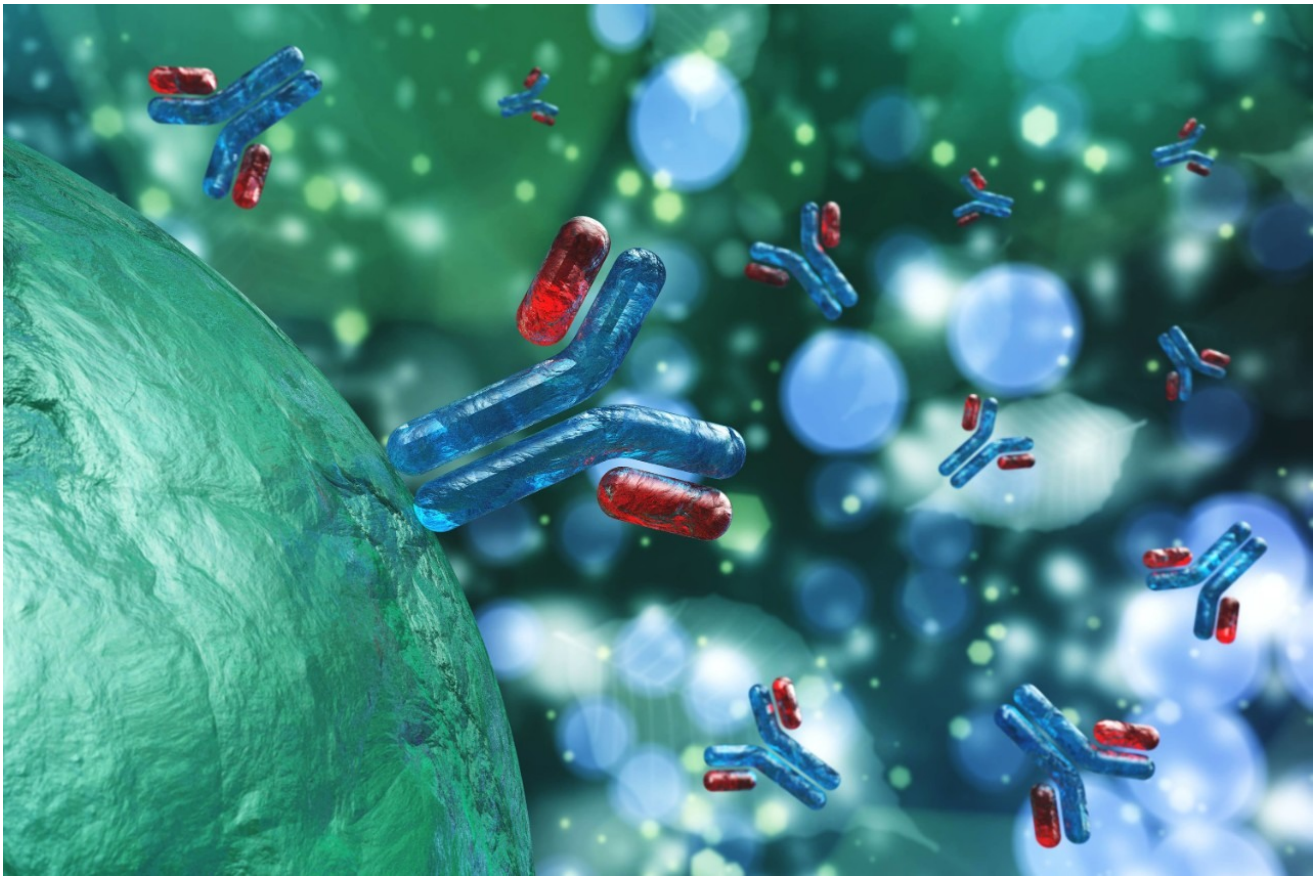

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates a size-exclusion chromatography method transfer for monoclonal antibody analysis from an Agilent 1260 Infinity Bio-Inert LC System to an ACQUITY Arc Bio System with ACQUITY 30 cm Single Zone Column Manager (CM-30S). By selecting the optimal SEC column based on LC system dispersions, comparable retention time, percent area of monomer, high molecular weight species and low molecular degradants, and repeatability were obtained.

Benefits

- Seamless SEC method transfer for a mAb across an ACQUITY Arc Bio System and Agilent 1260 Infinity Bio-inert System
- ACQUITY CM-30S allows running legacy LC methods and method development requiring columns greater than 25 cm

Introduction

Aqueous size-exclusion chromatography (SEC) is well established for the relative quantification of protein aggregates (high molecular weight species, HMWS).[1] Protein aggregates have been shown to correlate with undesired immunogenic effects as well as decreased efficacy.[2] Therefore, it is important that the SEC method can reproducibly quantify the amounts of HMWS along with other degradants during method transfer among different ultra high performance liquid chromatography (UHPLC) systems.

During method transfer, one important system characteristic that should be considered is the extra-column dispersion, also commonly called bandspread. A large extra-column dispersion always has a deleterious effect on the resolution of a separation, especially on SEC resolutions, since it is a diffusion-driven separation with minimal to no interaction with the stationary phase.[3] Selecting the optimal SEC column dimensions for an LC system can minimize the impact of extra-column dispersion.[4] To Illustrate this, we will demonstrate the transfer of an SEC method from an Agilent 1260 Infinity Bio-inert System to an ACQUITY Arc Bio System with CM-30S. CM-30S is a new extension of column managers for ACQUITY Arc systems.[5] It maintains the capability to run legacy methods requiring longer columns and expands column capacity up to 15 columns (with two CM-30S), allowing method development on larger dimension columns.

Results and Discussion

In liquid chromatography, the band broadening of a peak is approximately the sum of extra column and on-column dispersion volumes.[6] The extra column dispersion is the value that we can determine by the experiment, represented by $5\sigma_{ec}$, which the peak width at 4.4% peak height multiplies by the flow rate.[6,7] The measured $5\sigma_{ec}$ for the Agilent 1260 Infinity Bio-inert System is 31 µL, and 50 µL for the ACQUITY Arc Bio System with CM-30S. The key method transfer goal is to obtain equivalent quantification of percent area of HMWS. Following the guidance for selecting the optimal SEC column based on LC system dispersions,[4] we selected a 7.8 mm I.D. x 300 mm SEC column with the stationary particle size of 3.5 µm.

The SEC analysis of trastuzumab (post-expiration), an anti-HER2 IgG1 mAb used to treat breast cancer,[8] was run on both the Agilent 1260 Infinity Bio-inert System (Agilent 1260 Bio) and the ACQUITY Arc Bio System with CM-30S. Figure 1 showed that comparable chromatographic profiles with similar retention times and resolution were observed between the two systems. In both chromatograms, a single HMW and a low molecular weight (LMW) peak were baseline resolved from the main monomer peak. Adjacent to the monomer peak, a non-resolved shoulder (labeled as "*") was observed. For the purpose of method transferability, the monomer peak was integrated to include this shoulder.

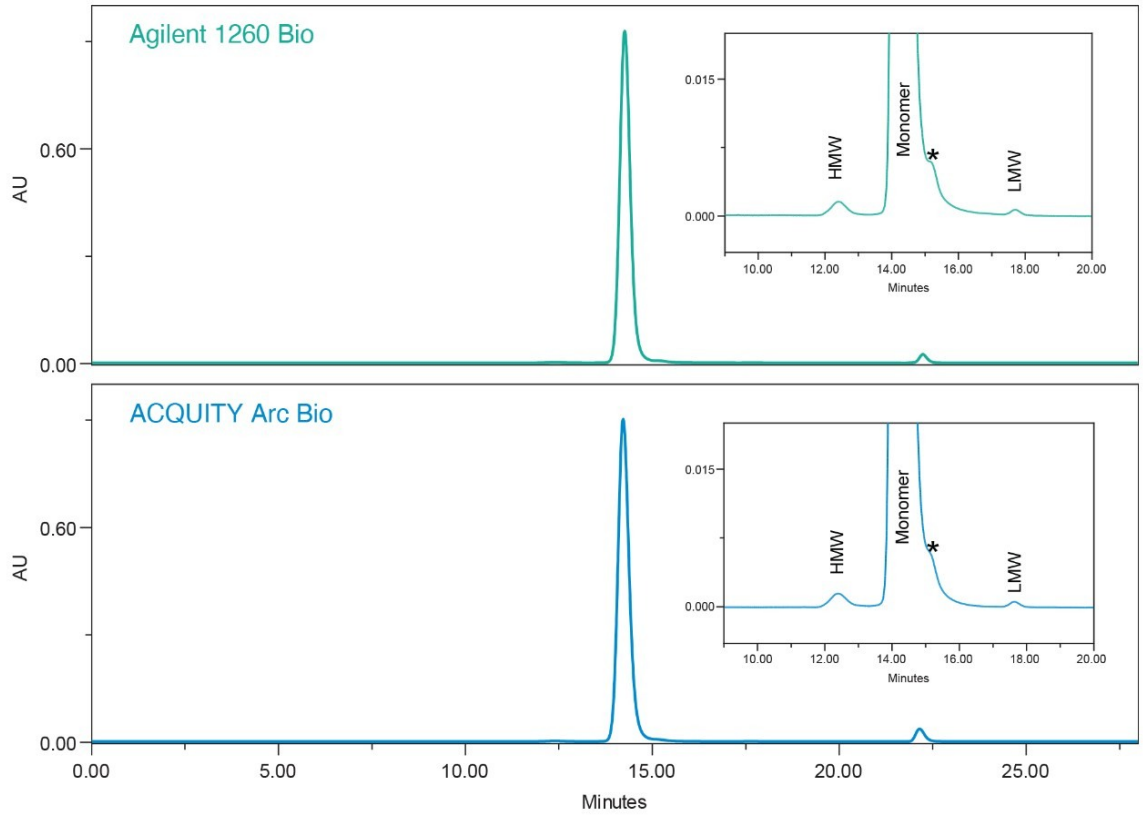

Figure 1. SEC chromatograms for trastuzumab acquired on an Agilent 1260 Infinity Bio-inert System (labeled as "Agilent 1260 Bio") and an ACQUITY Arc Bio System with CM-30S. (Method: XBridge Protein BEH SEC Column, 200 Å, 3.5 µm, 7.8 mm x 300 mm; 20 mM phosphate buffer/350 mM sodium chloride, pH 6.8; Trastuzumab, 10 mg/mL, injection volume, 10 µL; flow rate, 0.5 mL/min).

In the product life cycle of mAbs and other protein-based drugs, protein aggregation is a major problem since it can cause undesired immunogenic response and decreased efficacy. One of the primary goals of the SEC separation is to measure the percent of aggregates, i.e., HMW species. Table 1 showed the average resolution (Rs) between HMW and monomer, the peak width at 50% of peak height, and the peak width at 4.4% of peak of the HMW peak from both UHPLC systems. A negligible difference of the resolution between HMW and monomer and peak width of the HMW was observed across these two systems.

HMW peak	Rs	Width @ 50% (min)	Width @ 4.4% (min)
Agilent 1260 Bio	2.6	0.510	1.166
ACQUITY Arc Bio	2.5	0.524	1.195
Difference	**0.1**	**-0.01**	**-0.03**

Table 1. Comparison of average (n=6) resolution (Rs) between HMW peak and monomer peak, peak width at 50%, and peak width at 4.4% of the HMW peak between systems.

Table 2 summarizes the peak area percent and repeatability for the HMW, monomer peak along with LMW species. Comparison of the percent area showed no difference for the HMW, the monomer, and LMW, and percent RSD has up to two 0 digits 0.00% for all analytes across both systems.

% Area	HMW			Monomer			LMW		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
Agilent 1260 Bio	0.26	0.00	0.00	99.68	0.00	0.00	0.06	0.00	0.00
ACQUITY Arc Bio	0.26	0.00	0.00	99.68	0.00	0.00	0.06	0.00	0.00
Difference	**0.00**			**0.00**			**0.00**		

Table 2. Comparison of percent peak area (% Area) repeatability (n=6) of SEC separation of trastuzamab on both systems.

Even though the ACQUITY Arc Bio System with CM-30S has larger extra-column dispersion than the Agilent 1260 Infinity Bio-inert System, the impact on SEC separation is minimized by selecting an optimum SEC column. The extra-column dispersion is sufficiently small relative to the on-column dispersion; thus, an equivalent separation was obtained across these two UHPLC systems.

Conclusion

An SEC method for a mAb was transferred successfully from an Agilent 1260 Infinity Bio-Inert UHPLC System to an ACQUITY Arc Bio System with ACQUITY 30 cm Single Zone Column Manager (CM-30S). A critical quality attribute, the percent area of HMW species, was within 0.00% difference across both systems. The repeatability of percent area was within 0.00% RSD for all the species.

The ACQUITY Arc Bio System configured with the CM-30S will easily accommodate the 7.8 mm I.D. x 300 mm length columns often employed in legacy SEC separations. In this application brief we have demonstrated that the ACQUITY Arc Bio System reproduced high resolution separations with accurate quantification of aggregates, monomers, and fragments. In addition, with two CM-30S configured to ACQUITY Arc Bio systems, it can expand column capacity up to 15 columns, benefitting method development on larger dimension columns on a modern UHPLC system.

References

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