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Nota de aplicación

Size Exclusion Chromatography Method Transfer from a Standard HPLC to an Arc HPLC System

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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 (SEC) method transfer for monoclonal antibody (mAb) analysis from an industry-standard HPLC system to a Waters Arc HPLC System. Comparable retention time, percent area of monomer, high molecular weight species, and low molecular degradants and repeatability were obtained from both systems.

Benefits

- Seamless SEC method transfer across a standard HPLC system and Arc HPLC System
- Arc HPLC System provides tight RSD for SEC analysis for size variants of a monoclonal antibody (mAb)

Introduction

Size-exclusion chromatography is one of the common LC methods used to evaluate biotherapeutics in the biopharmaceutical industry. While bio-inert or biocompatible LC systems may be preferred, due to the high salt concentrations commonly used in SEC methods, this is not always possible due to instrument availability. Stainless steel LC systems can be successfully employed for SEC separations with proper care, such as flushing the system upon completion of analysis.

This study will demonstrate an SEC method transfer for a monoclonal antibody (mAb) biotherapeutic, trastuzumab, from an industry-standard HPLC system to an Arc HPLC System. Protein aggregation, including high molecular weight (HMW) species, has been shown to correlate with undesired immunogenic effects as well as decreased efficacy.[1] Therefore, it is important that the SEC method can reproducibly quantify the amounts of HMW species along with other degradants, regardless of the instrumentation. In this example, we will illustrate the transferability of an SEC method between a standard HPLC and an Arc HPLC System.

Results and Discussion

Protein aggregates, or HMW species, can potentially impact biotherapeutic product safety and efficacy.[1] In the product life cycle of mAbs and other protein-based drugs, SEC is a common separation method used to monitor the presence of both HMW and low molecular weight (LMW) degradants. LMW degradants usually are caused by non-enzymatic peptide bond hydrolysis.[2]

Trastuzumab is an anti-HER2 IgG1 mAb used to treat breast cancer.[3] The SEC analysis of trastuzumab (post-expiration) was run on two HPLC systems, an industry-standard HPLC system and an Arc HPLC System. Comparable chromatographic profiles with similar retention times and resolution were observed between the two systems (Figure 1). In both chromatograms, a single HMW and an LMW peak (labeled as “LMW2”) were baseline resolved from the main monomer peak. Adjacent to the monomer peak, a partially resolved shoulder (labeled as “LMW1”) was observed. For the purpose of method transferability, the monomer peak was integrated to include this shoulder.

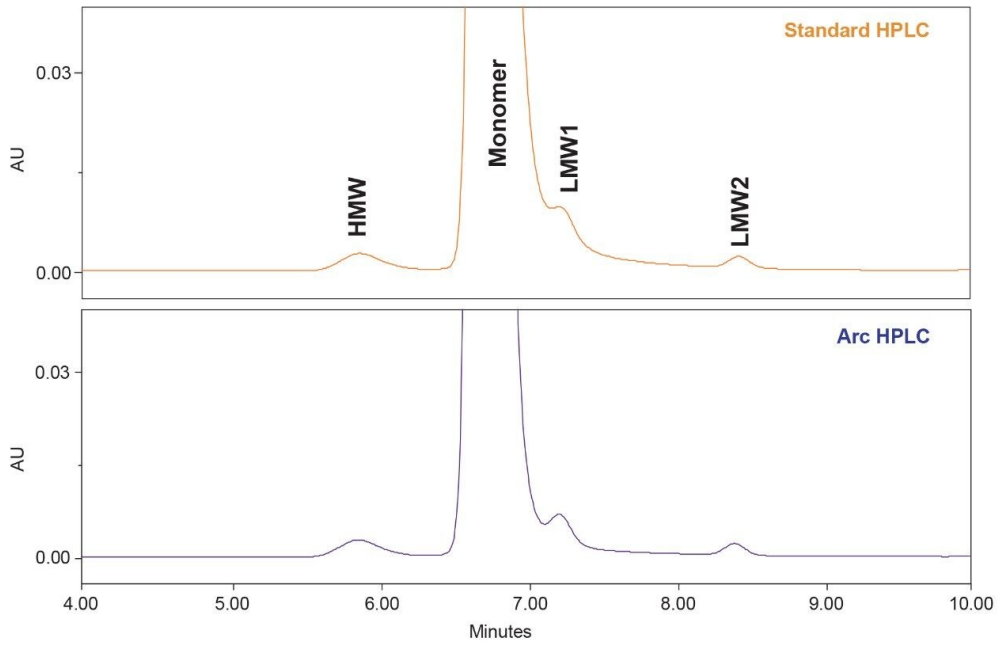

Figure 1. SEC chromatograms for trastuzumab acquired on the standard HPLC system (top) and the Arc HPLC System (bottom). (Method: XBridge Protein BEH SEC Column, 200 Å, 2.5 µm, 7.8 mm × 300 mm; 20 mM phosphate buffer/350 mM sodium chloride, pH 6.8; Trastuzumab, 10 mg/mL, injection volume, 10 µL; flow rate, 1.0 mL/min)

Tables 1 and 2 showed the average retention times (RT) and percent peak area for five replicate injections along with the corresponding standard deviations and percent RSD. The average RT of each of the three peaks across five injections and percent RSD on both these two systems were comparable. The shift in retention time was approximately 0.02 min while the RT repeatability for all the peaks was within 0.1% RSD.

Retention time (min)	Standard HPLC		Arc HPLC		Difference
	RT	%RSD	RT	%RSD	
HMW	5.89	0.07	5.87	0.06	0.02
Monomer	6.75	0.06	6.72	0.03	0.03
LMW 2	8.43	0.05	8.41	0.03	0.02
Average					**0.02**

Table 1. Comparison of retention time (RT) repeatability (n=5) of SEC separation of trastuzumab between systems.

% Area	HMW			Monomer			LMW2		
	Mean	SD	%RSD	Mean	SD	%RSD	Mean	SD	%RSD
Standard HPLC	0.52	0.01	1.71	99.29	0.01	0.01	0.18	0.00	0.00
Arc HPLC	0.53	0.01	1.04	99.28	0.00	0.00	0.19	0.00	0.00
Difference	**0.01**			**0.01**			**-0.01**		

Table 2. Comparison of percent peak area (%Area) repeatability (n=5) of SEC separation of trastuzamab on both systems.

One of the primary goals of the SEC separation is to measure the percent of aggregates (i.e., HMW species). Aggregation is a major problem in the development of protein therapeutics due to undesired immunogenic response and decreased efficacy. Table 2 summarizes the peak area percent and repeatability for the monomer peak and HMW and LMW2 species. Comparison of the percent area showed a difference within 0.01% for the HMW, the monomer, and LMW2, and percent RSD within 2% for all analytes across both systems.

From Figure 1, a slightly deeper valley between the main monomer peak and shoulder peak LMW1 can be visually observed on the Arc HPLC System than the standard HPLC system. By comparing the peak width at 50% of the peak height (width @ σ) and peak width at 4.4% of the peak height (width @ 5σ), the Arc HPLC System produces slightly narrower peak widths than the standard HPLC system (Table 3). The monomer peak on the Arc HPLC System also gave slightly lower tailing factor. Overall, the peak valley ratio (p/v) between the monomer

and LMW1 peak on the Arc HPLC System is 1.32 while it is 1.02 on the standard HPLC system. Empower Chromatography Data System (CDS) can integrate the peaks by dropping a line at the valley between the monomer and the LMW1 peak to assign a percent area to LMW1. The percent area of LMW1 was 1.58% on the standard HPLC system and 1.00% on the Arc HPLC System, while the summation of the monomer and LMW1 remains the same (Figure 2). Even though the chromatographic performance can vary from system to system, this case study demonstrated the same, if not better, performance for the SEC analysis can be achieved on an Arc HPLC System compared to a standard HPLC system.

LC systems	Monomer peak			p/v
	width @ σ	width @ 5σ	Tailing	
Standard HPLC	0.40	0.17	1.32	1.02
Arc HPLC	0.37	0.16	1.25	1.32

Table 3. Comparison of the monomer peak width (width @ σ and width @ 5σ), tailing, and p/v between the monomer peak and LMW1 peak on both HPLC systems.

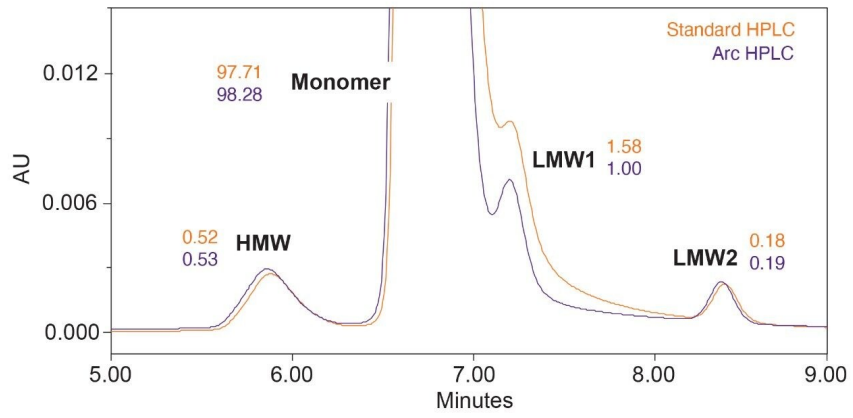

Figure 2. Area percent (average of five injections) comparison of all the species for trastuzumab on both HPLC systems.

Conclusion

An SEC method for trastuzumab was successfully transferred from an industry-standard HPLC system to an Arc HPLC System. The difference in retention times across the systems were within 0.02 min. A critical quality attribute, the difference of percent of HMW species, was within 0.01% across both systems. The repeatability of percent area was within 2% RSD for all the species. Furthermore, the Arc HPLC System produced slightly narrower peaks than the standard HPLC system, resulting in a higher p/v ratio between the main monomer and the shoulder LMW1 peak.

References

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3. Koza, S.; Hong, P.; and Fountain, K.J. Size-Exclusion UltraPerformance Liquid Chromatography Method Development for the Analysis of the Degradation Products of the Trastuzumab Antibody. Waters Application Note, 2012, 720004416EN <https://www.waters.com/waters/library.htm?cid=511436&lid=134693747> .

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