APPLICATIONS

Optimizing Monoclonal Antibody Separation using bioZen™ SEC-3 Column

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Introduction

Size exclusion chromatography is a non-adsorptive separation technique which fractionates analytes based on their hydrodynamic volume. It is commonly used for protein aggregation characterization in part as it is run under non-denaturing conditions. Monoclonal antibodies (mAbs) are the most common protein therapeutics with an estimation that by 2020 there will be over 70 mAb therapeutics in the global market.[1] Accurate characterization of monoclonal antibody (mAb) aggregates has huge significance in the development of new therapeutic agents. The presence of mAb aggregates can affect the efficacy of the therapeutic agent and cause safety implications. In biopharmaceutical development the quantitation of aggregate is a critical quality attribute with the determination of monomer, dimer as well as higher order structure commonly achieved with size exclusion chromatography.

Although this technique is generally used to determine the extent of aggregation of a protein we also present data showing the benefits of bioZen SEC-3 for the characterization of low molecular weight species (LMW) including protein fragments.

The introduction of high efficiency UHPLC instruments offering low system dwell volume coupled with sub-2 µm particles has offered significant improvements in the resolution and characterization of aggregates and fragments. In this technical note, four monoclonal antibodies were analyzed using sub-2 µm SEC columns under optimized mobile phase conditions. With bioZen SEC-3 and the introduction of BioTi™ hardware, identification of even lower concentration targets is now possible.

bioZen SEC-3, 1.8 µm, 300 x 4.6 mm (Phenomenex, Inc., Torrance, CA) was compared against a column from another leading manufacturer (ACQUITY® 1.7 µm Protein BEH SEC, Waters® Technologies Corp., Milford, MA) to demonstrate performance differences.

Materials and Methods

Trastuzumab and Rituximab biosimilars we obtained from Pall Corporation (MA, USA). Cetuximab and Trastuzumab were obtained from Myoderm® (Norristown, PA, USA). All applications were performed on an Agilent® 1260 Infinity II LC system equipped with a UV-Vis detector.

All chemicals were purchased from Sigma-Aldrich® (St Louis, MO)

HPLC Conditions

Conditions same for all samples:

Column:	bioZen 1.8 µm SEC-3 ACQUITY 1.7 µm Protein BEH SEC
Dimensions:	300 x 4.6 mm
Mobile Phase:	50 mM Potassium Phosphate + 300 mM Potassium Chloride (pH 6.8)
Flow Rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Samples:	As indicated

Comparative separations may not be representative of all applications.

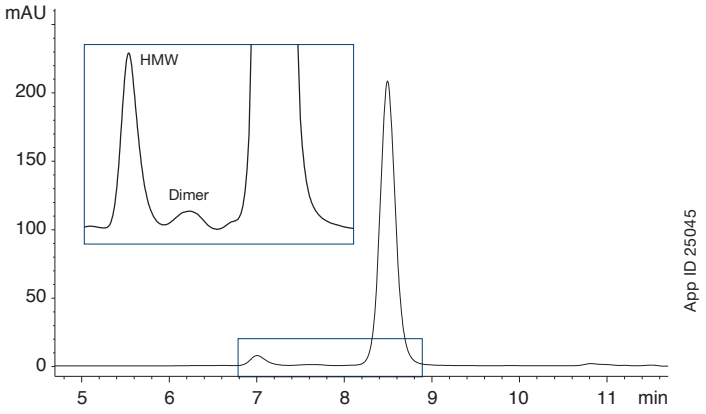
Results and Discussion

Using conditions developed by Fekete and coworkers,[2] the performance of the bioZen SEC-3 and its ability to resolve aggregate and fragment peaks of some common mAbs was evaluated. The advantages of bioZen SEC-3 over other columns is highlighted below and shows the improved recovery and separation we see from the bioZen column specifically for mAb fragments from their monomers. The BioTi hardware removes the need to extensively prime the column offering a reduction in analysis time and an improvement in reproducibility and recovery compared with traditional standard stainless steel hardware[3].

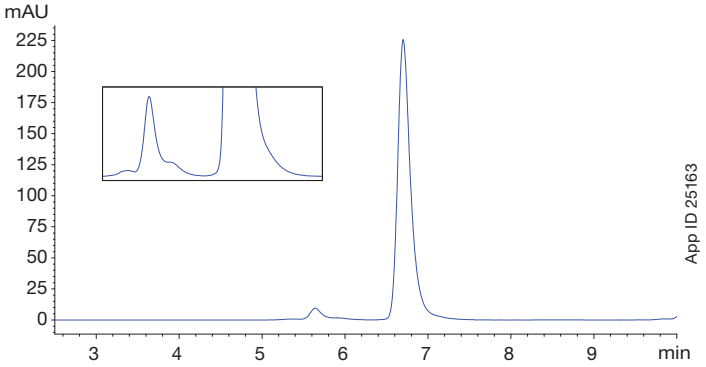
Rituximab Biosimilar

bioZen 1.8 µm SEC-3, when compared to the Waters ACQUITY 1.7 µm Protein BEH SEC column, showed excellent HMW separation for a Rituximab biosimilar with only limited HMW separation seen on the Waters BEH column. Separation and identification of all HMW species may be important for therapeutic development. In these cases, the bioZen SEC-3 column would be the preferred technology for rituximab HMW separation.

bioZen 1.8 µm SEC-3



Waters ACQUITY 1.7 µm Protein BEH SEC



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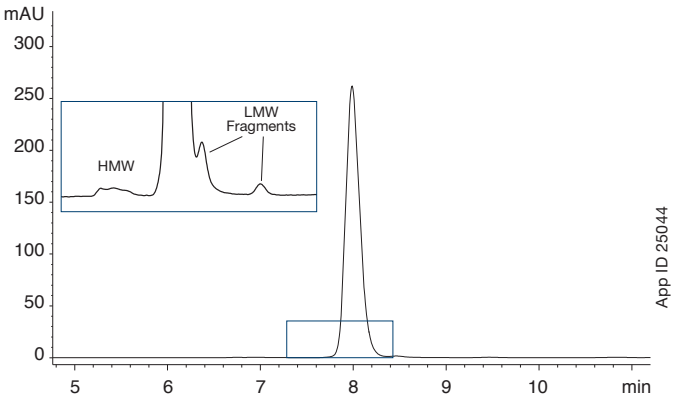
Results and Discussion *(cont'd)*

Cetuximab

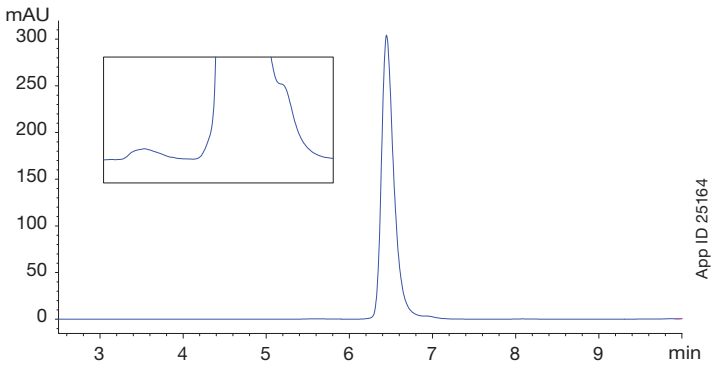
When analyzing Cetuximab, good separation of the HMW species was observed. In this case, we also detected fragment, or LMW peaks with excellent separation of the fragment peak on the tail of the main peak using the bioZen™ SEC-3. When comparing these mobile phase conditions to the Waters® ACQUITY® 1.7 µm Protein BEH SEC column, bioZen showed superior HMW definition and an improvement of resolution of the LMW fragment peaks.

Cetuximab

bioZen 1.8 µm SEC-3



Waters ACQUITY 1.7 µm Protein BEH SEC

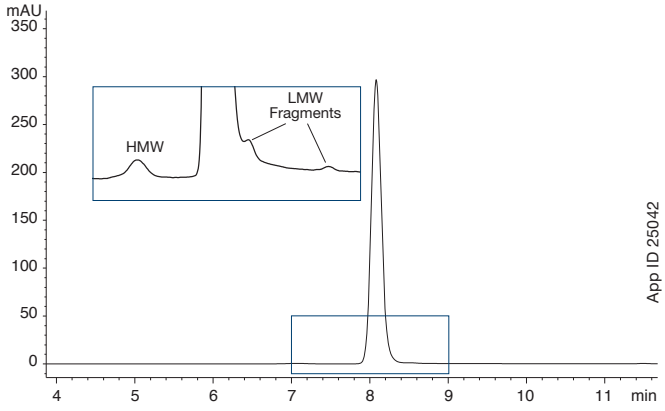


Trastuzumab and Trastuzumab Biosimilar

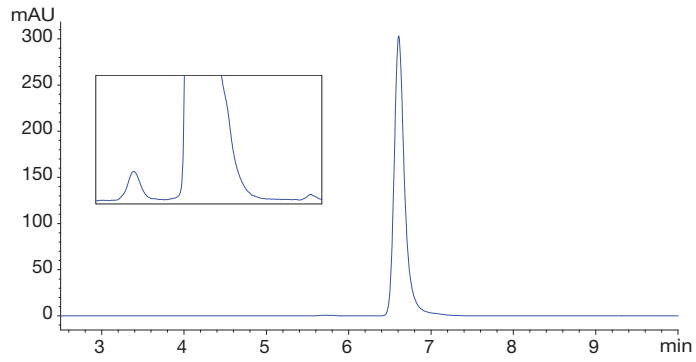
Next, we analysed trastuzumab and a biosimilar product. A biosimilar can be regarded as a therapeutic protein which is an approved replica of the original innovator product. Unlike generic small molecules, biologics have a higher molecular complexity and are sensitive to changes in their expression or manufacturing processes. Despite their differences biosimilars must maintain consistent quality and clinical performance to the innovator and size exclusion is an important technique in their characterization. For the analysis of trastuzumab and a biosimilar, again we saw superior separation of the fragment peak with bioZen SEC-3. Both columns partially resolved the fragment in the biosimilar and HMW separations were observed for both.

Trastuzumab

bioZen 1.8 µm SEC-3



Waters ACQUITY 1.7 µm Protein BEH SEC



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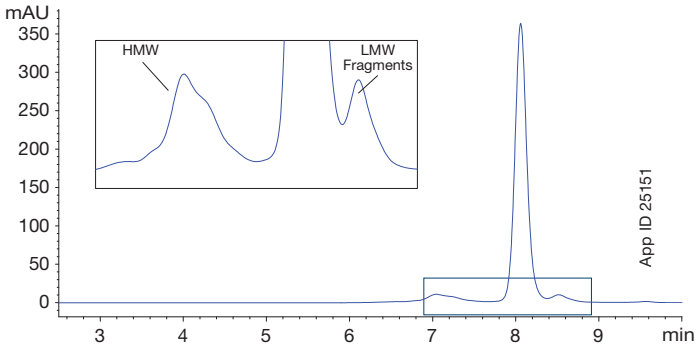
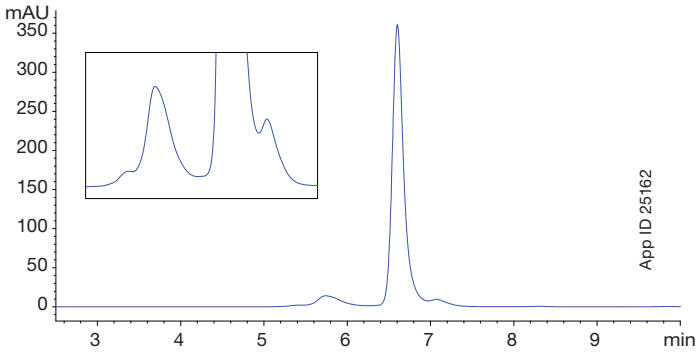
Results and Discussion *(cont'd)*

Trastuzumab Biosimilar

bioZen™ 1.8 µm SEC-3



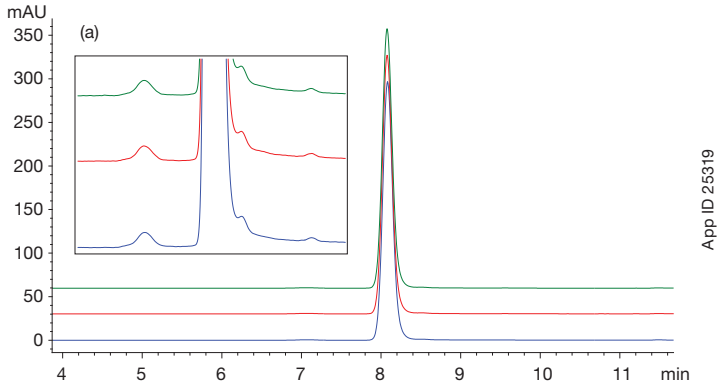
Waters® ACQUITY® 1.7 µm Protein BEH SEC

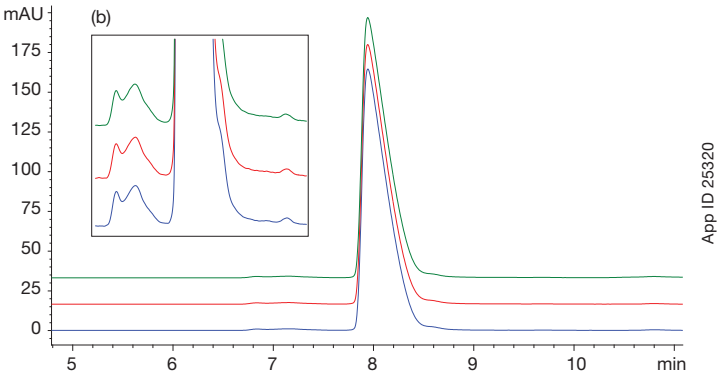


During our study we also investigated the reproducibility of the bioZen SEC-3 media and made triplicate injections of each mAb to determine the extent of recovery and retention time variation. In all cases no retention time shifts were observed and excellent correlation of peak area and recovery was seen in all cases with bioZen SEC-3. Looking at the triplicate comparison for trastuzumab and an infliximab biosimilar examples, there were no observed differences in the selectivity traces for both the aggregates and the fragments when repeat injections were made. Consistent resolution of the aggregate and fragment peaks was observed in both cases including a good correlation of recovery.

Results and Discussion *(cont'd)*

Overlay of injections of trastuzumab (Fig a) and an Infliximab biosimilar (Fig b) on bioZen SEC-3. In these examples a neat standard of mAb was injected onto a conditioned bioZen SEC-3 column. No retention time shifts were observed indicating reproducibility of the phase across multiple runs.





Having resolution of the aggregate and fragment species in a single run is critical and in all cases with the bioZen 1.8 µm SEC-3 column we found this was achievable using optimized mobile phase conditions with excellent sensitivity for all applications.

Conclusion

The unique bioZen 1.8 µm SEC-3 stationary phase combined with BioTi™ hardware provides exceptional recovery and increased separation of both high molecular weight aggregates and low molecular weight species from monomeric mAbs. As outlined previously, having resolution of antibody fragments is essential in the characterization of protein therapeutics and is achievable using the bioZen 1.8 µm SEC-3 column.

References

1. Ecker DM, Jones SD, Levine HL. *The therapeutic monoclonal antibody market. mAbs*. 2015;7(1):9-14. doi:10.4161/19420862 .2015.989042
2. Fekete, S., Beck, A., Veuthey, J.; *Theory and practice of size exclusion chromatography for the analysis of protein aggregates*; J Pharmaceutical and Biomedical Analysis 101 (2014) 161-173.
3. Bioinert Versus Biocompatible: *The Benefits of Different Column Materials in Liquid Chromatography Separations; LCGC*; Jun 01, 2018; Jason A. Anspach, Srinivasa Rao, Brian Rivera; Volume 36, Issue 6, pg 24–29

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Ordering Information bioZen™

bioZen Columns (mm)						Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
				—	—	/3pk	—	ea
bioZen 2.6 µm Glycan	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	AJ0-9800	—	AJ0-9000
				—	—	/3pk	—	ea
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	AJ0-9803	—	AJ0-9000
		—				/10pk	/10pk	ea
bioZen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	00B-4771-E0	00F-4771-E0	AJ0-7605	AJ0-7606	KJ0-4282
				—	—	/3pk	—	ea
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	AJ0-9806	—	AJ0-9000
						/3pk	/3pk	ea
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00B-4768-E0	00F-4768-E0	AJ0-9806	AJ0-9808	AJ0-9000
						/3pk	/3pk	ea
bioZen 3.6 µm Intact C4	00B-4767-AN	00D-4767-AN	00F-4767-AN	00B-4767-E0	00F-4767-E0	AJ0-9809	AJ0-9811	AJ0-9000
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	00B-4766-E0	00F-4766-E0	AJ0-9812	AJ0-9814	AJ0-9000
	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	300 x 4.6		for 4.6 mm	Holder
	—			—		—	/3pk	ea
bioZen 1.8 µm SEC-2	—	—	00F-4769-E0	—	00H-4769-E0	—	AJ0-9850	AJ0-9000
bioZen 1.8 µm SEC-3	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
					—	—	/10pk	ea
bioZen 6 µm WCX	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	—	AJ0-9400	KJ0-4282

Sample Preparation

bioZen Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit
bioZen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box

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