

APPLICATIONS

Aggregate Analysis of Monoclonal Antibodies using a bioZen™ 1.8 µm SEC-3 Column

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Overview

Monoclonal antibody (mAb) aggregates have significant implications in safety, immunogenicity, and efficacy for therapeutic drug development. Size exclusion chromatography (SEC) is one of the laboratory methods used to determine the extent of aggregation of a protein. In addition, this chromatographic method can detect low molecular weight (LMW) species including protein fragments. This method demonstrates three mAbs trastuzumab (Herceptin®), infliximab (Remicade®), and cetuximab (Erbix®) with varying aggregate and fragment amounts. The optimized method for aggregate analysis on the bioZen SEC-3 column is 50 mM KH₂PO₄, 250 mM KCl at pH 6.8. Under these conditions for trastuzumab a clear separation of the high molecular weight (HMW) is shown along with detection of two fragment or LMW species (**Figure 1**). The SEC analysis of infliximab is shown in **Figure 2**, which demonstrates the separation of two HMW species and detection of 2-3 LMW peaks. Finally, **Figure 3** shows an analysis of cetuximab where HMW aggregates are giving moderate separation into three peaks. In addition, the fragment peak is separating from the monomer.

Materials and Methods

All samples were injected directly onto the column as follows: trastuzumab (10 mg/mL) as 2.5 µL injection; infliximab (5 mg/mL) as 5 µL injection; cetuximab (2 mg/mL) as 15 µL injection.

HPLC Conditions

Conditions same for all samples:

Column:	bioZen 1.8 µm SEC-3
Dimension:	300 x 4.6 mm
Part No.:	00H-4772-E0
Mobile Phase:	50 mM Potassium Phosphate + 250 mM Potassium Chloride (pH 6.8)
Flow Rate:	0.35 mL/min
Temperature:	30 °C
Detection:	UV @ 280 nm
Sample:	As Noted

Results

Figure 1.
Trastuzumab aggregate analysis

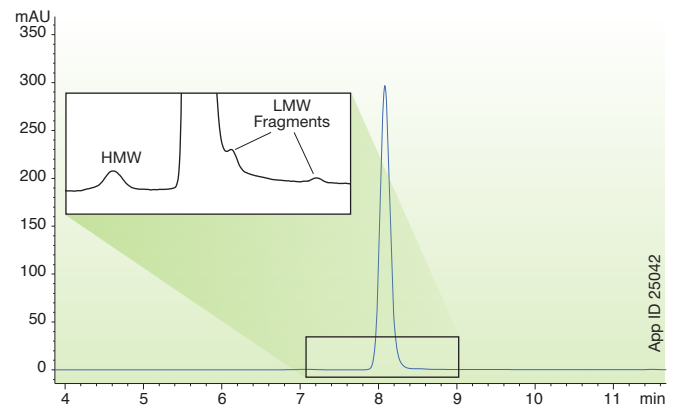


Figure 2.
Infliximab aggregate analysis

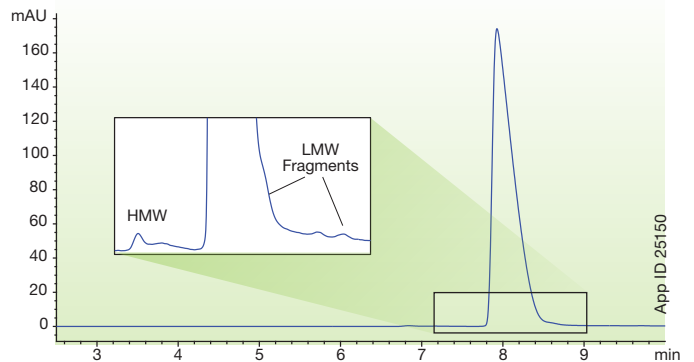
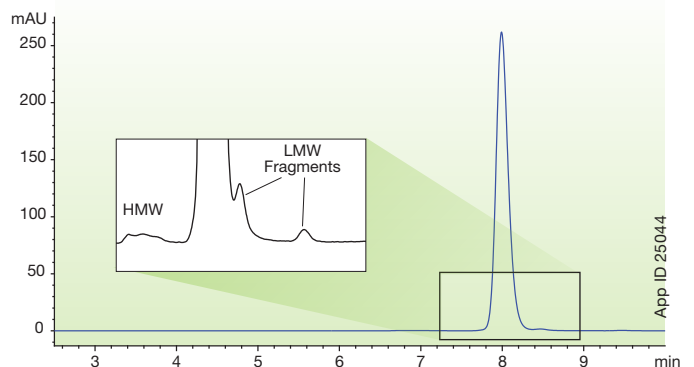


Figure 3.
Cetuximab aggregate analysis



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bioZen™ Ordering Information

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

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