APPLICATIONS

Improved Fragment Separation using bioZen™ 1.8 µm SEC-3

Helen Whitby[2], M. Christina Malinao[1], Brian Rivera[1], and Chad Eichman[1]
[1] Phenomenex Inc., 411 Madrid Avenue, Torrance, CA 90501 USA
[2] Phenomenex, Ltd., Queens Avenue, Hurdsfield Ind. Est., Macclesfield, Cheshire SK10 2BN UK

Introduction

Biopharmaceuticals have emerged as the next-generation in therapeutic molecules for the pharmaceutical industry, however biotherapeutics are not chemically synthesized like more traditional small molecule therapeutic compounds. Instead, they are produced recombinantly by engineered host cells. The complexity of analysis of biopharmaceuticals stems from the challenges associated with this process and during their recombinant production a multitude of post-translational modifications (PTMs) are generated. These modifications directly influence the function of the protein and are therefore the most critical aspect of their characterization. Identifying post-translational modifications is complex and analysts rely heavily on size exclusion chromatography (SEC), reversed phase (RP), and ion-exchange chromatography (IEX) to identify PTMs. These methods complement other techniques to provide a complete picture into the structure and function of biotherapeutic proteins.

Size exclusion chromatography (SEC) is widely used to characterize monoclonal antibodies (mAbs) and to quantify levels of aggregation, a critical quality attribute required for all therapeutic mAbs. The identification of fragments is also of prime importance during analysis as these fragments can significantly affect the efficacy of the protein and can have safety implications.

Monoclonal antibody (mAb) aggregates are often found at very low levels (<0.1 % by peak area compared to monomer) and to address this need for low level detection a robust set of bioZen SEC columns were developed which combine UHPLC efficiency and high sensitivity to drive resolution and identification of even lower level targets. Alongside this mAb fragment separation is often required adequate resolution, thus peak shape has become even more crucial to the successful outcome of the method.

In this technote we evaluate the benefits of the bioZen 1.8 µm SEC-3 column packed into BioTi™ hardware and compare it with a traditional size exclusion column to compare the efficiency, resolution and reproducibility of the two columns.

Materials and Methods

Cetuximab, rituximab, infliximab, and trastuzumab were purchased from Myoderm® (Norristown, PA) and injected directly onto the column. 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

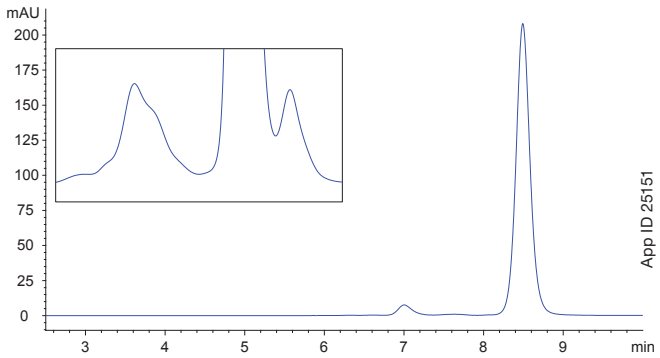
Column:	bioZen 1.8 µm SEC-3 TSKgel® 2 µm UP-SW3000
Dimensions:	300 x 4.6 mm
Mobile Phase:	50 mM KH_2PO_4 300 mM KCl, pH 6.8
Flow Rate:	0.35 mL/min
Detection:	UV @ 280 nm
Temperature:	30 °C
Samples:	As indicated (25 µL injection; 1 mg/mL concentration)

Results and Discussion

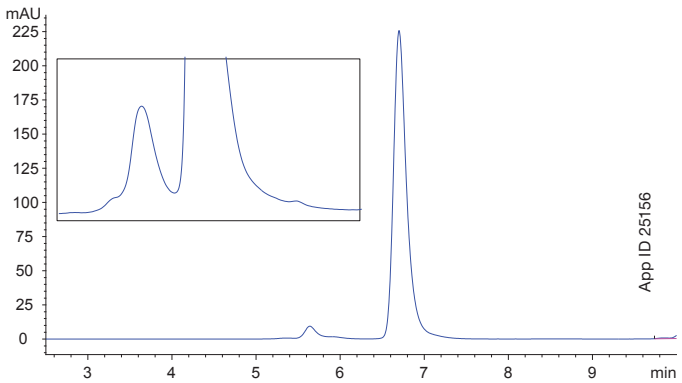
While ion-exchange and reversed phase chromatography are typically used for identifying many of the post-translational modifications of a protein, GFC (gel filtration chromatography) is exclusively used for identifying the aggregation state of most recombinant proteins and is a useful tool in the identification of fragment peaks. Here we present the results of a number of studies using bioZen 1.8 µm SEC-3 under conditions developed by Fekete and coworkers.[1] In this technote we evaluated bioZen SEC-3 together with the Tosoh Bioscience® TSKgel 2 µm UP-SW3000 for recovery of monoclonal antibodies, their aggregates, and also the mAb fragments.

Trastuzumab Biosimilar

bioZen 1.8 µm SEC-3



TSKgel 2 µm UP-SW3000



Comparative separations may not be representative of all applications.

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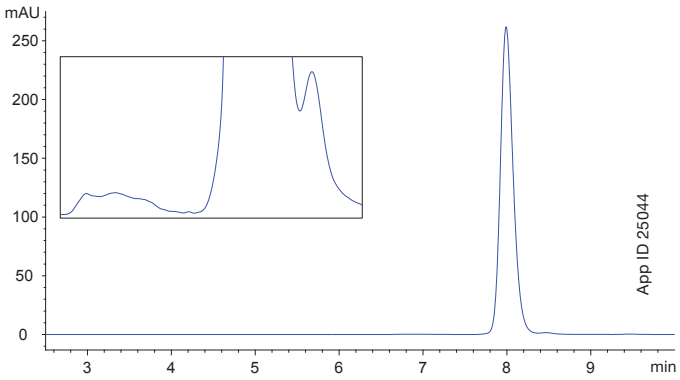
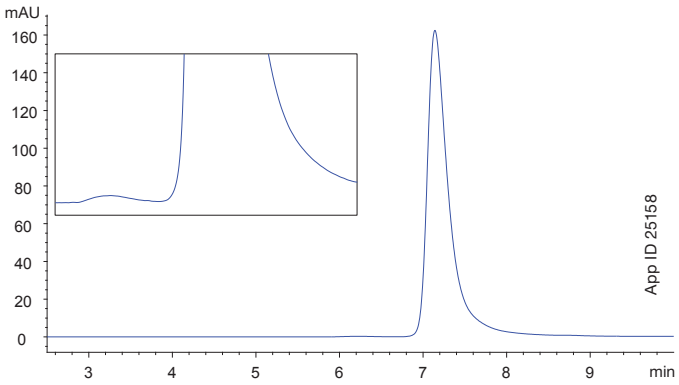
Cetuximab

bioZen™ 1.8 µm SEC-3

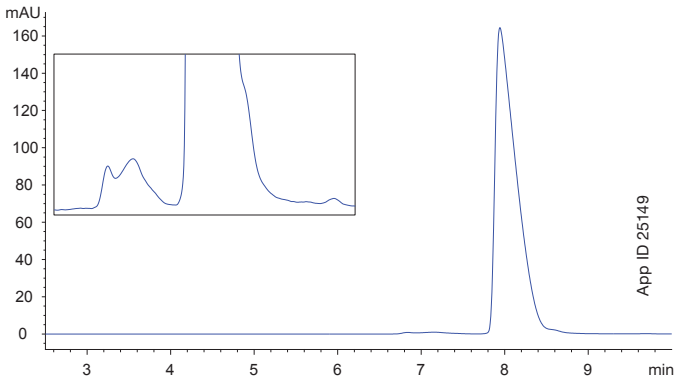


TSKgel® 2 µm UP-SW3000

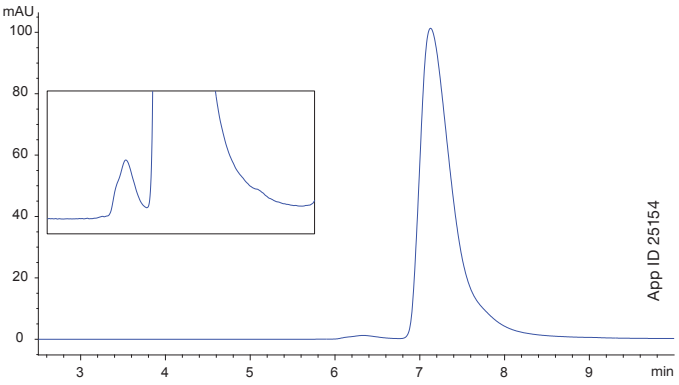


Infliximab Biosimilar

bioZen 1.8 µm SEC-3



TSKgel 2 µm UP-SW3000



Results and Discussion *(Cont'd)*

For trastuzumab peak shape is excellent; bioZen SEC-3 shows separation of both the fragment and aggregate peaks. There is also partial resolution of different aggregate peaks seen here which may be of benefit during development stages to identify the possible source of each aggregation product. When using the TSKgel column fragment identification was not possible due to tailing of the main peak. A similar situation was observed in the case of cetuximab with the TSKgel column failing to resolve any high molecular weight (HMW) or low molecular weight (LMW) species from the main peak under these mobile phase conditions.

For all mAbs, excellent separation of the HMW species was observed with bioZen 1.8 µm SEC-3. When compared to the TSKgel column, the narrow peaks achieved with bioZen SEC-3 allows for greater sensitivity especially for the main peak and additional separation of the different aggregate peaks is observed in the case of the infliximab biosimilar. Baseline separation is achieved here which is not apparent with the TSKgel column.

We also investigated the reproducibility of the media and triplicate injections of each mAb were run to investigate this. 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 seen in both cases and good correlation of recovery obtained too.

Comparative separations may not be representative of all applications.

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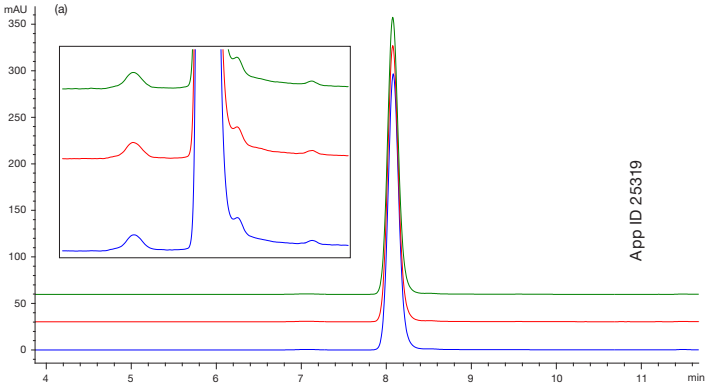
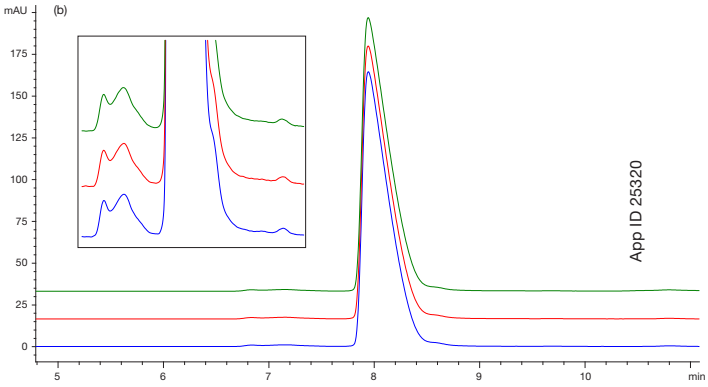
Results and Discussion *(Cont'd)*

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

Our unique 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. Having resolution of antibody fragments is critical and better separation of aggregates and improved fragment detection is demonstrated using the bioZen 1.8 µm SEC-3 column. 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.[2]

References

1. 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.

2. 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

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

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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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