The Effect of Column Internal Diameter for Robust Size Exclusion Chromatography Methods

Dr. Ivan Lebedev, Dr. Laurence Brill, Brian Rivera, and Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Size exclusion chromatography (SEC) is one of the primary methods for the quantitation of aggregates in a protein therapeutic. Because of its ease of use and method transferability, analytical SEC is used throughout the biotherapeutic development process.

Recently, the use of sub-2 µm particles for SEC have expanded analytical method for the characterization of biologics. This is especially noteworthy with monoclonal antibodies (mAbs), wherein methods developed on ultra high-performance SEC (UHP-SEC) columns can separate not only aggregates, but also fragments. However, system limitations in downstream labs may not be amenable to the sub-2 µm SEC columns which demand HPLC systems with minimal dwell volumes. Further, because they might be more prone to clogging, the UHP-SEC column format may not be the most practical choice.

A compromise to the sub-2 µm particle would be to utilize a 3 or 5 µm particle. Larger particles typically employ the traditional 7.8 mm internal diameter (ID), as larger IDs are more resistant to the diffusion or peak broadening associated with system dwell volume. SEC, being both isocratic and non-adsorptive, will undergo significant peak broadening with any extra column volume.

Some labs consider using the larger particle size with a smaller ID. This may be to save on mobile phase, as the 7.8 mm ID will require 3x the mobile phase as a 4.6 mm ID would. However, because the 4.6 mm ID format will be more prone to extra column volume , a performance decrease may be observed. Here we compare aggregate analysis results for a monoclonal antibody.

Figures 1 and **2** show the separation of Trastuzumab using standard mobile phase conditions. Although percent purity by peak areas and resolution of monomer/aggregate are relatively similar, the separation of fragment, especially the post peak, is only observed with the 7.8 mm ID. Chromatographic performance is summarized in **Table 1**.

It is important to note that the concentration and injection volume are identical with both columns. Based on similar peak widths of the 4.6 mm ID, the effect here is likely not due to overloading. Rather, because the 7.8 mm ID is more resistant to extra column volume, chromatographic performance will be superior. Even though the system used for this comparison was a relatively low dwell volume system (< 400 µL), this is still enough to impact the SEC separation.

The downside to the 7.8 mm ID is the need for more sample and mobile phase. However, if robustness and method transferability is prioritized, the 7.8 mm ID should be considered.

LC Conditions

Column:	Biozen™ 3 µm dSEC-2, 200 Å
Dimension:	300 x 7.8 mm 300 x 4.6 mm
Part No.:	00H-4788-K0 00H-4788-E0
Mobile Phase:	50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate:	1.0 mL/min (7.8 mm ID) 0.35 mL/min (4.6 mm ID)
Injection Volume:	10 µL
Temperature:	30 °C
Detection:	UV @ 280 nm
System:	Waters® UPLC®
Sample:	Trastuzumab, 10 mg/mL

Figure 1. SEC Profile for Trastuzumab (4.6 mm ID)

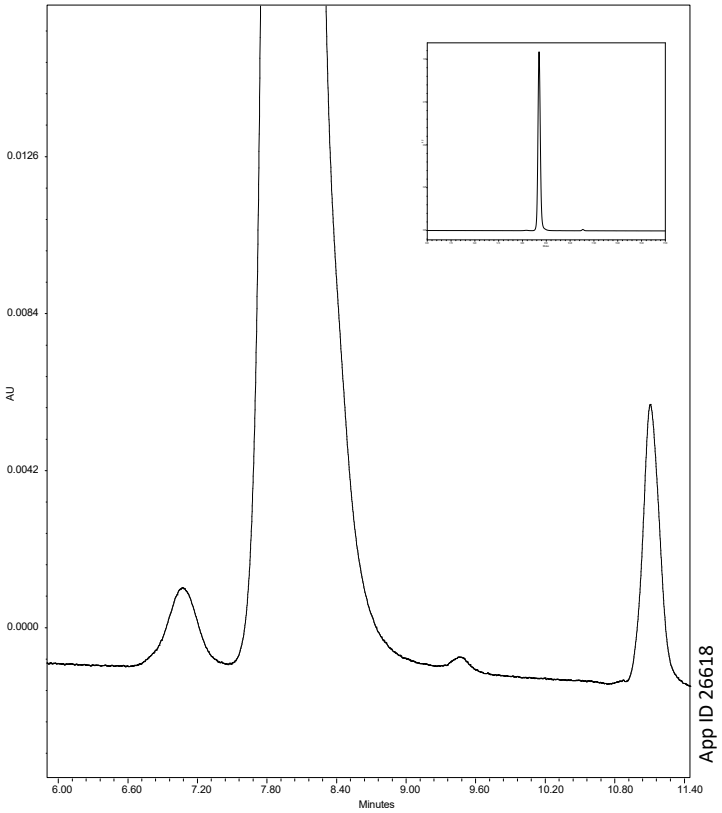

Figure 2. SEC Profile for Trastuzumab (7.8 mm ID)

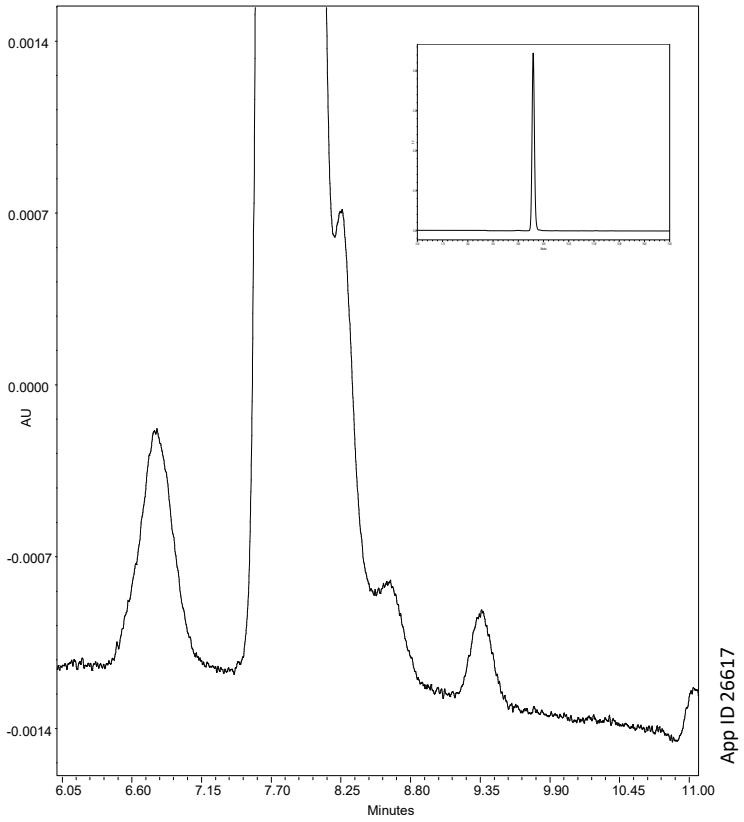

Table 1. Chromatographic Performance Comparison, 4.6 vs 7.8 mm ID

4.6 mm ID (Figure 1)					
Name	**Retention Time**	**% Area**	**USP Resolution**	**USP Tailing**	**USP Plate Count**
HMWS	7.07	0.27	-	1.05	3623
Monomer	7.984	99.3	2.36	1.1	10651
Fragment	11.107	0.43	10.56	1.15	26362
7.8 mm ID (Figure 2)					
Name	**Retention Time**	**% Area**	**USP Resolution**	**USP Tailing**	**USP Plate Count**
HMWS	6.799	0.18	-	1.29	6325
Monomer	7.804	99.73	2.86	1.09	11052
Fragment	12.049	0.09	15.22	1	36342

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

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

Czech Republic
t: +420 272 017 077
cz-info@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

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

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

Indonesia
t: +62 21 5010 9707
indoinfo@phenomenex.com

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

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

Japan
t: +81 (0) 120-149-262
jpinfo@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

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

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

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

Slovakia
t: +420 272 017 077
sk-info@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

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

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

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

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

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

Have questions or want more details on implementing this method? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*