Method Robustness Assessment for sub-2 µm Size Exclusion Columns

James Song, Brian Rivera, and Dr. Bryan Tackett
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of large molecules such as proteins and polymers based on their size in solution. It is one of the primary methods for the quantitation of aggregates within a protein sample. Consequently, SEC methods are commonly used for routine testing and quality control lot release for biotherapeutics, and most importantly monoclonal antibodies (mAbs).

As per the ICH Q2B guidelines, methods should be assessed for method robustness, that is, purposefully varied to assess their impact on assay results. Determining column-to-column variation is a common way to assess how performance is affected by differences in column packing, silica batch, and surface chemistry.

As the primary intent of aggregate analysis by SEC is quantitation of high molecular weight species (HMWS), it is critical that resolution is sufficient to allow for proper integration. Additionally, retention times should be within an acceptable window.

In this application note, NIST mAb RM 8671 was analyzed using 6 different batches of 150 mm length, 4.6 mm inner diameter columns packed with 1.8 µm 200 Å SEC media. The same mobile phase, sample, and UHPLC system was used for the analysis. To assess method performance, two parameters were prioritized: resolution of monomer and HMWS, as well as retention time. Additionally, overall sample recovery and peak areas were investigated as well.

Table 1 summarizes the results of the SEC analysis for NIST mAb RM 8671. Monomer and HMWS show good reproducibility for most chromatographic parameters measured. For monomer, retention time and peak areas show CVs less than 1%. USP resolution (i.e. resolution between monomer and HMWS) was less than 5%. Other chromatographic parameters for monomer, including USP tailing and plate count, also showed good reproducibility.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00F-4787-E0
Dimensions: 150 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate + 250 mM KCl, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 2 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: NIST mAb RM 8671, 10 mg/mL

HMWS retention time and peak areas also demonstrated good reproducibility, with retention time giving less than 1% CV and peak areas being less than 10% CV. However, as peak shape for HMWS is multi-modal for NIST mAb RM 8671, other chromatographic parameters such as efficiency show slightly more variation but are still within acceptable ranges.

Although HMWS and monomer show good reproducibility for the primary target attributes (retention time and peak areas), fragment does not, with %CVs above 10 for peak areas and unacceptably high values for most other chromatographic parameters. This is fundamental to the low abundance (thus, low peak areas) for fragment since they represent less than 0.2% of peak areas. Consequently, the recommendation is to either have an upper limit for fragment and/or orthogonal methods to identify any fragment or clipping (e.g. capillary gel electrophoresis).

In summary, when assessing column variation for robustness assessment for size exclusion chromatography methods, the primary target attributes to assess are peak areas, resolution, and retention times. Monomer should demonstrate excellent reproducibility for all chromatographic parameters, while HMWS should give good reproducibility for peak areas and recovery. Fragment may vary depending on the sample.

Table 1. Chromatographic Results for NIST mAb, 6 Batch Robustness Assessment

	Batch Number	Retention Time (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count
Monomer	1	3.041	979103	96.66	2.27	1.1	10322
	2	3.089	978862	97.35	2.17	1.16	9093
	3	3.089	978862	97.35	2.17	1.16	9093
	4	3.041	975646	95.37	2.31	1.1	10316
	5	3.03	974433	96.95	2.23	1.1	10036
	6	3.063	968101	96.91	2.37	1.09	10261
	Average	3.059	975835	96.8	2.3	1.1	9853.5
	%CV	0.9%	0.5%	0.8%	3.9%	3.1%	6.3%
Dimer/HMWS	1	2.668	32813	3.24		0.89	3073
	2	2.709	25246	2.51		0.99	2670
	3	2.694	28103	2.82		0.97	2474
	4	2.666	29413	2.93		0.9	3023
	5	2.68	29494	2.95		0.79	3247
	6	2.704	23879	2.41		1	3147
	Average	2.687	28158	2.81		0.92	2939
	%CV	0.7%	9.0%	8.8%		9.5%	11.2%
Fragment	1	3.821	1029	0.1	6.84	1.31	19962
	2	3.874	1390	0.14	6.31	1.33	14417
	3	3.844	1056	0.11	6.36	0.81	17957
	4	3.8	1197	0.12	6.28	1.36	15376
	5	3.836	1325	0.13	5.68	1.03	11326
	6	3.811	1060	0.11	6.25	1.97	17161
	Average	3.831	1176	0.12	6.29	1.30	16033
	%CV	0.8%	12.9%	11.0%	4.5%	33.6%	16.2%

Figure 1. Chromatogram Stack, NIST mAb

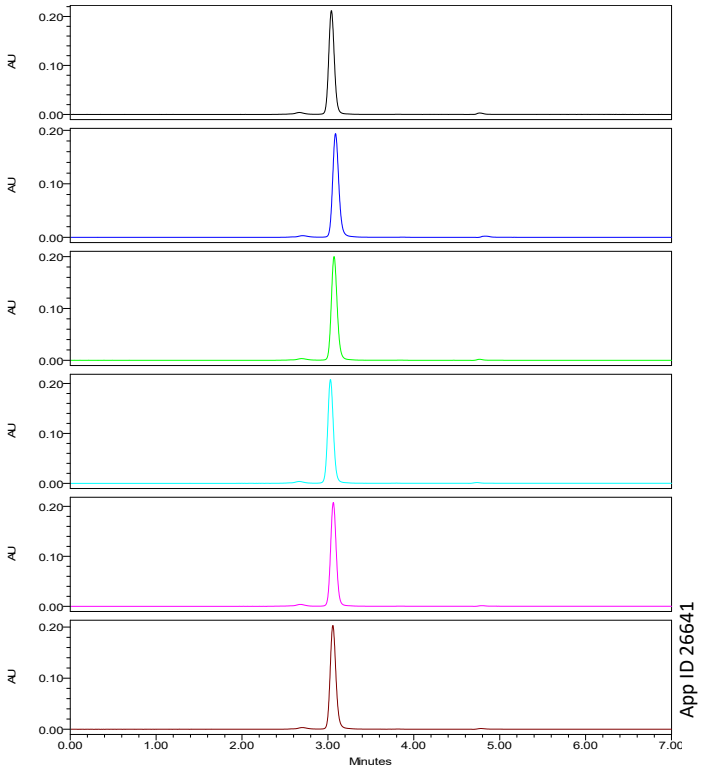
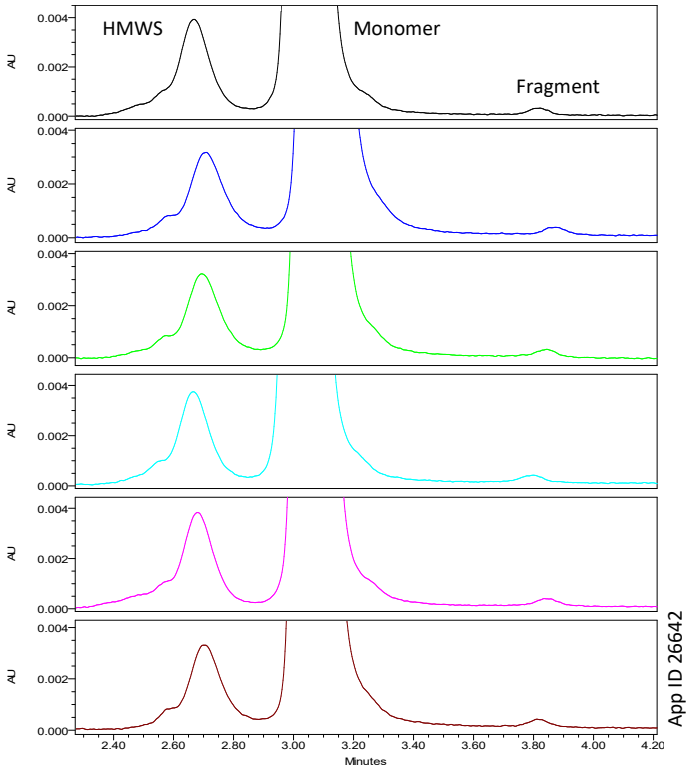


Figure 2. Chromatogram Stack, NIST mAb (Detail)



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*