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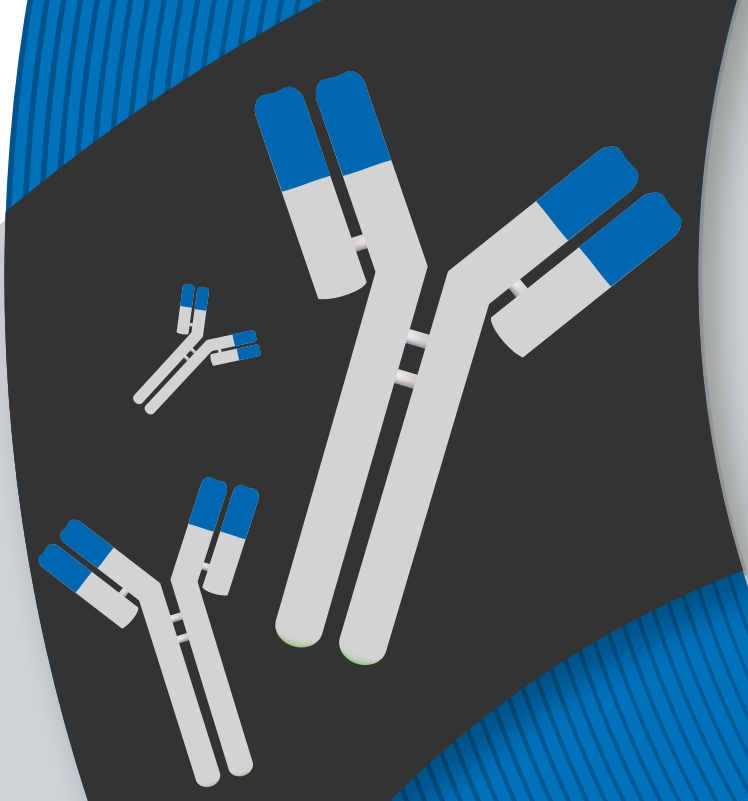
by Phenomenex

dSEC



Application Guide

Optimized Pore Controlled Technology for Characterizing Biomolecules with Biozen dSEC Columns

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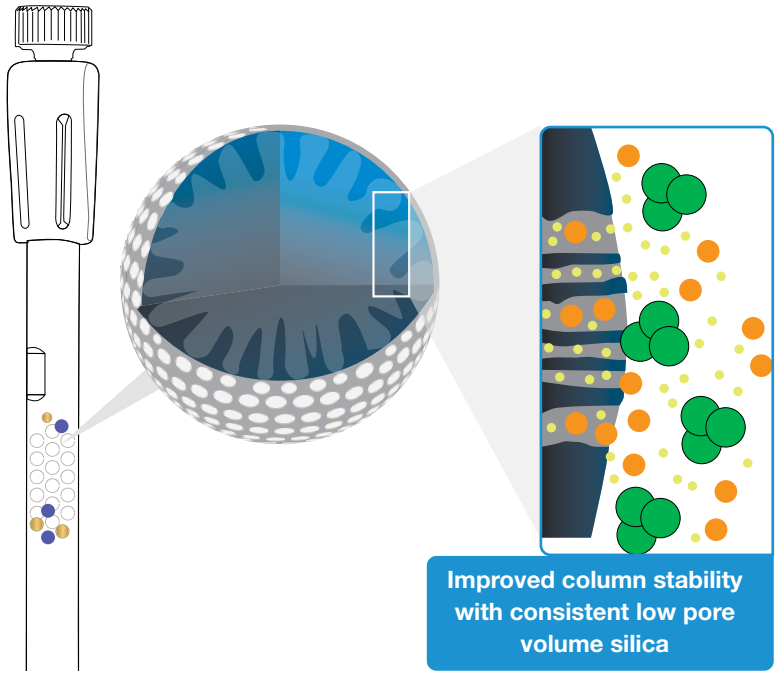
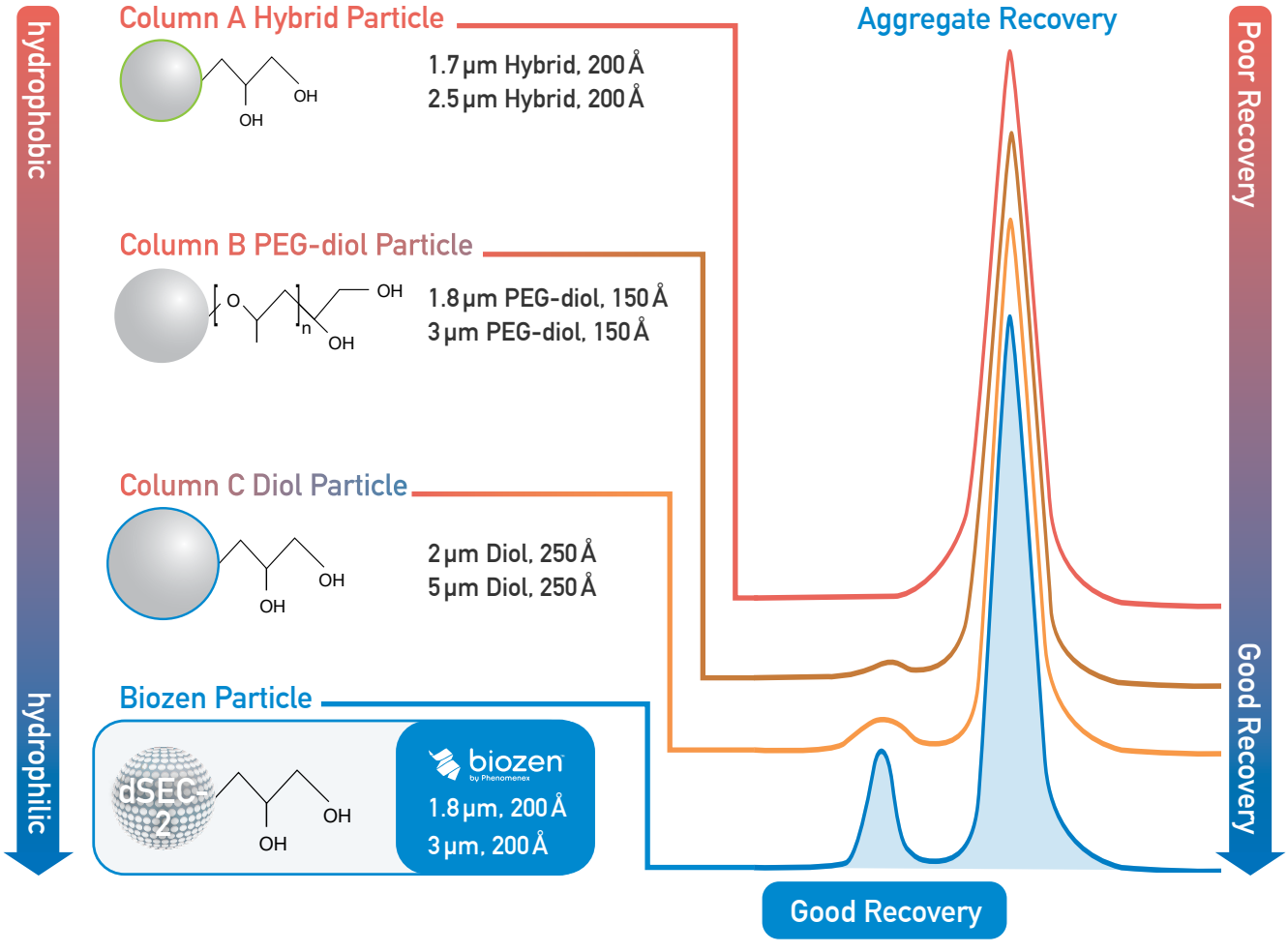
Advanced SEC Silica Particle Technology and Surface Chemistry for Characterizing Biomolecules

Biozen SEC Proprietary Silica Particle Technology

The Biozen dSEC columns are packed with low pore volume silica coupled with a proprietary hydrophilic diol-type bonded surface chemistry that prevents the silica surface from interacting with protein samples.



Biozen SEC Hydrophilic Surface Chemistry Improves Aggregate Analysis



Method Development

Effect of Mobile Phase pH in Aggregate Analysis of Monoclonal Antibodies by Size Exclusion Chromatography

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

Overview

Size Exclusion Chromatography (SEC) is a technique to separate biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, depending on the physicochemical properties of the analyte, sample recovery may be inconsistent, as non-specific interactions of sample to stationary phase might occur.

One possible mechanism for adsorption is hydrophobic interaction, which may be pronounced especially with moderately high concentrations of phosphate. This might be further exacerbated when mobile phase pH is closer to the isoelectric point (pI)of the protein. In this application note, the effect of mobile phase pH is explored for two IgG1 monoclonal antibodies (mAbs).

Figure 1 shows an overlay of NIST mAb, with mobile phase pH at 6.2, 6.8, and 7.4. It is important to note that the concentration of phosphate buffer and co-solvent (potassium chloride) remained consistent for all assessments. The inset shows chromatograms overlay almost identically; additionally, there are nominal changes in aggregate recovery and resolution of monomer and high molecular weight peaks.

The NIST mAb does have a relatively high isoelectric point (measured pI is typically 9-9.5). As such, the mobile phase is more than 1.5 pH units below the isoelectric point, thus theoretically in a state where hydrophobic interactions are minimized. As such, a less basic mAb (Adalimumab, pI ~8.25) was also investigated, with **Figure 2** showing a similar overlay of SEC chromatograms at three different pHs. Again, nominal differences in chromatography are observed, with the inset showing overlays are virtually identical.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH as indicated
Flow Rate: 0.35 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: NIST mAb (30 µg)
Adalimumab (30 µg)

In summary, protein adsorption may occur in SEC methods. As such, investigating the pH of the mobile phase is critical in understanding experimental design for method optimization and robustness studies. The two monoclonal antibodies investigated here show little difference in chromatographic performance when running moderately high phosphate buffers and varying pH levels- 6.2, 6.8 and 7.4.

Method Development

Figure 1. SEC chromatographic overlays of NIST mAb run with mobile phase at 3 different pH levels. There are negligible differences in aggregate by % peak area (~2.2%) as well as monomer and aggregate resolution (~2.8-2.9).

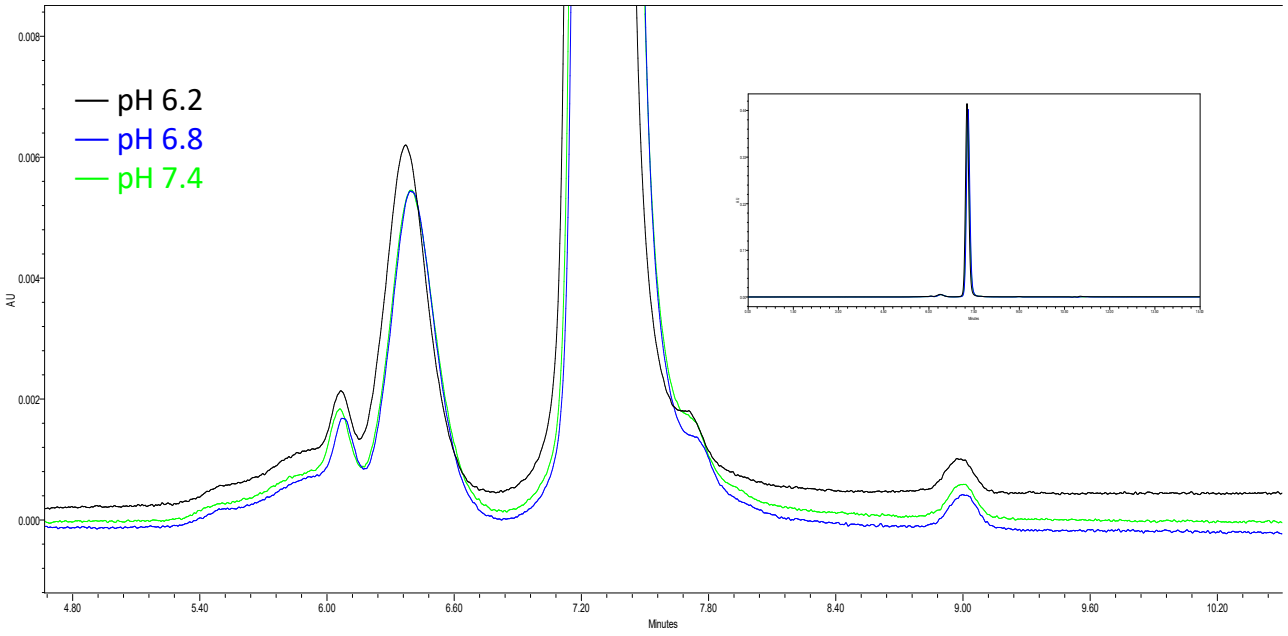
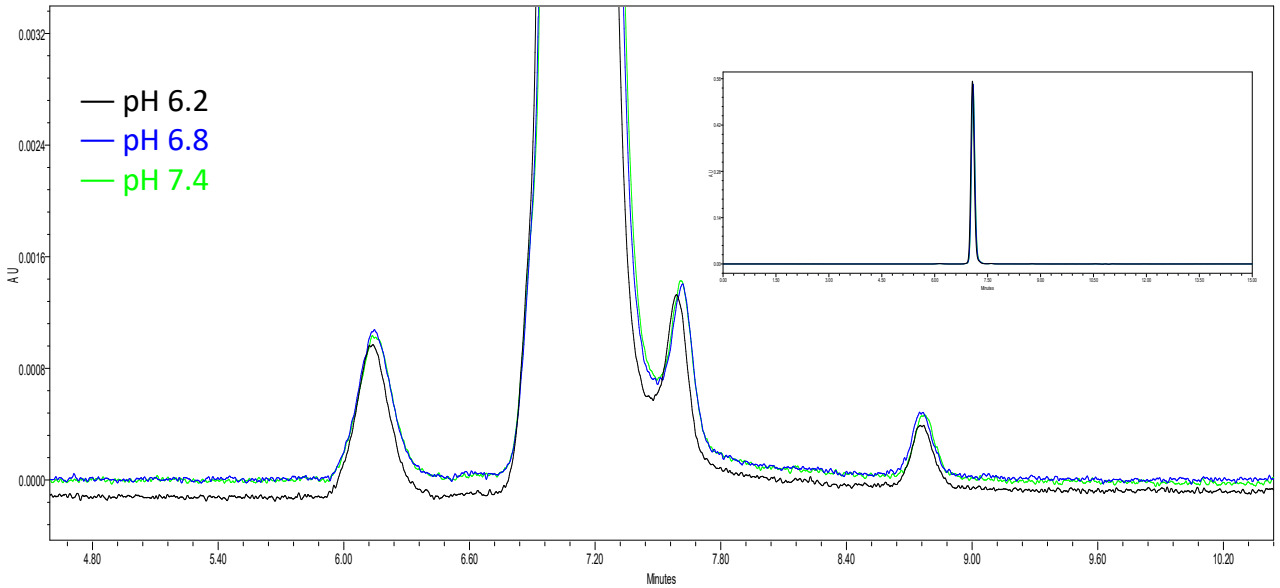


Figure 2. SEC chromatographic overlays of Adalimumab run with mobile phase at 3 different pH levels. Virtually no difference in peak area recoveries, resolution of monomer and dimer, and resolution of post peak fragment.



Improving Resolution for Size Exclusion Chromatography Methods by Optimization of Linear Velocity

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

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight (HMW) aggregates.

Flow rate is a common method parameter for most adsorptive LC methods. However, SEC is a unique separation modality in that lower flow rates often yield improvements in separation and resolution can improve quite considerably simply by running at a lower linear velocity.

Figure 1 demonstrates the utility of method improvements by running at a lower flow rate for the aggregate analysis of NIST mAb, a common standard protein. 0.4 mL/min gives a resolution (Rs) of 2.97 for monomer and HMW, with 0.35 mL/min giving Rs of 3.17 and 0.2 mL/min giving an Rs of 3.42. Results are summarized in **Table 1.**

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Catalog No: 00H-4787-E0
Mobile Phase: 0.2M Potassium Phosphate + 250 mM KCl, pH 6.2
Flow Rate: As Indicated
Injection Volume: 3 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: NIST mAb, 10 mg/mL

Although the method will effectively take twice as long, since resolution is often a requirement to meet system suitability, the superior result with lower flow rate value may give a wider margin of error prior to method failure. As such, if one is prioritizing performance and robustness over throughput, lower flow rate is one of the easiest and practical method parameters to implement.

Figure 1. SEC Chromatogram Overlays for NIST mAb Run at Varying Flow Rates

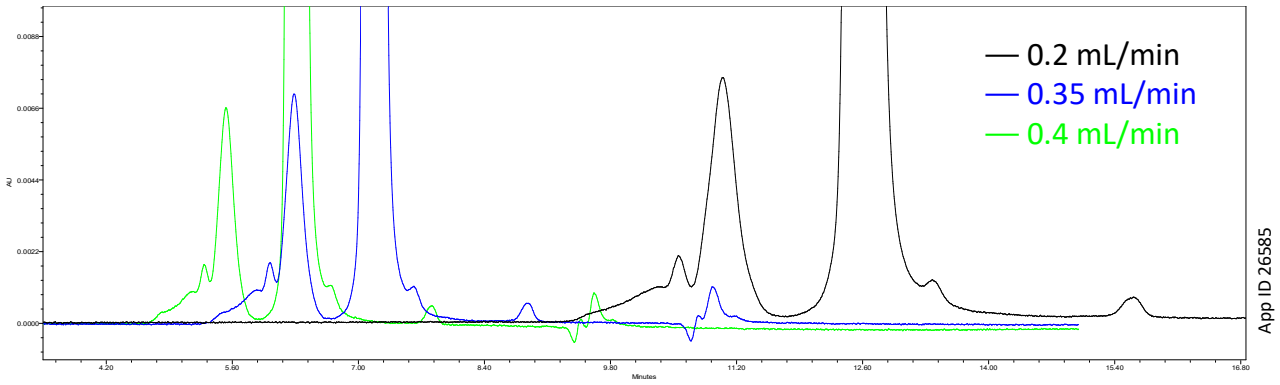


Table 1. Summary of Chromatographic Results with Modulating Flow Rate

Flow Rate (mL/min)	Rs 1,2 (HMW, Monomer)	RS 2,3 (Monomer, Fragment 2)
0.2	3.42	9.04
0.35	3.17	8.66
0.4	2.97	8.09

Arginine as a Mobile Phase Co-solvent to Improve High Molecular Weight Aggregate Recovery for Size Exclusion Chromatography

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 biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, depending on the physicochemical properties of the analyte, it may be prudent to investigate different mobile phase co-solvents to improve separation and sample recovery. One co-solvent that has been studied extensively is arginine, a common reagent used for protein refolding.[1] In this application note, we explore the impact of sample (i.e. aggregate) recovery on NIST mAb when modulating arginine concentration in mobile phase.

The mobile phase consisted of 200 mM Potassium Phosphate, pH 6.2, with arginine concentration assessed at 100 mM, 200 mM, and 250 mM. For NIST mAb, aggregate recovery was significantly less for 100 mM (80851 mAU) when compared to 200 and 250 mM (96818 and 97146, respectively). Further, percent high molecular weight (HMW) aggregate by peak area was 2.16% for 100 mM, which was again significantly lower than higher concentrations. Visually, post-peak fragment separation was slightly worse with 100 mM. HMW aggregate percentages and resolution values of monomer and dimer are summarized in **Table 1**.

In summary, arginine may be a useful co-solvent for SEC applications for large molecules. Particularly, arginine may improve high molecular weight aggregate recovery for monoclonal antibodies.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00H-4787-E0
Dimensions: 300 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate, Arginine as indicated, pH 6.2
Flow Rate: 0.35 mL/min
Detection: UV @ 280 nm
Temperature: 25 °C
Sample: NIST mAb (30 µg)

1. Arakawa, Tsutomu et al. "Suppression of protein interactions by arginine: a proposed mechanism of the arginine effects." *Biophysical chemistry* vol. 127,1-2 (2007): 1-8. doi:10.1016/j.bpc.2006.12.007

Figure 1. SEC chromatographic overlays for ADC mimic, demonstrating the effect of organic solvent to peak shape and recovery.

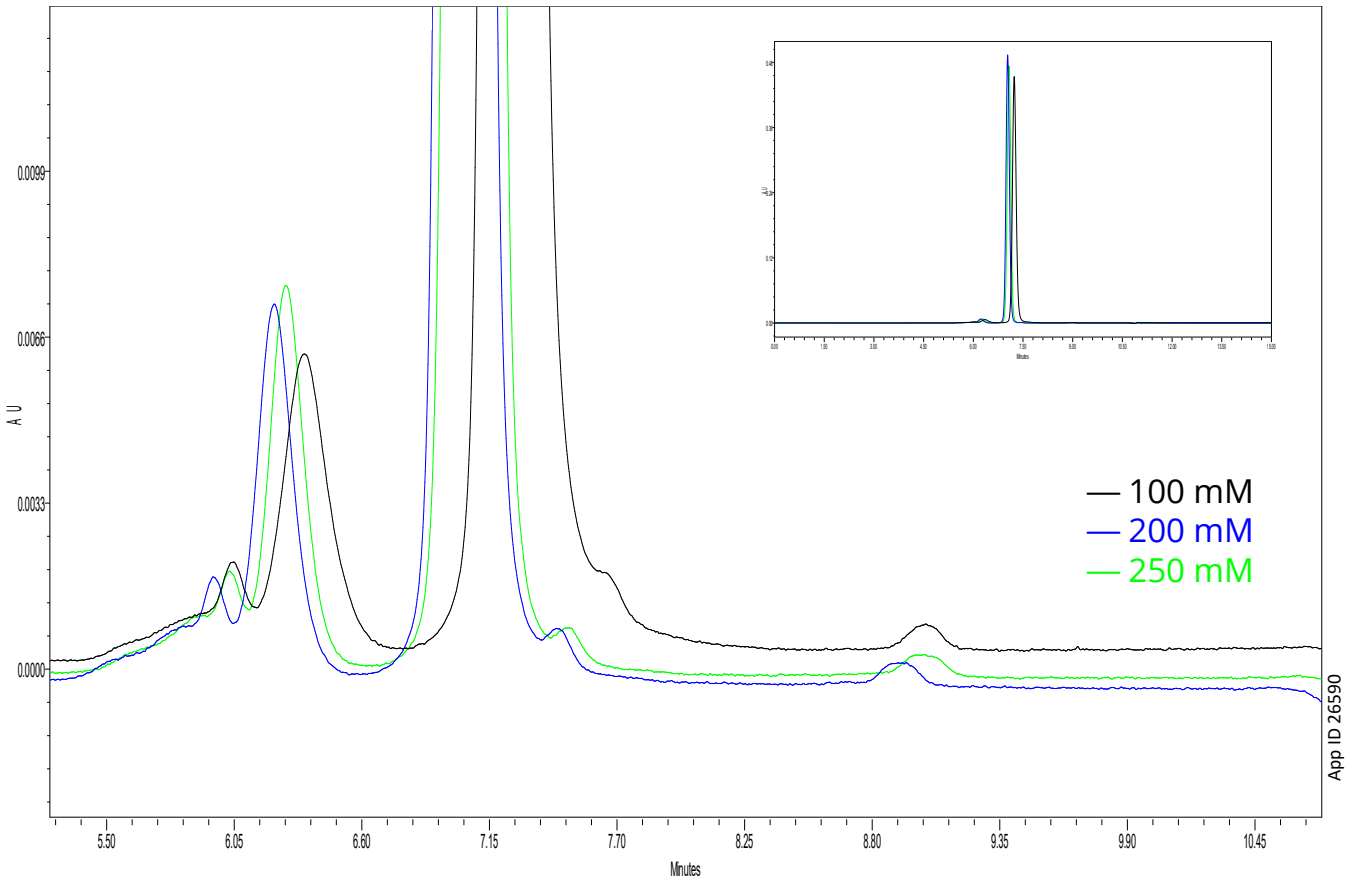


Table 1: Assessment of chromatographic parameters

Arginine Concetration	HMW Area Count (mAU)	Monomer Peak Height	% HMW	Rs 1,2
100 mM	80851	453324	2.16	3.02
200 mM	96818	493990	2.56	3.26
250 mM	97146	473197	2.58	3.25

Optimizing Phosphate Concentration for Size Exclusion Chromatography Aggregate Analysis

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 focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, depending on the physicochemical properties of the analyte, it may be prudent to investigate different mobile phase co-solvents to improve separation and sample recovery.

Because it is protein stabilizing and ubiquitous in biochemistry labs, phosphate is the common buffer used for SEC methods, especially for monoclonal antibodies. However, phosphate may also cause unwanted hydrophobic interactions. Consequently, exploring how phosphate concentration affects sample recovery and resolution of monomer and aggregate.

Figure 1 shows an overlay of separation of trastuzumab, modulating the concentration of potassium phosphate. As shown in **Table 1**, there is minimal impact to retention time, resolution of monomer and dimer, and percent purity. **Figure 2** shows NIST mAb RM8671 SEC chromatogram overlays; again, only nominal differences are observed for retention time, resolution and percent purity.

In summary, assessing phosphate concentration may be necessary to ensure that no differences are observed with modulating concentration. This ensures proper sample recovery and demonstrates robustness of the method.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: Potassium Phosphate (as indicated) + 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Trastuzumab (30 µg)
NIST mAb (30 µg)

Figure 1. SEC chromatographic overlays for Trastuzumab, modulating concentration of potassium phosphate.

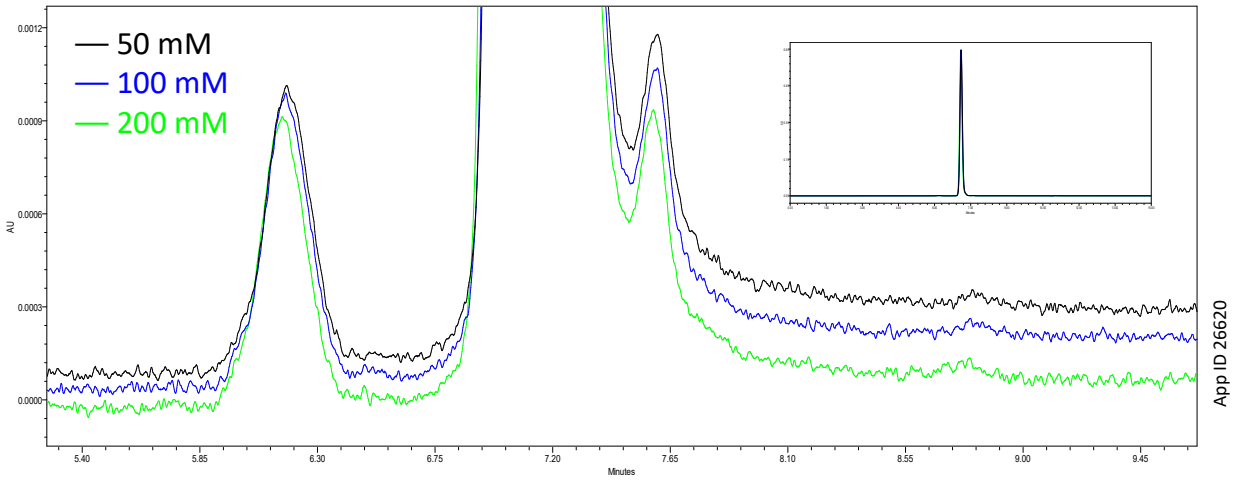
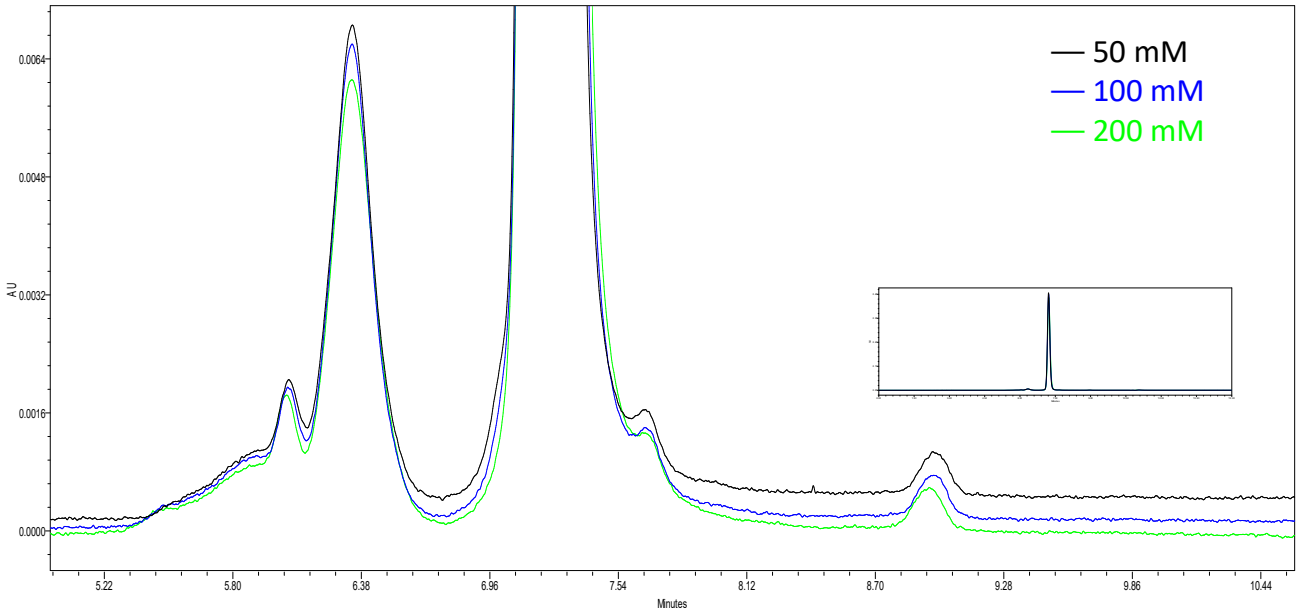


Figure 2. SEC chromatographic overlays for NIST mAb, modulating concentration of potassium phosphate.



App ID 26587

Table 1: Assessment of chromatographic parameters

Sample	Phosphate Concentration (mM)	Monomer Retention Time (min)	Resolution 1,2	% Purity Monomer
Trastuzumab	50	7.048	3.56	99.31
	100	7.058	3.55	99.39
	200	7.058	3.78	99.49
NIST mAb	50	7.214	3.01	96.81
	100	7.219	3.02	96.4
	200	7.224	2.91	96.47

Organic Solvent in Size Exclusion Chromatography of an Antibody Drug Conjugate Surrogate

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 focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Silica-based SEC columns are modified with a hydrophilic stationary phase, typically to minimize electrostatic interactions of positive moieties on proteins and other analytes. However, some analytes may present some challenges. For example, Antibody Drug Conjugates (ADCs), because of their hydrophobic nature, may have a propensity to interact with the stationary phase.

In this application note, we demonstrate the effect of organic solvent on SEC analysis of an ADC "mimic." This sample is a monoclonal antibody that has been conjugated to a dansyl group and functions as a surrogate for the typically cytotoxic agent commonly used for most ADC modalities.

Figure 1 demonstrates even with 5 % isopropanol (IPA) added to the mobile phase (black trace), hydrophobic adsorption is still observed for the ADC mimic, leading to poor peak shape and recovery. Overlay of 10 % IPA improves peak shape and recoveries.

Figure 2 shows full recovery of aggregate, and good peak shape for monomer. Percent aggregate by peak area was measured at 2 %. Additionally, post-peak, putative fragments which are common with cysteine-based ADCs, are partially separated.

In summary, ideal size exclusion chromatography separations should minimize secondary interactions. Although column selection is a consideration, some analytes such as ADCs may be too hydrophobic to run with phosphate containing mobile phases. Consequently, the addition of isopropanol may improve peak shape, thus allowing for the separation of monomer and aggregate for the calculation of purity by percent peak area.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2 + IPA
Flow Rate: 0.35 mL/min
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: ADC Mimic

Figure 1. SEC chromatographic overlays for ADC mimic, demonstrating the effect of organic solvent to peak shape and recovery.

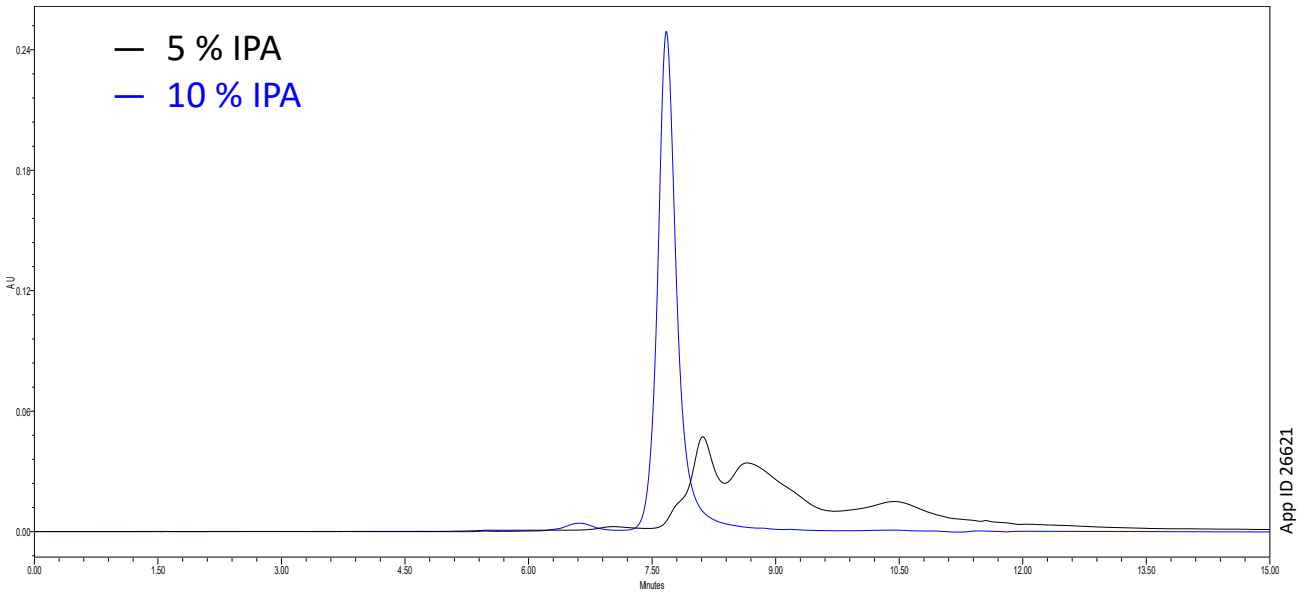
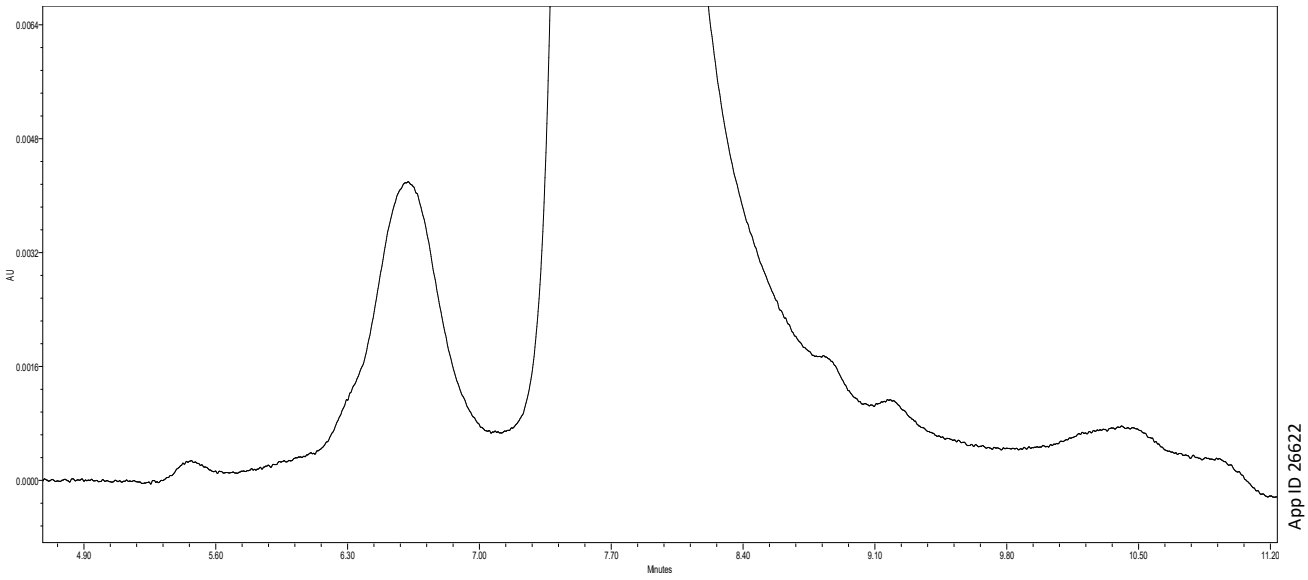


Figure 2. SEC chromatogram for 10% IPA. Sufficient separation of aggregate and monomer allows for integration to determine percent purity by peak area.



Method Development

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**.

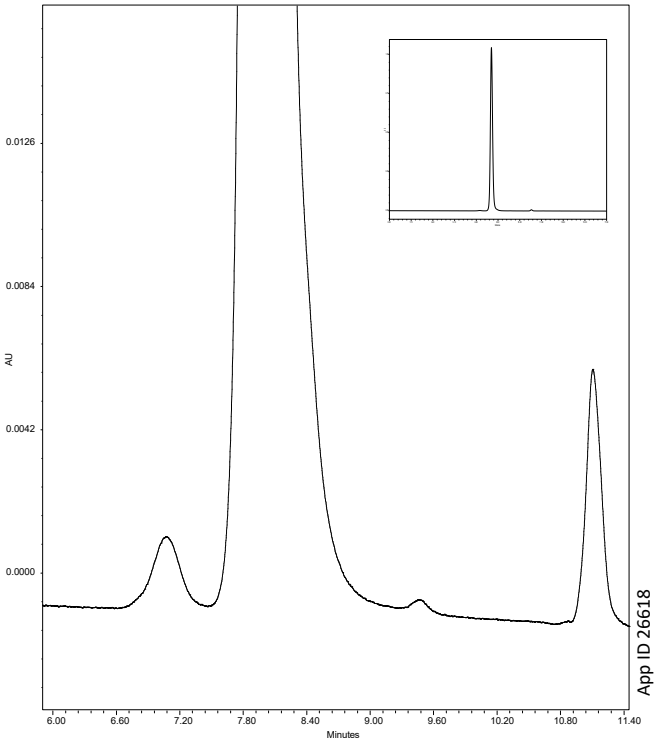
It is important to note that the concentration and injection volume are identical with both columns. Based on similar peak widths of the 4.6 mm ID, the effect here is likely not due to overloading. Rather, because the 7.8 mm ID is more resistant to extra column volume, chromatographic performance will 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

Method Development



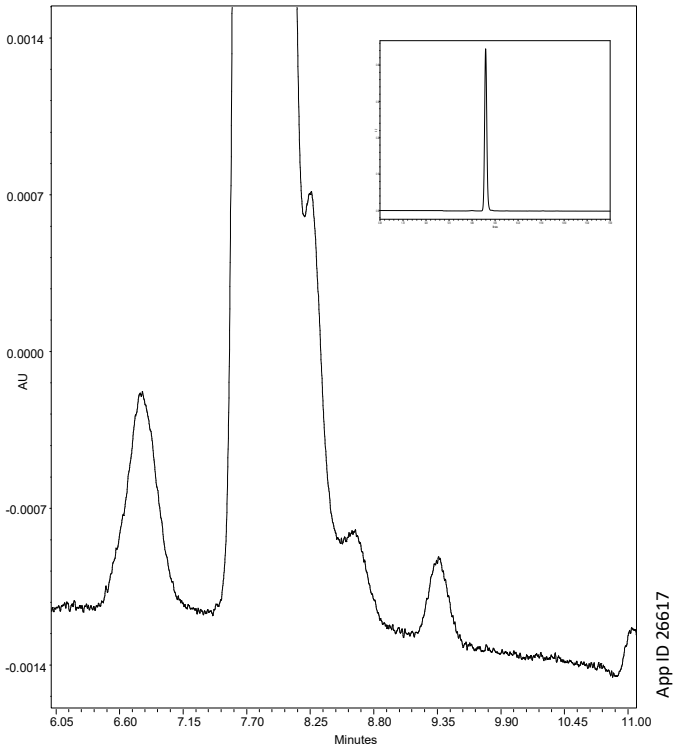


Table 1. Chromatographic Performance Comparison, 4.6 vs 7.8 mm ID

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

Antibodies and Recombinant Proteins

Aggregate Analysis of Fc-Fusion Proteins

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

Overview

Fc-fusion proteins are Fc homodimers linked to a polypeptide chain. From an analytical standpoint, Fc-fusion proteins present several challenges. Fc-fusion proteins may have much more complex glycosylation, including deviations from the biantennary fucosylated glycans associated with monoclonal antibodies, as well as O-glycans. As glycosylation directly impacts protein conformation or folding, analytical LC separations may be affected as well. Indeed, even with non-adsorptive separation modalities such as size exclusion chromatography (SEC), separation may require extensive method development for optimization to ensure a robust method.

Figure 2 shows an SEC profile for Etanercept, a heavily glycosylated Fc-TNFR conjugate. Because it often has sialylation, non-ideal SEC interactions can be observed. That is, so-called “ion exclusion” of the protein due to repulsion of the negatively charged protein and inherently negative silica. This can cause peak broadening and/or adsorption of the protein, which can prevent the proper quantitation of the protein. As such, the mobile phase used should have sufficient co-solvent (i.e. NaCl or other salt) to minimize this effect. Using a 2X Phosphate Buffered Saline as the mobile phase ensures that any electrostatic interaction is minimized. Thus, percent monomer and aggregate can be easily calculated, using standard integration parameters, with percent monomer being 96.9% by peak area.

This strategy can be implemented for other Fc-Fusion proteins which potentially can present similar issues. **Figure 3** shows a SEC profile for Aflibercept, an Fc-Fusion protein conjugated to VEGF receptor. Although not as complex as Etanercept, Aflibercept being a Fc-Fusion protein often has physicochemical properties that are not as predictable as a canonical IgG1 monoclonal antibody.

LC Conditions

Column: Biozen™ 3 µm dSEC-2, 200 Å
Dimension: 300 x 7.8 mm
Part No.: 00H-4788-K0
Mobile Phase: 50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate: 1.15 mL/min
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: As indicated

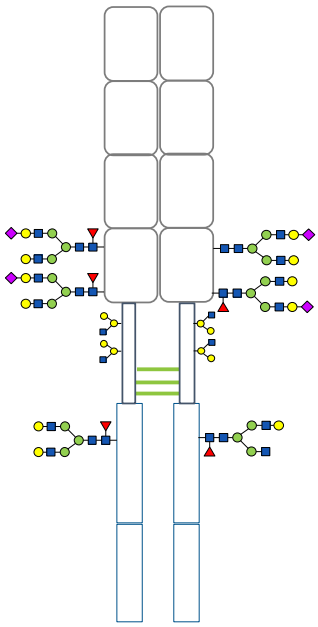


Figure 1. Structure of Etanercept

Antibodies and Recombinant Proteins

Figure 2. SEC Profile for Etanercept (25 mg/mL)

SEC Chromatogram for Etanercept, showing good separation of monomer and aggregate. Monomer purity is 96.9% by peak area.

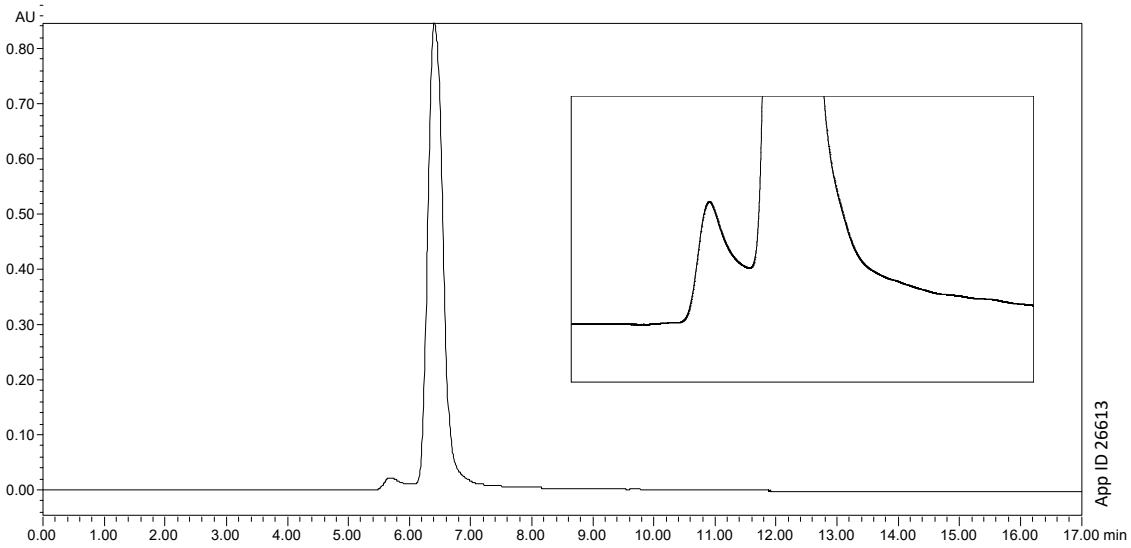
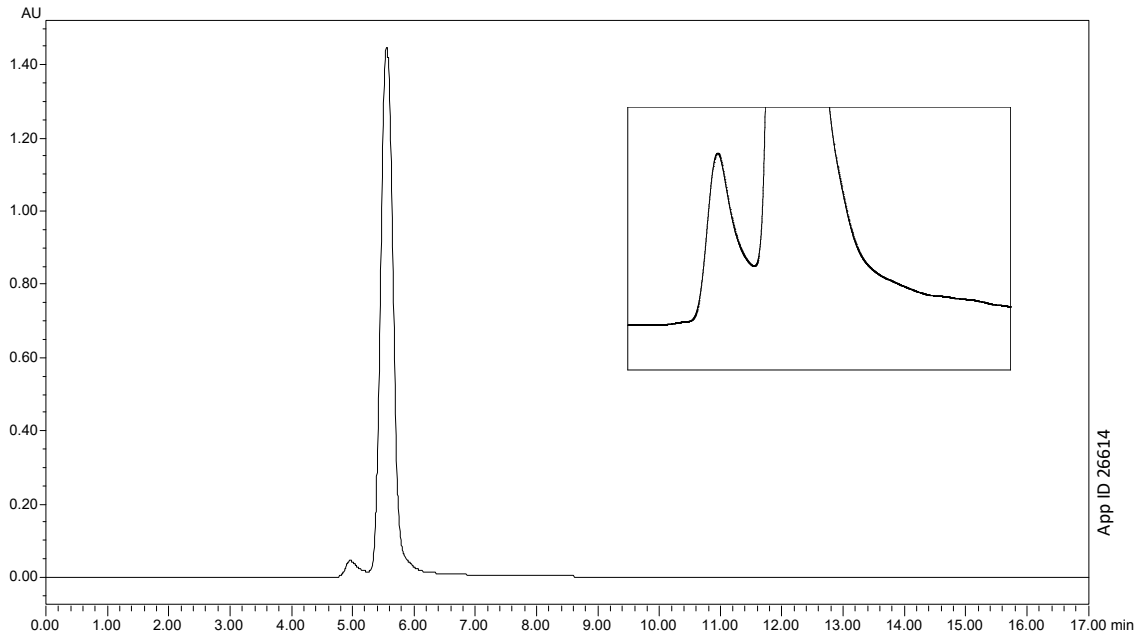


Figure 3. SEC Profile for Aflibercept (25 mg/mL)

SEC Chromatogram for Aflibercept, showing good separation of monomer and aggregate. Monomer purity is 96.8% by peak area.



Aggregate Analysis of an IgG2 Monoclonal Antibody

Dr. Ivan Lebedev, 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 biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. This is a common analytical method for monoclonal antibodies (mAbs), as aggregate can be quantitated by percentage of peak area.

In this application note, we explore the use of a standard phosphate containing buffer for aggregate analysis of an IgG2 mAb, Panitumumab, which has known recovery issues due to its hydrophobicity.[1] Using a moderately high ionic strength mobile phase, panitumumab exhibits good chromatography, as demonstrated by good USP tailing (0.99), and separation of dimer and pre-peak (putative full-length antibody + fragment). Percent dimer was determined to be 0.92 % by peak areas, with pre-peak determined 1.13 % by peak area.

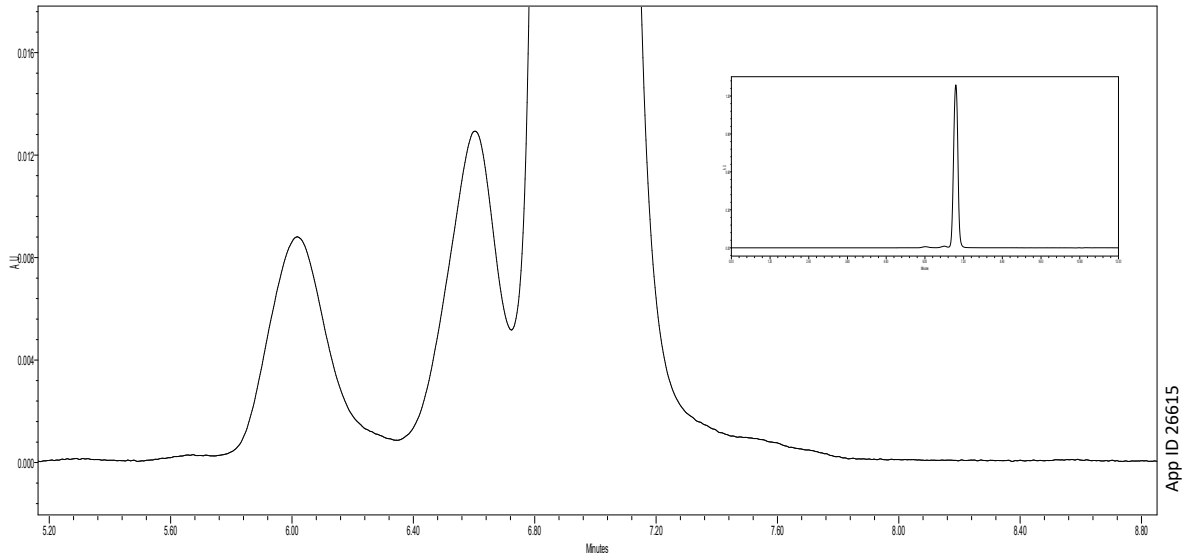
LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Panitumumab, 10 mg/mL

In summary, certain isotypes of antibodies (e.g. IgG2s) may present challenges when analyzing by a non-adsorptive separation modalities such as SEC. However, with optimal mobile phase conditions and high-resolution SEC columns, one can obtain good separation of dimer and other related high molecular weight impurities.

1. Fekete, Szabolcs et al. "Adsorption and recovery issues of recombinant monoclonal antibodies in reversed-phase liquid chromatography." *Journal of separation science* vol. 38,1 (2015): 1-8. doi:10.1002/jssc.201400996

Figure 1. SEC chromatogram for Panitumumab.



Antibodies and Recombinant Proteins

Aggregate Analysis of a Bispecific Antibody

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

Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. This is a common analytical method for monoclonal antibodies (mAbs), as aggregate can be quantitated by percentage of peak area.

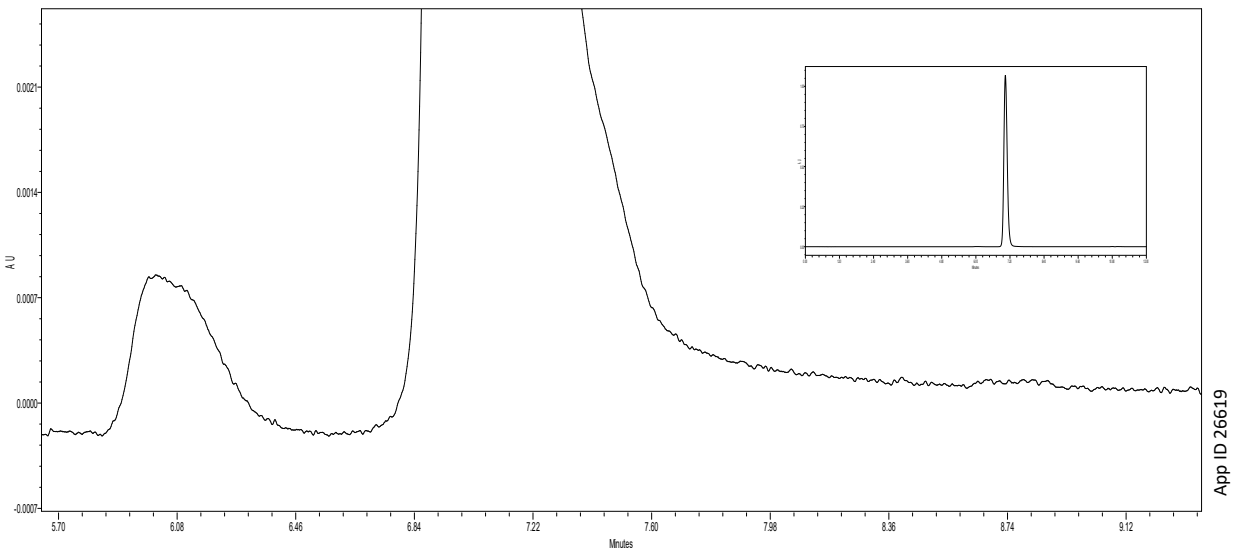
In this application note, we explore the use of standard phosphate containing buffers for aggregate analysis of a bispecific antibody, Emicizumab. Because aggregate is less than 0.2 % by peak areas, 100 µg of protein had to be loaded onto a 4.6 mm internal diameter (ID) SEC column to sufficiently quantitate. The use of a sub-2 µm column also was necessary as the additional improvements in efficiency help in detection.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 10 µL
Temperature: 25°C
Detection: UV @ 280 nm
Sample: Emicizumab, 10 mg/mL

In summary, SEC is an effective method for quantitation of mAb aggregate. Peak areas below 0.2 % can be obtained with sufficient mass load. With aggregate this low, it is prudent to investigate other orthogonal methods in confirming no aggregate was lost due to adsorption.

Figure 1. SEC chromatogram for Emicuzimab. 100 µg load was necessary to quantitate aggregate by peak areas. Peak asymmetry of 1.2 indicates column is not overloaded. Aggregate is measured as 0.19 % by peak area.



Aggregate Analysis of Recombinant Human Growth Hormone

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

Overview

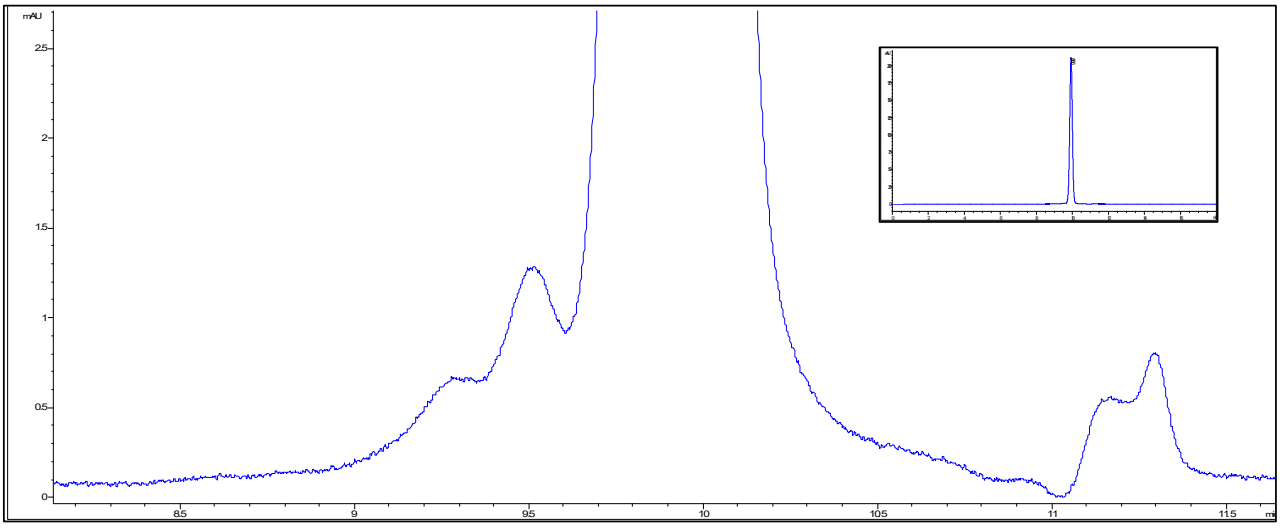
Aggregate analysis by size exclusion chromatography (SEC) of small, recombinant proteins can be challenging as they are more sensitive to non-specific interactions. That is, hydrophobic and/or ionic interactions may lead to poor chromatographic performance including peak tailing or analyte adsorption. Consequently, purity of monomer, as defined as a percentage by integrated peak areas, may be misreported.

In this application note, we demonstrate the SEC separation of recombinant human growth hormone (rhGH), somatotropin. Somatotropin is a recombinant polypeptide with a molecular weight of 22.1 kD. Using a mobile phase composition of moderate phosphate and sodium chloride concentrations, percent purity by peak area was determined to be 99.1%. Good peak shape and separation of high molecular weight aggregate was observed. Further optimization might include addition of organic or other additives to the mobile phase to modulate retention, if separation of excipients such as surfactant was necessary.

LC Conditions

Column: Biozen™ 3 µm dSEC-2, 200 Å
Dimension: 300 x 7.8 mm
Part No.: 00H-4788-K0
Mobile Phase: 50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate: 1 mL/min
Injection Volume: 10 µL
Temperature: 30 °C
Detection: UV @ 280 nm
Sample: Somatropin, USP Standard 1615708 (5 mg/mL)

Figure 1. SEC Profile for Somatropin (5 mg/mL)



Materials Science and Technology

Assessing Column Lifetime of a UHPLC Size Exclusion Column

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

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. One challenging aspect of SEC is column lifetime, with the main failure mode being peak broadening and/or loss of efficiency due to column bed collapse.

In this application note, we demonstrate the robustness of a sub-2 µm SEC column. Over 300 injections of a protein sample (Myoglobin) were performed. Peak symmetry was assessed to ensure consistent column performance.

Results are shown in **Table 1**. There was approximately 4 % decrease in peak symmetry, with percent purity (i.e. % area of main peak) remaining consistent throughout the method sequence. This strongly indicates little to no disruption of the column packing bed.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4787-E0
Mobile Phase: 50 mM Sodium Phosphate + 300 mM Sodium Chloride, pH 6.8
Flow Rate: 0.35 mL/min
Injection Volume: 5 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Myoglobin, 10 mg/mL

Some limitations in this methodology should be considered. The myoglobin samples were filtered standards, which may not be representative of all samples. Additionally, the method was run continuously; further assessment of column bed stability would include method shut down, and start up, as well as column storage. All these method parameters could potentially disrupt the SEC column packed bed.

Figure 1. SEC Chromatogram Overlays of Myoglobin

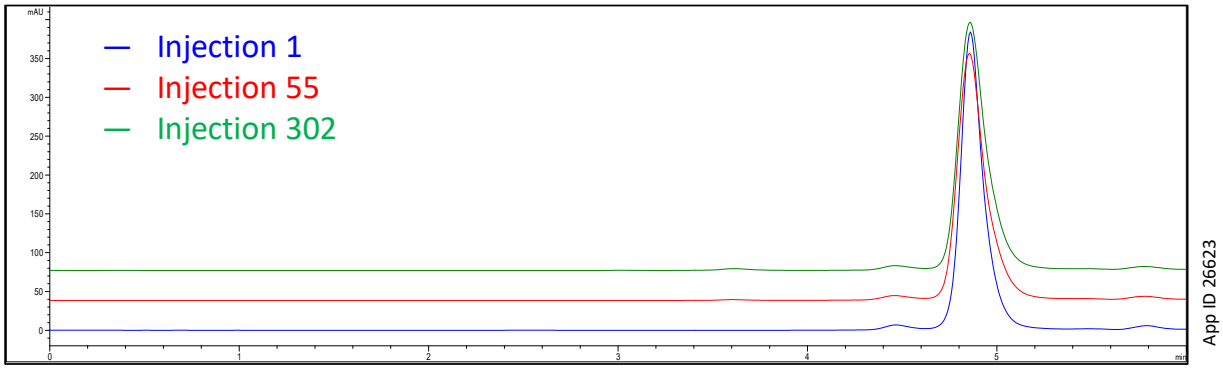


Table 1. Summary of Myoglobin Chromatographic Results

Injection No.	Time (min)	Peak Area	Peak Height	% Area	Peak Symmetry
1	4.866	3298.1	383.1	96.802	0.716
55	4.862	3308.4	342.8	96.839	0.725
302	4.860	3372.5	318.2	96.488	0.688

High-Throughput Methods for Size Exclusion Chromatography

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

Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. Flow rate is a common method parameter for most adsorptive LC methods. However, SEC is a unique separation modality in that lower flow rates often yield improvements in separation.

The use of sub-2 µm particles allows the method developer some flexibility in increasing flow rate while still maintaining acceptable chromatographic performance. Typically, a resolution from monomer and aggregates greater than 1.8 is desirable. If fragment is present in the sample, then maintaining resolution to allow for auto-integration is often preferred. **Figure 1** compares SEC chromatograms when running a flow rate of 0.35 mL/min, a standard linear velocity, and a flow rate of 0.6 mL/min. Acceptable resolution is obtained and a nominal change in percent purity is observed.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 150 x 4.6 mm
Part No.: 00F-4787-E0
Mobile Phase: 50 mM Sodium Phosphate + 300 mM NaCl, pH 6.8
Flow Rate: As Indicated
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Cetuximab, 2 mg/mL

In summary, the use of sub-2 µm particles for SEC methods may be a practical way to slightly increase throughput for applications that demand it. However, optimal chromatography for SEC methods is typically using at or below standard linear velocities.

Figure 1. SEC chromatogram overlays for Cetuximab run at varying flow rates.

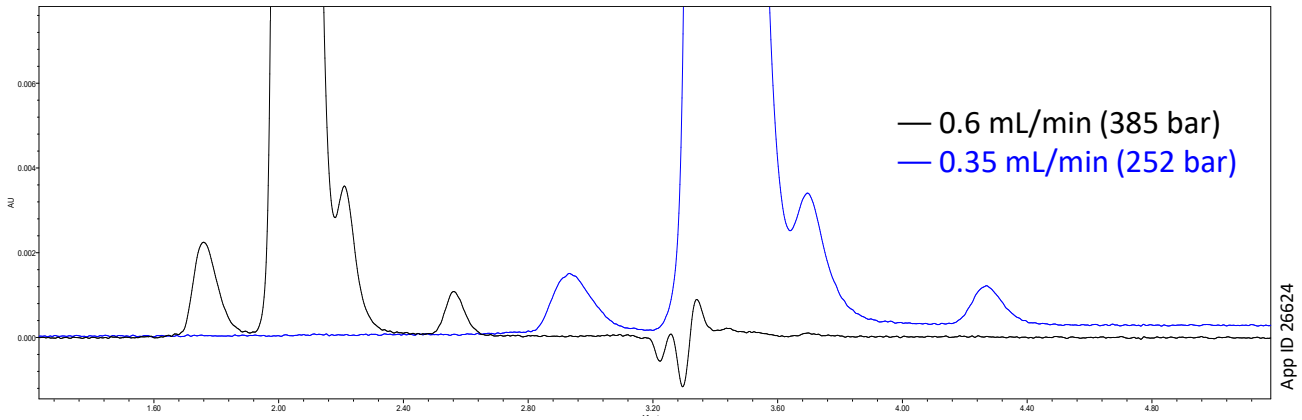


Table 1. Summary of chromatographic results with modulating flow rate

Flow Rate (mL/min)	Rs 1,2 (HMW, Monomer)	% Purity Monomer
0.35	2.20	98.4
0.6	2.15	98.8

Column Bed Stability During Routine Size Exclusion Chromatography

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 to separate large molecules such as proteins and polymers based on their size in solution. SEC methods are commonly used for routine testing application, most notably for the quantitation of aggregates in a biotherapeutic sample.

The primary failure mode for size exclusion columns is column voiding, wherein the column packed bed compresses and collapses. Column voids can occur because of spikes in backpressure, switching mobile phase (resulting in changes in solvent viscosity), or suboptimal method/sequence start up and shut down.

To demonstrate column method robustness against column voiding during routine testing, a 4.6 mm internal diameter (ID) by 150 mm length column was packed with sub-2 µm, 200 Å media and was run with a viscous mobile phase containing 10 % isopropanol. To further stress the column packed bed, flow rate was modulated between 0.35 mL/min and 0.45 mL/min, the latter resulting in a backpressure of roughly 320 bar. Finally, flow was intermittently stopped for 8 hours to replicate the system start up and shut down which can also result in chromatographic bed collapse. Overall, there was > 100 hours of run time, and 226 injections were performed. A summary of the sequence is indicated below. The robustness sequence was to be run until column failure, which was defined as a drop of 25 % efficiency for uridine, a small molecule which elutes in the total permeation volume.

Upon completion of the sequence, column efficiency showed a nominal drop in uridine efficiency (37231 N to 36257 N, or a 2.6 % drop). It is important to note that no protein samples were injected during the sequence, as the intent of the experiment was to confirm column bed stability. Additionally, peak shape and efficiency of uridine may not be representative of the performance of protein samples. Finally, because column failure was not reached, it is unclear as to when the 25% efficiency benchmark would have been reached.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00F-4787-E0
Dimensions: 150 x 4.6 mm
Mobile Phase: 0.1 M Sodium Phosphate, pH 6.8 + 10 % Isopropanol
Flow Rate: As indicated in Table 1
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Uridine

Table 1. Experimental Conditions and Sequence for SEC Column Robustness

Sequence	Mobile Phase	Flow Rate (mL/min)	Method Run Time (min)	Number of Injections
1	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	6.5	4
2	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.45	6.5	4
3	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	6.5	3
4	0.1 M Phosphate Buffer, pH 6.8 + 10 % IPA	0.35	90	1
5	N/A (no system flow)	0	480	0
Sequence repeated until 100 hours of run time was exceeded				

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Figure 1. Column Efficiency (N) For Uridine (0.35 mL/min Method)

Column efficiency for uridine over hours of run time. Sequence as shown in Table 1. Only a 2.6 % drop in efficiency is observed over the course of the sequence.

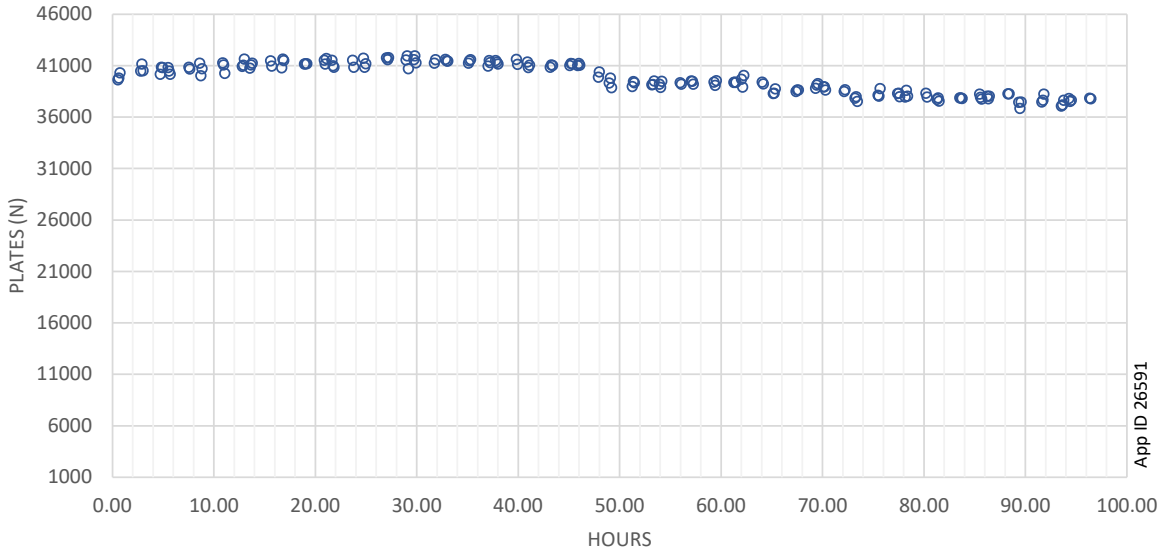
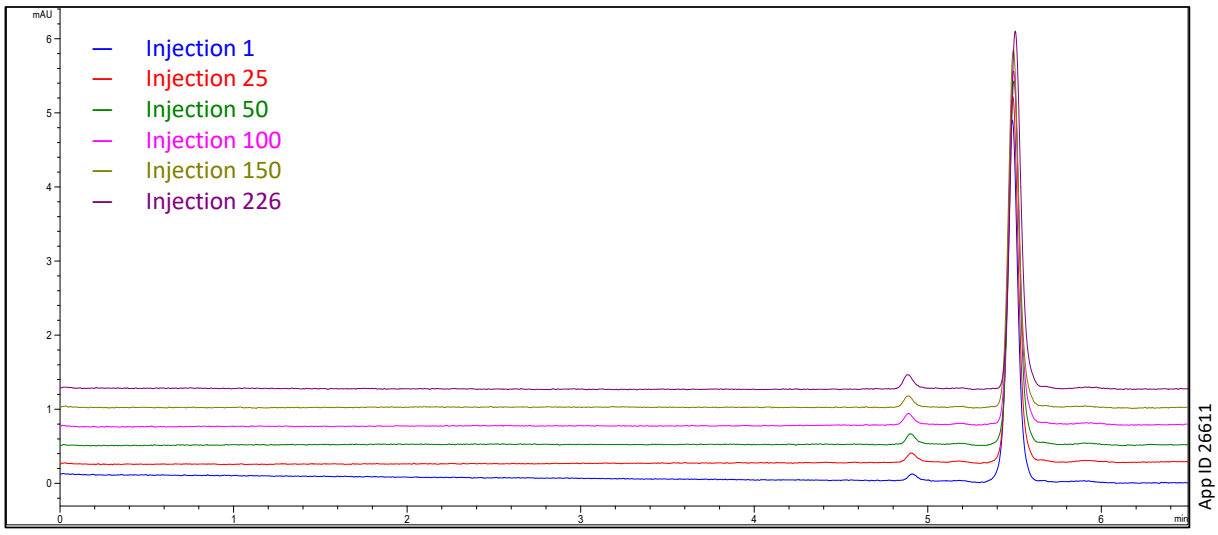


Figure 2. Chromatographic Overlay of Uridine Peak

Overlay of uridine chromatograms. Nominal changes in plates (37 k to 36 k) and retention time (5.4 min to 5.5 min) were observed through the assessment of bed stability during the sequence.



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.

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	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

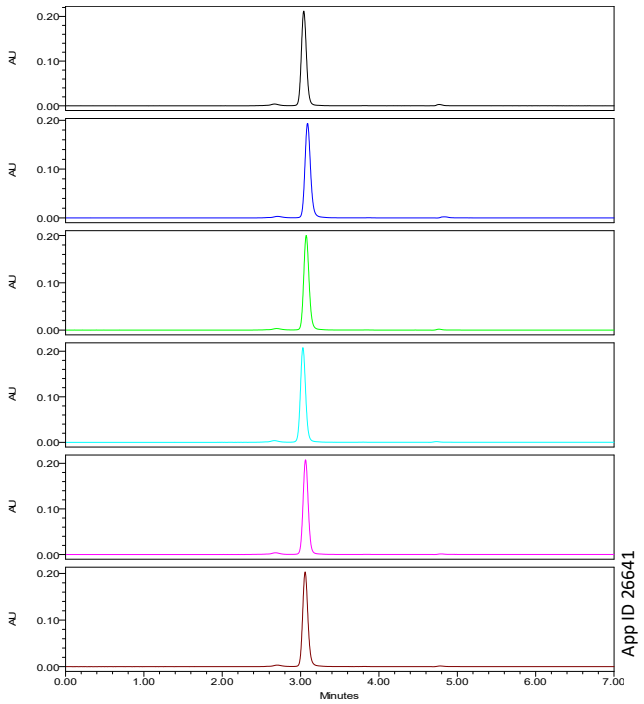
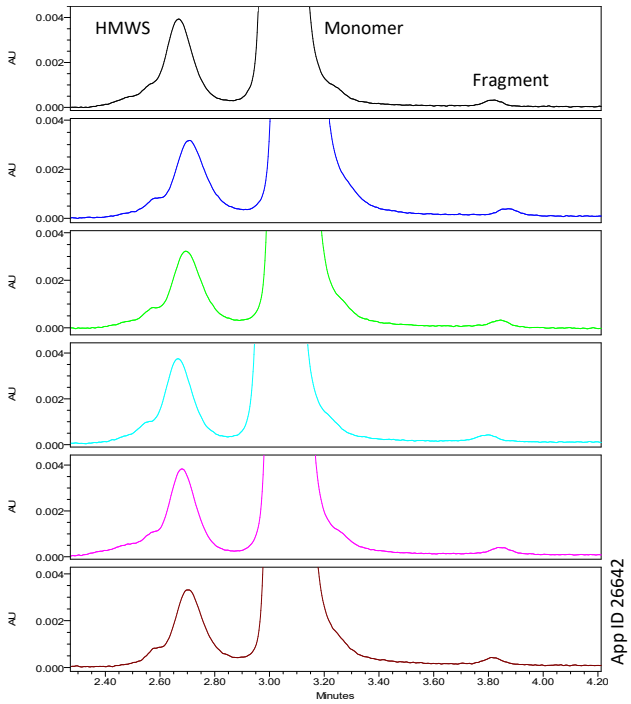


Figure 2. Chromatogram Stack, NIST mAb (Detail)



Assessment of Column Priming for sub-2 µm Size Exclusion Columns

Dr. Ivan Lebedev, 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. SEC methods are commonly used for the quantitation of aggregates in a biotherapeutic sample. Consequently, any non-specific adsorption to the column media or hardware could compromise data, as peak areas may be underreported.

As a failsafe for aggregate and sample adsorption to the column, column "priming" is often included in an analytical method sequence. This includes several injections of a protein; commonly an inert protein like bovine serum albumin (BSA) or reference standard. This helps minimize the chance for sample adsorption to occur.

In this application, we demonstrate a priming protocol, wherein 10 subsequent injections of BSA are injected onto a freshly packed 150 x 4.6 mm ID column with sub-2 µm particles. **Table 1** shows a summary of total peak areas for monomer and dimer. **Figure 1** shows a chromatographic overlay.

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: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Bovine serum albumin (10 mg/mL)

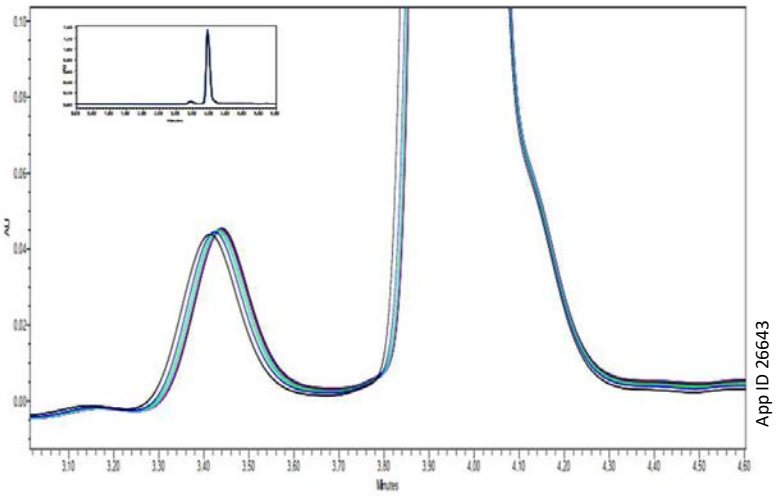
Upon initial injection, peak areas for both monomer and dimer show almost complete recovery. Additionally, as shown in the inset for **Figure 1**, only slight shifts in retention time for monomer and dimer are observed. This indicates that the extent of priming necessary for the Biozen dSEC-2 chemistry is nominal.

In summary, although the practice of column priming is typically performed, column selection may minimize the need for extensive priming.

Table 1. Retention Times for Protein Standards

Injection	Peak Area, Monomer	Peak Area, Dimer
1	10757707	462531
2	10774224	474567
3	10780383	475693
4	10775497	478940
5	10773430	480767
6	10779073	480131
7	10785349	481109
8	10785780	480578
9	10782926	481759
10	10771993	482644
average	10776636.2	477871.9
%CV	0.1%	1.3%

Figure 1. Chromatographic Overlays for BSA



Generating a Calibration Curve with UHPLC Size Exclusion Column

Dr. Ivan Lebedev, 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. To determine the hydrodynamic radius (R_h) of an unknown, a calibration curve can be generated with polymer standards. The relative retention times are plotted against the log MW for each respective standard, and a polynomial regression analysis is performed. Here we demonstrate the use of protein standards, with well-characterized hydrodynamic radii and molar masses, to generate calibration curves.

Figure 1 highlights the separation of common protein standards using a gel filtration standard for analytical and large-scale size exclusion analysis. **Table 1** shows a summary of relative elution times for these standards analyzed by SEC. Relative retention times plotted are then plotted against the log R_h for each respective standard, and a polynomial regression analysis is performed.

Figure 2 shows the calibration curve using a third order polynomial regression generated from standards and log R_h. Based upon the elution volume or retention time for NIST mAb (**Figure 3**), the measured R_h is 50.2 Å. This is within 97.2 % of the reported value of 5.2 Å for NIST mAb.

LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Part No.: 00H-4787-E0
Dimensions: 300 x 4.6 mm
Mobile Phase: 200 mM Potassium Phosphate, 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 10 µL
Temperature: 25 °C
Detection: UV @ 280 nm
Sample: Protein Standards

In summary, using a calibration curve generated by well-characterized standards can be used to determine the hydrodynamic radius of an unknown. Further, it may potentially be used for a stability indicating or biosimilarity method, as changes in post-translational modification such as deamidation and glycosylation can be detected with a well-developed analytical SEC method.

Table 1. Retention Times for Protein Standards

Analyte	Retention Time (min)	Relative Elution Volume (mL)	Theoretical R_h (Å)
Bovine Thyroglobulin	5.489	3.85	86
IgA	6.122	4.3	76
IgG	6.99	4.91	51
Ovalbumin	8.447	5.93	28
Myoglobin, horse heart	9.389	6.59	18.4
NIST mAb	7.078	4.97	52

Figure 1. SEC Chromatograms for Protein Standards

Chromatographic overlays of protein standards, y-axis normalized.

Black- Thyroglobulin, Teal- Gamma Globulin (IgA/IgG), Red- Ovalbumin, Green- Myoglobin

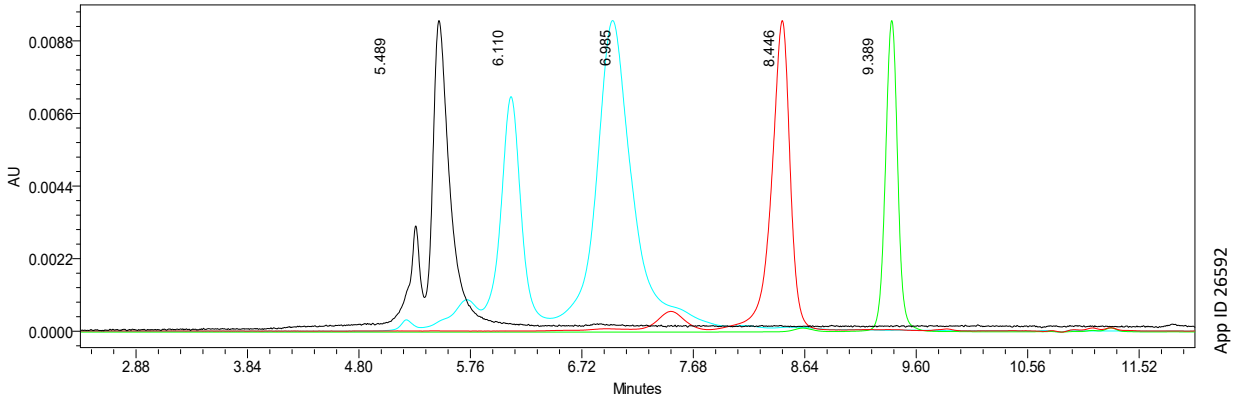


Figure 2. Calibration Curve, Hydrodynamic Radius (R_h)

Calibration curve generating using relative elution volume of protein standards, plotted against log R_h. A third order polynomial regression was used to calculate R_h of various analytical samples.

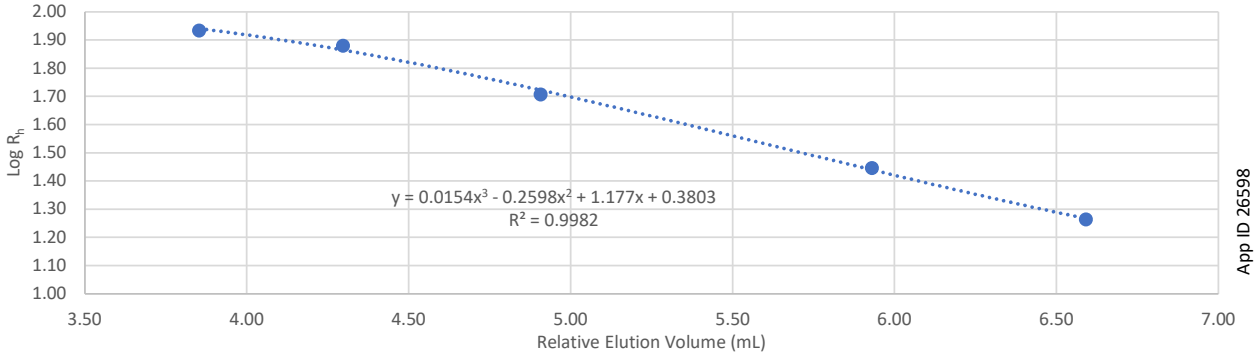
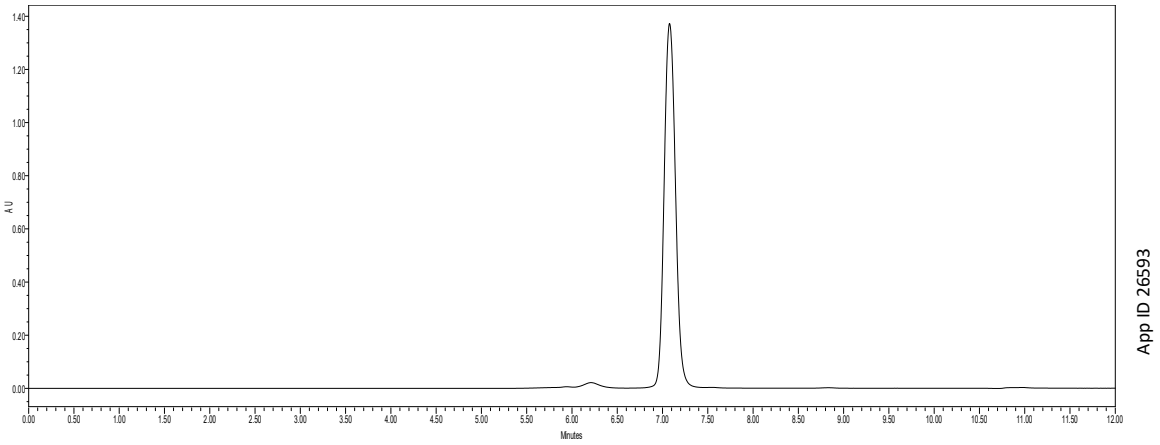


Figure 3. Representative SEC Chromatogram for NIST mAb

Retention time for NIST mAb is 7.078 min. Extrapolated hydrodynamic radius is 50.2 Å



SEC-MS

Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - Bispecific Antibody

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

Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called "Native MS" is especially useful for higher order structure (HOS) of monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the characterization of a bispecific antibody, Emicizumab. **Figure 1** shows the total ion chromatogram for Emicizumab. Good peak shape is observed, and the high molecular weight aggregate is separated, even using a relatively short column (150 mm). **Figure 2** shows the deconvoluted spectrum for the main peak. Glycoforms are identified with good mass accuracies, with most being less than 10 ppm.

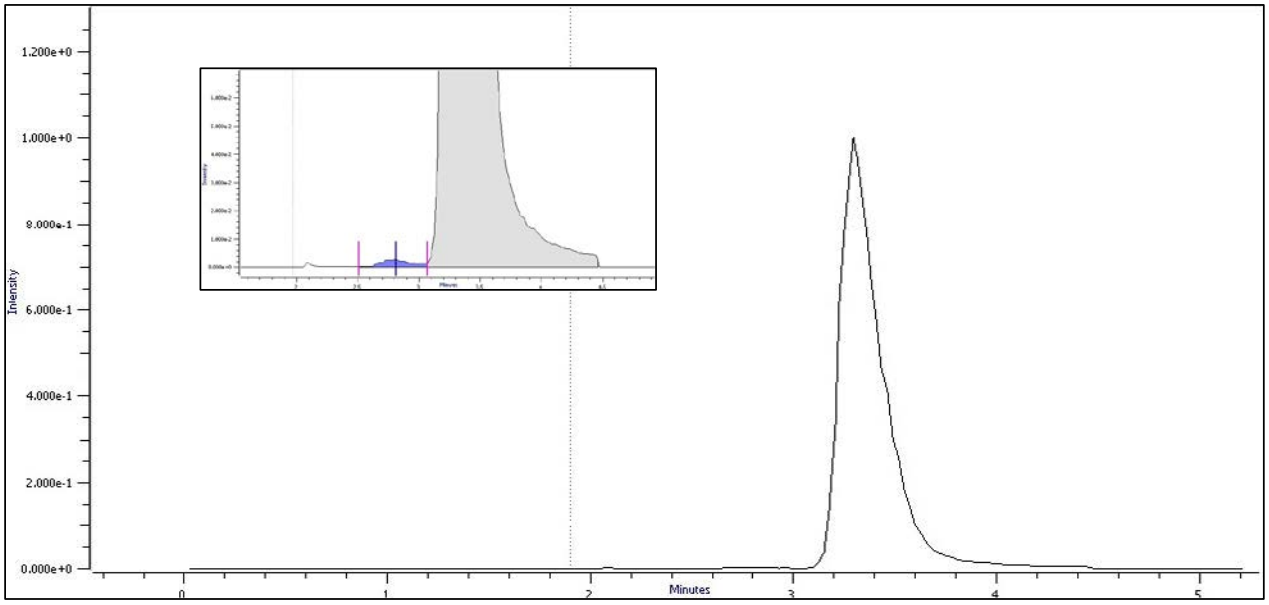
LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 150 x 2.1 mm
Part No.: 00F-4787-AN
Mobile Phase: 200 mM Ammonium Acetate
Flow Rate: 90 µL/min
Injection Volume: 2.5 µL
Temperature: 25 °C
Detection: HRMS
Sample: Emicizumab, 10 mg/mL

Figure 3 shows the deconvoluted spectrum for the high molecular weight peak, showing mostly dimer. The raw spectrum shows some denaturing of the mAb, which is likely dissociation in the gas phase. Dimer for the most abundant glycoform (G0F/G0F/G1F/G1F) is less than 40 ppm.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data, including confirming primary sequence and glycoforms, as well as confirmation of dimer and other high molecular weight species.

Figure 1. Total ion chromatogram (TIC) for Emicizumab, 25 µg



SEC-MS

Figure 2. Deconvoluted Spectrum, Main Peak - Emicizumab

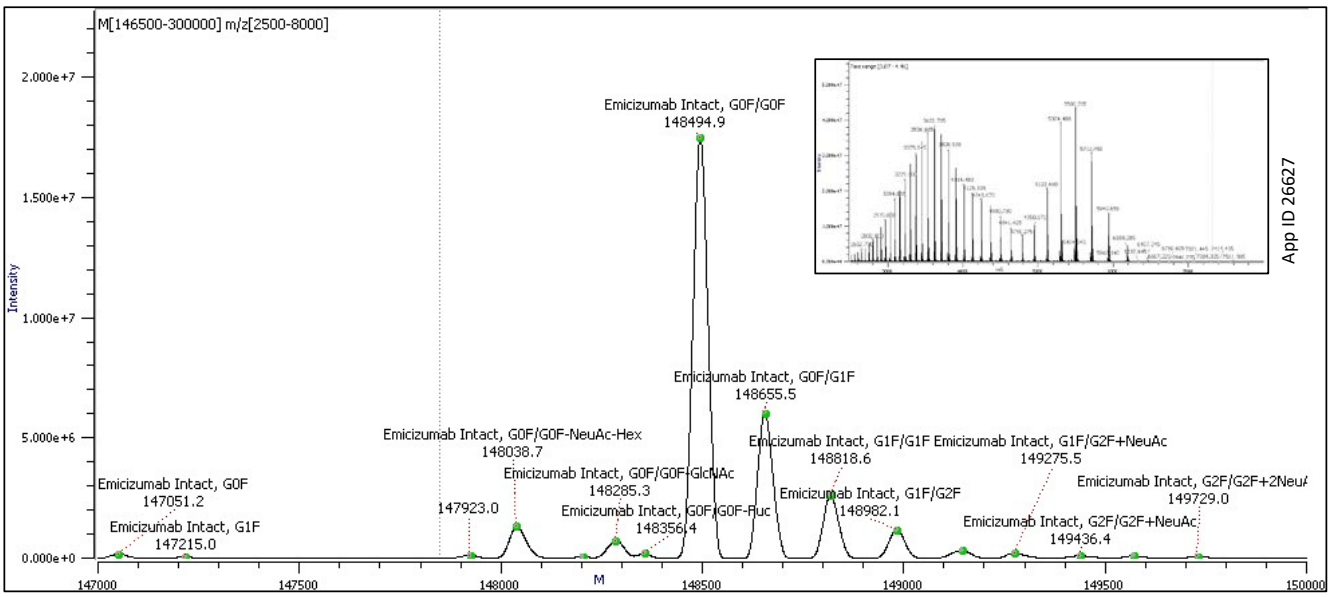
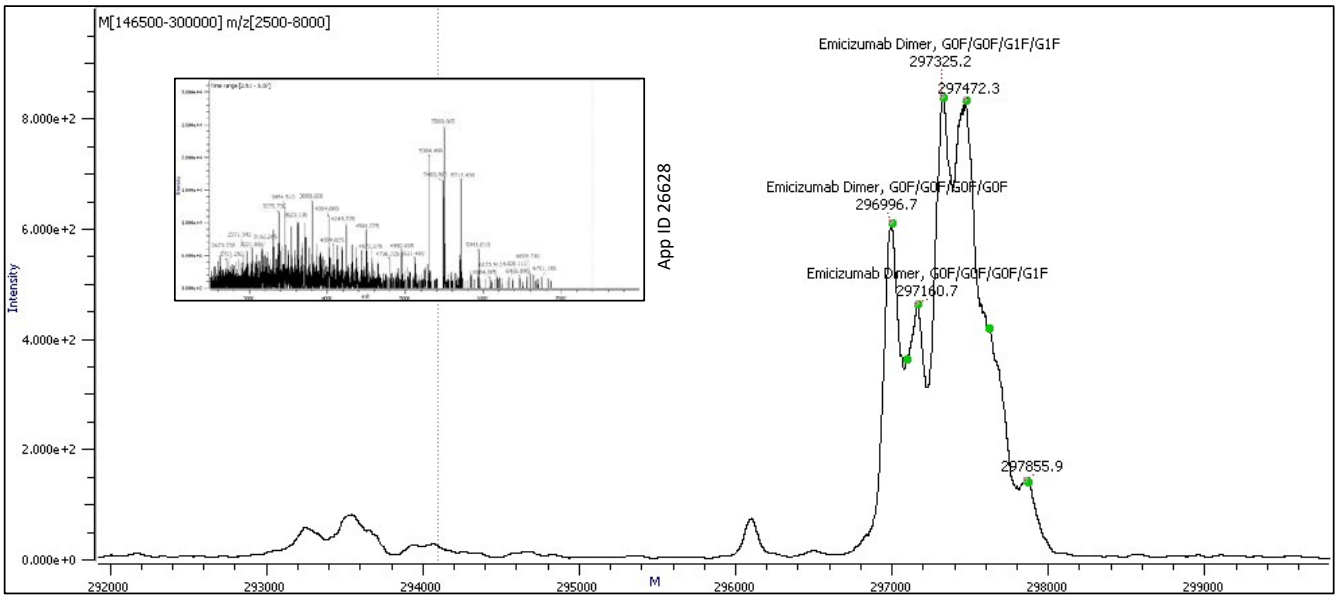


Figure 3. Deconvoluted Spectrum, Dimer/High Molecular Weight Aggregate Peak - Emicizumab



Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - IdeZ Digested Monoclonal Antibody

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

Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called "Native MS" is especially useful for monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the analysis of an IdeZ digested mAb. IdeZ is a cysteine protease which cleaves IgGs below the hinge region, yielding a Fab'2 and two Fc/2 fragments. This allows for specific identification of any post-translational modifications (PTMs) specific to the hypervariable region, as well as higher fidelity for Fc PTMs, including glycosylation.

Figure 1 shows the TIC for IdeZ digested trastuzumab. Good chromatographic separation was achieved for both Fab'2 and Fc/2, allowing for easier data interpretation for each respective fragment. **Figure 2** shows the Fab'2, which shows a glycosylation associated to the Fab region of the antibody.

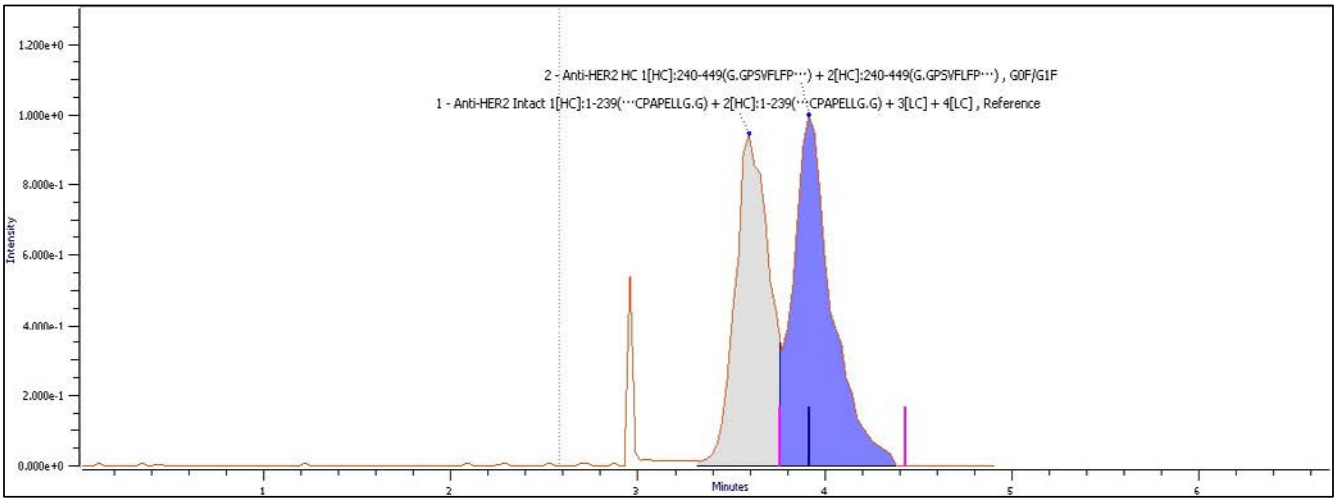
LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 150 x 2.1 mm
Part No.: 00F-4787-AN
Mobile Phase: 100 mM Ammonium Acetate
Flow Rate: 90 µL/min
Injection Volume: 2.5 µL
Temperature: 25 °C
Detection: HRMS
Sample: Trastuzumab, IdeZ digested, 10 mg/mL

Figure 3 shows the deconvoluted spectrum for Fc/2, identifying glycoforms commonly associated with trastuzumab; namely core-fucosylated, biantennary neutral glycans. Lysine variants are also observed.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data (including confirming primary sequence and glycoforms) and, when coupled with site specific proteases, can identify many post-translational modifications. This also shows a good proof of concept that smaller mAb formats are amenable to this workflow.

Figure 1. Total ion chromatogram (TIC) for IdeZ Digested Trastuzumab



App ID 26636

SEC-MS

Figure 2. Deconvoluted Spectrum, Fab'2- Trastuzumab

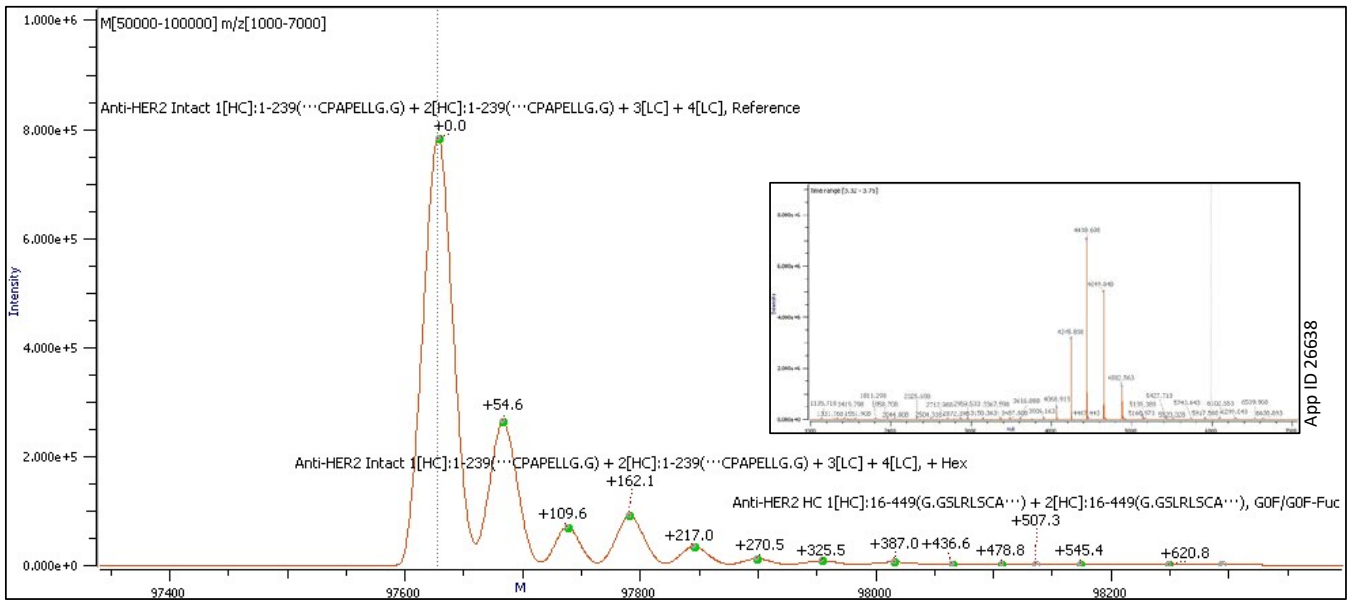
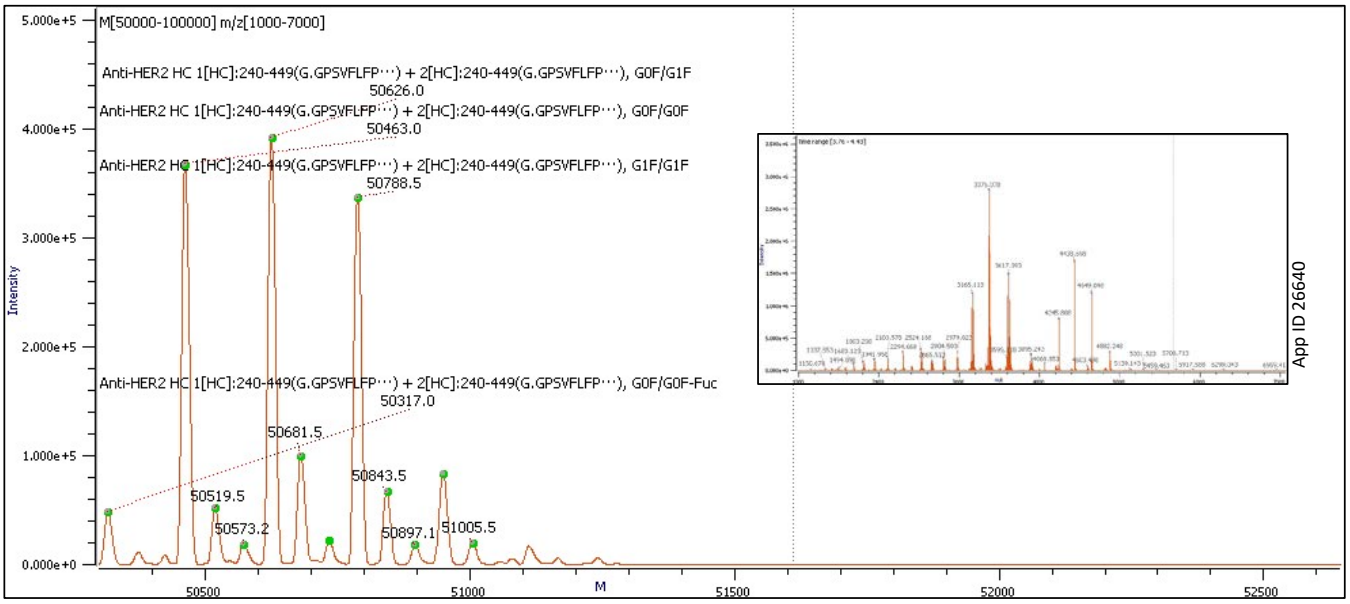


Figure 3. Deconvoluted Spectrum, Fc/2- Trastuzumab



Hyphenating Size Exclusion Chromatography to High Resolution Mass Spectrometry - NIST mAb

Dr. Laurence Brill, 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 biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. The hyphenation of SEC to high resolution mass spectrometry (HRMS) has enabled characterization of biologics. This non-denaturing, so-called "Native MS" is especially useful for higher order structure (HOS) of monoclonal antibodies (mAbs).

In this application, we demonstrate the use of SEC-HRMS for the characterization of NIST mAb RM 8671, a commonly used standard monoclonal antibody surrogate for method development. **Figure 1** shows the total ion chromatogram. Good peak shape is observed, and the low molecular weight fragment is separated even using a relatively short column (150 mm). **Figure 2** shows the deconvoluted spectrum for the main peak. Glycoforms are identified with good mass accuracies, with most glycoforms less than 10 ppm.

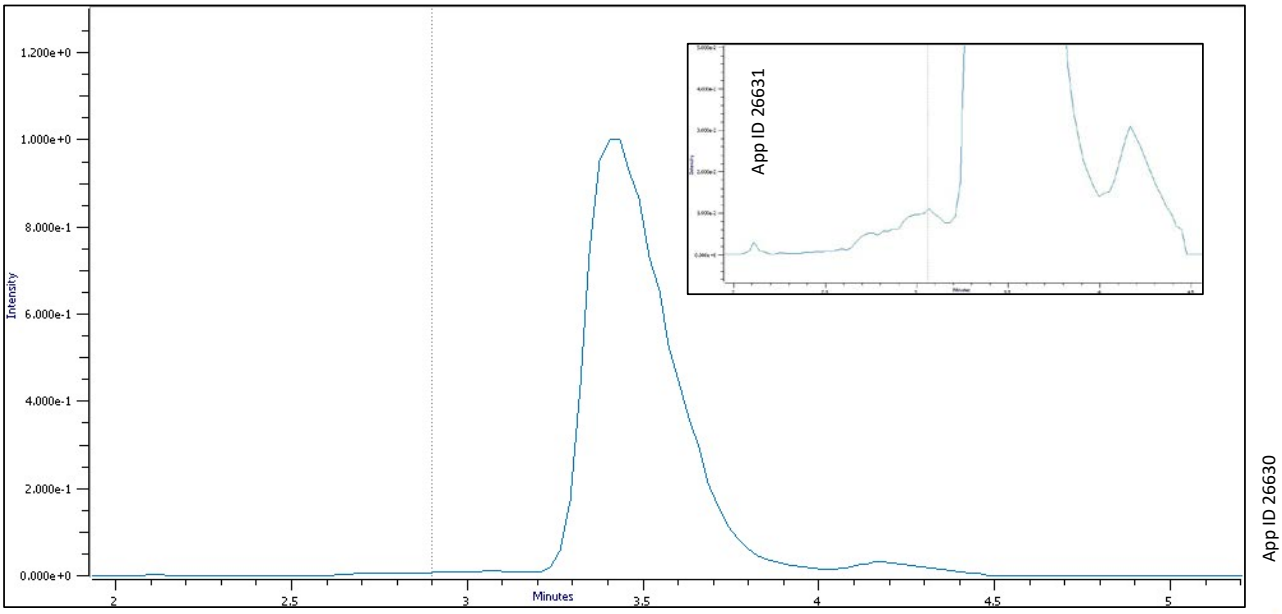
LC Conditions

Column: Biozen™ 1.8 µm dSEC-2, 200 Å
Dimensions: 150 x 2.1 mm
Part No.: 00F-4787-AN
Mobile Phase: 200 mM Ammonium Acetate
Flow Rate: 90 µL/min
Injection Volume: 2.5 µL
Temperature: 25 °C
Detection: HRMS
Sample: NIST mAb RM 8671, 10 mg/mL

Figure 3 shows the deconvoluted spectrum for the low molecular weight aggregate, including putative full-length antibody minus the Fab arm.

In summary, SEC-HRMS is a powerful technique that can provide valuable characterization data, including confirming primary sequence and glycoforms, as well as confirmation of dimer and other high molecular weight species.

Figure 1. Total ion chromatogram (TIC) for NIST mAb RM 8671, 25 µg



SEC-MS

Figure 2. Deconvoluted Spectrum, Main Peak- NIST mAb RM 8671

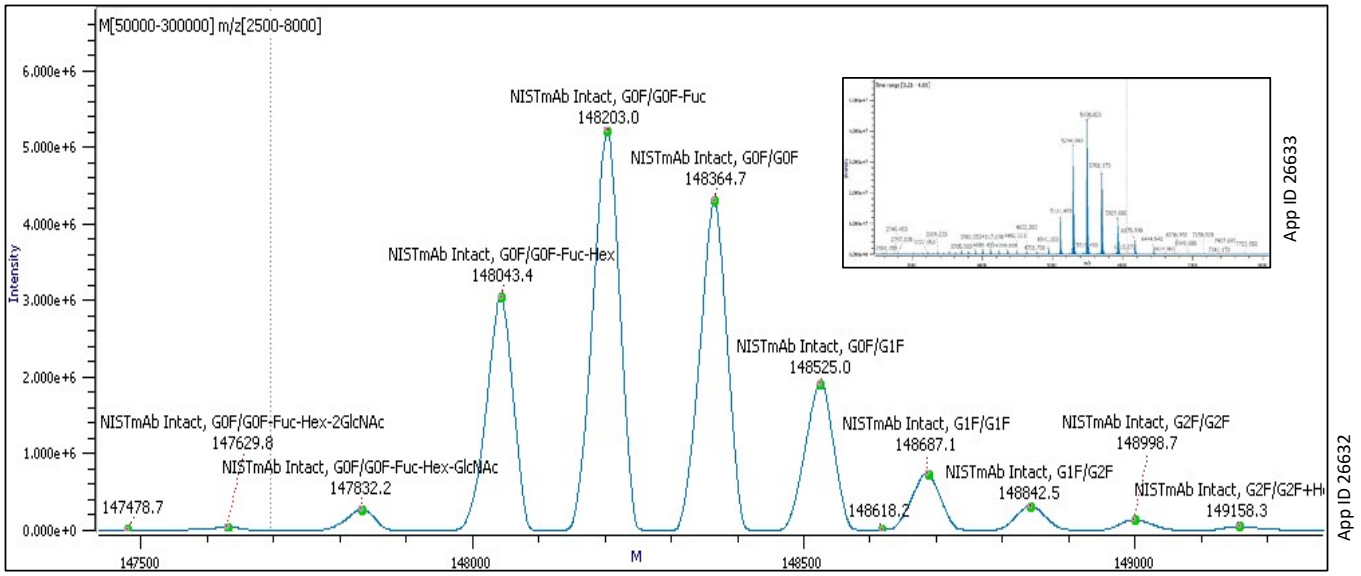
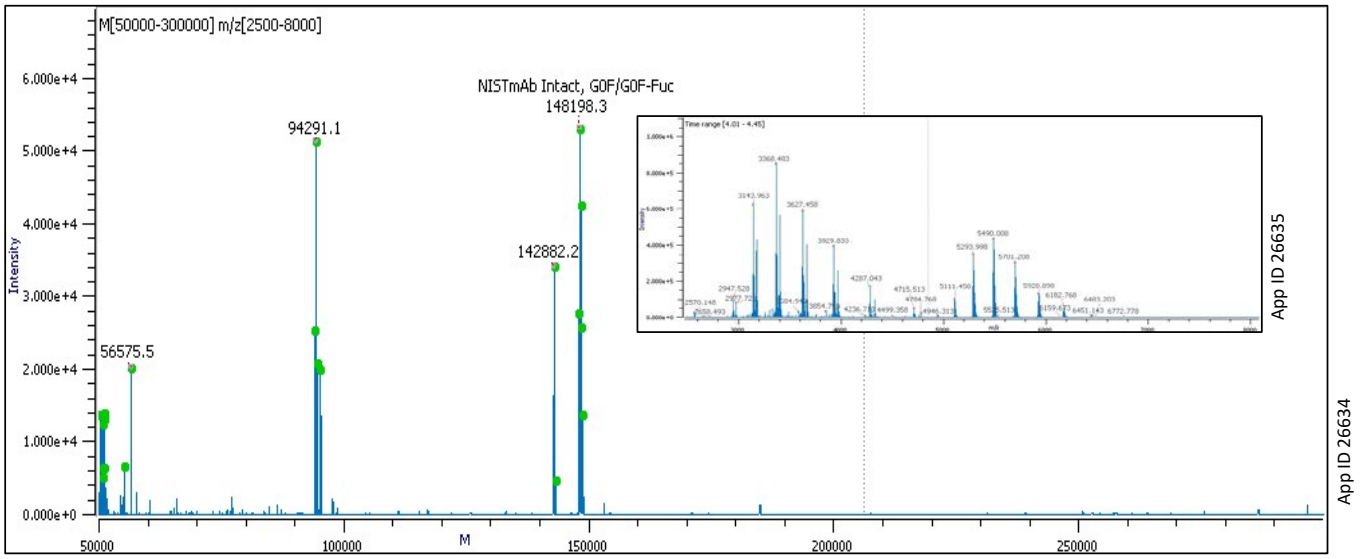


Figure 3. Deconvoluted Spectrum, Low Molecular Weight Fragment



Ordering Information

Biozen™ Products - Powered by Biocompatible Hardware

Biozen Columns (mm)								Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	250 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 2.6 µm WidePore C4	00B-4786-AN	00D-4786-AN	00F-4786-AN	00B-4786-E0	00D-4786-E0	00F-4786-E0	00G-4786-E0	AJ0-9809	AJ0-9811	AJ0-9000
								/3pk	/3pk	ea
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 2.1	150 x 2.1	150 x 4.6	300 x 4.6	150 x 7.8	300 x 7.8	for 4.6 mm	Holder
							/3pk	ea
Biozen 3 µm dSEC	—	—	00F-4788-E0	00H-4788-E0	00F-4788-K0	00H-4788-K0	AJ0-9850	AJ0-9000
Biozen 1.8 µm dSEC	00B-4787-AN	00F-4787-AN	00F-4787-E0	00H-4787-AN	—	—	AJ0-9851	AJ0-9000

	50 x2.1	100 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	for 4.6 mm	Holder
									/10pk	ea
Biozen 6 µm WCX	00B-4777-AN	00D-4777-AN	00F-4777-AN	00G-4777-AN	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	AJ0-9400	KJ0-4282

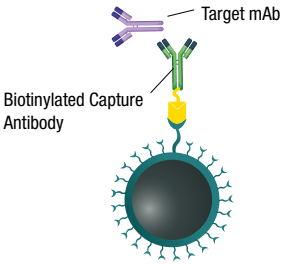
	50 x2.1	100 x 2.1	150 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	for 2.1 mm	for 4.6 mm	Holder
							/3pk	/3pk	ea
Biozen 1.7 µm Oligo	00B-4791-AN	00D-4791-AN	00F-4791-AN	—	—	—	AJ0-9820	AJ0-9822	KJ0-9000
Biozen 2.6 µm Oligo	00B-4790-AN	00D-4790-AN	00F-4790-AN	00B-4790-E0	00D-4790-E0	00F-4790-E0	AJ0-9820	AJ0-9822	KJ0-9000

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

Biozen MagBeads Streptavidin Coated

Formats	Part No.	Concentration	Bead Size
25 mg (≈50 samples) **50 mg (≈100 samples)** **500 mg (≈1000 samples)**	KS0-9531 KS0-9532 KS0-9533	20 mg/mL	1.0 µm



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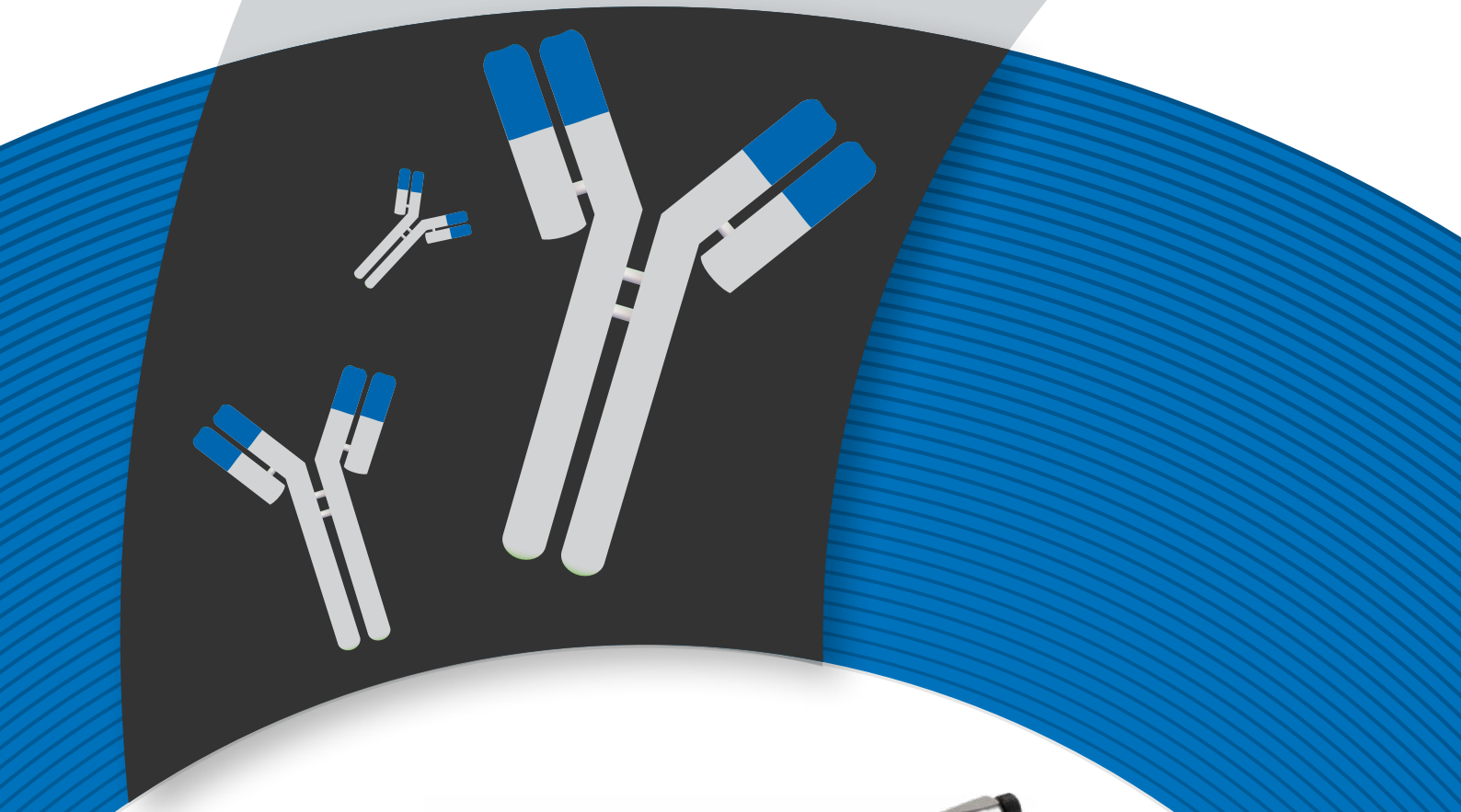
Application Guide

Optimized Pore Controlled Technology for Characterizing Biomolecules with Biozen dSEC Columns



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Australia
+61 (0)2-9428-6444
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Austria
+43 (0)1-319-1301
anfrage@phenomenex.com

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

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

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

Czech Republic
+420 272 017 077
cz-info@phenomenex.com

Denmark
+45 4824 8048
nordicinfo@phenomenex.com

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

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

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

Hong Kong
+852 6012 8162
hkinfo@phenomenex.com

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

Indonesia
+62 21 5010 9707
indoinfo@phenomenex.com

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

Italy
+39 051 6327511
italiainfo@phenomenex.com

Japan
+81 (0) 120-149-262
jpinfo@phenomenex.com

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

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

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

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

Norway
+47 810 02 005
nordicinfo@phenomenex.com

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

Portugal
+351 221 450 488
ptinfo@phenomenex.com

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

Slovakia
+420 272 017 077
sk-info@phenomenex.com

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

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

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

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

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

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

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

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

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