# Agilent AdvanceBio SEC for Peptides, Proteins & ADC Analysis

Don't lose your sample details to secondary interactions



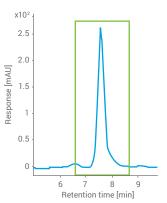
Secondary interactions with the SEC stationary phase lead to peaks eluting later than expected and separating into different forms. This can be confusing when you're trying to interpret your data!

Agilent AdvanceBio SEC columns minimize secondary interactions through a novel hydrophilic coating. There's no need to spend time modifying your method for stickier samples like antibody drug conjugates (ADCs), or worrying about the impact of mobile phase additives on your sample integrity.

Eliminate the confusion by eliminating non-specific secondary interactions.

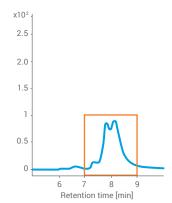
# Fewer secondary interactions: Agilent AdvanceBio SEC columns outperform the competition

Agilent AdvanceBio SEC 200 Å 1.9 µm



See page 3 for full chromatograms.

Waters Acquity BEH200 SEC 200 Å 1.7  $\mu m$ 





# Aggregate and fragment analysis solutions

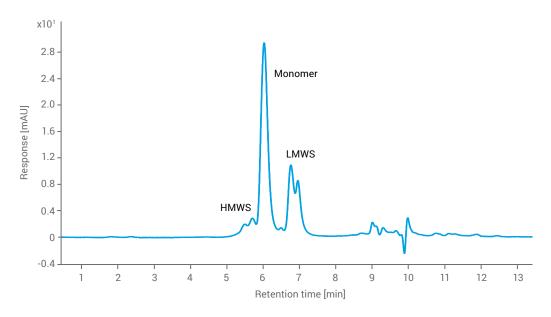
Download application compendium, access webinars, and more.

www.agilent.com/chem/aggregates

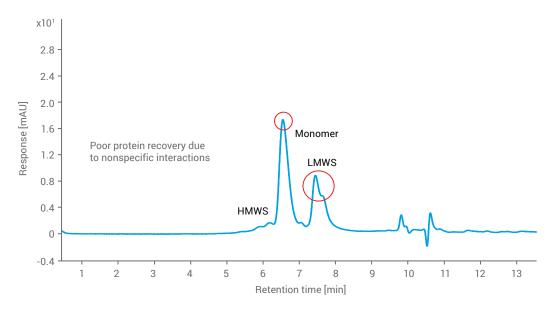


## Size exclusion characterization of human growth hormone

A: Agilent AdvanceBio SEC, 4.6 x 300 mm, 120 Å 1.9 μm



### B: Waters Acquity BEH125, 4.6 x 300 mm, 125 $\hbox{\AA}$ 1.7 $\mu m$



In this case, we tested human growth hormone by SEC. Nonspecific interactions on the Waters column leads to lower sample recovery and lost resolution between two low molecular weight species. The unique hydrophilic coating on the AdvanceBio SEC eliminates this problem, preserving information about your sample.

Instrument: Agilent 1260 Infinity II Bio-inert LC system

Software: Agilent OpenLab CDS

Flow rate: 0.35 mL/min
Eluent: 150 mM phosphate, pH 7

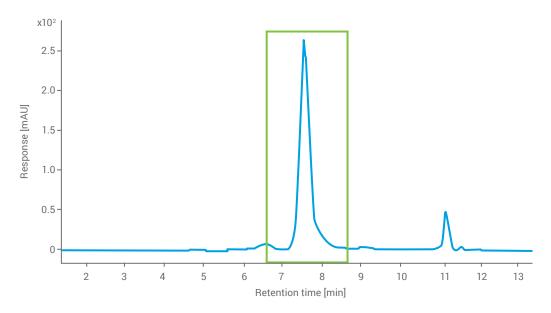
Sample: Concentration 1 mg/mL

 $\begin{tabular}{lll} Temperature: & 25 °C \\ Injection volume: & 2 $\mu L$ \\ \hline Detection: & UV, 220 nm \\ \end{tabular}$ 

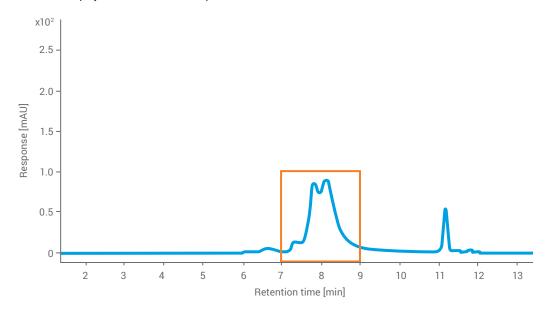
Column	Monomer RT (min)	Total Peak Area (Avg n=2)	Monomer Peak Tailing	Monomer Peak Width
Agilent AdvanceBio SEC	6.02	691.81	1.22	0.21
Waters Aquity BEH125, 4.6 x 300 mm, 125 Å, 1.7 μm	6.54	581.10	1.33	0.28

## Aggregate analysis of Sigma MAb ADC

#### A: Agilent AdvanceBio SEC 200 Å 1.9 μm



### B: Waters Acquity BEH200 SEC 200 Å 1.7 μm



Undesirable secondary interactions lead to the monomer peak being split into two forms, confounding relative quantitation of the monomer. Here we tested Sigma MAb ADC for aggregate analysis and found the monomer peak split in two on the Waters column, contributing to a loss in resolution between the monomer and dimer.

Instrument: Agilent 1260 Infinity II Bio-inert LC system

Software: Agilent OpenLab CDS

Flow rate: 0.35 mL/min

Eluent: 50 mM sodium phosphate, 200 mL NaCl, pH 7.0

Sample: Concentration 1 mg/mL

 $\begin{tabular}{ll} Temperature: & 25 °C \\ Injection volume: & 2 $\mu$L \\ \hline \end{tabular}$   $\begin{tabular}{ll} Detection: & UV, 220 nm \\ \end{tabular}$ 

## **Ordering Information**

AdvanceBio SEC 120 Å	Part Number	
AdvanceBio SEC 120 Å, 1.9 μm, 4.6 x 300 mm	PL1580-5250	
AdvanceBio SEC 120 Å, 1.9 μm, 4.6 x 150 mm	PL1580-3250	
AdvanceBio SEC 120 Å, 1.9 μm, 4.6 x 30 mm guard	PL1580-1250	
AdvanceBio SEC 120 Å, 1.9 μm, 2.1 x 150 mm, PEEK-lined SS hardware	PL1980-3250PK	
AdvanceBio SEC 120 Å, 1.9 μm, 2.1 x 50 mm, PEEK-lined SS hardware	PL1980-1250PK	
AdvanceBio SEC 200 Å	Part Number	
AdvanceBio SEC 200 Å, 1.9 μm, 4.6 x 300 mm	PL1580-5201	
AdvanceBio SEC 200 Å, 1.9 μm, 4.6 x 150 mm	PL1580-3201	
AdvanceBio SEC 200 Å, 1.9 μm, 4.6 x 30 mm guard	PL1580-1201	
AdvanceBio SEC 200 Å, 1.9 μm, 2.1 x 150 mm, PEEK-lined SS hardware	PL1980-3201PK	
AdvanceBio SEC 200 Å, 1.9 μm,	PL1980-1201PK	

Download the AdvanceBio SEC 1.9 µm column user guide.

# Be Agilent Sure in Your CQA Monitoring

Understanding the attributes of a biologic drug, and the processes used it create it, is critical to ensuring safety, efficacy, and pharmacokinetics.

Agilent AdvanceBio columns deliver results you can count on when analyzing complex biotherapeutic molecules. They can help you confidently monitor CQAs.

Agilent offers BioHPLC columns for the analysis of:

- Amino acids and cell culture
- Intact and subunits
- Intact using HIC
- Aggregates and fragments
- Charge variants
- Peptide mapping
- Glycans
- Protein titer

To learn more, visit www.agilent.com/chem/advancebio



Learn more about AdvanceBio SEC 1.9 µm columns. www.agilent.com/chem/advancebiosec

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This information is subject to change without notice.

