Helpful ‘Hints’ for Protein SEC Success

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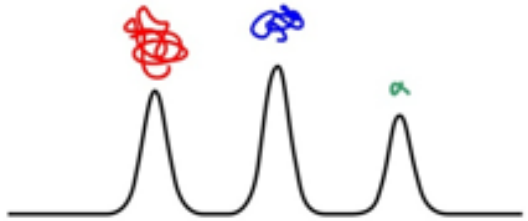
Applications Engineer
LC columns and consumables technical support
May 27, 20201



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Outline for Talk
Getting started

- SEC definition, common terminology, and why use it?
- Considerations for selecting an SEC column
  - Pain Points/Challenges for SEC
  - Importance of Pore Size and Particle Size Selection
- Mobile Phases and Buffer Considerations
- Common Detectors
- Considerations for the LC instrument

Protein Characterization

Testing Importance of Aggregates & Fragments

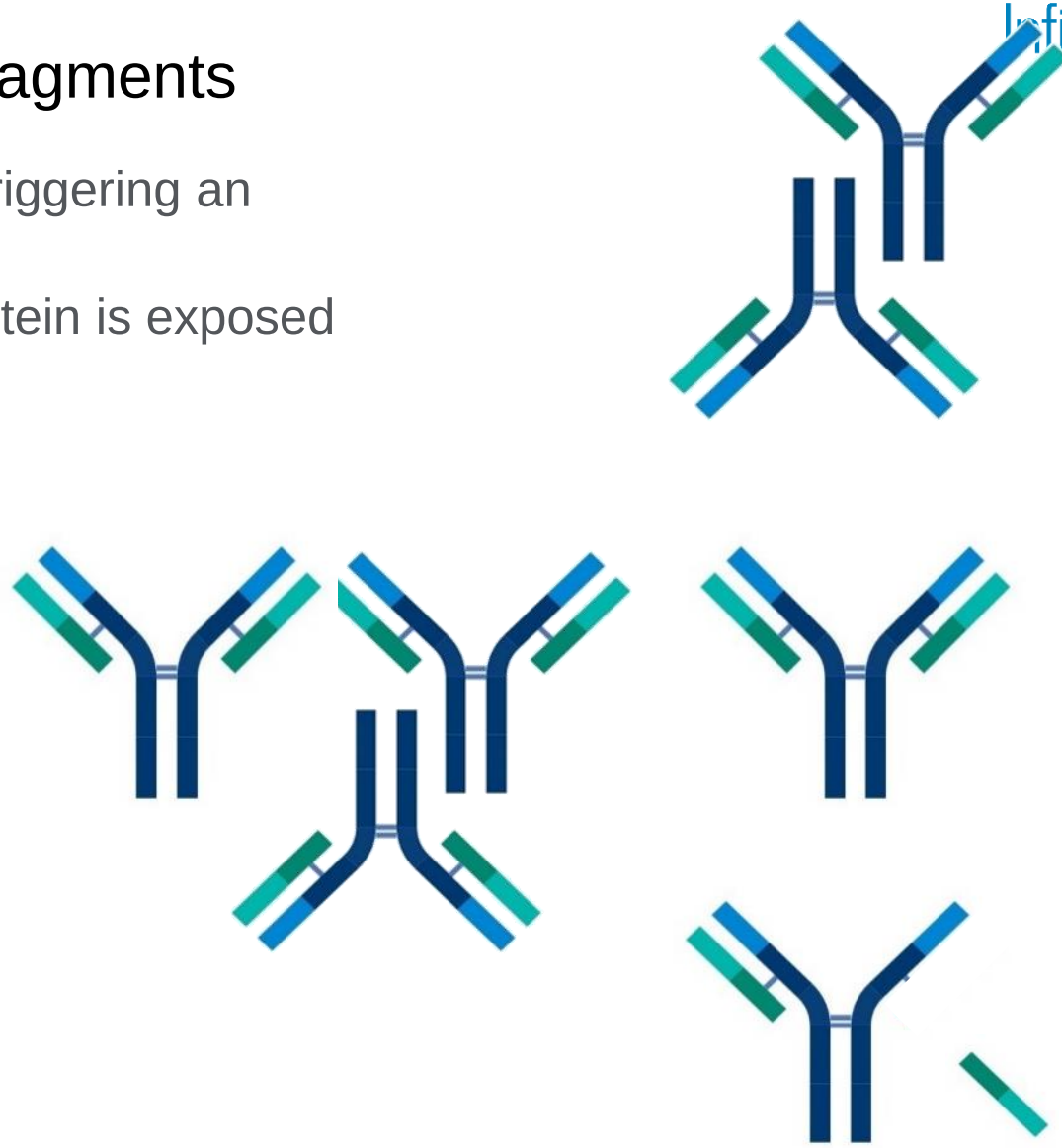
Aggregation is not only highly risky – possibly triggering an immunogenic response – it is very common.

Aggregation is a common response when a protein is exposed to stress conditions:

- Upstream or downstream processes
- Changes in concentration
- Changes in pH or temperature
- Exposure to surfaces or shear forces

Proteins are relatively unstable

Do not always adopt native conformation



SEC = Size Exclusion Chromatography

Separates (ideally) purely by sample size in solution

NOT by typical particle surface/sample interaction

SEC Size Exclusion Chromatography is for aqueous mobile phase conditions

GPC Gel Permeation Chromatography is for organic solvent mobile phase conditions

Separation Mechanism

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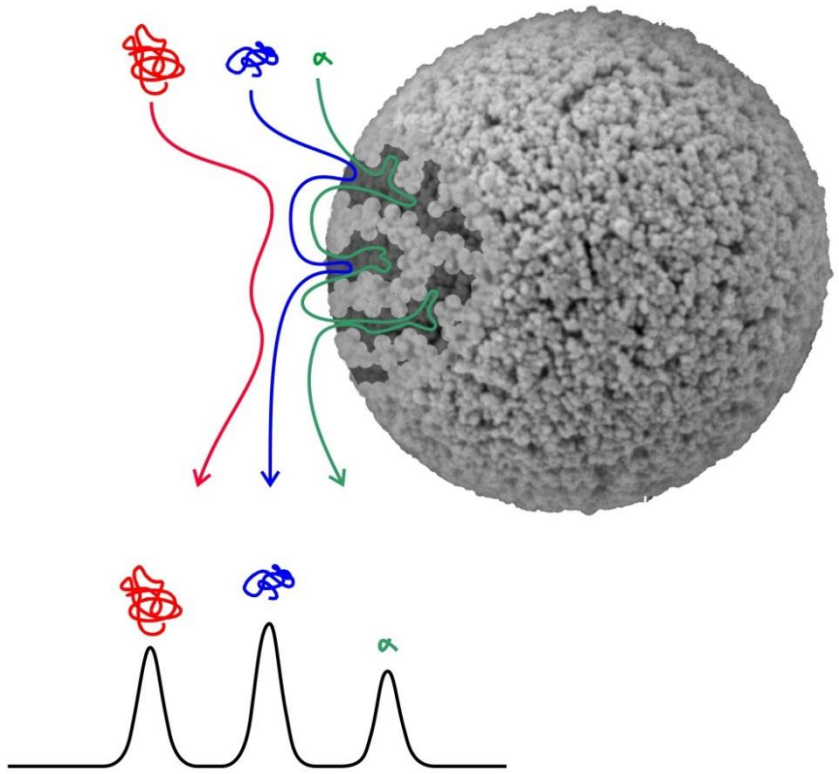
Largest molecules cannot penetrate the pores – they are excluded and elute first

Smallest molecules diffuse into all of the pores - elute from the columns last

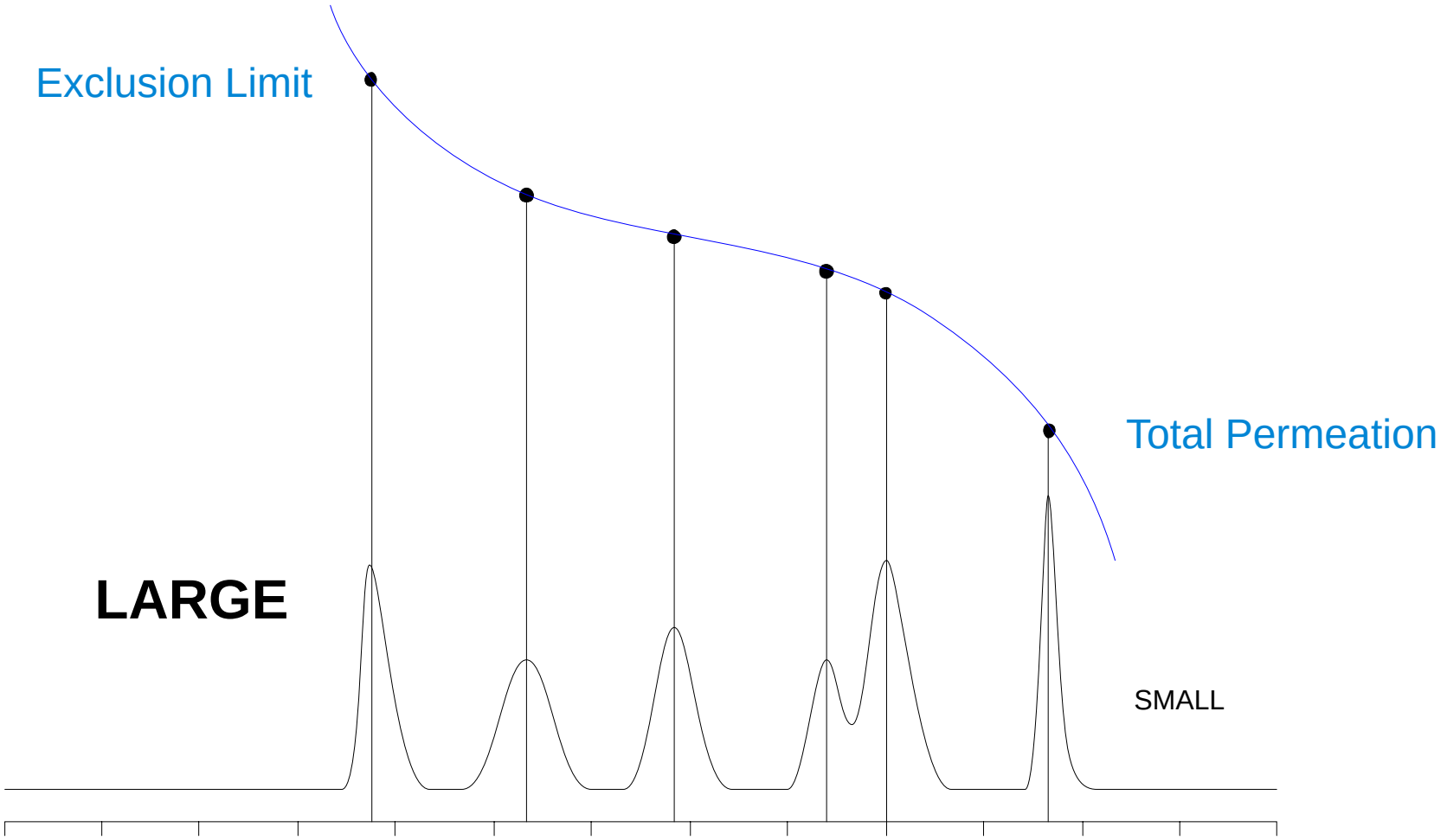
Resolution of the column is dependent on the pore volume in the column

To increase resolution, it is sometimes necessary to run multiple columns in series

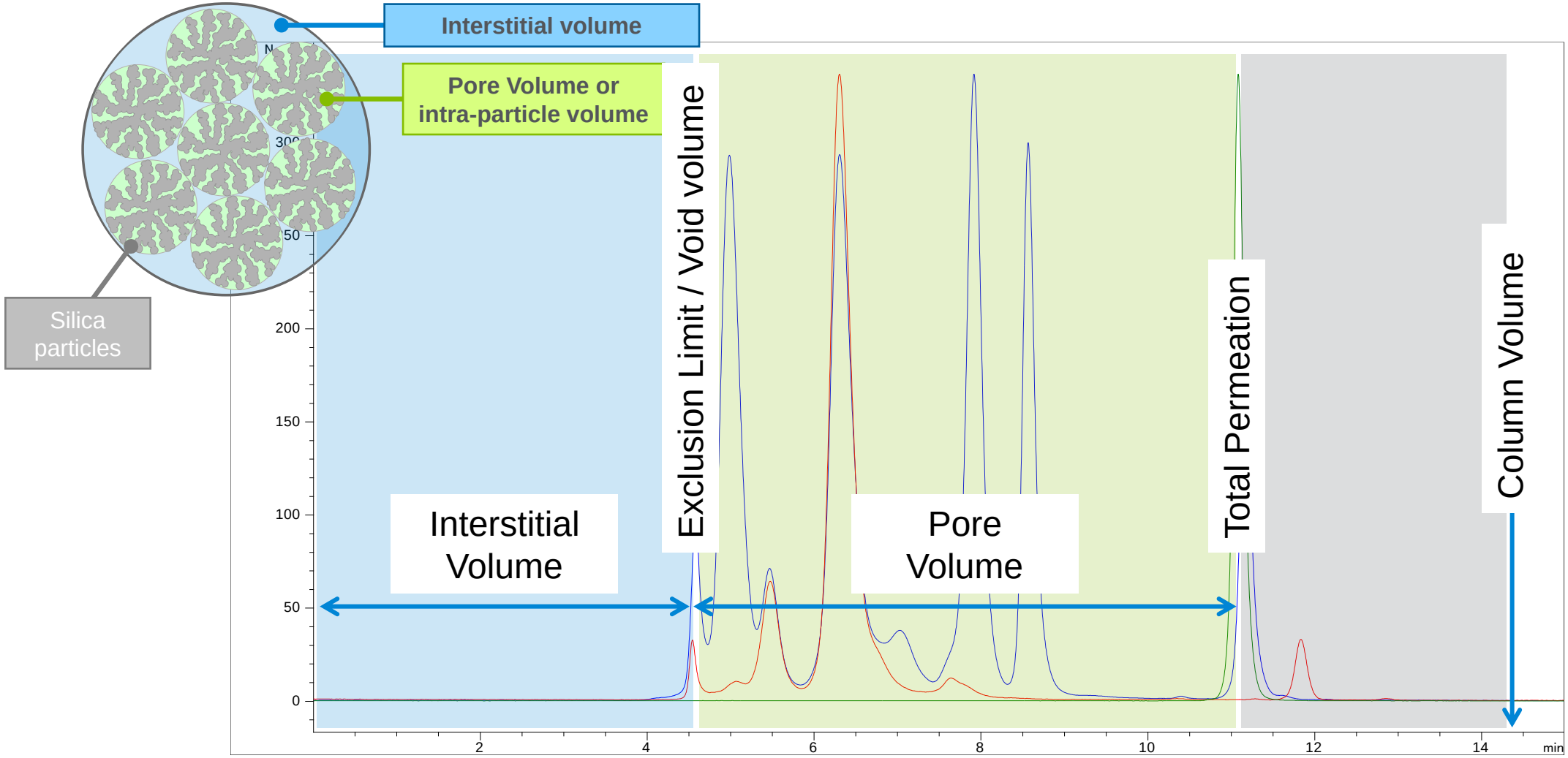


SEC: A Non-interactive isocratic technique; correct choice of pore size is critical for success

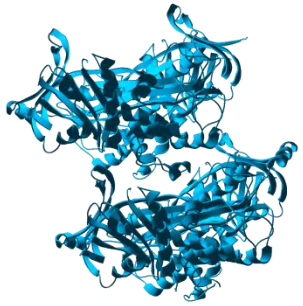
Making sense of Size Exclusion Chromatography



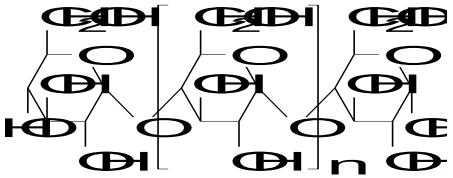
Common Terminology and chromatogram example noting common terms



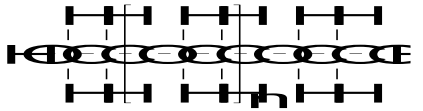
Molecular Weight or Size in Solution



Ovalbumin
MW 44,330D
385 amino acids

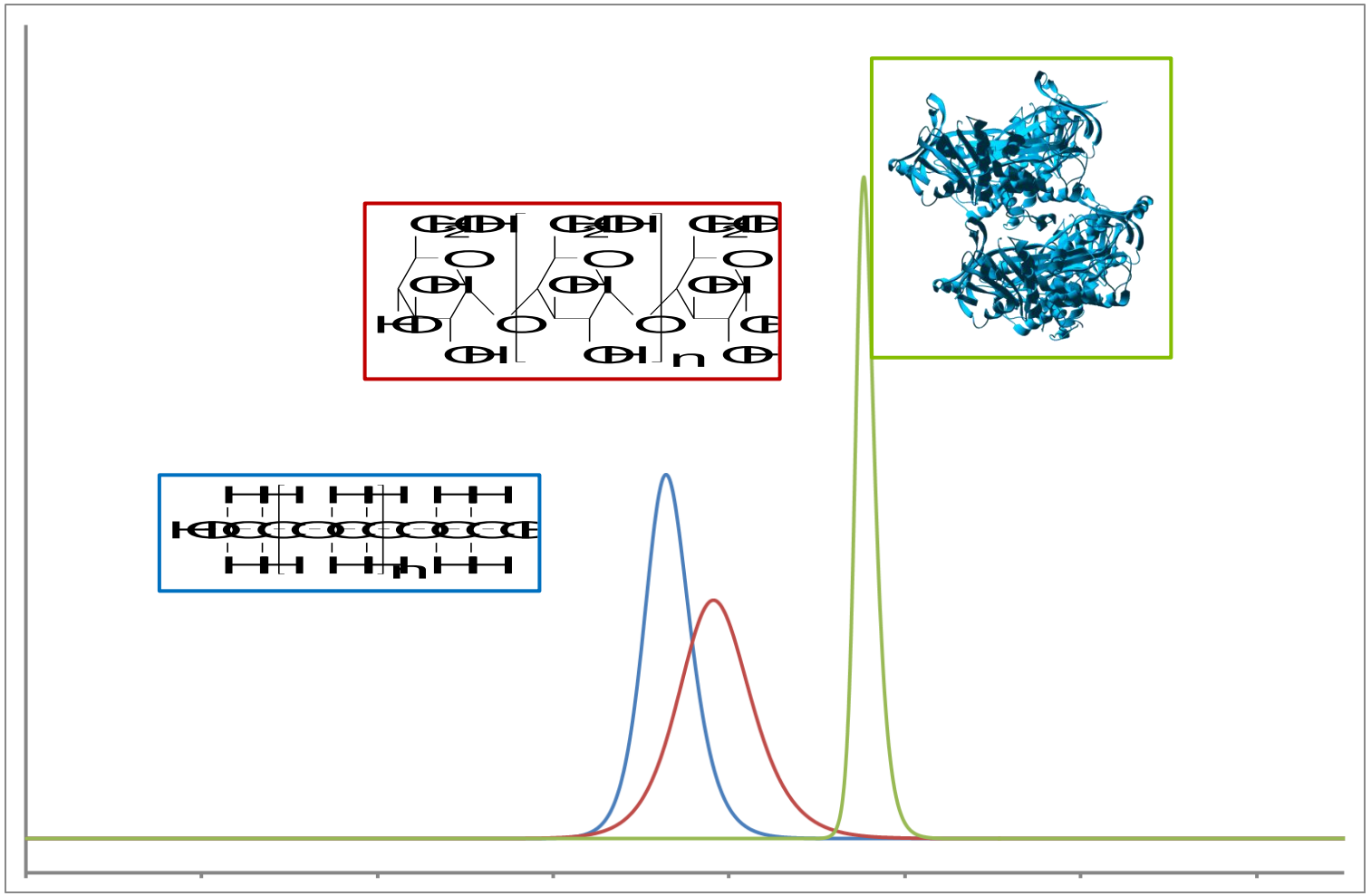


Pullulan
MW 45,000D
n ~ 275



PEO
MW 45,000D
n ~ 1000

Molecular Weight or Size in Solution



Column Selection Criteria

Pore size

- depends on molecular weight range of sample
- avoid exclusion of sample components
- maximize pore volume in required separation region

Particle size

- smaller particles for higher resolution

Number of columns/ length

- compromise between resolution and analysis time
- Smaller length column for increased thruput
- Longer length column for improved resolution

Column ID

- smaller column ID can be used for reduced solvent consumption, smaller injection volume, or improved sensitivity

Considerations for Column Selection
Pain points and common challenges for SEC

- Limited resolution - insufficient/incorrect pore sizes can reduce resolution
- Non-specific interactions contribute to loss of sample, lead to inconsistent results, rework
- Long analysis times - SEC is typically slow
- Poor pressure stability creates rework and increased cost
- Want consistent and reproducible results
- High salt conditions puts excessive wear on instrument, parts

Agilent Biomolecule Columns Portfolio

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Titer Determination	Aggregate Analysis	Intact Purity & PTM Analysis		Sequence Variant & PTM Analysis	Charge Variant Analysis	Glycan Analysis	Amino Acid / Cell Culture Media Analysis	
Affinity	Size Exclusion	Reverse Phase >150 Å	Hydrophobic Interaction	Reverse Phase < 150 Å	Ion Exchange	Hydrophilic Interaction	Reverse Phase < 150 Å	Hydrophilic Interaction
Bio-Monolith Protein A	AdvanceBio SEC	PLRP-S	AdvanceBio HIC	AdvanceBio Peptide Plus	Bio mAb	AdvanceBio Glycan Mapping	AdvanceBio Amino Acid Analysis (HpH)	AdvanceBio MS Spent Media
Bio-Monolith Protein G	Bio SEC-3	AdvanceBio RP mAb		AdvanceBio Peptide Mapping	Bio IEX (SAX, WAX, SCX, WCX)	ZORBAX RRHD 300-HILIC 1.8 µm	ZORBAX AAA	
	Bio SEC-5	ZORBAX RRHD 300 Å, 1.8 µm			PL SCX, SAX			
	ProSEC 300S	ZORBAX 300SB			Bio-Monolith (QA, DEAE, SO3)			
	ZORBAX GF250 & GF450	Poroshell 300						

Agilent Size Exclusion Columns

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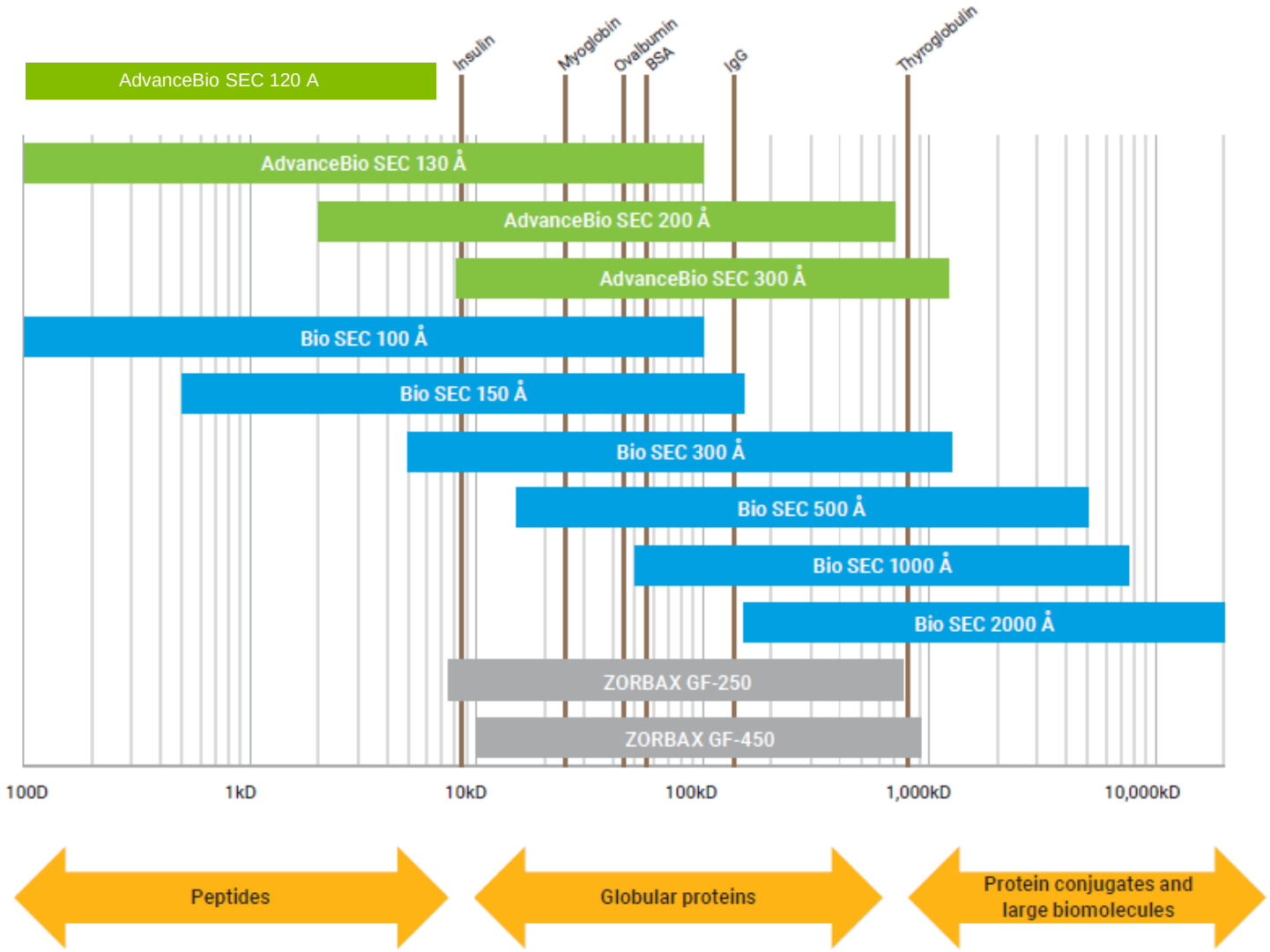
AdvanceBio SEC	AdvanceBio SEC	Bio SEC-3	Bio SEC-5	ProSEC 300S	ZORBAX GF-250 & GF-450
1.9µm	2.7µm	3µm	5µm	5µm	4µm, 6µm
200Å, 120Å	130Å, 300Å	100Å, 150Å, 300Å	100Å, 150Å, 300Å, 500Å, 1000Å, 2000Å	Nominal 300Å (linear resolving range)	150Å, 300Å
Coated silica (USP L59)	Coated silica (USP L59)	Coated silica (USP L59)	Coated silica (USP L59)	Silica Diol (USP L20)	Zirconium stabilized silica diol (USP L35)
• mAb and ADC analysis • Dimer/monomer • LMW mAb fragments	• mAb and ADC analysis • Higher-order aggregates • Dimer/monomer	• Polypeptide to small proteins • MS capable separations	• Broadest range of pore sizes for wide variety of biomolecules	• Unique linear resolving range • 30cm and 60cm column lengths	• Legacy product • Larger column dimensions • Ideal for GF-450 & GF-250 in series

Agilent Size Exclusion Columns



What to Look for in an SEC Column

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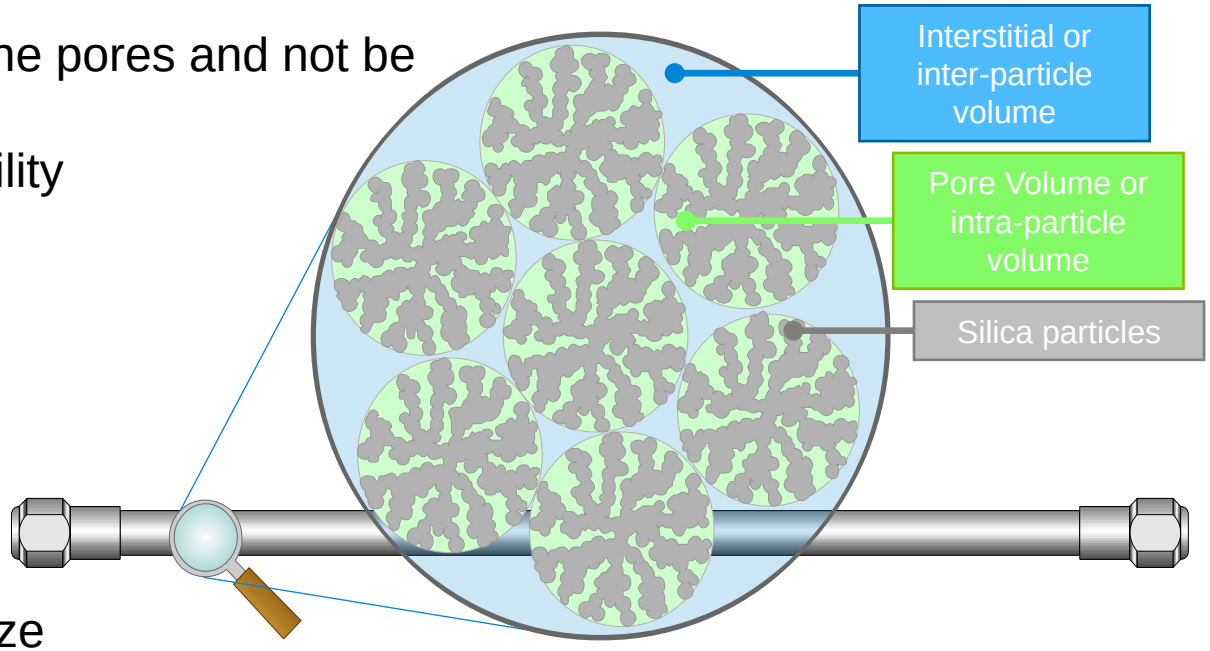
The Right Pore Size for Your Sample

- Pores must be large enough for sample to permeate the pores and not be excluded
- Pores that are too large will limit the separation capability
- Optimum pore size ~300Å for mAbs



Well Packed Column & Large Pore Volume

- Minimize interstitial volume
- Maximize pore volume

Inert Chemistry

- Ideal SEC separations are based only on molecular size
- Best to avoid secondary interactions between the sample and stationary phase

The Right Dimensions for Your Application

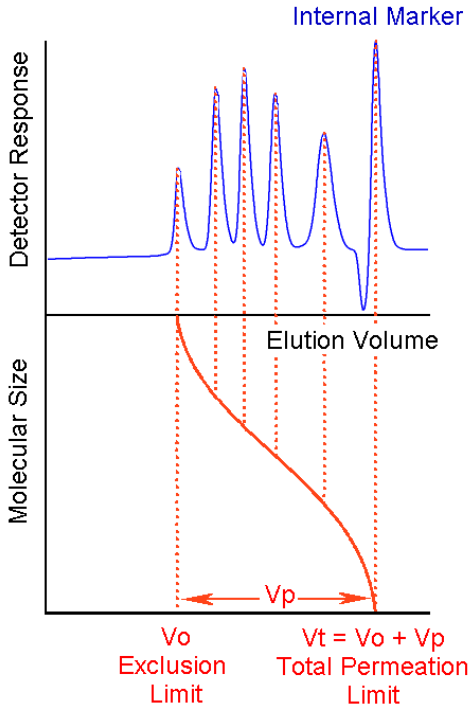
4.6 or 7.8 x 150 mm for higher throughput, faster separations

7.8 x 300 mm for higher resolution
4.6 x 300 mm for higher sensitivity

Column Selection:

Choose the right pore size

- It is essential to select a column that has pores sufficiently large enough to allow your molecule to permeate into the pore structure of the stationary phase and to not be excluded.
- Provides for complete coverage for the MW range of your sample and for your calibration standards
- Choose a pore size that allows you to work in the linear portion of the calibration curve
- It is also essential to choose a pore size that is not too large



Ex: For monoclonal antibodies the optimum pore size is around 300Å …

Column Selection
Agilent AdvanceBioSEC and BioSEC columns pore size options

Pore Size	MW Range (Globular Protein)	Typical Sample
100Å 120Å	100 – 100,000	Small proteins
150Å	500 – 150,000	Small proteins
200A	2,000 – 700,000	Monomer, dimer & LMW fragments – mAb & ADC
300Å	5,000 – 1,250,000	Monomer, dimer, higher order aggregates – mAb & ADC
500Å	15,000 – 5,000,000	Larger proteins and antibodies (IgM)
1000Å	50,000 – 7,500,000	Very large proteins
2000Å	> 10,000,000	Very large molecules – viruses Lower limit on this column is hard to define, Use only for special applications

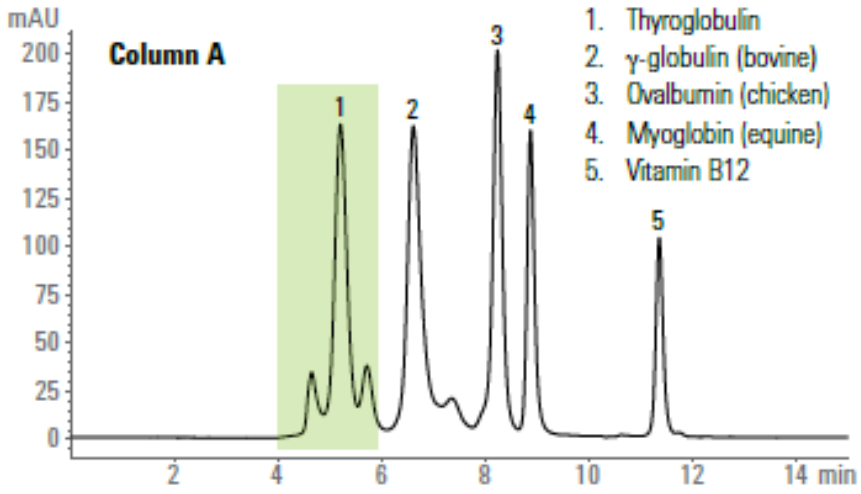
Importance of Pore Size Selection Calibrants

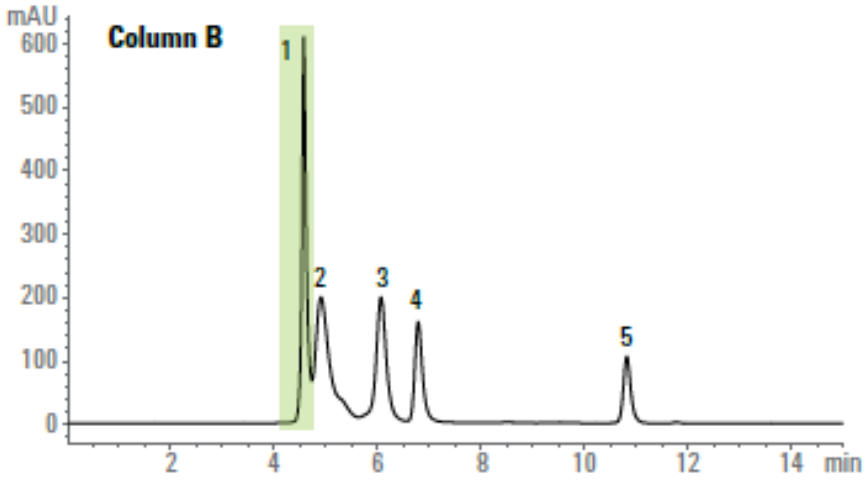
Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC System
Mobile phase:	150 mM phosphate buffer, pH 7.0
Flow rate:	0.35 mL/min
Detector:	UV, 220 nm
Sample:	BioRad gel filtration standards mix

Column A: **AdvanceBio SEC 300Å**
4.6 x 300 mm, 2.7 µm (p/n PL1580-5301)

Column B: **AdvanceBio SEC 130Å**
4.6 x 300 mm, 2.7 µm (p/n PL1580-5350)

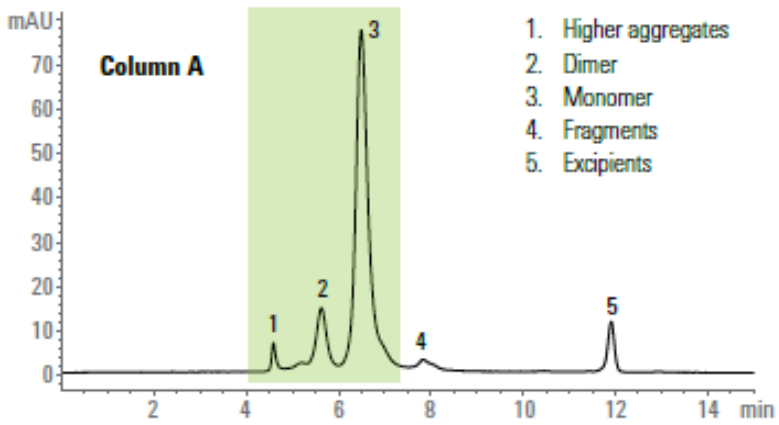
BioRad gel filtration standards mix

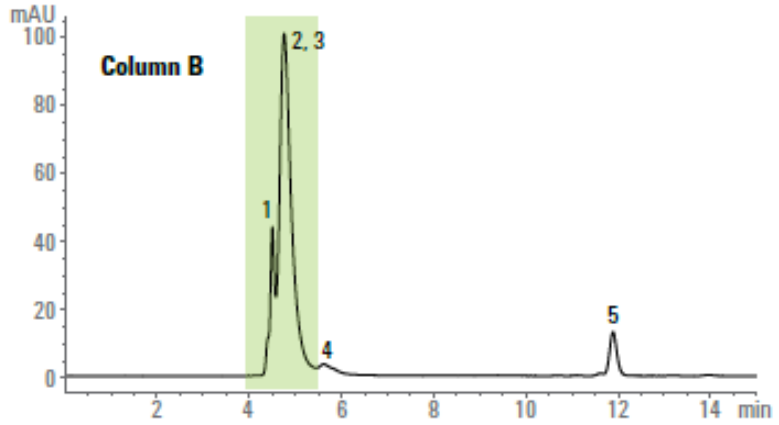




Importance of Pore Size Selection
Sample

Polyclonal IgG separation



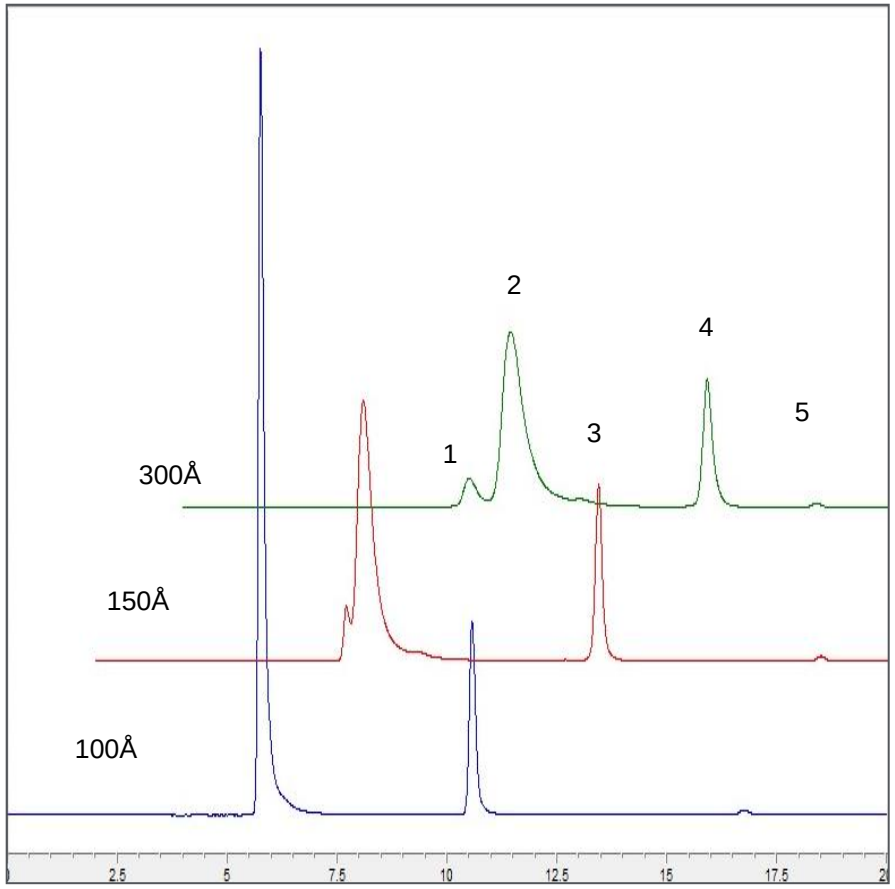


Column A: **AdvanceBio SEC 300Å**
4.6 x 300 mm, 2.7 µm (p/n PL1580-5301)

Column B: **AdvanceBio SEC 130Å**
4.6 x 300 mm, 2.7 µm (p/n PL1580-5350)

Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC System
Mobile phase:	150 mM phosphate buffer, pH 7.0
Flow rate:	0.35 mL/min
Detector:	UV, 220 nm
Sample:	Polyclonal IgG

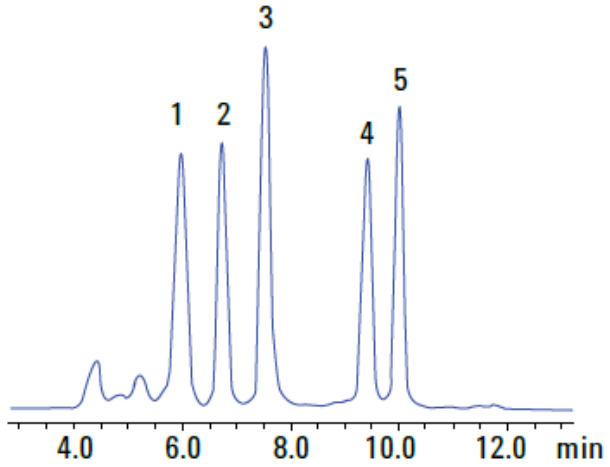
Pore Size Selection
Choice for Antibody Analysis



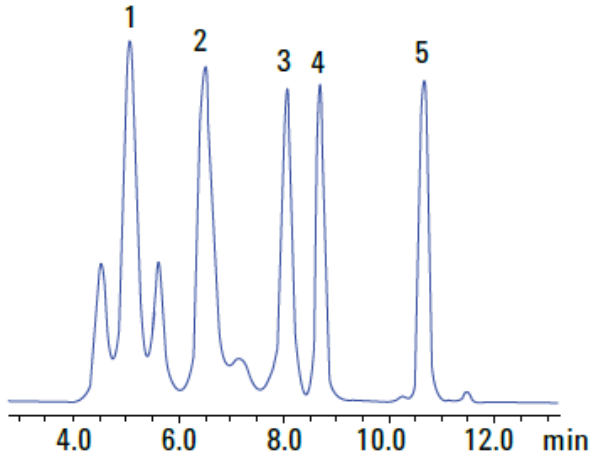
Eluent: 50mM NaH_2PO_4 + 0.15M NaCl, pH6.8
Columns: Bio SEC various pore sizes
Flow: 0.35ml/min
Detector: UV@220nm
System: Agilent 1260 Infinity Bio-Inert LC
Sample: Mouse IgG

1. Dimer
2. Monomer
3. Monomer Fragment
4. Azide
5. Retained Molecule

AdvanceBio SEC Protein Standards:



AdvanceBio SEC 130Å Protein Standard separation on AdvanceBio SEC 130Å column



AdvanceBio SEC 300Å Protein Standard separation on AdvanceBio SEC 300Å column

AdvanceBio SEC 130Å Protein Standard p/n 5190-9416, 1.5 mL vial)	
Analyte	**MW**
1. Ovalbumin	45,000
2. Myoglobin	17,000
3. Aprotinin	6,700
4. Neurotensin	1,700
5. Angiotensin II	1,000

AdvanceBio SEC 300Å Protein Standard (p/n 5190-9417, 1.5 mL vial)	
Analyte	**MW**
1. Thyroglobulin	670,000
2. γ-globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000

Features and Benefits of AdvanceBio SEC

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Particles:

- Large pore volume
- Optimized pore size and narrow pore size distribution
- Smaller particles

Chemistry:

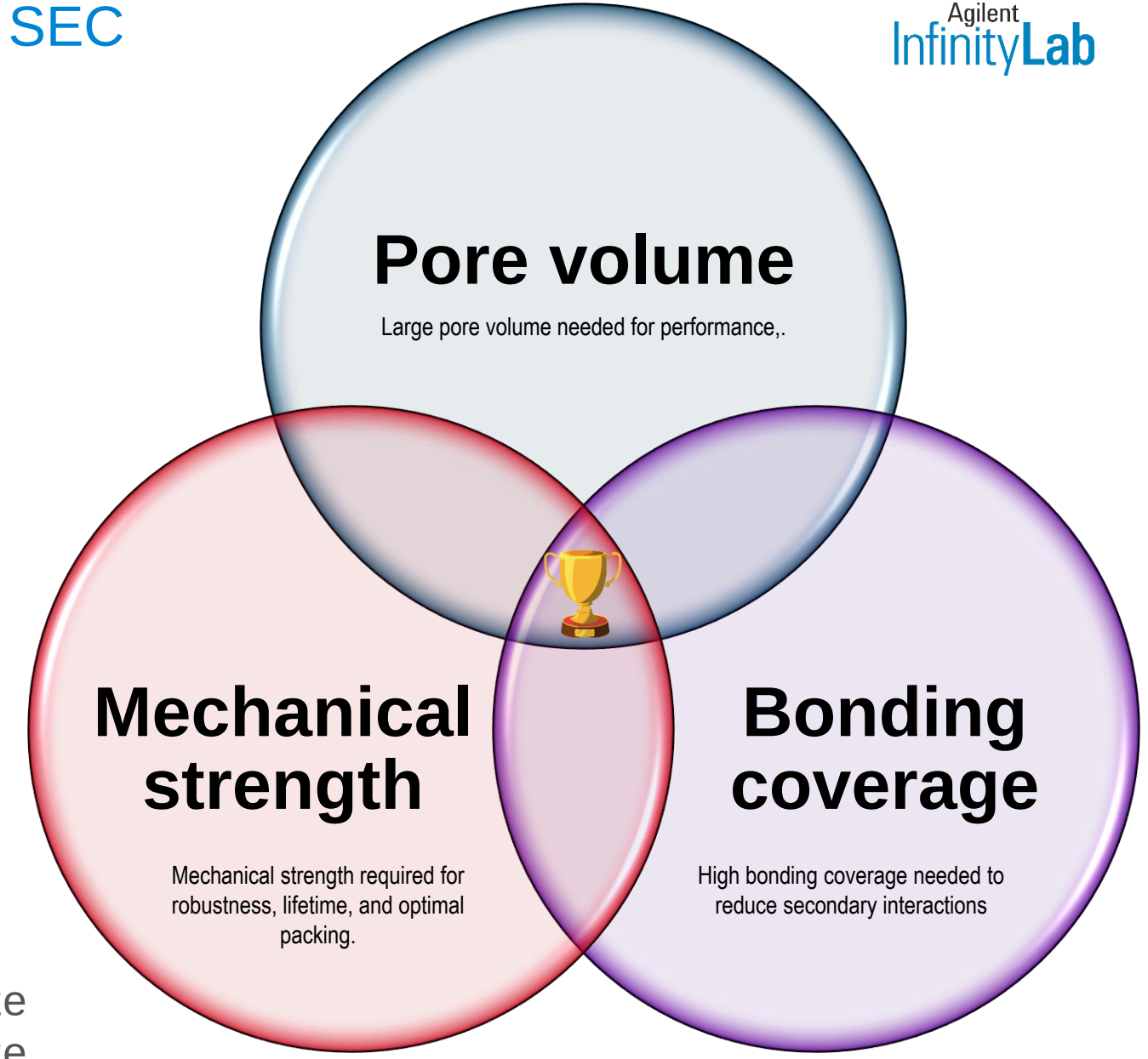
- Hydrophilic surface and inertness
- Stable chemistry (no retention time shift and peak shape change over injections)

Robustness:

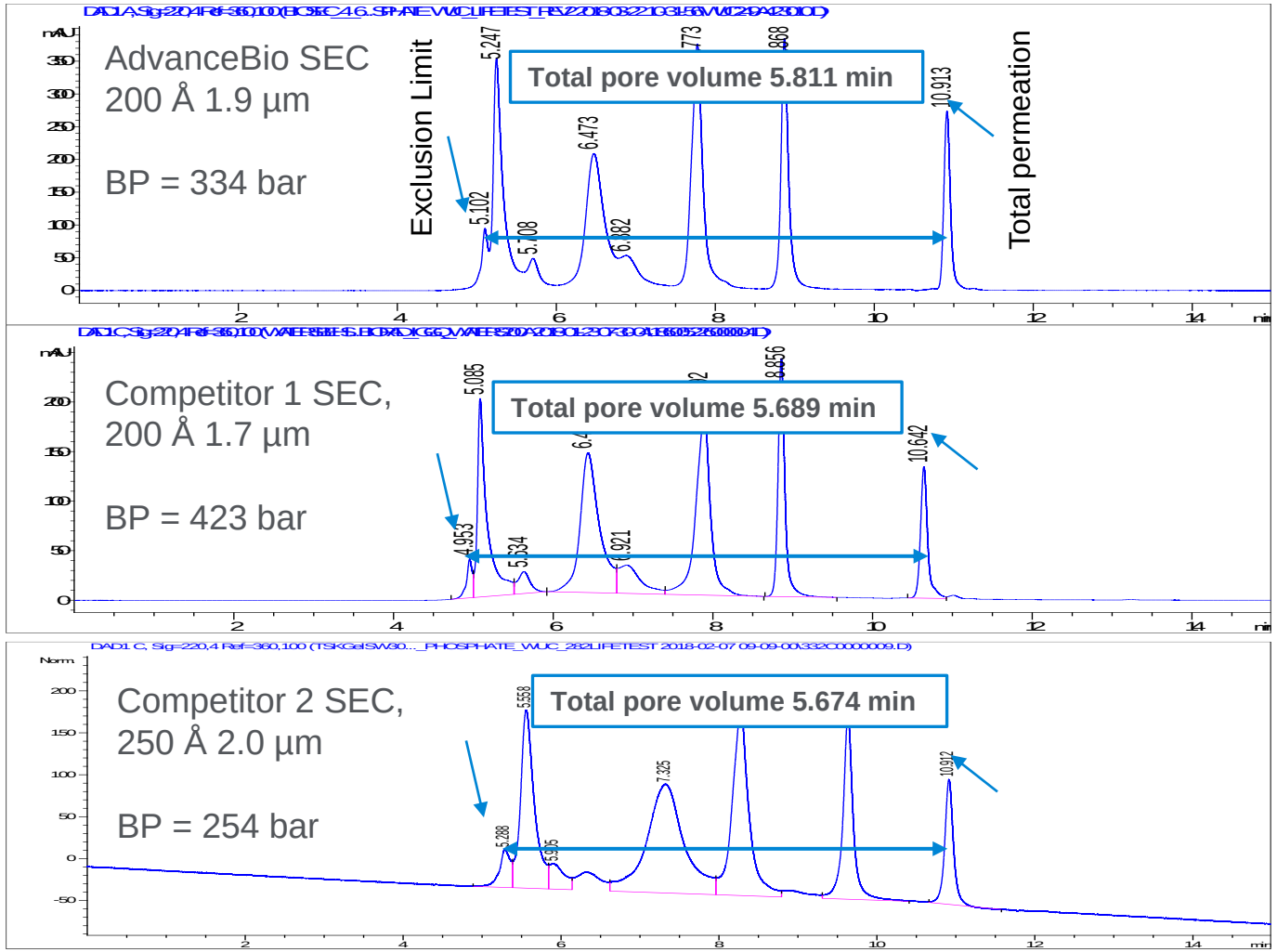
- Strong particles
- Stable packed bed

Available in:

2.7 µm particles 130Å & 300Å pore size
1.9 µm particles 120Å & 200Å pore size



Column Pore Volume Analysis
(4.6 x 300 mm, 0.35 ml/min, pH 7.0 Phosphate Buffer, BioRad sample)



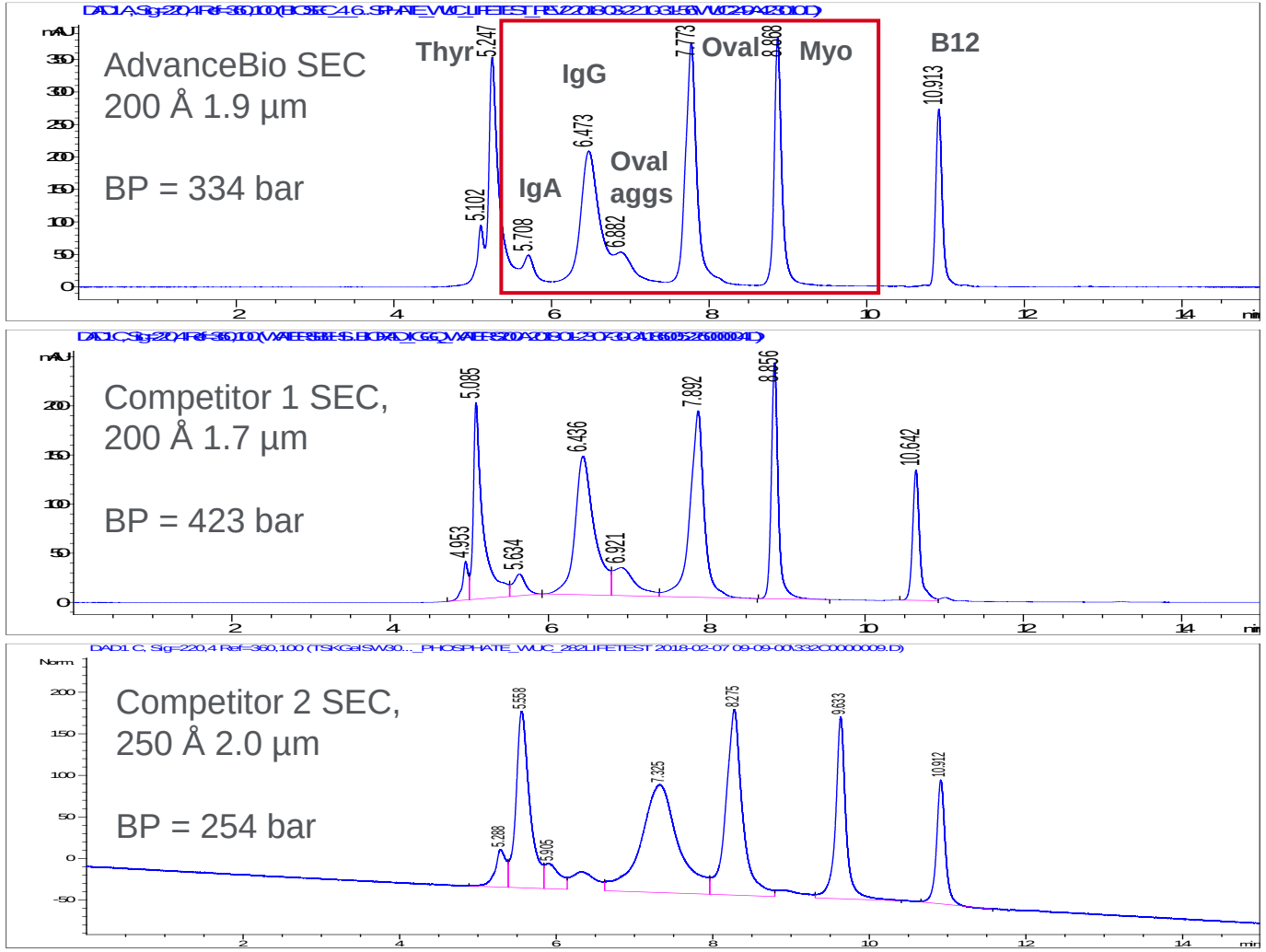
BioRad # 151-1901	Molecular Weight*
Thyroglobulin	670,000
γ-globulin	158,000
Ovalbumin	44,000
Myoglobin	17,000
Vitamin B12	1,350

Column	Exclusion limit (min)	Total Permeation (min)	Total Pore Volume (min)
AdvanceBio SEC 200 Å 1.9 µm	5.102	10.913	5.811
Competitor 1 SEC, 200 Å 1.7 µm	4.953	10.642	5.689
Competitor 2 SEC, 250 Å 2.0 µm	5.238	10.912	5.674

AdvanceBio SEC 200 Å 1.9 µm columns provide the widest separation window.

Optimized Performance in the Target Range

(4.6 x 300 mm, 0.35 ml/min, pH 7.0 Phosphate Buffer, BioRad sample)



BioRad # 151-1901	Molecular Weight*
Thyroglobulin	670,000
γ-globulin (IgG)	158,000
Ovalbumin	44,000
Myoglobin	17,000
Vitamin B12	1,350

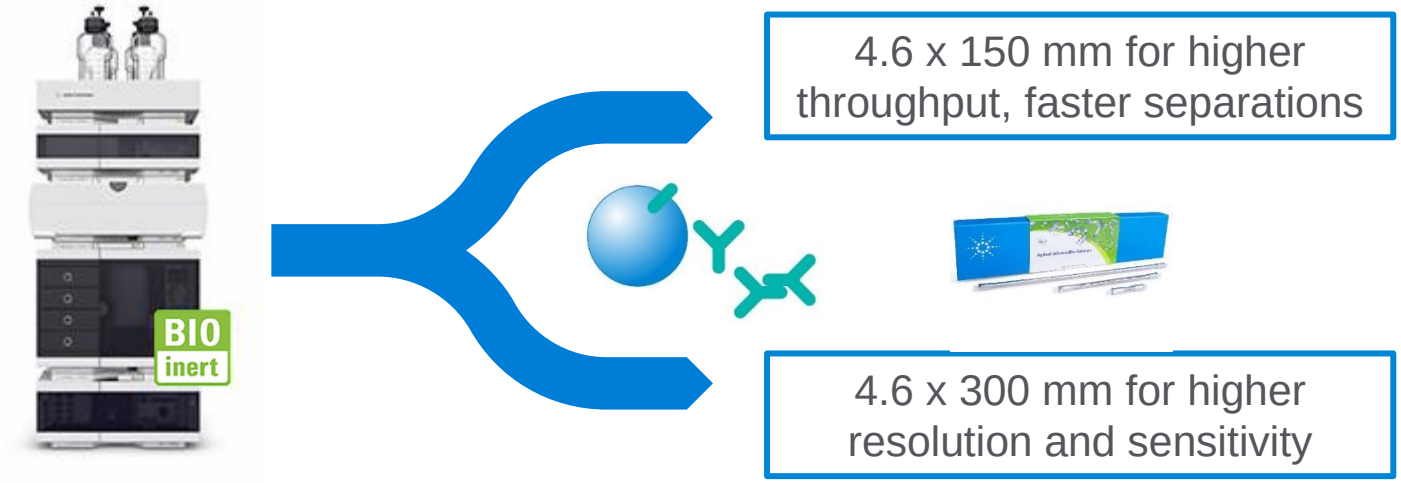
Column	N (B12)	Rs IgA/IgG	Rs IgG/Oval aggs	Rs Oval/Myo
AdvanceBio SEC 200 Å 1.9 µm	88839	2.56	1.00	5.47
Competitor 1 SEC, 200 Å 1.7 µm	79659	2.15	1.09	4.40
Competitor 2 SEC, 250 Å 2.0 µm	55558	1.73	NA	4.89

AdvanceBio SEC 200 Å 1.9 µm columns provide the best efficiency and resolution.

Product Description

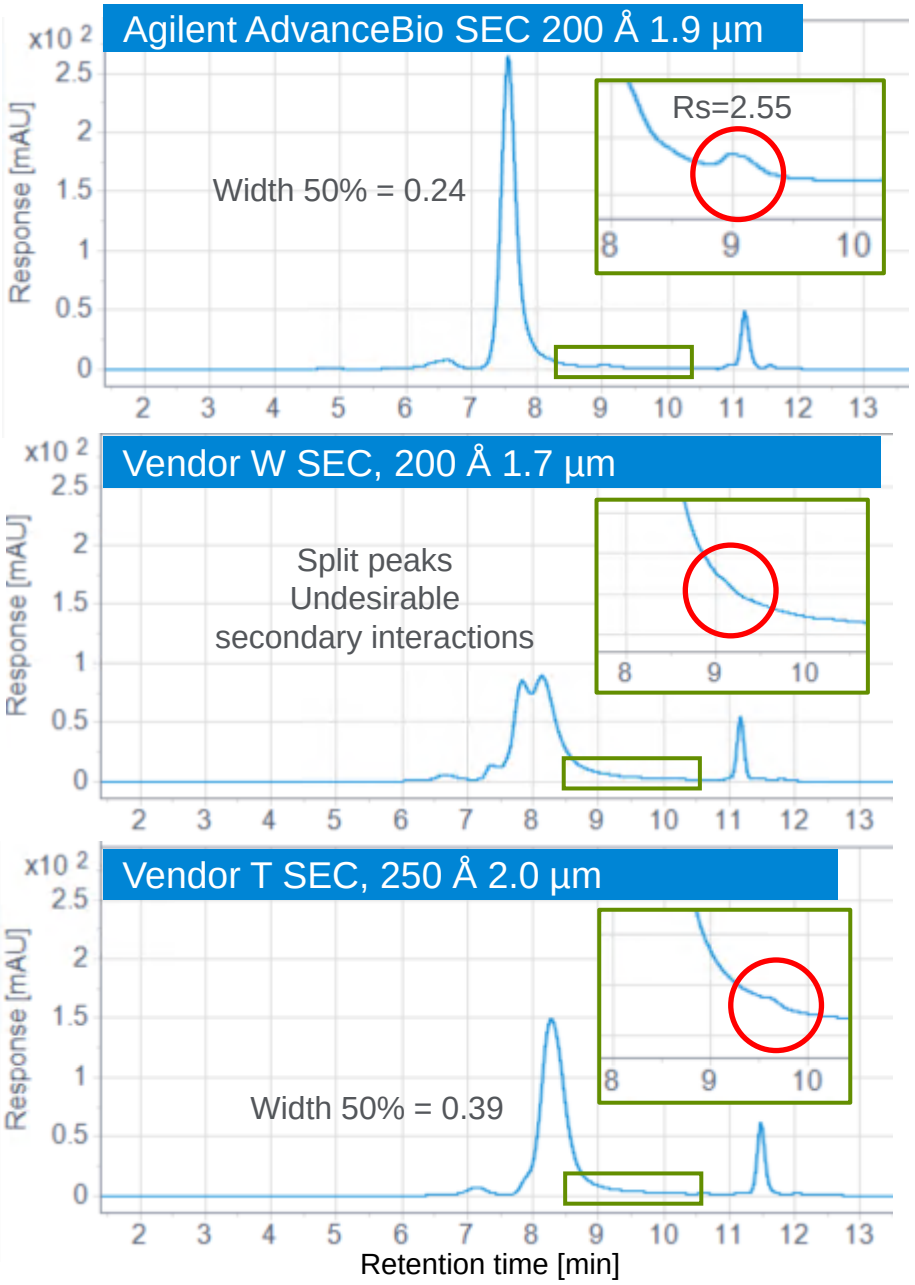
Product Name	Particle	Column Hardware	Column Dimensions	Part Number
AdvanceBio SEC 200Å 1.9 µm	1.9 µm 200Å (coated silica)	RRHD	4.6 x 300 mm 4.6 x 150 mm 4.6 x 30 mm guard	PL1580-5201 PL1580-3201 PL1580-1201
AdvanceBio SEC 120Å 1.9 µm	1.9 µm 120Å (coated silica)	RRHD	4.6 x 300 mm 4.6 x 150 mm 4.6 x 30 mm guard	PL1580-5250 PL1580-3250 PL1580-1250
AdvanceBio SEC 120Å 1.9 um MVK			3 lots of 4.6x300mm 3 lots of 4.6x150mm	PL1580-5250K PL1580-3250K

AdvanceBio SEC 1.9 µm 200Å, 120Å is designed for aggregates (monomer / dimer) and analysis of low MW mAb fragments



Resolving Range

200Å MW: 2,000 - 700,000 Da
120Å MW: 1,000 - 80,000 Da



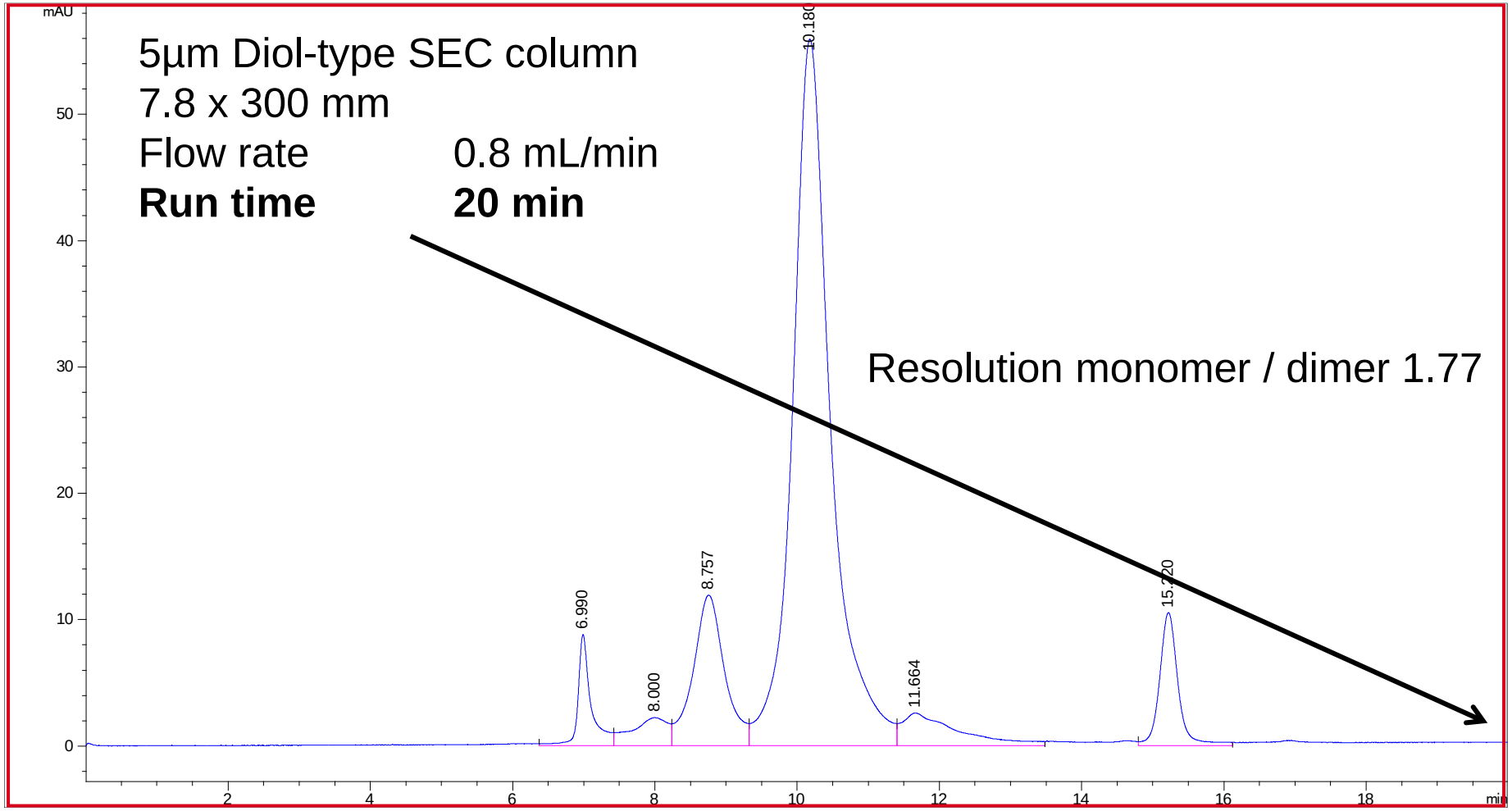
LC Conditions	1260 Infinity II Bioinert LC System
Column dimension	4.6 x 300 mm
Mobile phase	50 mM sodium phosphate, 200 mM NaCl, pH 7.0
Temperature	25 °C
Sample	SigmaMAb ADC Mimic
Flow rate	0.35 mL/min
UV detection	220 nm

	Monomer peak	Resolution of fragment peak
AdvanceBio SEC 200 Å 1.9 µm	Single peak (sharper)	Well resolved
Vendor W SEC 200 Å 1.7 µm	Split peaks	Not resolved
Vendor T SEC 250 Å 2.0 µm	Single peak	Partially resolved

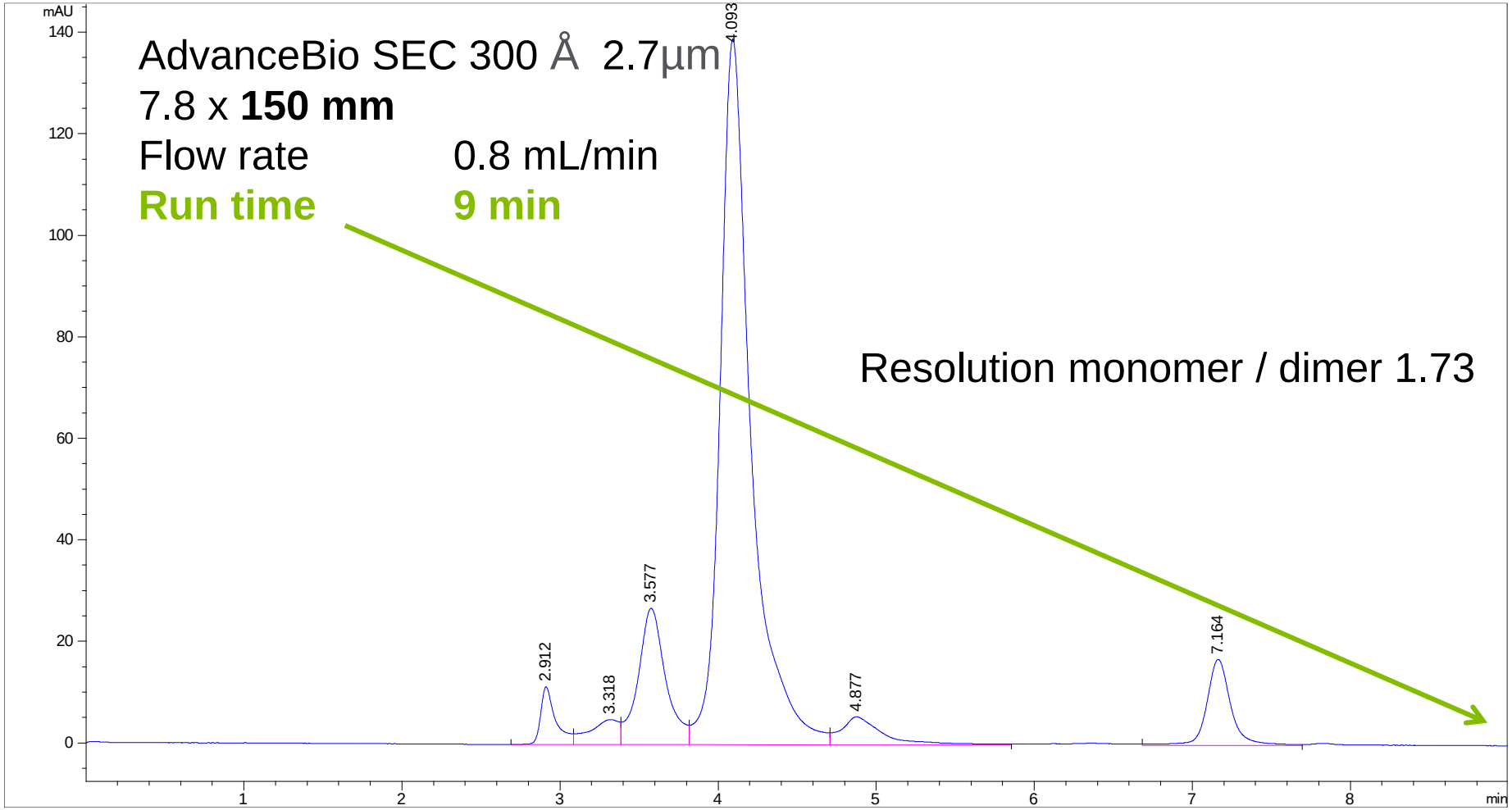
ADC aggregate and fragment separation

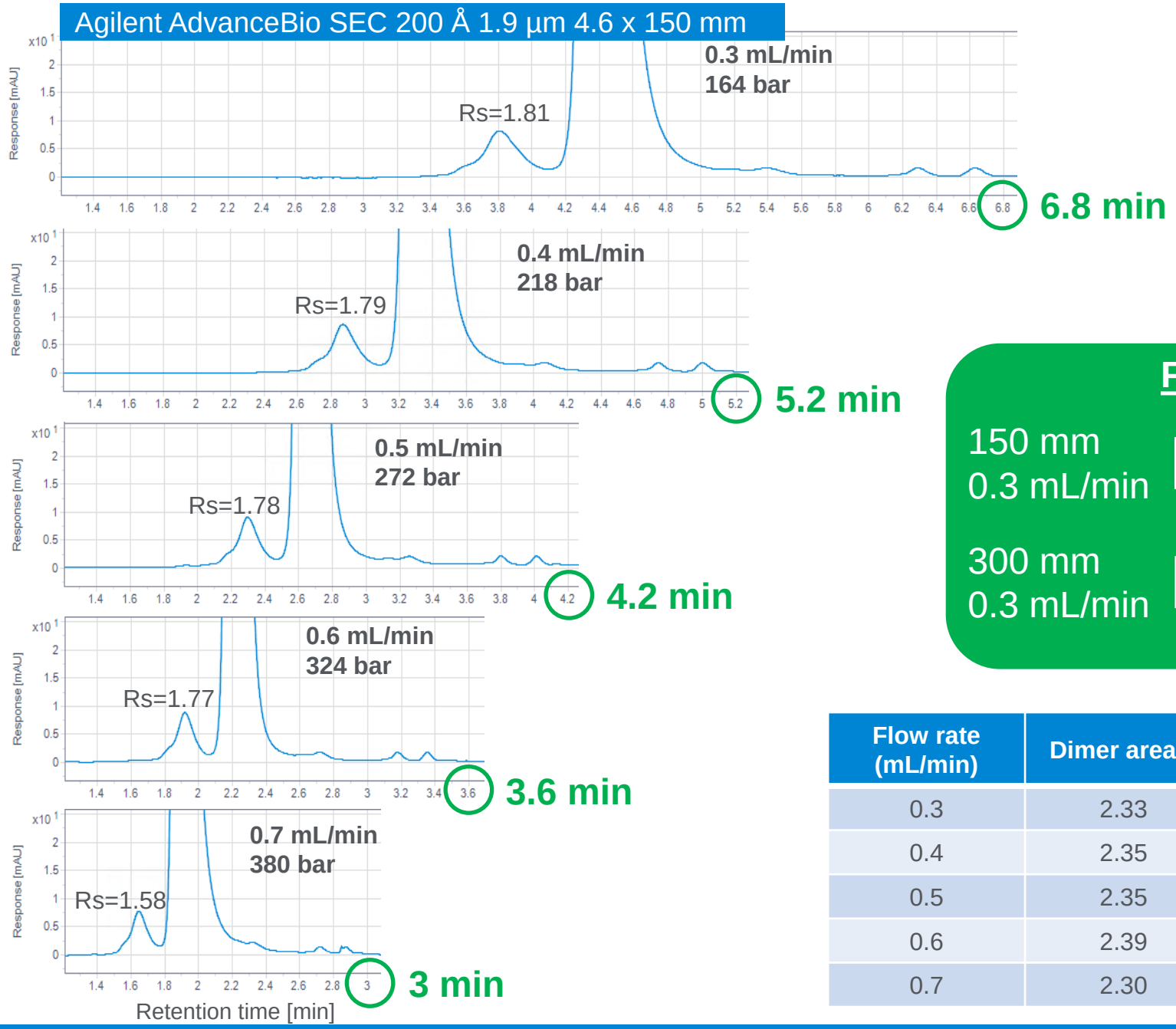
Greatly reduced undesirable secondary interactions resulting in a single, sharp monomer peak and well-resolved aggregate and fragment peaks.

Fast SEC
Initial Conditions



Same flow rate, 15cm column
Same resolution, much shorter run time





LC Conditions	1260 Infinity II Bioinert LC System
Column Dimensions	4.6 x 150 mm
Mobile phase	50 mM sodium phosphate, 200 mM NaCl, pH 7.0
Temperature	25 °C
Sample	SigmaMAb
UV detection	220 nm

Fast Analysis

150 mm, 0.3 mL/min → 2.3 times faster → 150 mm, 0.7 mL/min

300 mm, 0.3 mL/min → 4.6 times faster → 150 mm, 0.7 mL/min

Flow rate (mL/min)	Dimer area%	Samples per hour	Samples per day (24 h)
0.3	2.33	8-9	211
0.4	2.35	11-12	276
0.5	2.35	14	342
0.6	2.39	16-17	400
0.7	2.30	20	480

Buffers and SEC: criteria for optimal mobile phase

- Mobile phase should contain enough buffer/salt (to overcome ionic interactions).
- Mobile phase should not contain too much buffer/salt (to prevent hydrophobic interactions).
- Mobile phase should not alter the analyte (cause degradation / aggregation etc.).
- Mobile phase should be made up fresh and used promptly (bacterial growth is rapid in dilute buffer stored at room temperature).
- Buffer shelf life < 7 days unless refrigerated.
- Mobile phase should be filtered before use. Particulates may be present in water (less likely) or in buffer salts (more likely).

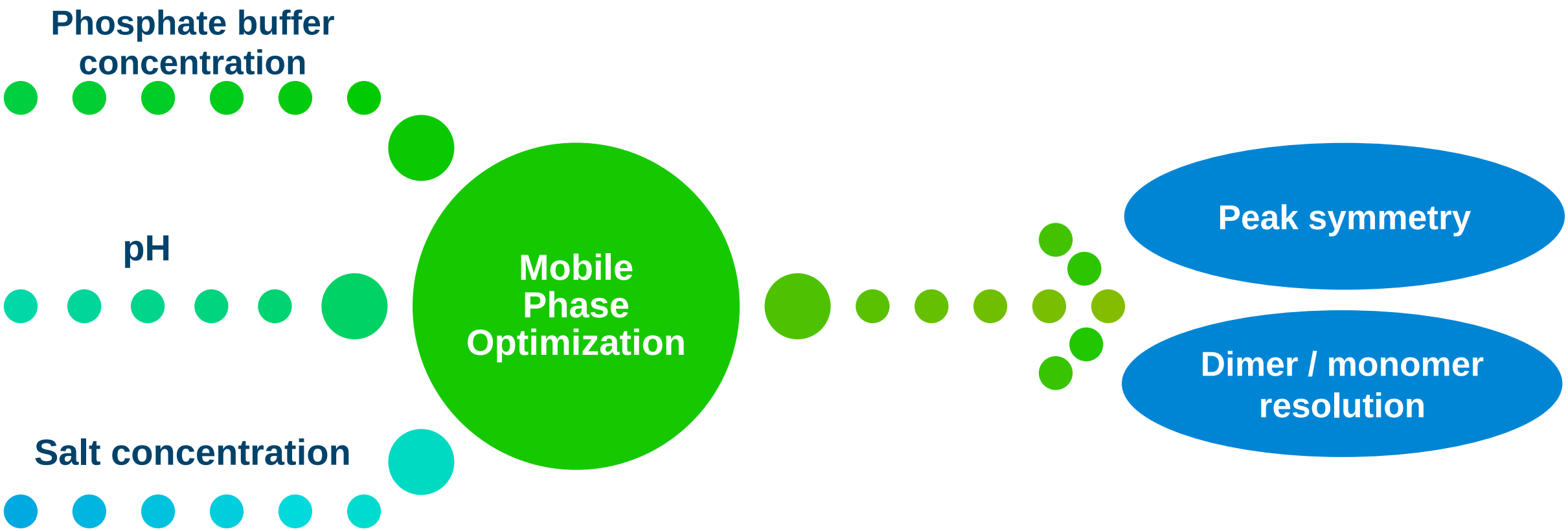
The optimal eluent for the separation should be determined by the characteristics of the column stationary phase and the proteins/polymers to be analyzed so that non specific interactions are minimized

Buffer Preparation
Good Practices

- Use HPLC grade water or Milli Q DI water
- Use HPLC grade reagents including salts and base and acid modifiers
- Use HPLC grade Organic mobile phase modifiers
- Always rinse pH electrode thoroughly when measuring/adjusting pH of mobile phase
- Prepare fresh buffers to avoid contaminants from the growth of bacteria or algae
- Do not top off your mobile phase bottle. Replace with a clean bottle
- Consider using amber solvent bottles to decrease light exposure
- Filter your mobile phase buffer with 0.2um or 0.45 µm filter before use
- Solvent filters installed at the end of solvent lines should be replaced periodically

Solvent Filtr

Buffer Considerations



Recommended Starting Conditions

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For AdvanceBio SEC Columns we recommend starting with 150mM Sodium phosphate, pH 7.0

Peptides, polypeptides, proteins, mAbs
MW >0.1-1,250 kDa

AdvanceBio SEC (2.7 µm)	
Pore Size	**MW Range (kDa)**
130Å	0.1-100
300Å	5-1,250

Recommended Initial Separation Conditions

Column: AdvanceBio SEC or Agilent Bio SEC-5

Mobile phase: 150 mM phosphate buffer, pH 7.0*

Gradient: Isocratic in 10-30 min range

Temperature: Recommended: 10-30 °C, Maximum: 80 °C

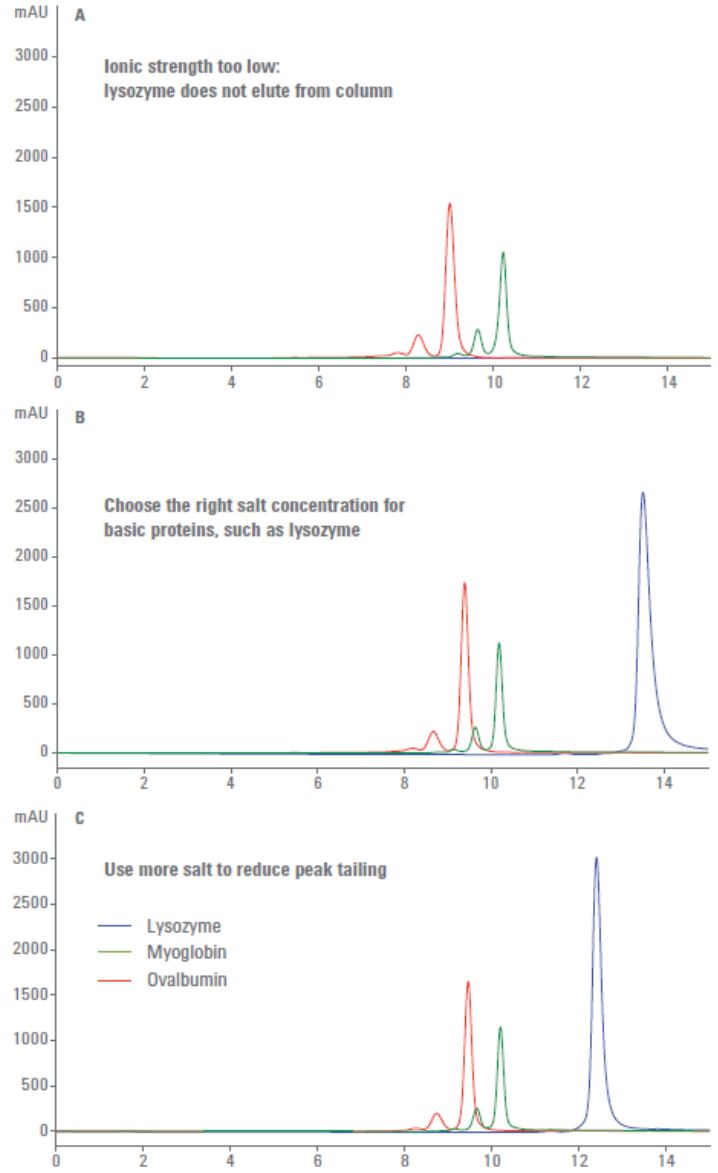
Flow rate: 0.1-0.4 mL/min for 4.6 mm id columns
0.1-1.25 mL/min for 7.8 mm id columns

Sample size: ≤ 5% of total column volume

*Other aqueous buffers with high and low salt can be used

Remember buffer concentration and ionic strength can impact retention time, peak shape, and resolution
Adjustments should be made depending on your sample requirements

Buffer Considerations



50 mM NaCl in 20 mM buffer

400 mM NaCl in 20 mM buffer

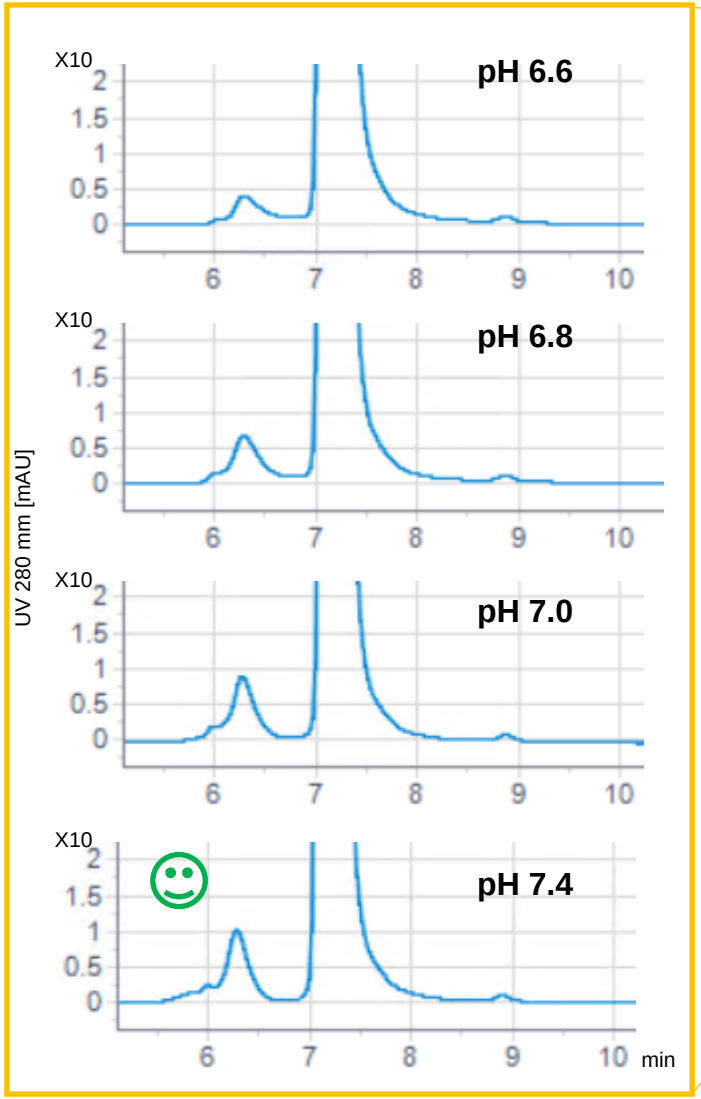
Column:	**Agilent Bio SEC-3 300Å** **4.6 mm x 300 mm, 3 µm** **(p/n 5190-2513)**
Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC System
Flow rate:	0.35 mL/min
Detector:	UV, 220 nm A: Eluent 20 mM phosphate buffer, pH 7 + 50 mM NaCl B: Eluent 20 mM phosphate buffer, pH 7 + 100 mM NaCl C: Eluent 20 mM phosphate buffer, pH 7 + 400 mM NaCl
Injection:	5 µL
Sample:	Protein (1 mg/mL 20 mM phosphate buffer, pH 7)

Pub No. 5991-3651EN

Mobile Phase Optimization - NISTmAb

Agilent InfinityLab

150 mM sodium phosphate



Buffer (mM)	NaCl (mM)	pH	Asymmetry (As)	Rs (dimer/monomer)
150	0	6.6	1.49	2.33
150	0	6.8	1.43	2.35
150	0	7	1.42	2.67
150	0	7.4	1.41	2.78
200	0	7.4	1.45	2.60
250	0	7.4	1.42	2.57
300	0	7.4	1.40	2.45
350	0	7.4	1.38	2.33

Sodium phosphate without NaCl

Lower pH, more tailing, less resolution

Higher buffer concentration, less tailing but worse resolution

Mobile Phase Optimization - NISTmAb

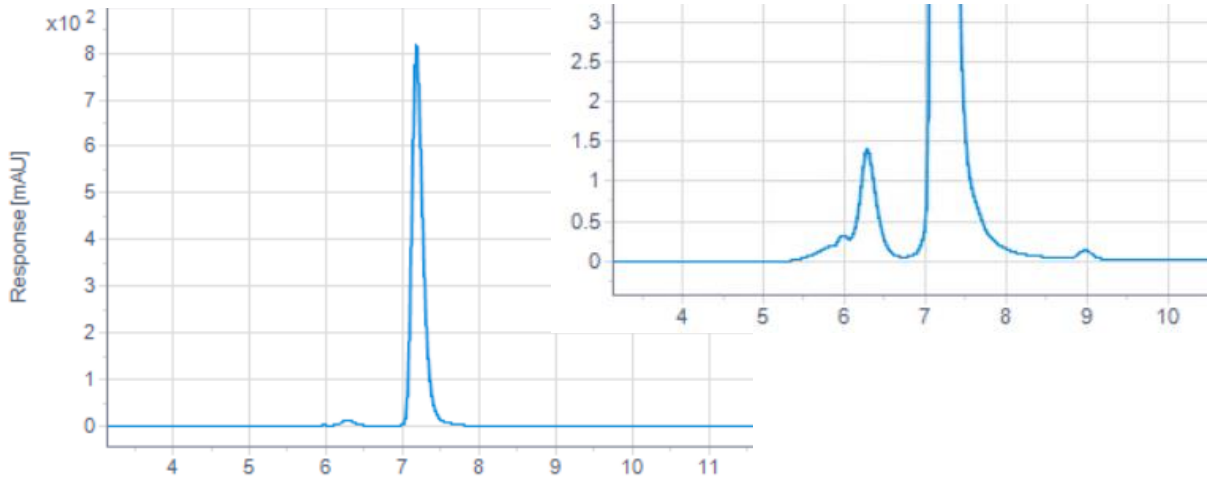
Agilent InfinityLab

Sodium phosphate concentration and pH screening

Buffer (mM)	NaCl (mM)	pH	As	Rs (dimer/monomer)
25	250	6.6	1.36	2.73
25	250	6.8	1.36	2.86
25	250	7	1.35	2.83
25	250	7.4	1.37	2.86

Buffer (mM)	NaCl (mM)	pH	As	Rs (dimer/monomer)
100	250	6.6	1.36	2.87
100	250	6.8	1.36	2.89
100	250	7	1.35	2.83
100	250	7.4	1.37	2.80

Buffer (mM)	NaCl (mM)	pH	As	Rs (dimer/monomer)
50	250	6.6	1.35	2.87
50	250	6.8	1.33	2.86
50	250	7	1.36	2.85
50	250	7.4	1.36	2.84



For some samples certain concentration of NaCl is needed in the mobile phase with optimized pH for a balance of good peak shape and resolution.

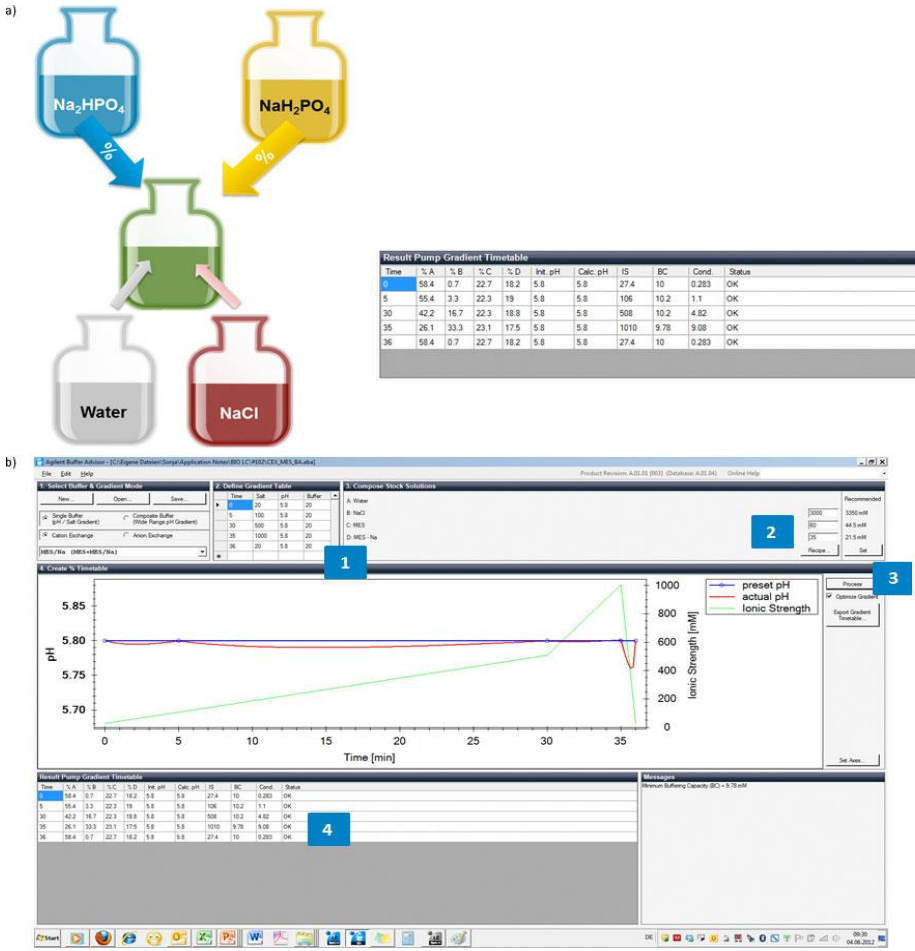
SEC Method Robustness
Agilent Buffer Advisor

Ease of use

- Automates preparation of mobile phases from stock solutions
- Eliminates the need to prepare multiple mobile phases for a method development/robustness study
- Provides recipes for buffer preparation

Increase of productivity

- Simplifies pH scouting studies
- Simplifies buffer concentration studies
- Simplifies salt concentration/mobile additive studies



The Advantage of Buffer Advisor

---------- vs. ----------

Manual method
One bottle for each mobile phase

Buffer Advisor method
Four bottles only

Buffer Advisor helps you achieve more in less time

Common Detectors

Choice of Detector

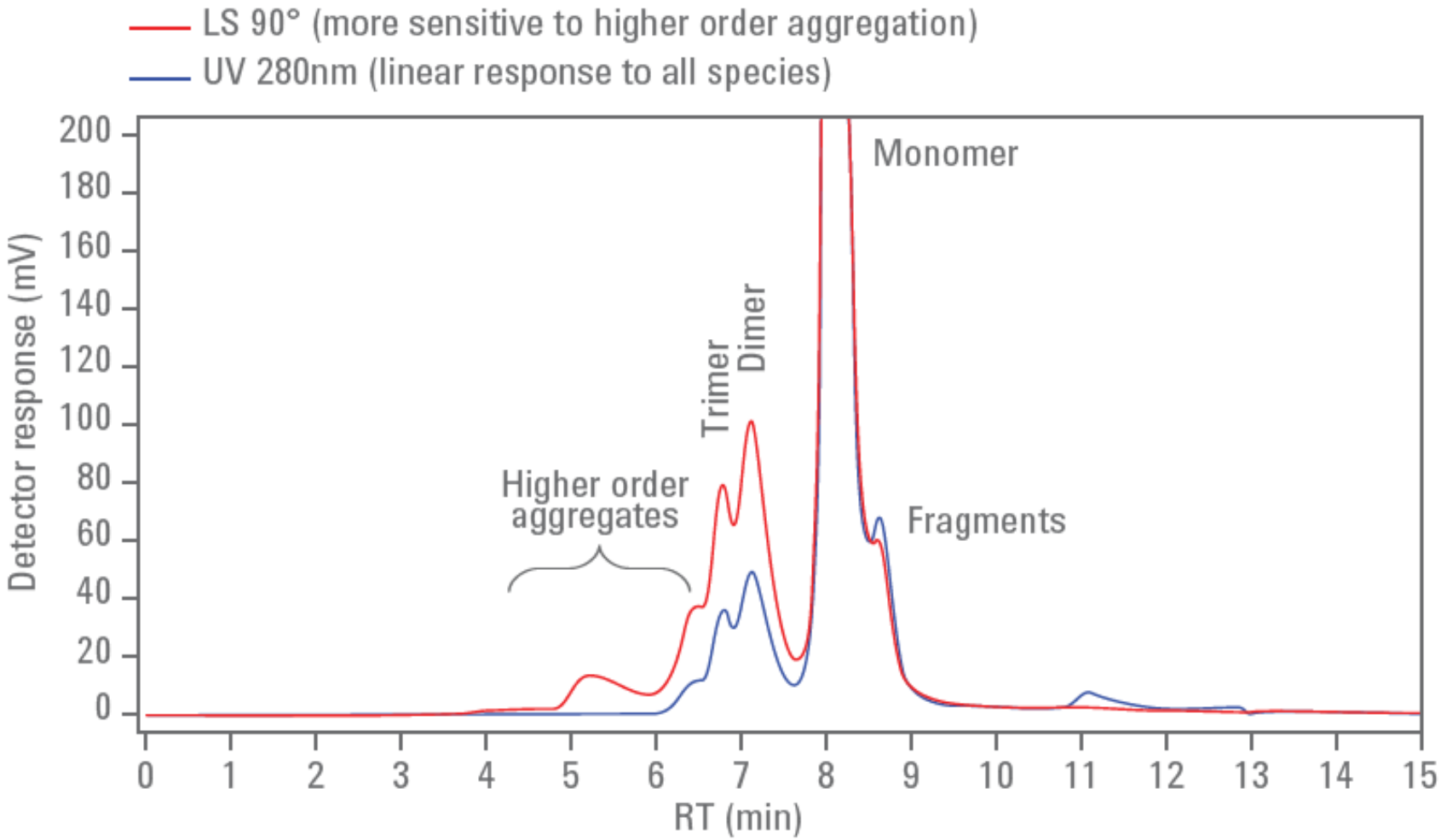
UV and Diode Array Detectors

Refractive Index Detector

Advanced Detection Techniques

- Light scattering detector
- Mass Spectometry

Detection for Size Exclusion Chromatography
UV and Light scattering



Light-scattering detection achieves much greater sensitivity to the presence of aggregates and is an important technique being increasingly used to study mAb aggregation

Agilent 1260 Infinity Bio-SEC System

Detection for Size Exclusion Chromatography

MDS more sensitive to light scattering of higher order aggregates

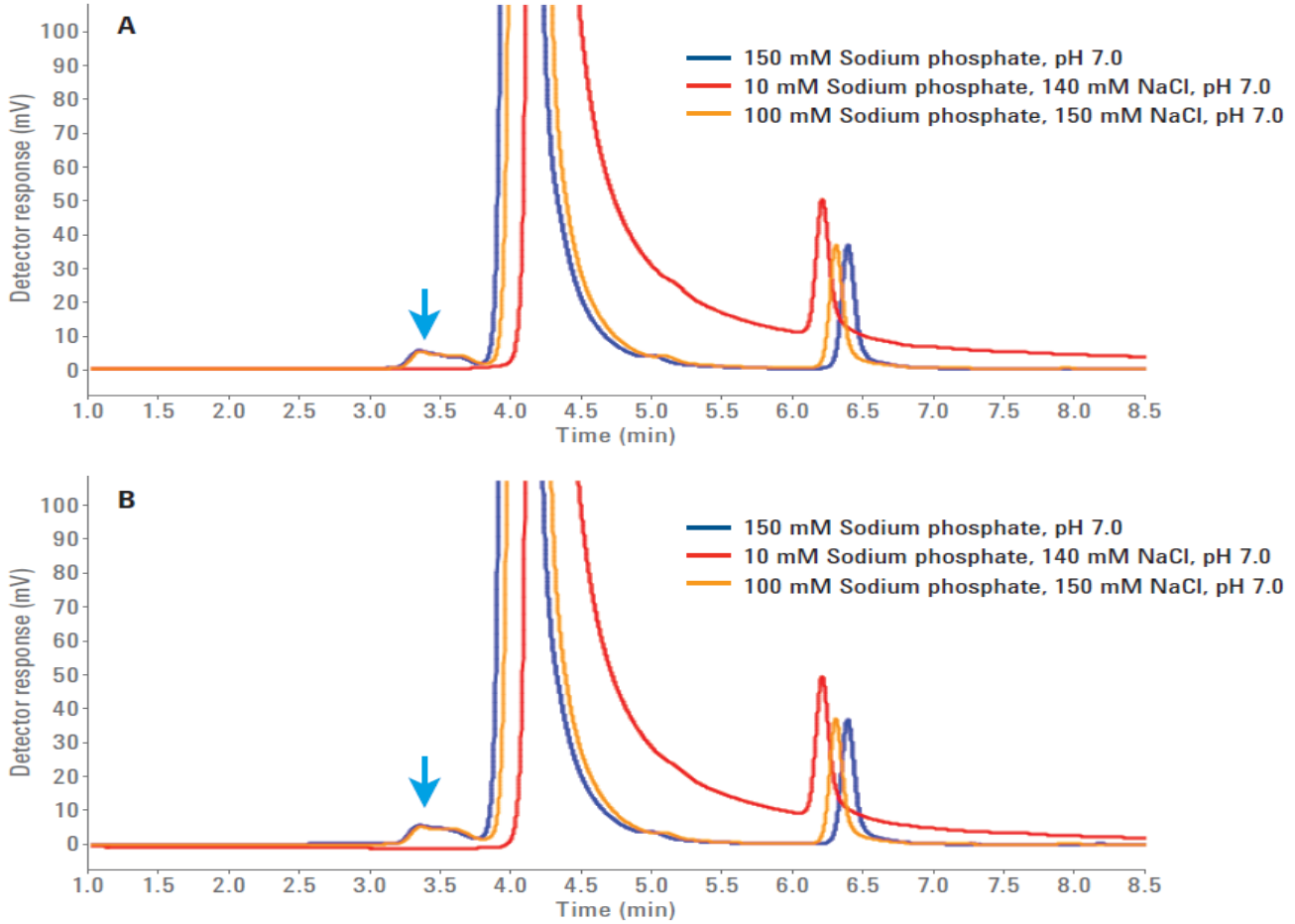


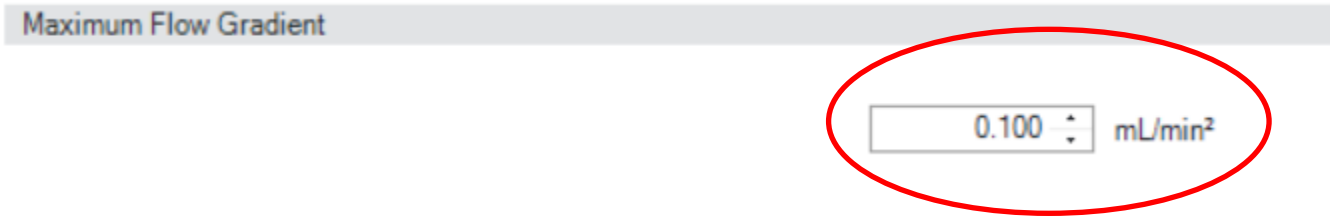
Figure 4. Baseline zoom of UV 220 nm signals of rituximab innovator (A) and rituximab biosimilar (B) run with different buffer salt concentrations at the optimized pH 7.0 (Experiments 3, 6, and 11 in Table 1).

Considerations for LC Instrument
Best Practices to keep in mind

Agilent InfinityLab

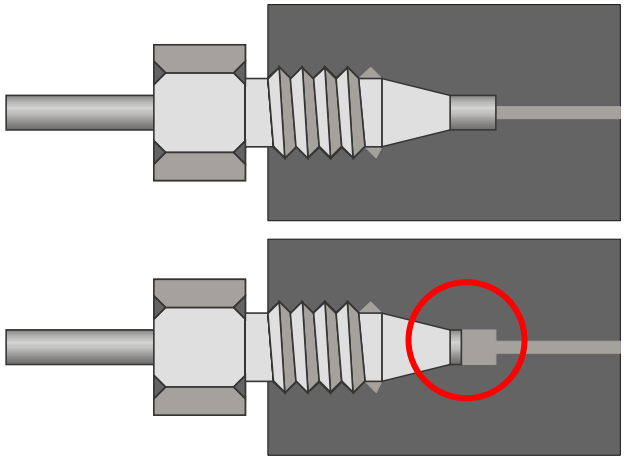
Low dispersion LC
Minimized tubing ID and length to reduce extra-column volume and band broadening (shortest possible 0.12 mm ID red tubing).

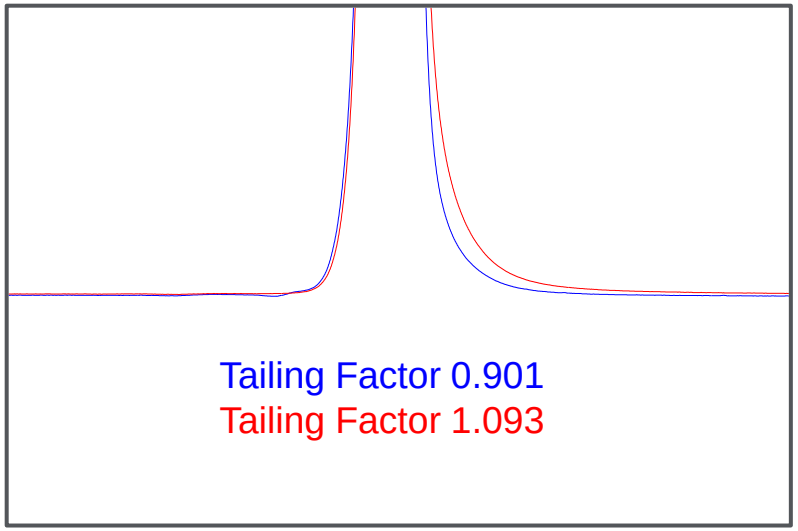
Start at a low flow rate such as 0.1 mL/min and gradually increase the flow with no more than 0.1 mL/min increments until you reach the intended operating flow rate.



Best Practices to keep in mind

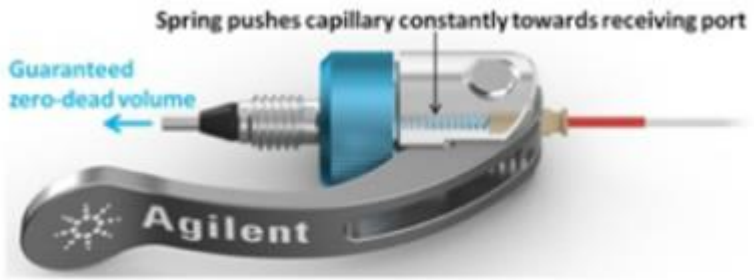
AVOID BAD CONNECTIONS !
Ensure column connections do not leave dead spots:





Use High Data Collection Rates
Data collection rates of 10 – 20 Hz could result in 4 – 5% reduction in column efficiency compared to 40 or 80 Hz.

Common LC consumables
Quick Connect and Quick Turn Fittings



Correct connection every time

- Spring loaded design
- Easy! **No tools needed**
- Works for all column types
- Reusable
- Consistent ZDV connection

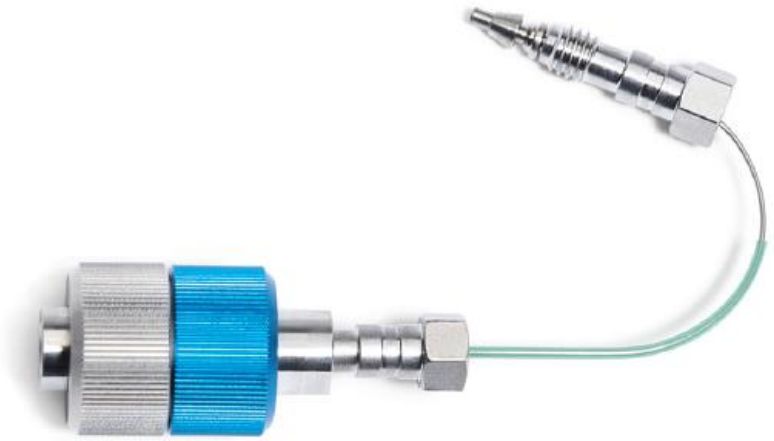
Quick Connect Fitting
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

Quick Turn Fitting
- Finger tight up to 600 bar
- Up to 1300 bar with a wrench
- Compact design

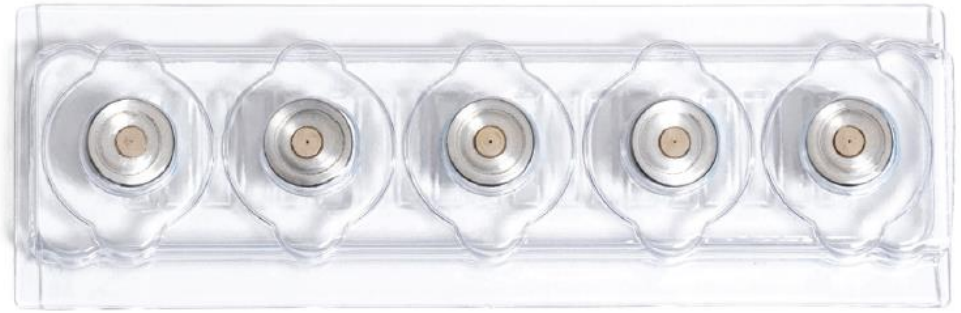
Common LC consumables

Inline Filters

Agilent InfinityLab Quick Change inline filter

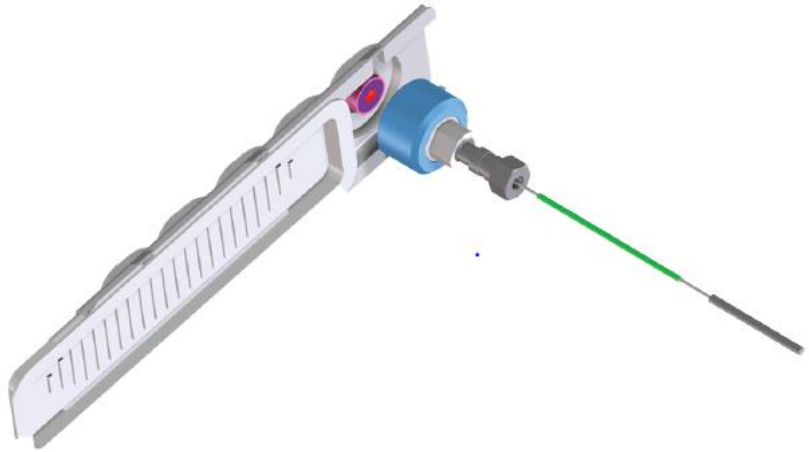


Touchless Packaging



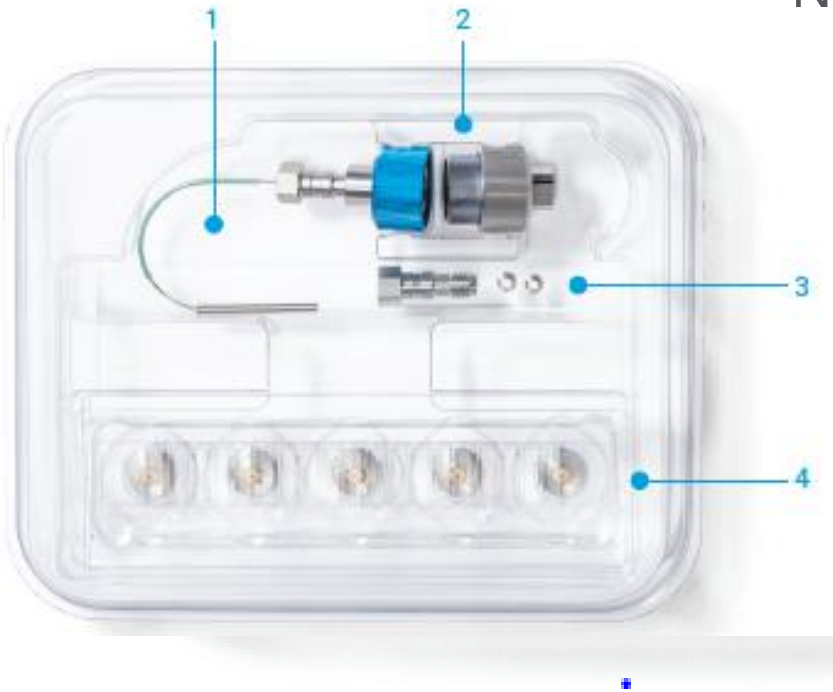
Dimensions and porosities of Filter Discs

Touchless insertion of filer disc to housing



InfinityLab Quick Change inline filter assembly

In-line filters can help extend the life of your column
Not intended to be a replacement for good sample cleanup



1. Capillary, SST, 90 mm length
2. Filter housing (two parts)
3. Loose fitting for non-swaged end of capillary
4. Filter discs in touchless packaging, 5/pk

For 2.1 mm ID – comes with 0.2um filter discs, 0.12 x 90mm SS capillary

For 4.6mm ID – comes with 0.5um filter discs, 0.17 x 90mm SS capillary

Parts available to purchase separately

InfinityLab Quick Change Inline Filters (agilent.com)
Pub No 5994-3028EN

Common LC consumables
Solvent Inlet filters

Use a Solvent inlet filter

- Glass solvent inlet filter (20 µm), 5041-2168
- Stainless steel solvent inlet filter, 01018-60028
- Stainless inlet filter recommended for LCMS

not designed to take the place of good mobile phase hygiene

AdvanceBio SEC User Guides

AdvanceBio SEC 1.9 µm		
Pressure limit	620 bar (9000 psi)	
Recommended flow rate	0.1 – 0.5 mL/min	
Flow rate limit	4.6 x 150 mm 4.6 x 300 mm	0.7 mL/min 0.5 mL/min
Shipping solvent	pH 6.7 100mM sodium phosphate buffer with 0.02% NaN_3	
Long term storage	Shipping solvent or 30% ACN in water	
pH stability	2 to 8	
Salt concentration	<= 0.5 M	
Operating temperature	20 – 40 °C (recommended) 80 °C (maximum)	

Agilent WebLinks:

[Bio LC Column User Guides | Agilent]

Working at extremes of the operating parameters may reduce column lifetime.

Troubleshooting your SEC method

Problem	Source	Solution
Peaks that appear when they should not, based on Molecular weight or peak tailing	Ionic Interactions or basic proteins	Increase the ionic strength – salt concentration at 50-100mM intervals, add to phosphate buffer
Poor peak shapes	Non specific adsorption	Increase salt concentration or try Agilent BioInert System
Poor resolution of analytes	Insufficient pore size for molecule size	Consider an alternative Advance BioSEC or BioSEC column choice
Lower than expected recovery or a broadening of the peaks	Hydrophobic analytes	Add a small amount (10-20%) organic modifier, ACN or MeOH to mobile phase
Increased back pressure	Inline filters, guard, or columns	Change frit for inline filter Replace guard column Remove and test guard column OR the individual columns used

Agilent WebLinks:

LC Troubleshooting Guide (agilent.com

LC Columns and Supplies Resources

Product catalog, ‘how to’ guides, and other resources

- BioHPLC column catalog: 5994-0974en-agilent.pdf
- SEC for biomolecules “How to” guide: 5991-3651EN_LR.pdf (agilent.com)
- Bio LC column user guides: Bio LC Column User Guides | Agilent
- InfinityLab supplies catalog: InfinityLab LC Supplies (agilent.com)
- LC troubleshooting poster: LC Troubleshooting Guide (agilent.com)
- App finder: Application Finder | Agilent
- Agilent University: http://www.agilent.com/crosslab/university
- YouTube: Agilent Channel
- Your local product specialists
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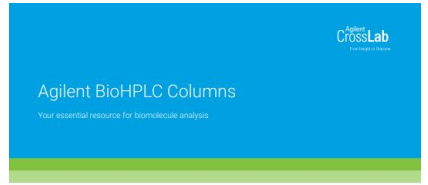

Contact Agilent Chemistries and Supplies Technical Support

Available in the USA and Canada 8-5pm all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for Prozyme products

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Thank you for attending

Any questions?

Agilent InfinityLab