END UNCERTAINTY

AdvanceBio SEC

Optimizing Quantification of mAb and ADC Aggregates: Speed versus Resolution

Optimizing Quantification of mAb and ADC Aggregates: Speed versus Resolution

What is size exclusion chromatography?

Why is resolution important?

How much resolution is needed?

How to speed up the analysis?

What is the benefit?

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Aggregation

Aggregates can be tangled clusters of denatured mAbs

- Irreversibly formed during production
- Expression, downstream processing
- Storage as drug substance or drug product

Aggregates can be ordered structures of native mAbs

- May be reversible or irreversibly
- Interaction may be hydophobic and/or electrostatic
- Dimer produced from association of two monomers

Aggregation is impacted by

- Biochemical and biophysical properties of the mAb
- Physicochemical environment

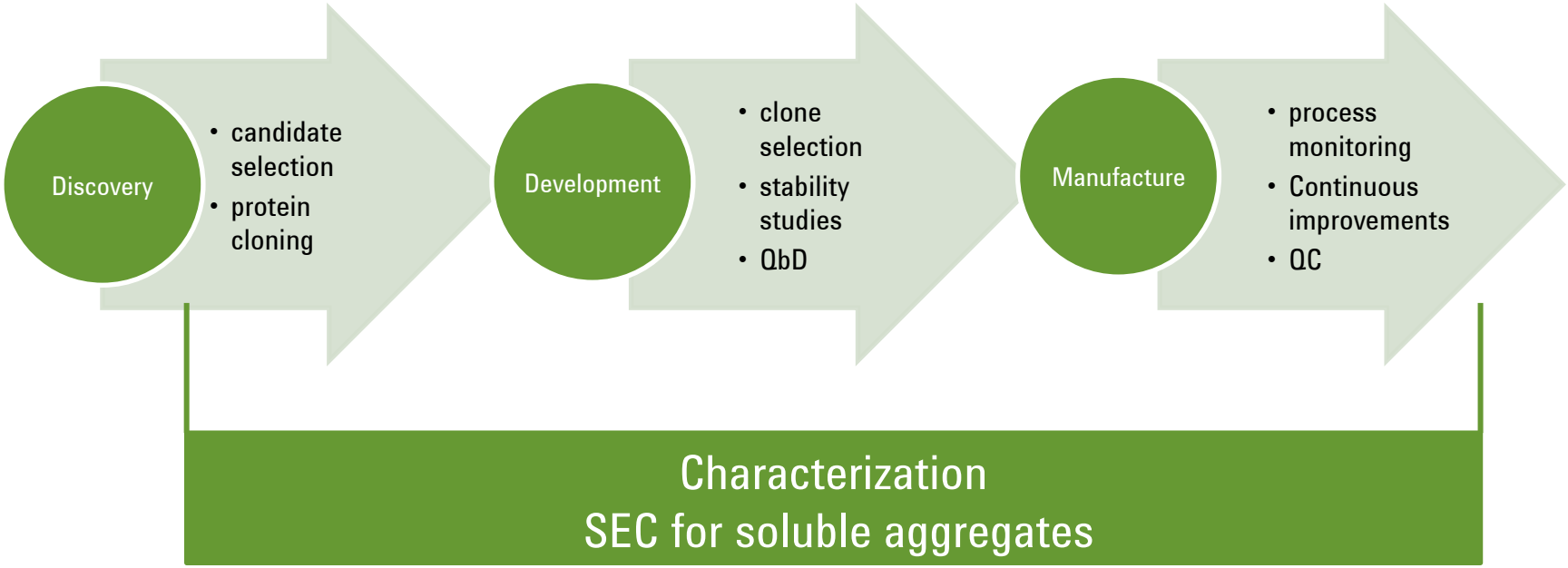
Aggregation impacts

- Process yield and economics
- Product immunogenicity

When does aggregation matter?

Aggregation is critical quality attribute

- size exclusion chromatography is required technique to quantify aggregation



100s of samples for analysis

Clone selection/development

How much is expressed	• Bio-Monolith Protein A/Protein G
How much is aggregated	• **AdvanceBio SEC**
What is the glycan form	• AdvanceBio Glycan Mapping

Formulation

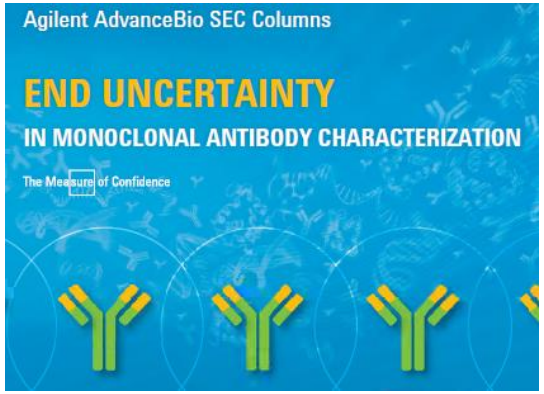
Drug product (DP) requires formulations that meet the target product profile (TPP)
Require analytical methods needed to determine stability and set shelf life
Requires high throughput assays to monitor multiple formulations as a function of time

Small number of samples

Candidate Characterization		Quality control
What is the candidate	• Multiple characterization techniques	Batches of drug product (DP) must meet the defined impurity profile
What are the critical quality attributes	• **Aggregation**	Require analytical methods with sensitivity and resolution to ensure batch complies with specification
Is it suitable to move forward	• Is the level of aggregation likely to cause late stage failure	Require robust analytical methods that deliver accurate data for every sample

What does that mean for SEC

- Columns must deliver accurate, precise quantitation for mAbs and next generation biologics
- High resolution for more accurate quantitation
- Faster analysis speeds for delivery to deadlines
- No change to sample integrity
- Sensitivity for quantitation at low levels - 1 to 5%
- Methods must be easily transferred to other locations

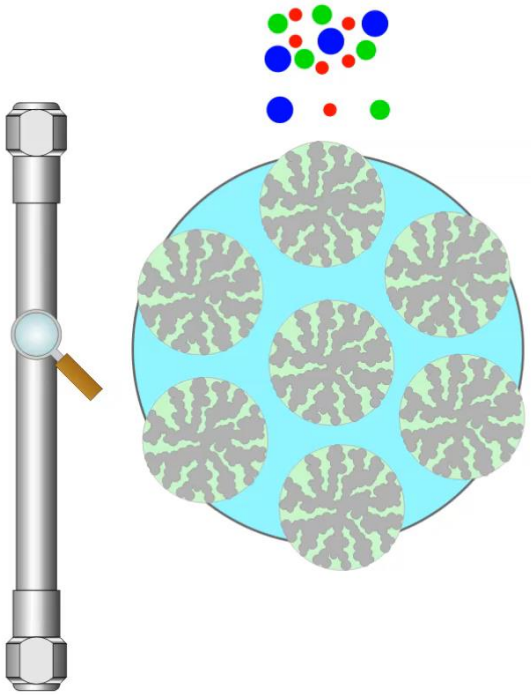


AdvanceBio SEC columns improve lab productivity by providing robust, reliable methods that eliminate sample re-analysis and increased sample throughput.

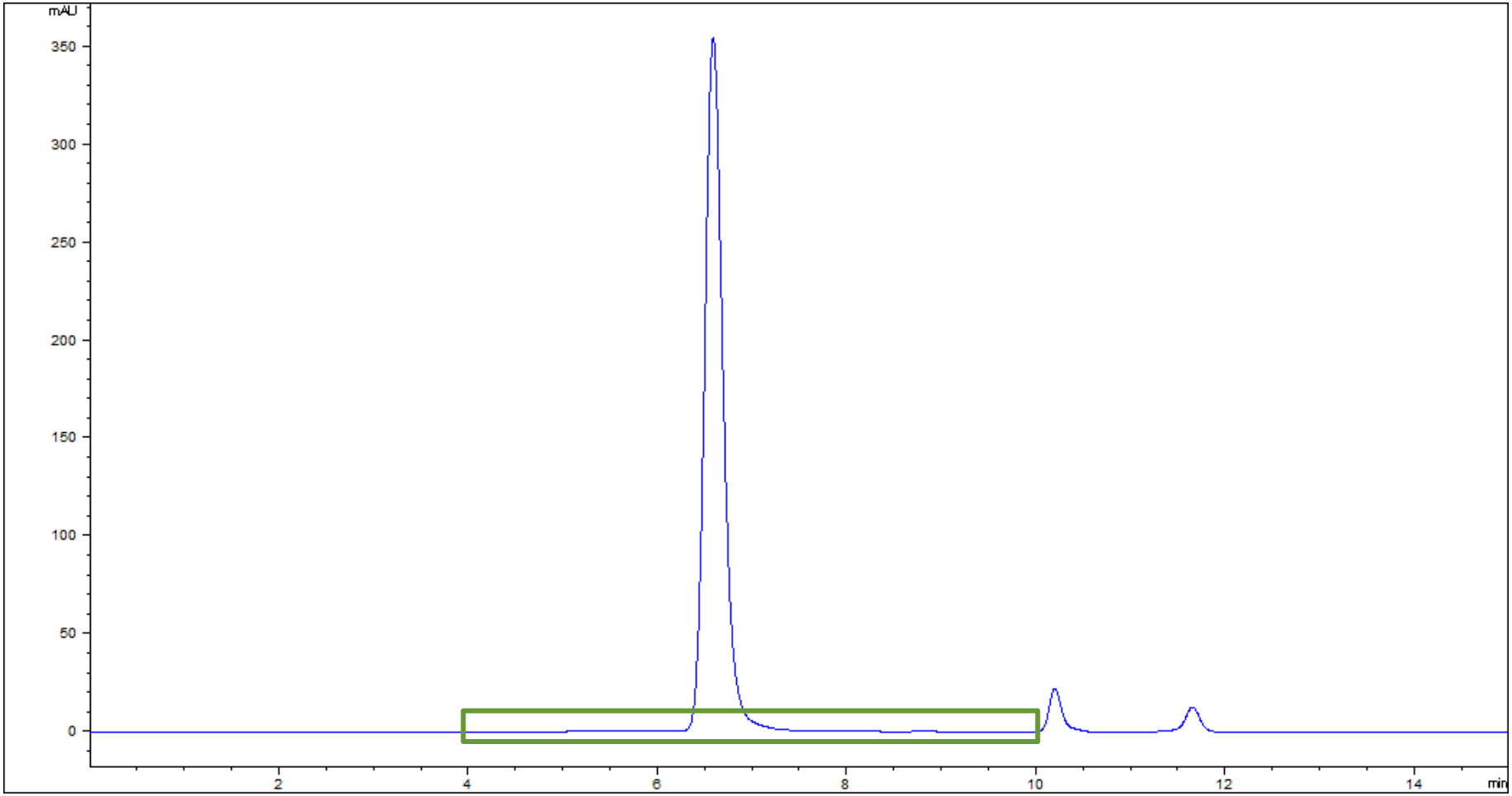
Size Exclusion Chromatography

- Size Exclusion Chromatography (SEC) allows the separation of molecules based on the size in solution.
- Larger molecules are excluded from the pores of the stationary phase and elute first.
- Smaller molecules diffuse into the pores and elute later.
- There is no interaction between the analyte and the stationary phase.
- **It is the method of choice for quantifying the amount of aggregates, dimers and larger oligomers, of therapeutic proteins such as monoclonal antibodies (mAbs).**

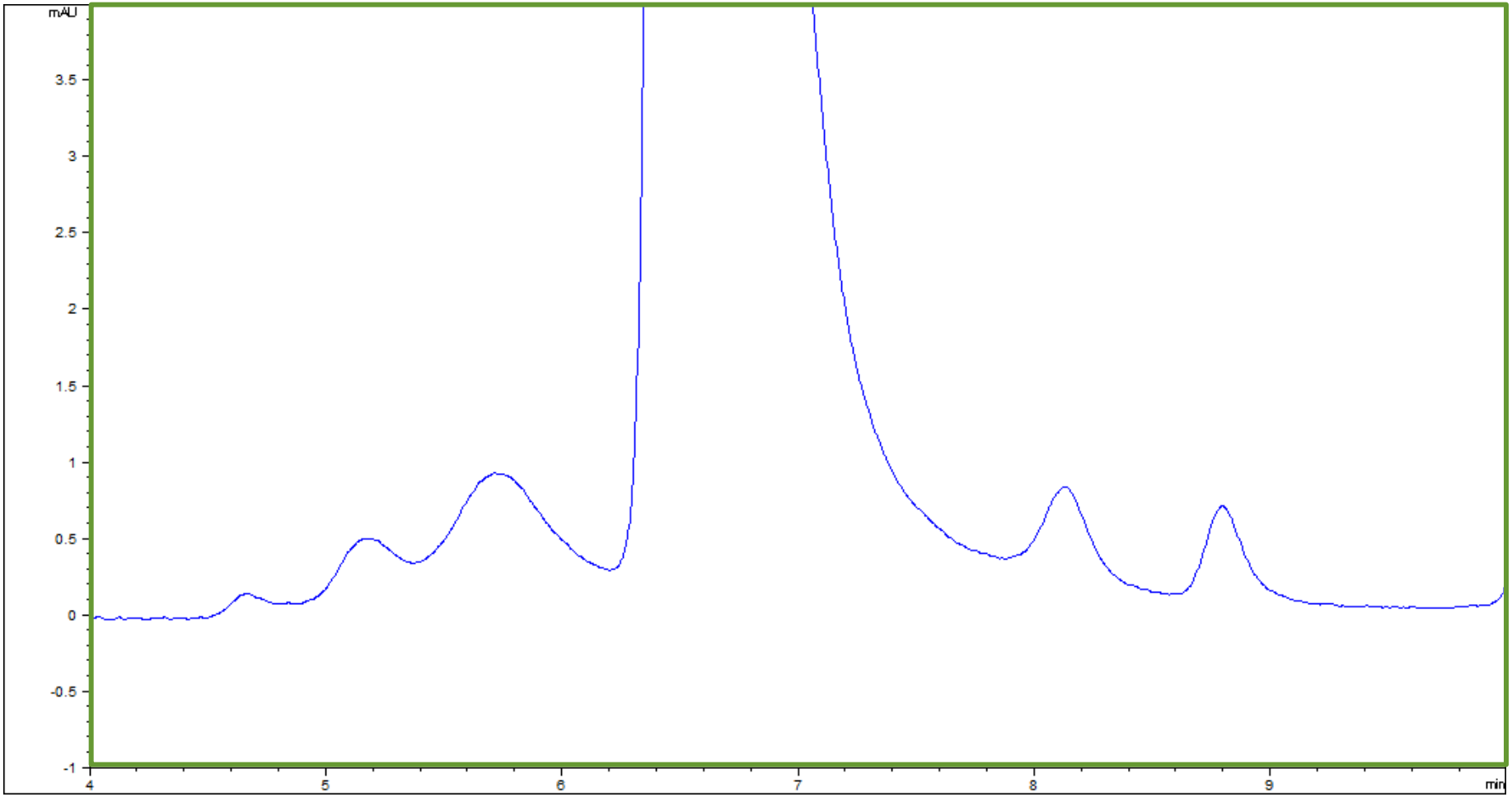
SEC animation



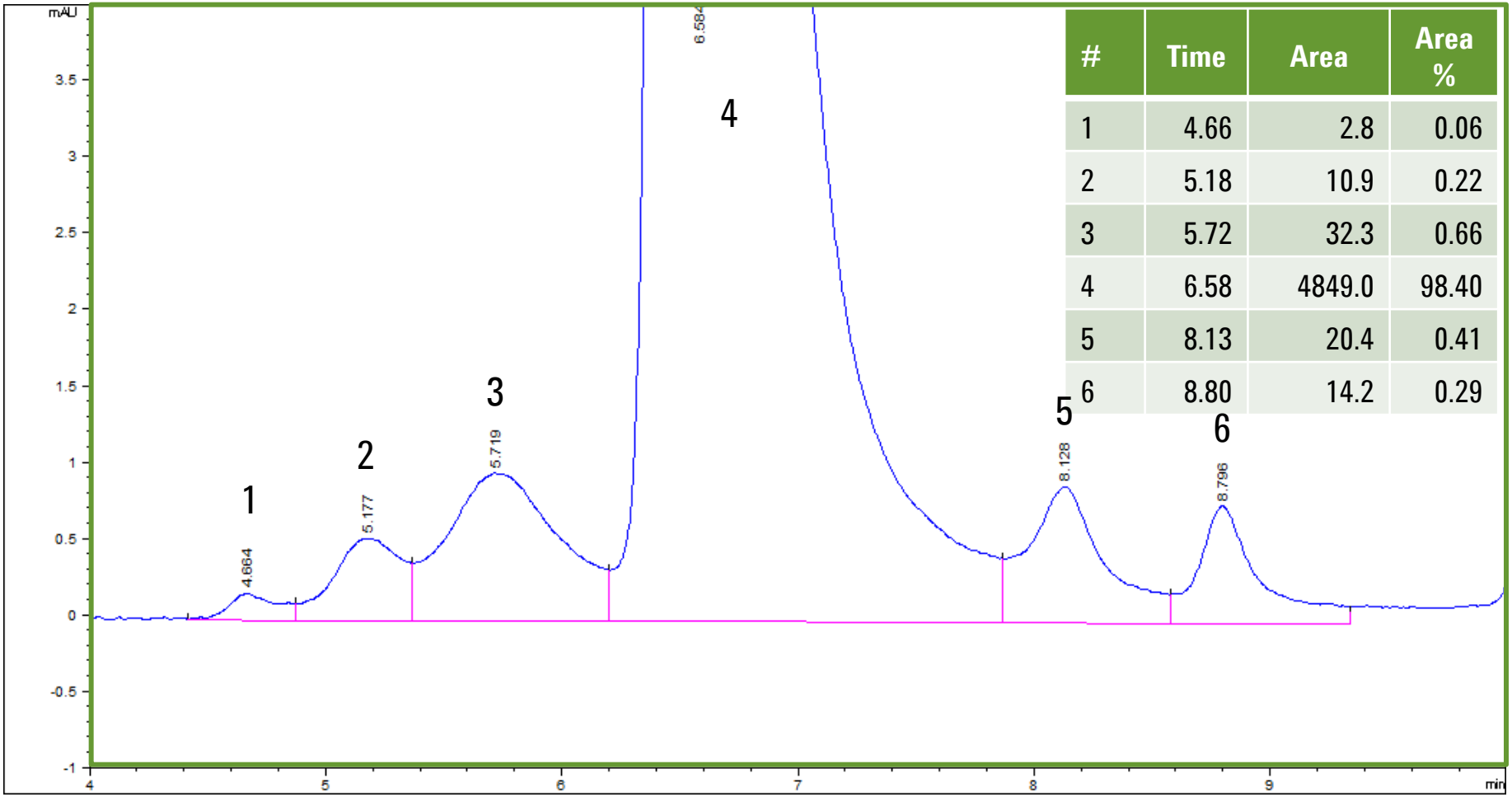
Detecting and quantifying mAb aggregation



Detecting and quantifying mAb aggregation



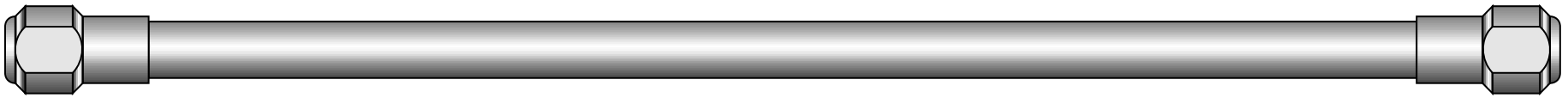
Detecting and quantifying mAb aggregation



#	Time	Area	Area %
1	4.66	2.8	0.06
2	5.18	10.9	0.22
3	5.72	32.3	0.66
4	6.58	4849.0	98.40
5	8.13	20.4	0.41
6	8.80	14.2	0.29

Some definitions

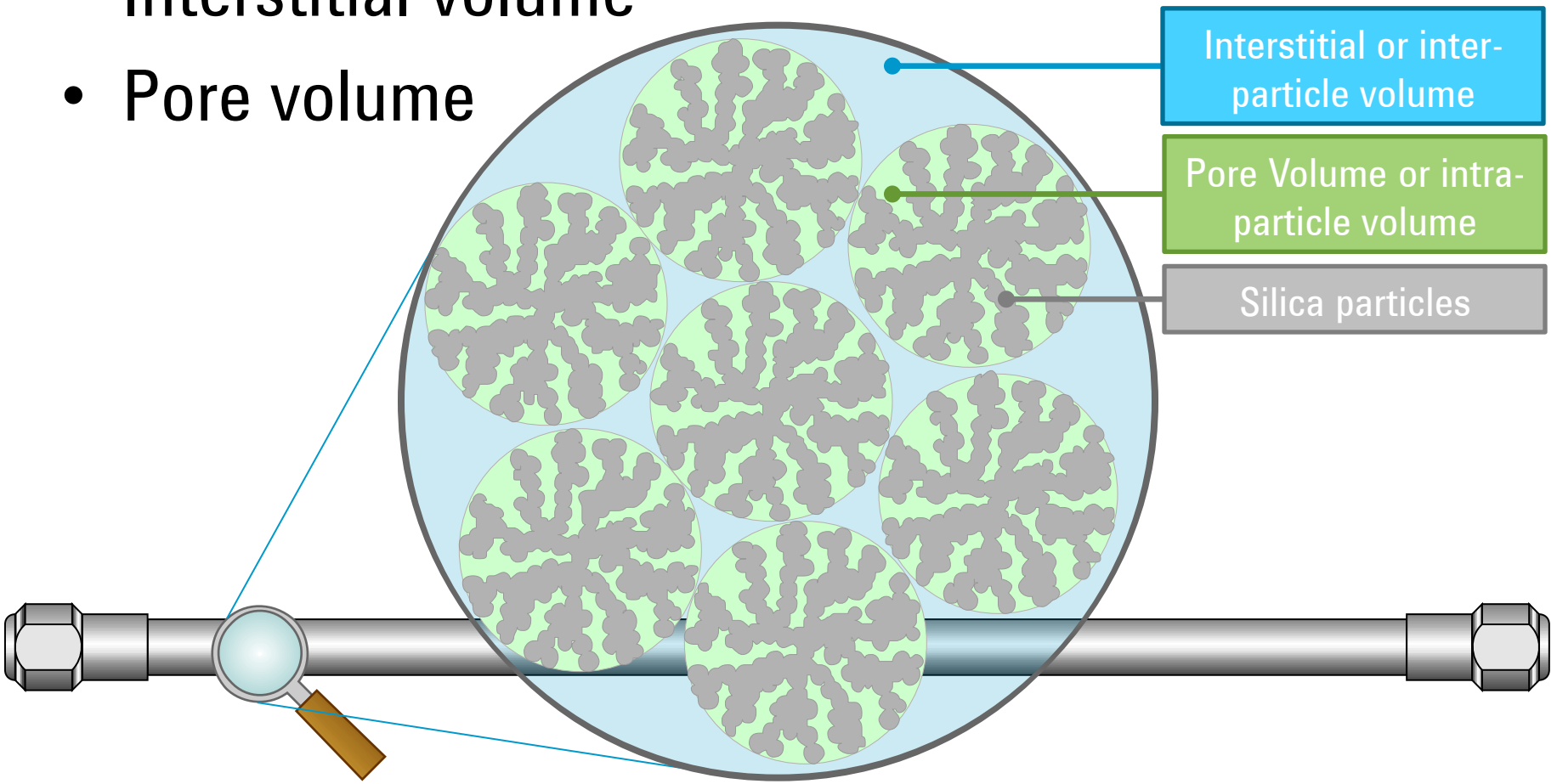
- Column volume
- Exclusion limit / Void volume
- Interstitial volume
- Pore volume
- Total permeation
- Non-specific interaction



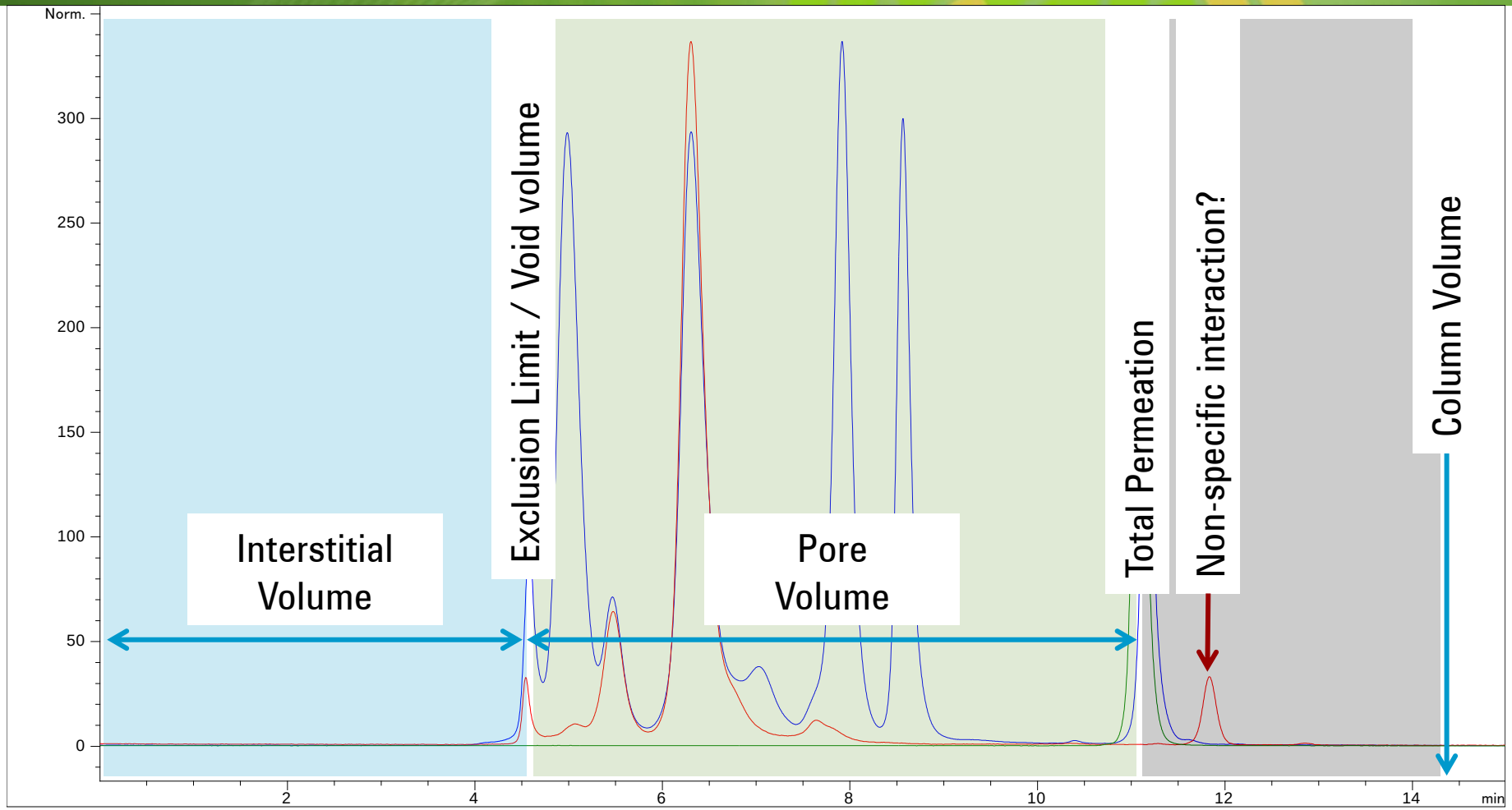
Column Dimensions: 7.8 x 300 mm
Column Volume = 14.3 mL

What are these inside the column?

- Interstitial volume
- Pore volume



What are these regions on a chromatogram?

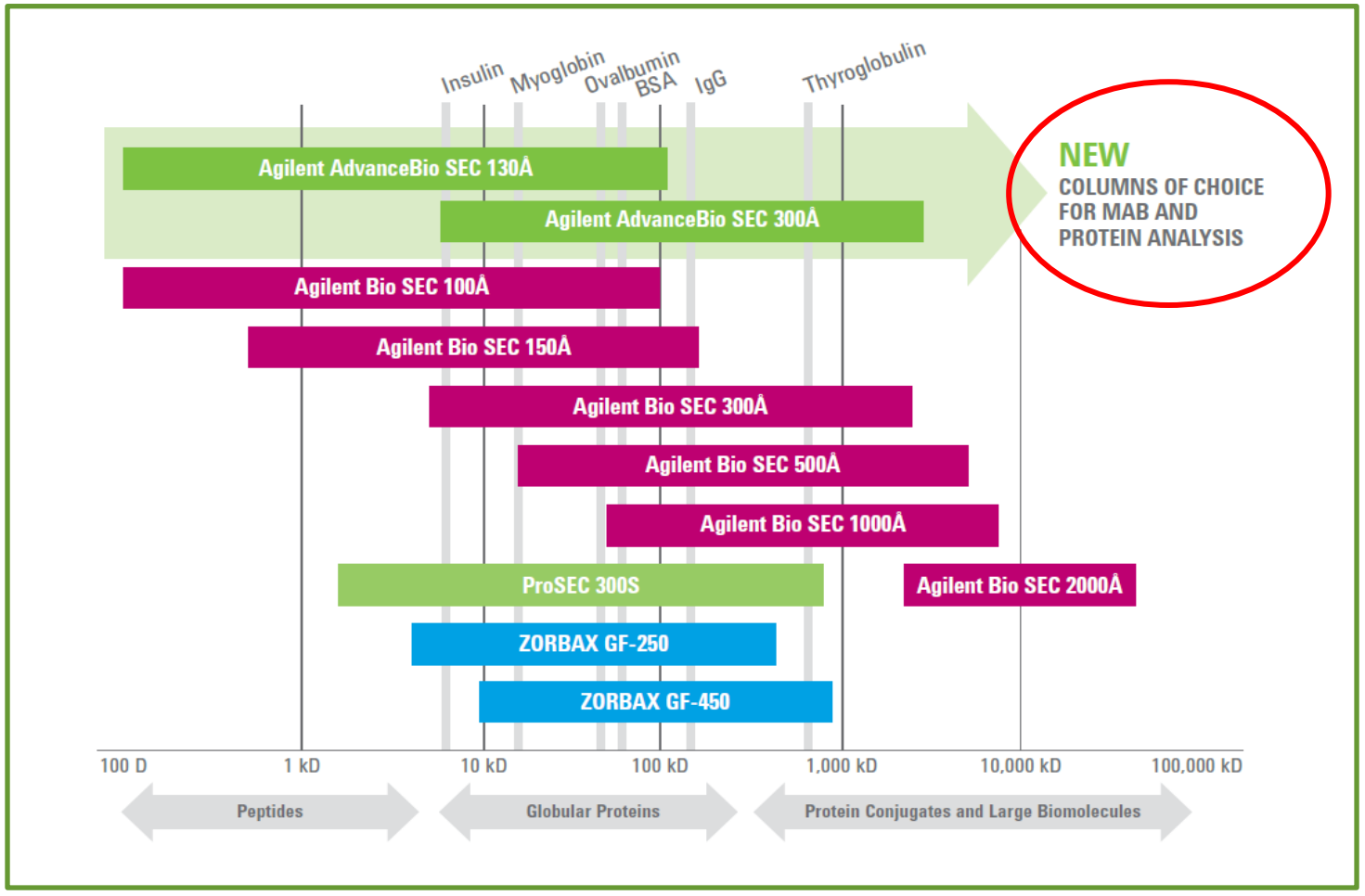


Rule Number #1

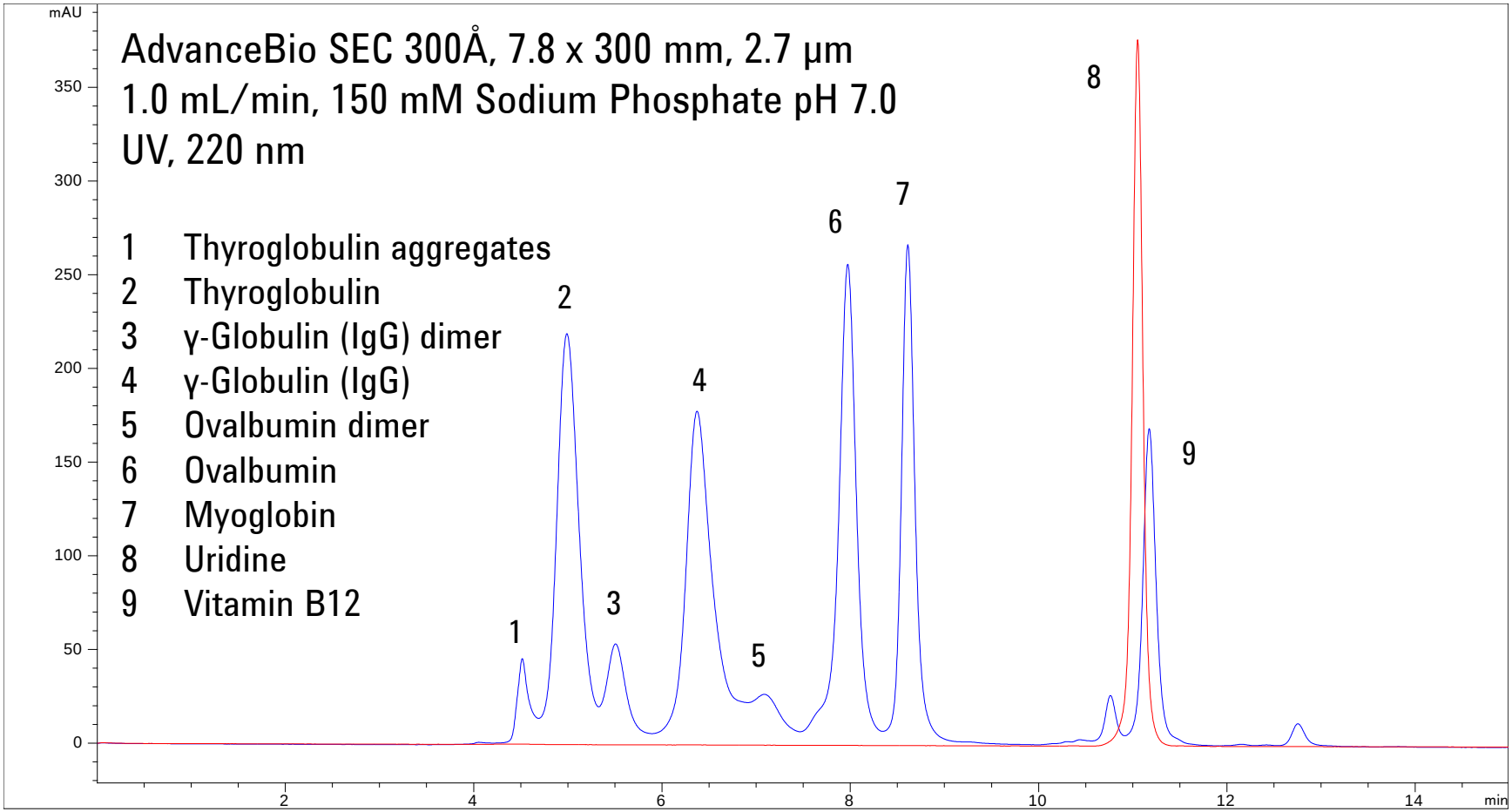
- Choose the right pore size
  - It is essential to select a column that has pores sufficiently large to allow your molecule to permeate into the pore structure of the stationary phase and not be excluded.
  - It is also essential to choose a pore size that is not too large.

For monoclonal antibodies the optimum pore size is around 300Å ...

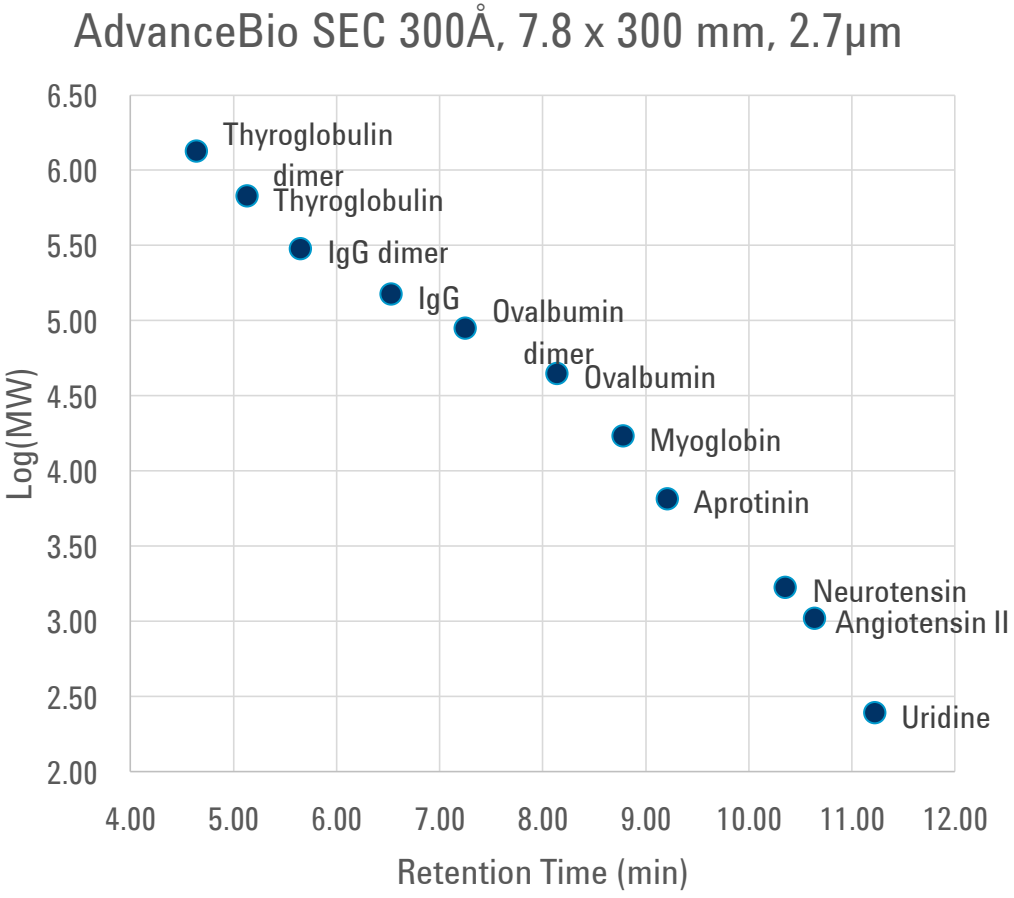
Agilent SEC pore size selection



SEC Chromatogram of proteins



Creating a calibration curve

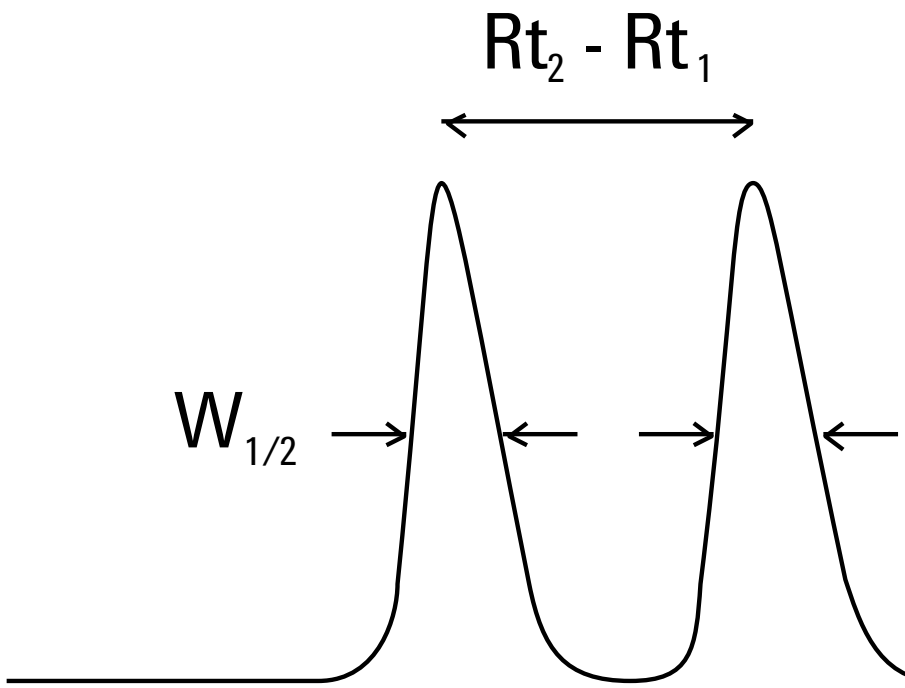


Protein or Peptide	MW	Log(MW)	RT (min)
Thyro Aggs	1340000	6.13	4.64
Thyroglobulin	670000	5.83	5.13
IgG Dimer	300000	5.48	5.65
γ-Globulin	150000	5.18	6.53
Oval Dimer	88600	4.95	7.25
Ovalbumin	44300	4.65	8.14
Myoglobin	16950	4.25	8.78
Aprotinin	6511	3.81	9.21
Neurotensin	1672	3.22	10.36
Angiotensin-II	1040	3.02	10.64
Uridine	244	2.39	11.22

What impacts resolution

- Resolution at half height

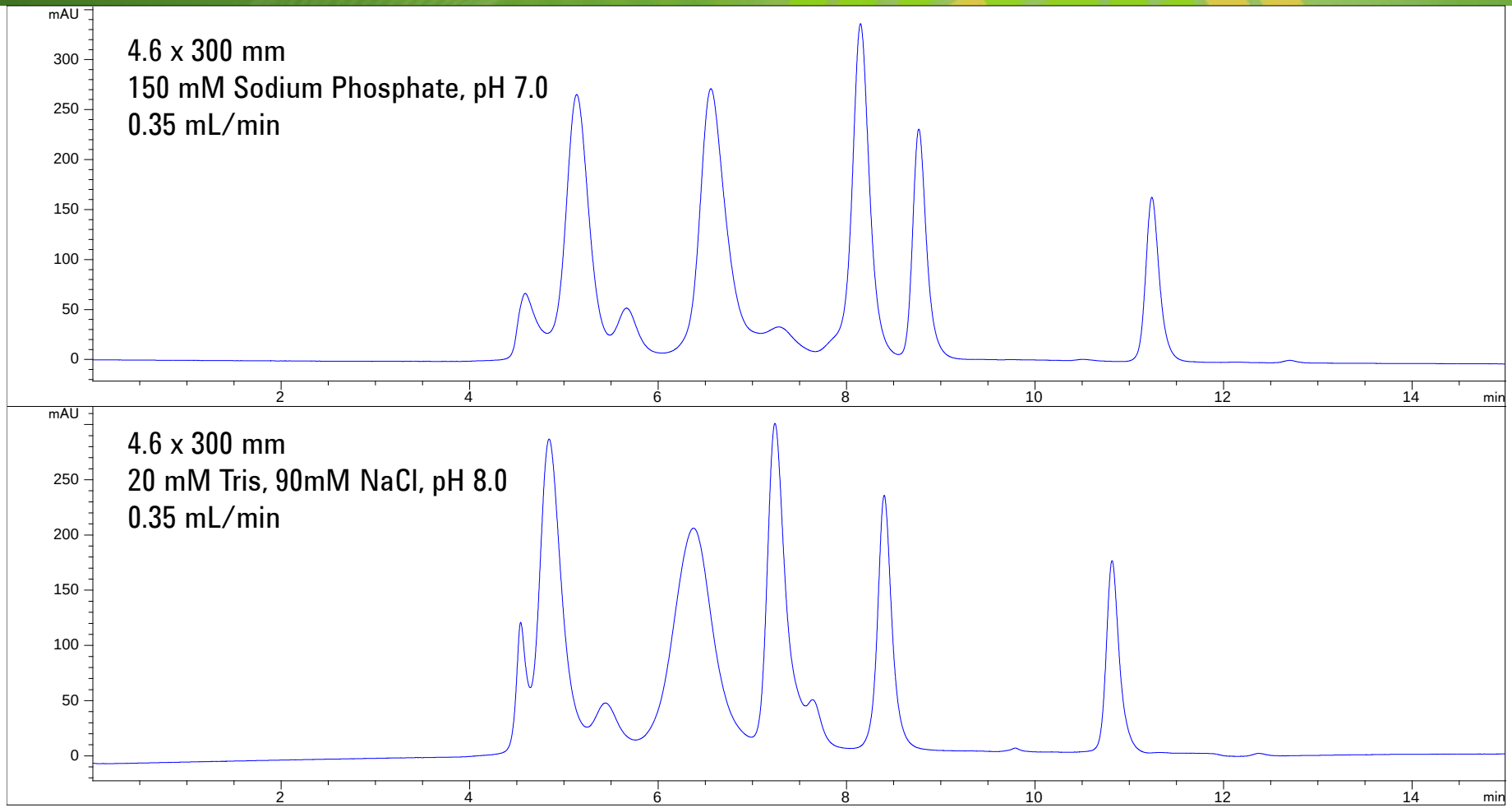
$$Rs = \frac{2\,(Rt_2 - Rt_1)}{1.7\,(W_{0.5\,1} + W_{0.5\,2})}$$



We have maximised Pore Volume in order to give greatest retention time difference ...

... we have optimised Particle Size and column loading in order to give sharper peaks (while retaining the ability to run on 400 bar instruments).

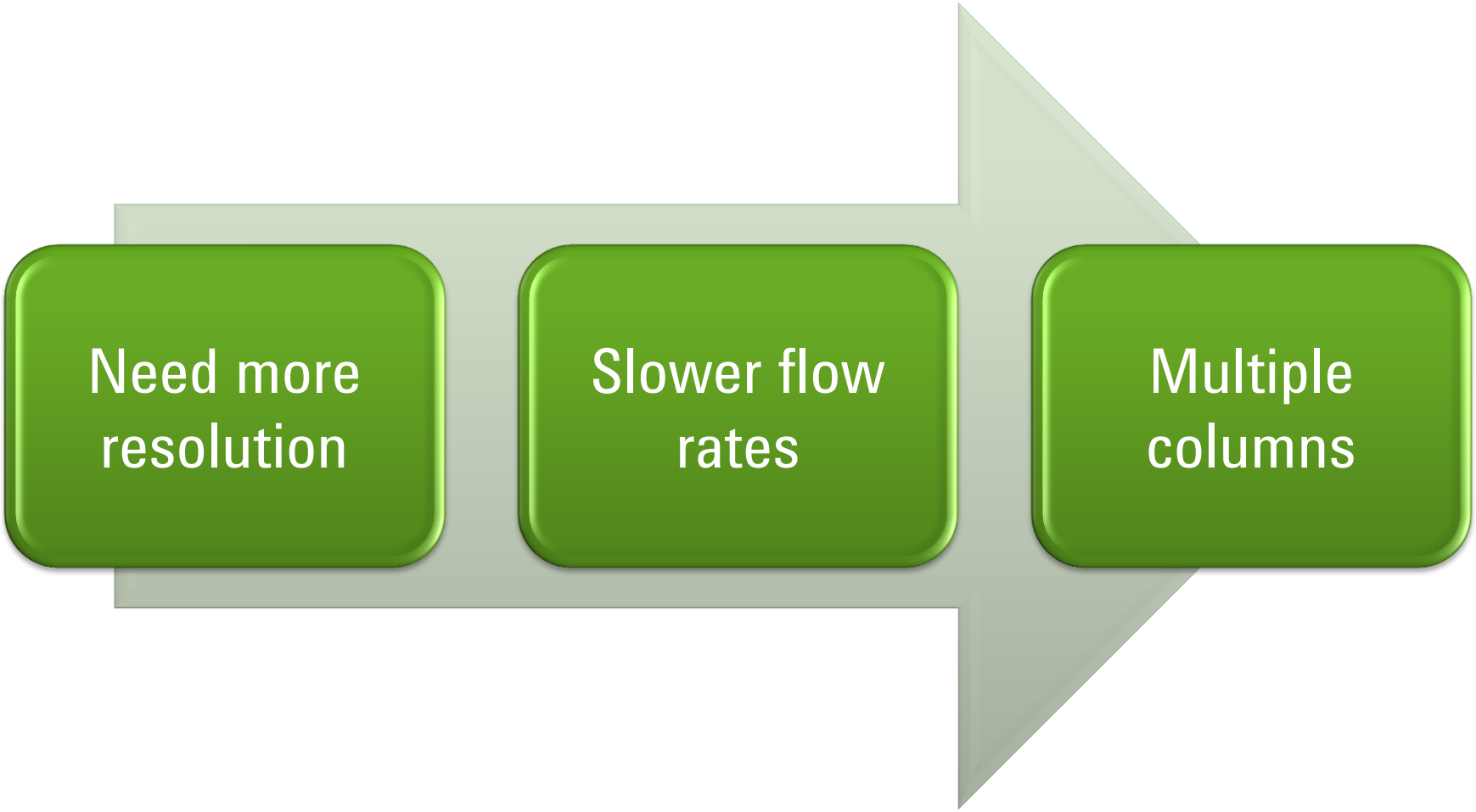
Mobile phase selection



AdvanceBio SEC columns

- Optimized pore size
  - 300Å for larger biotherapeutic proteins, mAbs and ADCs
  - 130Å covering a wide range of smaller proteins and polypeptides
- Optimized pore volume
  - Highest pore volume for maximum resolution
- Optimized particle size
  - 2.7 µm totally porous particles for high efficiency and applicability from 400 bar to UHPLC
- Optimized column loading
  - Multiple column dimensions for speed and resolution with maximum lifetime
- Optimized surface chemistry
  - Proprietary hydrophilic coating to reduce non-specific interactions

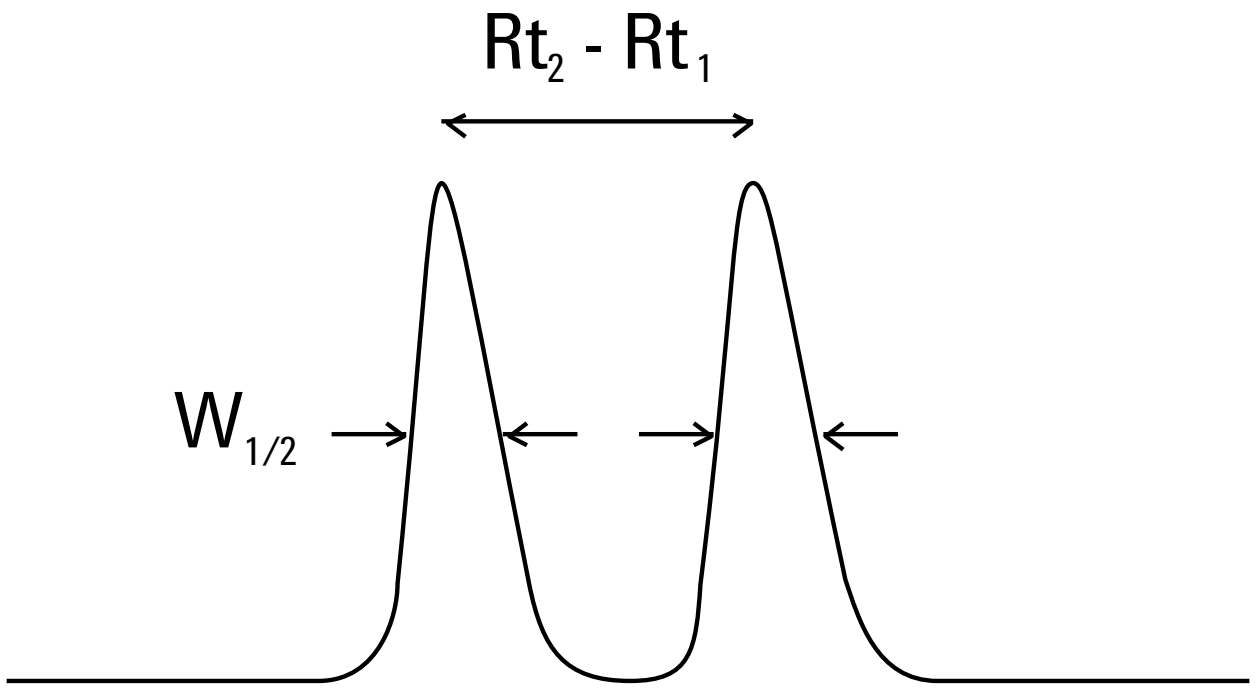
Characterization and QC



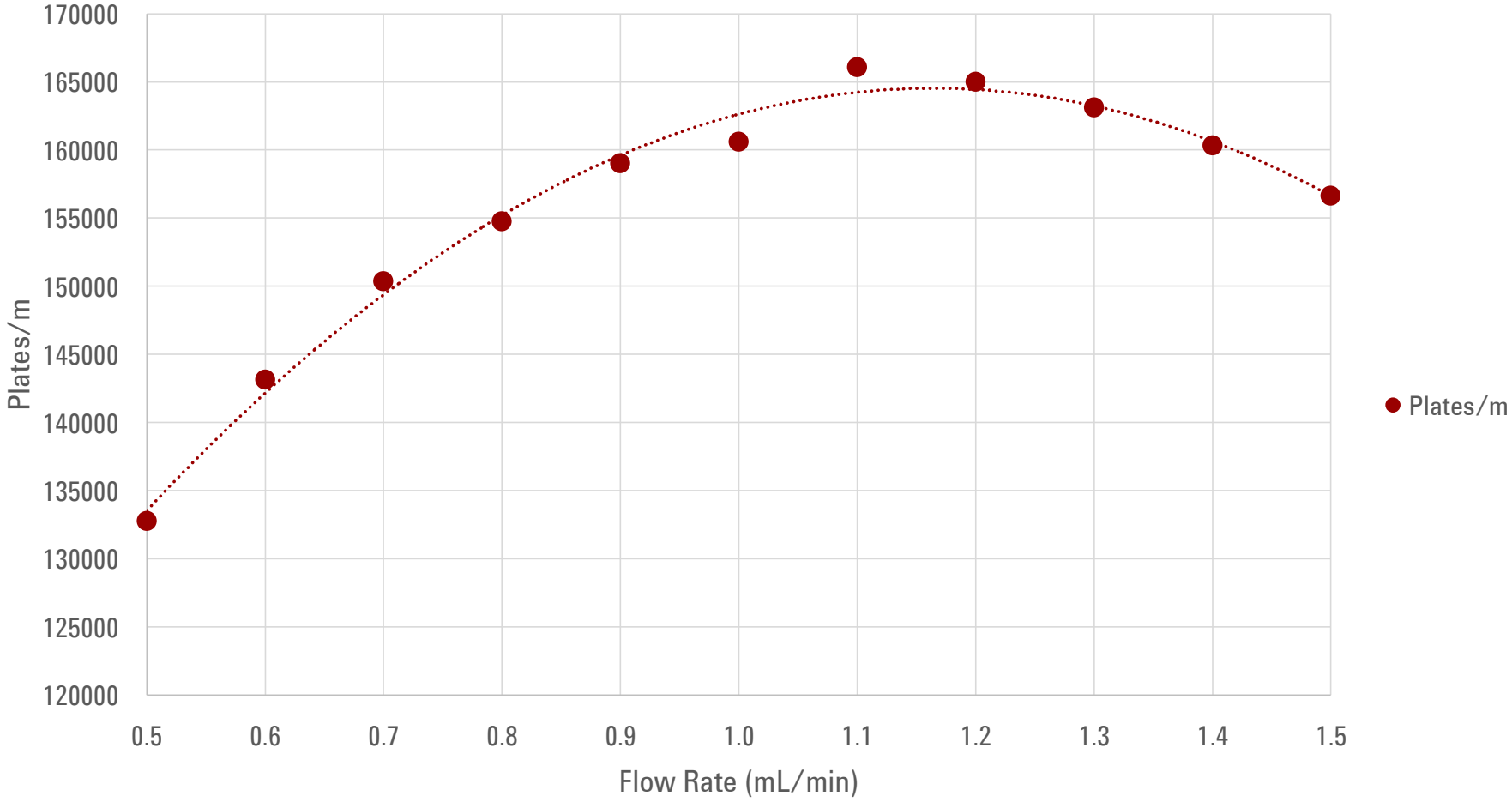
Need more resolution

- Resolution at half height

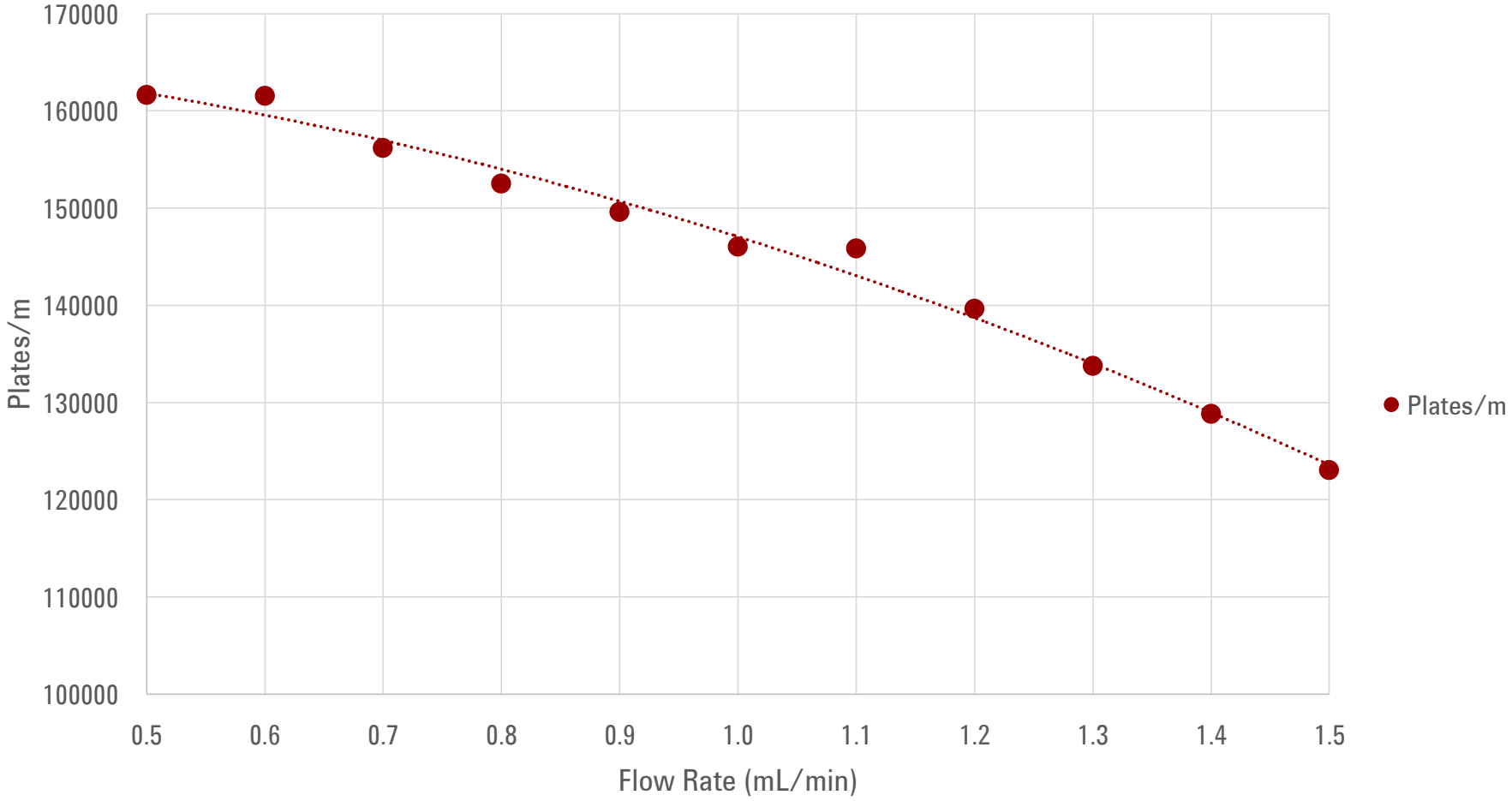
$$Rs = \frac{2\,(Rt_2 - Rt_1)}{1.7\,(W_{0.5\,1} + W_{0.5\,2})}$$



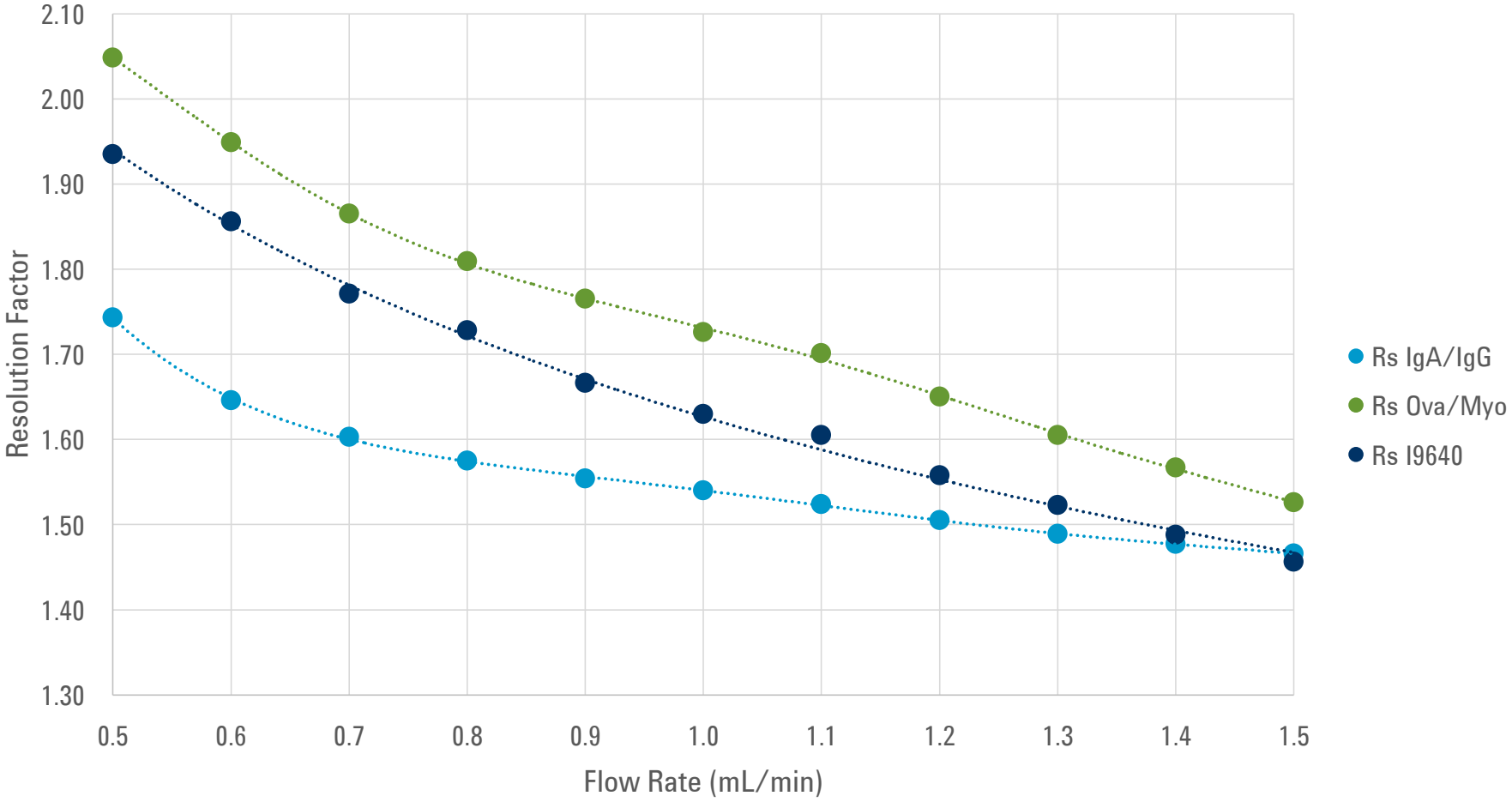
Column Performance
Uridine (small molecule)



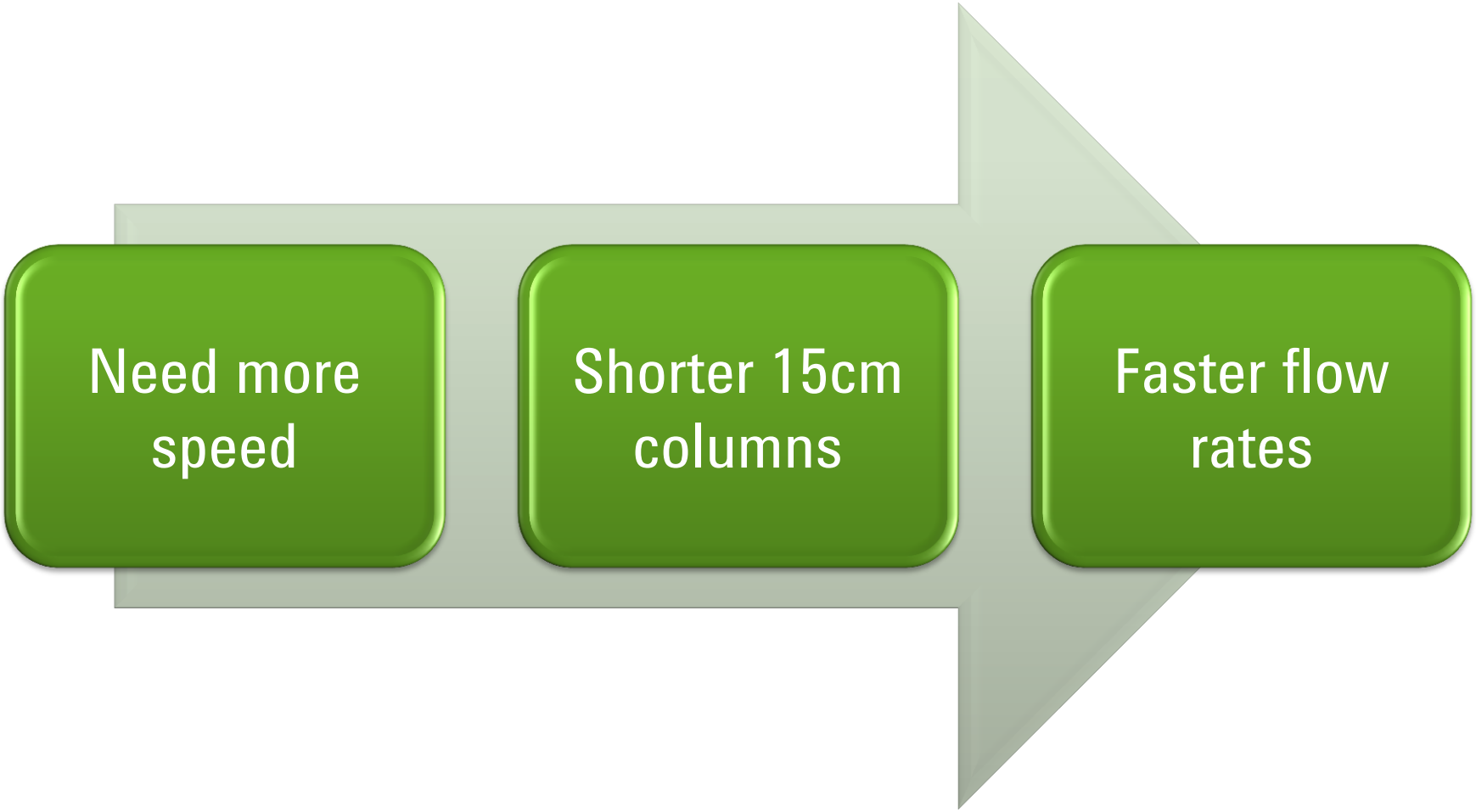
Column Performance
Vitamin B12



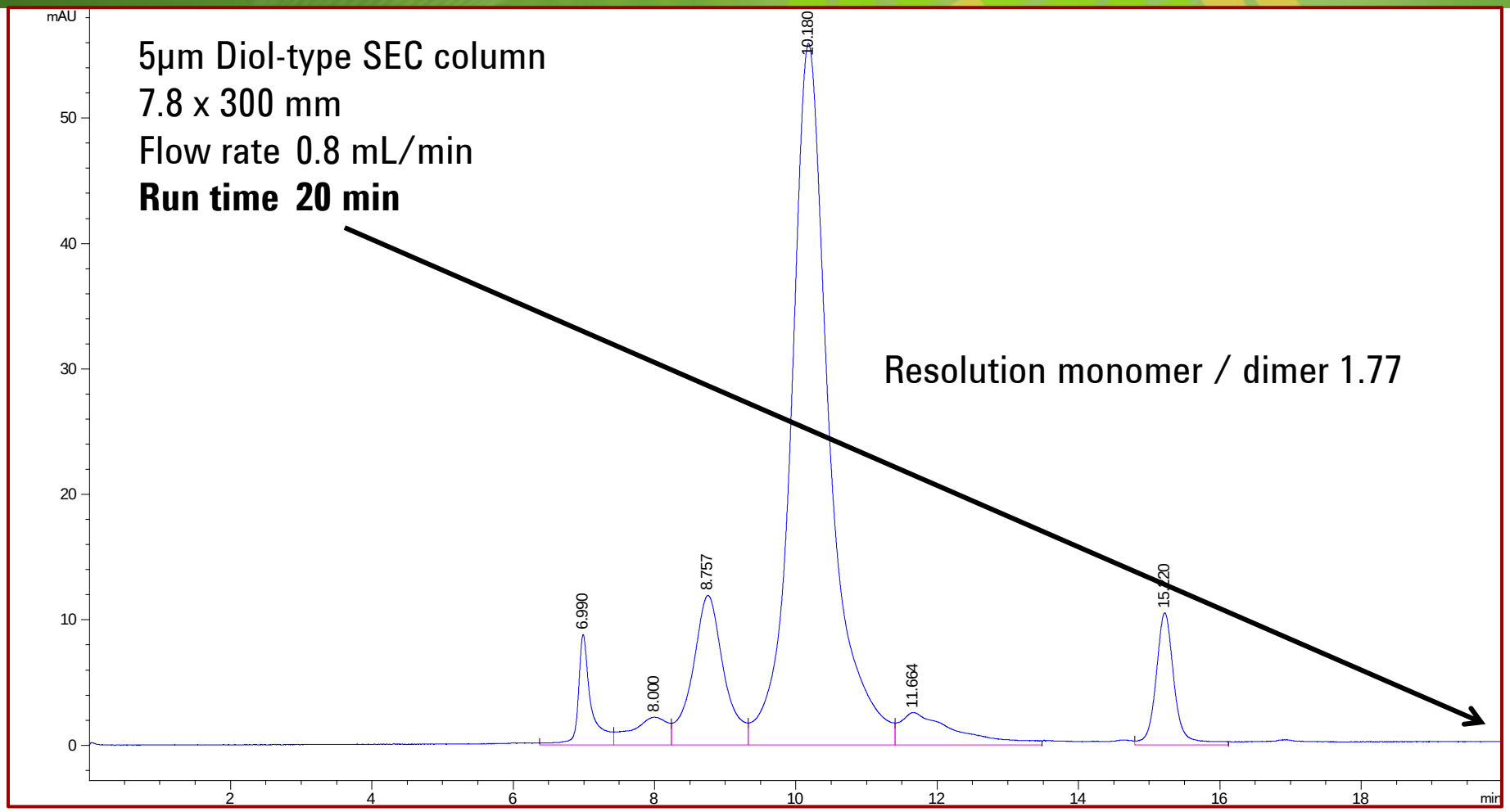
Column Performance
Resolution (BioRad and IgG I9640)



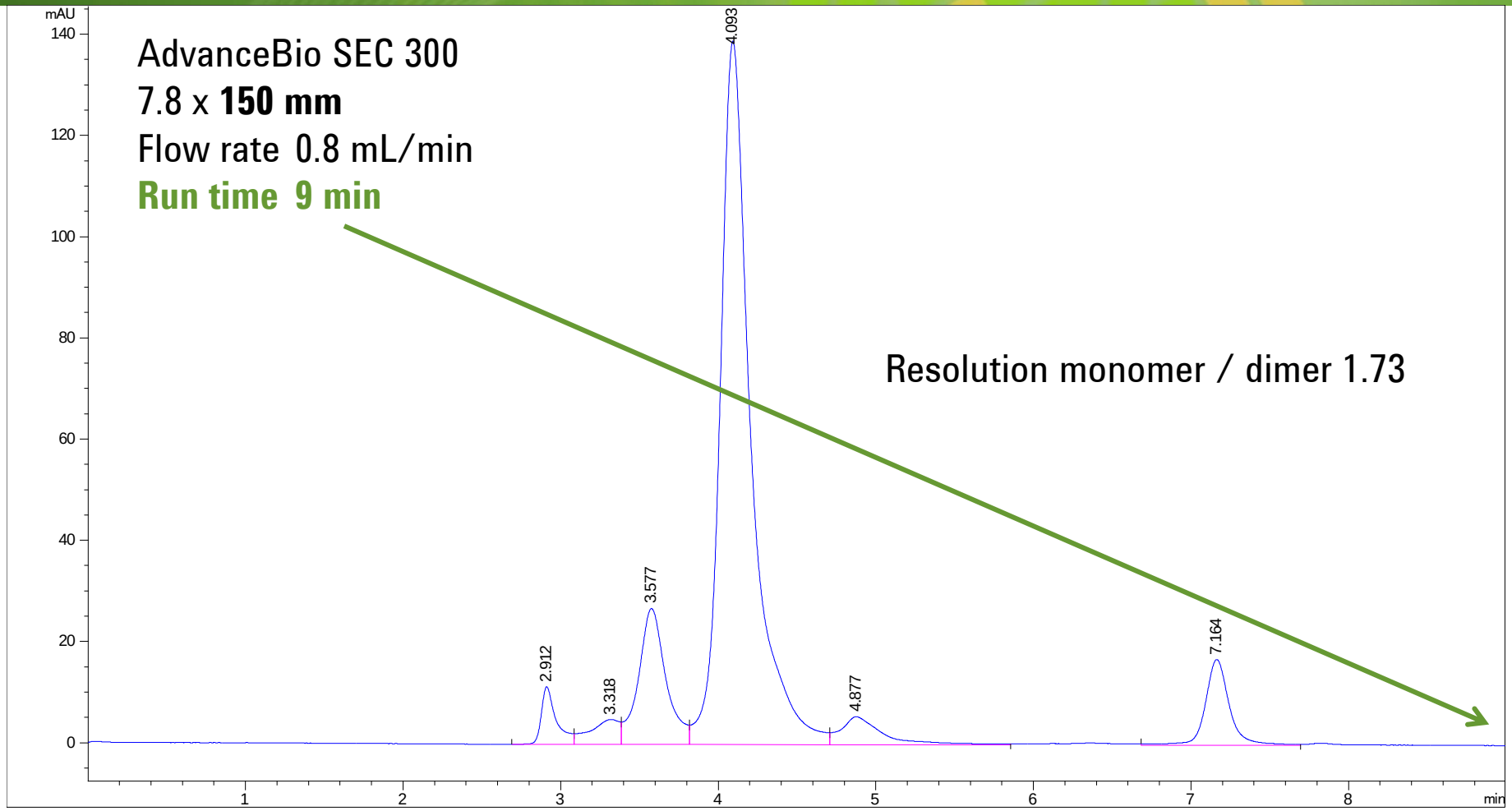
Increased sample throughput



Initial Conditions

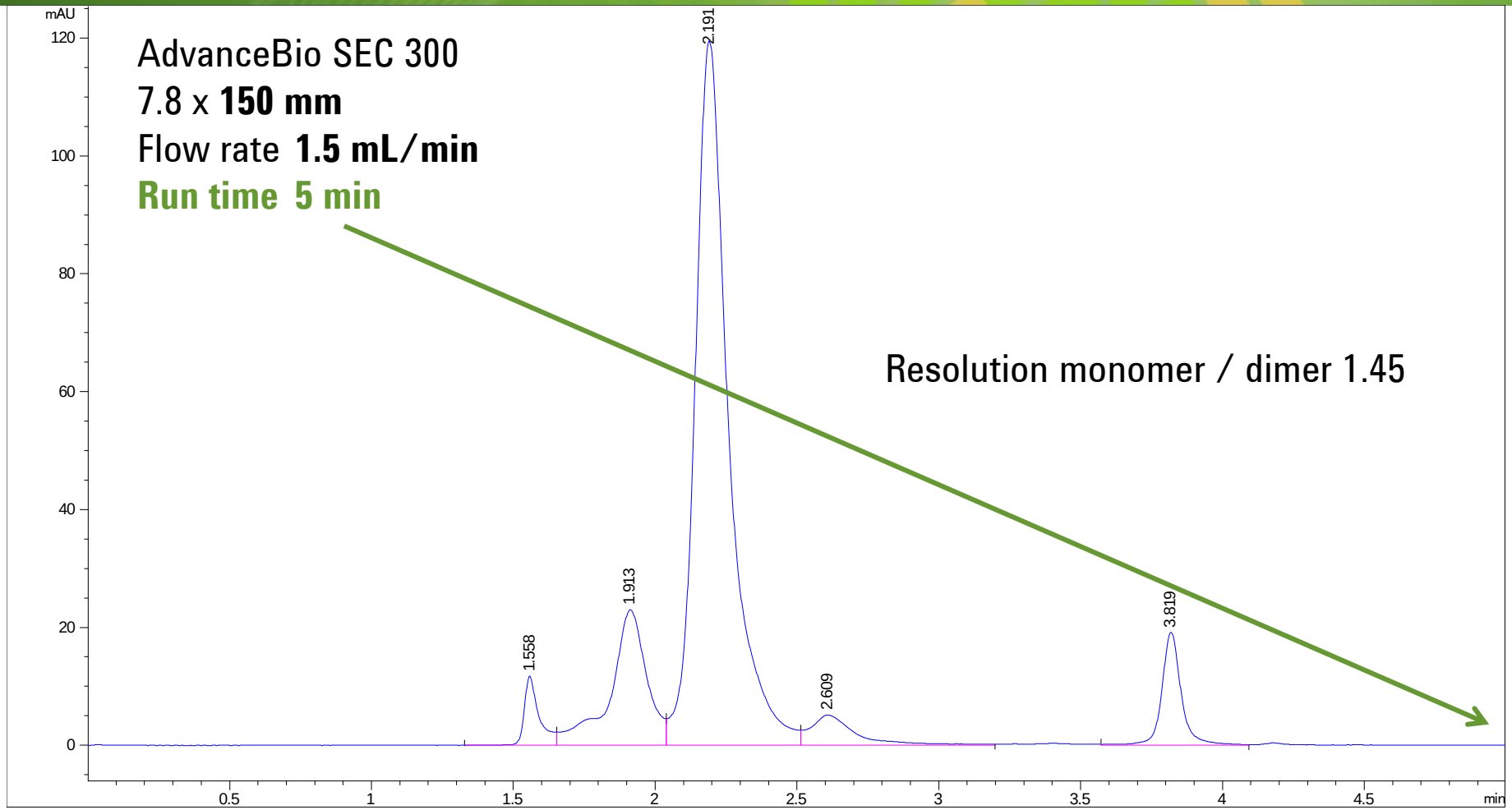


Same flow rate, 15cm column
Same resolution, much shorter run time

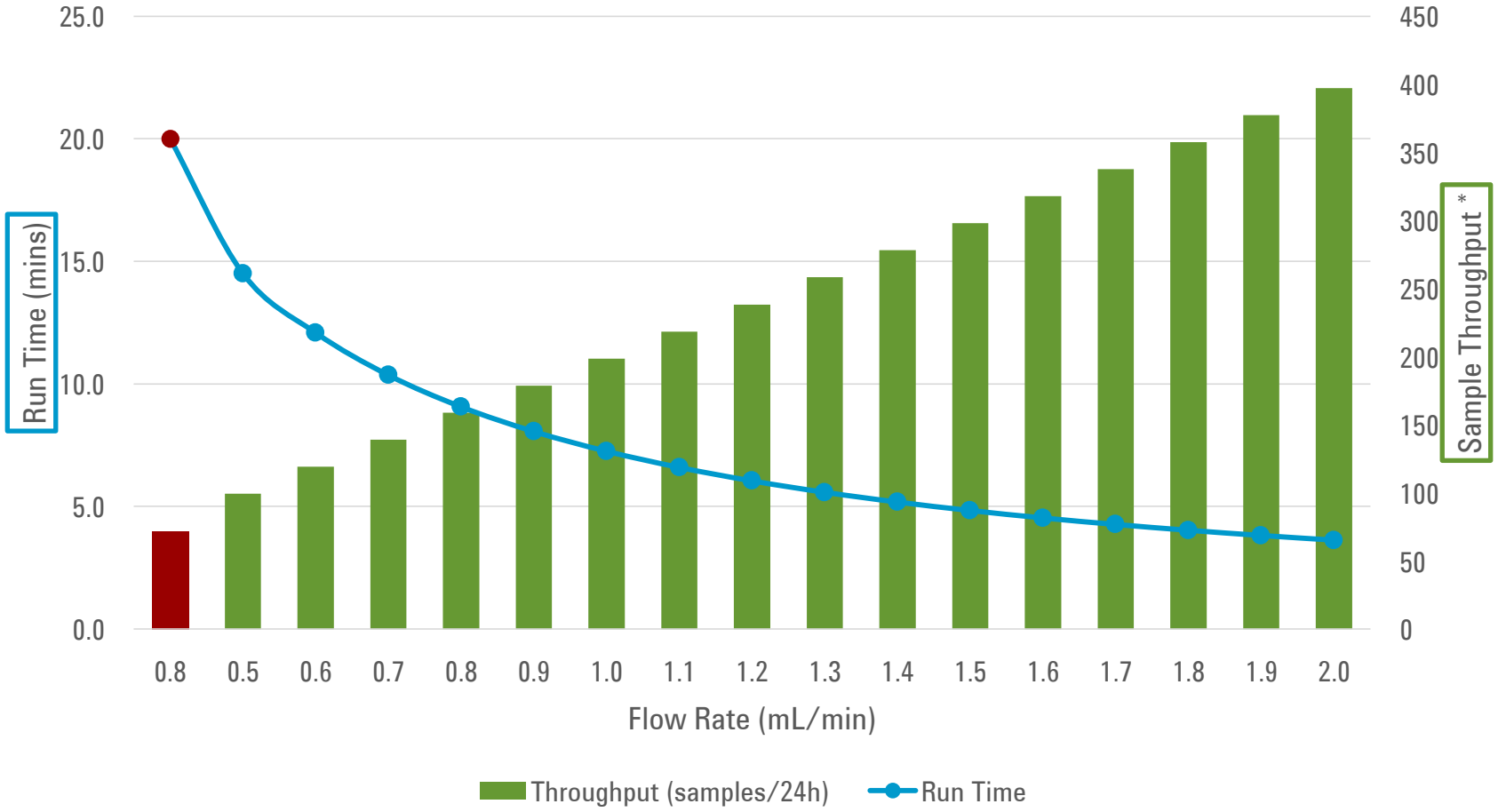


Highest flow rate tested, 15cm column

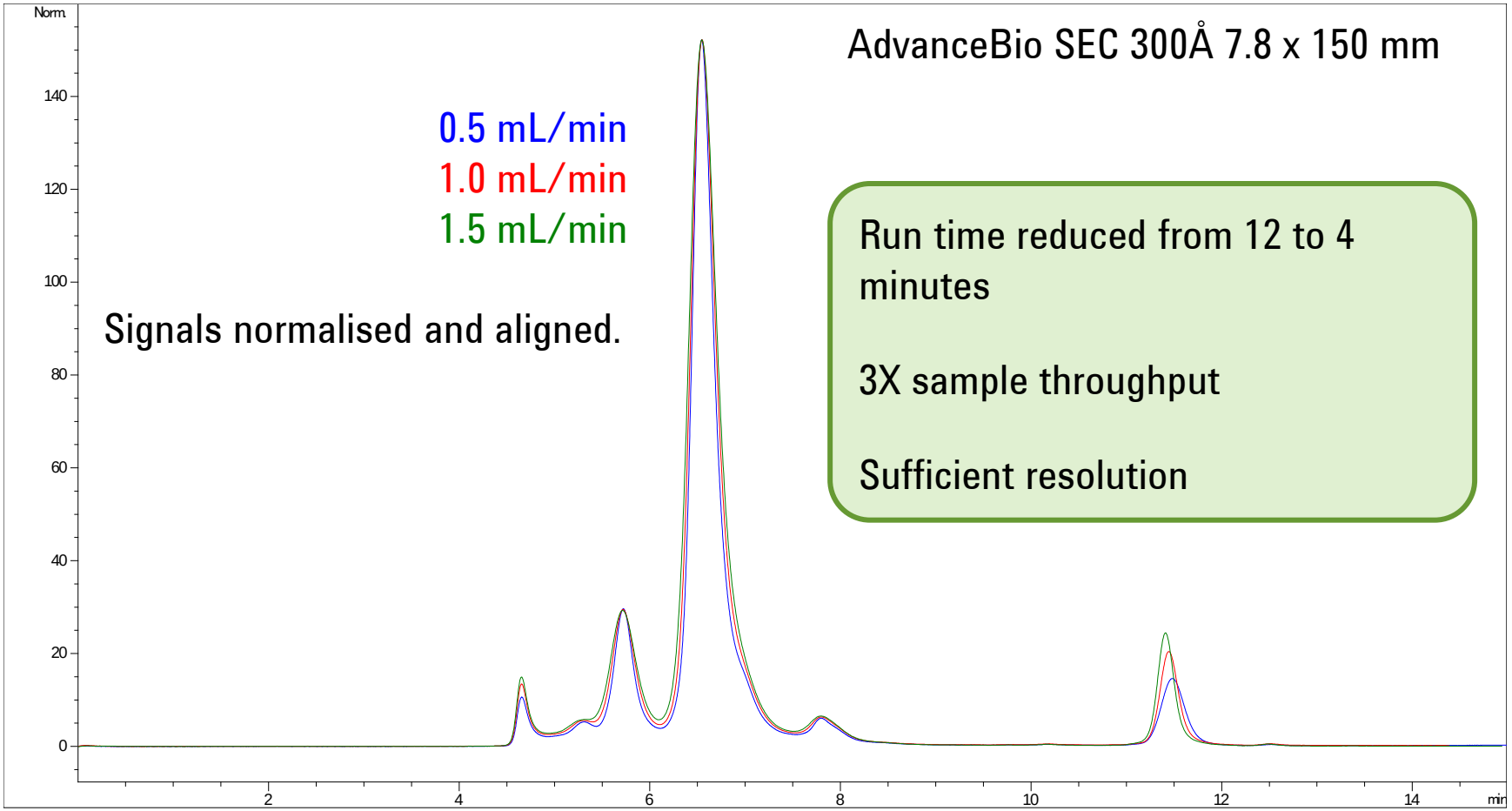
Resolution less than 1.5, but sufficient for rapid screening



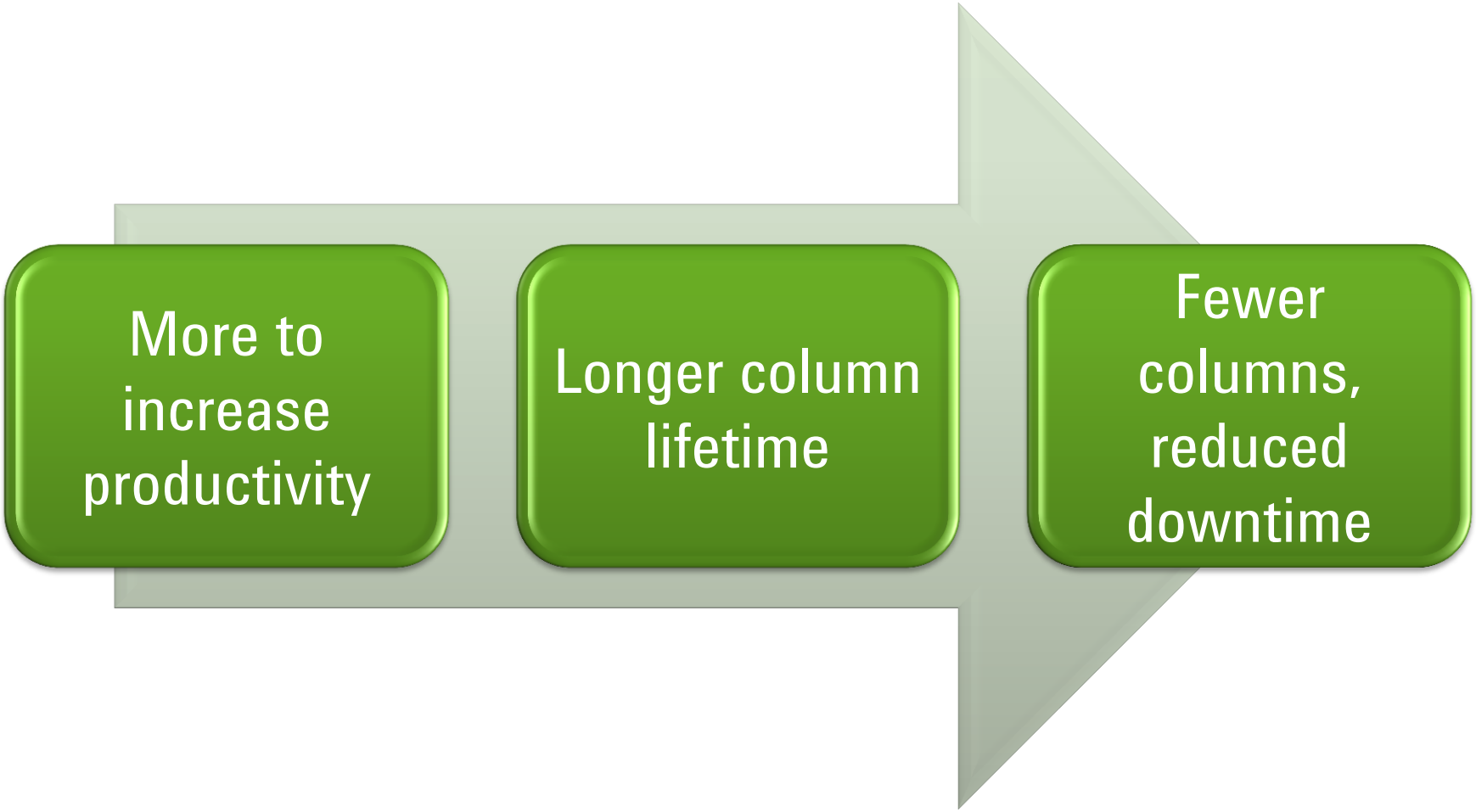
Flow rate impact on sample throughput



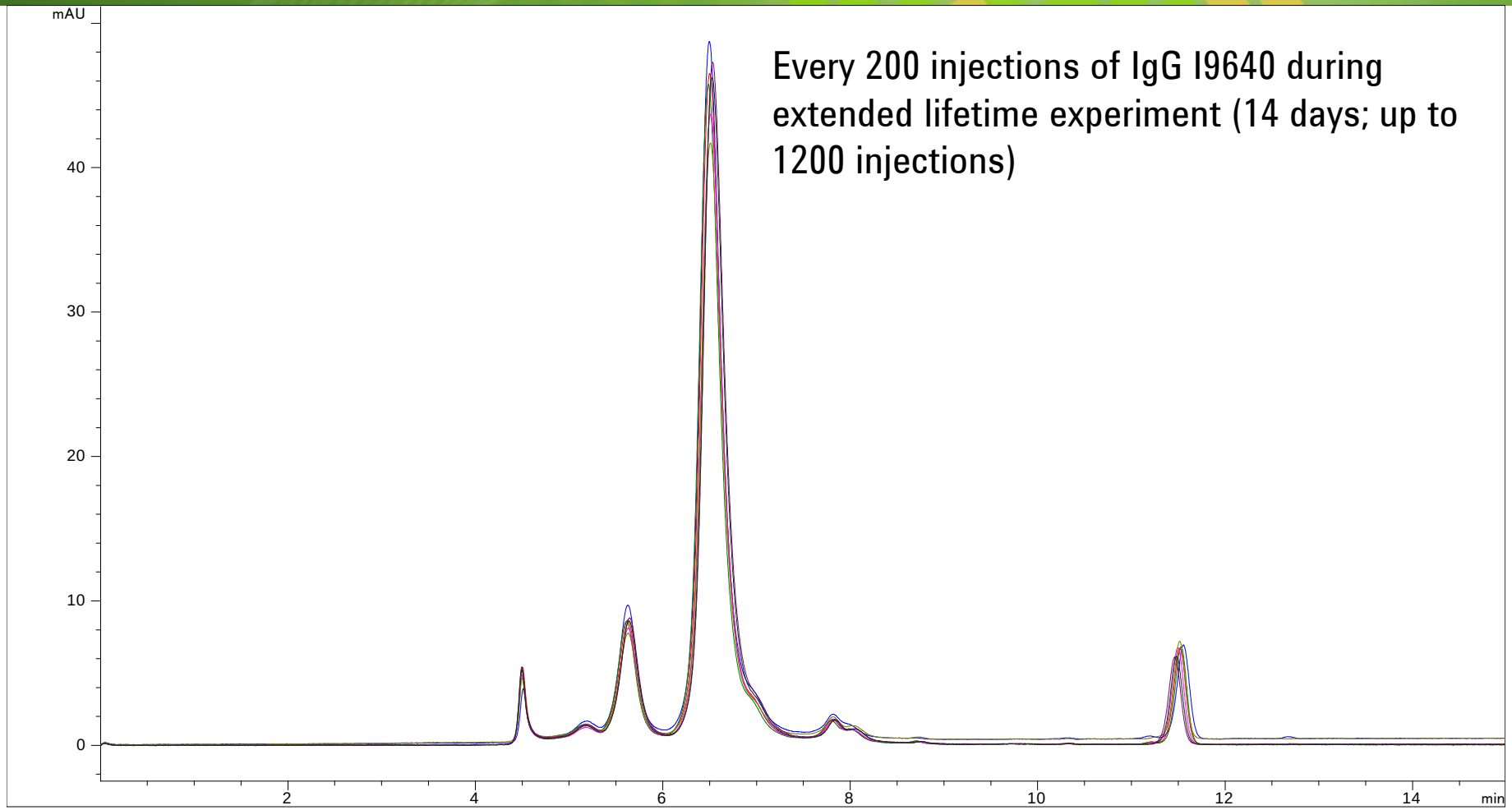
Increase speed of analysis



Reduce laboratory running costs



Extended Lifetime

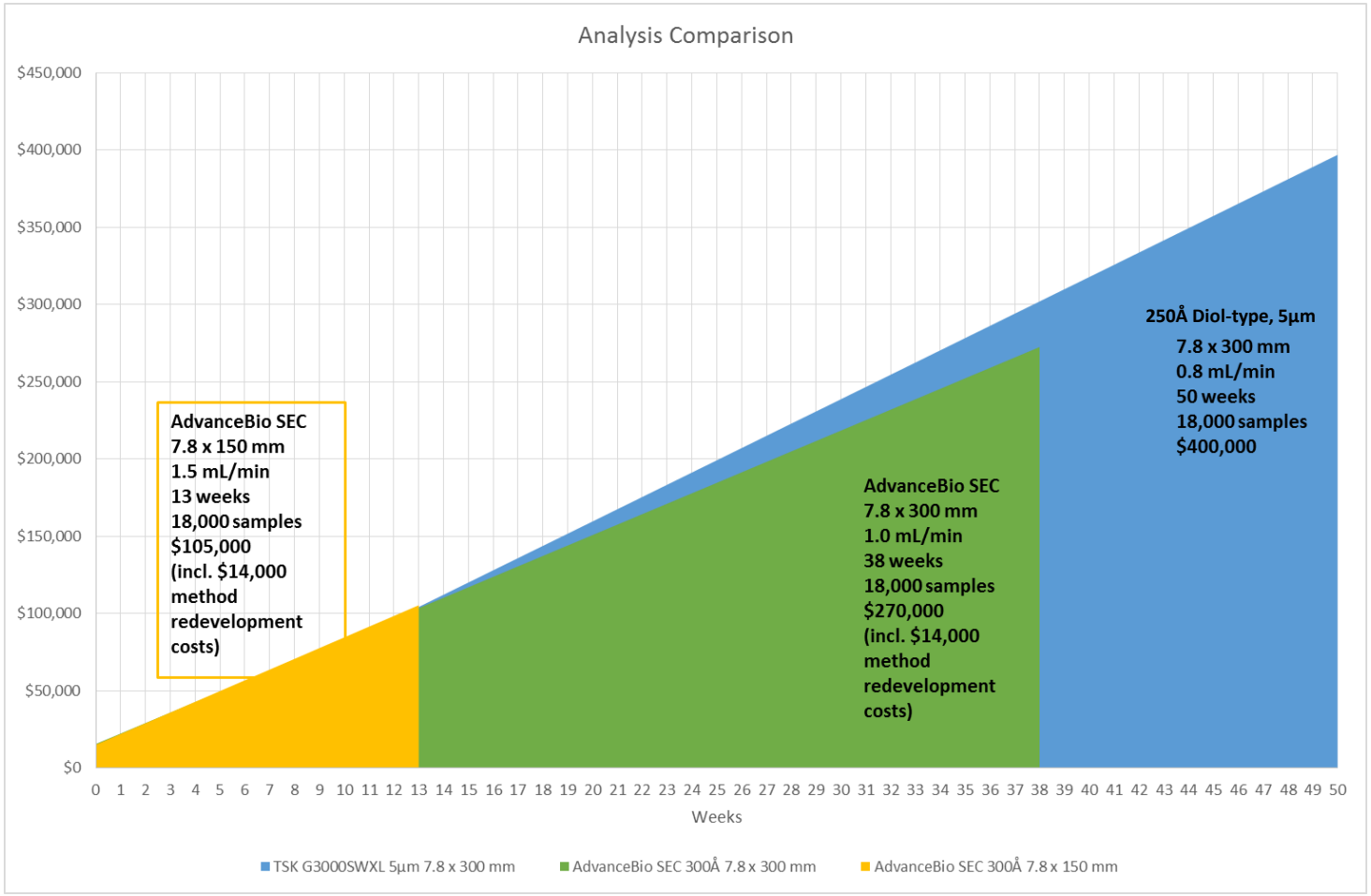


Every 200 injections of IgG I9640 during extended lifetime experiment (14 days; up to 1200 injections)

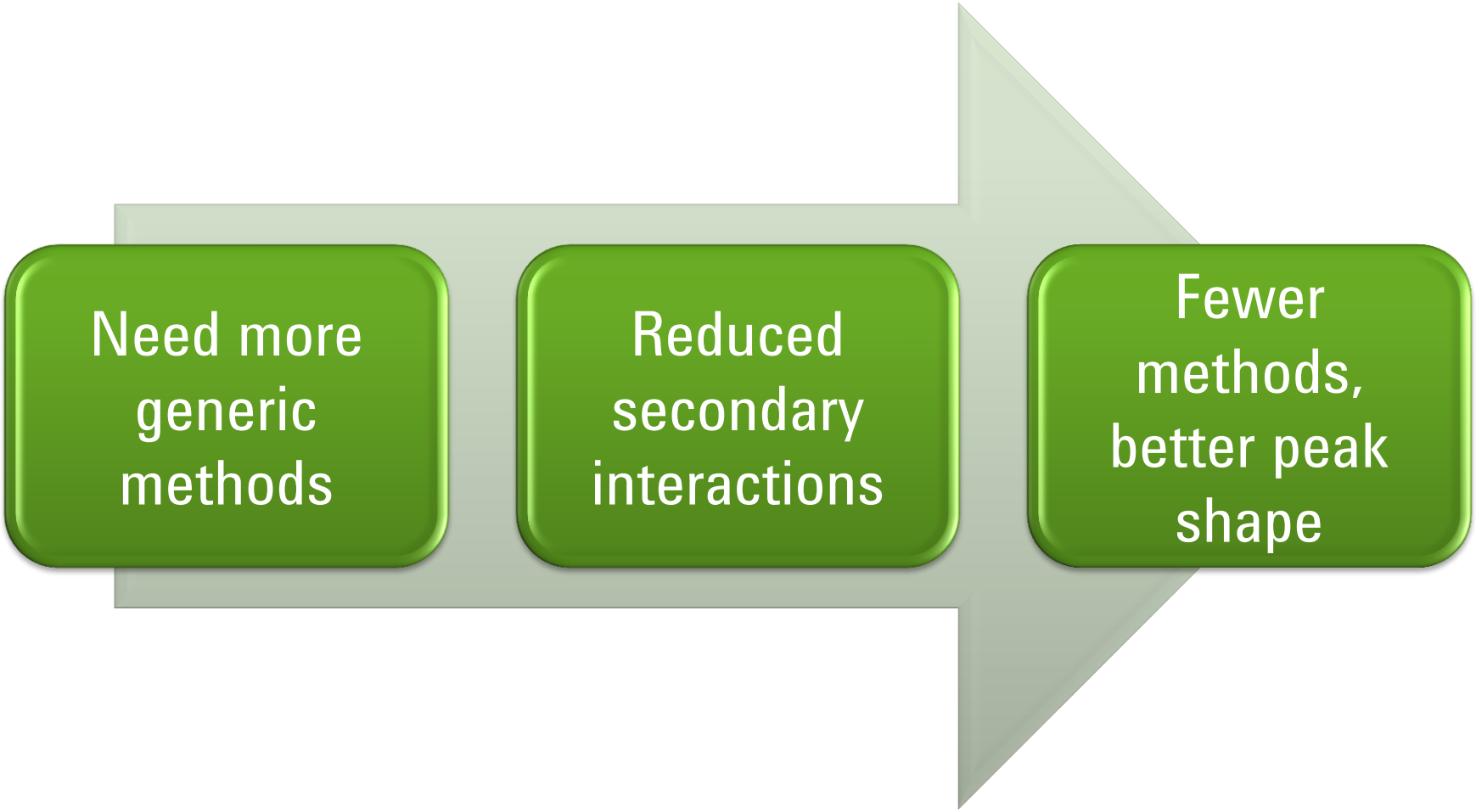
Economic Value

Business Inputs			Inputs
Lab Overhead per LC	125	$/hr	Outputs
Method Development Chemist Cost	175	$/hr	
Method Development Tim	2	weeks	
Solvent	0	$/Liter	
Buffer (cost+prep cost)	15	$/Liter	
Waste Disposal	0	$/Liter	
Total mobile phase cost	15	$/Liter	
For Reinvestment Section			
Sample Revenue	30	$/sample	
	Original	Transfer Option 1	Transfer Option 2
Column Variables			
Column Name	250Å Diol-type, 5µm 7.8 x 300 mm	AdvanceBio SEC 300Å 7.8 x 300 mm	AdvanceBio SEC 300Å 7.8 x 150 mm
Column Length (mm)	300	300	150
Column Diamter (mm)	7.8	7.8	7.8
Analysis Variables			
Column Price	$1,567.00	$1,719.00	$1,237.00
Average Column Lifetime	200	500	1000
Minutes per Analysis	20	15	5
mL Solvent per Analysis	16	15	7.5
Cost Per Injection			
Mobile Phase Cost	$0.24	$0.23	$0.11
Column Replacement	$7.84	$3.44	$1.24
Total	$8.08	$3.66	$1.35
Transition Costs			
Method Update	$0.00	$14,000.00	$14,000.00

Economic value calculation ...

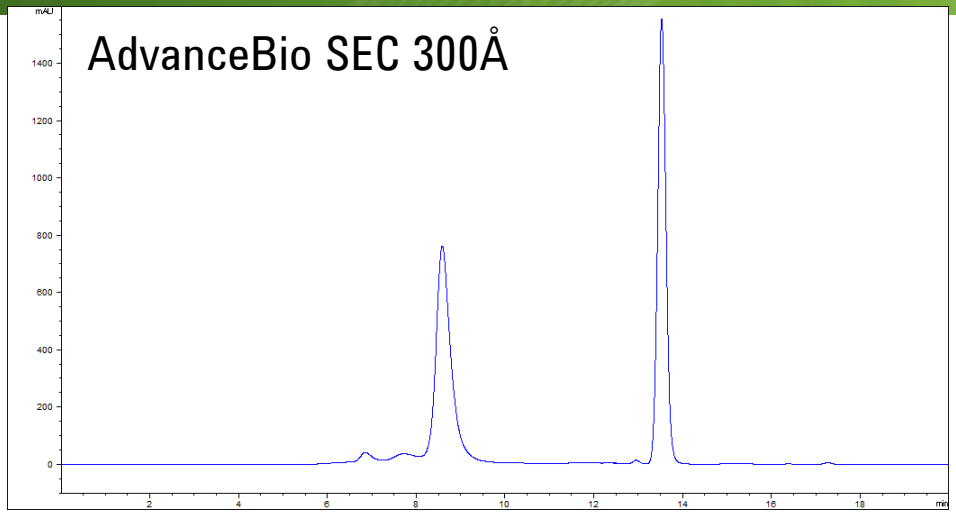


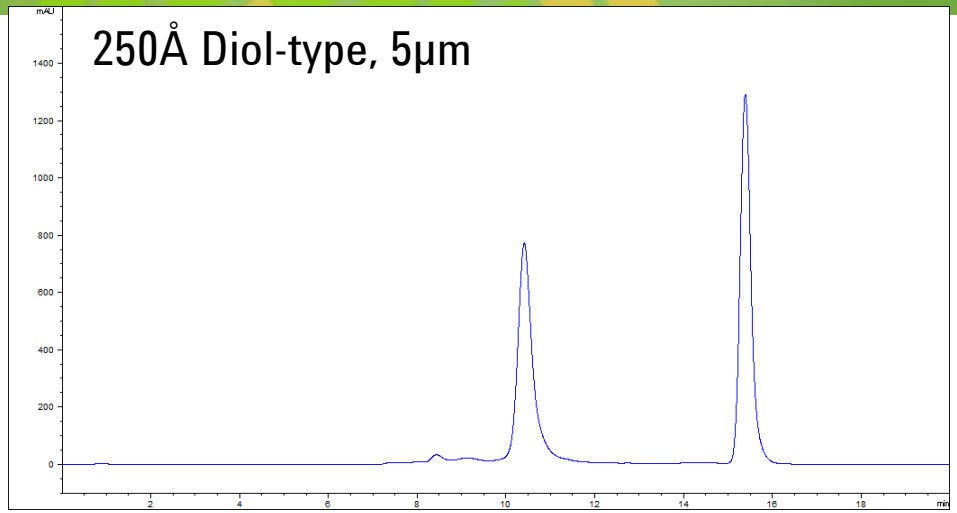
Multiple sample types
mAbs and next generation biologics

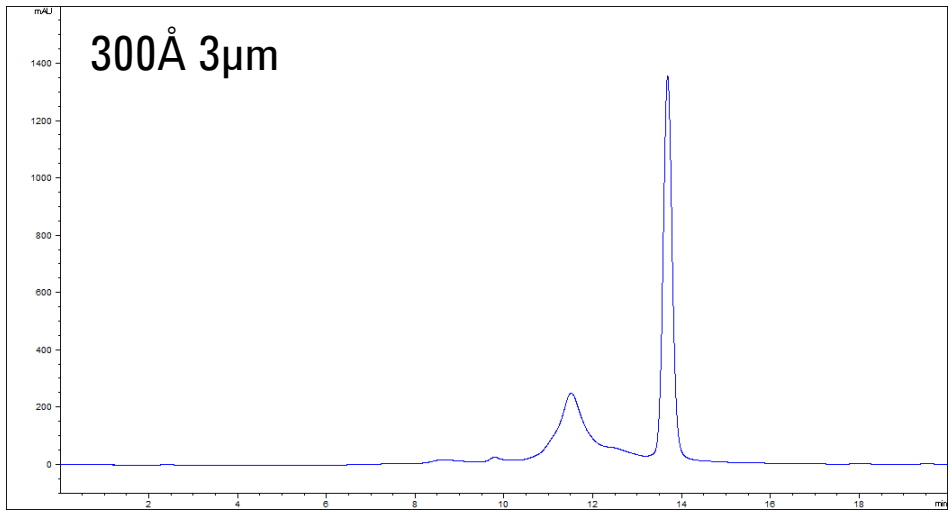


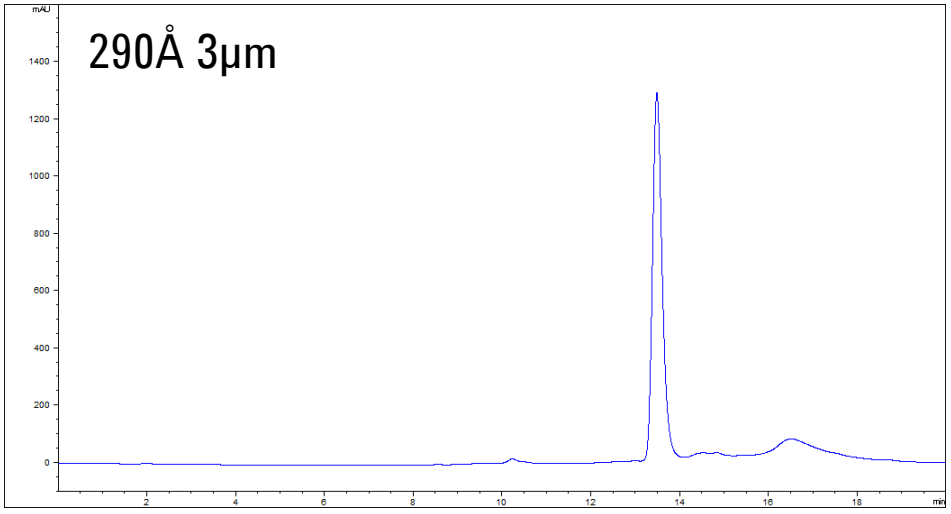
Difficult Samples

Problematical mAbs ...

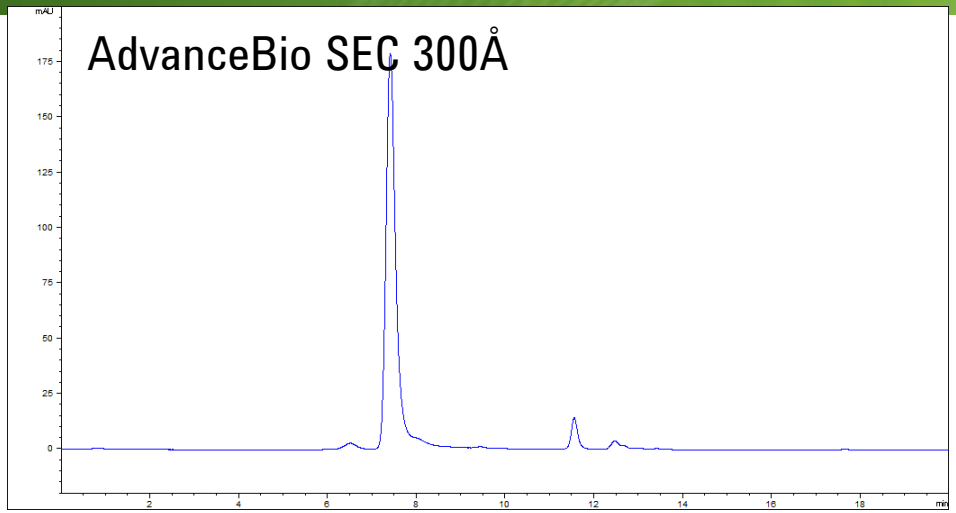


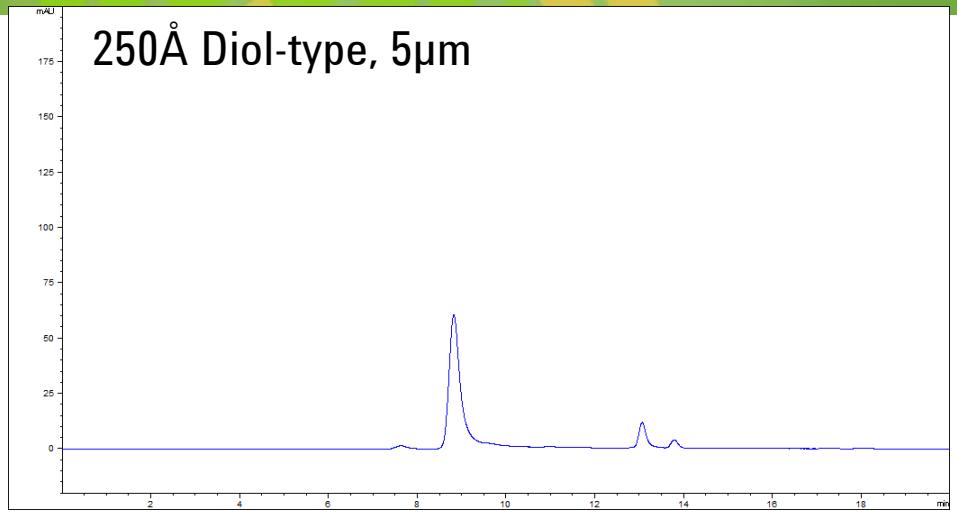


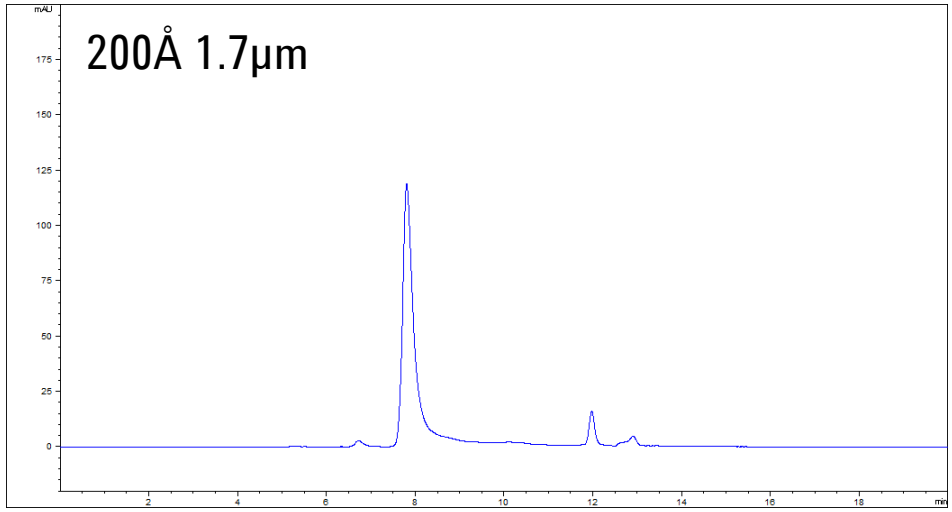


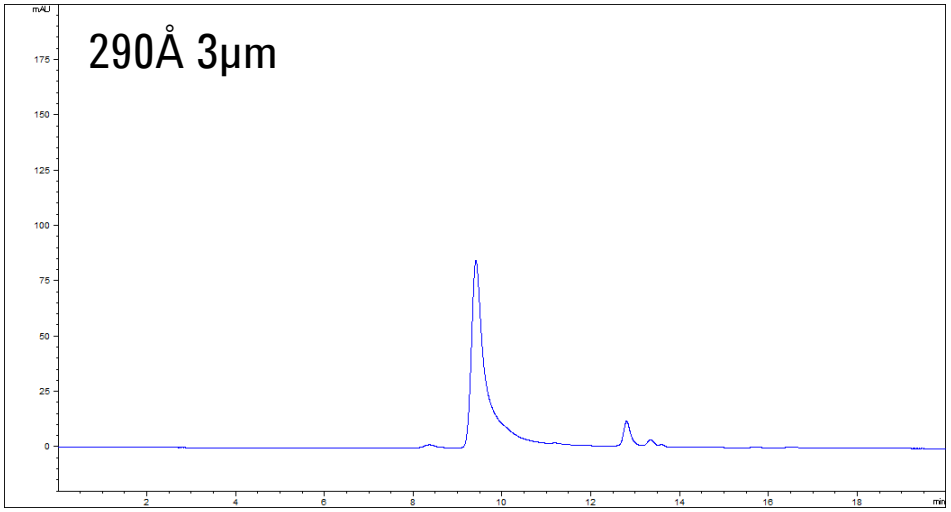


Difficult Samples
Antibody Drug Conjugates (ADCs)

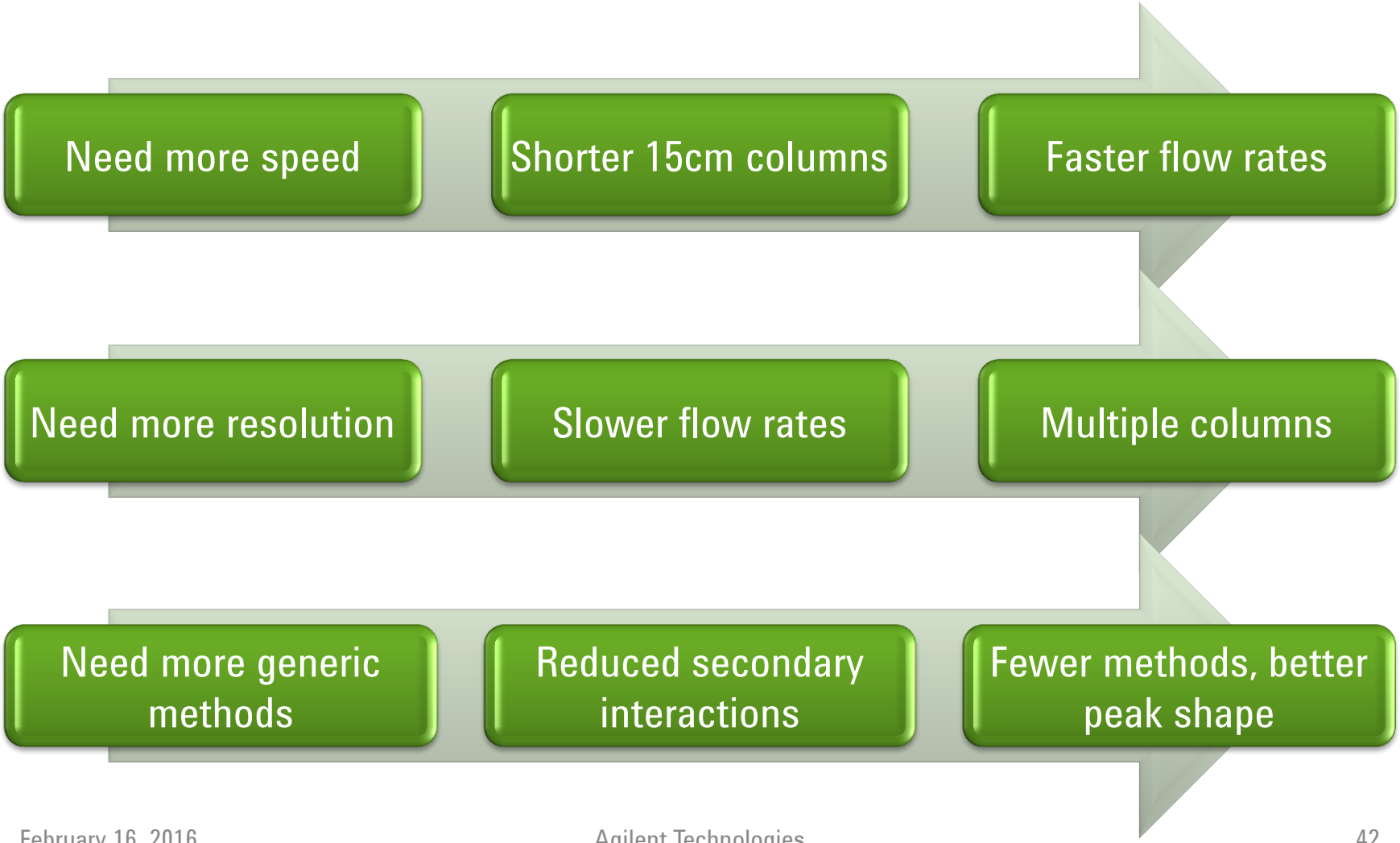




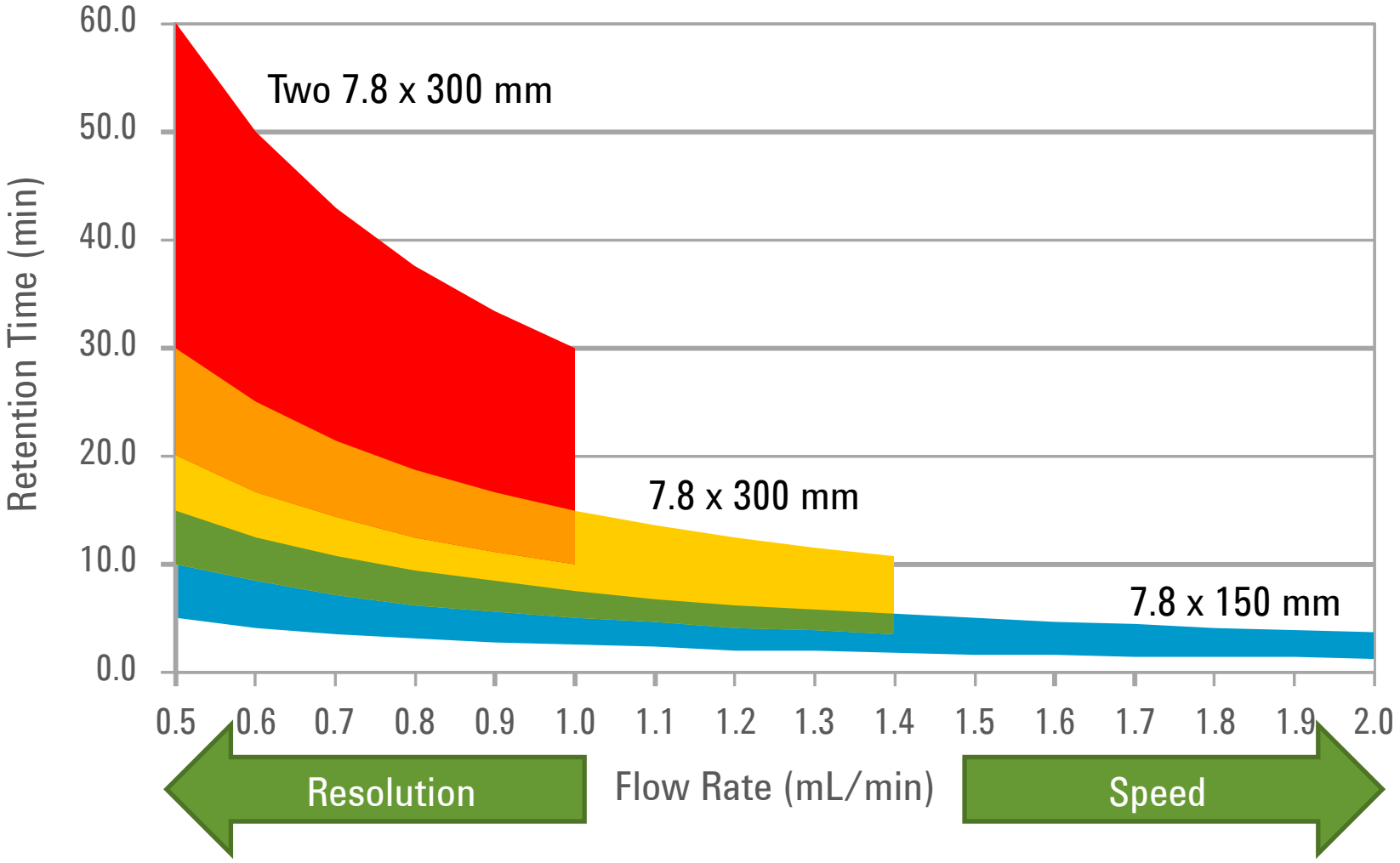




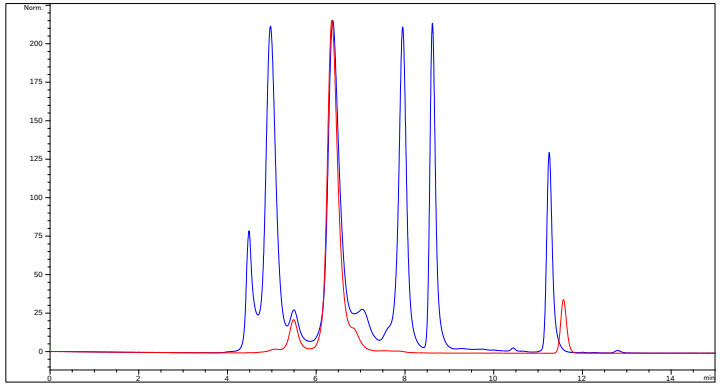
Recap


Need more speed
Shorter 15cm columns
Faster flow rates
Need more resolution
Slower flow rates
Multiple columns
Need more generic methods
Reduced secondary interactions
Fewer methods, better peak shape

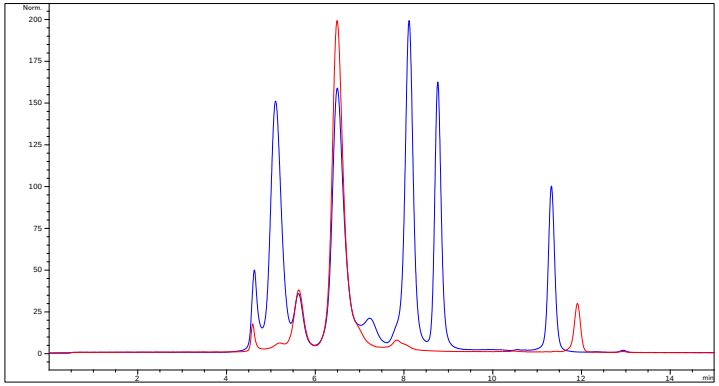
Column choice - resolution or speed - requires different column formats



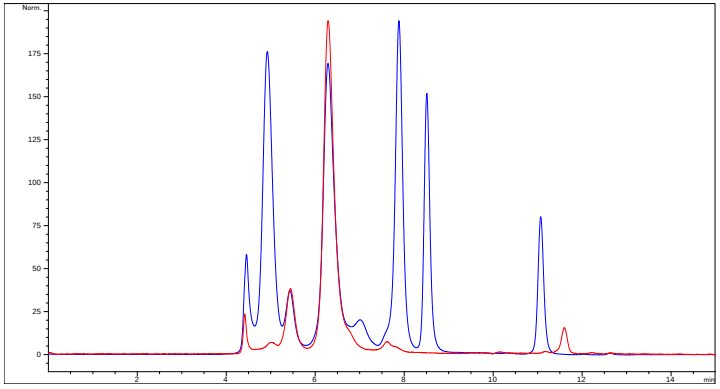
Separation of protein standards and IgG using different Agilent HPLC systems



Agilent 1100 LC System



Agilent 1260 Bio-inert LC System

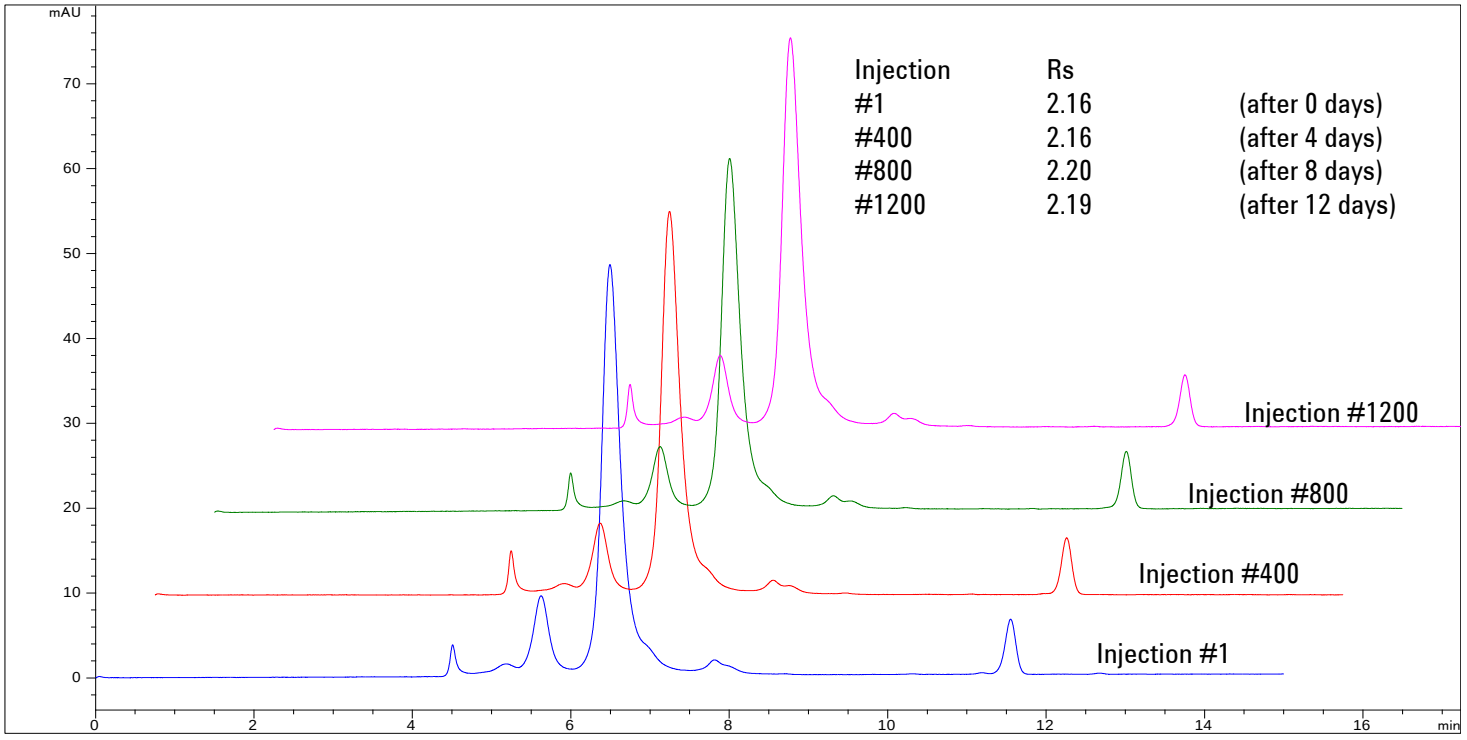


Agilent 1290 Infinity LC System

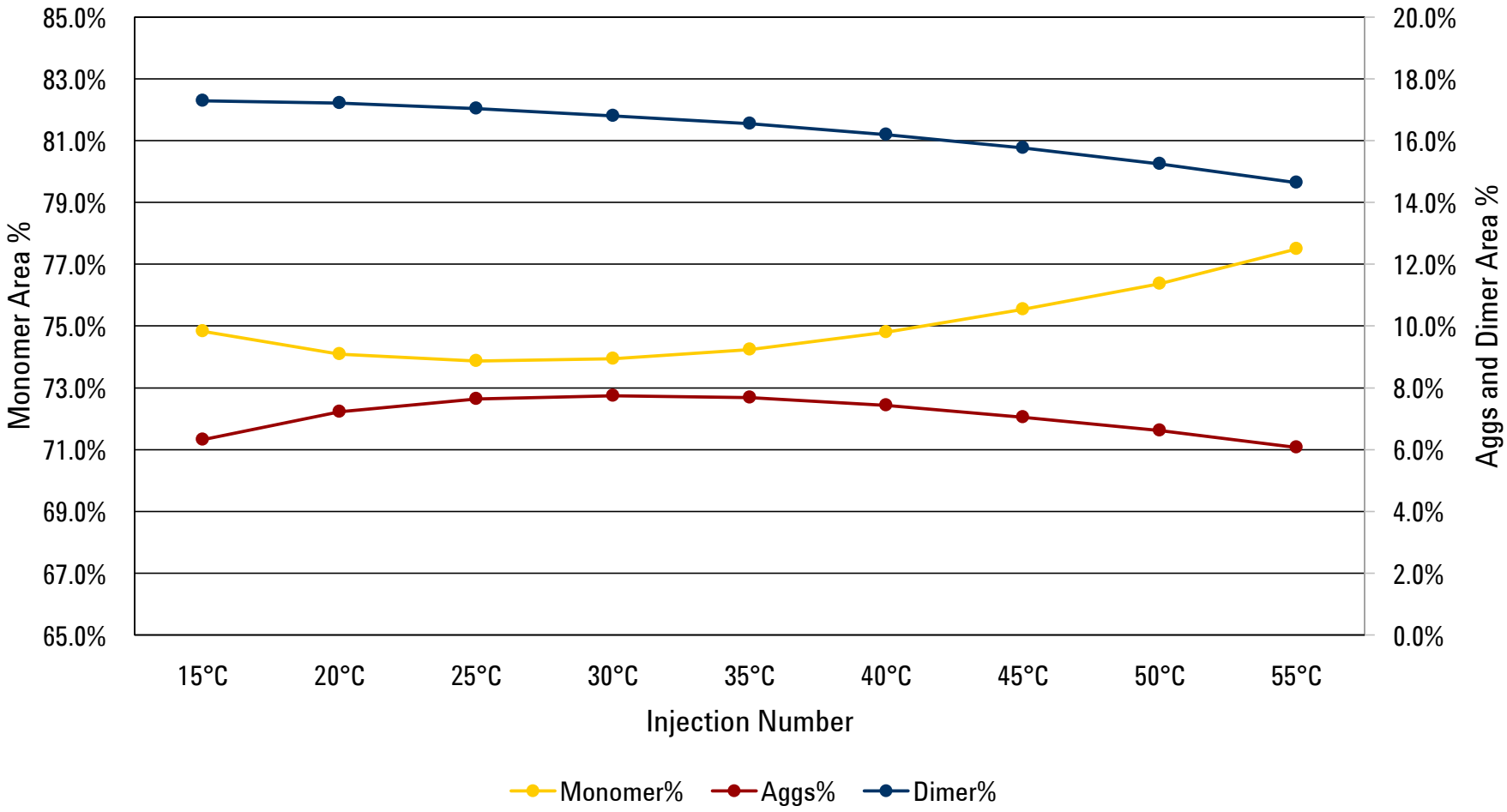
Conditions

Eluent	150 mM Sodium phosphate buffer, pH 7.0
Flow rate	1.0 mL/min (7.8 mm ID columns) or 0.35 mL/min (4.6 mm ID columns)
Temperature	Ambient
Sample	1 mg/mL protein concentration
Instrument	Agilent 1100 HPLC instrument (legacy, 400 bar)
	1260 Infinity Quaternary Bio-inert (600 bar)
	1290 Infinity Binary LC with G1315D DAD and Bio-inert flow cell (1200 bar)
Wavelength	220 nm

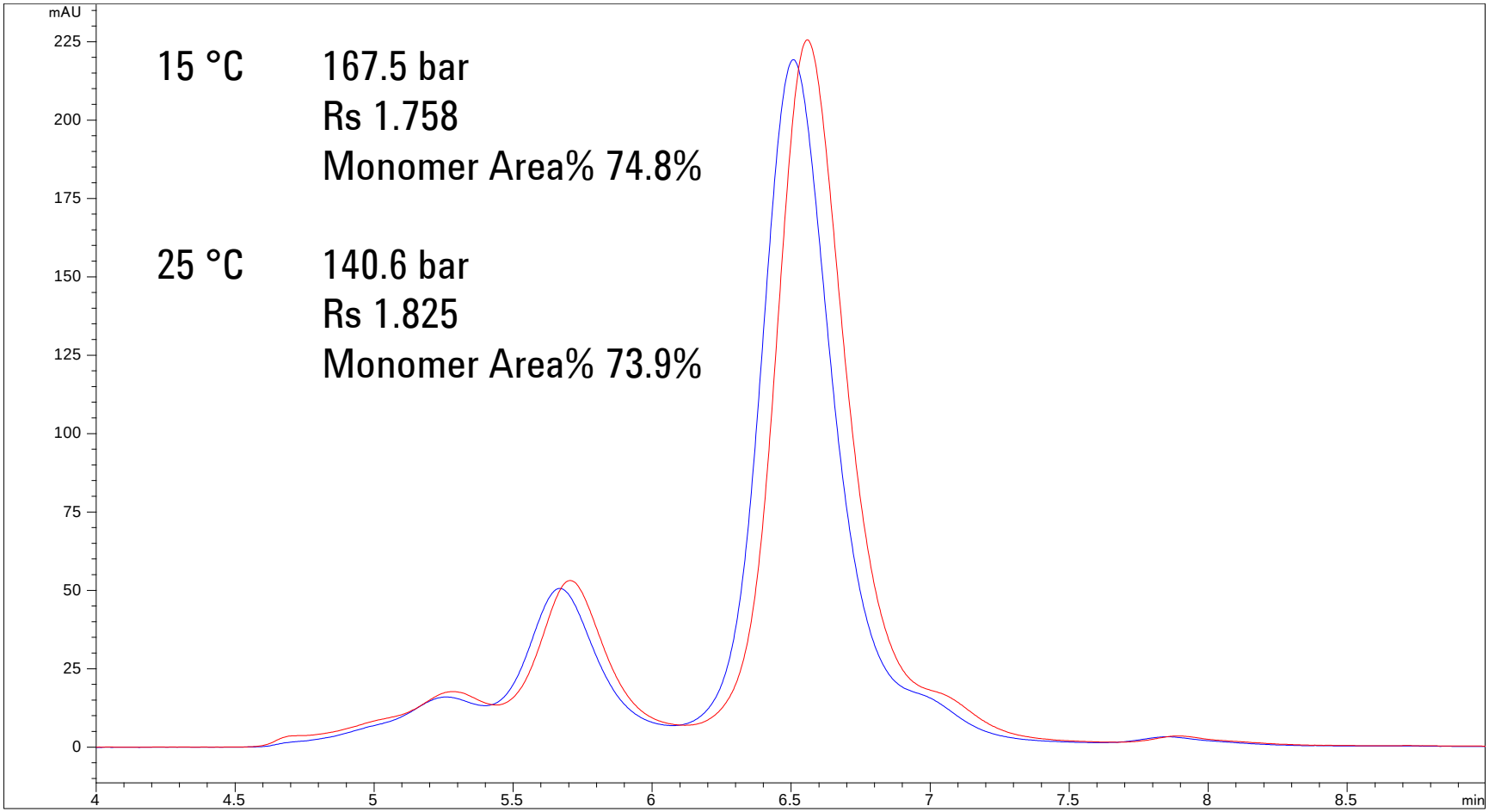
Agilent AdvanceBio SEC
Ensuring reliability



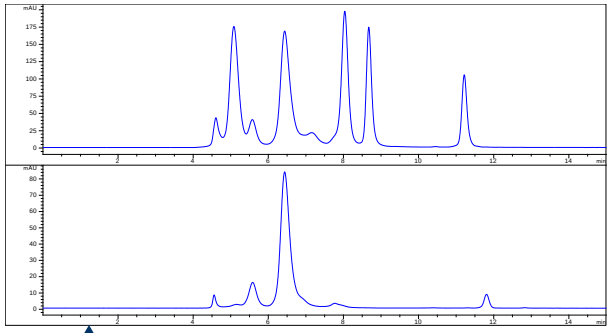
Effect of analysis temperature of on monomer / dimer / aggregate content of γ-Globulin



Control of temperature

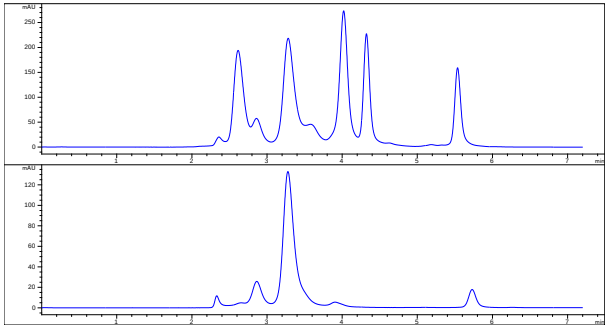


Column dimensions



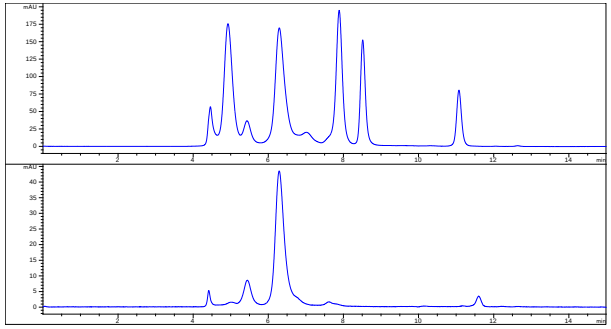
4.6 or 7.8 x 300 mm

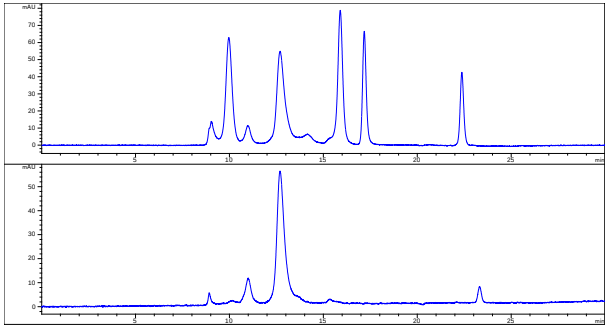
More speed



4.6 or 7.8 x 150 mm

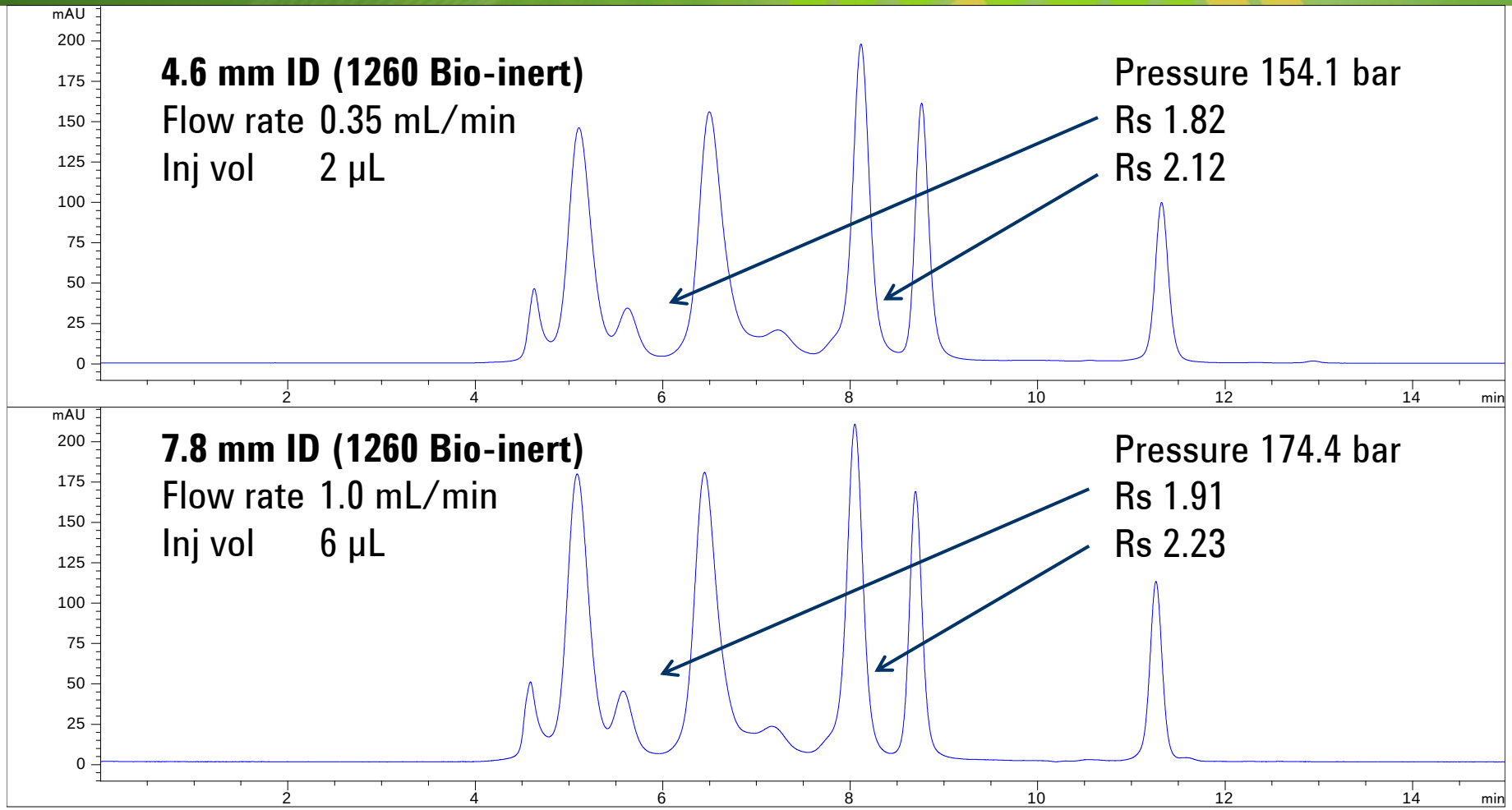
More resolution



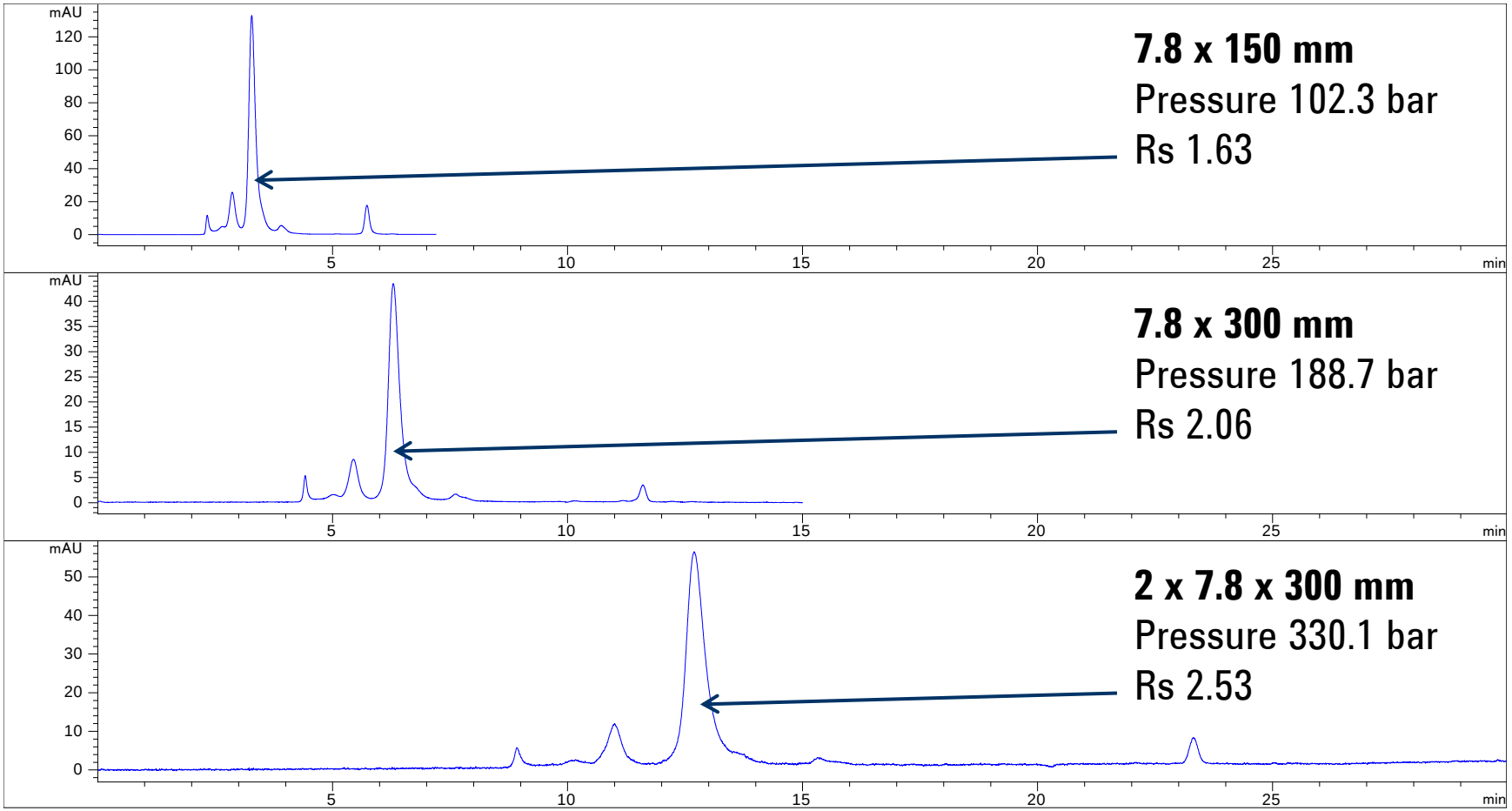


Two 4.6 or 7.8 x 300 mm

Robustness or sensitivity



Resolution 150 mm vs 300 mm vs 2 x 300 mm



Summary

- For resolution use longer column lengths 300 mm
  - Reduce flow rate
- For speed use shorter columns 150 mm
  - Increase flow rate
- Generic methods for wide range of sample types
  - Use an innovative SEC column that has minimal non-specific interactions

The AdvanceBio SEC columns provide solutions for all the above

Thank you !