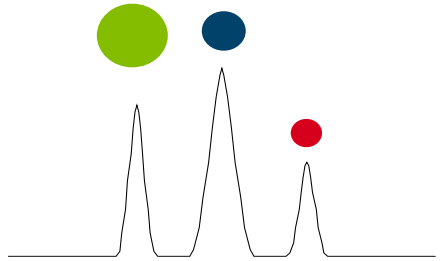
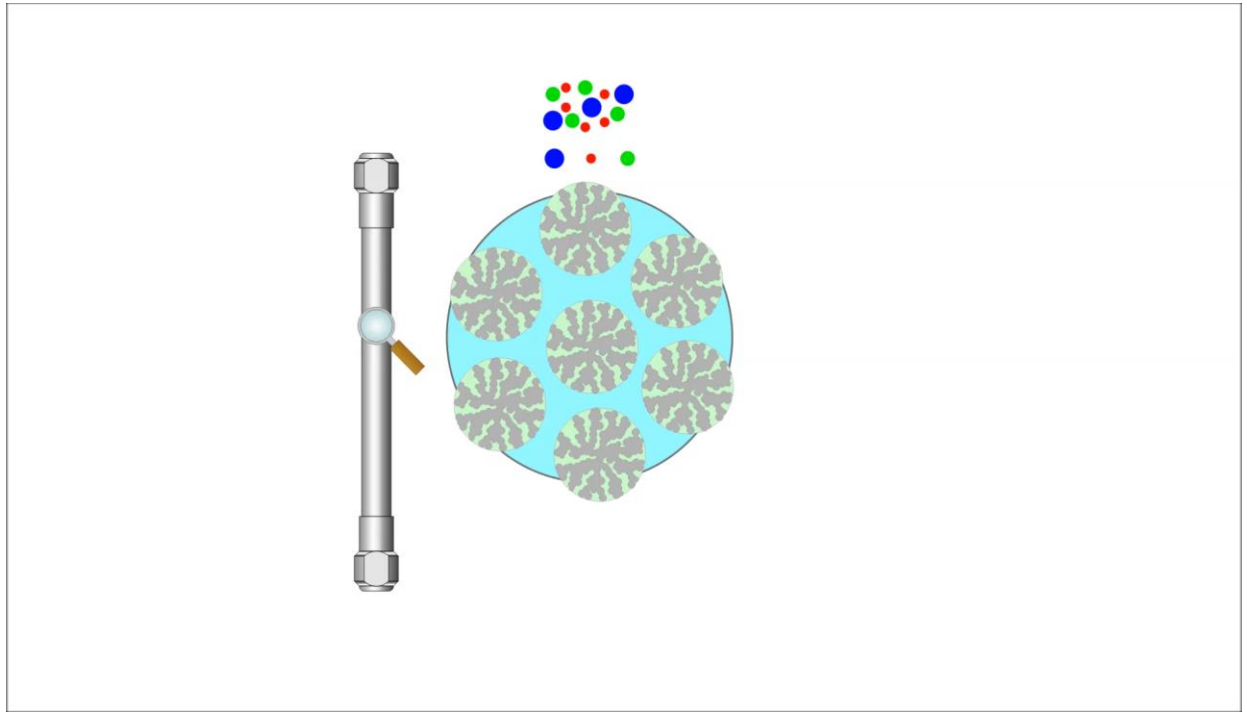
Maximizing Performance Through GPC Column Selection: Then and Now

Jean Lane
Application Engineer
LC columns and Consumables Technical Support
June 7, 2022



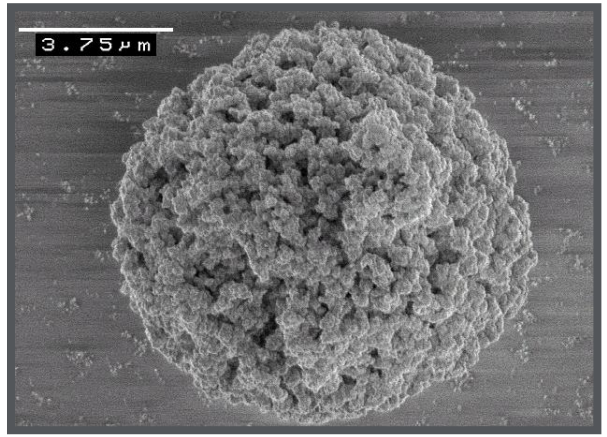
GPC/SEC Separation Mechanism

- A GPC/SEC column is packed with porous beads of controlled porosity and particle size
- A sample is prepared as a dilute solution in the eluent and injected into the system
- Large molecules are not able to permeate all of the pores and have a shorter residence time in the column
- Small molecules permeate deep into the porous matrix and have a long residence time in the column
- Sample molecules are separated according to molecular size, eluting the largest first and smallest last



Packings for GPC/SEC Columns: What are GPC Columns Made Of?

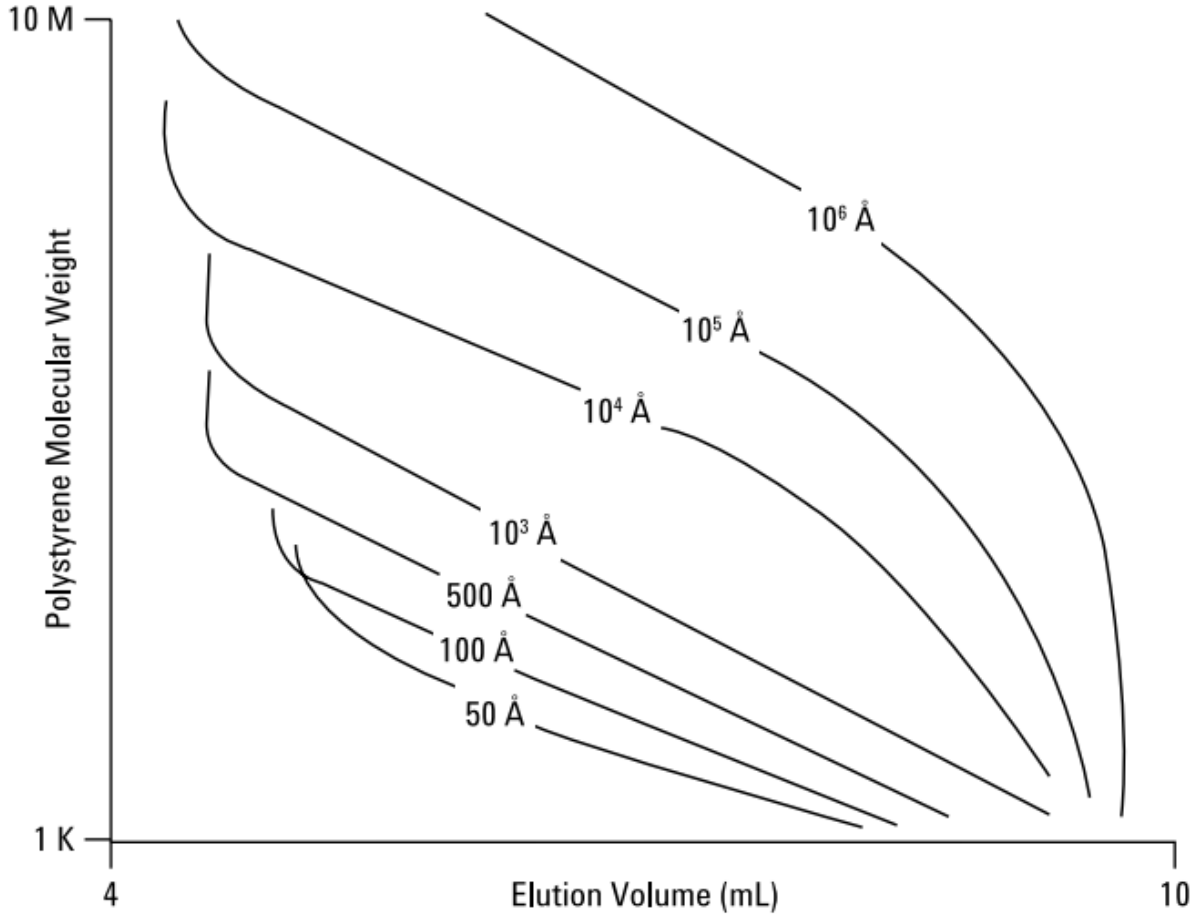
- Silica packings
  - Mechanically stronger
  - Exhibit enthalpic properties due to presence of silanols
  - Typically have lower pore volumes
- Polymeric packings
  - High pore volume and vendor specific differences in mechanical stability
  - Due to polarity of stationary phase, observed interactions are reduced



Column Type: Individual Pore Size

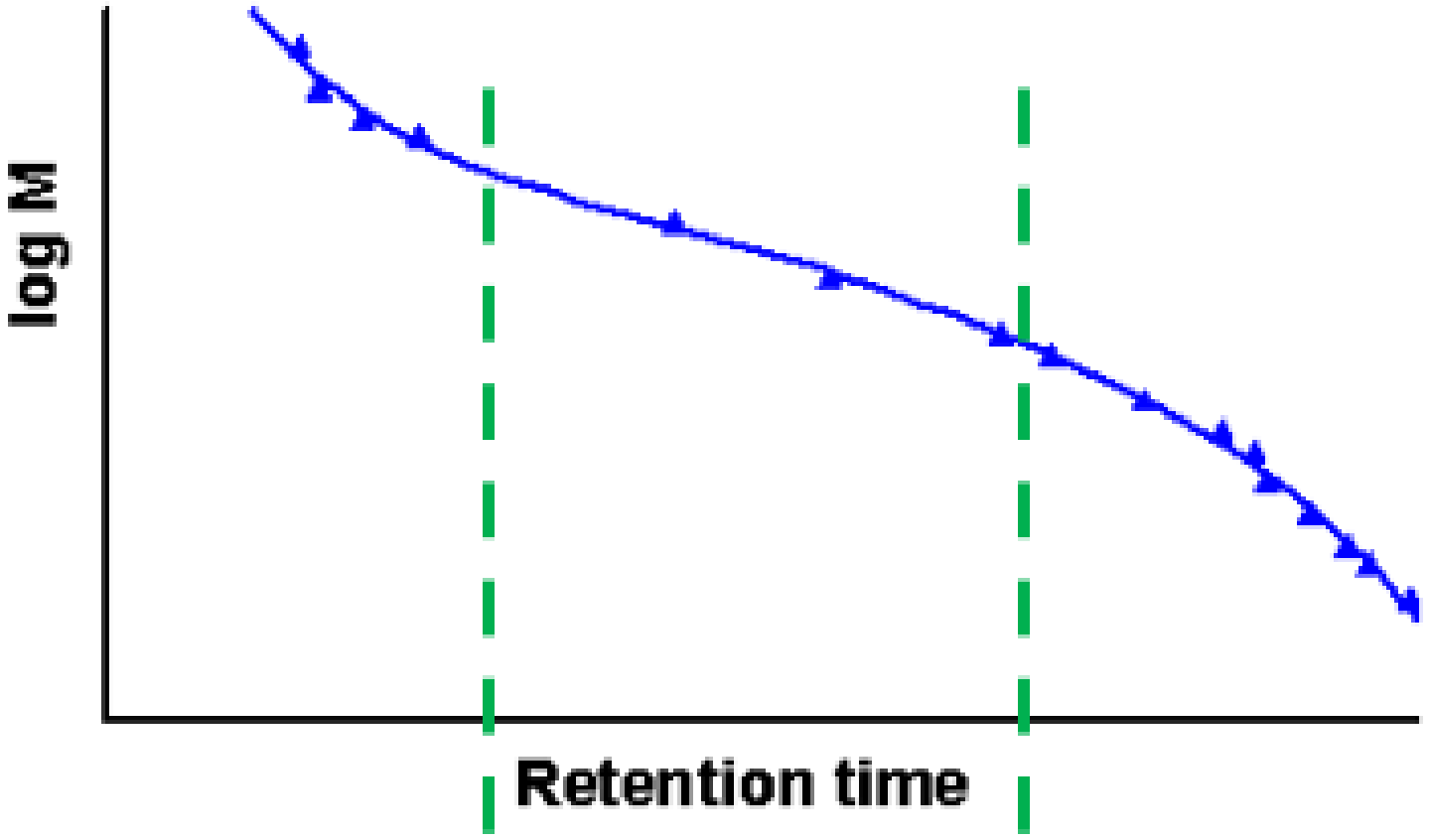
- All particles have the same pore size
- Good separation, but narrow range of molecular weight
- Very nonlinear curve; linear only over a narrow molecular weight range
- Oldest technology, but useful for separating very small and very large compounds
- Wider molecular weight range only possible by combining different columns in series, but need to select carefully to avoid column ‘mismatch’

Calibration curve



PLgel individual pore size calibration plots

Individual Pore Size



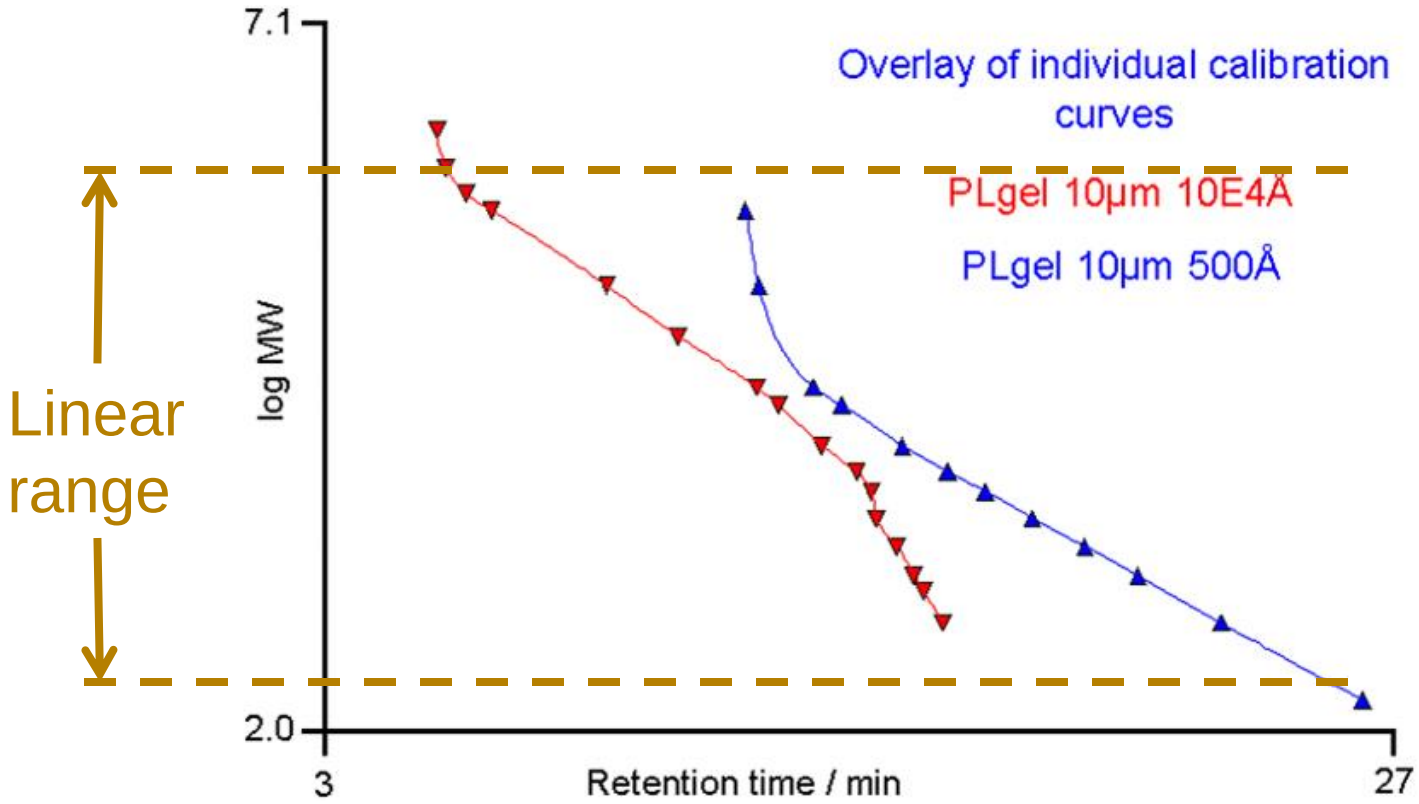
PLgel 5 µm, 10E4 Å

Mol wt range: 60 K – 400 K

Good resolution but only over a limited mol wt range

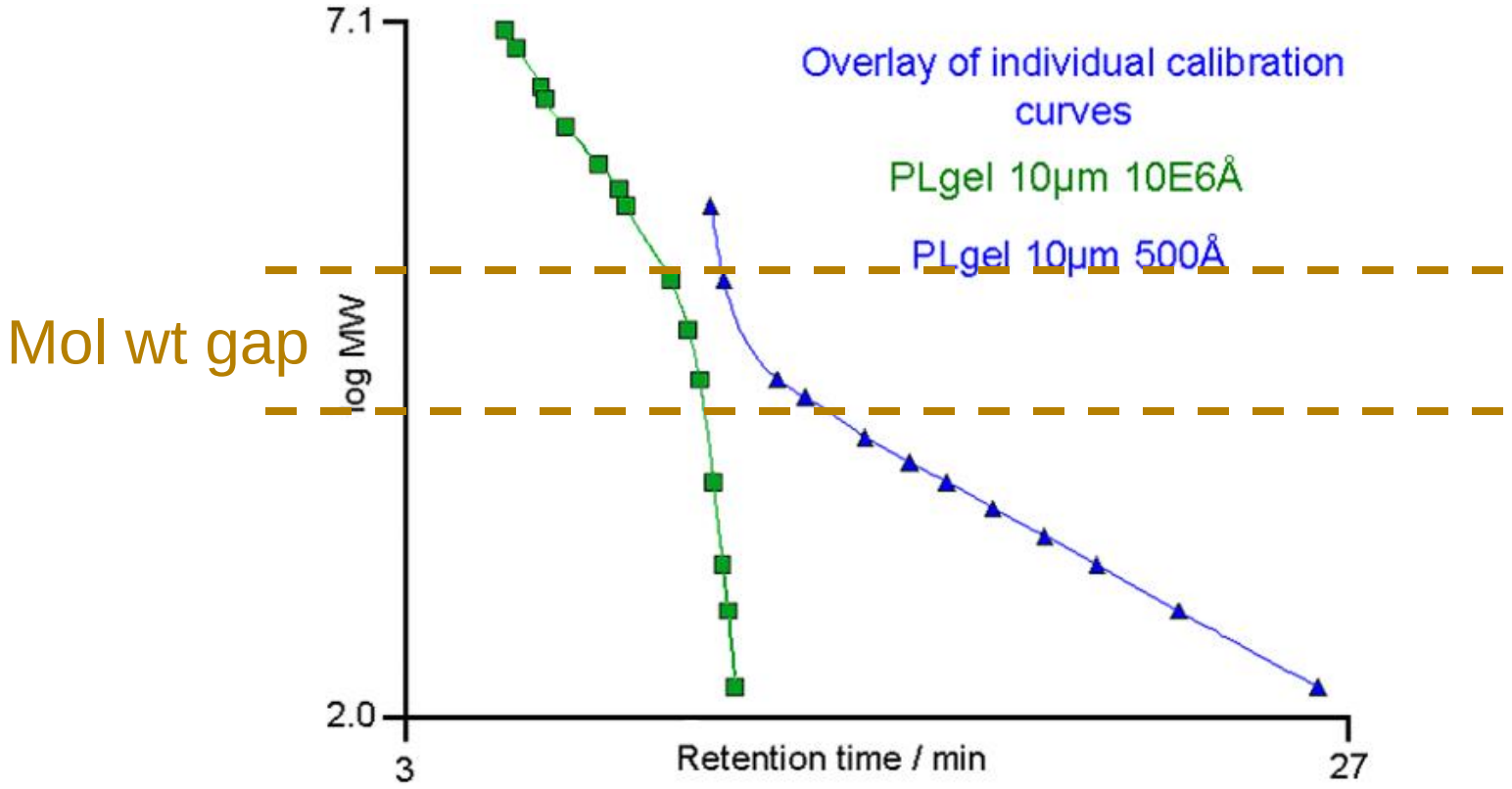
Linear range of the column limited

Increasing the Resolving Range



- Individual columns can be coupled in series
  - PLgel and PL aquagel-OH
- Need linear calibration ranges to complement without overlap

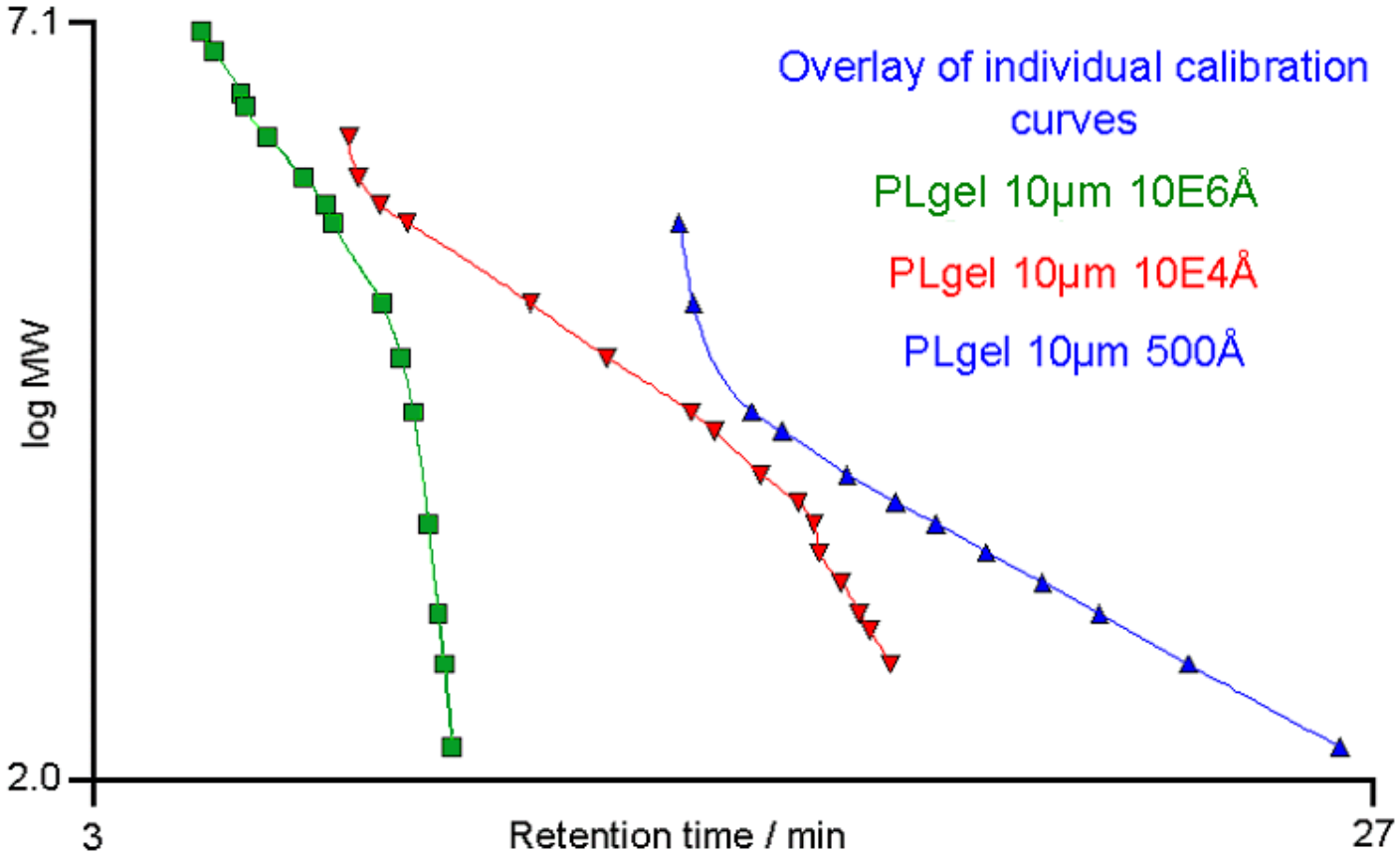
Wrongly Coupled Columns



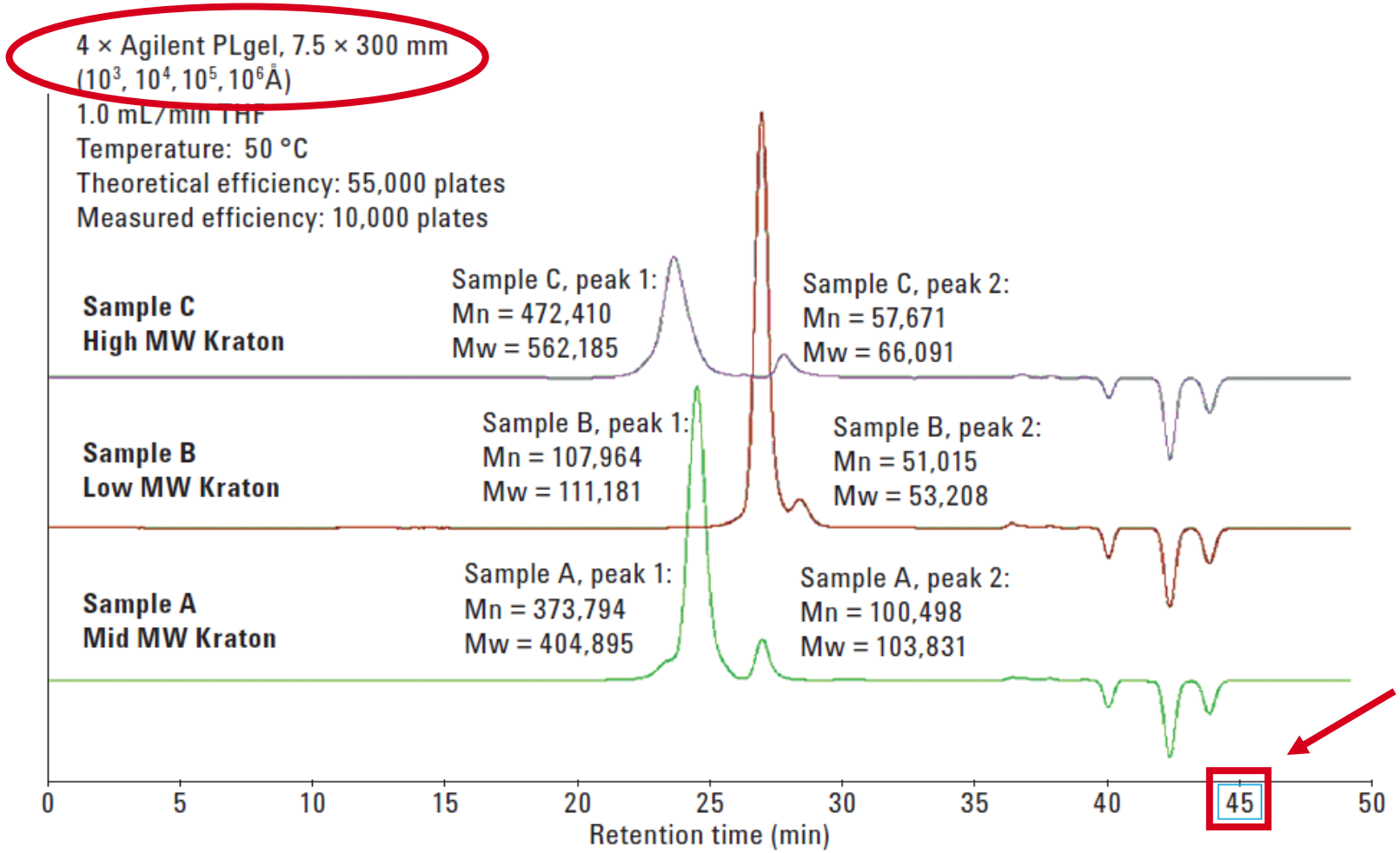
- Mol wt gap between linear ranges
- Changes retention and gives unusual peak shapes

Combination of Individual Pore Size Columns

Traditional approach to increasing mol wt operating range of column set



Conventional GPC Using a Standard GPC System
Individual Pore Columns



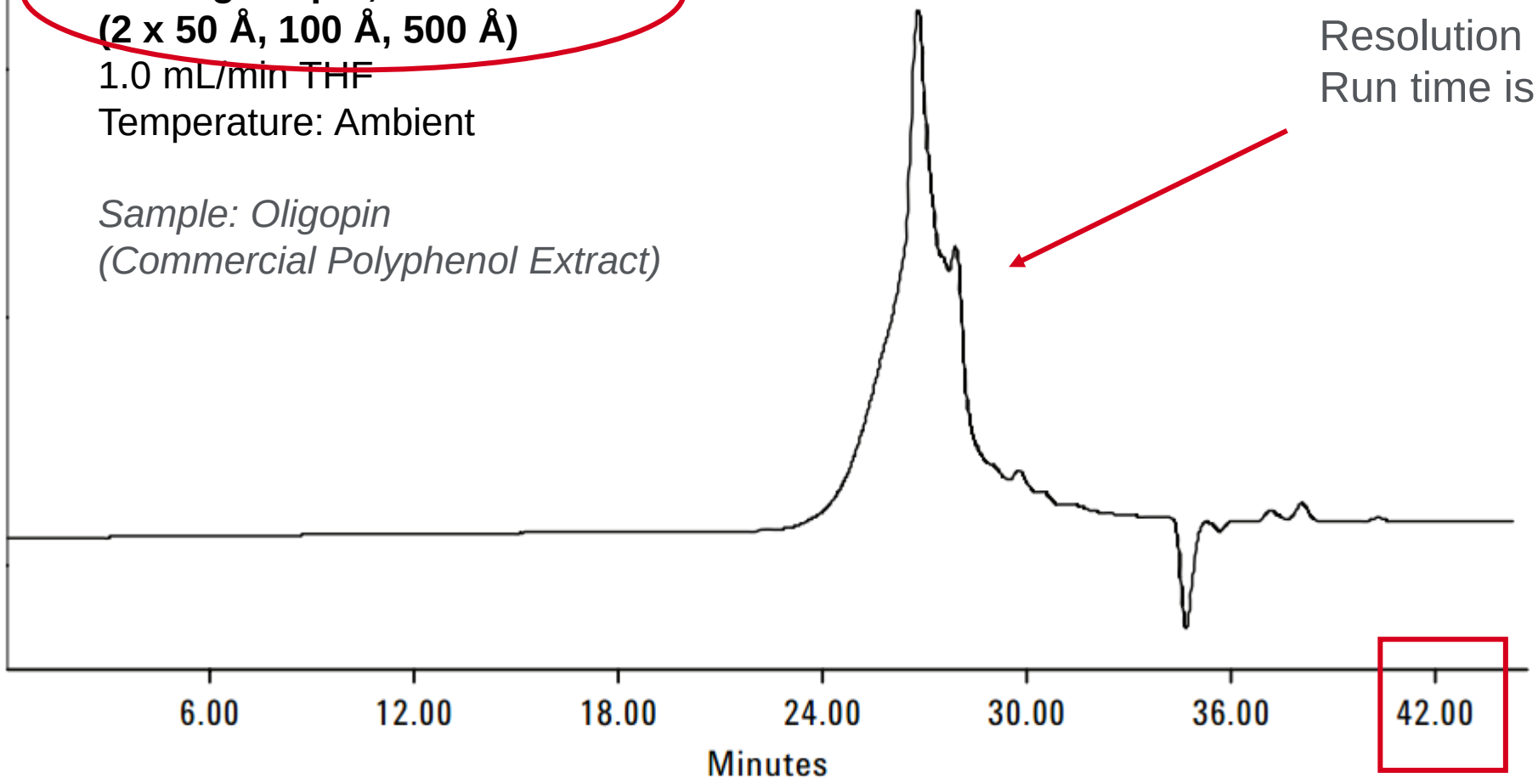
4 columns in series
Run time 45 minutes

Conventional GPC Using a Standard GPC System Individual Pore Columns

4 x PLgel 5 µm, 7.5 x 300 mm
(2 x 50 Å, 100 Å, 500 Å)
1.0 mL/min THF
Temperature: Ambient

Sample: Oligopin
(Commercial Polyphenol Extract)

4 columns in series
Resolution is only fair
Run time is 42 mins



Pain Points and Common Challenges for SEC

- Molecular weight ranges for the columns are limited
- Use of multiple columns in series can lead to mismatch/dislocations
- Need more resolution or it is insufficient
- Long analysis times reduce sample throughput
- Long analysis times increase solvent use and costs
- Nonspecific interactions contribute to loss of sample and lead to inconsistent results and rework
- Need consistent and reproducible results

Making Your GPC/SEC Column Selection

Key questions to ask

- What polymer are you analysing?
- What solvent is your polymer soluble in?
- What is the expected molecular weight range of your polymer?
- What is the requirement for your analysis or what would you like to improve about your existing GPC/SEC separation?
  - Resolution is important
  - Reproducibility of sample chromatography and results
  - Speed of analysis and/or sample throughput is something to improve on

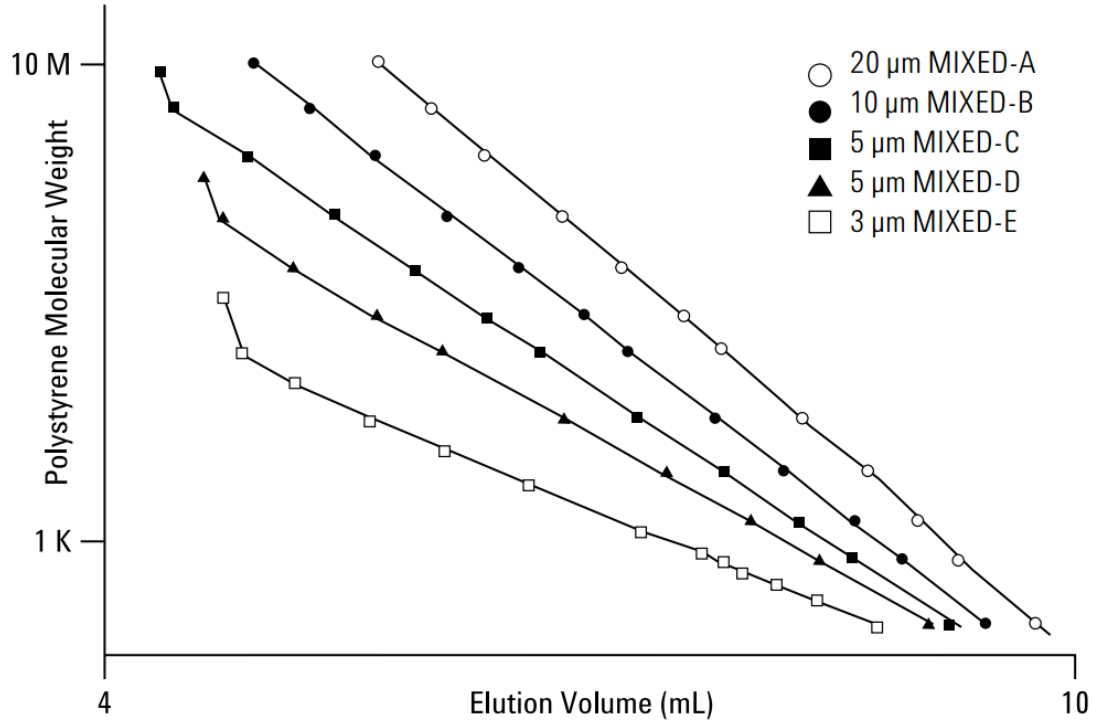
Considerations for Column Selection

- Column selection depends on:
  - Molecular weight
  - Polydispersity
  - Presence of additives
  - Solvents required
  - Temperature required
- Helpful to know the properties of the sample
- Necessary to understand the properties of the columns

Column Types: MIXED

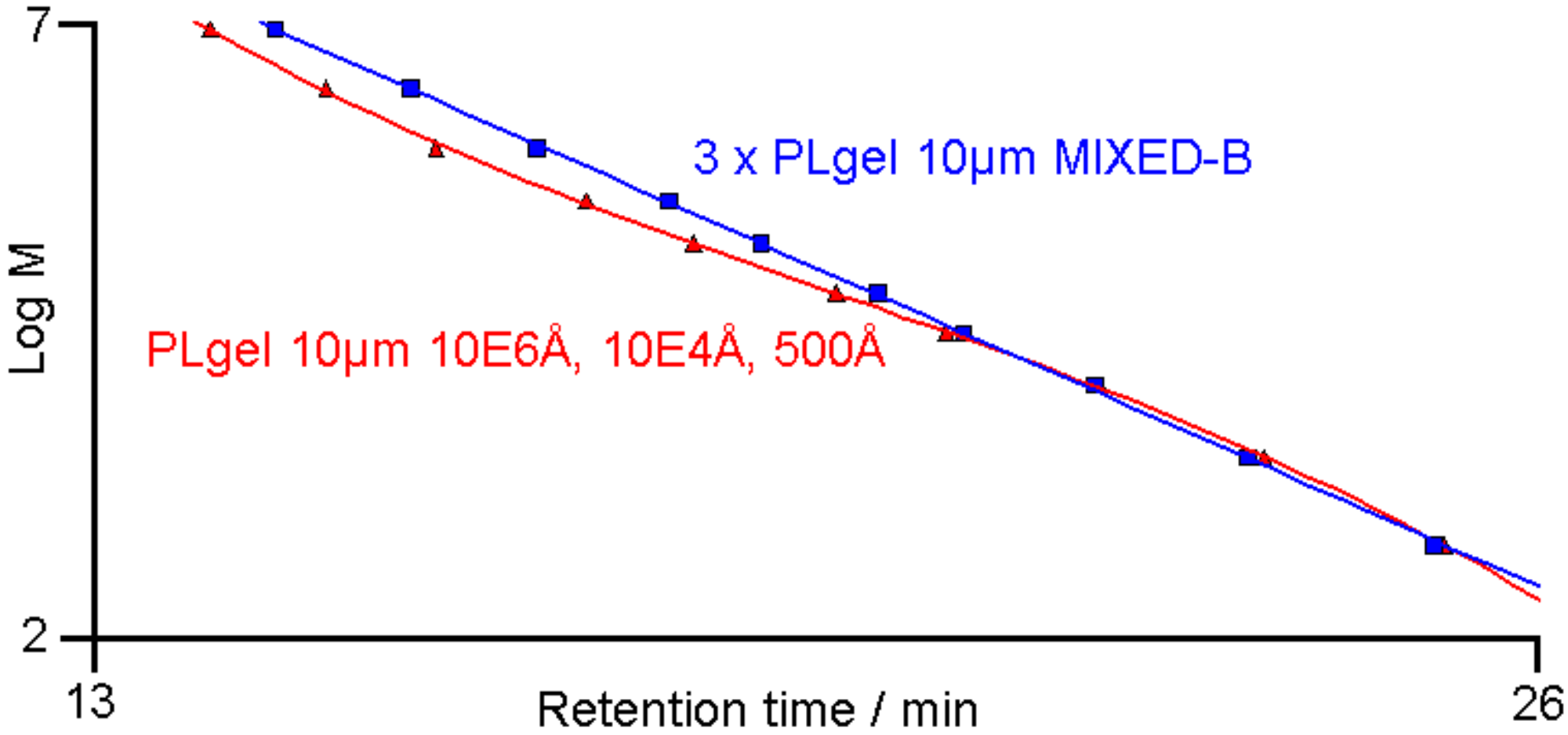
- Individual pore size particles are mixed together/blended to make a linear curve
- Very wide ranges possible, but only a small amount of separation of each mol wt
- Linear curve makes chromatogram easy to read and analyze
- Most popular technology; well established and widely used
- Columns in series of same type are still linear

Column family: PLgel



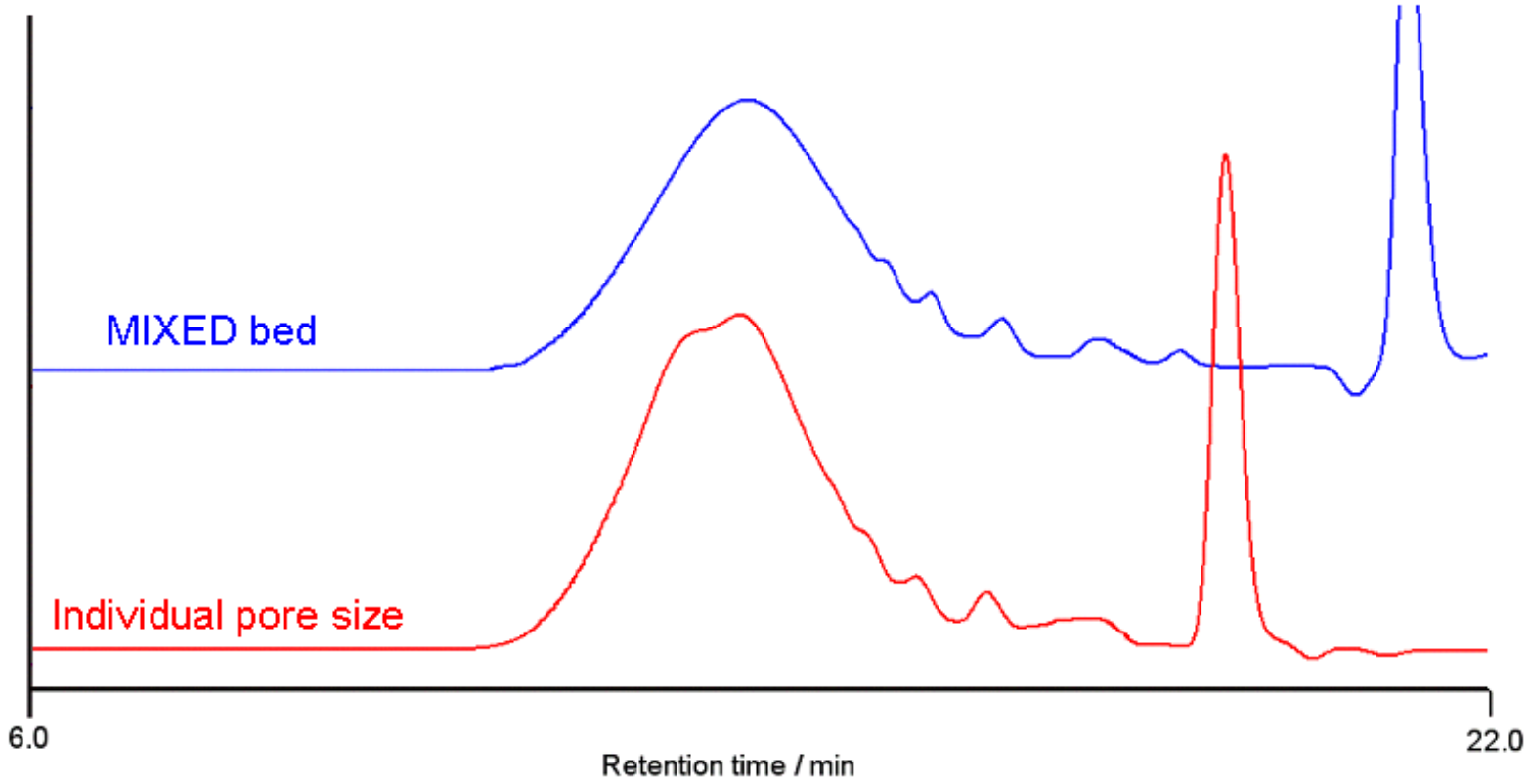
PLgel MIXED calibration plots

Comparison of Columns for Extend Mol Wt Range
Individual pore versus MIXED



Comparison of Columns for Extend Mol Wt Range

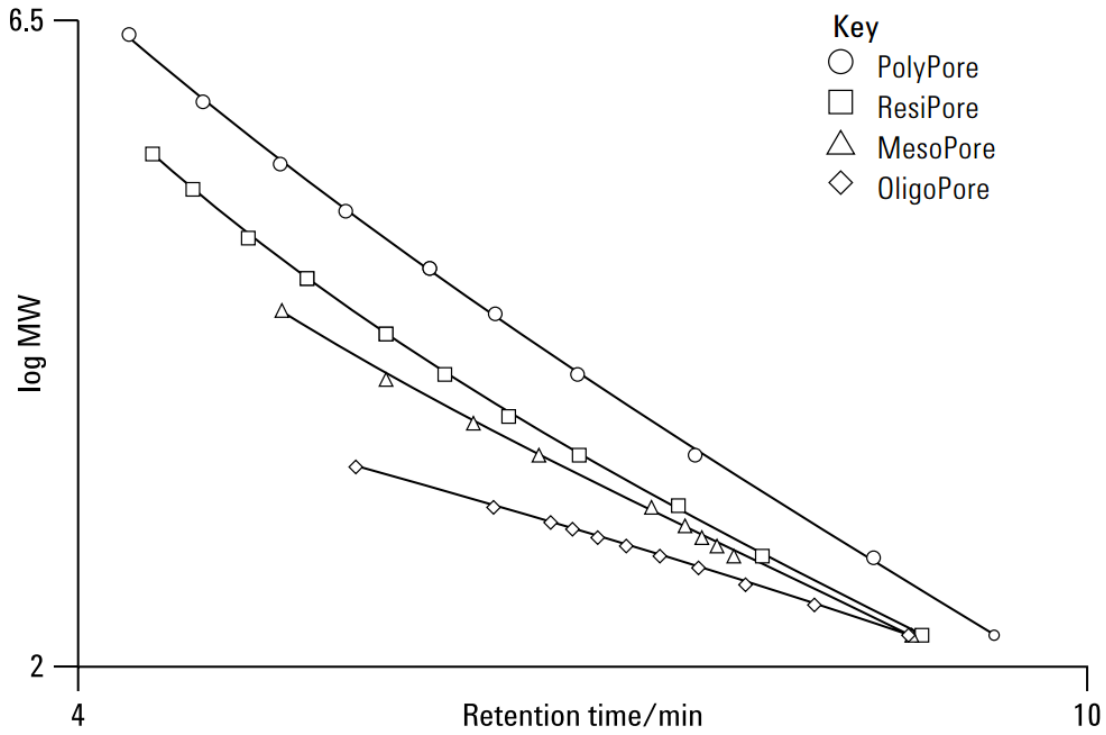
Individual pore versus MIXED for our sample



Column Types: Multipore Particle

- Newest, fastest growing technology
- Each particle has multiple pore sizes
- Increased pore volume
- Highest resolution and efficiency
- Best performance for most common mol wt ranges

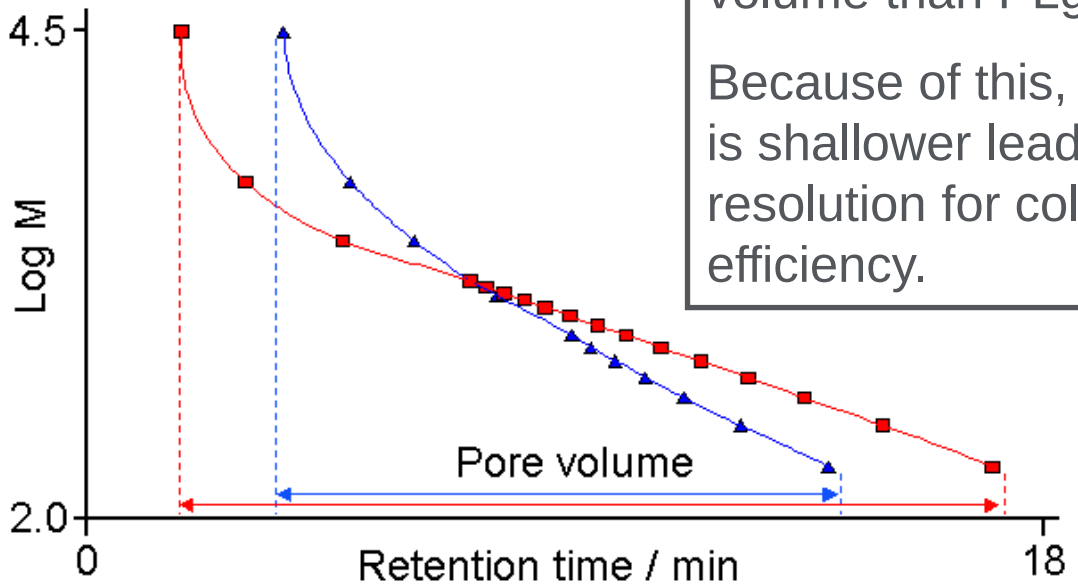
Column family: PlusPore



PlusPore calibration plots

Individual Pore versus MultiPorous Particle

Columns	2 x PLgel, 3 µm, 100 Å, 300 x 7.5 mm
	2 x OligoPore, 300 x 7.5 mm
Eluent	THF
Flow rate	1.0 mL/min



▲ - PLgel
■ - Oligopore

Both columns have a similar exclusion limit, but OligoPore has greater pore volume than PLgel 100 Å.

Because of this, the slope of the curve is shallower leading to greater resolution for columns of the similar efficiency.

Column Selection and Importance of Solvent Choice

Criteria for solvent selection

- The factor that principally controls which type of column is selected for a GPC analysis is the solvent
- Many polymer dissolve in only very limited numbers of solvents
- The columns used must be compatible with the solvent of choice
- Solvent choice permits adequate detection
- Most importantly, the size exclusion mechanism must be maintained

Column Selection – Solvent

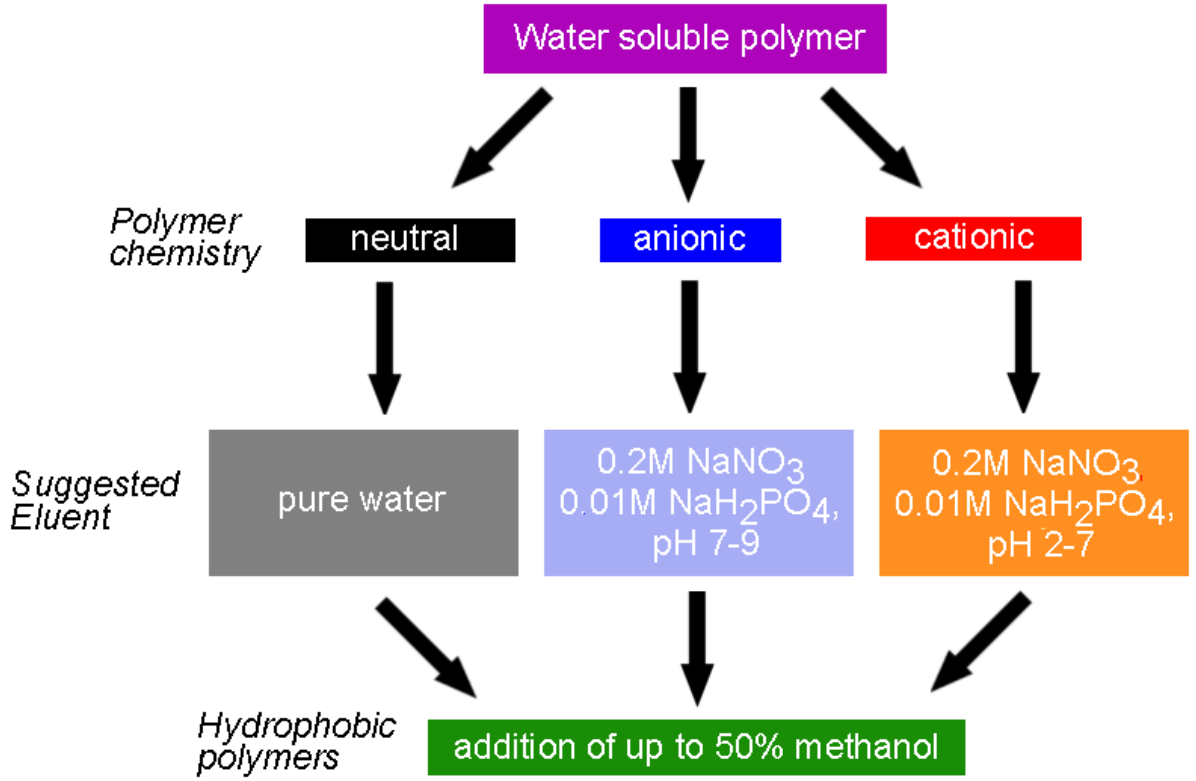
- Solvent determination is very simple

"What does the polymer dissolve in?"

- Organic – PLgel Individual/PLgel MIXED or PlusPore
- Aqueous – PL aquagel-OH
- Polar organic or organic/aqueous mixtures – PolarGel
- Aggressive solvents/temperatures – PLgel or specialist columns

Solvents for Organic and Aqueous SEC

Solvent polarity	Solvent
6.0	Perfluoroalkane
7.3	Hexane
8.2	Cyclohexane
8.9	Toluene
9.1	Ethyl acetate
9.1	Tetrahydrofuran (THF)
9.3	Chloroform
9.3	Methyl ethyl ketone (MEK)
9.7	Dichloromethane
9.8	Dichloroethene
9.9	Acetone
10.0	0-Dichlorobenzene (o-DCB)
10.0	Trichlorobenzene (TCB)
10.2	m-Cresol
10.2	o-Chlorophenol (o-CP)
10.7	Pyridine
10.8	Dimethyl acetamide (DMAc)
11.3	n-Methyl pyrolidone (NMP)
12.0	Dimethyl sulfoxide (DMSO)
12.1	Dimethyl formamide (DMF)



Column Selection - Solvent

- What solvent is your sample soluble in?

Type	Typical Solvents
Organic	• THF • Chloroform • Toluene • TCB
Mixed or Polar Organic	• THF/water • DMF • NMP
Aqueous	• Water • Buffer in water • Water/methanol (up to 50%)

Additives can be employed:

- Minimize non-size exclusion interactions between the sample and the column
- Stabilize the solution of the polymer (ionic aggregation)

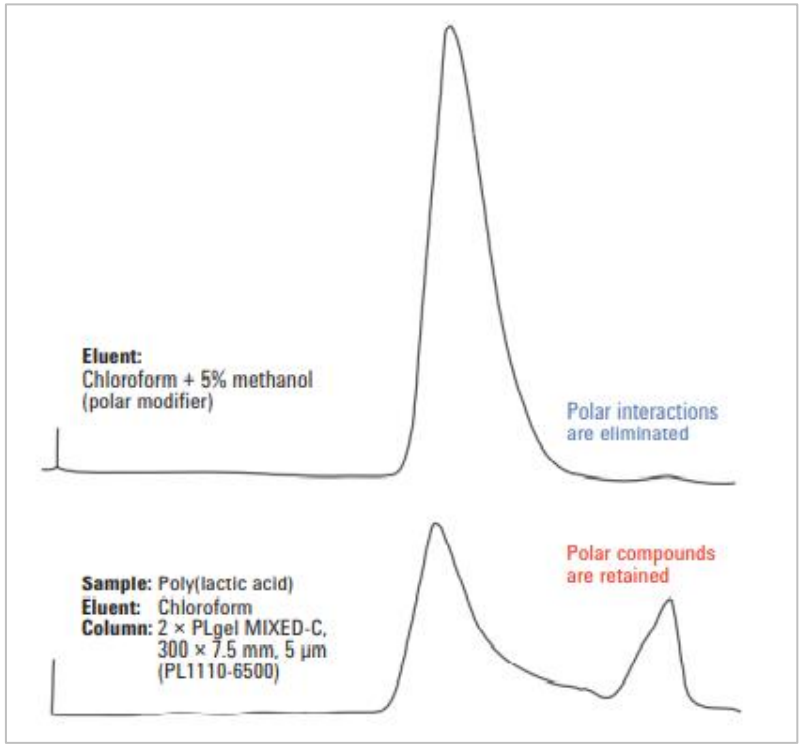
Polymer to Solvent Reference Table

Successful Solvent Choice

Agilent InfinityLab

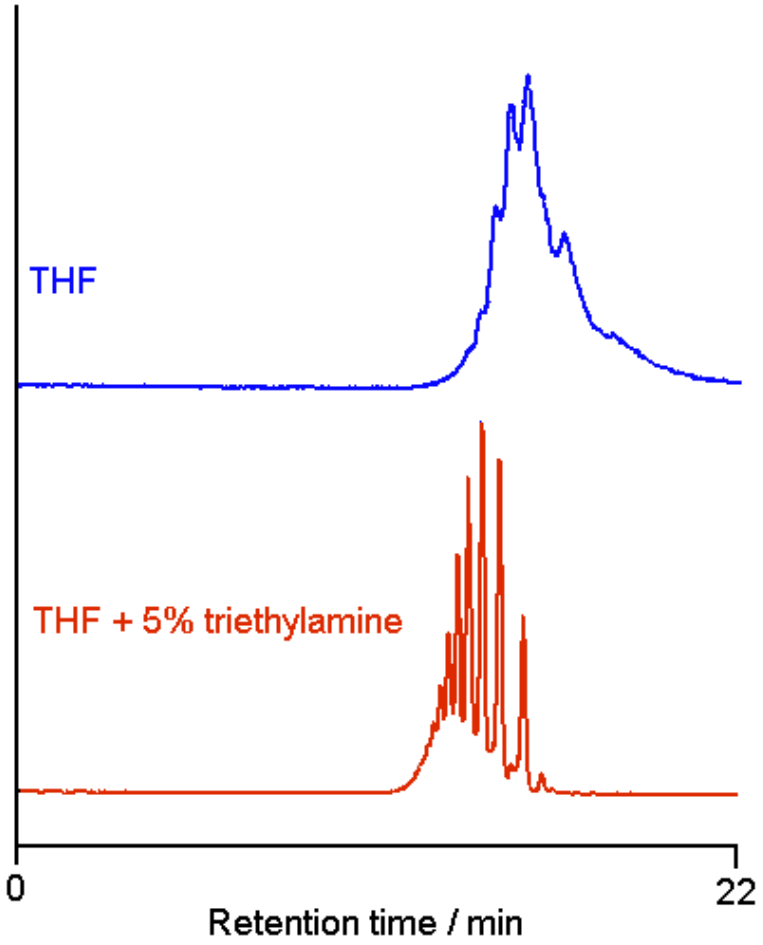
Tips for use of additives:

- Addition of salts to aqueous and polar organic solutions is the preferred method to eliminate polar interactions by electrostatic screening. **Salts should be flushed from the system after analysis.**
- For water-soluble polymers, interactions can also be minimized by addition of an organic solvent, such as methanol
- Lewis bases such as polyamines and polyamides may interact with polymeric media, but this can be eliminated by the addition of an amine to the mobile phase, such as triethylamine (TEA)



Polar interactions in the lower chromatogram are eliminated with 5% methanol addition to the eluent

Eluent Modification in Organic GPC



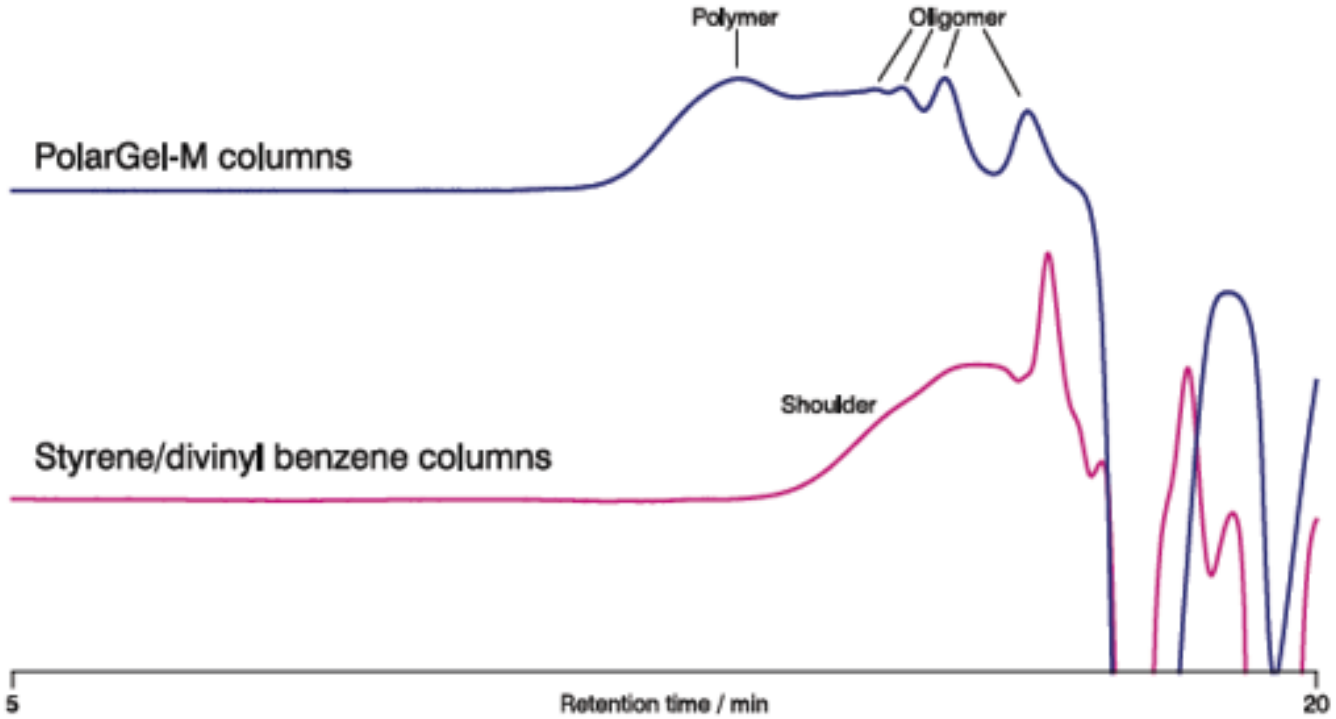
Hostavin N30

Polymeric UV stabilizer containing secondary amine groups

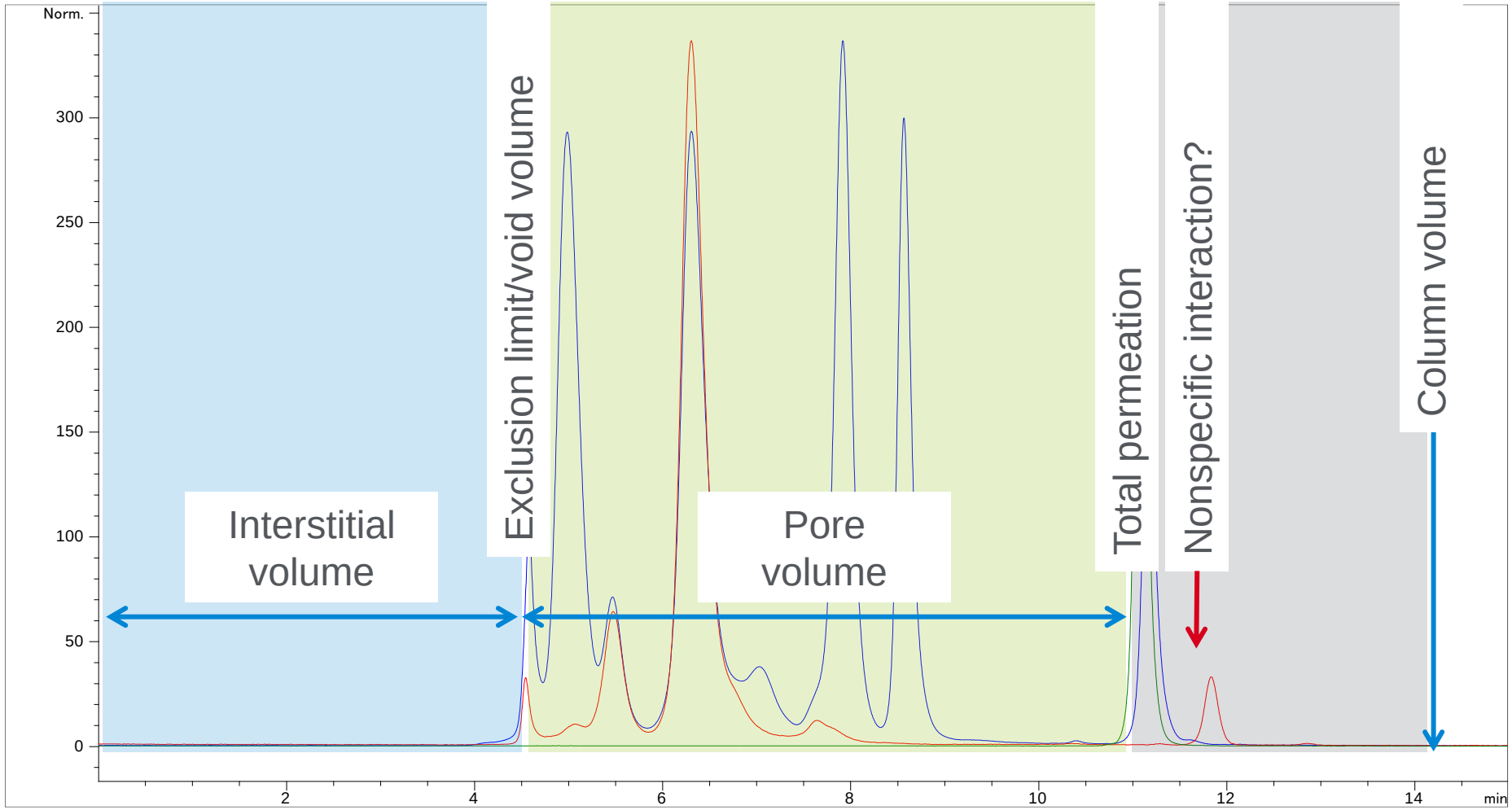
Column: 2 x PLgel 3µm MIXED-E
7.5 x 300 mm p/n PL1110-6300
Flow Rate: 1.0 mL/min
Detector: ELSD

Improve Peak Shapes of Polar Compounds

PolarGel GPC columns

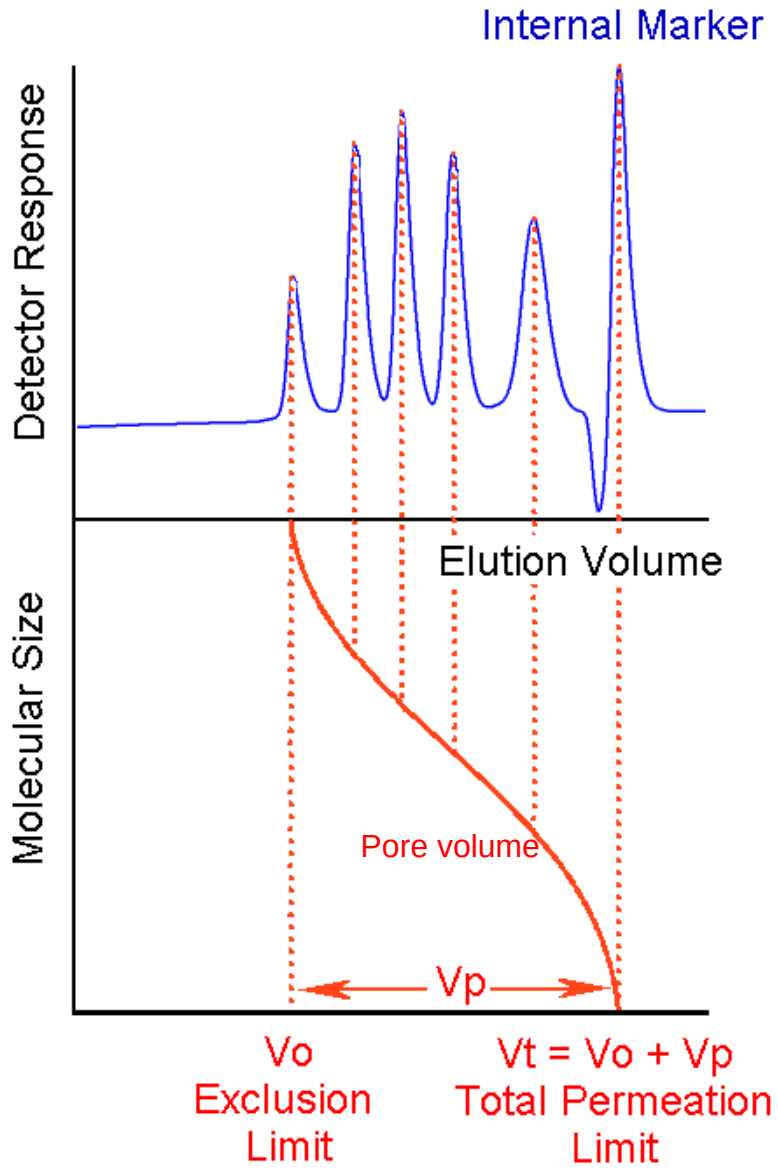


What Are These Regions on a Chromatogram?



Consider the Column's Mol Wt Range

- The example chromatogram and calibration curve illustrate how different size molecules elute from the column
- Choose a column that allows you to work in the linear portion of the calibration curve.



Considerations for Column Selection

- What is the expected molecular weight range of your polymer sample?

Mol wt	Mol wt range (g/mol or Da)
High	Up to several millions
Intermediate	Up to hundreds of thousands
Low	Up to tens of thousands
Very Low	A few thousand

GPC/SEC Columns – Making a Choice

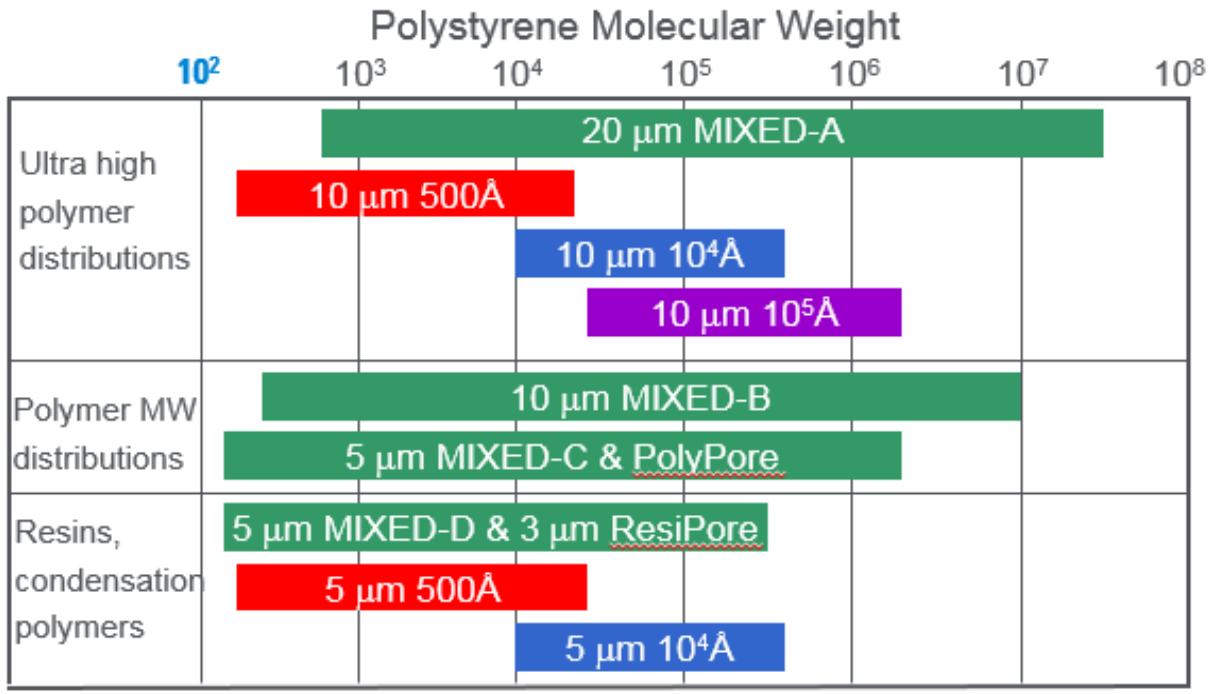
Agilent InfinityLab

Questions to consider:

- What mol wt range is needed for the column?
- Organic or Aqueous eluents being used
- What are your **key** requirements for your GPC/SEC analysis?
  - Resolution is important
  - Reproducibility of sample chromatography and results
  - Speed of analysis and/or sample throughput is something to improve on

Agilent GPC Columns

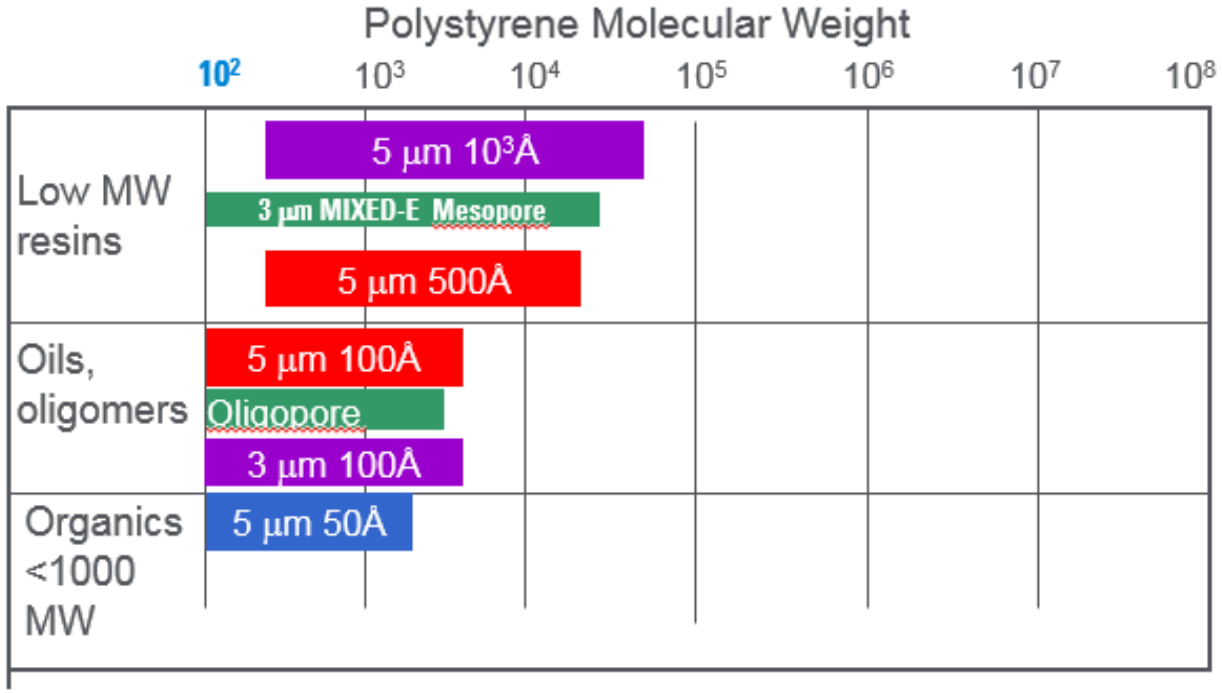
For organic soluble polymers



Column families:

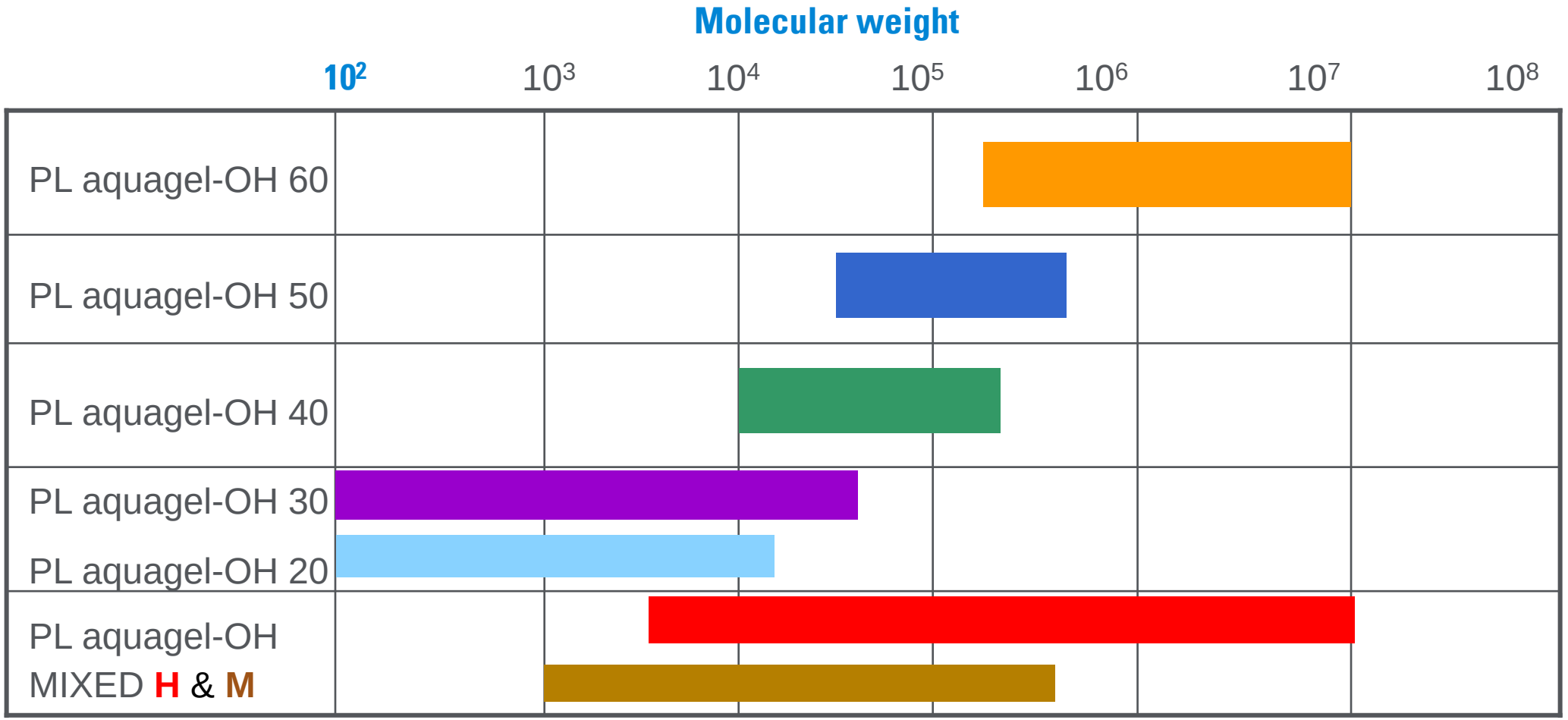
PLgel

PlusPore



Agilent SEC Columns

For aqueous soluble synthetic and natural polymers



GPC Column Selection

How many GPC/SEC columns to use

More than one column typically used
More columns = improved resolution

- The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution. More columns will be required to maintain the quality of the results.
- For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples.

Particle Size	Number of Columns
20 µm	4
13 µm	3
10 µm	3
8 µm	3
5 µm	2
3 µm	2

GPC Column Selection
Ways to improve resolution

Agilent InfinityLab

Running two columns in series using different pore sizes

- Extends the resolving range and enables analysis of multiple attributes in one run

Running two columns in series using the same pore size/same type

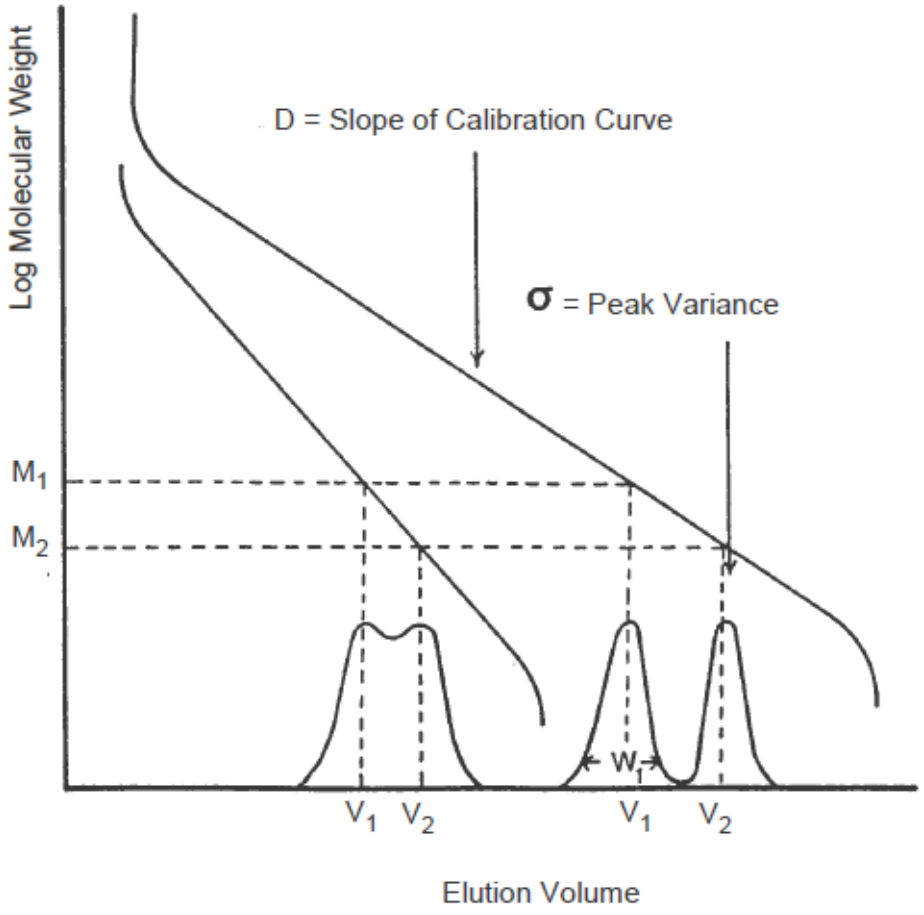
- Increasing pore volume increases the resolution

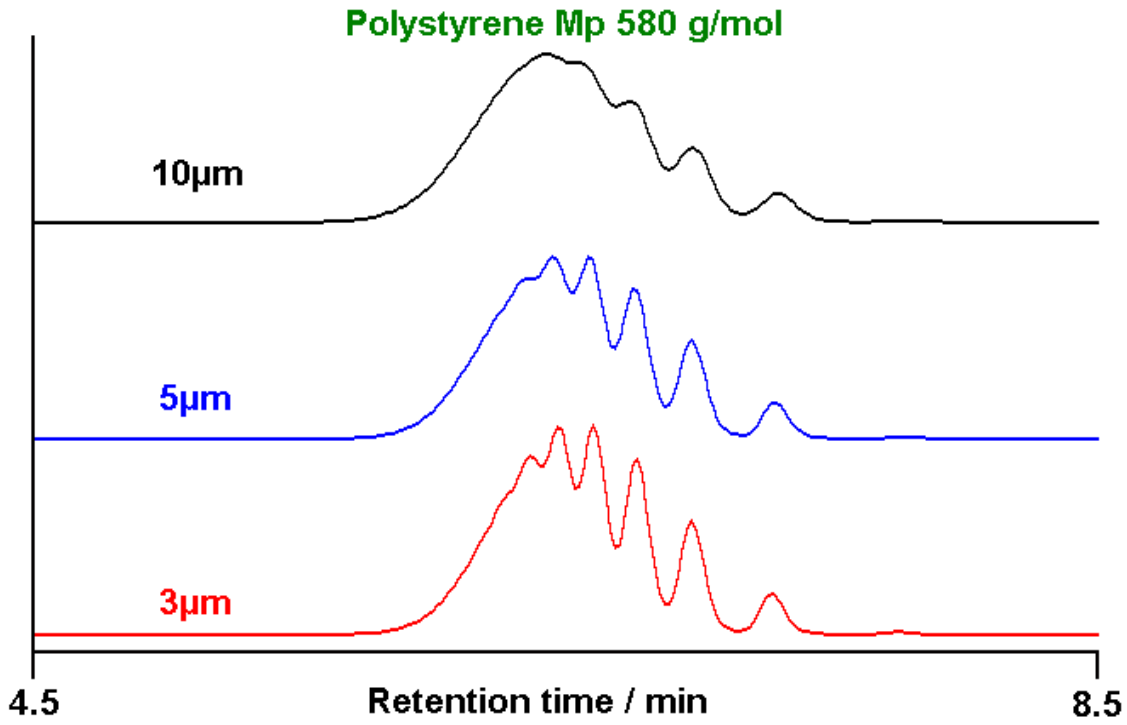
Use a packing with a smaller particle size

- Decreasing the particle size increases column efficiency

Resolution in GPC

Column length and particle size





Eluent:	THF
Flow rate:	1.0 mL/min
Inj vol:	20 µL
Detector:	DRI

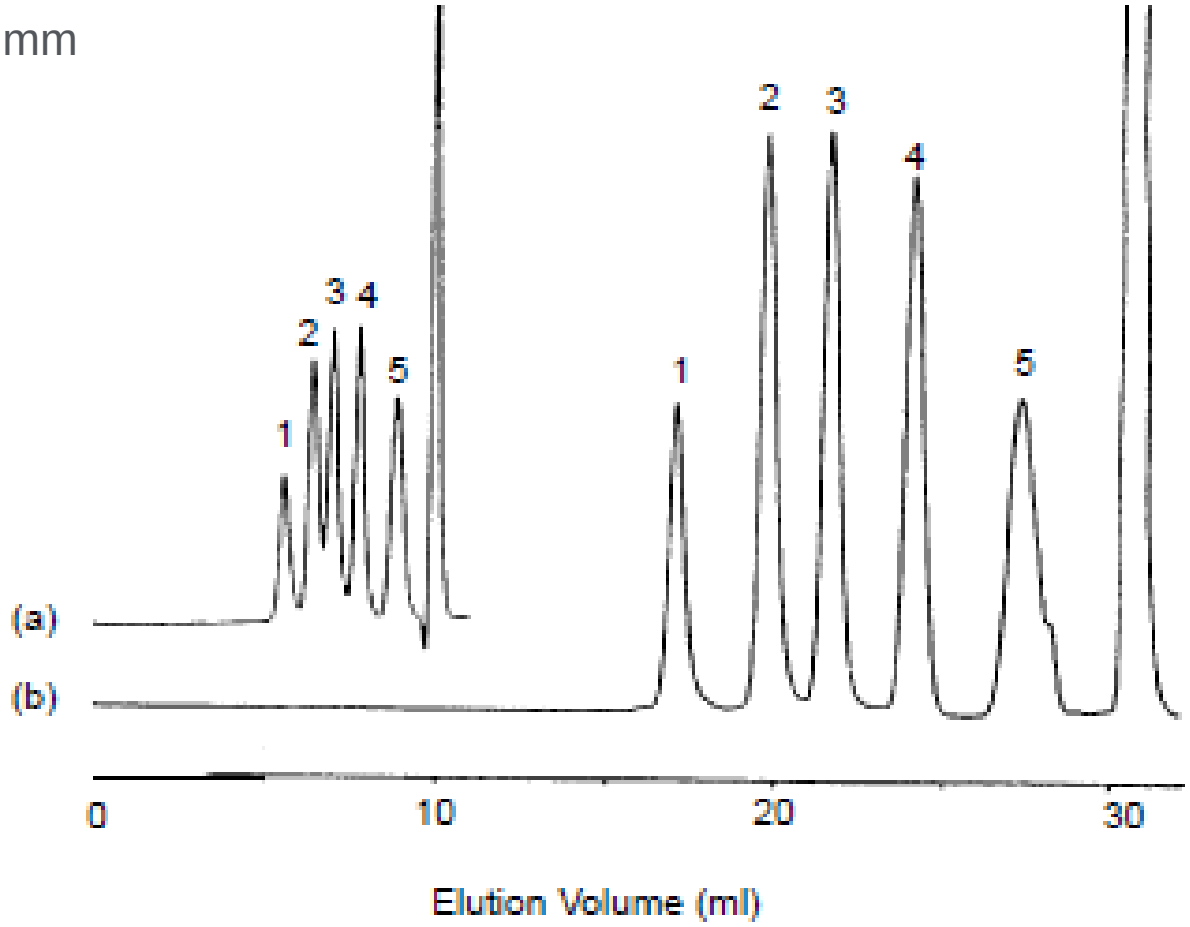
GPC Column Selection

Effect of column length on resolution

Columns: 1 x PLgel 10 µm MIXED-B 7.5 x 300 mm
p/n PL1110-6100
3 x PLgel 10 µm MIXED-B 7.5 x 300 mm
p/n PL1110-6100
Eluent: THF
Flow rate: 1 mL/min
Detector: RI

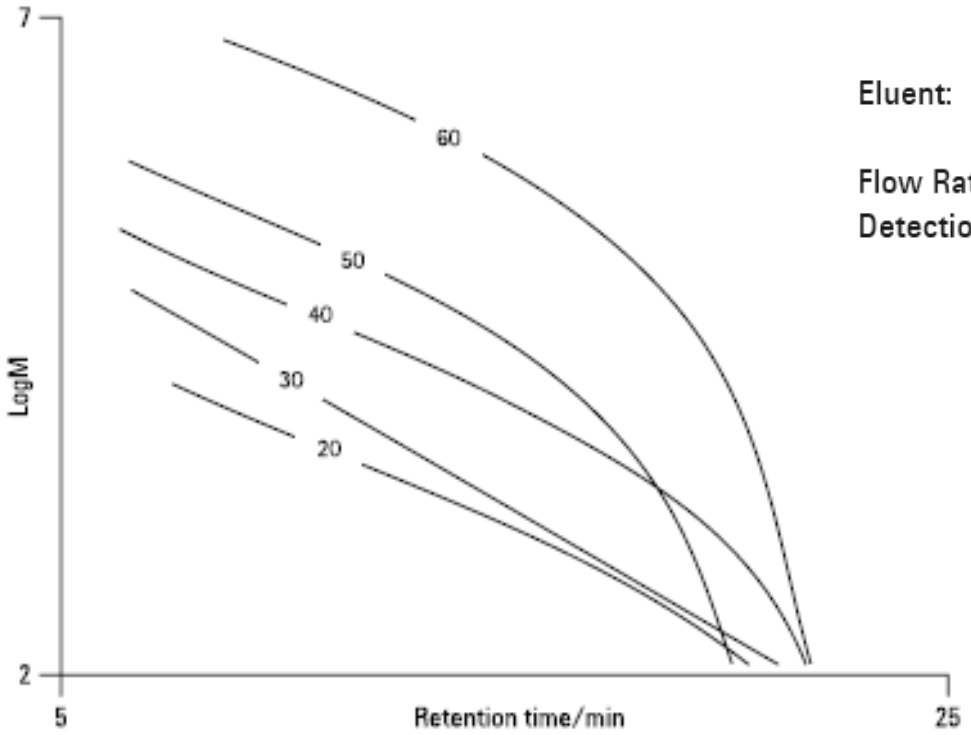
Polystyrene standards
Easical

1. 3,040,000
2. 330,000
3. 66,000
4. 9200
5. 580



Column in Series to Extend Resolving Range

PL aquagel OH columns
Individual pore Sizes



Conditions

Samples: Four samples of hyaluronic acid
Columns: 1 x PL aquagel-OH 60 15 µm, 300 x 7.5 mm (p/n PL1149-6260) + 1 x PL aquagel-OH 40 15 µm, 300 x 7.5 mm (p/n PL1149-6240)
Eluent: 0.2 M $NaNO_3$ + 0.01 M NaH_2PO_4 at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

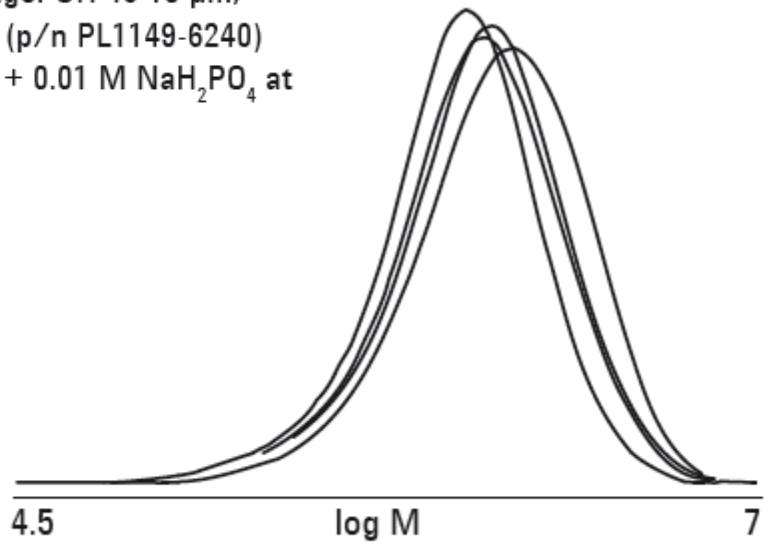
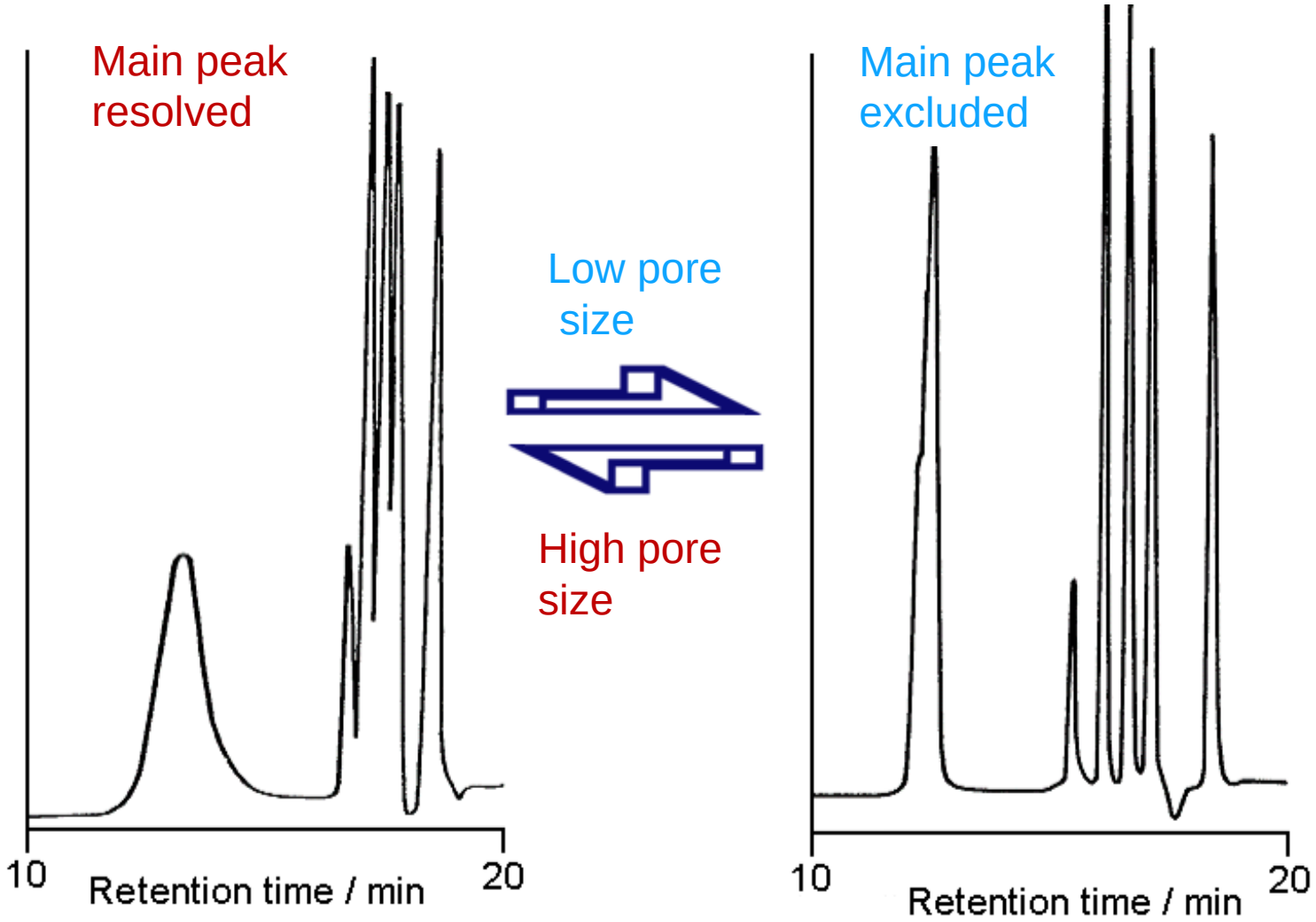


Figure 3. Overlay of the molecular weight distributions of four hyaluronic acid samples

Effect of Column Selection: Pore size



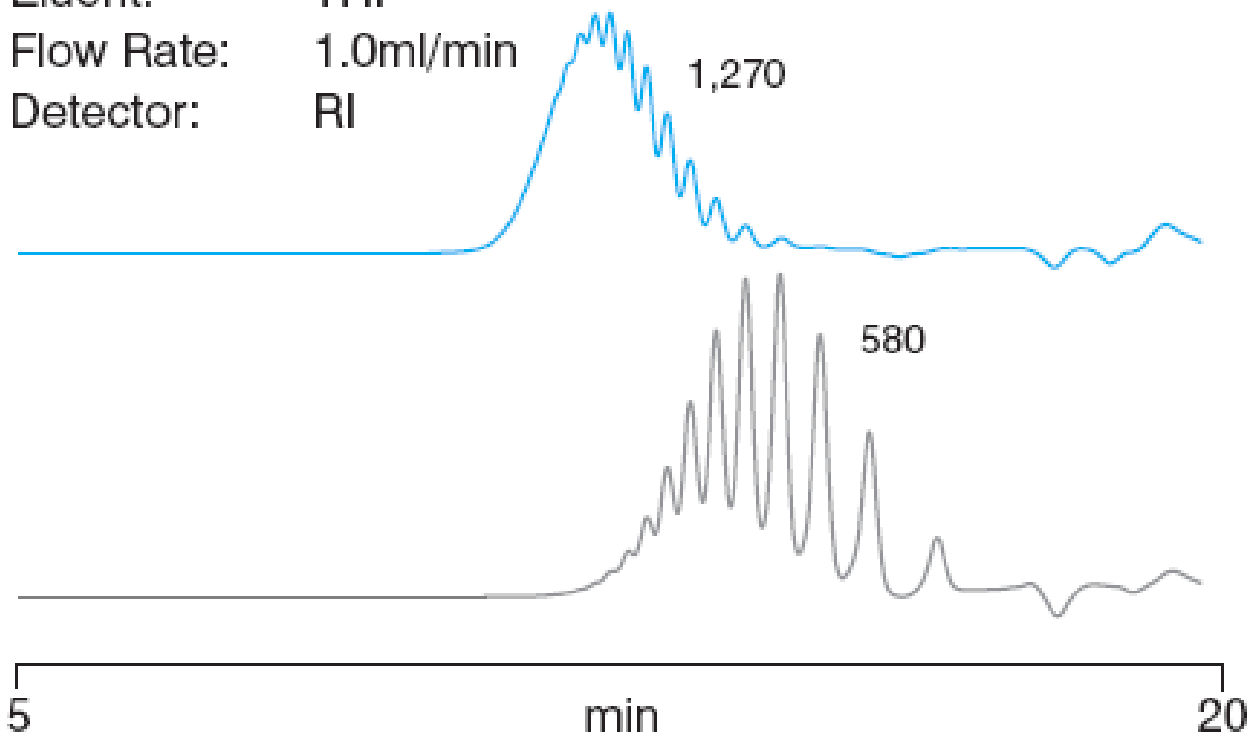
* Samples run using PLgel individual pore size columns

Importance of Pore Volume

Agilent InfinityLab

Polystyrene standards

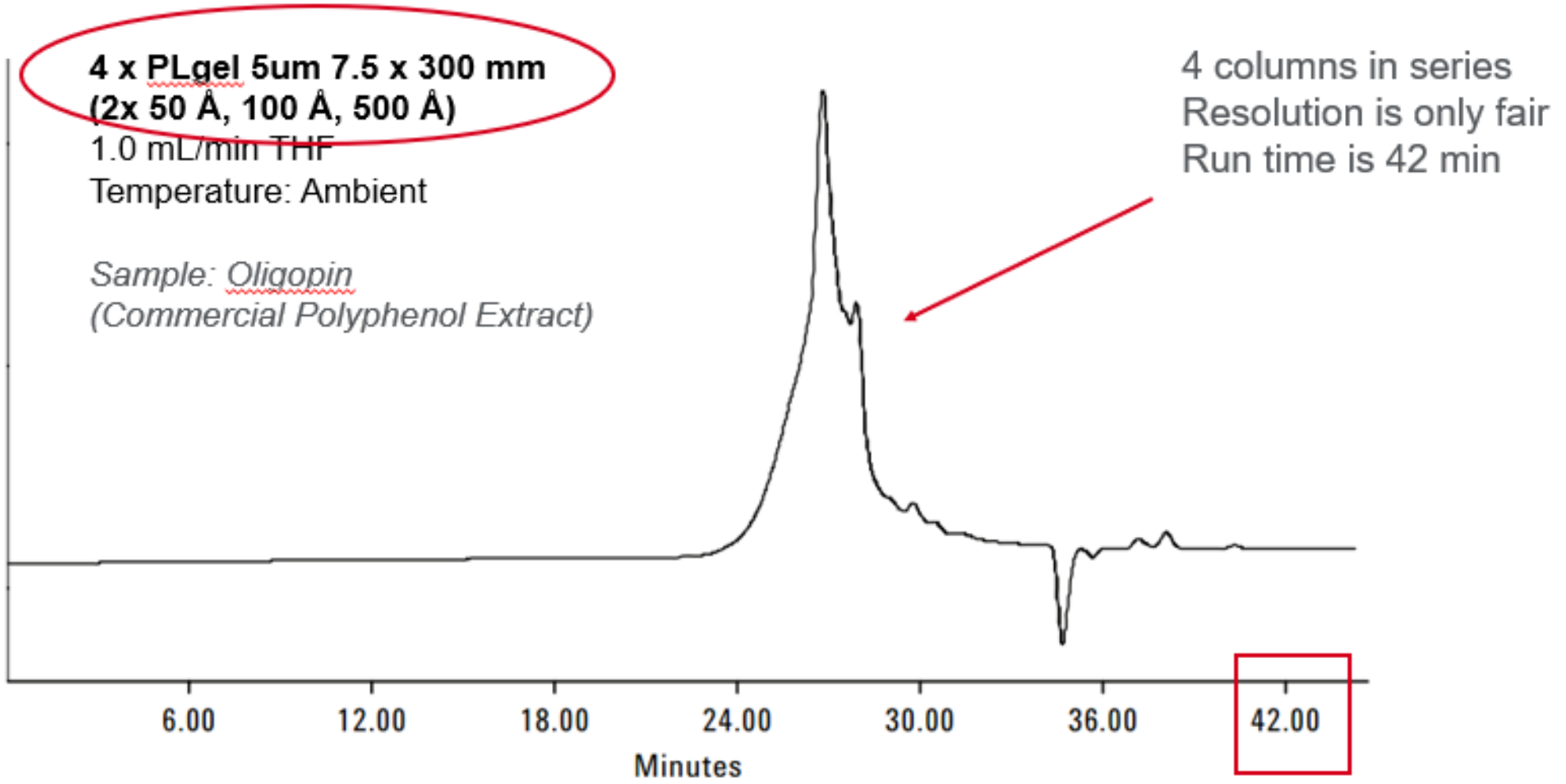
Columns: 2xOligoPore, 300x7.5mm (PL1113-6520)
Eluent: THF
Flow Rate: 1.0ml/min
Detector: RI



- With some columns it is possible to calibrate the column using the oligomers
- The molecular weights of the initiator fragment and the repeat unit of the polymer must be known

Improve Run time and Resolution

PlusPore columns

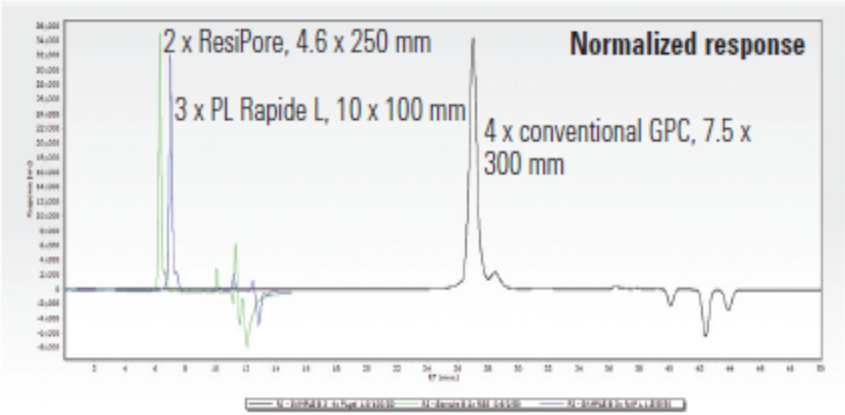


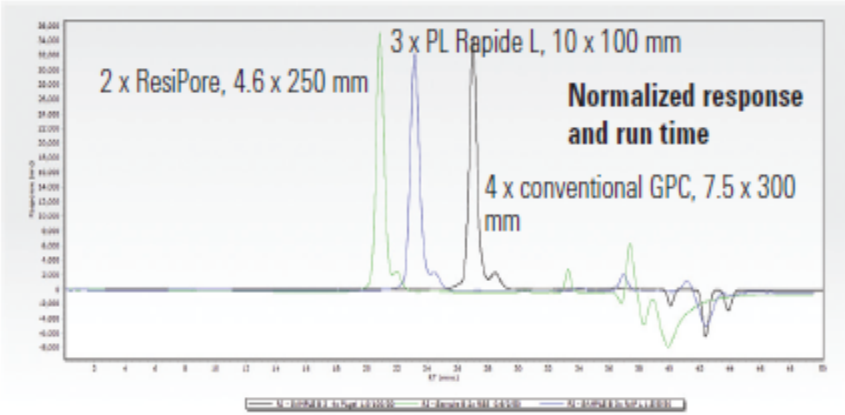
Column Selection

Fast GPC

Improving speed for analysis without sacrificing resolution

Comparison for conventional columns versus columns for fast GPC:





Throughput is increased by more than 3x

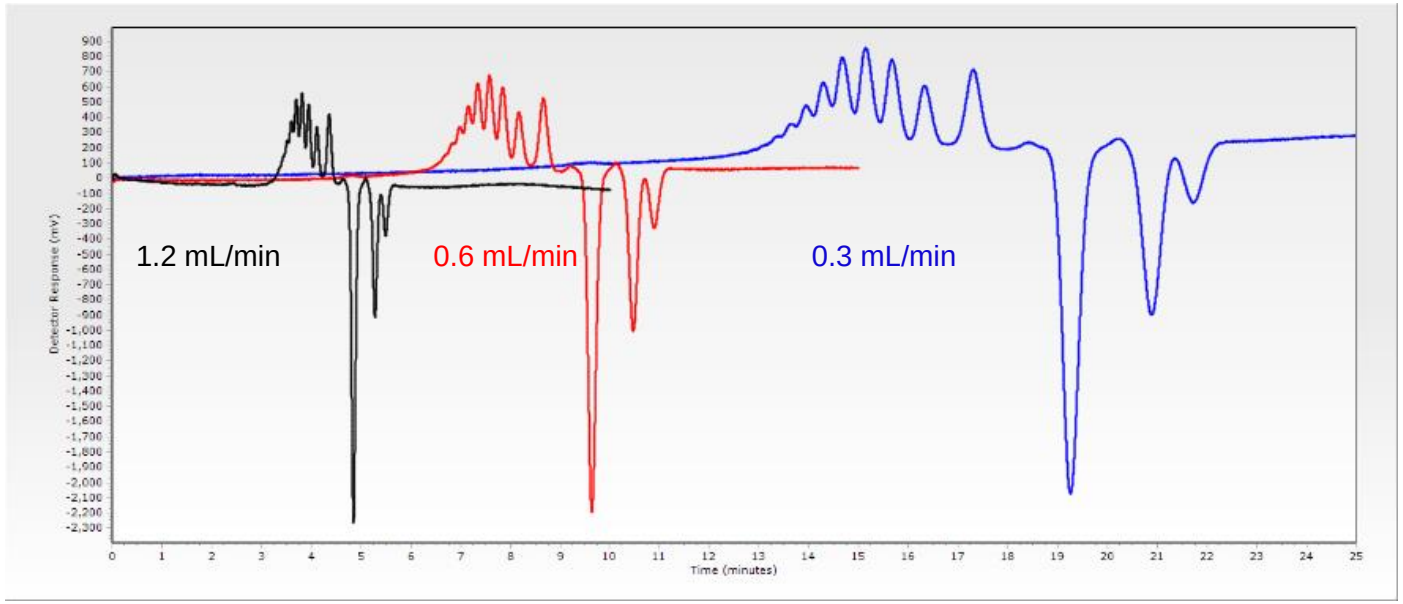
Columns	Peak 2 retention time (min)	Run time (min)
4 x conventional 7.5 x 300 mm	28.46	50
3 x PL Rapide L 10 x 100 mm	7.41	15
2 x ResiPore 4.6 x 250 mm	6.66	15

Without sacrificing separation quality

Columns	Resolution (Rs)	Selectivity (α)	Area %	Height %
4 x conventional 7.5 x 300 mm	1.2	1.05	8	7
3 x PL Rapide L 10 x 100 mm	1.1	1.06	7	7
2 x ResiPore 4.6 x 250 mm	1.1	1.05	8	8

Column Selection

Fast GPC

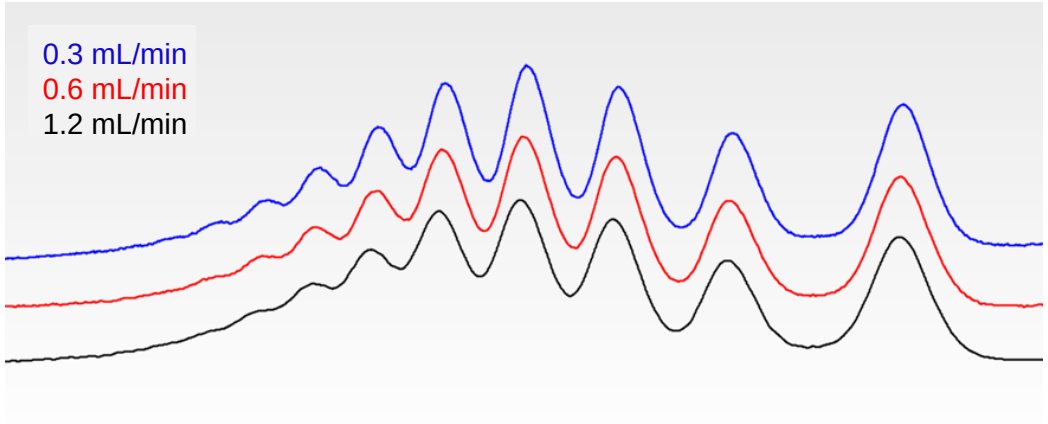


MW Range: up to 3,300 (g/mol)

Nominal Particle Size: 6 µm

Typical Efficiency: >55,000 p/m

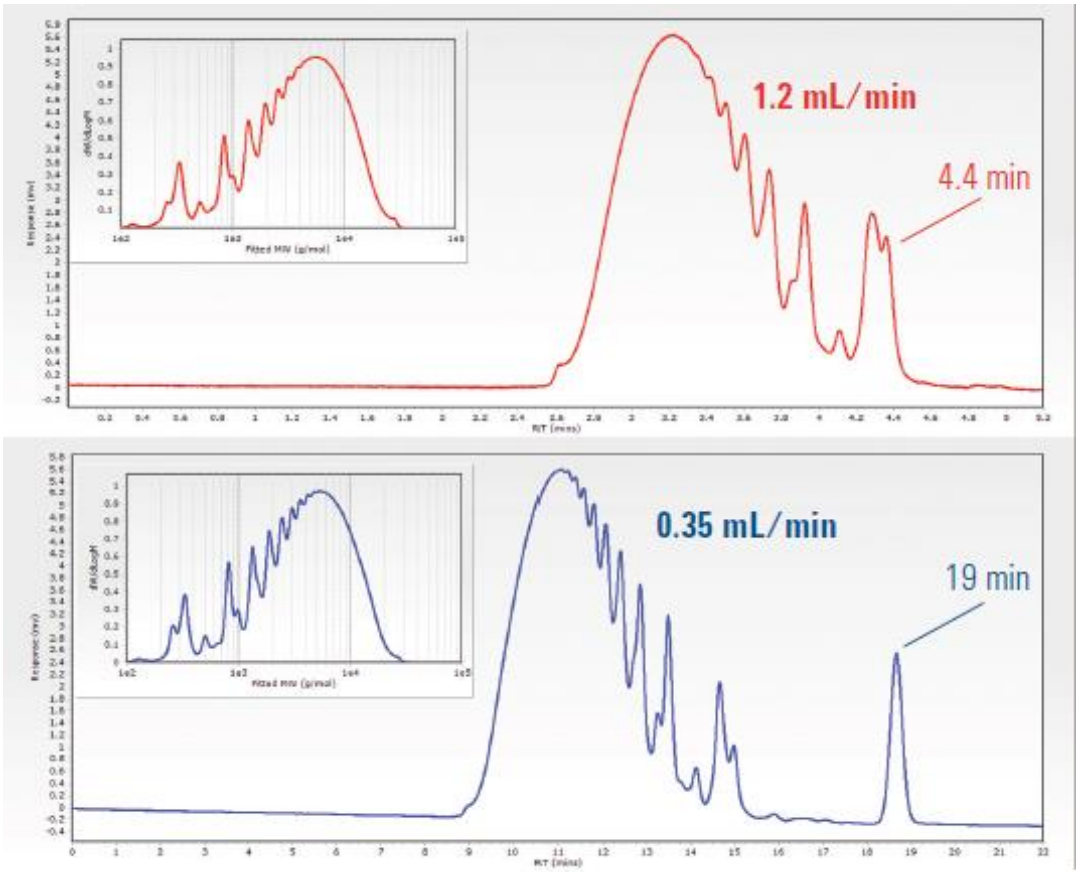
- Column: 2 x OligoPore, 4.6 x 250 mm, p/n PL1113-6520
- Flow Rate: 0.3, 0.6, 1.2 ml/min
- Sample: Polystyrene 580



Different flow rates overlaid to show that faster doesn't sacrifice resolution. The chromatograms have been normalized to better illustrate the differences.

Column Selection

Fast GPC



Conditions

Column:	2 x MesoPore, 4.6 x 250 mm (PL1513-5325)
Sample:	Epoxy resin
Eluent:	THF
Flow rate:	0.35 and 1.2 mL/min
Inj vol:	4 µL
System:	1260 Infinity GPC/SEC System, UV, 254 nm

Easy Method Transfer from Standard to rapid GPC on MesoPore 250x4.6mm GPC columns

MW Range: up to 25,000 (g/mol)

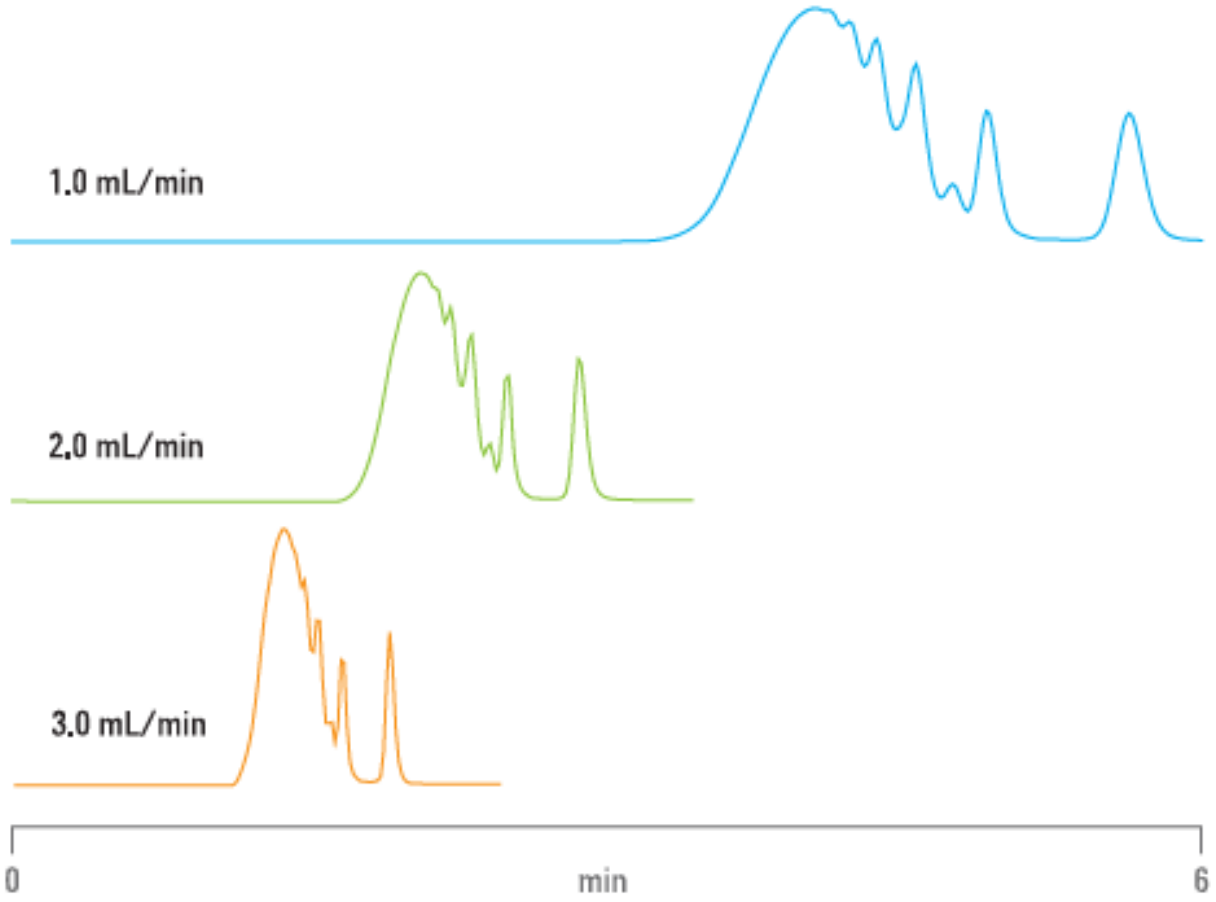
Nominal Particle Size: 3 µm

Typical Efficiency: >80,000 p/m

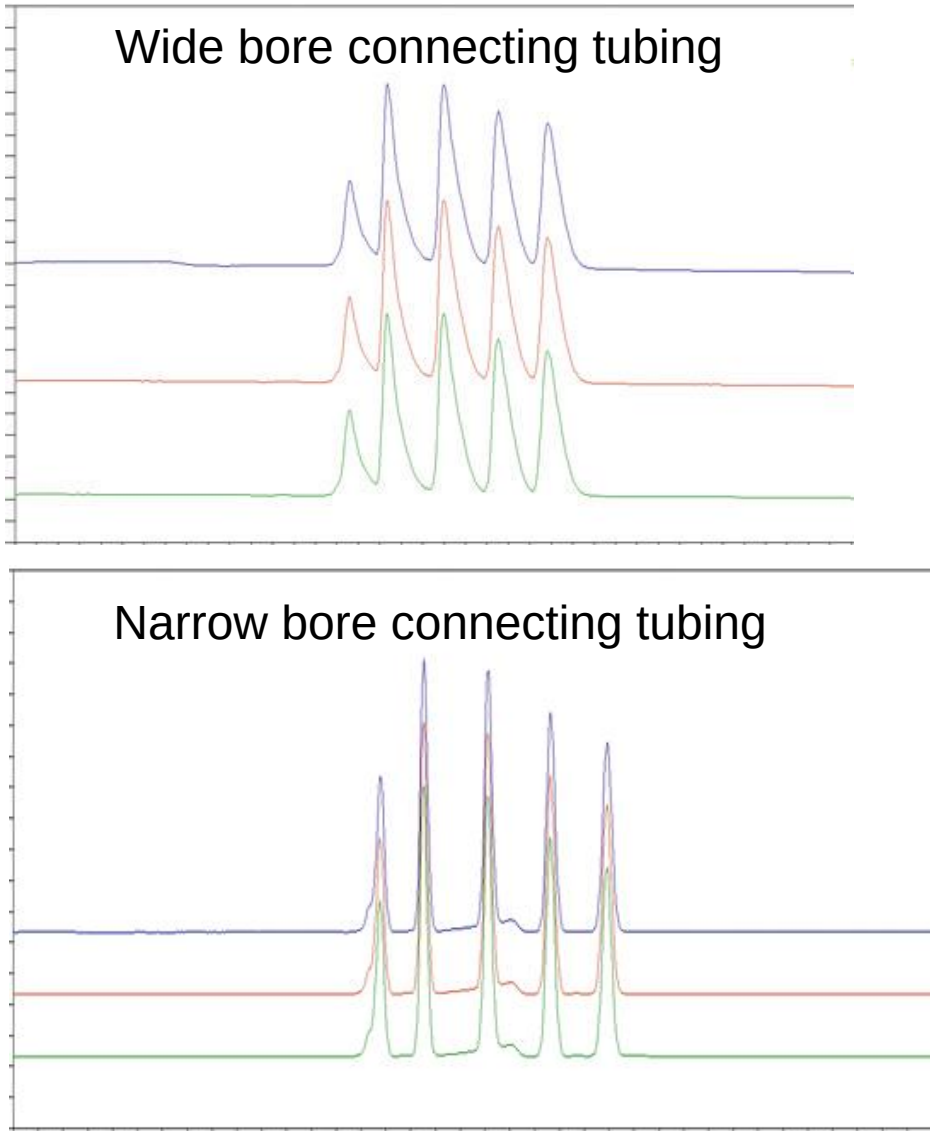
MesoPore Columns

Rapide Columns for Fast Trend Analysis

Column: PL Rapide L, 10 x 100 mm
Sample: Epoxy resin
Eluent: THF
Flow rate: as noted
Detector: UV, 254 nm



Reducing Dead Volume

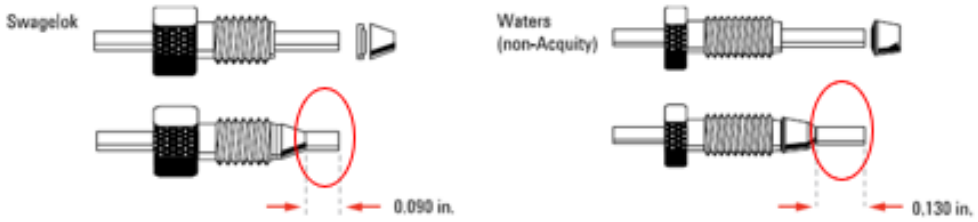


It is important to:

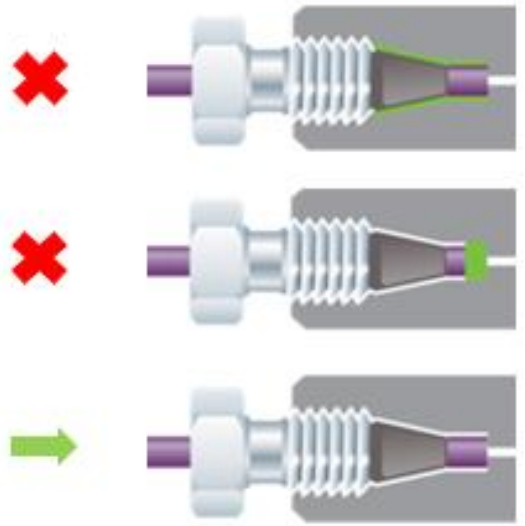
- Use tubing with an internal diameter that is as narrow as possible
- Keep tubing connections short
- Use proper fittings for connections

Proper Connections

- Problems with improper connections
  - Source of leaks
  - **Mistaken for chromatography issues**
- Making connections can vary with skill/technique
- Different manufacturers supply different types of fittings



Potential Fittings Issues

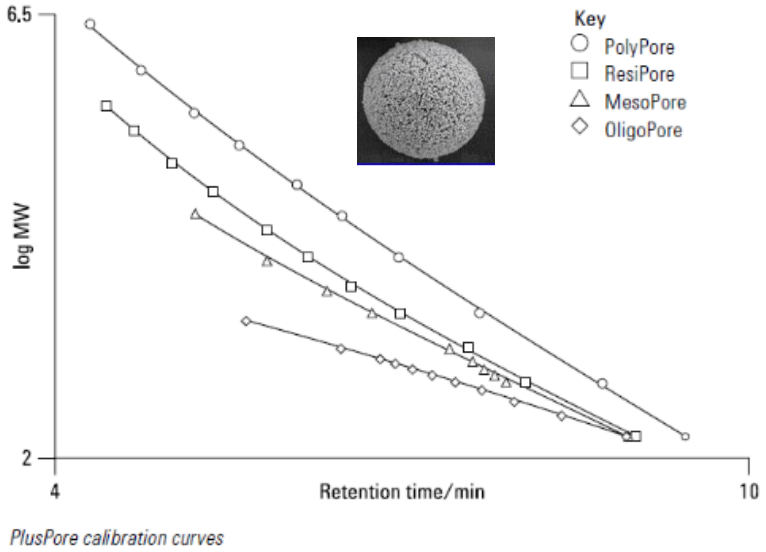


Pub no: 5991-8031EN
InfinityLab LC Supplies Guide

System Detection

Peak shape and resolution improvement

1290 Infinity II
GPC/SEC System

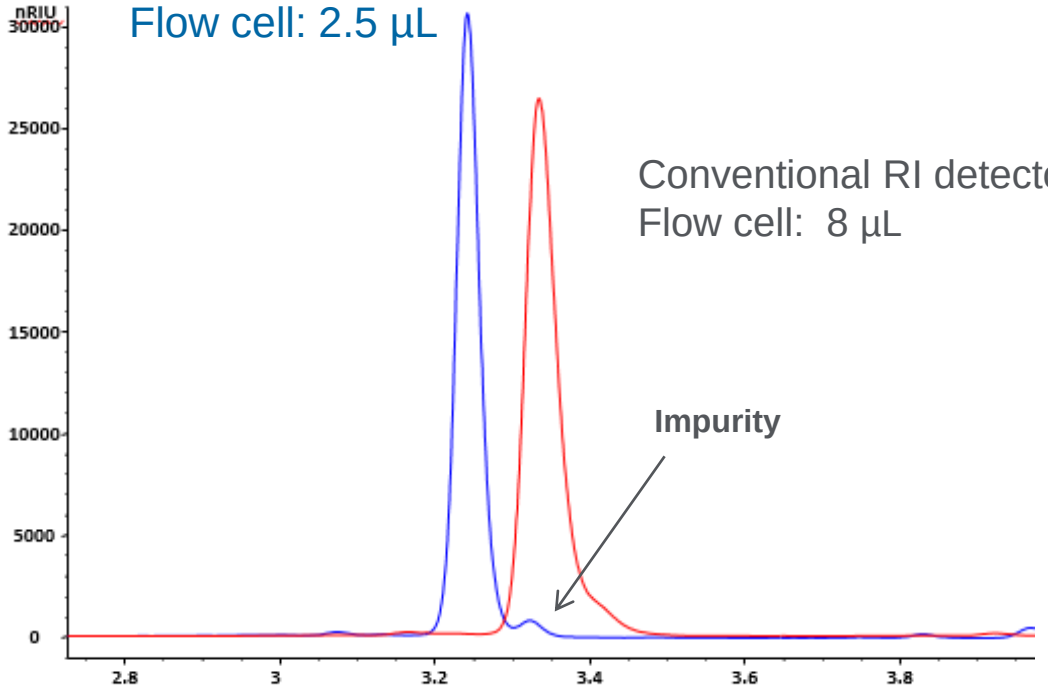


PlusPore calibration curves

Couple with the 1290 Infinity II Micro Refractive Index Detector (RID) to achieve excellent peak shapes and very high resolution

Polypore columns are a multiporous structure which give extremely linear calibrations.

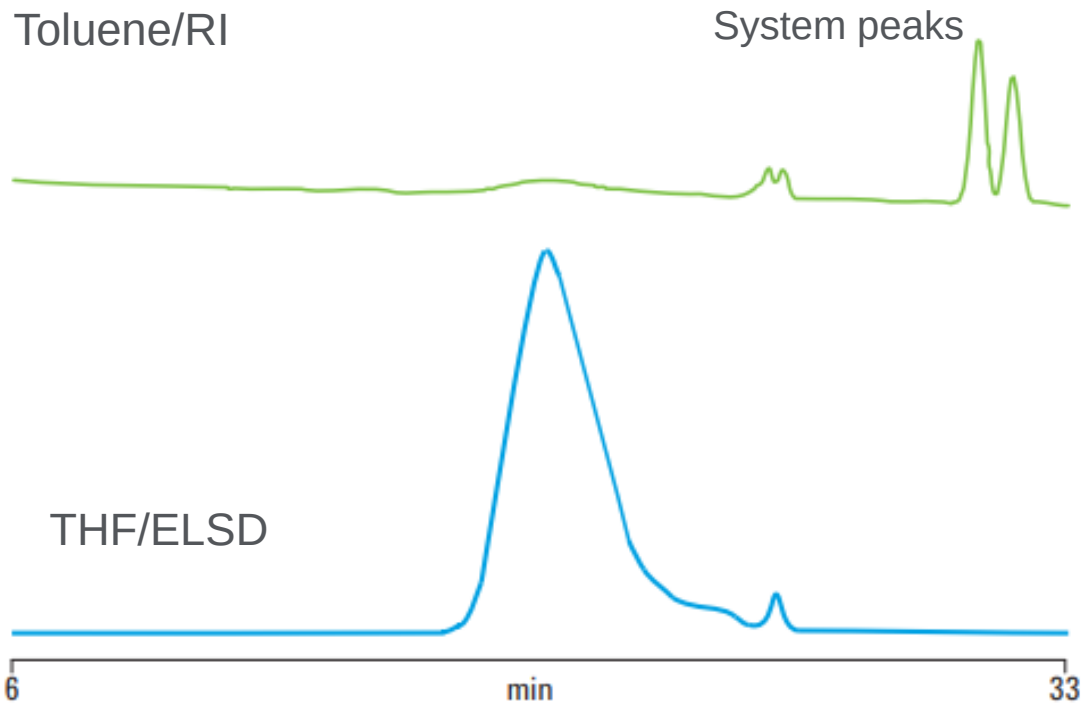
Micro RID
Flow cell: 2.5 µL



Conventional RI detector
Flow cell: 8 µL

System Detection

Choice of solvent and detection



Column:	3 x PLgel, 5 µm, MIXED-D
	7.5 x 300 mm, p/n PL1110-6504
Eluent:	Toluene or THF
Flow rate:	1.0 mL/min
Sample:	Polysiloxane, 0.2% w/v
Injection vol:	100 µL

Application note publication number: 5990-7897EN

Extending Experimental Data

Molecular weight sensitive detectors for GPC

Detector	Measures	Molecular Weight	Molecular Size	Information
Refractive Index Detector Only	Sample Concentration	Relative to the standards used for column calibration	No	Concentration
Viscometry	Intrinsic Viscosity	More accurate from the Universal Calibration	Yes, hydrodynamic radius (Rh).	Branching, density, aggregation.
Light Scattering	Scattered light intensity	Absolute determination	Yes, Radius of Gyration (Rg) directly.	Absolute Molecular Weight, size and structure.
Triple Detection	Concentration, viscosity, scattered light	Absolute determination	Yes, direct measure of Rg and Rh.	The ultimate configuration for comprehensive polymer characterization

Agilent GPC/SEC Columns

Agilent InfinityLab

Organic solvents

PLgel

- PLgel MIXED
- PLgel MiniMIX
- PLgel MIXED-LS
- PLgel [Pore Size]
- PLgel Olexis

PL HFIPgel

PL Rapide

EnviroPrep

Organic solvents

PlusPore

- PolyPore
- ResiPore
- MesoPore
- OligoPore

Polar solvents

PolarGel

Aqueous solvents

PL Aquagel-OH MIXED

PL Aquagel OH

PL Rapide Aqua

Agilent

GPC/SEC Columns and Supplies Resources

- Organic GPC Columns catalog: Organic GPC Columns
- Aqueous & Polar GPC/SEC Columns catalog: Aqueous & Polar GPC/SEC Columns
- GPC/SEC Polymer Standards catalog: GPC/SEC Polymer Standards
- GPC/SEC User Guide: GPC/SEC column user guide
- Polymer to Solvent Reference Table: Polymer to Solvent Reference Table
- GPC Troubleshooting poster: GPC Troubleshooting Guide
- InfinityLab Supplies catalog: InfinityLab LC Supplies (agilent.com)
- Consumables Community: Agilent Collection of Columns, Supplies, and Standards Resources - Consumables - Agilent Community
- App finder: Application Finder | Agilent
- Agilent University: Agilent University
- YouTube: Agilent Channel
- Your local product specialists
- Agilent Peak Tales podcasts: peaktales.libsyn.com
- Webinars, upcoming and recorded: LC & LC/MS Column Webinars | Agilent

Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8-5 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

pzi.info@agilent.com

Agilent InfinityLab

Thank you for attending

Any questions?

Agilent

Agilent

InfinityLab

Agilent