Don’t Lose It: Resolution and Reproducibility in GPC/SEC

Jean Lane
Application Engineer
May 7, 2020

Agilent InfinityLab

Agilent

Loss of Resolution and Reproducibility

Factors for consideration

Method-related factors

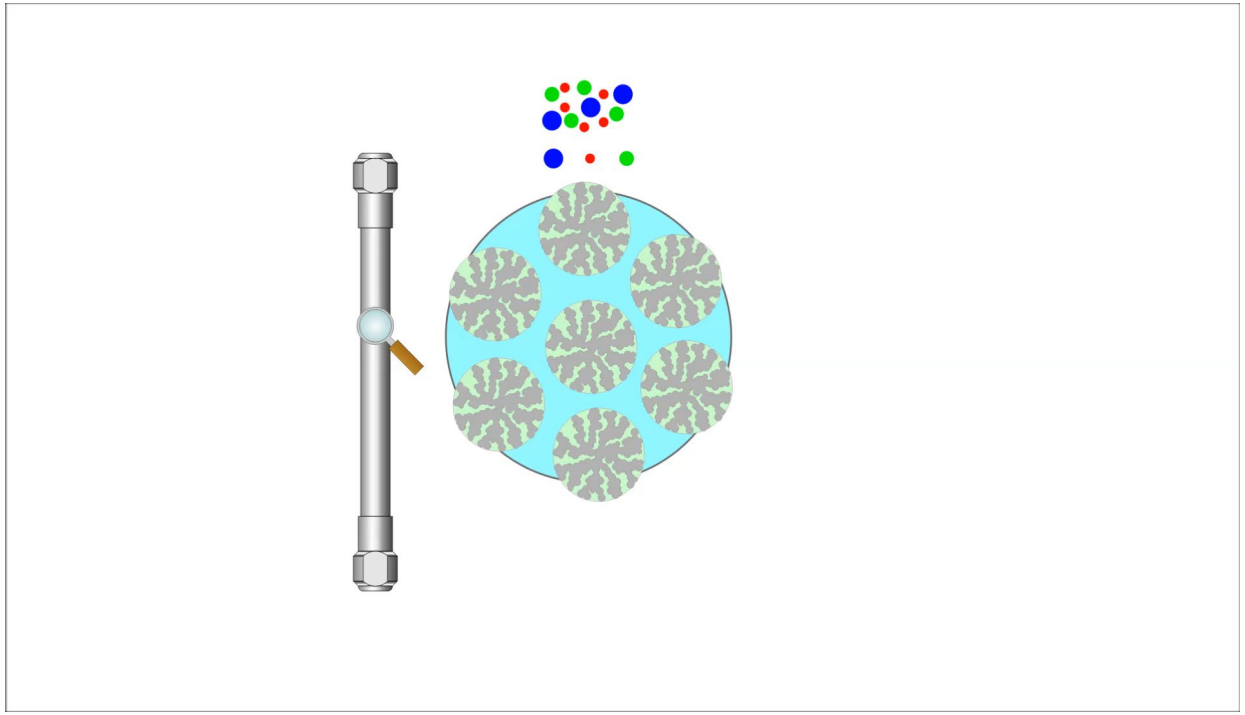
- Solvent selection
- Column selection
- Calibrant selection
- Detection

System-related factors

- Pump
- Autosampler/injector
- Column compartment
- Tubing/fittings
- Detection

GPC/SEC Separation Mechanism

- A GPC/SEC column is packed with porous beads of controlled porosity and particle size.
- The 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 largest first, smallest last.



Solvent Selection

Factors for consideration

Agilent InfinityLab

Remember, GPC/SEC is a noninteractive separations technique

When selecting the solvent or the mobile phase conditions for the sample and separation, the size exclusion mechanism must be maintained.

Just because a sample is soluble in a particular solvent, this does not mean it will be the suitable solvent to use for the GPC analysis.

Solvent polarity	Solvent
6.0	Perfluoroalkane
7.3	Hexane
8.2	Cyclohexane
8.9	Toluene
9.1	Ethyl acetate
9.1	Tetrahydrofuran (THF) (Stabilized only)
9.3	Chloroform (Stabilized only)
9.3	Methyl ethyl ketone (MEK)
9.7	Dichloromethane
9.8	Dichloroethene
9.9	Acetone
10.0	o-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 pyrrolidone (NMP)
12.0	Dimethyl sulfoxide (DMSO)
12.1	Dimethyl formamide (DMF)

Solvent Selection
Sample type

Agilent InfinityLab

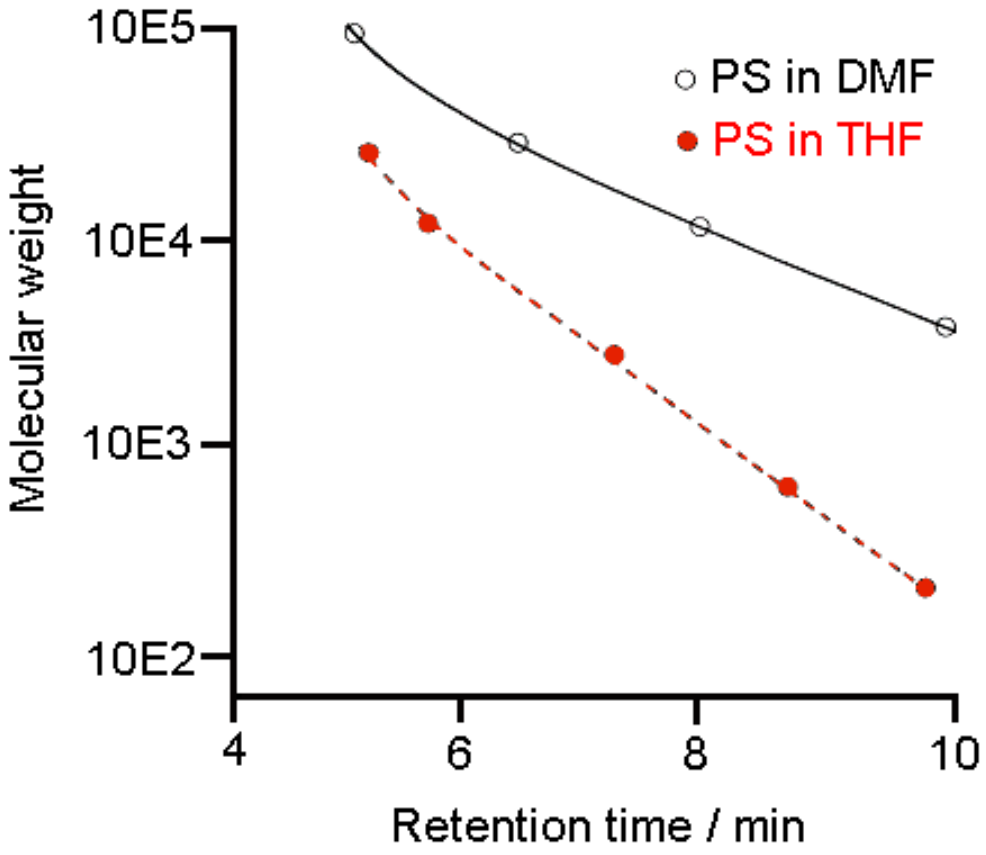
What solvent is your polymer soluble in?

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%)

Publication number: 5991-6802EN
Polymer to Solvent Reference Table for GPC/SEC

Solvent Selection

Comparison of calibration standard in two solvents

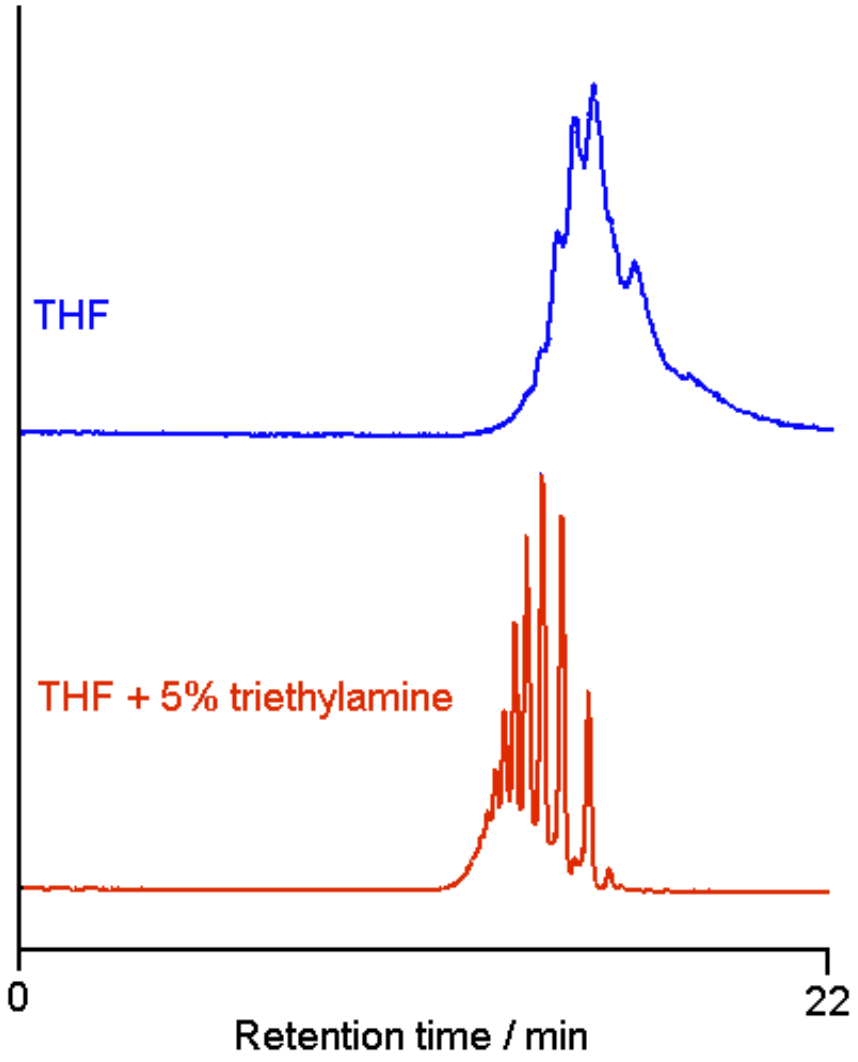


Column: PLgel, 5 µm, 500 Å
7.5 x 300 mm, p/n PL1110-6525

PS/DVB columns are excellent in many solvents, but although the column may be used in certain solvents this does not mean SEC will occur. The example here is polystyrene standards running in both THF and DMF.

Nonsize exclusion behavior seen for PS in DMF.

Solvent Selection
Modification of eluent



Hostavin N30

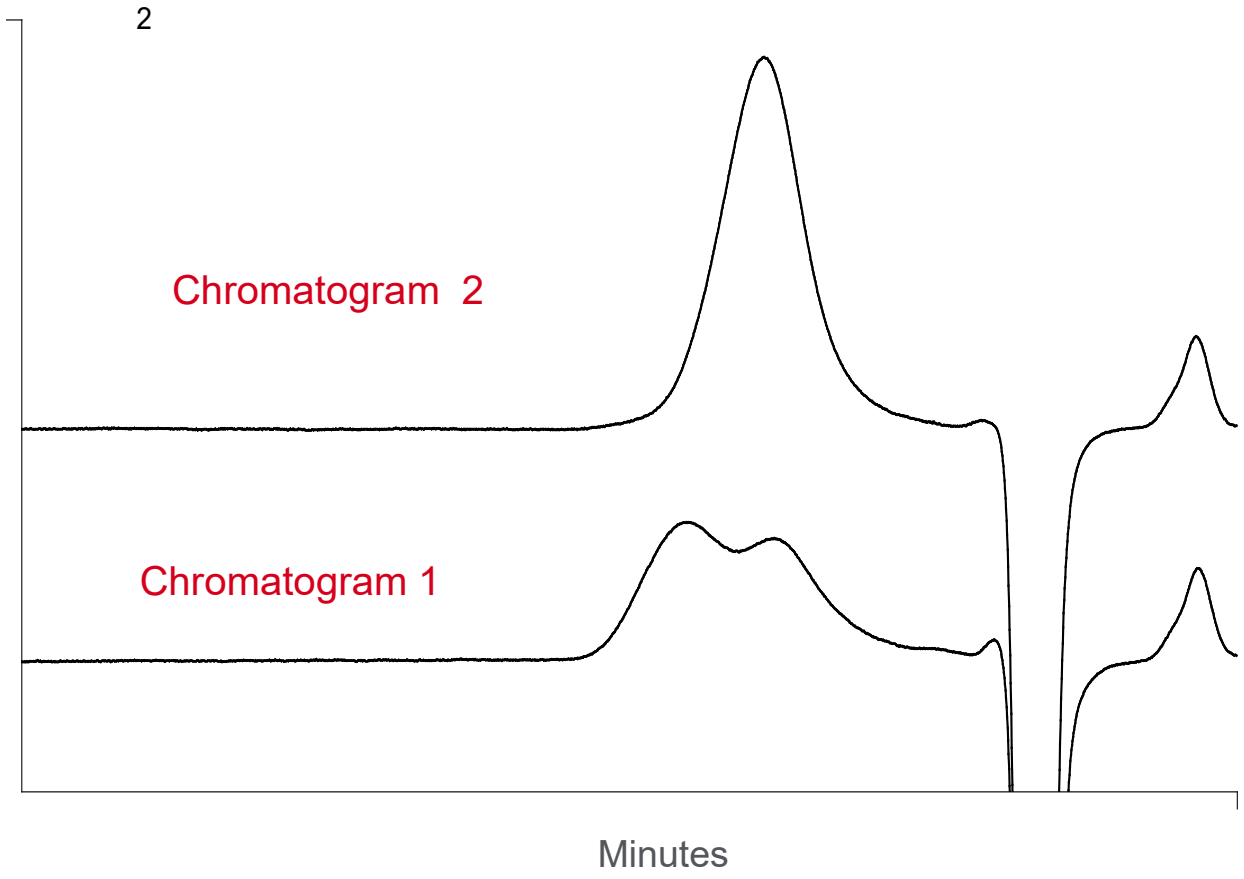
- Polymeric UV stabilizer containing secondary amine groups

Column: 2 x PLgel, 3 µm, MIXED-E
p/n PL1110-6300
Flow rate: 1.0 mL/min
Detector: ELSD

Improved resolution and reproducibility as a result of eluent modification.

Solvent Selection

Modification of eluent – Use of salt

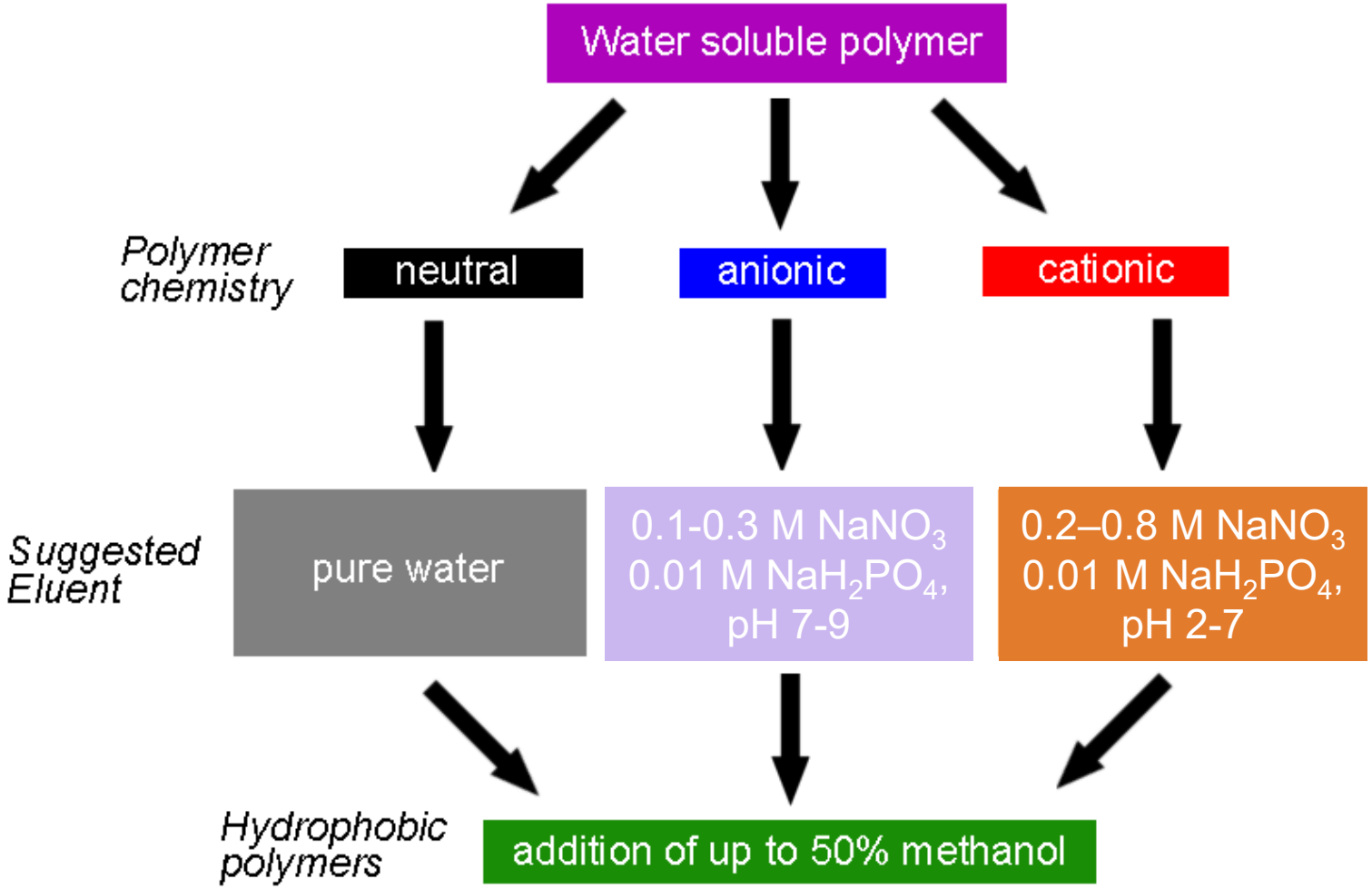


Columns:	4 x PLgel, 20 µm, MIXED-A 7.5 x 300 mm, p/n PL1110-6200
Eluent:	DMSO + 5 mM $NaNO_3$
Flow rate:	1.0 mL/min
Temperature:	80 C

Addition of salt is often required for polar organic solvents to suppress ionic interaction effects (chromatogram 2).

Solvent Selection

Factors for optimizing in Aqueous SEC



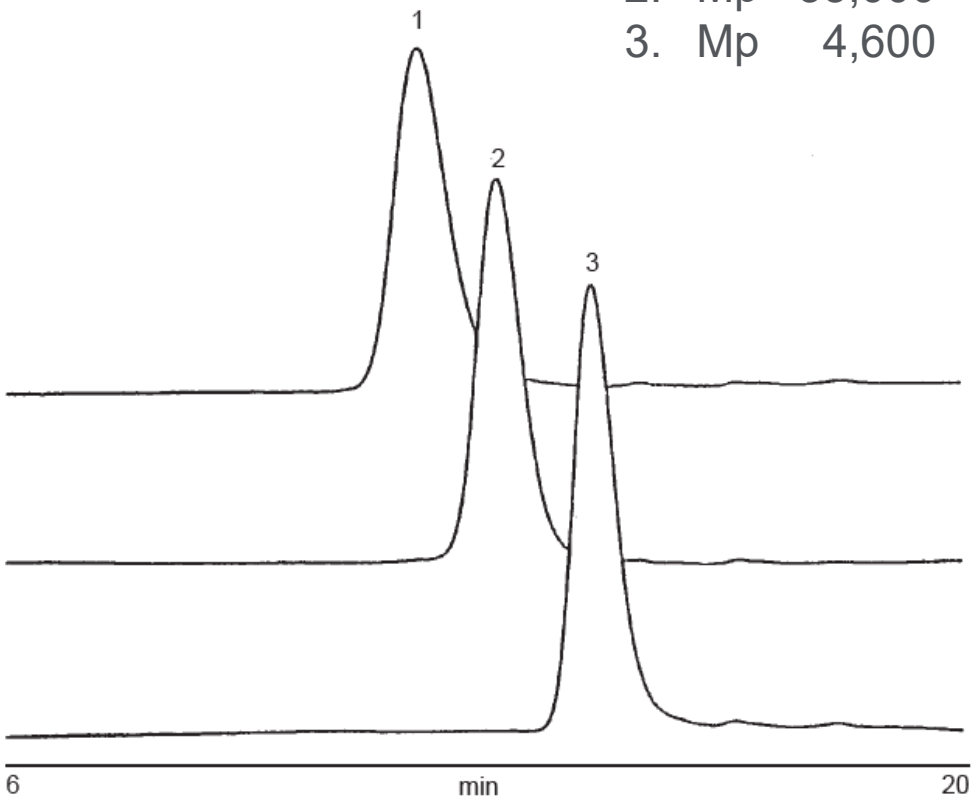
Guide to Eluent Selection for PL aquagel-OH Applications

Solvent Selection

Aqueous SEC application example

Polystyrene sulfonate

1. Mp 100,000
2. Mp 35,000
3. Mp 4,600



- Column: 2 x PL aquagel-OH 40, 8 µm
  7.5 x 300 mm, p/n PL1149-6840
- Eluent: 80% 0.3 M $NaNO_3$, 0.01M NaH_2PO_4, pH 9
  20% methanol
- Flow rate: 1.0 mL/min
- Detector: RI

These polymers are both ionic and relatively hydrophobic. The eluent conditions are chosen to minimize sample-to-column interaction, which would otherwise result in late elution times.

GPC Column Selection

How many GPC/SEC columns to use

Agilent InfinityLab

More than one column typically used
More columns = improved resolution

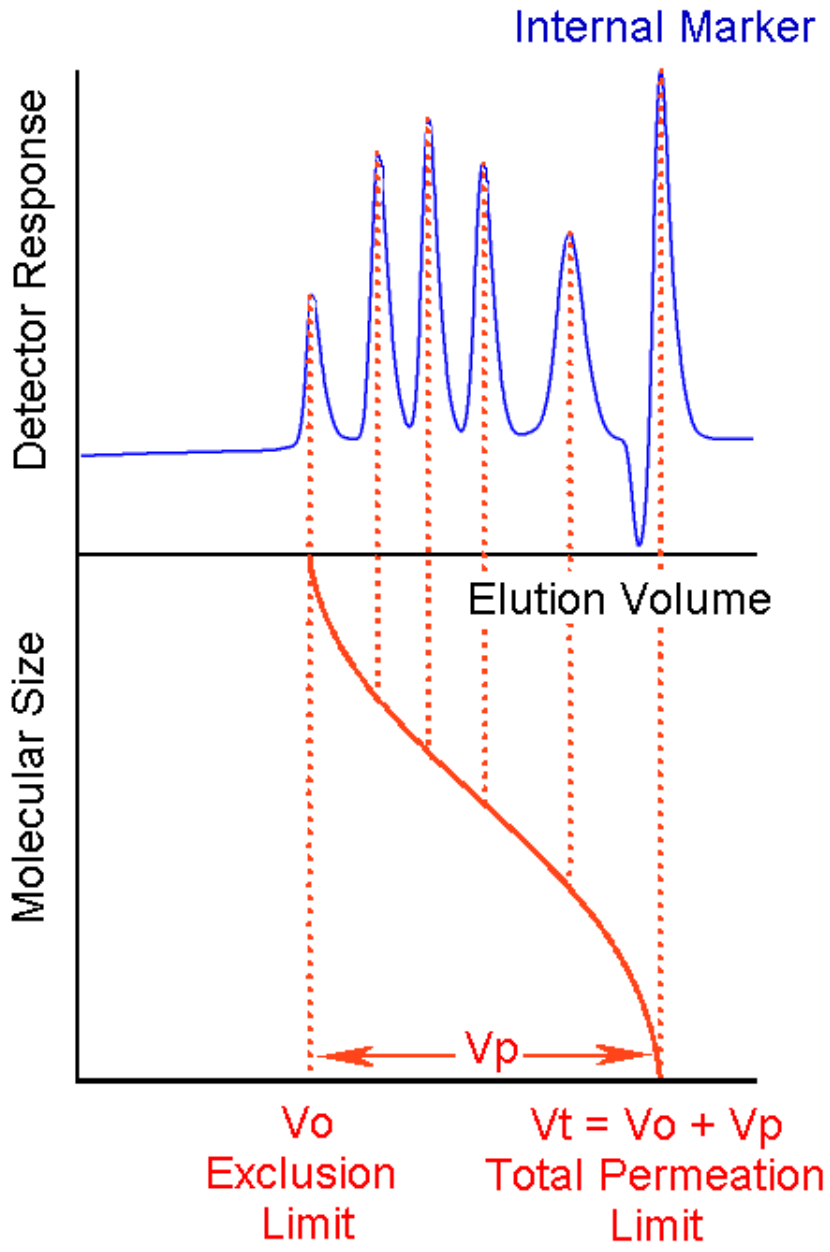
- The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution. More columns will be required to maintain the quality of the results.
- For higher molecular weight samples, larger particles are necessary to reduce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

Column elution profiles

- As a result of the GPC separation mechanism, polymer molecules elute from the column in order of size in solution.
- Largest elute first, smallest elute last.
- The separation is purely a physical partitioning, there is no interaction or binding.
- The separation is isocratic.
- If polymer molecules have the same molecular dimensions, they will co-elute by GPC and may not be separated by this technique.
- The calibration curve describes how different size molecules elute from the column.



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

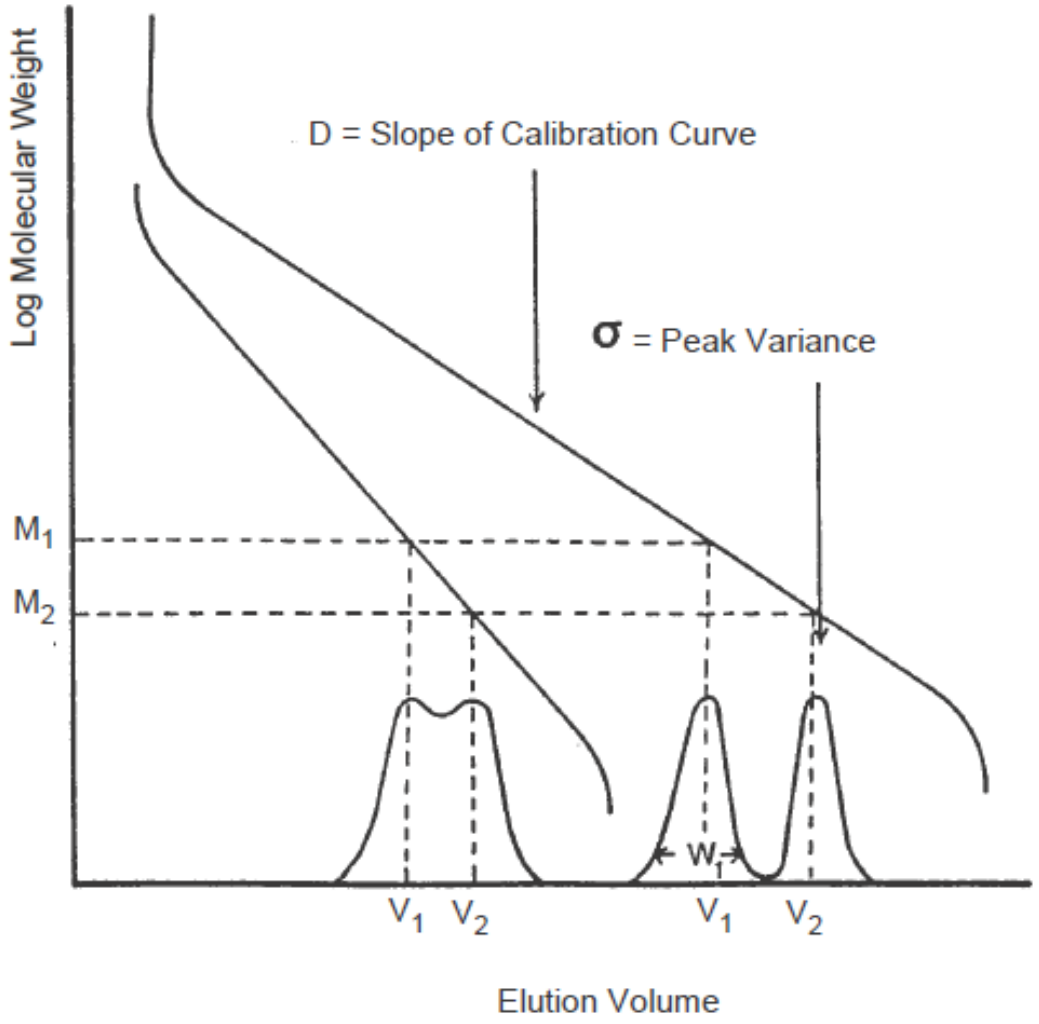
- Increasing pore volume increases the resolution

Use a packing with a smaller particle size

- Decreasing the particle size increases column efficiency

GPC Column Selection

Addition of column/pore volume to improve resolution

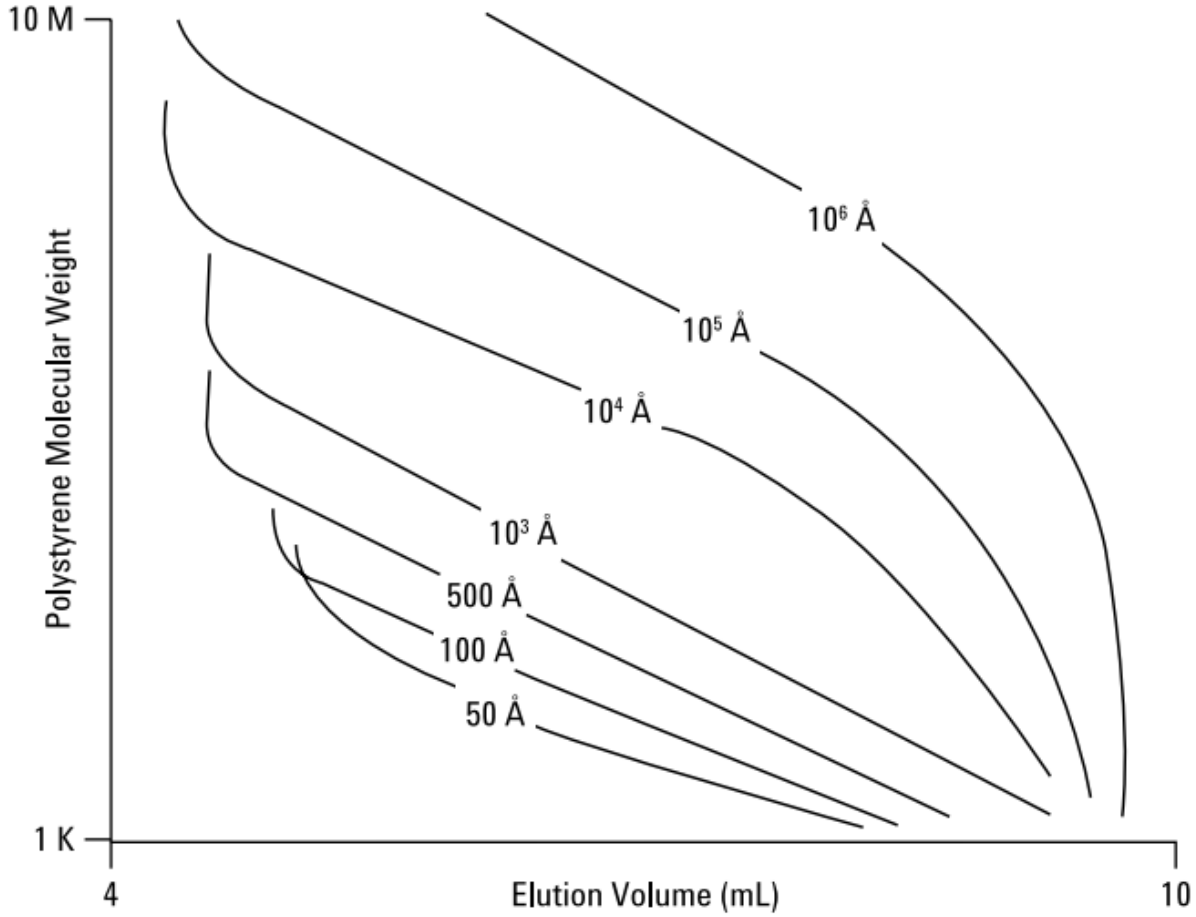


GPC Column Selection

Individual pore size

- All particles have the same pore size.
- Good separation, but narrow range of molecular weight (mol wt).
- Very nonlinear curve; linear only over a very narrow mol wt range.
- Oldest technology, but still popular, and useful for separating very small and very large compounds.
- A wider mol wt range is possible by combining different columns in series, but you need to select carefully so you do not create a column ‘mismatch’.

Calibration curve

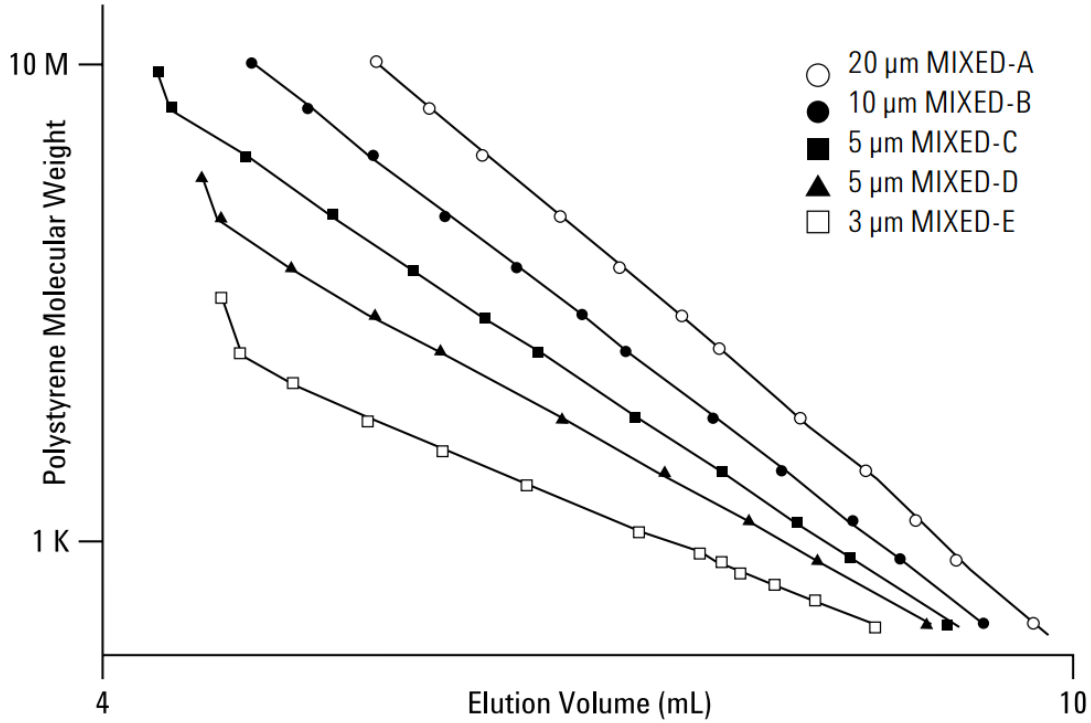


PLgel Individual Pore size calibration plots

GPC Column Selection
MIXED columns

- Individual pore size particles are mixed together/blended to make a linear curve.
- Very wide ranges are possible, but only a small amount of separation can be achieved for each mol wt.
- The linear curve makes the chromatogram easy to read and analyze.
- As the most popular technology, it is well established and widely used.
- The columns in series of same type are still linear.

Column family: PLgel

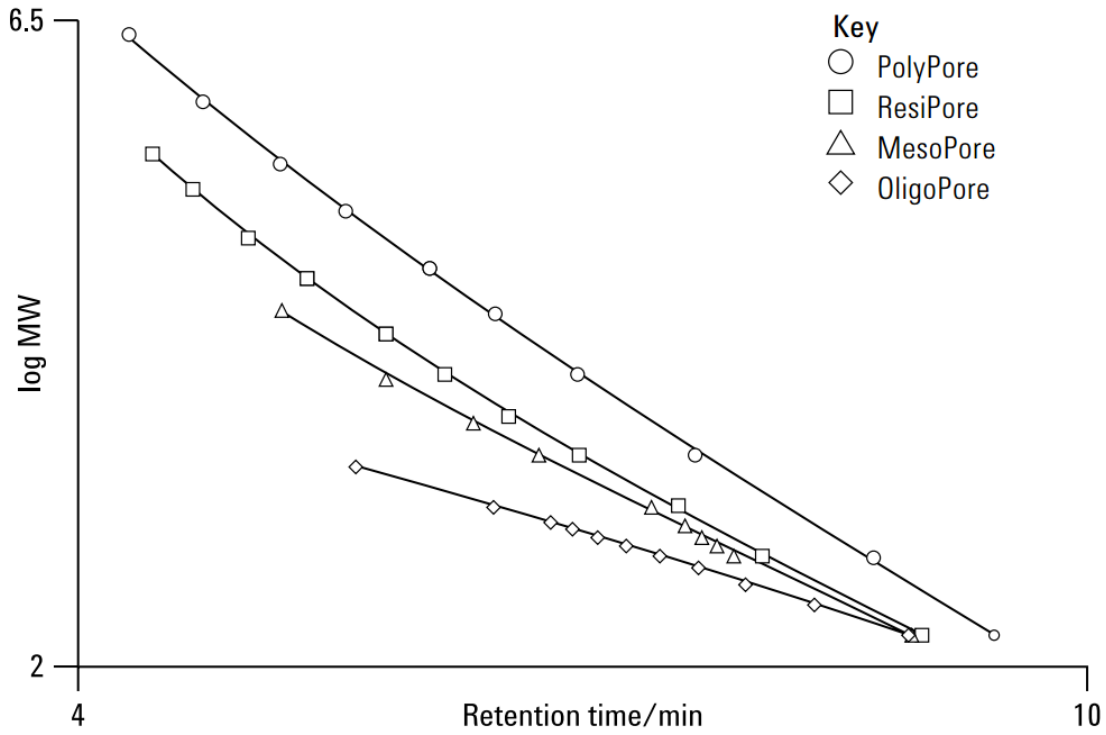


PLgel MIXED calibration plots

GPC Column Selection
Multiporous

- 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

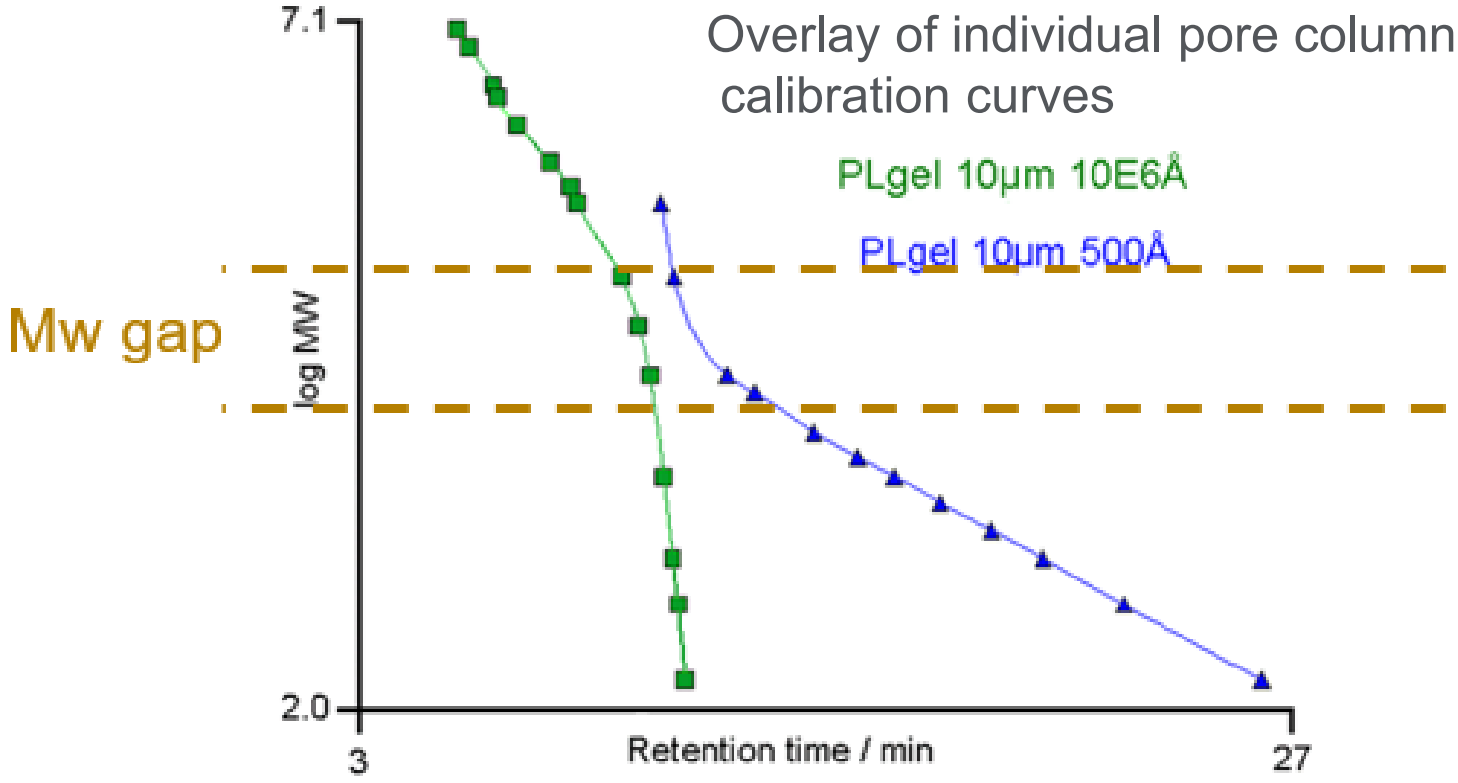
Column family: PlusPore



PlusPore calibration plots

GPC Column Selection

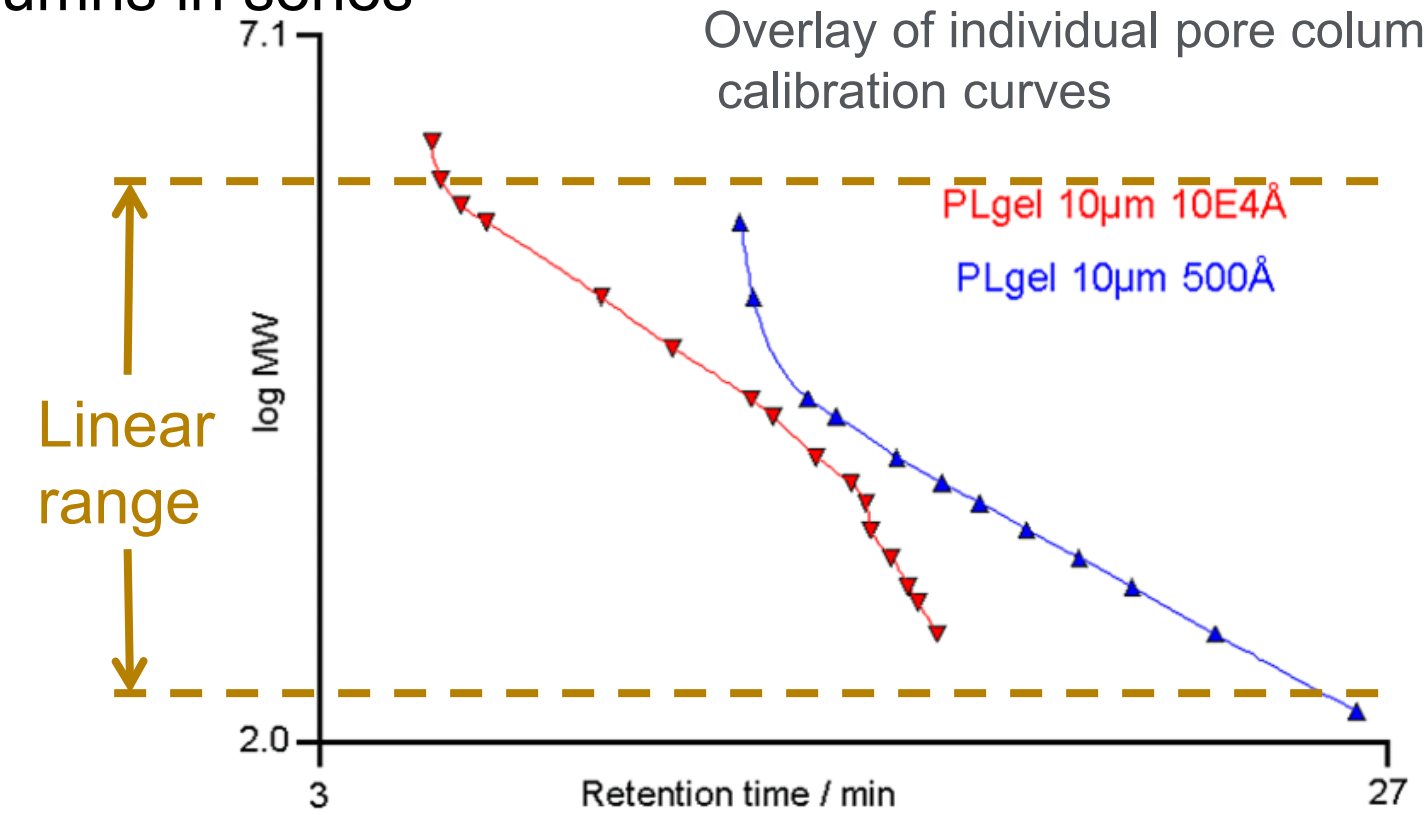
Combining columns in series



- Molecular weight gap between linear ranges
- Changes retention and gives unusual peak shapes

GPC Column Selection

Combining columns in series



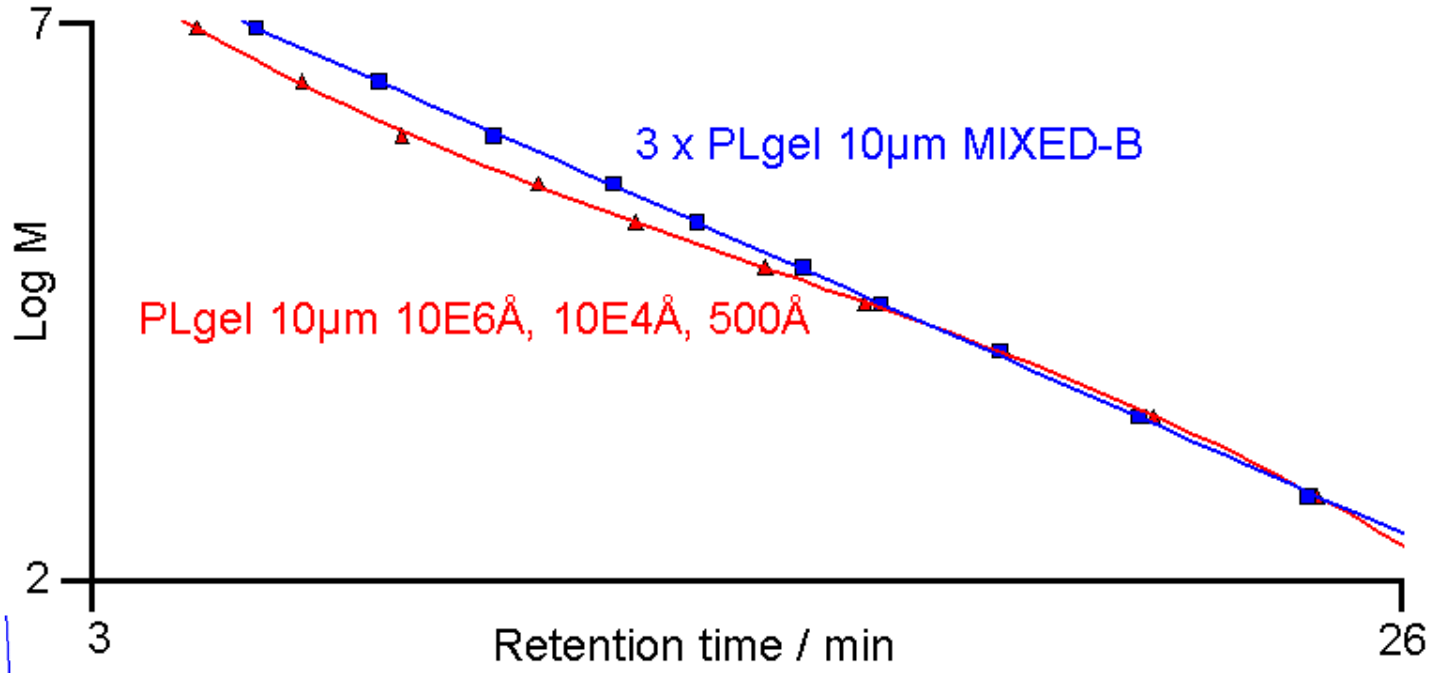
- Individual columns can be coupled in series
  - For example: PLgel and PL aquagel-OH
- The linear calibration ranges of the columns need to complement each other without overlap

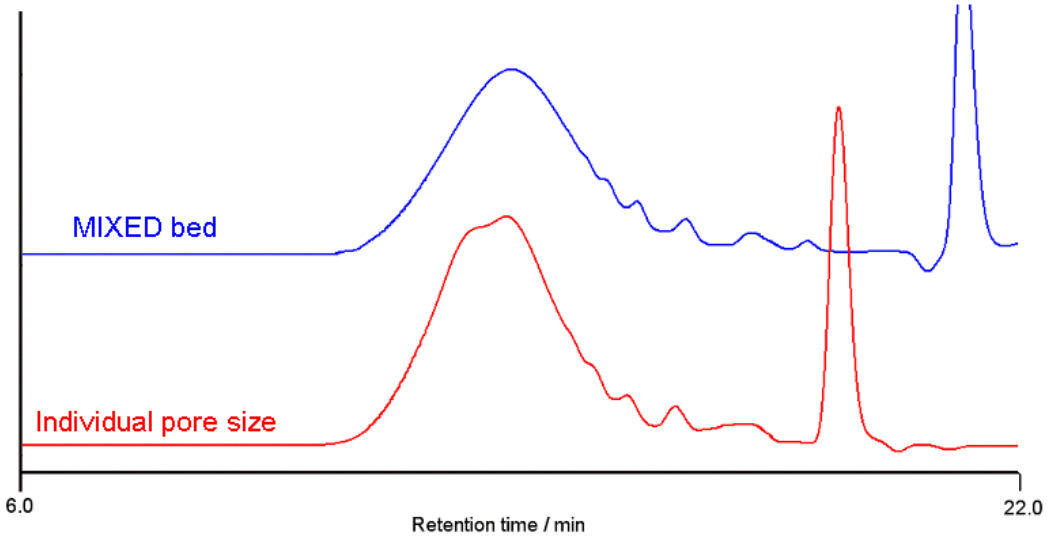
GPC Column Selection

MIXED columns preference

Agilent InfinityLab

Individual pore versus MIXED





GPC Column Selection

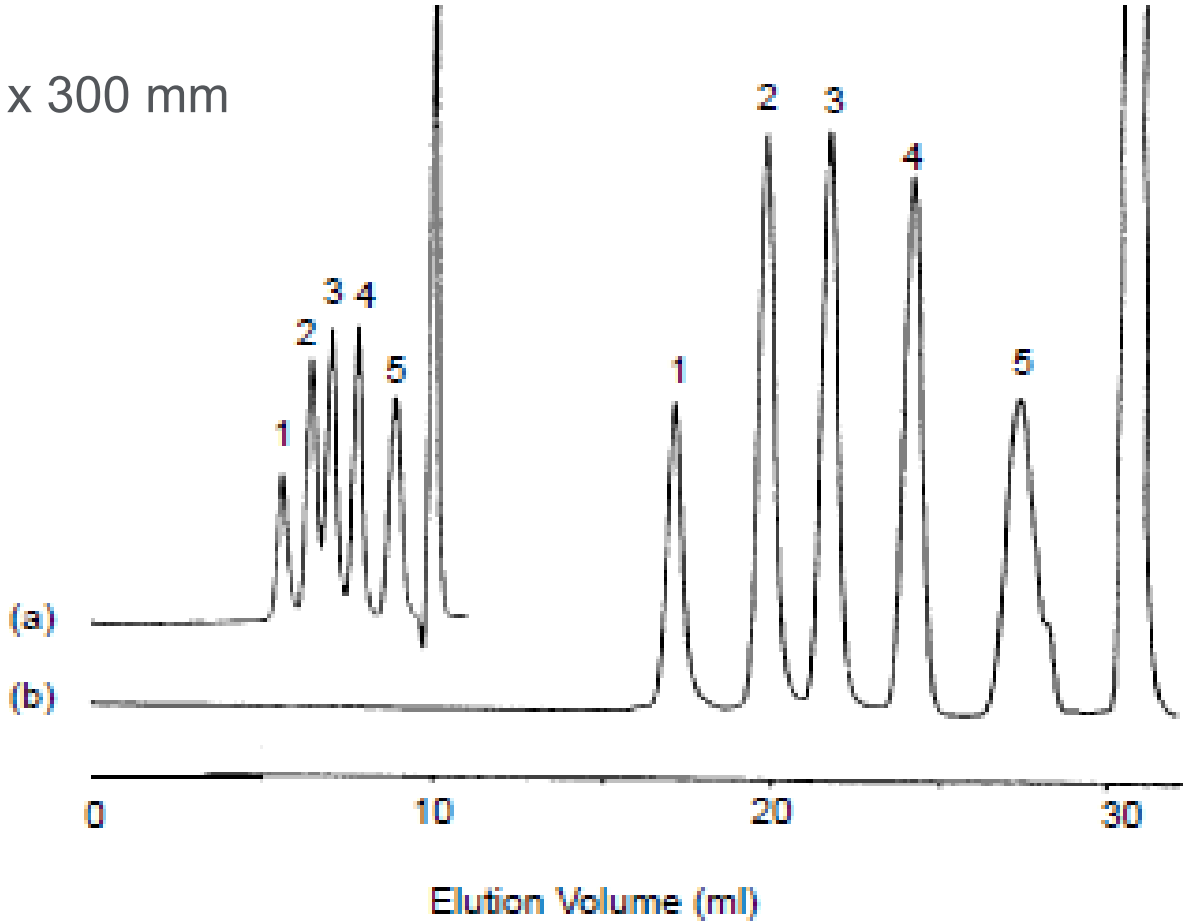
Effect of column length on resolution

Agilent InfinityLab

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

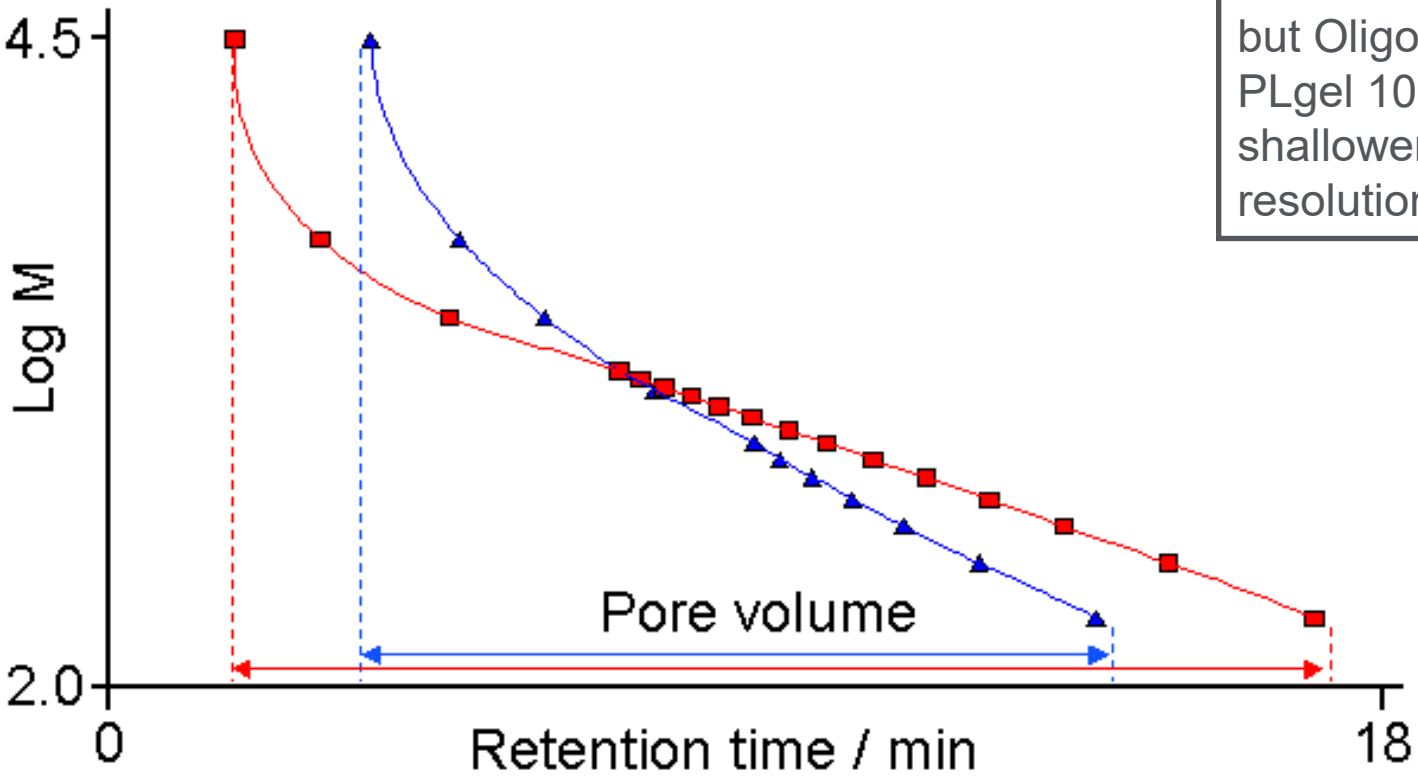
1. 3,040,000
2. 330,000
3. 66,000
4. 9200
5. 580



GPC Column Selection

Effect of increased pore volume

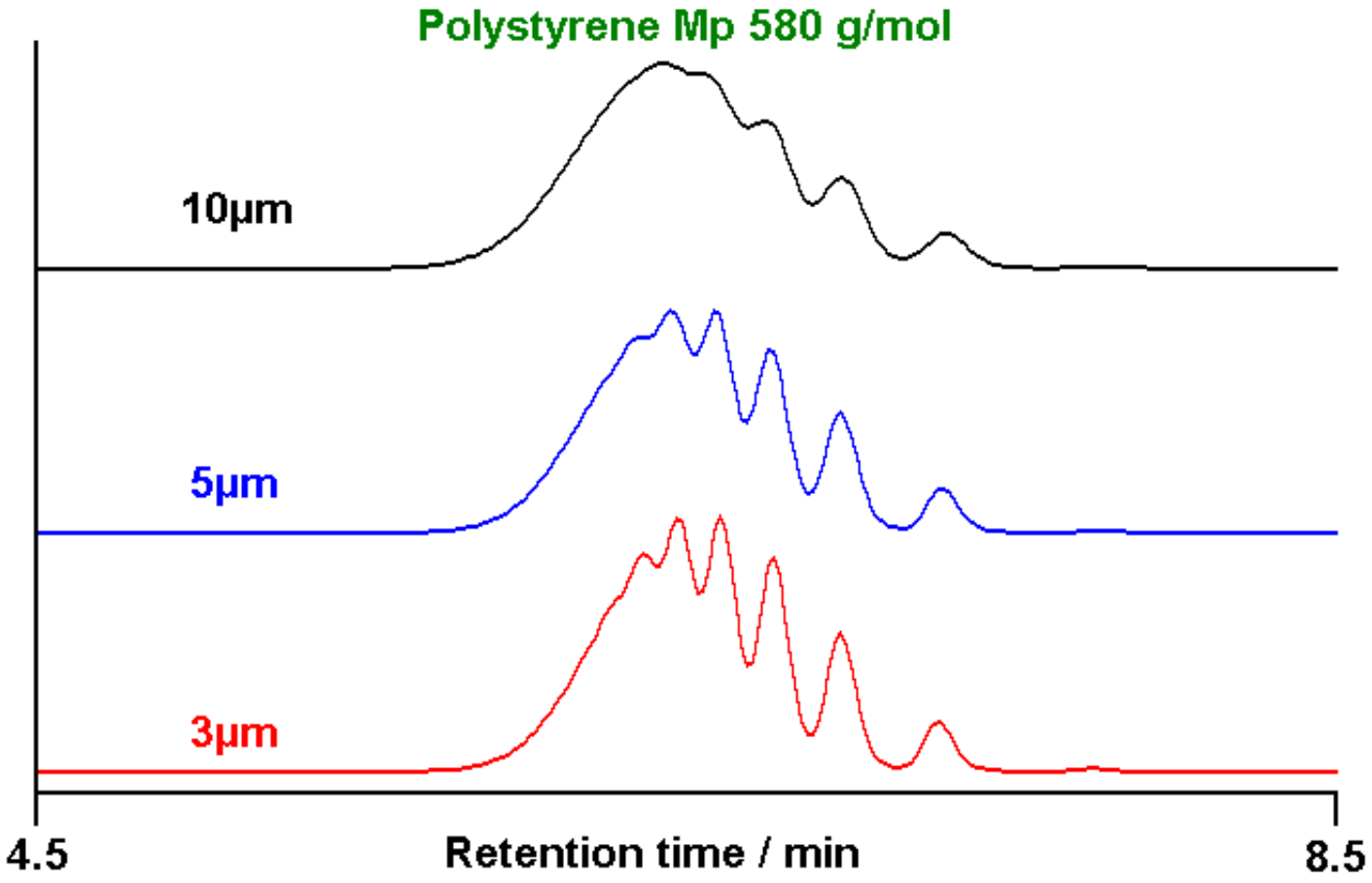
Columns	2 x PLgel, 3 µm, 100 Å, 7.5 x 300 mm, p/n PL1110-6320 ▲
	2 x OligoPore, 7.5 x 300 mm, p/n PL1110-6325 ■
Eluent	THF
Flow rate	1.0 mL/min



Both columns have a similar exclusion limit, but OligoPore has a greater pore volume than PLgel 100 Å. Hence the slope of the curve is shallower, leading to greater/improved resolution.

Column Selection

Effect of particle size on resolution



Eluent:	THF
Flow rate:	1.0 mL/min
Injection volume:	20 µL
Detector:	RI

Column Selection

Concentration - General guidelines

For **high mol wt** samples, use a lower concentration and if detector response requires it, increase the injection volume.

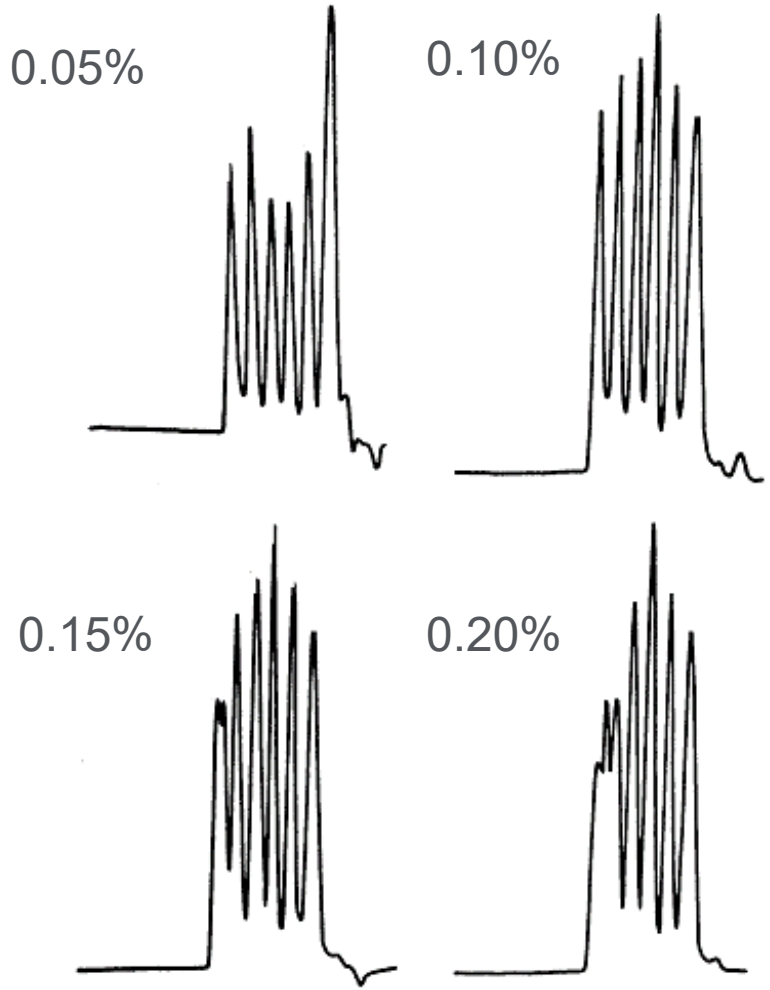
For **low mol wt** samples, use a higher concentrations and avoid larger injection volumes to maintain high resolution.

Mol Wt	Concentration (%)	Injection volume (µL)
<50,000	0.20 to 0.50	20 to 50
50,000 to 500,000	0.10 to 0.20	50 to 200
>500,000	0.01 to 0.10	50 to 200

Note: All values offered as guide only.

Column Selection

Effect of concentration on peak shape and resolution



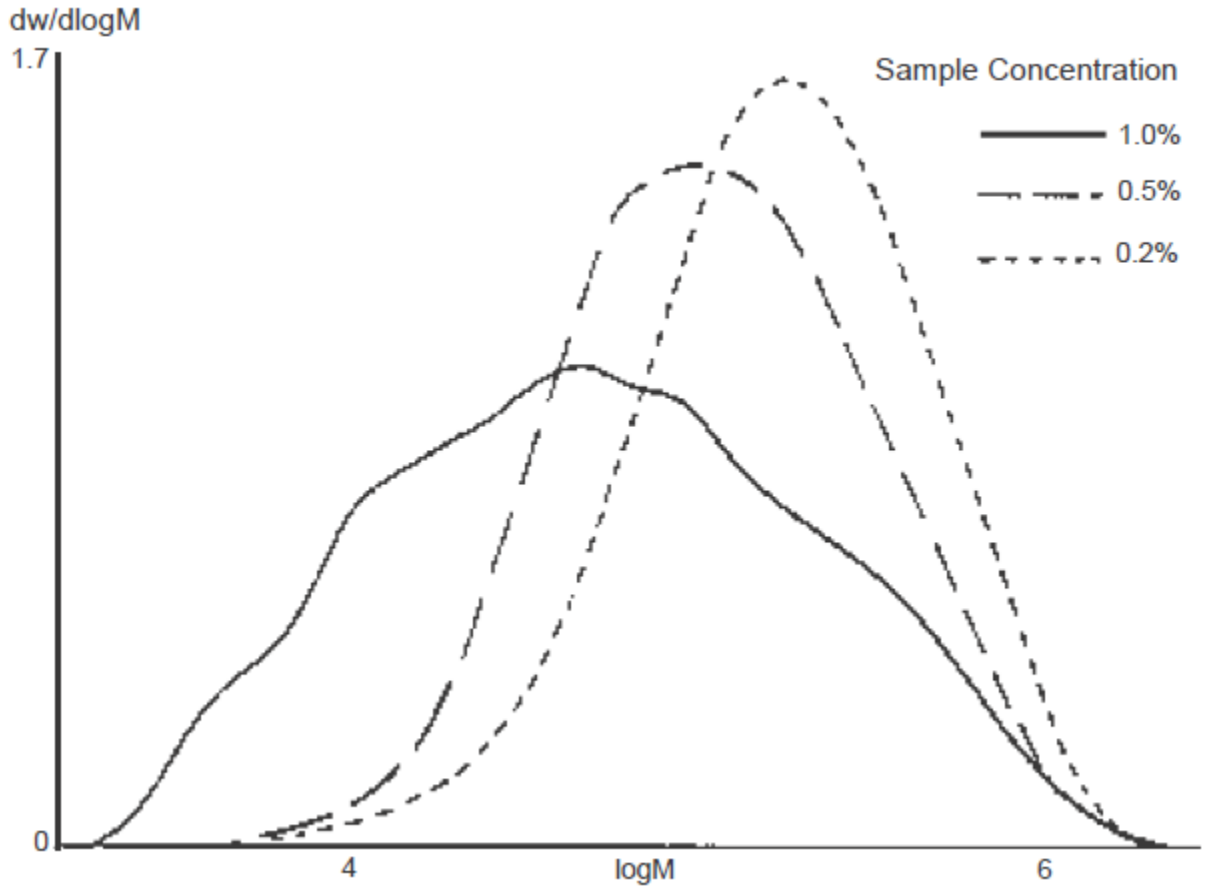
Column:	PLgel, 10 µm, MIXED-B
	7.5 x 300 mm, p/n PL1110-6100
Eluent:	THF
Flow rate:	1.0 mL/min
Detector:	UV

Polystyrene standards

1. 8,500,000	4. 34,500
2. 1,130,000	5. 5,100
3. 170,000	6. 580

Column Selection

Overloading effects



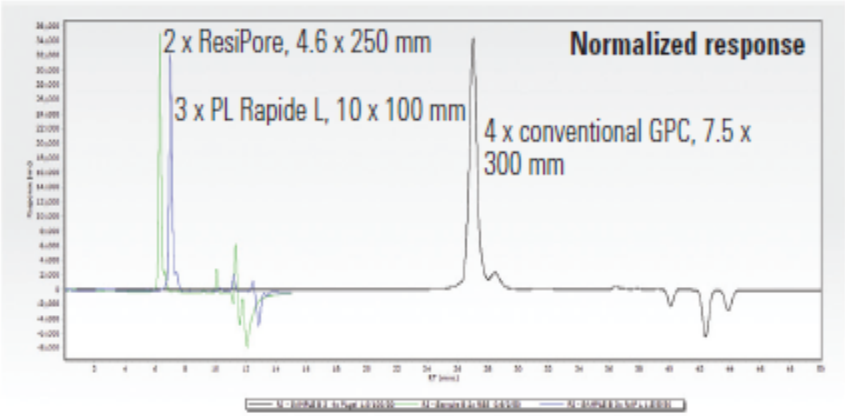
Sample:	Broad polystyrene
Column:	2 x PLgel, 5 µm, MIXED-C 7.5 x 300 mm, p/n PL1110-6500
Eluent:	THF
Flow rate:	1.0 mL/min
Injection volume:	200 µL
Detector:	RI

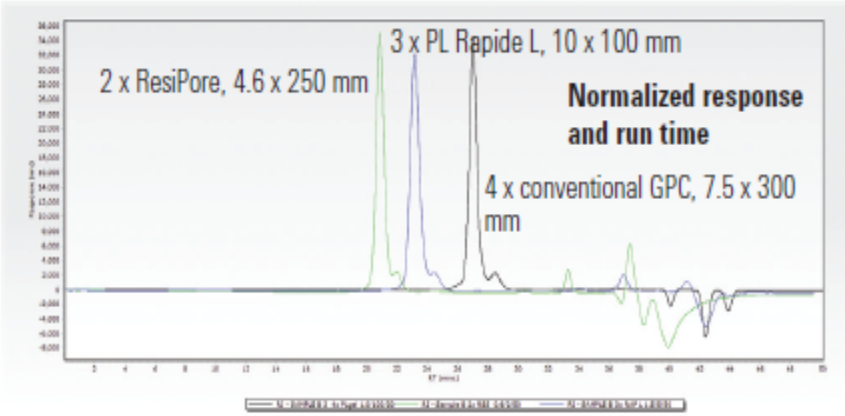
Column Selection

Fast GPC

Improving speed for analysis without sacrificing resolution

Comparison for Conventional Columns vs Cols for Fast GPC





Throughput is increased by more than 3x

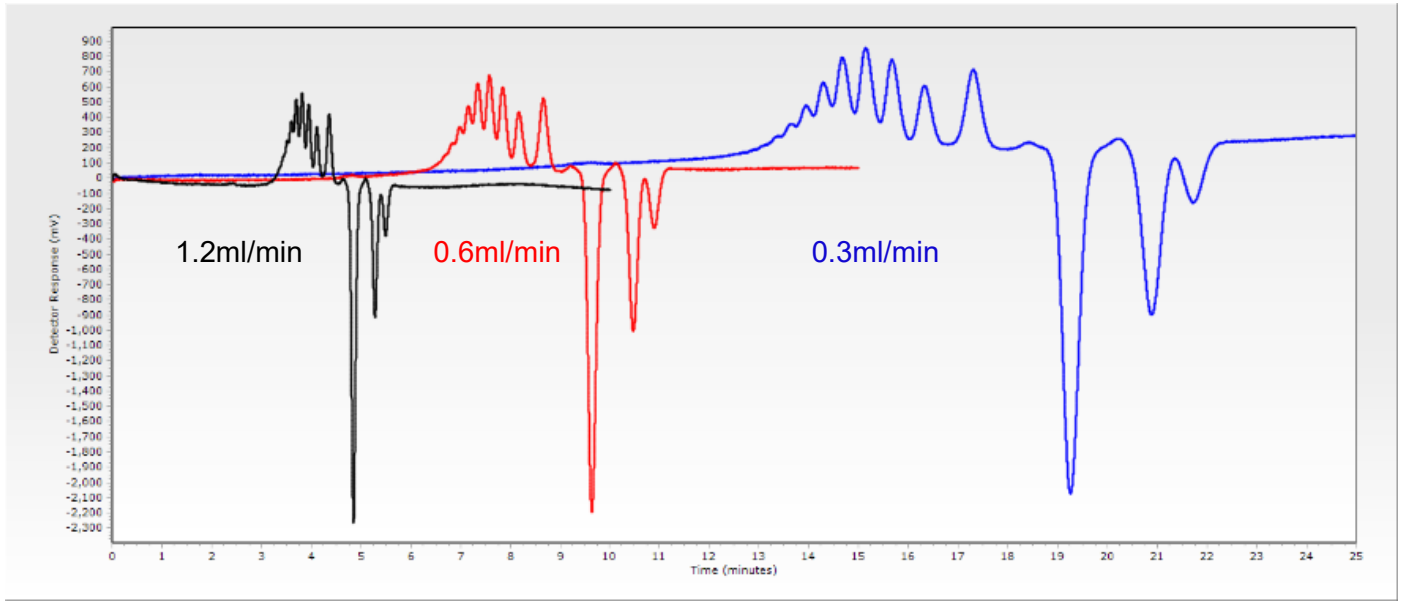
Columns	Peak 2 retention time (min)	Run time (min)
4 x conventional 7.5 x 300 mm	28.46	50
3 x PL Rapide L 10 x 100 mm	7.41	15
2 x ResiPore 4.6 x 250 mm	6.66	15

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

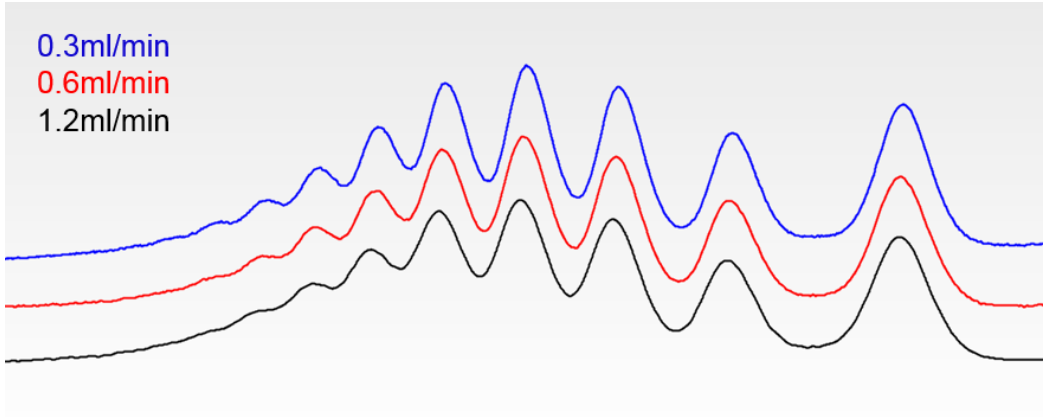


MW Range: up to 3,300 (g/mol)

Nominal Particle Size: 6 µm

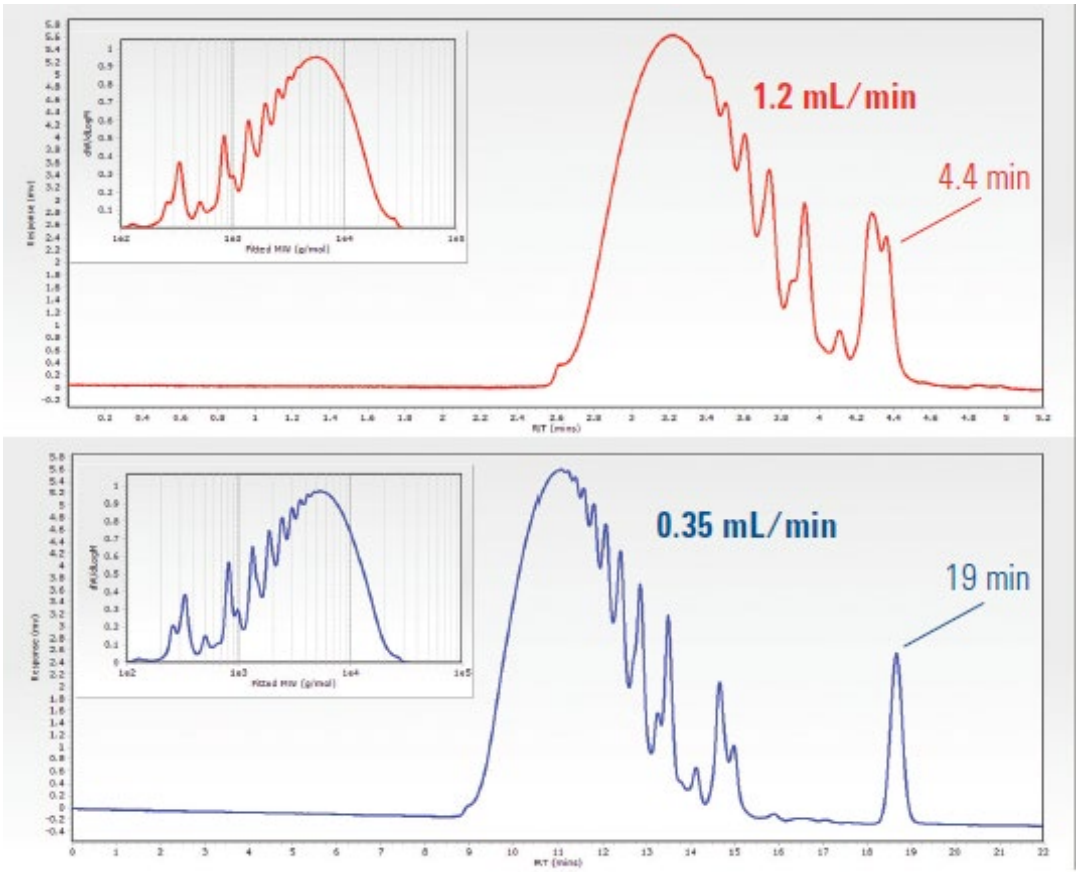
Typical Efficiency: >55,000 p/m

- 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 normalised 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

Column Selection

Agilent range of GPC/SEC columns

Agilent InfinityLab

Organic Solvents	Organic Solvents	Polar Solvents	Aqueous Solvents
PLgel	**PlusPore**	**PolarGel**	**PL aquagel-OH**
• PLgel MIXED	• PolyPore	• Polargel M	**PL Rapide Aqua**
• PLgel MiniMIX	• ResiPore	• Polargel L	
• PLgel MIXED-LS	• MesoPore		
• PLgel [Ind Pore]	• OligoPore		
• PLgel Olexis			
PL HFIPgel			
PL Rapide			
EnviroPrep			
PL MultiSolvent			

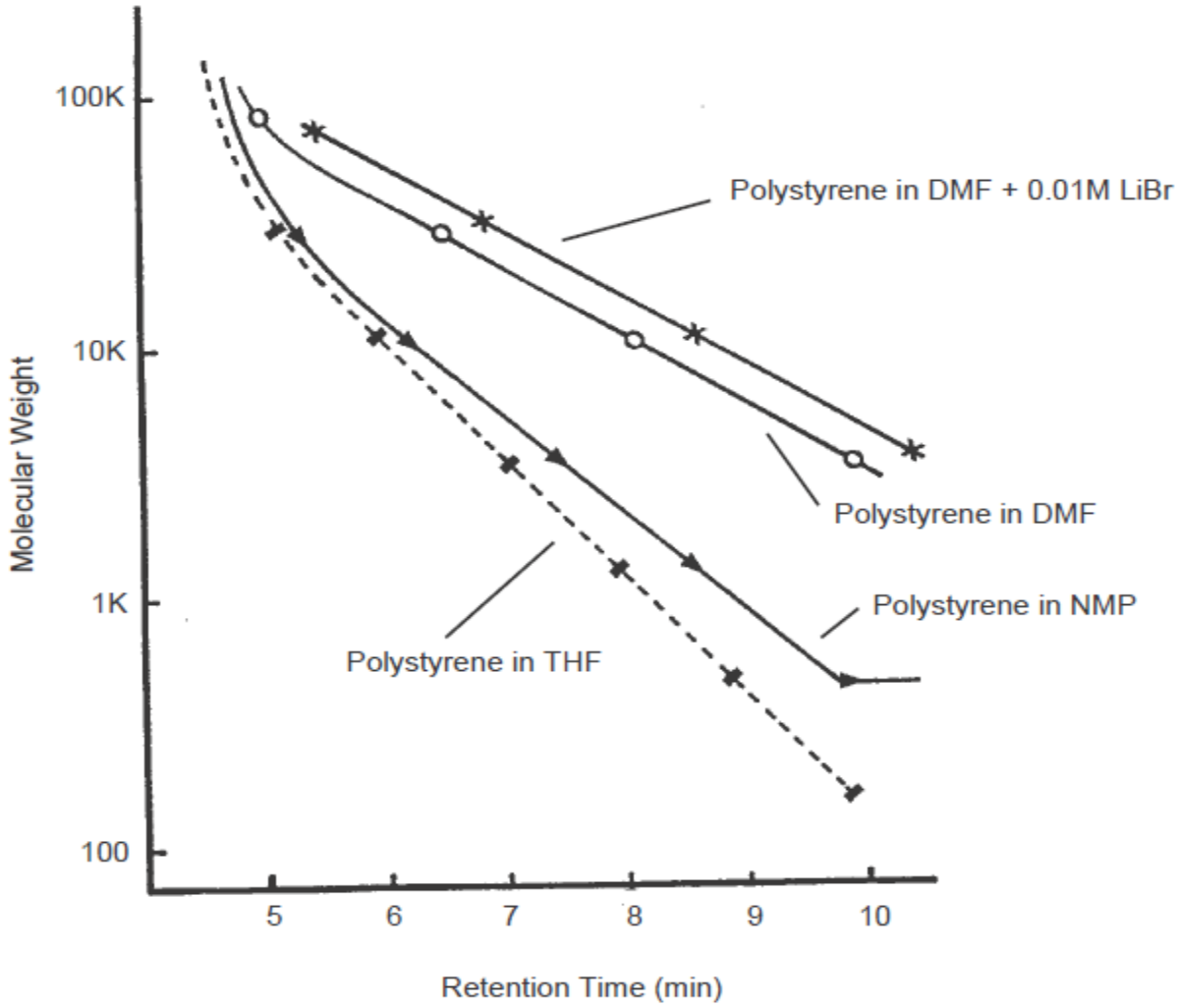
Calibrant Selection

What solvent is your polymer soluble in?

Answer	Recommendation	Comment
Water or water buffer with up to 50% methanol	• Polyethylene glycol/oxide (PEG/PEO) or • Polyacrylic acid	These standards perform well in all water-based systems. PEG/PEO available in convenient InfinityLab EasiVial format
Typical organic solvent, such as THF, chloroform, toluene	• Polystyrene (PS) or • Polymethylmethacrylate (PMMA)	Polystyrene is the most commonly used standard and is also available in convenient InfinityLab EasiVial format
Polar organics, such as DMF, DMSO, and NMP	• Polymethylmethacrylate (PMMA) or • Polyethylene glycol/oxide (PEG/PEO)	Polymethylmethacrylate is soluble in various polar organic solvents and is also available in InfinityLab EasiVial format

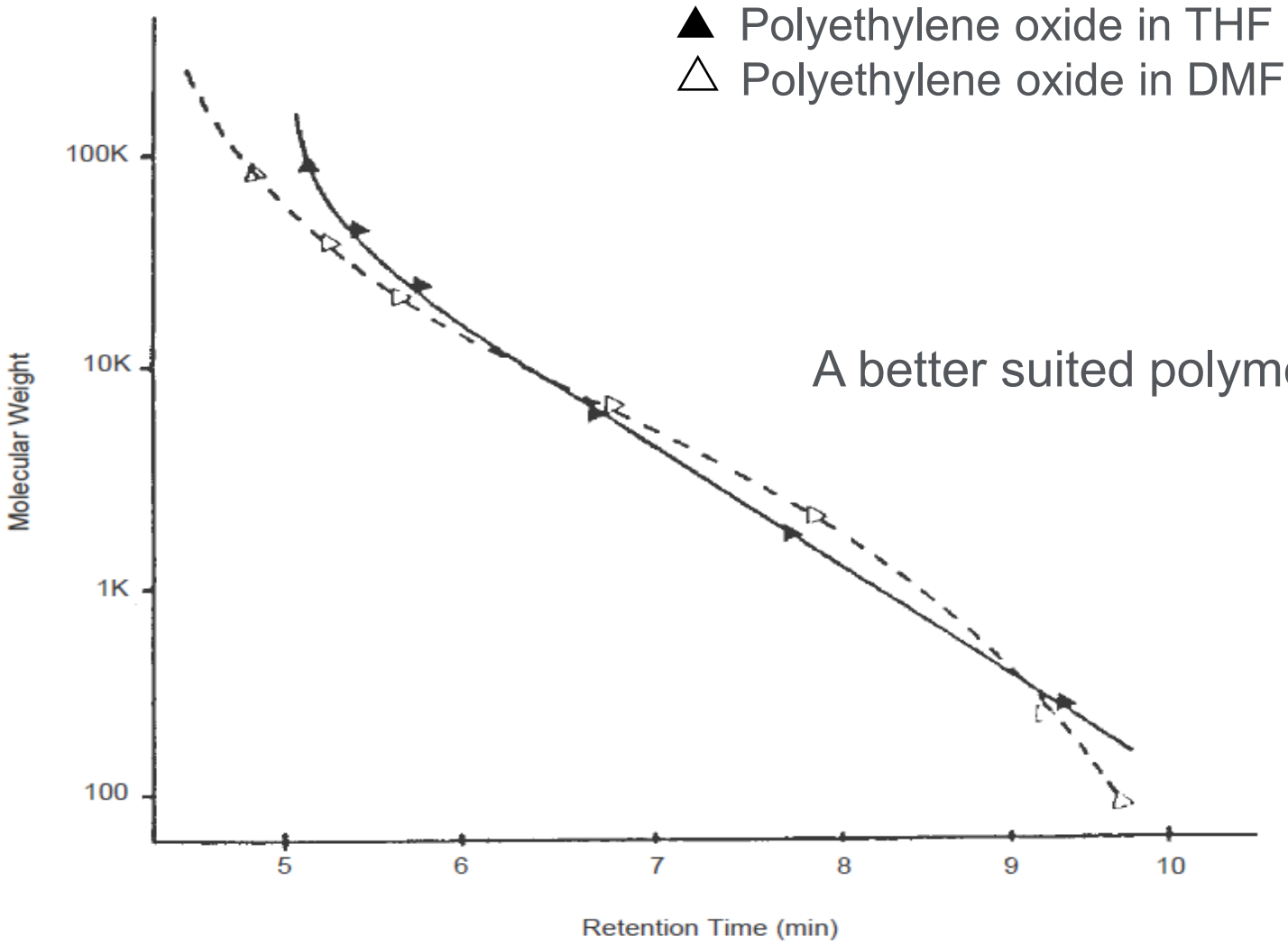
Calibrant Selection

Application example



Calibrant Selection

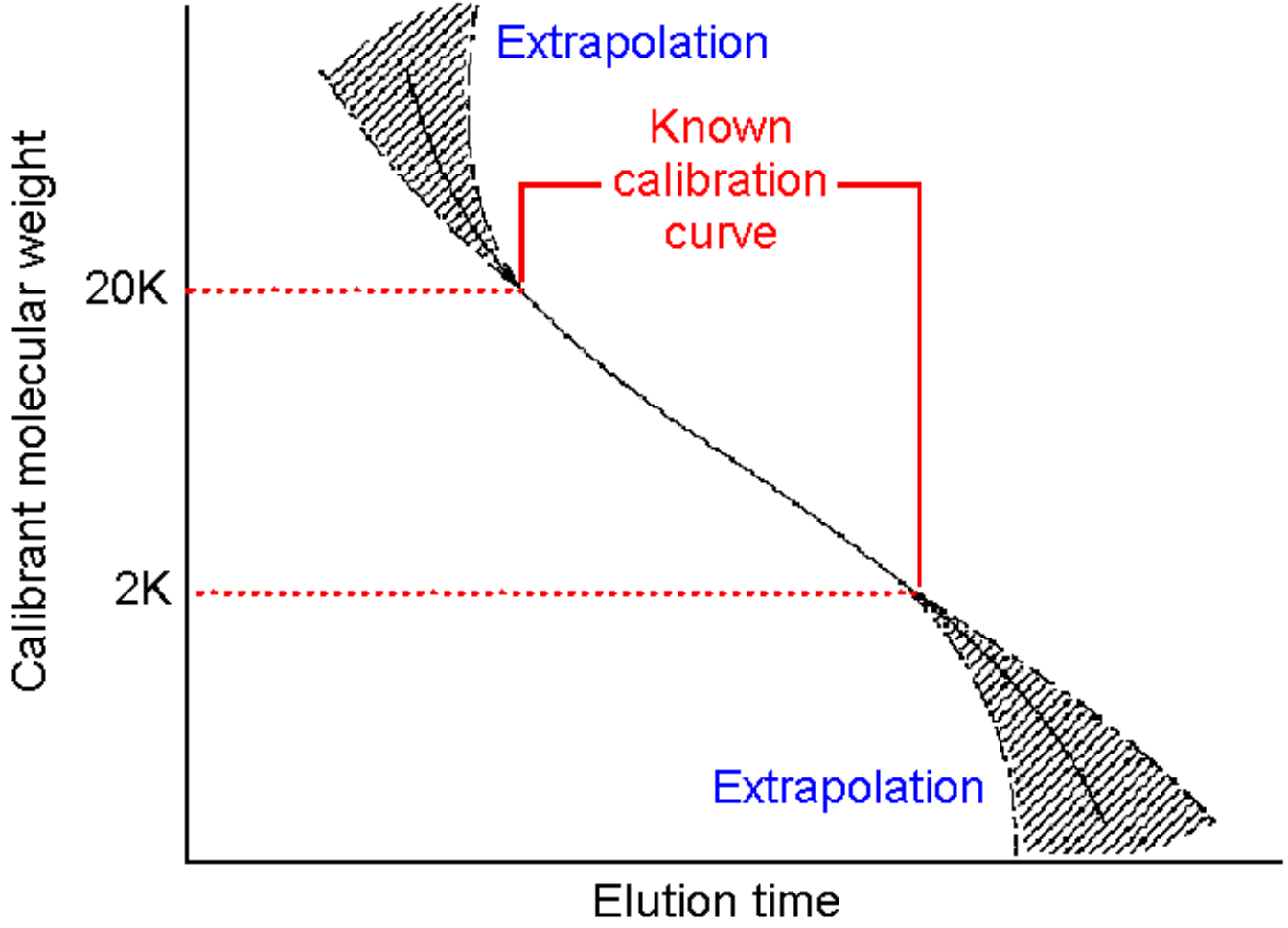
Application example



A better suited polymer standard selection

Calibrant Selection

Errors due to limited calibration region

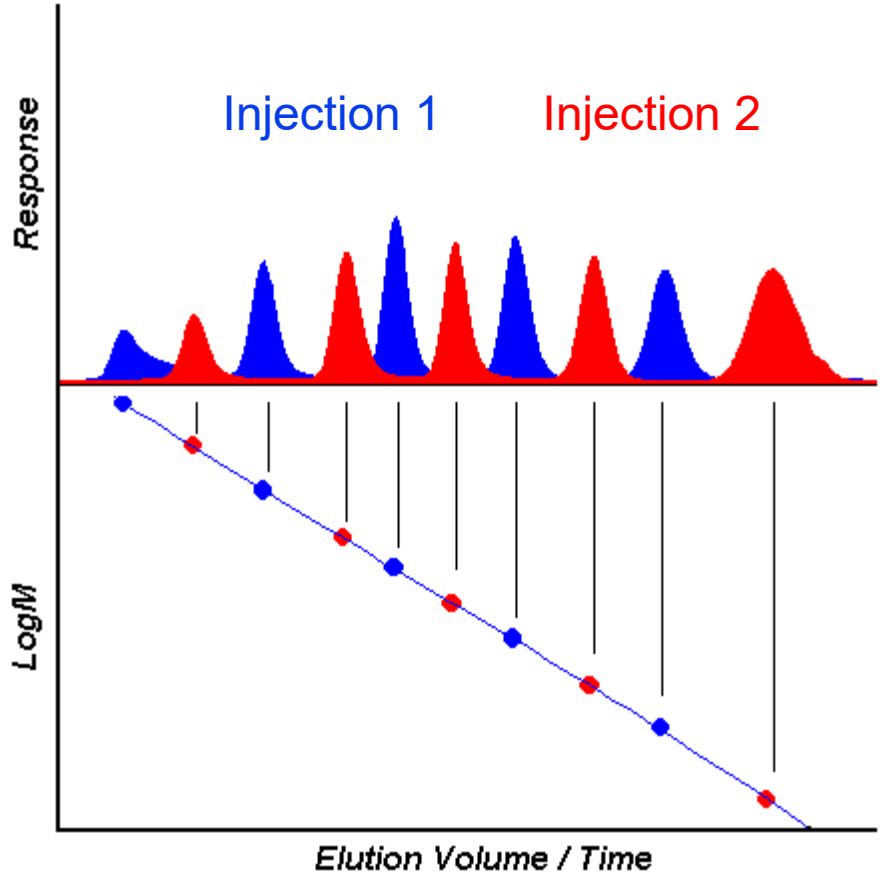


Important: The column calibration should cover the full elution time region of the sample to avoid errors due to extrapolation.

Calibrant Selection

Calibrating of GPC columns using narrow standards

- Chromatograph a series of well characterized, narrow polydispersity polymer standards. Injections of multiple narrow standards reduces the time taken to calibrate the system.
- Plot peak retention time (RT) versus peak log molecular weight (logM).
- Fit the data using a mathematical function (for example, polynomial order 1, 2, 3.)
- The calibration curve will be characteristic of the GPC column set used.



Standards peaks in each chromatogram should be fully resolved to obtain repeatable retention times

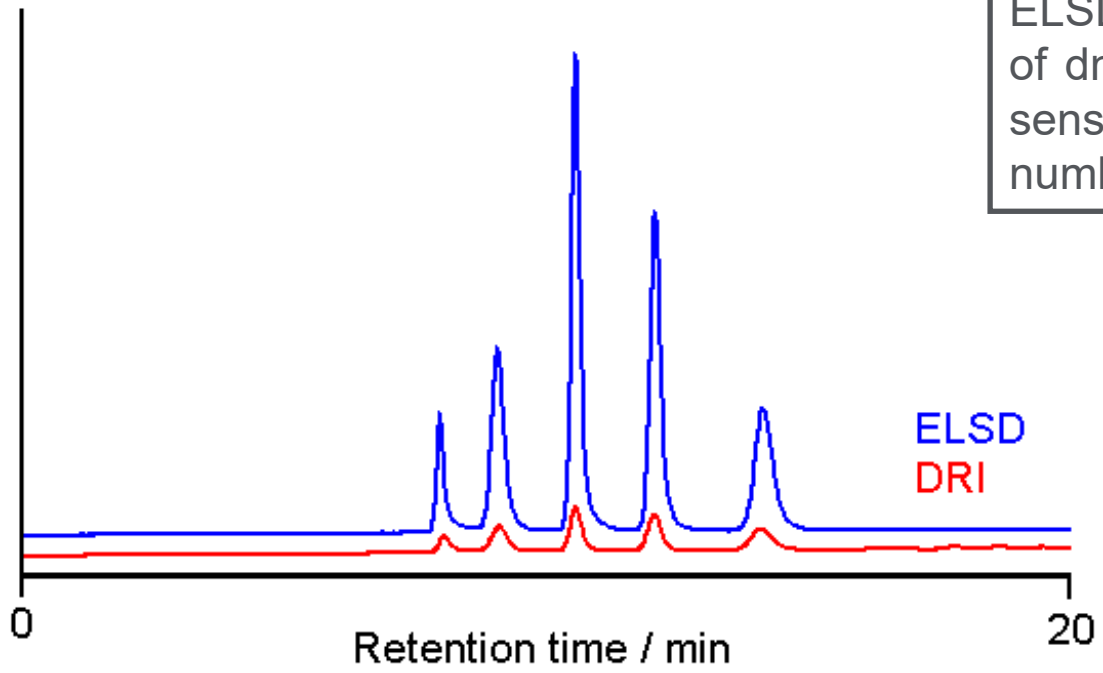
Detector Selection

Sensitivity of RID versus ELSD

Columns	2 x PLgel, 5 µm, MIXED-C, 7.5 x 300 mm p/n PL1110-6500
Eluent	THF
Flow rate	1.0 mL/min
Loading	0.1%, 20 µL

Mp values

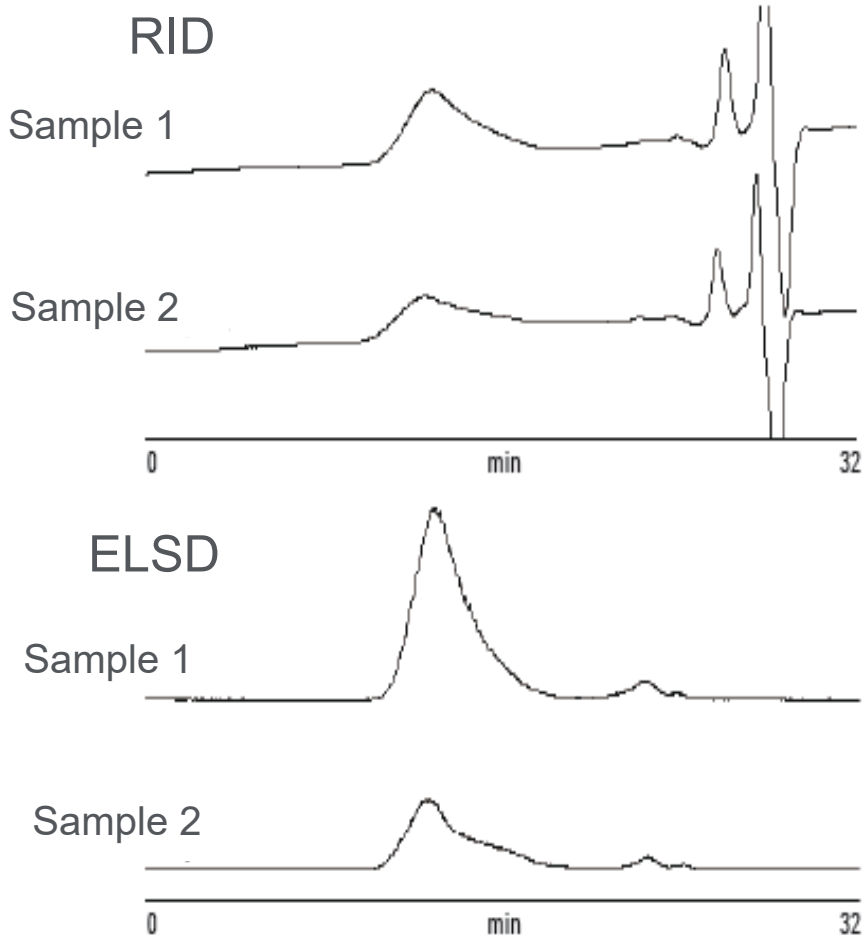
1. 7,500,000
2. 841,700
3. 148,000
4. 28,500
5. 2,930



ELSD is essentially independent of dn/dc, and an improvement in sensitivity will depend on a number of solute parameters.

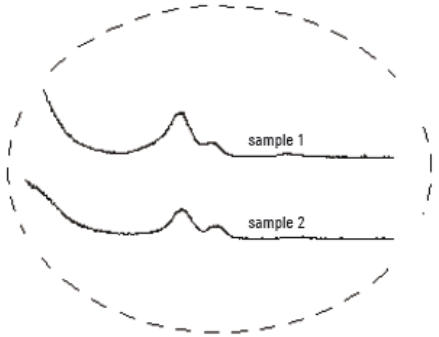
Detector Selection

Sensitivity of RID versus ELSD



RID:
Low response for sample
Unable to detect additives
System interference peaks present

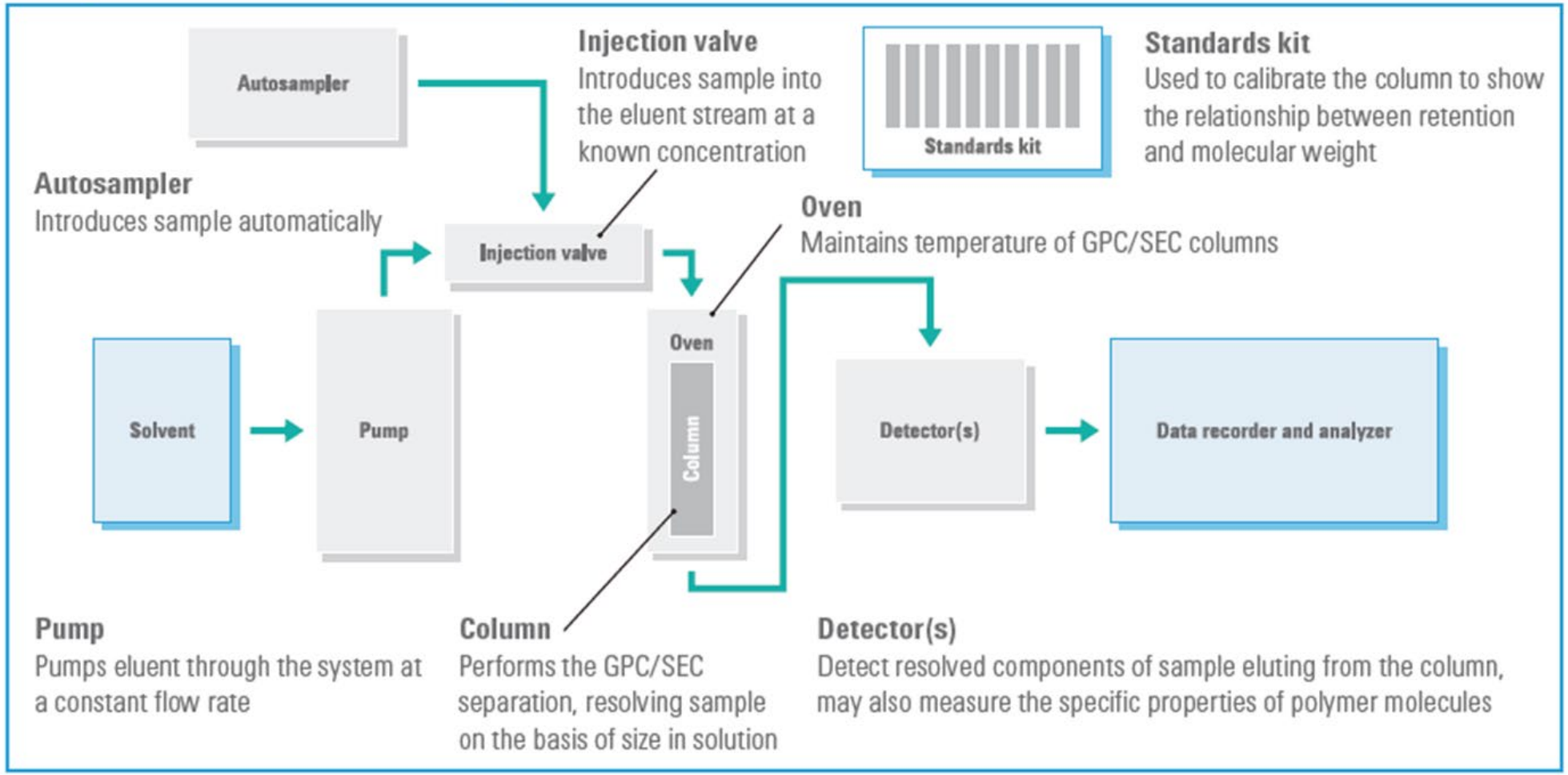
ELSD:
Improved response
Additives detected
No system interference peaks



System Considerations
Resolution and reproducibility

Agilent InfinityLab

Components of a GPC/SEC system



System Factors for Resolution and Reproducibility

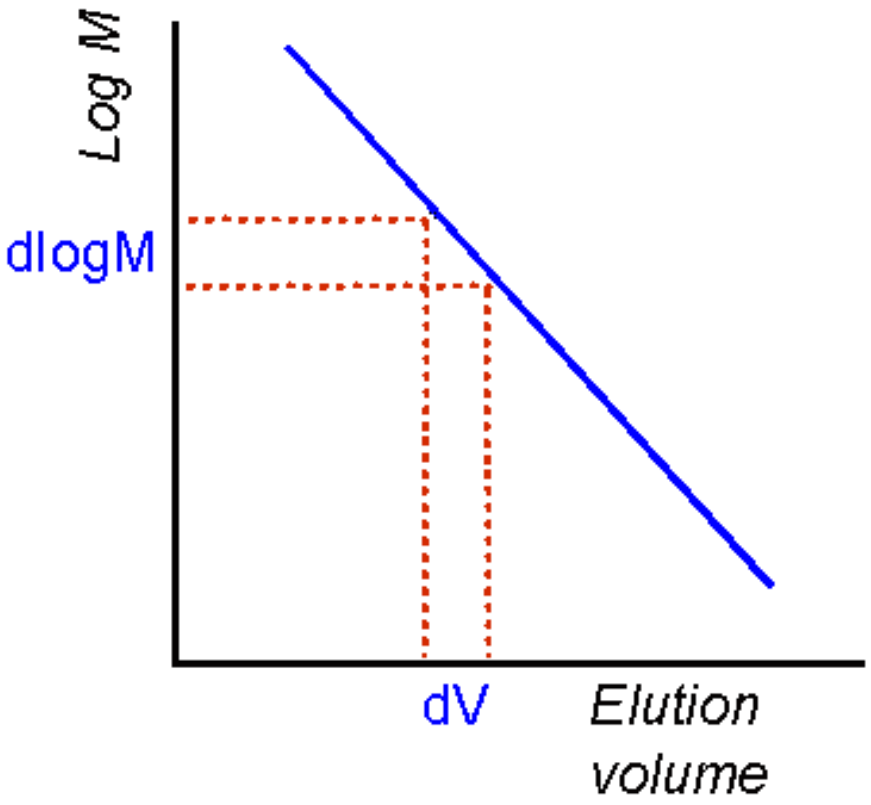
Pump considerations

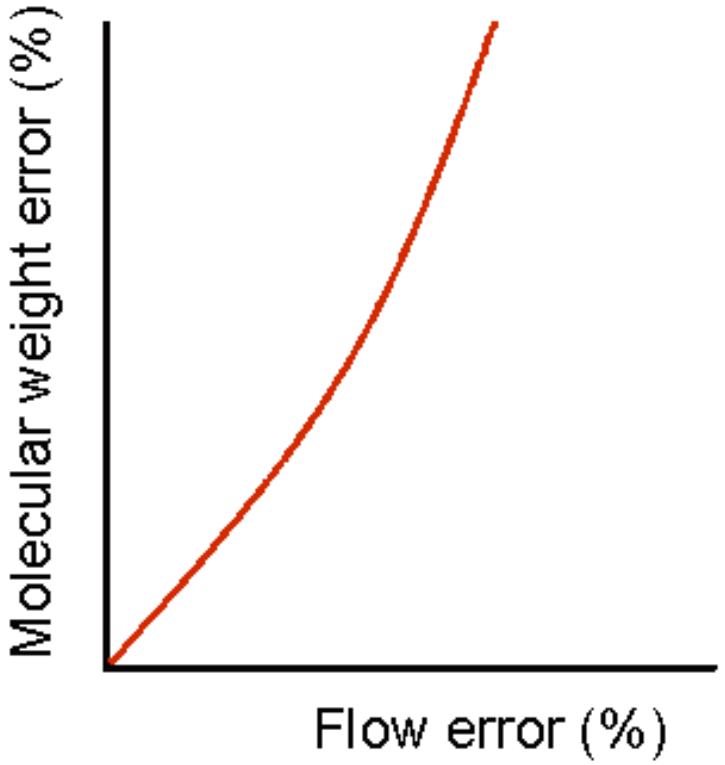
Common sources of retention time shifts are:

- Pump flow stability
- New connections
- Replaced parts

Pump Flow Rate and Reproducibility

Effect on molecular weight results



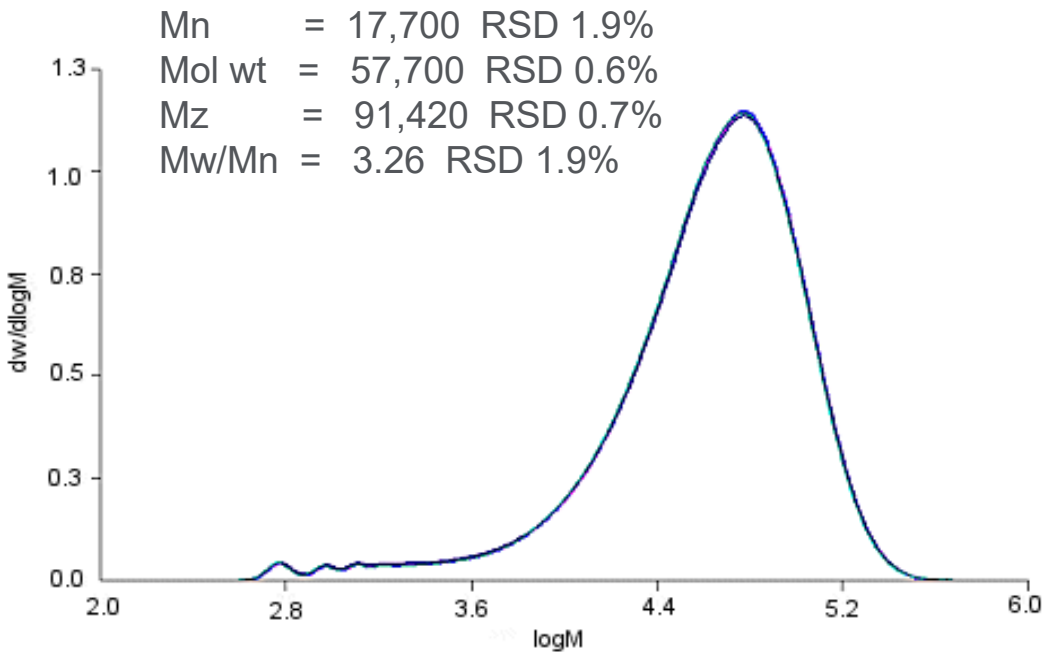


- A small change in flow rate can have a large effect on GPC molecular weight results

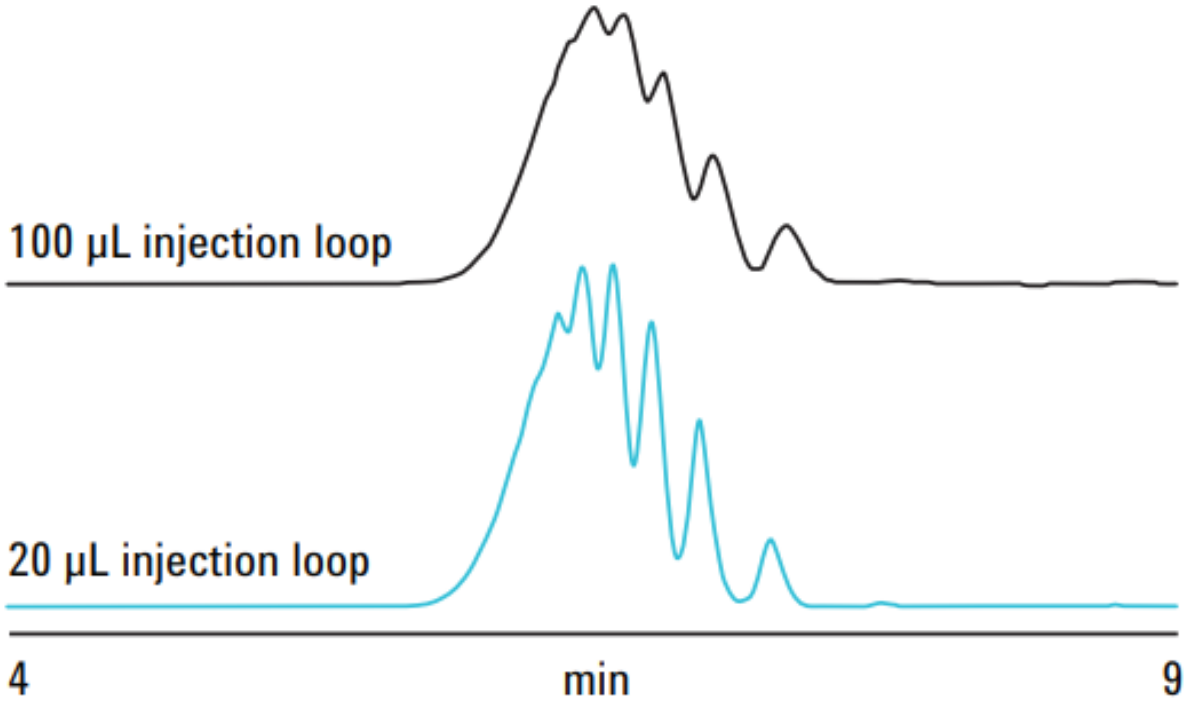
Pump Precision

The excellent flow precision of the 1260 Infinity II Isocratic Pump is ideal for accuracy in micro, analytical, and preparative GPC/SEC applications.

Overlay of molecular weight distribution plots for five injections of a commercial polycarbonate sample. Flow rate precision of <0.1% delivers repeatable calibration curves and accurately calculated polymer molecular weight data.



Effect of Injector Loop Size on Resolution



Column:	PLgel, 3 µm, MIXED-E
	300 x 7.5 mm, p/n PL1110-6300
Eluent:	THF
Flow rate:	1.0 mL/min
Sample:	Epikote 1001
	Epoxy resin

An injection loop can have a major contribution to system dead volume. Use reduced injection volume and increase concentration to maintain sensitivity.

System Temperature

Use of elevated temperature in column compartment

GPC applications employing elevated temperatures generally fall into these categories:

- To reduce solvent viscosity for improved mass transfer and improved chromatographic separation.
- To reduce system pressure and prevent column damage.
- To provide a stable thermal environment for GPC columns and detectors (especially RID).
- To achieve and maintain sample solubility.

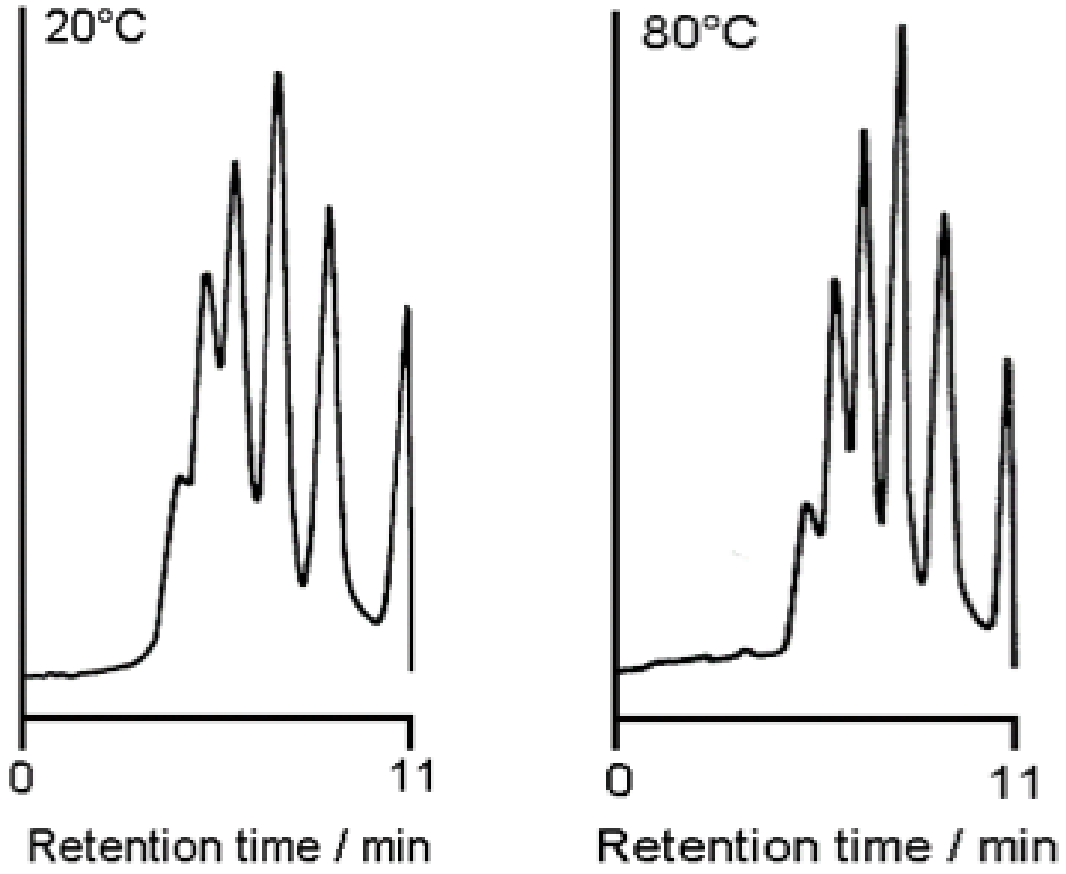
Eluent	Temperature (°C)
THF, Water, Chloroform	30 to 40
DMF, DMSO, DMAc	60 to 80
TCB	140 to 160

- All values offered as guide only

- Elevated temperature is a useful approach in GPC

System Temperature

Effect on resolution if using viscous solvents



Increased temperature:

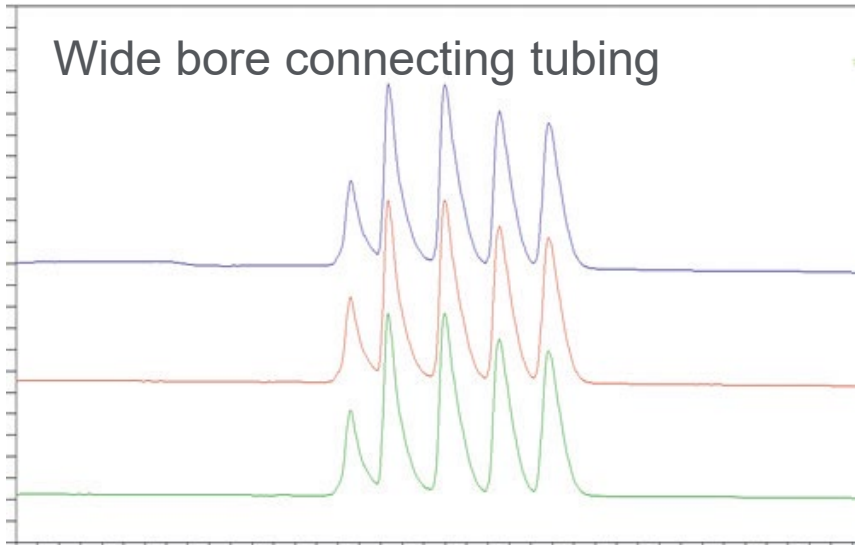
- Reduces operating pressure
- Improves resolution, particularly at high mol wt

Column: PLgel, 5 µm, MIXED-C
300 x 7.5 mm, p/n PL1110-6500
Eluent: DMF
Flow rate: 1.0 mL/min

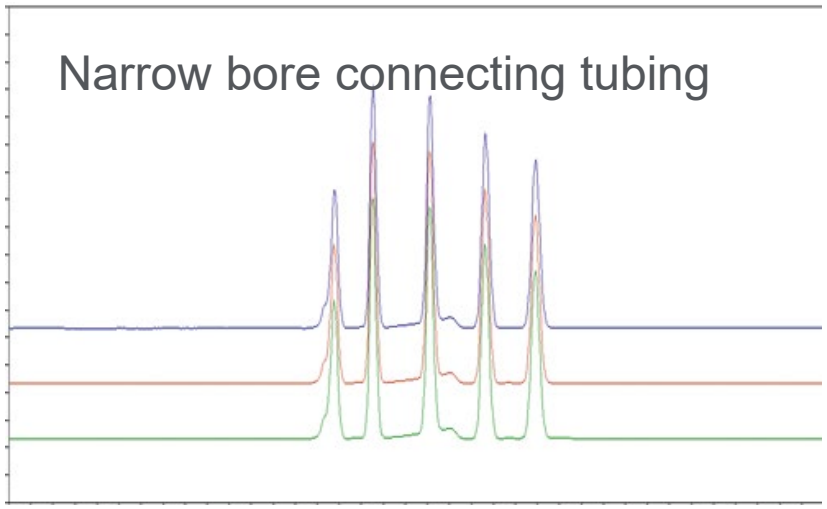
PEO/PEG standards

990,000	252,000
86,000	18,000
4,800	200

System Tubing
Reducing dead volume

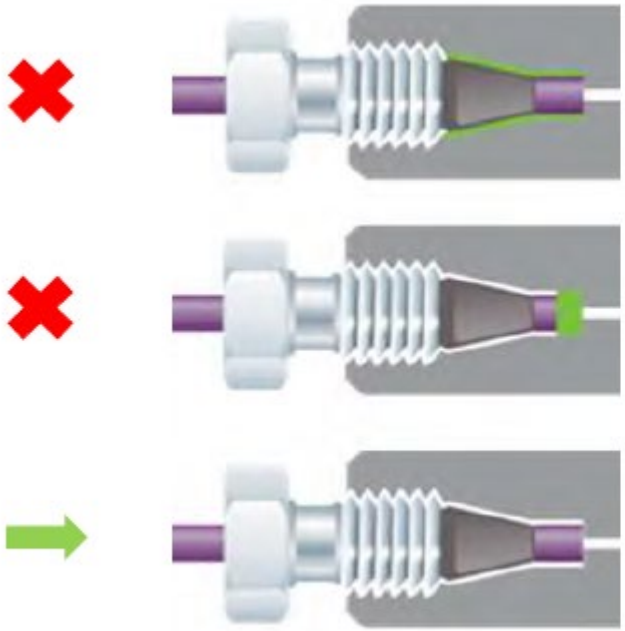


- Peak shape poor
- Resolution between peaks poor



- Use proper fittings
- Keep tubing connections short
- Inner diameter for tubing narrow as possible

System Fittings
Potential fittings issues



- Leak
- Peak shape problem
- No dead volume

Connection problems can lead to:

Poor chromatography
- Broad or tailing peaks
- Loss of resolution

Added maintenance costs
- Leaks, added troubleshooting
- Overtightening
- Column damage

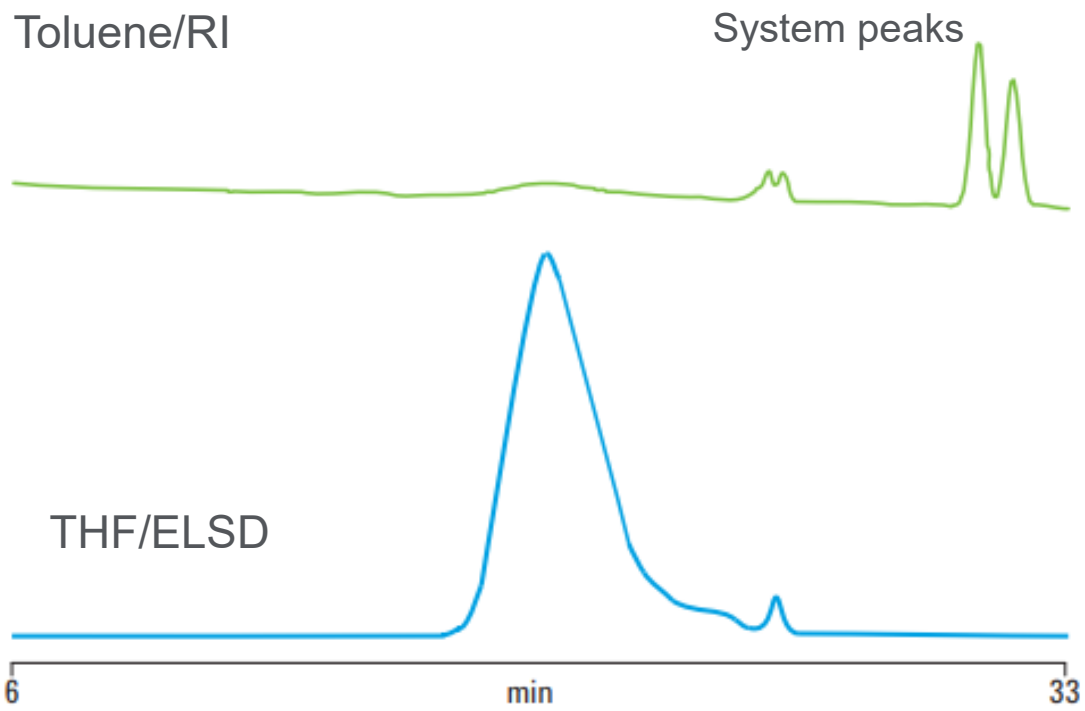
Suggested fitting

InfinityLab Quick turn fitting
Publication number 5991-5164EN

InfinityLab Supplies Guide
Publication number 5991-8031EN

System Detection

Choice of detection



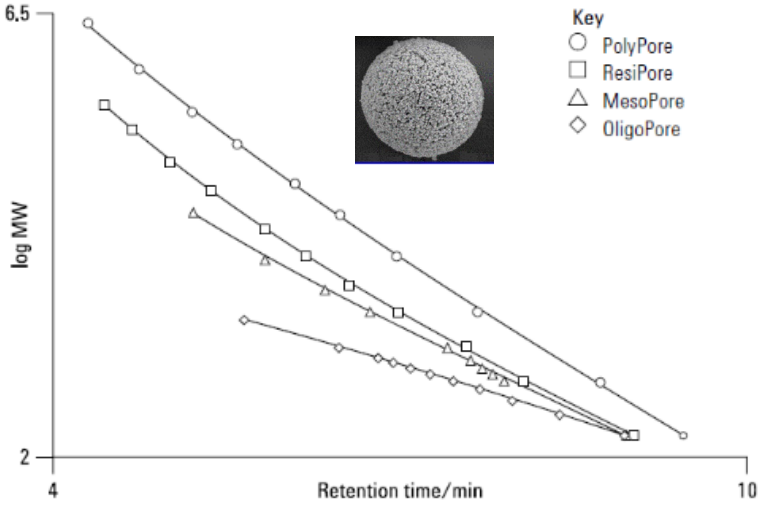
Column:	3 x PLgel, 5 µm, MIXED-D 7.5 x 300 mm, p/n PL1110-6504
Eluent:	Toluene or THF
Flow rate:	1.0 mL/min
Sample:	Polysiloxane, 0.2% w/v
Injection vol:	100 µL

Application note publication number: 5990-7897EN

System Detection

Peak shape and resolution improvement

1290 Infinity II
GPC/SEC System

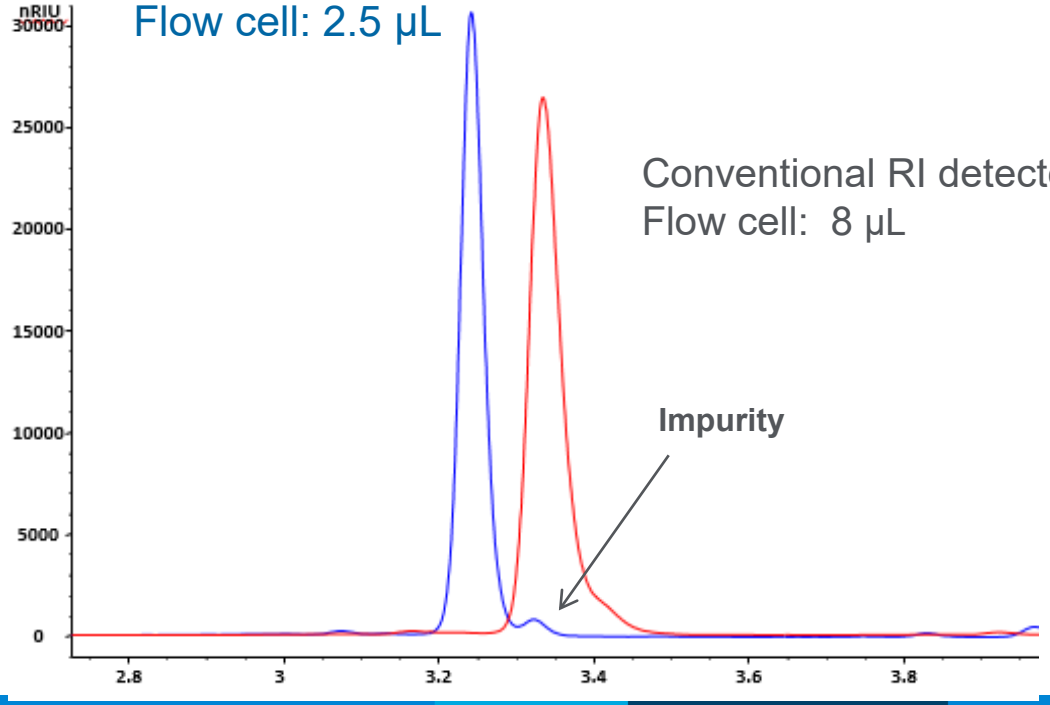


PlusPore calibration curves

Coupled 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.

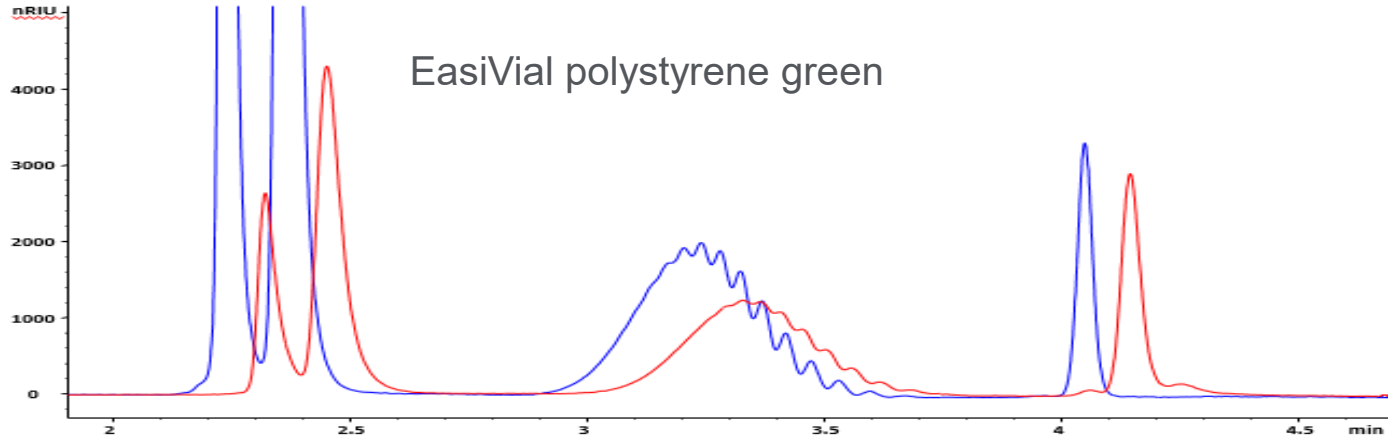
Micro RID
Flow cell: 2.5 µL

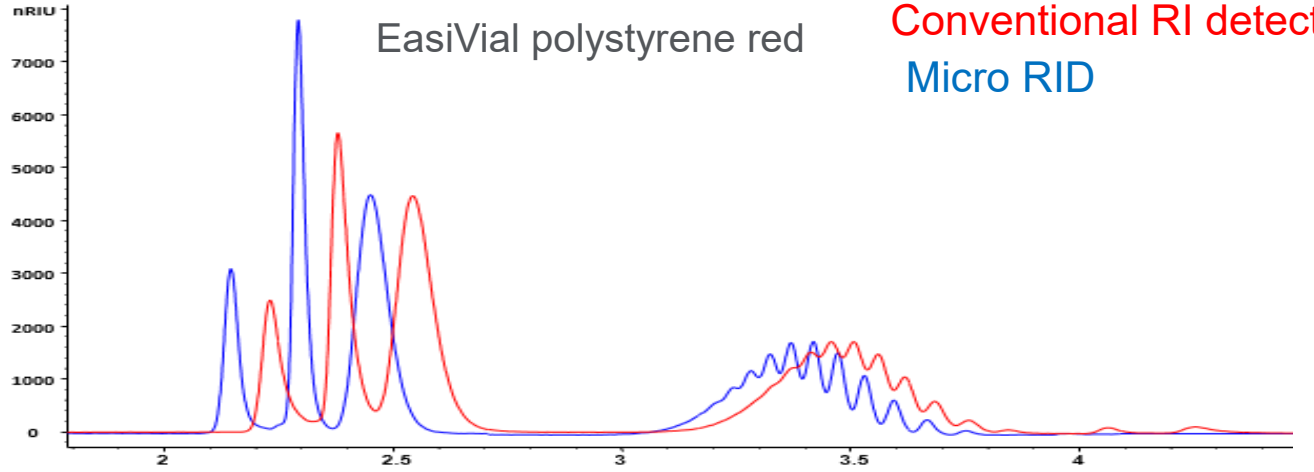


Conventional RI detector
Flow cell: 8 µL

System Detection

Ultralow dispersion for improved resolution





Conventional RI detector

Micro RID

Resource Slide

What polymer are you analyzing?

GPC/SEC Solutions for Accurate, Reproducible Polymer Analysis:
http://www.agilent.com/en-us/products/gpc-sec

- We have an extensive library of GPC/SEC applications

Applications compendia

- Chemicals and energy applications 5991-2517EN
- Pharma applications 5991-2519EN
- Food applications 5991-2029EN
- Engineering polymers 5990-6970EN
- Polyolefin analysis 5990-6971EN
- Analysis of elastomers by GPC/SEC 5990-6866EN
- Biodegradable polymers 5990-6920EN
- Low molecular weight resins and prepolymers 5990-6845EN
- Excipient analysis 5990-7771EN
- Analysis of food additives by GPC/SEC 5990-8634EN

Application notes

- Library Search

Resources for columns and consumables community at Agilent.com :

https://community.agilent.com/docs/DOC-1952-collection-of-consumables-resources

Agilent Peak Tales podcasts

http://peaktales.libsyn.com/

Resource Slide

All in one polymer analysis:
https://explore.agilent.com/all-in-one-gpc
GPC SEC Columns & Standards Selection Guide (poster):
https://www.agilent.com/cs/library/posters/public/poster-GPC-SEC-Columns-Standars-Selection-Guide-5994-1574EN-agilent.pdf
GPC SEC Troubleshooting Guide (poster):
https://www.agilent.com/cs/library/posters/public/poster-GPC-Troubleshooting-Guide-5994-1573EN-agilent.pdf
Complete GPC Solutions for the Polymer Scientist
https://www.agilent.com/cs/library/brochures/brochure-gpc-sec-portfolio-5994-0829EN-agilent.pdf

Product guides

- Organic GPC/SEC Columns: 5990-7994EN
- Aqueous and Polar GPC/SEC Columns: 5990-7995EN
- GPC/SEC Polymer Standards: 5990-7996EN
- Polymer to Solvent Reference Table: 5991-6802EN

Contact Agilent Chemistries and Supplies Technical Support

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

800 phone lines available 8-5 in all U.S. time zones

gc-column-support@Agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Thank you for attending

Any questions?