Practical Steps in GPC Method Development

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LC Columns & Consumables Technical Support

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Overview

Solvent

- Criteria for Solvent Selection
- Polymer Standards - choosing correctly
- Modifiers – why to use them?

Sample & Instrument Considerations

- Criteria for Dissolution and Concentration
- System Optimization – dead volume, fittings etc

Columns

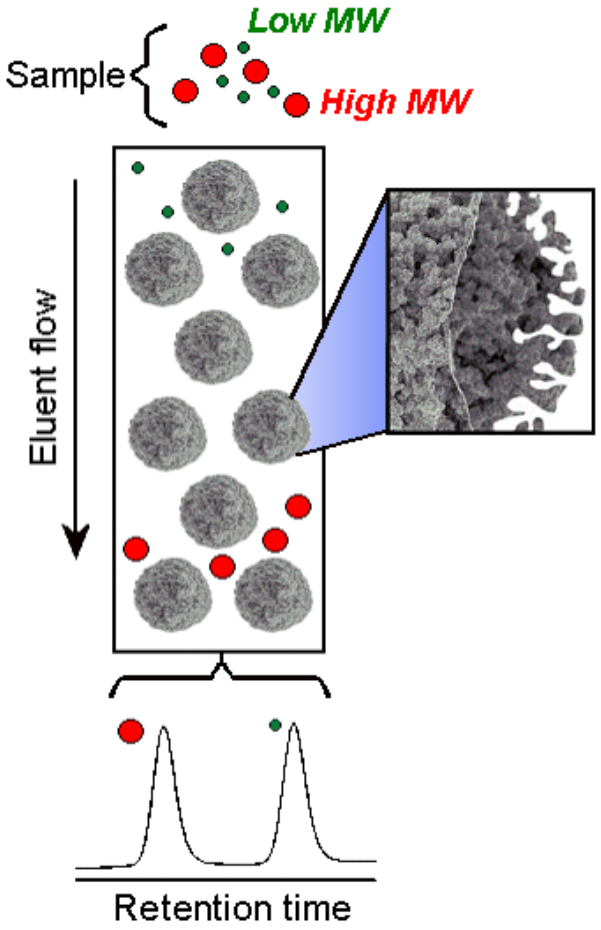
- Organic or Aqueous
- What exactly do I want from my analysis?
- Reproducibility
- Resolution
- Speed

Detectors

- Which is appropriate for my sample or my column?
- Is OTHER information for my sample needed?

GPC Separation Mechanism

- The GPC column is packed with porous beads of controlled porosity and particle size
- Polymer 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
- Polymer molecules are separated according to molecular size, eluting largest first, smallest last



GPC compare to HPLC

Isocratic elution, often single solvent systems

Typically uses organic eluents, not ACN, MeOH, IPA

Typically lower sample concentrations (0.1 – 0.2%)

Typically larger injection volumes (20 – 200 µl)

Primary use is to measure molecular weight distribution

Resolution provided by **non-interactive** mechanism

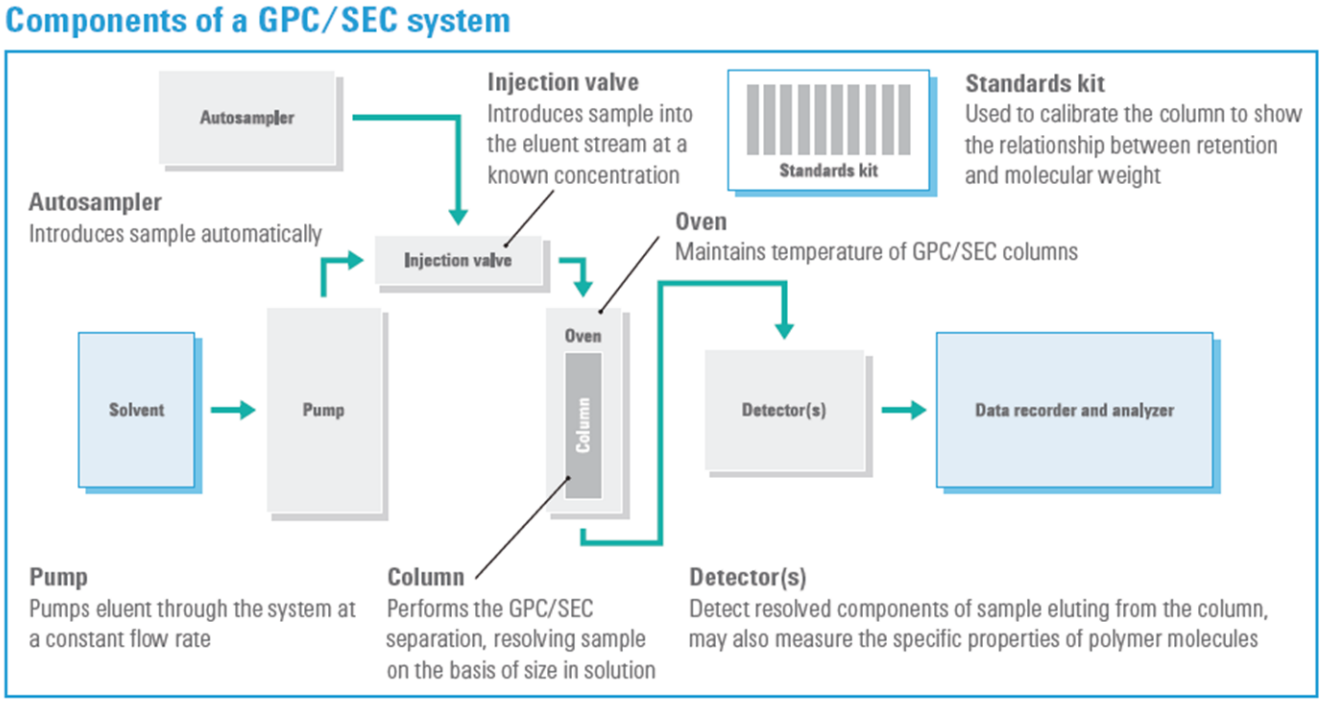
Larger columns (300 x 7.5 mm industry standard)

Use of multiple columns in series (2 – 4)

A Typical GPC System: Optimizing your GPC Method

- When establishing your GPC method and to ensure optimum **accuracy, reliability and throughput,** we need to consider ALL elements of our GPC/SEC system

  - Solvent
  - Sample
  - Flow Rate
  - Temperature
  - Column
  - Calibration
  - Detection



Criteria for Solvent Selection

Polymers are often employed due to their strength and toughness. Aggressive solvents and long dissolution times often required while ensuring:

- True sample solubility to avoid non-size exclusion effects
- Compatibility with columns
- Permit adequate detection (e.g. refractive index, UV cut off)
- Safety (e.g. toxicity, elevated temperature, etc.)

Additives can be employed

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

	Solvent Polarity	Solvent
Low	6.0	Perfluoralkanes
	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	Dichloroethane
	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 pyrolidone (NMP)
	12.0	Dimethyl sulphoxide (DMSO)
High	12.1	Dimethyl formamide (DMF))

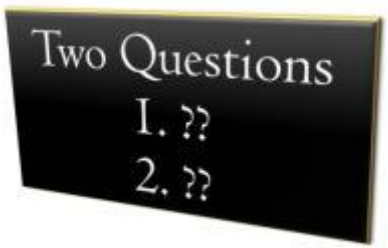
2 Key Questions

- 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%)

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

MW	MW 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



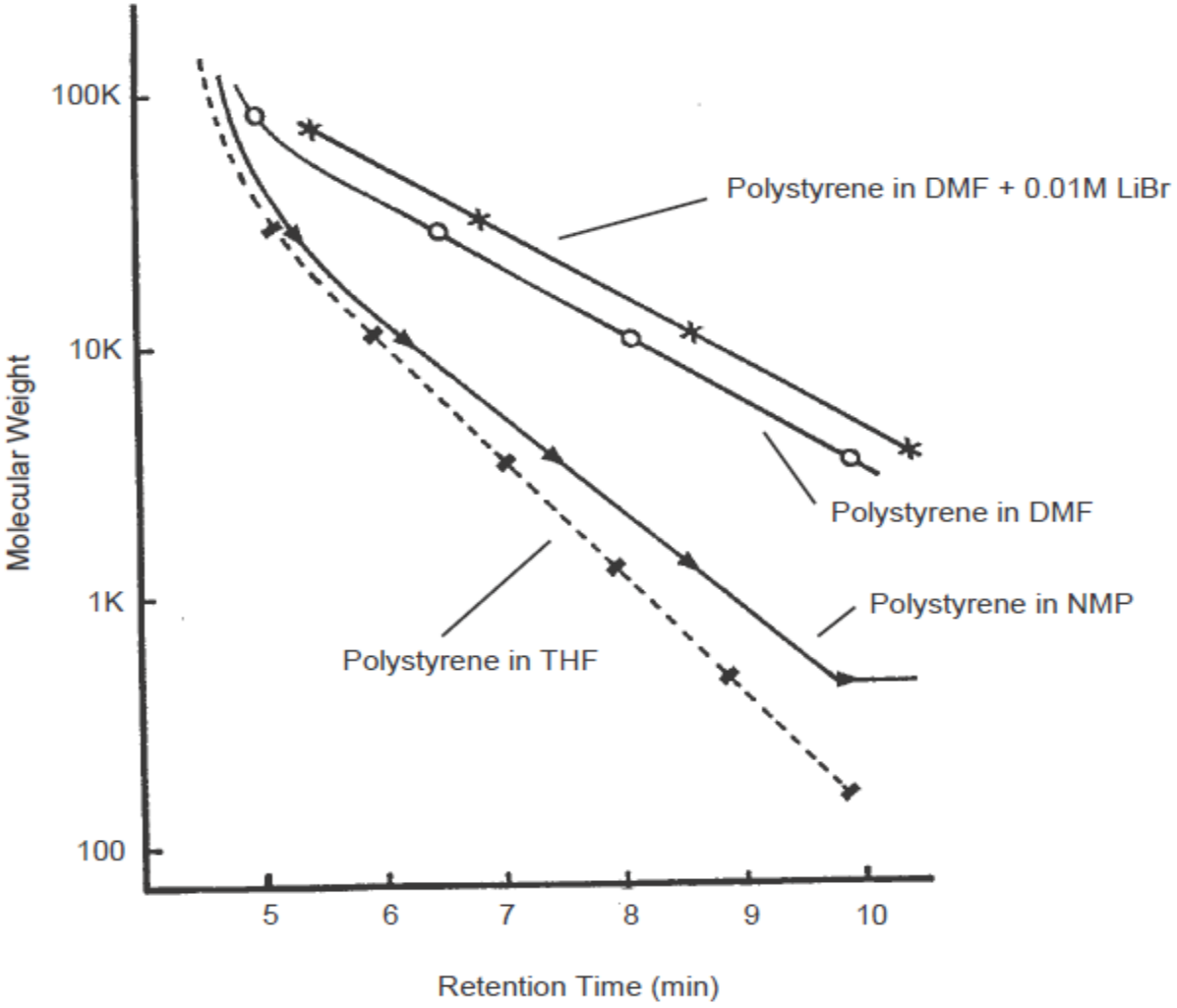
Polymer Standards – What is the Eluent/Mobile Phase?

Solvent Type	GPC/SEC Standards Type
Organic	• Polystyrene (PS) • Polymethylmethacrylate (PM)
Mixed or Polar Organic	• Polymethylmethacrylate (PM) • Polyethylene glycol/oxide (PEG/PEO)
Aqueous	• Polyethylene glycol/oxide (PEG/PEO) • Polysaccharide (SAC) • Polyacrylic acid (PAA)

- EasiVial – pre-prepared for fast and easy, accurate concentration, 12-point column calibration for organic and aqueous solvents
- EasiCal – easy 3-step process for accurate 10-point calibration, for organic solvents
- Calibration kits and individual standards – Polystyrene, PMMA, PEG/PEO, PAA, Polysaccharide

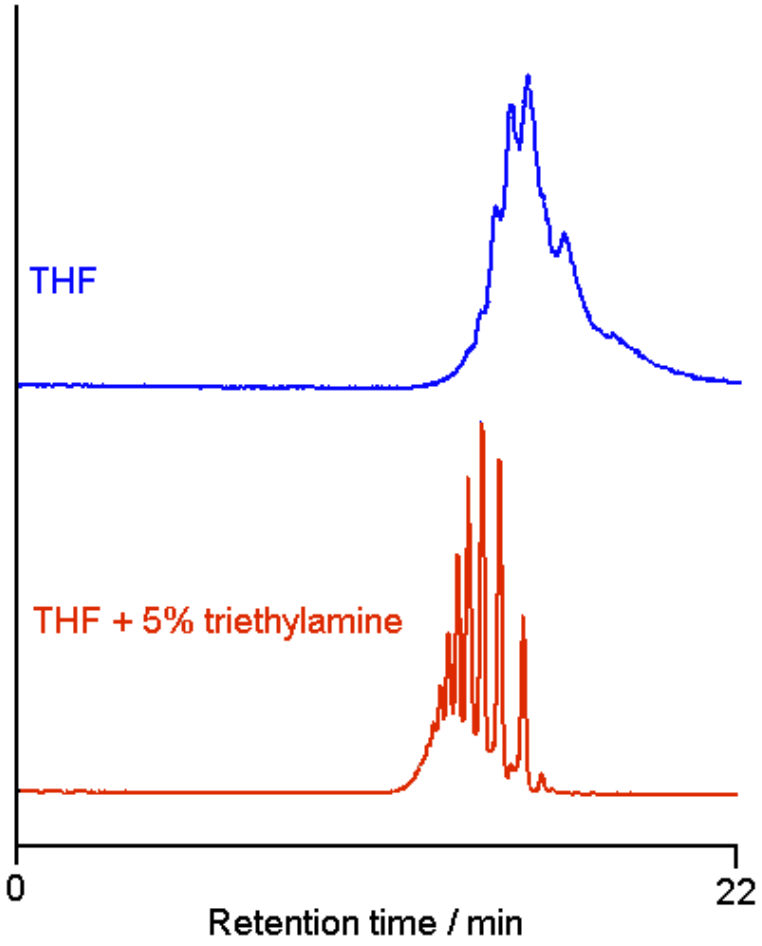
What TYPE of kits best suits my needs?

Calibrations Standards and Importance of Solvent Selection



Ex: PS/DVB columns are excellent in many solvents, but remember that although the column may be used in certain solvents this does not mean SEC will occur - the example here is polystyrene standards running in NMP, DMF, etc.

Eluent Modification in Organic GPC

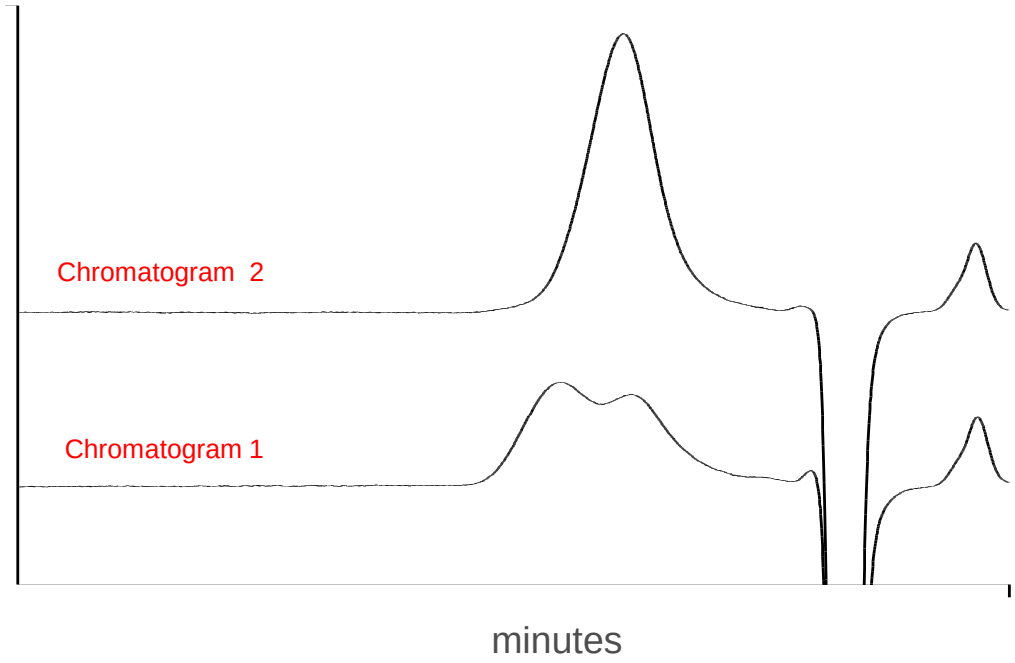


Hostavin N30

- Polymeric UV stabiliser containing secondary amine groups

Column: 2xPLgel 3µm MIXED-E
Flow Rate: 1.0ml/min
Detector: PL-ELS 1000

Solvent Modifiers



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

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

Sample Criteria for Dissolution

- Sample concentration depends on molecular weight
- Avoid high shear – stirring/sonication
- Dissolution time and temperature, if required, will depend on molecular weight and crystallinity of polymer
- Use an aliquot of the eluent to prepare the solution
- Use same solvent for reproducibility
  - Reduction of RI imbalance peak
- Filtering of samples to remove insoluble material (0.2 - 1.0 µm filters)

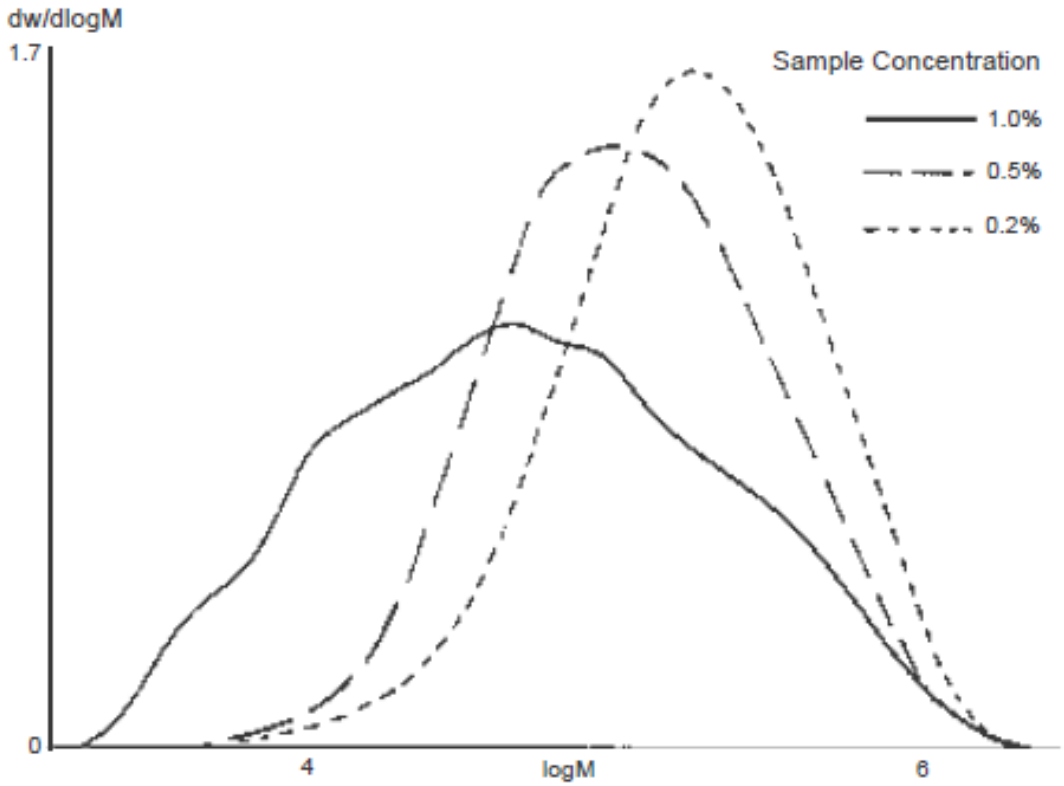
MW Range	Concentration	Time
100 – 10 k	3 – 5 mg/mL	30 mins
10 k – 500 k	2 mg/mL	1 – 4 hrs
500 k – 800 k	1 mg/mL	3 – 8 hrs
800 k – low millions	≤0.5 mg/mL	o/n - day
Millions	≤0.25 mg/mL	1 – 3 days

- All values offered as guide only
- Narrowly distributed samples require lower concentration

- In GPC sample preparation is an important consideration

Effect of Concentration on Peak Shape &Resolution

Column: 2 x PLgel 5 µm MIXED-C
7.5 x 300 mm
Eluent: THF
Flow Rate: 1.0 mL/min
Inj vol: 200ul
Detector: RI
Sample: broad Polystyrene



Temperature
Use of Elevated Temperature

- GPC applications employing elevated temperature 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

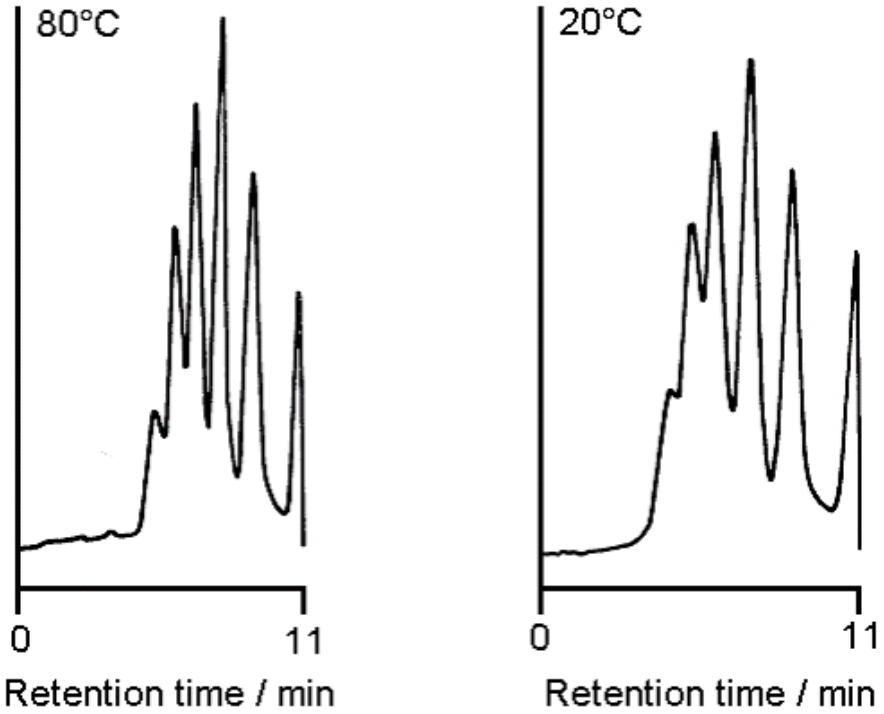
Eluent	Temp (°C)
THF, Water, Chloroform	30 - 40
DMF, DMSO, DMAc	60 - 80
TCB	140 - 160

- All values offered as guide only

- Elevated temperature is a useful approach in GPC

Temperature

Effect on Separations using Viscous Solvents



- Increased temperature:
  - Reduced operating pressure
  - **IMPROVED** resolution, particularly at high MW

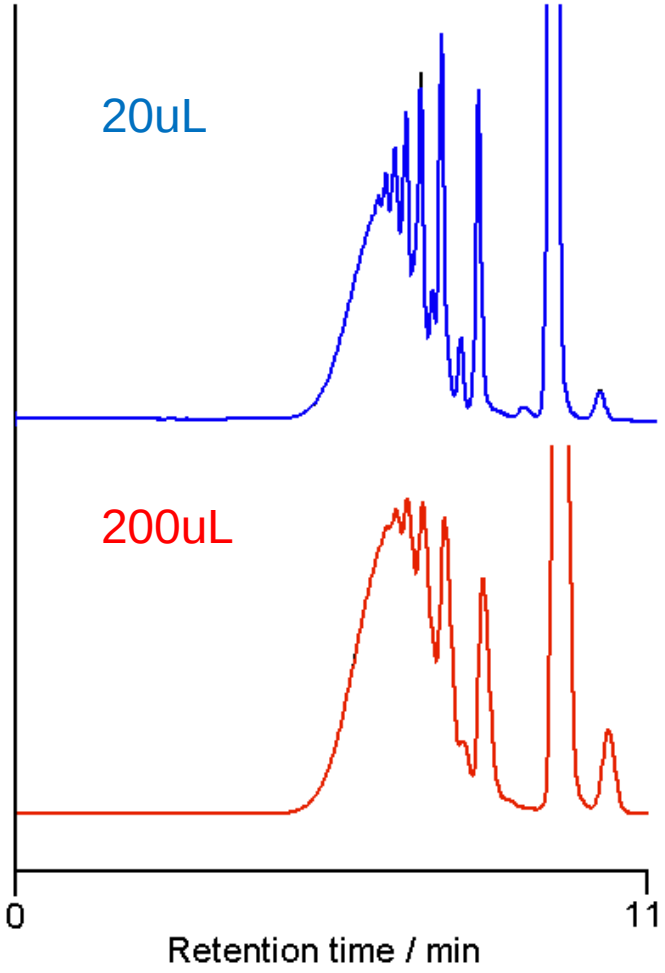
Column:	PLgel 5 µm MIXED-C
	300 x 7.5mm
Eluent:	DMF
Flow rate:	1.0 mL/min

PEO/PEG standards

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

Sample

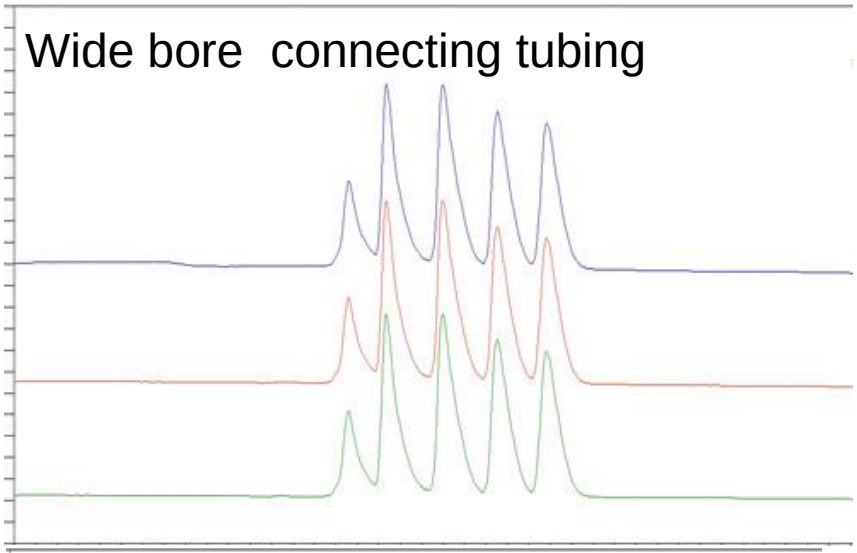
Effect of Injector Loop Size on Resolution

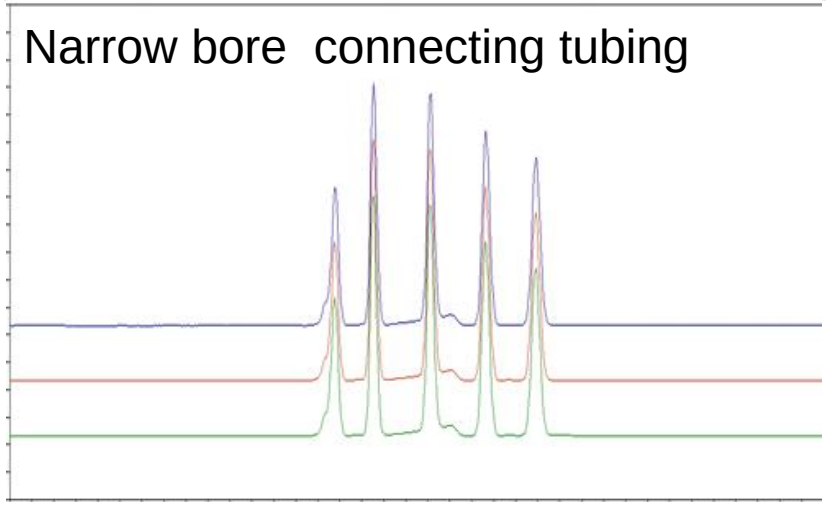


Column :	PLgel 3 µm MIXED-E
	300 x 7.5mm
Eluent :	THF
Flow rate :	1.0 mL/min
Sample :	Epikote 1001 epoxy resin

- Injection loop is a major contribution to system **dead volume**, use reduced injection volume and increased concentration to maintain sensitivity

Reducing Dead Volume

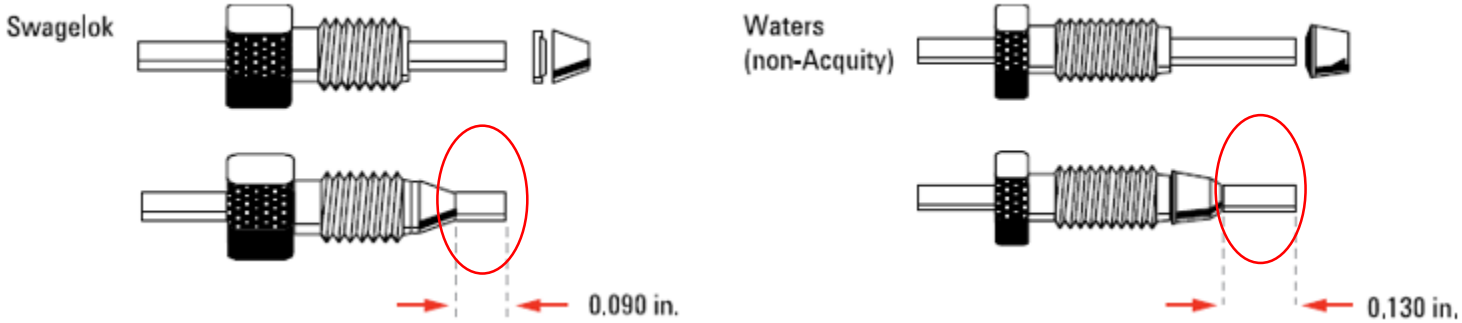




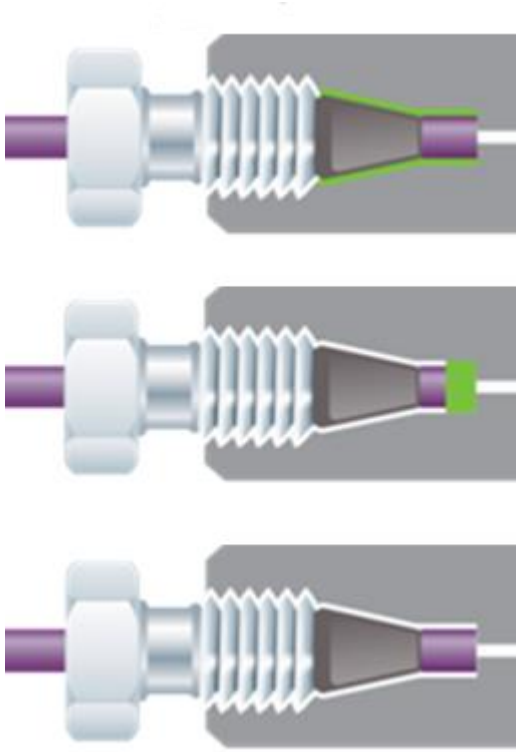
- ID for tubing narrow as possible
- Tubing Connections Short
- Use proper fittings for connections

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



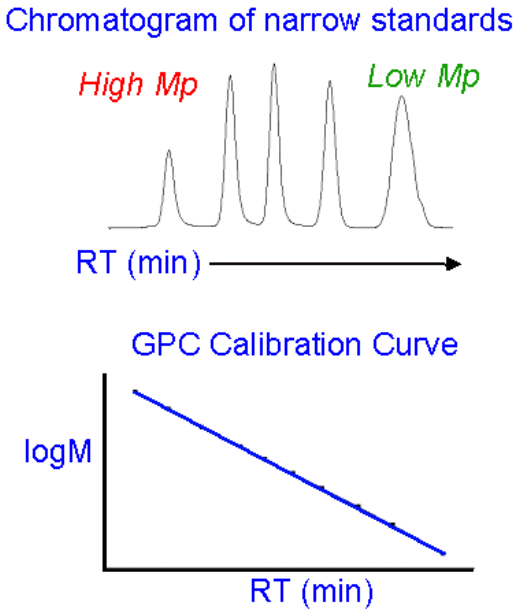
Potential Fittings Issues

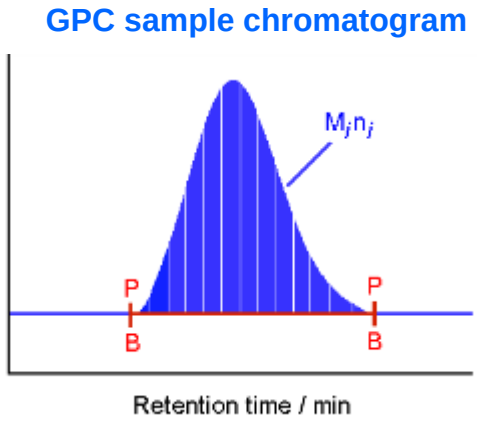


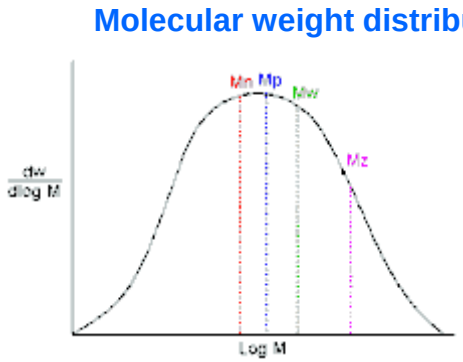
- Leak
- Peak shape problem
- No dead volume

Conventional GPC/SEC Workflow

- Calibrate the GPC column with a set of narrow polymer standards
- Plot retention time (RT) versus peak log molecular weight (logM)
- Calibration is used to generate molecular weight (averages and distribution) of unknowns run on the same system/column-set
- Molecular weights are relative to the standards used

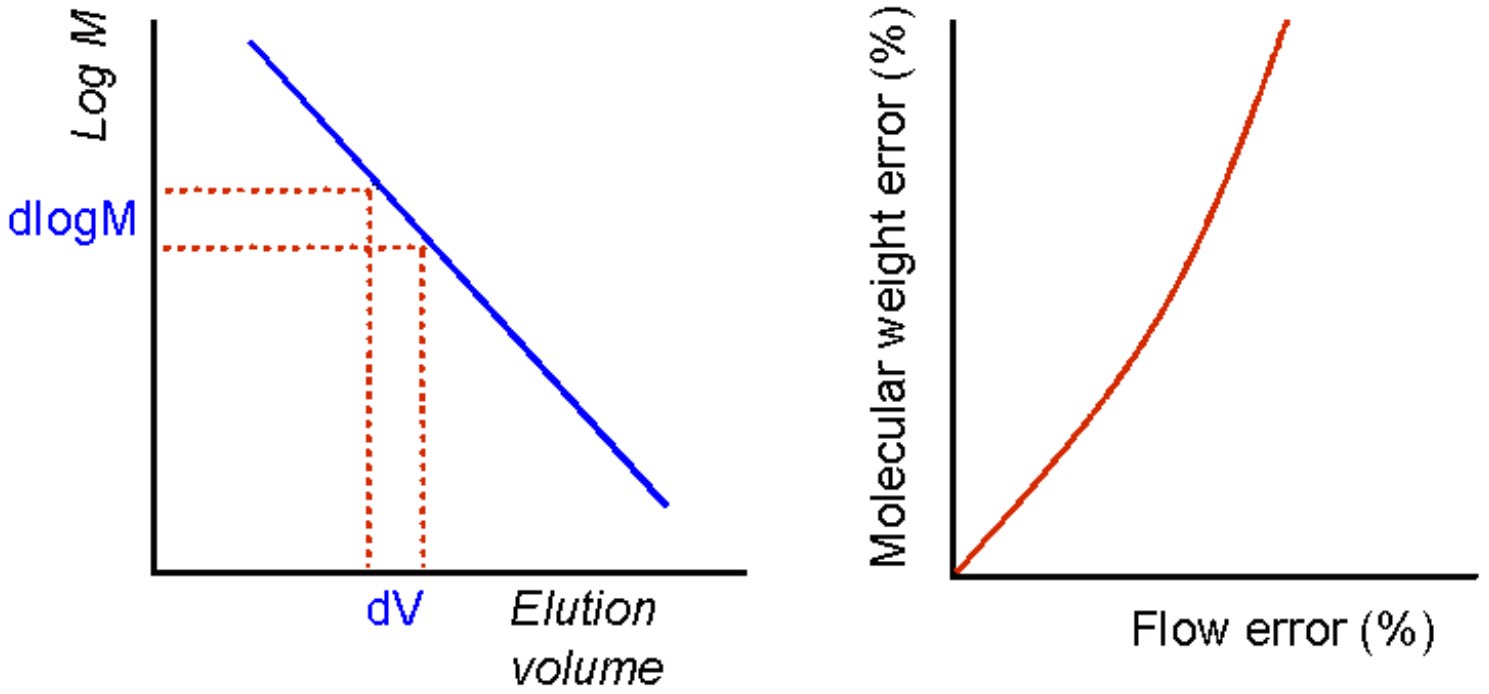






Pump Flow Rate & Reproducibility

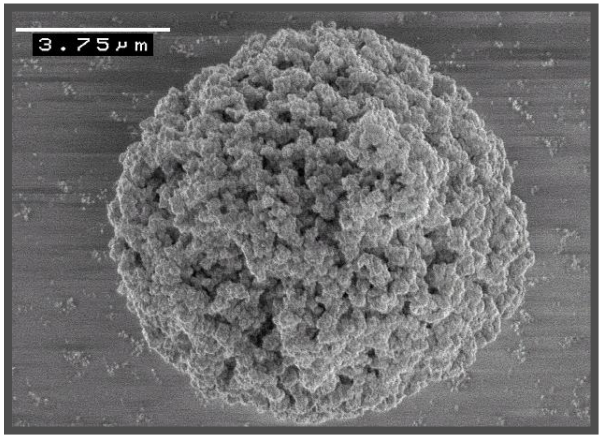
Effect on MW Results



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

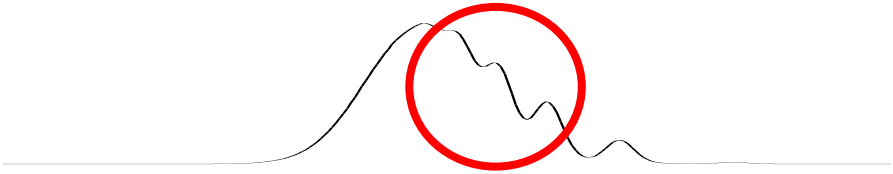
What are GPC Columns Made Of?

- *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

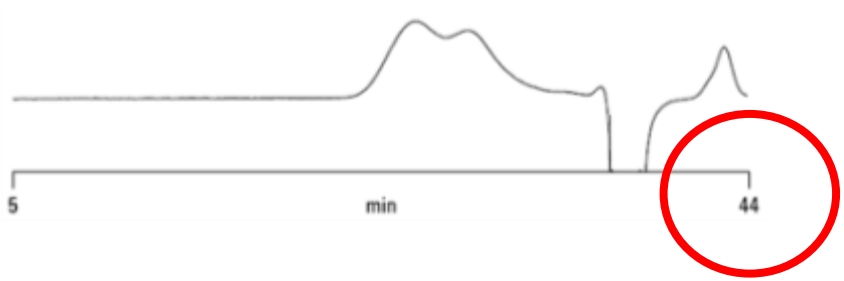


What would you like to improve about your GPC/SEC?

Resolution is too low

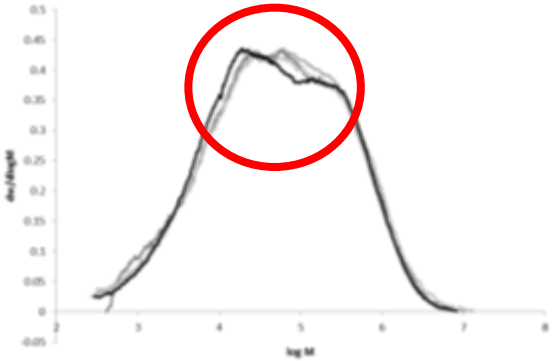


Analysis time is too long



Peak shapes are poor

Results are not reproducible

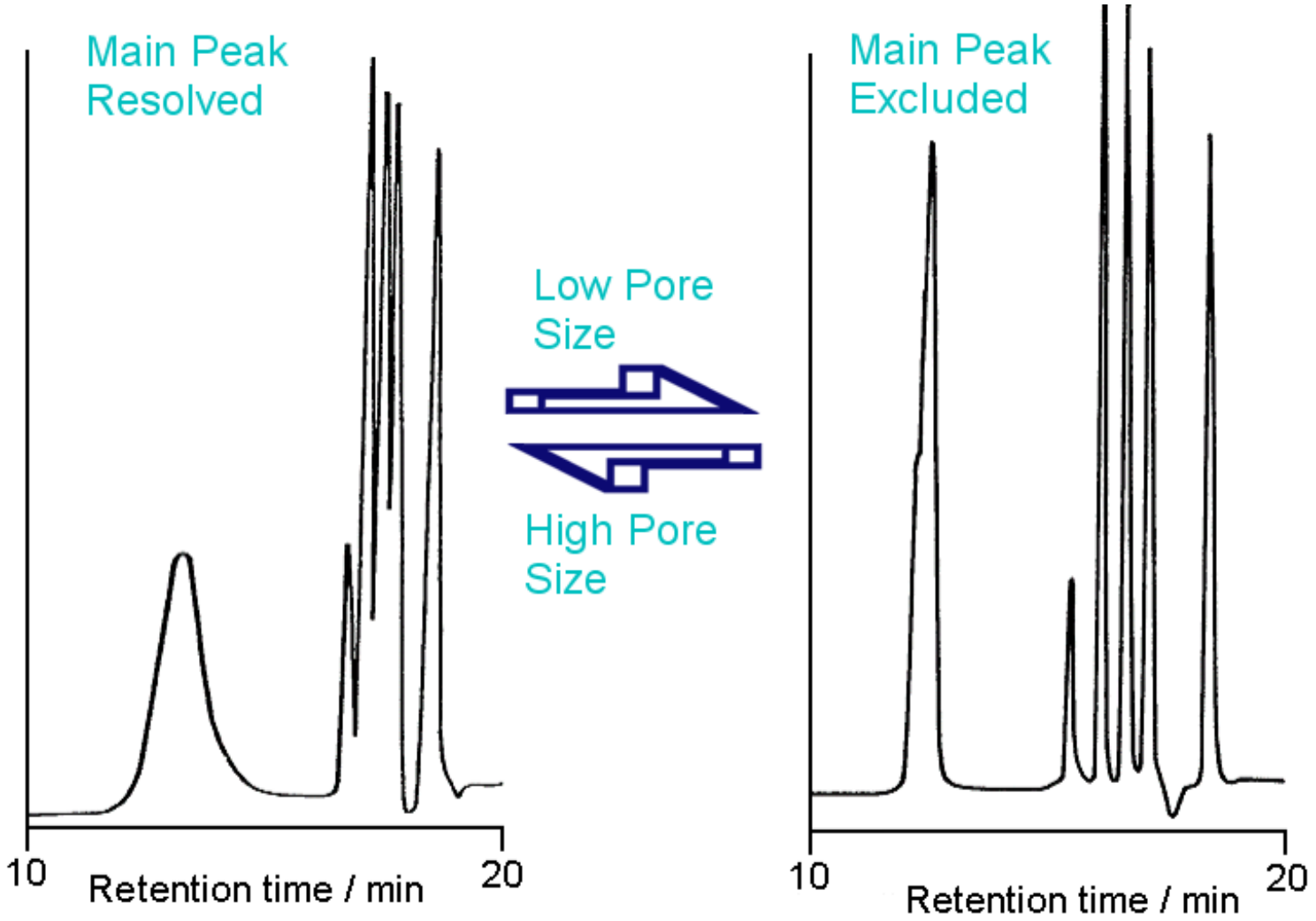


GPC Columns
Making a Choice

- What is the Molecular Weight range for my sample?
- Organic or Aqueous eluents being used
- What ARE your requirements for your GPC analysis?
  i. Resolution is important
  ii. Reproducibility of sample chromatography and results
  iii. Speed of analysis and/or sample throughput is something to improve on

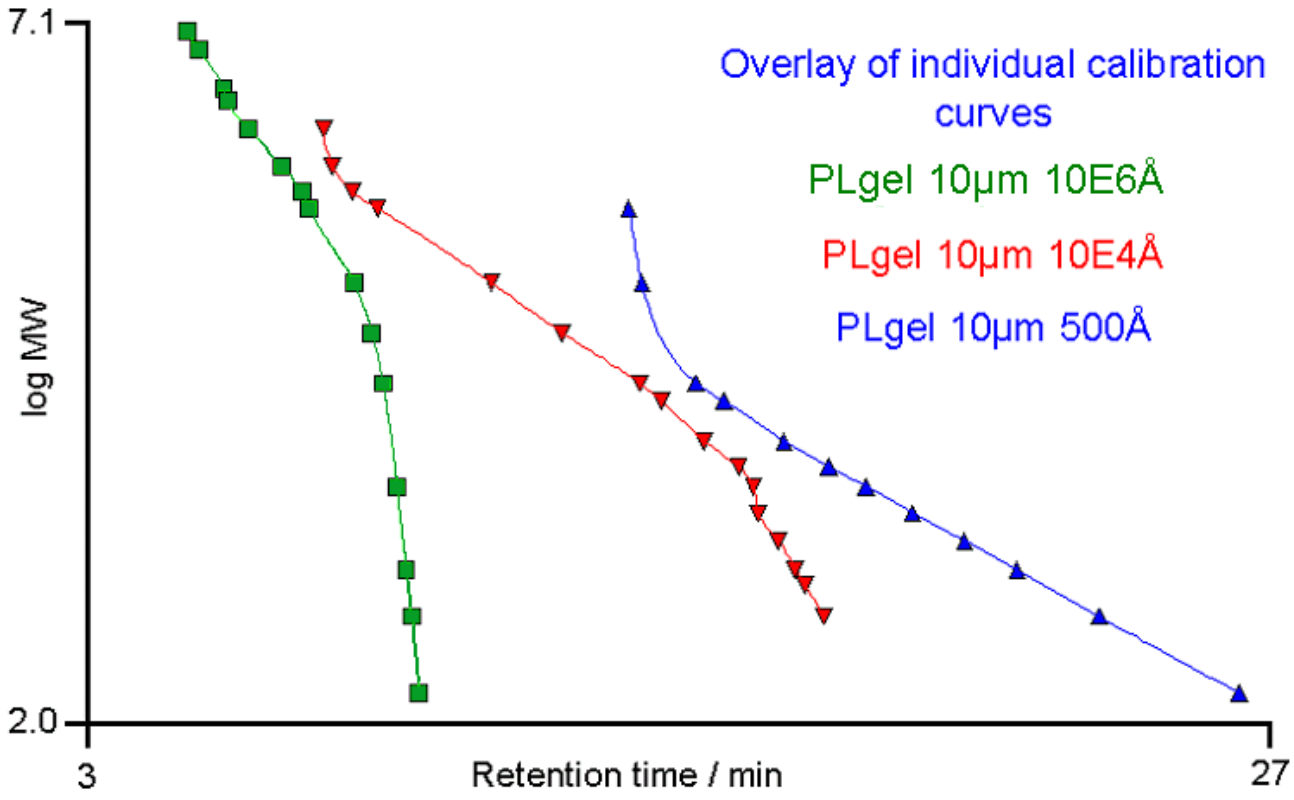
Choose a column(s) based on your KEY requirements

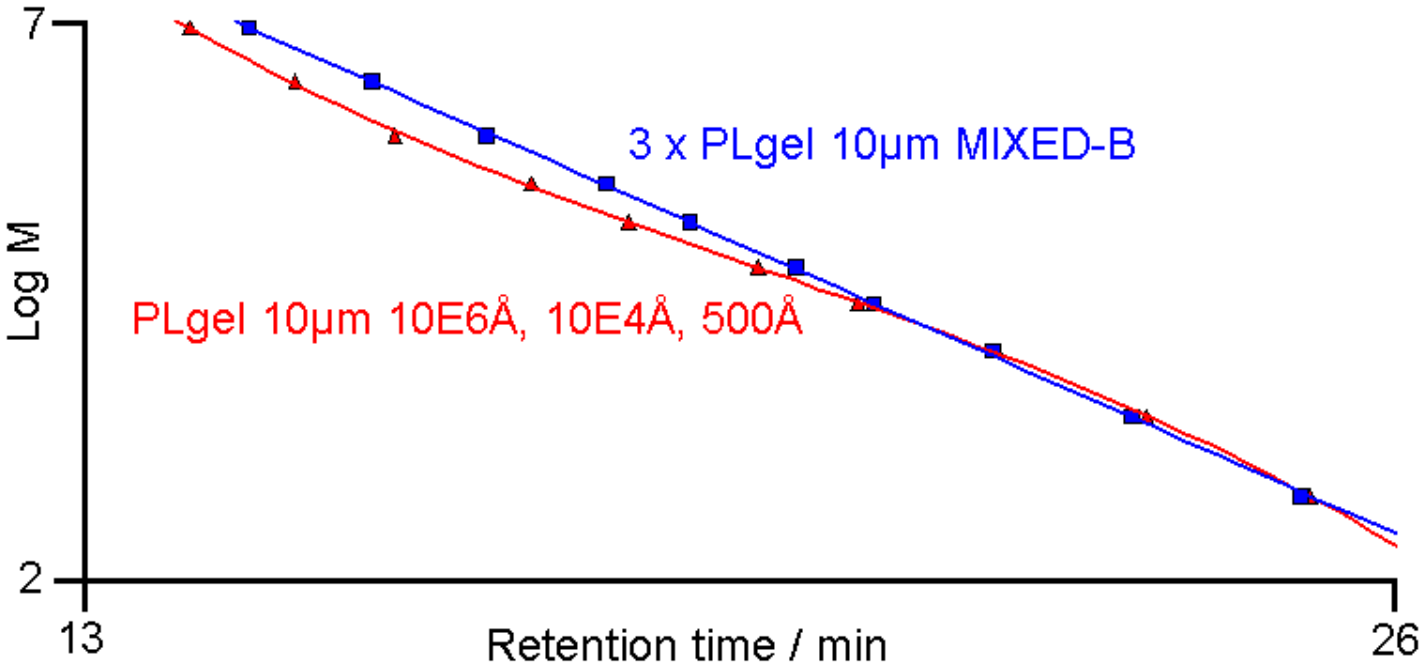
Effect of Column Selection: Pore size

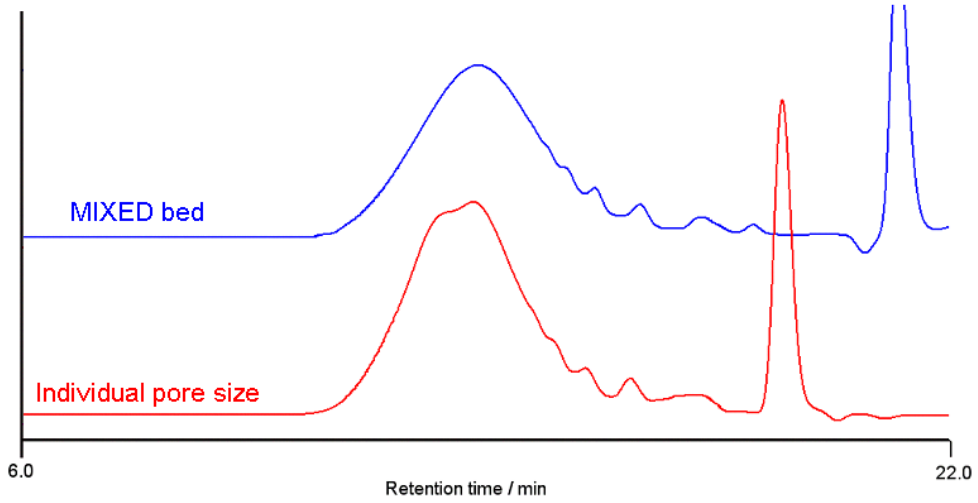


Combination of Individual Pore Size Columns

Traditional approach to increasing MW operating range of column set



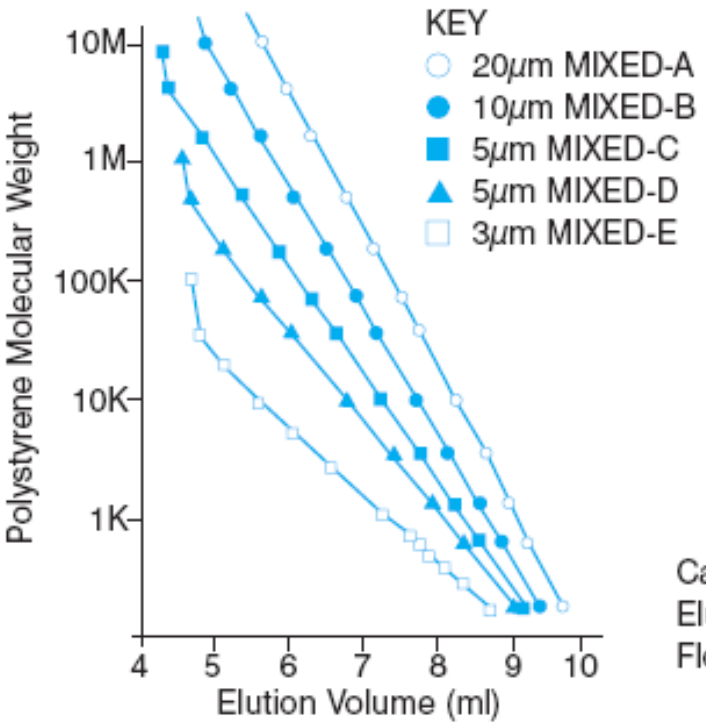

7
2
Log M
3 x PLgel 10µm MIXED-B
PLgel 10µm 10E6Å, 10E4Å, 500Å
13
Retention time / min
26


MIXED bed
Individual pore size
6.0
22.0
Retention time / min

PLgel MIXED Bed Calibrations

PLgel MIXED Gel

Calibration Curves

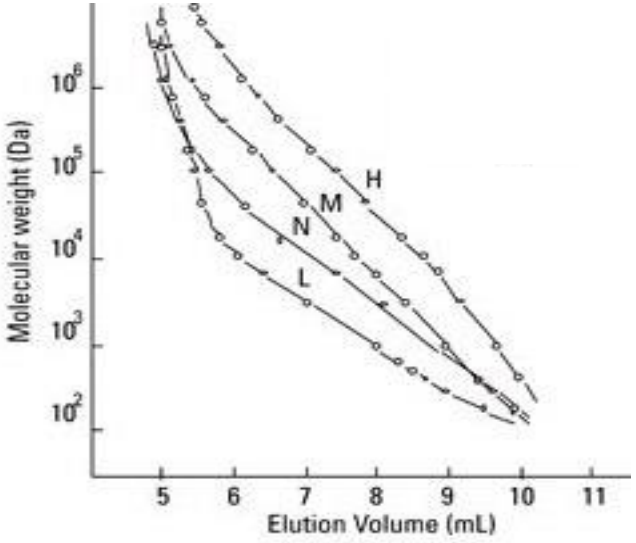


Specifications

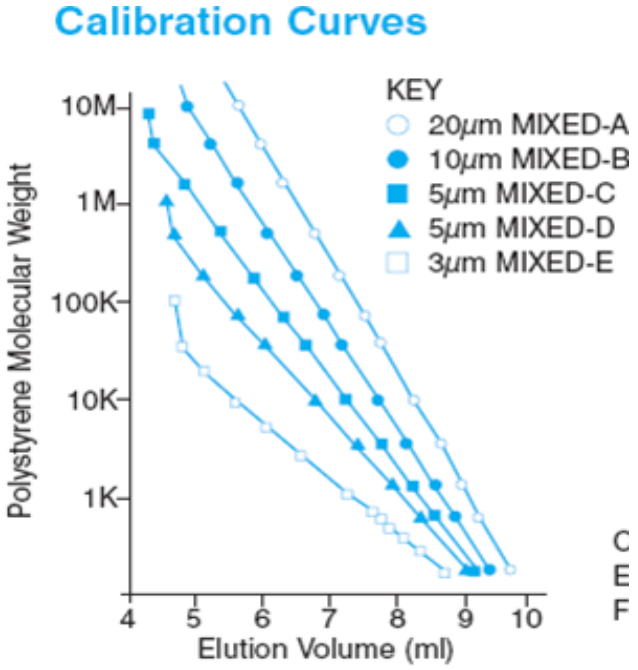
Column Type	Linear MW Range (PS)	Guaranteed Efficiency (p/m)
PLgel 20μm MIXED-A	2,000-40,000,000	>17,000
PLgel 10μm MIXED-B	500-10,000,000	>35,000
PLgel 5μm MIXED-C	200-2,000,000	>50,000
PLgel 5μm MIXED-D	200-400,000	>50,000
PLgel 3μm MIXED-E	up to 30,000	>80,000

Calibrants: Polystyrene
Eluent: THF
Flow Rate: 1.0ml/min

Difference in Linearity



Flow Rate: 1.0mL/min
Temperature: 25°C
Detection: UV @ 254nm



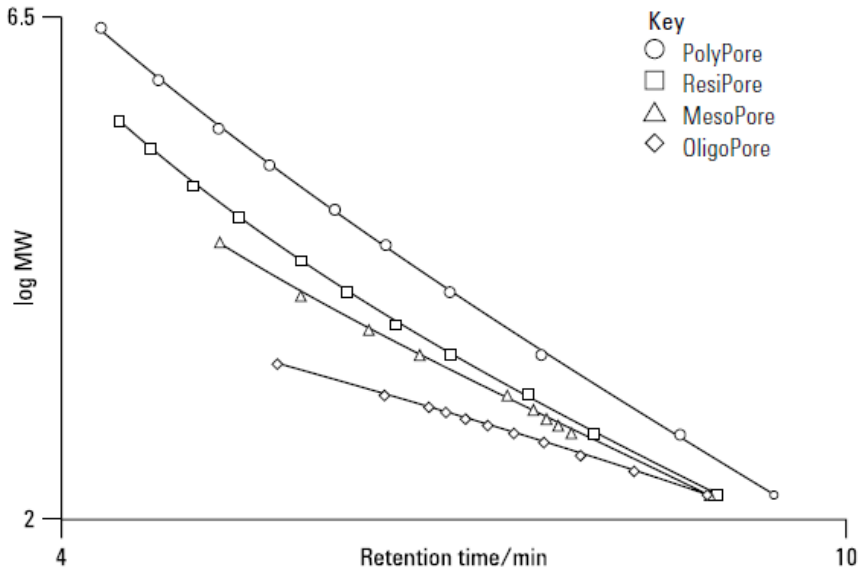
PlusPore PS/DVB Columns

The PlusPore series of columns has been specifically designed for *high resolution* GPC, and represents the very latest in GPC column technology. These novel packing materials are based on the industry standard, highly crosslinked polystyrene/divinylbenzene (PS/DVB), for the widest applicability and solvent compatibility. Each is made using a novel polymerization process to produce particles which exhibit a specific, controlled pore structure for optimum GPC performance.

- **PolyPore** for the routine analysis of general polymers
- **ResiPore** for the analysis of resins and condensation polymers
- **MesoPore** for the analysis of prepolymers and low MW resins
- **OligoPore** for the analysis of oligomeric samples

Features and Benefits of the PlusPore Range

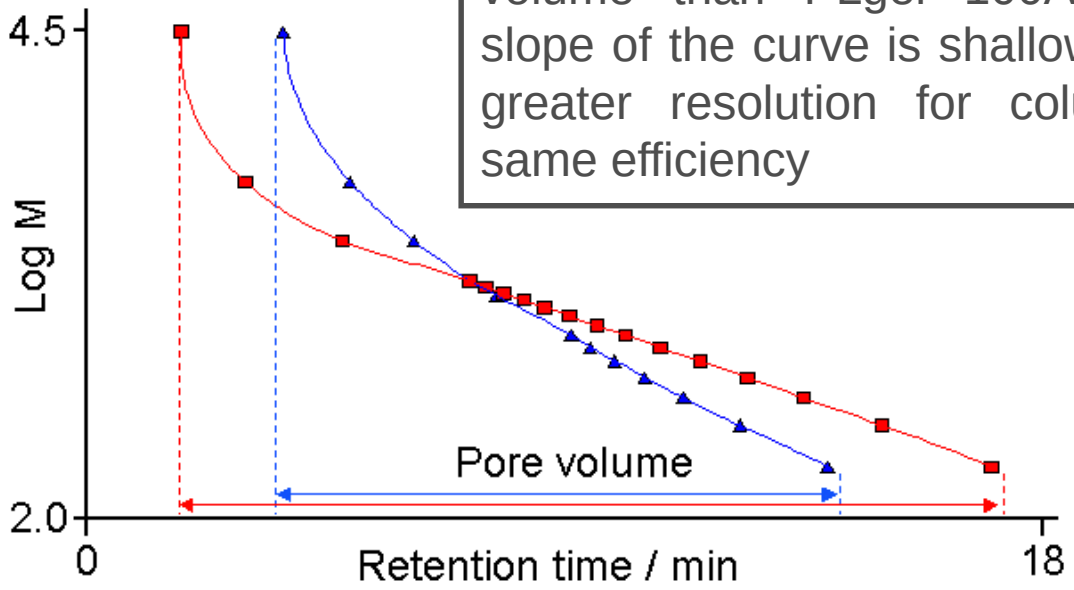
- High pore volume, high resolution
- Wide pore size distribution
- Optimized separation range
- Full solvent compatibility
- No MWD dislocations



Effect of Increased Pore Volume

Columns	2xPLgel 3µm 100Å 300x7.5mm 2xOligoPore 300x7.5mm
Eluent	THF
Flow rate	1.0ml/min

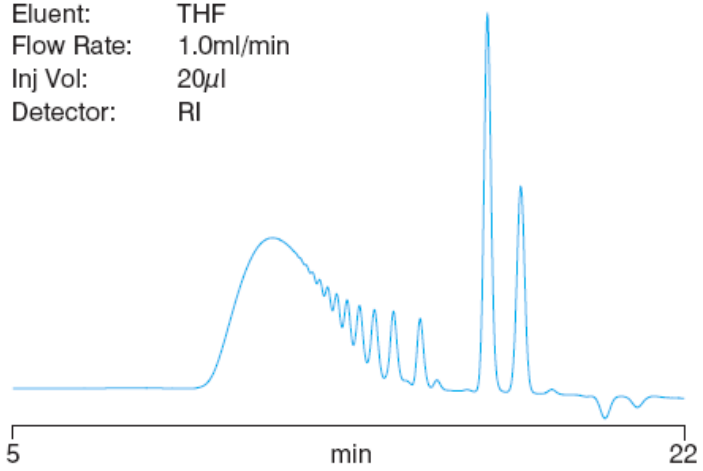
Both columns have a similar exclusion limit but OligoPore has greater pore volume than PLgel 100Å. Hence the slope of the curve is shallower leading to greater resolution for columns of the same efficiency



Examples of Resolution Using Pluspore Columns

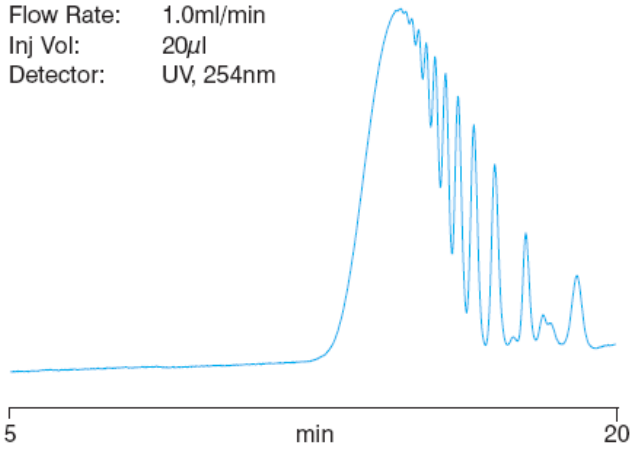
Polyurethanes

Columns: 2xMesoPore, 300x7.5mm (PL1113-6325)
Eluent: THF
Flow Rate: 1.0ml/min
Inj Vol: 20µl
Detector: RI



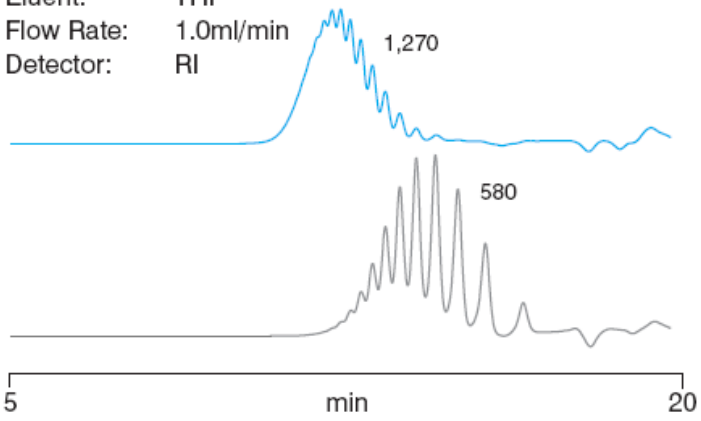
Polyester

Columns: 2xResiPore, 300x7.5mm (PL1113-6300)
Eluent: THF
Flow Rate: 1.0ml/min
Inj Vol: 20µl
Detector: UV, 254nm

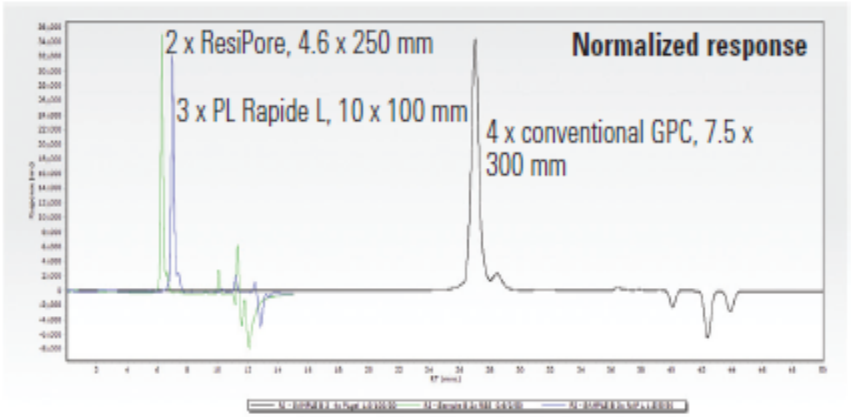


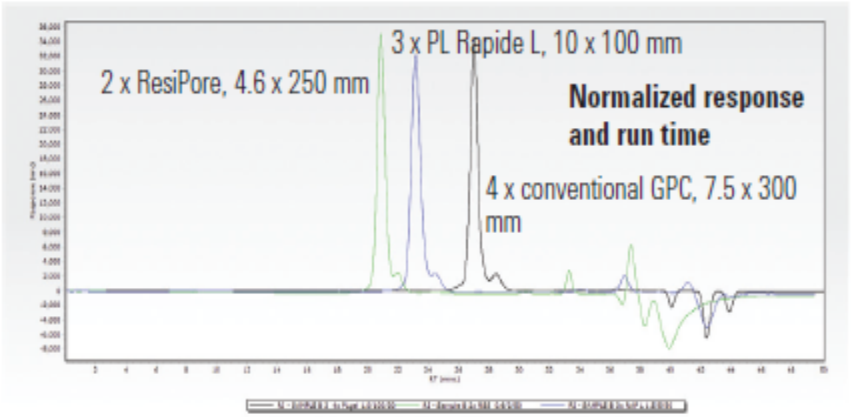
Polystyrene Standards

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



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

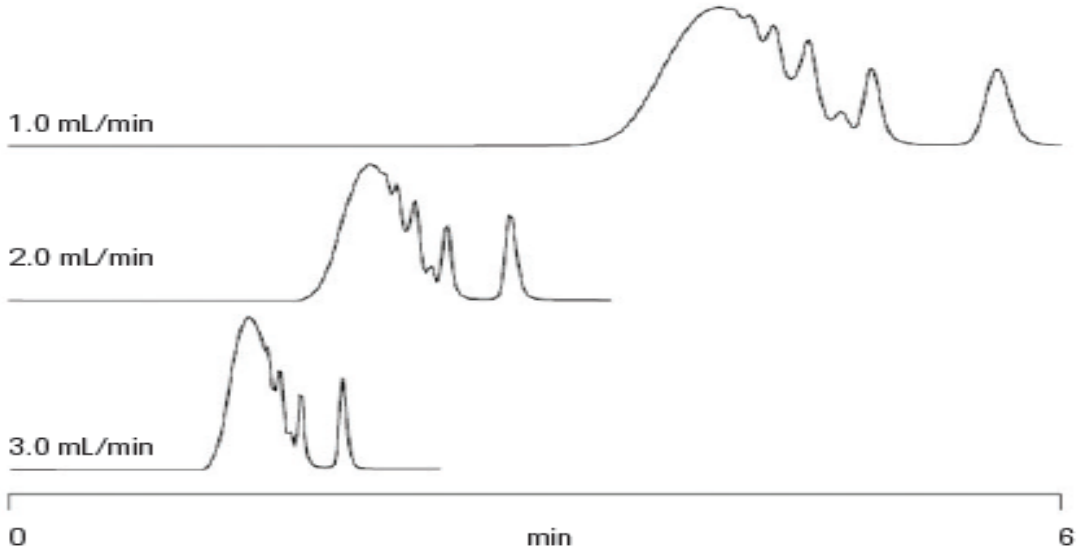
Columns	Resolution (Rs)	Selectivity (α)	Area %	Height %
4 x conventional 7.5 x 300 mm	1.2	1.05	8	7
3 x PL Rapide L 10 x 100 mm	1.1	1.06	7	7
2 x ResiPore 4.6 x 250 mm	1.1	1.05	8	8

If Speed of analysis is important……..

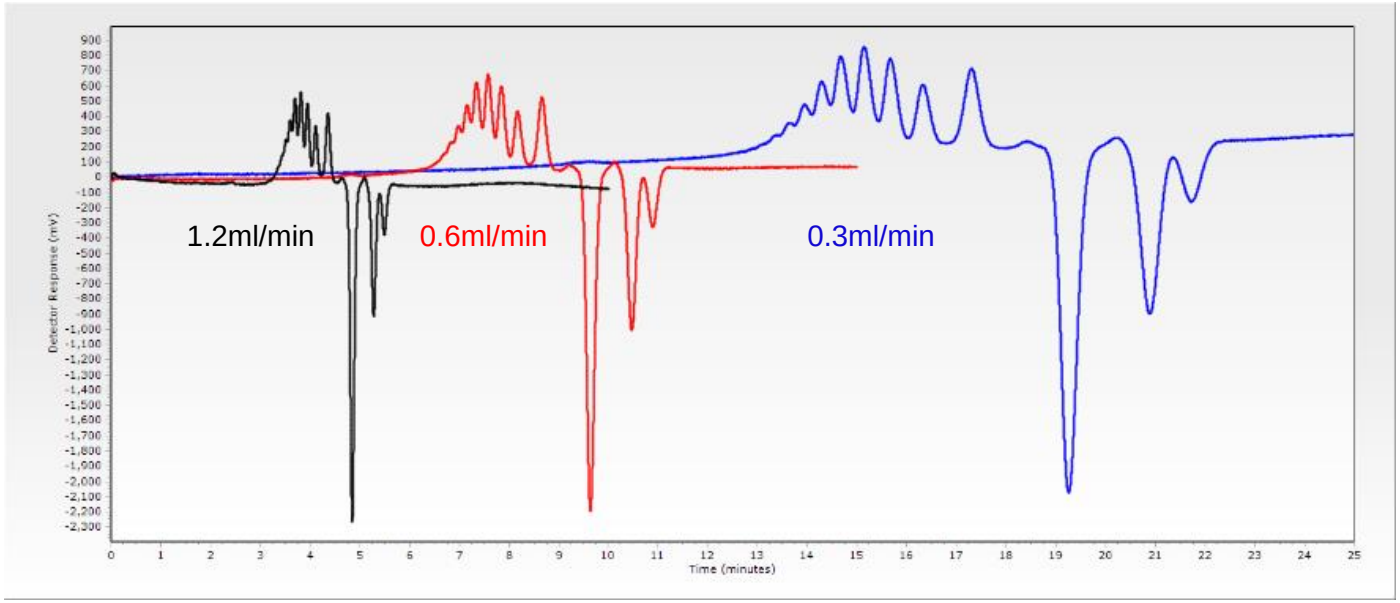
Conditions

Column:	PL Rapide L, 10 x 100 mm (PL1013-2300)
Sample:	Epoxy resin
Eluent:	THF
Flow rate:	1.0, 2.0 and 3.0 mL/min
System:	1260 Infinity GPC/SEC System, UV, 254 nm

Rapide columns reduce analysis times while maintaining the excellent solvent compatibility and mechanical stability



Polystyrene Mw 580 – Oligopore 250x4.6mm

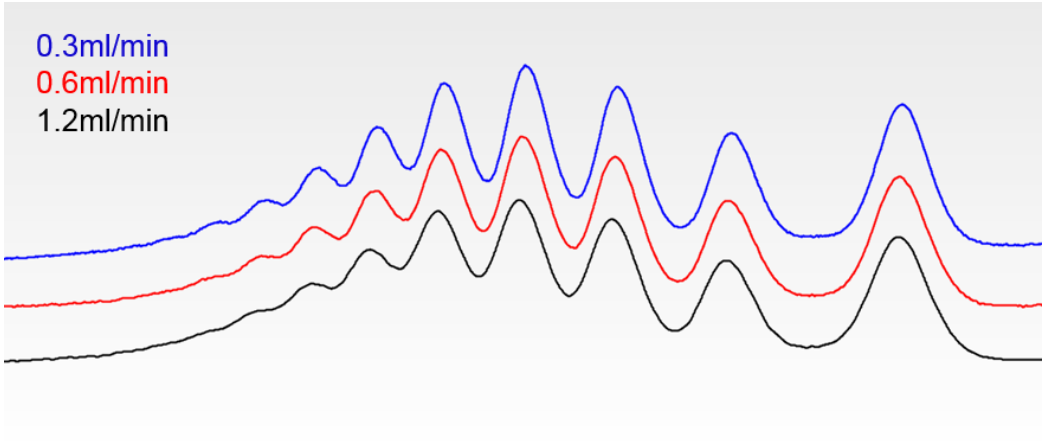


MW Range: up to 3,300 (g/mol)

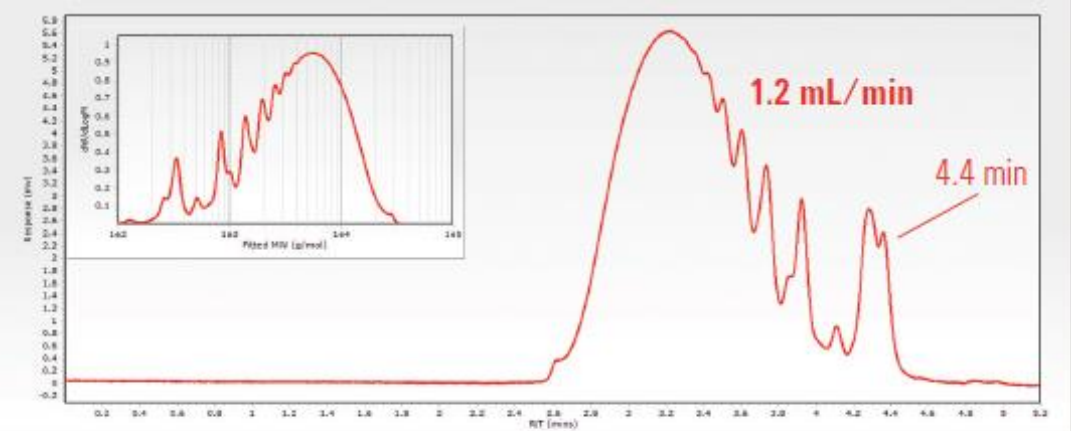
Nominal Particle Size: 6 µm

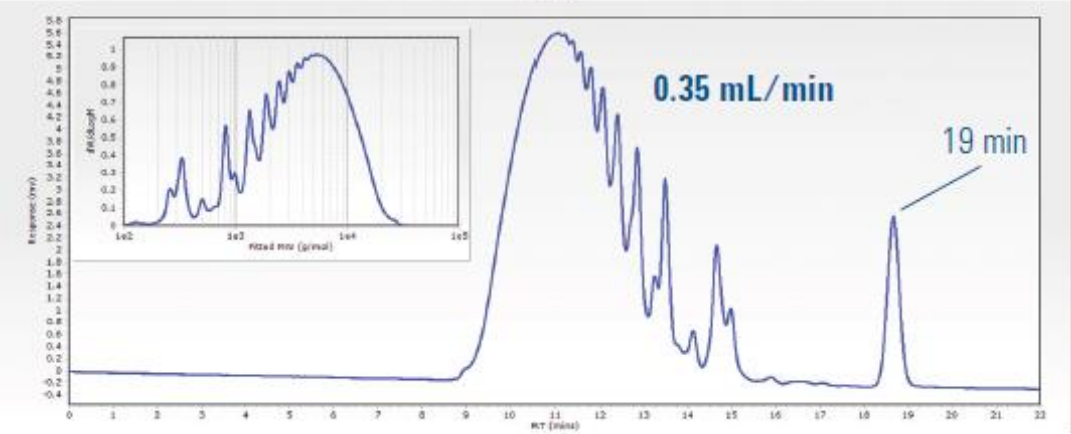
Typical Efficiency: >55,000 p/m

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



High Speed MesoPore Columns





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

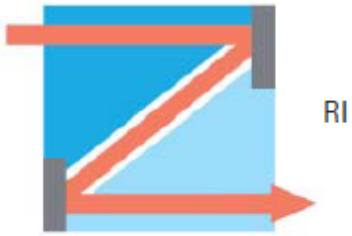
MW Range: up to 25,000 (g/mol)

Nominal Particle Size: 3 µm

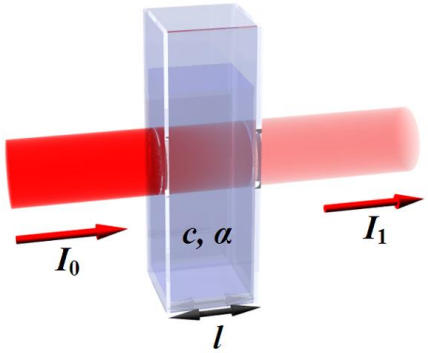
Typical Efficiency: >80,000 p/m

Detection
Concentration Detectors

- Most common detectors for GPC/SEC are *concentration* detectors:



RID

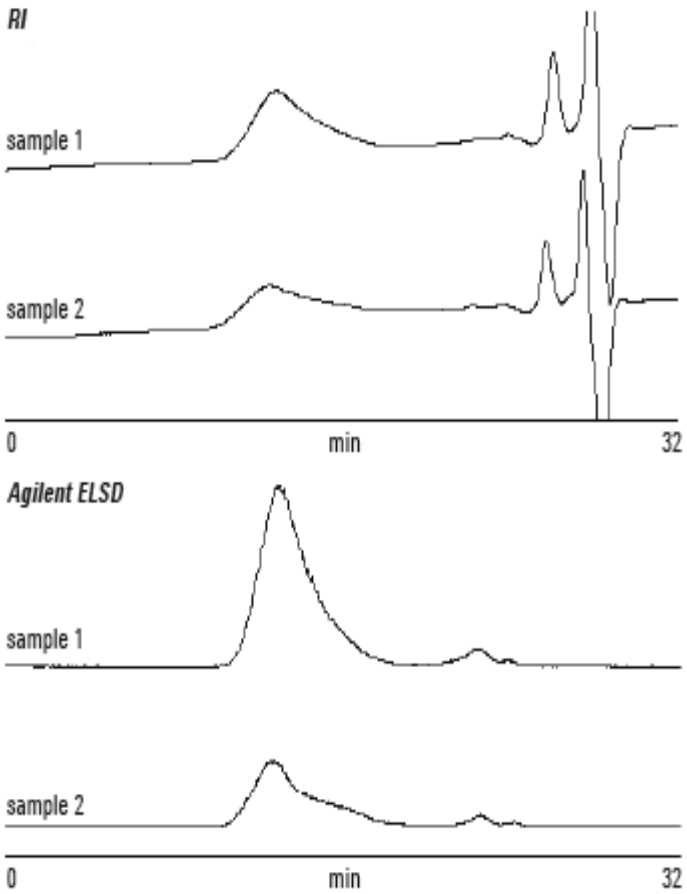


UV/DAD

ELSD

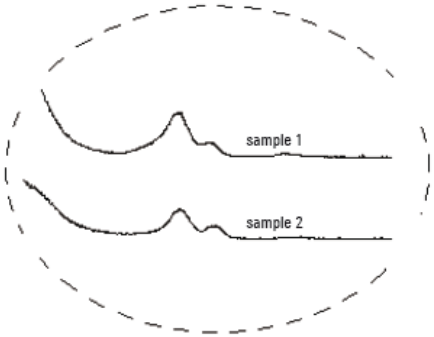
- These provide information on the amount of polymer eluting from the column at any given time

Detector Selection: ex RI vs ELSD



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

ELSD:
Improved response
Additives detected
No system interference peaks

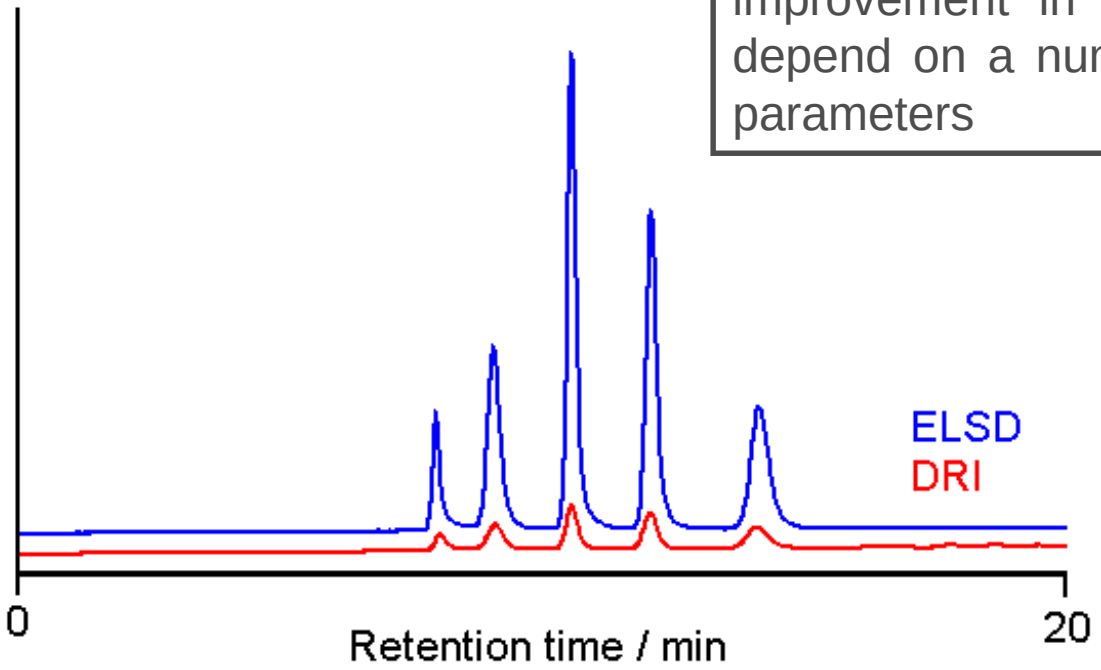


Sensitivity of DRI Versus ELSD

Columns	2 x PLgel 5µm MIXED-C 300x7.5mm
Eluent	THF
Flow rate	1.0ml/min
Loading	0.1%, 20µl

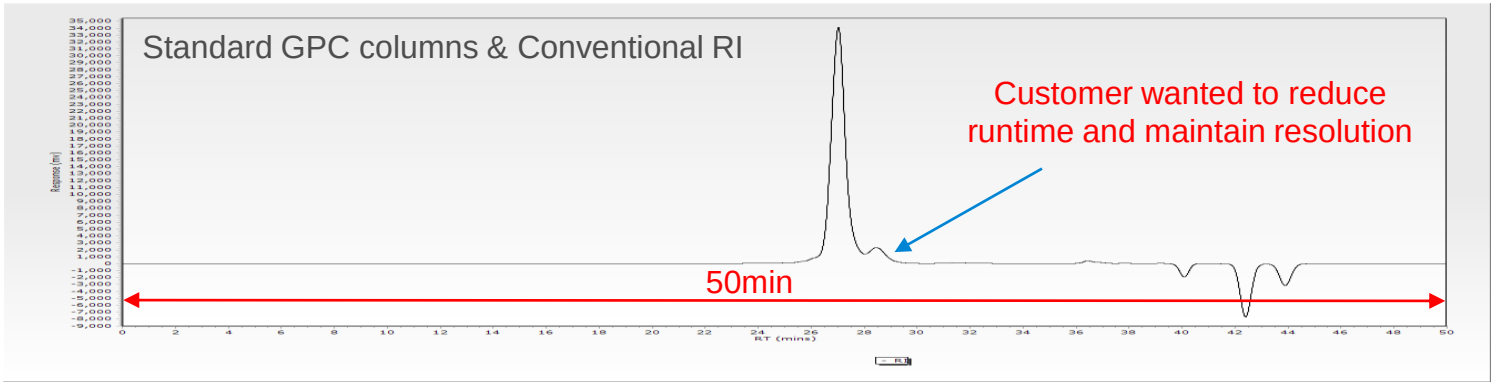
Mp values

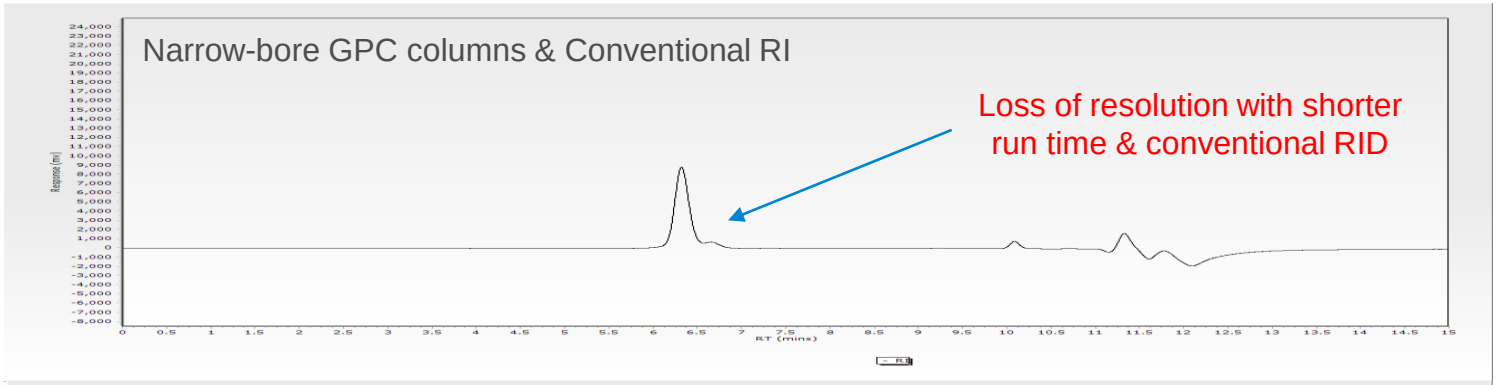
1. 7,500,000
2. 841,700
3. 148,000
4. 28,500
5. 2,930

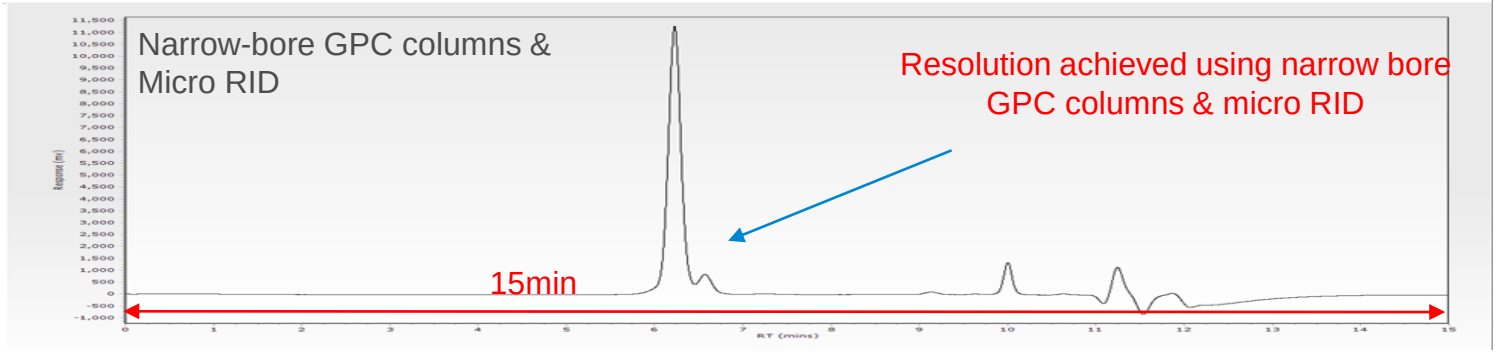


ELSD is essentially independent of dn/dc, improvement in sensitivity will depend on a number of solute parameters

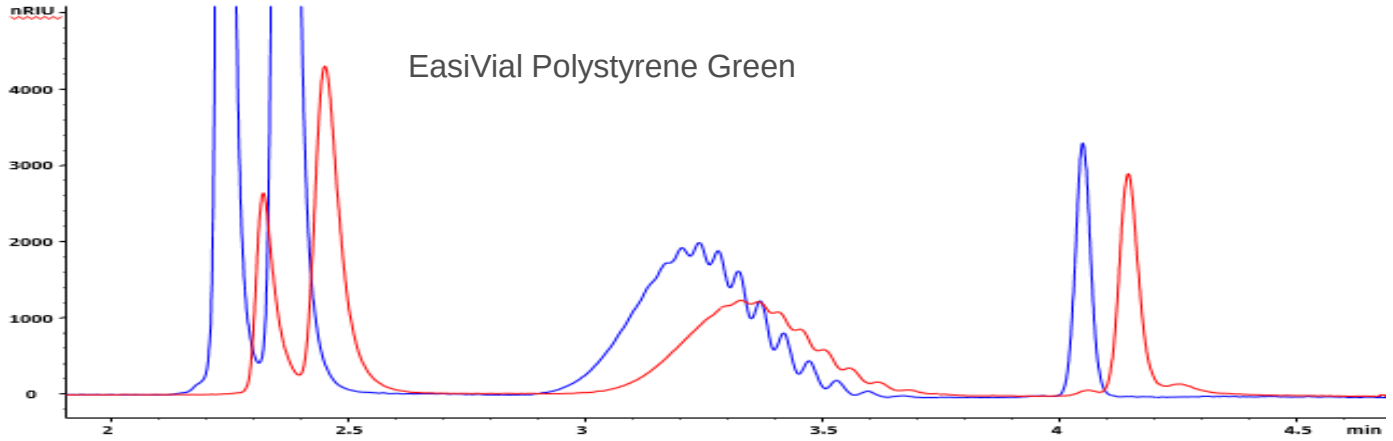
Case Study – Customer Sample “Kraton”

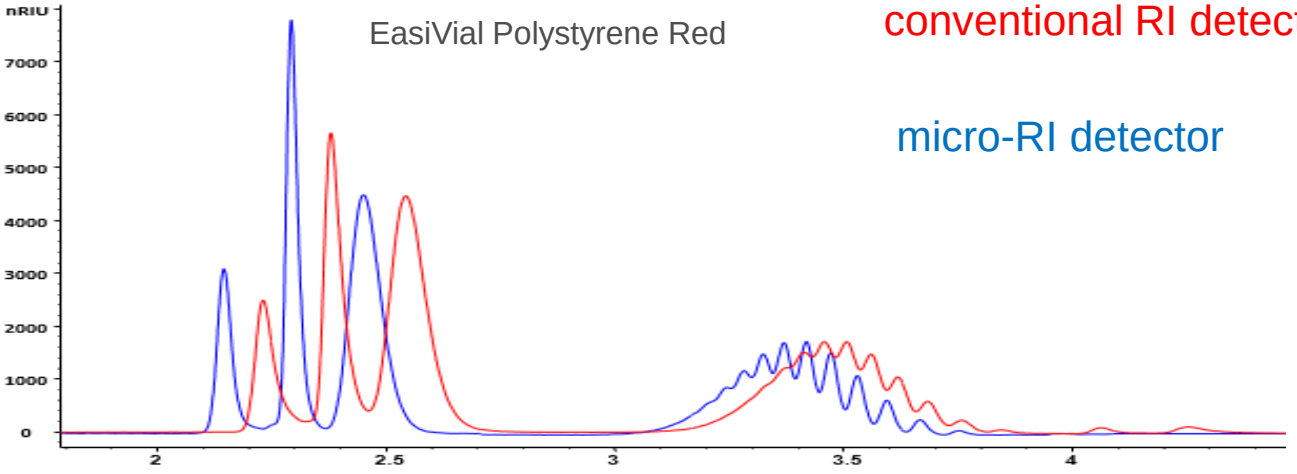






Ultra low dispersion for improved resolution





conventional RI detector

micro-RI detector

Increasing the information from GPC/SEC

GPC/SEC provides critical information for the polymer chemist:

Distribution of chain lengths

(Relative molecular weights)

Further parameters can be determined by employing advanced detectors

The molecular **weight** (accurate or absolute)

The polymer's **size**

The polymer's **shape**

Can be used to investigate polymer **branching**

Expanding Conventional GPC/SEC

Viscometer and Light Scattering Detectors

Advanced detectors give a greater understanding of the analyte as well as overcoming the limitations of Conventional GPC.

GPC/SEC Technique	Molecular Weight	Molecular Size	Information
Conventional (RI or UV)	Relative to standards used for calibration	No	Molecular weight distribution, concentration
Viscometry	More accurate from Universal Calibration	Yes, hydrodynamic radius (**Rh).**	Conformation, branching. Works with copolymers
Light Scattering	Absolute determination	Yes, Radius of Gyration (Rg) directly.	Conformation, branching.
Triple	Absolute determination	Yes, Rg and Rh, directly.	The ultimate configuration for comprehensive polymer characterisation

Summary

Solvent Selection	Consider you choice of solvent carefully for the type of sample, conditions, & columns required for analysis.
Sample & Instrument Considerations	Use appropriate concentrations & inj. volumes based on your sample's MW & check your system parameters to also optimize your analysis
GPC Column Selection	Look to make the appropriate selection based on expected MW, but also be sure to ask 'what is it that I want or need for my analysis?
Detectors	Concentration type detectors for Conventional GPC or look to Multi detector SEC to get additional information for your polymer

GPC/SEC Resources

Brochure	Agilent GPC/SEC solutions - comprehensively better polymer analysis	5990-8844EN
Application 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
Primers	Introduction to GPC/SEC	5990-6969EN
	A guide to multi-detector GPC	5990-7196EN
	Calibrating GPC columns – a guide to best practice	5991-2720EN
	An Automated System for Cleanup of Environmental Samples	5991-5321EN
Product Guides	1260 Infinity GPC/SEC System	5990-9920EN
	1260 Infinity Multi-Detector GPC/SEC System	5990-9921EN
	Agilent 1290 Infinity II Refractive Index Detector (Micro)	5991-6561EN
	Agilent PL-GPC 220	5990-9926EN
	Agilent GPC/SEC Software	5991-0478EN
	Aqueous and polar GPC/SEC columns	5990-7995EN
	Organic GPC/SEC columns	5990-7994EN
	GPC/SEC standards	5990-7996EN
Select Guide	Quick guide for selecting columns and standards for GPC/SEC	5990-6868EN
Quick Reference Guide	Agilent 1260 Infinity GPC/SEC System supplies	5990-9947EN
	Agilent PL-GPC 220 System supplies	5990-9946EN
Flyers	Agilent EnviroPrep GPC columns	5991-1588EN
	Agilent Fast GPC columns	5991-2785EN
Wall Chart	Achieve more with the Polymer Analysis People	5991-3802EN

Download at **www.agilent.com/chem/GPCresources**

THANK YOU FOR ATTENDING

ANY QUESTIONS??