

Tips and Tricks for GPC/SEC Method Development and Improvement

Agilent eSeminar

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Outline

**Part 1. Review of Modern GPC/SEC,
Agilent Resources, and New Technologies**

Part 2. Tips and Tricks



LABORATORY AND BUSINESS PERSPECTIVE

Goals of GPC/SEC

- **GPC/SEC analysis provides a full description of the MW fractions present in a polymer**
- **The molecular weight distribution determines most major physical and chemical properties of a polymer**
- **A powerful technique, GPC/SEC automates polymer characterization, eliminates physical tests, and reliably identifies failed batches**
- **Good GPC/SEC methods multiply the productivity of analysts, systems, and the lab as whole**

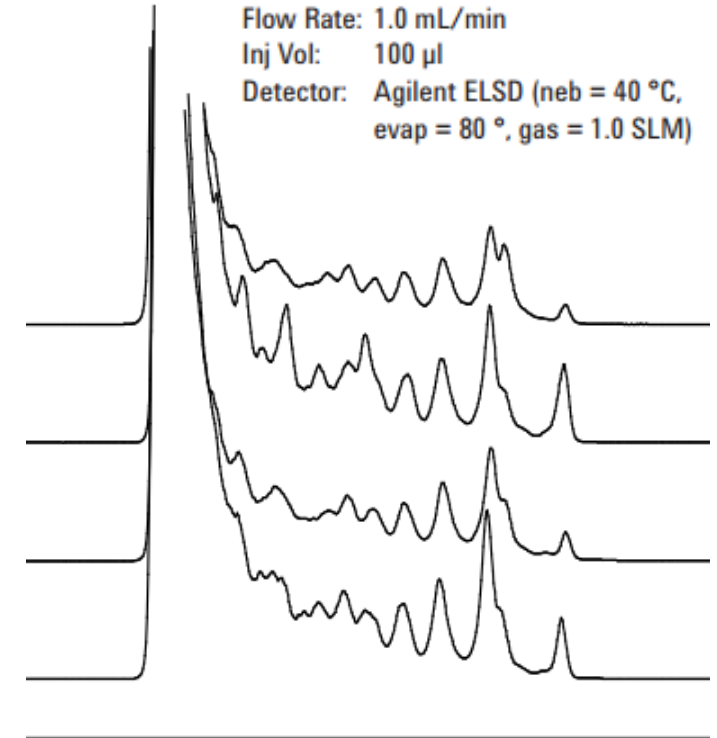


Using GPC/SEC Data

- **GPC/SEC provides a rich body of data beyond. This includes:**
 - **Polymer distribution values: Mw, Mn, Mz, PDI**
 - **Batch fingerprinting for quality record**
 - **Quantitation of high and low MW impurities**
 - **Quantitation of plasticizer, oligomer, and additive content**
- **Data can be used to predict properties such as strength, elasticity, solubility, and viscosity**
- **GPC/SEC is the only analytical technique that is able to provide this level of detail**



Samples: Epoxy resins
Columns: 2 x OligoPore, 300 x 7.5 mm
(Part No. PL1113-6520)
Eluent: THF + 250 ppm BHT
Flow Rate: 1.0 mL/min
Inj Vol: 100 μ l
Detector: Agilent ELSD (neb = 40 °C,
evap = 80 °, gas = 1.0 SLM)



MODERN GPC/SEC METHOD DEVELOPMENT

Ideal GPC/SEC Analysis

- **Fast**
 - Lab overhead averages \$100-\$250 per hour per analyst
 - Analysis time impacts project timelines and QA/QC turnaround time
- **Precise and Accurate**
 - Inaccurate values will not correlate to polymer properties
 - False-negatives can result in final product failure
- **Robust and Reliable**
 - Troubleshooting costs thousands of dollars in wasted analyst labor hours and sample re-runs



Innovations in GPC/SEC



Agilent 1200 Infinity II Series

- **Agilent 1260 Infinity II GPC/SEC System reaches new levels of speed and resolution**
- **1260 Infinity II High-Temperature GPC System brings new levels of reliability and performance to High-temp GPC**

Next-Generation Agilent Detectors

- **Agilent 1290 Infinity II RI and ELSD detectors reach new levels of sensitivity and linearity while eliminating peak broadening**

InfinityLab Columns

- **Pluspore pushes the limits of GPC efficiency and resolution**
- **PL Multisolvent offers high efficiency in solvents ranging from THF to water**

InfinityLab Standards

- **EasiCal and EasiVial Standards eliminate labor hours wasted on weighing and mixing**

A Modern GPC Separation

System: Agilent 1260 Infinity II GPC/SEC

Sample: InfinityLab EasiVial Polystyrene Standard

Column: InfinityLab Resipore 4.6x250mm

Solvent: THF

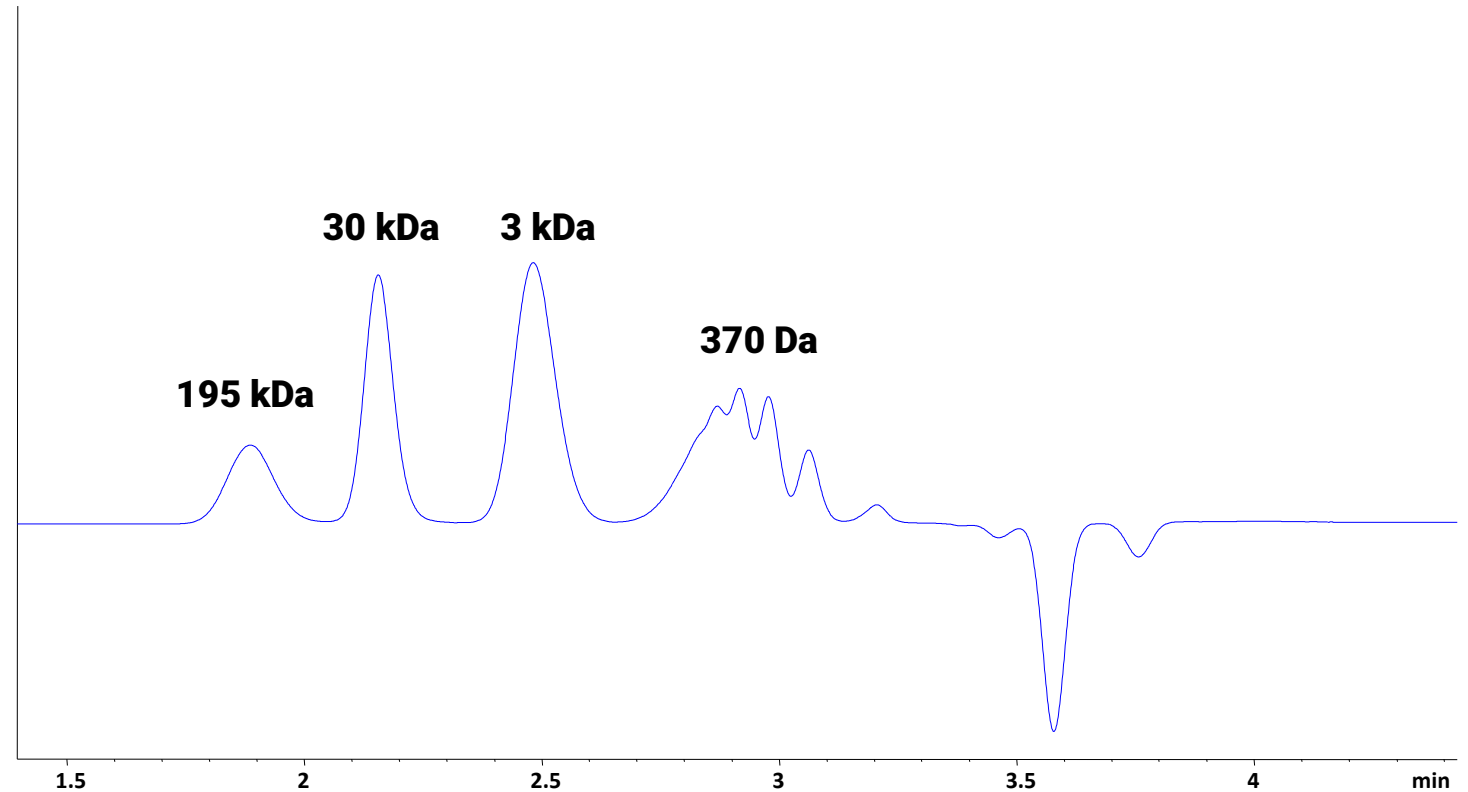
Flowrate: 1 mL/min

Temperature: 30 °C

Detector Temp: 35 °C

Frequency: 18.5 Hz

**5x Increase in productivity vs older
25 minute method**



A Modern SEC Separation

System: Agilent 1260 Infinity II GPC/SEC

Sample: Dextrin Polysaccharide

Column: InfinityLab PL Multisolvant 30 4.6x150mm

Solvent: 10 mM NaH₂PO₄ + 0.2 M NaNO₃ at pH 7

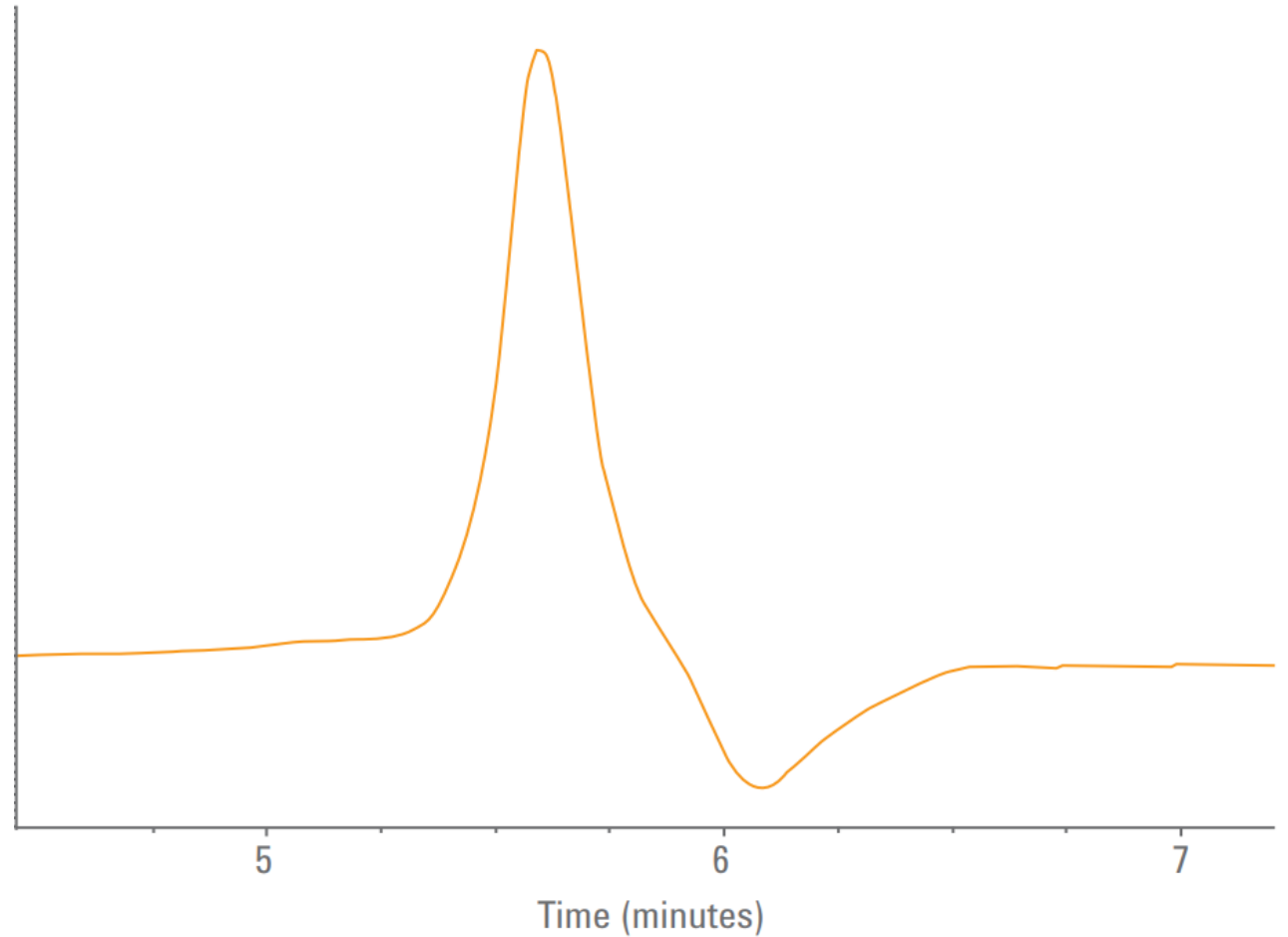
Flowrate: 0.3 mL/min

Temperature: 25 °C

Detector Temp: 30 °C

Frequency: 18.5 Hz

**3x Increase in productivity vs older
25 minute method**



ONLINE RESOURCES

Method Development Walkthroughs

Detailed method development walkthroughs are available online:

Step-By-Step Method Development for GPC/SEC

Publication number: 5991-7272EN

Method Development for Fast GPC/SEC

[agilent.com/en-us/method-development-for-fast-gpc-sec](https://www.agilent.com/en-us/method-development-for-fast-gpc-sec)

Method Development for Fast GPC/SEC

Method Development for Fast GPC/SEC
Part 1: Identifying Starting Parameters
Adam Bivens
Product Manager
Agilent Technologies

Method Development for Fast GPC/SEC
Part 2: Instruments for GPC/SEC
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Product Manager
Agilent Technologies

Method Development for Fast GPC/SEC
Part 1: Identifying Starting Parameters
Running time - 00:07:51
Reviews GPC as a technique for developing a method template. Method development resources.

Method Development for Fast GPC/SEC
Part 2: Instruments for GPC/SEC
Running time - 00:07:27

Step-by-Step Method Development for GPC/SEC

Technical Overview

Author
Adam Bivens
Agilent Technologies, Inc.

Abstract
Agilent offers a full suite of GPC/SEC solutions for the many different applications and samples measured with this technique. Resilient methods and correct product choice directly impact a laboratory's budget by maximizing throughput and reducing data errors. This technical overview reviews each step of method development from initial compound identification to column end-of-life.

Introduction
Gel Permeation Chromatography (GPC), also called Size Exclusion Chromatography (SEC) or Gel Filtration Chromatography (GFC), is the primary method of determining a sample's molecular weight distribution.
GPC columns are filled with porous beads of a well-defined pore diameter and large pore volume. After injection, small molecules drift into the pores, while large molecules are excluded and get swept along by the solvent. This separation mechanism causes analytes to elute from largest to smallest.
Precise method development is necessary to ensure that data are consistent and error-free, while also minimizing the time spent developing each analysis.

Agilent Technologies

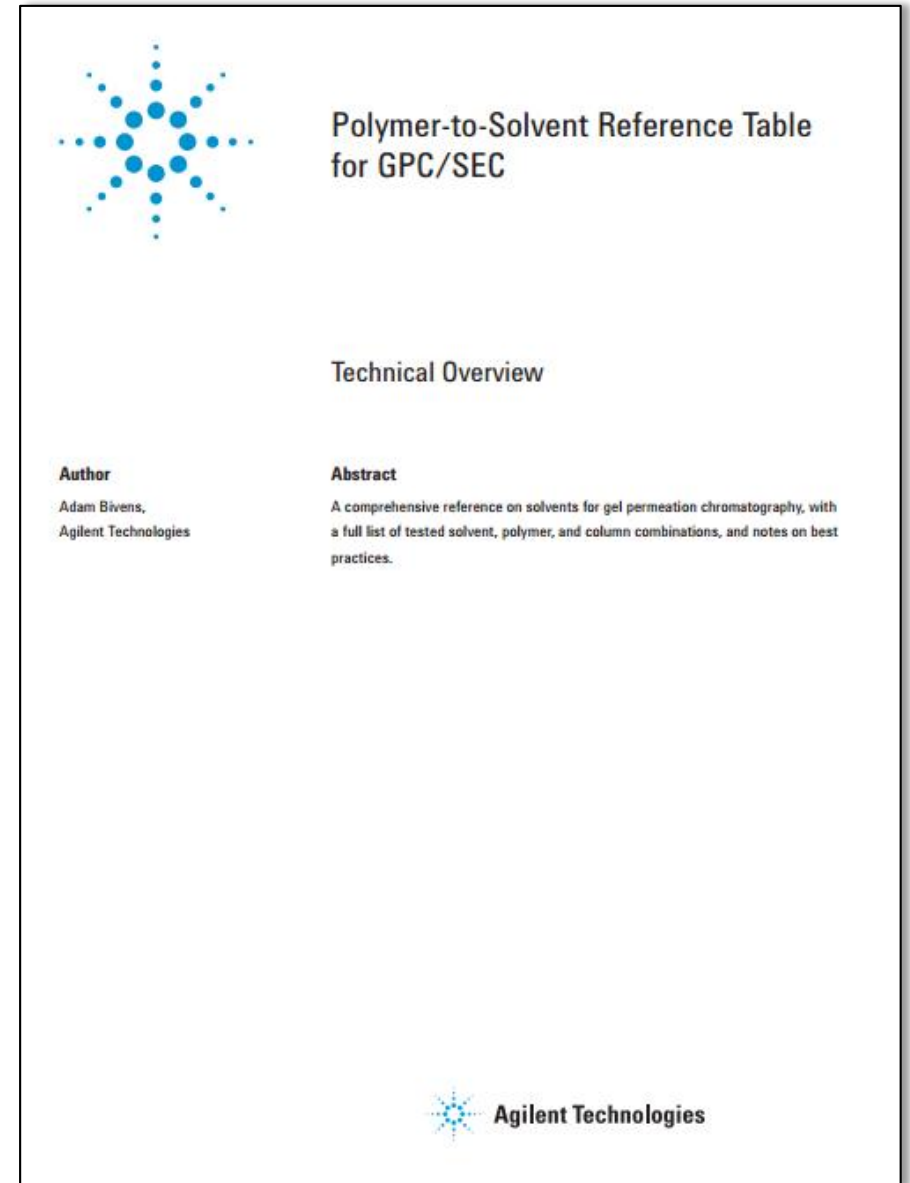
Finding Method Conditions

In its 40 years of experience in GPC, Agilent has tested a huge number of polymers, found here:

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

Includes:

- 1. Solvent**
- 2. Temperature**
- 3. Column Family**
- 4. Publication Number**



Optimizing a System

Narrow peaks can be severely broadened by a non-optimized system

Small changes to capillaries, fittings, and heat exchangers can drastically improve speed and resolution

Instrument Setup for Fast GPC/SEC (Publication number: 5991-7191EN)



Instrument Setup for Fast GPC/SEC

Technical Overview

Author

Adam Bivens,
Agilent Technologies Inc.

Abstract

Proper column choice and instrument setup together can vastly improve the speed and data quality that can be achieved when performing gel permeation chromatography (GPC)/size exclusion chromatography (SEC). Agilent manufactures a full range of consumable parts that enable the best possible results achieved with various types of instruments.

Introduction

Gel permeation chromatography (GPC) is an important technique for determining the molecular weight (MW) distribution of a polymer, and for comparing batch-to-batch polymer quality. With laboratories under increasing pressure to increase efficiency and reduce costs, Agilent is committed to offering GPC users innovative solutions to increase the speed and quality of their analyses.

Optimal system setup is key to reduce the cost per analysis for labs performing GPC, but systems vary widely in performance. This technical overview focuses on the different strategies to maximize performance on low and high dispersion instruments.

Low dispersion instruments, in combination with high efficiency columns, offer the highest possible speed and resolution. The columns separate samples quickly while the system transports the analytes from injector to column to detector without allowing these narrow peaks to broaden.

In practice, many analysts must use instruments that have too high dispersion to benefit from high efficiency columns. To help these customers improve their analysis, Agilent has designed PL Rapide columns to counter the dispersion in their instrument. Agilent PL Rapide columns offer a drop-in solution to significantly improve performance on systems that were previously dispersion-limited.



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CHOOSING A COLUMN

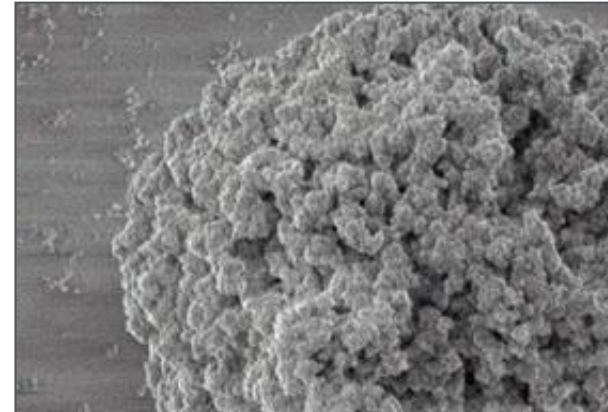
Types of GPC columns

Particle shape dictates separation

- **GPC particles offer different combinations of range, separation and efficiency**
- **A wider range particle will have less separation of individual peaks**
- **Small particles offer faster speed and higher resolution, but cannot support large pores for high MW analysis**

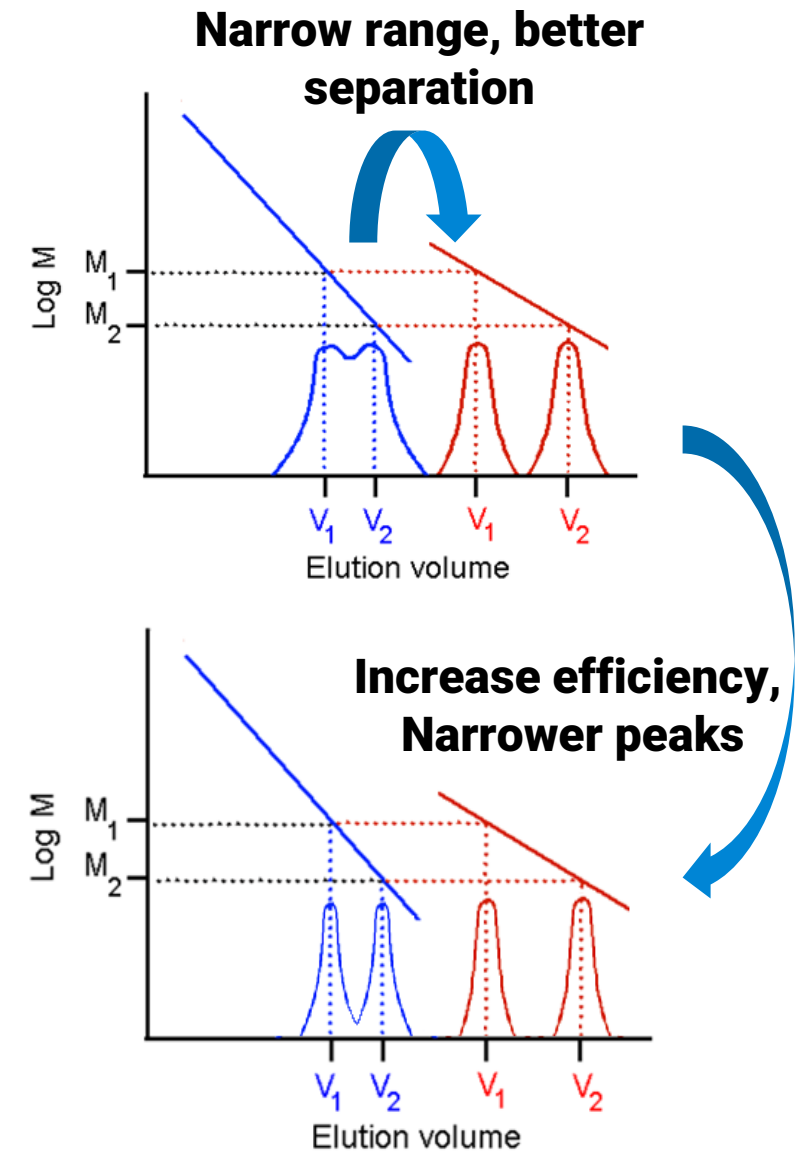
Types of Particles:

1. Individual Pore Size
2. MIXED
3. Multi-Pore Particle



Steps to choosing a GPC column

1. Identify the sample, solvent, temperature, and column type
2. Review the brochure of the appropriate column family
3. Identify all column candidates with correct MW ranges and temperature limits
4. Compare the efficiencies and the calibration curves
 - Large compounds should elute as early as possible, and small as late as possible.
 - Unimportant regions can go beyond the MW range, but the peaks will be highly distorted
 - High efficiency columns give better peak shape and faster analyses
 - Between better separation or higher efficiencies, separation is preferable
5. Choose a column, and determine the number in the stack
 - 1-2 columns for 3 μm , 2-3 for 5 μm , 3-4 for 10 μm and 13 μm , 4 for 20 μm



Example 1

Medical device extractables testing

Sample: PVC tubing

Goal: Quantitate plasticizer content

**Largest Plasticizer: Diisodecyl phthalate
(446.67 g/mol)**

System: Agilent 1260 Infinity II with UV

**Use Polymer Solvent Reference Table
(Publication 5991-6802EN)**

***=>Application Note 5991-5813EN
gives conditions for PVC analysis***

Conditions

- Solvent: THF
- Columns: PLgel 5um MIXED-C
- Temperature: 25 C

Potential Columns:

- 3 µm PLgel 100A 7.5 x 300 mm
- 5 µm PLgel 50A 7.5 x 300 mm (1-2x)
- Oligopore 7.5 x 300 mm



DEVELOPING A ROBUST SOP

Example Standard Operating Procedure

1. Warm up instrument, column, and detector to operating temperature
2. Prepare sample solvent from eluent plus 1% BHT
3. Dissolve polymer samples with gentle stirring (do not rapidly spin or sonicate) at a concentration of 1%.
4. Filter polymer samples into appropriate vials for autosampler
5. Load all samples and inject standards when instrument baseline stabilizes
6. Generate calibration curve
7. Run samples
8. Re-run polymer standards
9. Overlay calibration curves to verify consistency
10. Analyze Data

Startup

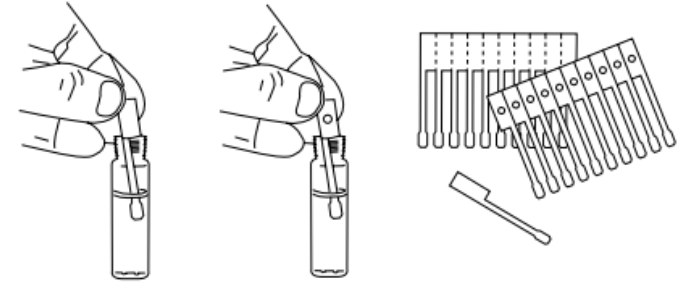
Sample Prep

Running Samples

Data Analysis

Sample Prep

- **Add a small molecule**
 - 1% BHT, toluene, ethylene glycol, etc.
- **Dissolve samples in the eluent**
- **Dissolution can be slow, overnight is recommended**
- **High MW samples can degrade to low MW fragments**
 - No rapid stirring or sonication
 - Add stabilizer to organic solvents
 - Keep cool and covered to prevent oxidation
- **Use a syringe filter just smaller than the frit size of the GPC column**



Running Samples

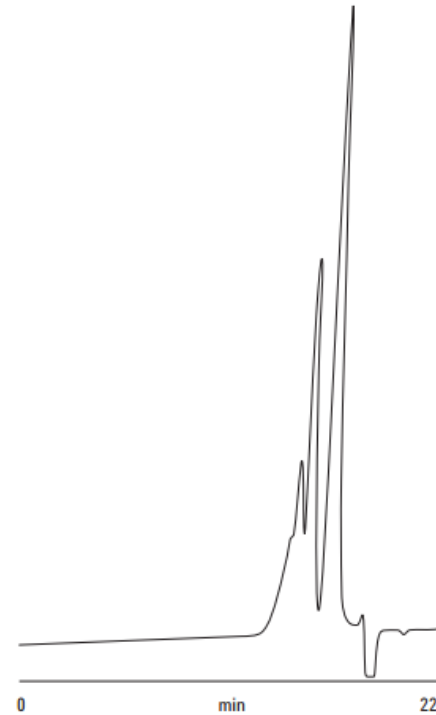
- **Run standards during warm-up to see when the system has stabilized**
- **Calibrate before and after a run to avoid inconsistent data**
- **Polymers are viscous, minimizing sample concentration improves chromatography**
- **Large polymers are better resolved at lower flowrates, use the calibration peaks for reference.**
- **For more details on running conditions, refer to the user guide**



Data Analysis

- **Calculate column efficiency and tailing using the small molecule peak**
- **Compare sample MW curves, not chromatograms (for nonlinear columns)**
- **Integration parameters impacts data**
 - **Do not exclude front or tail of peaks**
 - **Be consistent when including or excluding low MW peaks**
- **Mp, Mn, Mw, and Polydispersity are not very useful with unusual peak shapes**
 - **Alternatives: % impurity, % oligomers, % plasticizer, Peak area ratios, Ratio high/mid/low MW fractions**

Column	2 × Agilent PLgel 5 μm 100Å, 7.5 × 300 mm (p/n PL1110-6520)
Eluent	DMF
Flow rate	1.0 mL/min
Temp	80 °C
System	Agilent 1260 Infinity GPC/SEC Analysis System
Detector	RI



Melamine Resin

Dispersion and Peak Broadening

- **Peaks can broaden significantly as they travel through the injector, fittings, capillary, and detector**
- **For fast GPC, peaks must remain sharp, and low dispersion hardware is critical**
- **Increased speed reduces the cost of an analysis, and maximizes throughput**

For more information refer to:

**Instrument Setup for Fast GPC/SEC
5991-7191EN**



GPC COLUMN TROUBLESHOOTING

Bed degradation

Particles slowly crush with use, which shifts the pore size distribution, and reduces overall separation and efficiency.

Indication: Loss of efficiency and less separation between the highest and lowest MW calibration standards

Solution: Track separation and efficiency loss with regular calibration and use of a flowrate marker. Replace columns when the efficiency drops below acceptable levels (usually 80% of the original value).



Large pore collapse

Flowing solvent slowly crushes the largest pores, so that large molecules are no longer separated

Indication: High MW standards are not separated. The largest molecules begin to elute all at once as a peak or shoulder at the beginning of the chromatogram.

Solution: Regular calibration to track pore collapse and column replacement when data quality is impacted.



Chemical attack

Reactive compounds can alter the surface of the particles and cause the analyte to stick.

Common culprits: residual oxidizers, catalysts, radical initiators, reactive prepolymers (such as acyl chlorides), and solvent degradation products.

***Indication:* Over time the same samples become more retained, and peaks start to split or tail.**

***Solution:* Neutralize any strong acids or bases, use BHT to deactivate residual oxidizers and radical initiators, and use alcohols to destroy any strong nucleophiles and electrophiles.**



Shear

Large molecules can be sheared by small frits, strong agitation, or partially clogged capillary

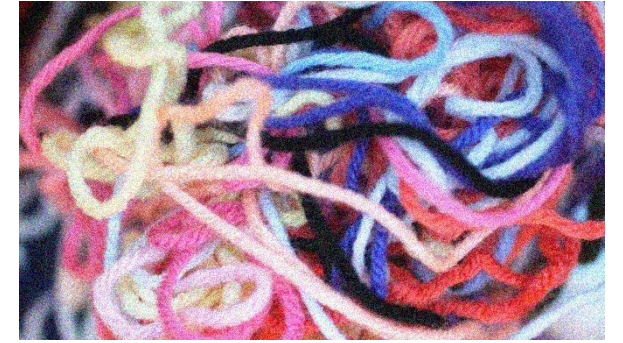
***Indication:* Disappearance of high MW compounds, long tail of low MW fragments**

Solution:

- **Use larger particle columns with frits larger than 10um**
- **Eliminate any strong shaking or sonication**
- **Replace components in the sample path starting with fittings, then sample loop, connecting capillaries, then autosampler needle.**



Partial Infiltration / Anchoring / Non-ideal separation



Large, branched molecules can be retained by small pores, causing secondary retention and inaccurate MW measurement

***Indication:* U-shaped light scattering or viscosity curve. Long tail for high MW sample.**

***Solution:* Switch wide-pore columns ($>10^3$ Å)
– Example: PLgel 10um $10^4 + 10^5 + 10^6$ Å**

SYSTEM AND WORKFLOW TROUBLESHOOTING

Calibration



Columns must be regularly calibrated with standards to obtain accurate MW values.

- **Pump drift, column aging, new capillaries, re-swaged fittings, and new column connections will all cause retention times to shift.**
- **Error increases exponentially in GPC/SEC, so a 1% inaccuracy in retention time can easily cause a 10% shift in measured MW.**

Solution:

- 1. Calibrate before and after any critical sample sets.**
- 2. For general use, systems should be calibrated at startup and weekly afterwards**

Sample Preparation

Sample preparation can significantly impact MW distribution measurements and experimental repeatability

Solution:

- **Samples should be left to dissolve overnight in the eluent, with only mild agitation**
- **Samples with a insoluble gel fraction have higher variability and risk of clogs.**
 - **Be consistent with dissolution time, temperature, and agitation.**
 - **Sample duplication is encouraged**
- **Prevent degradation. Avoid high temperatures, use amber bottles, keep samples covered, add a stabilizer**
- **Filtration prevents column clogging, but can cause sample loss. Use the largest frit that won't clog the column**



Data Processing

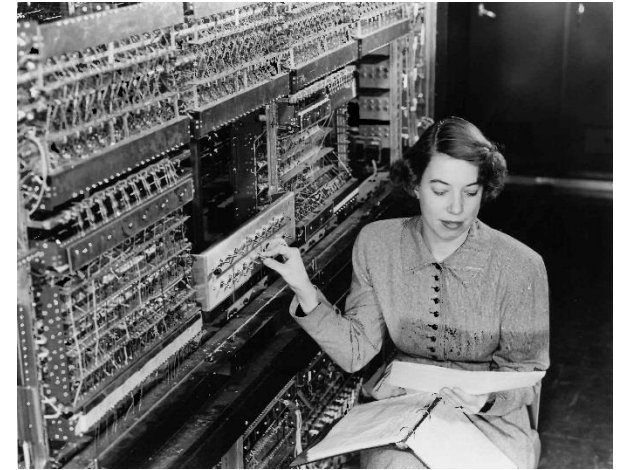
Software settings must be consistent. Values should reflect polymer properties.

Common sources of error for calculated values such as M_n , M_z , and M_w :

- **Excluding a peak's front or tail from peak integration**
- **Including the small molecule peak**
- **An unstable or slanted baseline**
- **Peak broadening**
- **Measuring beyond the columns' MW limits**

These values are also substantially less useful for bimodal or unusually shaped peaks.

- **Alternatives: % impurity, % oligomers, % plasticizer, Peak area ratios, Ratio high/mid/low MW fractions**



Dispersion

As much as 80% of peak broadening occurs outside the column. Severe dispersion artificially broadens MW distributions.

Solution:

- **Use short lengths of narrow capillary**
 - 0.17 mm minimum, 0.12 mm preferred, 0.075 mm for high speed, high resolution work
- **Use small flowcell detectors**
- **Increase flowrate***

***Shorter columns with higher flowrate often save solvent**



Thank You!

