User Guide

Agilent
Trusted Answers

Agilent Polymer Standards for GPC/SEC Column Calibration

Agilent polymer standards

Polymer standards from Agilent are produced in a specialized manner to offer specific characteristics not found in mass-produced counterparts. The use of stringent synthesis conditions and purified reagents results in polymers of the highest purity and the narrowest molecular weight distribution available. This is an important consideration when selecting polymer reference standards.

Agilent has 40 years of experience in the synthesis and characterization of polymers, employing a wide variety of proprietary techniques. With such extensive characterization, Agilent polymer standards are ideal reference materials for use in analytical techniques that require calibration methods or routine checks of performance.

The polymers selected for the kits have been chosen to complement Agilent GPC/SEC columns, and to produce the equidistant calibration plot necessary for accurate polymer analysis by GPC/SEC.

Quality and traceability

The Agilent philosophy is based on producing the most reliable, extensively characterized polymer standards available worldwide, and on ensuring continuity of supply and reproducibility of replacement batches.

Agilent polymer standards are reference materials that have been manufactured and tested under a Quality Management System registered to ISO 9001. Each polymer standard is assigned a unique batch number and is supplied with a Certificate of Analysis that details the methods used and the characterization results assigned. Certificates of Analysis are downloadable from https://www.agilent.com/en/ecertificates-of-performance.

At Agilent, we are totally committed to the quality, reliability, and accuracy of our range of polymer standards, giving our customers security in the knowledge that they have purchased the best reference materials available.

Characterization of Agilent polymer standards

Polymers consist of a distribution of many molecular species. The reported average molar masses (M) result from several possible methods of averaging the different species present. Agilent polymer standards are characterized by various well-defined methods.

Agilent polymer standards for GPC/SEC calibration

- Nuclear magnetic resonance to determine M_n
- Light scattering to determine M_w
- Viscometry to determine intrinsic viscosity
- High-resolution gel permeation chromatography (GPC) to determine M_n, M_w, M_w/M_n, and M_p

The applicable methods depend on the polymer chemistry and standard type.

Which standards to use

The chosen calibration standard must be readily soluble in the eluent that will be used. Details of polymer solubilities are given in Table A1 in the Appendix.

Column calibration procedure

Both conventional GPC/SEC using a single concentration detector and GPC/SEC viscometry using universal calibration rely on an initial calibration.

Old column calibration curves produce increasingly inaccurate data with time. Calibration at the start and end of a sample set is best practice. As a minimum, systems should be calibrated at startup, and weekly thereafter.

Use narrow molar mass distributed standards when constructing a GPC/SEC column calibration plot, and use the M_p value for the calibration. Operating conditions for different analytical techniques vary according to the type of polymer and the molecular weight. Full details of the analysis conditions are described in the Certificate of Analysis, together with the expiration date.

Appearance

Individual polymer standards: White powder, waxy lumps, or viscous liquid according to M_w and composition, see the note below.

Agilent EasiCal: The system for organic solvents consists of two different combs, each with 10 detachable spatulas supporting a mixture of five polymer standards. The thin film of polymer (approximately 5 mg) on the tip of the PTFE spatulas rapidly dissolves when immersed in eluent to provide two calibration solutions for GPC/SEC column calibration. A single pack provides 10 spatulas of each type, with molecular weights selected to provide equidistant calibration points for greater accuracy.

Agilent EasiVial/ReadyCal: Three sets of color-coded glass vials with a polymer film deposited on the inner surface or a lyophilized powder used for GPC/SEC column calibration. Polystyrene and PMMA EasiVials/ReadyCals are supplied in clear vials. PEG, PEG/PEO, Pullulan, Dextran, and Protein EasiVials/ReadyCals are supplied in amber vials.

Note: The color codes on the glass vials of EasiVial and ReadyCal are different. For detailed information please download your certficate of analysis from https://www.agilent.com/en/ecertificates-of-performance.

Note: Very low molecular weight polymers are liquid or semisolid at room temperature. Similarly, many low molecular weight standards will soften or liquefy if exposed to mild heat. Liquid standards are most easily handled by glass pipette. Softer polymer standards that have aggregated may be refrigerated until solid, carefully cut with clean shears, and handled as chips.

Agilent ReadyVLS: Two sets of color-coded glass vials with a film deposited on the inside. The vials are used for setup of advanced GPC/SEC detectors.

General preparation guidelines

The use of freshly prepared polymer standards is recommended. Standards should be dissolved in the same batch of mobile phase being run in the columns. Always allow at least three hours for molecular weight standards < 500,000 g/mol to fully dissolve (see Appendix Table A1). If an internal flow rate standard is being used, it needs to be monodisperse, eluted after the separation limit, and added to the polymer standard solution. An analytically pure small molecule is generally recommended.

High molecular weight polymers (> 500,000 g/mol) are also more likely to suffer from mechanical or thermal degradation during sample preparation. These polymer standards should be allowed to swell and dissolve slowly, for at least 24 hours, with only gentle stirring. The exact system conditions of concentration, injection loop size, and so forth, will vary per system setup.

If using molar mass-sensitive detectors such as viscometers or multi-angle light scattering detectors, precise calculation of the concentration is required. Weighing out the amount of reference material, as well as the added amount of solvent, is recommended.

GPC/SEC advanced detector setup

GPC/SEC systems using advanced detectors such as viscometry or laser light scattering with a concentration detector, must be set up first before any column calibration is undertaken. The detector setup involves determination of the interdetector delay to enable synchronization of the various detector signals, normalization of the detectors at different angles (for MALS detectors), calibration of viscometers, and determination of all detector constants.

A narrow, isotropic (< 120,000 Da) standard is used for the initial setup, typically at a concentration of 1 to 2 g/L, referencing the MW value of the standard. A broader standard (typically at a concentration of 2 to 4 g/L) can be used to fine tune viscometers' interdetector delays and to verify detector constants. The concentrations must be precisely known and weighing out the amount of reference materials, as well as the added amount of solvent, is recommended.

As well as the information supplied in the certificate of analysis, knowledge of the solvent refractive index, solvent density, and refractive index increment (dn/dc) of the calibrants in the solvent are required. Check the detector manufacturer's instructions and software documentation for setup requirements.

Preparation of Agilent ReadyVLS standards

Prepare ReadyVLS standards by adding a precise amount of solvent to each of the color-coded vials.

Table 1. Recommended solvent volumes.

Solvent System	2 mL Vials
Polystyrene in THF	1 mL
Pullulan in Water	1 mL
Dextran in Water	1 mL

Preparation of Agilent EasiVial/ReadyCal kits

Prepare EasiVial/ReadyCal standards by adding solvent to one of each of the color-coded vials. The concentration of the standards will depend on the dn/dc for the solvent system in use and the relative sensitivity of the detectors.

Table 2. Recommended solvent volumes.

Solvent System	EasiVial 2 mL Vials	EasiVial 4 mL Vials	ReadyCal 2 mL Vials	ReadyCal 4 mL Vials	ReadyCal 10 mL Vials
Polystyrene in THF	1.0 mL	2.0 mL	1.5 mL	4.0 mL	NA
Polystyrene in TCB	0.5 mL	1.0 mL	1.5 mL (PSS-PSKITR1HT)	NA	10 mL (PSS-PSKITR10HT)
PMMA in THF	1.0 mL	2.0 mL	1.5 mL	4.0 mL	NA
PEG/PEO in Water	1.0 mL	2.0 mL	1.5 mL	4.0 mL	NA
Pullulan in Water	NA	NA	1.5 mL	NA	NA
Dextrans in Water	NA	NA	1.5 mL	NA	NA
Proteins in Water	NA	NA	1.5 mL	NA	NA

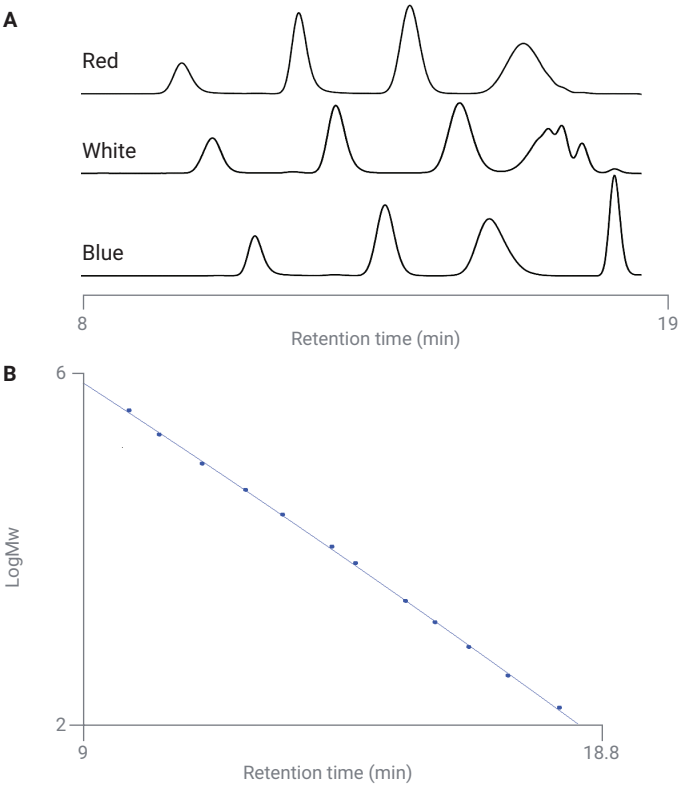

Figure 1. Typical Agilent EasiVial PS-M chromatograms and calibration plot.
Note: EasiVial and ReadyCal calibration standards use different color codes for the different molecular weight ranges of calibration standards. See the relevant CoAs.

Preparation of Agilent EasiCal standards

Prepare EasiCal standards by filling two vials with solvent, removing one of each type of spatula from the package, and stirring one spatula into either vial.

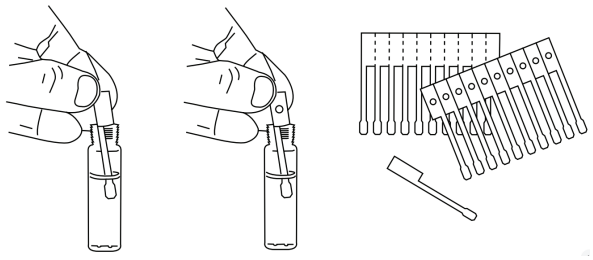

Figure 2. Preparation of Agilent EasiCal standards.

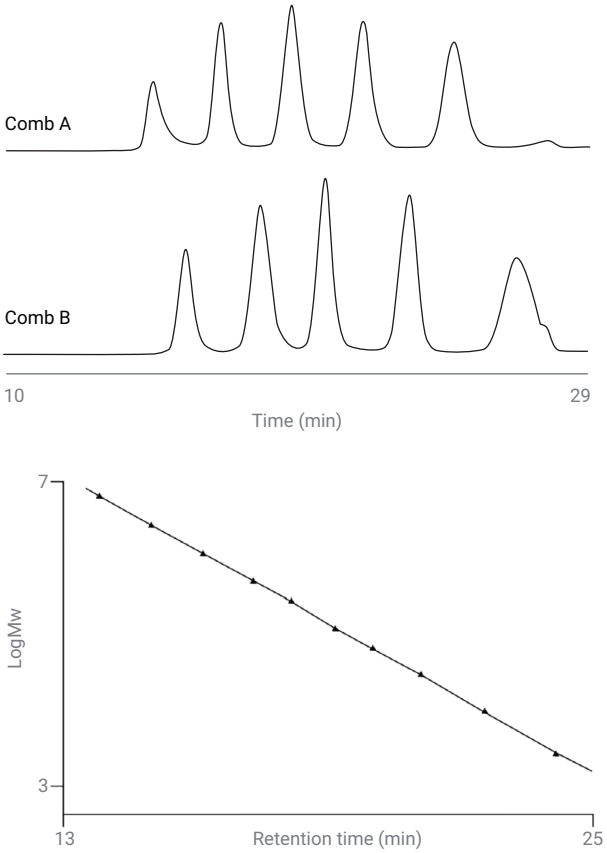

Figure 3. Typical Agilent EasiCal PS-1 chromatograms and calibration plot.

Table 3. Agilent EasiCal Standards.

Comb A M_p Mixture (g/mol)	Comb B M_p Mixture (g/mol)
8,500,000	3,040,000
1,030,000	330,000
156,000	66,000
28,500	9,200
3,250	580

Preparation of individually bottled standards

Weigh out individual standards and dissolve them in the eluent. Use the concentrations recommended in the relevant column user guide or the CoAs from the calibration standards. In the absence of this information, use the recommended concentrations in Table 4. For accurate results, follow the instructions given in the "General preparation guidelines" section.

Table 4. Recommended concentrations.

Peak Molecular Weight (M_p g/mol)	Concentration (mg/mL)
< 5,000	~ 1.0
5,000 to 400,000	< 1.0
400,000 to 2,000,000	< 0.5
> 2,000,000	< 0.1

Table 5. Example of mg/mL or a 0.1% wt/vol solution.

Mass (g)	Volume (mL)
0.001	1.0
0.01	10

The size of the injection loop also determines the quantity of relevant polymer being injected onto the column. Refer to the "Additional resources" section for a list of Agilent GPC/SEC column user guides.

Low molecular weight standards might resolve with multiple oligomeric peaks, depending on the obtained resolution of the column combination used. The highest peak is used for the M_p of the standard.

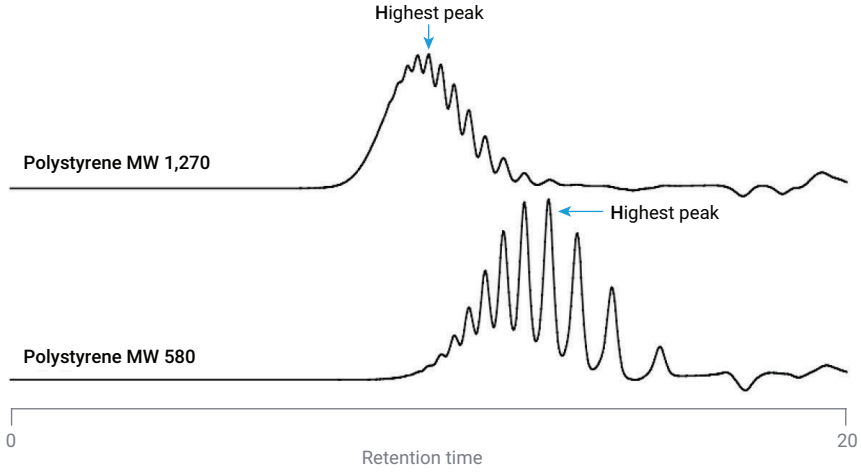

Figure 4. Typical chromatograms of low molecular weight standards showing oligomeric detail.

Summary

- Calibration curves lose accuracy over time, causing errors. Calibrate before and after critical sample sets. For standard work, calibration at startup and weekly thereafter is adequate.
- Verify that the molecular weight range of the polymer standards covers the complete resolving range of the columns listed in the selection guide.
- Accurately prepare fresh solutions as per Table 1, and allow the correct length of time, given the molecular weight, for complete dissolution.
- It is possible to generate a custom mixture of individual standards, provided they do not coelute. The molecular weights should vary by an order of magnitude, for example, for polystyrene: 1, 10, and 100 kDa. The convenient EasiVial/ReadyCal kit and EasiCal ranges have been specially selected to avoid coelution.
- Take care to avoid overloading the column. Refer to Table 2 as a guide for selecting concentrations, minimizing the sample load on-column where possible.
- Construct your GPC/SEC calibration using the M_p values stated on the certificates.

Storage

See the documentation and certificate of analysis supplied with the standard for detailed storage conditions.

Please also see the CoA, downloadable from https://www.agilent.com/en/library/certificates-of-performance.html.

Most standards should be stored under normal conditions (at room temperature, in a drawer or cupboard) and do not require special storage conditions. Once prepared, dilute polymer solutions are prone to degradation, and it is recommended that they are stored in a cool, dark environment for no longer than seven days.

Polyethylene oxide standards react slowly with oxygen to form peroxides along the backbone in a process that is facilitated by light. These peroxides can lead to chain cleavage. It is therefore recommended that polyethylene oxides be stored refrigerated under an inert gas (argon or nitrogen). Pullulans should also be stored refrigerated whereas proteins should be stored in the freezer.

Additional resources

- GPC/SEC Standards Product Guide, *Agilent Technologies*, publication number 5990-7996EN.
- Calibrating GPC Columns: A Guide to Best Practice, *Agilent Technologies*, publication number 5991-2720EN.
- GPC/SEC General Column User Guide, *Agilent Technologies*, publication number 5991-3792EN.
- Column-specific user guides:
  - GPC/SEC Column User Guide, *Agilent Technologies*, publication number 5991-3792EN.
  - SDV GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6522EN.
  - GRAM GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6514EN.
  - PolarSil GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6519EN.
  - PFG GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6518EN.
  - POLEFIN GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6520EN.
  - SUPREMA GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6523EN.
  - PROTEEMA GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6521EN.
  - NOVEMA Max GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6517EN.
  - MCX GPC/SEC Columns User Guide, *Agilent Technologies*, publication number 5994-6516EN.
- Step-by-Step Method Development for GPC/SEC, *Agilent Technologies*, publication number 5991-7272EN.
- A Guide to Multidetector Gel Permeation Chromatography, *Agilent Technologies*, publication number 5990-7196EN.

Technical support can also be obtained by contacting Agilent at: http://www.agilent.com/en-us/contact-us/page

Appendix

Table A1. Polymer standard solvent solubilities.

Solvent	Dextran	Poly(2-vinyl pyridine)	Poly(acrylic acid) Na+	Poly(α-methyl styrene)	Poly(butadiene1,2)	Poly(butadiene 1,4)	Poly(dimethyl siloxane)	Poly(ethylene glycol)	Poly(ethylene oxide)	Poly(ethylene terephthalate)	Poly(ethylene)	Poly(isobutylene)	Poly(isoprene 1,4)	Poly(isoprene 3,4)	Poly(lactide)	Poly(methacrylic acid) Na+	Poly(methyl methacrylate)	Poly(propylene glycol)	Poly(styrene sulfonate) Na+	Poly(styrene)	Poly(t-butyl methacrylate)	Proteins	Pullulan
Water	✓	(✓)	✓					✓	✓							✓			✓			✓	✓
Ethanol/methanol																					✓		
Trifluroethanol															✓								
Hexafluoroisopropanol										✓					✓		✓						
Dimethylformamide				✓				✓	✓								✓			✓	✓		
Dimethylacetamide				✓				✓	✓								✓			✓	✓		
Dimethylsulfoxide	✓									(✓)							✓						✓
Tetrahydrofuran		✓		✓	✓	✓	✓*	(✓)				✓	✓	✓			✓	✓		✓	✓		
Acetone																	✓				✓		
Chloroform				✓			✓					✓			✓		✓			✓	✓		
N-Methyl-2-pyrollidone				✓													✓			✓	✓		
Trichlorobenzene				✓							(✓)	✓								✓	✓		
Dichlorobenzene				✓	✓	✓					(✓)	✓								✓	✓		
Toluene				✓	✓	✓	✓					✓	✓	✓			✓			✓	✓		
EasiVial/ReadyCal	✓							✓	✓								✓			✓		✓	✓

✓ Standard is soluble in solvent
(✓) Standard is soluble under special conditions such as temperature, additives, or certain Mw ranges
✓* Standard is soluble but isorefractive

Learn more

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