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Application Note

Reproducibility and Reliability of GPC Analysis of Polystyrene on the Arc™ HPLC System

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Abstract

In this application note, we demonstrate the ability to adapt the Arc HPLC System for GPC analyses and demonstrate the compatibility with strong solvents, including THF. The reproducibility, reliability, and durability of the system were evaluated for polystyrene GPC analysis over five days. The resulting data demonstrated consistent retention times, peak areas, and calculated molecular weight values for the polystyrene sample, highlighting the reproducibility of the Arc HPLC System equipped with the strong solvent compatibility kit, and producing reliable GPC analyses results.

Benefits

- Reliable and reproducible Polystyrene GPC analysis over an extended period using the Arc HPLC System with strong solvent compatibility kit
- Integration of Arc HPLC System with Empower™ CDS GPC option for MW analysis of polystyrene sample

Introduction

In Gel Permeation Chromatography (GPC) reliable retention times are critical to determine the expected molecular weight of the sample. Given that the sample peak elution is compared to a calibration curve, reproducible retention times are required for the analyses, including both standards and samples, as changes in retention can affect sample characterization including M_n and M_w values. In addition, GPC requires mobile phases that solubilize polymers, and are typically strong organic solvents, such as tetrahydrofuran (THF). Many traditional HPLC systems contain materials that are incompatible with these strong organic solvents, from swelling or otherwise degrading over time. For GPC analysis, the instrument must contain parts durable under strong solvents conditions. To address this concern, the strong solvent compatibility kit for the Arc HPLC System replaces all components that can be adversely impacted by strong solvents, enabling extended use of the system for GPC analysis with strong organic solvents. In this study, system reproducibility using this strong solvent compatibility kit was examined for the GPC analysis of polystyrene over an extended analysis time. The resulting data demonstrated consistent retention times and calculated molecular weight values for the polystyrene sample. This highlights the flow rate precision of the Arc HPLC System equipped with the strong solvent kit, producing reliable GPC analyses results.

Experimental

Sample Description

Standard Solutions

Polystyrenes Standards, ReadyCal, (p/n: WAT058930 < https://www.waters.com/nextgen/global/shop/standards--reagents/wat058930-polystyrene-readycal-standards-4-ml-kit.html>) was obtained from Waters™ Corporation and contains three sets of narrow standards:

- M_p Range 1 (3,470 to 2,520,000)
- M_p Range 2 (1,306 to 1,210,000)
- M_p Range 3 (370 to 552,000)

ReadyCal standards were prepared by addition of 2 mL THF to each vial. The final concentrations were 1 mg/mL

for M_p = 2,520,000 and 1,210,000 and 2 mg/mL for the remaining standards. The vials were capped and left for three hours in the fume hood for standards to dissolve without any vigorous shaking.

Sample Solution

4 mg of the polystyrene sample was weighed and dissolved in 2 mL of THF in a similar way to the standards.

Method Conditions

System:	Arc HPLC with CHC column compartment and strong solvent compatibility kit (p/n: 205002572)
Mobile phase:	Tetrahydrofuran (stabilized)
Separation:	Isocratic
Columns:	All columns are 5 µm, 7.8 x 300 mm Styragel HR 5E, mixed bed, 2K - 4M, THF, (p/n: WAT044228) Styragel HR 4, 10,000 Å, 5 µm, 5K - 600K, THF (p/n: WAT044226) Styragel HR 3, 1000 Å, 500 - 30K, THF (p/n: WAT044223) Styragel HR 4E, mixed bed, 5 µm, 50 - 100K, THF (p/n: WAT044240)
Column temperature:	40 °C
Detection:	Refractive Index (RI) ▪ Sampling rate: 10 pts/sec ▪ Polarity: positive ▪ Flow cell temperature: 40 °C

Injection volume:	50 µL
Flow rate:	1.0 mL/min
Vials:	LCMS Certified Clear Glass 12 x 32 mm Screw Neck Total Recovery Vial, p/n: 600000671CV
Sample temp.:	25 °C
Wash solvents:	Purge Solvent: isopropyl alcohol Needle wash: tetrahydrofuran

Data Management

Chromatography software:	Empower 3.8.0 GPC option used for data processing and reporting

Results and Discussion

To assess the reproducibility of the Arc HPLC System with the strong solvent compatibility kit, GPC analysis of polystyrene was performed with THF mobile phase over a period of five days. Both polystyrene narrow standards and broad sample were analyzed using a bank of four GPC columns in a controlled temperature environment. The bank of columns, including two mixed bed columns, was selected to characterize a MW range from 4 M to 50 Da. The method conditions were optimized to resolve all peaks of the polystyrene narrow standards, as well as characterize the unknown polystyrene sample (Figure 1).

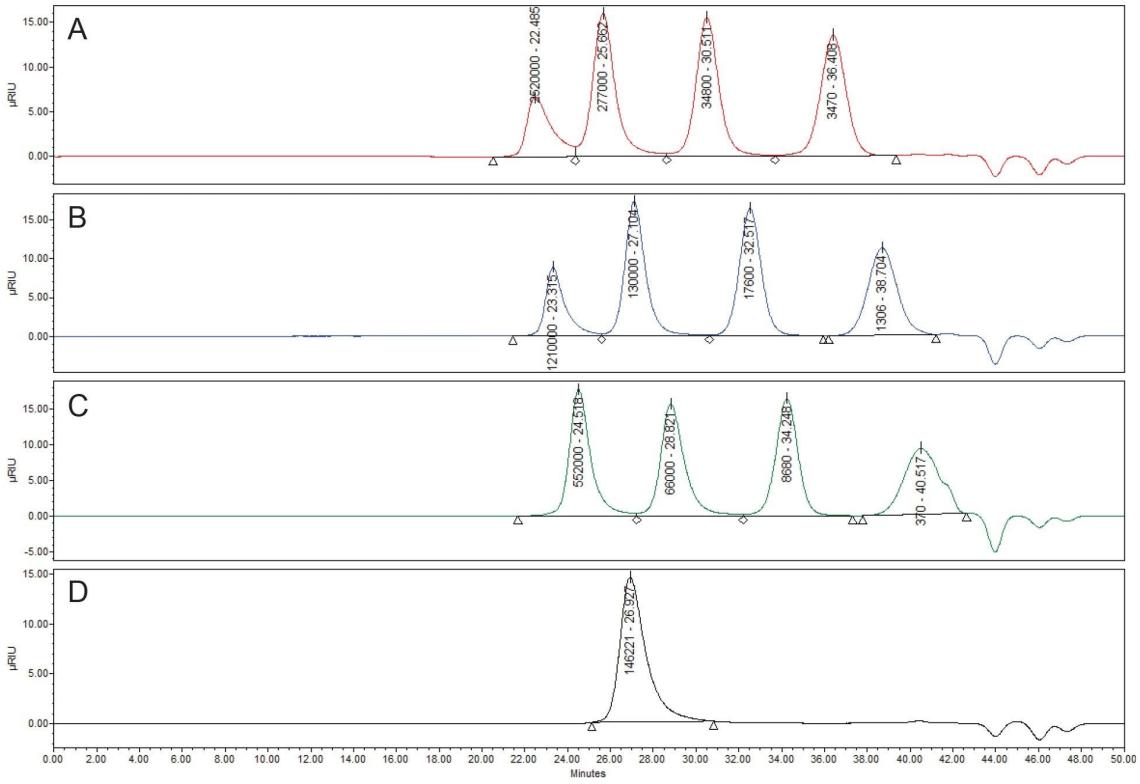

Figure 1. GPC analysis of narrow standards (A) M_p Range 1 (3,470 to 2,520,000) standards, (B) M_p Range 2 (1,306 to 1,210,000) standards, (C) M_p Range 3 (370 to 552,000) standards, and (D) the broad sample obtained on the Arc HPLC System equipped with strong solvent kit.

To analyze the separation, Empower CDS Software GPC option was used for data analysis and reporting.[1] As described earlier, a set of narrow polystyrene standards with the molecular weights (M_p) from 370 to 2,520,000 were analyzed each day to create the calibration curve. Correlation between the log molecular weight (MolWt) and retention time was achieved by applying a third order fit, producing a correlation coefficient of 0.9997, as shown for Day 5 (Figure 2).

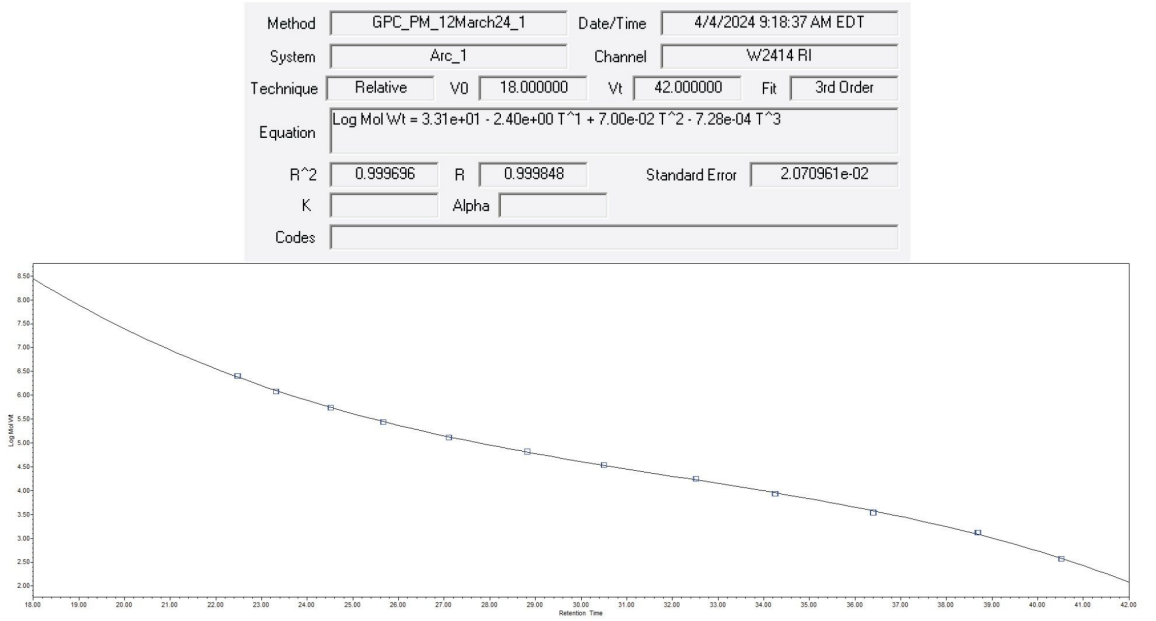

Figure 2. The calibration curve and the calibration data obtained from injection of narrow polystyrene standards on the Arc HPLC System equipped with strong solvent compatibility kit.

Reproducibility of Standards and Calibration Curve

As described earlier, the GPC analysis of polystyrene was continuously run for a period of five days, with five replicate injections of standards and samples analyzed daily. To show the consistency of the calibration, two standard peaks closest to the retention time of the broad sample (M_p = 277,000 and 130,000 respectively), were evaluated (Table 1). The intra-day retention time precision for each day ranged from 0.01–0.32% for the standard at 277,000 and was 0.01% for the standard at 133,000 Da for each day. Furthermore, the flow rate precision was assessed over an extended time and inter-day variability was evaluated. The mean retention times of these standards were within 0.01 minute, demonstrating reproducible performance over the course of the study.

Day	Retention time (min)		Area		Retention time (min)		Area	
	Standard peak at 277000Da				Standard peak at 133000 Da			
	Mean	RSD%	Mean	RSD%	Mean	RSD%	Mean	RSD%
1	25.66	0.02	1023044	1.6	27.10	0.01	1065613	1.0
2	25.66	0.03	1005116	1.7	27.10	0.01	1054886	1.4
3	25.66	0.03	1016529	1.2	27.09	0.01	1065940	1.4
4	25.66	0.02	1024523	0.5	27.09	0.01	1073151	1.1
5	25.66	0.01	1009689	1.7	27.10	0.01	1071585	1.0

Table 1. Analysis of polystyrene standards (2,770,000 and 133,000 Da) for five replicate injections on the Arc HPLC System over five days.

As described earlier, the standards were analyzed daily over the period of five days, with calibration curves created each day. During this period, the calibration curves for the analysis showed minimal variation and produced identical R^2 values, demonstrating the consistency of calibration (Table 2).

Day	Equation	R^2	Standard error
1	$\text{Log MolWt} = 33.2 - 2.40T + 0.0702T^2 - 0.000730\,T^3$	0.9997	2.057×10^{-2}
2	$\text{Log MolWt} = 33.2 - 2.40T + 0.0701T^2 - 0.000729\,T^3$	0.9997	2.072×10^{-2}
3	$\text{Log MolWt} = 33.2 - 2.41T + 0.0703T^2 - 0.000729\,T^3$	0.9997	2.068×10^{-2}
4	$\text{Log MolWt} = 33.2 - 2.41T + 0.0703T^2 - 0.000731\,T^3$	0.9997	2.071×10^{-2}
5	$\text{Log MolWt} = 33.1 - 2.40T + 0.0700T^2 - 0.000728\,T^3$	0.9997	2.070×10^{-2}

Table 2. Calibration curve data for polystyrene analysis on the Arc HPLC System. Standards were analyzed daily, with calibration curve information shown for each day.

Reproducible GPC Analysis of Polystyrene Sample

In addition to calibration curves, it is also critical that a system provide reproducible GPC characterization, including polydispersity. Analysis of a known polystyrene sample was performed daily, with calculations using the respective standards from each day. Using the GPC Option in Empower CDS, the sample was characterized

with reporting of M_n, M_w, M_p, M_z, M_{Z+1}, and polydispersity.

The results show comparable results obtained over the time period (Table 3). High retention time repeatability was observed with RSDs of less than 0.02% for each day. In addition, acceptable correlation was observed for the sample's polydispersity, M_n, M_w, and M_p values. All results were within 10% of the reported values for the broad sample (M_p=146,600, M_w=141,700, M_n=133,300). In addition, the polydispersity values were within 5% of the expected value. The day-to-day reproducibility is demonstrated by the overlay of the distribution plot obtained for the broad sample on each of the five days, as displayed in Empower (Figure 3).

Day	Retention time (min)		Area		Polydispersity	Mn		Mw		Mp		Mz	Mz+1
	Mean	RSD%	Mean	RSD%	NA	Mean	Deviation% from expected	Mean	Deviation% from expected	Mean	Deviation% from expected	Mean	Mean
1	26.92	0.01	1213682	2.7	1.10	122872	8.14	136081	4.04	146458	0.09	147386	157487
2	26.92	0.01	1229027	4.7	1.10	123467	7.65	136509	3.73	146164	0.29	147856	158424
3	26.92	0.01	1220568	1.8	1.10	123690	7.47	136447	3.77	146367	0.16	147500	157429
4	26.92	0.02	1221335	4.7	1.11	122565	8.39	136351	3.84	146445	0.10	148209	159295
5	26.92	0.02	1189850	2.7	1.09	125664	5.89	137333	3.13	146425	0.12	147872	157613
Expected value	NA		NA		1.06	133300		141700		146600		NA	NA

Table 3. Analysis of polystyrene broad sample on Arc HPLC System over a five day period. Sample M_W values were calculated in Empower CDS, using the calibration curve collected each day. The mean values for each day have been reported.

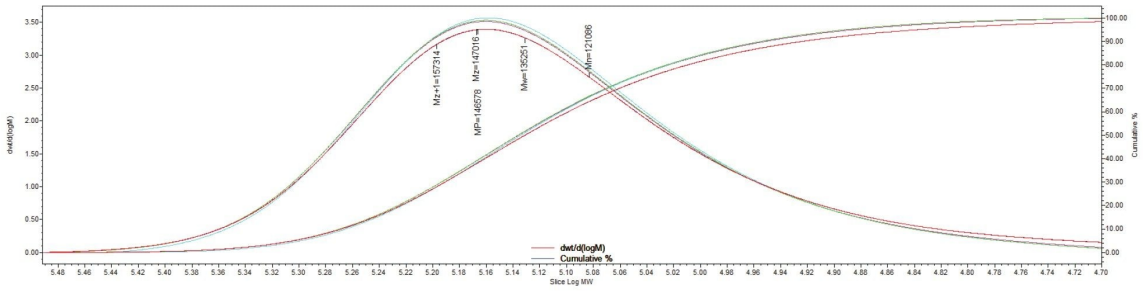

Figure 3. Overlay of the molecular weight distribution plots of a single injection from Day 1–5. The numerical values on the plot are the average of all five injections, one from each day.

Conclusion

GPC analysis of a polystyrene sample in THF was performed over five days on the Arc HPLC with strong solvent compatibility kit and Empower CDS with GPC option. Reproducible retention times were observed on the Arc HPLC System, demonstrating good flow rate precision. The system also produced consistent day-to-day results for both calibration curves and sample analysis, with molecular weight (M_n and M_w) values within 10% of expected value. These results demonstrate the Arc HPLC System equipped with the strong solvent compatibility kit, combined with Empower CDS, and GPC Option, is a reliable system for routine GPC analysis.

References

1. Empower GPC Software Getting Started Guide, Waters Corporation User Guide, 71500031303 < https://www.waters.com/waters/support.htm?lid=1852970>

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Arc HPLC System </nextgen/br/pt/products/chromatography/chromatography-systems/arc-hplc-system.html>

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