

Note d'application

Performance Assessment of the Waters Breeze QS HPLC System GPC Applications

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief assess the performance of Waters Breeze QS HPLC System for Gel Permeation Chromatography (GPC).

Benefits

- Waters Breeze QS HPLC System, running Empower QuickStart Software, delivers precise flow rate to obtain accurate, reproducible peaks in GPC tests.
- Waters most affordable GPC analysis using a Breeze QS HPLC System.
- Empower QuickStart (QS) Software: a simplified and intuitive workflow.
- Linear GPC separation to 7 million Daltons with a Waters Ultrahydrogel Linear Column.

Introduction

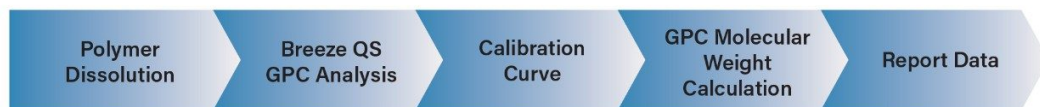


Gel permeation chromatography (GPC) requires a high performance liquid chromatography (HPLC) system that delivers precise flow and reproducible results. In GPC, the retention time is plotted against the log of the molecular weight. Thus, flow rate variations can affect peak reproducibility. The flow rate for this experiment is listed in the method parameters (Table 1). The molecular weight of experimental samples is calculated by comparing of unknown sample data to a calibration curve using narrow molecular weight polyethylene oxide (PEO) and polyethylene glycol (PEG) calibration standards (Figure 1).

The excellent flow rate precision of Breeze isocratic pumps is demonstrated in an overlay of six injections of a PEO/PEG mixed molecular weight standard, as shown in Figure 2. Relative standard deviations of the peak molecular weight (M_p) at <0.5% are obtained on a Waters Breeze QS HPLC System using two linear GPC columns and a 30-minute run time (Table 2).

Experimental

Breeze QS GPC Workflow



Method Parameters:	Column 1
Flow type:	Isocratic
Flow (mL/min.):	1
Mobile phase:	DI water (0.1 M Sodium nitrate)
Sample conc. (mg/mL):	1.5
Injection vol. (mL):	50
Column temp. (°C):	30
Column:	Ultrahydrogel linear (two columns are used)
Particle chemistry:	Hydroxylated polymethacrylate-based gel
Column diameter (mm):	7.8
Column length (mm):	300
Particle size (mm):	NA
Pore Size (Å):	Pore size blend, exclusion limit of 7M Daltons
Detector:	RI (30C)
Sample Information	
	Polyethylene glycol/Polyethylene oxide (PEG/PEO)
Molar mass (g/mol):β	Mp = 238–969 K Daltons

Table 1. Method parameters.

Results and Discussion

Empower QS Software provides easy-to-use fundamental GPC calculations. In this experiment, the narrow

PEO/PEG standards are used as calibration standards, as well as broad unknowns. The Empower QS Software's partial list of reportable peak result fields includes Mn, Mw, Mp, Mv, Mz, Mz+1, and polydispersity (Figure 3). Relative and modified universal calibration types employing point-to-point and first- through fifth-order fits are available, and an example of relative calibration is shown in Figure 1.

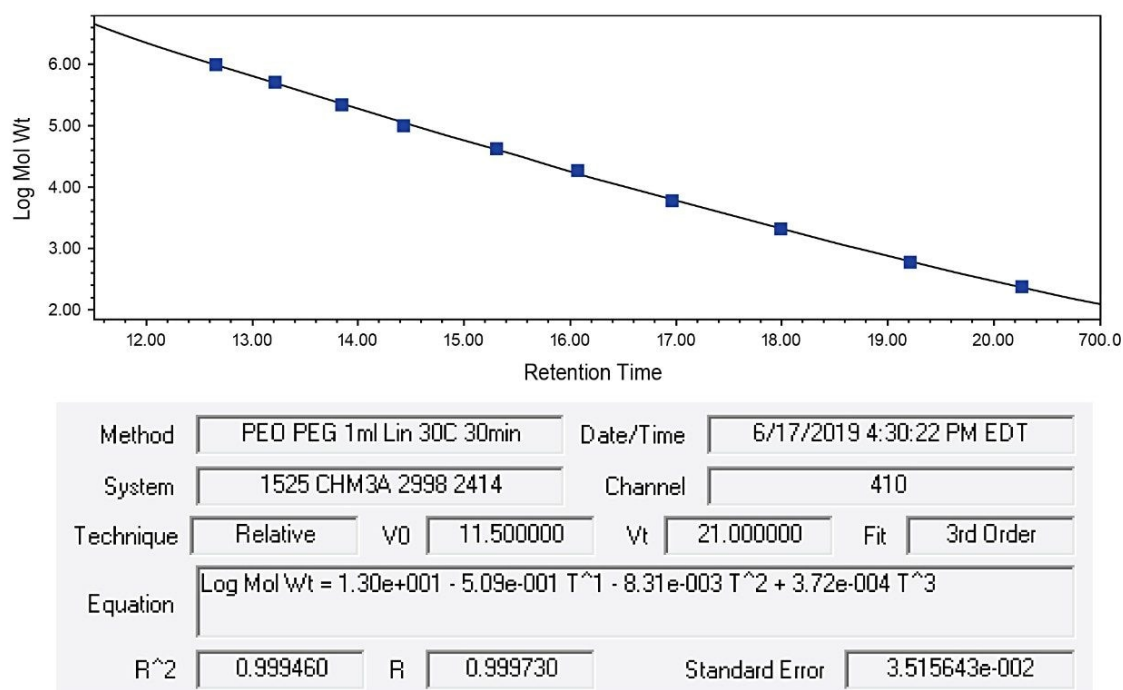
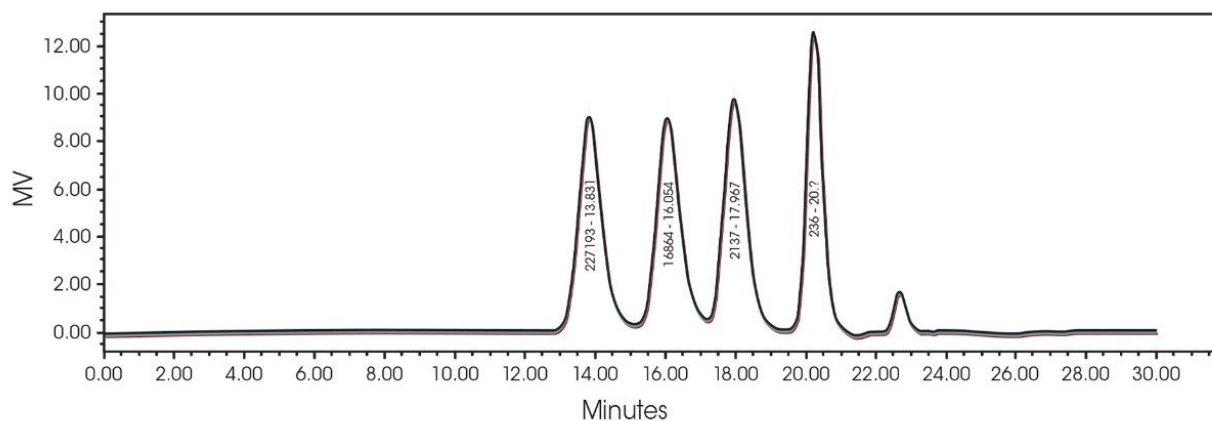


Figure 1. PEO/PEG calibration.



— SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 12:51:47 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410
 — SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 1:22:17 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410
 — SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 1:52:47 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410
 — SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 2:23:17 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410
 — SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 2:53:46 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410
 — SampleName: PEO/PEG Yellow cap Std 1.5 mg/ml Date Acquired: 6/15/2019 3:24:17 AM EDT Instrument Method Name: GPC PEO PEG 1ml UHGel In 30 C Channel: 410

Figure 2. Six injections of the PEO/PEG calibration standard.

MP Summarized by Name Channel: 110

	Sample Name	Inj	Y4 (Daltons)	Y4 (Daltons)	Y4 (Daltons)	Y4 (Daltons)	Y4 (Daltons)	Y4 (Daltons)
1	PEO/PEG Yellowcap Std 1.5 mg/ml	1	410	1:A4	227193	16854	2137	236
2	PEO/PEG Yellowcap Std 1.5 mg/ml	2	410	1:A4	225670	16686	2137	236
3	PEO/PEG Yellowcap Std 1.5 mg/ml	3	410	1:A4	227132	16738	2137	236
4	PEO/PEG Yellowcap Std 1.5 mg/ml	4	410	1:A4	224762	16690	2122	235
5	PEO/PEG Yellowcap Std 1.5 mg/ml	5	410	1:A4	226278	16728	2128	235
6	PEO/PEG Yellowcap Std 1.5 mg/ml	6	410	1:A4	226430	16791	2134	236
	Mean				226244	16748	2131	236
	Std. Dev				922	64	6	1
	%RSD				0.4076	0.3840	0.2650	0.2191

Table 2. Empower QuickStart data report from six injections of the yellow cap PEO/PEG calibration standard (Mp = peak molecular weight).

	Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw	Area ($\mu\text{V}\cdot\text{sec}$)	% Area	Height (μV)	USP Plate Count
1	BL1												
2	Y1	188759	226539	227193	263499	300964	1.200150	1.163151	1.328530	435837	25.49	9061	1980
3	Y2	14283	16662	16854	19126	21716	1.166542	1.147913	1.303366	435649	25.47	8947	2641
4	Y3	1909	2129	2137	2348	2568	1.115066	1.102648	1.206063	444052	25.97	9752	3660
5	Y4	226	234	236	243	252	1.038924	1.037944	1.076874	394591	23.07	12604	9818
Peaks / Distribution / MS Library Match													

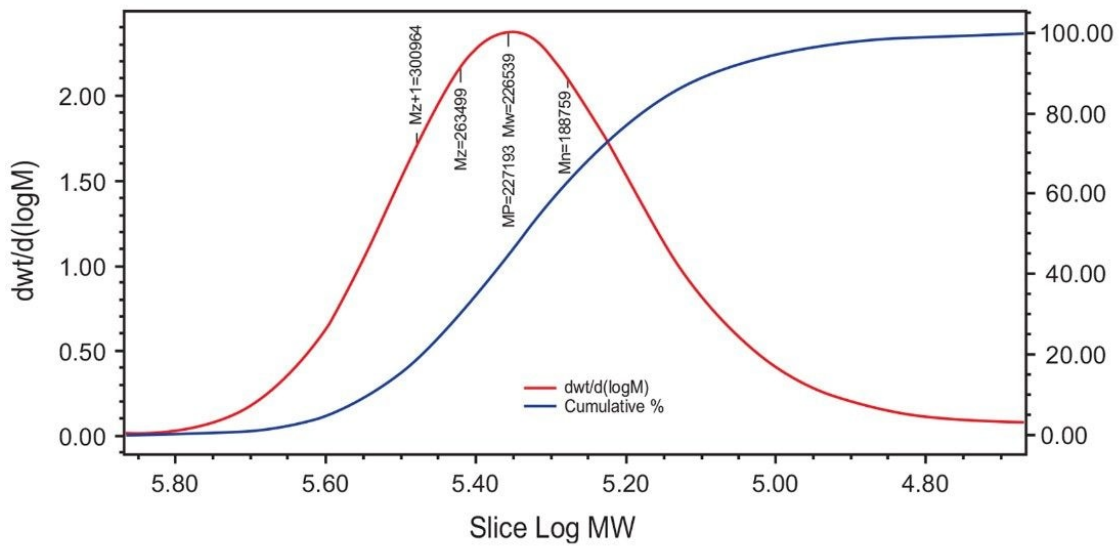


Figure 3. Calculated peak fields and distribution curve of PEO/PEG calibration standard.

Empower QS has many built-in features that enable sophisticated reporting of data usually done in spreadsheets. Two popular reporting areas are column plate counts and control charting. By reporting the column plate counts, the column efficiency can be charted and observed for any changes in performance (Table 3). Control charts can capture upper and lower limits of molecular weight ranges used in a polymer pre-manufacture notice (PMN) or for charting impurity levels in raw materials (Figure 4).

Name	Mn (Daltons)	Mw (Daltons)	MP (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity	Mz/Mw	Mz+1/Mw	Area ($\mu\text{V}\cdot\text{sec}$)	% Area	Height (μV)	USP Plate Count
1 BL1												
2 Y1	188759	226539	227193	263499	300964	1.200150	1.163151	1.328530	435837	25.49	9061	1980
3 Y2	14283	16662	16854	19126	21716	1.166542	1.147913	1.303366	435649	25.47	8947	2641
4 Y3	1909	2129	2137	2348	2568	1.115066	1.102648	1.206063	444052	25.97	9752	3660
5 Y4	226	234	236	243	252	1.038924	1.037944	1.076874	394591	23.07	12604	9818

Table 3. System suitability features in Empower QS include plate count calculations.

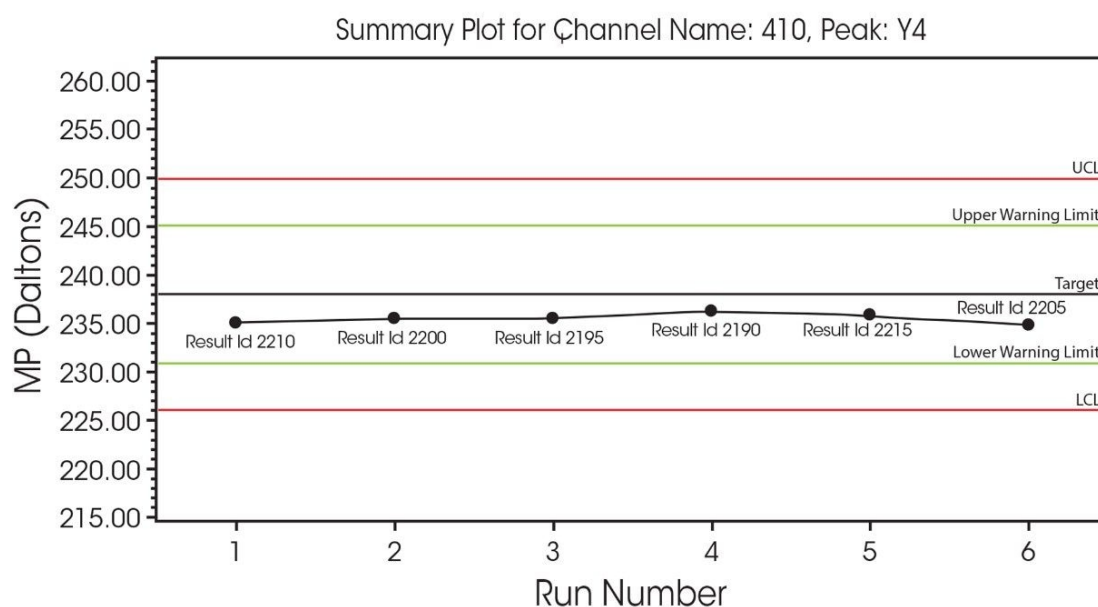


Figure 4. Example of an Empower QS control chart of calibration standard yellow cap peak 4.

Conclusion

- Breeze isocratic pumps provide the excellent flow precision necessary for high-quality gel permeation chromatography.

- All basic GPC calculations can be calculated using the easy-to-use interface included with Empower QS Software.
 - Short analysis time of 30 minutes was demonstrated using two linear columns.
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References

1. Waters Corporation. Gel Permeation Chromatography (GPC) Capabilities. <https://www.waters.com/waters/library.htm?cid=511436&lid=134697666>, August 2014.
 2. Waters Corporation. Empower GPC Software Getting Started Guide. <https://www.waters.com/waters/support.htm?lid=1852970>, May 2004.
 3. Waters Corporation. Empower Tips. <https://blog.waters.com/category/empower-tips>
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Featured Products

[Breeze QS HPLC <https://www.waters.com/135032418>](https://www.waters.com/135032418)

[Empower 3 Chromatography Data Software <https://www.waters.com/10190669>](https://www.waters.com/10190669)

720006668, October 2019

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