GPC Molecular Weight Calibration Standard Operating Procedure

1) Locate the PSS polystyrene (PS) or poly(methyl methacrylate) (PMMA) standards in the "Polymers/Oligomers" section in the inventory (black shelves near fridge).

PS Mw (Da)	mg quantity to weigh	Sample #
162 (liquid)	8 (10 µL)	A
9,550	8	
176,000	3.5	
953	8	B
21,200	6.5	
335,000	3.5	
2,090	8	C
42,600	6.5	
524,000	1	
4,840	8	D
90,900	5	
827,000	1	

Table 1: Amounts of polystyrene standards.

PMMA Mw (Da)	mg quantity to weigh	Sample #
643	8	A
21,200	6.5	
517,000	1	
2,130	8	B
40,300	6.5	
936,000	1	
5,930	8	C
86,700	5	
1,410,000	0.35	
12,800	6.5	D
214,000	3.5	
2,400,000	0.35	

Table 2: Amounts of high MW poly(methyl methacrylate) standards.

PMMA Mw (Da)	mg quantity to weigh	Sample #
200	8	A
2,130	8	
40,300	6.5	
300	8	B
5,930	8	
86,700	5	
400	8	C
12,800	6.5	
214,000	3.5	
643	8	D
21,200	6.5	
517,000	1	

Table 3: Amounts of low MW poly(methyl methacrylate) standards.

2) Weigh out the amounts described in the tables above into 4 separate 5 mL volumetric flasks and label each flask with a Sharpie marker (A, B, C, and D).

3) Dilute each volumetric flask with an appropriate amount of HPLC grade CHCl₃ (to the line, ~5 mL) using a Pasteur pipette.

4) Cap each of the volumetric flasks with the corresponding penny head glass stoppers and shake vigorously/vortex until the solid contents are fully dissolved. Larger molecular weight standards will take longer to dissolve, make sure they are fully dissolved before moving to next step. Do not sonicate as this may sheer larger molecular weight samples, leading to broadening of the observed peak.

5) Into 4 separate 2 mL sample vials, fill the vial to slightly above the 1.5 mL mark using separate Pasteur pipettes, making sure not to cross-contaminate samples.

6) Label each of the vials (A, B, C, D) with a Sharpie marker, cap, and place into the sample tray of the GPC. Discard all soiled glassware.

Sample Queue							
	Cup	Sample Name	Acquisition Method Name	Injection Volume [uL]	Repeat	Type	Individual Condition Name
1	2	PS calibration A – 9/28/2022	PS calibration 09-28-2022	20	1	Standard	Chloroform conditions
2	3	PS calibration B – 9/28/2022	PS calibration 09-28-2022	20	1	Standard	Chloroform conditions
3	4	PS calibration C – 9/28/2022	PS calibration 09-28-2022	20	1	Standard	Chloroform conditions
4	5	PS calibration D – 9/28/2022	PS calibration 09-28-2022	20	1	Standard	Chloroform conditions

Figure 1: Example of how Sample Queue should be populated for molecular weight calibration.

Running the calibration samples:

7) Open EcoSEC Elite-WS Acquisition.

8) Click on the "Sample Queue" icon at the bottom of the WS (⬛).

9) Input the calibration samples into the Sample Queue. There should be 4 samples corresponding to each of the mixtures of different molecular weight standards (**Figure 1**).

10) As shown in **Figure 1**, Sample Name should read "PS calibration X – MM/DD/YYYY" for polystyrene samples, "HMW PMMA calibration X – MM/DD/YYYY" for high molecular weight poly(methyl methacrylate), or "LMW PMMA calibration X – MM/DD/YYYY" for low molecular weight poly(methyl methacrylate), Injection Volume should be "20 μL", Repeat should be "1", Type should be "standard", and Individual Condition Name should be "Chloroform conditions" (30 minute run).

11) The Acquisition Method Name (yellow box, **Figure 1**) will need to be changed to create a new Acquisition Method for this MW calibration. Double click on one of the cells under Acquisition Method Name and a new window will appear (Edit Acquisition Method). Click "New..." and a new window will appear (Acquisition Method Settings).

12) In the Acquisition Method Settings fill the Molecular Weight column in the Calibration Curve Data with the following molecular weights going from high molecular weight (top) to low molecular weight (bottom) for both "RI" and "UV/EXT" (only for PS, PMMA does not have a UV signal) which can be changed using the drop down box under "Identification/Calibration Curve Conditions":

- *Polystyrene* - 827000, 524000, 335000, 176000, 90900, 42600, 21200, 9550, 4880, 2090, 953, 162 (12 in total, **Table 1**).
- *High molecular weight poly(methyl methacrylate)* - 2,400,000, 1,410,000, 936,000, 517,000, 214,000, 86,700, 40,300, 21,200, 12,800, 5,930, 2,130, 643 (12 in total, **Table 2**).
- *Low molecular weight poly(methyl methacrylate)* - 517,000, 214,000, 86,700, 40,300, 21,200, 12,800, 5,930, 2,130, 643, 400, 300, 200 (12 in total, **Table 3**).

Click OK and type "PS calibration MM-DD-YYYY" for poly(styrene) samples and "HMW/LMW PMMA calibration MM-DD-YYYY" for poly(methyl methacrylate) in the new popup window, click OK, then Setting. Change each of the 3 addition samples to this new Acquisition Method.

13) Click Setting on the Edit Sample Queue list, then click Yes to save over the sample queue being edited.

14) Click the Play button (⏻) to start analysis and allow the samples to run (120 minutes, 30 minutes per run).

Creating a calibration curve from calibration samples:

15) After the calibration samples have run, open EcoSEC Elite-WS Analysis.

16) Locate the batch where the calibration samples ran (may need to refresh page to see new bath [↻]), select "Manual Peak Edit" (✎), delete all autopicked peaks (✖), and draw a line (✎) for all of the polystyrene standard peaks (baseline to baseline). Following the completion of this, save (💾) the batch (if two or more batches were edited, select "Save all batches").

17) Click on analysis method (📄), under General – RI – Calculation Type, change "Column Test" to "Molecular Weight" via a drop-down box. Under UV/EXT – Calculation Type, change "Column Test" to "Molecular Weight" via a drop-down box.

18) Click on "Automatic Creation of Calibration Curve…" at the bottom of the Analysis Method Settings window. A new window (Automatic Creation of Calibration Curve) will appear. Click on "Register Data…" and a new window (Select Chromatogram Data) will appear. Select the samples corresponding to the standard you wish to calibrate. Click "OK" and you will see those samples populate the Automatic Creation of Calibration Curve window. Close the Select Chromatogram Data window. Click "Create from the registered data" and close the window. You will note that the data will have populated the Calibration Curve Data spreadsheet. Repeat this process for the UV/EXT. *Note that the elution times for the UV should be slightly smaller than the RI (by approximately ~0.9 sec) – this is because the sample is passed through the UV detector before the RI detector. Calibration of the UV only applies to polystyrene as PMMA does not have a UV signal.*

19) For both RI and UV/EXT, change the Approximation to "Cubic: At3+Bt2+Ct+D" by a dropdown menu. This is not necessary if you have a Correlation Coefficient < -0.980. Normally we have Correlation Coefficients of -1.000.

20) Click "OK" and a new window "Select Process" will appear. Click "Save as the other analysis method (No calculation)" and use the naming convention "PS calibration - MM/DD/YYYY" for polystyrene, "HMW/LMW PMMA calibration – MM/DD/YYY" for high/low MW PMMA, respectively.

21) A new window will appear "Reflection to the Acquisition Method". Click on the Acquisition Method Name that reflects the standard you are calibrating (polystyrene, HMW PMMA, or LMW PMMA). Click "OK", if a window saying "Is the selected acquisition method updated?" appears, click "Yes".