Gel Permeation Chromatography (GPC)
Waters Alliance 2695

A. Things to Remember
   1. Be sure there is enough THF mobile phase
   2. Be sure there is enough room in waste
   3. If instrument has been shut down, be sure to run 0000 GPC initialization method before injecting your samples.
   5. Computer password is 1234.
   6. Empower user name is: system.
   7. Empower password is: manager.

B. Instrument Start Up
   1. Turn on Alliance 2695.
   2. It takes a few minute to run through its initial calibrations and checks. Wait for main window to read Idle.
3. Turn on **RI detector**.

4. Sample carousel- Open sample compartment door, and see screen below. Press A, B, C, D, or E for that carousel to move to opening. Place vials in sample compartment. Close door.
5. Samples should be prepared **the day before** running. Dissolve **5 to 20mg in 2 mls THF** (The higher the PDI, the more sample you need. The lower the PDI, the less sample you need). Let sit at room temperature overnight. Do not sonicate or heat samples to go into solution.

6. Filter samples though **0.45 micron filter** into sample vial.

7. Place vials in **sample compartment** and shut door.

**C. Empower- Run Samples**

1. If computer is on and someone is running samples, skip to 9.
2. Turn on **Computer**. Password is **1234**.
3. Double Click on **Empower icon**
4. User name is **system**.
5. Password is **manager**.
6. Click on **OK**.

![Empower Login](image)
7. Empower interface appears. Choose **Run Samples** to run unknowns.

8. A new box opens up. Choose **GPC Measurements, RI GPC**, and then **OK**. The system may stay stuck on “Connecting to Empower Node Newinstall” for a few minutes.
9. Under **File**, choose **New Sample Set Method** and **Based on Existing Sample Set Method**.

10. Choose the first sample set **0000 GPC Initialization**.
11. This sample set should appear.

12. Under **File**, Choose **Save Sample Set Method as**.. and give name.
13. Enter Name and Choose **Save**.

14. Under **Inject**, choose **Run Sample Set**, then **Run**.
15. It will take 1 hour to run the initialization methods before your first sample is injected.

16. Under File, Choose **New Sample Set** and **Empty**.

17. Enter your samples here:
   a. vial #
   b. injection volume (recommend 100 to 200ul)
   c. Number of injections -1 typically
   d. Sample name
   e. Under **Function**, Choose **Inject Broad Samples**
   f. Under **Method Set**, search for **Earl Wendy GPC Shutdown 2**. This will shut flow off at end of run
   g. **Run time is 50.0min**.
   h. If more than one sample, click on next line to continue adding more samples.
18. Under **File**, **Save Sample method set as** and give name (i.e. 04142016 Wendy)

19. Under **Inject**, choose **Run Sample Set** and **Run**.

**D. Analysis**

1. When run is complete, go back to Empower Main window and Choose **Browse Project**.

2. Select **GPC Measurement** and then **OK**.
3. Under **Sample Set** tab, find your Sample Set.

4. Highlight injections of interest, right-click and chose **View as Channels**.

5. Highlight RI (410) of injection, right-click and choose **Review**.
6. Click on **Review Main Window icon** if the chromatogram is not seen.

7. Under **File**, choose **Open**, and then **Processing Method**. Choose processing method
   a. **2020 January**

8. Click on **Processing Wizard Icon**
   a. A dialog box will appear. Choose **Edit Existing GPC Processing Method and Keep the calibration**. Click **Ok** button.
9. The Integration-Integration dialog box will appear. Click and drag to Zoom in on region to integrate. Click **Next** when ready.
10. Check Integration. If correct click **Next** to go to GPC Calibration dialog box. If not use **Back** button to redo integration. You can fix integration after processing if necessary. Be sure only one peak is integrated, if you have only one peak.

11. Choose:
   a. What type of calibration will you be doing? **Relative**
   b. What calibration fit type do you use? **3rd order**
   c. Do you calibrate against time or volume? **Time**
   d. Click **Next** to go to GPC-Column Set.
12. Leave $V_0$ and $V_t$ blank or enter time of 17 and 34. Click **Next**.

13. In GPC-Broad Unknowns and Standards dialog box, leave names as peak names. Click **Next** to go to Processing Method Name dialog box.
14. Click on **Finish**

15. Click on **Copy Curves**.
16. View the integrated and quantitated broad unknown in review window.

17. If you need to fix the integration, click on the Integrate icon , and change the integration.
18. Click on the **quantitate icon** to quantitate. **You must hit this if you alter your integration!**

19. To save your result, go to **File, Save, Calibration**.
20. Hit Save

21. Hit Save.

22. Then go back to File, Save, Result.
23. Go under **File** and choose **Exit**.

24. Hit the **Update** button to update your results.

25. Choose **Results** tab.

26. Highlight Results, right-click and choose **Preview/Publisher**.
27. Choose **Use the following Report Method** and choose **Wendy GPC Report**. Hit **OK**.

28. Hit **Yes** to next question
29. Choose **Save Report** icon and save as pdf on your flash drive. Flash drive goes in back of computer tower.

30. Click on the **Next Report** icon and click **Save** again for next sample.
E. Shut down
   1. The method has a built in shut down of the mobile phase, however the detectors need to be manually shut down. Detectors have a limited lifetime. If no one is signed up to use GPC, please manually turn off all components.

F. Overlay of chromatograms
   - An overlay of your samples helps to tell the story.
   1. Hold the Ctrl button and click on each 410 of each sample you would like to overlay.
   2. Right click and Choose Review.
   3. Click on Autoscale icon.
4. Click on **Scale to Chromatogram** icon.

5. Right click on chromatogram and choose **Properties**.
6. Click on Tab labeled **Legend**.

7. Under **Sample Set**, click on **Sample**.
8. Click on **Sample name** under Sample.

9. Delete **1 Sample Name** under **Field Name**.
10. Hit **Apply**.

11. Right click and choose **Copy**.
12. Open **WordPad** and Paste into **WordPad**.

13. Under **File**, Choose **Save as**.