**HPLC Operation SOP**

*Updated 10/31/23 by Aaron Socha*

**Shimadzu HPLC Instrument with DAD and optional RID detection**

*How to run a sample or a sample sequence:*

1. Sample Preparation: Most materials for sample prep are located on the bench in Rogers 311.
2. Prepare sample at approximately 50 mg/mL using solvent(s) near to your initial conditions. For example, on reversed phase HPLC, initial conditions are approximately 20% MeCN or MeOH in 80% water or buffer. Try to dissolve your sample in the same solvent system. If necessary, add additional organic solvent (MeCN or MeOH) until soluble. Try not to exceed 100 mg/mL concentration.
3. Filter sample using a 2-micron Luer Lock syringe filter into an HPLC vial. Use compatible filter (PVDF for organic solvents, Nylon for aqueous solvents). You need to fill the HPLC vial at least 1/3 full (approximately 600 uL of sample volume).
4. Open the lowest door on the left side of the instrument, remove **sample rack** and place sample in empty space, noting location. When re-inserting sample rack, ensure that it “clicks” into place
5. Method Selection: Exact method will be determined by both sample (analyte) and column. However, some general operations can be applied.
6. Power on all components of HPLC (marked with red tape). White tape is RID (optional, use for non-UV active analytes, e.g sugars, etc.)
7. Open PC (Tucker / Tucker) and LC Solution Software (top left most icon Queens HPLC, Admin, no password).
8. A beep should sound when PC is interfaced with HPLC. A grinding sound also happens. This is a low-pressure pump that will eventually need to be replaced but is not an issue currently. The same grinding noise will sound during each injection.
9. Select the **Data Acquisition** tab on the bottom of the page
10. File 🡪 Open an old method from the C/LabSolutions/Data/ORGO\_LAB folder. A good starting method is: C18\_MeCN\_Formate\_101423
11. Make changes to the method as needed by selecting Advanced Tab. Some suggested changes include:
    1. Flow rate (0.5-1.5 mL/min range)
    2. Solvent gradient (A = organic, D = aqueous/buffer) in LC Time Program. Note the D conc is entered and the A conc is default. For example, if 40% D conc is entered, 60% A conc is assumed. The last row must always be Controller Off
    3. Column oven temperature (see column manufacturer notes for specific/recommended temps)
    4. ***PLEASE SAVE AS*** A NEW METHOD as to ***NOT*** overwrite the existing method.
12. To modify flow rate, or initial solvent conditions, use Normal Tab. This is valuable for purging pumps and flushing column (Reference valve open and closed, respectively)
13. Press Download button to send method to HPLC.
14. Wait until a steady pressure is reached before beginning the run.
15. Sequence Set-Up: The sequence is the program that tells the HPLC which sample to run (i.e. which position in the **sample rack** in Step A3 above), using the specified method. You may also program a SHUTDOWN Method into the sequence to avoid having to come to lab to shut off pump, lamp, and column oven. The SHUTDOWN Method can also be used to rinse the column with the appropriate storage solvent (See below). It is highly recommended that you first monitor/observe a successful SHUTDOWN method before leaving the instrument overnight.
16. From the same software used to modify the Method, Select the **Batch Table** tab on the bottom of the page.
17. Open SIELC sequence 092723 from C/LabSolutions/Data/ORGO\_LAB folder.
18. Scroll down to the last row and place the cursor on the far left column and left click so that the entire column is highlighted in BLACK
19. Right click 🡪 Add Row and enter the number of samples you plan to run.
20. Copy the entire row that you just highlighted in BLACK and paste the entire row into the first of the new rows.
21. Modify ONLY the following in the newly pasted row:
    1. VIAL # (this should match the vial position in the **sample rack**)
    2. Method File (this should match the method created for your sample in Section B above)
    3. Data File (this is the sample file name, be sure to name it well, and place in a folder where you will easily find it later!)
    4. Injection Volume (Inj. Vol.) between 1-10 uL. Start with 1-2 uL and work up if more signal is needed.
22. Data Processing: Once the sample is finished running, you will need to go back to the original desktop icon for LC Solutions and Open the Postrun section of the software. A new window will appear
23. File 🡪 Open to find and open your data file.
24. The (pink) top window is the UV profile and the bottom is the chromatogram
25. In the top window, you may scroll to select the appropriate wavelength for analysis using the blue arrow on the far right
26. Peaks will appear integrated in the bottom chromatogram, to view the integral values, select View 🡪 Peak Table.
27. You may copy and paste to MS Word, Powerpoint or Excel to process the data further. Use the designated thumbdrive to transfer your data to the common computer. Do NOT use external thumb drives for risk of virus.

General Information and Storage Conditions for Queens HPLC Columns:

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| Column | Use | Purchase Date | Operation notes | Long-Term Storage |
| Reversed Phase C18 Column: Phenomenex (150 x 4.6) | Most organic compounds | October, 2023 |  | 90% MeOH or MeCN 10% water (no buffer) |
| Primesep SB SIELC (150 x 4.6) | Charged aromatics (ionic liquids) | Jan 2023 |  | 75% MeCN: 25% water or formic/acetic acid buffer |
| Aminex HPX-87X  “H-column” | Organic acids |  |  |  |
| Aminez HPX-87P  “P-column” | Sugars |  |  |  |
| PL Gel Mixed-D  “Size Exclusion / Lignin column” | Polymer analysis |  |  |  |