HPLC Walkup Guide

1 Instrument Setup

1. Complete a sample request form at https://jmorim.github.io/forms/sample_request.
2. Install the necessary column for your method. Consult someone from Bioanalytics if you don't know what to use. See **Column Installation** below.
3. Change the solvents to those needed for the method if necessary. Channel A should always be for aqueous solvent; Channel B for isopropanol; Channel C for acetonitrile; Channel D for methanol. Do not use aqueous buffers for channels B, C, or D, or organic solvents in channel A to avoid precipitation and clogging the HPLC capillaries. Rinse the outside of Channel A's line in pure water if switching from a buffer.
4. Turn on power to all modules if they're not on. Each module should have a green lit power button.

2 ChemStation

1. 

Open Chemstation Online.
2. Navigate to **Method and Run Control** in the bottom left menu if not already open.
3. Select the method for your analyte by double clicking a method file on the left to load it to the instrument.
4. If the pumps have been idle for several hours or if the solvents are changed, see **Purging** the pump below.
5. Check that there's enough solvent to complete the analysis. Update the bottle fillings by right clicking the pump module and selecting **Bottle Fillings**. Enter the approximate volume left in each channel's bottle. Failure to do this step may result in pumping air into the LC or premature abortion of the sequence.

6. Click the 

button to start the pump, column thermostat, and detectors.

7. Load your personal sequence template (**Sequence → Load Sequence Template**) or create a new one with your name (**Sequence → New Sequence Template**).

8. Open **Sequence → Sequence Parameters**. Edit the subdirectory name to the format "[Researcher]_[Analyte]_YYYYMMDD". You will be prompted to create the directory if it's new. Don't overwrite other people's sequence templates.

9. Check that **Post Sequence command/macro** is checked and **STANDBY** or **SHUTDOWN** are selected.

10. In the **Sequence Output** tab, check **Print sequence summary report**. Select the **Use Intelligent Reporting** radio button. Select your report template or use **XLS** or **XLS+chromatograms**. Check **Report to XLS**.

11. Click **OK**.

12. Right click the autosampler module and select **Assign Wellplates**. Change each sampler container to the appropriate sample plate type (54 vial plates or Micronic 0.75 mL). Click **OK**.

13. Open **Sequence → Sequence Table**. Enter your sample locations, sample names, and the method into the sequence table. You can copy sample names and locations from Excel and filldown the method name.

14. Click 

to start the sequence.

3 Data Analysis

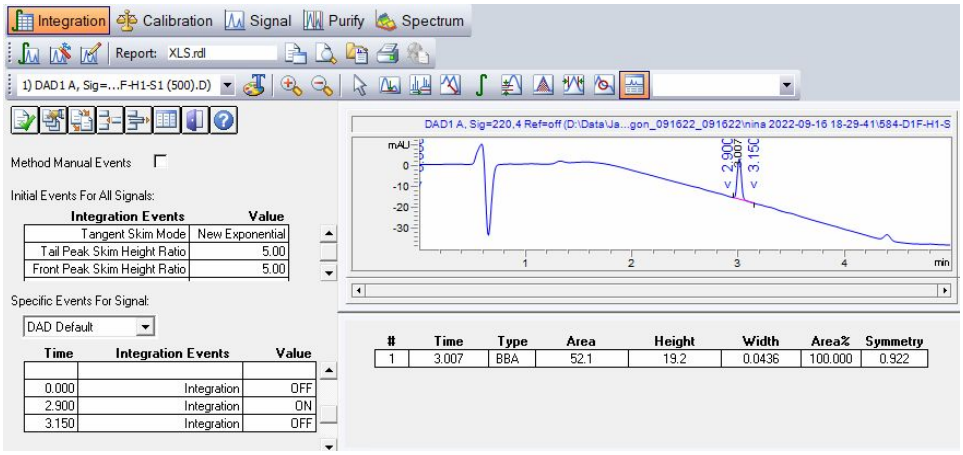
1. Open Chemstation Offline if not already open. 

2. Open **Data Analysis** from the bottom left menu.

3. Navigate to your sequence output folder and open your sequence.

4. Double click the data files and check that the integrations are correct. Otherwise, edit the integration parameters by selecting **Integration** and **Edit/Set Integration Events Table**.

5. Adjust the integration time windows and area/height thresholds to fit your data.



6. Click the green checkmark button to save the parameters. 

7. If the integration parameters had to be changed, click the green arrow to reprocess the sequence. 

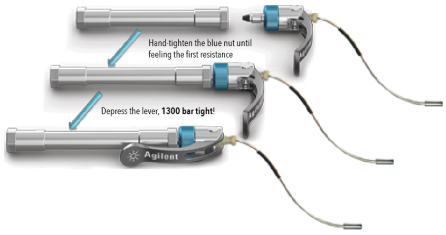
8. Navigate to your sequence folder in Explorer and collect your xls report for your data.

4 Column Installation

1. Open the column compartment module

2. If a column is already installed, remove it following instructions below depending on the fittings.

4.1 A-Line Quick Connects



(a) Unhinge the grey clamp so that it's perpendicular to the column.

(b) Unscrew the blue fingertight fitting so that the inlet capillary is freed.

(c) Unscrew the fingertight PEEK fitting at the outlet of the column.

PEEK fitting

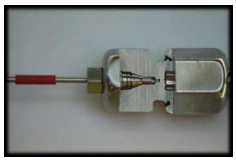
(d) Orient the new column so that the **Flow** $\rightarrow$ label is in the proper direction.

(e) Insert the inlet capillary into the column inlet and fingertighten the blue screw.

(f) Close the gray clamp.

(g) Insert the outlet capillary so it bottoms out in the column outlet and fingertighten the PEEK fitting. Check that the capillary doesn't come loose by pulling on it away from the column.

4.2 Swagelok fittings

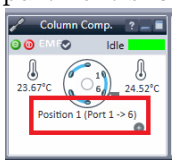


(a) Use a 1/4" wrench over the Swagelok fitting and an appropriate sized wrench to hold the column and unscrew the Swagelok fitting.

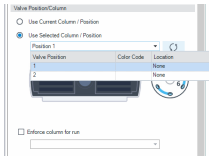
(b) Orient and install the new column so that the **Flow** → label is in the proper direction.

(c) Insert the inlet capillary into the column inlet so that it bottoms out. Fingertighten the Swagelok fitting. Use a wrench to tighten it further by 1/4 turn. Do not overtighten the fitting.

(d) Insert the outlet capillary so it bottoms out in the column outlet and fingertighten the PEEK fitting. Check that both capillaries don't come loose by pulling on them away from the column.

3. If the HPLC has a column switching valve, in ChemStation, check that the Column Compartment module is directing flow to the correct ports. The column compartment should be labeled with which side goes to which port. 

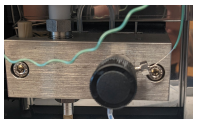
(a) If the valve needs to be switched, right click the column compartment module and select **Method**.

(b) Change the valve position to direct flow through the column. 

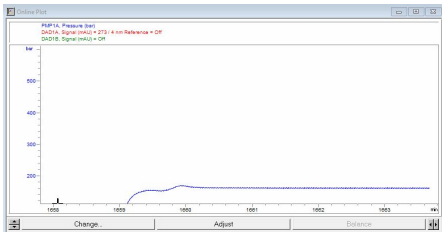
(c) Save the method. In the audit trail, enter "Valve switch."

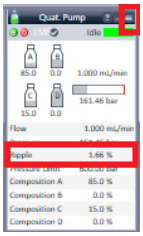
5 Purging

These steps are necessary when changing solvent reservoirs or if the pump has been idle for a few hours or more.

1.  Open the purge valve on the pump by turning counter-clockwise 1/2 to 1 full rotation. The valve may be in a different location depending on the specific pump model. The pressure reading for the pump in ChemStation should be < 1 bar when the purge valve is open.

2. Right click the pump module window in ChemStation and select **Method**
3. Edit the solvent composition parameters so the pump is using 100% of the changed channel. Channel A composition is calculated from the composition of the other channels.
4. Set the flow rate to 5 mL/min and click OK.
5. Turn the pump module on in the software with the small green button if not already on. 
6. Allow the pump to run until all air bubbles have been purged from the solvent line.
7. Repeat steps 2 – 6 for each channel that will be used.
8. Reload the method without saving or restore the flow rate and compositions to their original settings.
9. Close the purge valve by rotating clockwise. Check the pressure trace in the Online Plot in ChemStation for stable pressure. Expand the pump module window with the button in the top right corner and check for a pressure ripple $< 2\%$.





10. Continue with setting up your analysis.

6 Best Practices

- Run a 0 injection at the start of a sequence to let the instrument equilibrate, especially if using gradient methods. This can be done by leaving the **Sample Location** blank in the sequence table.

- Avoid injecting with solvents stronger than the initial mobile phase to prevent peak shape issues. Reduce injection volume if this is unavoidable.
- When removing a column for storage, flush with 50% pure water and 50% acetonitrile for 5 column volumes and store the column in this mixture.

7 Troubleshooting

Consult the Bioanalytics team for any issues with the HPLC before attempting to solve them yourself.

7.1 Leak detected

1. Hover over the red status indicator for each module in ChemStation to determine from where the leak is coming.
2. Investigate the appropriate module and check for leaking solvent. Dry any solvent around the leak sensor with a Kimwipe. Clear the leak error by clicking the **On** button for the module where the leak was detected.
3. Retighten fittings as necessary. If Swagelok fittings are leaking, tighten by no more than 1/4 turn at a time and check for leaks again.
4. If the leaking capillary is PEEK material, recut the end of the capillary. Dip the new end in acetone to remove manufacturing lubricants and retighten the fitting.

7.2 Pressure ripple >2% or no pressure when purge valve closed

This usually indicates that air has entered the pump heads after they have been sitting idle. If the system uses a binary pump, isolate which pump head is causing the issue by monitoring the pressure trace when using 100% of that channel.

1. Use a 1/4" wrench to loosen the outlet steel capillary on the pump head.
2. Cover the outlet with Kimwipes.

3. Change the mobile phase composition to use 100% of the problematic channel and pump head.
4. Increase the pump flow rate to 5 mL/min and turn the pump on.
5. Tap on the pump head with a screwdriver handle or adjustable wrench to release air bubbles.
6. Reduce the flow rate.
7. Retighten the steel outlet capillary fitting to the pump head.
8. Restore method settings, turn the pump on, and check if the pressure ripple has reduced to $< 2\%$.
9. If not, attach a syringe and PTFE tubing to the purge valve outlet.
10. Open the purge valve.
11. Draw 20 mL of solvent through the pump head by pulling the syringe plunger.
12. Close the purge valve with the syringe still attached.
13. Remove the syringe line and reattach the waste tubing.
14. Turn the pump on and check if the pressure ripple has reduced to $< 2\%$
15. If not, run isopropanol through the pump head for a few hours to dissolve any air.