

JAG Instrument Manual

Preparative HPLC

August, 2022

**FOR SAFETY, YOU SHOULD HAVE READ THE GENERAL HANDOUT FOR HPLC
BEFORE WORKING WITH THIS ANALYTICAL HPLC**

1. Purging the solvent pumps

- 1.1 Place the solvent intake lines for preparatory pumps A and B into the appropriate solvent containers.
- 1.2 Place the solvent drainage lines for prep pumps A and B into the waste container below the HPLC.
- 1.3 Turn on prep pumps A and B.
- 1.4 Press the green “pump” button and open the drainage valve from closed to open.
- 1.5 Observe the solvent drainage lines. The solvent should purge through the pump at 40 ml/min. (default setting). When solvent has been flowing out of the drainage line at a constant rate with no air bubbles for 1 min press the green “pump” button to stop the solvent flow.
- 1.6 Place the solvent drainage line from the pump back into the solvent container that contains the solvent intake line.
- 1.7 Press the green “pump” button to resume purging. (This allows you to purge the pump for an extended period of time without wasting lots of solvent.) Continue purging for at least 10 min.
- 1.8 Press the green “pump” button again to stop the solvent flow. Return the drain valve to the closed position.
- 1.9 At this time it is best to turn the pumps completely off by switching the power on/off switch.

2. Instrument Set-up

- 2.1 Install the appropriate preparatory column if necessary.
- 2.2 Remove the current column from the solvent line by unscrewing the connection above and below the column.
- 2.3 Screw in the solvent line connections (you may need to use a small wrench here) into the new column. The solvent should flow through the column from down to up. Make sure that the flow direction indicator on the column is pointing up.
- 2.4 Open the door on SPDM20A. The prep detector must be installed. If the analytical detector is in place then it must be exchanged with the prep detector. Ensure that the prep detector is connected to the solvent line from the preparatory column.
- 2.5 Turn on FCV20AH2, FCV20AH6, SPDM20A, and CBM-20A.

3. Setting Up a Method File

- 3.1 Open LC Solution from the computer desktop.
- 3.2 Choose Instrument **2** for **preparatory** experiments.
- 3.3 In the instrument parameter window that opens, click the “Advanced” tab. (**Fig. 1**)
- 3.4 Under the “Data Acquisition” tab, adjust the LC Stop Time time and the PDA (detector) time to fit the duration of your experiment. All other options should remain in the default settings.
- 3.5 Open the “LC Time Prog.” tab. In row 1 input “0.01” for time. Under Module select “Pumps”. Under Action select the letter (A or B) that corresponds to the correct solvent. Under value set the desired solvent ratio. Repeat this process in row 2 if an additional solvent will be used. In the final row input the experiment stop time. Under Module select “Controller”. Under action select “Stop”. (**Fig. 2**)
- 3.6 Open the “Pump” tab. Select “isocratic flow” (constant composition) or “low pressure gradient”. Select the flow rate (7.5 ml/min is good starting point) and initial solvent composition. (**Fig. 3**)
- 3.7 Open the “Controller” tab and ensure that the box for “Power On”. All others should be unchecked.
- 3.8 The contents under tabs “PDA”, “Autosampler”, and “Auto Purge” should remain in their default settings.
- 3.9 It is strongly recommended to not use the “fraction collector” until peak reproducibility on your sample has been proven. See section 5 below for more details.
- 3.10 Save the method file. (File-Save Method File As ...)
- 3.11 Click the “Download” button.

4. Running the experiment

- 4.1 Turn the preparatory pumps A and B back on.
- 4.2 Press the green “Pump On/Off” button *on the computer screen* to purge solvent through the column. (**Fig. 4**)
- 4.3 Check the entire HPLC system for solvent leaks.
- 4.4 Observe the solvent exit line that drains into the waste container. When the solvent has been flowing at a constant rate with no air bubbles for at least 5 minutes you may start your analysis.
- 4.5 Click “Single Start” button on the frame on the left. (“Acquisition Information” window will pop up.)
- 4.6 Fill in the Sample Name, Sample ID, and Data File name where the data will be saved in the “Acquisition Information” window.
- 4.7 Click Ok. A new window will open that says “Analysis will start by clicking “OK” or “Man Inj” (manual inject). DO NOT click anything in this window.

5. Manual Sample Injection

- 5.1 Verify that the manual sample injector is in the load position.
- 5.2 Rinse the injector port with 10 mL of solvent (this should be the same as the column eluant) using the appropriate syringe labeled “solvent”.
- 5.3 Inject your sample (max. 3-4 mL) slowly into the injection port using the syringe labeled “sample”.

- 5.4 Turn the manual injection port clockwise from “load” to “inject”. Analysis will automatically start. You will notice that the window mentioned in section 4.7 automatically disappears.
- 5.5 The analysis will automatically stop, however you need to press the green pump on/off button (**Fig. 4**) to stop the flow of solvents and avoid wasting them.

6. Fraction collection

- 6.1 For the first several runs, it is recommended that the user collects fractions manually by holding the solvent drainage line over a test tube rack while watching the chromatogram develop on the computer screen. As you do not yet know the retention times, peak intensities, or appropriate fraction volumes it is difficult to program the automatic fraction collector. Once you have done the experiment with reproducible results, the fraction collection procedures are as follows.
- 6.2 Click the “Fraction Collector” tab in the bottom window. (**Fig. 5**)
- 6.3 Load the data file of interest by clicking the “Load Data” button.
- 6.4 Type in the starting retention time of the peak of interest into the “Time” column on the spreadsheet.
- 6.5 Select “Valve Open” in the “Action” column.
- 6.6 Type in the ending retention time of the peak of interest into the second row of the “Time” column.
- 6.7 Select “Valve Close” in the “Action” column.
- 6.8 Click the “Simulate” button next to the “Load Data” button.
- 6.9 The fraction being collected will be shaded and vial number will be shown on the shaded area.
- 6.10 If you need multiple fractions, continue to fill in the time for “Valve Open” and “Valve close” in the following rows.
- 6.11 On the left side of this window, type in the Vial Volume (3.5 mL), the Delay Volume (540 μ L), and the Start Vial Number (the first vial on the rack is designated as **0**.)
- 6.12 Save the parameter file, and click download.
- 6.13 Start sampling by clicking the “Single Start” icon.
- 6.14 Follow the steps outlined in section 5 to run the experiment.

5. Data Analysis

- 5.1 Open the file where your experiment was saved.
- 5.2 You can export the data in ascii format and make your own plot using a third party program. (Go to File-Export data.)
- 5.3 You may print the “data analysis” screen, or click the “Data Report” icon to see and print the chromatogram.

6. Shut down procedure

- 6.1 Verify that no more peaks will show up on the chromatogram by purging the column with additional solvent.

6.2 Turn off all parts of the instrument and close the software.

7. Other Notes

- 7.1 For solvents, use HPLC grade only.
- 7.2 It is very important that you mention the last solvents that you used in the HPLC log book so that the next user can know how to purge the system. (ex. 1 - it is impossible to purge with methanol if the last user was running hexanes. The solvent immiscibility will overload the pressure on the column) (ex. 2 – all dichloromethane must be removed from the solvent lines before a chiral column is used as it will destroy the stationary phase.)
- 7.3 If you notice that the HPLC grade solvents in the yellow cabinet are running low, then let the person in charge know ASAP.
- 7.4 If you have trouble/questions ask the person in charge of the instrument before you modify anything on the instrument or the software.

Fig. 1. Click the “Advanced” button on the bottom window.

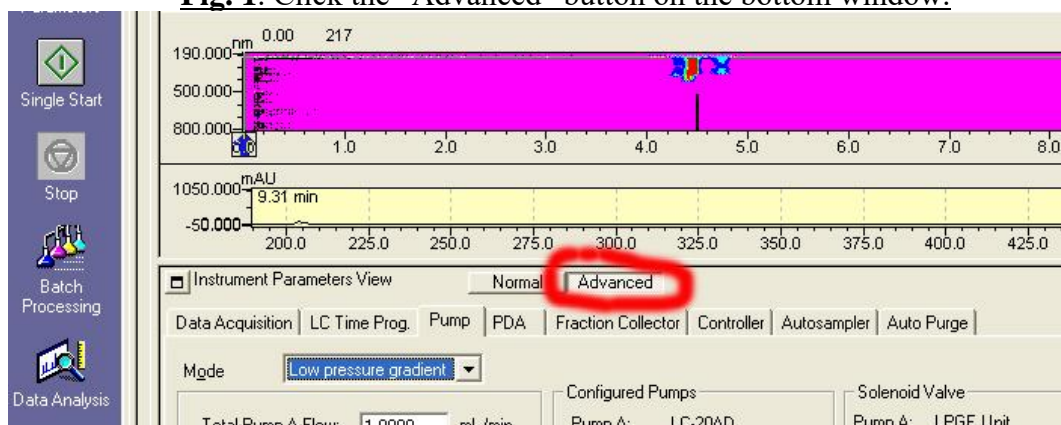


Fig. 2. Click the “LC Time Prog.” tab, type in the initial compositions, and type in the duration of the analysis.

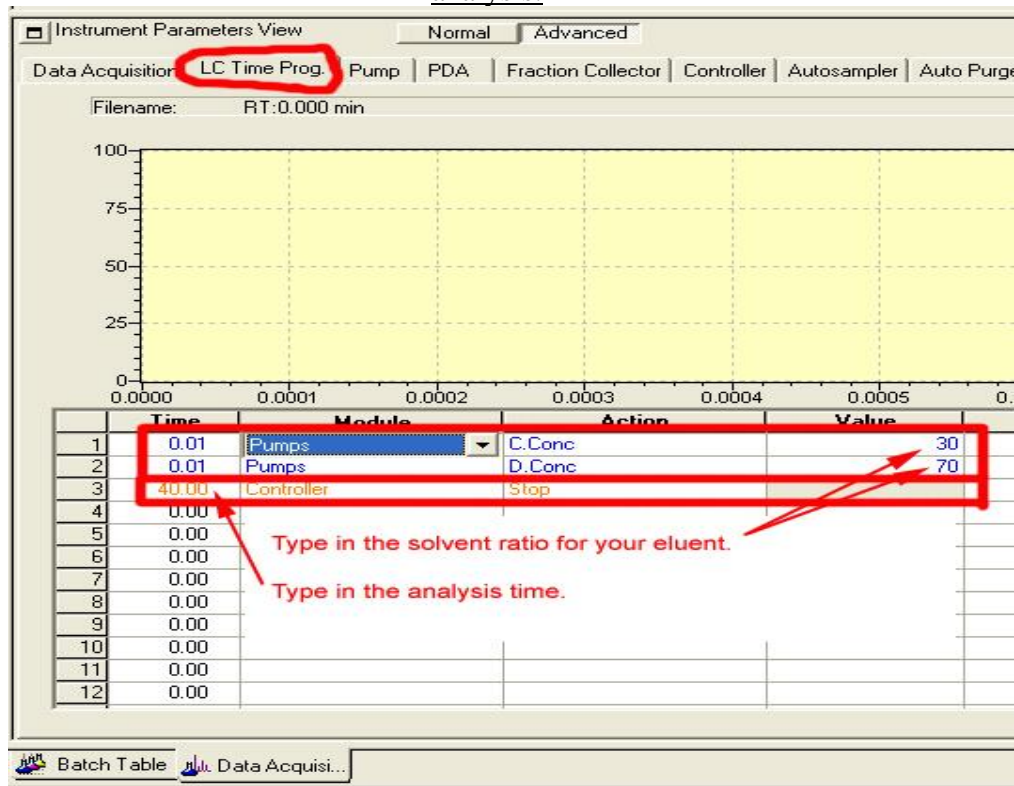


Fig. 3. Click the “pump” tab, choose the Mode, and type in the composition of the eluent.

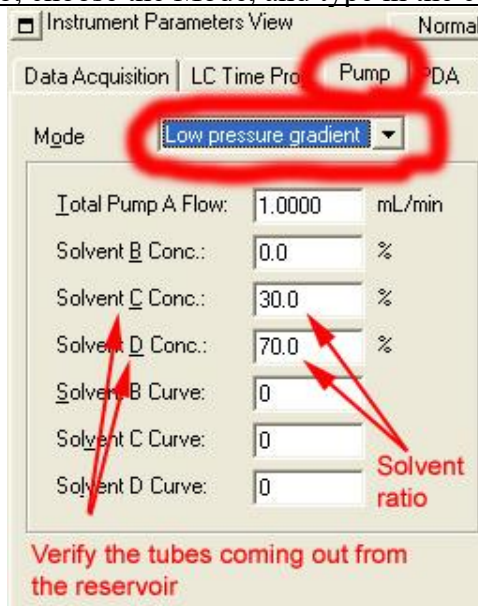


Fig. 4. Click the Pump On/Off icon to start the pump.



Fig. 5. Fraction Collector tab showing the selection for the Action column.

Instrument Parameters View Normal Advanced

Data Acquisition | LC Time Prog. | Pump | PDA | Fraction Collector | Controller | Autosampler | Auto Purge

Model: FRC-10A

Fraction collector

Width: 2 sec
 Slope: 100 uV/sec
 Level: 1000000 uV
 Vial Volume: 0.000 mL
 Response: 1.00 sec

Volume ▾

Auto Delay Offset

Delay Volume(D): 0 uL
 Sig. Delay Volume(G): 0 uL
 Flow Rate(F): 1.000 mL/min

Rack Type: Rack1 ▾

100 uV
 50
 0
 0.0000 0.0003 0.0004

Load data...

- Lock
- Slope
- Level
- Slope Override
- Valve Open
- Valve Close
- Initial Vial
- Final Vial
- Peak Number
- Divide Time
- Band
- H. Wave Ratio

| | Time | | Value |
|---|------|--|-------|
| 1 | 0.00 | | |
| 2 | 0.00 | | |
| 3 | 0.00 | | |
| 4 | 0.00 | | |
| 5 | 0.00 | | |