

JAG Instrument Manual

Analytical HPLC

Updated October 04, 2016

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

1. Purging the solvent pumps

- 1.1 Turn on LC20AD (**component 4**) and SIL20A (**component 3**).
- 1.2 Push the “conc” button on LC20AD. Use the keypad to type in the solvent composition for pumps A, B, C, and D. Make sure that you know which solvent is linked to which pump. (usually **A = water, B= methanol/iso-propanol, C= dichloromethane, D = hexanes** but always double check before starting experiment)
- 1.3 Turn the drain valve (black knob on LC20AD) counter-clockwise 180°
- 1.4 Press “Purge” on the keypad on LC20AD and SIL20A. (The screens on these instruments will show PURGING LINE when it is purging.)
- 1.5 During the purge, watch the drain line and make sure that solvent is flowing at a constant rate. The air bubbles that are generated at the beginning of the purge should disappear after a few minutes.
- 1.6 After the purging is complete (default 25 min.) turn the drain valve back to the original closed position.

2. Setting Up a Method File

- 2.1 Open LC Solution from the computer desktop.
- 2.2 Choose Instrument 1 for analytical experiments.
- 2.3 In the instrument parameter window that opens, click the “Advanced” tab. (**Fig. 1**)
- 2.4 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.
- 2.5 Open the “LC Time Prog.” tab. In row 1 input “0.01” for time. Under Module select “Pumps”. Under Action select the letter (A-D) 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**)
- 2.6 Open the “Pump” tab. Select “isocratic flow” (constant composition) or “low pressure gradient”. Select the flow rate (1.0 mL/min is a good starting point) and initial solvent composition. (**Fig. 3**)
- 2.7 Open the “Controller” tab and ensure that the boxes for “Power On”, “Event 1”, and “Event 2” are checked. All others should be unchecked.
- 2.8 The contents under tabs “PDA”, “Fraction Collector”, “Autosampler”, and “Auto Purge” should remain in their default settings.
- 2.9 Save the method file. (File-Save Method File As ...)
- 2.10 Click the “Download” button (**Fig. 4**).

3. Instrument Set-up

- 3.1 **Check the solvent availability!** Solvent should be at least half-full in the container.
- 3.2 Connect the solvent line that usually flows into the analytical column to a drainage tube.
- 3.3 Press the green “Pump On” button and purge solvent through this portion of the line for 5 minutes (**Fig. 5**)
- 3.4 Pause the pump by pressing the same green button now named “Pump Off”.
- 3.5 Install the appropriate analytical column.
- 3.6 Screw in the solvent line connections (finger tight) 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.
- 3.7 Open the door on SPDM20A. The analytical detector must be installed (**Fig. 6**). If the prep detector is in place then it must be exchanged with the analytical detector. Ensure that the analytical detector is connected to the solvent line from the analytical column.
- 3.8 Turn on FCV20AH2, FCV20AH6, SPDM20A, and CBM-20A.
- 3.9 Put your sample in the sample tray. ***Make sure that the tray is in the right position (you should hear a "click" sound).*** Take note of the number of your sample in the tray.

4. Running the experiment

- 4.1 Press the green “Pump On/Off” button to purge solvent through the column. (**Fig. 5**)
- 4.2 Check the entire HPLC system for solvent leaks.
- 4.3 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. *Also check the "LC chromatogram" to see pressure profile.*
- 4.4 Click “Single Start” button on the frame on the left (**Fig. 7**). (“Acquisition Information” window will pop up (**Fig. 8**)).
- 4.5 Fill in the Sample Name, Sample ID, and Vial# in the “Acquisition Information” window. (**Verify that the number in the sample tray and the vial# in the window match.**)
- 4.6 By clicking “OK” in the “Acquisition Info” window, analysis will start and the file will be saved automatically at the end of the run.
- 4.7 Analysis will automatically stop, however, you need to stop the pump by clicking the green “Pump On/Off button” to save the solvents.

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.
- 6.3 Remove the column from the solvent line and recap column with Teflon plugs.

7. Other Notes

- 7.1 For solvents, use HPLC grade only.
- 7.2 It is very important that we know the last solvents that were used in the HPLC instrument to purge the system for next run. (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.). Therefore,
 - 7.2.1 When you finish: note down the solvent system you used in the HPLC log book.
 - 7.2.2 When you start: before loading new method, check the existing method that pop-up when you start the program (this is the solvent system that was previously used). Take note of the solvent system from that method.
- 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.

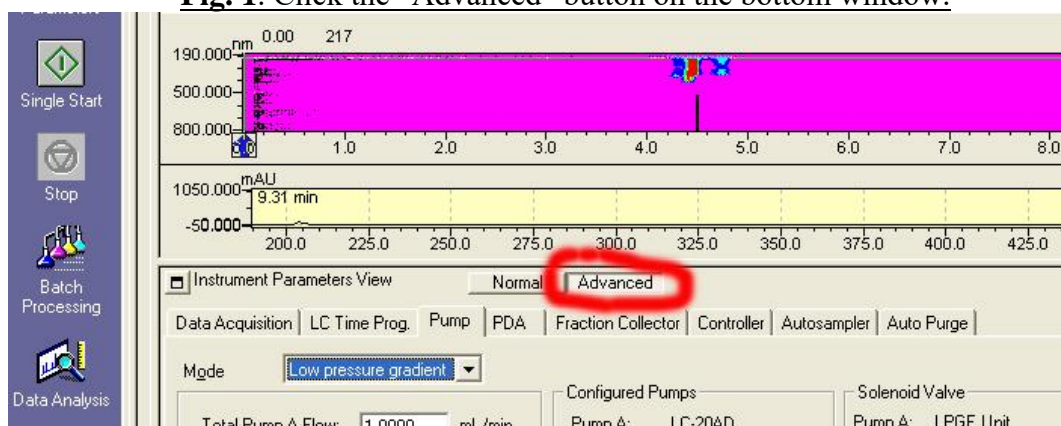


Fig. 2. Click the “LC Time Prog.” tab, type in the initial compositions, and type in the duration of the analysis.

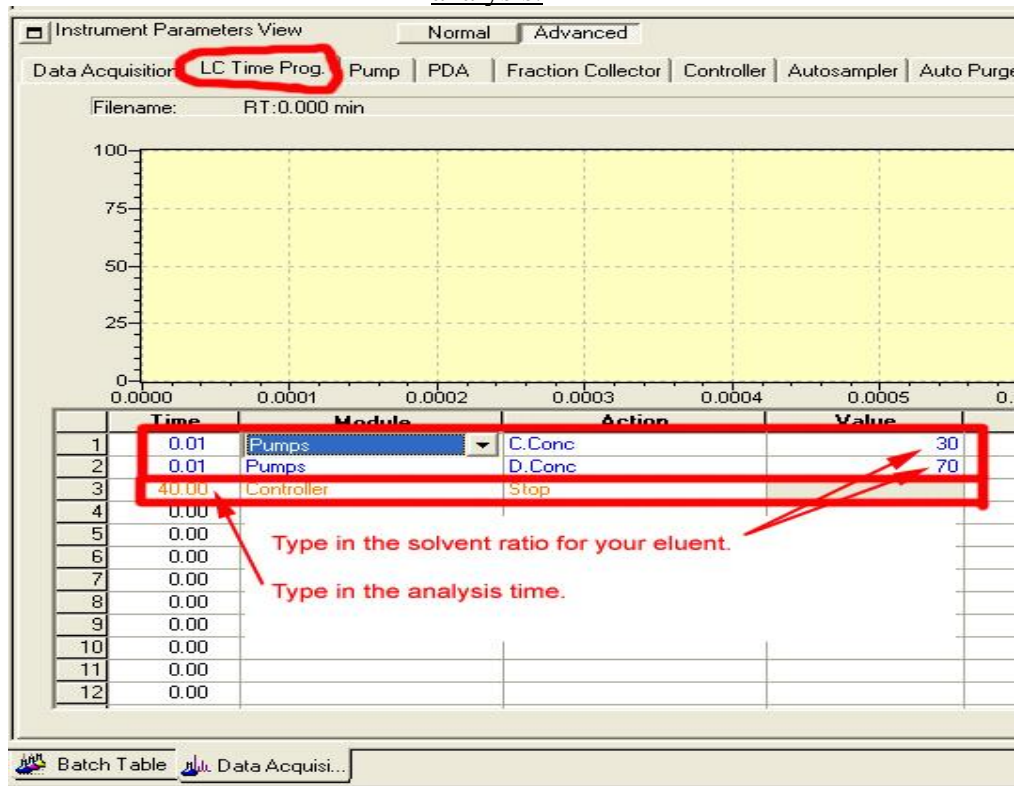


Fig. 3. Click the “pump” tab, choose the Mode, and type in the composition of the eluent.

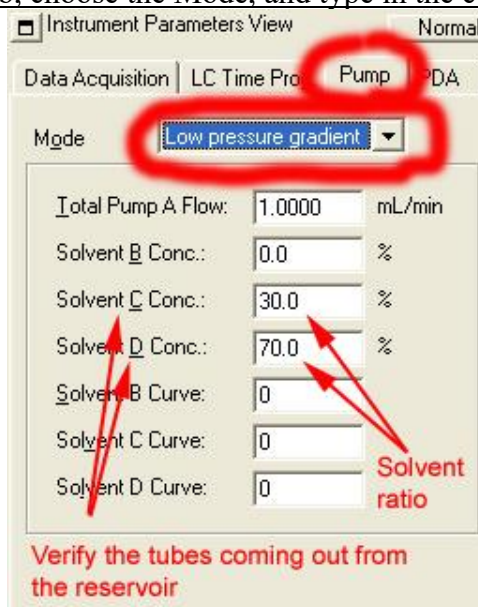


Fig. 4 "Download" button to activate method files

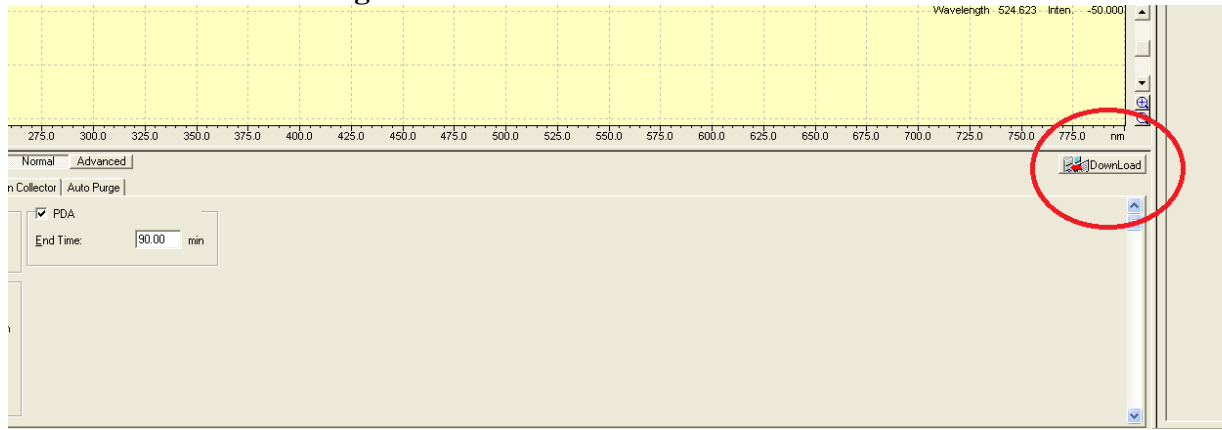


Fig. 5. Click the Pump On/Off icon to start the pump.



Fig. 6 Analytical detector cell in SPDM20A



Fig. 7 "Single start" buttons

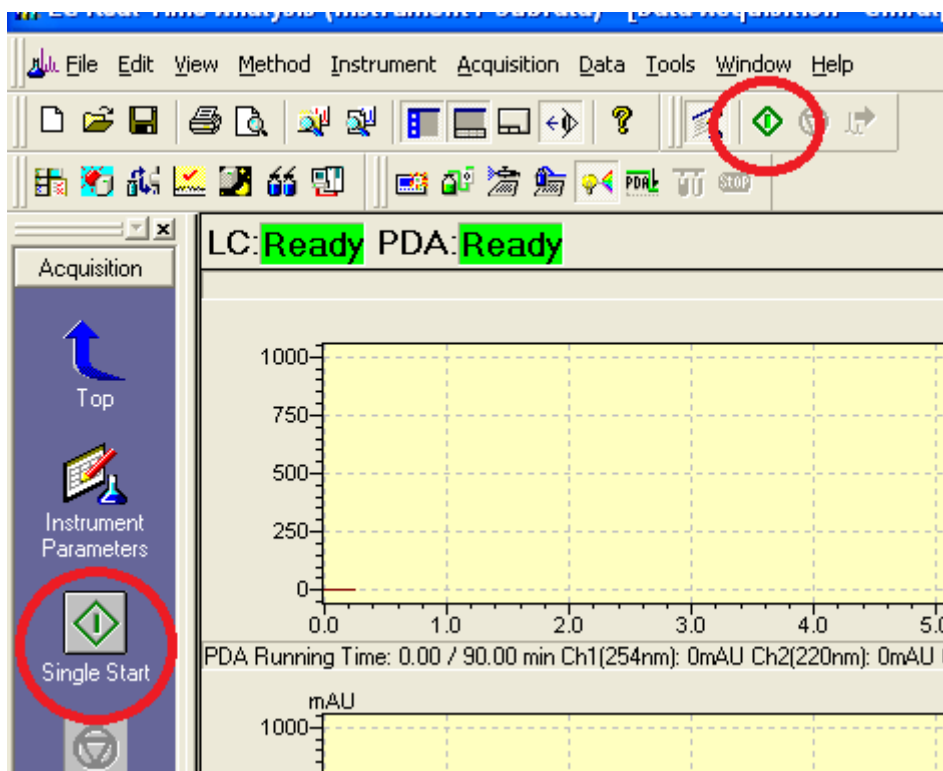


Fig. 8 Single run sample info window

The 'Single Run' dialog box is shown with the following fields and options:

- Acquisition Information:**
 - Sample Name: [Empty text box]
 - Sample ID: [Empty text box]
 - Method File: C:\LabSolutions\Data\Subrata\Chiral-AD\Chiralpak AD 9 [Browse button]
 - Data File: \LabSolutions\Data\Subrata\Chiral-AD\ [Browse button]
 - Auto Increment: 1, 2, ... [Dropdown menu]
 - Background File: [Empty text box] [Browse button]
 - Data Description: [Empty text box] [Scroll bars]
- Sampler:**
 - Vial#: 1 [Text box]
 - Tray#: 1 [Text box]
 - Injection Volume: 15 [Text box] uL
- Buttons:** Options..., Advanced >>, OK, Cancel, Help