ChemStation for Control and Data Analysis

Using the Agilent 1100 HPLC System

I am providing a copy of this document adapted from one Dr. Forkey wrote.

ChemStation provides real time control of the functioning of the Agilent 1100 HPLC system and control of the post-run processing of the data. This document is meant to provide a quick introduction for the CSUS student on how to run the system to provide useful analytical results. For this tutorial LC represents "left click" and means that the mouse pointer must be placed in the desired screen position and then the left button of the mouse is pressed. LCD represents "left click and drag" and means that the mouse cursor should be placed at the appropriate position on the screen and the left mouse button should be held down and the mouse is dragged to move the object to the desired location on the screen.

ChemStation has five main windows that you normally use as starting points for common tasks. These main windows include Method and Run Control, Data Analysis, and several others. **Figure 1** shows the Method and Run Control window.

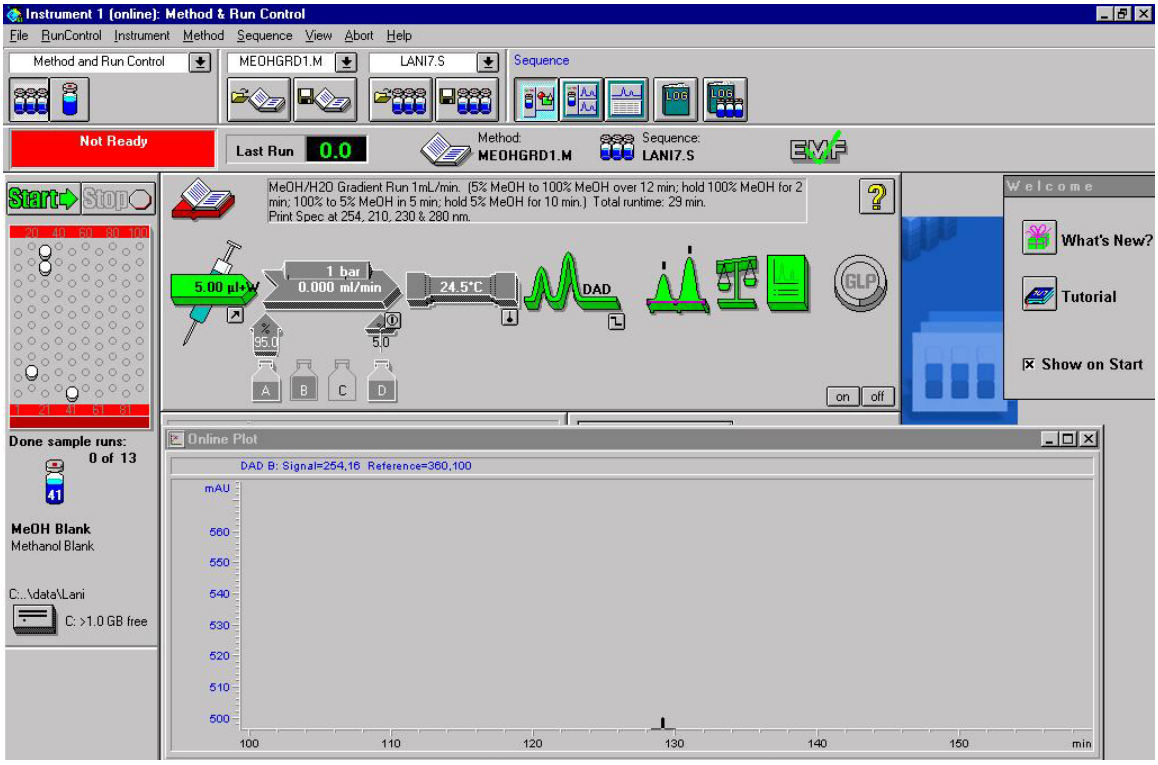


Figure 1. The ChemStation Method and Run Control window.

Method and Run Control Window Icons and Functioning

Figure 2 shows the status of the HPLC system. If the individual icon representing a HPLC component is green, then that component is ready for a run. If it is gray, the component is not ready for you to start a run. If it is yellow, the component is "on" but not yet ready for use. In **Figure 2** the pumping system and column heater are off, while the autosampler and diode array detector (DAD) are ready for a run.

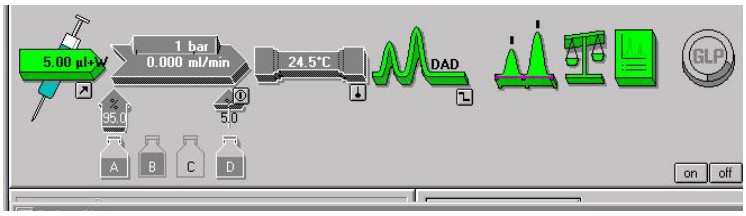
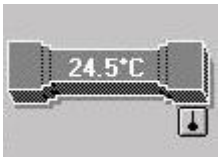


Figure 2. Status of the hplc system.

The following icons are components of the hplc status window in **Figure 2**.

This icon gets you to a window to check injector conditions including injection volume and whether a wash solvent is being used.

This icon shows the status of the hplc pumping system including the flow rate, pressure, % composition, and the state of the solvent level in each bottle.



This icon tells the type of column and the temperature of the column. If it appears gray, the temperature is not set. Clicking on this icon also allows you to select a column through the column switching valve.

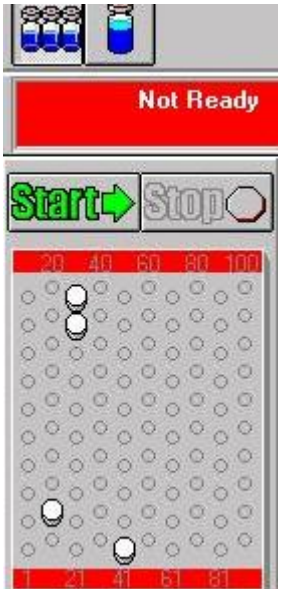
This icon gets you to the diode array detector (DAD) window.

This icon gets you to peak calibration.

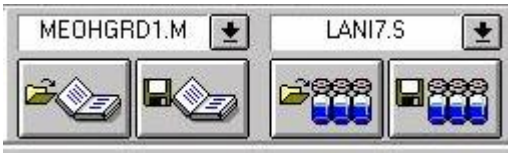
Takes you to the Specify Report window that you can also get to under *Method* and *Edit Entire Method.*

This icon gives you access to the "Good Laboratory Procedures" (GLP) report that is currently setup.

With the next icon you can choose between single injection and injection of the first sample in the sequence. The three bottle button is depressed so the currently set up sequence will start when you LC on Start.



The three-vial button and the single-vial buttons toggle between sequence and single sample for the next injection. Selection of the single sample sets the system up to inject the sample in the assigned vial, often #1. Selection of the sequence injection will set the system up to inject the first sample in the sequence. Pushing the start button will start the single or sequence depending on which one is selected.



The top-left side of this icon indicates the method that is currently loaded and the top-right side of the icon indicates the sequence file that is currently loaded. The buttons below are for loading and saving the method or sequence.

Once a Method has been loaded from the Method and Run Control window, you can edit a method by LC on *Methods* on the main menu and LC *Edit Entire Method*. LC on *OK* several times until the Setup Pump window in **Figure 3** appears. In this window note the flowrate is 1 mL/min and the Stop Time is 15.00 min. The Stop Time is the time you allotted to each run**. Note: This does not guarantee that a peak won't come out after 15 minutes.** Note the Pressure Limits where Max refers to the maximum allowed pressure.

This is often different from one column to another. **Do not exceed the maximum pressure prescribed by the column manufacturer as this could destroy the column.** Minimum pressure should be set to about 2 bar so that the pump will shut down if a leak occurs. To create a gradient elution or stepwise gradient use *Insert or Append* to add the necessary Timetable lines to create the time based changes.

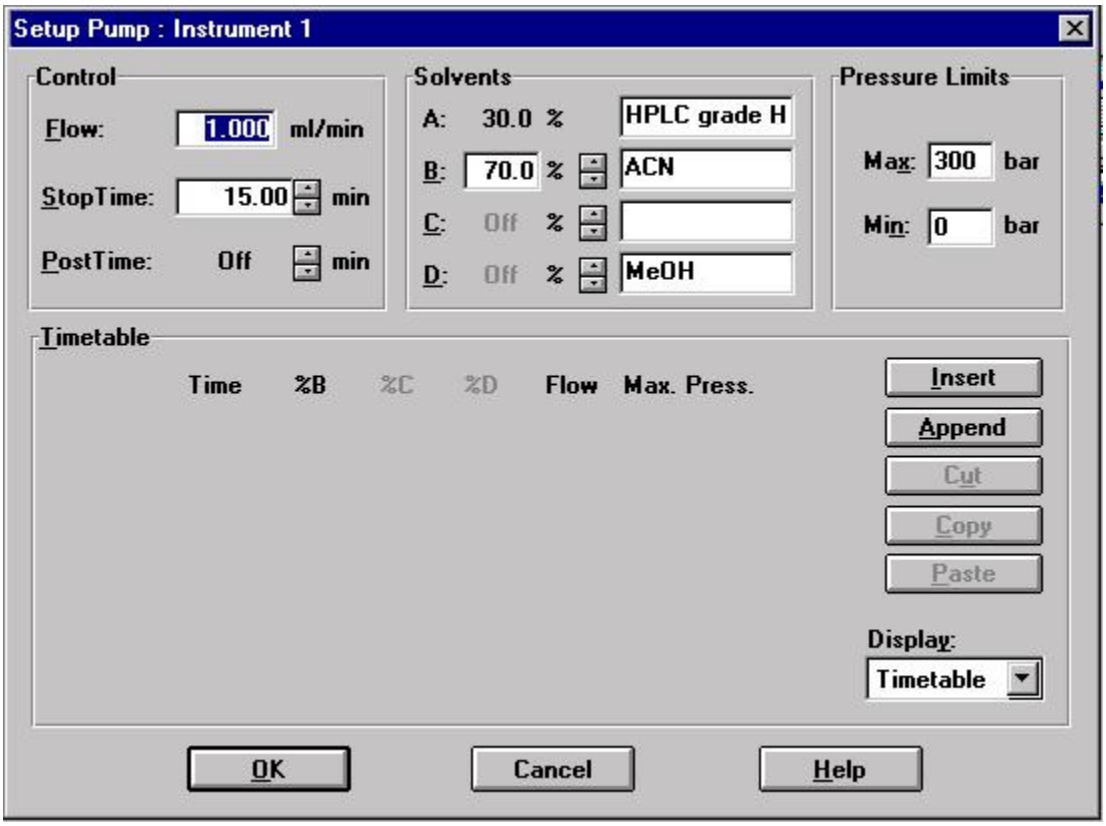


Figure 3. Setup pump window accessed from *Edit Entire Method* under *Method* on the main menu.

Diode Array Detector

Continue on in *Edit Entire Method* by continuing to LC on *OK* until you come to the DAD Signals window as in **Figure 4**. In **Figure 4** the signals A-E that have been chosen for storage on the disk by your method are indicated by the X in the box. Note that Signal B is to use 254 nm with a bandwidth of 16 nm. Thus the A signal that will be stored ranges from 246-262 nm. The reference wavelength for signal B is 360 nm with a bandwidth of 100 nm. Under spectrum the current choice is *None* which means the spectrum will not be stored. It is possible to choose to store DAD spectra during the run. The system gives you several choices for storing spectra. Since full spectra consume considerable disk space, it is important to be conservative in saving spectra. A reasonable choice is to save all spectra in a peak.

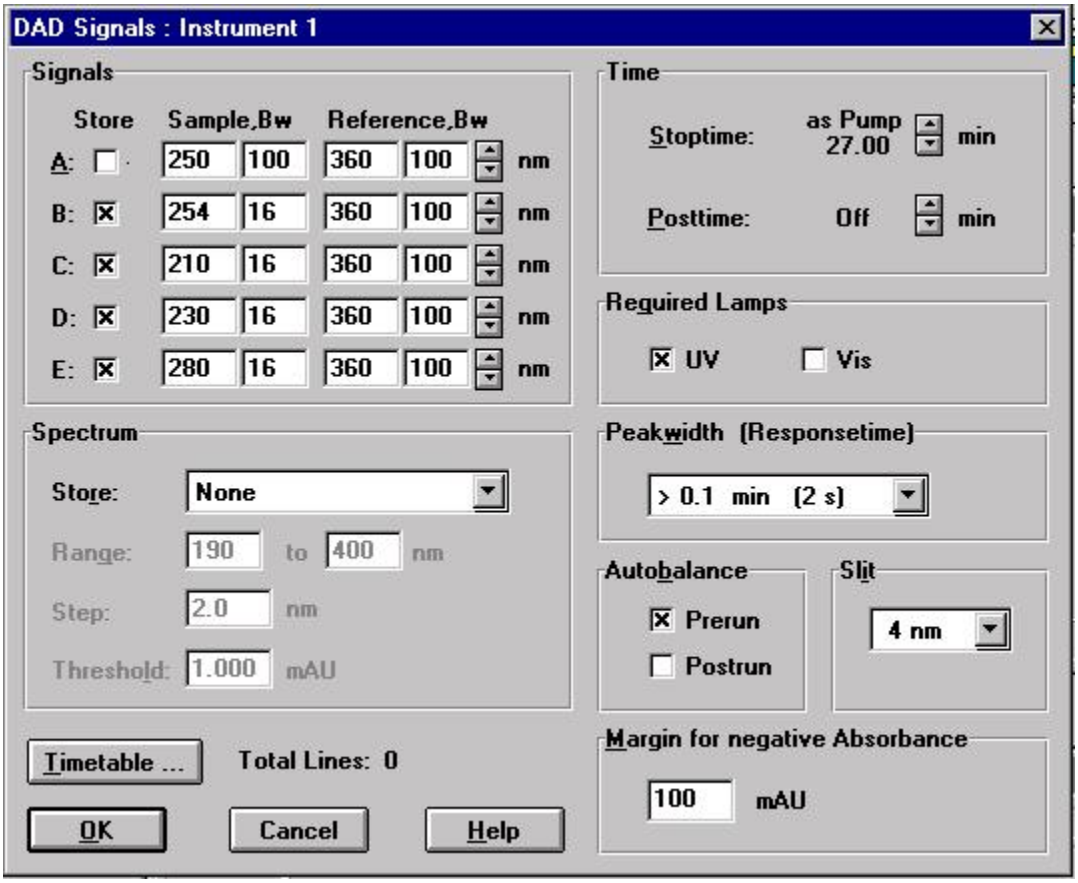


Figure 4. The Diode Array Signals table.

Sequence Table

Under *Sequence* on the main menu of the Method and Run Control window you can get to the sequence table by LC on *Sequence Table*. This gives the table in **Figure 5** that needs to be filled in with information for each sample that will be injected in the sequence. In **Figure 5,** note that Vial 30 on line 1 is highlighted. It is important to place descriptive information in the window above the table for each sample line. Several days later you will not remember details of each sample. All samples will be injected in the order indicated by the line number. The column labeled *Inj/Location* indicates how many replicates of each sample must be run. Note that sequence samples can be run with different methods. In this sequence you can override the injection volume specified by the method file by entering a µL volume in the sample volume column. Use Append to add a blank line at the end of the table for another sample. Use Insert to insert a blank line above a highlighted line.

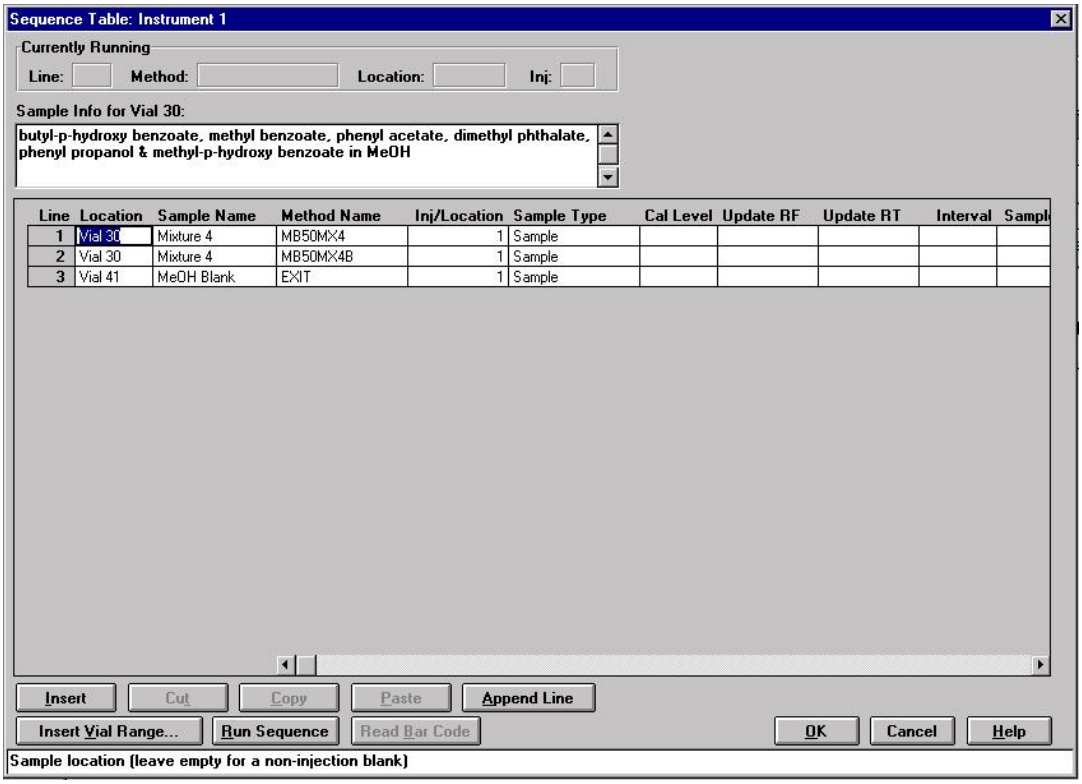


Figure 5. Sequence Table window.

Note that the last line in the sequence should be the one indicated in **Figure 5** because the method "Exit" has a macro that is executed at the end of the run. This method runs 100% acetonitrile for 15 minutes to clean off the column. Then the pump, column heater, and diode array detector UV lamp are turned off.

Figure 6 shows the Sequence Parameters window that is accessed by LC on *Sequence* and LC on *Sequence Parameters*. Enter your name in the Operator Name box. It is important to specify the information that is necessary to save your run data in a place that you can find and one that keeps it separate from that of others in the class. Enter your name in the Prefix box and your directory in the Subdirectory box. Your data will be saved by the system with the path: C:\HPCHEM\1\DATA\[*your directory*] with file name starting with *run80001*.

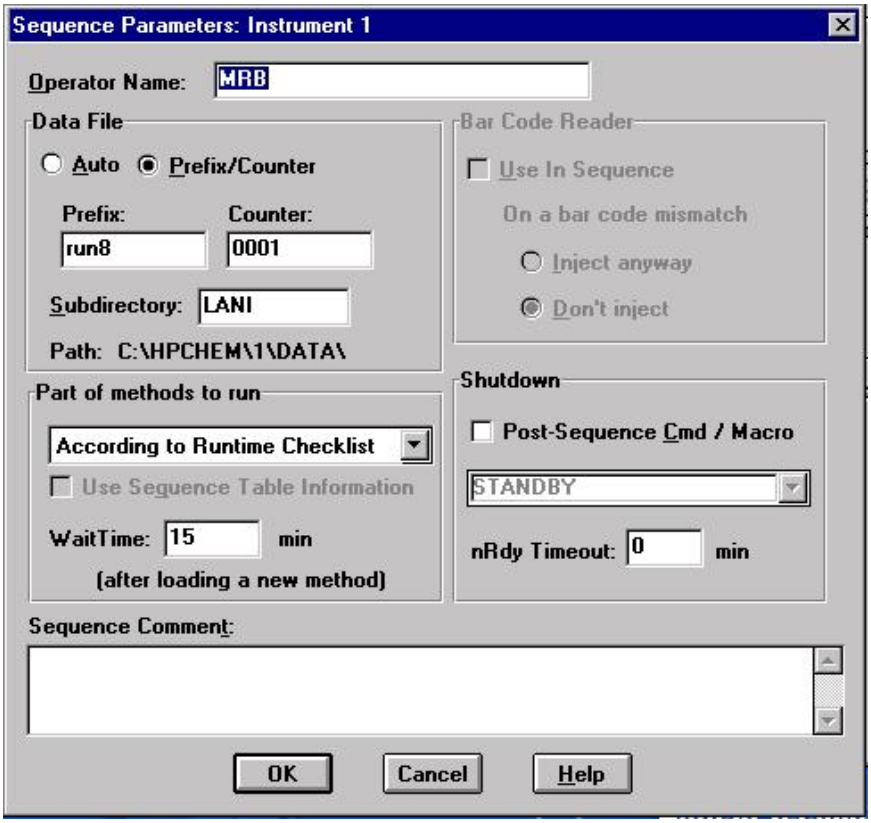


Figure 6. Sequence Parameter window.

Data Analysis Window Icons and Functioning

The Data Analysis main window is accessed by choosing Data Analysis out of the box just below *File, Graphics, and Integration* on the top menu using the ↓. This often is done using the offline version of Chemstation so that you can analyze samples on a separate window from the window controlling the analysis underway.

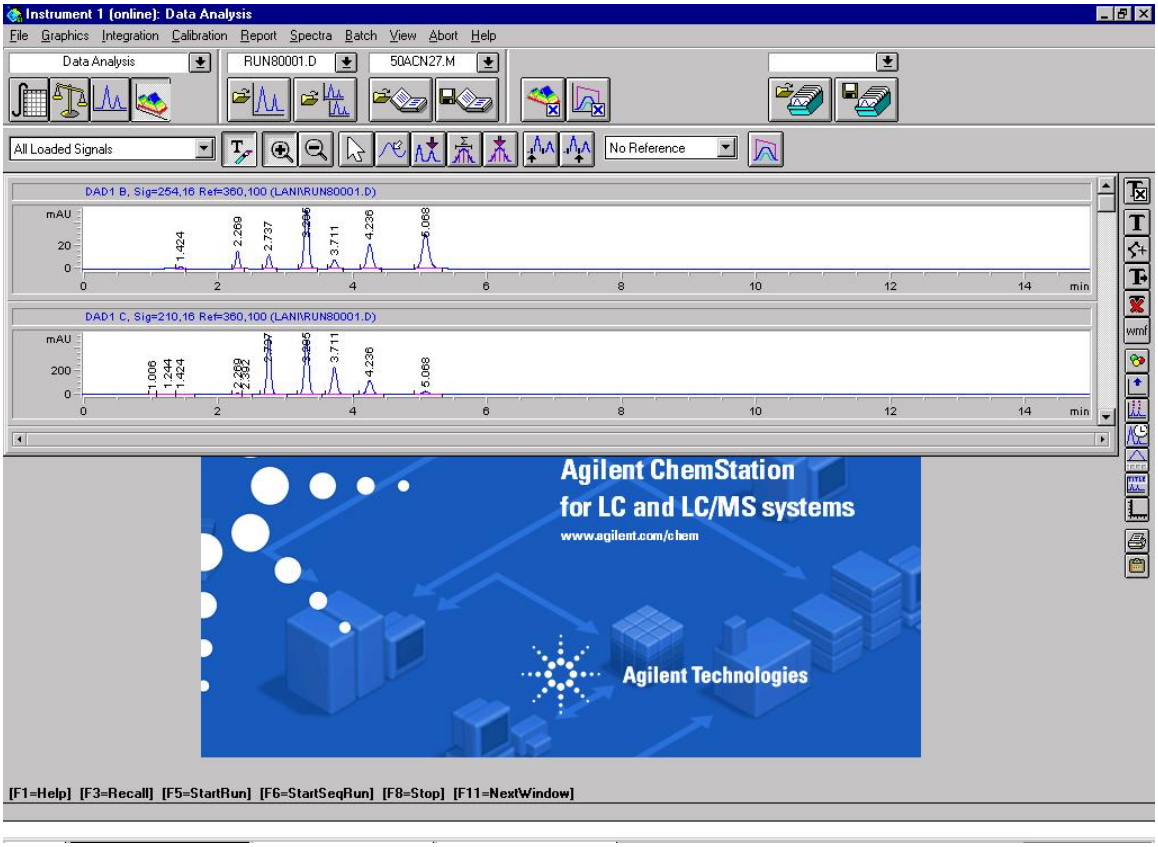


Figure 7. An example of the main Data Analysis window.

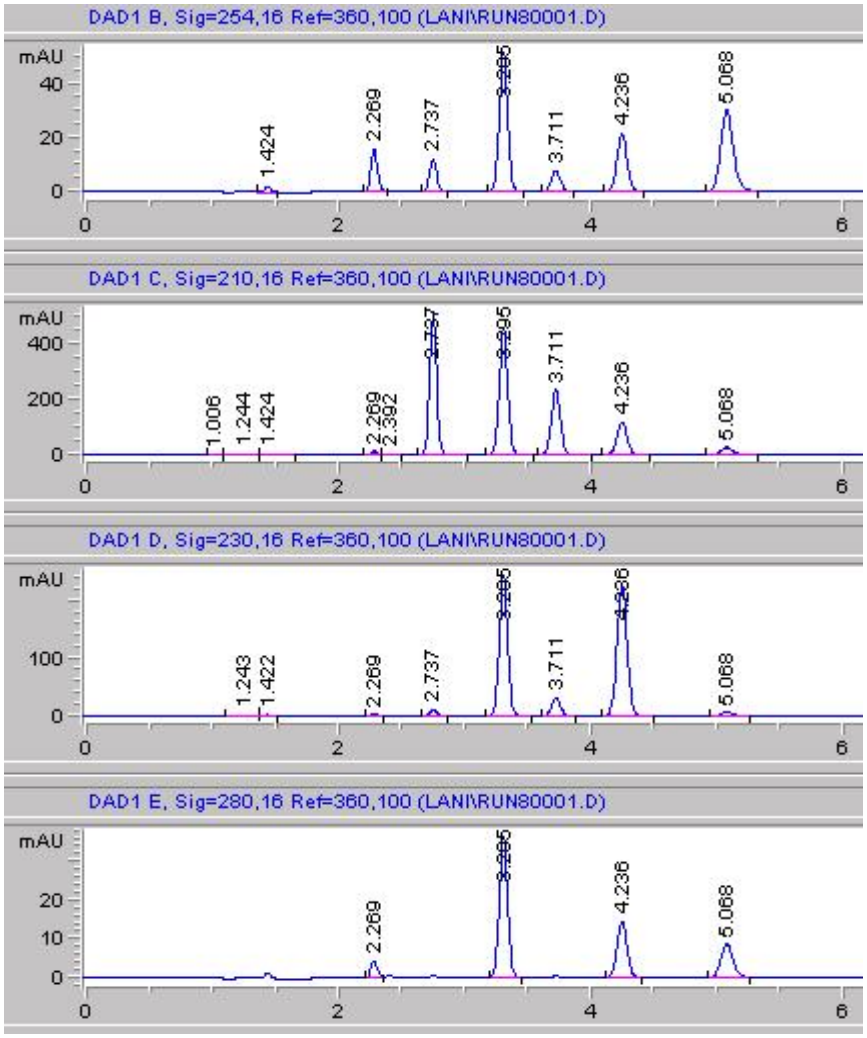


Figure 8. An example of four windows that can be displayed at one time where they differ in the wavelength that is being monitored.

If you look at the top margin of the first chromatogram in **Figure 8**, DAD 1 says that this is a diode array signal on chromatography system 1. ChemStation can control several systems at one time. However, each additional system adds cost for the use of ChemStation. The wavelength is indicated in *Sig = 254,16* to be 254 nm with a bandwidth of 16 nm. This means that signals at all wavelengths from 246 to 262 nm were used. The wider the band width, the more universal is the detector. A general purpose detector wavelength is 254 nm with a bandwidth of 100 nm. *Ref=360,100* indicates that the reference wavelength is 360 nm with a bandwidth of 100 nm. A reference wavelength should be selected where the sample will not absorb light.

Notice that the retention times for peaks are listed at the apex of the peak. If you have previously identified peaks with the name of a compound at a particular retention time, then you may get the assigned name printed at the apex of the peak.

Signal Option buttons

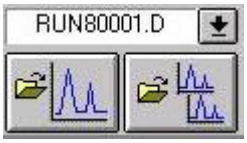
1. Overlays chromatograms on one time axis.
2. Stacks chromatograms as in **Figure 8**.
3 and 4. Adjust y-axes to the same max or different max values.

1 2 3 4

Data Analysis Task Buttons

1. Integration
2. Calibration
3. Signal
4. Spectral

Data File icons



1 2

1. Load Signal(s) and spectra of a data file.
2. Load Signal(s) of a data file and overlay with current signals.

Method File icons

1 2

1. Load a method.
2. Save current method.

Spectral Tasks

1 2 3 4 5 6 7 8 9 10

1. Toggles the display of the Graphics Toolbar
2. Zoom in on window
3. Zoom out
4. Pointer tool (Set to selection mode)
5. Select spectrum at any time position
6. Select spectrum at peak apex
7. Average a selected set of spectra
8. Select a set of spectra of a peak
9. Select spectrum to set as first reference
10. Select spectrum to set as second reference

Graphics Toolbar

Edit Annotation Options (to set default parameters for your annotation)
Add annotation to window (create a new annotation)
Draw a line annotation in the window
Move (Drag) /Change (LC) annotation
Delete annotation from the window
Add new "WMF" annotation to the window
Toggles between Separate and Overlay of signals
Toggles between all signals of the same scale and each full-scale
Toggles between compound names and no compound names at apex of peak
Toggles between retention time display and no retention time display Toggles
Toggles between baseline and no baseline to display
Toggles between Object Titles and no Object Titles to display
Toggles between Axis and No Axis to display
Print Window
Copy currently selected window to the clipboard

Integration of a Chromatogram

Chromatograms are integrated according to parameters put in the method. This is one of the last windows encountered when going through the Edit Entire Method routine (see p. 3). Integration parameters can just as easily be changed after carrying out an analysis. This can be done by going to the *Integration* menu and choosing *Integration Events*.

The parameters that are most frequently changed are 1) slope sensitivity, 2) peak width, and 3) area and height rejects. A high slope sensitivity will result in fewer peaks being detected. Slope sensitivity should be increased when the software is integrating noise peaks. Slope sensitivity should be decreased when actual chromatographic peaks are not being picked up. The peak width should be set similar to typical chromatographic peak width. Area and height rejections are set to avoid retention of data from small peaks (peaks with area and peak height less than the limits in the box). Once parameters are changed you can click on the right most icon to reintegrate the chromatogram. If the changes are not satisfactory, you can re-select *Integration Events* and continue to adjust the integration parameters.

Sometimes you can not consistently set parameters so that the integration is satisfactory. You also can manually integrate chromatograms using the commands in the *Integration* menu at and below the *draw baseline* command.

Gradient Elution-Setup Pump

Figure 12 shows the *Setup Pump* window that is accessed from the Method and Run Control window. You can edit a method by LC on *Methods* on the main menu and LC *Edit Entire Method*. LC on *OK* several times until you get to the Setup Pump window. See **Figure 13** for the plot of the solvent composition versus time. **Figure 14** shows a chromatogram with the mobile phase gradient profile superimposed.

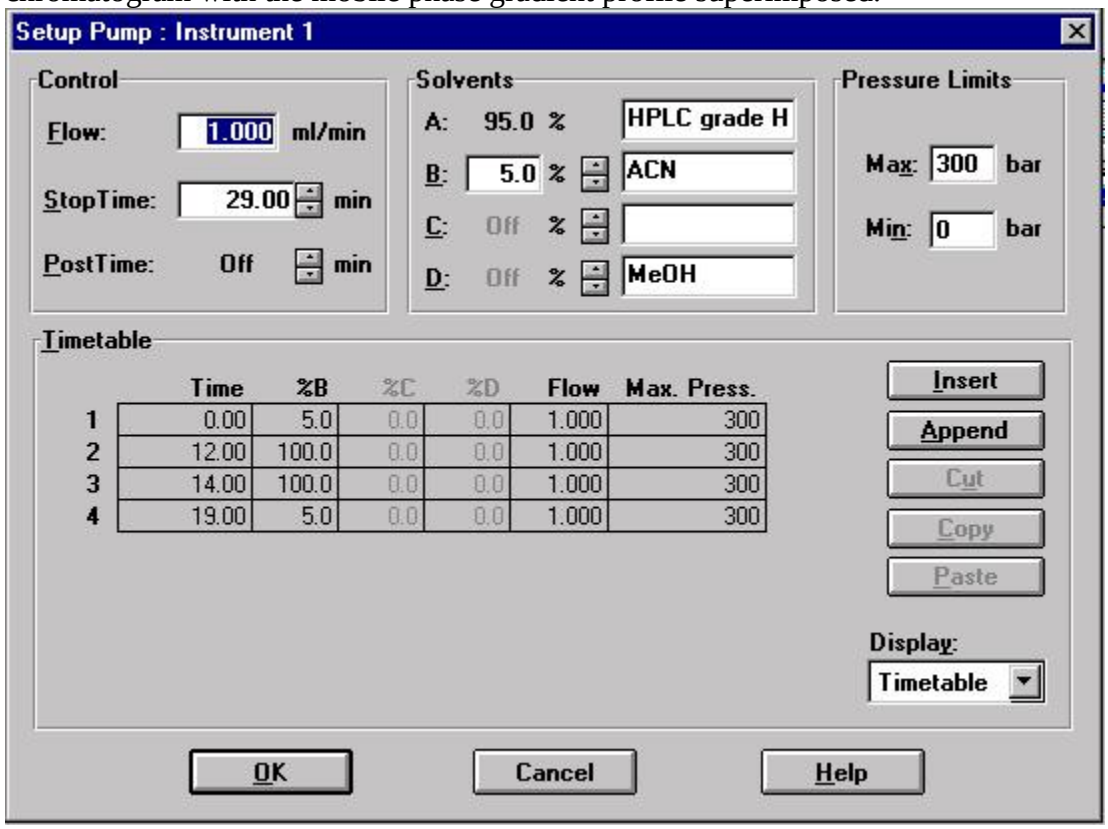


Figure 12. Setup Pump window showing the time program table for a gradient elution run starting at 95% water and 5% acetonitrile.

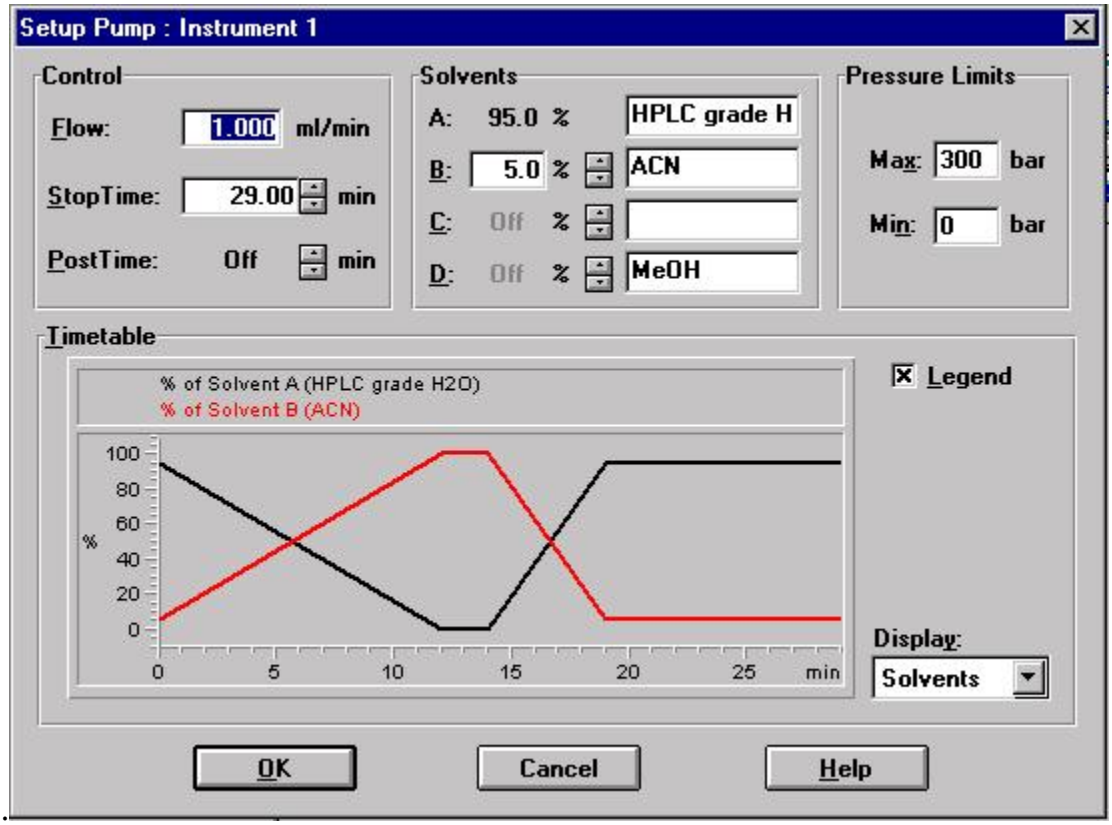


Figure 13. Setup Pump window displaying the solvent composition versus time plots.

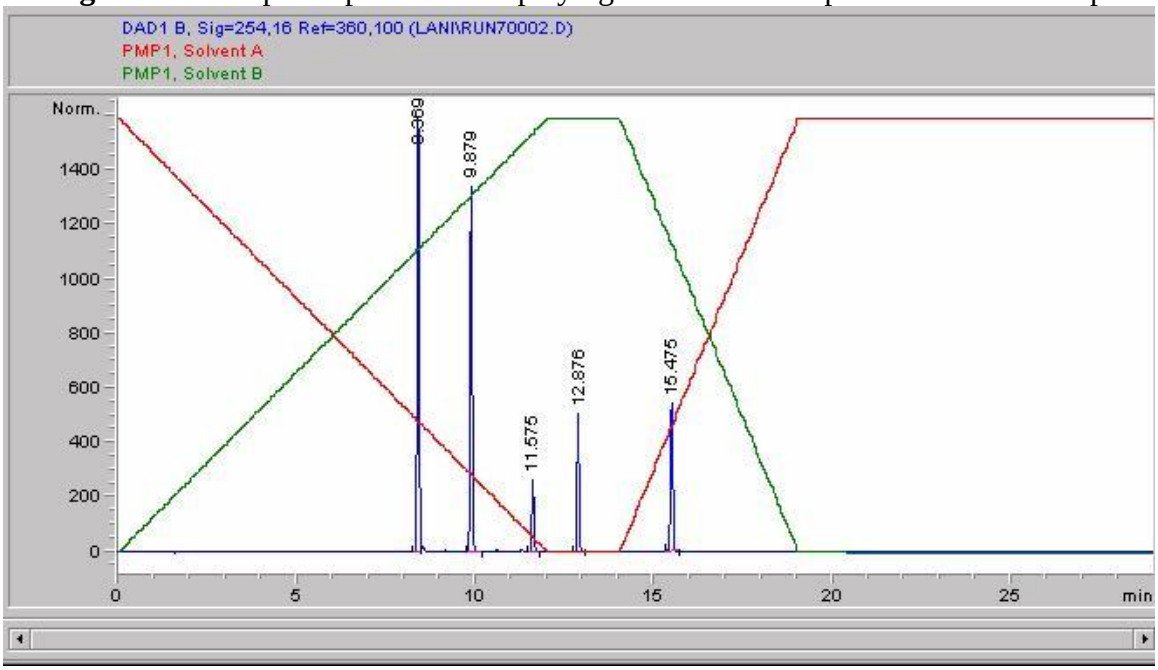


Figure 14. Chromatogram of a five component mixture with the mobile phase compositions superimposed.

Every HPLC instrument that does gradient elution has its own unique gradient dwell time. This is the time required for a change in composition to go from the proportioning valves where the composition change is made to the detector where it's effect will be seen. This

time is not the same for different vendors' instruments. Because of this, duplicating gradient elution results from one manufacturer's instrument to another is difficult.

Getting the System Going Before a Single Injection or Sequence Run

1. Check the solvent waste bottle for sufficient volume for the intended runs.
2. Go to the Main Run Control window.
3. Check the level below the pump icon on the Run Control Window. If the volume is too low, add the appropriate **hplc grade** solvent, and update the system tracking of the actual volumes by LC on the solvent bottle icons and LC on Update Solvent Volumes, etc.
4. LC on *File* and LC on *Open.* Choose *Method* and select the method you want to start with. Immediately save this method in your method directory:
   C:\hpchem\1\method\[your name]\[file name].
   By doing this you edit your own method and don't alter the examples.
5. To turn on the pump, column thermostat, and DAD UV lamp, look for the *On* button on the lower right side of the Main Run Control window just below the GLP icon. LC on *On.*
6. The icons for the pump and column should turn green. The DAD should start warming up as indicated by the yellow color. It is ready for a qualitative run when the icon is green. For quantitative work it should warm up at least 30 minutes.

Injection of a Single Sample

1. LC on the Single Bottle icon below the *START* button. LC *sample information.* Enter your name in the *Operator box*. Enter your last name in the *Subdirectory* box. For *Prefix* enter your first name. For *Location* enter Vial 1 or whatever vial the sample is in. Fill in some very descriptive information about the sample to be run. This ought to include a reference to the page in your lab book, because after fifty samples it is impossible to keep track of them without this documentation. After entering this information, LC on *OK*.
2. LC on the injector icon. LC on *Set Up Injector.* Note where the methanol wash vial is located in the autosampler and check to see that it has methanol for the programmed runs.
3. LC on the *START* button and your run should begin as soon as the autosampler has injected it. The start of the run is indicated on the chromatogram by a vertical red line at 0 time.

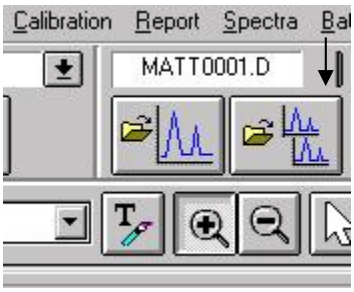
Starting of a Sequence Run

Under the Main Method and Run Control window LC on *Sequence*, LC on *Sequence Table* and enter the data for your runs in the sequence. For runs that do not use buffer, make sure that the last run uses the method "Exit" if the system needs to be shutdown at the end of the sequence. You can start the run on the Sequence Table or on the Main Method and Run Control window by first LC on the three bottle icon and LC on Start.

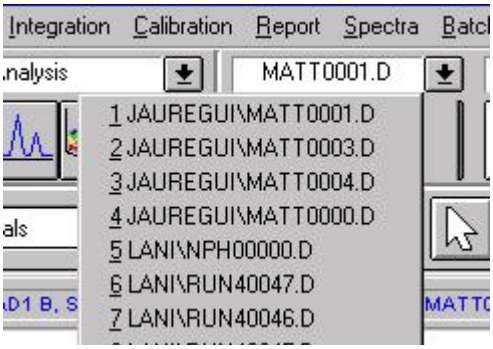
Stop or Abort?

Abort will end the run without saving the data file. Stop will end the run and will save the data file.

Looking at Previously Run Data



Go to the Main Data Analysis window. Data file names are in the form [Prefix]0001.D. Find the Data File List and LC on the ↓.



A dropdown list of recently run file names will appear. LC on the file you want.

To look at older data files LC on *File*, LC on *Open Signal*, and choose the data file you are looking for. Normally, the first step will be making sure that the chromatogram is properly integrated. Once this has been accomplished, you should specify the format for the report. This is found under *Report* as *Specify Report*. Typically this is specified as the "short" format under *Report Style*. If you need to estimate the noise level, you can use the "performance + noise" format.