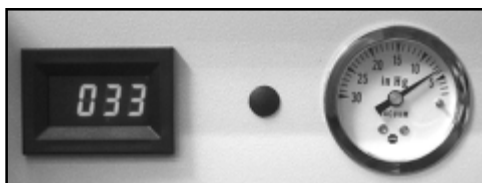
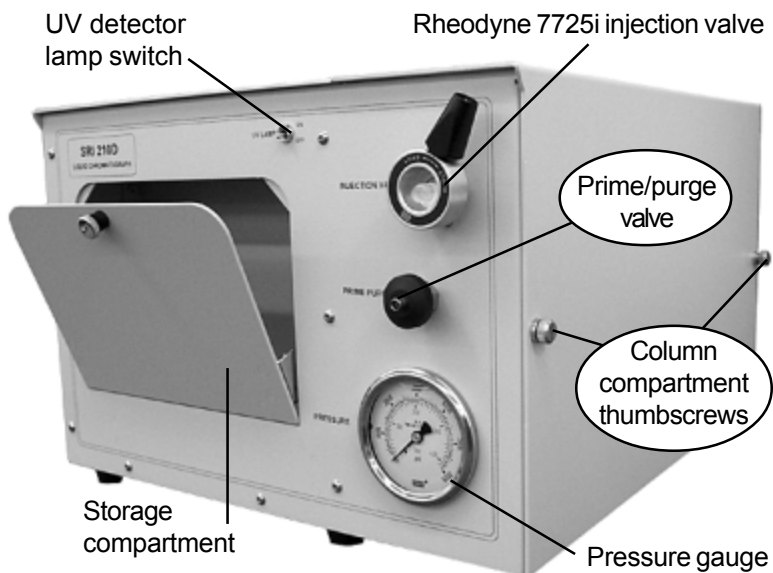


1. Overview

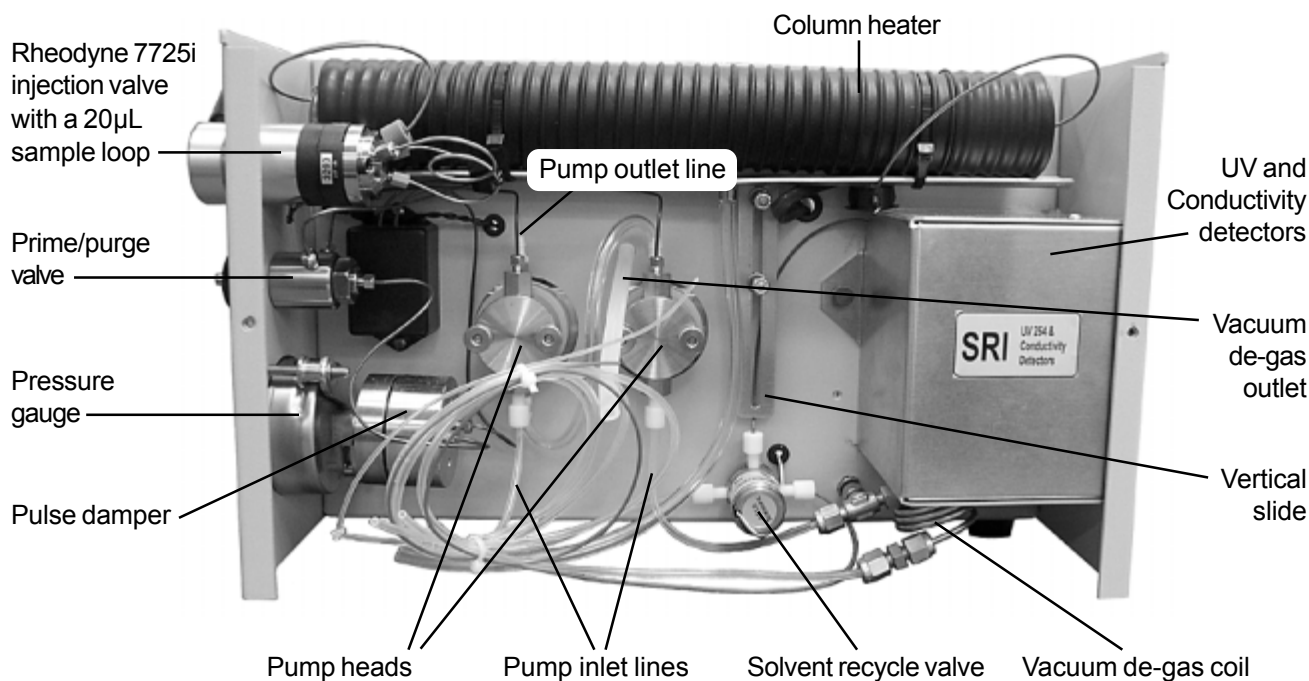
The Model 210D front panel houses the UV detector lamp switch, Rheodyne injection valve, prime/purge valve, pressure gauge, and the storage compartment. In the storage compartment are the LED display for the column temperature, and the vacuum degas pressure



gauge (shown above). An accessories kit is included with your 210D. In the kit are two PEEK finger tight nuts (Alltech part # 32233) for column connection, a spare 1/16" stainless steel fitting (Valco part ZN1) and ferrule (Valco part ZF1S6), a flange fitting (Upchurch part 203X) and ferrule (Upchurch part 240X) for the solvent recycle valve, a 20mL priming syringe (Sigma Aldrich part Z248037), and a 100µL glass injection syringe (SGE part 005300).



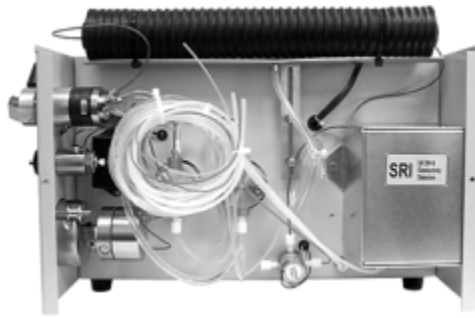
On the right hand side of the 210D is the column compartment, which gives you access to the column heater, pump head(s), solvent recycle valve, and the detector housing. Open the column compartment by loosening the two captive thumbscrews and lifting off the cover. Use the picture below to familiarize yourself with the interior of the column compartment.



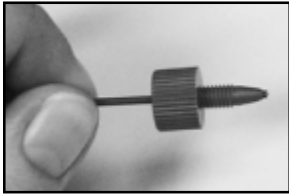
HPLC Model 210D

2. Installing a Column

Slide the column heater up.

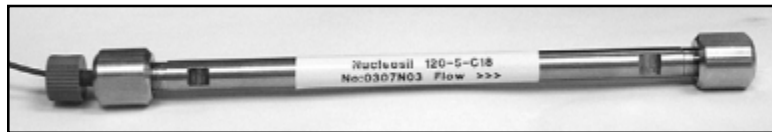


Slide the column heater up on its aluminum slider.

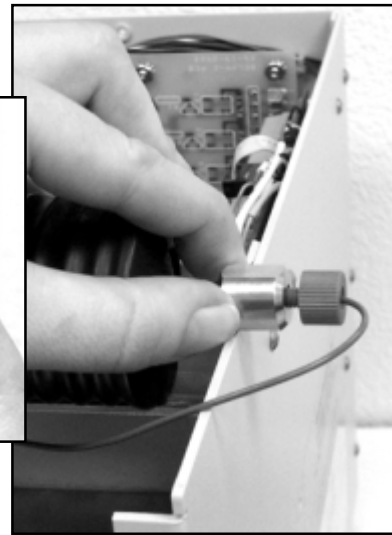
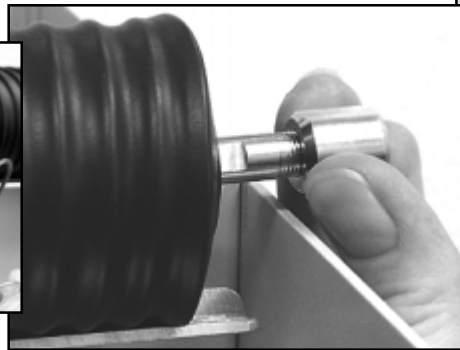
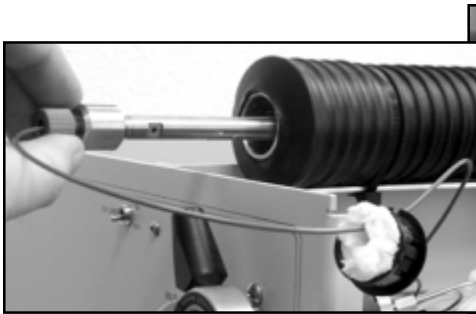


Install the column of your choice using the included PEEK finger tight nuts. Slide the nuts onto the PEEK tubing, letting about 1 mm of PEEK tubing protrude.

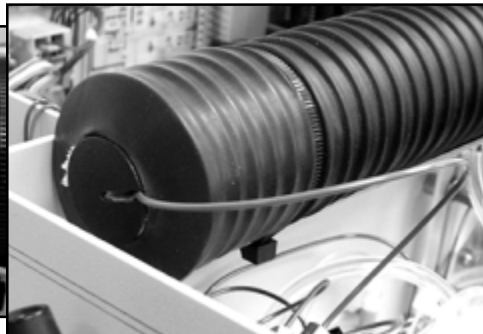
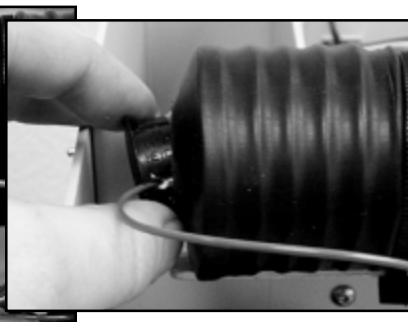
Secure one nut to the column.



Slide the column through the column heater so you can reach the other end, and attach the second nut. Roughly center the column in the column heater.

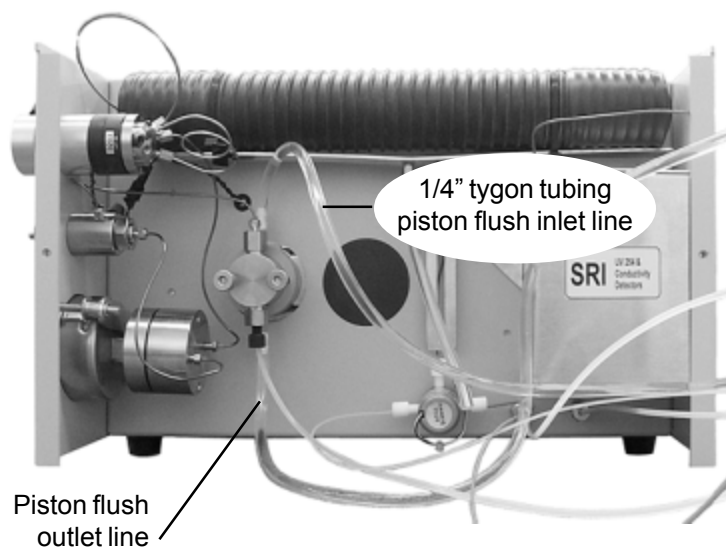
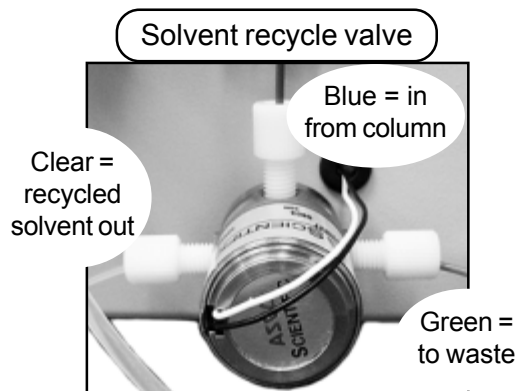


Replace the black column heater end caps, which have notches in them for the PEEK tubing. Pack in any loose insulation. When you have replaced both end caps as shown below, slide the column heater back down into the operating position.



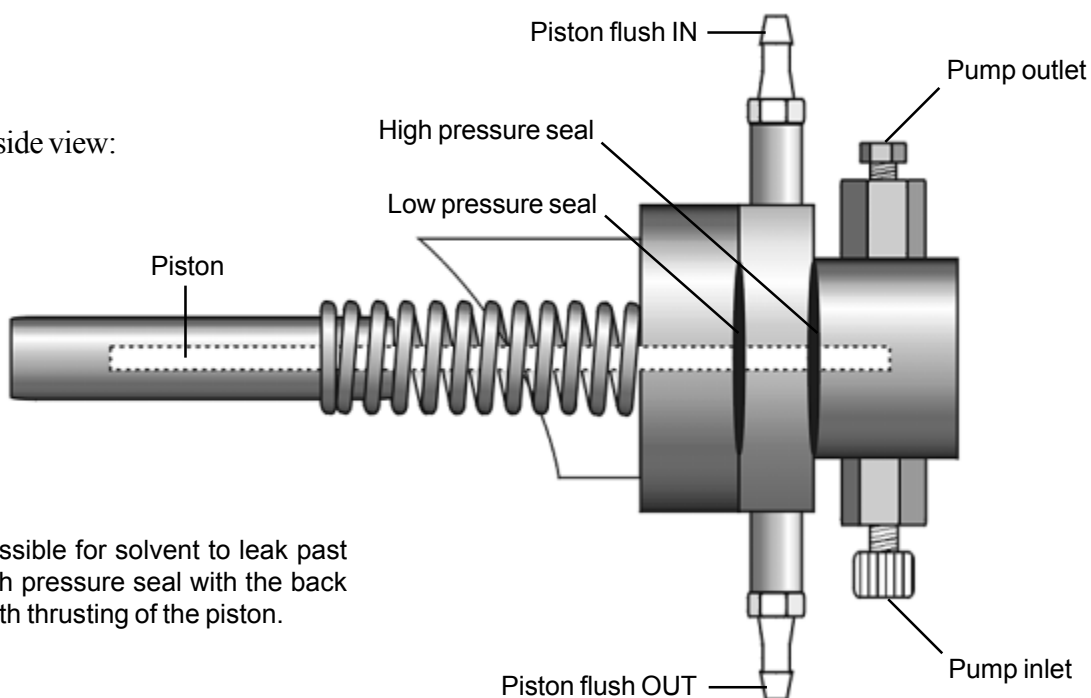
3. Solvent Set-up

The pump heads each have an 1/8" Teflon solvent inlet line. Place the ends of these lines in your solvent bottle. The solvent recycle valve has two outlet lines. The 1/16" clear line is recycled solvent. Put it into the solvent bottle, or into its own container if you wish to keep it separate from your fresh solvent. Place the end of the 1/16" green line into a waste bottle. A simplified way to remember: all the clear lines go to the solvent bottle. In default mode, the solvent recycling valve sends the effluent out the clear tube; when activated, it directs the effluent out the green waste tube.



The 1/4" Tygon tubing attached to the pump head(s) is the piston flush line. Piston flushing helps keep the high pressure seal(s) clean, minimizing wear and tear from crystallized and abrasive buffer deposits that leak through the seal, and would otherwise dry on the piston. There are inlet and outlet lines for piston flushing. Place the ends of both into a bottle containing at least 50% organic solvent (usually diluted with deionized water). Binary gradient pump head piston flush lines are daisy chained, and share the same in and out lines.

210D pump, side view:



It is possible for solvent to leak past the high pressure seal with the back and forth thrusting of the piston.

HPLC

Model 210D

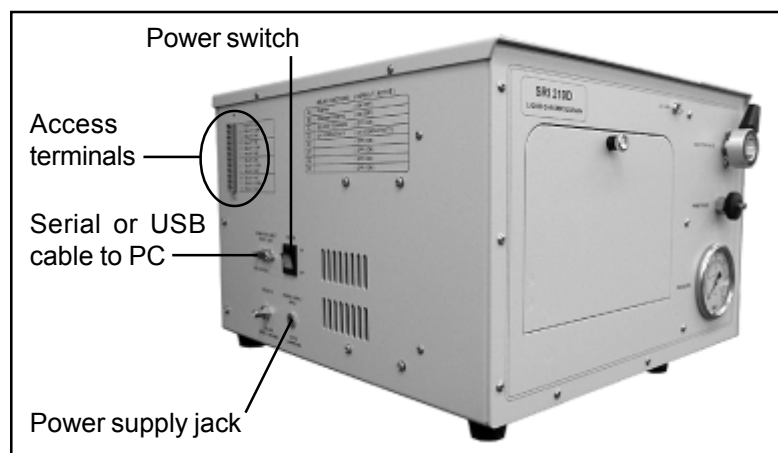
4. Vacuum De-gas

While not all HPLC users degas their solvent, it is a good idea to do so. The 210D is equipped with an air pump for vacuum degassing. A length of tygon tubing is provided to connect the pump to the degas fitting on the left-hand side of the HPLC. The tubing is equipped with a nut for easy connection to the degas fitting. With the 210D power ON, connect the pump tubing to the degas fitting, then plug the pump into a wall outlet. Open the storage compartment to see the vacuum degas gauge, which should read 7psi with the pump ON and solvent lines attached.



Simply screw this nut onto the degas fitting to connect the vacuum pump to the 210D.

The vacuum degas gauge should read 7psi during operation.



5. Computer Connection

Connect your 210D to your Windows™ computer with the provided serial or USB cable. The cable connection port is located on the left-hand side of the 210D.



6. Power Supply

Your 210D comes with a universal (115V or 230V) benchtop 12 volt power supply (shown at right). Plug the small power source cord into the jack on the left hand side of the LC. Plug the larger power cord into a wall outlet. Turn the power ON with the power switch, located above the power supply jack. The 210D can be powered with any regulated or unregulated voltage in the range of 11-15 volts at 80 watts (approximately 7 amps), including a car battery via the cigarette lighter.

7. Installing PeakSimple software



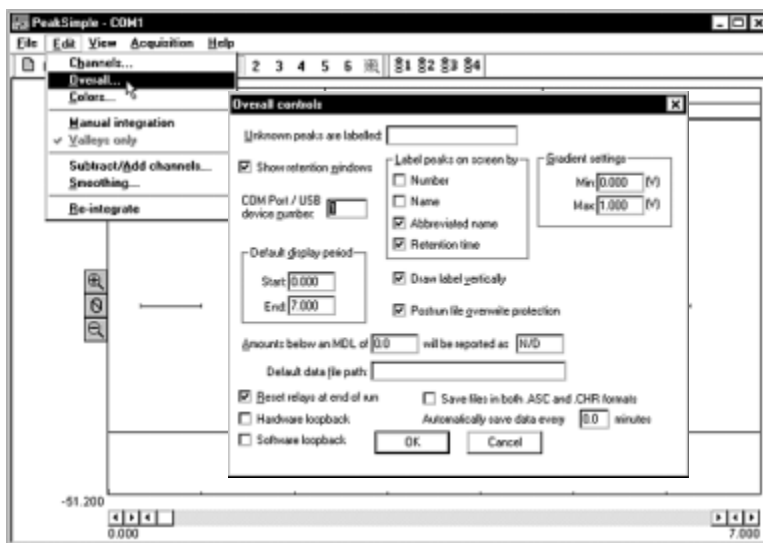
You will find your PeakSimple installation disk just inside the front cover of your manual. Install the PeakSimple software by inserting the PeakSimple CD into the CD-ROM drive of your Windows™ computer. Double-click on My Computer, then open the CD-ROM drive. Double-click on the “setup.exe” program icon and follow the instructions.



8. Double-click on the PeakSimple icon to launch the program. Verify that communication is established between the computer and the pump. An error message (shown at left) will appear if communication is not established.

9. Load the LC.CON control file from your PeakSimple program folder. This control file contains all the necessary information to configure PeakSimple for HPLC.

10. Verify that the correct I/O port is specified in the Edit/Overall screen. By default, COM 1 is entered in the Edit/Overall screen because many Windows™ computers have COM 1 designated as the serial port. Other computers may use COM 2, COM 3, or COM 4. You may have to examine the My Computer/System screen to determine what serial port numbers Windows has assigned to the hardware in your particular computer. If you upgraded to the 6 channel USB data system, enter the USB device ID number in the box instead. USB device ID numbers are unique to each instrument, and the number for your 210D is printed on the HPLC under the cable port, and also on your PeakSimple disk. Once you have successfully gotten the data system to sign on, click Save All so you don't have to repeat this step the next time you open PeakSimple.



HPLC

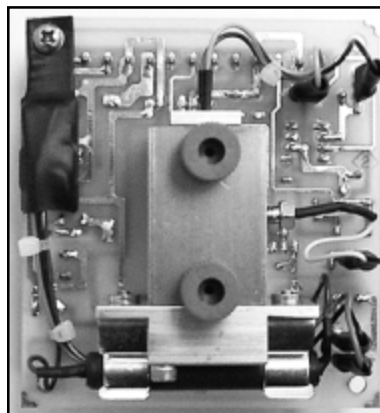
Model 210D

11. UV Detector

The UV detector signal is connected to channel 1. The detector uses a low volume flow-through cell. The cell temperature is factory set to 40°C, which is suitable for a wide range of common applications.

Note for those who have the 4 or 6 channel data system upgrade: Unless you have connected an external detector to the 210D, the column temperature will display on channel 3, and the detector cell temperature will display on channel 4.

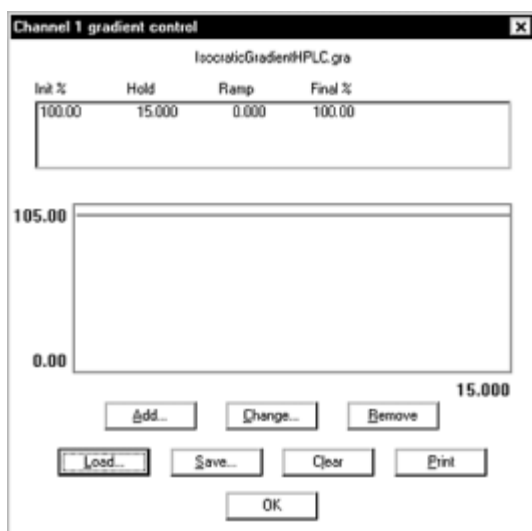
Both detectors are housed in the cell in the middle of this board.



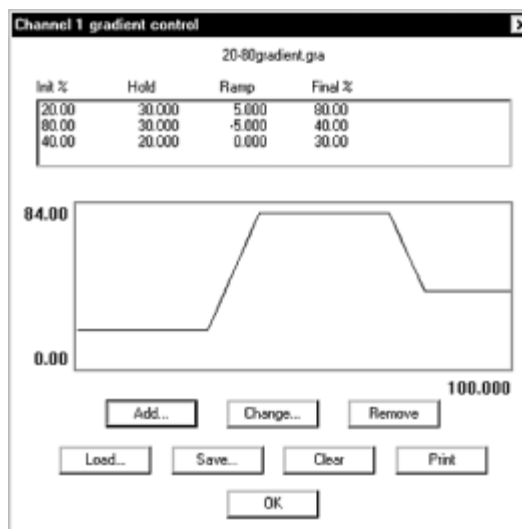
12. Setting a Gradient Program

Click on the Gradient button for channel 1. If your system is isocratic, the Initial and Final percentages are 100%. The only variable is the Hold time period, which is determined by the length of your analytical run. Setting a gradient program automatically starts the pump at a very low speed. For binary gradient systems, load a gradient program from your PeakSimple program folder (version 3.19 and later) by clicking on the Load button in the Channel 1 gradient control window. Use the Add button to create a new gradient.

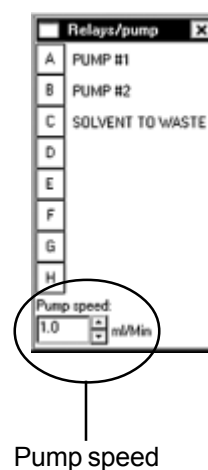
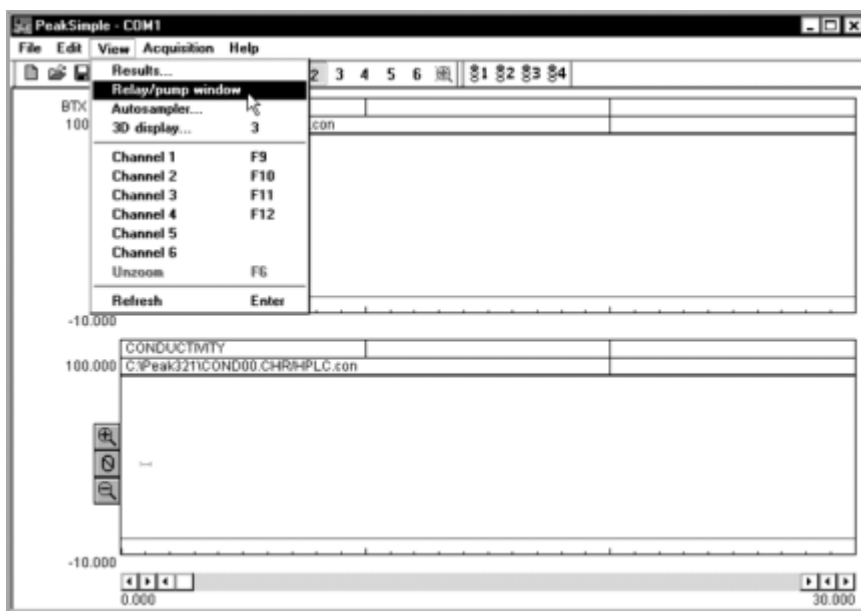
Isocratic gradient



Binary gradient



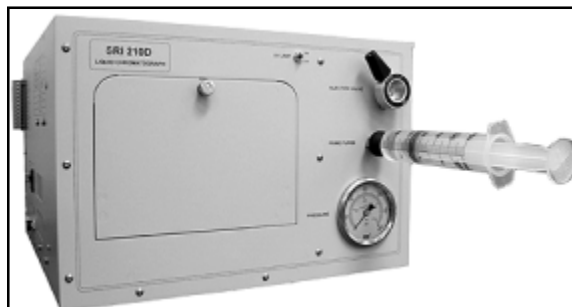
13. Click View and choose Relay/pump window. In the Relays/pump window, click on Relay A to turn the pump OFF, since you haven't primed it yet. The flow rate of the pump (pump A in a binary gradient system) can be adjusted from 0.1 to 5.0mL/minute, in increments of 0.1mL/minute, using the dialog box at the bottom of the Relays/pump window. In a binary gradient system, the gradient program determines how the flow is split between the two pumps.



14. Priming the Pump

Note: Do not open the prime/purge valve until pressure is at zero, or very close. Otherwise, system pressure could damage the pulse damper or cause the column adsorbent bed to shift.

Open the prime/purge valve by turning it to the left, then start the pump by clicking on Relay A in the pump window. Insert the priming syringe into the prime/purge valve, then slowly and gently pull back the plunger. When you can pull a bubble-less stream out of the prime/purge valve, close the valve by turning it back to the right. The pressure should begin building to operating range, which is dependent upon the flow rate, solvent viscosity, and the back pressure of the column selected. When the system stabilizes at the operating pressure range, you may inject your sample. It is normal for the pressure gauge needle to fluctuate with the pump stroke.



HPLC

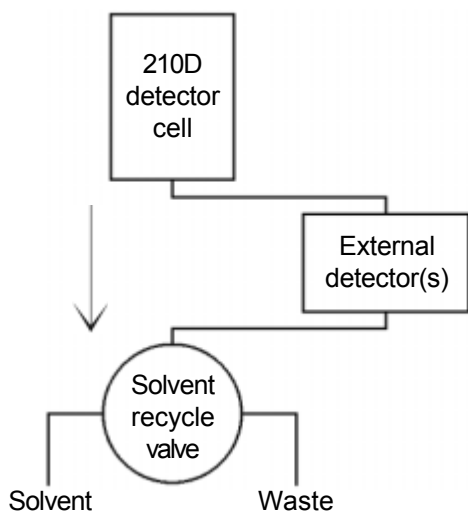
Model 210D

15. Solvent Recycle Control

Relay C controls the solvent recycle valve. When Relay C is ON, the solvent is directed to waste (green exit tube). Turn Relay C ON before any peaks appear onscreen, and turn it OFF at the end of the run. Click on Relay C in the Relays/pump window to turn it ON and OFF, or use an event table to do it automatically. The solvent recycle valve may also be used as a single peak fraction collector by turning it ON/OFF at specific times during the run.

Time	Event
0.500	C ON (SOLVENT TO WASTE)
15.000	C OFF (SOLVENT TO WASTE)

Example event table for the solvent recycle valve



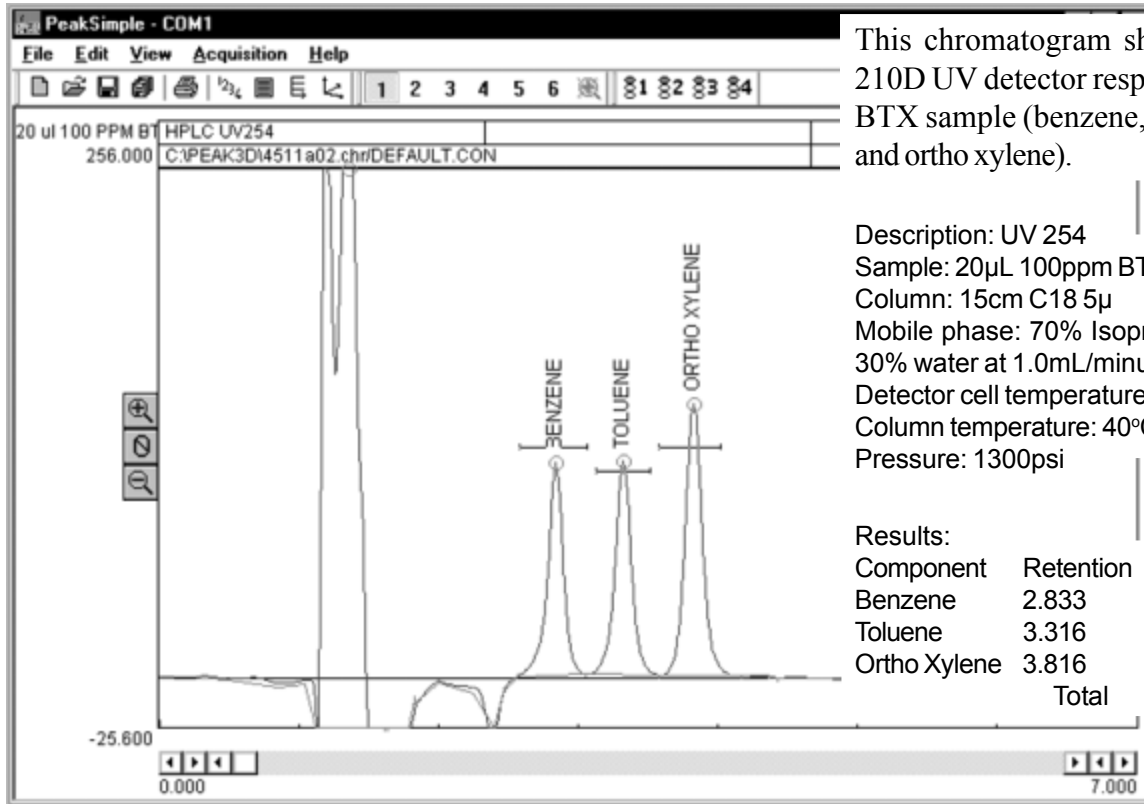
The detector exit tube that connects to the top of the solvent recycle valve may be disconnected, and connected to external detectors, like refractive index or fluorescence detectors. The external detector's exit tubes are then connected back to the solvent recycle valve.

16. Injecting a Sample and Starting the Run

The Rheodyne injection valve comes with its own blunt-tipped flushing needle, which is shipped inserted into the valve under the protective red cap. Remove the protective cap and tape, then pull out the needle. The provided 100 μ L glass injection syringe should suit most injection applications. For the best results, prepare your sample in the same solvent you are using for your mobile phase. Fill the syringe with sample, and eliminate any air bubbles. Insert the syringe into the Rheodyne injection valve, pushing gently until the needle hits the stop. Depress the syringe plunger to fill the 20 μ L sample loop. Leave the syringe in the while you turn the Rheodyne injection valve knob to the INJECT position. The run will automatically start. See the Rheodyne documentation in your SRI manual under HPLC Injectors for more information about operating the injection valve.



Expected Performance

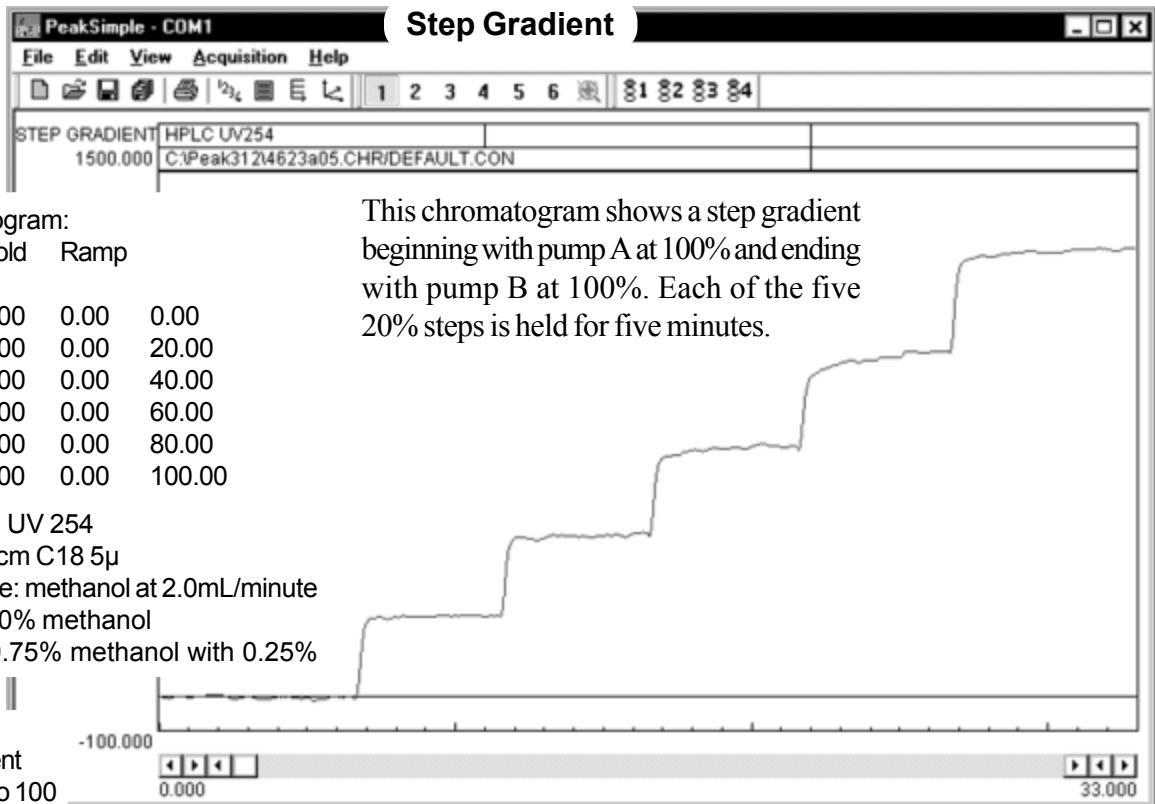


This chromatogram shows the 210D UV detector response to a BTX sample (benzene, toluene, and ortho xylene).

Description: UV 254
 Sample: 20µL 100ppm BTX
 Column: 15cm C18 5µ
 Mobile phase: 70% Isopropyl Alcohol, 30% water at 1.0mL/minute
 Detector cell temperature: 40°C
 Column temperature: 40°C
 Pressure: 1300psi

Results:

Component	Retention	Area
Benzene	2.833	851.1640
Toluene	3.316	912.9170
Ortho Xylene	3.816	1207.1795
Total		2971.2605



Step Gradient

Gradient program:

%pumpA	Hold	Ramp	%pumpB
100.00	5.00	0.00	0.00
80.00	5.00	0.00	20.00
60.00	5.00	0.00	40.00
40.00	5.00	0.00	60.00
20.00	5.00	0.00	80.00
0.00	5.00	0.00	100.00

This chromatogram shows a step gradient beginning with pump A at 100% and ending with pump B at 100%. Each of the five 20% steps is held for five minutes.

Description: UV 254
 Column: 15cm C18 5µ
 Mobile phase: methanol at 2.0mL/minute
 Pump A: 100% methanol
 Pump B: 99.75% methanol with 0.25% acetone

Events:

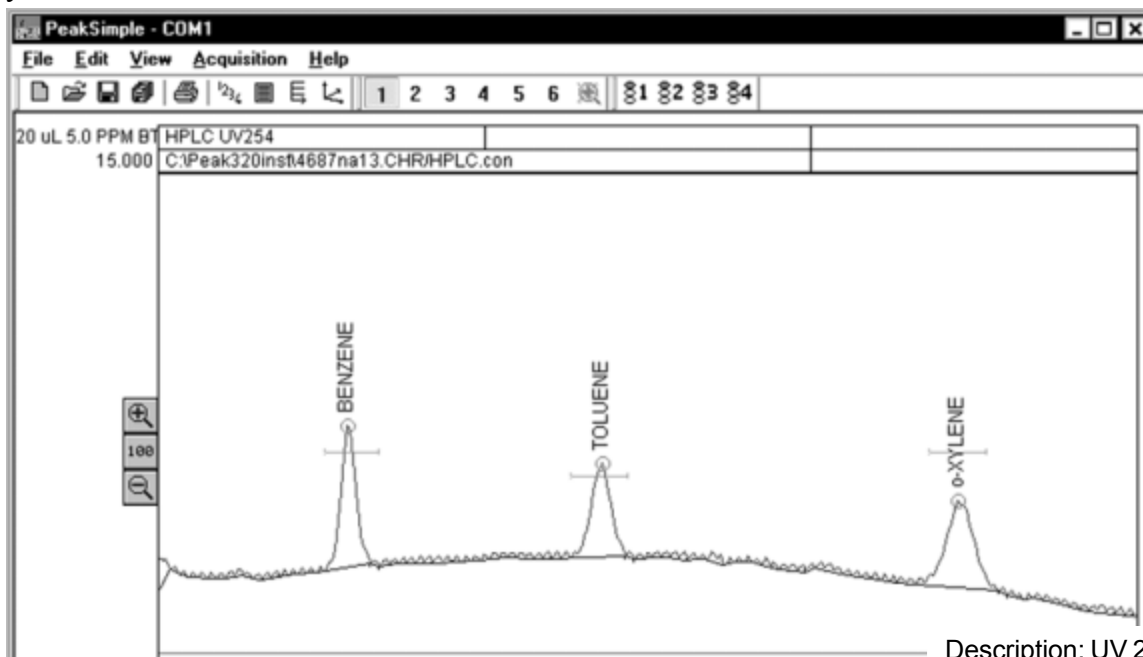
Time	Event
0.00	Zero 100

HPLC

Model 210D

Expected Performance

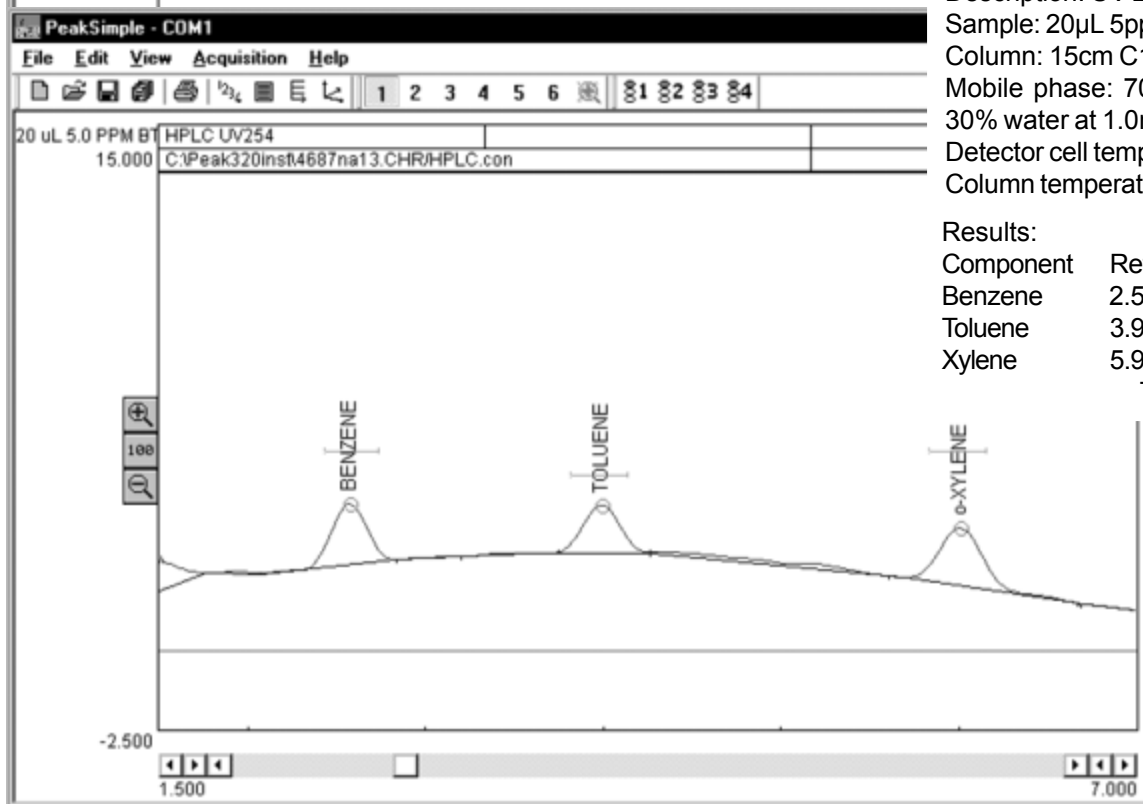
The first chromatogram is a UV separation of 5ppm BTX with an isocratic 210D system. The second chromatogram shows the same run after using PeakSimple's data smoothing function. Short term up/down noise is a function of pump refill. Binary systems are therefore noisier than isocratic systems. PeakSimple's smoothing feature can reduce the pump noise for a clearer picture of your data. The data in any channel can be smoothed automatically using Post-run actions for that channel. See the "PeakSimple Software" section of your manual for more information.



Description: UV 254
Sample: 20µL 5ppm BTX
Column: 15cm C18 5µ
Mobile phase: 70% Methanol,
30% water at 1.0mL/minute
Detector cell temperature: 40°C
Column temperature: 40°C

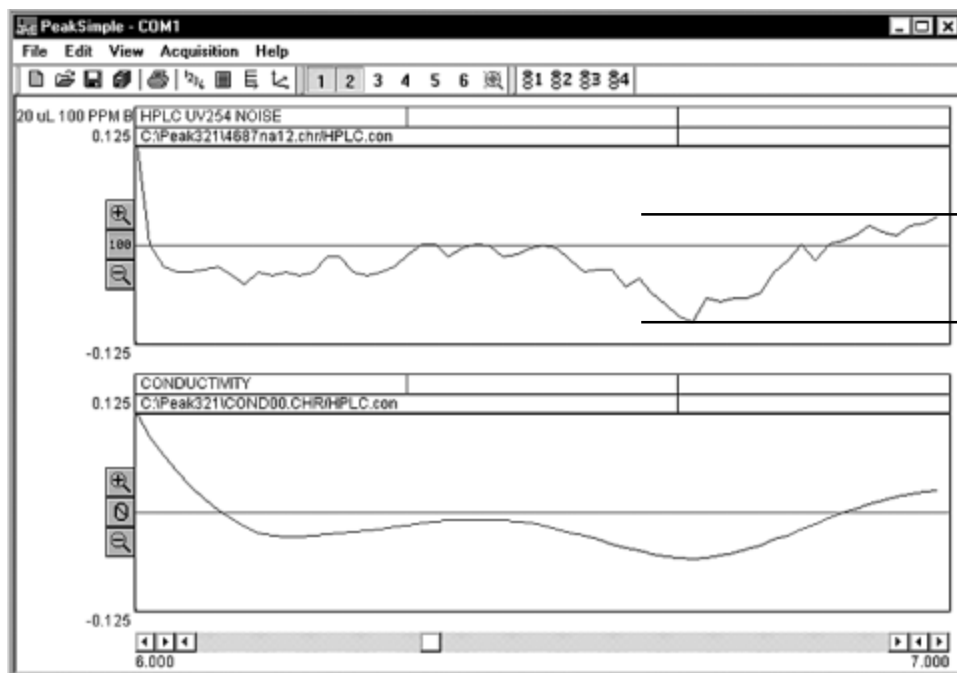
Results:

Component	Retention	Area
Benzene	2.550	26.01
Toluene	3.983	22.69
Xylene	5.983	29.90
Total		78.60



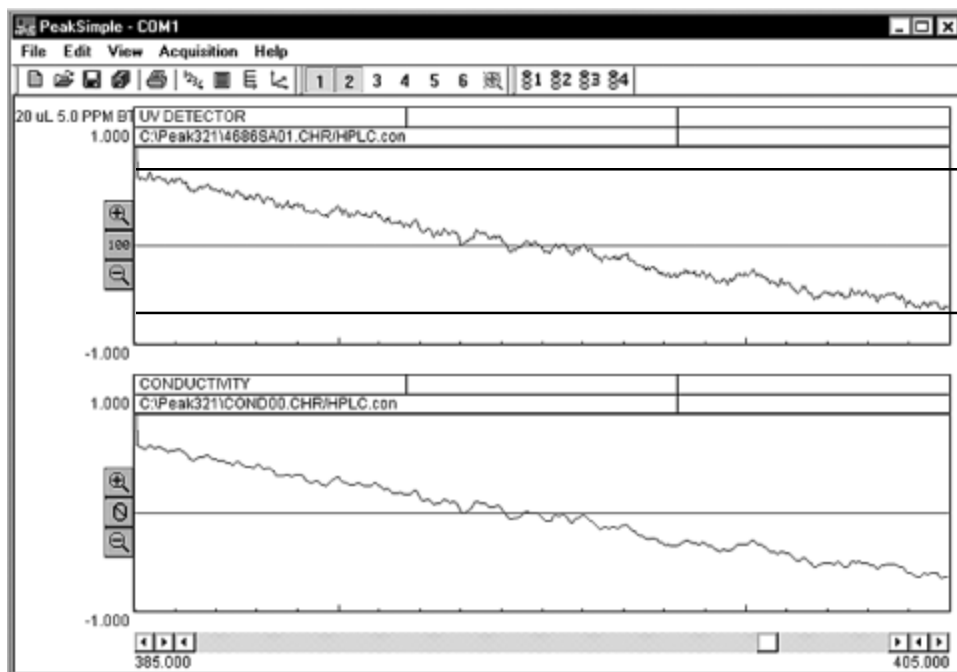
Expected Performance
UV Detector Noise

This pair of chromatograms shows a short-term, one minute UV detector noise run on an isocratic 210D system. The first chromatogram is the raw data, and the second shows it after smoothing.



UV detector noise averages approximately $100\mu\text{V}$ from peak to peak, or 10^{-4} a.u. (absorbance units).

This pair of raw and smoothed chromatograms shows 20 minutes of a UV detector noise run on an isocratic 210D system. In the longer time segment, the signal drift is visible.



This UV detector signal drift averages approximately 1.3mV in 20 minutes, or 0.0013 a.u.

HPLC

Model 210D

General Information

Smoothing: Why Use It?

Short term up/down noise is a function of pump refill. Most other manufacturers smooth data inside detector. SRI does not, so pump noise is visible. Binary systems are therefore noisier than isocratic systems. PeakSimple's smoothing feature can reduce the pump noise so you can more clearly picture your data. Pump noise peaks are much narrower than data peaks, and do not obscure, esp after smoothing is applied.



To smooth data, open the chromatogram of your choice in channel 1. Any channel will work, this is just an example. Open the **Data smoothing** window by clicking on the **Edit** pull-down menu, and choosing **Smoothing...** Specify the source channel (the channel in which your chromatogram is open), then specify the channel in which you want the smoothed chromatogram to display. Even if you have a single channel data system, PeakSimple gives you 6 channel screens in which you can open, compare, and edit your chromatograms. Next, choose one of the three available smoothing methods by clicking in the radio button beside it. If you choose Savitzky-Golay, enter the order. Type in the desired filter width and iterations (you may need to experiment to find the best combination for your data). Click the Apply button, and your smoothed chromatogram will appear in the destination channel you designated.

Absorbance VS Transmittance

Other manufacturers make the conversion from transmittance to absorbance in the hardware. To help keep products affordable and portable, SRI does the conversion through PeakSimple software. With the UV detector lamp OFF, the data system signal should be at or very close to zero. Click on the Auto Zero button on the channel 1 chromatogram window to zero the signal at 0% transmittance (0%T). Turn ON the UV lamp switch. The signal should jump to 3-6mV. This signal is 100% transmittance, or 0 absorbance units. Click on the Zero 100 button to zero the signal with just the solvent flowing (this is 100% transmission). Keep in mind that light absorbance is exponential. For example, twice as much toluene does not equal twice as much absorbance. See the conversion chart below:

% Transmittance	Absorbance units	Millivolt signal readings
100%	0	0mV
10%	1	1000mV
1%	2	2000mV
0.1%	3	3000mV
0.01%	4	4000mV
0.001%	5	5000mV