Chemistry 306a
High Performance Liquid Chromatography
Perkin-Elmer Series 400

Operation:

1) Fill solvent reservoirs with the desired solvent and degas. Make sure that caps for the reservoirs are not cracked, that "O" rings are properly seated, and that cover is placed squarely on reservoir. Degasing is accomplished by bubbling a fine stream of helium through the solvent.

To degas a particular reservoir, open the SEC-4 door and pull the plungers to the corresponding reservoirs. Turn on the helium at the tank and set the regulator to approximately 60 psi. Shut and latch door at conclusion of degassing.

2) Turn on the Series 400 by pressing the ON/OFF rocker switch located on the lower rear panel of the instrument.

3) Set solvent pressure limitations [P-Lim]. Press this key to set upper and lower pressure limitations. Upon pressing P-Lim, "Max" is displayed on right side of display and the current pressure is displayed on left side of display under "Pressure". Type a desired pressure and press [Enter]. Pressing [Enter] without making the requested maximum entry, accepts the displayed pressure. Press [Toggle] to display the minimum current denoted by "Min" displayed on right side of display. The minimum pressure must have a non-zero value (50 psi).

4) Prime the Series 400 by connecting the priming syringe to the FLUSH PORT and "RUN" Method 0. Connect the supplied 30 mL lever-lock syringe to the FLUSH PORT on the front of the Series 400. Turn the PRIME/PURGE VALVE above the FLUSH PORT approximately one turn counterclockwise to open the valve.

Press the [Recall] key, then press 0 followed by pressing [Enter] to make Method 0 your current method, if it is not your current method. Press [Run] to start pump.

When "Ready" is displayed press [Run] again and collect ~8-10 mL of Reservoir A. Press the forward arrow [-->] to purge the next reservoir (Reservoir B).

NOTE: 100 is displayed below the reservoir (A%, B%, C% or D%) you
When the syringe is full, press [Stop] to stop the pump and empty the syringe in the solvent waste container. Press [Run] to restart the pump (noting where the 100 is displayed). Press [Run] to restart the pump (noting where the 100 is displayed). Press [Run] again to start the method and press the forward arrow [-->] to remove the 100 until it is under your desired reservoir.

After all the reservoirs are purged, press [Stop] to stop the pump, and turn the PRIME/PURGE VALVE clockwise until closed (snug).

5) Generate and store a method.

The following functions are applicable when you are generating or modifying a method:

After typing a value, press [Enter] to retain the entry. You must type a value in a blank field. If you do not wish to use a particular reservoir, then type 0, and press enter. Typing a flow of "0" will stop the pump during that Step for the amount of time you entered for the step.

If you type a value and wish to change it, before you press [Enter] press [CE] and type the new value.

Press the backspace arrow to review values in a displayed Step.

To generate a new method press [Prog] key then press the [CE] key to clear the display and start you at the beginning (Step 0) of a new method. "Step 0" is generally used to equilibrate the system before running gradients (A prompt bar is displayed over "Time", the parameter where you make your first entry. Also, the instrument status "Program" is displayed and "Store" is blinking.)

Type the required "Time" for "Step 0" and press [Enter]. The acceptable range is 0.1 to 99.9 m. Five minutes is a good equilibration time. Once you press [Enter], the prompt bar is displayed above "Flow."

Type in the required "Flow" rate for "Step 0," then press the [Enter] key. The acceptable range is from 0.01 to 10 mL/min.

Type in the required percentage of Reservoir A, then press [Enter]. If you do not wish to use this reservoir, press "0," followed by pressing the [Enter] key.
Type the required percentage of Reservoir B, then press [Enter]. If you do not desire B then press "0" followed by [Enter].

Likewise for Reservoir C. The compliment percentage to total 100% is automatically entered and displayed under Reservoir D.

The timed events are displayed to the right of %D. "TE1" indicates that the time event is inactive. Press [Toggle] to display "TE1 On" (active), this puts the prompt bar over "Time" and sets the "Time" display to "0.0". Type the desired time for the timed event to switch on, then press [Enter]. A time of 0 causes the timed event to switch on at the beginning of the Step. After pressing [Enter], "On" is displayed to remind you that "TE 1" is active during this Step. The range is 0.00 to 99.9 minutes. "TE 2" is set in the same way.

Remember to press [Toggle] to display time event options (on and off) and press [Enter] to accept your options and move to the next parameter. If you do not want a timed event in that Step, then simply press [Enter] when "TE 1" to "TE 2" is displayed.

The next entry is "Time" for "Step 1". Follow the same procedure as previously mentioned. After the required percentage of Reservoir C is entered, the linear gradient prompt (Lin Grad) is displayed to the right of %D. If a linear gradient from "Step 1" to "Step 2" is desired, press [Enter]. If a Step change is desired, press [Toggle] when "Lin Grad" is displayed to display "Step Grad." Press [Enter] to program a step change from "Step 1" to Step 2." Press [Enter] to ignore timed events. Continue the method generation procedure for the remaining Steps in your method. When your method is complete and you wish to exit the method generation, type a Time of "0" followed by pressing [Enter] or exiting by pressing [Prog].

After generating a new method it is advisable to store the method in memory for future use. Nine methods may be stored in the Series 400.

Before storing, insure that the pump is not running by observing that "Run" is not displayed on screen next [Run]. A running method cannot be stored. Press [Store]. This causes a blank display under "Method" and "Store" stops blinking.

Type a desired method number (from 1 to 9) which will appear under method then press [Enter]. If the prompt "Program Exists" is displayed, select another number, and press [Enter]. If you wish to overwrite a stored method press [Enter] when
Another way to store a current method is to press [Store] then [Enter] and the program will be stored under the next available number. When "Store" is displayed pressing [Store] will always exit that routine.

To recall a method, press [Recall] then type number of method when "Recall" is displayed and press [Enter].

To modify a method once it has been recalled, use the Forward Arrow [-->] to move from Step to Step and [Enter] to move to the right from parameter to parameter within a Step. The Backspace Arrow [<--] moves one parameter to the left until prompt bar is over "Time," then pressing it decreases the "Steps" by one.

Pressing [Prog] at any time will retain all the values and exit from modifying. You must "Store" method if it is modified.

7) Press [Run] to start the pump and equilibrate the system.

8) When "Ready" is displayed, press [Run] to start method.

9) Press [Stop] at any time to stop the pump.

10) To shut down
   Remove harmful mobile phases - buffers and acid forming organics such as chloroform.
   Flush with isopropanol after harmful mobile phases have been removed.
   Flush with 60%/40% MeOH/water.
   Print methods you wish to retain.

   The current method can be printed on the LCI-100 integrator.
   Press [Store] then [.] to print method.

   Turn OFF pump, detector and integrator power.