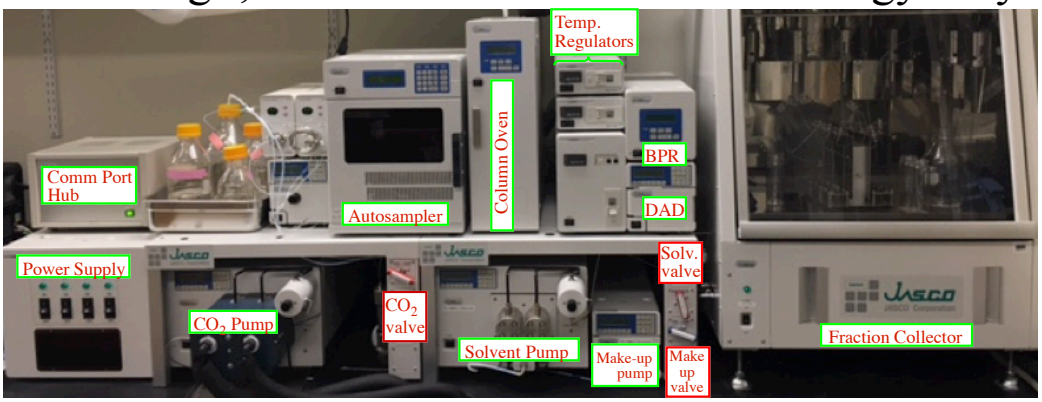
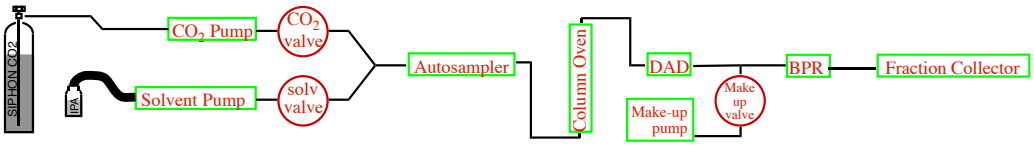
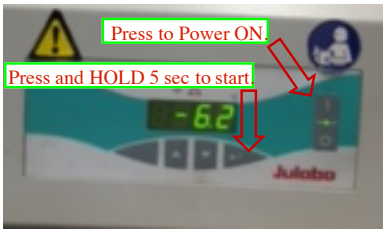
Jasco Preparative SFC System and SF-NAV Instructions

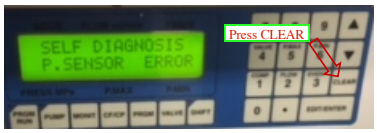
-Scott Virgil, California Institute of Technology May 2016



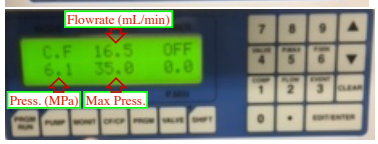




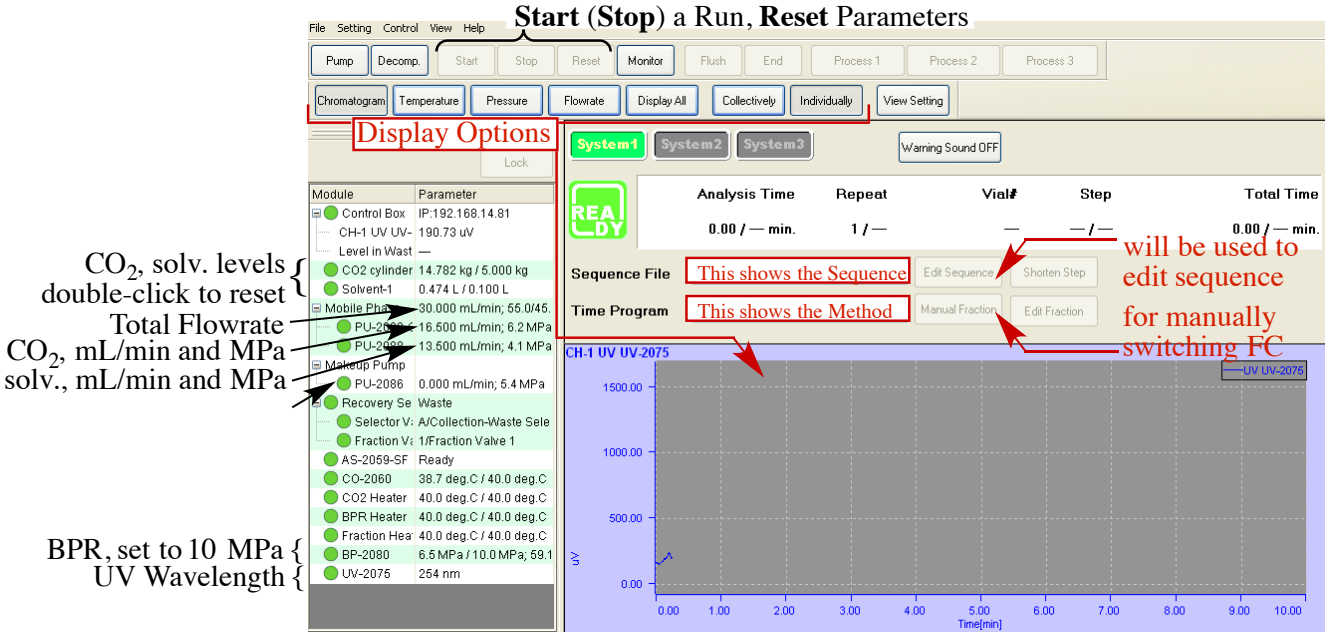
<u>Step 1:</u> Open CO_2 tank and Check solvent levels. Turn on Julabo Chiller. The chiller needs to cool to -5 °C so that the CO_2 pump can draw the liquid CO_2 efficiently. Open CO_2 Valve, Solvent Valve and Make-up Valve (if the Make-up Pump will not be used it can remain closed)**.**



<u>Step 2:</u> Turn on all power switches for each individual component. If any component reads "**Self Diagnosis P. Sensor Error**" then press the "**CLEAR**" Button. **Then press the "MONIT" button to give the reading of pressure, max pressure and flowrate. 10 MPa = 100 bar.** So, it can be seen that the max pressure at right is 350 bar.

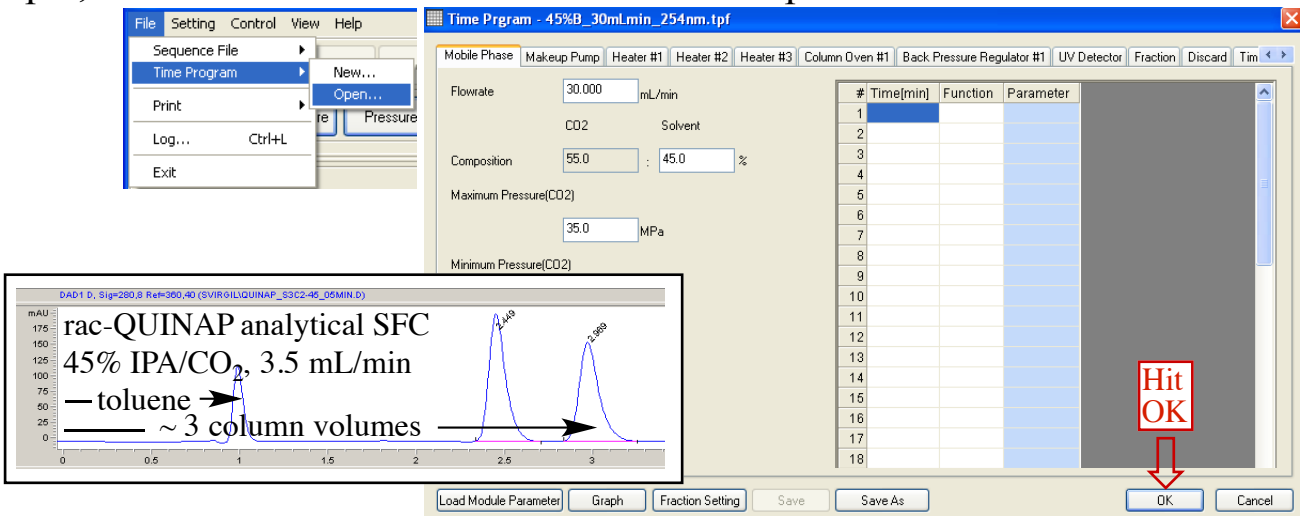


<u>Step 3:</u> Open SF-NAV Monitor. (All components should have a green dot.)



Step 4: LOAD TIME PROGRAM with desired flowrate and %B.

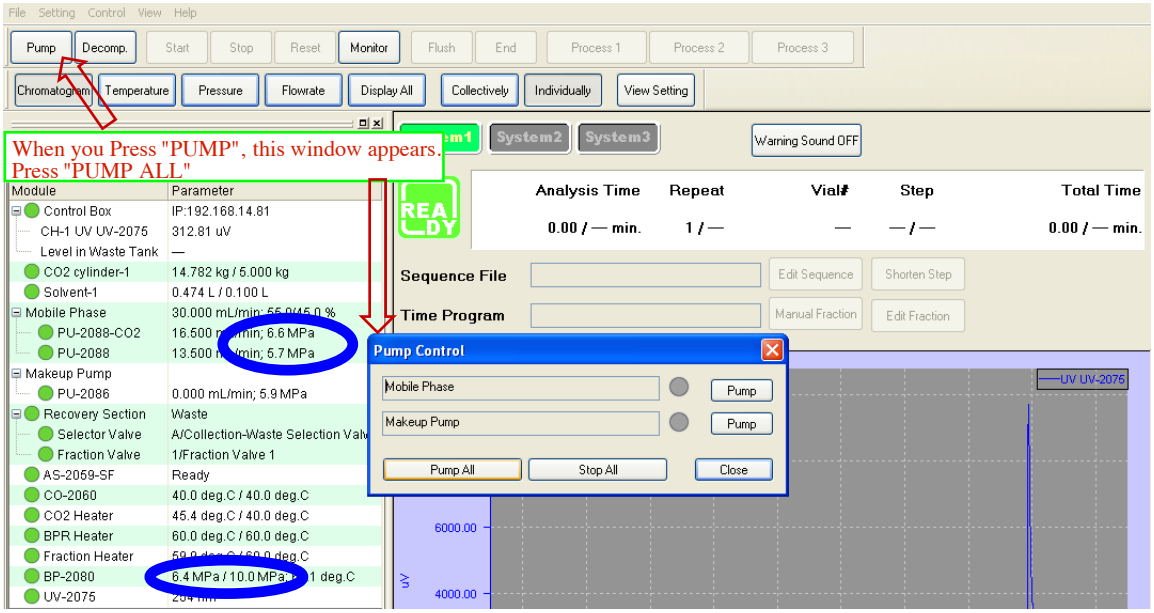
A set of starter time programs for various flowrates and %B are saved in the "METHODS" folder and write-protected to ensure reliability. If you have a compound that requires a UV wavelength other than 254nm, you will need to change the UV detector wavelength and **Save As** under a new method name. In this example, 45% IPA was chosen which elutes both peaks in 3 column volumes.



Before continuing, make sure that there is a column in the Column Oven. Also be sure that no detrimental solvents (EtOAc, CH_2Cl_2) remain in the system that could damage particular DAICEL columns (AD-H, OD-H).

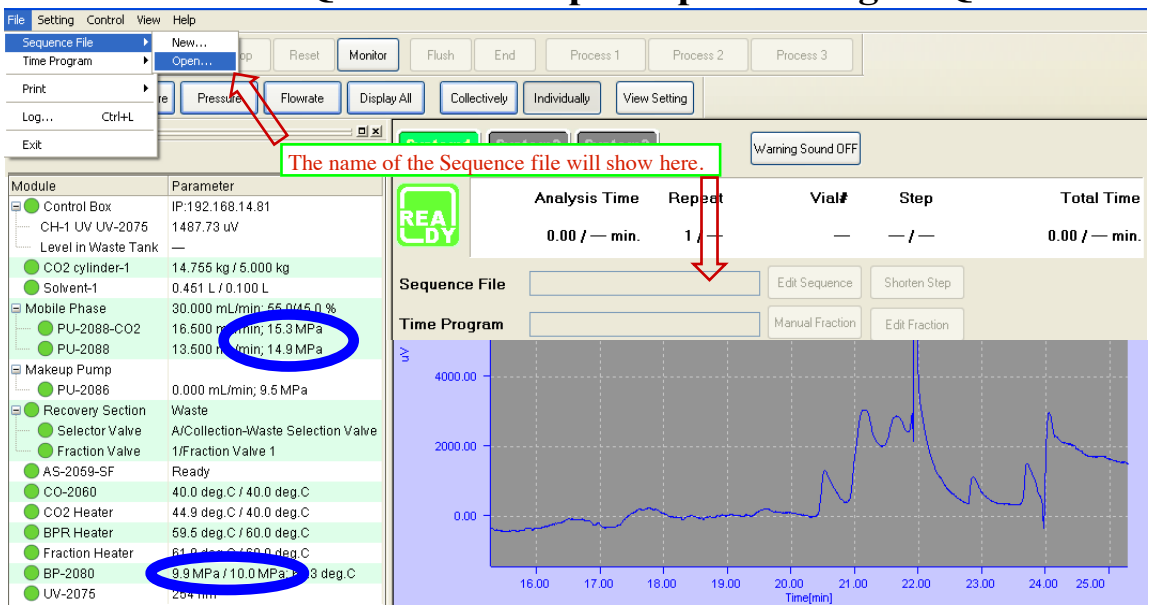
Step 5: Press "PUMP" to open window. Press "PUMP ALL" to start pumps.

You will see both dots turn green. The pumps are very quiet and are flowing very fast. You should see the Pressures increase on the two pumps and on the Back-Pressure Regulator (BPR). You will also see the CO_2 and Solvent levels decrease.

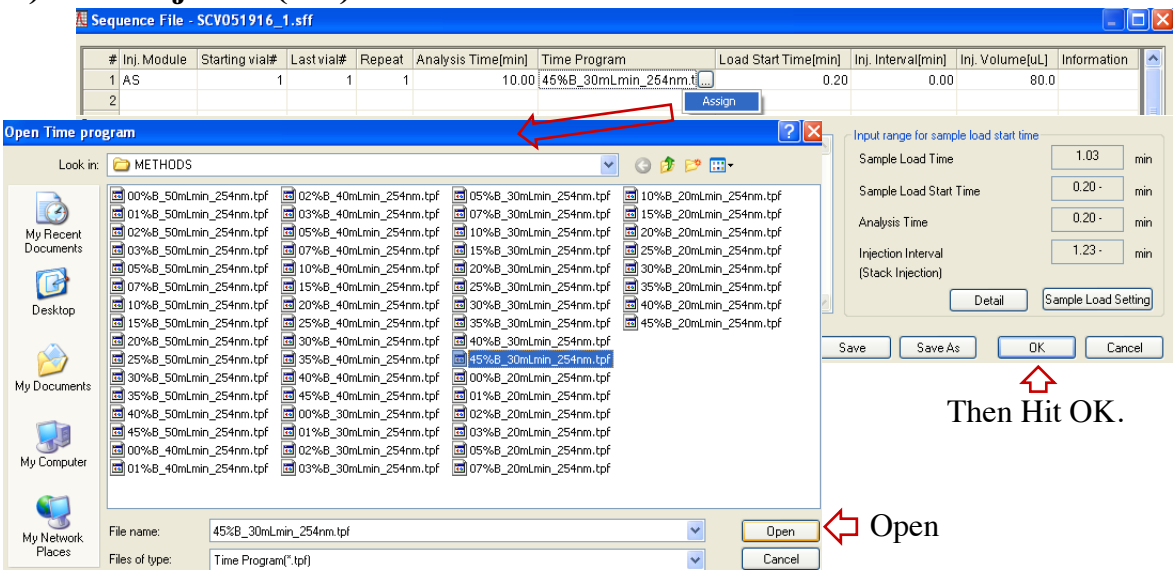


As shown below, the pressures at the two pumps rise towards ~15 MPa (150 bar) and the Back Pressure Regulator rises above 10 MPa (100 bar) and then the BPR proceeds to regulate the pressure at the 10.0 MPa setpoint selected in the Method.

Step 6: Choose NEW SEQUENCE or open a preexisting SEQUENCE FILE.



Step 7: Choose the Time Program by clicking "Assign" and enter Run Time (minutes) and Inj. Vol(uL).

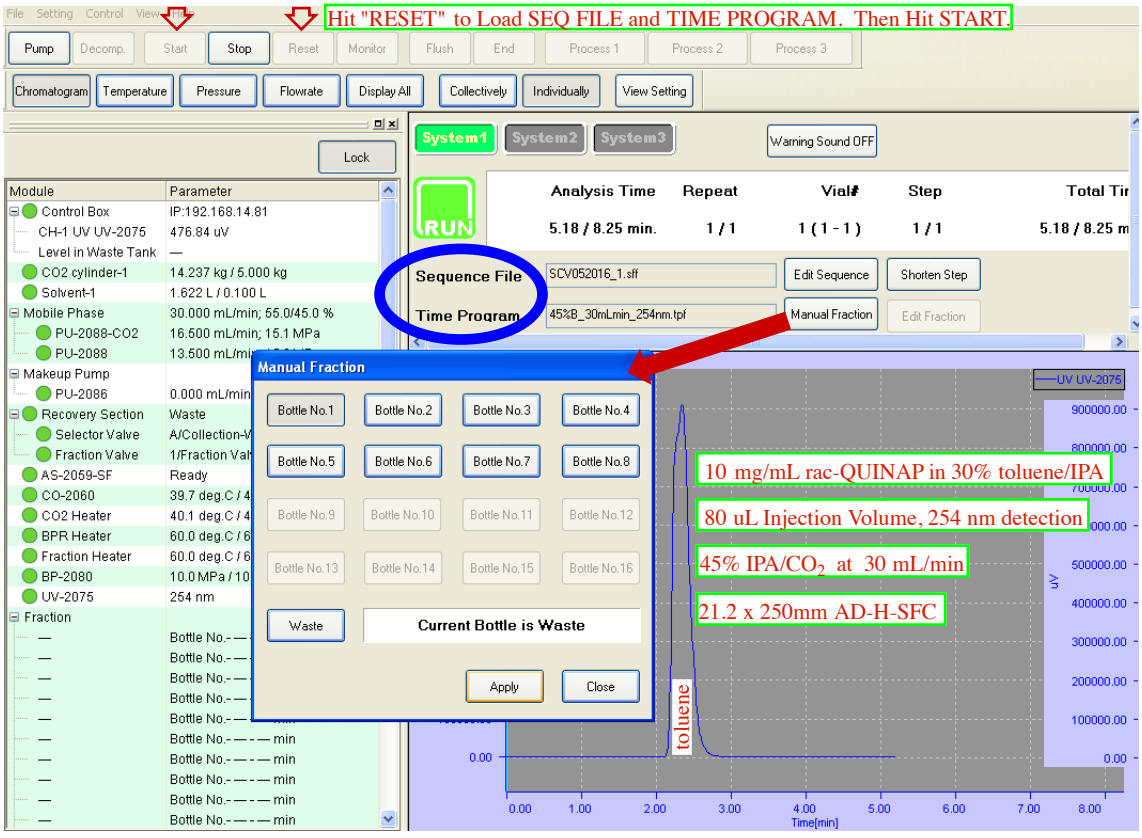


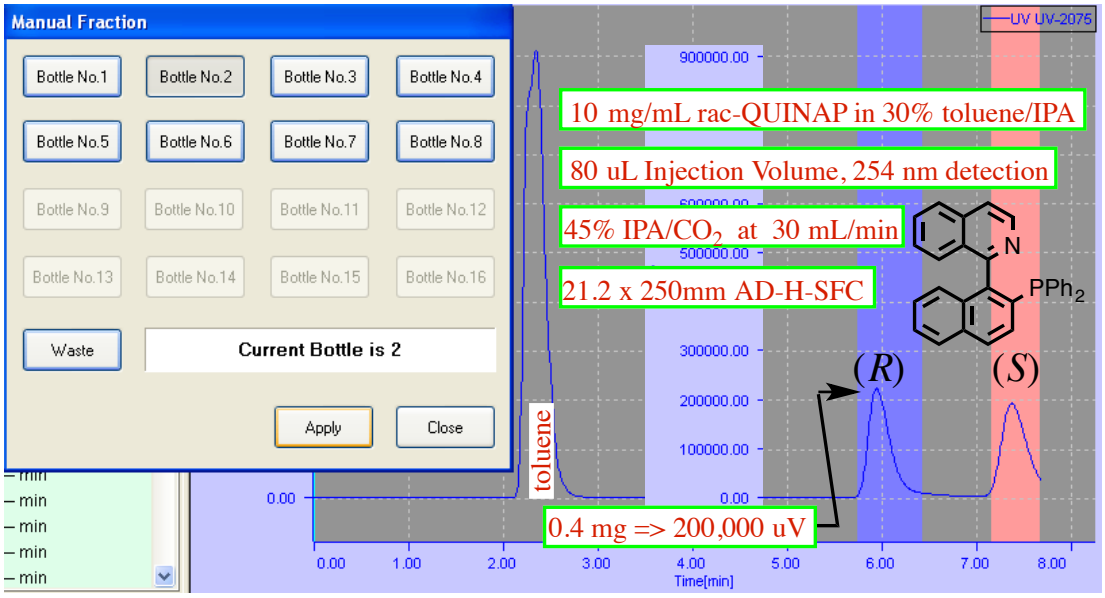
Step 8: When you return to the main Acquisition Screen, Hit "RESET" to load the Time Program and Sequence File and to set the parameters for each module.

Step 9: Hit Start.

The Autosampler will inject the sample and the Chromatogram will show the UV signal in units of **uV** (microvolts). In this sample, the injection solvent contained 30% toluene which elutes at 2.4 minutes (x 30 mL/min = ~70 mL column volume). The toluene signal registers near the detector max at 900,000 uV ~ 1800 mAu.

When the chromatogram approaches the desired peaks, **click "Manual Fraction"** and wait for the first peak to elute. Then click **Bottle 1**. When you click "**Apply**", the fraction collector will switch to bottle 1. Switch back to **Waste** then to **Bottle 2**.



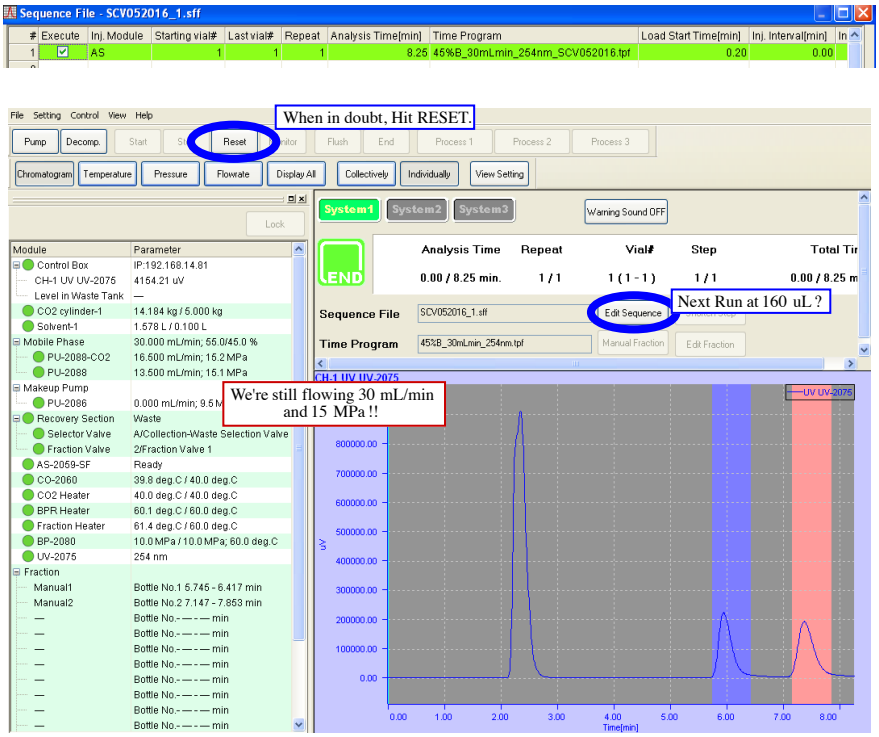


Your Run Is Complete !

If you hit "**Edit Sequence**", you will see your completed run in green. To run it again without changes, hit "**RESET**" and then "**Start**".

You can also hit **Reset** and then go to "**Edit Sequence**" to set a higher injection volume and then when you get back, hit "**RESET**" to load the new settings and "**Start**".

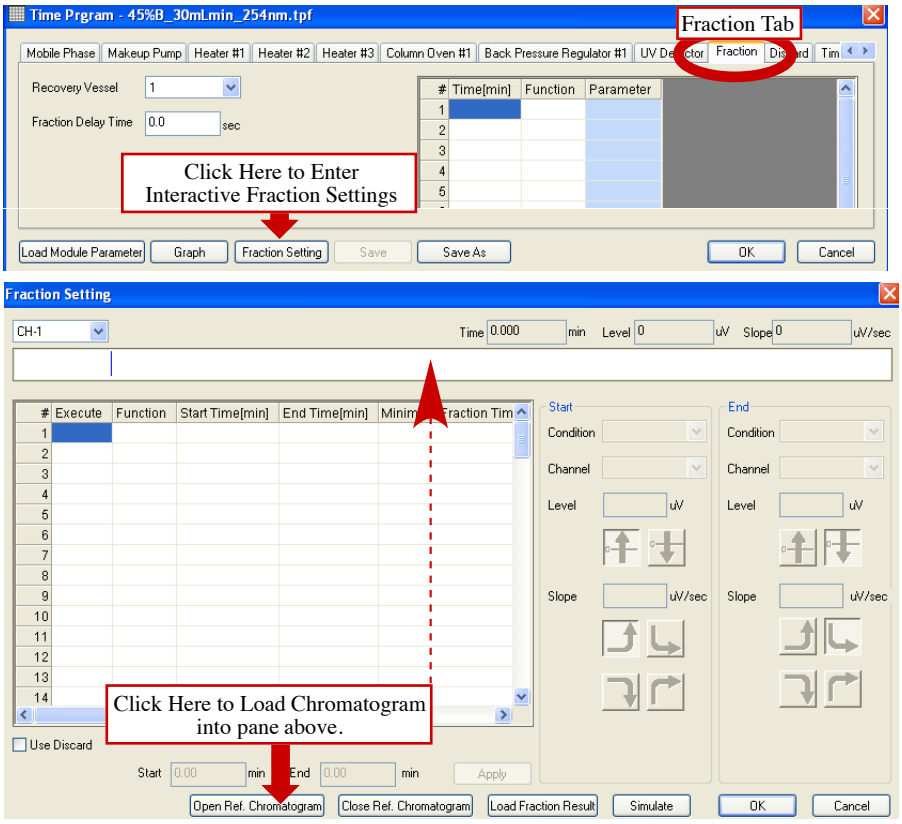
If you're going for a congratulatory coffee break, don't forget to turn off the pumps !



Step 10: Setting up Auto-Fraction Collection

LOAD the Time Program as described in Step 4, but before hitting OK you will select your desired **Fraction Settings** and **SAVE** it as your own Time Program.

Under the Fraction Tab, click on "**Fraction Setting**". Then Click on "**Open Ref. Chromatogram**" and find your recent run.



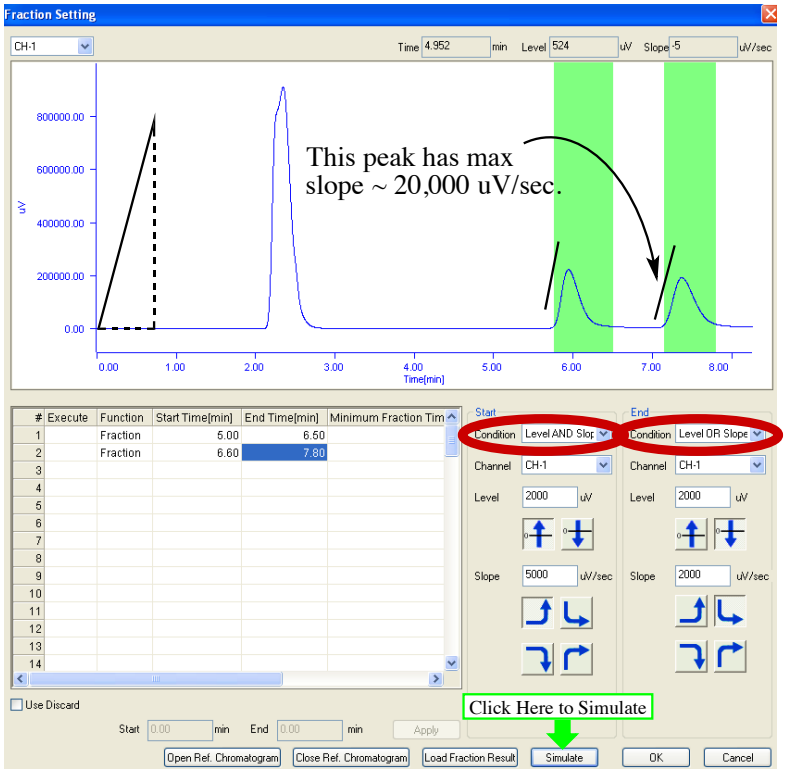
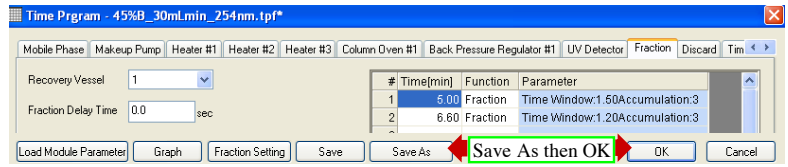
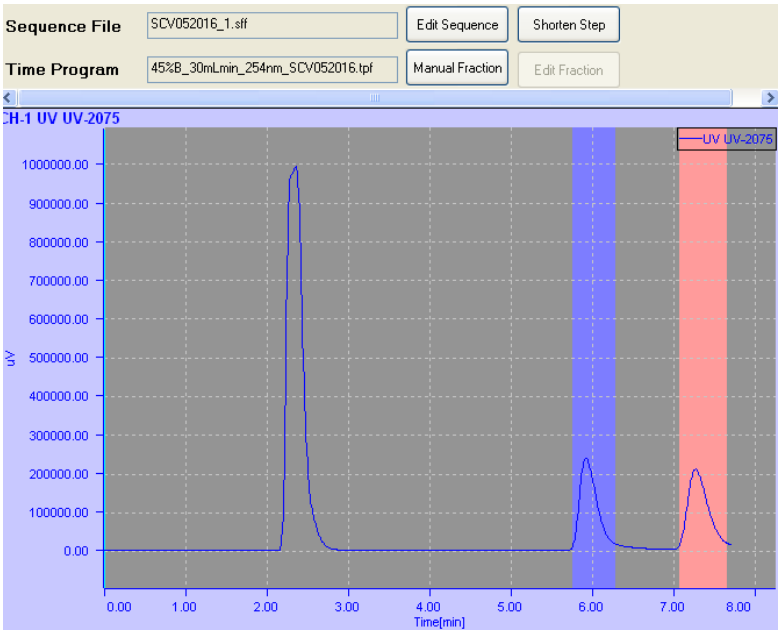
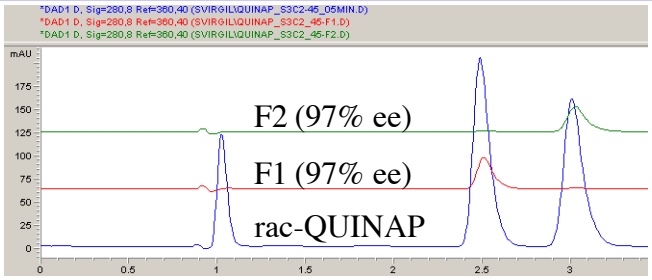
Step 10: Setting up Auto-Fraction Collection (continued)

When your chromatogram appears, build a timetable to instruct the fraction collector to look for a peak in each time range where you want a peak to be collected.

You can set up the fraction collector by **Level** or **Slope** alone or a combination of the two (shown at right - **Start** collecting when Level **AND** Slope are above our settings and **end** collection when Level **OR** Slope fall below our settings).

Estimating the slope setting:

We can expect that a 0.33 min wide peak of 200,000 uV intensity would likely have a max upslope of (200,000/10sec ≈ 20,000 uV/sec). So we set the upslope trigger to ~5000 uV/sec and the downslope trigger to ~2000 uV/sec noticing that the peak usually tails off with a lower downslope.

Each time range entry will allow **only one peak collection**. (It would be nice if JASCO fixed this.) So you need a separate line in the timetable for each peak that you want to collect.

When satisfied, click OK. You will see the timetable changes populated under the **Fraction Tab** of the Time Program. Then **Save As** and give the Time Program a name and then go call it up in your sequence table.