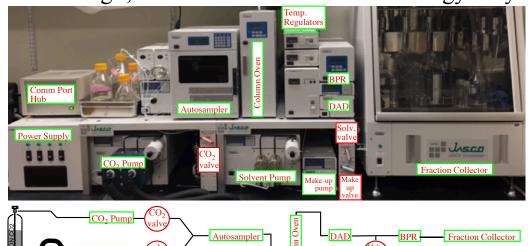
# Jasco Preparative SFC System and SF-NAV Instructions -Scott Virgil, California Institute of Technology May 2016



**Step 1:** Open CO<sub>2</sub> tank and Check solvent levels. Turn on Julabo Chiller. The chiller needs to cool to -5 °C so that the CO<sub>2</sub> pump can draw the liquid CO<sub>2</sub> efficiently. **Open CO<sub>2</sub> Valve, Solvent Valve and Make-up Valve** (if the Make-up Pump will not be used it can remain closed).

ent Pump

Step 2: 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.

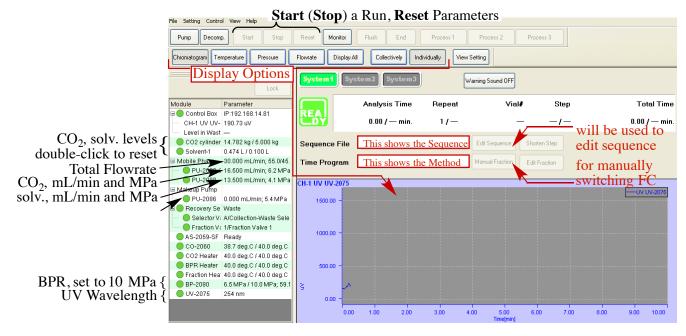






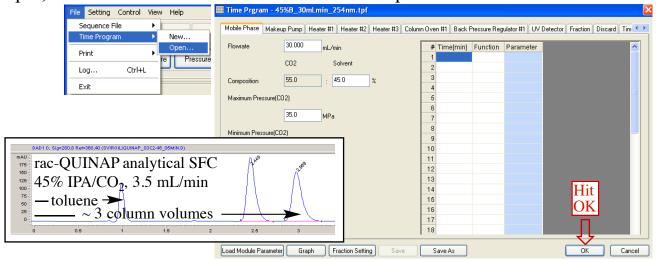
**<u>Step 3:</u>** Open SF-NAV Monitor. (All components should have a green dot.)

Make-up



## **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<sub>2</sub>Cl<sub>2</sub>) remain in the system that could damage particular DAICEL columns (AD-H, OD-H).

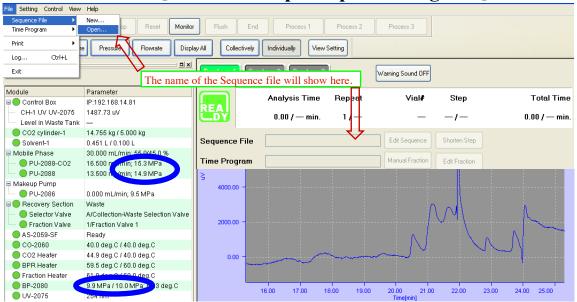
**<u>Step 5:</u>** Press "PUMP" to open window. Press "PUMP ALL" to start pumps.

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.

Pump Decomp.	Start Stop Reset Mon	itor Flush End	Process 1	Process 2	Process 3					
Chrometogen Temperature Pressure Flowrate Display All Collectively Individually View Setting										
				li i						
When you Press	"PUMP", this window a	appears.	stem2 System3	J	Warning Sound OFF					
Press "PUMP AL	L"									
Module	Parameter		Analysis Time	Repeat	Vial#	Step	Total Time			
🖃 🔵 Control Box	IP:192.168.14.81		0.001				0.001			
CH-1 UV UV-2075	312.81 uV		0.007 — min.	17-	_	-/-	0.00 / — min.			
Level in Waste Tank										
CO2 cylinder-1	14.782 kg / 5.000 kg 0.474 L / 0.100 L	Display All Collectively Individually View Setting		Shorten Step						
Mobile Phase	30.000 mL/min: 55.0445.0 %	Time Deserves				E D E - V				
PU-2088-CO2	16.500 m min; 6.6 MPa	Time Program			Manual Hacton	Edit Fraction				
PU-2088		Pump Control			×					
🗏 Makeup Pump		A comment								
- OPU-2086	0.000 mL/min; 5.9 MPa	Mobile Phase			P					
🖃 🔵 Recovery Section	Waste	Makeup Pump								
📃 🔵 Selector Valve	A/Collection-Waste Selection Valu									
Fraction Valve	1/Fraction Valve 1	Pump All	Stop All		<b></b>					
AS-2059-SF	Ready	FumpAir								
CO-2060	40.0 deg.C / 40.0 deg.C									
CO2 Heater	45.4 deg.C / 40.0 deg.C 60.0 deg.C / 60.0 deg.C	6000.00					ļļ			
Fraction Heater	59.9 deg.C / 60.9 deg.C									
BP-2080	6.4 MPa / 10.0 MPa > 1 deg.C									
UV-2075	204 mm	S 4000.00								

As shown below, the pressures at the two pumps rise towards  $\sim 15$  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).

AL Se	quence File -		l.sff										
	# Inj. Module 1 AS 2	Starting vial# 1	Lastvial# 1	Repeat 1	Analysis Time[min] 10.0	Time Program (46%B_30mL	min_254nm.t	Load Start Time	min] 0.20	Inj. Interval(min) 0.00	Inj. Volume[uL] 80.0	Informatio	n 🛆
Open Time prog Look in:	gram				<	~	G 🗊 📂 🛙	<b>?</b> ≥		Input range for samp Sample Load Time	ole load start time	1.03	min
My Recent Documents	00%8_50mLn 01%8_50mLn 02%8_50mLn 03%8_50mLn 03%8_50mLn	nin_254nm.tpf nin_254nm.tpf nin_254nm.tpf	02%8_40n 03%8_40n 05%8_40n 05%8_40n 07%8_40n 10%8_40n	hLmin_254nr hLmin_254nr hLmin_254nr	n.tpf 🖬 07%B_30ml n.tpf 🖬 10%B_30ml n.tpf 🖬 15%B_30ml	Lmin_254nm.tpf Lmin_254nm.tpf Lmin_254nm.tpf Lmin_254nm.tpf Lmin_254nm.tpf	10%8_20mLmi 15%8_20mLmi 20%8_20mLmi 25%8_20mLmi 30%8_20mLmi	in_254nm.tpf in_254nm.tpf in_254nm.tpf		Sample Load Start Analysis Time Injection Interval	Time [	0.20 - 0.20 - 1.23 -	min   min   min
Desktop	00 %8_50mLn 07%8_50mLn 10%8_50mLn 15%8_50mLn 20%8_50mLn	nin_254nm.tpf nin_254nm.tpf nin_254nm.tpf	10 %8_40n 15%8_40n 20%8_40n 25%8_40n 30%8_40n	nLmin_254nr nLmin_254nr nLmin_254nr	n.tpf 🖬 25%B_30ml n.tpf 🖬 30%B_30ml n.tpf 🖬 35%B_30ml	Lmin_254nm.tpf Lmin_254nm.tpf Lmin_254nm.tpf	35%B_20mLmi 35%B_20mLmi 40%B_20mLmi 45%B_20mLmi	in_254nm.tpf in_254nm.tpf		(Stack Injection)		ample Load S	
My Documents	25%B_50mLn 30%B_50mLn 35%B_50mLn 40%B_50mLn	nin_254nm.tpf nin_254nm.tpf nin_254nm.tpf nin_254nm.tpf	35%B_40n 40%B_40n 45%B_40n 00%B_30n	nLmin_254nr nLmin_254nr nLmin_254nr nLmin_254nr	n.tpf 💼 <mark>45%8_30m</mark> n.tpf 💼 00%8_20ml n.tpf 💼 01%8_20ml n.tpf 💼 02%8_20ml	min_254nm.tpf Imin_254nm.tpf Imin_254nm.tpf Imin_254nm.tpf				ave Save A	』しい Chen Hi		ncel
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My Network Places	File name: Files of type:	45%B_30mLm Time Program					<b>v</b>	Open Cancel	¢	Open			

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.

- 1100

– min

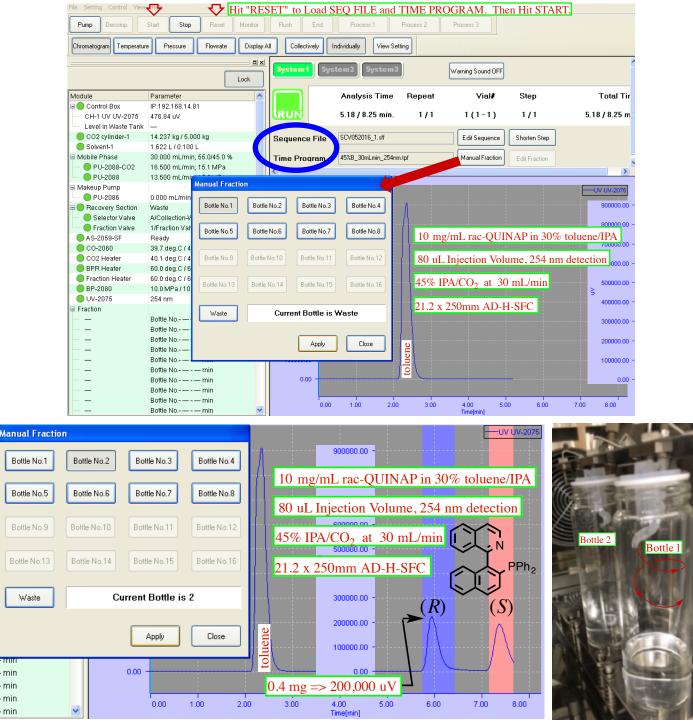
– min

– min

– min

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 =  $\sim$ 70 mL column volume). The toluene signal registers near the detector max at 900,000  $\text{uV} \sim 1800 \text{ mAu}$ .

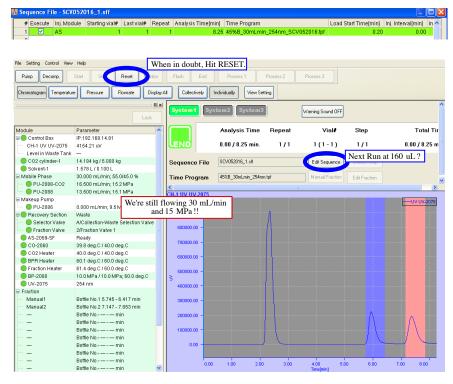
When the chromatogram approaches the desired peaks, click "Manual Fraction" and wait for the first peak to elute. Then click **Bottle 1**. When you click "Apply", the fraction collector will switch to bottle 1. Switch back to Waste then to Bottle 2.



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**".

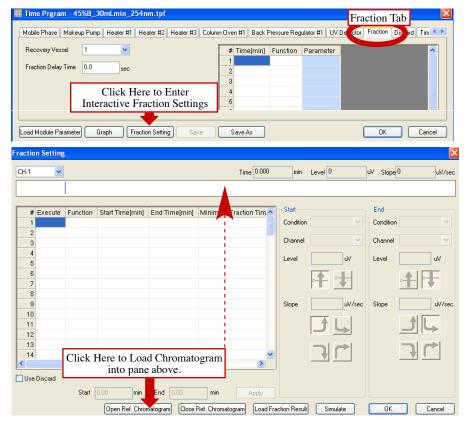
If you're going for a congratulatory coffee break, don't forget to turn off the pumps !



#### **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.

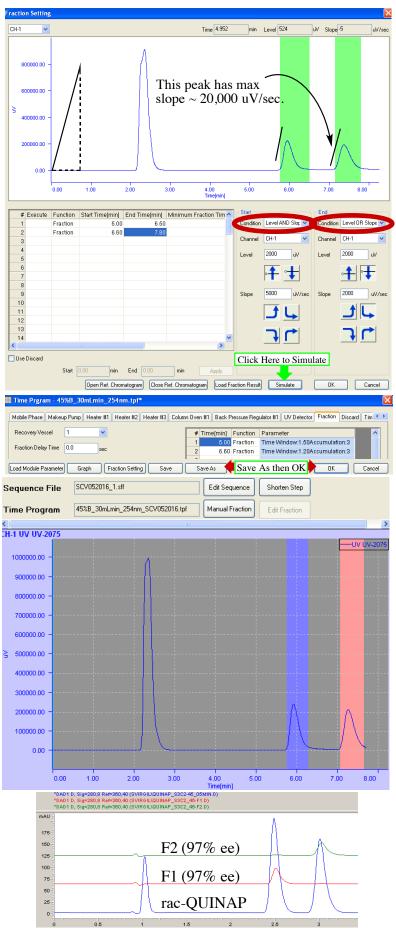
You can set up the fraction collector by **Level** or **Slope** alone or a combination of the two (shown at right - **Start** collecting when Level **AND** Slope are above our settings and **end** collection when Level **OR** Slope fall below our settings).

## **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  $(200,000/10 \text{sec} \approx 20,000 \text{ uV/sec})$ . 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.



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