IOWA STATE UNIVERSITY
Chemical Instrumentation Facility

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Prep LC Training Manual

(Kamel 11-6-2013)

Location: 1238 Hach Hall

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Safety:

All researchers working in 1238 Hach Hall must complete the EH&S courses: *"Fire Safety and Extinguisher Training"*, and *"Lab Safety: Compressed Gas Cylinders"*. When preparing samples in 1238A, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available.
Properly dispose of waste solvents and glass pipettes in the containers provided in 1238A. All of the data processing computers and many of the data acquisition computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Introduction:



The Agilent PrepStar SD-1 preparative HPLC is a student operated instrument. Students (and other researchers) are given an appropriate training in order to affect the prep experiments themselves. The instrument can be operated in analytical, semi-prep, and prep mode. Three C18

column sizes are available: analytical (4.6*50 mm, 1.8 um), semi-prep (9.4*250 mm, 5 um), and prep (21.2*250 mm, 7 um). Approprite guard columns are also available and must be used in all circumstances.

Two Rheodyne manual injectors are mounted next to the pumps. The top one is for analytical and semi-prep with 500 ul sample loop. The bottom one is for the Prep experiments and is equipped with a 10 ml sample loop.

Sample collection tubes are provided. Larger size collection tubes or beakers can be used for large collection volumes. The training will include, among other hardware pluming issues, how to remove and install LC columns.

Overview:

The SD-1 PrepStar is a dual pump (binary) capable of drawing from to separate solvent reservoir A and B. The A reservoir is dedicated to aqueous solutions while the B is always reserved for the organic solutions. The solvent delivery is system a dual interchangeable pump heads that provide pumping capacity to 3.2 L per minute at pressures of up to 375 psi for high throughput microparticulate columns.

The PrepStar SD-1 is an innovative HPLC solvent delivery system engineered with preparative chromatographers in mind. A powerful drive unit and dual interchangeable pump heads provide pumping capacity to 3.2 L per minute at pressures of up to 2.6 MPa (375 psi) for superior throughput with large high-resolution microparticulate columns. For bench-scale prep, 200 mL per minute and 50 mL per minute heads provide flow at pressures up to 41.4 MPa (6000 psi) and 68.9 MPa (10,000 psi), respectively. Corrosion-resistant titanium pump heads address the needs of biochemists for compatibility with salt-containing buffers and freedom from unwanted metal ions. Each pump head has a piston washing chamber to prevent deposition of abrasive salt residues behind the high-pressure seal, thereby greatly extending seal life.

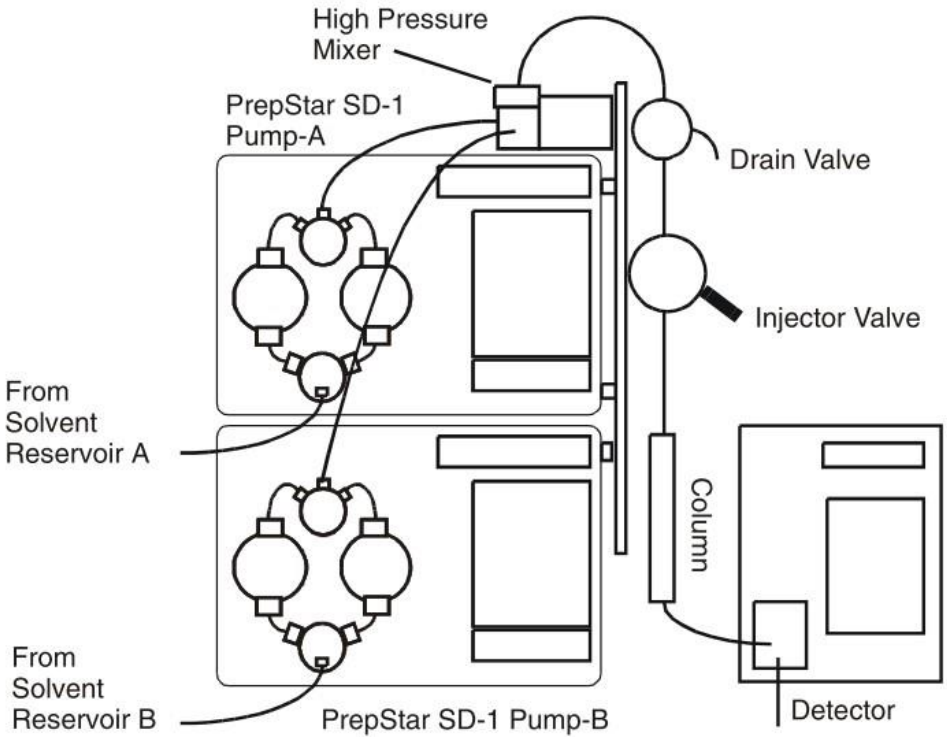


Table 1 illustrates general considerations for column size, flow rates, injection volumes, and sample load.

Column ID	Flow Rate	Sample load (mg)	Injection volume
4.6 mm (analytical) 9.4 mm (semi-prep) 21.2 mm (prep)	1 ml/min 4.2 ml/min 21.0 ml/min	2-10 mg 10-50 mg 50-200 mg	1-20 ul 100-400 ul 500ul-5 ml

Table 1 **Operating**

Instruction:

Before starting an experiment, make sure there is enough solvents in reservoir A and reservoir B. Choose which manual injector to use. Top injector is for analytical and bottom one is for the prep separation. Also, make sure that the purge line and waste line tubing are inserted in their respective bottles.

Operating the instrument consists of:

1- Setting up the method

2- Starting and stopping an experiment

!!!!Pay attention to the Flow rate. It must be 0.000 ml/min when there is no intention of having solvent flow!!!!

For existing method, simply open the desired method from the pull down menu Method. Click on ON button. Verify that the flow rate is the desired one. 

Allow the flow of solvent for sometime until the back pressure is stable.

Direct Control:

You can control each device directly. Simply right click on the field of the device and set the desired conditions.



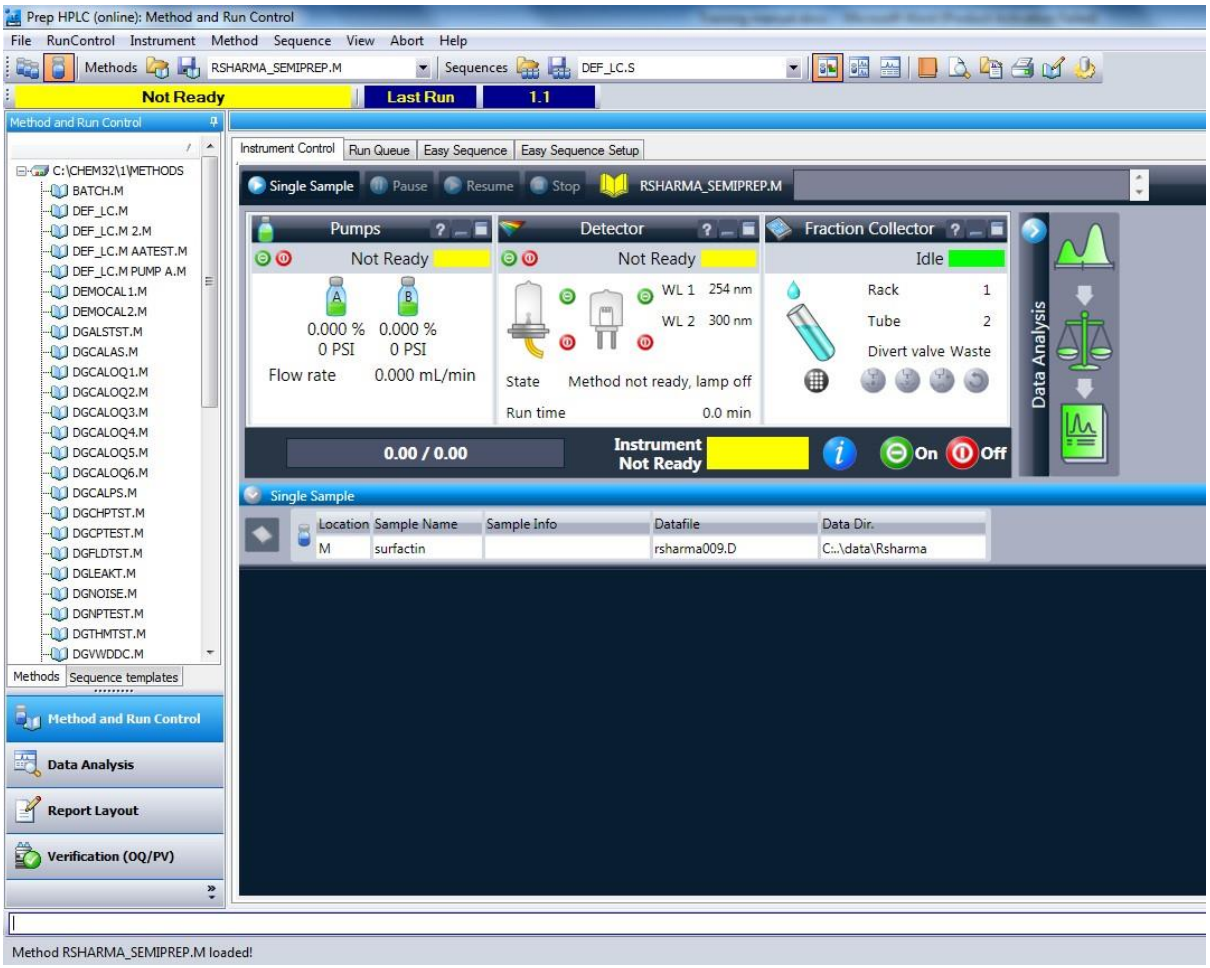
Setting up new method:

All methods are located in C:\Chem32\1\METHODS directory.

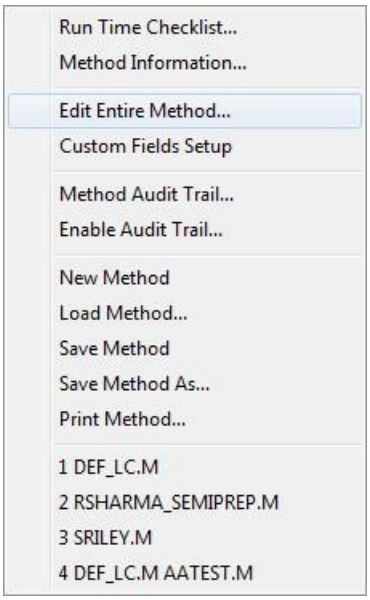
We prefer that each user possesses only one method under ISU username (e.g. C:\Chem32\1\METHODS\usename).

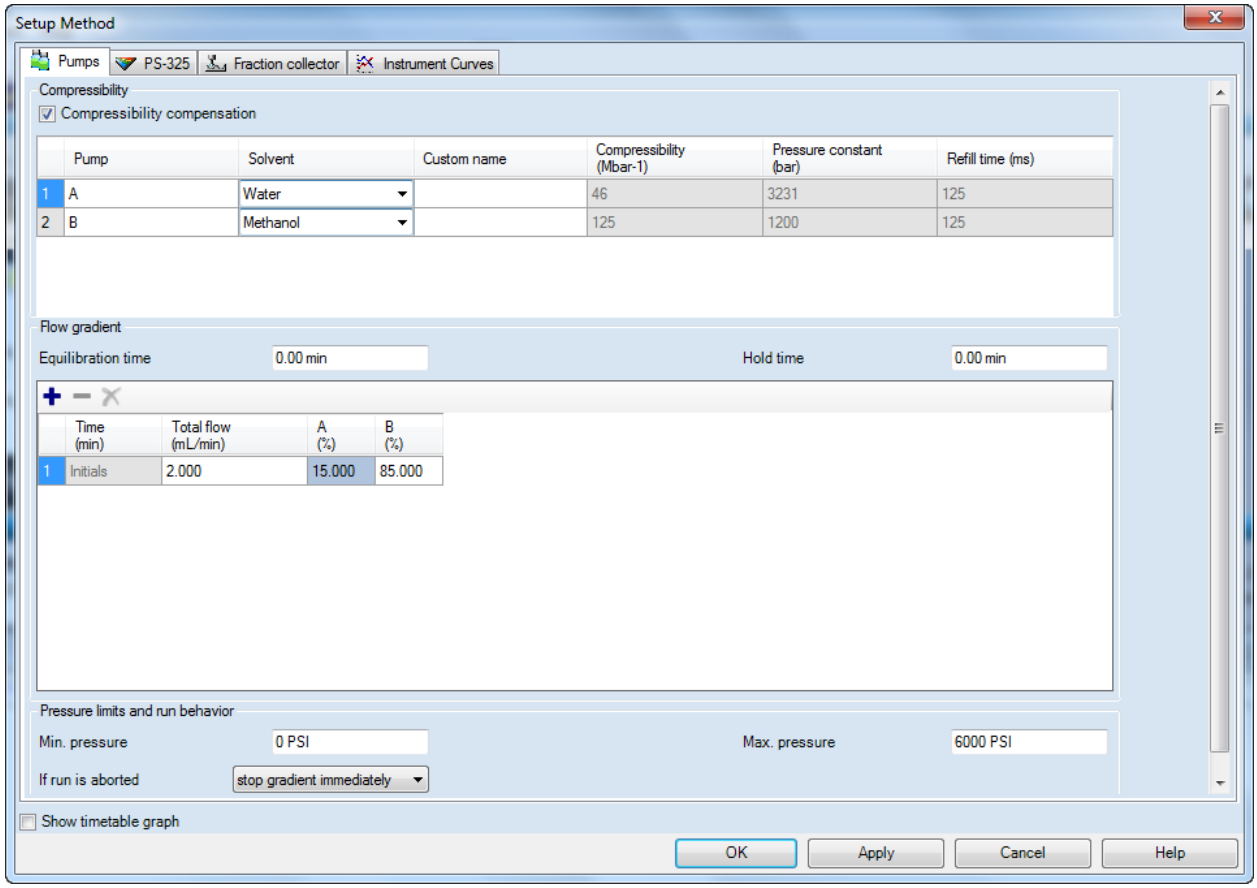
Files are always located in C:\Chem32\1\DATA\filname directory. Filnames must be in the form of usernameXXX, where XXX is the incrementing number.

Open the prep LC program: 



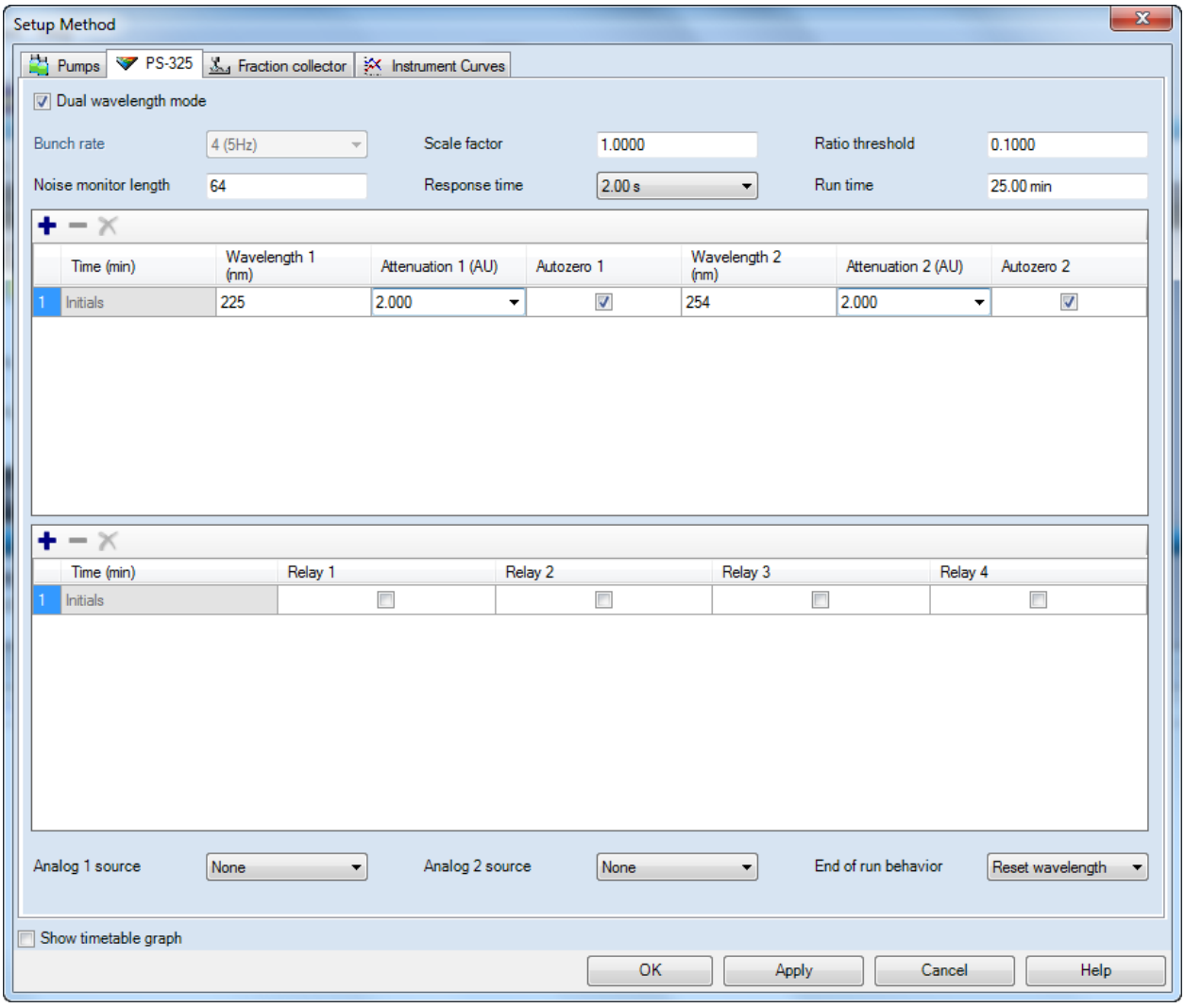
From the Method drop down menu click on Edit Entire Method:





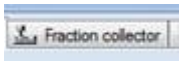
Set up flow rate and solvent composition. Click Apply.

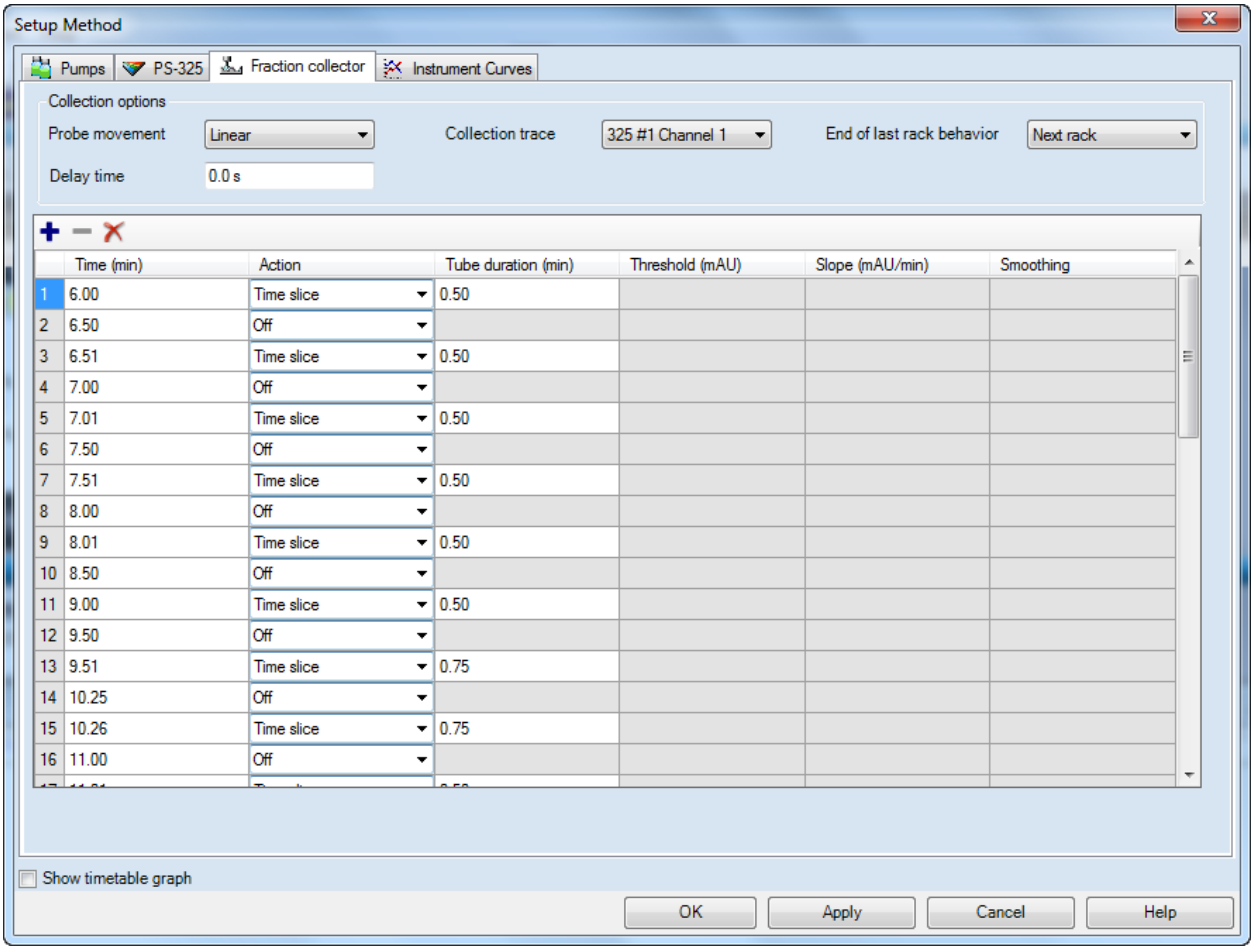
Set up detector conditions by clicking on the PS-325

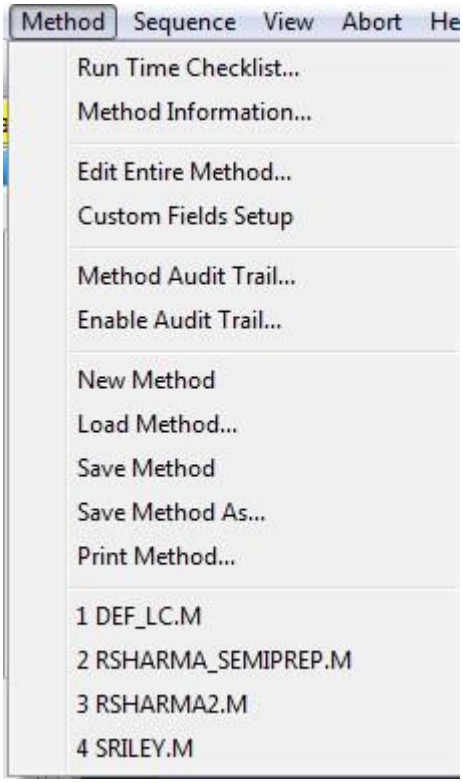


Enter wavelengths values. Run time or experiment time is entered in this page.



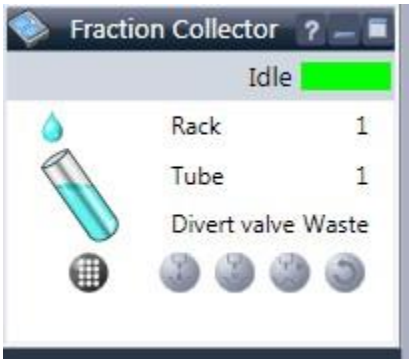
Set up Fraction Collector by clicking on 


Setup Method
Pumps
PS-325
Fraction collector
Instrument Curves
Collection options
Probe movement
Linear
Collection trace
325 #1 Channel 1
End of last rack behavior
Next rack
Delay time
0.0 s
Time (min)
Action
Tube duration (min)
Threshold (mAU)
Slope (mAU/min)
Smoothing
1 6.00 Time slice 0.50
2 6.50 Off
3 6.51 Time slice 0.50
4 7.00 Off
5 7.01 Time slice 0.50
6 7.50 Off
7 7.51 Time slice 0.50
8 8.00 Off
9 8.01 Time slice 0.50
10 8.50 Off
11 9.00 Time slice 0.50
12 9.50 Off
13 9.51 Time slice 0.75
14 10.25 Off
15 10.26 Time slice 0.75
16 11.00 Off
Show timetable graph
OK
Apply
Cancel
Help

Click OK and save the method: 

Alternatively you can collect samples automatically by entering the Action (time slice) or you can collect fraction manually.

If you want to manually collect sample simply use the buttons on the main instrument page:

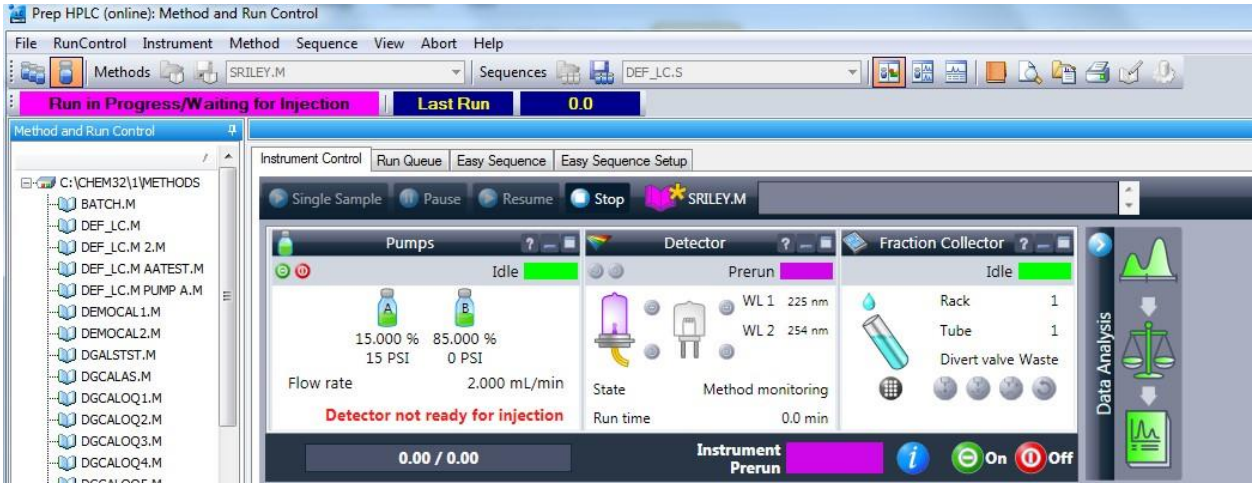


The divert valve waste contain 4 buttons. The left one allows collection. The next one to the right stops collection.

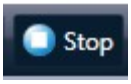
Data Acquisition:

From Run Control---Sample info tabs you can set sample name data file etc…

Proceed to acquisition by clicking Single sample.







Test Mix Standard Experiments:

Operating Conditions:

Semi-Prep: Eclipse XDB-C18 9.4*250 mm, 5 um

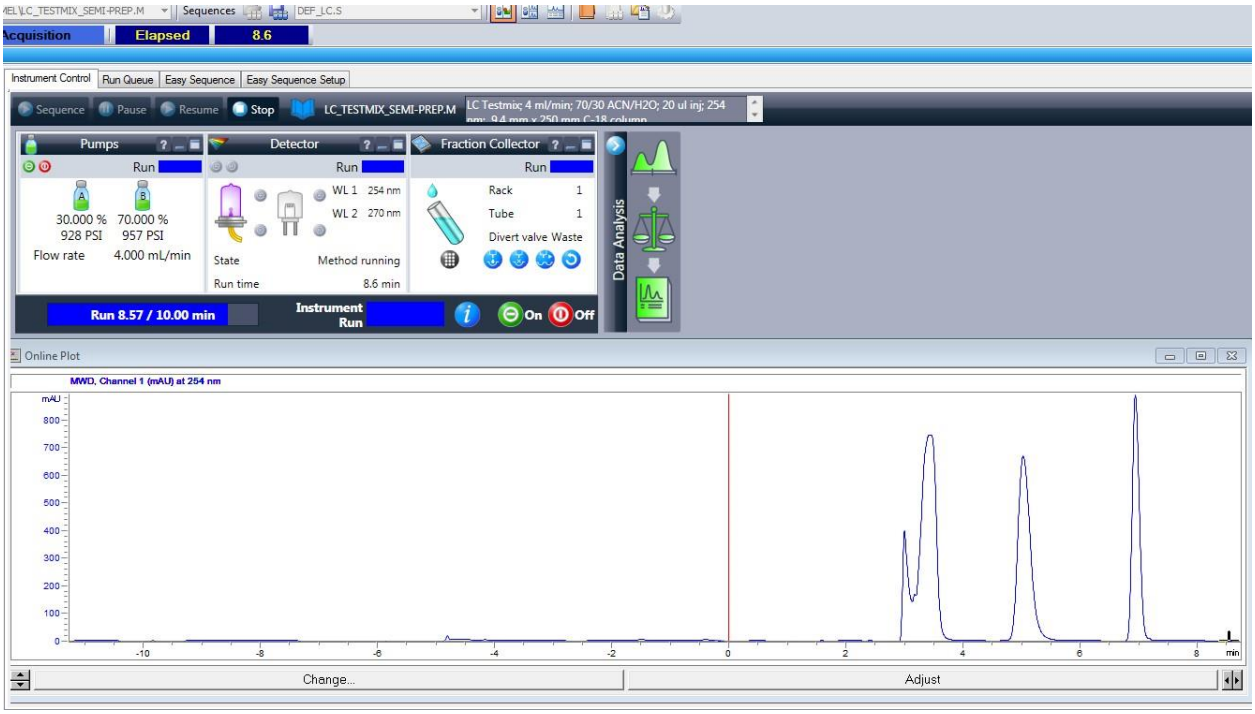
Mobile Phase: 70% Acetonitrile/30% Water

Flow Rate: 4 ml/min

Injection Volume: 50 ul

Column back pressure: 1000 PSI

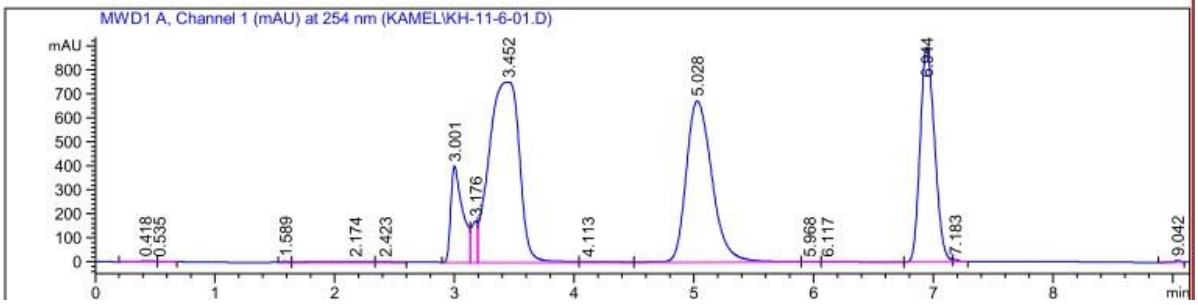
Peak#	Conc (ug/ml)	Compound
1	100	Uracil
2	4000	Phenol
3	500	4-Chloro Nitrobenzene
4	800	Naphthalene



Data File C:\CHEM32\1\DATA\KAMEL\KH-11-6-01.D
Sample Name: testmix-semiprep

```
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : Prep HPLC                    Location :   -
Injection Date  : 11/6/2013 10:11:32 AM
                                             Inj Volume : Manually
Method          : C:\CHEM32\1\METHODS\KAMEL\LC_TESTMIX_SEMI-PREP.M
Last changed    : 11/6/2013 10:06:17 AM by SYSTEM
Method Info     : LC Testmix; 4 ml/min; 70/30 ACN/H2O; 20 ul inj; 254 nm;  9.4 mm x 250 mm C-
                  18 column

Sample Info     : 20 ul injected of testmix
```



Data File C:\CHEM32\1\DATA\KAMEL\KH-11-6-01.D
Sample Name: testmix-semiprep

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount: : 20.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: MWD1 A, Channel 1 (mAU) at 254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.418	BV	0.1057	30.36943	3.90675	0.0878
2	0.535	VB	0.0776	12.47189	2.15286	0.0361
3	1.589	VV	0.0571	19.64657	5.08334	0.0568
4	2.174	VV	0.4885	125.15381	3.12316	0.3618
5	2.423	VV	0.1796	39.30242	3.08592	0.1136
6	3.001	VV	0.0977	2772.42505	400.95438	8.0145
7	3.176	VV	0.0507	612.84119	171.54732	1.7716
8	3.452	VV	0.2345	1.31466e4	749.88831	38.0038
9	4.113	VV	0.2348	80.03748	4.32674	0.2314
10	5.028	VV	0.2383	1.00962e4	672.05432	29.1859
11	5.968	VV	0.1184	27.24593	3.22995	0.0788
12	6.117	VV	0.3015	56.66071	2.30723	0.1638
13	6.944	VV	0.1341	7516.56787	894.24475	21.7287
14	7.183	VB	0.0481	31.77199	9.98686	0.0918
15	9.042	VV	0.0494	25.50265	7.96090	0.0737
Totals :				3.45928e4	2933.85278	