	Chemistry Department Bldg. 8 Research Group	SOP #	4
		Revision #	4
Prepared by	Sidney G. Coombs/ Lauren Heese	Implementation Date	1/15/2014
Page Number	1 of 8	Last Reviewed Date	1/15/2014
SOP Owner	Tom Allston	Approval	

I. Purpose

To promote the effective use of the Agilent HPLC to collect scans. To educate students in the basic technique of measurement through high pressure liquid chromatography.

II. Scope

This SOP is intended for in-group use by trained and certified personnel in the Chemistry Department.

III. Prerequisites

The experimenter must be trained in proper instrument techniques before using this SOP.

IV. Responsibilities

The responsibility for this instrument lies with Tom Allston

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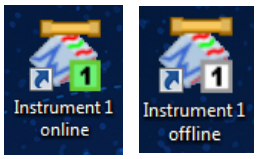
V. Procedures and information

1) Turn on the power buttons on each instrument module, computer, and monitor.

2) Select the Instrument 1 online icon from the desktop.
   i) (The offline version may be used for data analysis during a run)
   ii) The autosampler will go through its boot-up process.



3) If you wish to only view data you may open the offline icon shown.

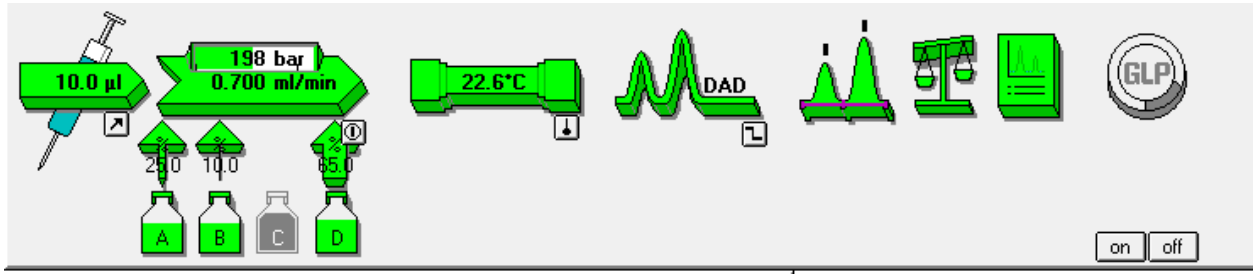
4) On the lower left of the screen you will see four menu choices. To control the instrument you will need to be in the online mode (above icon) and choose "method and run control". To view data using the on-line or off-line window choose "data analysis".

5) To get the instrument and sampling diagrams on the screen, select "view," then select "system diagram" and "sampling diagram"

6) The diagram shows exactly what the instrument is:
   i) From left to right the icons represent:
   Injector → pump → column → variable wavelength detector → data analysis

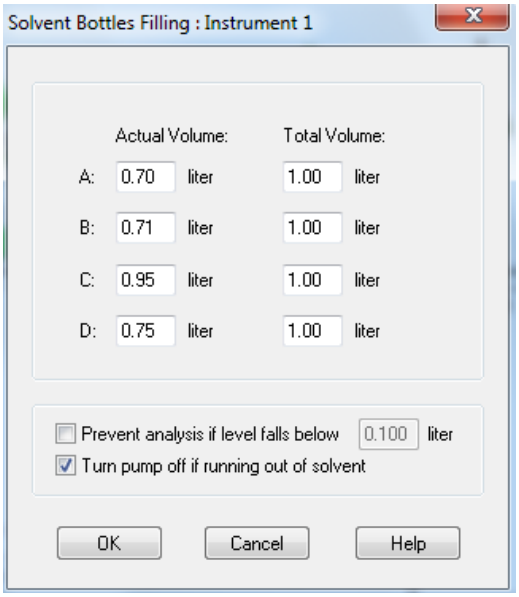
7) On the bottom right of the diagram there is a button that says "on." Click it to activate all components of the system.
   i) Typically there are always two ways to activate a command, one from the menu bar and one from the icons in the system diagram. In this case you can also click "system on" under "instrument" in the menu bar.

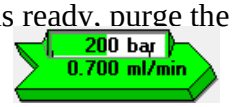
8) The components will initialize as indicated by the icons turning yellow. When each module is ready to go, the corresponding icon will turn green. When all modules are green this means the HPLC is ready to analyze your samples.

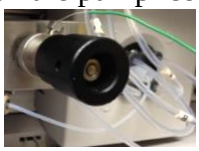


9) During initialization, look at the waste container:
   i) Predict how much waste will runoff during your experiment based upon the flow rate and total time of analysis you will be using.
   ii) If needed, get a new waste bottle from the stockroom. Be sure to turn in the waste notification slip for the current bottle.

10) Check that the volumes entered for the solvent containers are accurate.
    i) Click on the bottle icons then select **solvent bottles**. Change the values for each bottle to reflect the actual volume available for each. Don't forget to go through this process any time that you refill a solvent bottle.



11) Once the pump is ready, purge the system as needed by clicking on the pump icon followed by **setup pump**. 



Adjust the flow rate, stop time, and solvent selection manually.
    i) Turn the black bypass valve counter-clockwise to open it.
    ii) To purge the pumps and degasser pump the solvent through at a high rate to get any contaminants out of the system.
        **Recommended: 3mL/min of Solvent A (aqueous buffer, ie. 1% acetic acid) for 3 min followed by 3mL/min of Solvent B (methanol) for 3 min.
    iii) After the purge is completed, close bypass valve until finger-tight
    iv) **VERY IMPORTANT**: If prompted, do **not** save! The only time you should be saving a method is if you have purposefully gone to **save as**. Never overwrite another operator's method.

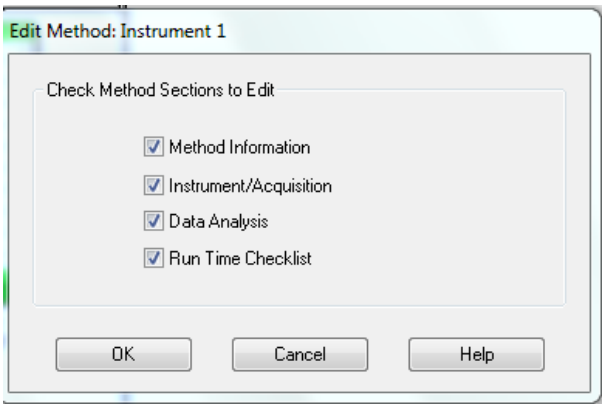
12) Load the method you will be using by selecting Method → "Load Method" → "(filename)". You can use this directly in a sequence or modify it. When you load this the pumps will start to equilibrate the column with the initial conditions.

13) If you do not have a method, pick any appropriate method (.M)
    i) **Method edit → edit entire method**
        i) At the end of the edit, you will save the modified method under a new name.
        ii) For additional notes on setting up your own method, see your lab instructor or research advisor.

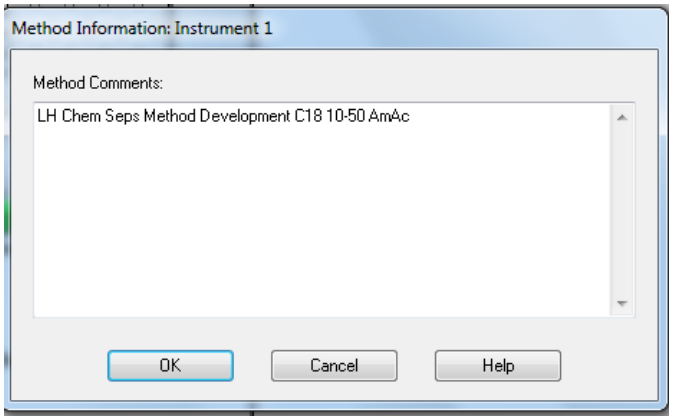
iii) Again VERY IMPORTANT: If prompted, do not save! **Only use "save as"** under "method" when you finish editing and you will never run the risk of overwriting a program that you do not intend to change.

14) To create or modify a method, choose method → "edit entire method". The following pictures illustrate each window as it appears as you click "OK".

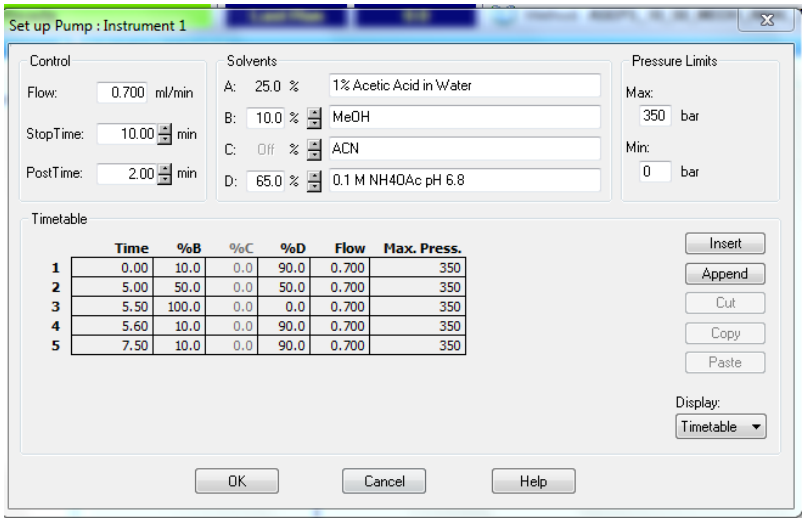
1.



2.

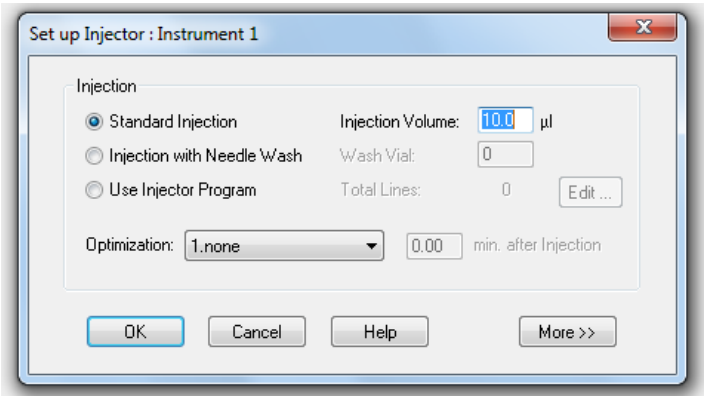


3.

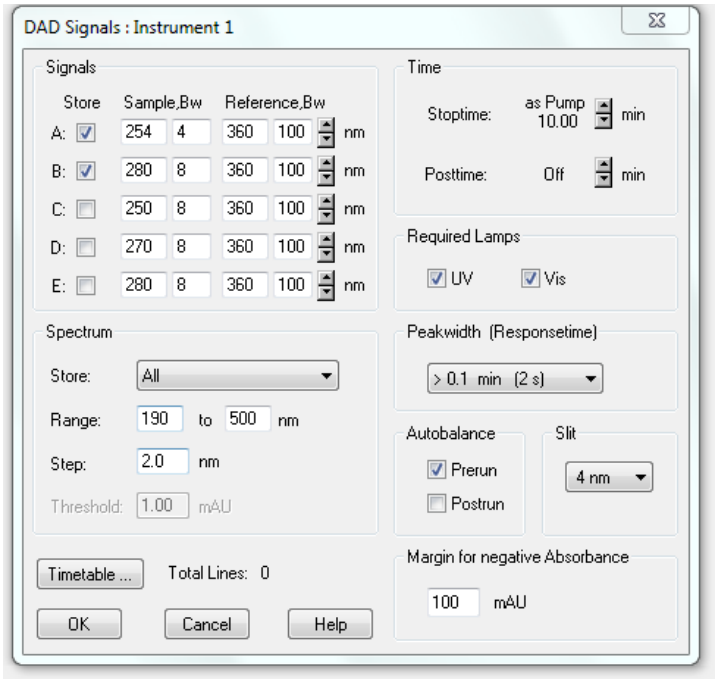


	Time	%B	%C	%D	Flow	Max. Press.
1	0.00	10.0	0.0	90.0	0.700	350
2	5.00	50.0	0.0	50.0	0.700	350
3	5.50	100.0	0.0	0.0	0.700	350
4	5.60	10.0	0.0	90.0	0.700	350
5	7.50	10.0	0.0	90.0	0.700	350

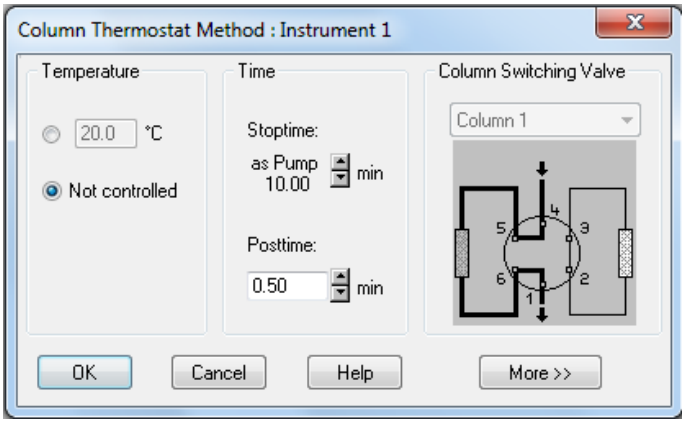
4.



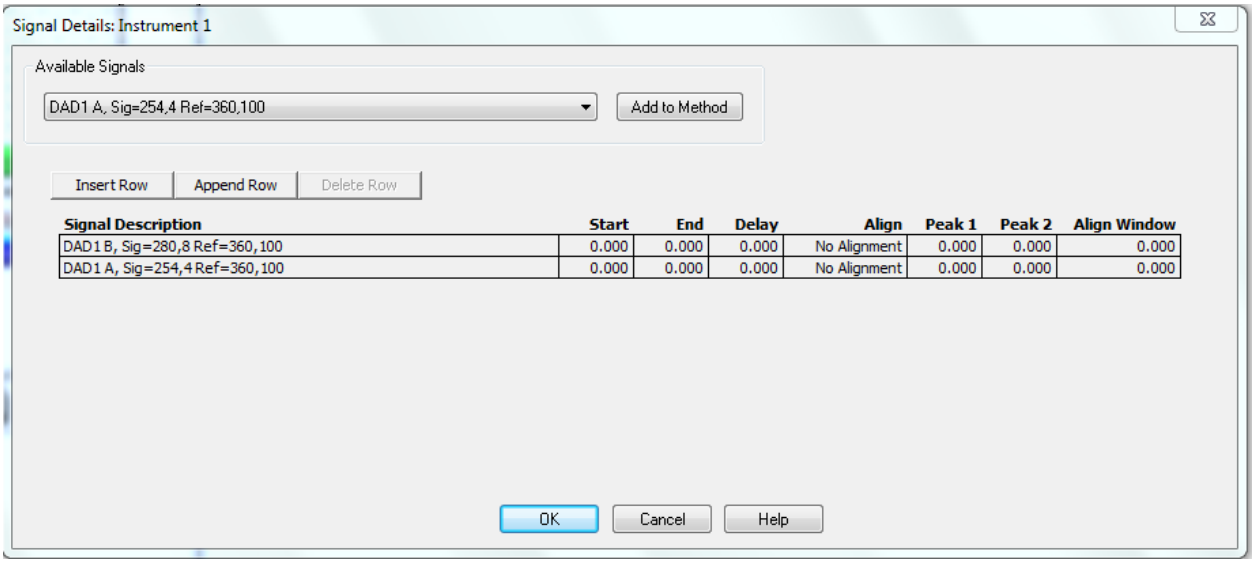
5.



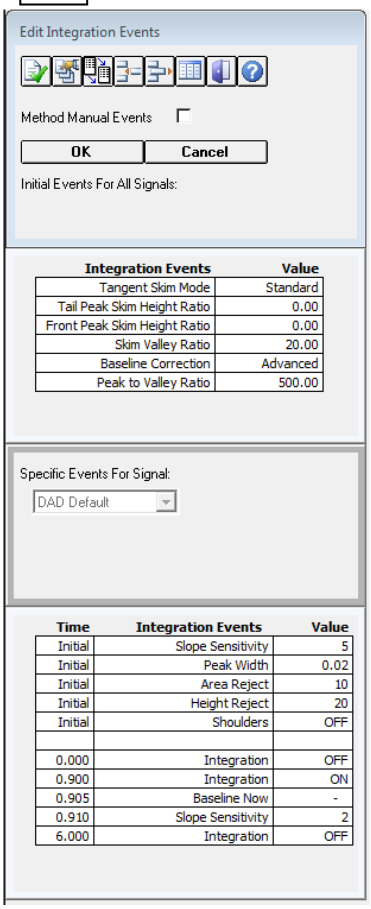
6.



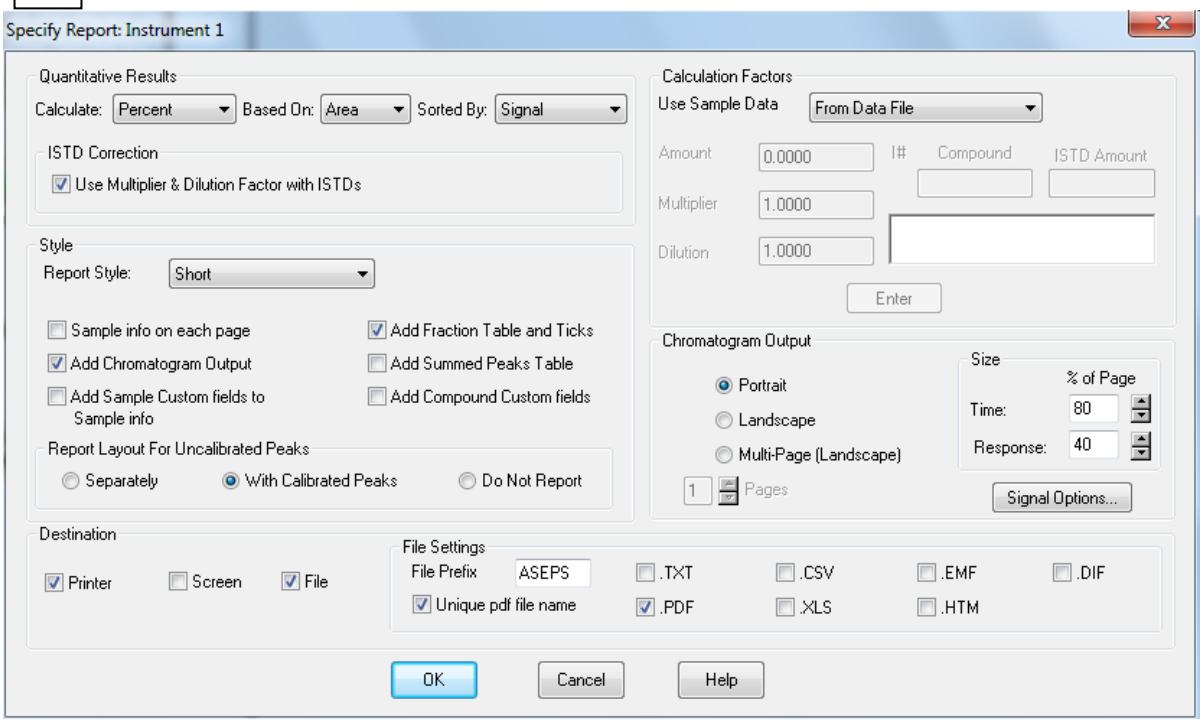
7.


Signal Details: Instrument 1
Available Signals
DAD1 A, Sig=254,4 Ref=360,100
Add to Method
Insert Row
Append Row
Delete Row
Signal Description
Start
End
Delay
Align
Peak 1
Peak 2
Align Window
DAD1 B, Sig=280,8 Ref=360,100 0.000 0.000 0.000 No Alignment 0.000 0.000 0.000
DAD1 A, Sig=254,4 Ref=360,100 0.000 0.000 0.000 No Alignment 0.000 0.000 0.000
OK
Cancel
Help

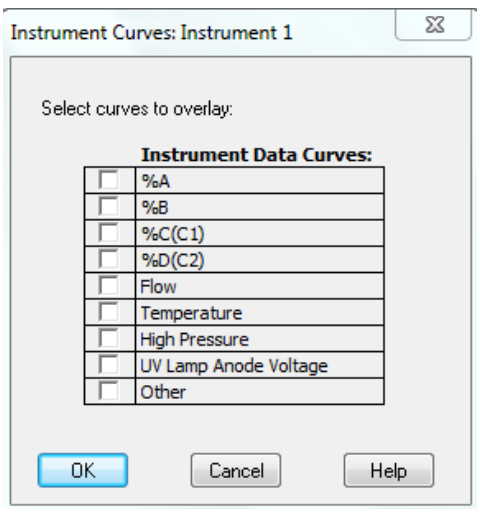
8.


Edit Integration Events
Method Manual Events
OK
Cancel
Initial Events For All Signals:
Integration Events
Value
Tangent Skim Mode Standard
Tail Peak Skim Height Ratio 0.00
Front Peak Skim Height Ratio 0.00
Skim Valley Ratio 20.00
Baseline Correction Advanced
Peak to Valley Ratio 500.00
Specific Events For Signal:
DAD Default
Time
Integration Events
Value
Initial Slope Sensitivity 5
Initial Peak Width 0.02
Initial Area Reject 10
Initial Height Reject 20
Initial Shoulders OFF
0.000 Integration OFF
0.900 Integration ON
0.905 Baseline Now -
0.910 Slope Sensitivity 2
6.000 Integration OFF

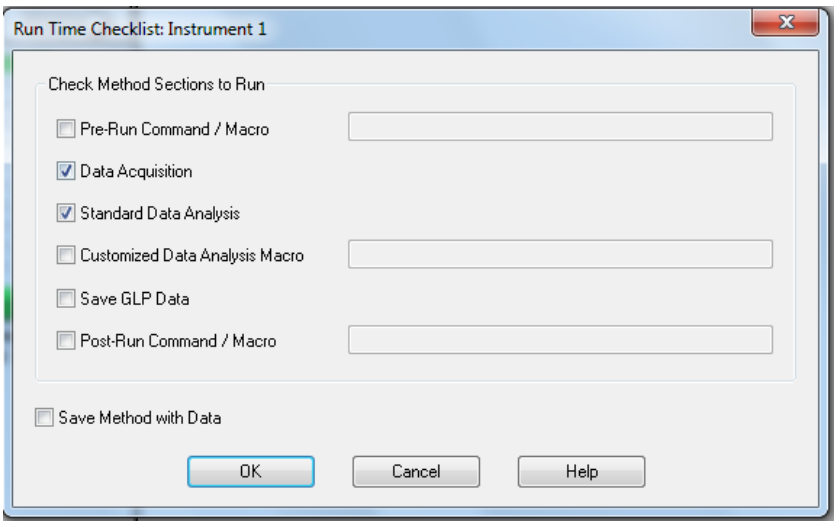
9.


Specify Report: Instrument 1
Quantitative Results
Calculate: Percent
Based On: Area
Sorted By: Signal
ISTD Correction
Use Multiplier & Dilution Factor with ISTDs
Style
Report Style: Short
Sample info on each page
Add Chromatogram Output
Add Sample Custom fields to Sample info
Add Fraction Table and Ticks
Add Summed Peaks Table
Add Compound Custom fields
Report Layout For Uncalibrated Peaks
Separately
With Calibrated Peaks
Do Not Report
Destination
Printer
Screen
File
Calculation Factors
Use Sample Data
From Data File
Amount 0.0000
Multiplier 1.0000
Dilution 1.0000
I#
Compound
ISTD Amount
Enter
Chromatogram Output
Portrait
Landscape
Multi-Page (Landscape)
Pages
Size
% of Page
Time: 80
Response: 40
Signal Options...
File Settings
File Prefix ASEPS
Unique pdf file name
.TXT
.CSV
.EMF
.DIF
.PDF
.XLS
.HTM
OK
Cancel
Help

10.


Instrument Curves: Instrument 1
Select curves to overlay:
Instrument Data Curves:
%A
%B
%C(C1)
%D(C2)
Flow
Temperature
High Pressure
UV Lamp Anode Voltage
Other
OK
Cancel
Help

11.


Run Time Checklist: Instrument 1
Check Method Sections to Run
Pre-Run Command / Macro
Data Acquisition
Standard Data Analysis
Customized Data Analysis Macro
Save GLP Data
Post-Run Command / Macro
Save Method with Data
OK
Cancel
Help

Instructions for "**Edit Entire Method…**"

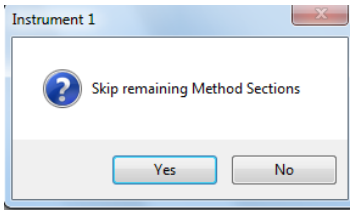
When the following windows pop up, do the following:

1→Click "OK"
2→Change the comment, Click "OK"
3→Change flow if necessary, Click "OK"

Option 1=gradient starting percentage
for %(first letter) and 100-%(first letter)
is what you put for the %(second letter)
Option 2=stays the same as in picture
Option 3=stays the same as in picture
Option 4= same as entered for Option 1
Option 5=same as entered for Option 1

4→Click "OK"
5→Check wavelengths, Click "OK"
6→ Click "OK"
7→ Click "OK"
8→ Click "OK"
9→Make sure printer is selected, Click "OK"
10→Click "OK"
11→ Click "OK"

You may hit **cancel** after you check the wavelengths as shown in picture 5. The following window will pop up and you select "**YES**"



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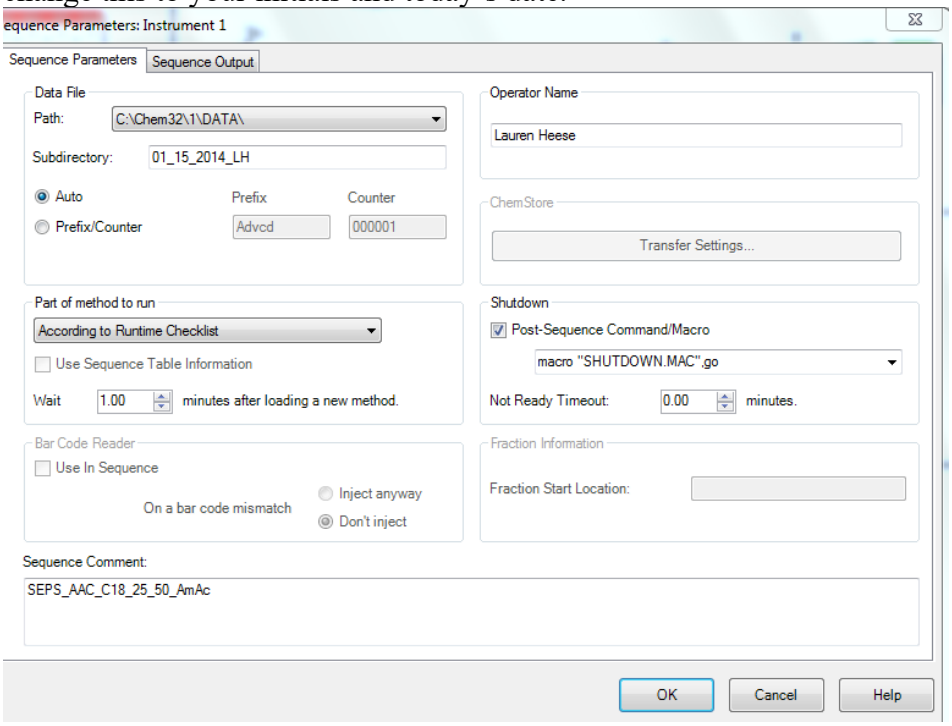
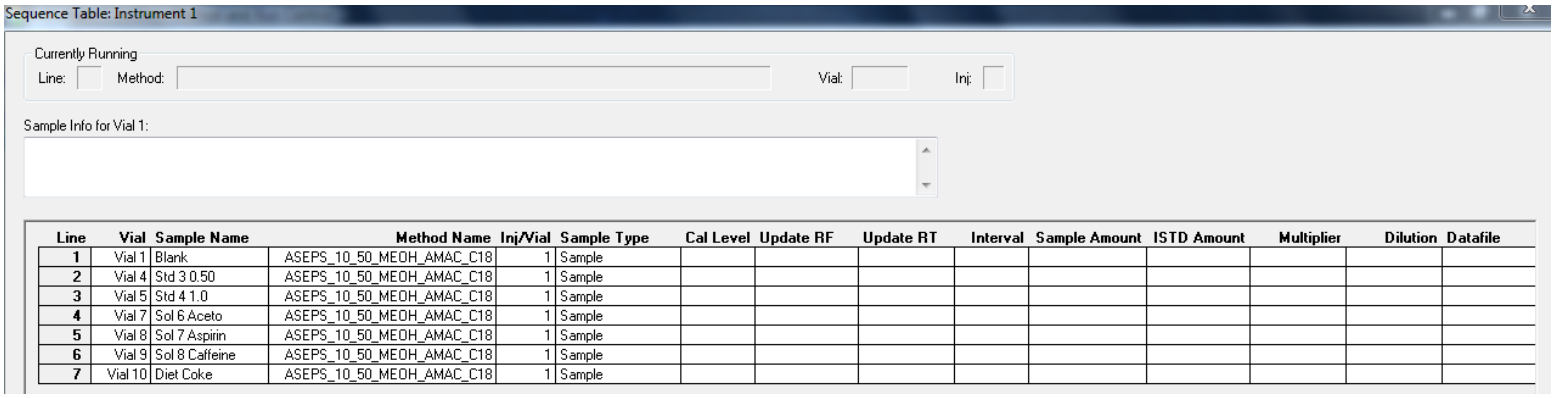
15) Load the sequence you will be using by selecting Sequence→ "Load Sequence Template" →and search for desired "(filename)".
   i) If you do not have a sequence or one does not already exist go to **Sequence→ Sequence Parameters** and in the box "Subdirectory" there will already be a date and initials, change this to your initials and today's date.



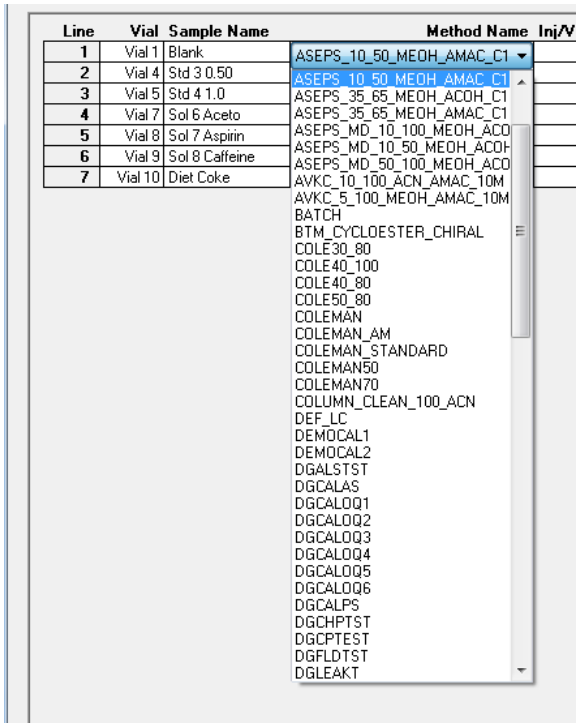
i) At the end of the edit, you will save the modified sequence under a new name using Sequence→ **Save Sequence Table as**.

ii) Then go to **Sequence→ Sequence Table**

- Go through the columns and fill in the data
- Be sure that you indicate the method that you wish to use under "method"



Line	Vial	Sample Name	Method Name	Inj/Vial	Sample Type	Cal Level	Update RF	Update RT	Interval	Sample Amount	ISTD Amount	Multiplier	Dilution	Datafile
1	Vial 1	Blank	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
2	Vial 4	Std 3 0.50	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
3	Vial 5	Std 4 1.0	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
4	Vial 7	Sol 6 Aceto	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
5	Vial 8	Sol 7 Aspirin	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
6	Vial 9	Sol 8 Caffeine	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									
7	Vial 10	Diet Coke	ASEPS_10_50_MEOH_AMAC_C18	1	Sample									



- Sample names need to be under eight characters with no special characters.
- Duplicate sampling can be prompted by changing the values in the "Inj/location" column to reflect the desired number of repetitions.

iii) After your samples, add a row with the following information:

i) *Location:* Vial 1, *Method:* purgesop.M, *Inj/location:* 1

ii) This is a purge method that will ensure that the column is clear of any residual samples.

iv) Clicking "ok" does not save your sequence. This must be done manually by going to Sequence→ **Save Sequence Table as** mentioned above.

16) Press **Start** to begin the run once all above steps have been completed and the pressure on the column has stabilized.
    i) The sequence table header will go from green to blue to indicate that the sequence is running.

17) Before leaving the workstation:
    i) Make sure that you have booked the instrument for the length of time the run will take.
    ii) Check that there is enough of your solvent to last throughout the run. If need be, plan to come refill the solvent reservoir during very long experiments. If the instrument runs out of solvent, it will automatically stop the run!
        i) Follow step 10 (i) to change the solvent bottle data.

18) Once the sequence is complete:
    i) Remove all samples from the tray and workstation. Any samples left behind will be disposed of!
    ii) Manifest the waste runoff from the sequence on the log sheet before leaving the lab.