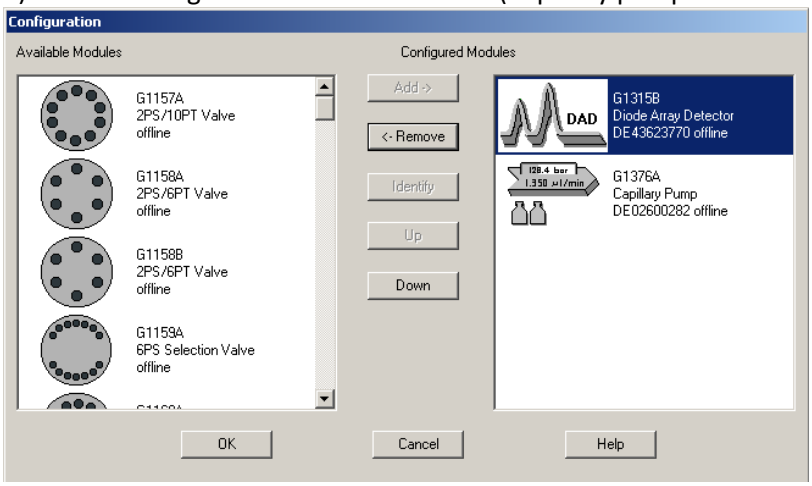
Agilent 1100 HPLC ChemStation

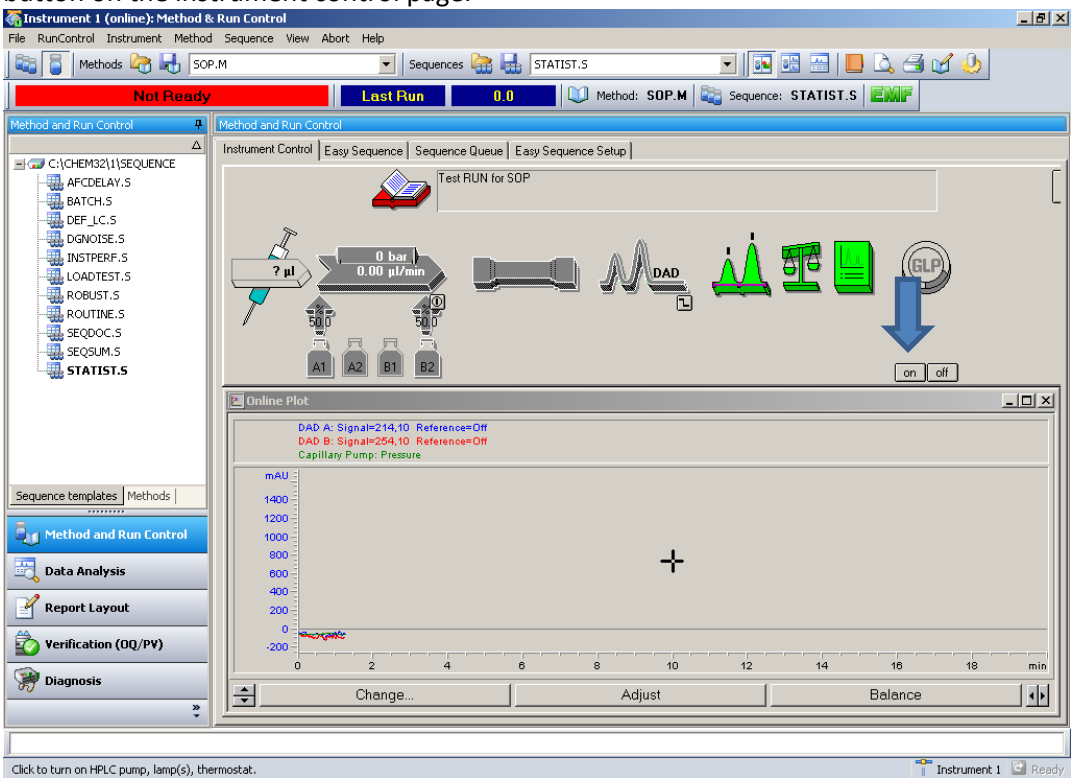
1) Turn on all the modules for HPLC system (CapPump G1376A, DAD G1315B) and allow for system to initialize.

2) Turn on the computer, open the ChemStation software by clicking Start --->Programs ---> Agilent Chemstation ---> Instrument 1 online

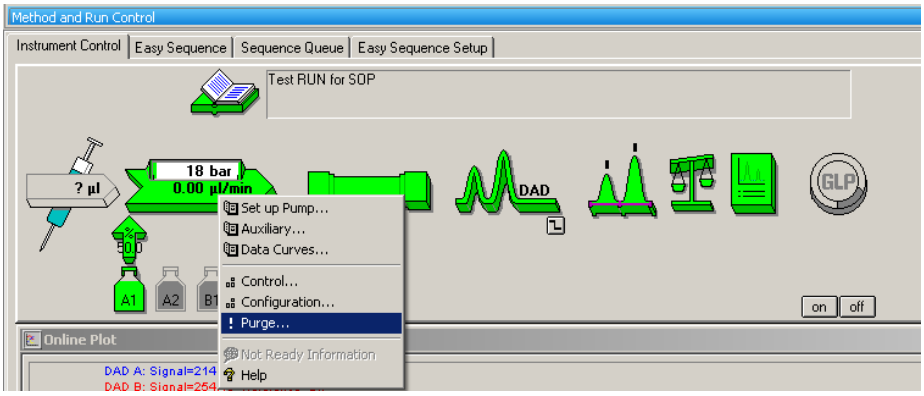
3) Choose configuration that will be used (Capillary pump G1376A and DAD G1315B) and click OK.

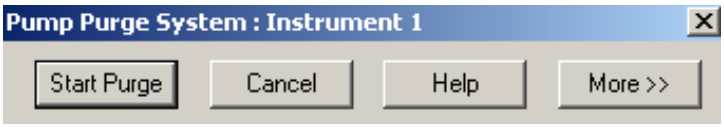


4) Under the "Instrument" menu, choose "System On" to turn on pump and DAD or use the shoutcut button on the instrument control page.

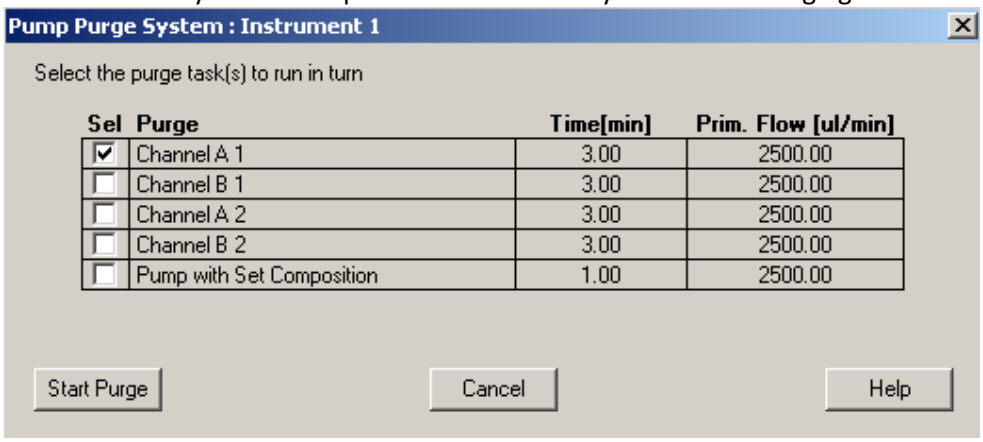


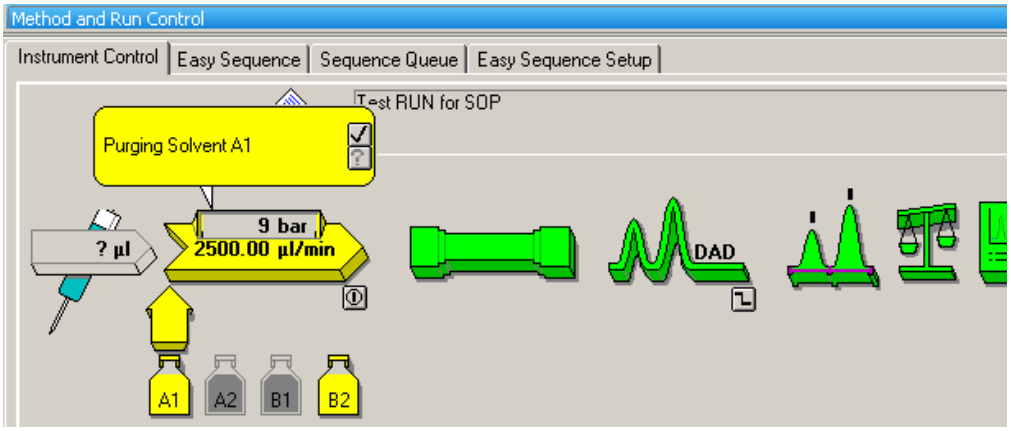
5) It is recommended to flush the reservoir lines before your column is connected to the HPLC system. Click at the pump interface ---> Purge… ---> More>>





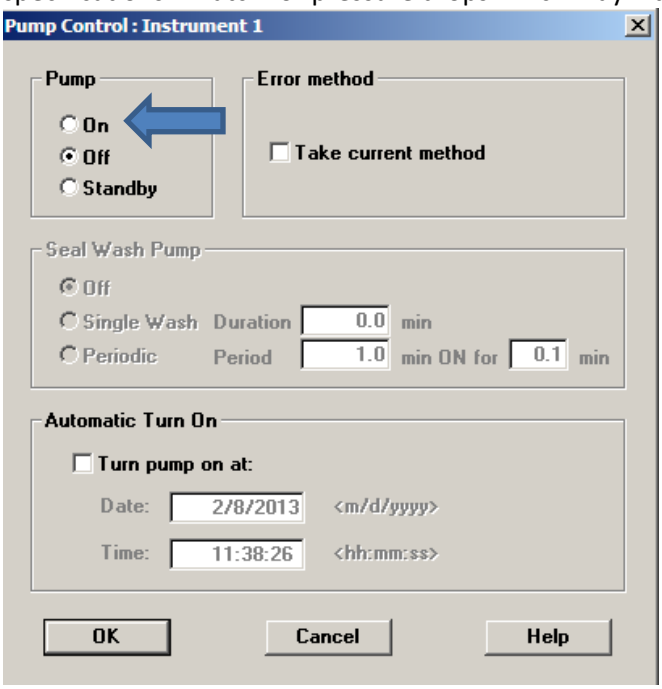
- Choose your mobile phase reservoirs that you will use. Purging the reservoir one by one.



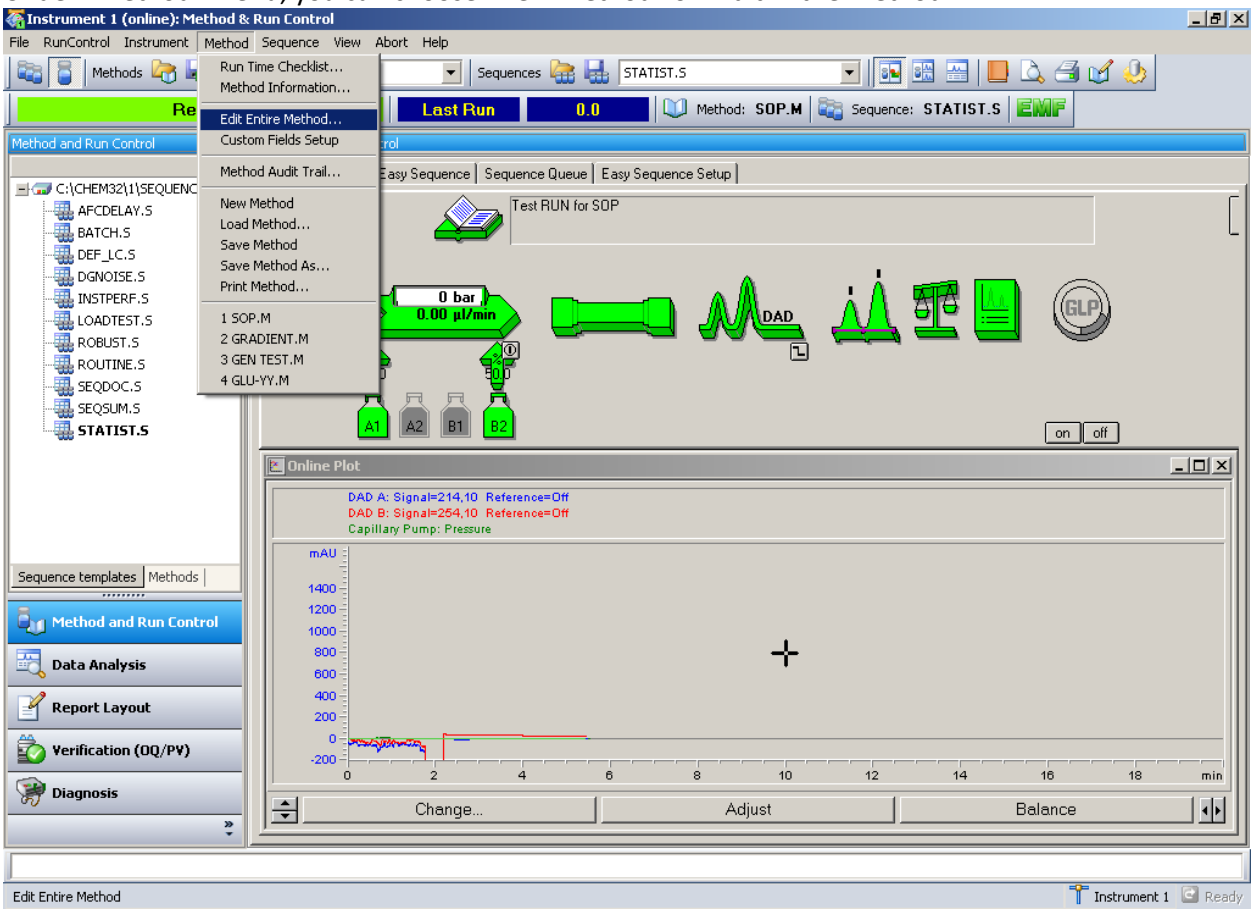


6) To connect your column to the instrument, turn off pump and all connections should be finger tight (do not over tighten)

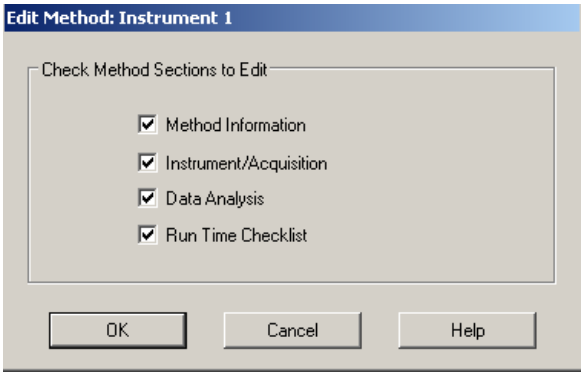
7) Turn the pump on and monitor your pressure and ensure that your pressure is within your column's specifications. Watch for pressure drops which may indicate a leakage problem.

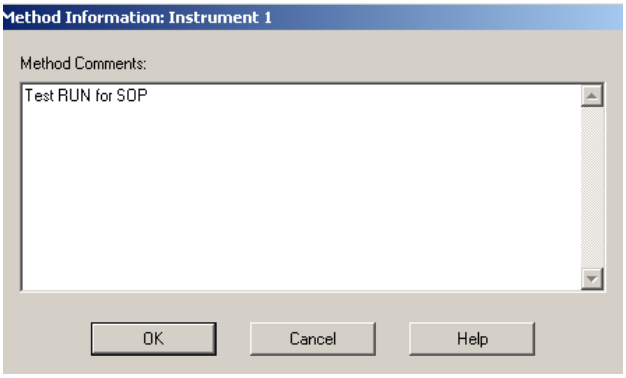


8) Set up a **method** by either starting a New Method or Edit Entire Method.
Under "Method" menu, you can choose "New Method" or "Edit Entire Method…"

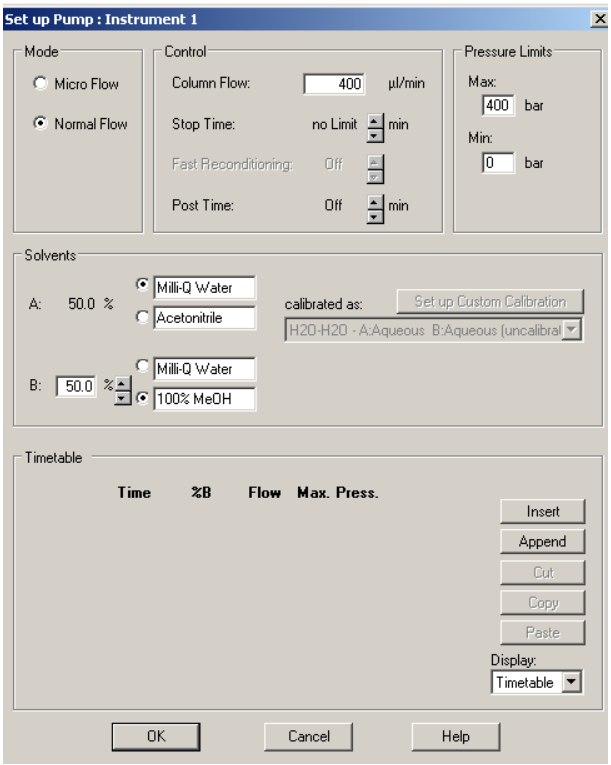


- Click method sections to edit

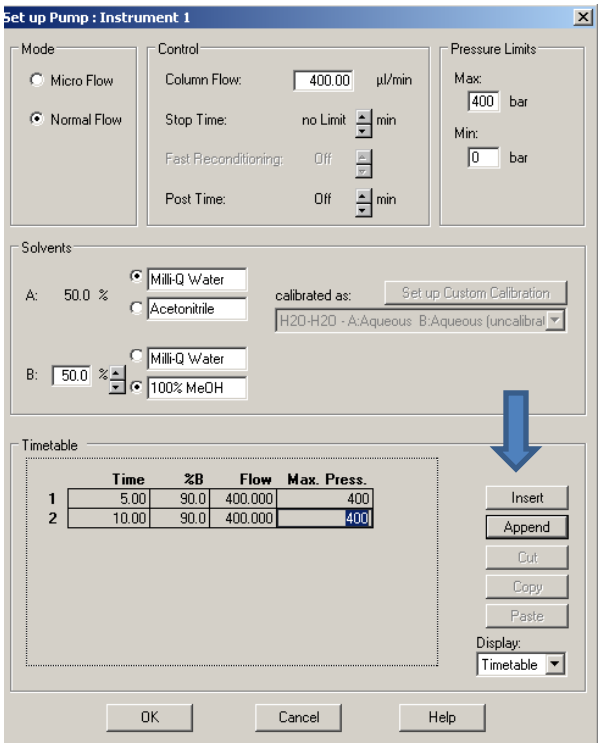


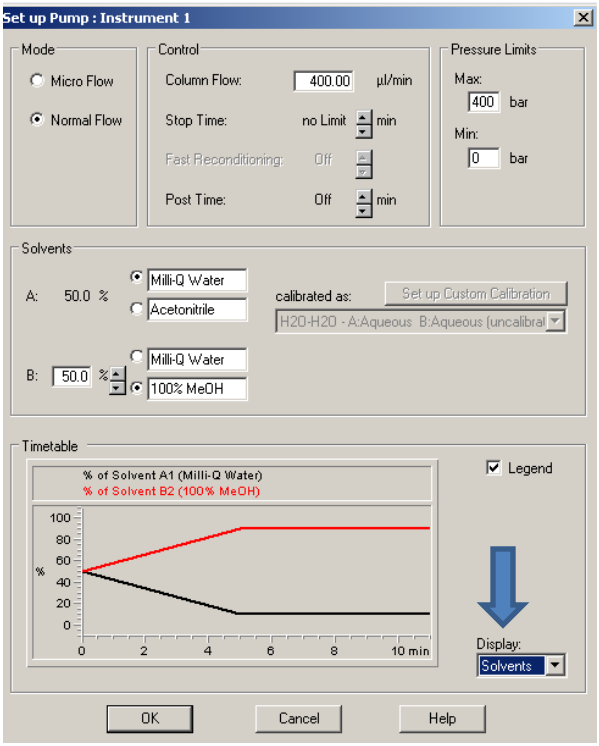


- Choose pump mode to use (Micro Flow / Normal Flow), set the flow rate as well as compositions of mobile phase to use. This setting is isocratic elution.

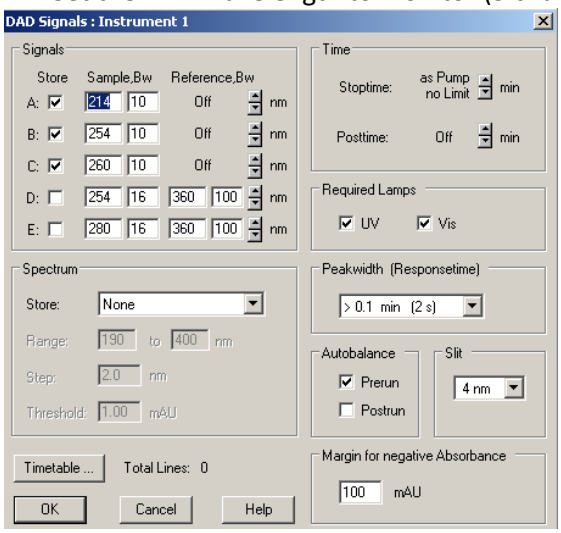


- If gradient elution will be used, set the timetable of the mobile phase.

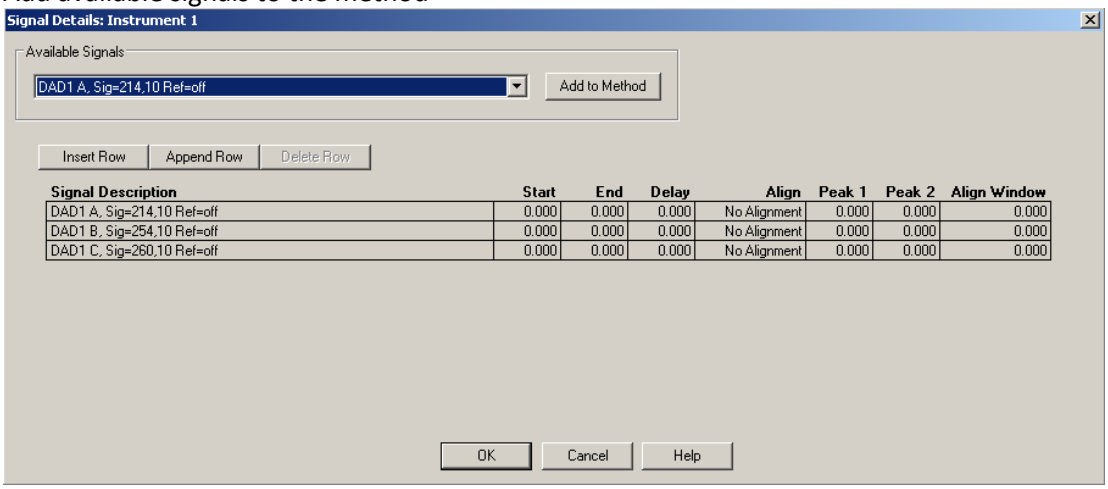




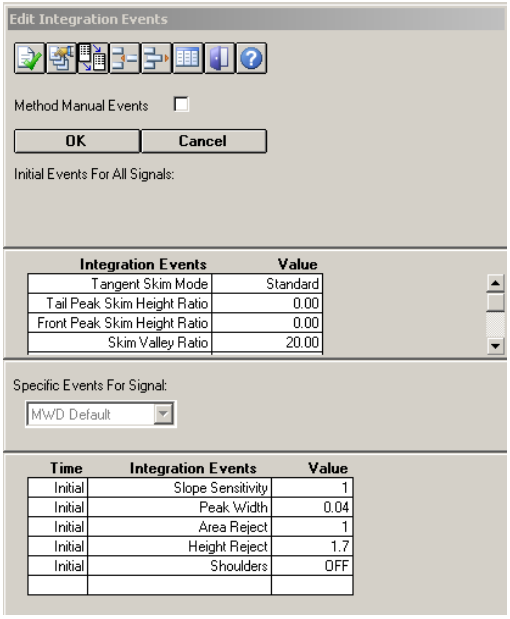
- Set the DAD wavelength to monitor (5 channels are available simultaneously.)



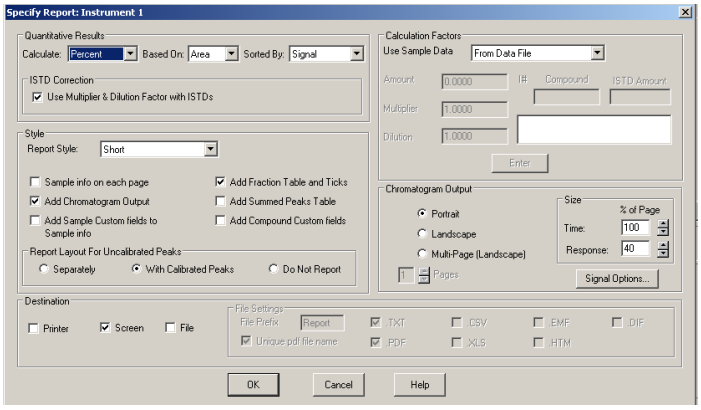
- Add available signals to the method



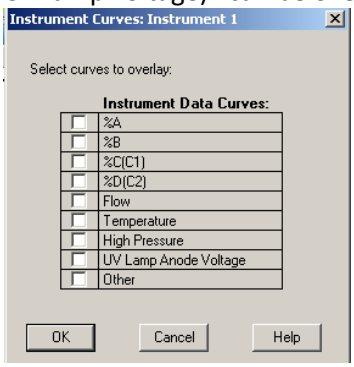
- Edit integration events for chromatogram



- Set Specification for the report and instrument curve.



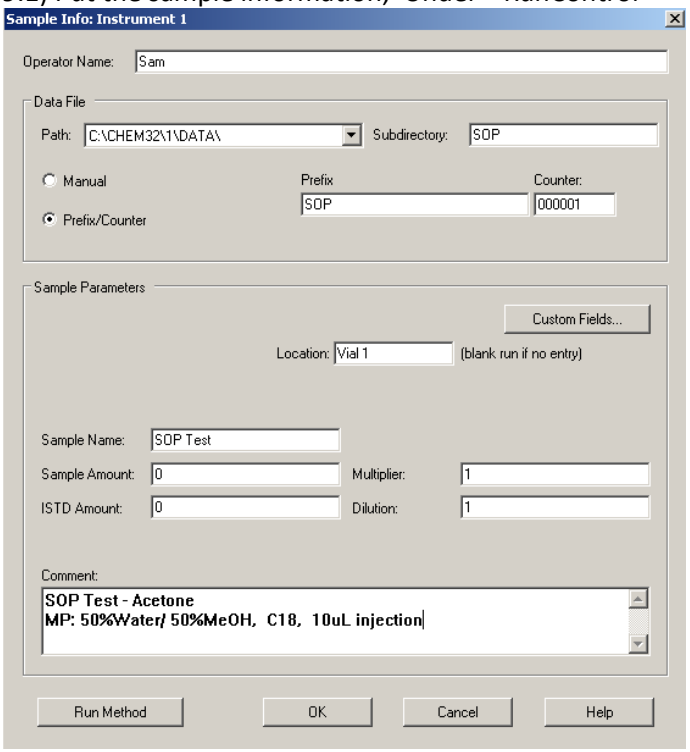
- The instrument data curve (i.e. mobile phase compositions, Flow rate, Temterature, Pressure or UV lamp voltage) can be overlaid to the chromatogram.



- After Method is set, save the method. Under “File” menu ---> “Save as” ---> “Method”
- Put the method name

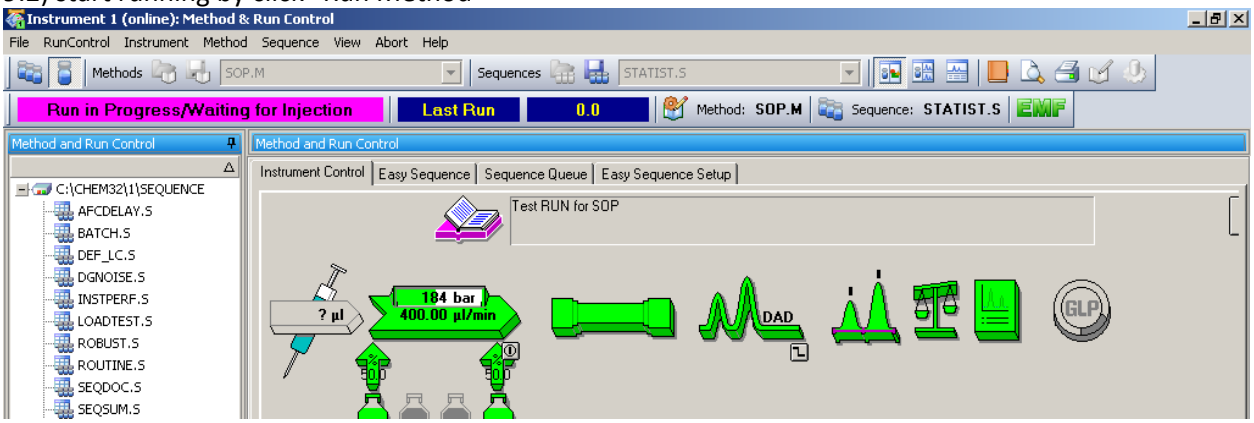
9) Run a single sample

9.1) Put the sample information; Under “RunControl” ---> “Sample Info…”



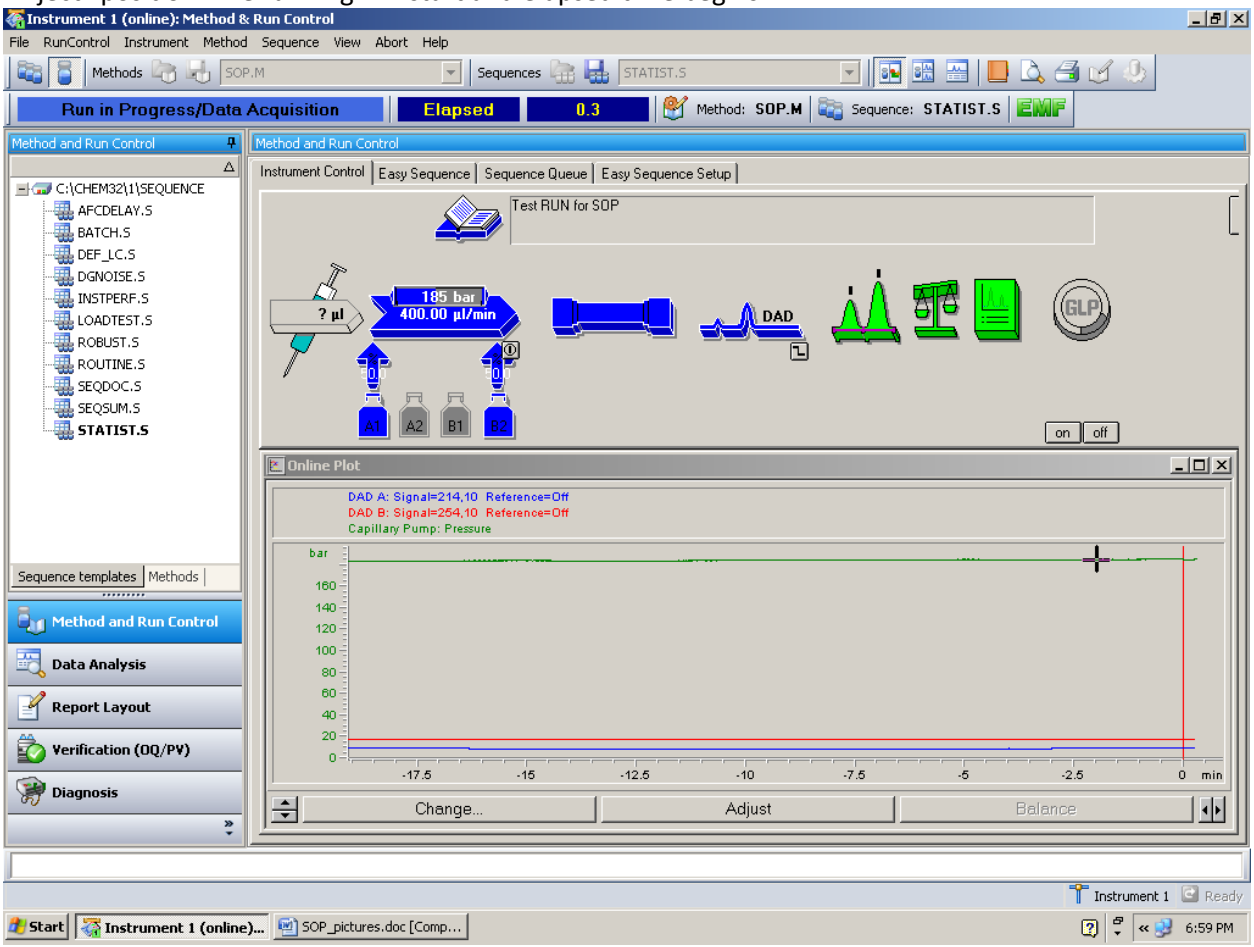
Insert sample information i.e. Operator name, Subdirectory, File name (prefix/counter can be used.), Sample name, and Comments (The running condition can be entered in this field such as, sample information, mobile phase composition, Flow rate, Injection volume)

9.2) start running by click “Run Method”



9.3) Let the column to be equilibrated until the smooth baseline is obtained.

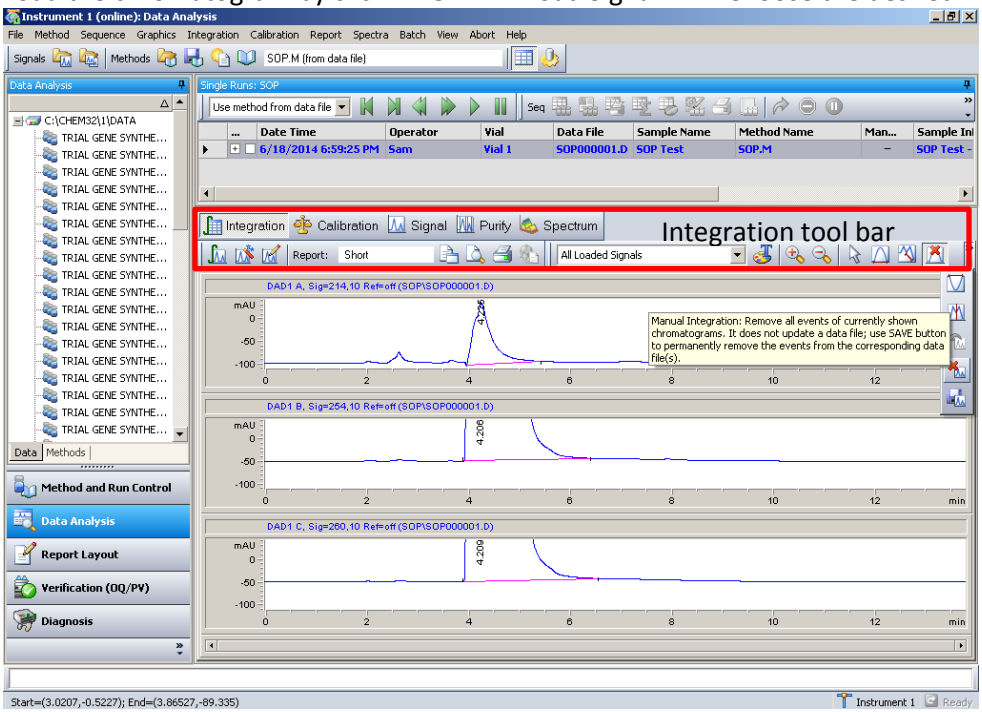
9.4) inject the sample by using HPLC syringe into injection valve and then switch injection valve to “inject” position. The running will start and elapsed time begins.



9.5) If you want to stop the run, Under “Run Control” Menu, click “Stop Run/Inject/Sequence” or shortcut F8

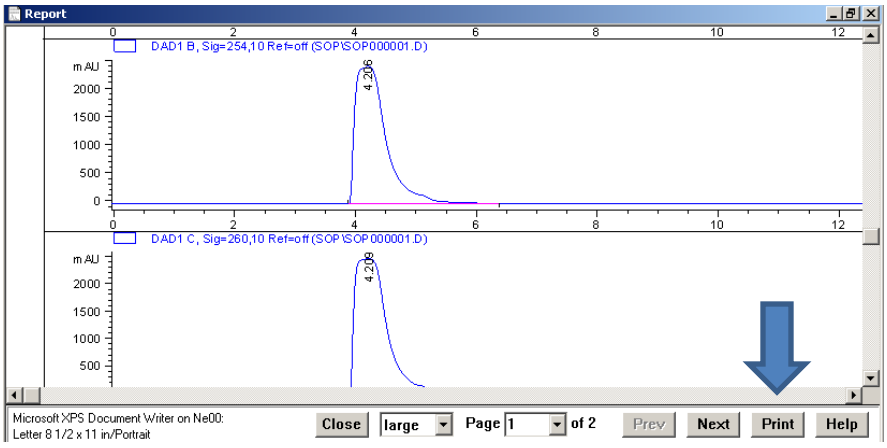
10) Data Analysis

10.1 The chromatogram is processed in the “Data Analysis” Tab (second tab on the left panel).
Load the chromatogram by click “File” ---> “Load Signal” ---> Choose the desired file name



The peaks on the chromatogram can be manually integrated or deleted by using “integration tool bar”.

10.2 The chromatogram can be printed out
Under File menu ---> Print Preview ---> Report and Click Print



11) Once the running is finished, Flushing the analytical column with high percent of methanol or acetonitrile and keep it in storage solution (see the column manual).

12) Turn off the pump and the lamp by click the DAD ---> “Control” and click off for both UV and Vis lamps. You can close out the software, and turn off the instrument (Micropump and DAD).