Shimadzu LC-MS 2020 Standard Operation Procedure

Rochester Institute of Technology

Department of Chemistry and Material Science

SOP Prepared by William Charbonneau on 4/4/19

SOP Owned by Tom Allston

I. Purpose

To promote the effective use of the Shimadzu LCMS-2020 Liquid Chromatography Mass Spectrometer to establish an instrumental method and analyze the chromatogram obtained.

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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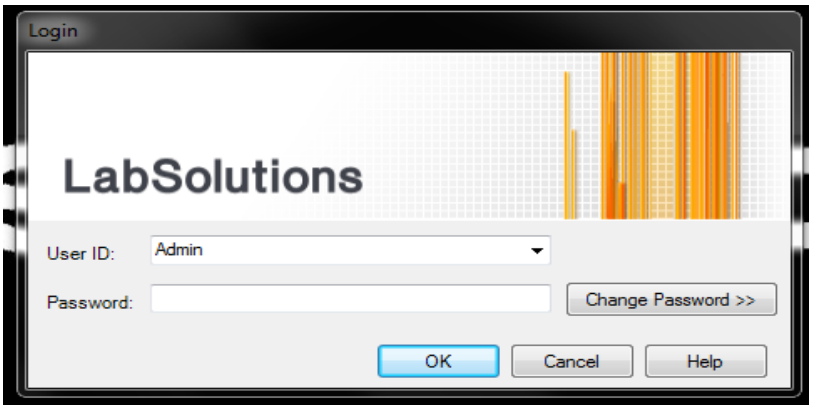
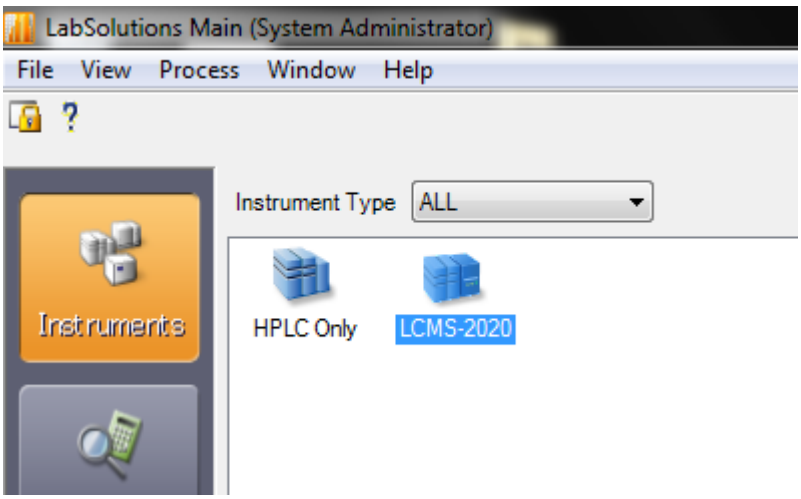
V. Procedures and information

1. Turn on the spectrometer (switch on the front of the LC instrument), computer, and monitor.
   a. Check the Nitrogen tank in the corner of the room to ensure it is on (and has pressure).

2. Connect and Start the Instrument
   a. On the Windows Desktop, double click (with the left button of the mouse) on the "LabSolutions" icon. LabSolutions will ask for a password, there is no password, just hit the "OK" icon.

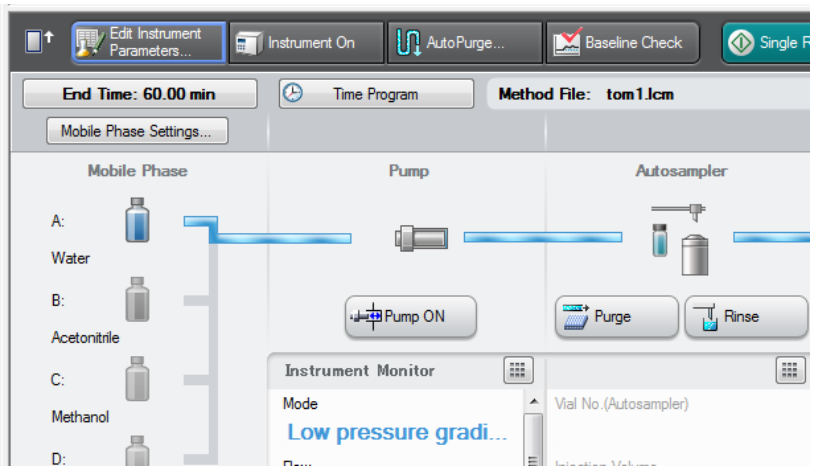


   b. Click on the "Instrument" icon, then double click on the "LCMS-2020" icon. This will open up a new window called "Realtime Analysis…".



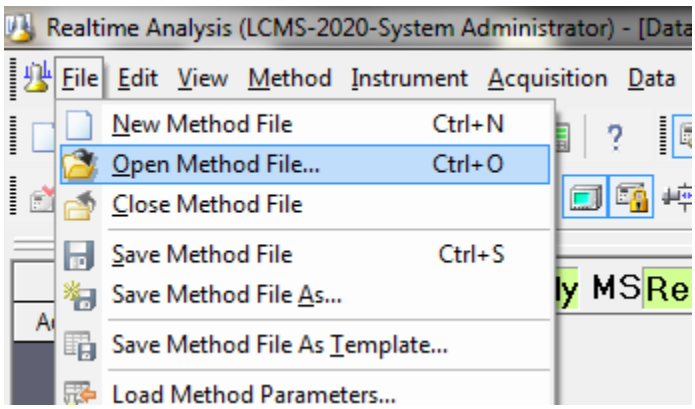
   c. Once the new window pops up, on the left hand side, click the "acquisition" Tab

d. Click on the “Instrument On” icon and wait till the status bar at the top of the screen shows “ready” for all three components.

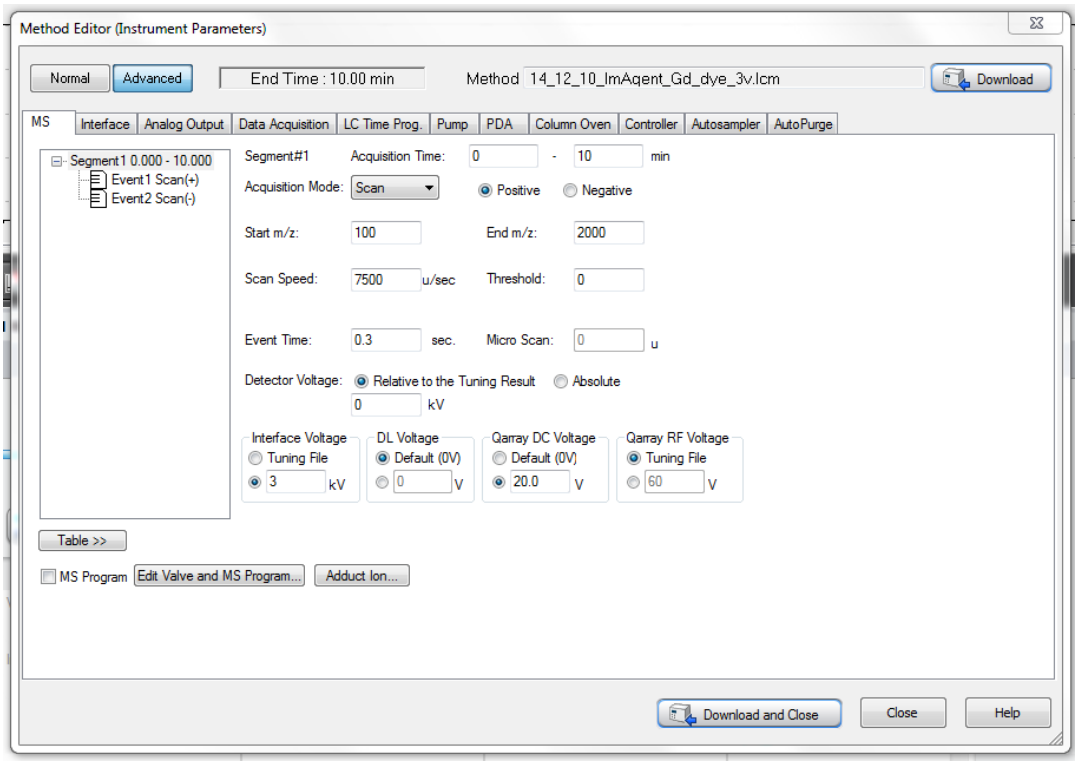


3. Loading a Pre-existing Method

a. Go to “File” \ ”Open Method…”, then select the previously created method file.



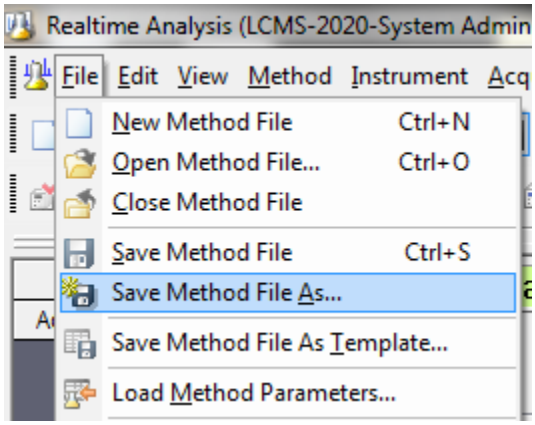
b. The “Method Editor” window will appear. Edit the parameters as you wish. The click “Download and Close”.



c. Resave your method by going to, “File” / “Save Method As…”

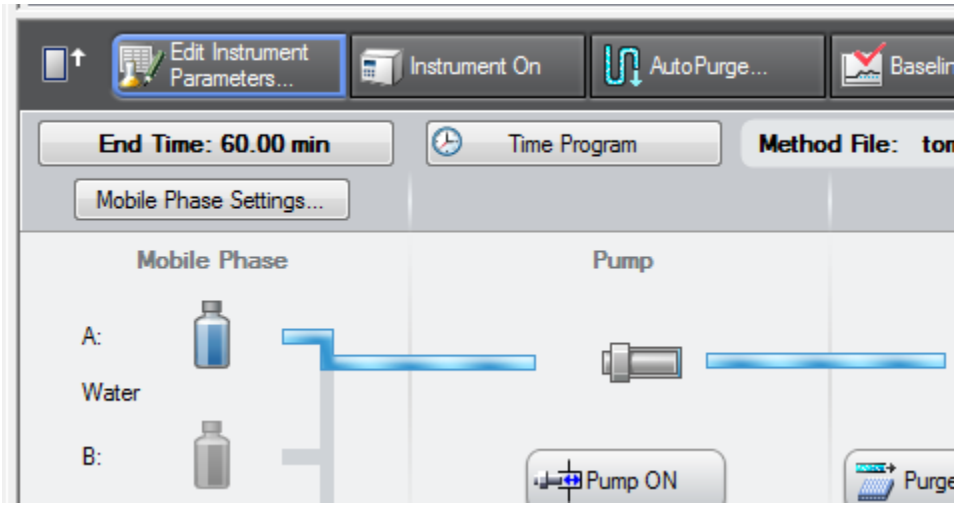
   i. Be sure to create YOUR OWN folder within the default save locations (“C:” \ “Lab Solutions” \ “Data”) folder. This will ensure all your data will be kept in once location.

   ii. Note: The chemistry department takes not responsibility for your data, you must back up any data file that you do not wish to be deleted.



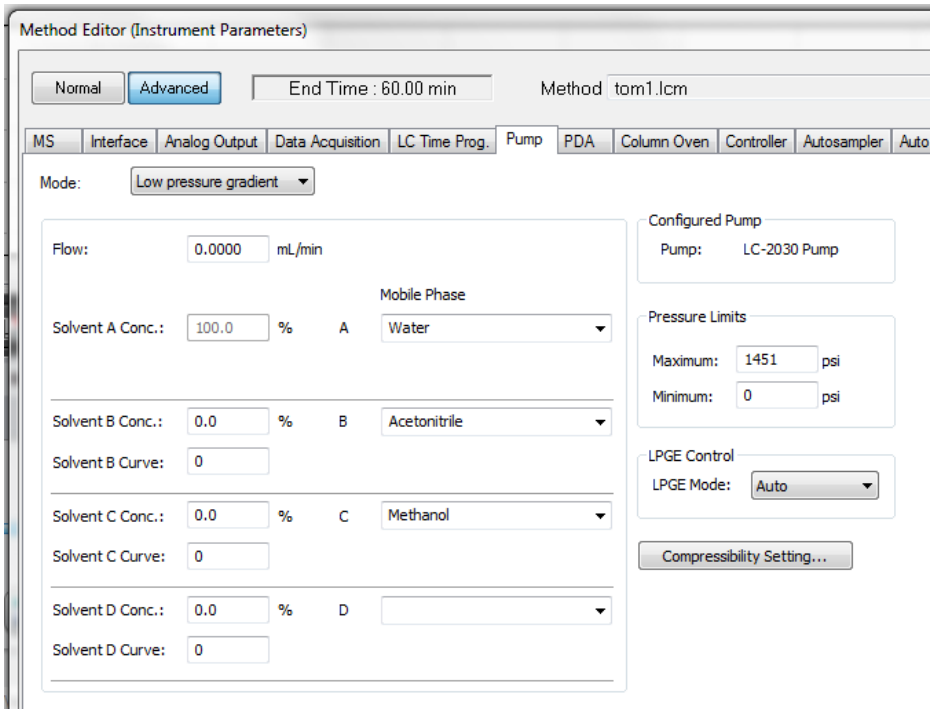
4. Creating a New Method.

a. Click on “Edit Instrument Parameters…”, this will open up the Method Editor.

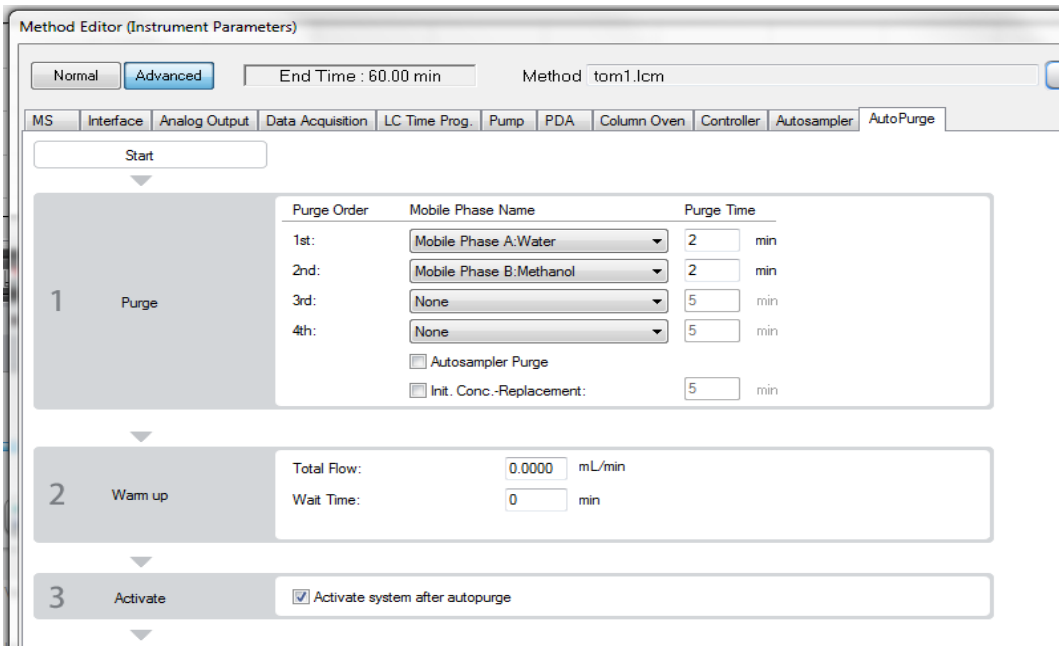


b. First select the “Pump” tab. Chose the “Low Pressure Gradient” mode if you want to slowly change from one solvent to another, or choose “Isocratic” if you want one solvent at a set flow rate. Then ensure all the solvents are correct and input your starting concentration for each solvent. (Be sure the solvent lines on top of the LC unit match what you have entered.)

   i. By clicking on the “Compressibility setting” button, and making sure that compressibility is checked, it will create more of an uniform retention time for sample.



c. Next click on the “AutoPurge” tab, Set your purge order and time for your solvents.

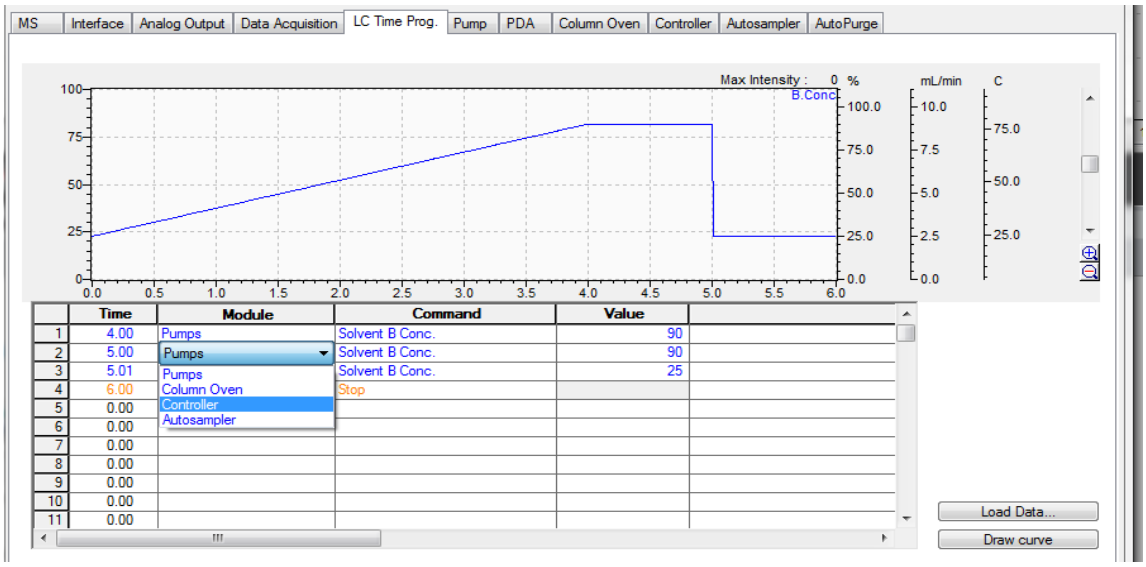

Method Editor (Instrument Parameters)
Normal
Advanced
End Time : 60.00 min
Method tom1.lcm
MS
Interface
Analog Output
Data Acquisition
LC Time Prog.
Pump
PDA
Column Oven
Controller
Autosampler
AutoPurge
Start
1
Purge
Purge Order
Mobile Phase Name
Purge Time
1st:
Mobile Phase A:Water
2
min
2nd:
Mobile Phase B:Methanol
2
min
3rd:
None
5
min
4th:
None
5
min
Autosampler Purge
Init. Conc.-Replacement:
5
min
2
Warm up
Total Flow:
0.0000
mL/min
Wait Time:
0
min
3
Activate
Activate system after autopurge

d. Next click on the “LC Time Prog.” Tab. Enter the desired gradient for the run if using “low pressure gradient”.

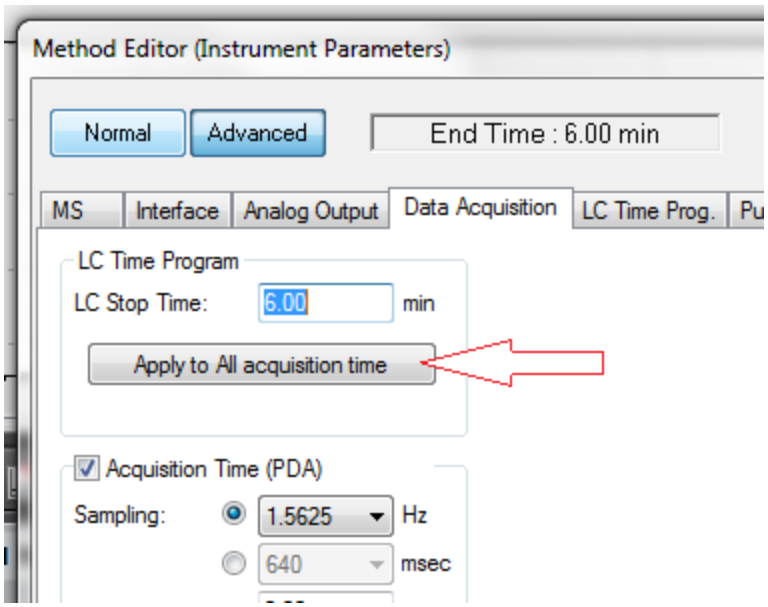
   i. “Pumps”: will create a pumping gradient to change the percentage of the solvent over a course of time

   ii. “Column Oven”: will change the temperature of the oven at a selected time

e. Be sure to stet a “Controller, Start” in the beginning of the run. At the end of the run, ste a “Controller, Stop” function. The “Controller, Stop” function should be set to 0.01 seconds after the final module. After the table is set, click on the “Draw curve” icon and double check it is as you want it.

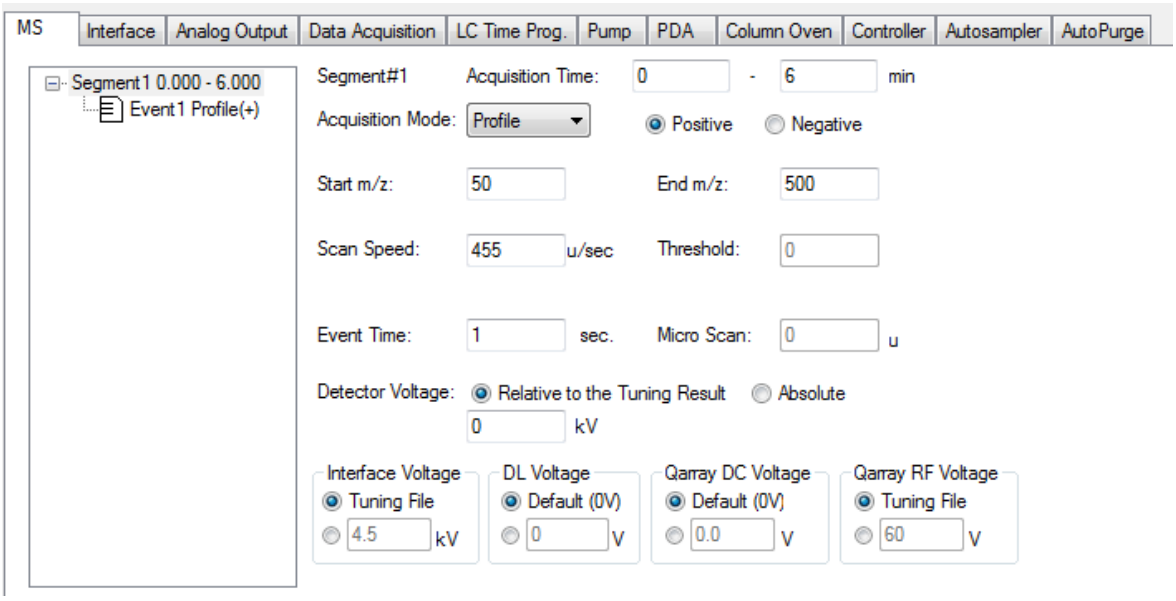


f. Next go to the “Data Acquisition” tab, select the “Apply to all acquisition time” icon. This will set all run times to match the LC Time Prog.’s run time.



g. Next go into the "MS" tab.

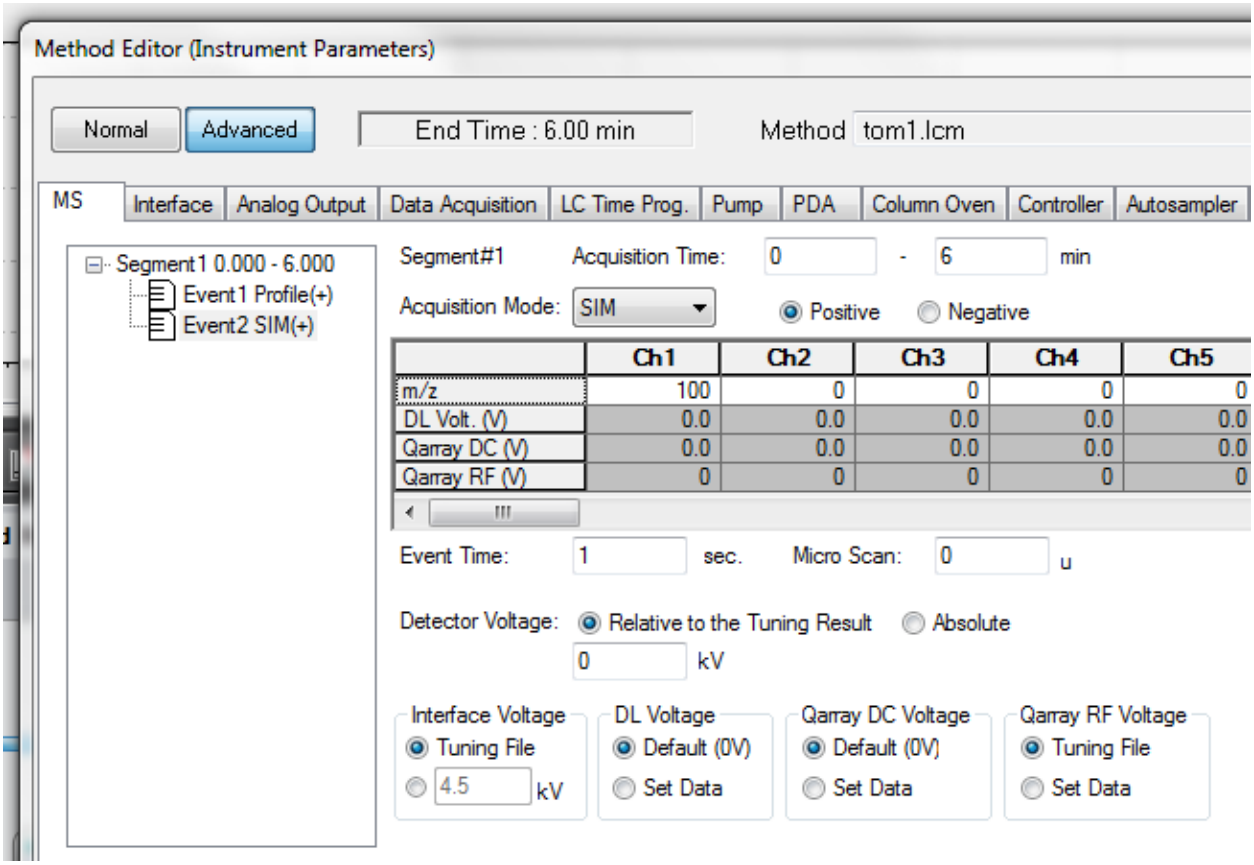
   i. Click on Event 1 on the left hand side of the window to start editing the event. Then set the "Acquisition Mode". SIM which looks at specified mass to charge ratio (m/z), Scan which scans the who m/z range. Then select the charge (either "+" or "–") and set the m/z range.



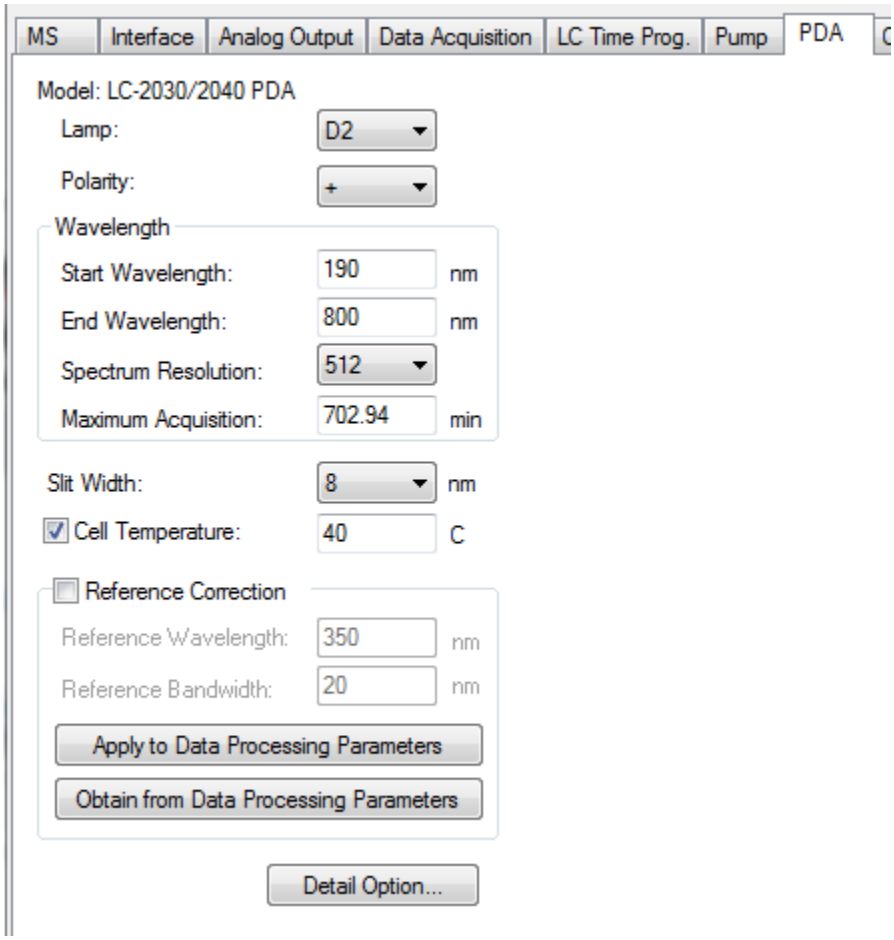
   ii. To add another event, right click on "Event 1", and then click "Event Add". This will create an "Event 2" which can be edited in the same manner as

"Event 1" was. these events can be set to run after one another by setting the event start time after the first event.

iii. When creating an event that is in the "SIM" (or Single Ion Measurement) acquisition mode, you will need to set each "Ch #" for the number of "m/z"'s you wish to measure.



h. Next, If you wish to use the PDA (photodiode array detection) component of the LCMS, select on the “PDA” tab. Set to your desired parameters.



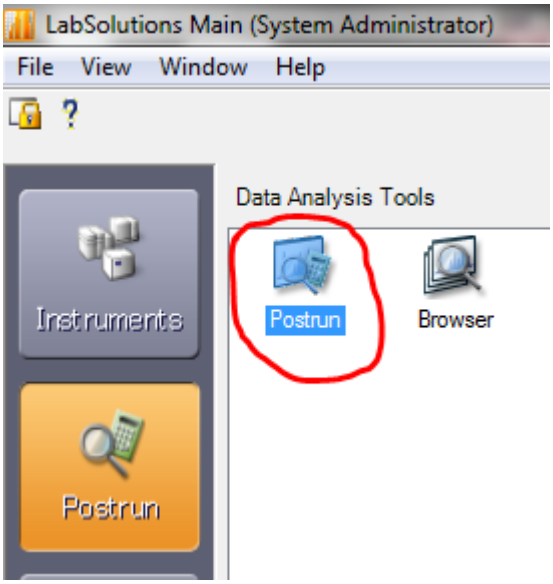
i. Finally, select the “Download and Close” icon. Save your method by going to “File” / “Save Method As…”. Save your file in a place that can be easily retrieved at a later date.

5. Running Samples
    a. Insert your samples into Tray 1.
    b. On the left hand side, click on “Realtime Batch”, this is where you can set up a list of runs for the samples

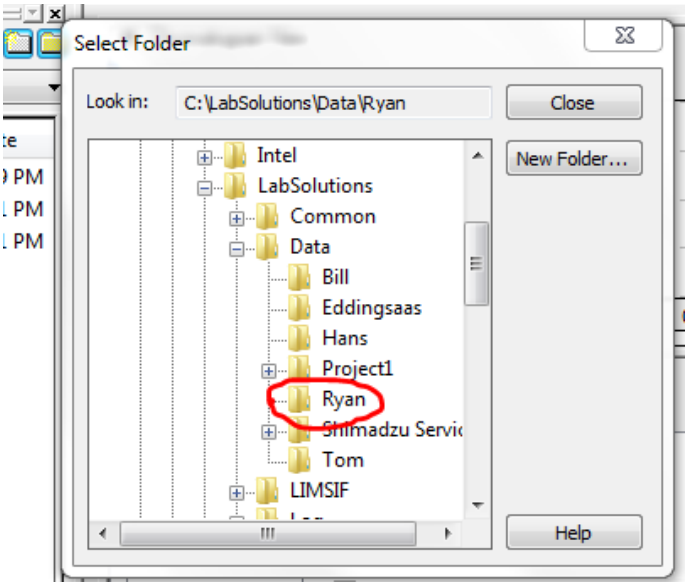
Analysis	Vial#	Tray Name	Sample Name	Sample ID	Sample Type	Analysis Type	Method File	Data File
1	1	1		1	0:Unknown		21_2185\method03.lcm	ection3D\vial1grpD.lcd
2	2	1		2	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD001.lcd
3	3	1		3	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD002.lcd
4	4	1		4	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD003.lcd
5	5	1		5	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD004.lcd
6	6	1		6	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD005.lcd
7	7	1		7	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD006.lcd
8	8	1		8	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD007.lcd
9	9	1		9	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD008.lcd
10	10	1		10	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD009.lcd
11	11	1		11	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD010.lcd
12	12	1		12	0:Unknown		21_2185\method03.lcm	tion3D\vial1grpD011.lcd

    c. Start by right clicking the first row, and select “Add Row” to add the desired amount of rows for the samples.
    d. In the “Sample name” column, set name of each of your samples. They should be filled in corresponding to the position of the sample vial in the tray.
    e. In the “Method File” column set the method file created for each sample by clicking the down arrow, and then choosing the method file from the folder it was saved. Then click and drag down to highlight each empty row, right click, and “Fill Down”.
    f. Then fill in the “Data File” column with the name of each sample corresponding to the row the sample is in. Once done click the down arrow and select the folder to which the data will be saved under.
    g. The injection volume can be set to the desired amount of each sample to be injected into the column.
    h. Once completed, click the “Start Realtime Batch” button on the left hand side to start the run.
    i. 

6. Data Analysis (qualitative) for MS
    a. In the “LabSolutions” window, select the “Post Run” tab, then select “Post Run”



    b. Click On “Layout \ Qualitative”
    c. Open one of your data files by going to “folder” window within LabSolutions, Then click on the folder icon and click on the folder where you saved your data and close out of the window.



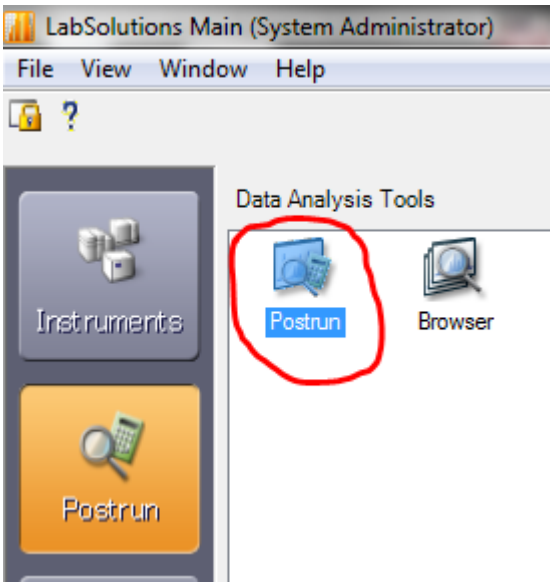
    d. Then double click the data file chosen to bring up the chromatogram and the Mass spec of the sample.

e. Then you can double click on the peaks in the chromatogram to bring up the Mass Spec for that peak. This allows for the determination of the speed at which each compound moved through the column. The Mass spec will allow for the determination of which compound corresponds to each peak.

f. Right click the Mass Spec of the peak and click on library search. This will search a known library and try to determine the compound based on the Mass Spec.

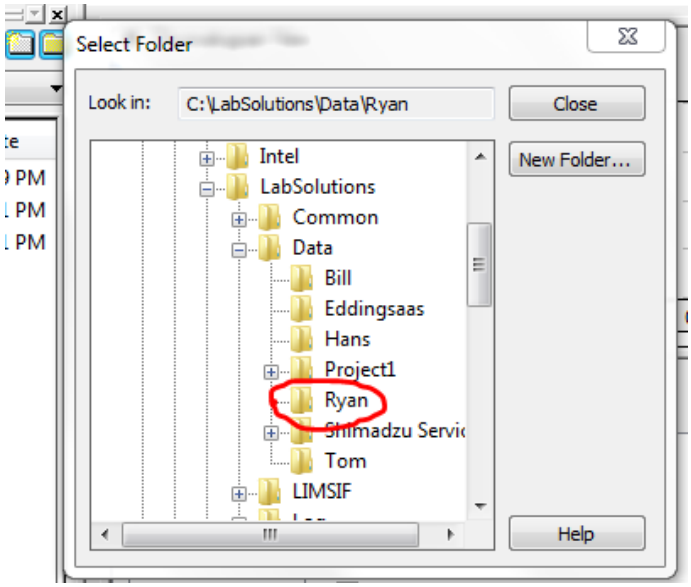
7. Data analysis of samples (quantitative)

a. In the “LabSolutions” window, select the “Post Run” tab, then select “Post Run”



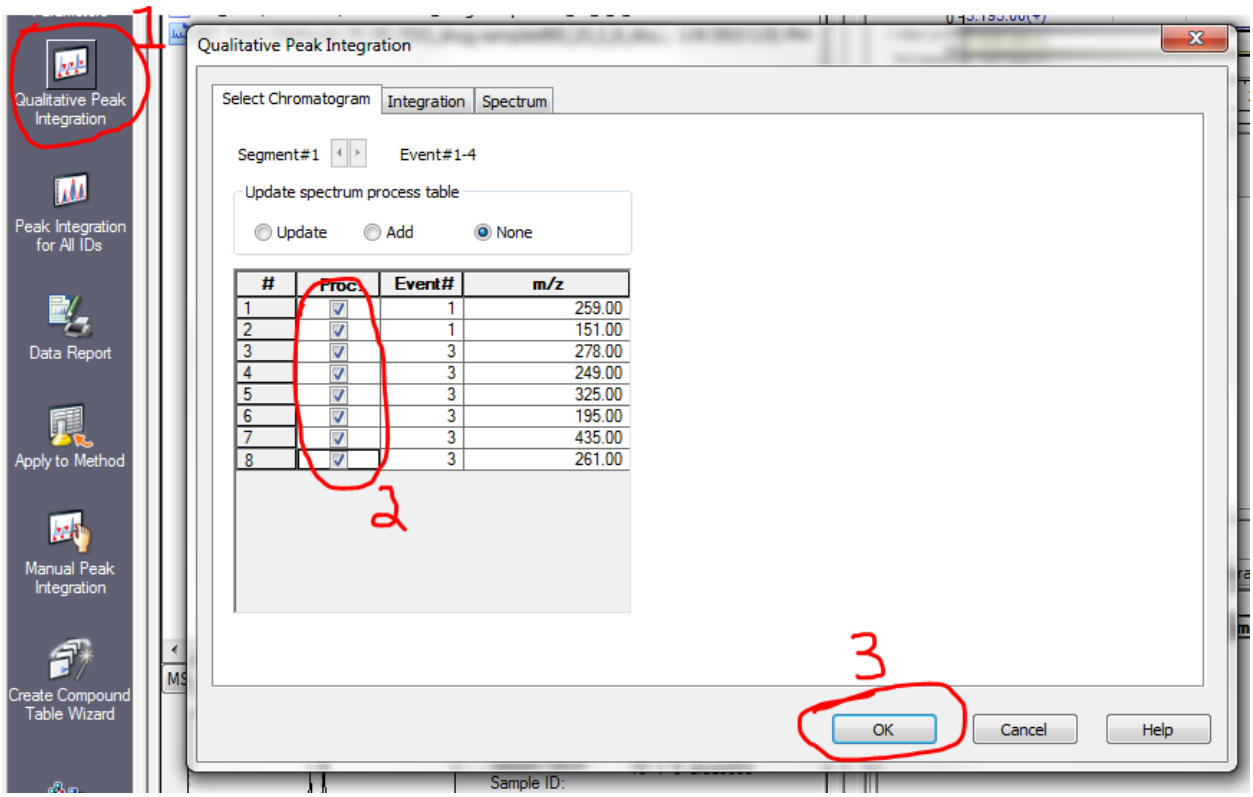
b. Click on “Layout” \ “Quantitative”

c. Open one of your data files by going to “folder” window within LabSolutions, Then click on the folder icon and click on the folder where you saved your data and close out of the window.



d. Select a standard from your data and double click it to bring it onto the screen. (The better defined the peaks are in this scan the better the rest of your data will turn out.)

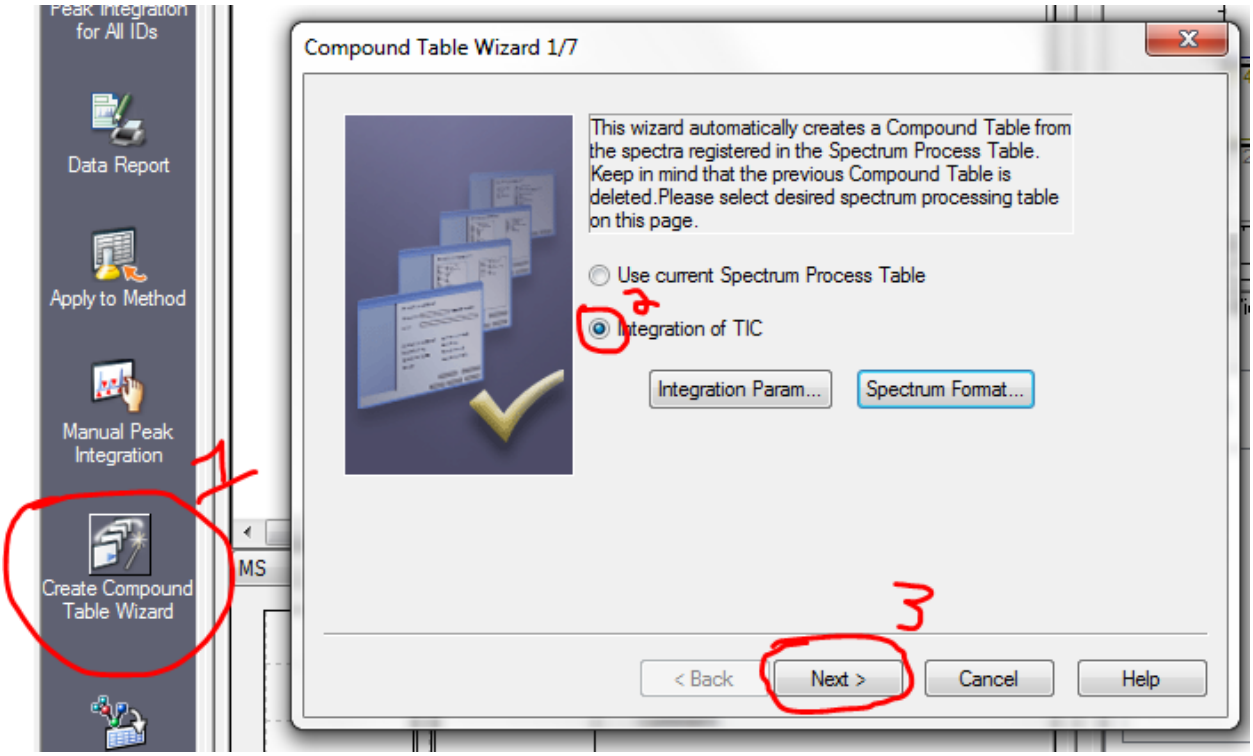
e. [1] Select on the “qualitative Peak Integration” icon (its on the left hand side of the screen). All peaks of Interest should be labeled at this point.



f. [2] Click on each molecular weight specified (only for SIM) and make sure they correspond to an actual peak. [3] Click “Ok”

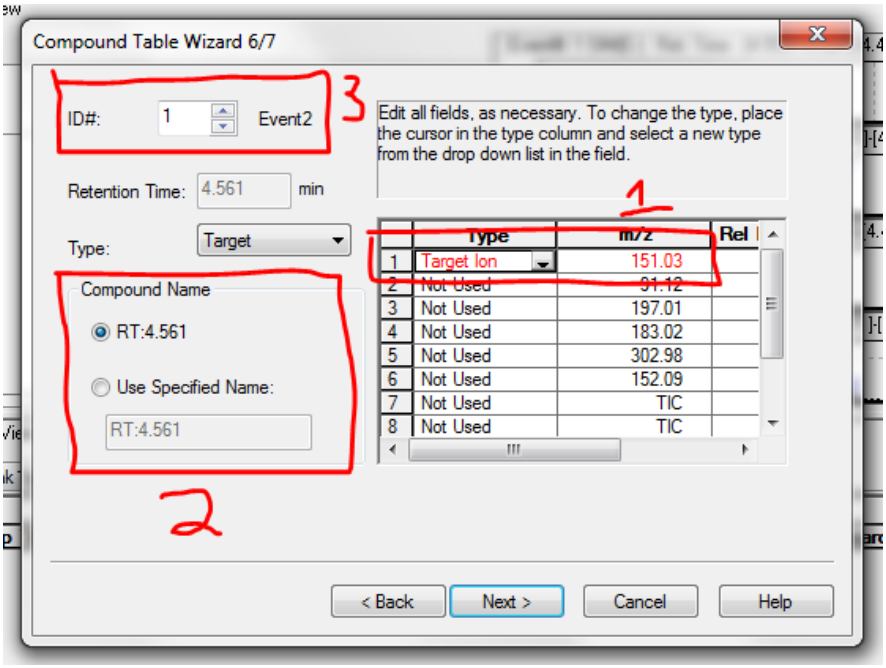
g. On the side of the screen select the “Create Compound Wizard” icon.

   i. Select “Integration of TIC”, then hit “next”.



   ii. Input the number of peaks in your spectrum.

   iii. Use your controls to label each peak to the corresponding compound within the wizard. At this point you may also enter the concentrations of the standards [1,2].



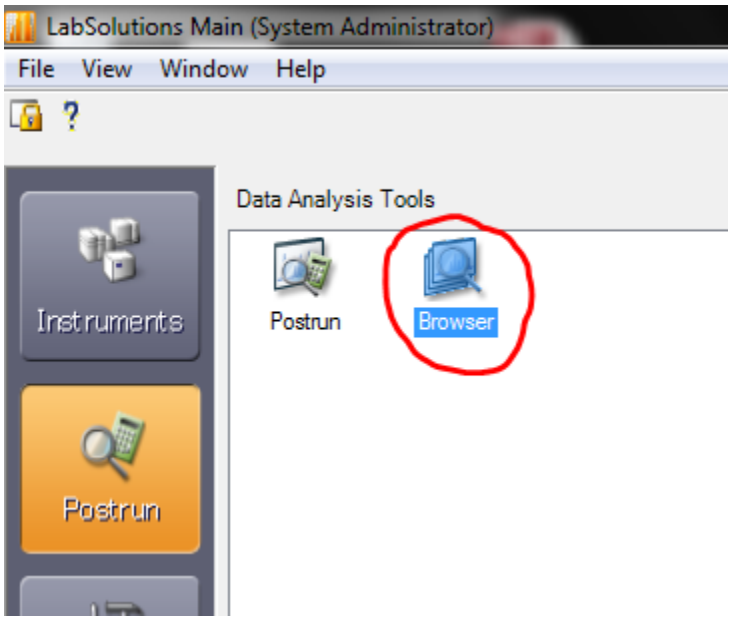
iv. Move from peak to peak using the up arrow in the wizard [3].

v. Finish by clicking view on the right side and then click on yes for integration the peaks again (trust me, it's better this way).

h. Click on "Save Data and Method File"

i. Close the "Postrun Analysis" window completely.

j. In the "LabSolutions" window, select the "Post Run" tab, then select "browser"



k. Select “File” \ “Open Method File”
l. Select your standards and unknowns for the “data” window and drag them into the center window.
m. Select “Peak Integration for All Data”
n. Click on each component and write down the concentration levels used in the compound tab on the right.
o. Check that all calibration points are good and reject those that are not by unchecking them.

8. Turning Off the Instrument and Logging off
   a. Simple excite out of the __ window, it will prompt you asking if you what components you wish to shut off.