LCMS Notes – complete as of 16/10/2010

See a summary on google docs

https://docs.google.com/document/edit?id=10hwYxW_4T9P2or6RIBCho-ysyWuSkV2jtjrODhSgXeg&hl=en&authkey=CJ6R8vsF&pli=1#

Italicised and initialled text was done by Fargol. If you have any questions or want to make editions feel free to ask Fargol or update it online (above link) and initial the edited text.

Things to do/ask: October 2010:

- ask technician to train users again and identify the problem with ionisation of the mass spec. (i.e. doesn't show the right ions) F.T

-another problem that needs resolving is the auto stopping and shutting down of a run or sequence with an error message saying: 'insufficient IG vacuum' even though the IG vacuum is a bit higher in value than the one on the ShimadzuLCMS2020 in the separations lab (i.e. 7.2 E-4 Pascals on this machine rather than 5.9E-4 on the other machine in the separations lab)Yves and Brenden have not encountered this problem on the other Shimadzu. F.T

- put the column guard on

- change the solvent c reservoir (which is for rinsing the autosampler tip and is currently ACN with 0.1% formic acid) to one with isopropyl alcohol or MeOH because Cedric said to do so) - F.T

-ask techinician if you can take out a sample or add one in another row after your sample is withdrawn via the autosampler whilst the sample is in the MS and/or running through the column? F.T

- I had a problem getting an ionisation of any kind after one run (the last day I started using it) because I accidentally left the tap on the pump open to waste so the compound did not go through the column or the MS and hence did not show ions. I highlighted this crucial step in closing the tap on the pumps A and B before starting a run in the procedure and it seems a few trained users are not sure of the operation of the tap and so I believe a follow-up user training session is crucial for us once the above hardware problems have been resolved. F.T

Things to do: August 2010:

Guard column. Quote to do.

Snapshots & screengrabs in this document and place this on the PC.

Section Each - sel-assign.

Initial to indicate that you are in charge of a section.

Filters.

Cotton wool or membrane.

Need make and model for the filter. Used a few times is OK. Sabrina to forward details to Mat.

Samples made up in 1mg per 10 mL. Standard organic phase is: Acetonitrile.

Degas: sonication. do this for the solvent bottles. Start of each refil. Standard flow is 0.2 ml per minute.

MilliQ Level 3 - check OK?

Common account for things?

Charge central account.
For ease, recommend using group login as you would for the NMR machines. One person from each group to set up.

Equilibrate column.

Things to check: How the Instrument should be when you come to it:

Solvent Preparation

- solvents quality: HPLC or MS grade; water: fresh Milli-Q water (from brown glass bottles, no plastic container- filtered and degassed by 10 minutes of sonification)
- recommended rinse solution (reservoir C) for rinsing the syringe: 10% iPrOH or MeOH in water
- buffers: TFA, formic acid, ammonium acetate, ammonia - use only volatile buffers (no phosphate buffer)!

Solvent A: Milli-Q H_2O:Formic Acid (0.1%)

Solvent B: HPLC grade Acetonitrile:Formic Acid (0.1%)

Solvent C: 10% IPA: MeOH (Cedric said isopropyl alcohol or MeOH is fine too)

Starting procedure: by Vicky

- is the vacuum pump running (should run all time, otherwise it'll need 4-5 hours to have a adequate vacuum for the MS) *otherwise something has gone wrong- F.T*
- Remove plug from ion inlet of mass spec making sure to hold needle at the end (Figure 1)

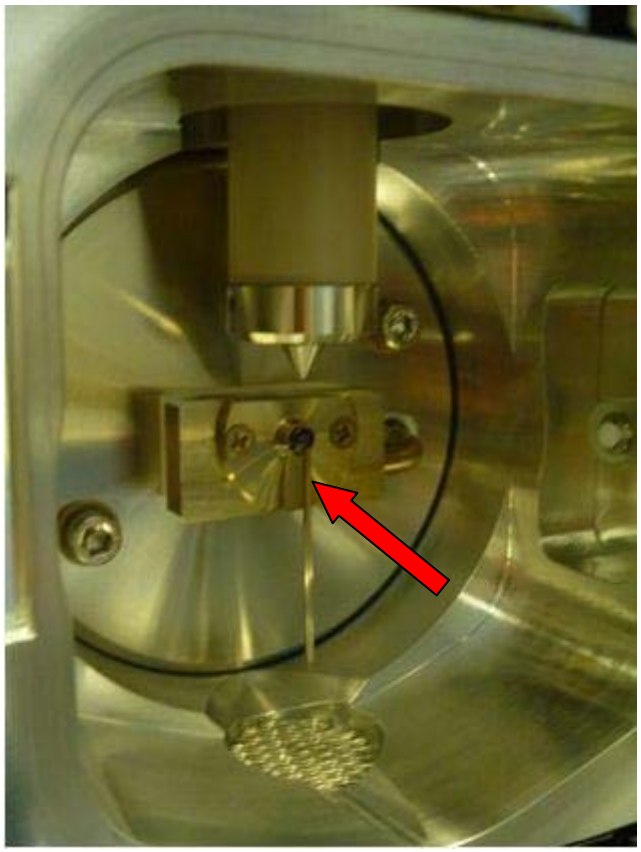


Figure 1: Plug in ion inlet of mass spec

- Turn on power buttons on all machine components (Figure 2) starting from the right top side (**red arrow)** and finishing at the interface (**blue arrow).**

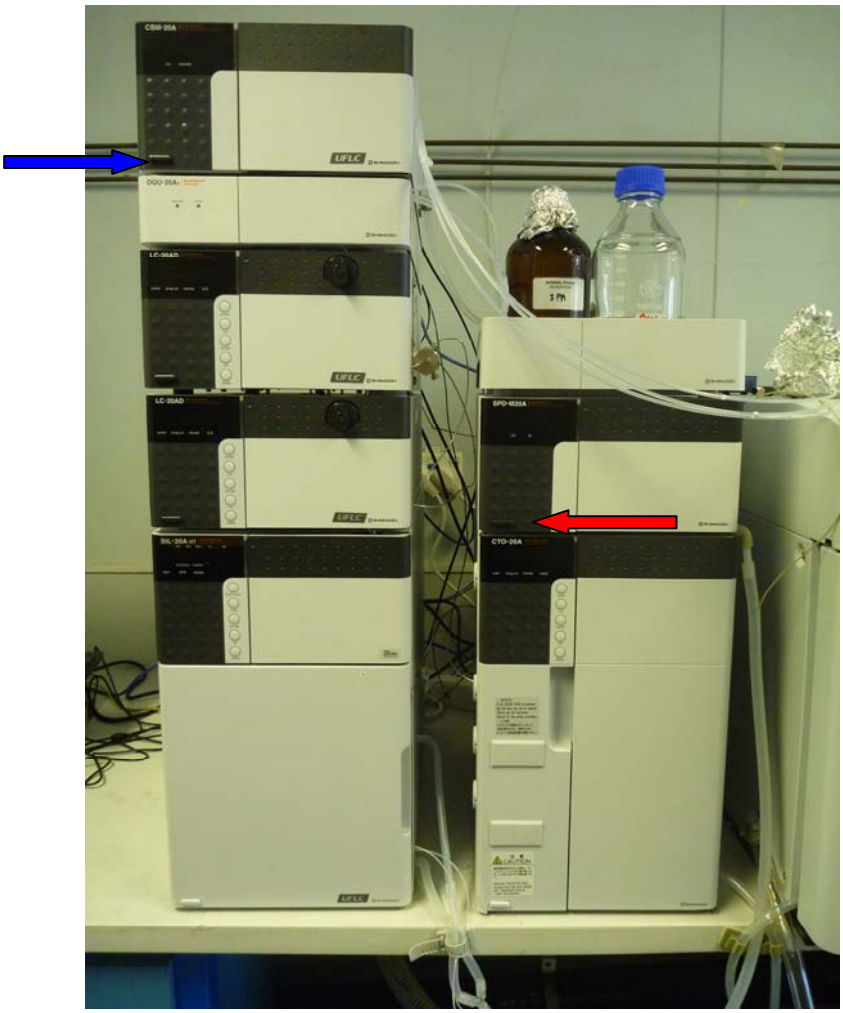


Figure 2: Turning on computer components from right to left

- Turn on computer (Login: admin, password: admin)
- Click on software icon – labsolutions (login: admin, no password)
- Click on LCMS – 2020 (Figure 3) – Make sure a beep is heard when software opens (indicating a connection between LC-MS and software)

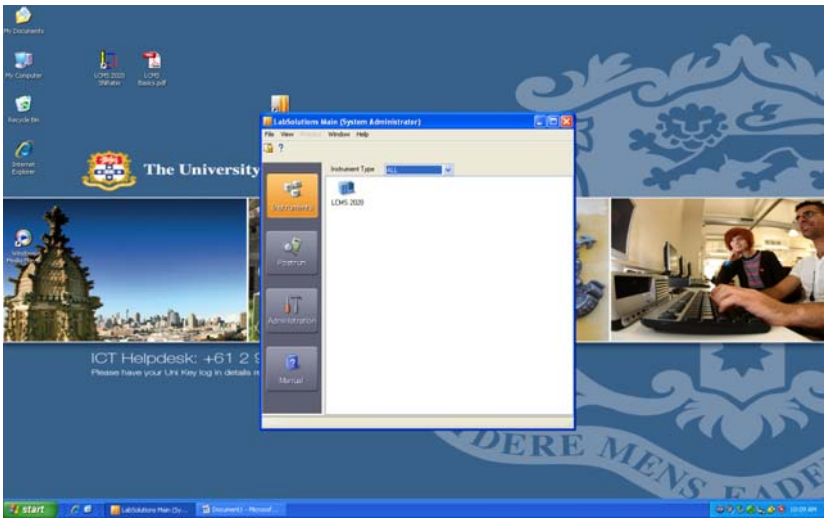


Figure 3: Labsolutions software – LCMS 2020

- Turn nebulizer gas on (Gas on icon – Figure 3), make sure nebulizing gas says 1.5L per min in right hand window (Figure 3)

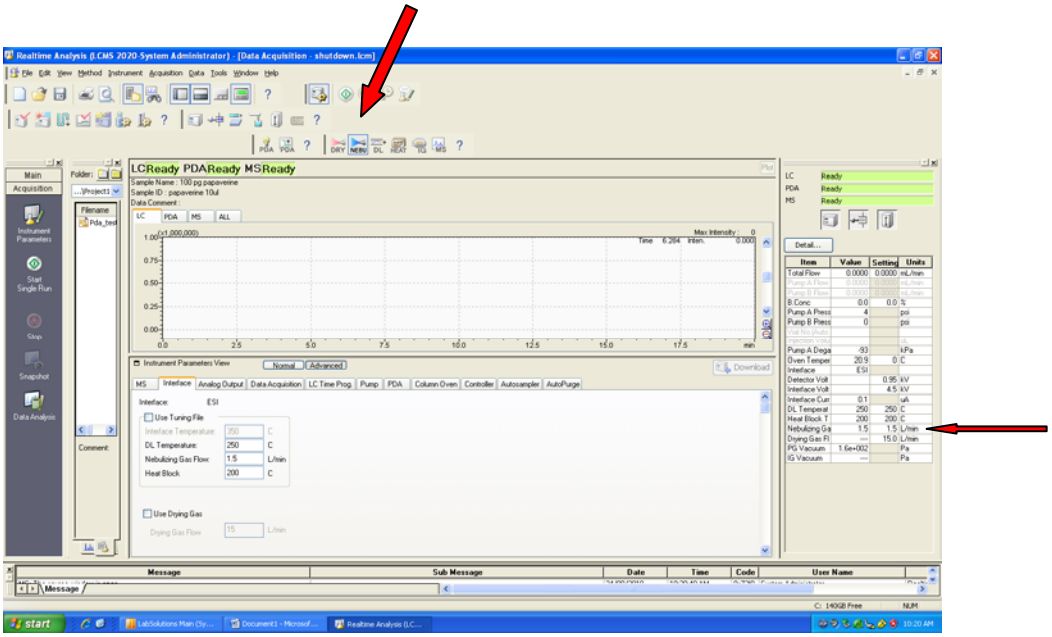


Figure 3: Press NEB icon on, make sure gas reads 1.5L/min

Purge:

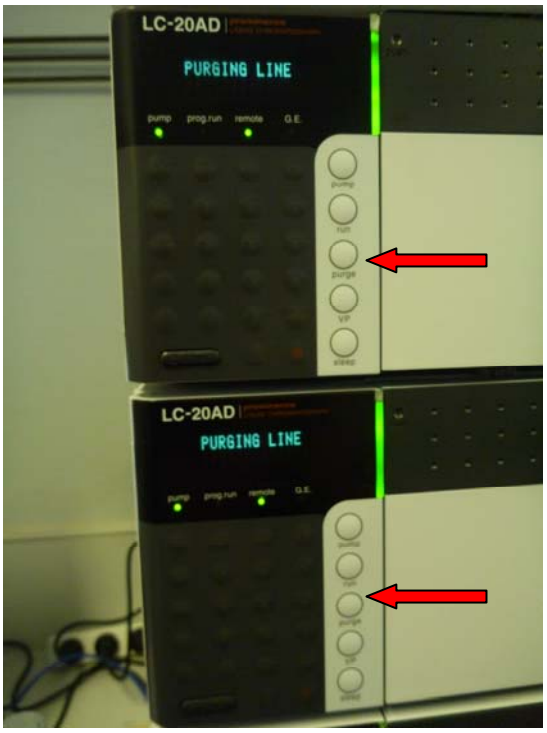
- Manual purge - Only necessary if machine has been idle for 2 days or more, you can check this by visually checking solvent lines for air bubbles. Turn black wheel 90° anticlockwise on Pump A and B, remove about 10 - 15 mL solvent with the syringe from both solvent lines A and B at the bottom of the left tower. (Figure 4) NB: When finished purging manually and purging via pressing the machine's button (see 'auto purge' instructions below) turn the black wheel back to the closed position. (Figure 5), *Otherwise your compound will go through the waste bin (happened to me) & you won't get a spectrum of your compound as it would not have passed through the column. Please do not tighten the tap too tight, Cedric said that's not good- F.T*

Figure 4: Turn black wheels on Pump A and Pump B 90° to the left

Figure 5: Syringe out 10-15 mL solvent form line A and line B

- Auto Purge tubes/pumps - To be done every morning before start-up. (*The nebulizer gas must be on otherwise machine will not allow purging as the computer has been turned on. The typical flow setting for the gas is 1.5 mL/min - F.T) usually it's good to have drying gas on too when you are using the MS.* (Figure 6).

- Turn black wheel 90° anticlockwise on pump A and pump B. Press "purge" and let run for about 3-4 min. Then press "purge" again to stop it (*unless it has stopped itself already*). (Default value is 8 mins but 3-4mins is enough). NB: When finished turn the black wheel back to closed position.



- **Figure 6: Press purge on Pump A and Pump B**

- Purge the auto sampler (default 25 min, but 10 - 15 min should be enough): press “purge” on front of machine and leave running for about 10-15 mins. NB: Make sure there is enough rinsing solution (Solvent C) before purging. (Figure 7),

- **Figure 7: Press purge on front of autosampler**

Note: Cedric said the pumps can withhold pressures up to 6000 psi. Usually the pump pressure is way below 4000 psi and if it is at an instance > 4000psi then the pump is blocked. Usually, if solvent is not flowing through the column yet, the pressure is about 200-300 psi but when the solvent is flowing through the column the pressure is between ~300 and ~2500 psi depending on the solvent used and column density - F.T.

Mass Spec: It is essential to remove the plug from ion inlet before using the MS (don't touch plug / ion source plug at the tip), check that the vacuum light is green on MS (after closing the warning window, which appears every time when the MS chamber is opened).
Turn Nebulizer gas on (*if off - F.T)*, (Gas on icon). Click download. Click instrument icon to activate pumps and column oven. Green lights will be seen on front of machine parts when activated.

Sample preparation

This is absolutely crucial. The instrument will block up if a sample is badly prepared
- use only HPLC/MS grade solvents to dissolve sample
- *Any solvents that are miscible with the solvent used for the run can be used. E.g. MeOH - F.T.*
- centrifuging and/or filtering the sample (pipette with cotton wool plug) to remove particles / solids *(the best way to filter solvents is to centrifuge first and then filter using the 0.2uM filters- F.T)*

How much to inject?
If in doubt always start with a sample that is very dilute. If your signal is weak, you can increase the concentration or injector volume. Standards (things that ionize well) can be analysed at concentrations of XXX. As a guide: maximum concentration that should be employed is: 1mg / 10 mL
- minimum concentration: 10 pg/µL (1 mg / 100 mL)

Creating a method – by Fargol

Creating a new method file on the Shimadzu 2020 LCMS:

You can create or open files whilst the machine is off (see the right hand side window in **Image 1** which shows the computer is not connected to the system).

Note: It is not possible to view or create a new method file whilst running a sample. The sample must be stopped and then a method can be modified/created. You can only brwose your previously collected data whilst a sample is running.

Sometimes there is no option of opening or creating a new method file when you click on ‘file’, and instead there will be an option to create some other file (e.g. batch). For example, if the instrument was left on with a batch run, it will not allow you to create a new ‘method file’ but a new ‘batch file’ instead. If this occurs, click on the ‘All’ tab below the viewable filenames (next to the batch tab). This is circled in **Image 1**.

To open a method file, click ‘File’, ‘Open’ (**Image 1**).

Step 1: Click ‘File’, ‘New’ and then ‘Method file’.

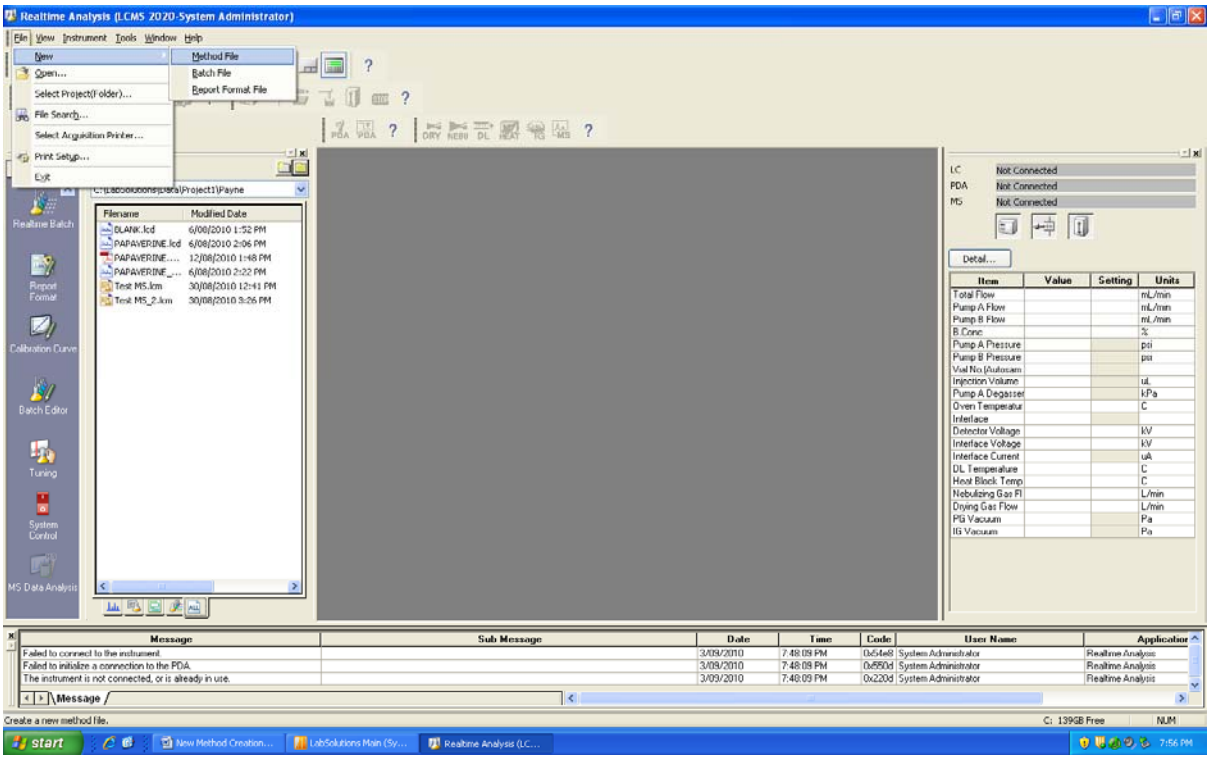


Image 1. First step.

If you still have problems opening/creating a new method file tryclosing whatever dialog box is open and then click file, new, method file.

Step 2: Select the parameters for your run (in the **MS tab**). (**Image 2**)

Click the 'Scan' mode (**image 2**) or scroll to select the desired spectrometry mode. Choose the desired mass range (in scan mode). Scan speed need not be changed. 'Event time' = sampling time (no. of scans per second), select '1' seconds (between 0.2 – 1 s is fine). The higher the event time, the larger the data file.

Leave the detector voltage determination up to the machine by selecting 'relative to tuning result' and, as in image 2, leave the detector voltage at 0 kV. Therefore, the machine will optimise the detector voltage. As in image 2, leave the other voltage settings (e.g. 'Tuning File') as is.

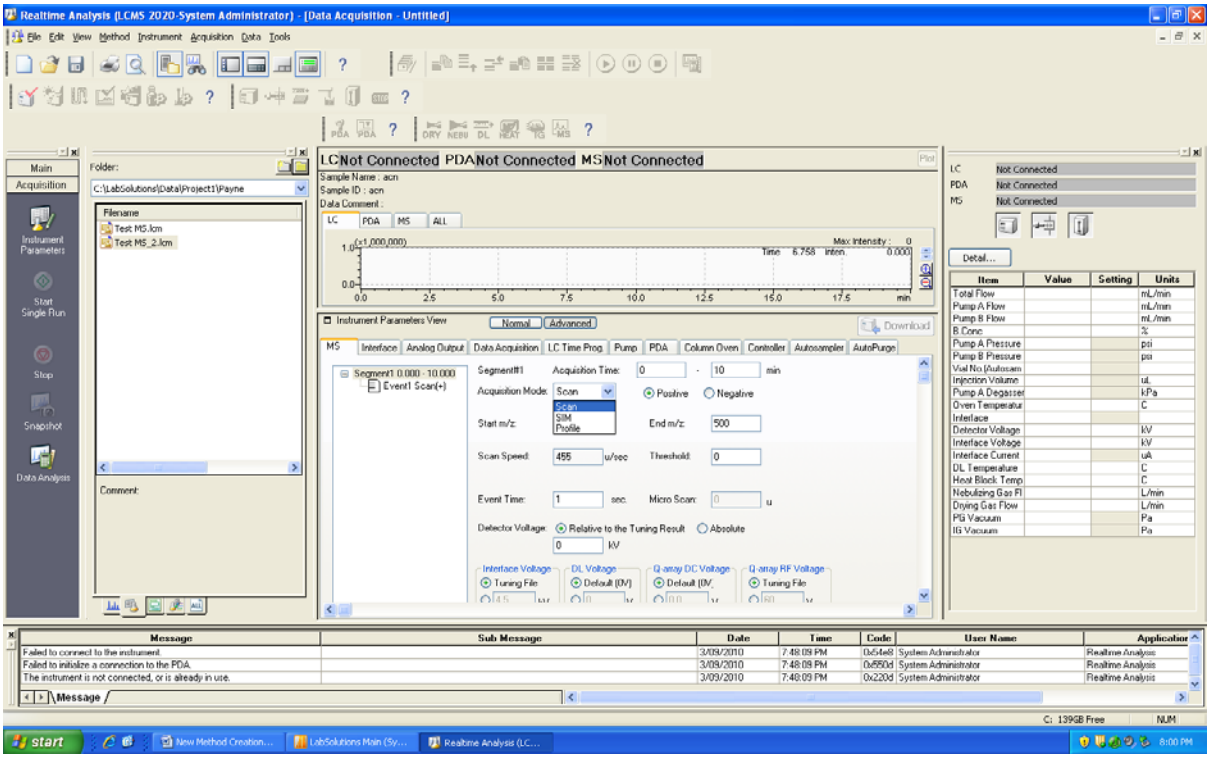


Image 2.

A time range for 'acquisition time' should be entered. E.g. for a half an hour run, select 0 - 30 min.

According to Cedric, there can be up to about 50 events running at the same time with different mass parameters (e.g. 50 different mass spec. ranges can be detected in one run). To create a different 'event' right click on 'Event 1 Scan(+)' (stay on the same MS tab) and click 'event add' (**Image 3**).

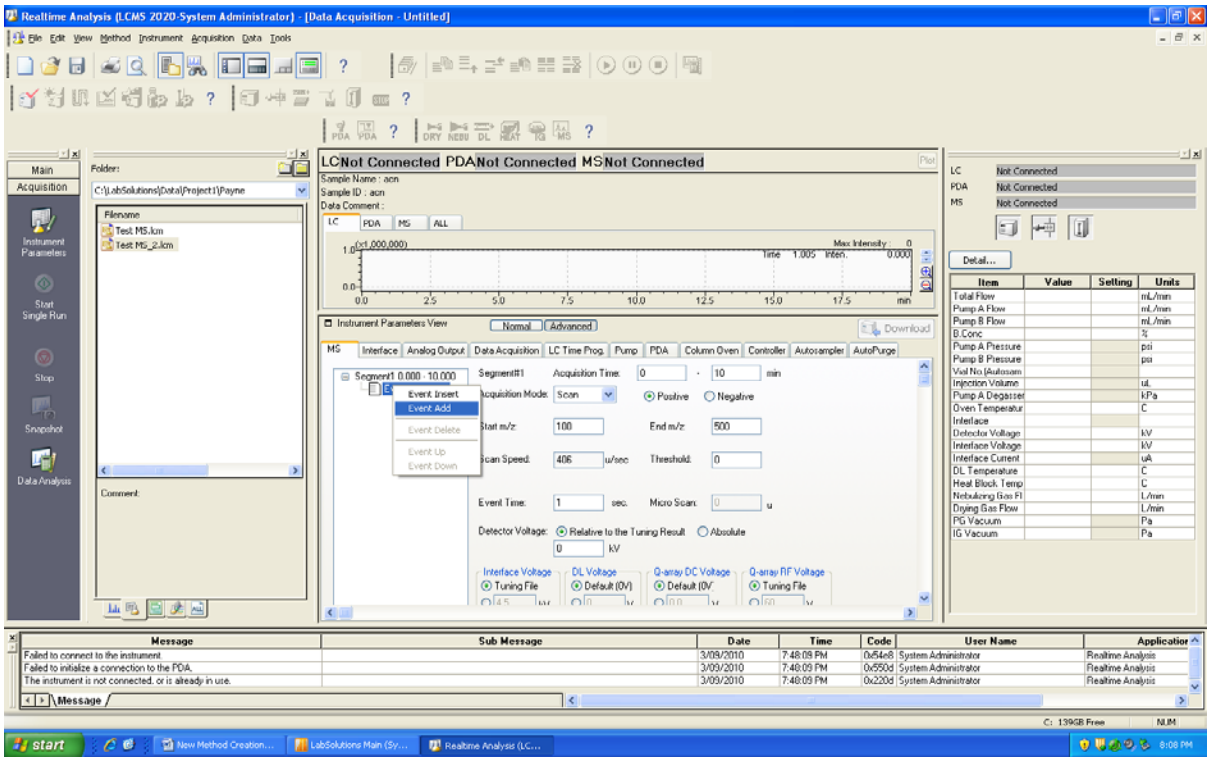


Image 3. Creating new events with new MS parameters

Step 3: Create parameters in the **Interface tab**. (**Image 4**)

Check the box next to ‘use tuning file’

(Tuning file conditions are fine, only vary IF really needed)

DL temperature: 250
Drying gas flow: 15 L / min
Detector voltage: -0.95 kV
Heat block temperature: 200
nebulizer gas flow: 1.5 L / min

and leave the box next to ‘use drying gas’ unchecked (sometimes if too much water is sprayed out of the MS then you can use the drying gas?)

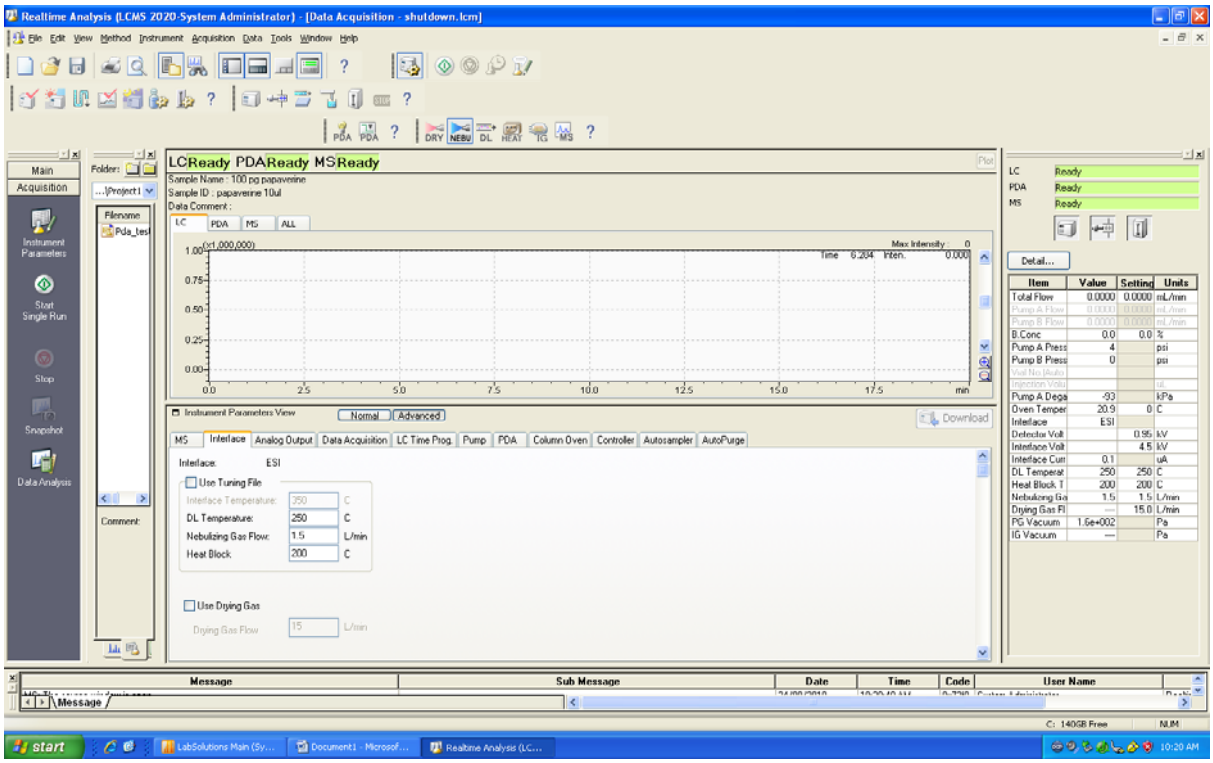


Image 4.

Analogue output tab: N/A (**Image 5**)

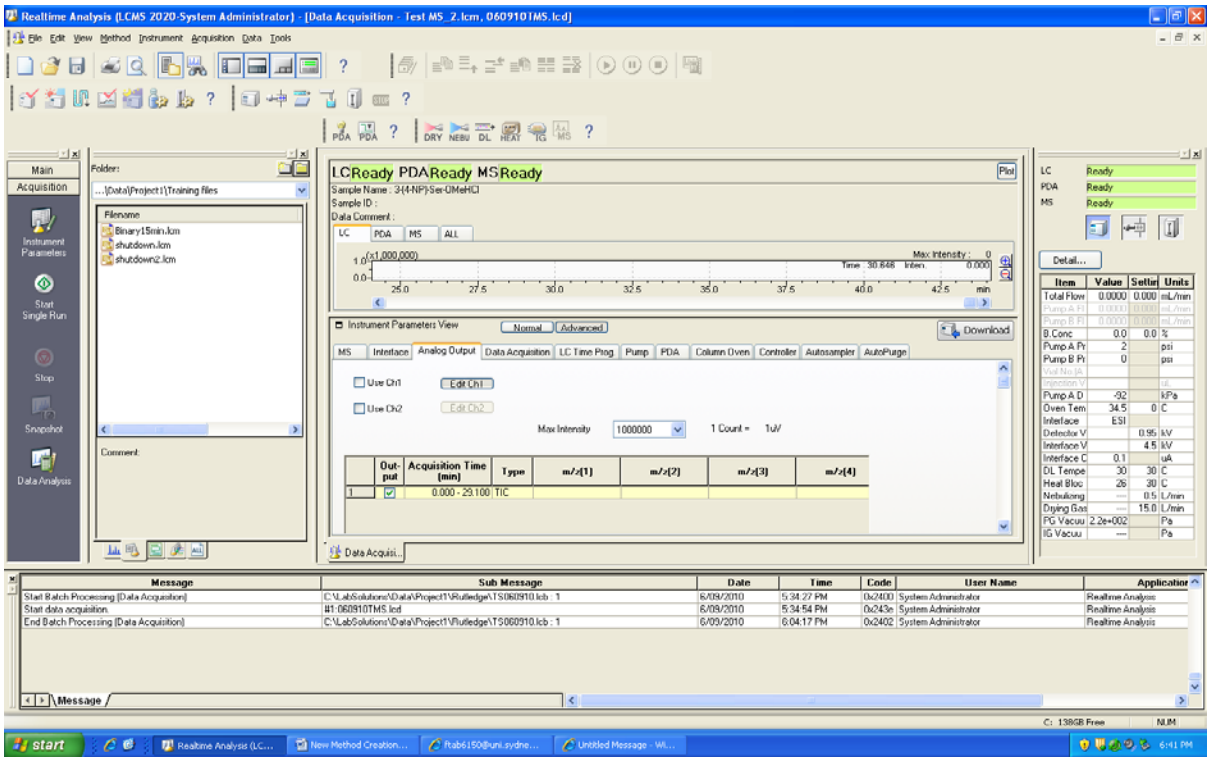


Image 5.

Step 4: **Data Acquisition** tab: LC stop time at 30 mins (for example), click the button ‘apply to all acqusition time”. (**Image 6**)

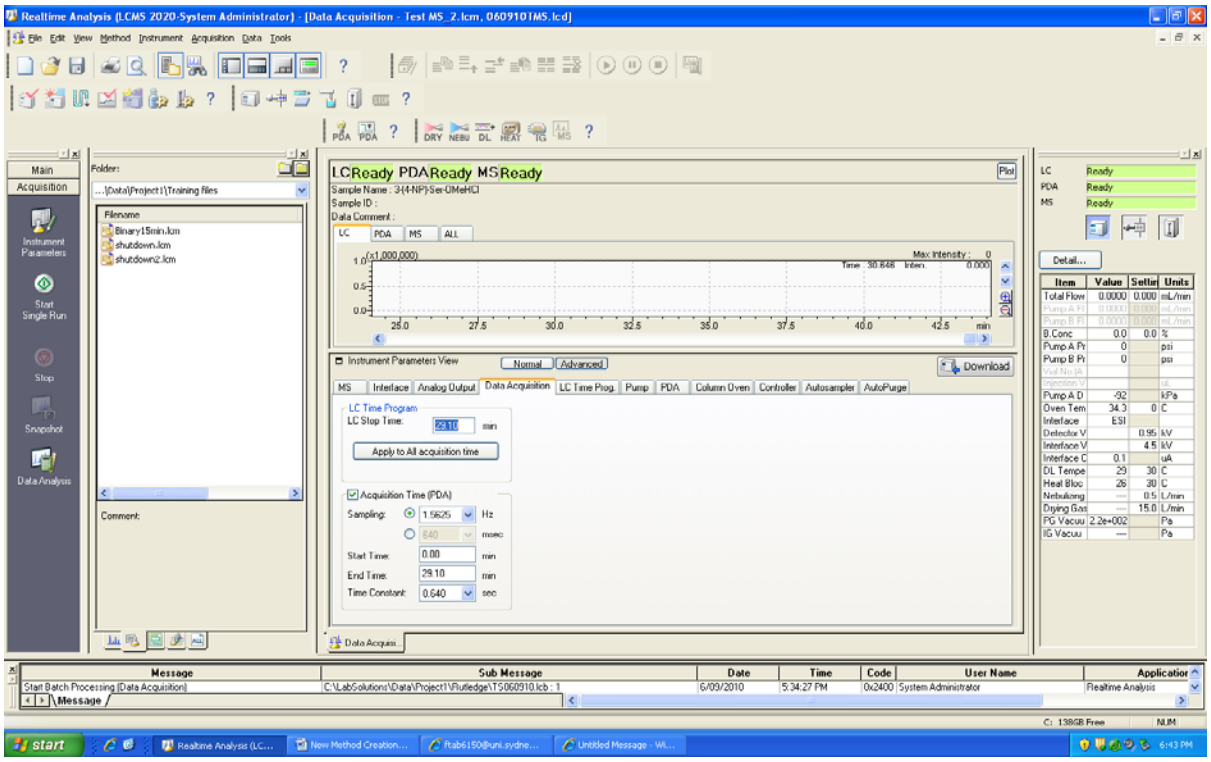


Image 6.

Step 5: **LC time prog** tab: Acquisition time = 30 mins for run-time (for example) (**Image 7**).

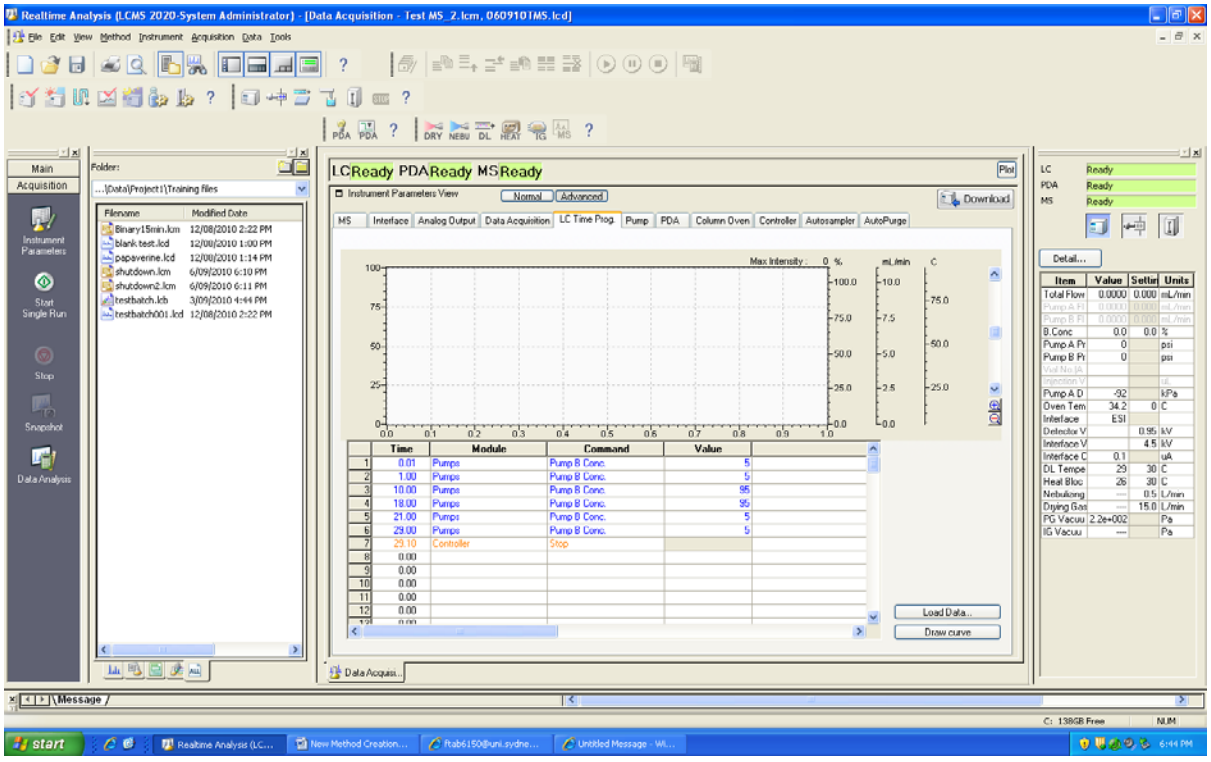


Image 7.

	Time	Module	Command	Value
1.	0.01	pumps	Pump B conc.	5
2.	1.00	Pumps	Pump B conc.	5
3.	10.00	Pumps	Pump B conc.	95
4.	18.00	Pumps	Pump B conc.	95
5.	21.00	Pumps	Pump B conc.	5
6.	29.00	Pumps	Pump B conc.	5
7.	29.10	Controller	stop	

The table above shows the LC pump settings that were used for running Anh's and Tim's samples previously (from the method called 'Test MS_2' which Rob created with us).

This gradient is a typical method used for most samples. 5:95 $CH_3CN:H_2O$ - 95:5 $CH_3CN:H_2O$. This can modified as needed for your specific purpose. Must have the higher proportion of the organic phase to flush out the column at the end of the run.

Step 6: **Pump** tab: Change the mode, Select isocratic or gradient (users should use a binary gradient). Pump B Conc = 5% (this is what you start off with usually), flow = 0.2 mL / min

pump curve = 0 (**Image 8**)

For pressure maximum: 1451 psi was selected and the minimum was 0 psi.

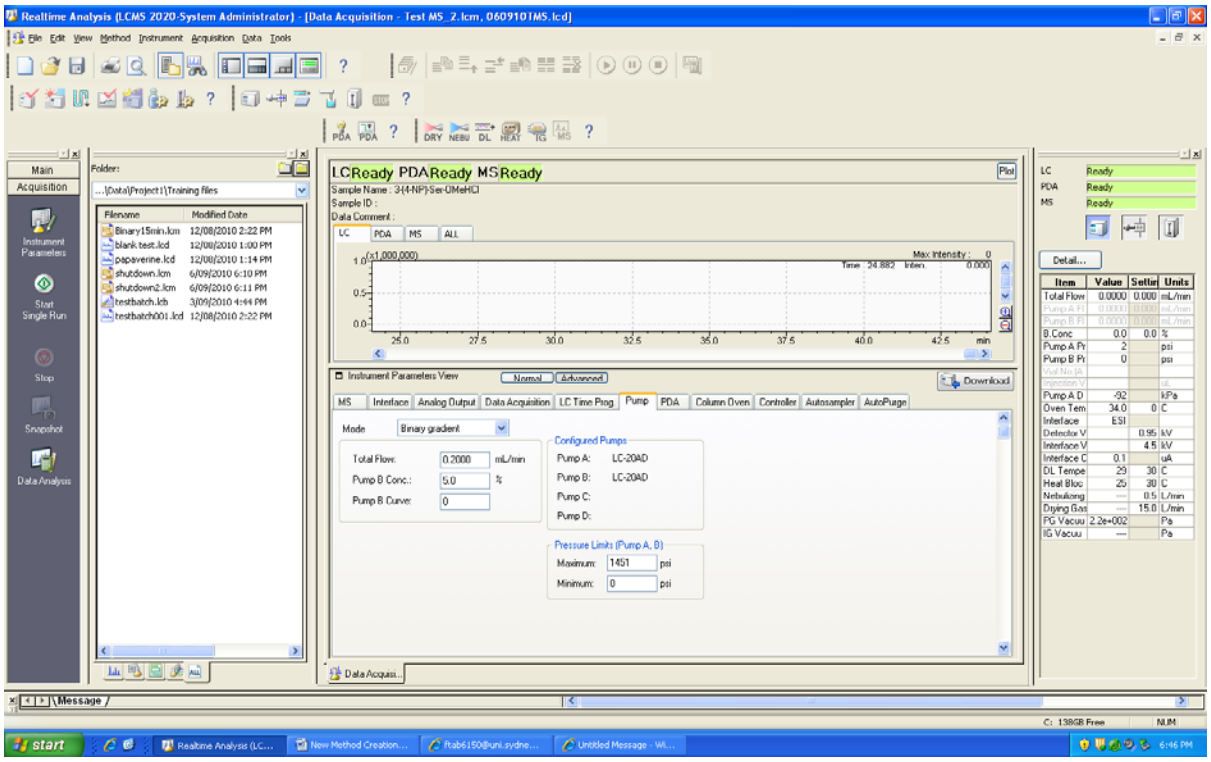


Image 8.

Step 7: **PDA** tab: Select 190 – 800 nm for the wavelength range if using entire range with both lamps (D2&W). (Note: lamp lifetime is 2000 hours, please turn off the lamps when not in use)

Cell temperature = 40 degrees

Slit width = 1.2 nm (**Image 9**)

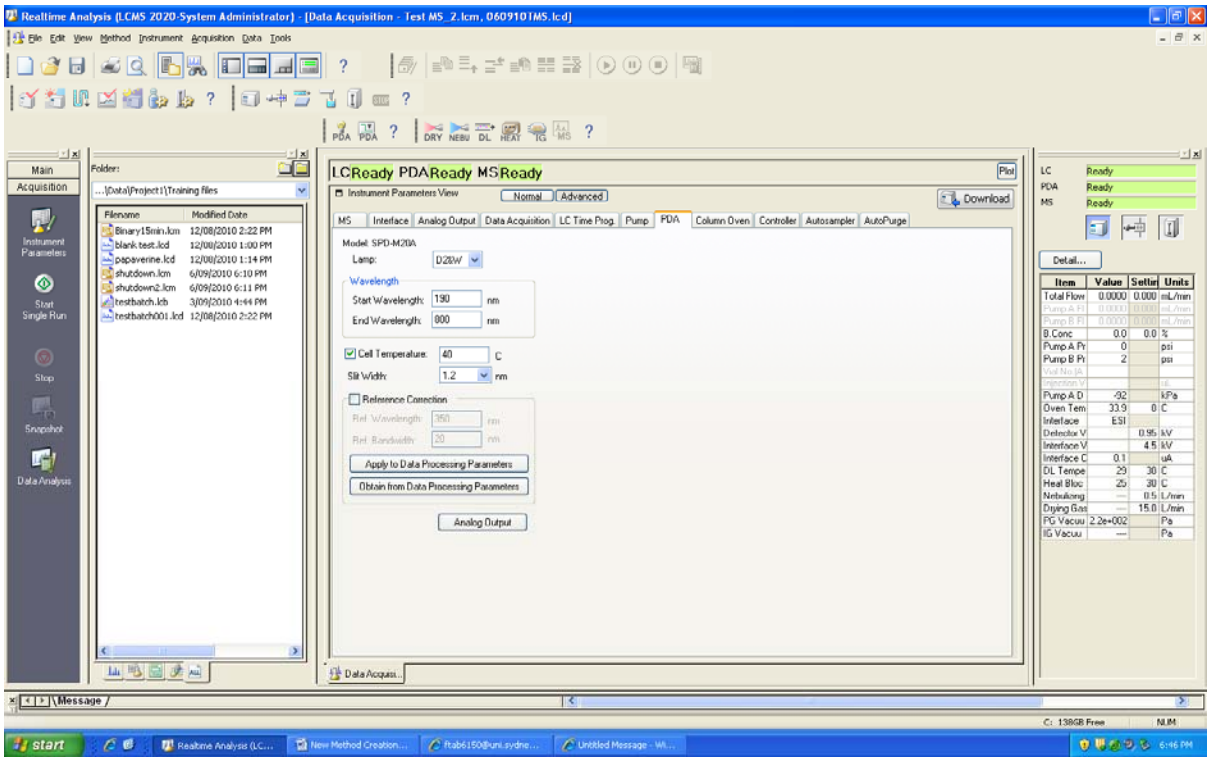


Image 9.

Step 8: **Column oven** tab: use default values (column oven ticked, so column oven is on and is set to 40 degrees) (**Image 10**)

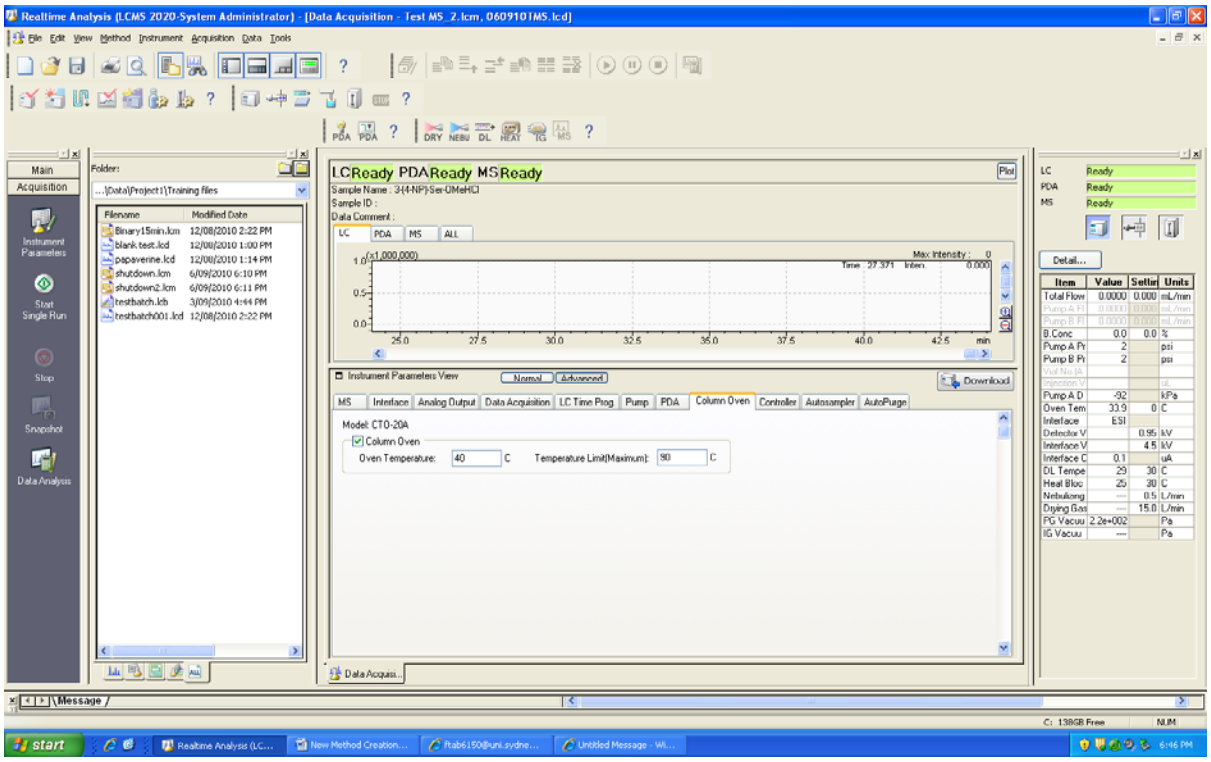


Image 10.

Step 9: **Controller** tab: Settings not changed (leave 'power on' checked) **(Image 11)**

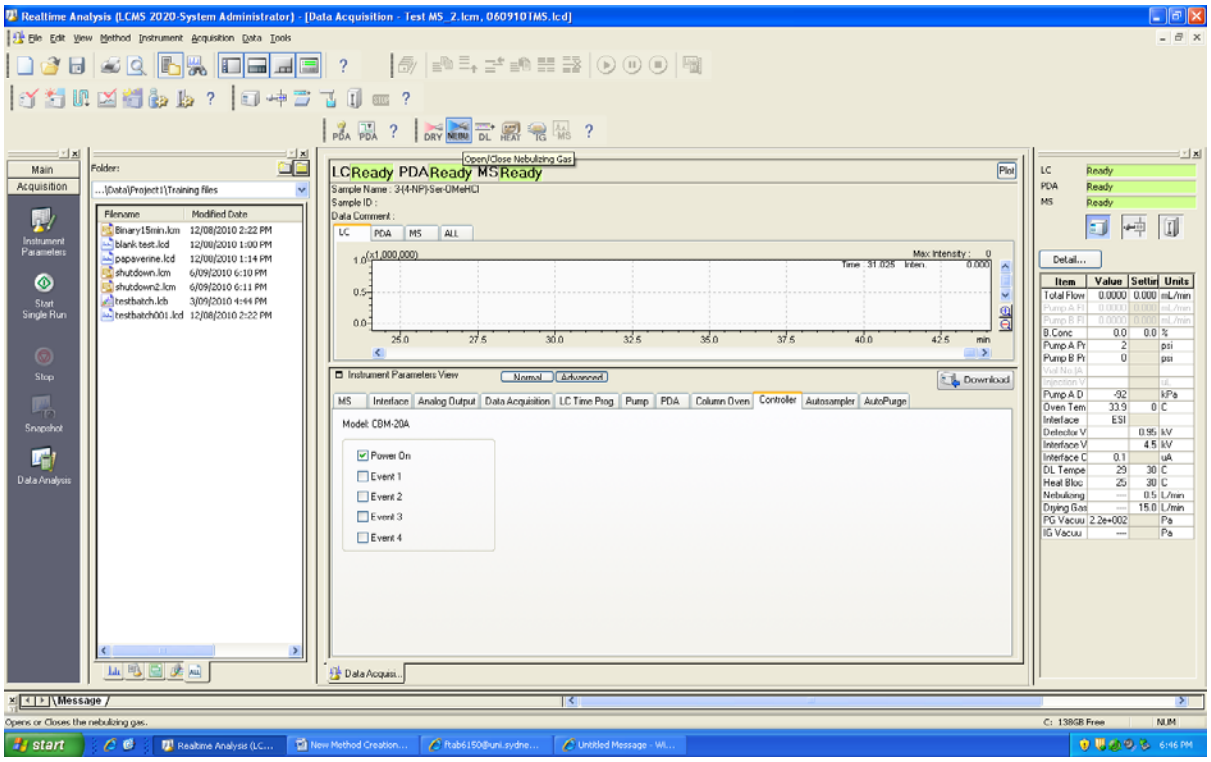


Image 11.

Step 10: **Autosampler** tab: Check the needle stroke value. Default is 52 mm but you must change this to 49 mm if using vials with inserts (this is the usual case). (**Image 12**). The 'control vial needle stroke' settings are only used for another small rack which we do not use. Leave rinsing speed at 35 uL/sec ad Sample suction speed at 15 uL/sec. For the 'rinse mode', 'before aspiration' rinse was selected. 'Rinse dip time' = 0 seconds. Rinsing volume is typically about 500uL.

Note: you must hear a click when you slide the autosampler rack in the machine in order to place it in correctly.

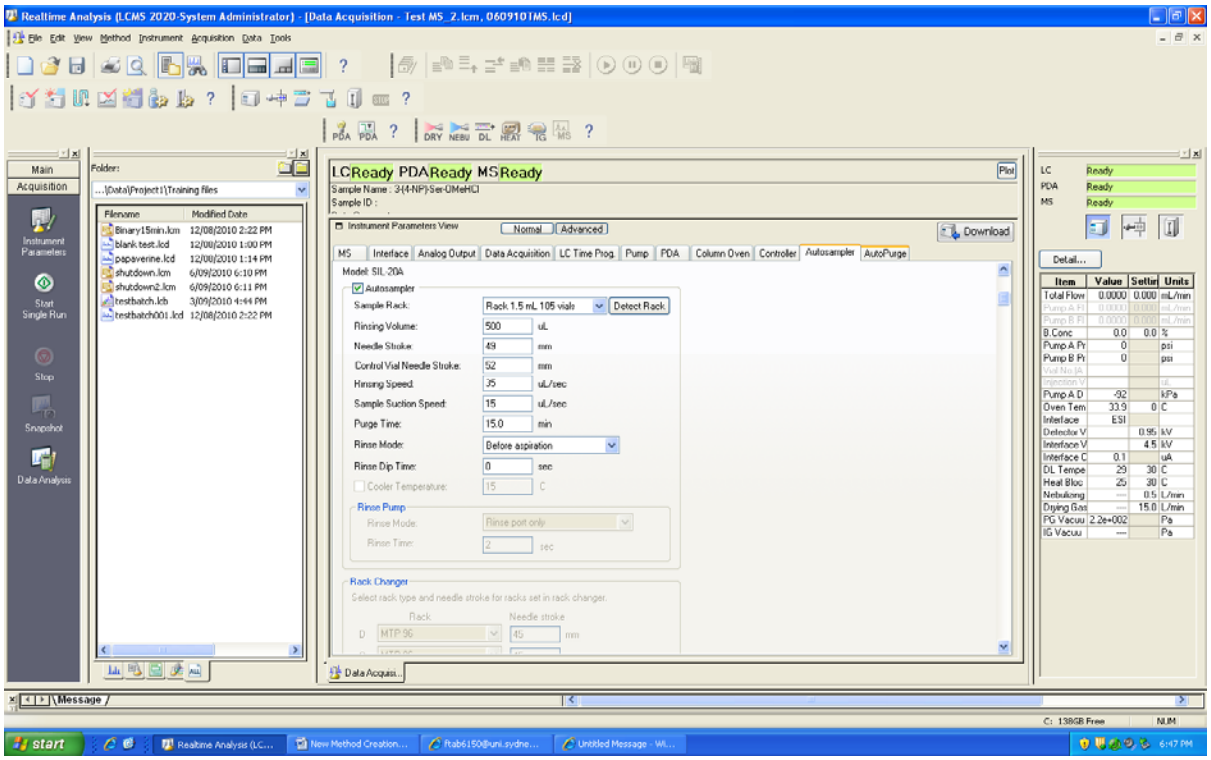


Image 12.

Step 11: Leave these settings as default (only thing that is checked/ticked is 'activate system after autopurge' (**Image 13**).

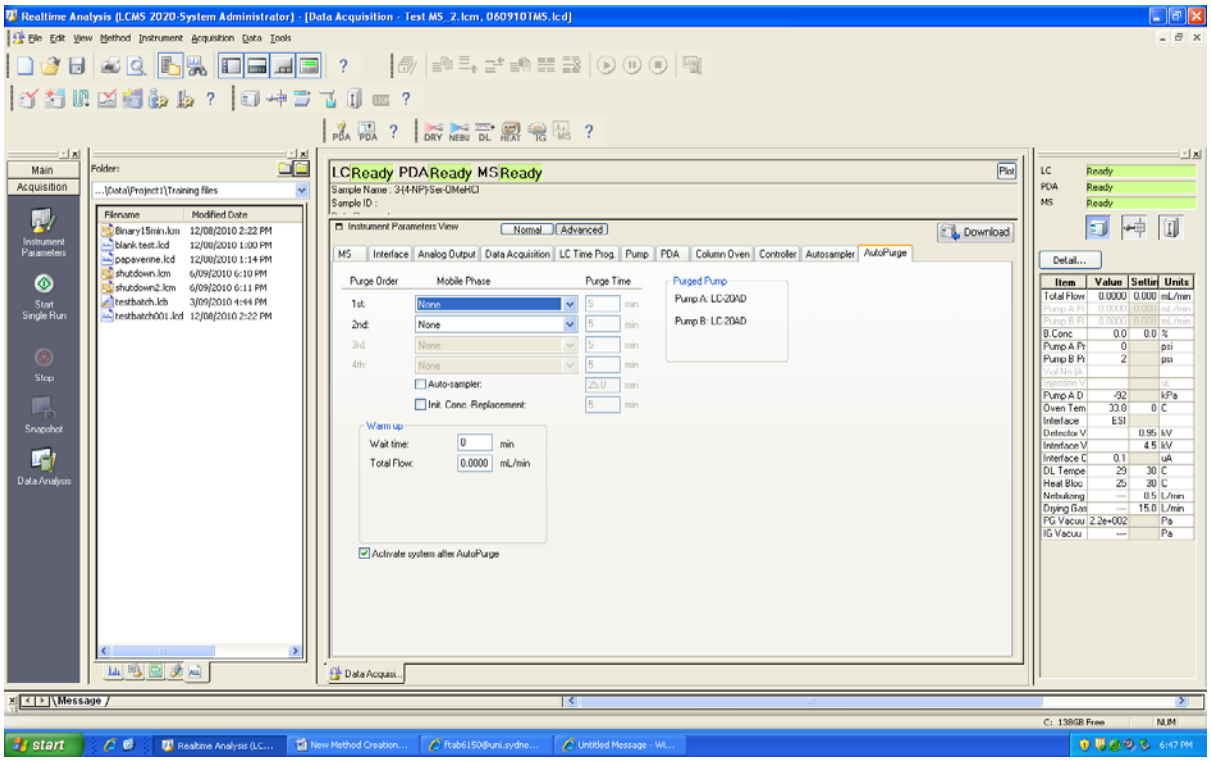


Image 13.

Step 12: Save in your folder in c:/Labsolutions/data/Project1/*YourGroupName* (**Image 14**).

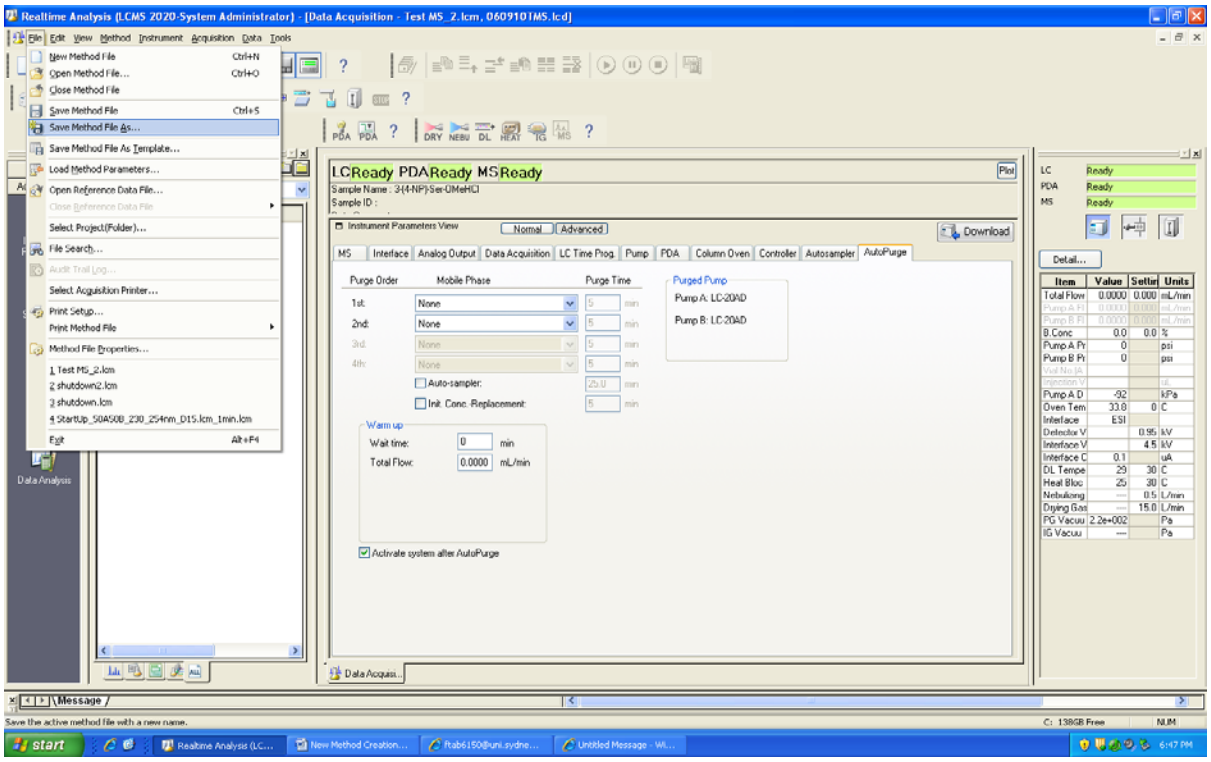


Image 14.

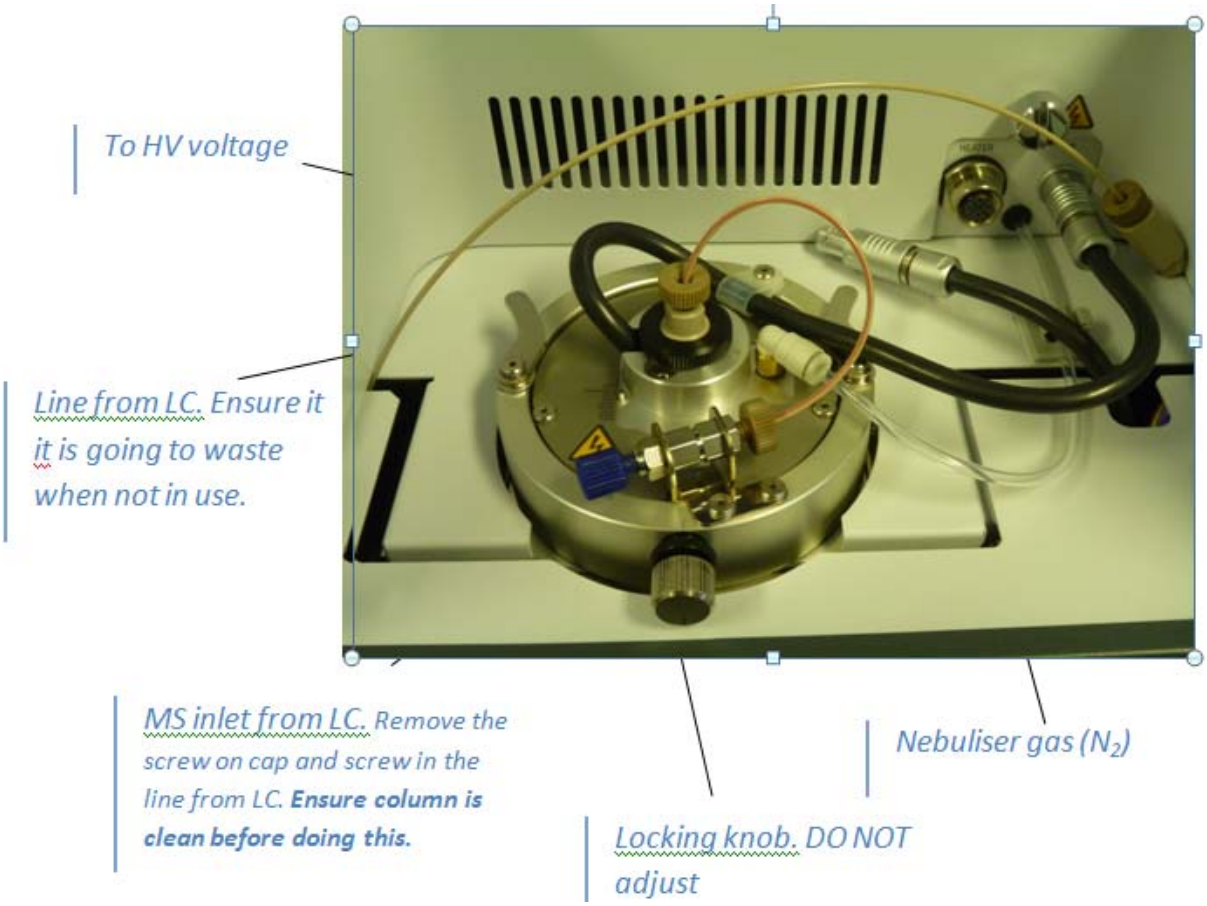
Congratulations you have created a new method file for repeated use☺.

Injecting a sample (using the autosampler) and acquiring data

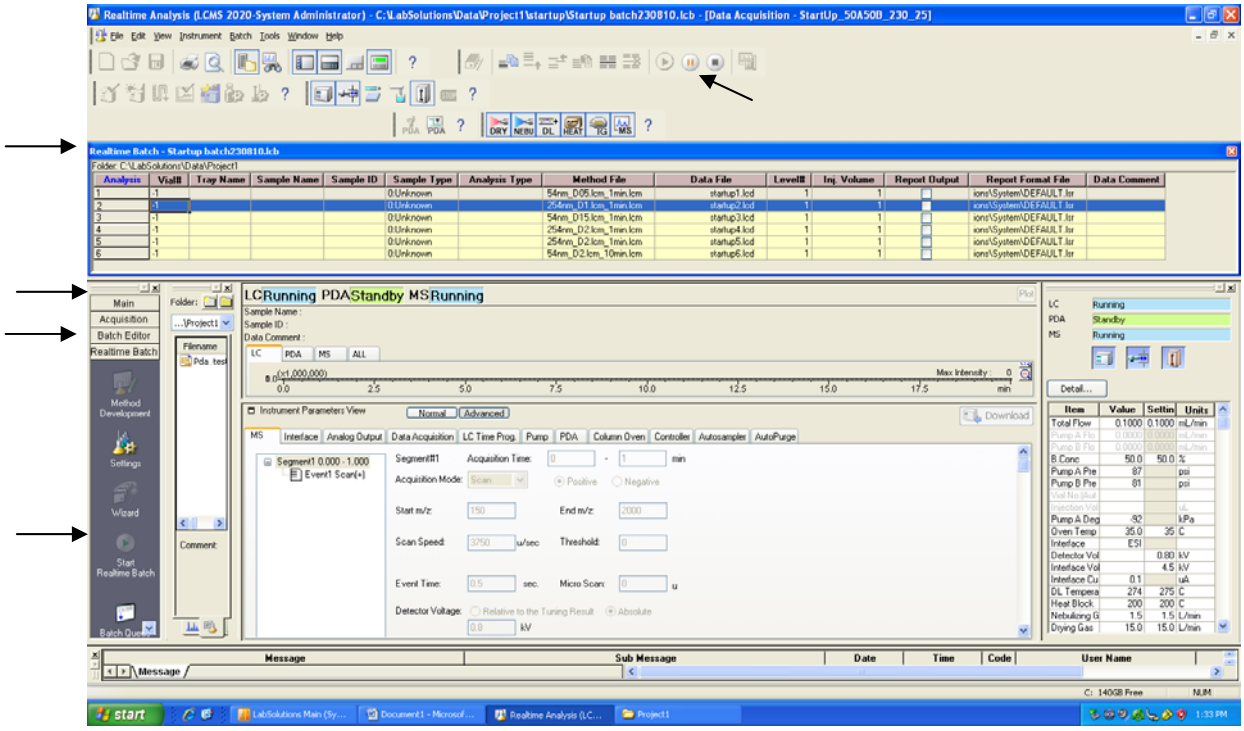
If you are not using a batch sequence you can click on single run on the left hand side panel (to do this click on acquisition first) then enter the 'vial number', 'injection volume' and 'data file' (the name of the file)- F.T

Setting up a batch sequence and helpful diagrams of ESI probe-by Fatiah

- APCI (atmospheric pressure chemical ionisation) probe used for non polar compounds
- ESI probe used for polar compounds. ESI probe is pictured below and is useful for most applications



- Data can be collected in SIM or SCAN mode (see method development section) using ESI or APCI probes. However sample injection should be approx 500 times less concentrated when collecting in SIM.
- flow rates of 0.2 -0.5 mL/min ideal for ESI



To queue experiments in a batch:

1. Click on **main** icon
2. click **realtime batch**
3. If you started up the machine, ensure that you load ~~*startup batch020910*~~*.lcb* batch file. 6 sequential startup rows should be automatically loaded. *I couldn't find this file so I crossed it*

out. You can just insert the method files in the folder: 'startup' in order into the appropriate row(in order) if you wanta comprehensive wash but if not just open 'startup' folder and then select '95A...D2—10min' and this method file will wash the column with 5% ACN for 10 min. - F.T

4. Check that:
    a. vial# is set to -1 (i.e. no sample for injection)
    b. tray name is set to 1
    c. sample name
    d. method files are in sequential order as shown in diagram
    e. data files are selected as shown (this data file (in each row) will be the name of your file)
5. highlight first row and click **start realtime batch**
6. select **all rows**, click OK
7. After startup batch has run to completion, close the startup batch file.
8. To create a new batch sequence: in file menu, click new batch file. A new batch sequence window is opened. *Alternatively you can create a new batch sequence through the wizard*.
9. In each row (run) set:
    a. Vial# corresponding to location of your sample vial. When placing vials in rack ensure rack clicks back into place.
    b. Tray name to 1
    c. Sample name
    d. Select a saved method file (if no suitable method file exists, see section XXX-creating new method files (FARGOL)) by clicking on arrow in RHS of cell.
    e. Select data file
    f. Select injection volume
10. Repeat for other rows.
11. Start realtime batch
12. Save the batch sequence (e.g give it today's date). The batch sequence is thus set up for the day.
13. Go back and highlight the first row and click **start realtime batch**
14. If additional rows need to be added to the day's batch sequence after starting the realtime batch, click the **pause** icon in top of realtime analysis window. Right click in the row immediately before the shutdown run, and select **add row** from drop down menu. Add details to the new row (see step 5). Repeat for additional samples, and then click **pause** icon again (to unpause) to continue the batch run. Your samples have now been placed in the queue.
15. If you are the last user for the day, add shutdown file (see the last shutdown procedure section to find the shutdown file location) to end of batch queue.

How to leave the instrument

If the batch sequence has been created with a shut-down method at the end then this will ensure that the pump flow will go to zero, the uv lamps will shut off, the column oven will turn off and the nebulizer gas will run on the minimum setting.

See Anh's procedure below:
LC-MS shutdown steps

1. From real time batch window, press 'Edit table (Pause)' at the top, followed by 'OK'
2. Right click, select 'add row'
3. Change method file to 'shutdown2' in 'training files' folder (optional: you can also modify sample name and data file name)
4. Click 'Edit table' tab again to resume batch.

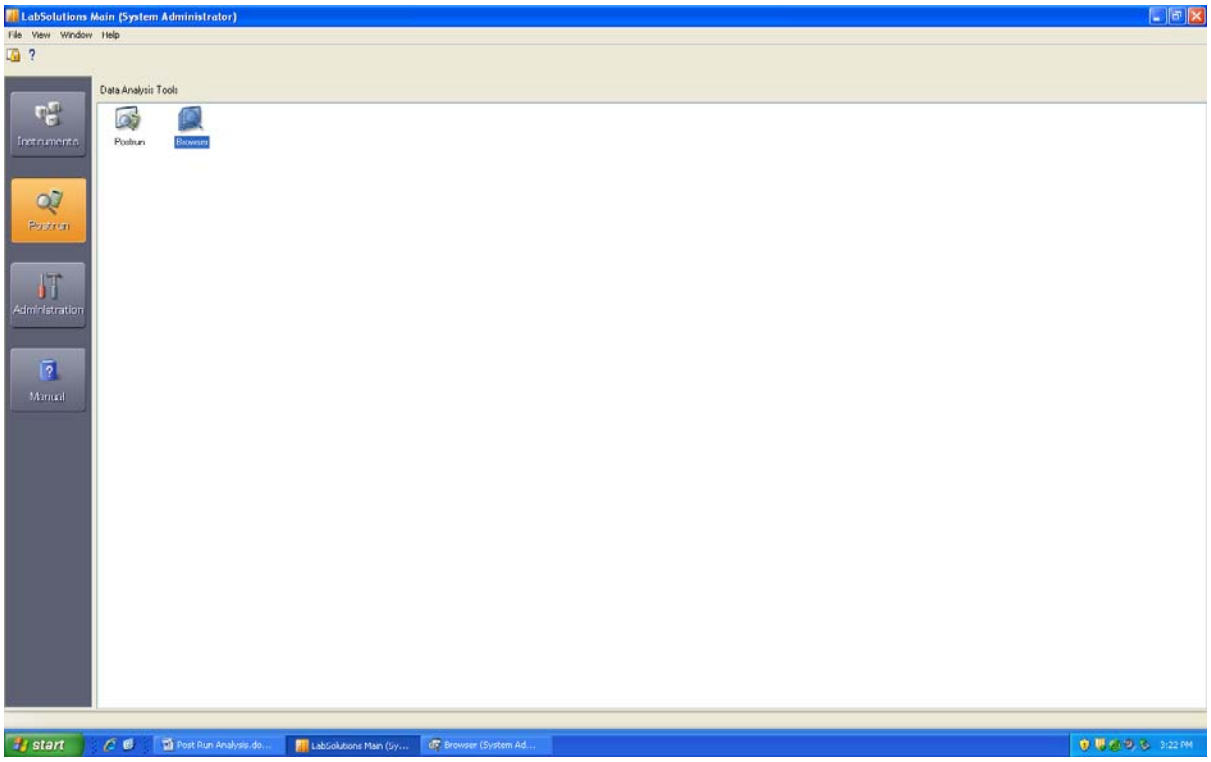
If manually switching off machine then reverse the order of the start-up power sequence. Turn off the instrument: reverse order of the starting procedure .Cap or metallic plug must be placed on the mass spectrometer. (usually it's kept in a sleeve on the top of the machine next to the solvents.

You can also just press the shutdown button on the top of the page and then close the software instead of inserting a shutdown method file if you are not running any samples and it will immediately shutdown everything after that. - *F.T*

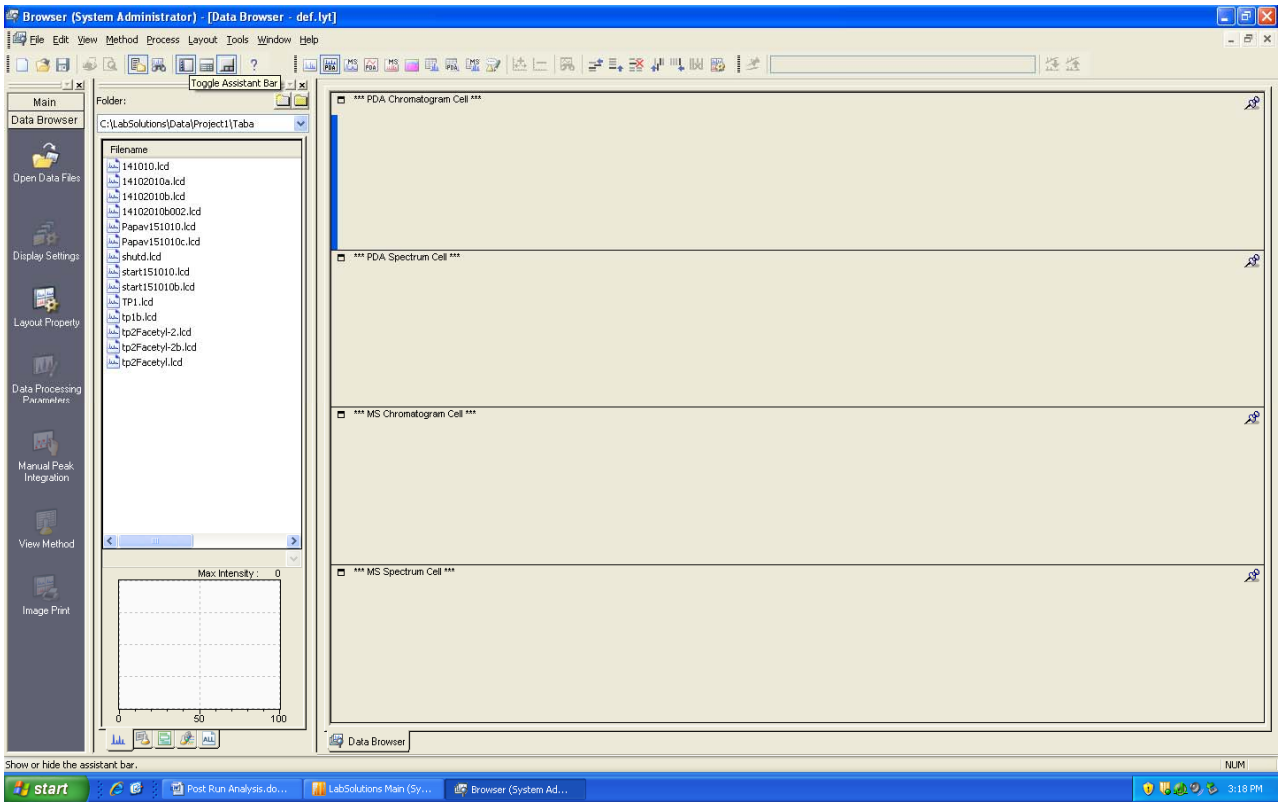
Post-Run Analysis/Processing

Basic Post Run Analysis method: Oct 17th 2010 by Fargol

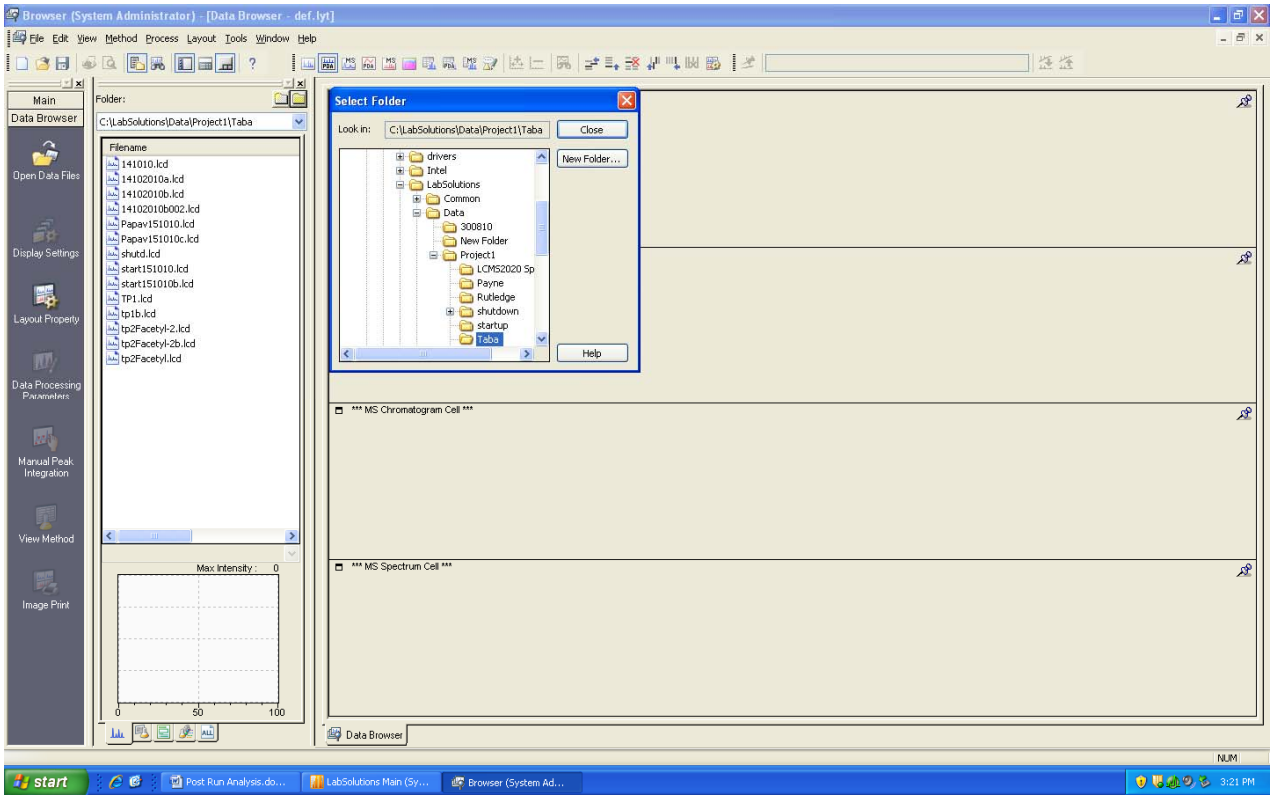
From main LabSolutions window, click Postrun from the left hand side toolbar and double click the Browser icon



Select top cell (here it's the PDA chrom. Cell):

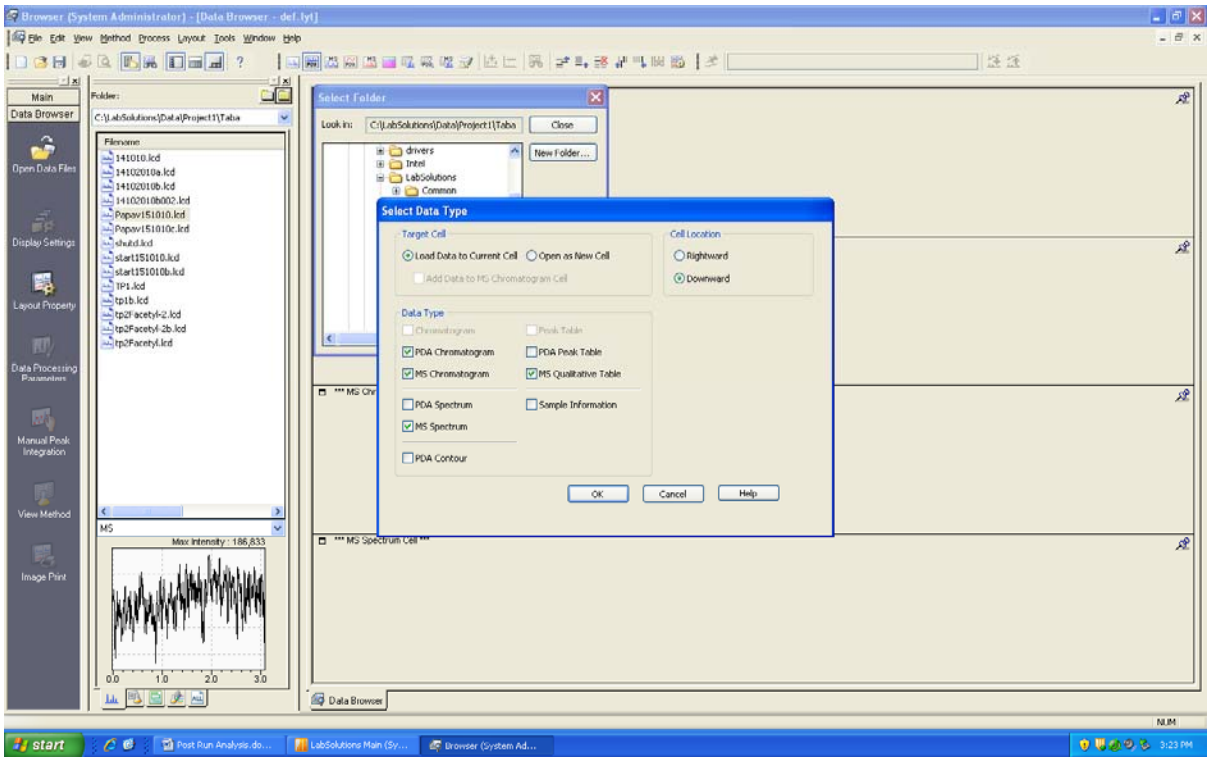


Select file (if it’s not in the file toolbar then select the yellow folder by double clicking it (see where the cursor is on the next image) and then select your folder by clicking on it so that your files ar visible on the file toolbar above.



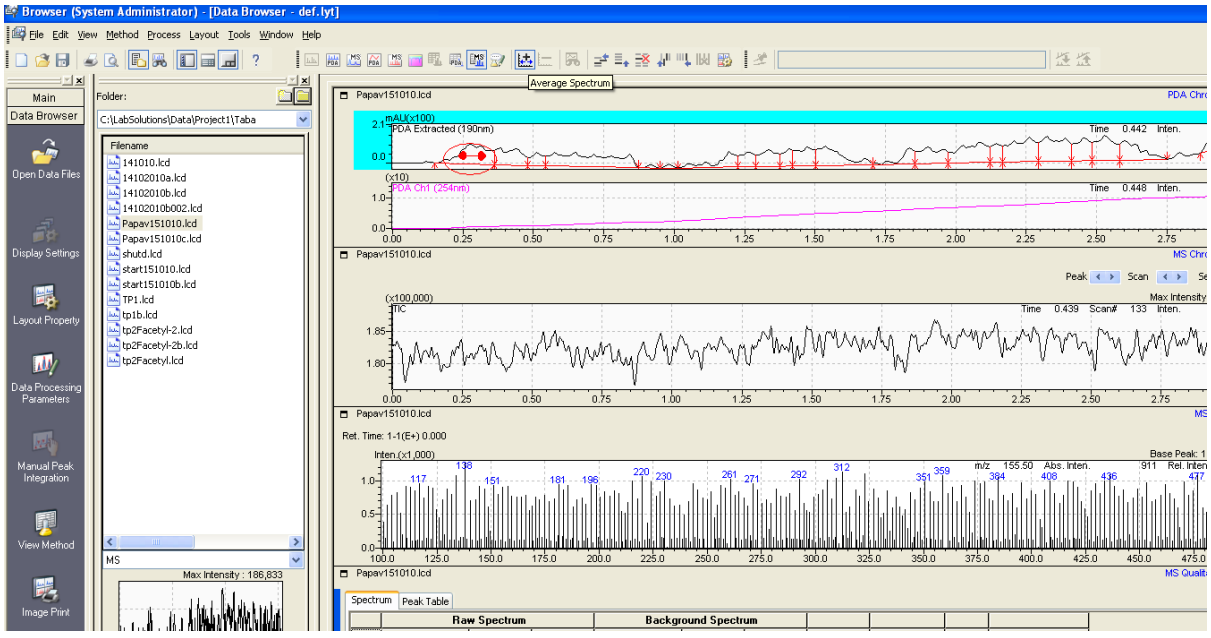
Double-click your filename and select data type as in the picture (usually the PDA spec. Is not used so that is not ticked)

& click OK

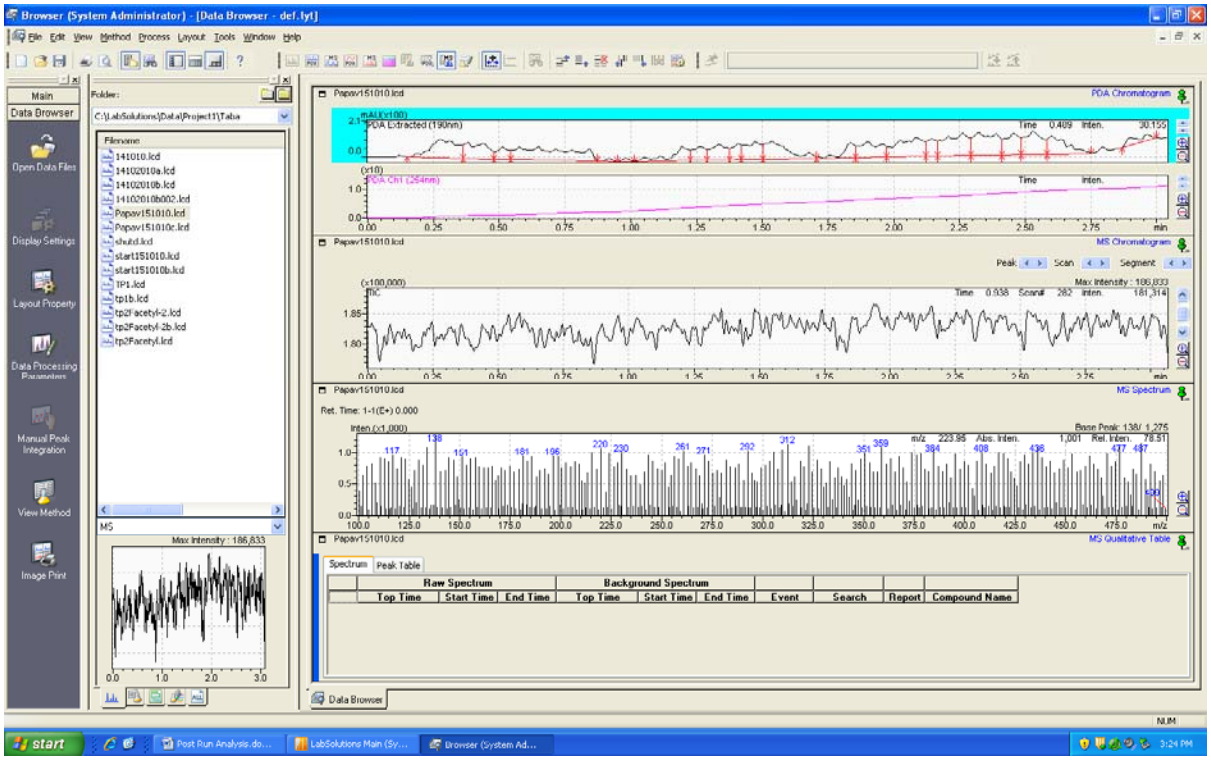


Click ‘average spectrum’ button depicted in the picture below:

and then select the range in the spectrum which you want to see the ions in (by left clicking and dragging in that region - you can do it for the PDA or the total ion count) .E.g look at the circled red arrow region in the print screen
below:



but if you want to look at a different area then you have to click that ‘average spectrum’ button again.



You can copy the spectrum (by right clicking on the spectrum desired) and paste it in word like this:

E.g see the copied PDA below from Taliesha’ run: 151010