<u>Creating a new method file on the Shimadzu 2020 LCMS:</u>

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.

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'.

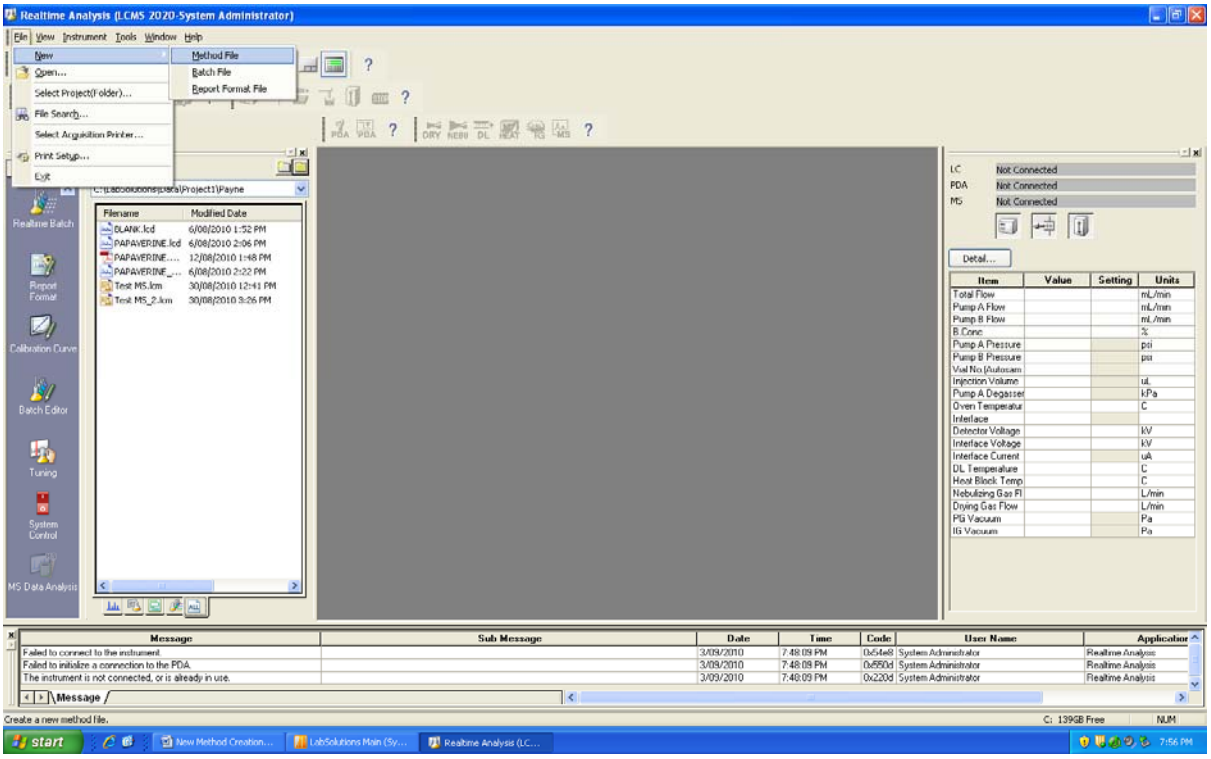

Image 1. First step.

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.

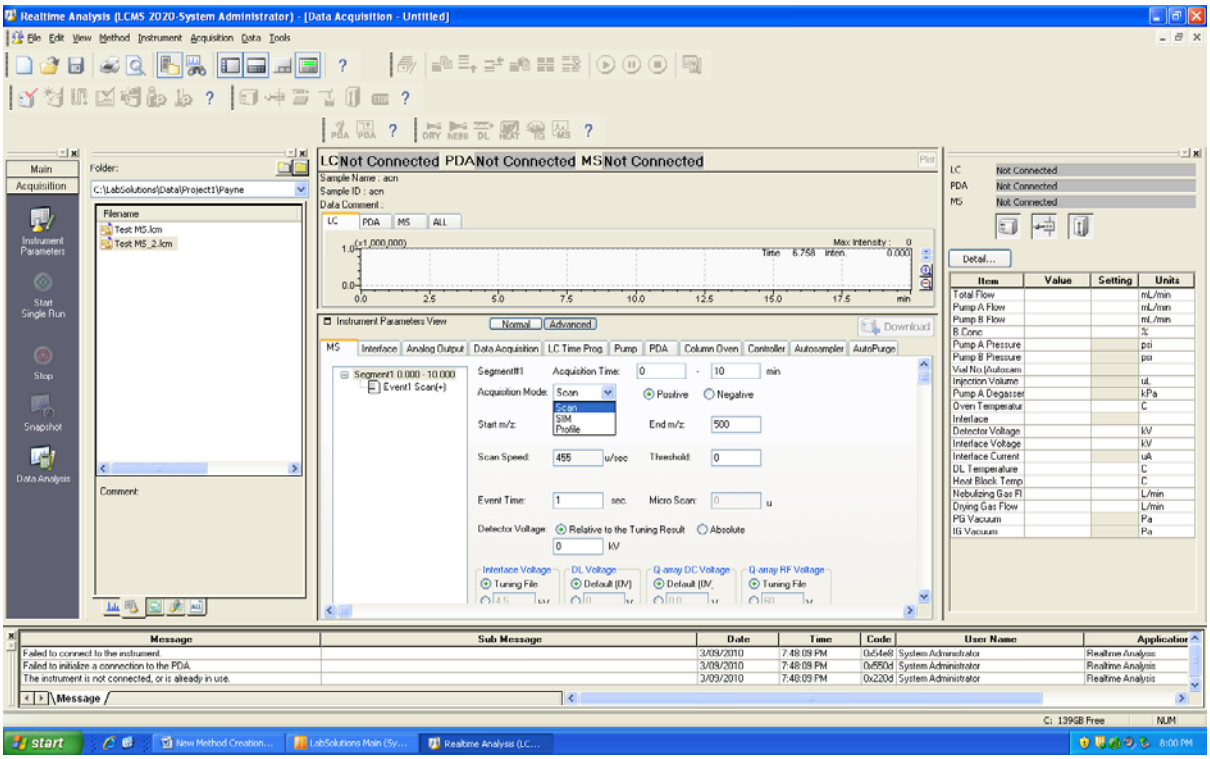

Image 2.

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**).

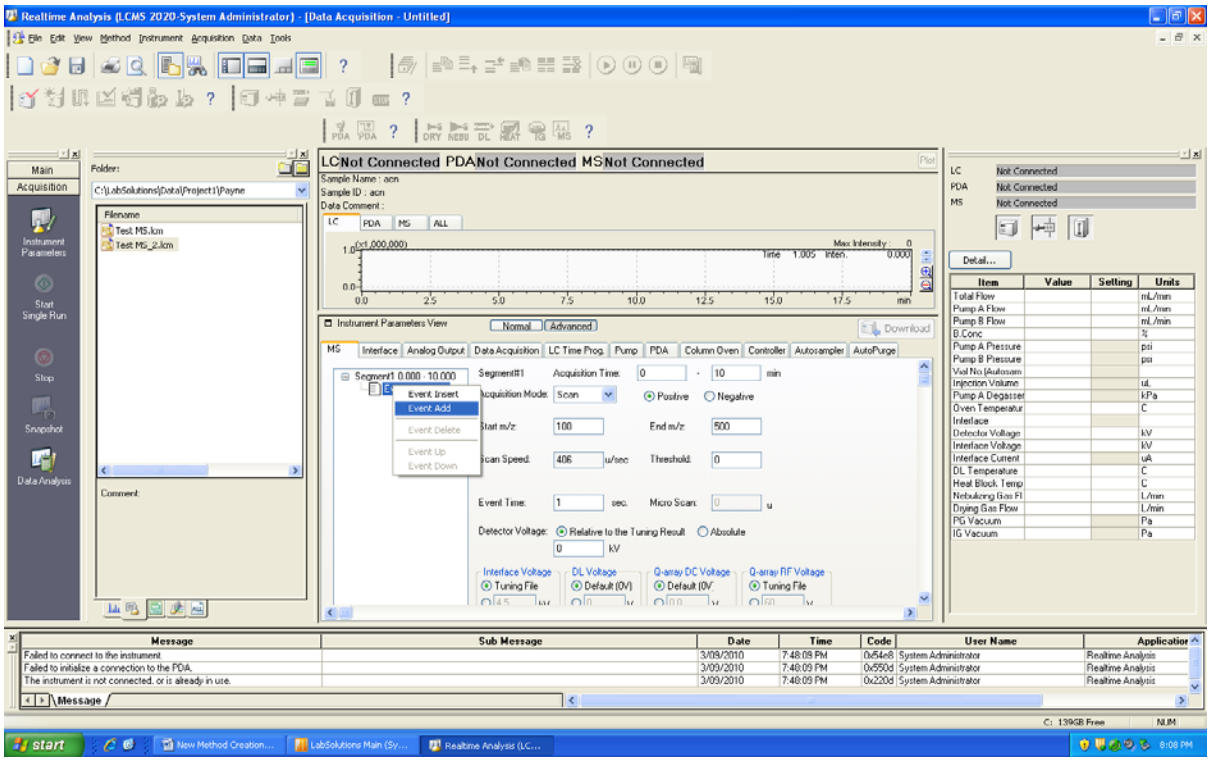

Image 3. Creating new events with new MS parameters

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?)

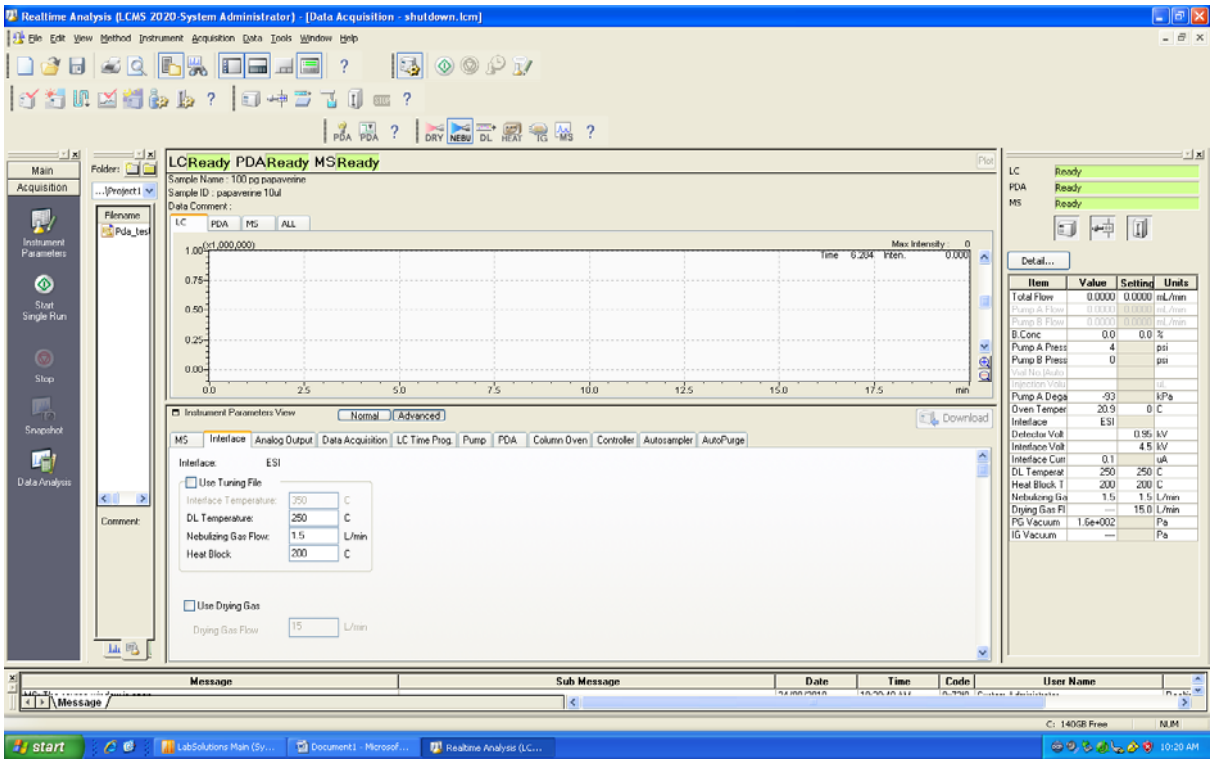

Image 4.

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

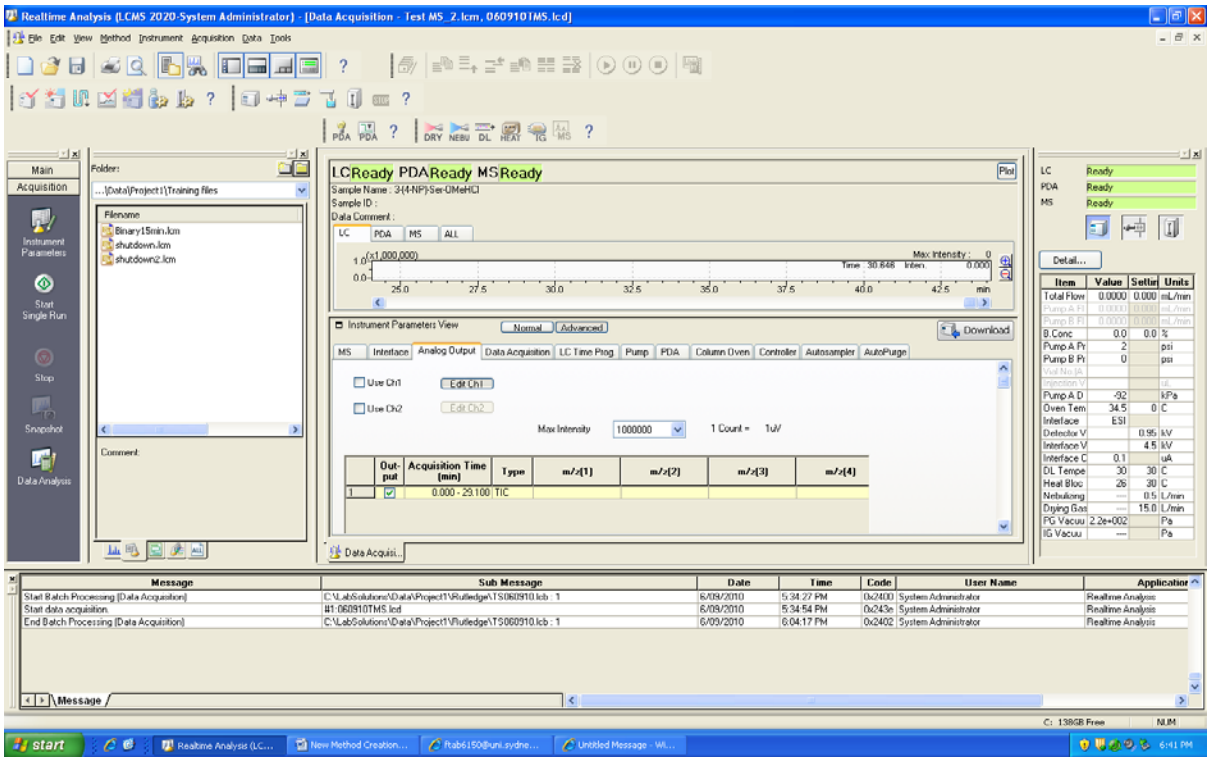

Image 5.

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

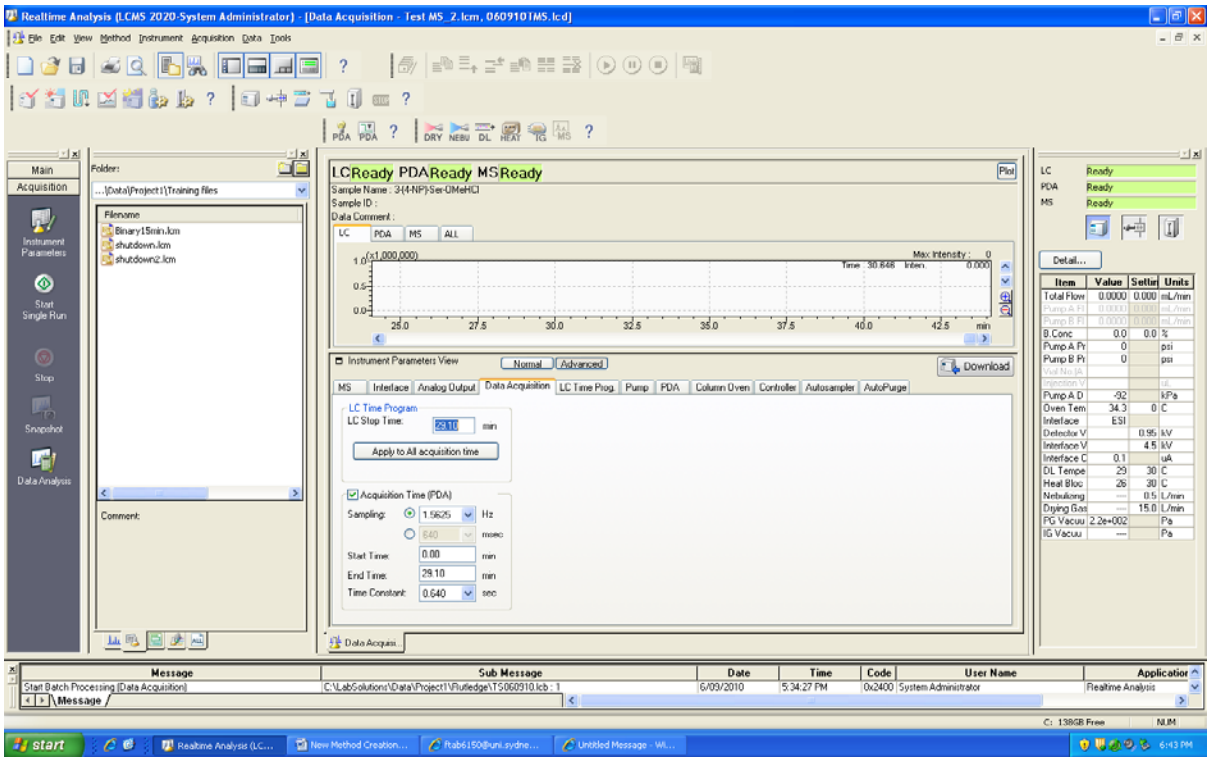

Image 6.

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

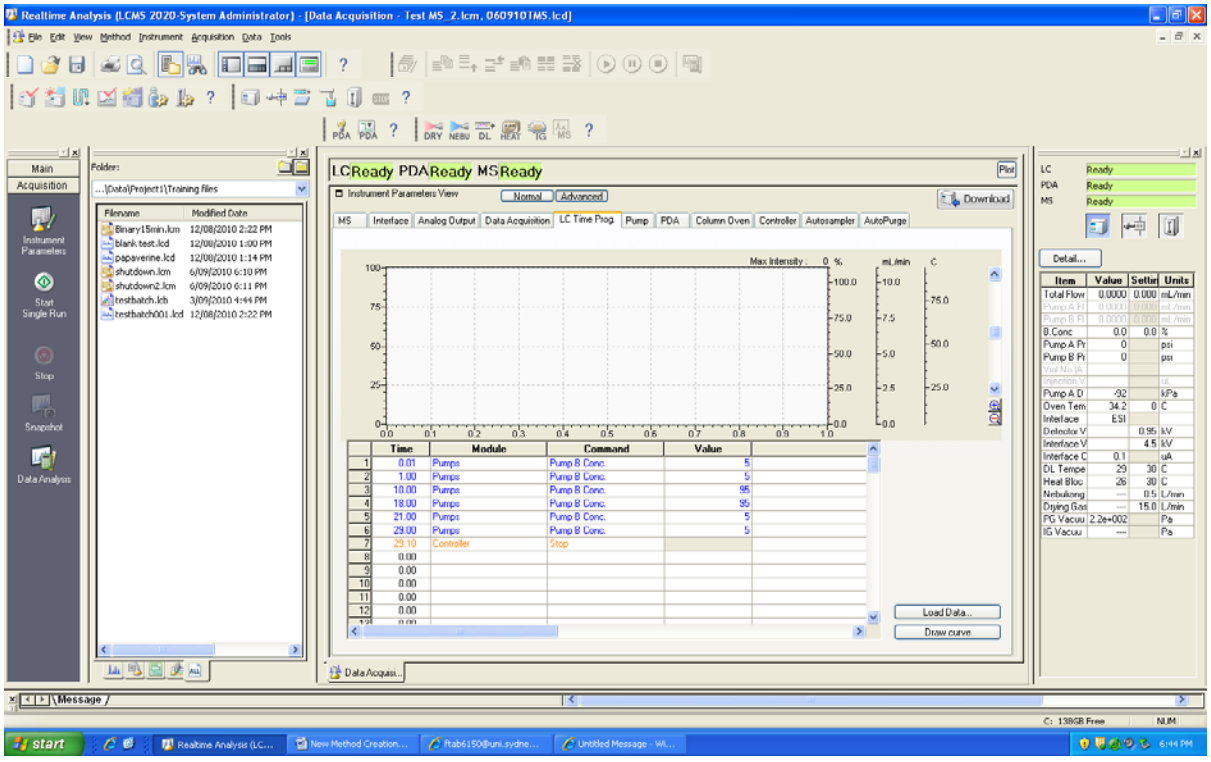

Image 7.

	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).

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.

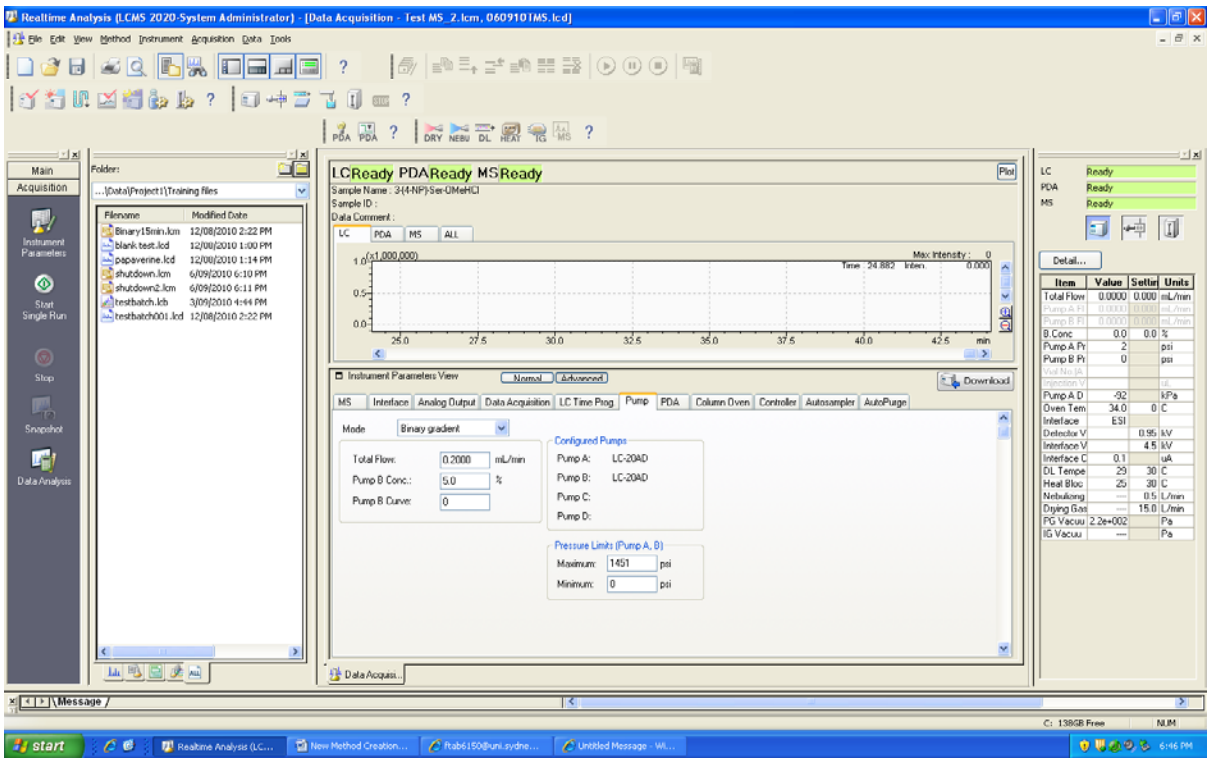

Image 8.

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**)

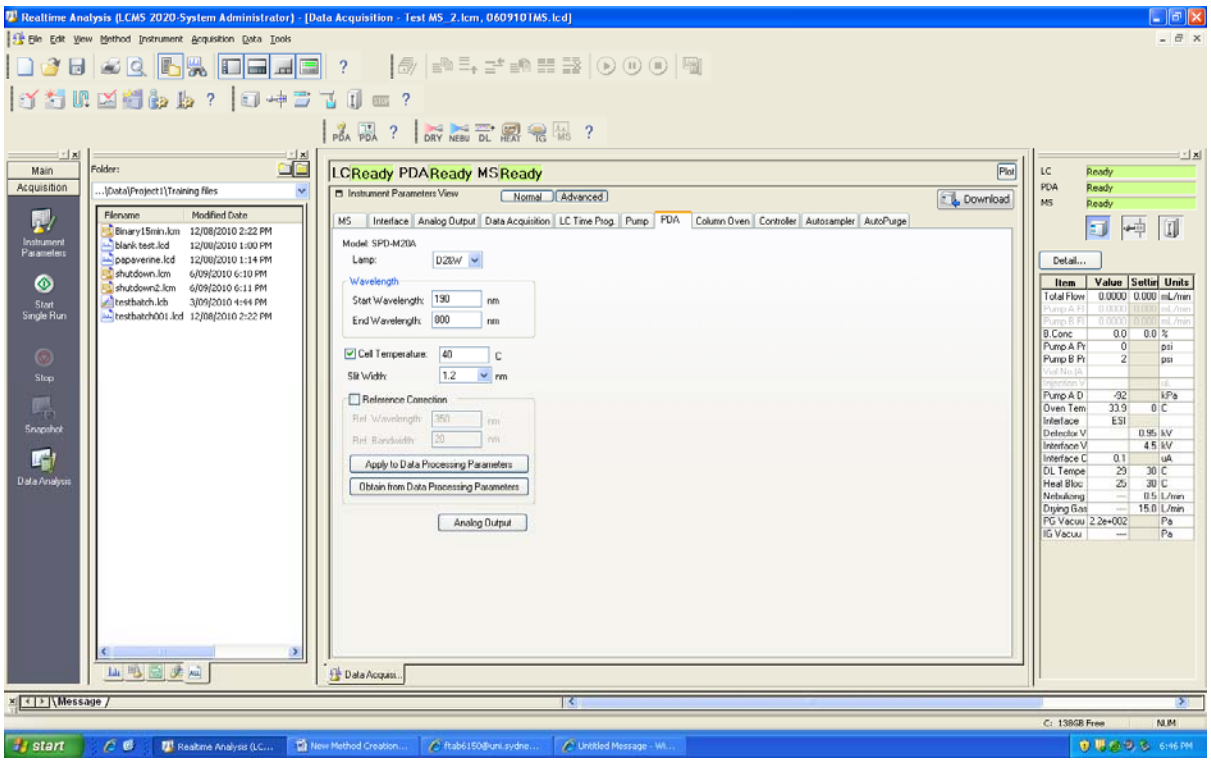

Image 9.

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

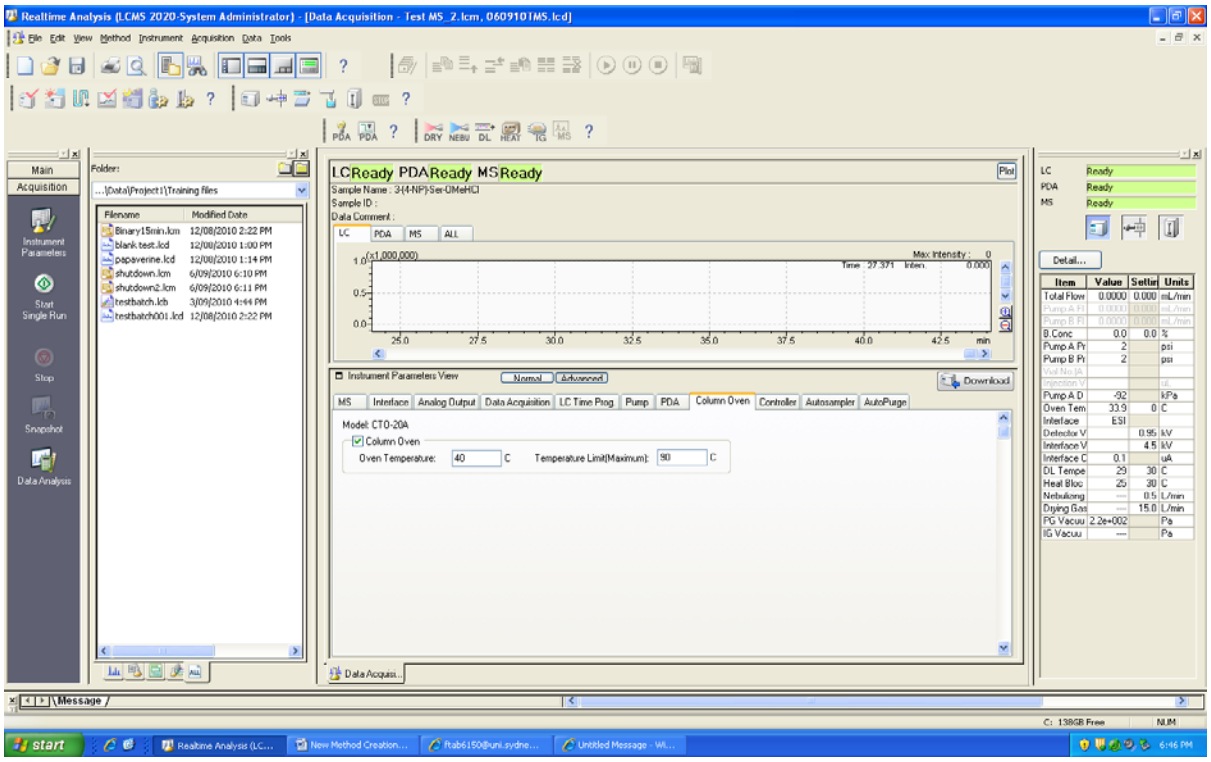

Image 10.

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

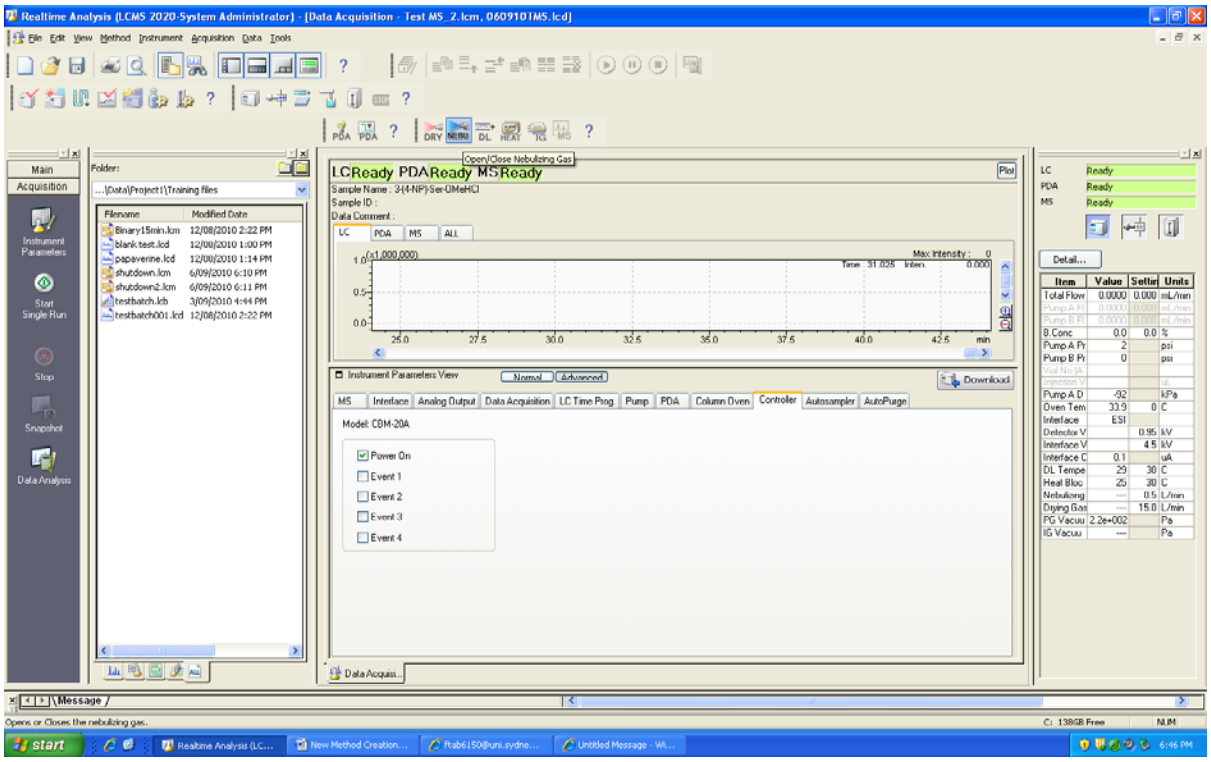

Image 11.

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.

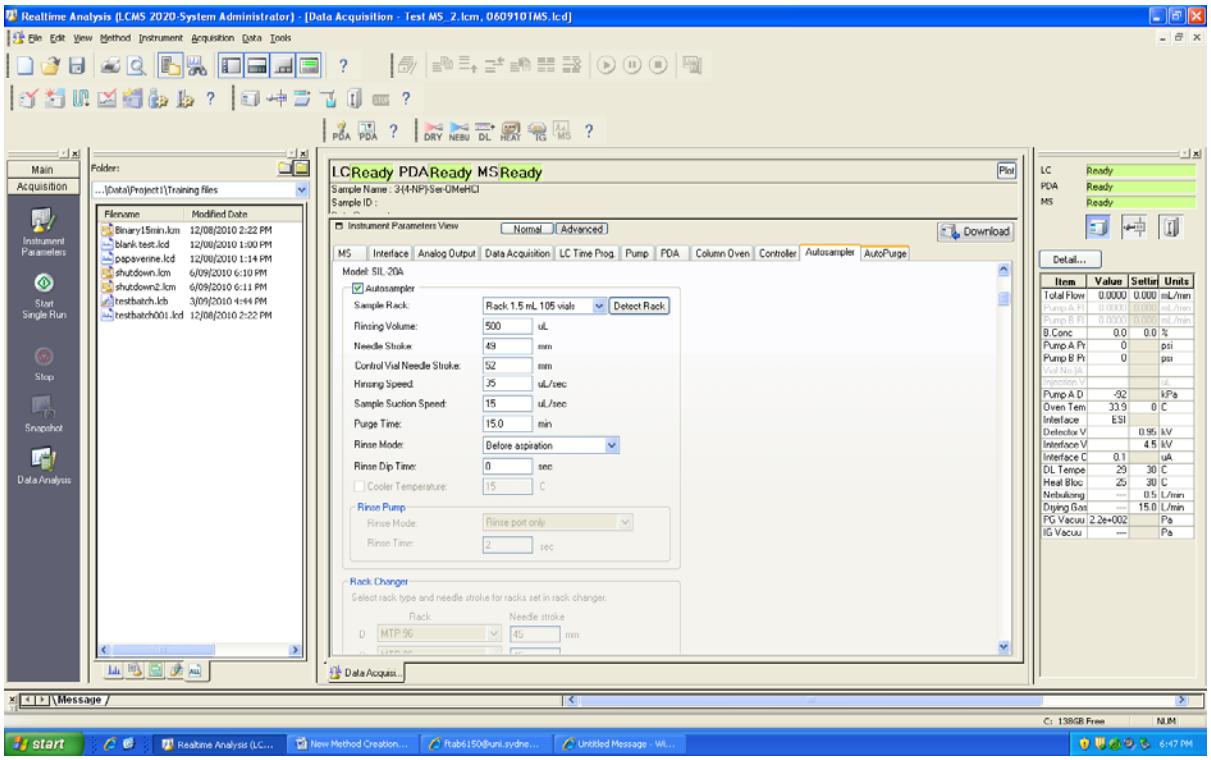

Image 12.

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

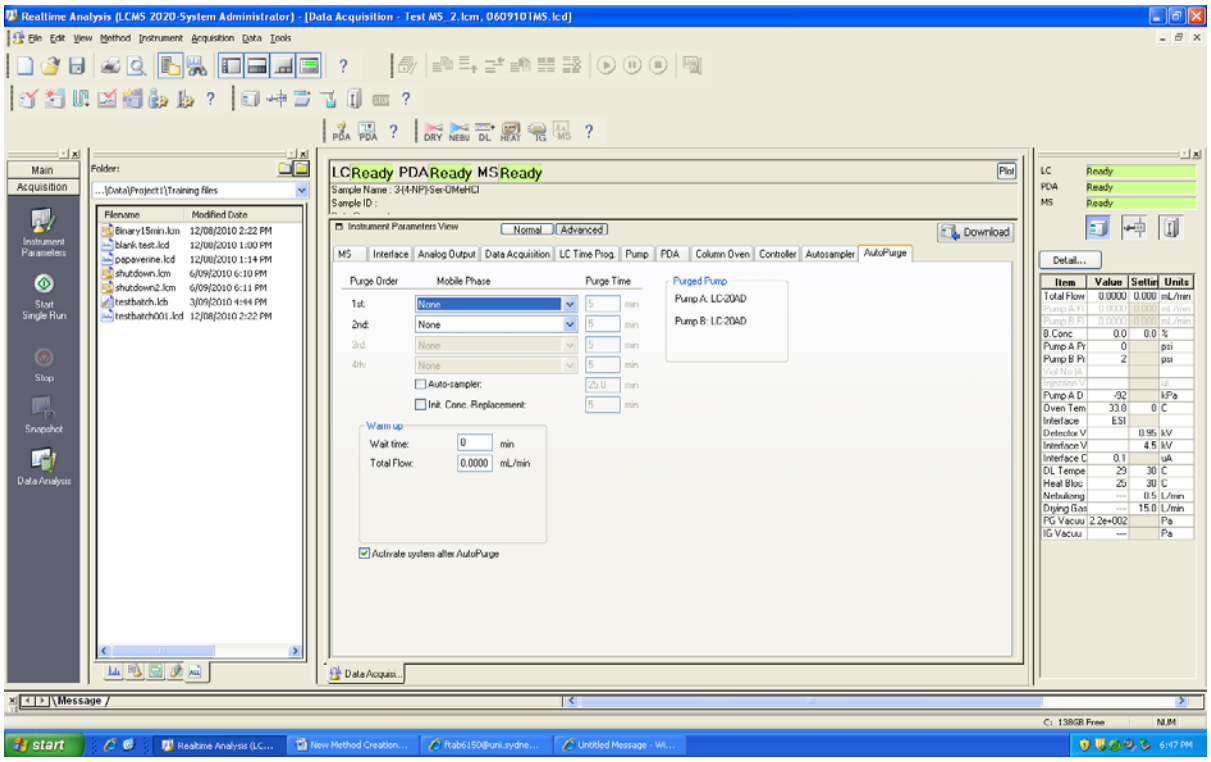

Image 13.

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

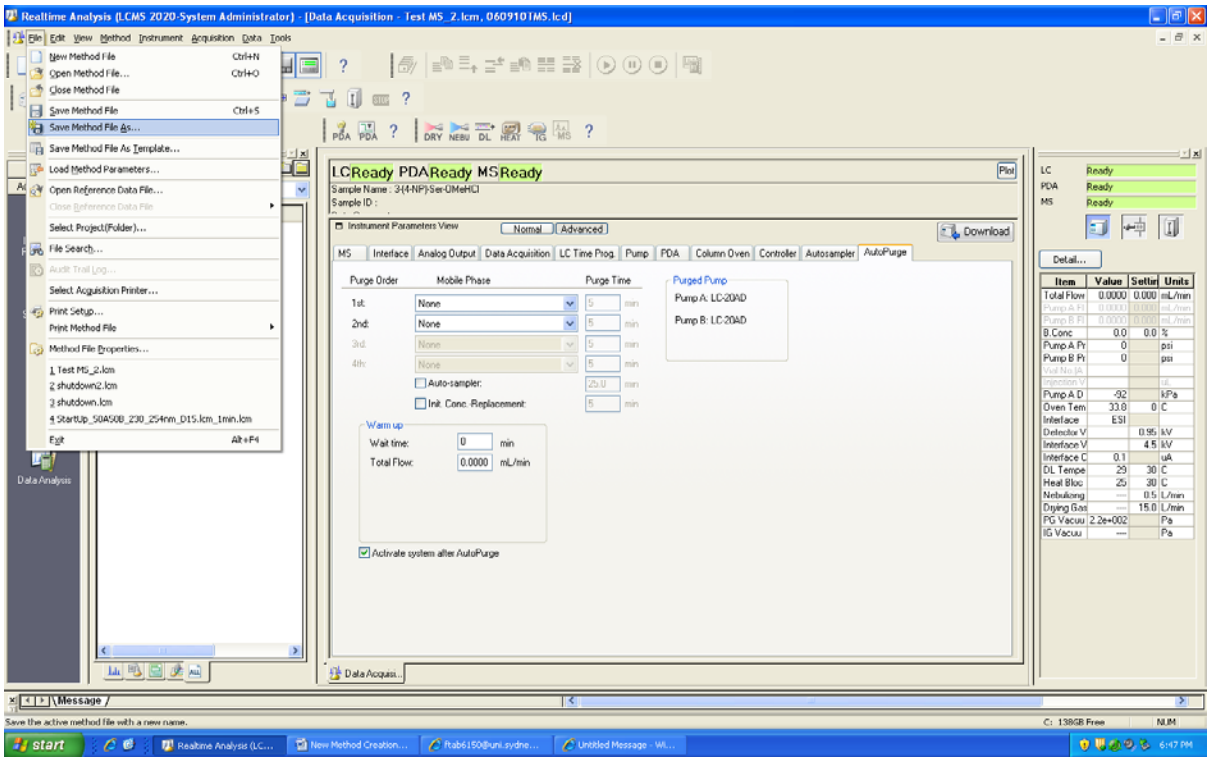

Image 14.

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