Organic Geochemistry Method Manual for operations onboard the Ryan Chouest following the Deepwater Horizon oil spill May - September 2010.

Introduction

This manual outlines the Geochemical, and GC MS methods used during the Deepwater Horizon oil spill. A team from CSIRO was asked to help with the surface water monitoring using the Hydrocarbon Sensor Array and the team was deployed very quickly with very little time to prepare all of the experiments needed to be conducted. As expected, in a time of crisis there were many operational issues that had to be solved whilst at sea in conditions not normally encountered in normal laboratory.

This method is a summary of the work carried out during and after the response to collect geochemical data and apply corrections after the sea mission was finished.

In this document, only the basic operation of the instrument and software is described. There is a certain level of assumed knowledge in the use and operation of the equipment. The instrument manufacturer can provide detailed documents and training on how to carry out simple and complex processes.

Equipment

An Agilent 7890A GC connected to a 5975C MSD was set up on board the Ryan Chouest. The system was fitted with a 2 uninterruptible power supply (UPS) to condition the power which was supplied by the ship. Ultra high Purity Helium (UHP) was used as the carrier gas delivered in G size bottles. 6 Cylinders were connected to a manifold which allowed easy transportation onto the ship. All connections were made using ¼" copper tubing and the gas was filtered through an all in one Helium purifier which removed moisture, and hydrocarbons which may be present in the carrier gas.

Standards

D_8 Toluene, d_8 naphthalene, d_{10} phenanthrene and deuterated 2,6,10,15,19,23-hexamethyltetracosane were combined in a mixed composite solution and used as surrogates for different groups of compounds with a known amount added to each sample before extraction.

Standards 1,1-binapthyl and p-Terphenyl were combined in a second mixed composite solution and used as internal standards. A known amount was added to each sample at the end of the extraction just before injecting into the GC-MS.

The stock solutions were prepared in DCM and diluted to 100 mL in a volumetric flask and kept in a fridge.

Standard	Weight (mg)	Vol (mL)	Final Conc (µg/mL)
Toluene d_8	44.25	100	442.5
Naphthalene d_8	50.93	100	509.3
Phenanthrene d_{10}	51.46	100	514.6
2,6,10,15,19,23-Hexamethyltetracosane - d_{62}	53.39	100	533.9
1,1-binaphthyl	51.01	100	510.1
p-terphenyl	53.19	100	531.9

Because there were no facilities on board to weigh standards, only one stock solution was used throughout the whole voyage. Each day, the standards were allowed to come to room temperature and topped up to the volumetric mark. The remaining concentration in the mixed standard was calculated by subtracting the amount added to each sample from the total concentration, the results were kept on a spreadsheet.

Bottle preparation

Amber glass sample bottles were rinsed three times with tap water, followed by three times with DI water to remove sea salts. The bottles were further rinsed with methanol, (2 x 20ml) and DCM (2 x 20 ml). Once the DCM was evaporated, the bottles were put in the oven (150 °C) for a few hours, cooled and covered with aluminium foil and capped. The lids were rinsed with the same procedure but were air dried.

Sample collection and Liquid/Liquid extraction

1 L samples were collected at different intervals and their details recorded (UTC time, GPS position and observations). Samples were collected either by sub-sampling the outflow stream from the sensor array box or directly from the sea surface by lowering the bottle attached to a pole. A 20 mL aliquot was sub sampled into a scintillation vial. Aluminium foil was placed on the mouth of the bottles and vials before capping and the scintillation vials were wrapped with Parafilm ® and stored in a fridge.

Once all samples were sub sampled, 20 µl of the deuterated surrogate standard mix was added to each 1 L bottle followed by 15 mL of DCM with a dispenser. Aluminium foil was placed on the mouth of the bottle and capped. This was then vigorously shaken for 30 s. The samples were rested and shaken again for 1 min, before allowing the sample to rest for at least 30 min or longer until the DCM separated out to the bottom of the bottle. The DCM phase was removed with a long Pasteur pipette and dried over a minimum amount of anhydrous sodium sulfate in a scintillation vial.

A 1 mL aliquot was transferred to a GC vial and 2 µL of internal standard mix was added.

GC-MS conditions

The Samples were analysed on an Agilent 7890A GC interfaced to a 5975C MSD. Samples were injected (0.5µl) onto a DB-5MS GC column (J&W, 60 m, 0.25 mm I.D., 0.25µm film thickness) via a split/splitless injector at a constant carrier gas pressure of 25 psi and a temperature of 310 °C. The temperature program started at 40 °C (hold for 2 min.) with an 8 °C min^{-1} ramp to 310 °C (hold for 20 min.).

The MS was run on SIM mode with three different groups for selected compounds as shown in the table below with their approximate retention times.

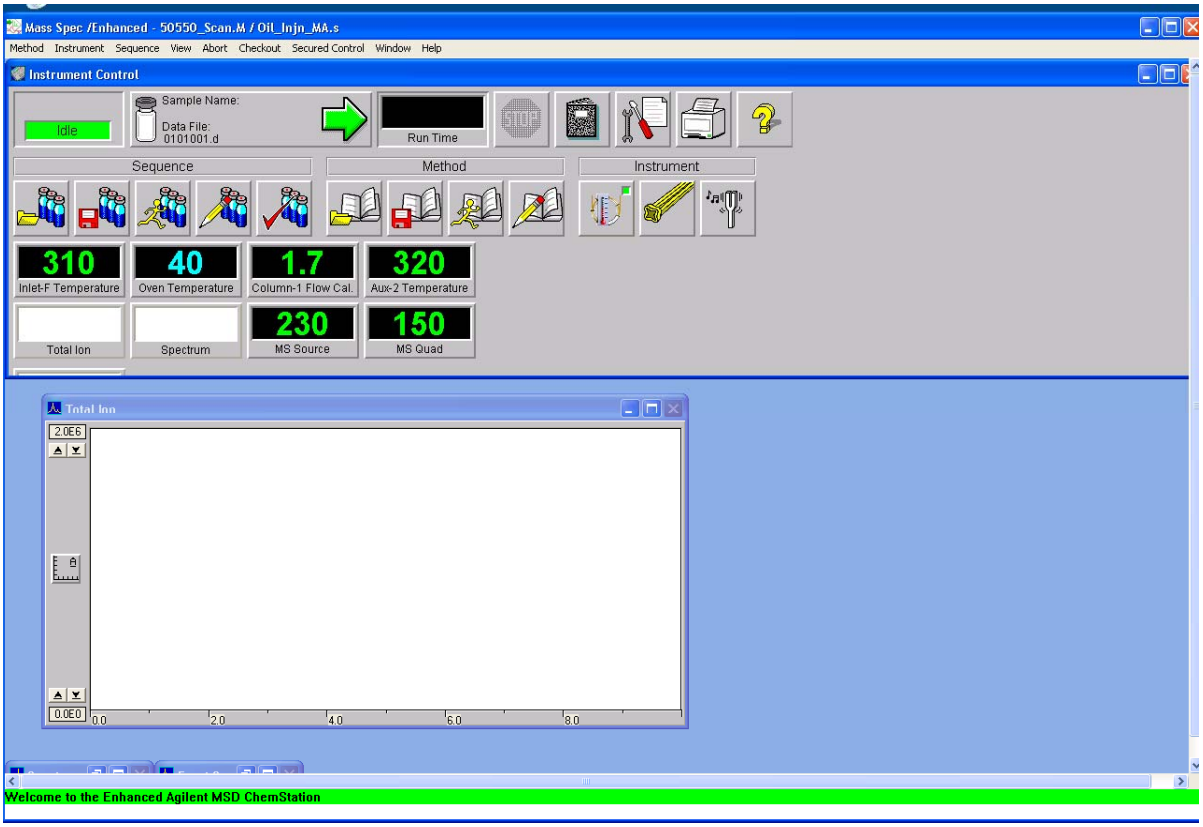
Group 1			Group 2			Group 3		
m/z	**target**	RT (min.)	**m/z**	**target**	RT (min.)	**m/z**	**target**	RT (min.)
57	aliphatics		57	aliphatics		57	aliphatics	
92	Toluene	7.7	128	naphthalene	17.7	178	phenanthrene	28.5
100	d8-toluene (std)	7.6	136	d8-naphthalene (std)	17.6	184	dibenzothiophene	28.1
106	et-benzene	9.9	142	methylnaphthalenes	19.9-20.4	188	d10-anthracene (std)	28.4
	m/p-xylene	10.2	156	C2-naphthalenes	21.8-23	192	methylphenanthrenes	30-30.6
	o-xylene	10.7	170	C3-naphthalenes	23.2-25.3	198	methyldibenzothiophenes	29.4-30.2
120	C3-benzenes	12.2-14	184	C4-naphthalenes	24.1-27.6	206	C2-phenanthrenes	31.4-32.2
						212	C2-dibenzothiophenes	30.7-31.7
						230	p-terphenyl (std)	33.7

Quantification was carried out by comparison of the areas of individual components with the appropriate surrogate standard.

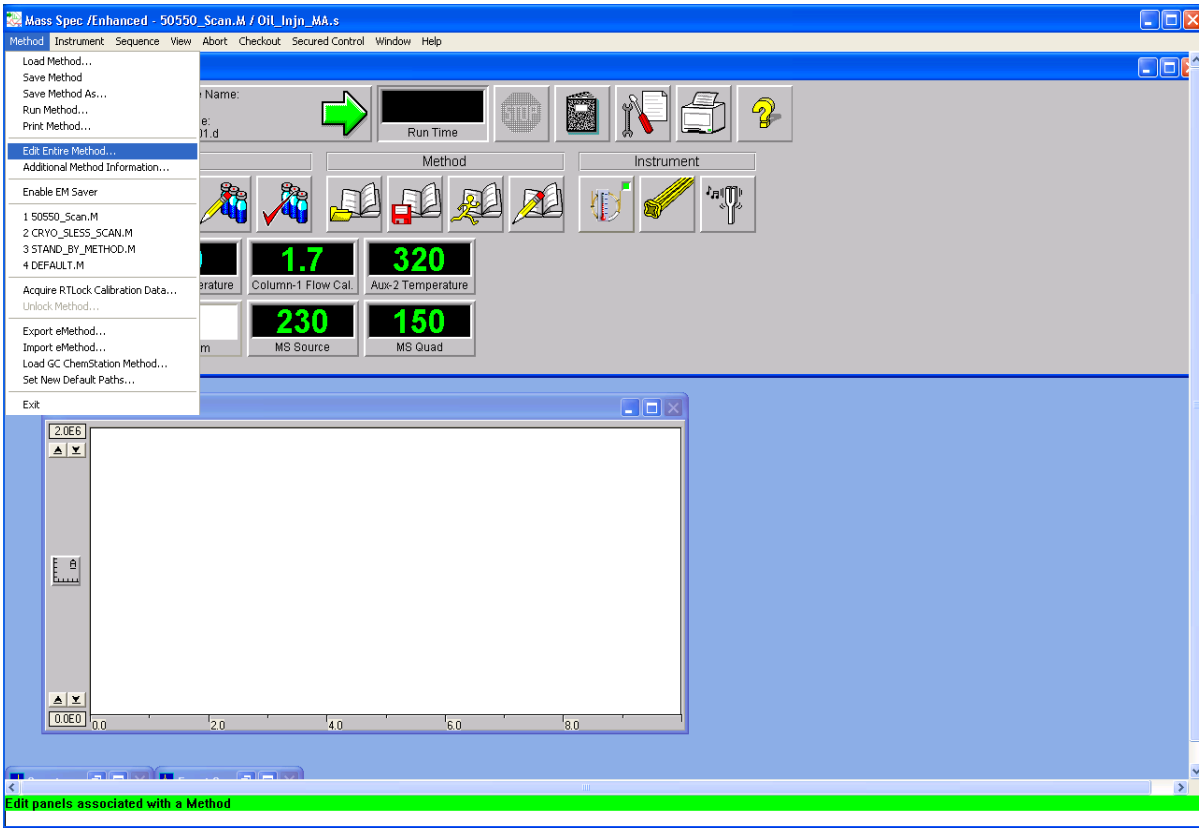
Basic operation of the GC-MS

Creating a method

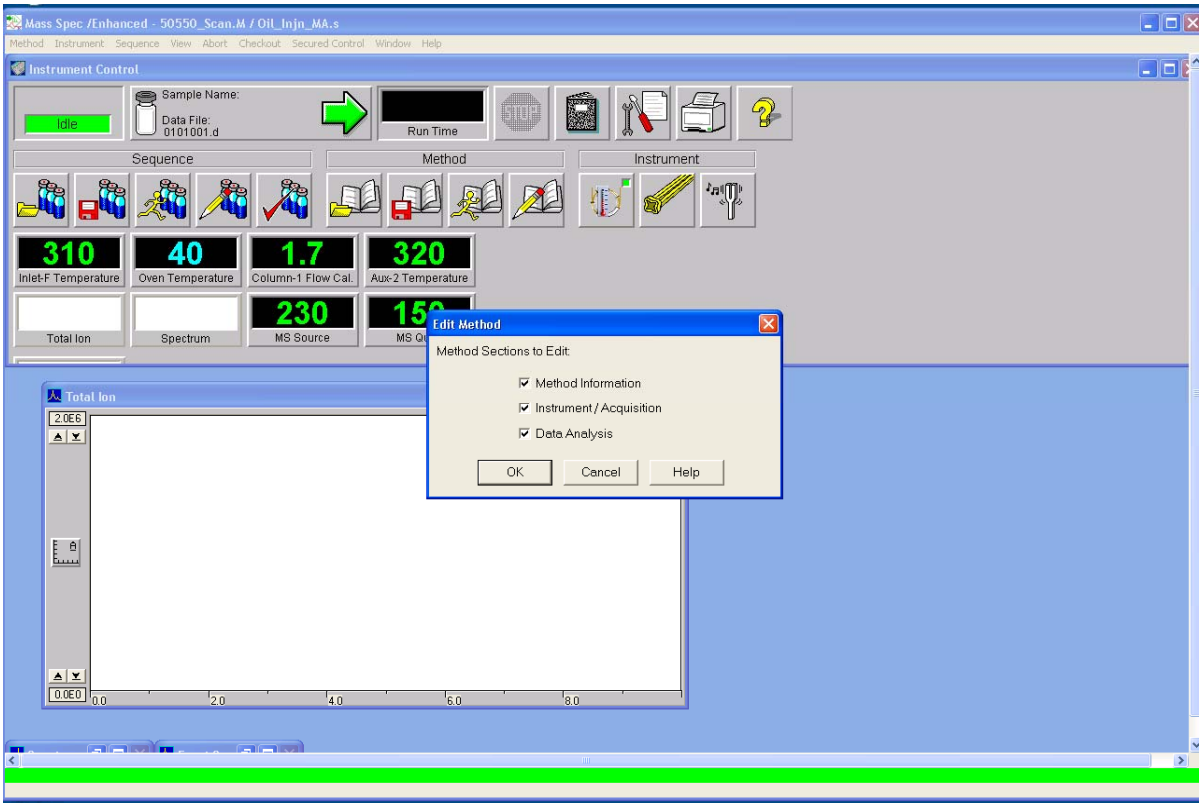
Load the Default method



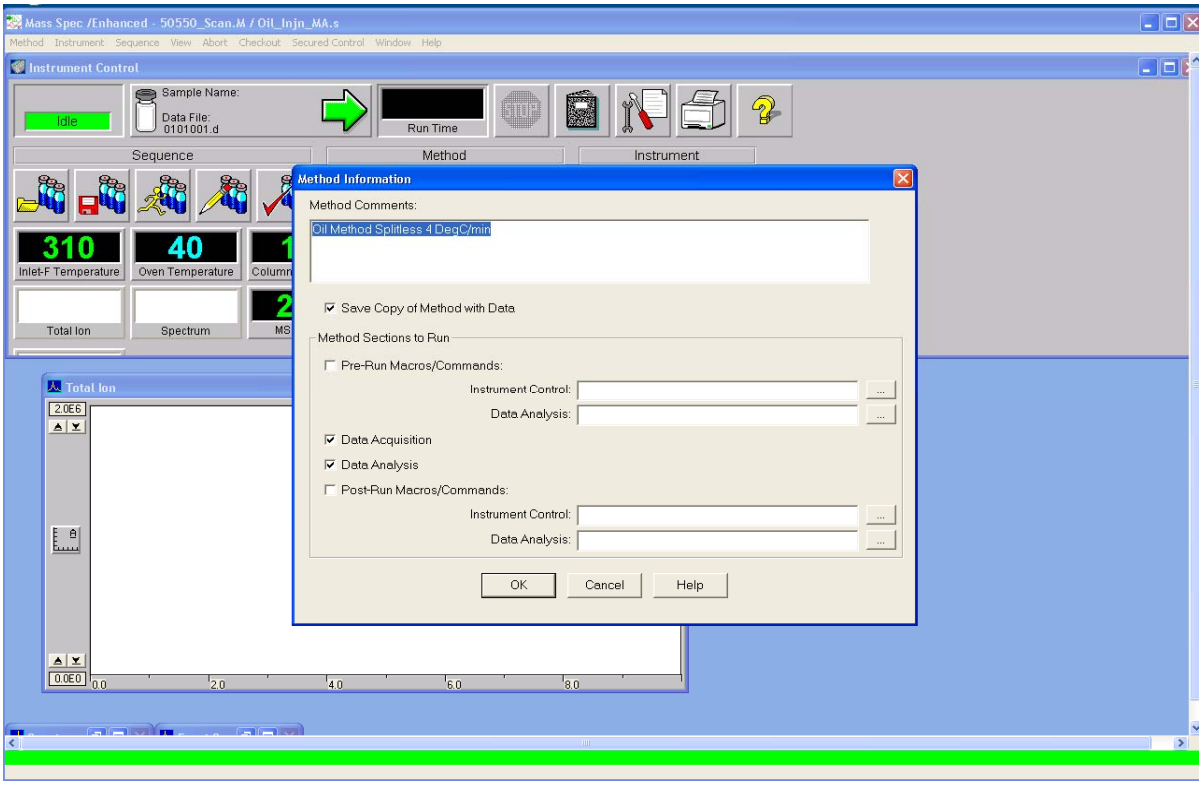
Load the Default method from the Method Menu, and then click on “Edit Entire Method”. The short cut buttons on the screen can also be used for this purpose



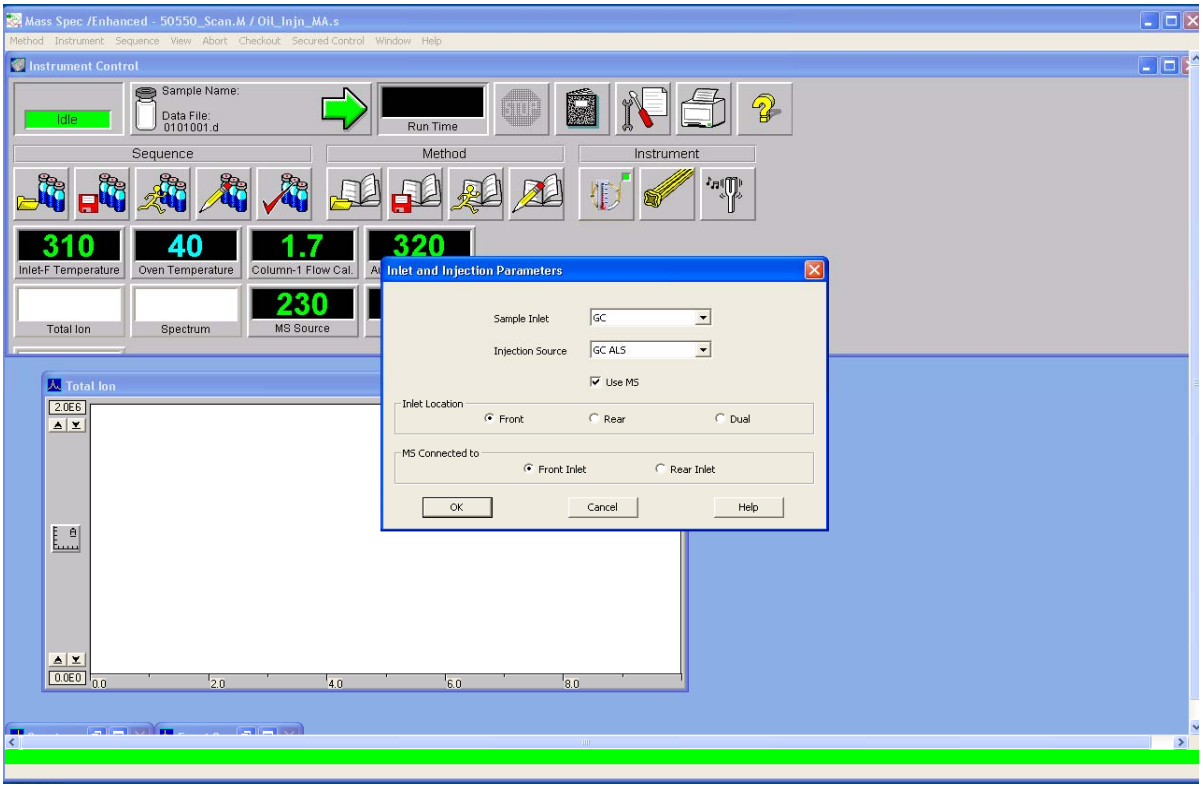
The software will now go through all method parameters that can be edited



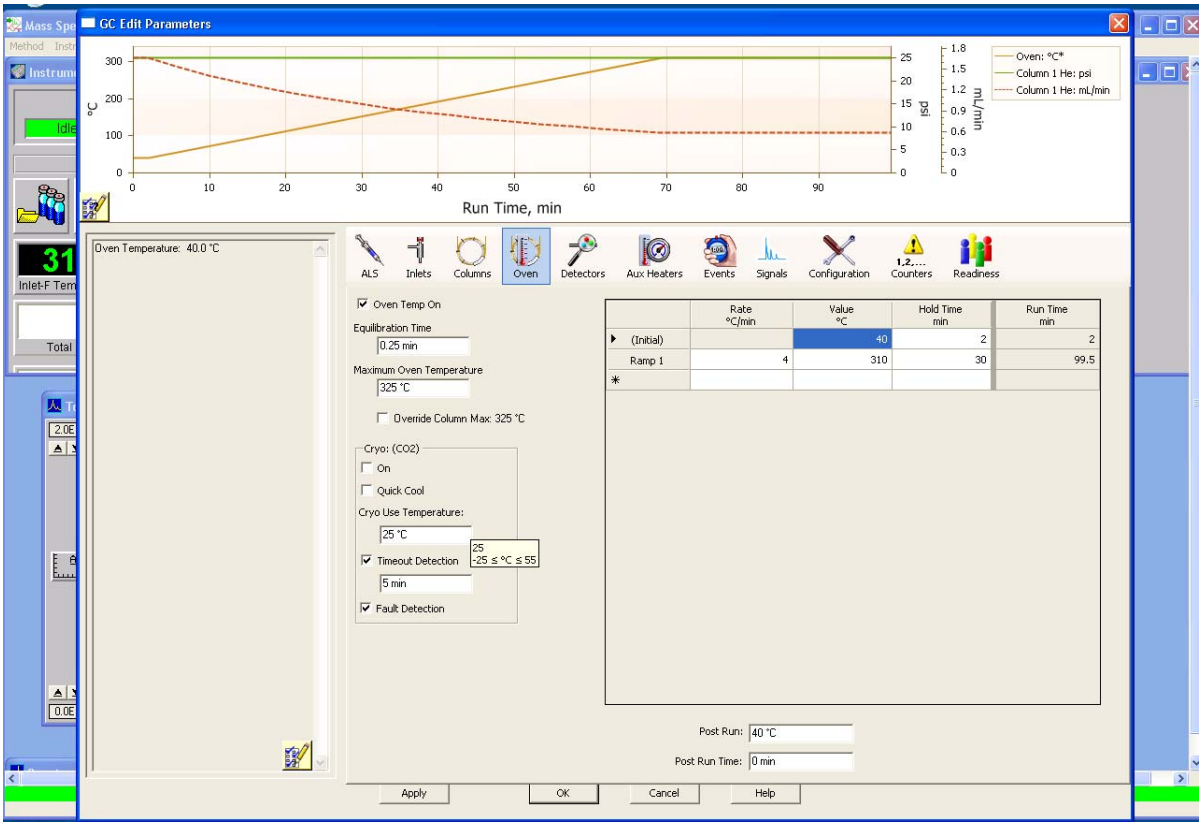
On the screen below the software allows for a quick method description. Make sure the “Save copy of method with data” box is ticked



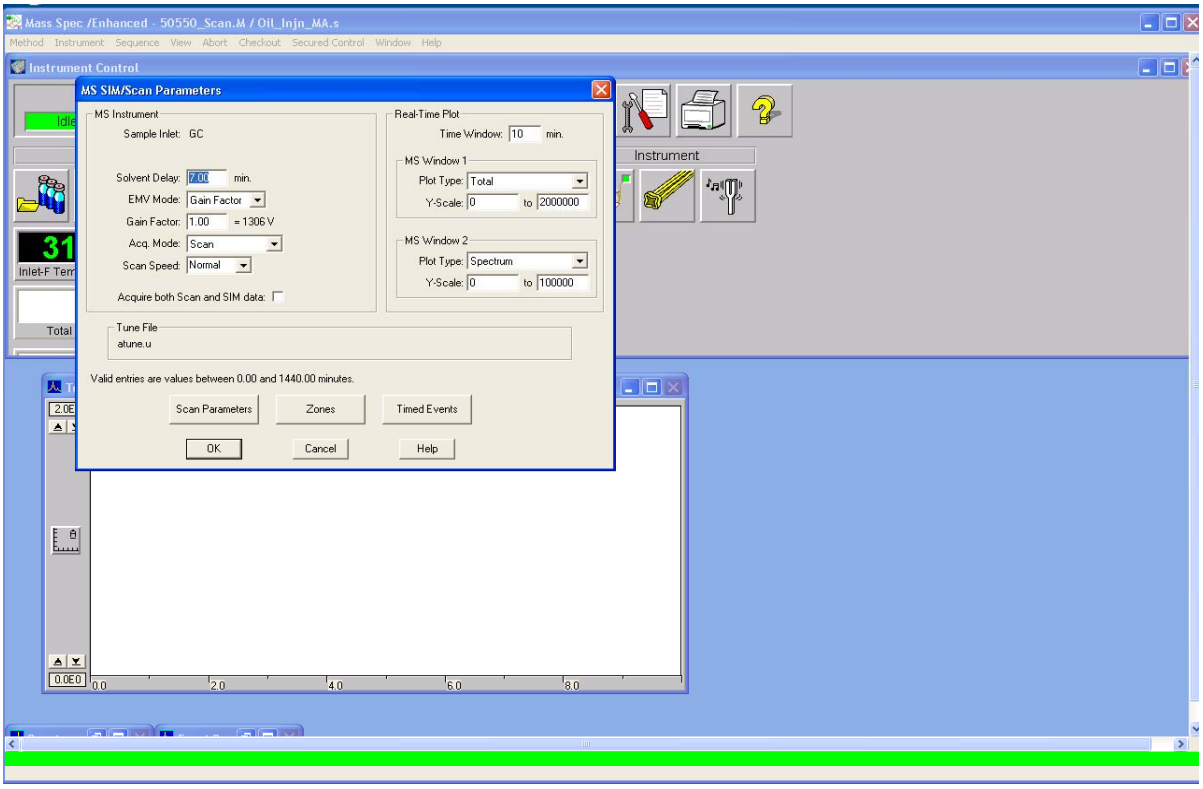
Choose injector position and ALS if there is an Autosampler. Manual injections can also be selected



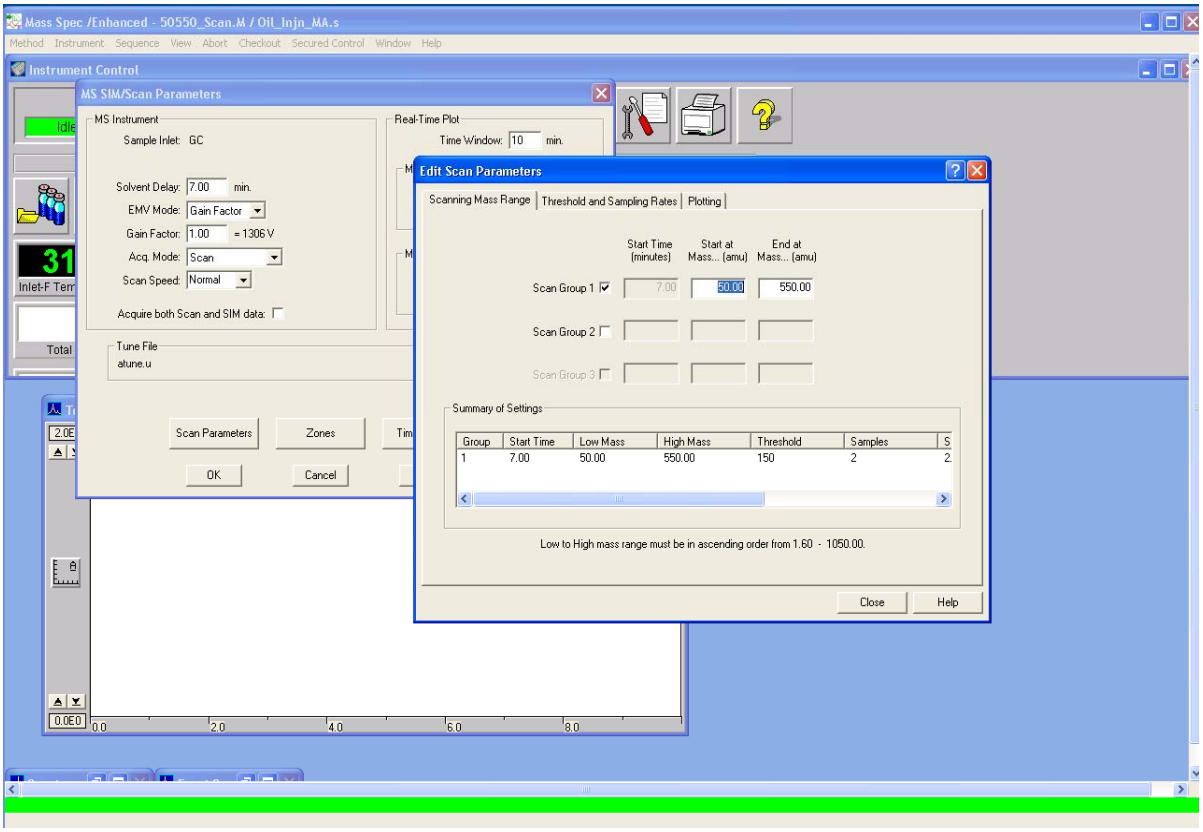
The next screen will allow the user to edit all aspects of the instrument starting from the injector settings to the oven settings and other peripherals attached to the instrument. Ensure that all tabs are checked if the method is being edited before proceeding to the next step.



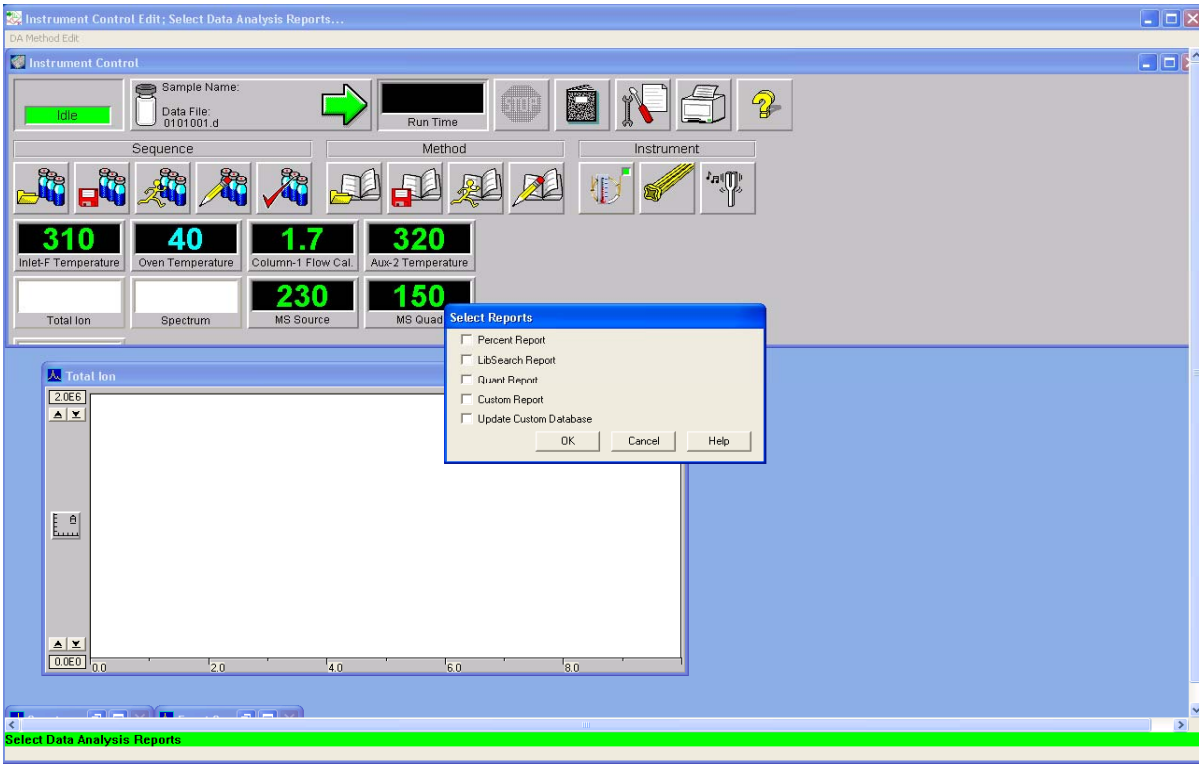
The next window allows the user to choose solvent delay time, scan or SIM mode



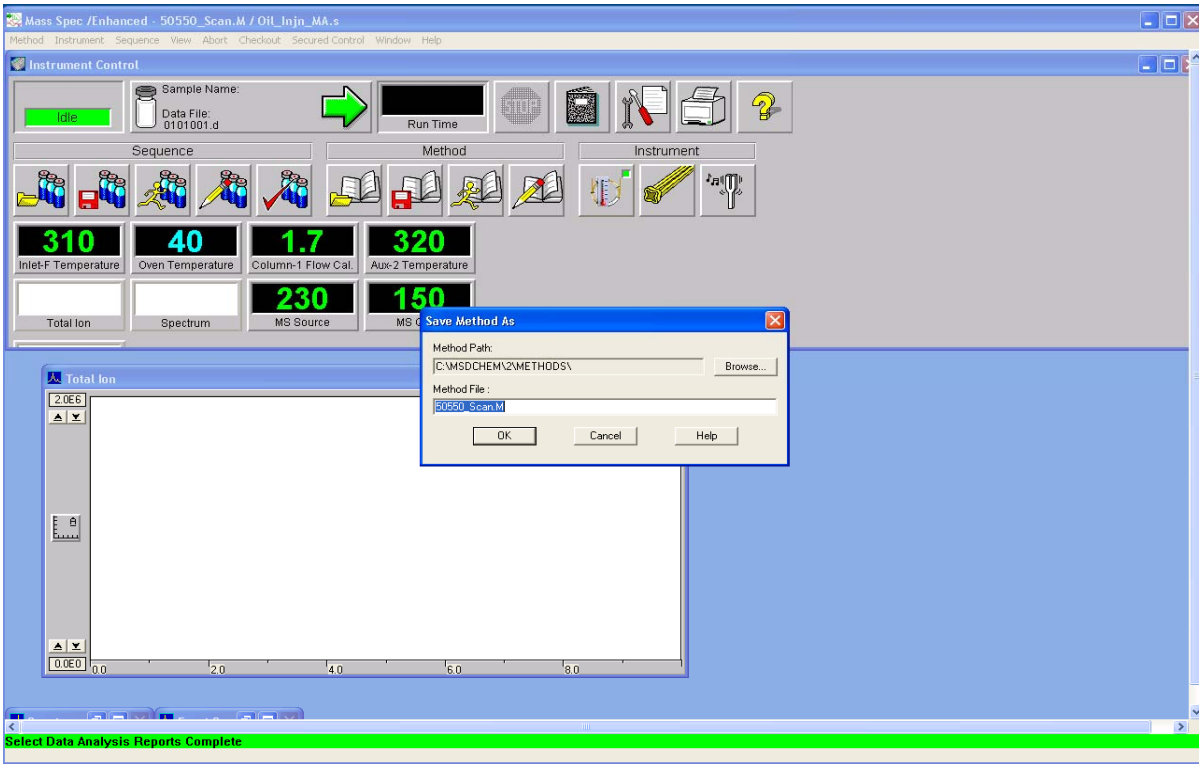
The Scan and / or SIM parameters can be edited by clicking the “Scan parameters” or “SIM parameters” button.



The next screen allows the user to select the report type in which the final data will be compiled. For this project, no report was selected as all the processing was done after the conclusion of the sea operations once back on land.

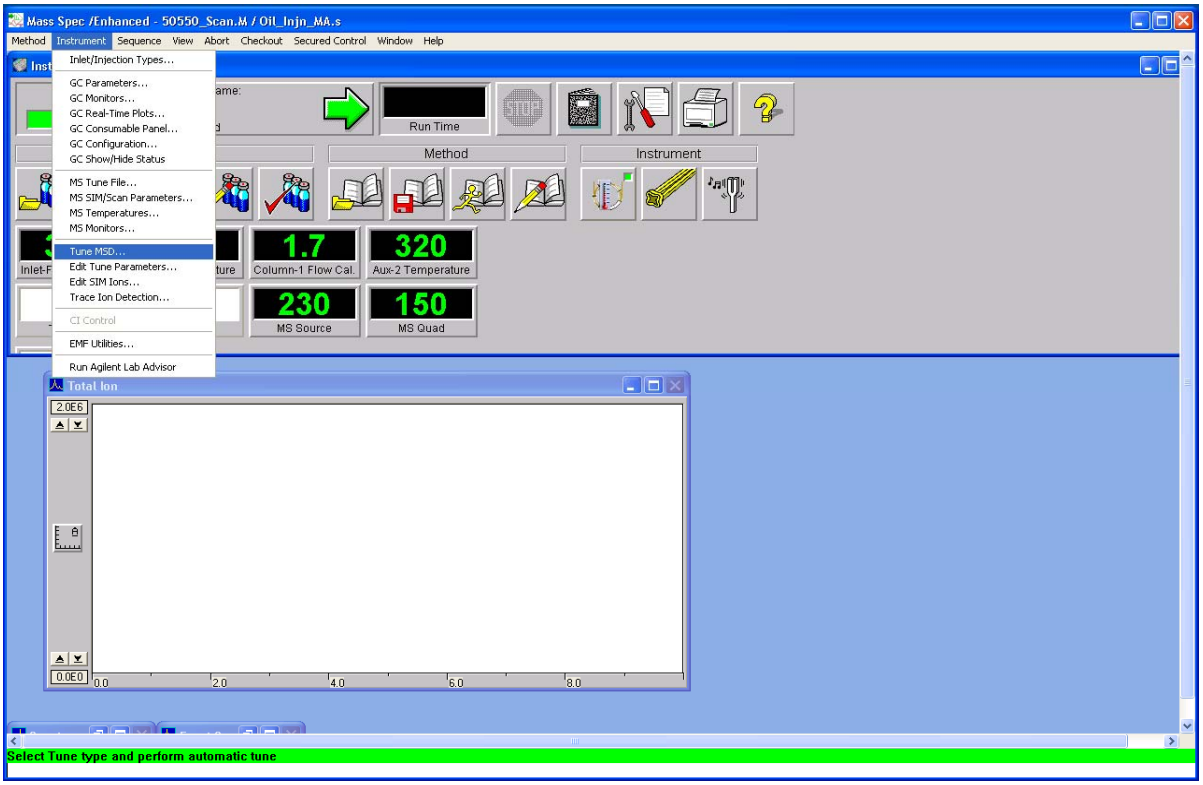


Save the method after editing. The method can be used for analysing samples

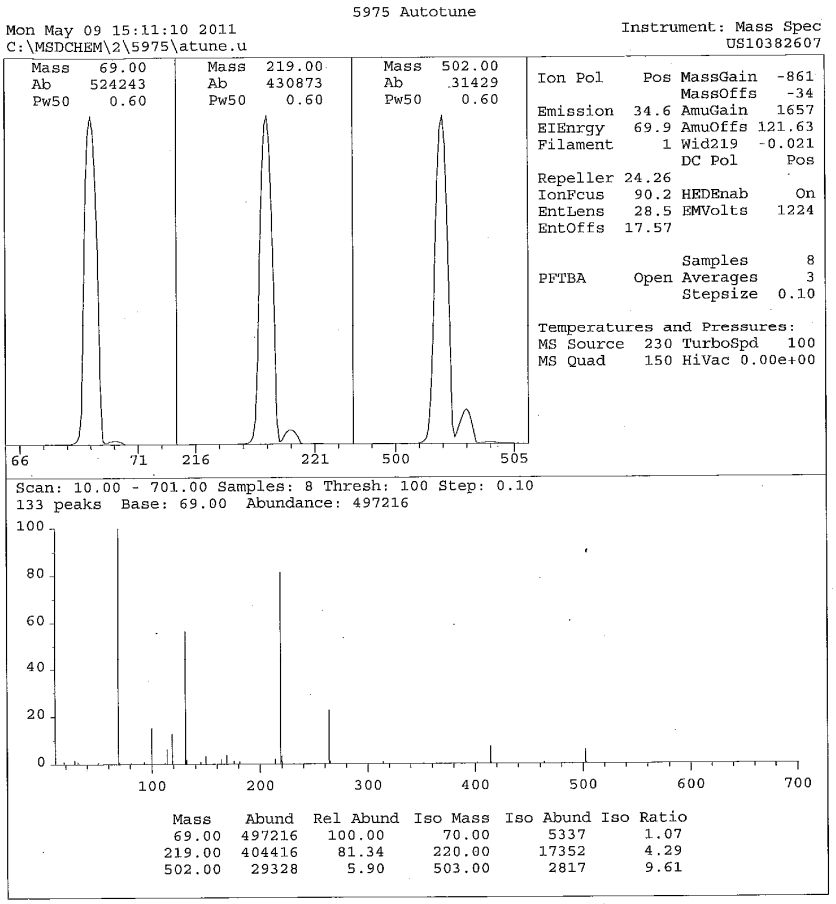


Tuning the GC-MS

MS Tuning is done when a column is changed and /or when the sources is cleaned. Select “Instrument” then “Tune MSD”. The MS will then tune, the process takes approximately 4-5 minutes. Tuning can also be done by clicking on the tuning fork on the main screen.



The tune report looks like the image below. Ensure the instrument tunes as per Agilent's specified standards. The main things to look for are air and water peaks which will affect the instrument sensitivity. If the system is not leak free, corrective action must be taken to eliminate any leaks into the system.



Air/Water Check: H20~1.15% N2~1.59% O2~0.52% CO2~0.13% N2/H2O~138.97%

Column(1) Flow: 1.66 Column(2): -1.79769e+308 ml/min. Interface Temp: 320

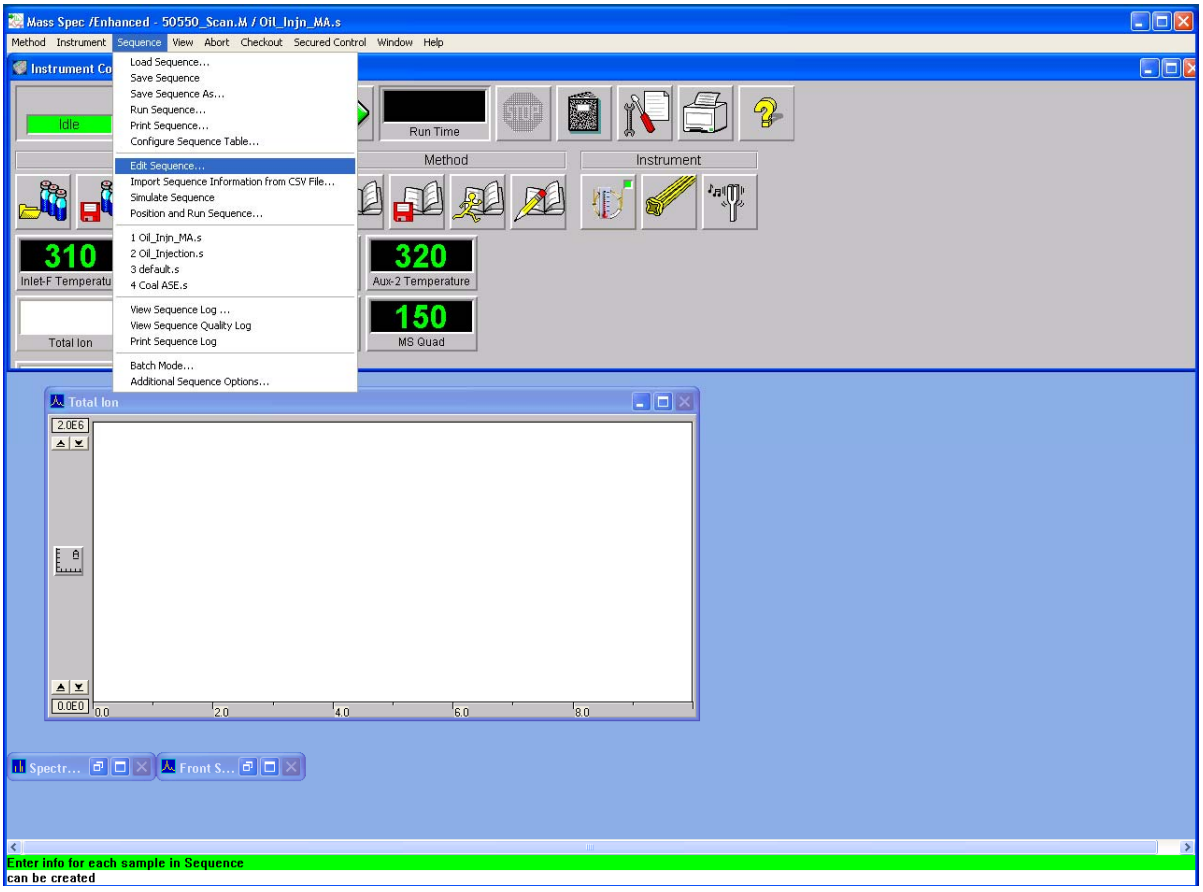
Ramp Criteria:
Ion Focus Maximum 90 volts using ion 502; EM Gain 49400
Repeller Maximum 35 volts using ion 219; Gain Factor 0.49

MassGain Values(Samples): -853(3) -849(2) -830(1) -805(0) -761(FS)

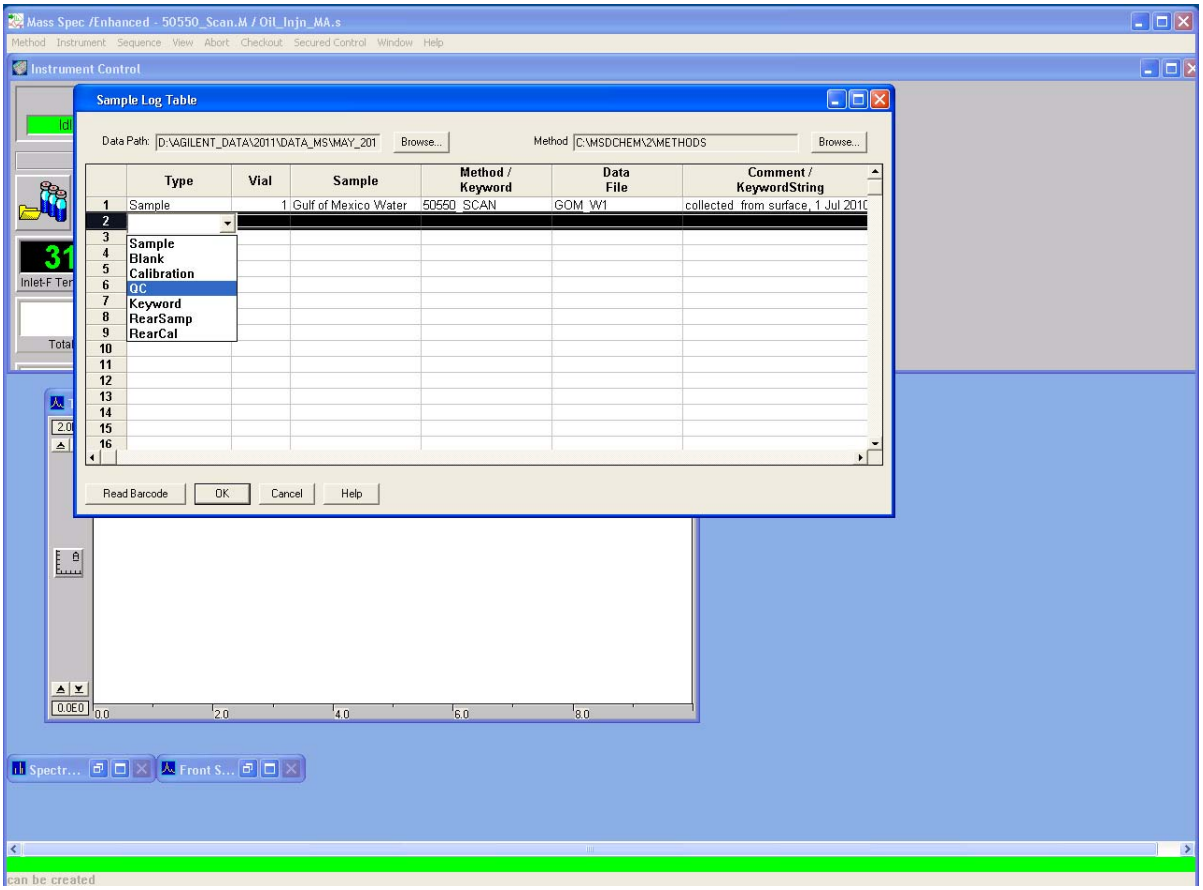
TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset:	121.6	121.6	121.6	121.6	121.6	121.6	121.6
Entrance Lens Offset:	17.6	17.6	17.6	17.6	17.6	17.6	17.6

Creating and Running a Sequence

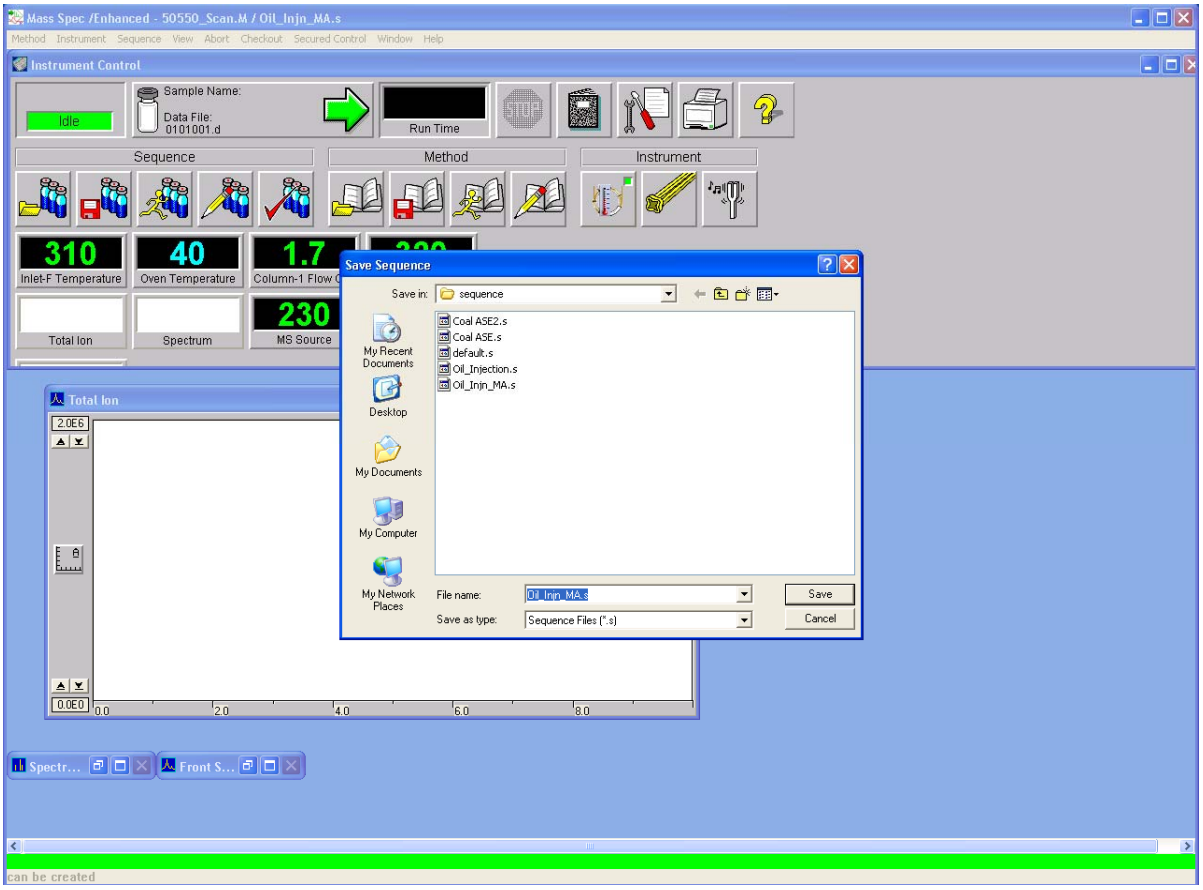
A sequence can be created for running samples through the day. It is important to save each sequence as this makes the process of re calculation and analysis much faster. The sequence can be loaded, edited, saved and run by using the short cut buttons on the screen or by using the menus.



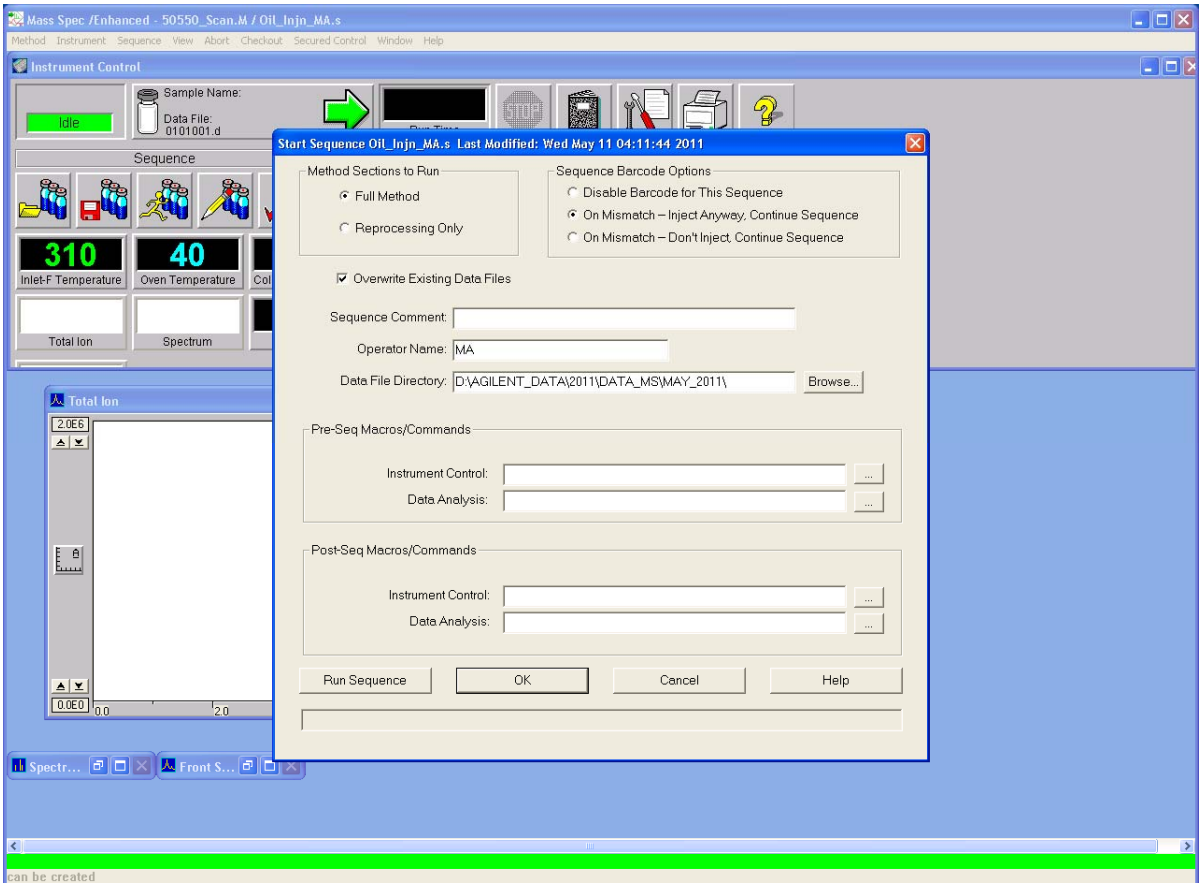
Write in the relevant sample information for each sample, enter sample information, select method and vial positions



Once the sequence table is filled in, save the sequence and give it a name



Press the Run sequence button and the sequence dialogue will come up. Press run sequence and the instrument will go through the steps to inject multiple samples.



Processing of Data Files

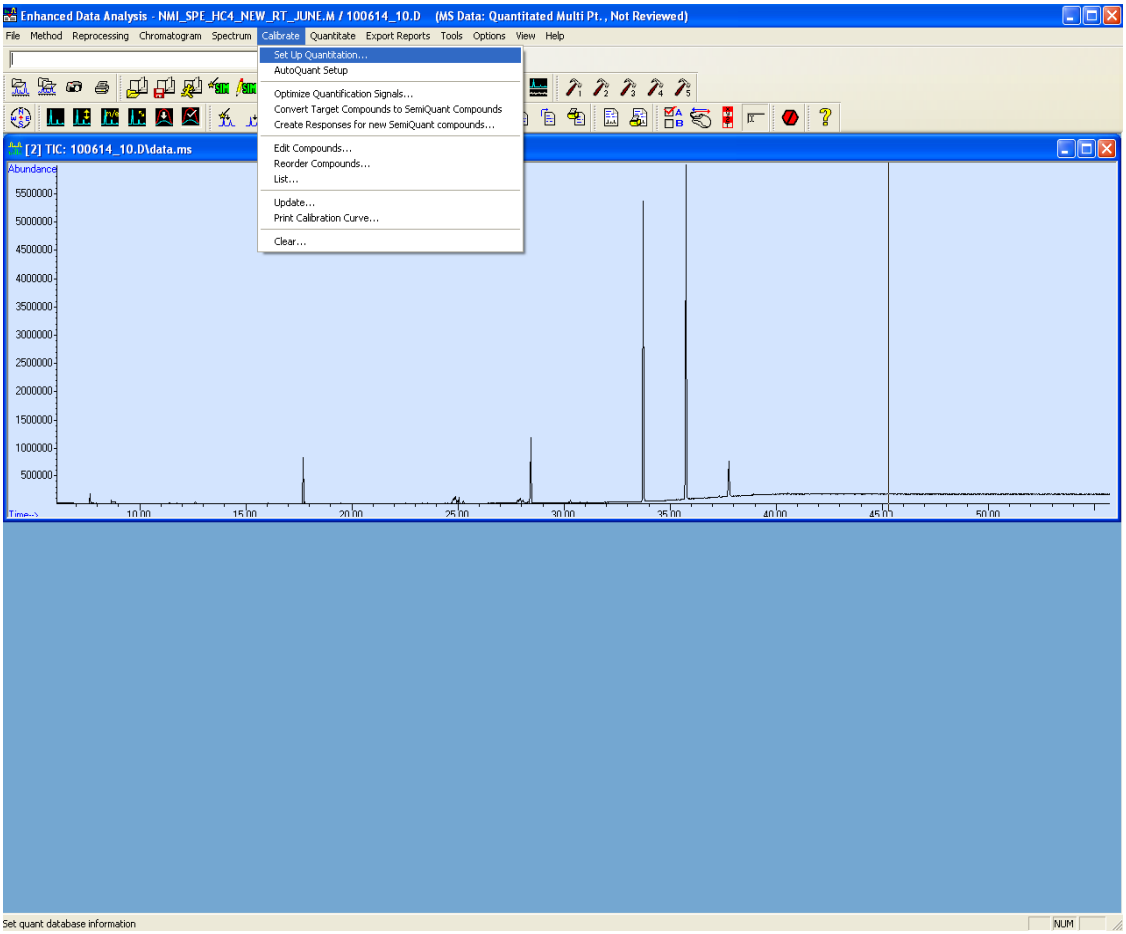
After the validation process was finished, all GC data was analysed using an automatic method using the Chemstation software. The basic steps to generate a processing method and analysing a data file are described below

Developing a method for Scan/SIM analysis

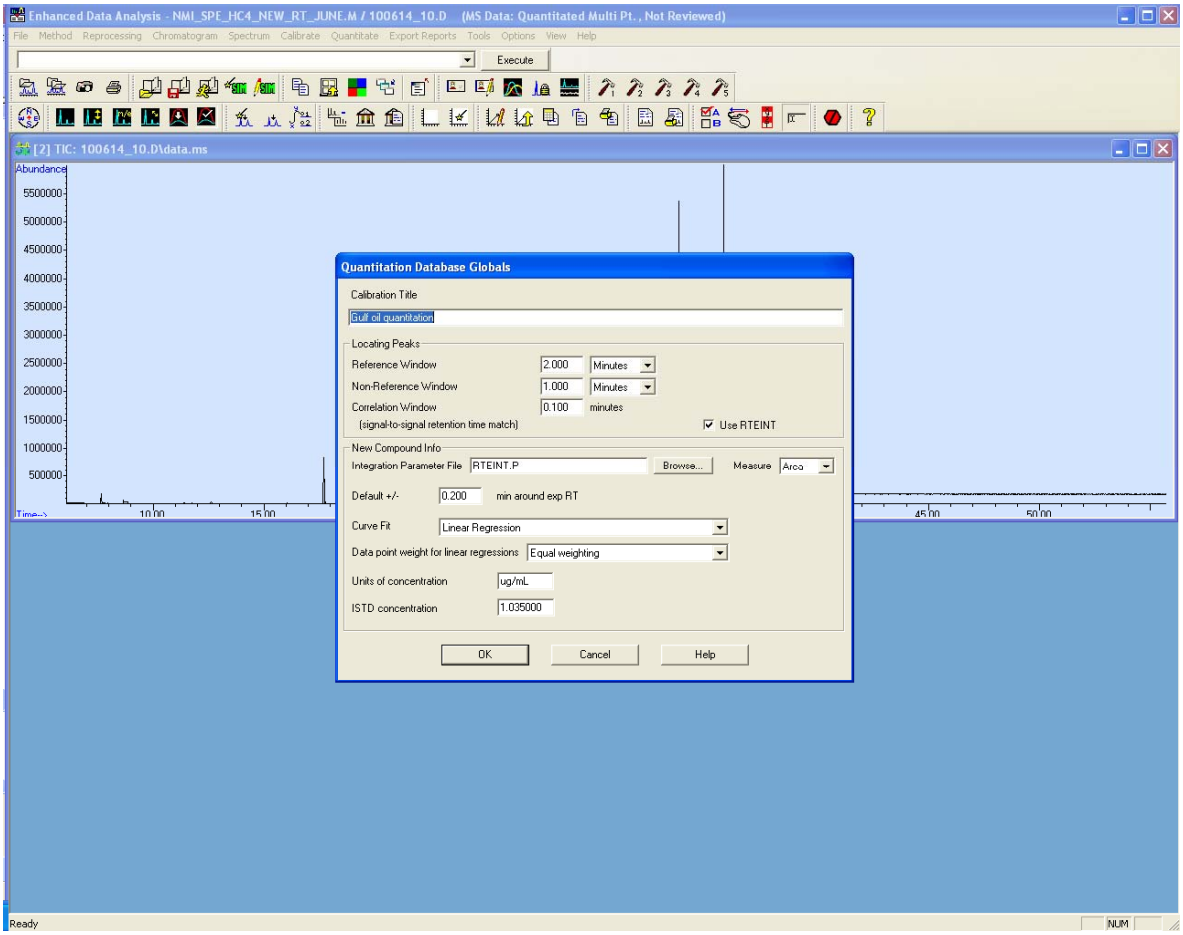
Open Chemstation, choose and load a file that contains all standards that will be used for the quantitation. Normally a low to medium level standard is chosen to adjust the integration parameters making sure all peaks are integrated correctly

Setting up Quantitation method

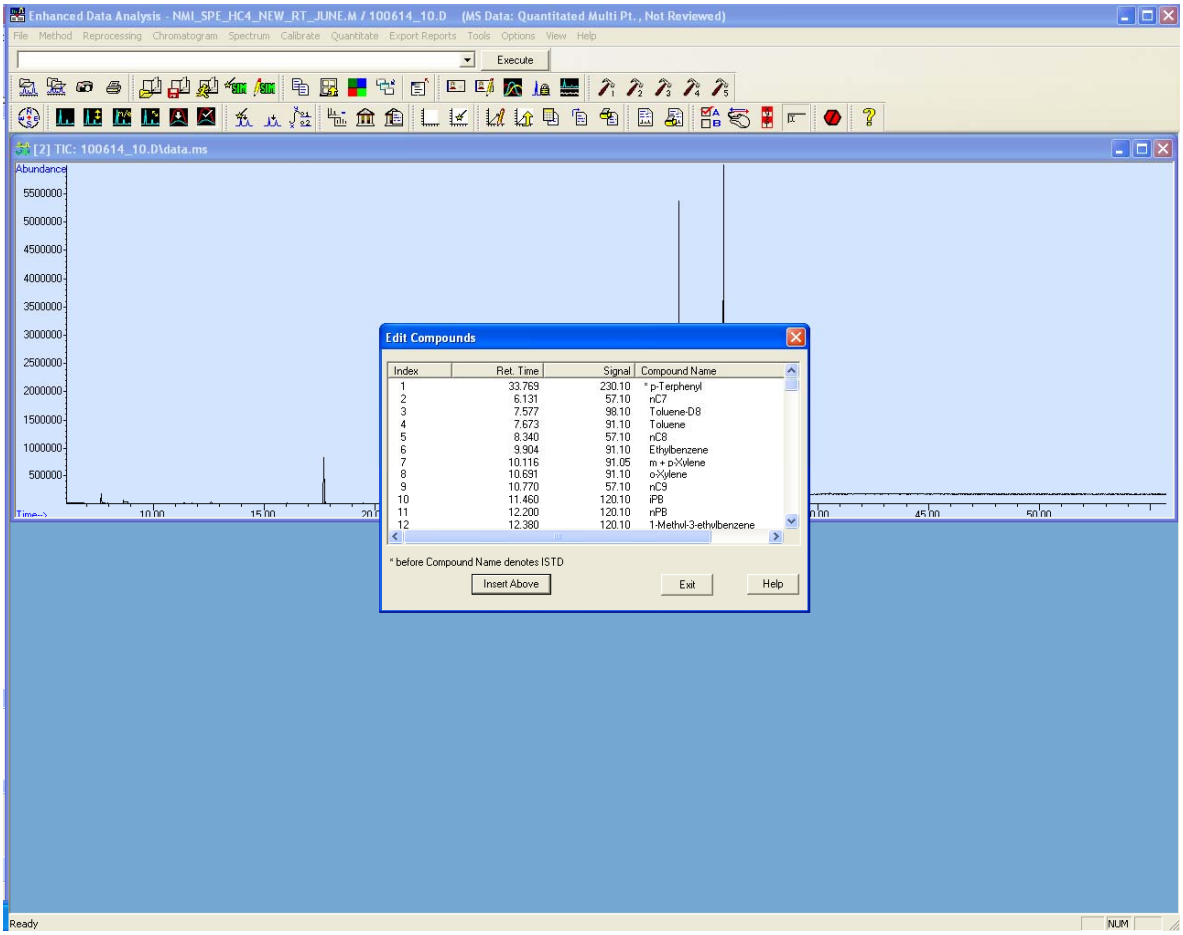
Select “Calibrate” and choose “Set up quantitation”



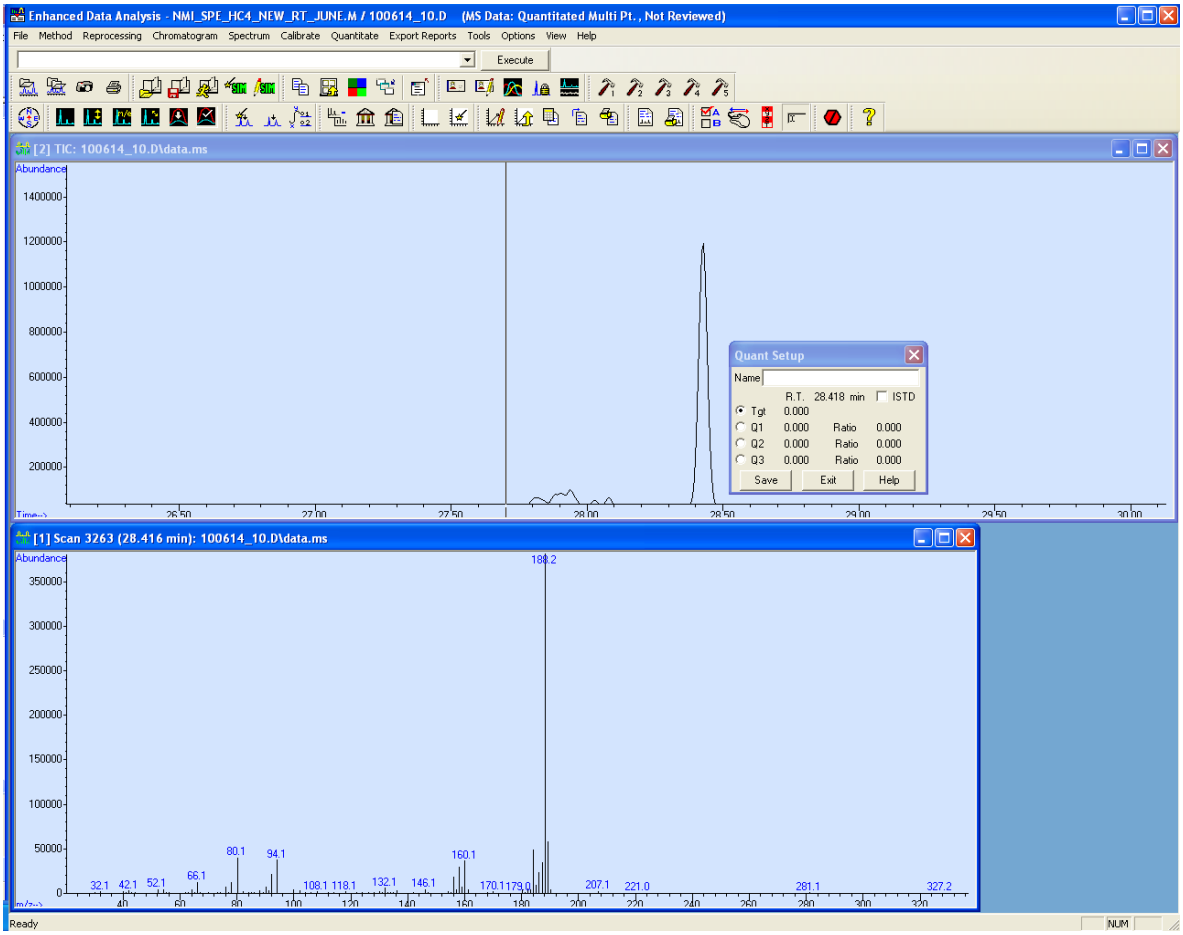
Set your locating peak conditions, enter the units of the internal standard and the amount of ISTD. Give a title to the Calibration.



The internal standard used for quantitation must be the first one on the list. If ISTD is not the first compound then Chemstation will not recognize it and quantitation will not be possible.

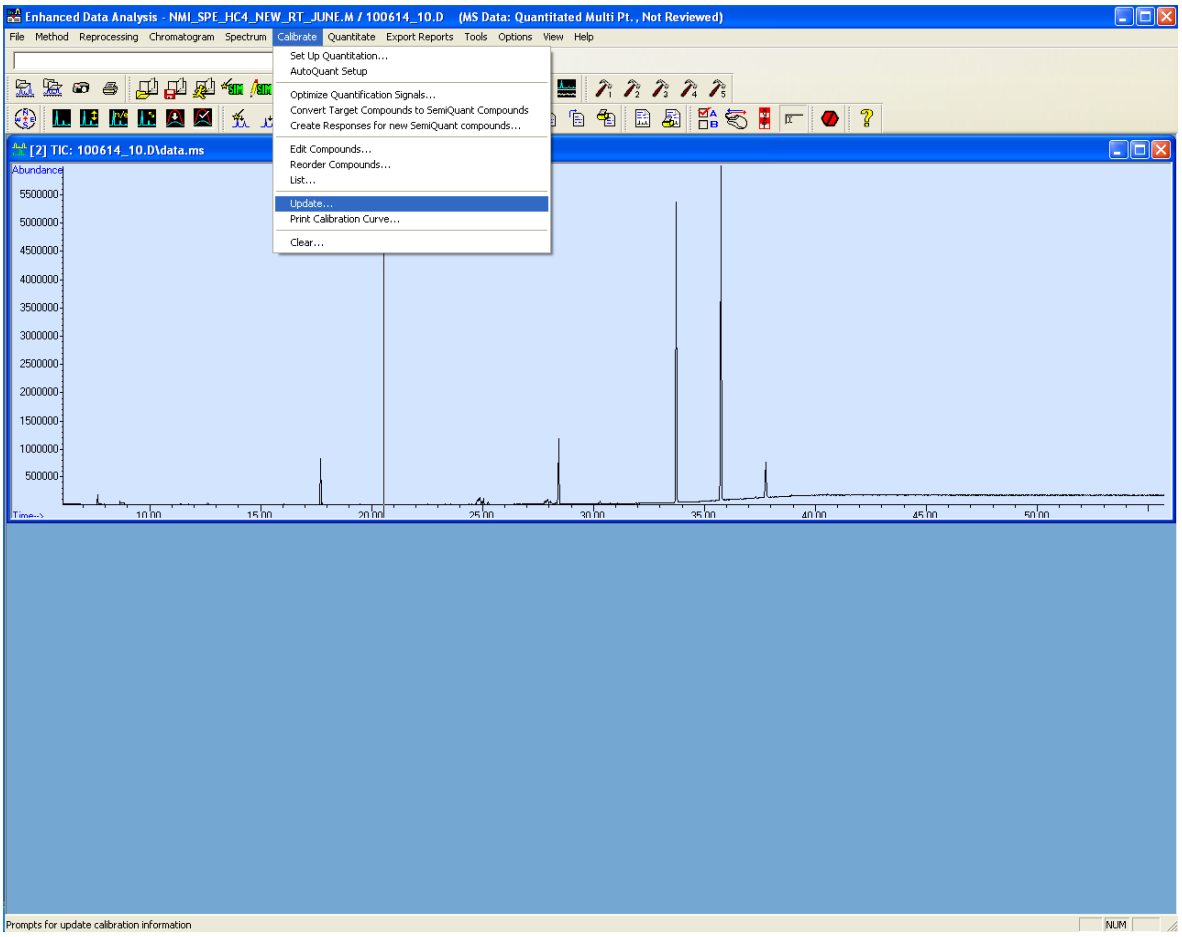


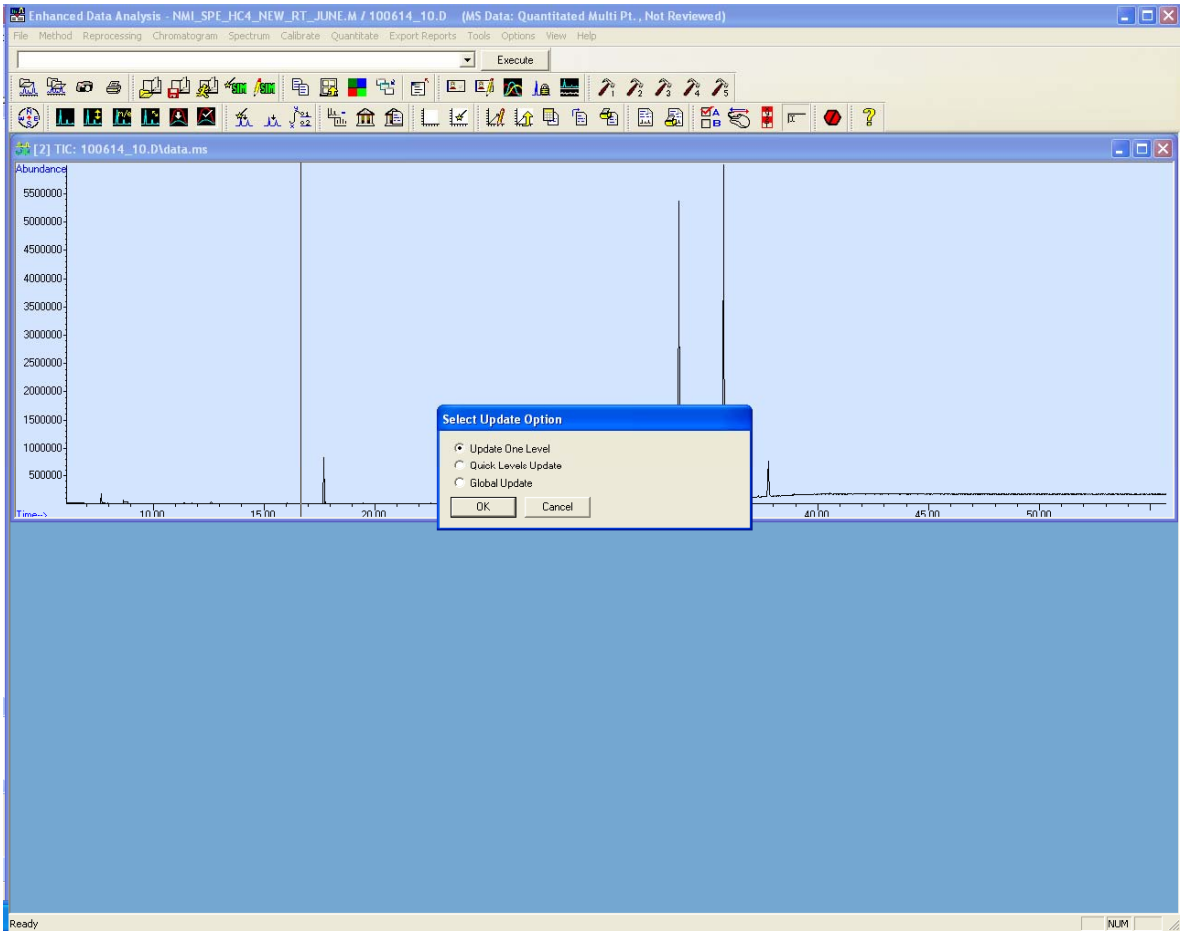
Choose “Insert Above” Enter the name of the compound and right click on the corresponding peak. A spectral window will appear. Click on the target and qualifier ion for the compound. Click “Save” and the peak information will be entered into the compound list. Repeat this process for each compound of interest



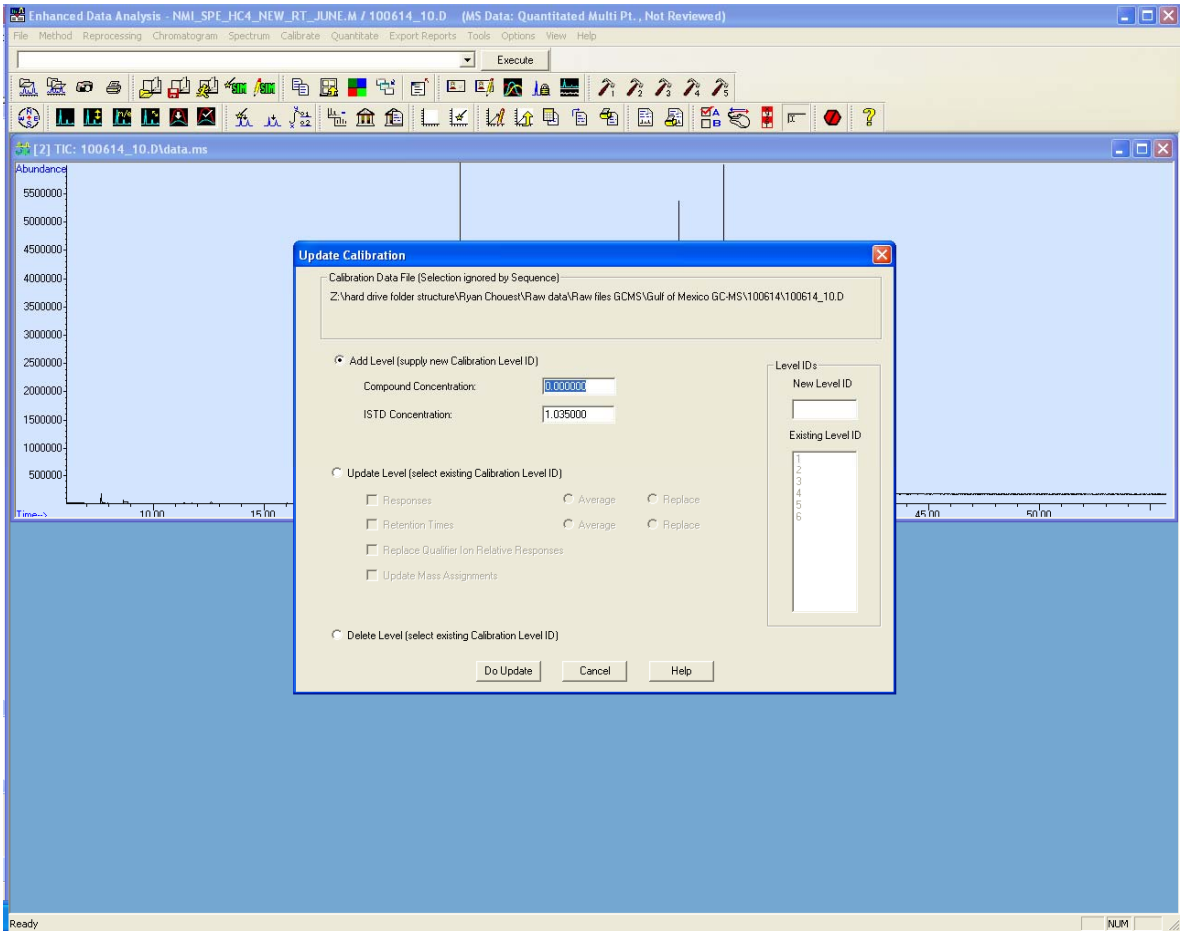
Once all compounds have been entered a calibration level update can be performed.

To calibrate, choose and open a file with the lowest standard concentration and update the level as described below

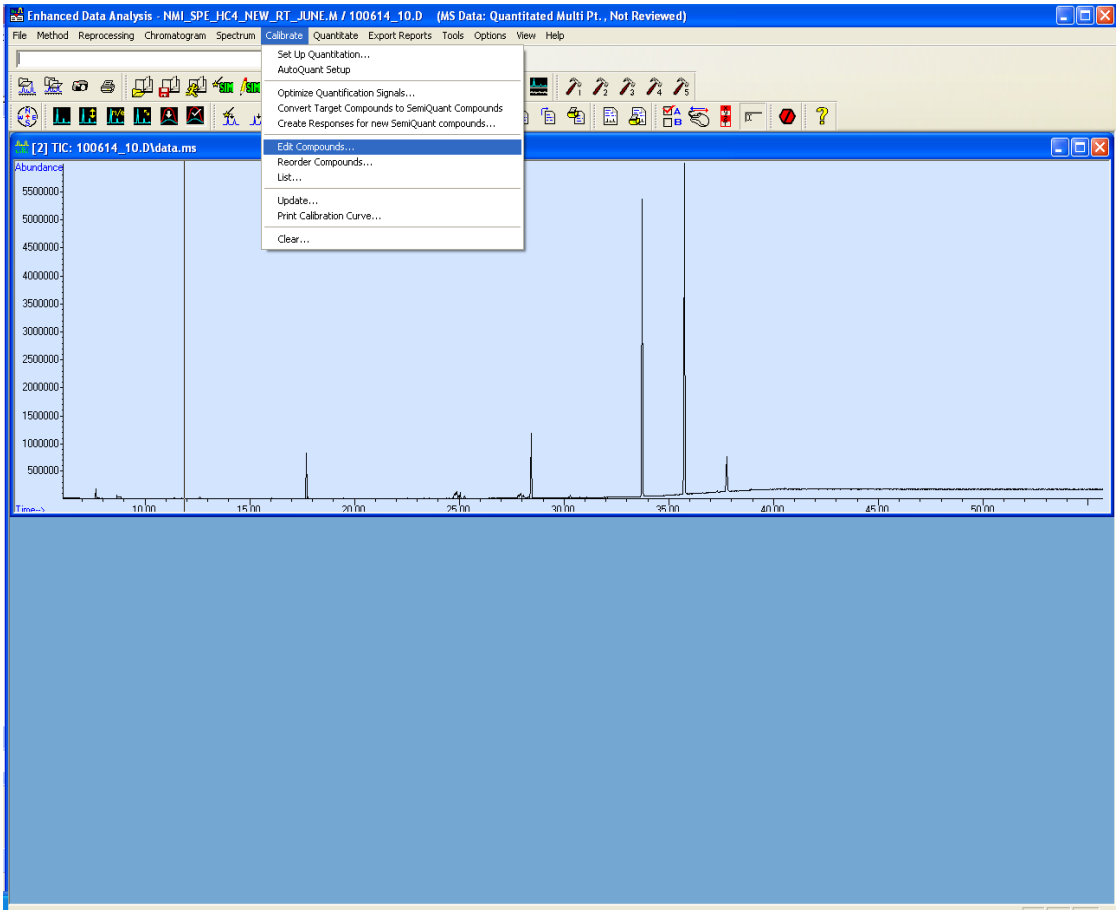



Enhanced Data Analysis - NMI_SPE_HC4_NEW_RT_JUNE.M / 100614_10.D (MS Data: Quantitated Multi Pt. , Not Reviewed)
File Method Reprocessing Chromatogram Spectrum Calibrate Quantitate Export Reports Tools Options View Help
Execute
[2] TIC: 100614_10.D\data.ms
Abundance
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
10.00
15.00
20.00
40.00
45.00
50.00
Select Update Option
Update One Level
Quick Levels Update
Global Update
OK
Cancel
Ready
NUM

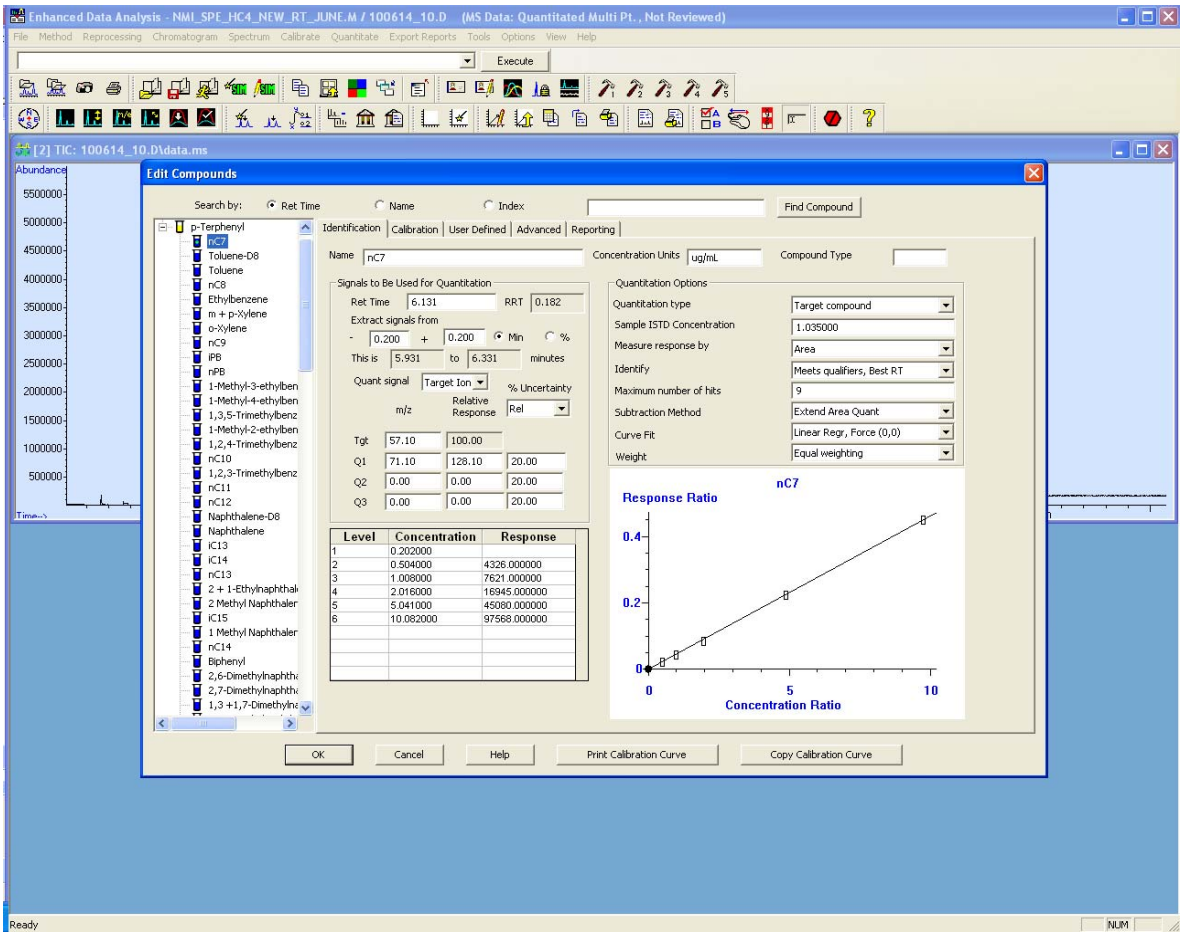
Update the calibration level. Once this is done, repeat the calibration process for all the levels required and check the calibration curves under “Edit compounds”



Editing quantification method



On this screen more compounds can be manually added. All compounds entered come from a file where a standard or sample containing all analytes are found. In this window, retention time windows, target ions and other parameters are added. Also the calibration curves for each compound can be viewed. If the calibration curve does not fit the standards, try a different type of curve fit. All curves are forced through zero.



For quantitation of compounds without a standard, a standard of similar chemical behaviour was chosen, and the same calibration curve was used. For Example there was no standards for methyl naphthalenes so the chosen standard was Naphthalene.

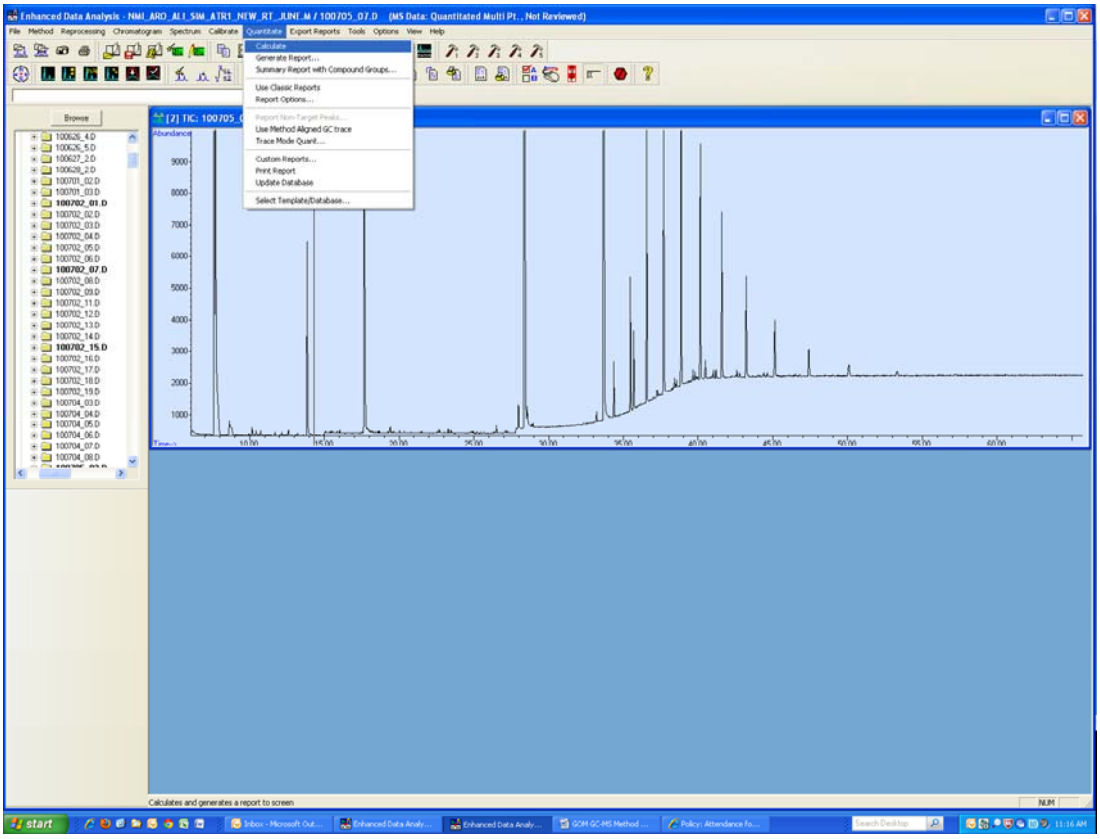
Quantitating a data file

If the Retention Times (RT) of the compounds have changed due to a column maintenance or other parameter, they need to be adjusted.

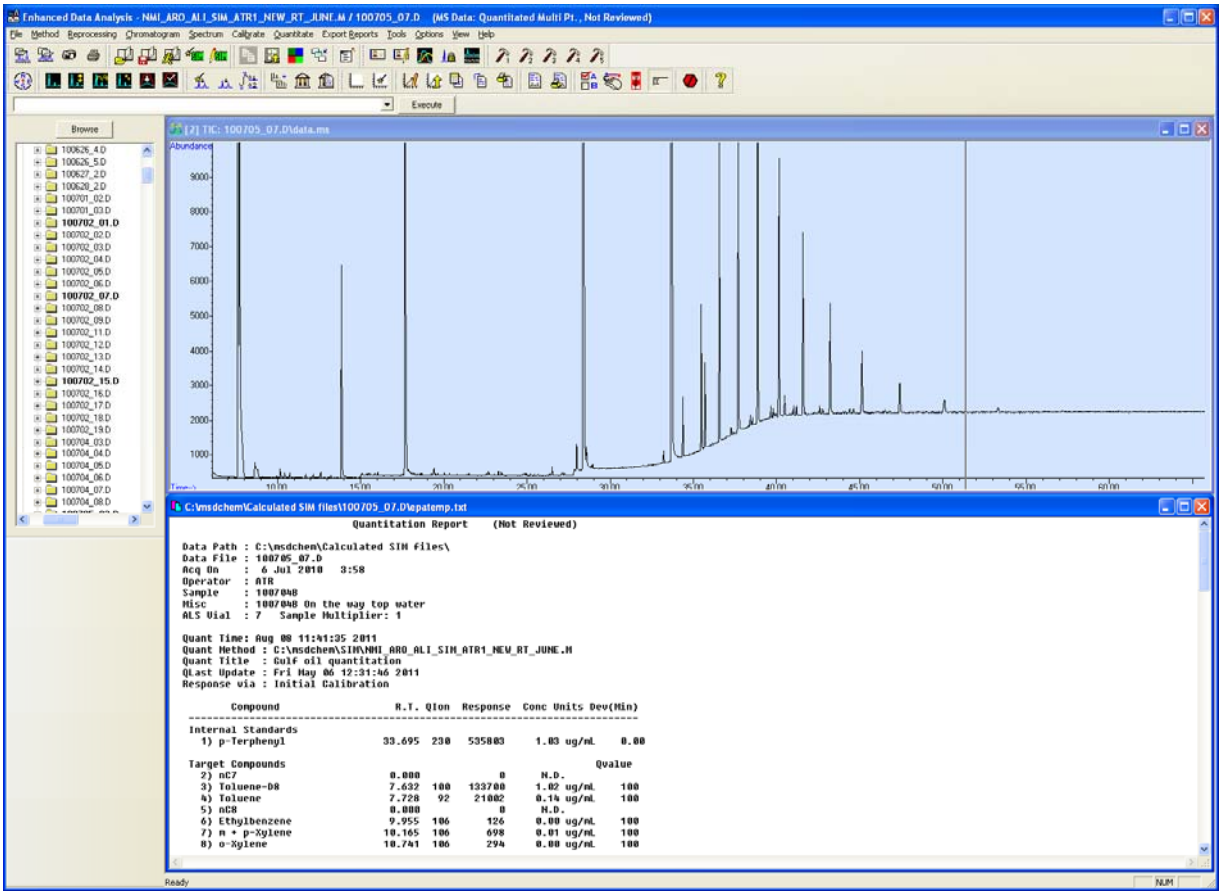
It is preferable to open a more concentrated sample for easy identification of compounds.

Load the method created before for the calibration. To check that the RT fit the right peak, you'll need to run the method like a normal calculation

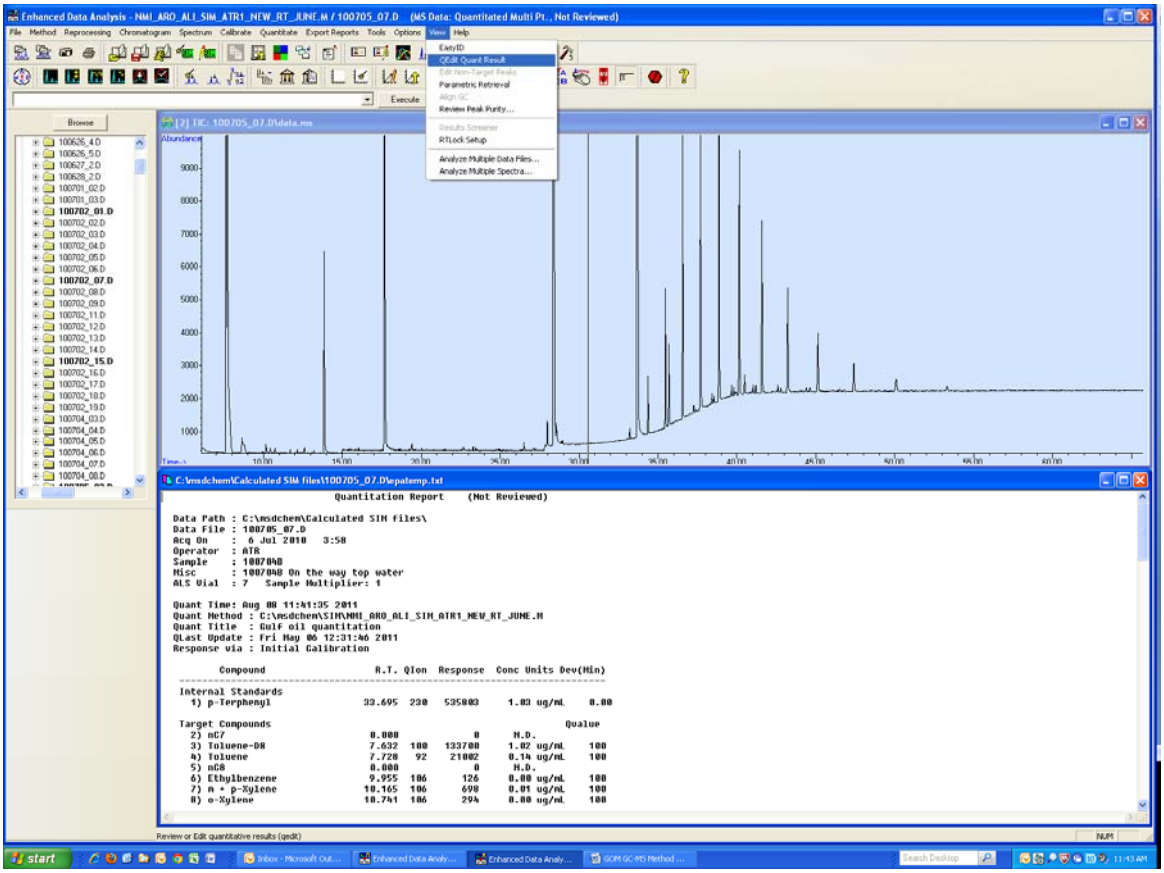
Click on "Quantitate", then "Calculate"



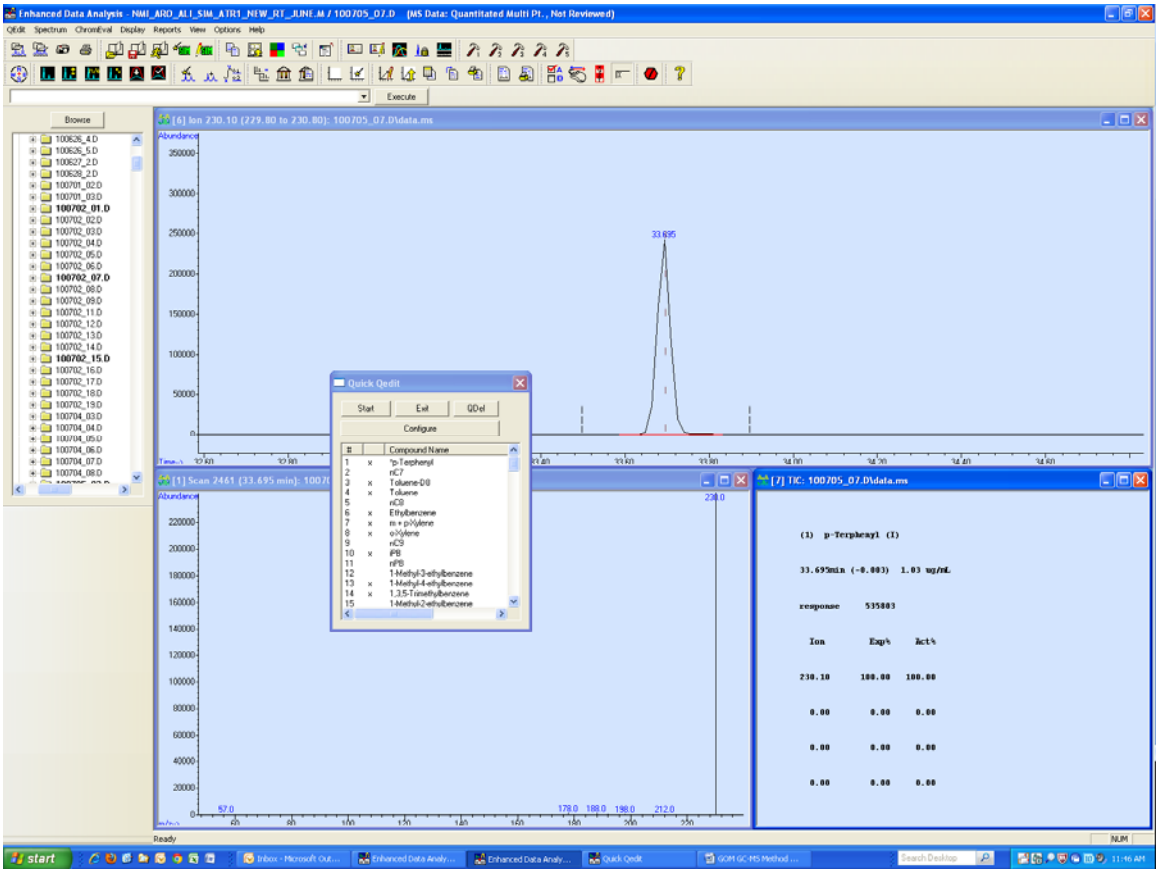
A new window with the list of results will appear.



To check the integration of each peak, click on “View” and “Qedit Quant Results”. This process should be carried out for each peak in each sample.



The following window will be displayed.



The small Quick Qedit window in the middle lists the compounds in the method.

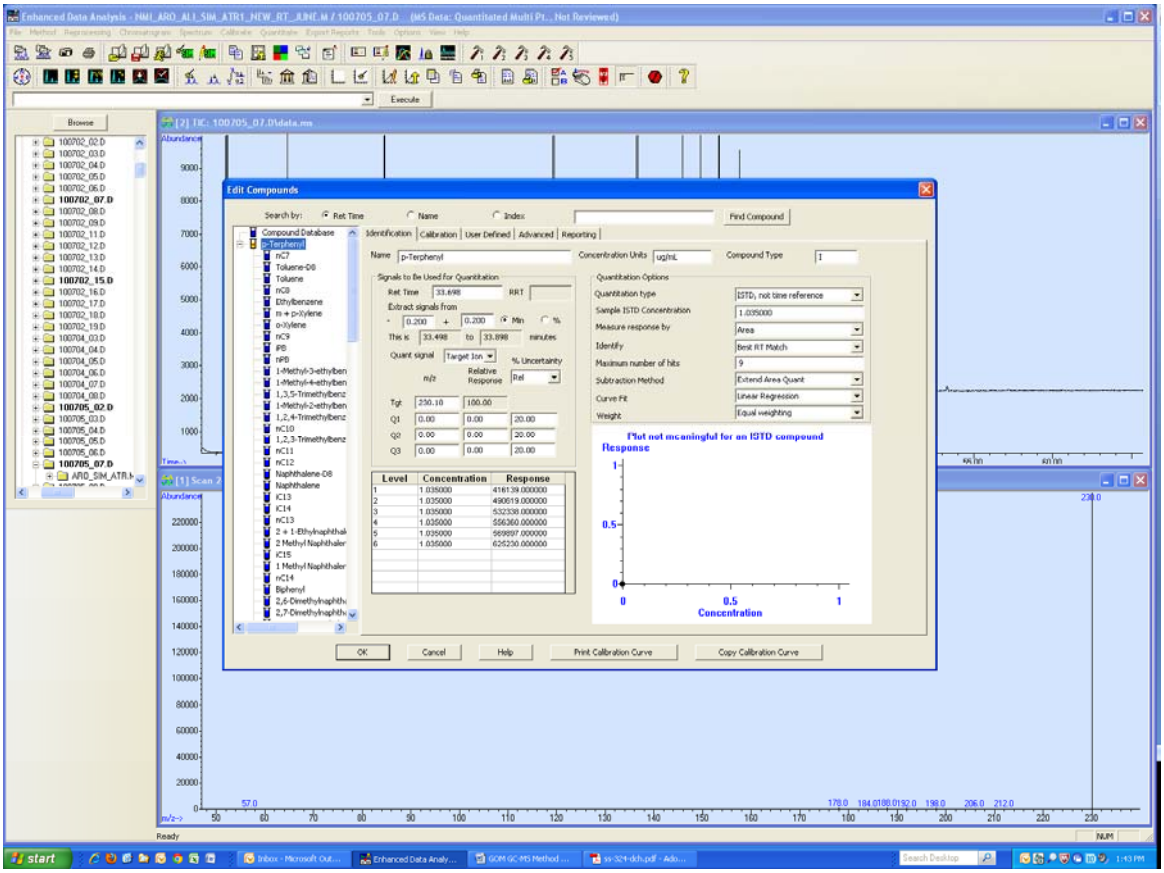
The integrated peak (if found) is in the top window [6]. The 3 dash lines are the RT window width where the peak should appear (fixed in “Edit Compounds”). The spectra of each compound is given in the bottom left window.

To check every compound, ‘double click’ on the bottom right window [7] to go down the list or select one from the list in the Quick Qedit window.

As you go down the list, check that each peak is appearing in the right RT window, otherwise note what the new RT is. It will need to be changed again in the “Edit compounds” section.

When finished, click “Exit” and don’t save yet if you need to change some RT.

To change the RT, click on “Calibrate” and “Edit Compounds”.

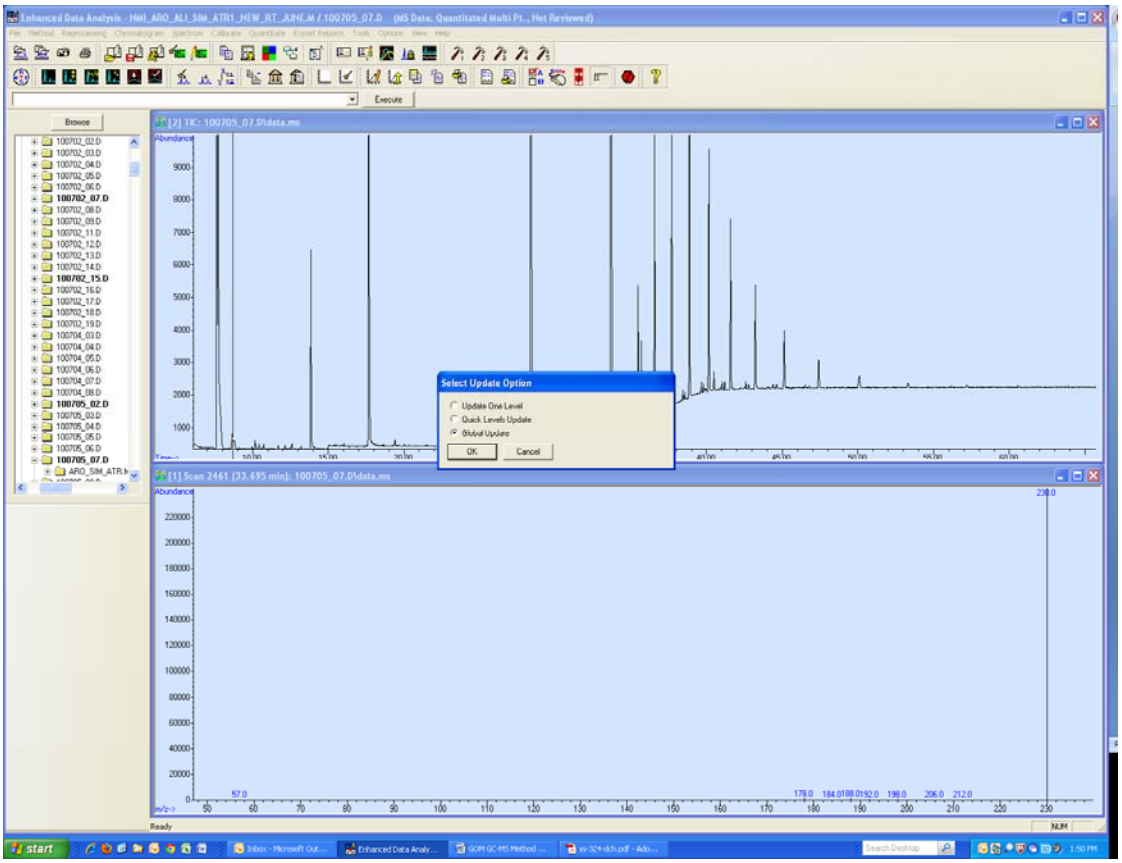


If you click on the + on p-Terphenyl, the RT time for each compound can be adjusted. Note that you can change the width of the RT window to find the peak by changing “Extract signals from”. In this project, they have been set at - 0.2 to + 0.2 min. Save the new method and give it a name.

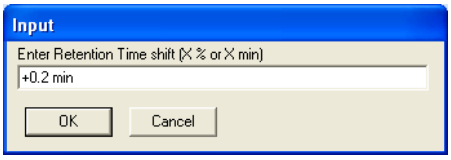
Important note: on this “Edit Compounds” picture, you can see that on the right you can check what is the “Sample ISTD Concentration”, which is the concentration of Internal Std (here #1 p-Terphenyl). This number is to be set for the whole batch or changed. It can be globally set Calibrate>update>global update>set other (by command) and type “Istd_Amt=1.035” (for example), and save.

When maintenance on the GC column is carried out, every RT will be changed. You can do a global update of all the RT:

Click on “Calibrate” then “Update…”

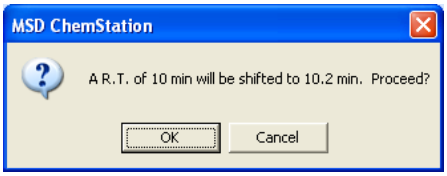


Choose “Global Update”, and then choose “Shift Retention Times”. That is where the time shift for the global RT can be changed, for example:



Note: NO space after + or –

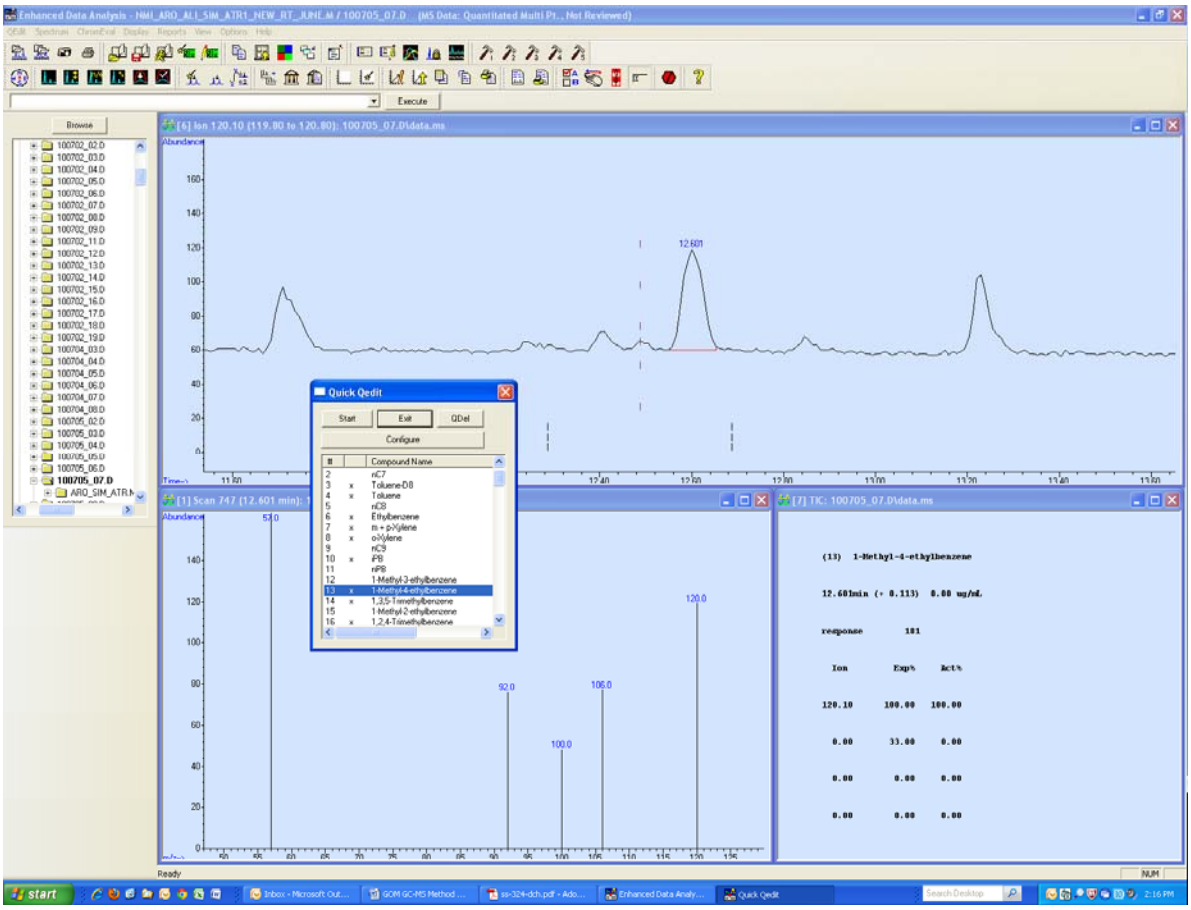
It will prompt you, to confirm:



When completed, save the new method under a new name and recalculate your sample to check.

Final Results

After doing “Calculate”, click on “Quantitate”, click on “View” and do the “Qedit Quant Result”. To go through all the compounds to check the peak identification and selection, you can press “Start” and all the compounds will animate and be displayed one every second. If you see a problem, press “Stop” and come back to it to check the selection (by double clicking the left button – double click on the right goes forward down the list).

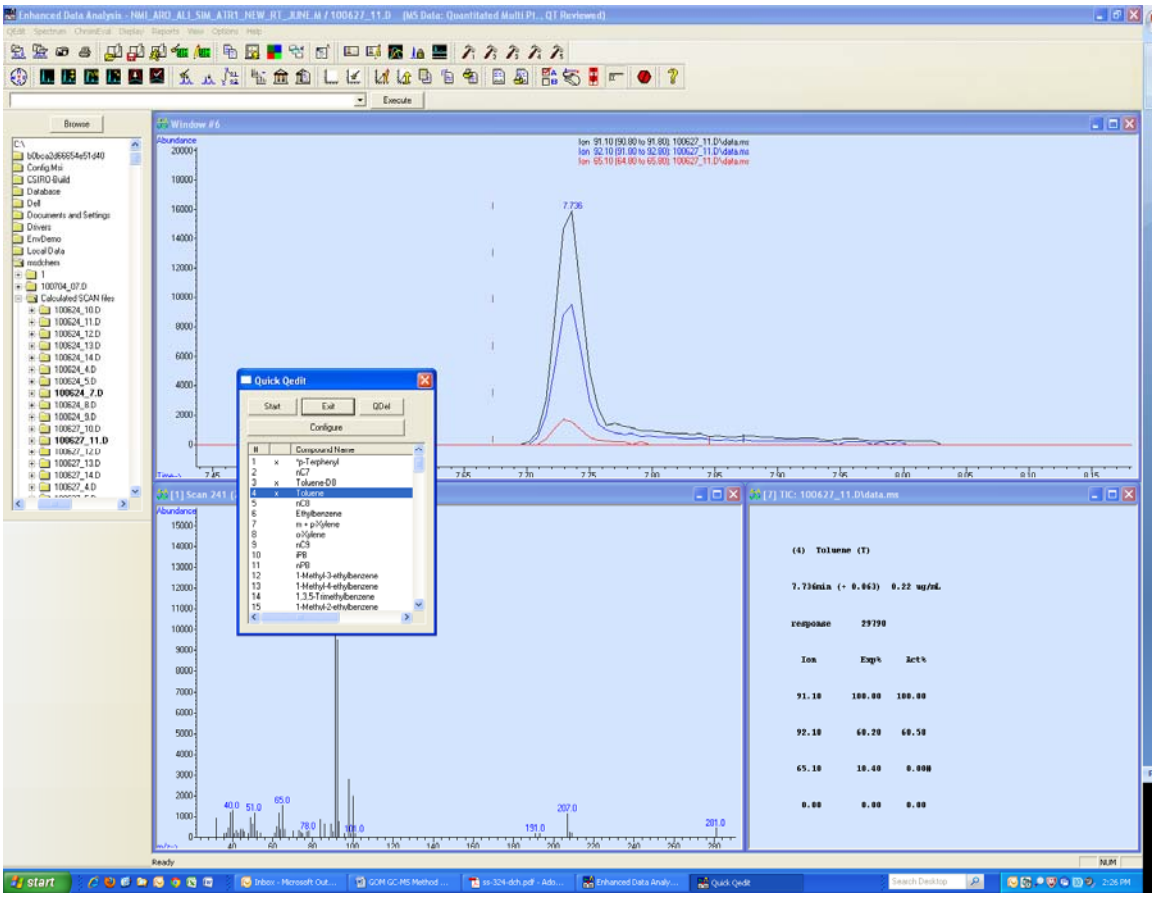


A peak can be zoomed in by clicking and dragging with the left button.

To integrate the peak, click and drag at the base of the peak with the right button, a red line will appear.

Window [7] shows all the integration details of the selected peak. Un-zoom by double clicking on the graph with the left button.

Note that in SIM mode you will only have one peak to check (one ION selected), compared to the SCAN mode where there will be few Ions chromatograms to check:

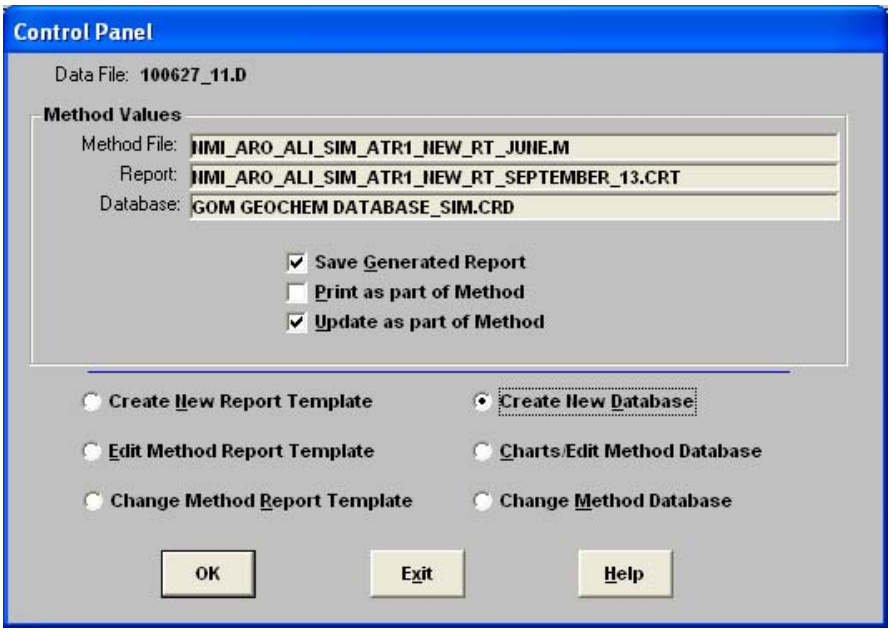


Once finished, click “Exit” and Save. The file will now have in memory the calculated results and peak selection. It is worth keep this file separate from the raw one.

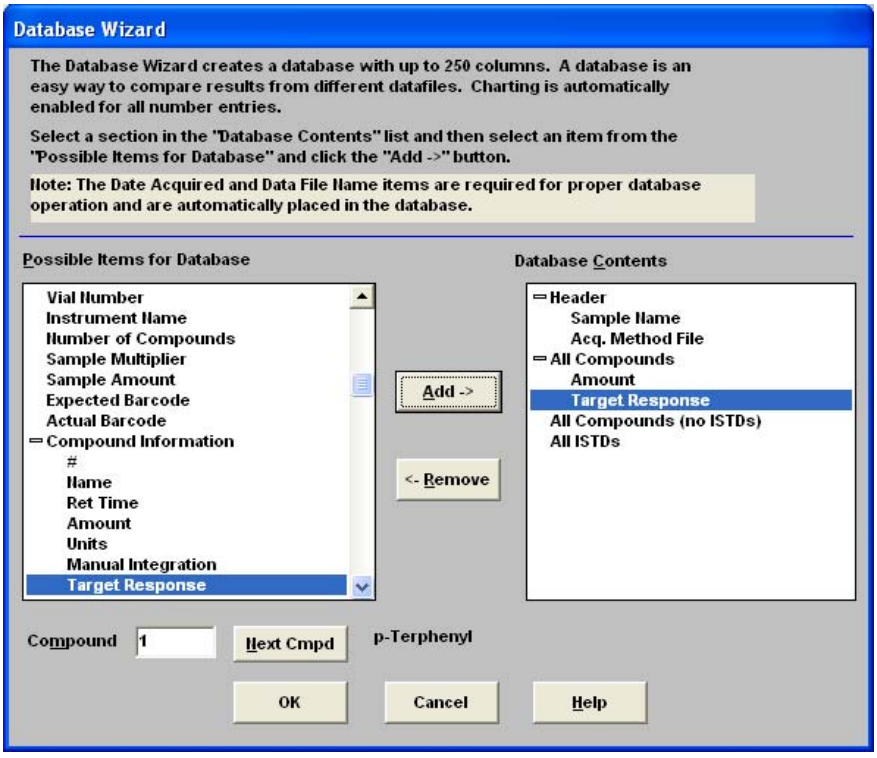
Make sure you keep the different calculating methods under an accessible folder so they can be re-opened in the future if needed.

To save the results, a “Database” and a “Custom Report” need to be created.

Click on “Quantitate” then “Custom reports…”



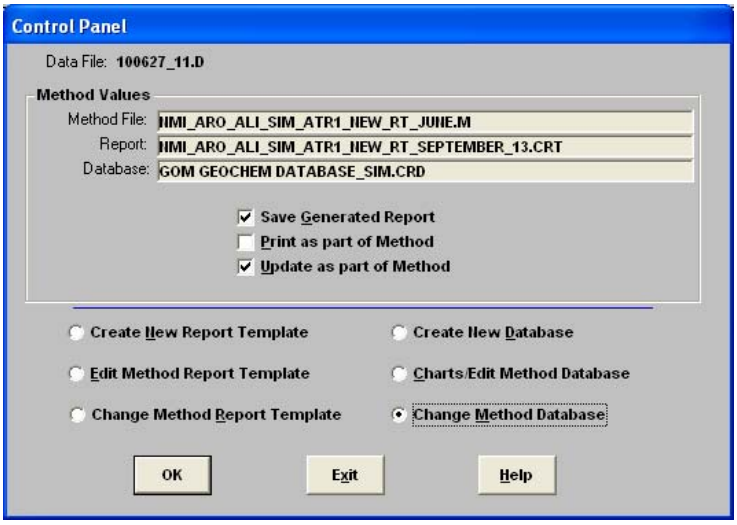
Click on “Create New Database” on the right and “OK”



Under “Header”, add “Sample Name” and “Acq. Method File” to the right. Under “All compounds” add “Amount” and “Target Response”. Press OK and save. Note that this format of database is a horizontal table with a limited number of columns. Each compound will have a parameter (here: Amount and Target Response) so it is limited in details you can save.

This Database table is opened only by Chemstation and should be saved as an Xcel sheet. At each calculated sample, it can be updated and a line is added at the bottom of it.

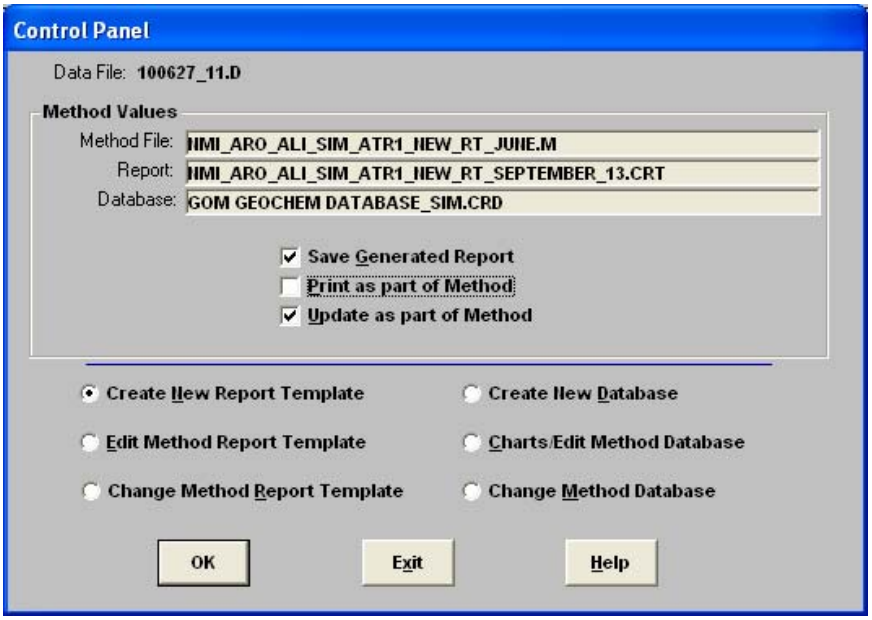
Save the Database with a name, and it can be recalled so you can be used as a template:

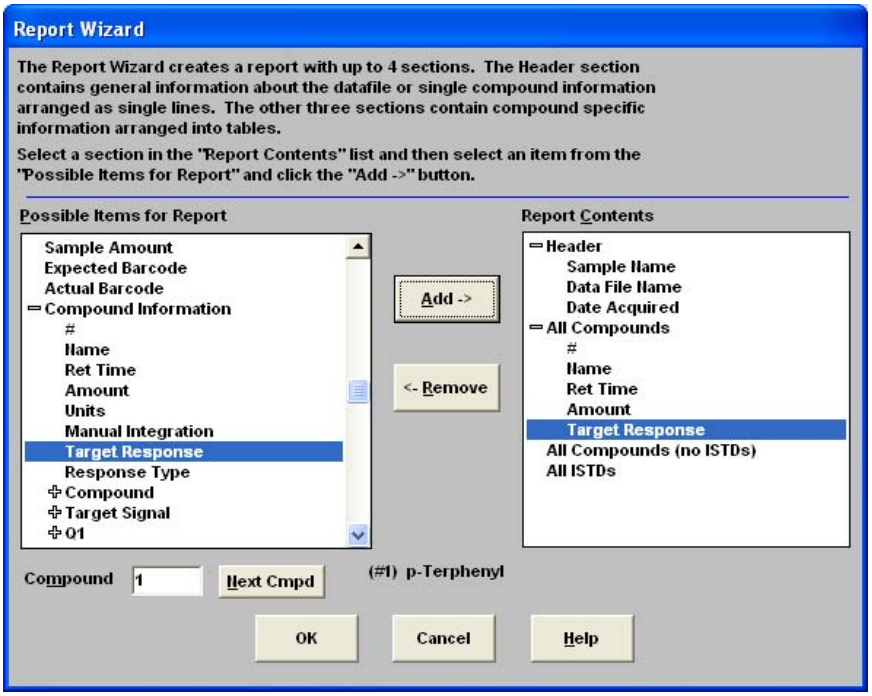


Otherwise you’ll have to change to another template

To Create a New Custom Report Template:

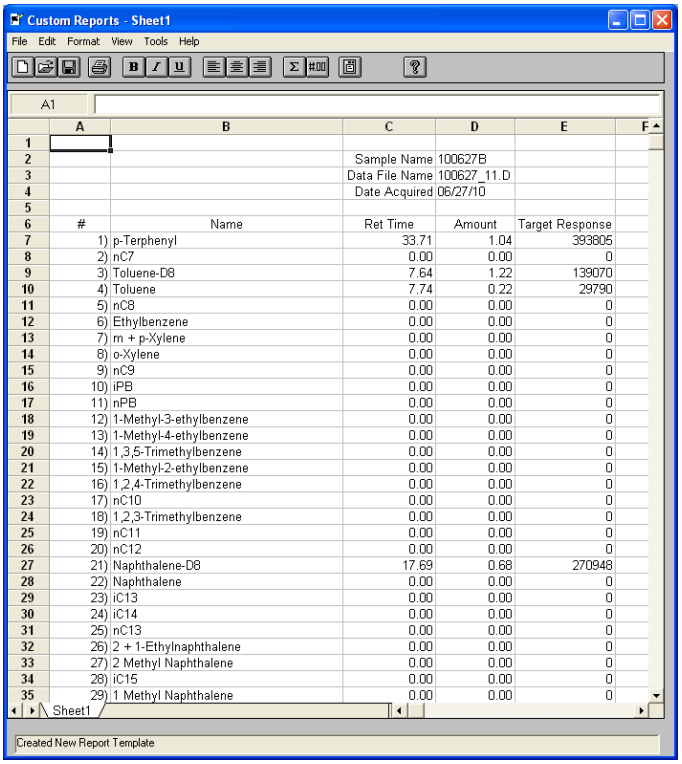
Click on “Quantitate” then “Custom reports…”, then on “Create New Report Template”, Ok.





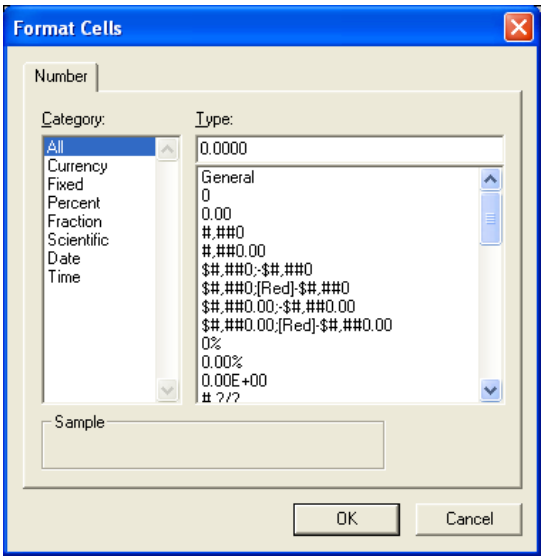
In the same way, add from the left parameters under “Header”: “Sample Name, Data File Name and Date Acquired”

“All Compounds”: “# (number of peak), Name, Ret Time, Amount and Target Response”

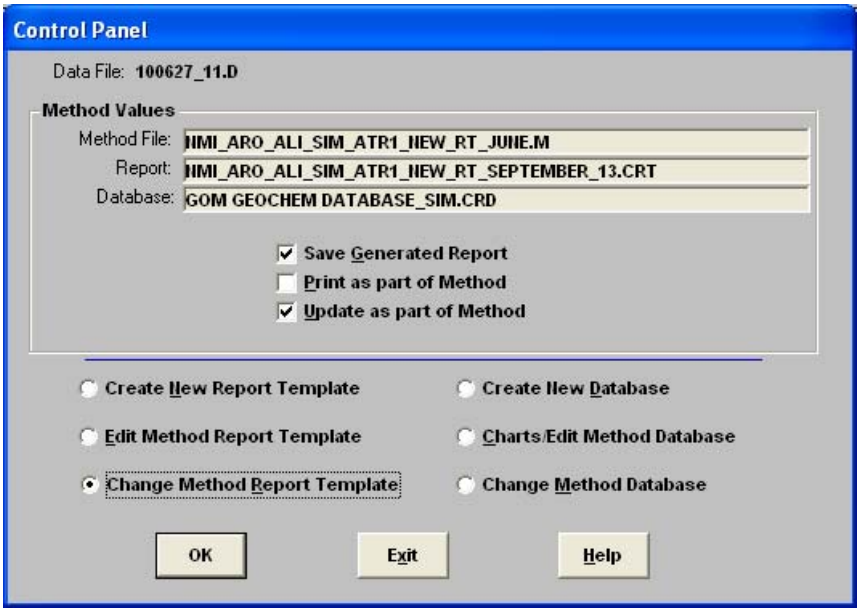


The Custom Report will look like this and can be copied into an Xcel worksheet. There is one report per GC run, so it is possible to add many parameters on it compared to the Database which has only 2 possible (limited by number of columns).

At this stage it is important to set the decimal places you are interested in having your results. Select the “Amount” column and click on the tab with “#.00” then type 0.0000 if you want 4 decimal points.



Save it with a name, and it can be recalled so you can use it as a template:



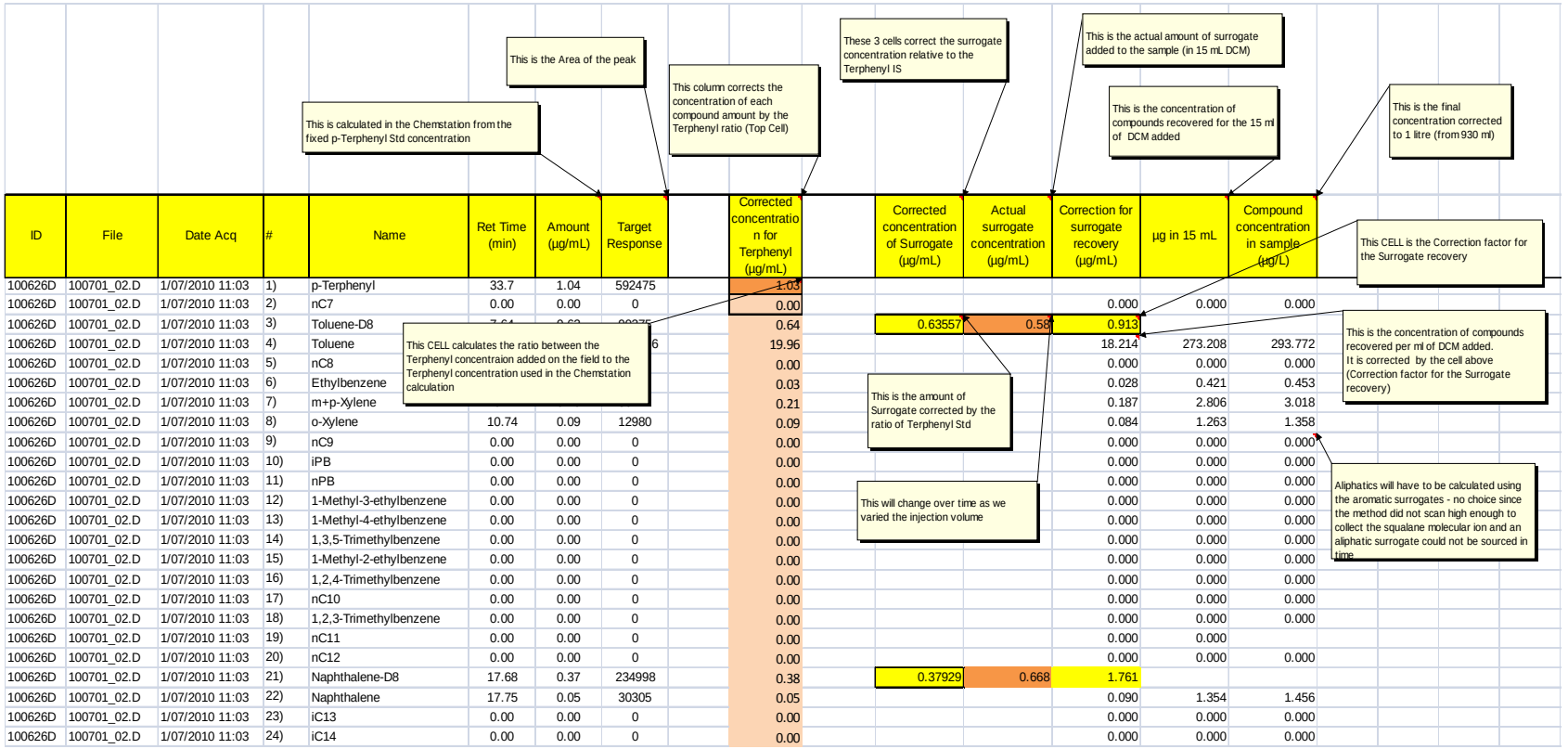
Otherwise you'll have to change to another template

Calculation correction for final results

Because of different amounts of p-Terphenyl internal standards used on the field compared to the calibration curves done in the laboratory, it was necessary to correct for the discrepancies.

Each sample was corrected to account for the difference in the internal standards. From this value, the surrogate concentrations were calculated and these were used to correct for analyte recovery.

A Sample calculation is given below



ID	File	Date Acq	#	Name	Ret Time (min)	Amount (µg/mL)	Target Response	Corrected concentration for Terphenyl (µg/mL)	Corrected concentration of Surrogate (µg/mL)	Actual surrogate concentration (µg/mL)	Correction for surrogate recovery (µg/mL)	µg in 15 mL	Compound concentration in sample (µg/L)
100626D	100701_02.D	1/07/2010 11:03	1)	p-Terphenyl	33.7	1.04	592475	1.03					
100626D	100701_02.D	1/07/2010 11:03	2)	nC7	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	3)	Toluene-D8				0.64	0.63557	0.58	0.913		
100626D	100701_02.D	1/07/2010 11:03	4)	Toluene			6	19.96			18.214	273.208	293.772
100626D	100701_02.D	1/07/2010 11:03	5)	nC8				0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	6)	Ethylbenzene				0.03			0.028	0.421	0.453
100626D	100701_02.D	1/07/2010 11:03	7)	m+p-Xylene				0.21			0.187	2.806	3.018
100626D	100701_02.D	1/07/2010 11:03	8)	o-Xylene	10.74	0.09	12980	0.09			0.084	1.263	1.358
100626D	100701_02.D	1/07/2010 11:03	9)	nC9	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	10)	iPB	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	11)	nPB	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	12)	1-Methyl-3-ethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	13)	1-Methyl-4-ethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	14)	1,3,5-Trimethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	15)	1-Methyl-2-ethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	16)	1,2,4-Trimethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	17)	nC10	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	18)	1,2,3-Trimethylbenzene	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	19)	nC11	0.00	0.00	0	0.00			0.000	0.000	
100626D	100701_02.D	1/07/2010 11:03	20)	nC12	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	21)	Naphthalene-D8	17.68	0.37	234998	0.38	0.37929	0.668	1.761		
100626D	100701_02.D	1/07/2010 11:03	22)	Naphthalene	17.75	0.05	30305	0.05			0.090	1.354	1.456
100626D	100701_02.D	1/07/2010 11:03	23)	iC13	0.00	0.00	0	0.00			0.000	0.000	0.000
100626D	100701_02.D	1/07/2010 11:03	24)	iC14	0.00	0.00	0	0.00			0.000	0.000	0.000