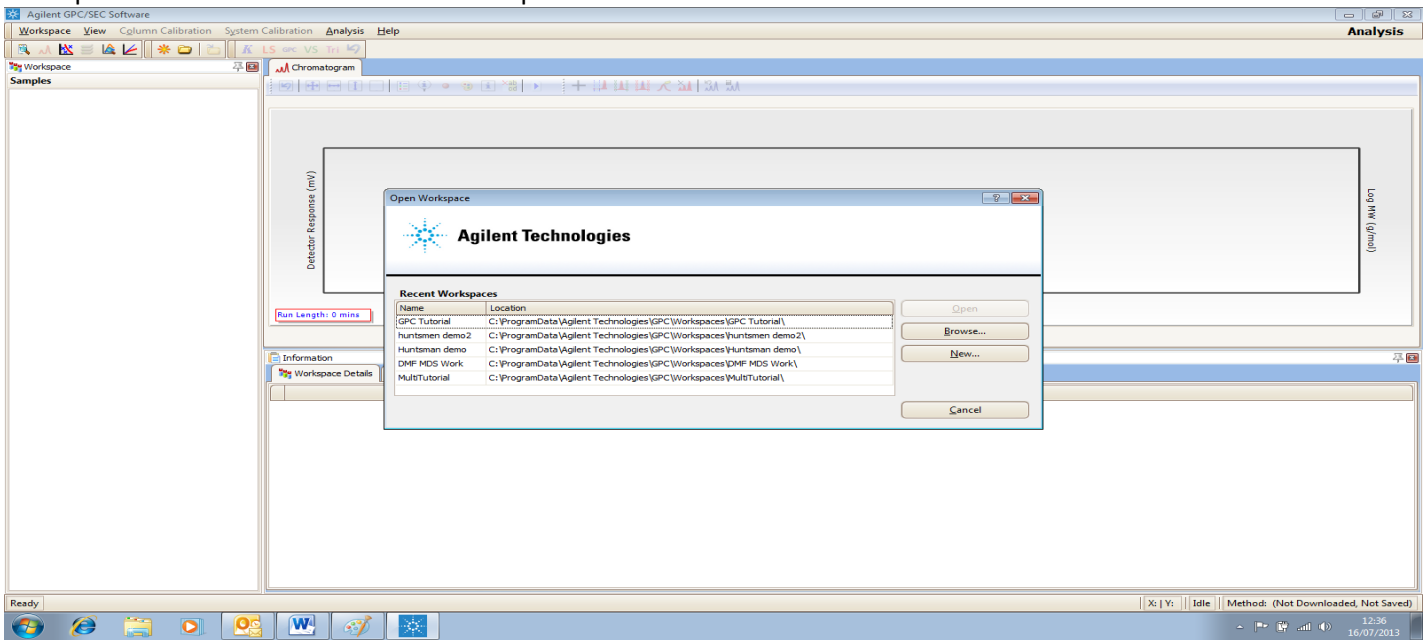
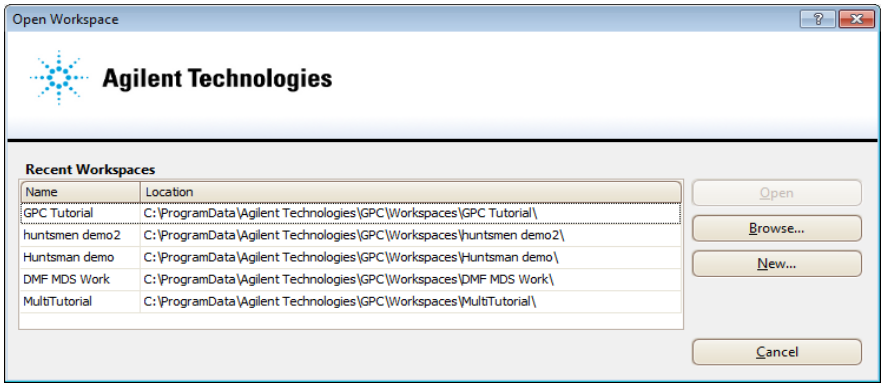
GPC SOP

1) open the Agilent GPC by going to start all programs, then to the Agilent technologies , Agilent GPC and select

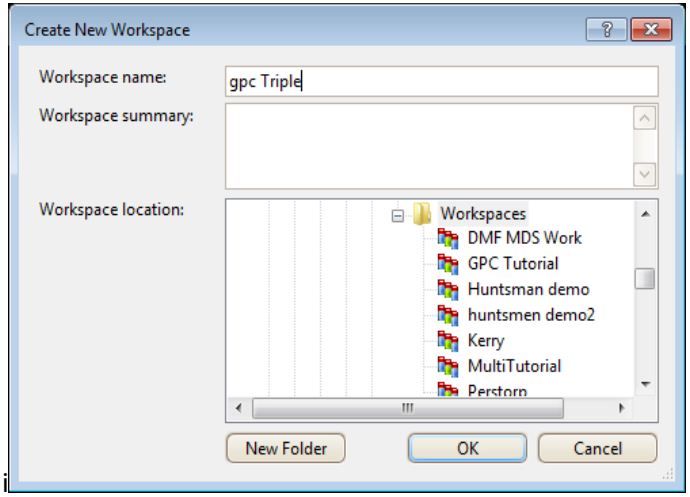
This opens the deafult screen for the workspace



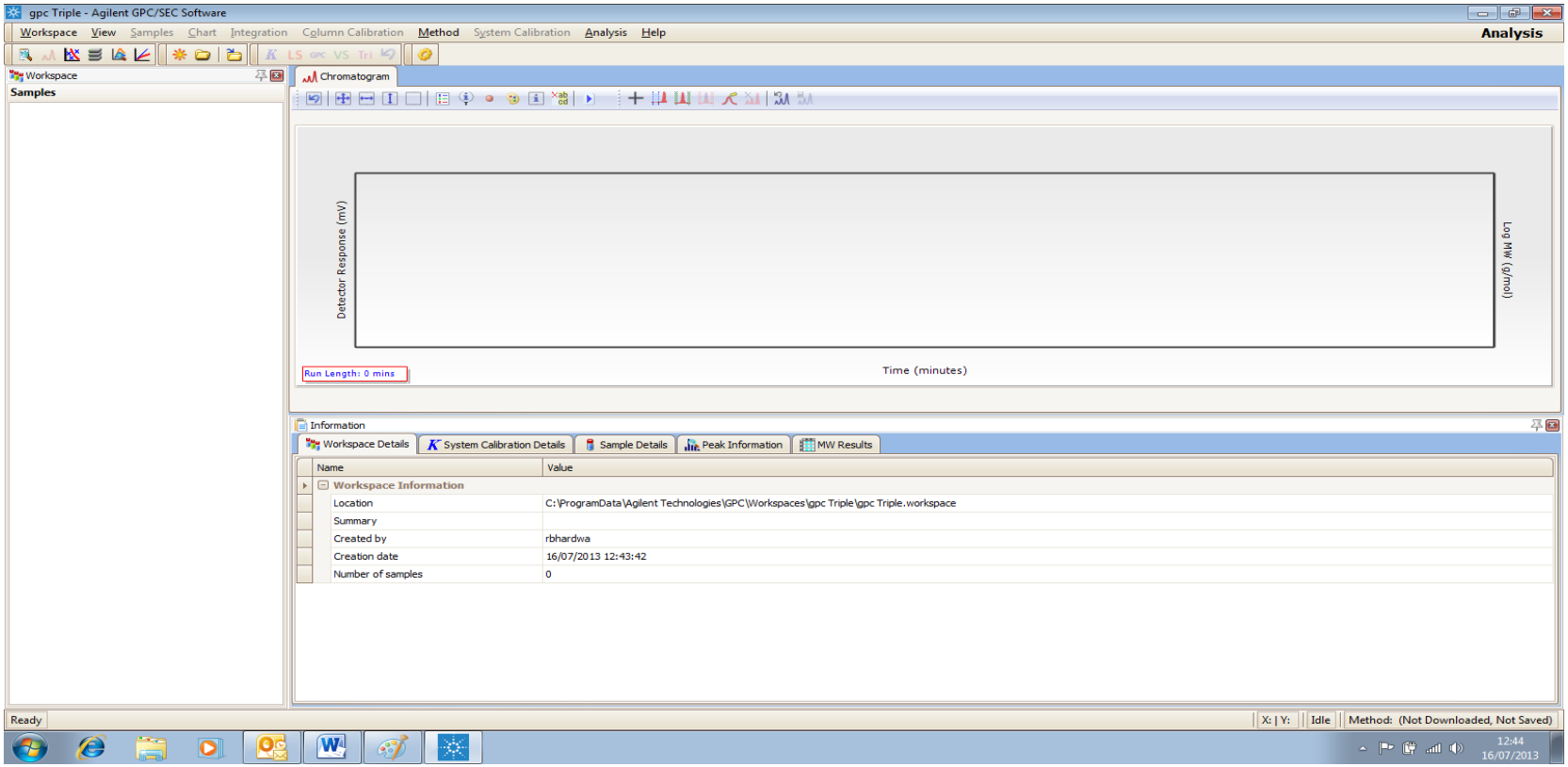
To create a new workspace for your analysis, click the tab New



You will now be prompted with the window below and you can give a name to your workspace e.g. gpc Triple.



Click OK


gpc Triple - Agilent GPC/SEC Software
Workspace View Samples Chart Integration Column Calibration Method System Calibration Analysis Help
Analysis
Workspace
Samples
Chromatogram
Detector Response (mV)
Log MW (g/mol)
Run Length: 0 mins
Time (minutes)
Information
Workspace Details
System Calibration Details
Sample Details
Peak Information
MW Results
Name
Value
Workspace Information
Location
C:\ProgramData\Agilent Technologies\GPC\Workspaces\gpc Triple\gpc Triple.workspace
Summary
Created by
rbhardwa
Creation date
16/07/2013 12:43:42
Number of samples
0
Ready
X: | Y:
Idle
Method: (Not Downloaded, Not Saved)
12:44
16/07/2013

Instrument Control

The *Instrument Control* View is accessed from the *View* menu, Figure 3.

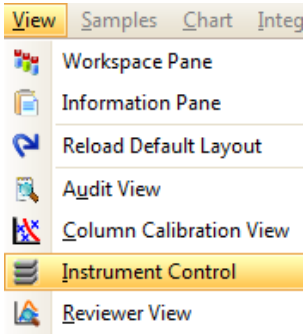


Figure 3 Accessing the Instrument Control view from the menu

Alternatively,

Now Click on the Icon  and this opens the above window. Now select your instrument by high lighting the Icon gpc Triple & OK

This will take a few moments to populate with the instrument control window as seen below (Figure 4).

Select the Instrument to connect to and click ok, upon connection the software will automatically synchronize with the current system parameters.

2.1 Overview of Instrument Control View

The Instrument Control View has two main sections, the **Instrument Dashboard** and the **Injection Sequence**.

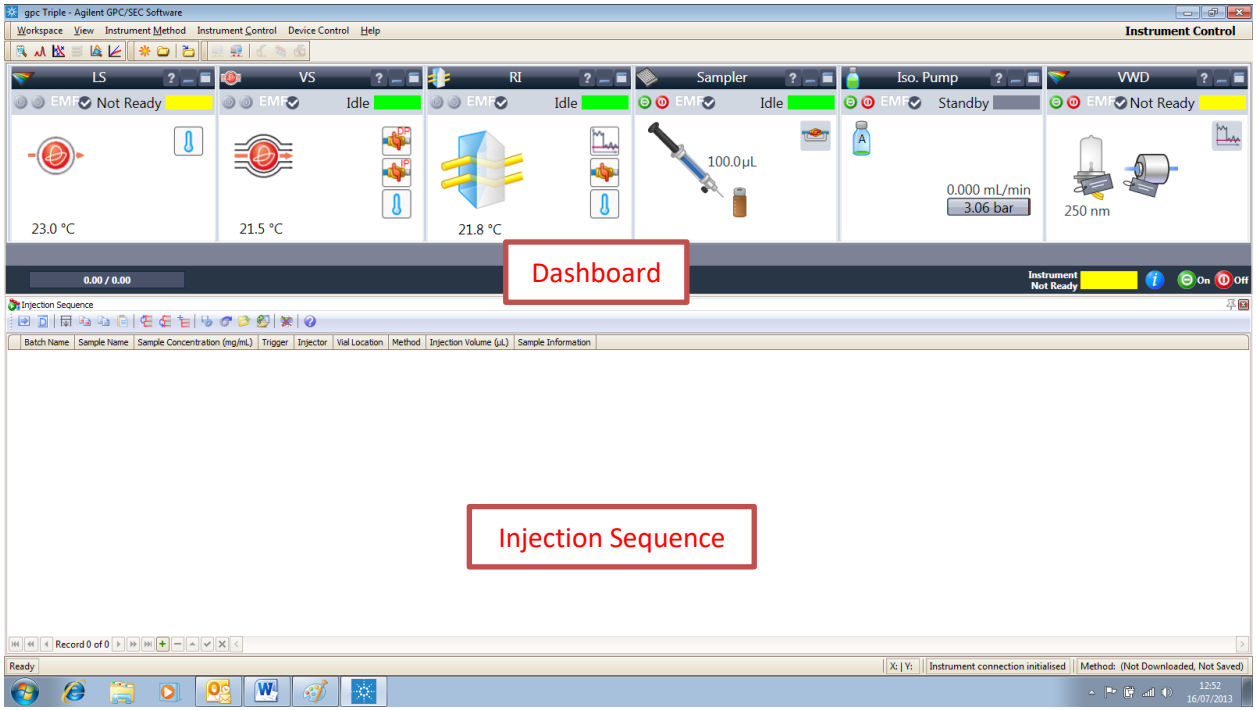
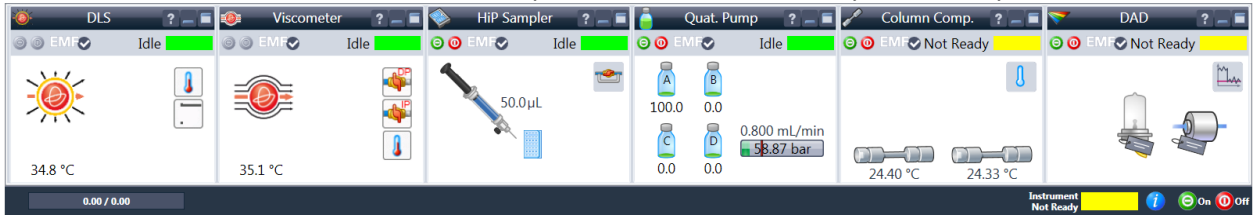


Figure 4

Instrument Dashboard

The Instrument dashboard will show all components of the 1260 Infinity Bio-MDS System with their current status.



The dashboard will highlight the component status with a colored status bar. When in a **Ready** state a green bar will appear, a **Not Ready** state is yellow; if there is an **Error** they will appear red. The global status is shown on the bottom right.

Placing the mouse cursor over the yellow bar will provide information on why the system is **Not Ready**, i.e. if the column oven is heating.

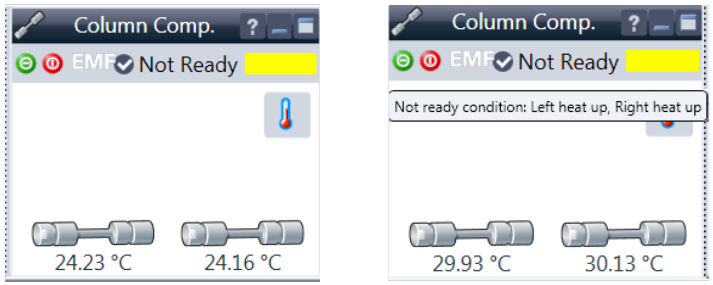


Figure 1 Dashboard tiles in *Not Ready* state

Icons show what action(s) the detector is currently performing:

 Heat off

 Heat on

 UV lamp off

 UV lamp initializing

 UV lamp on

 Laser off

 Laser on

 Purge off

 Purge on

 Balance off

 Balance on

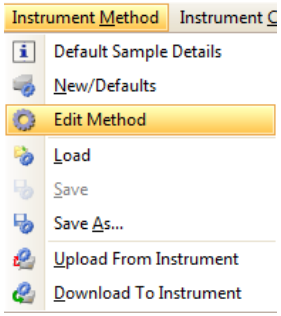
More detailed information can be seen by maximizing the dashboard tiles

Purge the pump line to remove old solvent. (Necessary when changing the solvent/ Bubble in line)

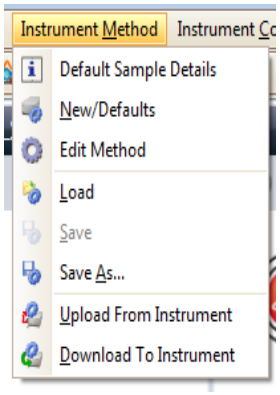
1) Open the pump purge valve anticlockwise.(Only loosen it, don't open it complete.
2) Right click on the Quat pump and give flow of 5 ml, select all 4 ports and increase the flow gradient to 100 from advance menu➔ Click OK.
3) While purging the pressure of the pump should be near to Zero, if pressure of the pump is high during the purge, frit needs replacement.
4) Select port one by one and purge at least for 2 minutes each port.
5) Once purging is done reduce the flow to 0.1 ml then reduce the flow gradient to 0.1

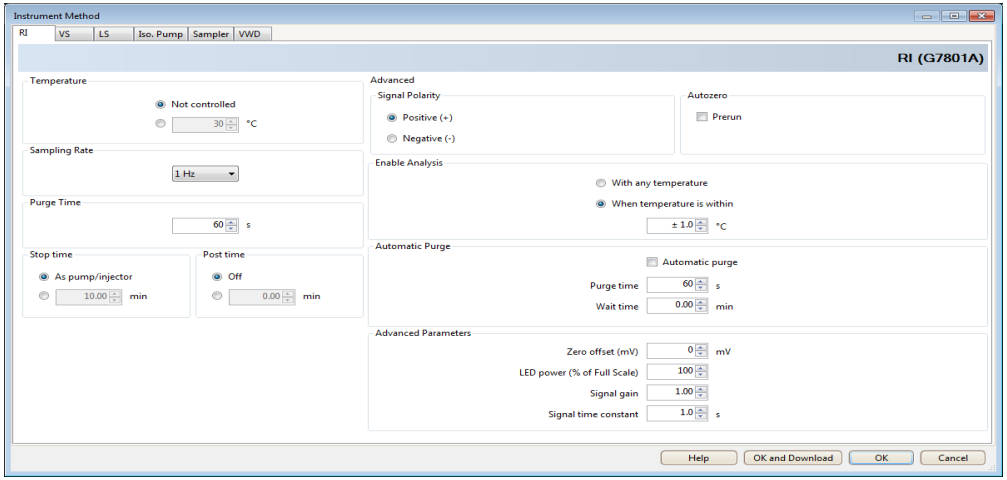
Creating instrument methods/ Loading any existing method.

Upon connection the software will automatically synchronize with the current system parameters. Edit the method from the *Instrument Method – Edit Method*



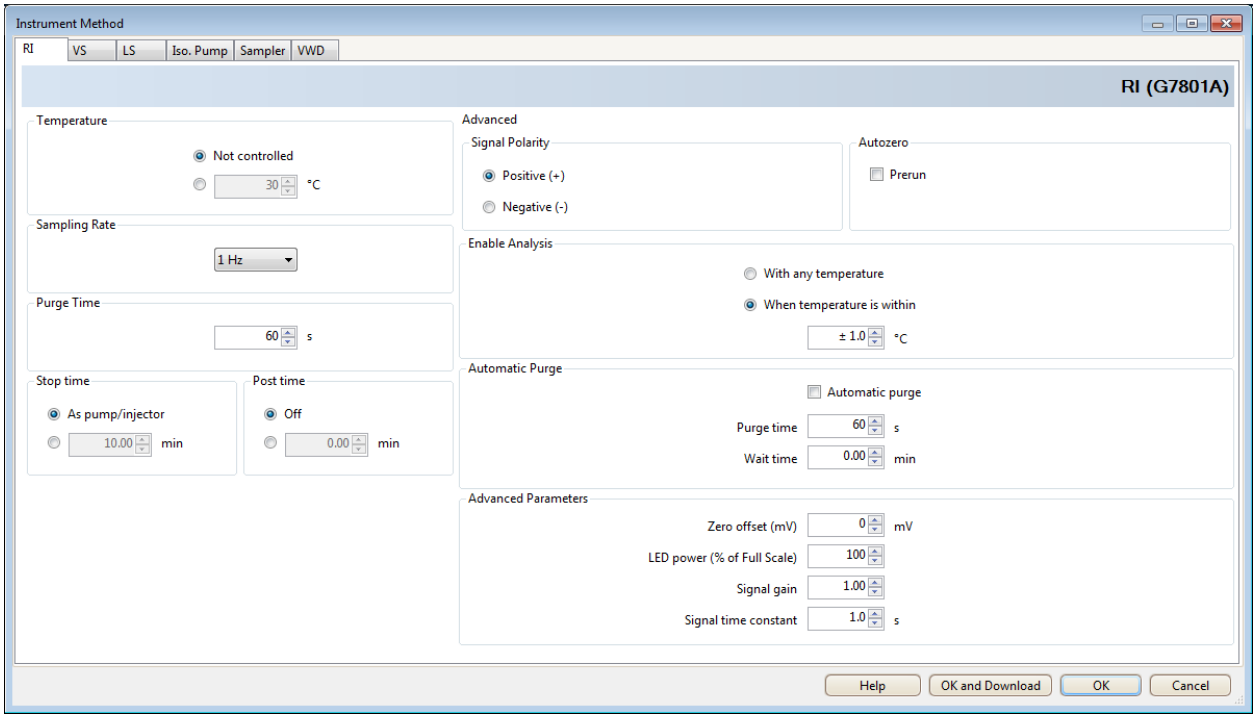
Or in this case Select New Method; you'll now be prompted with a new window as seen below:





Now populate each instrument module starting from the RI detector.

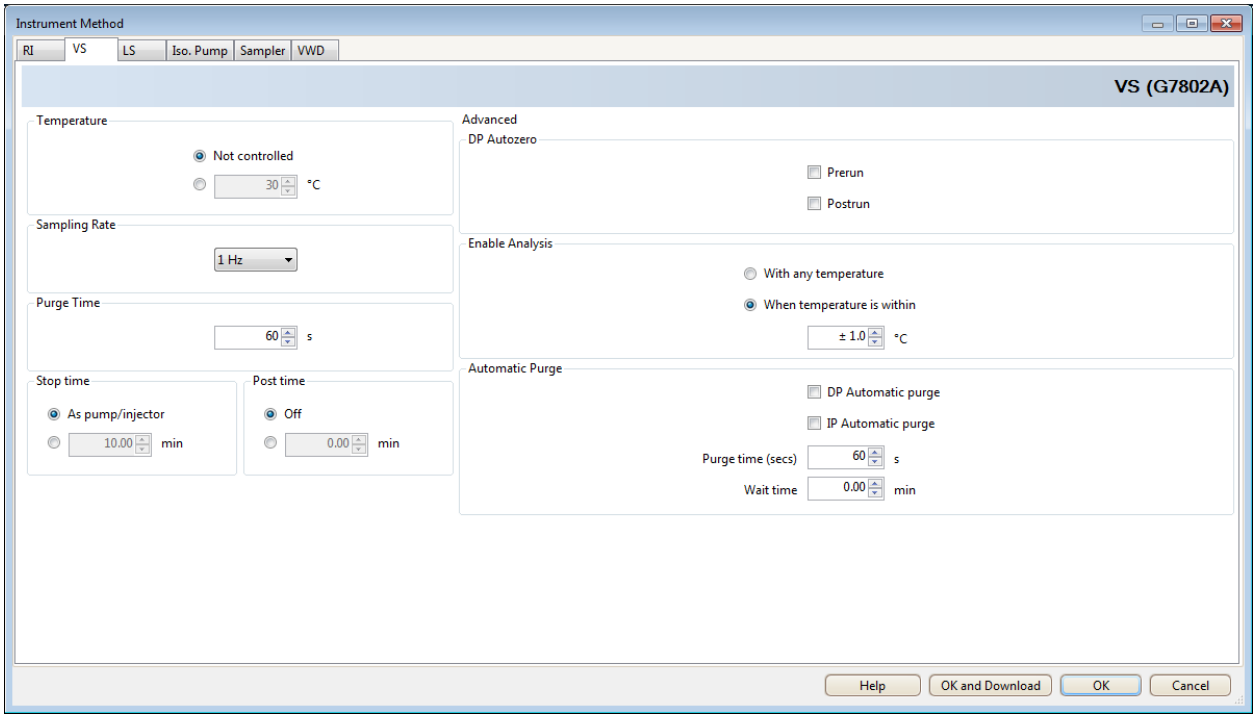
Refractive Index Detector (RI)



Important parameters

Setting	Comments
Temperature	*Not controlled* will turn the heaters off. Setting a temperature will turn the heaters on and the RI will start to heat to that temperature, 30 – 60 °C
Stoptime	Set *As Pump/Injector* to ensure collection time synchronization
Enable Analysis	If set to *When temperature is within* then the run will not start until the RI detector temperature is within those limits

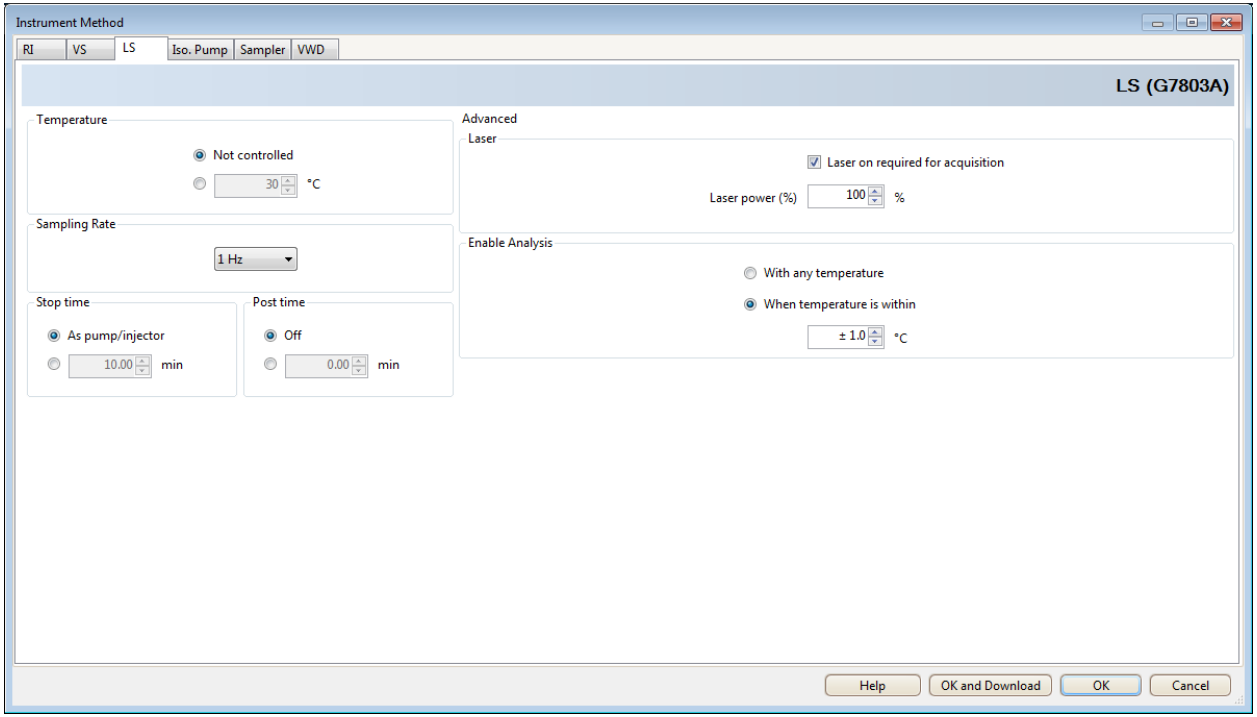
Viscometer (VS)



Important parameters

Setting	Comments
Temperature	*Not controlled* will turn the heaters off. Setting a temperature will turn the heaters on and the Viscometer will start to heat to that temperature, 30 – 60 °C
Purge Time	A Viscometer has larger internal volumes than standard detectors, will require longer purges
Stoptime	Set *As Pump/Injector* to ensure collection time synchronization
Enable Analysis	If set to *When temperature is within* then the run will not start until the Viscometer detector temperature is within those limits

Light Scattering Detector (LS)

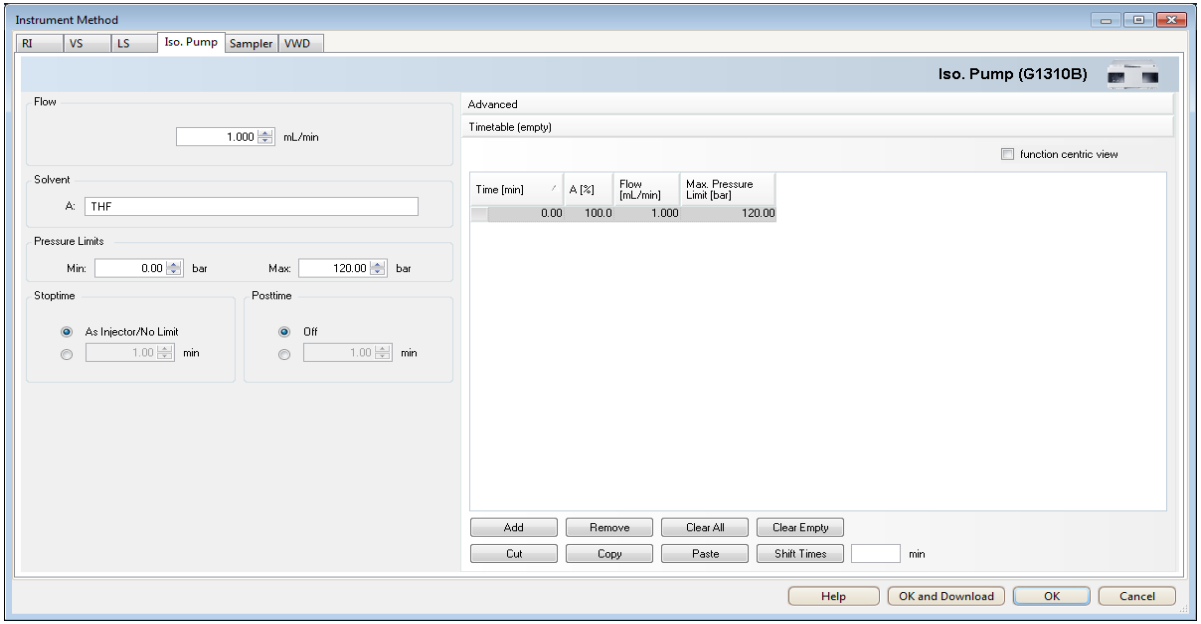


Important parameters

Setting	Comments
Temperature	*Not controlled* will turn the heaters off. Setting a temperature will turn the heaters on and the LS will start to heat to that temperature, 30 – 60 °C
Stoptime	Set *As Pump/Injector* to ensure collection time synchronization
Laser	If selected the run will not commence until the laser is on and ready. The laser power can be adjusted to suit analysis. **Important**, the detector constants must be recalculated if the laser power is changed

Enable Analysis	If set to *When temperature is within* then the run will not start until the LS detector temperature is within those limits

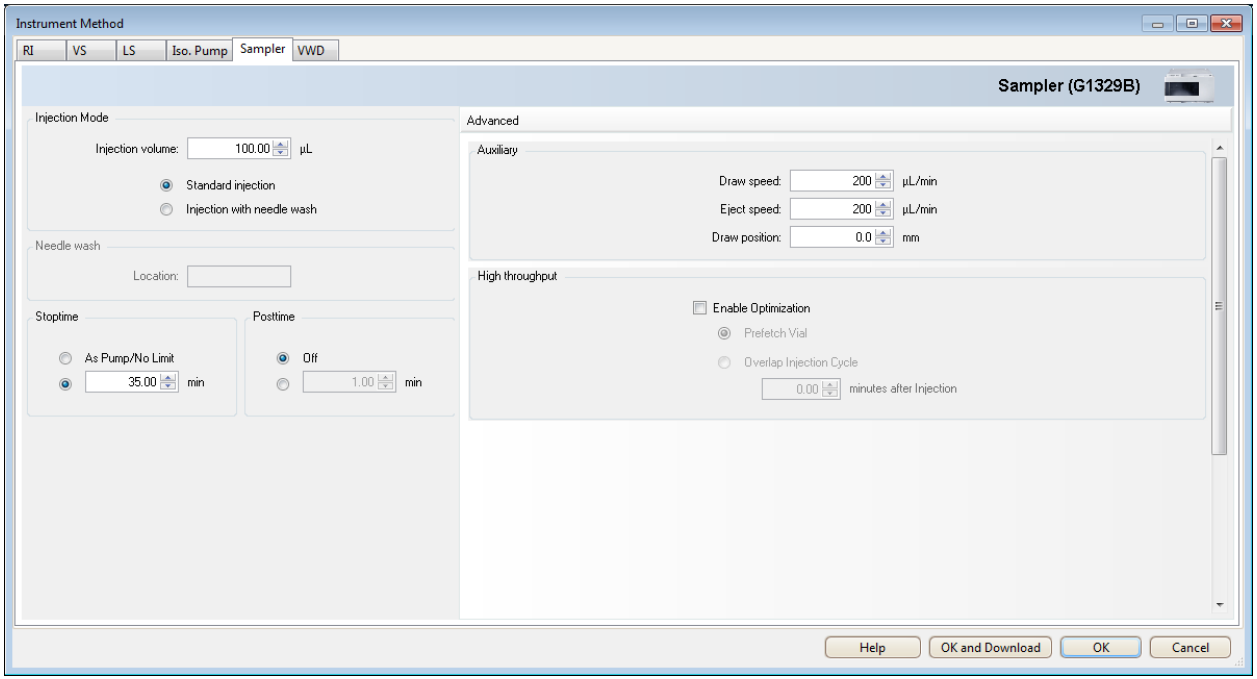
Now the pump (Quat Pump)



Setting	Comments
Flow	Set the flow rate (mL/min)
Solvents	Set the solvent composition and what channels should be employed. **Important**, SEC is always isocratic. Enter the buffer name and it will be stored with the sample.
Pressure Limits	SEC columns typically have lower pressure limits than standard HPLC. Refer to the column guide.
Stoptime	The length to collect data
Posttime	The length after the data is collected before the next injection starts. This is useful when using a Viscometer to allow the delay peak through.

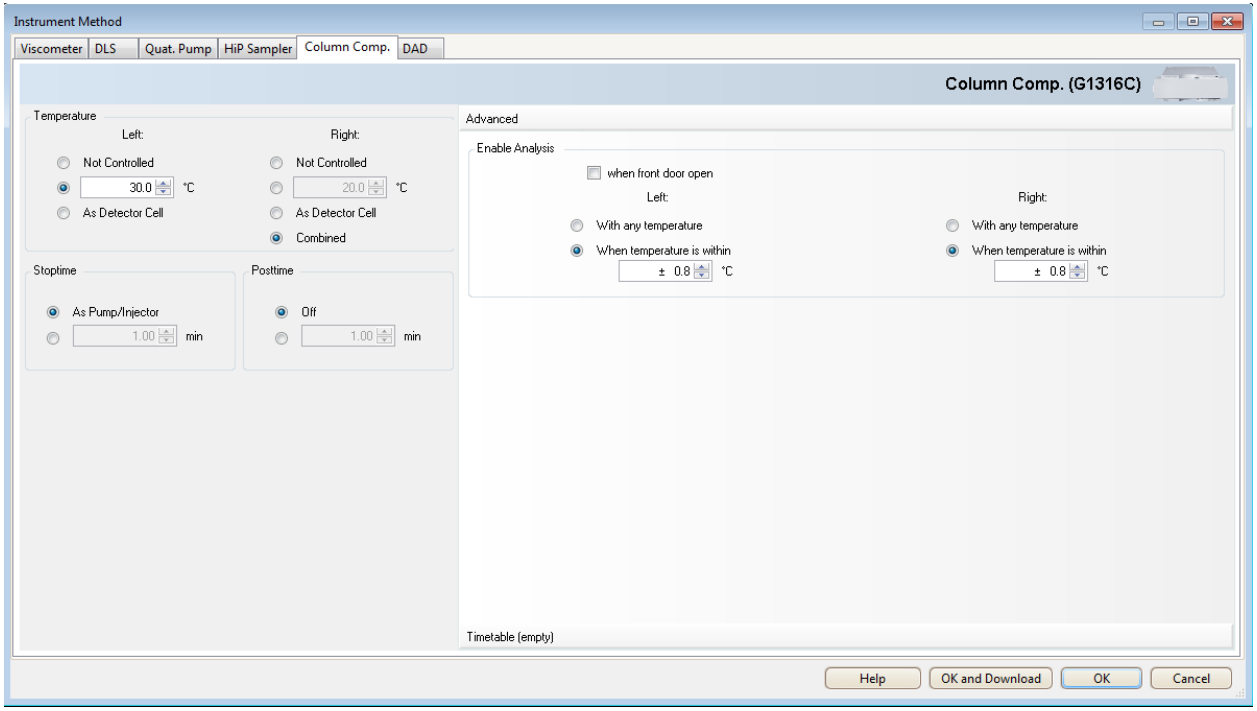
Compressibility	To ensure a smooth flow, enter the correct compressibility setting. For aqueous 46 is recommended.
Maximum Flow Gradient	Reduce this to 0.5 mL/min^2 to increase column lifetime and ensure detector stability.

Now the Auto Sampler (Sampler)



Setting	Comments
Injection volume	Set the volume to be injection onto the column
Stoptime	Set *As Pump/Injector* to ensure collection time synchronization

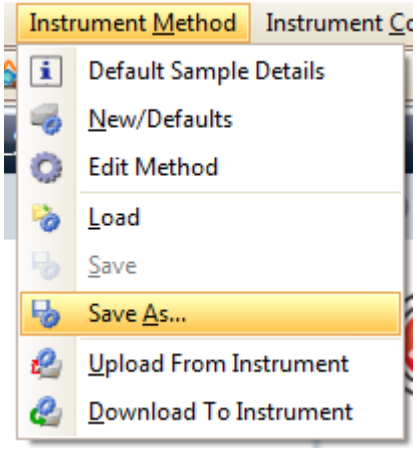
Column compartment (TCC)



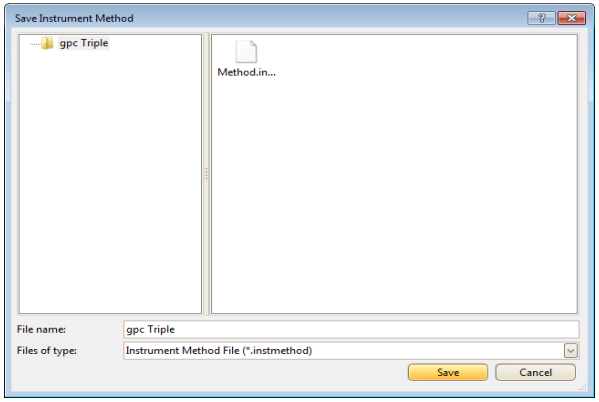
Important parameters

Setting	Comments
Temperature	If a temperature is set, the oven still requires turning on. On the dashboard tile right-click and turn oven on. Select *Combined* to ensure column oven temperature consistent across oven
Stoptime	Set *As Pump/Injector* to ensure collection time synchronization
Enable Analysis	If set to *When temperature is within* then the run will not start until the column compartment temperature is within those limits

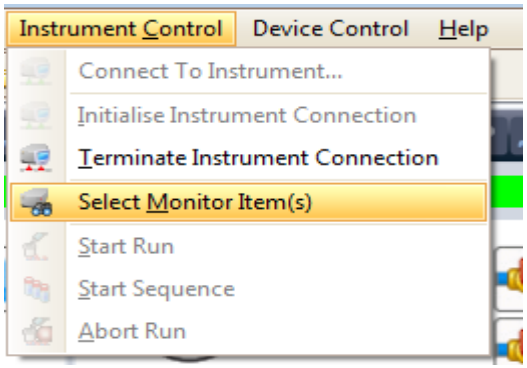
Once all parameters have been set the parameters can be sent to the instrument by selecting *OK and Download*. The method can then be saved *Instrument Method – Save As*. The method that is currently on the system is displayed on the lower right of the screen “see below”



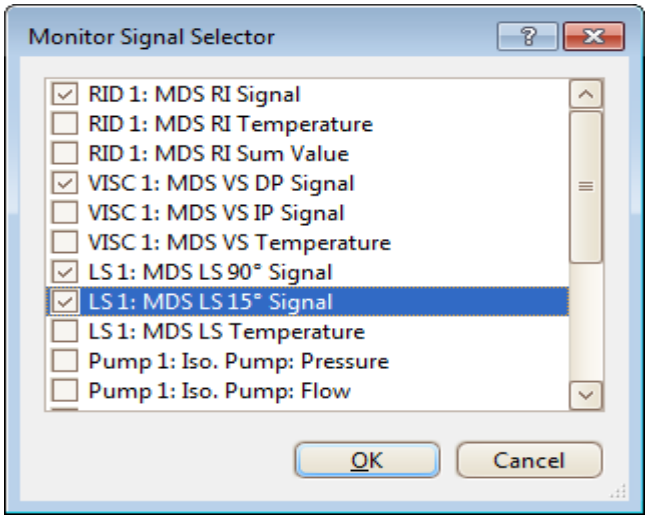
Now go to Instrument Method and click Save As..



High Light the gpc Triple Method and type in the file name and Save.



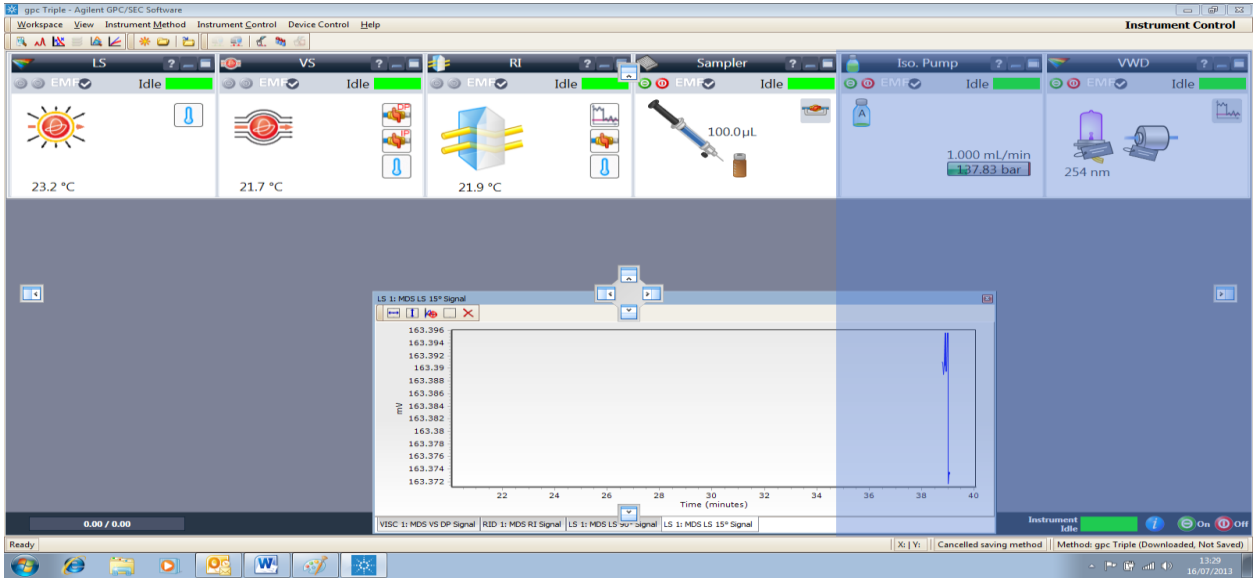
Now go to Instrument Control and select Monitor Items(s)



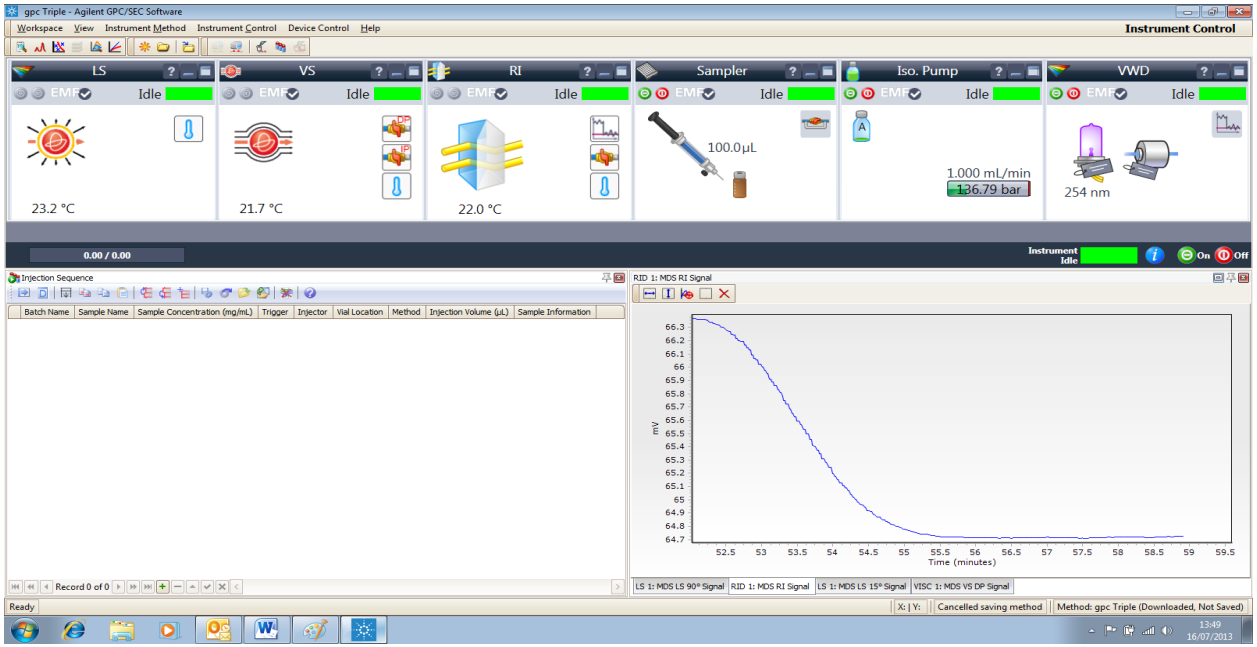
Place a tick in the appropriate box(s) to see the signals from these modules. Then drag the monitor signals screen to the bottom right hand corner by clicking on the left hand side of the mouse and fixing the screen to allocation tabs in the centre of the window below.

Preparing the Detector

- Once the desired pump flow is reached and column is saturated, now RID and Visco Detector needs to be stabilised.
- Maximise the RID, LS and Visco Pane to see the detector values.
- Right click on the RI and purge the RI Detector reference cell for 10 minutes and repeat this step 3 times.
- Purge the Visco DP and Purge Visco IP 3 times to 10 minutes each.
- Autozero the RI and Visco both.
- Once the detectors and all the modules are ready and baseline is stabilised, system is ready for further use.
- If the system is completely stabilised and no run is in progress, Waste flow can be recycled if necessary.
- Reminder: remove the waste tubing from the Solvent bottle before starting the sequence. (if Recycle is done)

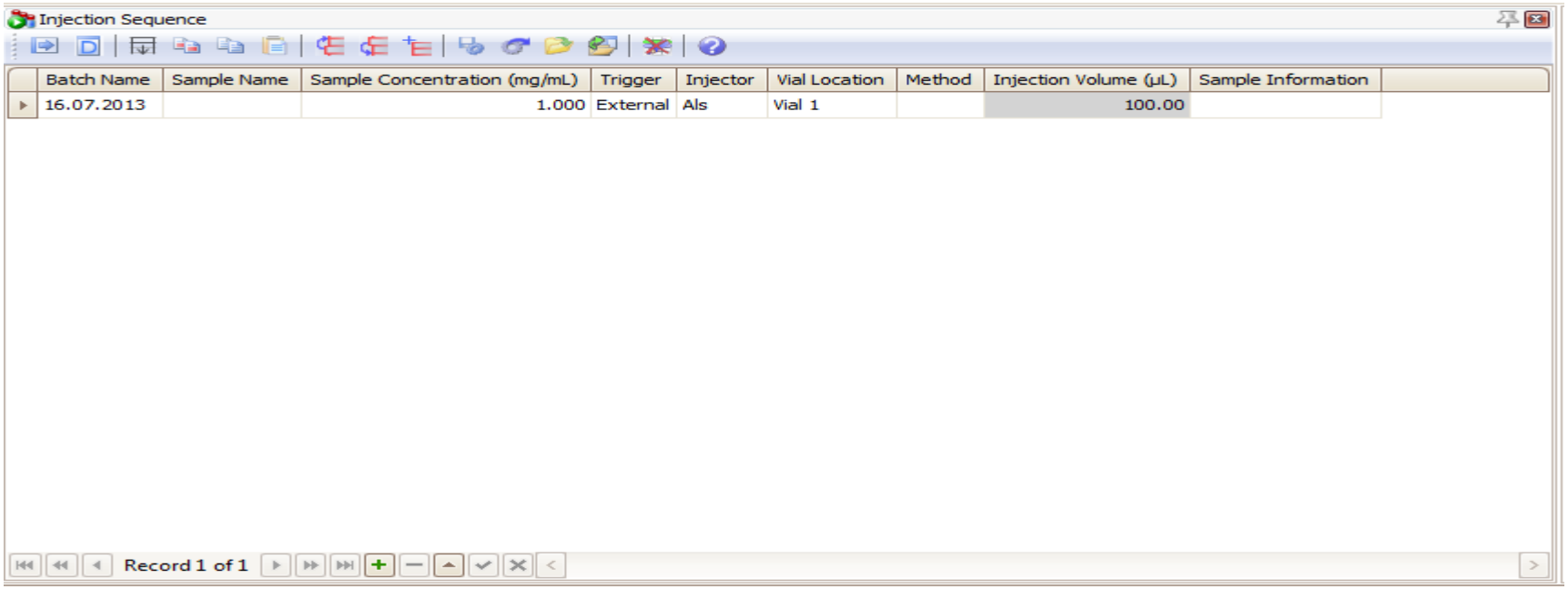


Creating the Sequence.

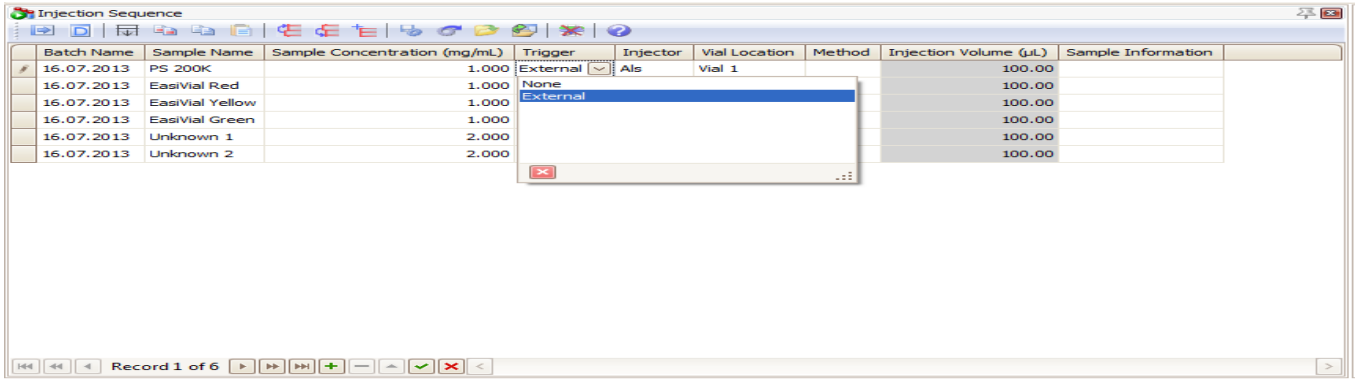


Now we need to input the samples that are going to be collected. This is done in the Injection Sequence Screen.

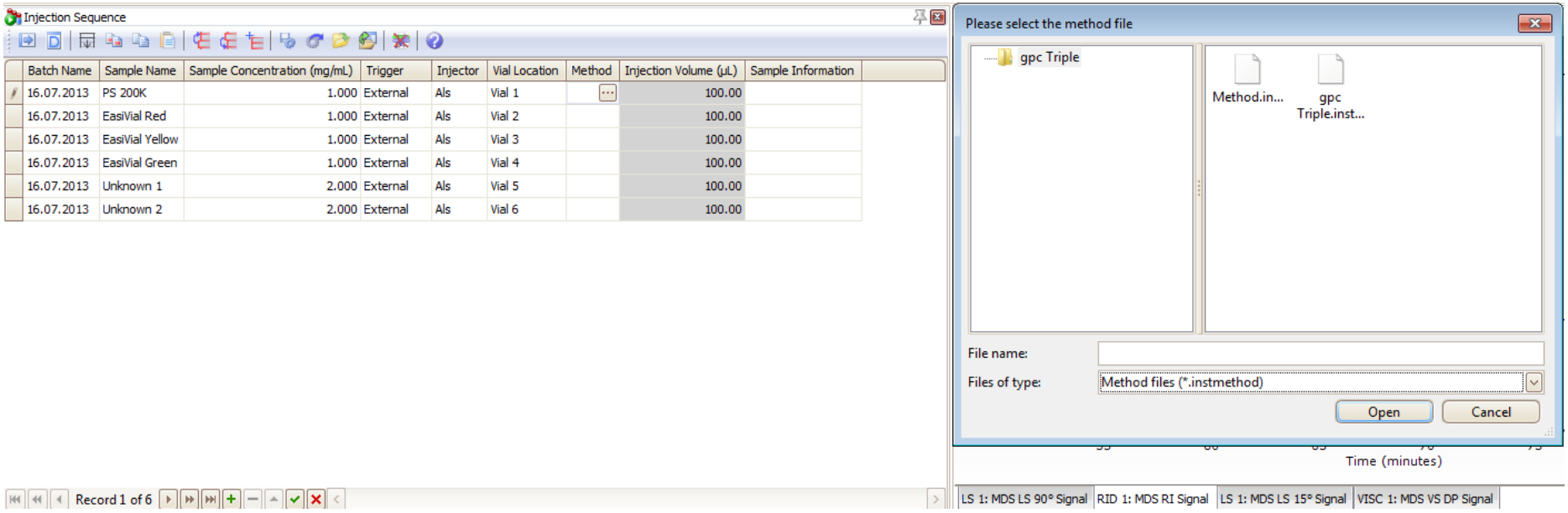
Click on the green button  at the bottom of the Injection Sequence screen and the following window pops up.



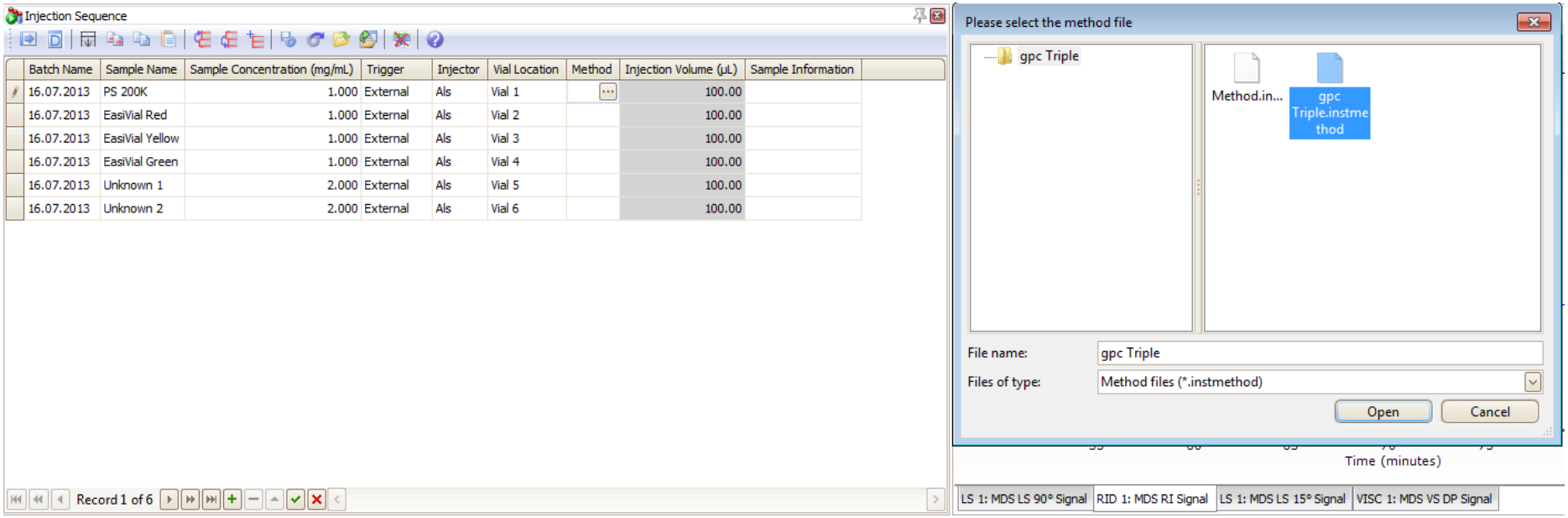
This can be populated by the number of samples that are going to be collected, in this case 6 samples by pressing the + button 6 times.
Click under the Trigger tab and select External if autosampler is connected for each sample.

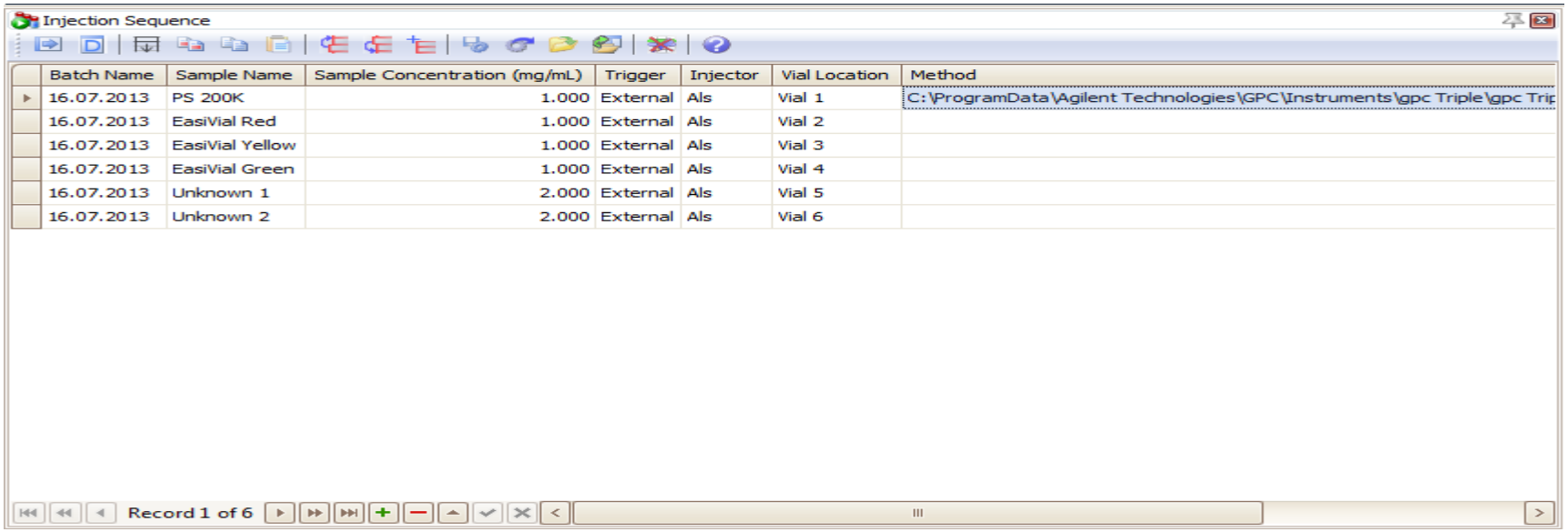


Then Click under the Method Tab next to the Vial location tab and the screen next to the injection sequence below will pop up.

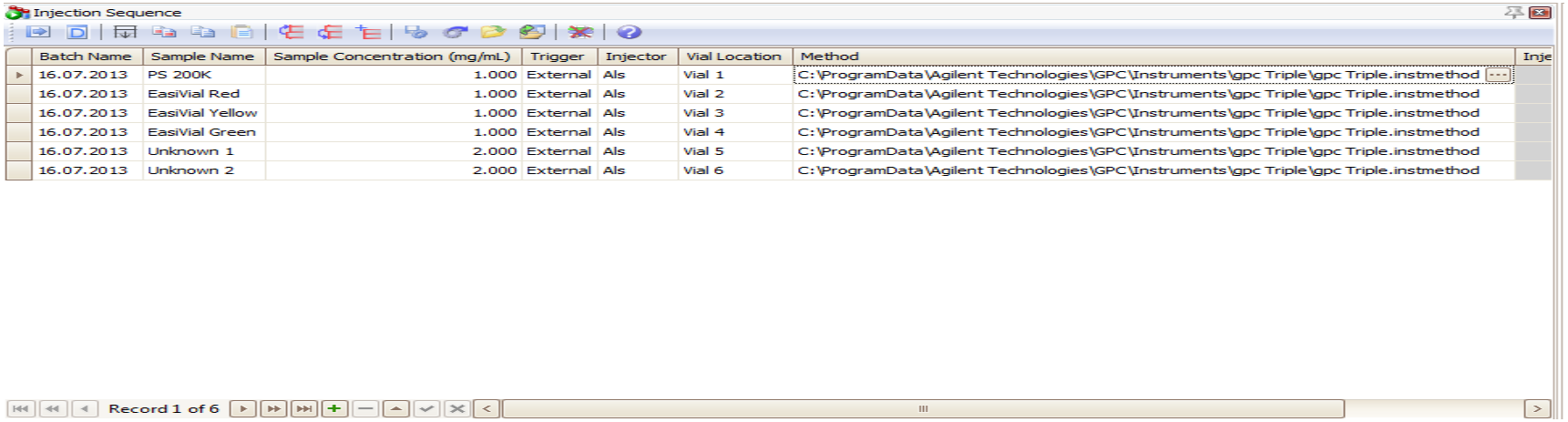


High Light the gpc Triple Instrument Method and click Open to load the method.


Injection Sequence
Batch Name
Sample Name
Sample Concentration (mg/mL)
Trigger
Injector
Vial Location
Method
Injection Volume (µL)
Sample Information
16.07.2013 PS 200K 1.000 External Als Vial 1 100.00
16.07.2013 EasiVial Red 1.000 External Als Vial 2 100.00
16.07.2013 EasiVial Yellow 1.000 External Als Vial 3 100.00
16.07.2013 EasiVial Green 1.000 External Als Vial 4 100.00
16.07.2013 Unknown 1 2.000 External Als Vial 5 100.00
16.07.2013 Unknown 2 2.000 External Als Vial 6 100.00
Record 1 of 6
Please select the method file
gpc Triple
Method.in...
gpc Triple.instmethod
File name:
gpc Triple
Files of type:
Method files (*.instmethod)
Open
Cancel
Time (minutes)
LS 1: MDS LS 90° Signal
RID 1: MDS RI Signal
LS 1: MDS LS 15° Signal
VISC 1: MDS VS DP Signal



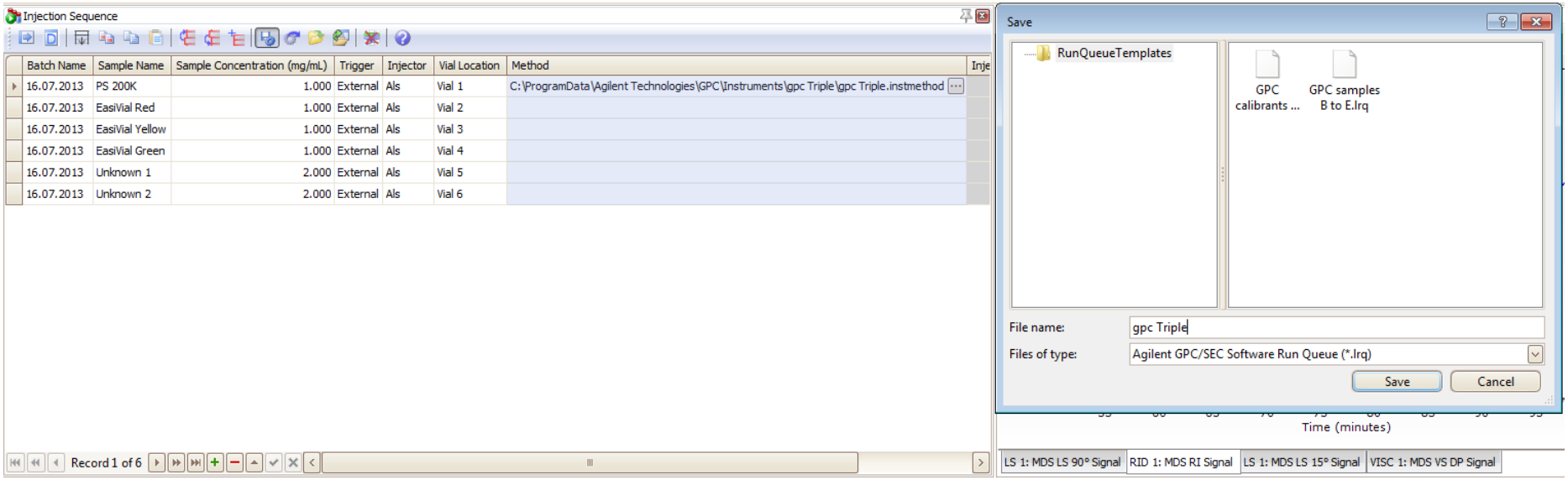
Copy down the method to each sample as shown below and select Icon 



Injection Sequence

This injection sequence can now be saved by clicking on Icon  and typing the file name gpc Triple as shown below
Click Save

This section enables definition of all samples and/or calibrants to be injected into the system. It is good practice to include as much information as is known as this information in stored within the sample data file and can always be referred to at a later date

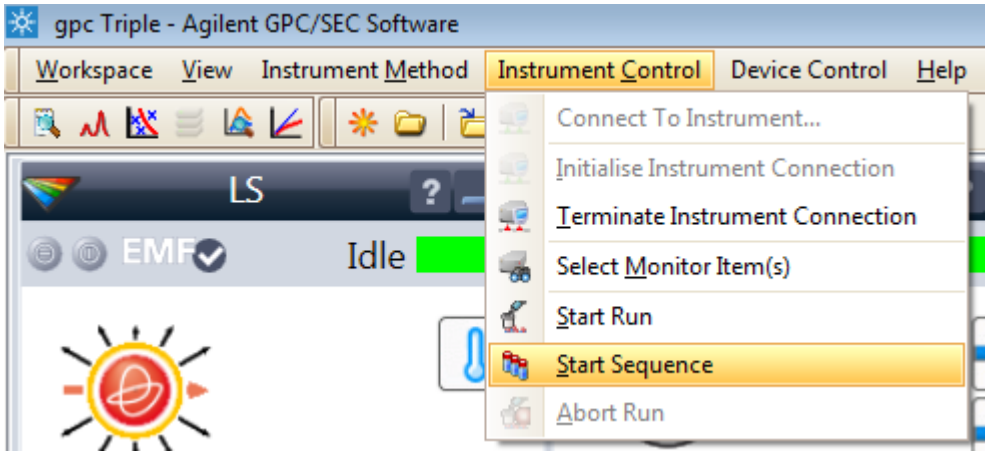


Example Injection Sequence

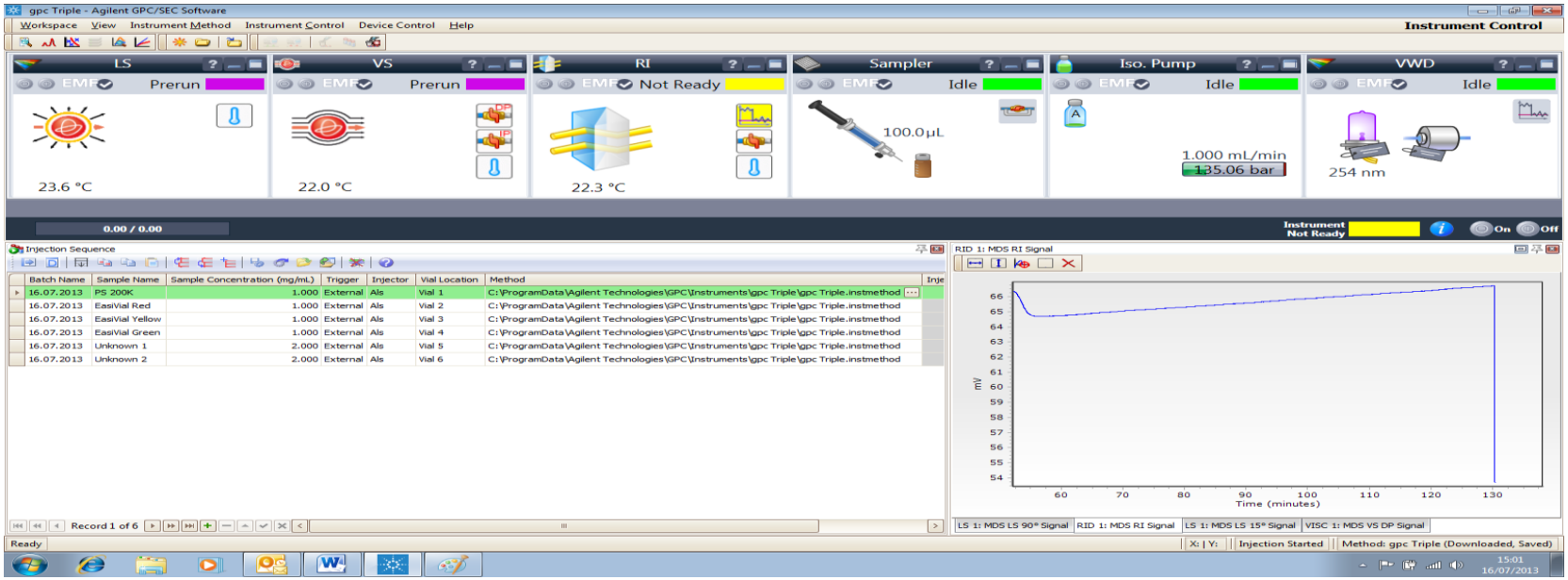
Key icons

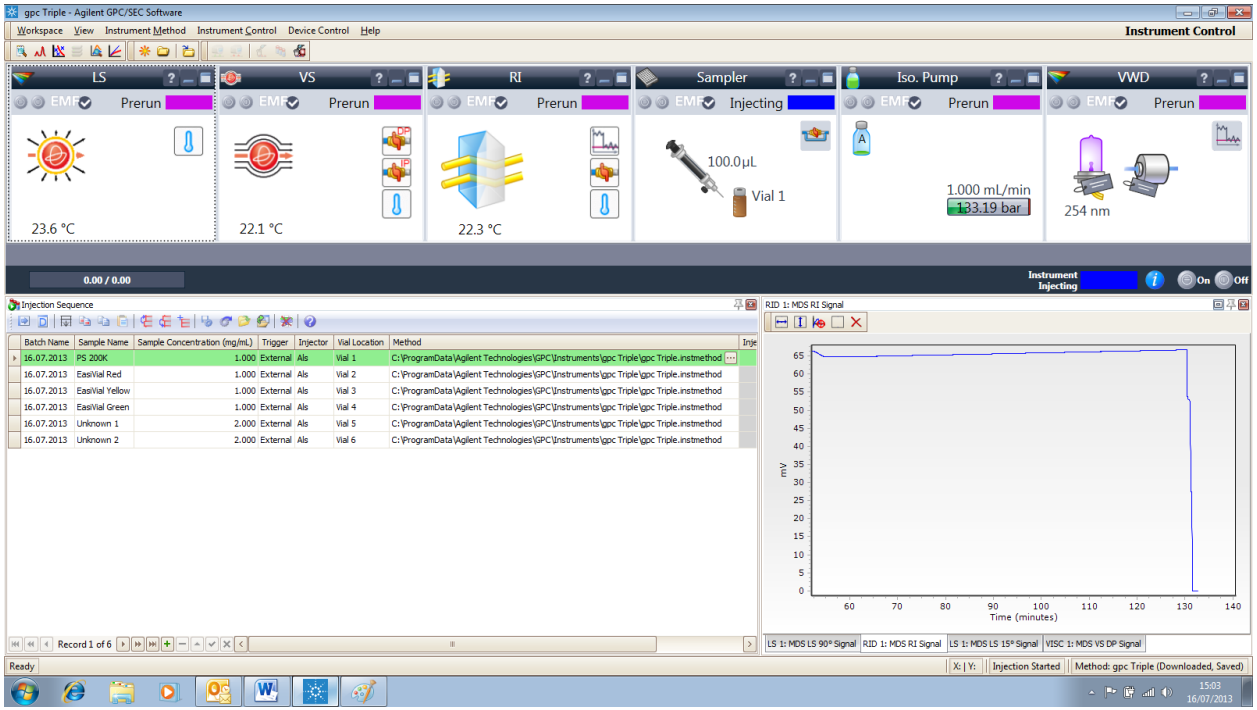
Icon	Command	Description
	Append	Add a single line to injection sequence
	Delete	Delete highlighted row from injection sequence
	Add Multiple Runs	Displays dialog to define number of samples to add to sequence
	Promote Run	Moves the selected run up one row in the sequence
	Demote Run	Moves the selected run down one row in the sequence
	Save Injection Sequence	Displays Dialog in which the sequence can be named and saved
	Open Injection Sequence	Load an existing injection sequence - replacing the current

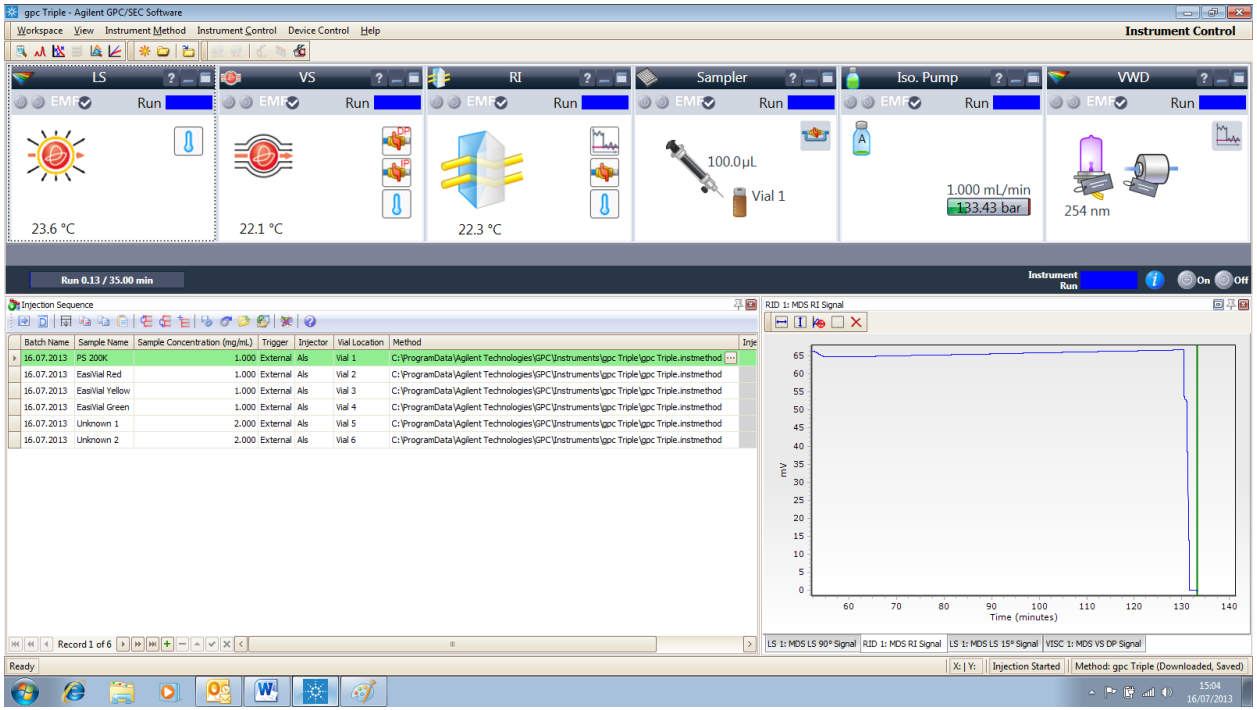
Now go to Instrument Control tab and click Start Sequence. This will start the data collection of the 6 samples.



The following screen will be shown


gpc Triple - Agilent GPC/SEC Software
Workspace View Instrument Method Instrument Control Device Control Help
Instrument Control
LS
EMF
Prerun
23.6 °C
VS
EMF
Prerun
22.0 °C
RI
EMF
Not Ready
22.3 °C
Sampler
EMF
Idle
100.0µL
Iso. Pump
EMF
Idle
1.000 mL/min
135.06 bar
VWD
EMF
Idle
254 nm
0.00 / 0.00
Instrument Not Ready
On
Off
Injection Sequence
Batch Name | Sample Name | Sample Concentration (mg/mL) | Trigger | Injector | Vial Location | Method
16.07.2013 | PS 200K | 1.000 | External | Als | Vial 1 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Red | 1.000 | External | Als | Vial 2 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Yellow | 1.000 | External | Als | Vial 3 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Green | 1.000 | External | Als | Vial 4 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | Unknown 1 | 2.000 | External | Als | Vial 5 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | Unknown 2 | 2.000 | External | Als | Vial 6 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
Record 1 of 6
RID 1: MDS RI Signal
mV
Time (minutes)
LS 1: MDS LS 90° Signal
RID 1: MDS RI Signal
LS 1: MDS LS 15° Signal
VISC 1: MDS VS DP Signal
Ready
Injection Started
Method: gpc Triple (Downloaded, Saved)
15:01
16/07/2013


gpc Triple - Agilent GPC/SEC Software
Workspace View Instrument Method Instrument Control Device Control Help
Instrument Control
LS
VS
RI
Sampler
Iso. Pump
VWD
EMF
Prerun
Injecting
23.6 °C
22.1 °C
22.3 °C
100.0µL
Vial 1
1.000 mL/min
133.19 bar
254 nm
0.00 / 0.00
Instrument Injecting
On
Off
Injection Sequence
Batch Name
Sample Name
Sample Concentration (mg/mL)
Trigger
Injector
Vial Location
Method
16.07.2013 PS 200K 1.000 External Als Vial 1 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 EasiVial Red 1.000 External Als Vial 2 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 EasiVial Yellow 1.000 External Als Vial 3 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 EasiVial Green 1.000 External Als Vial 4 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 Unknown 1 2.000 External Als Vial 5 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 Unknown 2 2.000 External Als Vial 6 C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
Record 1 of 6
RID 1: MDS RI Signal
mV
Time (minutes)
LS 1: MDS LS 90° Signal
RID 1: MDS RI Signal
LS 1: MDS LS 15° Signal
VISC 1: MDS VS DP Signal
Ready
Injection Started
Method: gpc Triple (Downloaded, Saved)
15:03
16/07/2013


gpc Triple - Agilent GPC/SEC Software
Workspace View Instrument Method Instrument Control Device Control Help
Instrument Control
LS
VS
RI
Sampler
Iso. Pump
VWD
EMF
Run
23.6 °C
22.1 °C
22.3 °C
100.0µL
Vial 1
1.000 mL/min
133.43 bar
254 nm
Run 0.13 / 35.00 min
Instrument Run
On
Off
Injection Sequence
Batch Name | Sample Name | Sample Concentration (mg/mL) | Trigger | Injector | Vial Location | Method
16.07.2013 | PS 200K | 1.000 | External | Als | Vial 1 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Red | 1.000 | External | Als | Vial 2 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Yellow | 1.000 | External | Als | Vial 3 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | EasiVial Green | 1.000 | External | Als | Vial 4 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | Unknown 1 | 2.000 | External | Als | Vial 5 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
16.07.2013 | Unknown 2 | 2.000 | External | Als | Vial 6 | C:\ProgramData\Agilent Technologies\GPC\Instruments\gpc Triple\gpc Triple.instmethod
Record 1 of 6
RID 1: MDS RI Signal
mV
Time (minutes)
LS 1: MDS LS 90° Signal
RID 1: MDS RI Signal
LS 1: MDS LS 15° Signal
VISC 1: MDS VS DP Signal
Ready
Injection Started
Method: gpc Triple (Downloaded, Saved)
15:04
16/07/2013

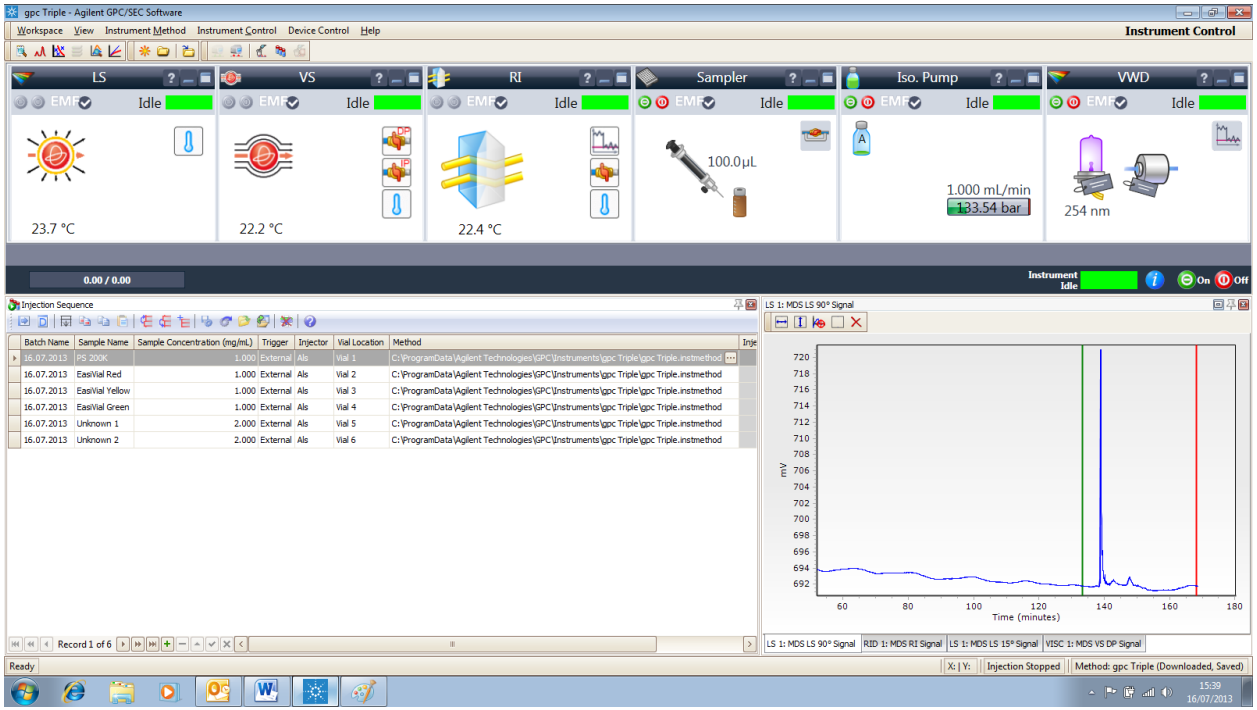


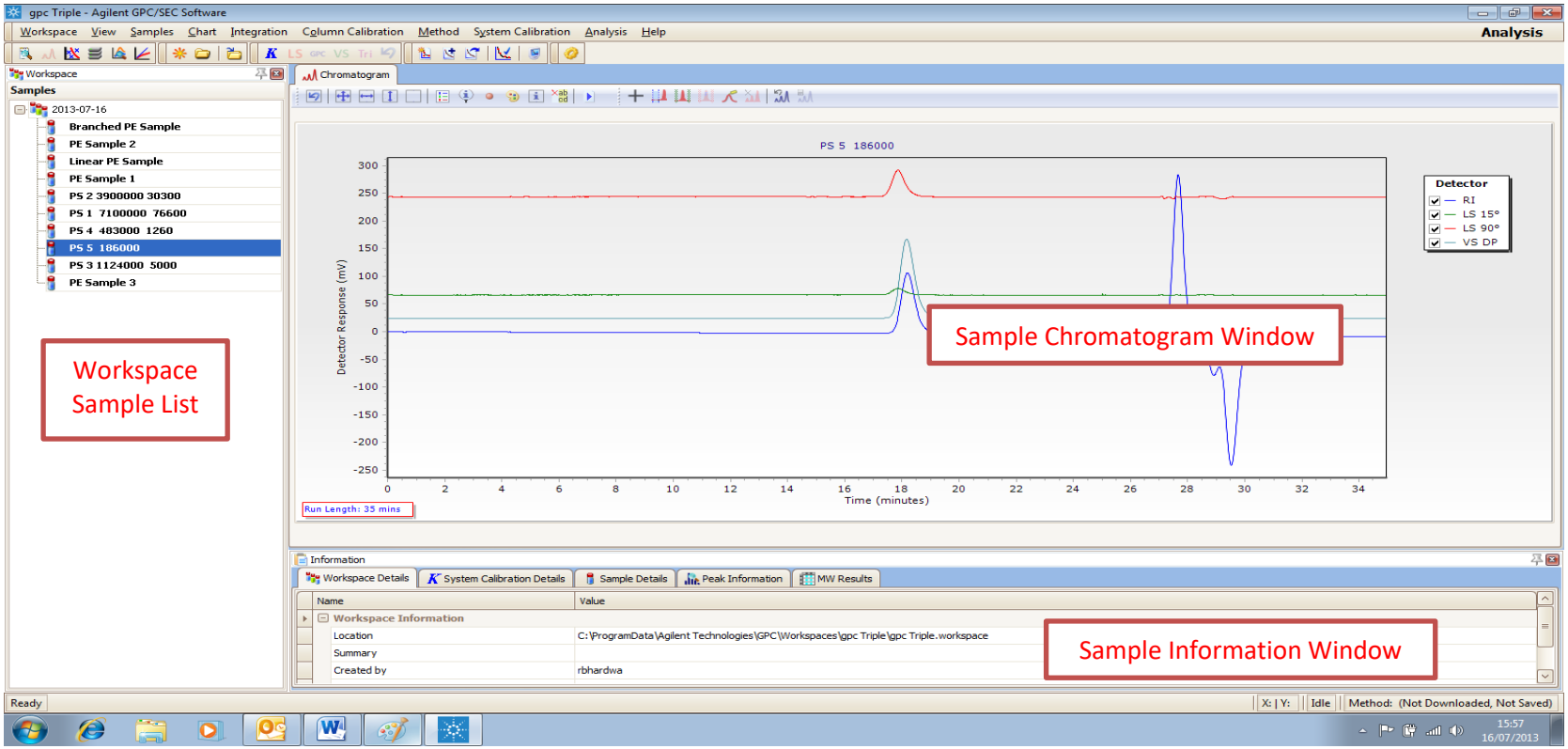
Figure2

The 1st sample has finished highlighted in grey. The system will continue until the last sample has been injected.

The *Injection Sequence* will always run in order, starting from the first non-completed run.
Figure 2 shows an example sequence, the initial run has been completed and are now grayed out, the data will be viewable in the **Analysis View**. These lines cannot be rerun, a new line will need to be added and the details can be copy and pasted if required. To remove these completed lines from the *Injection Sequence* highlight and *Delete*.
The next run will highlighted in green while running, the data for this is being collected and will be viewable only once the run has completed. No cells can be edited at this point.
The lines with a white background are the next to be injected, all entered information can still be edited. If a sample is added at the end of the sequence but is required to run sooner, *Promote run* to the top available slot.

Processing of GPC Triple data

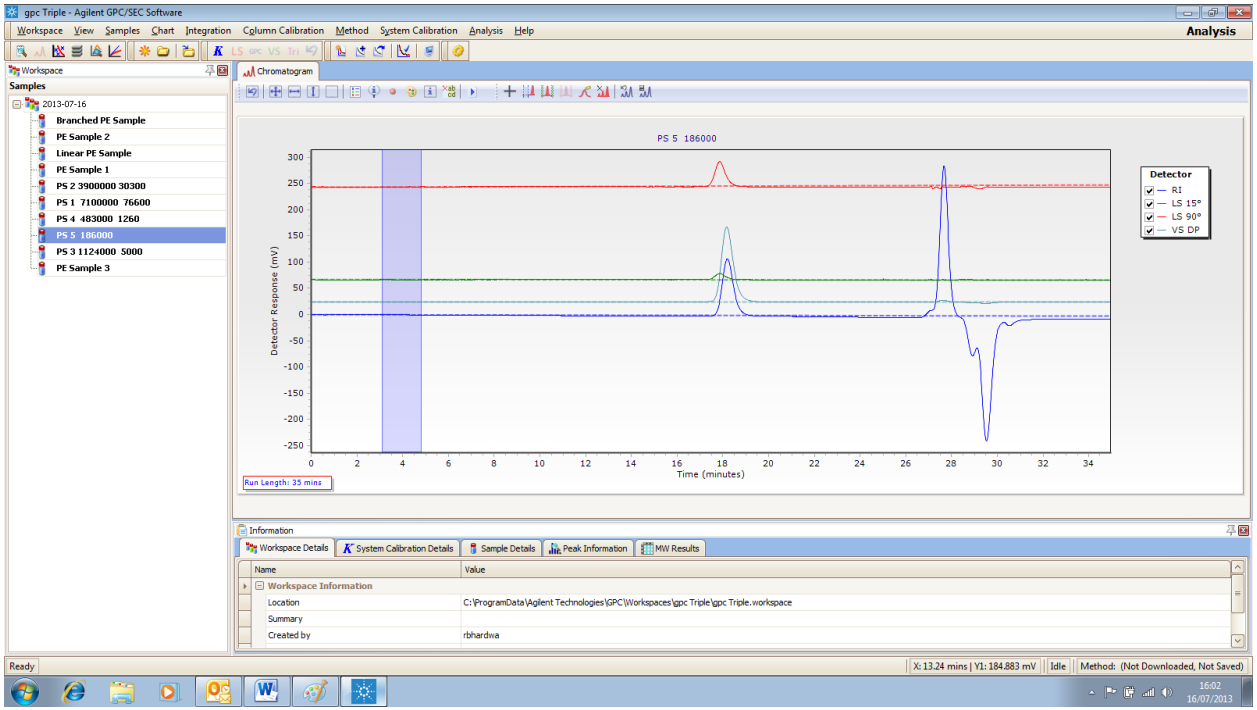
Click on the Icon  and your data for the 6 samples will be in the workspace under samples ready to be processed.
Alternatively you can import samples collected previously or load other workspaces in this analysis View.



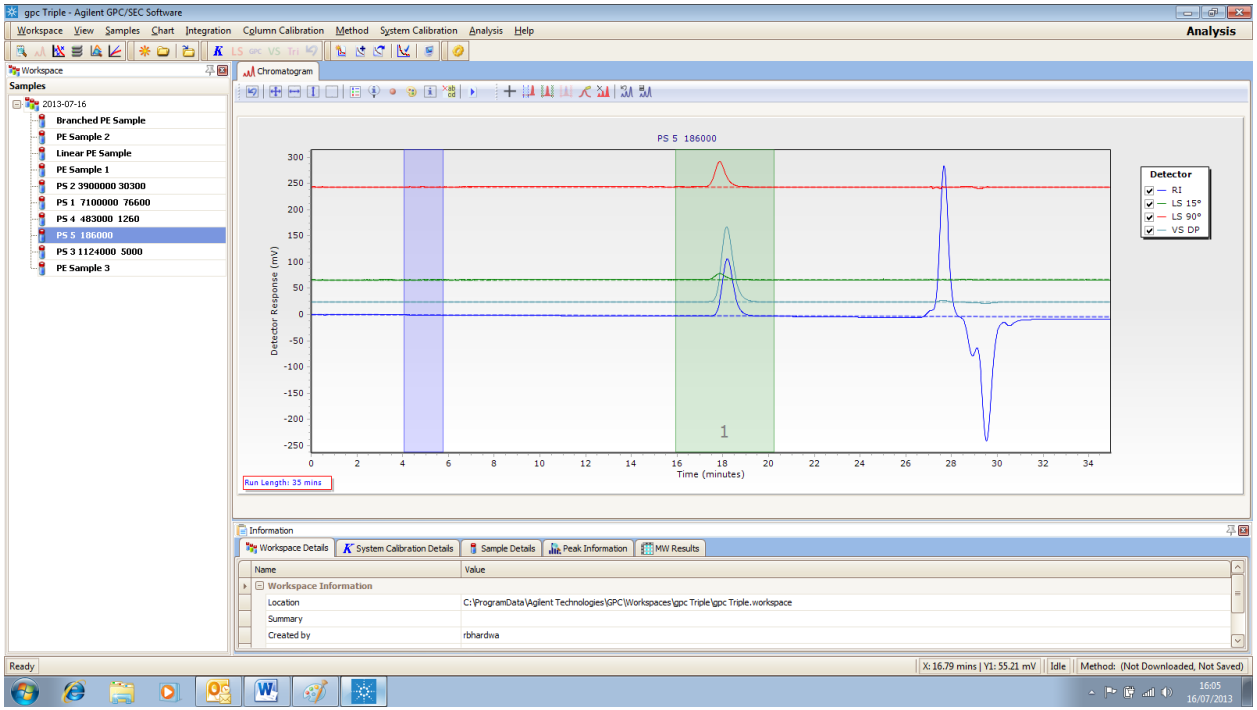
The *Analysis View* has three main sections, the **Workspace Sample List**, **Sample Chromatogram Window** and the **Sample Information Window**. Once a sample has been analyzed a fourth section will be added, the **Data Results Window.**

Highlight the sample PS 186,000, as shown above, and the right side of the screen will show the raw data. This sample will be used to measure the Detector Constants for the RI/LS & Viscometer and the Inter-detector Delay (IDD).In general this sample should be between Mp 60,000 to 200,00.

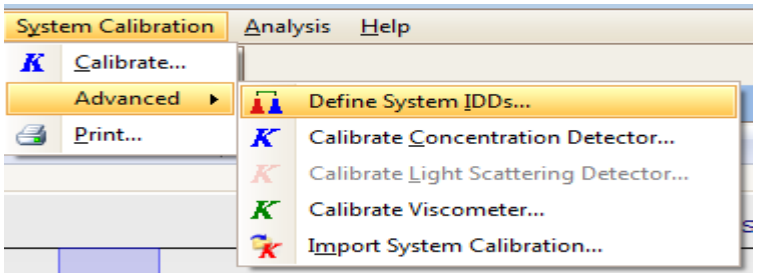
Now put in the base line by clicking on the Icon  and clicking on the raw data



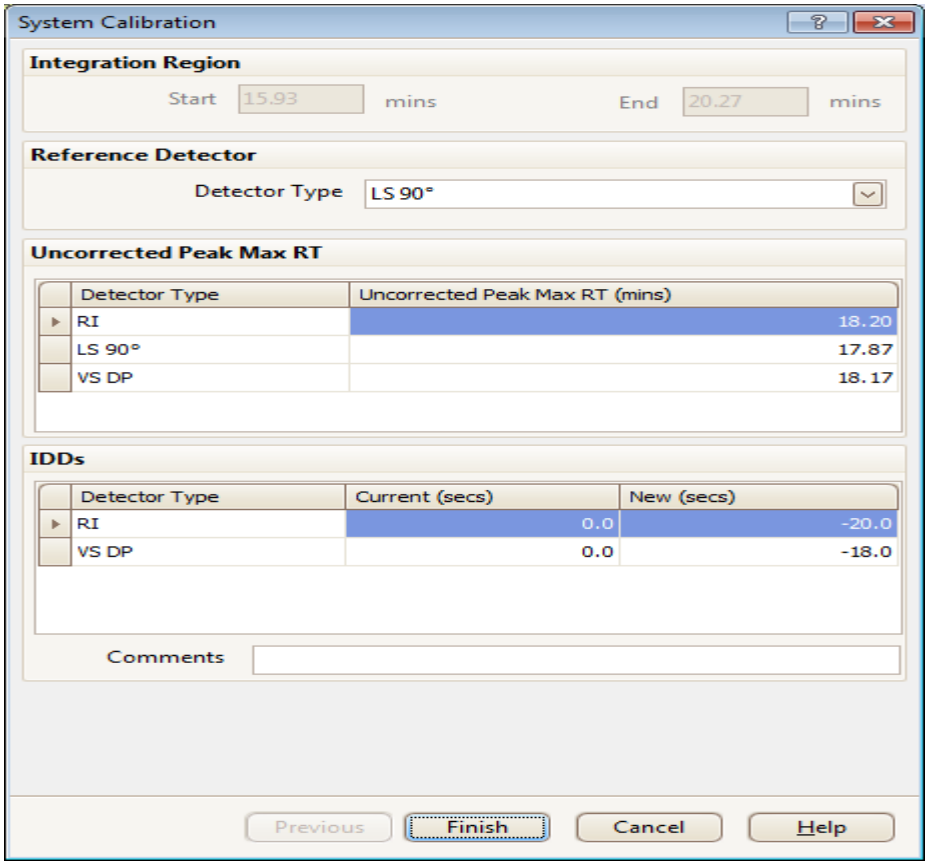
Then put the integration area by clicking on the Icon  and click and drag the area around the sample peak.



Now click on System Calibration, Advanced, Define System IDDs

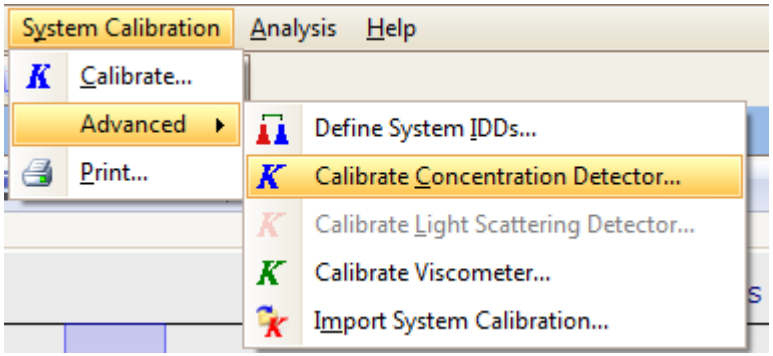


And the following window comes up

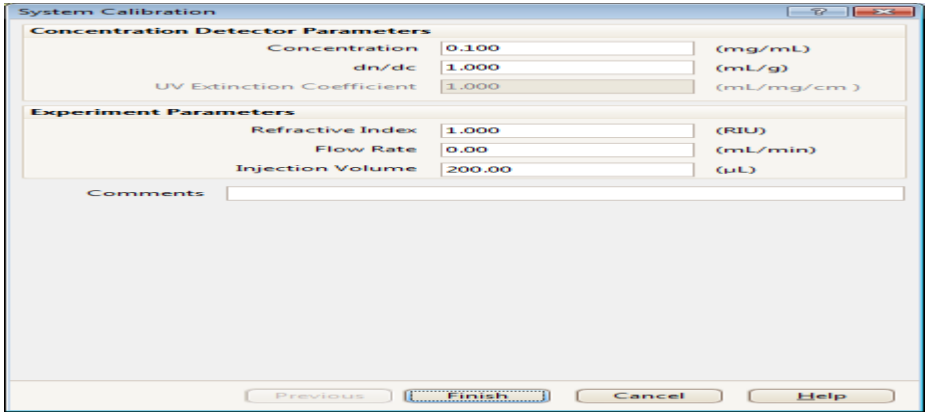


Click the Finish tab

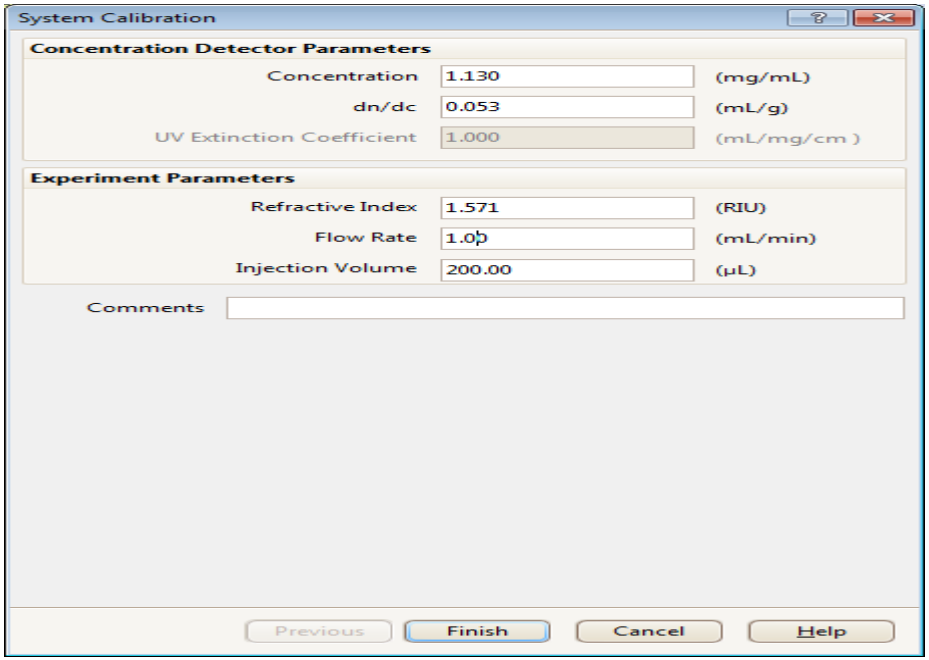
Now we need to generate from this sample the detector constants for each of the detectors
Click System Calibration, advanced & Calibrate Concentration Detector as shown below



The following screen pops up

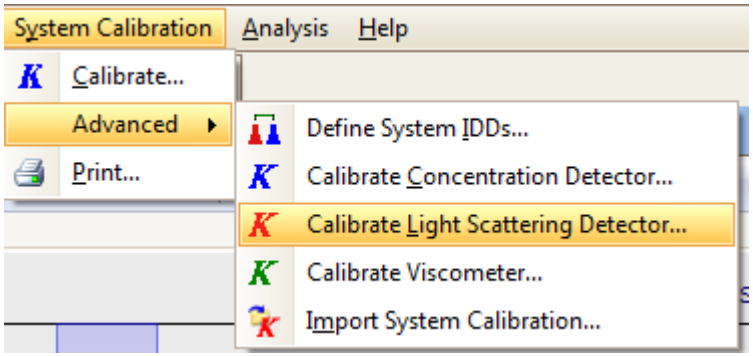


This screen needs to be populated with the following information for this sample.
Conc of the sample, dn/dc of PS in TCB is 0.053, RI of TCB is 1.571, Flow rate of Pump and injection volume

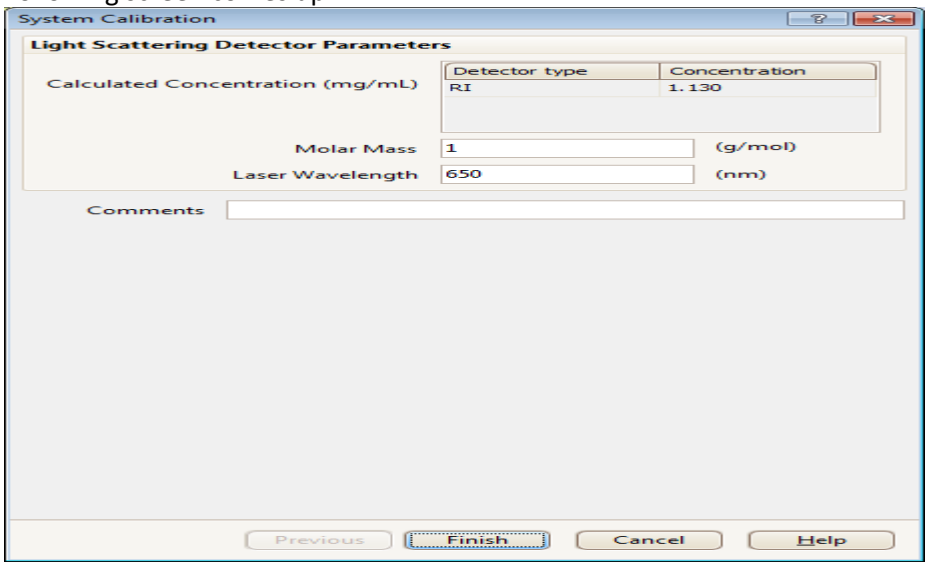


Click Finish now

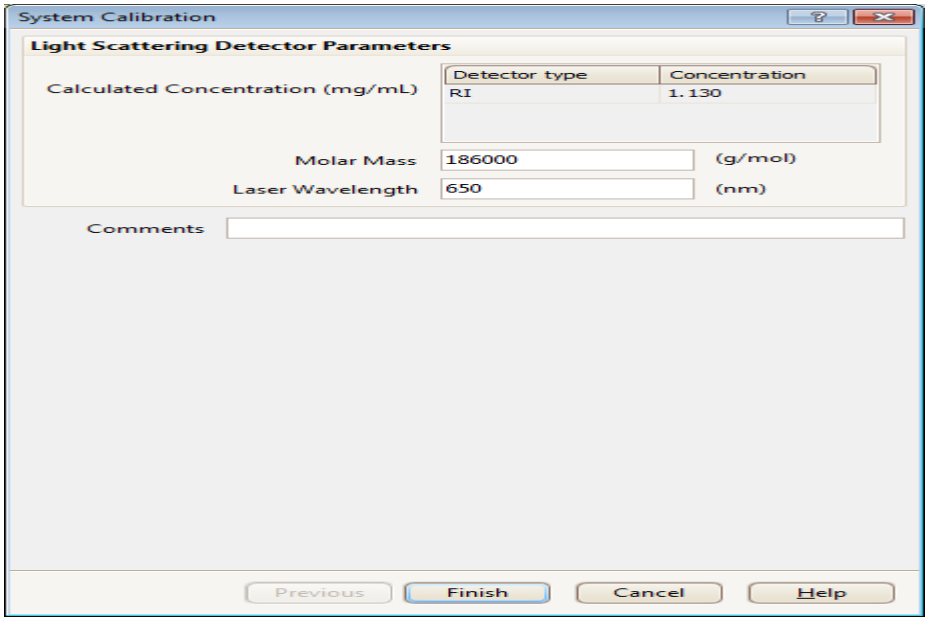
Now we need to calibrate the LS detector
Click System Calibration, Advanced & Calibrate Light Scattering Detector



Following screen comes up



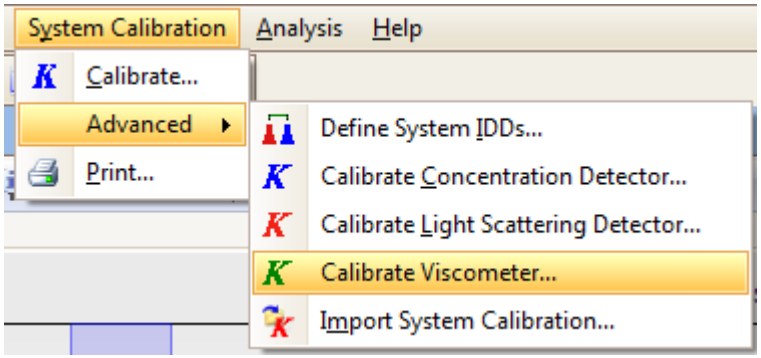
This screen now needs to be populated with the Mp 186,000 as shown below



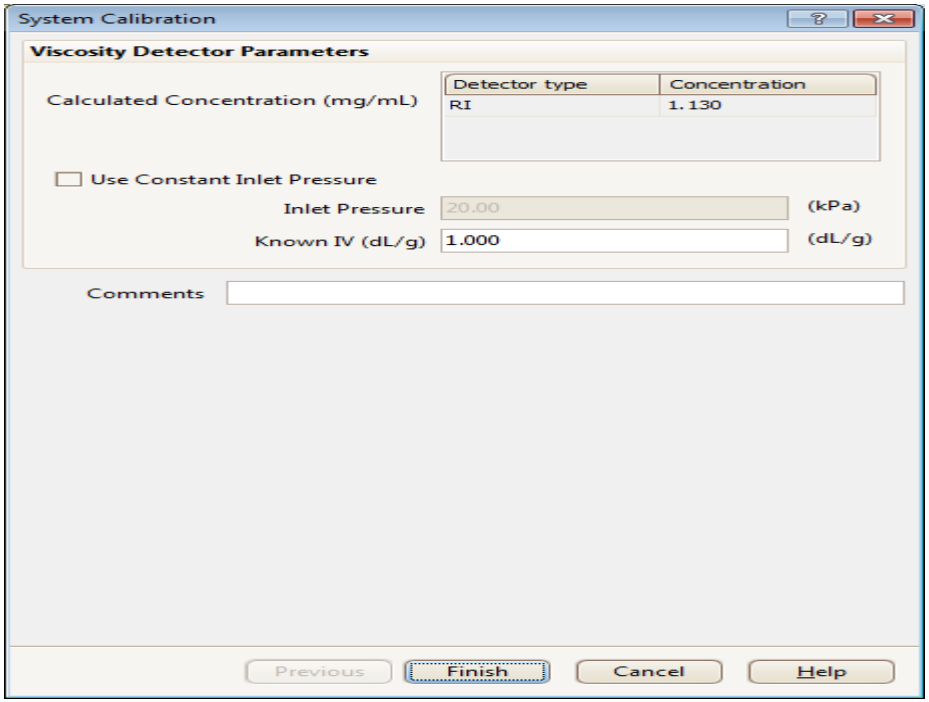
Click Finish

Now we need to calibrate the Viscometer

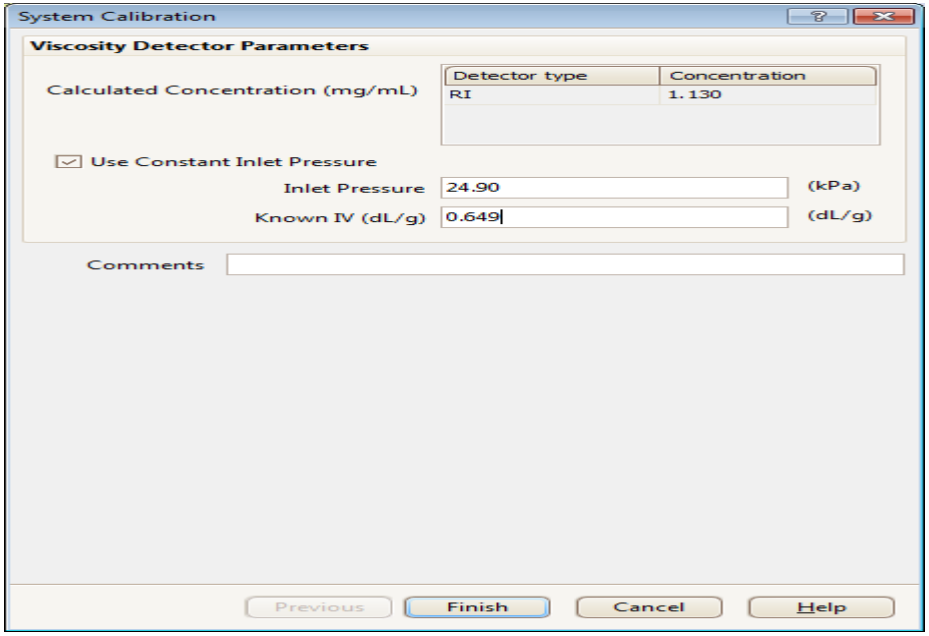
Click System Calibration, Advanced & Calibrate Viscometer



The following window pops up

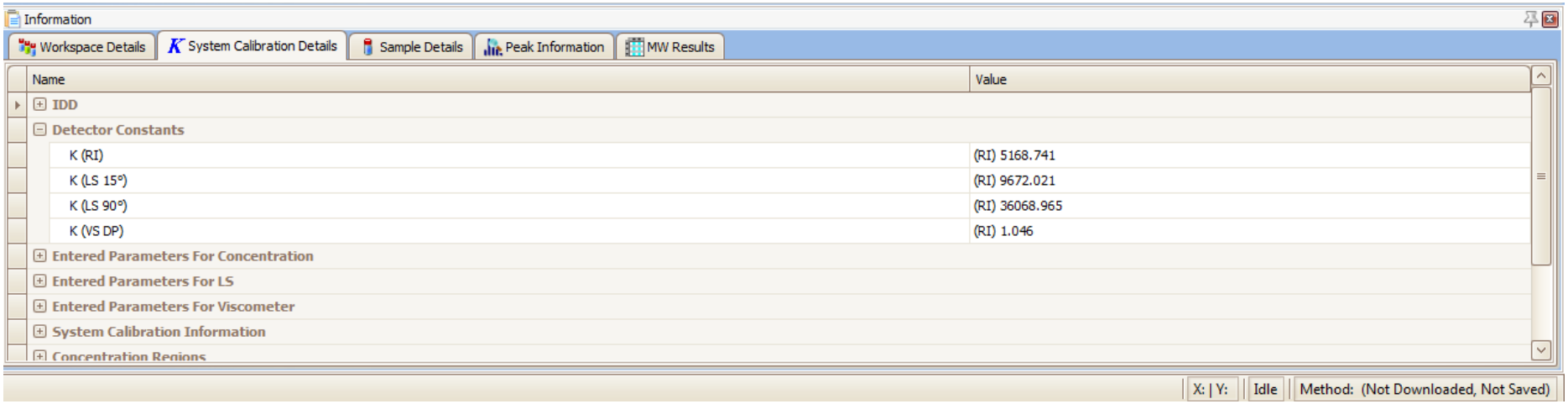


This screen needs to be populate as :
IV is provided for this sample as 0.649 and the Inlet Pressure reading is obtained from the instrument read out (24.9KPa).

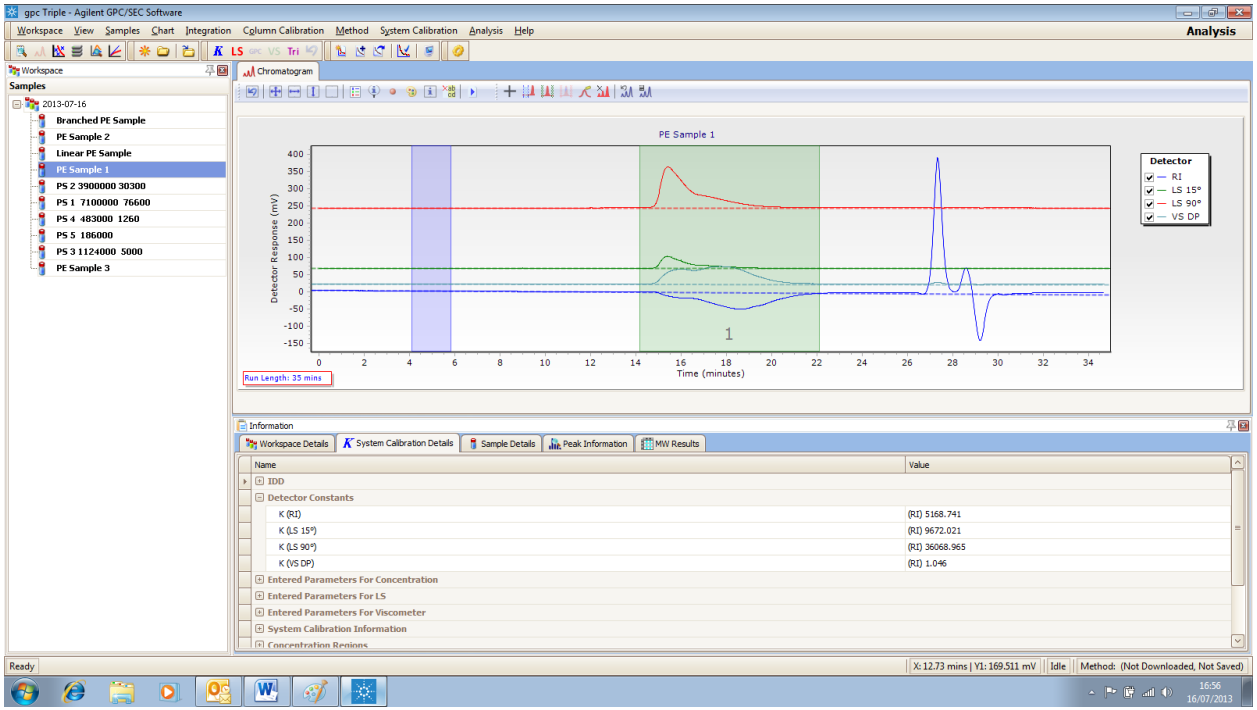


Click Finish

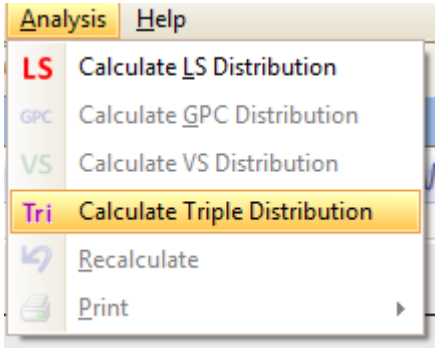
We can now look at the constants generated in the Information window below



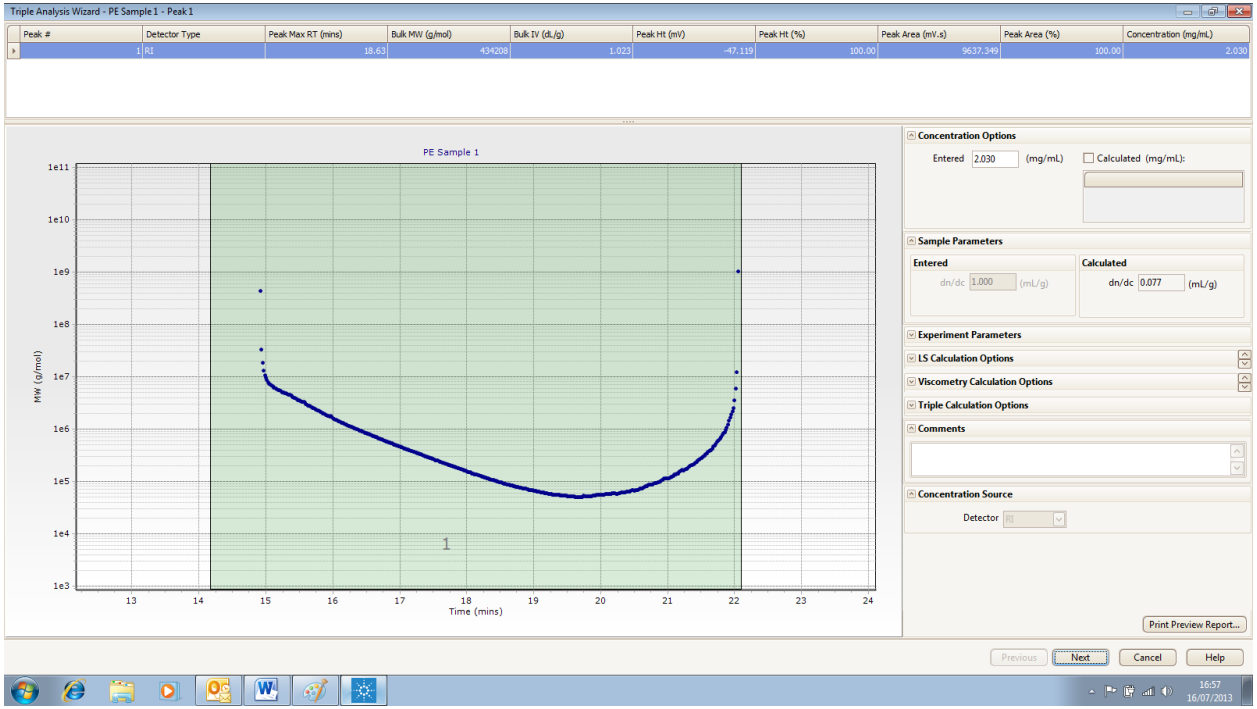
Now let's process the unknown samples and start with PE Sample 1
Highlight this sample and set base line and integration area as before:



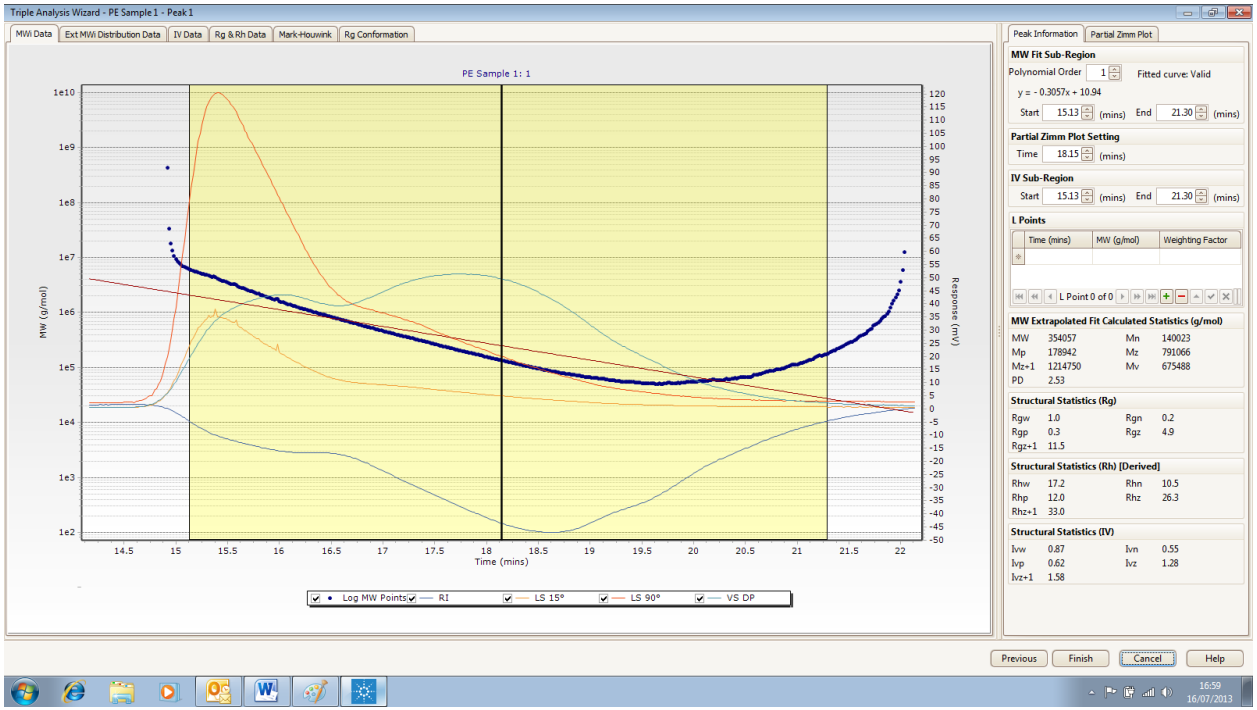
Now go to Analysis, Calculate Triple Distribution as shown below



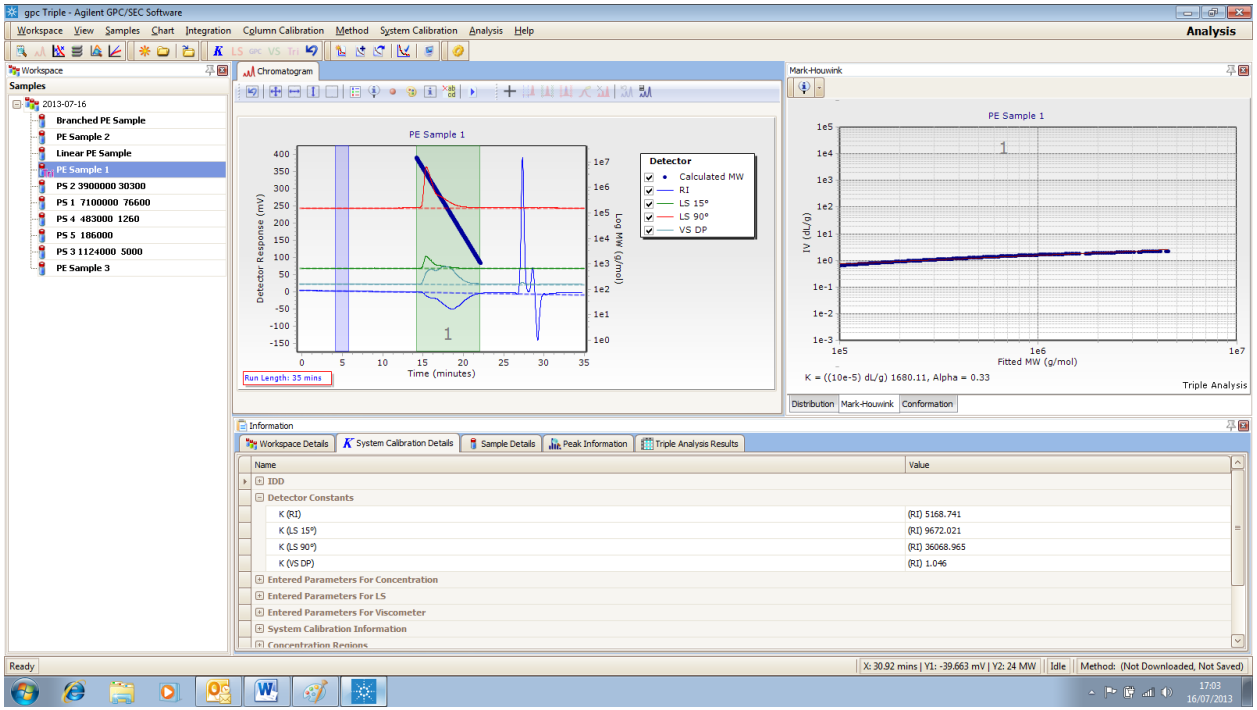
The following screen pops up



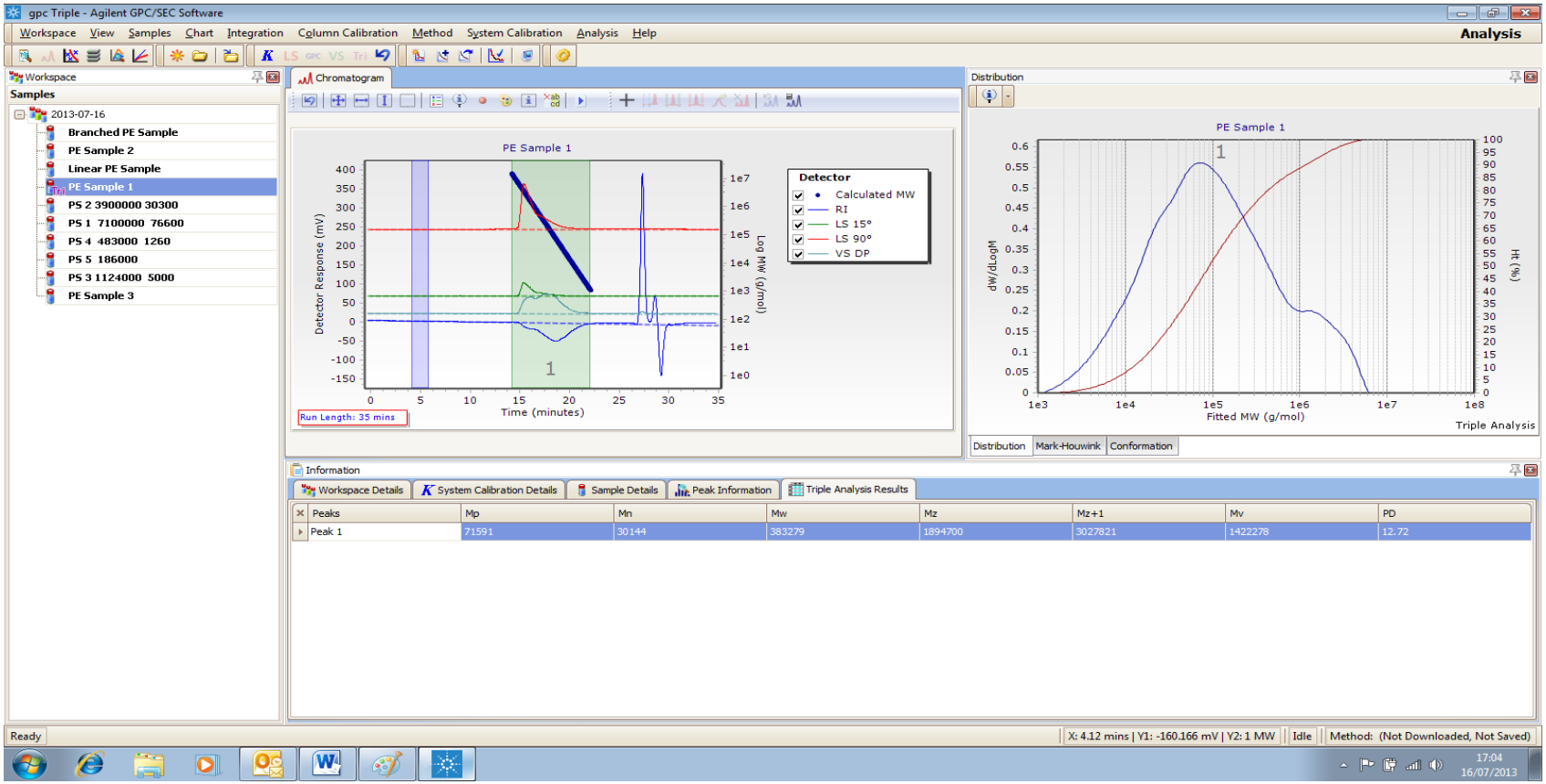
Click the Next button and the following screen pops up



Set the data fit limits – do not include the upturned part of the data which is created by weak signal from the LS detectors, Move the limits on the above screen so that the blue line is close to the linear red as above. Data is processed in the yellow area.
Then click Finish button and the following screen will be shown.



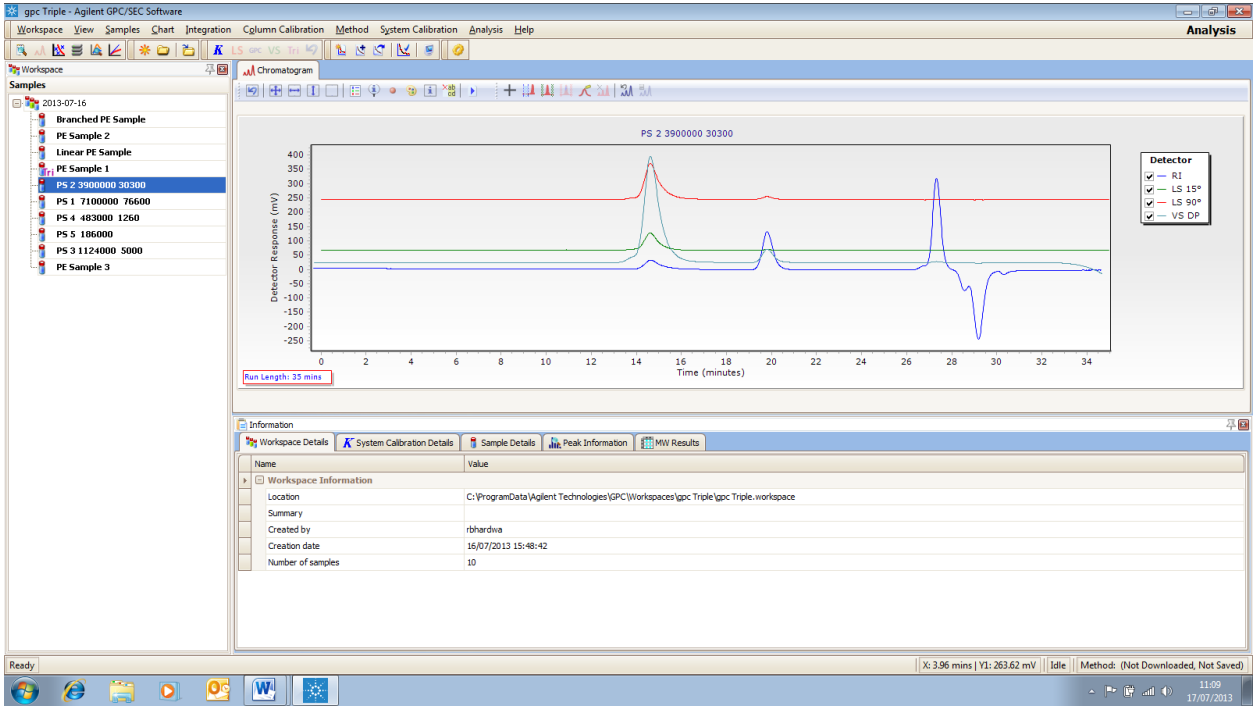
The Triple GPC analysis results are shown as below.



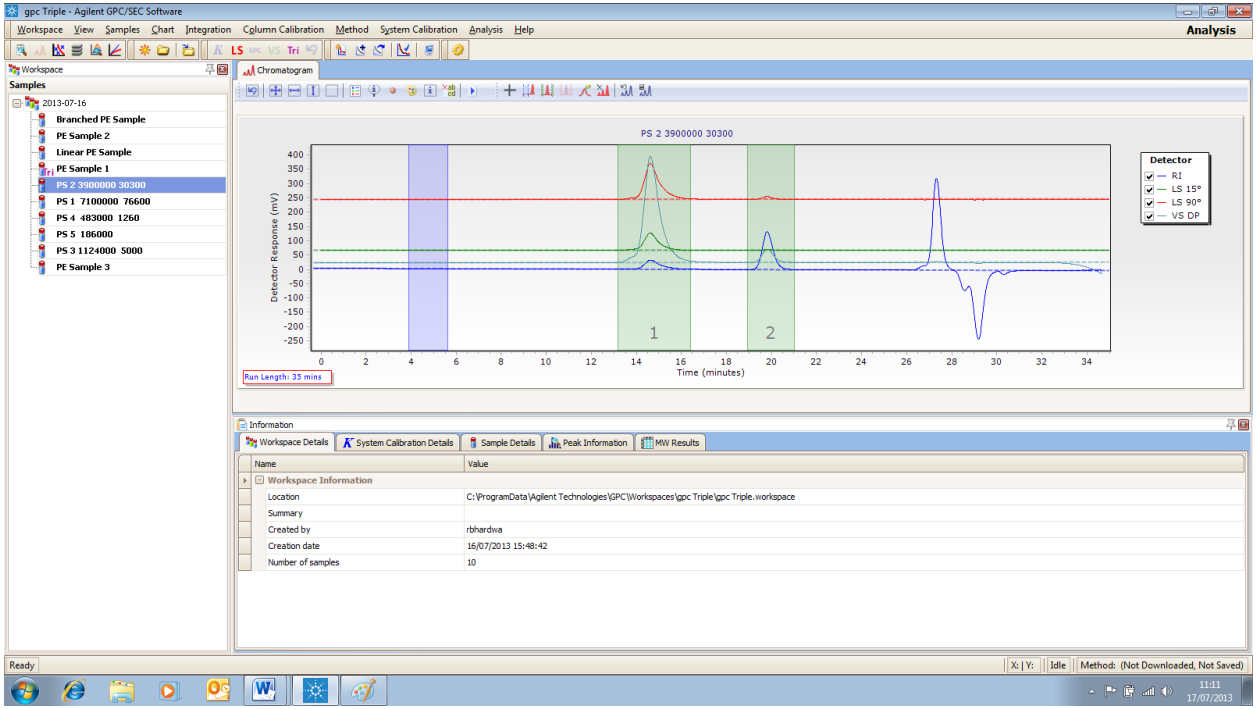
The above procedure is also applicable for RI/LS detector options also.

Generating Universal Calibration

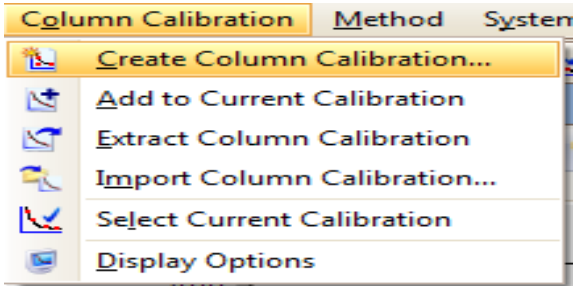
For generating a Universal Calibration we need to perform a column calibration using well chacterised polymer standards.
In this example we will use four PS EasiVial Mix standards.
Highlight the first standard file to be processed, as shown below.



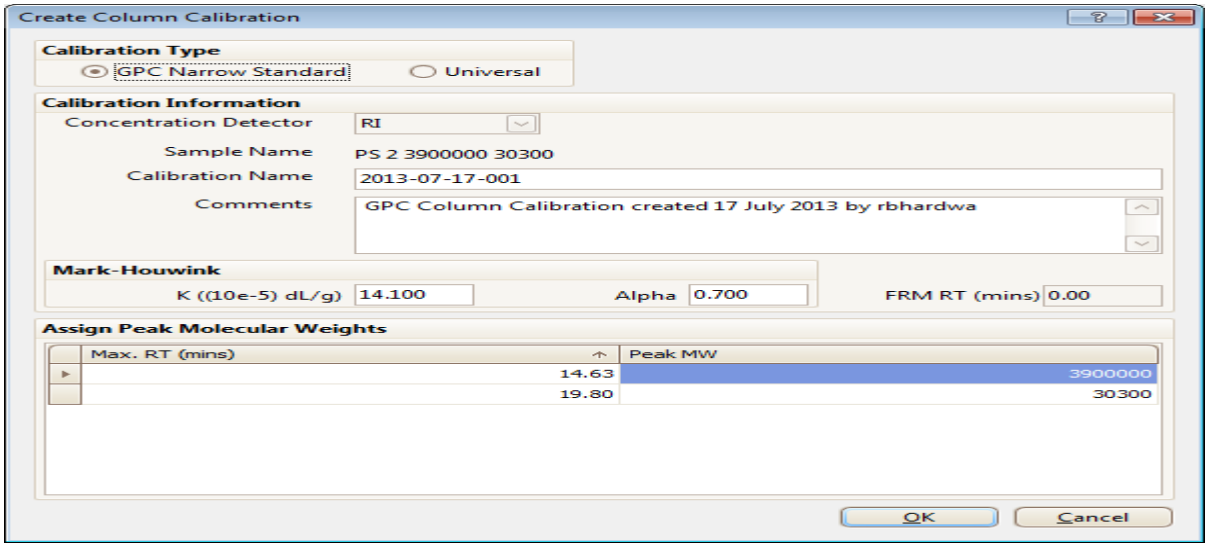
Now set the base line and the integration areas on the peaks as shown below



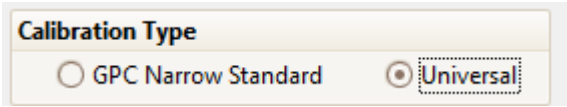
Then go to Column Calibration and Select Create Column Calibration



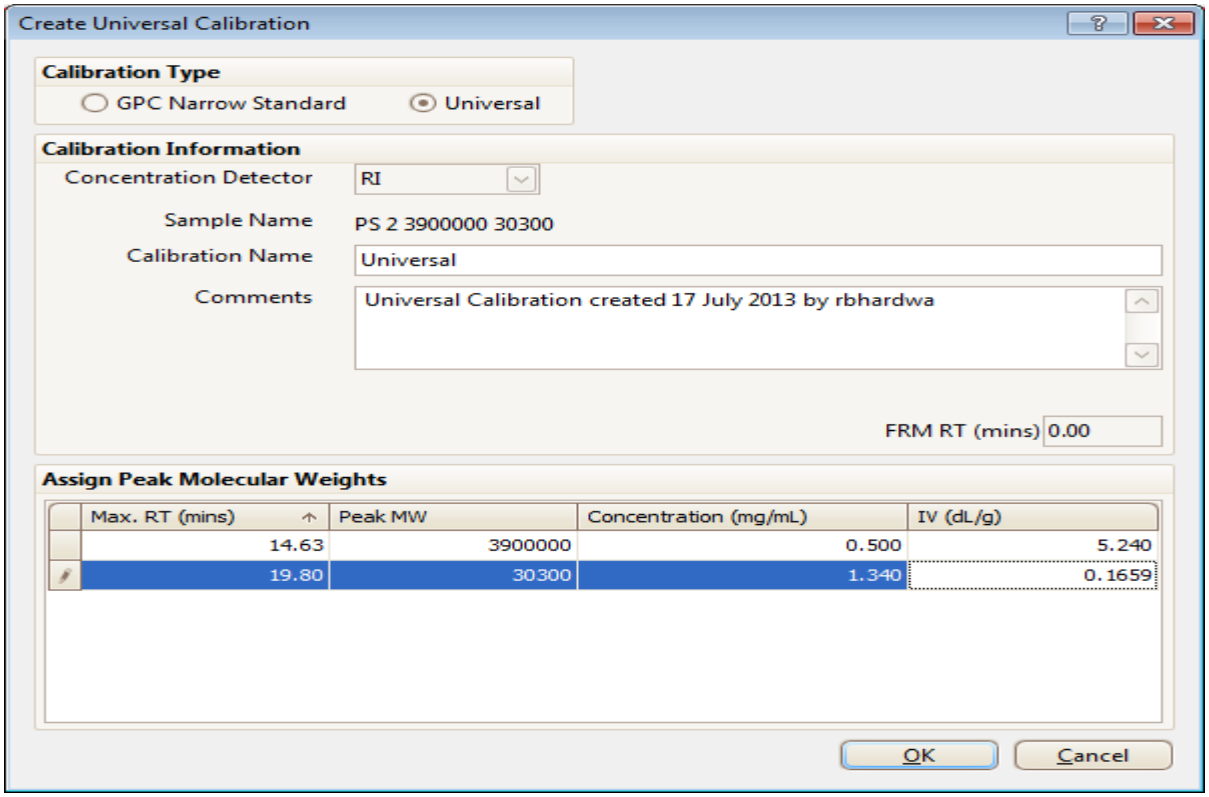
The window below will pop up



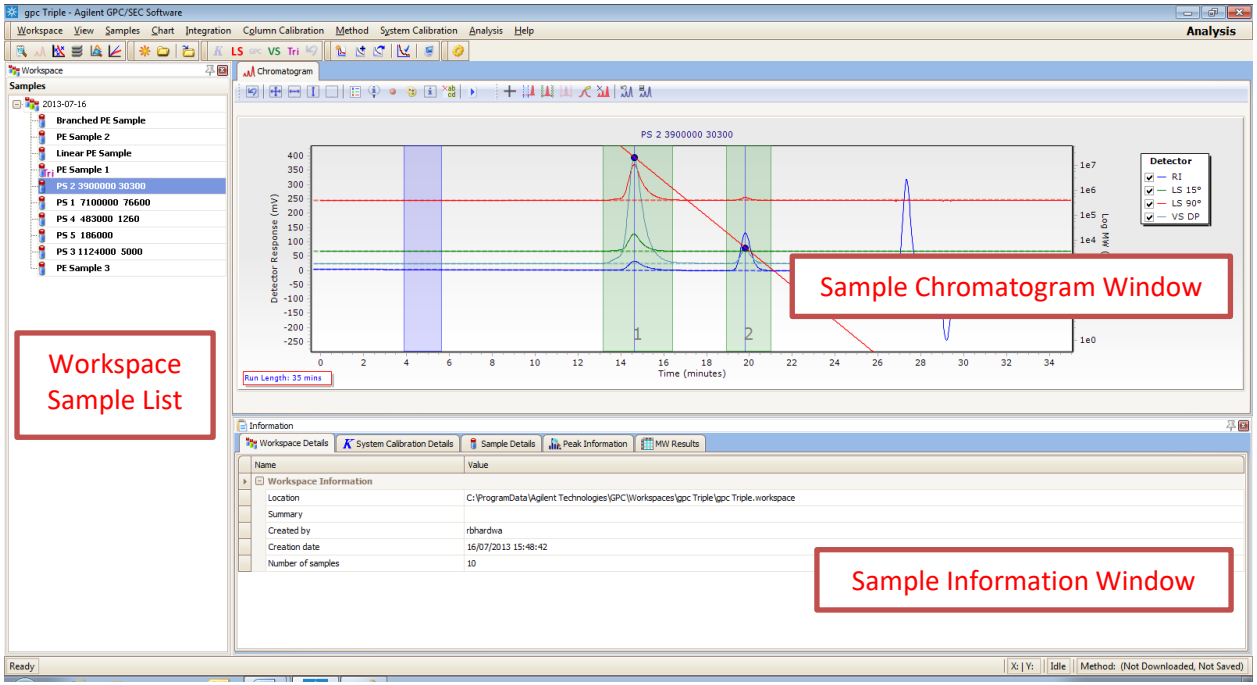
Select Universal Calibration as shown below



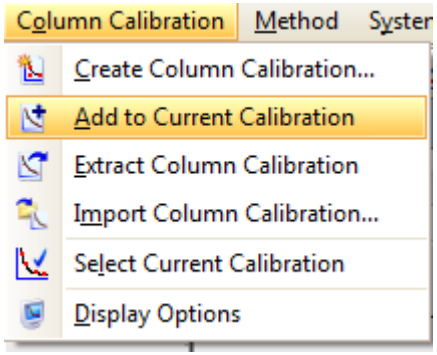
Then populate as below by inputting the Mw values for both peaks with their corresponding accurate Molecular weights.
This will automatically generate the IV values for both peaks.
At this stage you can type in a Calibration name for this calibration. In this example we have called it Universal.



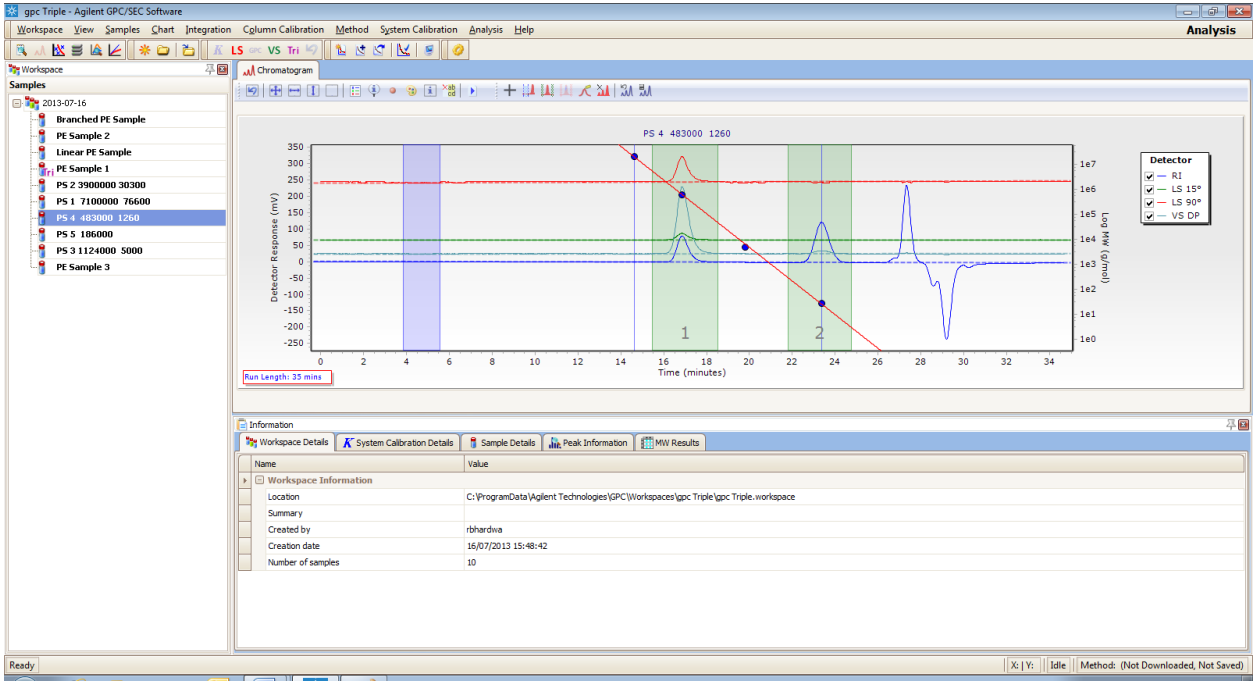
Then Click OK tab to show the window below



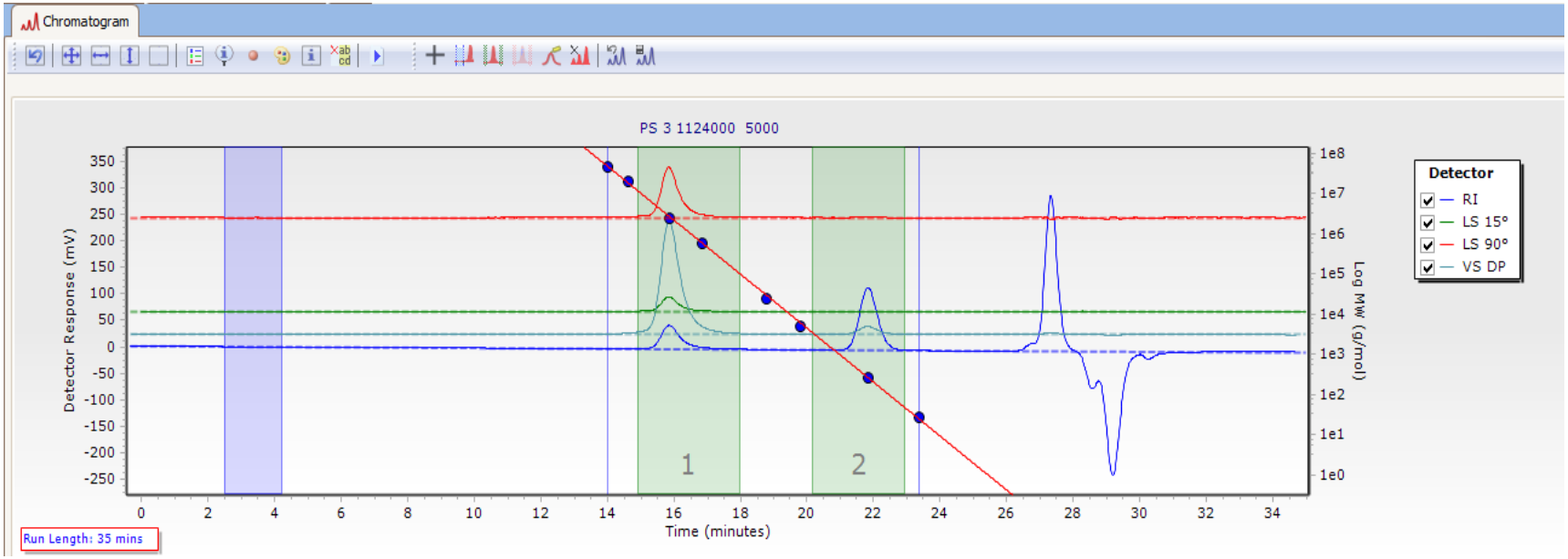
Now highlighting the 2nd sample and put base line and area integration for both peaks as with the 1st sample, and then go to Column Calibration tab and select Add to current Calibration



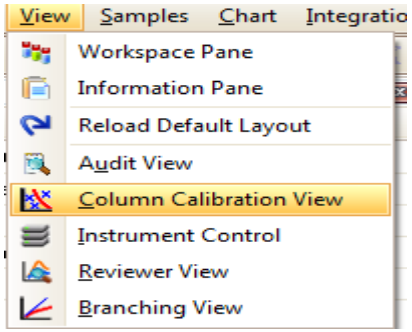
This will generate the following window

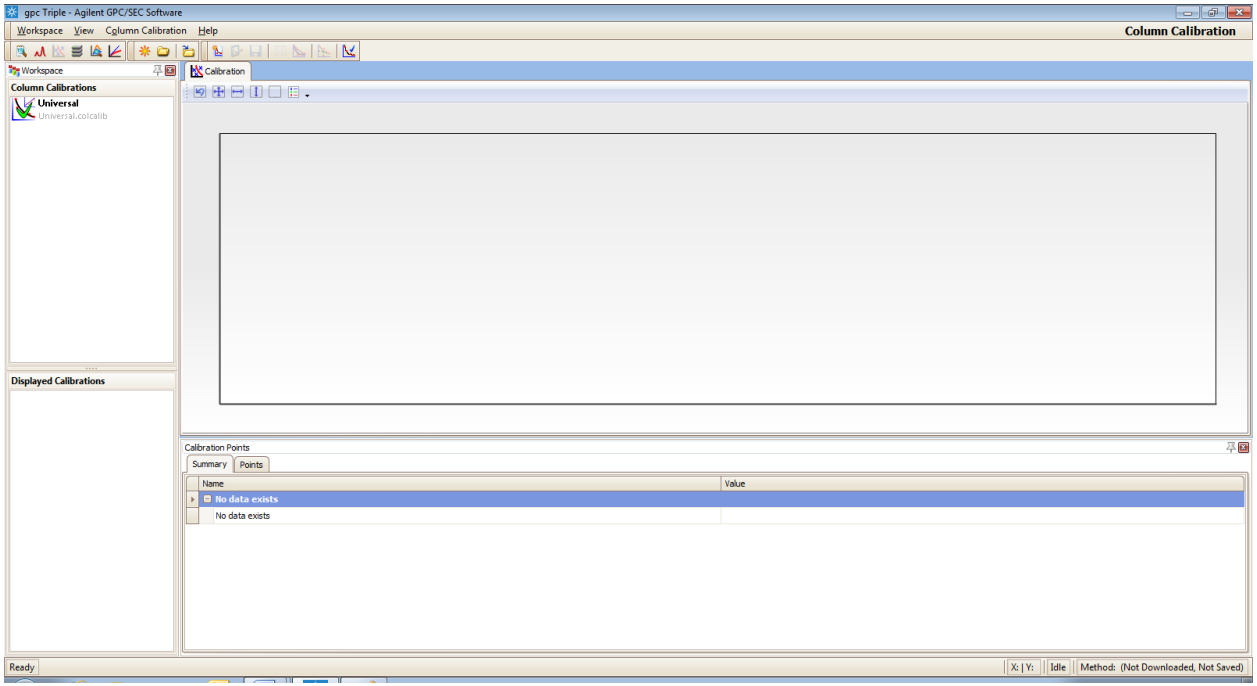


As seen above the 4 point calibration has been performed. Repeat for the next 2 standard runs to generate the following 8 point calibration

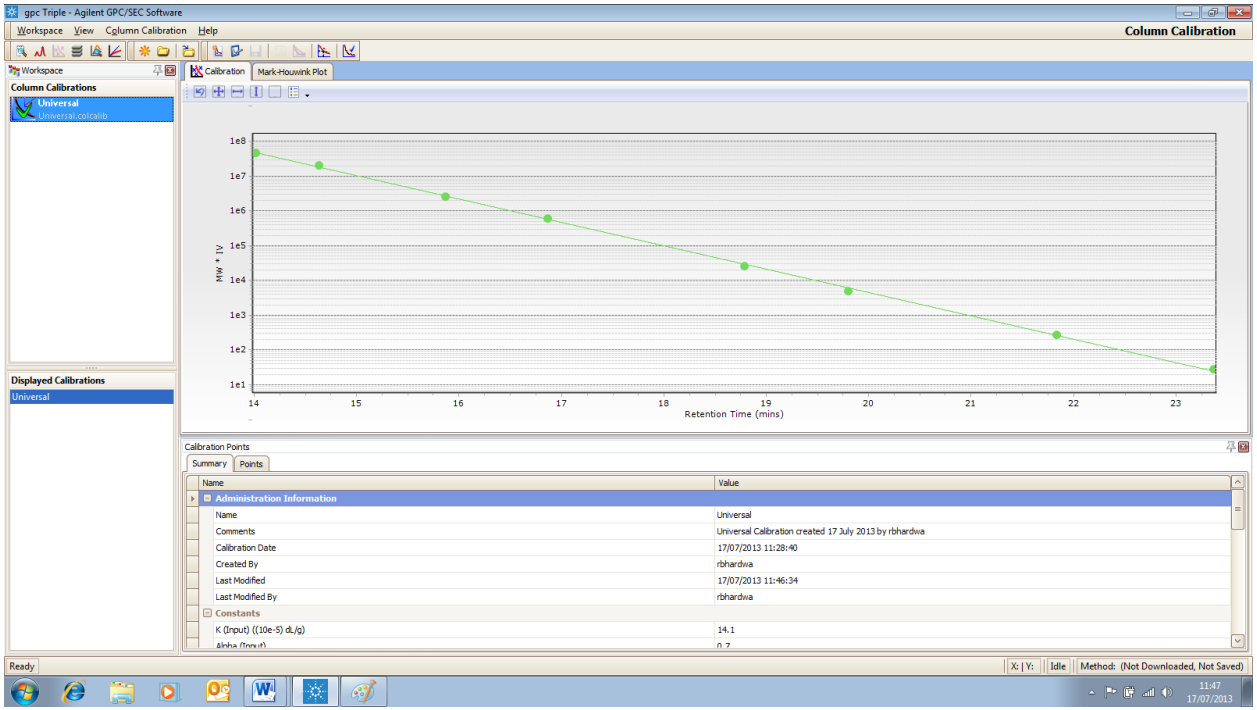


To view the created 8 point Universal Calibration go to View ,Select Column Calibration View

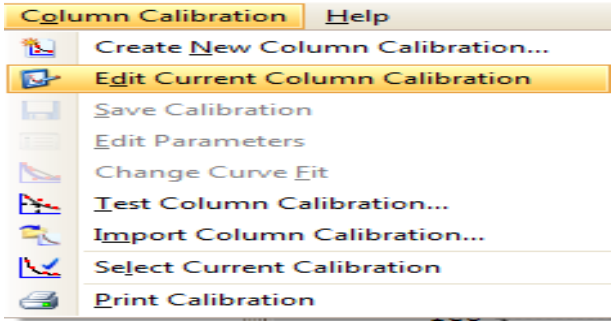




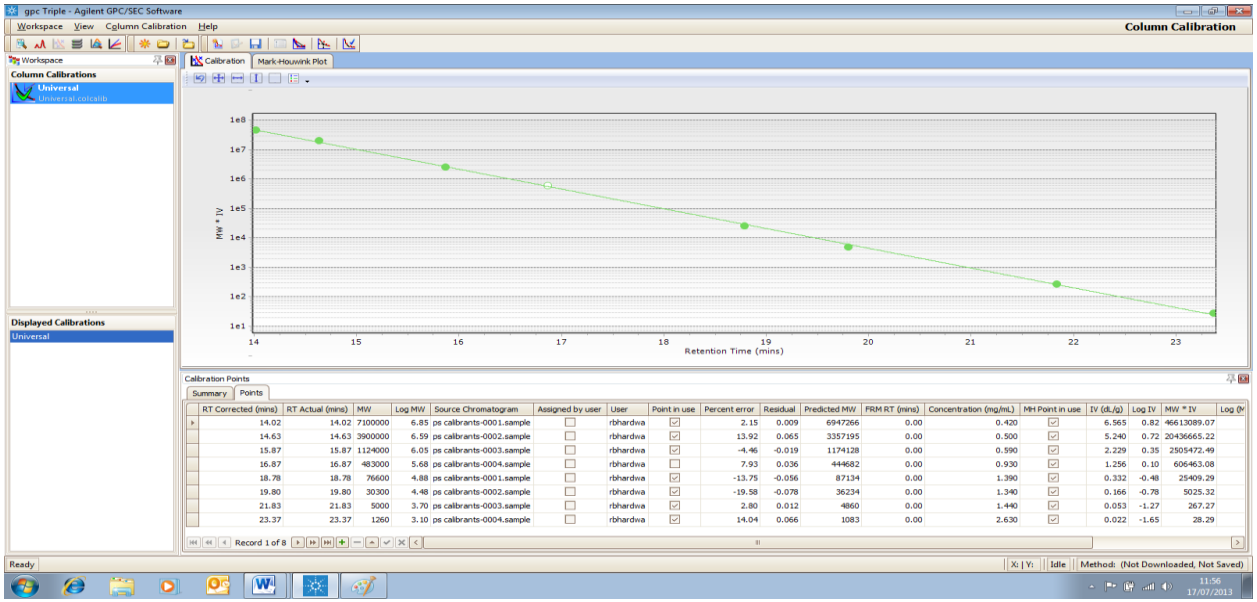
Click on the  and the following calibration window pops up


gpc Triple - Agilent GPC/SEC Software
Workspace View Column Calibration Help
Column Calibration
Workspace
Column Calibrations
Universal
Universal.colcalib
Displayed Calibrations
Universal
Calibration
Mark-Houwink Plot
MW * IV
1e8
1e7
1e6
1e5
1e4
1e3
1e2
1e1
14
15
16
17
18
19
20
21
22
23
Retention Time (mins)
Calibration Points
Summary
Points
Name
Value
Administration Information
Name
Universal
Comments
Universal Calibration created 17 July 2013 by rbhardwa
Calibration Date
17/07/2013 11:28:40
Created By
rbhardwa
Last Modified
17/07/2013 11:46:34
Last Modified By
rbhardwa
Constants
K (Input) ((10e-5) dL/g)
14.1
Ready
X: | Y:
Idle
Method: (Not Downloaded, Not Saved)
11:47
17/07/2013

You can edit the generated calibration (add or remove points) go to
Column Calibration tab and select Edit Current Column Calibration

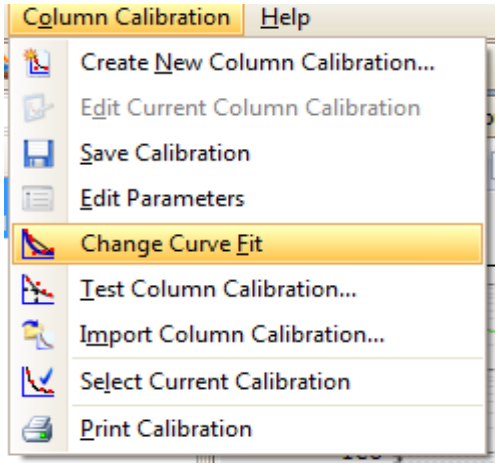


And click on any point to remove it from the calibration.In this example we have deselected the 4th point (Mp 48,3000) as shown below.
This can be selected again by simply clicking onto the point to reactive it.

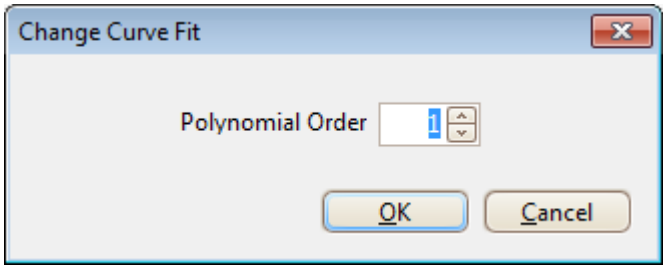


RT Corrected (mins)	RT Actual (mins)	MW	Log MW	Source Chromatogram	Assigned by user	User	Point in use	Percent error	Residual	Predicted MW	FRM RT (mins)	Concentration (mg/mL)	MH Point in use	IV (dL/g)	Log IV	MW * IV	Log (M
14.02	14.02	7100000	6.85	ps calibrants-0001.sample		rbhardwa		2.15	0.009	6947266	0.00	0.420		6.565	0.82	46613089.07	
14.63	14.63	3900000	6.59	ps calibrants-0002.sample		rbhardwa		13.92	0.065	3357195	0.00	0.500		5.240	0.72	20436665.22	
15.87	15.87	1124000	6.05	ps calibrants-0003.sample		rbhardwa		-4.46	-0.019	1174128	0.00	0.590		2.229	0.35	2505472.49	
16.87	16.87	483000	5.68	ps calibrants-0004.sample		rbhardwa		7.93	0.036	444682	0.00	0.930		1.256	0.10	606463.08	
18.78	18.78	76600	4.88	ps calibrants-0001.sample		rbhardwa		-13.75	-0.056	87134	0.00	1.390		0.332	-0.48	25409.29	
19.80	19.80	30300	4.48	ps calibrants-0002.sample		rbhardwa		-19.58	-0.078	36234	0.00	1.340		0.166	-0.78	5025.32	
21.83	21.83	5000	3.70	ps calibrants-0003.sample		rbhardwa		2.80	0.012	4860	0.00	1.440		0.053	-1.27	267.27	
23.37	23.37	1260	3.10	ps calibrants-0004.sample		rbhardwa		14.04	0.066	1083	0.00	2.630		0.022	-1.65	28.29	

The curve fit can also be changed if required. Highlight Column Calibration tab and select Change Curve Fit



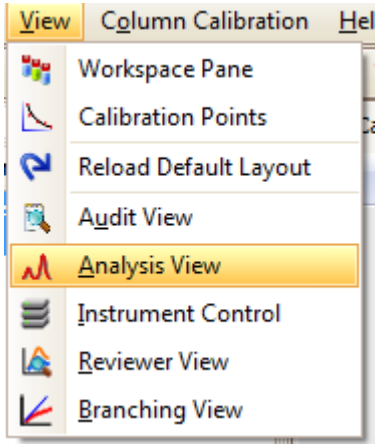
This will show the following window



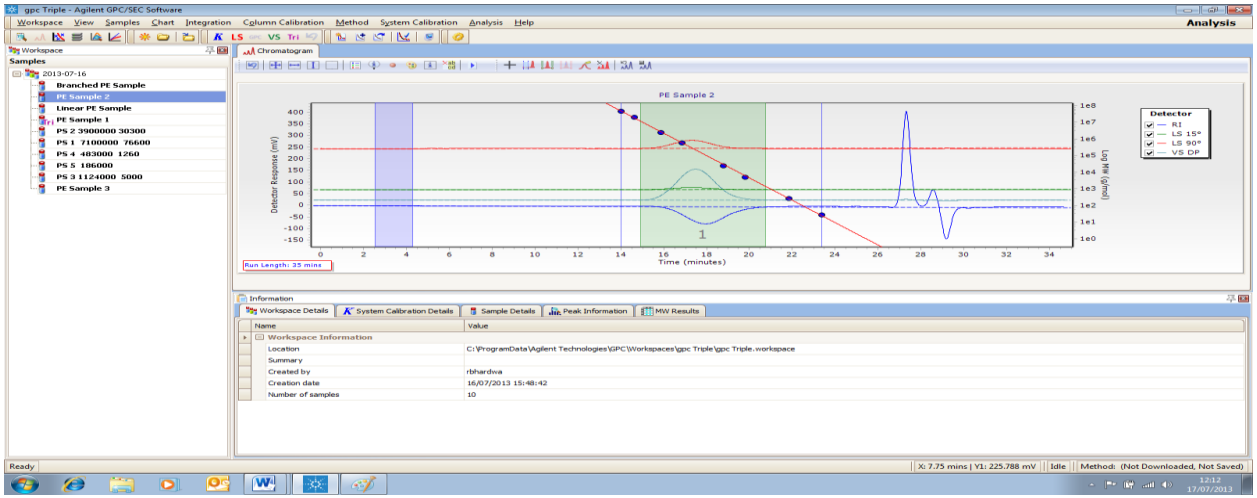
Change the order fit 1-5,but generally a good fit is 1-2. And select OK

This will now save your calibration.

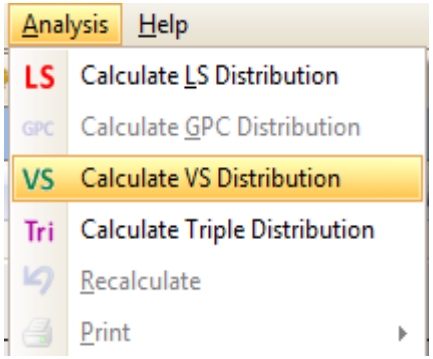
Know proceed to Analysis View as below to process the unknown samples



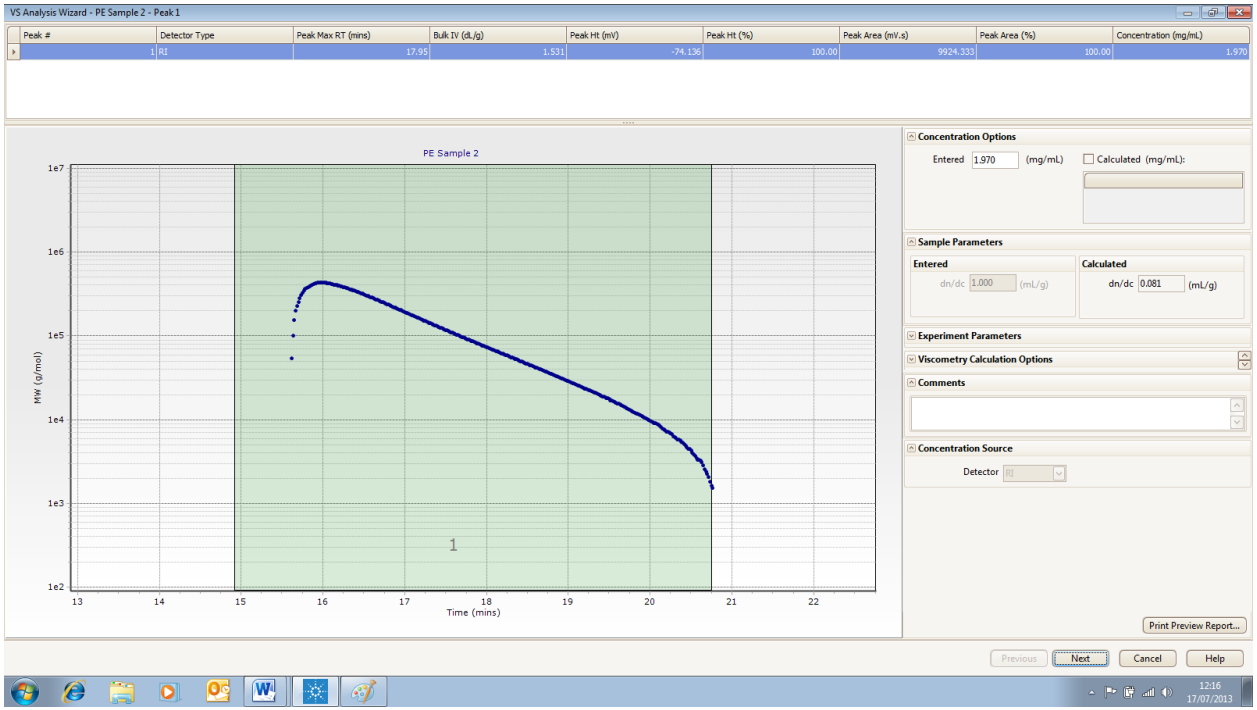
Highlight sample PE Sample 2 put in base line and area integration as before and the following window is seen



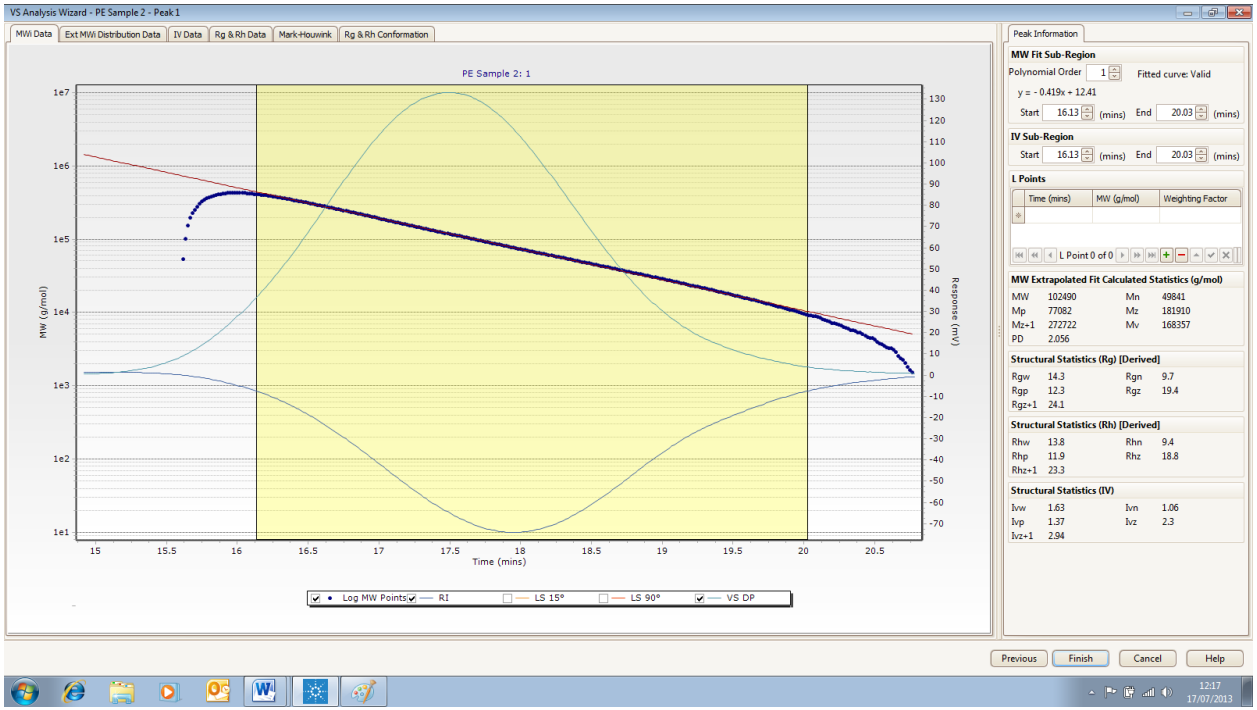
Now go to Analysis lab and select Calculate VS Distribution



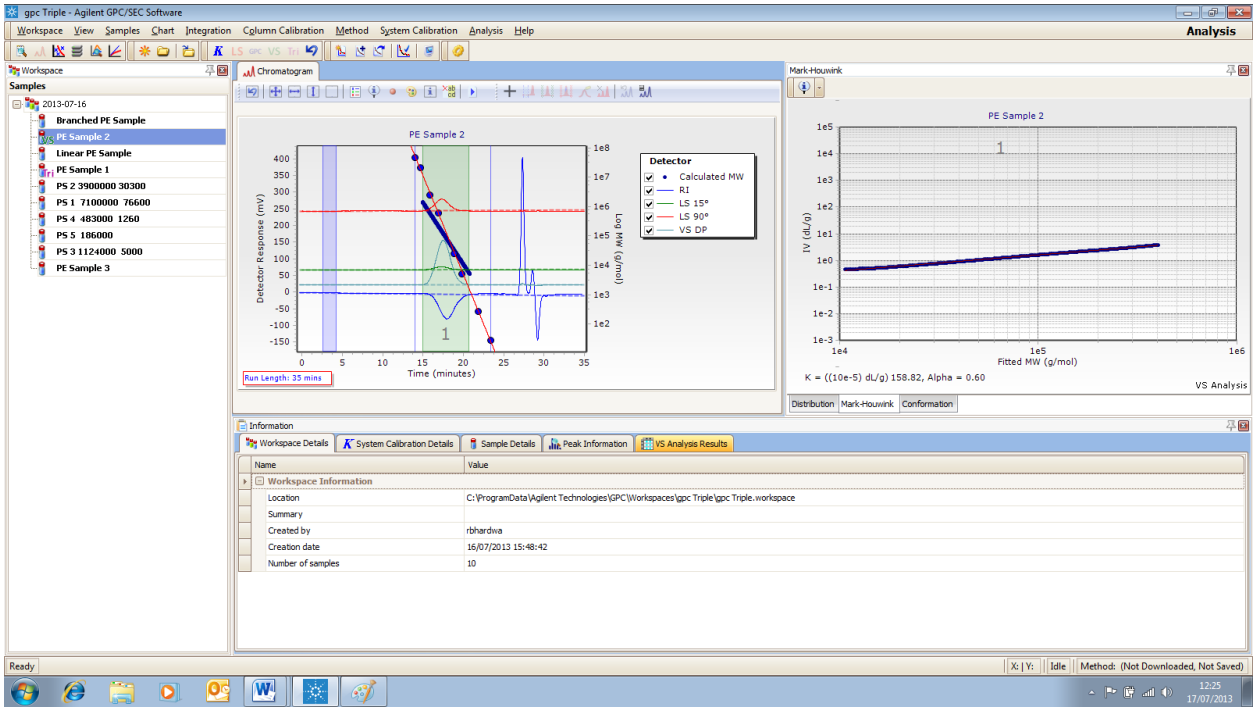
The following screen pops up



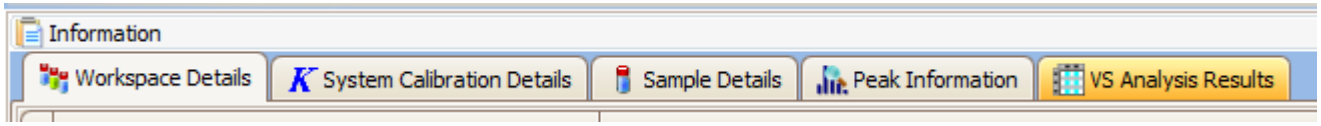
Click Next to go to the following screen



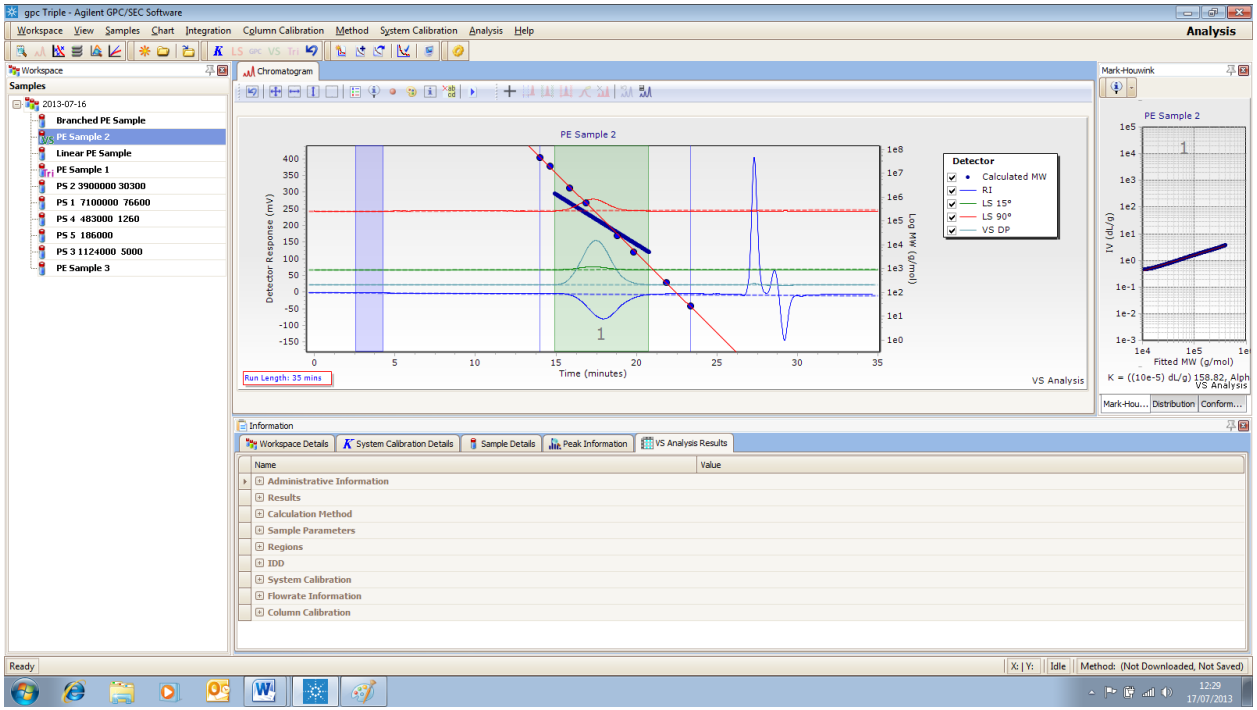
Set the data fit limits – do not include the upturned part of the data which is created by weak signal from the detectors, Move the Mw Fit start cursor and Mw Fit End Cursor to define the liner part of the curve fit.
Once you have done this Click Finish and the following screen is displayed



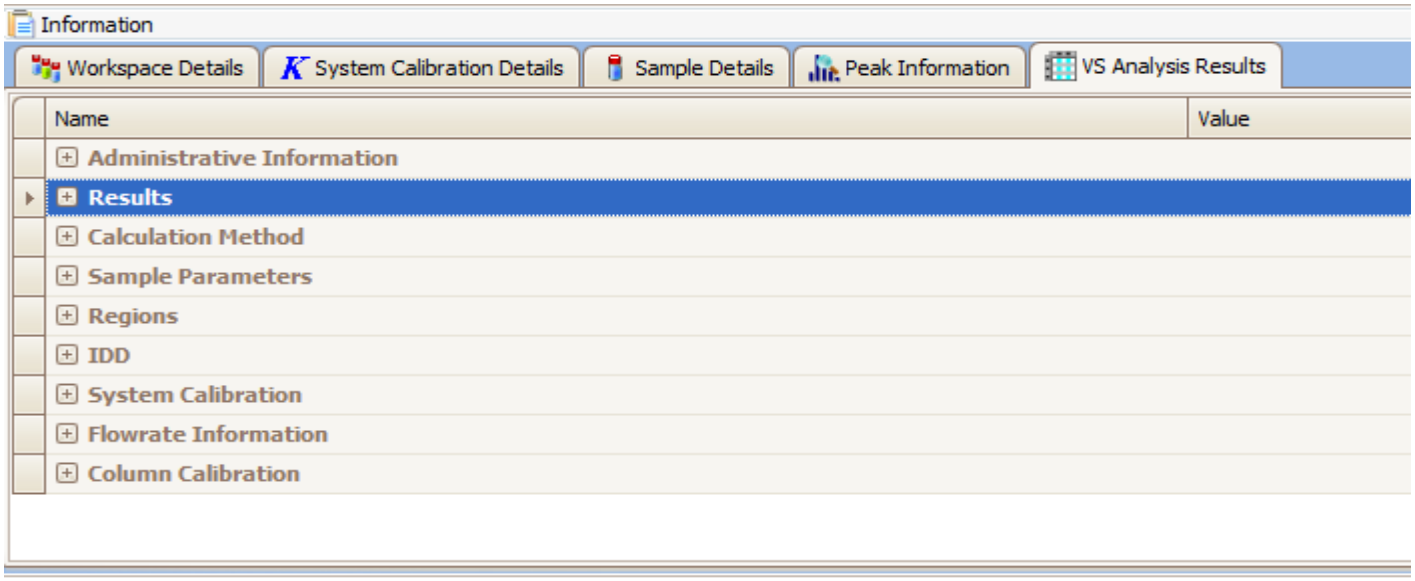
Now to see the data for this sample select VS Analysis Results tab



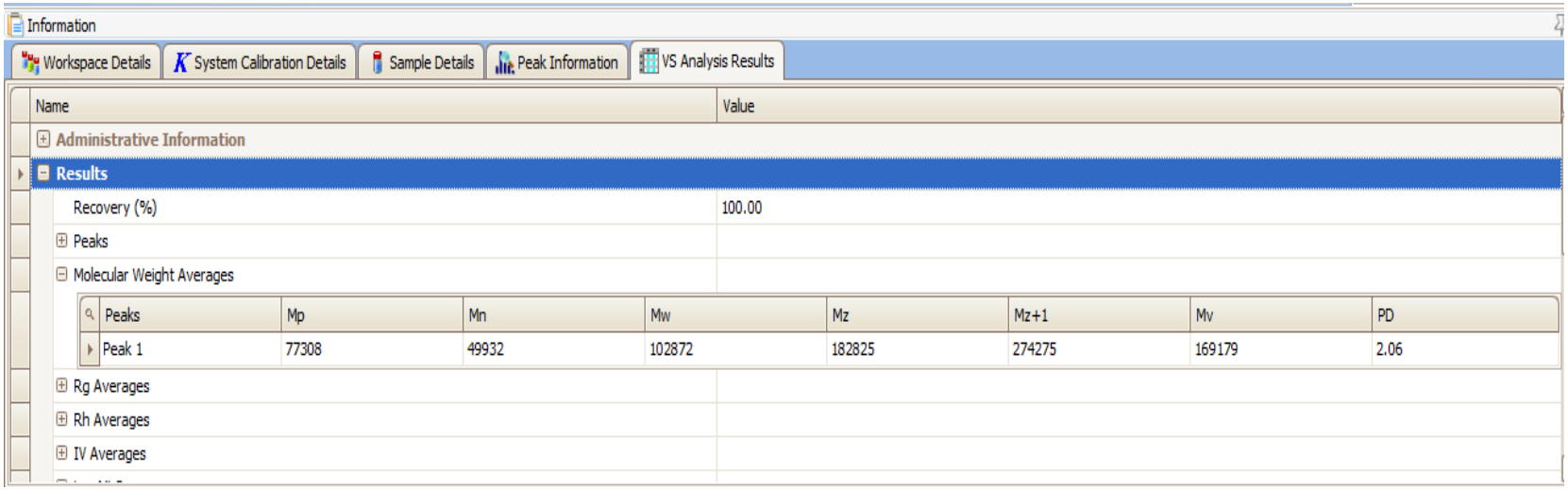
The following screen pops up



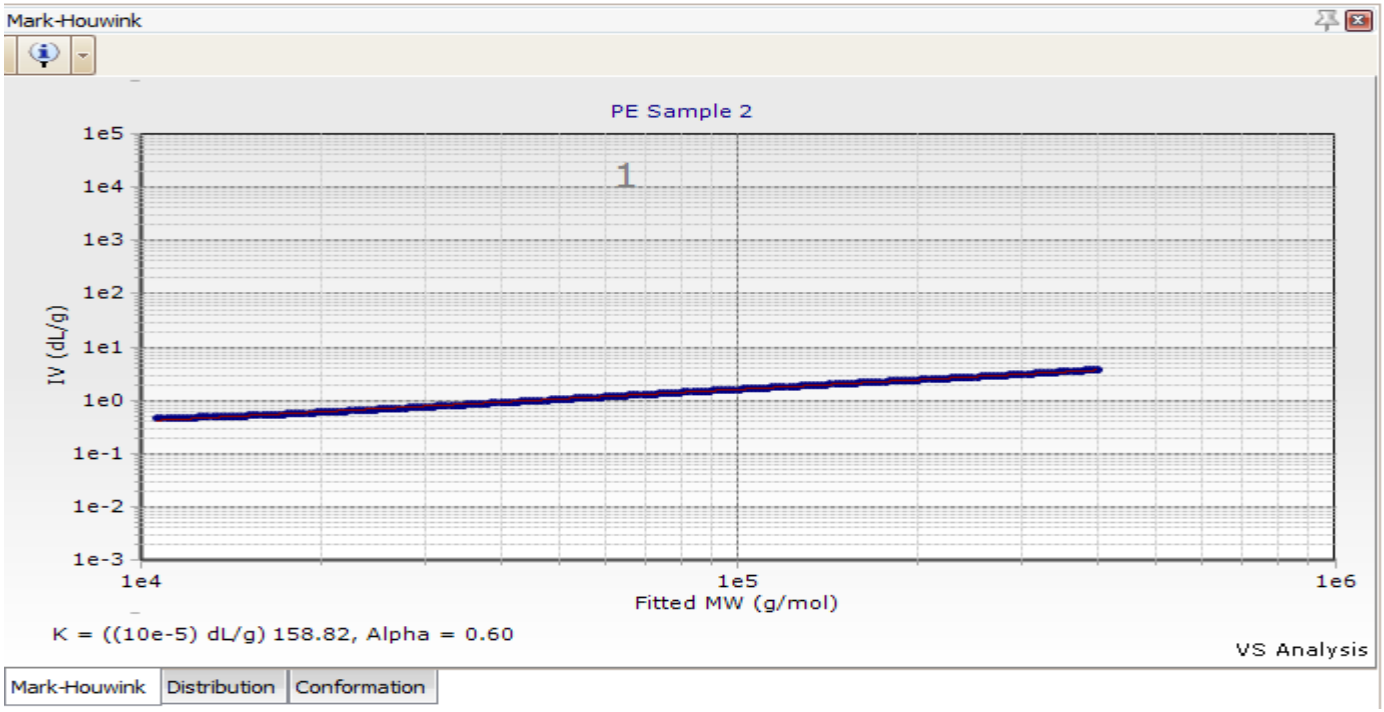
Now Click on the +Results tab, as highlighted below.

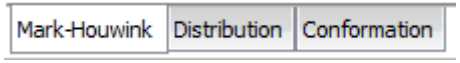


The results can be seen as below.



In addition to the molecular weight data, Mark-Houwink , Distribution and Conformation plots can also be displayed, as shown below.



Each plot can be displayed by selecting their individual tabs 

Branching In Polymer Analysis

Introduction

Branching can be measured by LS/Vic by looking at changes in the molecular size (Rg) or Intrinic Viscosity (IV) as a function of increasing Molecular Weight. Branched polymers always have lower Rg & IV values than linear analogs, this is due to presence of branched points. The calculations can be done by either IV (measured or calculated) or radius of gyration (Rg measured or calculated) data.
The quality of the results depends on the quality of the source data e.g Rg or IV.
Contraction factors are determined from Mark-Houwink (log IV Vs log Mw) or Conformation (log Rg Vs log Mw) plots using the relationship below:

$$g = (Rg_{branched}/Rg_{linear})_{Mw}$$

Were g is Radius of gyration contraction factor or branching Ratio

==========

For many polymers and applications this is as far as the branching analysis can be taken
This is especially true if the nature of the polymer is not known or if it is complex, or if the nature of the branches is not certain
At this point a qualitative indication of the level of branching is obtained
The analysis can only be advanced to give values if the exact repeat unit structure of the polymer is understood and the nature and rough distribution of the branching is known
Many of the methods that are used when measuring branching numbers only really apply to polyolefins
This is because polyolefins have a very simple structure and also because the presence of branching has proved of great commercial significance

The ratio of the intrinsic viscosity or radius of gyration of a branched polymer compared to a linear polymer of the same molecular weight is known as a contraction factor:

$$g = \frac{Rg\ (branched)}{Rg\ (linear)} \qquad g' = \frac{IV\ (branched)}{IV\ (linear)}$$

At any given molecular weight:

- The Rg contraction factor measures a contraction in size
- The IV contraction factor measures an increase in molecular density

and they are not equivalent

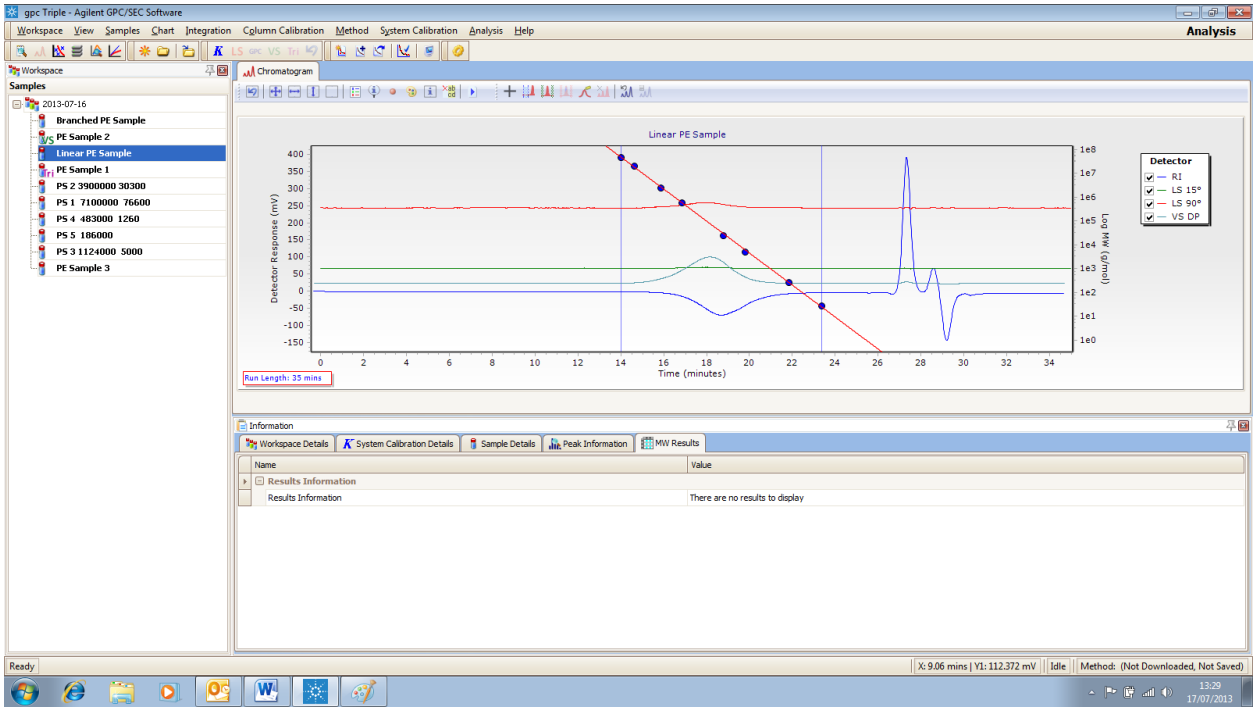
The value of g can be obtained from g' using the following relationship where ε is the structure factor, a value between 0.5 and 1.5

$$g = g'^{(1/\varepsilon)}$$

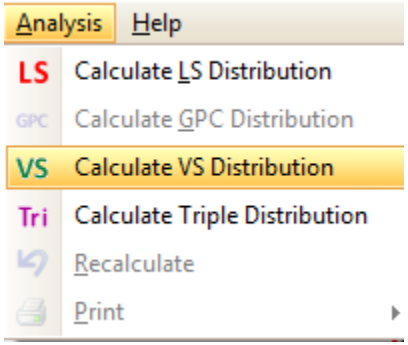
All branching models are based on g, therefore for accurate determination of Branching numbers and frequency using a Viscometer, a robust value of ε is required.

Doing Branching Calulations on collected data

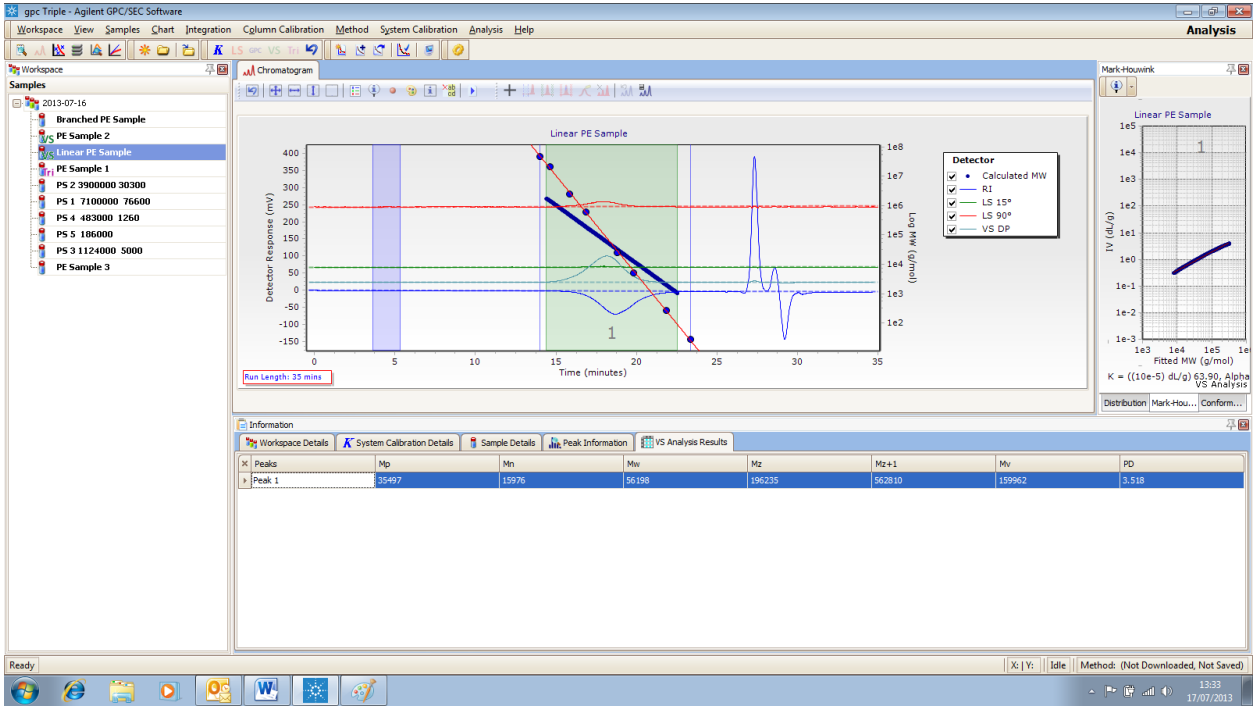
Highlight the Linear analogue (Linear PE Sample)



Draw the base line and area Integration and then calculate the results by selecting Calculate Vs Distribution

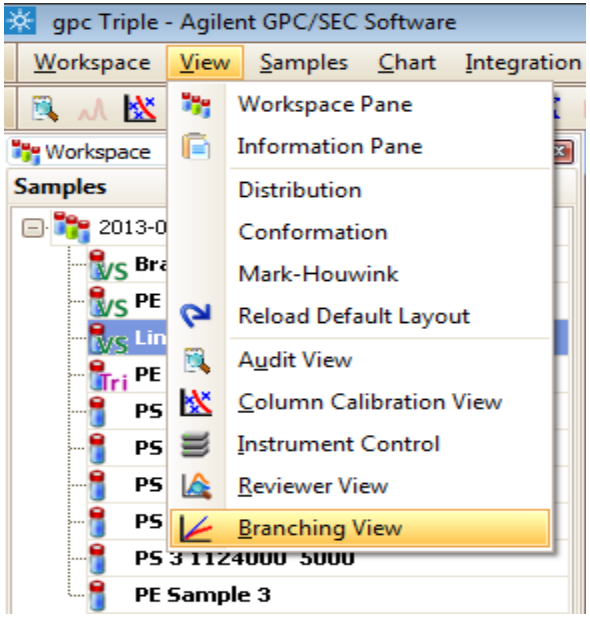


This will give the results molecular weight data for the linear sample, as seen below.

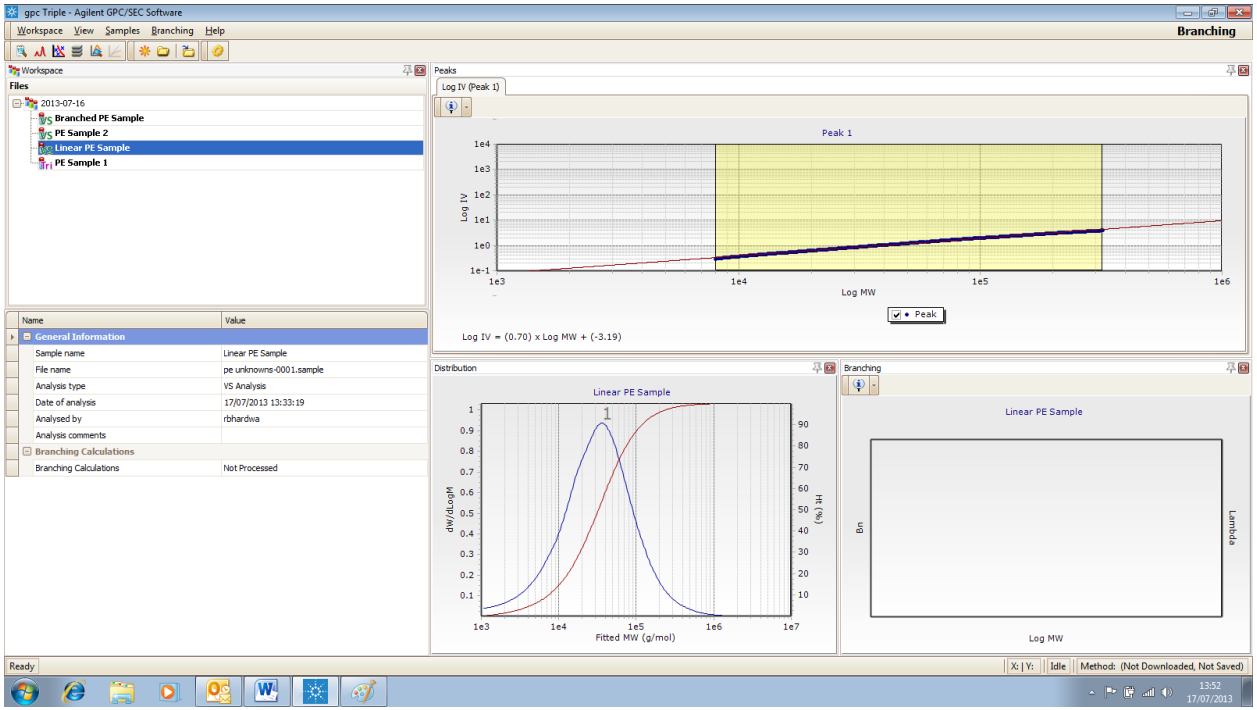


Now repeat the above steps for the branched PE Sample.

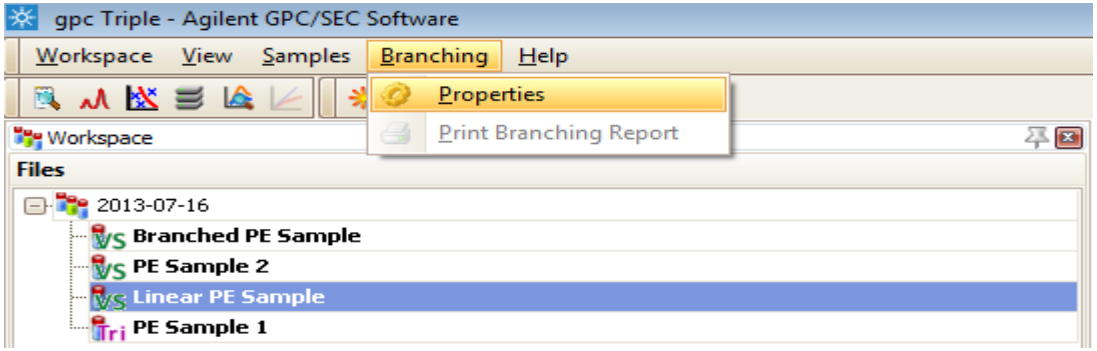
Once you have done this highlight the linear sample again and go to View & select the Branching View



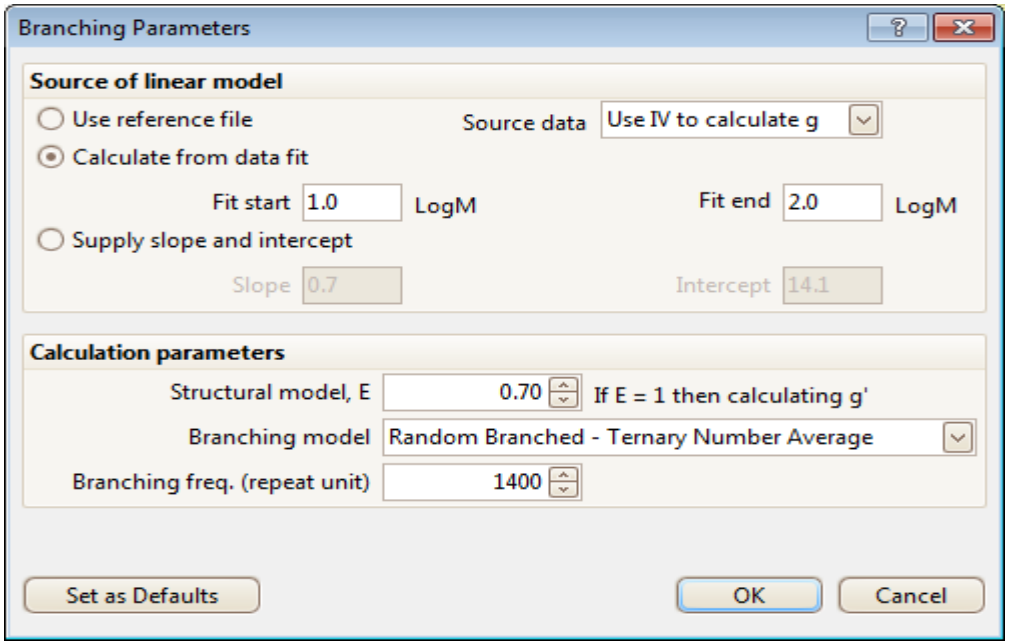
The following screen pops up



Then go to Branching Tab and select Properties

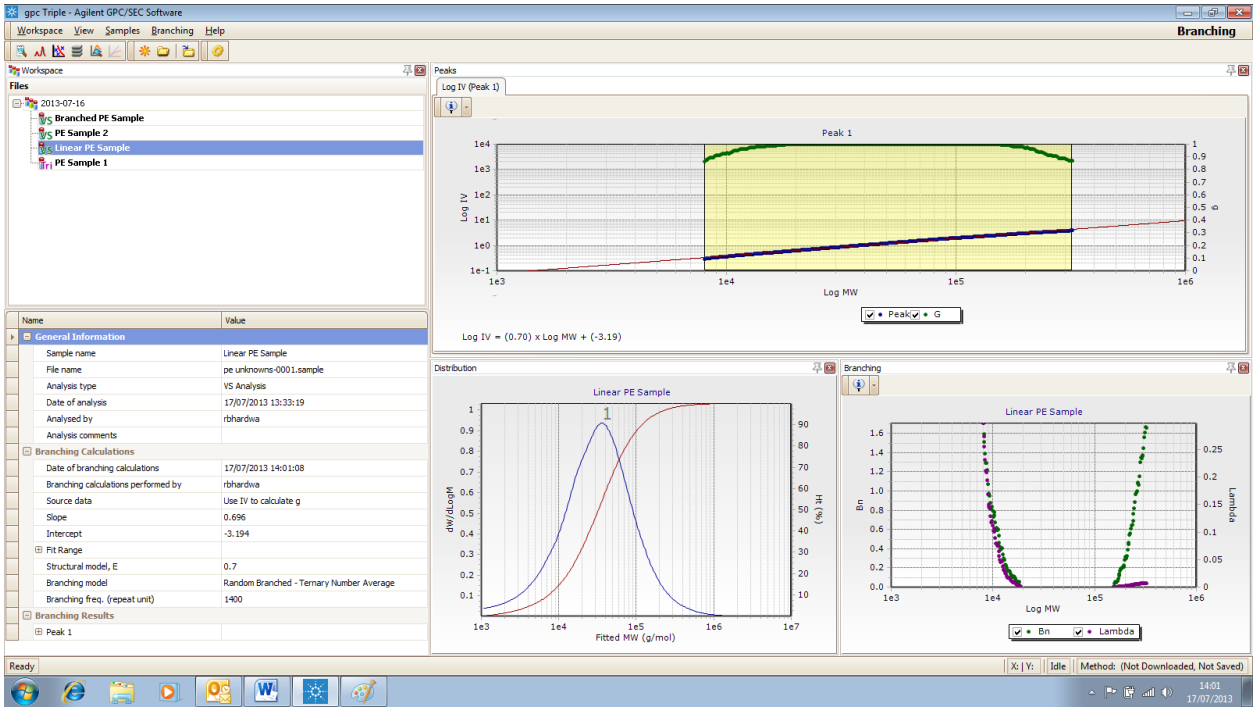


The window below will pop up. Edit the window with the appropriate information as shown.

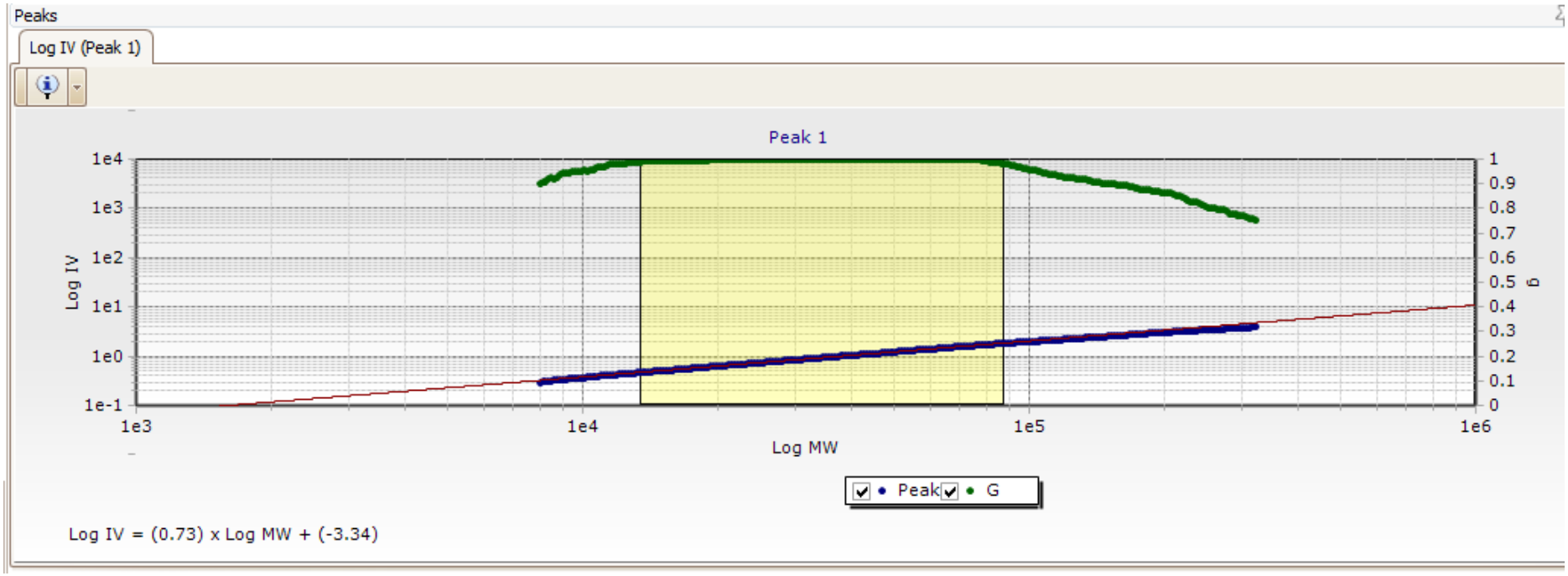


Click OK

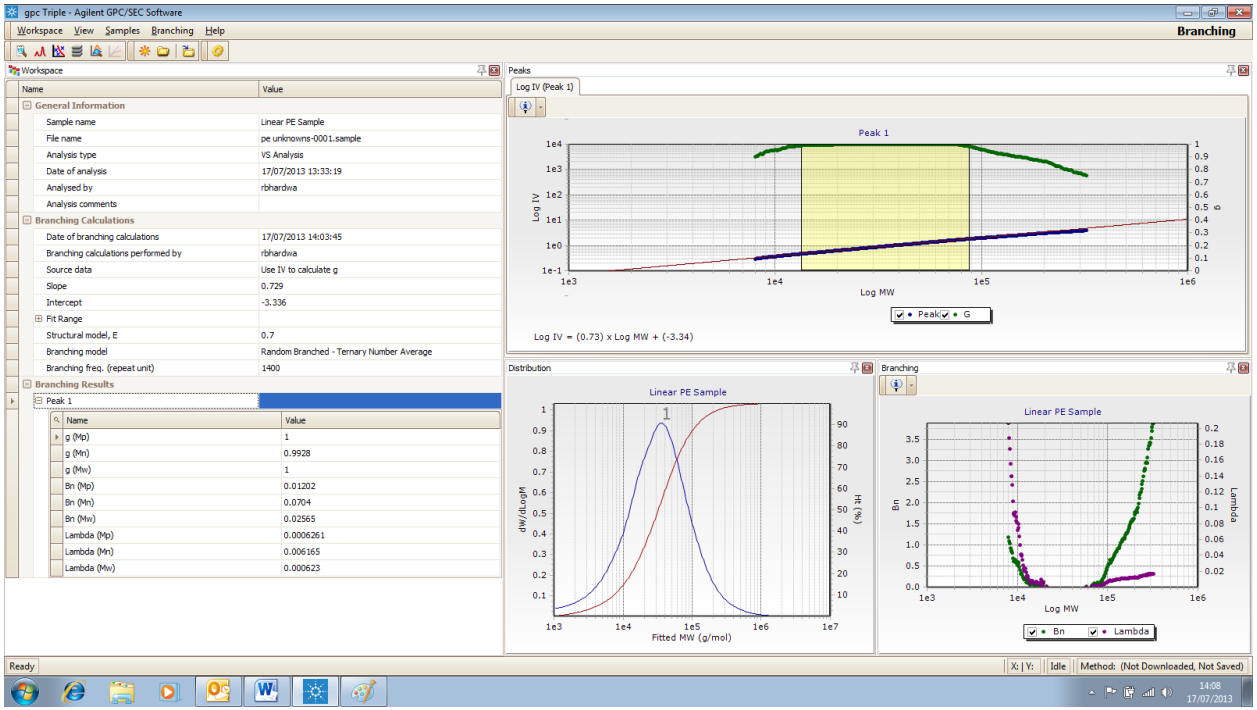
And the following screen will pop up.



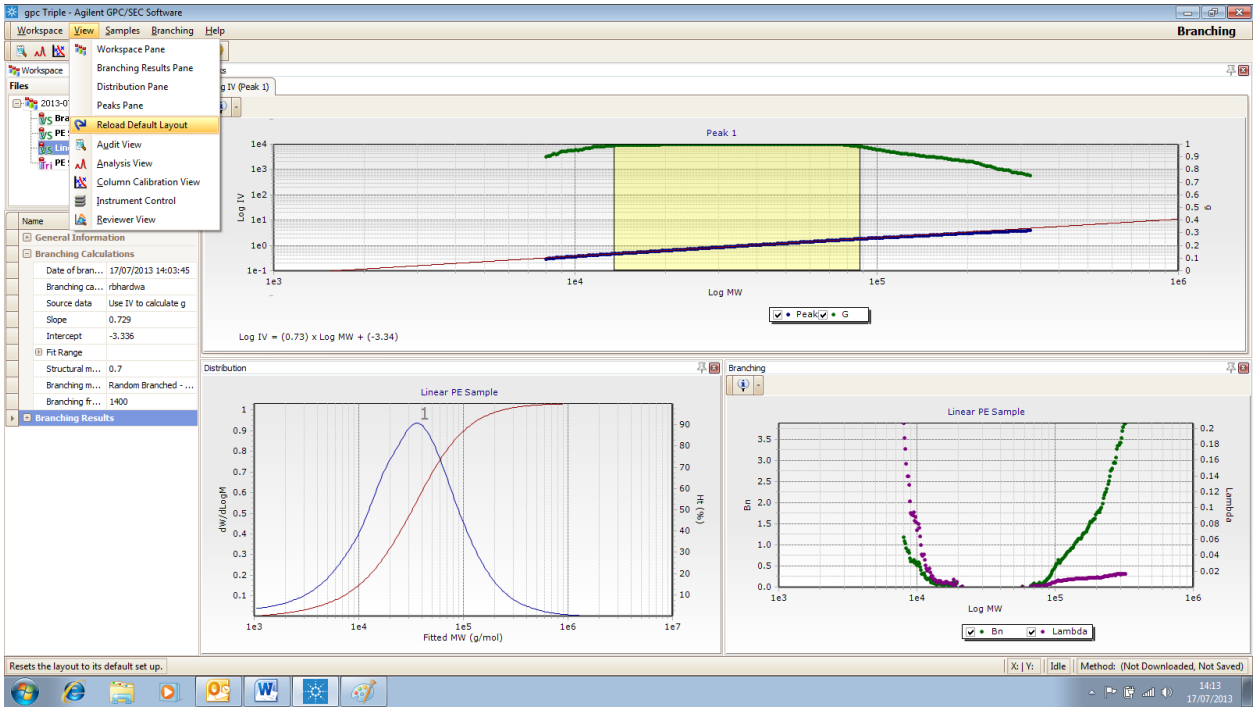
Move the highlighted region in yellow to get a good linear fit (as shown below)



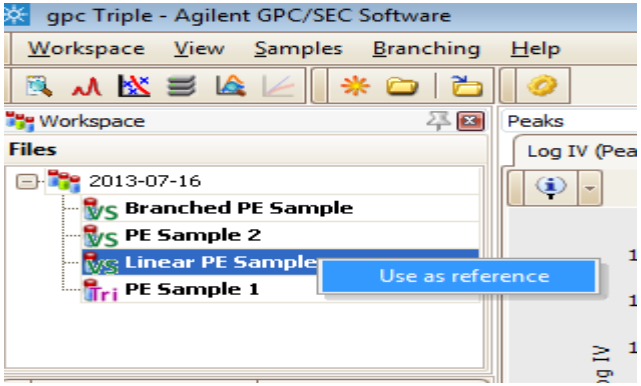
To see the results for the linear sample go to the branching Results window and click on + Peak 1, as shown below.



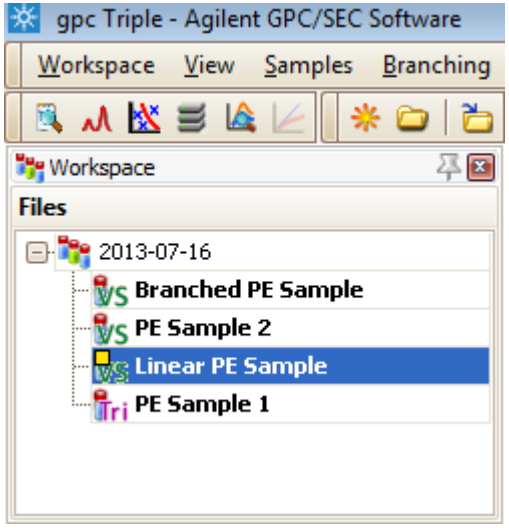
Now in order to view the linear highlighted sample go to View tab and select Reload Default layout.
This will show the following window



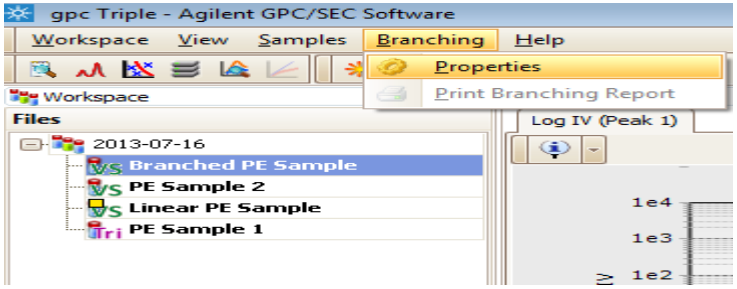
Then Right Click on the Linear PE Sample



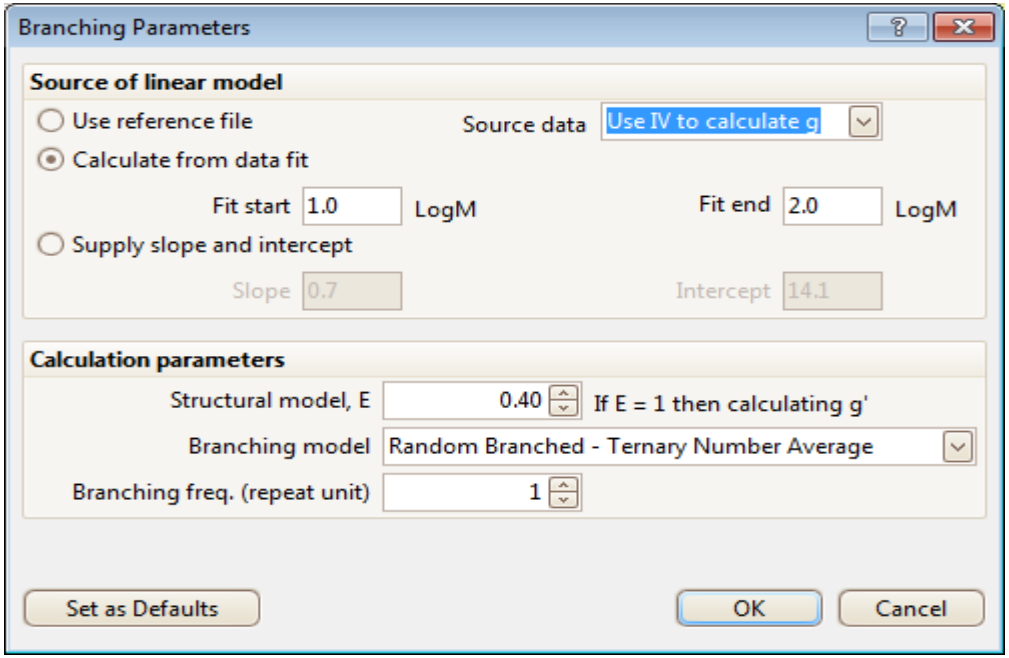
Then left click on the Use as reference button to set this sample as the reference model that you will be using.



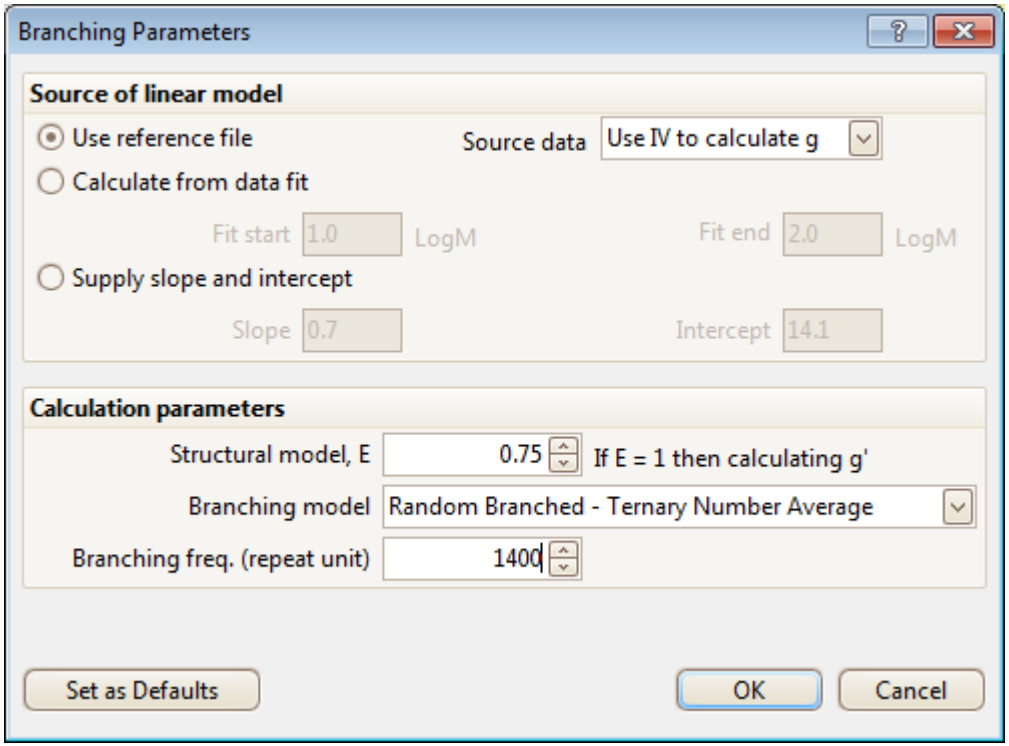
Now highlight the Branched sample and go to Branching tab and select Properties



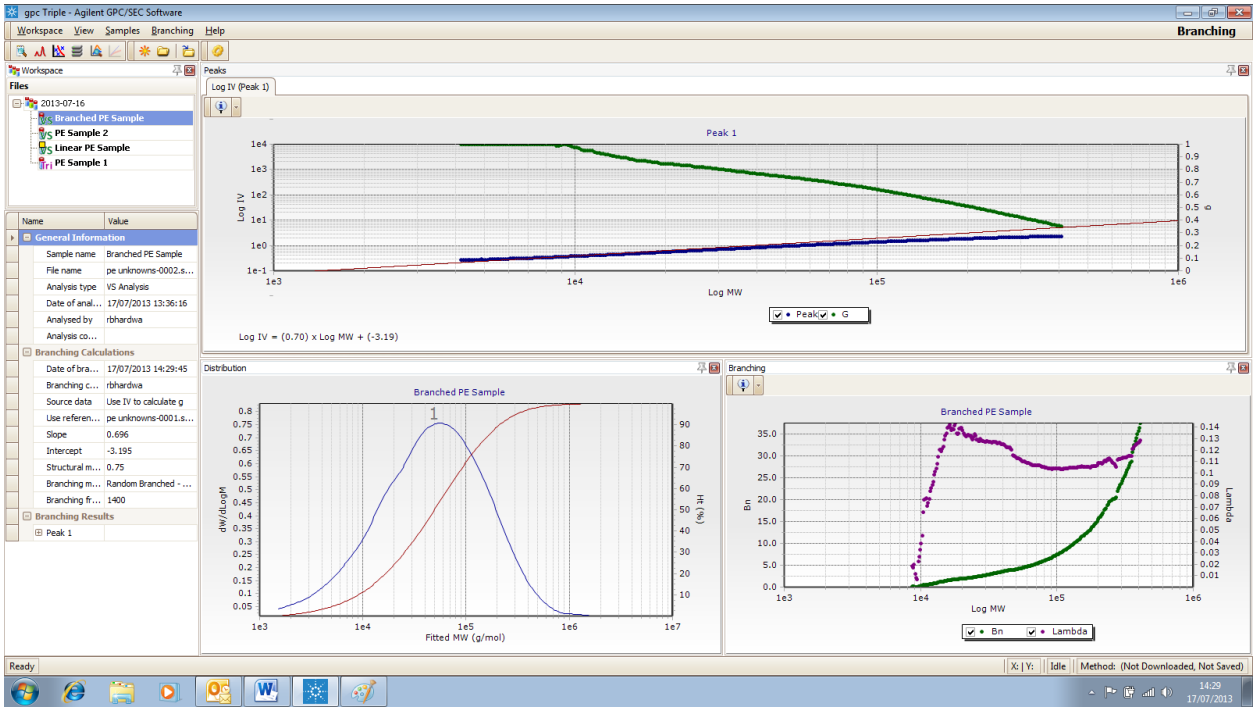
Then the following window pops up



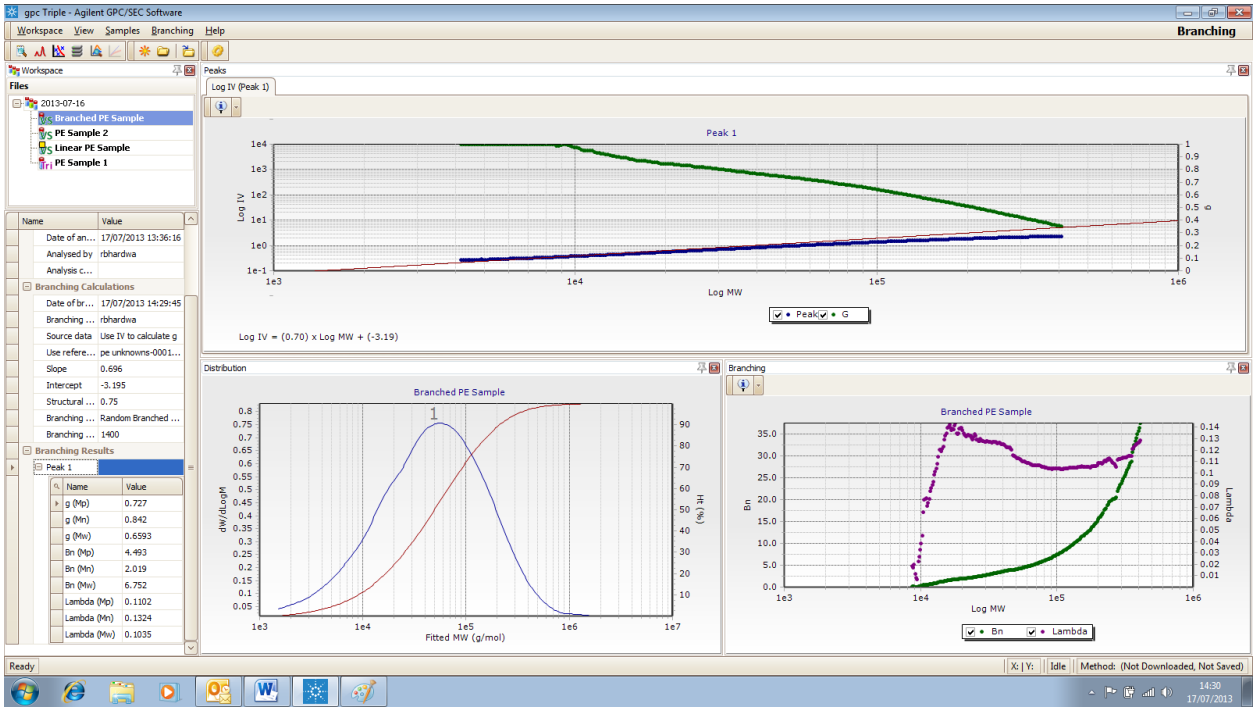
Now we need to populate the above window as;



Click OK and the screen below pops up

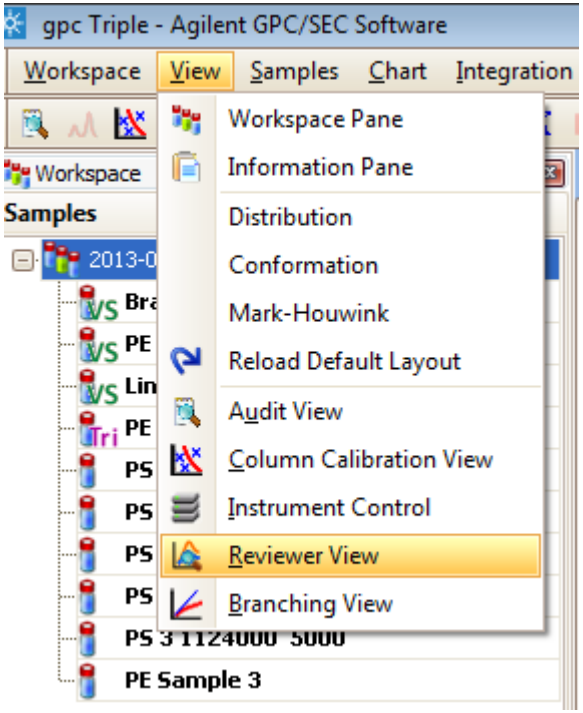


Click + Peak 1 tab to get branching data results;

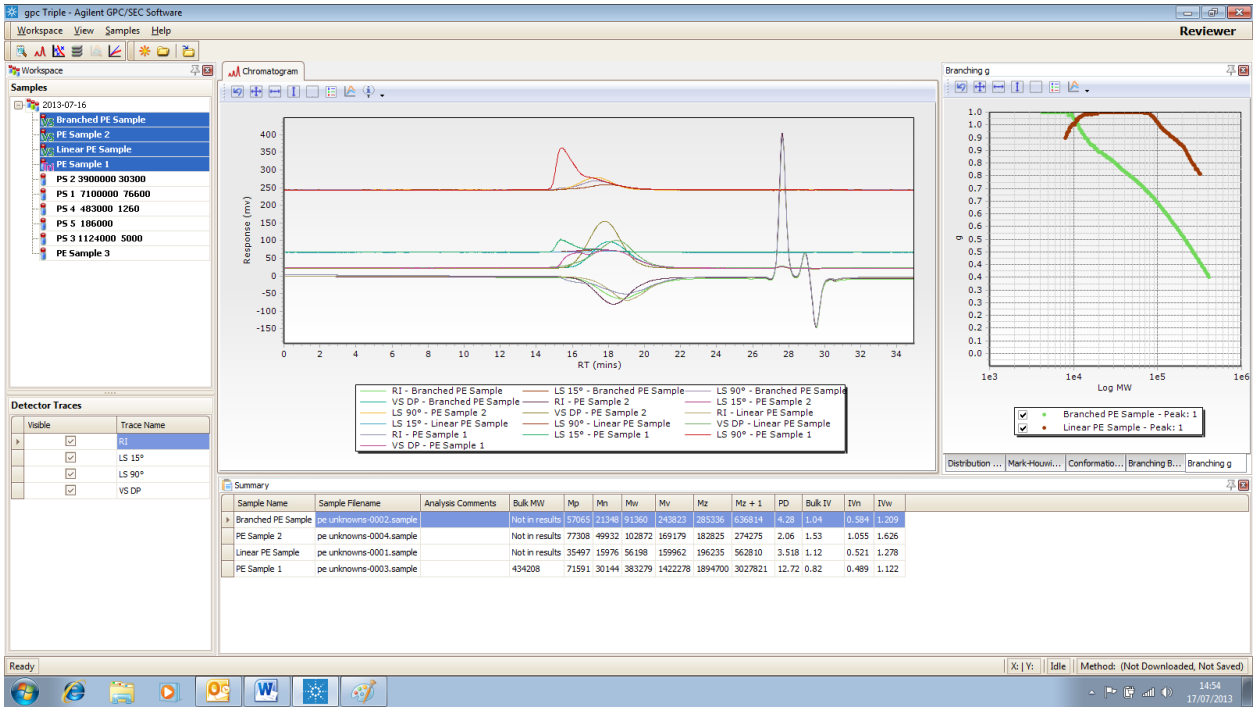

gpc Triple - Agilent GPC/SEC Software
Workspace View Samples Branching Help
Branching
Workspace
Files
2013-07-16
Branched PE Sample
PE Sample 2
Linear PE Sample
PE Sample 1
Name Value
Date of an... 17/07/2013 13:36:16
Analysed by rbhardwa
Analysis c...
Branching Calculations
Date of br... 17/07/2013 14:29:45
Branching ... rbhardwa
Source data Use IV to calculate g
Use refere... pe unknowns-0001...
Slope 0.696
Intercept -3.195
Structural ... 0.75
Branching ... Random Branched ...
Branching ... 1400
Branching Results
Peak 1
Name Value
g (Mp) 0.727
g (Mn) 0.842
g (Mw) 0.6593
Bn (Mp) 4.493
Bn (Mn) 2.019
Bn (Mw) 6.752
Lambda (Mp) 0.1102
Lambda (Mn) 0.1324
Lambda (Mw) 0.1035
Peaks
Log IV (Peak 1)
Peak 1
Log IV
Log MW
g
Peak G
Log IV = (0.70) x Log MW + (-3.19)
Distribution
Branched PE Sample
dW/dLogM
Fitted MW (g/mol)
Int (%)
Branching
Branched PE Sample
Bn
Log MW
Lambda
Bn Lambda
Ready
X: | Y:
Idle
Method: (Not Downloaded, Not Saved)
14:30
17/07/2013

Reviewer

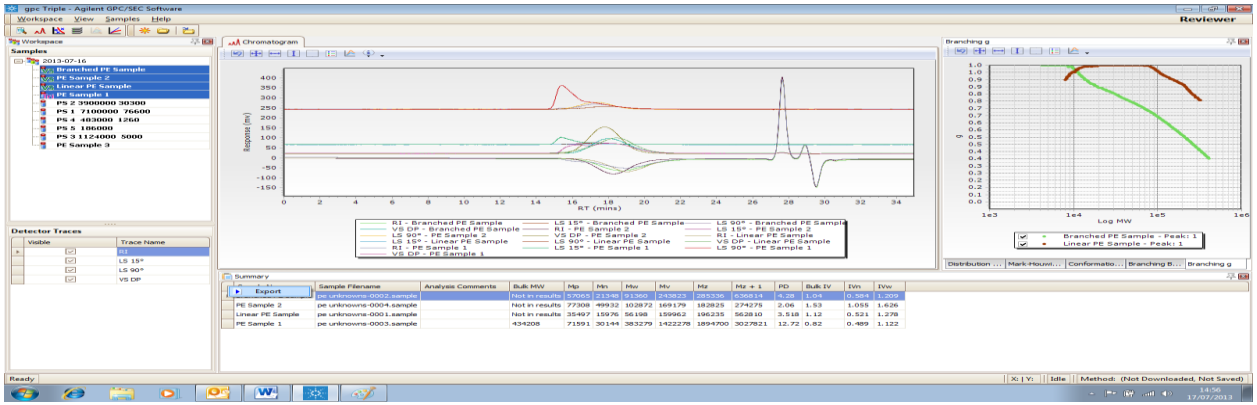
Once the samples have been collected they can all be viewed in the Reviewer window.
Go to the View tab and select Reviewer.

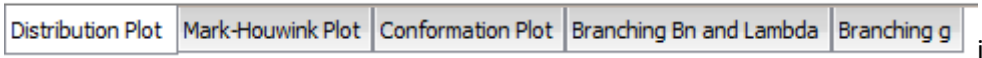


This will open the window below where you can select the files that you want to look at and the detector signals can also be selected by going to the detector traces.


gpc Triple - Agilent GPC/SEC Software
Workspace View Samples Help
Reviewer
Workspace
Samples
2013-07-16
Branched PE Sample
PE Sample 2
Linear PE Sample
PE Sample 1
PS 2 3900000 30300
PS 1 7100000 76600
PS 4 483000 1260
PS 5 186000
PS 3 1124000 5000
PE Sample 3
Chromatogram
Response (mv)
RT (mins)
RI - Branched PE Sample
VS DP - Branched PE Sample
LS 90° - PE Sample 2
LS 15° - Linear PE Sample
RI - PE Sample 1
VS DP - PE Sample 1
LS 15° - Branched PE Sample
RI - PE Sample 2
VS DP - PE Sample 2
LS 90° - Linear PE Sample
LS 15° - PE Sample 1
LS 90° - Branched PE Sample
LS 15° - PE Sample 2
RI - Linear PE Sample
VS DP - Linear PE Sample
LS 90° - PE Sample 1
Branching g
g
Log MW
Branched PE Sample - Peak: 1
Linear PE Sample - Peak: 1
Distribution ...
Mark-Houwi...
Conformatio...
Branching B...
Branching g
Detector Traces
Visible
Trace Name
RI
LS 15°
LS 90°
VS DP
Summary
Sample Name
Sample Filename
Analysis Comments
Bulk MW
Mp
Mn
Mw
Mv
Mz
Mz + 1
PD
Bulk IV
IVn
IVw
Branched PE Sample pe unknowns-0002.sample Not in results 57065 21348 91360 243823 285336 636814 4.28 1.04 0.584 1.209
PE Sample 2 pe unknowns-0004.sample Not in results 77308 49932 102872 169179 182825 274275 2.06 1.53 1.055 1.626
Linear PE Sample pe unknowns-0001.sample Not in results 35497 15976 56198 159962 196235 562810 3.518 1.12 0.521 1.278
PE Sample 1 pe unknowns-0003.sample 434208 71591 30144 383279 1422278 1894700 3027821 12.72 0.82 0.489 1.122
Ready
X: | Y:
Idle
Method: (Not Downloaded, Not Saved)
14:54
17/07/2013

The results can be exported into excel or word by right clicking the forward arrow in the summary tab.



Also by selecting the individual tabs 

information generated for the samples can be displayed.