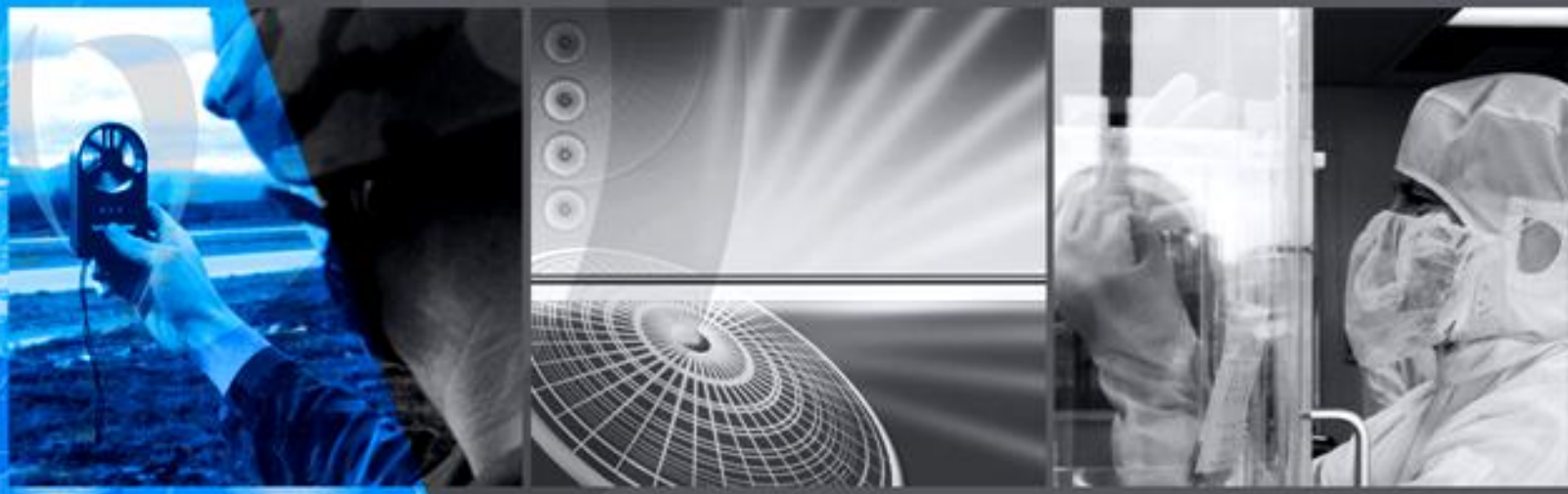
29th August 2014

Lauren Brown

THE data processing tutorial



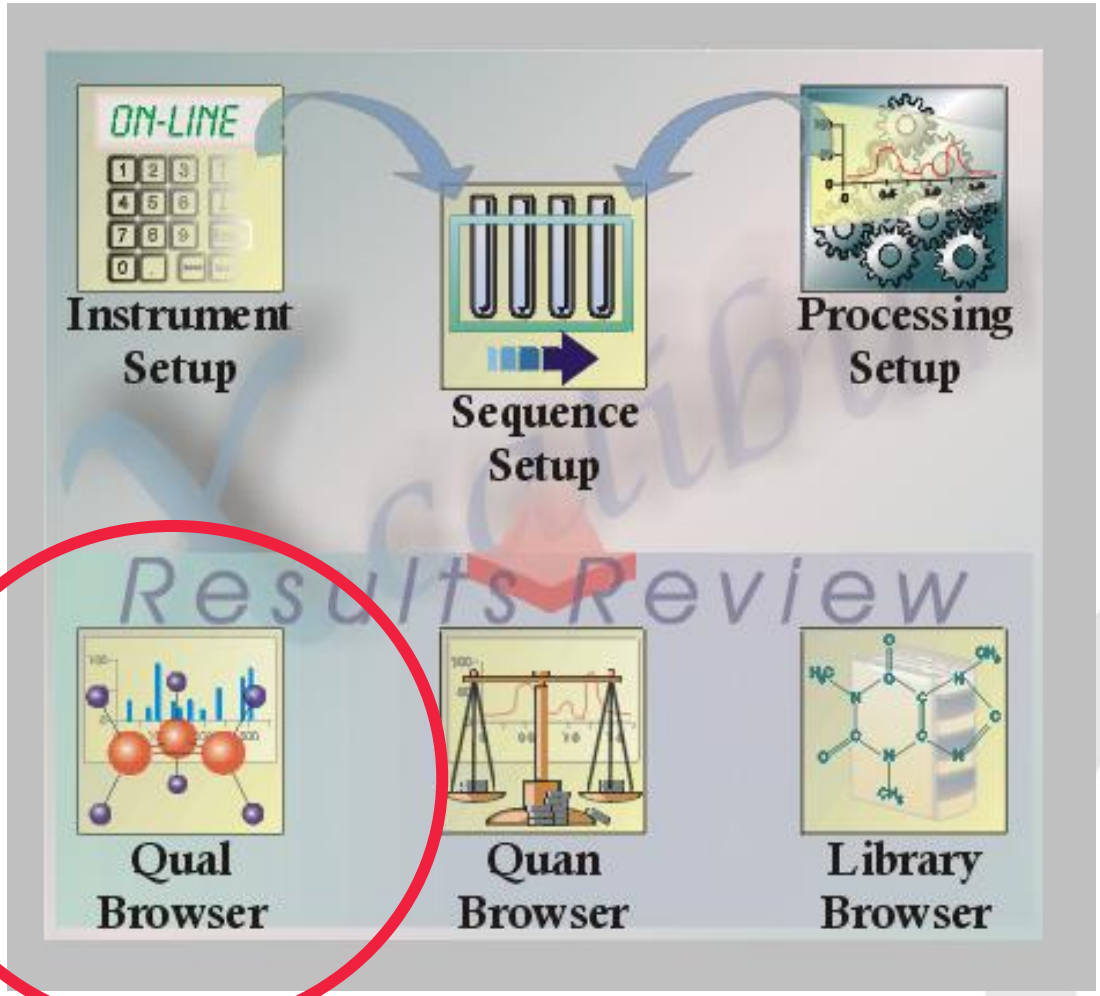
NEXT GENERATION DETECTION

Aim

- This tutorial will describe the steps required to export THE ultraFAIMS data files from Xcalibur and convert into the format required for viewing/processing data in Excel.

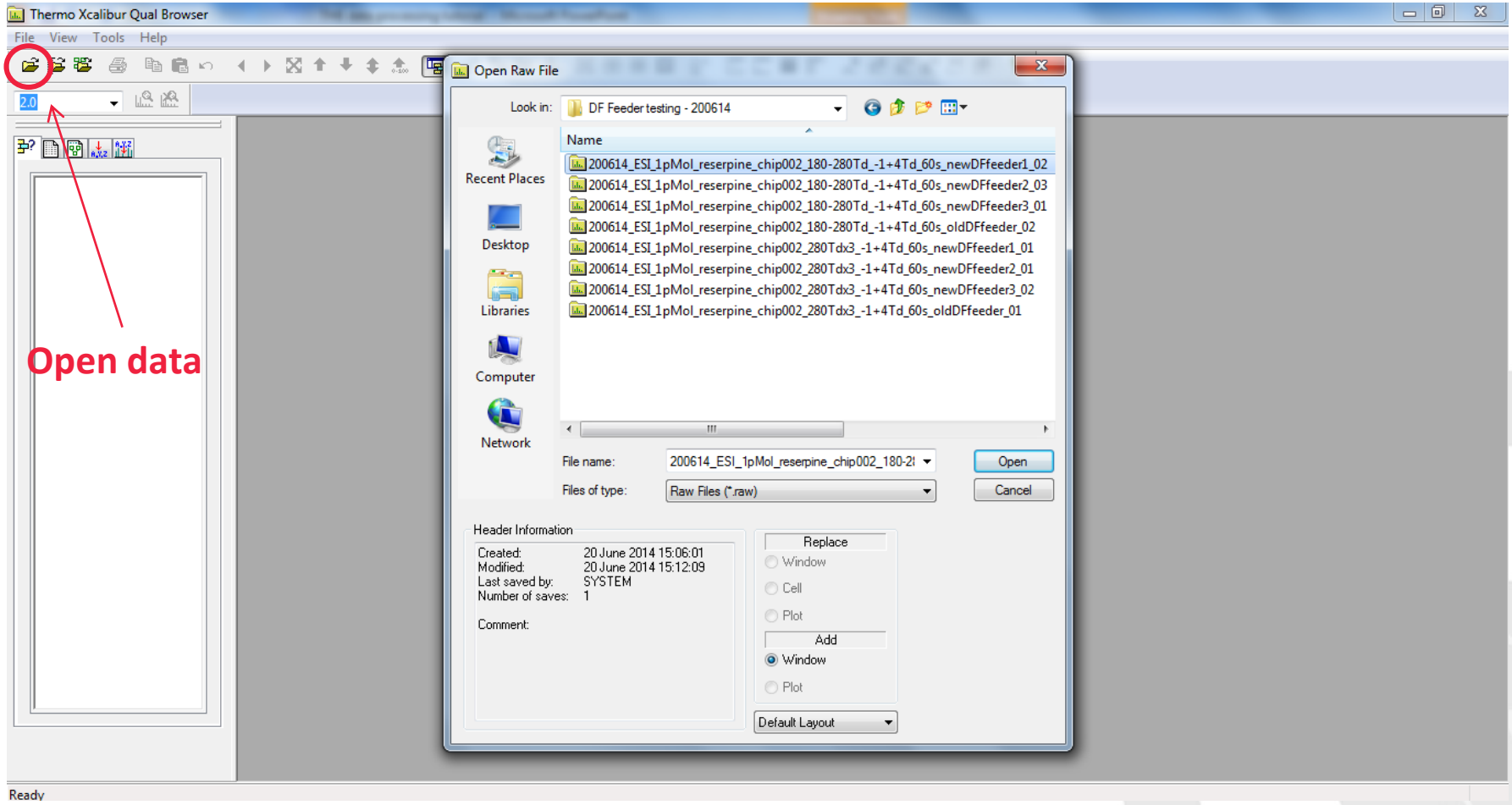
View data in Qual Browser

- Open Qual Browser from the Xcalibur Roadmap window.



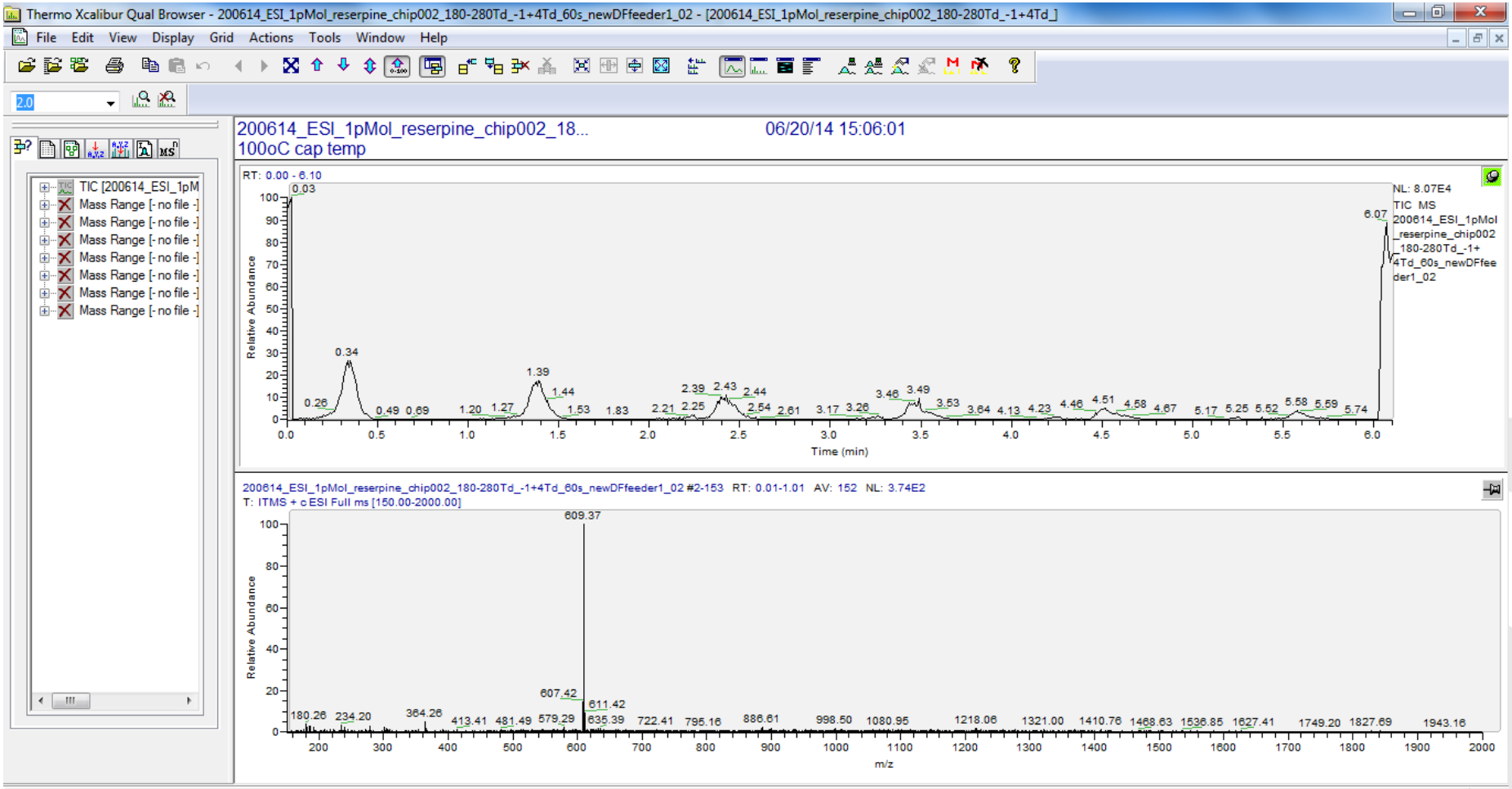
Load RAW file in Qual Browser

- Click on the ‘open data’ button in the top left corner of the screen
- Select data file.



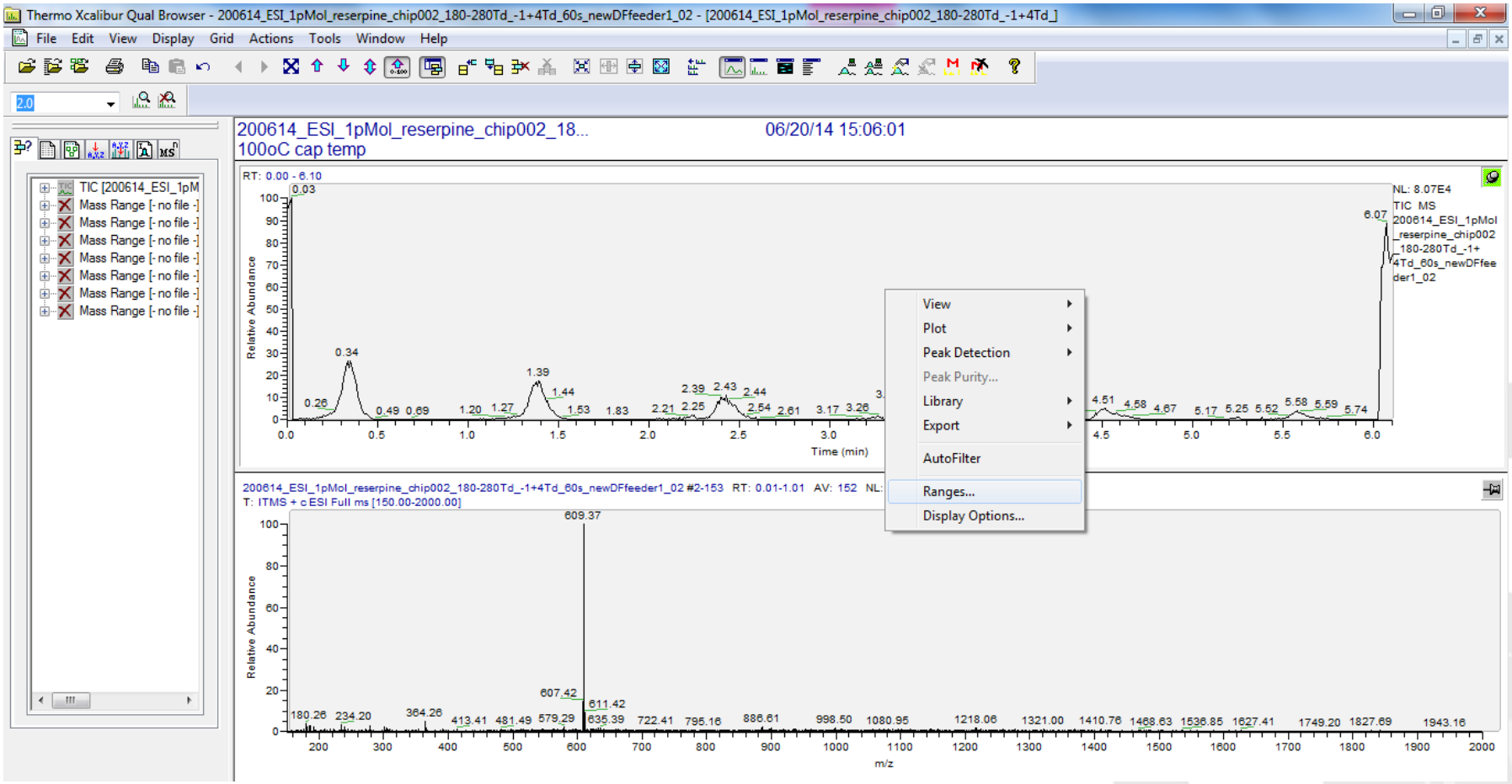
View data in Qual Browser

- TIC loads in chromatogram window as standard.



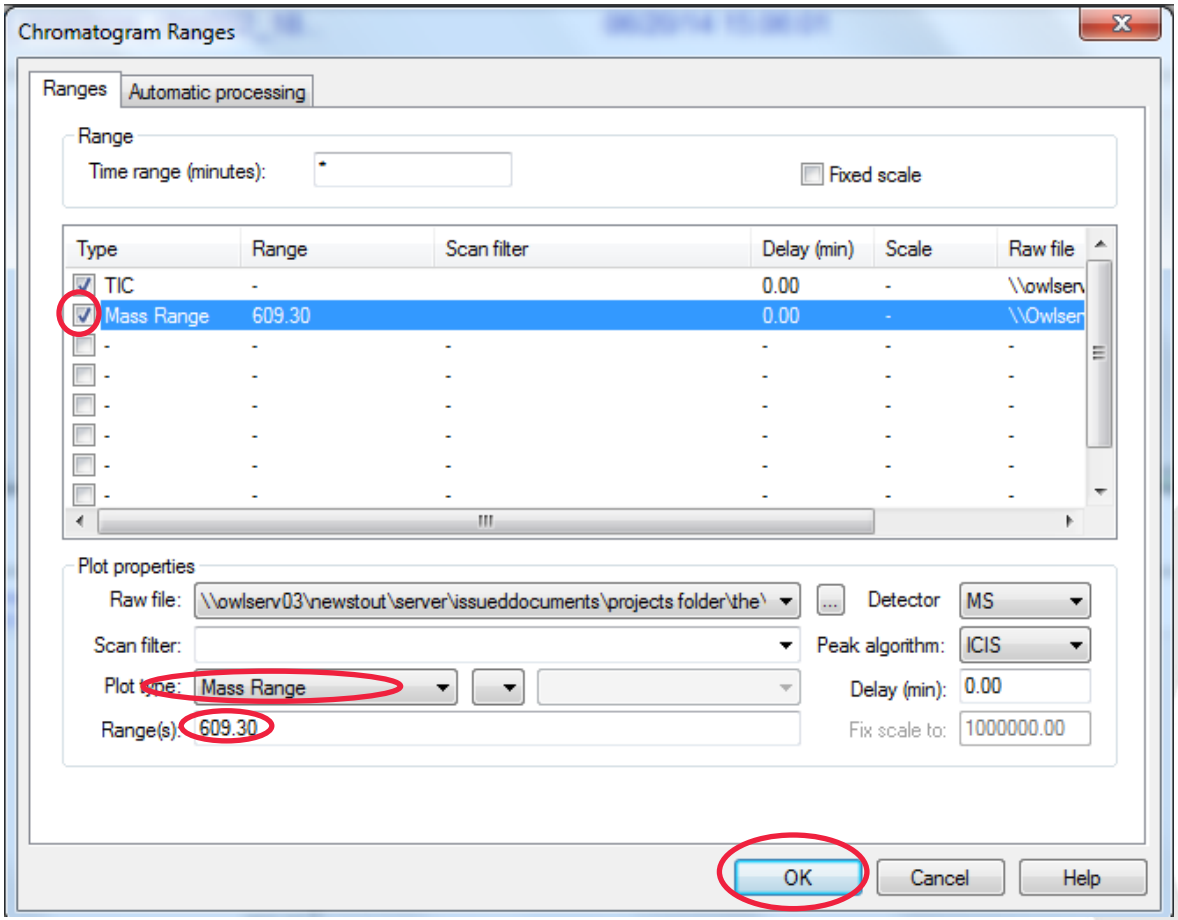
View data for ion of interest

- Ensure chromatogram window is pinned (green square indicates active window).
- Right click and select ‘Ranges’.



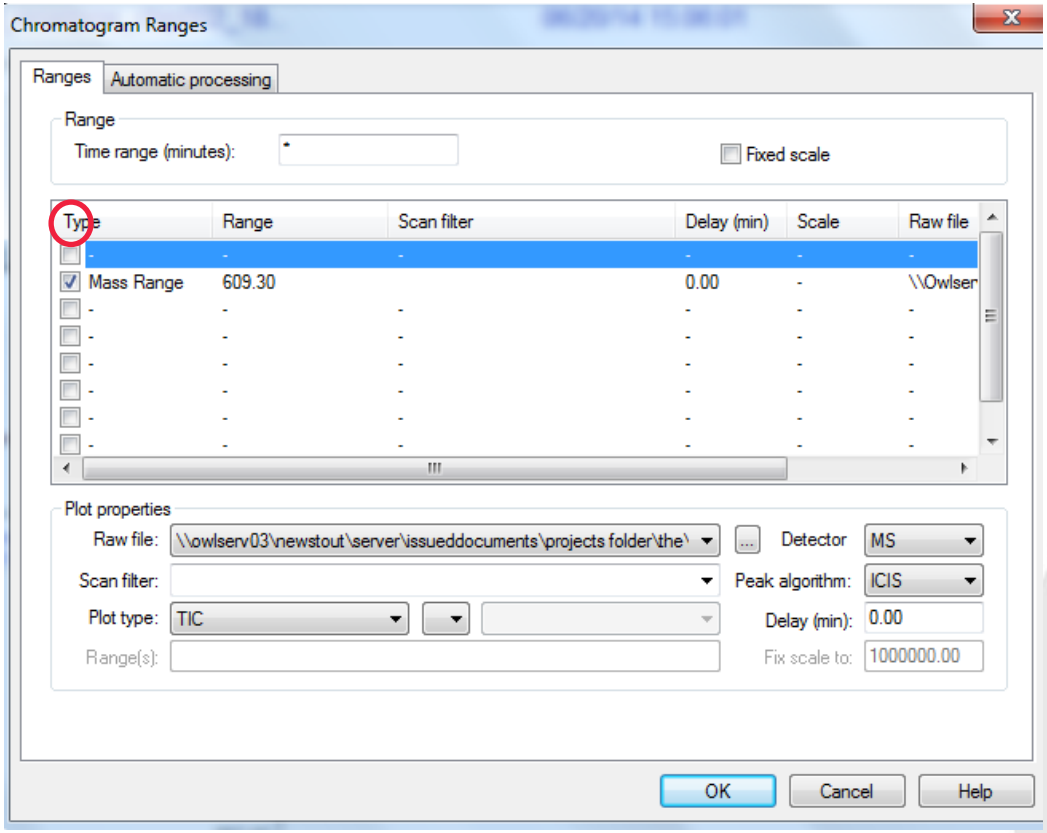
View data for ion of interest

- Click the box under TIC.
- Change ‘Plot type’ to ‘Mass Range’ from the drop down menu.
- Enter the m/z of the ion of interest in the ‘Range(s)’ box
- Press ‘OK’.



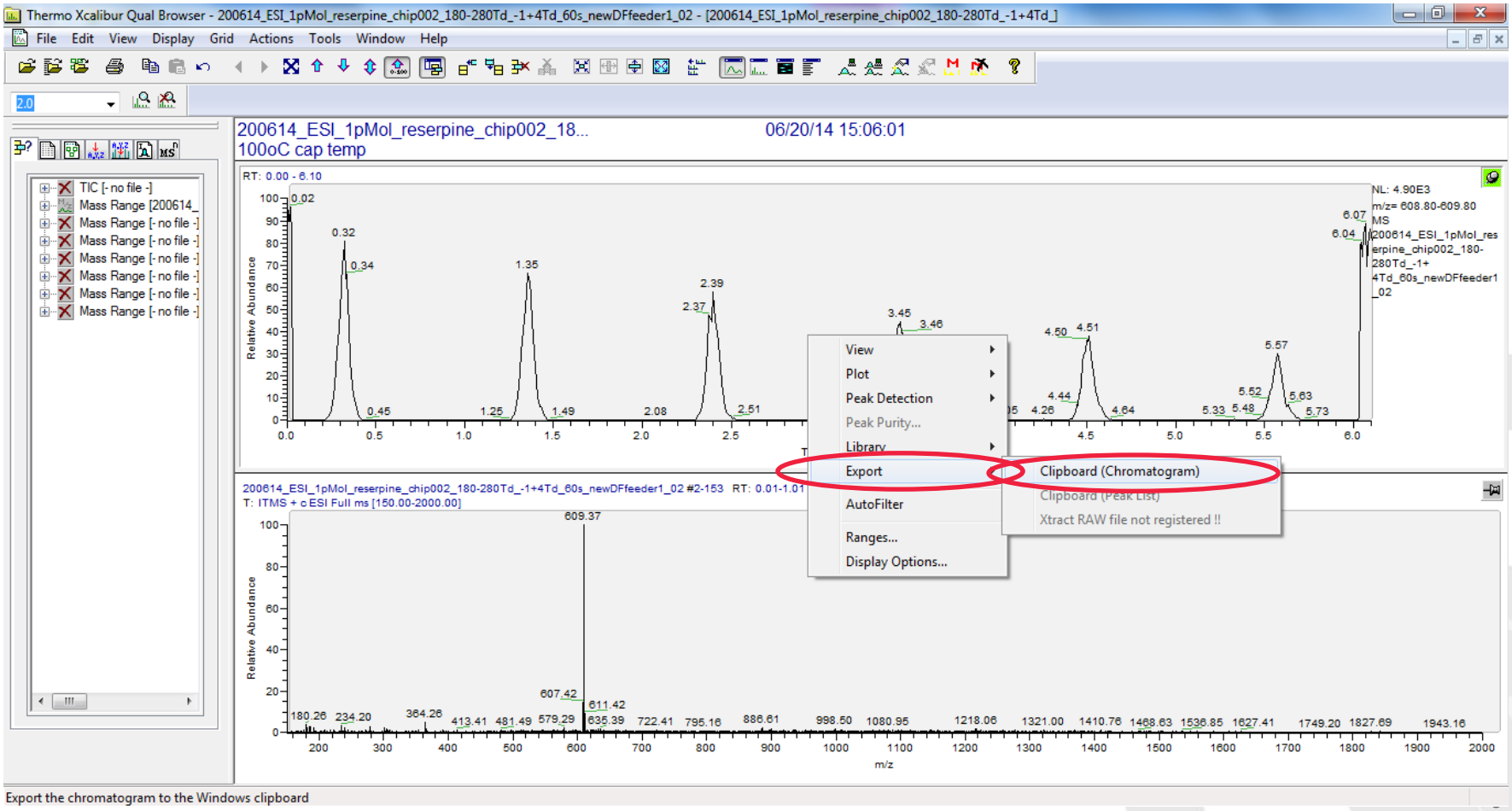
View data for ion of interest

- Xcalibur will only allow the export of one set of data at a time.
- By default it selects the first chromatogram range.
- TIC therefore has to be de-selected to allow the ion of interest data to be exported.
- Re-load the Chromatogram Ranges window, as the previous step, and de-select the TIC.
- Hit ‘OK’.



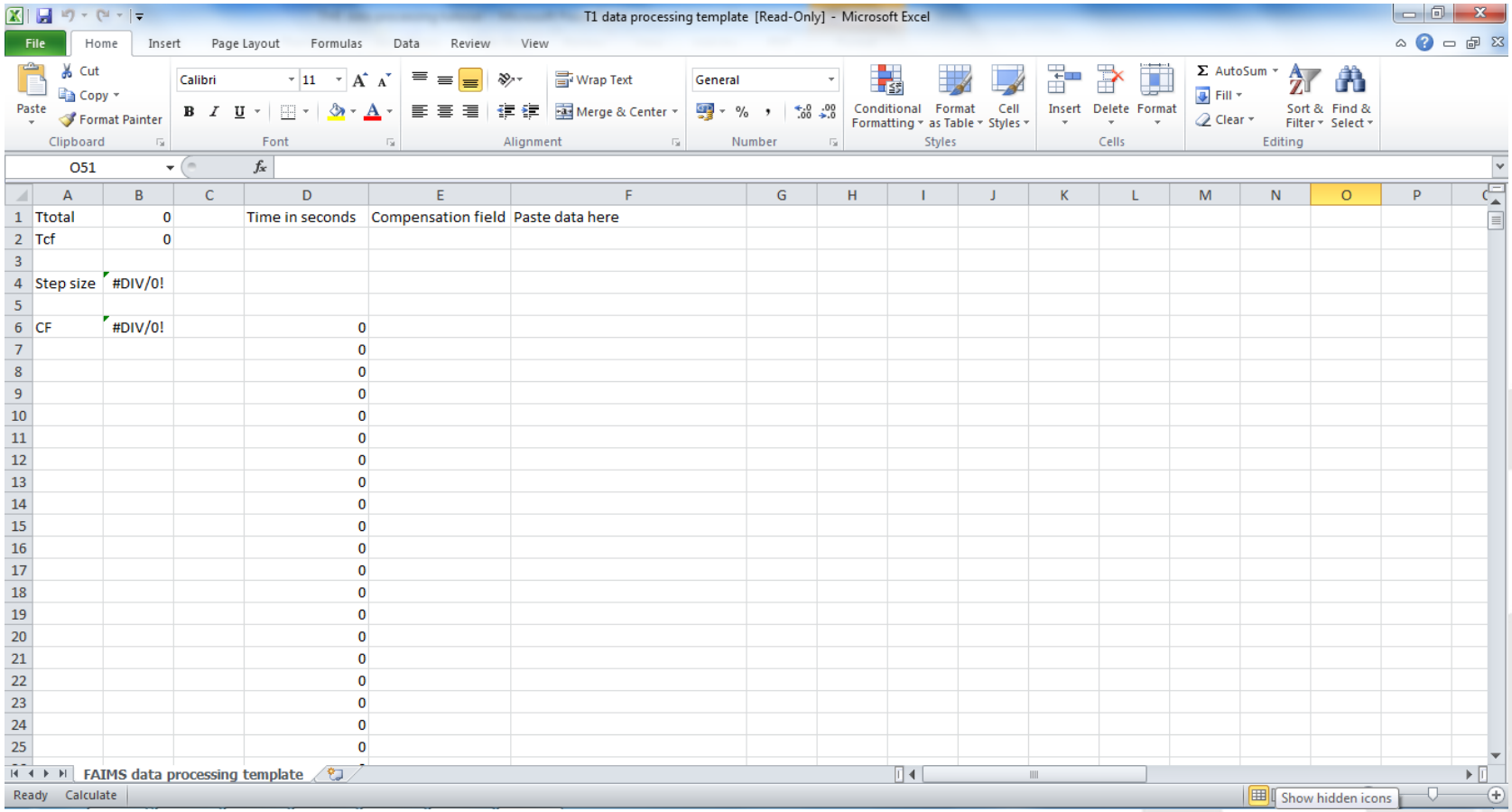
Export data to Clipboard

- Right click on the chromatogram window and click ‘Export’.
- Select ‘Clipboard (Chromatogram)’. This ‘copies’ the data and allows it to be pasted into another program.



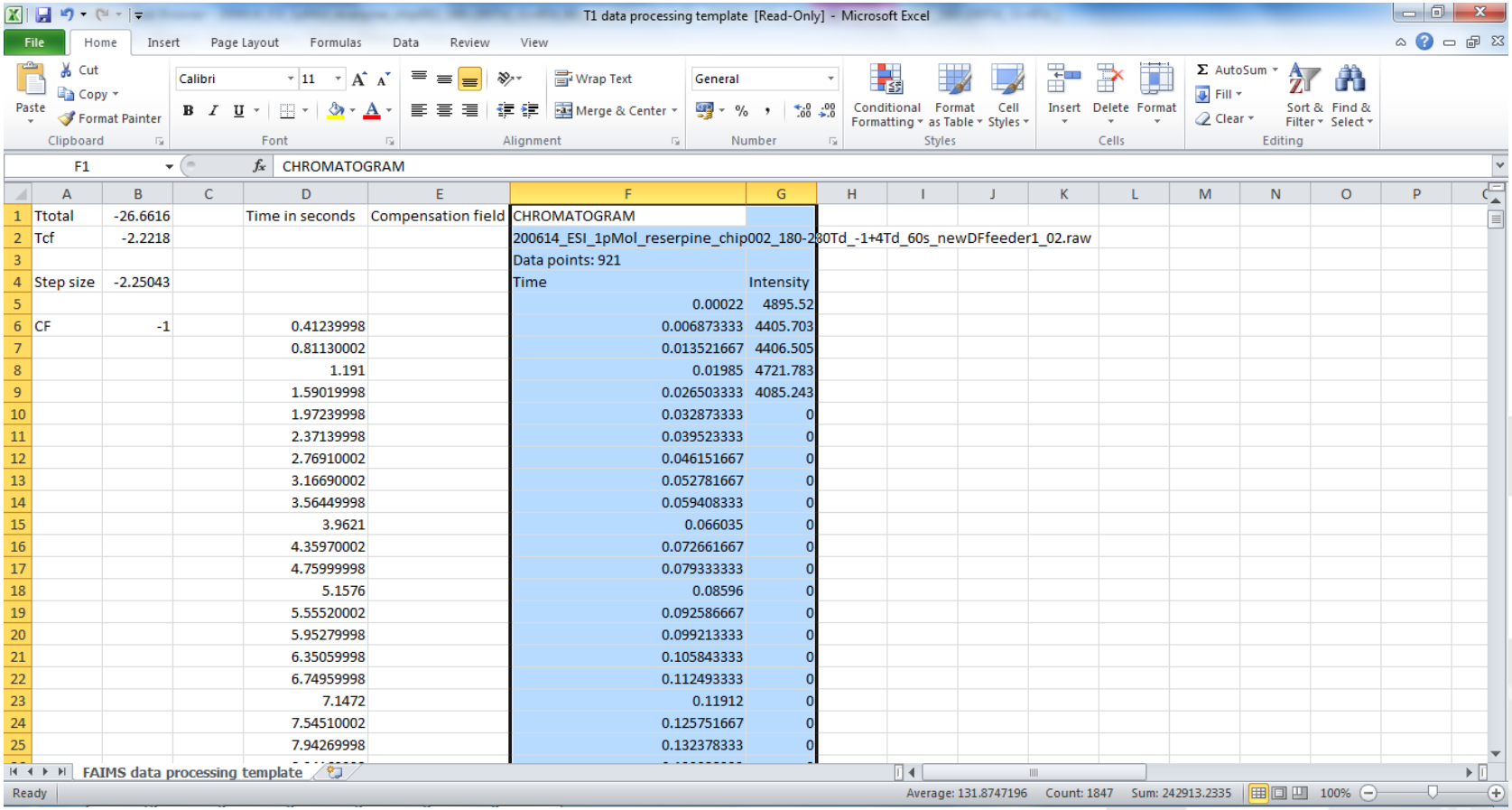
Paste data into Excel

- Owlstone will provide an Excel Spreadsheet that can be used to paste the exported data into to allow data to be viewed/processed with a compensation field axis.



Paste data into Excel

- Right click on the ‘Paste data here’ cell and paste in the exported data.



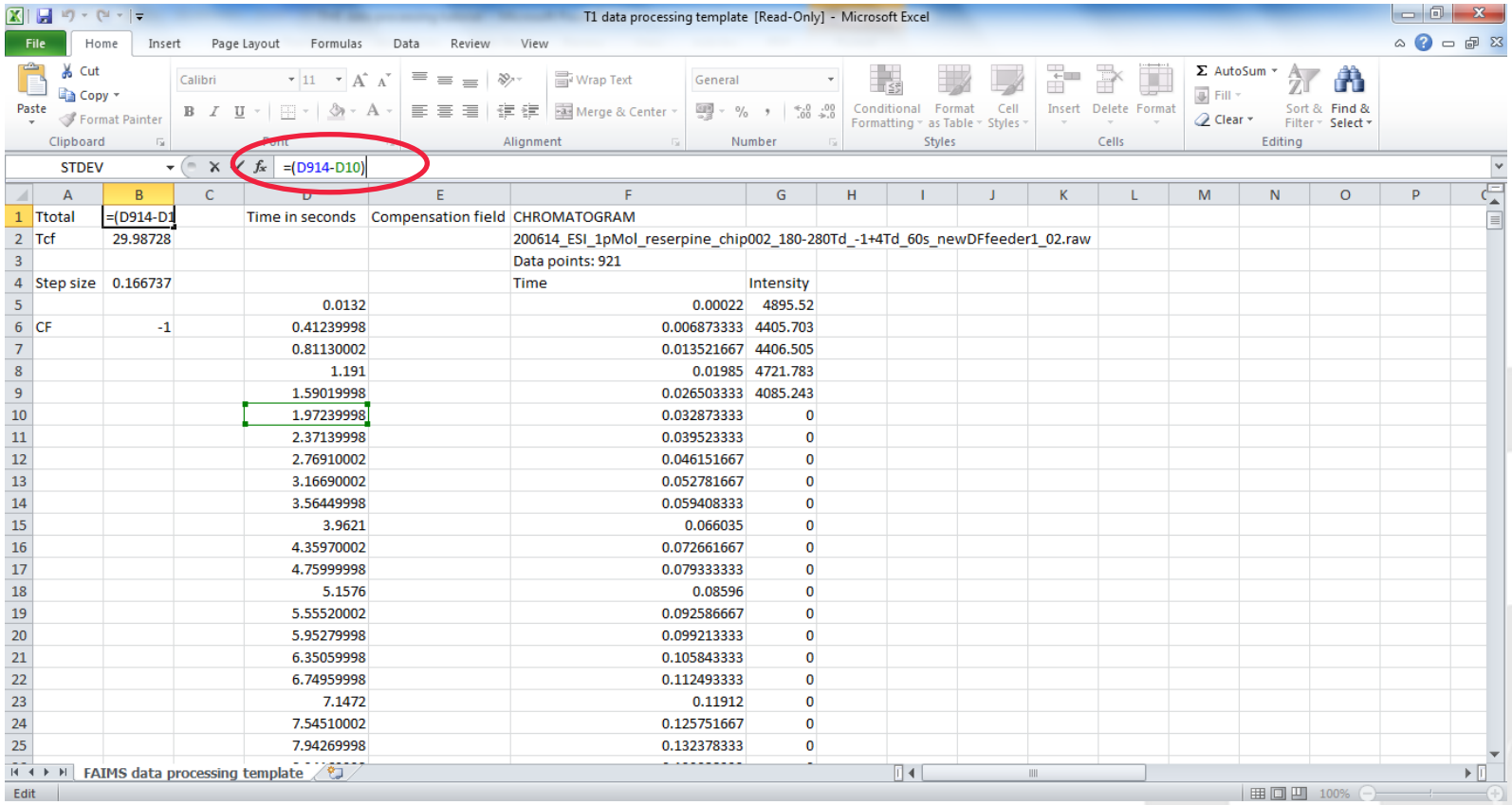
	A	B	C	D	E	F	G
1	Ttotal	-26.6616		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	-2.2218				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	-2.25043				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998		0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

Convert time axis into compensation field

- The spreadsheet will automatically convert the time in minutes into time in seconds (column D).
- Manual input is then required to convert the time in seconds to compensation field (CF).
- This is due to the non-linear nature of ion-trap-type data acquisition.
- Automatic gain control means that the ion trap will fill for different amounts of time dependent on the number of ions present.
- However, the CF is ramped at a linear rate over a scan.
- This means the step in CF will not always be the same between different data points.
- However, CF across the compensation field scan can be back calculated using the steps in the following slides.

Convert time axis into compensation field

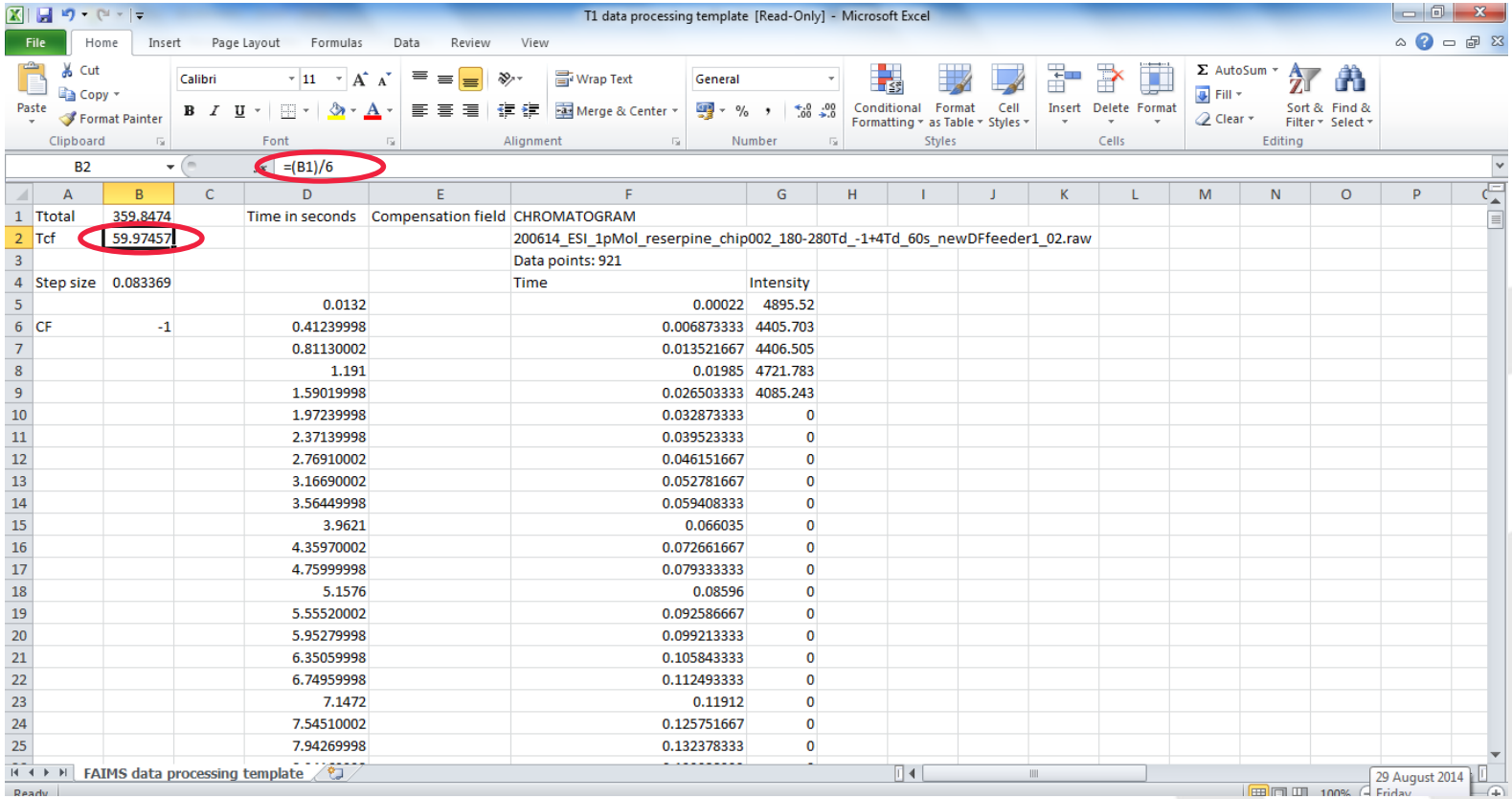
- First, the time taken for the total FAIMS scan must be calculated.
- In cell ‘B1’ change the equation to the cell where the FAIMS scan ends (in the example shown D914) minus the start of the FAIMS scan (D10 in the example shown).



	A	B	C	D	E	F	G
1	Ttotal	=(D914-D1		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	29.98728				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.166737				Time	Intensity
5				0.0132		0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998		0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

Convert time axis into compensation field

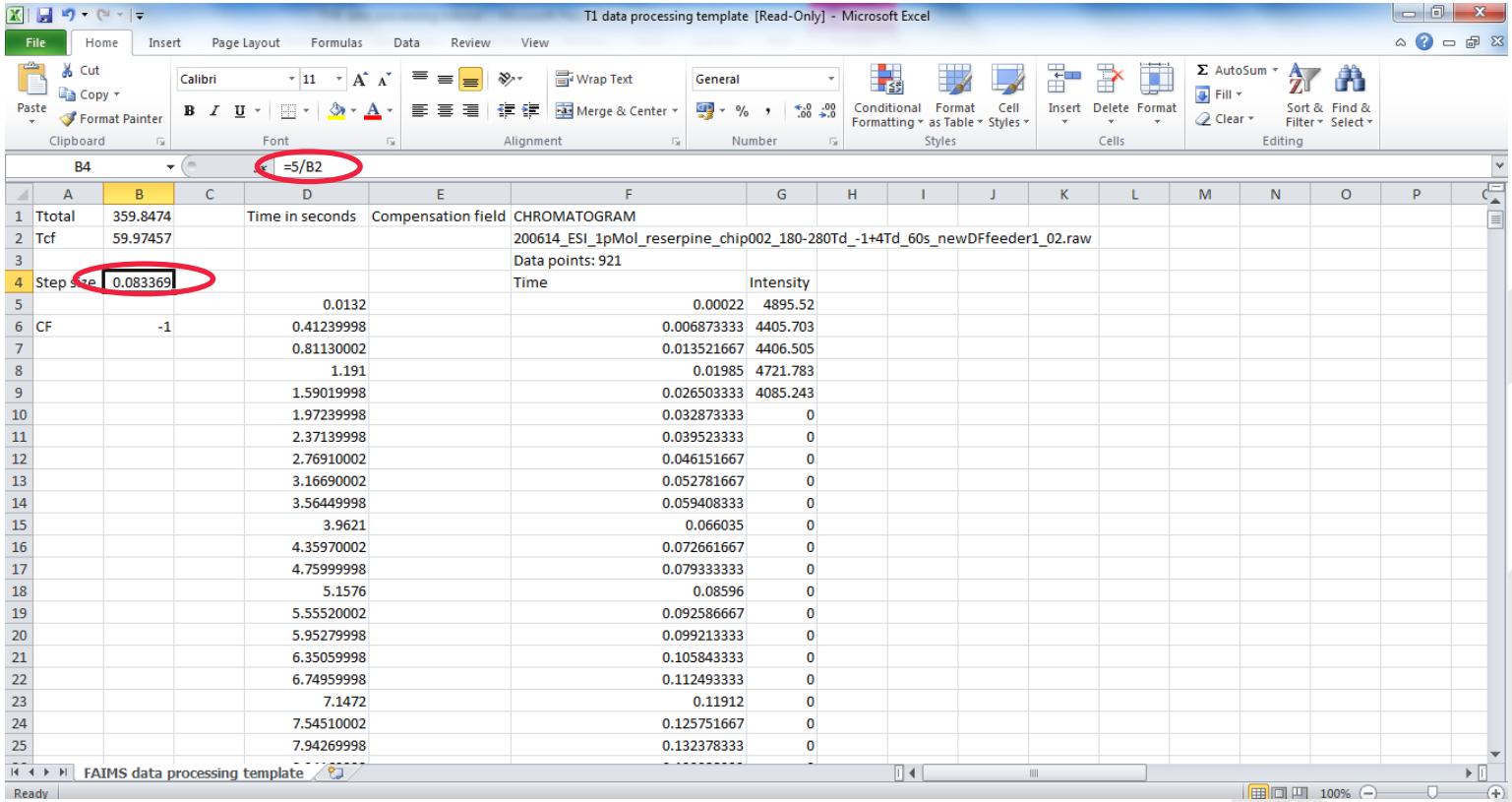
- Next, the time taken for each CF scan should be calculated.
- Divide the time taken for the FAIMS scan by the number of dispersion fields (DF) used.
  - 6 DFs were used in the example shown (cell B2).
- The number should be approximately the CF scan time inputted into the ultraFAIMS software when the data was acquired (in the example below this was 60s).



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5				0.0132		0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998		0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

Calculate compensation field step size

- Next, the time taken for each CF step should be calculated, based on the total time for the CF scan and the CF scan range.
- Divide the number of Td for the CF scan by the time taken to perform the CF scan, calculated in the previous step.
  - A 5 Td range (-1 Td to +4 Td) was used in the example shown (cell B4).



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step Size	0.083369				Time	Intensity
5				0.0132		0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998		0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

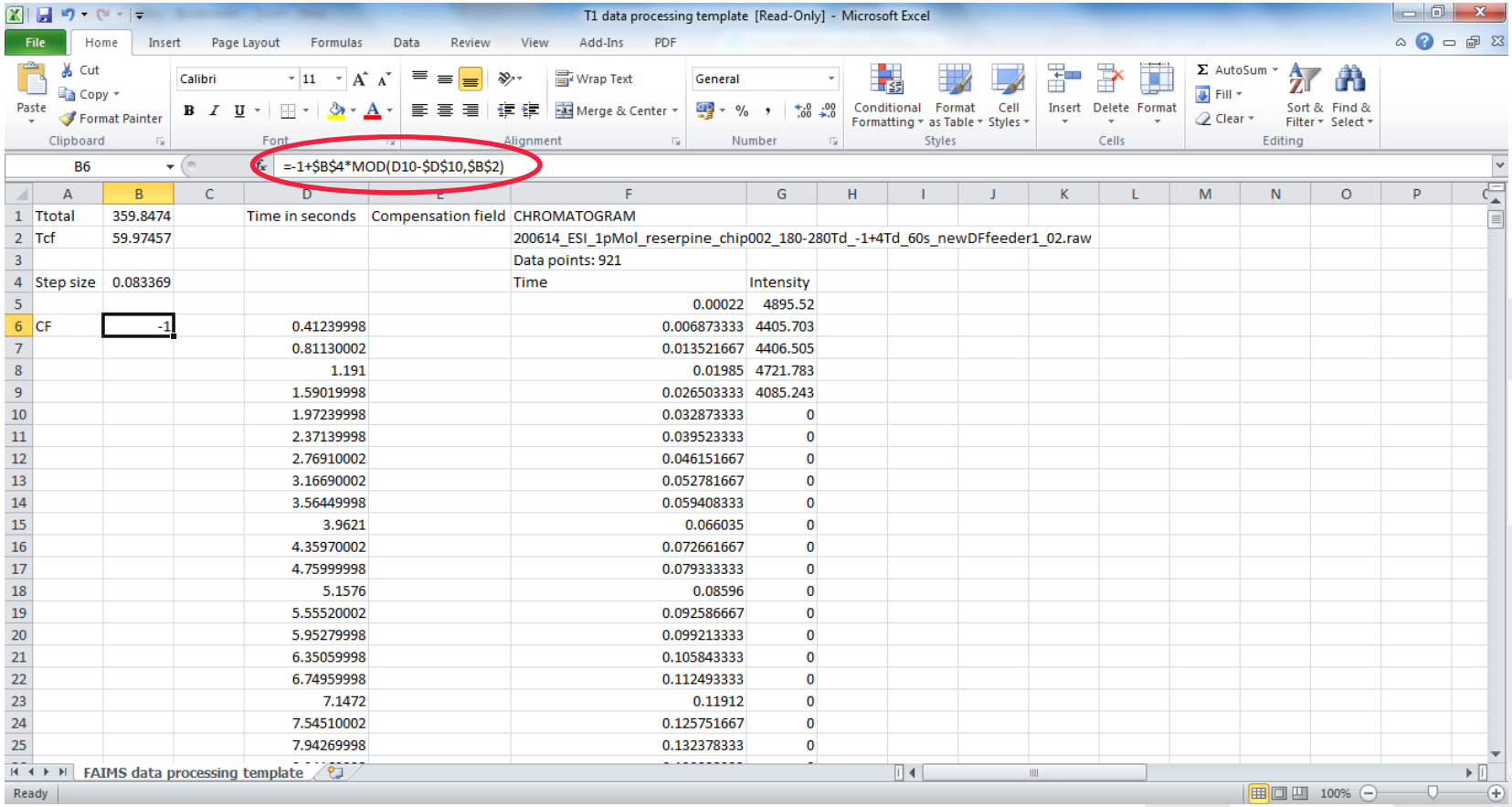
Calculate compensation field for a given time point

- A formula is inputted into cell B6 to allow the calculation of the CF at a given time point.
- This formula uses the MOD function which tells you what is the remainder after dividing one number with another.
- This allows CF to be calculated at a particular time point based on how much time has elapsed since the start of the scan.
- The CF is calculated by taking the starting CF plus the step size (in CF s^{-1}) and multiplying it by the elapsed time.

E.g. -1+B4*MOD(D10-D10,B2)

Calculate compensation field for a given time point

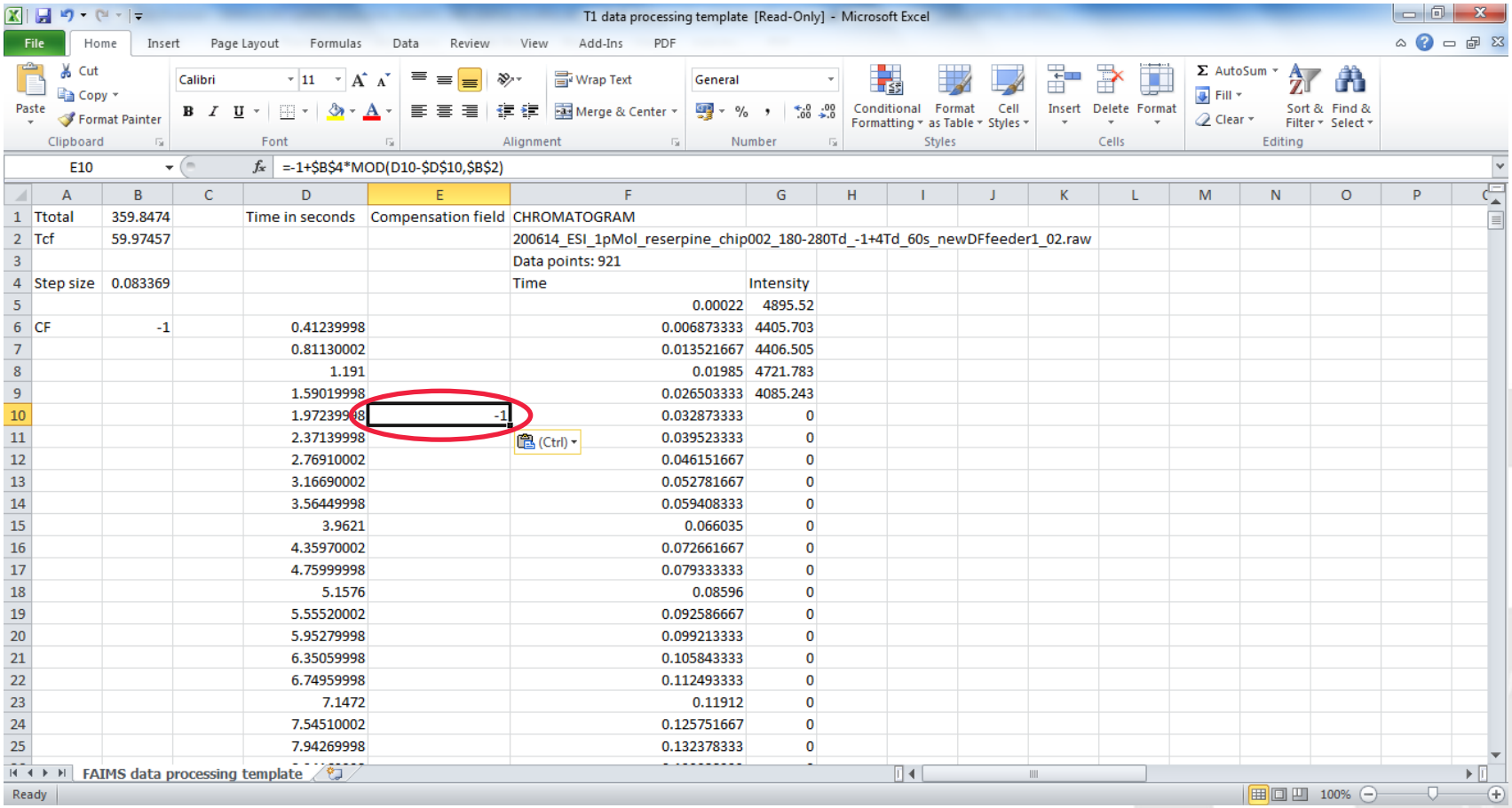
- It is important to enter the correct starting CF and step size, as well as the correct starting time. This must be changed for each exported data file.
- In the e.g. shown below, the CF scan starts at -1 Td, the step size is calculated in cell B4. The start time of the scan is in cell D10 and the total time taken is calculated in cell B2.



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998		0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

Convert each time point to compensation field

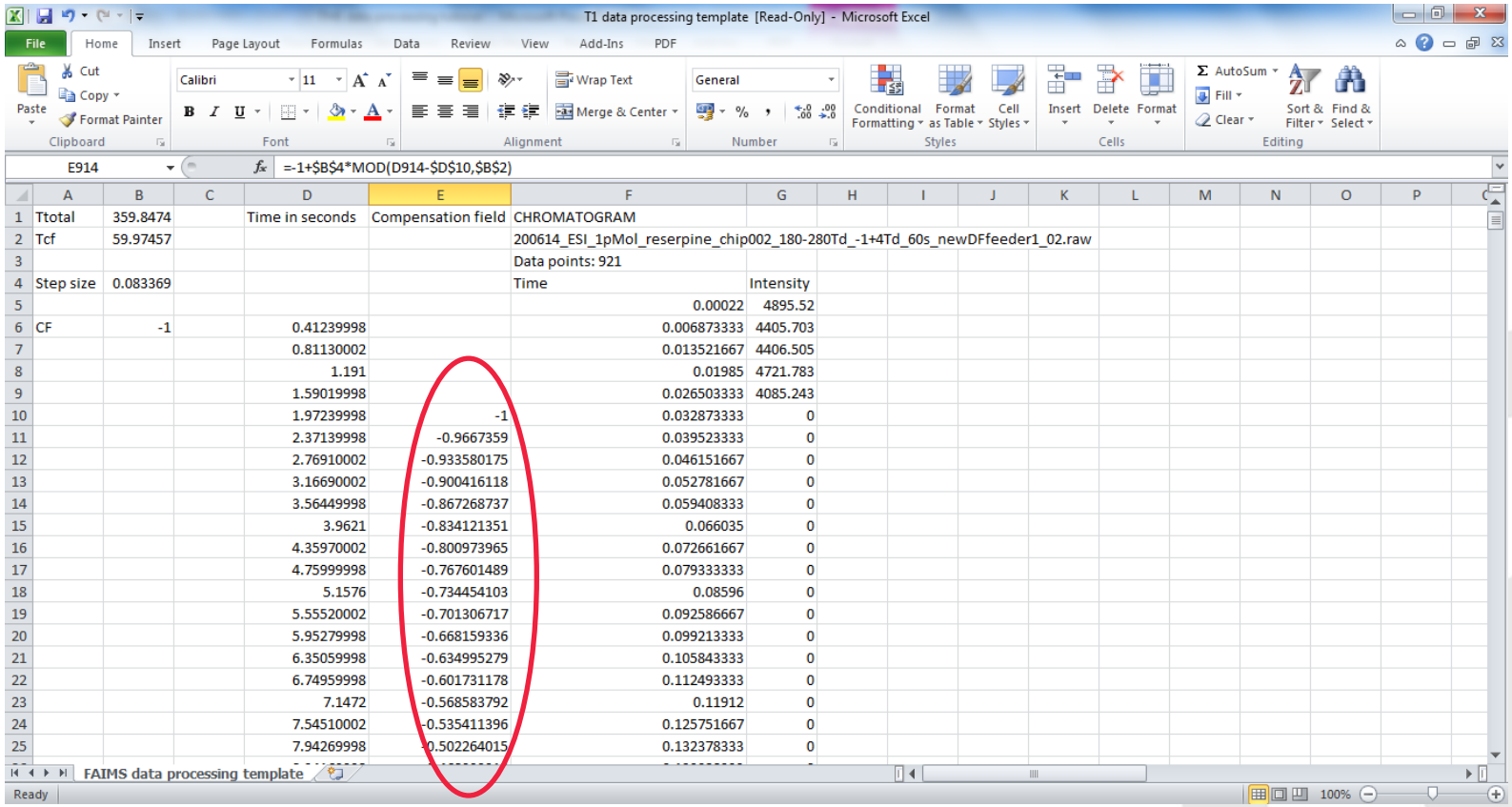
- The equation can be copied from cell B6 and pasted into the CF cell correlating to the start of the CF scan (E10 in this example).



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998	-1	0.032873333	0
11				2.37139998		0.039523333	0
12				2.76910002		0.046151667	0
13				3.16690002		0.052781667	0
14				3.56449998		0.059408333	0
15				3.9621		0.066035	0
16				4.35970002		0.072661667	0
17				4.75999998		0.079333333	0
18				5.1576		0.08596	0
19				5.55520002		0.092586667	0
20				5.95279998		0.099213333	0
21				6.35059998		0.105843333	0
22				6.74959998		0.112493333	0
23				7.1472		0.11912	0
24				7.54510002		0.125751667	0
25				7.94269998		0.132378333	0

Convert each time point to compensation field

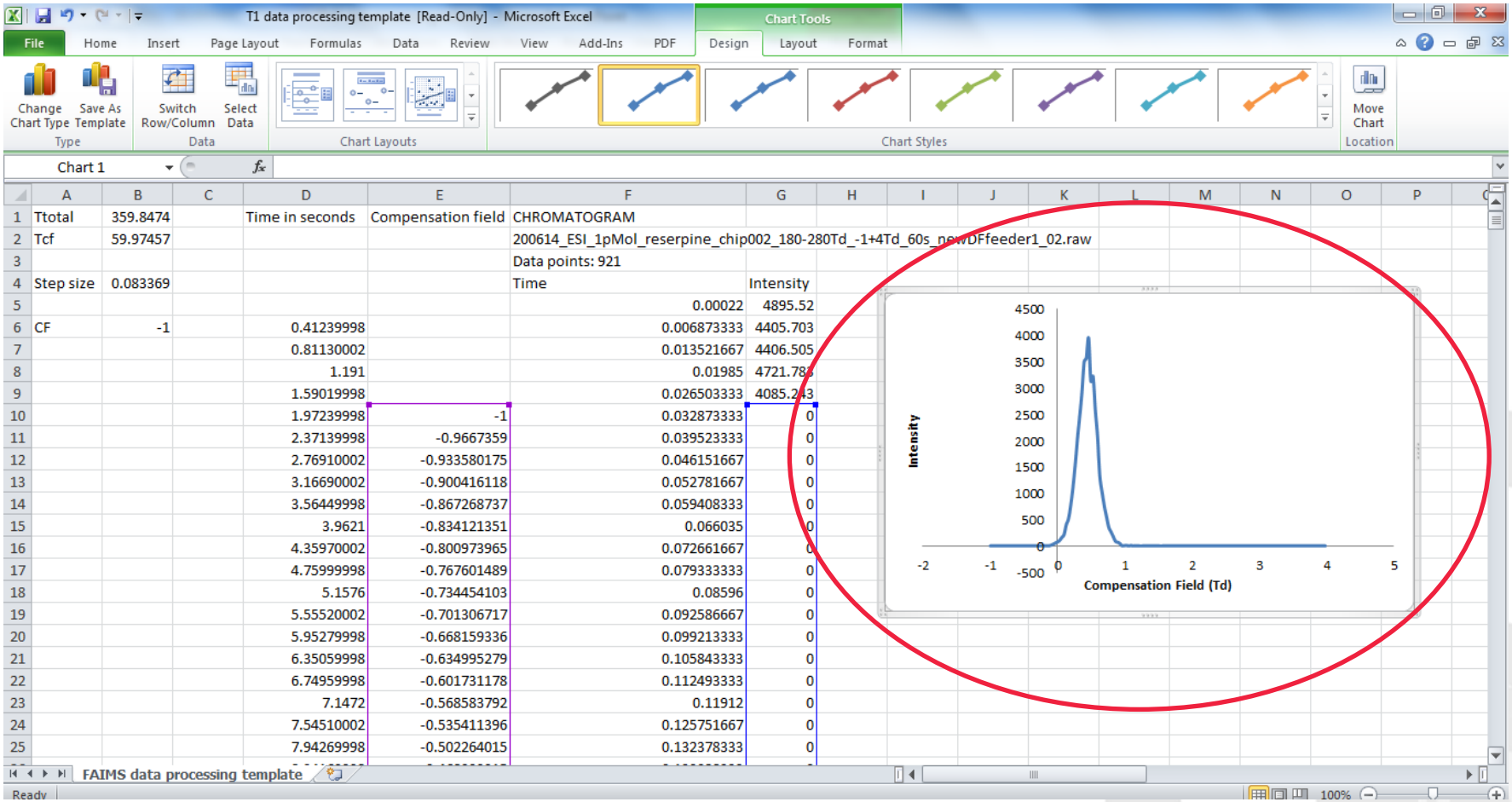
- This can then be dragged to each subsequent cell – in the example below cells D10 to D914, covering the total CF scan range.
- This then gives the data needed to be able to plot CF against intensity (Column E *vs.* column G).



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998	-1	0.032873333	0
11				2.37139998	-0.9667359	0.039523333	0
12				2.76910002	-0.933580175	0.046151667	0
13				3.16690002	-0.900416118	0.052781667	0
14				3.56449998	-0.867268737	0.059408333	0
15				3.9621	-0.834121351	0.066035	0
16				4.35970002	-0.800973965	0.072661667	0
17				4.75999998	-0.767601489	0.079333333	0
18				5.1576	-0.734454103	0.08596	0
19				5.55520002	-0.701306717	0.092586667	0
20				5.95279998	-0.668159336	0.099213333	0
21				6.35059998	-0.634995279	0.105843333	0
22				6.74959998	-0.601731178	0.112493333	0
23				7.1472	-0.568583792	0.11912	0
24				7.54510002	-0.535411396	0.125751667	0
25				7.94269998	-0.502264015	0.132378333	0

Plot intensity against compensation field

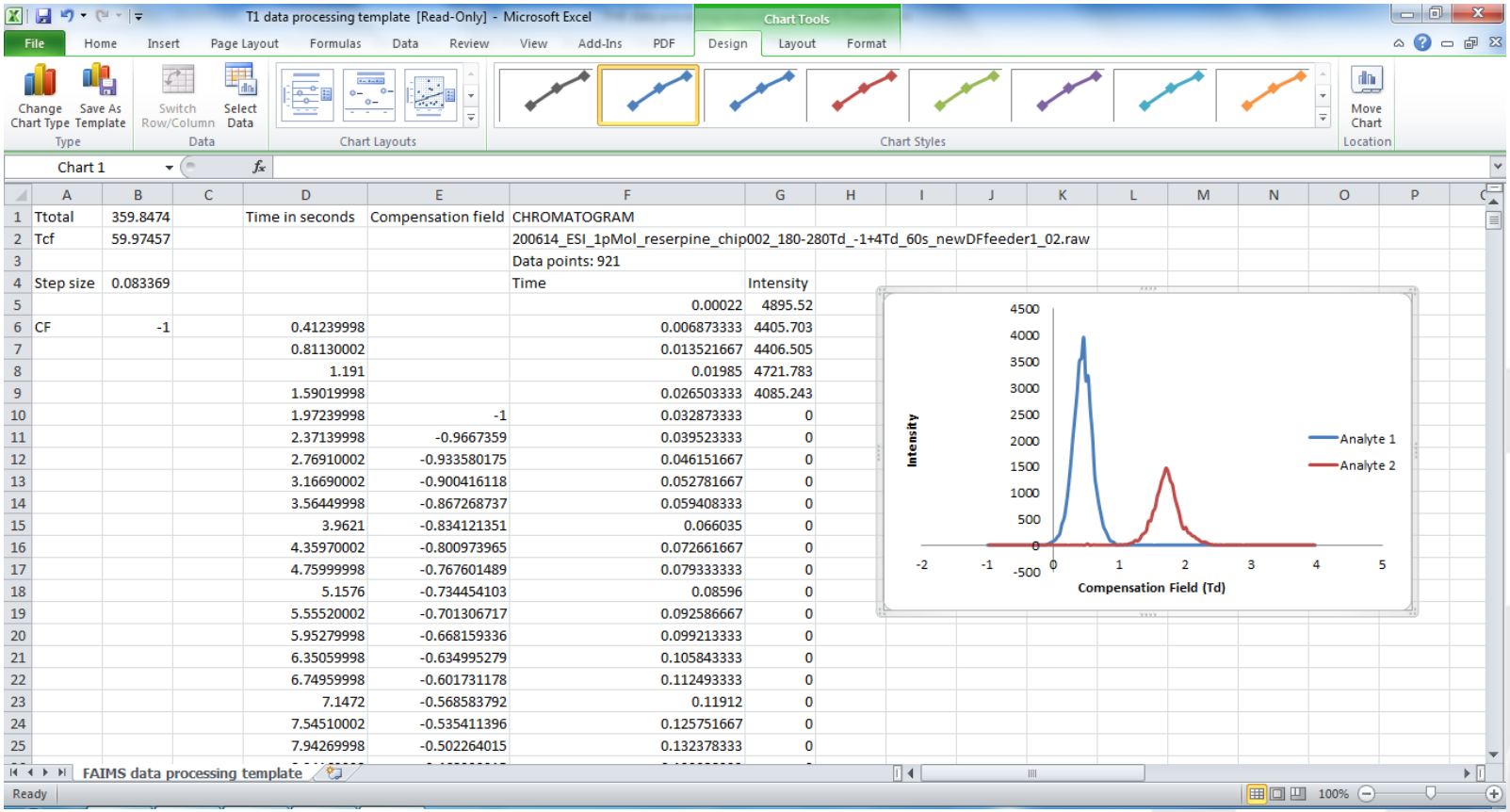
- Plotting intensity against CF allows the CF scan data to be visualised.



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.78
9				1.59019998		0.026503333	4085.243
10				1.97239998	-1	0.032873333	0
11				2.37139998	-0.9667359	0.039523333	0
12				2.76910002	-0.933580175	0.046151667	0
13				3.16690002	-0.900416118	0.052781667	0
14				3.56449998	-0.867268737	0.059408333	0
15				3.9621	-0.834121351	0.066035	0
16				4.35970002	-0.800973965	0.072661667	0
17				4.75999998	-0.767601489	0.079333333	0
18				5.1576	-0.734454103	0.08596	0
19				5.55520002	-0.701306717	0.092586667	0
20				5.95279998	-0.668159336	0.099213333	0
21				6.35059998	-0.634995279	0.105843333	0
22				6.74959998	-0.601731178	0.112493333	0
23				7.1472	-0.568583792	0.11912	0
24				7.54510002	-0.535411396	0.125751667	0
25				7.94269998	-0.502264015	0.132378333	0

Compare compensation field scans

- Different CF scans can then be plotted on the same graph to allow a comparison of their CF spectra.
- E.g.



	A	B	C	D	E	F	G
1	Ttotal	359.8474		Time in seconds	Compensation field	CHROMATOGRAM	
2	Tcf	59.97457				200614_ESI_1pMol_reserpine_chip002_180-280Td_-1+4Td_60s_newDFfeeder1_02.raw	
3						Data points: 921	
4	Step size	0.083369				Time	Intensity
5						0.00022	4895.52
6	CF	-1		0.41239998		0.006873333	4405.703
7				0.81130002		0.013521667	4406.505
8				1.191		0.01985	4721.783
9				1.59019998		0.026503333	4085.243
10				1.97239998	-1	0.032873333	0
11				2.37139998	-0.9667359	0.039523333	0
12				2.76910002	-0.933580175	0.046151667	0
13				3.16690002	-0.900416118	0.052781667	0
14				3.56449998	-0.867268737	0.059408333	0
15				3.9621	-0.834121351	0.066035	0
16				4.35970002	-0.800973965	0.072661667	0
17				4.75999998	-0.767601489	0.079333333	0
18				5.1576	-0.734454103	0.08596	0
19				5.55520002	-0.701306717	0.092586667	0
20				5.95279998	-0.668159336	0.099213333	0
21				6.35059998	-0.634995279	0.105843333	0
22				6.74959998	-0.601731178	0.112493333	0
23				7.1472	-0.568583792	0.11912	0
24				7.54510002	-0.535411396	0.125751667	0
25				7.94269998	-0.502264015	0.132378333	0