HINTS & TIPS

Guidance for users

How to Setup and Optimise Target Enhancement on a SYNAPT G2-Si MS and HDMS

This guidance document is directed at analysts who are planning to set up acquisition methods that require the increased sensitivity offered with the Target Enhancement capabilities (Tune page trapping options and EDC acquisition method options) provided with the SYNAPT G2-Si MS or HDMS mass spectrometer.

Guidance is provided on how to optimise instrument parameters in order to obtain the best enhancement across the entire mass range.

1. What is and Why use Target Enhancement

In normal Time of Flight operation, 5-20% of the ions from a continuous ion beam reach the detector from the Tof pusher region. Target Enhancement can be used to maximize the duty cycle for a targeted m/z range, increasing the number of ions that reach the detector.
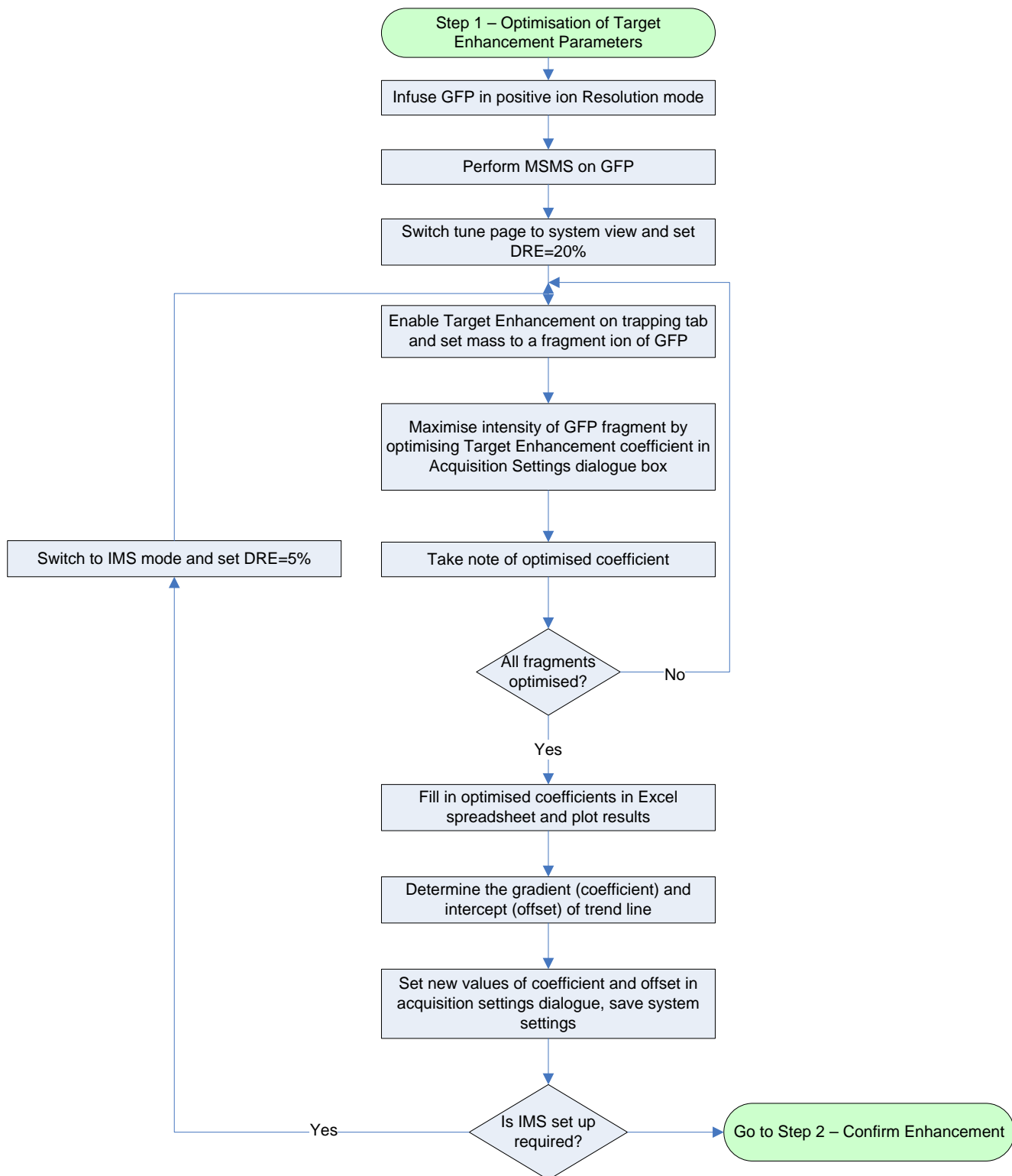*A Travelling waveform is used to split the ion beam into packets, which undergo a short time of flight separation as they travel through the transfer lens assembly. The Tof pusher is then synchronised with the arrival of ions having the targeted m/z. This will provide a 100% duty cycle, resulting in an increase of response between x5 and x15 depending on the m/z ratio of the ions of interest – larger gains in response are achievable for low m/z ions (typically less than m/z 300).*
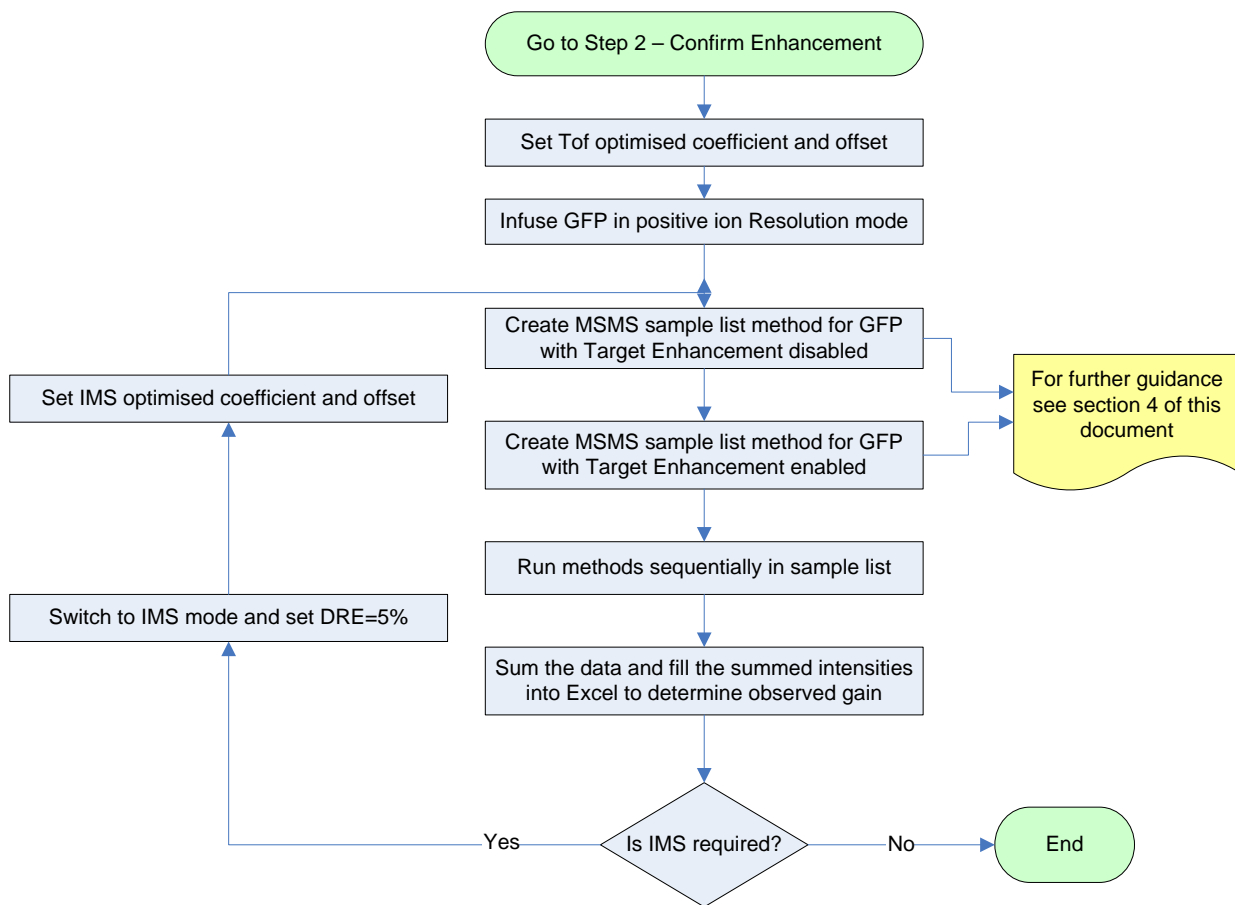
2. The Basics Steps of Target Enhancement Setup

Target Enhancement is optimized by adjusting the offset and coefficient values so that the intensity of the selected target mass is maximised. The offset and coefficient are time of flight calibration parameters used to define the motion of ions in the gap between the end of the Transfer Twave and the Pusher and are instrument specific. For a specified target mass the time of flight is always the same unless you change the trap voltage, extraction voltage, transfer wave speed or transfer wave voltage of the Transfer Twave. Creating a calibration sets the time at which the Pusher pushes to be equal to the time of flight of the target. As a result if any of the above conditions are changed a new calibration is required. Adjusting the calibration will not change the time that the targeted ions take to get to the pusher but instead change the timings of the Pusher.
The Calibration curve is a straight line (y=ax+b). However, the time of flight is not effectively described by this below about mass 100, so gains may become unpredictable below this mass.
It is necessary to determine the offset and coefficient in both Tof and IMS modes as the trap voltage is different between the two modes thus requiring different parameters for the two modes.

Step 1 – Optimisation of Target Enhancement Parameters

Infuse GFP in positive ion Resolution mode

Perform MSMS on GFP

Switch tune page to system view and set DRE=20%

Enable Target Enhancement on trapping tab and set mass to a fragment ion of GFP

Maximise intensity of GFP fragment by optimising Target Enhancement coefficient in Acquisition Settings dialogue box

Switch to IMS mode and set DRE=5%

Take note of optimised coefficient

All fragments optimised? — No

Yes

Fill in optimised coefficients in Excel spreadsheet and plot results

Determine the gradient (coefficient) and intercept (offset) of trend line

Set new values of coefficient and offset in acquisition settings dialogue, save system settings

Is IMS set up required? — Yes

Go to Step 2 – Confirm Enhancement

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                        ┌─────────────────────────────┐
                        │ Go to Step 2 – Confirm      │
                        │        Enhancement          │
                        └─────────────┬───────────────┘
                                      ▼
                        ┌─────────────────────────────┐
                        │ Set Tof optimised coefficient│
                        │        and offset           │
                        └─────────────┬───────────────┘
                                      ▼
                        ┌─────────────────────────────┐
                        │ Infuse GFP in positive ion  │
                        │      Resolution mode        │
                        └─────────────┬───────────────┘
                                      ▼
┌──────────────────────┐  ┌─────────────────────────────┐  ┌──────────────────┐
│                      │  │ Create MSMS sample list     │  │ For further      │
│                      │  │ method for GFP with Target  │──│ guidance         │
│                      │  │ Enhancement disabled        │  │ see section 4 of │
│ Set IMS optimised    │  └─────────────┬───────────────┘  │ this document    │
│ coefficient and      │                ▼                  └──────────────────┘
│ offset               │  ┌─────────────────────────────┐
│                      │  │ Create MSMS sample list     │
│                      │  │ method for GFP with Target  │
│                      │  │ Enhancement enabled         │
└──────────────────────┘  └─────────────┬───────────────┘
                                      ▼
┌──────────────────────┐  ┌─────────────────────────────┐
│ Switch to IMS mode   │  │ Run methods sequentially in │
│ and set DRE=5%       │  │        sample list          │
└──────────────────────┘  └─────────────┬───────────────┘
                                      ▼
                        ┌─────────────────────────────┐
                        │ Sum the data and fill the   │
                        │ summed intensities into     │
                        │ Excel to determine observed │
                        │ gain                        │
                        └─────────────┬───────────────┘
                                      ▼
          Yes ◄─────────────◇ Is IMS required? ◇──────── No ──────►  End
```

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3. Optimise Target Enhancement Parameters

Start optimisation by infusing a calibrant. The calibrant needs to have regularly spaced ions across the mass range of interest; for small molecule and proteomics applications Glu-fibrinopeptide B (GFP) is a suitable calibrant.

1. Infuse GFP at a concentration of 100 fmol/µL in 1:1 acetonitrile : water +0.1% formic acid at a flow rate of 5 µL/min.
2. Switch to Resolution positive ion mode.
3. Perform MSMS on the [M+2H]$^{2+}$ ion at *m/z* 785.8426 using a collision energy of 30V.
4. Ensure you have a stable beam.
5. Switch the tune page into system view.
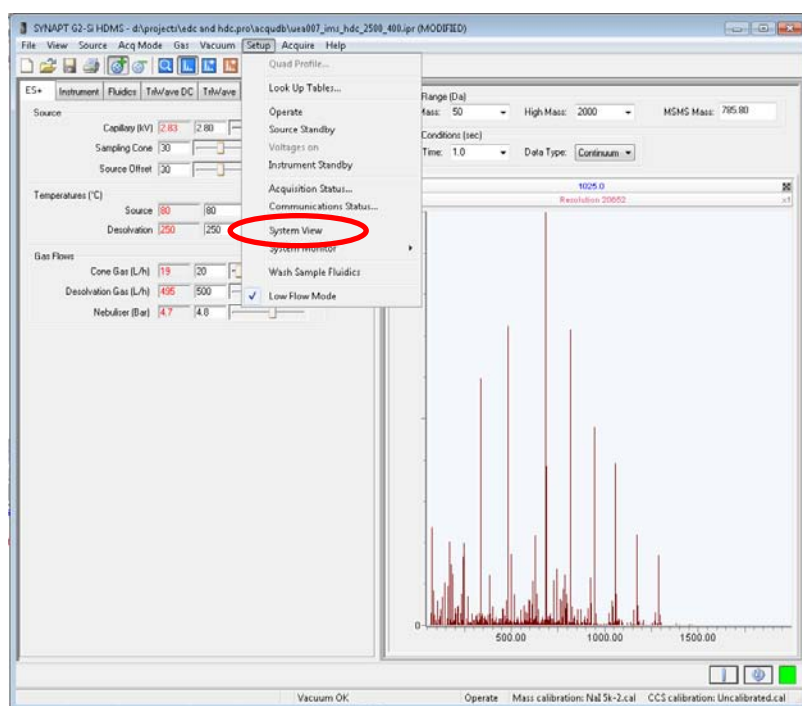
6. Set the DRE lens to 20% on the 'system 2' tab of the tune page.
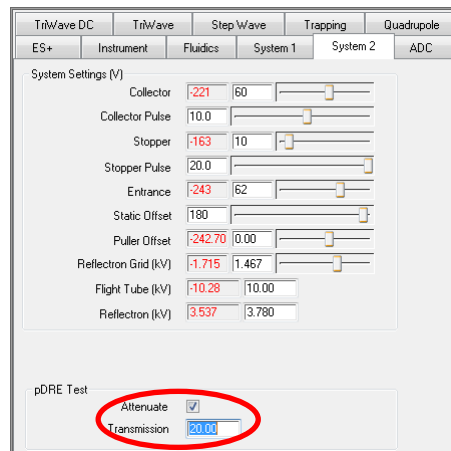
7. On the tune page 'trapping' tab set Target enhancement mass to 684.3 and enable 'Use Target Enhancement'.
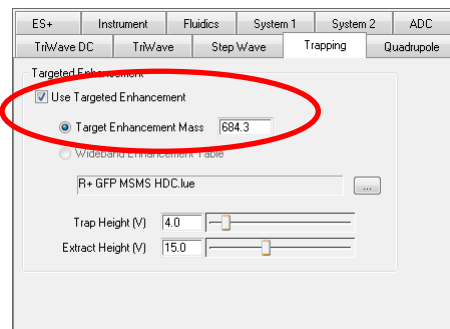
8. Under the 'system' menu select 'acquisition settings' make a note of the offset value (do not change) in the table opposite.

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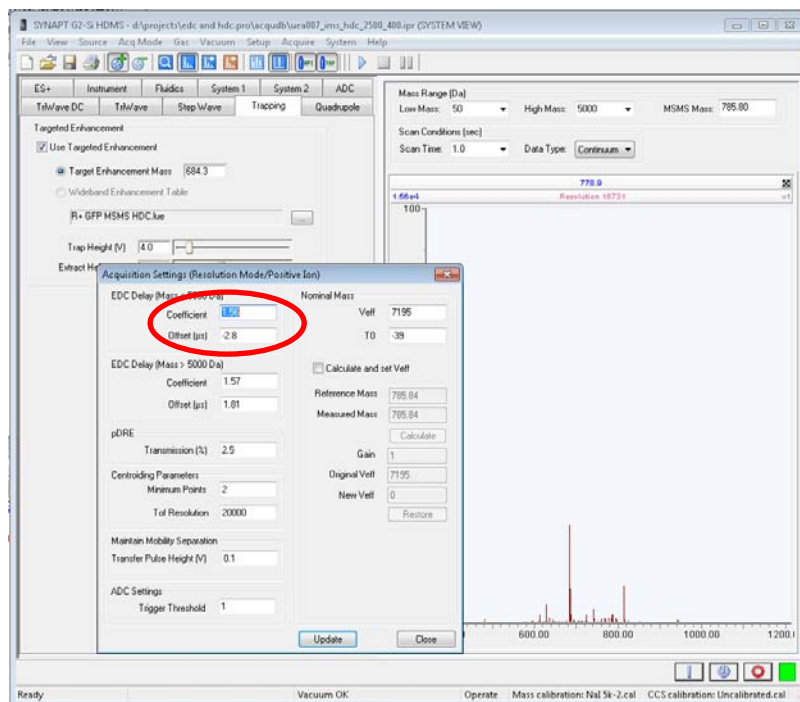
9. Change the coefficient value to optimise the intensity of the *m/z* 684.3 ion. Steps of ±0.03 should be sufficient to determine the optimised value (deselecting the normalisation of the tune page display may help). Make a note of the optimised coefficient for the *m/z* 684.3 ion in the table opposite.
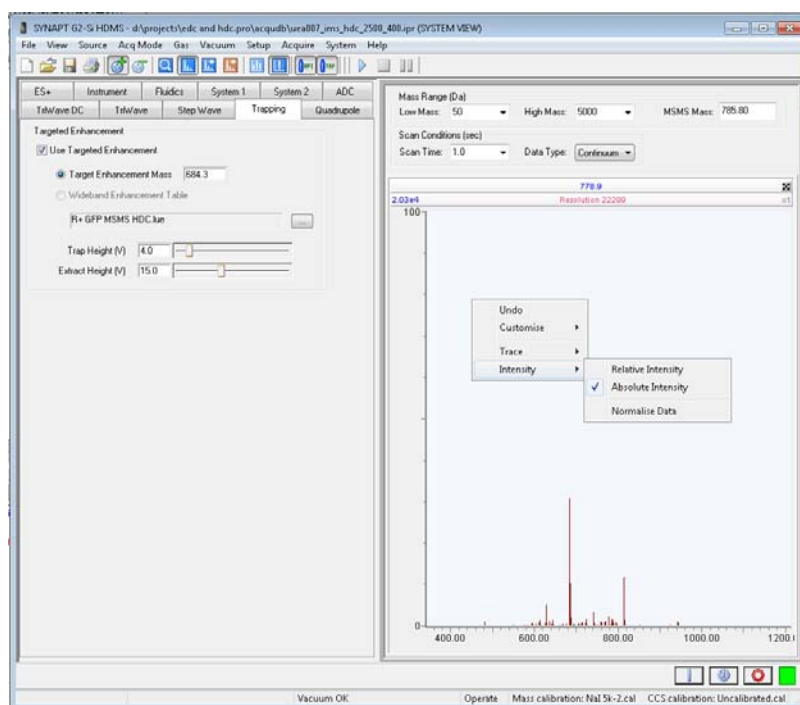
10. Repeat steps 7 to 9 on GFP fragments *m/z* 72.1, 120.1, 175.1, 246.2, 333.2, 382.2, 480.3, 627.3, 813.4, 942.4, 1056.5, 1171.5 and 1285.5 making a note of the optimised value of the coefficient for each ion in the table below.

GFP fragment ion / m/z	Offset Coefficient
72.1	
120.1	
175.1	
246.2	
333.2	
382.2	
480.3	
627.3	
684.3	
813.4	
942.4	
1056.5	
1171.5	
1285.5	

11. In the attached Excel spreadsheet (Target Enhancement Calibration tab, SAP part number 721005998) fill in the offset value and the values of the coefficient for each mass in the Target Enhancement Calibration. The gradient of the resulting trend line is the coefficient and the intercept is the offset.

12. The values of the coefficient and offset determined in Excel should now be set in the 'acquisition settings' dialogue box in the tune page. These parameters are equally applicable for resolution and sensitivity, positive and negative ion modes. It is important to note that these values will have to be set on each tune as the 'acquisition settings' are independent for each mode. Ensure that you save these new system settings.

13. Repeat in IMS mode (where applicable). It may be necessary to set the DRE lens to 5% (or lower) on the 'system 2' tab of the tune page to prevent saturation. Unfortunately, the 'acquisition settings' are not independent between Tof and IMS modes. You must, therefore, remember to manually change the Target Enhancement values used when you change between Tof and IMS modes.

4. Confirm Gain Enhancement Across the Mass Range

Once calibrated the gain should be optimised across the mass range. This can be determined by acquiring GFP fragments with the target enhancement enabled and then again with the target enhancement disabled and comparing the observed gain to the theoretical gain.

1. Infuse GFP at a concentration of 100 fmol/µL in 1:1 acetonitrile : water +0.1% formic acid at a flow rate of 5 µL/min.
2. Ensure you have a stable beam.
3. Create a GFP MSMS method in the sample list:

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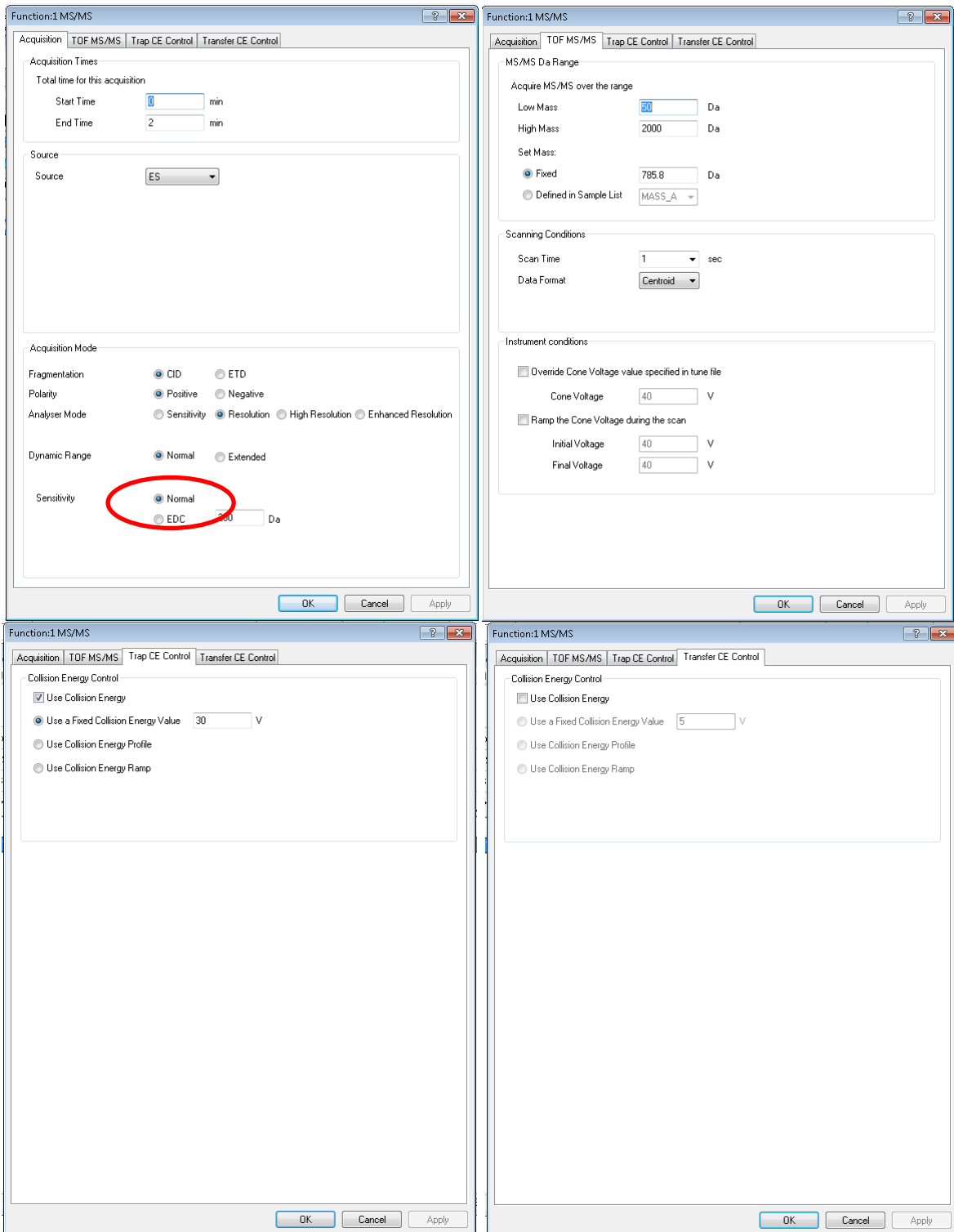
4. Create a second GFP MSMS method in the sample list, but with Target Enhancement enabled with a time resolved function for each GFP fragment ion (a total of 14 functions):

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Function number	Target Enhancement mass (EDC)
1	72.1
2	120.1
3	175.1
4	246.2
5	333.2
6	382.2
7	480.3
8	627.3
9	684.3
10	813.4
11	942.4
12	1056.5
13	1171.5
14	1285.5

5. Ensure the optimised values of coefficient and offset are saved in the 'acquisition settings' dialogue box.

6. Run the methods by creating a sample list with the Target Enhancement disabled, repeat with it enabled and finally repeat with it disabled again. The target enhancement disabled data are acquired twice as these are used to calculate the gain and an average value is used to compensate for spray stability / changing conditions.

	File Name	File Text	MS File	Inlet File	
1	MP091613_012	100fmol/µL GFP MSMS	R+ MSMS GFP		
2	MP091613_013	100fmol/µL GFP MSMS EDC coefficient/offset=1.56/-2.8	R+ MSMS EDC GFP		
3	MP091613_014	100fmol/µL GFP MSMS	R+ MSMS GFP		
4					

7. Combine the data and fill the summed intensities into the attached Excel spreadsheet (Target Enhancement Gain tab, SAP part number 721005998) to determine the Target Enhancement gain.

8. This will provide an indication of the gains achieved with Target Enhancement at any given *m/z*. Typical gains should be in the region x5 to 10 improvement.

9. Repeat in IMS mode (where applicable). It may be necessary to set the DRE lens to 5% (or lower) on the 'system 2' tab of the tune page to prevent saturation. Unfortunately, the 'acquisition settings' are not independent between Tof and IMS modes. You must, therefore, remember to manually change the Target Enhancement values used when you change between Tof and IMS modes.

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