HINTS & TIPS

Guidance for users

How to perform quantitation using Tof-MRM on a SYNAPT G2-*Si* system and MassLynx Desktop (SCN901)

This guidance document is directed at analysts who are planning to perform accurate quantitation using the Tof-MRM functionality available on a SYNAPT G2-Si mass spectrometer and process the data using the Targetlynx applications manager supplied with MassLynx Desktop (SCN901).

This document provides a step by step guide along with an example of a typical experiment including materials, methods and results.

1. Why use Tof-MRM for quantitation?

The new MRM method makes use of the unique SYNAPT G2-Si ion optics to deliver very low limits of detection and quantification with 100% Tof duty cycle for transitions of interest. The new method also incorporates RADAR (m/z, retention time and intensity), which provides full scan accurate mass information at regular time points during a chromatographic run to assist in method development and the evaluation of matrix effects. The Tof-MRM method editor delivers simple MS acquisition and processing method set up and TargetLynx software provides automated data processing and review.

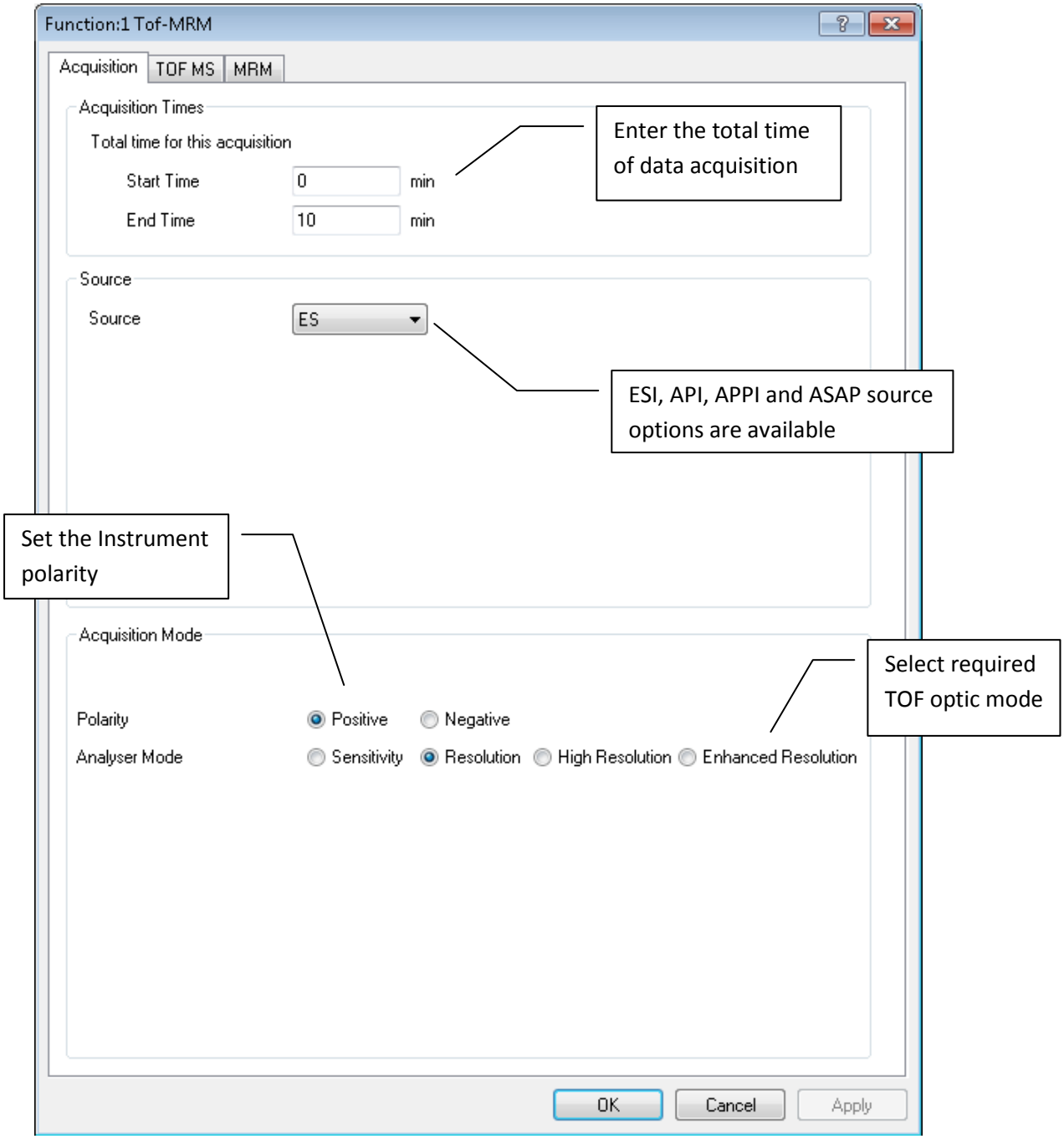
2. The Basics of Tof-MRM on Synapt G2-*Si*

MRM acquisitions on tandem quadrupole instruments work by mass selecting a target ion in the first scanning quadrupole (Q1) followed by fragmentation with Collision Induced Dissociation in a T-wave collision cell, after which a target fragment ion (MRM transition) is isolated with the second scanning quadrupole (Q2) and the associated ion current recorded with a detector. The Tof-MRM acquisition mode on the SYNAPT G2-Si works in a similar fashion. The chosen parent ion is selected by the quadrupole and fragmented to a known target ion in either the trap or the transfer collision cell. The QuanTof analyzer is then used to provide quantitative measurement of that MRM transition, together with the benefits of high resolution Time of flight and exact mass measurement. Like tandem quadrupole MRM, this method greatly increases selectivity and sensitivity resulting in decreased limit of detections compared to traditional scanning modes. In addition Target Enhancement can be used to preferentially monitor the target fragment ion, boosting sensitivity further.

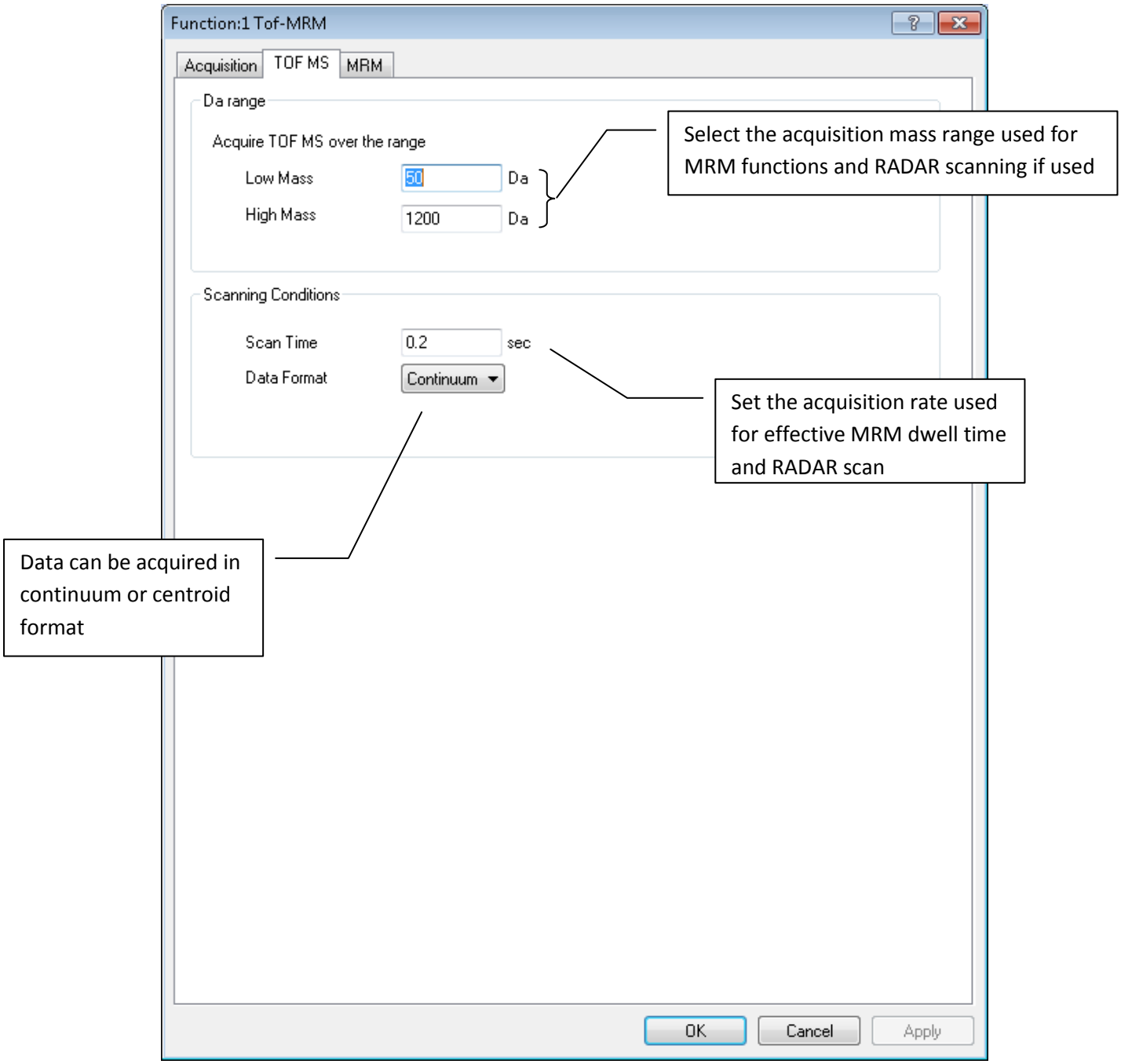
3. How to setup a Tof-MRM Experiment

To set up a Tof-MRM experiment the Tof-MRM acquisition type must be chosen in the MS method editor window. Specifying the required parameters for the experiment then proceeds as follows:

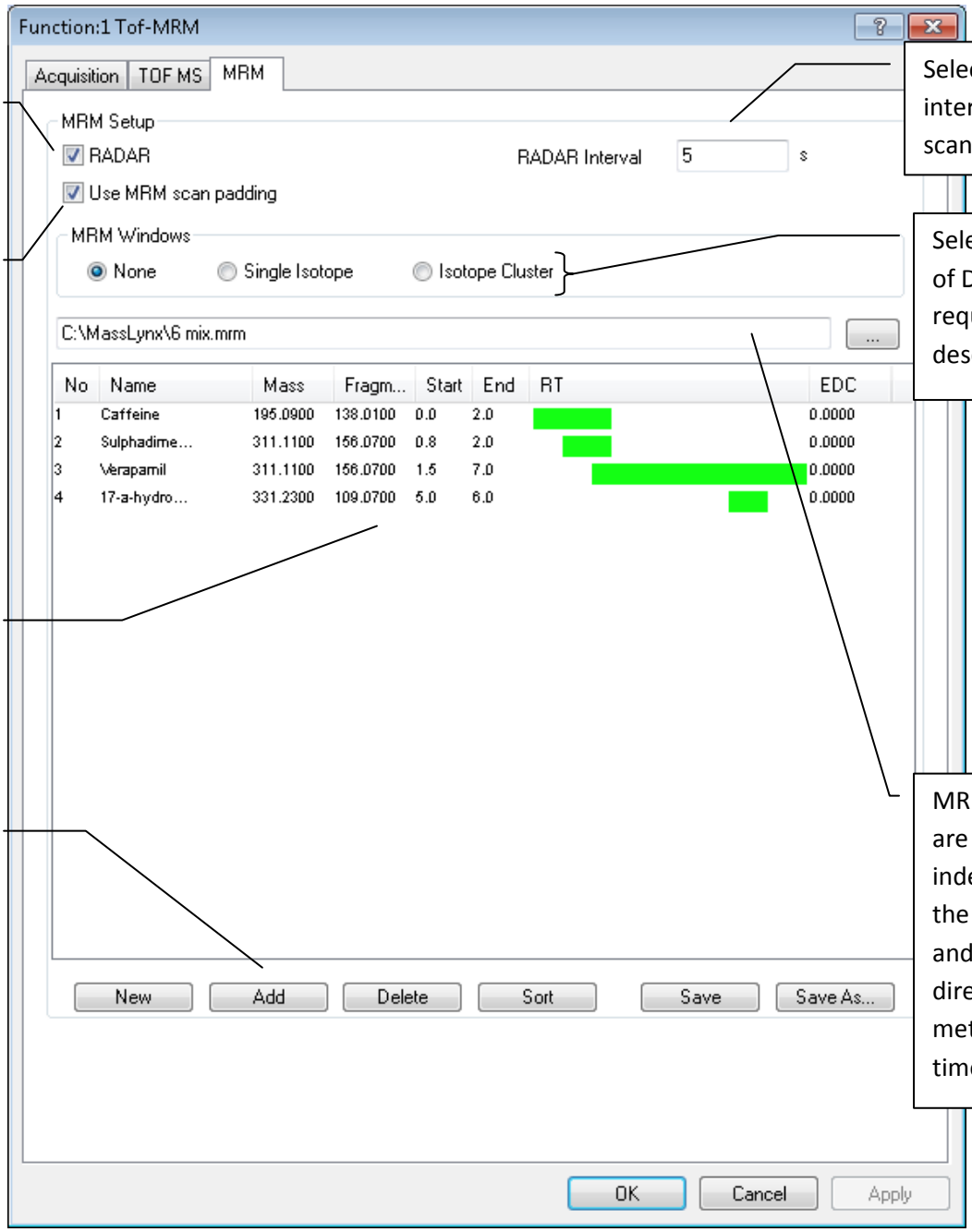
1) In the first tab, specify the basic method parameters including time length, source type, polarity and Tof mode.



2) In the second tab, input parameters specific to the Tof analyzer including mass range, scan time and data format.



3) The third tab, contains parameters that are specific to the MRM experiment to be performed. MRMs can be scheduled manually by inputting start and end times, masses of precursor and fragment ions, collision energies, cone voltage and a Target Enhancement mass if used. MRM schedules can also be imported directly as a *.mrm file from a previous experiment or from an excel spreadsheet to save time. Other options on the MRM tab include the toggling of RADAR, scan padding and data stripping if required (see descriptions below).



Select RADAR if required (see description below)

Select Scan Padding if required (see description below)

Populate this box with the MRM schedule for the experiment.

MRMs are added to the schedule by selecting Add. This opens the 'MRM Editor' window (see next page)

Select the time interval for RADAR scanning if selected

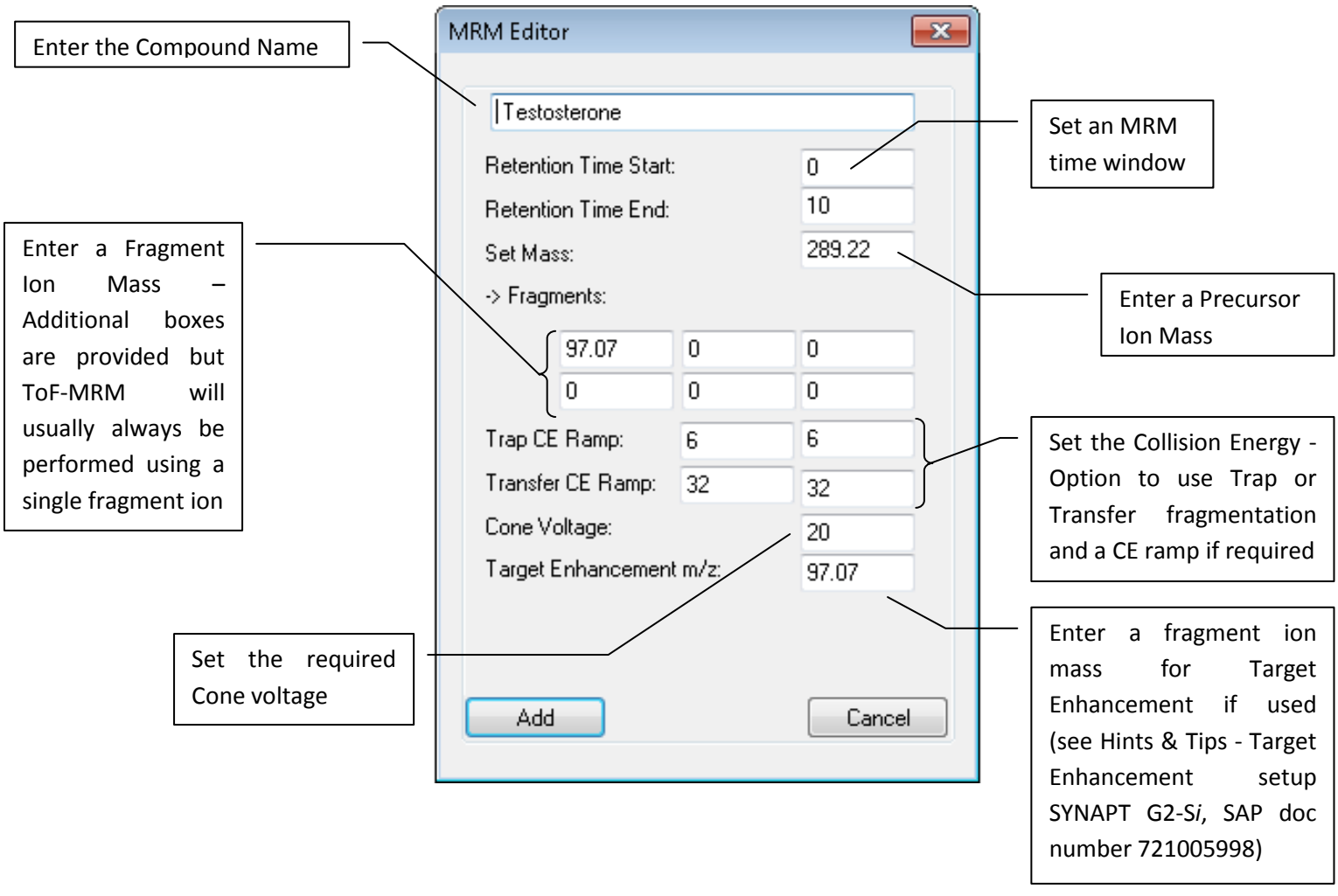
Select the degree of Data Stripping required (see description below)

MRM schedules are saved independently of the MS Method and can be loaded directly into a new method to save time

RADAR – If so desired it is possible to schedule a full spectrum Tof MS scan to provide additional data on what else is present in the sample outside of the MRM transitions that are the principle focus of the experiment.

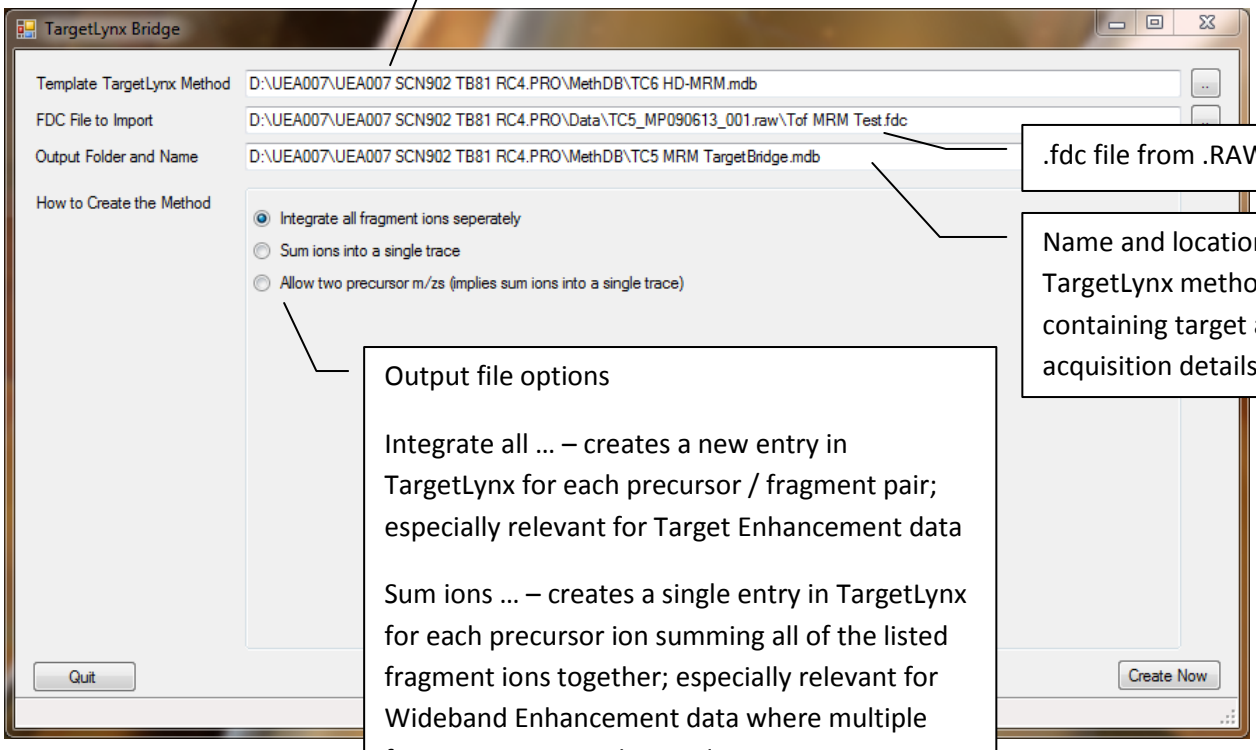
Scan Padding – If multiple overlapping MRMs are scheduled such that the number of simultaneous MRMs changes as the run proceeds it is advisable to enable Scan Padding. This feature inserts dummy functions into regions where the number of consecutive MRMs drops such that the amount of time spent looking for any given transition is consistent over the lifetime of the experiment. This is important for accurate quantitation.

Data Stripping – MassLynx will store data that covers the entire acquisition mass range specified in the Tof MS tab by default. For ease of viewing and to greatly reduce file size, data stripping can be used to limit the Tof MS data that is written to disk. The Isotope Cluster option retains data within a window of -1/+5 Da around the target fragment ion mass while the Single Isotope option retains data within a +/- 0.2 Da window centred on the target fragment ion mass.



4. Data Processing

Data should have lock mass applied either during or post acquisition. It is recommended that the data be centered (use AFAMM or AutoAFAMM in MassLynx) prior to processing in TargetLynx. When Tof-MRM data is acquired a file (.fdc) is created within the .RAW folder, this file contains details of the compound including name, precursor and fragment ion *m/z*, collision energy, expected retention time and the data channel in which this transition can be found. This file can be imported into TargetLynx Bridge (part of SCN901 MassLynx desktop) and merged with a template TargetLynx method file to populate a new TargetLynx method file with the target analyte acquisition details.



TargetLynx template – a 'blank' method file containing appropriate integration / processing parameters

.fdc file from .RAW folder

Name and location of new TargetLynx method file containing target analyte acquisition details

Output file options

Integrate all … – creates a new entry in TargetLynx for each precursor / fragment pair; especially relevant for Target Enhancement data

Sum ions … – creates a single entry in TargetLynx for each precursor ion summing all of the listed fragment ions together; especially relevant for Wideband Enhancement data where multiple fragment ions are observed

Allow two … – creates a single entry in TargetLynx combining two precursors that have common fragment ions, for example, a peptide of 2+ and 3+ charge states fragmenting to *m/z* 300 would be combined

5. Example Experiment

The following section outlines an example of a Tof-MRM experiment for the quantitation of testosterone in solvent standards. Testosterone is injected as calibration standards, QCs and a sample of 'unknown' concentration. All necessary instrument setup is performed prior to running the experiment including a detector gain test, resolution setup, mass calibration and LockSpray setup. The data are processed using the Targetlynx applications manager within MassLynx Desktop (SCN901).

5.1 Method

5.1.1. Samples

Stock solutions:	1µg/µl of Testosterone in MeCN
Standards:	Testosterone in 90:10 H2O/MeCN @ 1fg/µl, 5fg/µl, 10fg/µl, 50fg/µl, 100fg/µl, 500fg/µl, 1pg/µl, 5pg/µl, 10pg/µl, 50pg/µl and 100pg/µl
QCs:	50fg/µl and 5pg/ul testosterone in 90:10 H2O/MeCN
'Unknown':	500fg/µl testosterone in 90:10 H2O/MeCN

5.1.2. MS Source Conditions

ESI+, Resolution mode

Capillary voltage:	0.8 kV
Cone Voltage:	30 V
Desolvation Temperature:	600 °C
Source Temperature:	120 °C
Desolvation Gas:	1000 L/Hr
Cone Gas:	0 L/Hr

5.1.3. MS Acquisition Parameters

Tof MRM with EDC applied.

MRM transitions:	Testosterone - 289.22 m/z > 97.07 m/z
MSMS collision energy:	20 eV
Scan time:	0.1 s
Start mass:	50 Da
End mass:	1200 Da
EDC set mass:	97.07 m/z
Profile stripping:	Isotope cluster

5.1.4. LockMass Setup

Leucine Enkephalin:	200 pg/µL in 1:1 aqueous 0.1% formic acid : ACN
Lockmass:	m/z 556.2771
Scan time:	0.1 s
Flow rate:	20 µL/min
Capillary voltage:	2 kV
Collision energy:	4 eV
Frequency	20s

5.1.5. LC Conditions

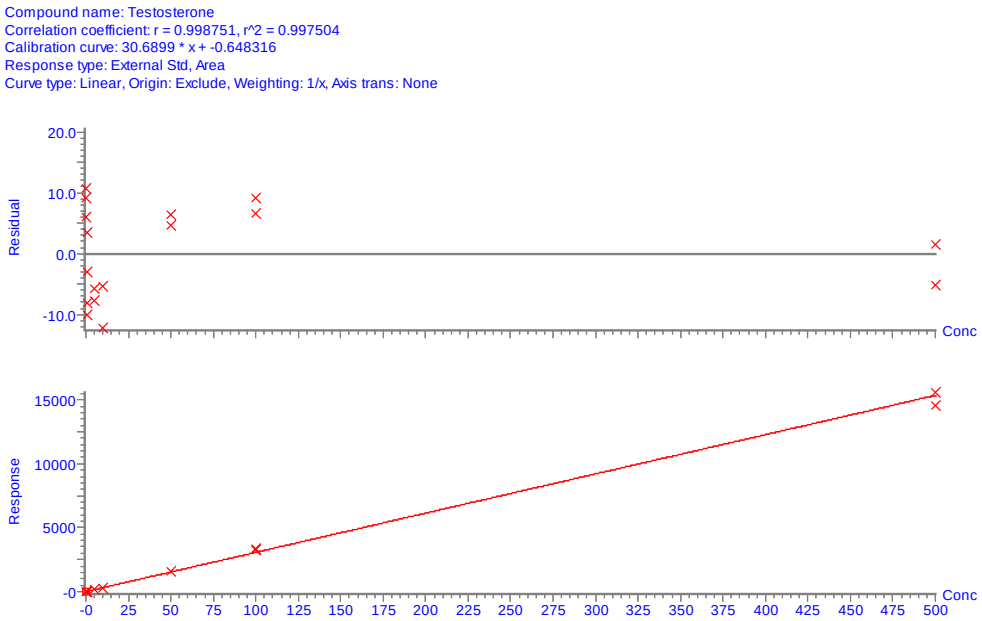
Analytical column:	2.1 x 50 mm Acquity BEH C18 1.7 µm
Column temp:	45 °C
Sample temp:	5°C
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid
Strong wash:	Acetonitrile + 0.1% formic acid
Weak wash:	90/10 Water/Acetonitrile
Seal wash:	90/10 Water/Acetonitrile
Flow rate:	0.6 mL/min
Injection volume:	10 µL
Purge wash:	600 µL
Wash solvent:	600 µL
Run Time:	4.0 min

Gradient:

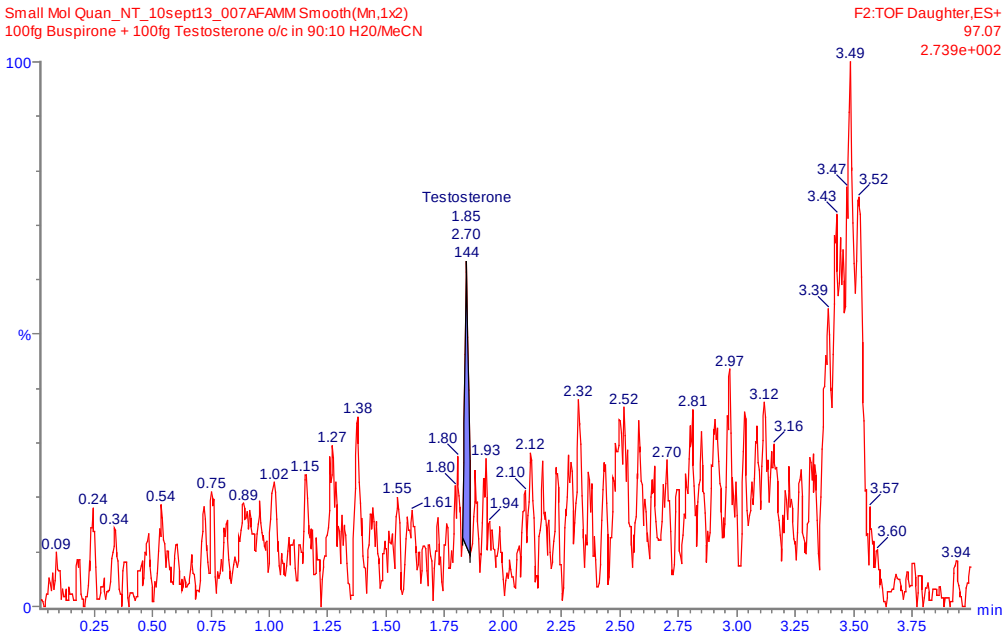
Time (min)	%A	%B	curve
0.00	95	5	6
3.0	15	85	6
3.1	2	98	6
3.2	2	98	6
3.3	95	5	6
4.0	95	5	6

5.2 Results

Results are presented in this section from data collected using the above method. The figure below shows a calibration line for testosterone showing over 3 orders of linear dynamic range.



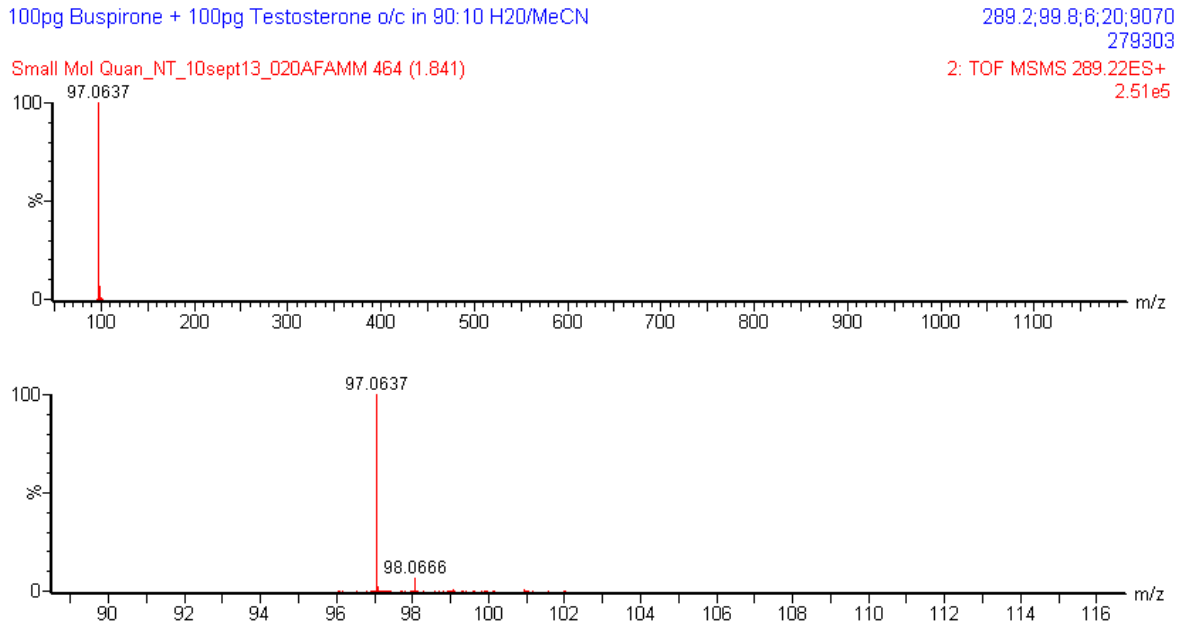
The chromatogram below shows the EIC for 100fg of testosterone on column.



The following table shows responses and % deviations for a typical sample set containing calibration standards, QCs and an unknown sample.

	#	Name	Type	Std. Conc	RT	Area	IS Area	Response	Primar...	Conc.	%Dev
1	1	Small Mol Quan_NT_10sept13_001AFAMM	Blank		1.86	0.204		0.204	bb	0.0	
2	2	Small Mol Quan_NT_10sept13_002AFAMM	Blank		1.84	1.092		1.092	bb	0.1	
3	3	Small Mol Quan_NT_10sept13_003AFAMM	Standard	0.010	1.83	0.087		0.087	bbX	0.0	139.6
4	4	Small Mol Quan_NT_10sept13_004AFAMM	Standard	0.010	1.84	0.145		0.145	bdX	0.0	158.5
5	5	Small Mol Quan_NT_10sept13_005AFAMM	Standard	0.050	1.85	0.979		0.979	bb	0.1	6.0
6	6	Small Mol Quan_NT_10sept13_006AFAMM	Standard	0.050	1.85	1.051		1.051	bb	0.1	10.7
7	7	Small Mol Quan_NT_10sept13_007AFAMM	Standard	0.100	1.85	2.698		2.698	bb	0.1	9.0
8	8	Small Mol Quan_NT_10sept13_008AFAMM	Standard	0.100	1.84	3.739		3.739	bbX	0.1	43.0
9	9	Small Mol Quan_NT_10sept13_009AFAMM	Standard	0.500	1.84	15.226		15.226	bb	0.5	3.4
10	10	Small Mol Quan_NT_10sept13_010AFAMM	Standard	0.500	1.84	14.248		14.248	bb	0.5	-2.9
11	11	Small Mol Quan_NT_10sept13_011AFAMM	Standard	1.000	1.84	27.530		27.530	bb	0.9	-8.2
12	12	Small Mol Quan_NT_10sept13_012AFAMM	Standard	1.000	1.85	26.925		26.925	bb	0.9	-10.2
13	13	Small Mol Quan_NT_10sept13_013AFAMM	Standard	5.000	1.84	140.956		140.956	bb	4.6	-7.7
14	14	Small Mol Quan_NT_10sept13_014AFAMM	Standard	5.000	1.84	144.051		144.051	bb	4.7	-5.7
15	15	Small Mol Quan_NT_10sept13_015AFAMM	Standard	10.000	1.84	268.751		268.751	bb	8.8	-12.2
16	16	Small Mol Quan_NT_10sept13_016AFAMM	Standard	10.000	1.84	289.860		289.860	bb	9.5	-5.3
17	17	Small Mol Quan_NT_10sept13_017AFAMM	Standard	50.000	1.84	1631.345		1631.345	bb	53.2	6.4
18	18	Small Mol Quan_NT_10sept13_018AFAMM	Standard	50.000	1.85	1604.616		1604.616	bb	52.3	4.6
19	19	Small Mol Quan_NT_10sept13_019AFAMM	Standard	100.000	1.84	3349.926		3349.926	bb	109.2	9.2
20	20	Small Mol Quan_NT_10sept13_020AFAMM	Standard	100.000	1.84	3269.433		3269.433	bb	106.6	6.6
21	21	Small Mol Quan_NT_10sept13_021AFAMM	Standard	500.000	1.84	15579.671		15579.671	bb	507.7	1.5
22	22	Small Mol Quan_NT_10sept13_022AFAMM	Standard	500.000	1.84	14537.532		14537.532	bb	473.7	-5.3
23	23	Small Mol Quan_NT_10sept13_023AFAMM	Blank		1.85	0.970		0.970	bd	0.1	
24	24	Small Mol Quan_NT_10sept13_024AFAMM	Blank		1.85	1.834		1.834	bb	0.1	
25	25	Small Mol Quan_NT_10sept13_025AFAMM	QC	0.500	1.85	15.683		15.683	bb	0.5	6.4
26	26	Small Mol Quan_NT_10sept13_026AFAMM	QC	0.500	1.84	15.730		15.730	bb	0.5	6.7
27	27	Small Mol Quan_NT_10sept13_027AFAMM	QC	0.500	1.84	13.572		13.572	MM	0.5	-7.3
28	28	Small Mol Quan_NT_10sept13_028AFAMM	Blank		1.83	0.184		0.184	bb	0.0	
29	29	Small Mol Quan_NT_10sept13_029AFAMM	Blank		1.86	1.130		1.130	bb	0.1	
30	30	Small Mol Quan_NT_10sept13_030AFAMM	QC	50.000	1.84	1718.284		1718.284	bb	56.0	12.0
31	31	Small Mol Quan_NT_10sept13_031AFAMM	QC	50.000	1.84	1708.651		1708.651	bb	55.7	11.4
32	32	Small Mol Quan_NT_10sept13_032AFAMM	QC	50.000	1.84	1678.885		1678.885	bb	54.7	9.5
33	33	Small Mol Quan_NT_10sept13_033AFAMM	Blank		1.84	1.207		1.207	bb	0.1	
34	34	Small Mol Quan_NT_10sept13_034AFAMM	Blank		1.84	0.132		0.132	bb	0.0	
35	35	Small Mol Quan_NT_10sept13_035AFAMM	Analyte	5.000	1.84	145.375		145.375	bb	4.8	-4.8
36	36	Small Mol Quan_NT_10sept13_036AFAMM	Analyte	5.000	1.84	153.802		153.802	bb	5.0	0.7
37	37	Small Mol Quan_NT_10sept13_037AFAMM	Analyte	5.000	1.84	151.695		151.695	bb	5.0	-0.7
38	38	Small Mol Quan_NT_10sept13_038AFAMM	Analyte	5.000	1.84	147.353		147.353	bb	4.8	-3.6
39	39	Small Mol Quan_NT_10sept13_039AFAMM	Analyte	5.000	1.84	150.976		150.976	bb	4.9	-1.2
40	40	Small Mol Quan_NT_10sept13_040AFAMM	Blank		1.85	0.152		0.152	bb	0.0	
41	41	Small Mol Quan_NT_10sept13_041AFAMM	Blank		1.84	0.606		0.606	bb	0.0	

The following spectrum and the expansion underneath shows what is achieved when isotope cluster data stripping is applied.



The following spectrum and the expansion below is an example of a RADAR scan taken near to the chromatographic peak of interest. Note the number of additional peaks present that would not have been detected without the use of RADAR.