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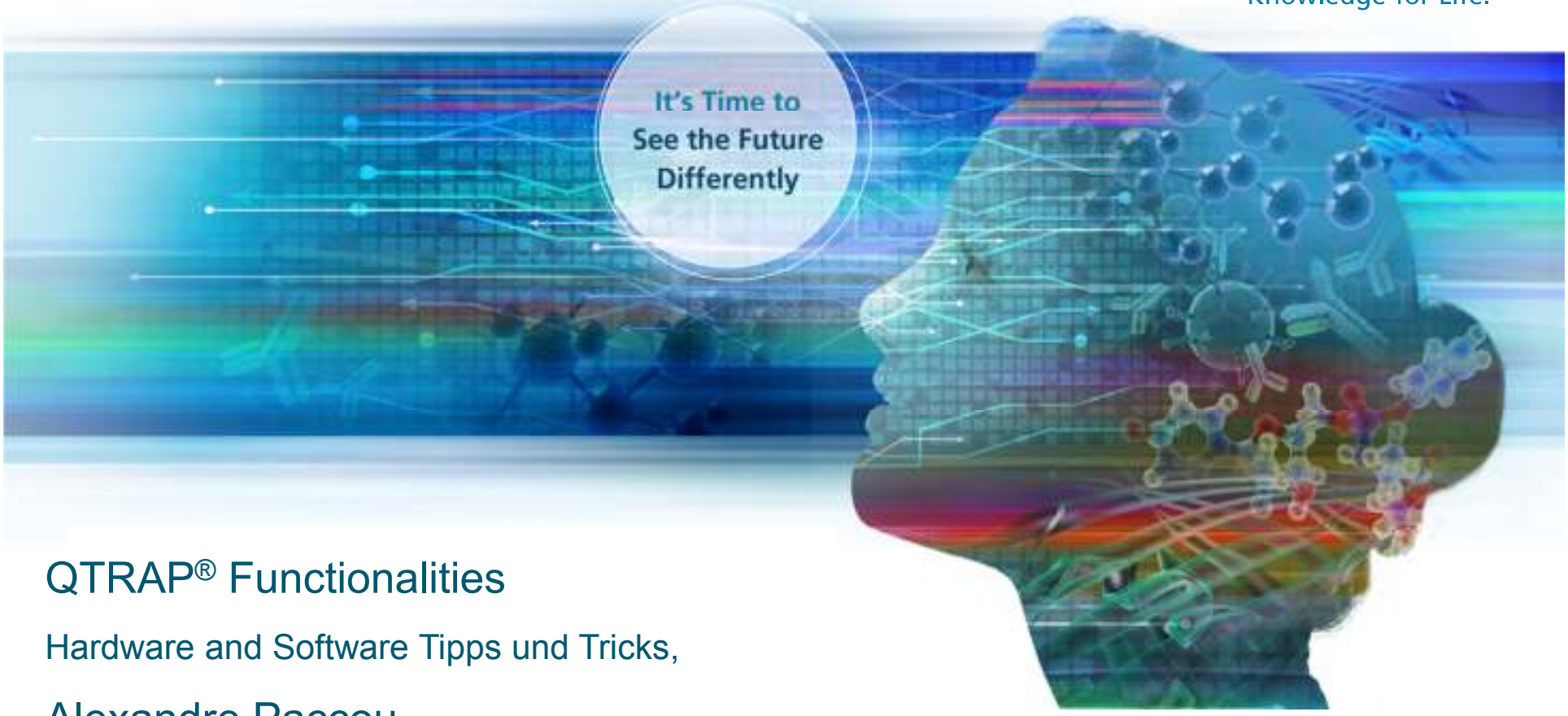

QTRAP® Functionalities

Hardware and Software Tipps und Tricks,

Alexandre Paccou

RUO- MKT-11-5404-A

- The QTRAP® instrument
- Calibration Check
- The workflow design
  - What is it?
  - How to set up?
  - Application examples and what can go wrong…
  - Live MasterView™ software training
- Questions / Discussion

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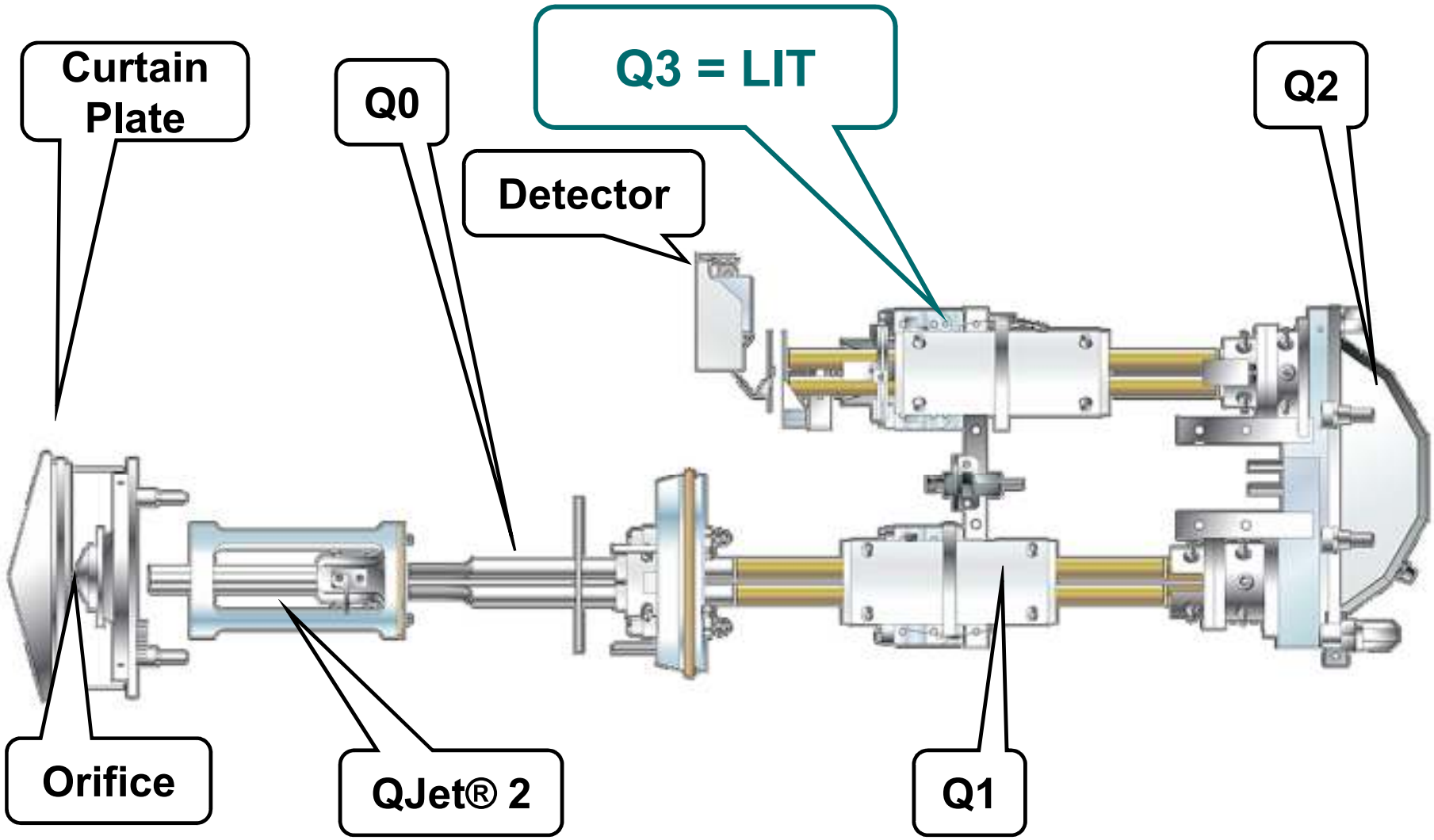
General setup of the newer SCIEX QTRAP® LC/MS/MS systems



Principle of a Linear Ion Trap

An ion trap is working sequently (MS in time)
while an QqQ operates continuously (MS in space)

- **0. Dynamic Fill Time determination**

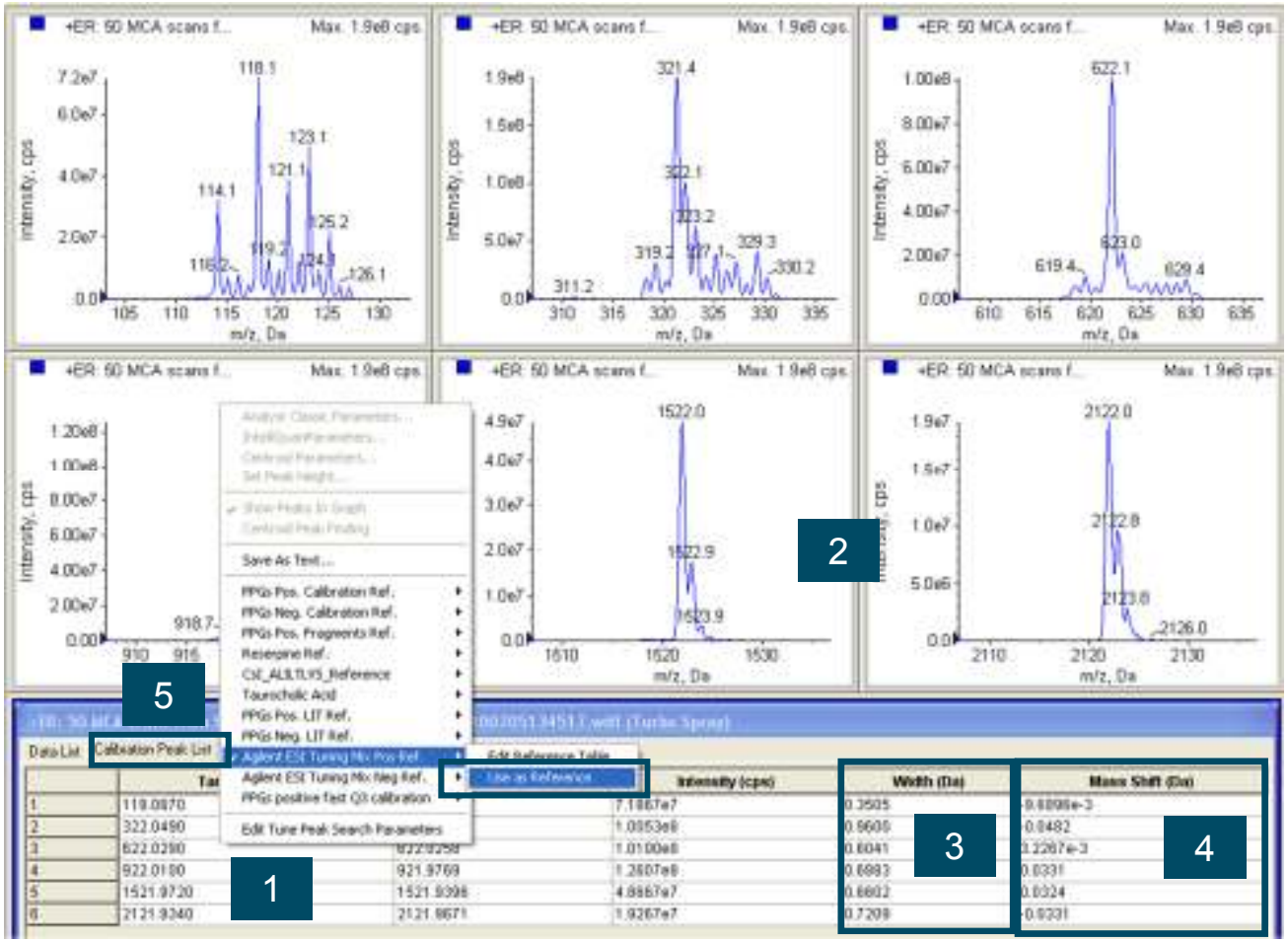
- **1. Trapping**
  - Ions enter the linear ion trap
  - Ions are trapped radially by quadrupole fields (RF)
  - Ions can not leave trap due to Cell Exit Potential (CXP) and Exit Barrier (EXB)

- **1.5. keep the ions happy**

- **2. Scanning**
  - New ions can not enter the trap due to high potential applied at IQ3
  - Ramp of auxiliary frequence (AF3) and EXB

Please Note : For all LIT experiments on QTRAP® 3200/4000 the Collision Gas (CAD) is usually set to “high”. It is used for damping and cooling of the ions. Not necessary for QTRAP® 5500/4500/6500+

LIT Calibration – please check from time to time



1. Right click into the table
2. Choose correct reference masses
3. Control Width and compare with specification
4. Control Mass shift <|0.1|
5. If calibration peak list tab is not present=>

=>Tools/settings /appearance options/Tab : miscellaneous/select «show mass calibration peak list »

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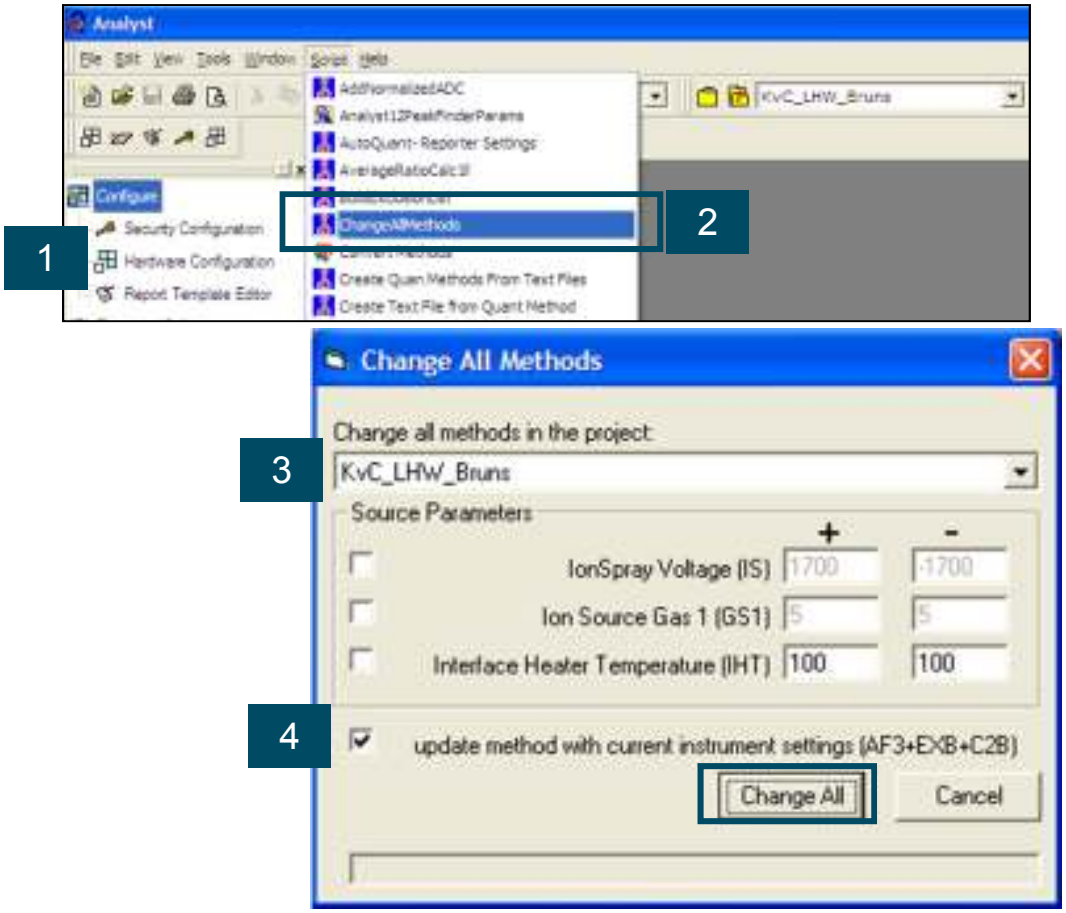
IMPORTANT: Updating trap method settings after calibration of LIT (2) - using the script

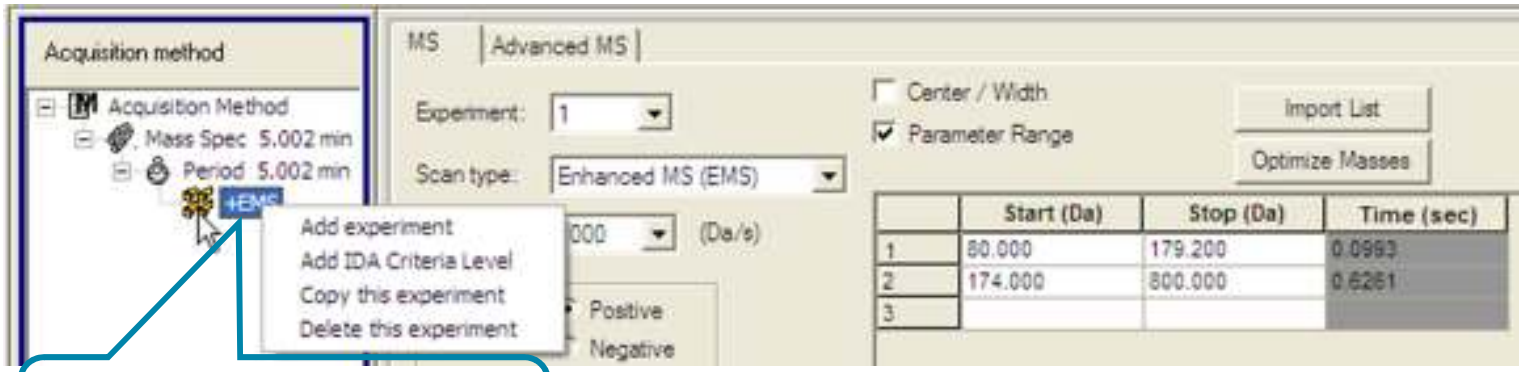


1. Confirm presence of LC in active hardware configuration !!!!
2. Go to script menu and select script
3. Select the project to update
4. Select update method

- Click on Change All
- This script will update the AF3 and EXB for all methods in the selected project

Manual Setup is easy – use the right mouse button

- 1:



„Click“ right mouse button

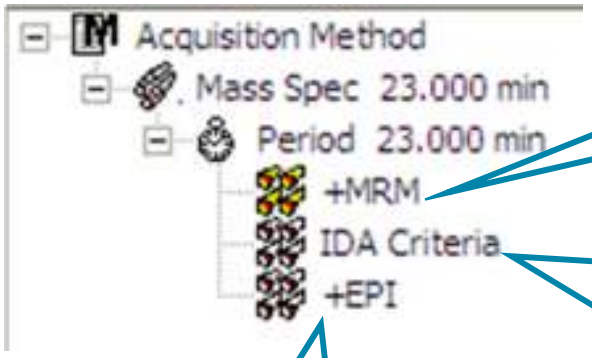
Add IDA Criteria Level

- 2:

„Click“ right mouse button

Add experiment (EPI)

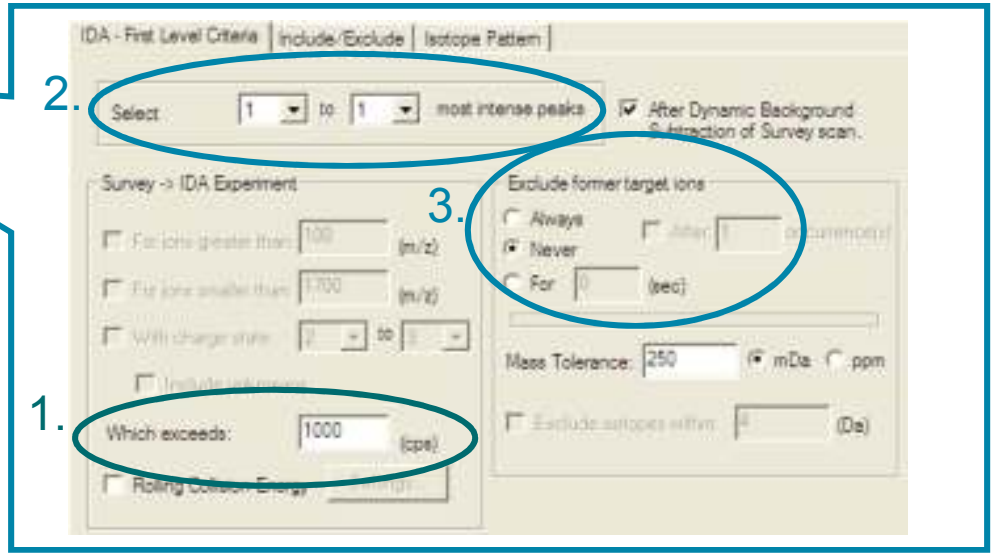
How is IDA working?



Survey Scan:

This experiment runs always

The results of this experiment are checked by the IDA criterium

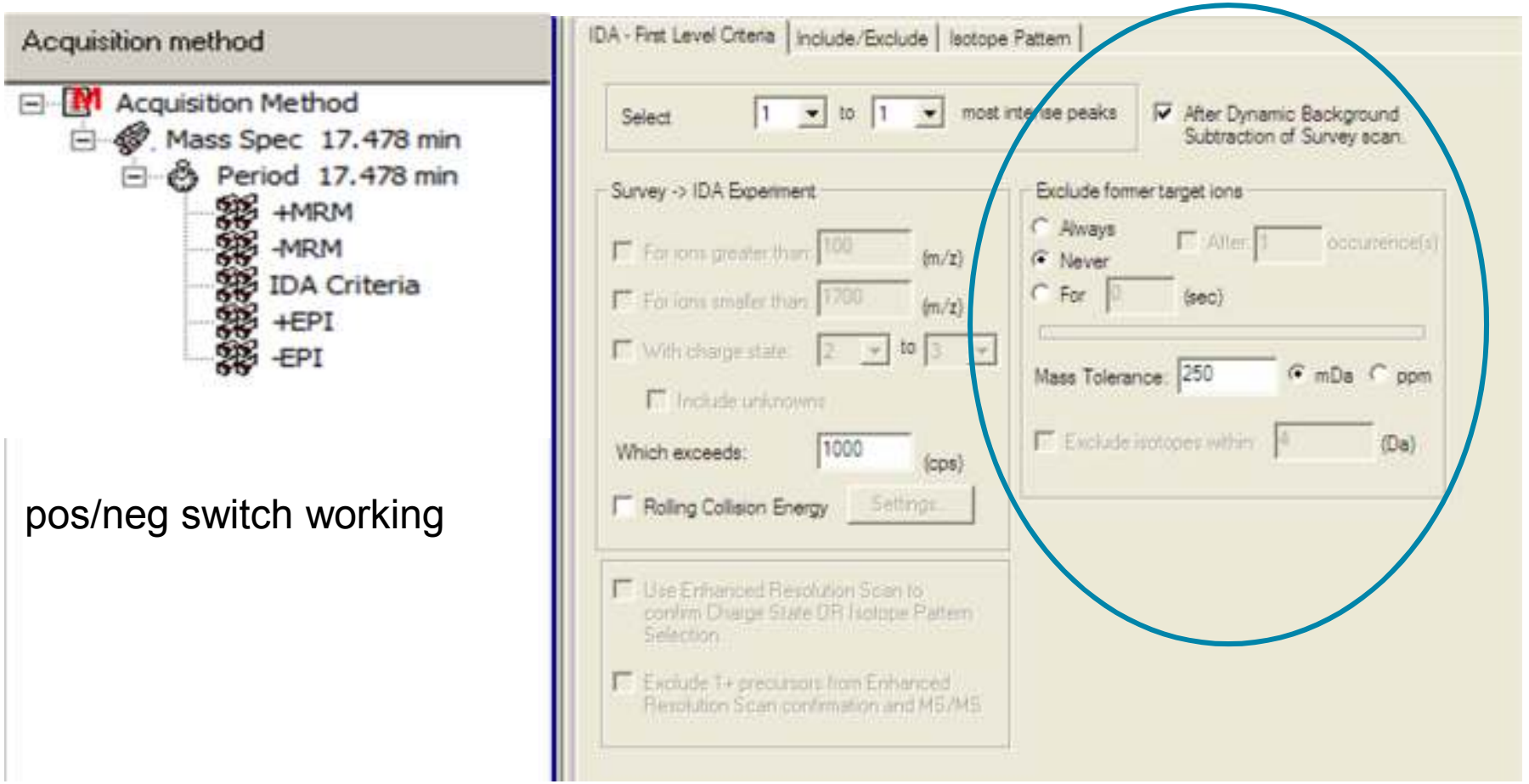


Dependant Scan:

Only performed when criteria is fulfilled

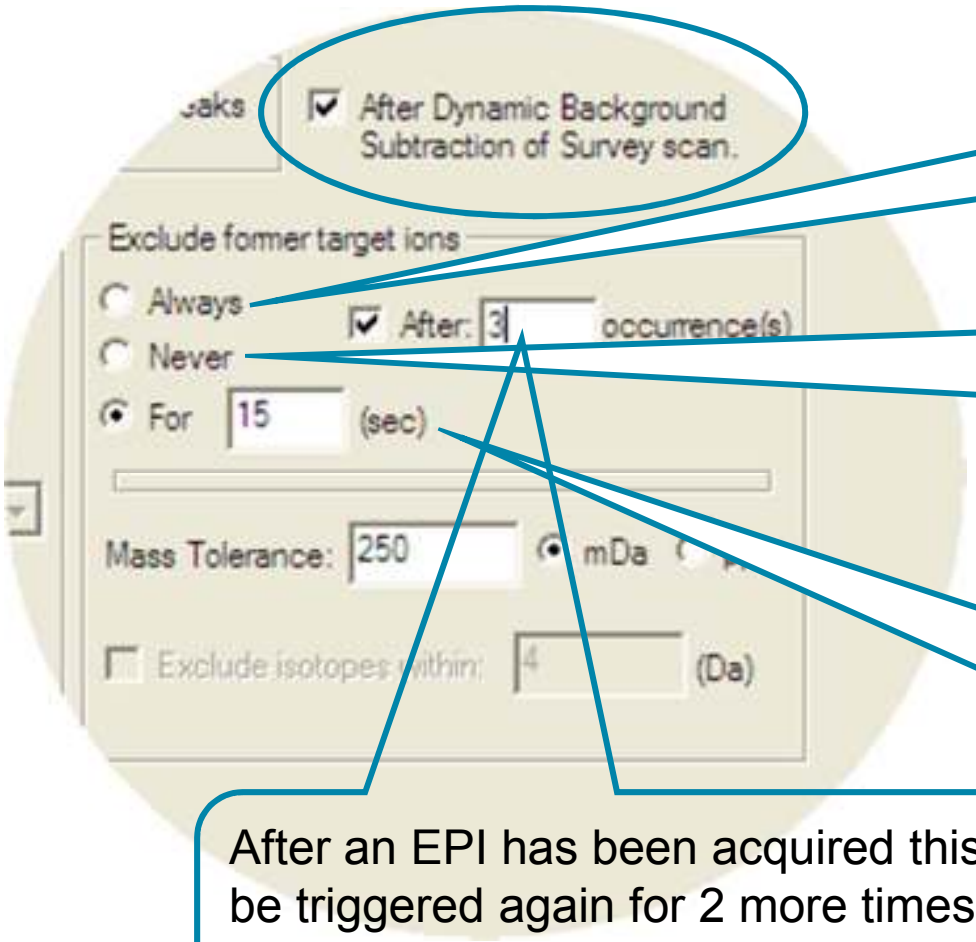
Return to survey scan

What does this part of IDA mean?



pos/neg switch working

Dynamic Exclusion



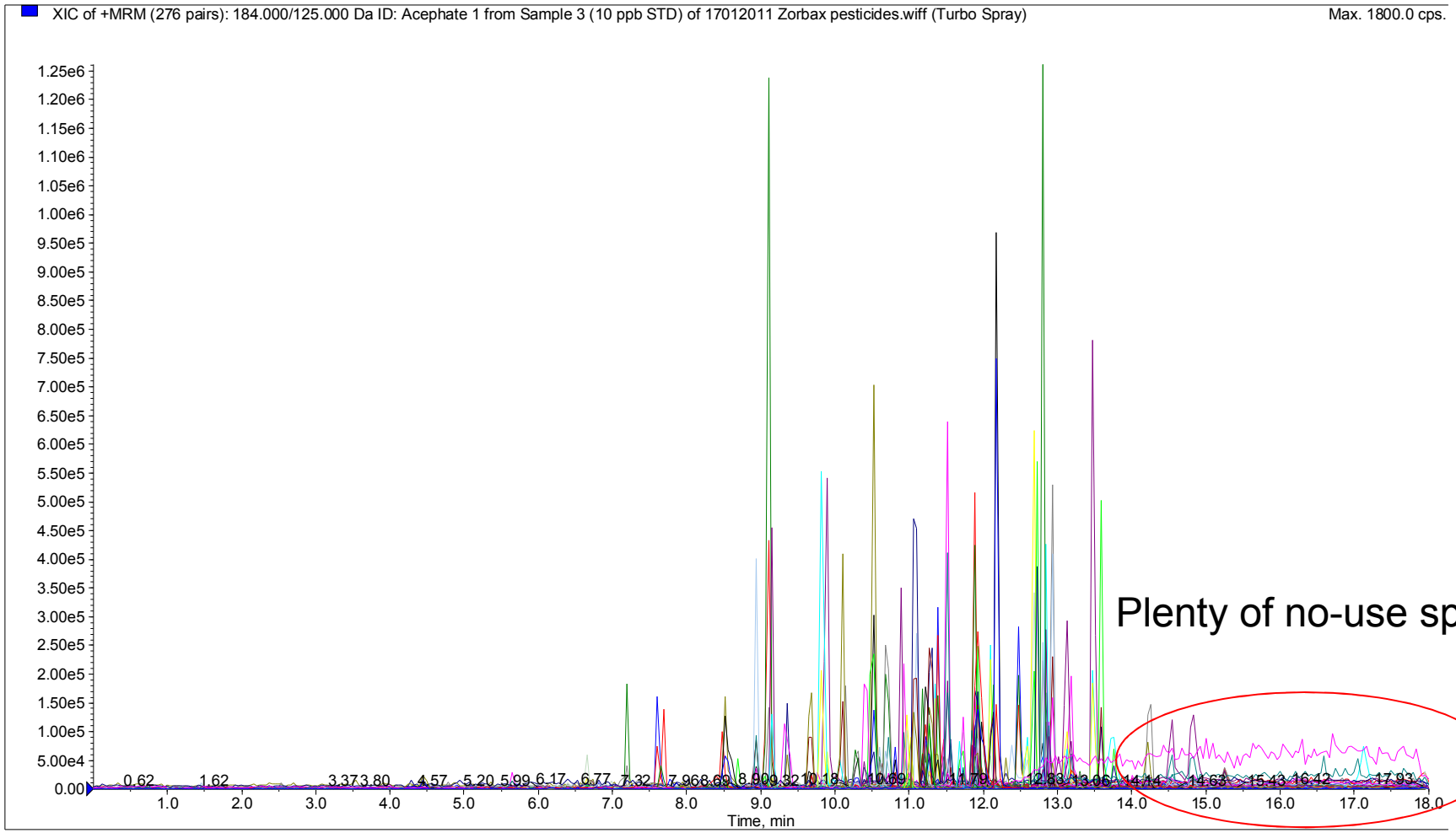
After an EPI has been acquired this mass will not be triggered again for the entire run

After an EPI has been acquired this mass will be triggered again as long as it is the most abundant.

After an EPI has been acquired this mass will not be triggered again for the following 15s, then it can be triggered again.

After an EPI has been acquired this mass can be triggered again for 2 more times if it is the most abundant ion; then it is excluded for the following 15s; then it can be triggered again.

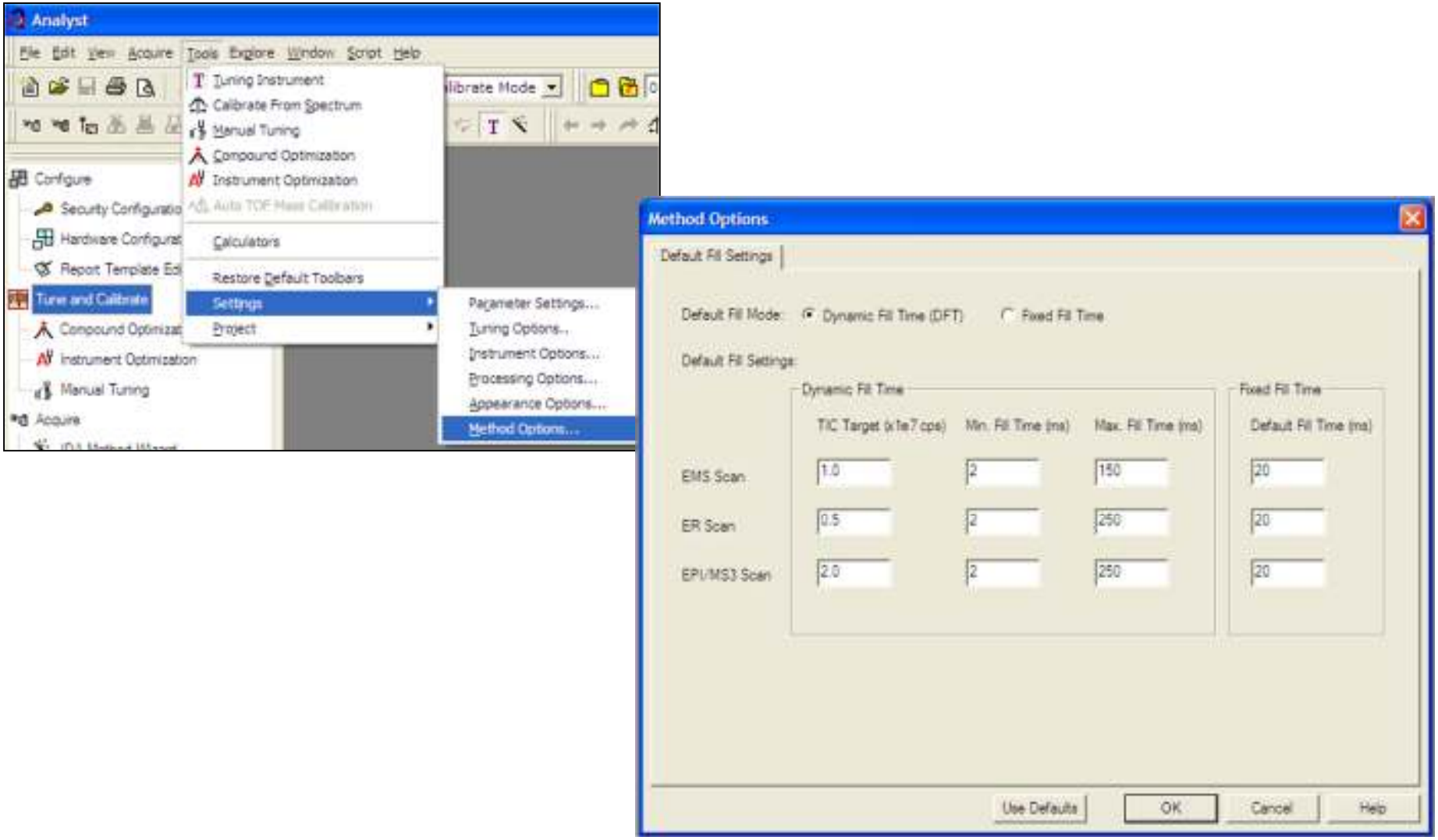
Dynamic background substract (DBS)



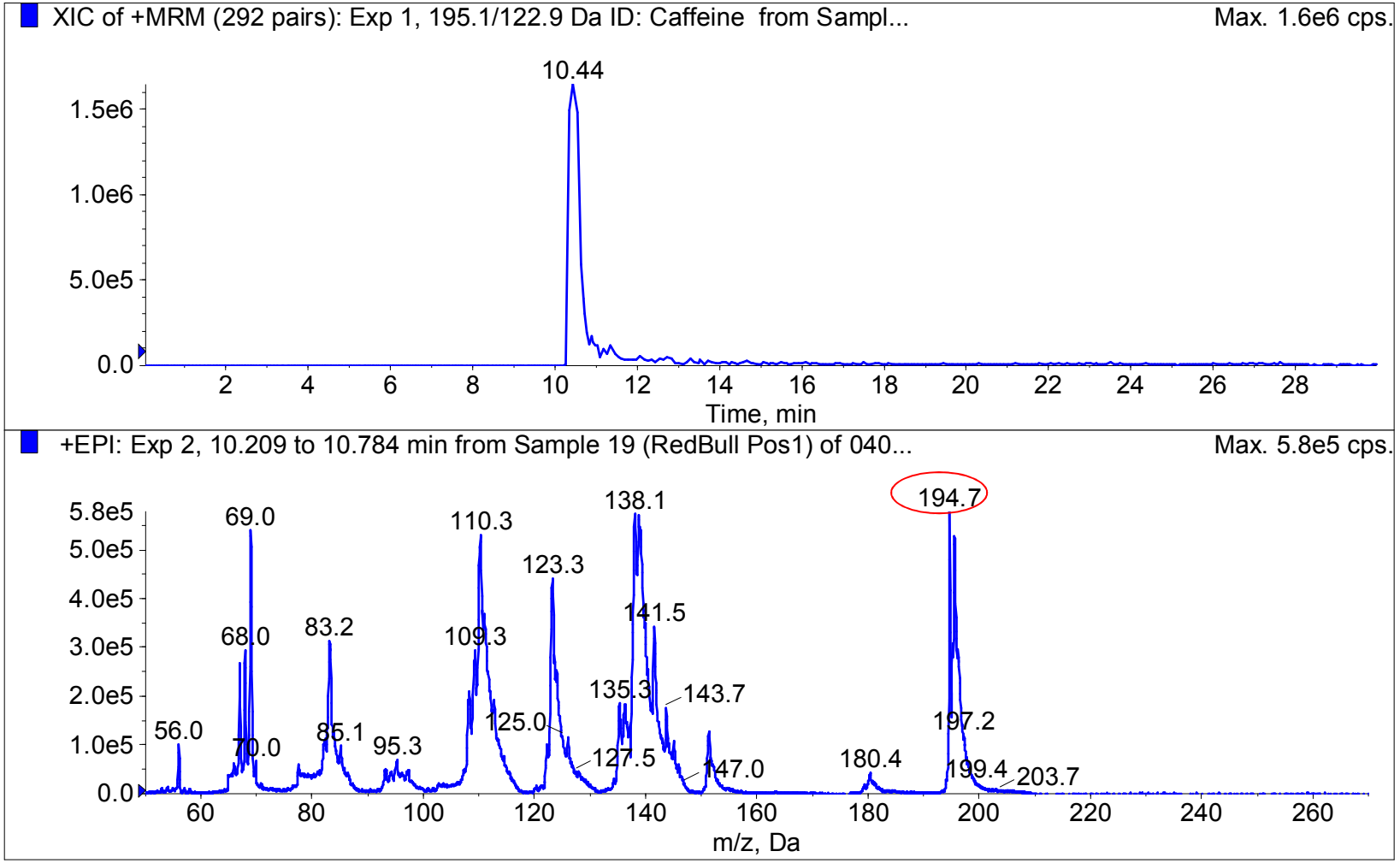
(Dynamic) fill time – (D)FT

- Controls the number of ions entering the trap
- Avoids space charge effects
  - (ions influencing each other due to lack of space)
- Space charge effects can cause:
  - Disturbed peak shape (often „fronting“, broadening)
  - (Partial) mass shift
- Min. Fill time:
  - QTRAP® 3200/4000: 1 ms
  - QTRAP® 5500/6500+: 50 µs
- *Tip: Use the DFT Tracker when running experiments with „Dynamic Fill Time“ to find out what values are used for certain scan situations. Use this values as start point for determining suitable „Fixed Fill Times“ if desired*
- *Tip: Particular on an QTRAP® 3200 „Fixed Fill Time“ & Q0 Trapping increases sensitivity*

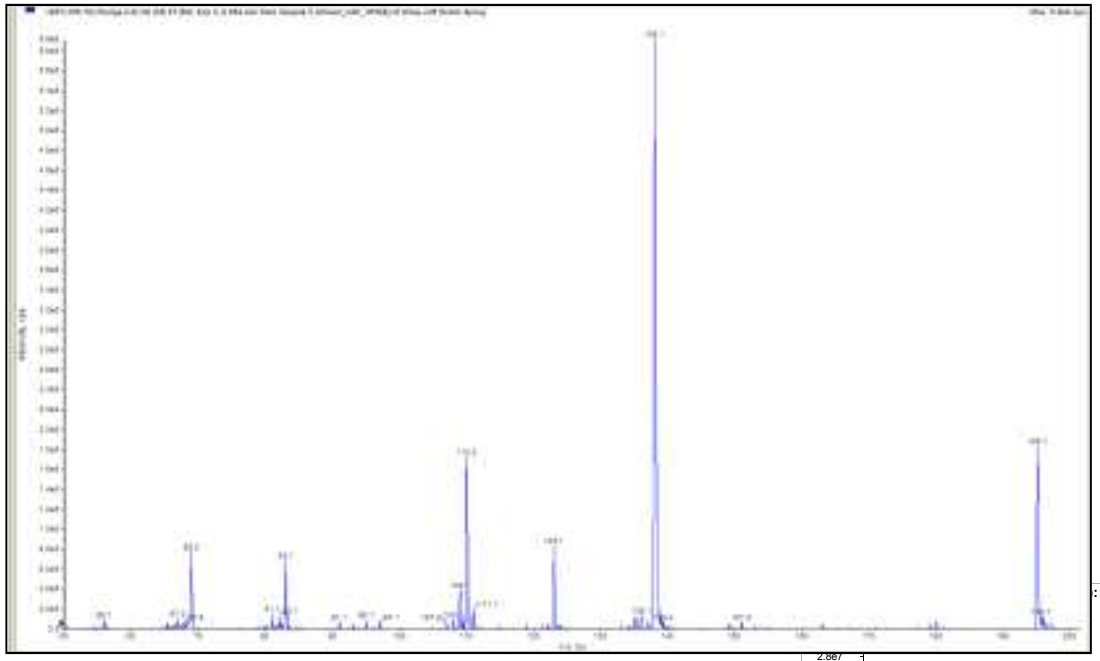
(Dynamic) fill time – (D)FT



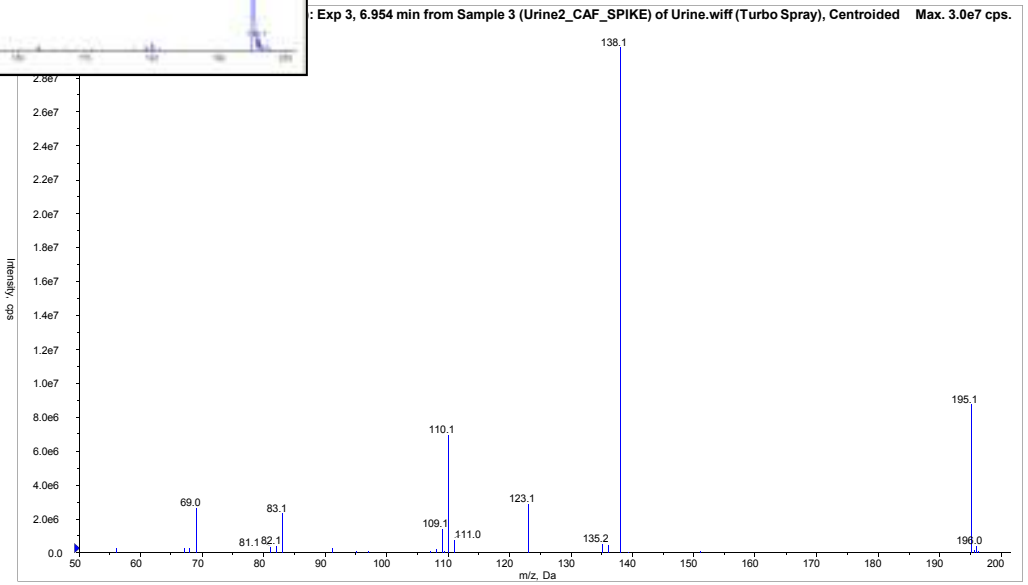
and here is my first nice aquired spectrum…or?



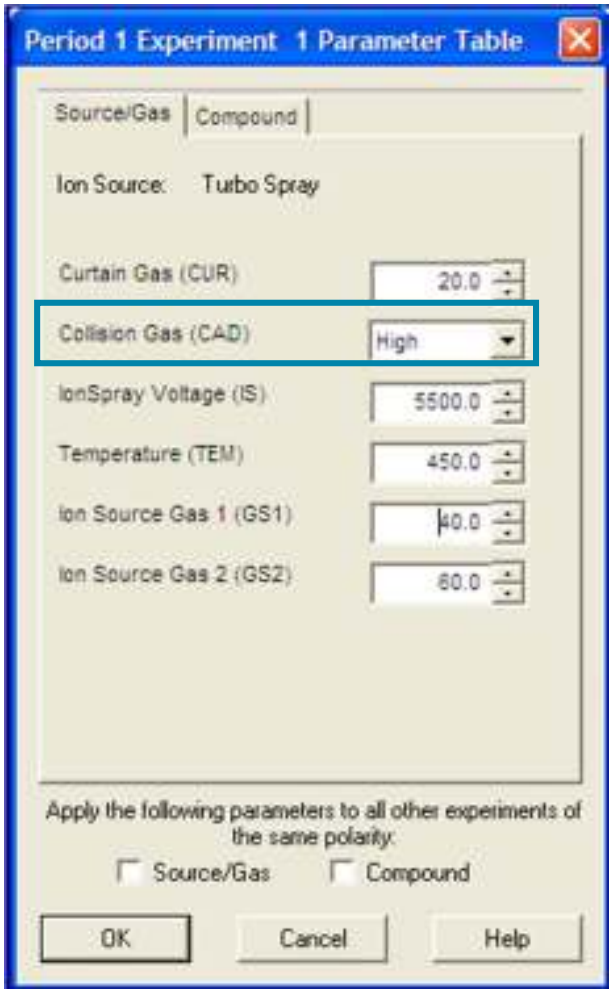
(Dynamic) fill time – (D)FT – as it should be



centroid

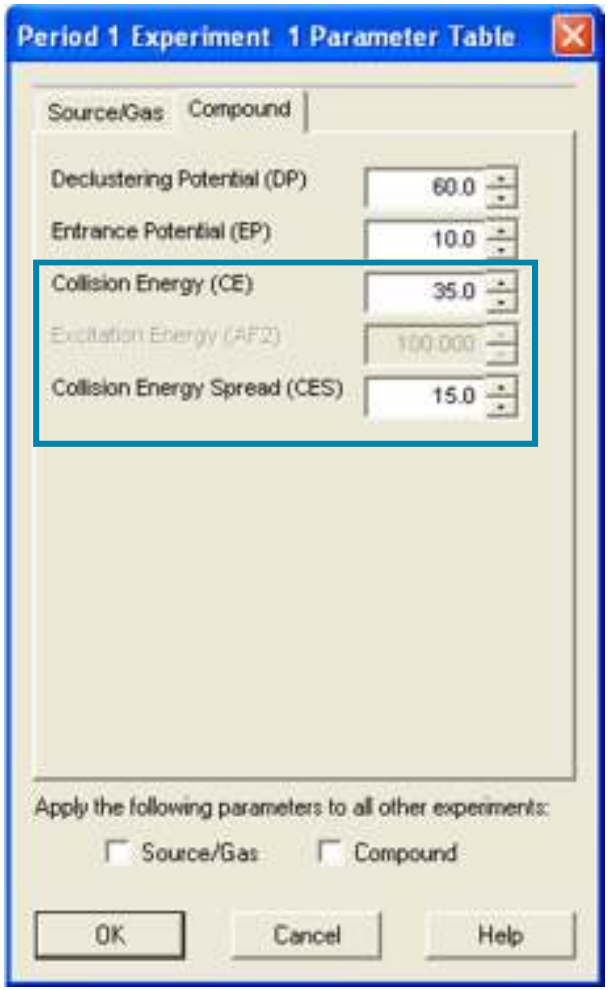


CAD and CE in Enhanced Product Ion Scan (EPI)



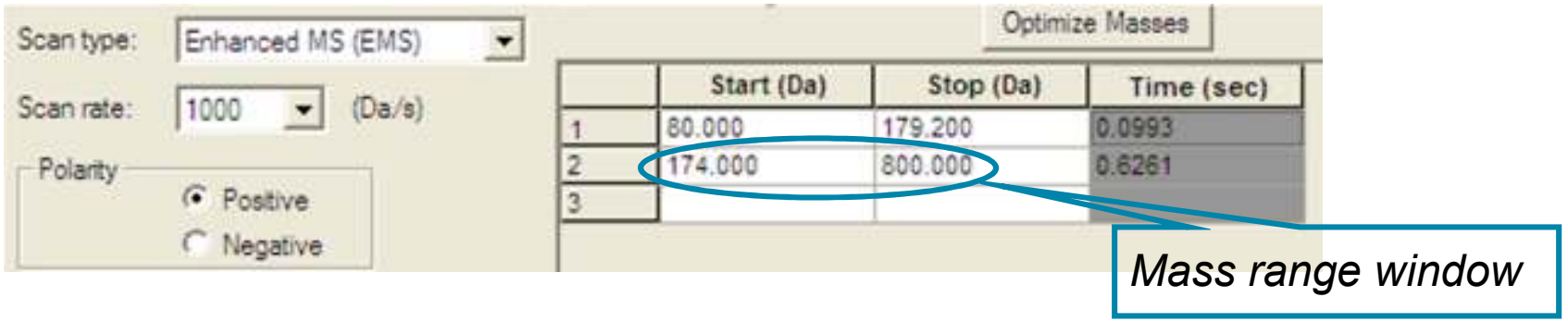
vacuum/pressure

should be in the range
3.8 to 4.3e^{-5} Torr



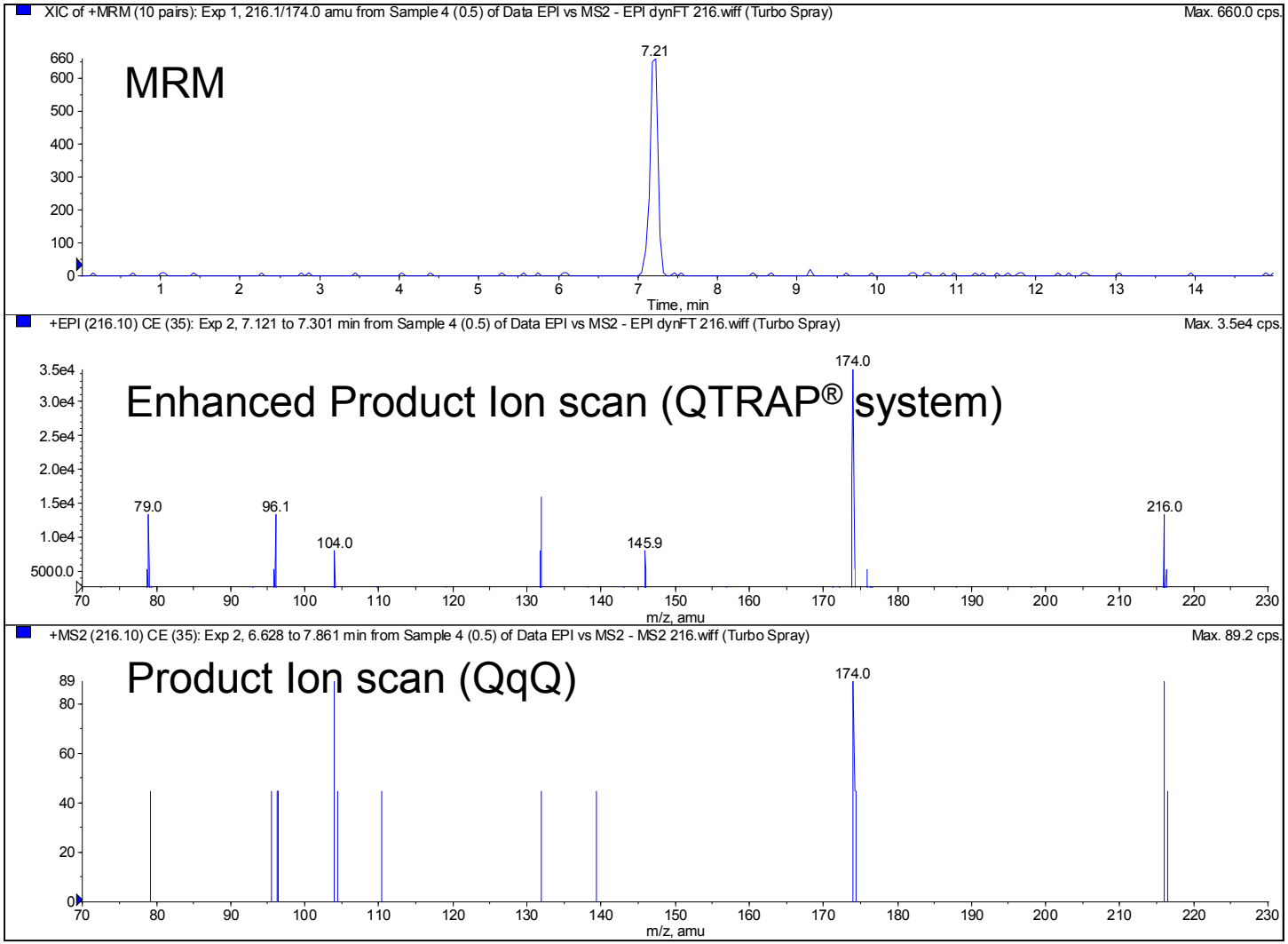
What is the advantage of a LIT over a QqQ

- Speed and sensitivity for all experiments is higher
  - Q1/Q3 vs. EMS and MS^2 vs. EPI
- LIT collects all Ions in a „mass window“ simultanously
  - Having a fill time of 50ms means that ALL ions are collected for 50 ms
  - This compares to a dwell time of 50 ms for EACH ion in QqQ
  - QqQ would need 313.000 msec for this experiment



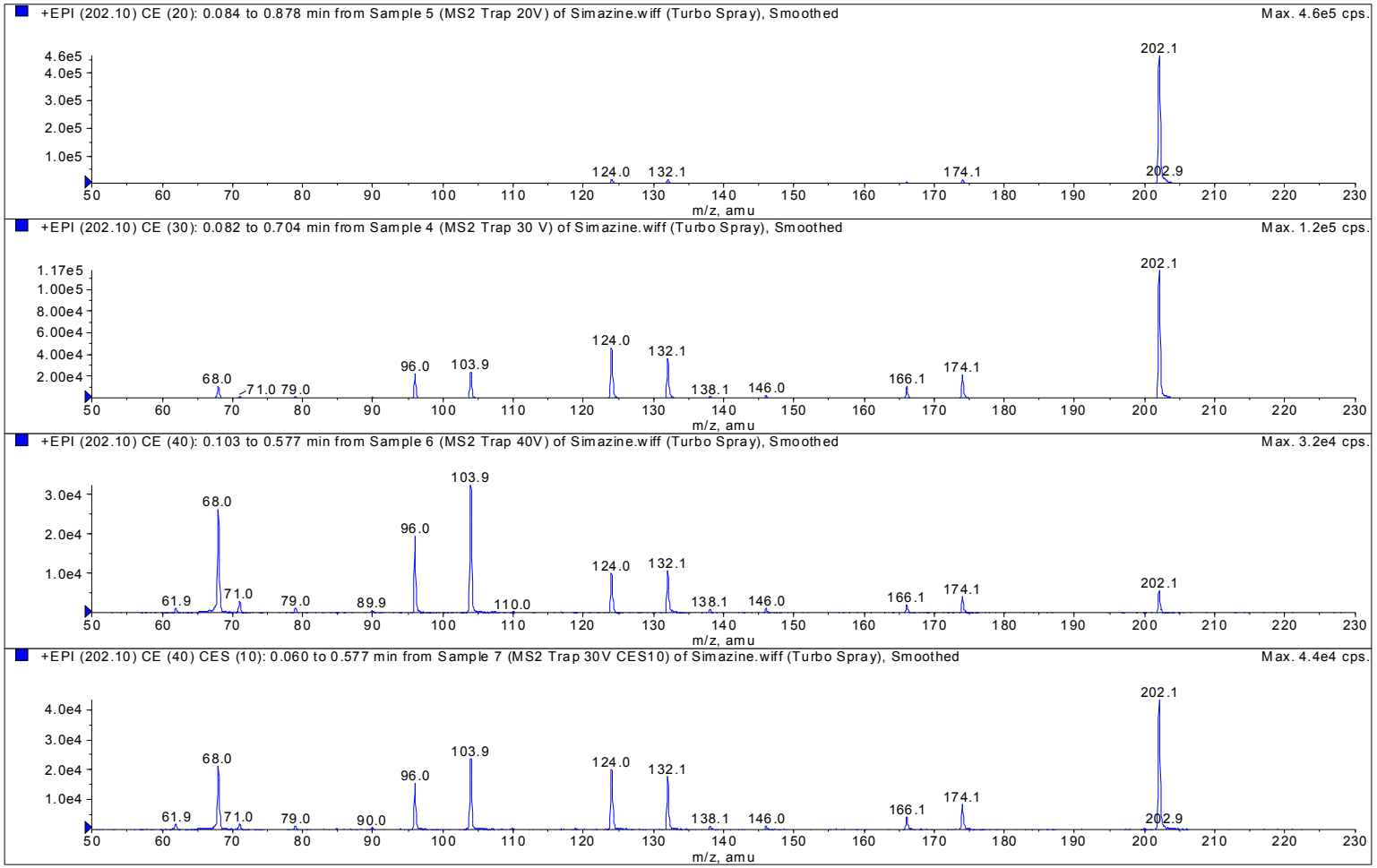
  - EPI experiment up to 20.000 da/sec and in addition change CE while LIT is trapping the ions => CES

Confirmation by QTRAP® or QqQ (0.5 ng/mL Atrazine)



EPI Simazine at 35 eV with CES of +/- 15 eV

Simultaneous trapping of fragment ions coming from 3 different CE (20, 35 & 50 eV)



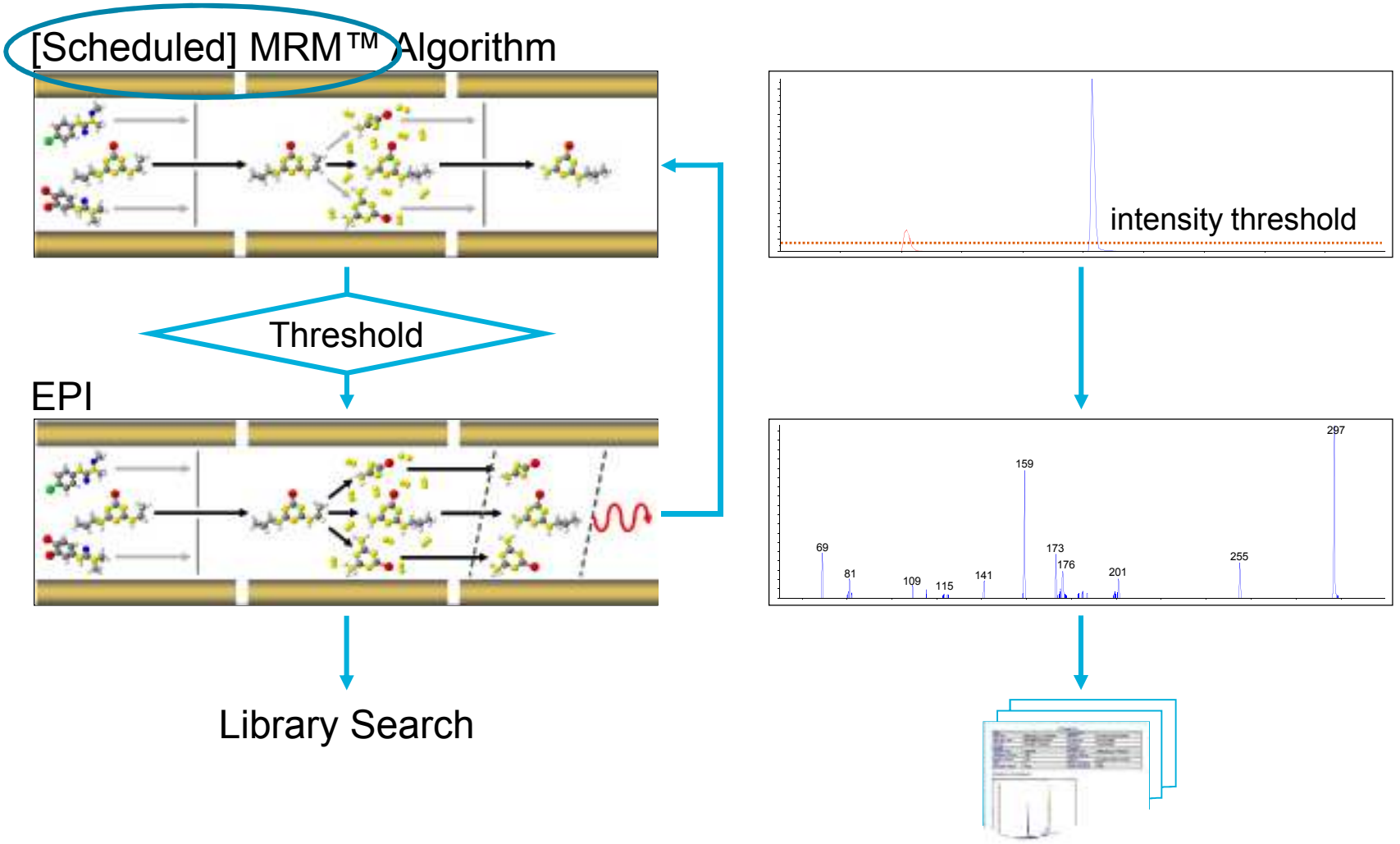
20eV

35eV

50eV

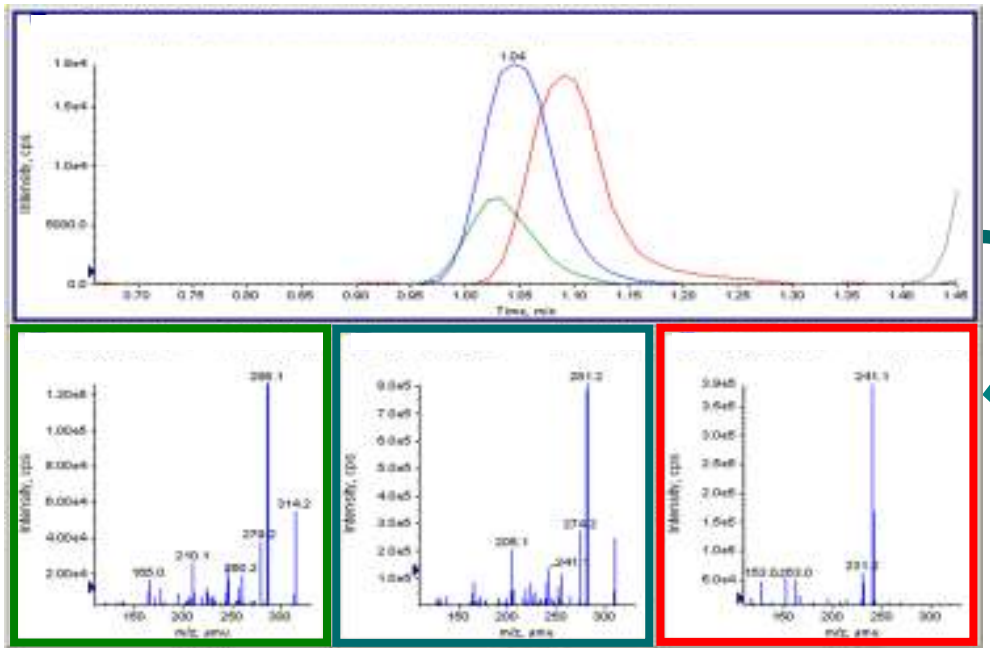
35eV with CES 15eV

Fast Screening with Quantitation for various applications



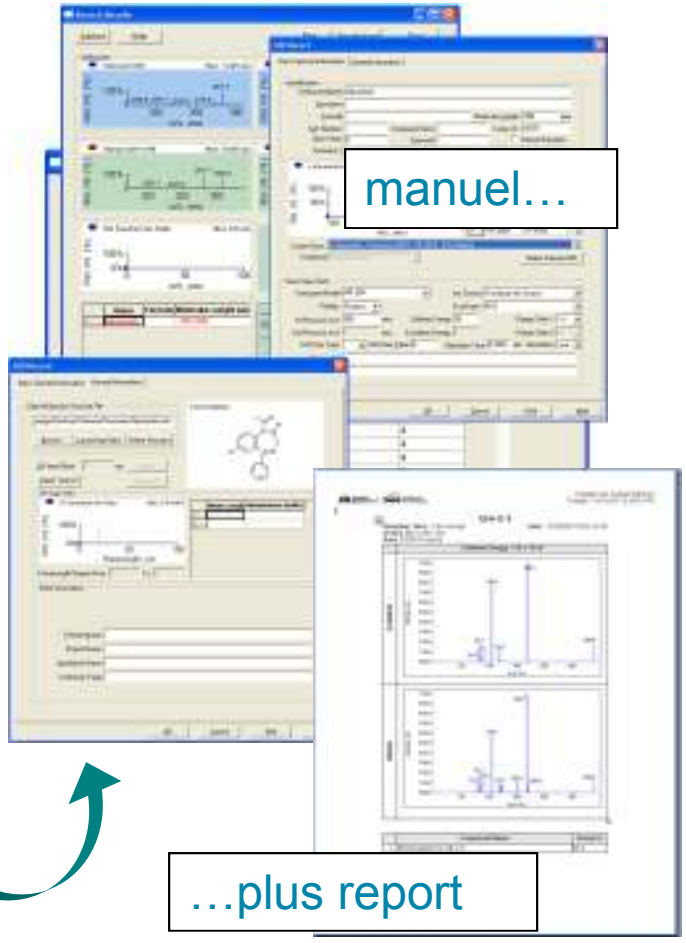
Screening and Confirmation:

MRM Survey (quantitation)



Collection of MS/MS
(full scan confirmation)

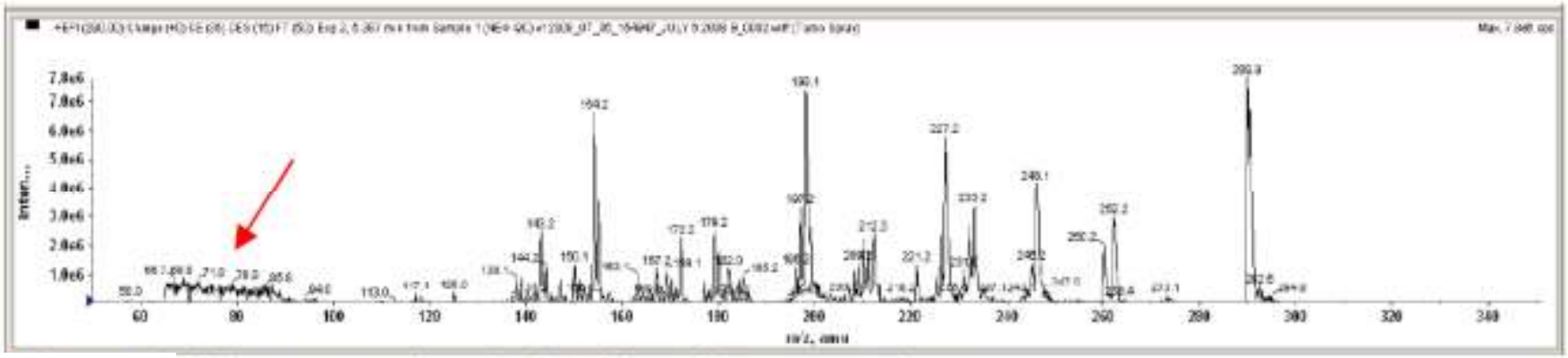
Library Search Results…



manuel…

…plus report

Some more examples for troubleshooting [1]



Noise appearing in the 65 to 90 amu region

What can be done to solve it?

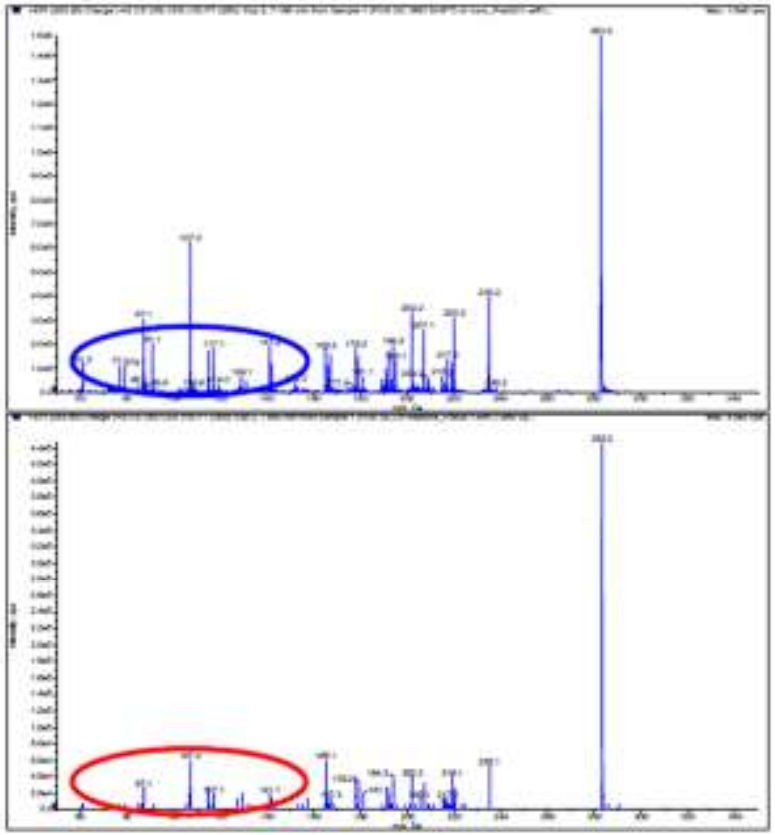
Some more examples for troubleshooting [1]

Cause: EXB is too high so ions are held in the trap too long causing ghost peaks or noise to appear in the low end (50 – 80 amu) of the spectrum.

Corrective Action: Instrument should be re-tuned in LIT scan mode (appropriate polarity) to reduce noise in the low mass region while still meeting specifications at all masses. Follow the steps below.

1. Infuse instrument with appropriate TRAP tuning solution (PPGs or ES- Tuning Solution depending on instrument) at 5 – 10 uL/min
2. In Tune Method Editor Window, select:
    - ER scan type
    - Add mass at 59 Da if tuning 3200 QTRAP with PPGs —— or 60 Da if tuning 4000 QTRAP with Agilent solution – DP start and stop can be set between 100 – 130
    - Scan rate at 4000 Da/s (or appropriate scan rate).

Some more examples for troubleshooting [2]



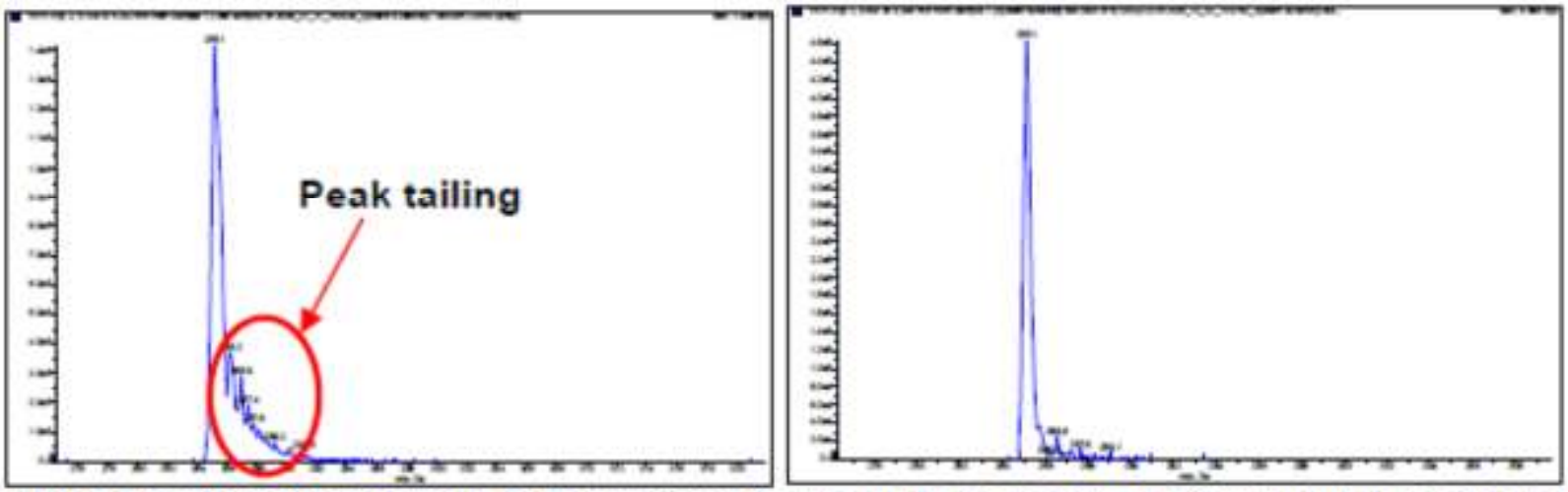
Using the same compound in both spectra, top panel shows expected peak detection at Da < 140 and bottom panel shows absence of expected peaks in the same low mass region.

Cause: EXB is too low so ions are not held in the trap long enough resulting in the absence of peaks in the low end (typically < 140 Da) of the spectrum.

Corrective Action: Instrument should be re-tuned in LIT scan mode (appropriate polarity) to increase peak detection in the low mass region while still meeting specifications at all masses. Follow the steps in the previous section (section 1) to adjust EXB intercept until peak detection is acceptable in the low mass region.

Some more examples for troubleshooting [3]

Cause: Incorrect AF3 value. Typically, an incorrect AF3 value will manifest itself as tailing on spectral peaks This is most common when AF3 is too low.

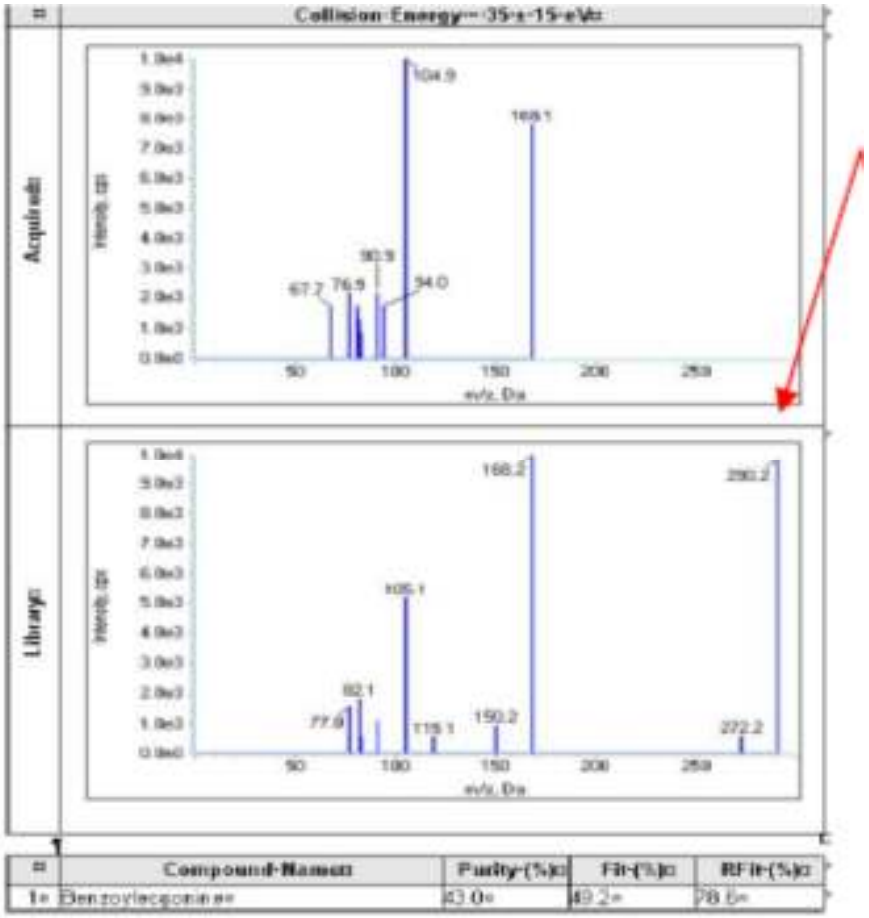


An EPI scan with Q1 resolution set to unit resolution. All fragment ions should be monoisotopic, and free from artifacts. Notice the abundant tailing on the m/z 285 ion in the left panel. By adjusting the AF3 value, this artifact was eliminated.

Corrective Action: Instrument should be re-tuned in LIT scan mode (appropriate polarity) to reduce artifacts. If the artifact cannot be reduced by normal tuning procedures, try tuning AF3 using an EPI scan where Q1 is at unit resolution and CE is set to 10. You will likely have to increase the fill time to achieve reasonable peak statistics for judging appropriate AF3 values. See Figure 9 below for example of well tuned AF3.

Some more examples for troubleshooting [4]

Library search filters out relevant ions. If the ion trap is too sensitive such that peak widths of tuning compounds are below 0.4 at full width half height, then the library search algorithm sees the peaks as noise and does not integrate them.



Some more examples for troubleshooting [4]

Cause: Peak widths too narrow on LIT at 4000 Da/s scan rate.

Correction Action: Instrument should be re-tuned in LIT ER scan mode (appropriate polarity) at 4000 Da/s to ensure that peaks in the tuning solution have a peak width at half height in the range of 0.4 and 0.7. It is recommended to increase EXB to achieve this, however keep in mind not to increase it too high which could lead to the problem in section 1.

Things to please keep in mind:

Section	Potential Issue	Corrective Action
1	Low Mass Noise	EXB too high, re-tune the instrument.
2	Reduced Low Mass Ion Intensity	EXB too low, re-tune the instrument.
3	Peak Tailing	Incorrect AF3, re-tune the instrument.
4	Library Search Filters Out Relevant Ions	Peak widths too narrow on LIT at 4000 Da/s scan rate, re-tune the instrument.

QTRAP® 4500/5500/6500+ systems with Analyst® advanced sMRM algorithm

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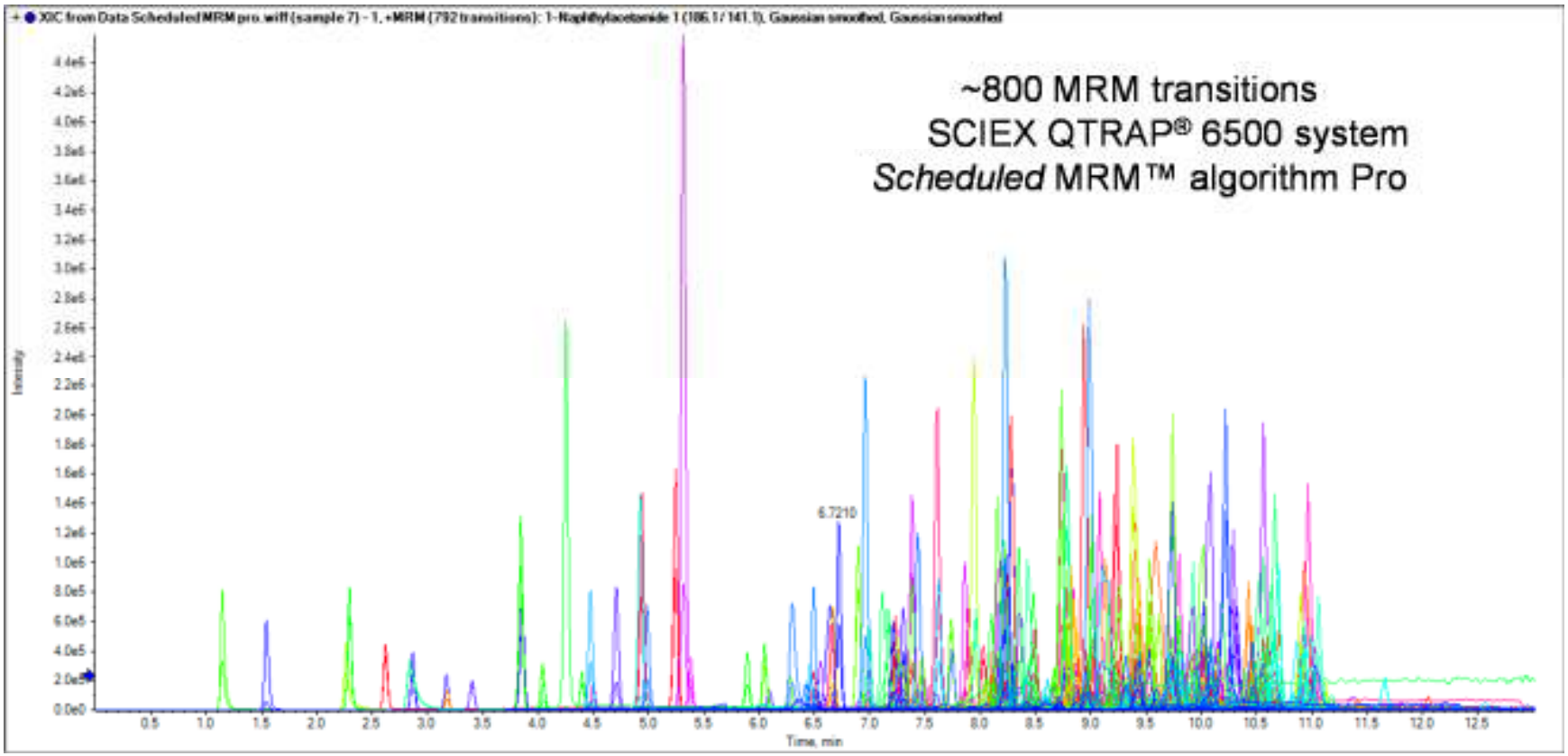
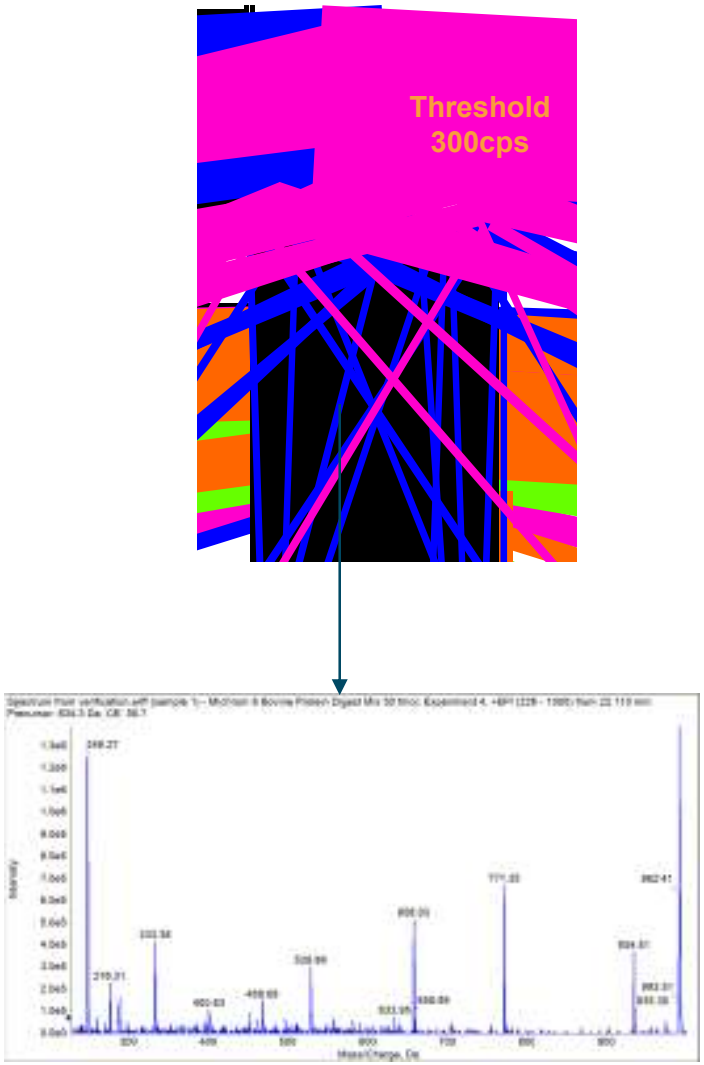
Sensitivity, Selectivity, and new Analyst 1.6.3 software

Quantitation and Identification of ~ 400 agro chemical

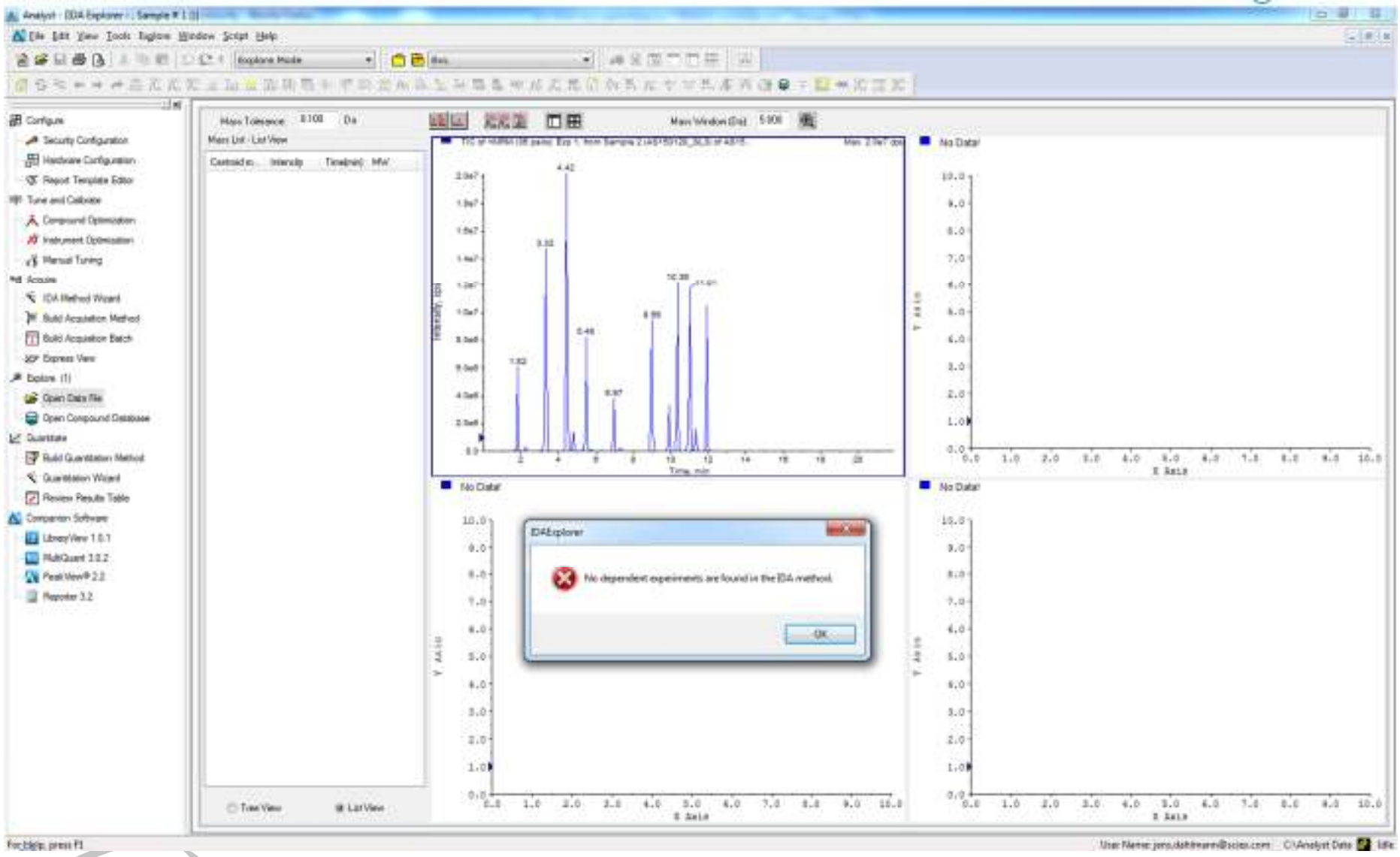


MRM-Triggered MRM + Group MRM-Triggered MS/MS

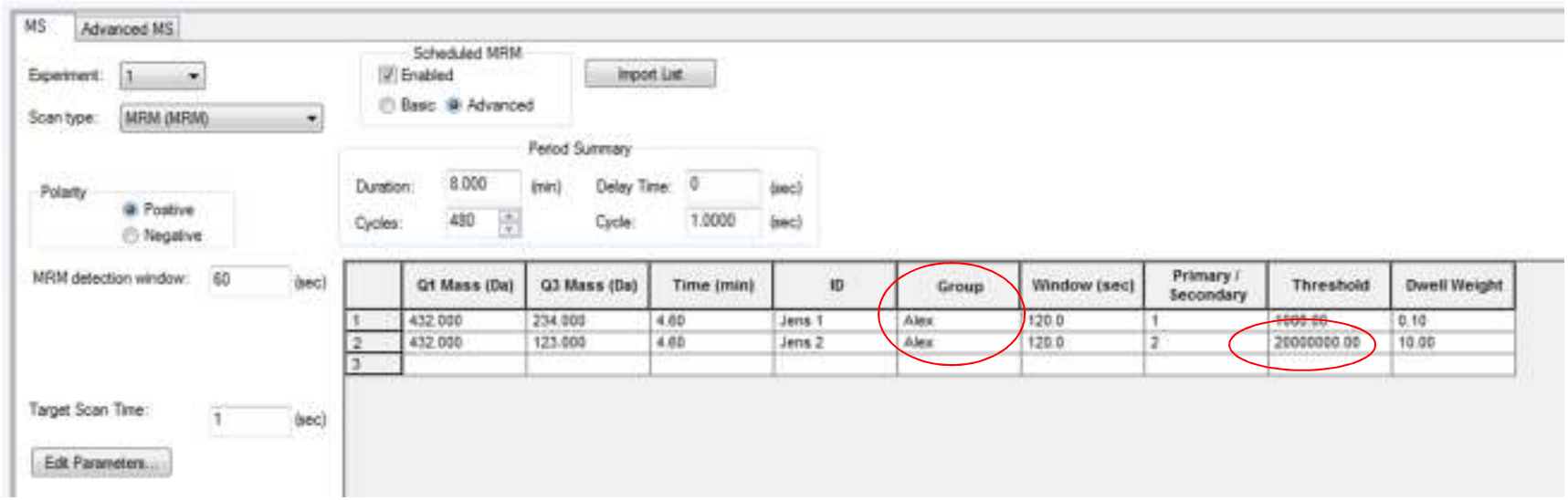
- MRM Triggered MRM can be combined with Group Triggered MS/MS
- Primary MRM are used to trigger secondary MRM
- Only when all primary and all secondary MRM are above the thresholds will IDA be triggered
- Thresholds can be set individually
  - Can be set to zero for weak transitions
  - Could be set based on known ion ratios



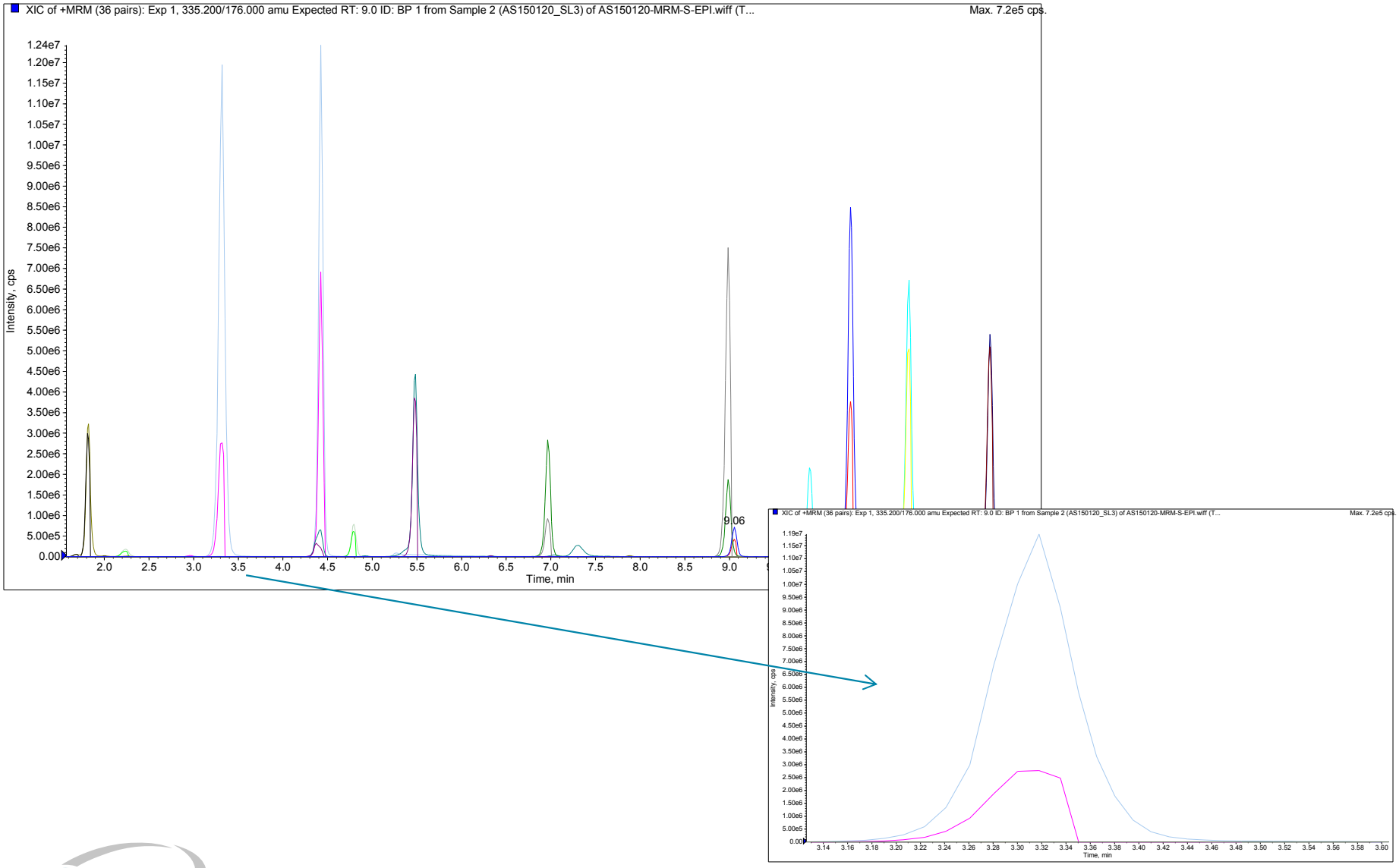
What is this….?



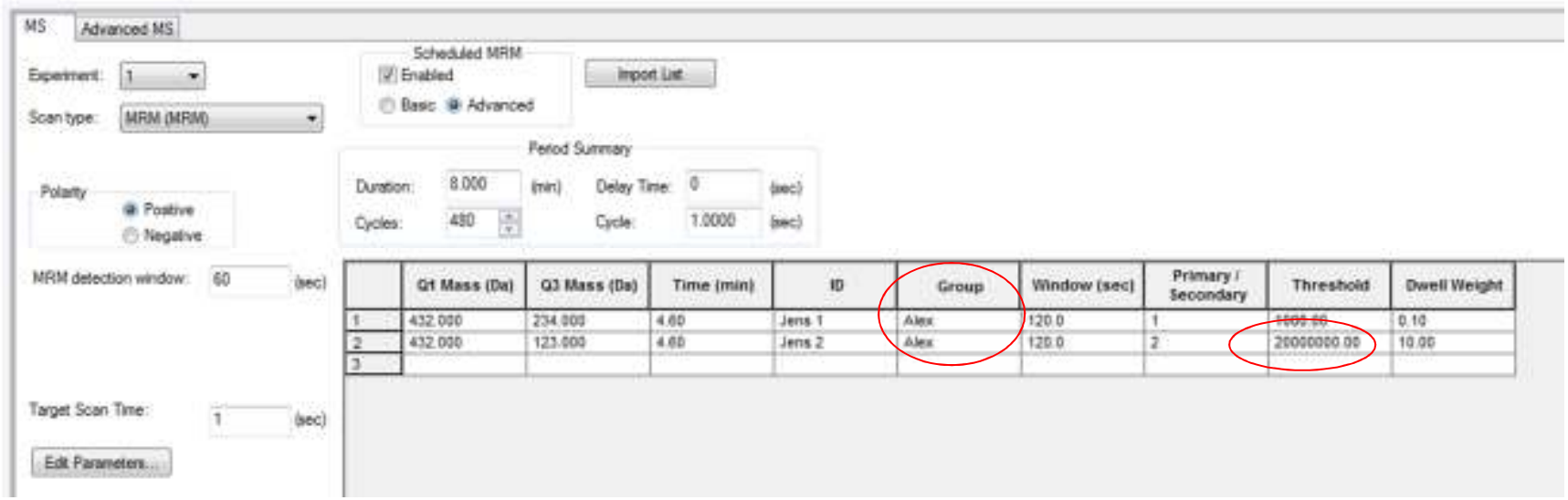
….that is why!



…ohh what happens with the 2. MRM?



Watch the settings in the method carefully

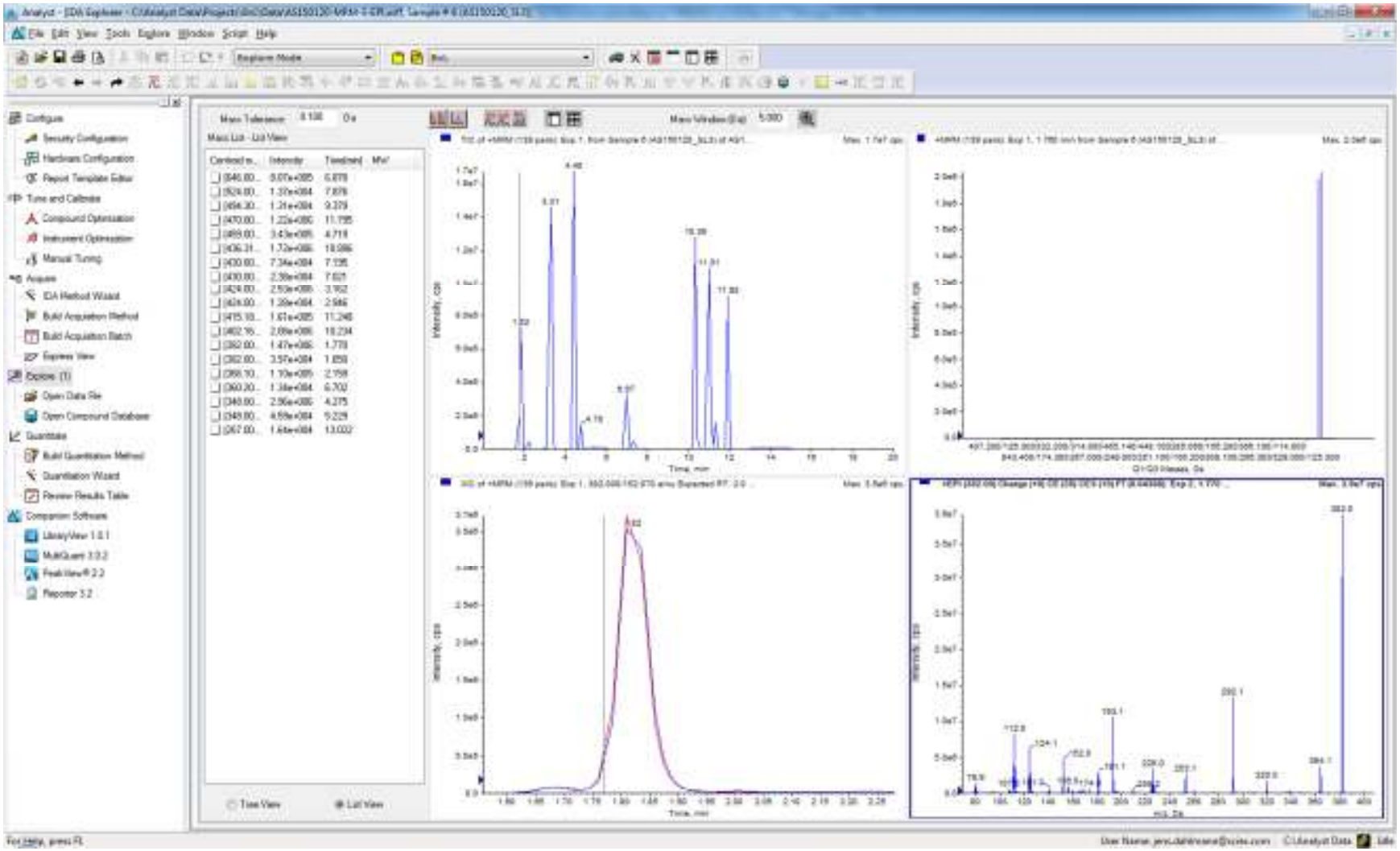


sMRM Advanced Settings to avoid issues

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	Group	Window (sec)	Primary / Secondary	Threshold	Dwell Weight
1	180.100	105.100	2.90	3,4-Methylenedioxyamphetamine			1		1.00
2	208.100	163.200	4.71	3,4-Methylenedioxyethylamphetam			1		1.00
3	194.100	163.100	4.44	3,4-Methylenedioxymethamphetam			1		1.00
4	194.100	105.100	4.44	3,4-Methylenedioxymethamphetam			1	100000000.00	1.00
5	328.200	165.200	0.00	6-O-Monoacetylmorphine 1			1		10.00
6	328.200	211.200	0.00	6-O-Monoacetylmorphine 2			1	100000000.00	10.00
7	136.100	91.000	4.11	Amphetamine 1			1		1.00
8	136.100	65.000	4.12	Amphetamine 2			1	100000000.00	1.00
9	290.100	168.200	4.56	Benzoylecgonine 1			1		1.00
10	290.100	105.100	4.56	Benzoylecgonine 2			1	100000000.00	1.00
11	468.300	396.300	4.52	Buprenorphine			1		1.00
12	304.200	182.200	5.77	Cocaine			1		1.00
13	300.200	165.200	4.57	Codeine 1			1		1.00
14	300.200	215.200	4.57	Codeine 2			1	100000000.00	1.00
15	177.100	80.000	3.48	Cotinine			1		1.00
16	302.200	199.200	4.57	Dihydrocodeine			1		1.00
17	200.100	182.200	0.80	Ecgoninemethylester			1		10.00
18	278.200	234.200	6.66	EDDP 1			1	10000.00	1.00
19	278.200	186.200	6.66	EDDP 2			1	100000000.00	1.00
20	166.100	115.100	3.78	Ephedrine			1		1.00

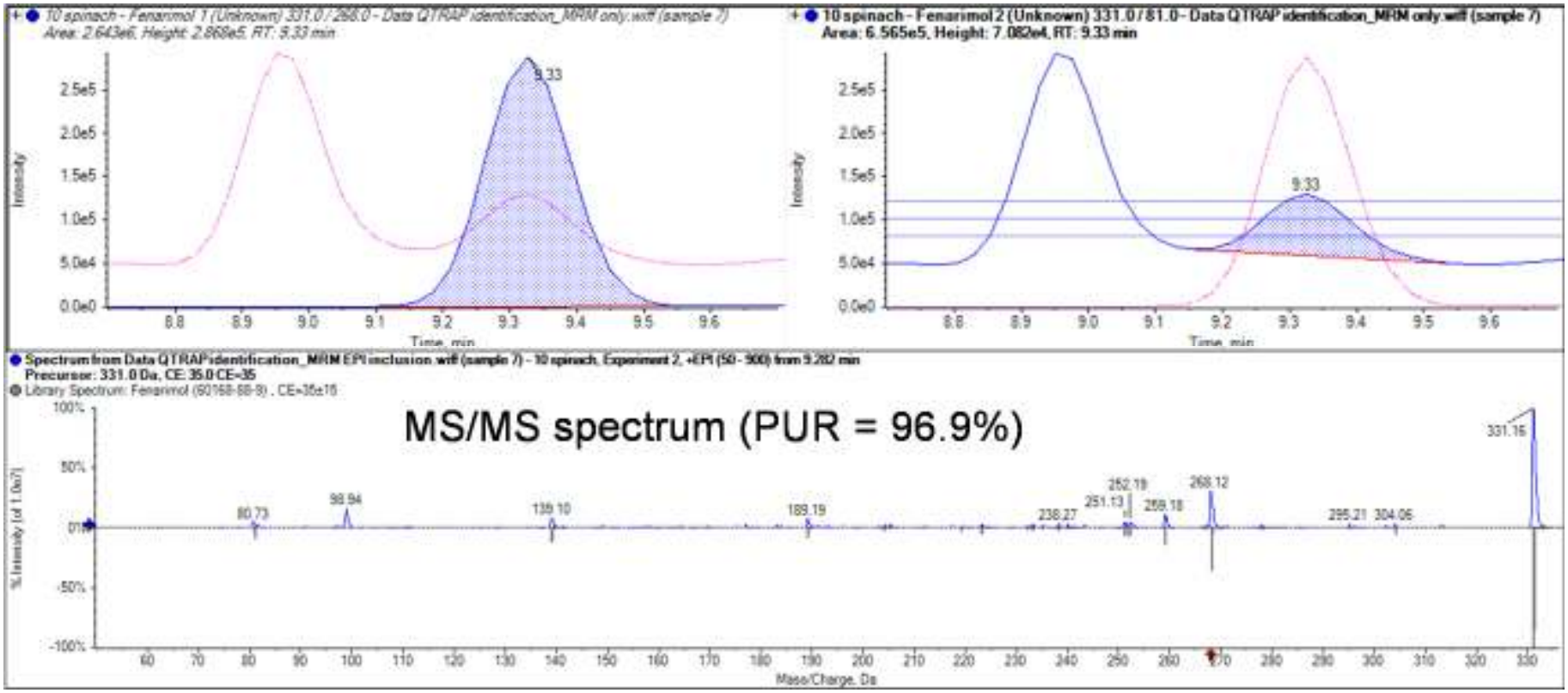
- Do not use groups in acquisition method or primary secondary to avoid what we saw
- Set a very high threshold for the second MRM of quantitation compounds so that we never get IDA EPI on the second MRM
- Threshold set for each ‘primary’ compound to get good MRM ratios and IDA EPI when we want it

Finally !



Questionable ID using only MRM Ratio, MS/MS Confirmation

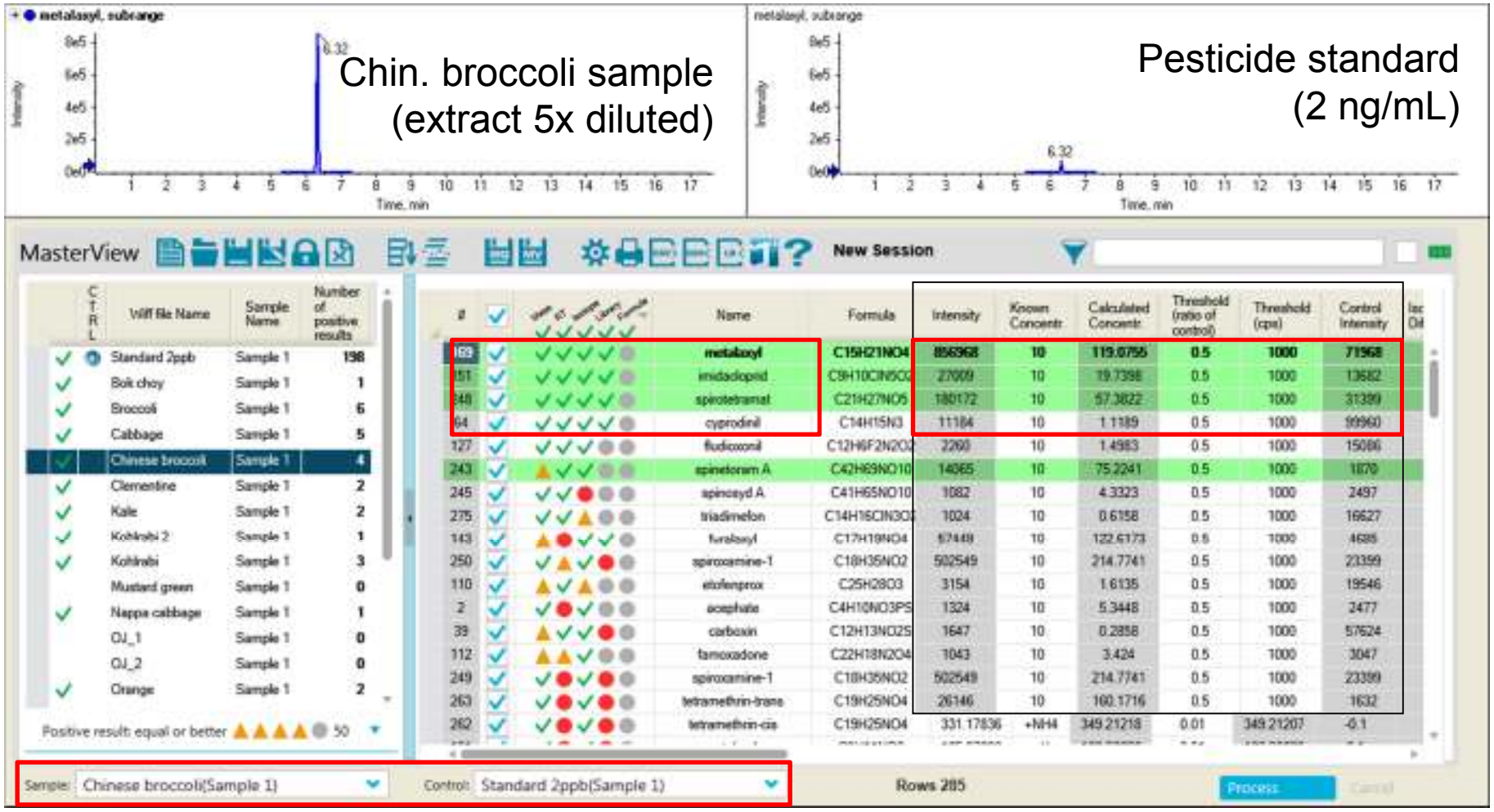
10 µg/kg Feniramol in Spinach (MRM ratio error = 32.0)



Processing in MultiQuant™ and MasterView™ Software

QTRAP® Targeted Screening using MasterView™

Confirmation and Quantitative Comparison



Identification of residues above maximum residue limit (MRL) in multiple samples

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Thank you for Your Attention!

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