



Answers for Science.
Knowledge for Life.™

The background of the slide features a large, stylized silhouette of a human head in profile, facing left. The head is filled with a complex, colorful pattern of molecular structures, including various atoms and bonds, rendered in shades of blue, green, and red. The background also includes horizontal bands of light blue and green, with faint, glowing lines and dots that suggest a digital or scientific environment. A circular callout box is positioned in the upper left quadrant of the head silhouette.

It's Time to
See the Future
Differently

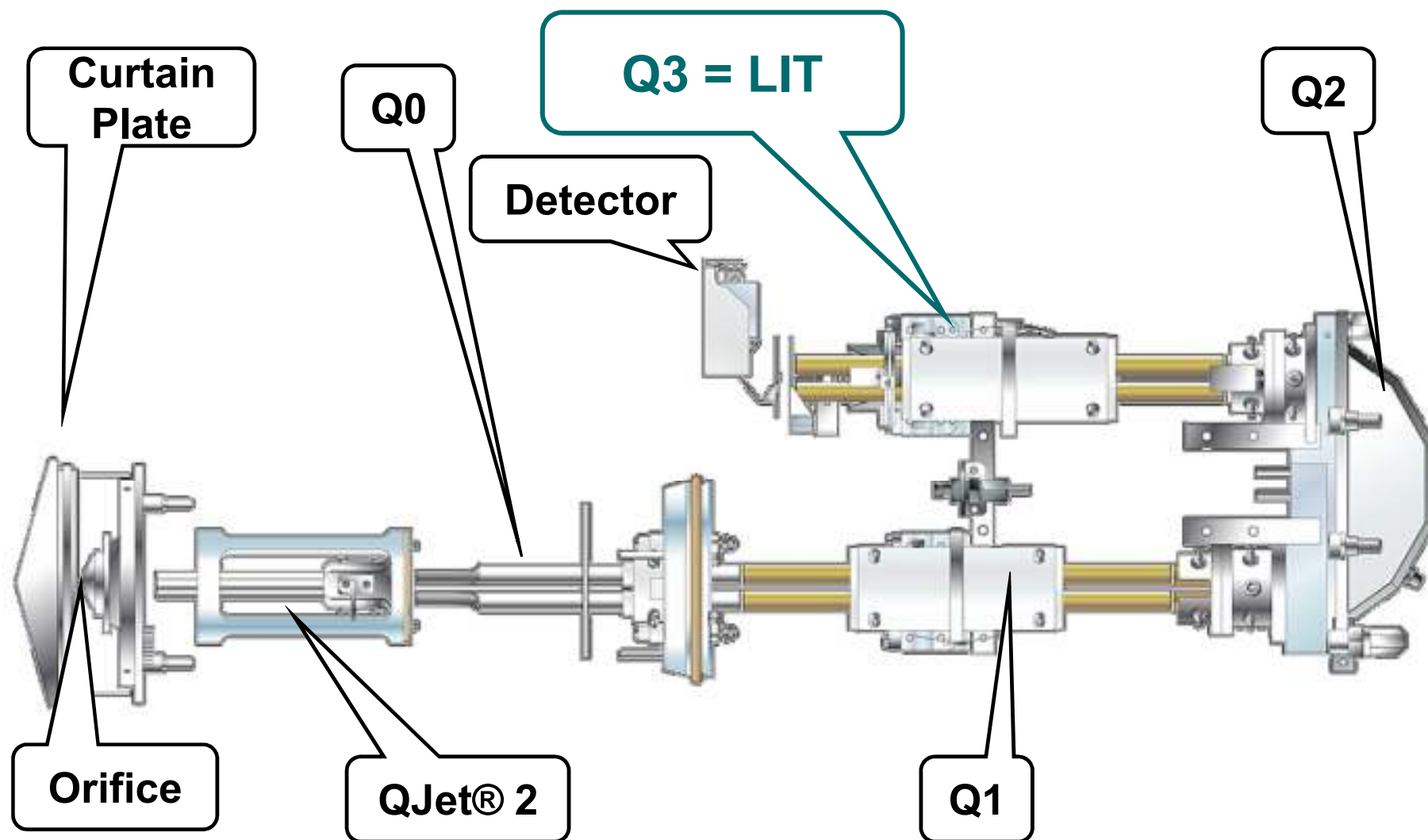
QTRAP® Functionalities

Hardware and Software Tipps und Tricks,

Alexandre Paccou

- 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

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



Principle of a Linear Ion Trap



An ion trap is working sequentially (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)

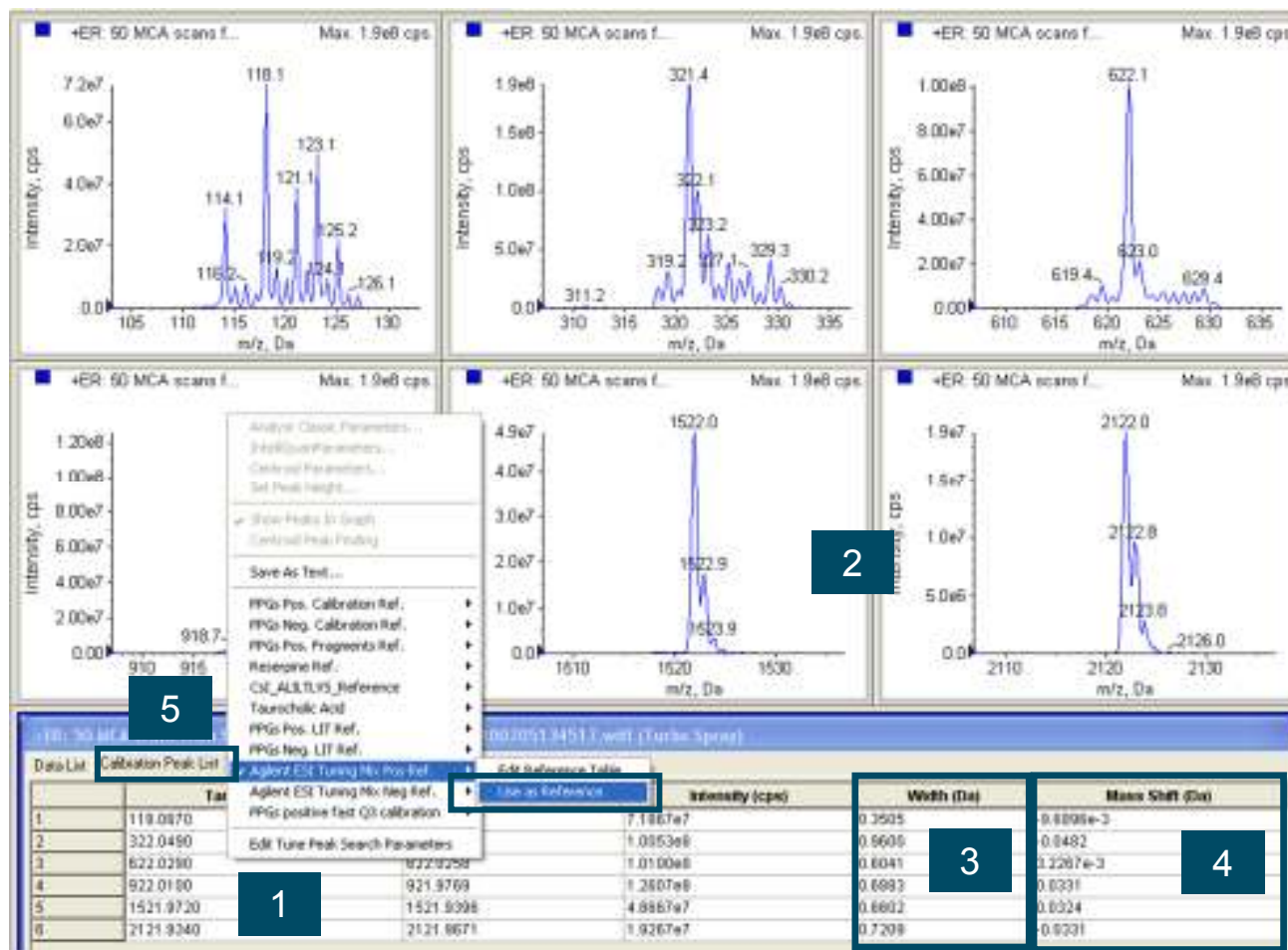
– 1.5. keep the ions happy

– 2. Scanning

- New ions can not enter the trap due to high potential applied at IQ3
- Ramp of auxiliary frequency (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+

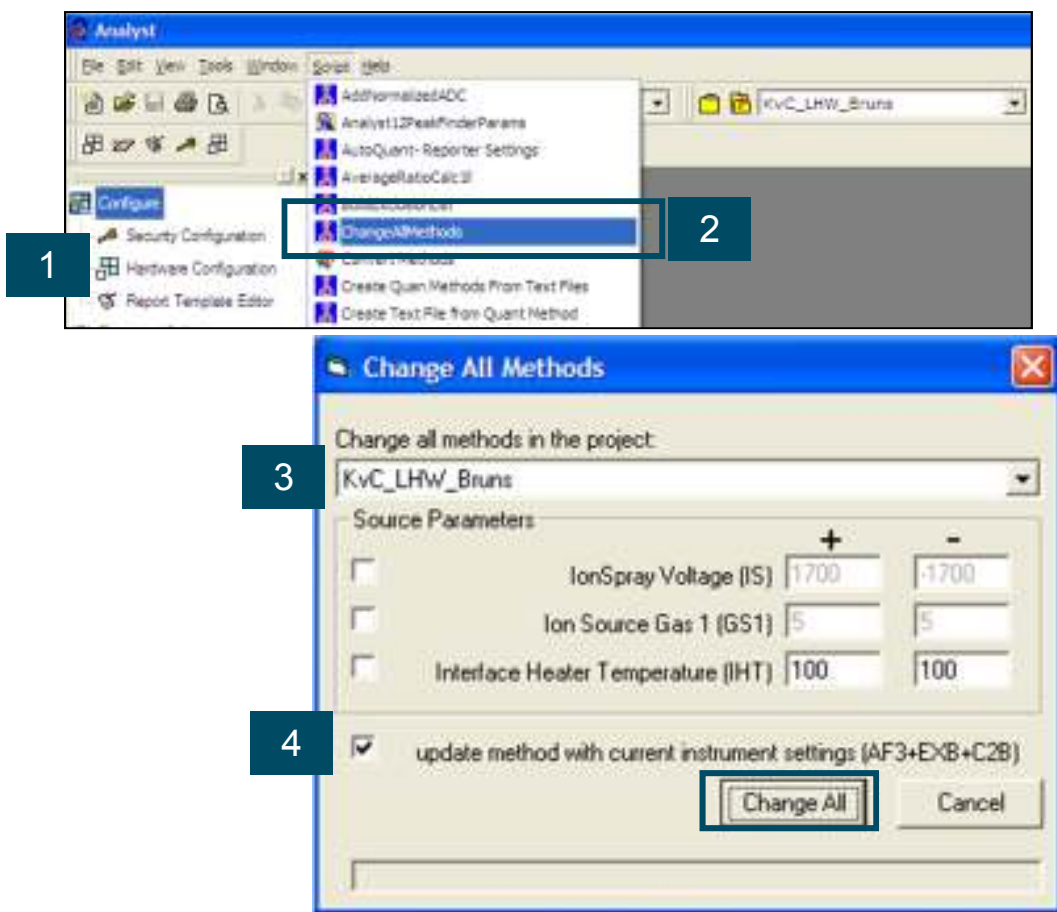
LIT Calibration – please check from time to time



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=>

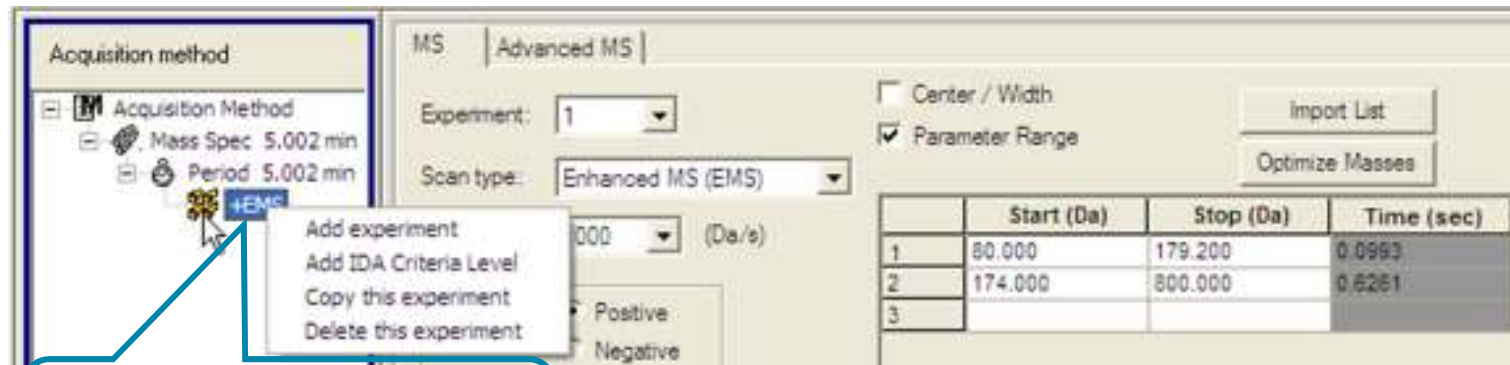
=>Tools/settings /appearance options/Tab : miscellaneous/select «show mass calibration peak list »

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



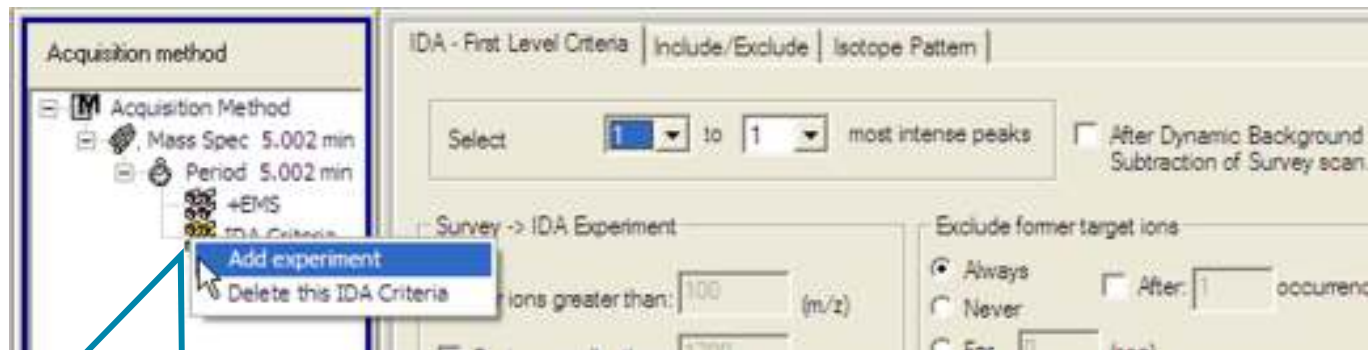
Manual Setup is easy – use the right mouse button

• 1:



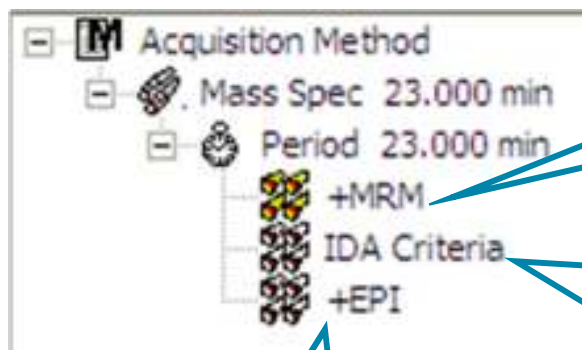
„Click“ right mouse button
Add IDA Criteria Level

• 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

IDA - First Level Criteria | Include/Exclude | Isotope Pattern

1. Which exceeds: 1000 (cps)

2. Select: 1 to 1 most intense peaks

3. Exclude former target ions

☐ For ions greater than 100 (m/z)

☐ For ions smaller than 1700 (m/z)

☐ With charge state: 1 to 1

☐ Include unknowns

☒ After Dynamic Background Subtraction of Survey scan.

Survey -> IDA Experiment

☐ Exclude former target ions

☐ Always ☐ Never ☐ For 0 (sec)

Mass Tolerance: 250 mDa

☐ Exclude isotopes within 0 (Da)

What does this part of IDA mean?

Acquisition method

- [-] Acquisition Method
 - [-] Mass Spec 17.478 min
 - [-] Period 17.478 min
 - +MRM
 - MRM
 - IDA Criteria
 - +EPI
 - EPI

IDA - First Level Criteria | Include/Exclude | Isotope Pattern

Select to most intense peaks ☒ After Dynamic Background Subtraction of Survey scan.

Survey -> IDA Experiment

- ☐ For ions greater than: (m/z)
- ☐ For ions smaller than: (m/z)
- ☐ With charge state: to
- ☐ Include unknowns
- Which exceeds: (cps)
- ☐ Rolling Collision Energy

Exclude former target ions

- ☐ Always
- ☒ Never
- ☐ For (sec)
- ☐ After occurrence(s)

Mass Tolerance: ☒ mDa ☐ ppm

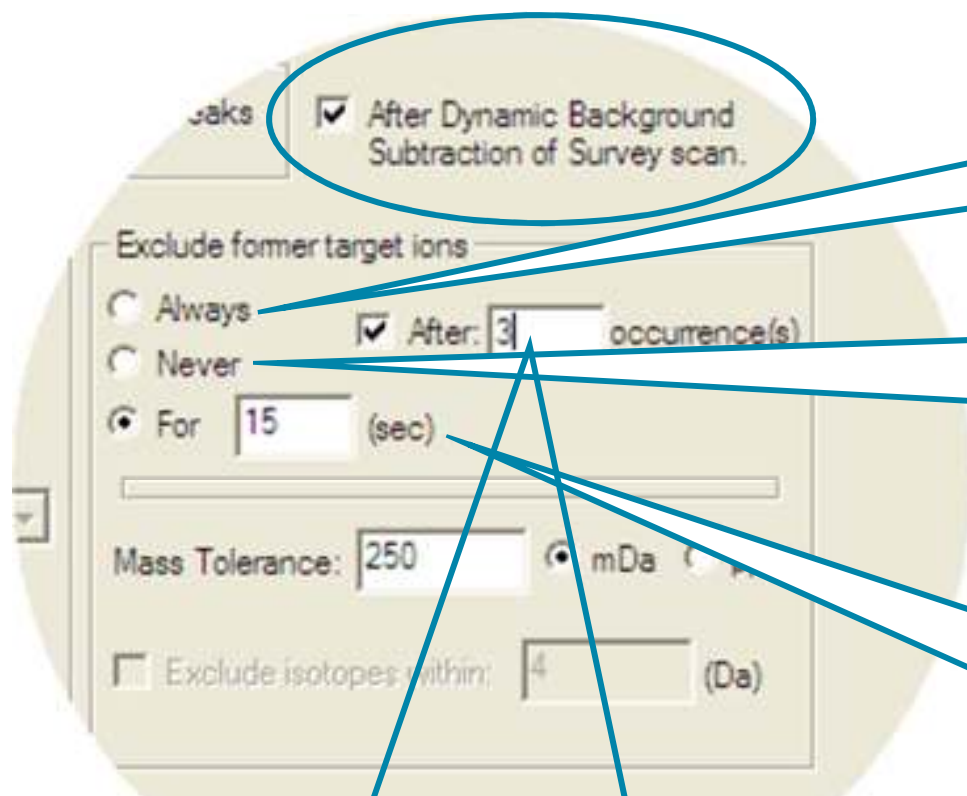
☐ Exclude isotopes within (Da)

☐ Use Enhanced Resolution Scan to confirm Charge State OR Isotope Pattern Selection

☐ Exclude T+ precursors from Enhanced Resolution Scan confirmation and MS/MS

pos/neg switch working

Dynamic Exclusion



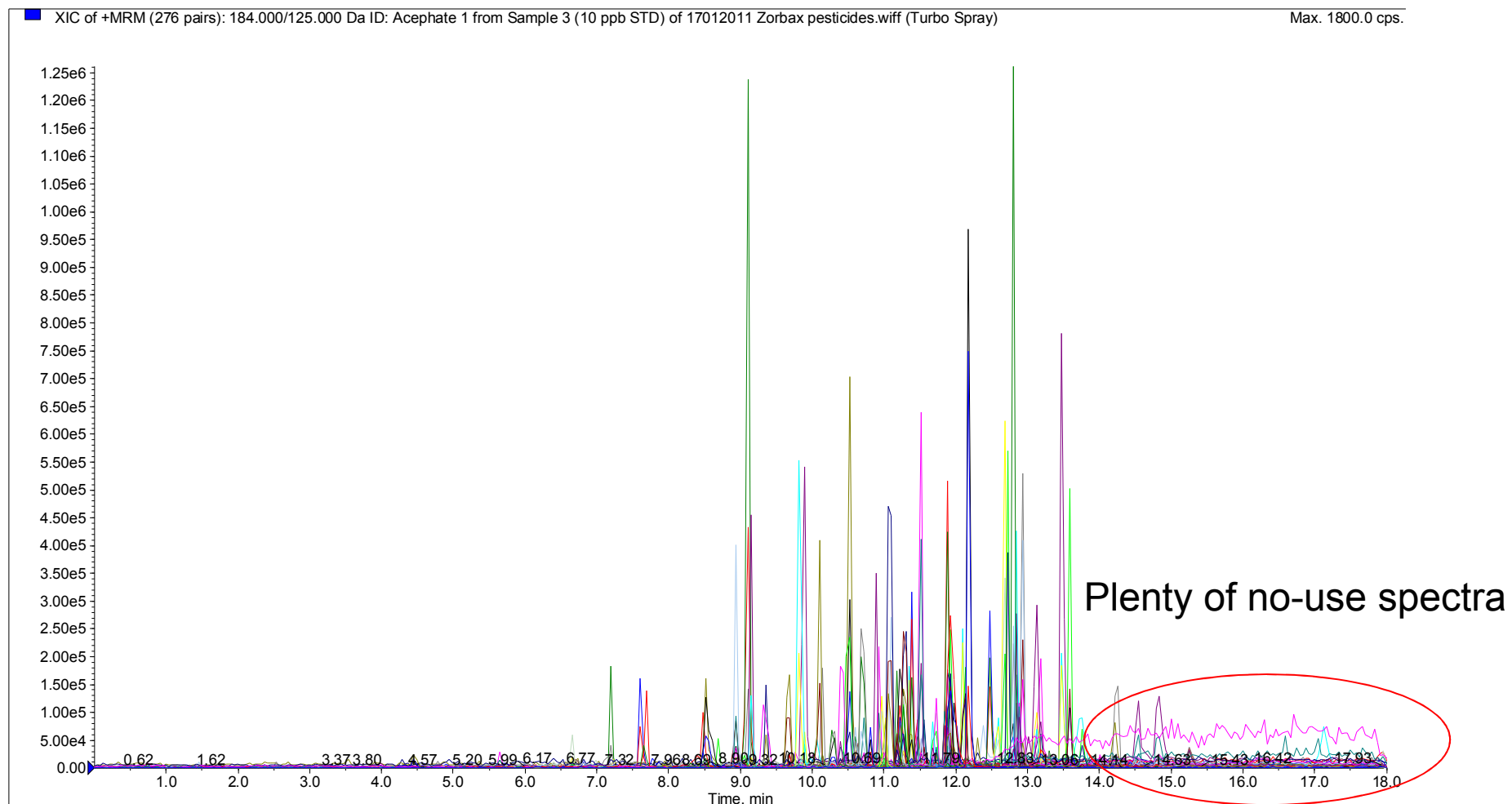
After an EPI has been acquired this mass will not be triggered again for the entire run

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 subtract (DBS)

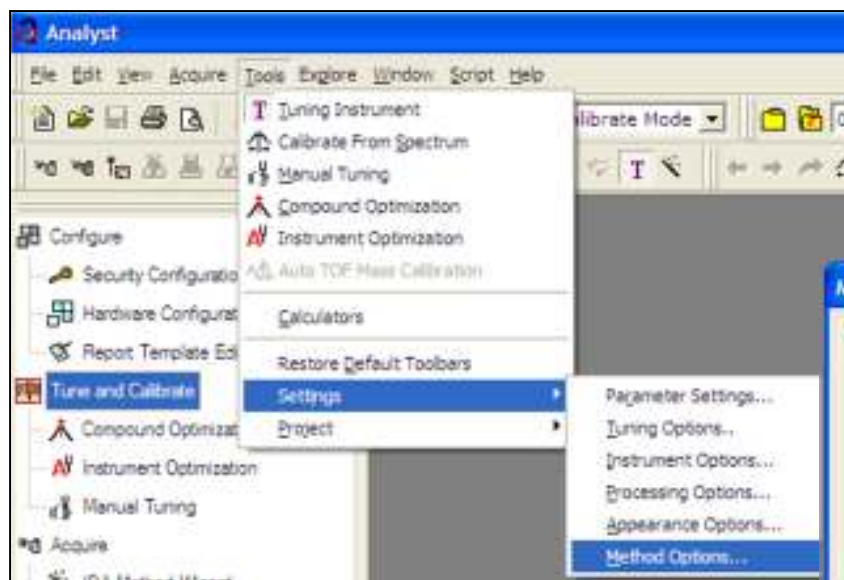


(Dynamic) fill time – (D)FT



- 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*

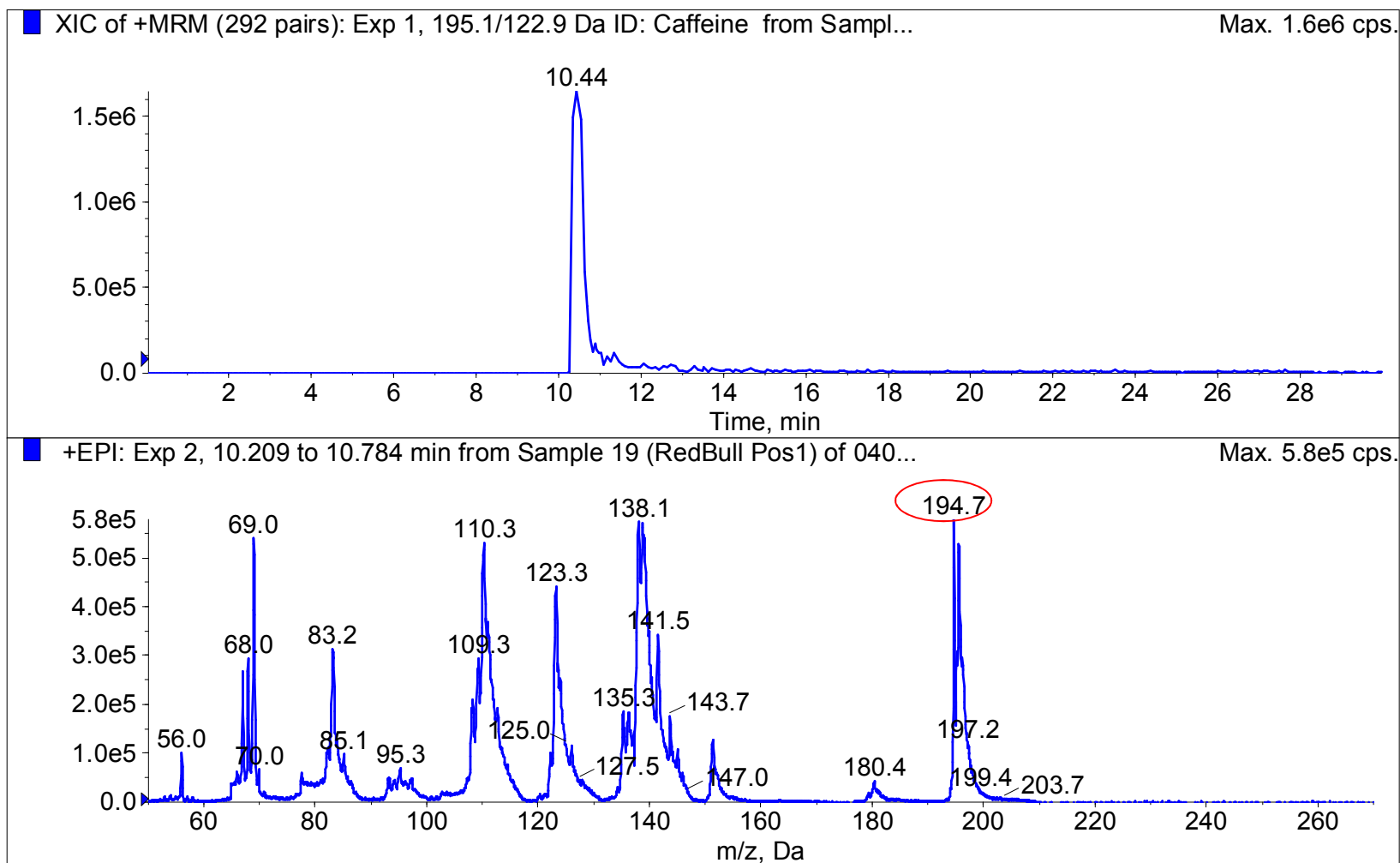
(Dynamic) fill time – (D)FT



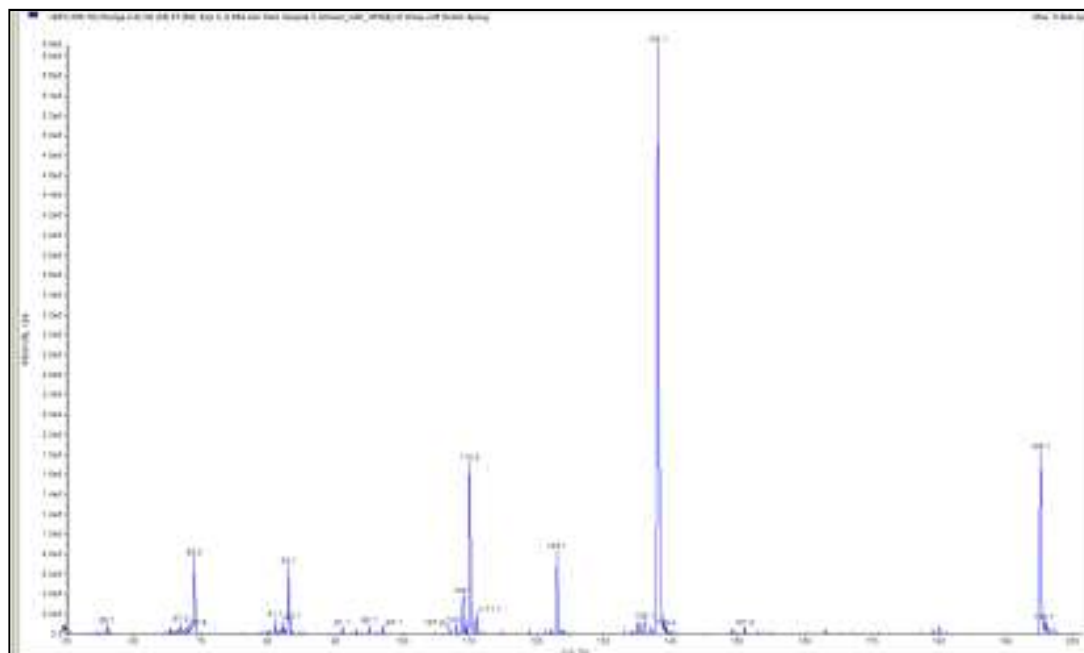
The 'Method Options' dialog box is shown, with the 'Default Fill Settings' tab selected. The 'Default Fill Mode' is set to 'Dynamic Fill Time (DFT)'. The 'Default Fill Settings' section contains a table with columns for 'TIC Target (x1e7 cps)', 'Min. Fill Time (ns)', 'Max. Fill Time (ns)', and 'Default Fill Time (ns)'. The table has three rows: 'EMS Scan', 'ER Scan', and 'EPI/MS3 Scan'. The 'Dynamic Fill Time' section is active, and the 'Fixed Fill Time' section is inactive.

	TIC Target (x1e7 cps)	Min. Fill Time (ns)	Max. Fill Time (ns)	Default Fill Time (ns)
EMS Scan	1.0	2	150	20
ER Scan	0.5	2	250	20
EPI/MS3 Scan	2.0	2	250	20

and here is my first nice aquired spectrum...or?

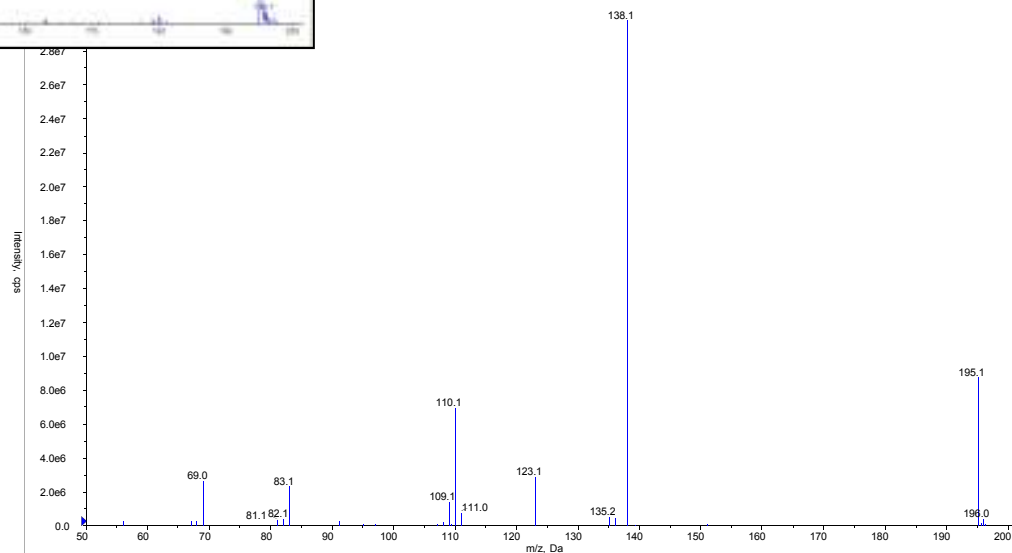
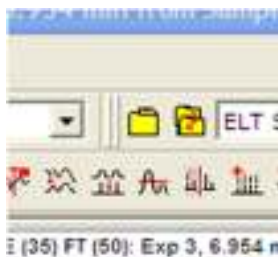


(Dynamic) fill time – (D)FT – as it should be



Exp 3, 6.954 min from Sample 3 (Urine2_CAF_SPIKE) of Urine.wiff (Turbo Spray), Centroided Max. 3.0e7 cps.

centroid



CAD and CE in Enhanced Product Ion Scan (EPI)



Period 1 Experiment 1 Parameter Table

Source/Gas	Compound
Ion Source: Turbo Spray	
Curtain Gas (CUR)	20.0
Collision Gas (CAD)	High
IonSpray Voltage (IS)	5500.0
Temperature (TEM)	450.0
Ion Source Gas 1 (GS1)	40.0
Ion Source Gas 2 (GS2)	60.0

Apply the following parameters to all other experiments of the same polarity:

☐ Source/Gas ☐ Compound

OK Cancel Help

vacuum/pressure

should be in the range
 3.8 to 4.3×10^{-5} Torr

Period 1 Experiment 1 Parameter Table

Source/Gas	Compound
Declustering Potential (DP)	60.0
Entrance Potential (EP)	10.0
Collision Energy (CE)	35.0
Excitation Energy (AF2)	100.000
Collision Energy Spread (CES)	15.0

Apply the following parameters to all other experiments:

☐ Source/Gas ☐ Compound

OK Cancel Help

What is the advantage of a LIT over a QqQ

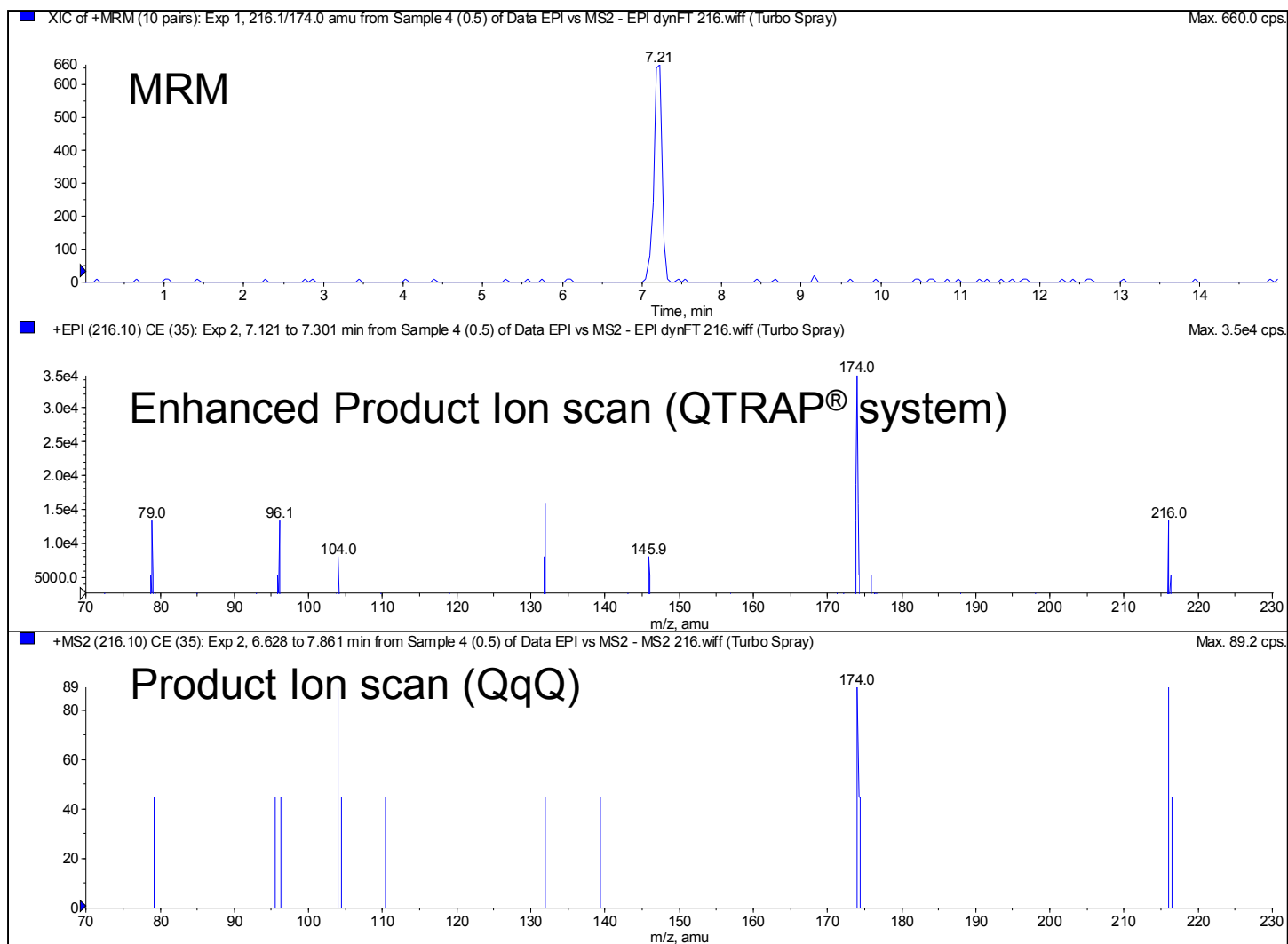
- **Speed and sensitivity** for all experiments is higher
 - Q1/Q3 vs. EMS and MS² vs. EPI
- LIT collects all ions in a „mass window“ simultaneously
 - Having a fill time of 50ms means that ALL ions are collected for 50 ms
 - This compares to a dwell time of 50 ms for EACH ion in QqQ
 - QqQ would need 313.000 msec for this experiment

The screenshot shows a control interface for a mass spectrometer. On the left, there are settings for 'Scan type' (Enhanced MS (EMS)), 'Scan rate' (1000 Da/s), and 'Polarity' (Positive). On the right, there is a table with columns 'Start (Da)', 'Stop (Da)', and 'Time (sec)'. The table contains three rows of data. A blue oval highlights the second row, which represents a mass range window from 174.000 to 800.000 Da, with a time of 0.6261 seconds. A callout box points to this row with the text 'Mass range window'.

	Start (Da)	Stop (Da)	Time (sec)
1	80.000	179.200	0.0993
2	174.000	800.000	0.6261
3			

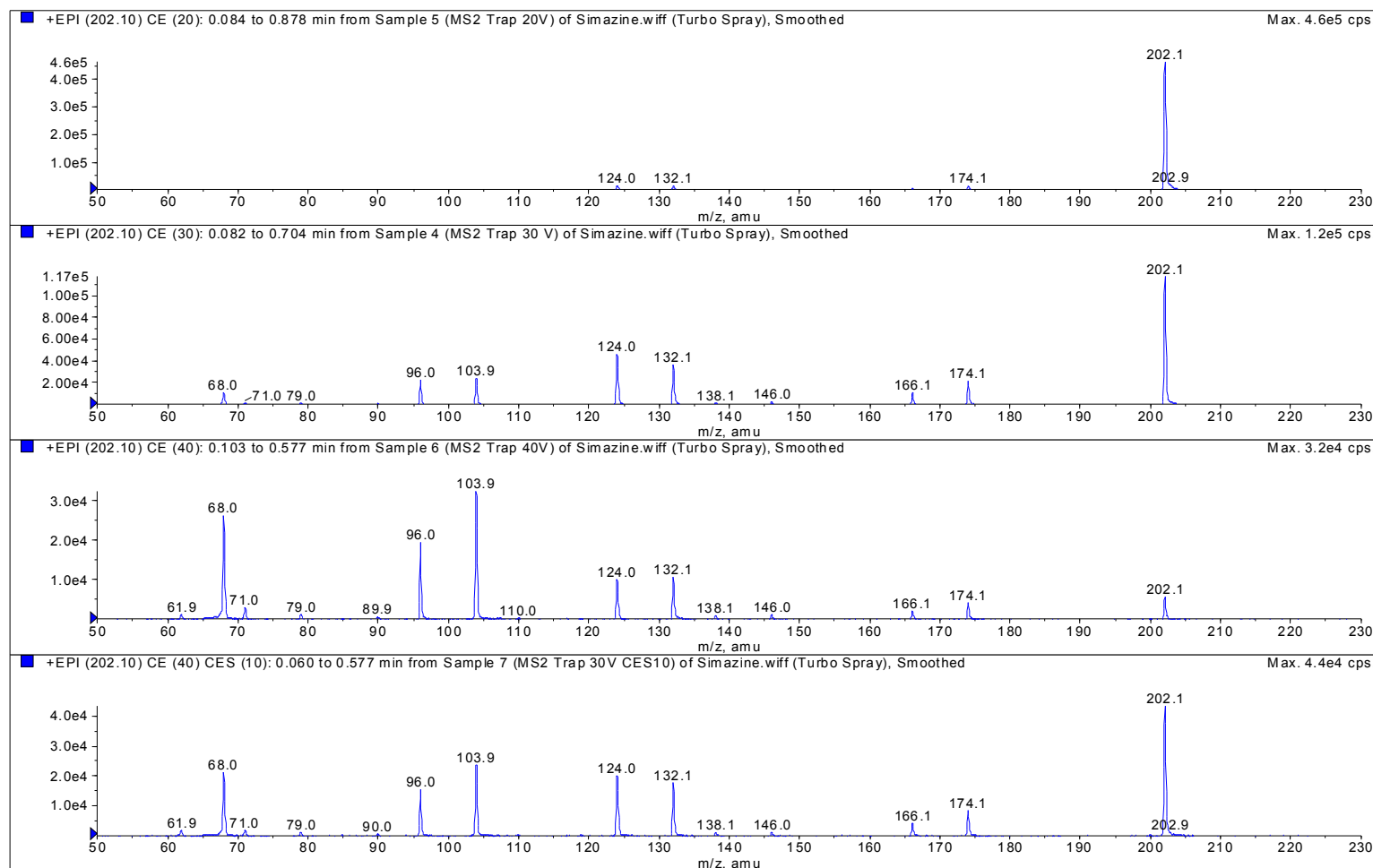
- EPI experiment up to 20.000 da/sec and in addition change CE while LIT is trapping the ions => CES

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

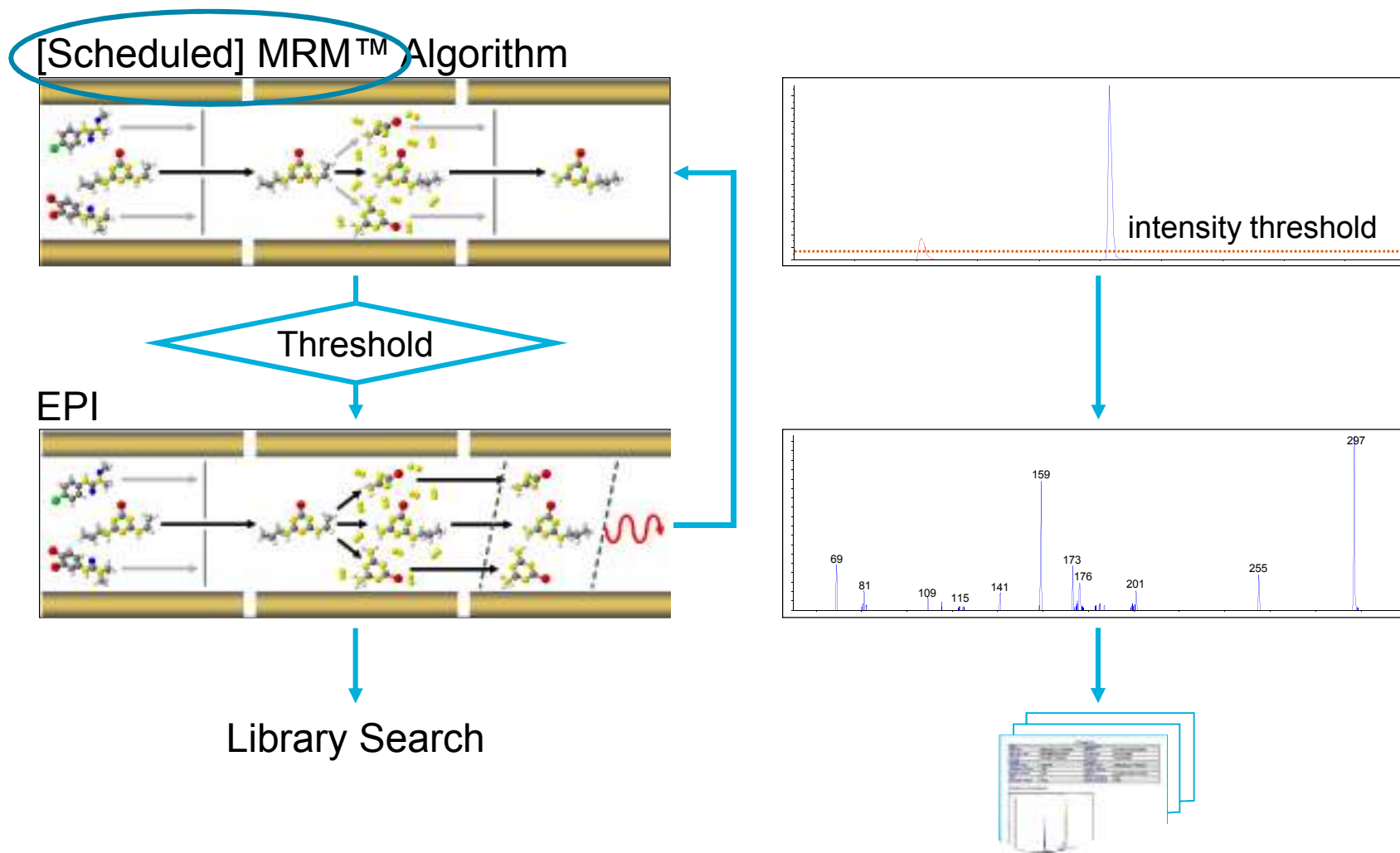
**35eV with
CES 15eV**



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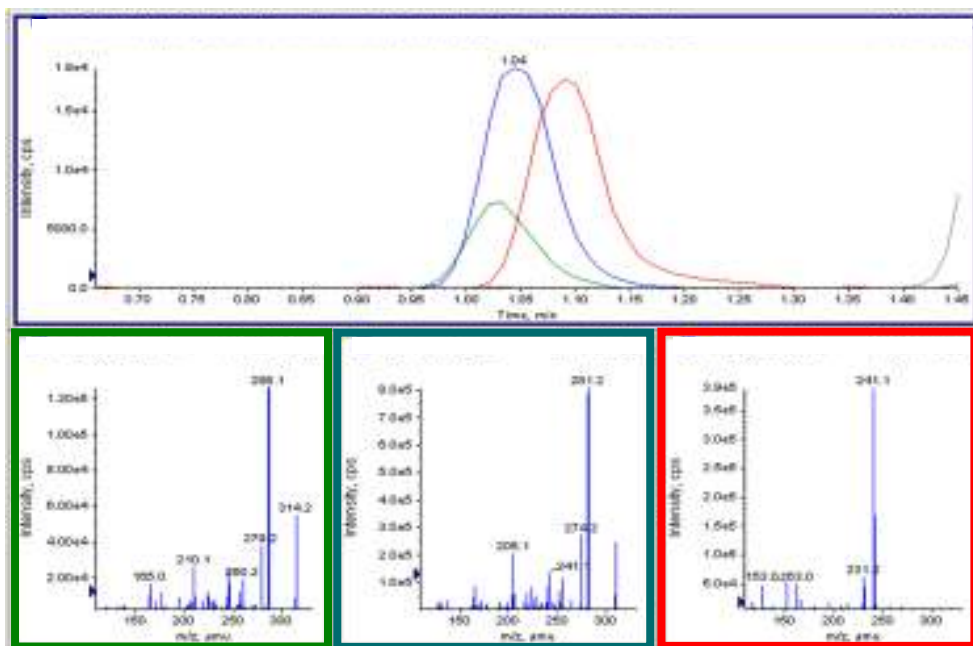
Fast Screening with Quantitation for various applications



Screening and Confirmation:

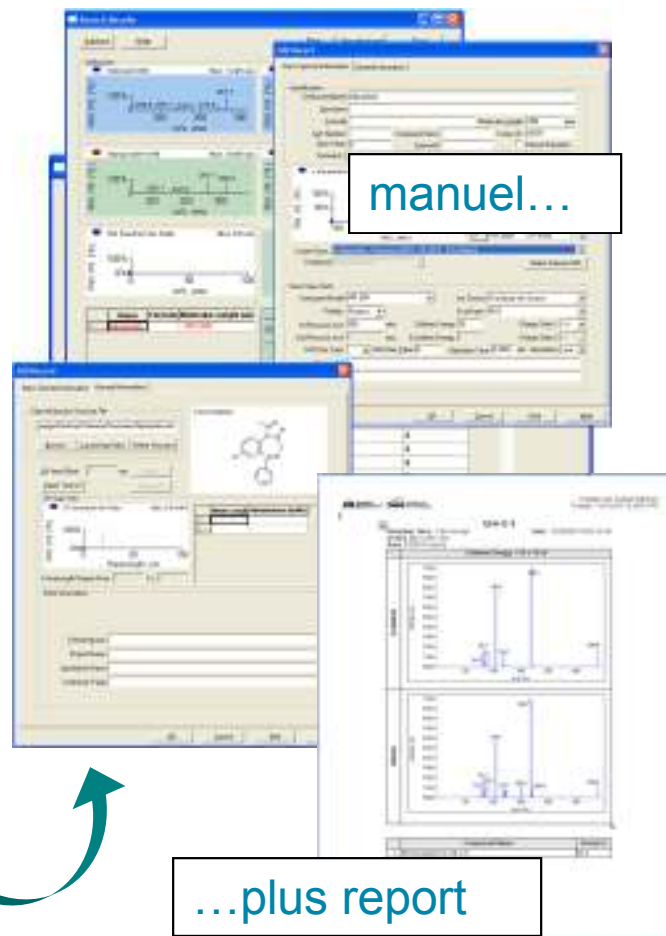


MRM Survey (quantitation)

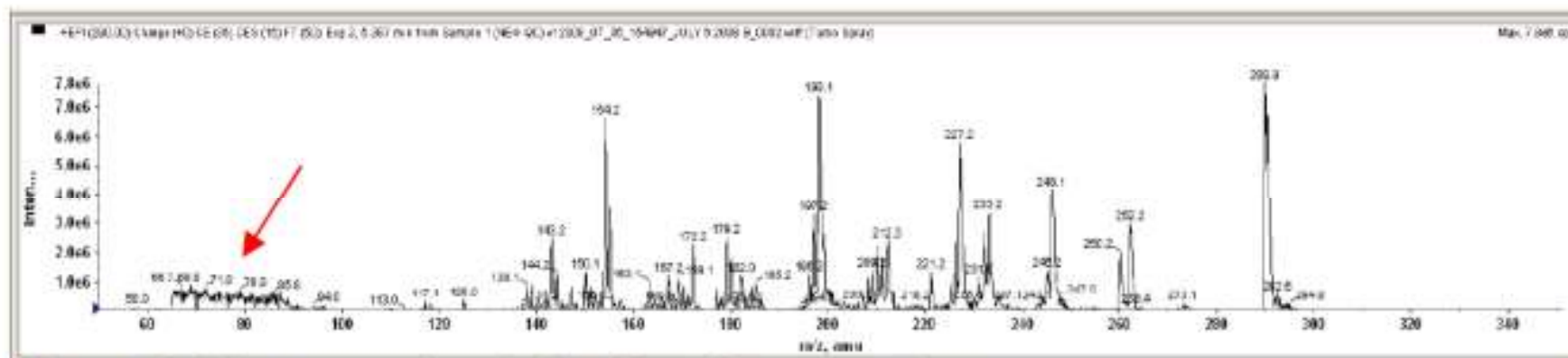


Collection of MS/MS
(full scan confirmation)

Library Search Results...



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]

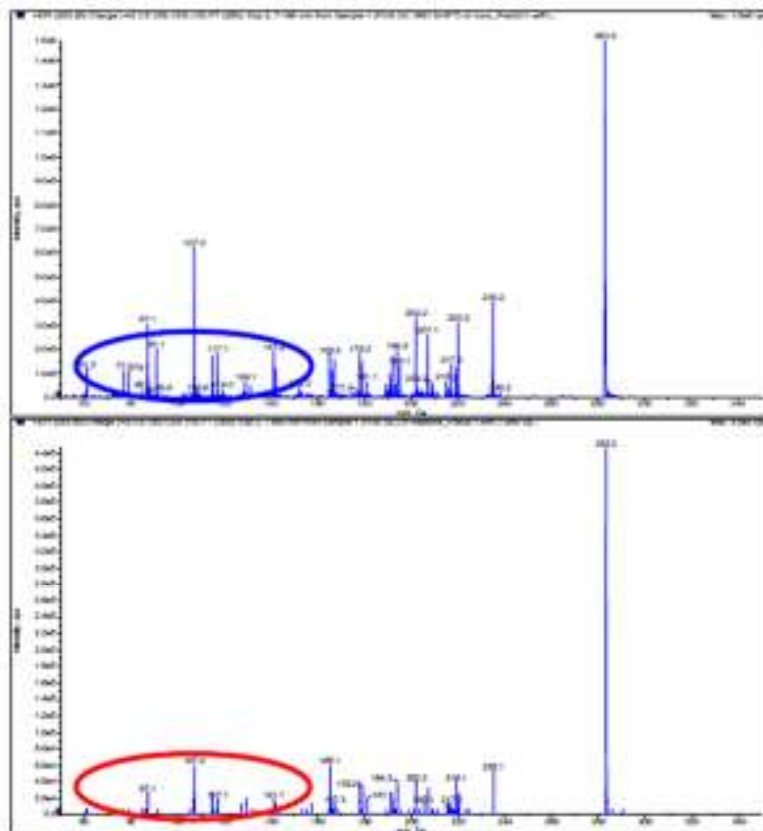


Cause: EXB is too high so ions are held in the trap too long causing ghost peaks or noise to appear in the low end (50 – 80 amu) of the spectrum.

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 μ L/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]



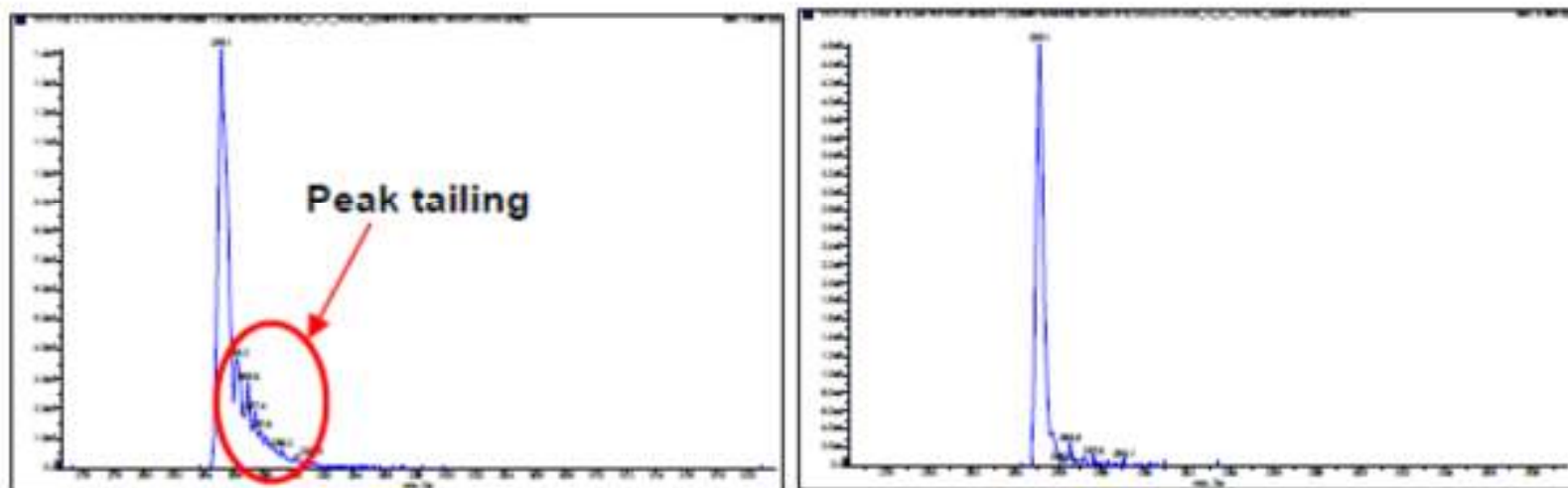
Using the same compound in both spectra, top panel shows expected peak detection at $m/z < 140$ and bottom panel shows absence of expected peaks in the same low mass region.

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.

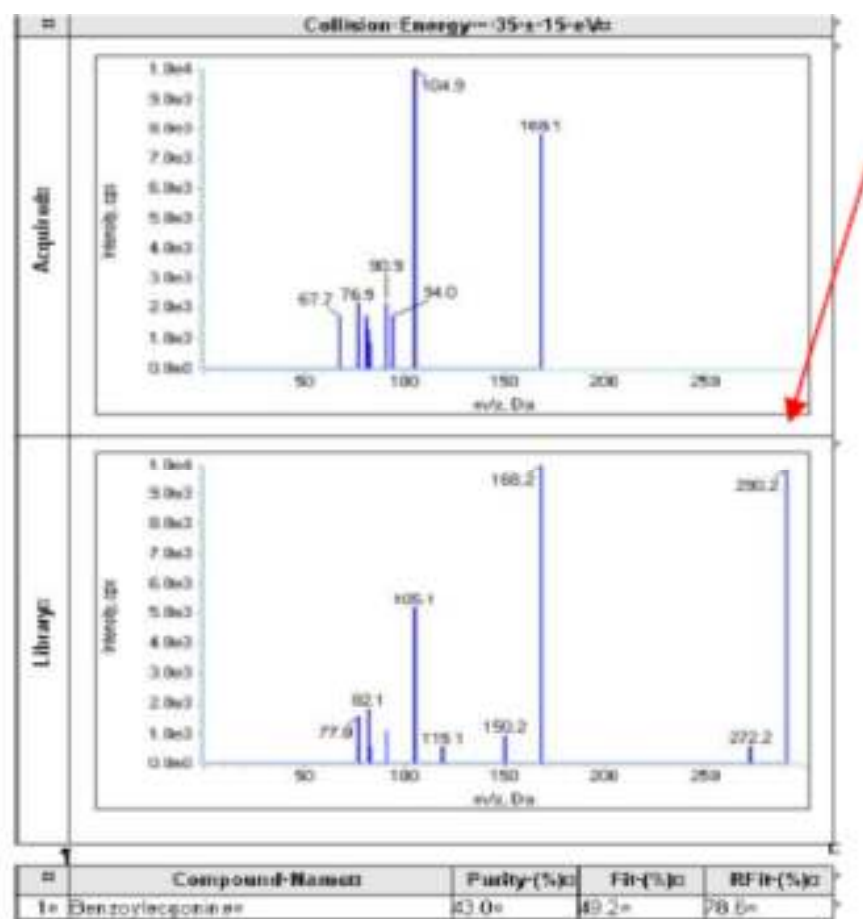


An EPI scan with Q1 resolution set to unit resolution. All fragment ions should be monoisotopic, and free from artifacts. Notice the abundant tailing on the m/z 285 ion in the left panel. By adjusting the AF3 value, this artifact was eliminated.

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:



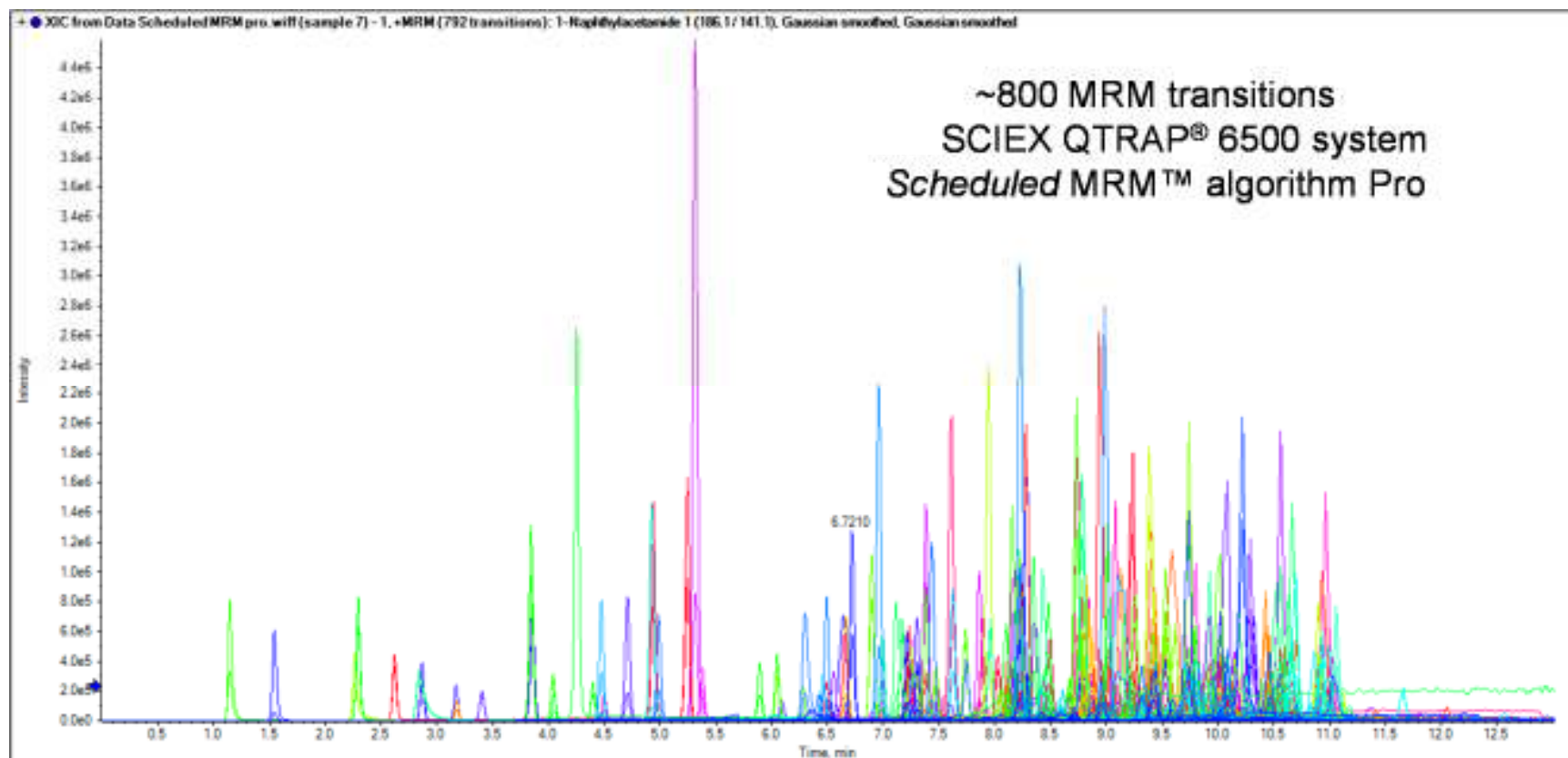
Section	Potential Issue	Corrective Action
1	Low Mass Noise	EXB too high, re-tune the instrument.
2	Reduced Low Mass Ion Intensity	EXB too low, re-tune the instrument.
3	Peak Tailing	Incorrect AF3, re-tune the instrument.
4	Library Search Filters Out Relevant Ions	Peak widths too narrow on LIT at 4000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

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



Sensitivity, Selectivity, and new Analyst 1.6.3 software

Quantitation and Identification of ~ 400 agro chemical

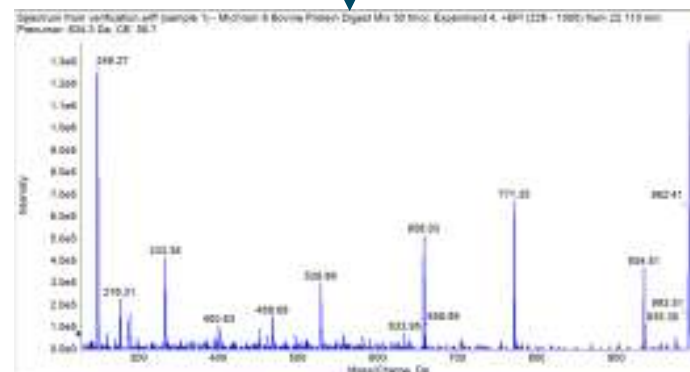


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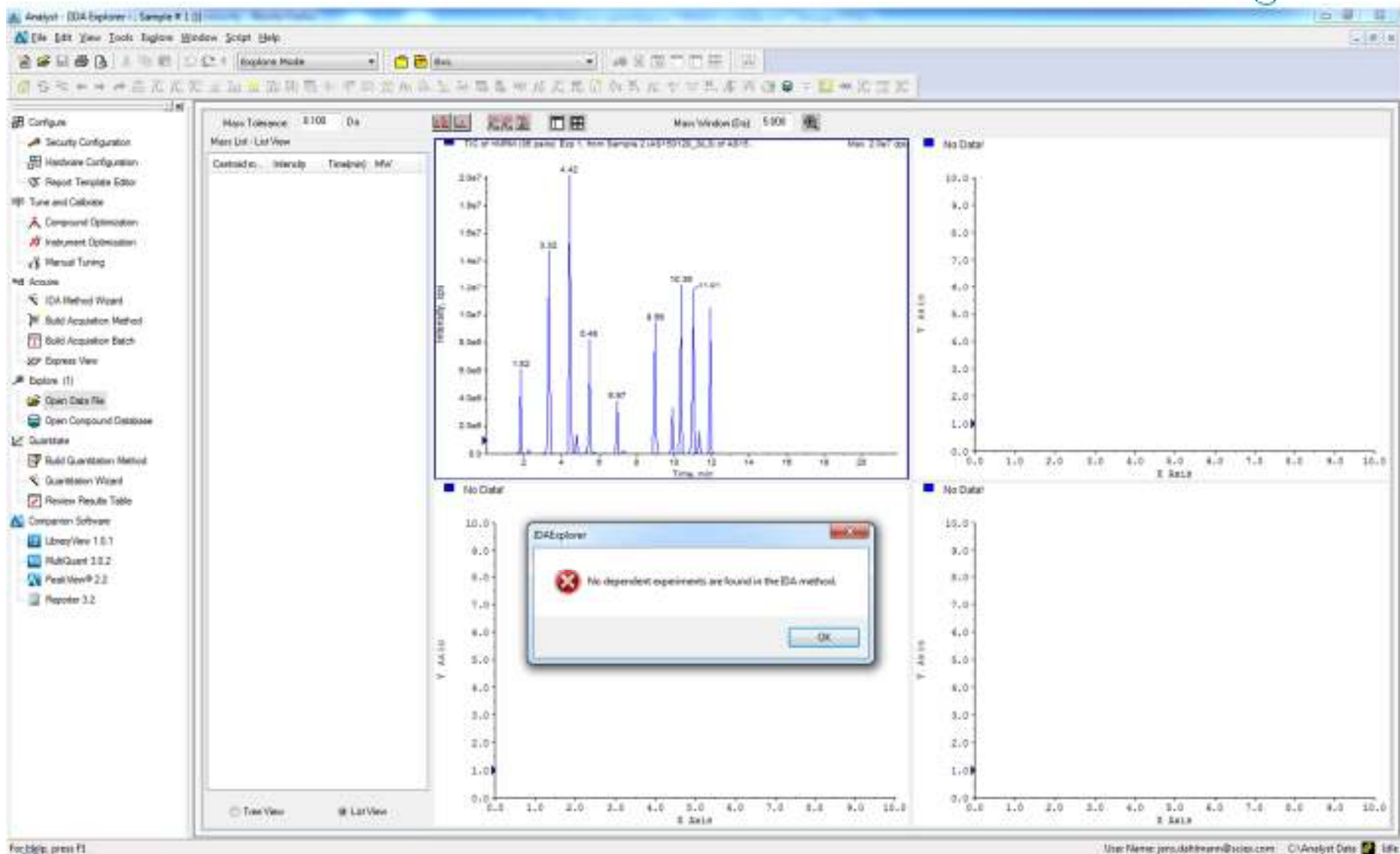
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MRM-Triggered MRM + Group MRM-Triggered MS/MS

- 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!



MS Advanced MS

Experiment: 1

Scan type: MRM (MRM)

Polarity: ☒ Positive ☐ Negative

MRM detection window: 60 (sec)

Target Scan Time: 1 (sec)

Edit Parameters...

Scheduled MRM

☒ Enabled ☐ Disabled

☐ Basic ☒ Advanced

Import List

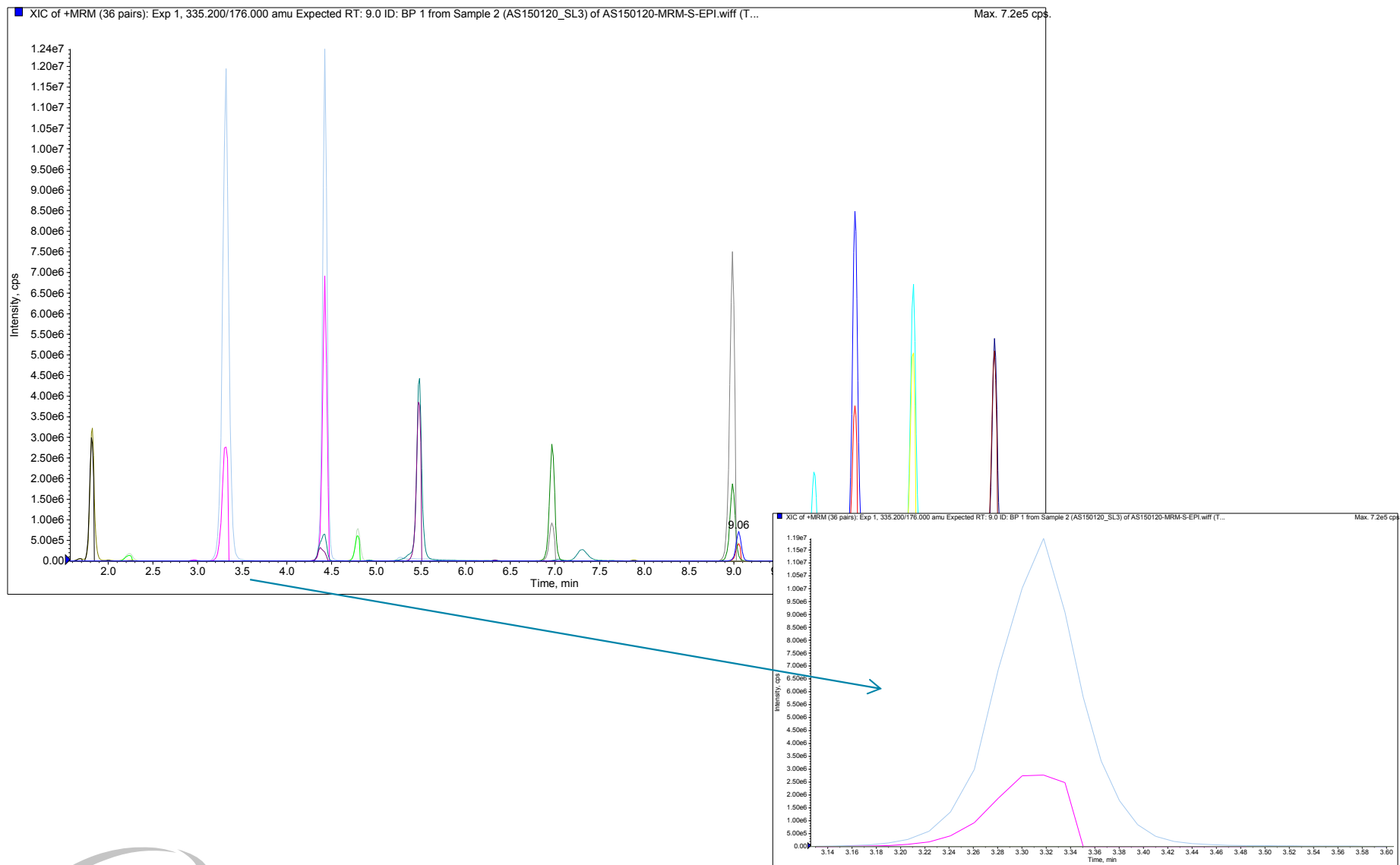
Period Summary

Duration: 8.000 (min) Delay Time: 0 (sec)

Cycles: 480 Cycle: 1.0000 (sec)

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	Group	Window (sec)	Primary / Secondary	Threshold	Dwell Weight
1	432.000	234.000	4.60	Jens 1	Alex	120.0	1	1000.00	0.10
2	432.000	123.000	4.60	Jens 2	Alex	120.0	2	20000000.00	10.00
3									

...ohh what happens with the 2. MRM?



Watch the settings in the method carefully

MS Advanced MS

Experiment: 1

Scan type: MRM (MRM)

Polarity: ☒ Positive ☐ Negative

MRM detection window: 60 (sec)

Target Scan Time: 1 (sec)

Scheduled MRM

☒ Enabled ☐ Disabled

☐ Basic ☒ Advanced

Import List

Period Summary

Duration: 8.000 (min) Delay Time: 0 (sec)

Cycles: 480 Cycle: 1.0000 (sec)

	Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	ID	Group	Window (sec)	Primary / Secondary	Threshold	Dwell Weight
1	432.000	234.000	4.60	Jens 1	Alex	120.0	1	1000000.00	0.10
2	432.000	123.000	4.60	Jens 2	Alex	120.0	2	20000000.00	10.00
3									

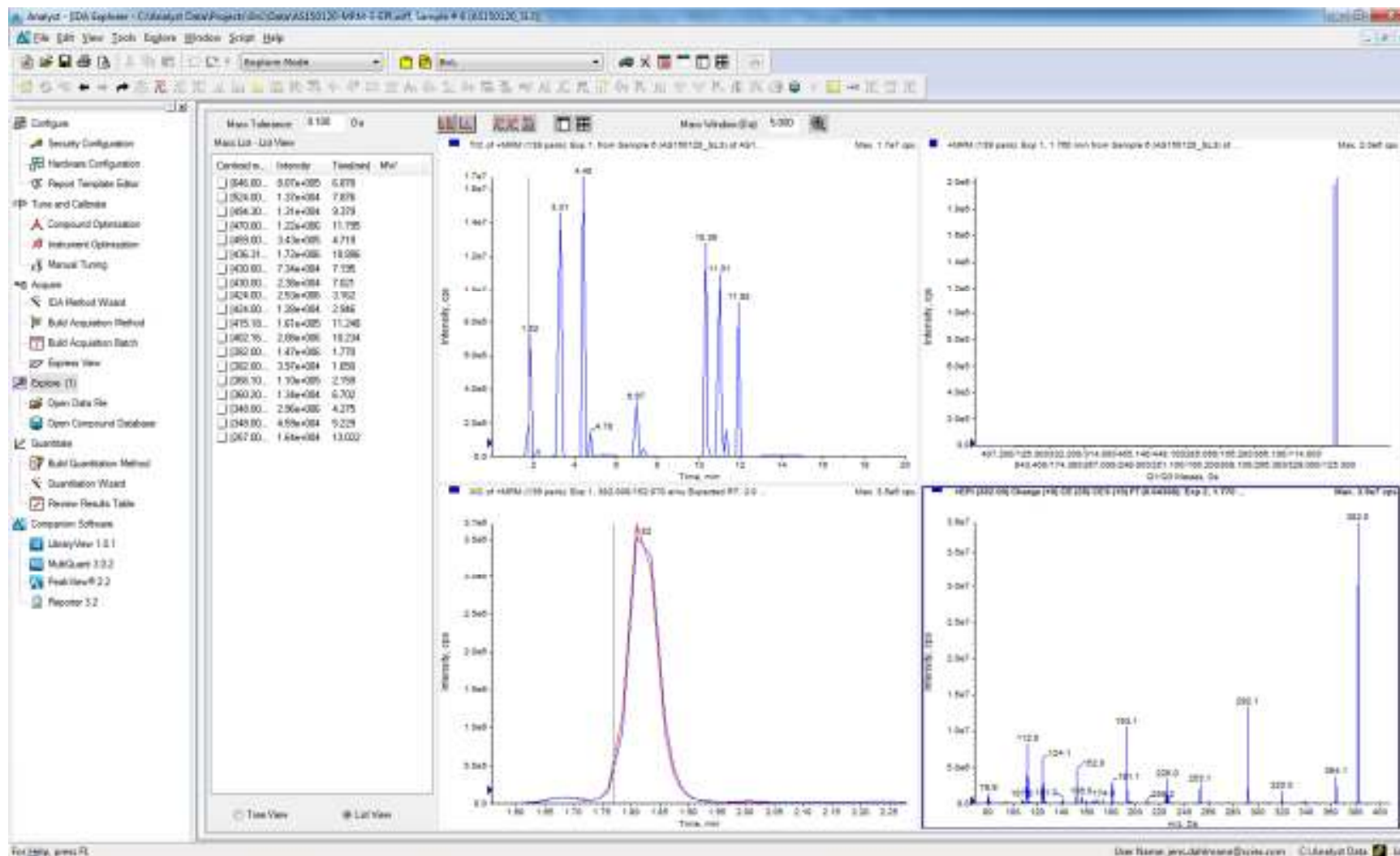
Edit Parameters...

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-Methylenedioxymphetamine			1		1.00
2	208.100	163.200	4.71	3,4-Methylenedioxyethylamphetamine			1		1.00
3	194.100	163.100	4.44	3,4-Methylenedioxymethamphetamine			1		1.00
4	194.100	105.100	4.44	3,4-Methylenedioxymethamphetamine			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	Benzoylcegonine 1			1		1.00
10	290.100	105.100	4.56	Benzoylcegonine 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

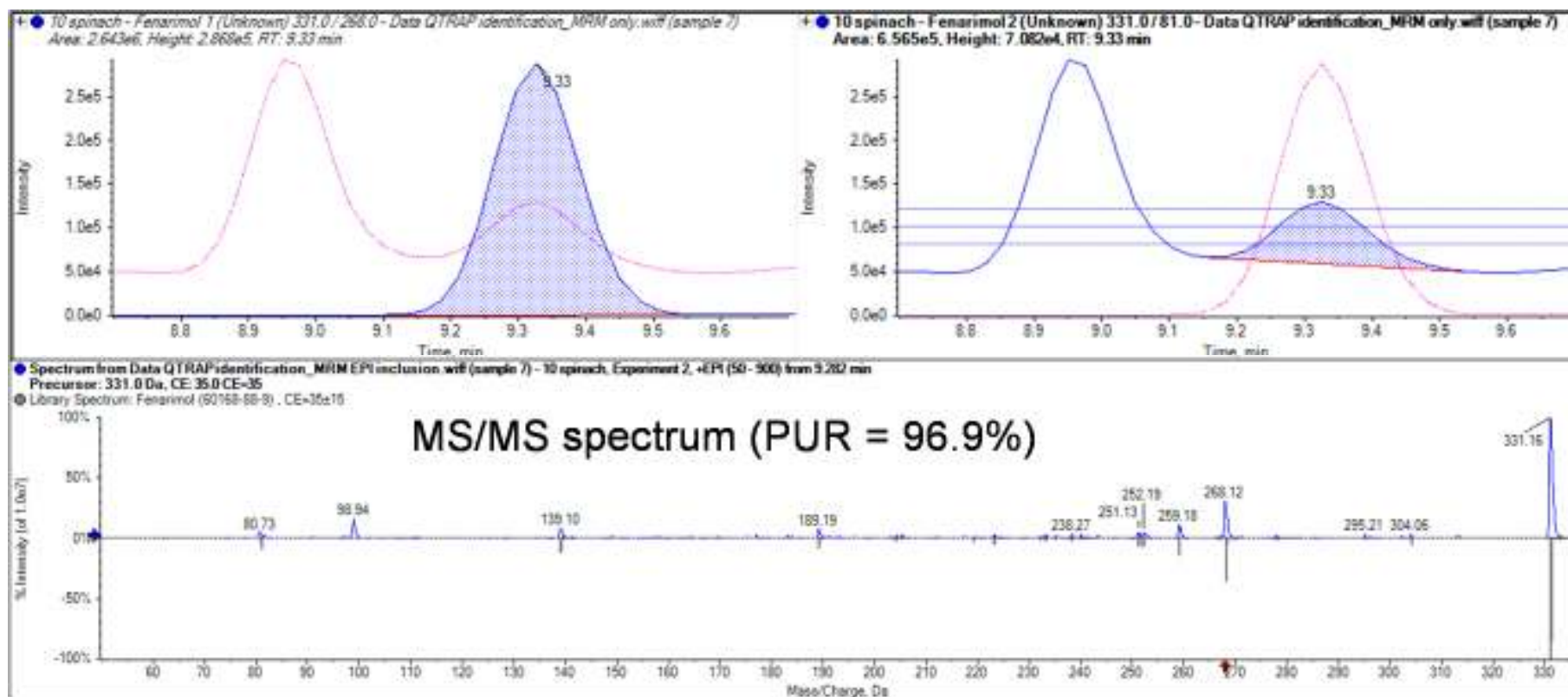
- Do not use groups in acquisition method or primary secondary to avoid what we saw
- Set a very high threshold for the second MRM of quantitation compounds so that we never get IDA EPI on the second MRM
- Threshold set for each 'primary' compound to get good MRM ratios and IDA EPI when we want it

Finally !



Questionable ID using only MRM Ratio, MS/MS Confirmation

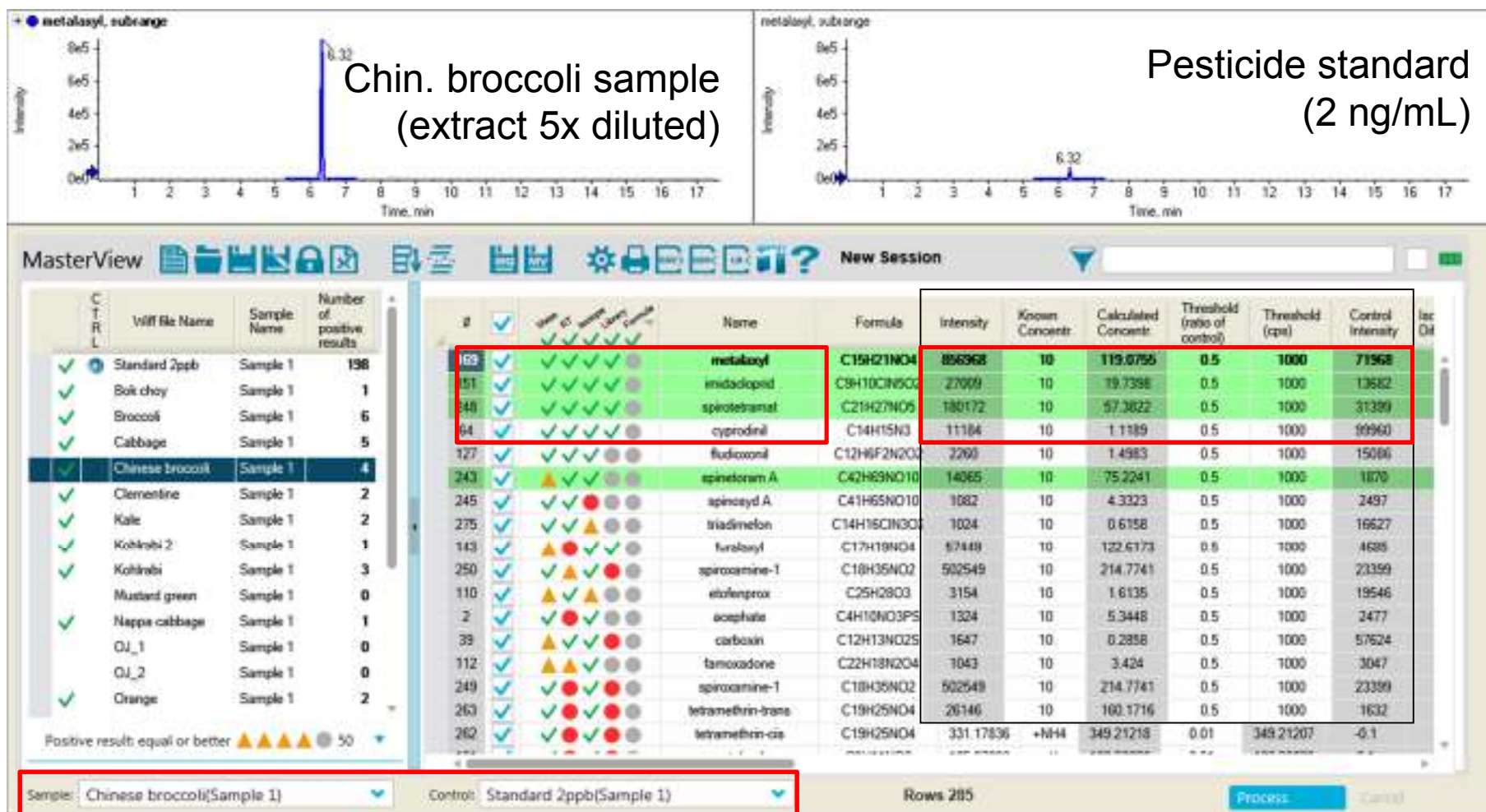
10 µg/kg Feniramol in Spinach (MRM ratio error = 32.0)



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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