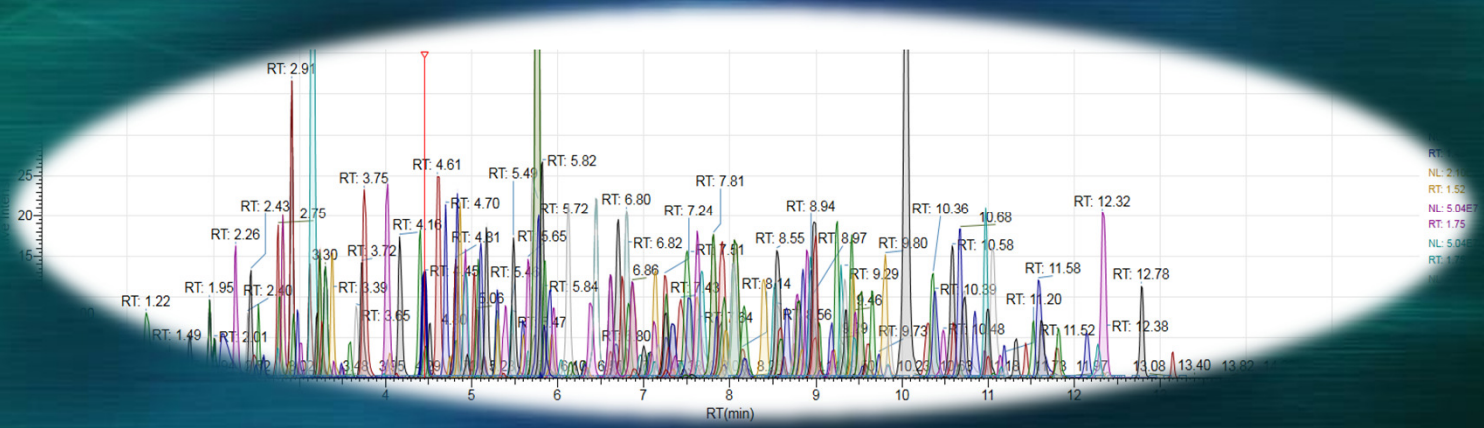
Use of HRAM with LC, GC and IC for the analysis of pesticide residues in food

Choosing the best tools for the job at hand



Jim Garvey

Summary

Introduction

Multi residue method – extraction

GC Method

Validation data

LC- Method

MS^2 options

Data

IC Methods

PRM

Data

Screening

Introduction

- HRAM methods offer a number of advantages over current QQQ methods
- They allow for unlimited expansion of analysis scope
- No loss of data
- Multiple MS2 options depending on what we want to do
- Comparable sensitivity to other techniques

Sample preparation

Extraction

15g of homogenised sample is taken

Acetone (30ml) is added and the sample is homogenised

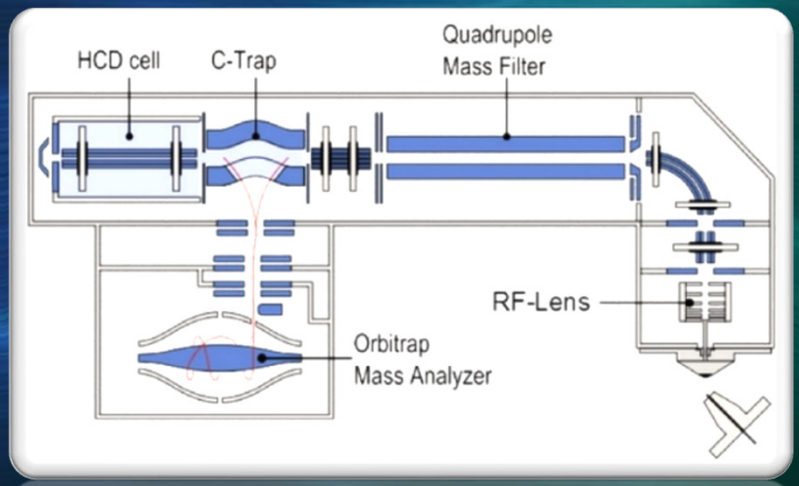
DCM (30ml), Petroleum Ether 40-60°C (30ml) and Sodium Sulphate (30g) is added and the sample homogenised again

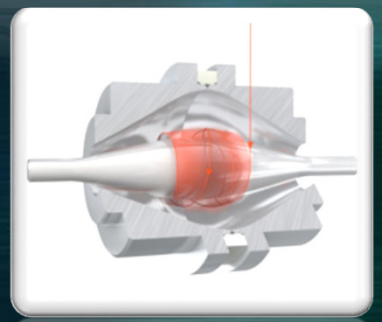
The extract is centrifuged, 60ml of the extract is reduced and reconstituted in Ethyl Acetate to a final volume of 10ml

The neat extract is injected on the GC-MS and a 1/20 dilution with methanol is injected onto the LCMS

Orbitrap – basic components

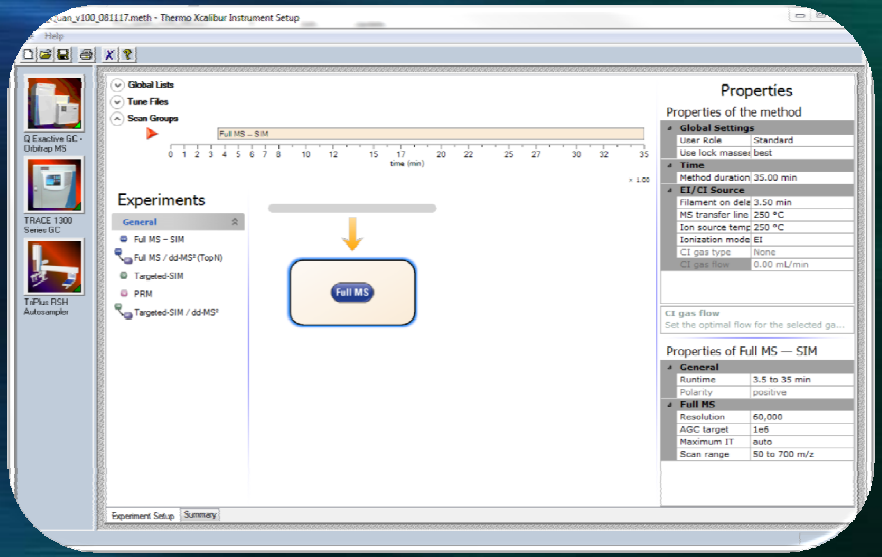
1. Ion source – EI / HESI
2. Focussing lenses
3. Quadrupole - Parallel Reaction Monitoring
4. C-Trap
5. HCD Cell – Secondary ionisation
   - All ions fragmentation – AIF
   - Data dependent analysis – ddMS2
   - Variable data independent analysis vDIA
6. Orbitrap mass analyser



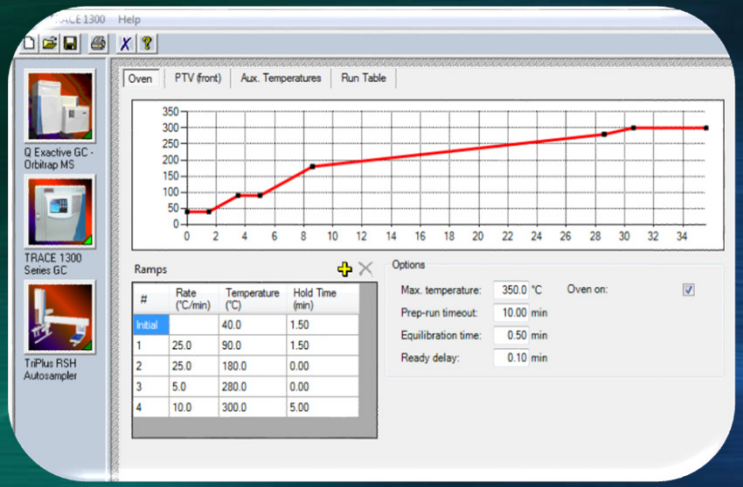


GC Method

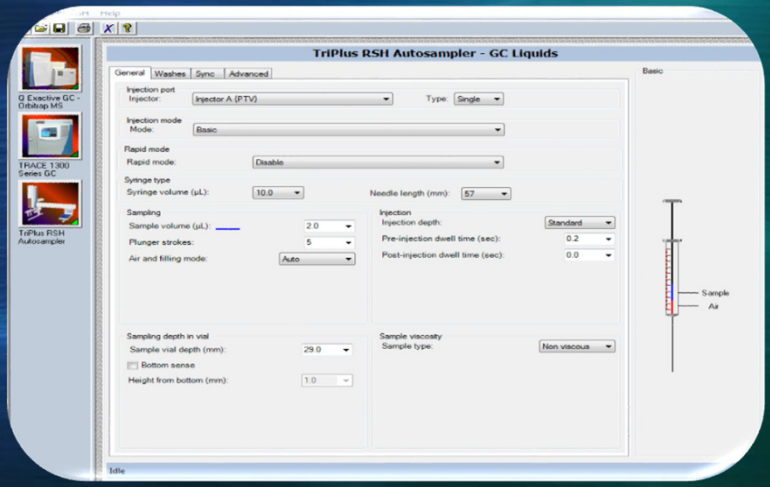
- Ion source – EI
- Quadrupole left open
- Ions are allowed accumulate in the C-trap before being sent to the orbitrap
- Full scan data
- No MS^2 options used
- The simplest way to use this system



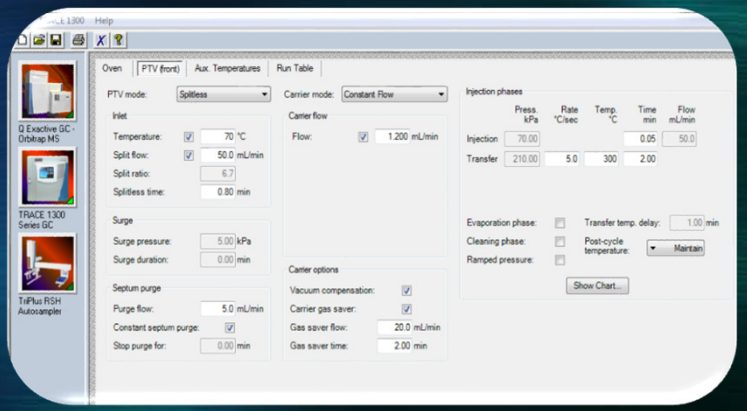
GC Method



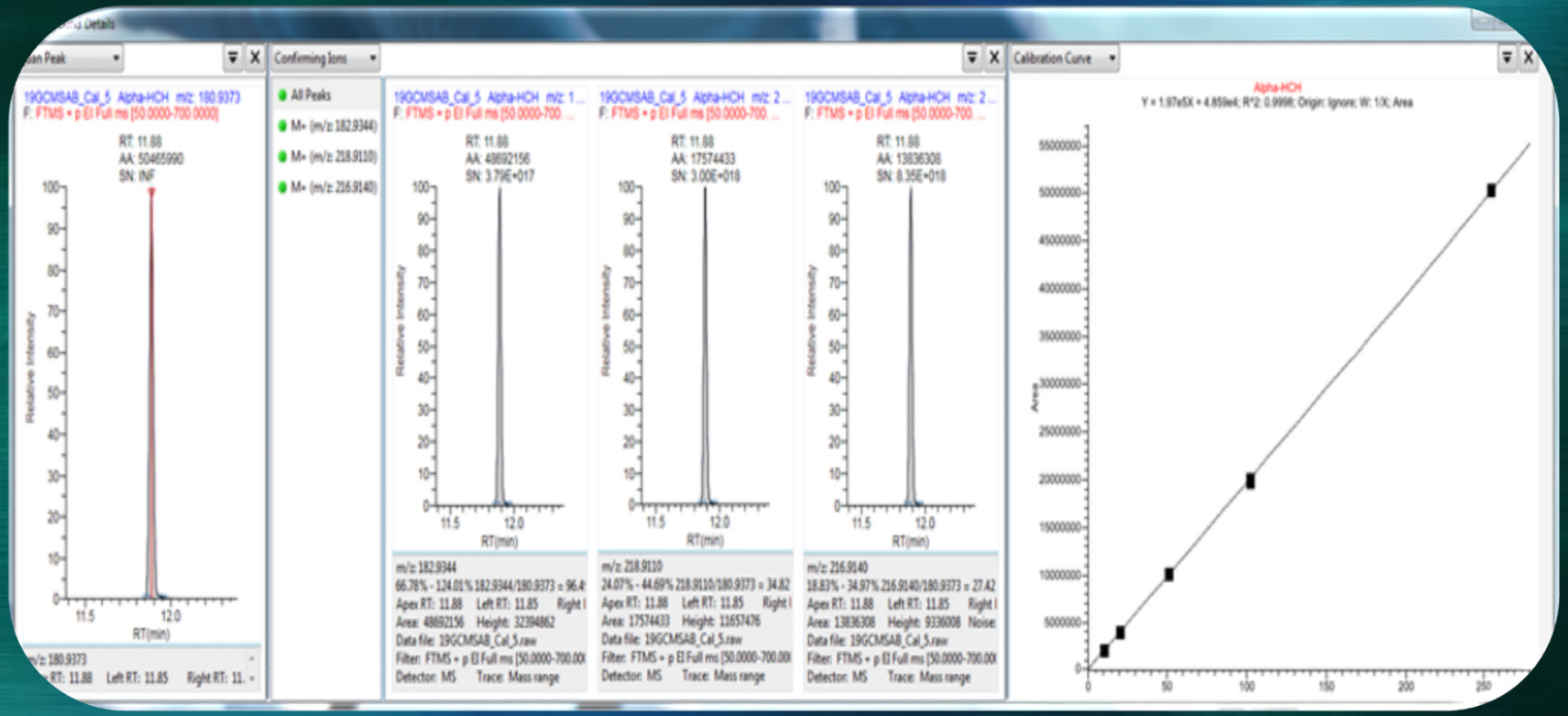
#	Rate (°C/min)	Temperature (°C)	Hold Time (min)
Initial		40.0	1.50
1	25.0	90.0	1.50
2	25.0	180.0	0.00
3	5.0	280.0	0.00
4	10.0	300.0	5.00



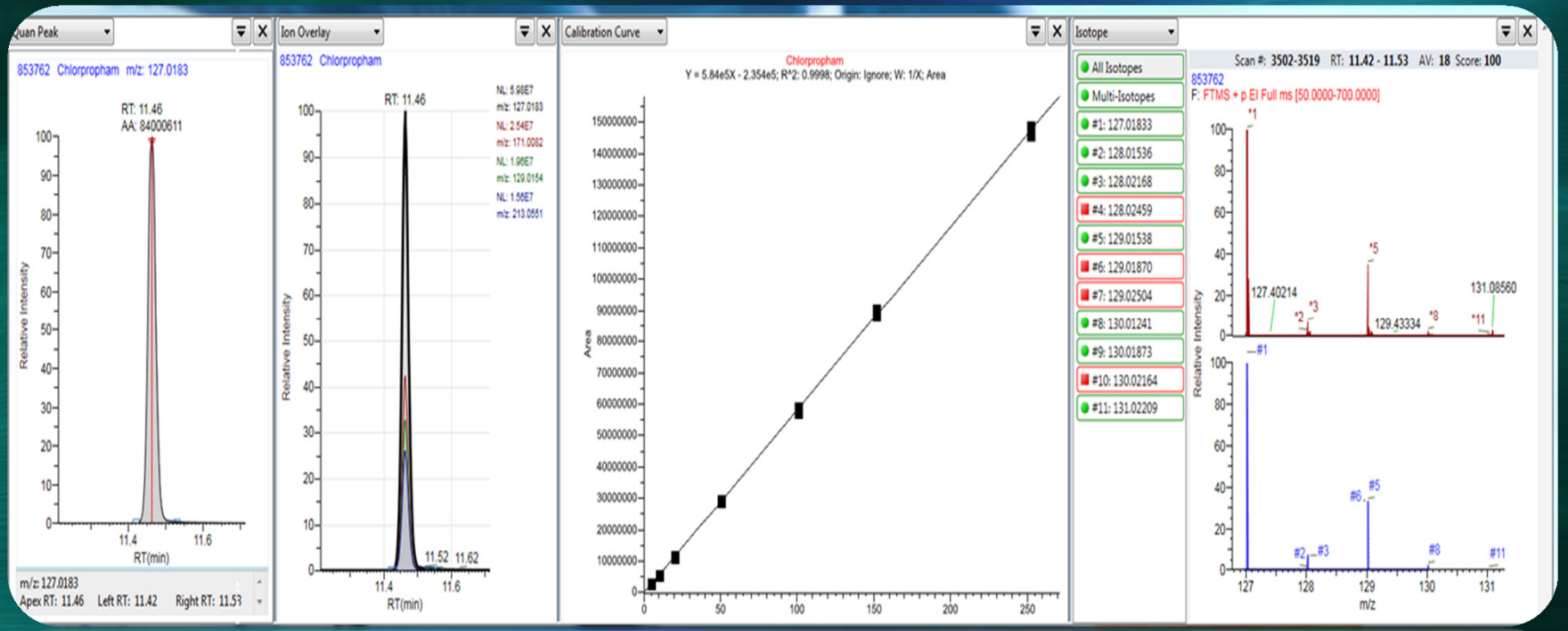
- **Column = TG-SilMS with 5m safeguard – 5% diphenyl, 95% dimethyl polysiloxane**
- **0.25mm x 0.25µm**
- **Max Temp 320/350°C**



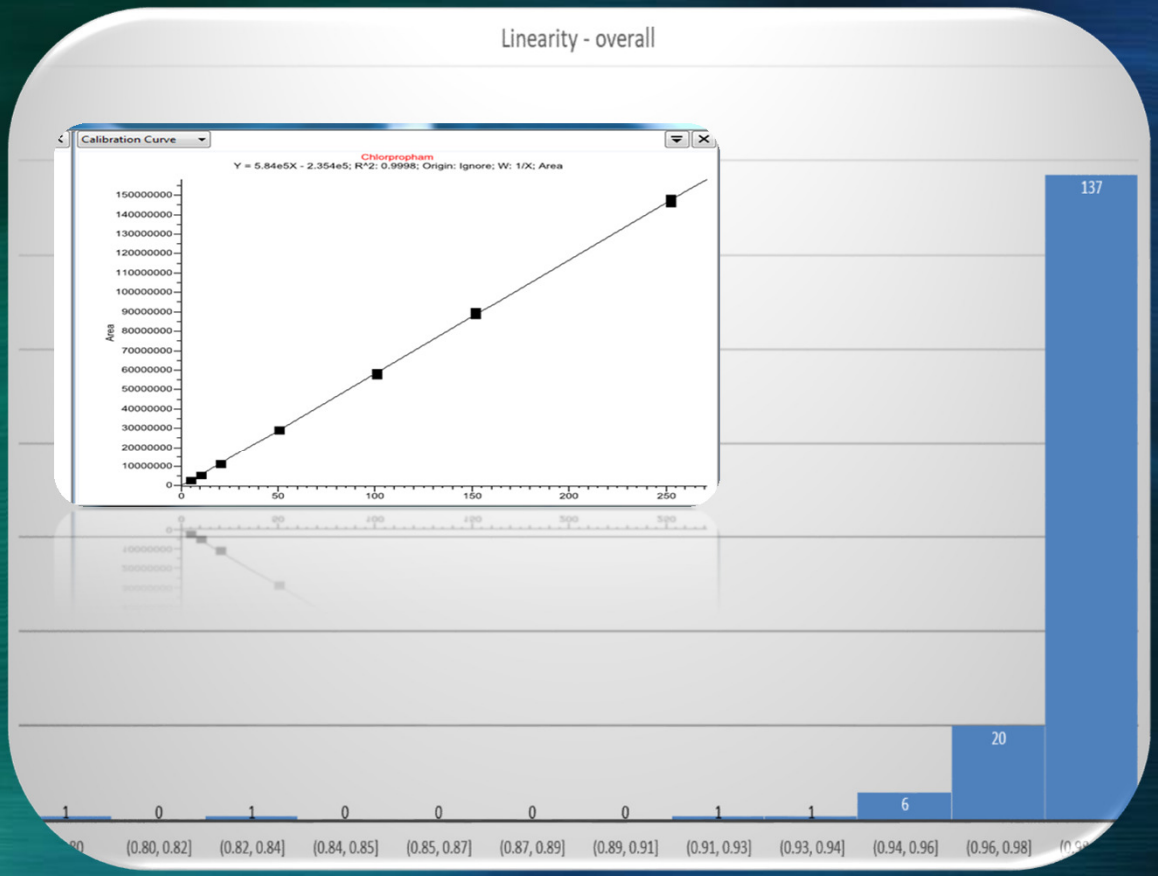
Raw data – α-HCH



Raw data - Chlorpropham



Linearity

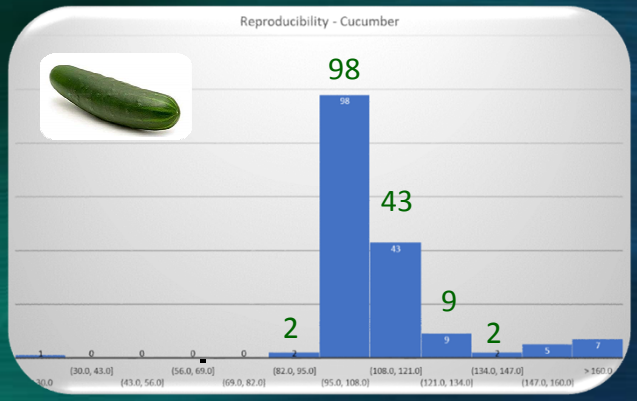


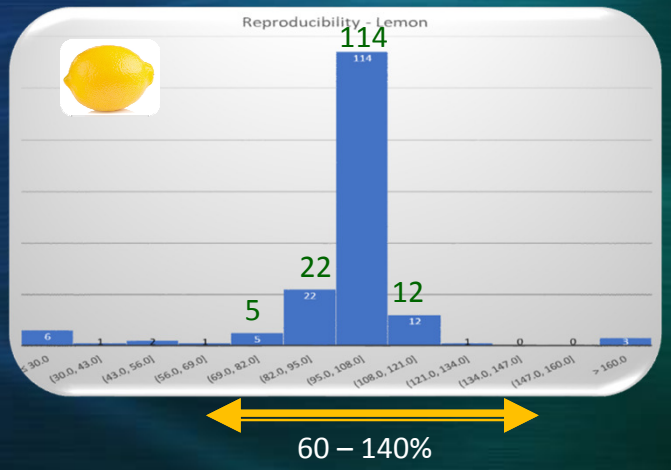
7 point calibration curve between 5 and 250 µg/l

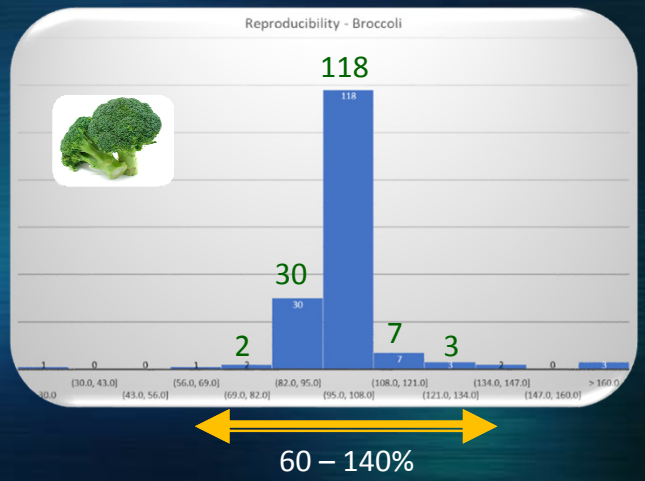
Spikes bracketed between calibration standards

0.95 - 1

Recovery data - reproducibility

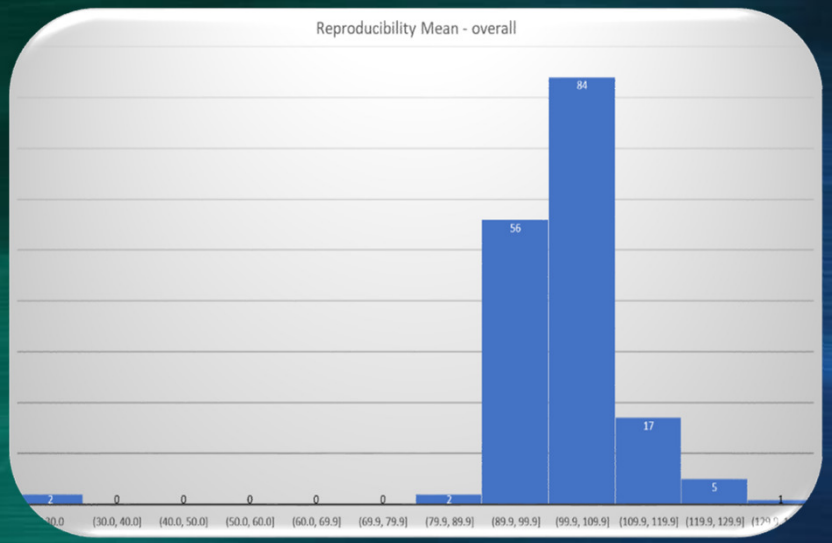




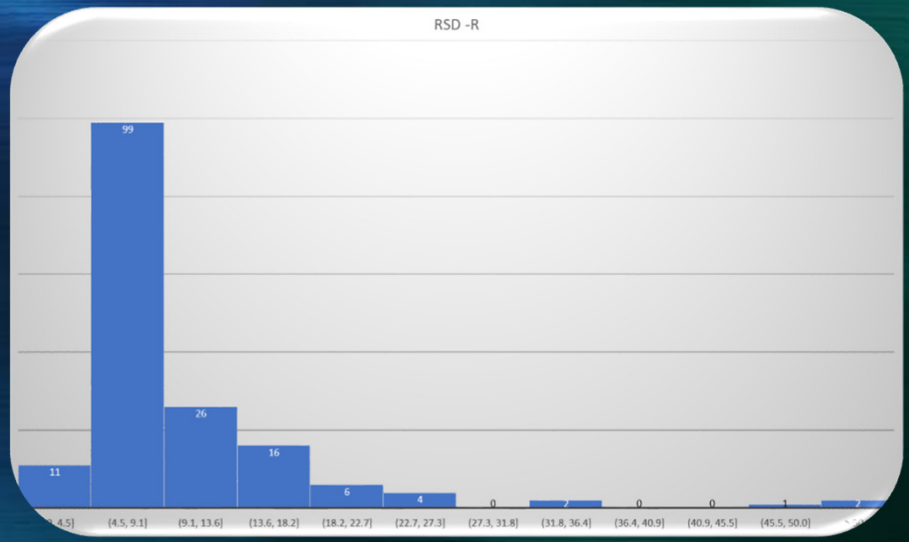


93.2% of the compounds in the method met the reproducibility criteria

Overall reproducibility

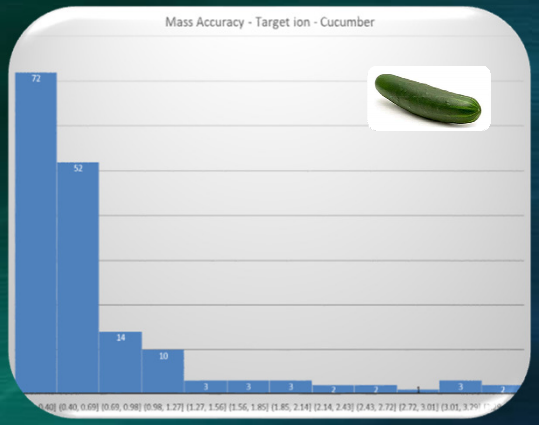


60 – 140%

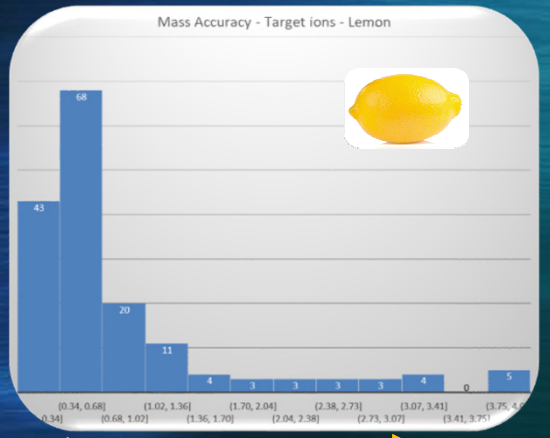


0 – 25%

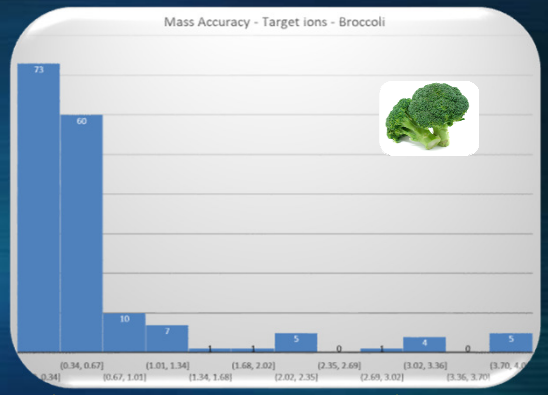
Mass Accuracy by matrix



0 – ±3ppm

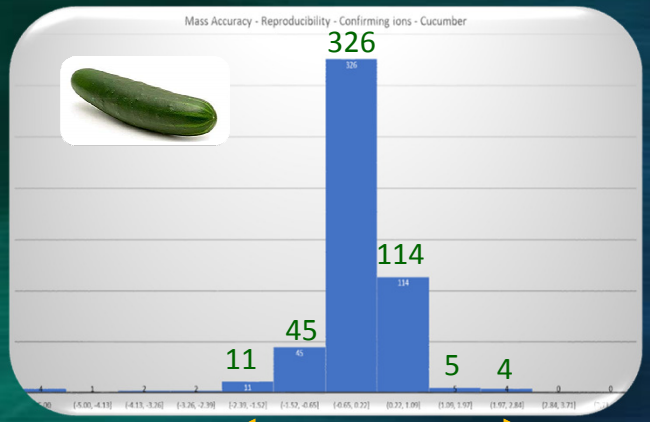


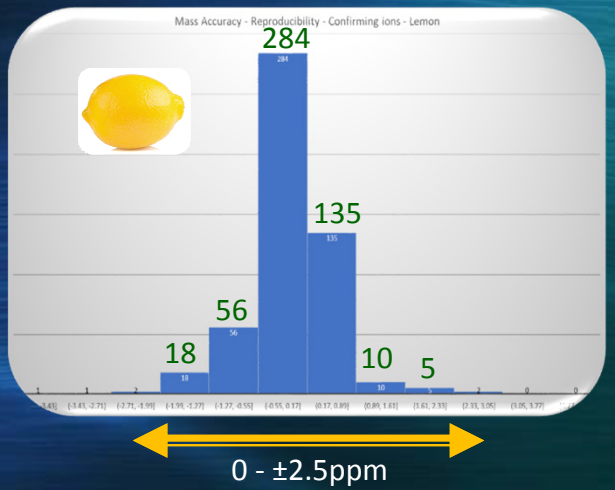
0 - ±3ppm

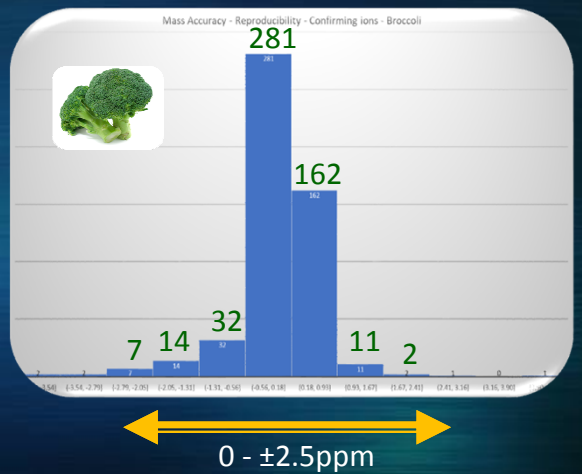


0 – ±3ppm

Mass Accuracy – confirming ions







Ion Ratio data

- **HRAM : Ion ratio 30% Tolerance**
- **Analyte peaks for Target Ion and Confirming Ion must fully overlap.**
  - **We Measured a total of 515 Ion Ratio's For Each Matrix!**
  - **No easy way to *summ*arise as the ion ratio tolerance is slightly different from batch to batch**
  - **The mean ratio at each level is compared with the mean tolerance across all levels**
  - **93.2% of the mean ratio's meet the tolerance**

Table 4. Identification requirements for different MS techniques[2]

MS detector/Characteristics		Acquisition	Requirements for identification	
Resolution	Typical systems (examples)		minimum number of ions	other
	Single MS quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	S/N ≥ 3[d] Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within **±30% (relative)** of average of calibration standards from same sequence
	triple Q, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass [illegible] for precursor-ion [illegible] to or better than [illegible]	2 product ions	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions with mass accuracy ≤ 5 ppm[a, b, c]	S/N ≥ 3[d] Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap. Ion ratio: see D12

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

Problematic compounds

Problem	Compounds
Poor	Captafol, Phorate,folpet,
Inconsistent recoveries	Azinphos-methyl, pp-ddt, omethoate
Poor extraction from lemon	Pirimicarb, Pirimicarb-desmethyl, Prochloraz & Triflumizole
High recovery, except in broccoli	Acephate
Enhancement in broccoli	Dichlofluanid (chlorthalonil)
Suppression in lemon	Metribuzin
Enhancement in cucumber	Phosmet
Enhancement, except in lemon	Resmethrin
Some poor recoveries in lemon	Trifluralin

Overall conclusion

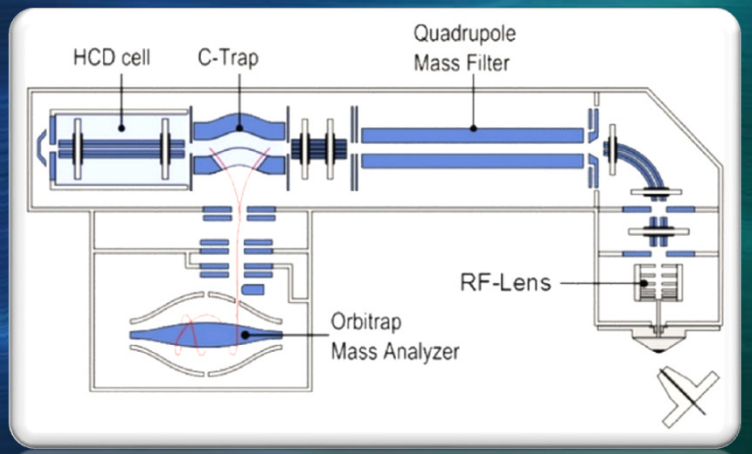
- Method was successfully validated for more than 93% of the 167 pesticides & PCBs.
- The method has been used successfully in parallel with existing methods.
- PT results excellent
- The screening capability of the technology has been successfully tested
- Next steps - Validation of other matrices, cereals, IF, Milk, eggs, honey.

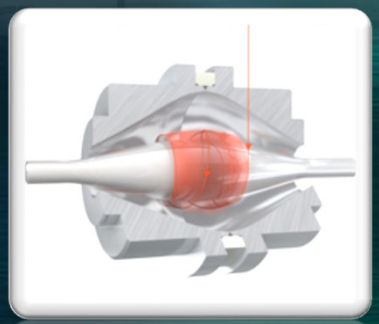




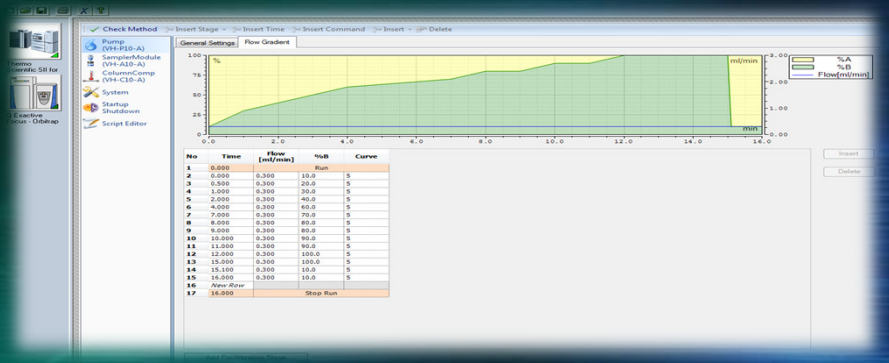
LC Orbitrap

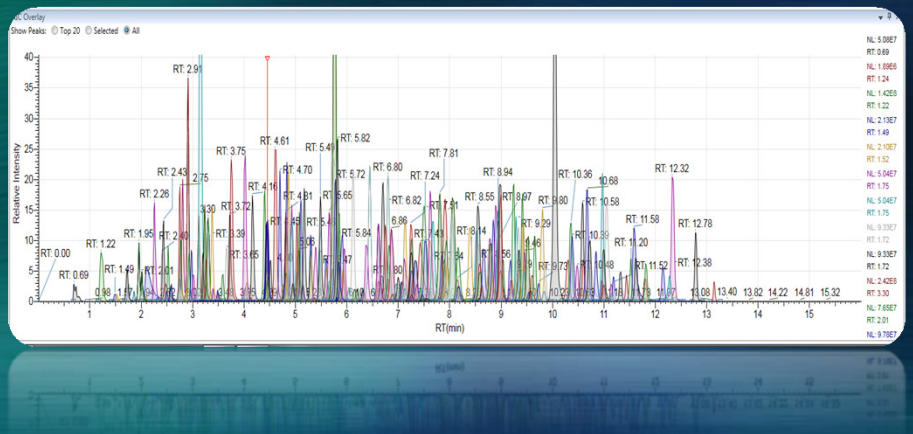
1. Ion source – HESI
2. Focussing lenses
3. Quadrupole - Parallel Reaction Monitoring
4. C-Trap
5. HCD Cell – Secondary ionisation
   - All ions fragmentation – AIF
   - Data dependent analysis – ddMS2
   - Variable data independent analysis vDIA
6. Orbitrap mass analyser





LC Method

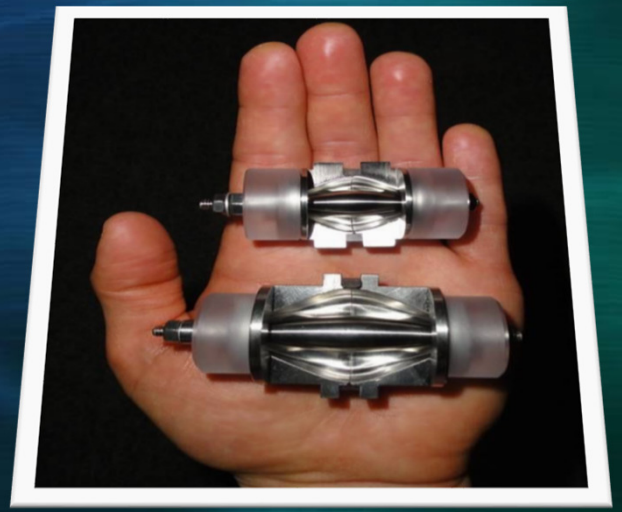




- Column = Accucore Vanquish C18+
- 100 x 2.1mm
- Particle size = 1.5µm
- Mobile phase – 5mmol Ammonium Formate : Methanol
- Injection volume = 1.0µl

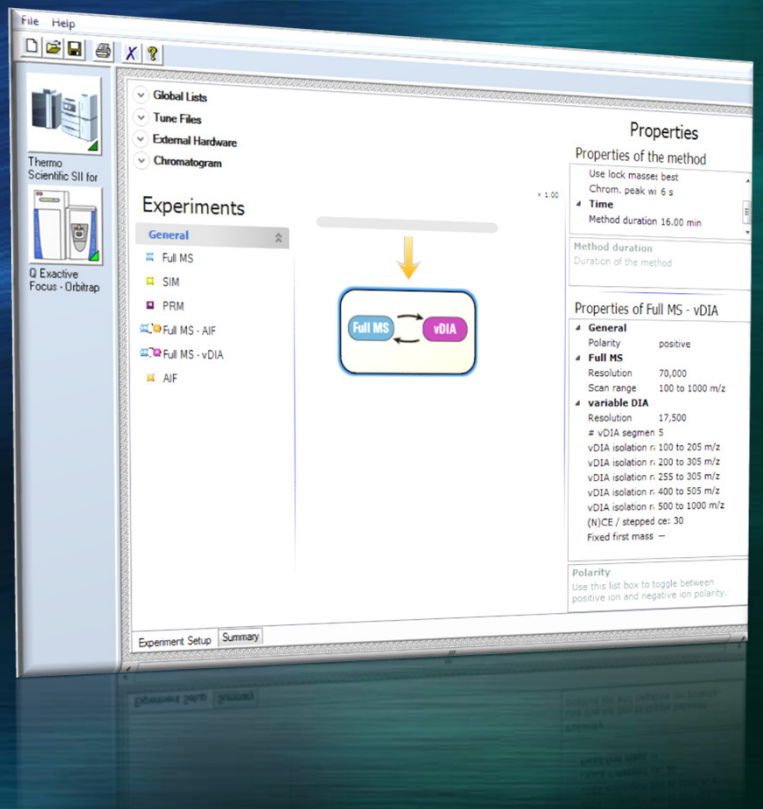
Quantitation

- In all cases the initial experiment is a full scan experiment across the full mass range covered by the method.
- From this experiment a target ion is chosen and this is used to construct a calibration curve
- This target ion is also used to calculate the theoretical isotope ratio's, when this function is enabled



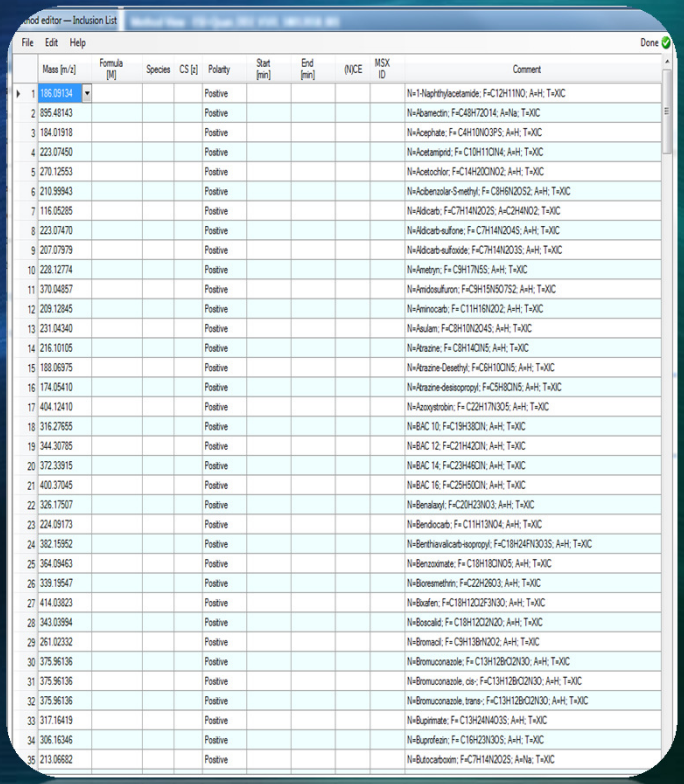
Confirmation options – MS2

1. All ions fragmentation – AIF
2. Data dependent MS^2 analysis – $ddMS^2$
3. Variable data independent analysis – vDIA
4. $ddMS^2$ / AIF



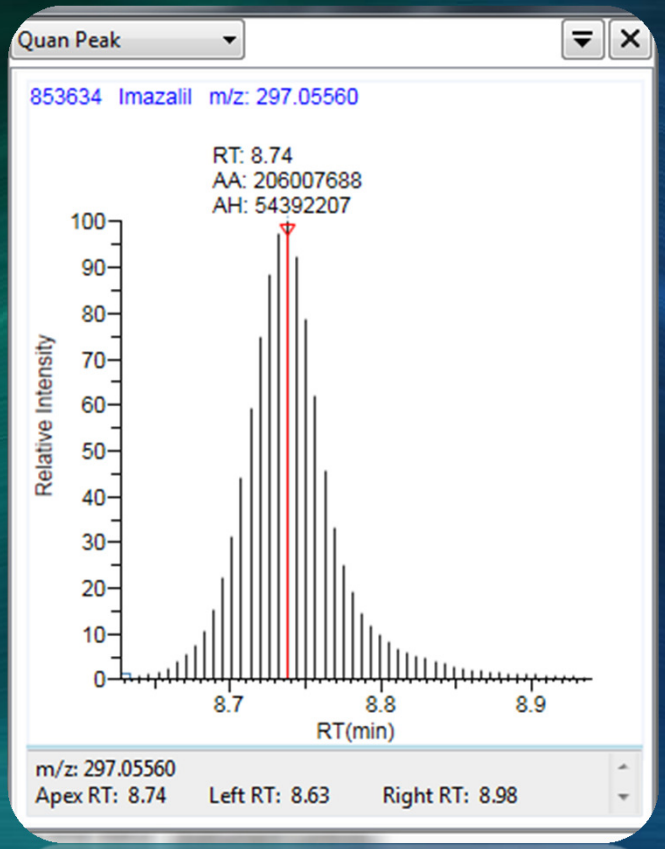
Data dependent analysis – ddMS2

1. **Requires an inclusion list**
2. **Once a compound from the inclusion list is found this is fragmented in a secondary experiment – one scan**
3. **MS^2 fragments are used for confirmation of presence**
4. **Efficient use of cycle time**
5. Secondary fragmentation doesn't always fire on the apex of the peak and sometimes doesn't fire at all



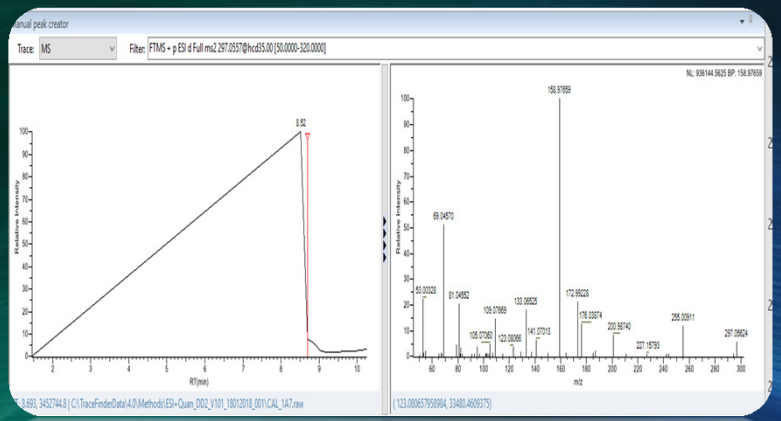
	Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID	Comment
1	186.09134				Positive					N=1-Naphthylacetamide; F=C12H11NO; A=H; T=XIC
2	895.48143				Positive					N=Abamectin; F=C48H72O14; A=Na; T=XIC
3	184.01918				Positive					N=Acephate; F=C4H10NO3PS; A=H; T=XIC
4	223.07450				Positive					N=Acetamiprid; F=C10H11ClN4; A=H; T=XIC
5	270.12553				Positive					N=Acetochlor; F=C14H20ClNO2; A=H; T=XIC
6	210.99943				Positive					N=Aclbenzolar-S-methyl; F=C8H6N2OS2; A=H; T=XIC
7	116.05285				Positive					N=Aldicarb; F=C7H14N2O2S; A=C2H4NO2; T=XIC
8	223.07470				Positive					N=Aldicarb-sulfone; F=C7H14N2O4S; A=H; T=XIC
9	207.07979				Positive					N=Aldicarb-sulfoxide; F=C7H14N2O3S; A=H; T=XIC
10	228.12774				Positive					N=Ametryn; F=C9H17N5S; A=H; T=XIC
11	370.04957				Positive					N=Amidosulfuron; F=C9H15N5O7S2; A=H; T=XIC
12	209.12845				Positive					N=Aminocarb; F=C11H16N2O2; A=H; T=XIC
13	231.04340				Positive					N=Asulam; F=C8H10N2O4S; A=H; T=XIC
14	216.10105				Positive					N=Atrazine; F=C8H14ClN5; A=H; T=XIC
15	188.06975				Positive					N=Atrazine-Desethyl; F=C6H10ClN5; A=H; T=XIC
16	174.05410				Positive					N=Atrazine-desisopropyl; F=C5H8ClN5; A=H; T=XIC
17	404.12410				Positive					N=Azoxystrobin; F=C22H17N3O5; A=H; T=XIC
18	316.27655				Positive					N=BAC 10; F=C19H38ClN; A=H; T=XIC
19	344.30785				Positive					N=BAC 12; F=C21H42ClN; A=H; T=XIC
20	372.33915				Positive					N=BAC 14; F=C23H46ClN; A=H; T=XIC
21	400.37045				Positive					N=BAC 16; F=C25H50ClN; A=H; T=XIC
22	326.17507				Positive					N=Benalaxyl; F=C20H23NO3; A=H; T=XIC
23	224.09173				Positive					N=Bendiocarb; F=C11H13NO4; A=H; T=XIC
24	382.15952				Positive					N=Benthiavalicarb-isopropyl; F=C18H24FN3O3S; A=H; T=XIC
25	364.09463				Positive					N=Benzoximate; F=C18H18ClNO5; A=H; T=XIC
26	339.19547				Positive					N=Bioresmethrin; F=C22H26O3; A=H; T=XIC
27	414.03823				Positive					N=Bixafen; F=C18H12Cl2F3N3O; A=H; T=XIC
28	343.03994				Positive					N=Boscalid; F=C18H12Cl2N2O; A=H; T=XIC
29	261.02332				Positive					N=Bromacil; F=C9H13BrN2O2; A=H; T=XIC
30	375.96136				Positive					N=Bromuconazole; F=C13H12BrCl2N3O; A=H; T=XIC
31	375.96136				Positive					N=Bromuconazole, cis-; F=C13H12BrCl2N3O; A=H; T=XIC
32	375.96136				Positive					N=Bromuconazole, trans-; F=C13H12BrCl2N3O; A=H; T=XIC
33	317.16419				Positive					N=Bupirimate; F=C13H24N4O3S; A=H; T=XIC
34	306.16346				Positive					N=Buprofezin; F=C16H23N3OS; A=H; T=XIC
35	213.06682				Positive					N=Butocarboxim; F=C7H14N2O2S; A=Na; T=XIC

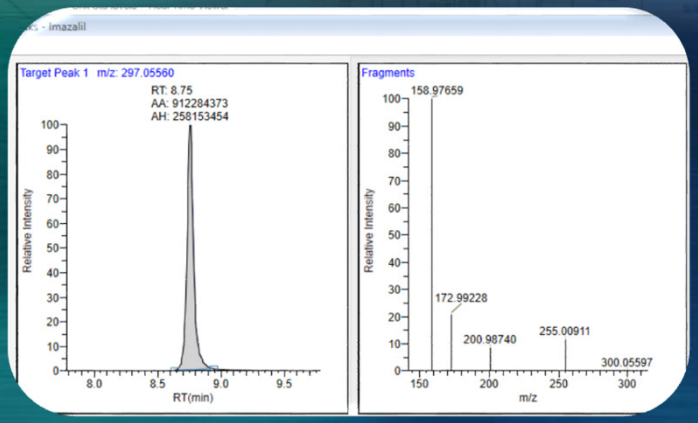
Data dependent analysis – ddMS2



- The total cycle time or scan rate will now be dependent on the two experiments carried out
- Imazalil elutes at 8.74 minutes and therefore gives the average number of points across the peak for this method
- In this case approximately 30 points are being collected across the peak which gives a cycle time of ~ **0.35secs**

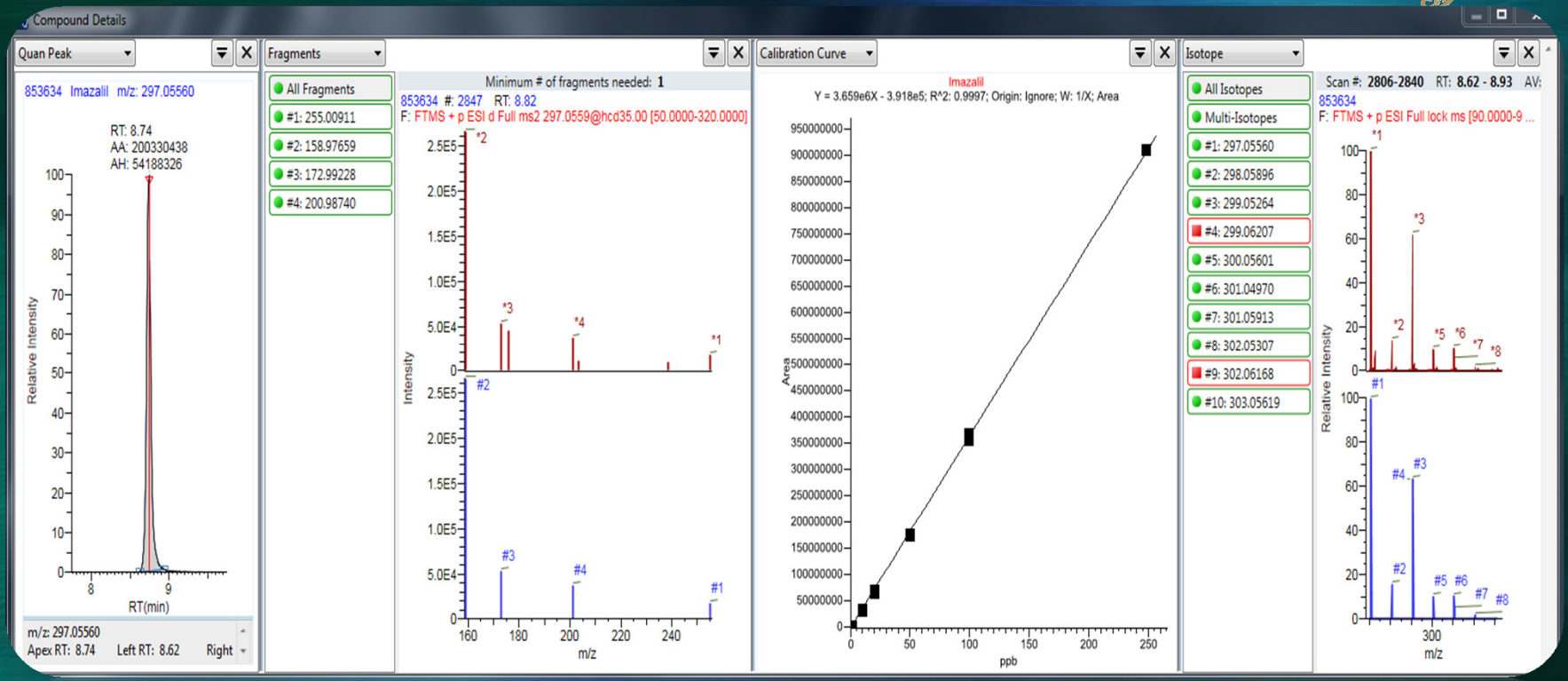
Data dependent analysis – ddMS2





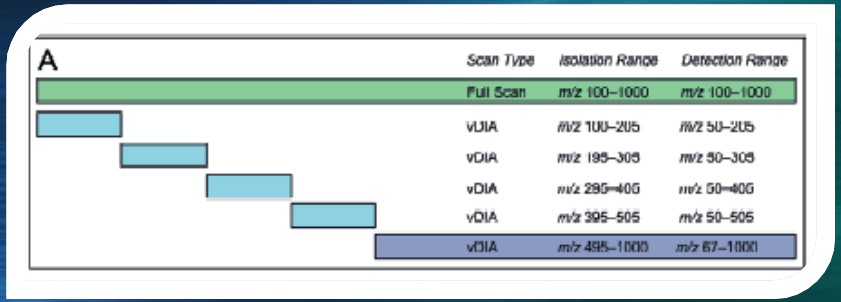
- When a mass from the inclusion list is found one MS^2 scan is carried out
- Because only one scan is carried out across the peak centroid data is collected

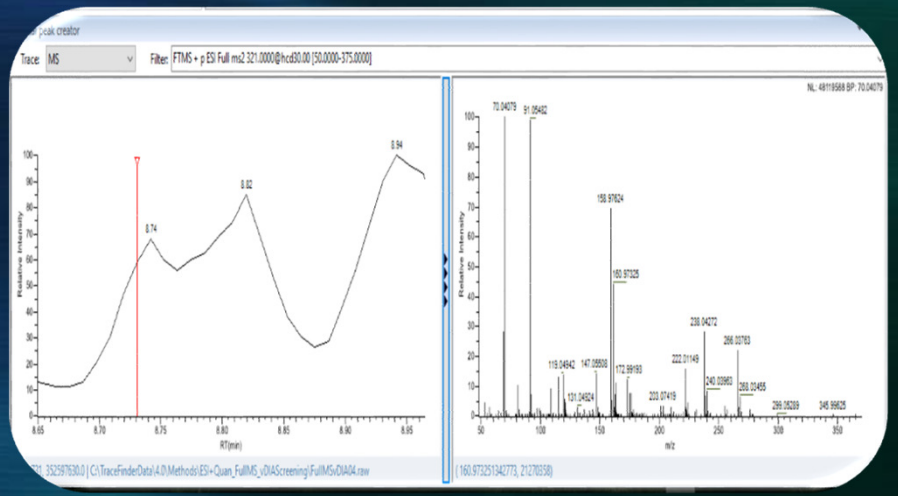
Data dependent analysis – ddMS2



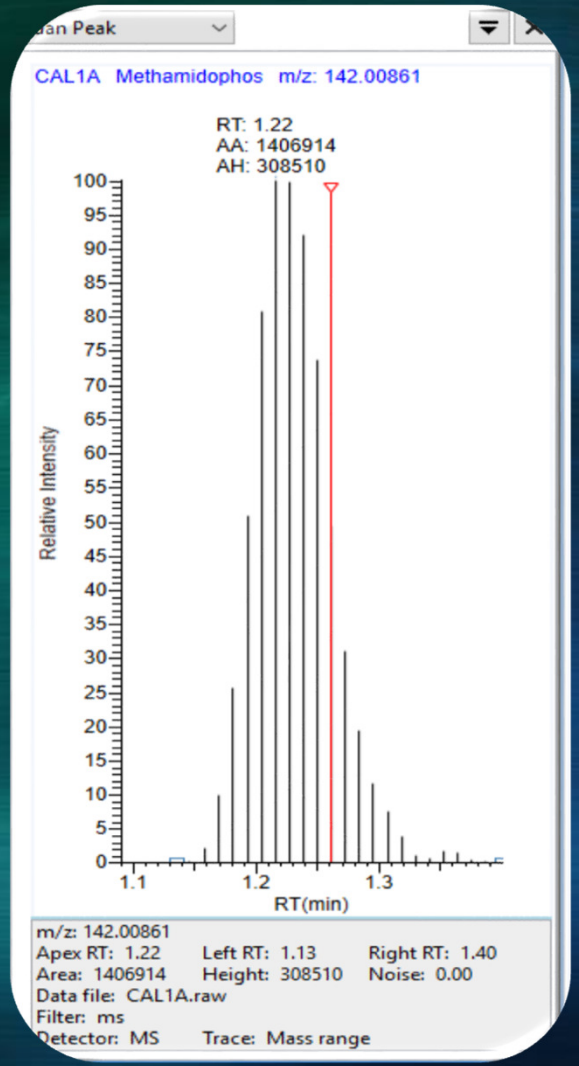
Data independent analysis - vDIA

1. The mass window is broken up into a number of mass ranges
2. Secondary fragmentation is carried out on each range independently
3. MS^2 fragments used for confirmation
4. Reduces the risk of common fragments causing interference
5. Heavy on cycle time – **0.8sec**





Peak definition



Methamidaphos elutes at a retention time of 1.2min

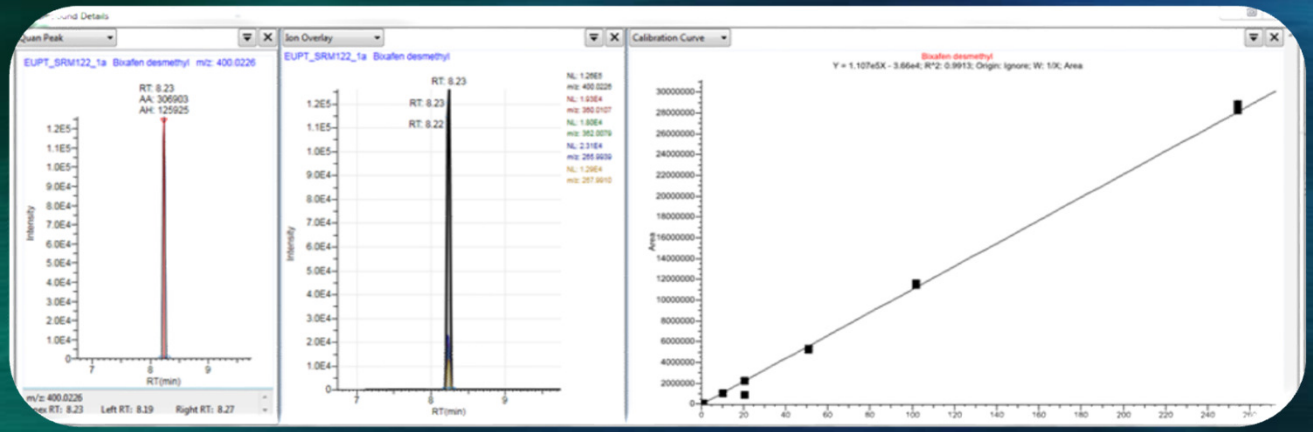
The peak width is approximately 9secs

The number of points collected across the peak is approximately 15

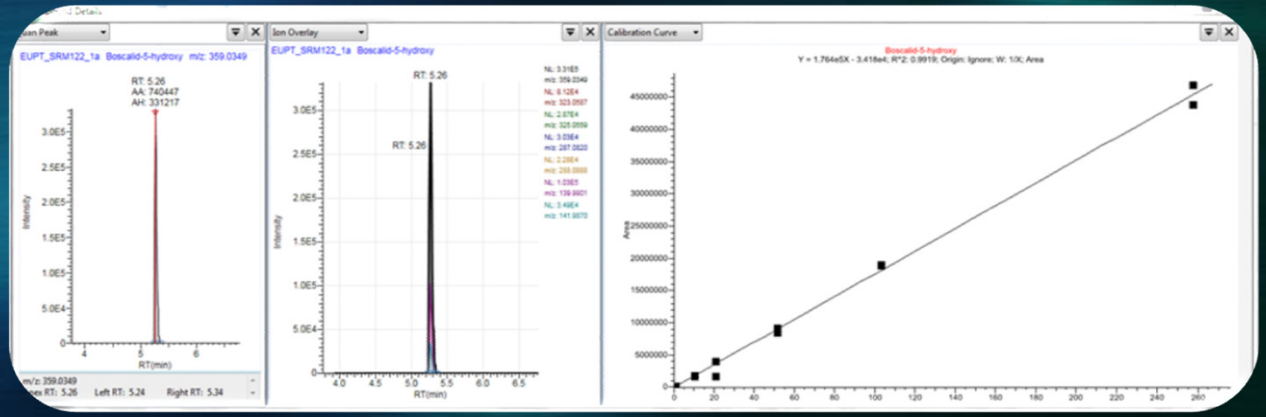
This is the worst case scenario as the peak width will increase with increasing retention time

SRM 14 – Bovine liver – Ad hoc method

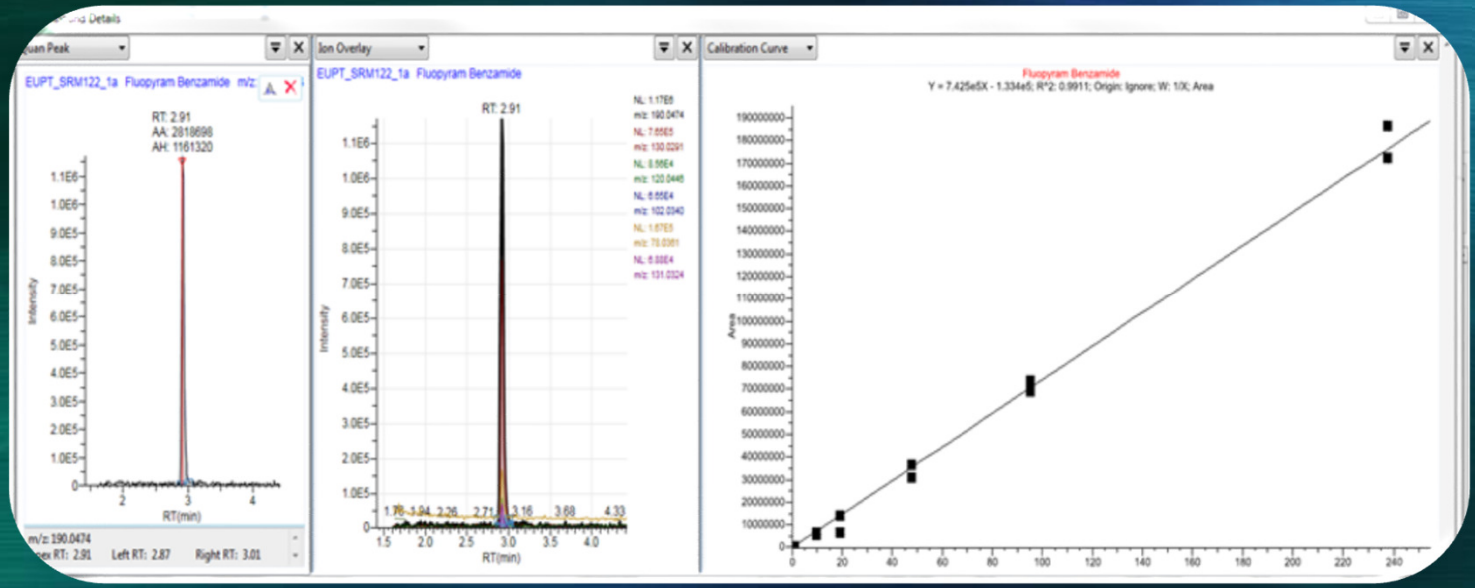
Bixafen-desmethyl in Liver– 56 ppb



Boscalid-5-hydroxy in Liver – 90 ppb

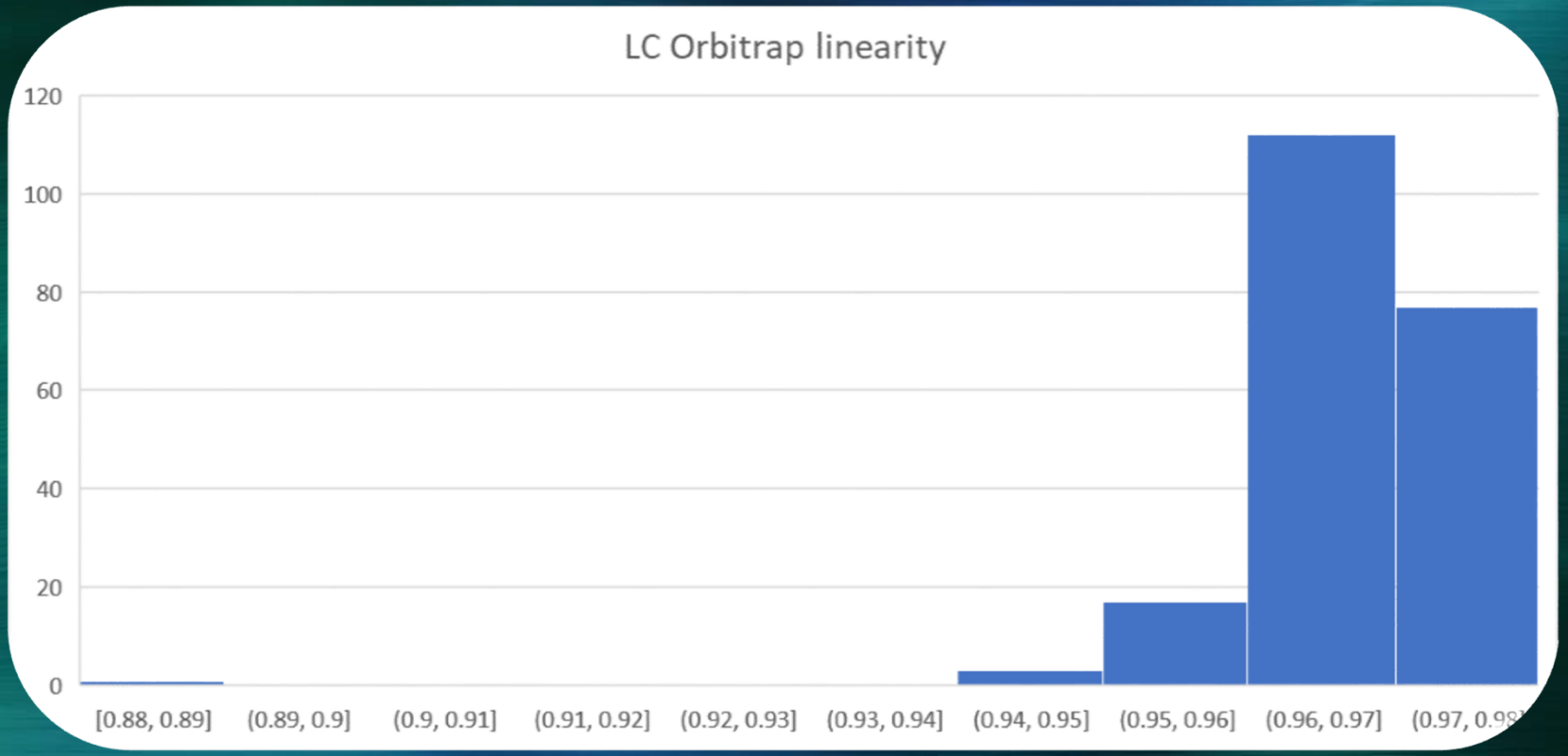


Fluopyram benzamide in Liver– 83.5 ppb



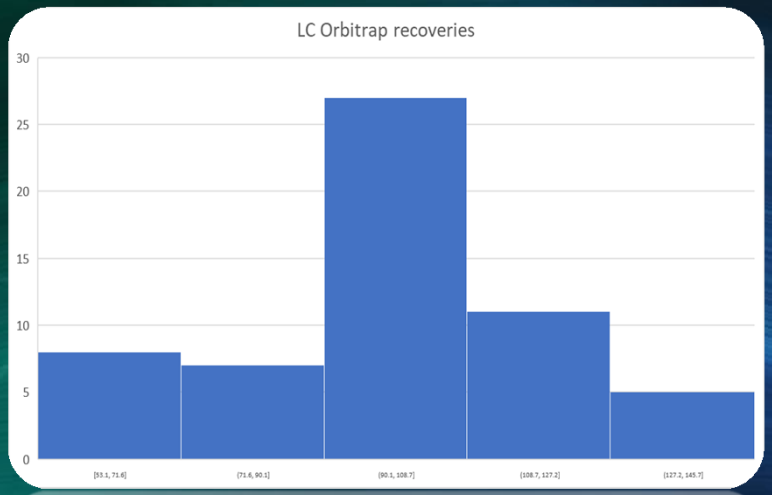
SRM 14	Reported	Assigned value	Z-score
Bixafen desmethyl	56.0	50	0.5
Boscalid-5-ydroxy	90.2	81	0.5
Fluopyram benzamide	83.5	101	-1.0

Linearity 0.5 – 250ppb



0.95 - 1

LC Orbitrap recoveries

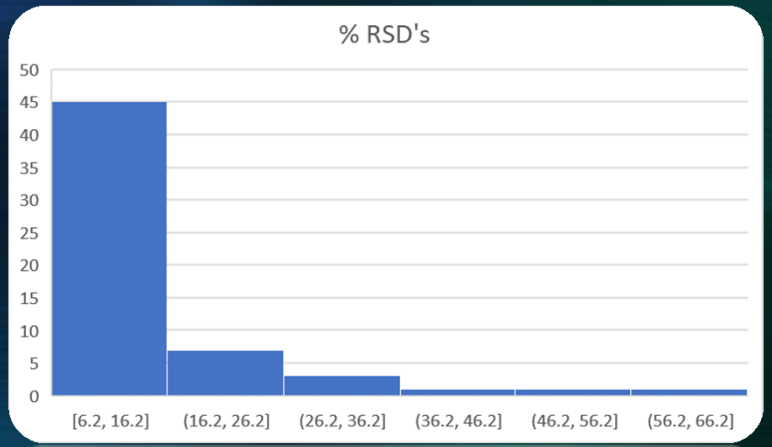


60 – 140%

Average recovery for fruit and veg matrices

In most cases the recoveries are within the AQC criteria

% RSD's



0 – 25%

% RSD's are less than 25% in most cases

Screening PT EUPT SM11

Designed to test the laboratory's screening capability

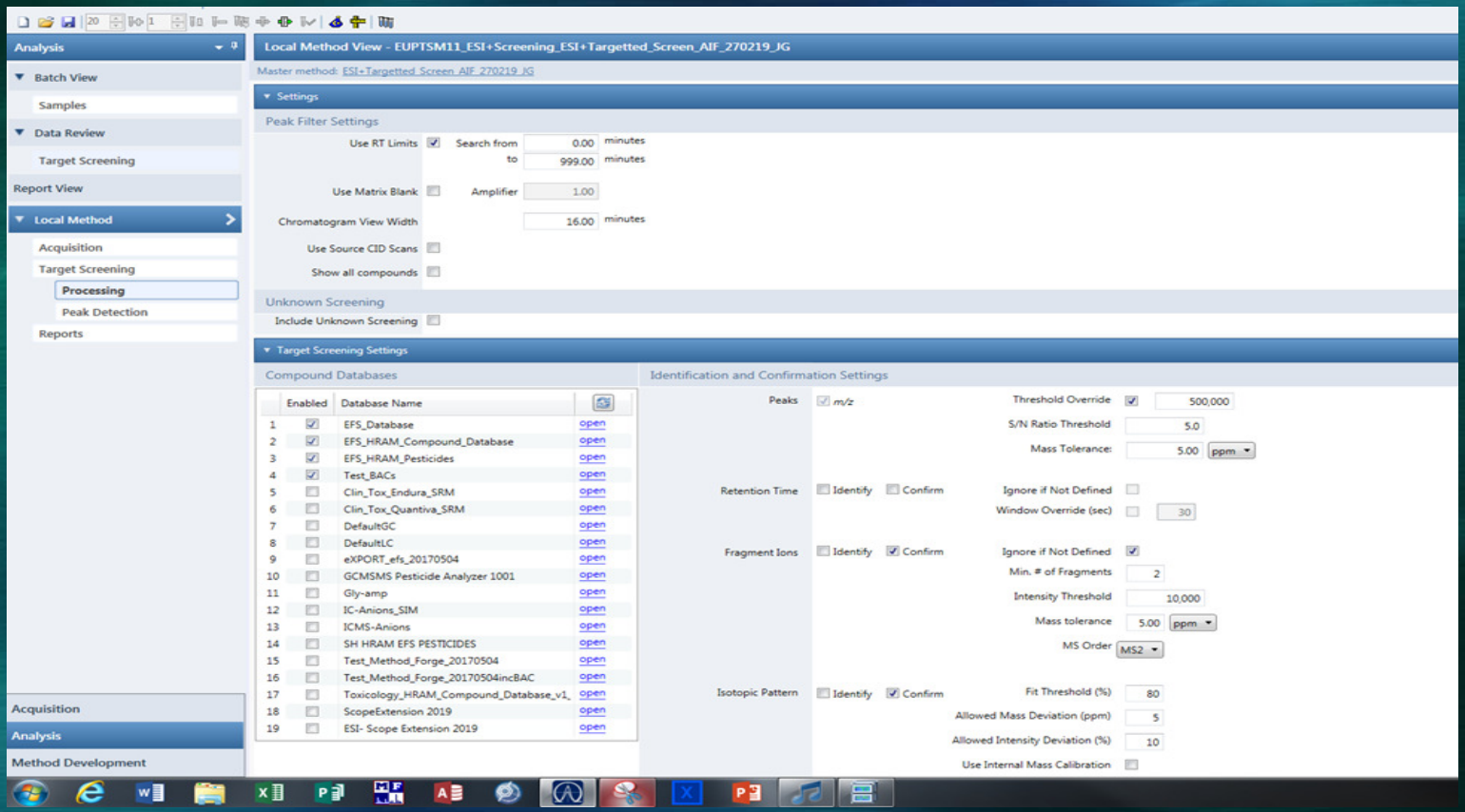
Sample has to be analysed and results reported within 72 hours of receipt of sample

No target list – anything could be present !!

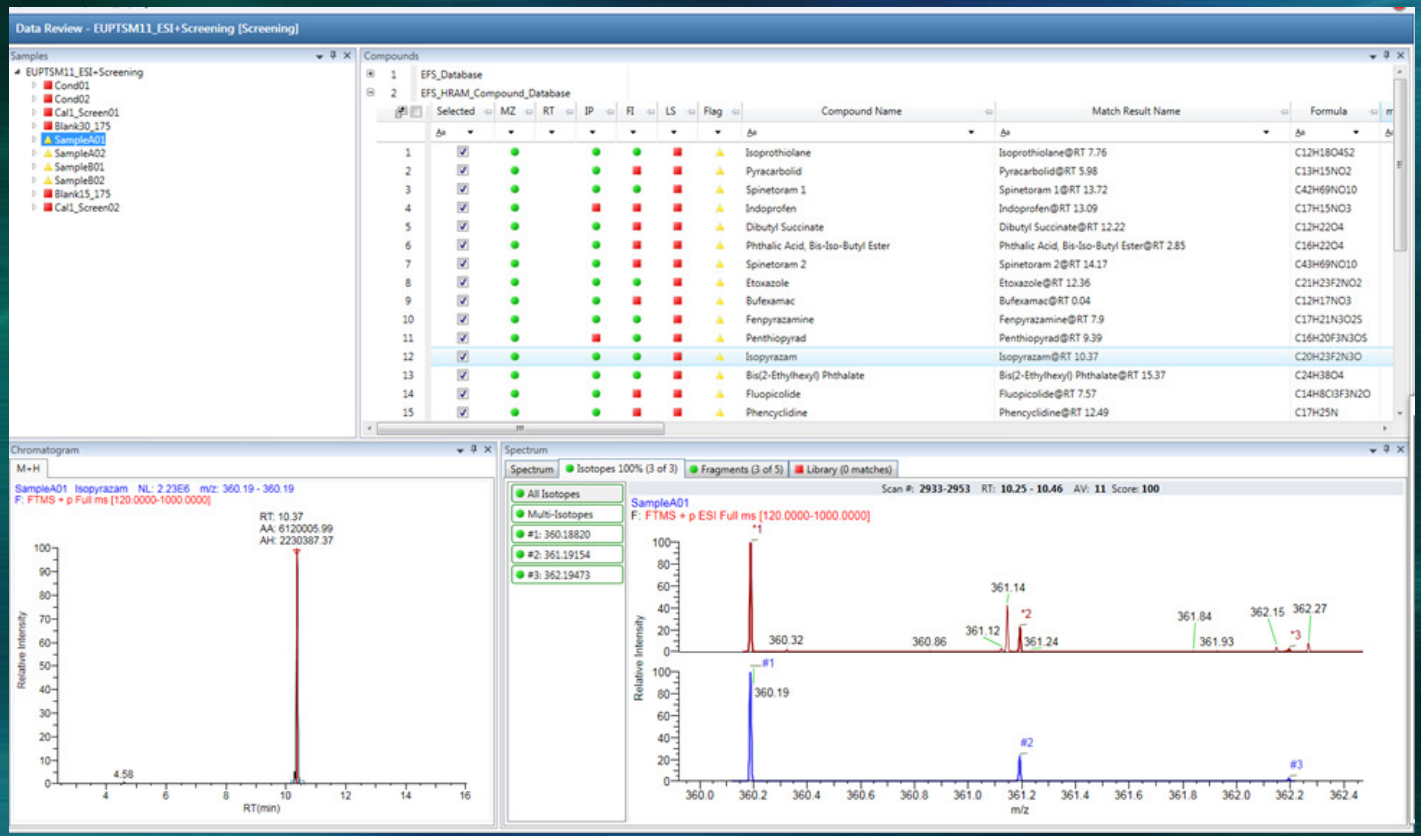
Semi-quantitative

Sample analysed using a targeted method on GC and LC-Orbitrap

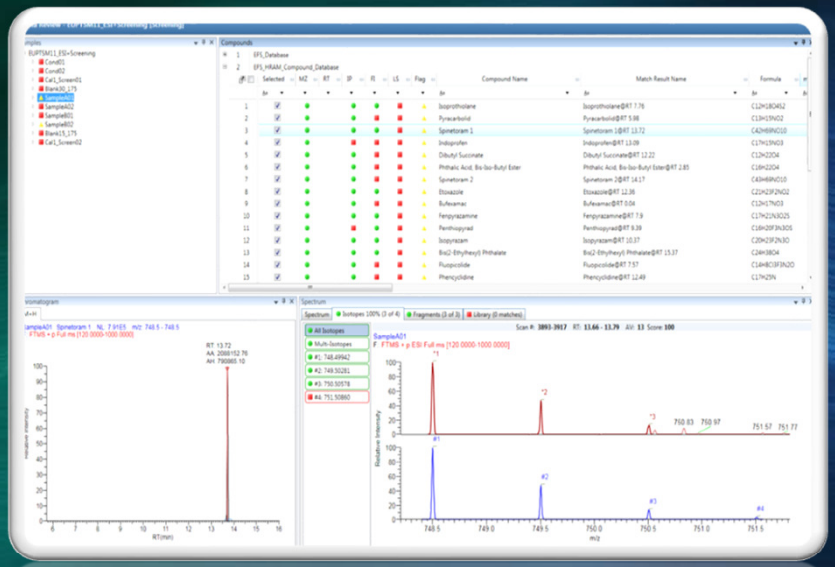
Targeted screening



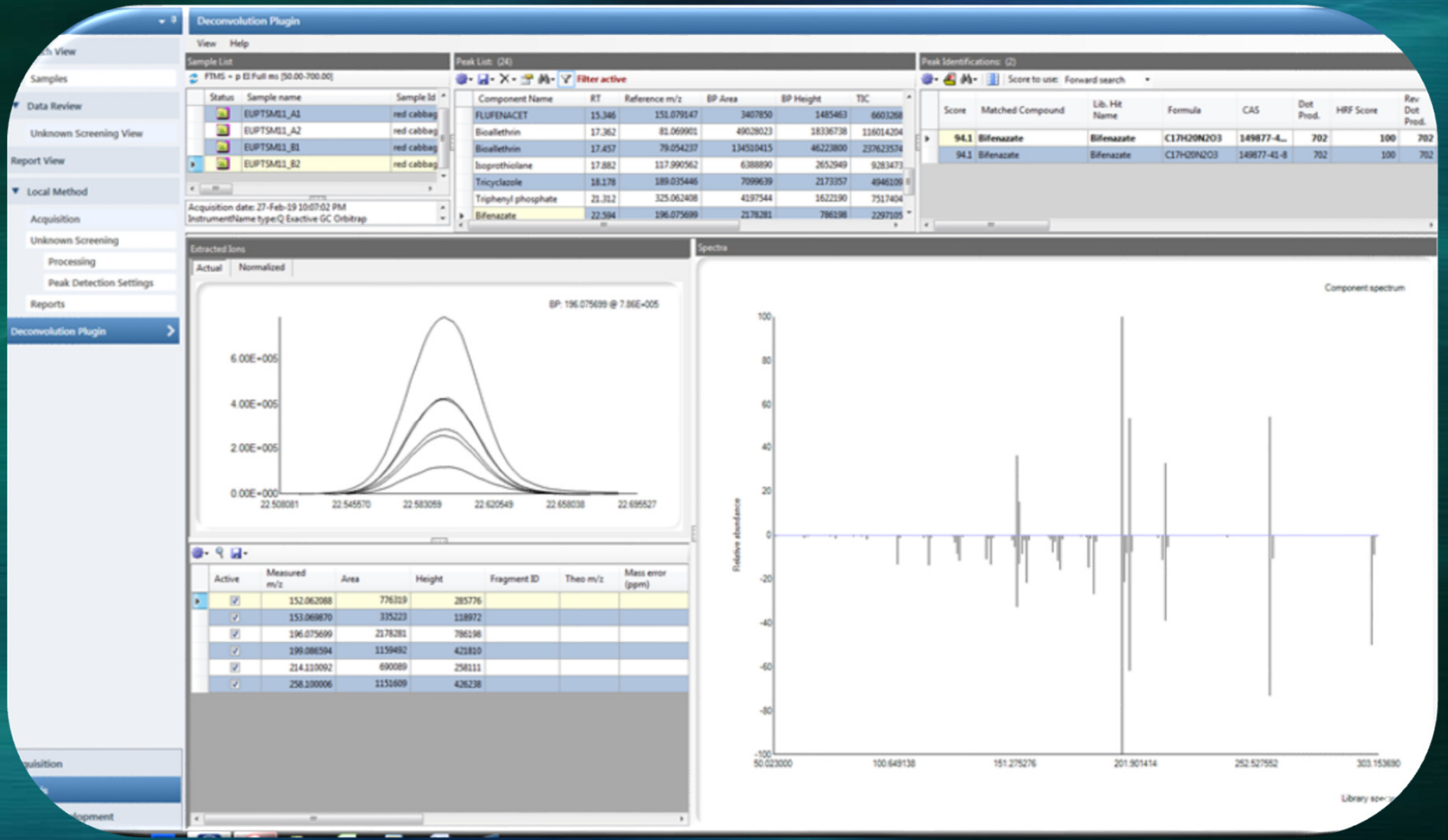
Isopyrazam


Data Review - EUPTSM11_ESI+Screening [Screening]
Samples
EUPTSM11_ESI+Screening
Cond01
Cond02
Cal1_Screen01
Blank30_175
SampleA01
SampleA02
SampleB01
SampleB02
Blank15_175
Cal1_Screen02
Compounds
1 EFS_Database
2 EFS_HRAM_Compound_Database
Selected MZ RT IP FI LS Flag Compound Name Match Result Name Formula
1 Isoprothiolane Isoprothiolane@RT 7.76 C12H18O4S2
2 Pyracarbolid Pyracarbolid@RT 5.98 C13H15NO2
3 Spinetoram 1 Spinetoram 1@RT 13.72 C42H69NO10
4 Indoprofen Indoprofen@RT 13.09 C17H15NO3
5 Dibutyl Succinate Dibutyl Succinate@RT 12.22 C12H22O4
6 Phthalic Acid, Bis-Iso-Butyl Ester Phthalic Acid, Bis-Iso-Butyl Ester@RT 2.85 C16H22O4
7 Spinetoram 2 Spinetoram 2@RT 14.17 C43H69NO10
8 Etoxazole Etoxazole@RT 12.36 C21H23F2NO2
9 Bufexamac Bufexamac@RT 0.04 C12H17NO3
10 Fenpyrazamine Fenpyrazamine@RT 7.9 C17H21N3O2S
11 Penthiopyrad Penthiopyrad@RT 9.39 C16H20F3N3OS
12 Isopyrazam Isopyrazam@RT 10.37 C20H23F2N3O
13 Bis(2-Ethylhexyl) Phthalate Bis(2-Ethylhexyl) Phthalate@RT 15.37 C24H38O4
14 Fluopicolide Fluopicolide@RT 7.57 C14H8Cl3F3N2O
15 Phencyclidine Phencyclidine@RT 12.49 C17H25N
Chromatogram
M+H
SampleA01 Isopyrazam NL: 2.23E6 m/z: 360.19 - 360.19
F: FTMS + p Full ms [120.0000-1000.0000]
RT: 10.37
AA: 6120005.99
AH: 2230387.37
4.58
Relative Intensity
RT(min)
Spectrum
Spectrum Isotopes 100% (3 of 3) Fragments (3 of 5) Library (0 matches)
All Isotopes
Multi-Isotopes
#1: 360.18820
#2: 361.19154
#3: 362.19473
Scan #: 2933-2953 RT: 10.25 - 10.46 AV: 11 Score: 100
SampleA01
F: FTMS + p ESI Full ms [120.0000-1000.0000]
360.32
360.86
361.12
361.14
361.24
361.84
361.93
362.15
362.27
360.19
Relative Intensity
m/z

Spinetoram I and II



Bifenazate


Deconvolution Plugin
View
Help
Samples
Data Review
Unknown Screening View
Report View
Local Method
Acquisition
Unknown Screening
Processing
Peak Detection Settings
Reports
Deconvolution Plugin
Sample List
Peak List: (24)
Filter active
Peak Identifications: (2)
Component Name
RT
Reference m/z
BP Area
BP Height
TIC
FLUFENACET
Bioallethrin
Isoprothiolane
Tricyclazole
Triphenyl phosphate
Bifenazate
Score
Matched Compound
Lib. Hit Name
Formula
CAS
Dot Prod.
HRF Score
Rev Dot Prod.
Extracted Ions
Actual
Normalized
Spectra
Component spectrum
Active
Measured m/z
Area
Height
Fragment ID
Theo m/z
Mass error (ppm)

Conclusion

Compound found, not yet on PCL scope

Fenpyrazamine

Penthiopyrad

Spinetoram I and II

Metrafenone

Isopyrazam

Bifenazate

To find that needle in the haystack you just need to use the right tools


THANK
YOU!