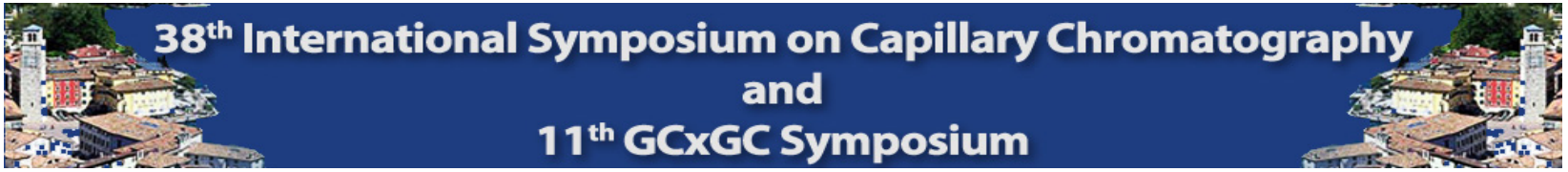


Riva del Garda 23th May 2014

APPLICATION OF NEW GC AND LC-MS APPROACHES TO OVERCOME CURRENT ANALYTICAL DIFFICULTIES IN PESTICIDE RESIDUE ANALYSIS

Amadeo R. Fernández-Alba

A NEW ANALITYCAL APPROACH……

Illustration used with permission of Arts Parts

IS

COMING!

ROUTINE LABORATORIES FOR PESTICIDE FOOD CONTROL

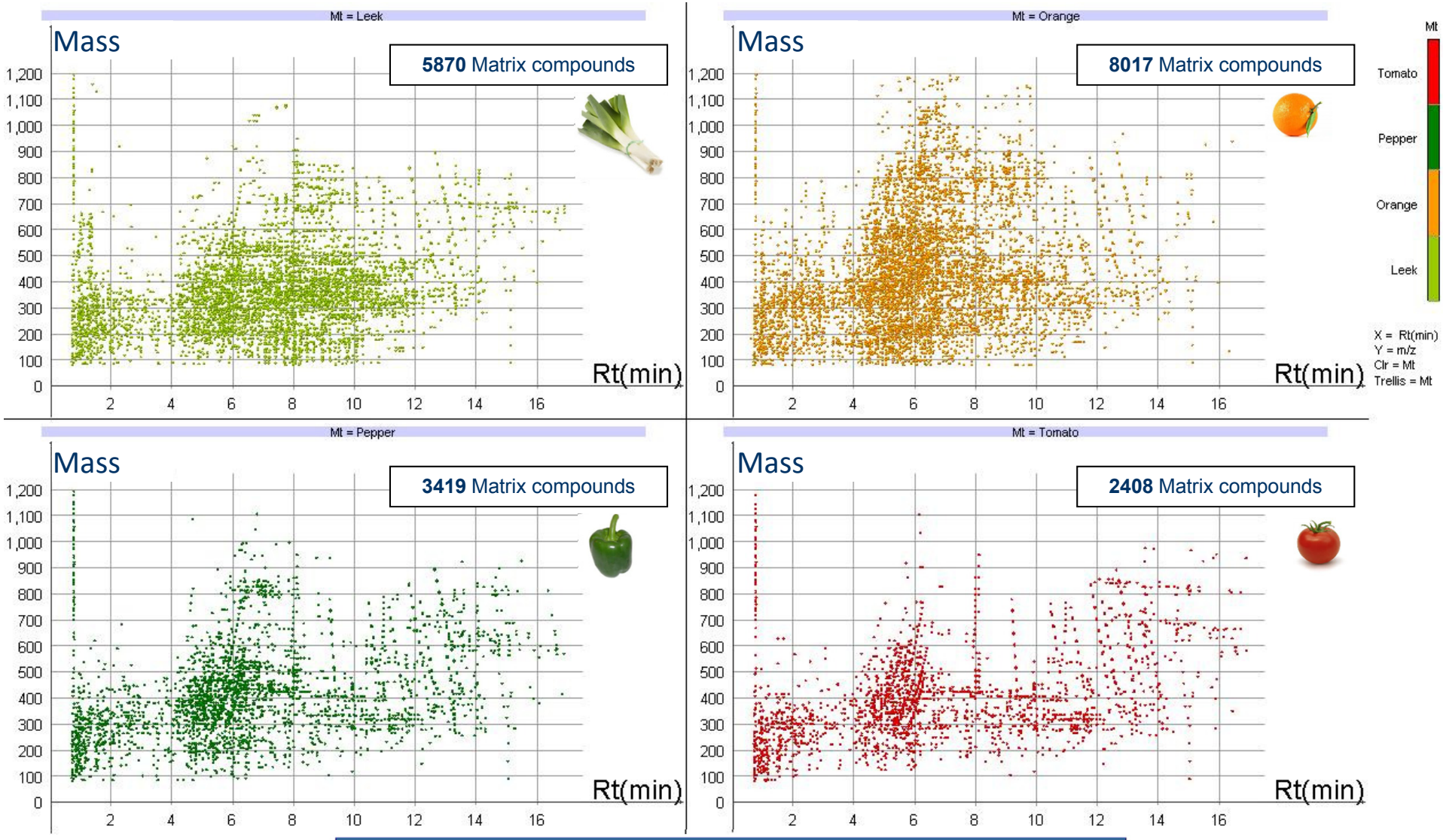
Regulated QC Procedures (17025)
Productivity (10-500 samples/day)
Robustness
Fast response (24-48 h.)

LC-MS/MS
Low Resolution

HIGH SENSITIVE LC-QqQ-MS/MS

Co-extracted matrix components Agilent LC-TOF-MS

Injected: 1g sample/mL extract

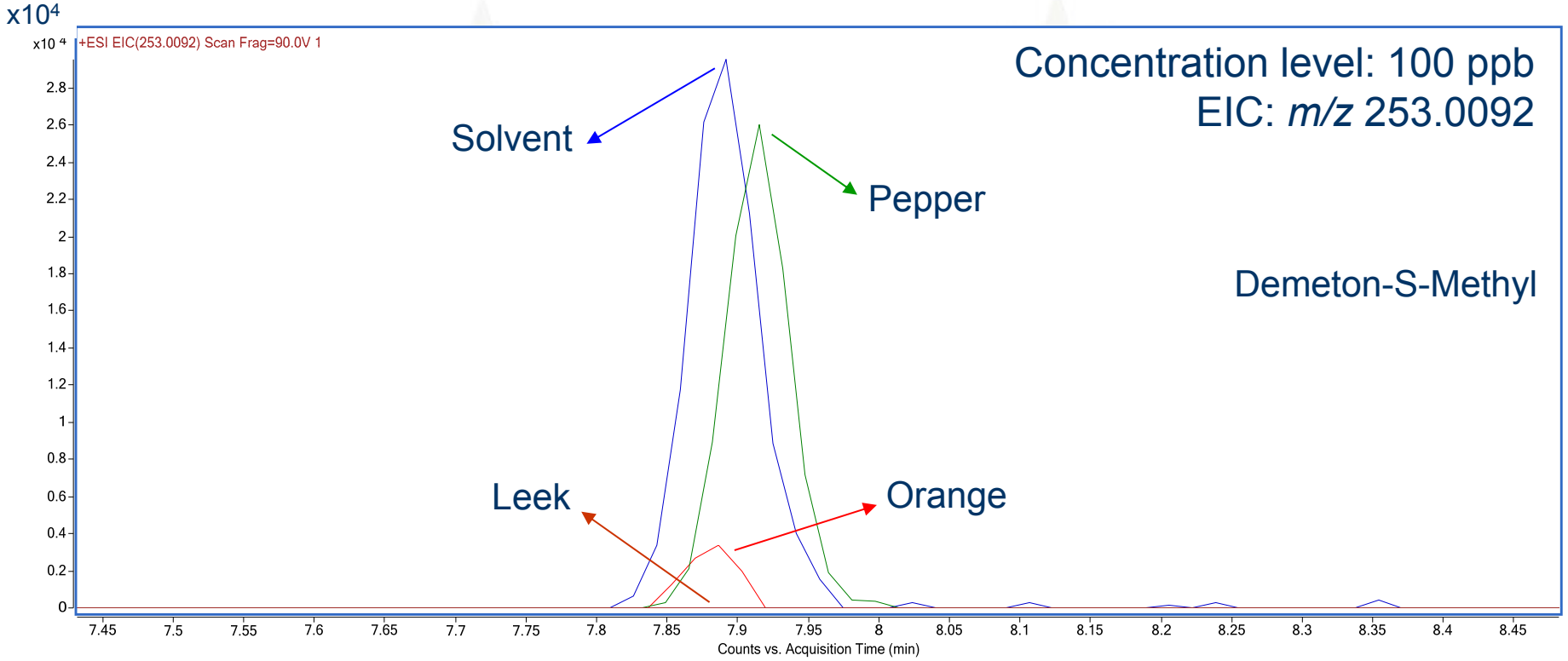


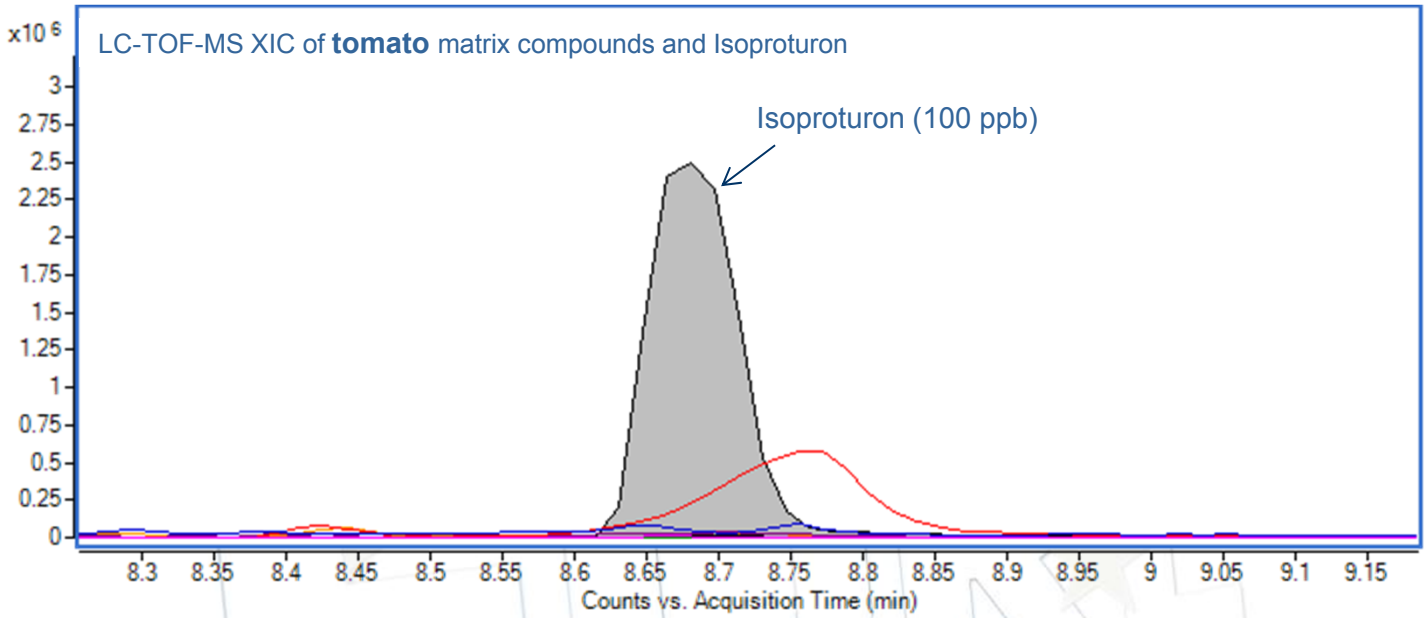
Miner 3D Enterprise

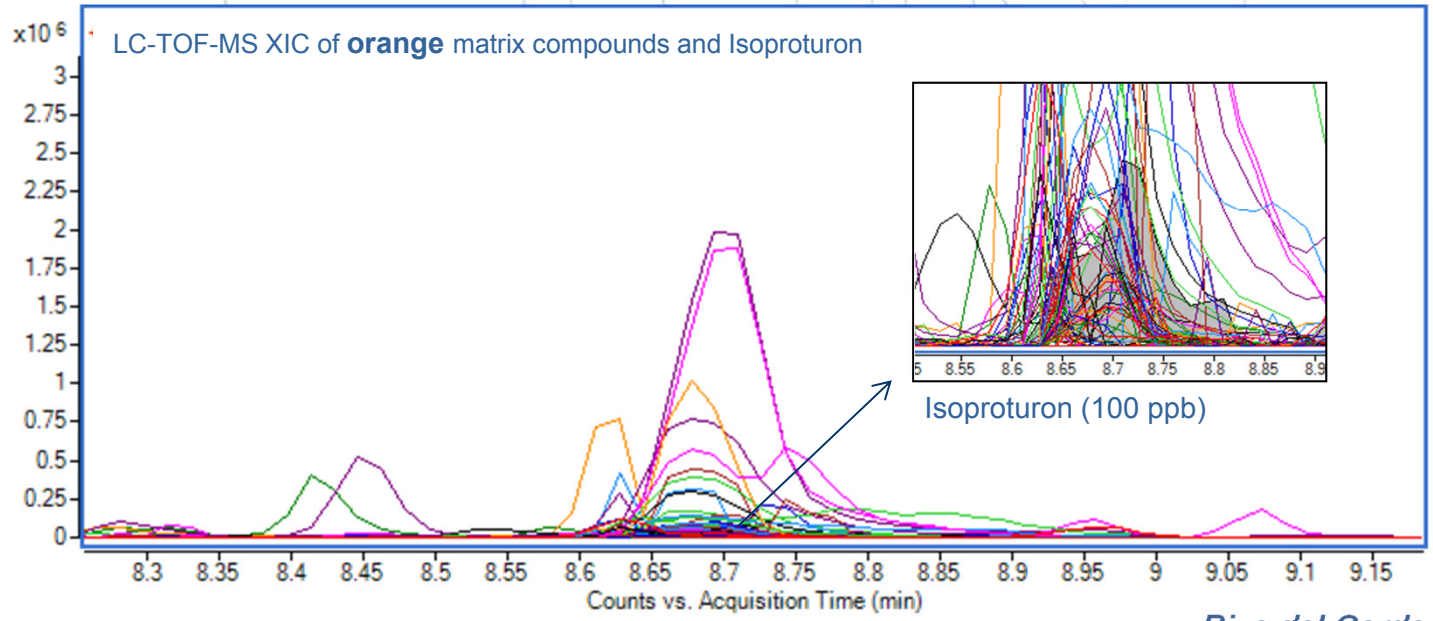
Compounds with absolute height ≥ 10000 counts

Signal supression due to matrix effects

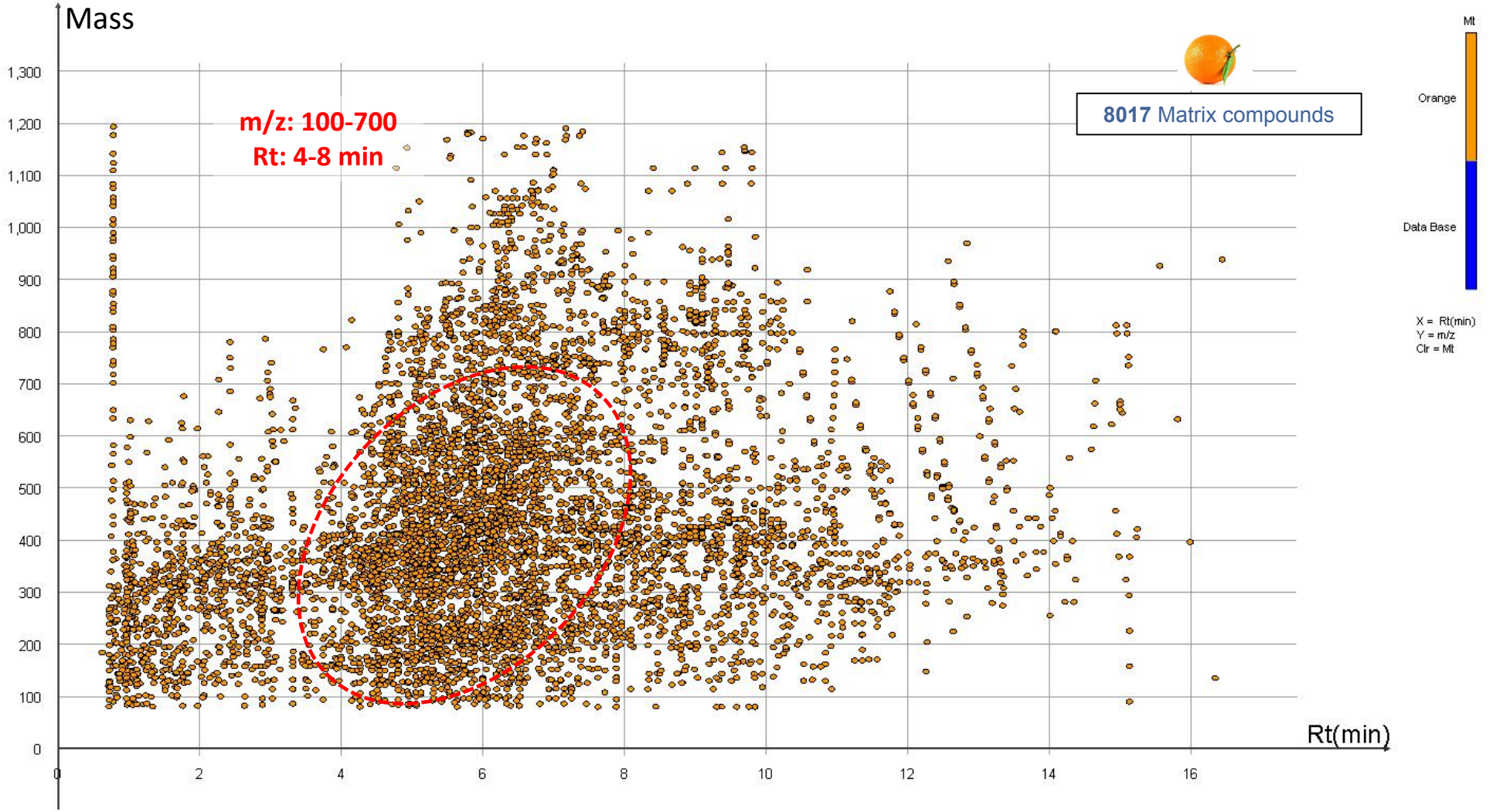
Injection 1g matrix/ml




LC-TOF-MS XIC of **tomato** matrix compounds and Isoproturon
Isoproturon (100 ppb)
x10 6
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
8.3 8.35 8.4 8.45 8.5 8.55 8.6 8.65 8.7 8.75 8.8 8.85 8.9 8.95 9 9.05 9.1 9.15
Counts vs. Acquisition Time (min)

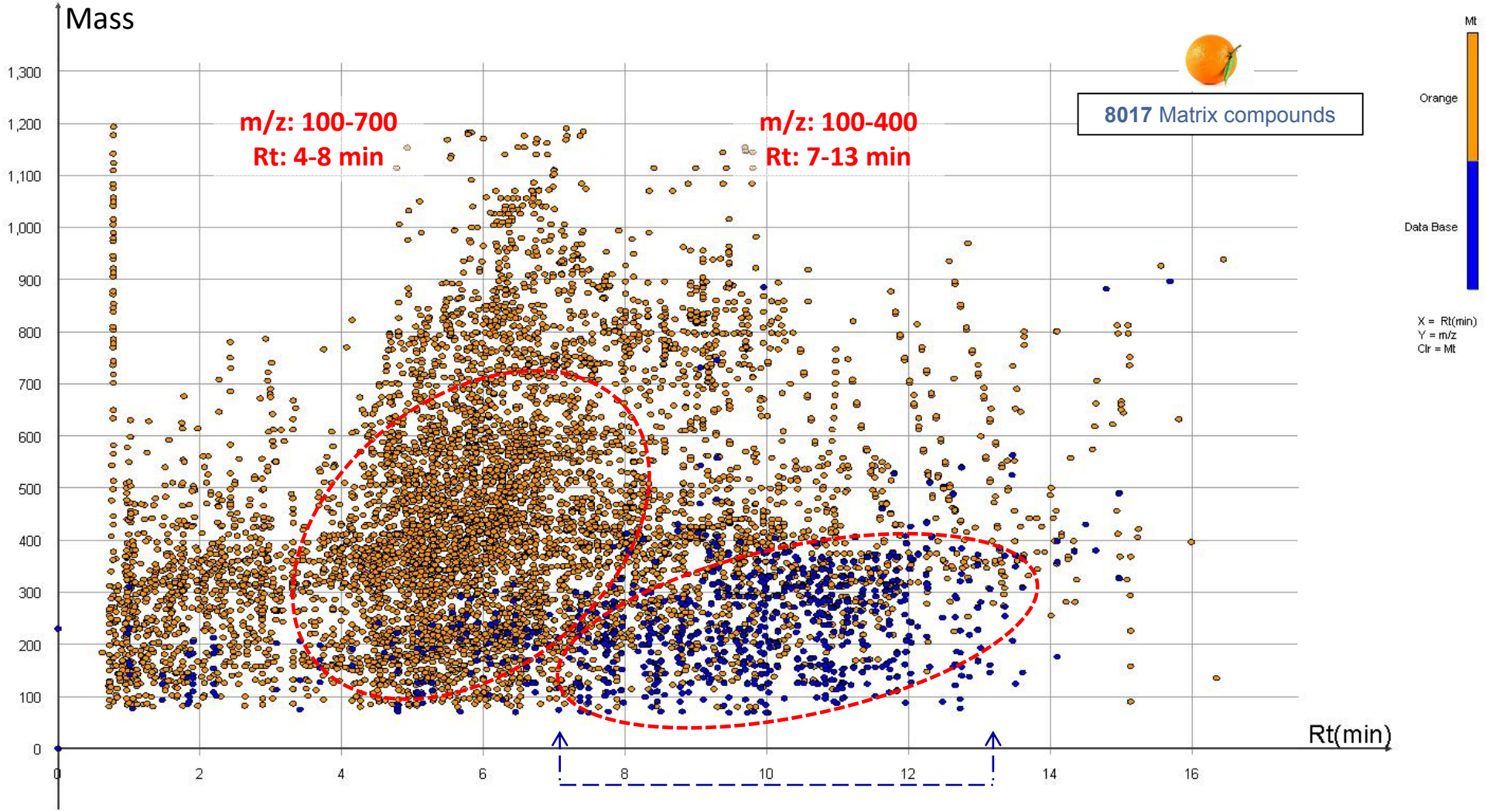

LC-TOF-MS XIC of **orange** matrix compounds and Isoproturon
x10 6
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
8.3 8.35 8.4 8.45 8.5 8.55 8.6 8.65 8.7 8.75 8.8 8.85 8.9 8.95 9 9.05 9.1 9.15
Counts vs. Acquisition Time (min)
Isoproturon (100 ppb)

Co-extracted matrix components LC-TOF-MS of orange



Miner 3D Enterprise

Data base components- **orange** matrix compounds

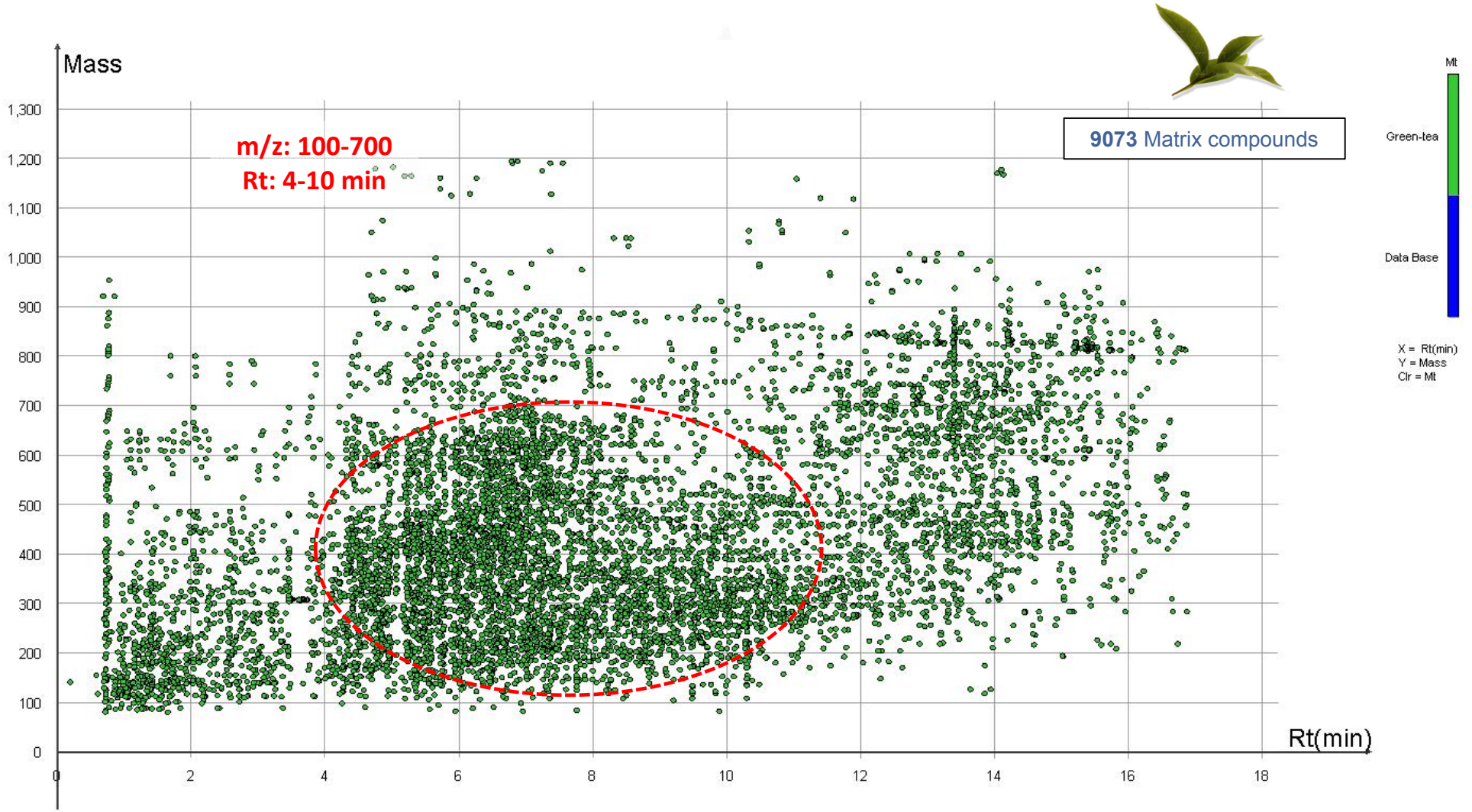


Miner 3D Enterprise

DB: 750 components

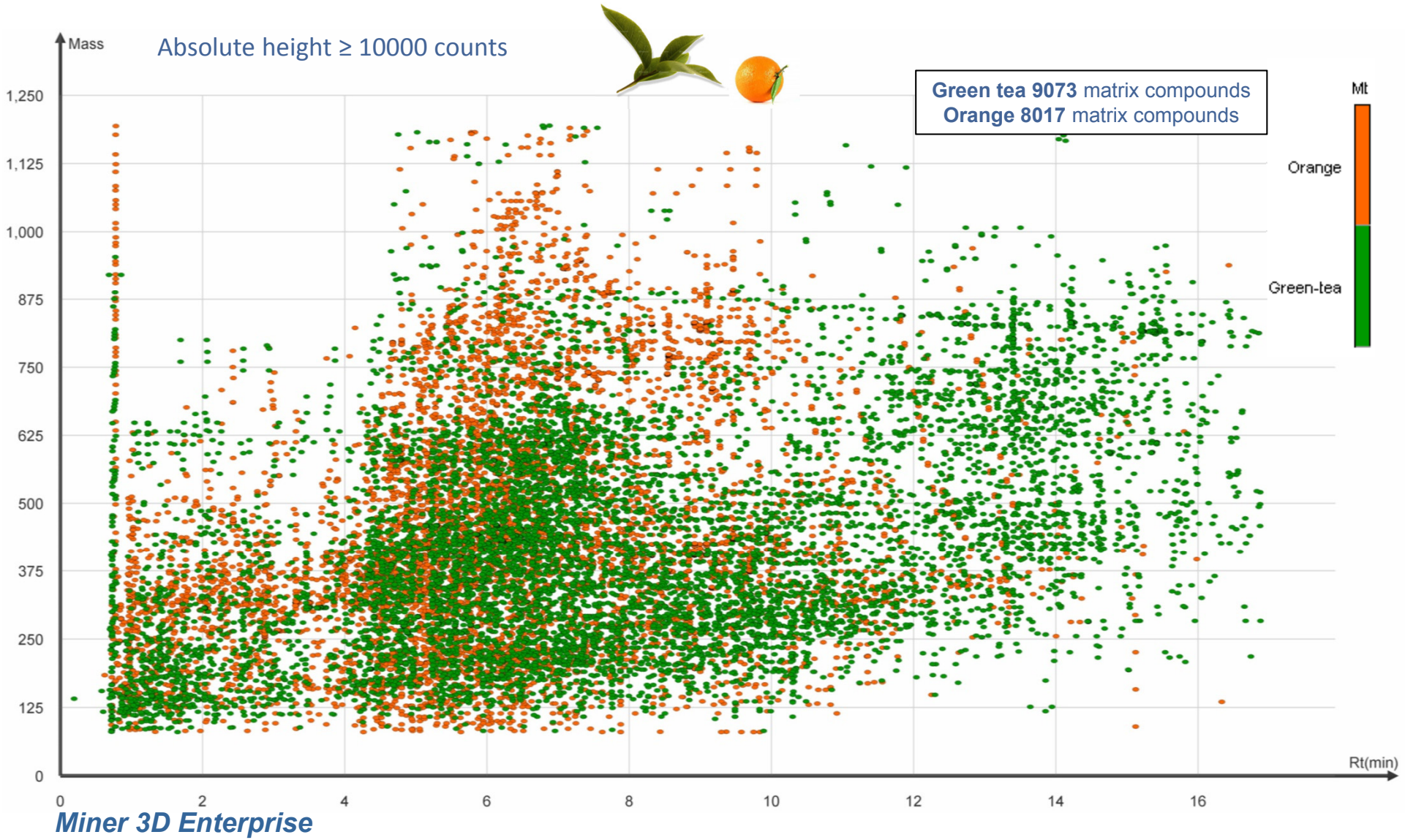
Orange: 2743 matrix compounds

Co-extracted matrix components of green-tea

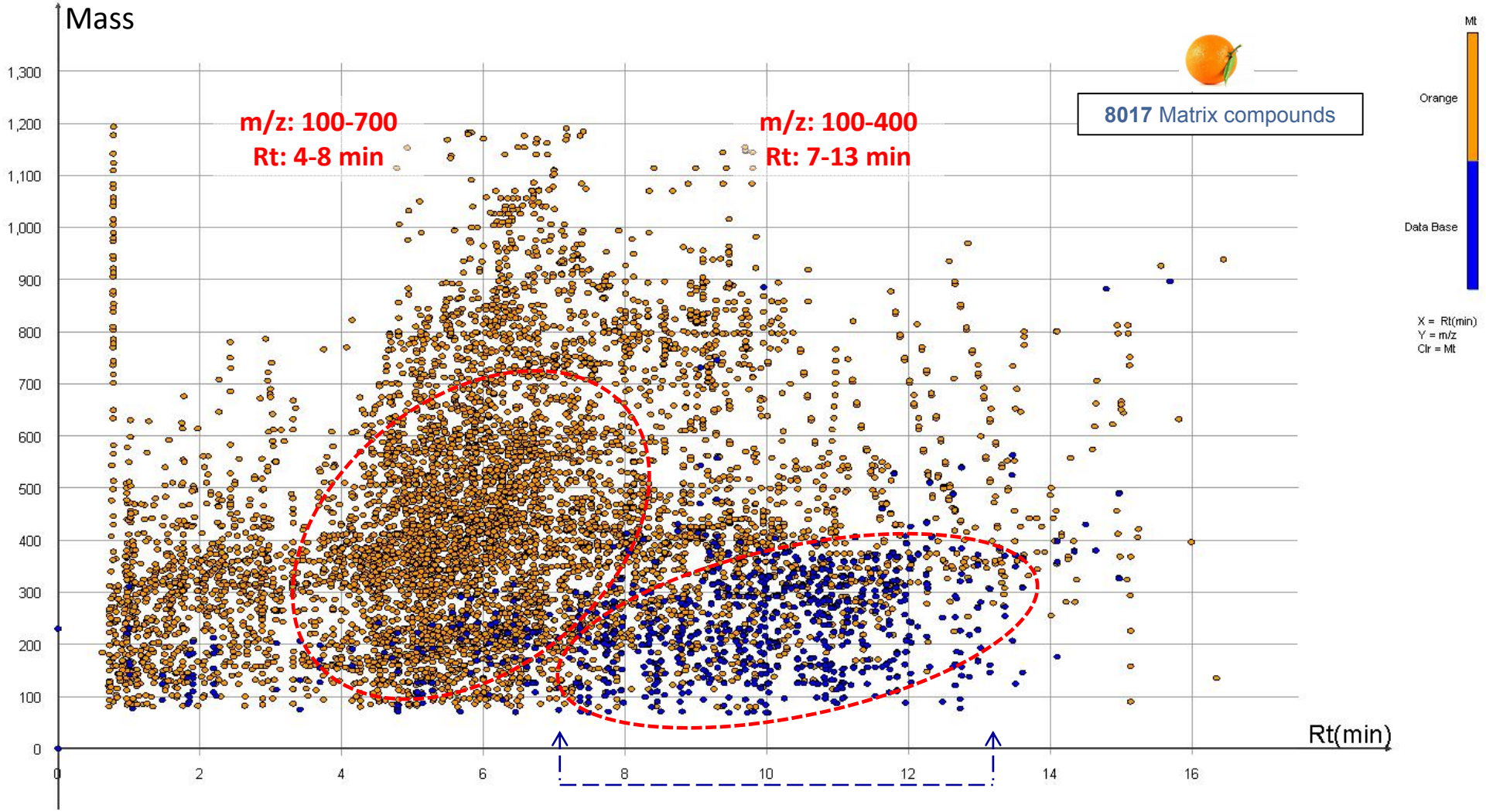


Miner 3D Enterprise

Co-extracted matrix components of green-tea and orange. LC-TOF/MS



Data base components- **orange** matrix compounds (1 g/mL)

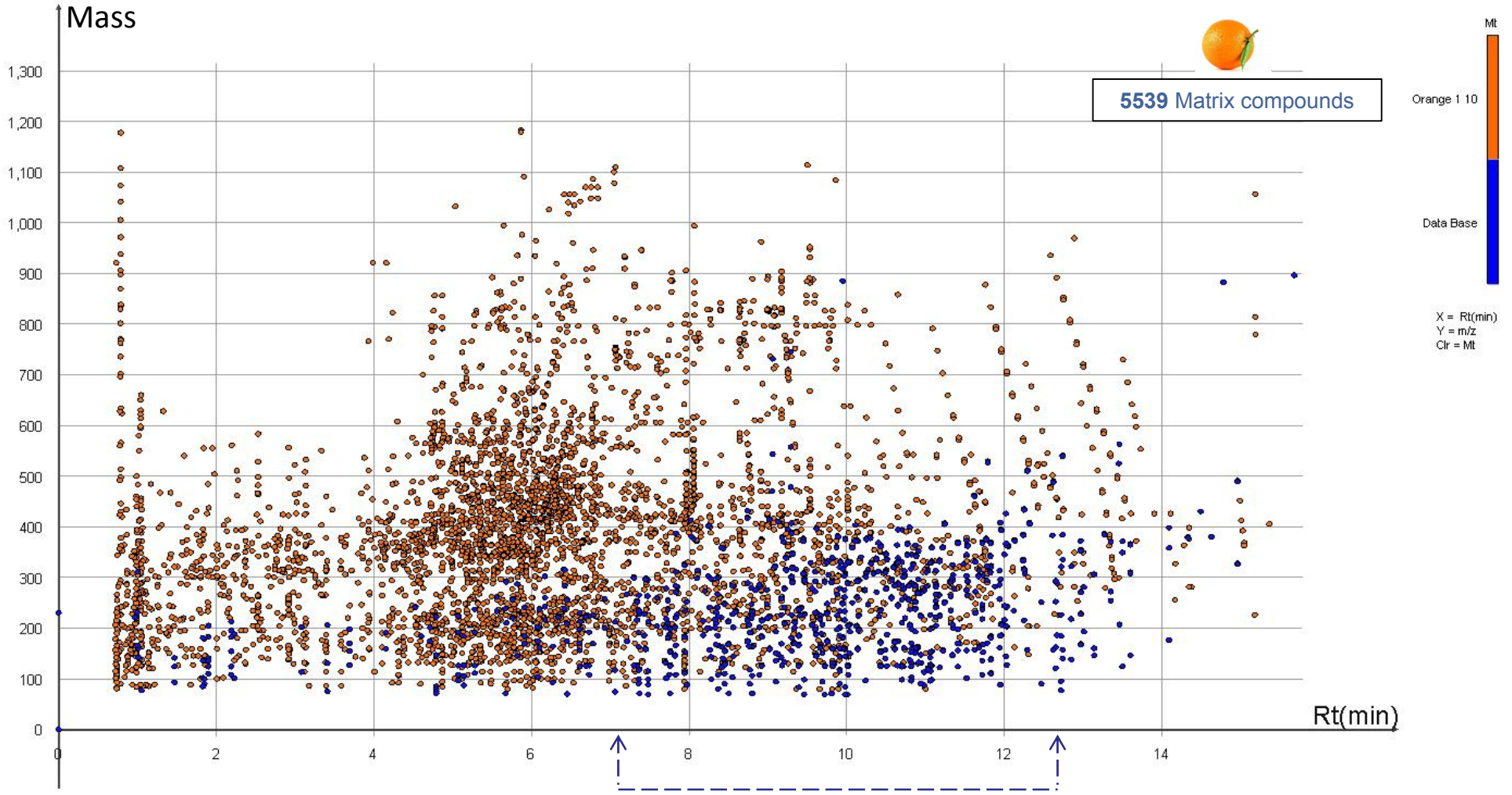


Miner 3D Enterprise

DB: 750 components

Orange: 2743 matrix compounds

Data base components- **orange** matrix compounds. **Dilution 1:10**



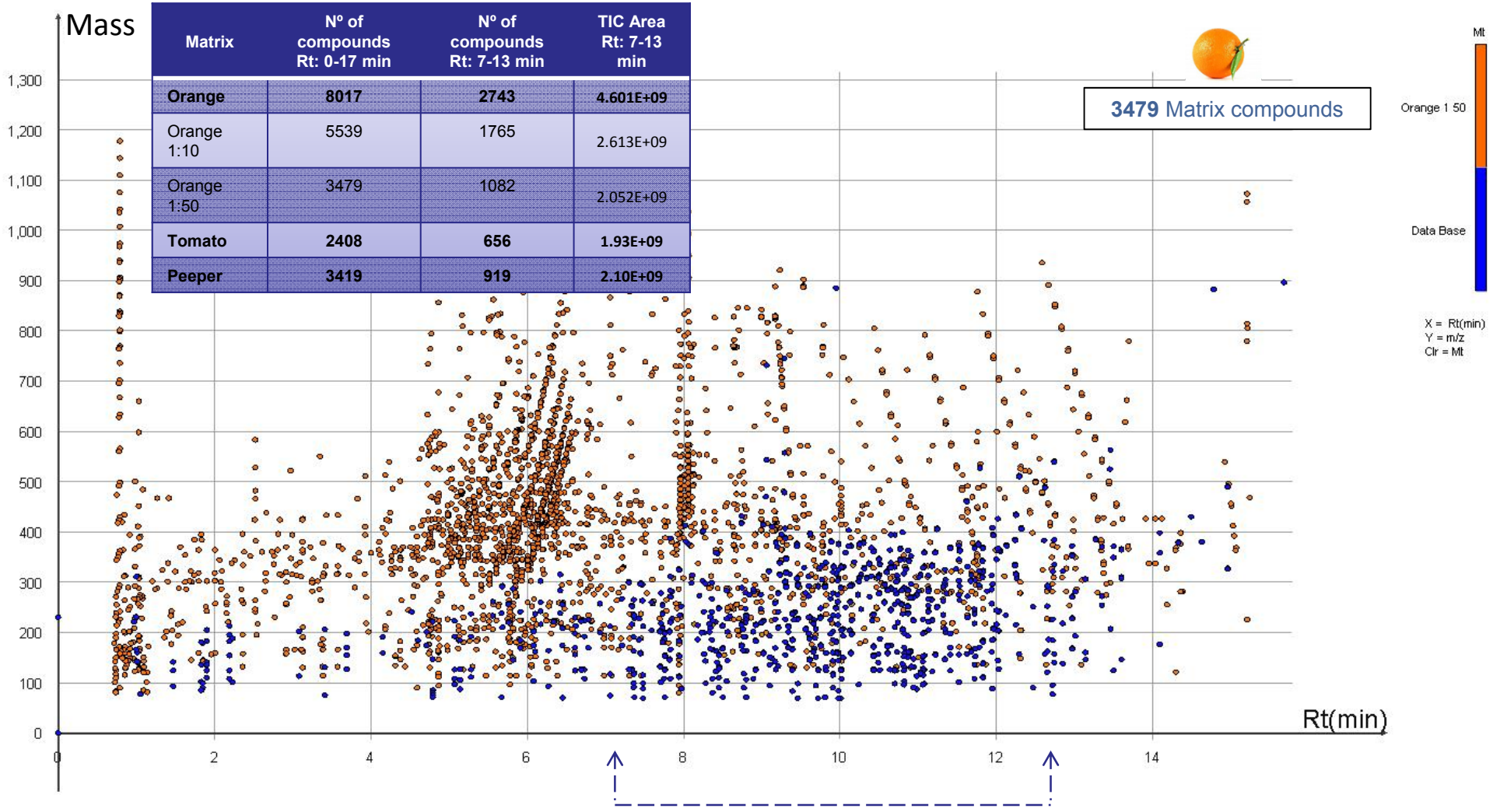
Miner 3D Enterprise

DB: 750 components

Orange: 1765 matrix compounds

Data base components- orange matrix compounds. Dilution 1:50

Matrix	Nº of compounds Rt: 0-17 min	Nº of compounds Rt: 7-13 min	TIC Area Rt: 7-13 min
Orange	**8017**	**2743**	**4.601E+09**
Orange 1:10	5539	1765	2.613E+09
Orange 1:50	3479	1082	2.052E+09
Tomato	**2408**	**656**	**1.93E+09**
Peeper	**3419**	**919**	**2.10E+09**

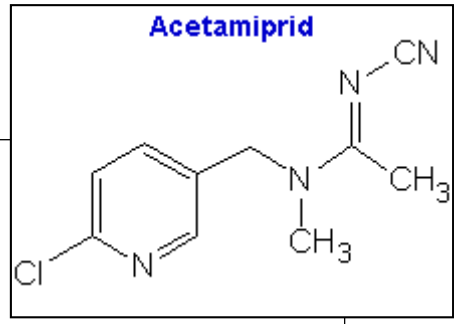


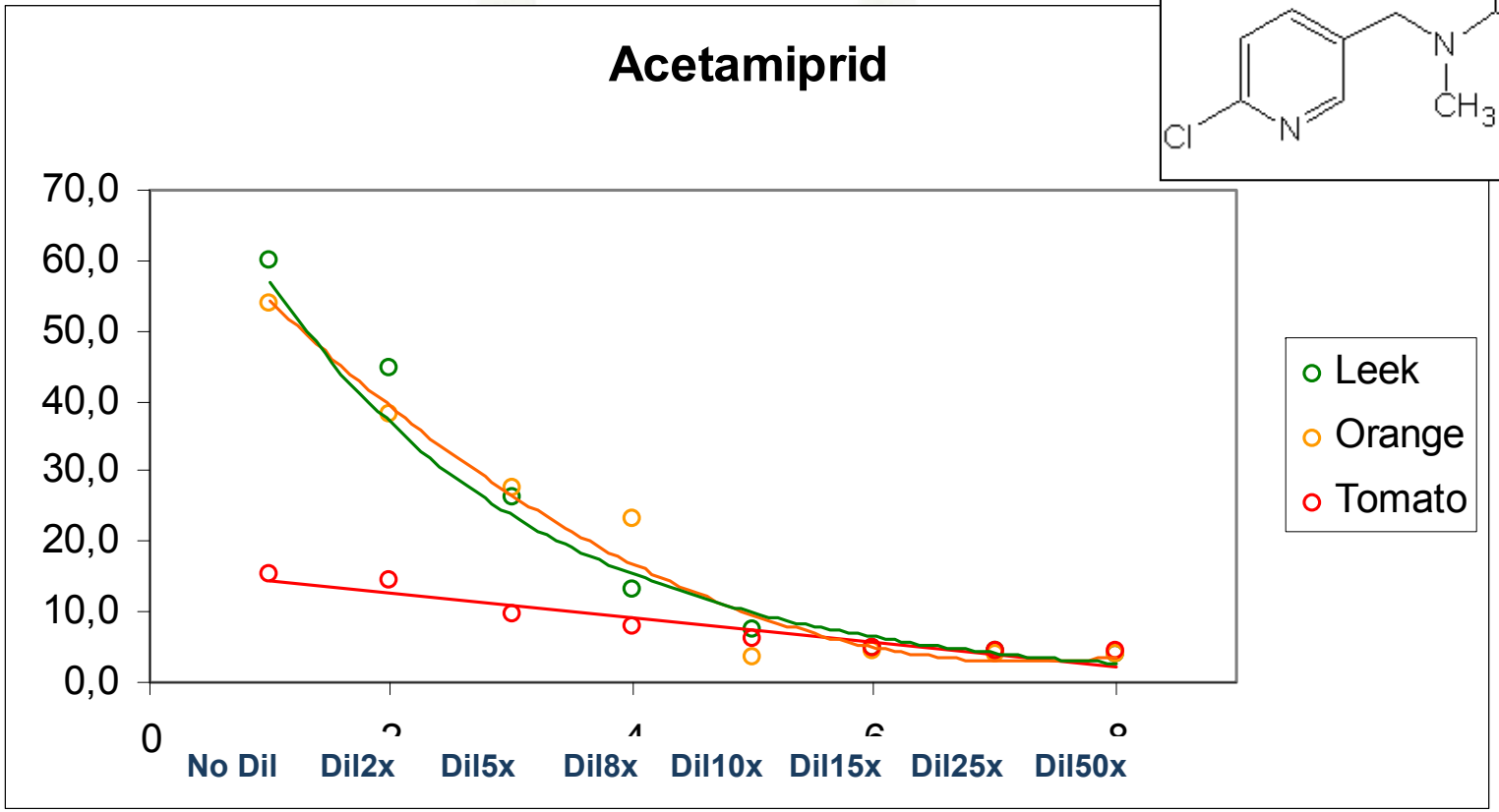
Miner 3D Enterprise

DB: 750 components

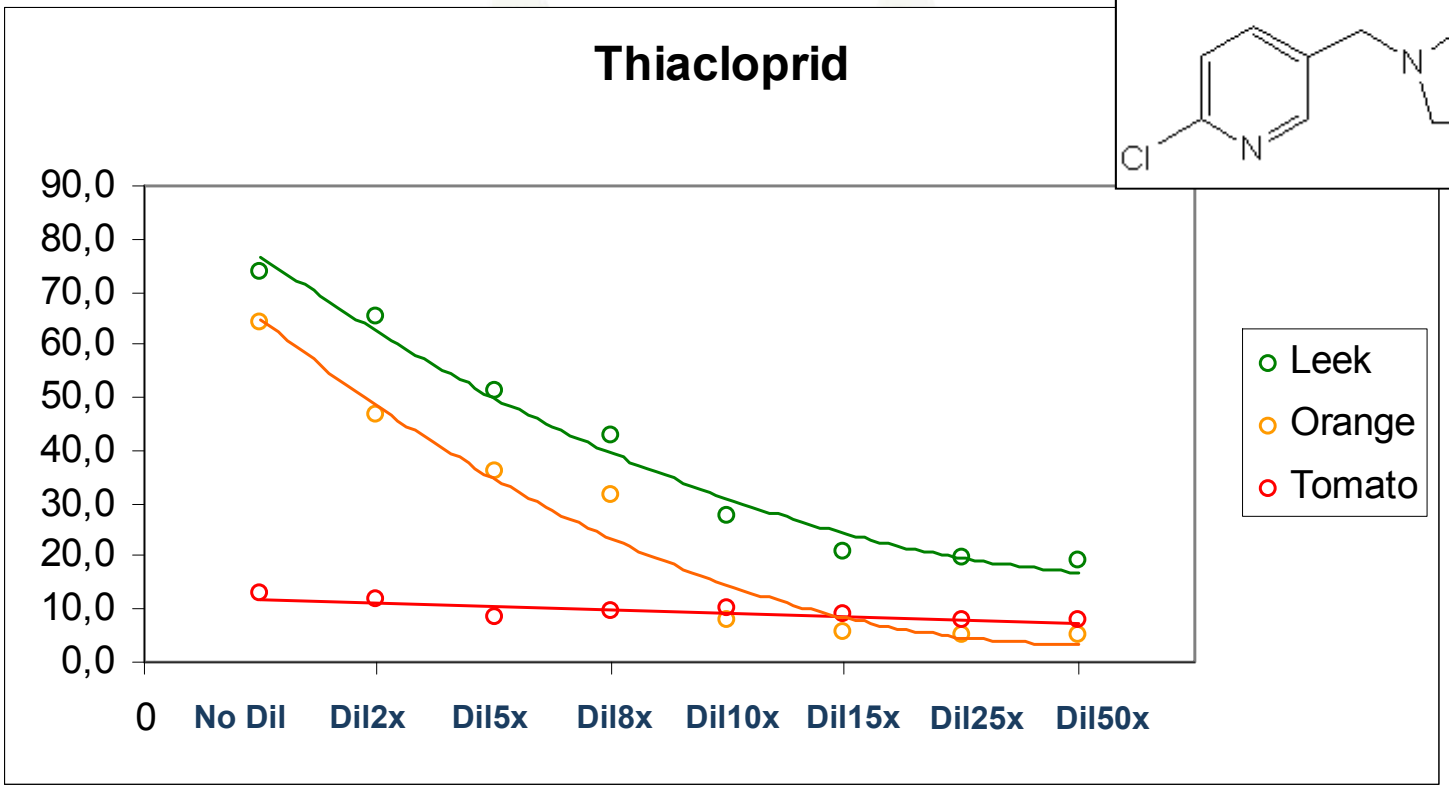
Orange: 1082 matrix compounds

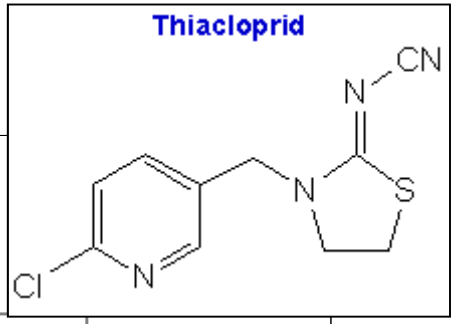
Matrix effect

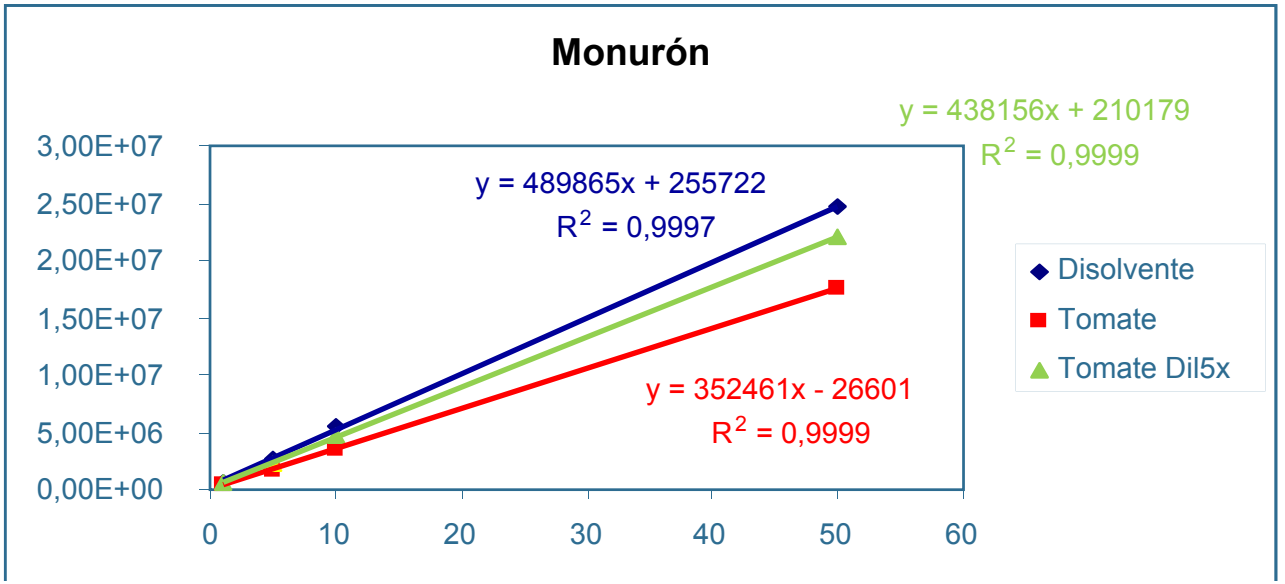




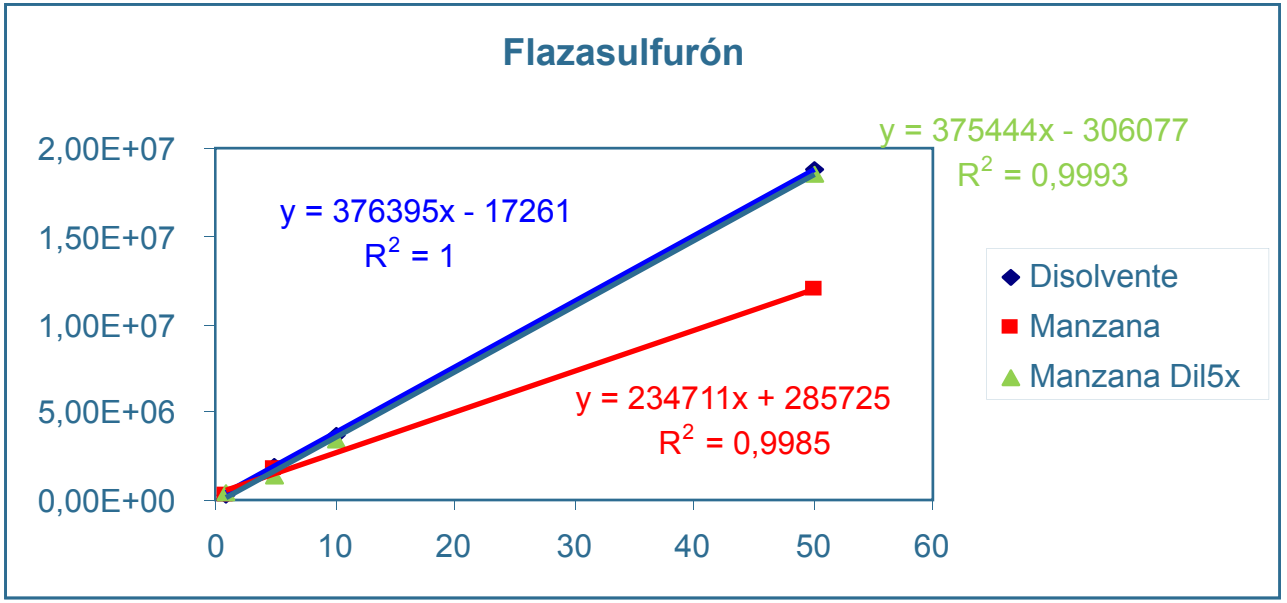
Matrix effect







Tomato

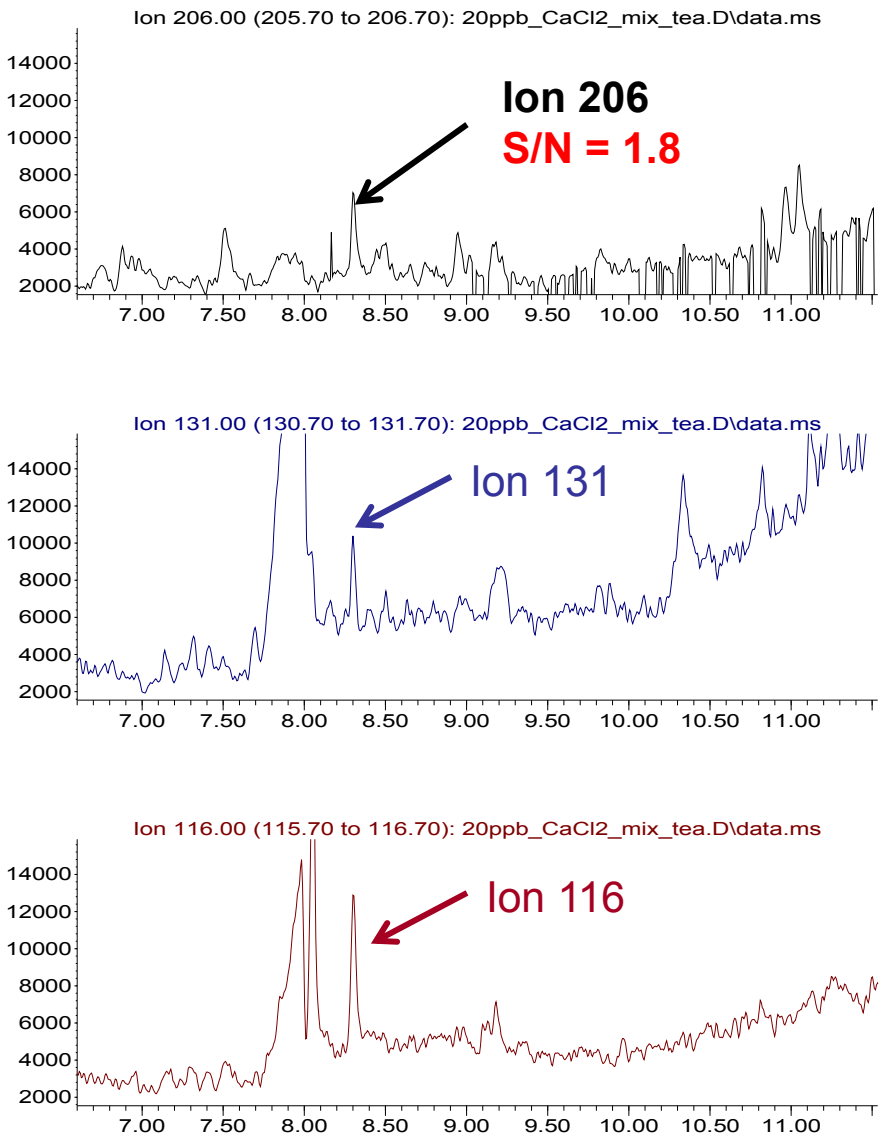


Apple

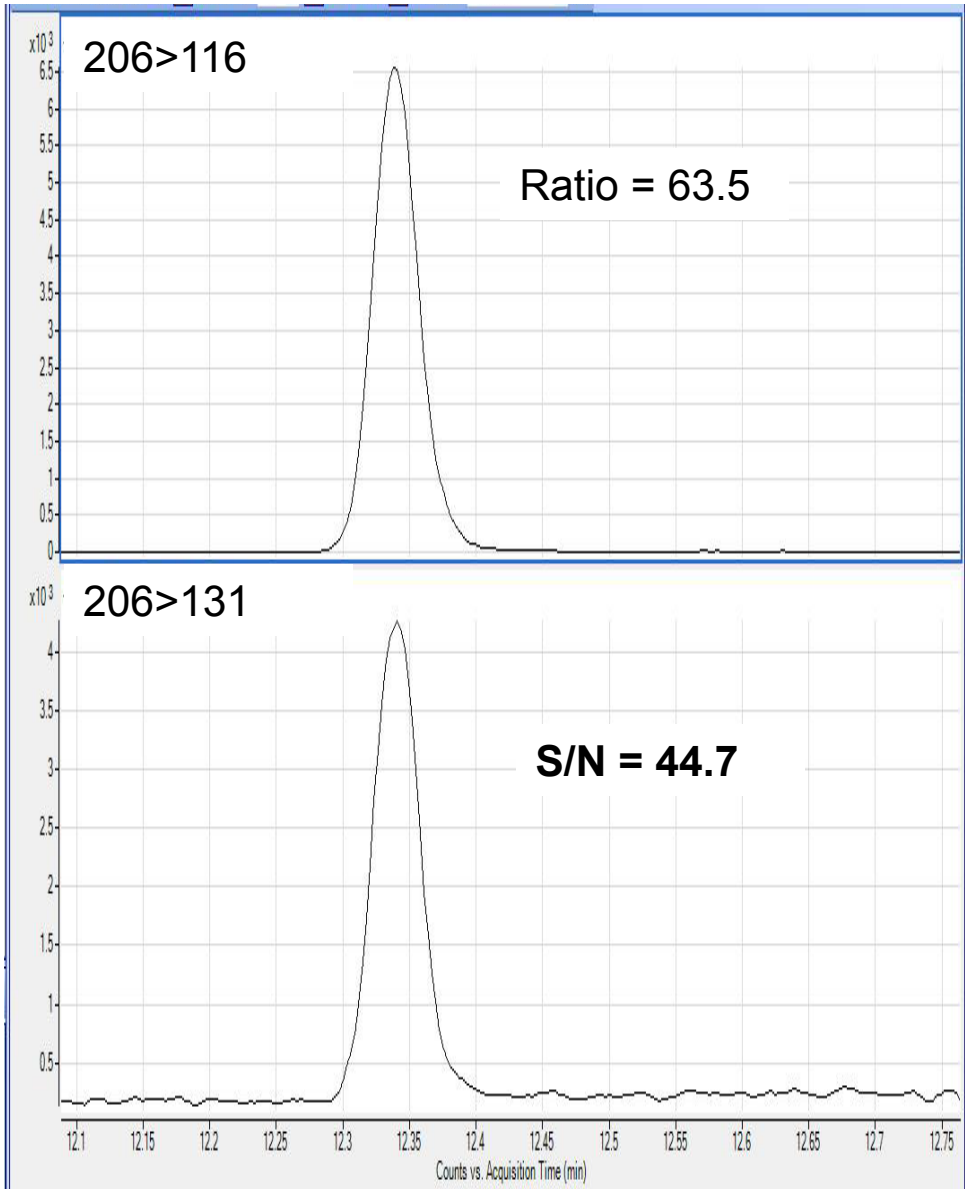
INSTRUMENTATION

Kresoxim-Methyl
20 µg/L in Green Tea

Agilent 5975 GC-Q-MS



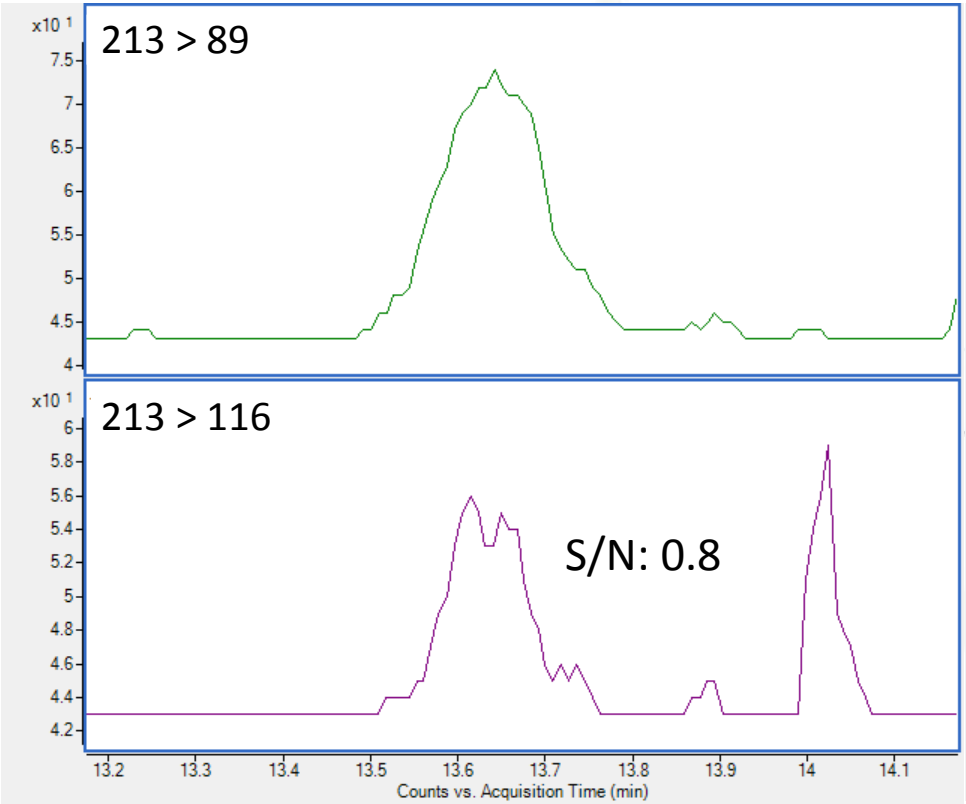
Agilent 7000 GC-QqQ-MS/MS



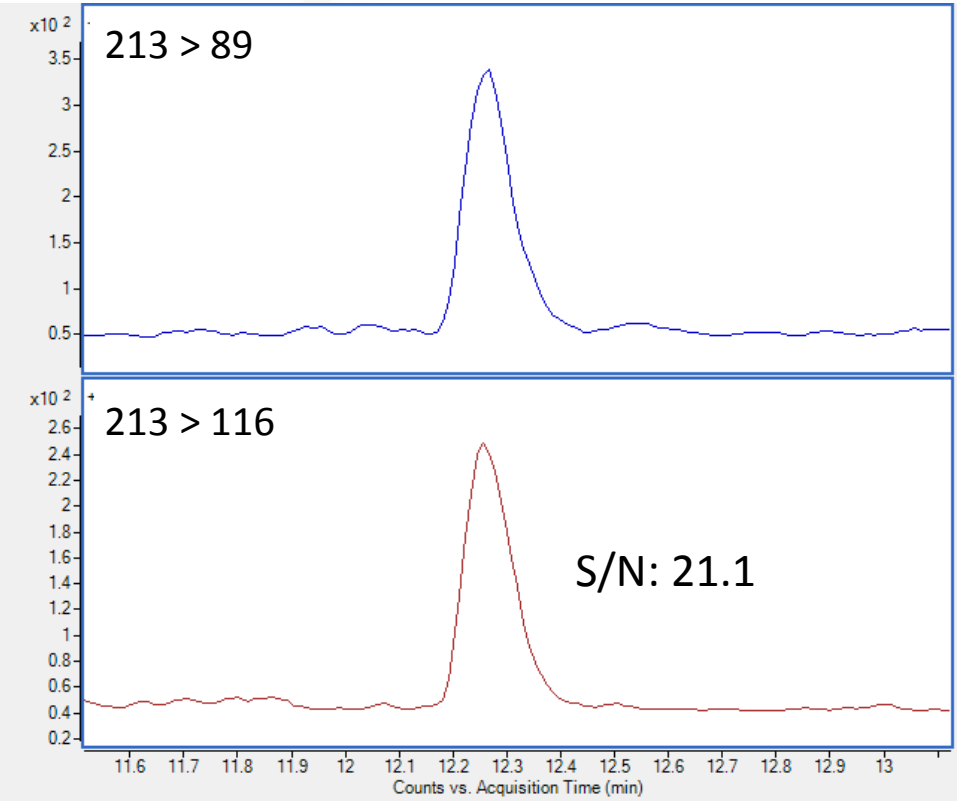
INSTRUMENTATION

Aldicarb

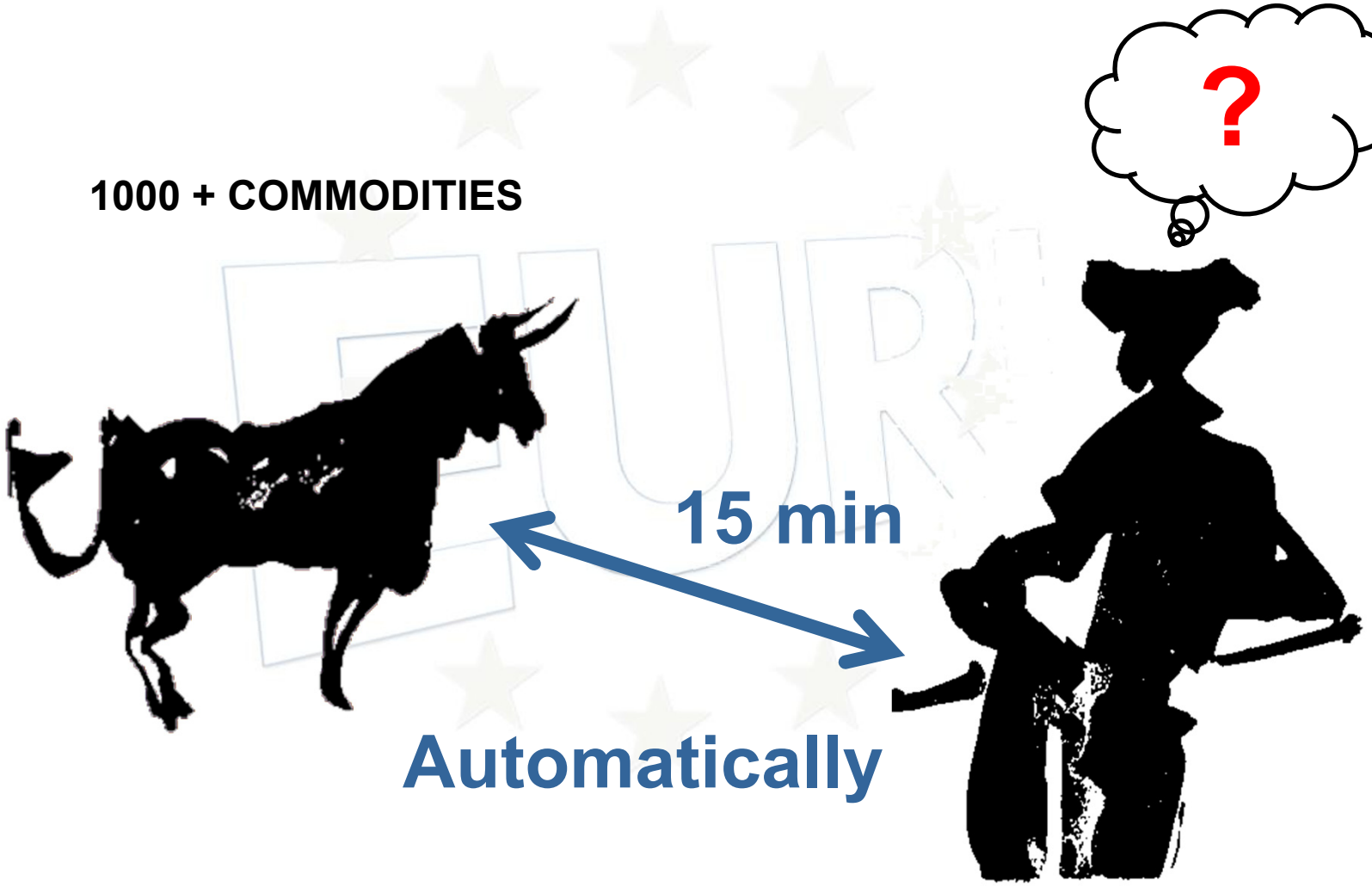
Orange at 10ppb
LC-QQQ-MS/MS Agilent **6410**



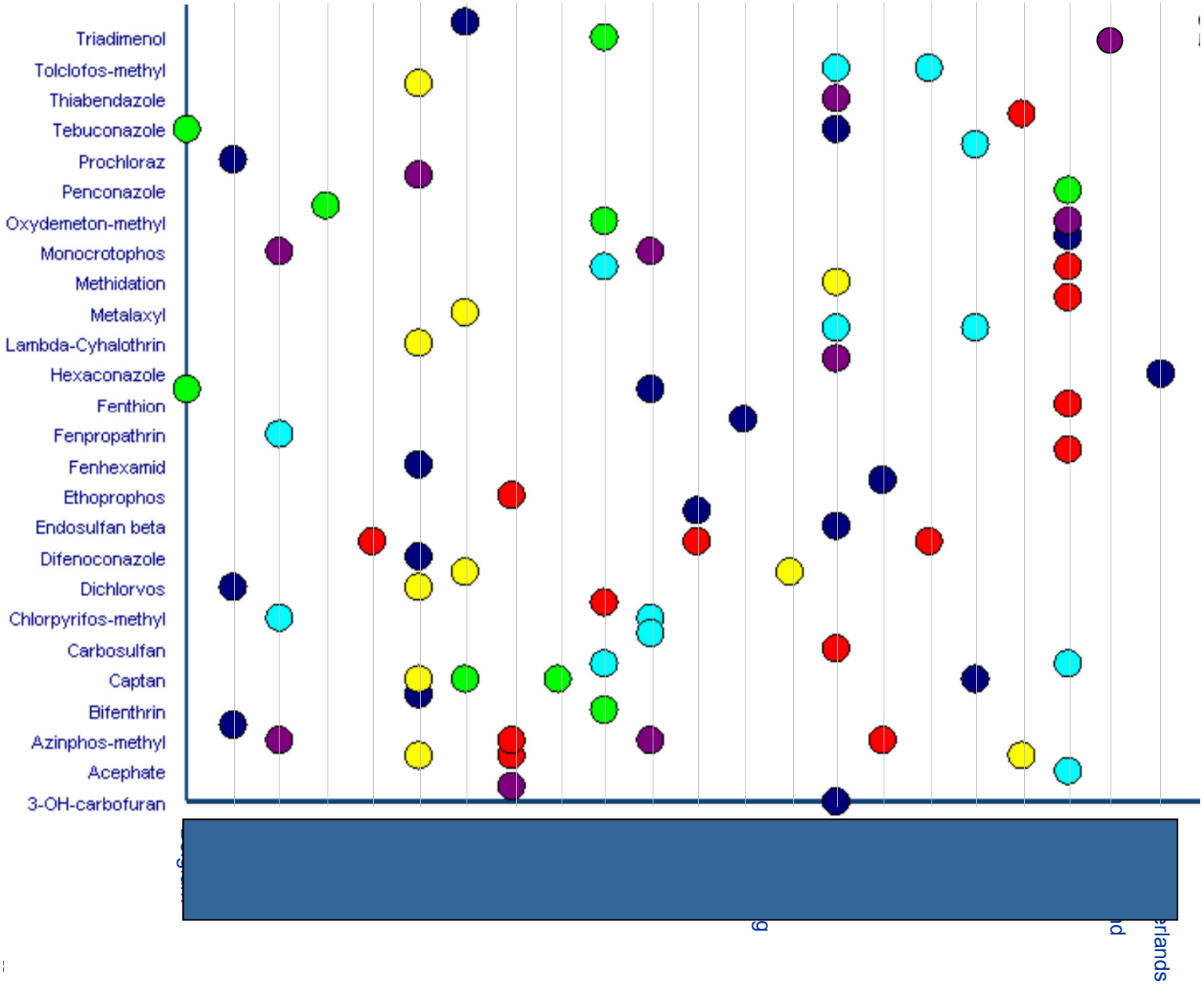
Orange at 10ppb **dilx10**
LC-QQQ-MS/MS Agilent **6490**



1000 + COMMODITIES



False +



EUPT No.

- FV9
- FV10
- FV11
- FV12
- FV13
- FV14

77 results
0.4%

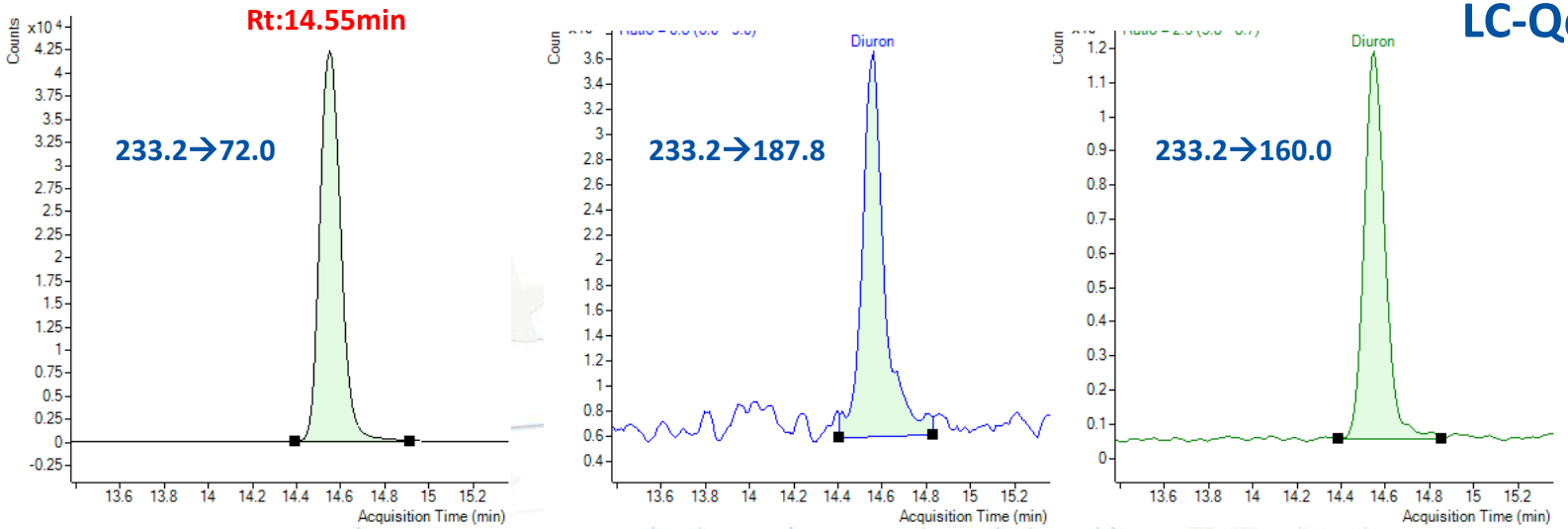
Difficulties in Identification

Other reported pesticides

Diuron Standard in solvent

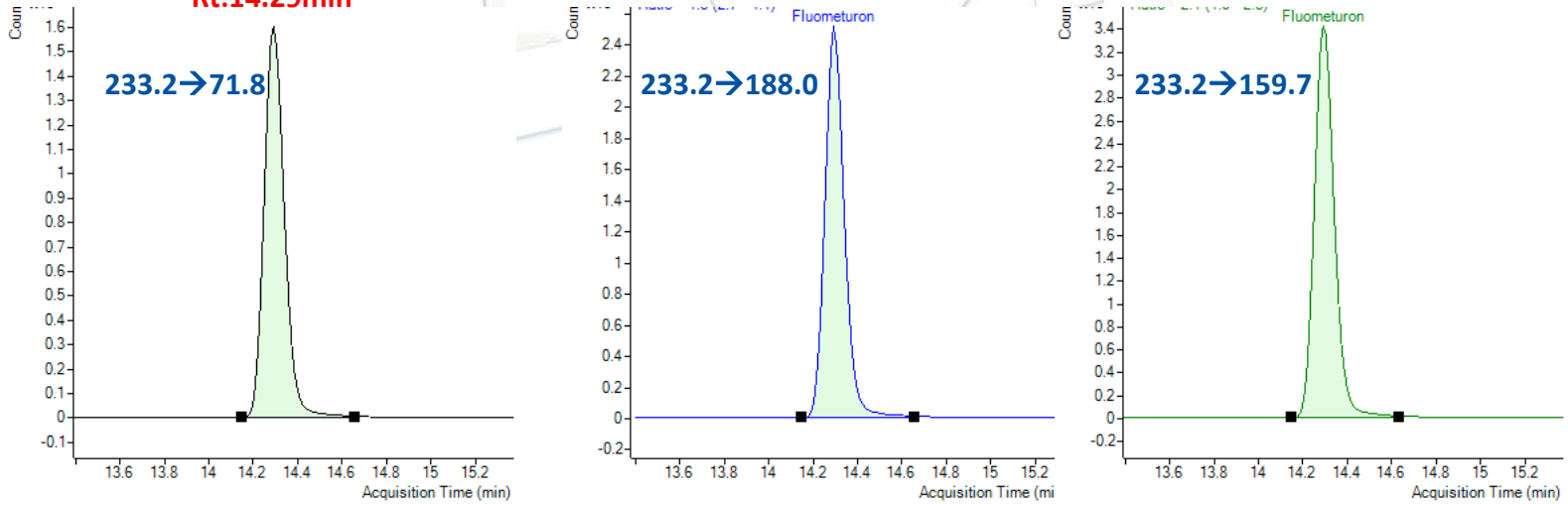
Rt:14.55min

LC-QqQ-MS



Fluometuron Standard in solvent

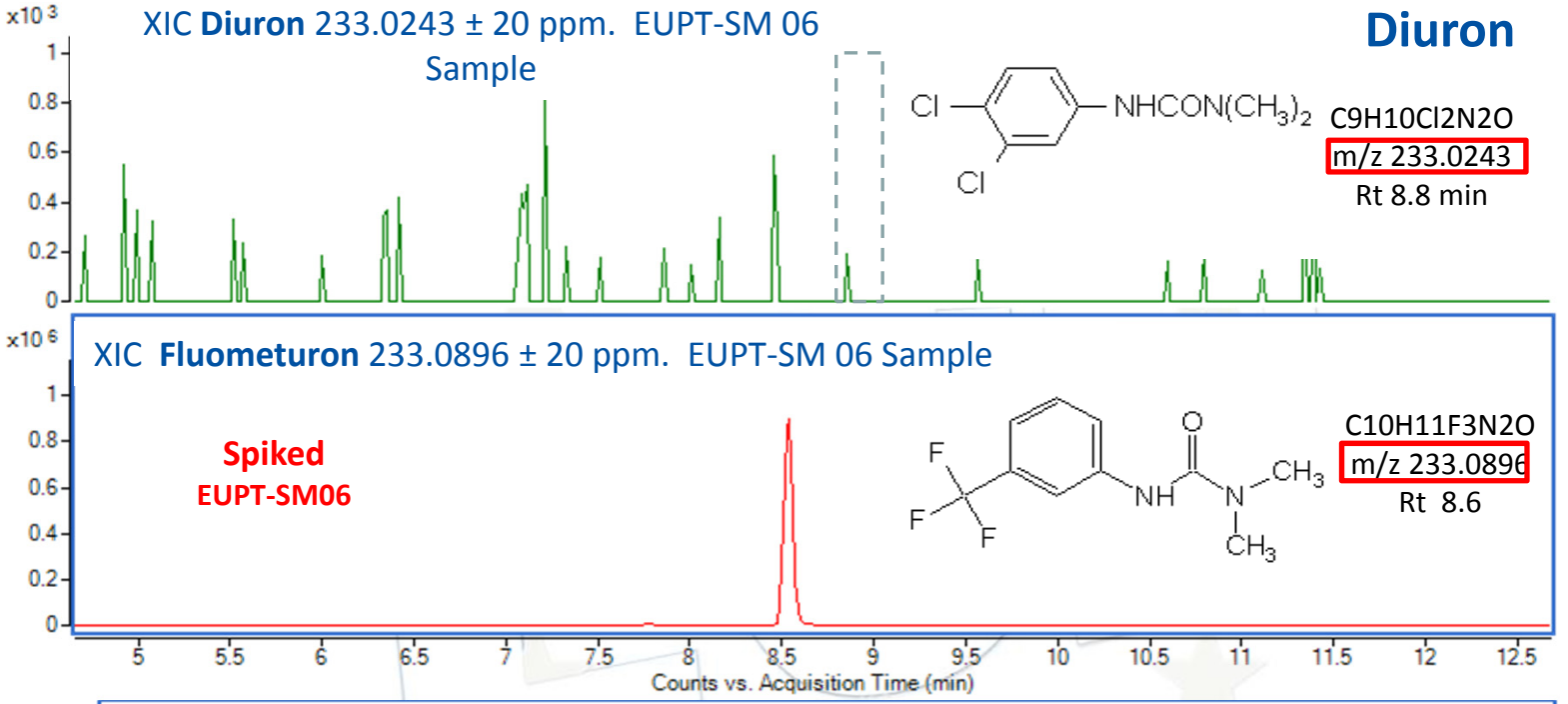
Rt:14.29min



Other reported pesticides

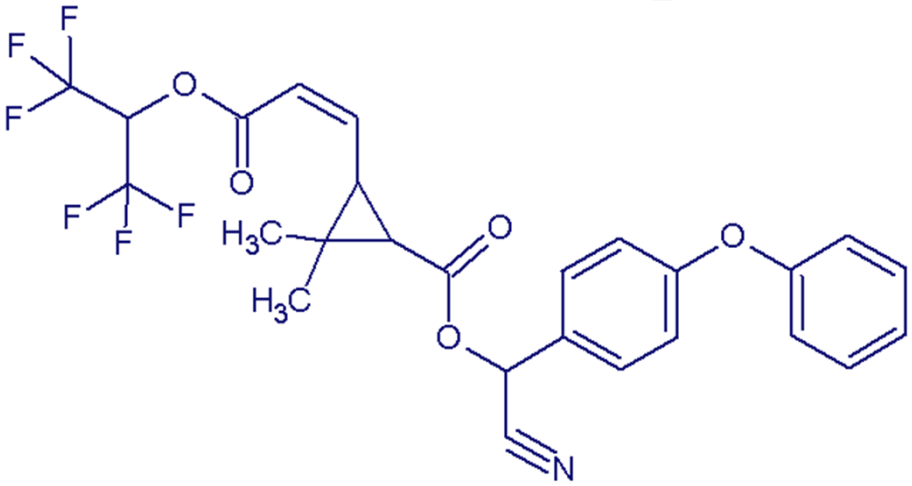
LC-TOF -MS

Diuron



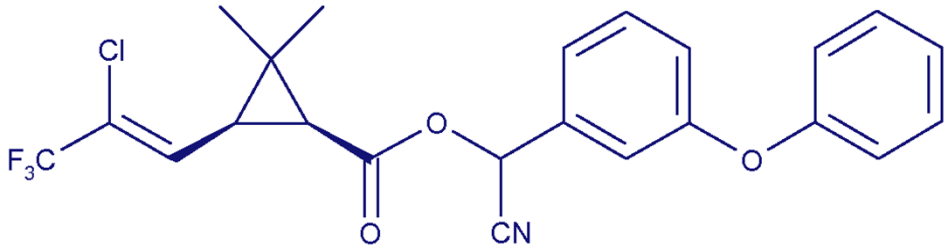
Best	Formula	Species	m/z	Diff (ppm)	Score
●	C10 H11 F3 N2 O	(M+H)+	233.0901	-1.84	98.48
○	C13 H10 F2 N2	(M+H)+	233.0901	-6.71	86.02
○	C12 H12 N2 O3	(M+H)+	233.0901	8.72	82.21

Acrinathrin



$C_{26}H_{21}F_6NO_5$
Mw 541,4

Lambda Cyhalothrin

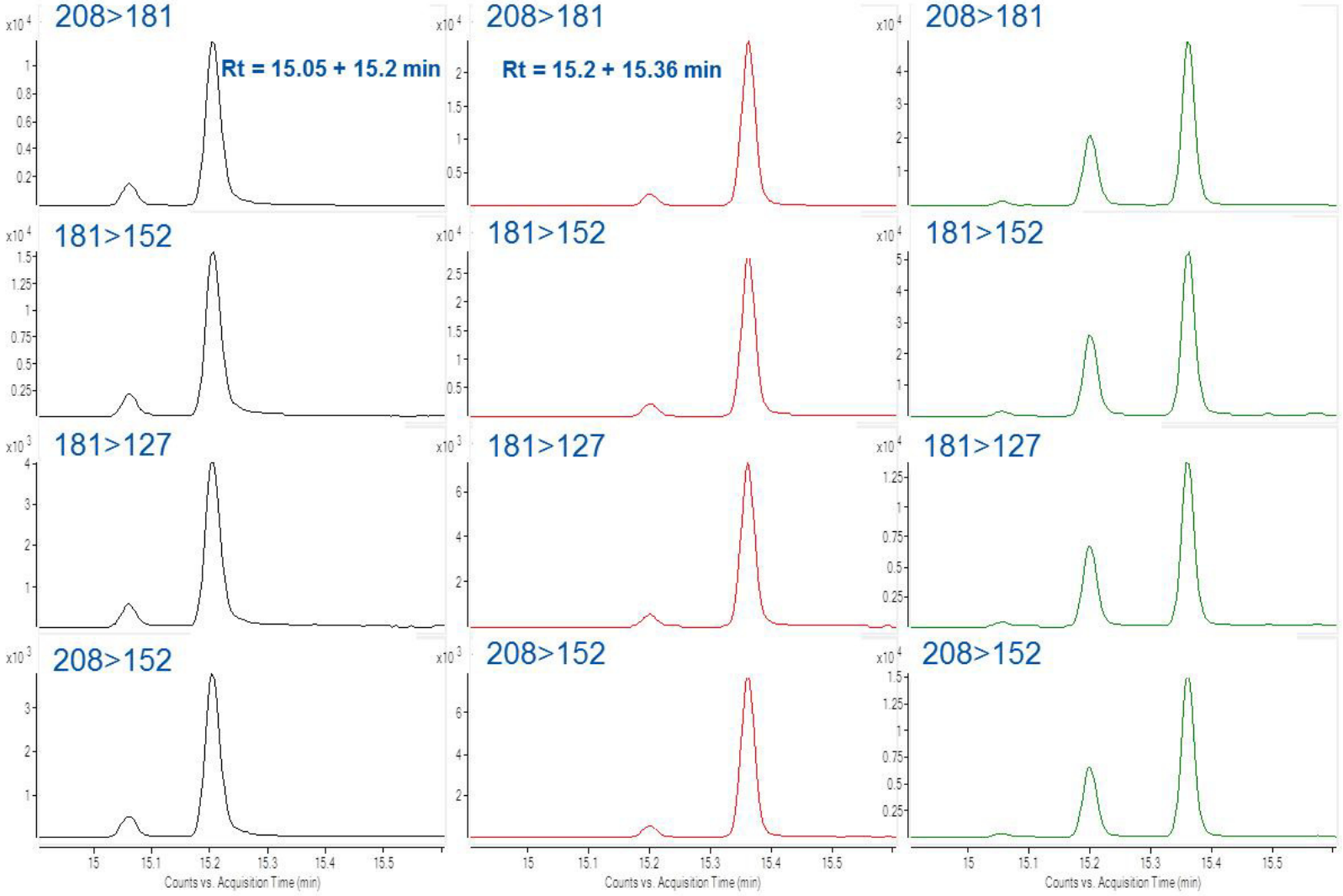


$C_{23}H_{19}ClF_3NO_3$
Mw 449,9

GC-QQQ-MS/MS

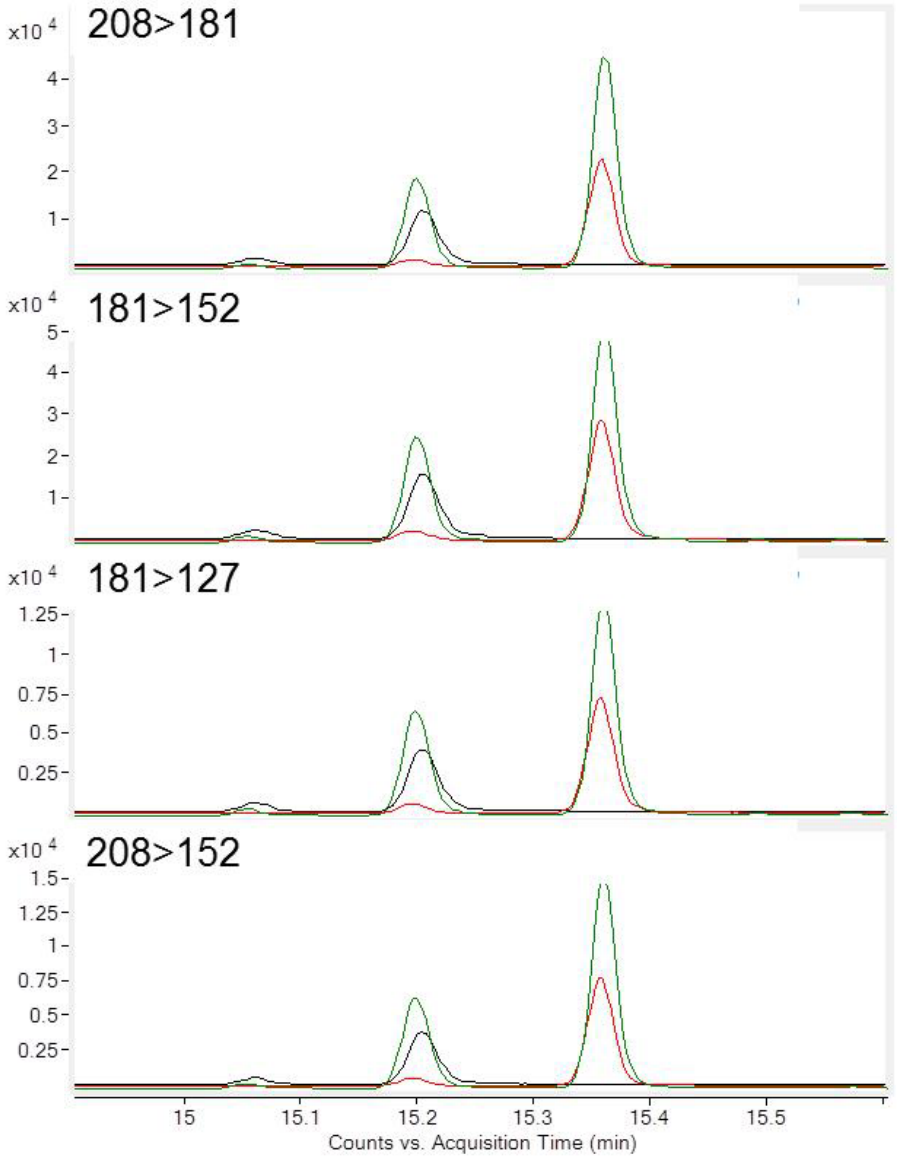
λ-Cyhalothrin **Acrinathrin**

Pepper Sample
(EUPT-FV16 Sample 015)



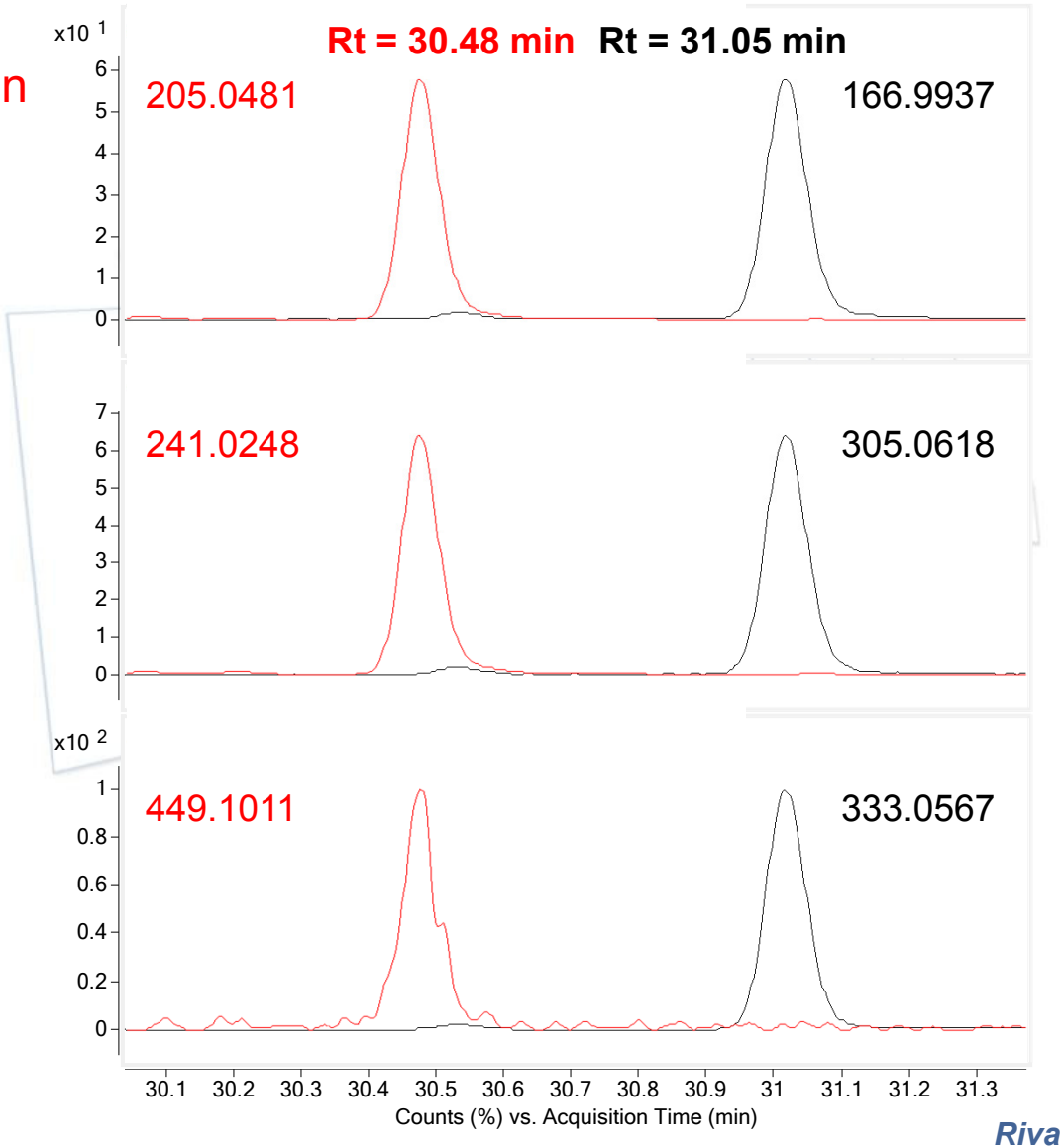
GC-QQQ-MS/MS-Overlapped

λ-Cyhalothrin
Acrinathrin
Pepper Sample
(EUPT-FV16
Sample 015)

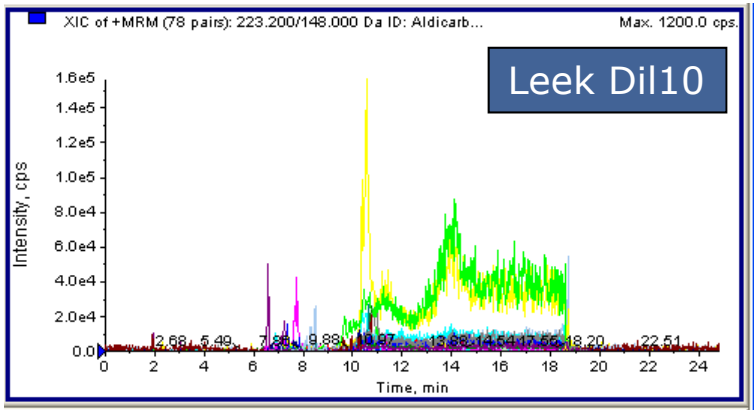


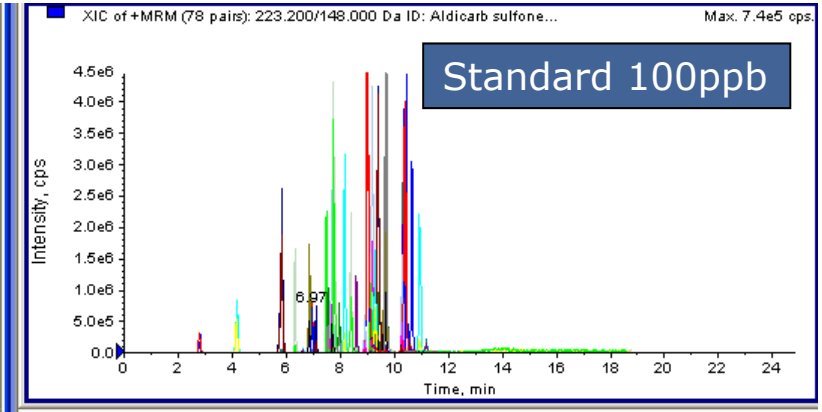
GC-QTOF (NCI) Pepper Sample (EUPT-FV16 Sample 015)

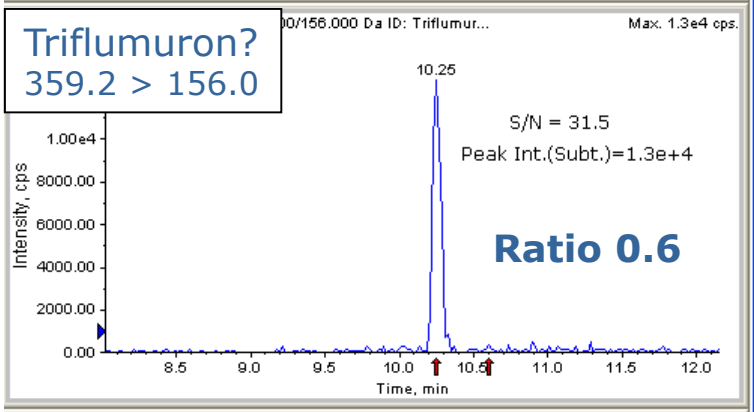
λ-Cyhalothrin

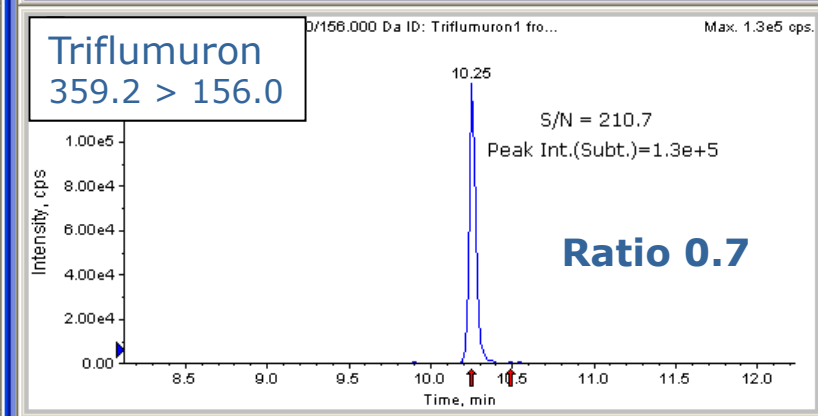


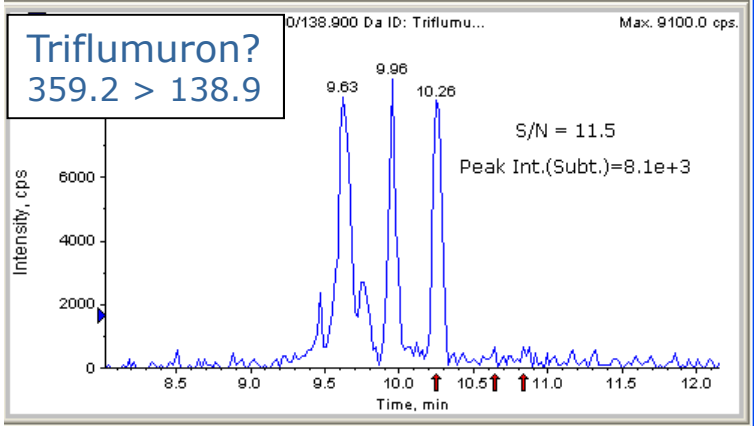
Acrinathrin

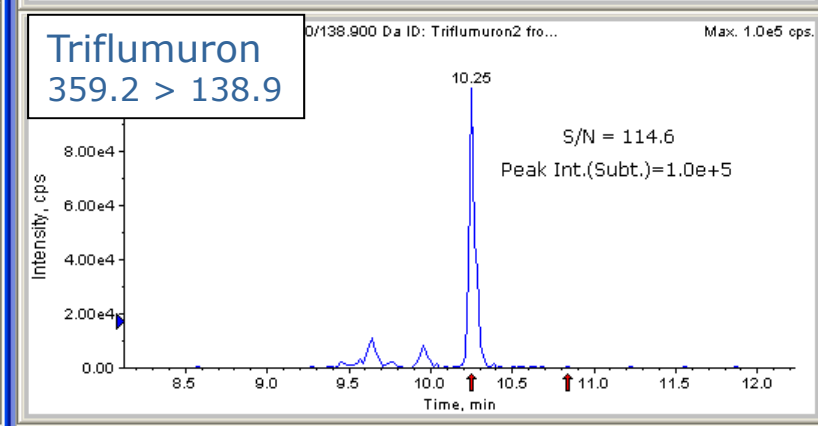

XIC of +MRM (78 pairs): 223.200/148.000 Da ID: Aldicarb...
Max. 1200.0 cps.
Leek Dil10
Intensity, cps
Time, min


XIC of +MRM (78 pairs): 223.200/148.000 Da ID: Aldicarb sulfone...
Max. 7.4e5 cps.
Standard 100ppb
6.97
Intensity, cps
Time, min


Triflumuron?
359.2 > 156.0
Max. 1.3e4 cps.
10.25
S/N = 31.5
Peak Int.(Subt.)=1.3e+4
Ratio 0.6
Intensity, cps
Time, min

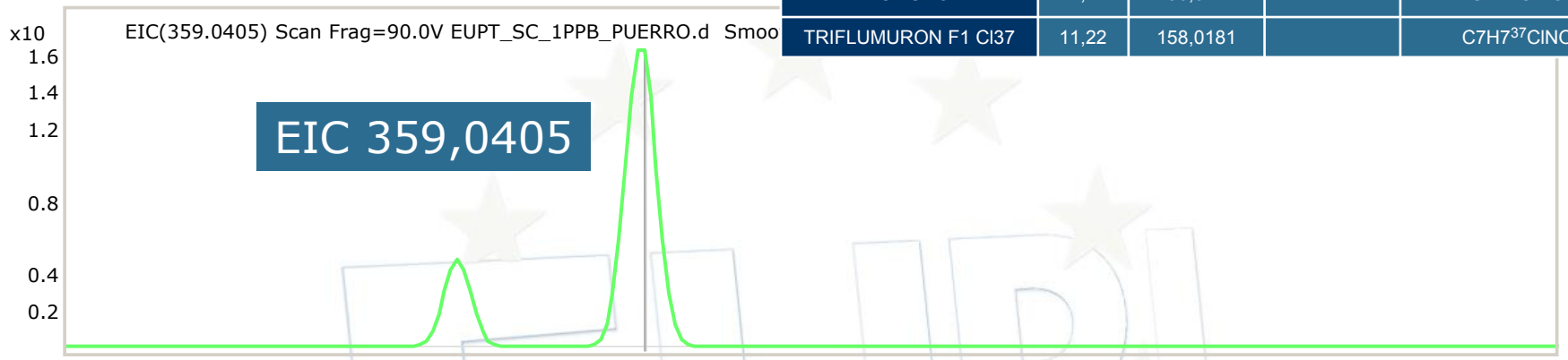

Triflumuron
359.2 > 156.0
Max. 1.3e5 cps.
10.25
S/N = 210.7
Peak Int.(Subt.)=1.3e+5
Ratio 0.7
Intensity, cps
Time, min

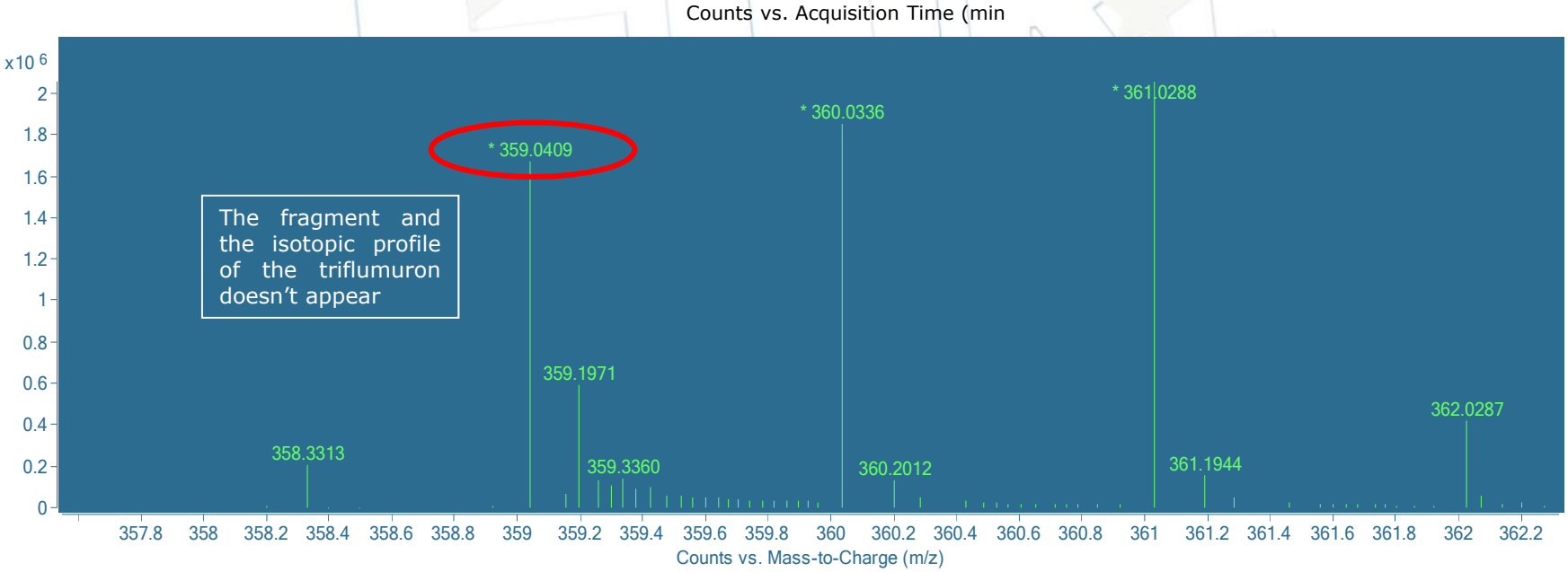

Triflumuron?
359.2 > 138.9
Max. 9100.0 cps.
9.63
9.96
10.26
S/N = 11.5
Peak Int.(Subt.)=8.1e+3
Intensity, cps
Time, min


Triflumuron
359.2 > 138.9
Max. 1.0e5 cps.
10.25
S/N = 114.6
Peak Int.(Subt.)=1.0e+5
Intensity, cps
Time, min

TRIFLUMURON in LC-TOF-FULL SCAN

TRIFLUMURON	11,22	359,0405	$[M+H]^+$	$C_{15}H_{11}ClF_3N_2O_3$
TRIFLUMURON Cl37	11,22	361,0375	$[M+H+2]^+$	$C_{15}H_{11}{}^{37}ClF_3N_2O_3$
TRIFLUMURON F1	11,22	156,0211		C_7H_7ClNO
TRIFLUMURON F1 Cl37	11,22	158,0181		$C_7H_7{}^{37}ClNO$





FOOD ANALYSIS

IDENTIFICATION

QUANTIFICATION

OVERCOMING Identification Dificulties

MS

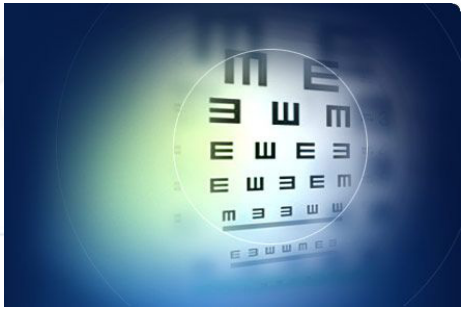
HRMS

Typical Poblems in screening LC-ESI-HRMS for Routine application (Document Nº SANCO/1257/2013)

Mass accuracy	**≤ 5 ppm (NO PROBLEM)**
Sensitivity	**0.01 mg/kg**
Linearity	**saturation**
Reproducibility	**≤ 20%**
Software+ Resolution	**False + and False -**
HRMS and HRMS/MS	
Low ion ratio rep.	**False positives**

Full Scan Mode

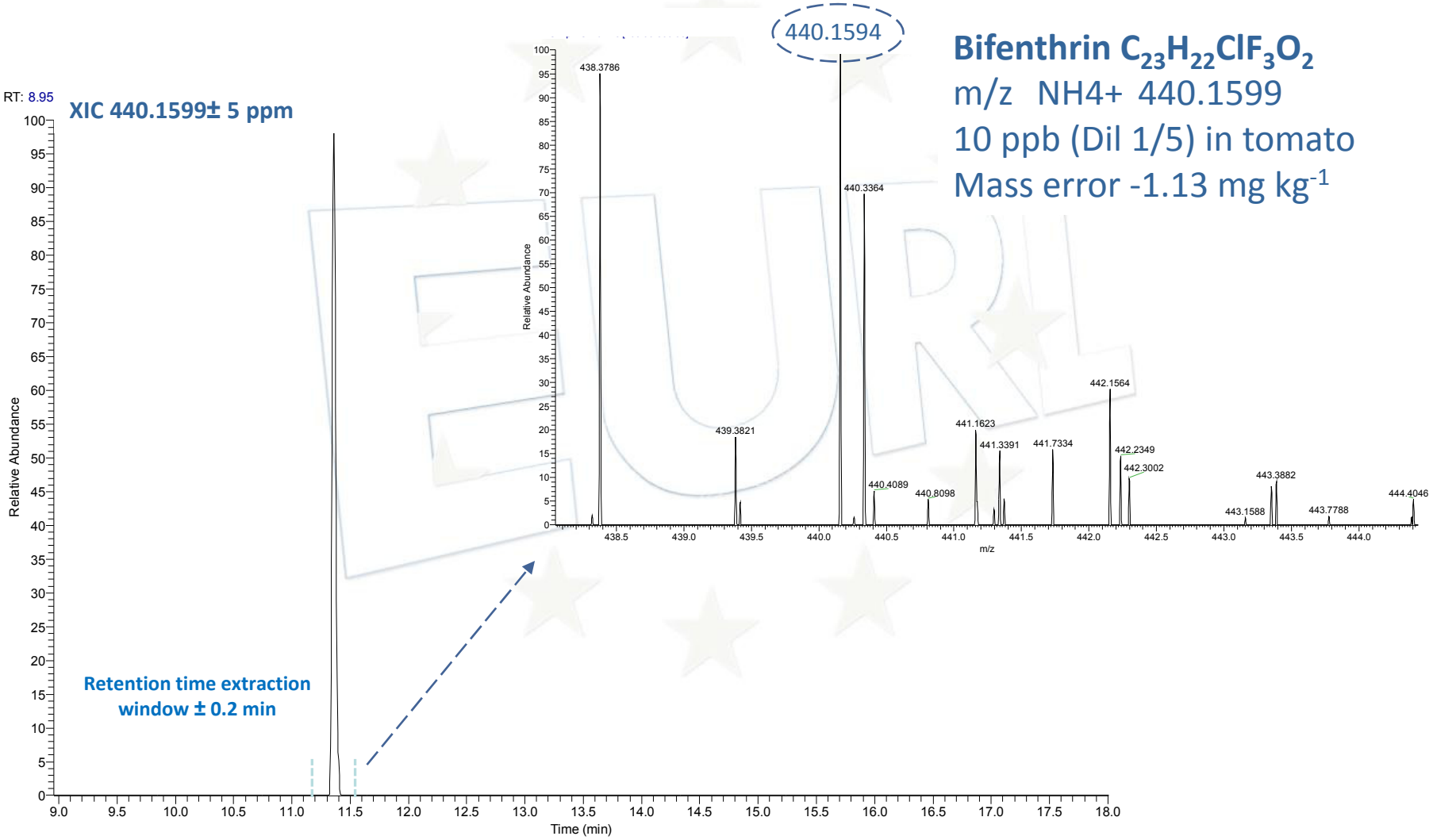
SENSITIVITY + REPRO.



RESOLUTION

LINEARITY

LC-HRMS Full Scan mode R 35000 (m/z 200)



Bifenthrin $C_{23}H_{22}ClF_3O_2$

m/z NH4+ 440.1599

10 ppb (Dil 1/5) in tomato

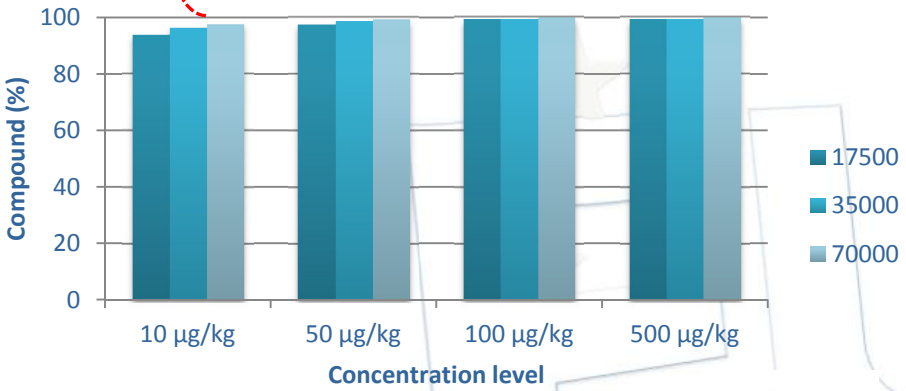
Mass error -1.13 mg kg^{-1}

Detected pesticides *vs* resolution
Orbitrap *Full Scan*

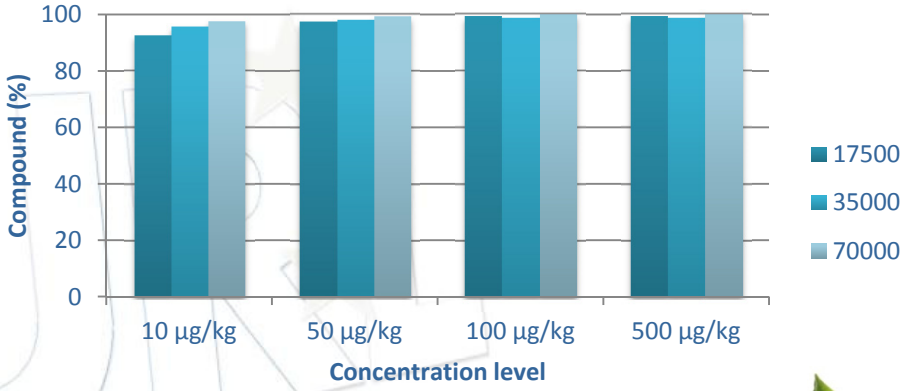
Low sensitivity compounds

Aldicarb	Prothiophos
Fenitrothion	Oxydemethon-methyl

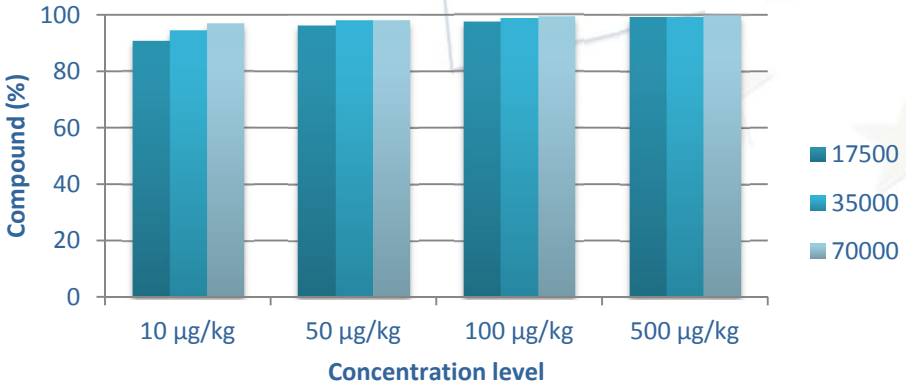
Tomato



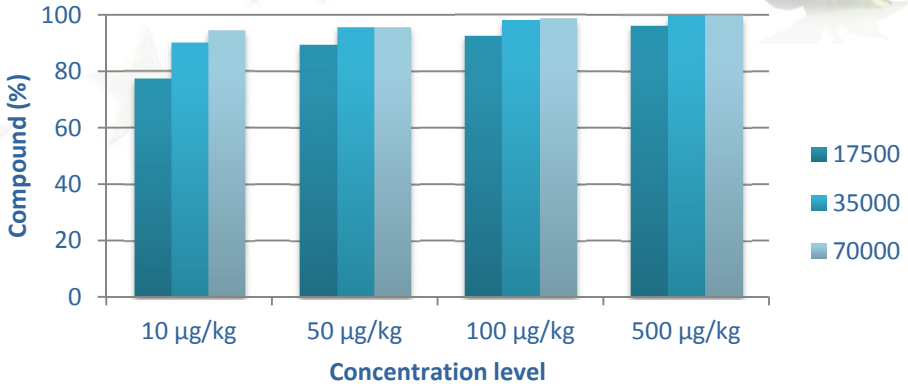
Pepper



Orange



Green Tea



Numbers of points at different resolutions

XIC Bromuconazole 100 µg/kg m/z 375.9613 ± 5 ppm

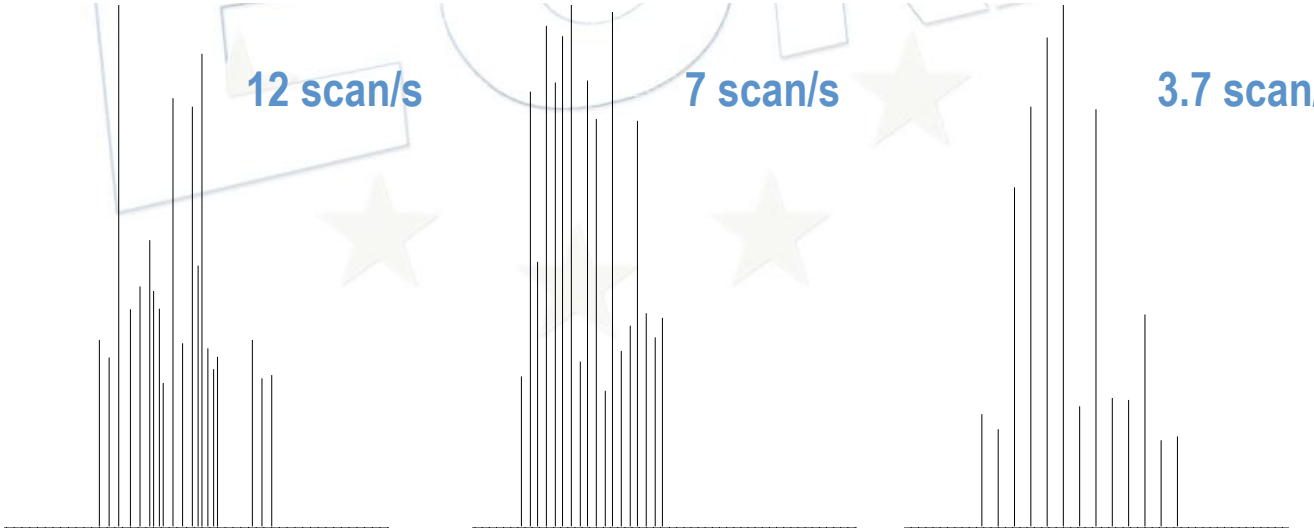
Difficult matrix

Orange matrix

Scan Rate - / + Resolution + / -

Due to better mass accuracy

Pepper matrix



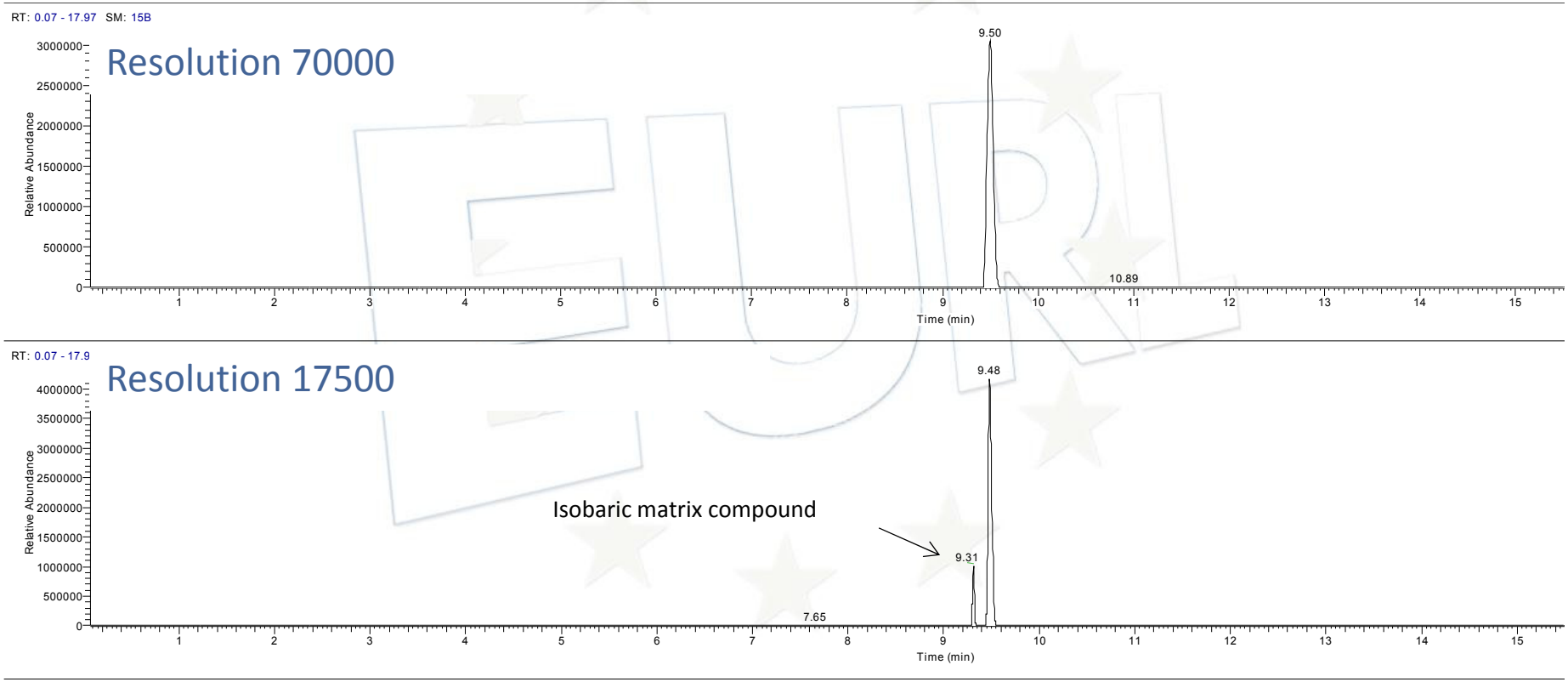
Resolution **17500 FWHM**

Resolution **35000 FWHM**

Resolution **70000 FWHM**

Isobaric compounds at different resolutions

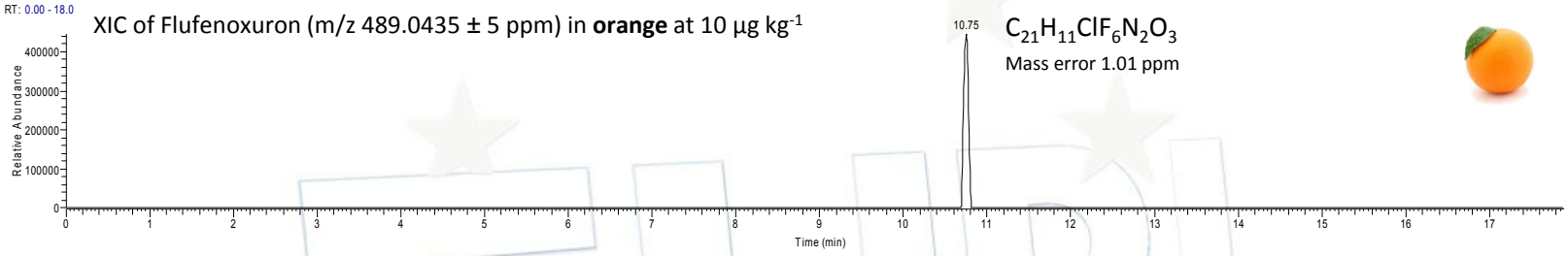
Bupirimate (m/z 317.1642 ± 5 ppm) in orange at 10 µg kg^{-1}



Isobaric compounds in different matrices

Flufenoxuron (m/z 489.0435 ± 5 ppm) at 10 µg kg^{-1}
Resolution 70000

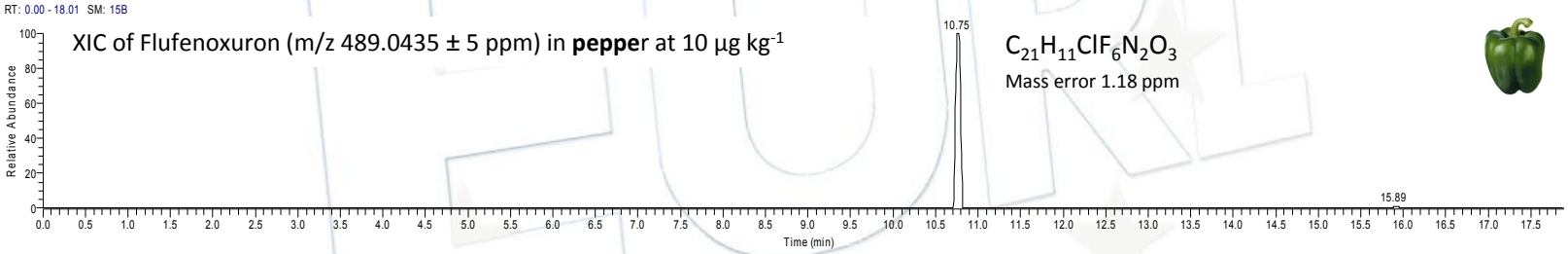
XIC of Flufenoxuron (m/z 489.0435 ± 5 ppm) in **orange** at 10 µg kg^{-1}



$C_{21}H_{11}ClF_6N_2O_3$

Mass error 1.01 ppm

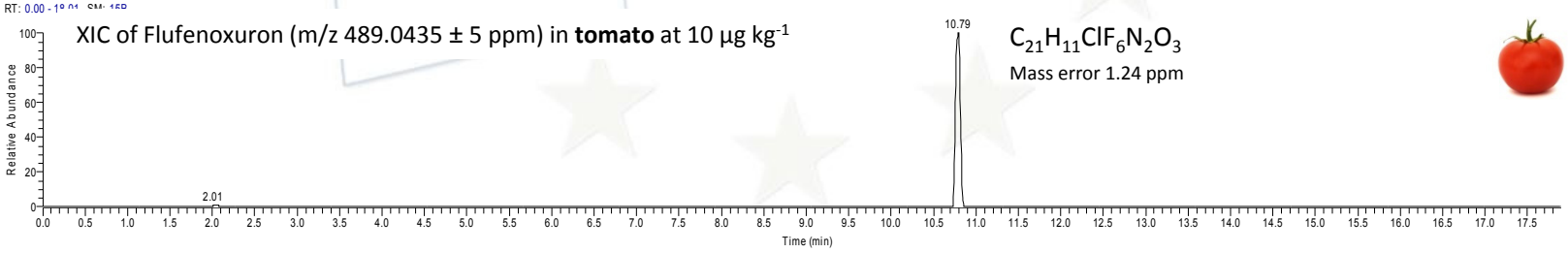
XIC of Flufenoxuron (m/z 489.0435 ± 5 ppm) in **peppe**r at 10 µg kg^{-1}



$C_{21}H_{11}ClF_6N_2O_3$

Mass error 1.18 ppm

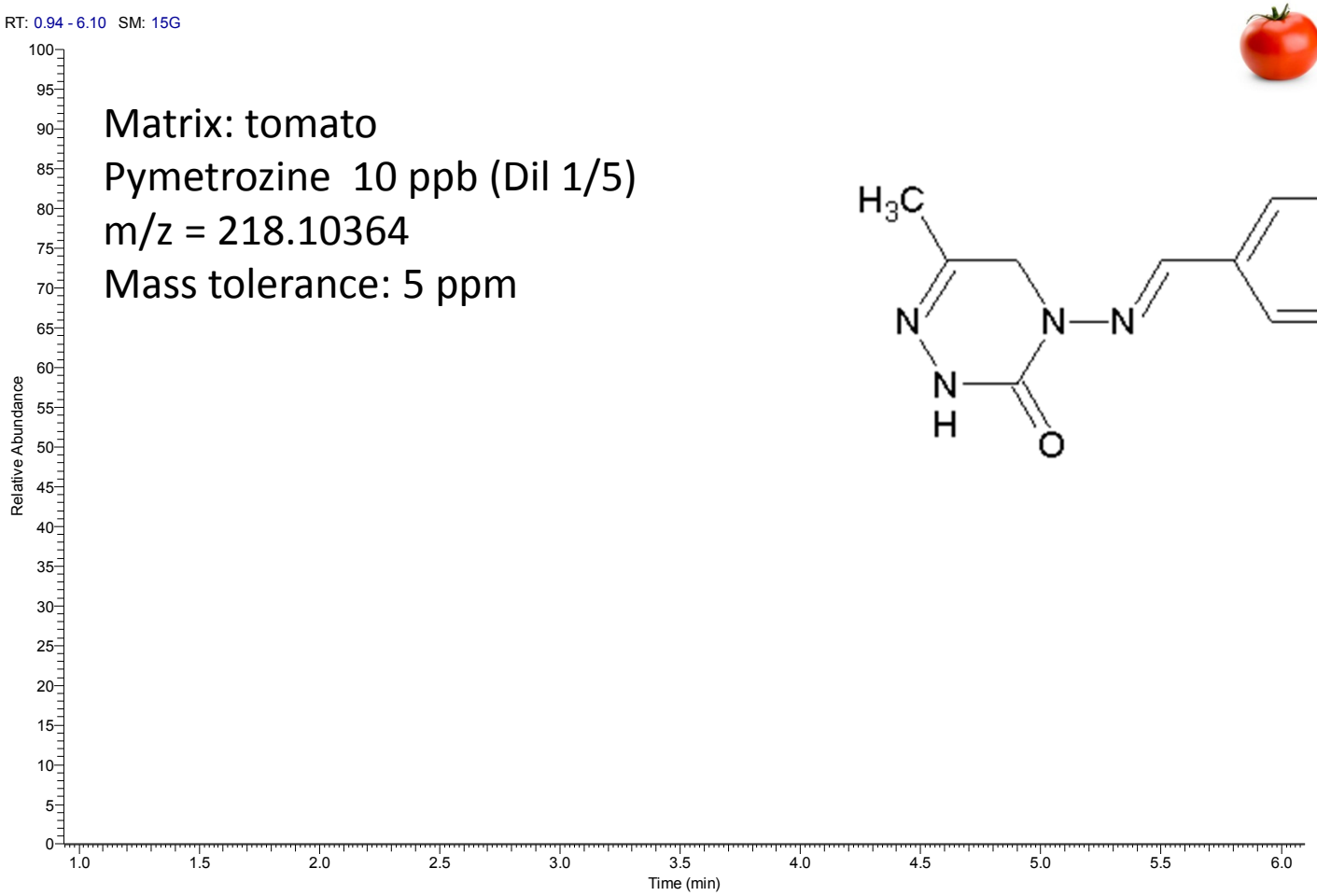
XIC of Flufenoxuron (m/z 489.0435 ± 5 ppm) in **tomato** at 10 µg kg^{-1}



$C_{21}H_{11}ClF_6N_2O_3$

Mass error 1.24 ppm

Influence of resolution on peak shape
Resolution 17500



Matrix: tomato

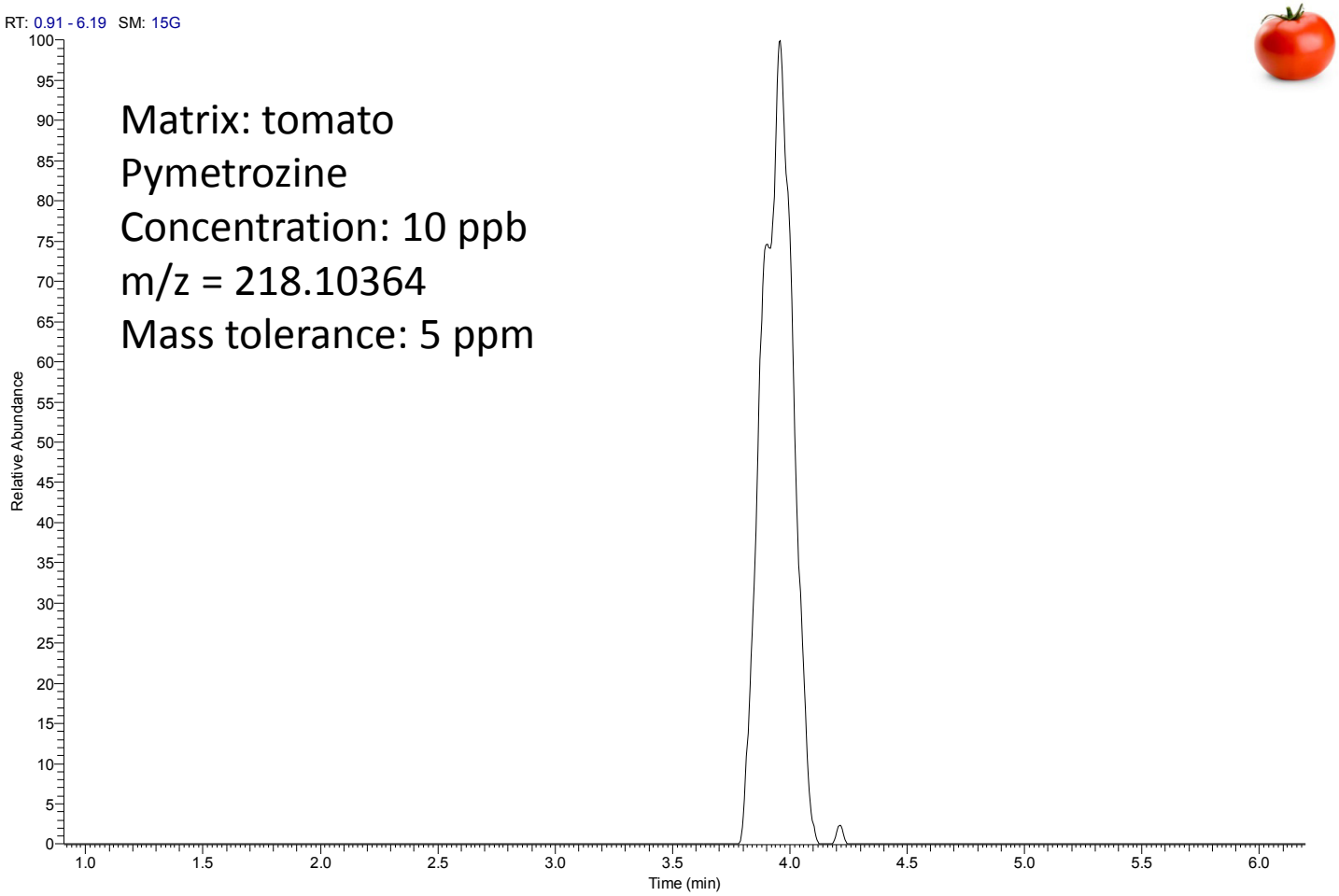
Pymetrozine 10 ppb (Dil 1/5)

m/z = 218.10364

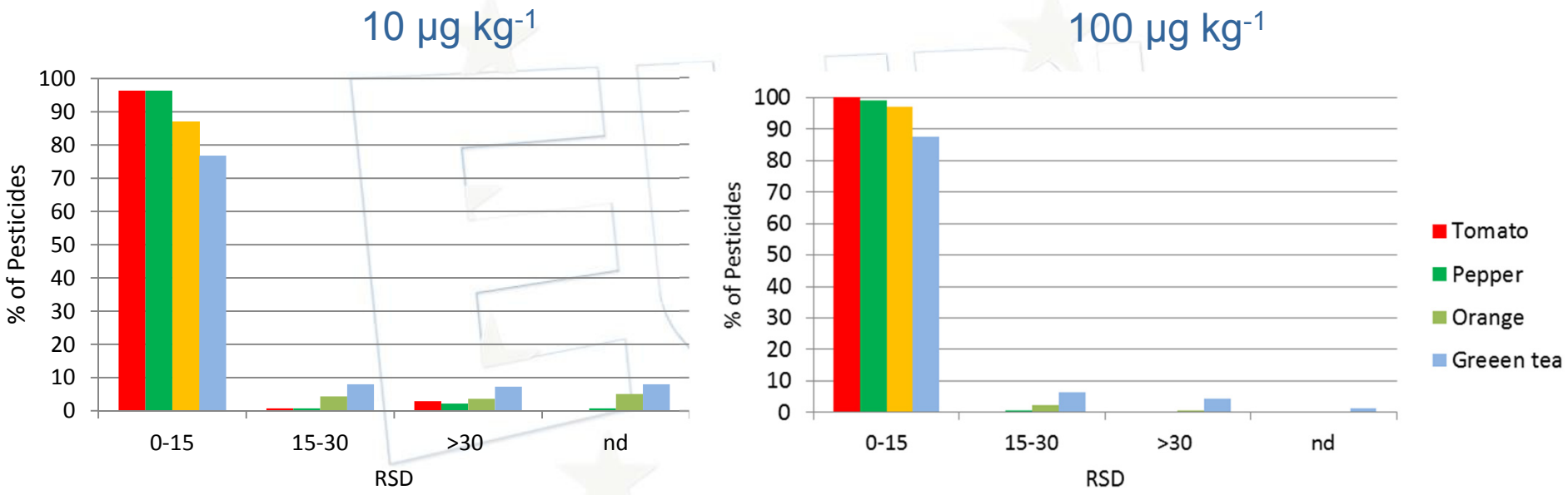
Mass tolerance: 5 ppm

Influence of resolution on peak shape
Resolution 35000

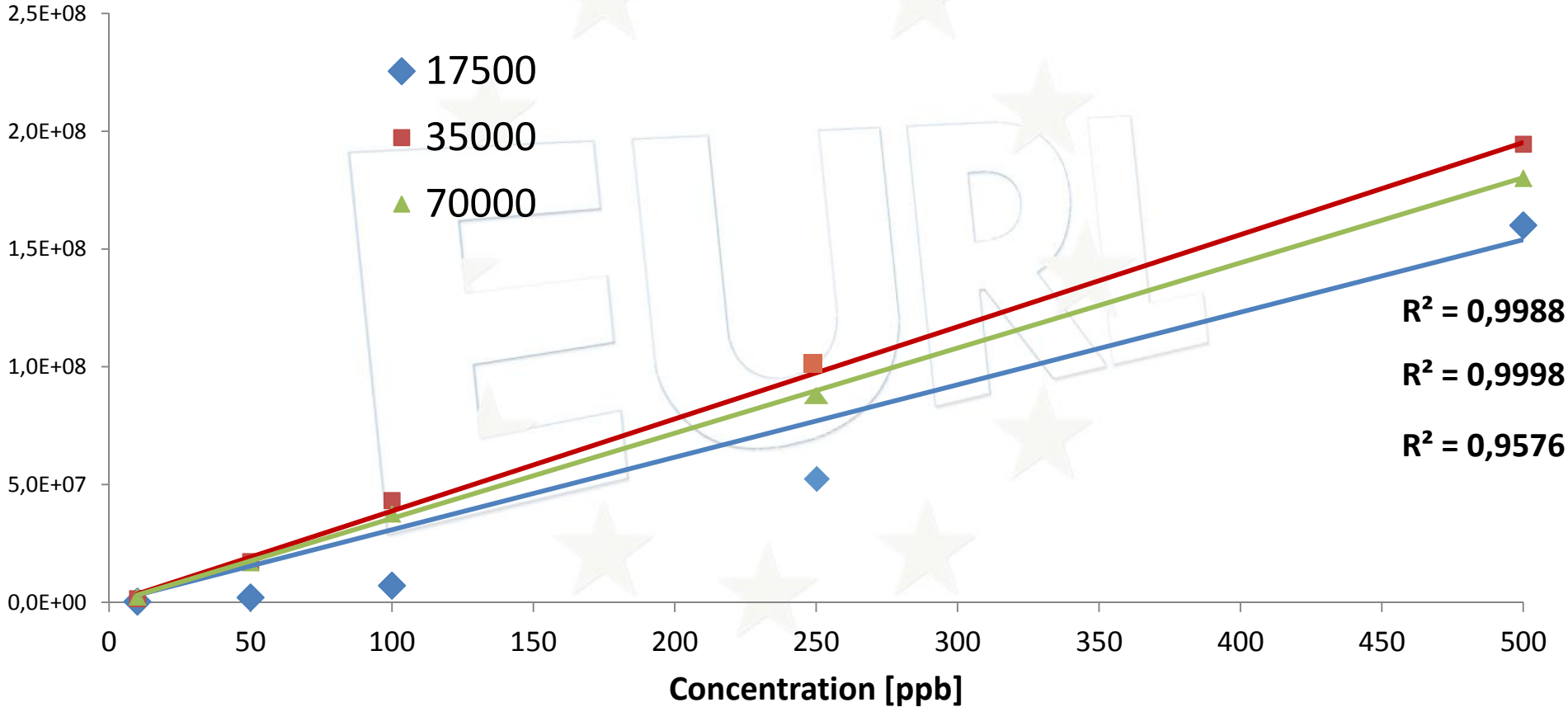
Influence of resolution on peak shape
Resolution 70000



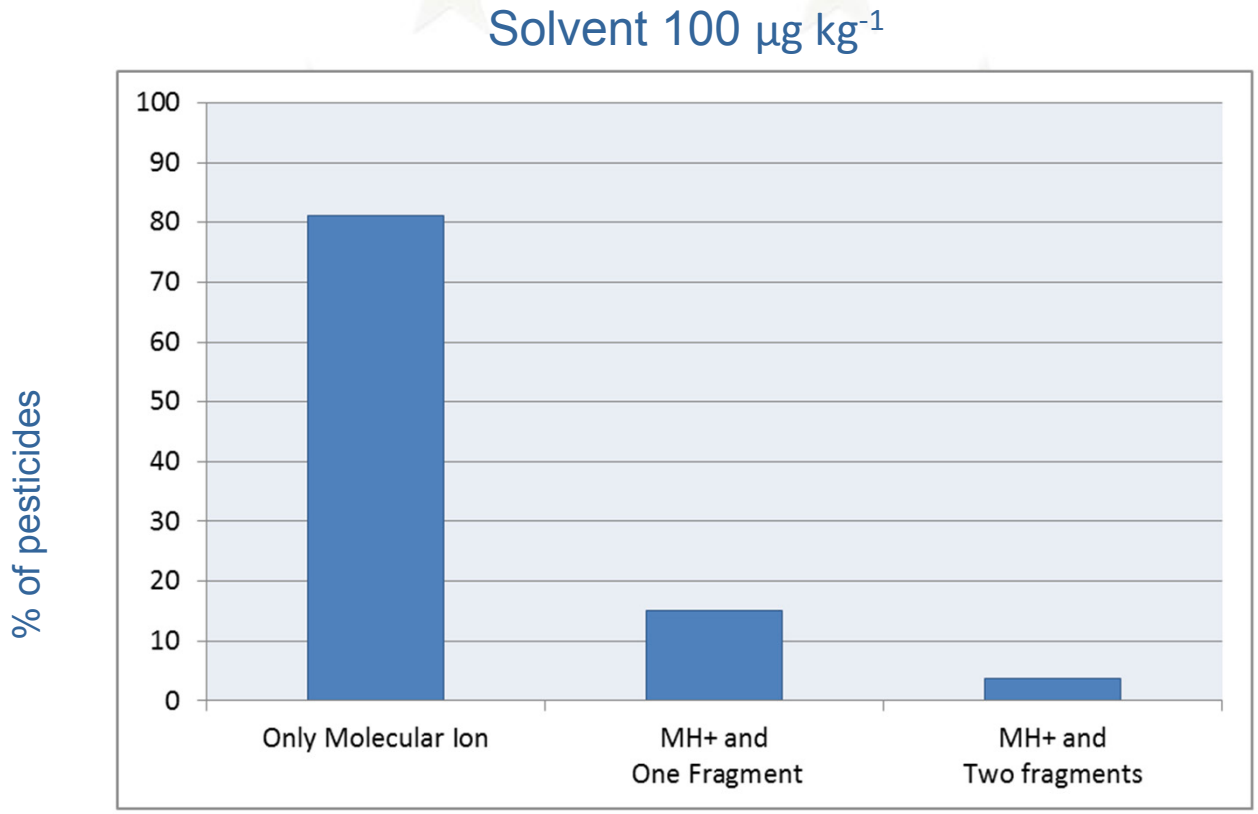
Area Reproducibility



Calibration lines of Fenbuconazol at different resolutions



Compounds with fragments > 10%
LC-HRMS *Full Scan mode*

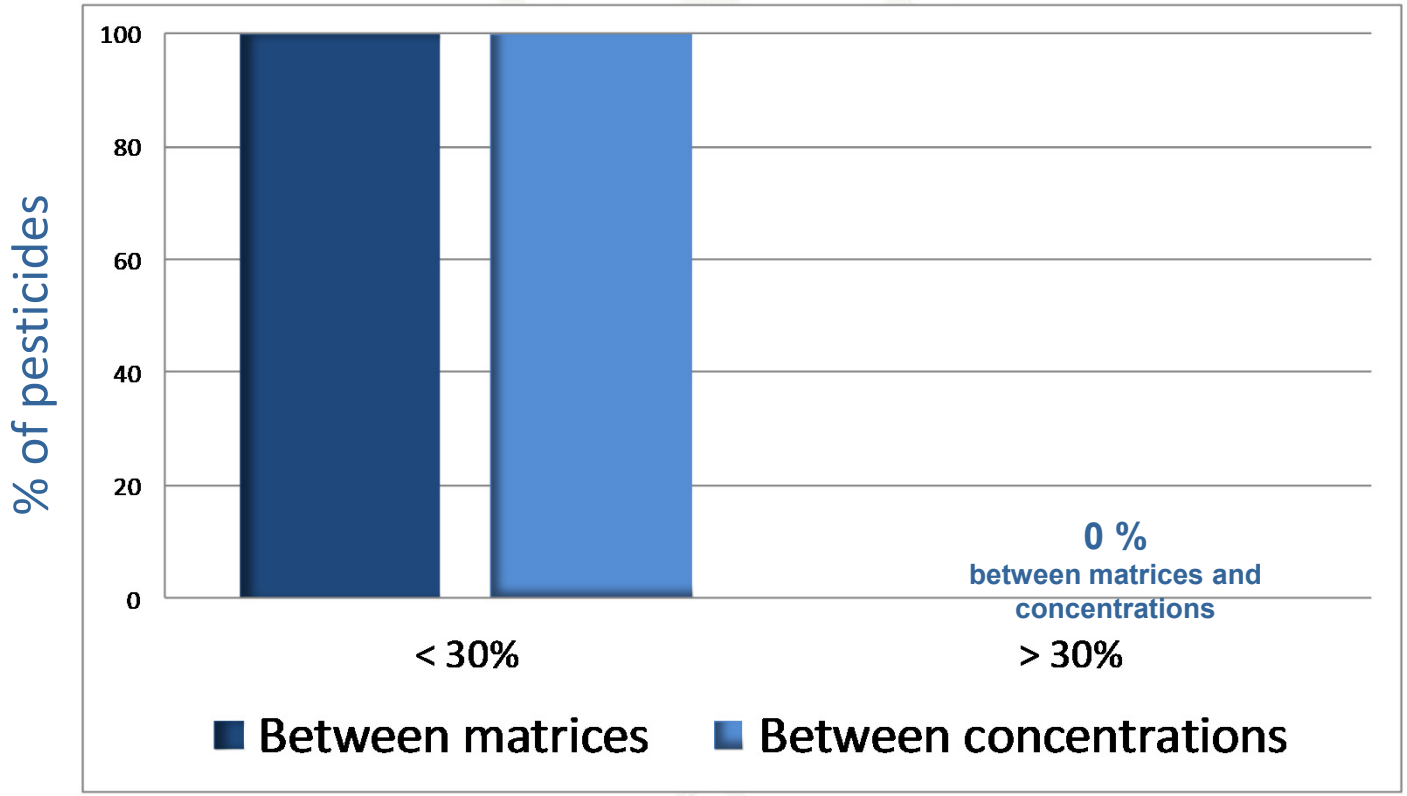


MS and MS^2 Mode Simultaneously

Pesticide residue method MS^2 data dependent mode (Full Scan/dd-MS^2)

- **Full Scan**
  - Detection/Identification (mass and retention time)
  - Quantitation
- **MS/MS**
  - Identification (at least one fragment)

Ion ratio variation (%)
Orbitrap MS^2 mode

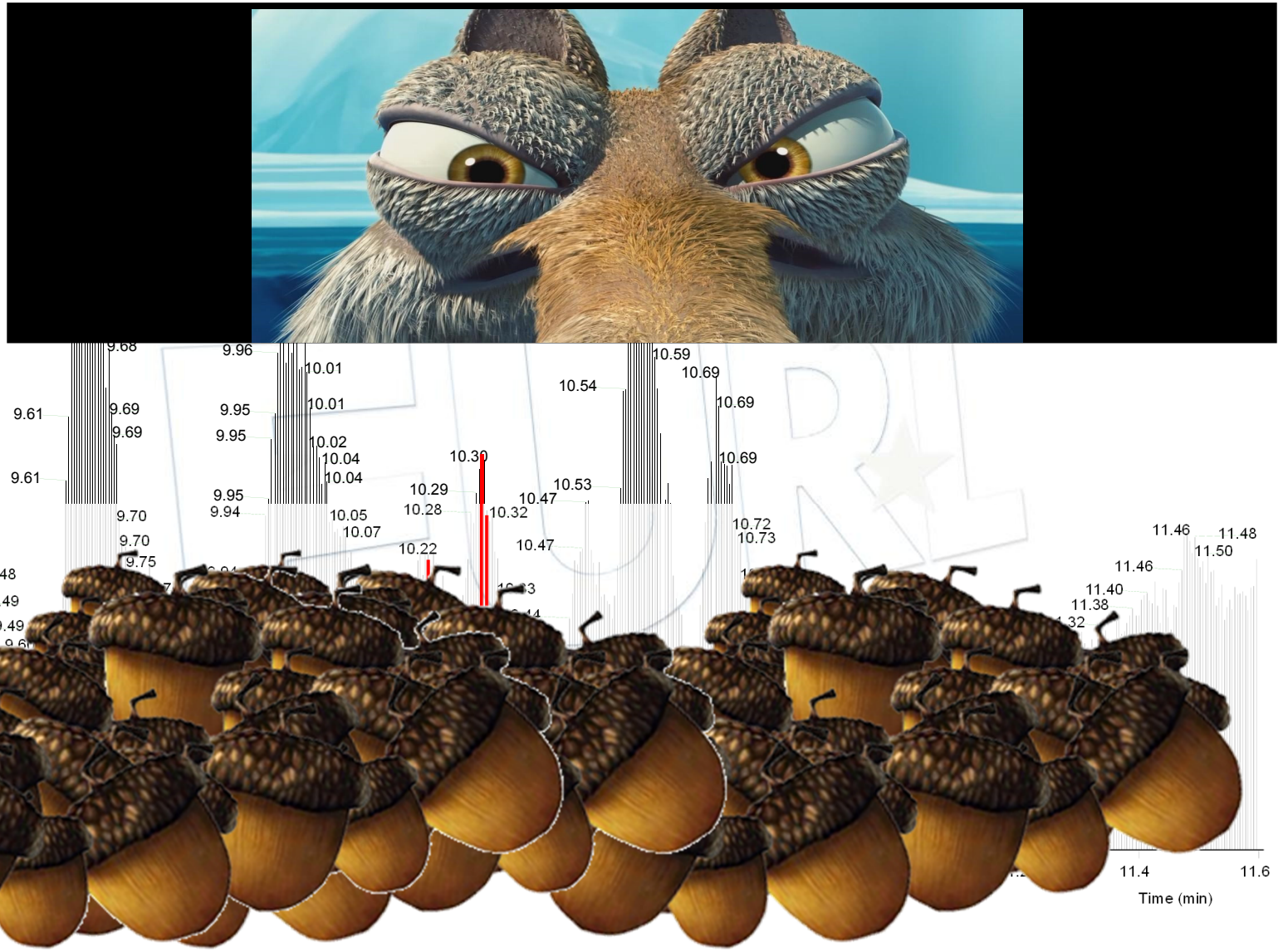


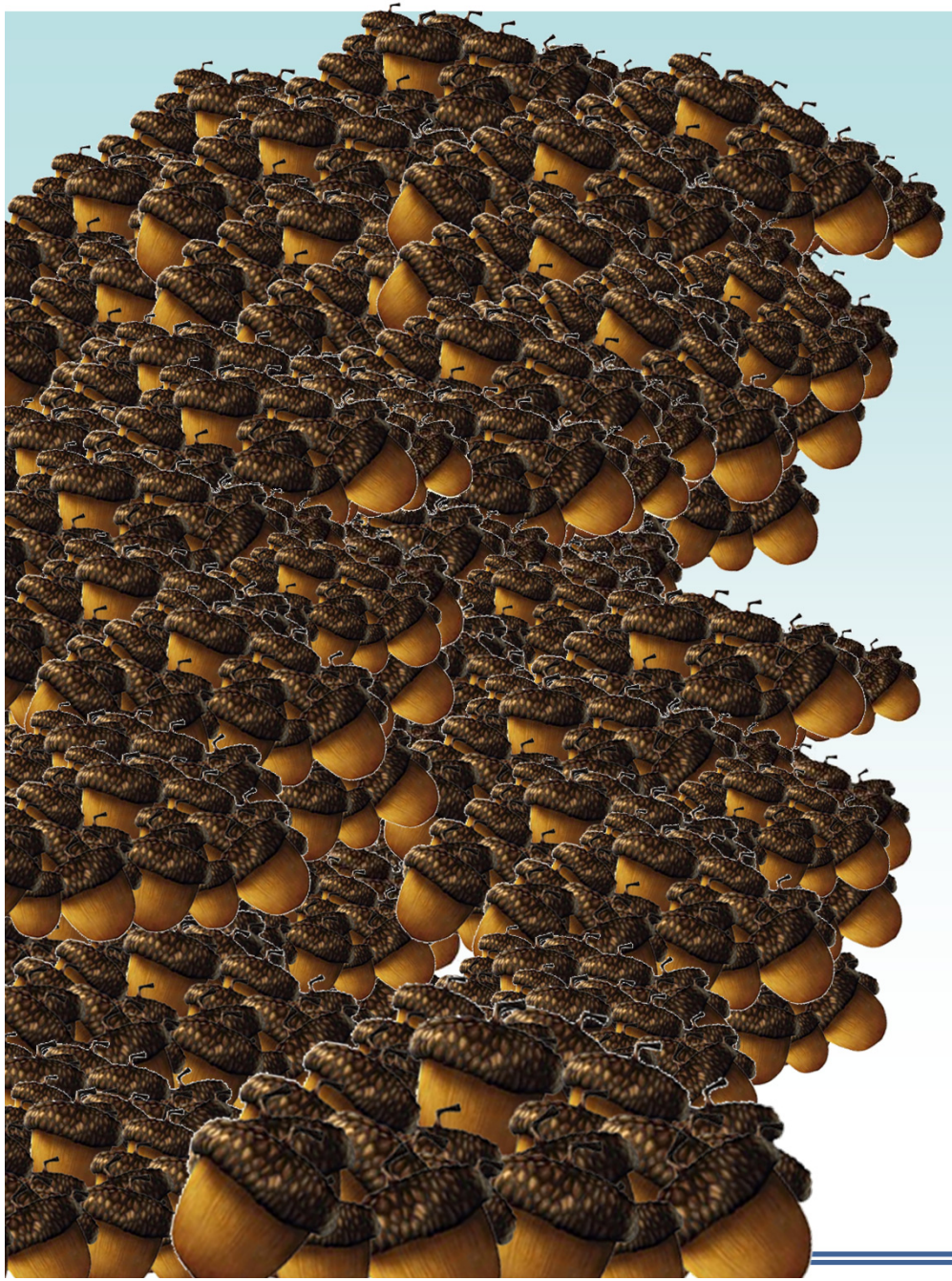
- Tomato 10 µg kg^{-1} Dil 1/5
- Orange 10 µg kg^{-1} Dil 1/5
- Tomato 100 µg kg^{-1} Dil 1/5
- Tomato 10 µg kg^{-1} Dil 1/5

HRMS/MS

Typical Poblems in screening LC-ESI-HRMS for Routine application

Mass accuracy	**≤ 5 ppm (NO PROBLEM)**
Sensitivity	**0.01 mg/kg (default value)**
Linearity	**saturation**
Reproducibility (area)	**≤ 20%**
Software+ Resolution	**False + and False -**
HRMS and HRMS/MS	***Robustness***

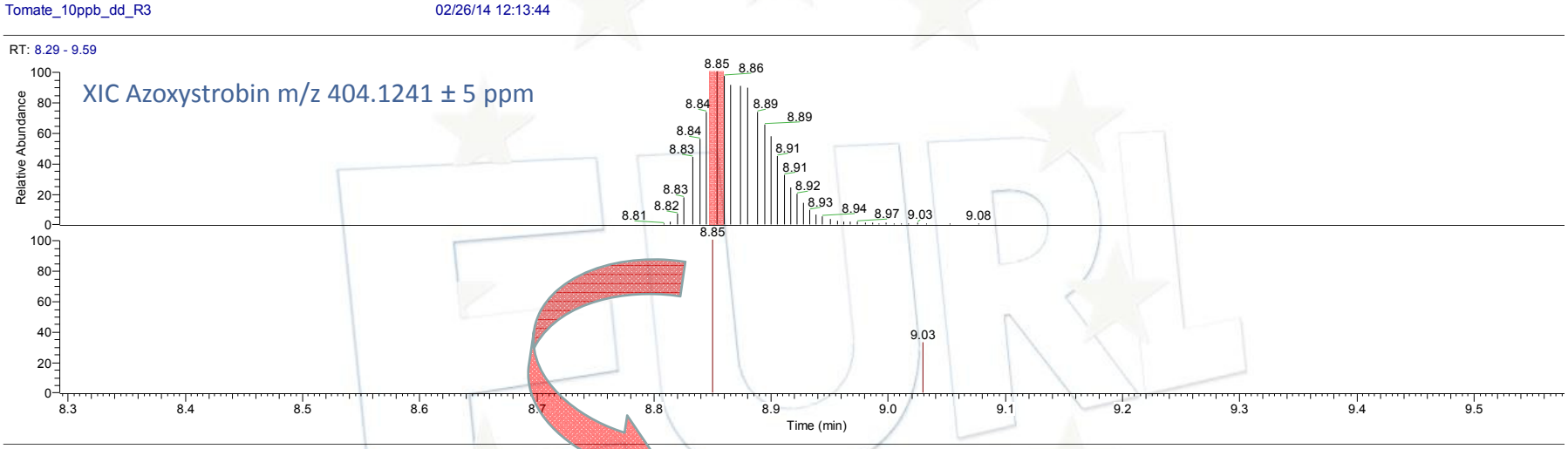

Relative Abundance
Time (min)
11.4
11.6



MS² Identification

Injection 1: Tomato matrix 10 µg/kg

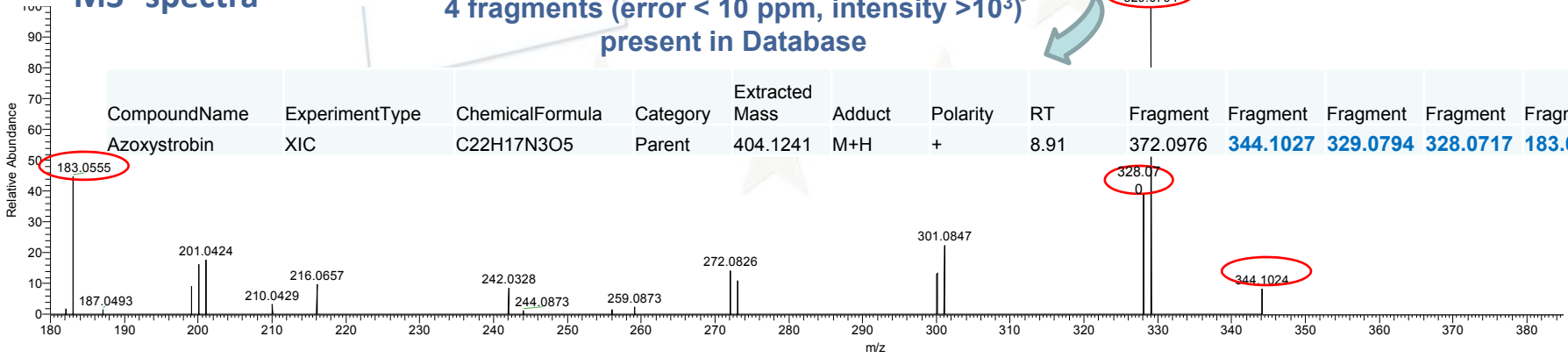
Identification of Azoxystrobin



MS² spectra

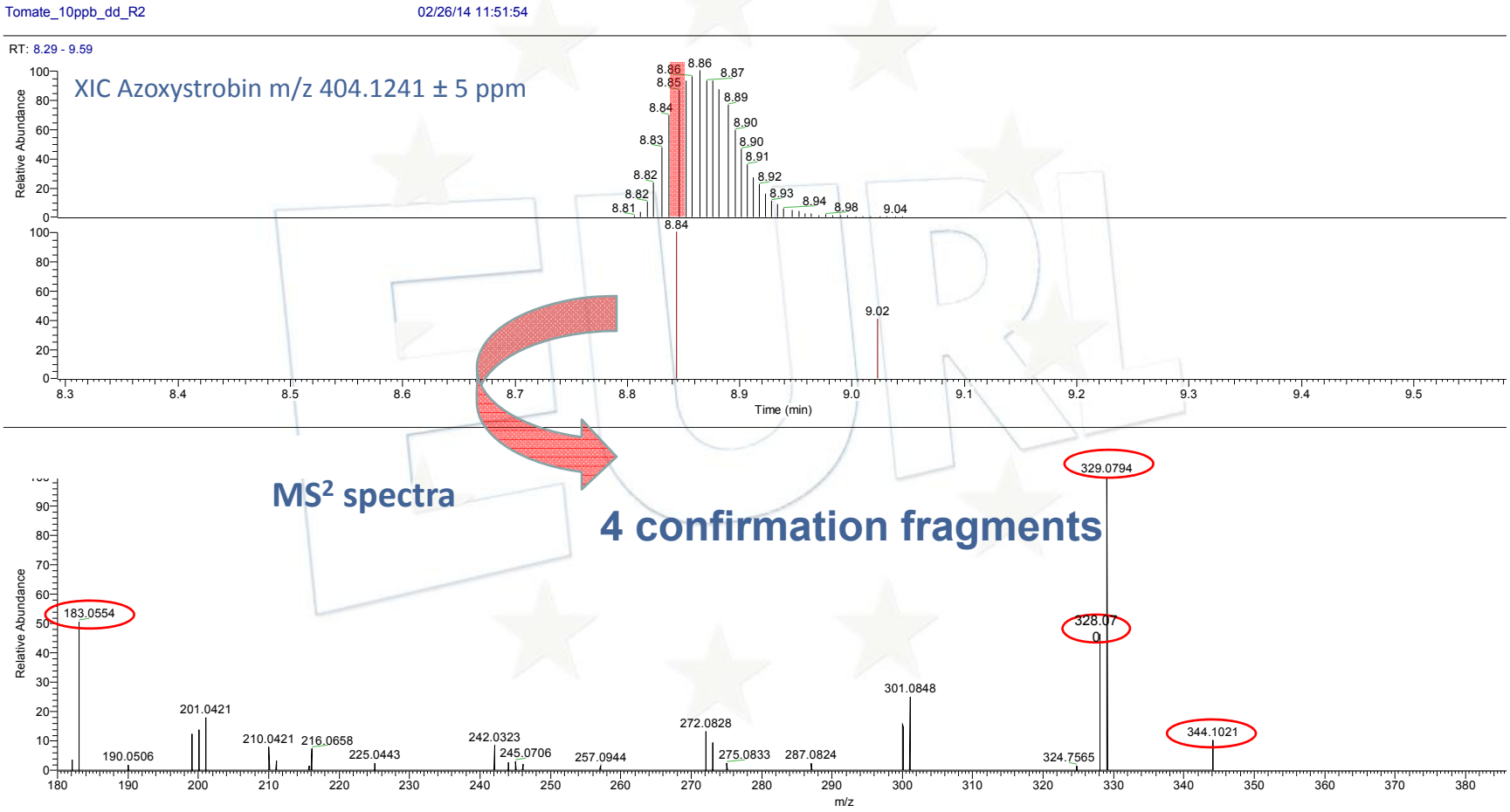
4 fragments (error < 10 ppm, intensity >10³) present in Database

CompoundName	ExperimentType	ChemicalFormula	Category	Extracted Mass	Adduct	Polarity	RT	Fragment	Fragment	Fragment	Fragment	Fragment
Azoxystrobin	XIC	C22H17N3O5	Parent	404.1241	M+H	+	8.91	372.0976	**344.1027**	**329.0794**	**328.0717**	**183.0553**



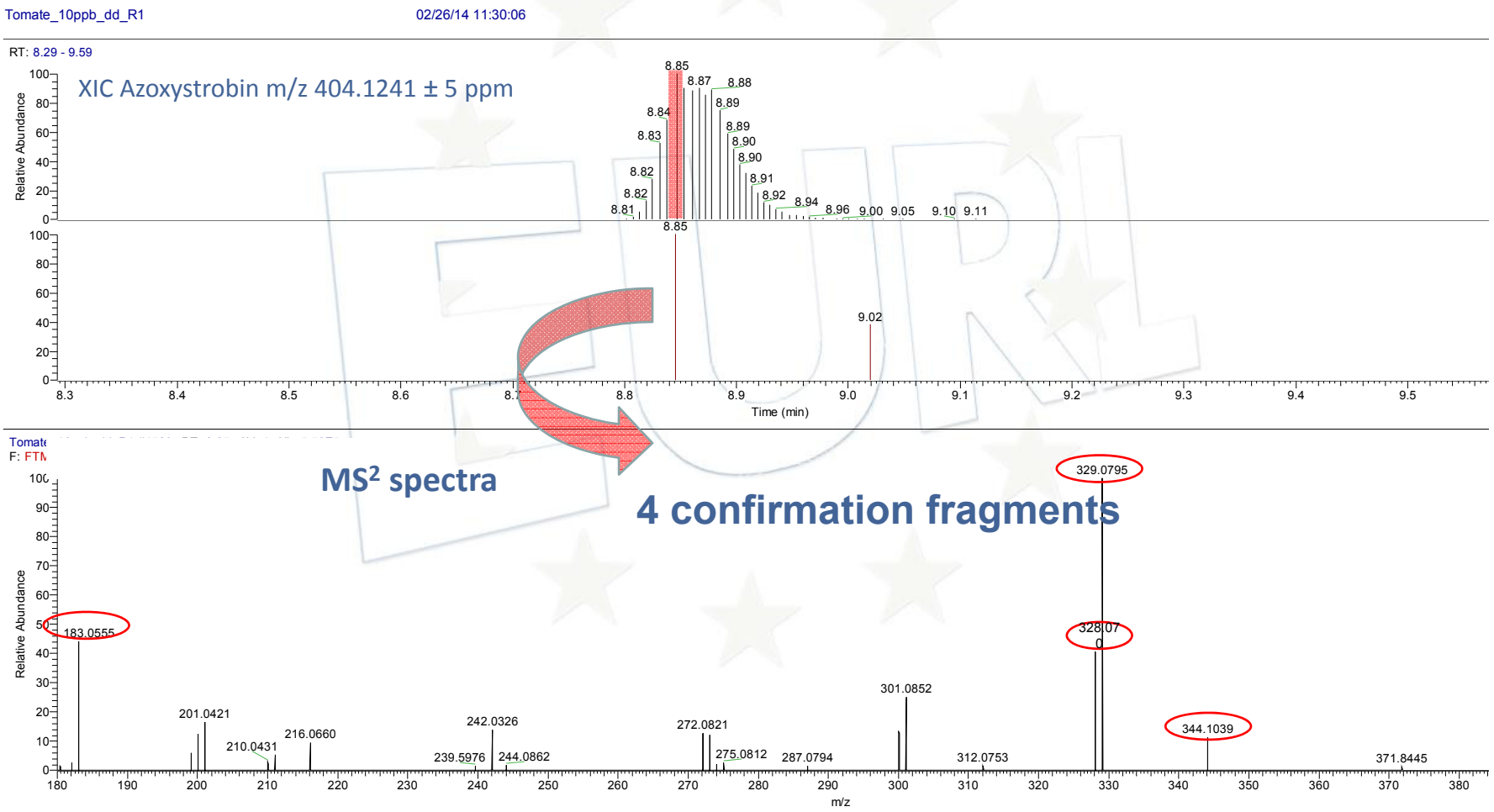
Injection 2: Tomato matrix 10 µg/kg

Identification of Azoxystrobin



Injection 3: Tomato matrix 10 µg/kg

Identification of Azoxystrobin

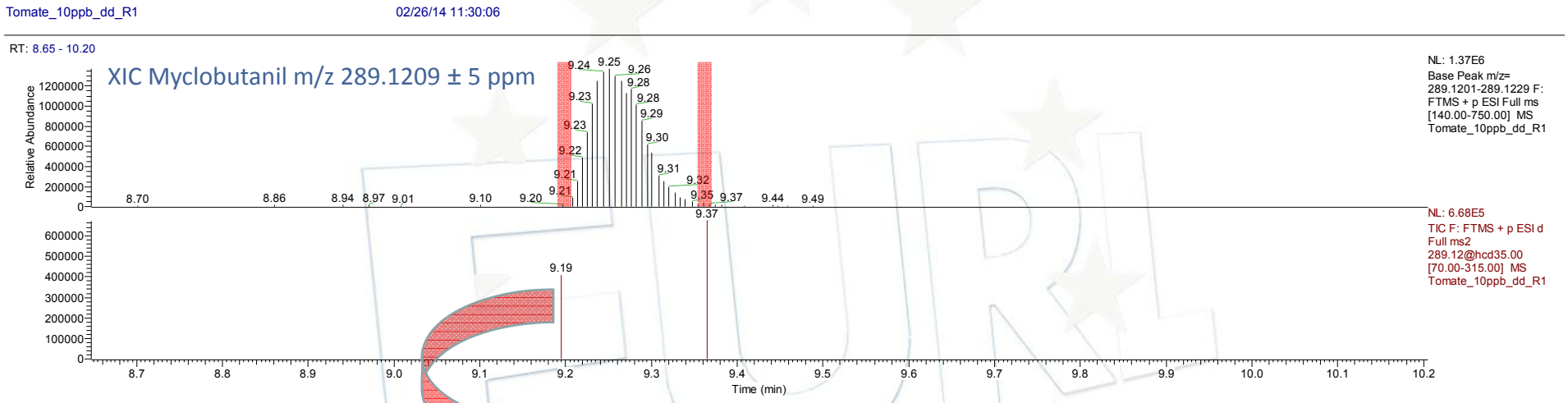


Cases of Software failure

2. Location of MS/MS data acquisition not correct

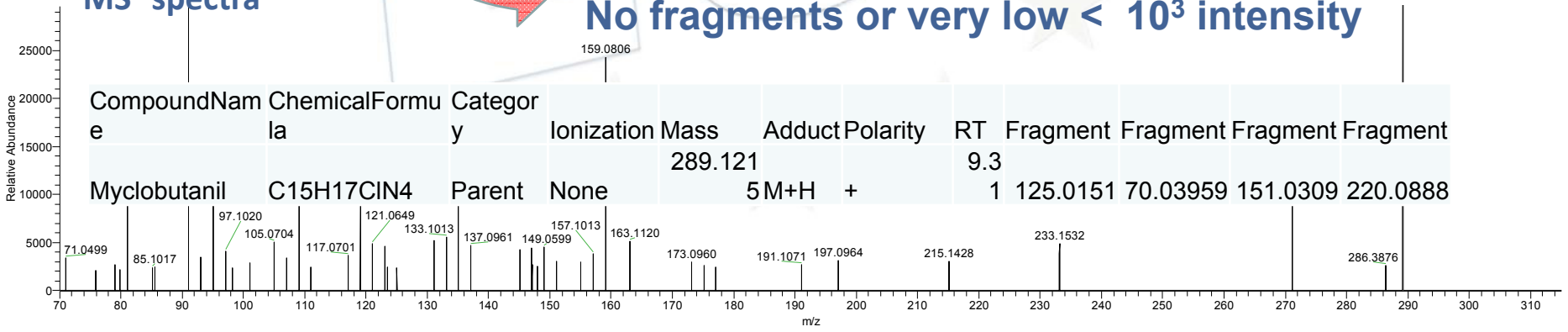
Injection 1: Tomato matrix 10 µg/kg

No identification of Myclobutanil



MS² spectra

No fragments or very low < 10^3 intensity



CompoundName	ChemicalFormula	Category	Ionization	Mass	Adduct	Polarity	RT	Fragment	Fragment	Fragment	Fragment
Myclobutanil	C15H17ClN4	Parent	None	289.1215	M+H	+	9.31	125.0151	70.03959	151.0309	220.0888

Analyitcal Method applied to:

Real samples

LC-MS/MS and LCHRMS/MS

- **20 analyzed samples**
  - 70 Identified and confirmed pesticides by MS/MS
  - 1 False negatives → 1 pesticide Fenpropathrin
  - 2 False positives detected by MS/MS

Identified Pesticides	Melon 1	Melon 2	Cucumber 1	Cucumber 2	Potato 1	Potato 2	Apple	Cauliflower	Peach	Banana	Pepper 1	Pepper 2	Papaya	Onion	Lettuce	Green Beans	Strawberry	Lima	Grapes 1	Grapes 2
Acetamiprid	61		130																	
Azoxystrobin	143	3.9	290		295											19			0.6	103
Boscalid																			134	
Bupirimate	386		791																	
Buprofezin	138		334																	
Carbendazim	108		253															12		101
Chlorpyrifos									31	48									2.2	59
Cyprodinil																			1104	242
Cyproconazole																				133
Diazinon	17		35		235															
Difenoconazole																				374
Diethofencarb									74											
Epoxiconazole										3.8										
Ethirimol	27		38																	
Ethoprophos																				0.7
Fenhexamid									804											169
Fenpropathrin									32	48										14
Fosthiazate					106															
Imazalil	79		121						2	171								5		
Imidacloprid	71	22	156						127										499	286
Iprovalicarb					100			93												
Kresoxim-methyl			302																	241
Methiocarb					238															
Penconazole			3.5																	50
Pencycuron					424															
Phenthoate								175												
Prochloraz					56													1708		
Pyraclostrobin																				146
Propiconazole									0.6											
Pyrimethanil	576		1268																1662	70
Quinoxyfen																				67
Tebuconazole	190		445																	253
Thiophanate-methyl													1142							
Triadimefon													2.6							1.4

Screening LC-ESI-HRMS for Routine application (70,000)

Mass accuracy	**≤ 5 ppm**
Sensitivity	**0.01 mg/kg**
Linearity	**no saturation**
Reproducibility + linearity	**≤ 20% +10-500 ppb**
Low ion ratio rep.	**≤ 30%**

Further improvements

Software

Evaluation in process

Robustness

DG SANCO - EUROPEAN COMMISSION - 2013

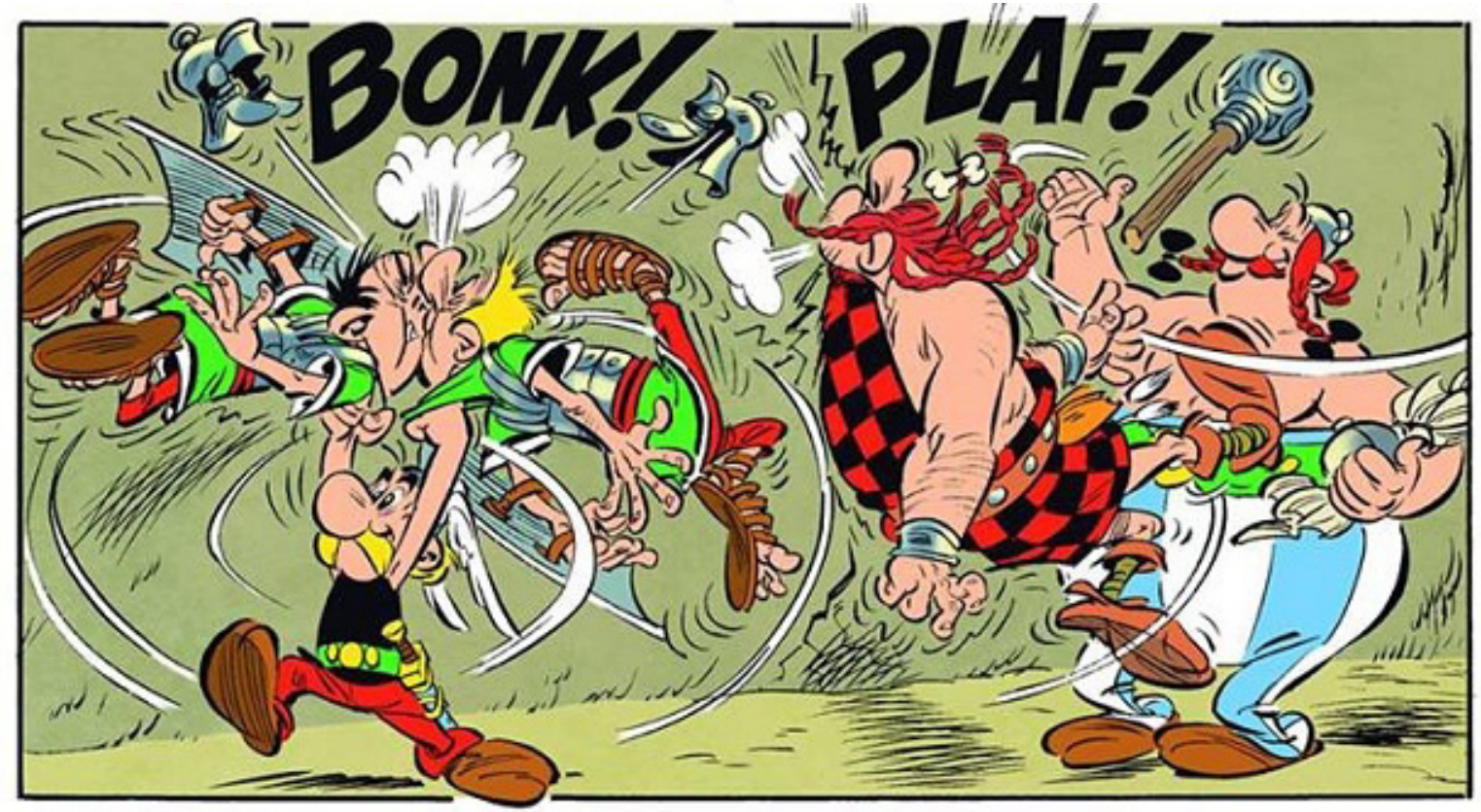
European Commission

Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed

Document N° SANCO/12571/2013

LC-MS/MS
Low Resolution

LC-HRMS
High Resolution


BONK!
PLAF!

eurl-pesticides.eu

Thank You
for Your Attention