EURL-FV EXPERIENCES ON THE EVALUATION OF ACCURATE MASS PLATFORMS FOR PESTICIDES RESIDUES ANALYSIS IN FRUITS AND VEGETABLES

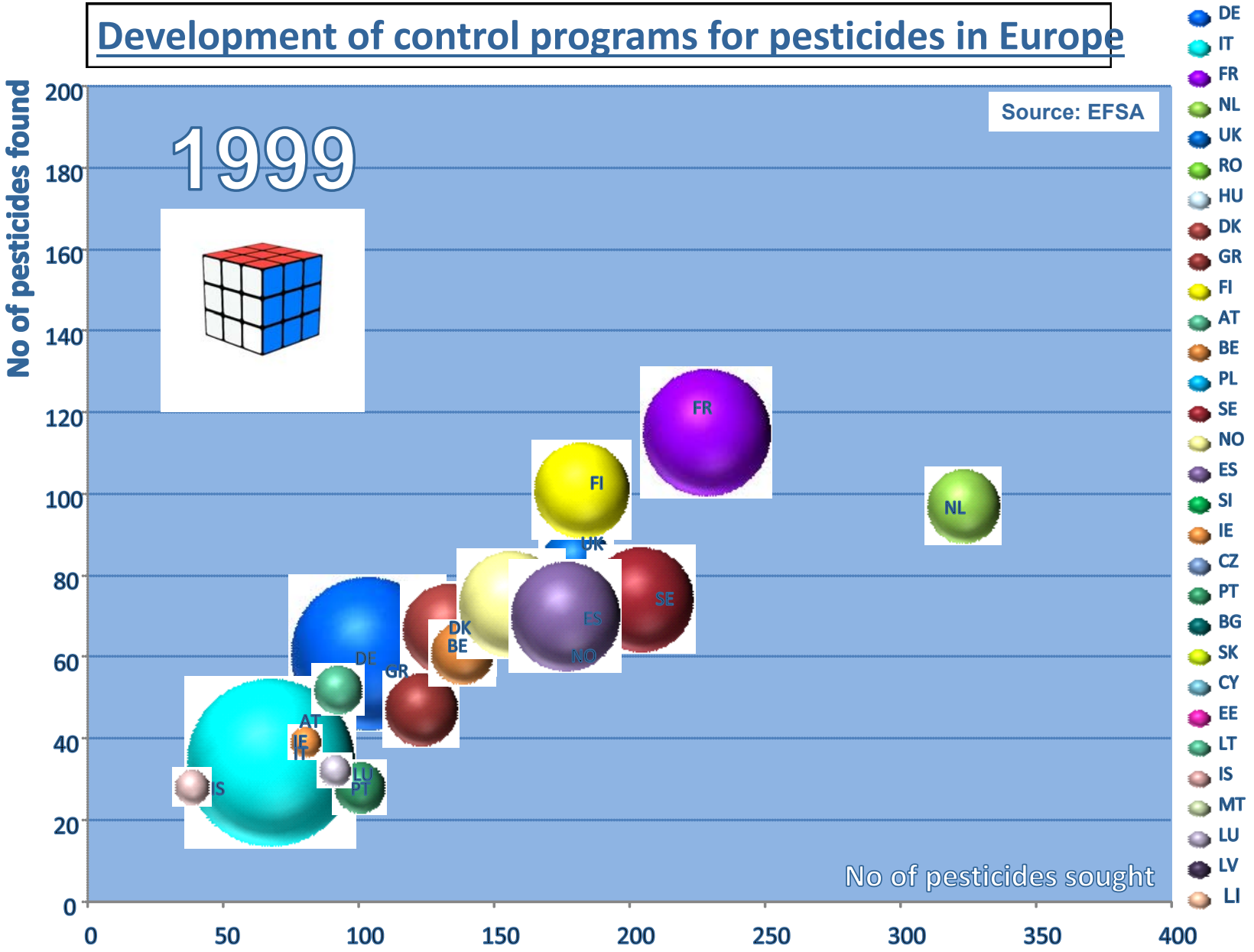
Limassol CYPRUS
26th May 2016

AMADEO R. FERNÁNDEZ-ALBA

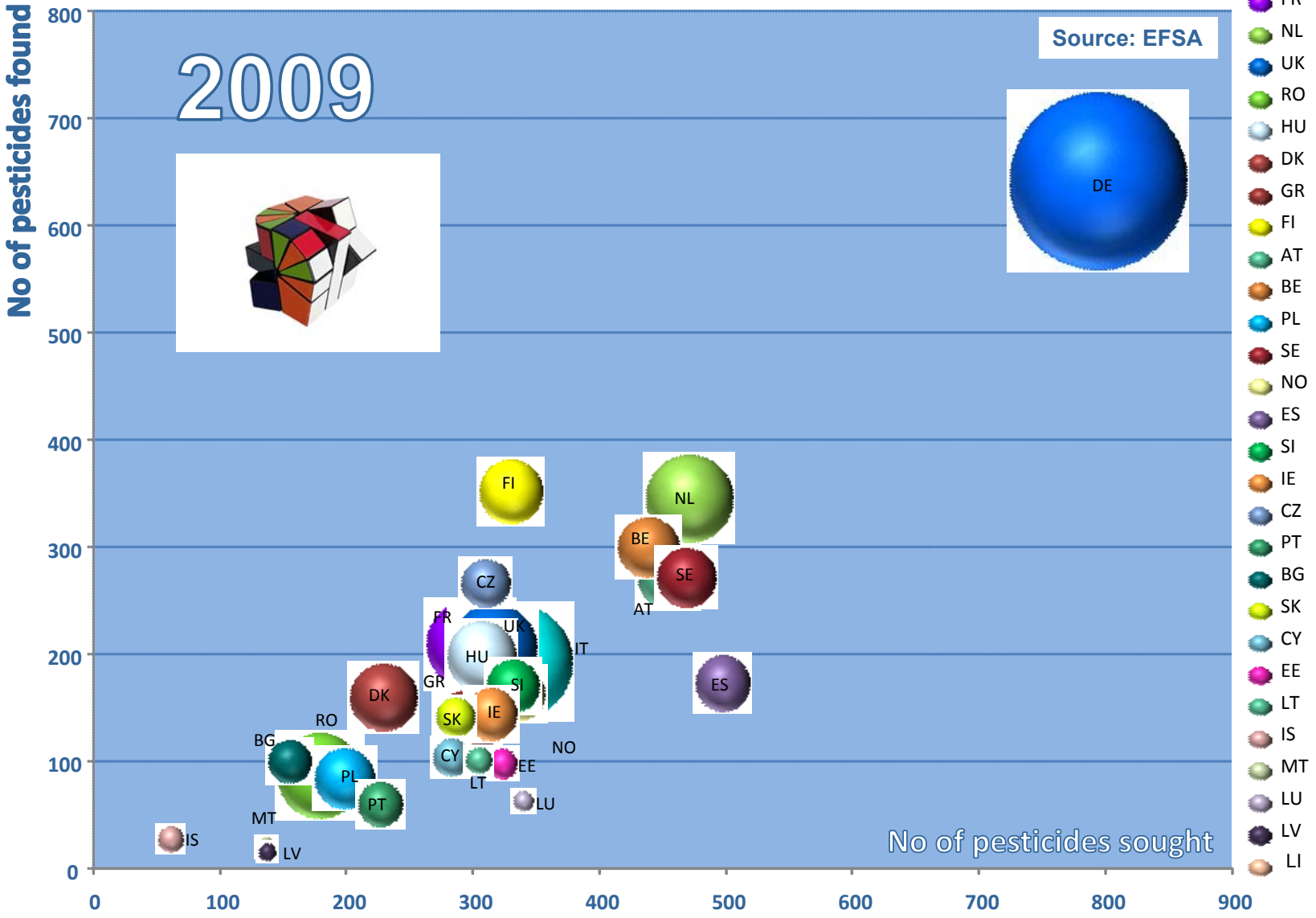
LC-HRAMS

WHY ?
BENEFITS ?

Development of control programs for pesticides in Europe



Development of control programs for pesticides in Europe



Target list: 62 pesticides

EUPT-FV07 (2005)

PESTICIDES	Nº of reported results	Nº of NA (not sought)	False negatives	% of NA results from the total 125
Acetamiprid	56	67	2	54
Carbaryl	101	24	0	19
Cyprodinil	99	24	2	19
Diazinon	123	2	0	2
Dimethoate	119	2	4	2
Fenhexamid	89	36	0	2
Fludioxonil	85	36	4	29
Imidacloprid	64	60	1	48
Iprodione	113	8	4	6
Kresoxim-methyl	104	20	1	16
Methomyl	71	45	9	36
Monocrotophos	89	30	5	24
Procymidone	121	4	0	3
Pyrimethanil	98	25	2	20
Tetraconazole	70	49	6	39
Thiabendazole	104	17	3	14

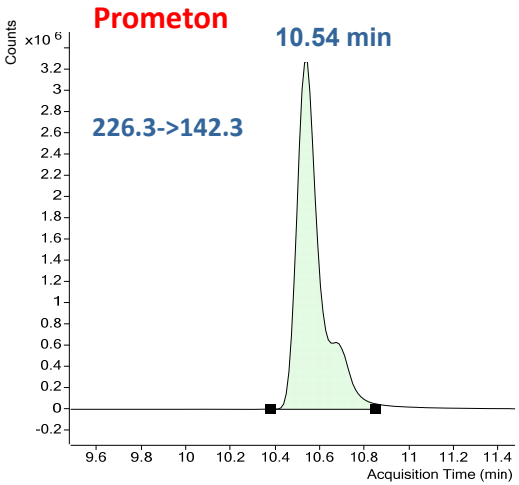
EUPT-FV07 (2005)

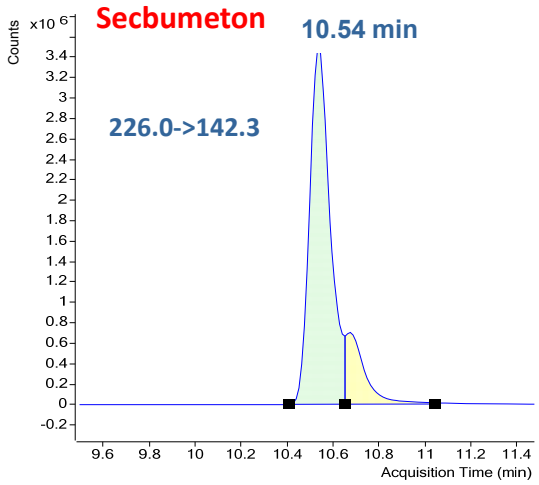
Laboratories that reported false positives

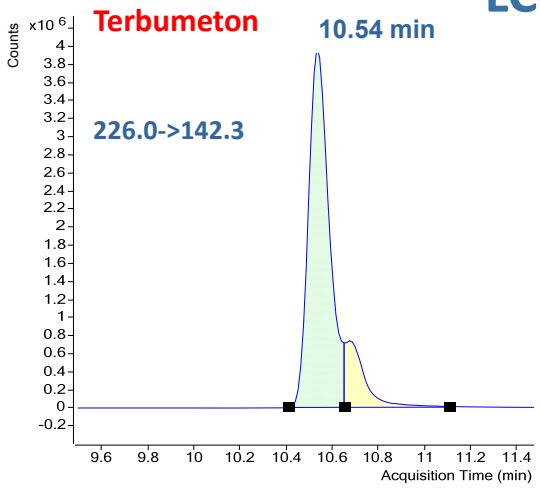
Pesticide	Lab Code	Sample	Concentration (mg/kg)	RL (mg/kg)	MPRL (mg/kg)
Chlorpyrifos	EUPT-7 116	Test	0.0016	0.01	0.05
Dichlofluanid	EUPT-7 097	Test	0.013	0.05	0.05
Dichlofluanid	EUPT-7 122	Test	0.103	0.006	0.05
Endosulfan	EUPT-7 125	Test	0.106	0.0034	0.05
Methidathion	EUPT-7 126	Test	0.176	-	0.02
Myclobutanil	EUPT-7 049	Test	0.26	0.01	0.02
Myclobutanil	EUPT-7 125	Test	0.005	0.0006	0.02
Vinclozolin	EUPT-7 097	Test	0.022	0.05	0.05

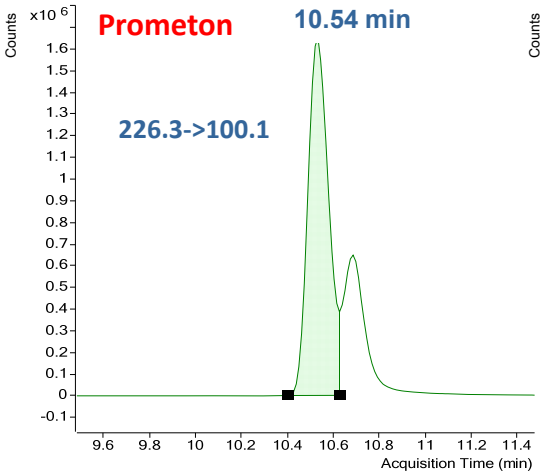
Common Transitions

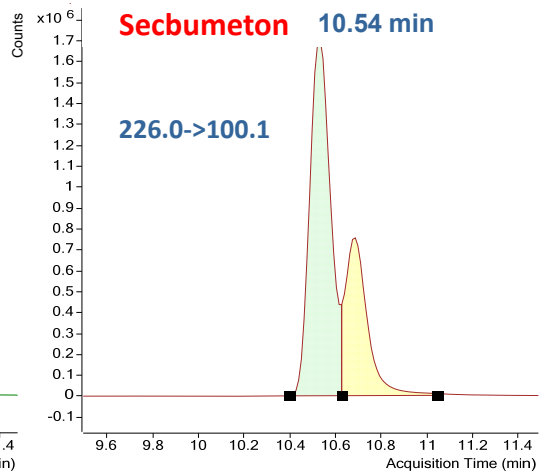
LC-QqQ-MS

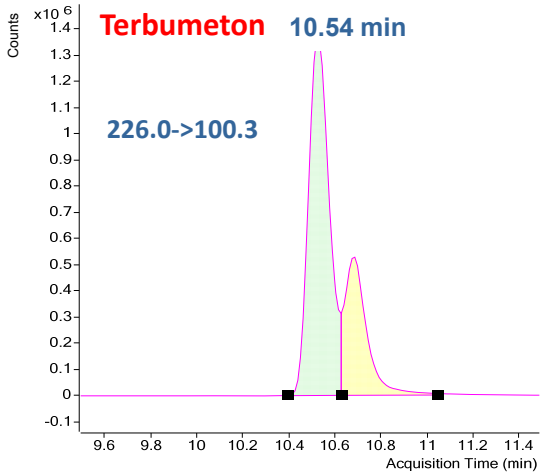




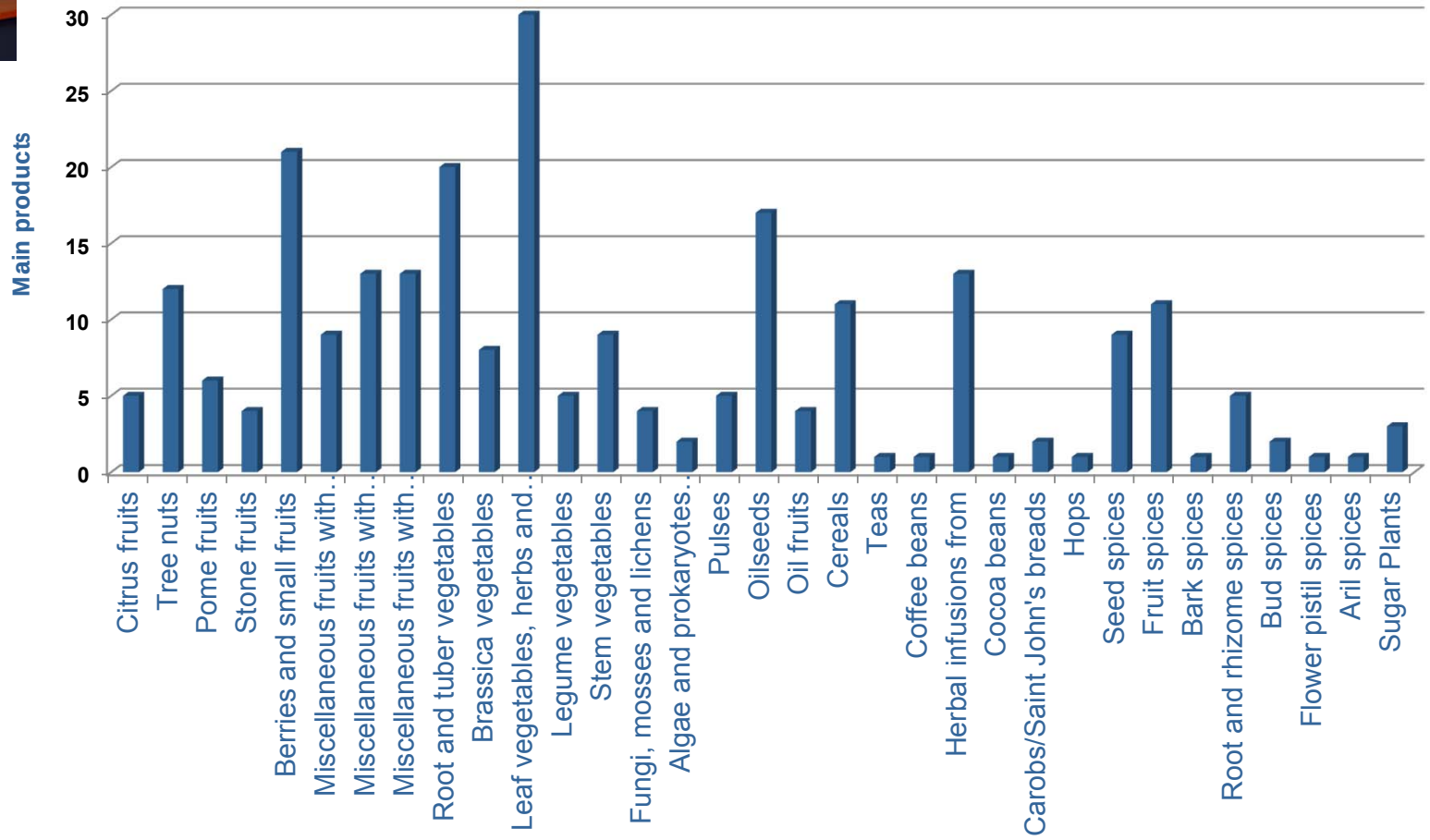






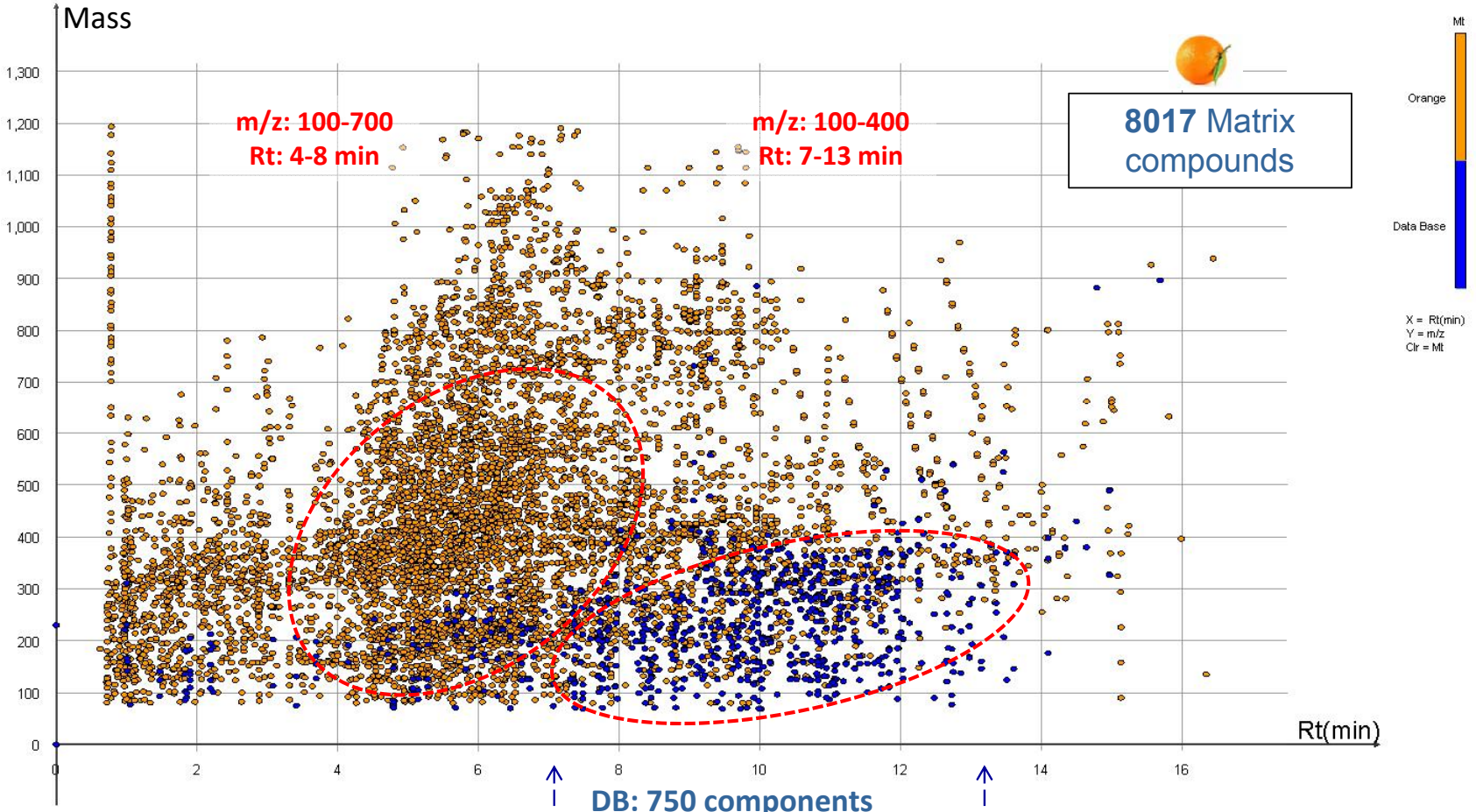


Plant Origin Matrix Groups



Total number: 250 matrices

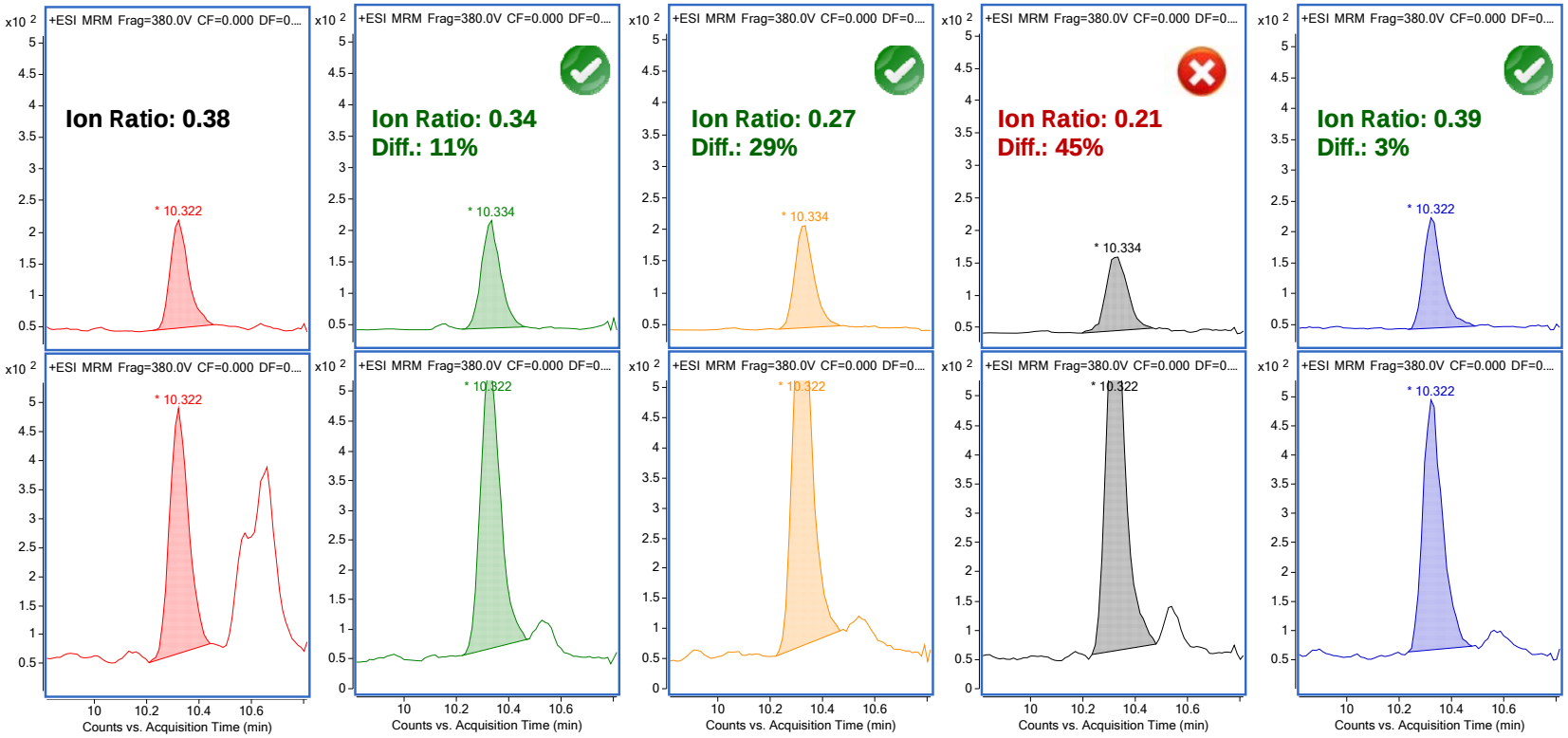
Data base components- **orange** matrix compounds (1 g/mL)



Miner 3D Enterprise

Orange: 2743 matrix compounds

Haloxyfop at 0.01 mg/kg in Aubergine. LC-MS/MS

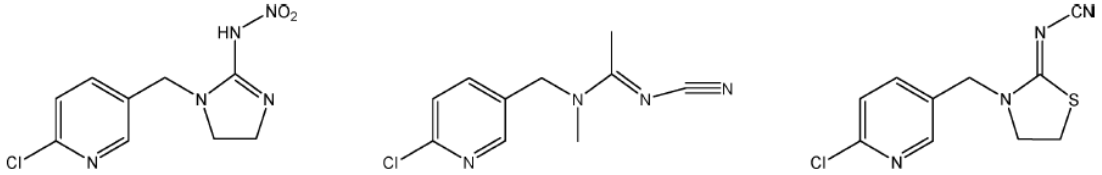


Anal. Chem. **2005**, *77*, 2818−2825

Quantitation and Accurate Mass Analysis of Pesticides in Vegetables by LC/TOF-MS

Imma Ferrer,* E. Michael Thurman, and Amadeo R. Fernández-Alba

Pesticide Residue Research Group, University of Almeria, 04120 Almeria, Spain



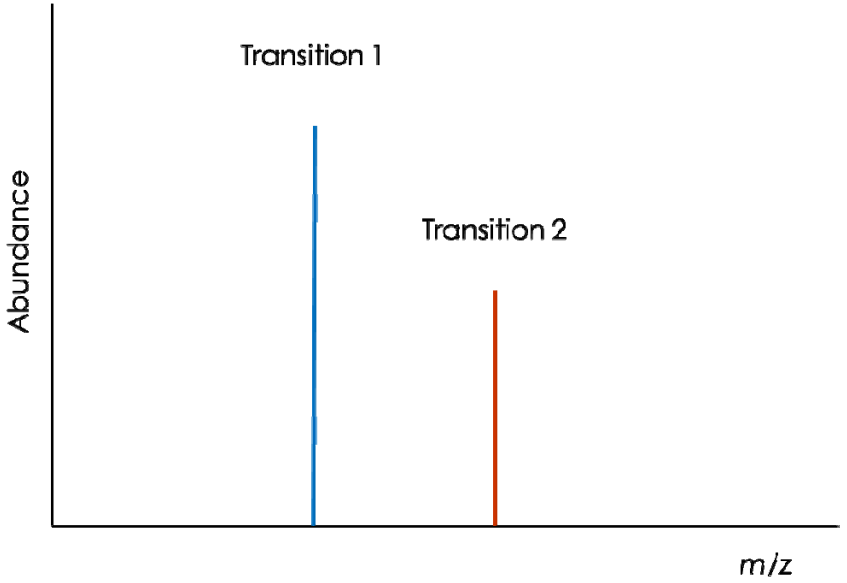
Imidacloprid m/z 256 Acetamiprid m/z 223 Thiacloprid m/z 253

Figure 1. Chemical structures of imidacloprid, acetamiprid, and thiacloprid.

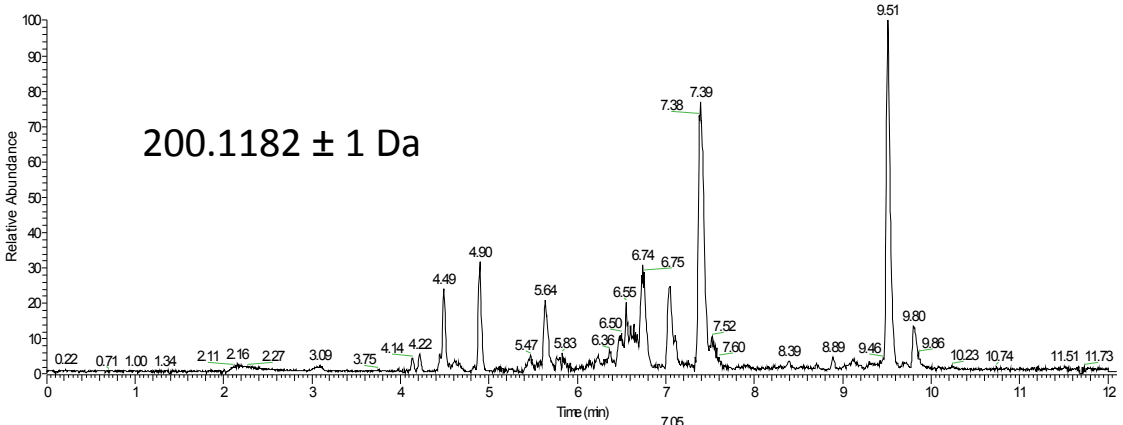
Table 2. LC/TOF-MS Accurate Mass Measurements for the Chloronicotinyl Pesticides and Their Fragments in a Tomato-Matched Matrix

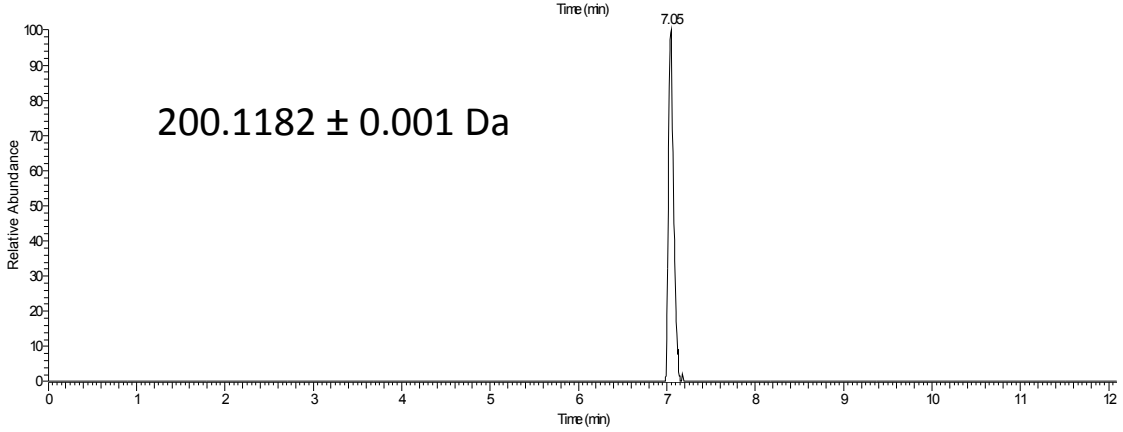
			concentration (0.05 mg/kg)		concentration (0.5 mg/kg)	
compound	elemental composition	theoretical mass	measured mass	error (ppm)	measured mass	error (ppm)
imidacloprid	$C_9H_{11}N_5O_2Cl$	256.0596	256.0596	0.1	256.0597	0.5
	$C_9H_{11}N_4Cl$	210.0667	210.0663	−1.8	210.0664	−1.3
	$C_9H_{10}N_4Cl$	209.0589	209.0587	−0.7	209.0587	−0.7
	$C_9H_{11}N_4$	175.0978	175.0983	2.7	175.0977	−0.7
acetamiprid	$C_{10}H_{12}N_4Cl$	223.0745	223.0746	0.5	223.0749	1.8
	C_6H_5NCl	126.0105	126.0106	0.8	126.0105	0.0
thiacloprid	$C_{10}H_{10}N_4SCl$	253.0309	253.0311	0.7	253.0313	1.5
	C_6H_5NCl	126.0105	126.0107	1.6	126.0103	−1.6

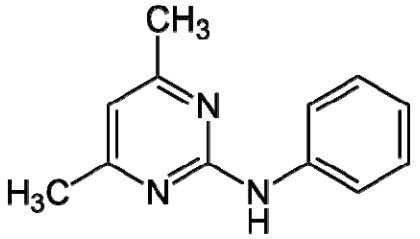

1 Da


Transition 1
Transition 2
Abundance
m/z

Pyrimethanil at 0.01 mg/kg

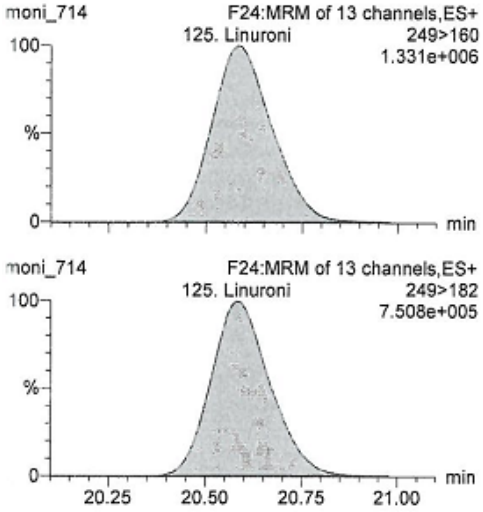




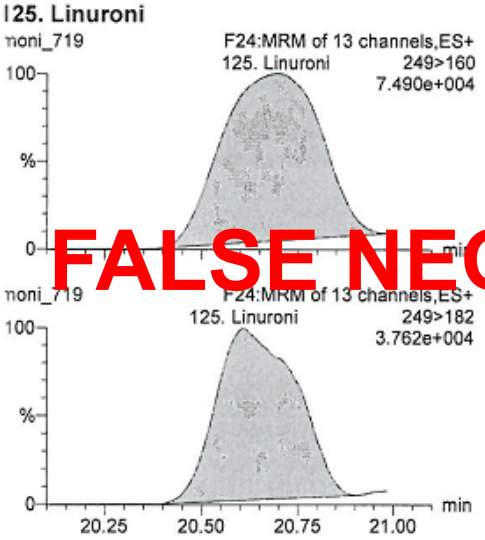


Pyrimethanil
[M+H$^+$] m/z 200.1182
0.01 mg/ kg in onion
RT = 7.05 min
Dilution 5x

EUPT Sample: coriander



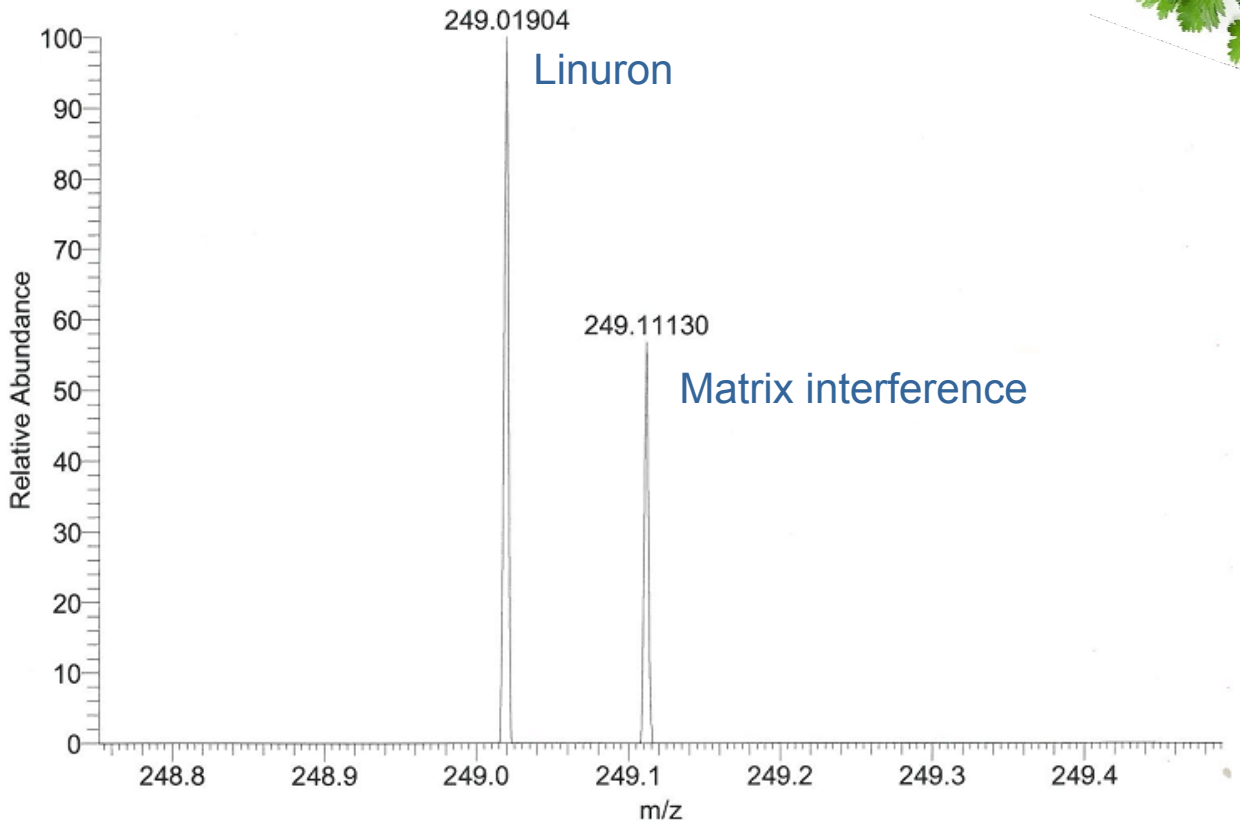
Linuron
Standard in solvent
Ion ratio: 1.8



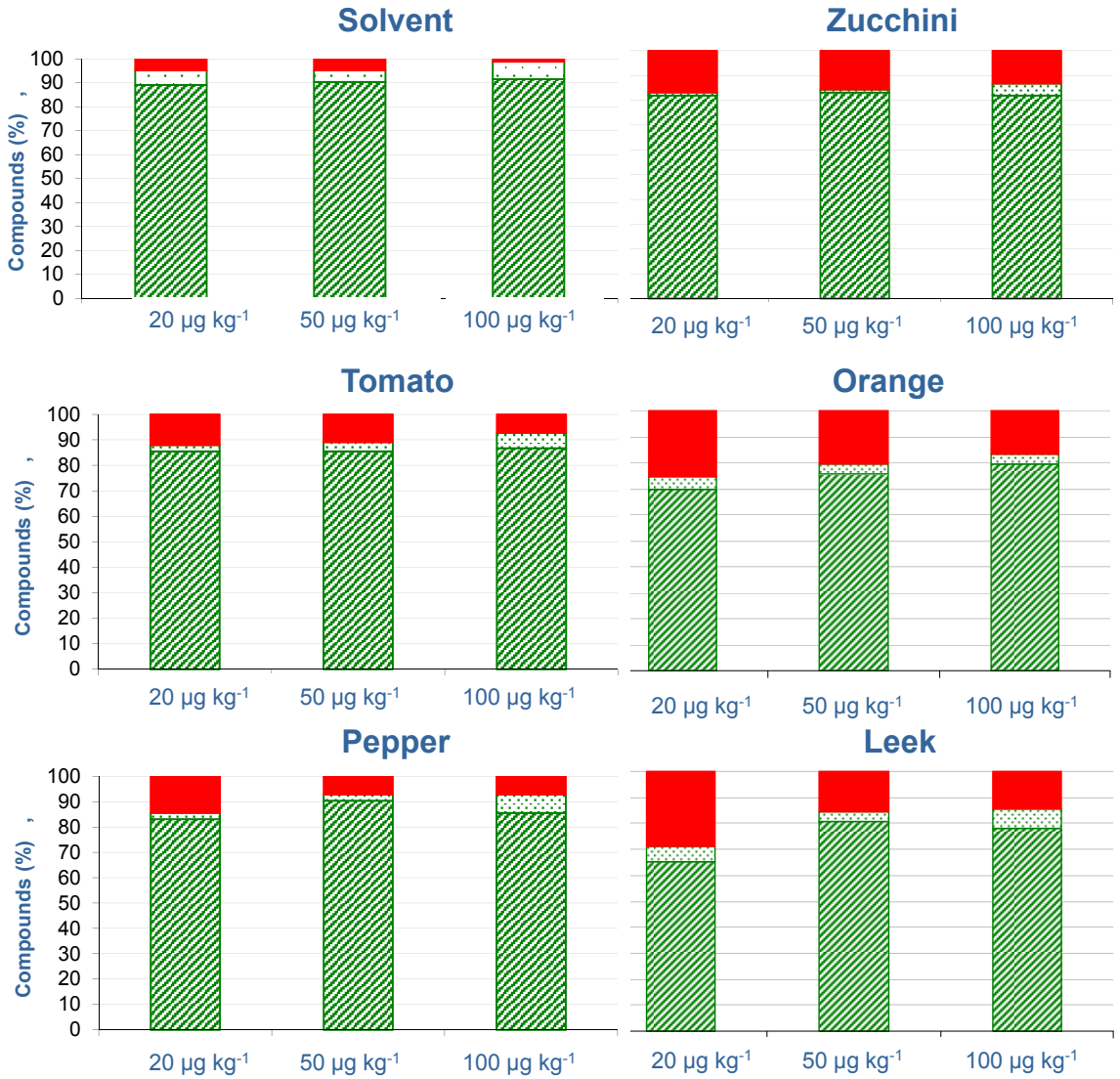
FALSE NEGATIVE

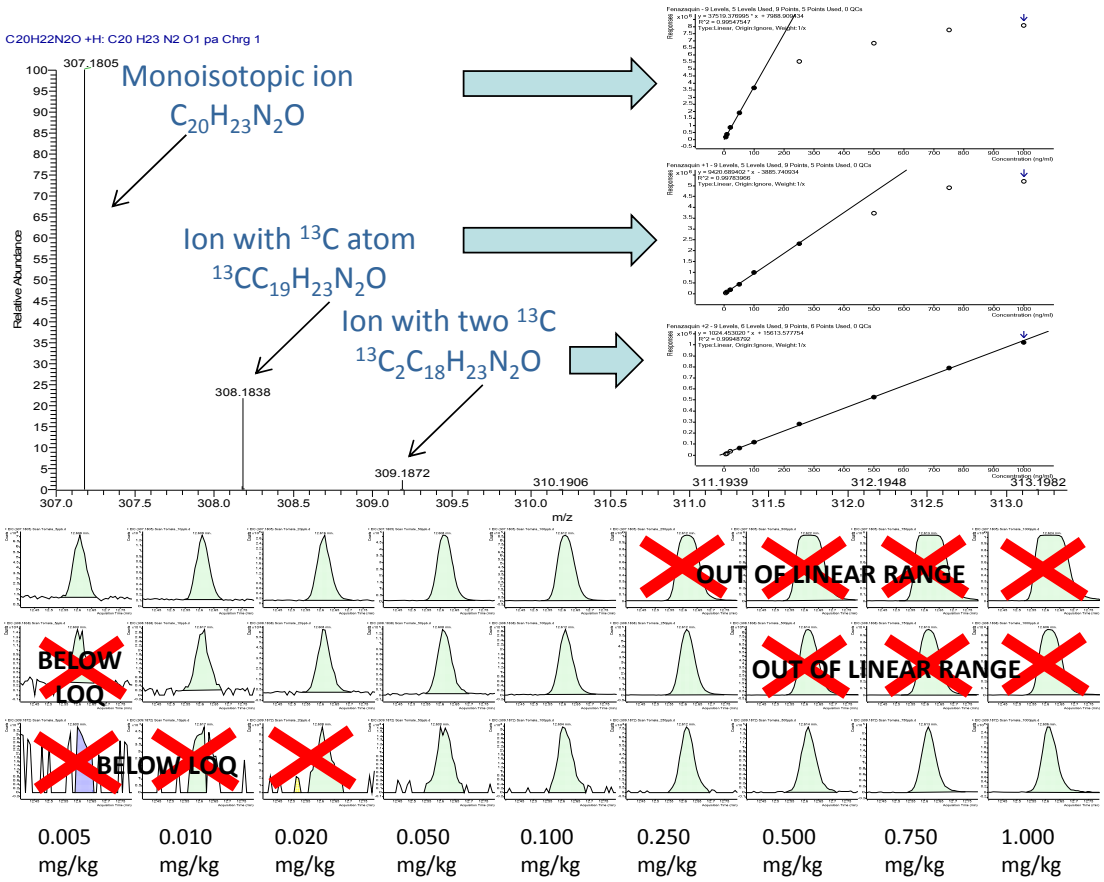
Linuron (0.125 mg/kg)
Real sample of coriander
Ion ratio: 2.4

EUPT Sample of coriander



BUT…..

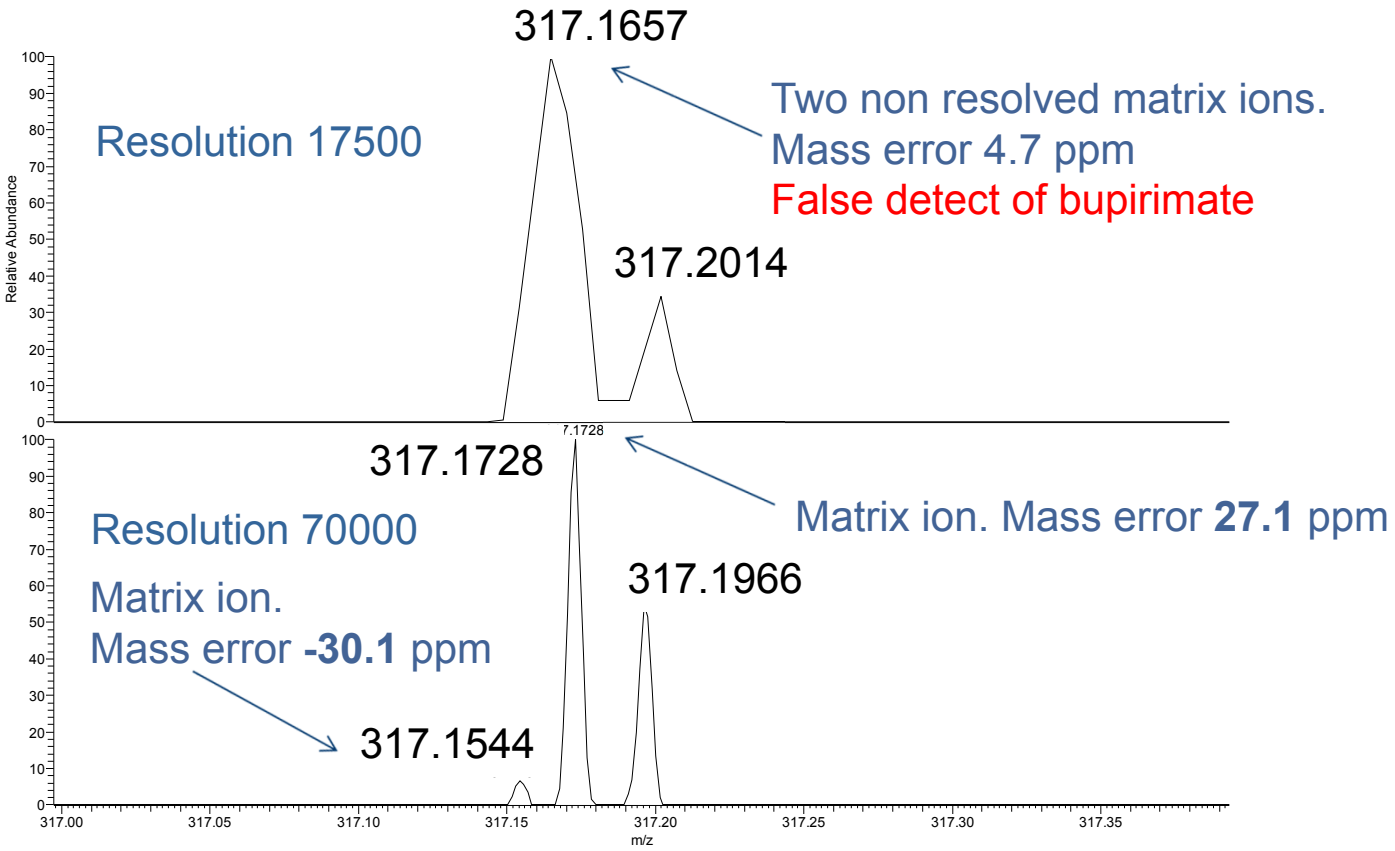

Solvent
Zucchini
Tomato
Orange
Pepper
Leek
Compounds (%)
100
90
80
70
60
50
40
30
20
10
0
20 µg kg-1
50 µg kg-1
100 µg kg-1
Automatic Detection
Manual Detection
Not detected



Fenazaquin in tomato extract, calibration with monoisotopic ion, with ion containing one ^{13}C atom and with ion containing two ^{13}C atom. Quantitation with monoisotopic ion possible up to 0.100 mg/kg. Higher concentration levels have to be quantified with ion containing two ^{13}C atoms

Influence of resolution on detection

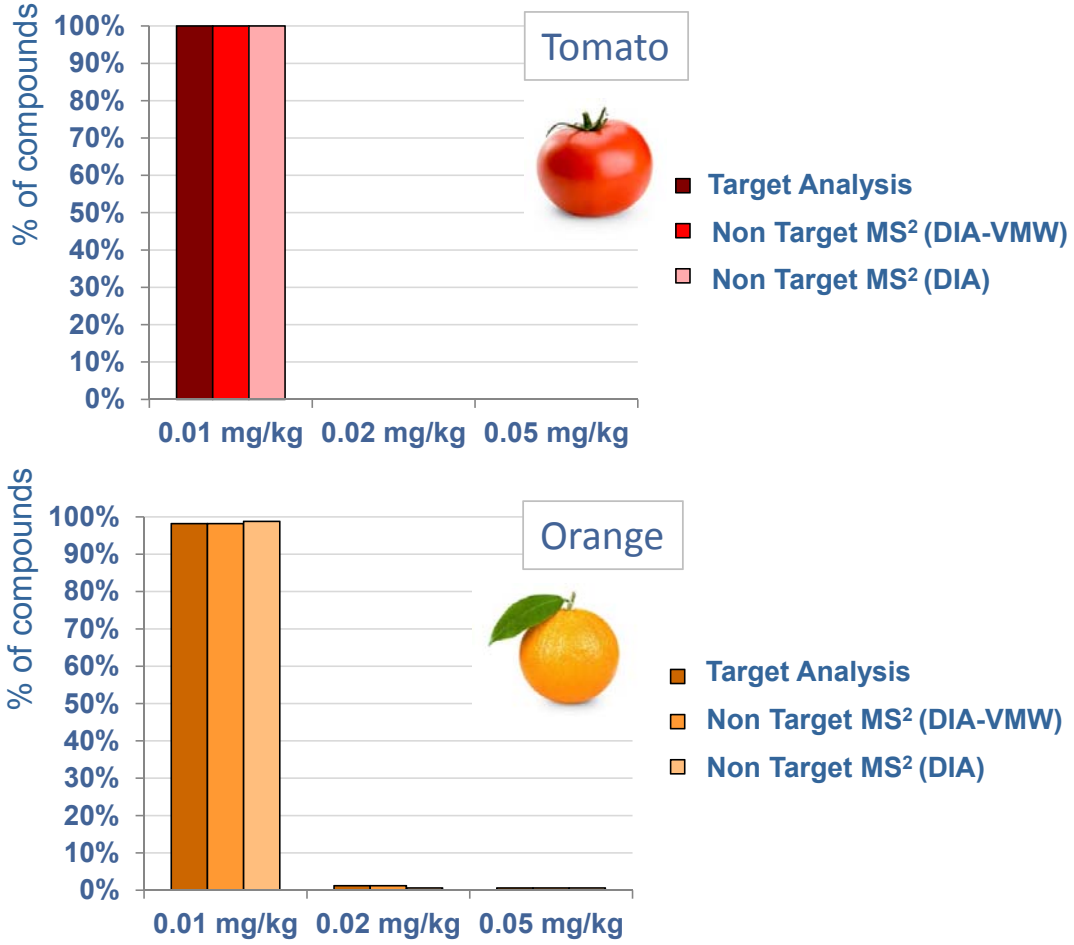
Exact mass of bupirimate: 317.1642

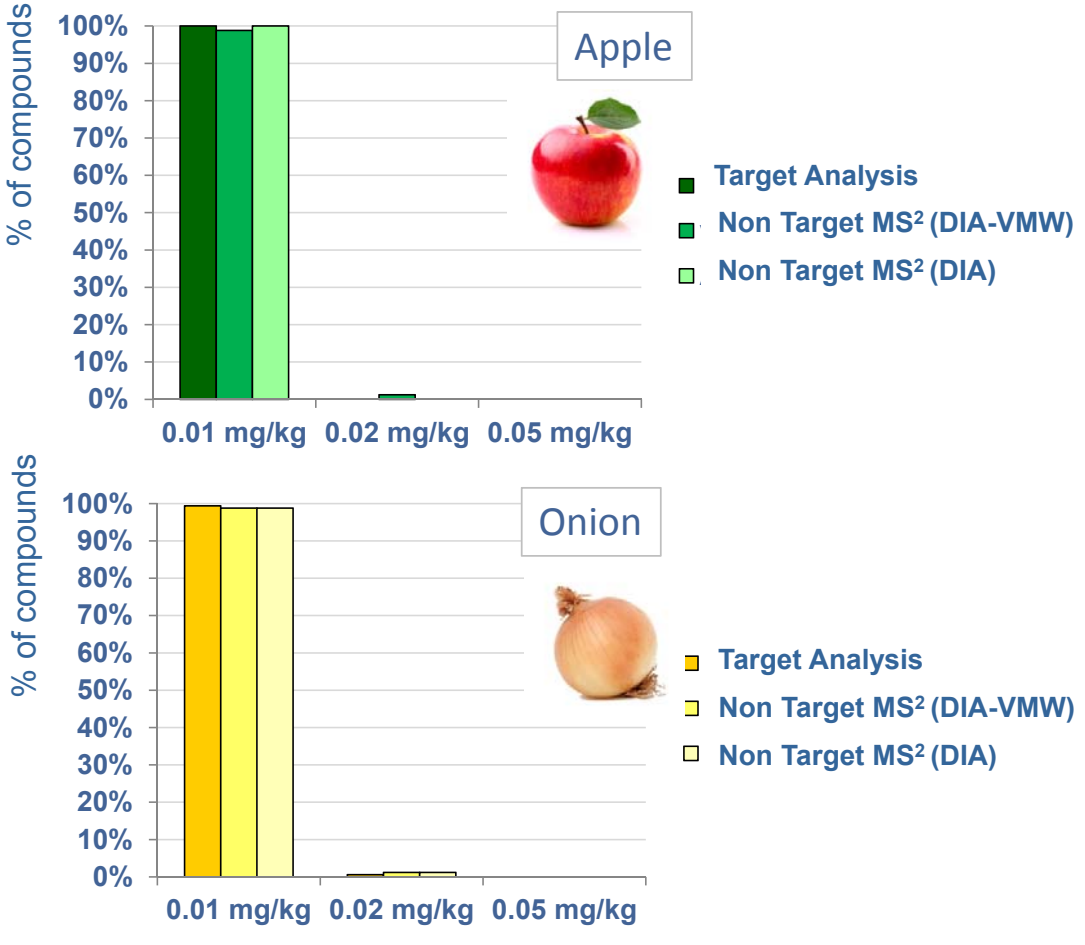



2005
LOOKING FOR
PERFORMANCE


2016
ACHIEVED

LOQ


Tomato
% of compounds
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.01 mg/kg
0.02 mg/kg
0.05 mg/kg
Target Analysis
Non Target MS² (DIA-VMW)
Non Target MS² (DIA)

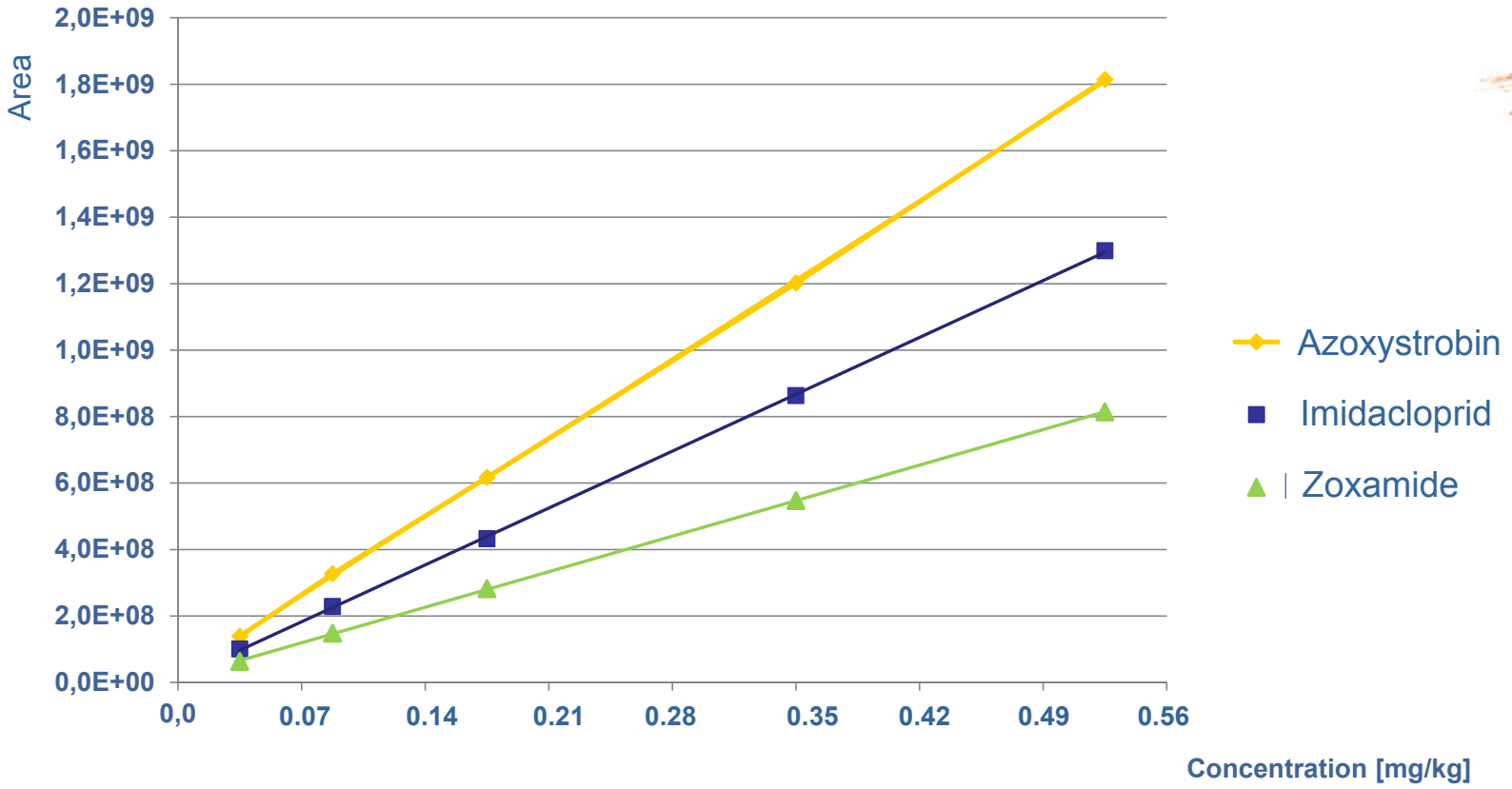

Apple
% of compounds
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.01 mg/kg
0.02 mg/kg
0.05 mg/kg
Target Analysis
Non Target MS² (DIA-VMW)
Non Target MS² (DIA)

Orange
% of compounds
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.01 mg/kg
0.02 mg/kg
0.05 mg/kg
Target Analysis
Non Target MS² (DIA-VMW)
Non Target MS² (DIA)

Onion
% of compounds
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.01 mg/kg
0.02 mg/kg
0.05 mg/kg
Target Analysis
Non Target MS² (DIA-VMW)
Non Target MS² (DIA)

Linearity LC-HRAMS

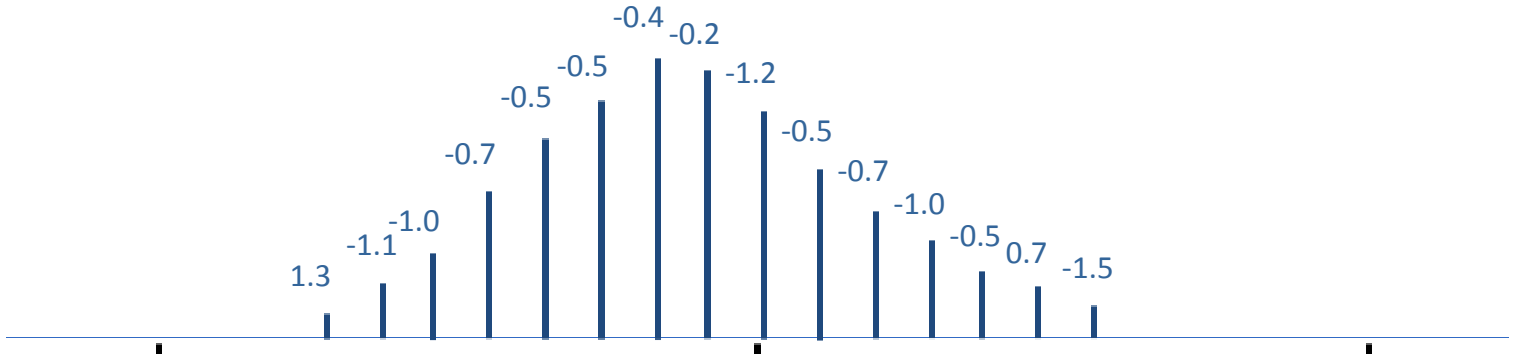
Spinach



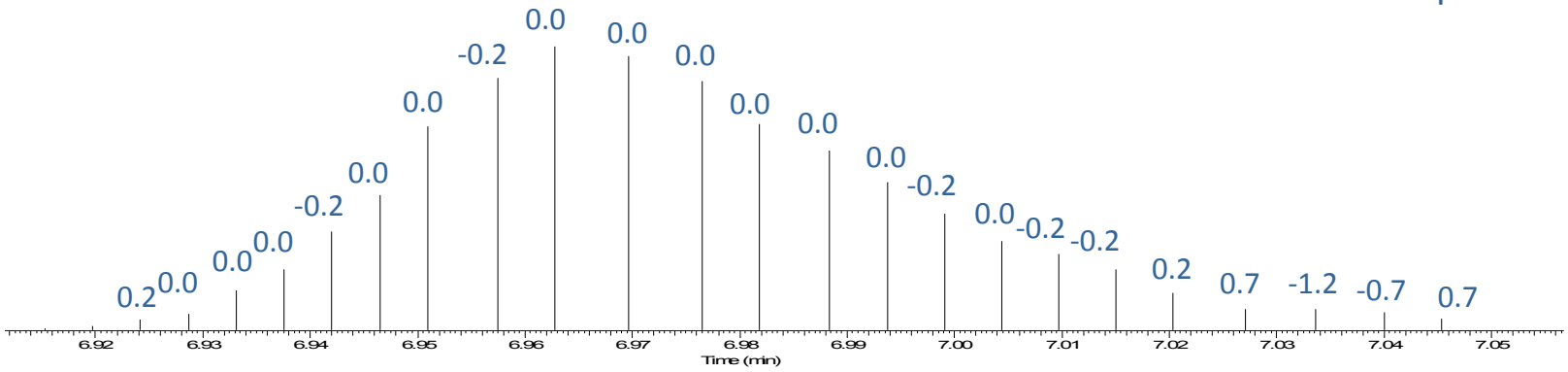
Azoxystrobin 0.01 mg/kg in tomato

Dilution 5x (mass errors in ppm)

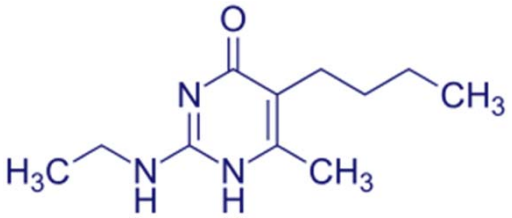
QTOF

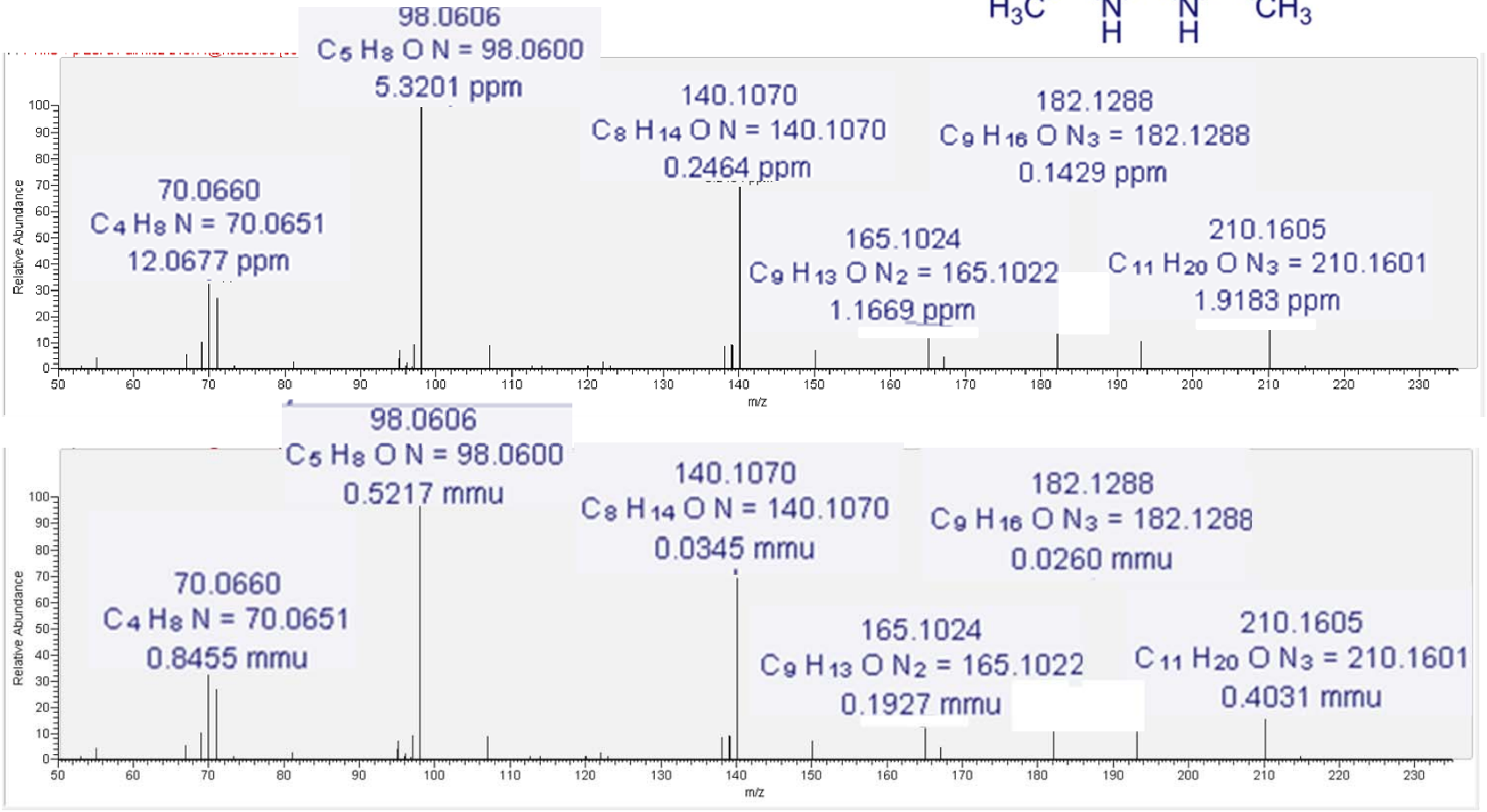


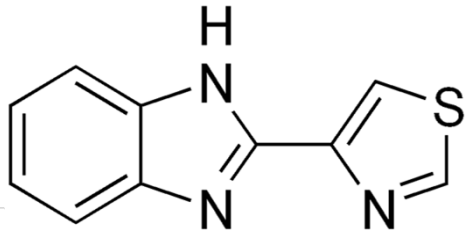
Orbitrap



Ethirimol 0.1 mg/kg in tomato, MS^2 spectrum

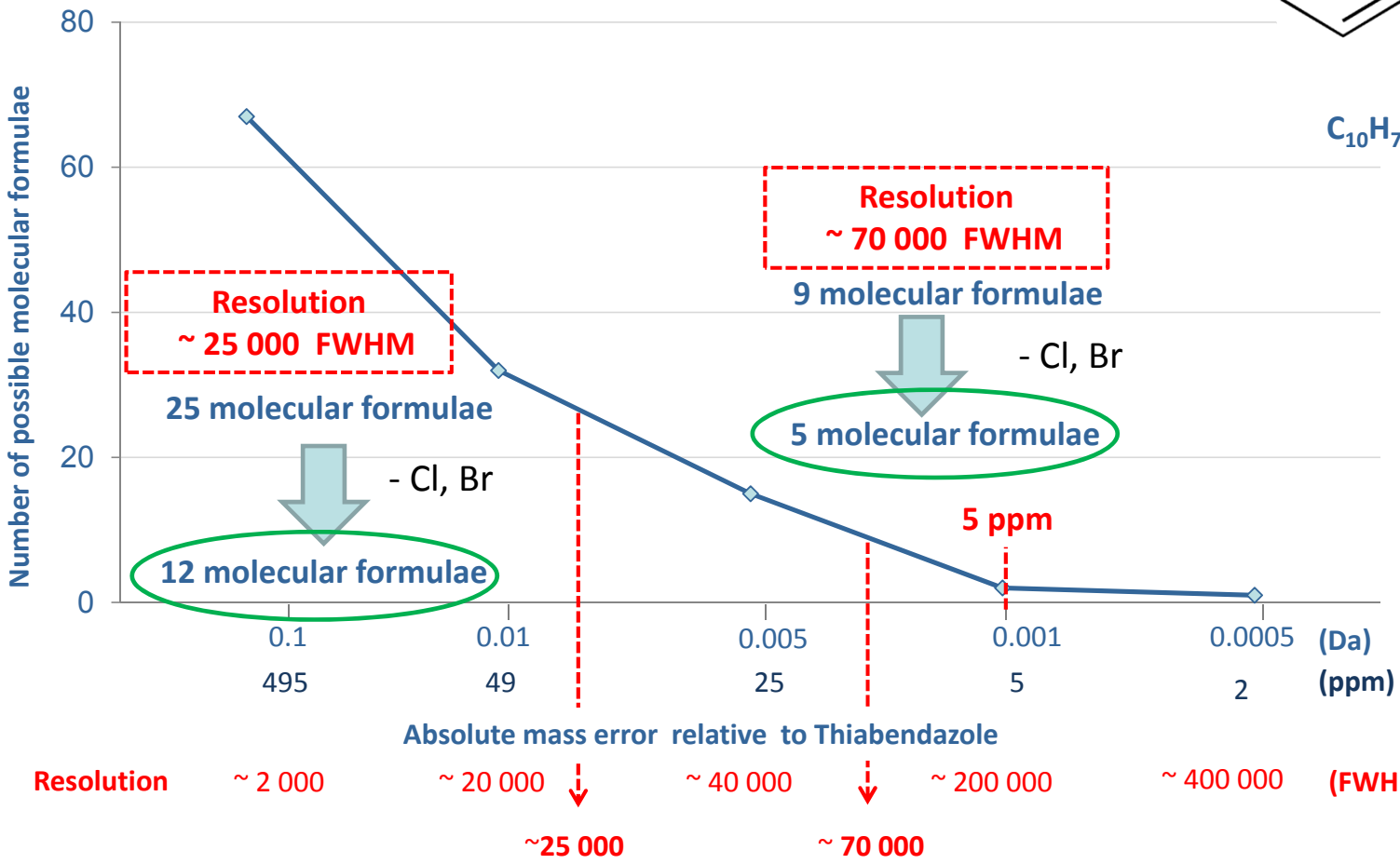




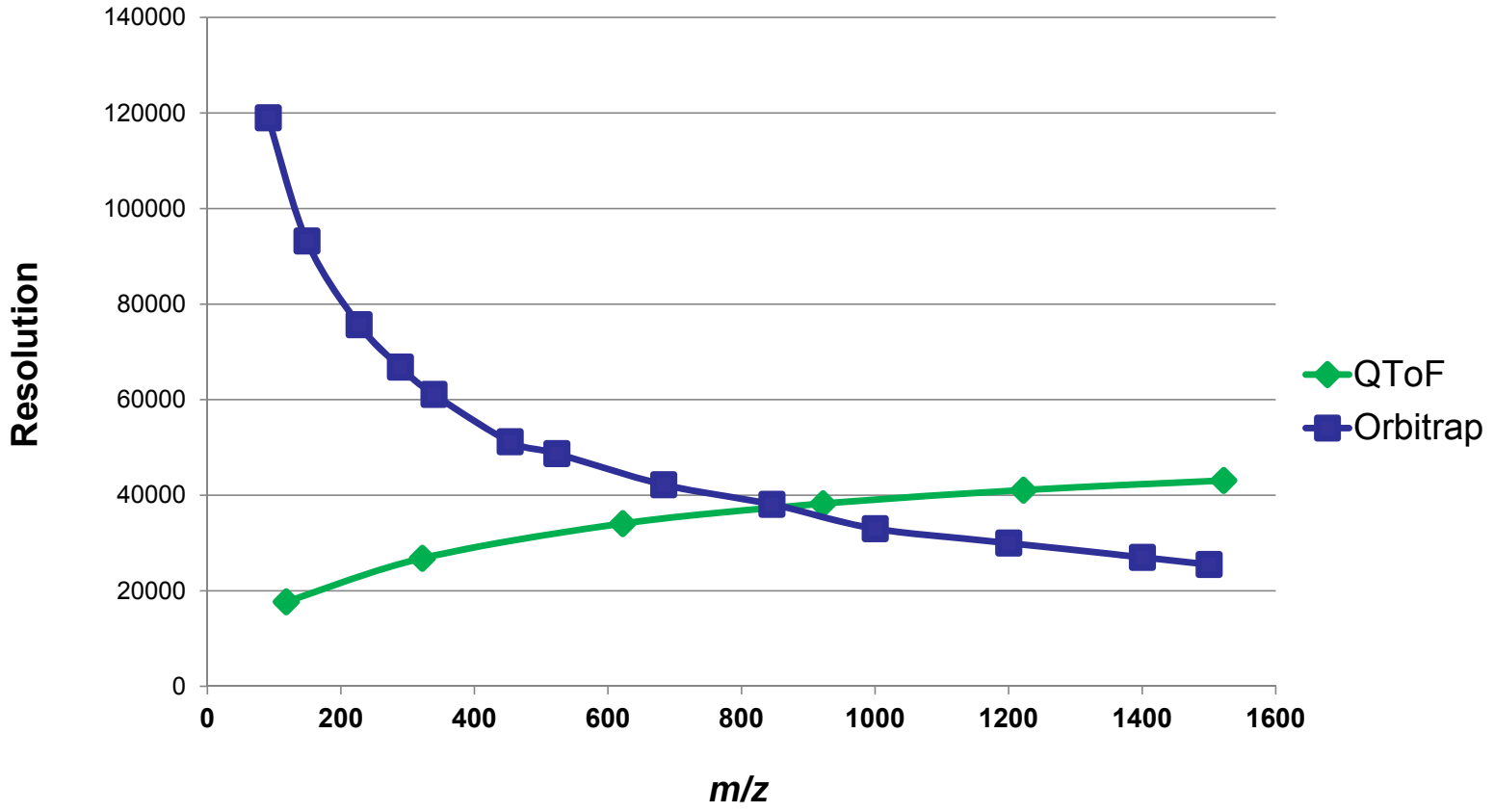


Thiabendazole
$C_{10}H_7N_3S$ (*m/z* 202.0433)

$C_{0-25}H_{0-50}N_{0-5}O_{0-5}Cl_{0-5}Br_{0-5}S$

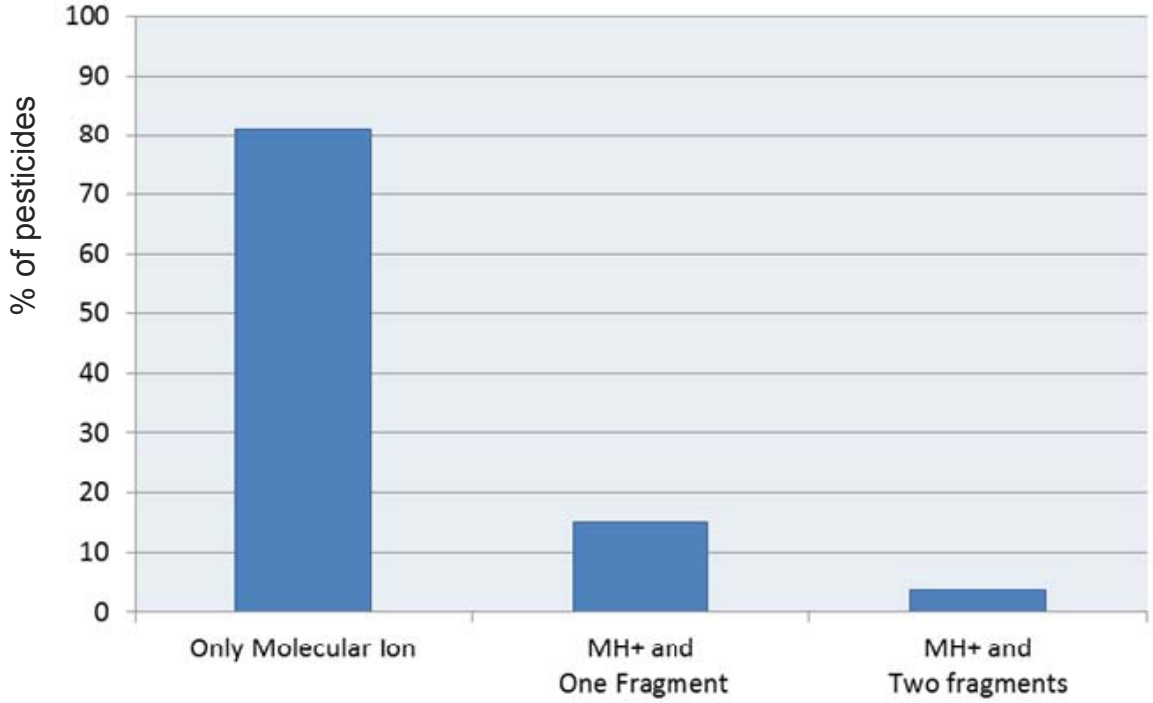


Resolution dependence on m/z
Experimental data



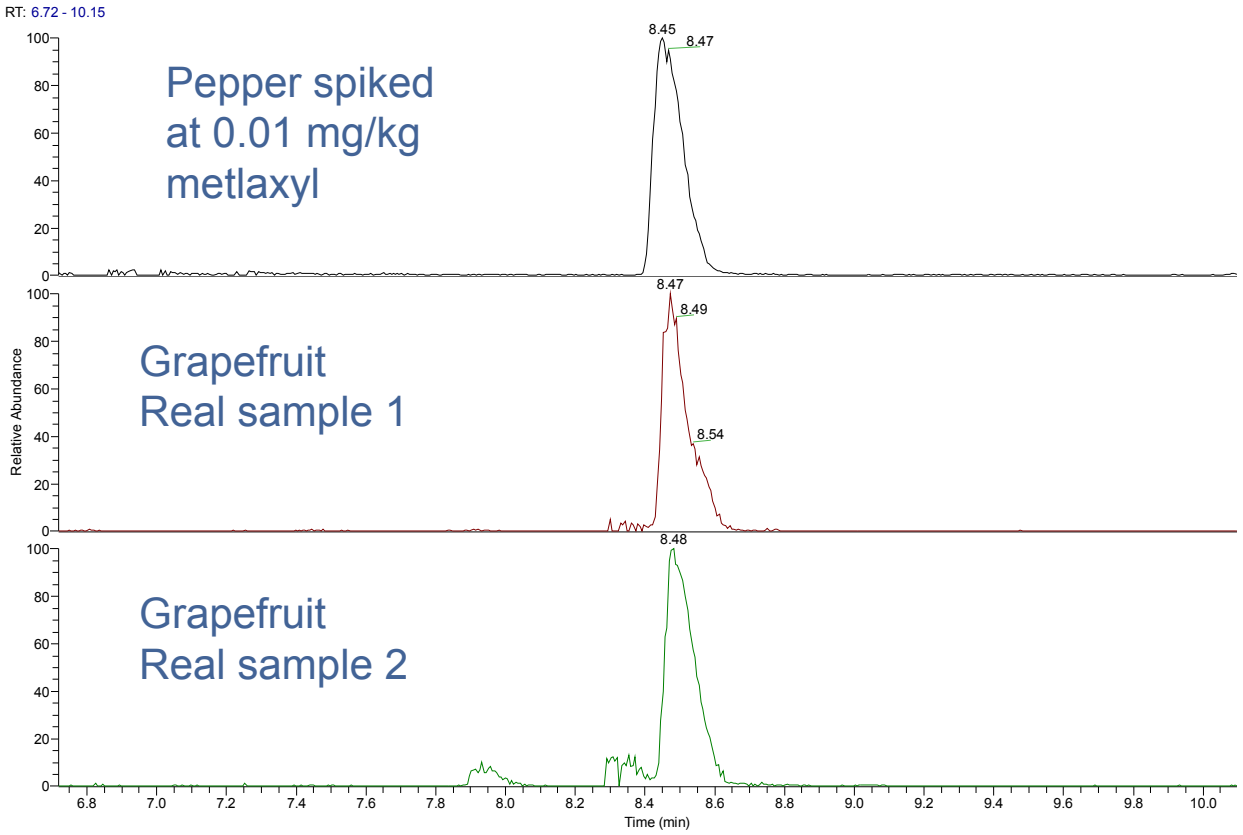
Number of Pesticides with fragments using Orbitrap in *Full Scan mode*

Solvent 0.10 mg/kg



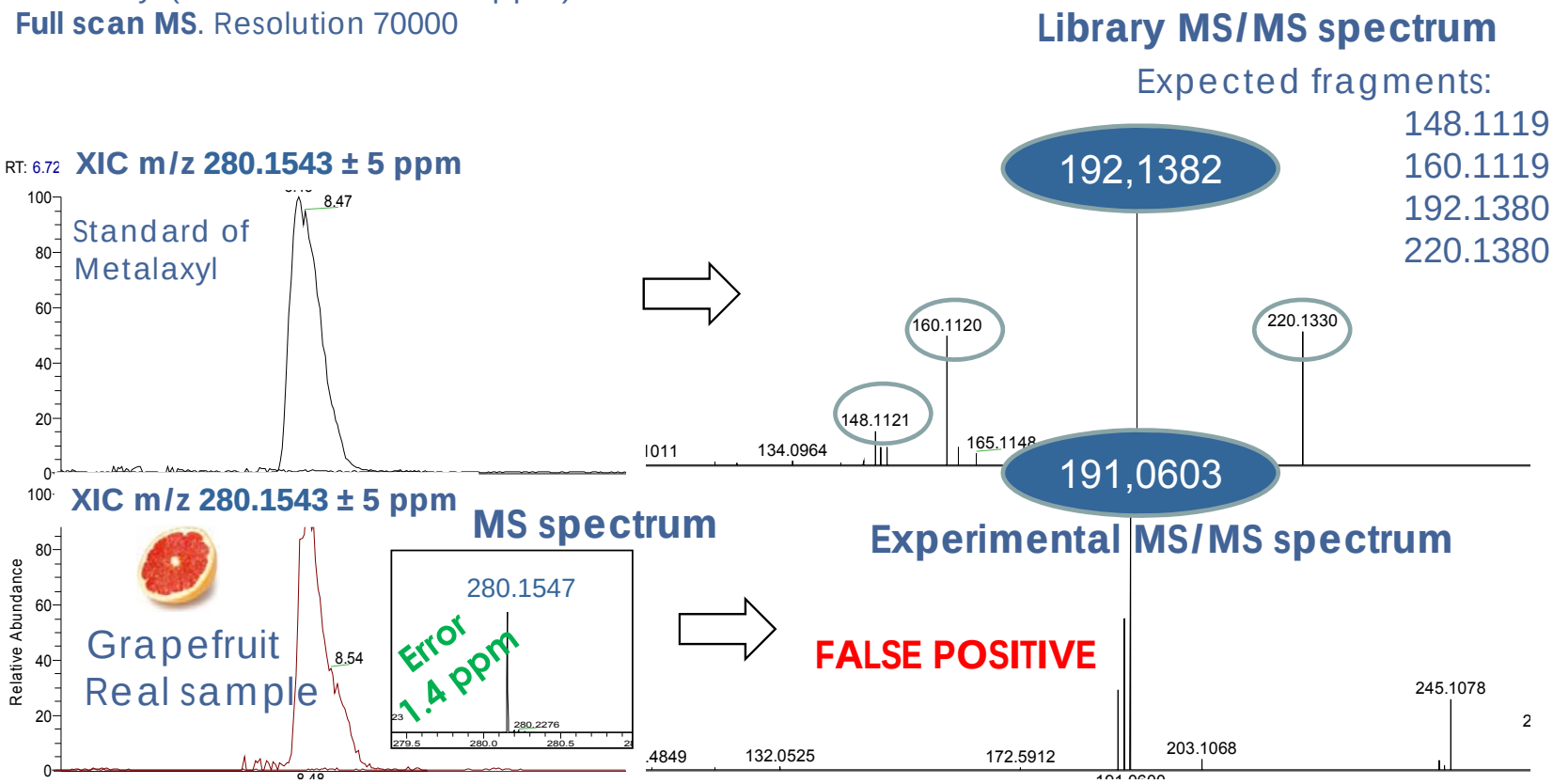
Analysis of real samples by LC-HRAMS MS^2

Metalaxyl (XIC m/z 280.1543 ± 5 ppm). **Full scan MS**. Resolution 70000



LC-QOrbitrap

Metalaxyl (XIC m/z 280.1543 ± 5 ppm).
Full scan MS. Resolution 70000



Combining Full Scan MS and MS^2 Mode

Pesticide residue method MS^2

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

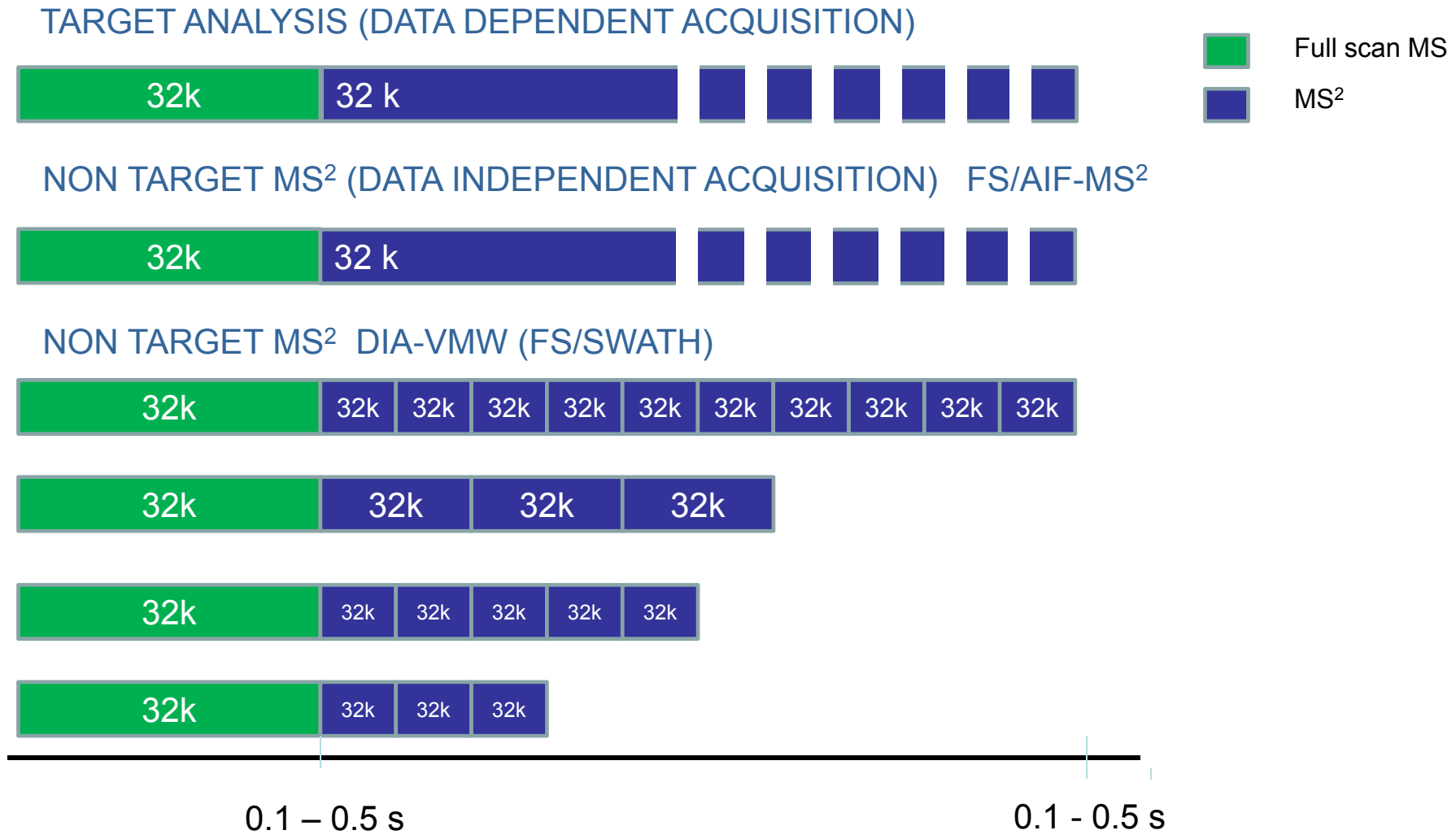
Tomato 0.01 mg/kg

Pesticides	Full scan	QTOF	Orbitrap	MS^2	QTOF	Orbitrap
Azoxystrobin	404.1241	-1.5	0.0	372.0979	0.6	-0.3
Diazinon	305.1083	-1.5	0.3	169.0794	0.9	0.0
Dichlorvos	220.9532	0.5	0.5	109.0047	-1.9	1.6
Fenamiphos - sulfone	336.1029	-0.2	0.6	266.0246	-1.6	0.4
Profenofos	372.9424	1.9	0.5	302.8642	1.0	-0.3

Orange 0.01 mg/kg

Pesticides	Full scan	QTOF	Orbitrap	MS^2	QTOF	Orbitrap
Azoxystrobin	404.1241	0.4	0.0	372.0979	1.4	0.3
Diazinon	305.1083	1.2	-0.3	169.0794	-1.1	-0.6
Dichlorvos	220.9532	1.9	-0.5	109.0047	-2.3	2.4
Fenamiphos - sulfone	336.1029	-0.2	0.0	266.0246	1.5	0.0
Profenofos	372.9424	-0.4	0.0	302.8642	1.1	-1.0

TOF


TARGET ANALYSIS (DATA DEPENDENT ACQUISITION)
32k
32 k
Full scan MS
MS2
NON TARGET MS2 (DATA INDEPENDENT ACQUISITION) FS/AIF-MS2
32k
32 k
NON TARGET MS2 DIA-VMW (FS/SWATH)
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
32k
0.1 – 0.5 s
0.1 - 0.5 s

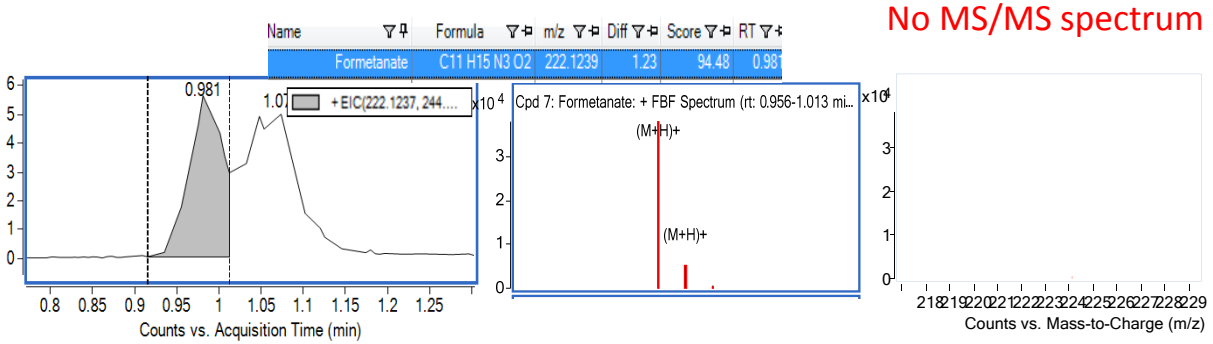
LC-QTOF-MS

Formetanate Tomato 0.02 mg/kg

Auto MS/MS mode

Full scan detection

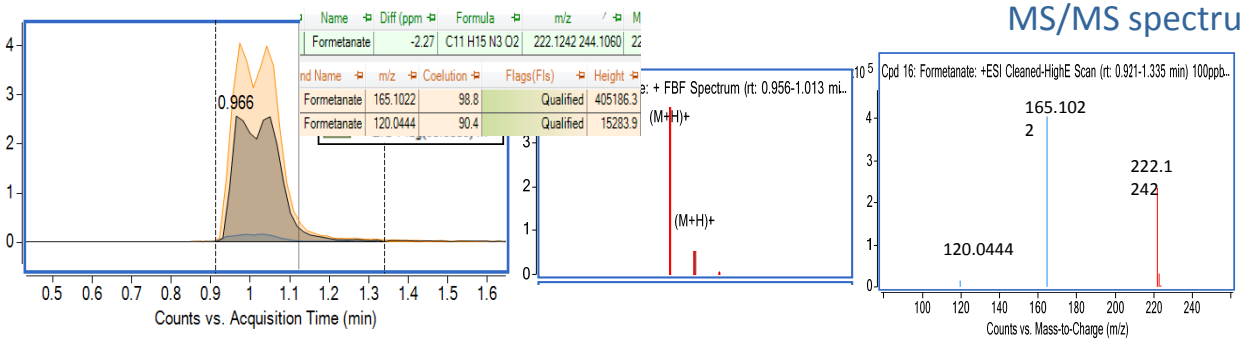
No MS/MS spectrum



All ions mode

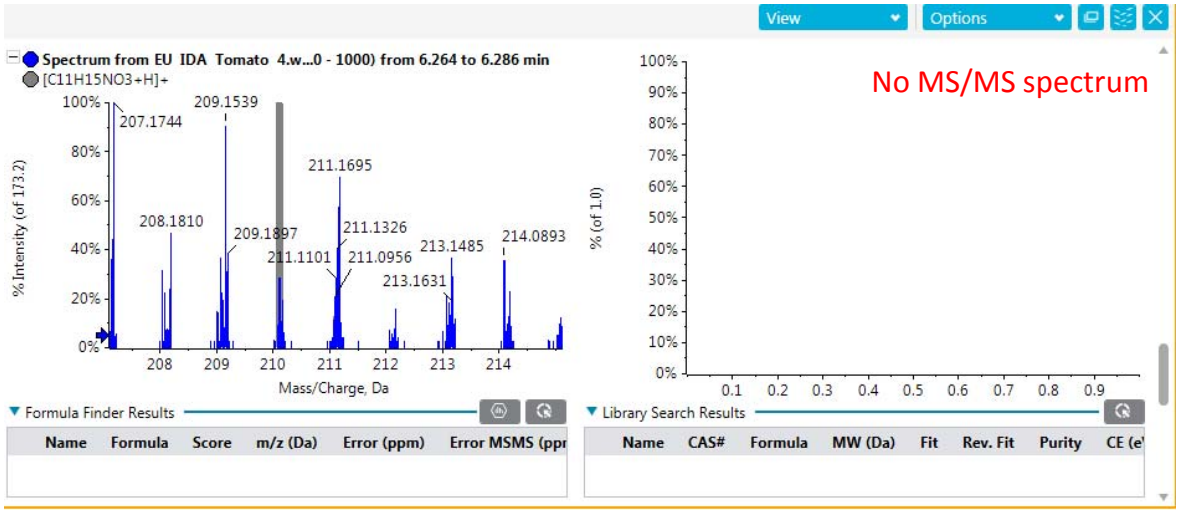
Formetanate Identified with two fragments ions

MS/MS spectrum



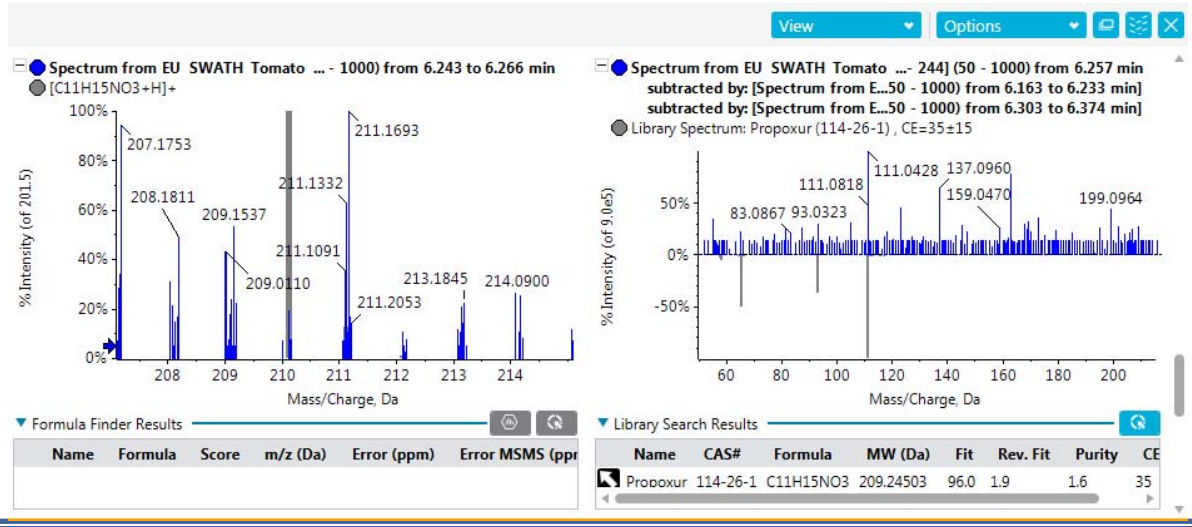
IDA mode

Full scan detection

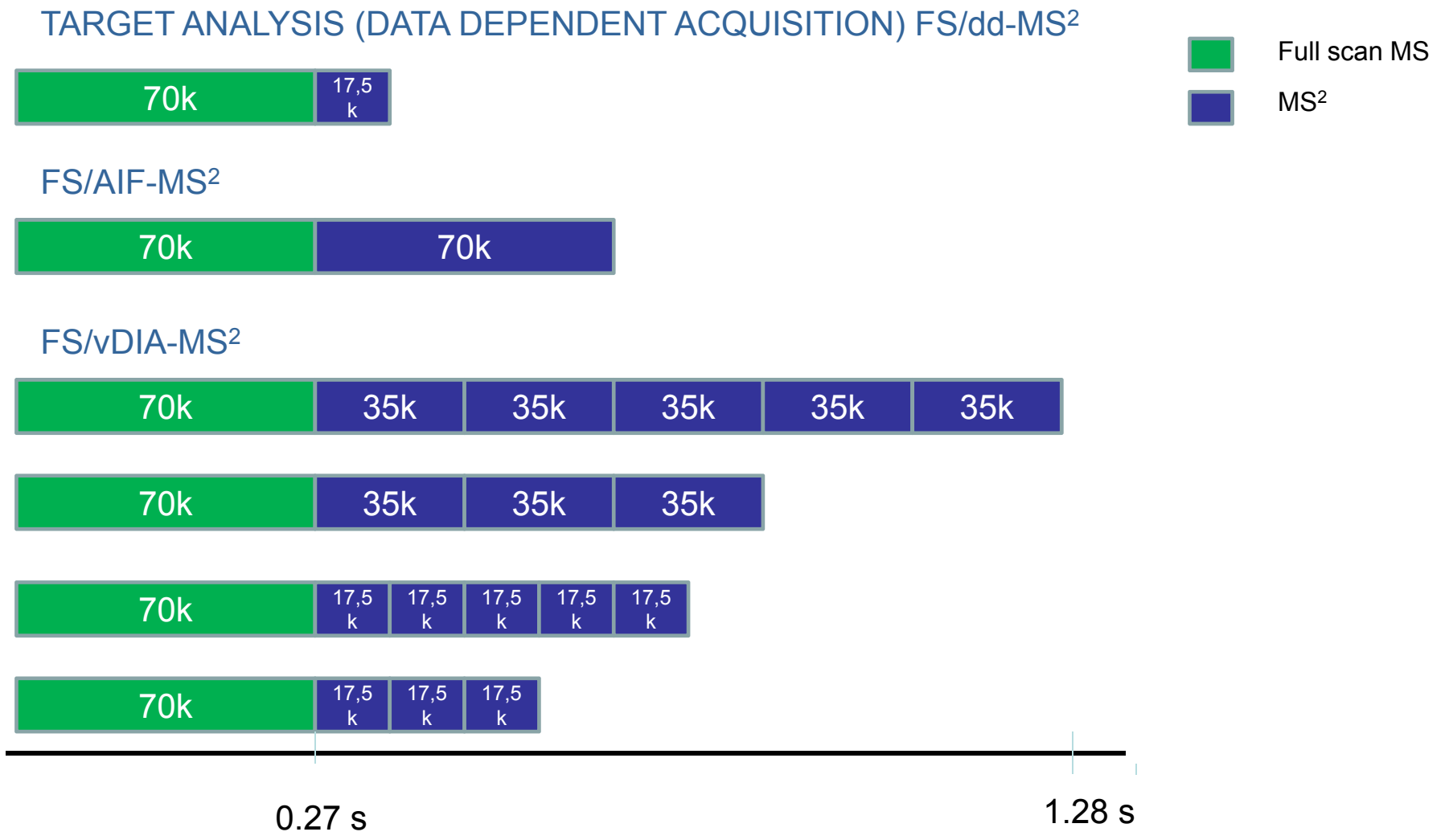


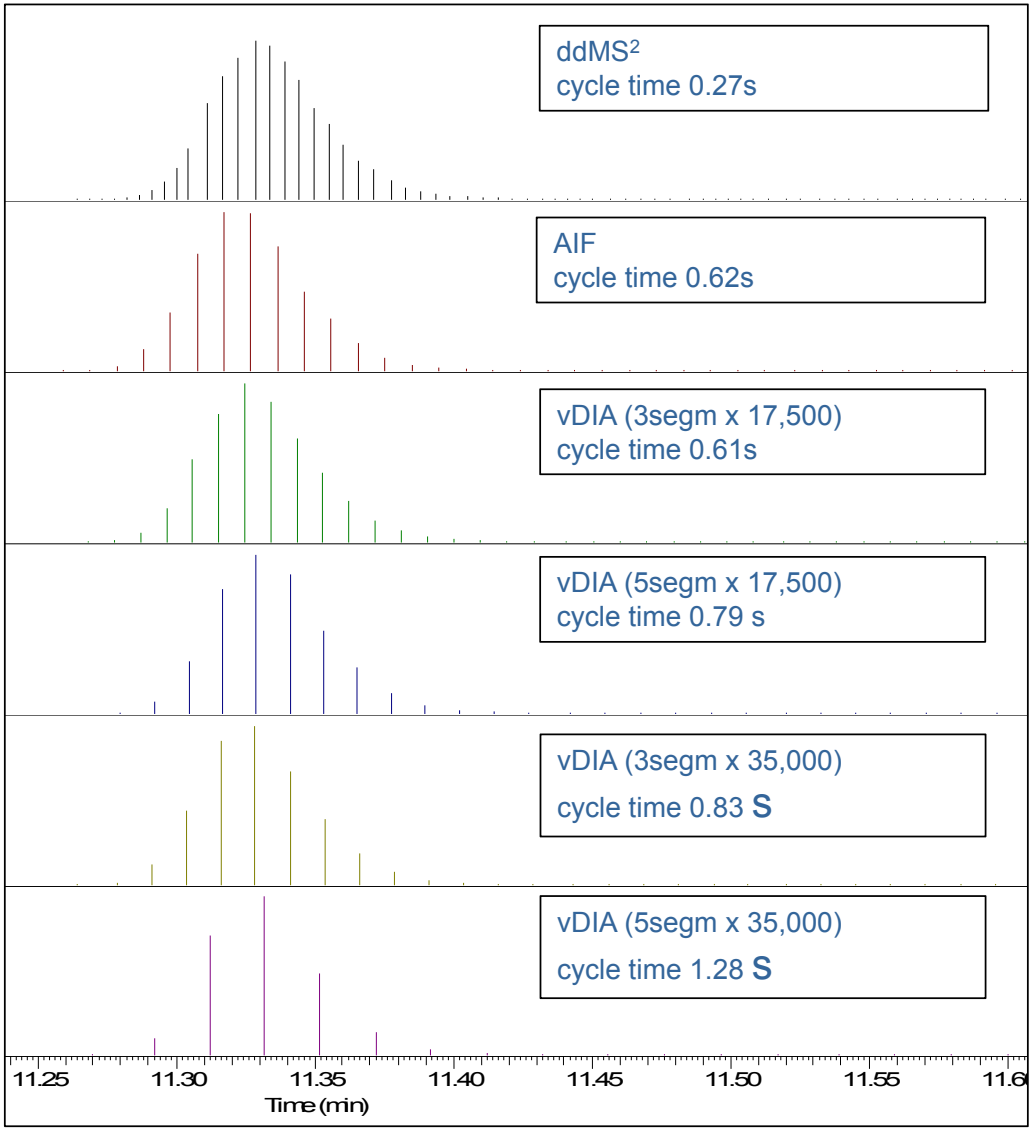
SWATH mode

Propoxur Identified by fragments ions



Orbitrap


TARGET ANALYSIS (DATA DEPENDENT ACQUISITION) FS/dd-MS²
70k
17,5 k
Full scan MS
MS²
FS/AIF-MS²
70k
70k
FS/vDIA-MS²
70k
35k
35k
35k
35k
35k
70k
35k
35k
35k
70k
17,5 k
17,5 k
17,5 k
17,5 k
17,5 k
70k
17,5 k
17,5 k
17,5 k
0.27 s
1.28 s

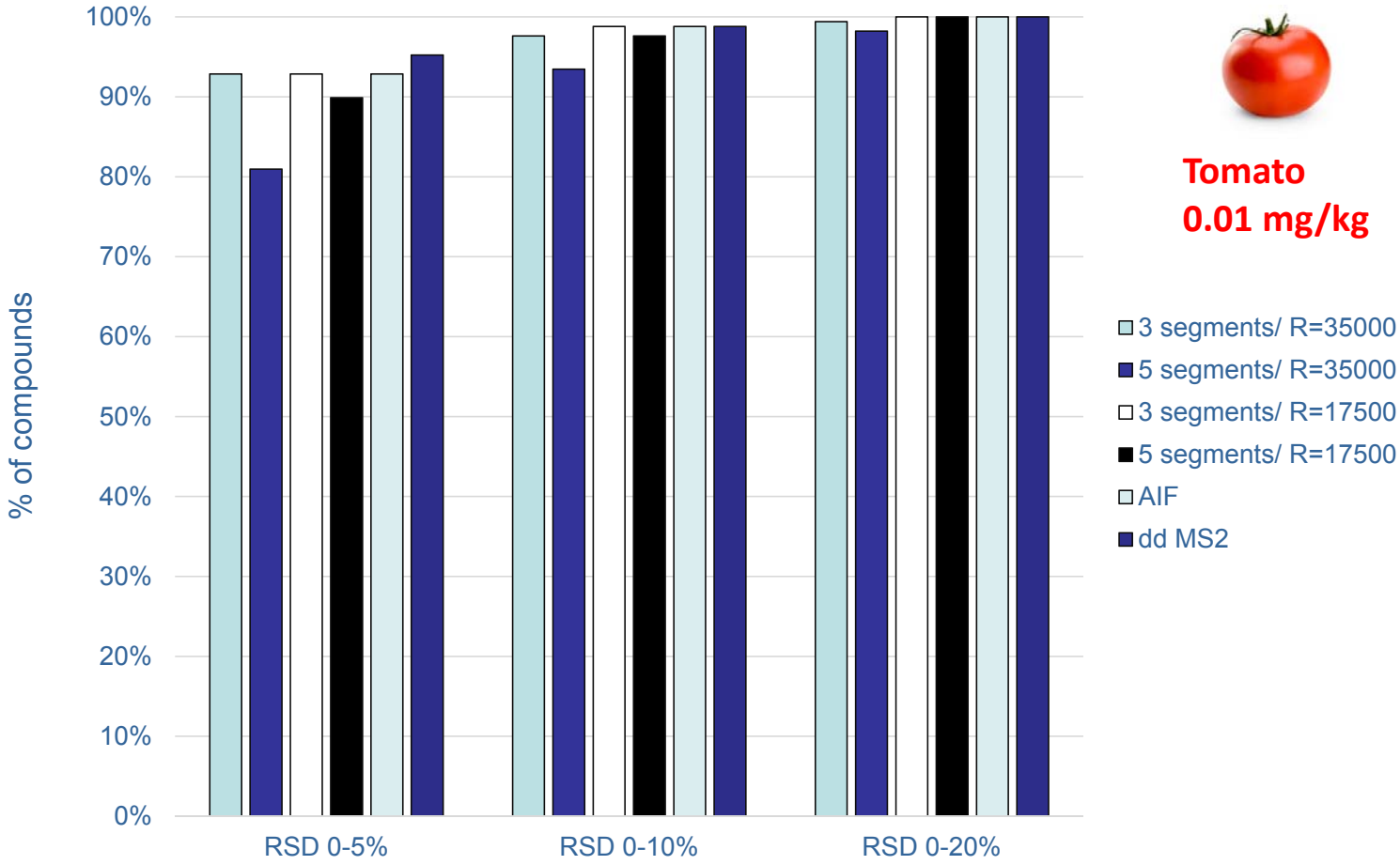


Fenazaquin

0.02 mg/L in solvent

Repeatability

(Five Analyses)



Tomato
0.01 mg/kg

11 Matrices

Lettuce

Cucumber

Orange

Green bean

Onion

Apple

Parsley

Leek

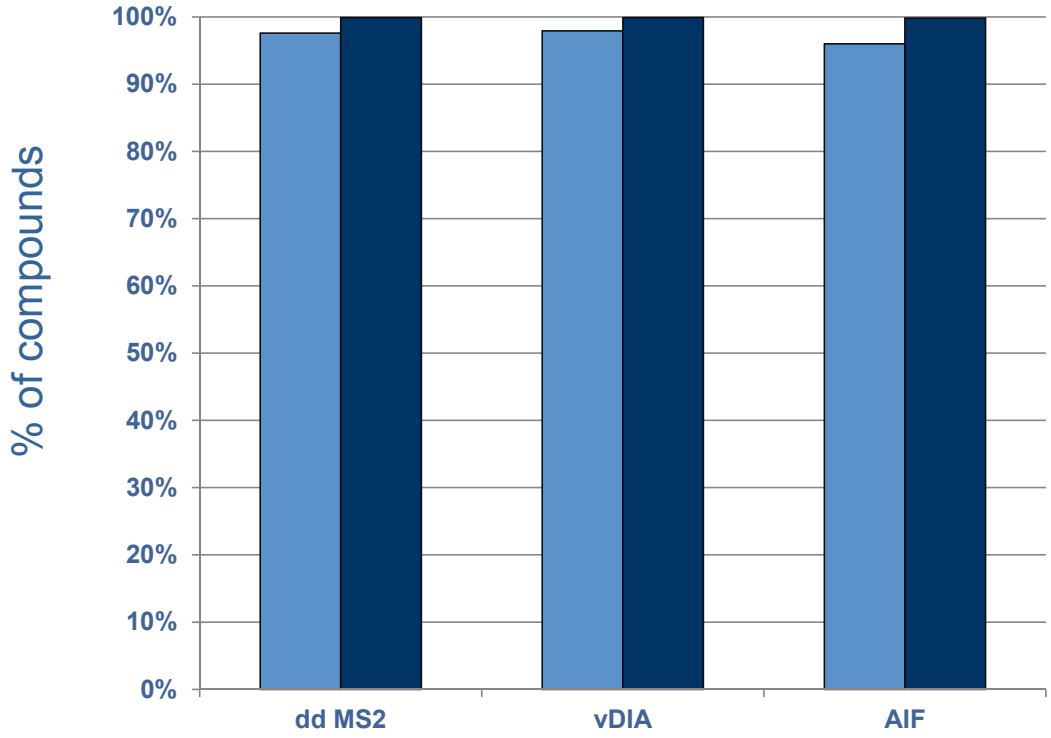
Tomato

Garlic

Spinach

Identification

166 pesticides x 11 matrices = 1826 results



0.01 mg/kg

0.1 mg/kg

EUPT-FV-17 Broccoli 2015

Pesticide	Asigned value (mg/kg)	Obtained value (Difference)		
		AIF	dd MS^2	vDIA
Bupirimate	0,165 (±50%)	0,157 (-5%)	0,152 (-8%)	0,160 (-3%)
Carbendazim	0,512 (±50%)	0,415 (-19%)	0,404 (-21%)	0,404 (-21%)
Diazinon	0,071 (±50%)	0,051 (-28%)	0,051 (-28%)	0,051 (-28%)
Difenoconazol	0,53 (±50%)	0,625 (18%)	0,631 (19%)	0,610 (15%)
Diflubenzuron	0,319 (±50%)	0,367 (15%)	0,332 (4%)	0,354 (11%)
Metoxyfenozide	0,349 (±50%)	0,300 (-14%)	0,304 (-13%)	0,293 (-16%)
Spinosaad	0,051 (±50%)	0,044 (-14%)	0,044 (-14%)	0,044 (-14%)
Thiabendazole	1,90 (±50%)	2,07 (9%)	2,01 (6%)	2,00 (5%)
Trifloxystrobin	0,466 (±50%)	0,592 (27%)	0,592 (27%)	0,559 (20%)
Pendimethalin	0,062 (±50%)	0,070 (13%)	0,059 (-5%)	0,065 5%)

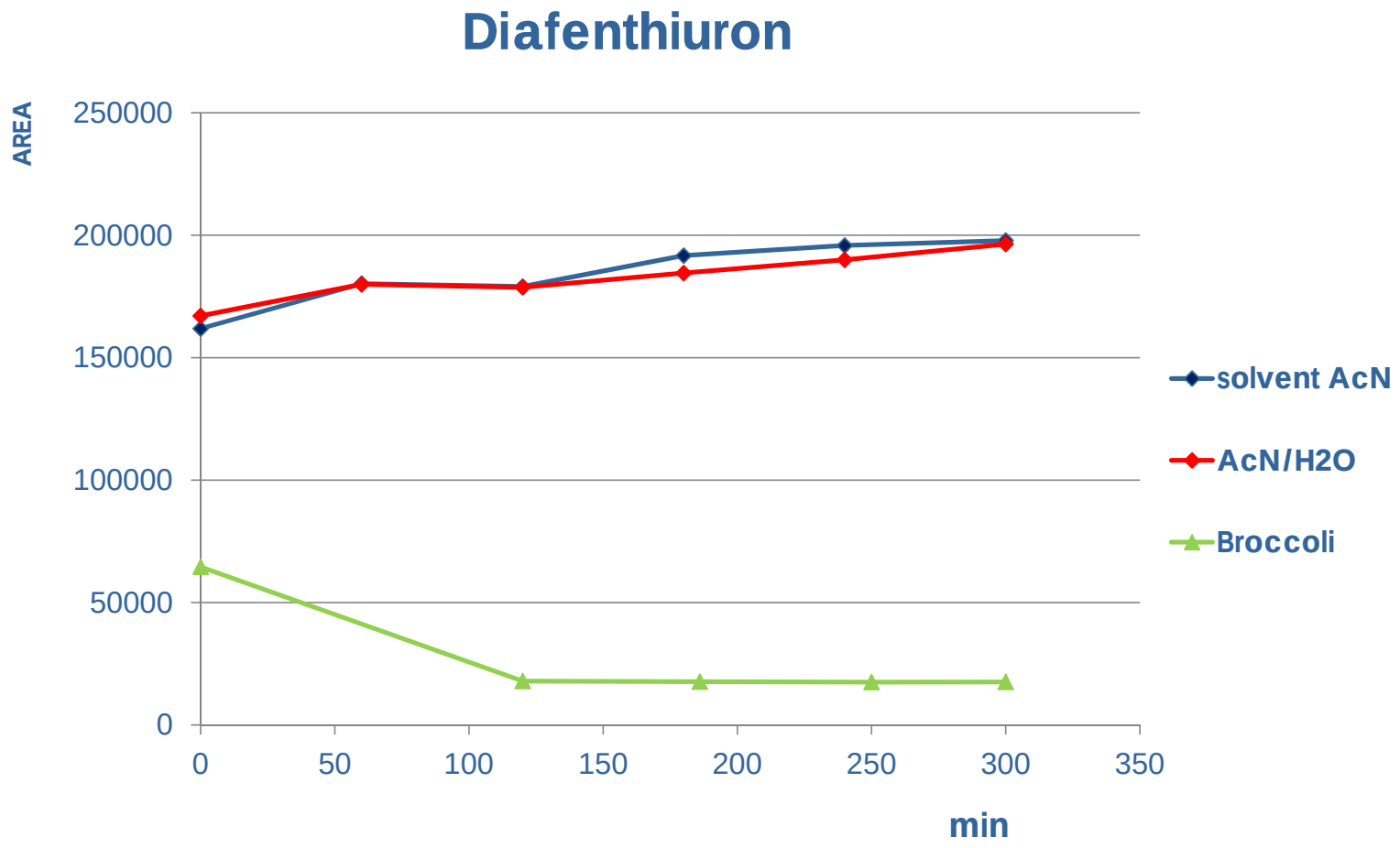
- **“Old” or rarely detected compounds**

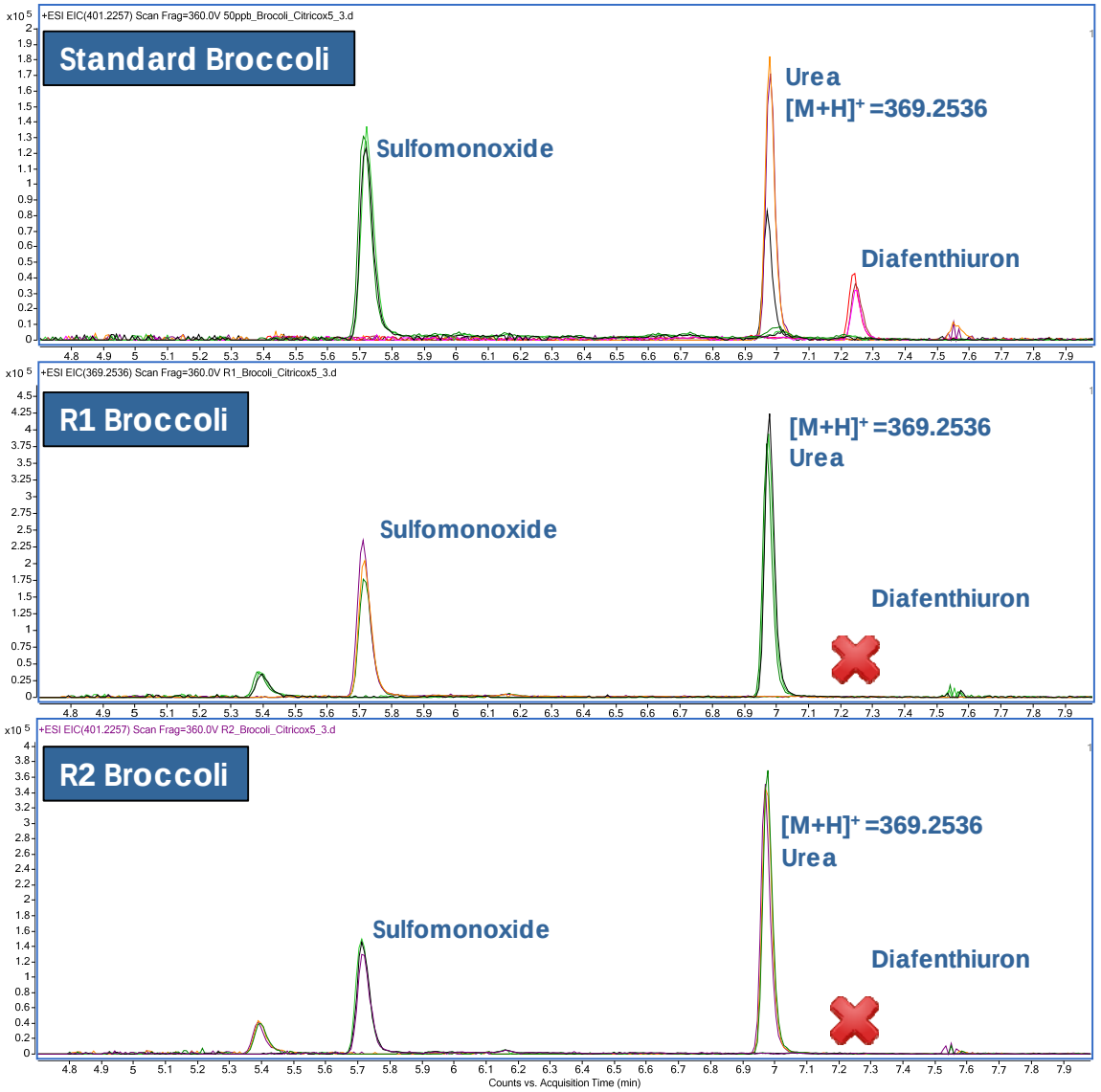
- **Complex residue definition**

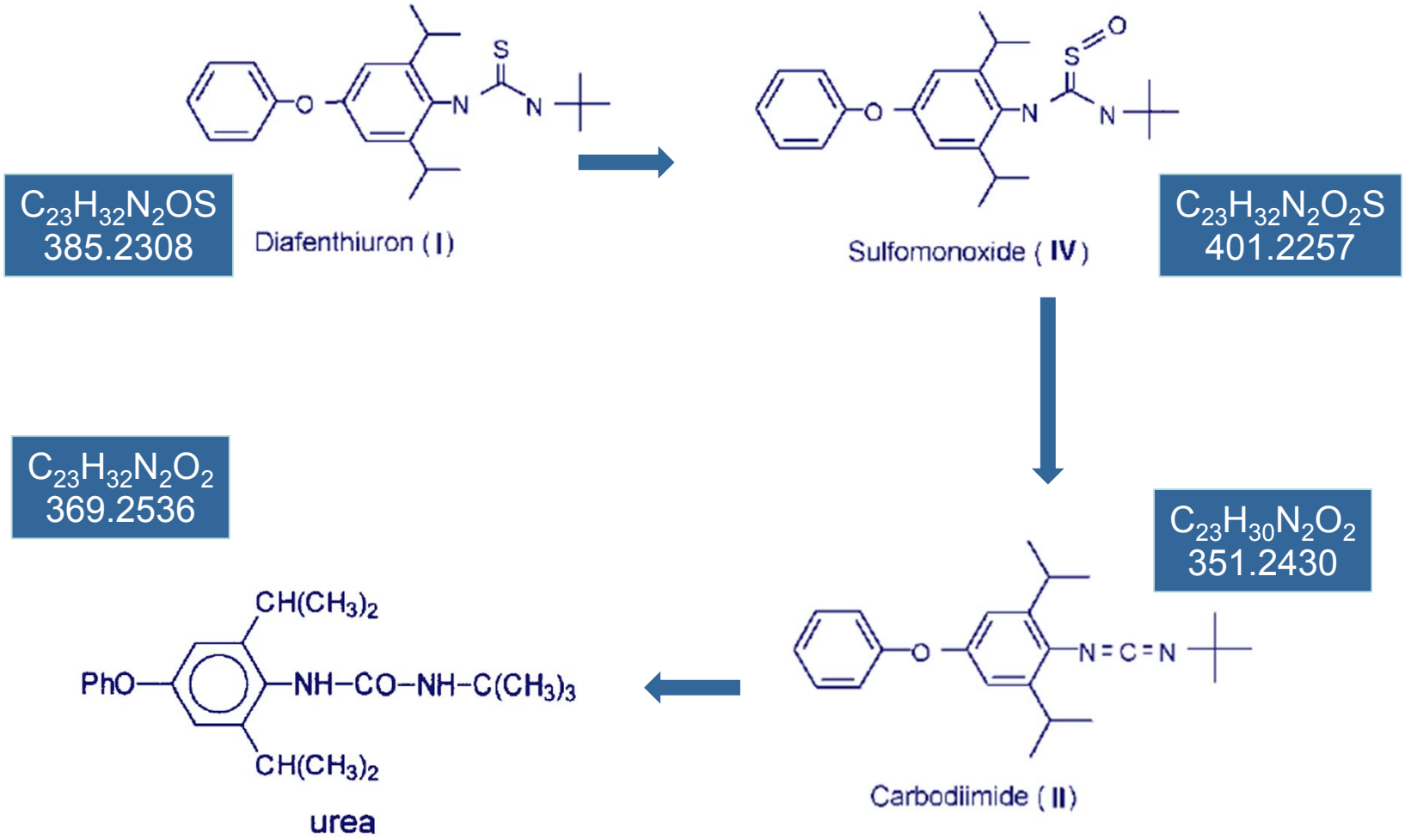
Cycloxydim including degradation and reaction products which can be determined as 3-(3-thianyl)glutaric acid S-dioxide (BH 517-TGSO$_2$) and/or 3-hydroxy-3-(3-thianyl)glutaric acid S-dioxide (BH 517-5-OH-TGSO$_2$) or methyl esters thereof, calculated in total as cycloxydim

- **“Very expensive” analytical standards**

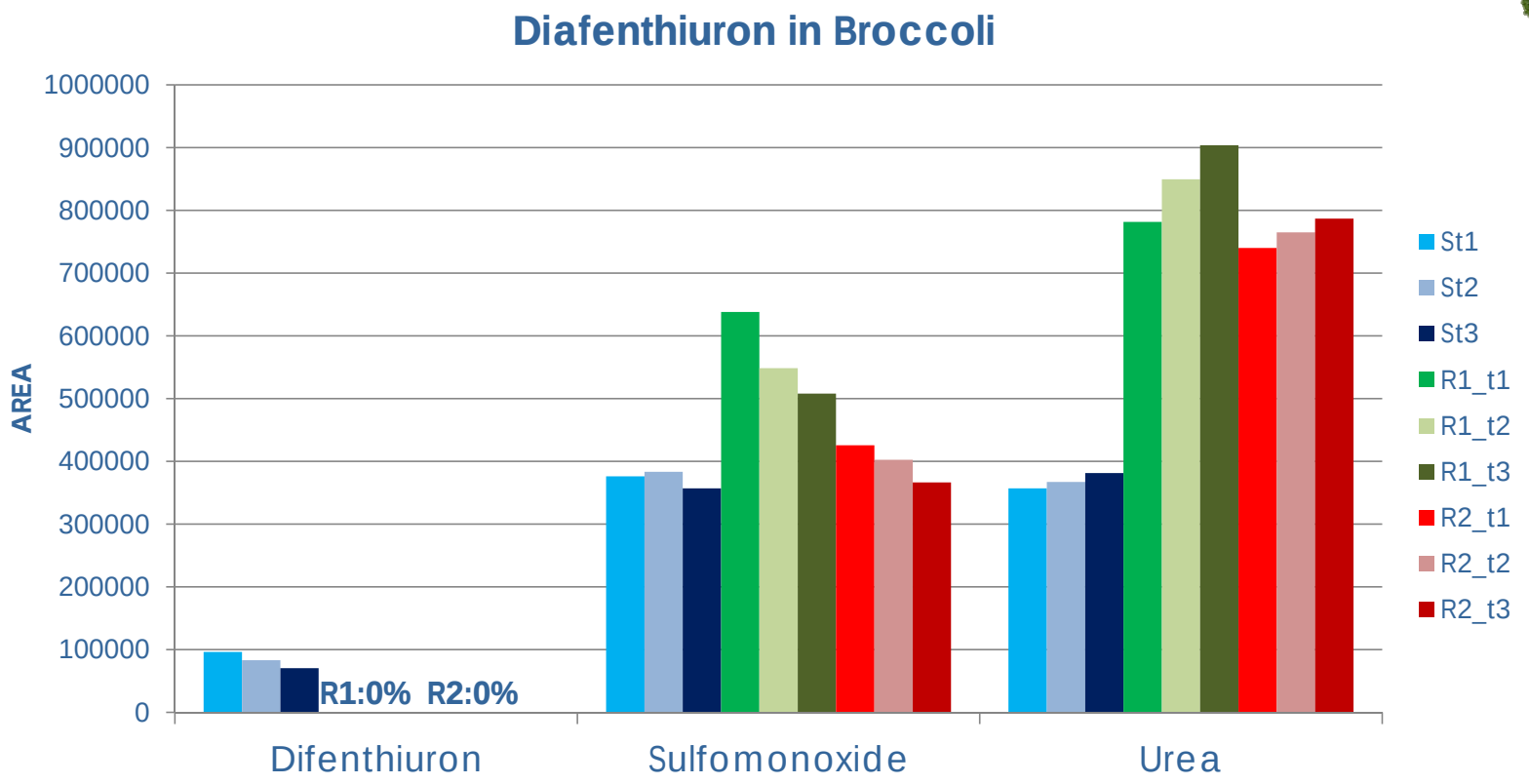
- **Compounds “produced” during the analysis**


Diafenthiuron
AREA
250000
200000
150000
100000
50000
0
0
50
100
150
200
250
300
350
min
solvent AcN
AcN/H2O
Broccoli

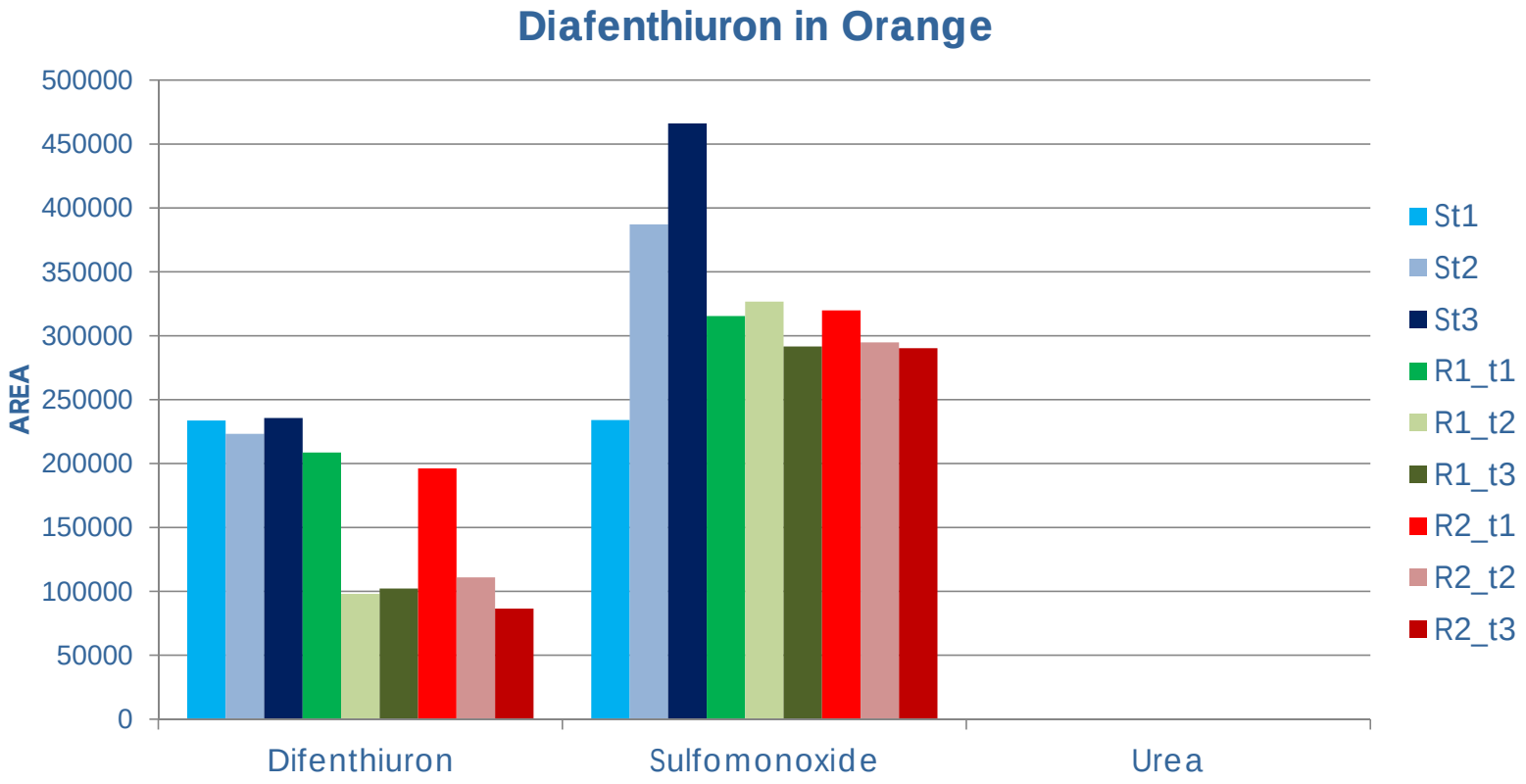

+ESI EIC(401.2257) Scan Frag=360.0V 50ppb_Brocoli_Citricox5_3.d
Standard Broccoli
Urea
[M+H]+ =369.2536
Sulfomonoxide
Diafenthiuron
+ESI EIC(369.2536) Scan Frag=360.0V R1_Brocoli_Citricox5_3.d
R1 Broccoli
[M+H]+ =369.2536
Urea
Sulfomonoxide
Diafenthiuron
+ESI EIC(401.2257) Scan Frag=360.0V R2_Brocoli_Citricox5_3.d
R2 Broccoli
[M+H]+ =369.2536
Urea
Sulfomonoxide
Diafenthiuron
Counts vs. Acquisition Time (min)

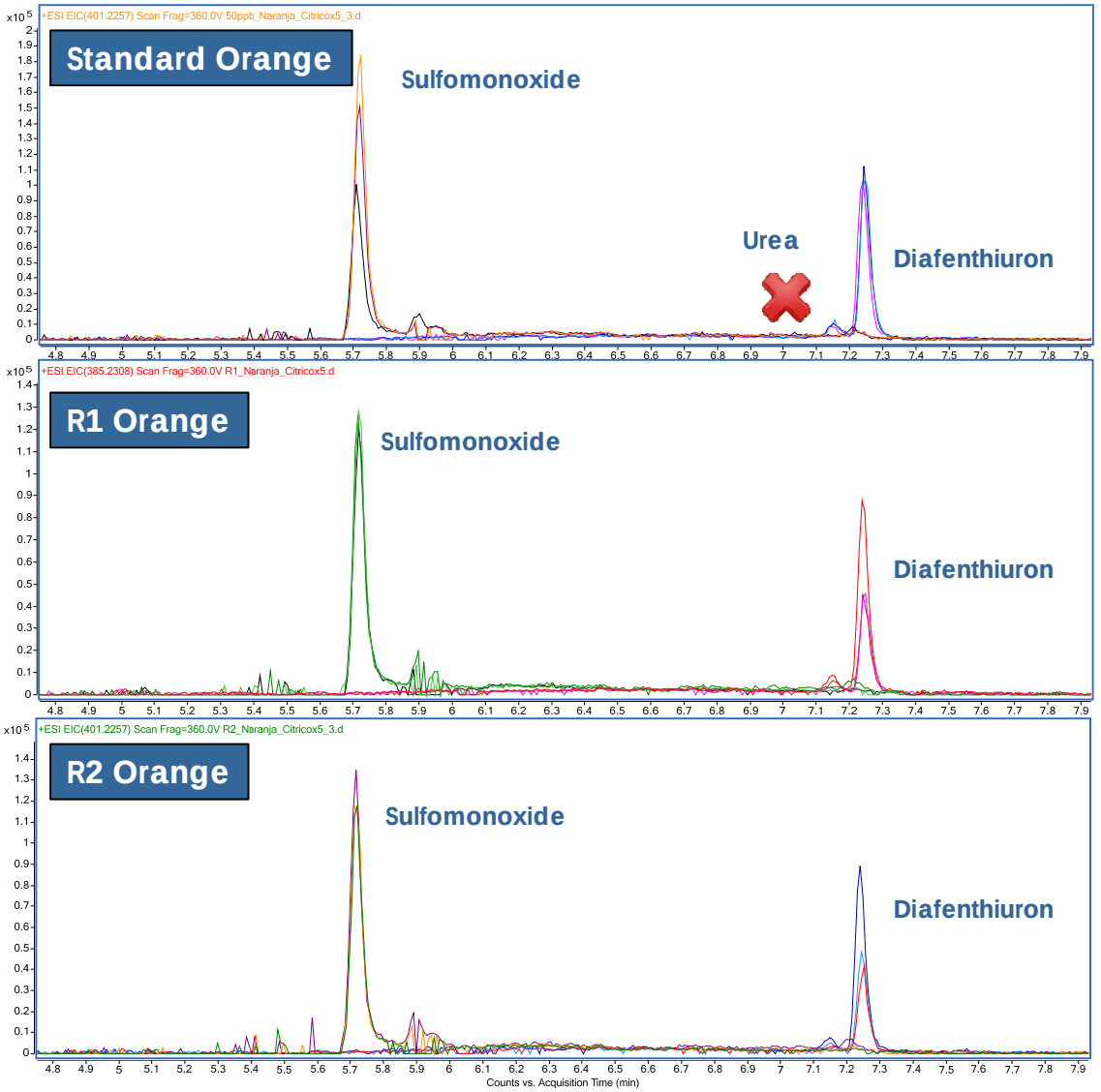

$C_{23}H_{32}N_2OS$
385.2308
Diafenthiuron (I)
Sulfomonoxide (IV)
$C_{23}H_{32}N_2O_2S$
401.2257
$C_{23}H_{30}N_2O_2$
351.2430
Carbodiimide (II)
$C_{23}H_{32}N_2O_2$
369.2536
urea

LC-QTOF-MS

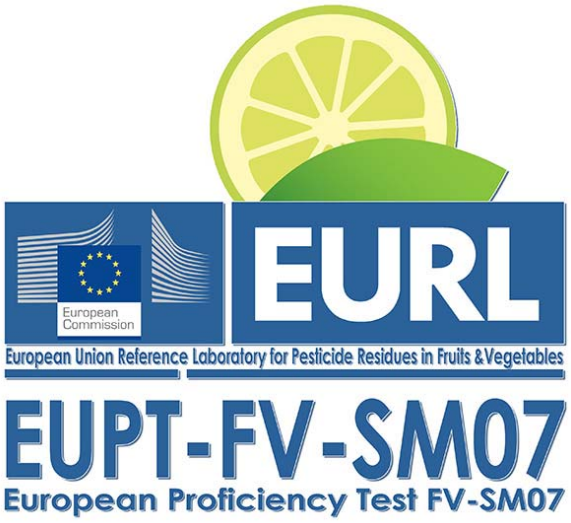

Diafenthiuron in Broccoli
AREA
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
R1:0% R2:0%
Difenthiuron
Sulfomonoxide
Urea
St1
St2
St3
R1_t1
R1_t2
R1_t3
R2_t1
R2_t2
R2_t3

LC-QTOF-MS


Diafenthiuron in Orange
AREA
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
Difenthiuron
Sulfomonoxide
Urea
St1
St2
St3
R1_t1
R1_t2
R1_t3
R2_t1
R2_t2
R2_t3

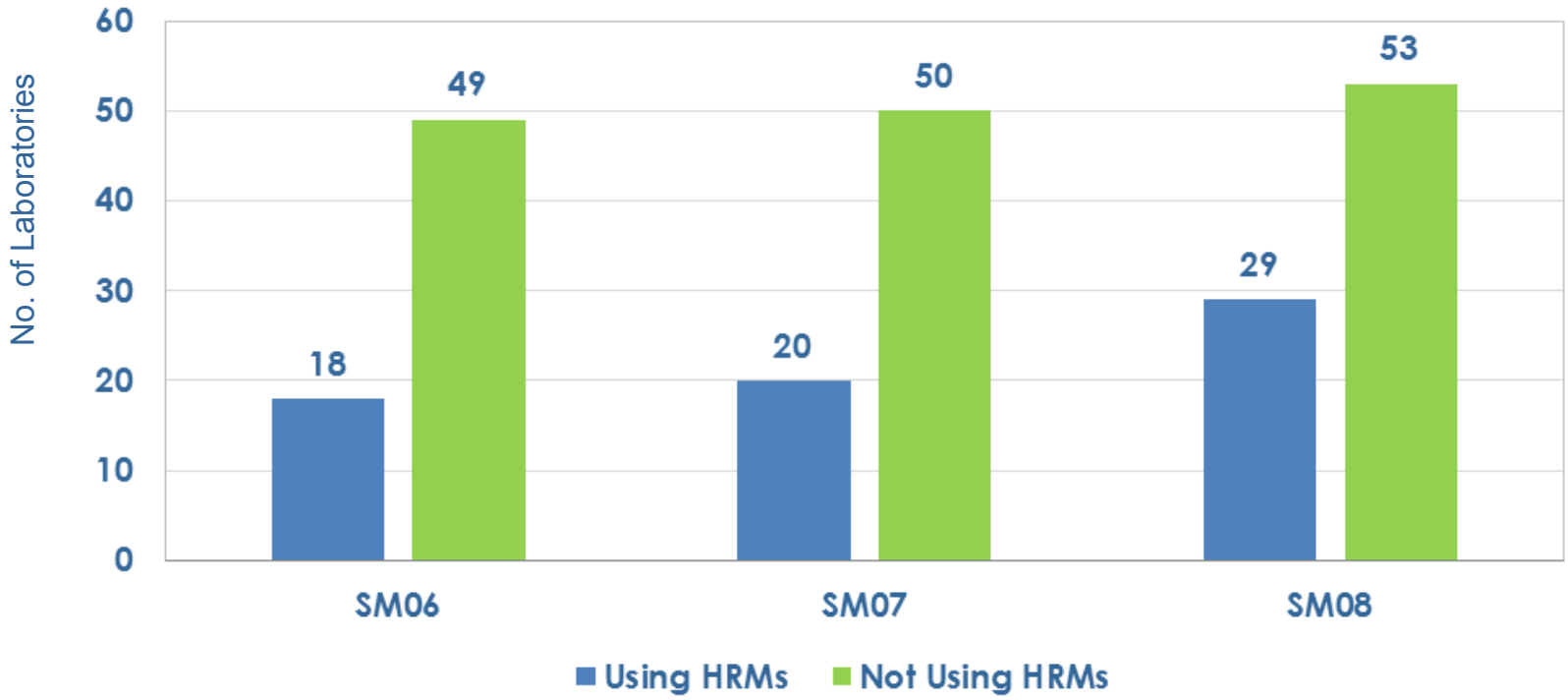

+ESI EIC(401.2257) Scan Frag=360.0V 50ppb_Naranja_Citricox5_3.d
Standard Orange
Sulfomonoxide
Urea
Diafenthiuron
+ESI EIC(385.2308) Scan Frag=360.0V R1_Naranja_Citricox5.d
R1 Orange
Sulfomonoxide
Diafenthiuron
+ESI EIC(401.2257) Scan Frag=360.0V R2_Naranja_Citricox5_3.d
R2 Orange
Sulfomonoxide
Diafenthiuron
Counts vs. Acquisition Time (min)


EURL
European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables
EUPT-FV-SM06
European Proficiency Test FV-SM06

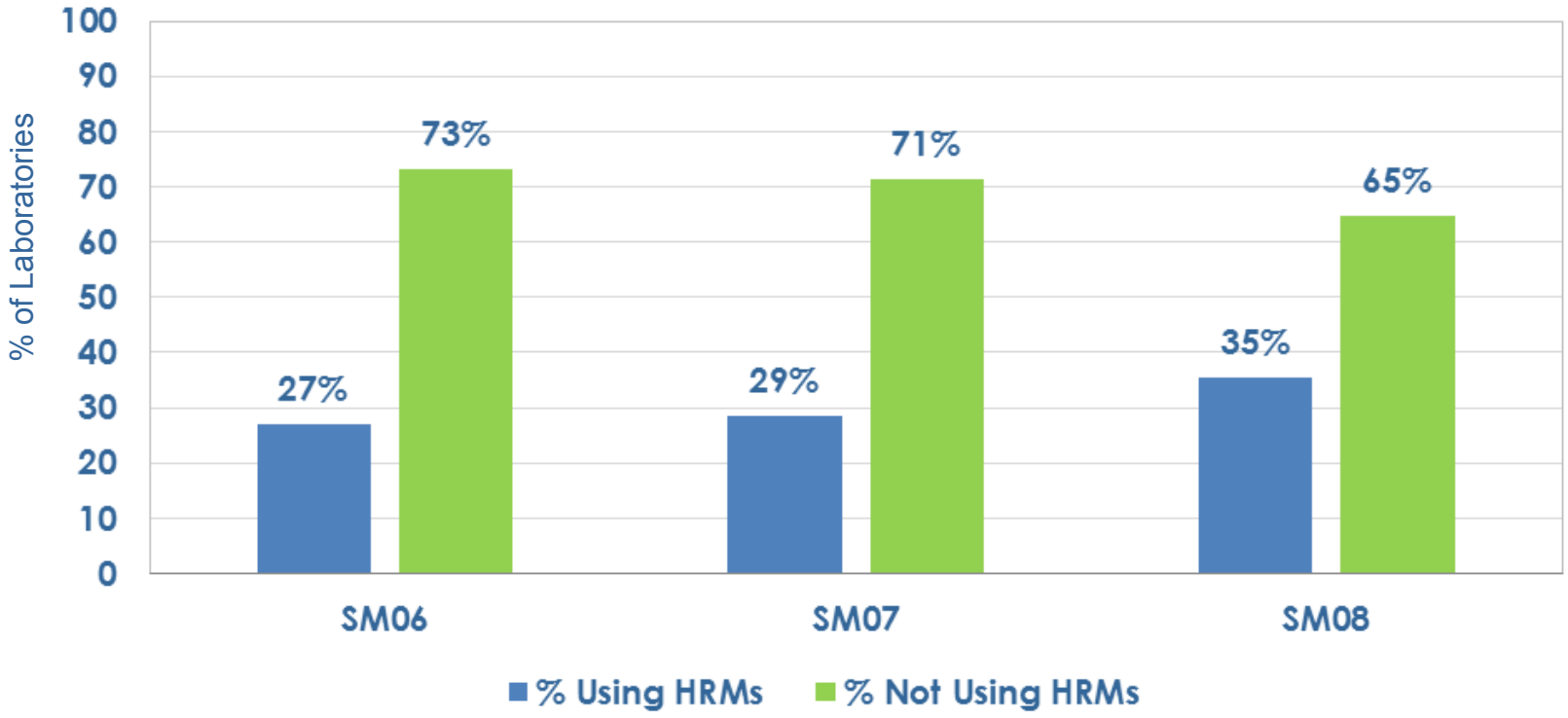

European Commission
EURL
European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables
EUPT-FV-SM07
European Proficiency Test FV-SM07


European Commission
EURL
European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables
EUPT-FV-SM08
European Proficiency Test FV-SM08

Number of Laboratories analising by HRAMS in the last three EUPTs-FV-SM



% of Laboratories analising by HRAMS in the last three EUPTs-FV-SM



Participation EUPT-FV-SM08

Austria
Belgium
Bulgary
Croatia
Cyprus
Czech Republic
Denmark
Estonia
Finland
France
Germany
Greece
Hungary
Italy
Ireland
Latvia
Netherlands
Norway
Romania
Serbia
Slovenia
Spain
Sweden
Switzerland
United Kingdom

24 EU/EFTA Countries

HRAMS

Czech Republic
Denmark
France
Germany
Greece
Italy
Netherlands
Norway
Romania
Spain
Sweden
United Kingdom

Thank You
for Your Attention