

6th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS

November 5 - 8, 2013 • Prague, Czech Republic

RAFA
2013

***NEW LC-MS APPROACHES TO
OVERCOME ANALYTICAL
DIFFICULTIES DERIVING FROM THE
APPLICATION OF EU
PESTICIDE REGULATIONS FOR FRUITS
AND VEGETABLES***



EURL-FV

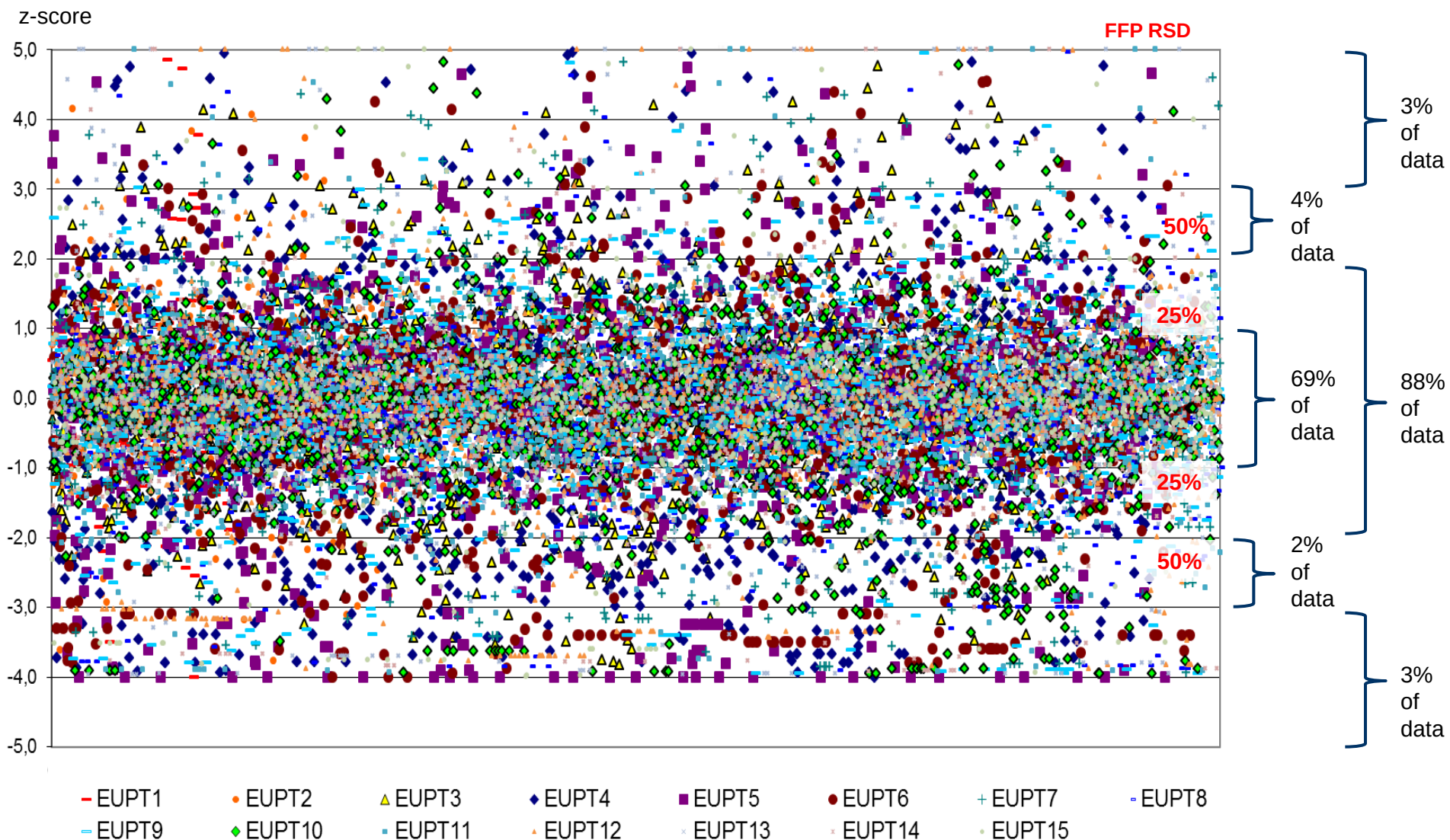


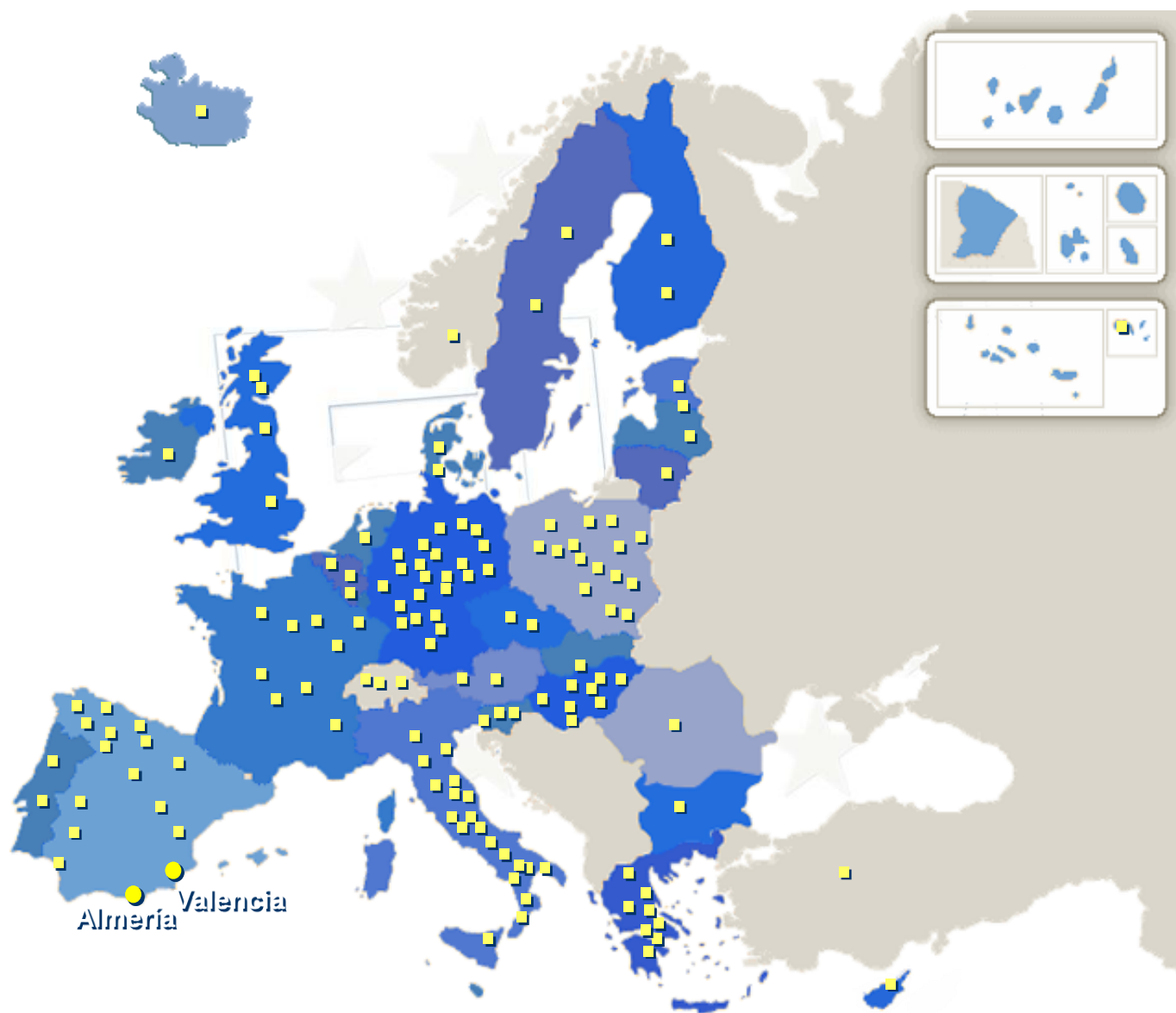
AMADEO R. FERNÁNDEZ-ALBA

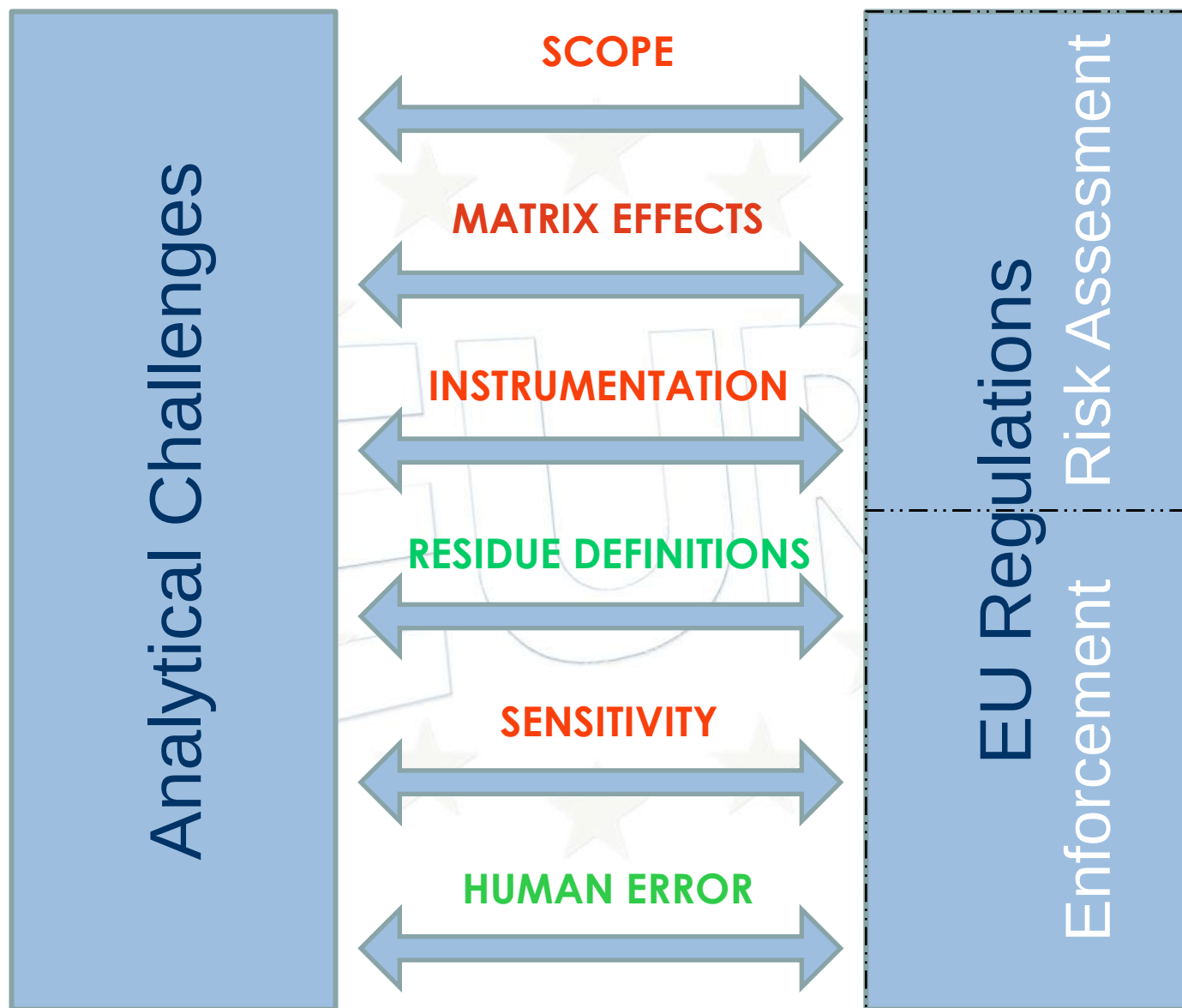
7th November 2013

Summary of Results

z-score results: 23764 points

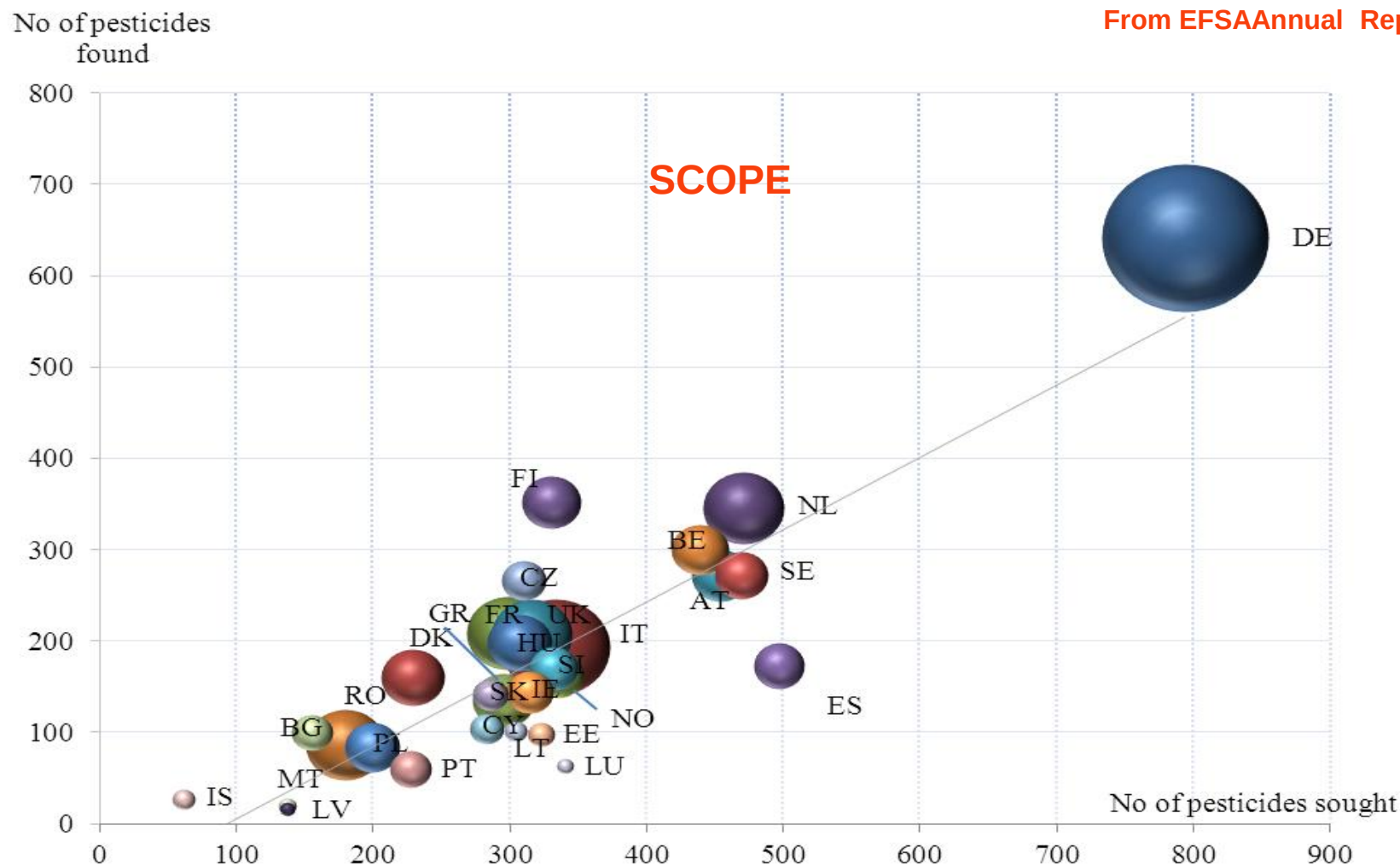






National and EU programmes

From EFSA Annual Report 2009

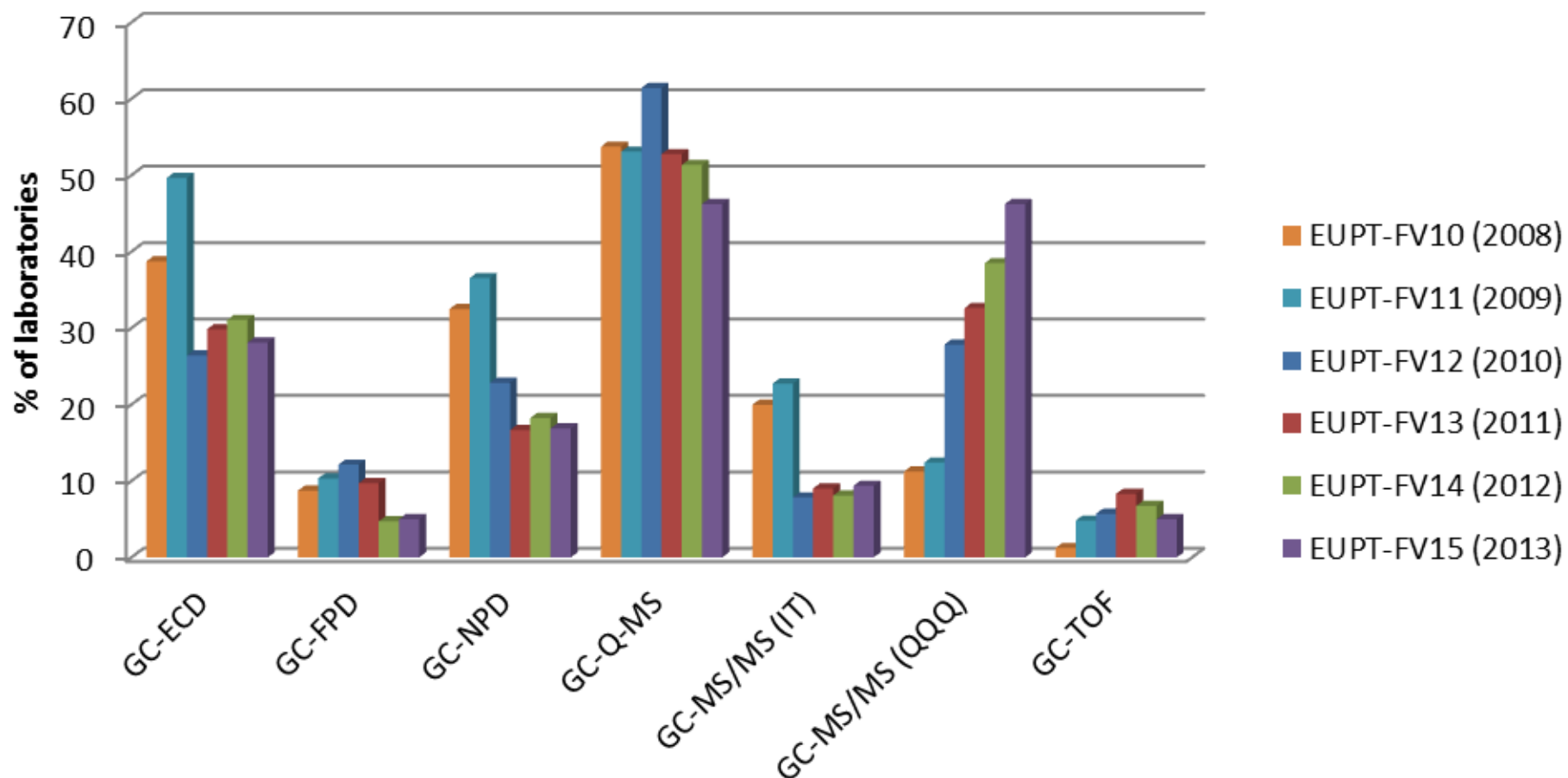


Number of samples analysed per reporting country (size of the dot), in combination with scope of the analytical methods used (total number of pesticides sought) and the number of different pesticides found.

Overview of the analytical techniques used by the EU official laboratories 2008-2013

INSTRUMENTATION

GC

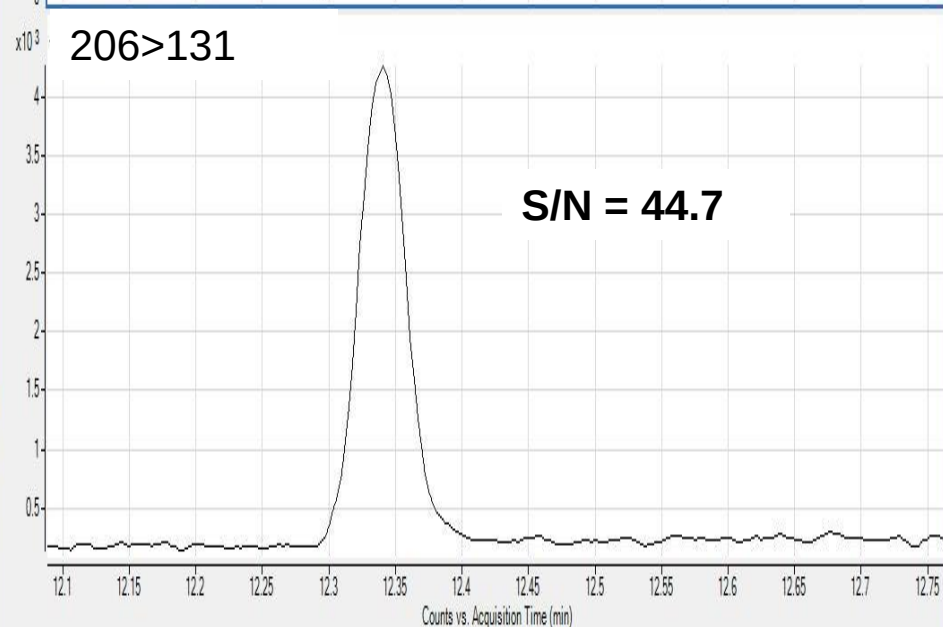
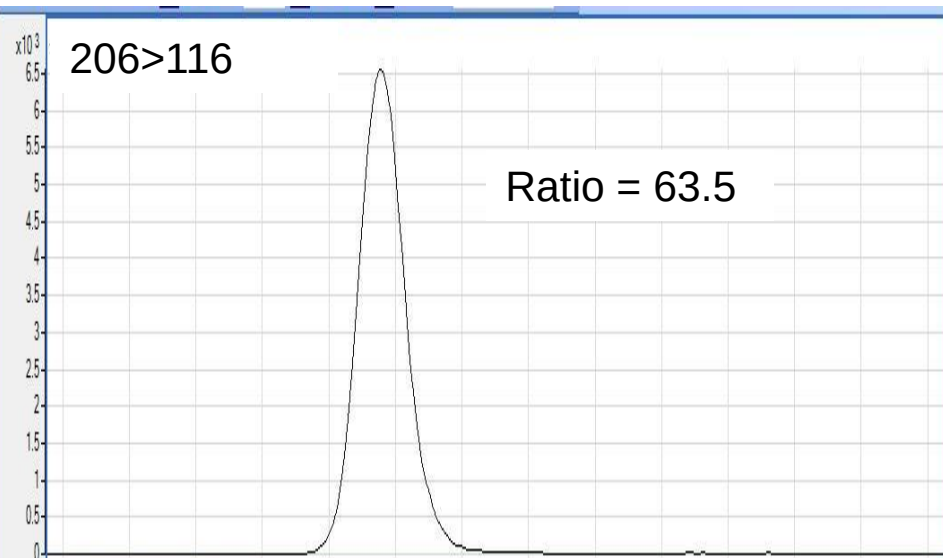
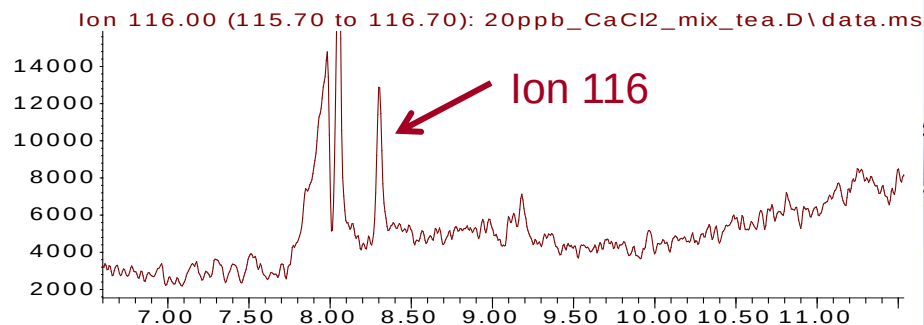
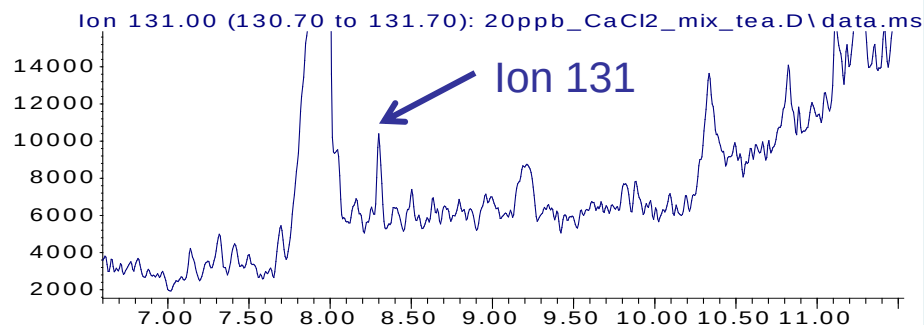
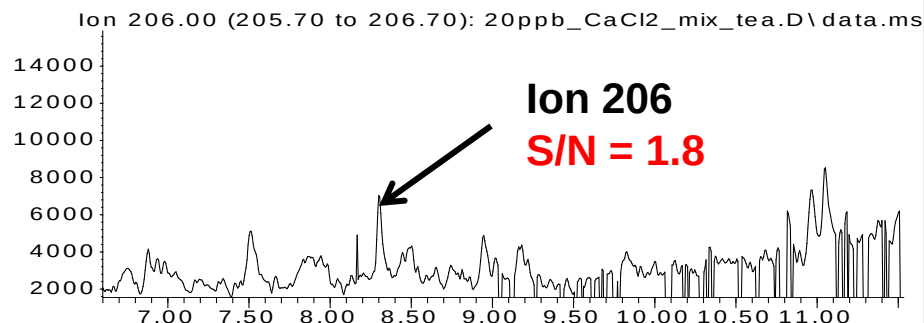


INSTRUMENTATION

Kresoxim-Methyl 20 µg/L in Green Tea

GC-Q-MS

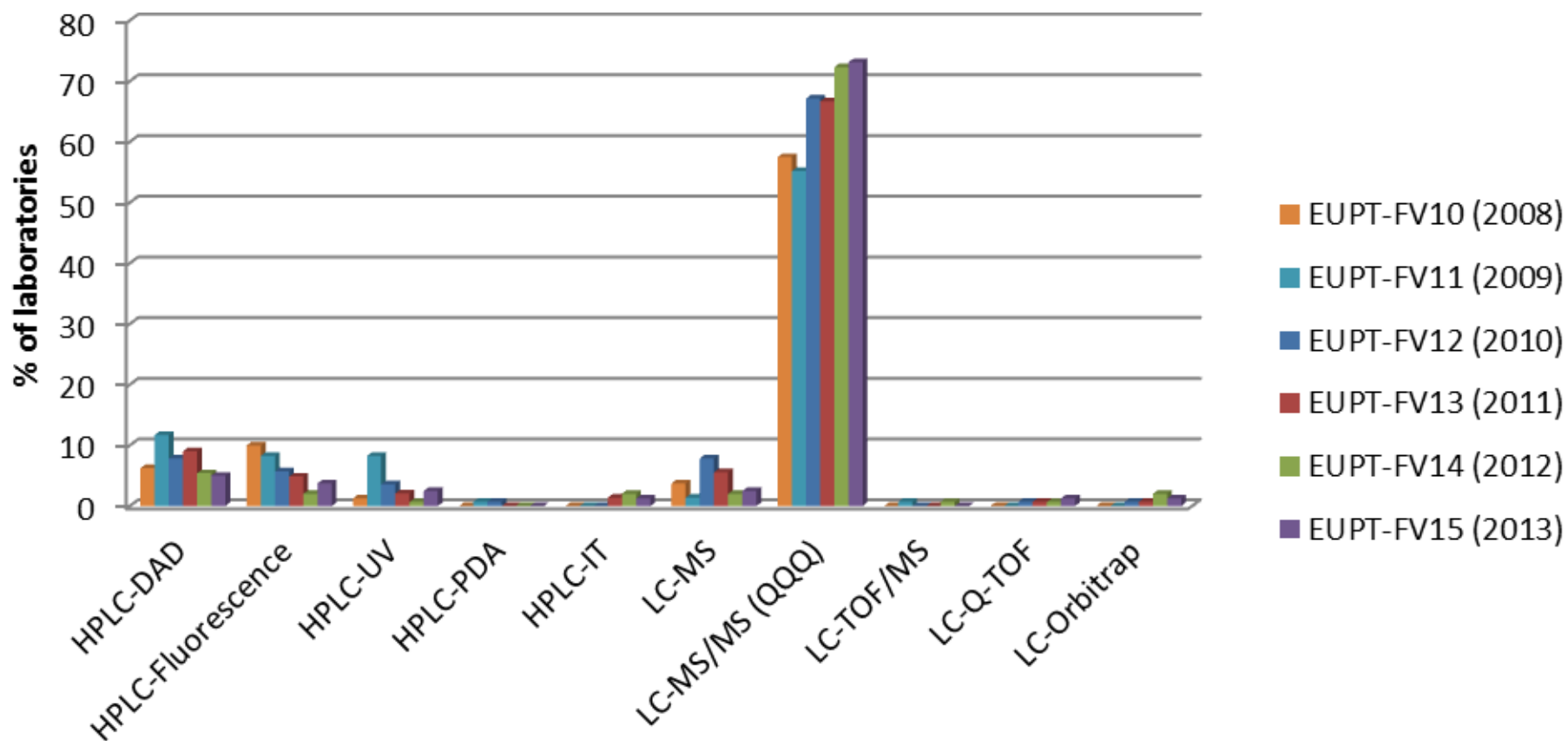
GC-QqQ-MS/MS



Overview of the analytical techniques used by the EU official laboratories 2008-2013

INSTRUMENTATION

LC

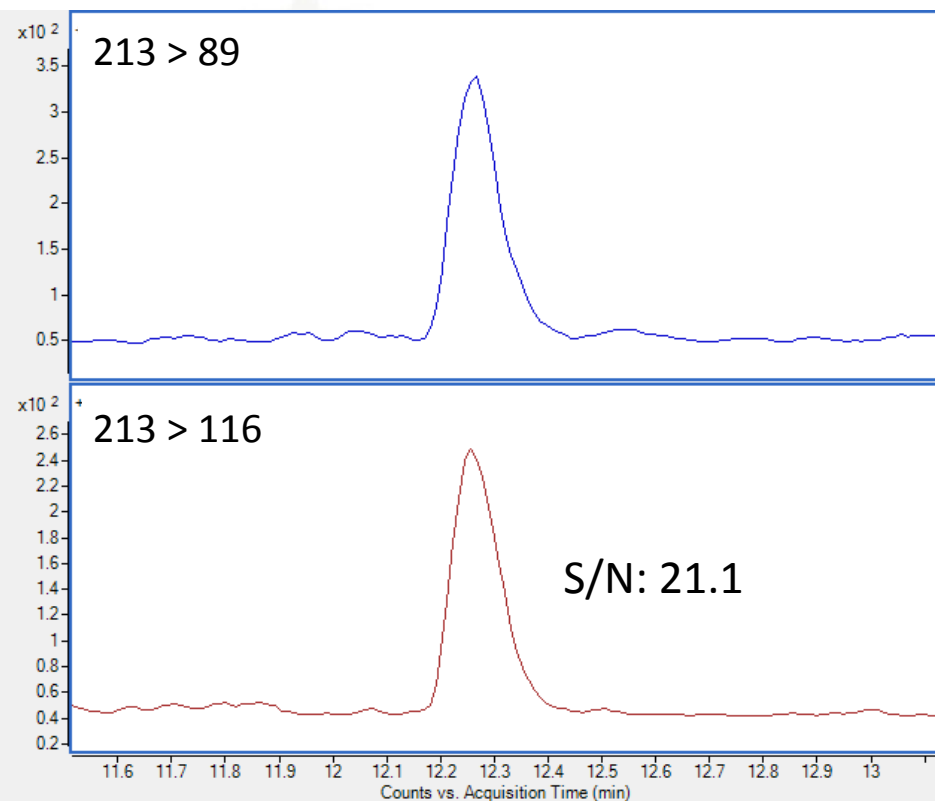
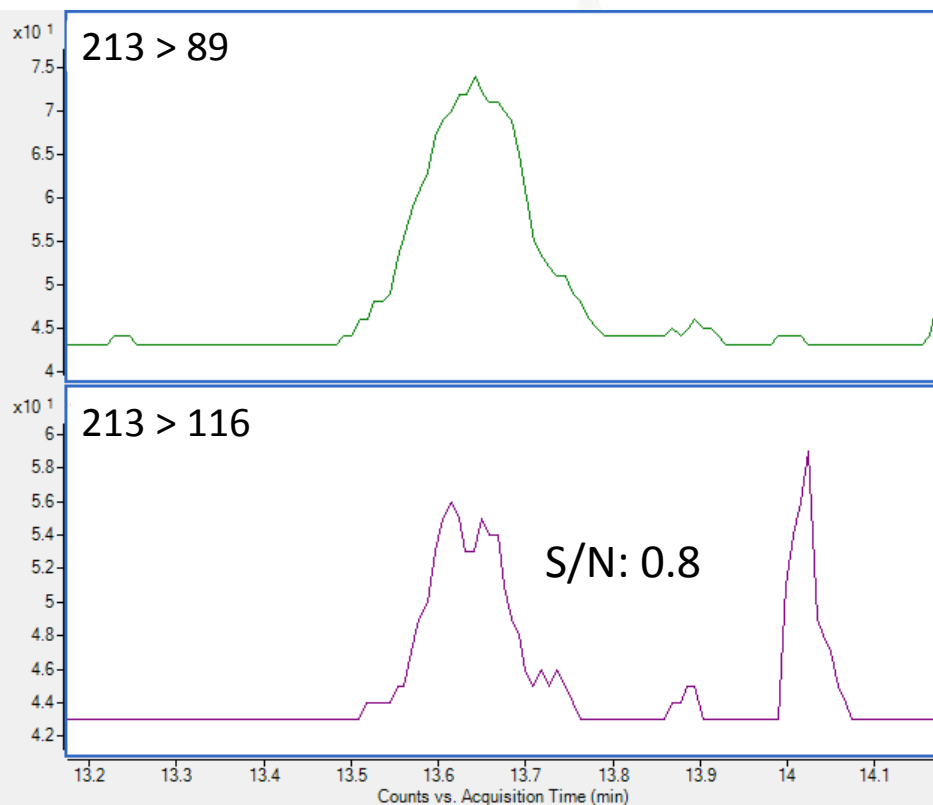


INSTRUMENTATION

Aldicarb

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

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



INSTRUMENTATION

For carbofuran/lettuce (0,02 mg/kg) and chlorfenvinphos/apples (0,02 mg/Kg) residues at the LOQ caused an exceedance of the ARfD. The MRLs for substances with extremely low toxicological reference values like carbofuran and chlorfenvinphos should be set at the lowest level achievable from an analytical point of view. Therefore it should be explored if a further lowering of the LOQs for these two substances is feasible.

From EFSA, EU 2010 Annual Report

What is the meaning of difficult here?

- Something unexpected
- Something out of our control
- Something beyond our **knowledge**
- Something beyond our capabilities

But can become feasible if:

- we identify exactly **what** the difficulty is
- we can control or avoid it



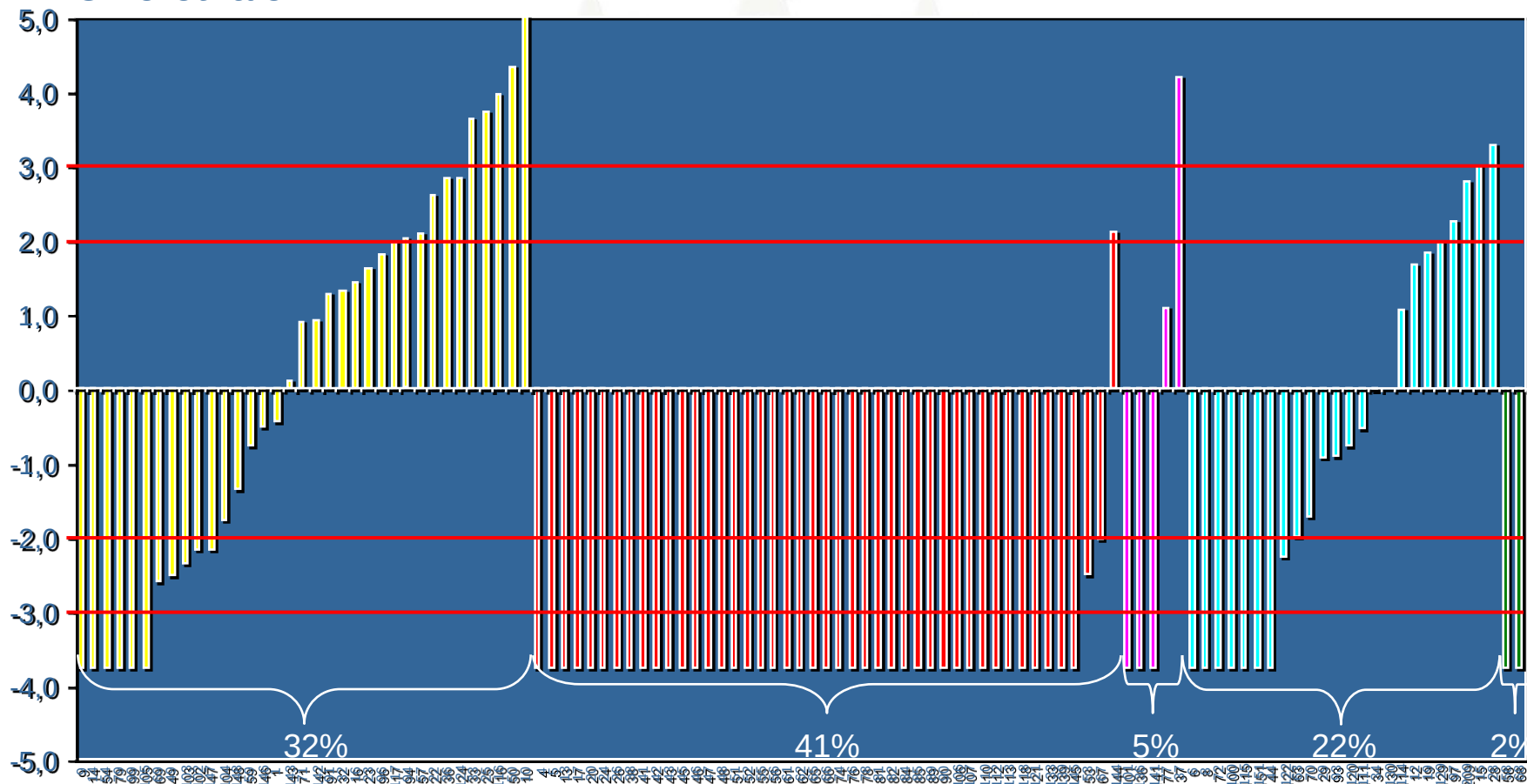
EUPT FV 12 – EPRW 2010 Strassbourg

EUPT-FV12 - Leek

35% of Reported Results
40% of False Negative Results
25% of Not Analysed Results

$|z| \leq 2$ Acceptable
 $2 < |z| \leq 3$ Questionable
 $3 < |z|$ Unacceptable

Chlorothalonil

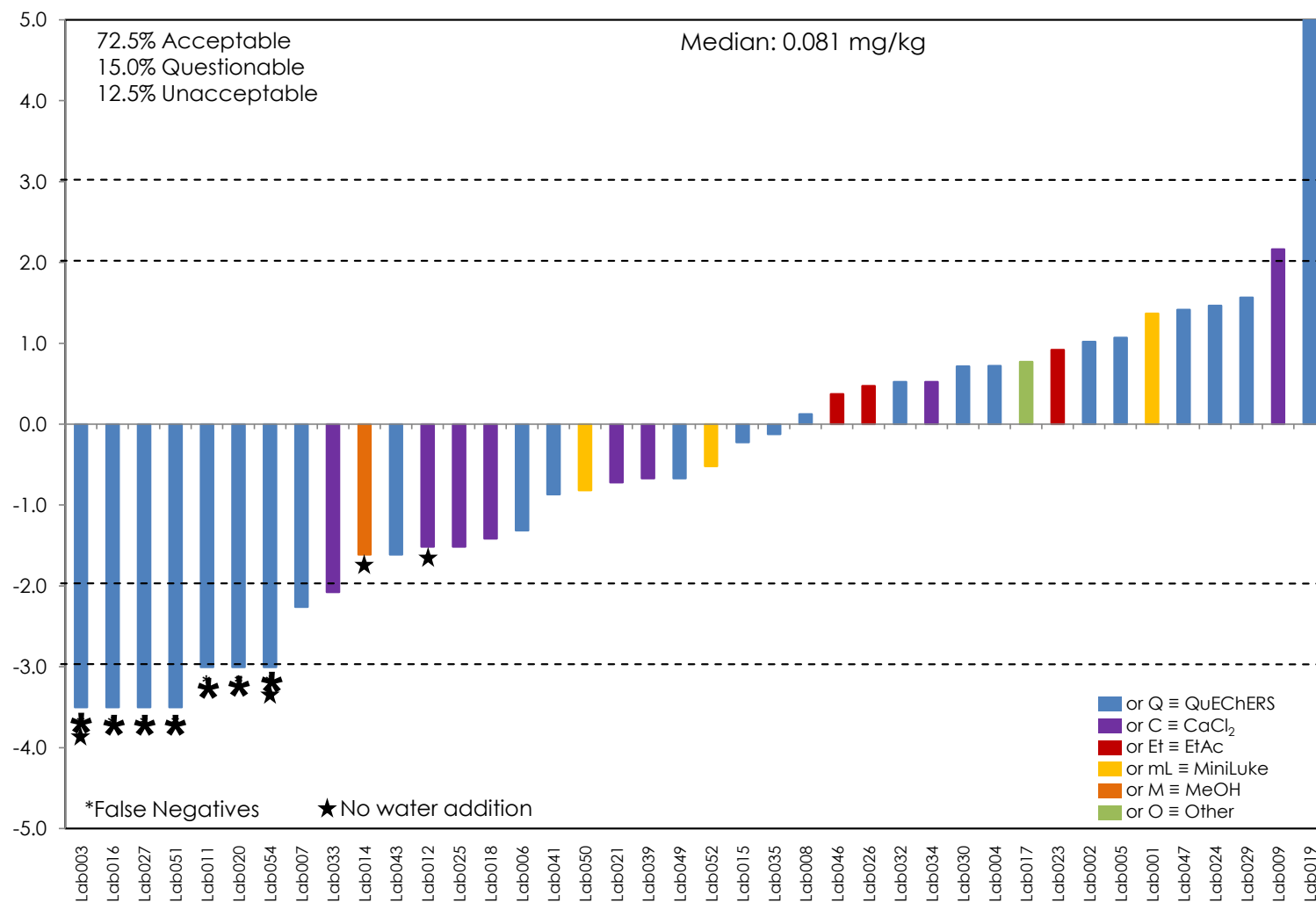


Acetone Acetonitrile Dichloromethane Ethyl Acetate Methanol

EUPT-T01 – Tea

Z-Scores

Imidacloprid



False negatives EUPT-T01

Laboratory Code	Acetamiprid	Buprofezin	Chlorpyrifos	Cypermethrin (cypermethrin incl. other mixtures of constituent isomers (sum of isomers))	Difenoconazole	Endosulfan beta	Etofenprox	Fenpropathrin	Imidacloprid	Methomyl	Parathion-ethyl	Pyridaben	Tebuconazole	Thiophanate- methyl
Lab003									ND	ND				
Lab006	ND													
Lab007				ND						ND				ND
Lab010		ND												
Lab011									ND	ND				
Lab013			ND											
Lab016				ND		ND			ND	ND				
Lab018				ND										
Lab020									ND					
Lab022											ND	ND	ND	
Lab025				ND						ND				
Lab026										ND				
Lab027	ND								ND	ND	ND			
Lab033		ND												
Lab034		ND						ND						
Lab035							ND							
Lab039				ND										
Lab043				ND		ND	ND							
Lab047						ND								
Lab050														ND
Lab051	ND								ND	ND				
Lab054	ND				ND				ND	ND				

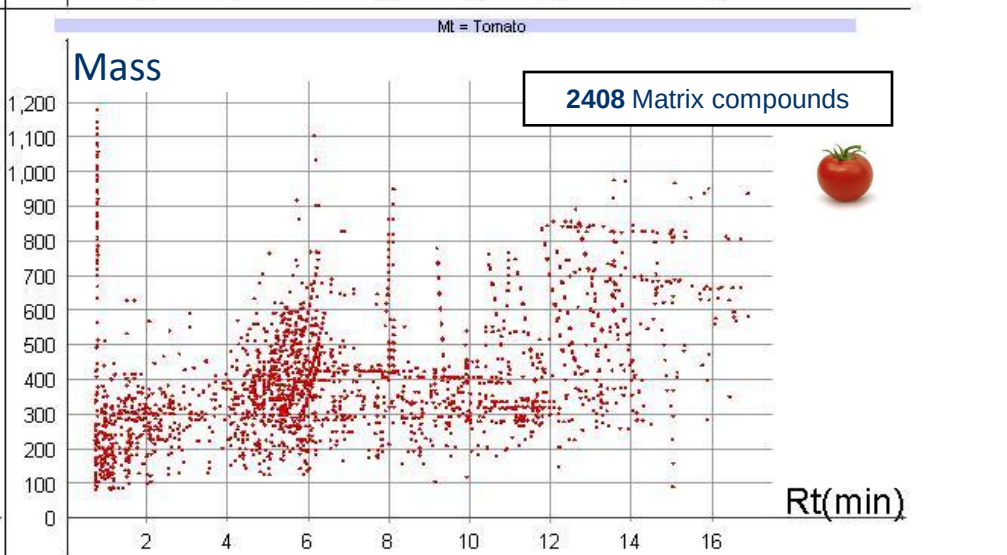
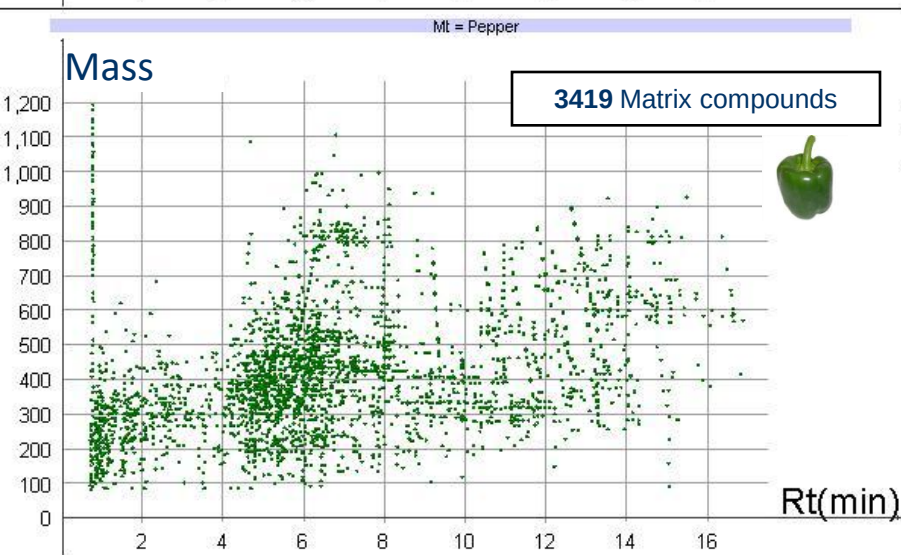
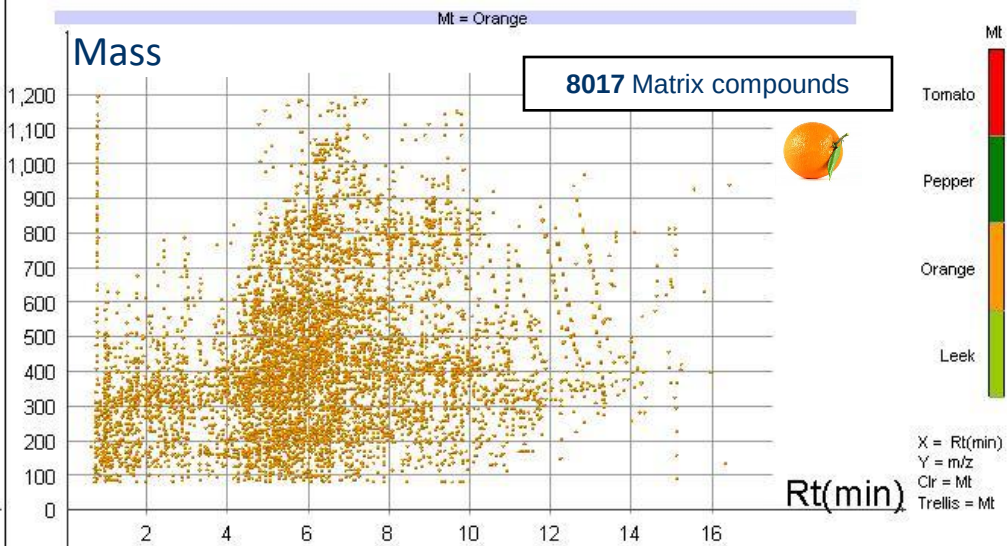
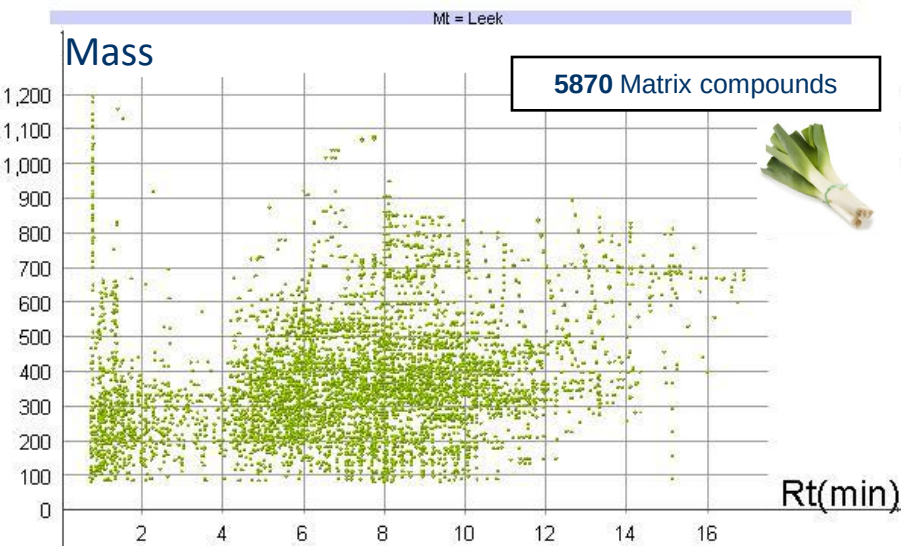
■ QuEChERS
■ CaCl₂
■ EtAc
■ MiniLuke
■ MeOH
■ Other

False negatives (%) over
the total number of
results per extraction
method

QuEChERS: 6.9%
 CaCl₂: 6.3%
 Ethyl Acetate method: 2.3%
 MeOH extraction: 6.7%
 miniLuke: 6.1%
 Other methods: 2.2%



Co-extracted matrix components Agilent LC-TOF-MS



Miner 3D Enterprise

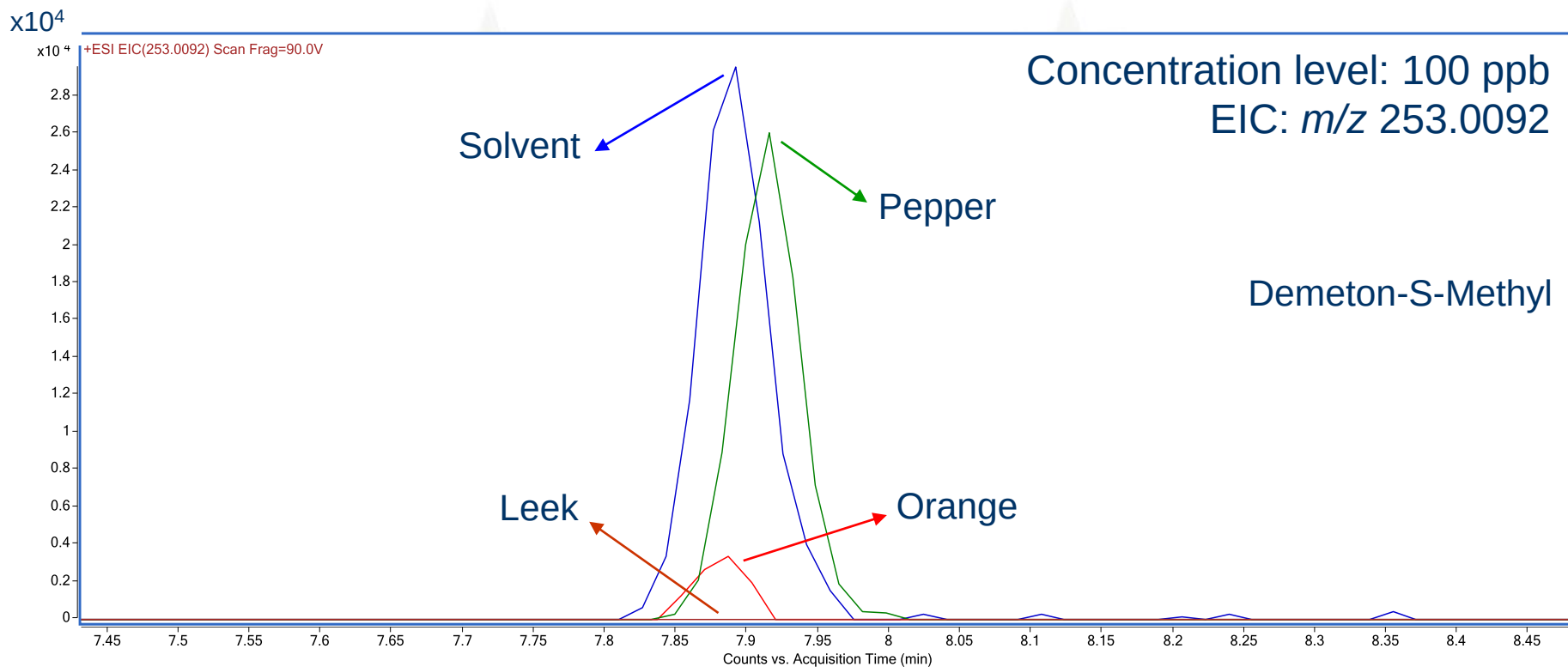
Compounds with absolute height ≥ 10000 counts

Injected: 1g sample/mL
extract

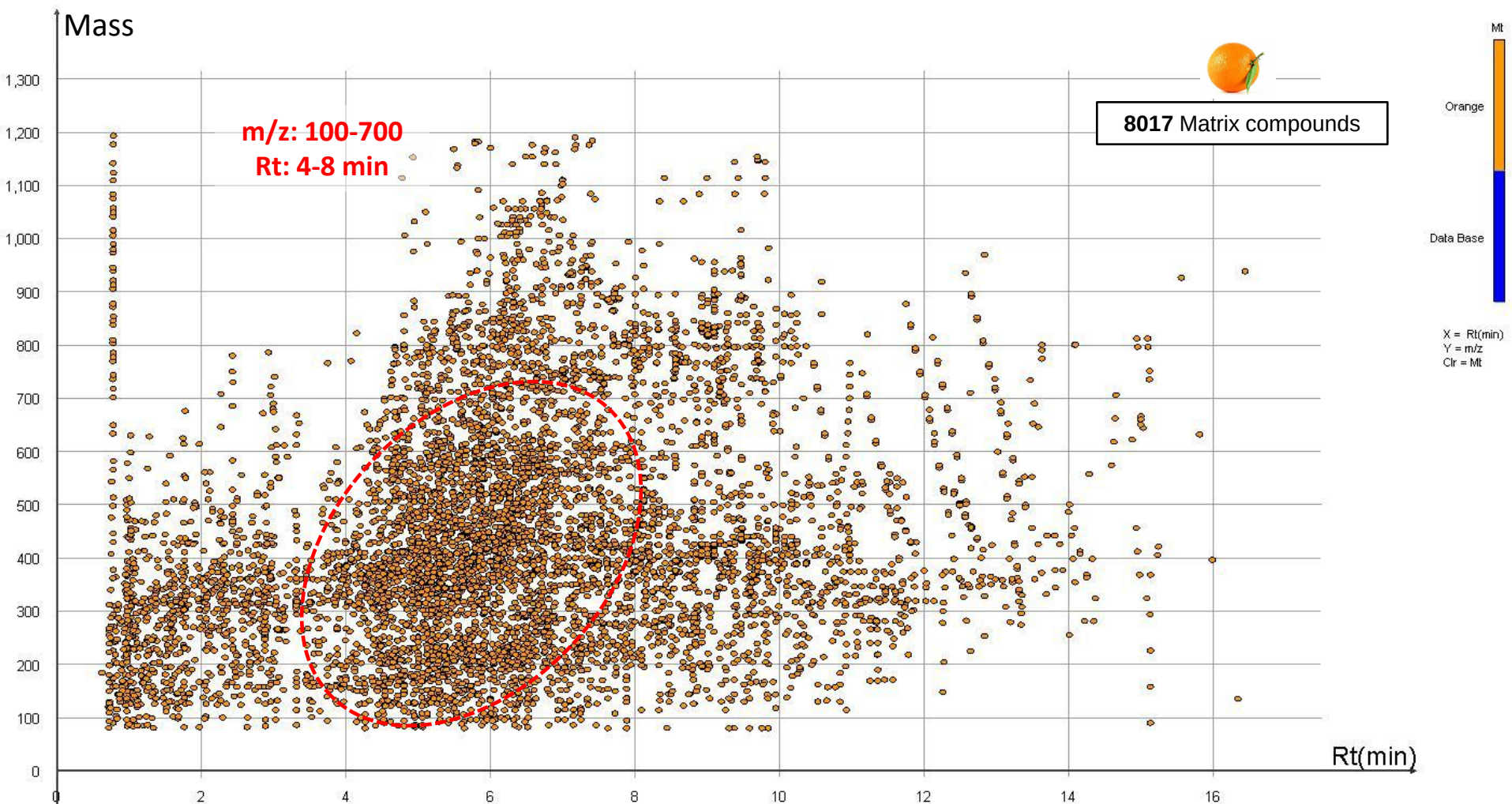
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Signal suppression due to matrix effects

Injection 1g matrix/ml

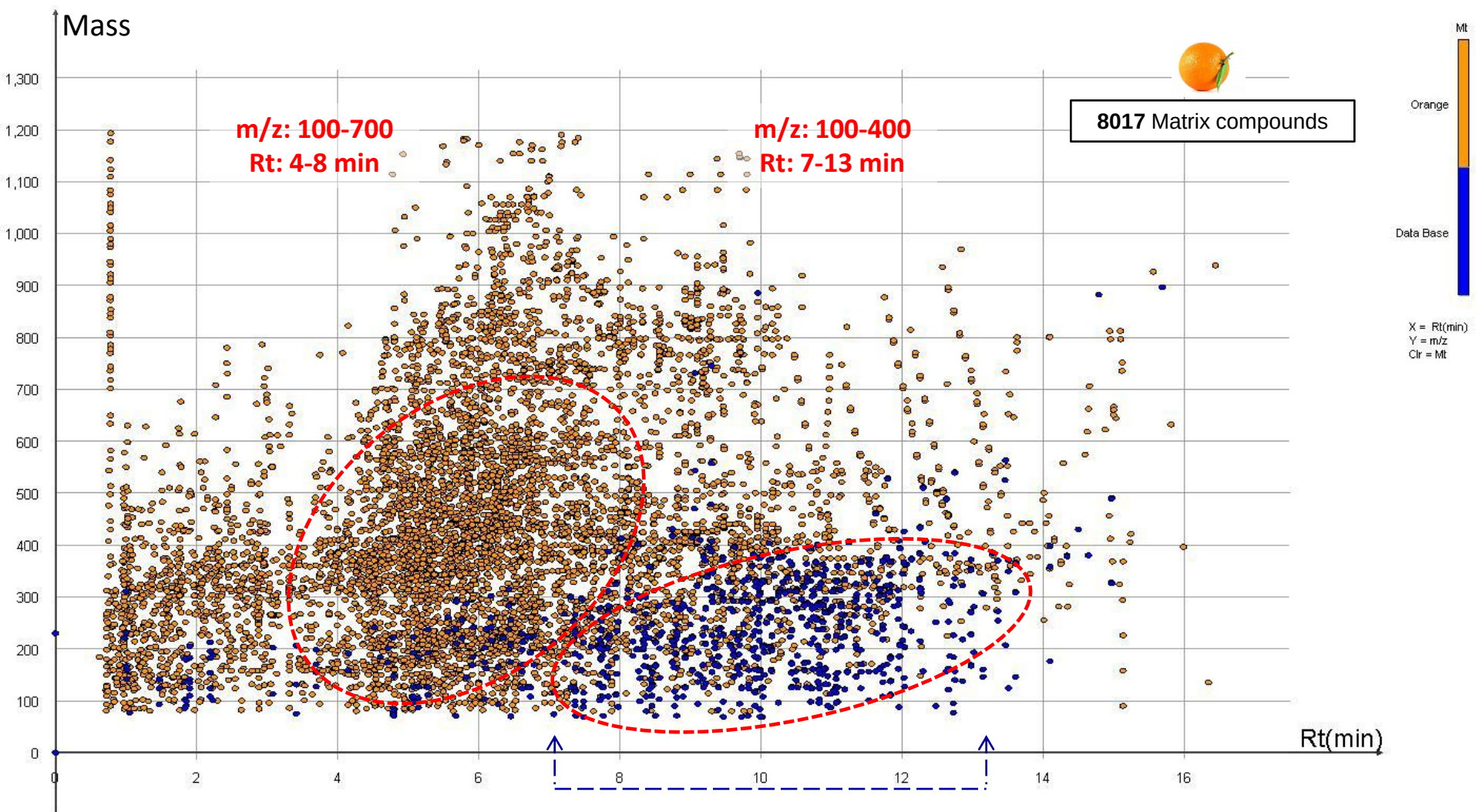


Co-extracted matrix components LC-TOF-MS of orange



Miner 3D Enterprise

Data base components- orange matrix compounds

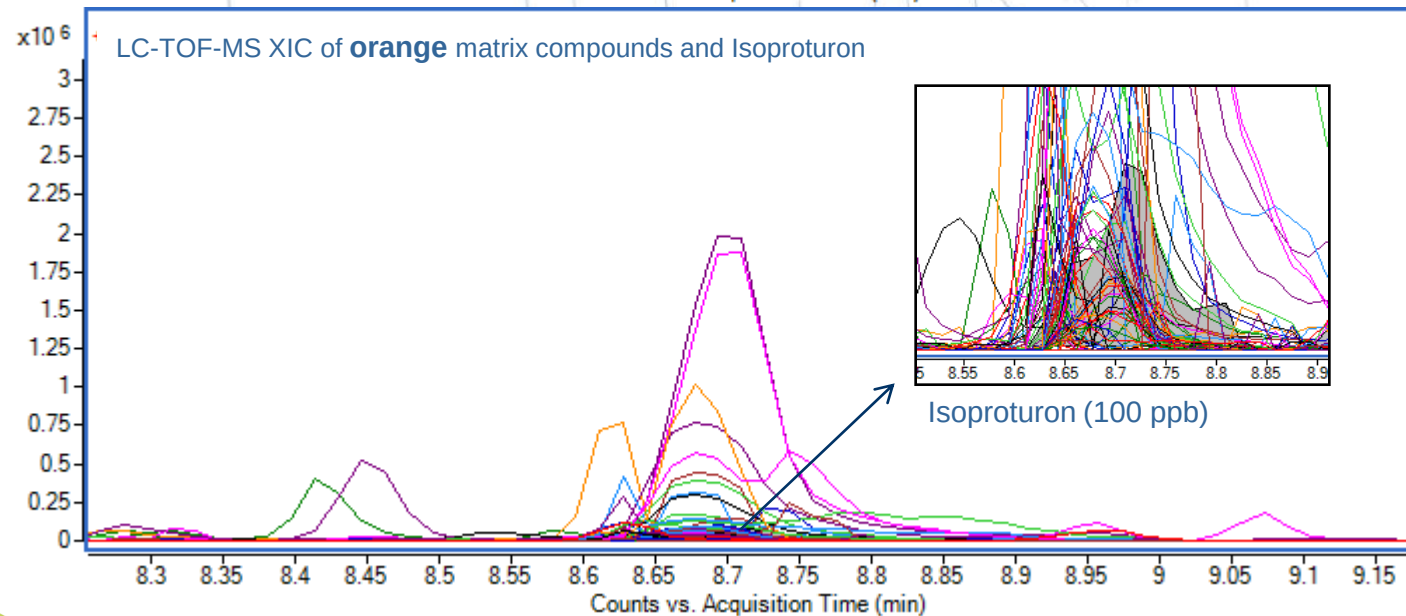
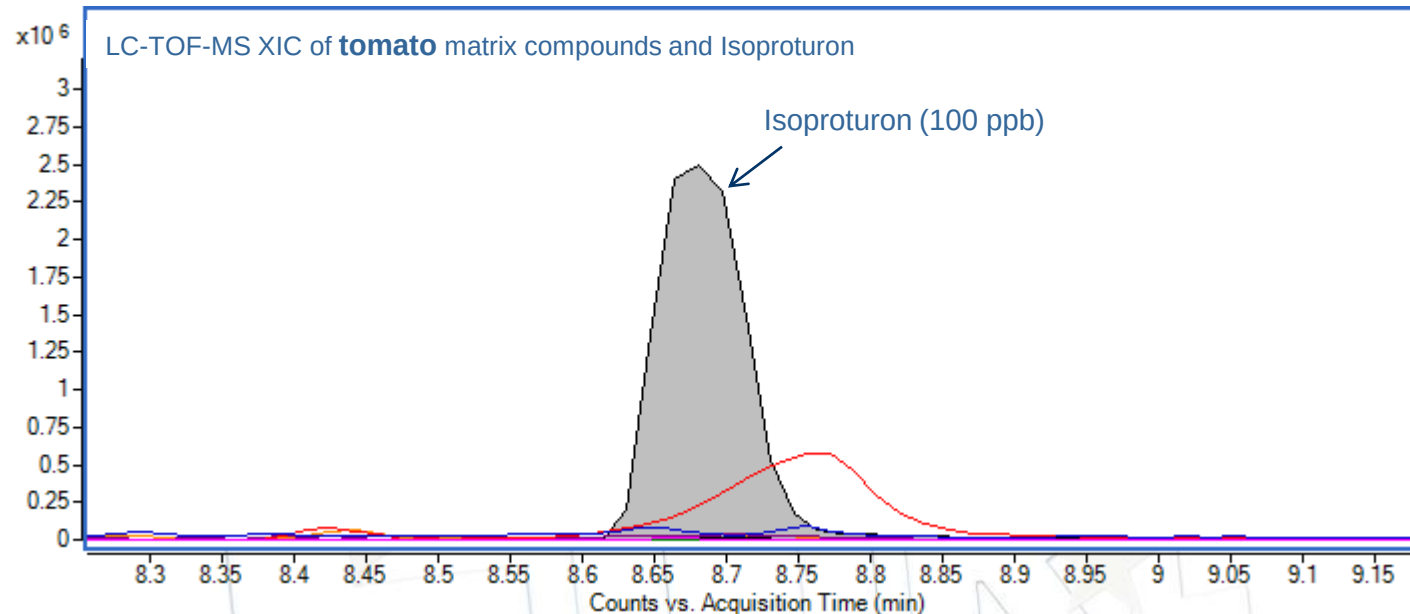


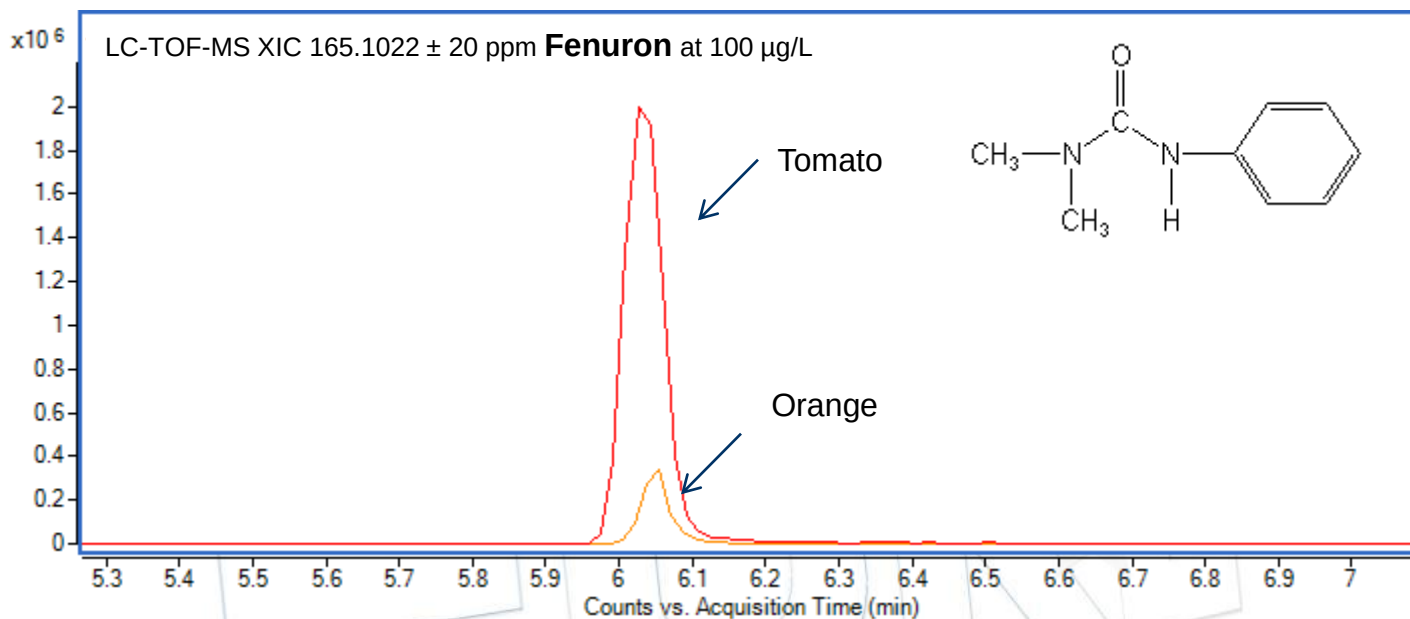
Miner 3D Enterprise

DB: 750 components

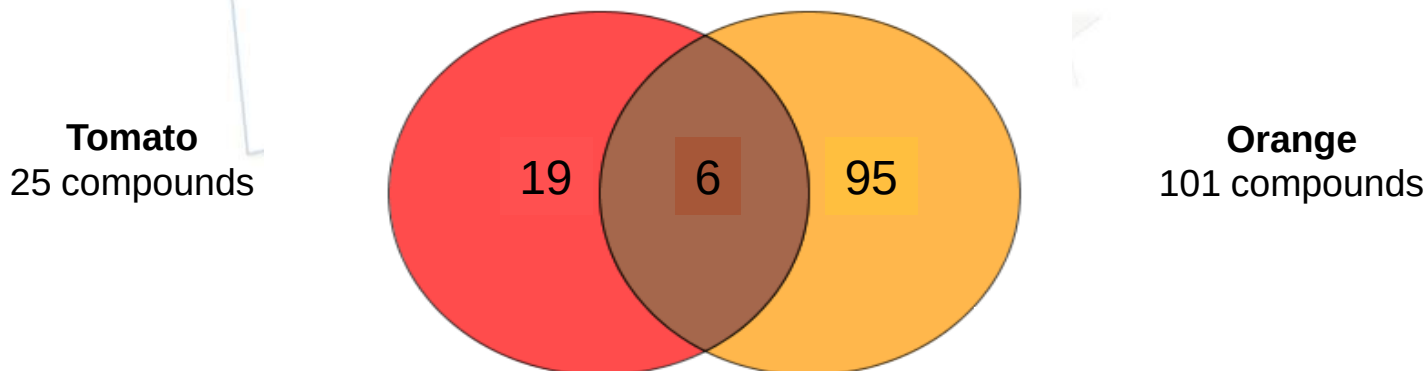
Orange: 2743 matrix compounds

Amadeo R. Fernández-Alba



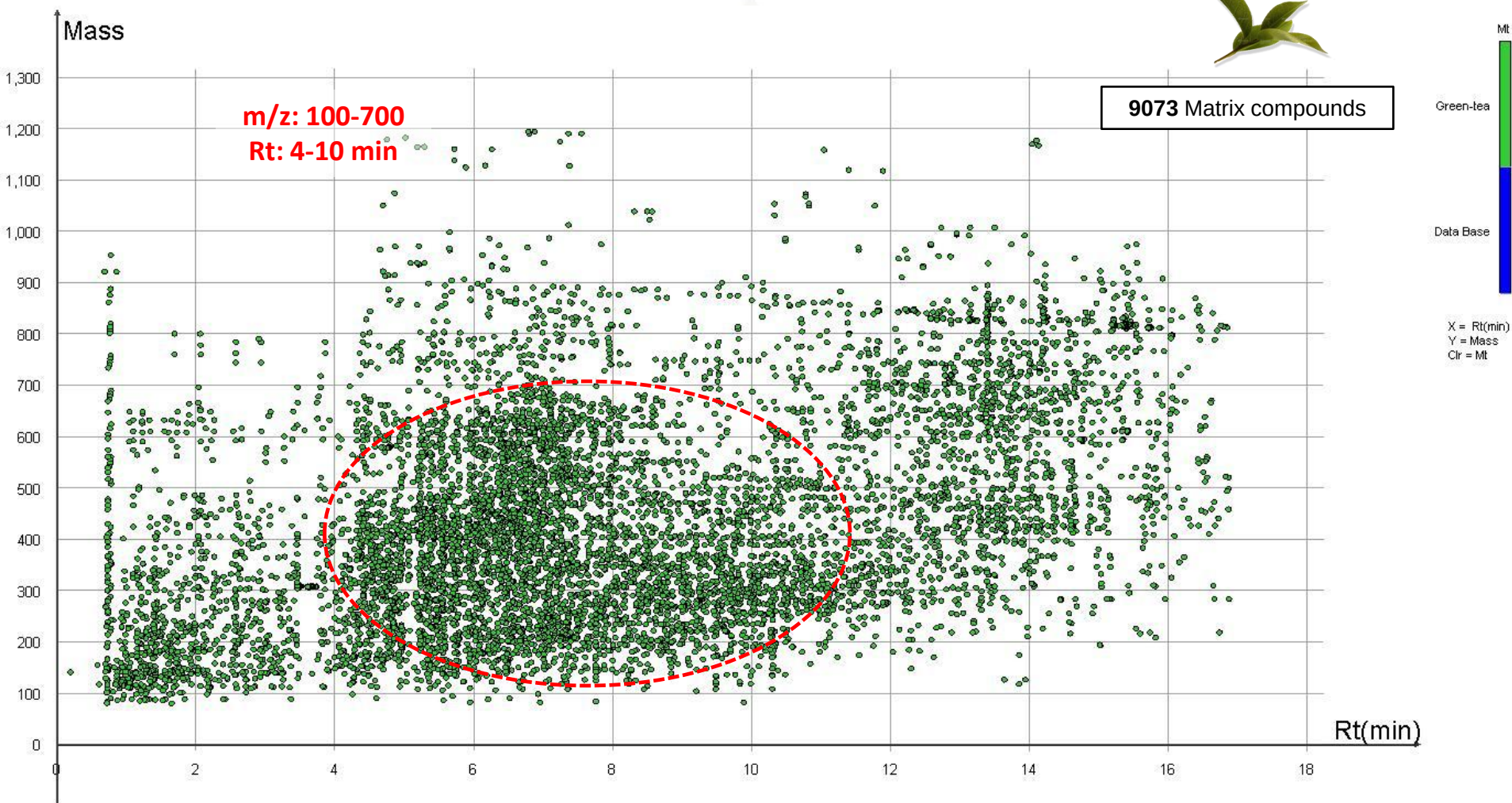


Number of co-extracted matrix compounds Rt: 5.99-6.08 min



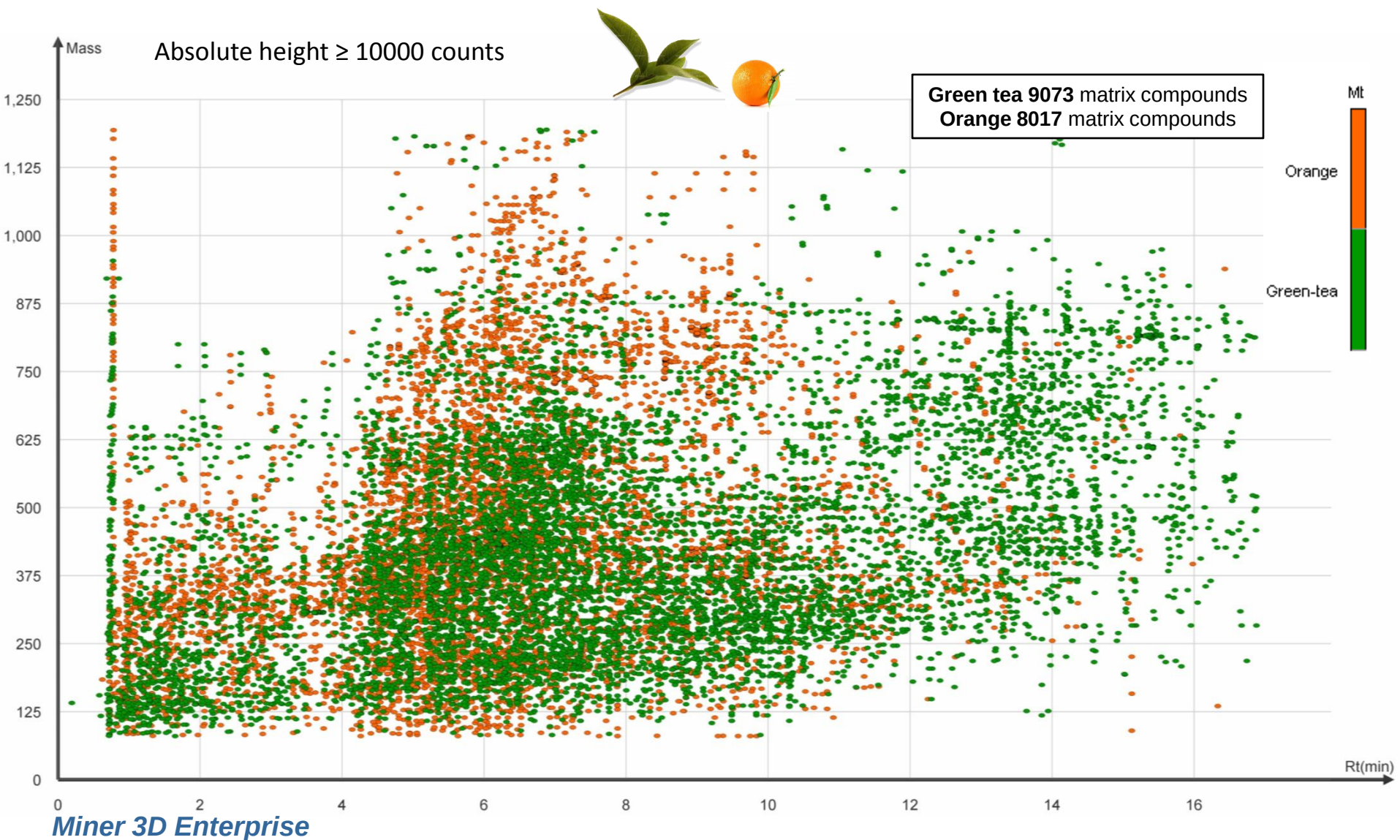
Mass Profiler Professional 12.1. Agilent Tech.

Co-extracted matrix components of green-tea

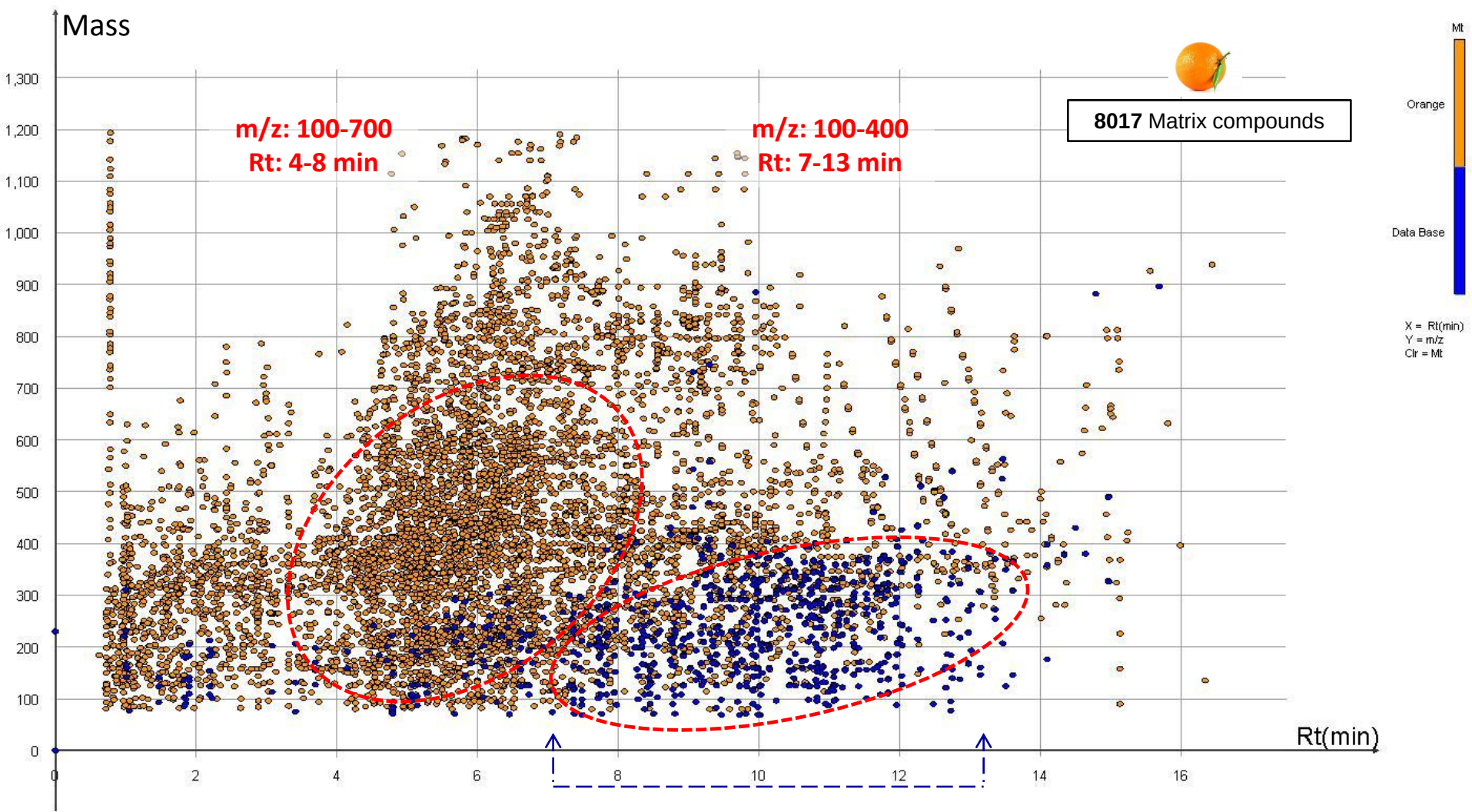


Miner 3D Enterprise

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



Data base components- orange matrix compounds



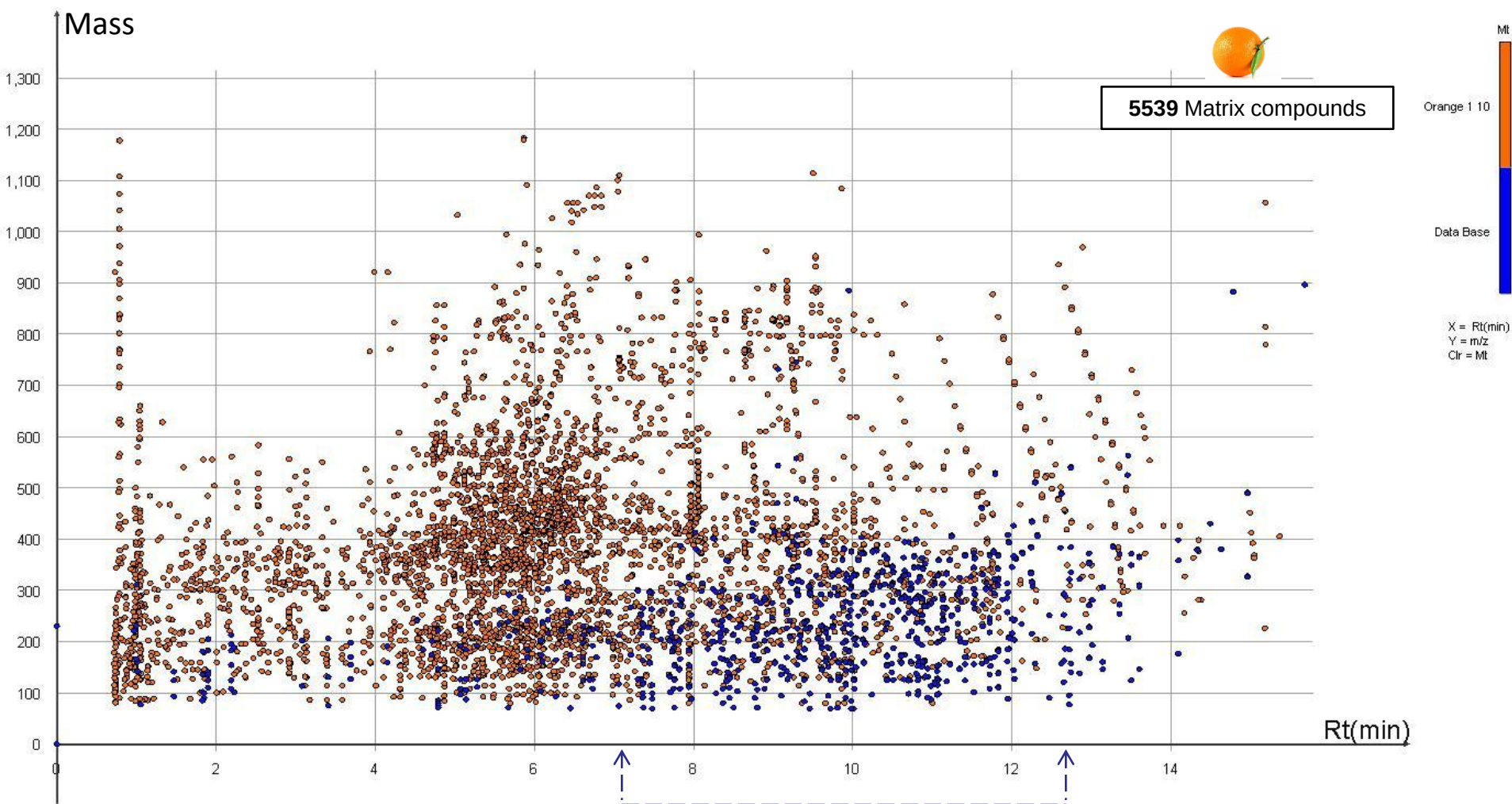
Miner 3D Enterprise

DB: 750 components

Orange: 2743 matrix compounds

Amadeo R. Fernández-Alba

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



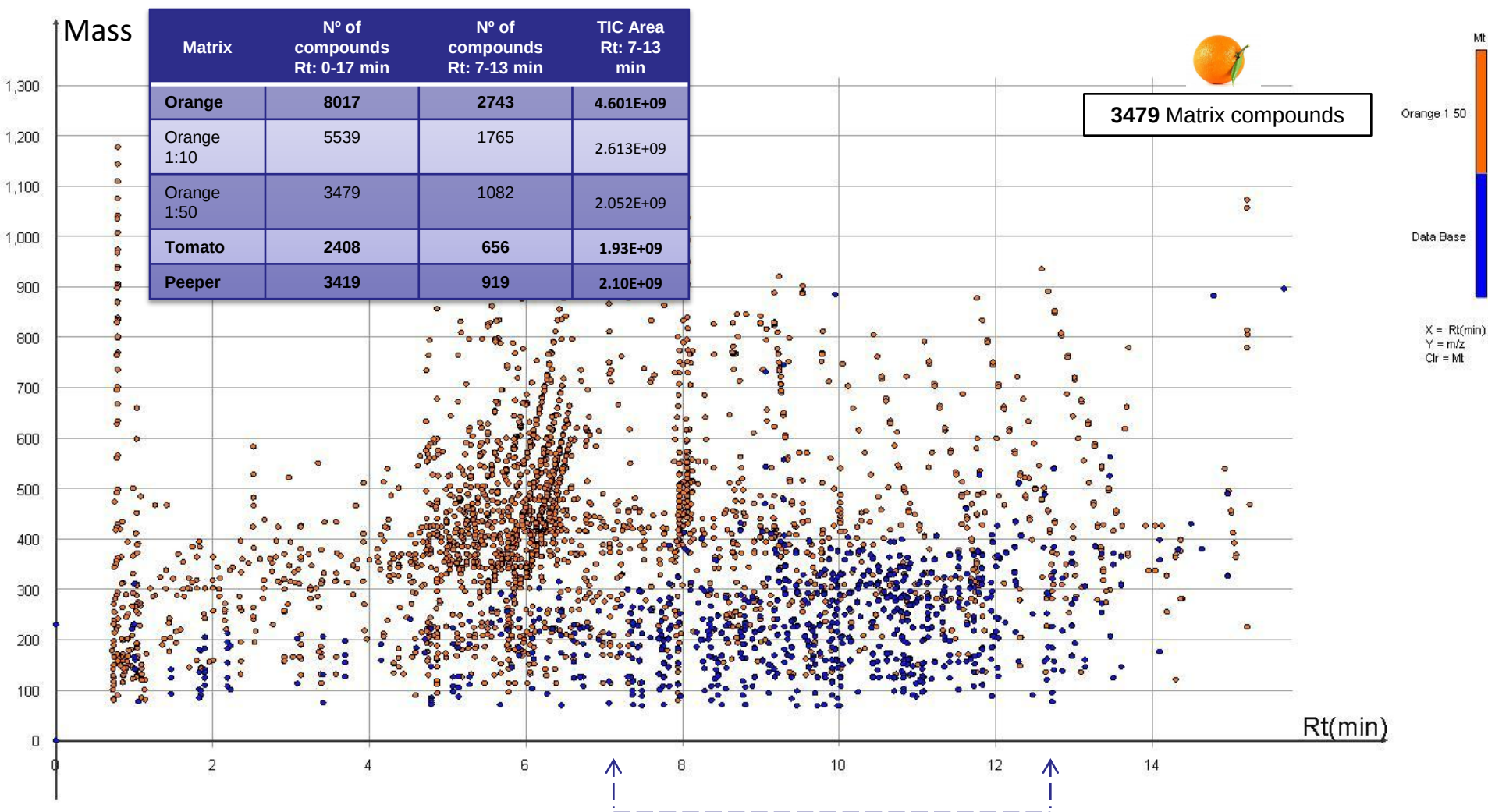
Miner 3D Enterprise

DB: 750 components

Orange: 1765 matrix compounds

Amadeo R. Fernández-Alba

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



Miner 3D Enterprise

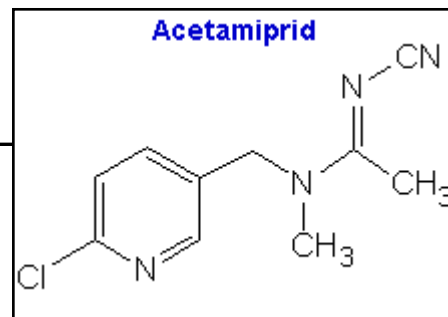
DB: 750 components

Orange: 1082 matrix compounds

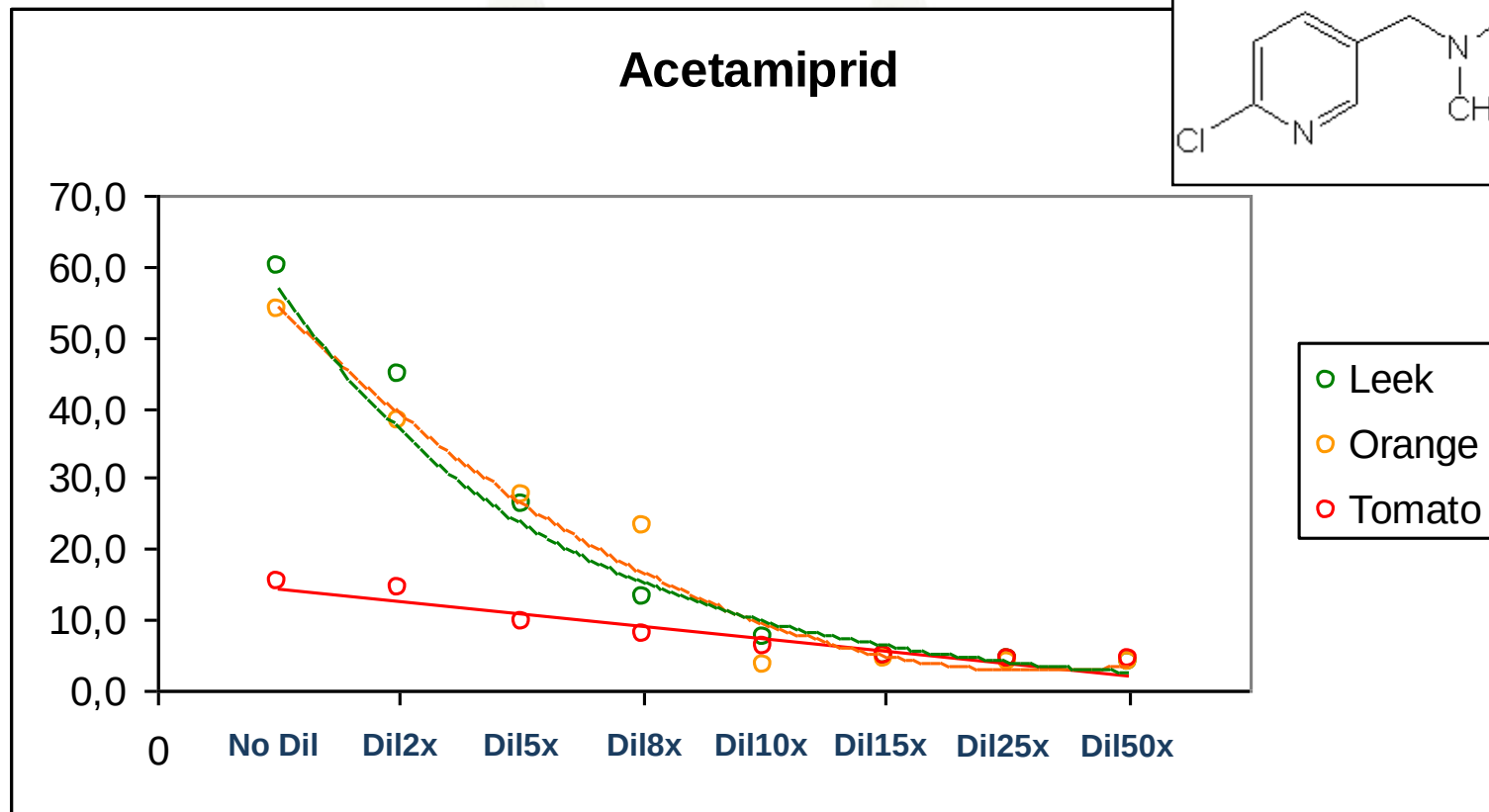
Amadeo R. Fernández-Alba

Matrix effect

Acetamiprid

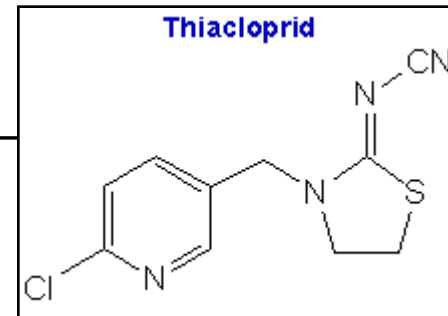


% Signal Suppression

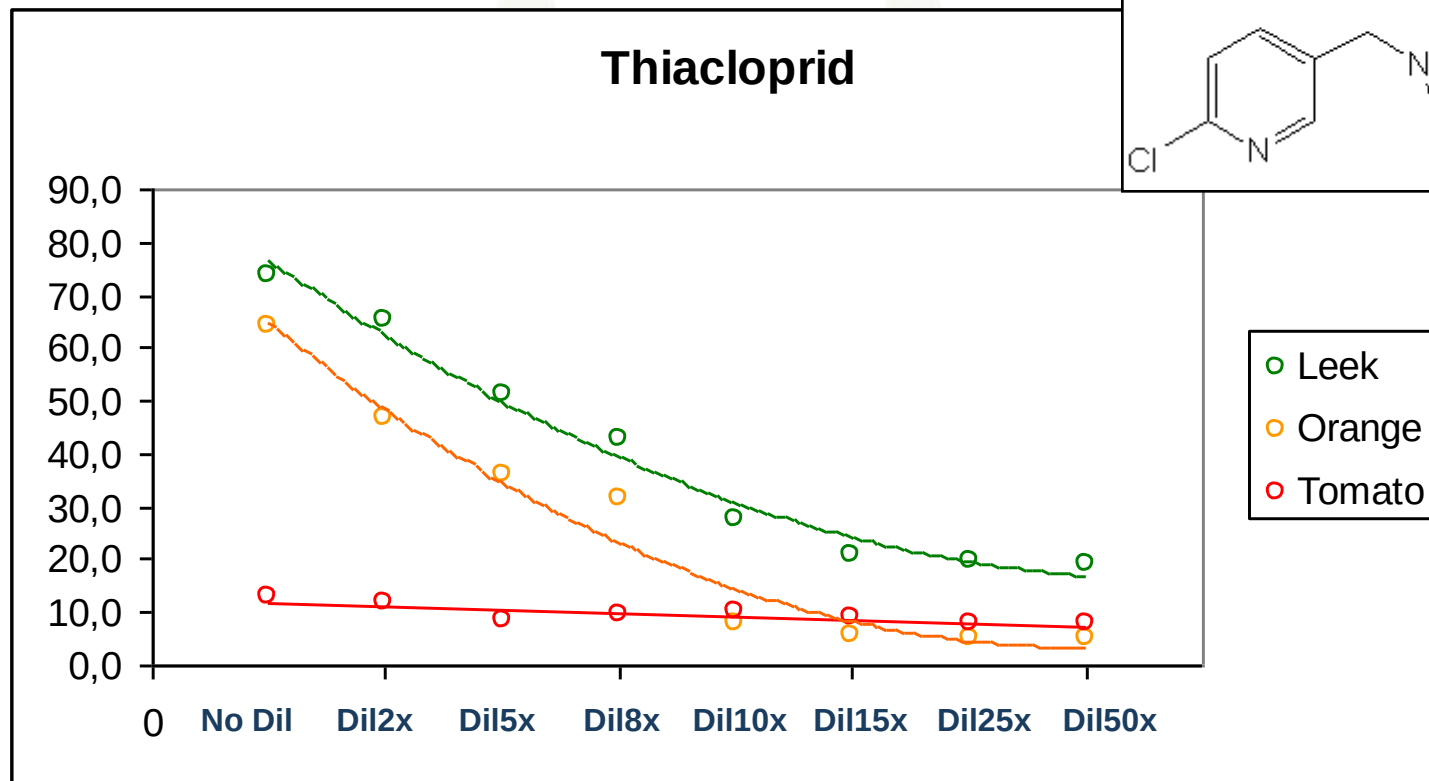


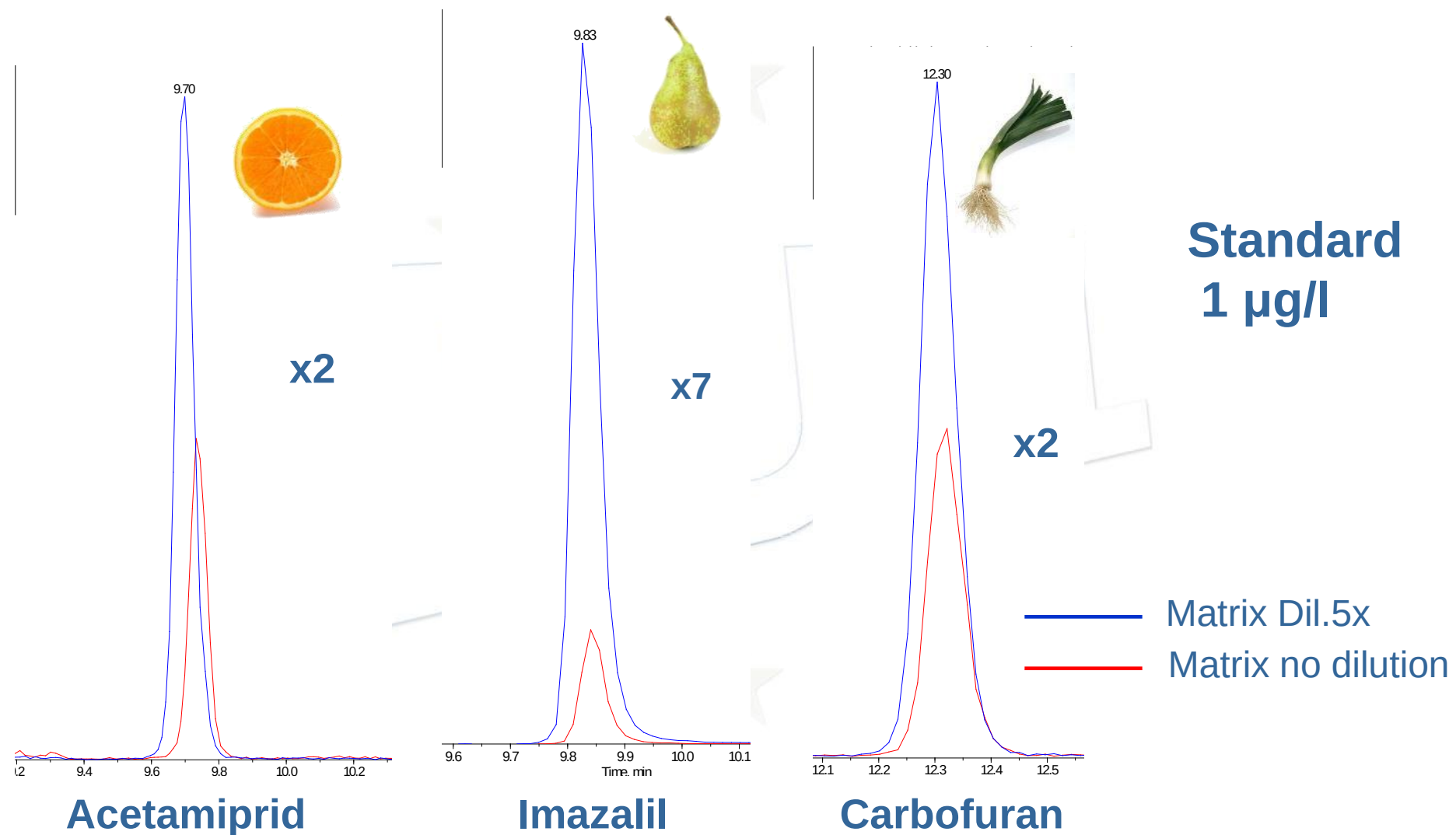
Matrix effect

Thiacloprid



% Signal Supression





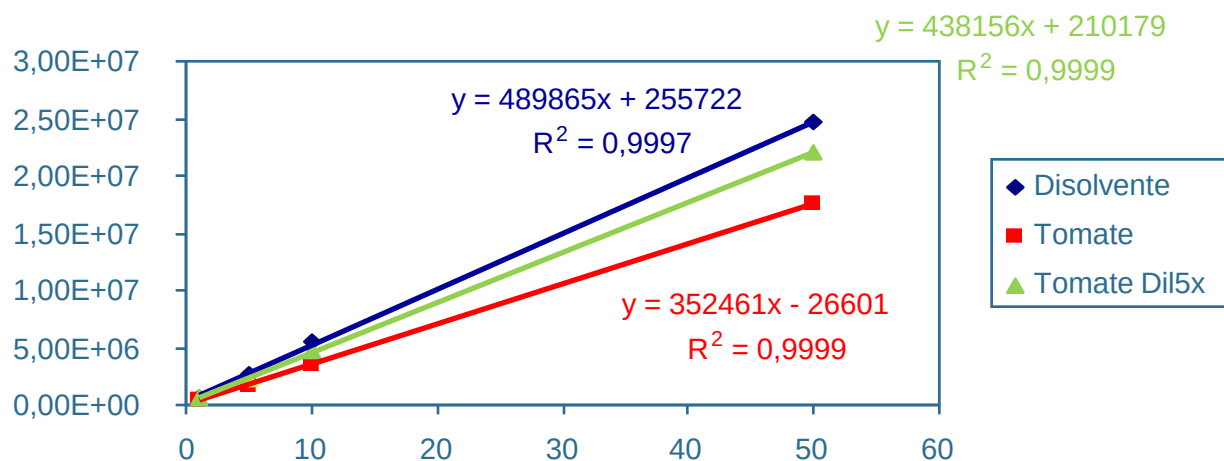
Signal Supression or Enhancement

Ratio Slope matrix / Slope solvent

	Orange	Orange Dil5x	Orange Dil10x
Chloroxuron	0,56	0,90	0,98
Diuron	0,15	0,30	0,55
Ethiofencarb	0,18	0,36	0,70
Fluometuron	0,32	0,49	0,86
Flazasulfuron	0,20	0,55	1,16
Isoproc carb	0,57	0,77	1,06



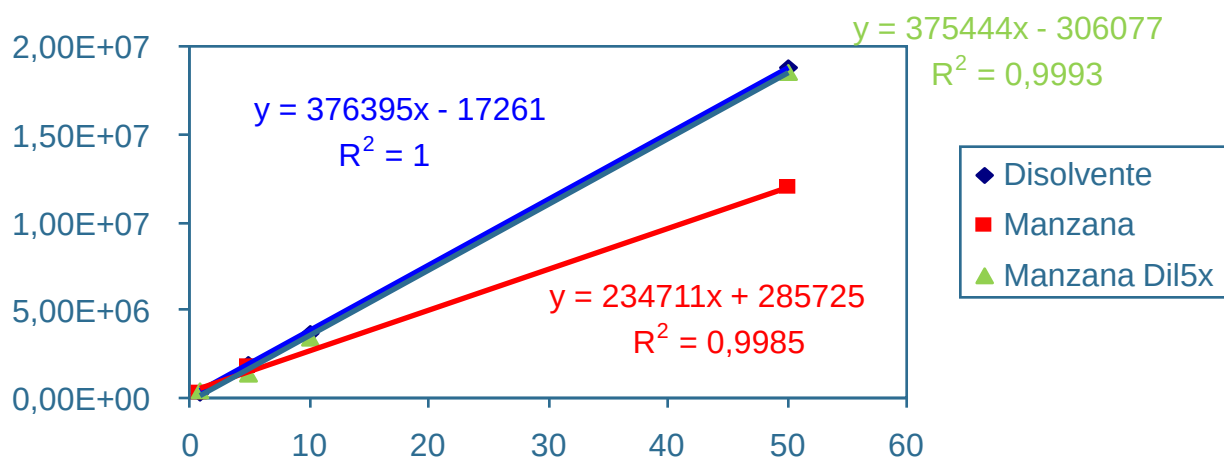
Monurón



Tomato



Flazasulfurón

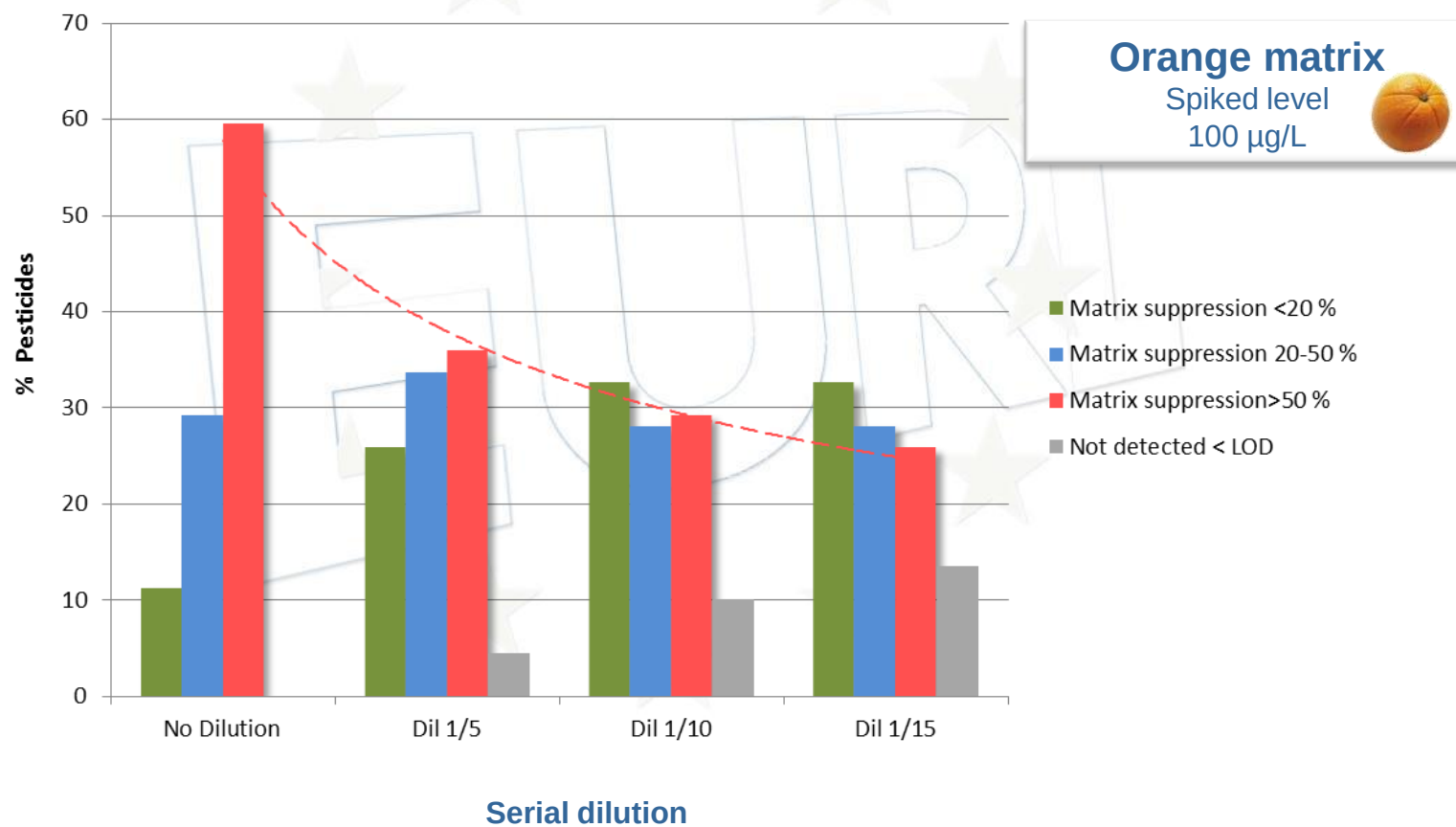


Apple

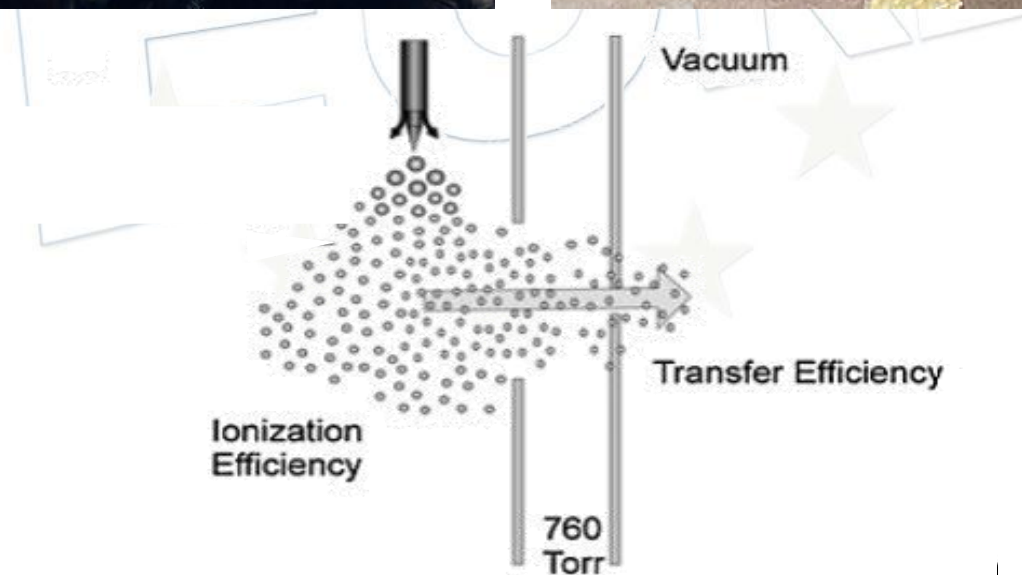


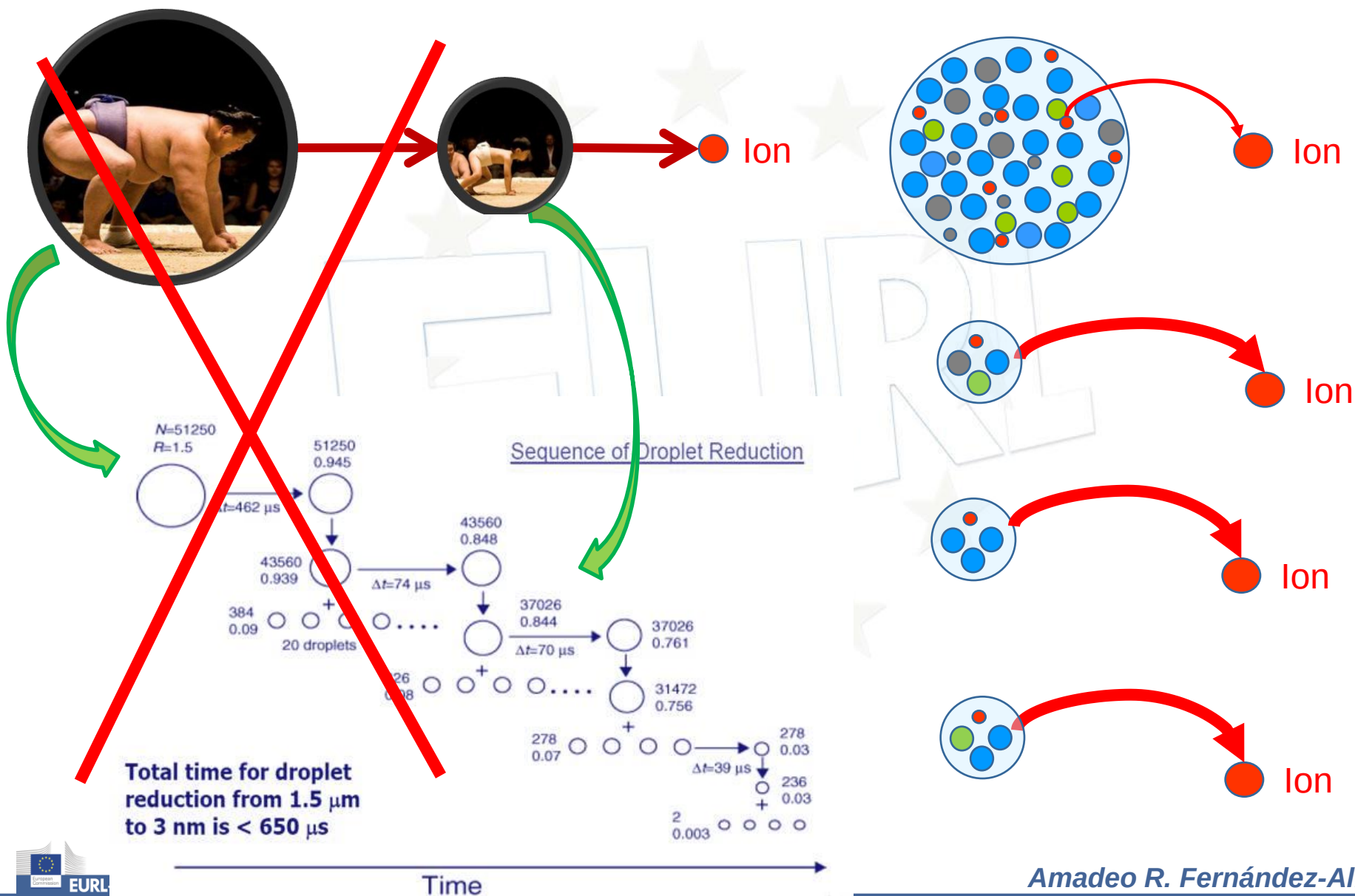
Dilution effect on matrix suppression Agilent LC-TOF-MS

100 pesticides

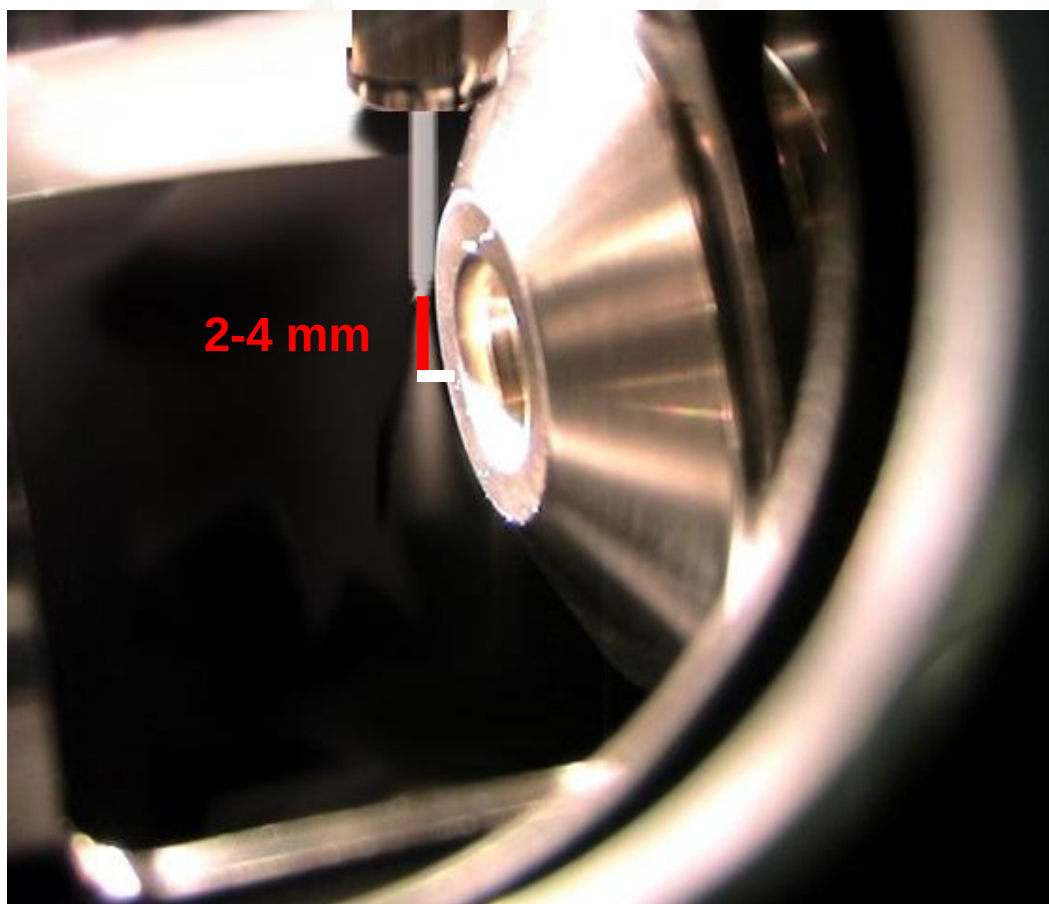


Sampling efficiency





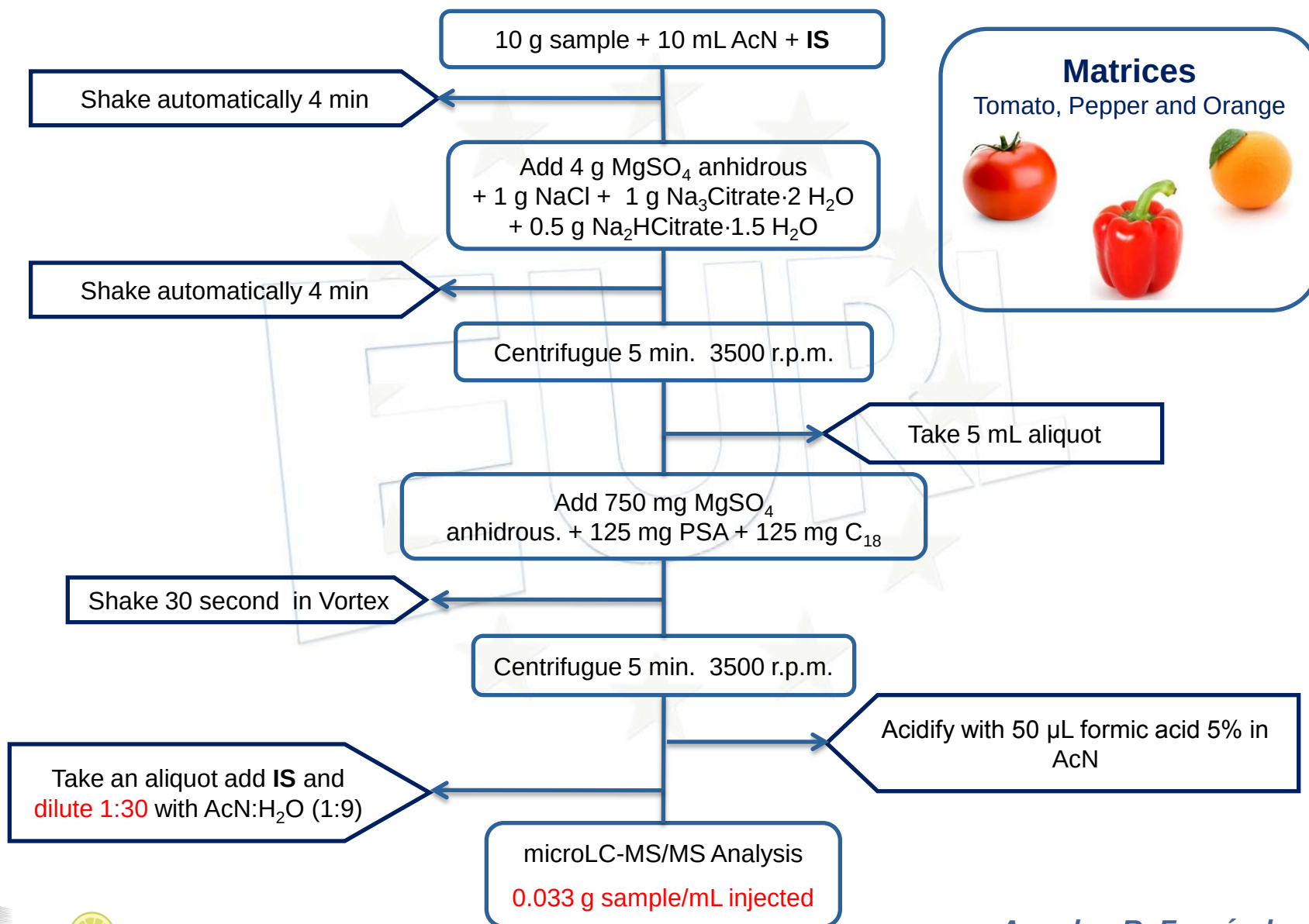
Observing Thermal Focusing



Temperature = 550 °C

Amadeo R. Fernández-Alba

QuEChERS Extraction Method



AB SCIEX microLC-QqQ-MS

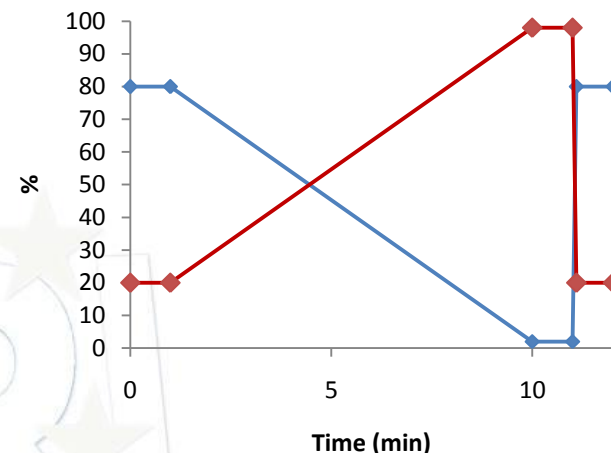
Liquid chromatography

- Column: HALO C18 2.7 μm 90 Å 0.5x50mm
- Mobile phases: Acetonitrile and water (0.1% formic acid)
- Injection volume: 5 μl
- Flow: 30 $\mu\text{l}/\text{min}$
- Total run time: 12 min

QqQ-MS

- Source: ESI (+) with microFlow electrode
- Pesticide Identification: 2 transitions
- Source Parameters:
 - ✓ Nebulizer and collision gas: nitrogen
 - ✓ Collision gas (CAD): Medium
 - ✓ Gas temperature: 400°C (+)
 - ✓ Curtain gas (CUR): 20
 - ✓ IonSpray Voltaje (IS): 5000V (+)
 - ✓ Schedule MRM software features

Gradient

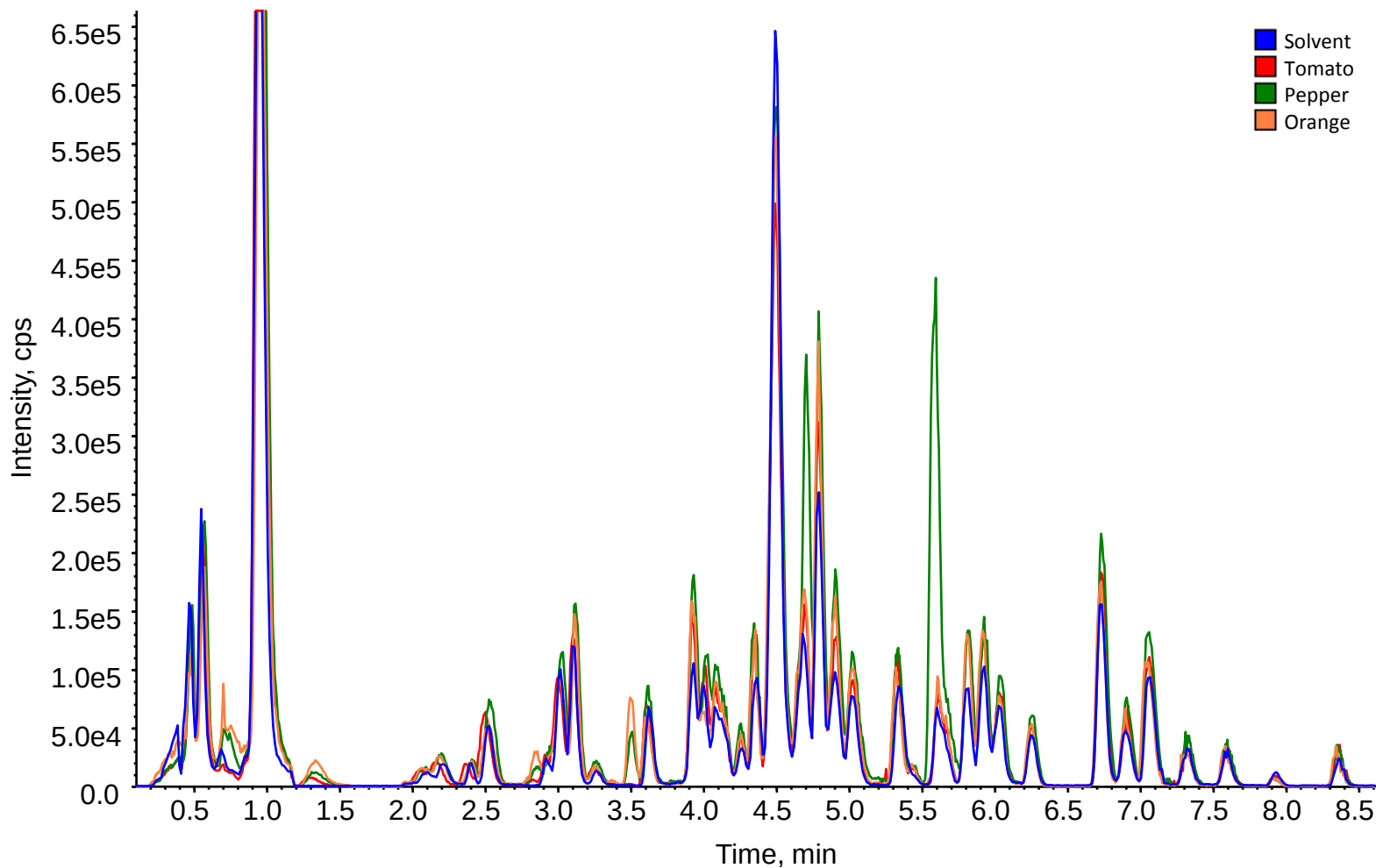


Compounds in the method

1 Azinphos-methyl	23 Fenitrothion	45 Methiocarb	67 Prothiofos
2 Azoxystrobin	24 Fenpropathrin	46 Methiocarb Sulfone	68 Pyraclostrobin
3 Bitertanol	25 Fenpropimorph	47 Methiocarb Sulfoxide	69 Pyrethrins
4 Boscalid	26 Fenpyroximate	48 Methomyl	70 Pyridaben
5 Bromuconazole	27 Fenthion	49 Omethoate	71 Pyrimethanil
6 Bupirimate	28 Fenthion Oxon	50 Oxadixyl	72 Pyriproxyfen
7 Carbaryl	29 Fenthion Oxonsulfone	51 Oxydemeton-methyl	73 Quinoxifen
8 Cyprodinil	30 Fenthion Oxonsulfoxide	52 Paclobutrazole	74 Rotenone
9 Diclorvos-d6 (I.S)	31 Fenthion Sulfoxide	53 Parathion	75 Tebuconazole
10 Dicrotophos	32 Flusilazole	54 Parathion-Methyl	76 Tebufenpyrad
11 Diethofencarb	33 Flutriafol	55 Penconazole	77 Tetraconazole
12 Dimethoate	34 Fosthiazate	56 Pencycuron	78 Thiodicarb
13 Dimethoate-d6 (I.S)	35 Hexaconazole	57 Pendimethalin	79 Thiophanate-methyl
14 Diniconazole	36 Imazalil	58 Phenthoate	80 Tolclofos-methyl
15 Dodine	37 Imidacloprid	59 Phosalone	81 TPP (I.S)
16 Epoxiconazole	38 Iprodione	60 Phoxim	82 Triadimefon
17 Ethirimol	39 Iprovalicarb	61 Pirimiphos-methyl	83 Triadimenol
18 Ethofenprox	40 Kresoxim-methyl	62 Prochloraz	84 Triticonazole
19 Ethoprophos	41 Malathion-d10 (I.S)	63 Propargite	85 Zoxamide
20 Fenamidone	42 Mandipropamid	64 Propiconazole	
21 Fenarimol	43 Metconazole	65 Propoxur	
22 Fenbuconazole	44 Methidathion	66 Propyzamide	

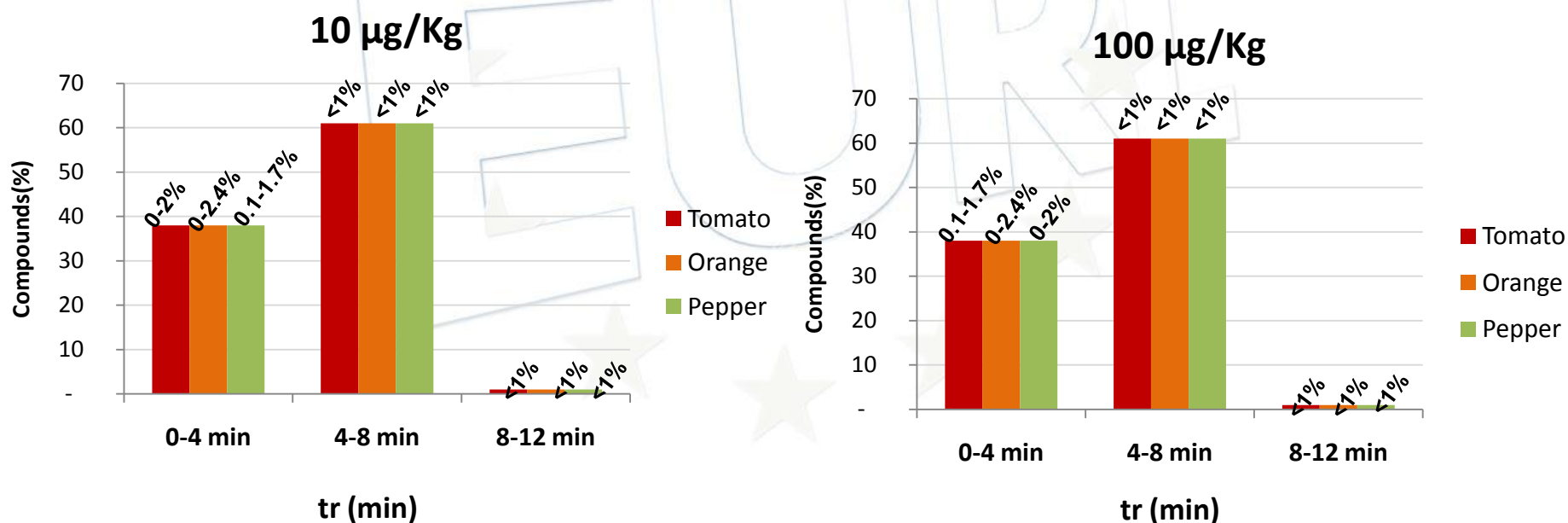
Total Ion Chromatogram

10 µg/Kg Tomato, Pepper and Orange diluted 30 times



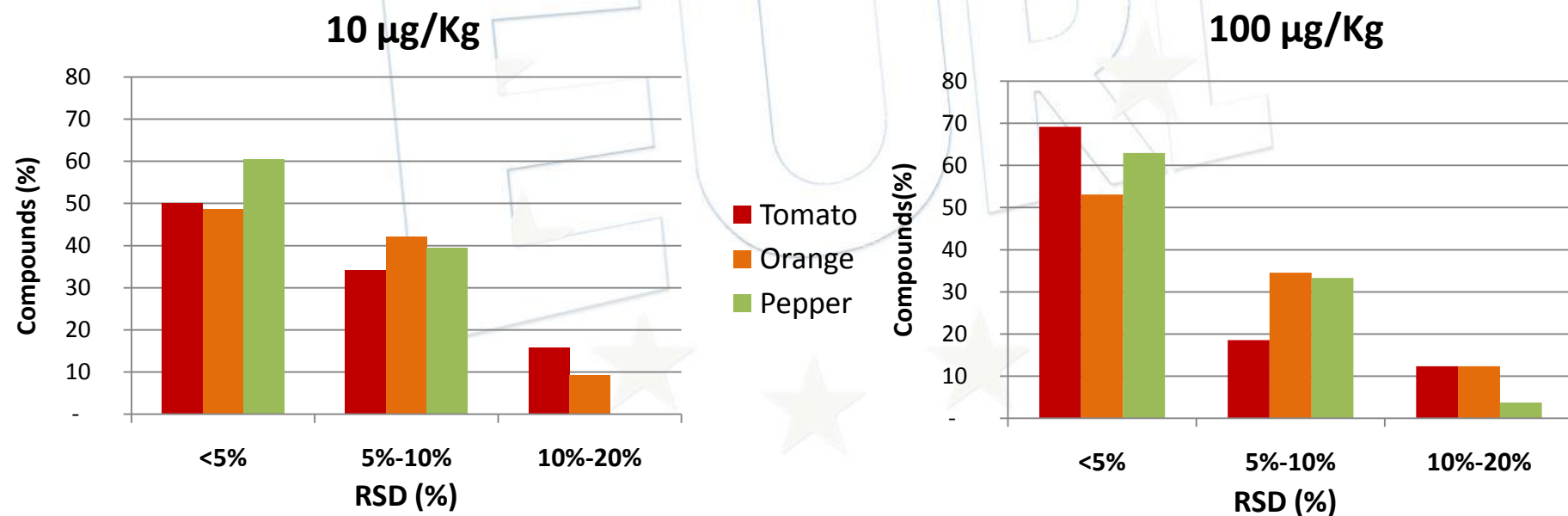
Retention time reproducibility

5 consecutive injection over 3 days of a tomato, orange and pepper spiked at 10 µg/Kg and 100 µg/Kg and diluted 30 times.

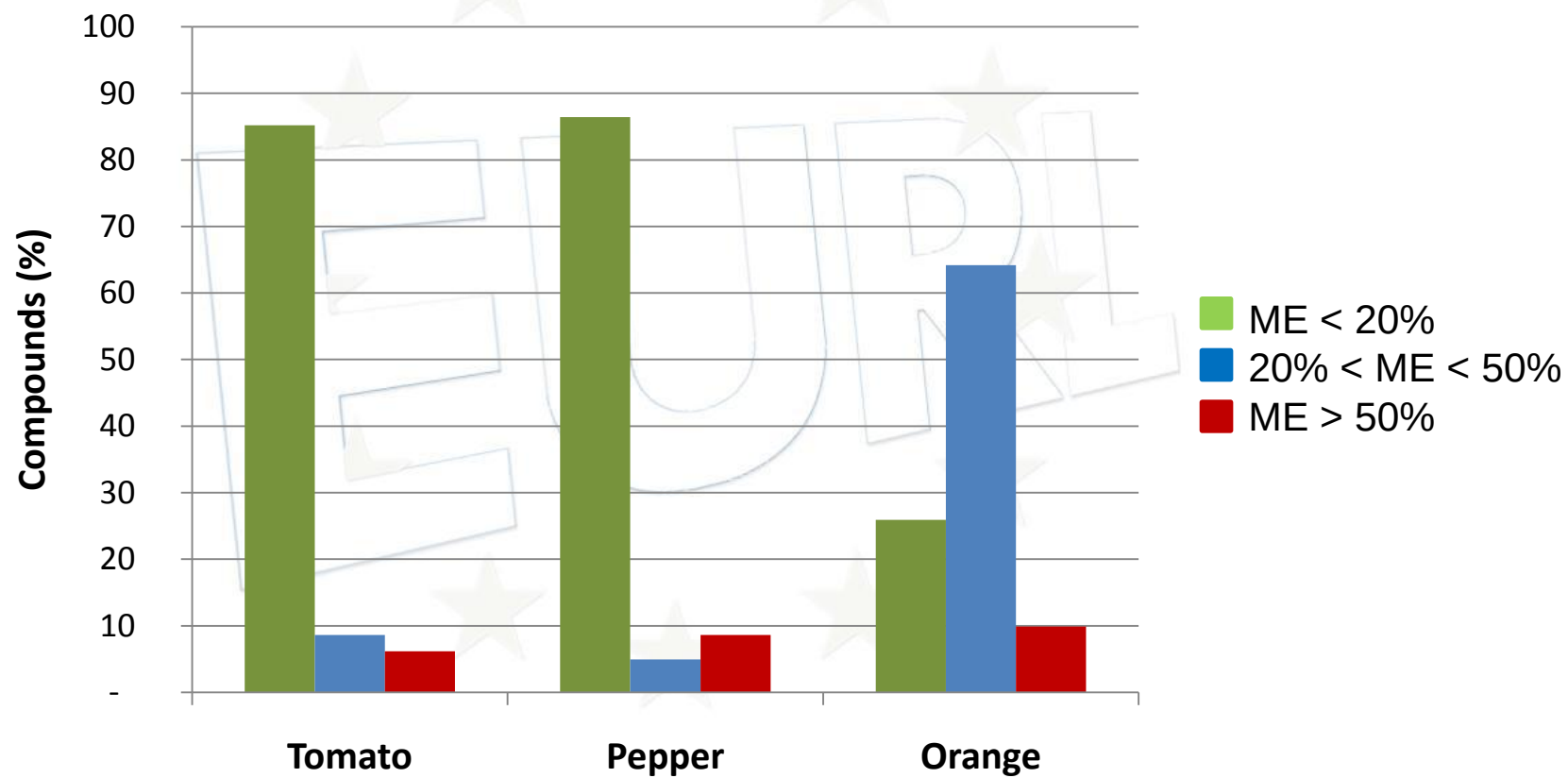


Peak area reproducibility

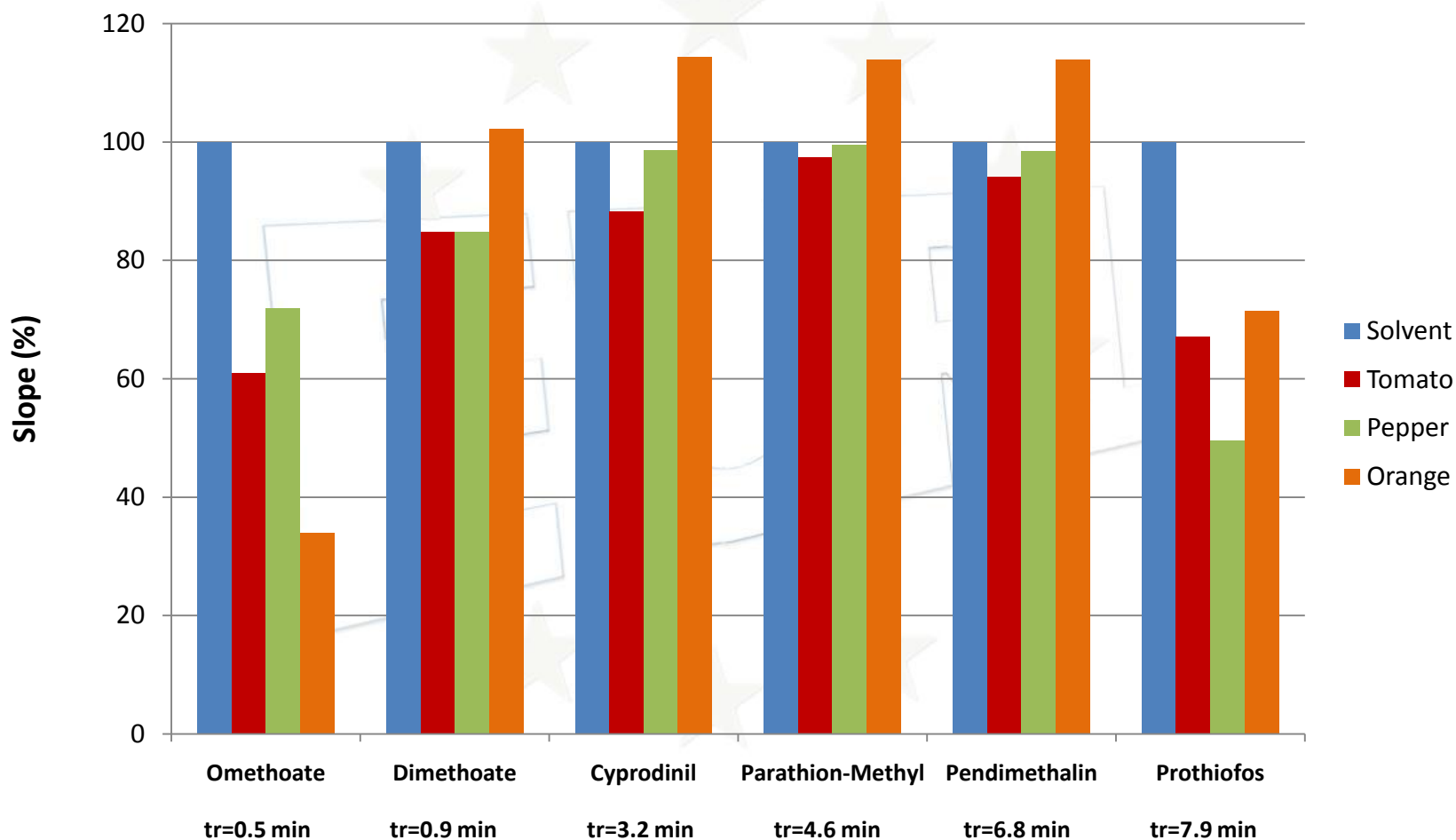
5 consecutive injection over 3 days of a tomato, orange and pepper spiked at 10 µg/Kg and 100 µg/Kg and diluted 30 times.



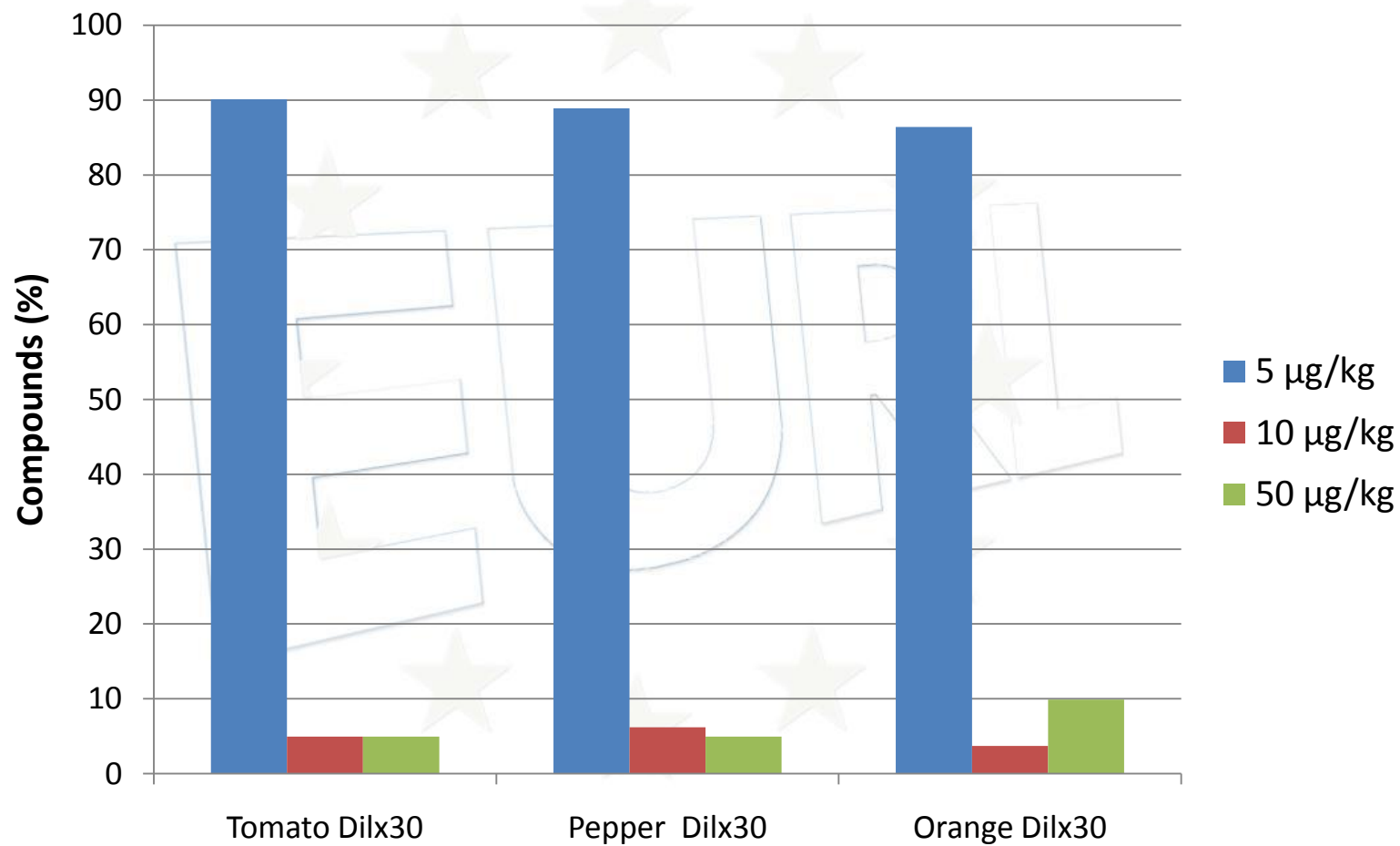
Matrix Effects



Slope variability of different compounds in solvent, tomato, pepper and orange matrices diluted 30 times



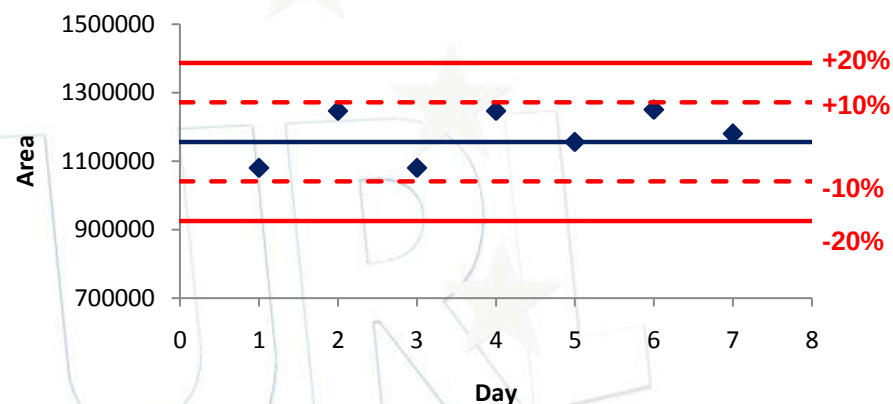
Reporting limits



Isotopically Labelled Internal Standard

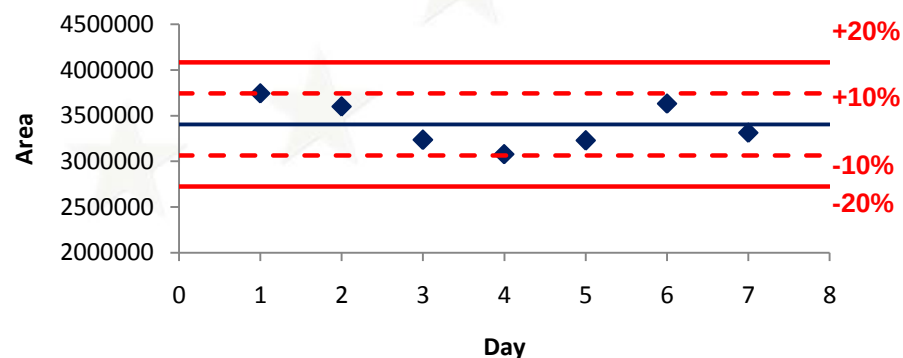
Malathion-d10

Added at the before the extraction procedure



Dimethoate-d6

Added before sample dilution



Samples from different european proficiency tests (EUPTs) diluted 30 times

Pesticides	Pear		Mandarin		Leek	
	Concentration (µg/Kg)	Assigned value in EUPT (µg/Kg)	Concentration (µg/Kg)	Assigned value in EUPT (µg/Kg)	Concentration (µg/Kg)	Assigned value in EUPT (µg/Kg)
Azinphos-methyl					n.a	48
Boscalid	213	177				
Cyprodinil	248	247				
Fenpropathrin					247	62
Imazalil			1556	1300		
Imidacloprid					262	249
Iprodione	385	334				
Kresoxim-methyl					384	316
Methidathion			792	730		
Methomyl			190	201		
Pendimethalin			605	583		
Phosalone			375	280		
Prochloraz			396	301		
Prothiofos					367	279
Pyraclostrobin	168	172				
Pyrimethanil	128	461				
Pyriproxyfen			392	443		

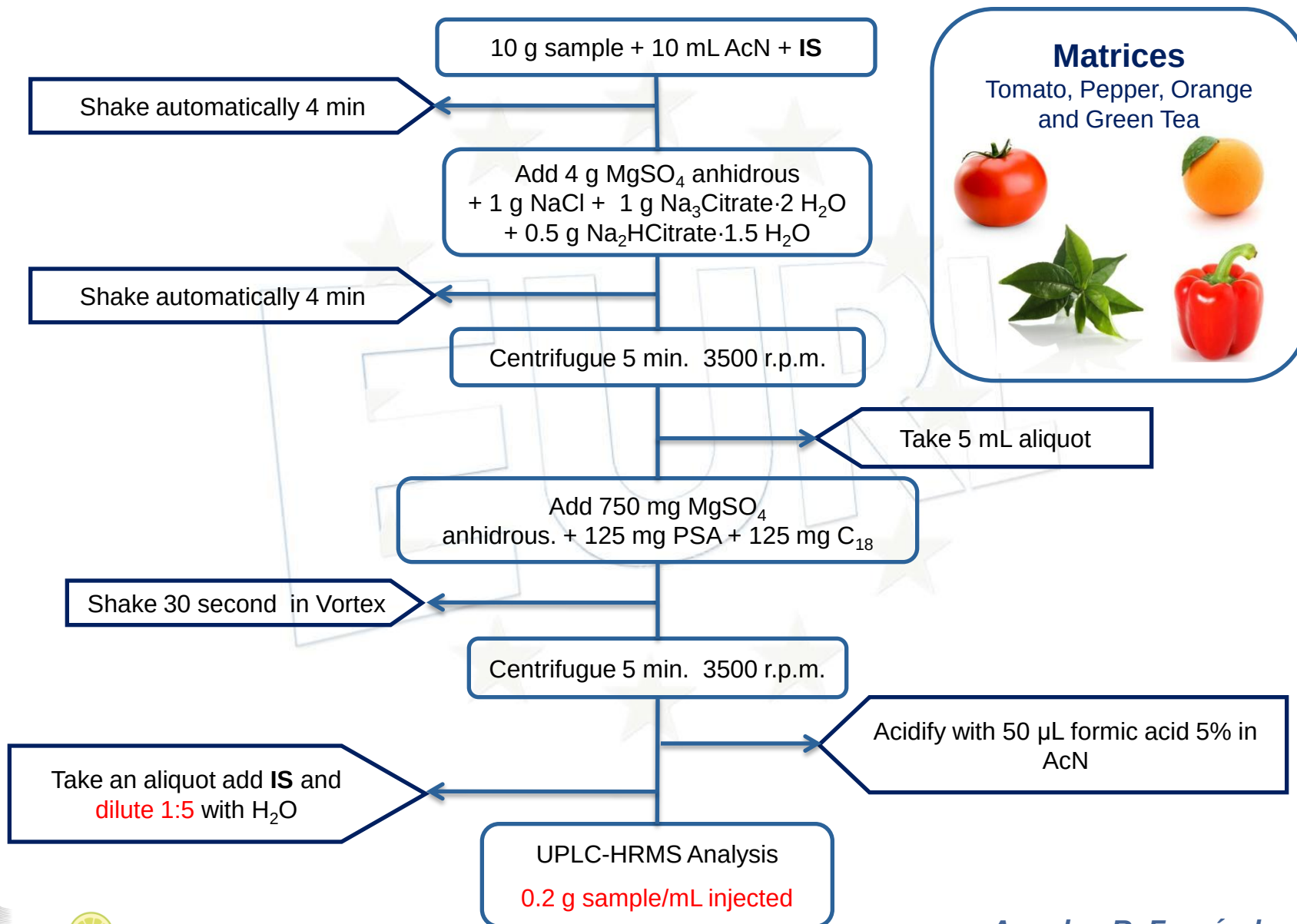
SENSITIVITY

MASS ACCURACY

RESOLUTION

SOFTWARE

QuEChERS Extraction Method



List of Pesticides

1	2,4-D
2	3-hidroxycarbofuran
3	Acephate
4	Acetamidrid
5	Aldicarb
6	Aldicarb Sulfone
7	Aldicarb Sulfoxide
8	Azinphos-methyl
9	Azoxystrobin
10	Benomyl
11	Bitertanol
12	Boscalid
13	Bromuconazole
14	Bupirimate
15	Buprofezin
16	Carbaryl
17	Carbendazim
18	Carbofuran
19	Chlorantraniliprole
20	Chlorfenvinphos
21	Chlorpyrifos
22	Chlorpyrifos-methyl
23	Clofentezine
24	Clothianidin
25	Cymoxanil
26	Cyproconazole
27	Cyprodinil
28	Cyromazine
29	Demeton-S-methylsulfone
30	Desmethyl Pirimicarb
31	Diazinon
32	Dichlorvos-d6 (I.S.)
33	Diclotophos

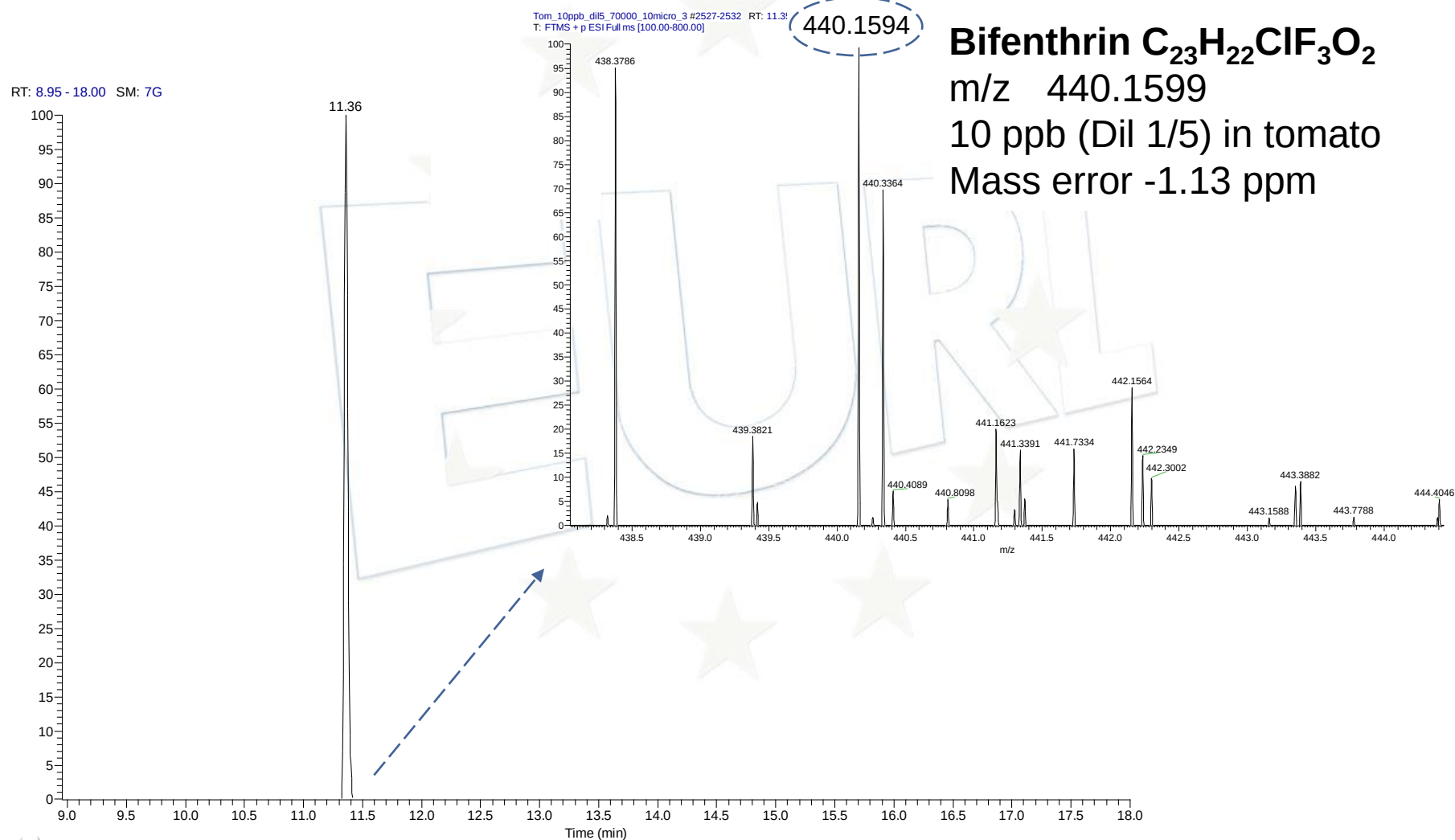
34	Diethofencarb
35	Difenoconazole
36	Diffubenzuron
37	Dimethoate
38	Dimethomorph
39	Diniconazole
40	Dithianon
41	Dodine
42	Epoxiconazole
43	Ethion
44	Ethirimol
45	Ethoprophos
46	Fenamidone
47	Fenamiphos
48	Fenamiphos Sulfone
49	Fenamiphos Sulfoxide
50	Fenarimol
51	Fenazaquin
52	Fenbuconazole
53	Fenhexamid
54	Fenitrothion
55	Fenoxycarb
56	Fenpropathrin
57	Fenpropimorph
58	Fenpyroximate
59	Fenthion
60	Fenthion Oxon
61	Fenthion Oxon Sulfone
62	Fenthion Oxon Sulfoxide
63	Fenthion Sulfone
64	Fenthion Sulfoxide
65	Fipronil
66	Flonicamid

67	Fluazifop
68	Flubendiamide
69	Fludioxonil
70	Flufenoxuron
71	Fluopyram
72	Fluquinconazole
73	Flusilazole
74	Flutriafol
75	Formetanate
76	Fosthiazate
77	Haloxypop
78	Hexaconazole
79	Hexythiazox
80	Imazalil
81	Imidacloprid
82	Indoxacarb
83	Iprovalicarb
84	Isofenphos-methyl
85	Isoprocab
86	Kresoxim-methyl
87	Linuron
88	Lufenuron
89	Malaoxon
90	Malathion
91	Malathion-d10 (I.S.)
92	Mandipropamid
93	Metalaxyl-M
94	Metconazole
95	Methamidophos
96	Methidathion
97	Methiocarb
98	Methiocarb Sulfone
99	Methiocarb Sulfoxide

100	Methomyl
101	Methomyl
102	Methoxyfenozide
103	Metobromuron
104	Monocrotophos
105	Myclobutanil
106	Nitenpyram
107	Omethoate
108	Oxadixyl
109	Oxydemeton-methyl
110	Paclobutrazol
111	Paraaxon Methyl
112	Penconazole
113	Pencycuron
114	Pendimethalin
115	Phenthoate
116	Phosalone
117	Phosmet
118	Phosmet Oxon
119	Phoxim
120	Pirimicarb
121	Pirimiphos-methyl
122	Prochloraz
123	Profenofos
124	Propamocarb
125	Propiconazole
126	Propoxur
127	Propyzamide
128	Prothioconazole
129	Prothioconazole-desthio
130	Pymetrozine
131	Pyraclostrobin
132	Pyrethrin

133	Pyridaben
134	Pyrimethanil
135	Pyriproxyfen
136	Quinoxifen
137	Rotenone
138	Spinosad
139	Spirodiclofen
140	Spiromesifen
141	Spiroxamine
142	Tebuconazole
143	Tebufenozide
144	Tebufenpyrad
145	Teflubenzuron
146	Terbutylazine
147	Tetraconazole
148	Thiabendazole
149	Thiacloprid
150	Thiamethoxam
151	Thiodicarb
152	Thiophanate-methyl
153	Tolclofos-methyl
154	TPP (I.S.)
155	Triadimefon
156	Triadimenol
157	Triazophos
158	Trichlorfon
159	Trifloxystrobin
160	Triflumuron
161	Triticonazole
162	Zoxamide

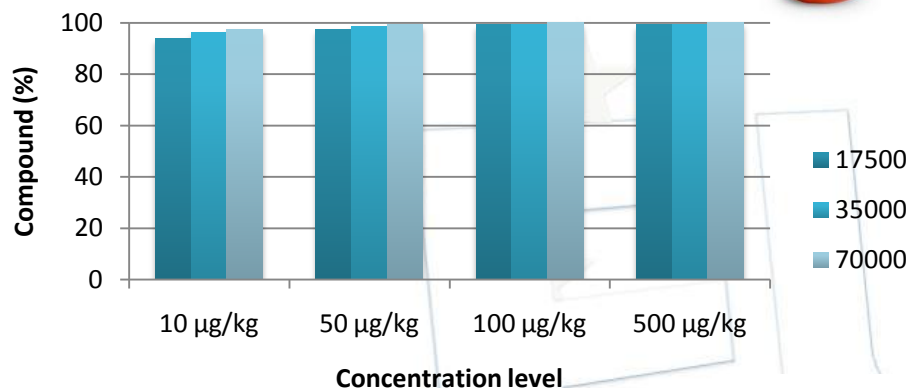
LC-Orbitrap Full Scan mode R 35000 (m/z 200)



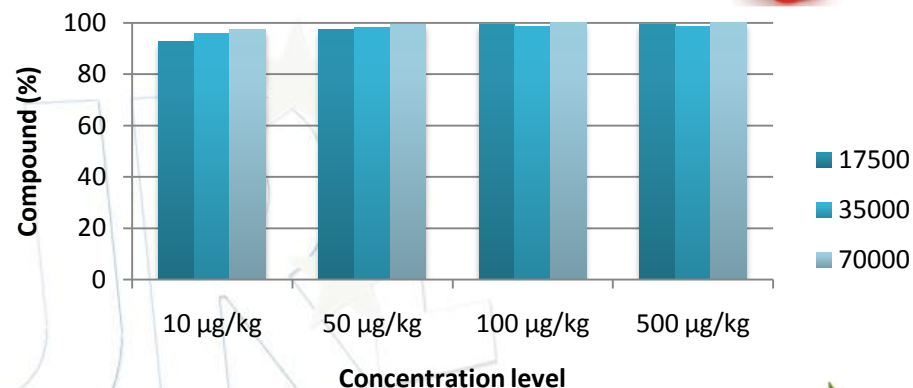
Detected pesticides vs resolution

Orbitrap Full Scan

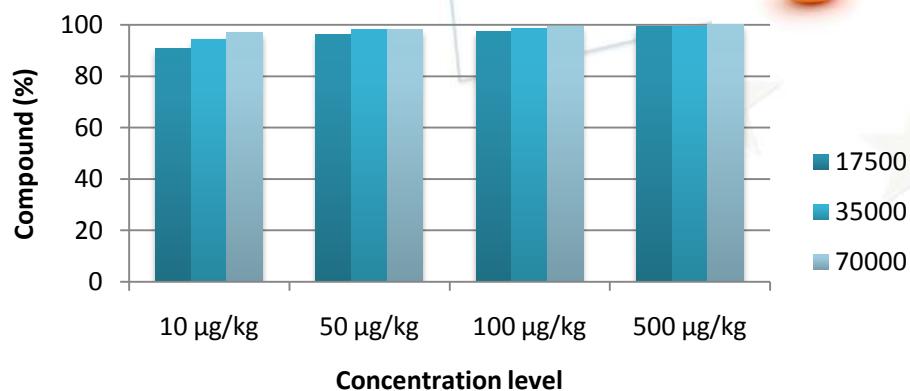
Tomato



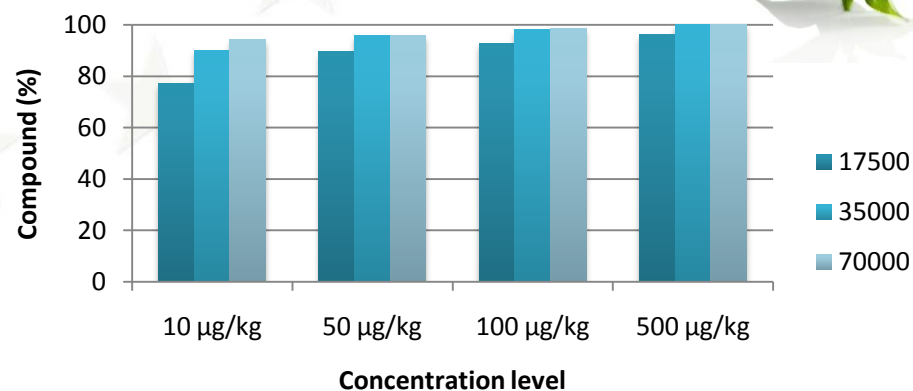
Pepper



Orange

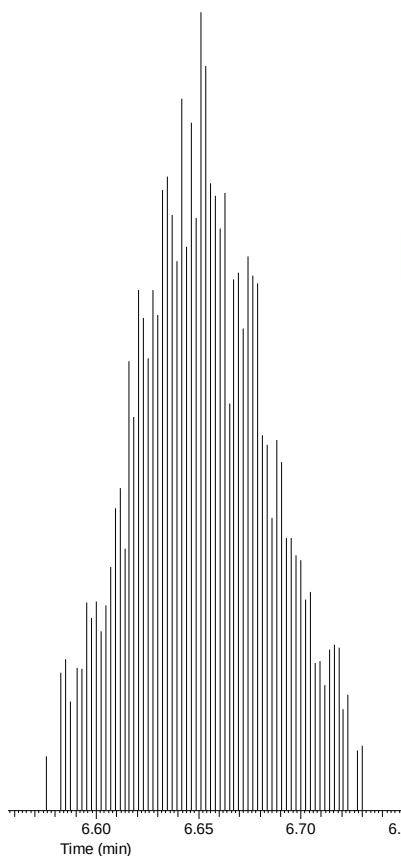


Green Tea

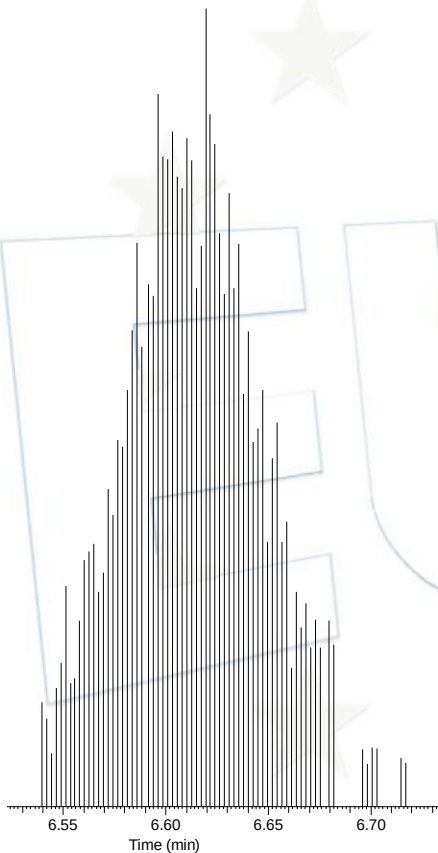


Numbers of points per peak in different matrices

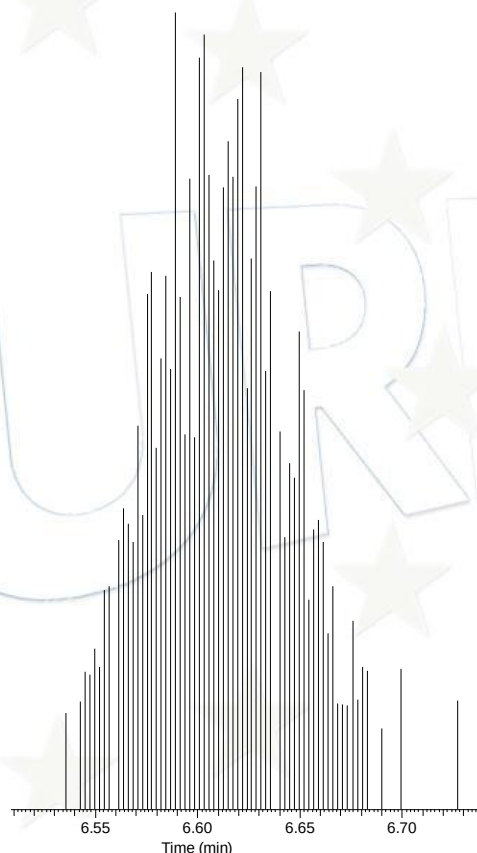
Acetamiprid 10 ppb, Resolution 35000



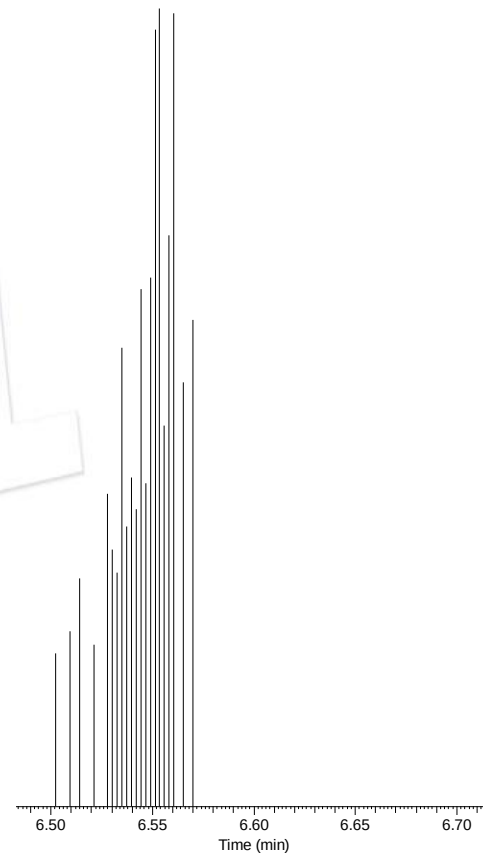
Tomato



Pepper



Orange

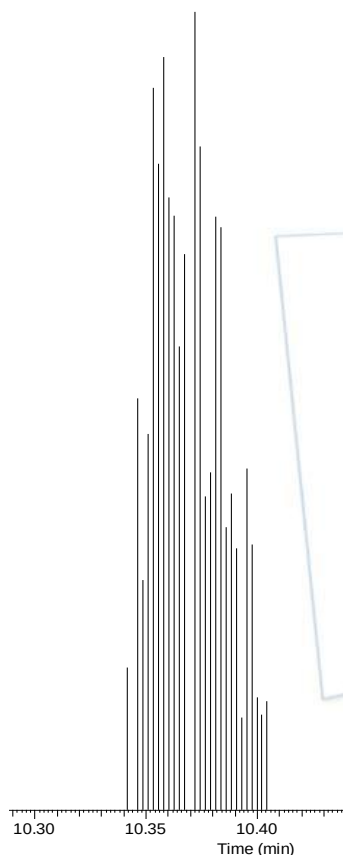


Green tea

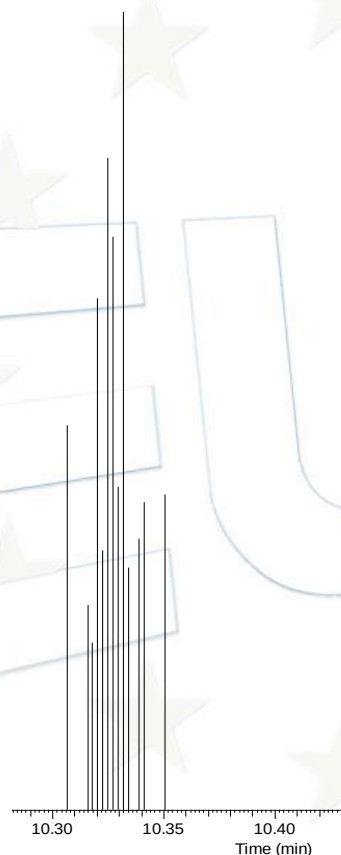


Numbers of points per peak in different matrices

Clofentazine 10 ppb, Resolution 35000



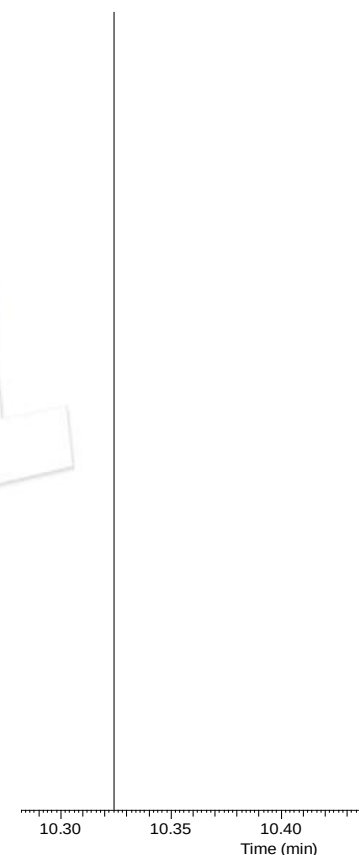
Tomato



Pepper



Orange



Green tea

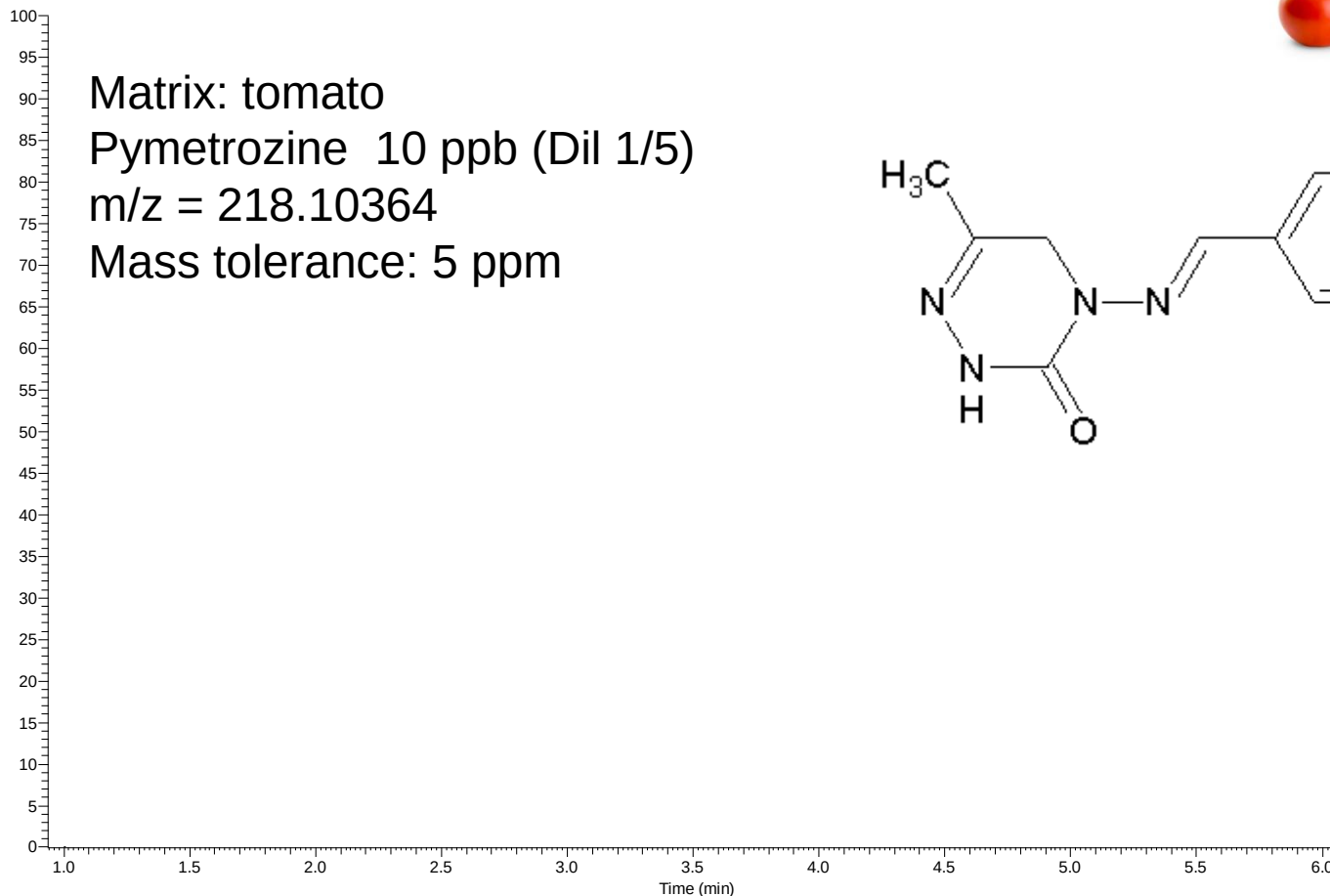


Influence of resolution on peak shape

Resolution 17500



RT: 0.94 - 6.10 SM: 15G

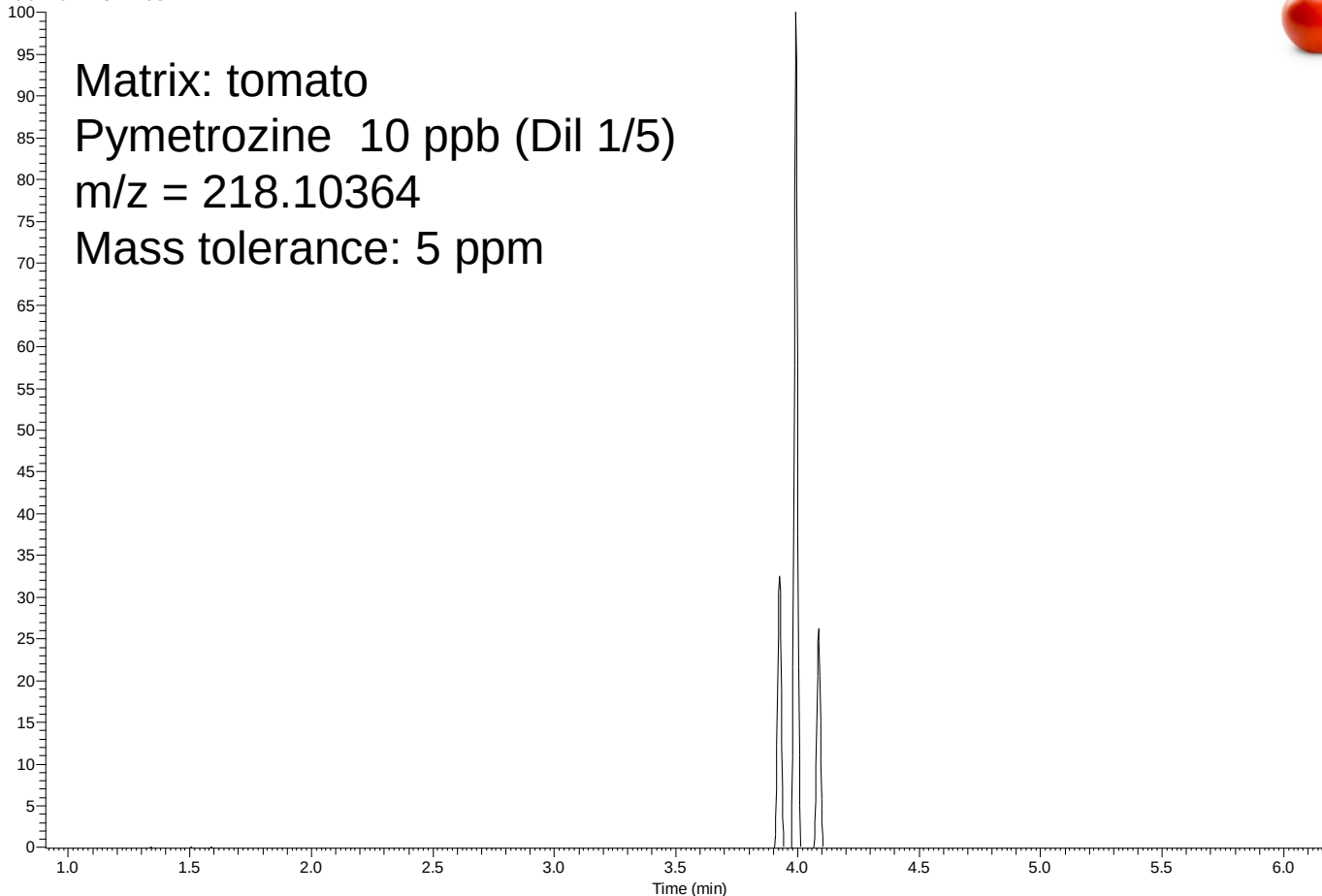


Influence of resolution on peak shape

Resolution 35000



RT: 0.91 - 6.21 SM: 15G

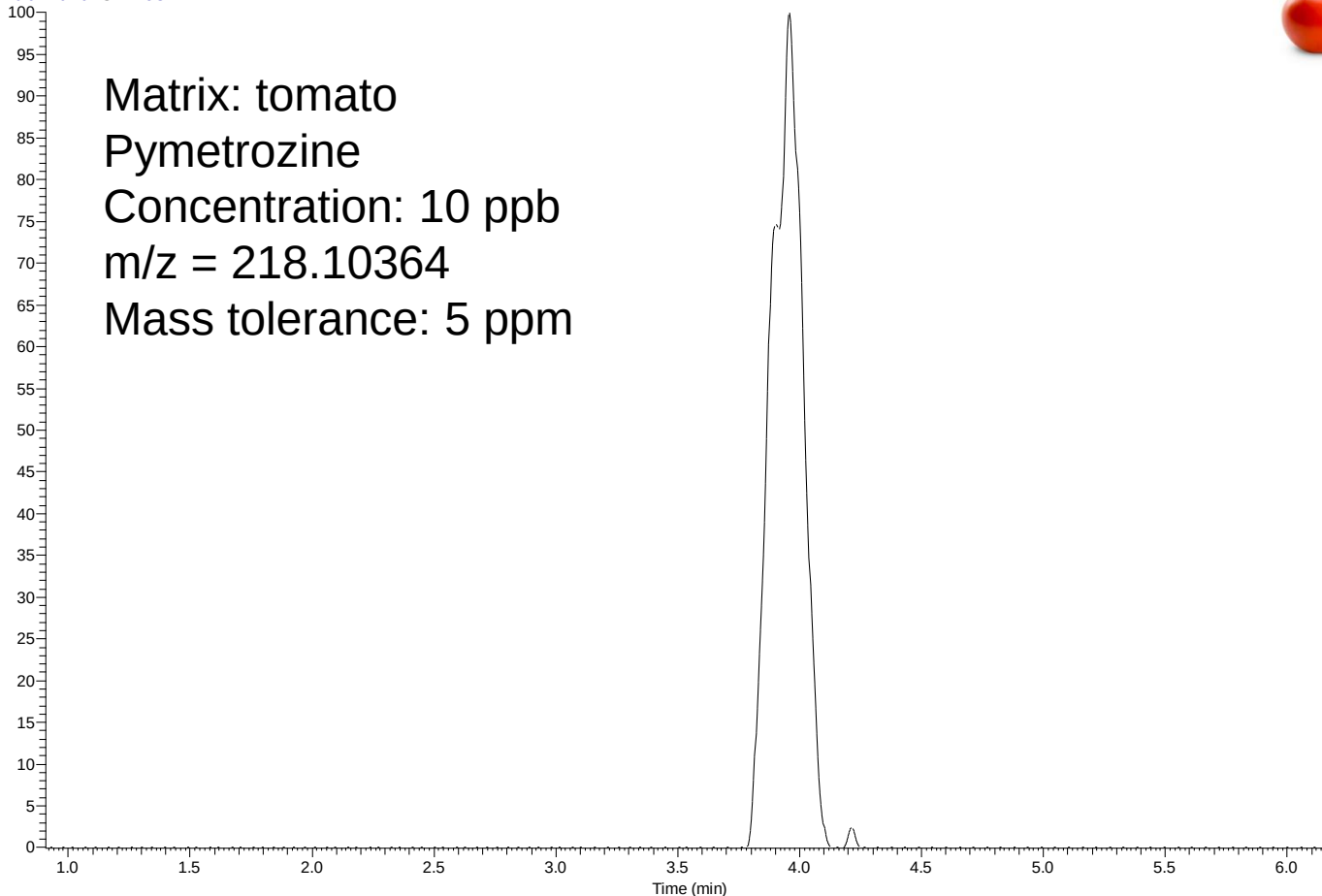


Influence of resolution on peak shape

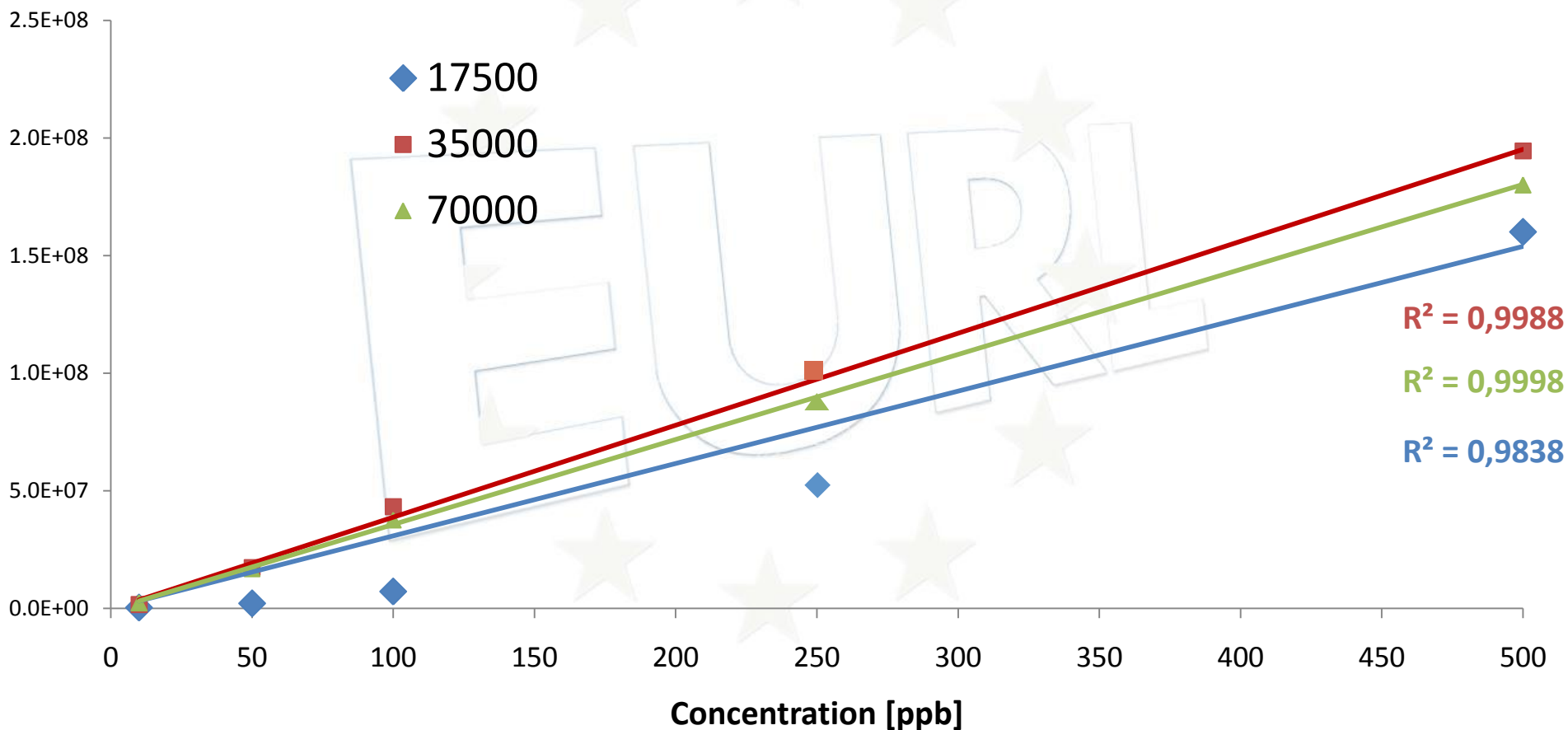
Resolution 70000



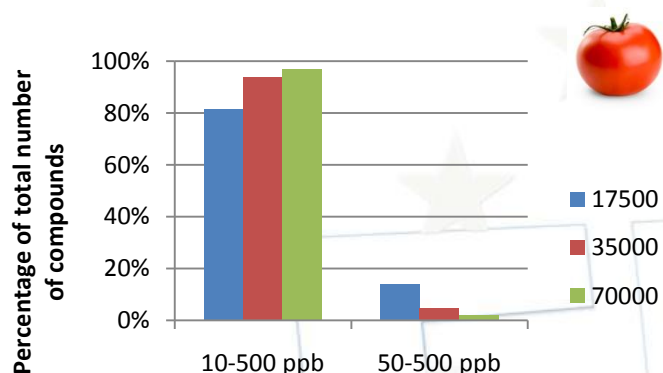
RT: 0.91 - 6.19 SM: 15G



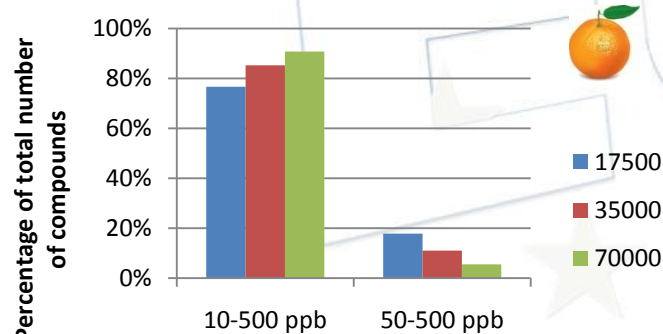
Calibration lines of fenbuconazol in different resolution



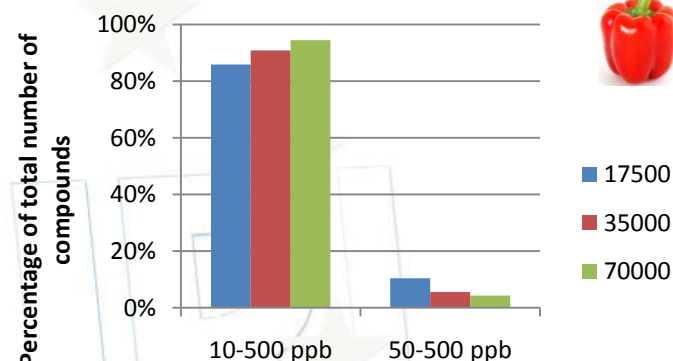
Linearity range vs resolution



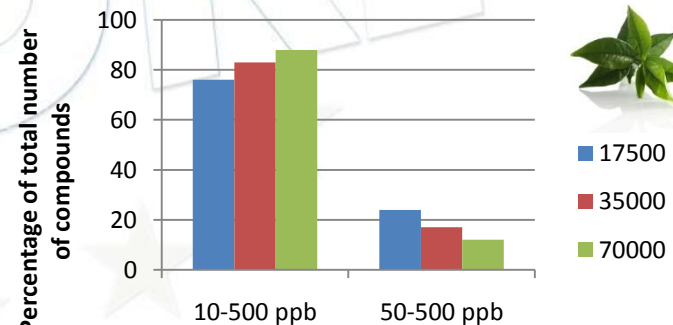
17500
35000
70000



17500
35000
70000



17500
35000
70000



17500
35000
70000

In all cases calibration curves were linear up to 500 ppb
Saturation effect was not observed.

Application of Orbitrap Proficiency Test samples

FV-13 Mandarin			
Pesticide	Measured concentration [mg/kg]	Median from PT [mg/kg]	
Carbendazim	1,24	1,25	0%
Chlorpyrifos	0,92	0,79	17%
Diazinon	0,18	0,19	-5%
EPN	0,57	0,42	30%
Imazalil	1,52	1,30	17%
Indoxacarb	0,88	0,79	11%
Malathion	0,45	0,38	18%
Methidathion	0,86	0,73	18%
Methomyl	0,21	0,20	3%
Oxamyl	0,16	0,13	22%
Pendimethalin	0,72	0,58	23%
Phosalone	0,31	0,28	11%
Prochloraz	0,35	0,30	16%
Pyriproxyfen	0,52	0,44	17%
Spinosad	0,70	0,61	15%
Thiabendazole	0,96	0,81	19%

Full scan mode. Resolution 35000
1 extraction x 3 injections



Analytical + ges



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**Thank You
for Your Attention**

