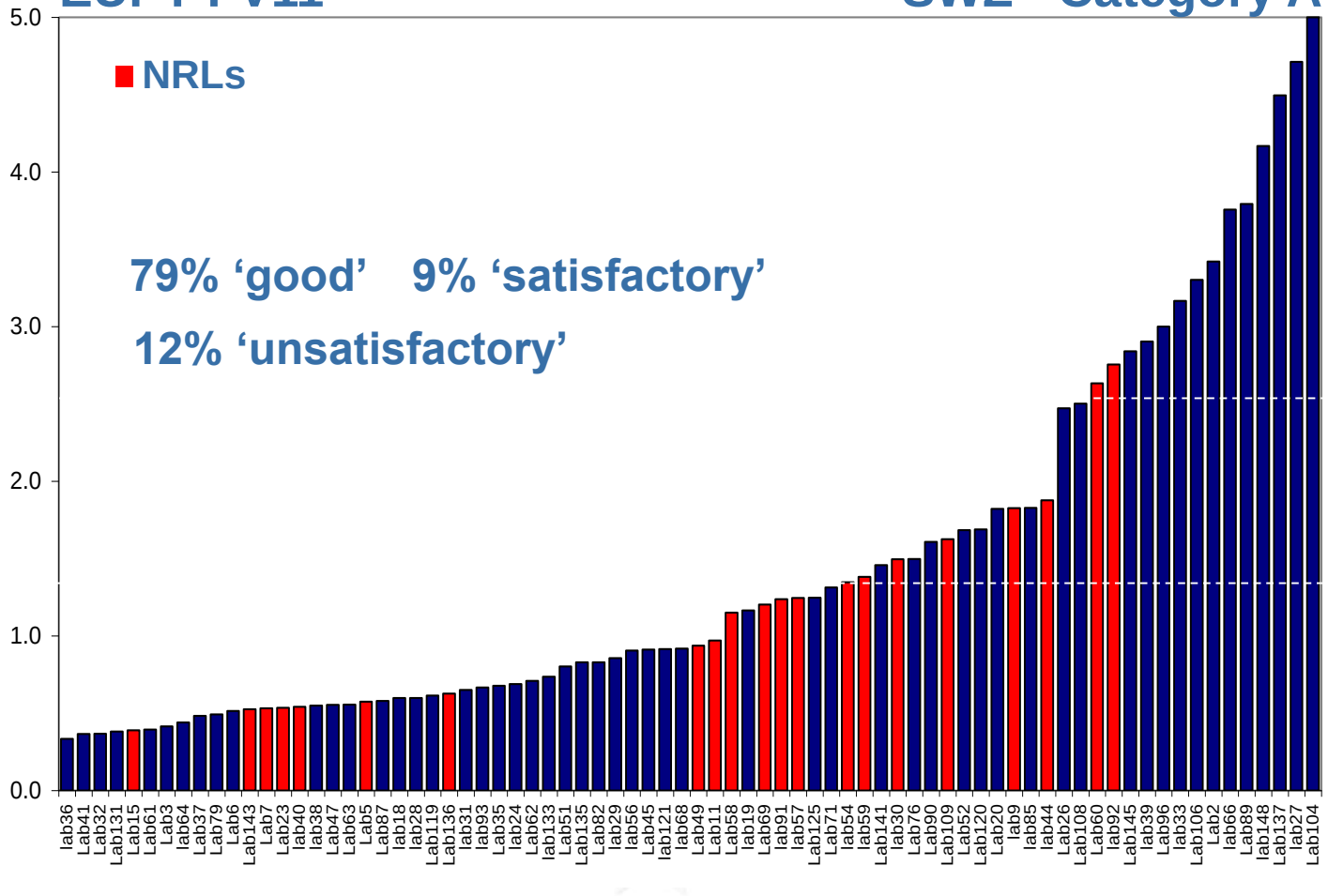
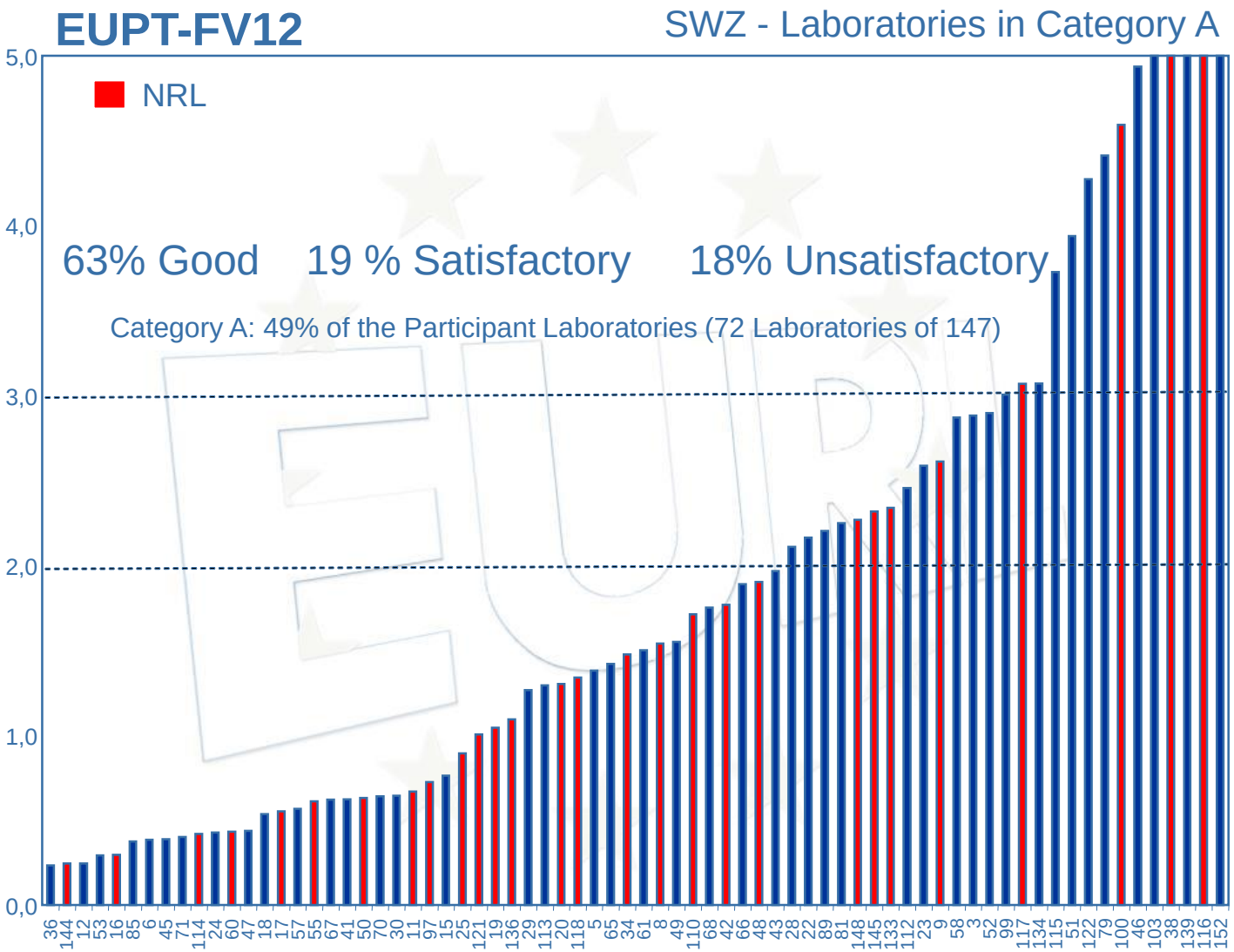
How to deal with difficult matrices? Current strategies and results from EUPTs

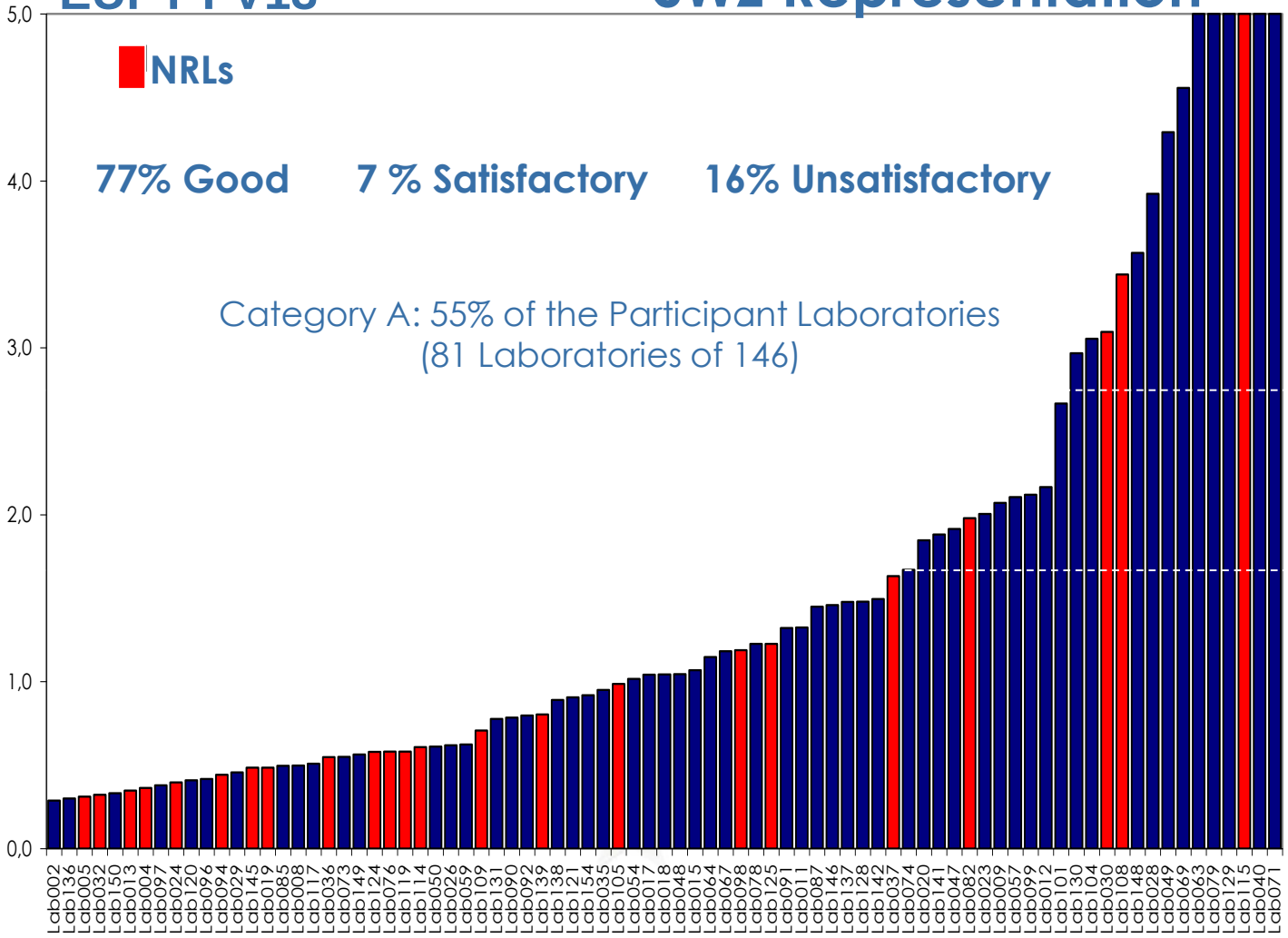
AMADEO R. FERNÁNDEZ-ALBA

EUPT-FV11 SWZ - Category A



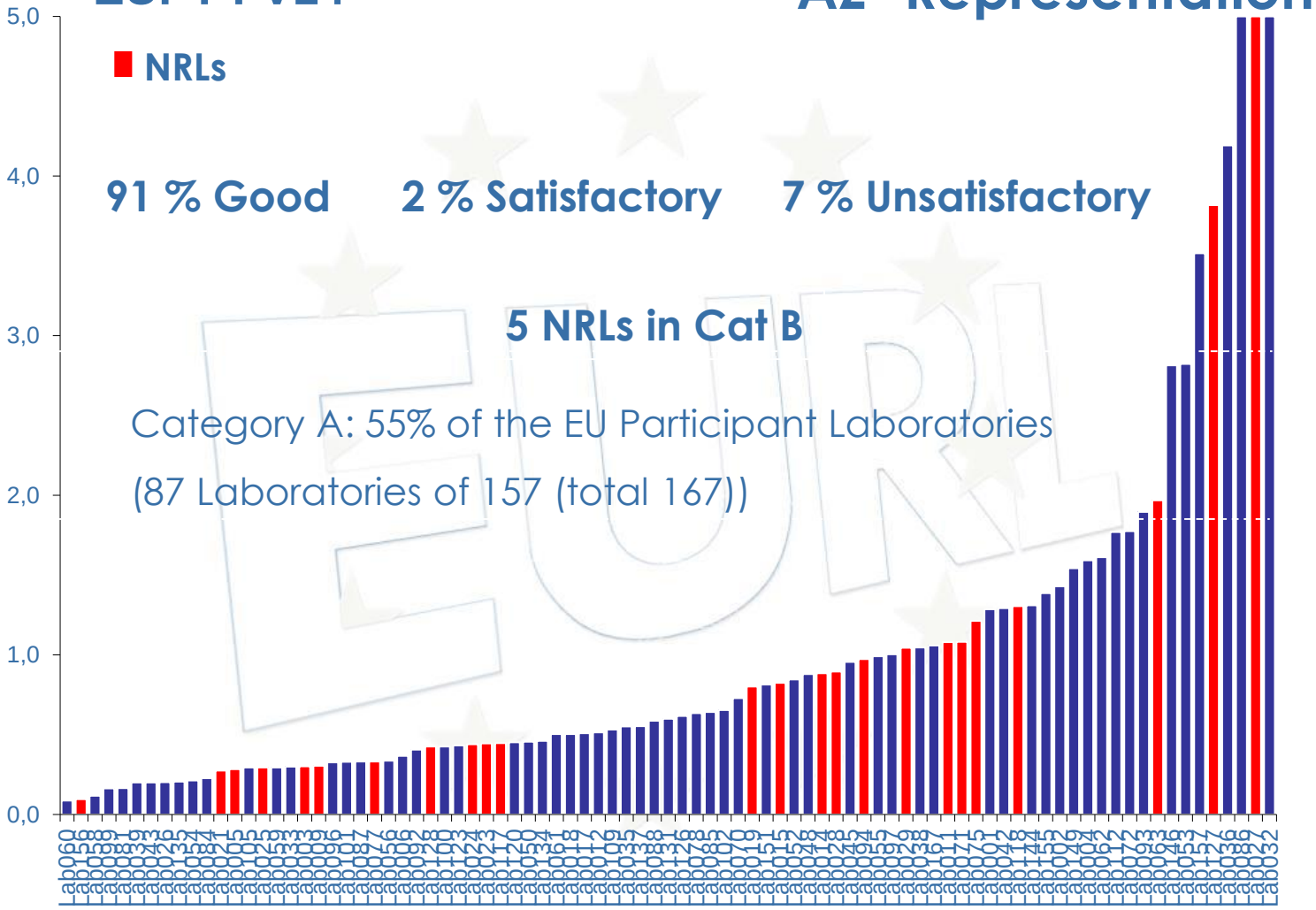

EUPT-FV12
SWZ - Laboratories in Category A
NRL
63% Good
19 % Satisfactory
18% Unsatisfactory
Category A: 49% of the Participant Laboratories (72 Laboratories of 147)
5,0
4,0
3,0
2,0
1,0
0,0
36 144 12 53 16 85 6 45 71 114 24 60 47 18 17 57 55 67 41 50 70 30 11 97 15 25 121 19 136 29 113 20 118 5 65 34 61 8 49 110 68 42 66 48 43 28 22 89 81 148 145 133 112 23 9 58 3 52 99 117 134 115 51 122 79 100 46 103 38 139 116 152

EUPT-FV13 SWZ Representation



EUPT-FV14

AZ² Representation



91 % Good **2 % Satisfactory** **7 % Unsatisfactory**

5 NRLs in Cat B

Category A: 55% of the EU Participant Laboratories

(87 Laboratories of 157 (total 167))

HOW MATRIX EFFECTS AFFECTS TO GC AND LC-MS ANALYSIS?

-Signal changes

-Abundance of specific fragment ions/trans

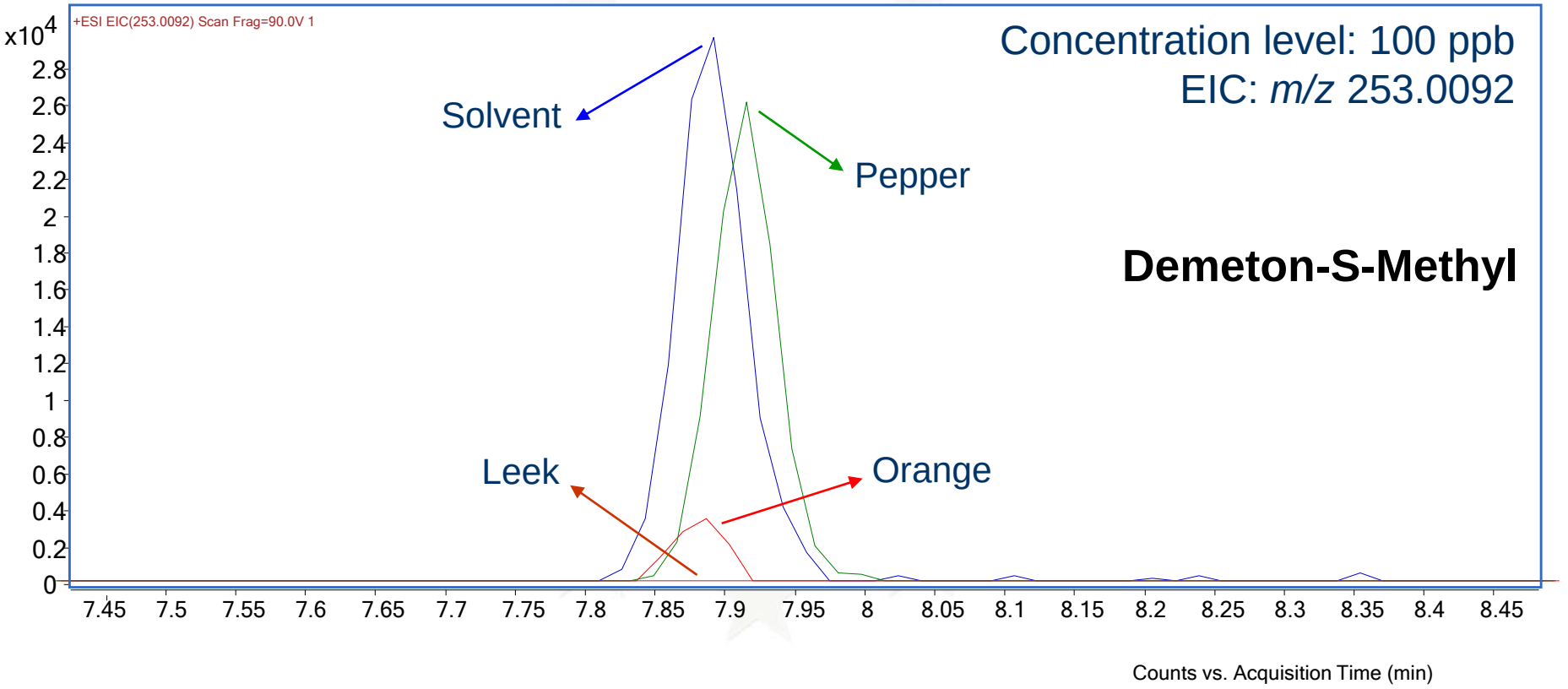
-Instrument maintenance

LC-QqQ-MS/MS
Skimer
After 30 tea injections

GC-QqQ-MS Liner after 30 tea injections

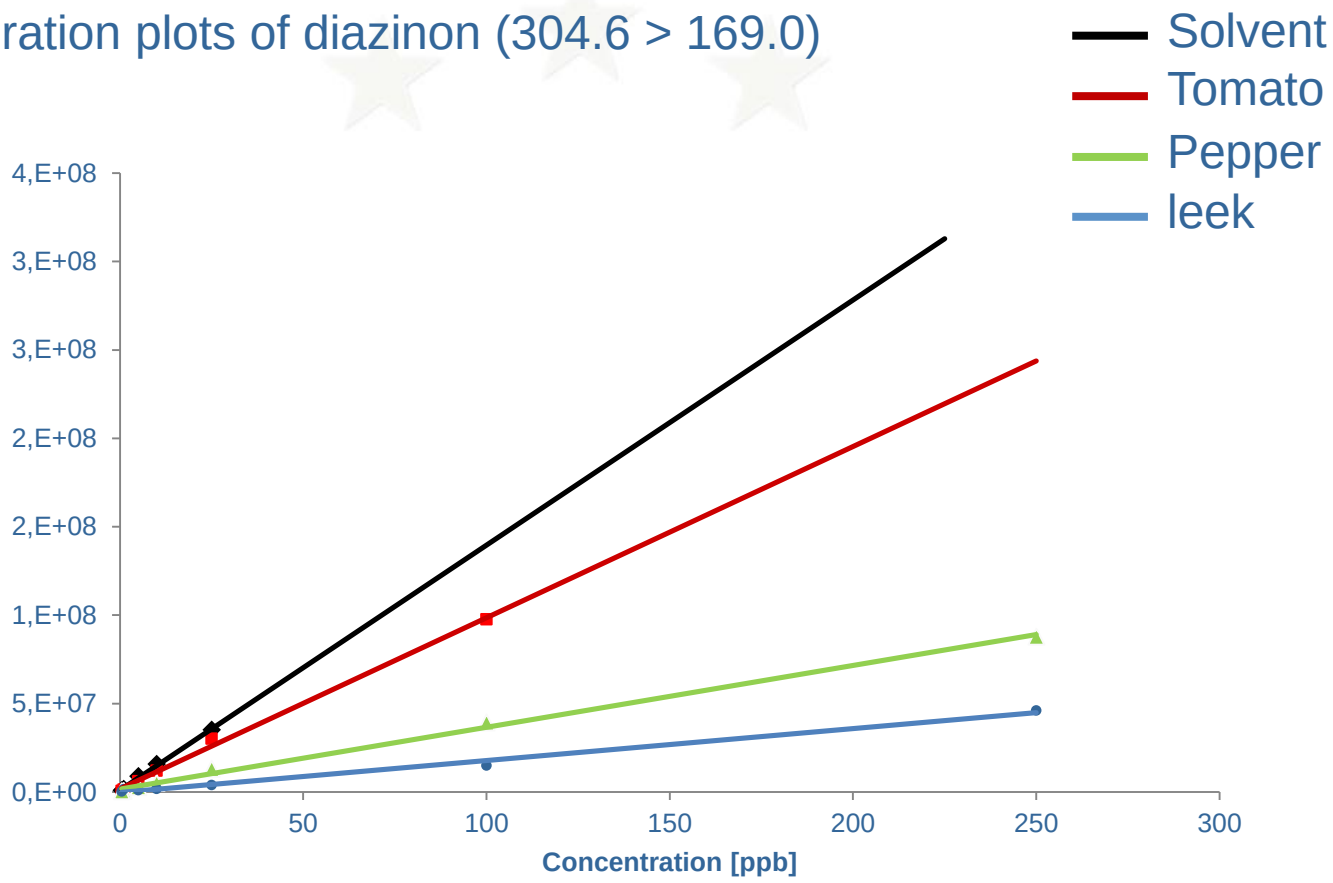
Signal supression due to matrix effects

Injection 1g matrix/ml



LC-MS/MS

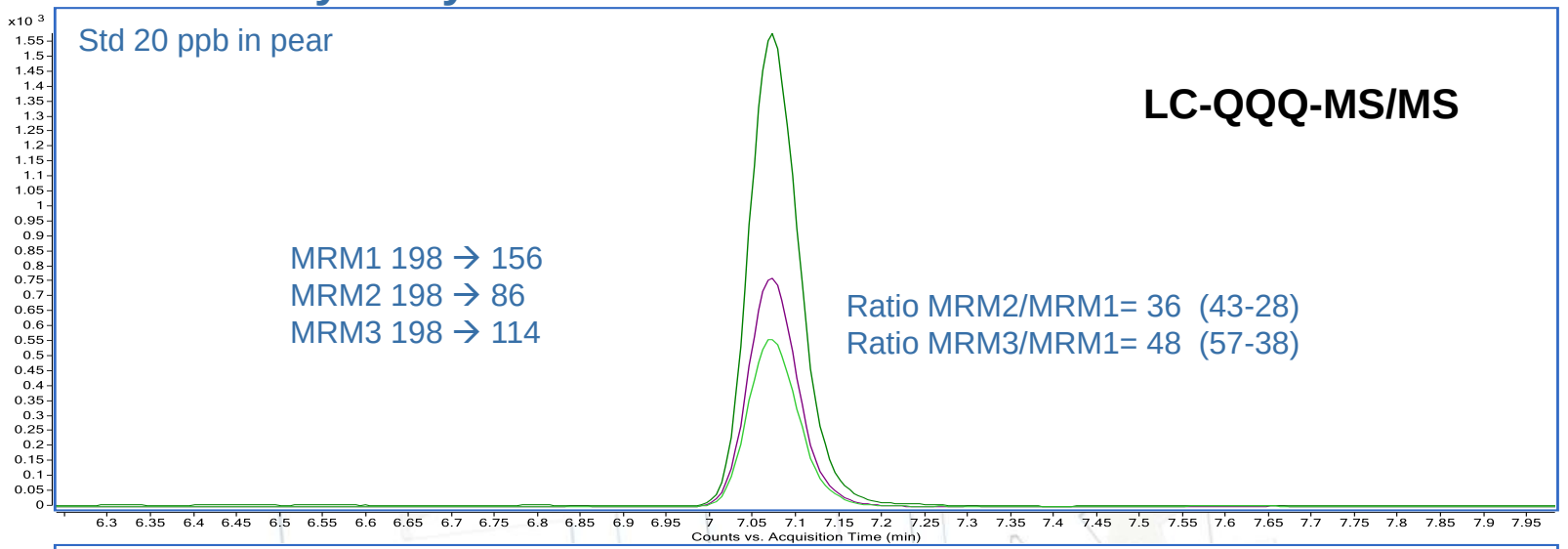
Calibration plots of diazinon (304.6 > 169.0)

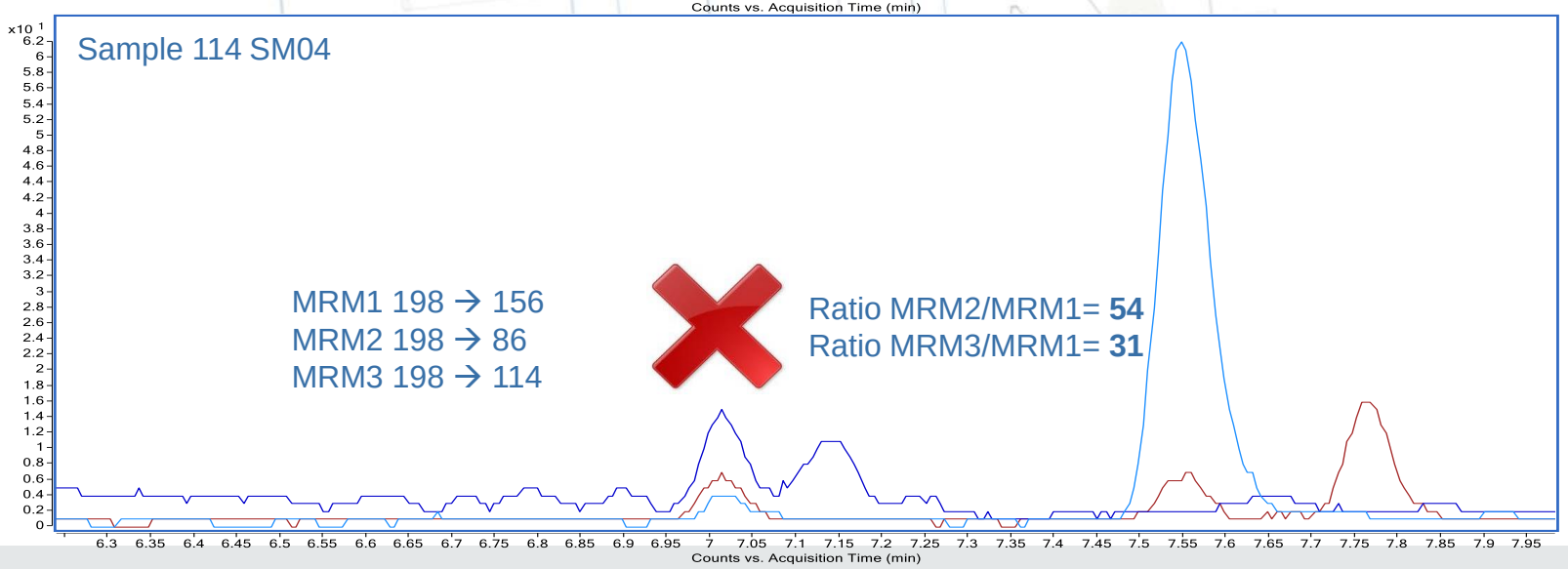


Common transition with matrix

Pear matrix

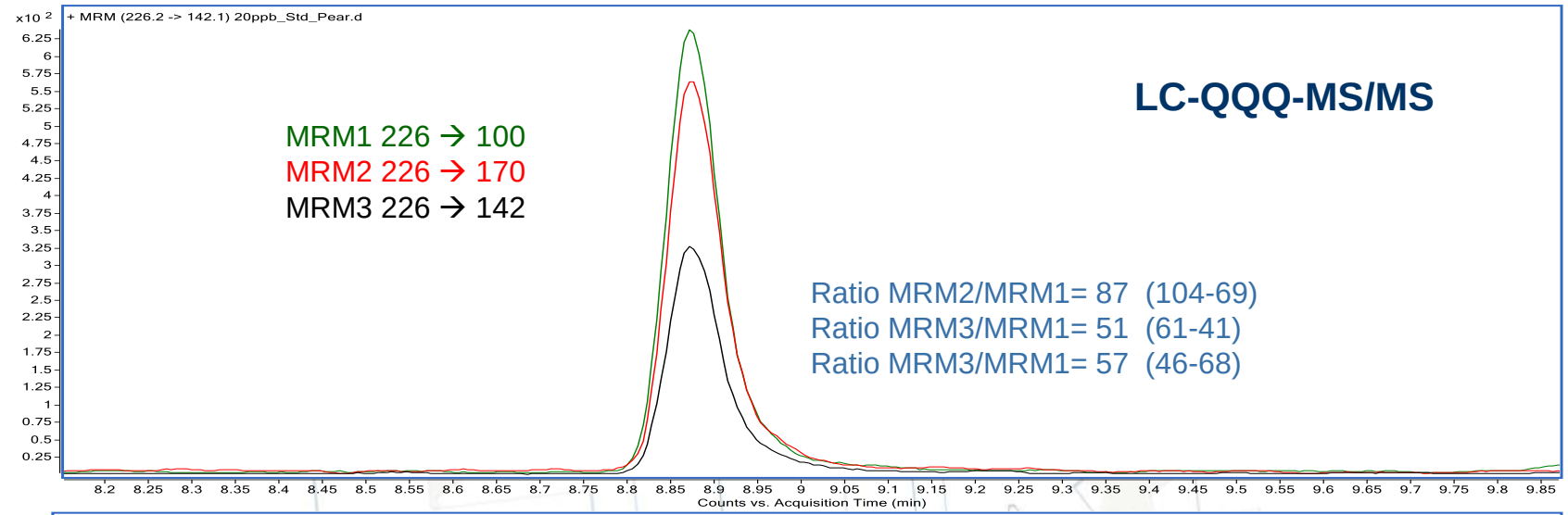
Atrazine-2-hydroxy

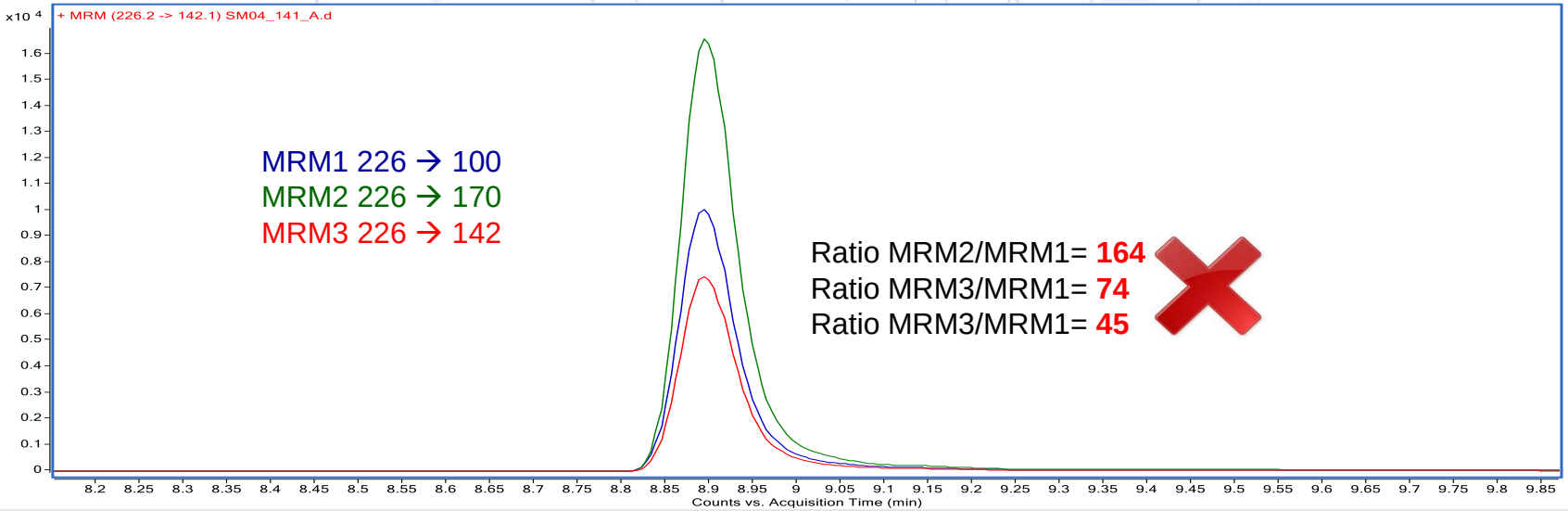


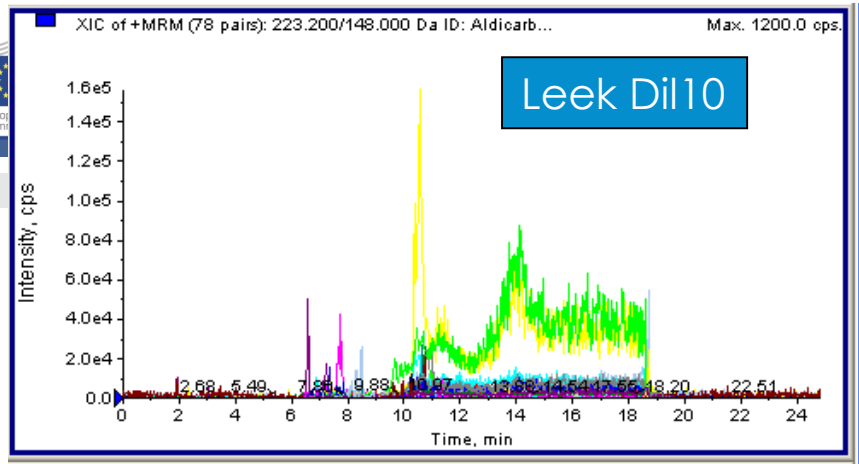


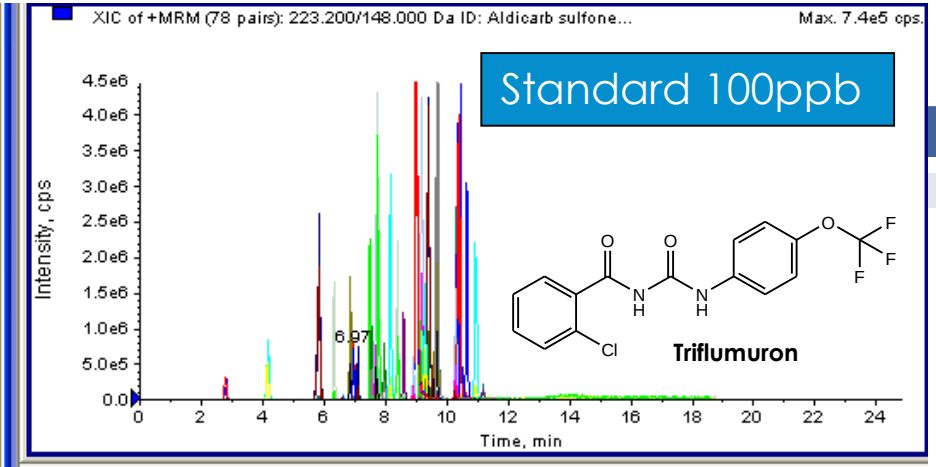
Pear matrix

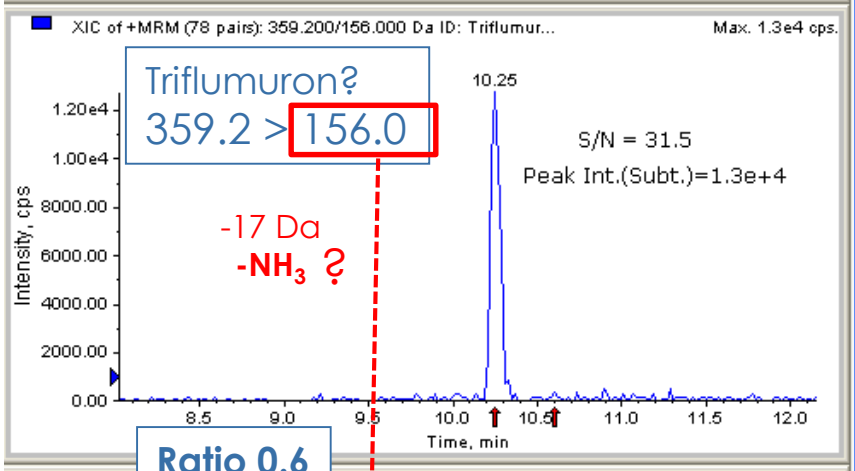
Secbumeton

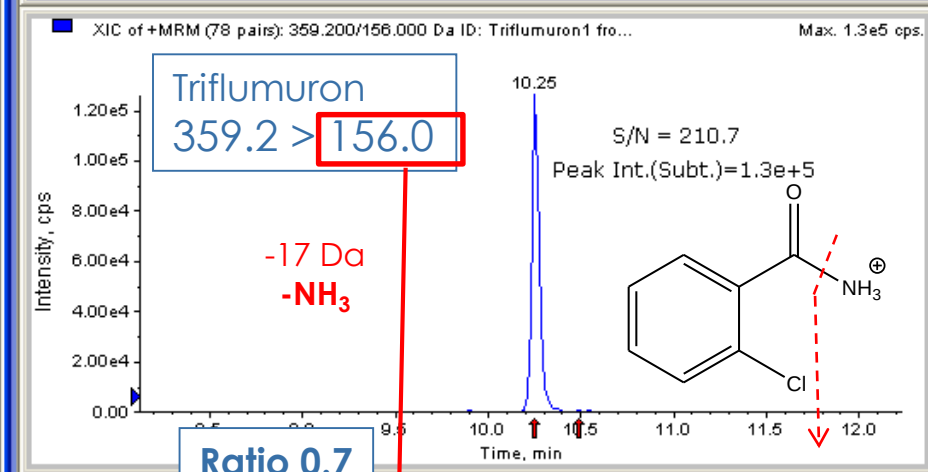


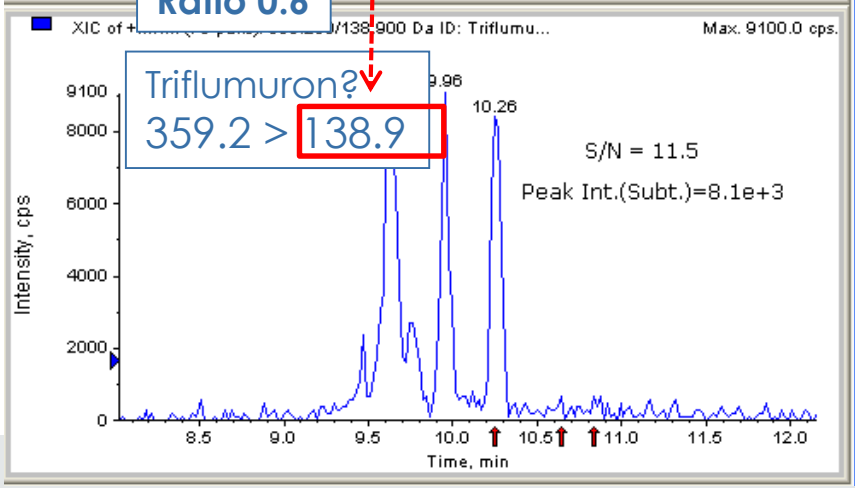


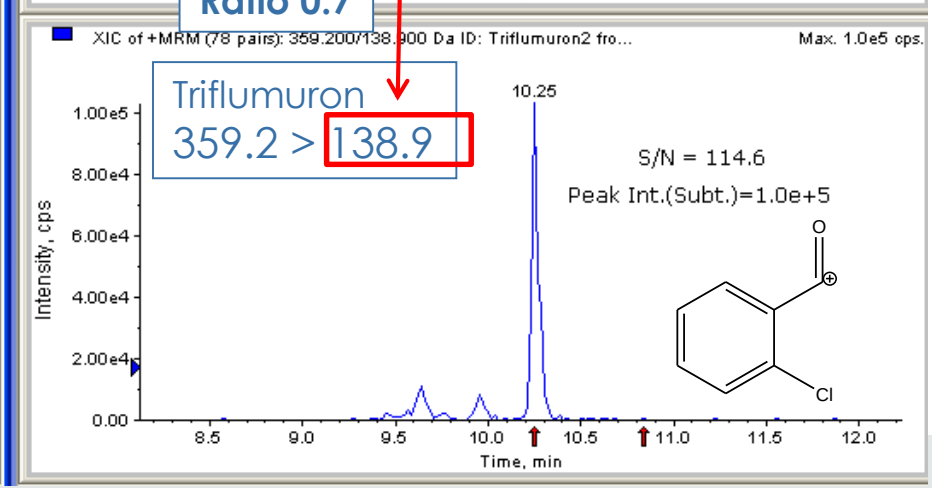

XIC of +MRM (78 pairs): 223.200/148.000 Da ID: Aldicarb...
Max. 1200.0 cps.
Leek Dil10
Intensity, cps
Time, min


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

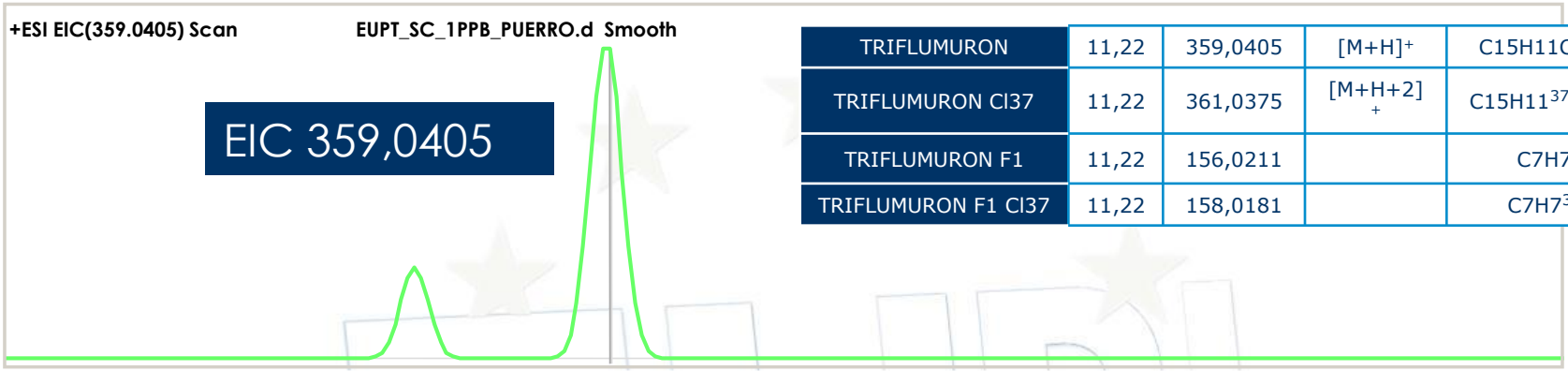

XIC of +MRM (78 pairs): 359.200/156.000 Da ID: Triflumur...
Max. 1.3e4 cps.
Triflumuron?
359.2 > 156.0
S/N = 31.5
Peak Int.(Subt.)=1.3e+4
-17 Da
-NH3 ?
Ratio 0.6
Intensity, cps
Time, min


XIC of +MRM (78 pairs): 359.200/156.000 Da ID: Triflumuron1 fro...
Max. 1.3e5 cps.
Triflumuron
359.2 > 156.0
S/N = 210.7
Peak Int.(Subt.)=1.3e+5
-17 Da
-NH3
Ratio 0.7
Intensity, cps
Time, min

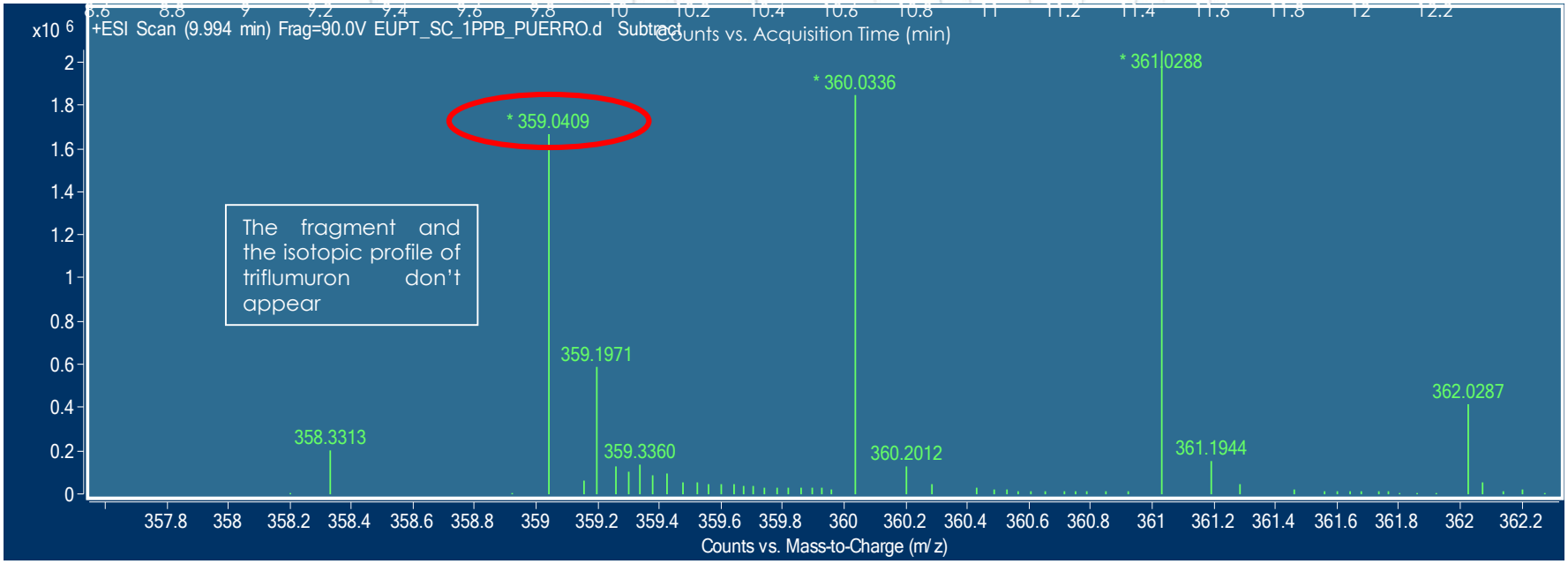

Max. 9100.0 cps.
Triflumuron?
359.2 > 138.9
S/N = 11.5
Peak Int.(Subt.)=8.1e+3
Intensity, cps
Time, min


XIC of +MRM (78 pairs): 359.200/138.900 Da ID: Triflumuron2 fro...
Max. 1.0e5 cps.
Triflumuron
359.2 > 138.9
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		$C_7H_7{}^{37}ClNO$

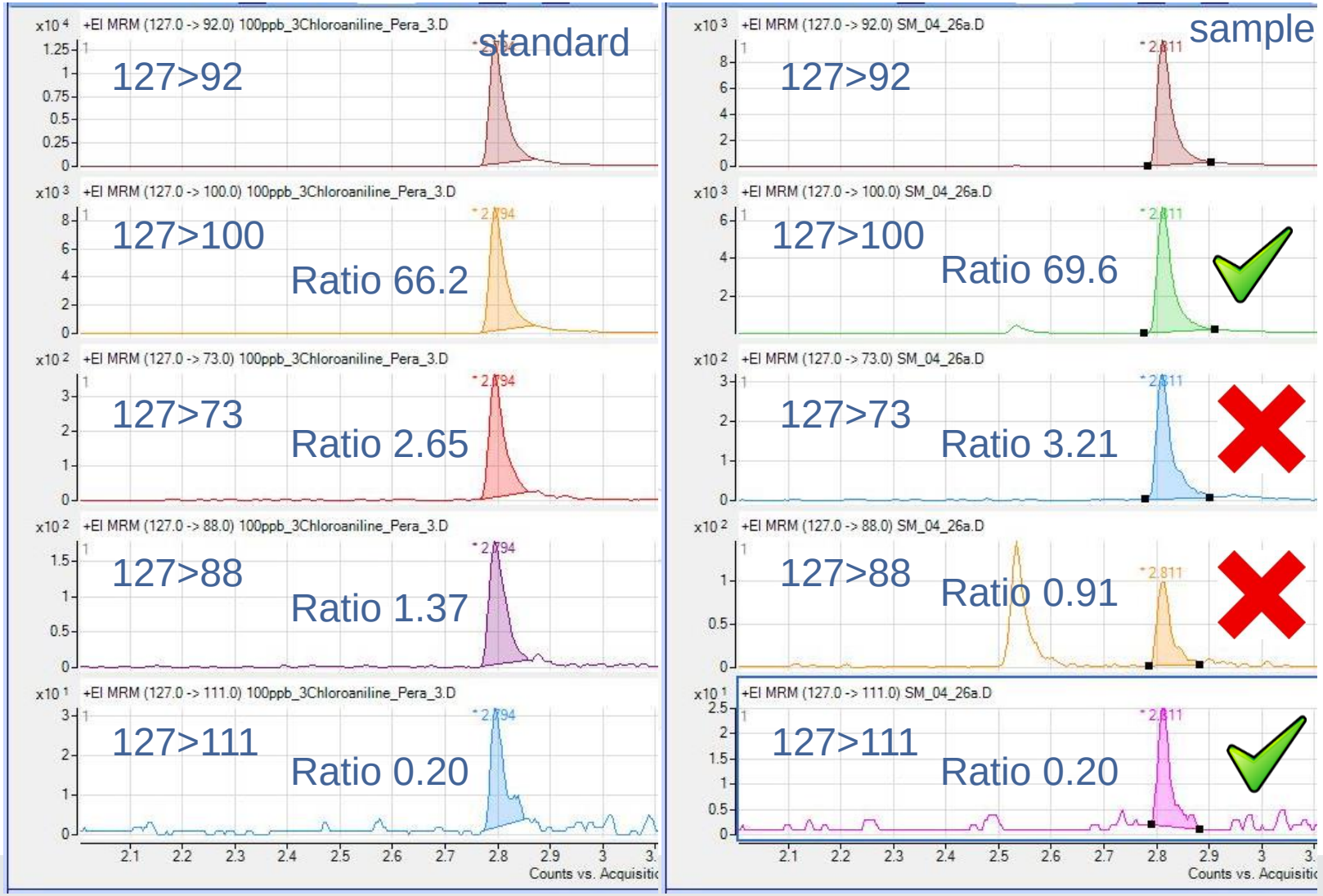


3-Chloraniline

100 ppb Pear
RT = 2.79 min

SM-04 (67 ppb?)
RT = 2.81 min

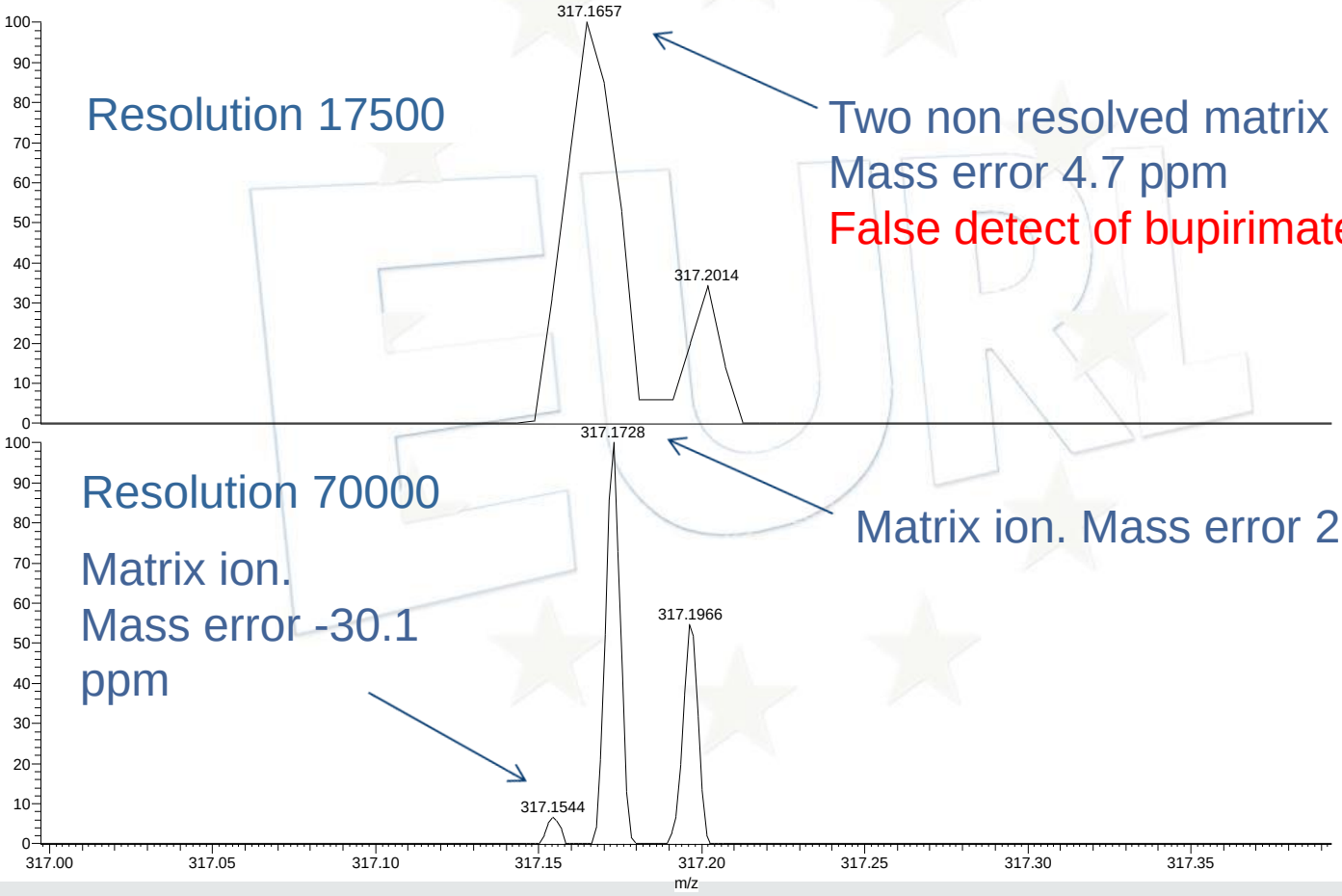
GC-QQQ-MS/MS



Influence of resolution on detection

Exact mass of bupirimate 317.1642

Orange



Resolution 17500

Two non resolved matrix ions.
Mass error 4.7 ppm
False detect of bupirimate

Resolution 70000

Matrix ion.
Mass error -30.1 ppm

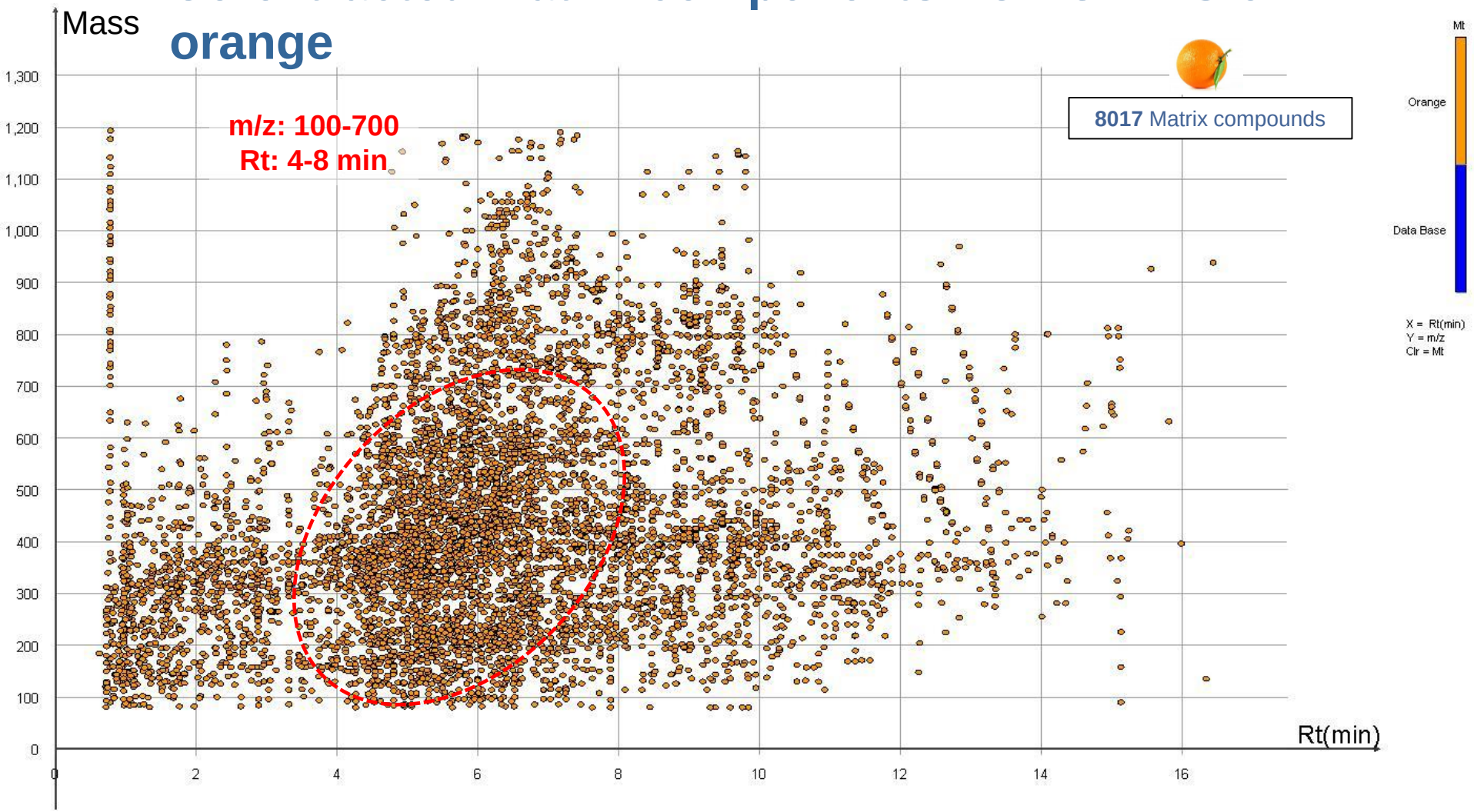
Matrix ion. Mass error 27.1 ppm

MATRIX STUDY

mapping of natural components by

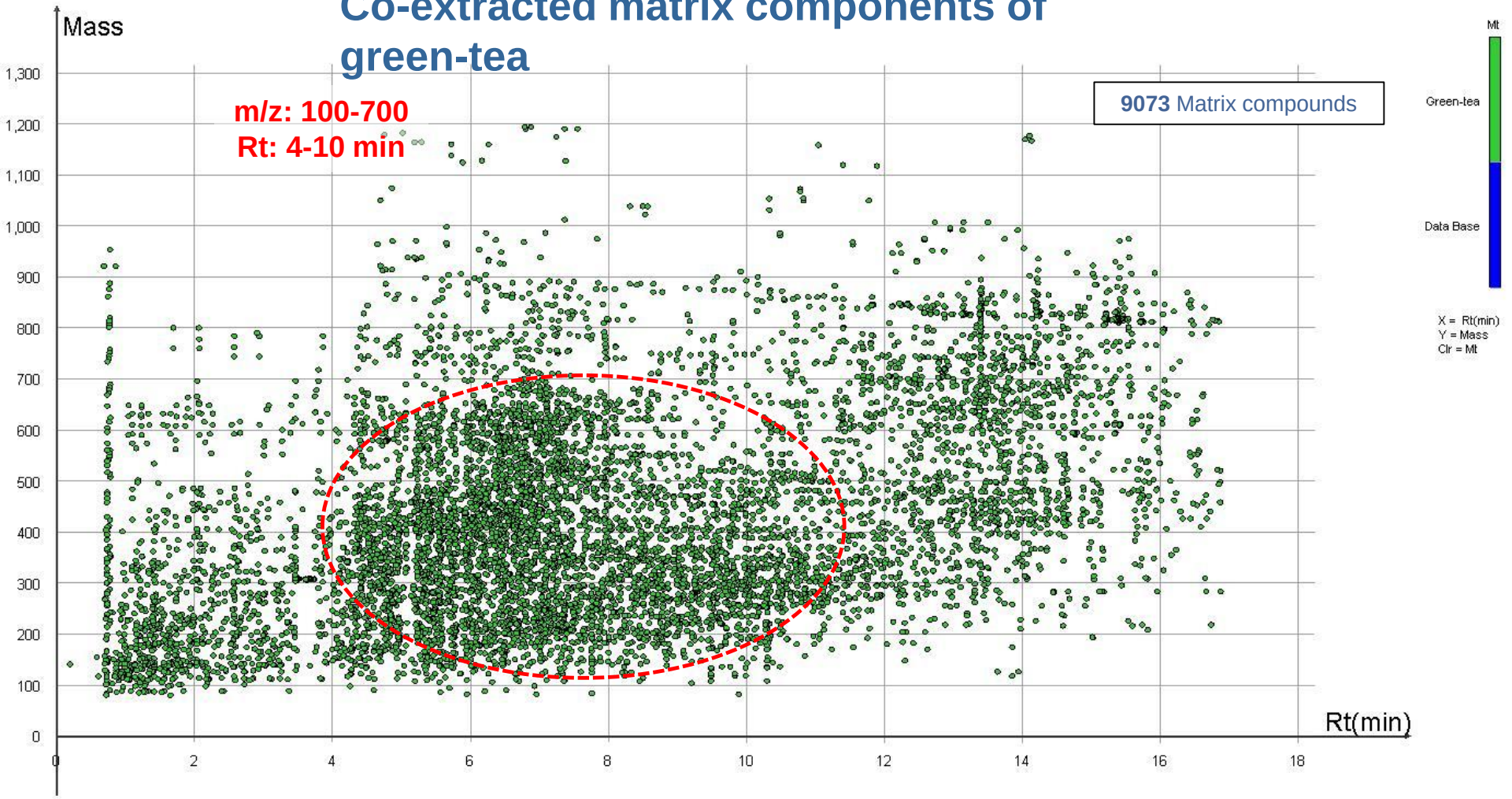
LC-TOF-MS ANALYSIS FULL SCAN MODE

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



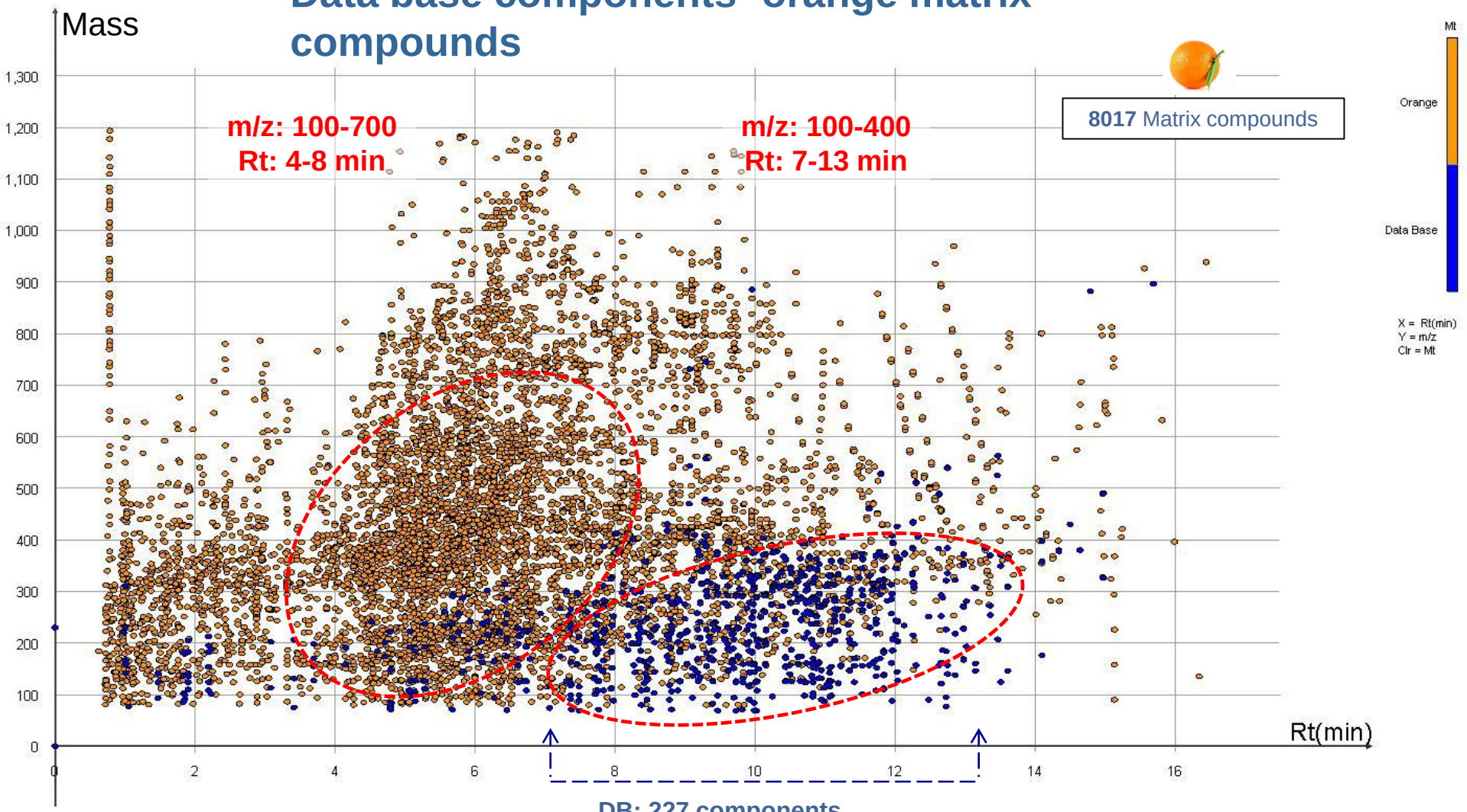
Miner 3D Enterprise

Co-extracted matrix components of green-tea



Miner 3D Enterprise

Data base components- orange matrix compounds



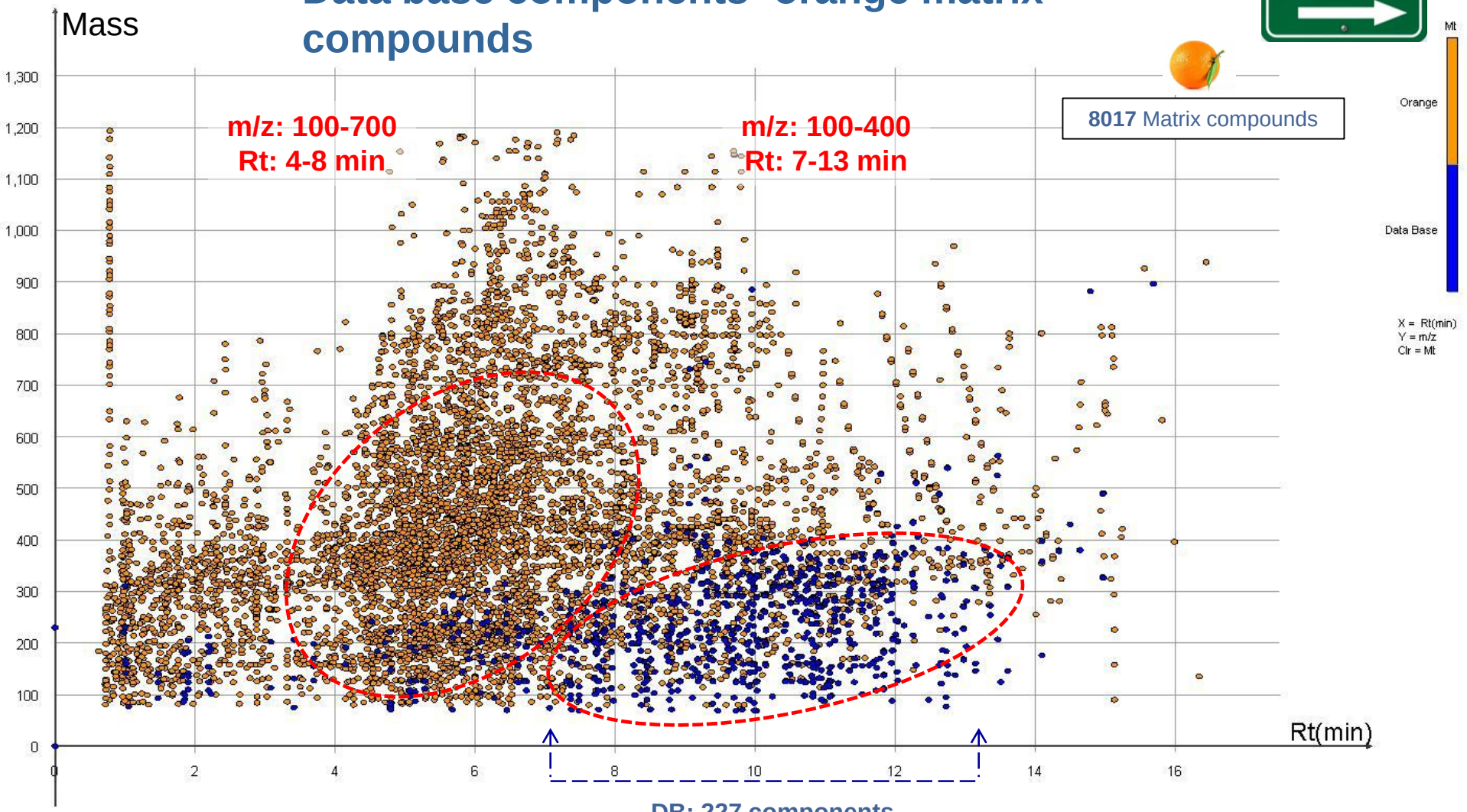
Miner 3D Enterprise

DB: 227 components
Orange: 2743 matrix compounds

How can we control or avoid them?

Highly Sensitive LC-MS Analysis

Data base components- orange matrix compounds

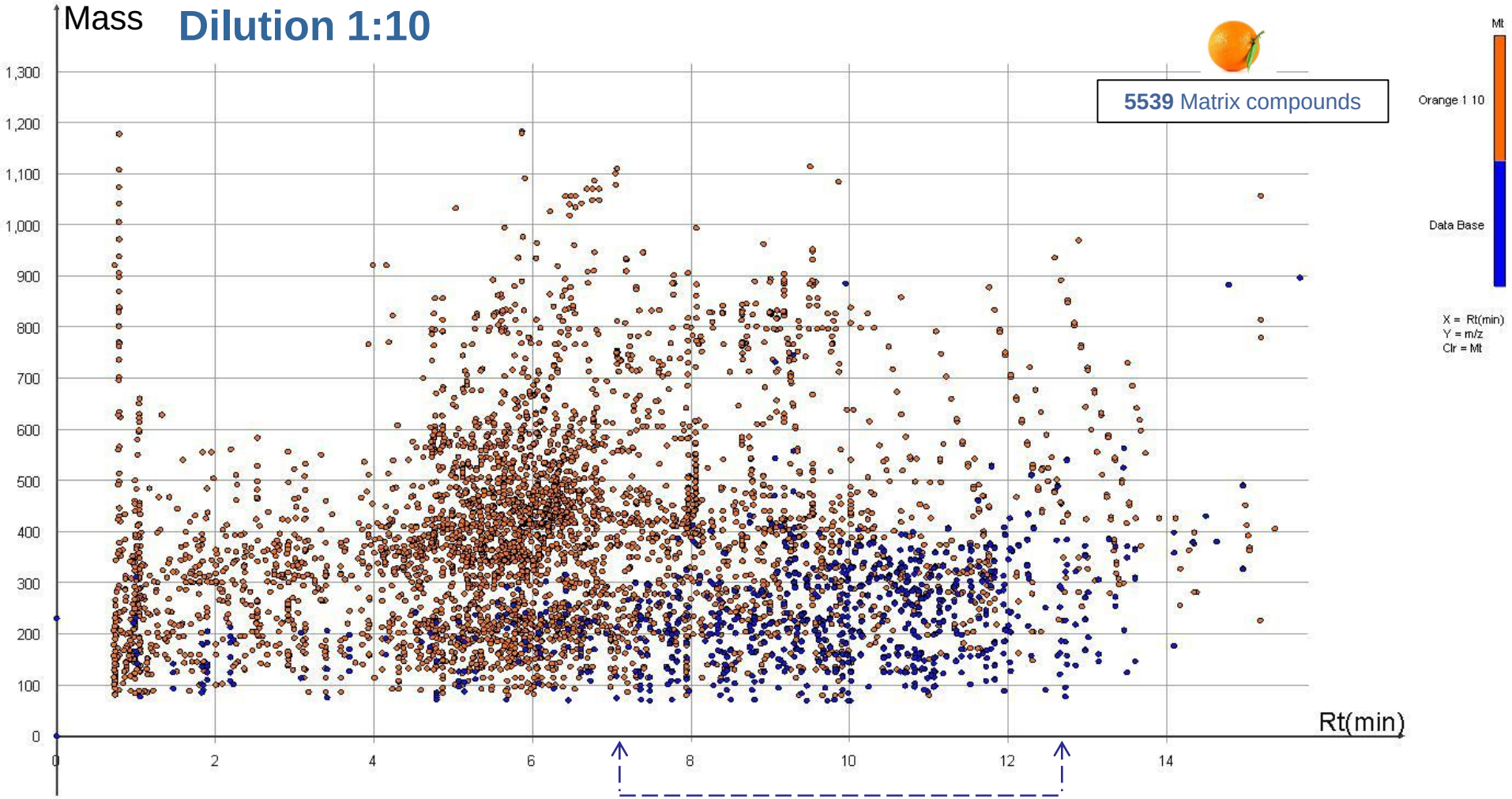


Miner 3D Enterprise

DB: 227 components
Orange: 2743 matrix compounds

Highly Sensitive LC-MS Analysis

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



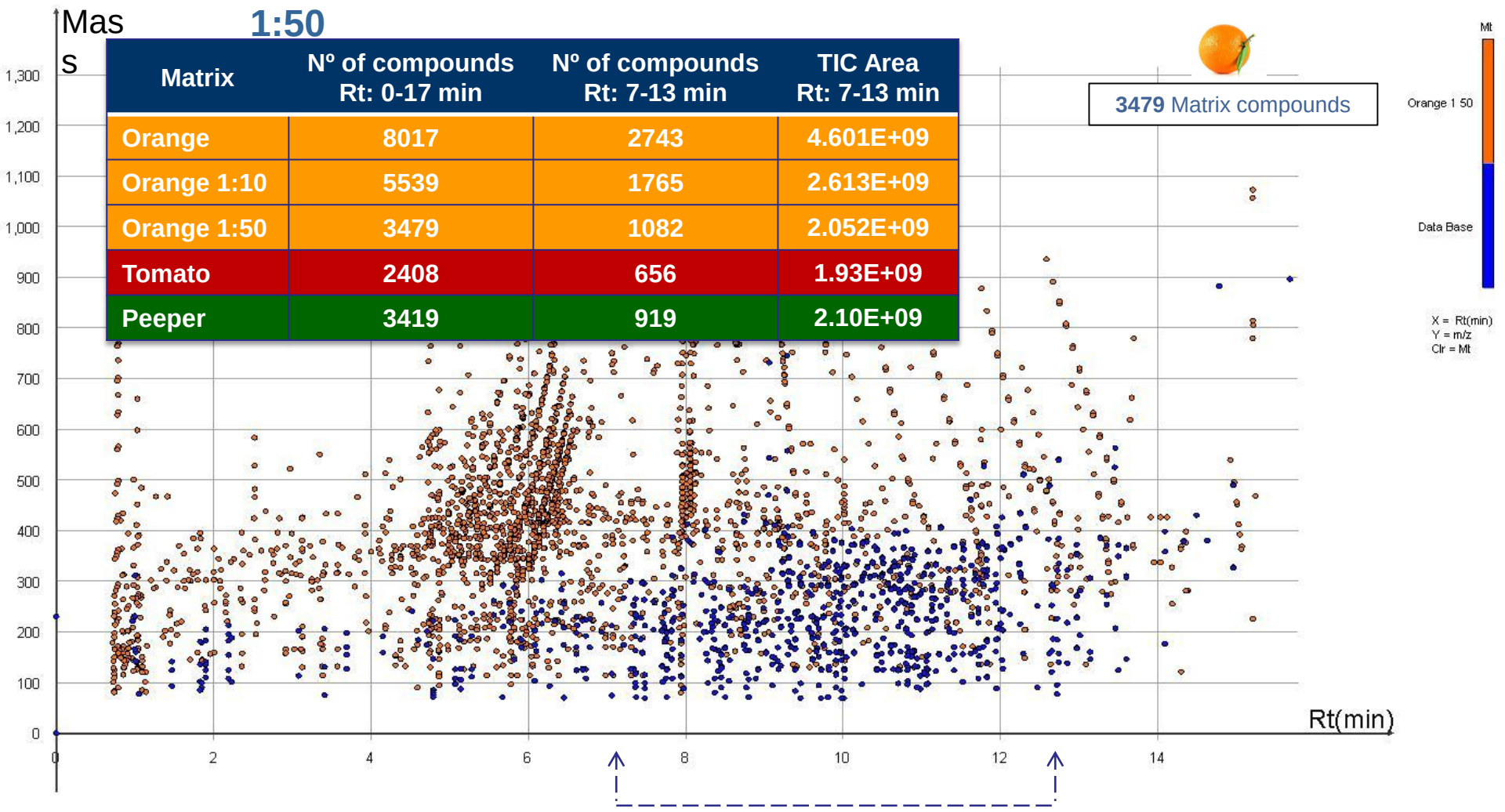
DB: 227 components
Orange: 1765 matrix compounds

Miner 3D Enterprise

Highly Sensitive LC-MS Analysis

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

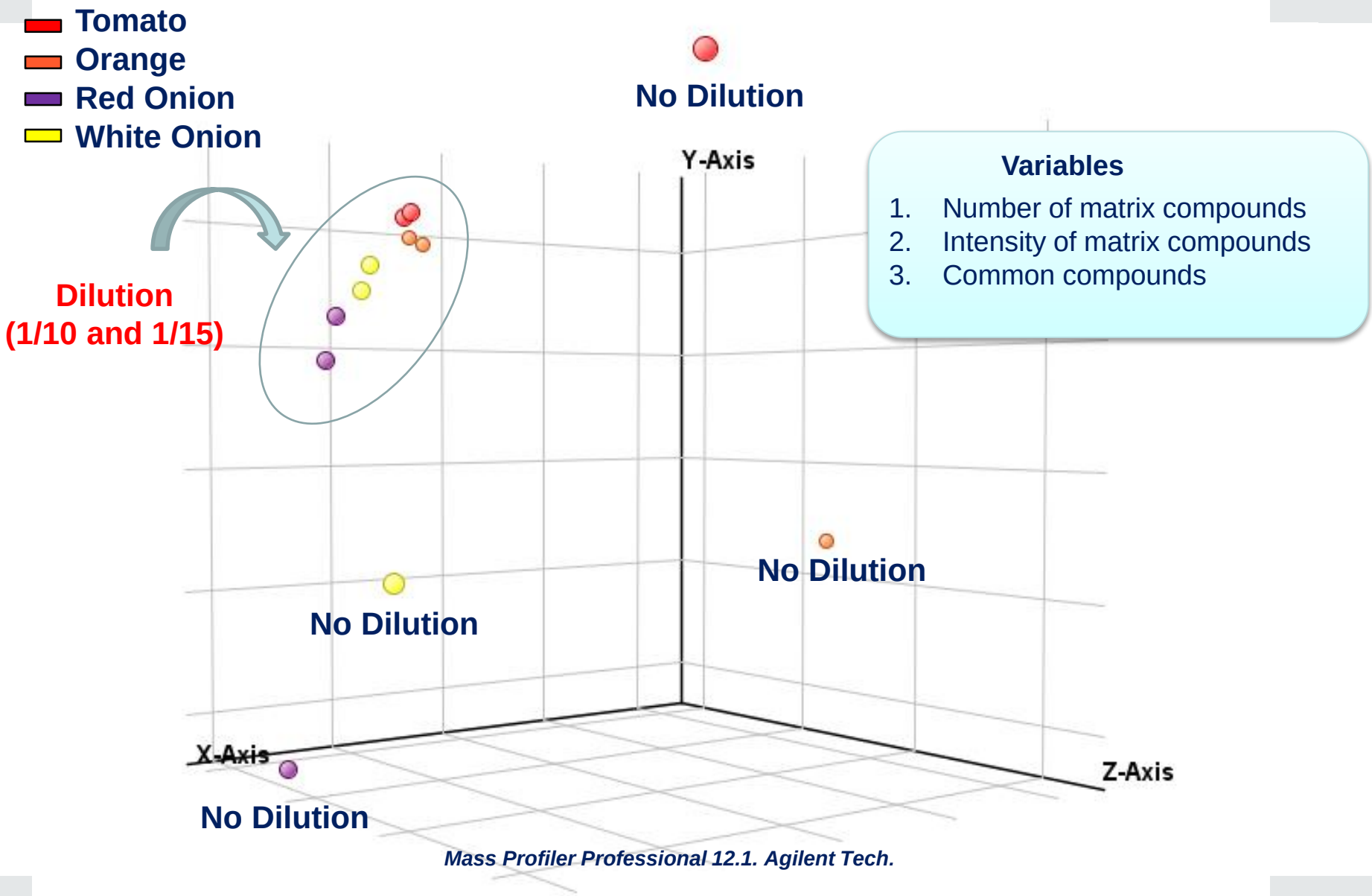
Matrix	Nº of compounds Rt: 0-17 min	Nº of compounds Rt: 7-13 min	TIC Area Rt: 7-13 min
Orange	8017	2743	4.601E+09
Orange 1:10	5539	1765	2.613E+09
Orange 1:50	3479	1082	2.052E+09
Tomato	2408	656	1.93E+09
Peeper	3419	919	2.10E+09



Miner 3D Enterprise

DB: 227 components
Orange: 1082 matrix compounds

Principal Components Analysis (PCA)



Mass Profiler Professional 12.1. Agilent Tech.

QuEChERS Extraction Method

10 g sample + 10 mL AcN + **IS**

Shake automatically 4 min

Add 4 g $MgSO_4$ anhidrous
+ 1 g NaCl + 1 g $Na_3Citrate \cdot 2\ H_2O$
+ 0.5 g $Na_2HCitrate \cdot 1.5\ H_2O$

Shake automatically 4 min

Centrifugue 5 min. 3500 r.p.m.

Take 5 mL aliquot

Add 750 mg $MgSO_4$
anhidrous. + 125 mg PSA + 125 mg C_{18}

Shake 30 second in Vortex

Centrifugue 5 min. 3500 r.p.m.

Acidify with 50 µL formic acid 5% in AcN

Take an aliquot add **IS** and
dilute 1:30 with AcN:H_2O (1:9)

microLC-MS/MS Analysis

0.033 gr sample/mL injected

Matrices

Tomato, Pepper and Orange

Compounds in the method

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

Diclorvos-d_6
Malathion-d_{10}: Extraction
TPP

Linuron-d_6: Dilution

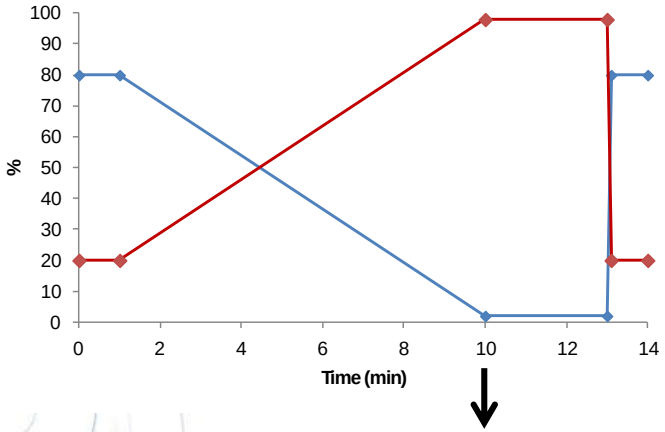
Dimethoate-d_6: Injection

microLC-QqQ-MS/MS

Liquid chromatography

- Column: HALO C18 2.7 µm 90 Å 0.5x50mm
- Mobile phases: Acetonitrile and water (0.1% formi
- Injection volume: 3µl
- Flow: 30 µl/min
- Total run time: 14 min

Gradient

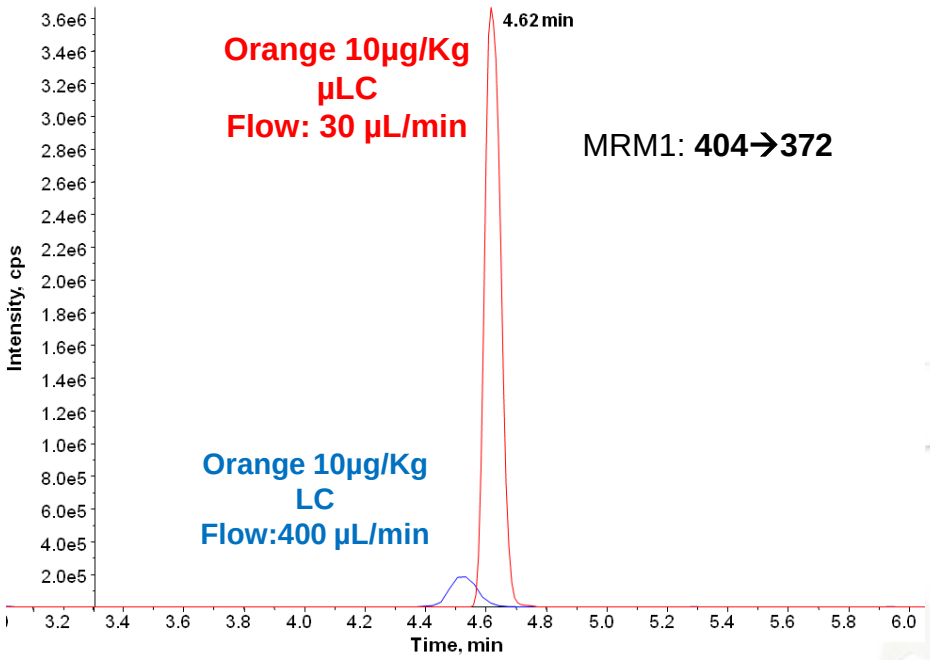


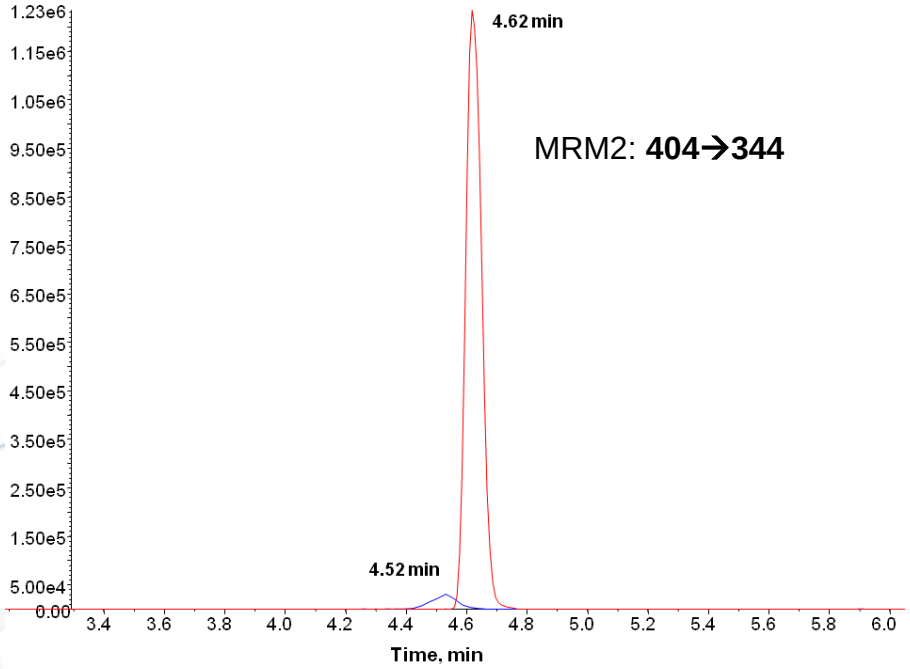
10 min Gradient

QqQ-MS

- Source: ESI (+) with microFlow electrode
- Pesticide Identification: 2 transitions
- Source Parameters:
  - ✓ Nebulizer and collision gas: nitrogen
  - ✓ Collision gas (CAD): Medium
  - ✓ Gas temperature: 300ºC
  - ✓ Curtain gas (CUR): 20
  - ✓ IonSpray Voltaje (IS): 5000V
  - ✓ Schedule MRM software features

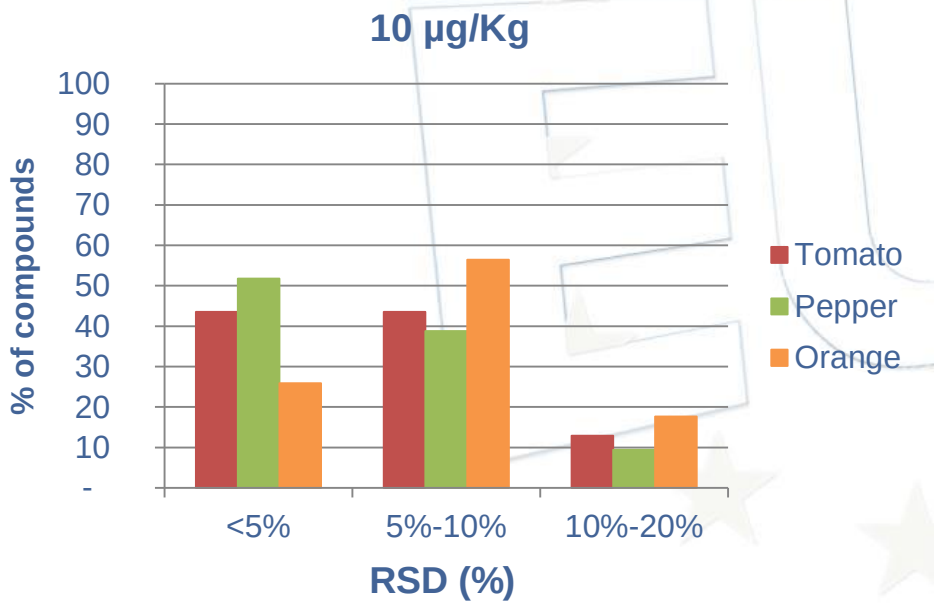
Comparison between µLC and LC: Azoxystrobin in an orange matrix at 10µg/Kg

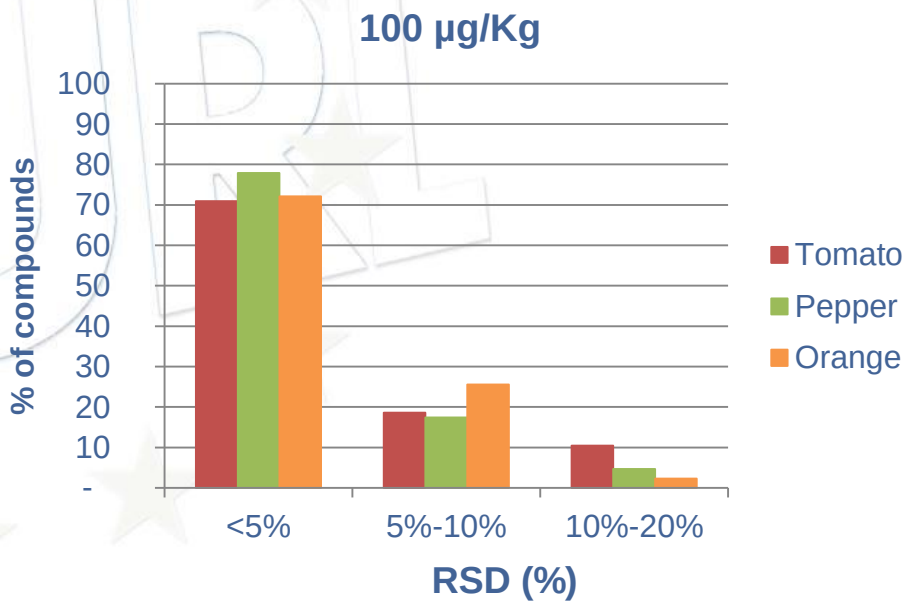




Peak Area Reproducibility

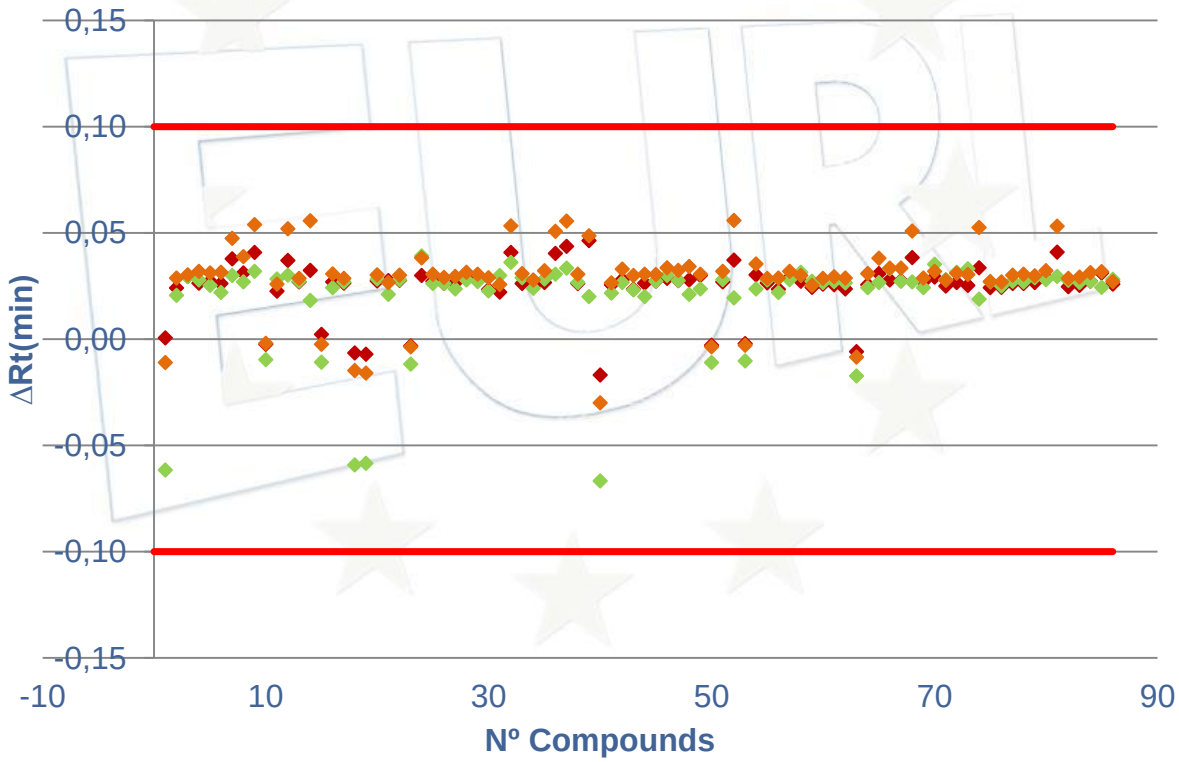
5 consecutive injections over 3 days of a tomato, orange and pepper spiked at 10 µg/Kg and 100 µg/Kg and diluted 30 times.



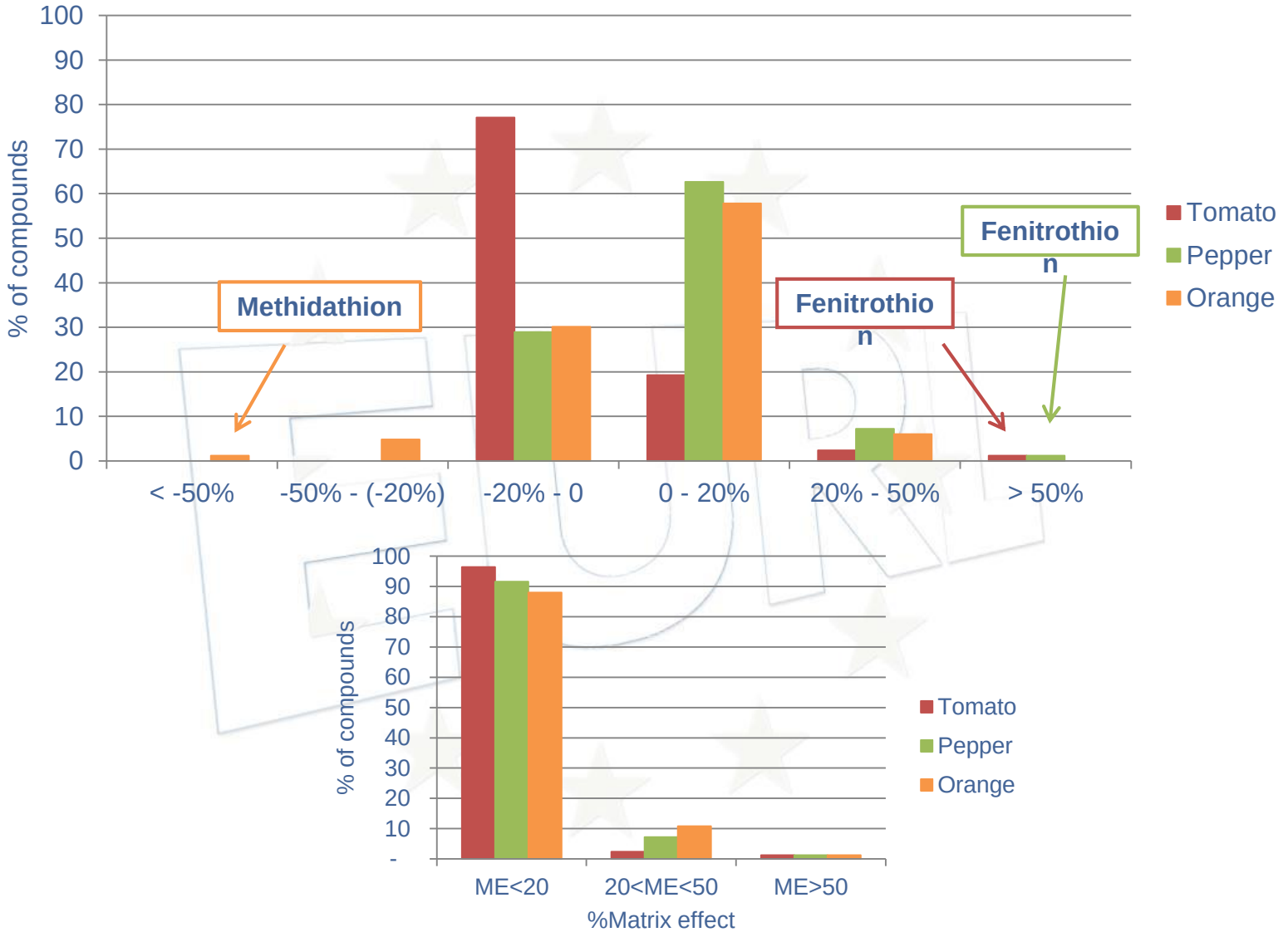


Retention Time Reproducibility

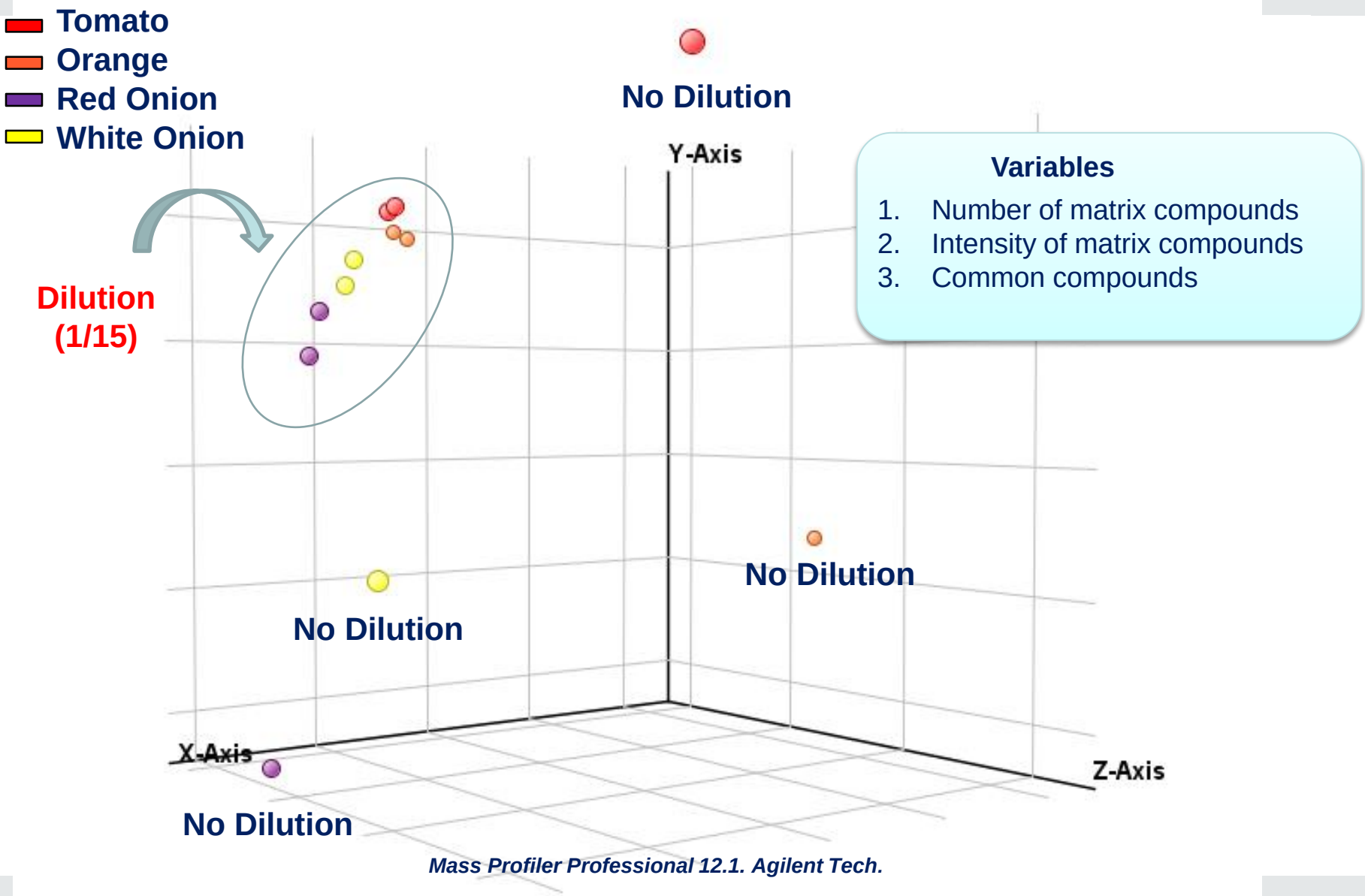
Difference between consecutive injections of tomato, pepper and orange and standard in solvent spiked at different concentrations diluted 30 times



Matrix Effects

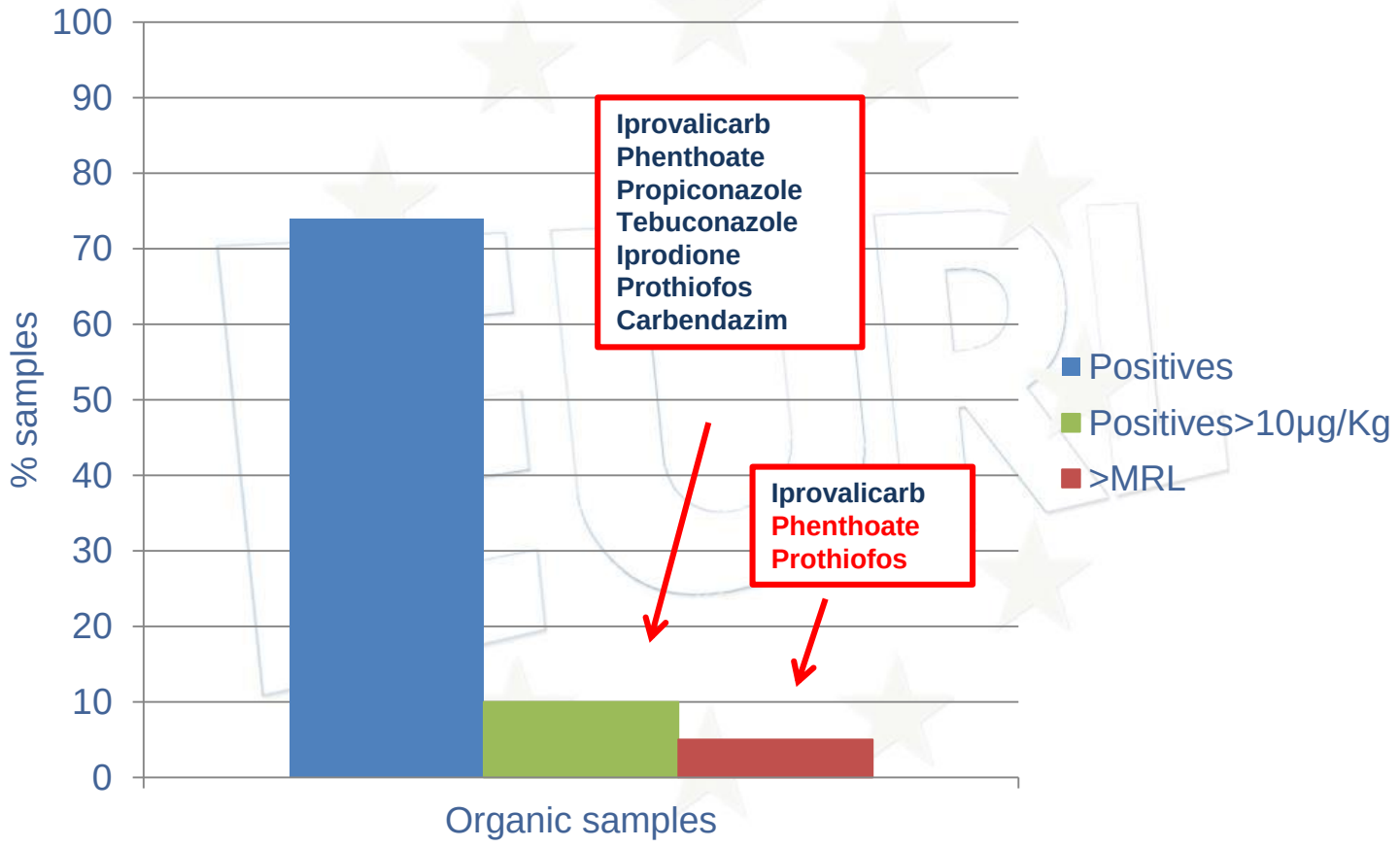


Principal Components Analysis (PCA)

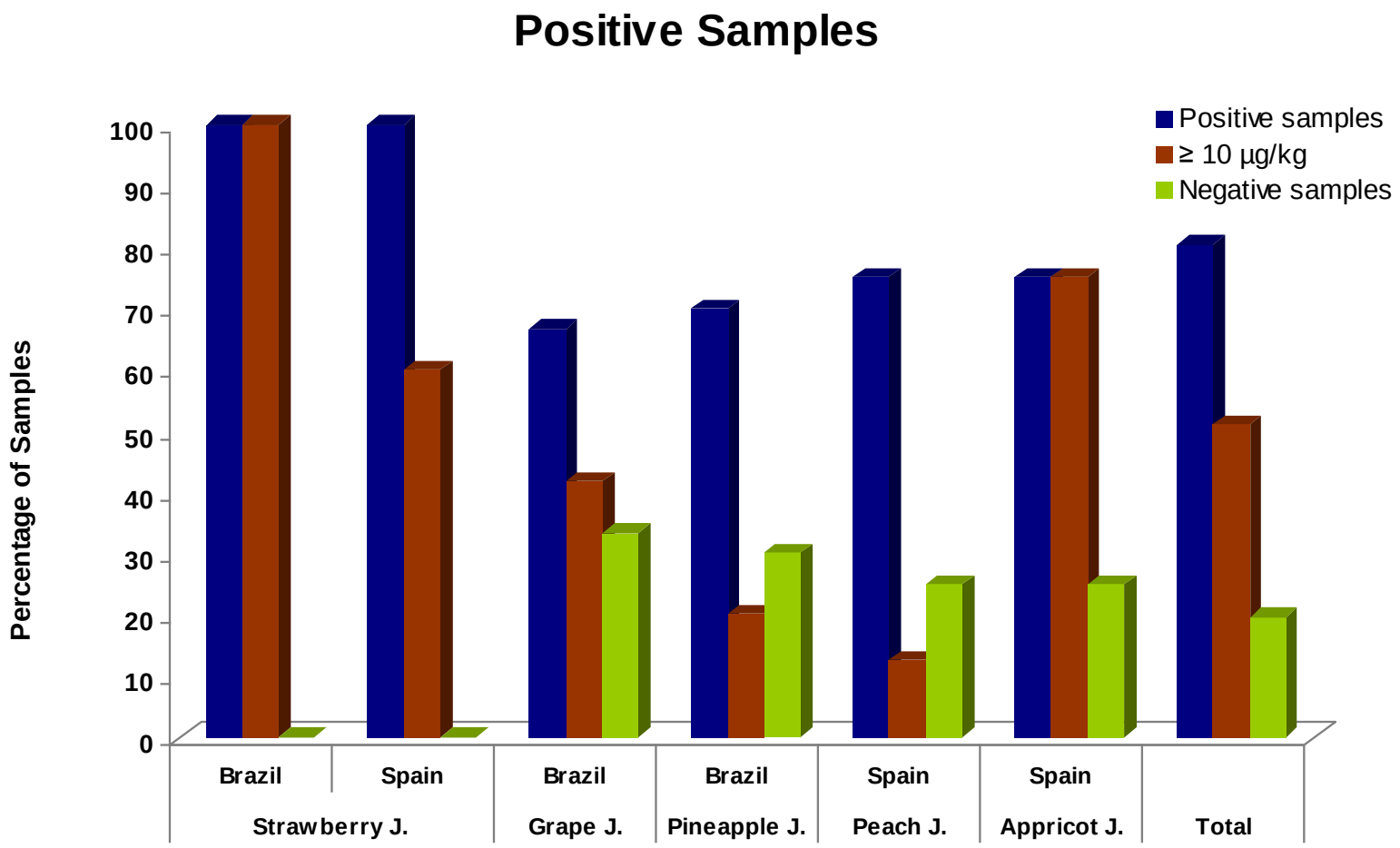


Mass Profiler Professional 12.1. Agilent Tech.

Real samples from different supermarkets
50 Organic Samples



Fruit jam samples (51) from various supermarkets

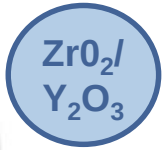


Zirconium oxide-coated Silica Particles

Particle size: 22 µm= 22000 nm
Relative surface area: 310 m^2/g

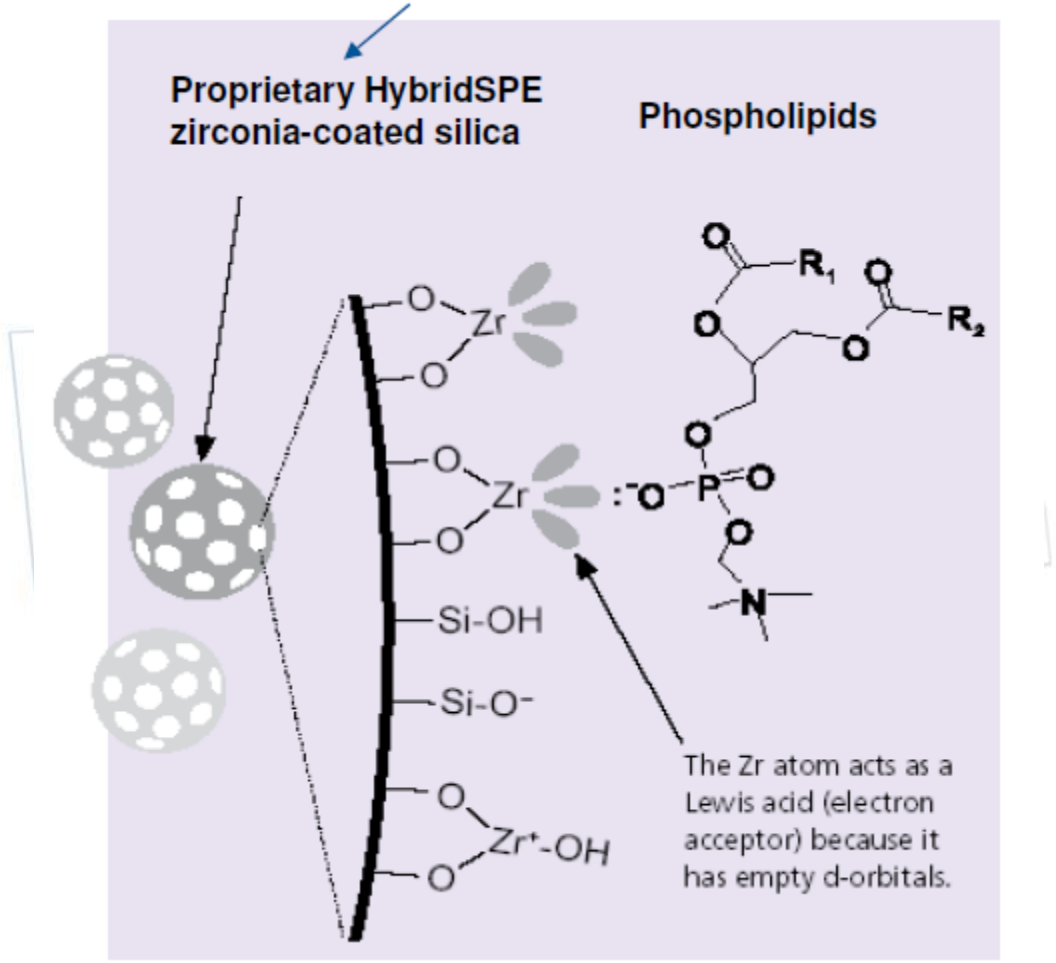
$Zr0_2$

Particle size: 100 nm
Relative surface area: ≥25 m^2/g



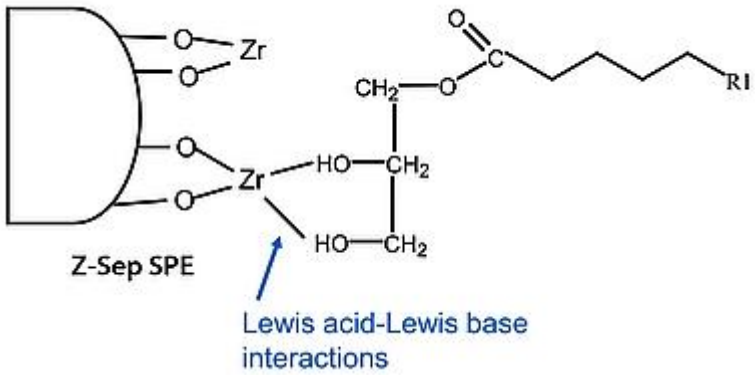
Particle size: 100 nm
Relative surface area: >100 m^2/g

Z-Sep


Proprietary HybridSPE
zirconia-coated silica
Phospholipids
Zr
Si-OH
Si-O⁻
Zr⁺-OH
The Zr atom acts as a Lewis acid (electron acceptor) because it has empty d-orbitals.

ZSEP-SIN C18 (55418-U SUPELCO)

The patented zirconia-coated silica particles of Supel QuE Z-Sep

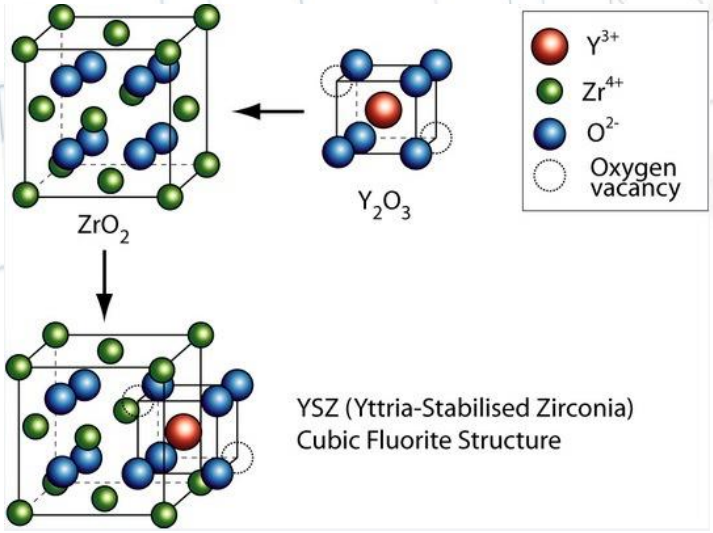


particle size	22 µm
pore size	14 nm pore size
surface area	310 m^2/g

Zirconium(IV) oxide-yttria stabilized (8 mol % yttria as stabilizer)

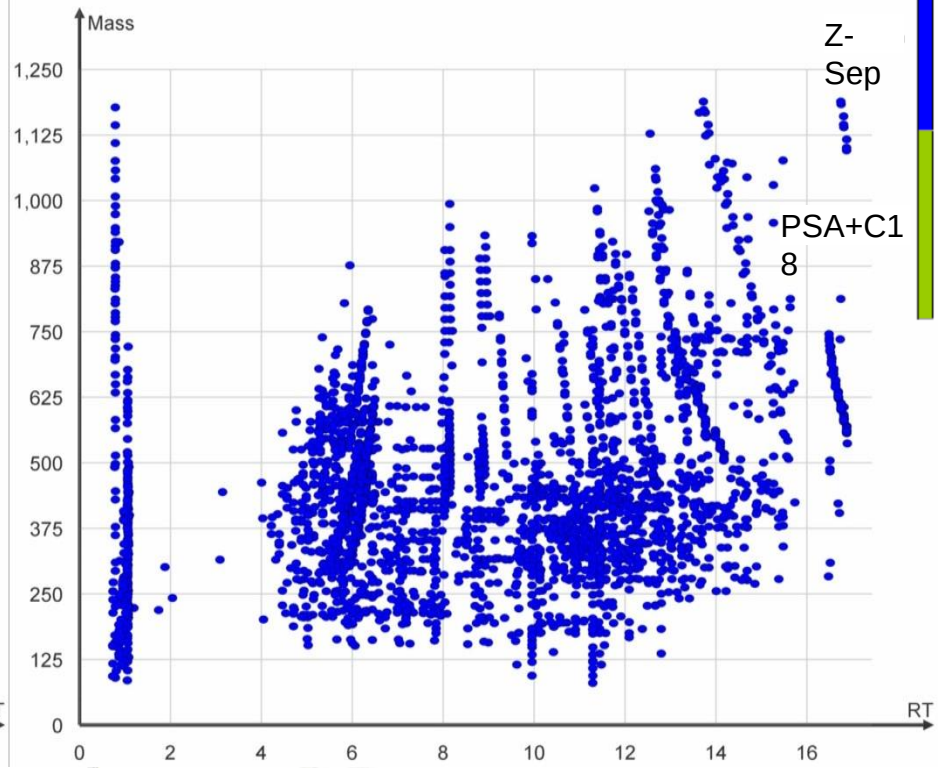
$$ZrO_2 / Y_2O_3$$

composition	$(ZrO_2)_{0.92}(Y_2O_3)_{0.08}$
particle size	**≤100 nm**
surface area	**>100 m²/g**



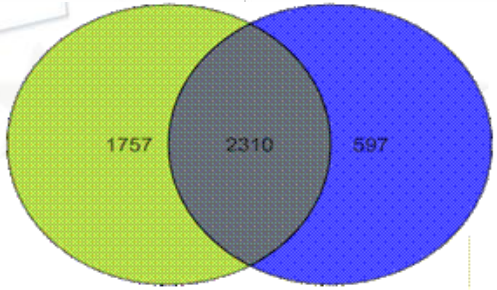
Co-extracted matrix components of avocado with Z-Sep and PSA+C18. LC-TOF/MS

Absolute height ≥ 10000 counts



Miner 3D Enterprise

PSA+C18
4067 matrix compounds



Z-Sep
2907 matrix compounds

Mass Profiler Professional 12.1. Agilent Tech.

FRESH HERBS

European Commission
EURL-FV

10 Fresh Herbs

Flat parsley

Curly parsley

Chive

Coriander

Dill

Mint

Rosemary

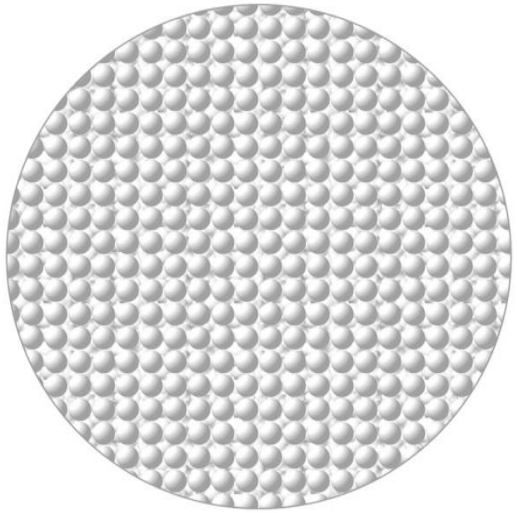
Basil

Oregano

Thyme

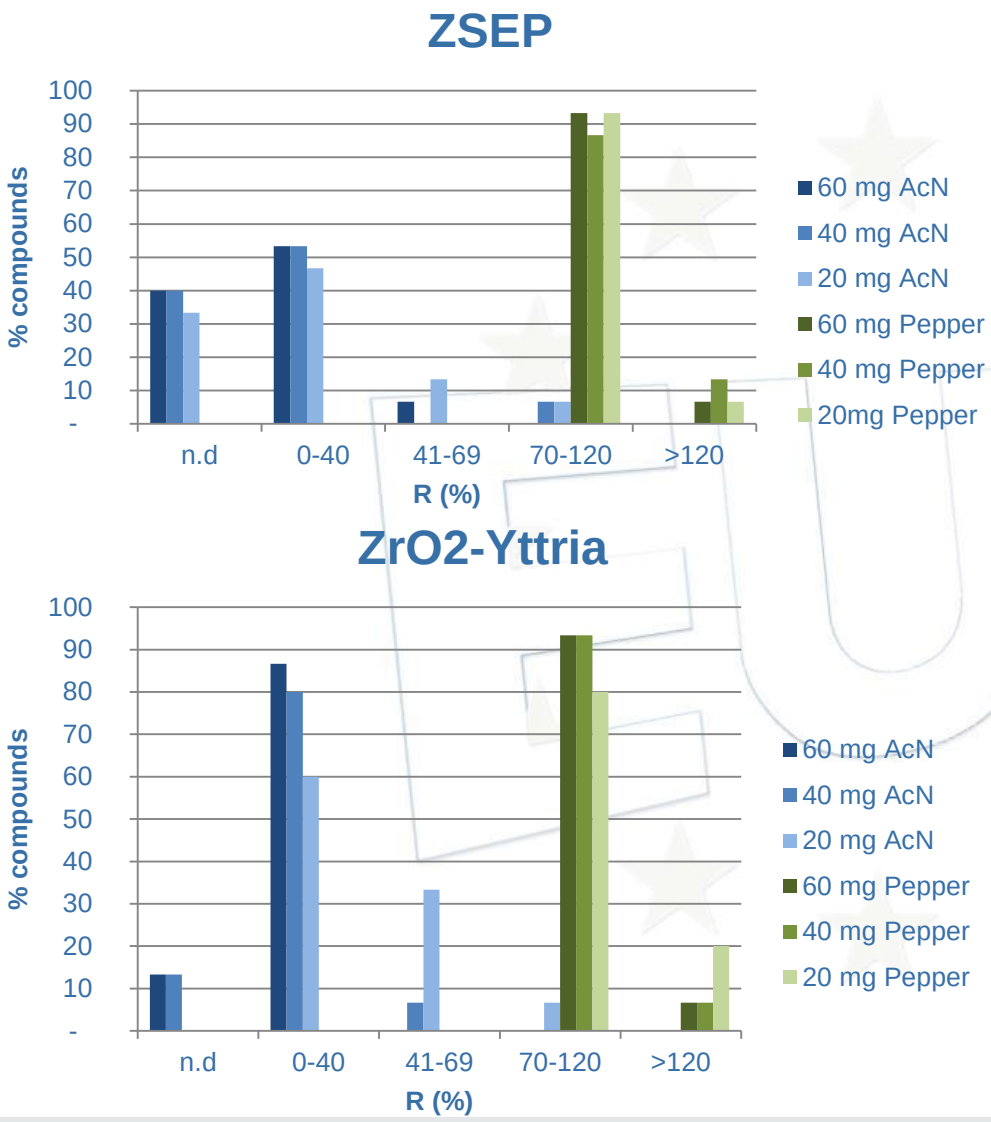
1mL sample + 50 mg
ZSEP
Cartridge format

Disc format



Particle size: 22 µm= 22000 nm
Relative surface area: 310 m2/g

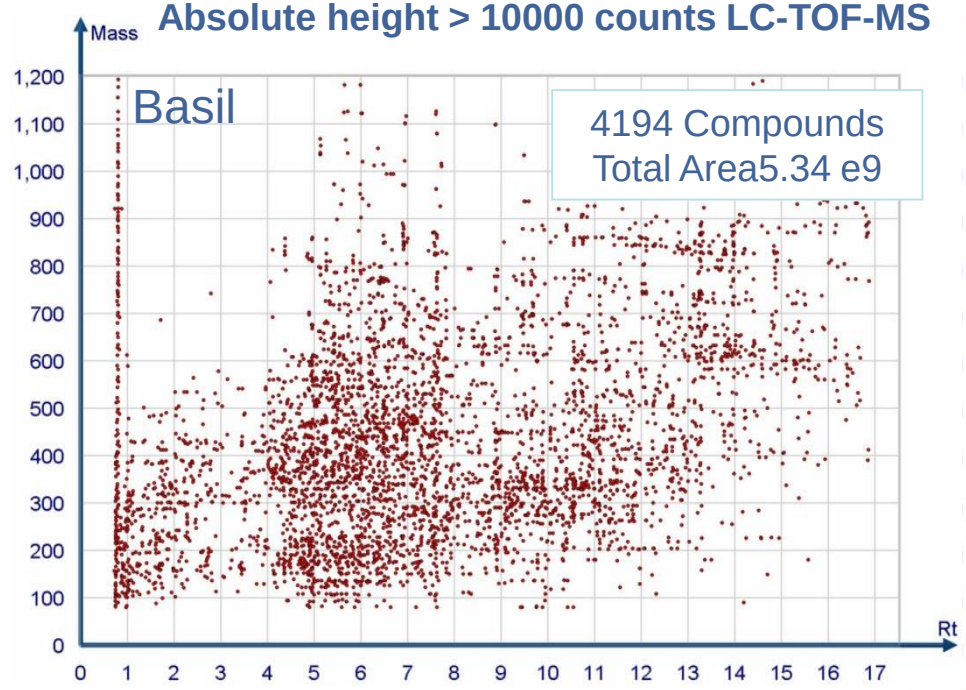
Triazoles

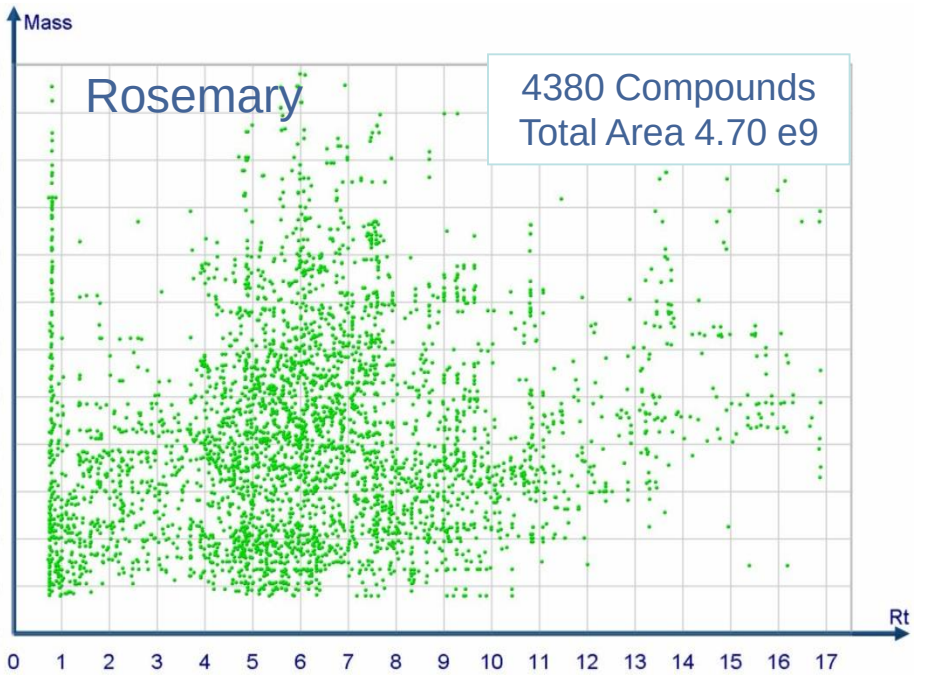


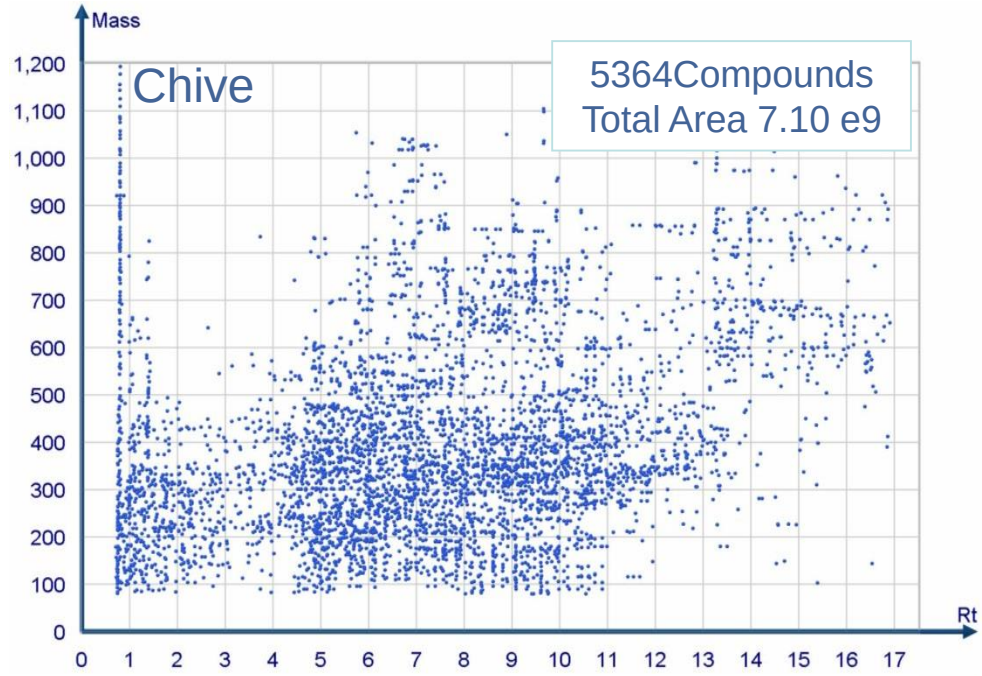
Triazole compounds
Bromuconazole
Cyproconazole
Difenoconazole
Diniconazole
Epoxiconazole
Fenbuconazole
Fluquinconazole
Hexaconazole
Metconazole
Penconazole
Propiconazole
Prothioconazole
Tebuconazole
Tetraconazole
Triticonazole

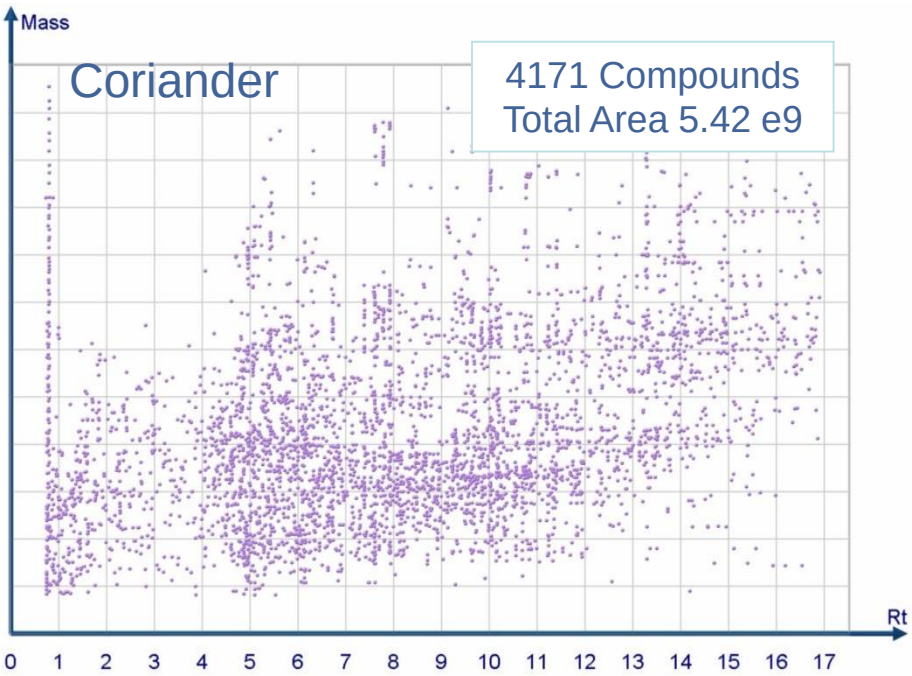
Co-extracted matrix components of Fresh Herbs

Absolute height > 10000 counts LC-TOF-MS









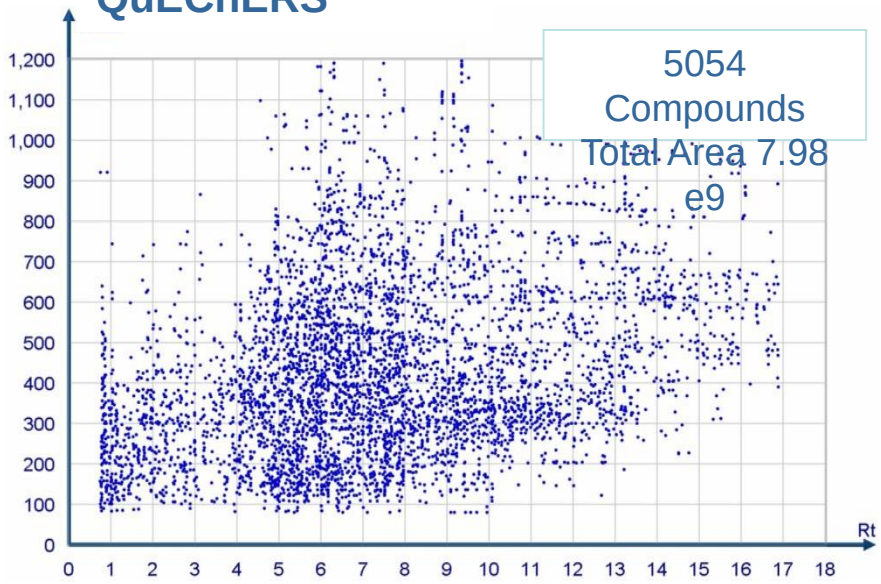
LC-TOF-MS Analysis Full Scan Mode

Matrix	QuEChERS	
	No. compounds	Total Area
Chive	5364	7.10E+09
Mint	5100	8.58E+09
Flat Parsley	5070	6.76E+09
Thyme	5054	7.98E+09
Curly Parsley	4843	5.80E+09
Oregano	4644	5.20E+09
Rosemary	4380	6.00E+09
Basil	4194	5.34E+09
Coriander	4171	6.93E+09
Dill	3574	4.51E+09

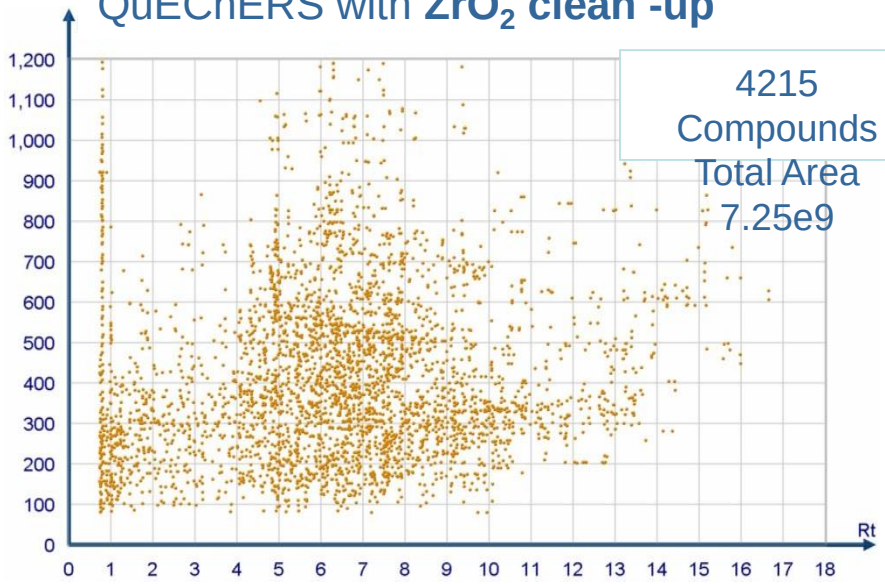
Co-extracted matrix components of Thyme

Absolute height > 10000 counts LC-TOF-MS

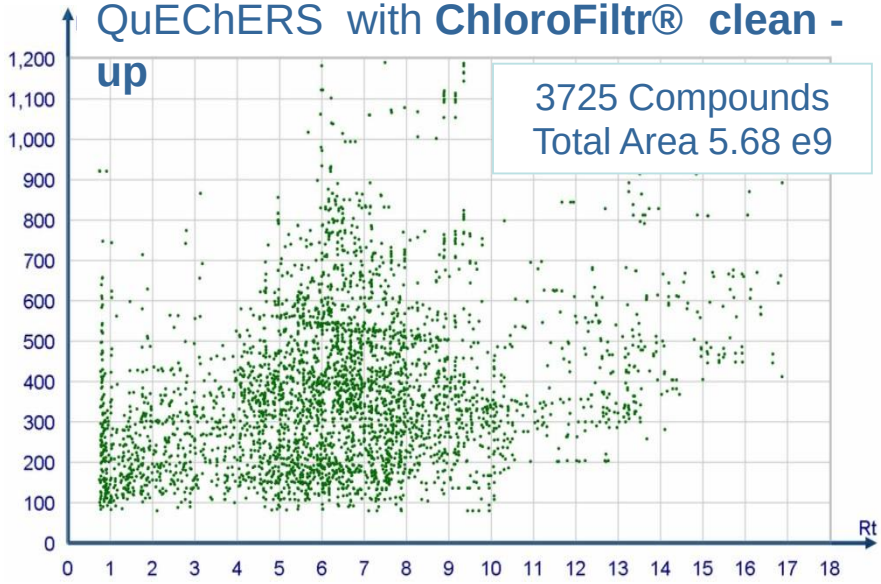
QuEChERS



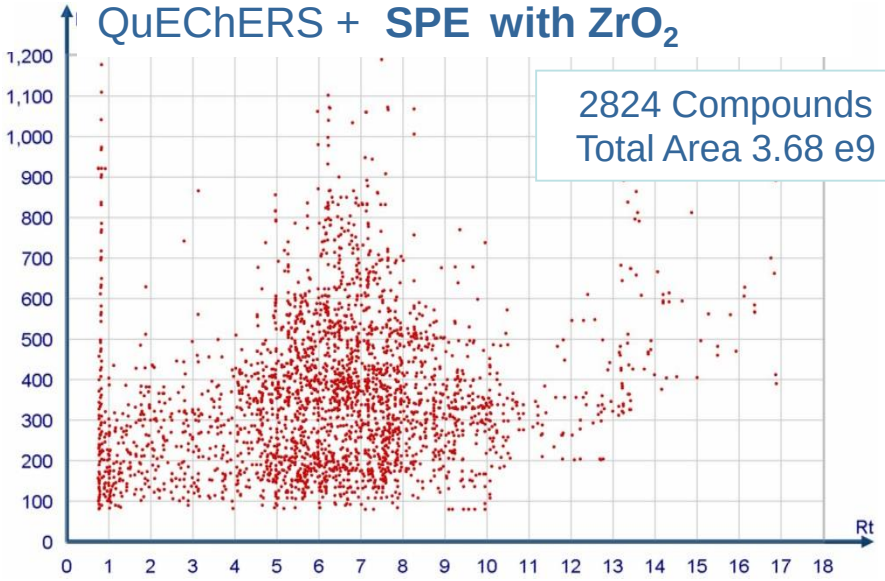
QuEChERS with ZrO_2 clean -up



QuEChERS with ChloroFiltr® clean -up



QuEChERS + SPE with ZrO_2

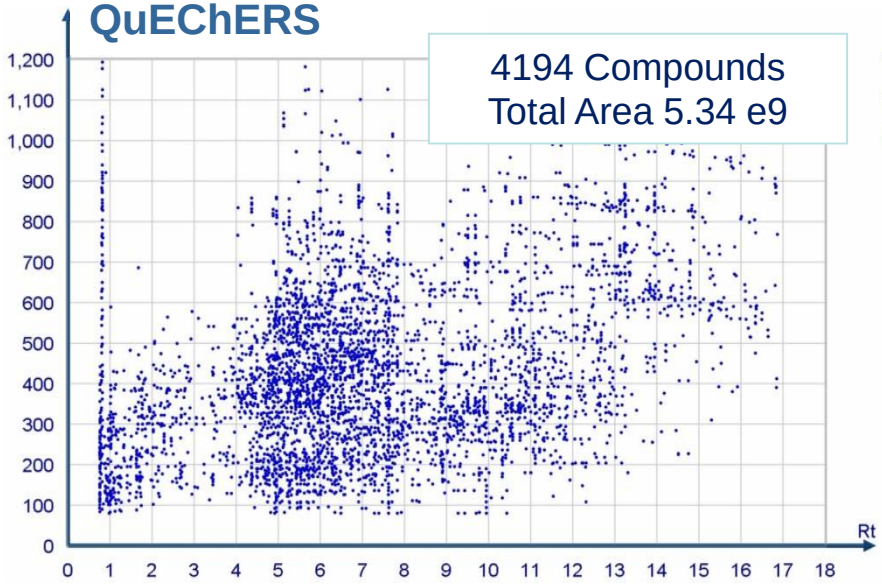


Co-extracted matrix components of Basil

Absolute height > 10000 counts LC-TOF-MS

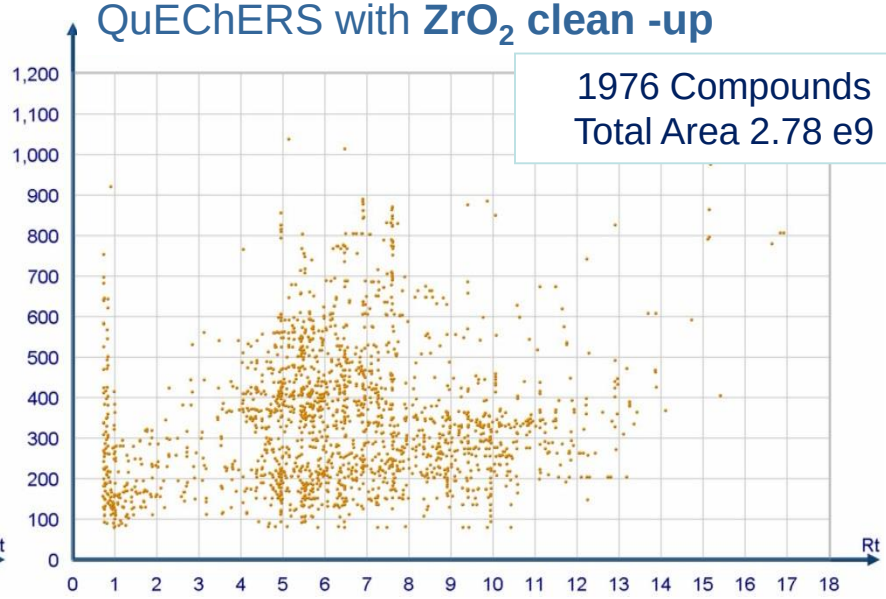
QuEChERS

4194 Compounds
Total Area 5.34 e9



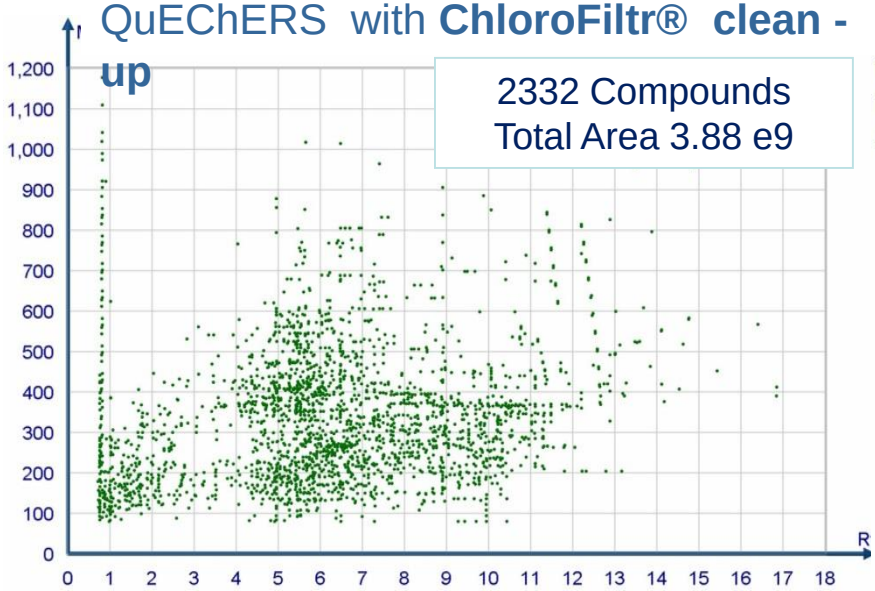
QuEChERS with ZrO_2 clean -up

1976 Compounds
Total Area 2.78 e9



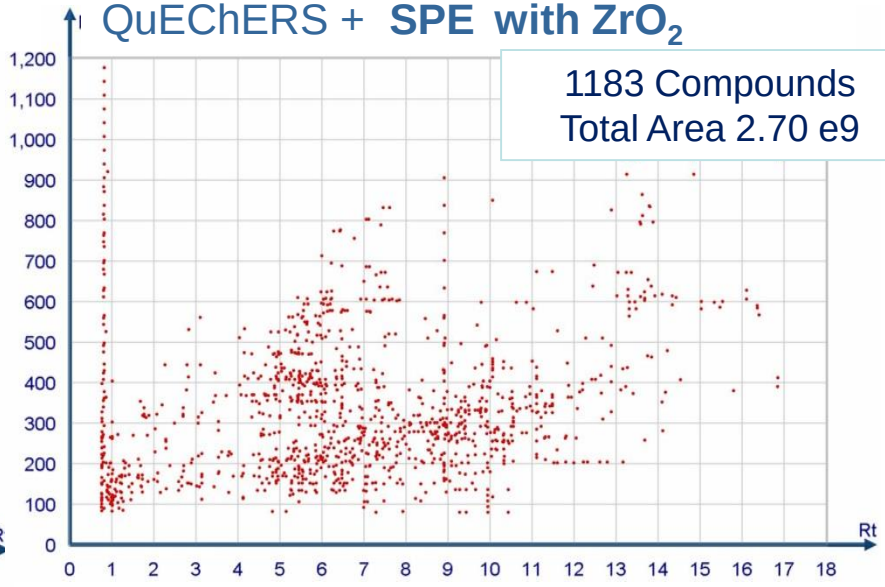
QuEChERS with ChloroFiltr® clean -up

2332 Compounds
Total Area 3.88 e9



QuEChERS + SPE with ZrO_2

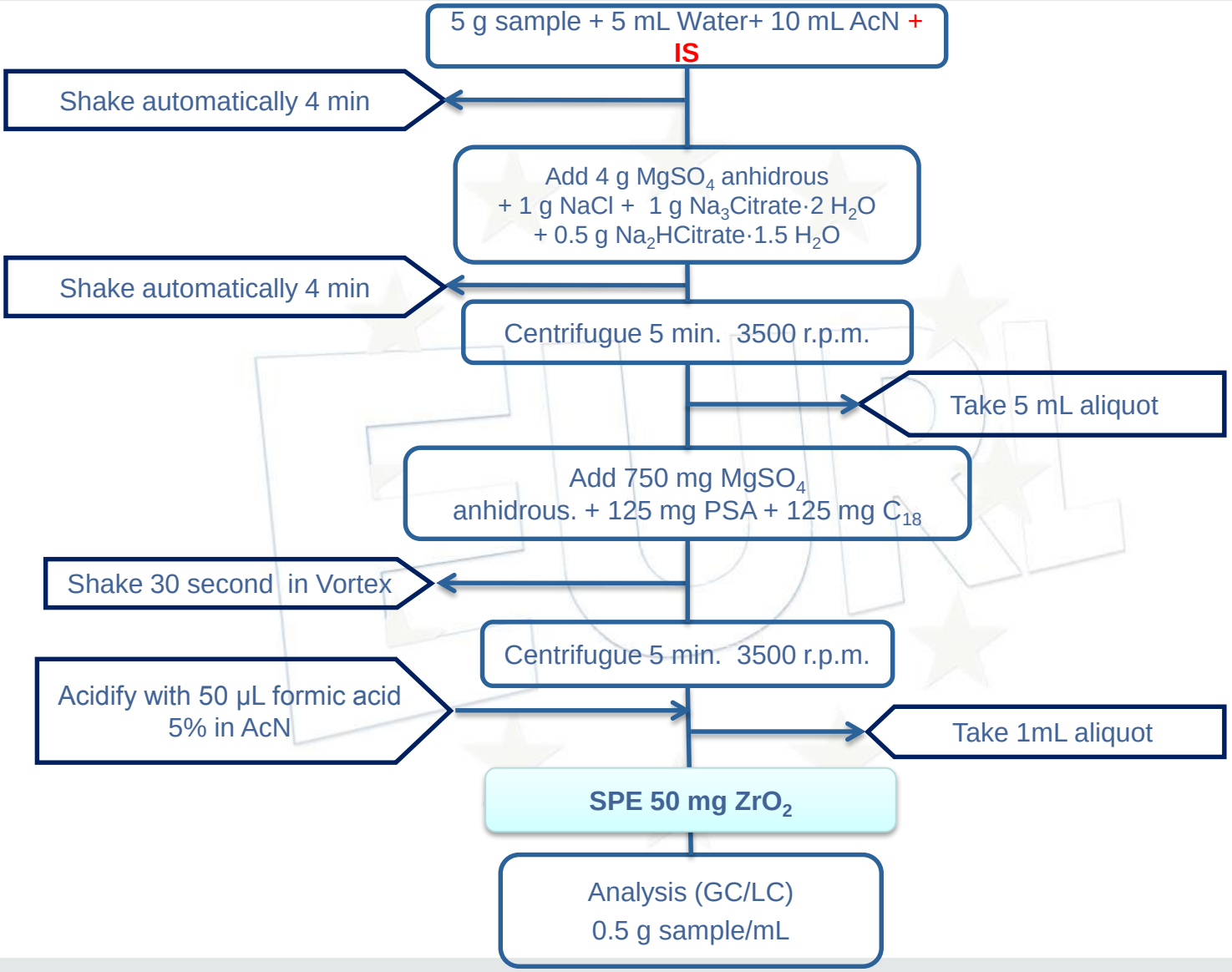
1183 Compounds
Total Area 2.70 e9



LC-TOF-MS Analysis Full Scan Mode

Matrix	QuEChERS		QuEChERs + SPE ZrO_2		QuEChERS (ZrO_2 instead PSA and C18)		QuEChERS ChloroFiltr® clean-up	
	Nº compounds	Total Area	Nº compounds	Total Area	Nº compounds	Total Area	Nº compounds	Total Area
Chive	5364	7.10E+09	3820	5.17E+09	4906	7.57E+09	4431	5.91E+09
Mint	5100	8.58E+09	2365	3.36E+09	4614	7.638E+09	3305	4.475E+09
Flat Parsley	5070	7.44E+09	2955	4.14E+09	5014	7.32E+09	4085	5.341E+09
Thyme	5054	7.98E+09	2824	3.68E+09	4215	7.25E+09	3725	5.681E+09
Curly Parsley	4843	5.80E+09	2136	3.03E+09	3485	5.13E+09	3538	4.907E+09
Oregano	4644	5.20E+09	1741	2.22E+09	3016	4.831E+09	2821	3.771E+09
Rosemary	4380	6.00E+09	3030	3.97E+09	4293	6.685E+09	4377	5.676E+09
Basil	4194	5.34E+09	1183	2.69E+09	1976	2.779E+09	2332	3.876E+09
Coriander	4171	6.93E+09	2224	3.88E+09	4041	6.593E+09	3546	6.136E+09
Dill	3574	4.51E+09	1428	2.29E+09	2682	3.842E+09	2388	3.664E+09

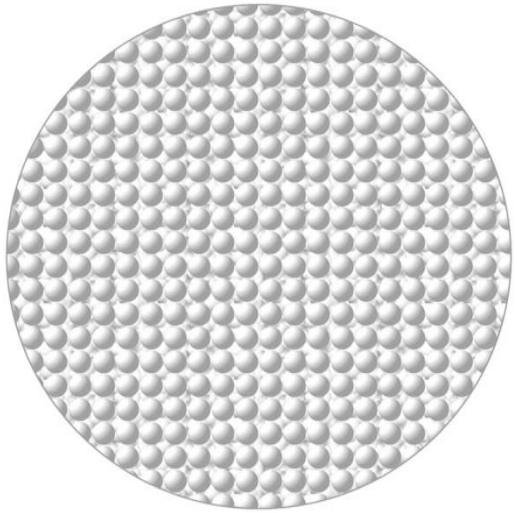
QuEChERS Extraction Method



1mL sample + 50 mg ZSEP

Cartridge format

Disc format



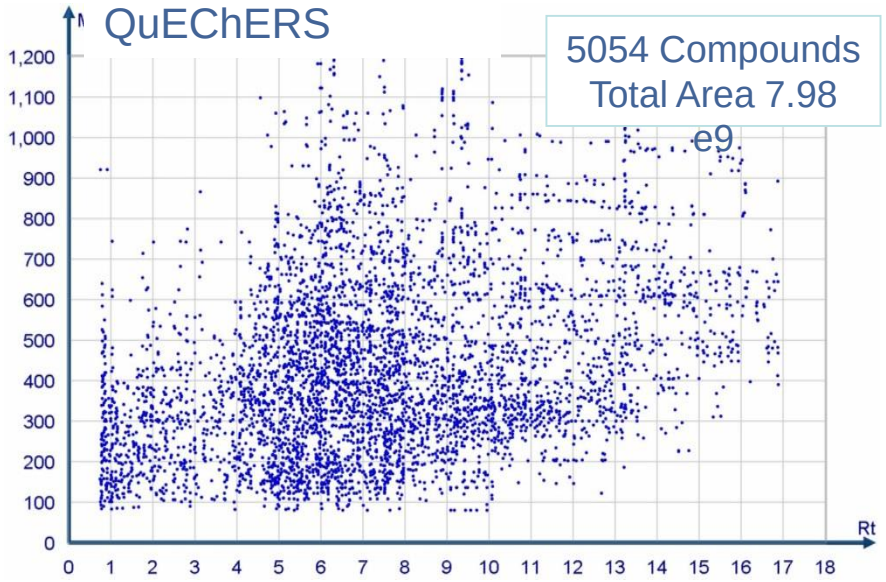
Particle size: 22 µm= 22000 nm

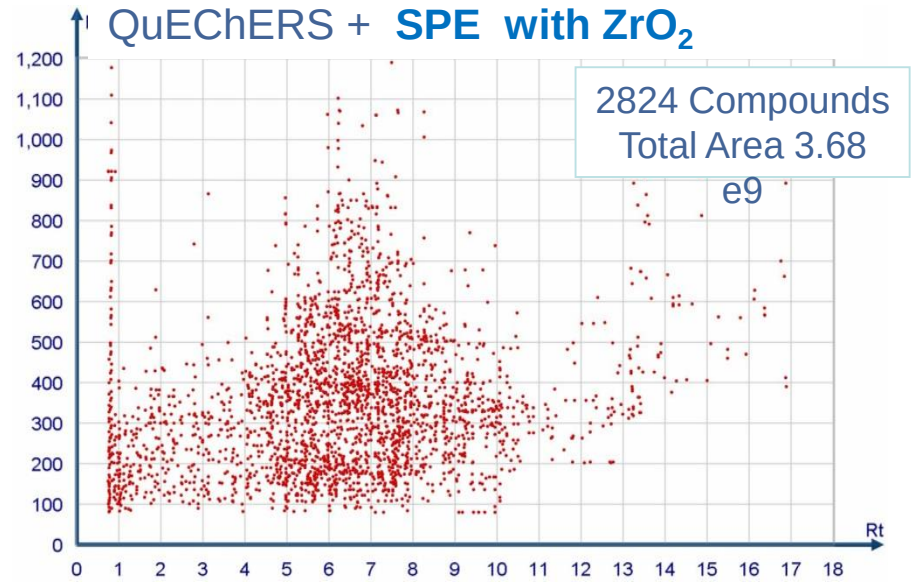
Relative surface area: 310 m2/g

Matrix	QuEChERS		QuEChERs + SPE ZrO_2	
	No. compounds	Total Area	No. compounds	Total Area
Chive	5364	7.10E+09	3820	5.17E+09
Mint	5100	8.58E+09	2365	3.36E+09
Flat Parsley	5070	6.76E+09	2955	4.14E+09
Thyme	5054	7.98E+09	2824	3.68E+09
Curly Parsley	4843	5.80E+09	2136	3.03E+09
Oregano	4644	5.20E+09	1741	2.22E+09
Rosemary	4380	6.00E+09	3030	3.97E+09
Basil	4194	5.34E+09	1183	2.69E+09
Coriander	4171	6.93E+09	2224	3.88E+09
Dill	3574	4.51E+09	1428	2.29E+09
Orange	4418	6.15E+09	1958	3.91E+09
Tea (Dil 1/5)	4576	9.19E+09	3982	6.49E+09
Tomato	2833	5.28E+09		
Lettuce	1586	3.42E+09		

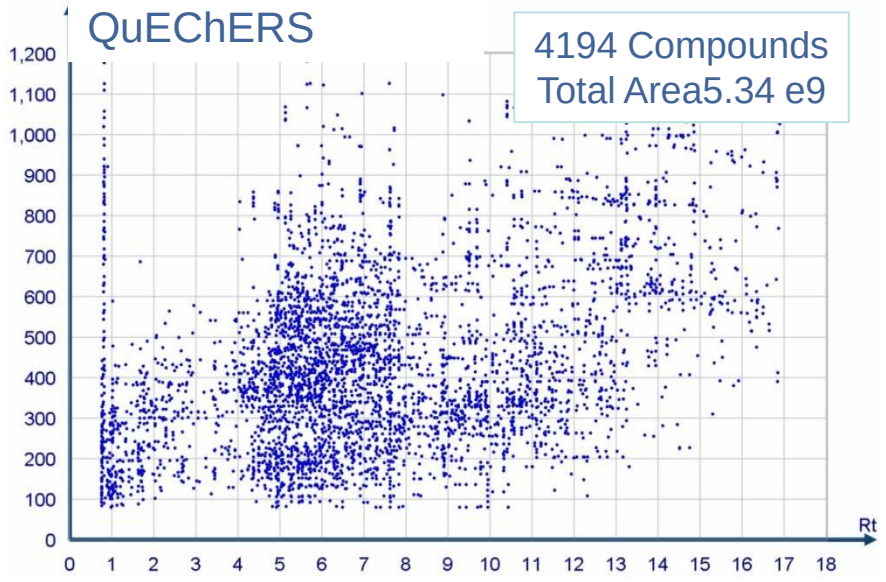
Co-extracted matrix components
Absolute height > 10000 counts LC-TOF-MS

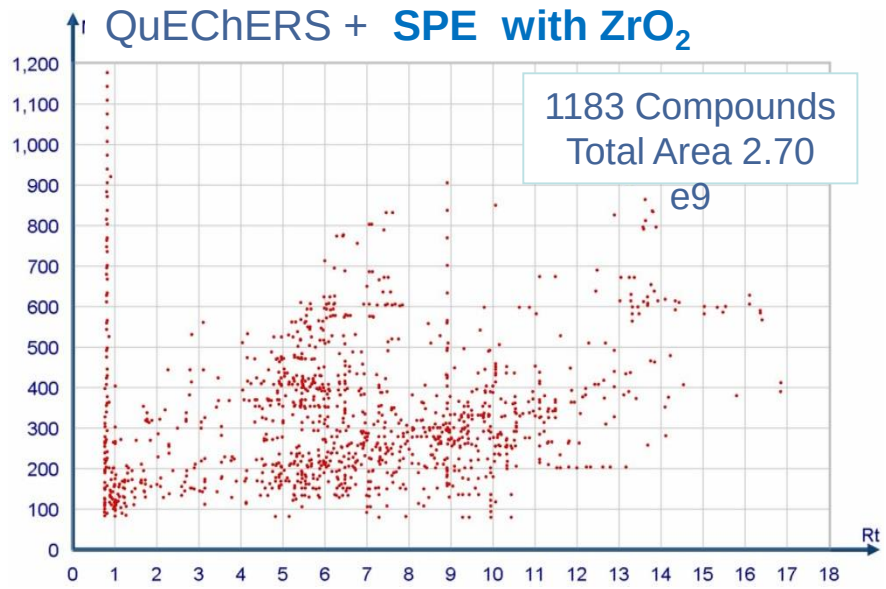
Thyme





Basil



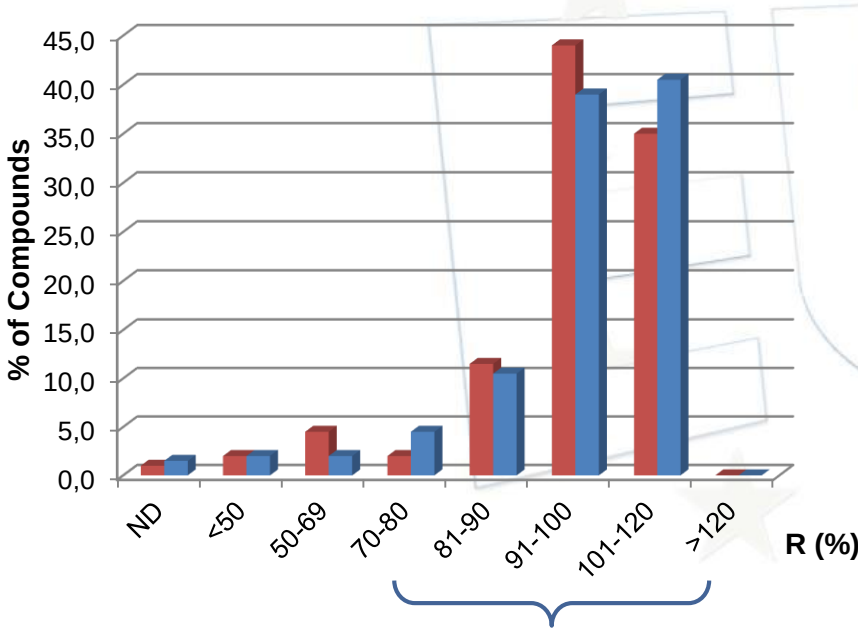


GC (203 Pesticides)

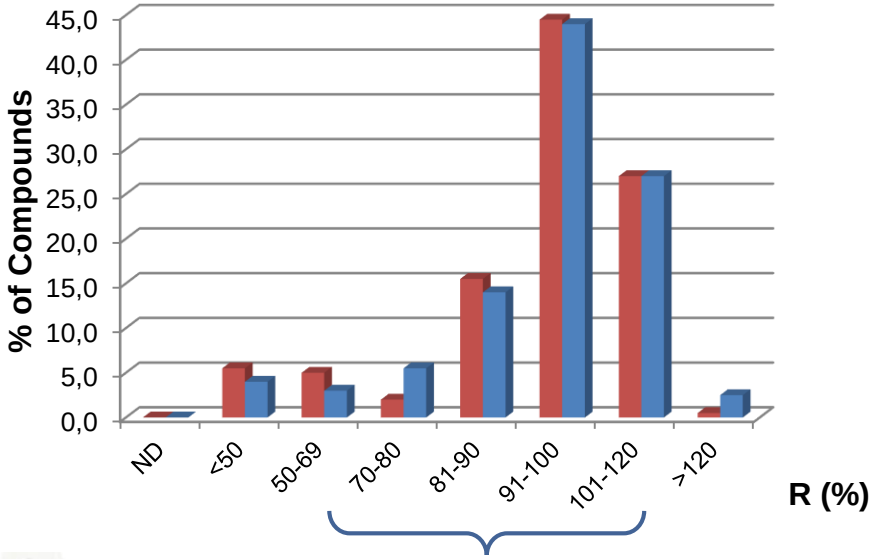
RECOVERIES at 50 µg/kg QuEChERS (+ZrO_2)

QuEChERS
QuEChERS + SPE ZrO2

Thyme



Basil

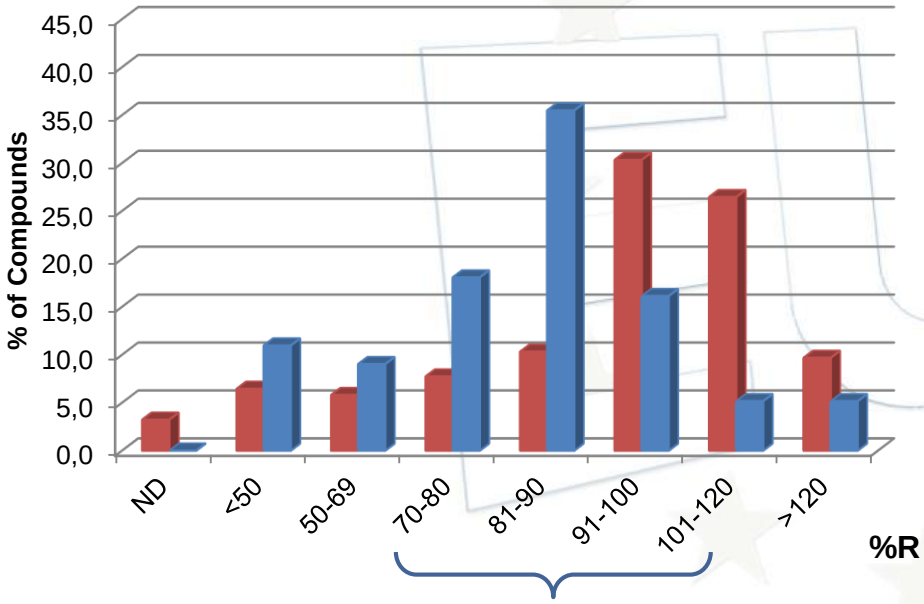


LC (155 Pesticides)

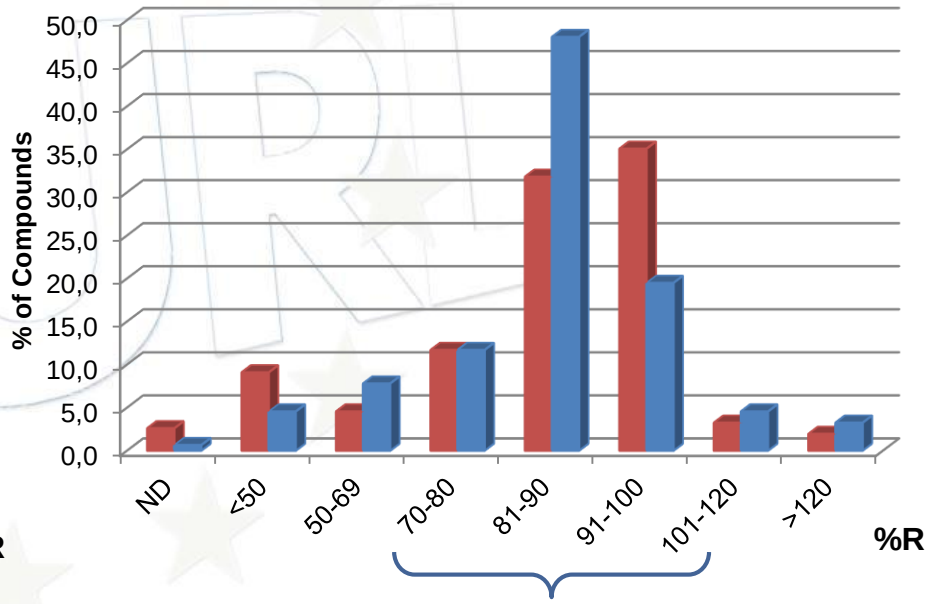
RECOVERIES at 50 µg/kg
QuEChERS (+ZrO_2)

QuEChERS
QuEChERS + SPE ZrO2

Thyme

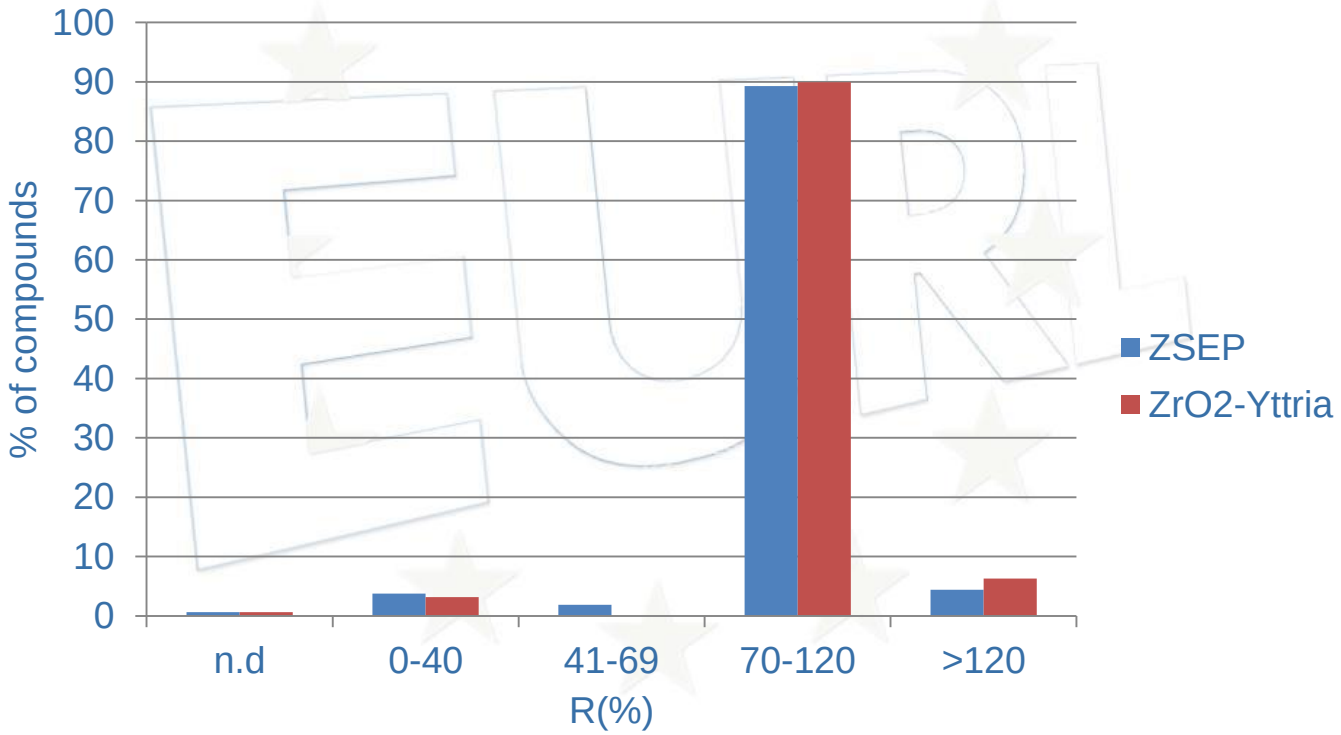


Basil

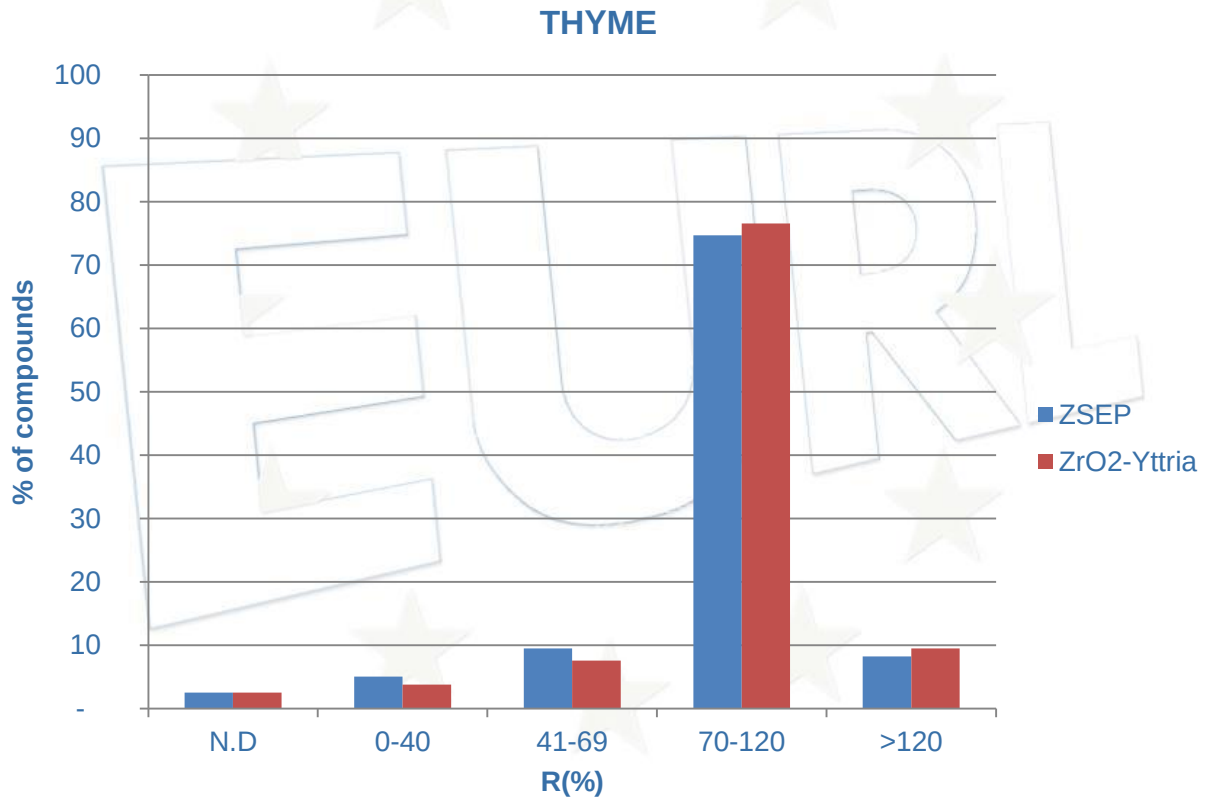


Recoveries at 50 µg/Kg
1 mL spiked orange extract + 40 mg ZSEP/ZrO2-Yttria cartidges
(conditioning cartidge with 1 mL AcN)

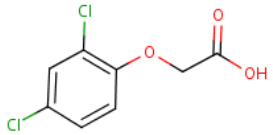
ORANGE



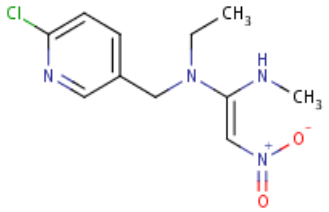
Recoveries at 50 µg/Kg
1 mL spiked thyme extract + 40 mg ZSEP/ZrO2-Yttria cartidges
(conditioning cartidge with 1 mL AcN)



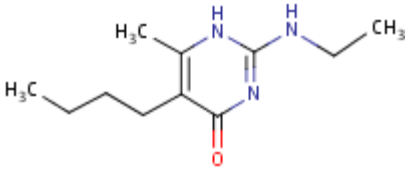
2,4-D
(0% ZSEP, ZrO2-Yttria)



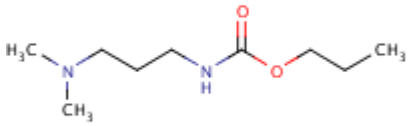
Nitenpyram
(0% ZSEP)



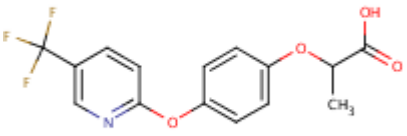
Ethirimol
(1% ZSEP)



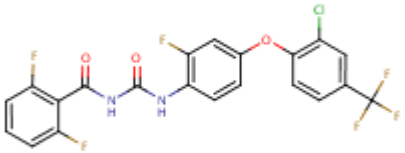
Propamocarb
(15% ZSEP)



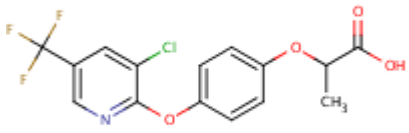
Fluazifop
(1% ZSEP, ZrO2-Yttria)



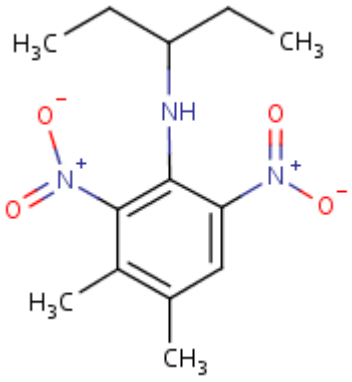
Flufenoxuron
(40% ZSEP, ZrO2-Yttria)



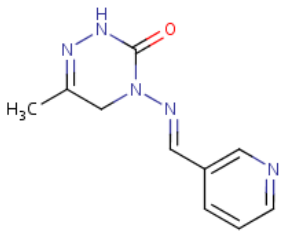
Haloxyfop
(0% ZSEP, ZrO2-Yttria)



Pendimethalin
(15% ZSEP, ZrO2-Yttria)

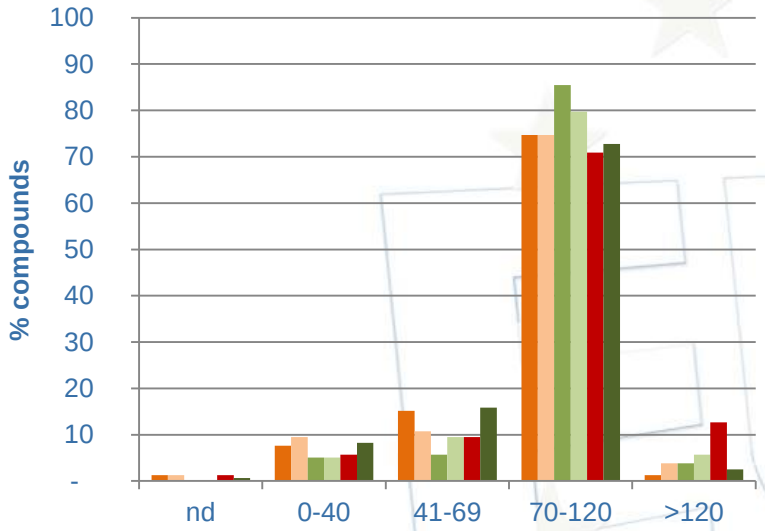


Pymetrozine
(40% ZSEP, ZrO2-Yttria)

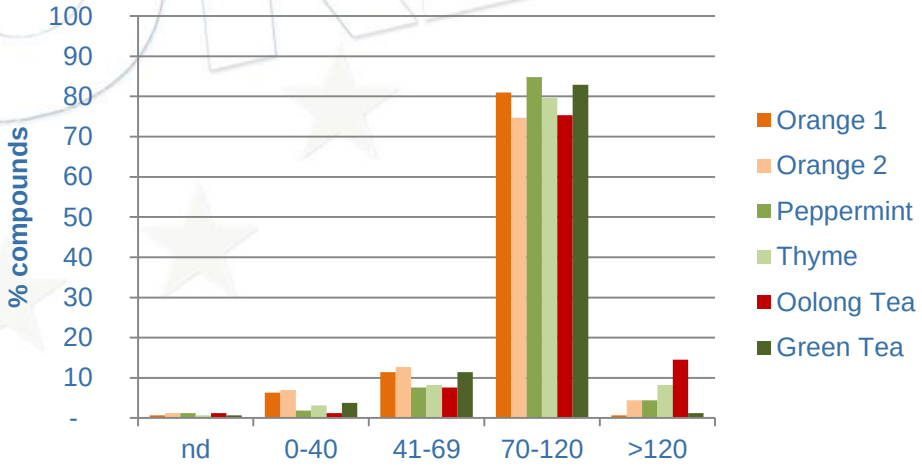


Recoveries at 50 µg/Kg
1 mL spiked extract + 40 mg ZSEP/ZrO2-Yttria cartidges
(conditioning cartidge with 1 mL AcN)

ZSEP



ZrO2-Yttria



Thank You
for Your Attention

EURL EUROPEAN UNION REFERENCE LABORATORY