EVALUATION OF ANALYTICAL PROCEDURES IN PESTICIDE MULTIRESIDUE METHODS TO OVERCOME MATRIX EFFECTS IN FRUITS AND VEGETABLES.

European Commission
EURL-FV
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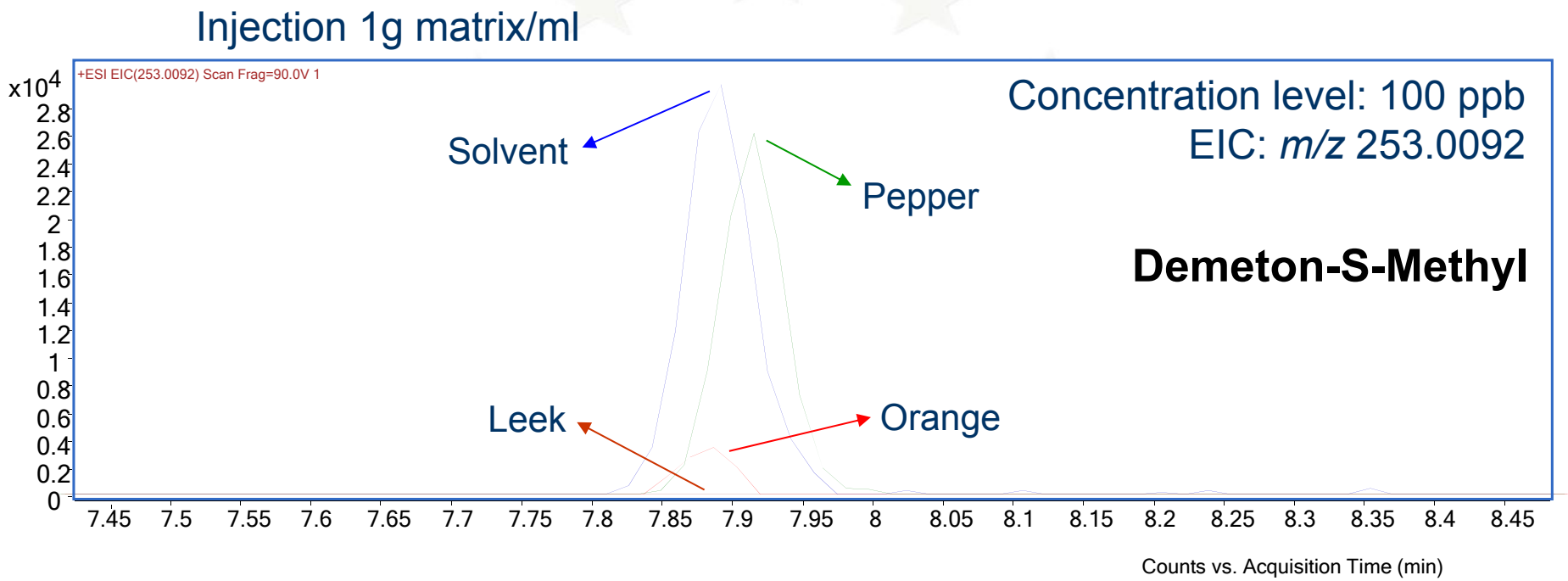
HOW MATRIX EFFECTS AFFECTS TO GC AND LC-MS ANALYSIS?

-Signal changes

-Abundance of specific fragment ions/trans

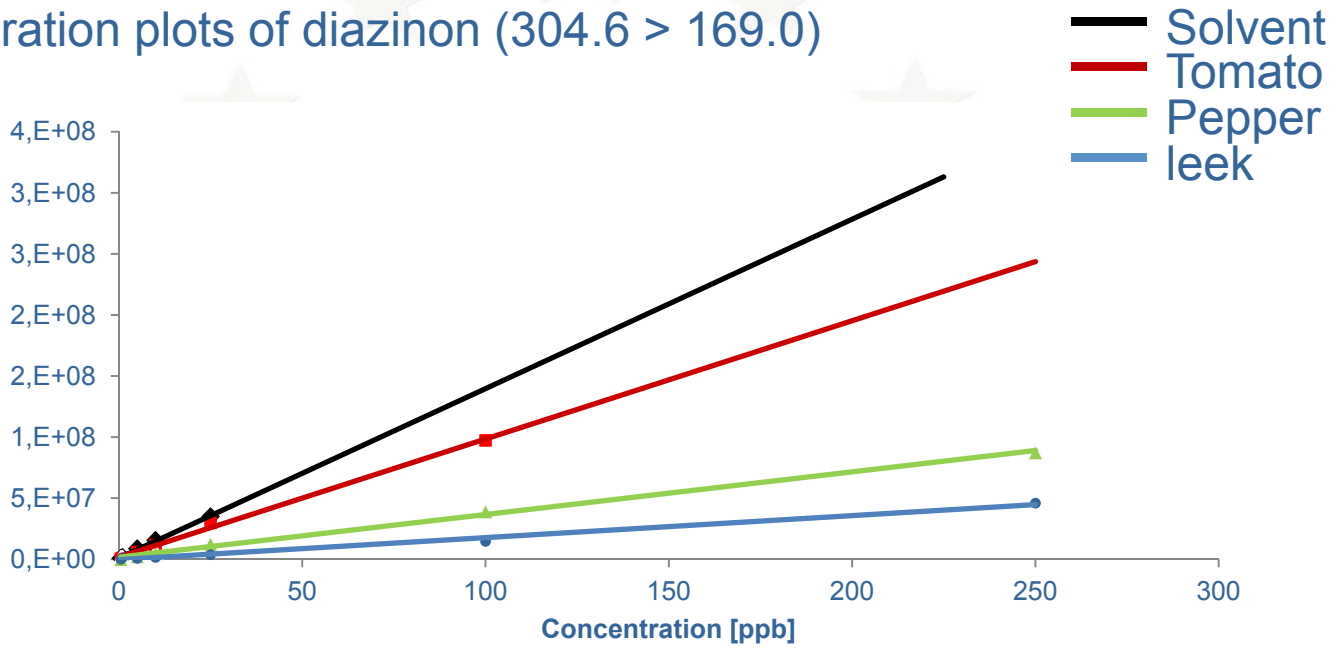
-Instrument maintenance

Signal supression due to matrix effects



LC-MS/MS

Calibration plots of diazinon (304.6 > 169.0)

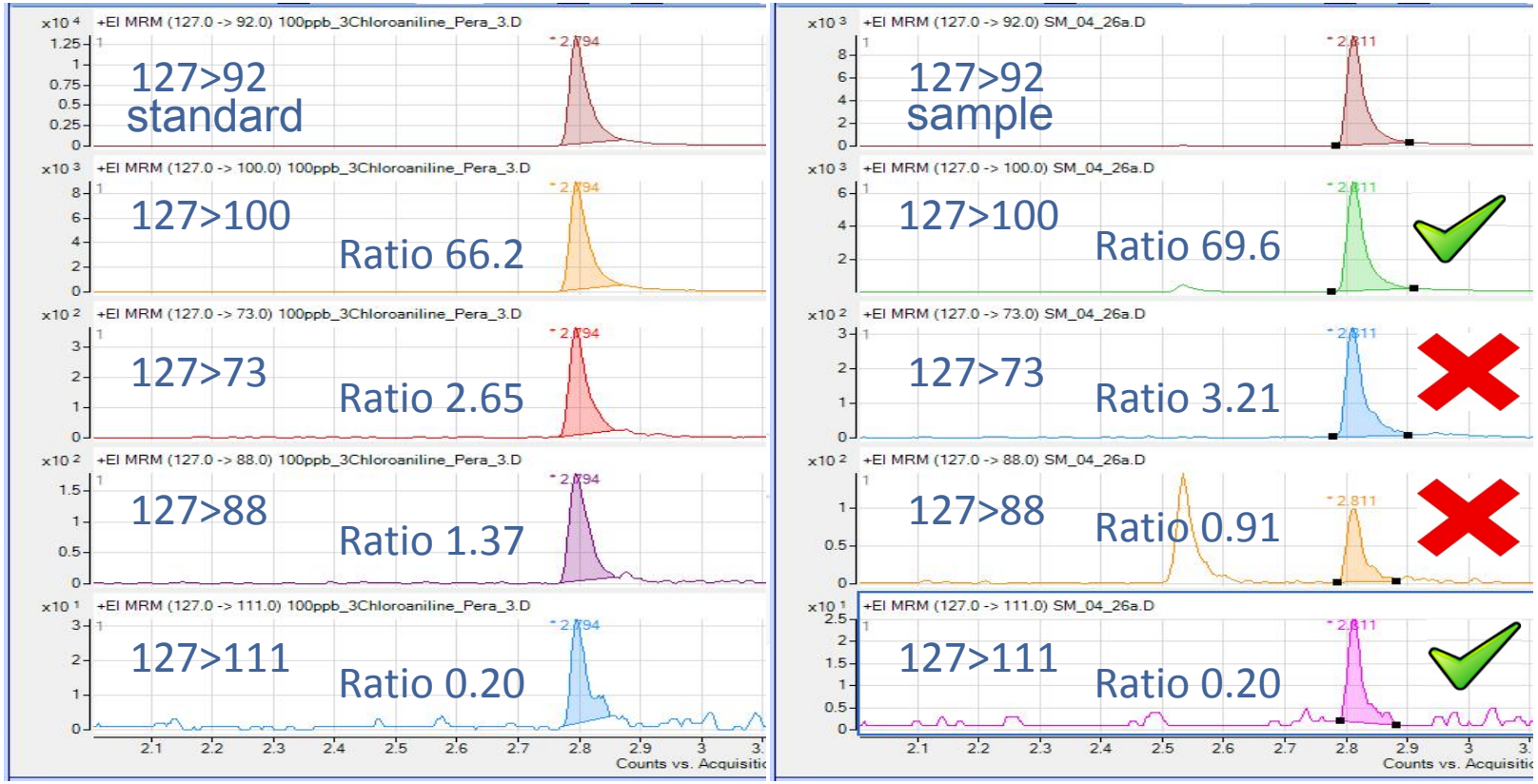


3-Chloraniline

100 ppb Pear
RT = 2.79 min

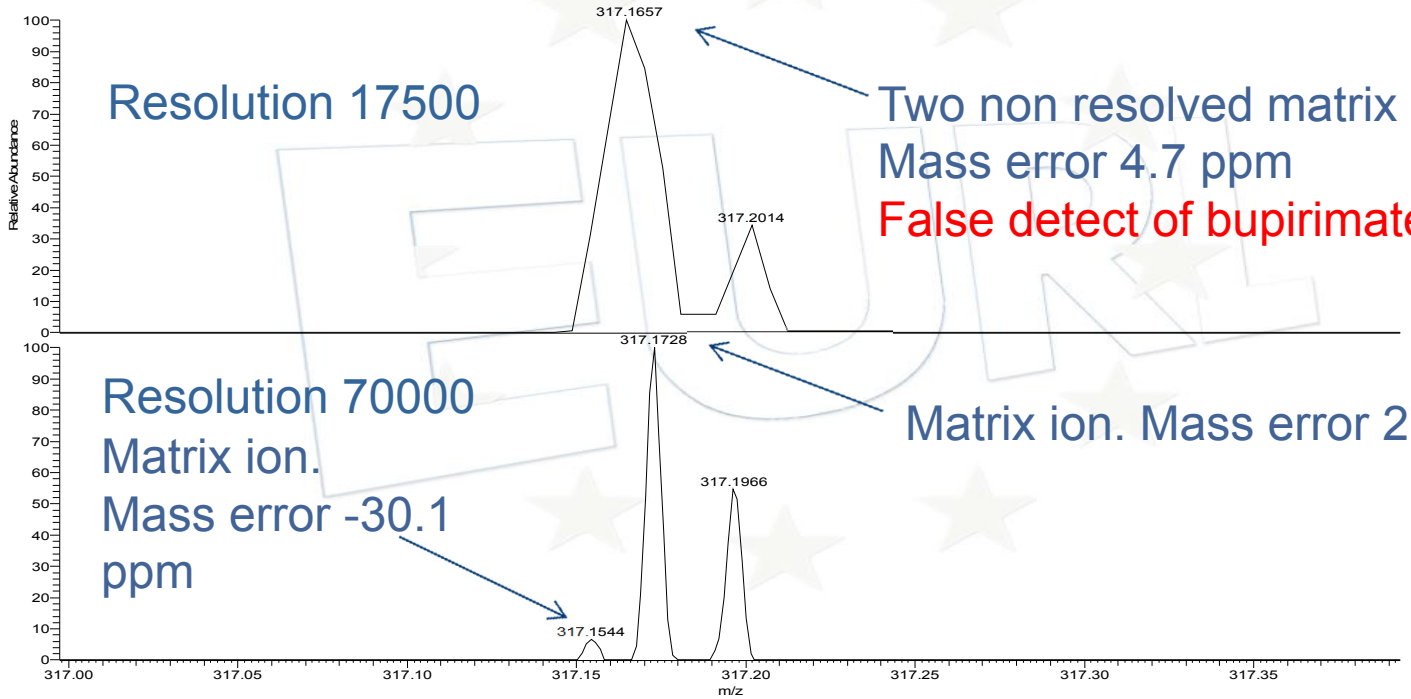
SM-04 (67 ppb?)
RT = 2.81 min

GC-QQQ-MS/MS

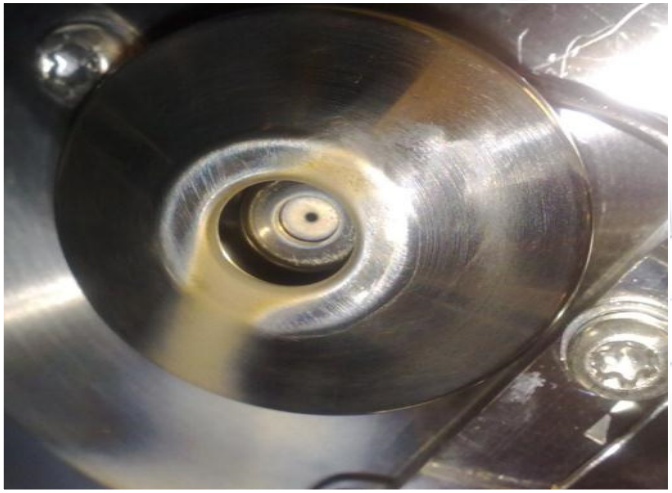


Influence of resolution on detection

Exact mass of bupirimate 317.1642



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



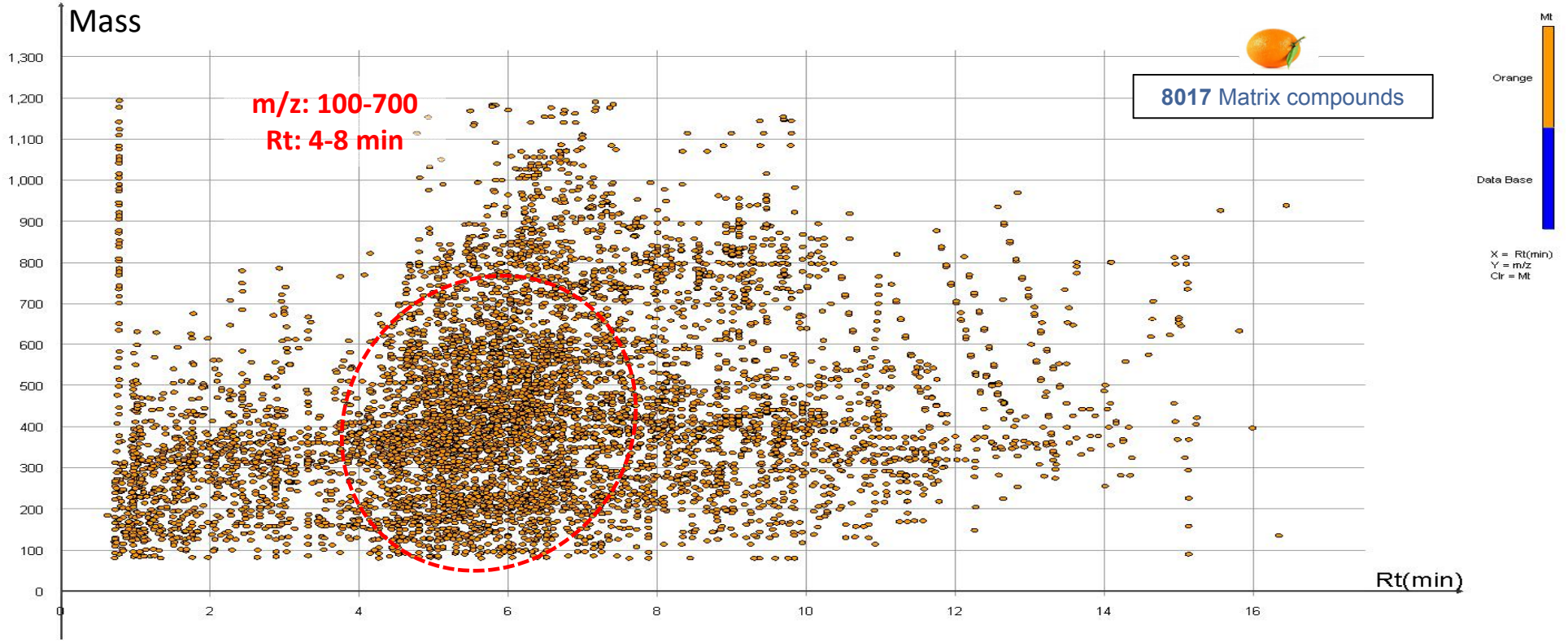
GC-QqQ-MS Liner after 30 tea injections

MATRIX STUDY

mapping of natural components by

LC-TOF-MS ANALYSIS FULL SCAN MODE

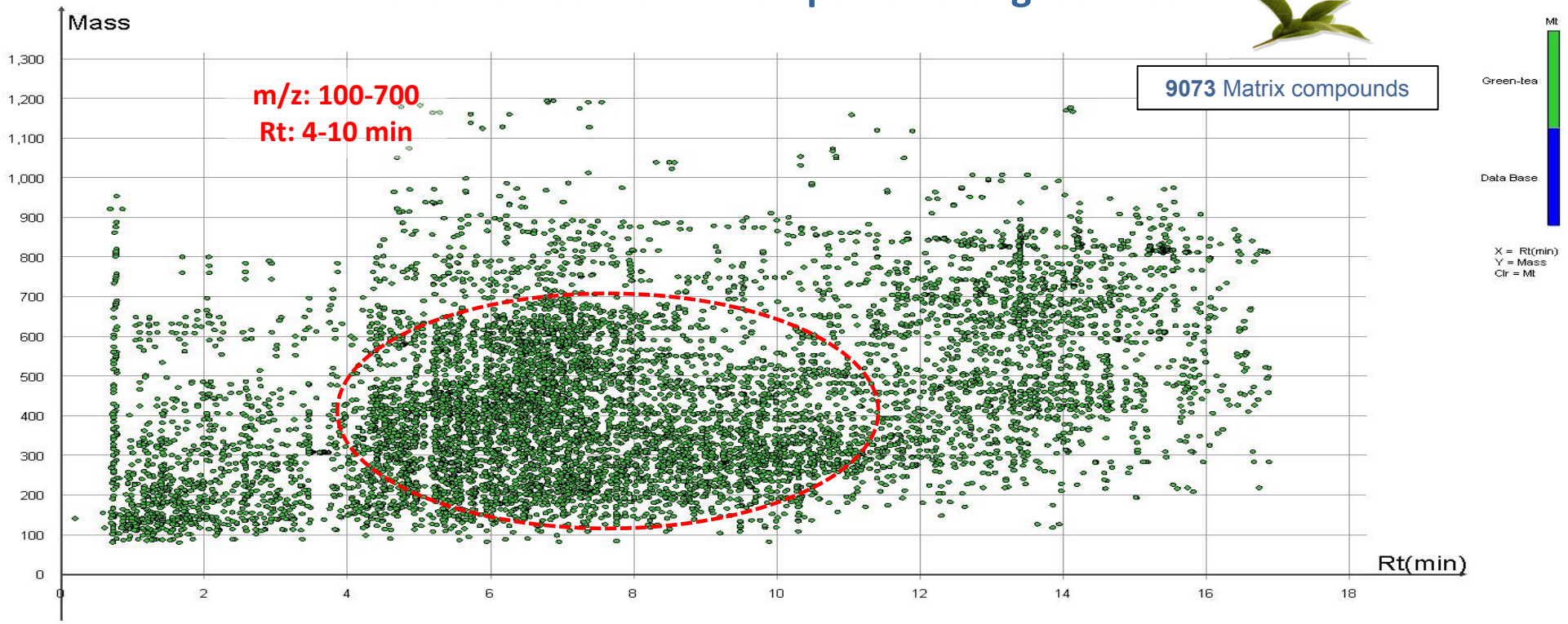
Co-extracted matrix components LC-TOF-MS of orange



Miner 3D Enterprise

MATRIX EFFECTS

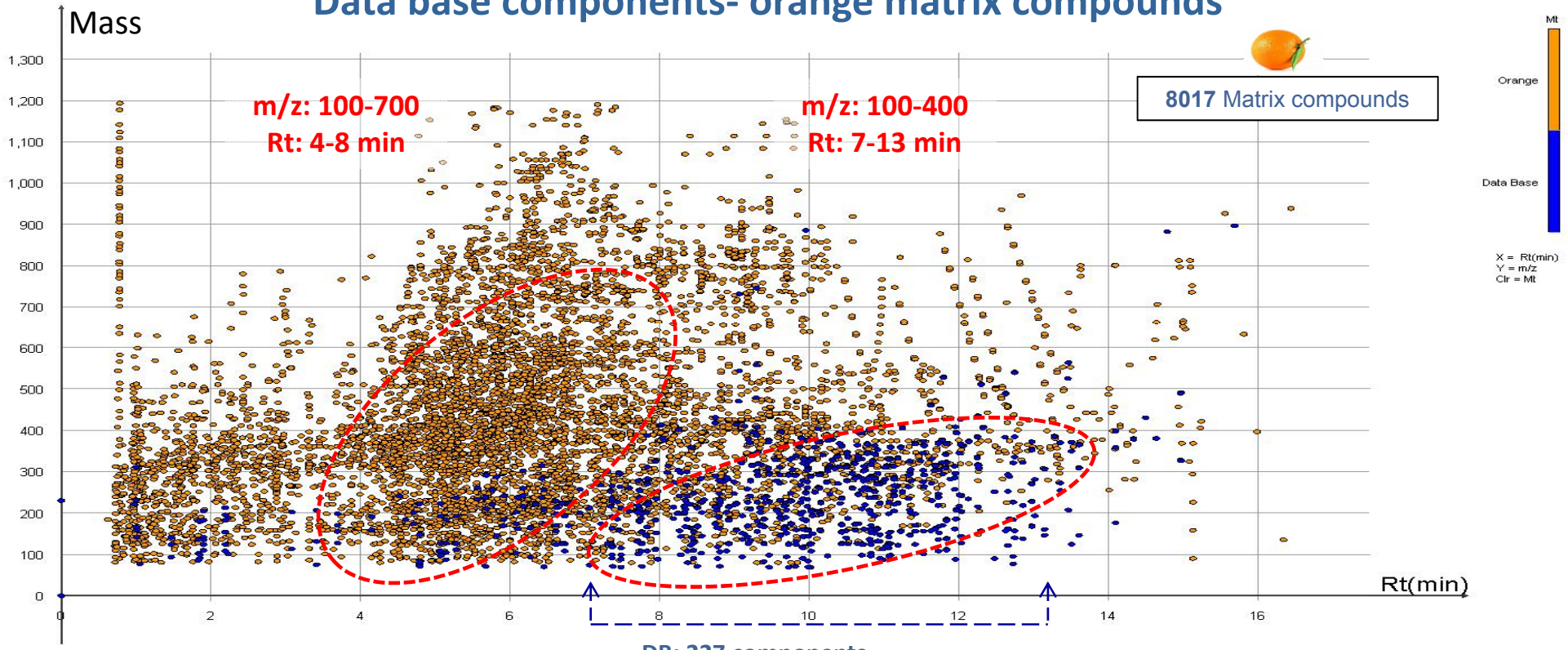
Co-extracted matrix components of green-tea



Miner 3D Enterprise

MATRIX EFFECTS

Data base components- orange matrix compounds



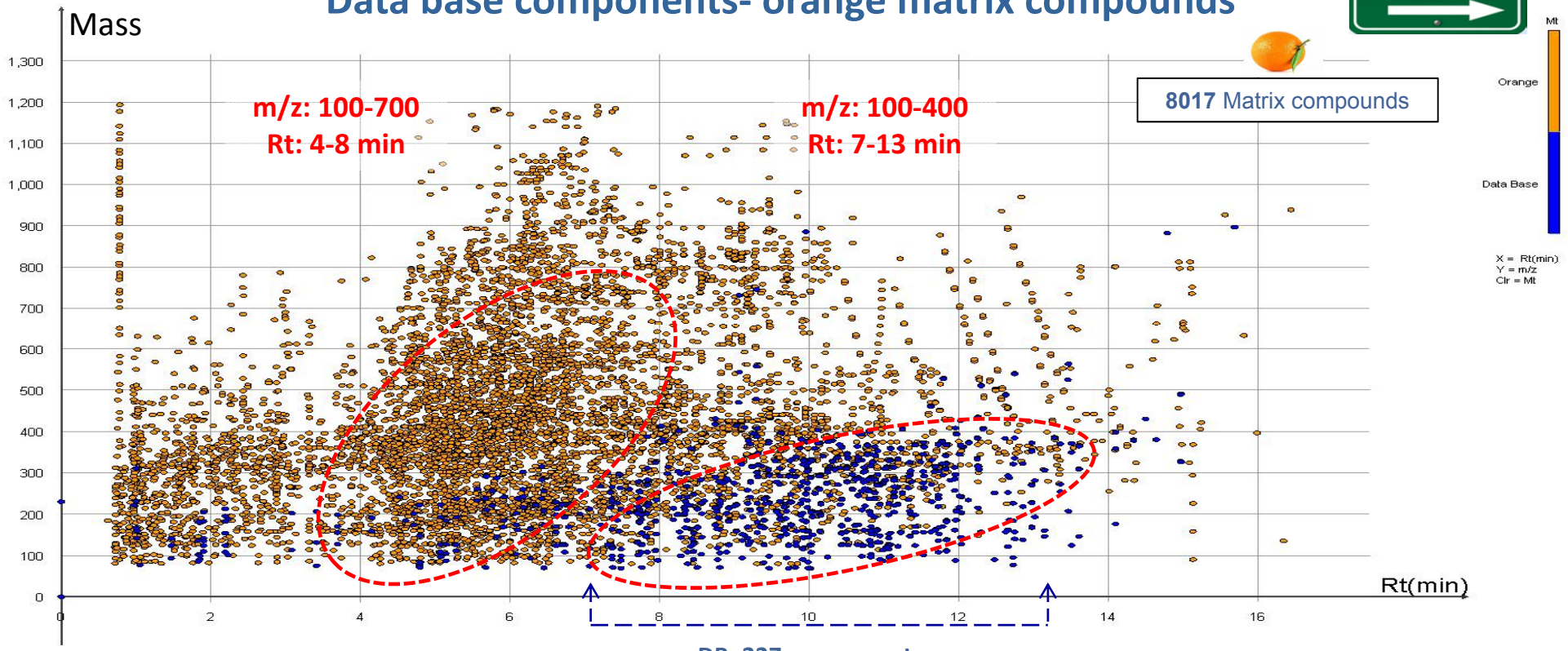
Miner 3D Enterprise

DB: 227 components
Orange: 2743 matrix compounds

How can we control or avoid them?

Data base components- orange matrix compounds

Highly Sensitive LC-MS Analysis

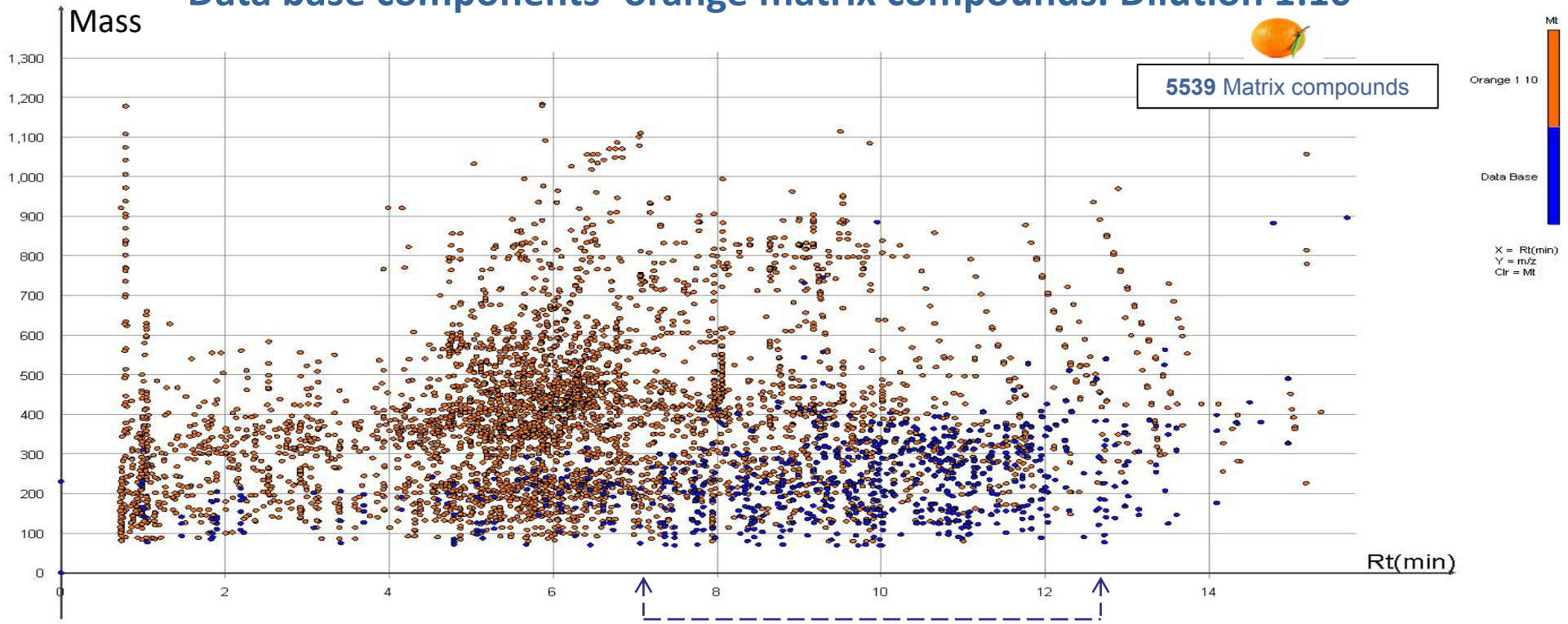


Miner 3D Enterprise

DB: 227 components
Orange: 2743 matrix compounds

Highly Sensitive LC-MS Analysis

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

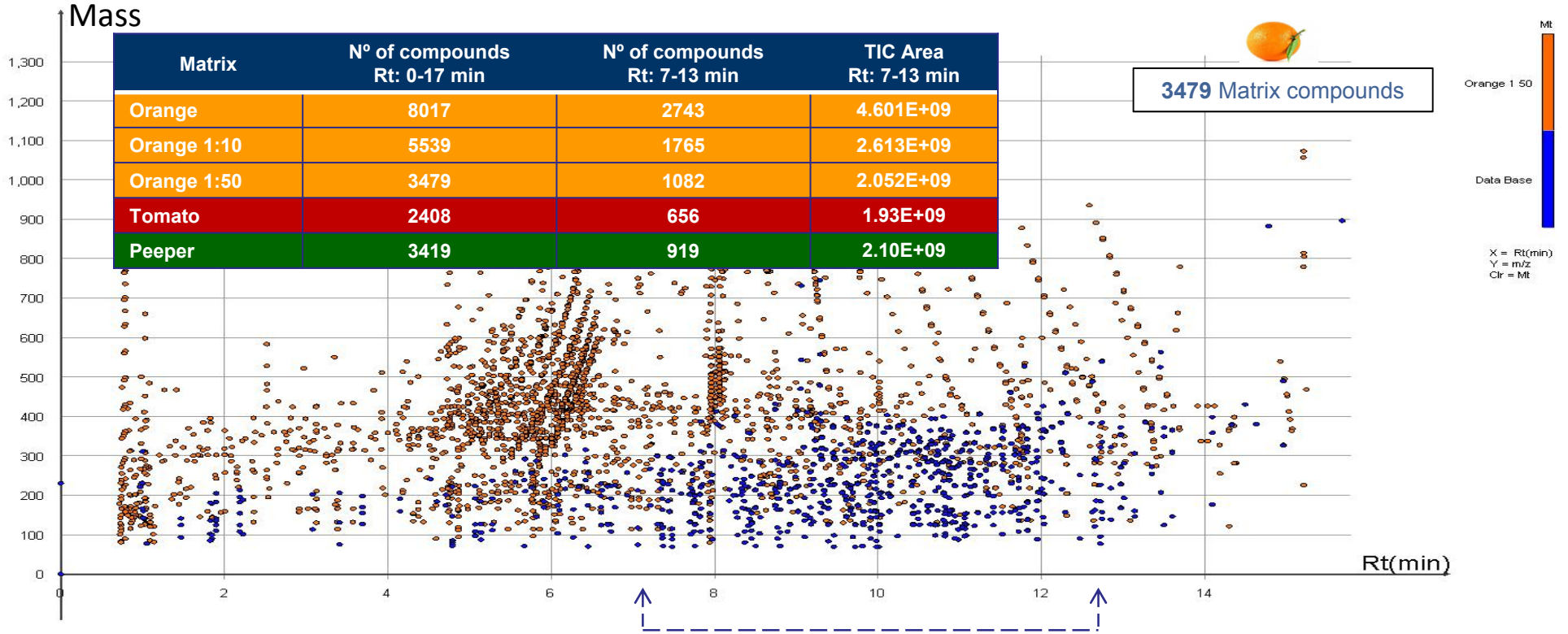


DB: 227 components
Orange: 1765 matrix compounds

Miner 3D Enterprise

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

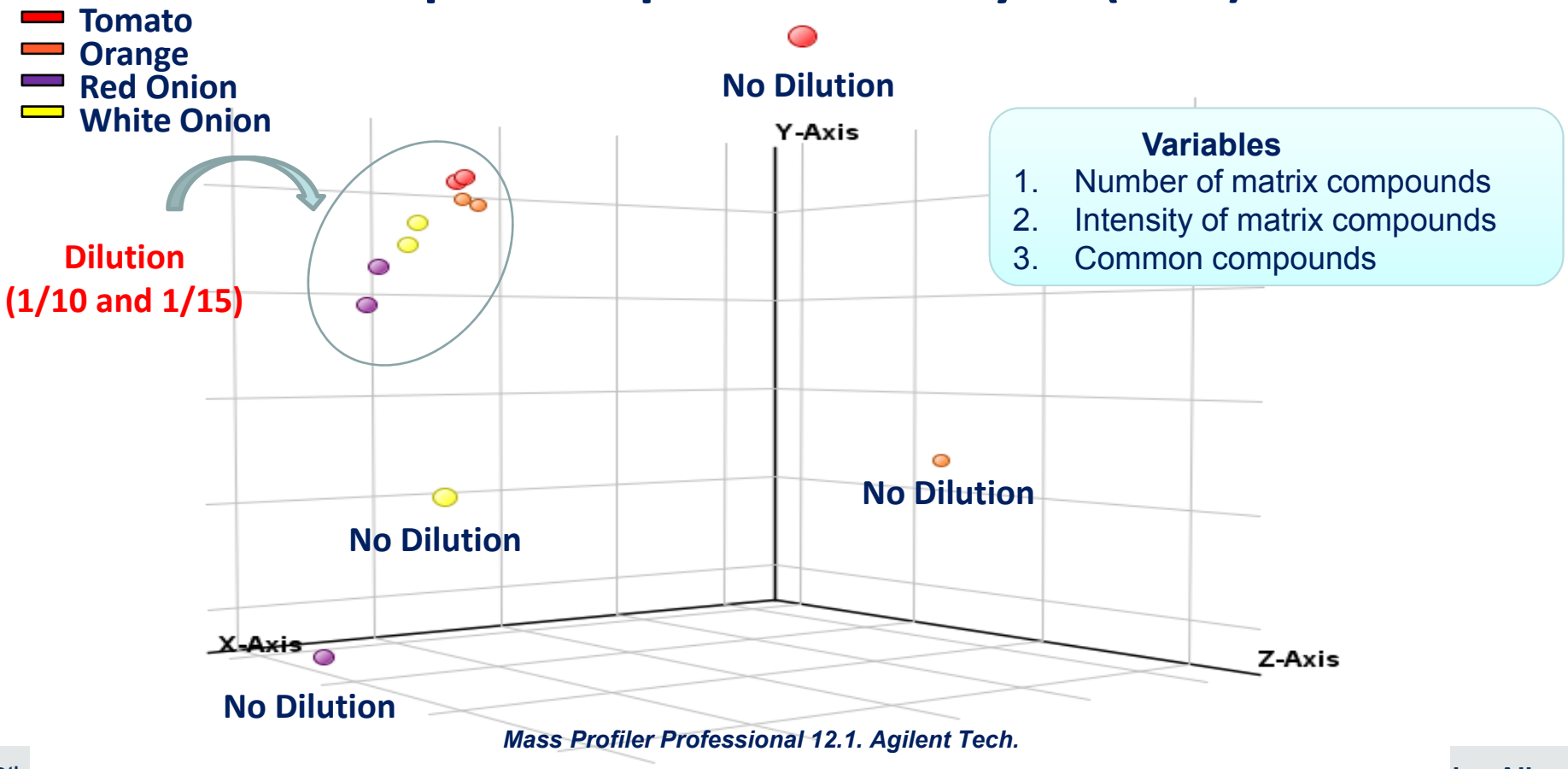
Matrix	Nº of compounds Rt: 0-17 min	Nº of compounds Rt: 7-13 min	TIC Area Rt: 7-13 min
Orange	8017	2743	4.601E+09
Orange 1:10	5539	1765	2.613E+09
Orange 1:50	3479	1082	2.052E+09
Tomato	2408	656	1.93E+09
Peeper	3419	919	2.10E+09



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

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

Linuron-d_6: Dilution

Dimethoate-d_6: Injection

microLC-QqQ-MS/MS

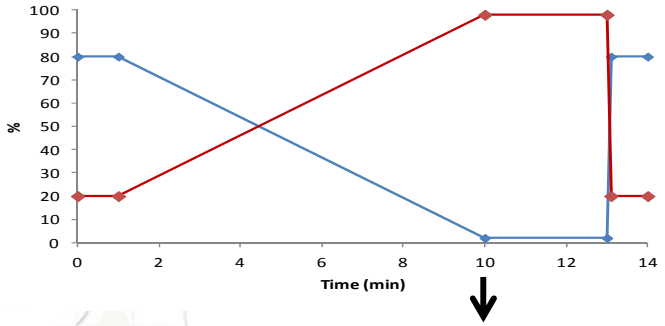
Liquid chromatography

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

QqQ-MS

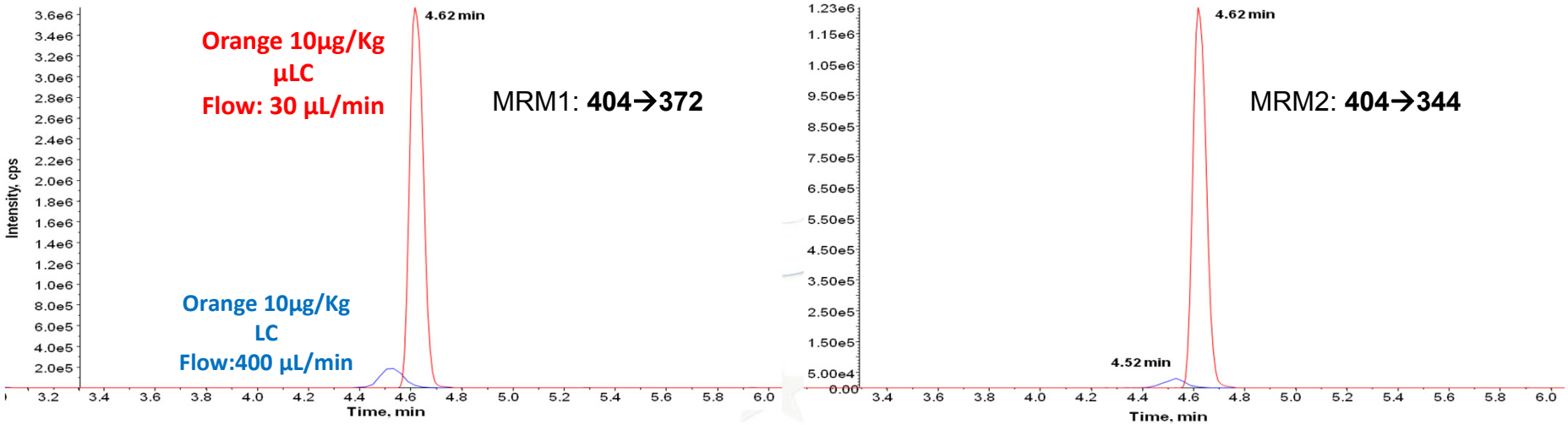
- Source: ESI (+) with microFlow electrode
- Pesticide Identification: 2 transitions
- Source Parameters:
  - ✓ Nebulizer and collision gas: nitrogen
  - ✓ Collision gas (CAD): Medium
  - ✓ Gas temperature: 300ºC
  - ✓ Curtain gas (CUR): 20
  - ✓ IonSpray Voltaje (IS): 5000V
  - ✓ Schedule MRM software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

Gradient



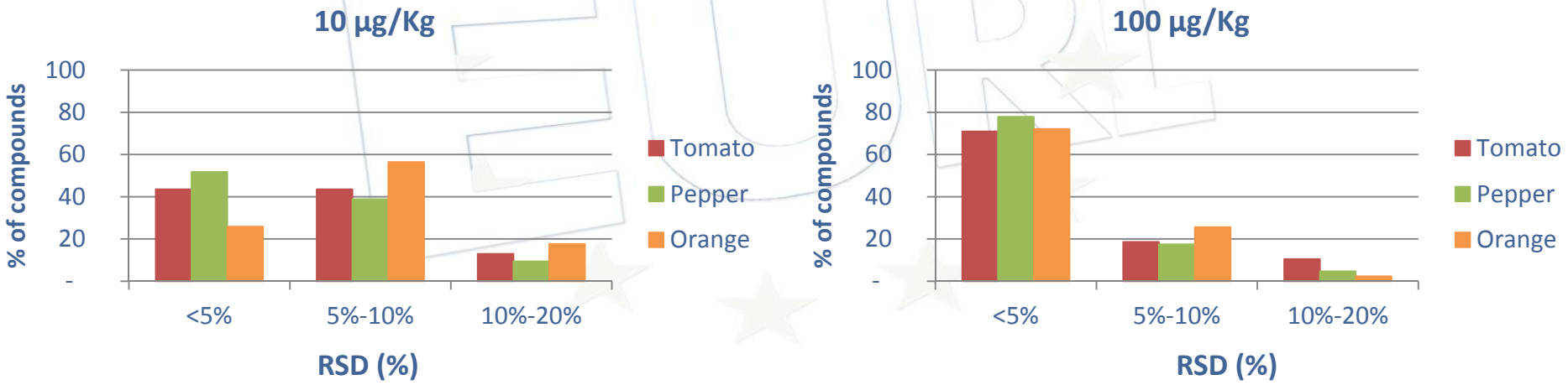
10 min Gradient

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



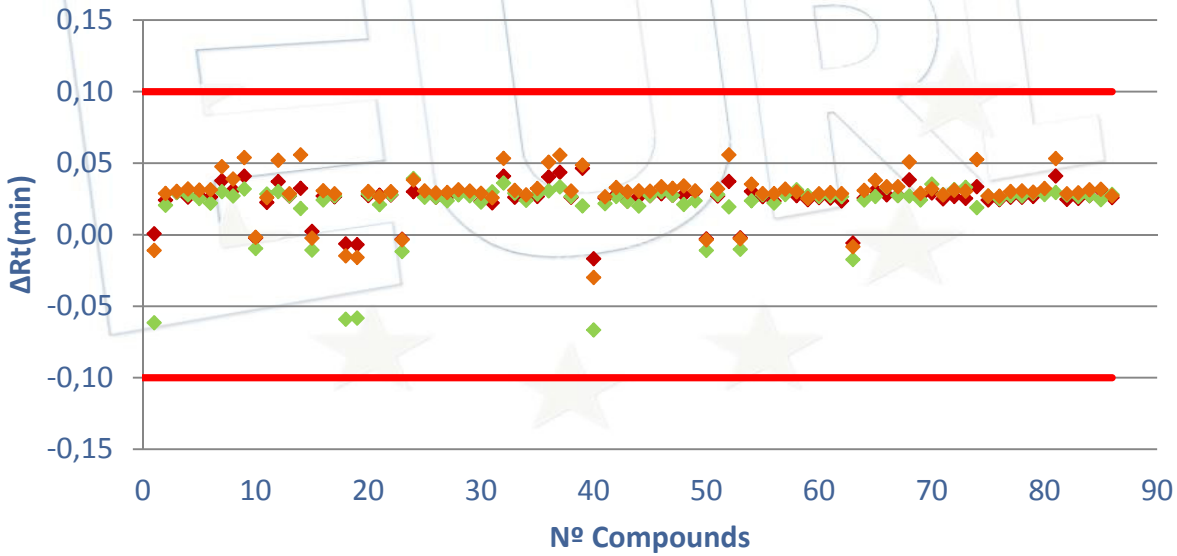
Peak area reproducibility

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.

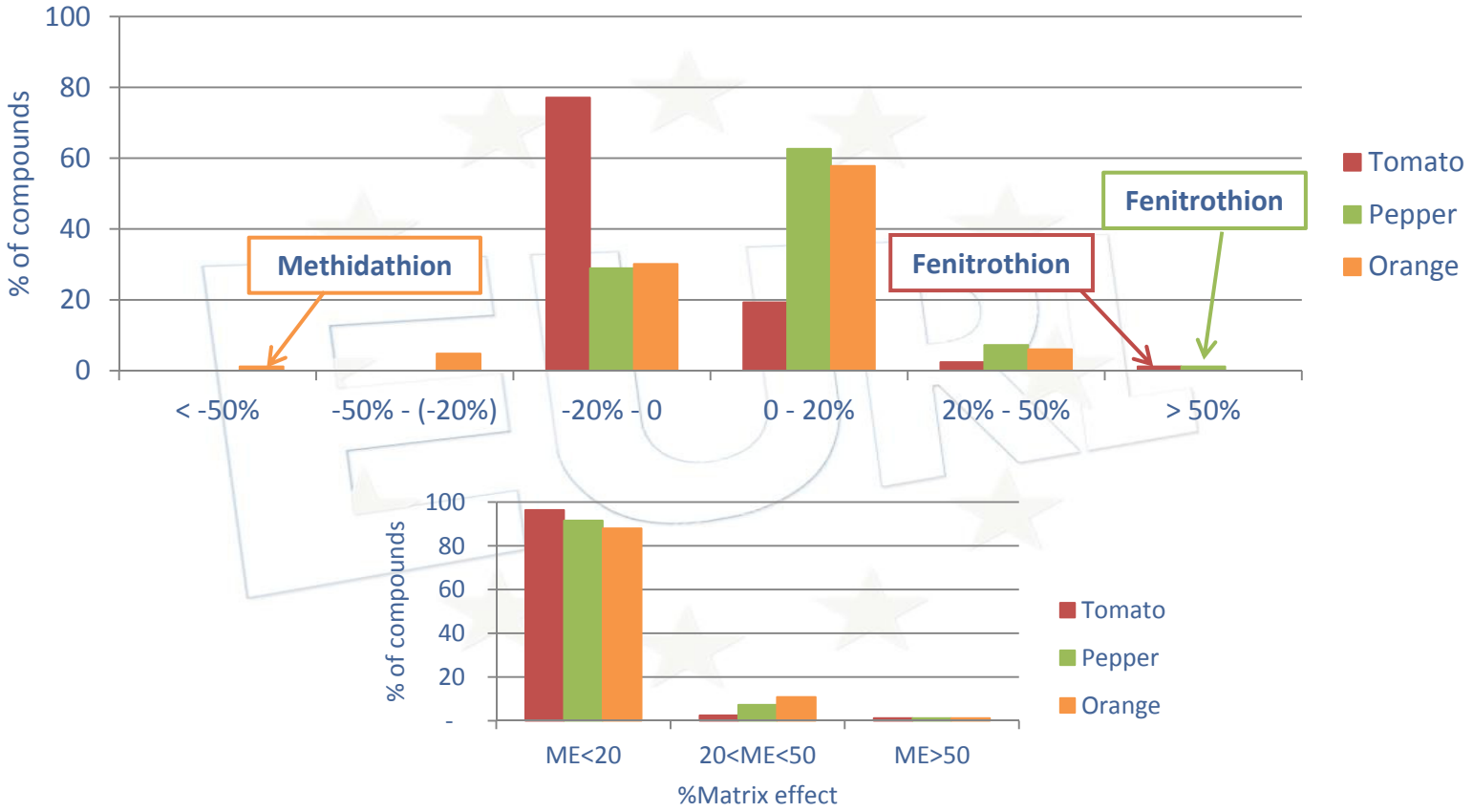


Retention time reproducibility

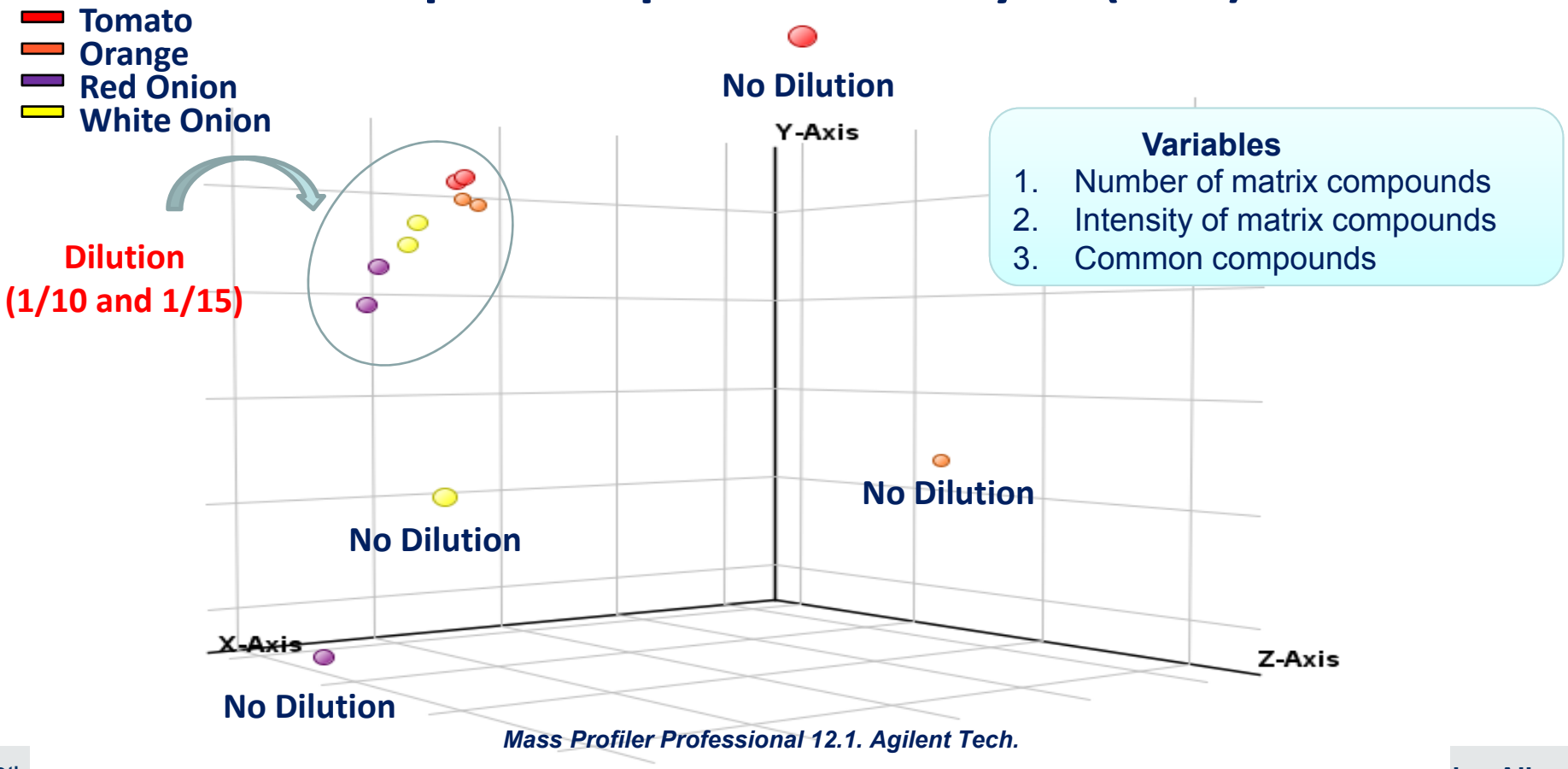
Difference between consecutive injections of tomato, pepper and orange and standard in solvent spiked at different concentrations diluted 30 times



Matrix Effects



Principal Components Analysis (PCA)



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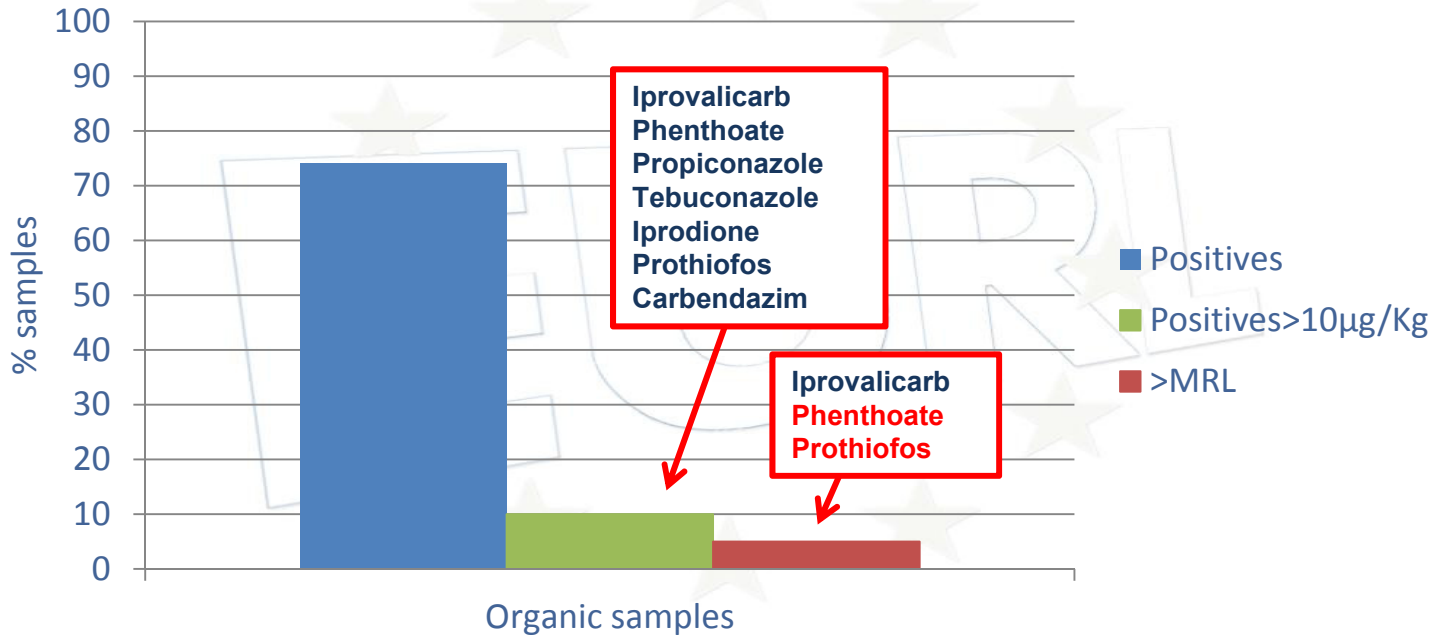
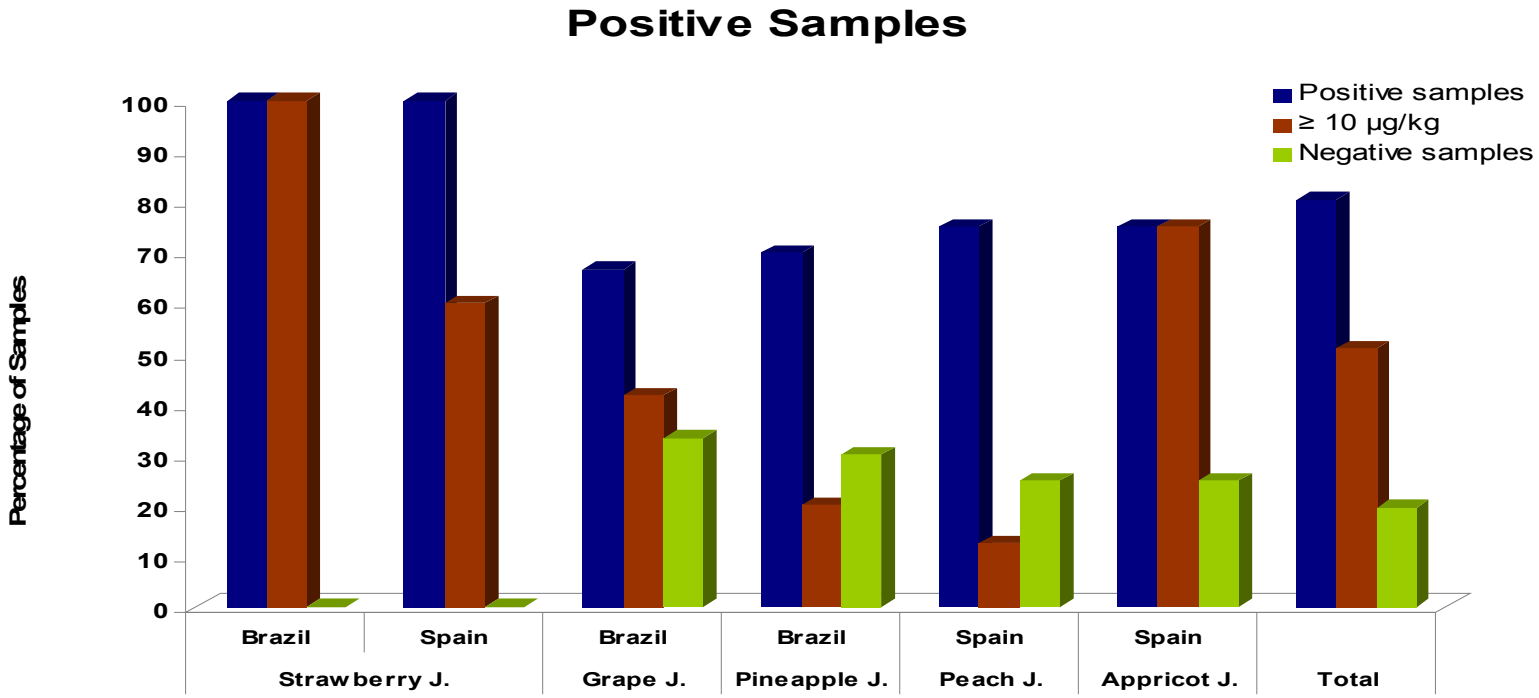
Real samples from different supermarkets
50 Organic Samples



Fruit jam samples (51) from various supermarkets

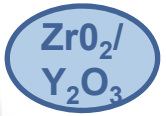


Zirconium oxide-coated Silica Particles

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

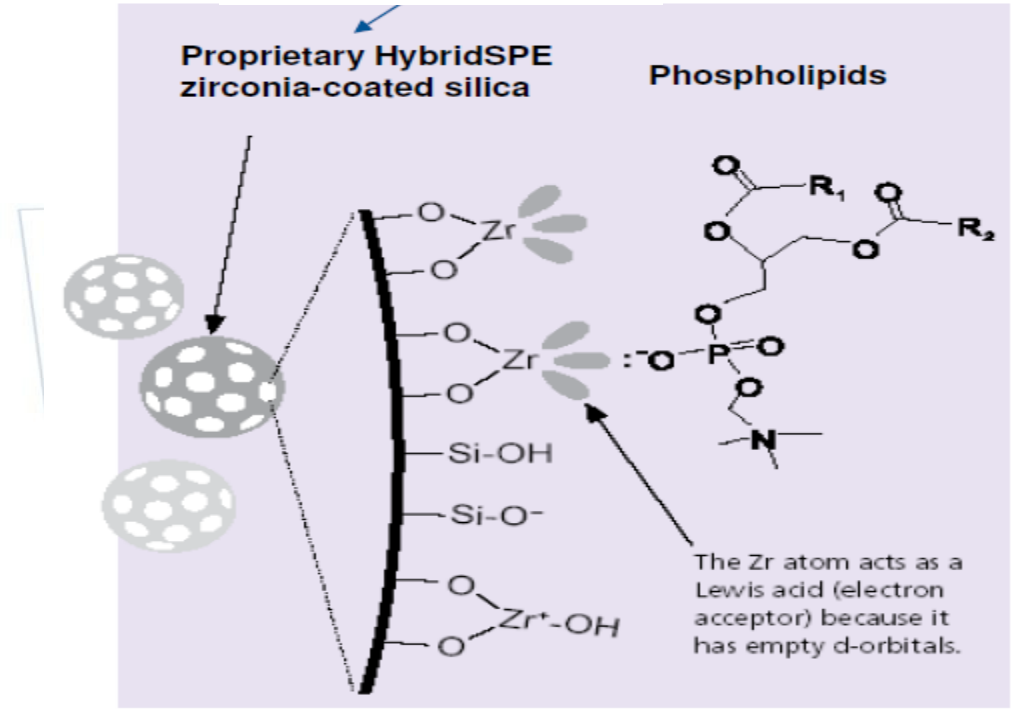
ZrO_2

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



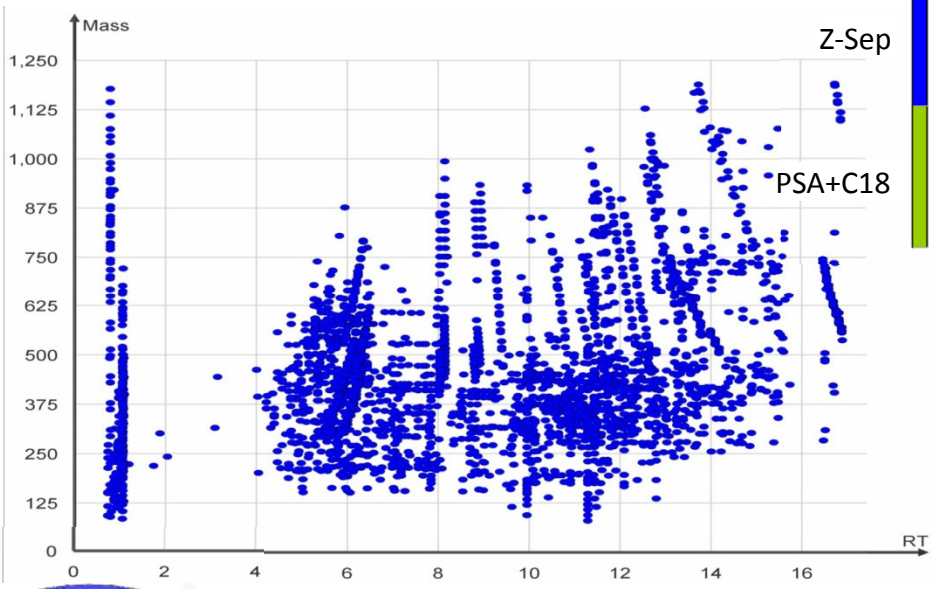
Particle size: 100 nm
Relative surface area: >100 m^2/g

Z-Sep


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

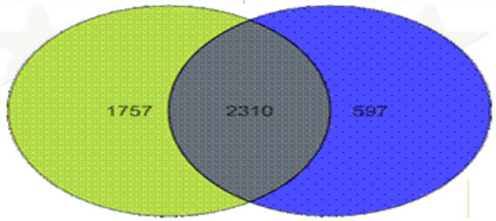
Co-extracted matrix components of avocado with Z-Sep and PSA+C18. LC-TOF/MS

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

10 Fresh Herbs

Falt parsley

Curly parsley

Chive

Coriander

Dill

Mint

Rosemary

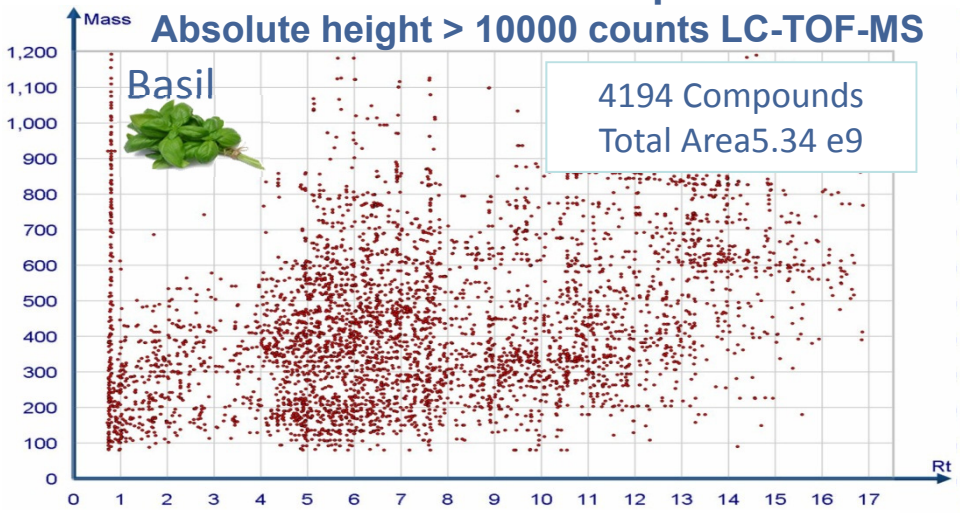
Basil

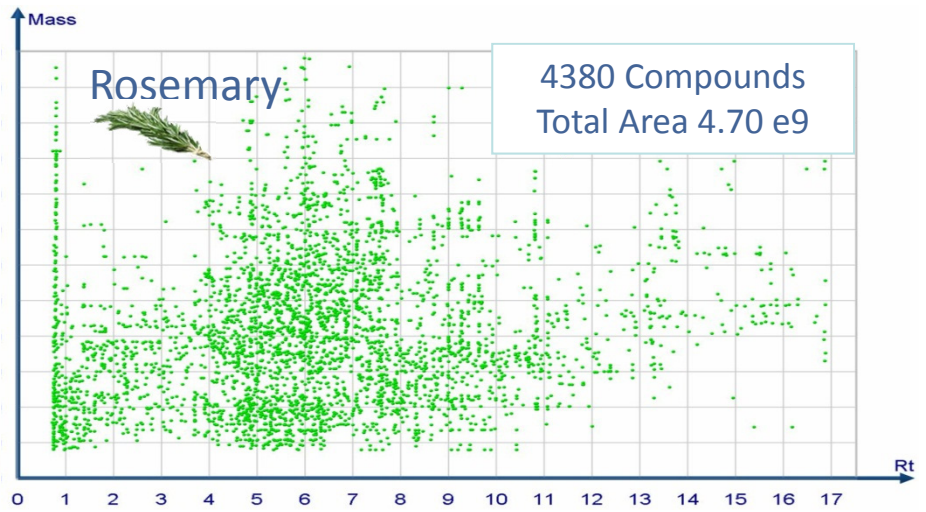
Oregano

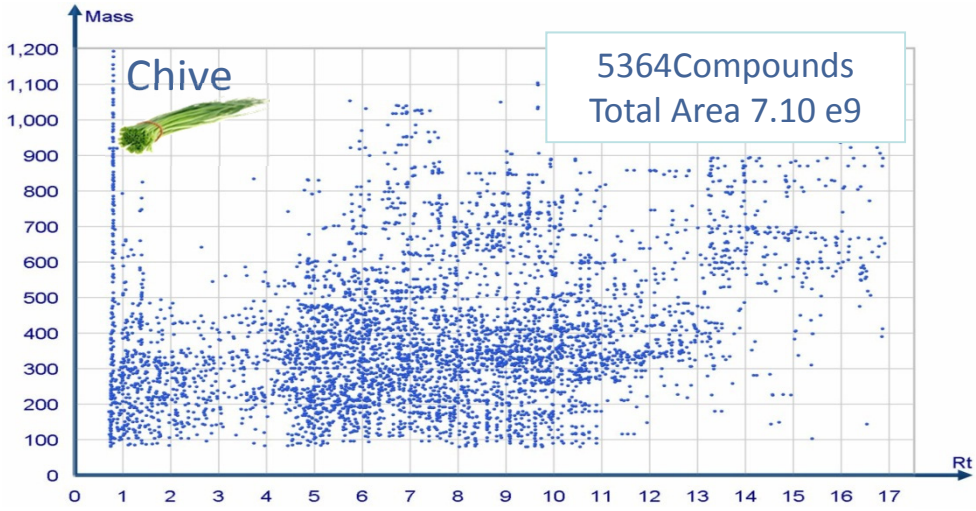
Thyme

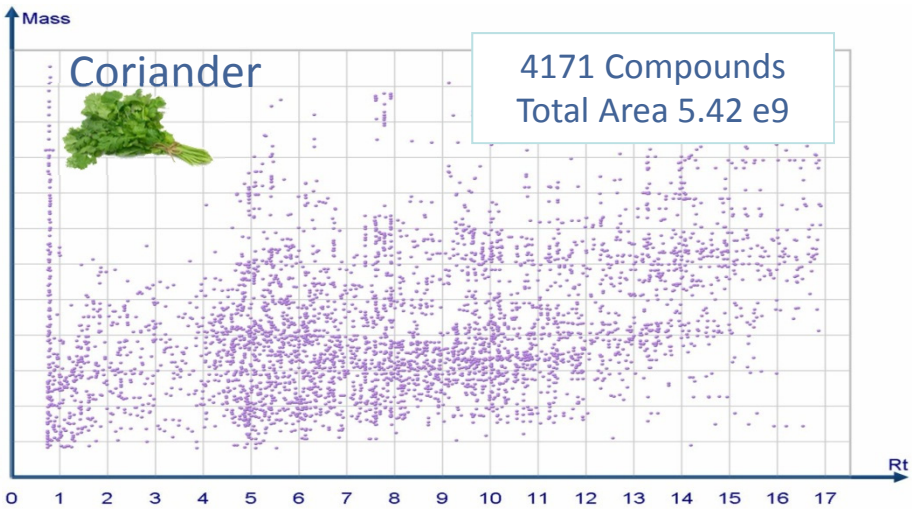
Co-extracted matrix components of Fresh Herbs

Absolute height > 10000 counts LC-TOF-MS









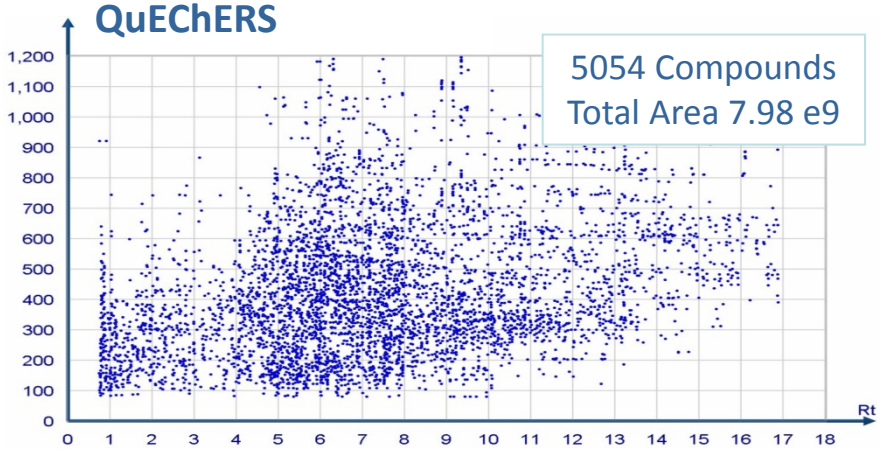
LC-TOF-MS Analysis Full Scan Mode

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

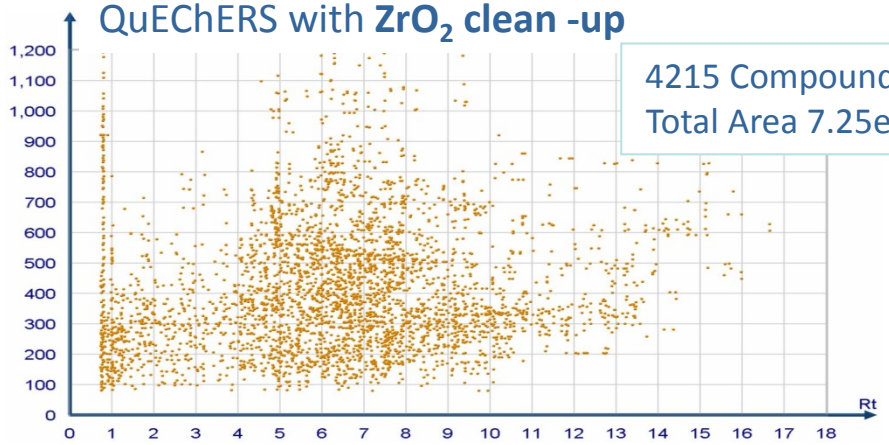
Co-extracted matrix components of Thyme

Absolute height > 10000 counts LC-TOF-MS

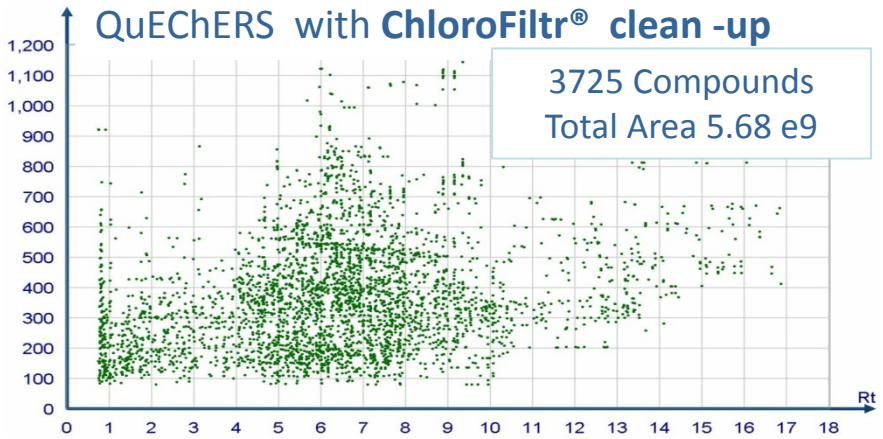
QuEChERS



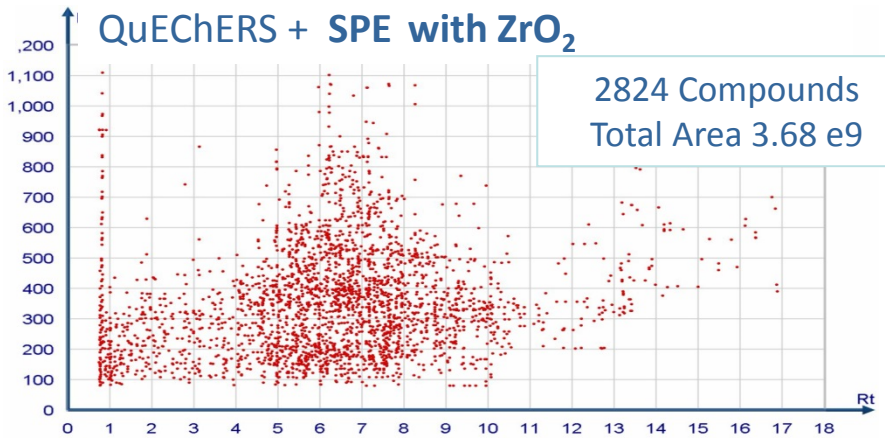
QuEChERS with ZrO_2 clean -up



QuEChERS with ChloroFiltr® clean -up



QuEChERS + SPE with ZrO_2

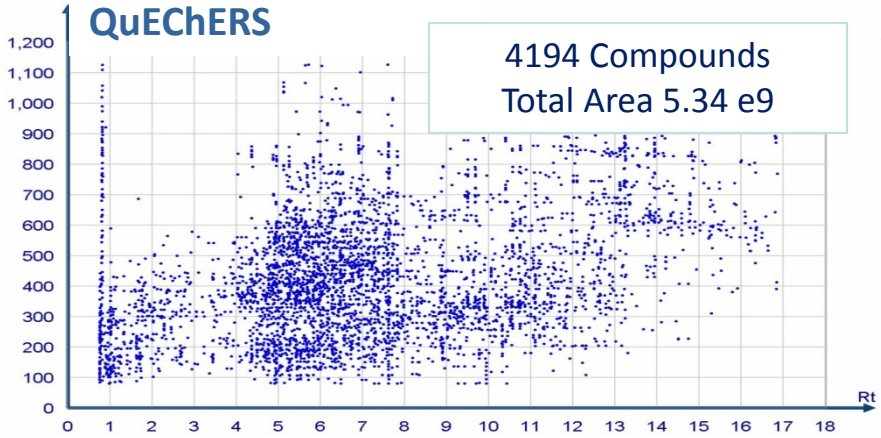


Co-extracted matrix components of Basil

Absolute height > 10000 counts LC-TOF-MS

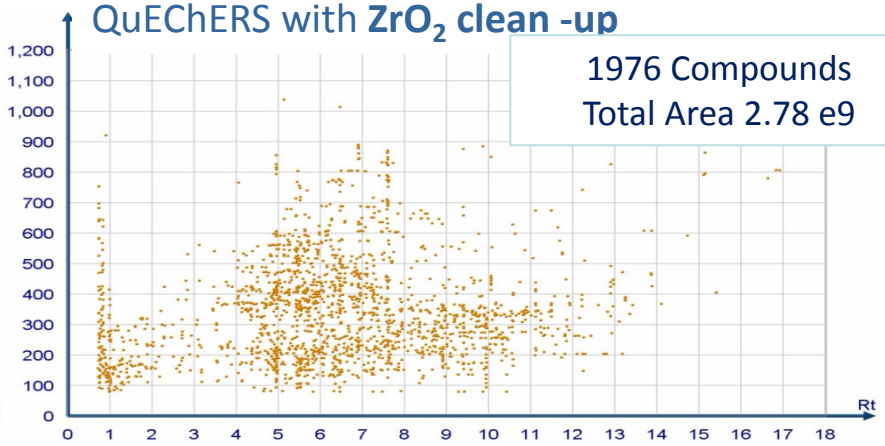
QuEChERS

4194 Compounds
Total Area 5.34 e9



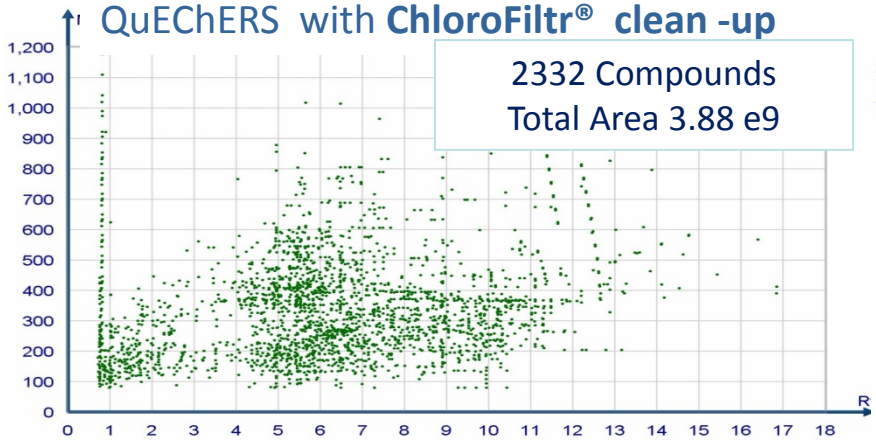
QuEChERS with **ZrO_2 clean -up**

1976 Compounds
Total Area 2.78 e9



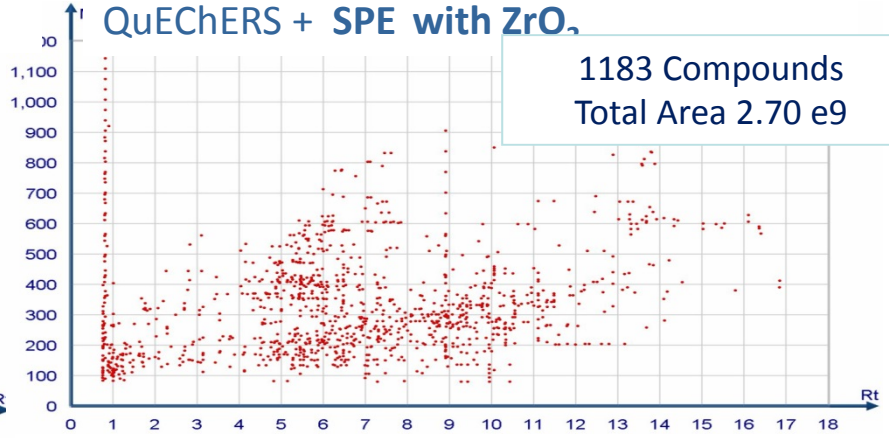
QuEChERS with **ChloroFiltr® clean -up**

2332 Compounds
Total Area 3.88 e9



QuEChERS + **SPE with ZrO_2**

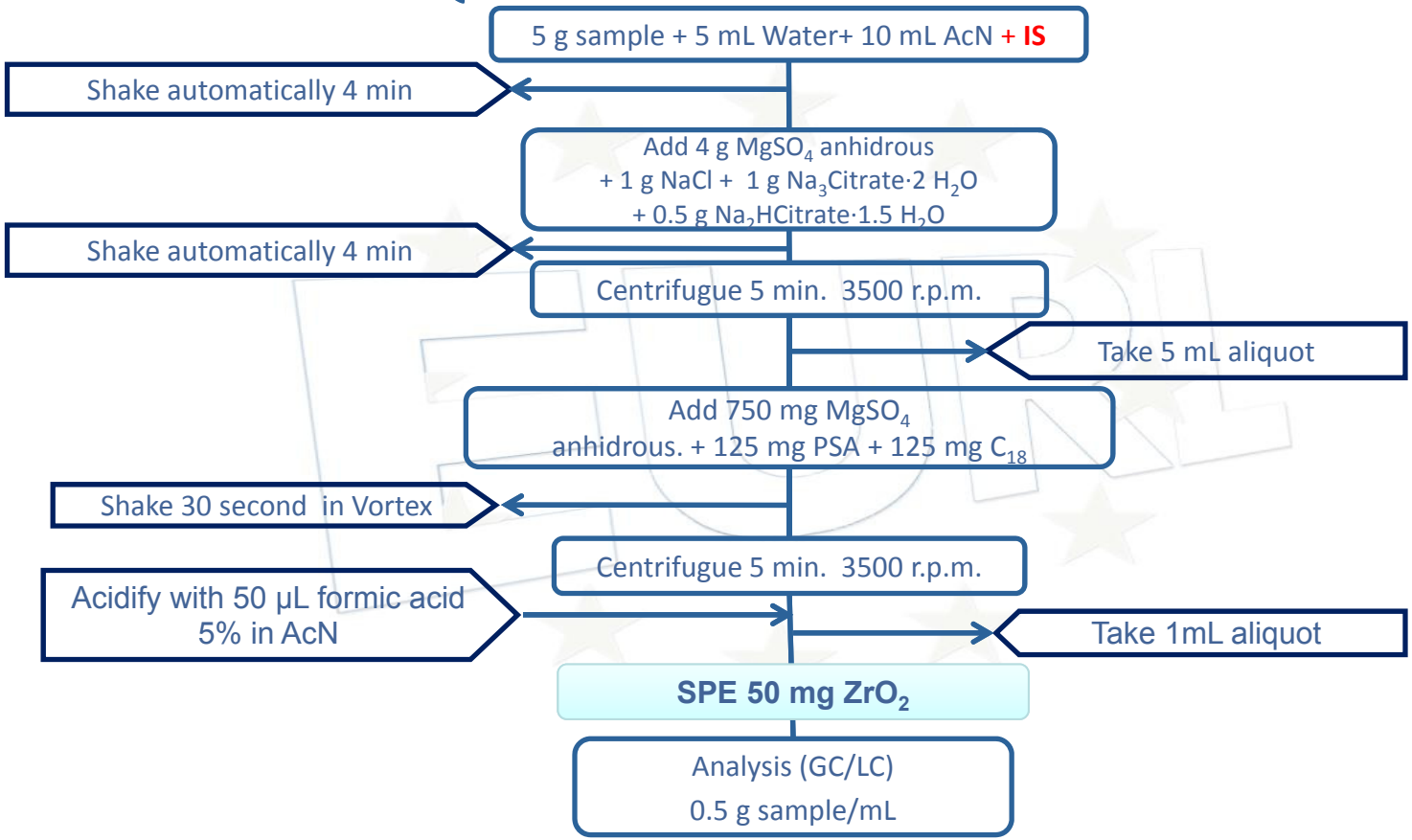
1183 Compounds
Total Area 2.70 e9



LC-TOF-MS Analysis Full Scan Mode

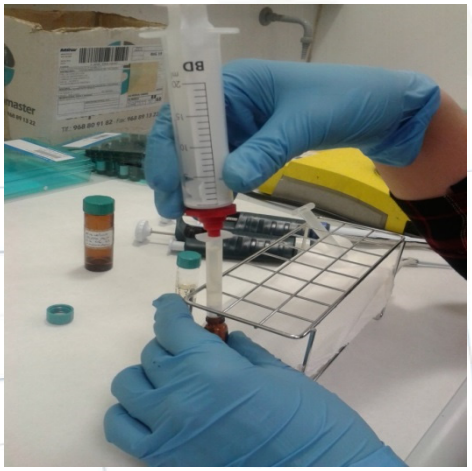
Matrix	QuEChERS		QuEChERs + SPE ZrO_2		QuEChERS (ZrO_2 instead PSA and C18)		QuEChERS ChloroFiltr® clean-up	
	Nº compounds	Total Area	Nº compounds	Total Area	Nº compounds	Total Area	Nº compounds	Total Area
Chive	5364	7.10E+09	3820	5.17E+09	4906	7.57E+09	4431	5.91E+09
Mint	5100	8.58E+09	2365	3.36E+09	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

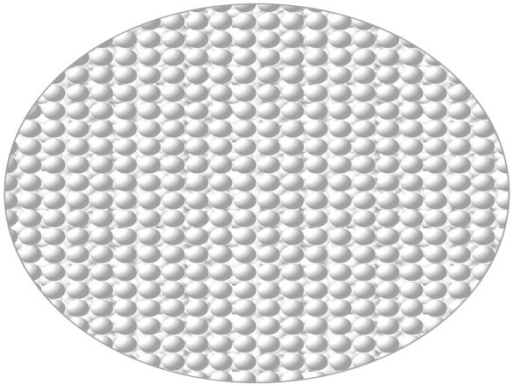


1mL sample + 50 mg ZSEP

Cartridge format



Disc format



Particle size: 22 µm= 22000 nm

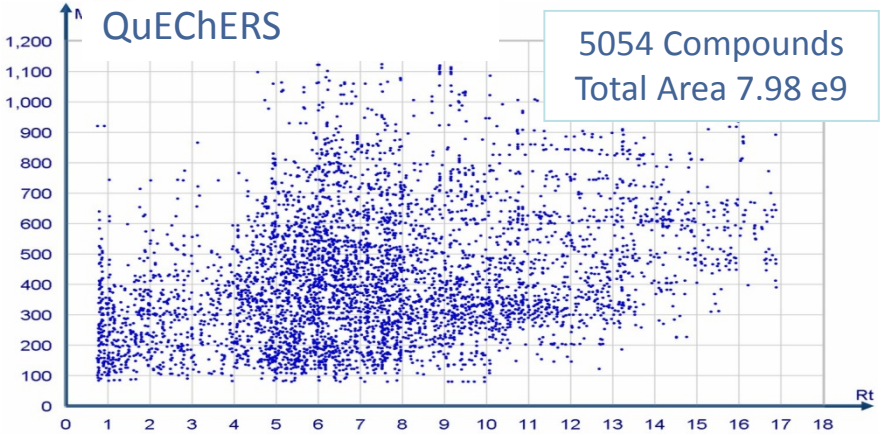
Relative surface area: 310 m2/g

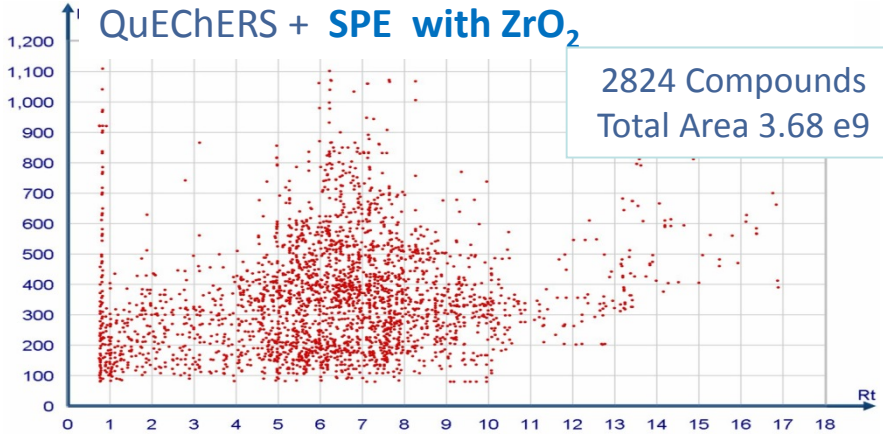
Matrix	QuEChERS		QuEChERs + SPE ZrO_2	
	No. compounds	Total Area	No. compounds	Total Area
Chive	5364	7.10E+09	3820	5.17E+09
Mint	5100	8.58E+09	2365	3.36E+09
Flat Parsley	5070	6.76E+09	2955	4.14E+09
Thyme	5054	7.98E+09	2824	3.68E+09
Curly Parsley	4843	5.80E+09	2136	3.03E+09
Oregano	4644	5.20E+09	1741	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

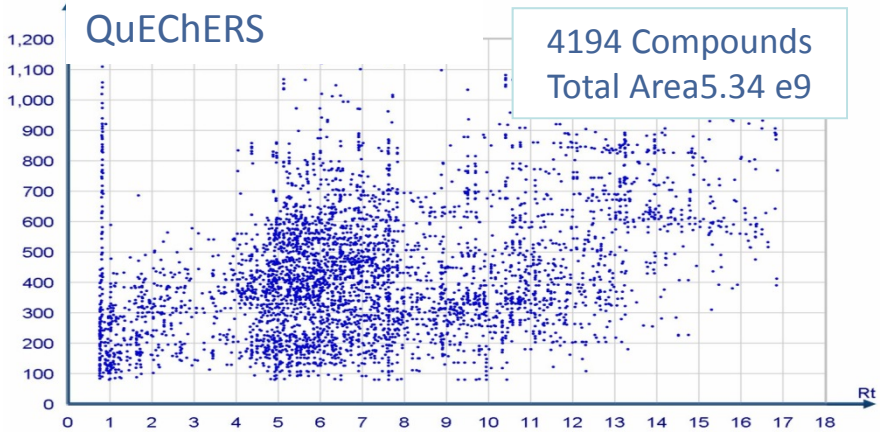
Absolute height > 10000 counts LC-TOF-MS

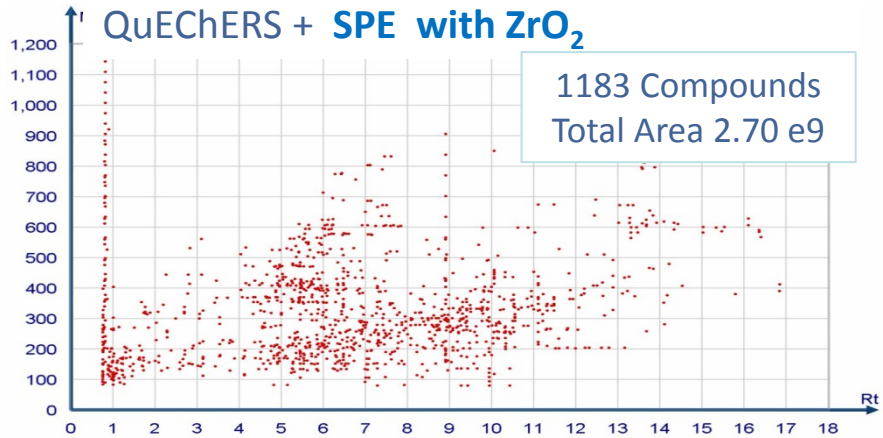
Thyme





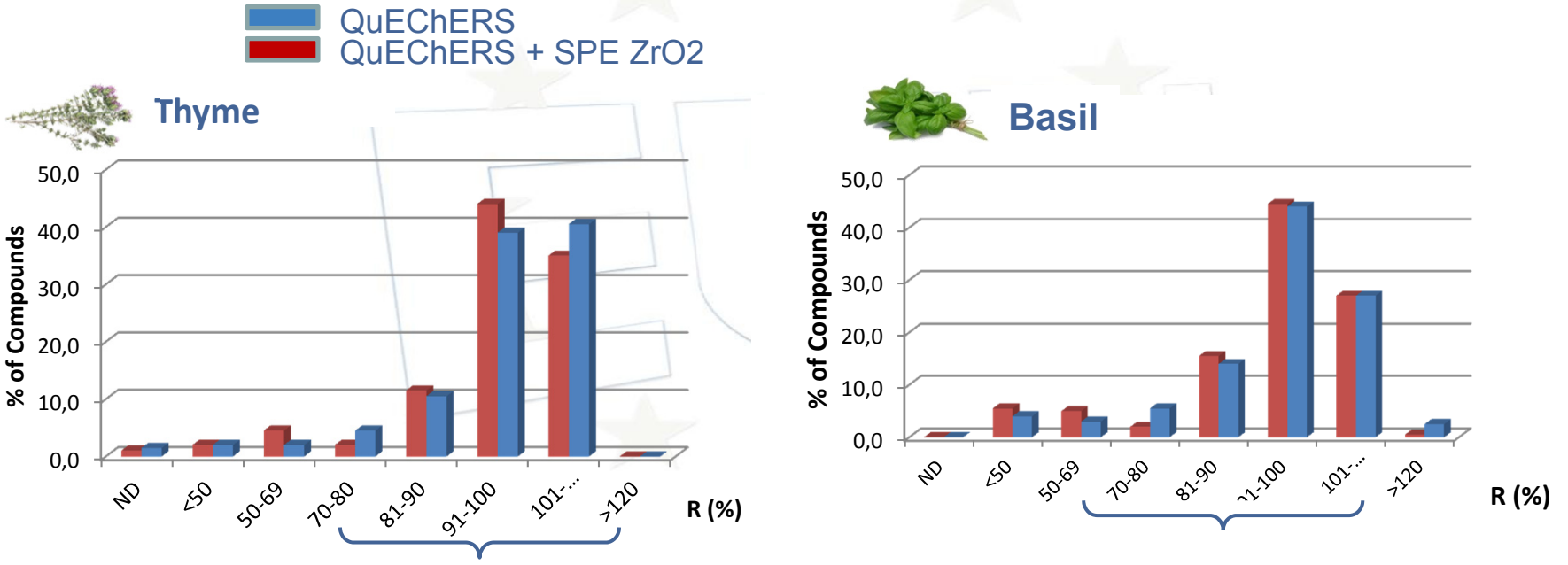
Basil





RECOVERIES at 50 µg/kg QuEChERS (+ZrO_2)

GC (203 Pesticides)

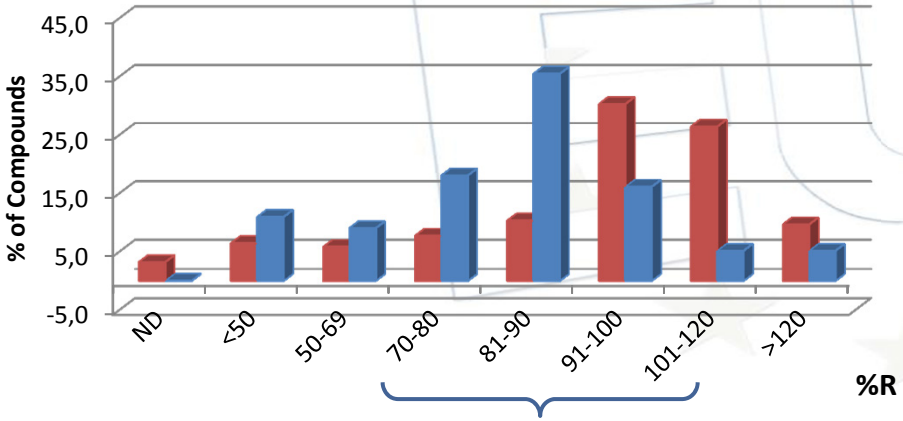


RECOVERIES at 50 µg/kg QuEChERS (+ZrO_2)

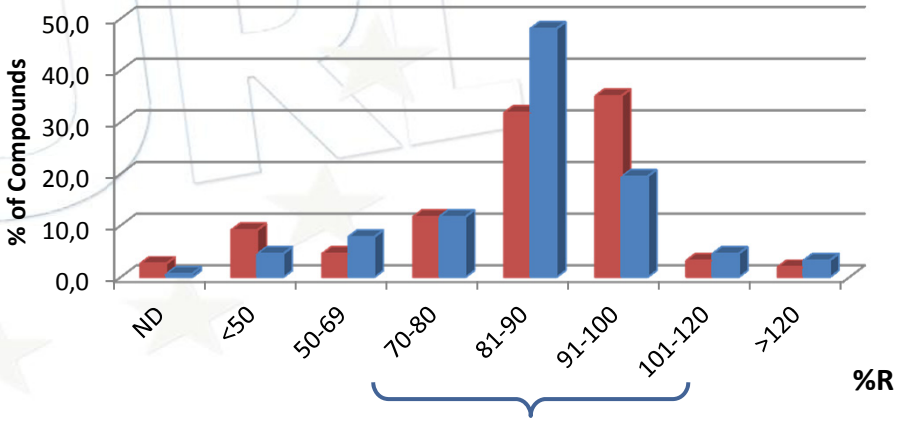
LC (155 Pesticides)

QuEChERS
QuEChERS + SPE ZrO2

Thyme

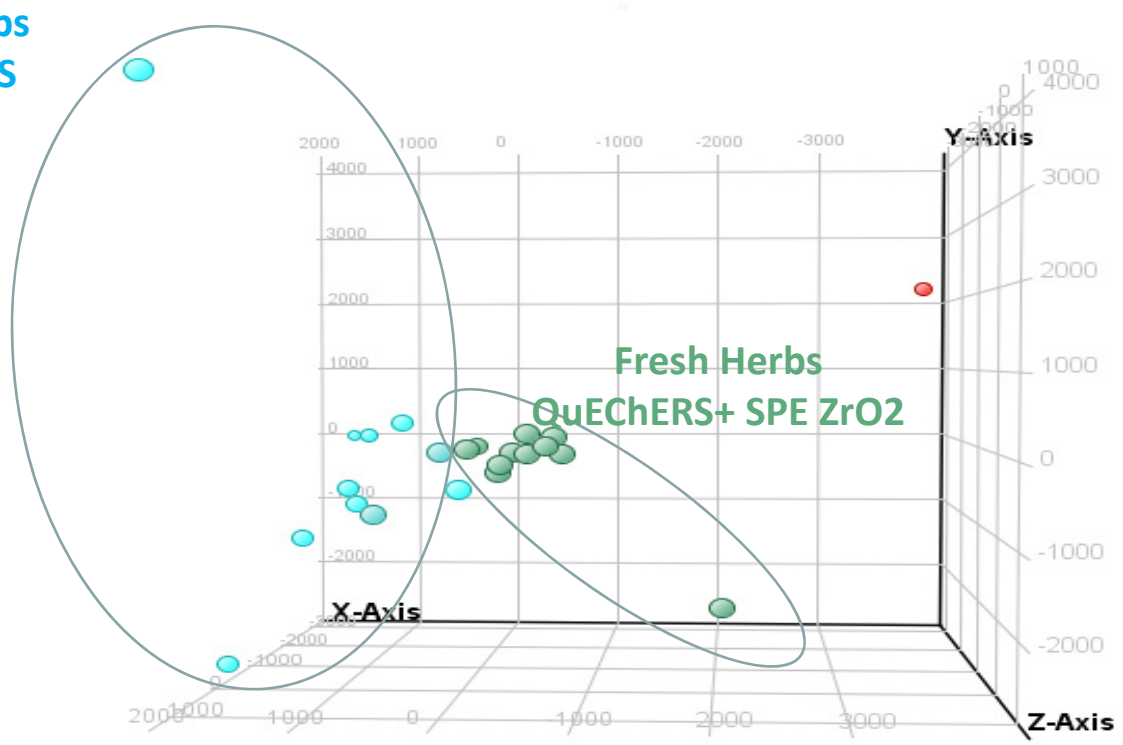


Basil



Principal Components Analysis (PCA)

Fresh Herbs
QuEChERS



Fresh Herbs

Chive
Mint
Flat Parsley
Curly Parsley
Thyme
Oregano
Rosemary
Basil
Coriander
Dill

Mass Profiler Professional 12.1. Agilent Tech.

How can we control or avoid them?

Acknowledgement

Carmen Ferrer

Milagros Mezcua

Ana Lozano

Łukasz Rajski

Thank You
for Your Attention

EURL EUROPEAN UNION REFERENCE LABORATORY