



EUROPEAN UNION REFERENCE LABORATORY
FOR PESTICIDE RESIDUES IN FRUITS AND VEGETABLES
(EURL-FV)

EVALUATION OF ANALYTICAL PROCEDURES IN PESTICIDE MULTIRESIDUE METHODS (MRMs)



50 CONGRESO
LATINOAMERICANO DE
RESIDUOS DE PLAGUICIDAS
5TH LATIN AMERICAN PESTICIDE RESIDUE WORKSHOP

LAPRW2015
ALIMENTOS Y MEDIO AMBIENTE / FOOD AND ENVIRONMENT

Stgo Chile
Casa Central
de la Pontificia
Universidad
Católica de Chile



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Overview

Mapping approach:

- Evaluation of Commodities
- Dilution effect
- Evaluation of MRMs

MRMs vs Identification

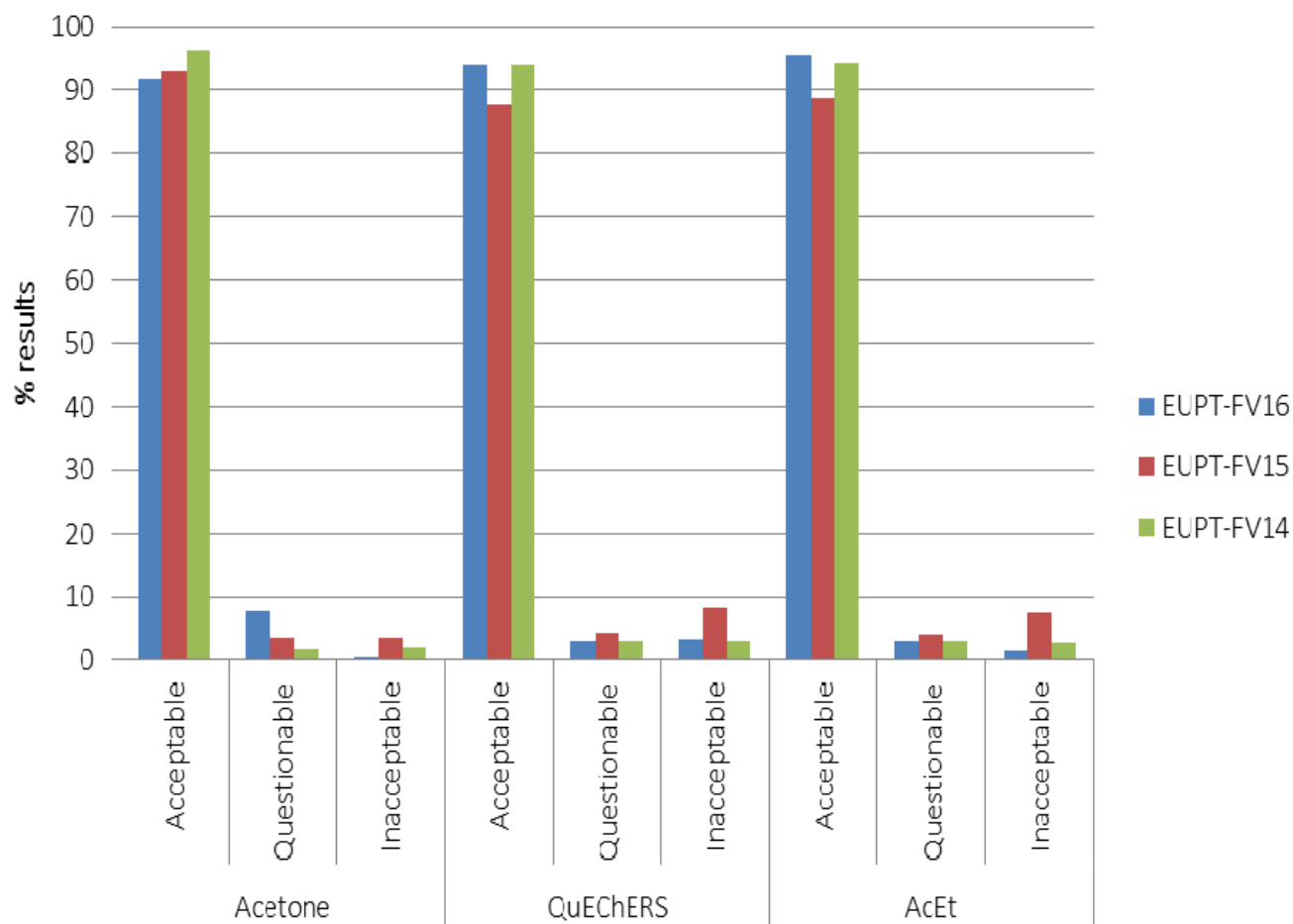
MRM vs Quantification

Extractability

Matrix Effects

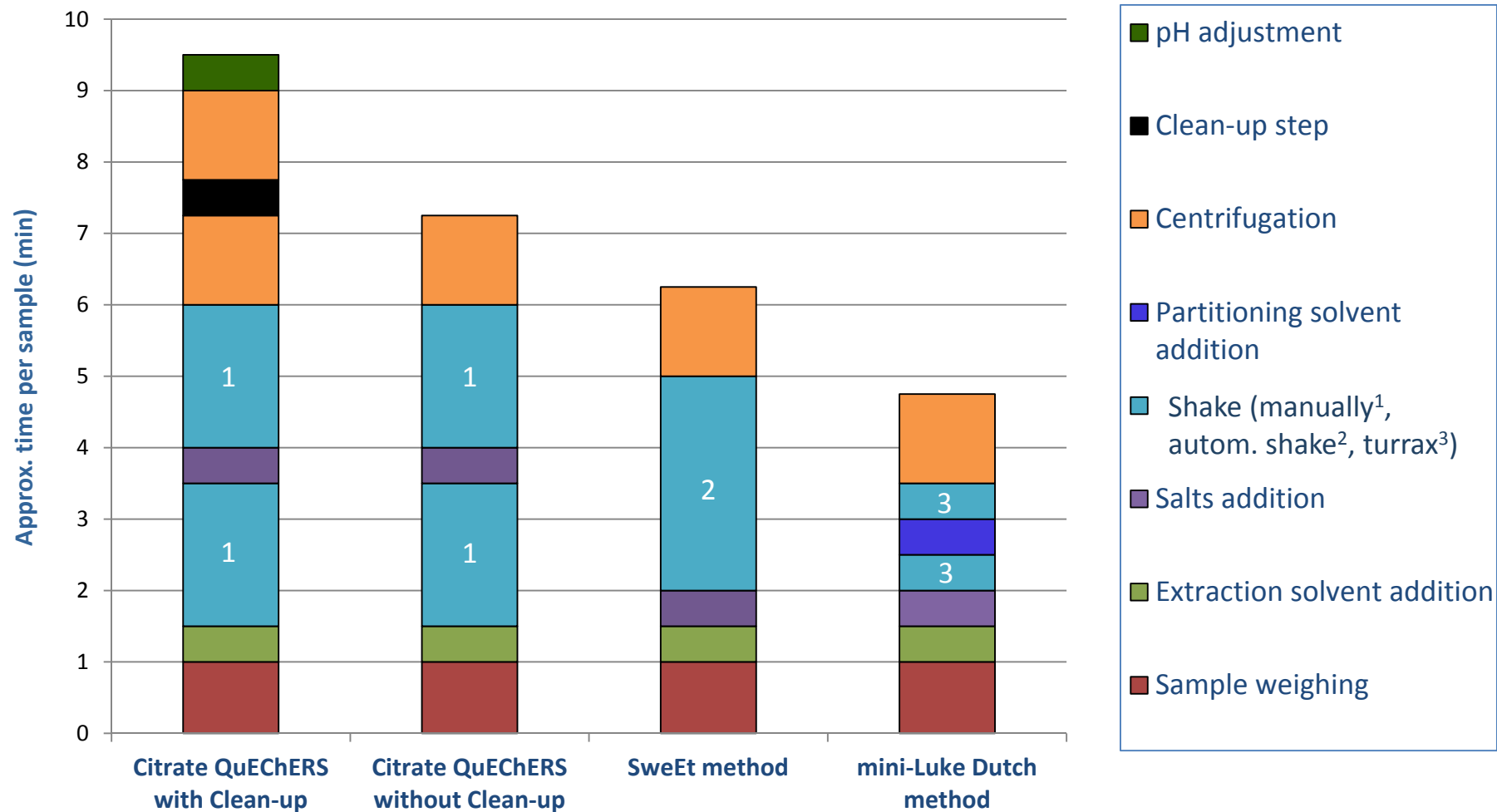


	Acetone (Luke-NL Method)			Acetonitrile (QuEChERS)			Ethyl Acetate (SweEt)		
	Acceptable	Questionable	Inacceptable	Acceptable	Questionable	Inacceptable	Acceptable	Questionable	Inacceptable
EUPT-FV16	91,7	7,7	0,6	93,9	2,9	3,2	95,5	2,9	1,6
EUPT-FV15	93,0	3,4	3,6	87,7	4,2	8,2	88,6	3,9	7,5
EUPT-FV14	96,2	1,8	2	93,9	3,1	3	94,3	3	2,7





Multiresidue extraction methods

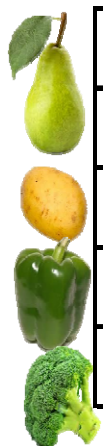


Solubility of fatty acids with various organic solvents

No of C atoms	Solubilities at 20° C		
	Ethyl acetate	Acetone	Acetonitrile
10	289	407	66
11	425	706	185
12	52	60,5	7,6
13	70	78,6	5,8
14	15,3	15,9	1,8
15	15,4	13,8	1,1
16	6,1	5,38	0,4
17	5,3	4,28	0,2
18	0,5	1,54	<0,1

Matrix Effects





	Compounds Evaluated	% F-	% F+
EUPT-FV14	17	1,8	0,9
EUPT-FV15	18	1,4	0,3
EUPT-FV16	22	1,3	0,1
EUPT-FV17	11	0,8	0,5

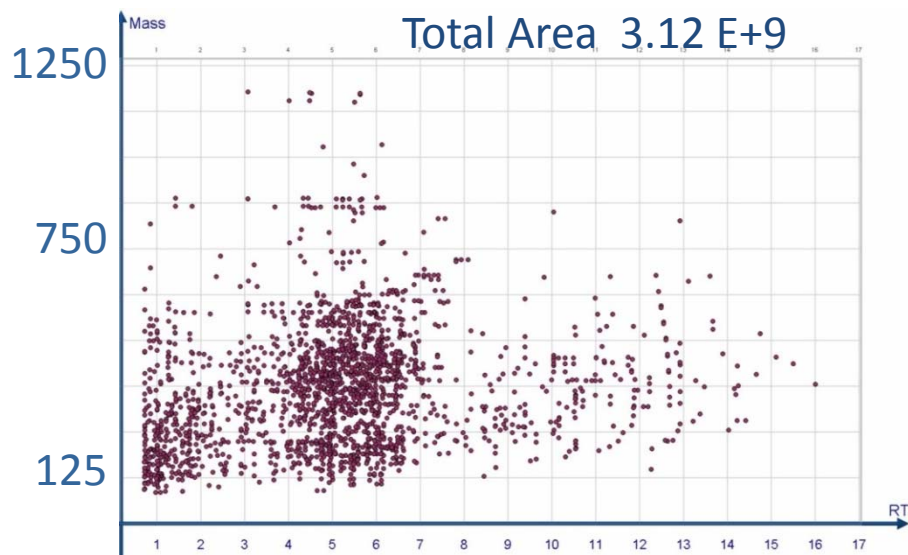
EVALUATION OF MATRIX EFFECTS

Mapping Natural Compounds by Chromatography and Accurate mass

LC-ToF-MS

Plum 2008 Matrix compounds

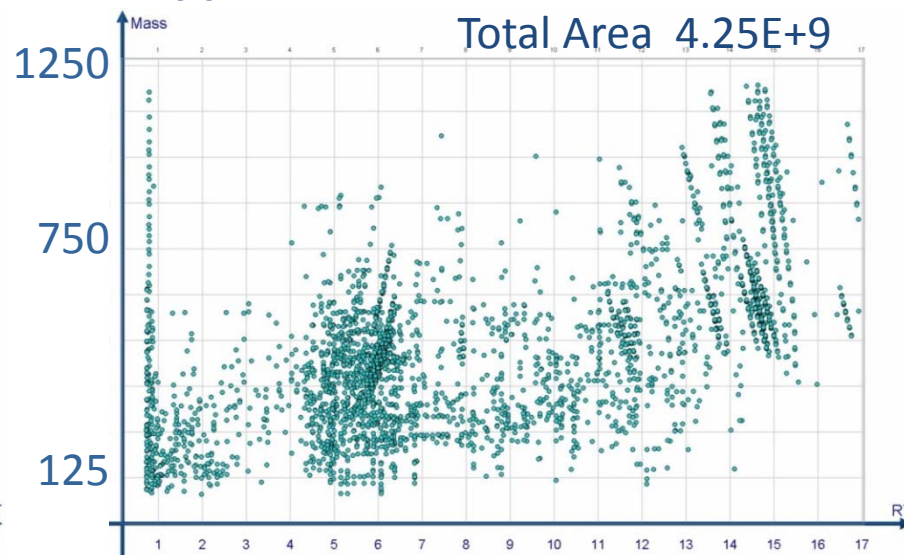
Total Area 3.12 E+9



Apple

3047 Matrix compounds

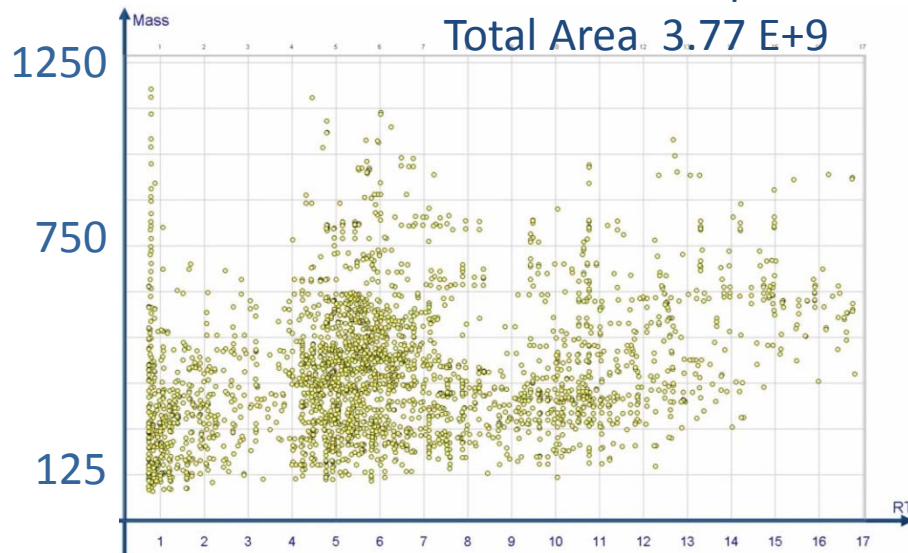
Total Area 4.25E+9



Mango

2649 Matrix compounds

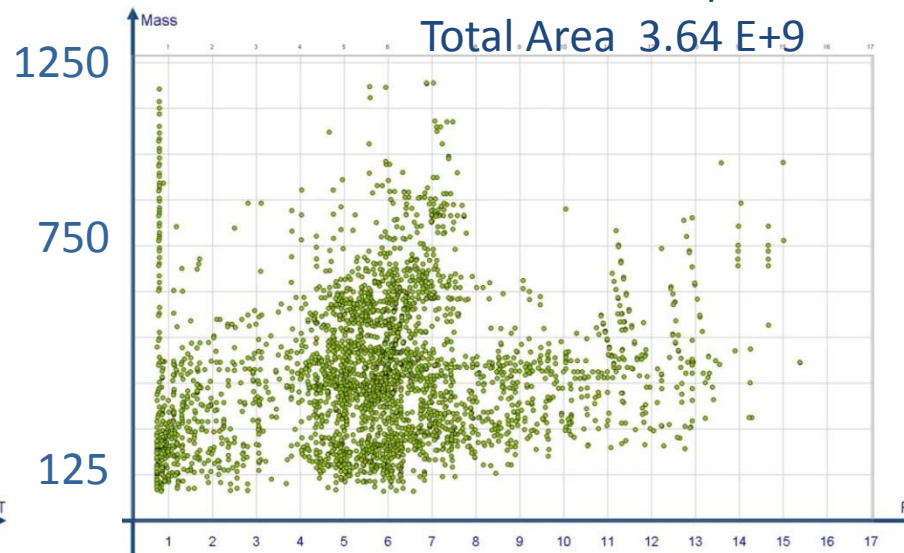
Total Area 3.77 E+9



Pear

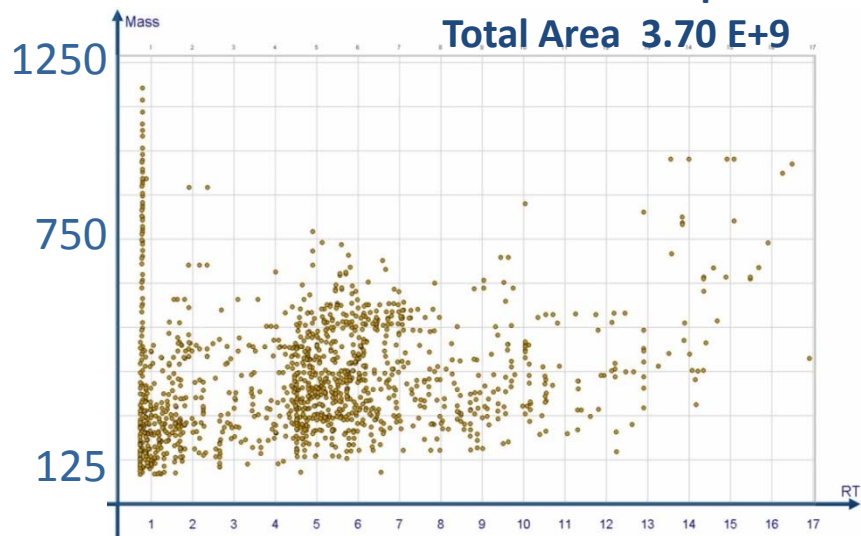
2919 Matrix compounds

Total Area 3.64 E+9



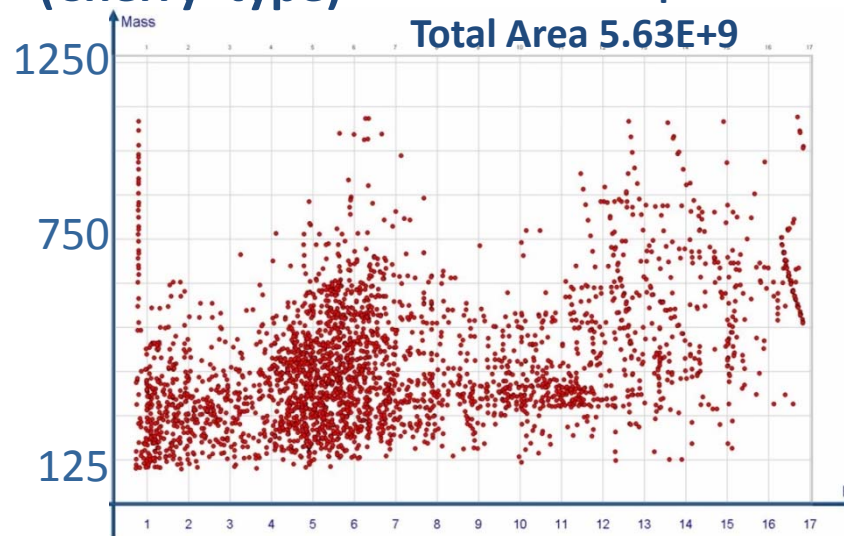
Papaya 1270 Matrix compounds

Total Area 3.70 E+9



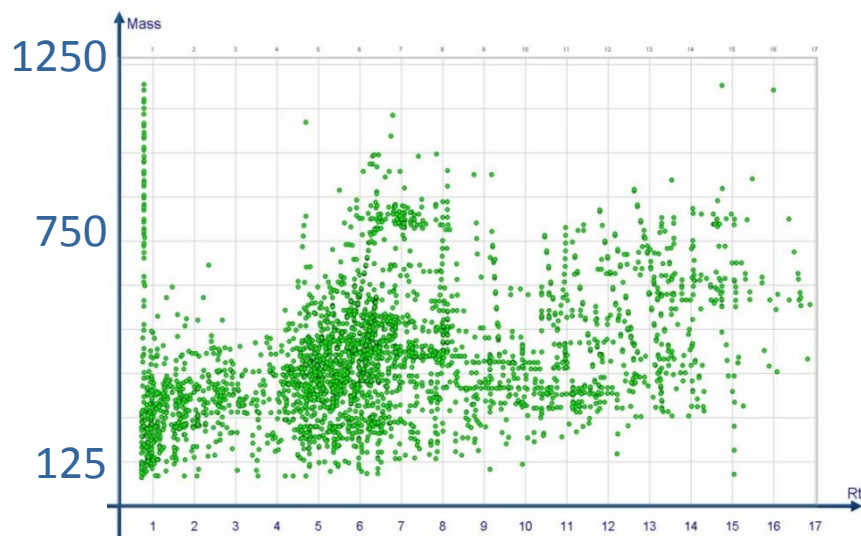
Tomato (Cherry type) 2833 Matrix compounds

Total Area 5.63E+9



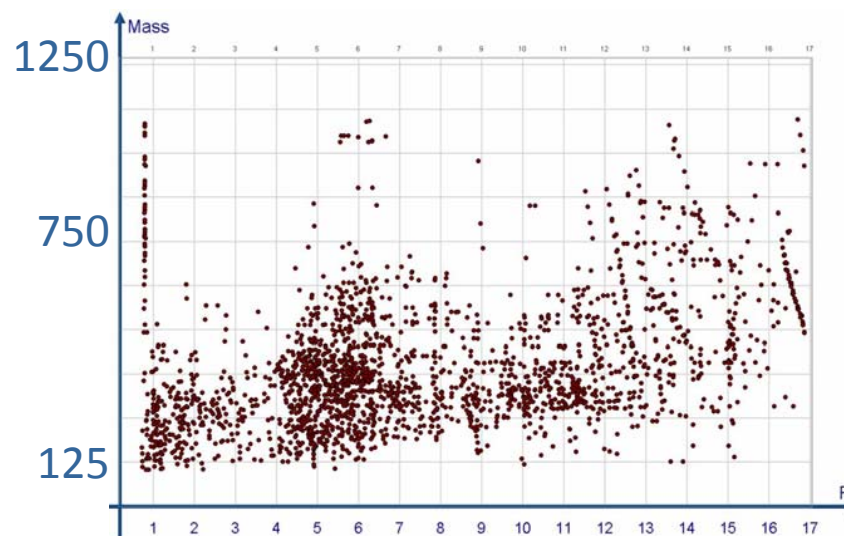
Pepper 3419 Matrix compounds

Total Area 5.51 E+9

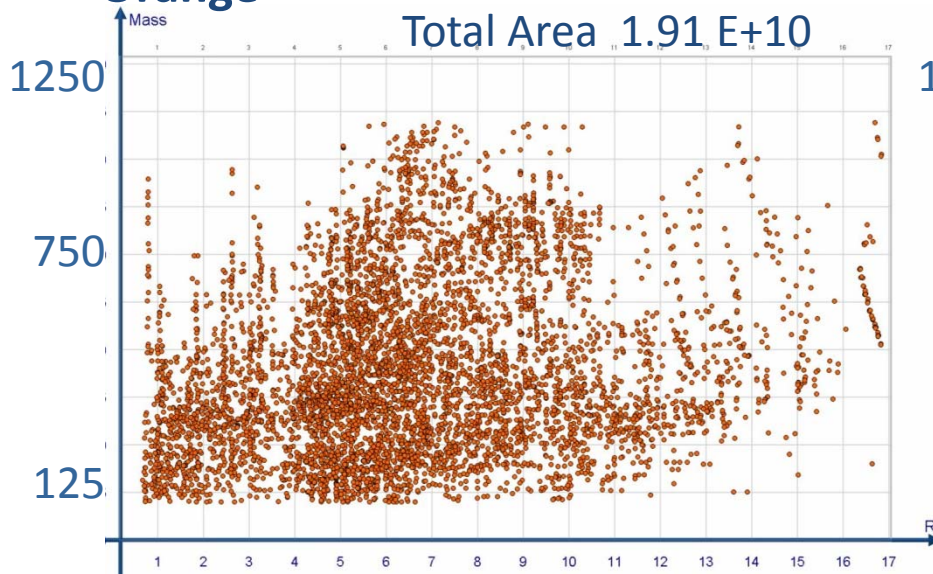


Tomato (Kumato type) 2155 Matrix compounds

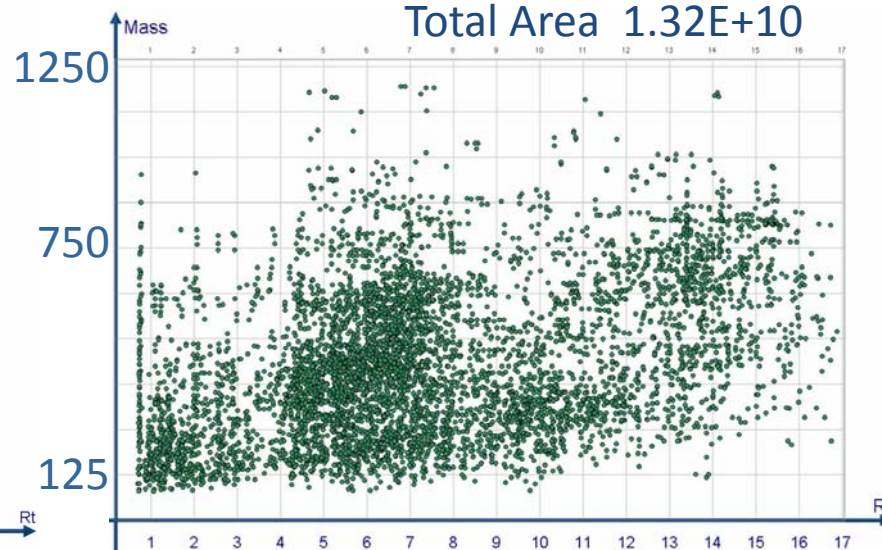
Total Area 4.18 E+9



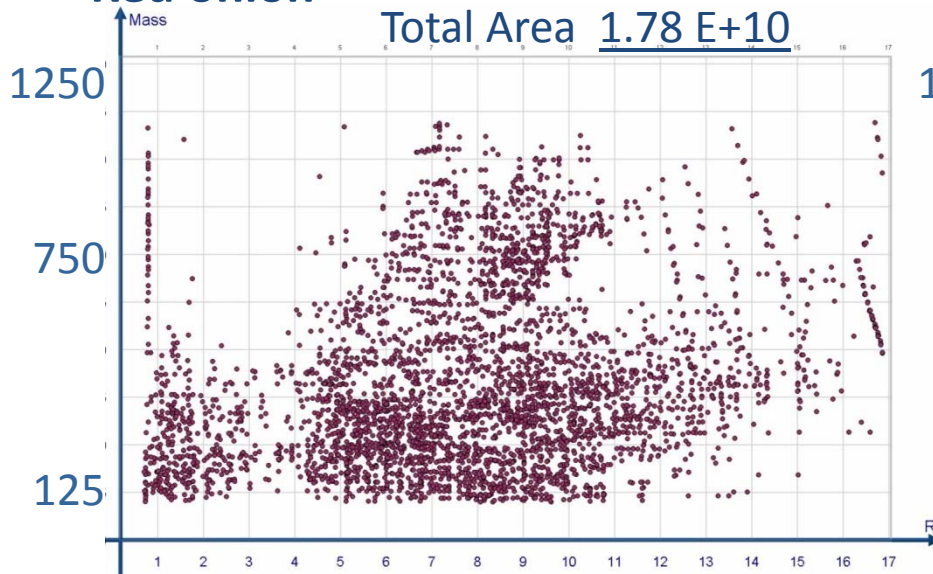
Orange 5768 Matrix compounds
Total Area $1.91 \text{ E}+10$



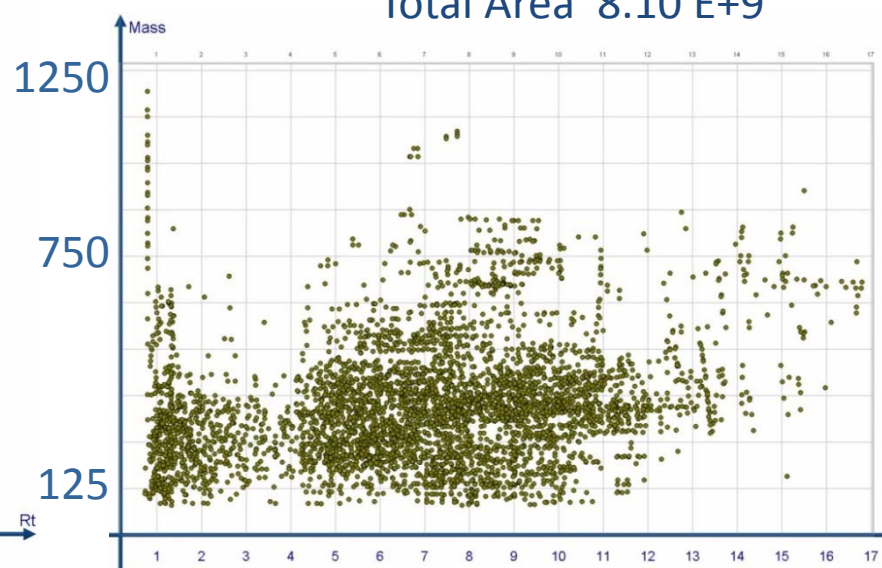
Green tea 5793 Matrix compounds
Total Area $1.32 \text{ E}+10$



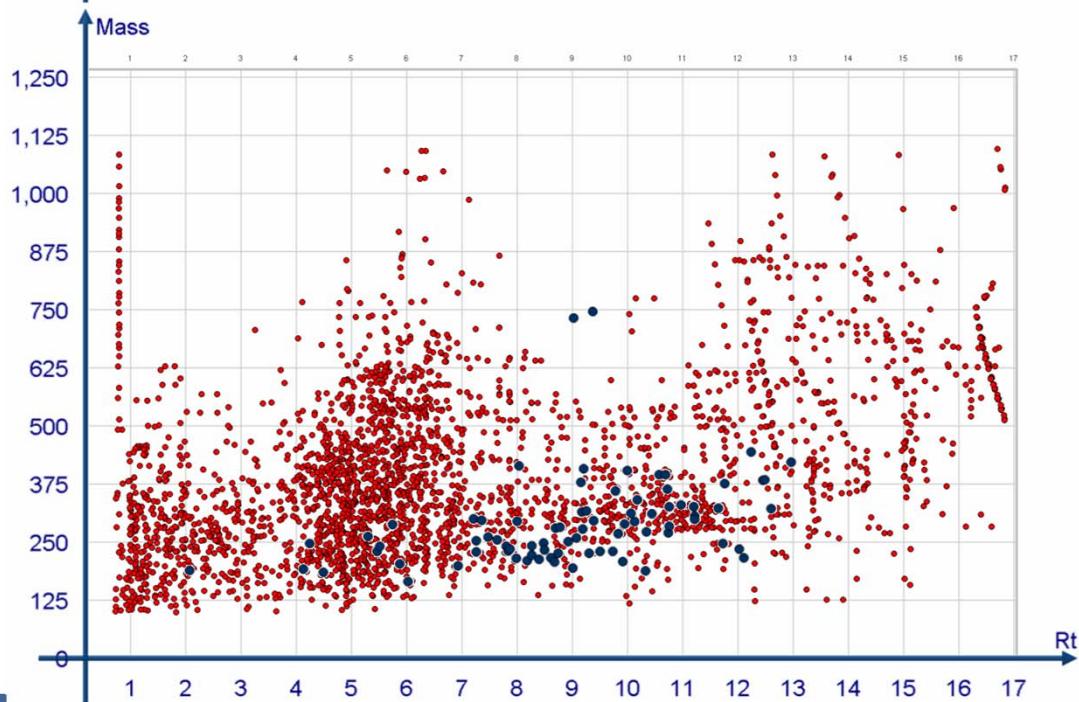
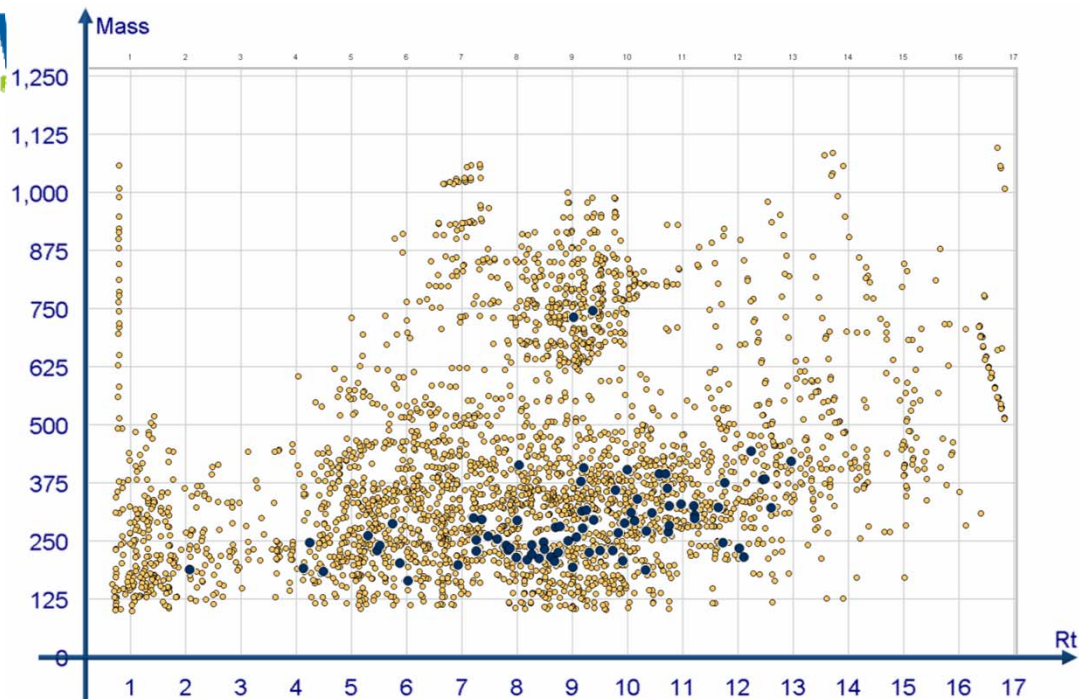
Red onion 3823 Matrix compounds
Total Area $1.78 \text{ E}+10$

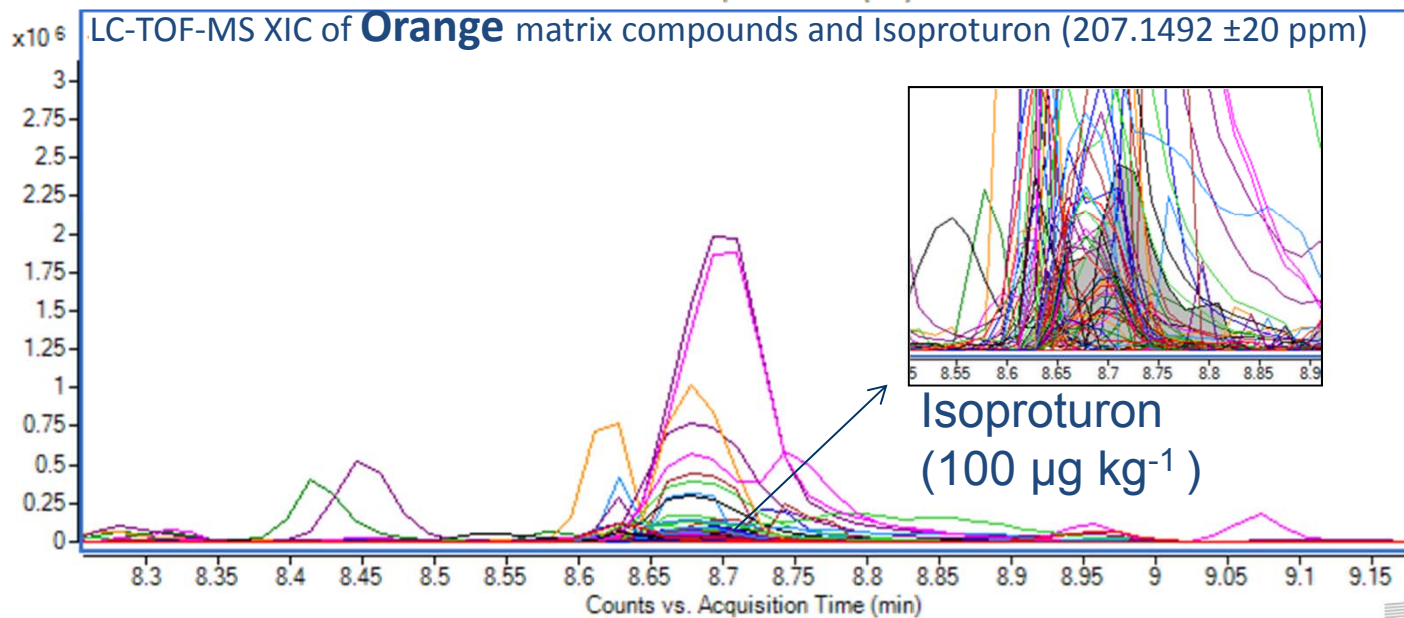
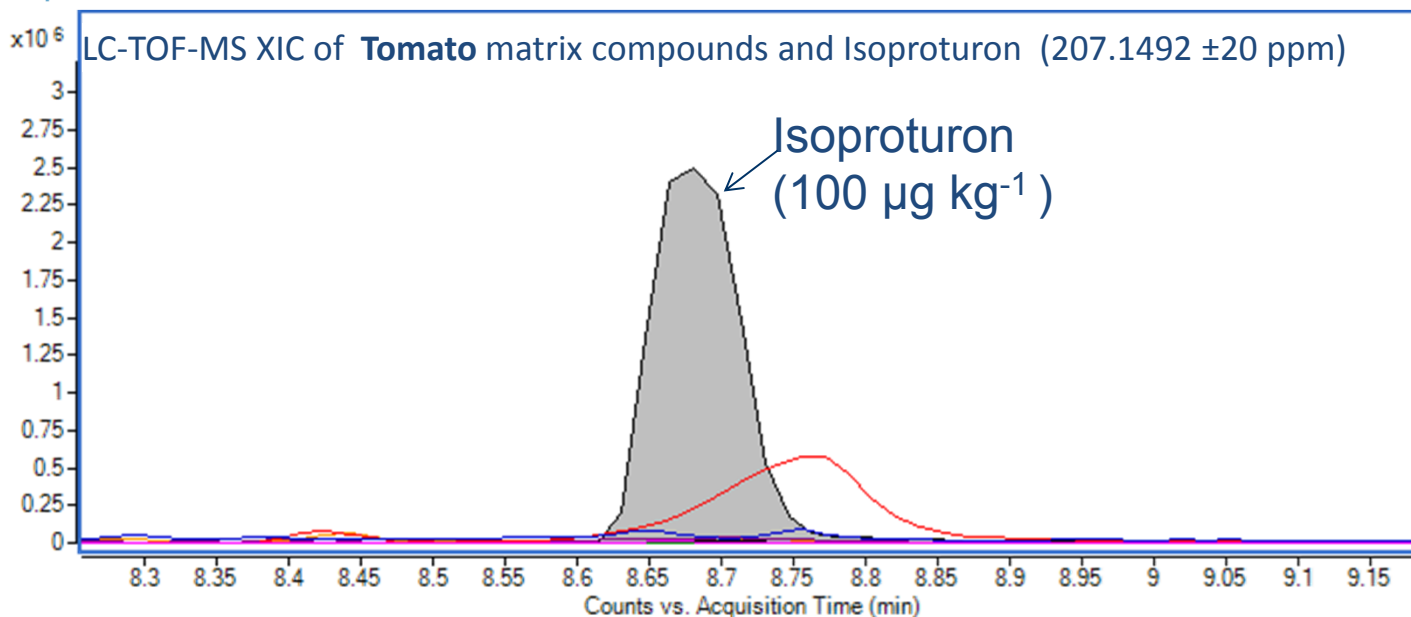


Leek 4381 Matrix compounds
Total Area $8.10 \text{ E}+9$



Commodity Groups	Matrix	N° of co-extracted compounds			TIC (Counts)	% of Pesticides with High (>50%), Medium (20-50%) and Low Signal Suppression (<20%)		
		Retention Time: 0-17min	Retention Time: 7-13 min	MW>500 Retention Time: 7-13 min		High	Medium	Low
High water content	Papaya	1270	214	38 (18%)	3.70E+09	--	--	--
	Aubergine	1400	573	36 (6%)	3.00E+09	--	--	--
	Plum	2008	260	51 (20%)	3.12E+09	--	--	--
	Lettuce	1586	625	208 (33%)	3.70E+09	--	--	--
	Tomato (Kumato type)	2155	668	147 (22%)	4.18E+09	0	10	90
	Tomato (Cherry type)	2833	746	201 (27%)	5.63E+09	0	16	84
	Pear	2919	708	173 (24%)	3.64E+09	--	--	--
	Apple	3047	726	270 (37%)	4.25E+09	--	--	--
	Mango	2649	765	199 (26%)	3.77E+09	--	--	--
	Pepper	3419	919	329 (36%)	5.51E+09	0	23	78
	Green bean	2398	913	88 (10%)	3.92E+09	--	--	--
	Asparagus	2277	1053	174 (17%)	3.33E+09	--	--	--
	Cucumber	2258	1068	181 (17%)	5.73E+09	--	--	--
	Cauliflower	2560	1101	160 (15%)	3.66E+09	--	--	--
	Zucchini	3115	1466	510 (35%)	5.78E+09	6	25	69
	Broccoli	3397	1666	359 (22%)	4.58E+09	--	--	--
	Onion (white type)	2925	1656	543 (33%)	1.05E+10	18	34	49
	Onion (red type)	3823	2113	765 (36%)	1.78E+10	56	33	11
Leek	4381	2155	382 (18%)	8.10E+09	--	--	--	
High acid content and high water content	Orange	5768	2072	879 (42%)	1.91E+10	55	33	13
	Mandarin	6060	2738	1345 (49%)	1.57E+10	--	--	--
Difficult or unique commodities	Chamomile	3784	1770	282 (16%)	1.36E+10	--	--	--
	Black tea	4910	1828	402 (22%)	1.18E+10	--	--	--
	Green tea	5793	2019	726 (36%)	1.32E+10	--	--	--
	Red tea	4504	1753	497 (28%)	1.18E+10	--	--	--





Pesticide	Mass	Rt (min)	Suppression	Nº of co-eluting matrix compounds (± 0.05 min)	Σ Compounds signal height of co-eluting compounds (counts)
Cinosulfuron	413.1005	8.09	-6	63	3E+06
Propoxur	209.1052	8.23	0	44	4E+06
Carbofuran	221.1046	8.32	0	34	3E+06
Forchlorfenuron	247.0512	8.54	-16	32	1E+06
Fluometuron	232.0823	8.54	-11	33	1E+06
Isoproturon	206.1419	8.73	-88	84	2E+07
Metalxyl	279.1465	8.74	-74	84	2E+07
Ofurace	281.0819	8.84	1	39	2E+06
Heptenophos	250.0162	8.97	-8	65	7E+06
Isoprocab	194.1176	9.01	-100	94	2E+07
Spinosyn A	731.4603	9.02	-61	99	2E+07
Flazasulfuron	407.0511	9.22	-93	83	2E+07
Metazachlor	277.0982	9.25	-83	93	3E+07
Bupirimate	316.1563	9.27	-83	125	4E+07
Cypodiril	226.126	9.35	-87	76	2E+07
Triadimenol	295.1082	9.42	-18	45	9E+06
Promecarb	207.1259	9.95	-15	42	3E+06
Azoxystrobin	403.1162	10.03	-4	29	5E+06

> 75

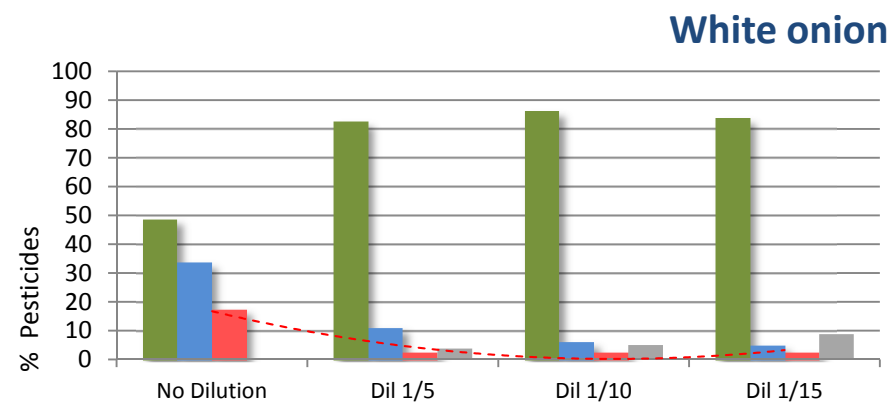
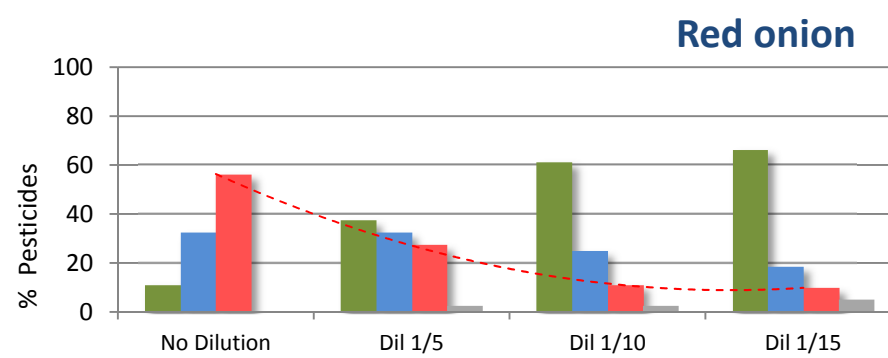
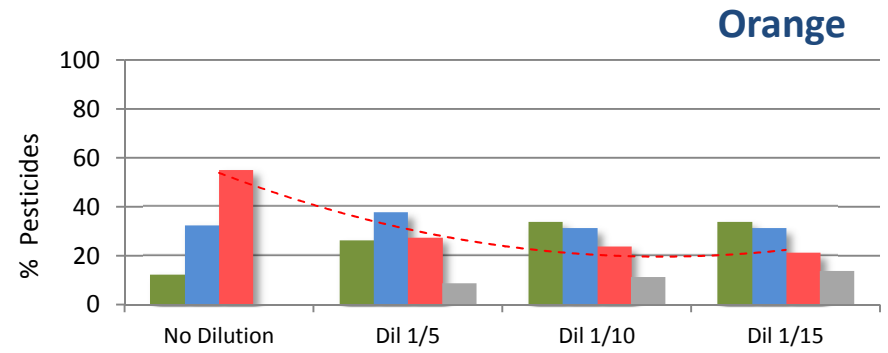
Evaluation of the effect of the Amount of Matrix Injected (Dilution)

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



**GC-Q-MS Liner after
40 tea injections**



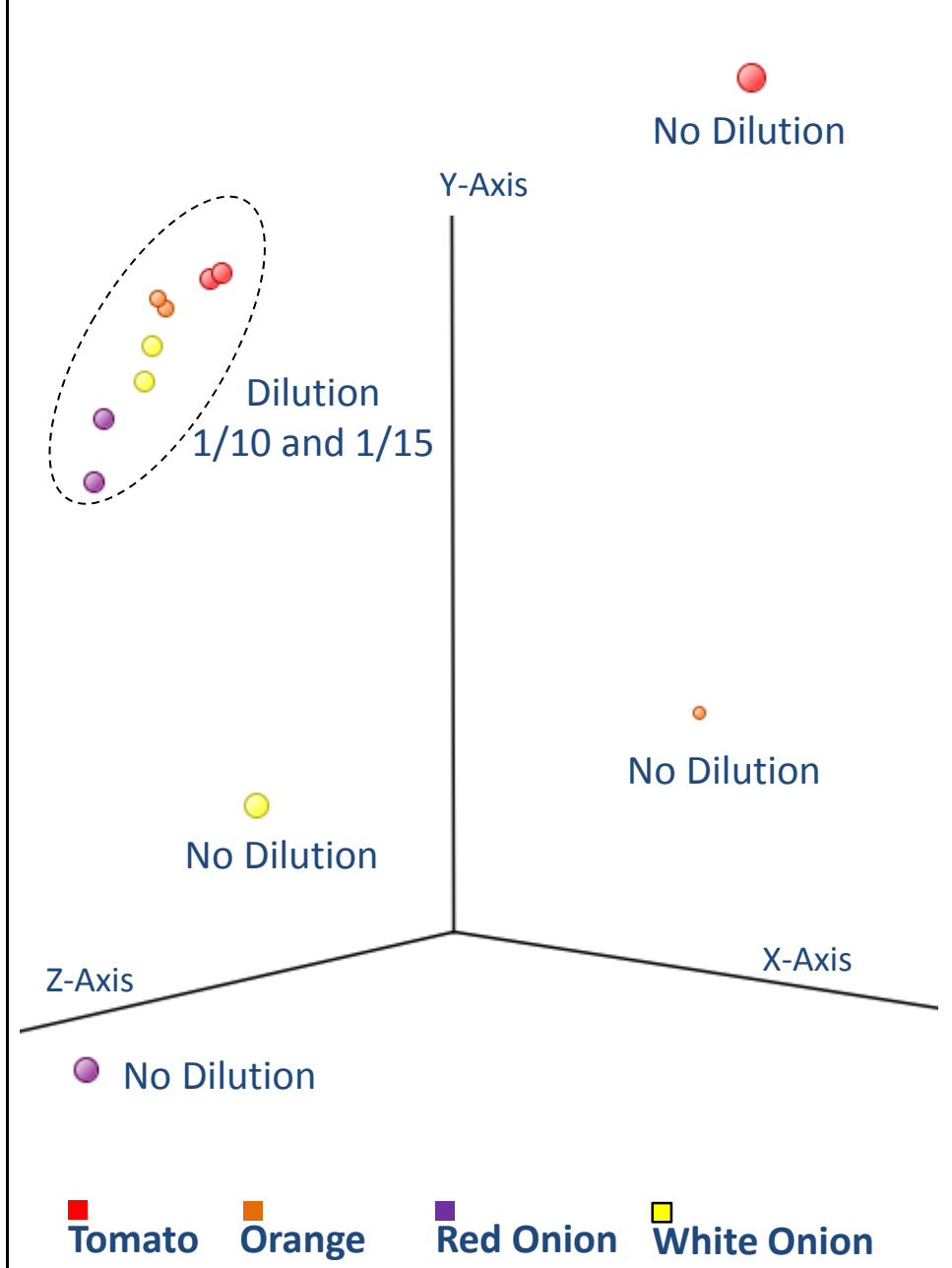


1g/mL 0.2g/mL 0.1g/mL 0.07g/mL

■ <20 % ■ 20-50 % ■ >50% ■ Nd

Matrix Suppression

Principal Components Analysis (PCA)

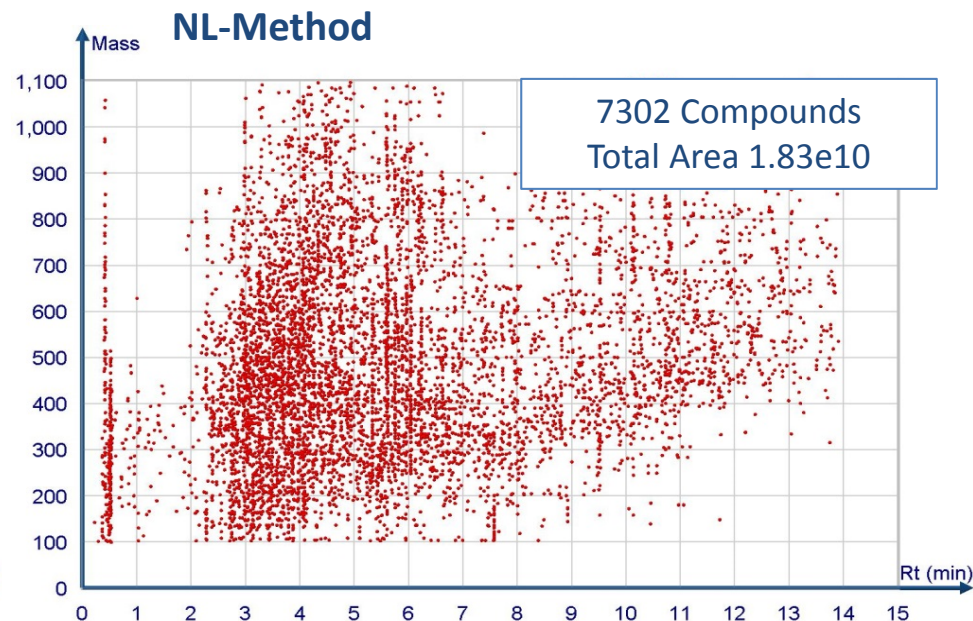
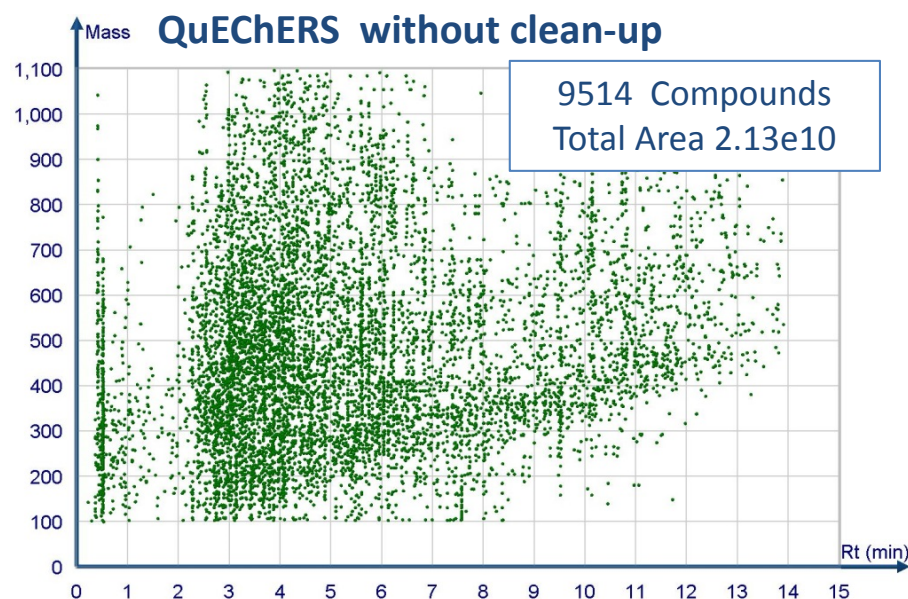
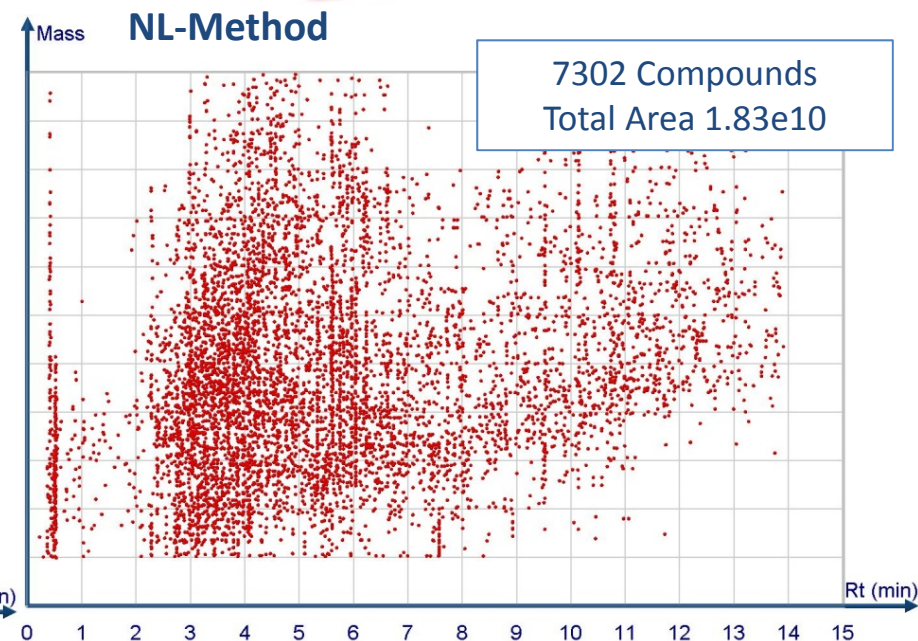
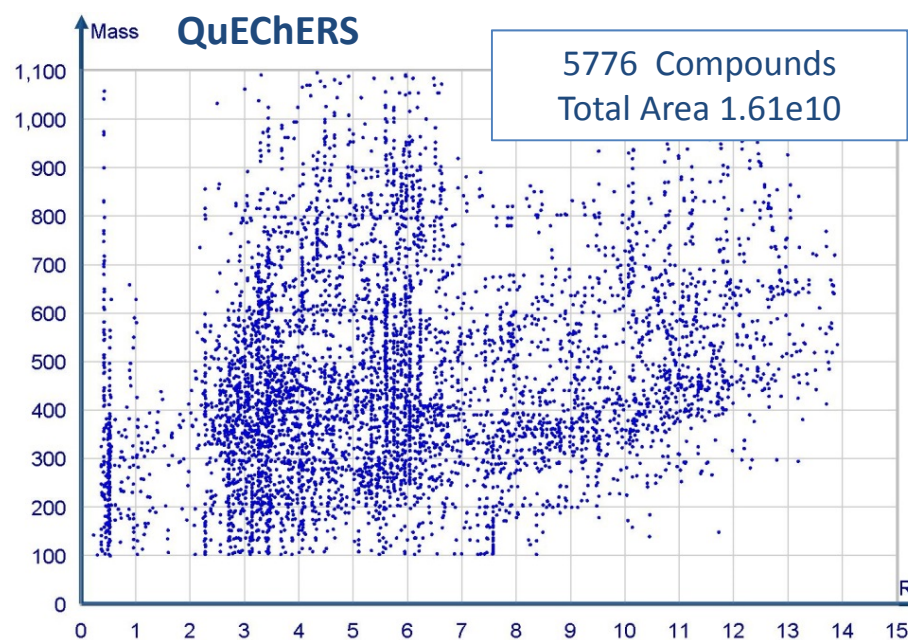


Co-extracted matrix components of orange Dilution 1/5

Absolute height > 10000 counts LC-TOF-MS



0.2 g sample/ mL

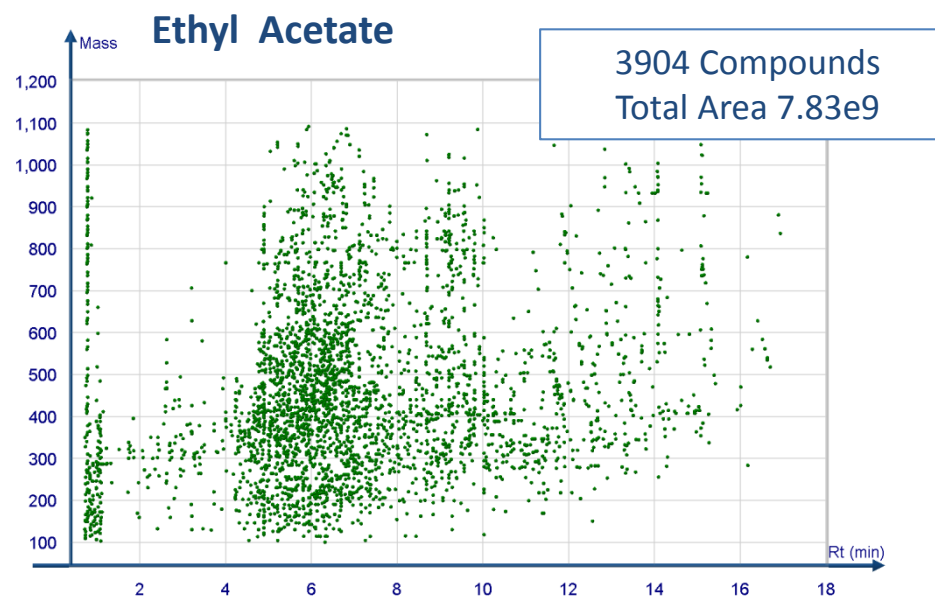
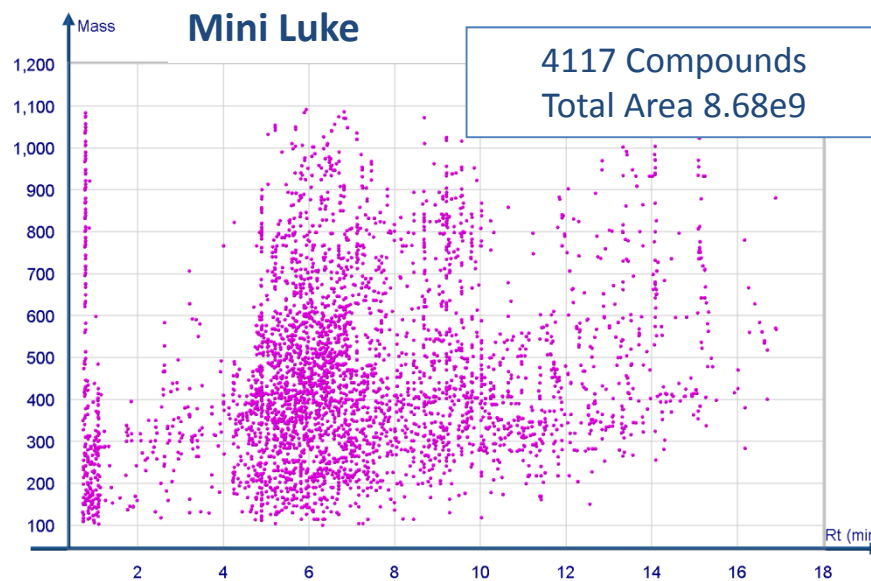
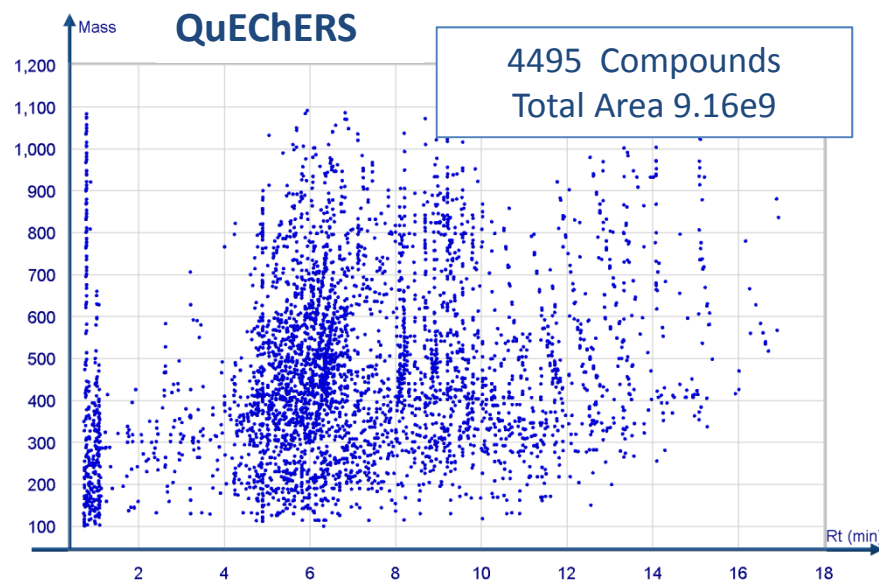


Co-extracted matrix components of orange Dilution 1/10

Absolute height > 10000 counts LC-TOF-MS



0.1 g sample/ mL





Evaluation of Matrix effects VS Multi Residue Methods

Influence on Identification (false positives and negatives)



Pear



Cucumber



Potato



Orange



Green bean



Melon



Lemon



Apple



Lettuce



Strawberry



Cauliflower



Cherry



Onion



Pumpkin



Broccoli



Aubergine



Celery



Avocado



Leek



Banana



Pepper



Green tea



Parsley



Tomato



Carrot

25 Commodites

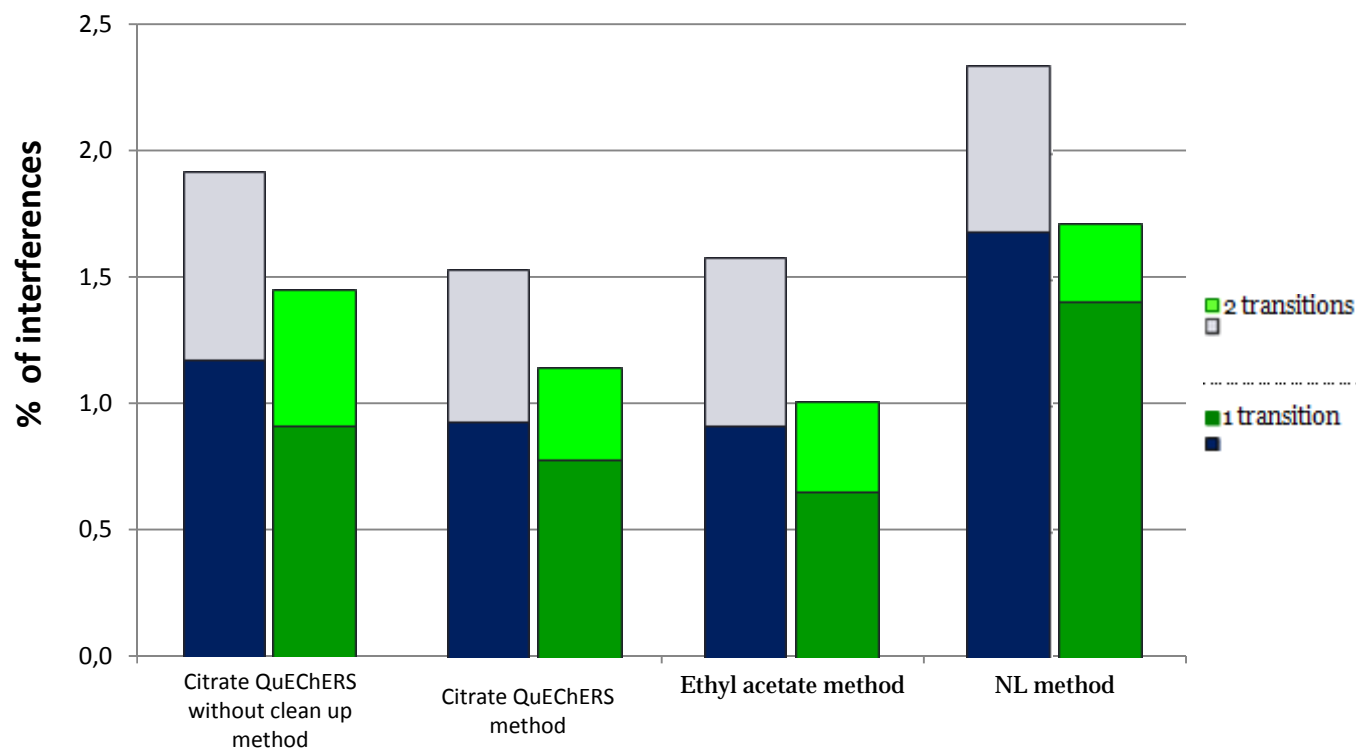
Methods

- **Citrate QuEChERS with clean-up**
 - Sample weigh: 10 g
 - Extraction solvent: Acetonitrile
 - Final extract: 1g sample/mL extract
- **Citrate QuEChERS without clean-up**
 - Sample weigh: 10 g
 - Extraction solvent: Acetonitrile
 - Final extract: 1g sample/mL extract
- **Ethyl acetate method**
 - Sample weigh: 10 g
 - Extraction solvent: Ethyl Acetate
 - Final extract: 1g sample/mL extract
- **NL method**
 - Sample weigh: 15 g
 - Extraction solvent: Acetone
 - Final extract: 0.3 g sample/mL extract

Total number of results= 225 compounds · 25 commodities = 5625 results

GC-QqQ-MS/MS

Percentage of Common Transitions by method
(same transition(s) at different retention time windows)

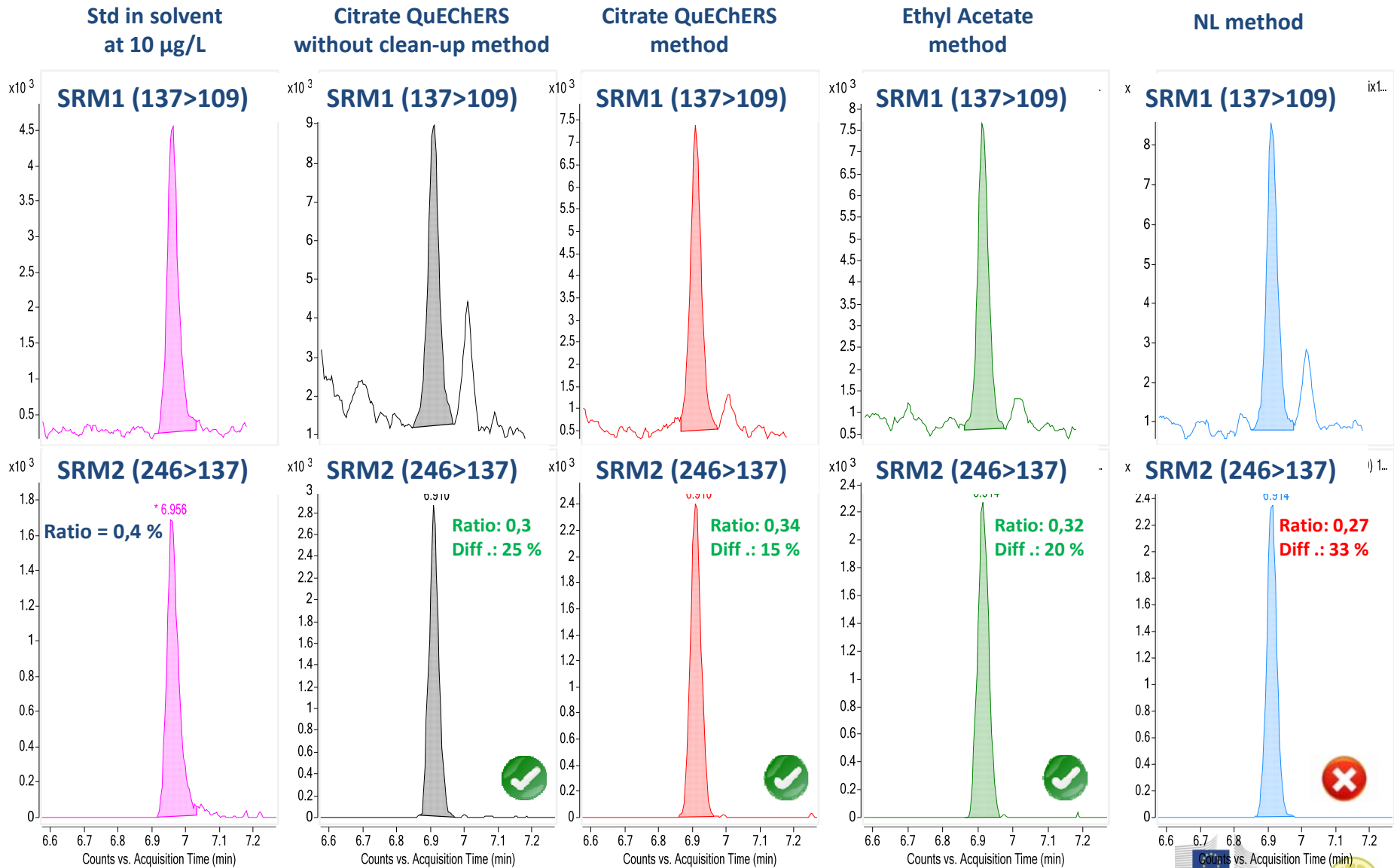


Blue 0.2 min. Green 0.1 min.

1g sample /mL extract

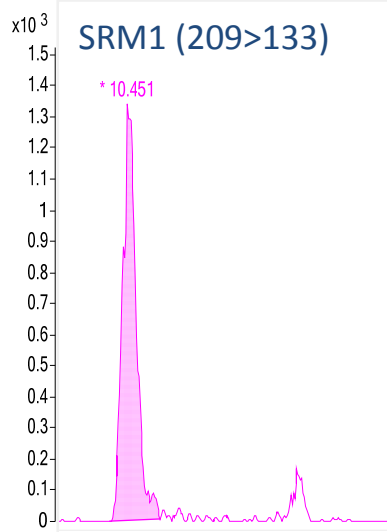
Amadeo R. Fernández-Alba

Fonofos at 10 µg/kg in Avocado. GC-MS/MS

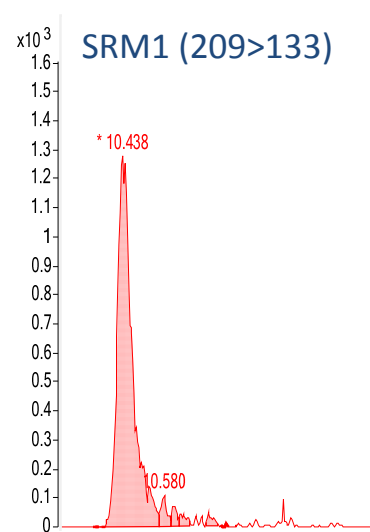


Metazachlor at 10 µg/kg in Celery. GC-MS/MS

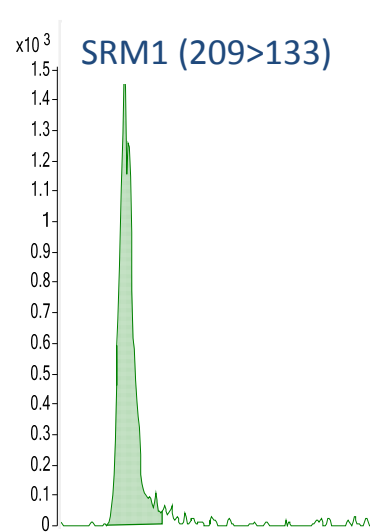
Std in solvent at
10 µg/L



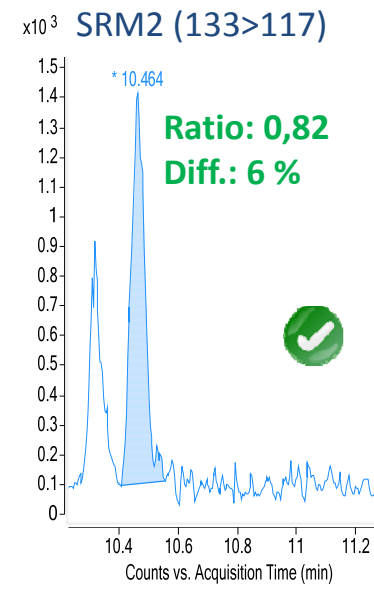
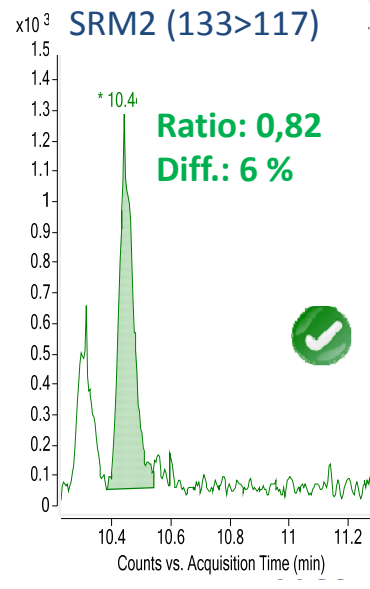
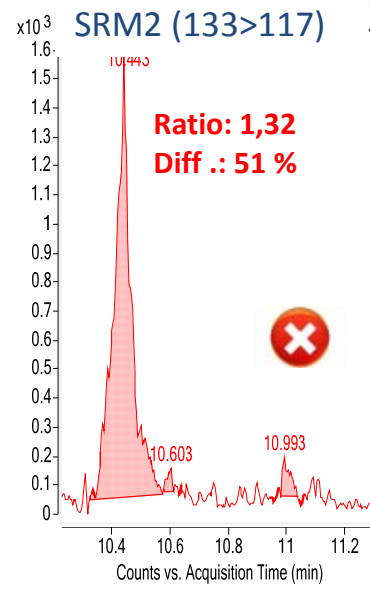
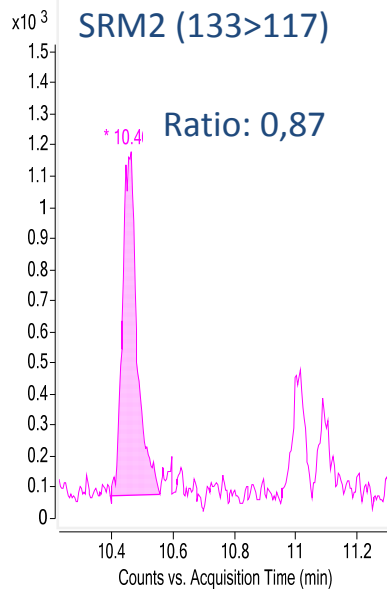
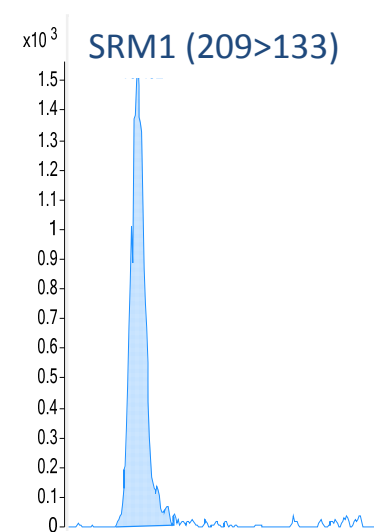
Citrate QuEChERS
method



Ethyl Acetate
method

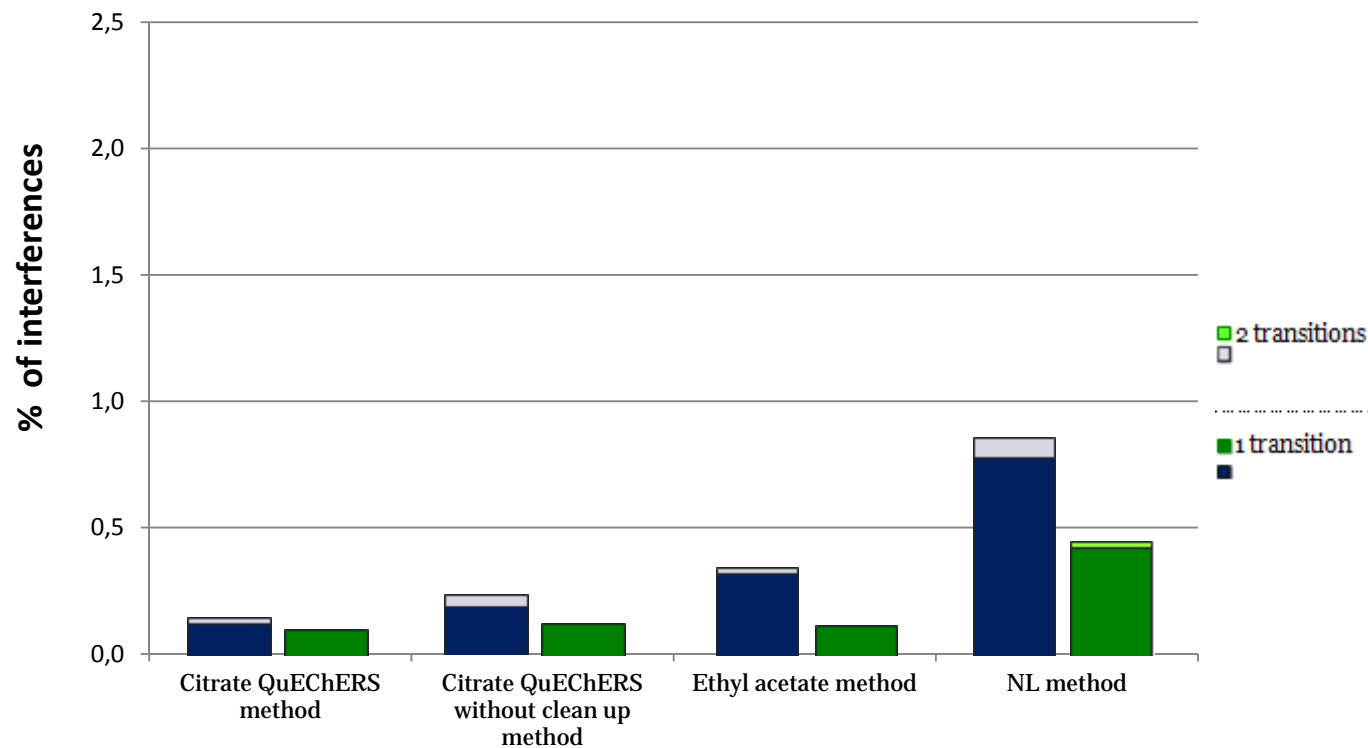


NL method



LC-QqQ-MS/MS

Percentage of Common Transitions by method
(same transition(s) at different retention time windows)



Blue 0,2 min. Green 0,1 min.

Haloxyfop at 10 µg/kg in Aubergine. LC-MS/MS

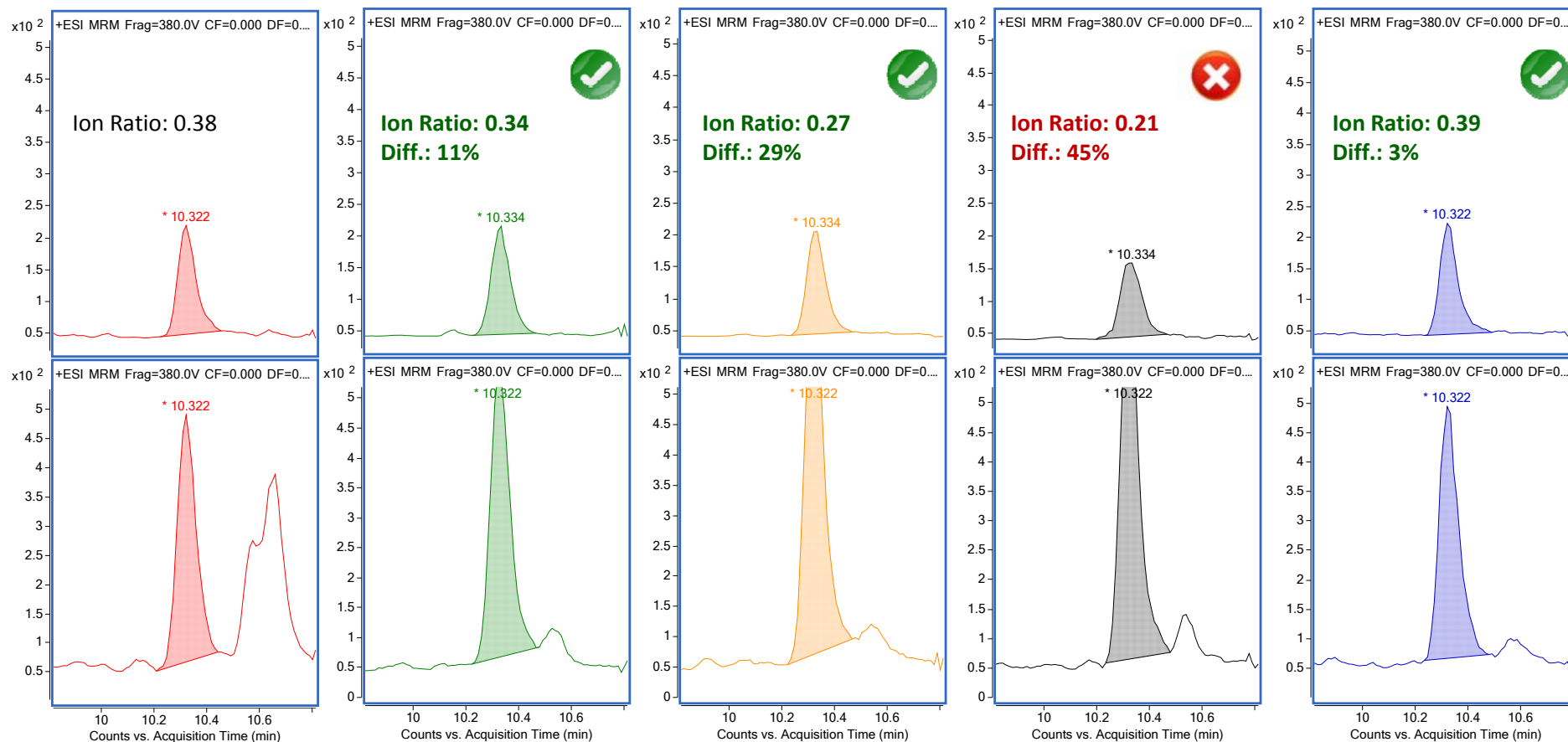
Std in solvent at
10 µg/L

Ethyl Acetate
method

NL method

Citrate QuEChERS without
clean-up method

Citrate QuEChERS
method



Azinphos methyl at 10 µg/kg in Onion. LC-MS/MS

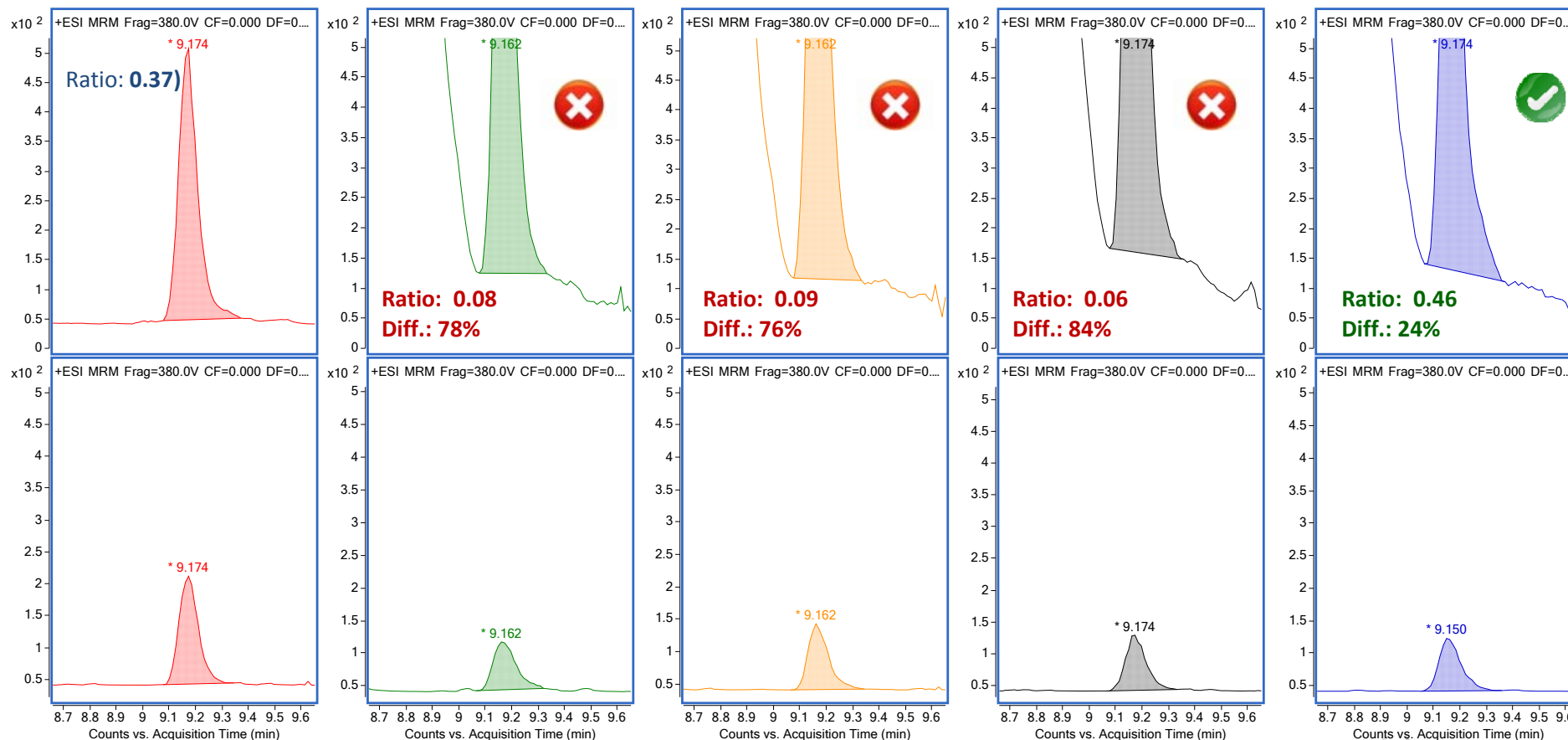
Std in solvent at
10 µg/L

Ethyl Acetate
method

NL mehod

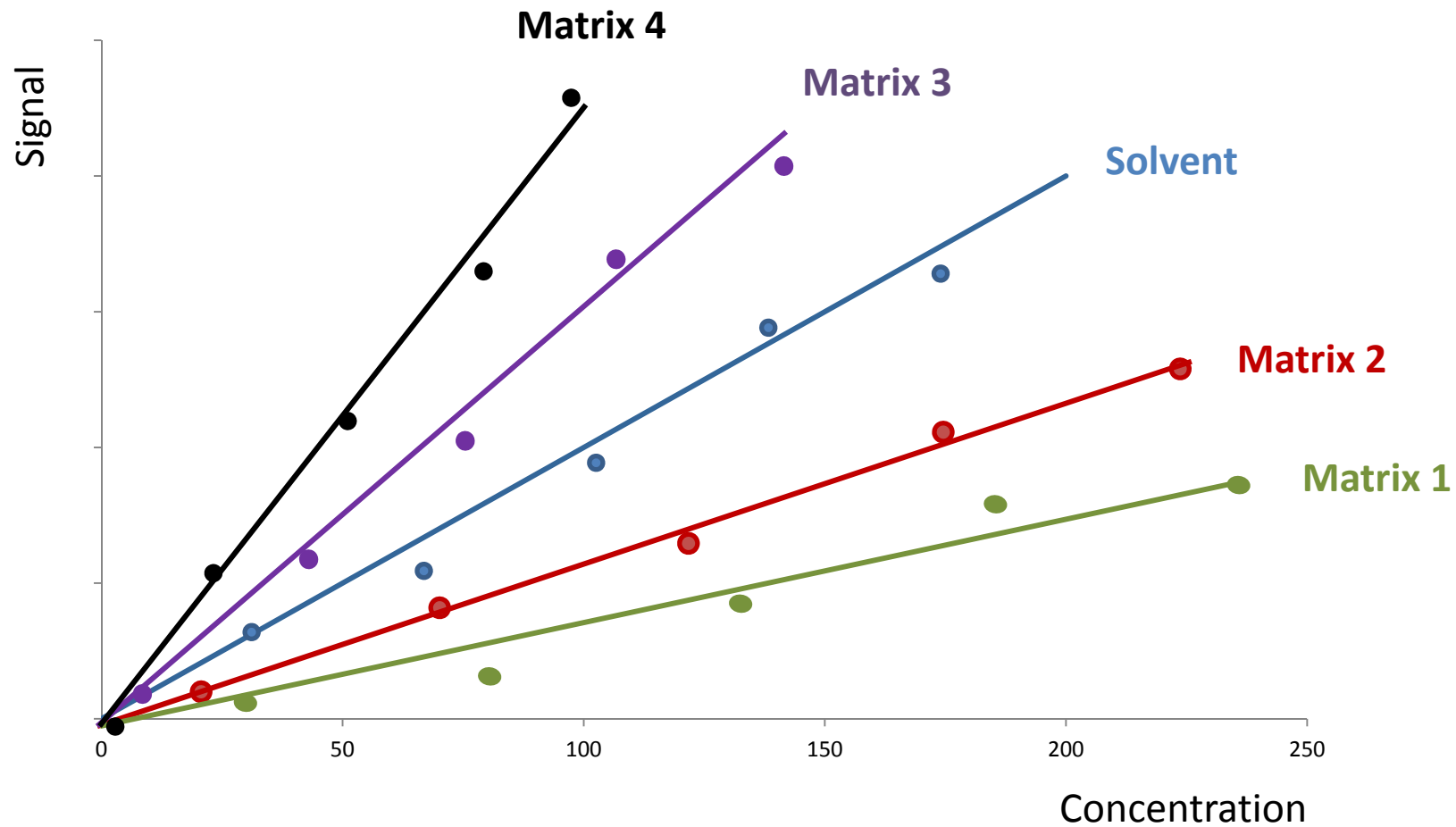
Citrate QuEChERS without
clean-up method

Citrate QuEChERS
method



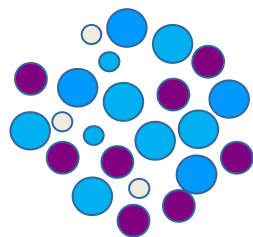
Evaluation of Matrix effects VS Multi Residue Methods

Influence on Quantification



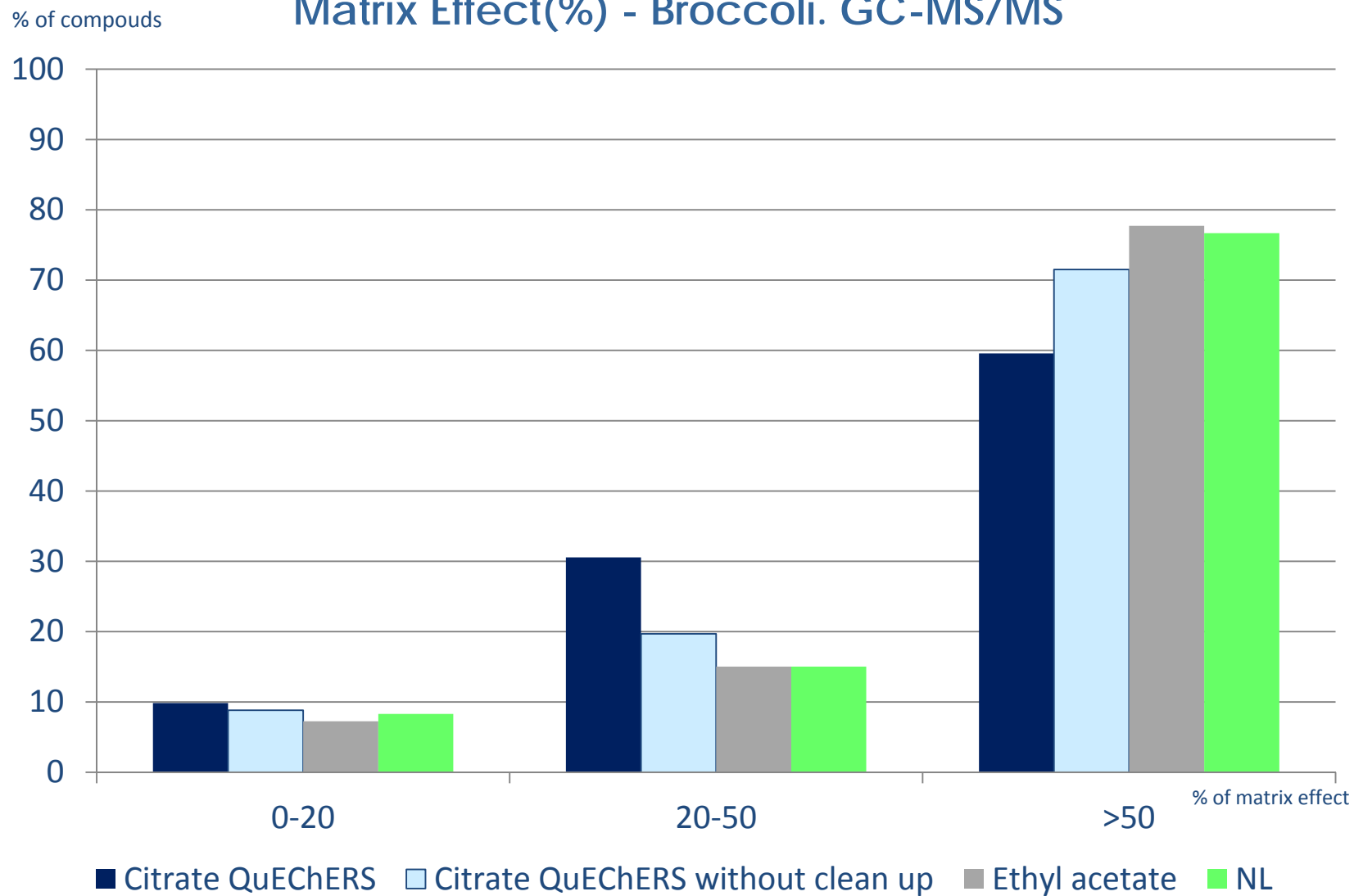


GC-QqQ-MS/MS

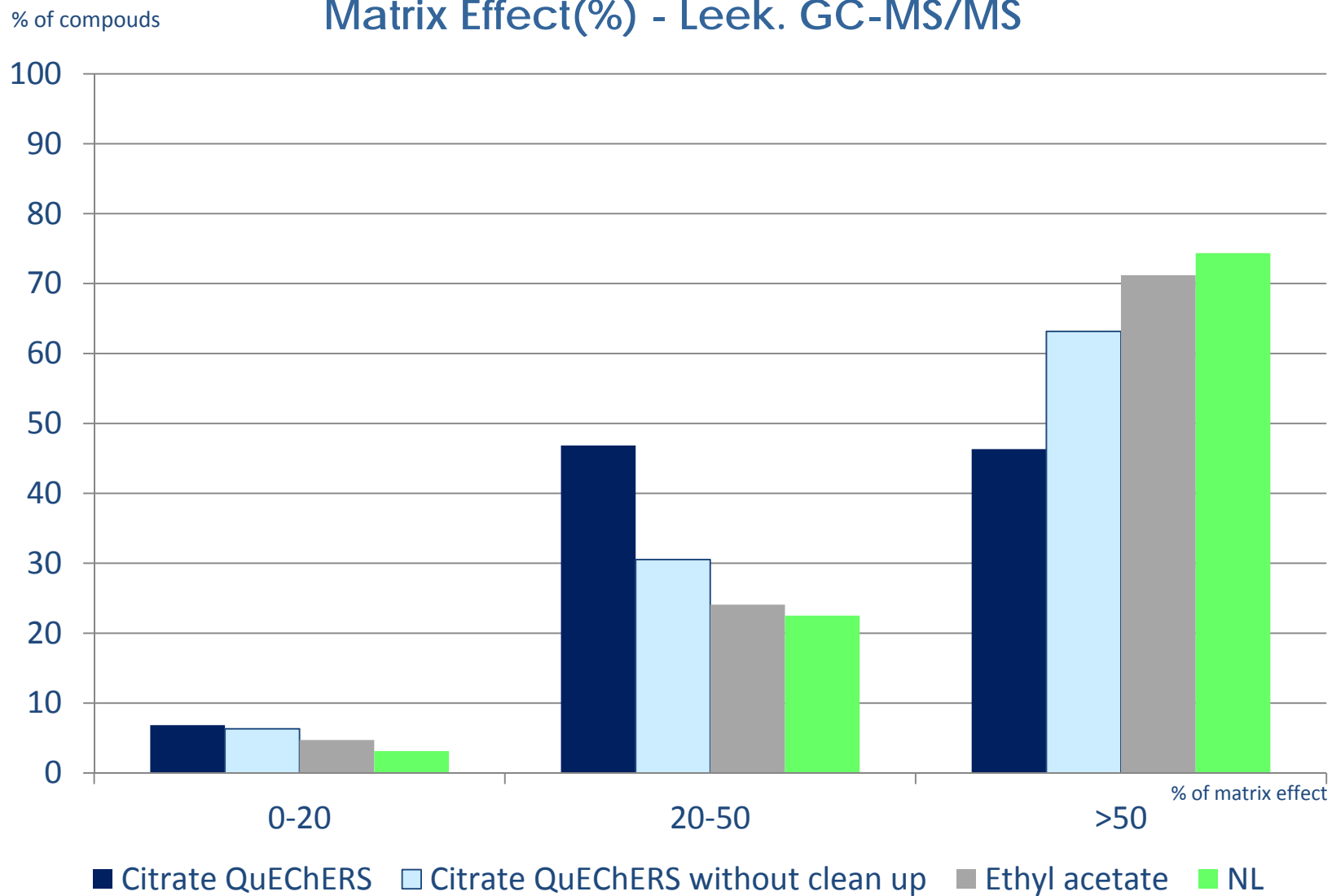


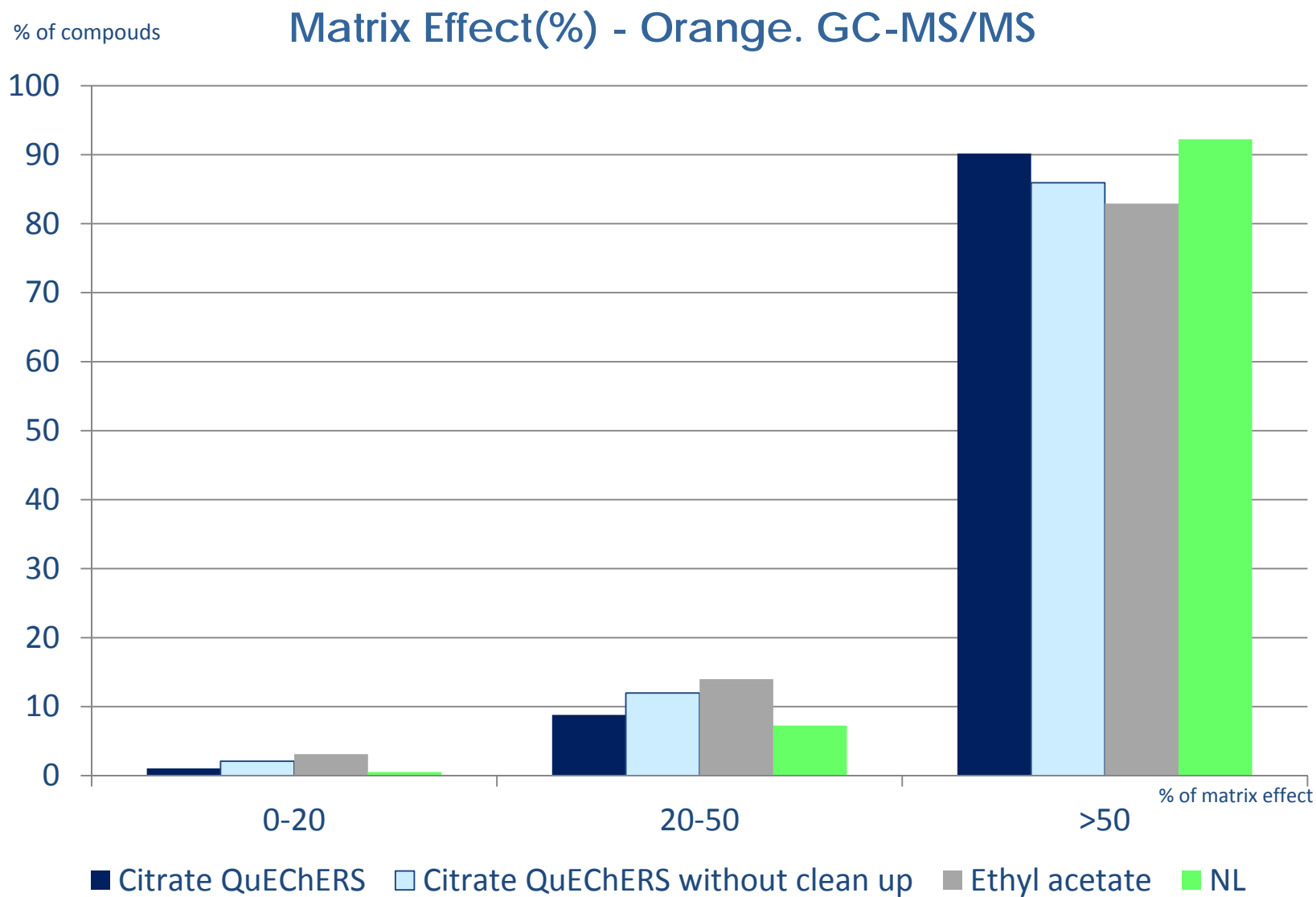
- Solvent
- Pesticide
- Matrix

Matrix Effect(%) - Broccoli. GC-MS/MS



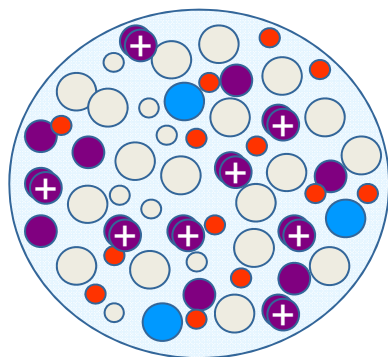
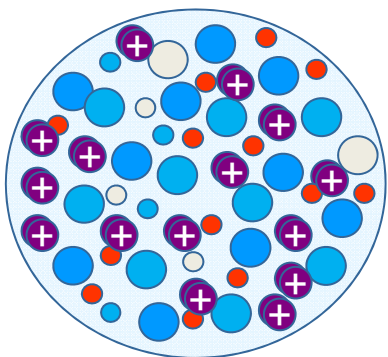
Matrix Effect(%) - Leek. GC-MS/MS





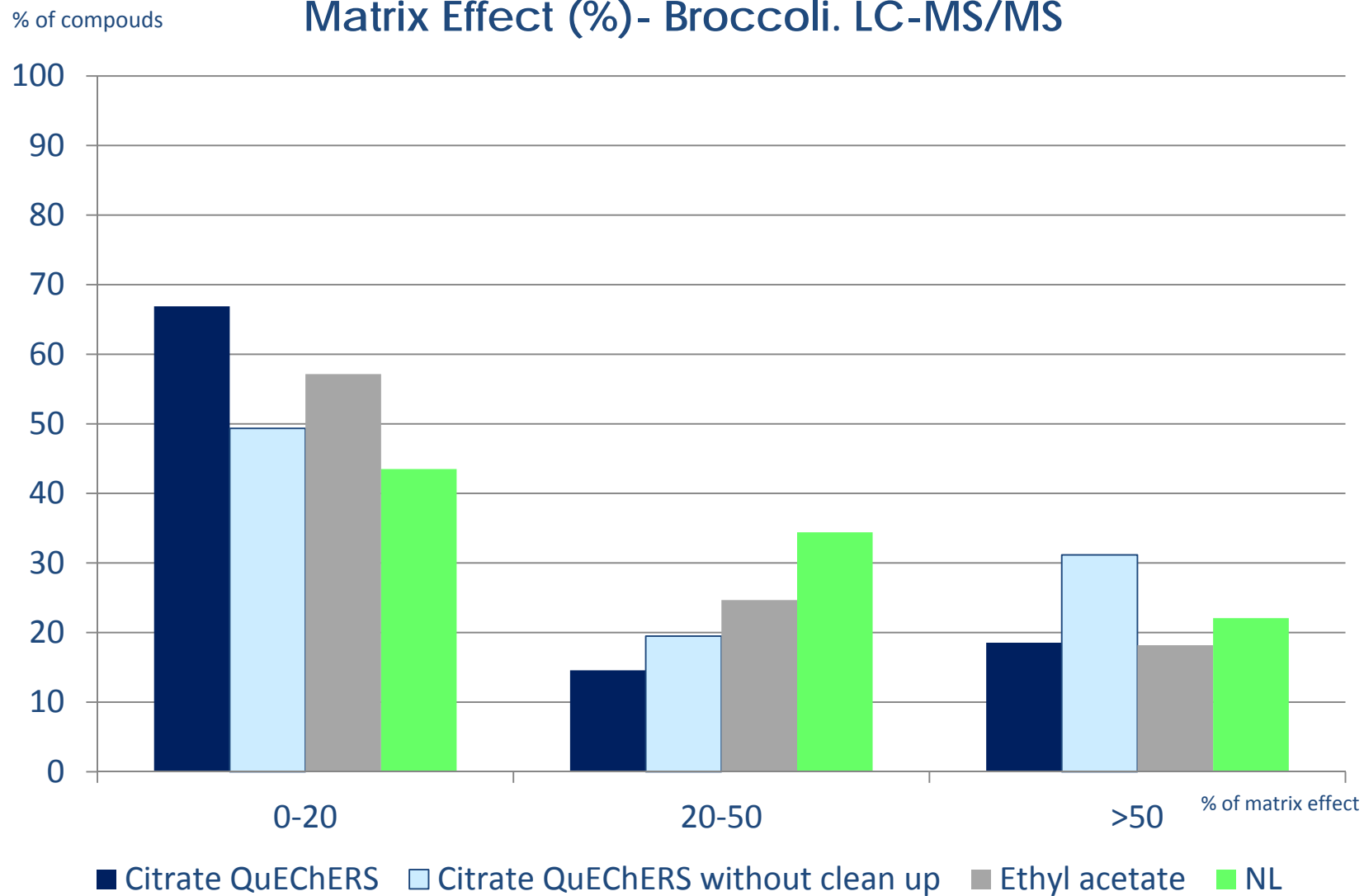


LC-QqQ-MS/MS

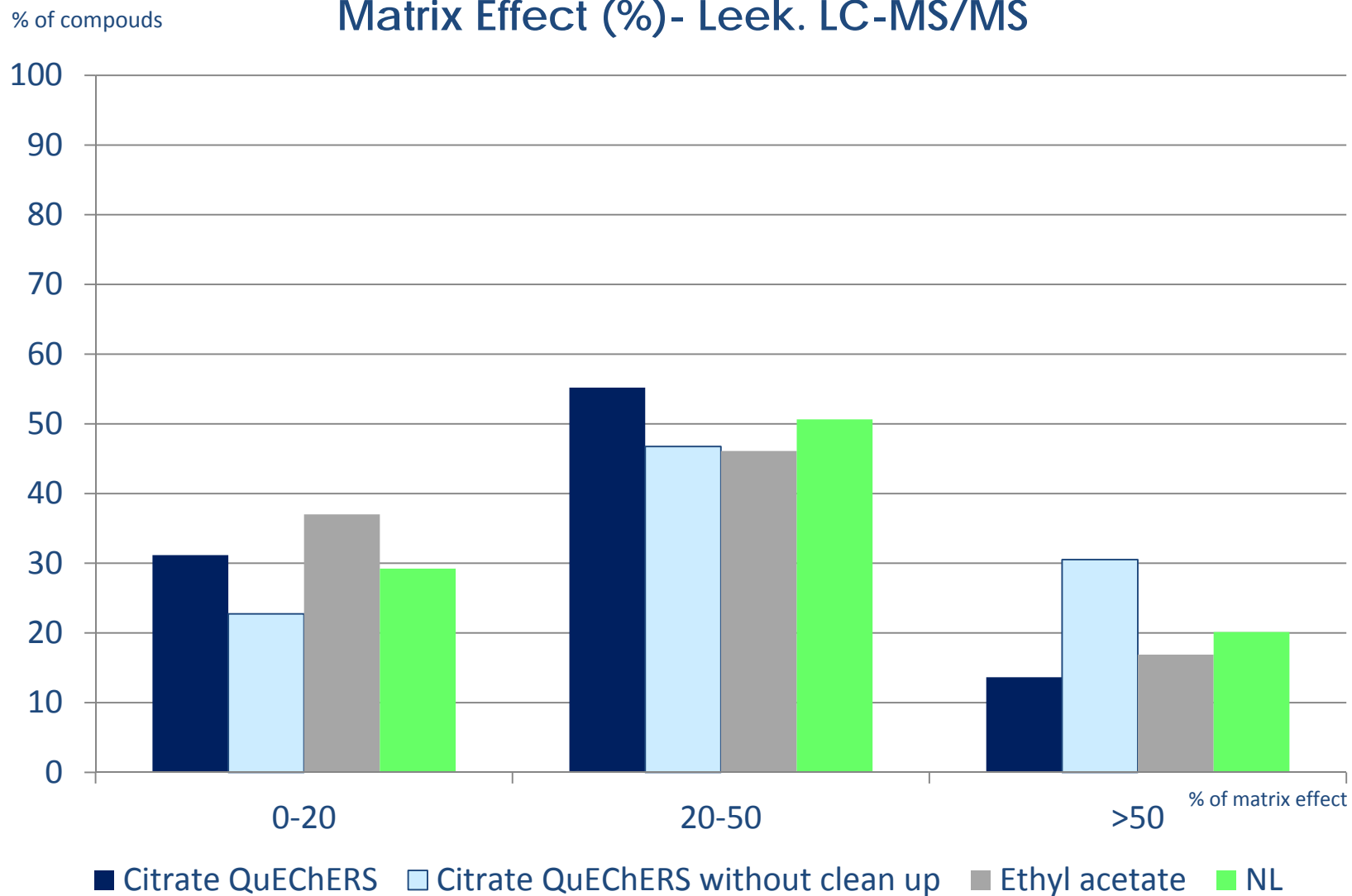


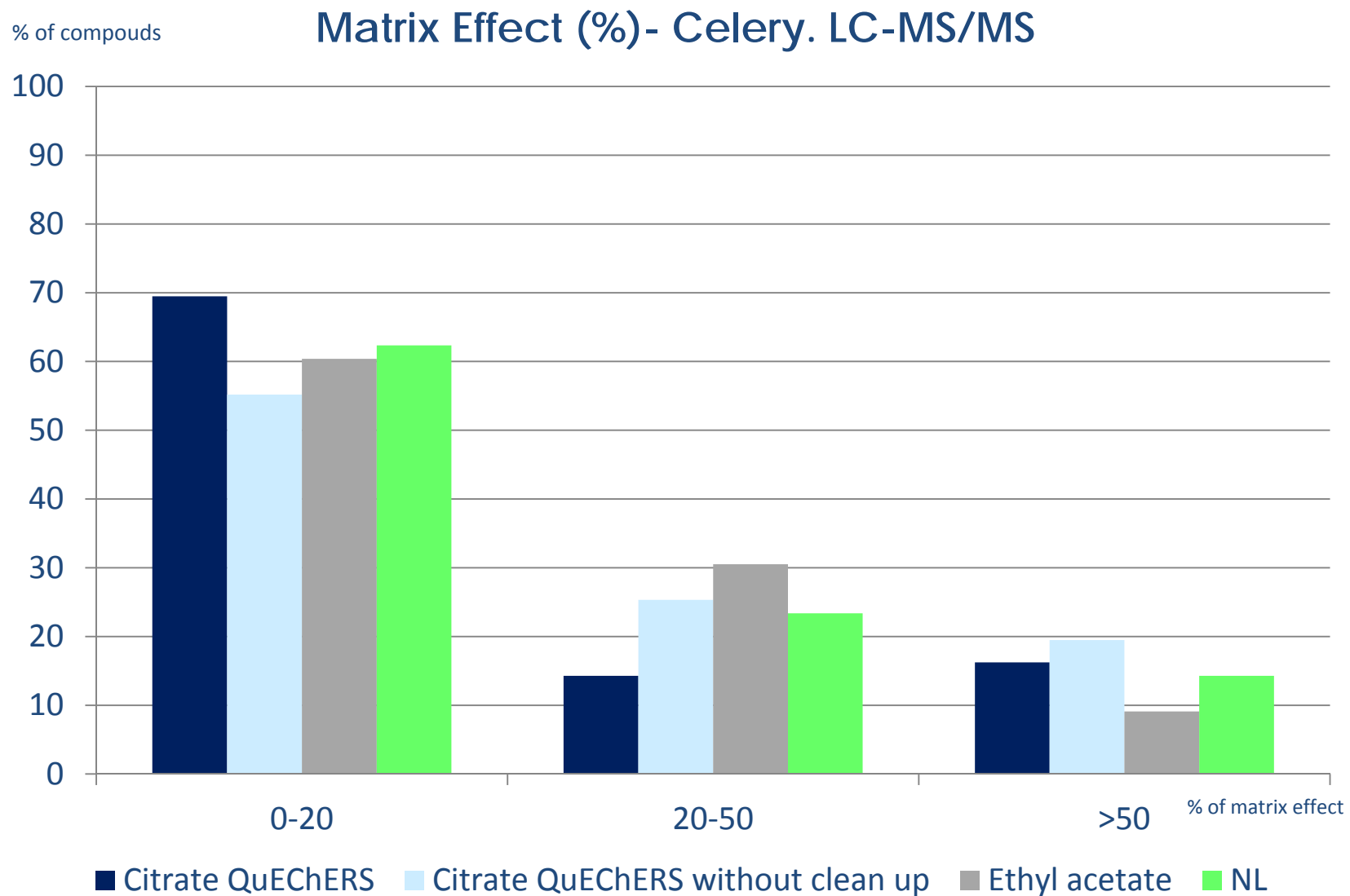
- Solvent
- Pesticide
- Matrix
- Compound that interacts with the pesticide

Matrix Effect (%) - Broccoli. LC-MS/MS



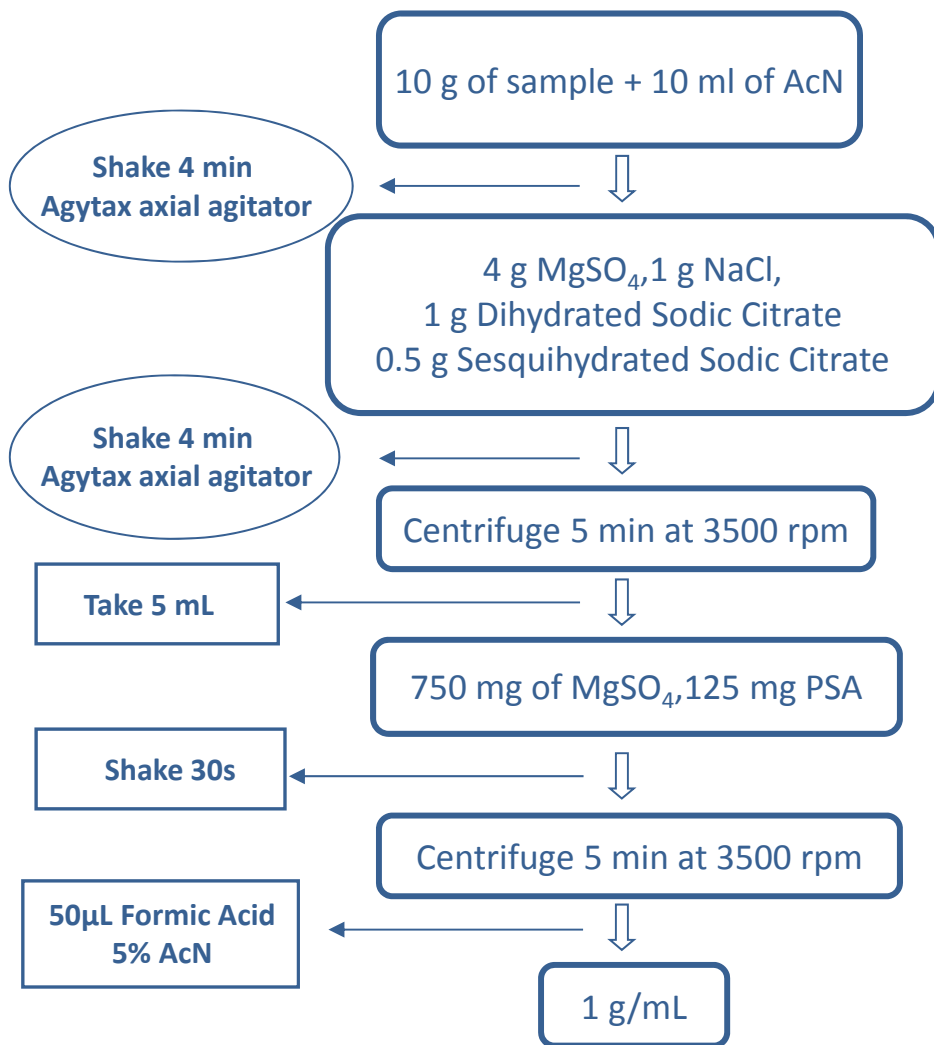
Matrix Effect (%)- Leek. LC-MS/MS



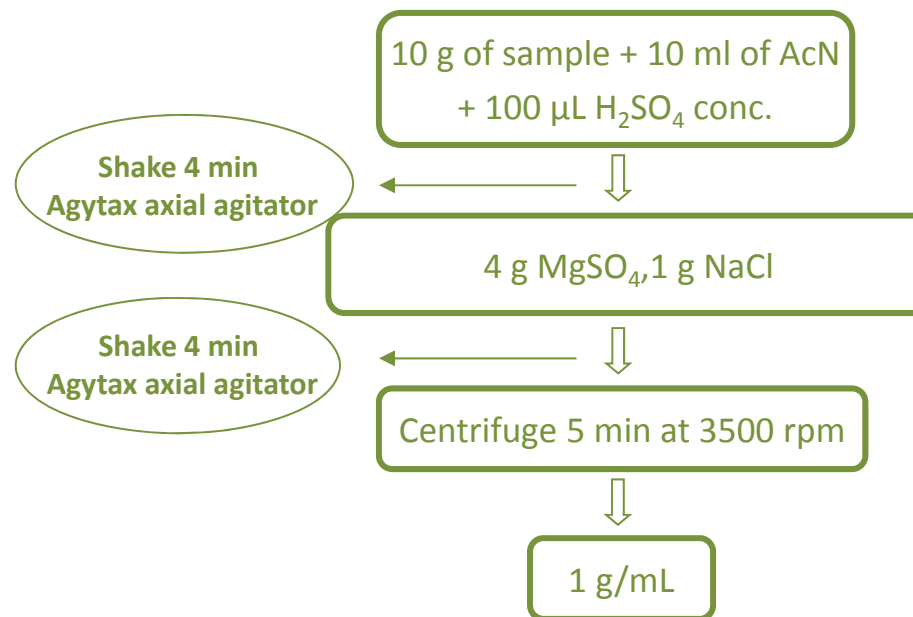


Extractability

Citrate QuEChERS

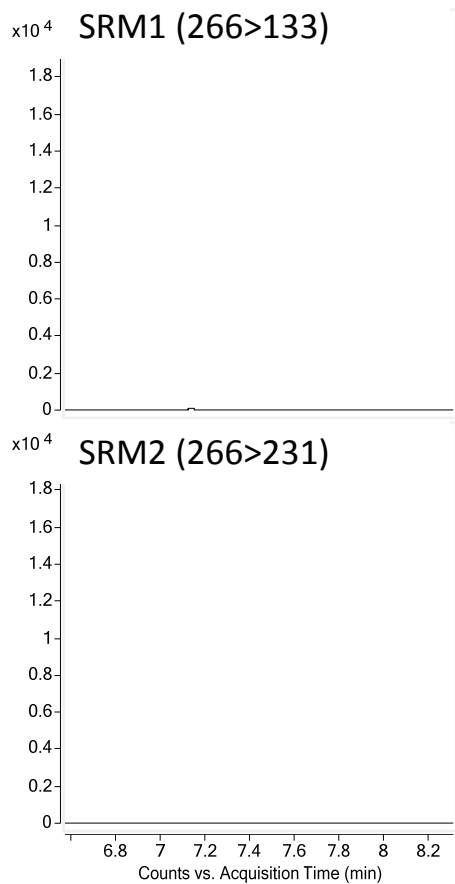


QuEChERS with acid hydrolysis

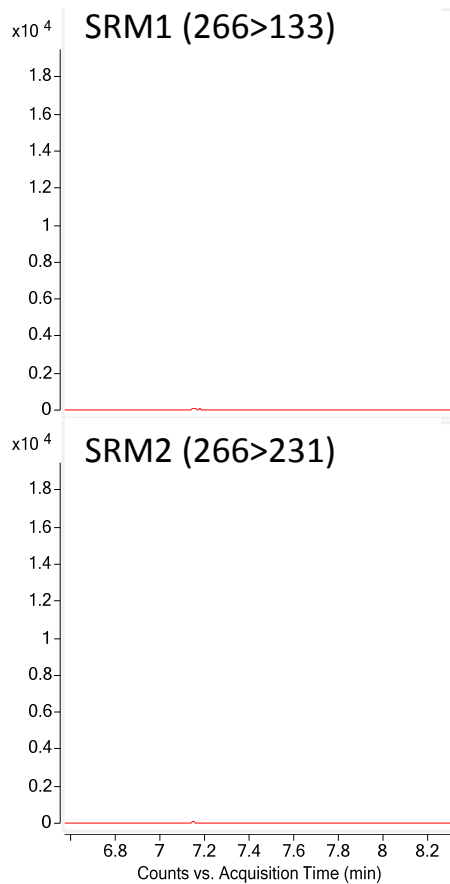


Citrate QuEChERs

**100 µg/kg
Std-Chlorothalonil
in Broccoli**

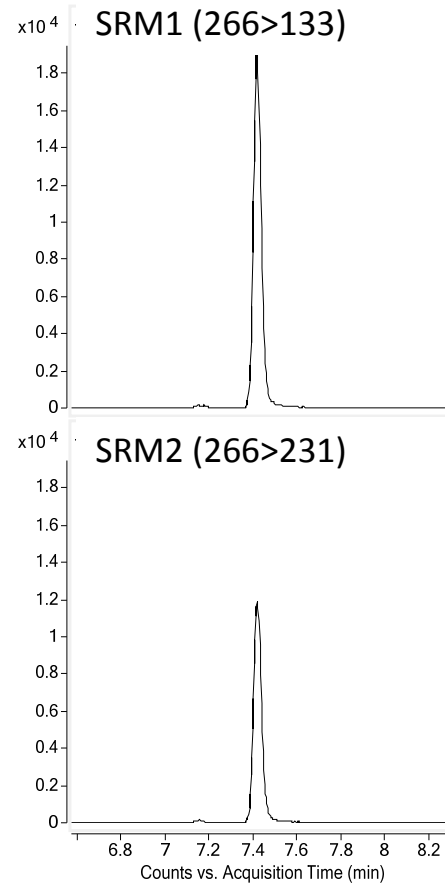


**Recovery at 100 µg/kg
Chlorothalonil in Broccoli**

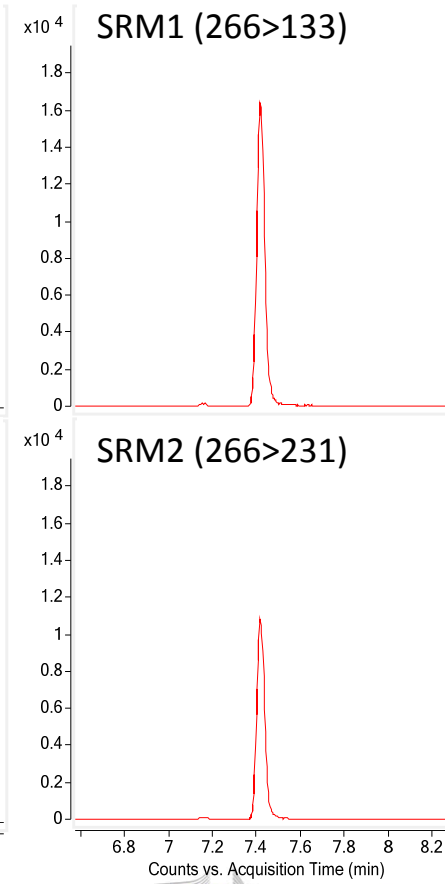


QuEChERs with acid hydrolysis

**100 µg/kg
Std-Chlorothalonil
in Broccoli**



**Recovery at 100 µg/kg
Chlorothalonil in Broccoli**



Mapping approach:

- Evaluation of Commodities
- Dilution effect
- Evaluation of MRMs



MRMs vs Identification
MRM vs Quantification



Extractability



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**Thank You
for Your Attention**



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