4th Joint Workshop of the EUROPEAN UNION REFERENCE LABORATORIES

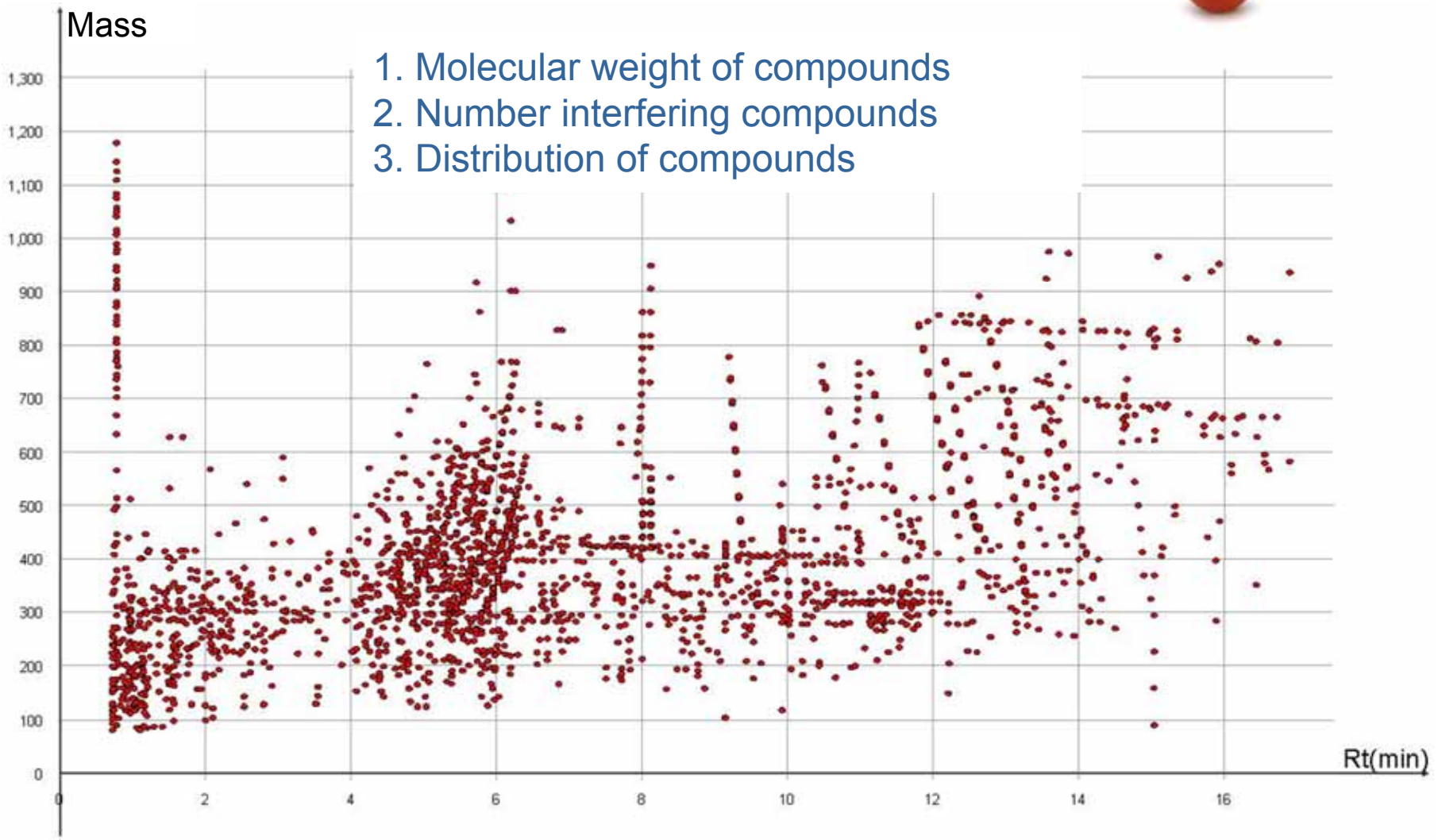
EURL
European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables

Almería (SPAIN) 23rd-25th OCT 2013

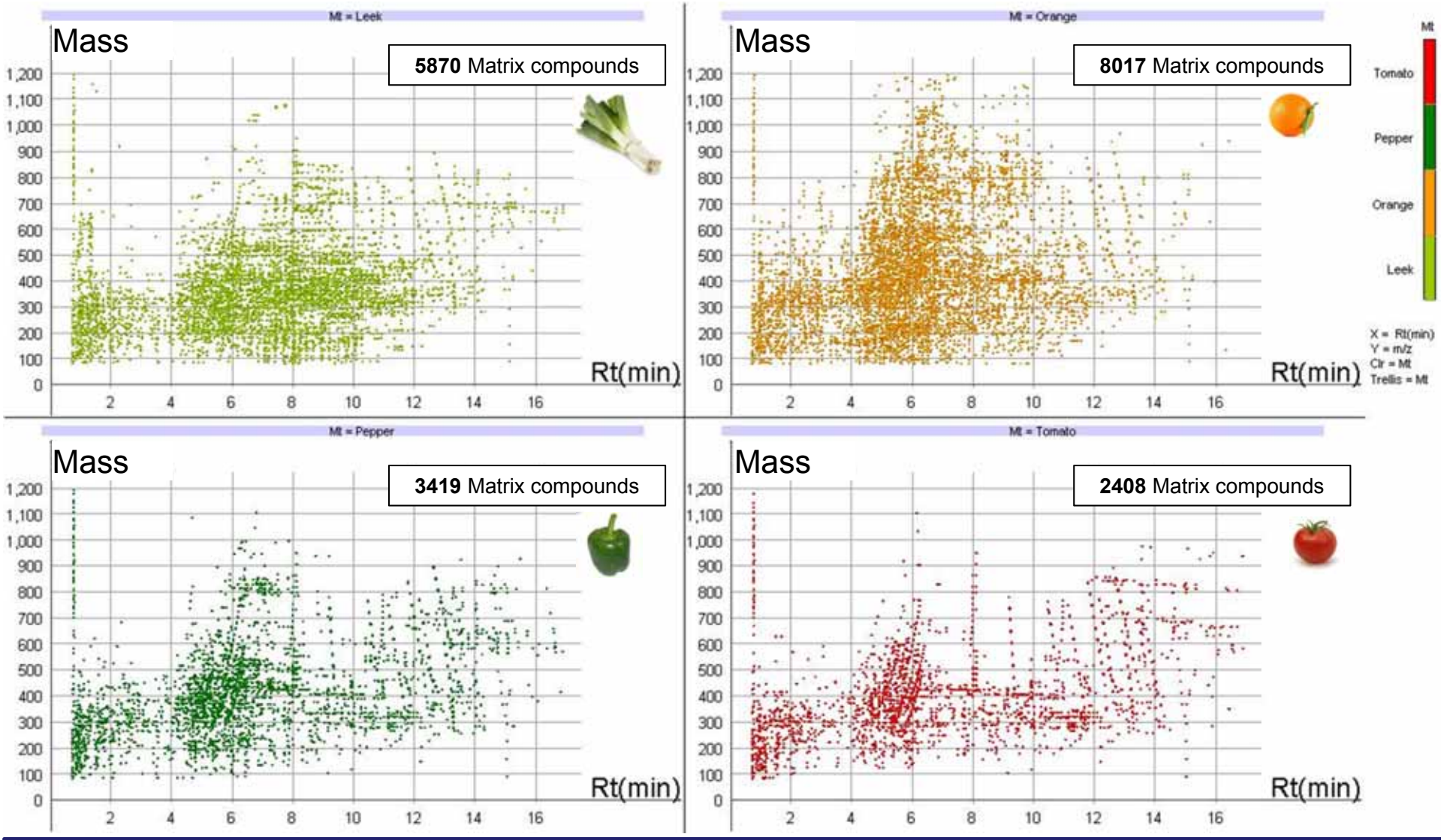
EVALUATION OF MATRIX EFFECTS BY LC-HRMS

European Commission
EURL
EUROPEAN UNION REFERENCE LABORATORIES

Co-extracted matrix components of tomato. LC-TOF-MS



Co-extracted matrix components LC-TOF-MS

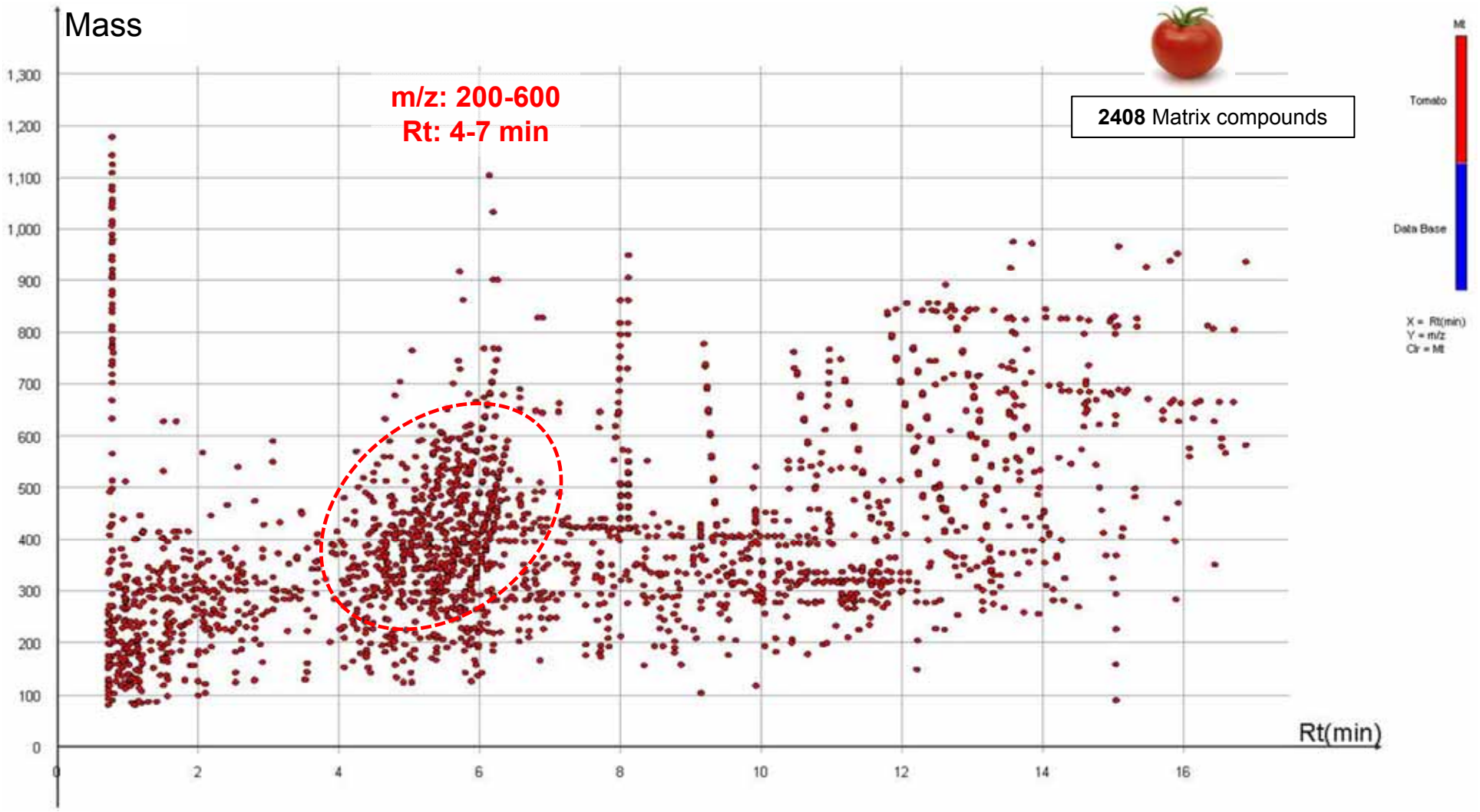


Compounds with absolute height ≥ 10000 counts

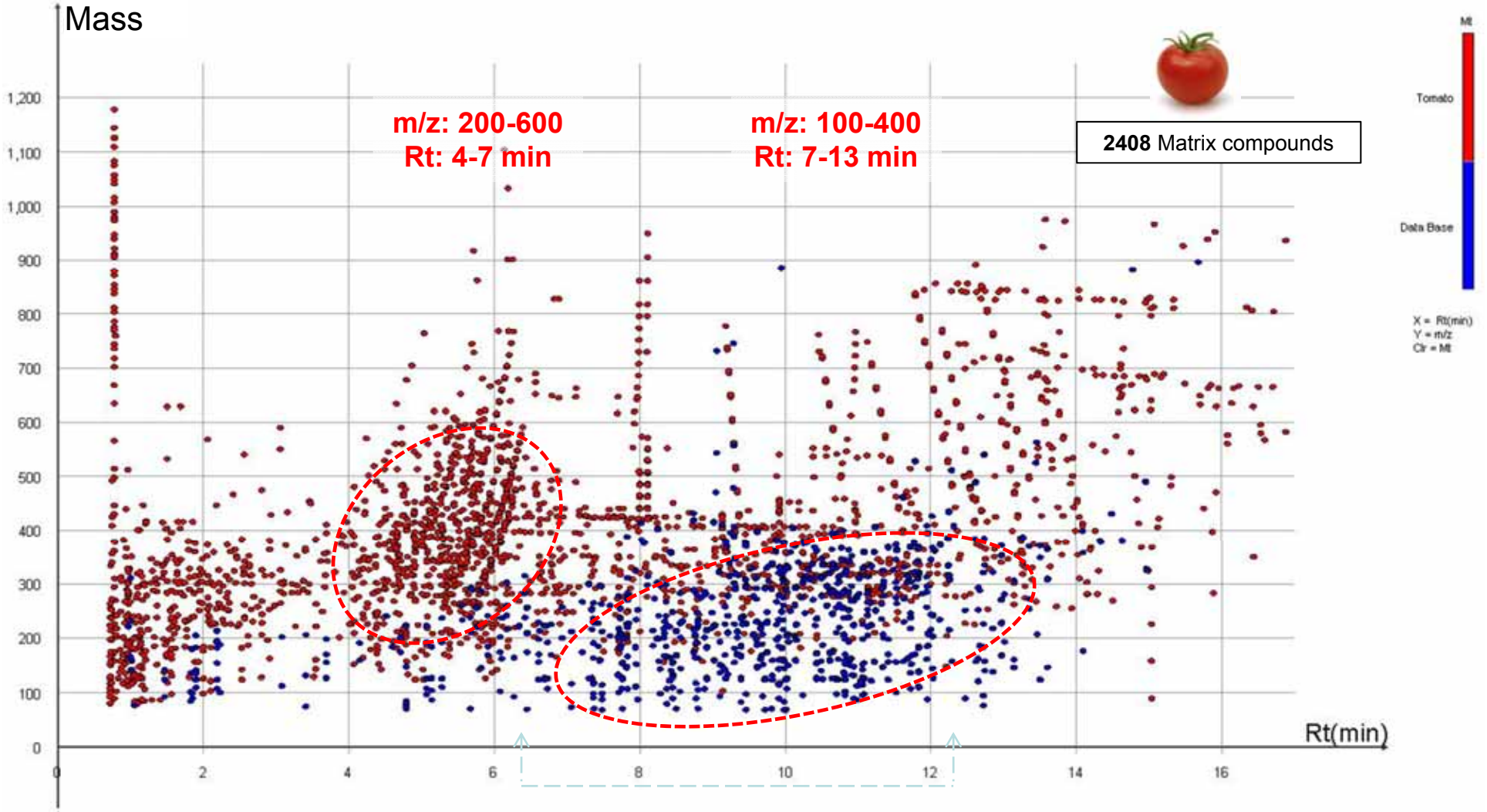
LC-QqQ-MS/MS
Skimer
After 30 tea injections

GC-Q-MS Liner after
40 tea injections

Co-extracted matrix components of tomato. LC-TOF-MS



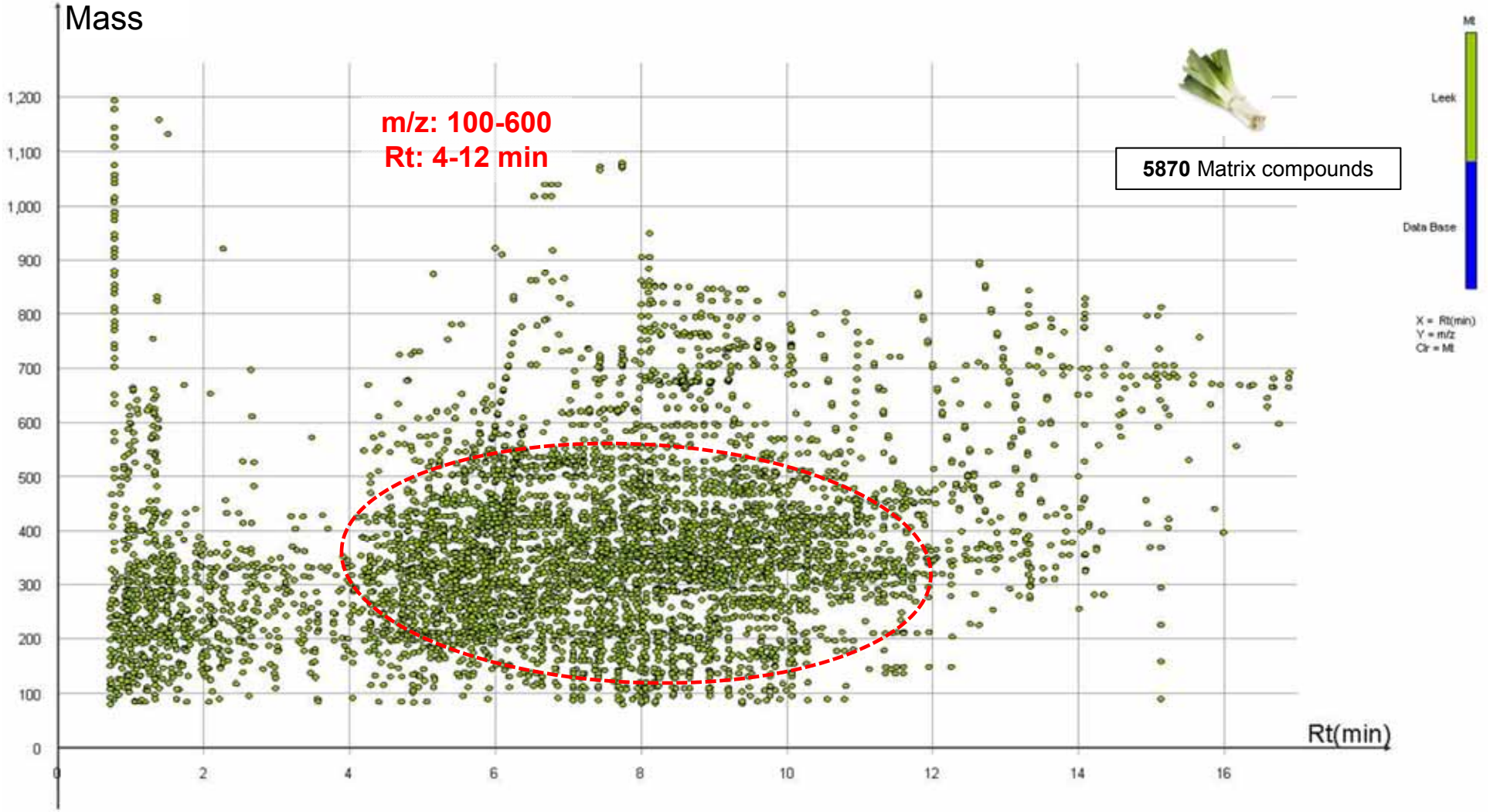
Data base components- **tomato** matrix compounds



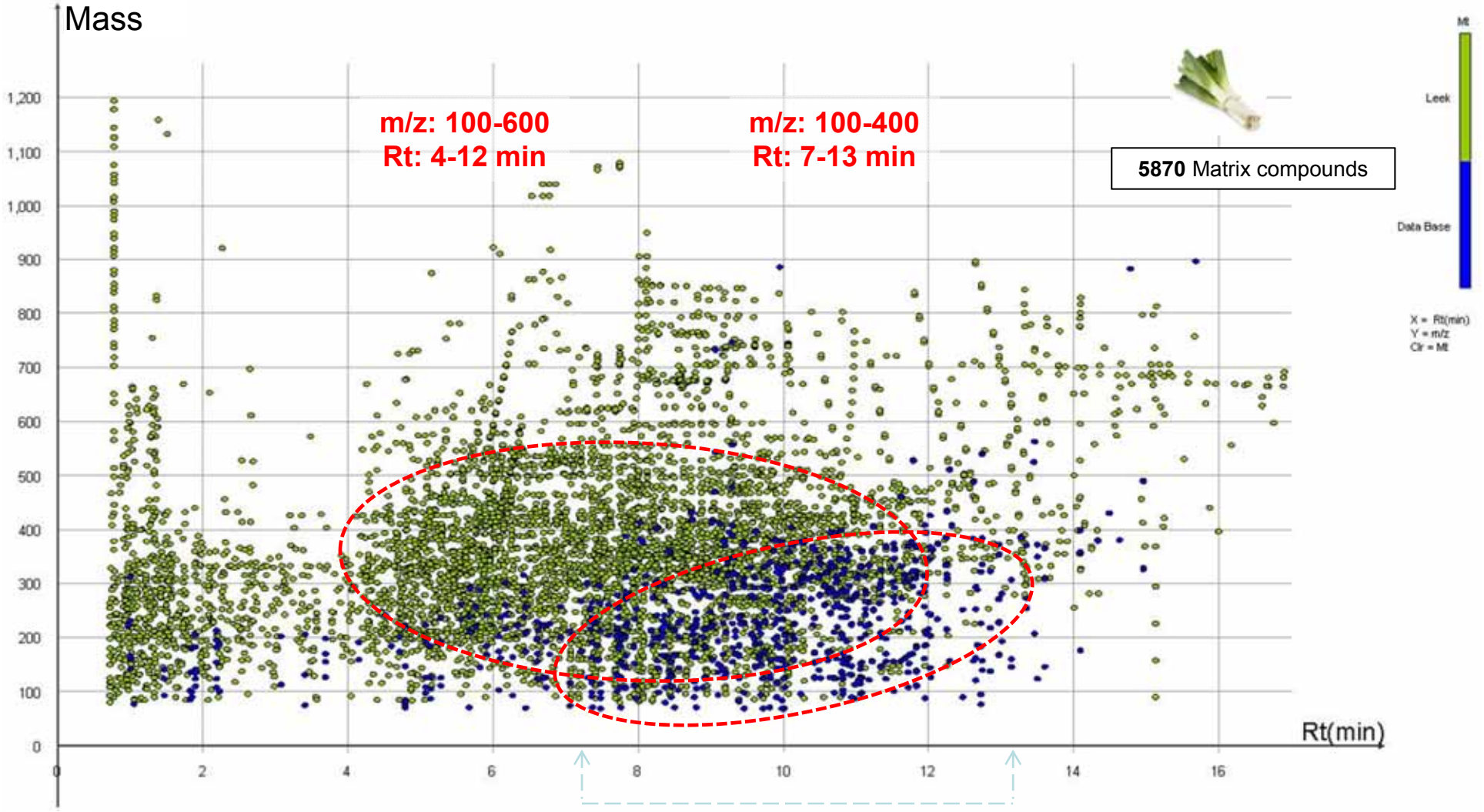
DB: 750 components

Tomato: 656 matrix compounds

Co-extracted matrix components of leek. LC-TOF-MS



Data base components- **leek** matrix compounds



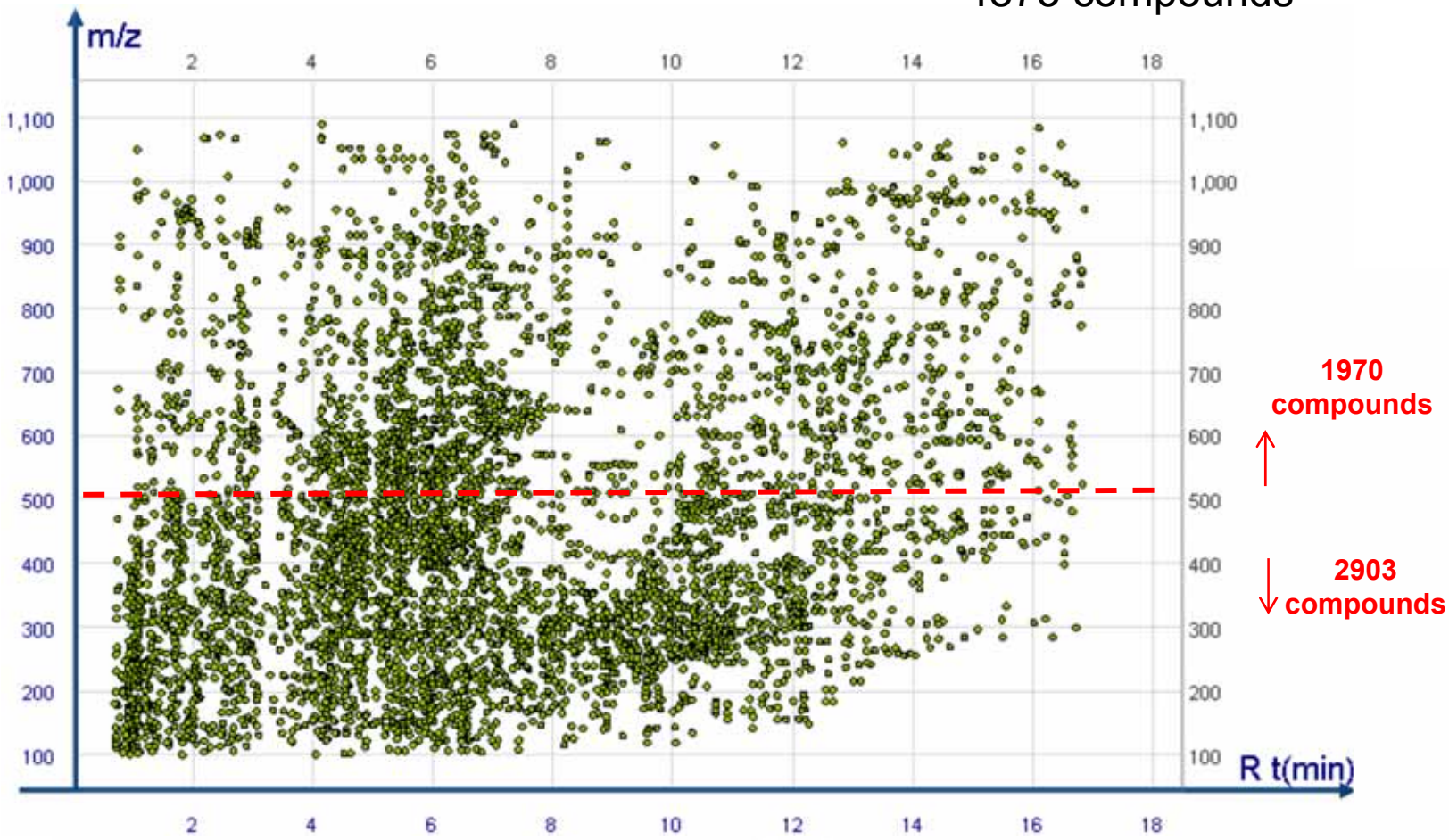
DB: 750 components

Leek: 3032 matrix compounds

Matrix	Nº of co-extracted compounds	
	Rt: 0-17 min	Rt: 7-13 min
Green tea	9073	3535
Orange	8017	2743
Chamomille	6630	3490
Leek	5870	3032
Pepper	3419	919
Tomato	2408	656

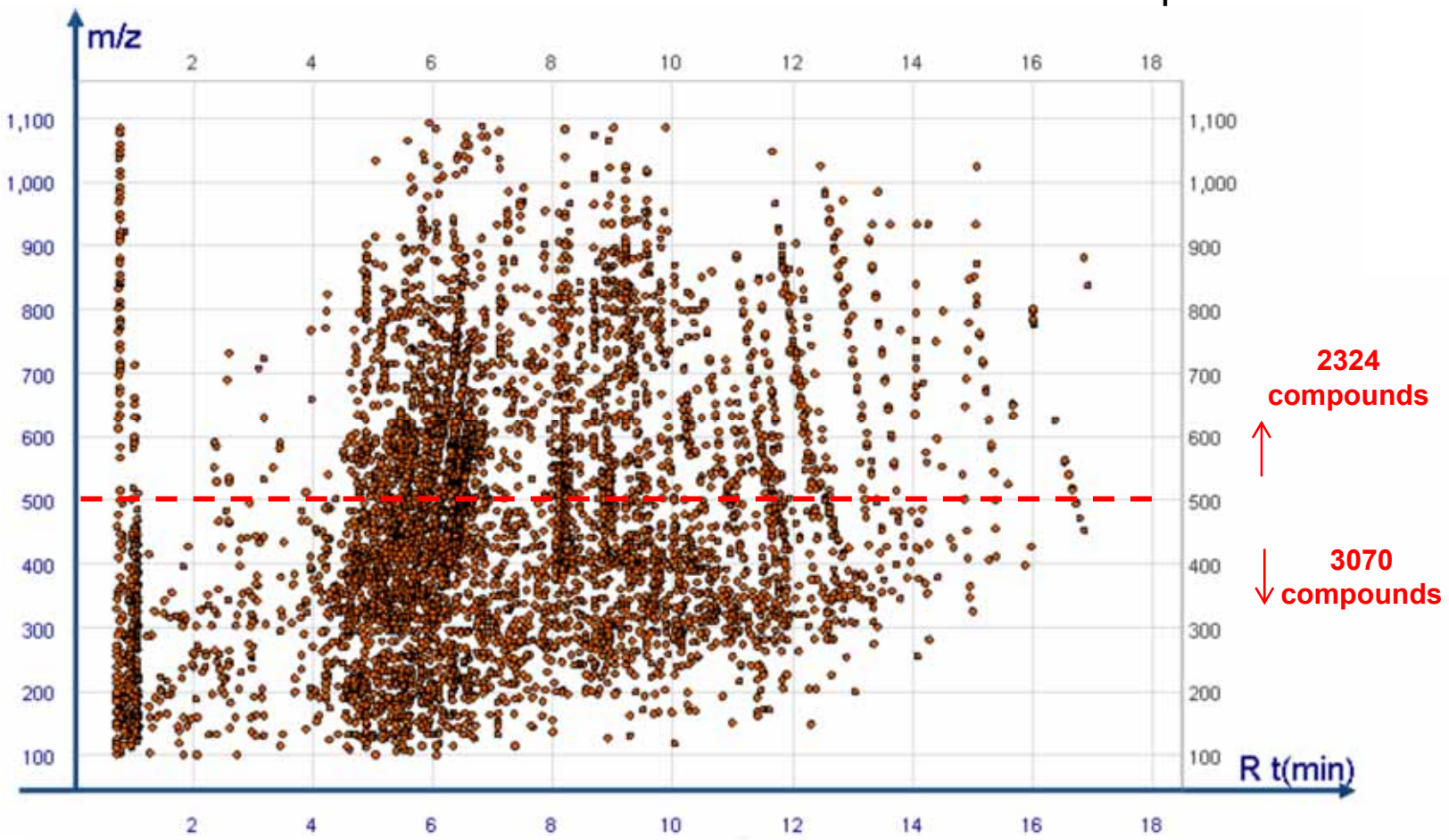
Green tea matrix compounds Dil 1/5 . **LC-TOF-MS**
Absolute height ≥ 10000 counts

4873 compounds

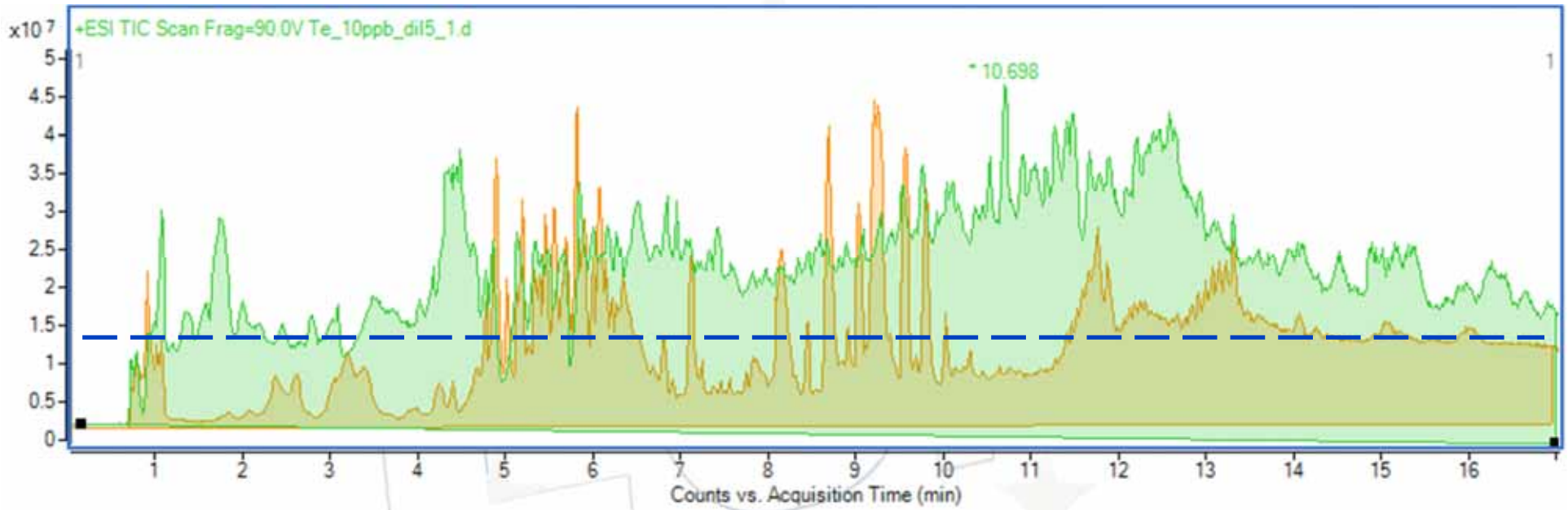


Orange matrix compounds Dil 1/5 . **LC-TOF-MS**
Absolute height ≥ 10000 counts

5394 compounds



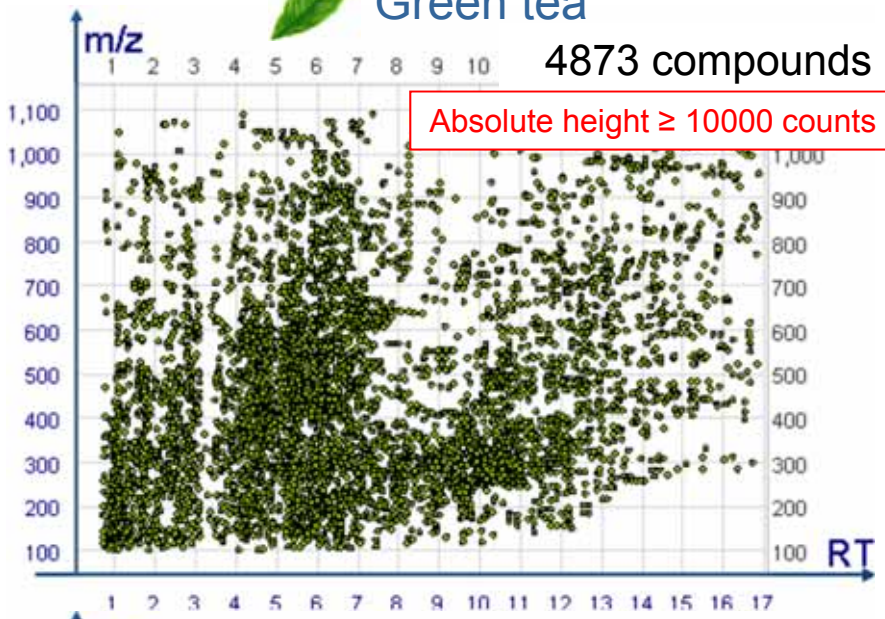
TIC LC-TOF-MS. Orange and Green tea. Dil 1/5



Green tea

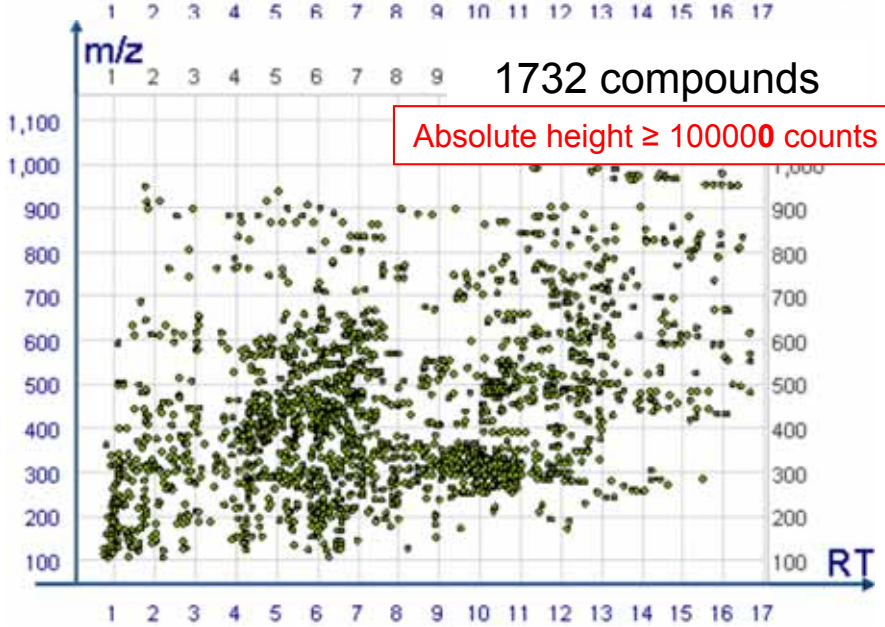
4873 compounds

Absolute height ≥ 10000 counts



1732 compounds

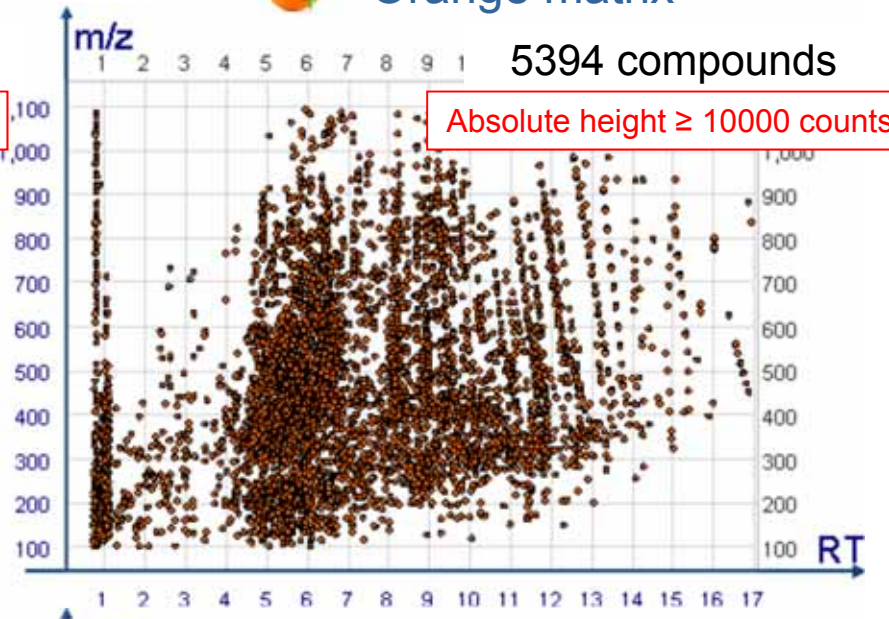
Absolute height ≥ 100000 counts



Orange matrix

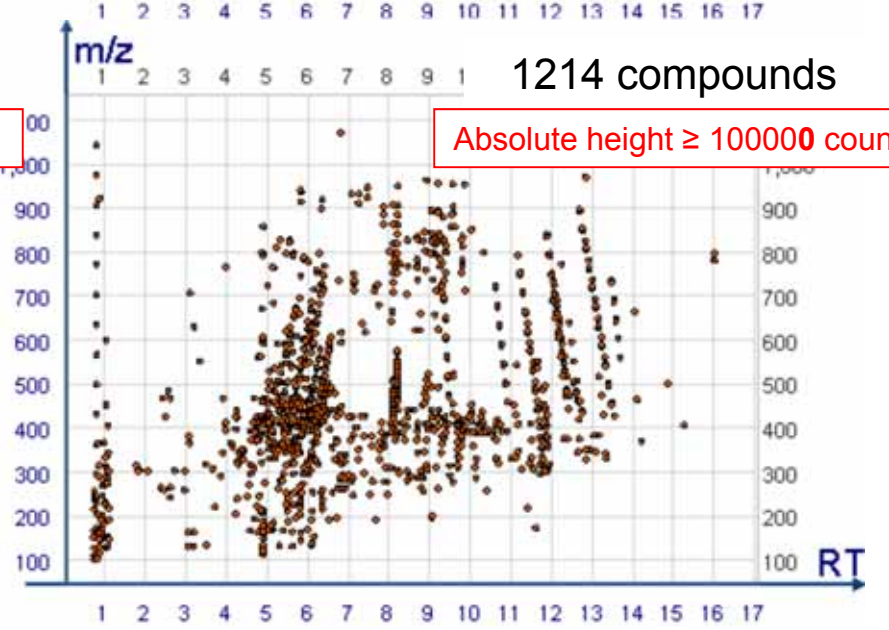
5394 compounds

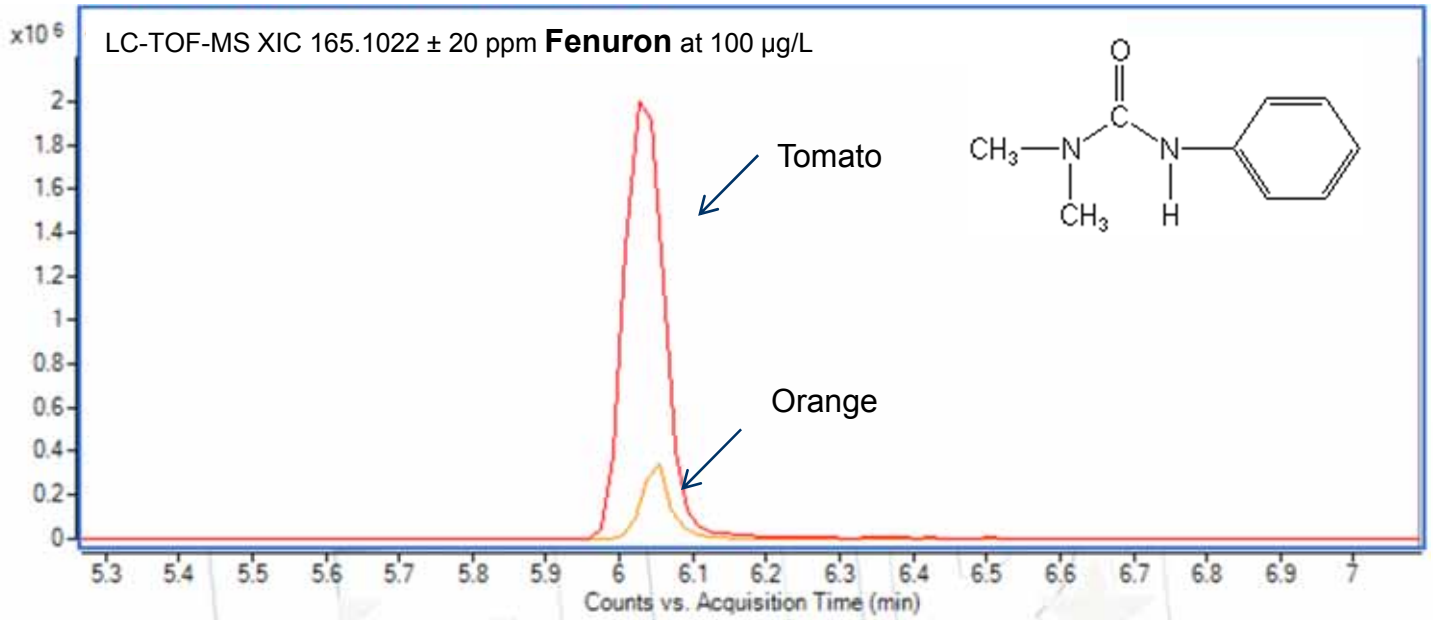
Absolute height ≥ 10000 counts



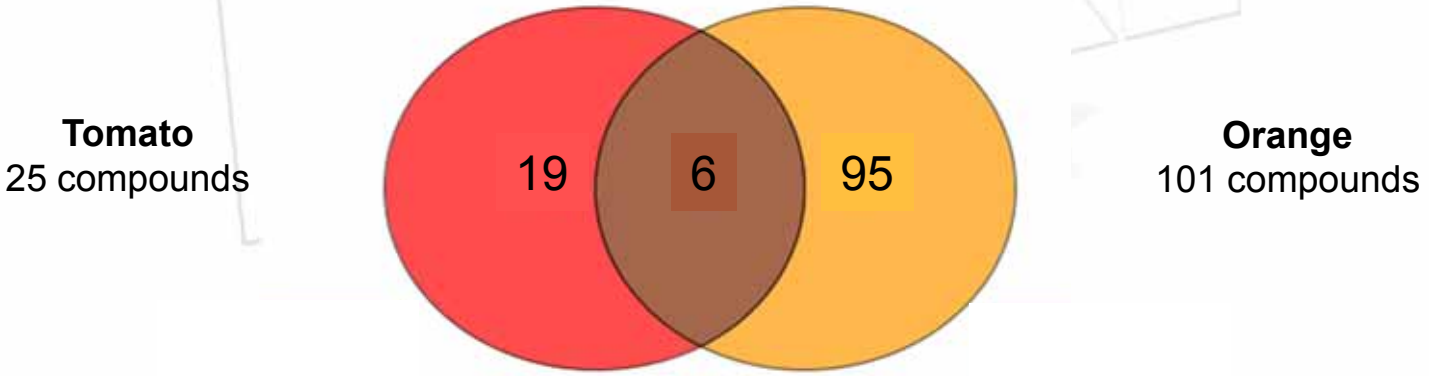
1214 compounds

Absolute height ≥ 100000 counts

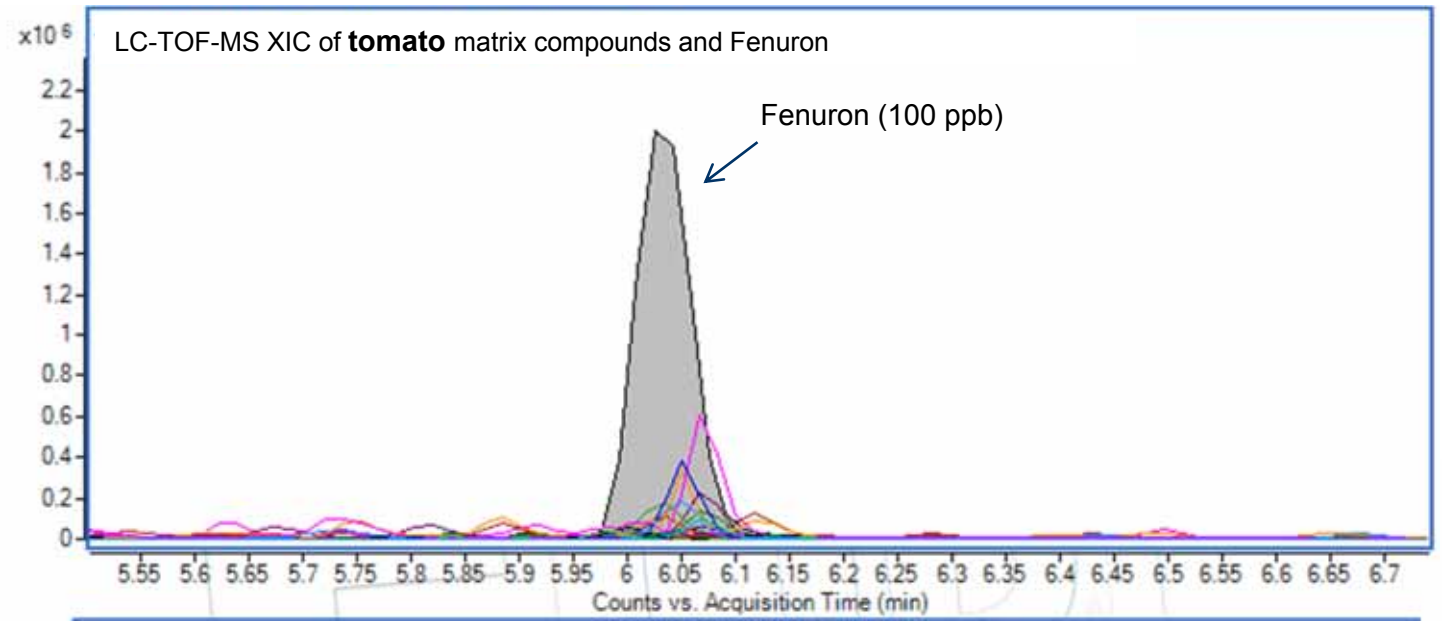


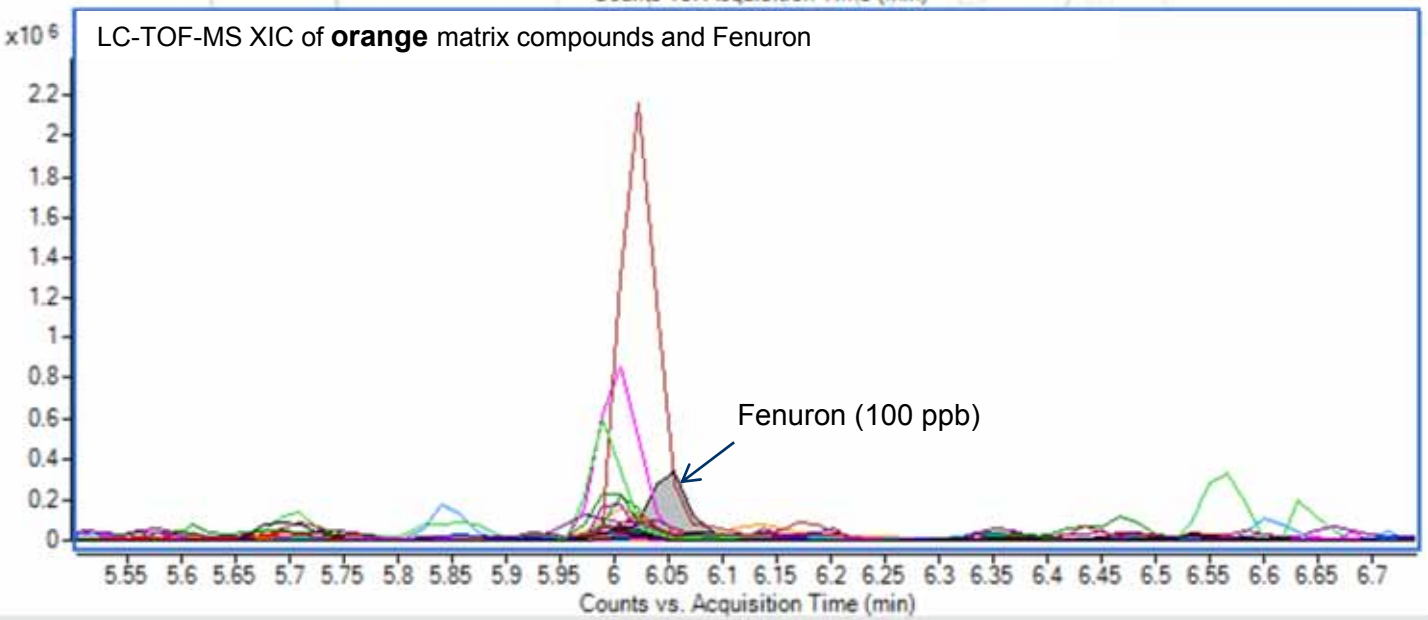


Number of co-extracted matrix compounds Rt: 5.99-6.08 min



Mass Profiler Professional 12.1. Agilent Tech.

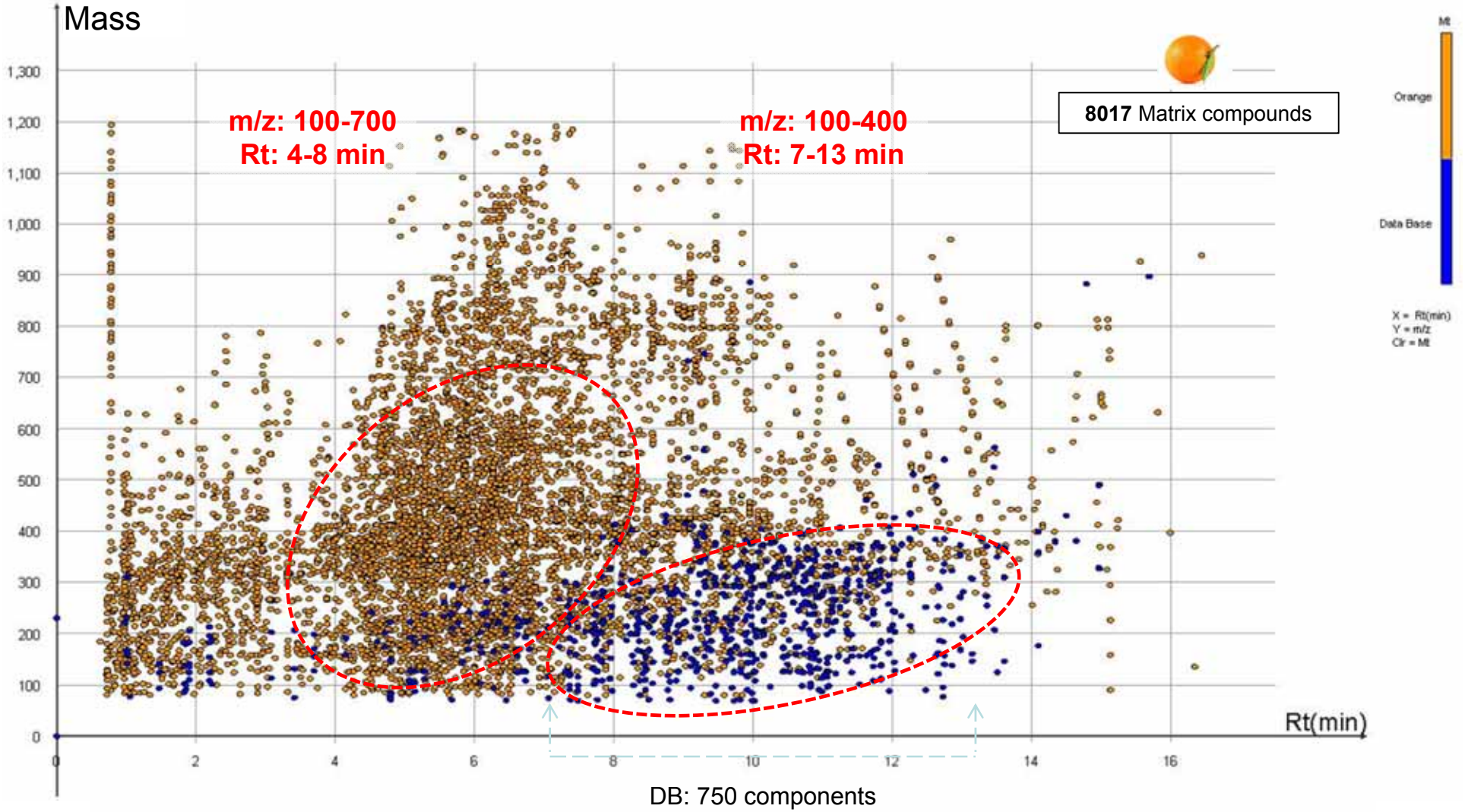

x10 6
LC-TOF-MS XIC of **tomato** matrix compounds and Fenuron
Fenuron (100 ppb)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5.55 5.6 5.65 5.7 5.75 5.8 5.85 5.9 5.95 6 6.05 6.1 6.15 6.2 6.25 6.3 6.35 6.4 6.45 6.5 6.55 6.6 6.65 6.7
Counts vs. Acquisition Time (min)


x10 6
LC-TOF-MS XIC of **orange** matrix compounds and Fenuron
Fenuron (100 ppb)
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5.55 5.6 5.65 5.7 5.75 5.8 5.85 5.9 5.95 6 6.05 6.1 6.15 6.2 6.25 6.3 6.35 6.4 6.45 6.5 6.55 6.6 6.65 6.7
Counts vs. Acquisition Time (min)

Number of matrix coeluting compounds *vs* matrix suppression
Orange matrix Dilution 1/30

Pesticide	Rt (min)	Suppression	Nº of coleuting compounds (± 0.05 min)	Σ Compounds height (counts)
CINOSULFURON	8.09	-6	63	3.46E+06
FENPROPIDIN	8.16	-18	62	1.20E+07
PROPOXUR	8.23	0	45	4.12E+06
CARBOFURAN	8.32	0	34	3.20E+06
FORCHLORFENURON	8.54	-16	32	9.85E+05
FLUOMETURON	8.54	-11	33	1.05E+06
ISOPROTURON	8.72	-88	84	2.49E+07
METALAXYL	8.74	-74	84	2.49E+07
OFURACE	8.84	1	39	2.13E+06
HEPTENOPHOS	8.97	-8	65	6.86E+06
SPINOSYN A	9.02	-61	99	2.39E+07
METAZACHLOR	9.25	-83	93	3.06E+07
BUPIRIMATE	9.27	-83	125	4.00E+07
CYPRODINIL	9.35	-87	76	1.98E+07
TRIADIMENOL	9.42	-18	48	9.73E+06
FLAZASULFURON	9.42	-76	58	9.78E+06
PROMECARB	9.95	-15	42	2.68E+06
AZOXYSTROBIN	10.03	-4	29	5.33E+06

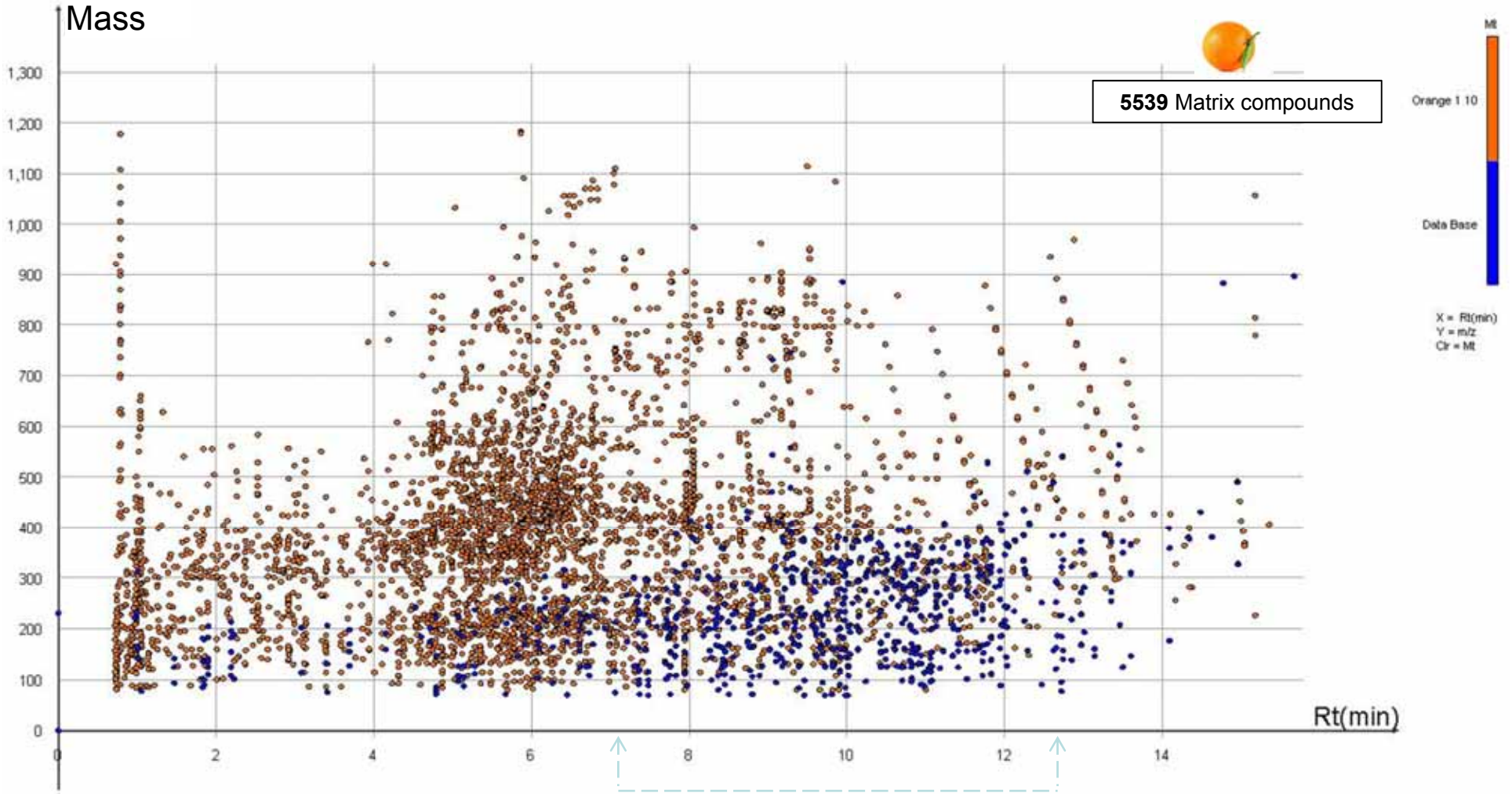
Data base components- **orange** matrix compounds



DB: 750 components

Orange: 2743 matrix compounds

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

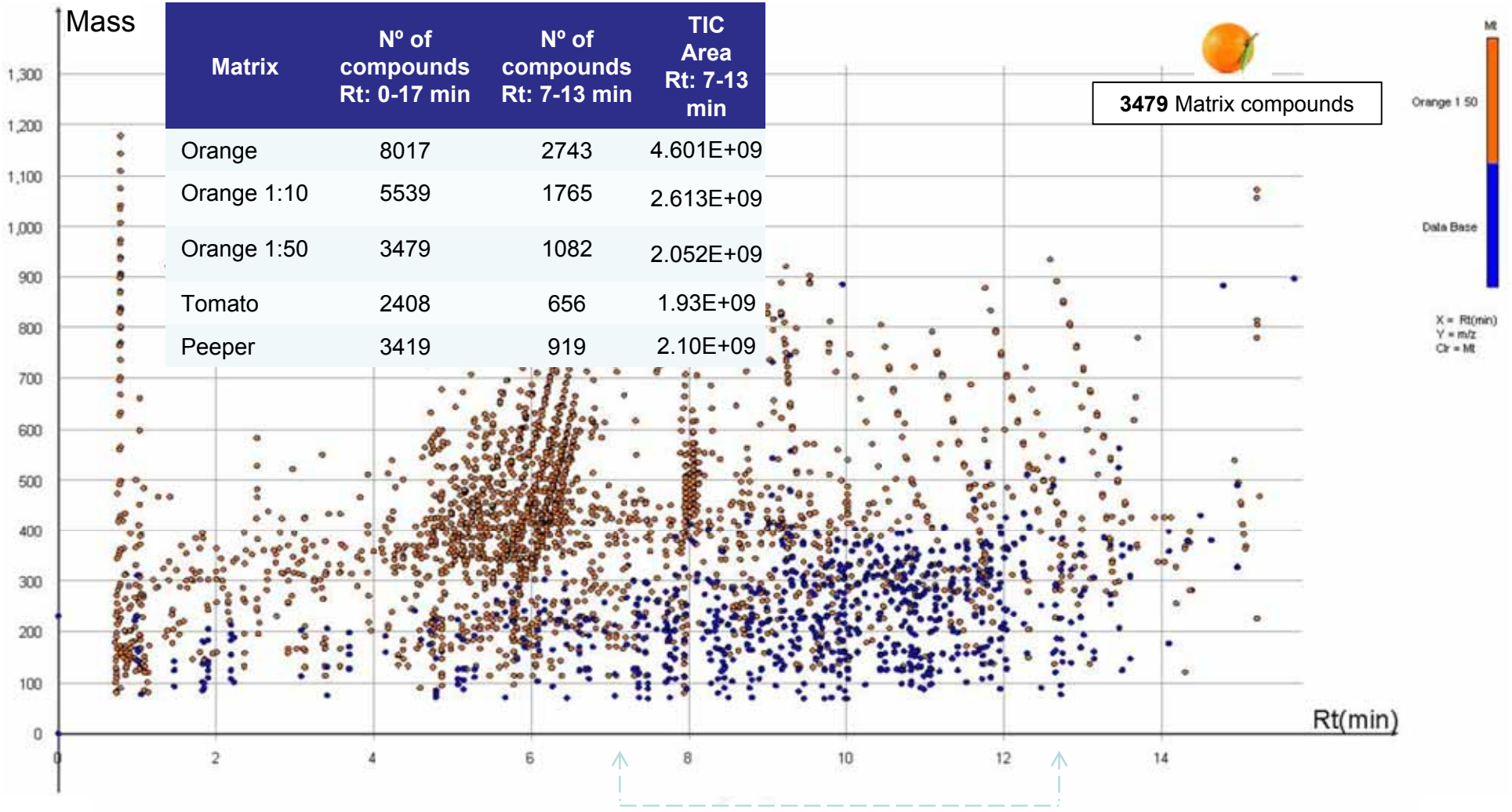


DB: 750 components

Orange: 1765 matrix compounds

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

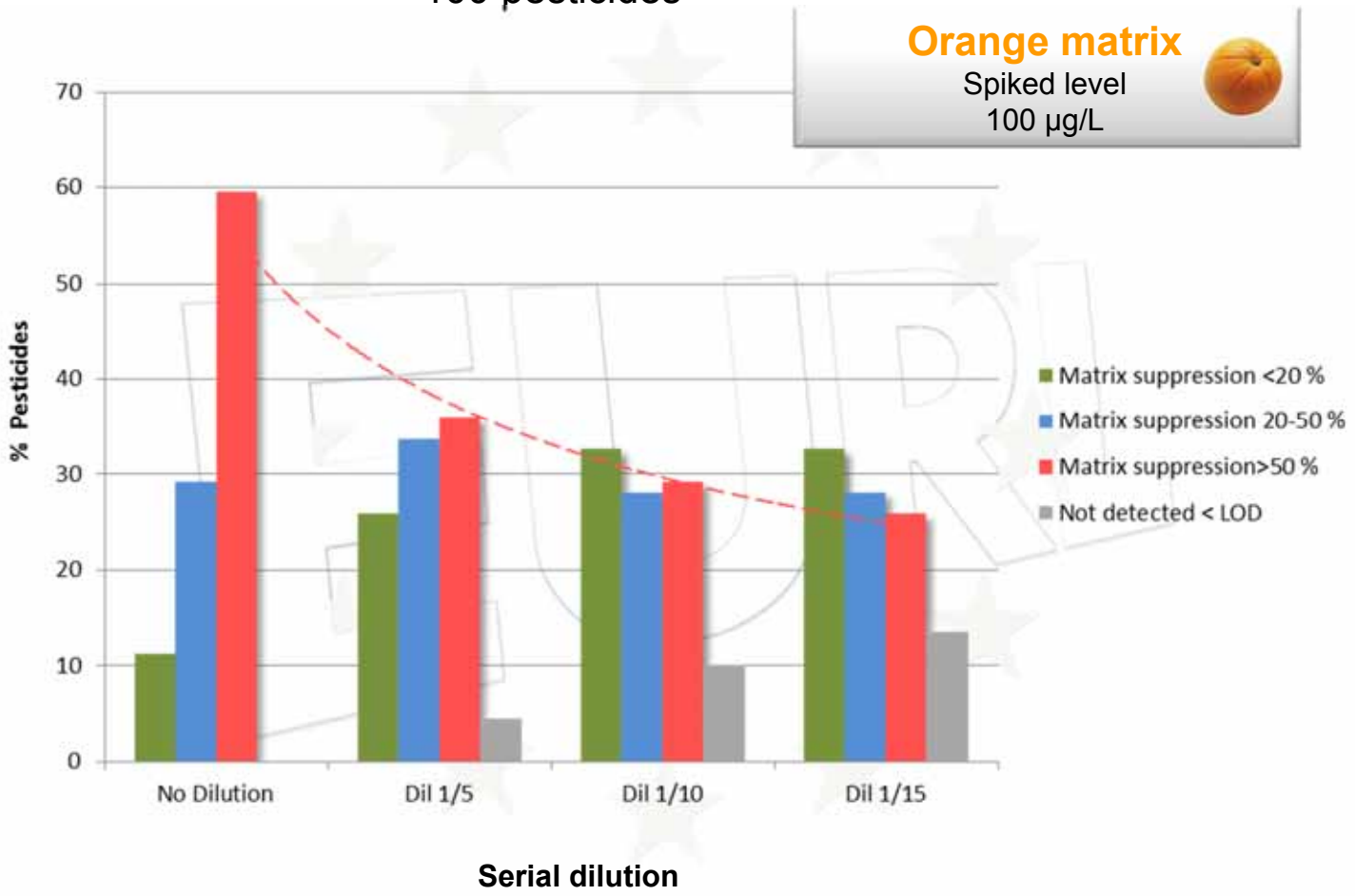


DB: 750 components

Orange: 1082 matrix compounds

Dilution effect on matrix suppression
LC-TOF-MS

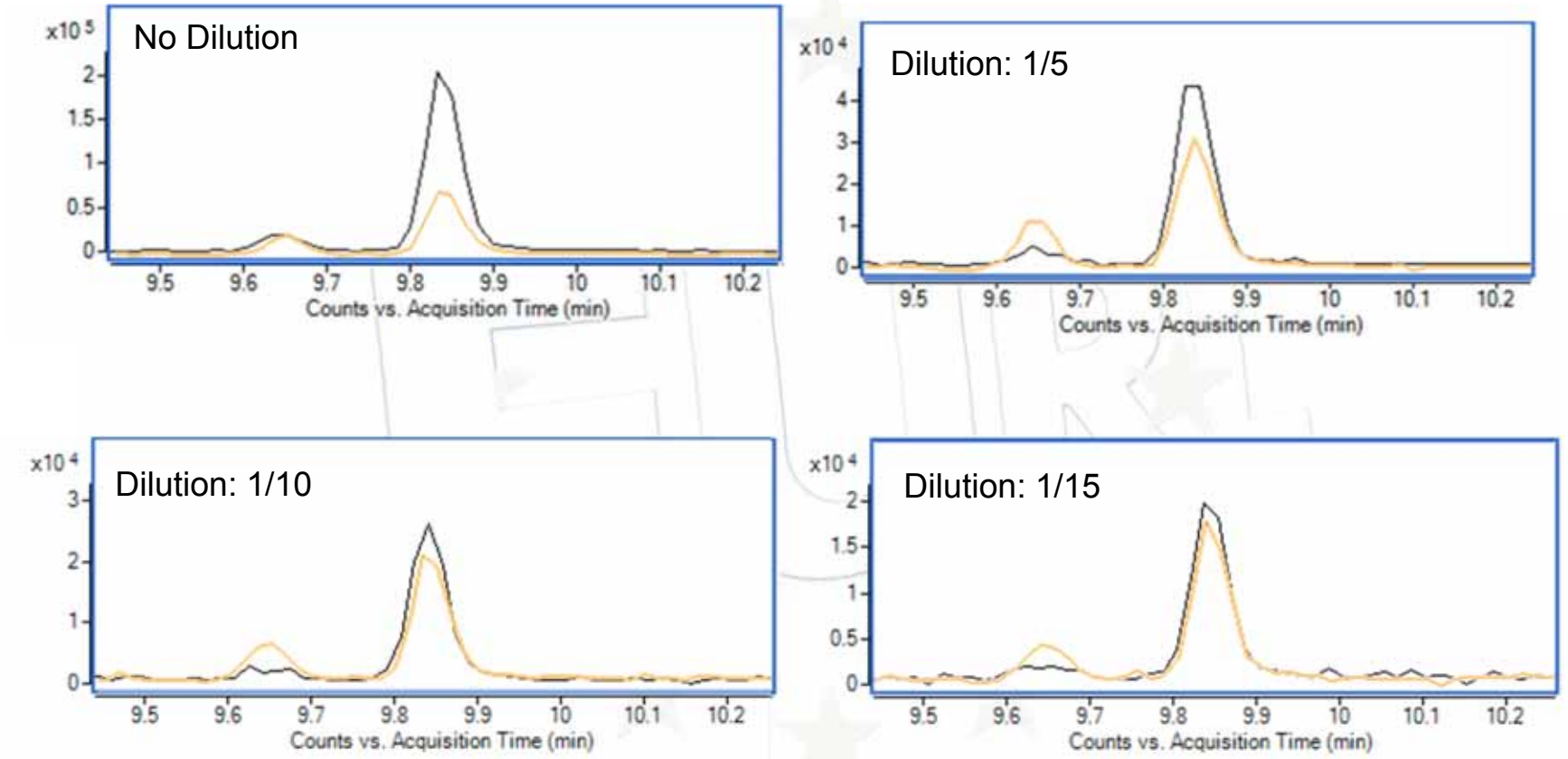
100 pesticides



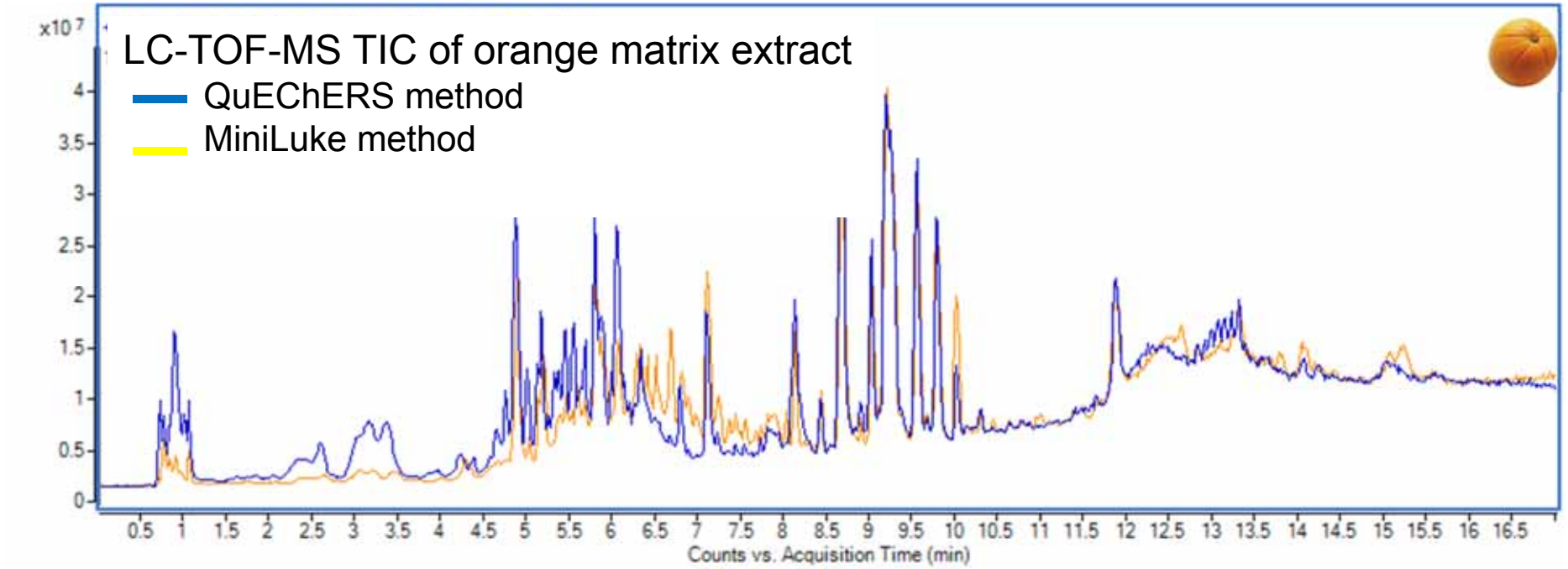
Dilution effect on suppression decrease

LC-TOF-MS XIC 268.1543 ± 20 ppm **Diethofencarb** at 100 µg/L

Solvent
Orange matrix

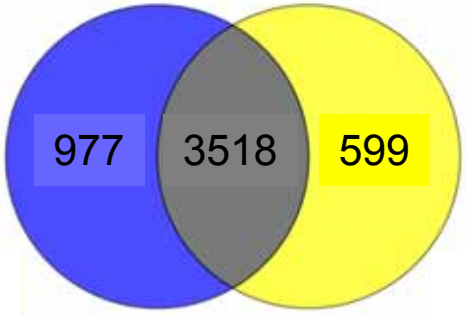


Orange matrix Dil 1/10. QuEChERS vs MiniLuke. **LC-TOF-MS**



Number of co-extracted matrix compounds

QuEChERS
4495 compounds



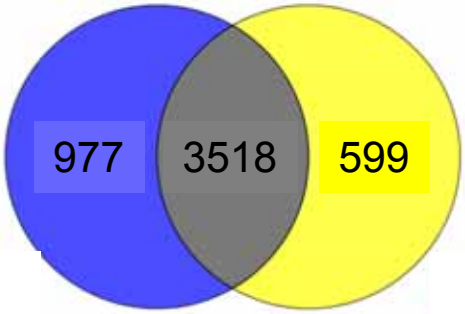
MiniLuke
4117 compounds

Mass Profiler Professional 12.1. Agilent Tech.

Orange matrix Dil 1/10. QuEChERS vs MiniLuke. **LC-TOF-MS**

Number of co-extracted matrix compounds
Rt 0-17 min

QuEChERS
4495 compounds



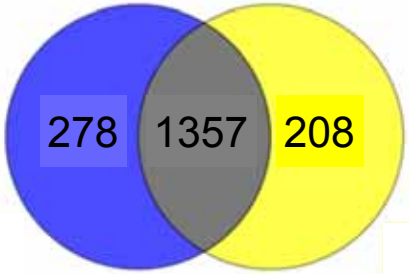
MiniLuke
4117 compounds

Mass Profiler Professional 12.1. Agilent Tech.

Rt < 6 min

QuEChERS
1635 compounds

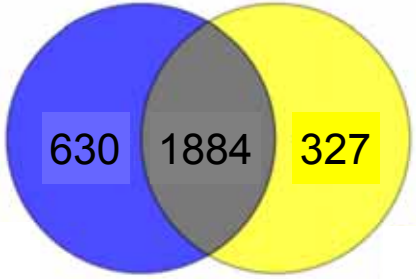
MiniLuke
1565 compounds



Rt 6-12 min

QuEChERS
2514 compounds

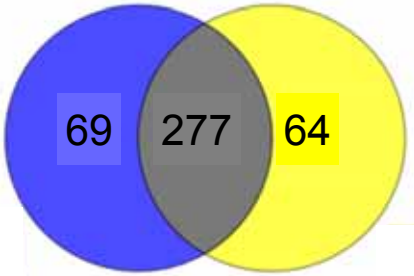
MiniLuke
2211 compounds



Rt > 12 min

QuEChERS
346 compounds

MiniLuke
341compounds



Citrate QuEChERS

2 g sample

Add 10 mL acetonitrile + TPP
Shake

Add 4 g $MgSO_4$ + 1 g NaCl + 1 g NaAc·2 H_2O + 0.5 g NaAc·3/2 H_2O

Shake automatically

Centrifugue 5 min. 3700 r.p.m.

Take 3 mL aliquot

Add 750 mg $MgSO_4$+ 125 mg PSA + 125 mg C18
Shake

Centrifugue
Acidify

Analysis

0.2 g sample/mL

Modified QuEChERS method

2 g sample + 4 mL H_2O
Shake 30 sec, wait 30 min

Add 10 mL acetonitrile + TPP
Shake

Add 4 g $MgSO_4$ + 1 g NaCl + 1 g NaAc·2 H_2O + 0.5 g NaAc·3/2 H_2O

Shake automatically

Centrifugue 5 min. 3700 r.p.m.

Take 3 mL aliquot

Add 150 mg $CaCl_2$ anh. + 150 mg PSA
Shake

Centrifugue
Acidify

Analysis

0.2 g sample/mL

AOAC method

5 g sample

Add 15 mL acetonitrile
Shake

Homogenize with Polytron
Centrifuge and take the supernatant

Re-extractact and combine extracts
Concentrated to about 1 mL

SPE with TPT Cartridge
Evaporate to about 0.5 mL

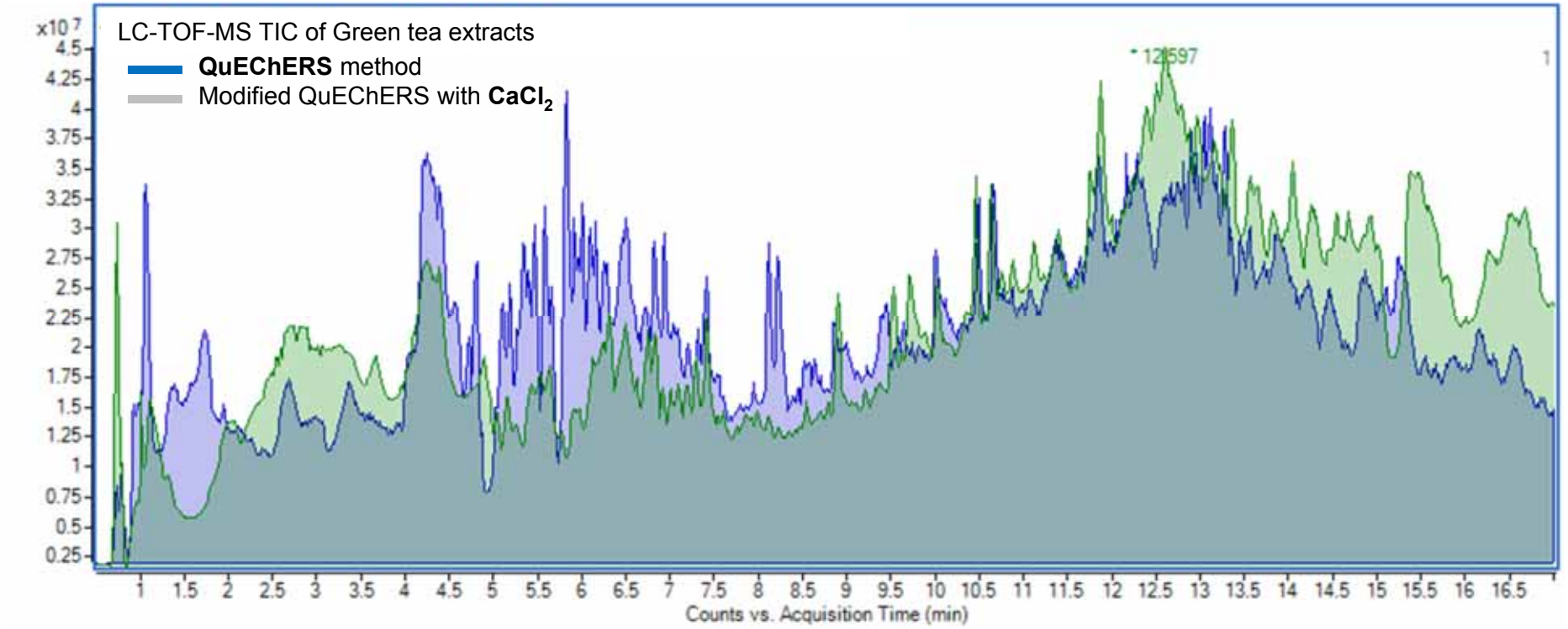
Add IS
Evaporate to dryness

Make up to 1.5 mL with adecuate solvent

Analysis

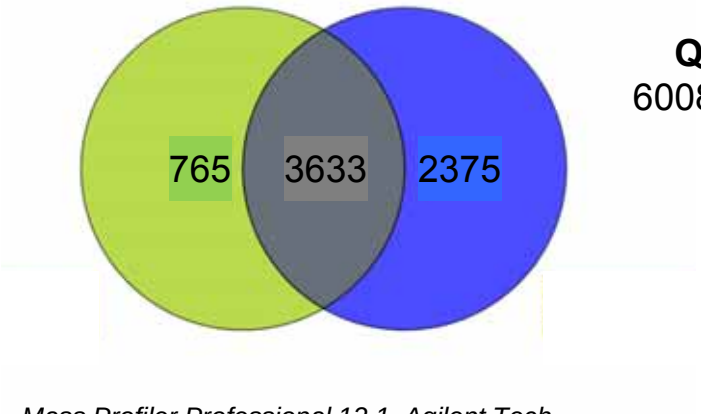
3.33 g sample/mL

Green tea extracts. CaCl2 *vs* QuEChERS method. LC-TOF-MS



Number of co-extracted matrix compounds

CaCl2
4398 compounds



QuEChERS
6008 compounds

Mass Profiler Professional 12.1, Agilent Tech.

Green tea extracts. CaCl2 *vs* QuEChERS method. LC-TOF-MS

Number of co-extracted matrix compounds

CaCl2
4398 compounds

QuEChERS
6008 compounds

Mass Profiler Professional 12.1. Agilent Tech.

Rt < 6 min

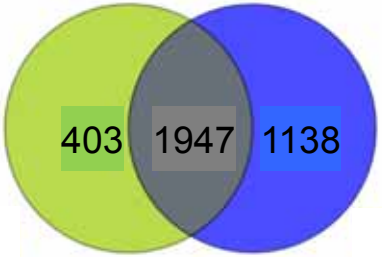
CaCl2
1159 compounds

QuEChERS
1926 compounds

Rt 6-12 min

CaCl2
2350 compounds

QuEChERS
3085 compounds

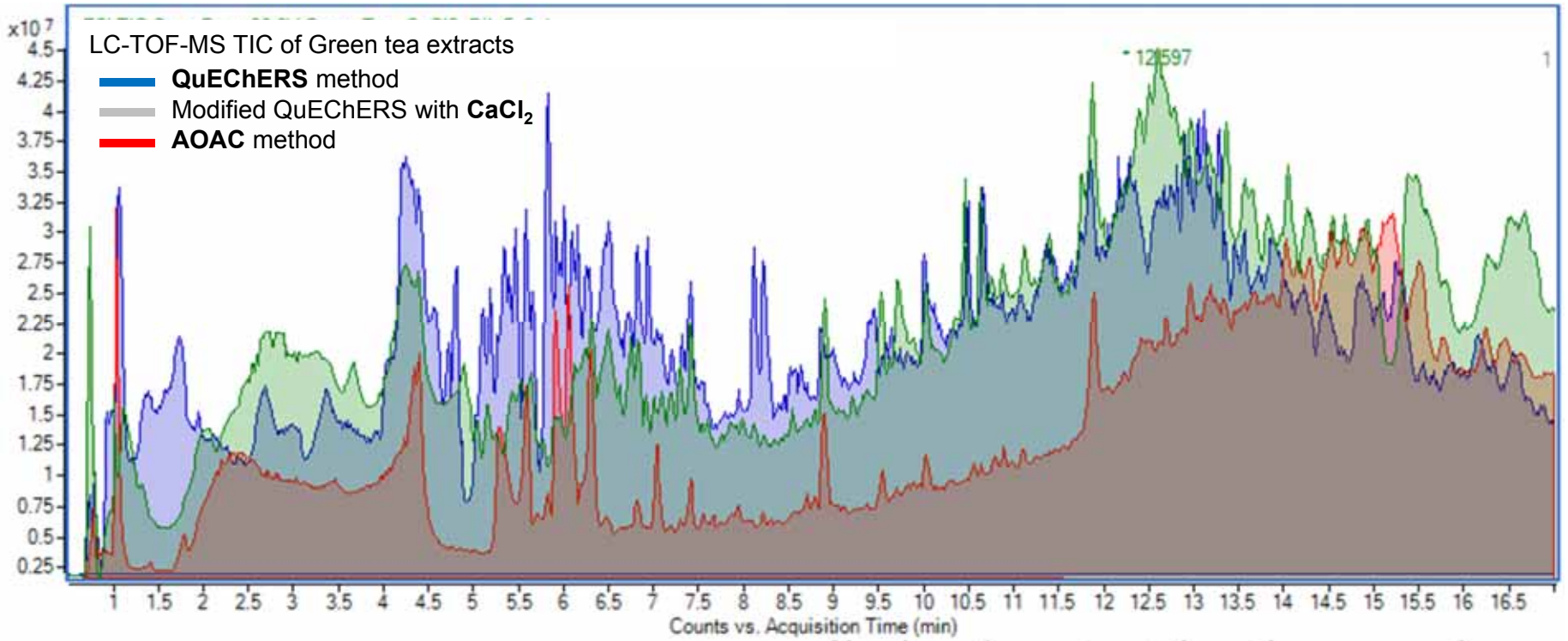


Rt > 12 min

CaCl2
889 compounds

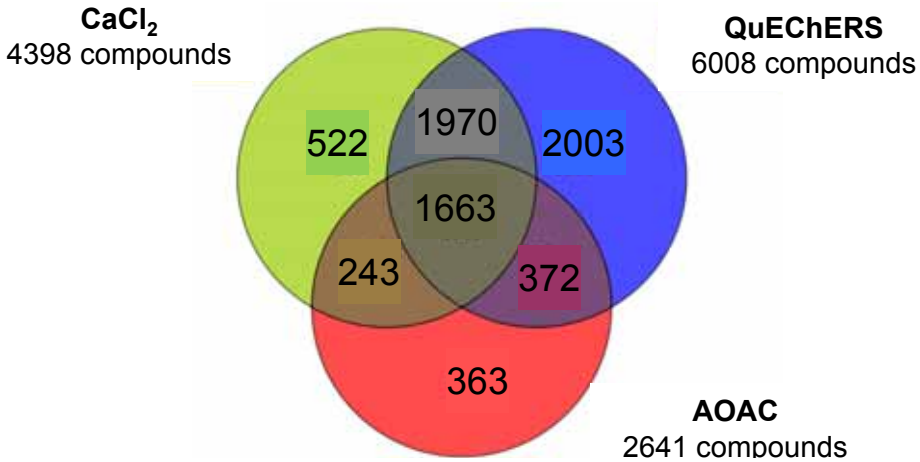
QuEChERS
997 compounds

Green tea extracts. LC-TOF-MS

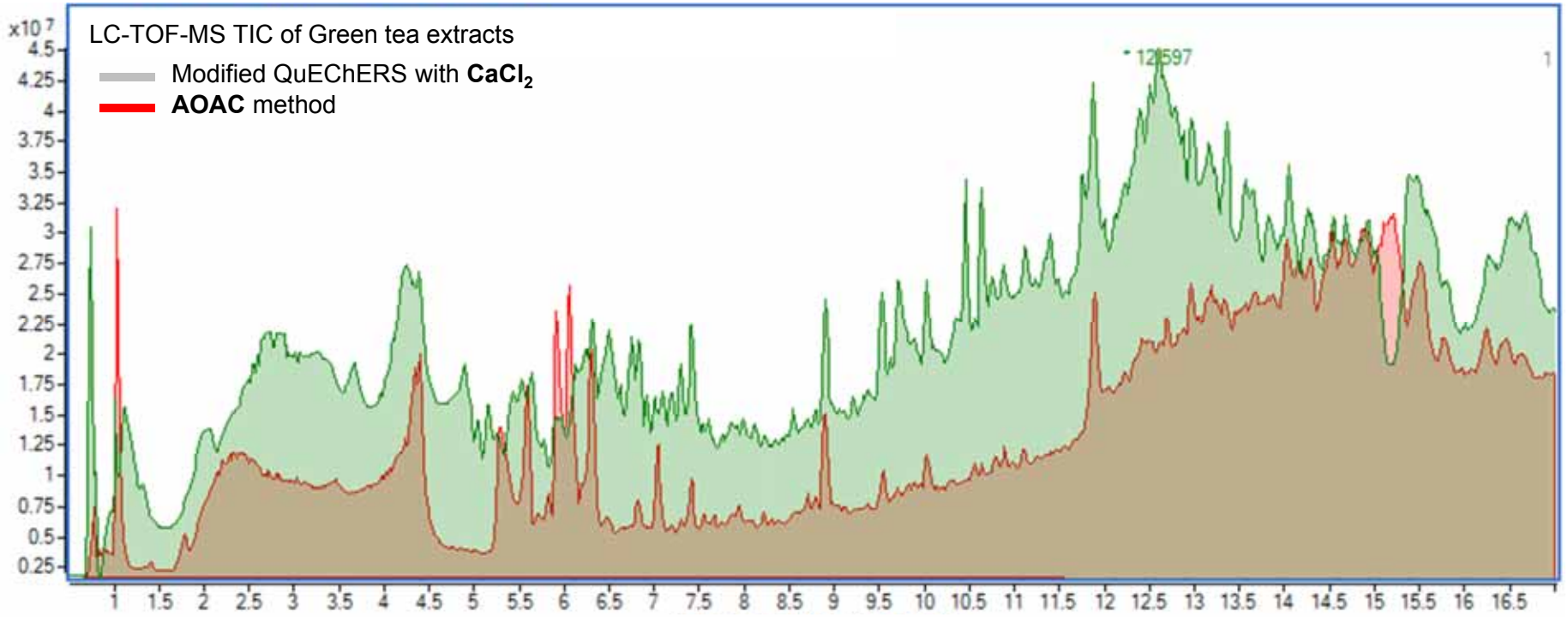


Methods	Σ Area
$CaCl_2$	8.34E+09
AOAC	2.67E+09
QuEChERs	9.87E+09

Number of co-extracted matrix compounds

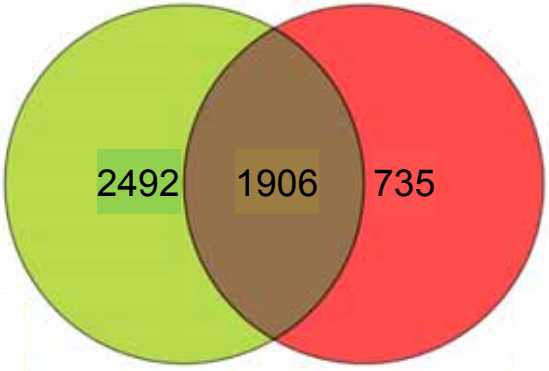


Green tea extracts. CaCl2 *vs* AOAC method. LC-TOF-MS



Number of co-extracted matrix compounds

CaCl2
4398 compounds

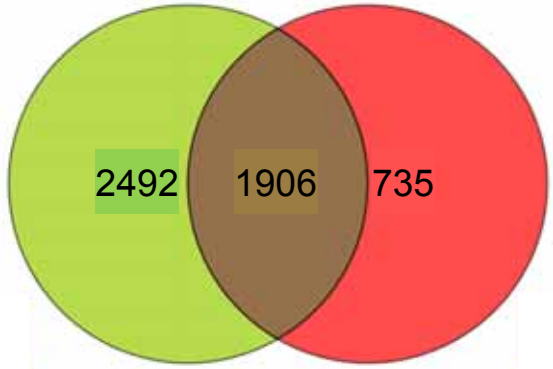


AOAC
2641 compounds

Mass Profiler Professional 12.1. Agilent Tech.

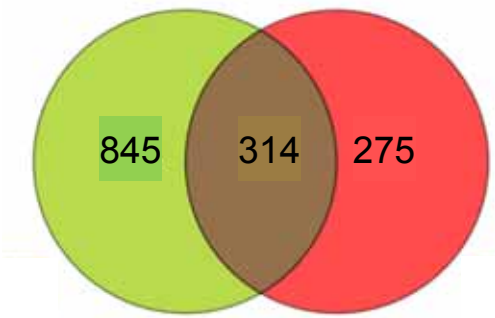
Green tea extracts. CaCl2 *vs* AOAC method. LC-TOF-MS

Number of co-extracted matrix compounds
Rt 0-17 min

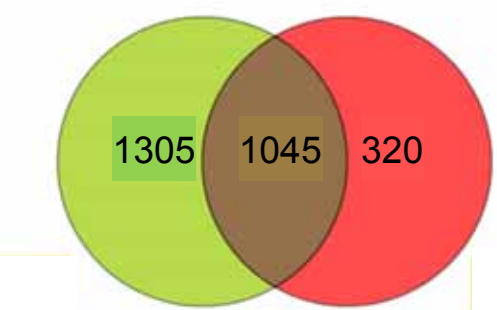


Mass Profiler Professional 12.1. Agilent Tech.

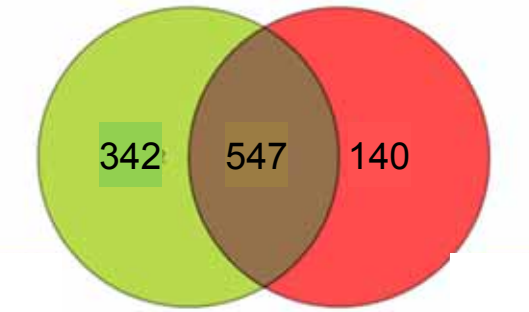
Rt < 6 min

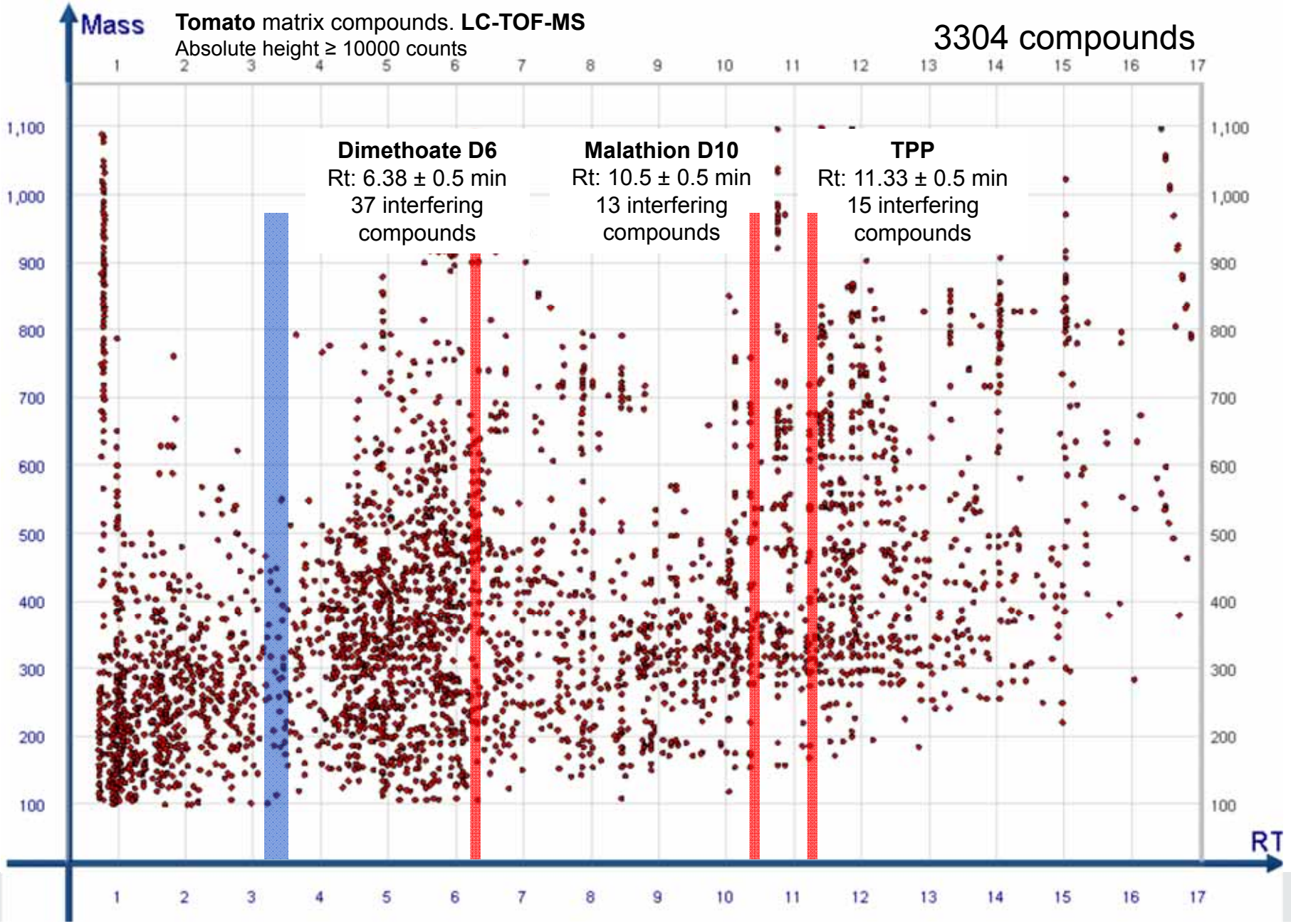


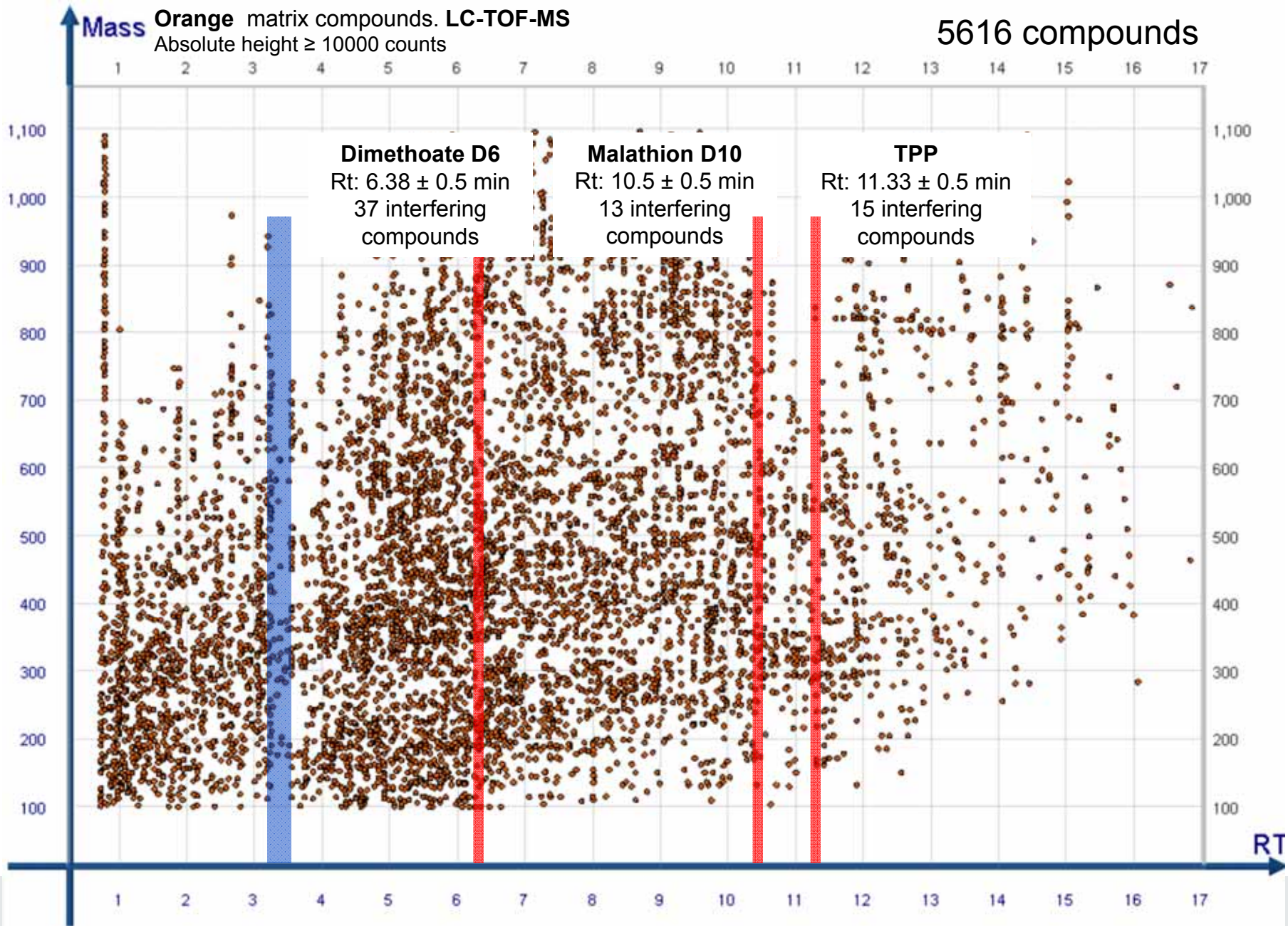
Rt 6-12 min

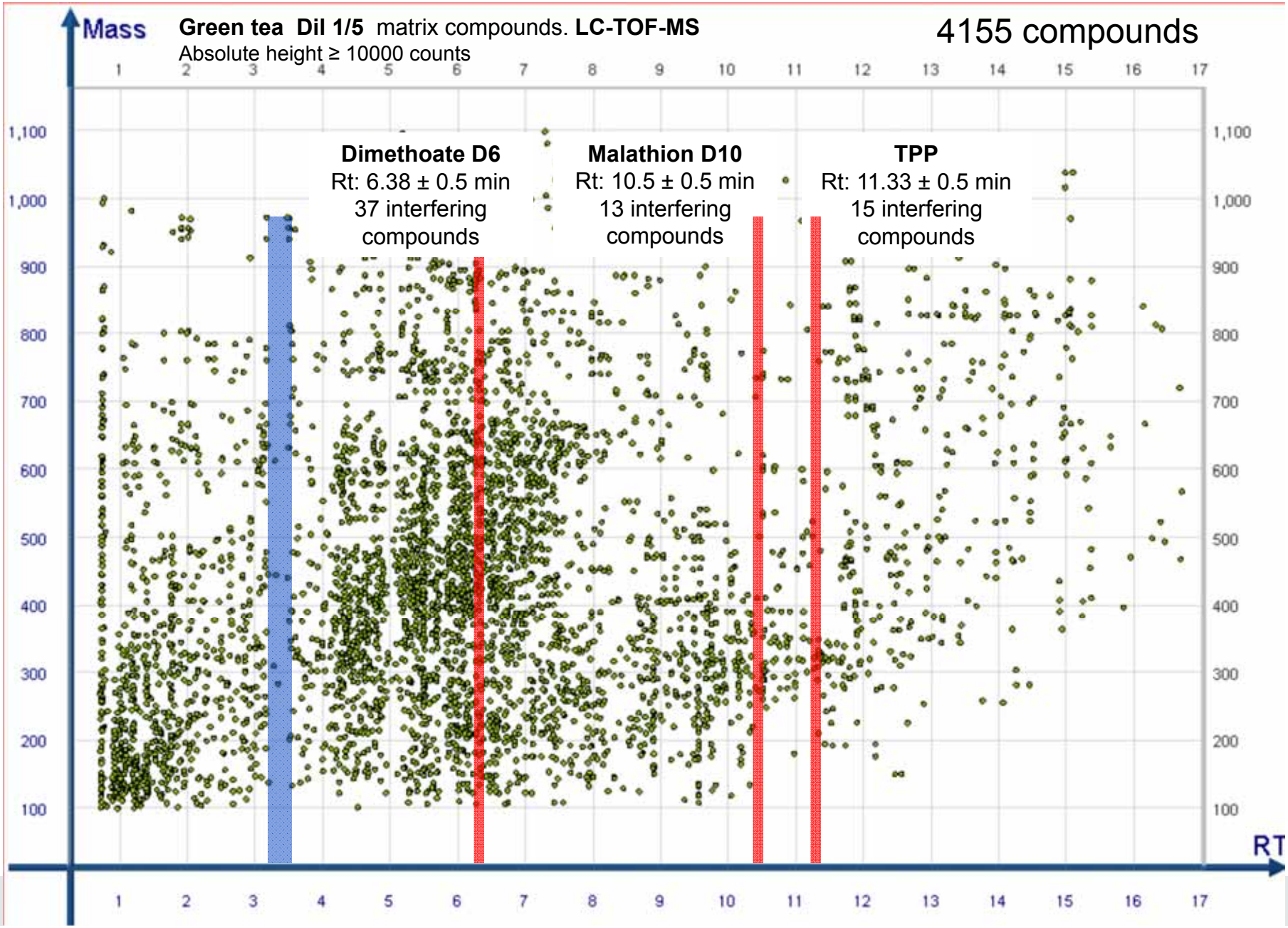


Rt > 12 min



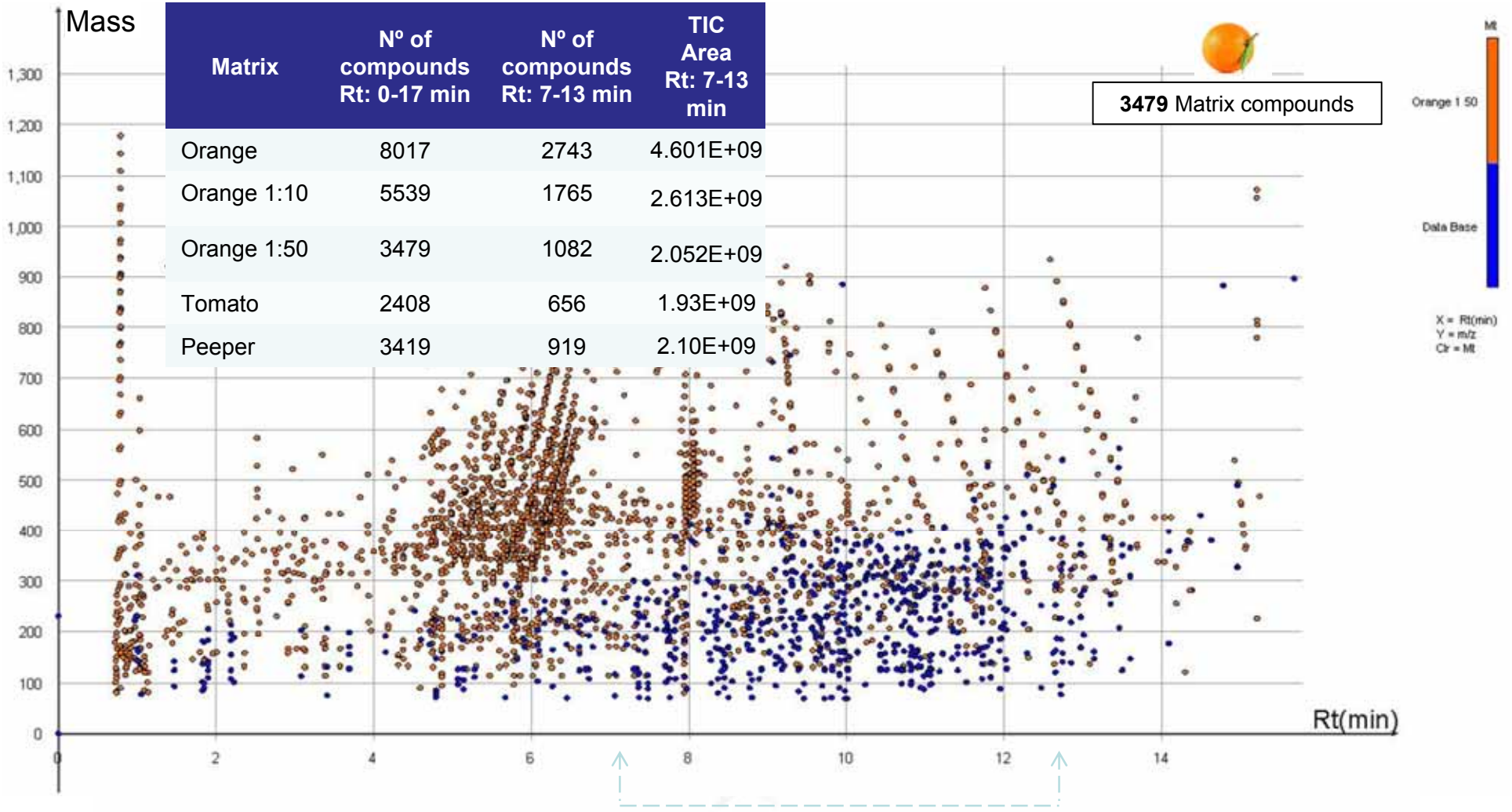

Mass
Tomato matrix compounds. LC-TOF-MS
Absolute height ≥ 10000 counts
3304 compounds
Dimethoate D6
Rt: 6.38 ± 0.5 min
37 interfering compounds
Malathion D10
Rt: 10.5 ± 0.5 min
13 interfering compounds
TPP
Rt: 11.33 ± 0.5 min
15 interfering compounds
RT


Mass
Orange matrix compounds. LC-TOF-MS
Absolute height ≥ 10000 counts
5616 compounds
Dimethoate D6
Rt: 6.38 ± 0.5 min
37 interfering compounds
Malathion D10
Rt: 10.5 ± 0.5 min
13 interfering compounds
TPP
Rt: 11.33 ± 0.5 min
15 interfering compounds
RT


Mass
Green tea Dil 1/5 matrix compounds. LC-TOF-MS
Absolute height ≥ 10000 counts
4155 compounds
Dimethoate D6
Rt: 6.38 ± 0.5 min
37 interfering compounds
Malathion D10
Rt: 10.5 ± 0.5 min
13 interfering compounds
TPP
Rt: 11.33 ± 0.5 min
15 interfering compounds
RT

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

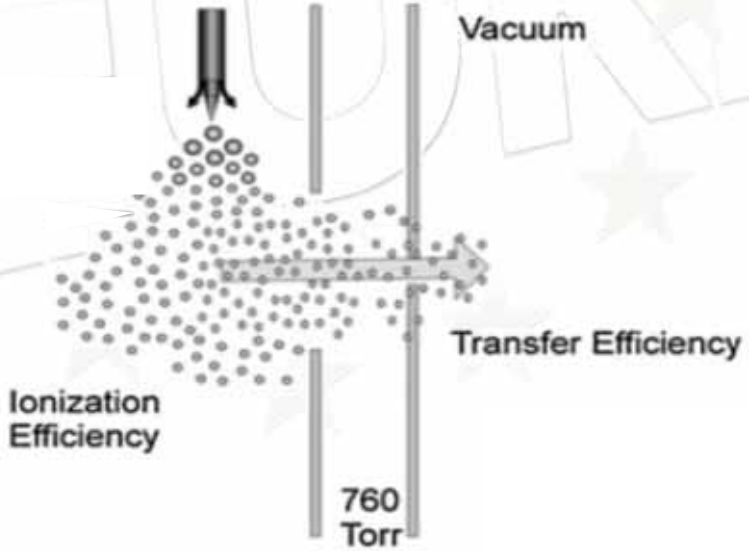
Matrix	Nº of compounds Rt: 0-17 min	Nº of compounds Rt: 7-13 min	TIC Area Rt: 7-13 min
Orange	8017	2743	4.601E+09
Orange 1:10	5539	1765	2.613E+09
Orange 1:50	3479	1082	2.052E+09
Tomato	2408	656	1.93E+09
Peeper	3419	919	2.10E+09



DB: 750 components

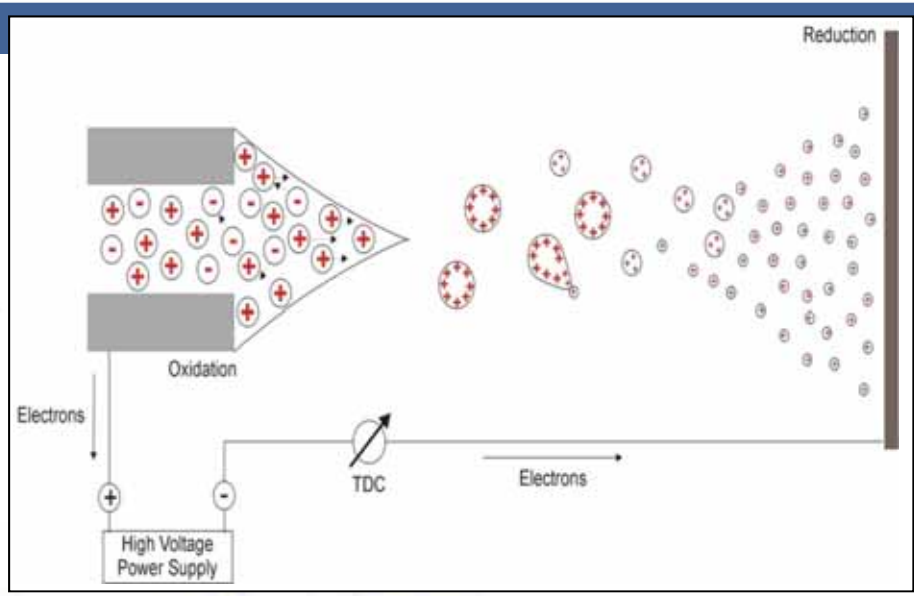
Orange: 1082 matrix compounds

Sampling efficiency

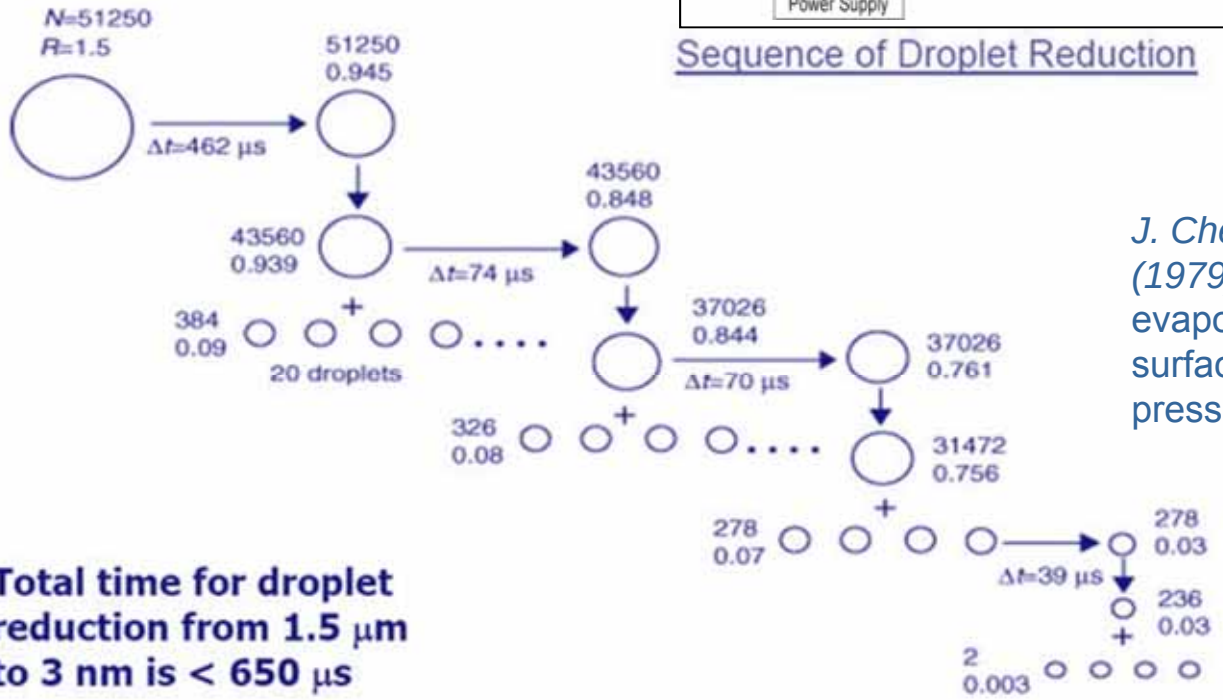


High temperature: 500ºC
Nitrogen flow: 40 L/min at 8 kg pressure
Gas velocity: 100 – 300 m/sec (1000 km/h, almost Match1)

Time → Ion



Sequence of Droplet Reduction

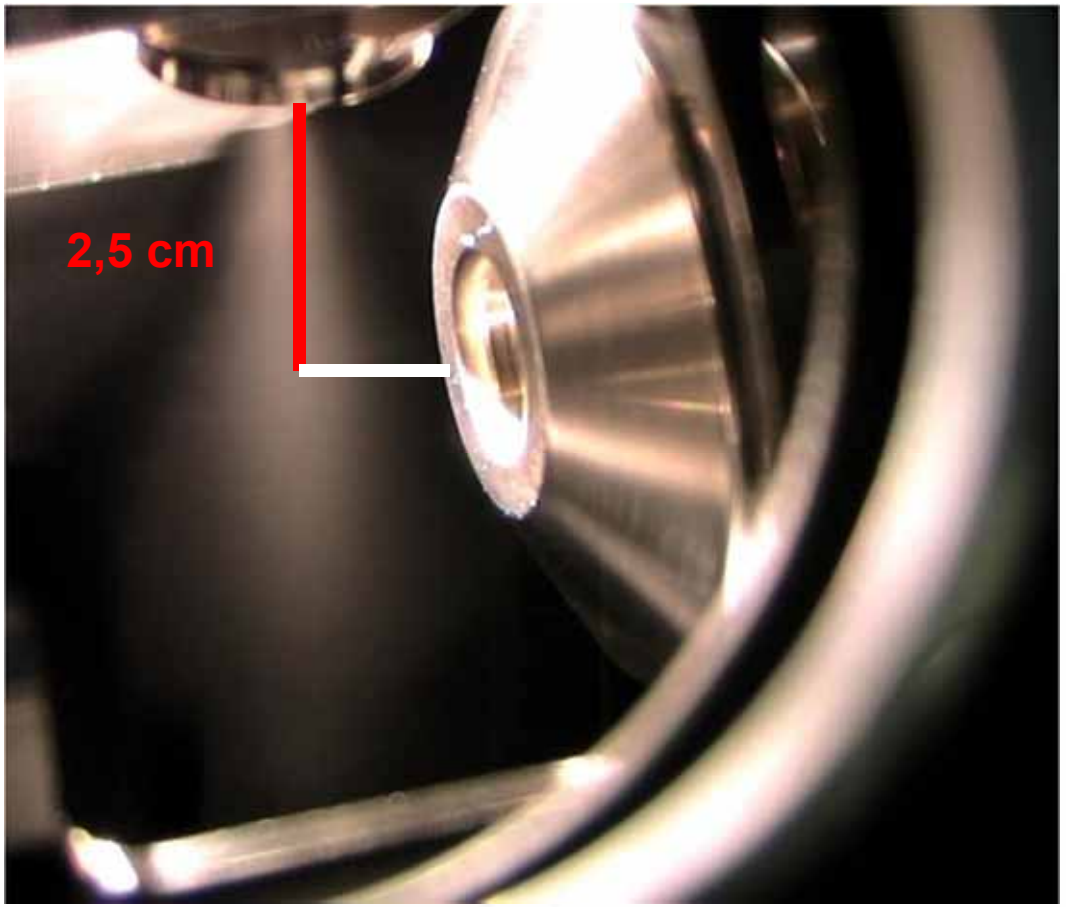


Total time for droplet reduction from 1.5 µm to 3 nm is < 650 µs

J. Chem. Phys. 71 4451 (1979) Field induced ion evaporation from liquid surfaces at atmospheric pressure

Highly Effective µLC-MS/MS Analysis

Observing Thermal Focusing



Temperature = 550 ºC

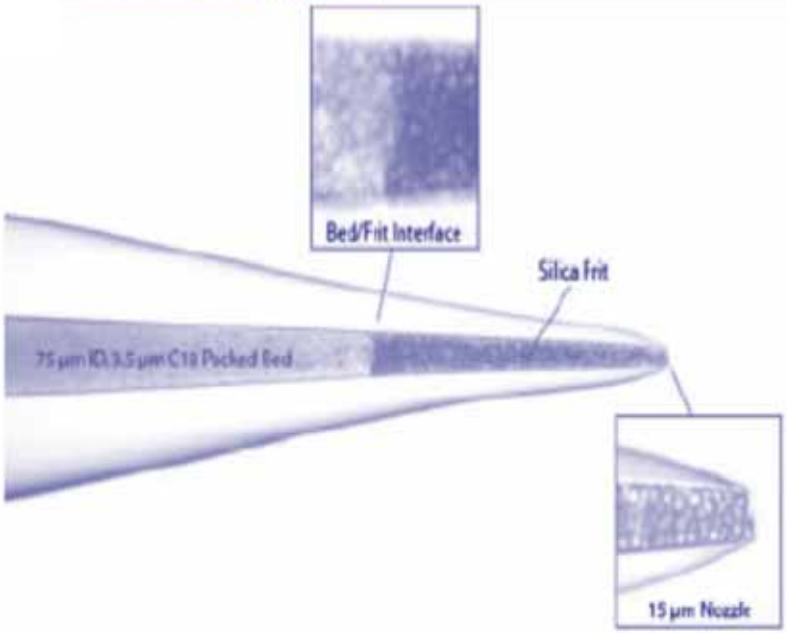


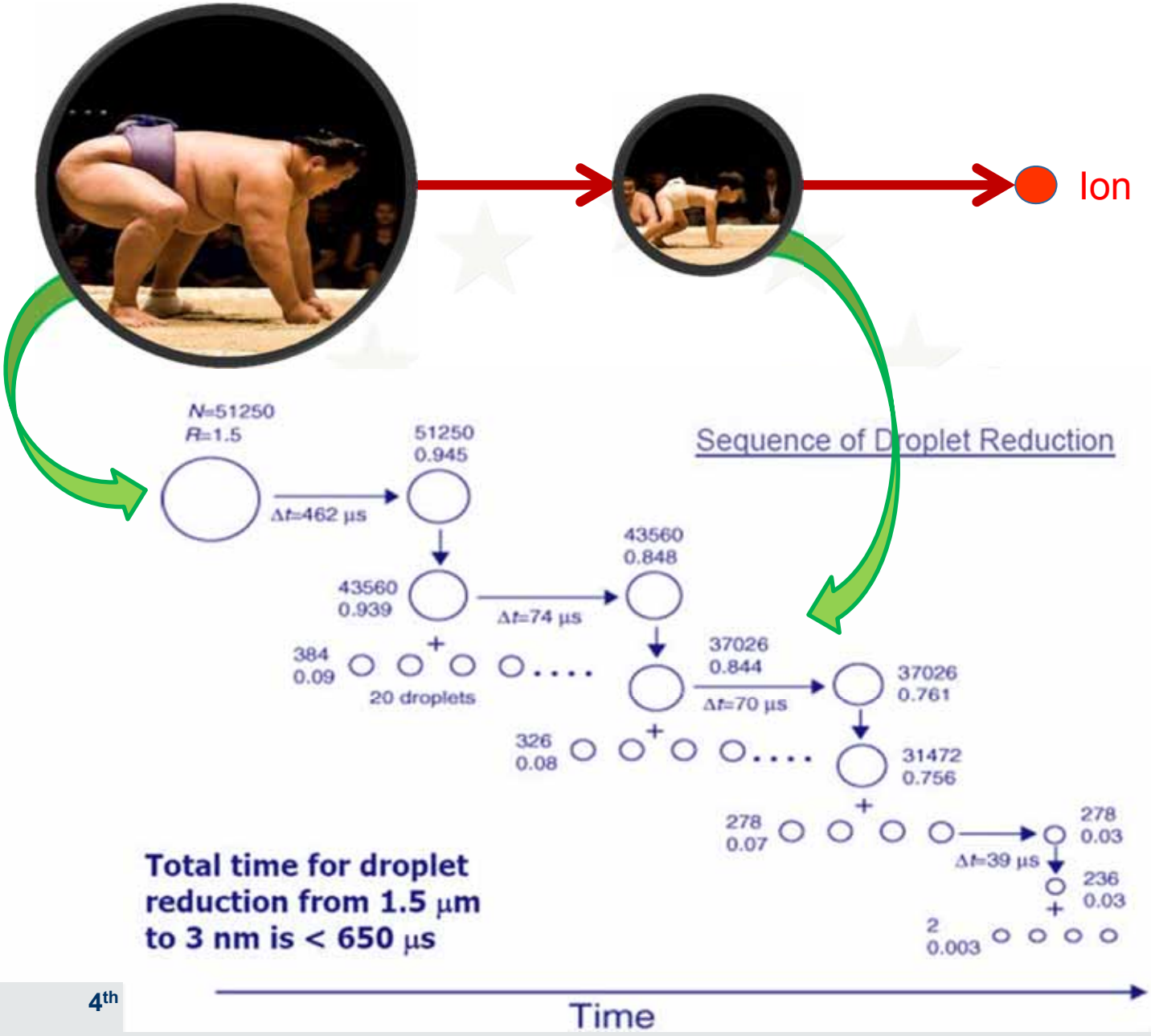
Table 2 Gas flows generated with different GC and LC columns and solvent flows.

Column	Carrier	Carrier flow-rate (ml min^{-1})	Gas flow atmosphere (ml min^{-1})	Gas flow (10^{-6} Torr) (× 1000 l s^{-1})
GC capillary	Helium	1	1	12.5
GC packed/GC (CI)	Helium	20	20	250
LC analytical	Hexane	1	184	2300
	MeOH	1	593	7400
	Water	1	1240	15500
LC capillary	Hexane	0.010	1.8	23
	Water	0.010	12	155

J. Abian *et* al. *J. Mass Spectrom*. 34, 157-168 (1999)

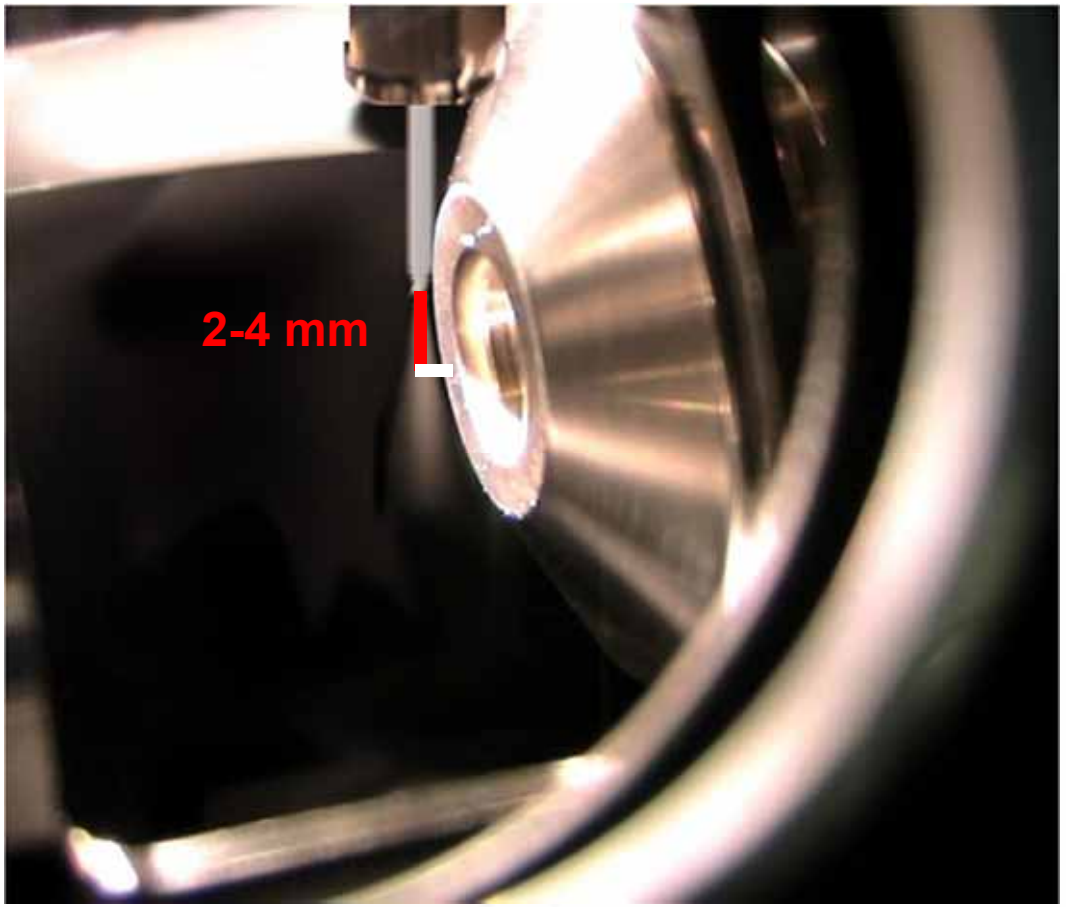
Sampling efficiency

Highly Effective µLC-MS/MS Analysis



Highly Effective µLC-MS/MS Analysis

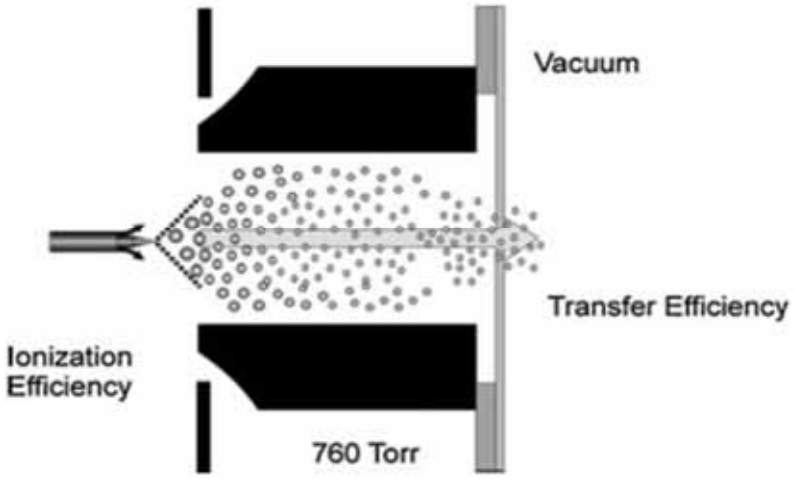
Observing Thermal Focusing



Temperature = 550 ºC

Highly Effective µLC-MS/MS Analysis

Sampling efficiency



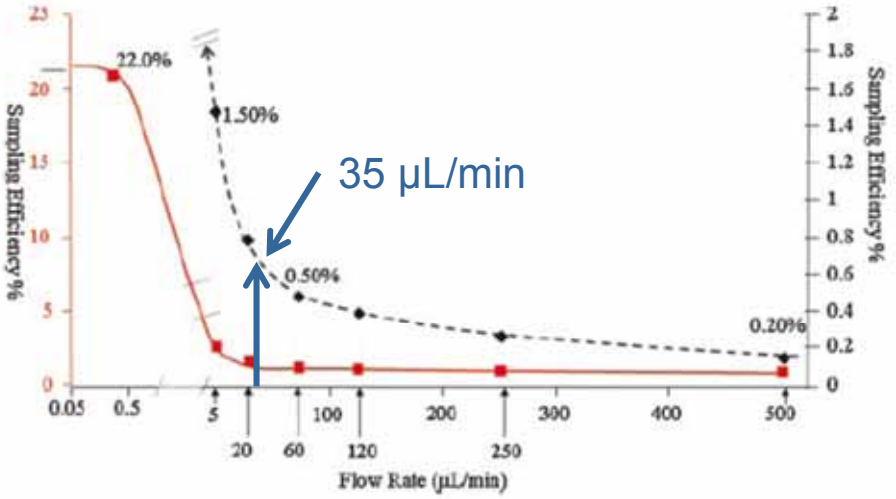
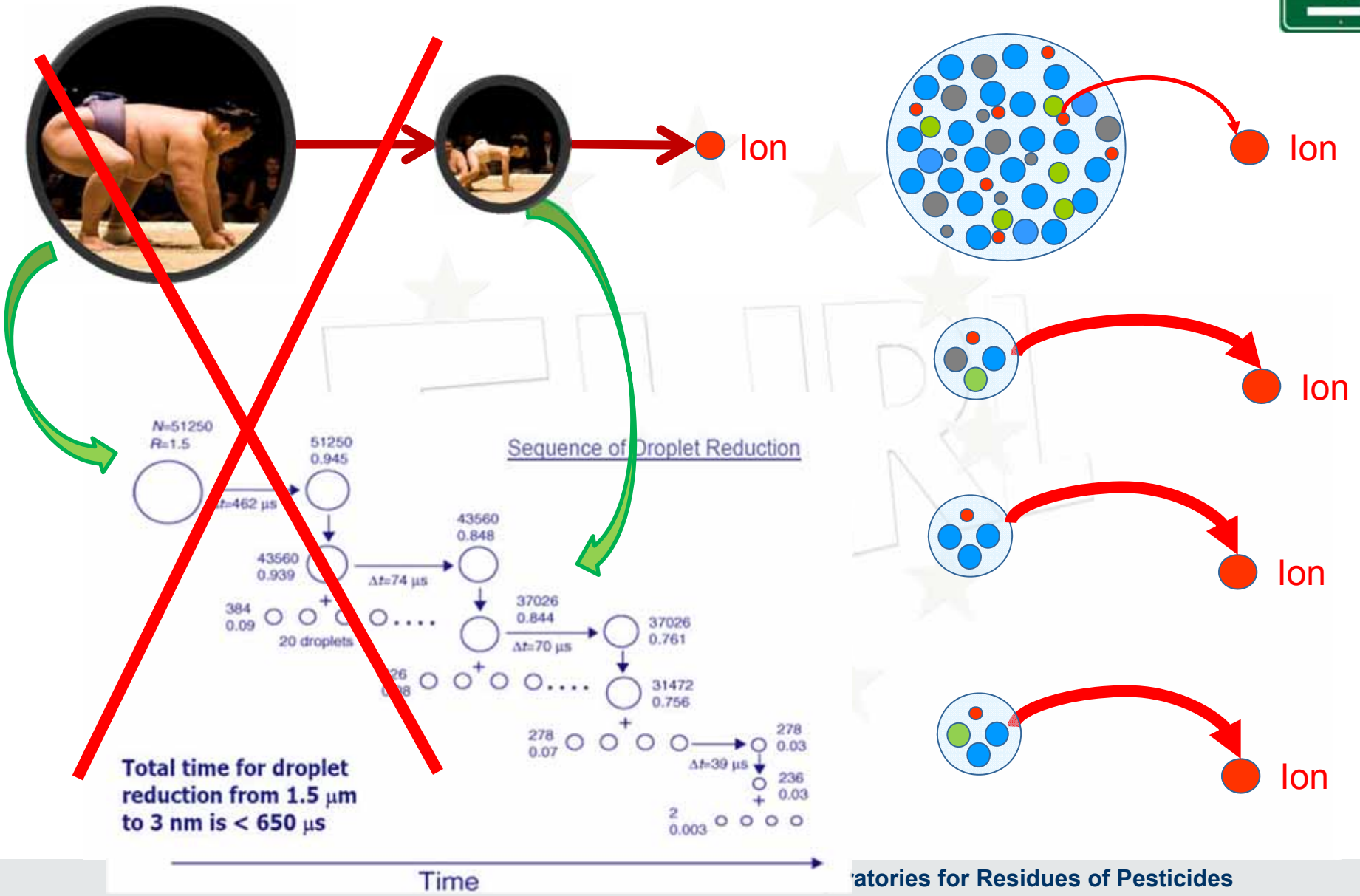


FIGURE 9. Sampling efficiency versus flow rate. The data in the upper dashed trace (black), read from the right *Y* axis, is an expansion of the data in the lower solid trace (red) read from the left *Y* axis. For each flow the optimum source and interface configuration was used for those conditions. For high flow rates above 1 µL/min a heated TurboIonSpray® source with a conical pinhole aperture interface (standard interface) on an API 5000™ triple quadrupole instrument. For points below 1 µL/min a nanospray source with a 15 µm aperture fused silica capillary and an interface optimized for nanoflow introduction (PDI interface, See Ion Transport Section, Declustering, PDI interface) was used.

Atmospheric pressure ion sources. Thomas R. Covey *et al.* *Mass spectrometry Reviews*, **2009**, 28, 870-897


Highly Effective
µLC-MS/MS
Analysis
Ion
Ion
Ion
Ion
Ion
Sequence of Droplet Reduction
N=51250
R=1.5
51250
0.945
Δt=462 µs
43560
0.939
43560
0.848
Δt=74 µs
384
0.09
20 droplets
37026
0.844
Δt=70 µs
37026
0.761
31472
0.756
278
0.07
278
0.03
Δt=39 µs
236
0.03
2
0.003
Total time for droplet reduction from 1.5 µm to 3 nm is < 650 µs
Time

Standard-LC vs micro-LC

Most used

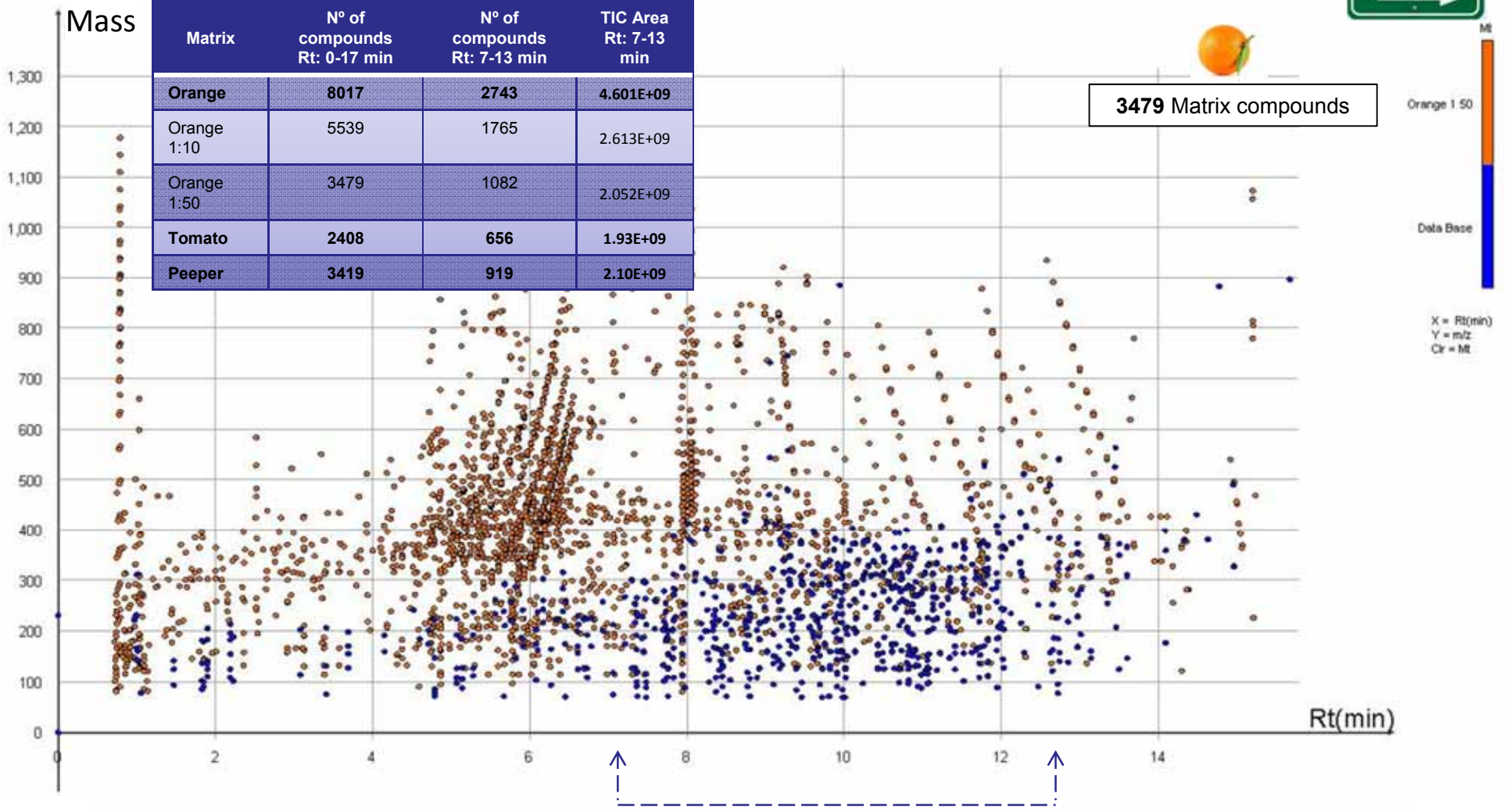
	I.D. Column (mm)	Flow (µL/min)	Nebulizer	I.D. column (mm)	Flow (µL/min)	Sensitivity Theoretically Gain
Standard LC	8.0 – 1.6	200 - 5000	Standard	4.6	400	1
				2.1	200	5
micro LC	0.3 – 1.0	5 - 200	Micro	1	40	20
				0.8	20	30
				0.5	35	≈ 30

Thanks for your Attention

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

Highly Effective LC-MS/MS Analysis

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