NEWS ON MRM METHODS

Amadeo R. Fernández-Alba

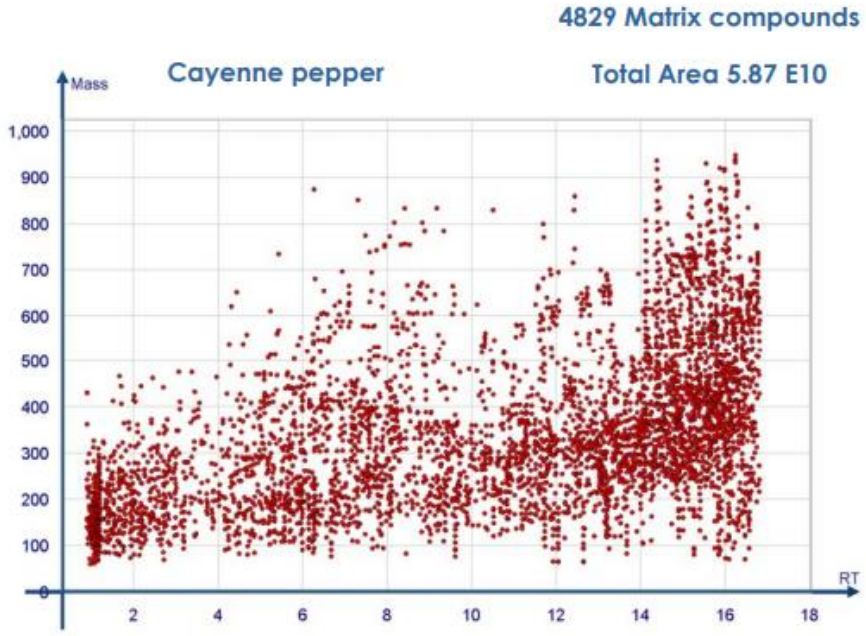
EURL-FV

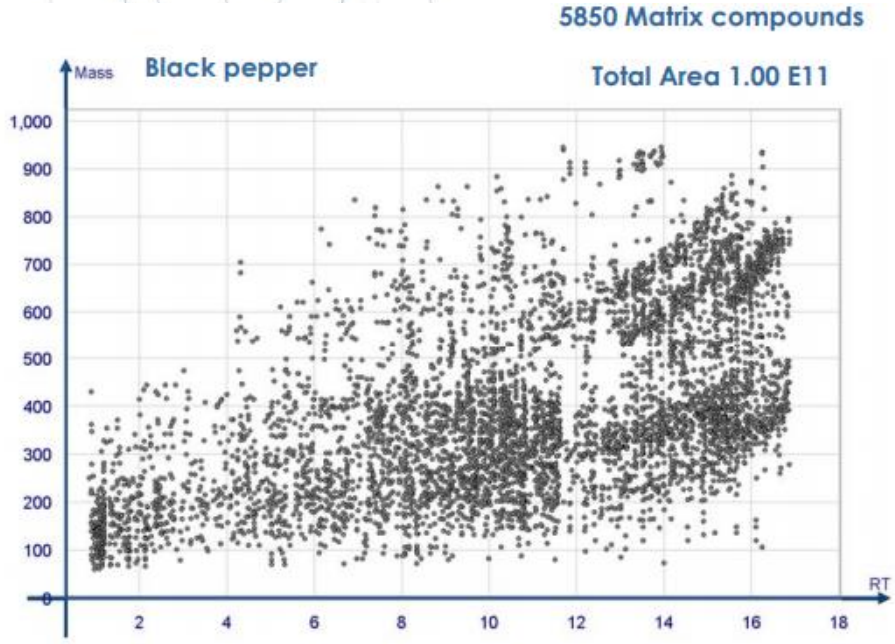
Adding of new compounds to the routine methods from the working document SANCO/12745/2013 of 21st – 22nd November 2017 rev. 9(1)

48 compounds by LC and GC, fully validated at 0.005 and 0.050 mg/kg in tomato, orange and avocado

Ametoctradin (LC)	Novaluron (LC)
Anthraquinone (GC)	Penflufen (LC)
BAC10 (LC)	Penthiopyrad (LC)
BAC12 (LC)	Phenthoate (GC)
BAC8 (LC)	Picolinafen (GC)
Benalaxyl (GC)	Propaquizafop (LC)
Chlorfluazuron (GC)	Proquinazid (LC)
Clomazone (LC)	Prothioconazole (LC)
Cyazofamid (LC)	Prothioconazole-desthio (LC)
Cyflufenamid (GC)	Prothiofos (GC)
Etoxazole (LC)	Pyridalil (LC)
Fenpyrazamine (LC)	Pyridate (LC)
Flufenacet (LC)	Pyriofenone (GC)
Fluopicolide (GC)	Quinoclamine (LC)
Fluxapyrosad (LC)	Quintozene (GC)
Heptachlor (GC)	Rotenone (LC)
Heptachlor endo-epoxide (GC)	Spinetoram (LC)
Heptachlor exo-epoxide (GC)	Spirotetramat (LC)
Ioxynil (LC)	Sulfoxaflor (LC)
Isopyrazam (GC)	Tetramethrin (GC)
Isoxaflutole (LC)	Triallate(GC)
Lufenuron (LC)	Tricyclazole (LC)
Metconazole (LC)	Triticonazole (LC)
Metrafenone (LC)	Tritosulfuron (LC)
Molinate (GC)	

Molecular Components Map of representative matrices of commodity groups (48 matrices)





Exact Mass database for LC-HRMS (with 188 compounds)

Compound	Molecular Formula	Neutral mass (Da)	Adduct	Exact mass (m/z)
Acephate	C4H10NO3PS	183.0119	[M+H]+	184.0192
Acephate_F1	C2H8NO3PS			142.9928
Acephate_F2	C4H4NOP			113.0025
Acetamiprid	C10H11ClN4	222.0672	[M+H]+	223.0745
Acetamiprid_F1	C6H5ClN			126.0105
Acetamiprid_F2	C6H4N			90.0338

Enlarge of the scope of the Accurate Mass Database by GC-HRMS (150

Compound	Molecular Formula	Retention Time (min)	Theoretical Mass
1-Naphthol	C9H7	10.51	115.0548
1-Naphthol F1	C10H8O	10.51	144.0575
1-Naphthol F2	C7H5	10.51	89.0391
2,3,5-Trimethacarb	C8H9O	13.49	121.0653
2,3,5-Trimethacarb F1	C9H12O	13.49	136.0888
2,3,5-Trimethacarb F2	C7H7	13.49	91.0548
2,3,5-Trimethacarb F3	C6H5	13.49	77.0391

Validation of the MRM methods at low concentration levels (0.002 mg/kg in GC-MS/MS and at 0.005 mg/kg in LC-MS/MS)

203 compounds by GC-MS/MS

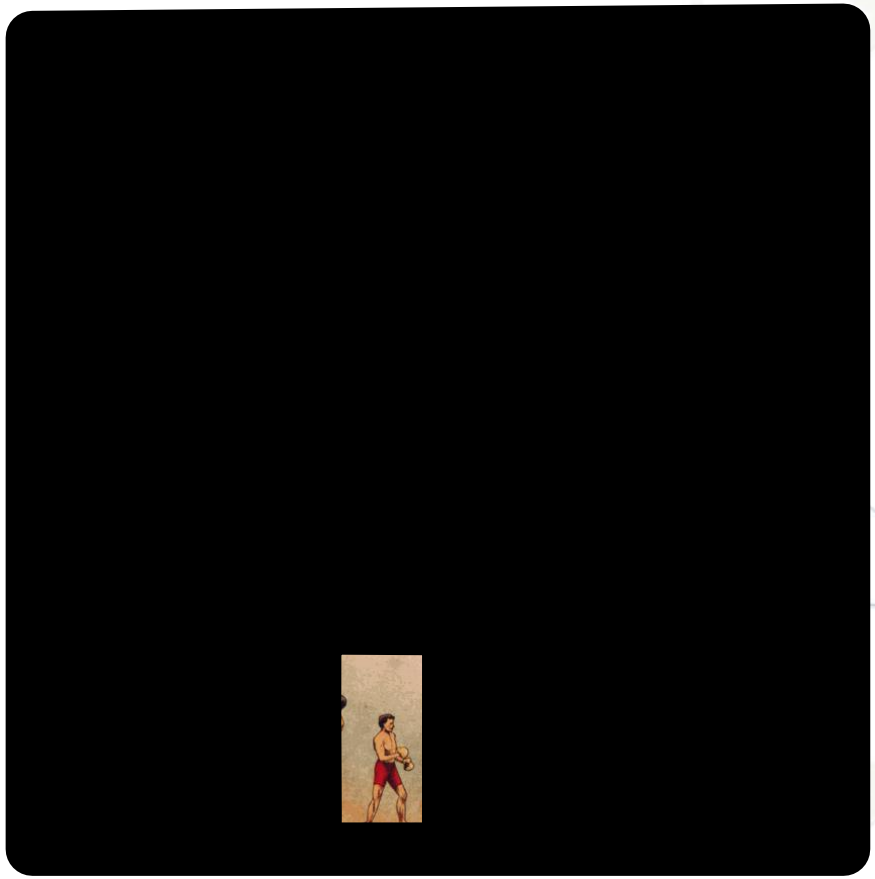
195 compounds by LC

All of them, fully validated in tomato, orange, apple, avocado and tea

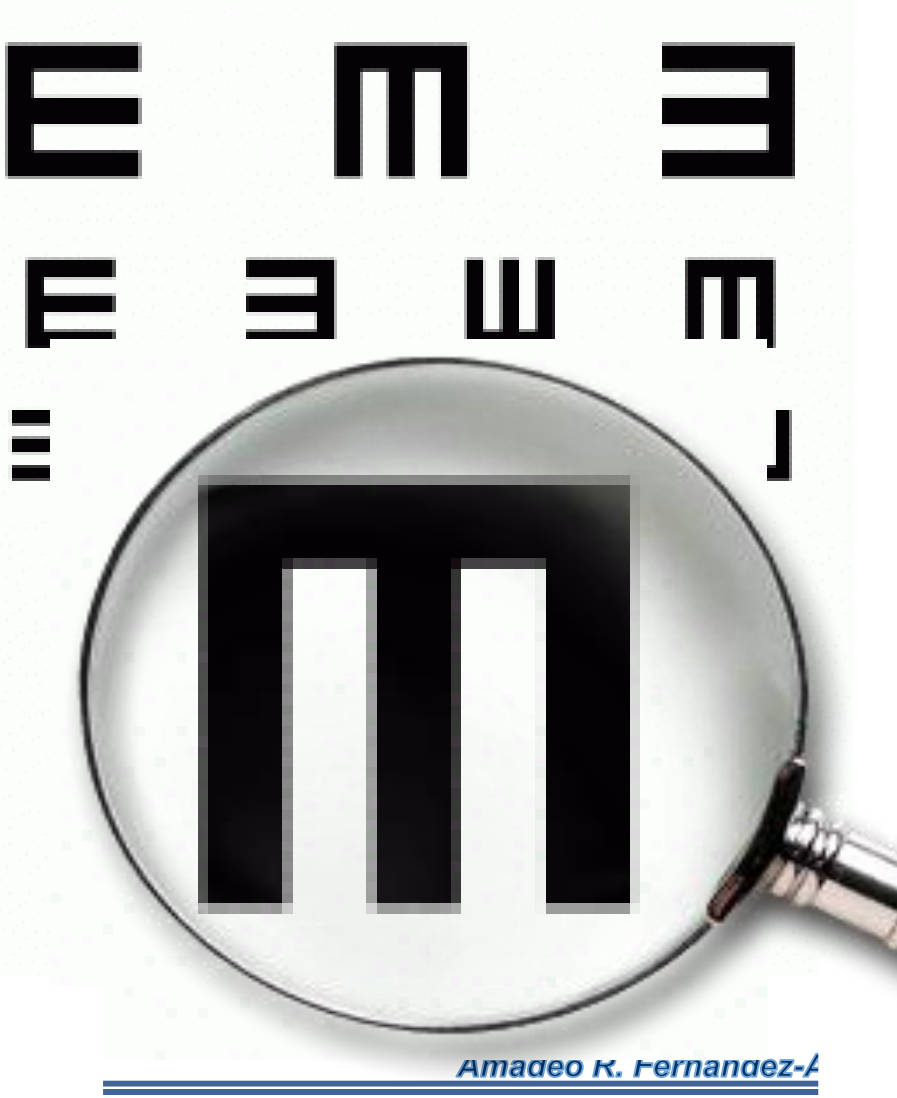




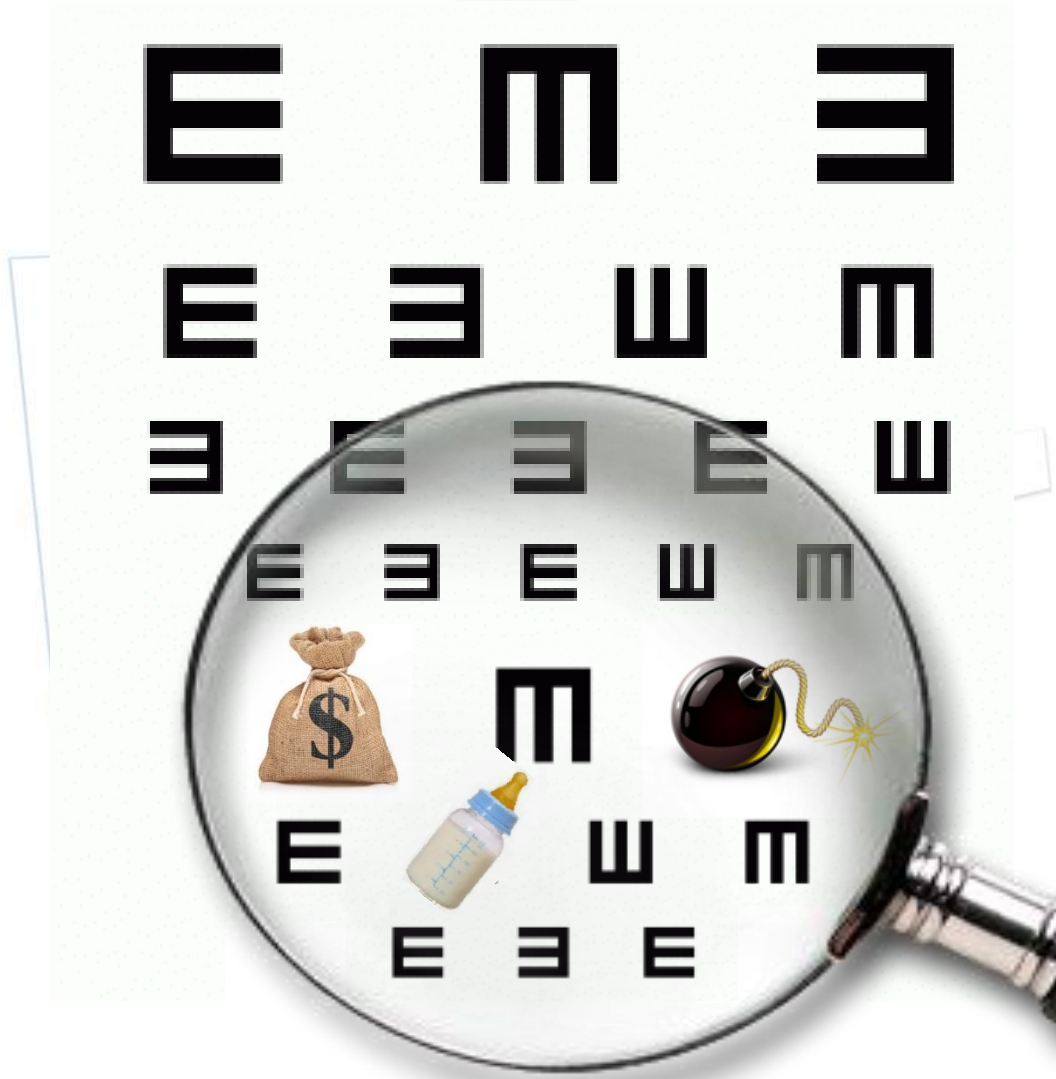
SELECTIVITY



SENSITIVITY



Sensitivity



$scCO_2$ AS MOBILE PHASE

Liquid-like density

Gas-like viscosity

Low critical point

T_c = 304.1 K

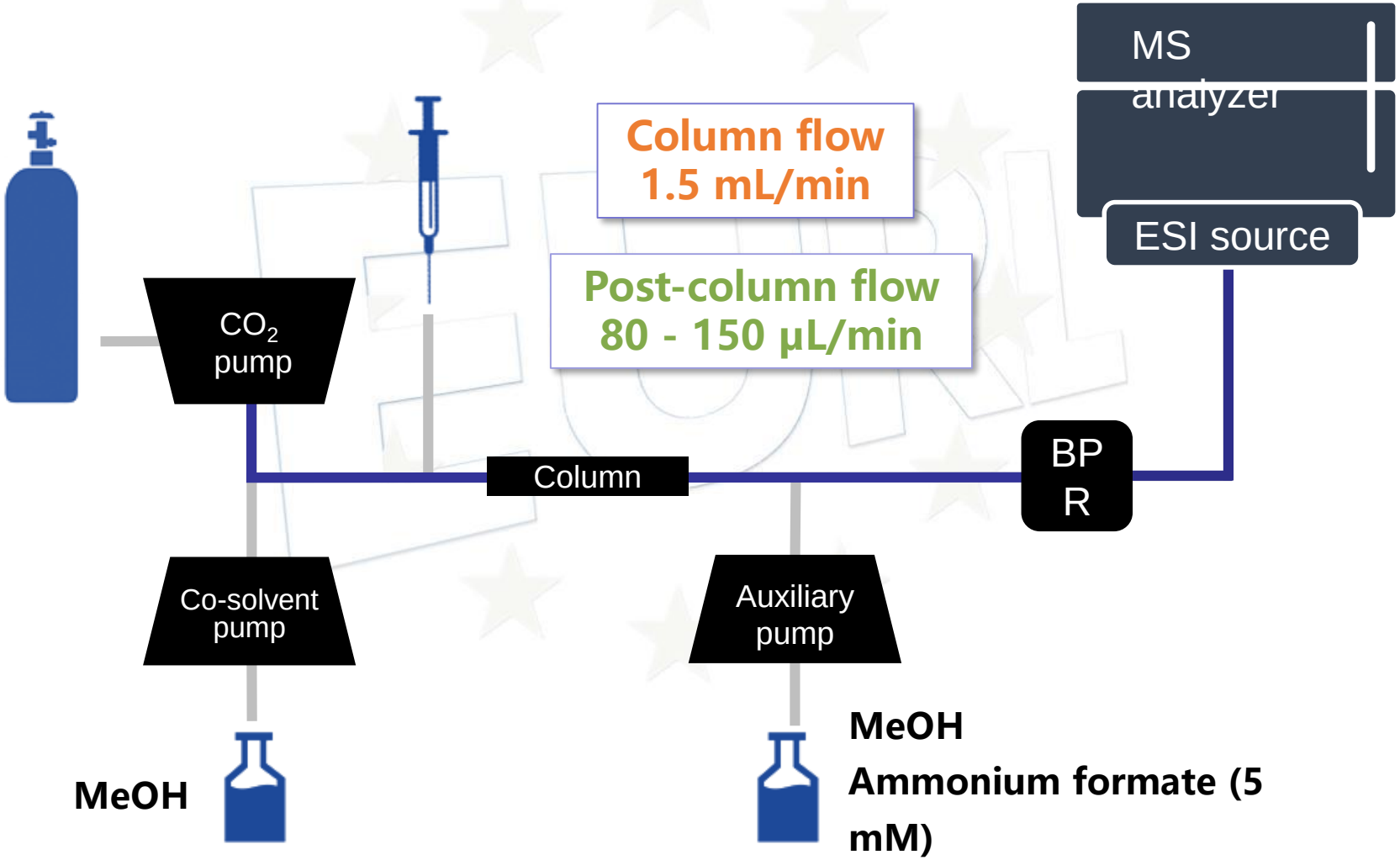
P_c = 73.8 bar

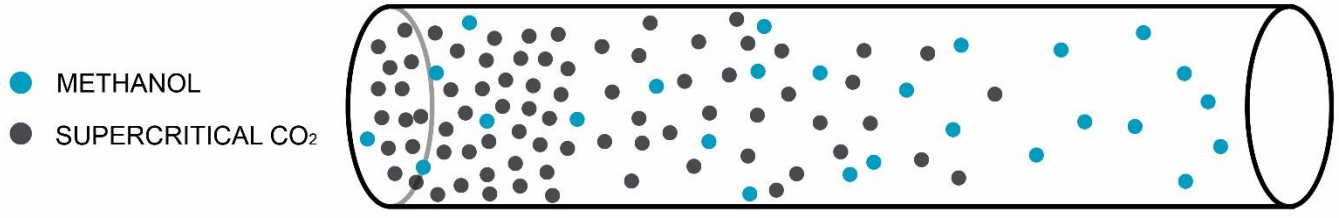
High diffusivity

Widely available, safe to use

Capability to mix with a wide range of liquid solvents

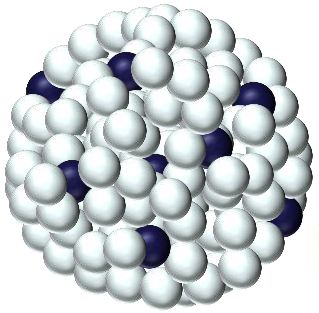
SFC-MS/MS Systems





SMALL AMOUNT OF ORGANIC SOLVENT REACHING THE SOURCE

IONIZATION EFFICIENCY



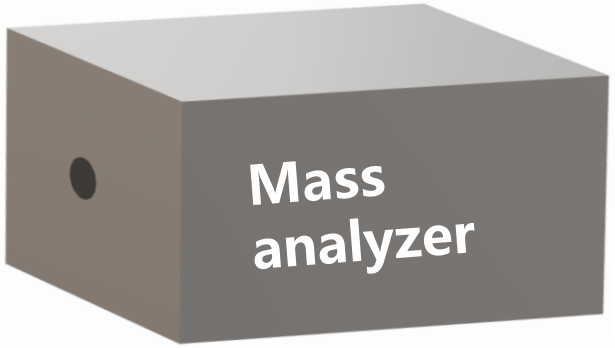
Microdroplet in **LC** (containing water)

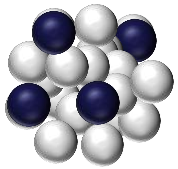
Absence of water

- Lower surface tension
- Lower polarity

CO_2 evaporation

- Lower flow in the MS detector
- Reduced microdroplet size





Microdroplet in **SFC** (only MeOH)

More analyte reaching the gas phase and the mass analyzer.

Increase of sensitivity

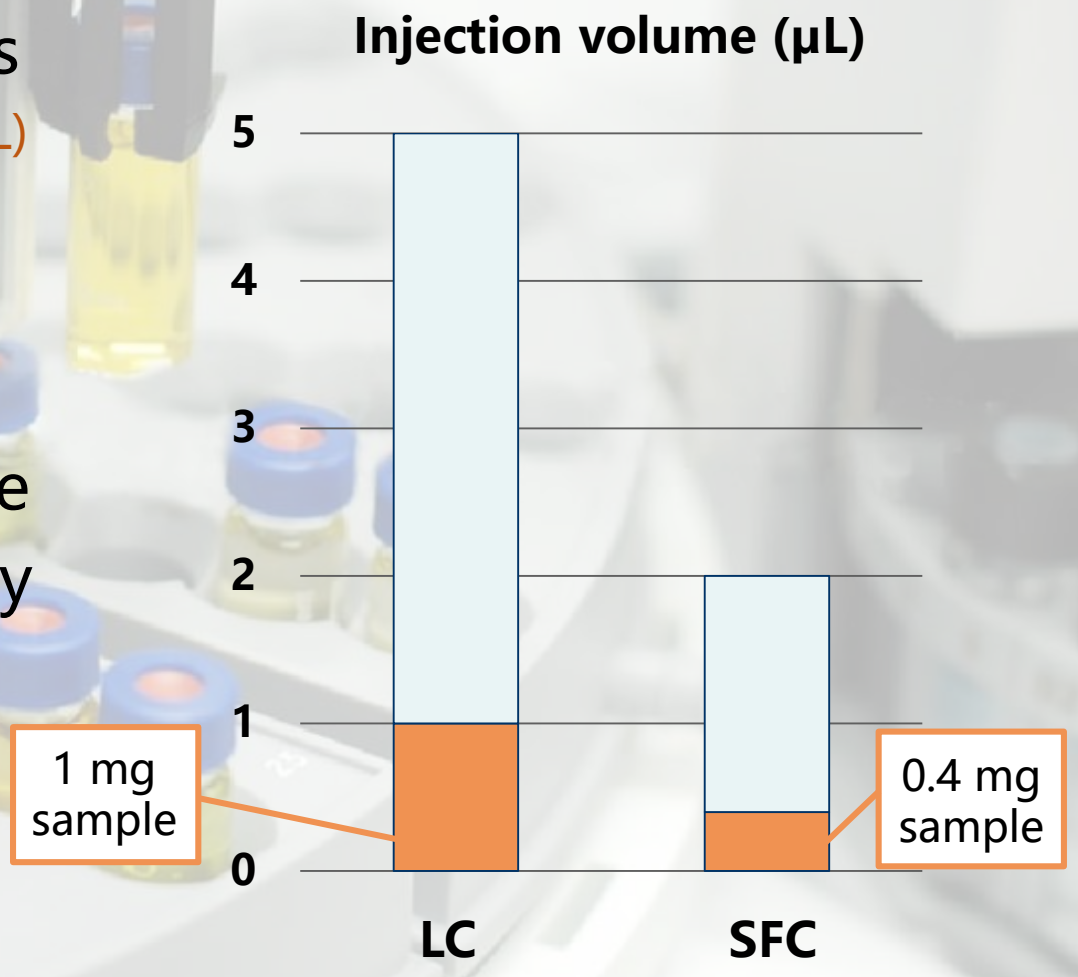
INCREASE OF SENSITIVITY

Lower injection volumes

(matrix concentration 0.2 mg/uL)

Lower amount of sample with no loss of sensitivity

Reduced matrix effect



EURL-FV

FRUITS AND VEGETABLES

Application of SFC-MS/MS for the detection of pesticides in fruits and vegetables

164 pesticides (log K_{ow} -0.8 to 6.9)

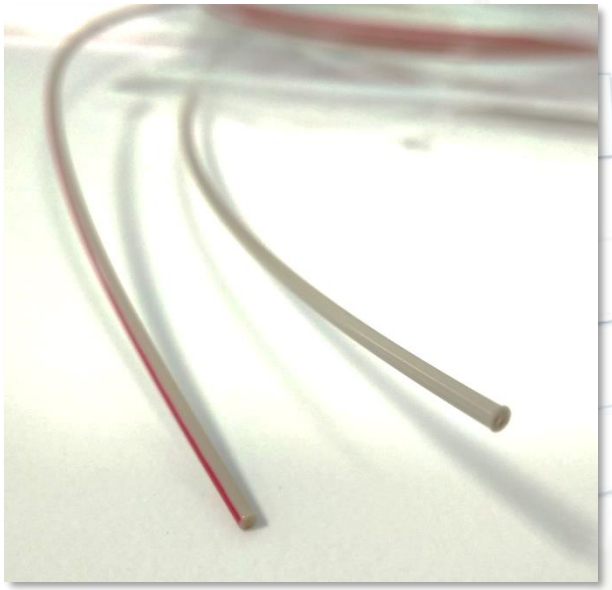
Tomato High water content

Orange Acidic matrix

Leek Interfering compounds

MAKE-UP FLOW

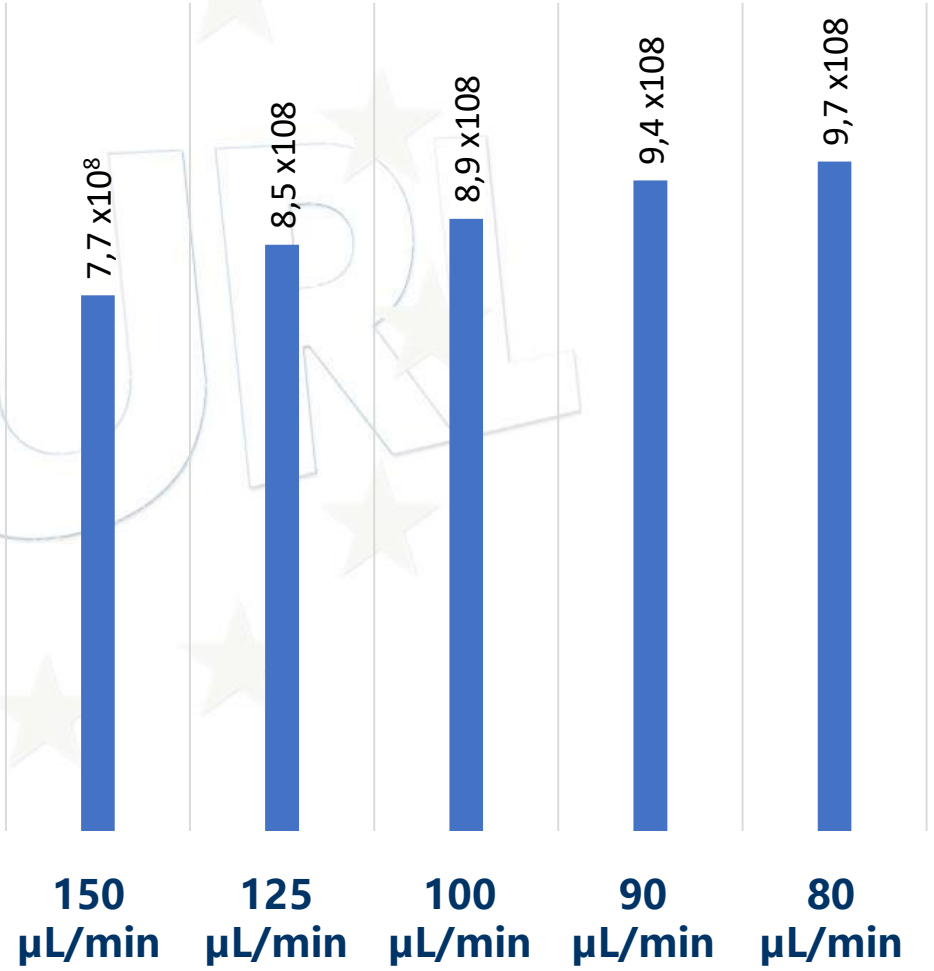
PEEK DIAMETER



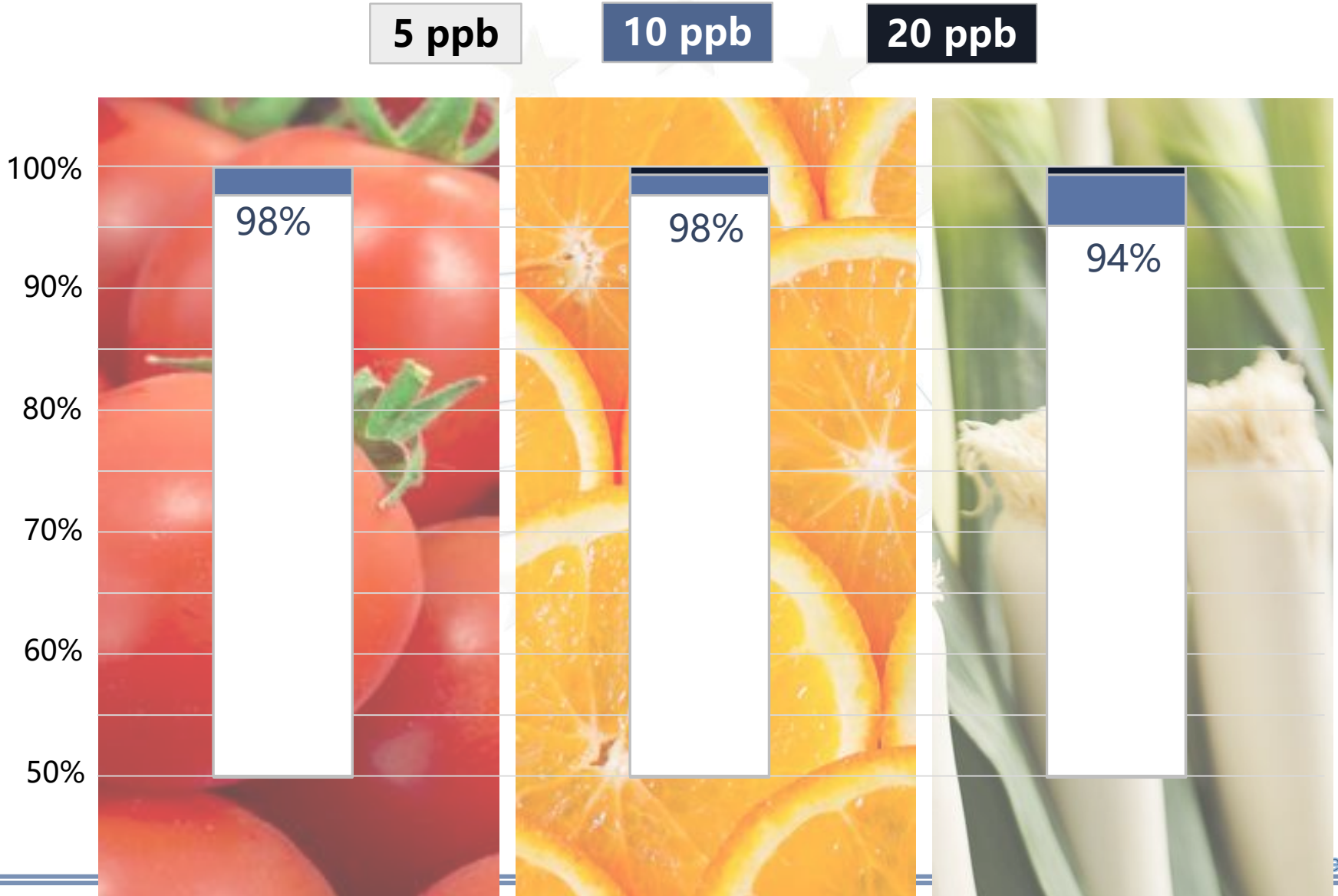
I.D: 127µm

I.D: 64 µm

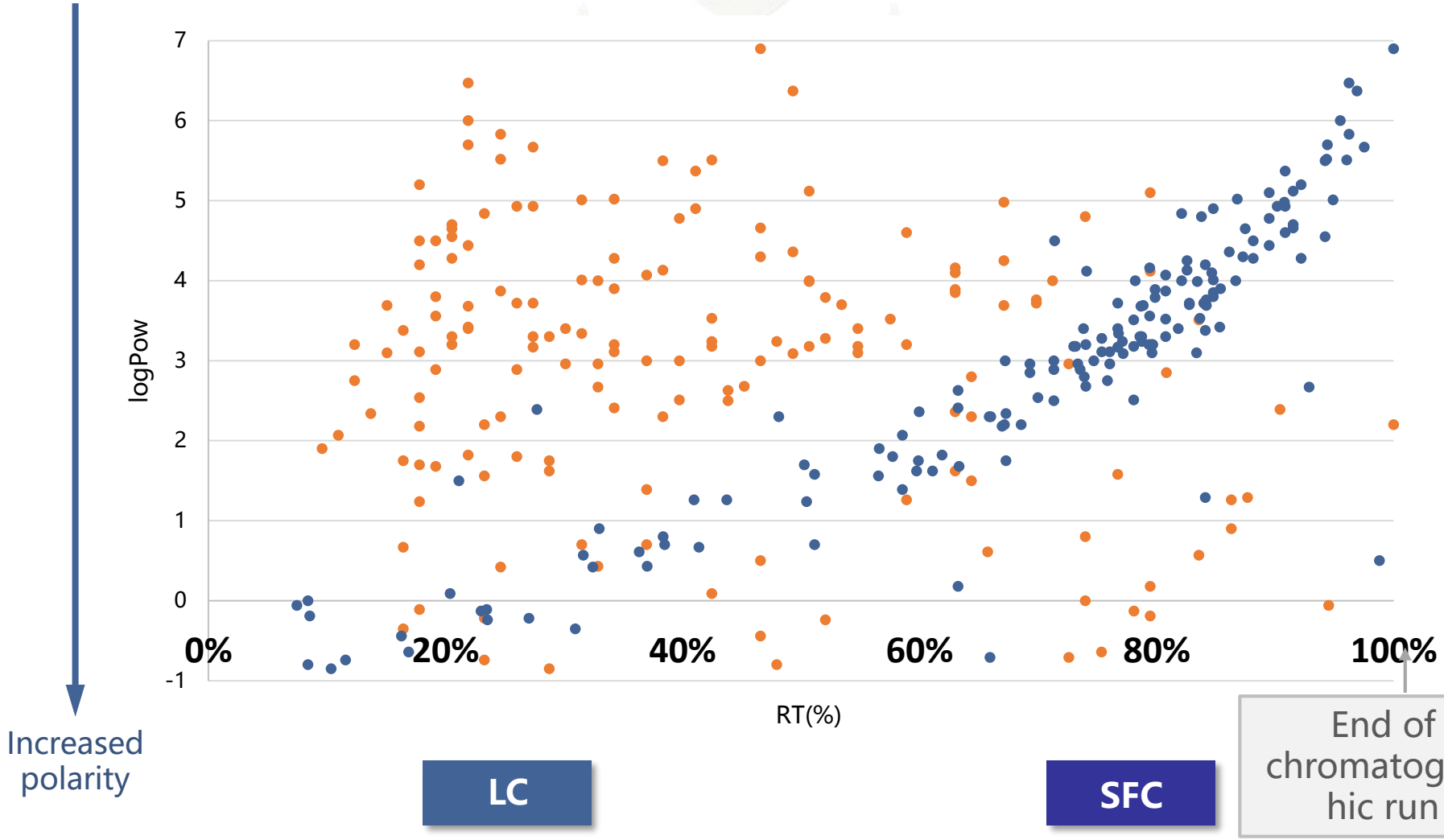
TOTAL AREA OF COMPOUNDS



SENSITIVITY - IDENTIFIED COMPOUNDS



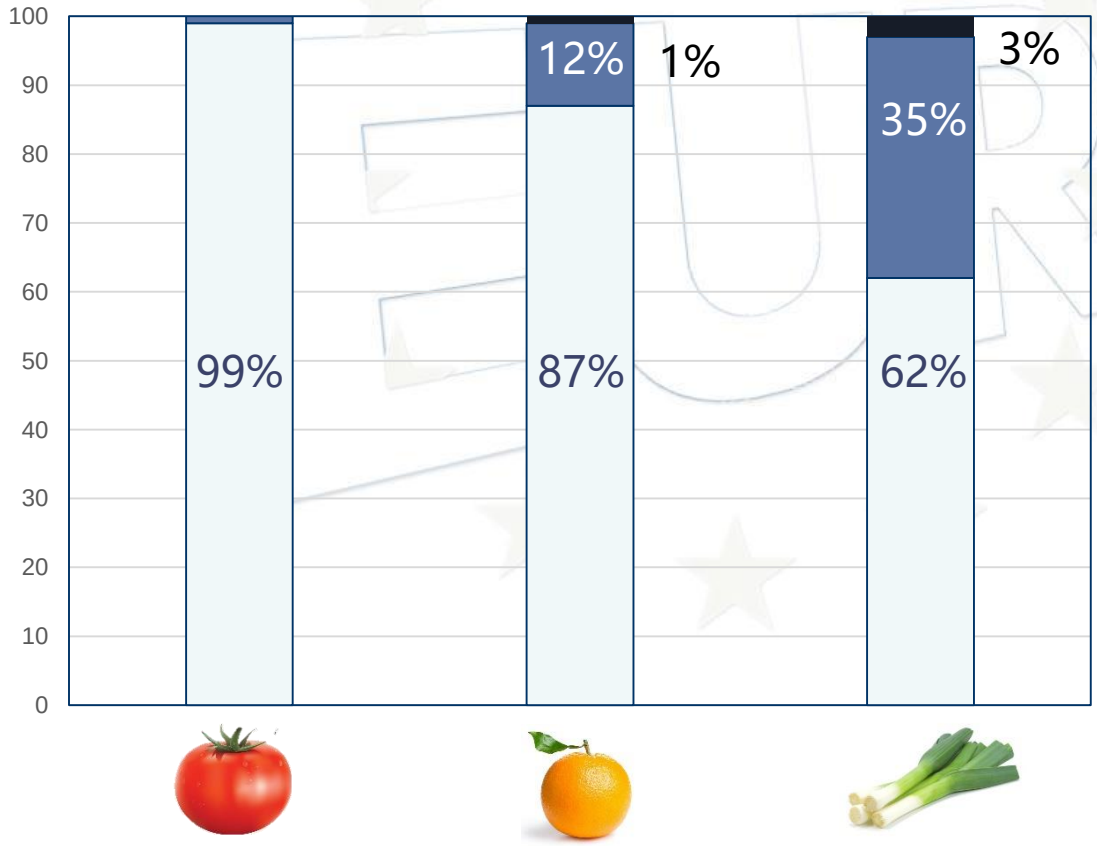
ELUTION ORDER



MATRIX EFFECT

Evaluation

Comparison between calibration curves built in matrix and solvent (considered as no suppression)

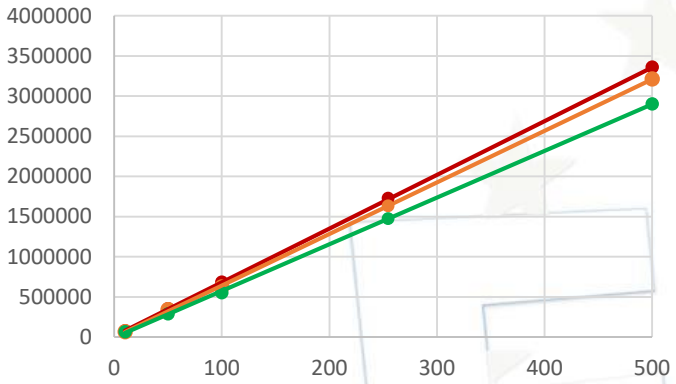


Significant matrix effect (>50% suppression)

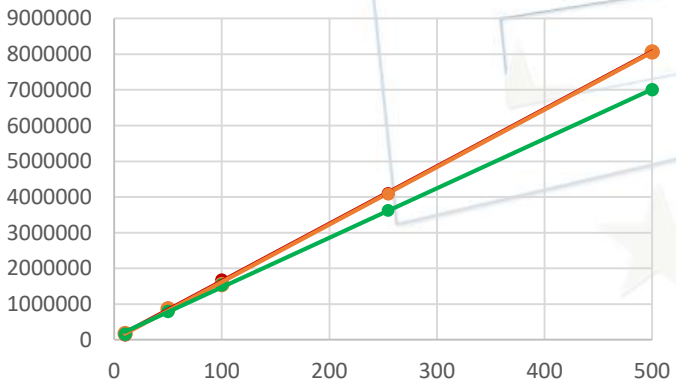
Low matrix effect (20-50% suppression)

Irrelevant matrix effect (<20% suppression)

MATRIX EFFECT



Carbaryl



Profenofos

Tomato

Orange

Leek

“

Matrix effect is more intense in samples with a high number of interfering compounds.

Similar calibration curves for these three different matrices illustrate the low matrix effect.

”

ISOBARIC INTERFERENCES

The use of high flow rates promotes the coelution of matrix compounds and analytes, which leads to modification of ion ratios (>30%).

Tomato

Isoprocarb

Fenhexamid

Orange

Isoprocarb

Lufenuron

Leek

Epoxiconazole

Penthion-sulfone

Fenoxycarb

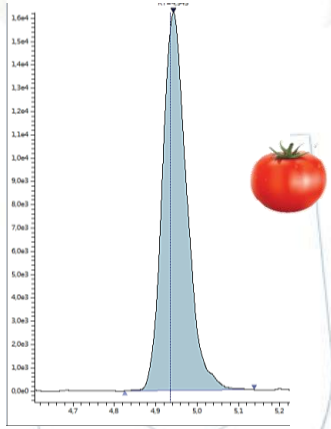
Flutriafol

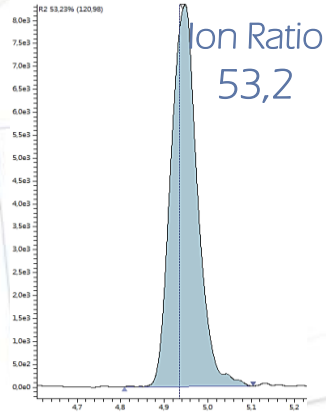
Imazalil

Fenarimol

Phoxim

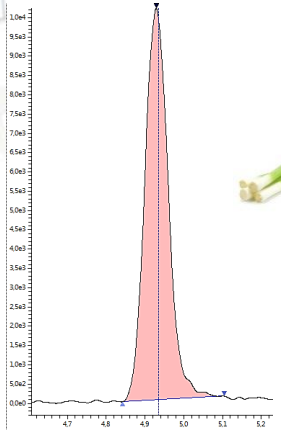
Trichlorfon

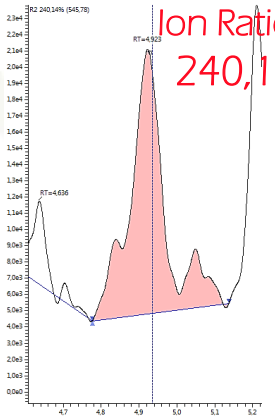


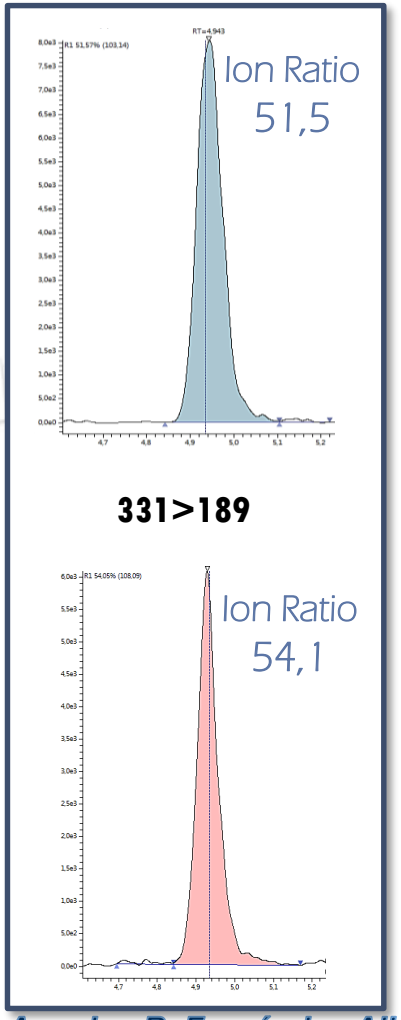


331>268

331>110







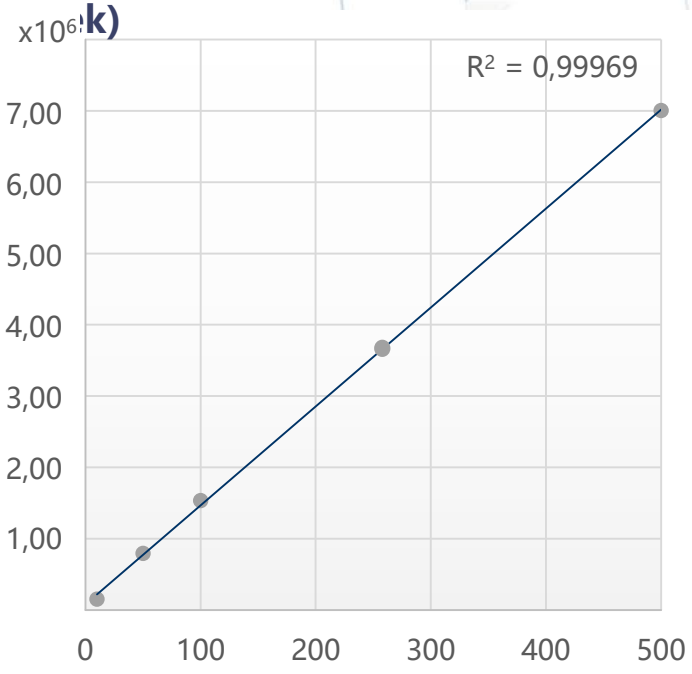
331>189

METHOD VALIDATION

Linearity

Matrix-matched standards in the range of 1-100 µg/L
(5-500 µg/kg in samples)

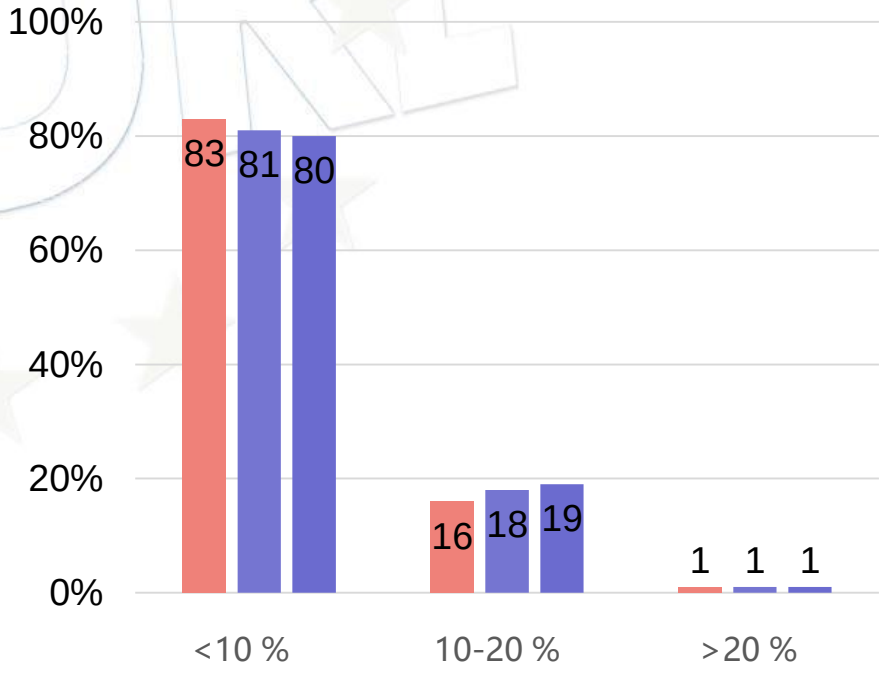
Calibration curve (Profenofos – k)



Reproducibility

5 replicates of each matrix extract

RSD of compounds (5ppb)



SPICES

Application of SFC-MS/MS for the detection of pesticides in spices

Interferring matrix components

Strong matrix effect

162 pesticides (log K_{OW} -0.9 to 6.9)

Cayenne

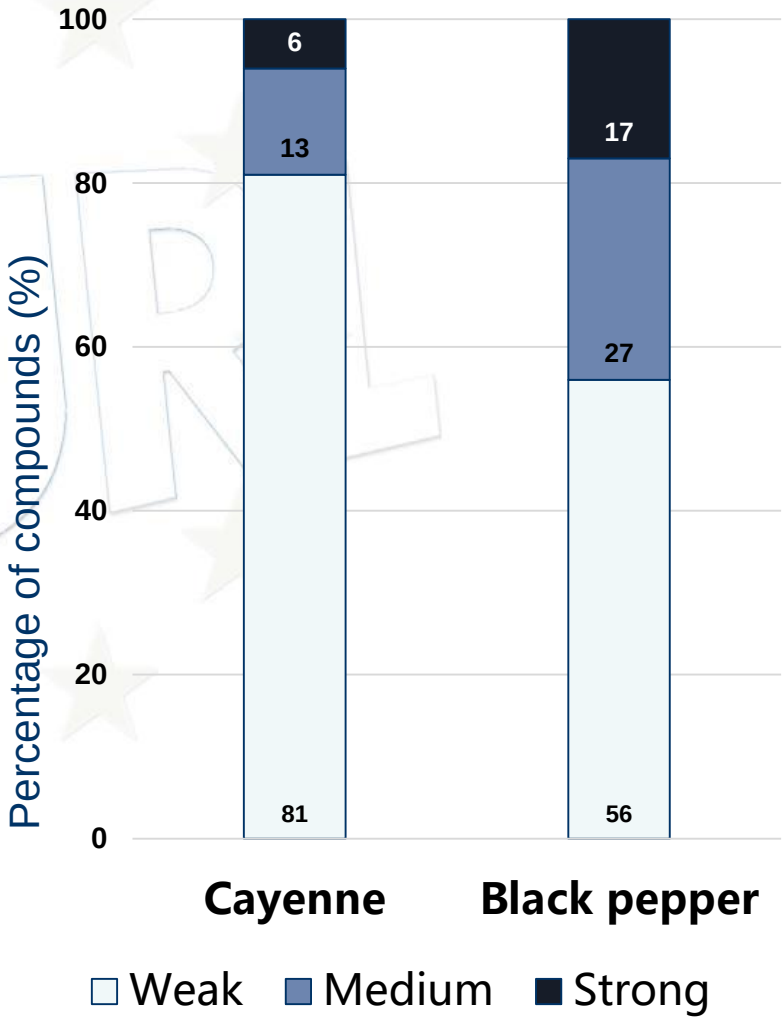
Black pepper

MATRIX EFFECT

Low flow rate in the source
Reduced microdroplet size

Absence in water in the mobile phase
Lower polarity and surface tension

Smaller amount of sample injected
Fewer interferences

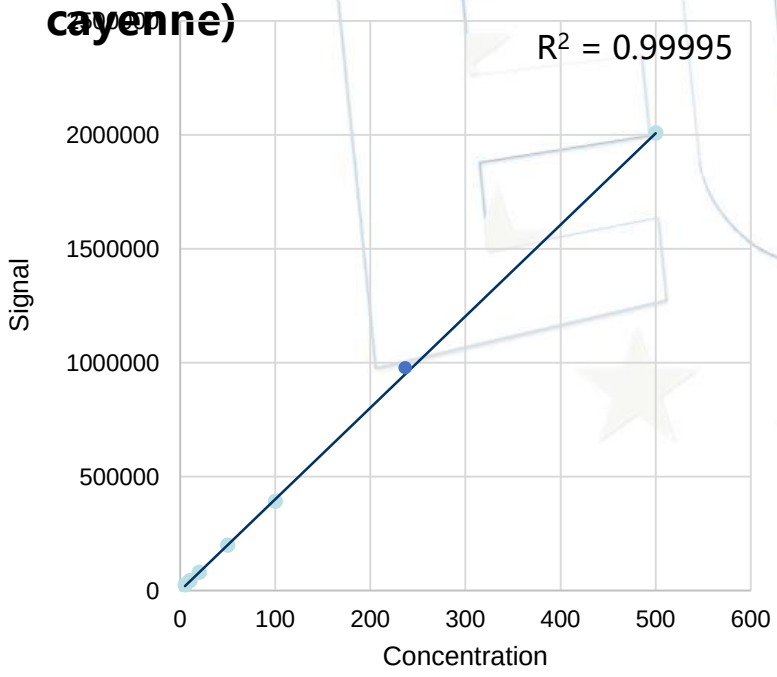


METHOD VALIDATION

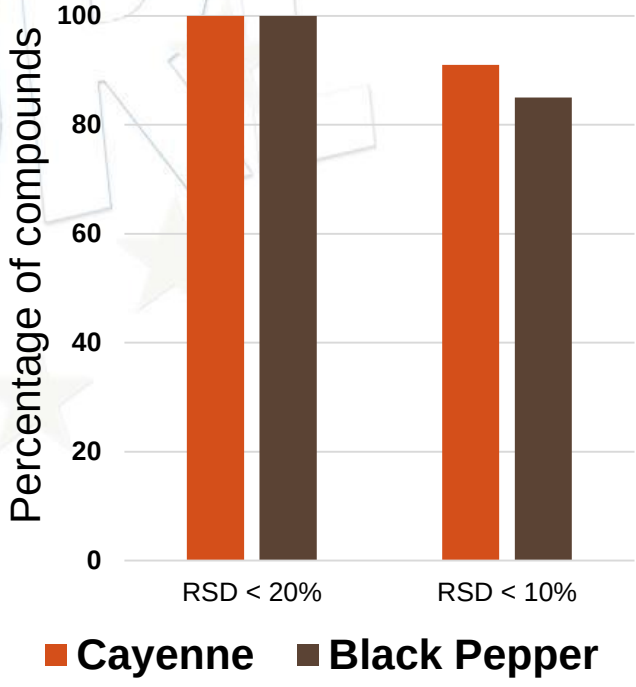
Linearity

Matrix-matched standards in the range of 5-500 µg/L
(50-5000 µg/kg in samples)

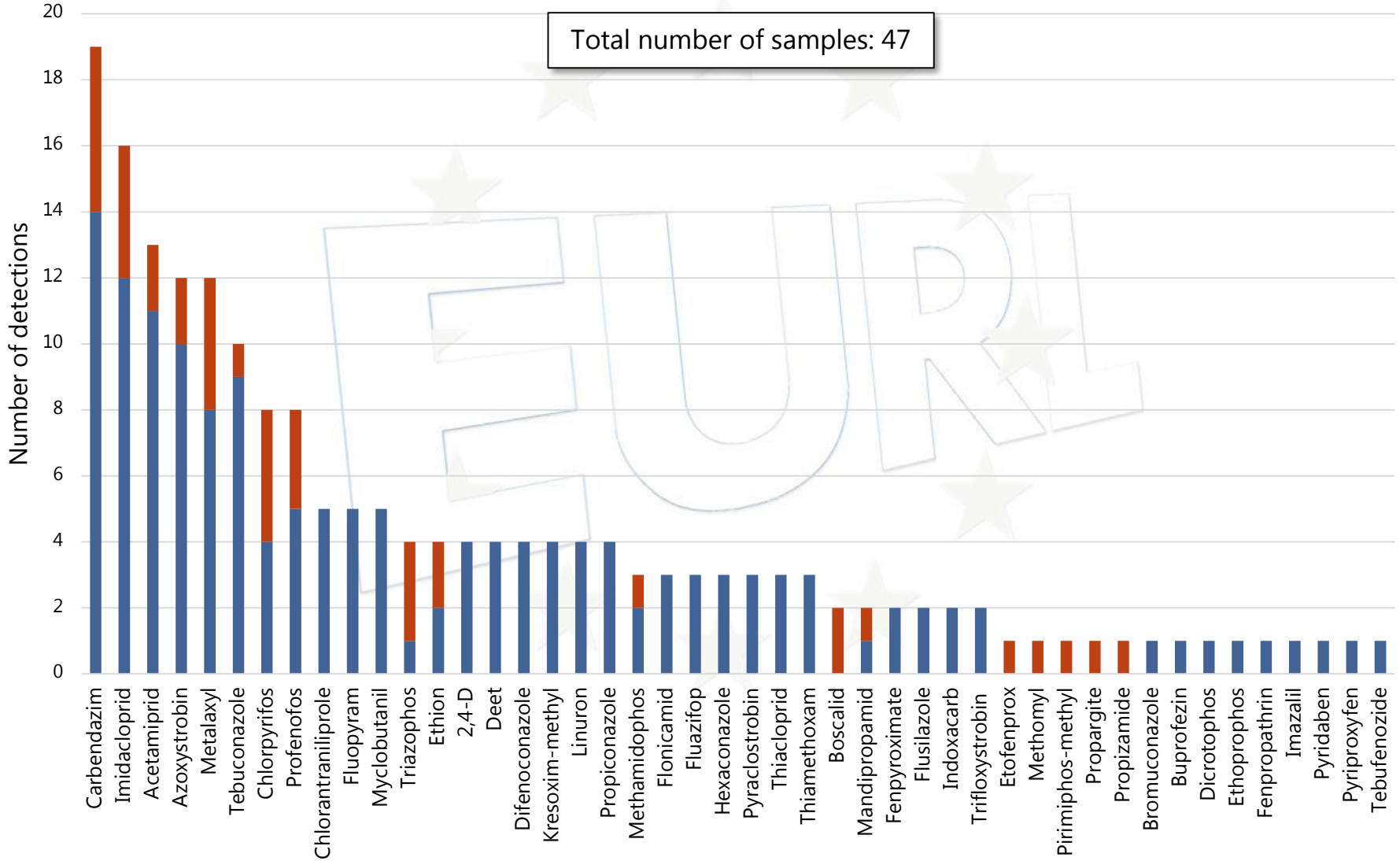
Calibration curve (Isoprocarb in cayenne)



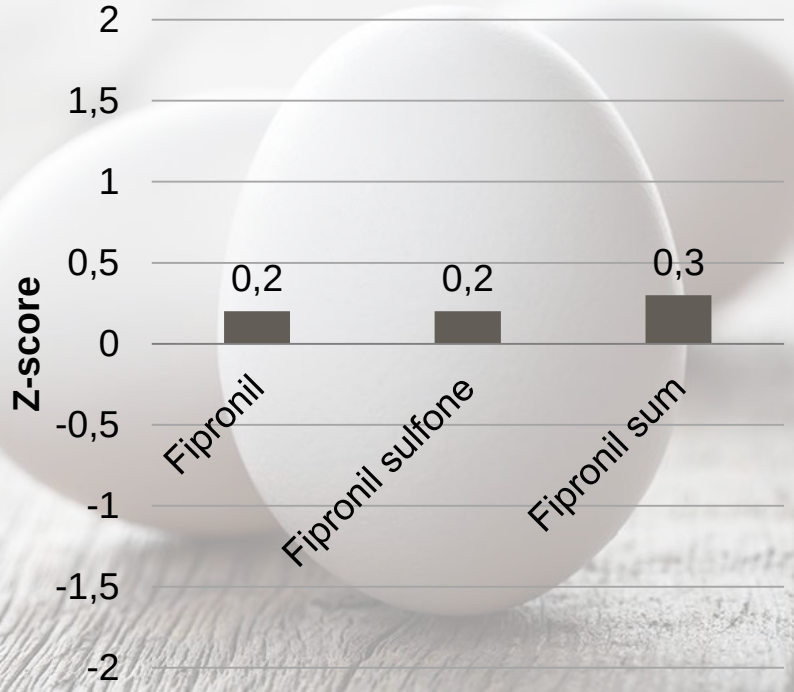
Reproducibility



REAL SAMPLES

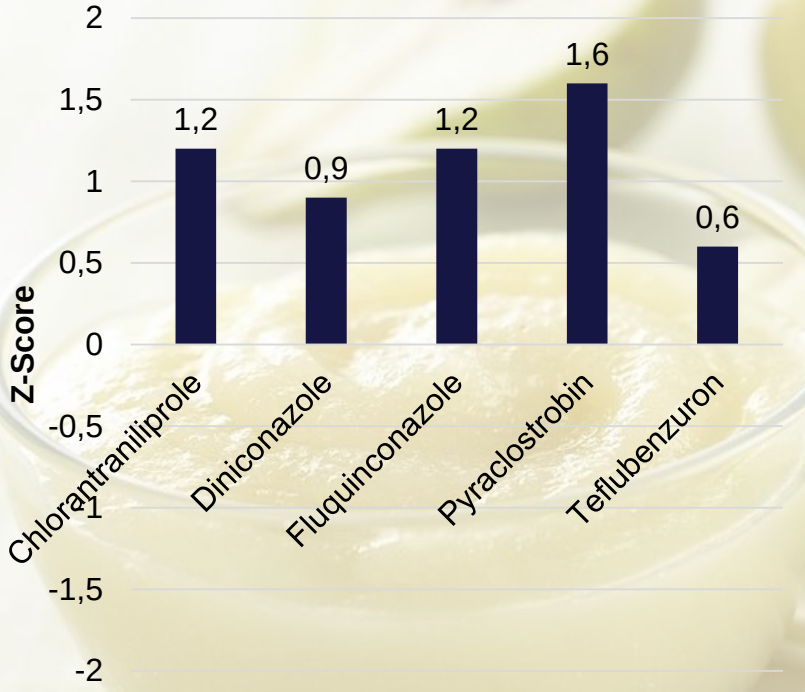


PROFICIENCY TESTS



JRC-GEEL (2017)

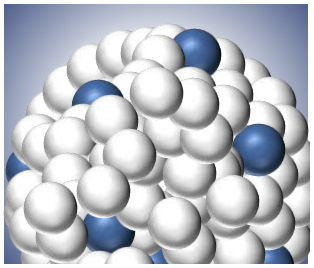
Fipronil in eggs

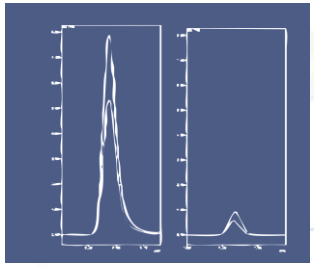


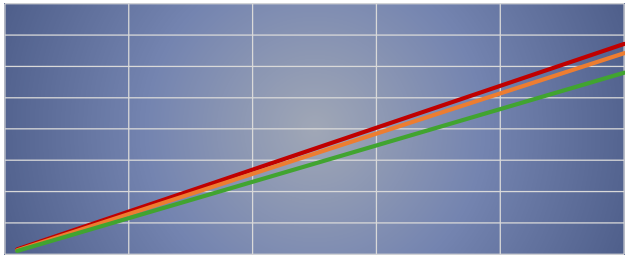
FAPAS (2017)

Pesticide Residues in pear purée

CONCLUSIONS







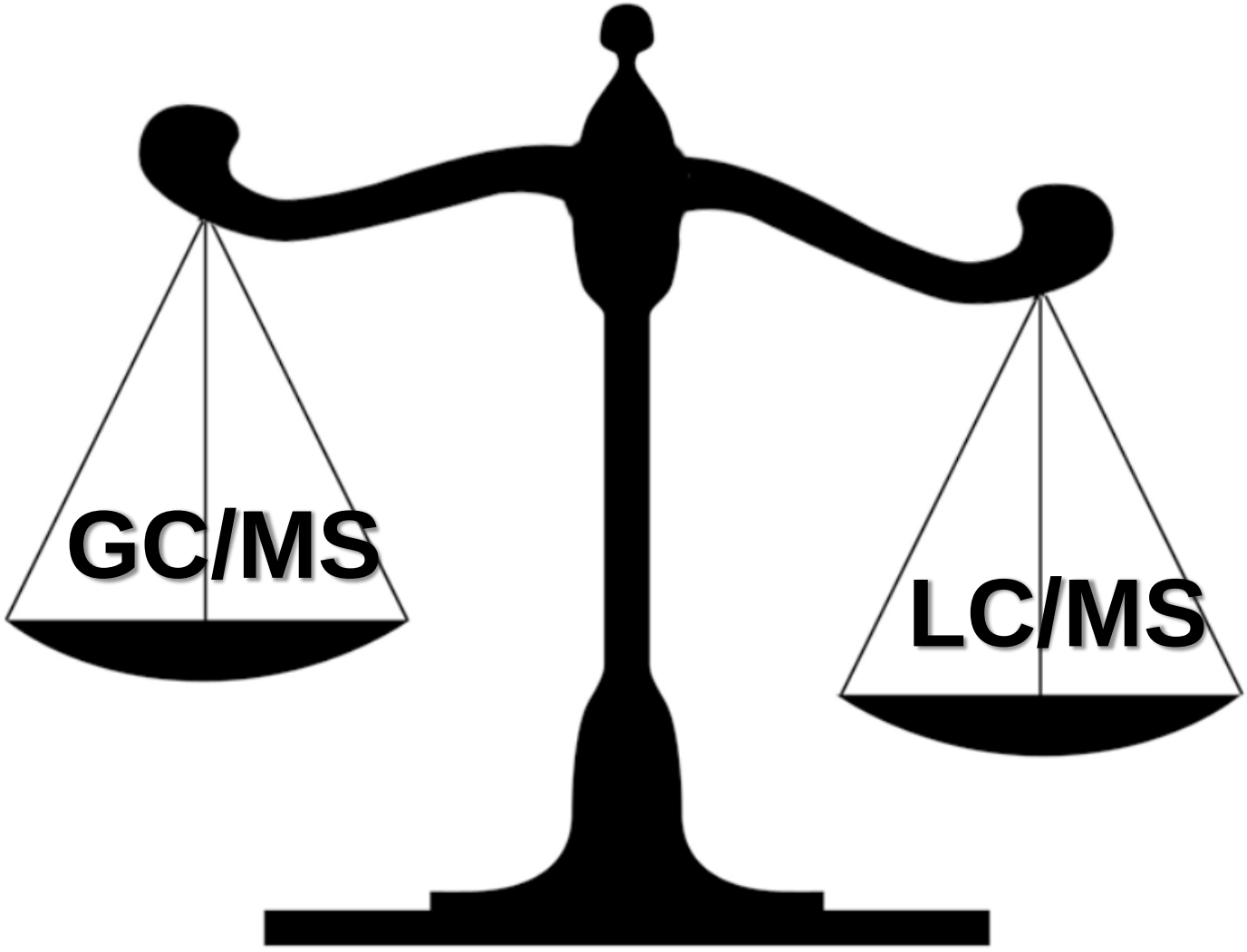
SFC provides a high sensitivity of the analysis even with small amounts of sample injected:

- Absence of water.
- Evaporation of CO_2 in the mobile phase.

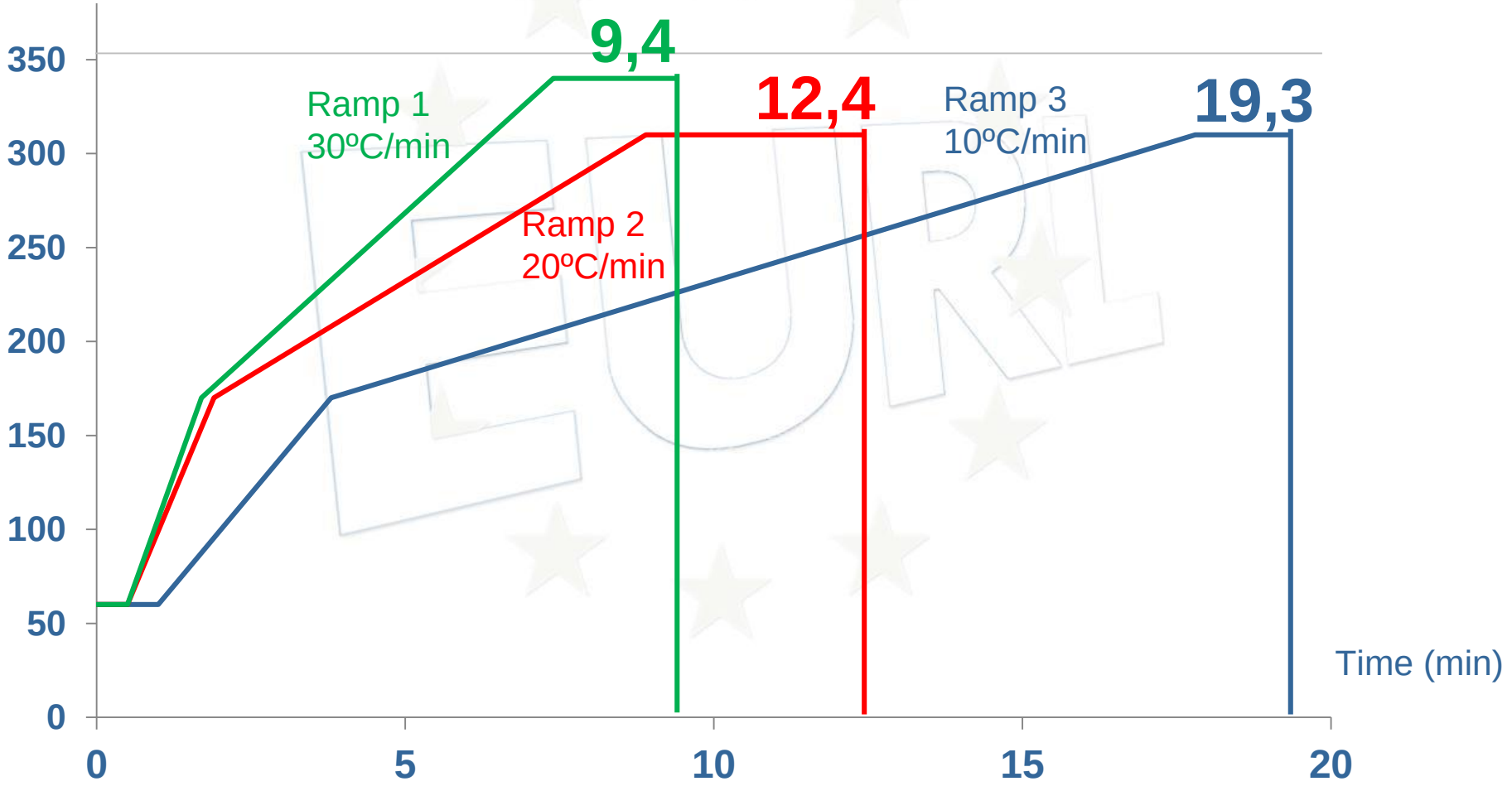
The use of SFC-MS/MS allowed the identification of the majority of 164 pesticides in tomato (98%), orange (98%) and leek (94%) at 5 µg/kg. Good recoveries (70-120%) were obtained in cayenne (89%) and black pepper (93%) at 50 µg/kg.

Some polar and acidic compounds underwent an increase of their signals. Their peak shapes could also be improved.

Very low matrix effect was observed for most compounds in all matrices studied.


GC/MS
LC/MS

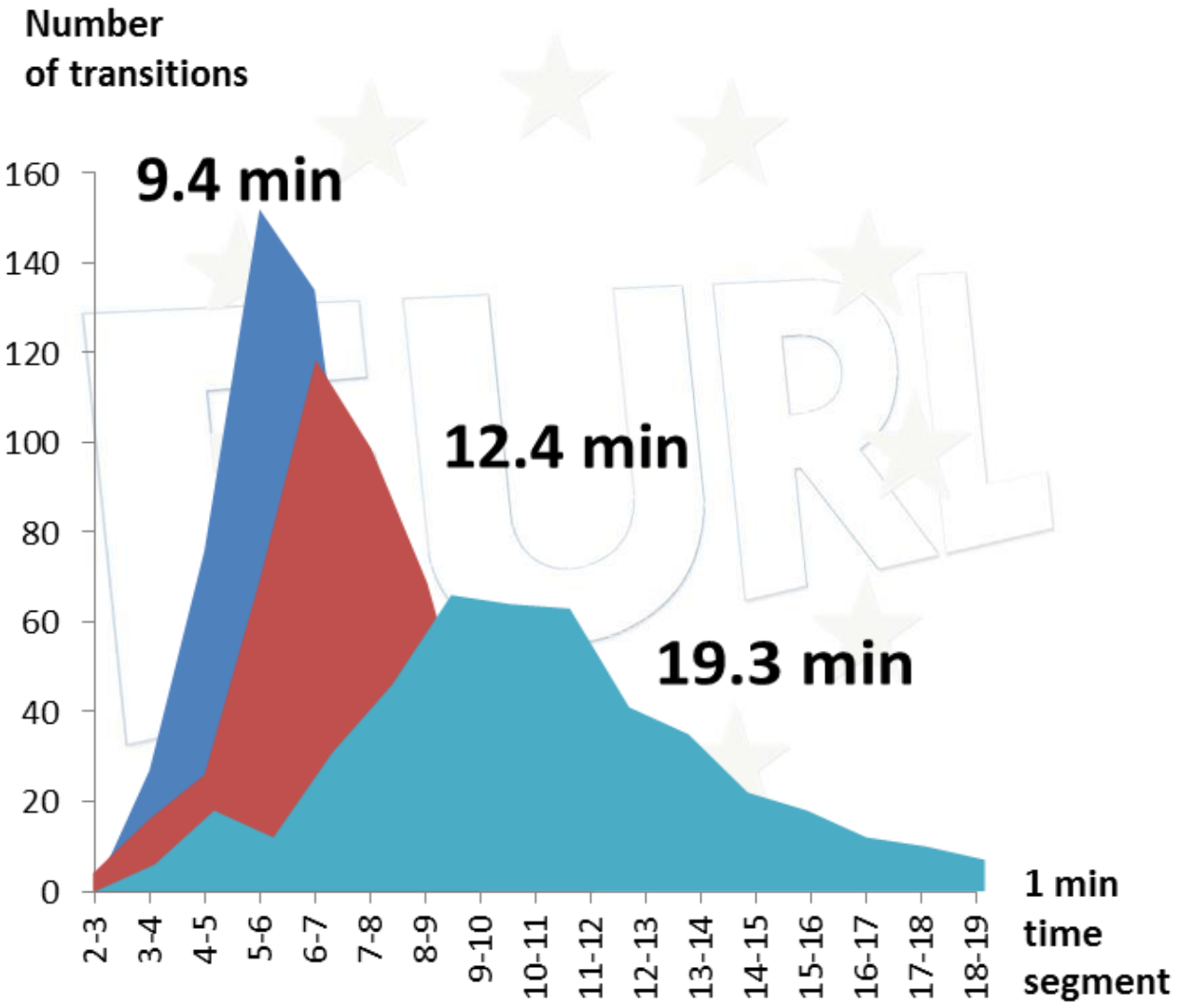
Oven temperature program with different runtime methods



203 GC compounds

2,4'-DDE
2-Phenylphenol
4,4'-DDD
4,4'-DDE
4,4'-DDT
Acrinathrin
Alachlor
Aldrin
Ametryn
Atrazine
Azoxystrobin
Benalaxyl
Bifenox
Bifenthrin
Biphenyl
Bixafen
Boscalid
Bromopropylate
Bupirimate
Buprofezin
Butralin
Butylate
Cadusafos
Carbofuran
Carbophenothion
Chinomethionate
Chlorbromuron
Chlordane
Chlorfenapyr
Chlorobenzilate
Chlorothalonil
Chlorpropham
Chlorpyrifos
Chlorpyrifos-methyl
Chlorthal-dimethyl
Chlozolinate
Coumaphos
Cyfluthrin
Cypermethrin
Cyproconazole
Cyprodinil
Deltamethrin
Desmethyl-pirimicarb
Diazinon
Dichlofluanid
Dichloran
Dichlorobenzophenone
Dichlorvos
Diclobutrazol
Dicofol
Dieldrin
Diethofencarb
Dimethenamid
Dimethipin
Diphenylamine
Disulfoton
Disulfoton-sulfoxide
Dodemorph
Endosulfan sulfate
Endosulfan-alpha
Endosulfan-beta
Endrin
EPN
Epoxiconazole
Ethion
Ethofenprox
Ethofumesate
Ethoprophos
Ethoxyquin
Etrimfos
Fenamidone
Fenarimol
Fenazaquin
Fenbuconazole
Fenchlorphos
Fenhexamid
Fenitrothion
Fenpropathrin
Fenpropidin
Fenpropimorph
Fenthion
Fenvalerate
Fipronil
Fipronil sulfone
Fipronil-desulfinil
Flamprop-isopropyl
Flamprop-methyl
Fluacrypyrim
Fluazifop-p-butyl
Flucythrinate
Fludioxonil
Fluopicolide
Fluopyram
Fluquinconazole
Flusilazole
Flutolanil
Flutriafol
Fluvalinate-tau
Fonofos
Formothion
Fosthiazate
HCB
HCH-alpha
HCH-beta
Heptachlor
Heptachlor endo-epoxide
Heptachlor exo-epoxide
Heptenophos
Hexaconazole
Indoxacarb
Iprodione
Iprovalicarb
Isazofos
Isocarbophos
Isofenphos
Isofenphos-methyl
Isoprothiolane
Isopyrazam
Kresoxim-methyl
λ-Cyhalothrin
Lindane
Malaoxon
Malathion
Mecarbam
Mepanipyrim
Merphos
Metalaxyl
Metazachlor
Metconazole
Methidathion
Methiocarb
Methoxychlor
Metolachlor
Mevinphos
Molinate
Myclobutanil
Napropamide
Nuarimol
Ofurace
Oxadixyl
Paclobutrazol
Paraoxon-methyl
Parathion
Parathion-methyl
Penconazole
Pendimethalin
Pentachloroaniline
Permethrin
Phenothrin
Phenthoate
Phorate
Phorate sulfone
Phosmet
Phthalimide
Picolinafen
Picoxystrobin
Pirimicarb
Pirimiphos-methyl
Procymidone
Profenofos
Prometon
Prometryn
Propaphos
Propazine
Propiconazole
Propyzamide
Prosulfocarb
Prothiofos
Pyraclostrobin
Pyrazophos
Pyridaben
Pyrifenox
Pyrimethanil
Pyriproxyfen
Quinalphos
Quinoxyfen
Quintozene
Secbumeton
Spirodiclofen
Spiromesifen
Sulfotep
Sulprofos
Tebuconazole
Tebufenpyrad
Tecnazene
Tefluthrin
Terbufos
Terbumeton
Terbutryn
Tetrachlorvinphos
Tetraconazole
Tetradifon
THPI
Tetramethrin
Thiobencarb
Tolclofos-methyl
Tolylfluanid
Triadimefon
Triazophos
Trifloxystrobin
Trifluralin
Vinclozolin

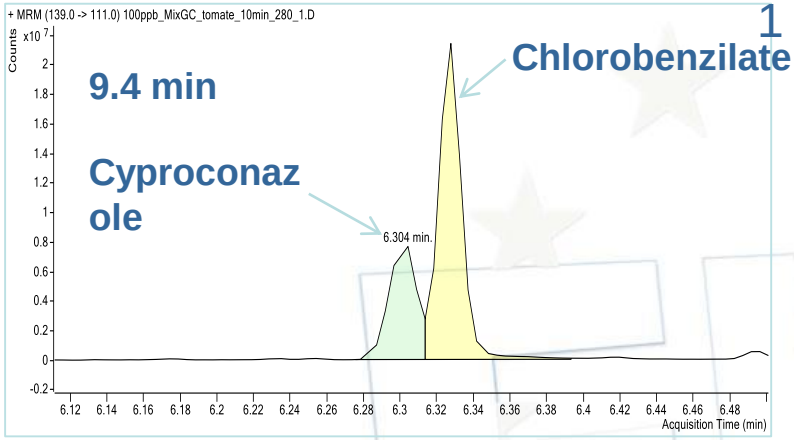
Azoxystrobin (data points per peak with the 5 runtime methods)

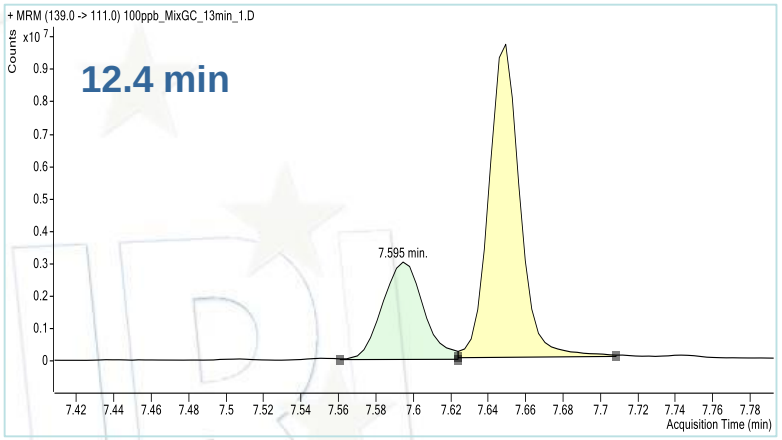

Number
of transitions
9.4 min
12.4 min
19.3 min
160
140
120
100
80
60
40
20
0
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-10
10-11
11-12
12-13
13-14
14-15
15-16
16-17
17-18
18-19
1 min
time
segment

Overlapping peaks

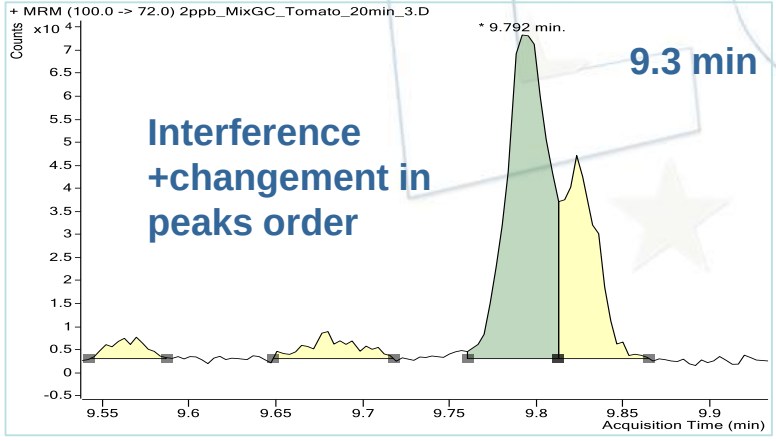
139>11

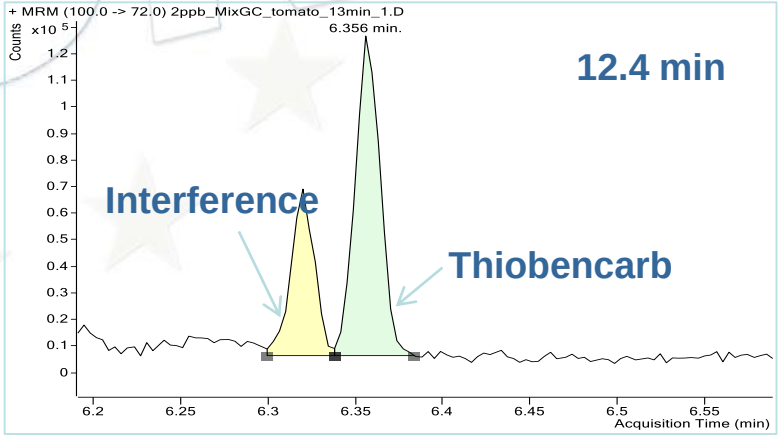
1





100>72





Extraction Method

Citrate buffered QuEChERS

10 g of Sample + 10 ml of AcN

Shake 4 min

4 g $MgSO_4$ + 1 g NaCl +
1 g NaCit·$2H_2O$ +0.5 g NaCit·$1.5H_2O$

Shake 4 min

Centrifuge 5 minutes at 3700 rpm

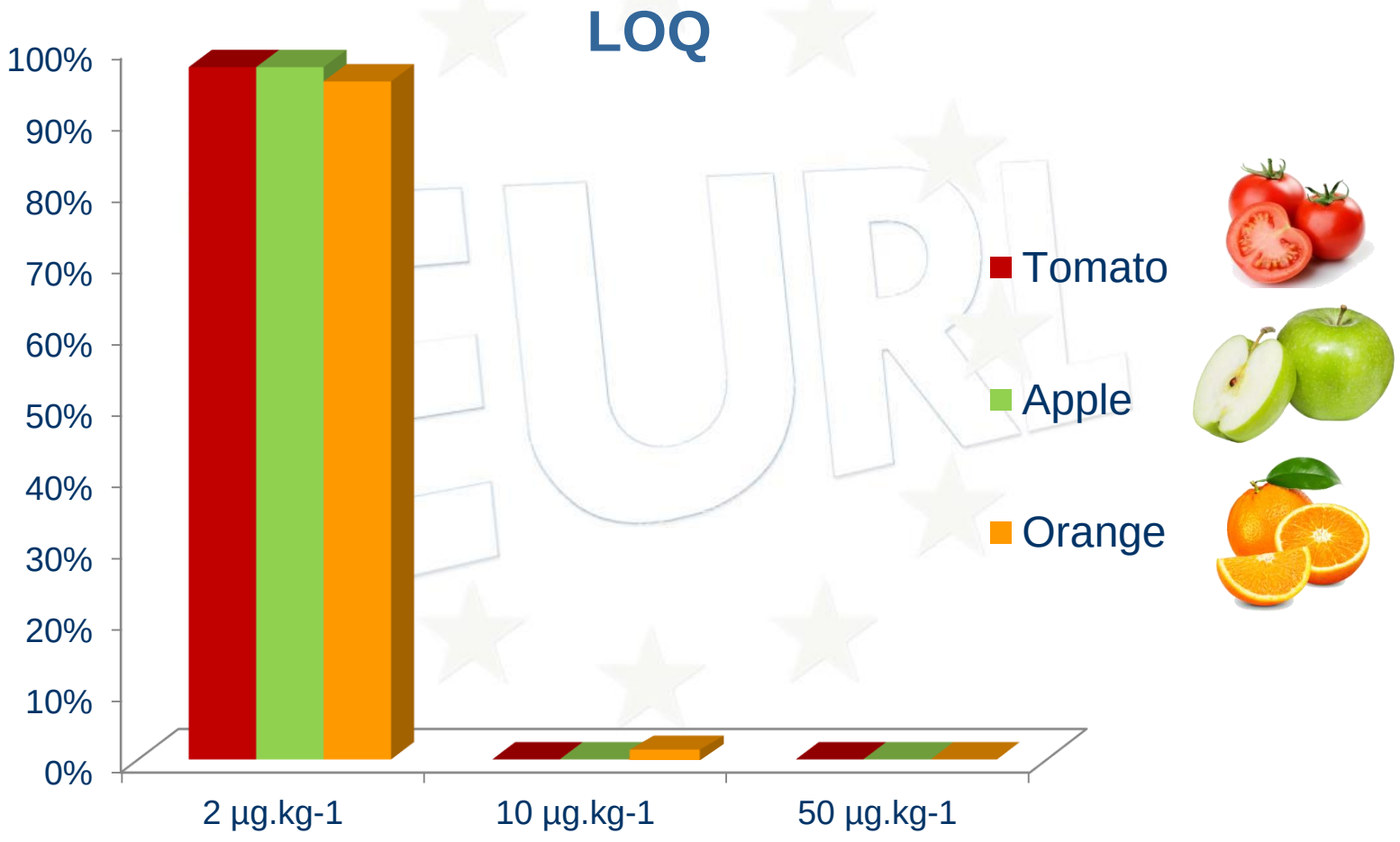
Take 5 mL + 750 mg $MgSO_4$
+ 125 mg PSA

Centrifuge 5 minutes at 3700 rpm

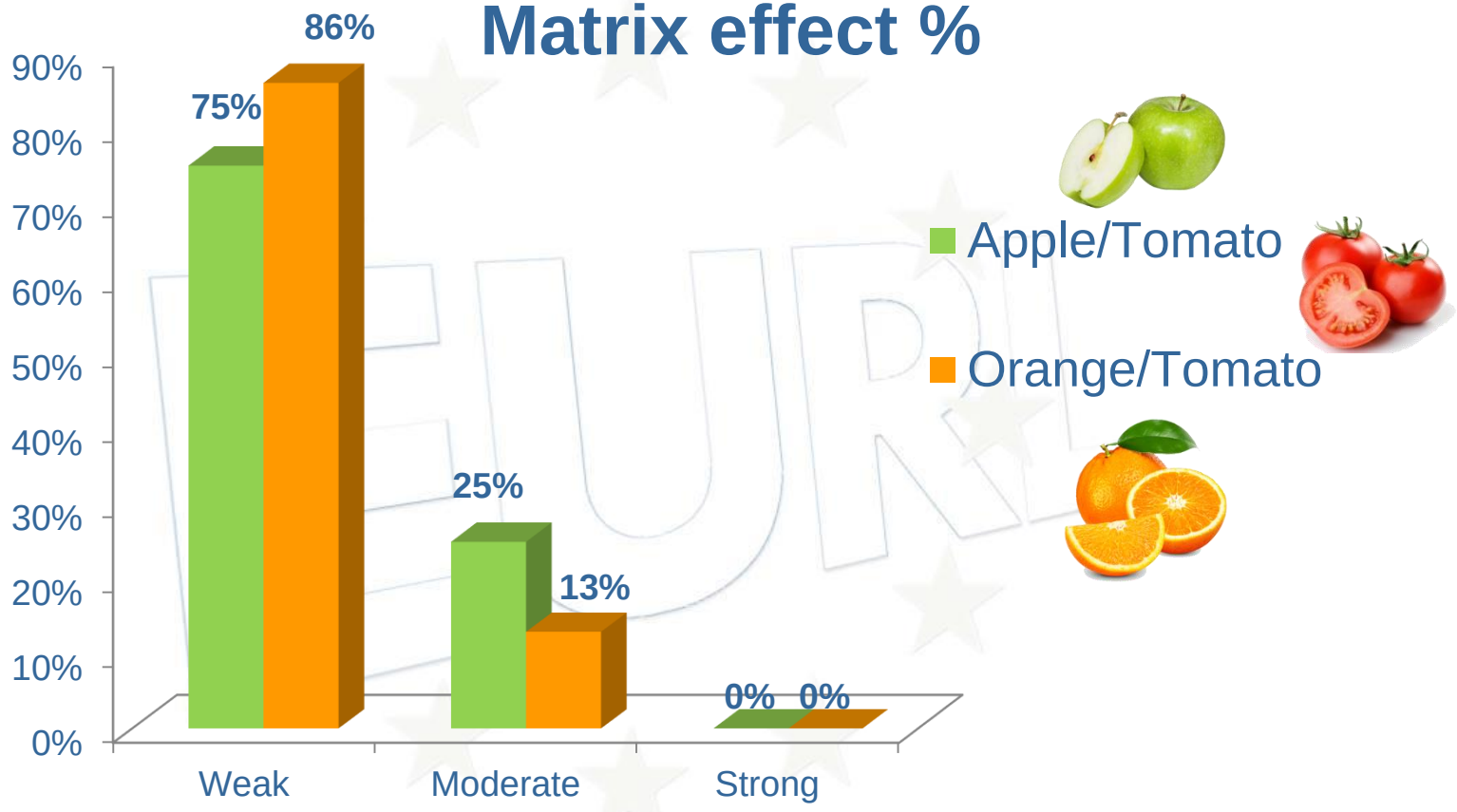
Acidify with 10 uL formic acid 5 %
per mL of extract

Solvent exchange (EtAc)

Analysis
1 g Sample/mL


LOQ
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2 µg.kg-1
10 µg.kg-1
50 µg.kg-1
Tomato
Apple
Orange

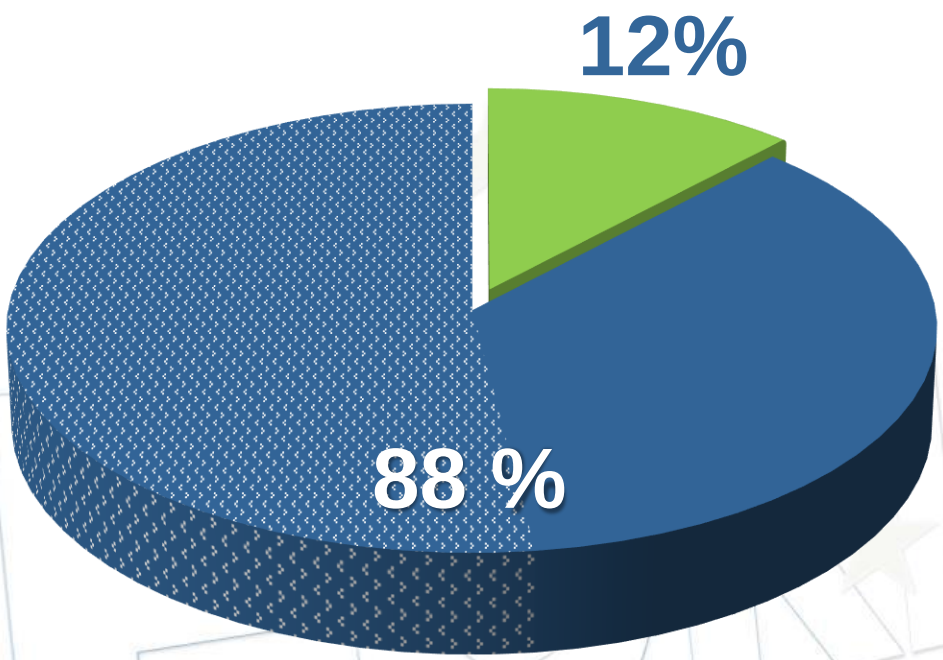
Matrix effect %



-20 to 20 %

-20 to -50 % and 20 to 50 %

<-50 and >50 %



- Samples with no pesticide residues
- Samples with detections > 2 µg.kg-1
- Samples with detections > 10 µg.kg-1


Enjoy!!!

Extraction Method

Modified Citrate buffered QuEChERS

2 g of Sample + 7 ml H_20 (30 min soaking time)

Add 10 mL ACN — 5 times dilution

Shake 7 min

Add 4 g $MgSO_4$ + 1 g NaCl +
1 g NaCit·$2H_2O$ +0.5 g NaCit·$1.5H_2O$

Shake 7 min
Centrifuge 5 minutes at 3700 rpm

Activate EMR-Lipid by adding 5 mL of water
Transfer 5 mL of the supernatant into EMR-Lipid tubes

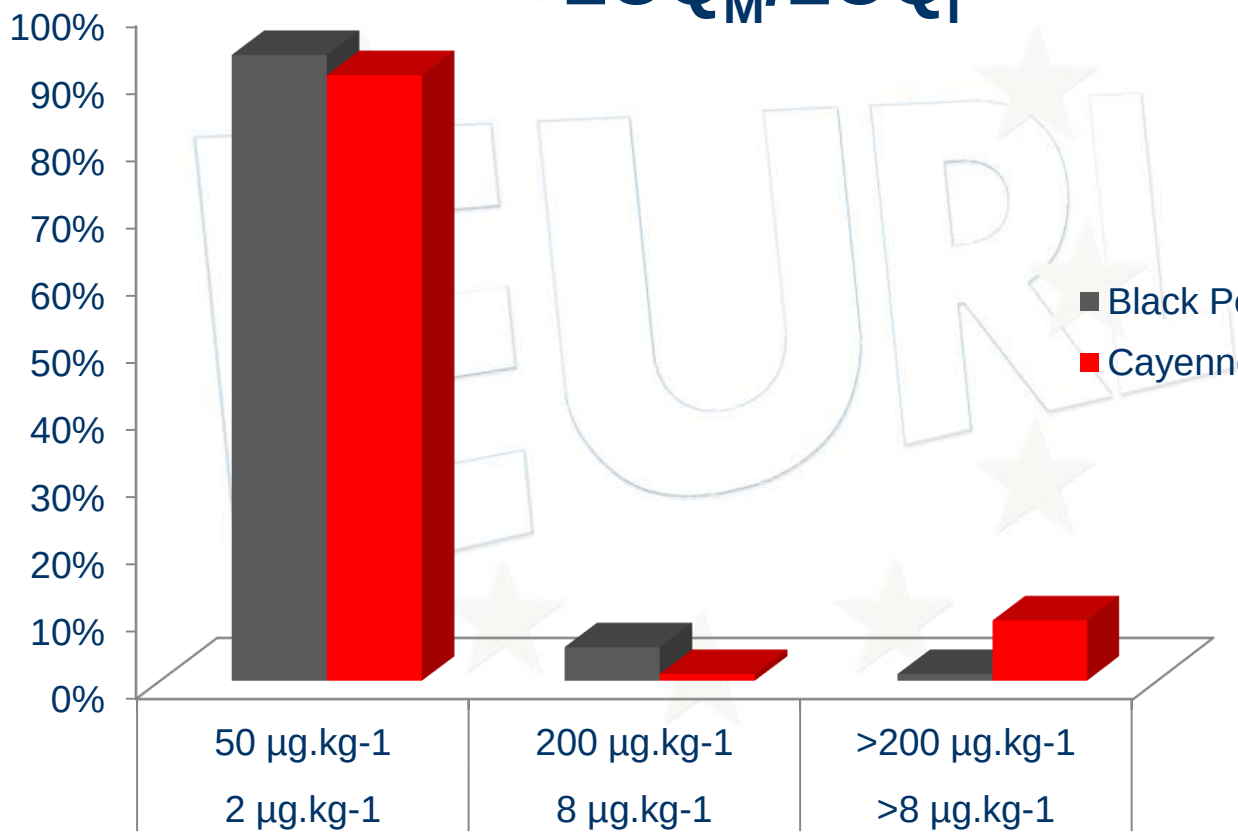
Mix and centrifuge for 5 minutes at 3700 rpm

Transfer 5 mL of the supernatant into Polish tubes (0.4 g NaCl+ 1.6 g $MgSO_4$)

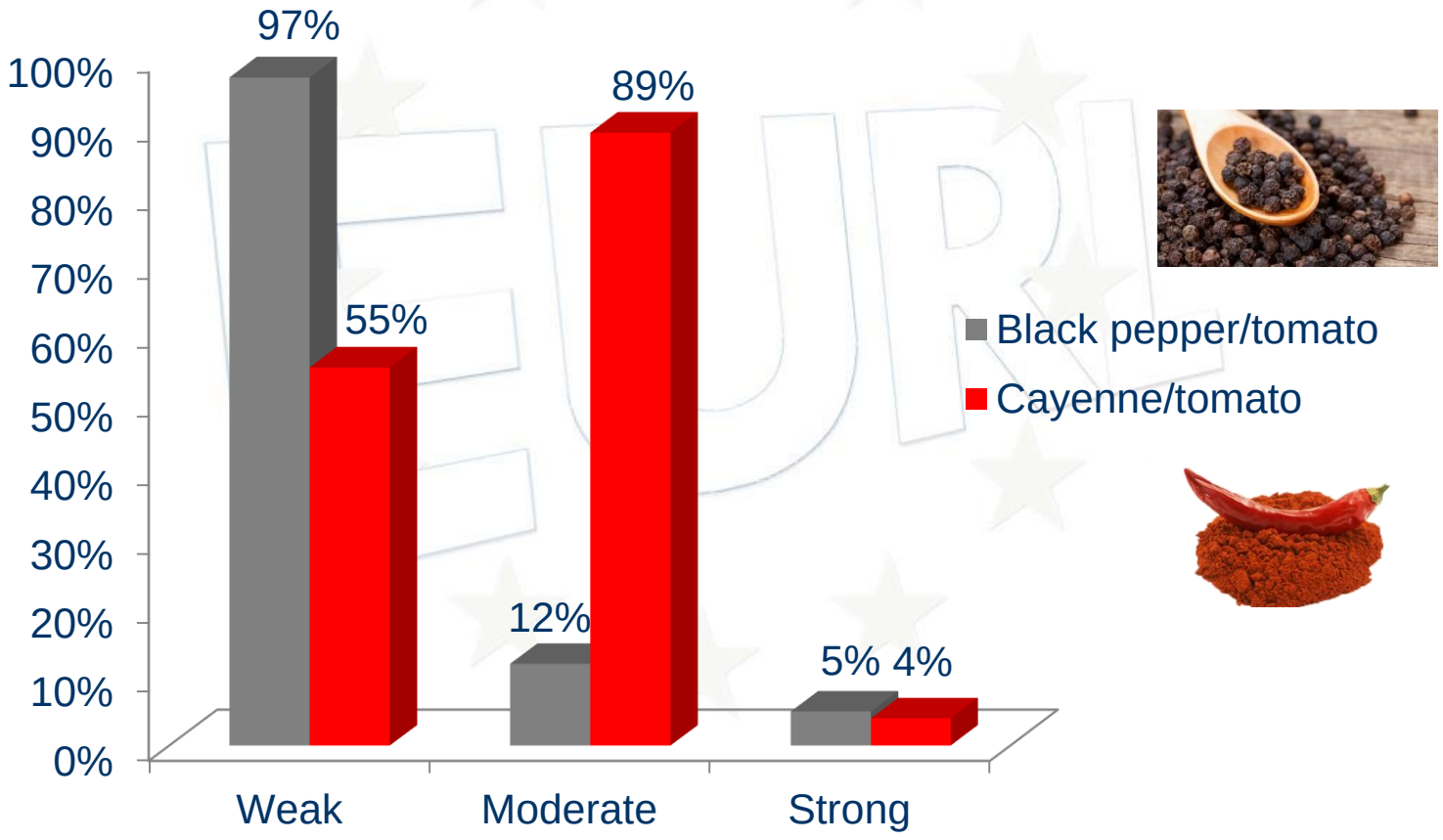
Mix and centrifuge for 5 minutes at 3700 rpm

Solvent Exchange (EtAc) — 5 times dilution

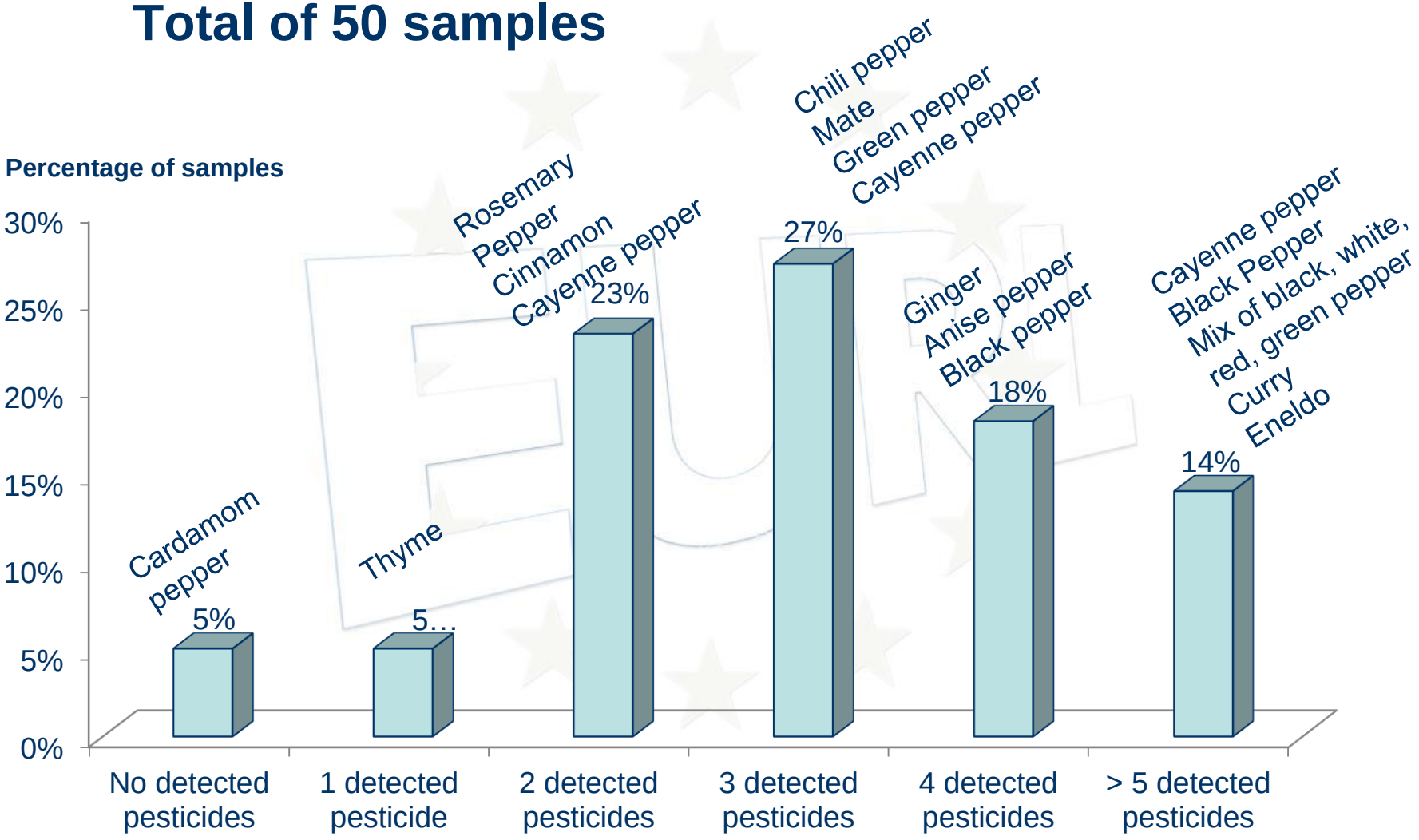
Analysis of
0,04 g Sample/mL


LOQM/LOQI
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
50 µg.kg-1
2 µg.kg-1
200 µg.kg-1
8 µg.kg-1
>200 µg.kg-1
>8 µg.kg-1
Black Pepper
Cayenne Pepper

Matrix effect



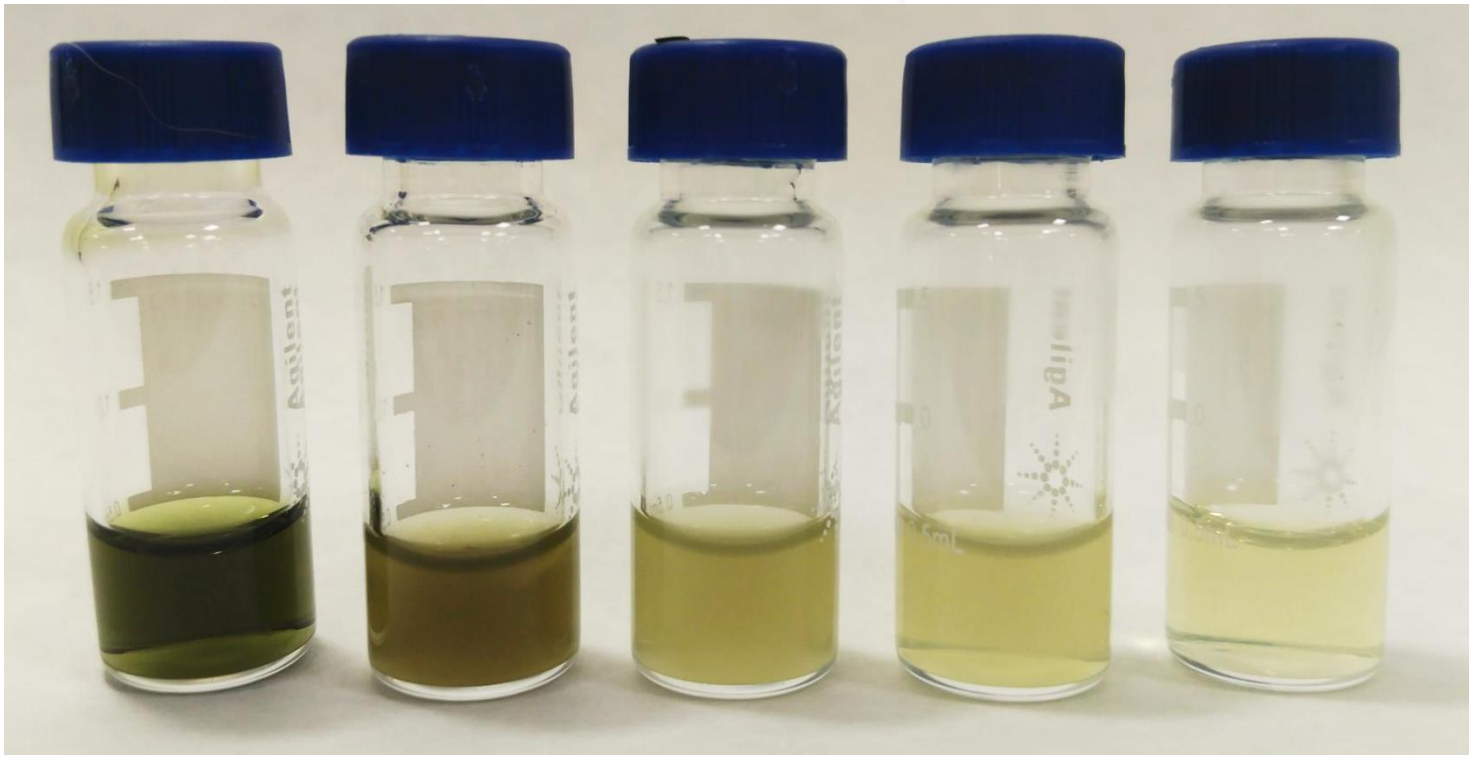
Total of 50 samples




Dilute and Win!!!

Injection volume	Matrix injected in column				
LC: 5 µL	5 mg	2.5 mg	1 mg	0.5 mg	0.25 mg
GC: 1 µL	1 mg	0.5 mg	0.2 mg	0.1 mg	0.05 mg

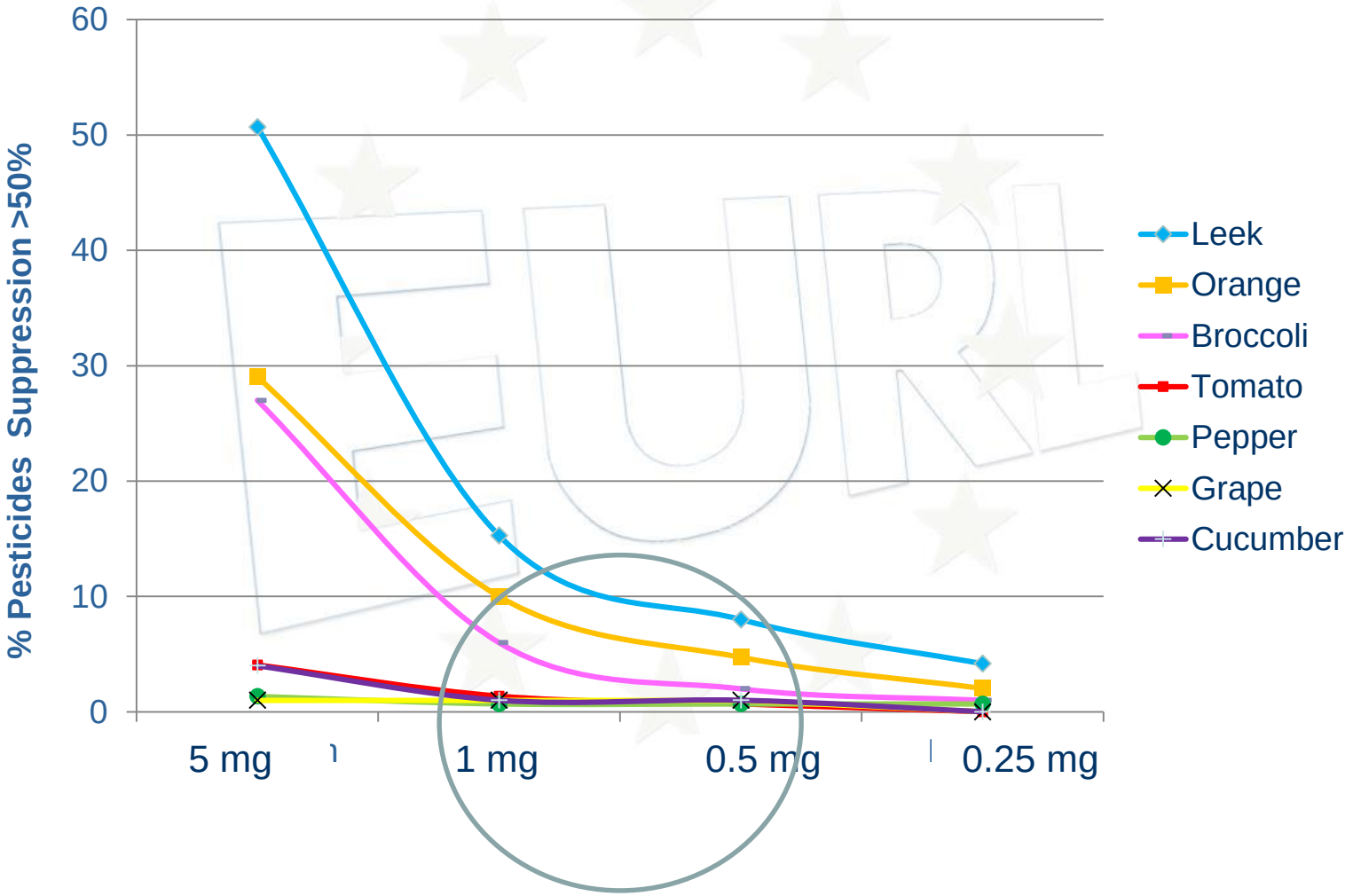
Green Tea



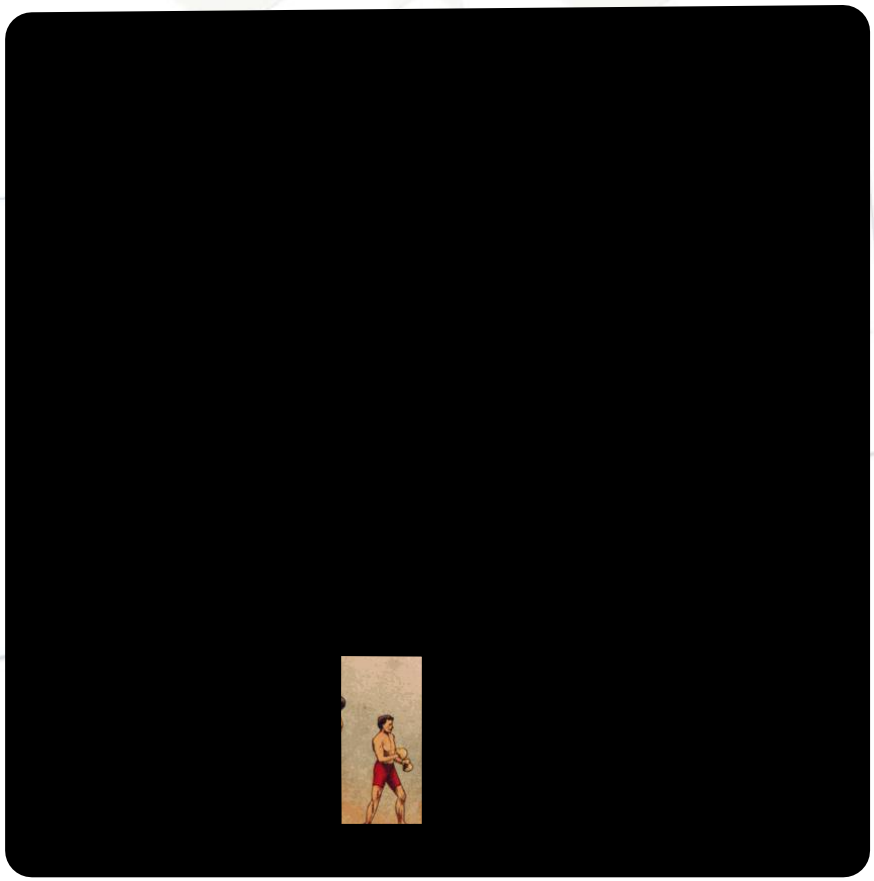
Dilx0	Dilx2	Dilx5	Dilx10	Dilx20
1 g/mL	0.5 g/mL	0.2 g/mL	0.1 g/mL	0.05 g/mL

LC-TOF-MS

EFFECT OF DILUTION IN "EASY-MEDIUM" COMPLEX MATRICES

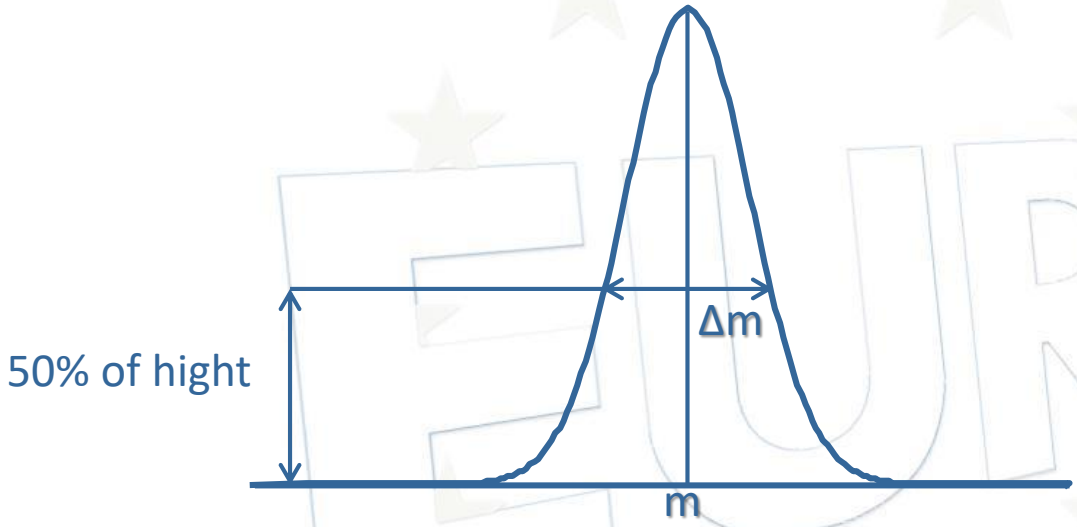


RESOLUTION



Resolution at FWHM - full-width half maximum

(Full Width of the peak at Half its Maximum height)

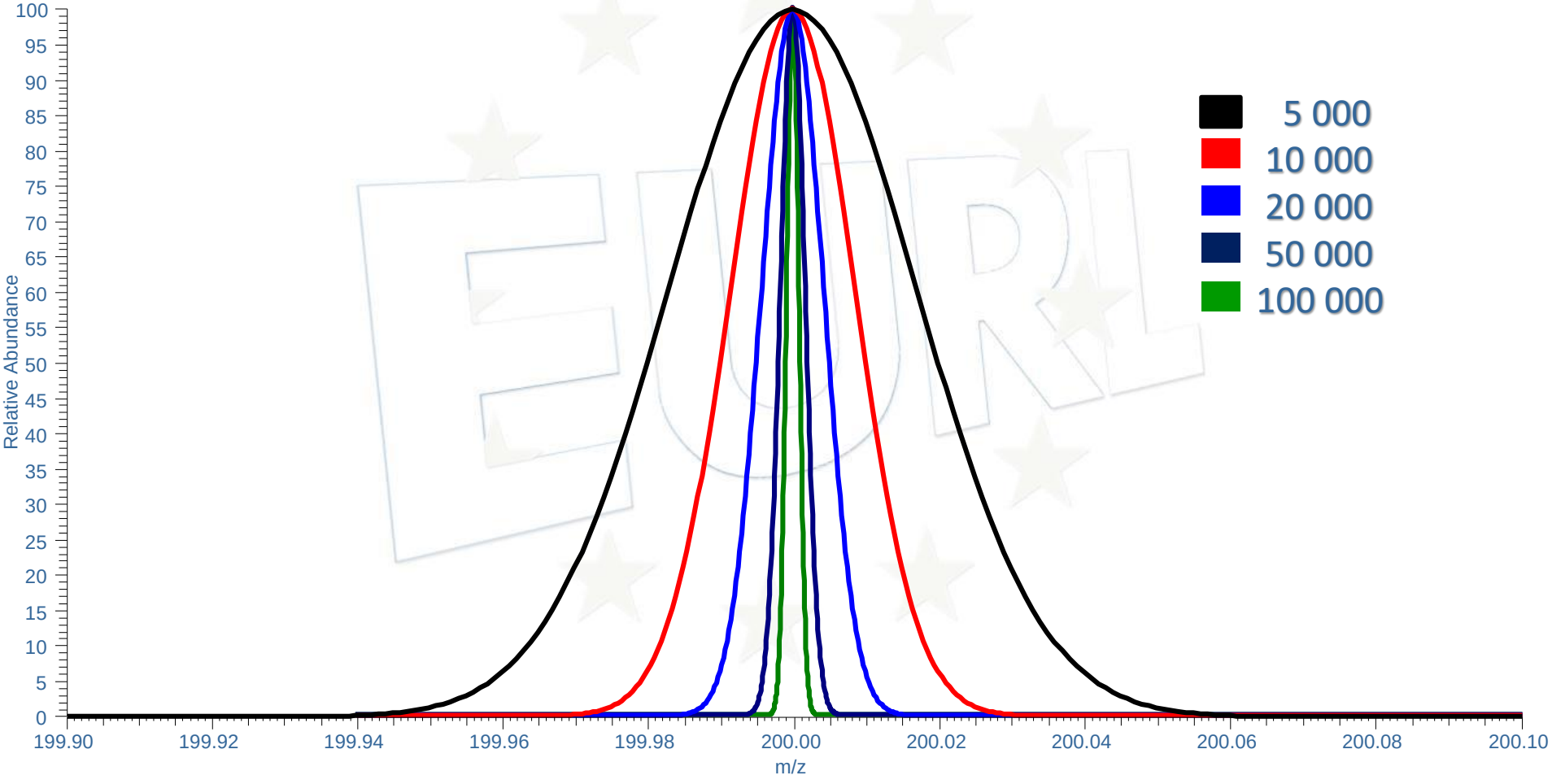


$$R = \frac{m}{\Delta m}$$

For example:

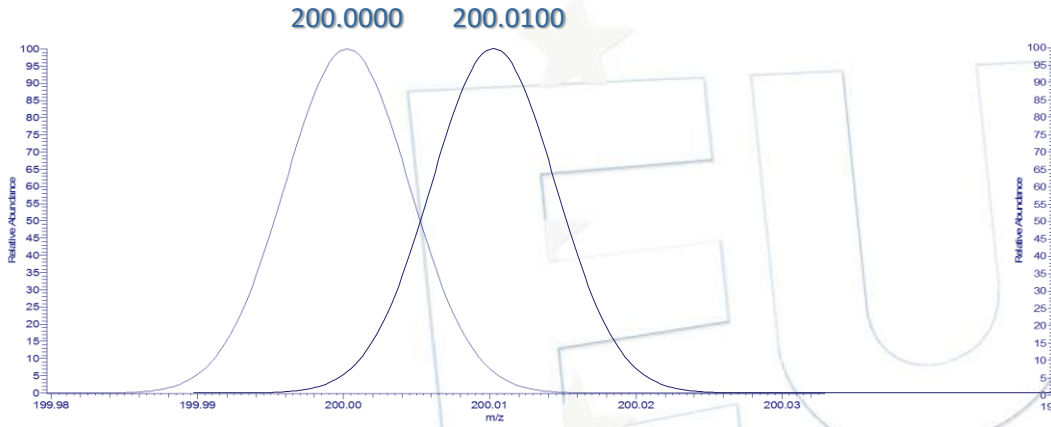
$$\frac{200}{0.4} = 500 \qquad \frac{200}{0.04} = 5000 \qquad \frac{200.}{0.004} = 50000$$

Effects of increased resolution

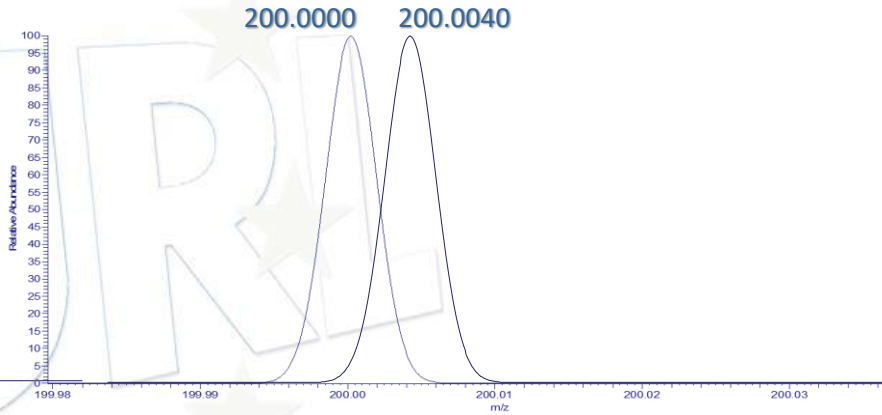


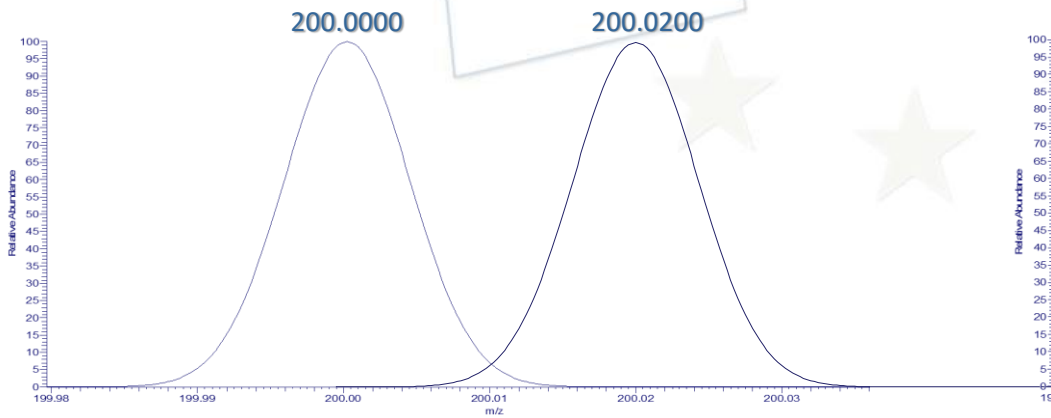
Effects of increased resolution

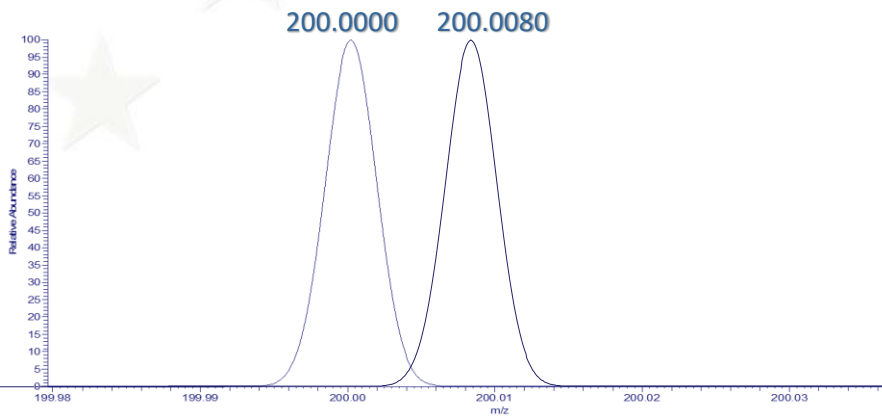
Resolution: 20 000



Resolution: 50 000



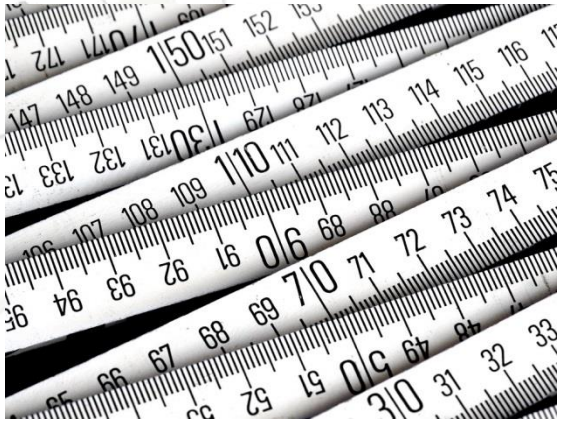




RESOLUTION (30,-50,000)

+

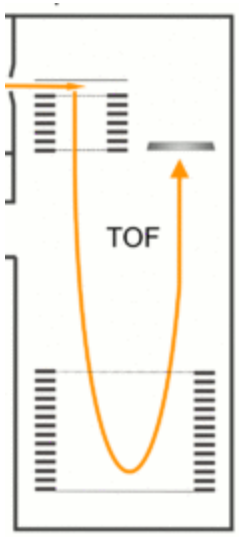
MASS ACCURACY (5ppm)

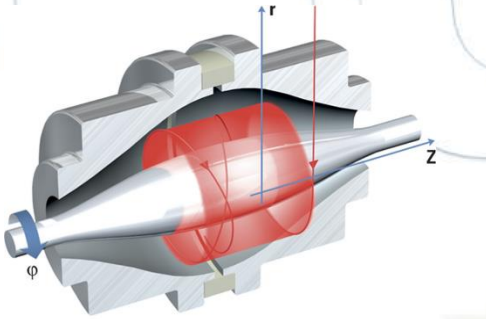


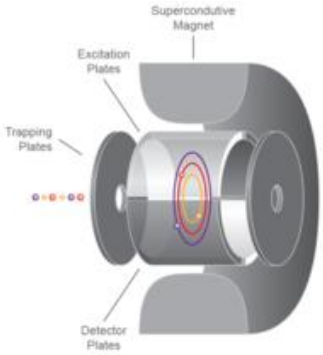
Nominal Mass----Exact Mass

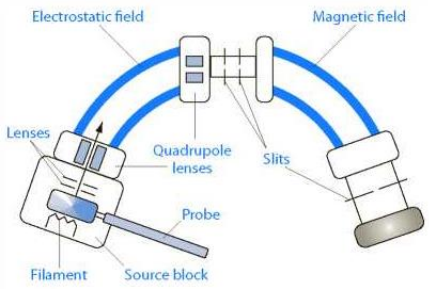
INSTRUMENTATION:

- Time of flight
- Orbitrap
- Fourier transform ion cyclotron
- (electric/magnetic) sector instrument

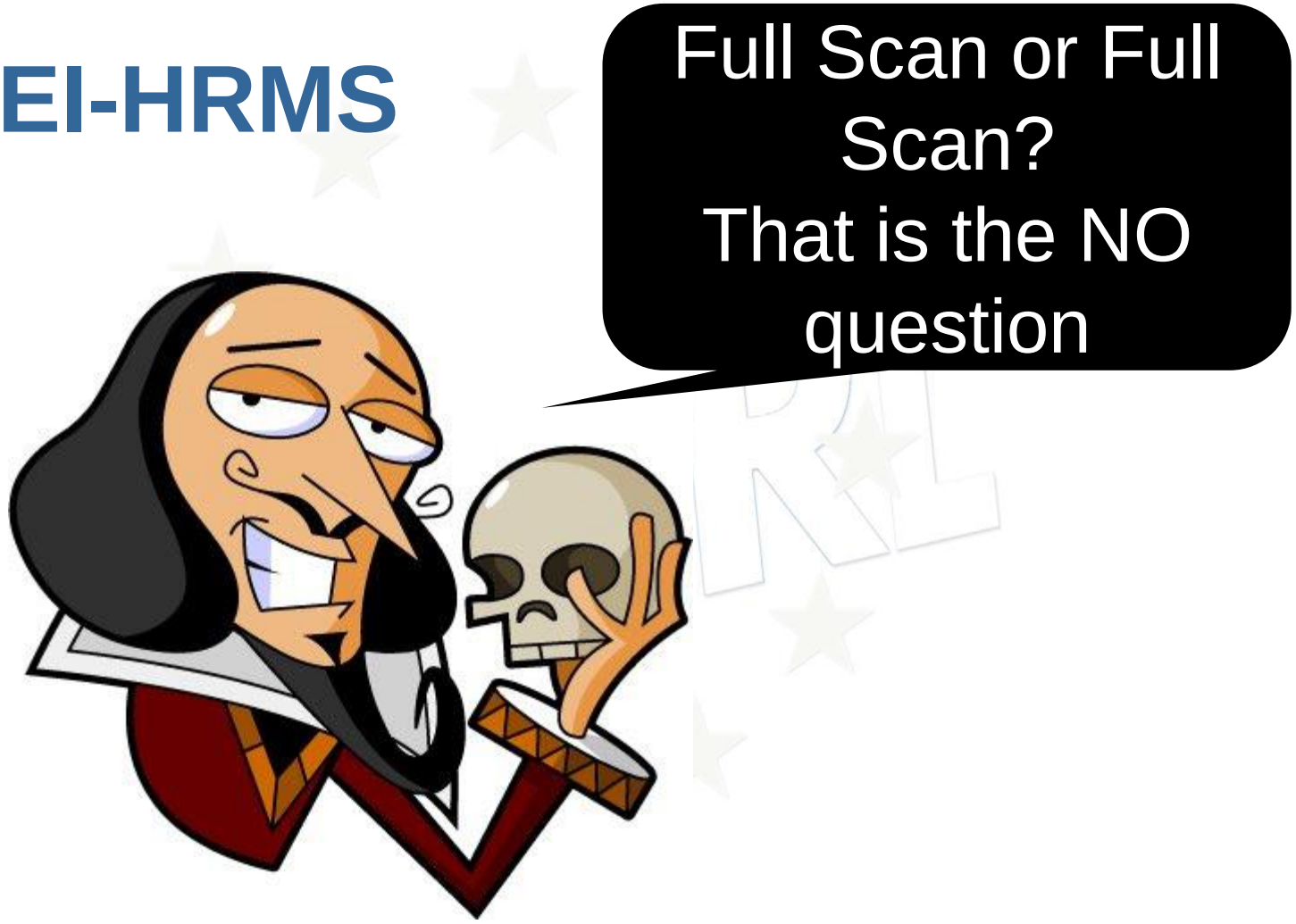




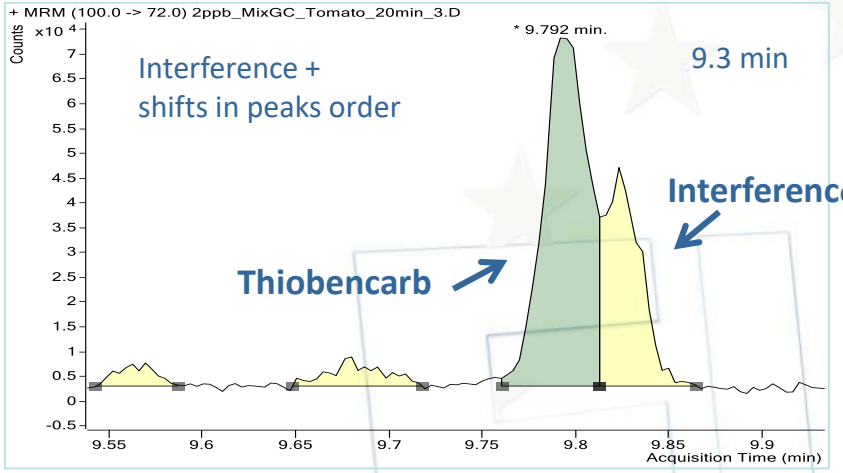




GC-EI-HRMS

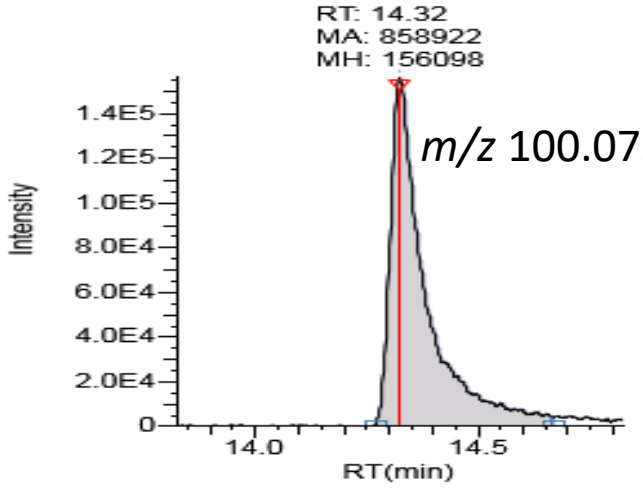


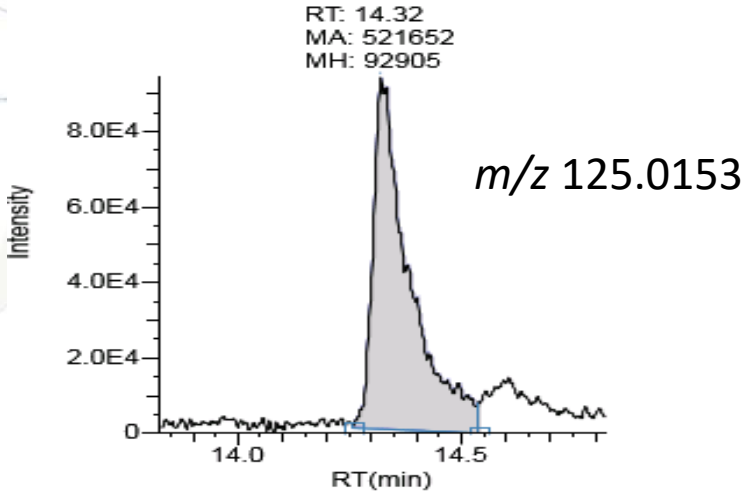
Thiobencarb
0.01 mg/kgin Tomato



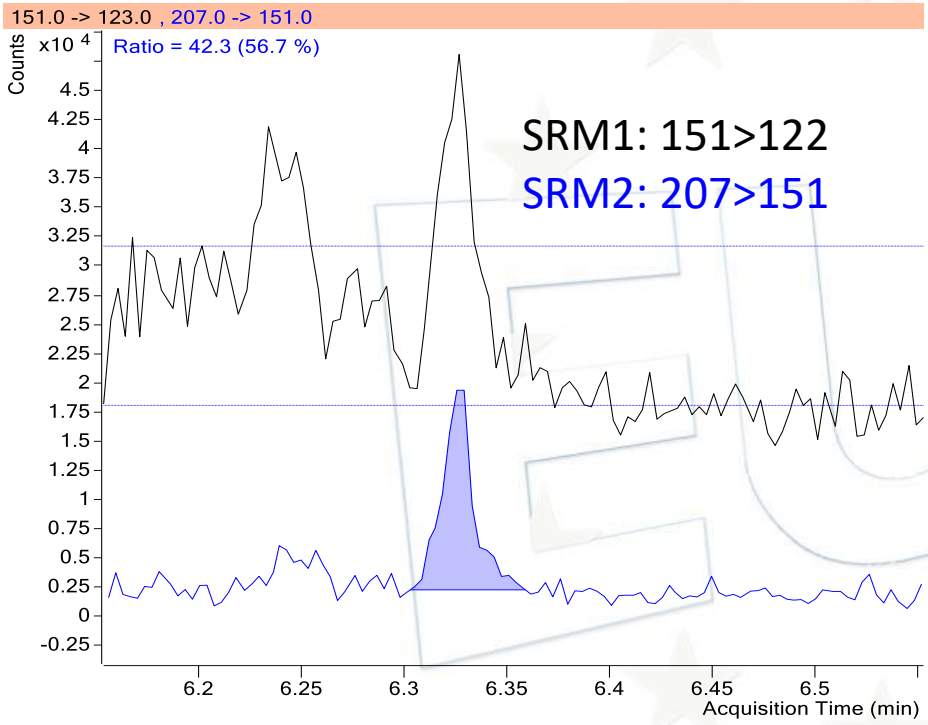
GC-Intuvo MS/MS (100>72)

GC-Orbitrap MS

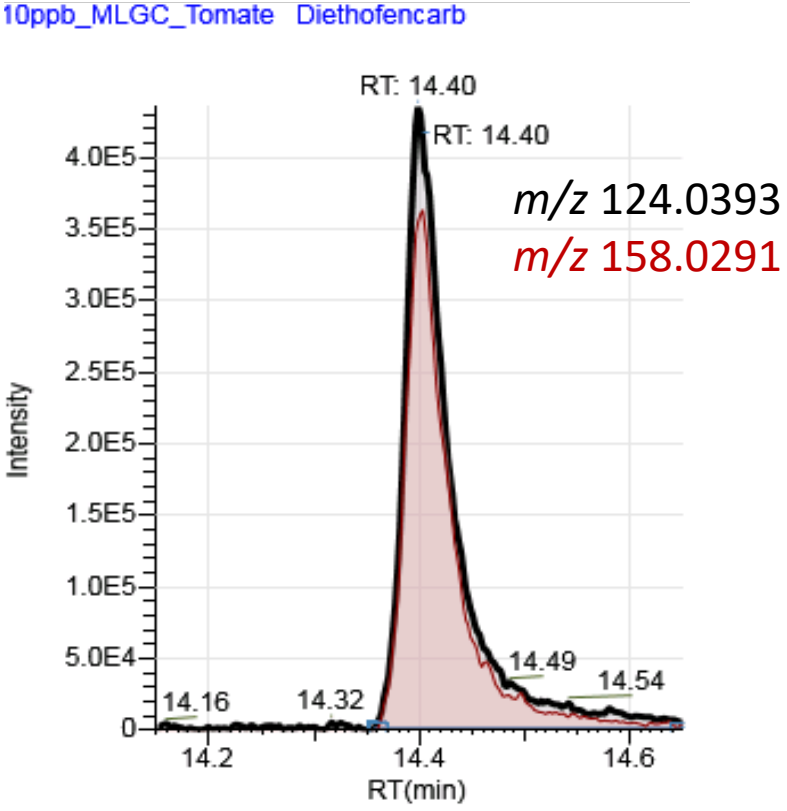




Diethofencarb
0.010 mg/kg in Tomato

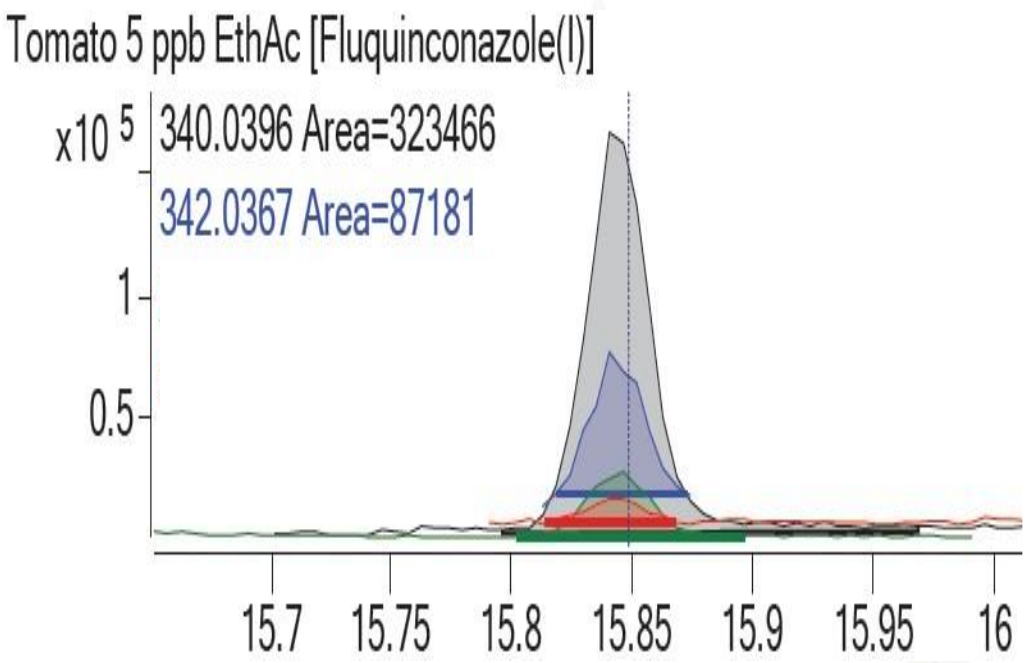


GC-Intuvo-MS/MS

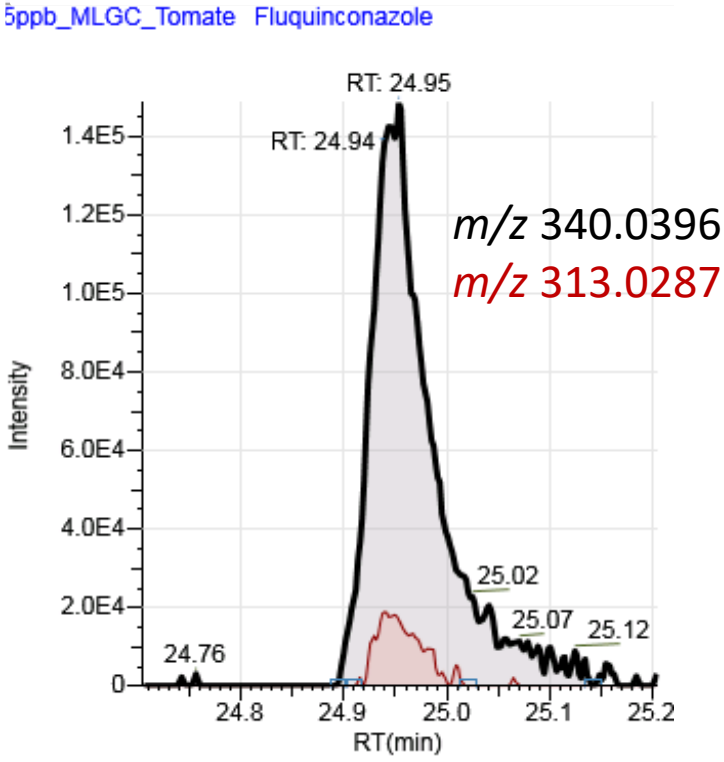


GC-Orbitrap MS

Fluquinconazole
0.005 mg/kg in Tomato



7250 GC-QToF-MS



GC-Orbitrap-MS

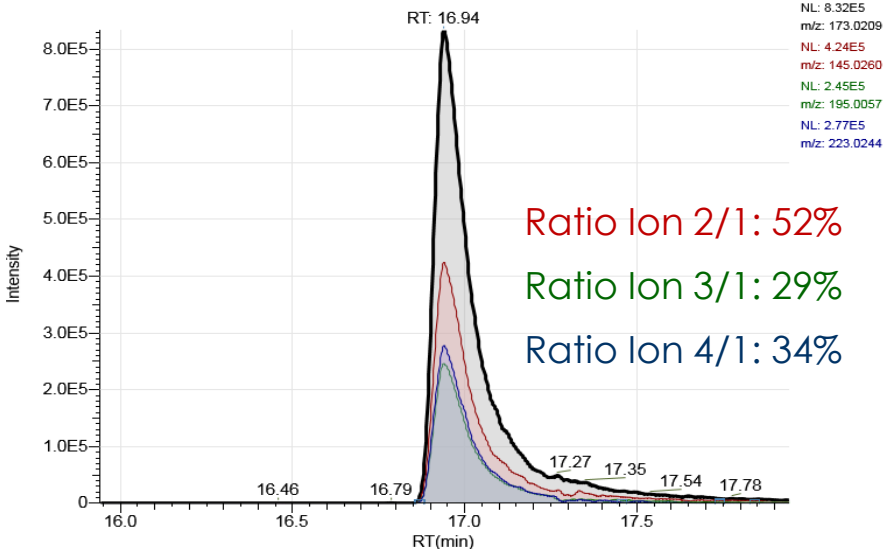
GC-EI-QExative

60k Full scan MS

Orbitrap GC-EI-MS System

Real Samples: Fluopyram

50 µg/kg Std in Tomato



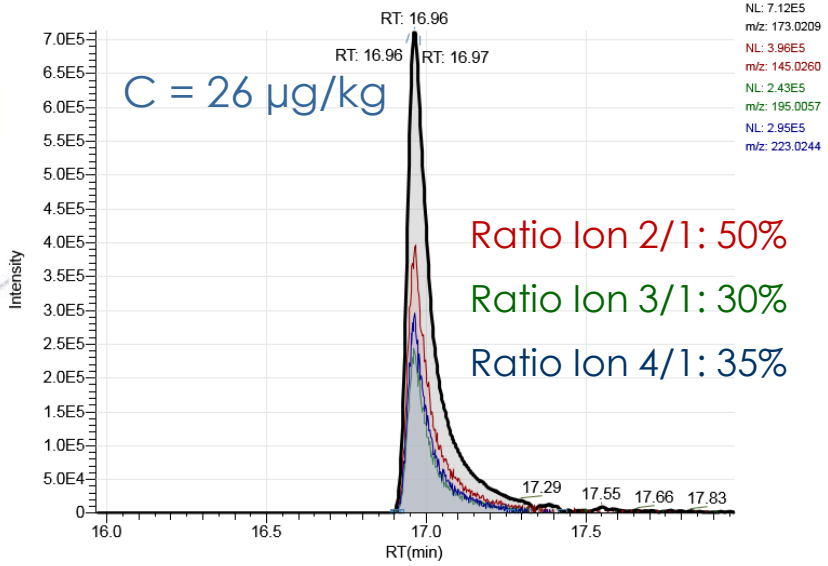
Ratio Ion 2/1: 52%

Ratio Ion 3/1: 29%

Ratio Ion 4/1: 34%

Real Sample: Pear

C = 26 µg/kg



Ratio Ion 2/1: 50%

Ratio Ion 3/1: 30%

Ratio Ion 4/1: 35%

The **ion ratios** are within **± 4%** with respect to the standard and mass accuracy below **0.2 mDa**

Ion 1 (173.0209): 0.01 mDa

Ion 2 (145.0260): 0.14 mDa

Ion 3 (195.0057): -0.08 mDa

Ion 4 (223.0244): 0.02 mDa

SENSITIVITY (10 ug/kg)

LINEARITY (2 orders) + REPRODUCIILITY (< 20%)

LC-HR-ESI-MS/MS

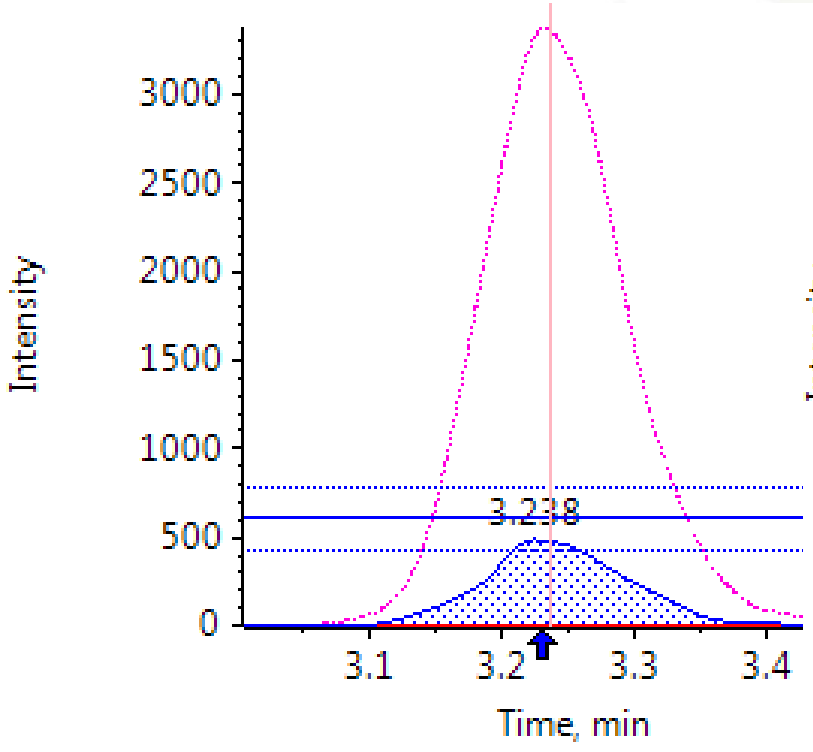
All Ion Fragmentation or NOT AIF? That is the question

	MS	MS2
targeted	SIM	Narrow quadrupole window with or without triggering
non-targeted	Full Scan	Wide quadrupole window(s)

Carbendazim in Carrot

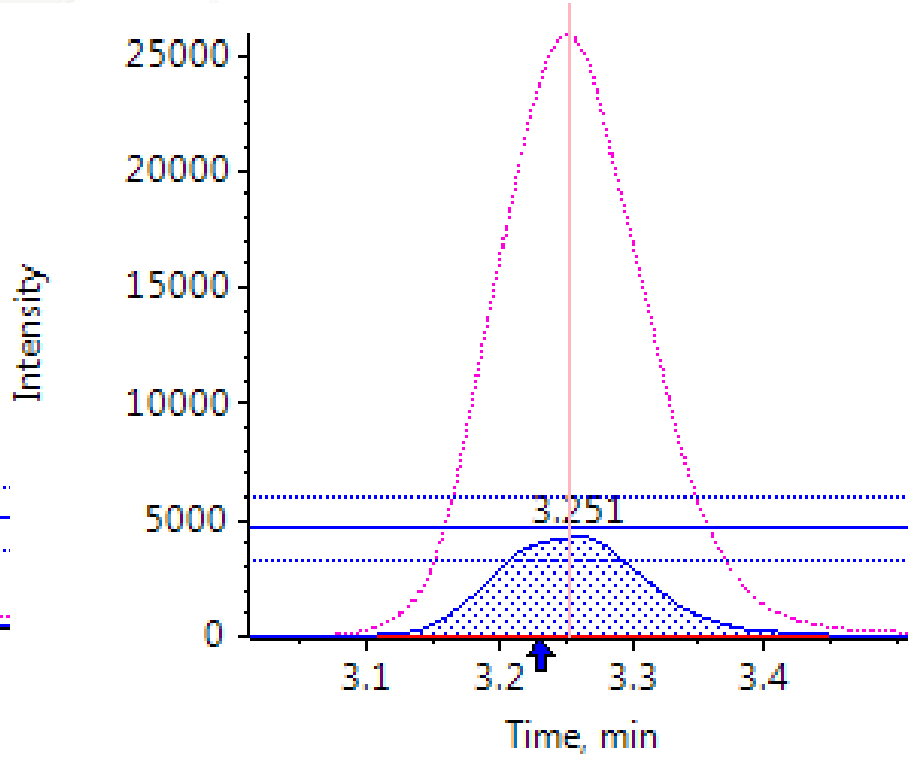
MRM-LC-HR-MS/MS, Isolation Mass Window: 0.7 Da

0.01 mg/kg



Mass accuracy precursor: -1.0 ppm
Mass accuracy fragment: -4.9 ppm
Ion ratio: 0.14

0.1 mg/kg



Mass accuracy precursor: -0.1 ppm
Mass accuracy fragment: -4.4 ppm
Ion ratio: 0.17

Precursor Ion: 192.0768
Fragment Ion: 160.0495

Ratio Difference: -18%

SENSITIVITY (10 ug/kg)

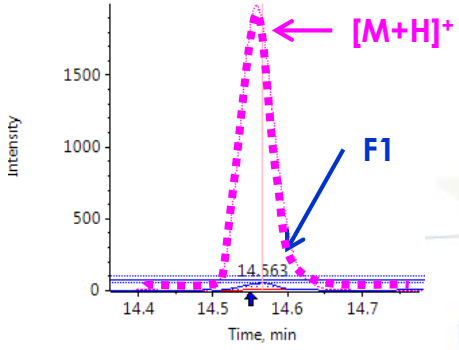
LINEARITY (2 orders) + REPRODUCIILITY (< 20%)

INSTRUMENTAL ISSUES ASSOCIATED WITH LC-HRMS WORKING IN ALL ION FRAGMENTATION

Flufenoxuron – AIF 1 window

0.010 mg/kg

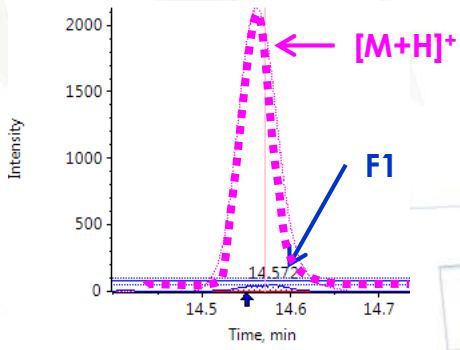
Aubergine



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.56	0.0222

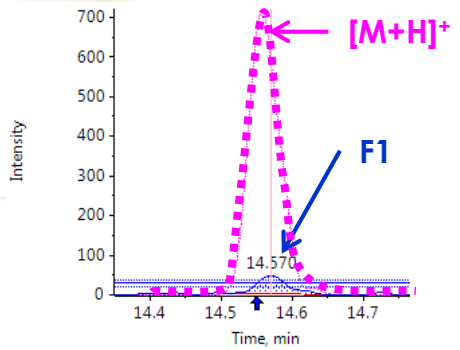
Broccoli



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	0.0250

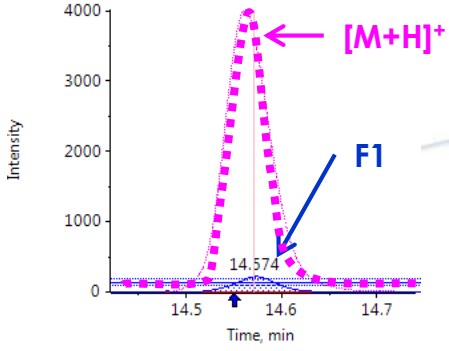
Lemon



Peak Details

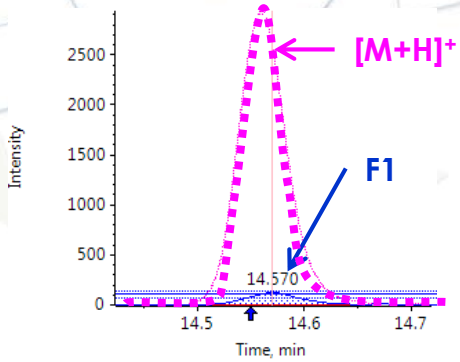
Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	0.0625

0.050 mg/kg



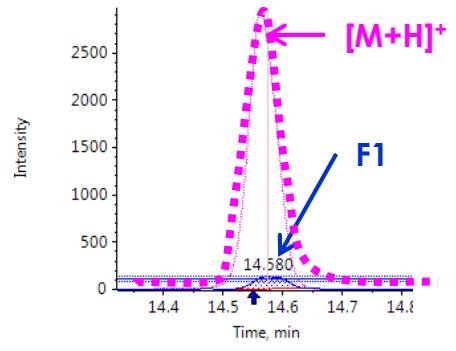
Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	0.0521



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	0.0471



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.58	0.0497

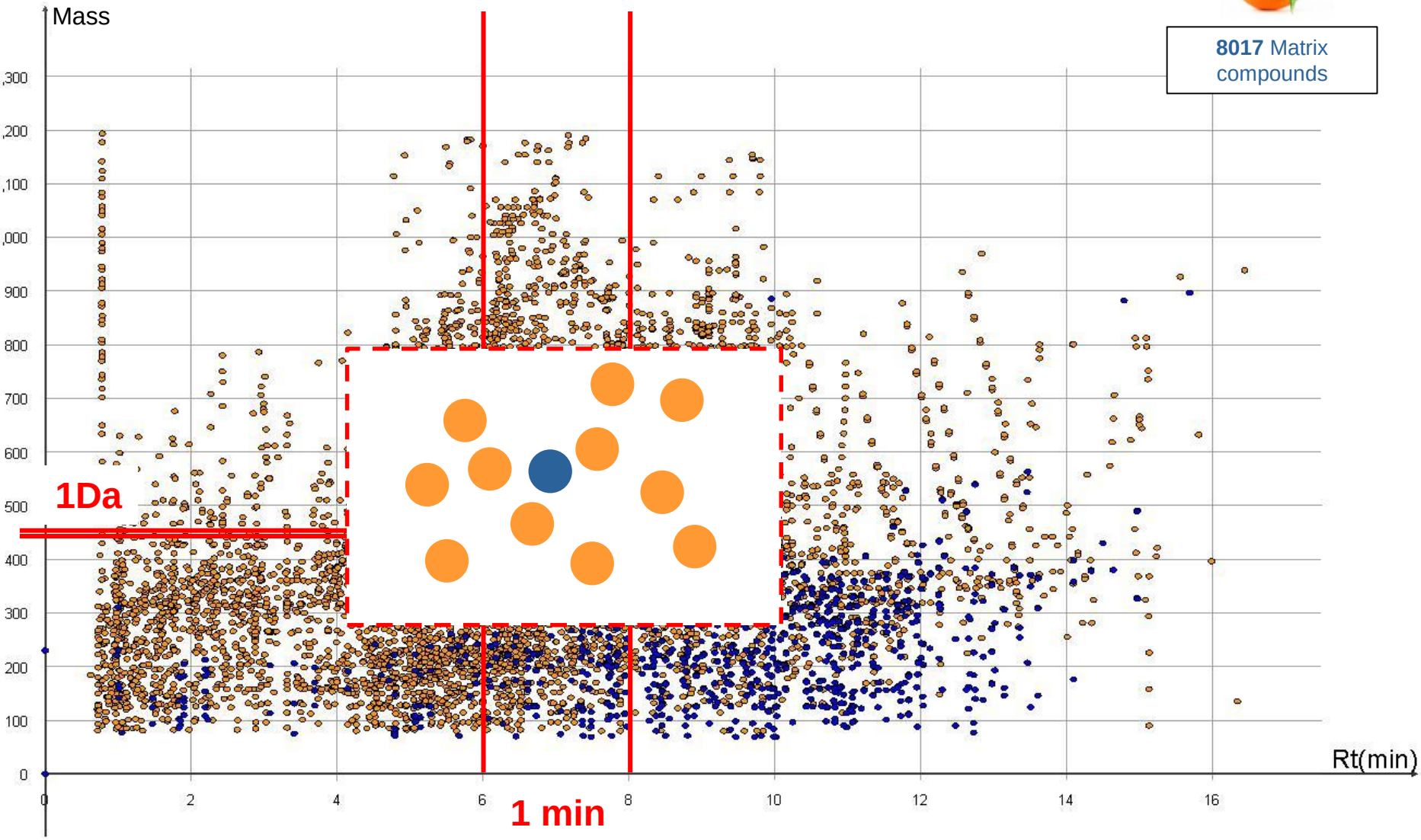
Ratio difference within ± **65 %**

Peticides - tomato matrix compounds

2408 Matrix compounds

Pesticides - **orange** matrix compounds

8017 Matrix compounds

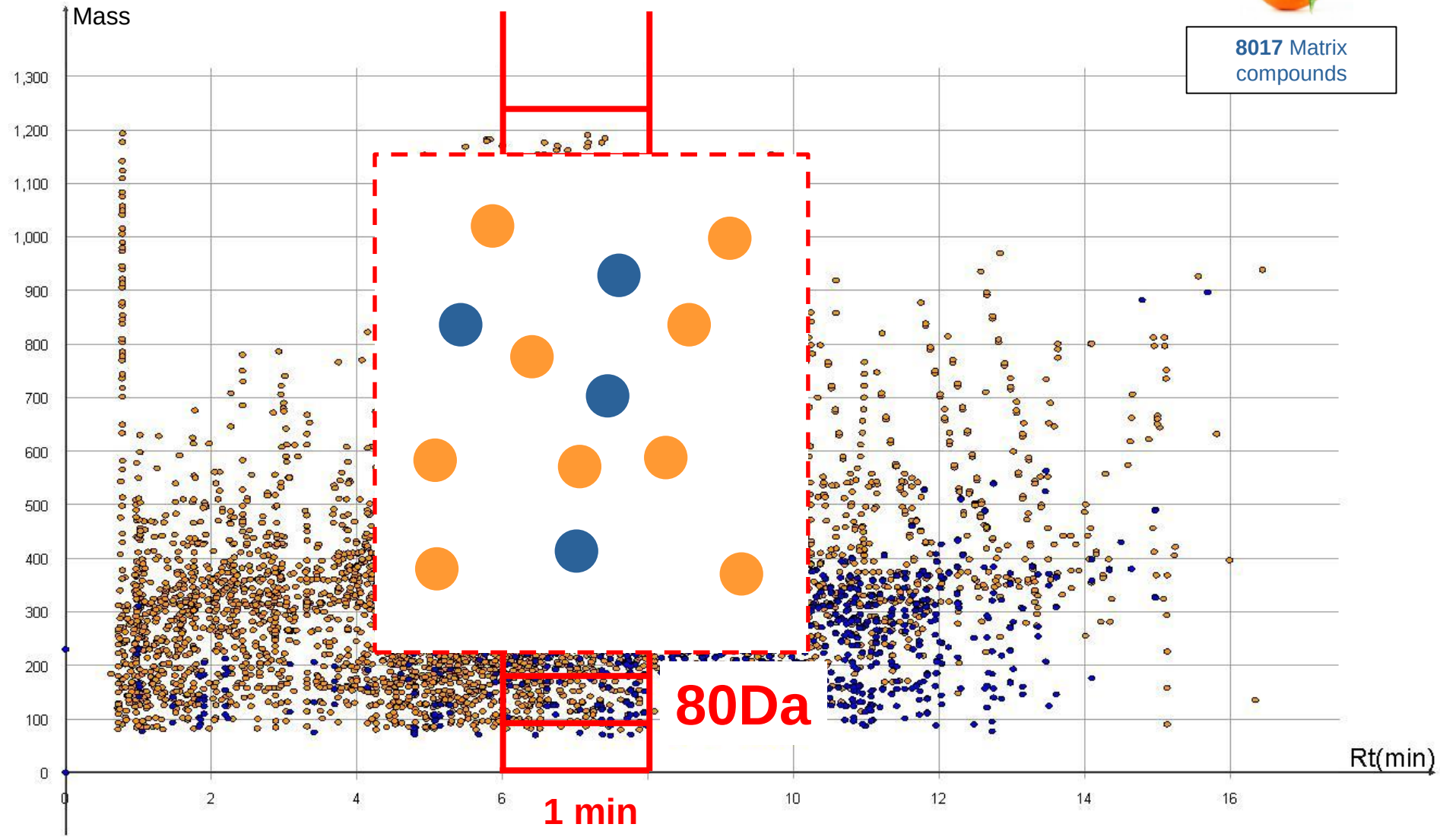


Pesticides - **orange** matrix compounds

8017 Matrix compounds

Pesticides - **orange** matrix compounds

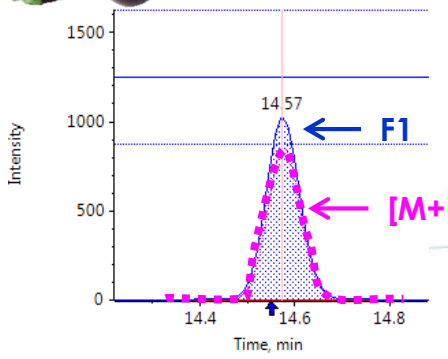
8017 Matrix compounds



Flufenoxuron – AIF 10 windows

0.010 mg/kg

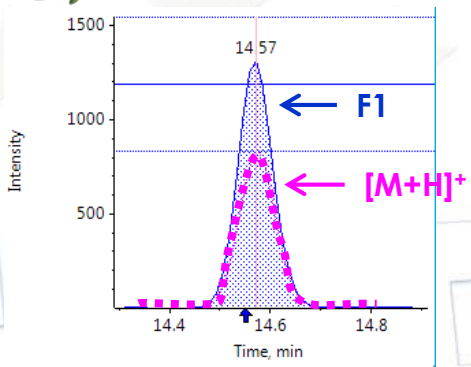
Aubergine



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	1.2377

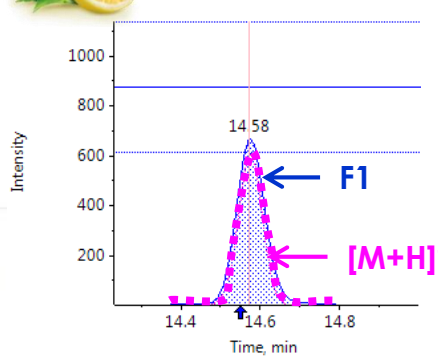
Broccoli



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	1.5142

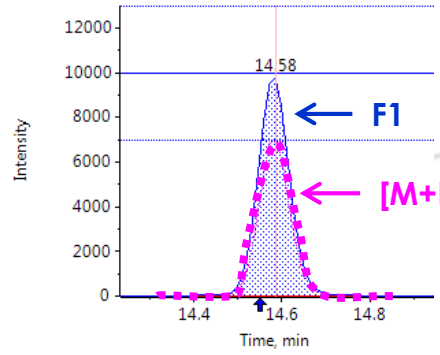
Lemon



Peak Details

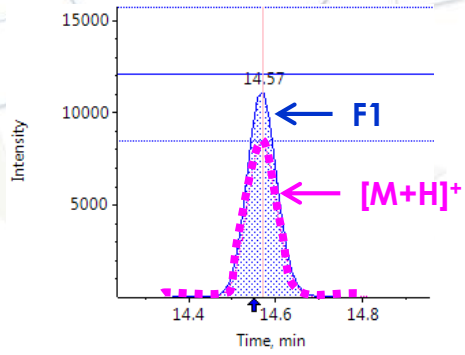
Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.58	1.3335

0.050 mg/kg



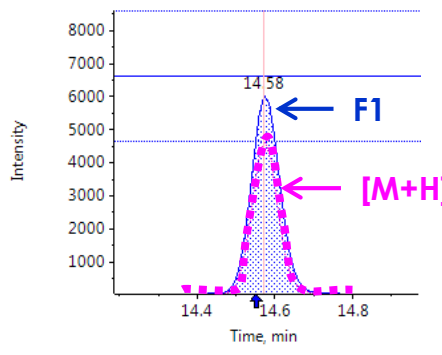
Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.58	1.4337



Peak Details

Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.57	1.3440



Peak Details

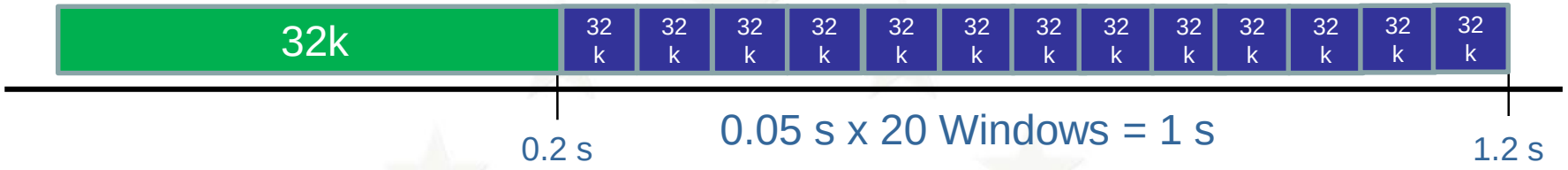
Precursor m/z	Retention Time (min)	Ion Ratio
489.044	14.58	1.4191

Ratio difference within ± **18 %**

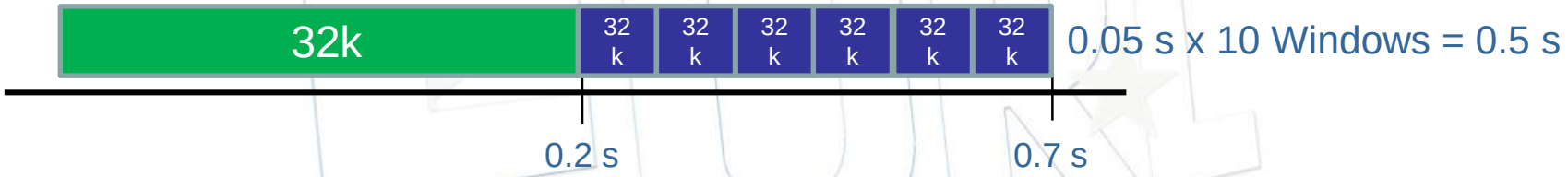
BUT.....

Cycle Time

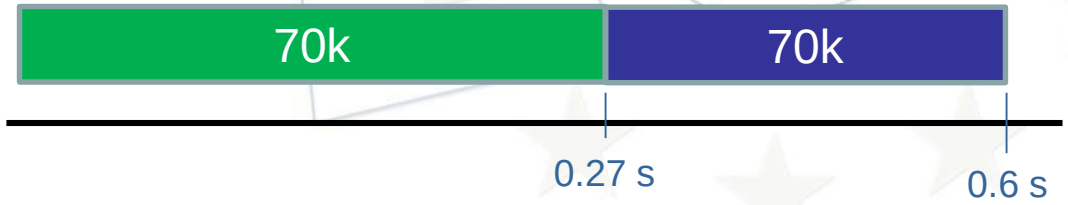
LC-QTOF-MS X500R (AIF -20 Windows)



LC-QTOF-MS X500R (AIF -10 Windows)



LC-QOrbitrap-MS (AIF)



LC-TQ-MS

MS1

MS2

Retrospective Analysis combined with the use of databases and libraries. ?

"Old" or very rare detected compounds

OR

"Very expensive" analytical standards

Compounds "produced" during the analysis

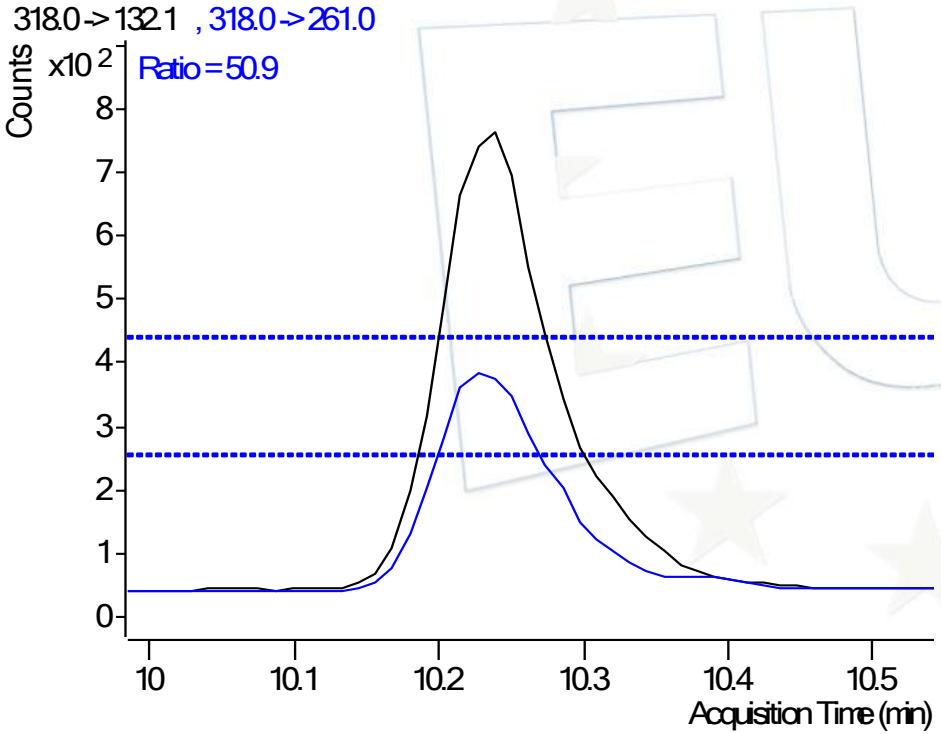
SELECTIVITY **SENSITIVITY**

Green Tea

Azinphos- methyl at 0.010 mg kg^{-1}

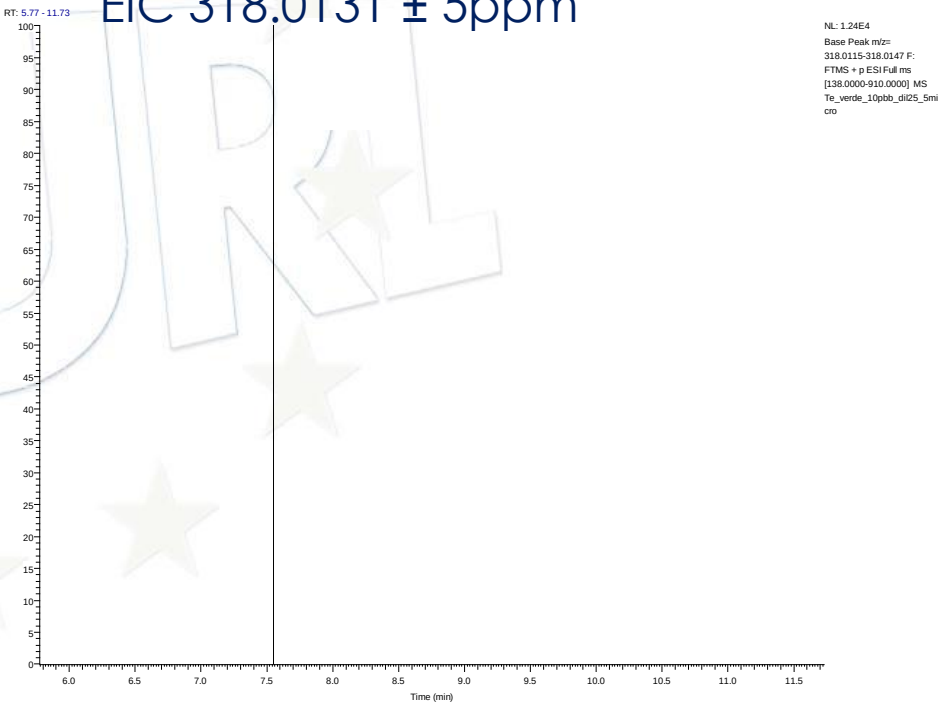
Injected amount:0.2 mg sample

LC-QqQ-MS/MS



LC-Q-Exactive-Orbitrap-MS/MS

EIC 318.0131 ± 5ppm



CONCLUSIONS

GC and LC-HRMS provide very accurate results improving the identification confidence (10 ug/kg). In cases of limitation in achieving analytical standards prediction of presence/ no presence can be provided.

In special GC-EI-HRMS can be easily implemented in the laboratories very effectively.

However, an increase of the sensitivity around 5-10 times should be necessary to be applied with the same workflows than triple quadrupoles

Thank You
for Your Attention

www.eurl-pesticides.eu