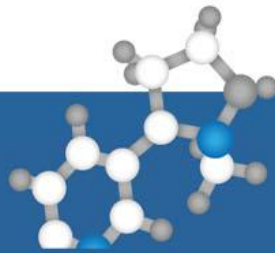


EURL



FV and SRM



25th-26th OCT
2016

European Union Reference Laboratories for Residues of Pesticides

MS² WORKFLOWS IN LC AND GC QTOF AND ORBITRAP SYSTEMS. EXAMPLE



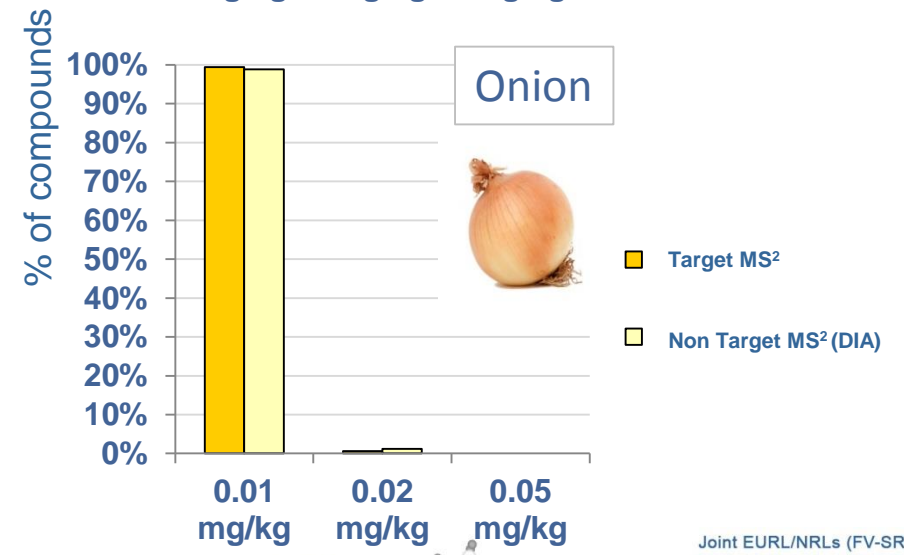
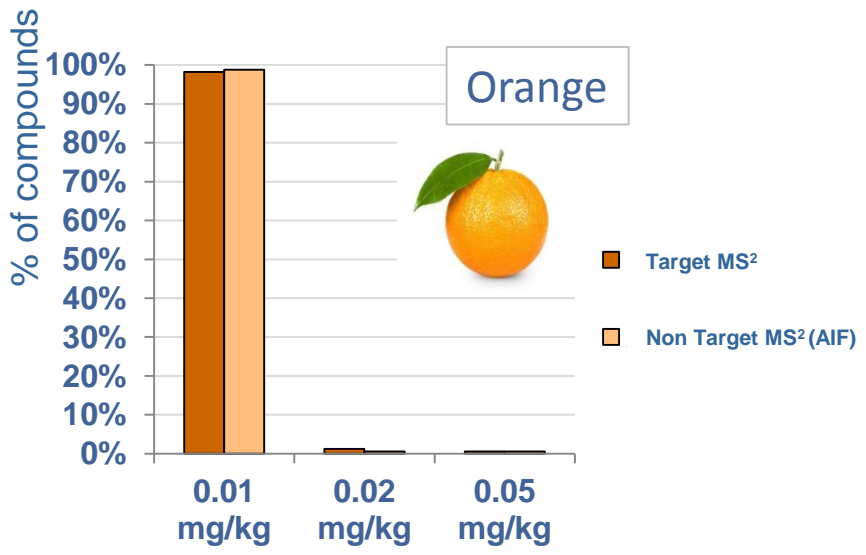
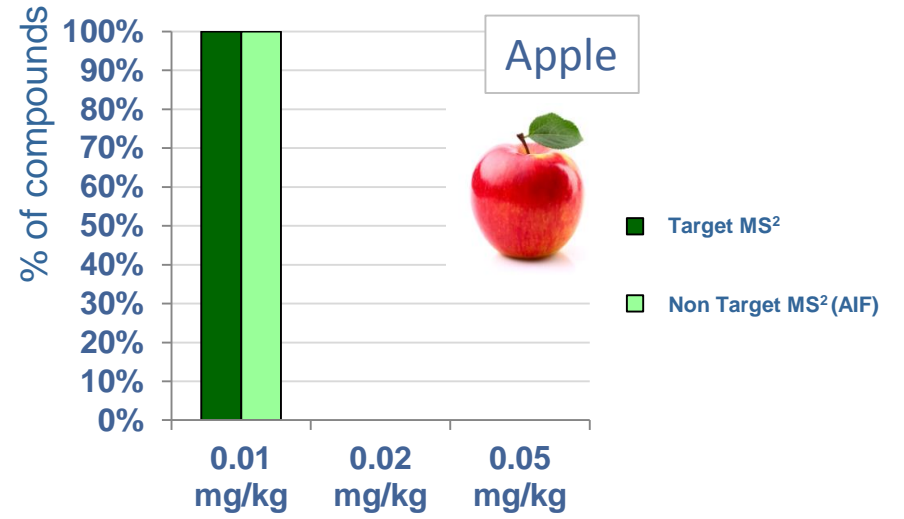
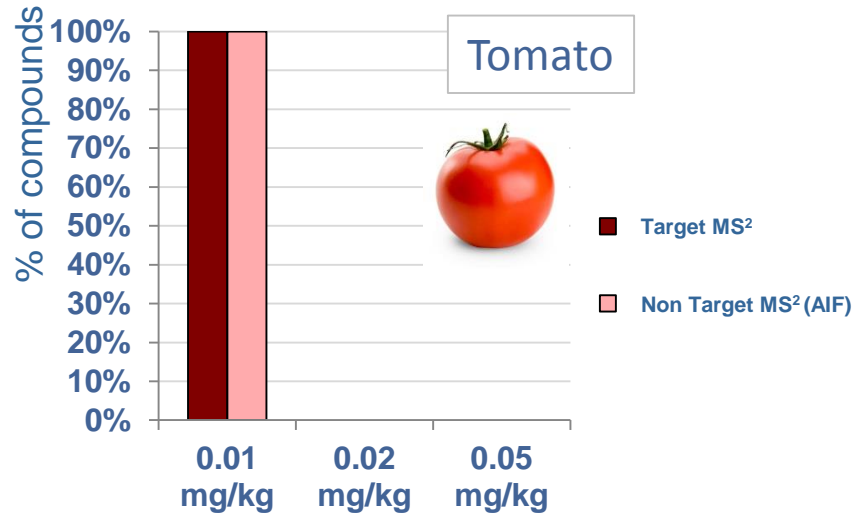
EURL-FV



AMADEO R. FERNÁNDEZ-ALBA

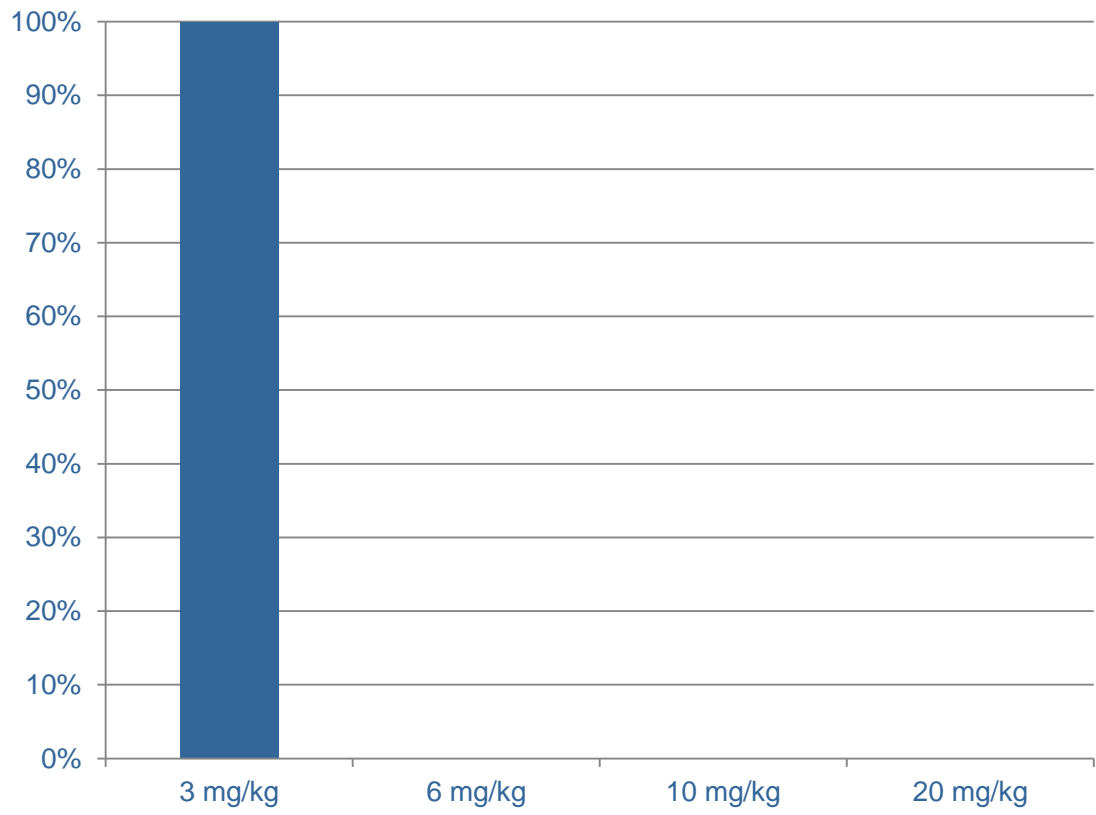
LC-Orbitrap QExactive Focus

Instrumental LOQ



Instrumental LOQs

LC-Orbitrap
QExactive Focus

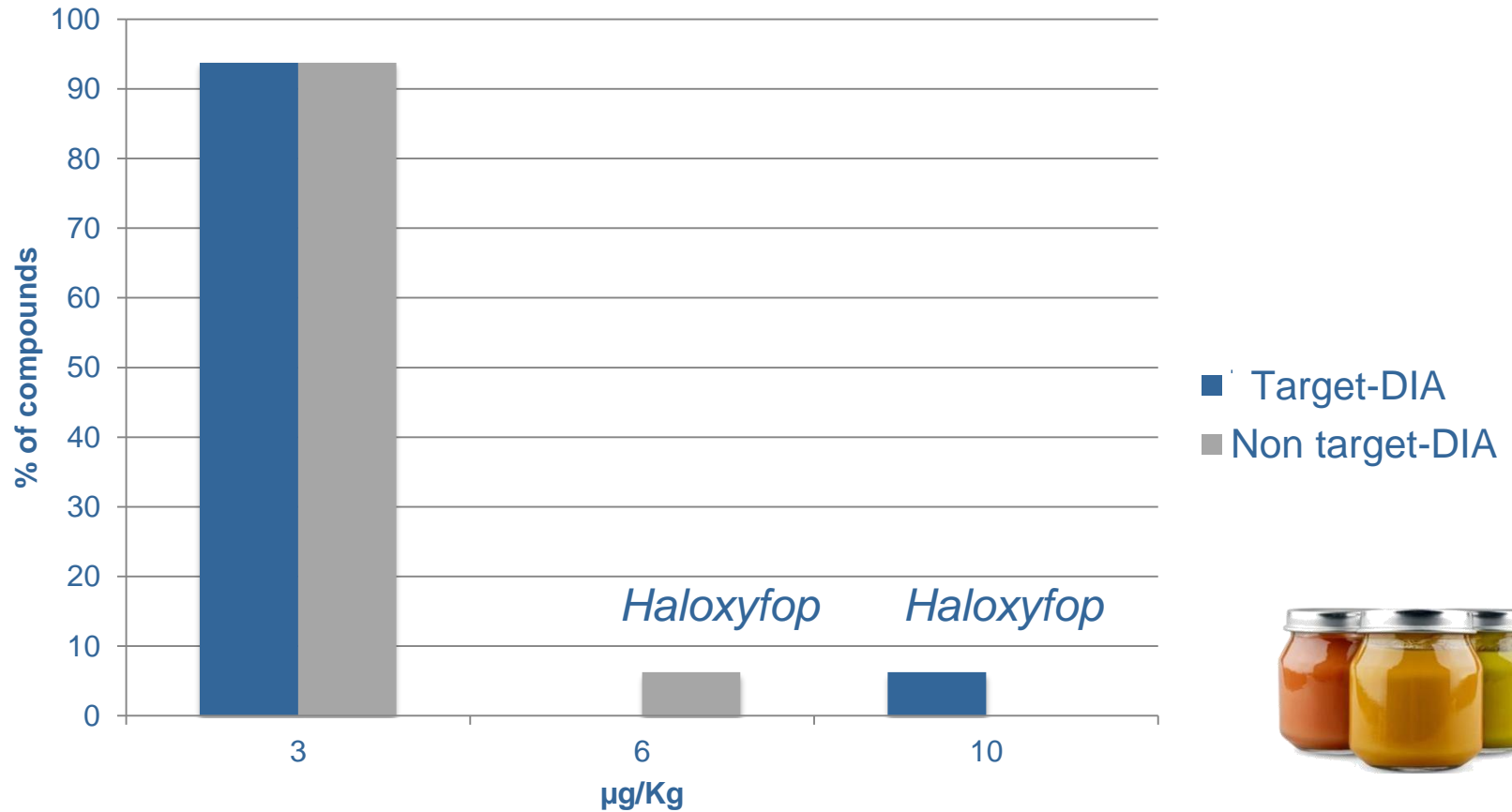


■ Target-DDA



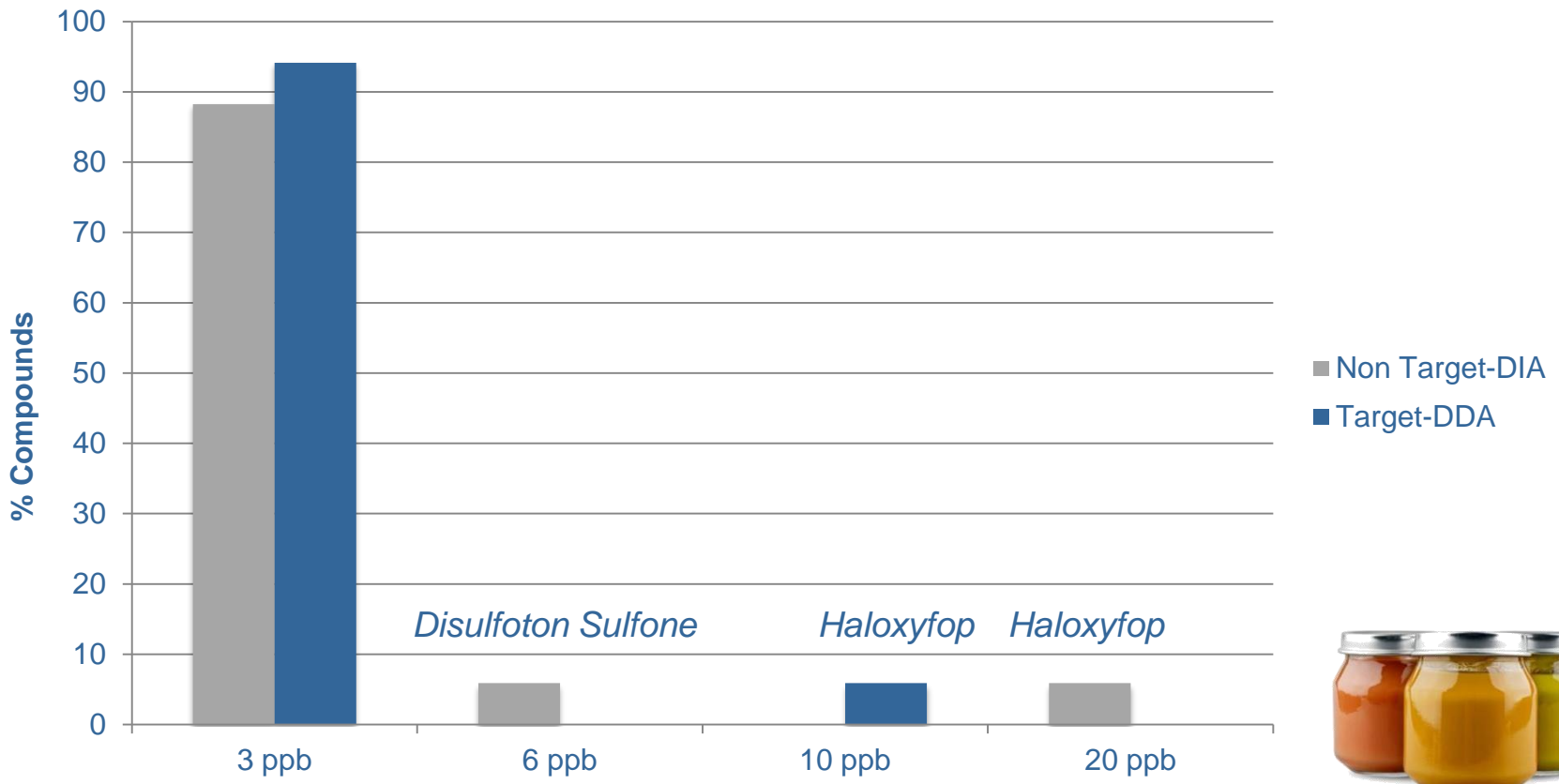
LC-QToF
X500R

Instrumental LOQs



LC-QToF
6550

Instrumental LOQs

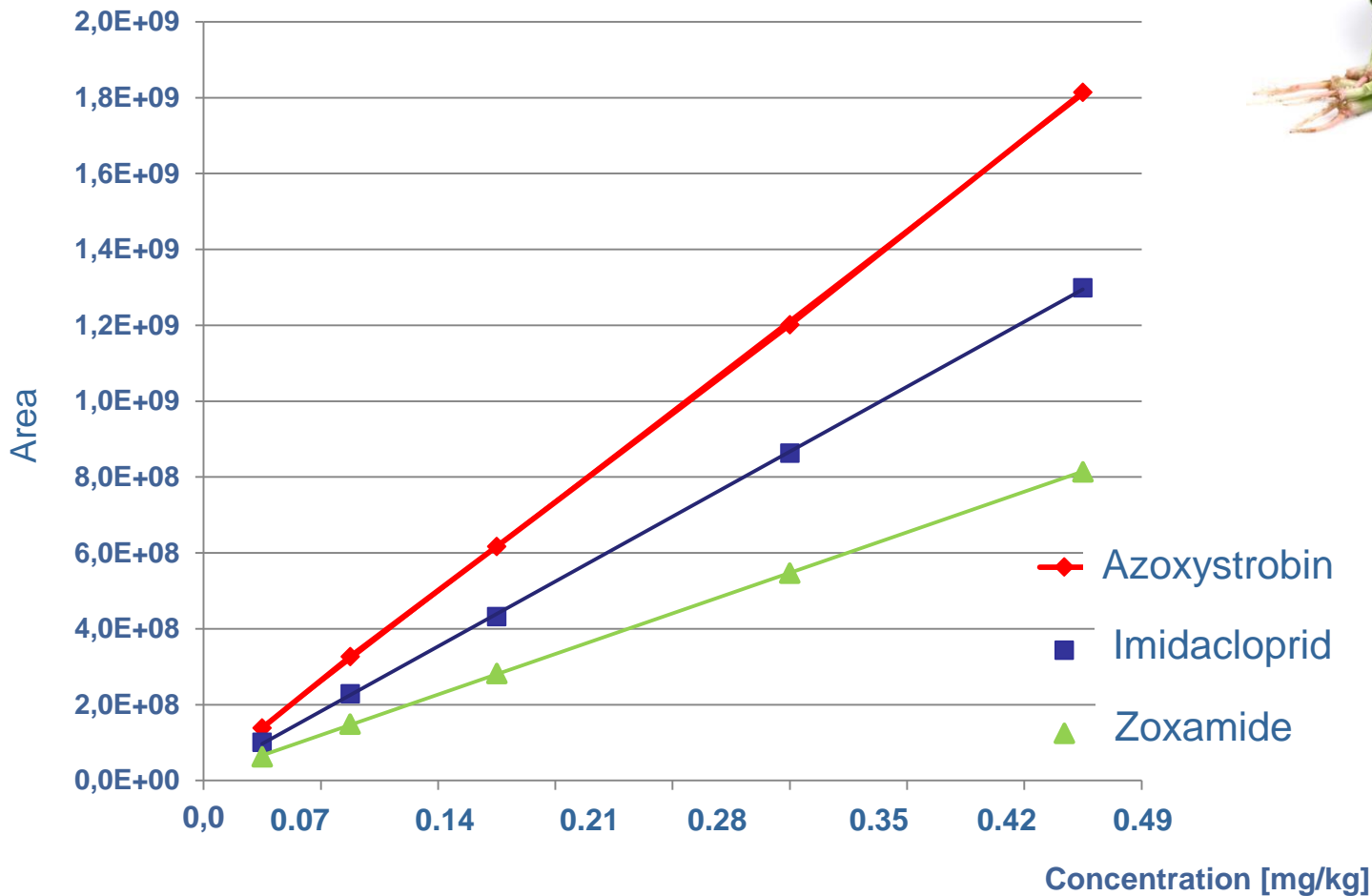


Linearity

LC-Orbitrap
QExactive Focus



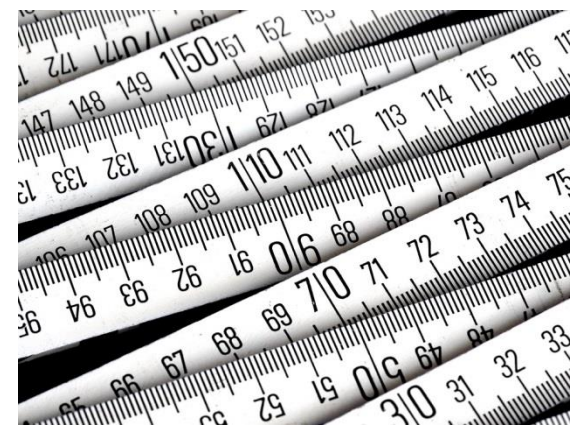
Spinach



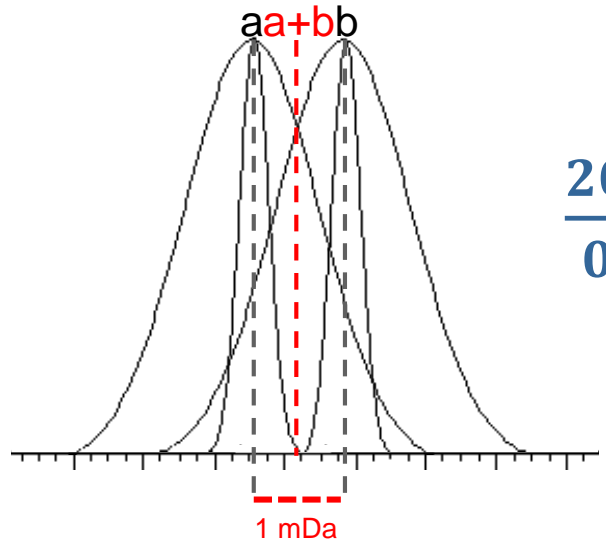
Qual.

MASS ACCURACY (5 ppm)

$$\frac{200.001 - 200.000}{200.000} = \frac{0.001}{200.000} = 5 \cdot 10^{-6}$$

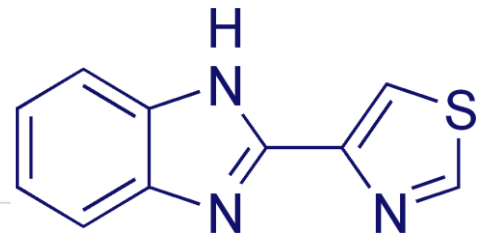


RESOLUTION (¿?)

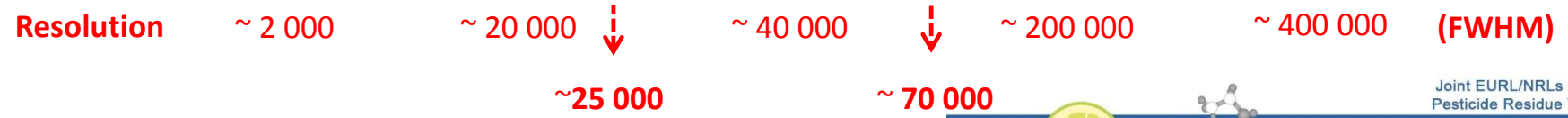
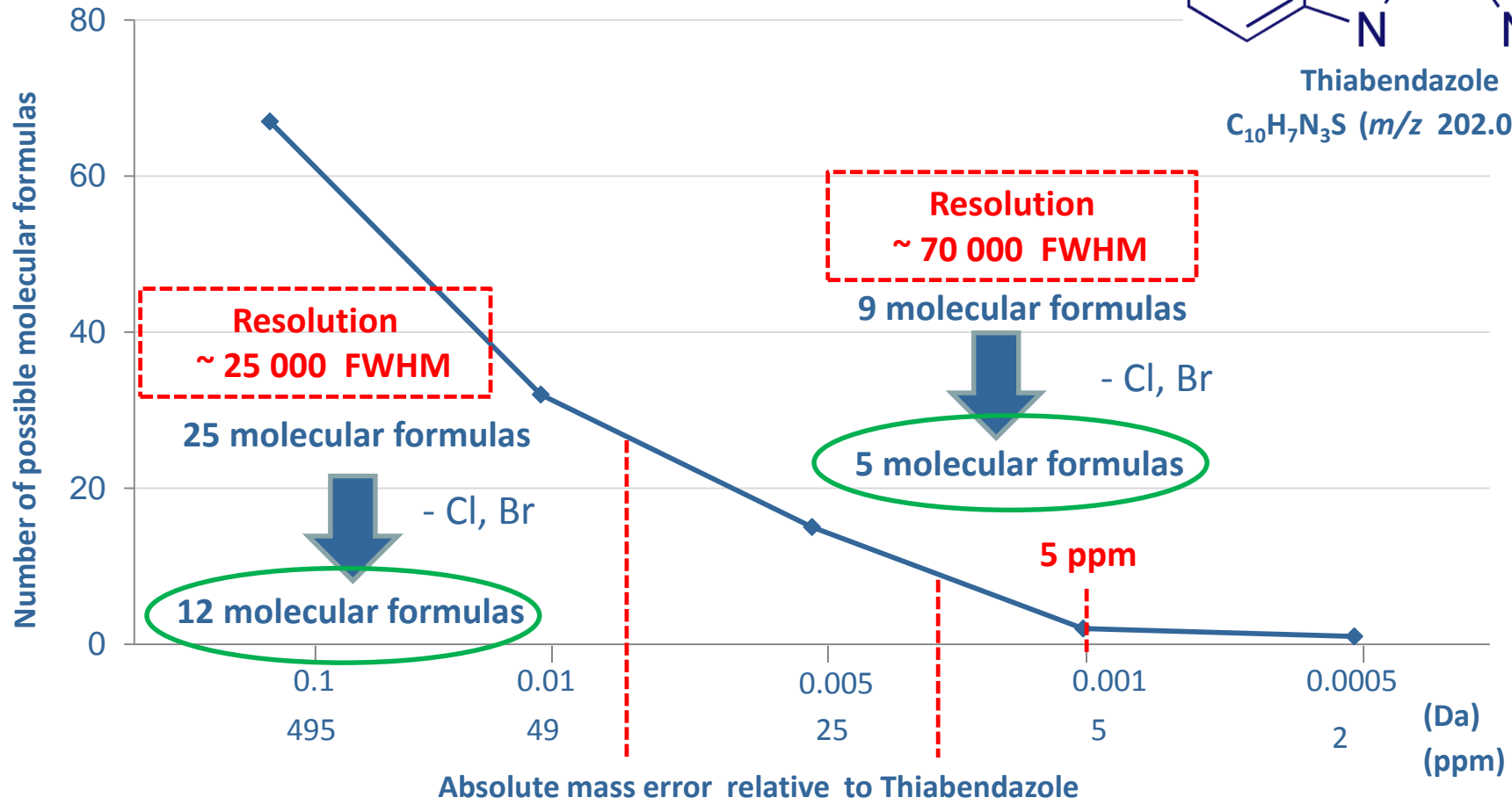


$$\frac{200.000}{0.0001} = 200,000$$





Thiabendazole
 $C_{10}H_7N_3S$ (m/z 202.0433)



ADVANTAGES IN USING A HIGH RESOLUTION ($> 25,000$)

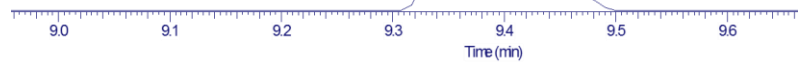
Clofentezine 0.01 mg/kg in leek



17 500

70 000

Full scan MS
303.0199 ± 5 ppm



57 RT: 9.36 AV: 1 N: 1.35EB
0.000-1000.0000]

1 RT: 9.37 AV: 1 N: 2.51EB
0.0000-1000.0000]

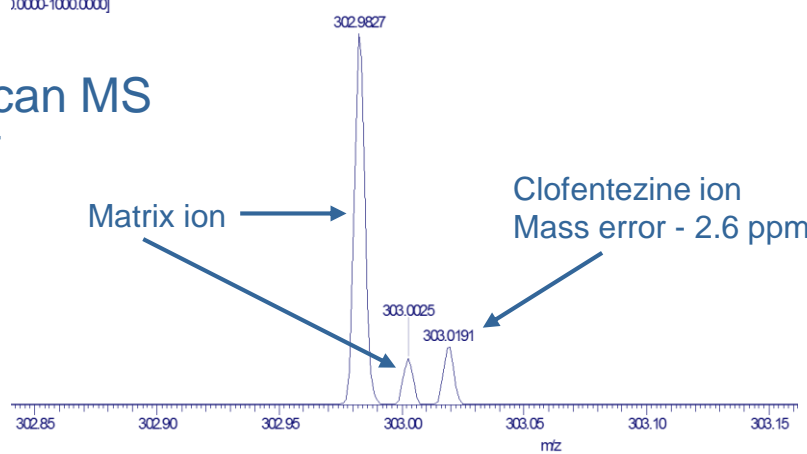
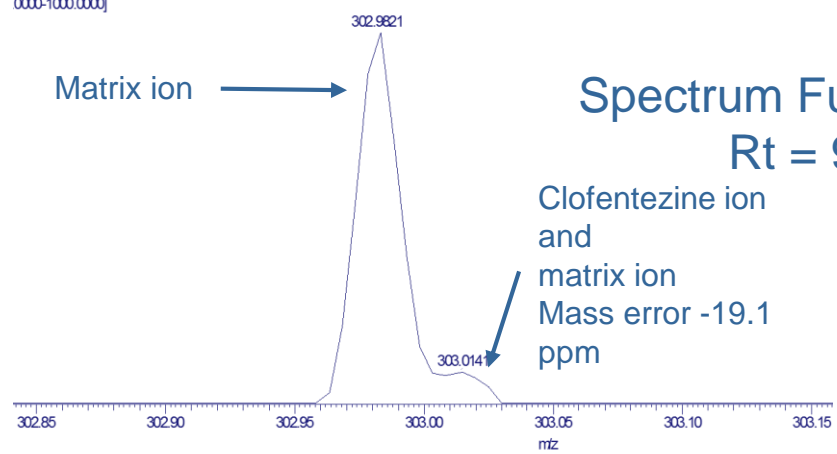
Matrix ion →

Spectrum Full scan MS
Rt = 9.37

Clofentezine ion
and
matrix ion
Mass error -19.1
ppm

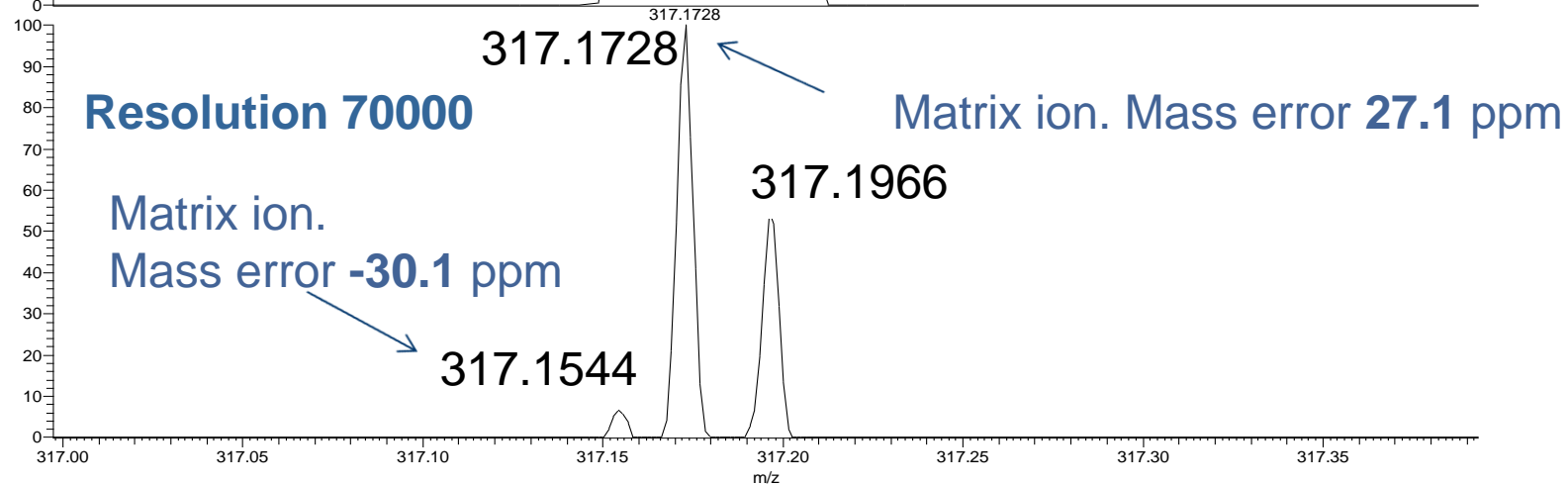
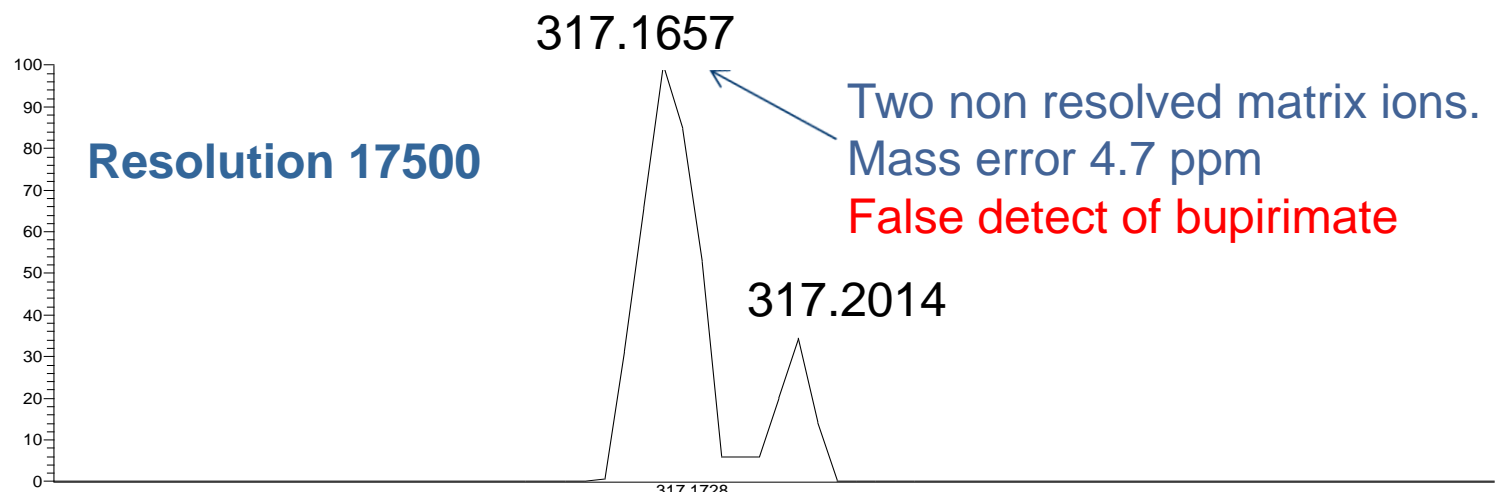
Matrix ion →

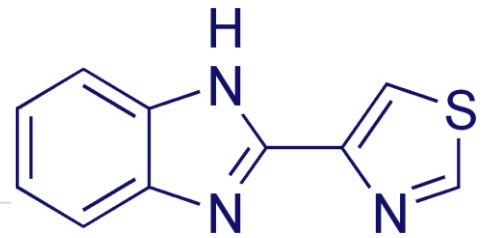
Clofentezine ion
Mass error - 2.6 ppm



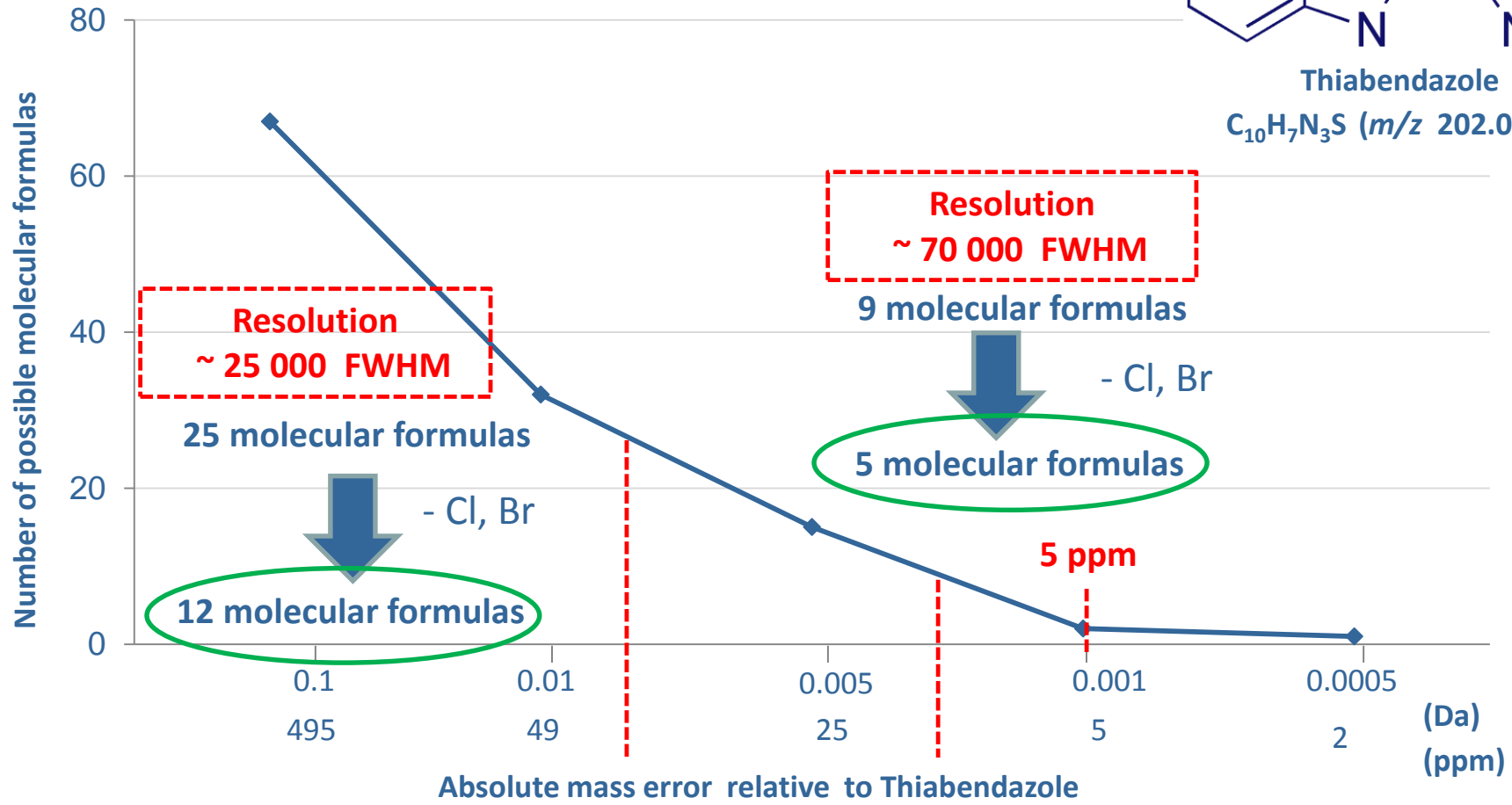
Influence of resolution on detection

Exact mass of bupirimate: 317.1642





Thiabendazole
 $C_{10}H_7N_3S$ (m/z 202.0433)



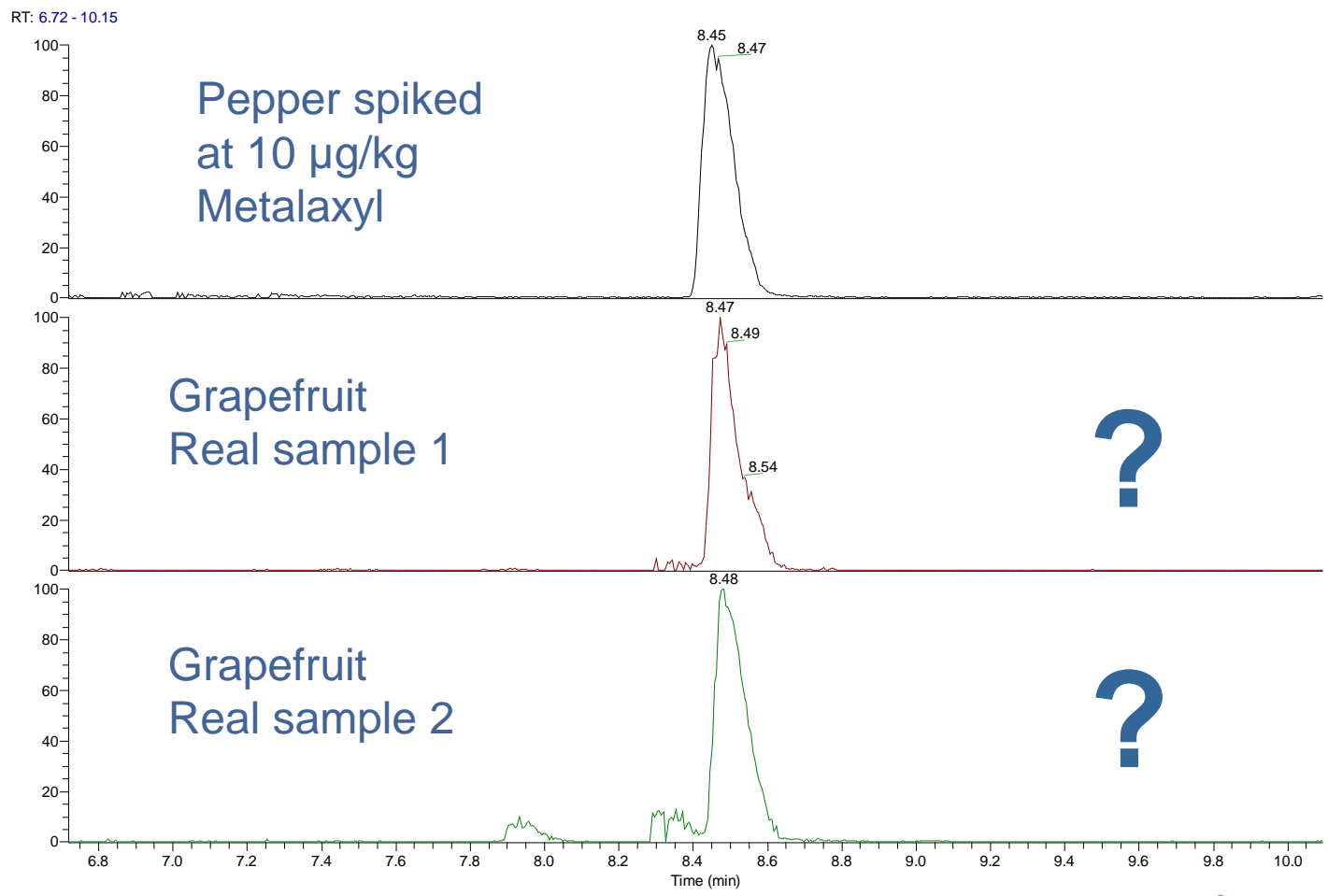
Resolution ~ 2 000 ~ 20 000 ~ 40 000 ~ 200 000 ~ 400 000 (FWHM)

~25 000 ~ 70 000

BUT...

Analysis of real samples by LC Q-Exactive MS²

Metalaxyl (XIC m/z 280.1543 ± 5 ppm). Full scan MS. Resolution 70000



LC-QOrbitrap (QExactive)

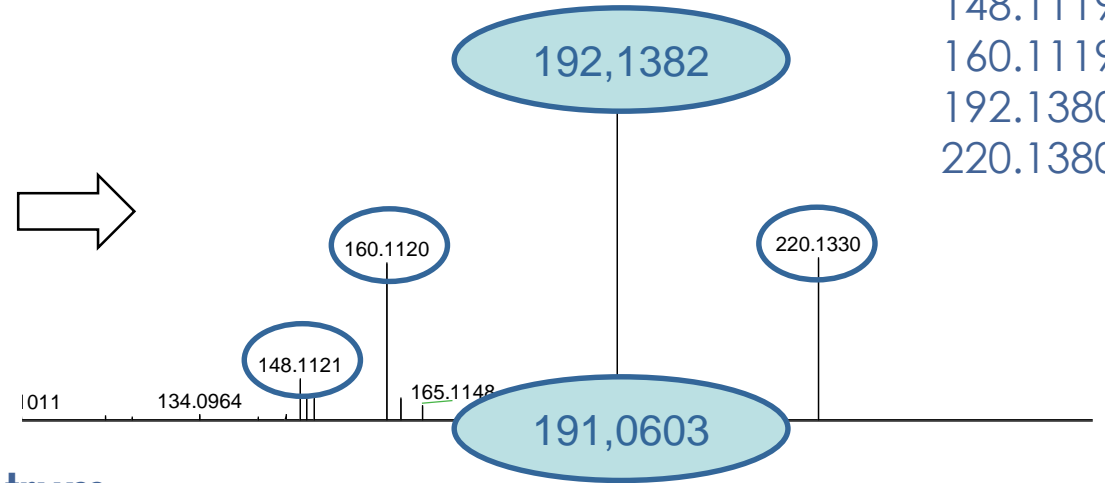
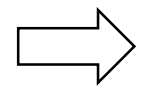
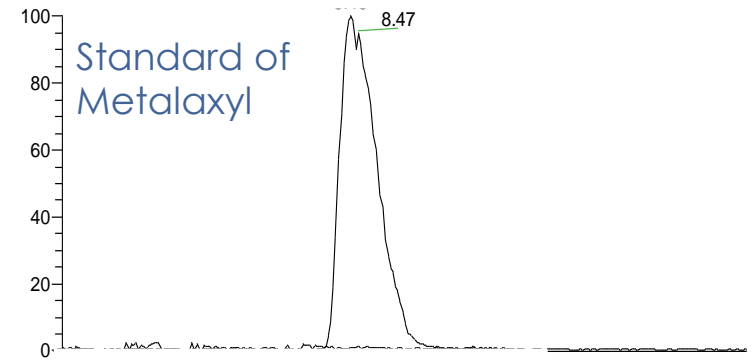
Metalaxyl (XIC m/z 280.1543 ± 5 ppm).
Full scan MS. Resolution 70000

Library MS/MS spectrum

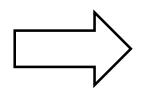
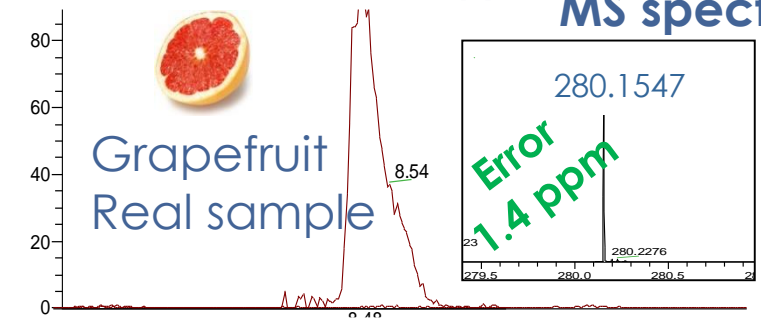
Expected fragments:

- 148.1119
- 160.1119
- 192.1380
- 220.1380

RT: 6.72 XIC m/z 280.1543 ± 5 ppm

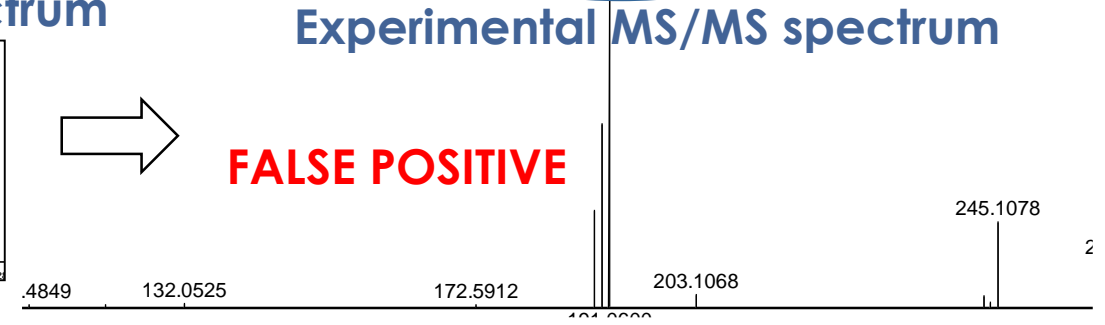


XIC m/z 280.1543 ± 5 ppm



Experimental MS/MS spectrum

FALSE POSITIVE



Identification criteria (EU)

MS detector / characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions with mass accuracy ≤ 5 ppm ^{a,b,c}	$\geq 3^e)$ peaks in the selected ion chromatograms must overlap.
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. ≤ 5 ppm ^{a,c} <u>plus</u> 1 MS/MS product ion ^{d)}	one within (relative) charge distribution from same ionance
		combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution		

^{a)} preferably including the molecular ion, (de)protonated molecule

^{b)} including at least one fragment ion

^{c)} < 1 mDa for $m/z < 200$

^{d)} no specific requirement for mass accuracy

^{e)} in case noise is absent, a signal should be present in at least 5 subsequent scans

GC-EI-HRAMS

60k

Full scan MS

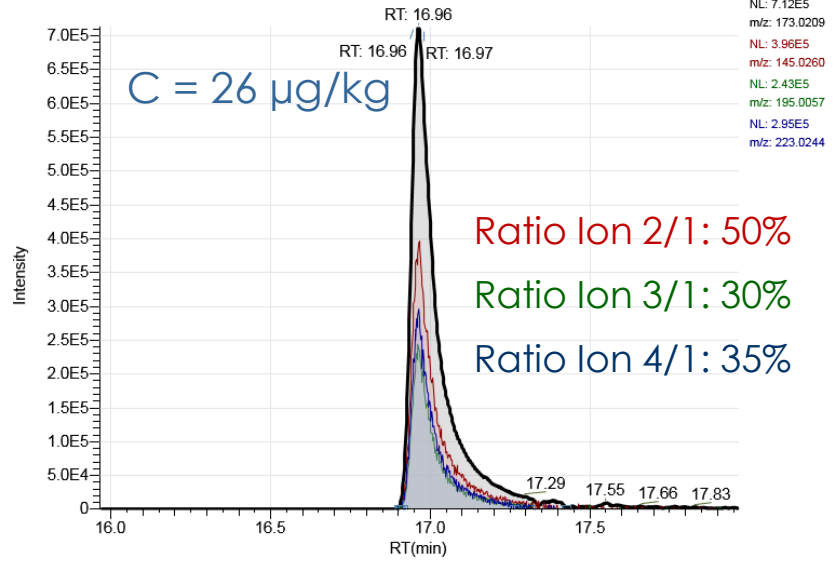
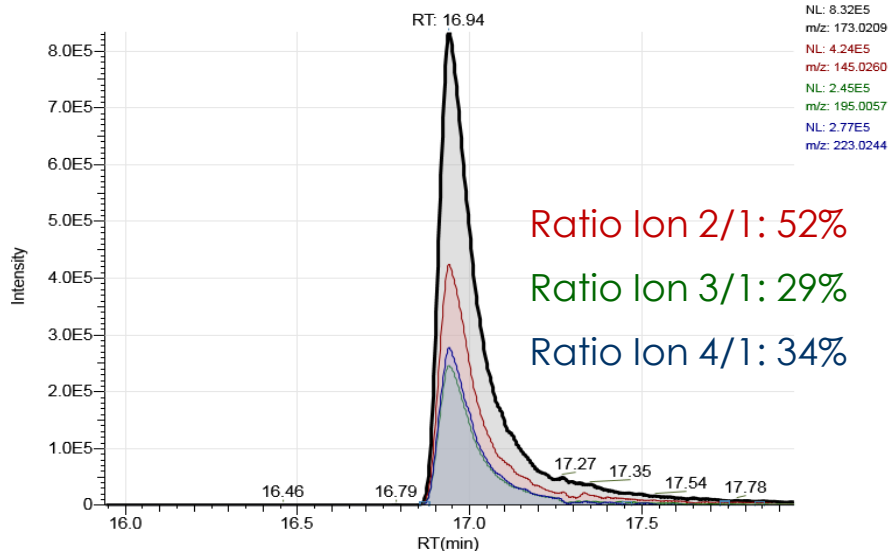
Orbitrap GC-EI-MS System



Real Samples: Fluopyram

50 µg/kg Std in Tomato

Real Sample: Pear

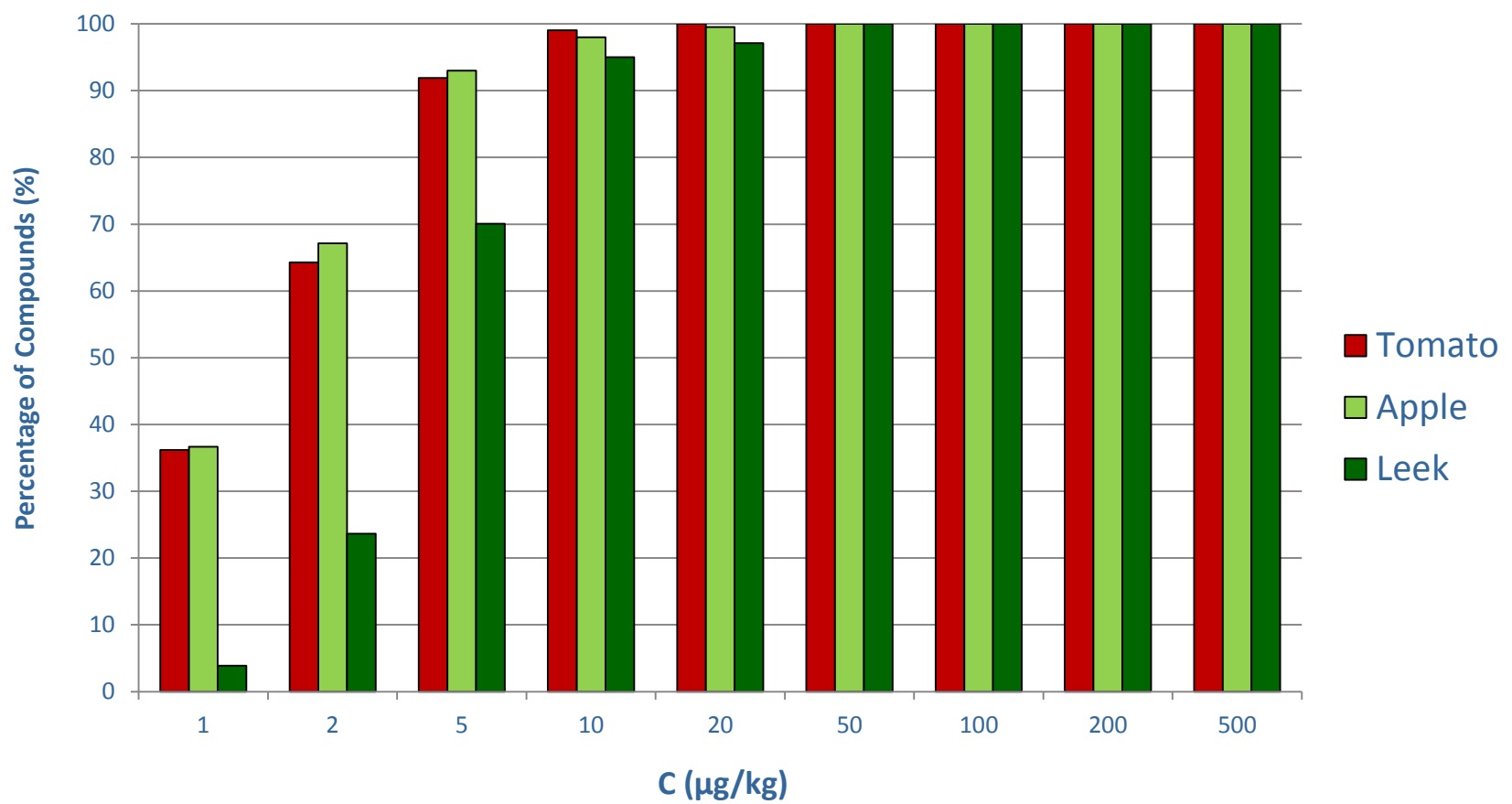


The **ion ratios** are within $\pm 4\%$ with respect to the standard and mass accuracy below **0.2 mDa**

Ion 1 (173.0209): 0.01 mDa Ion 3 (195.0057): -0.08 mDa
 Ion 2 (145.0260): 0.14 mDa Ion 4 (223.0244): 0.02 mDa

GC-Orbitrap QExactive

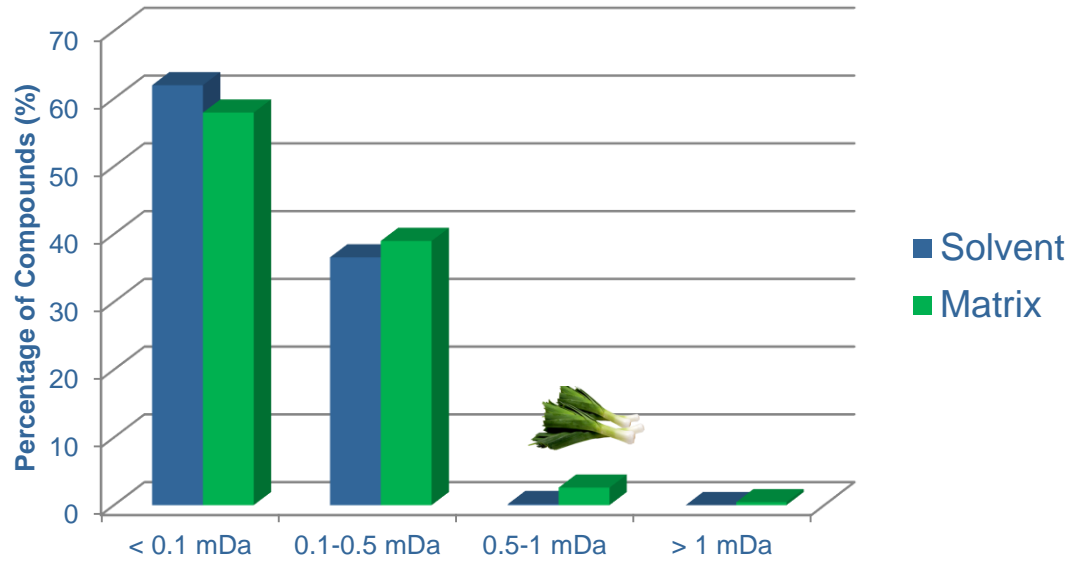
Instrumental LOQ



GC-Orbitrap
QExactive

Mass Error

Full Scan



Matrices:



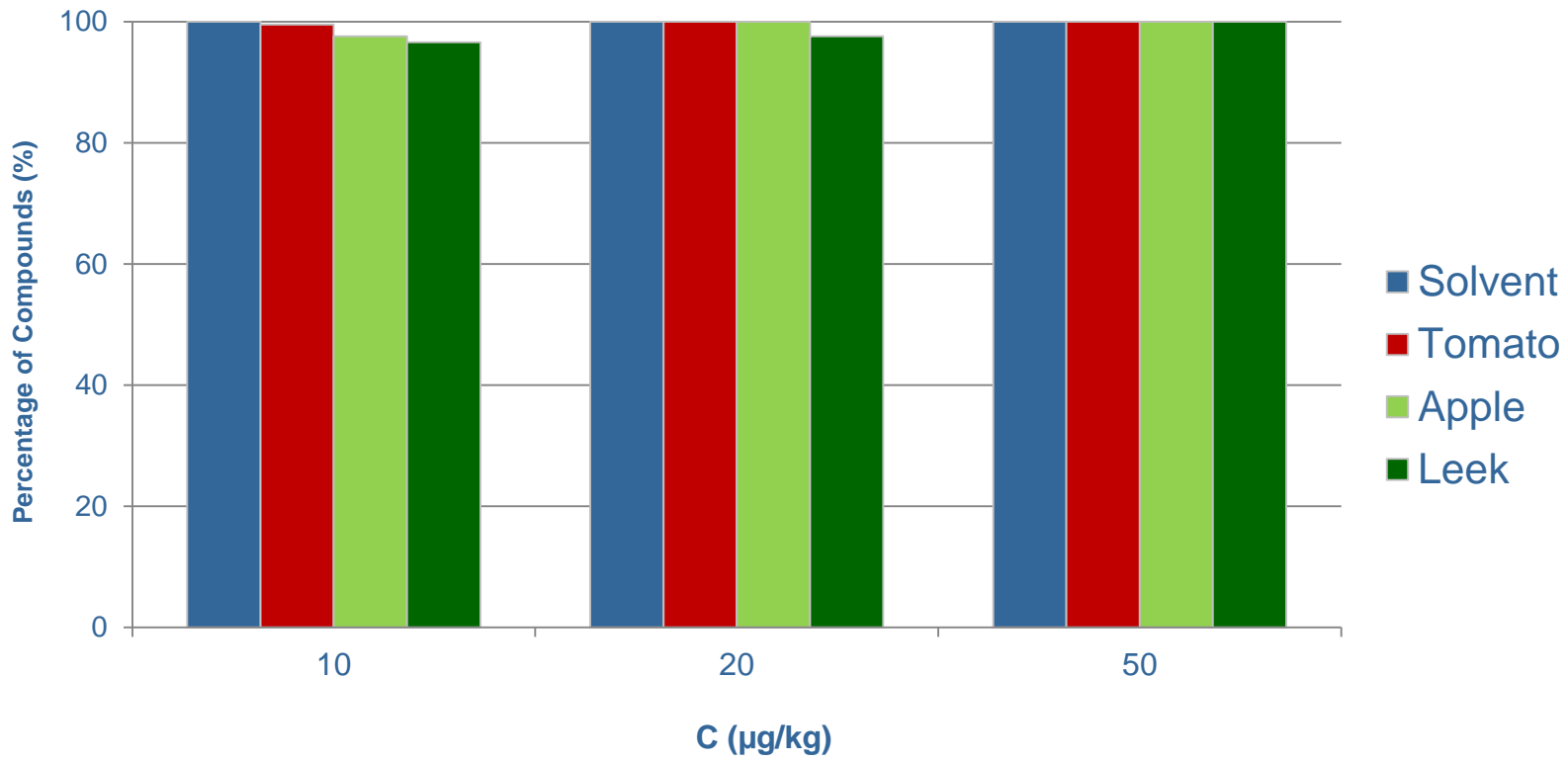
at 0.01, 0.02 and 0.05 mg/kg

Solvent: at 0.01, 0.02 and 0.05 mg/kg

Ion Ratio

GC-Orbitrap
QExactive

Ion Ratio within $\pm 30\%$



LC-ESI-HRAMS

TARGET ANALYSIS

m/z
t_R
CE

DDA MS² (*Data Dependent Acquisition*)
Restricted to inclusion list of m/z

DIA MS² (*Data Independent Acquisition*)
Always



DDA	dd-MS ²	AutoMS ² Preferred List	IDA Inclusion List (<i>Information Dependent Acquisition</i>)	AutoMS
DIA	PRM (<i>Parallel Reaction Monitoring</i>)	Targeted MS ²	MRM-HR (<i>MRM-High Resolution</i>)	MRM

NON TARGET ANALYSIS

~~m/z~~
~~t_R~~

1-3 CE

DDA MS² (*Data Dependent Acquisition*)
2-10, X more abundant ions

DIA MS² (*Data Independent Acquisition*)
Always



Agilent Technologies



DDA	dd-MS² (Data Dependent)	Auto MS/MS	IDA (Information Dependent Acquisition)	AutoMS
DIA	AIF-MS² (All Ion Fragmentation) vDIA (variable Data Independent Acquisition)	AI (All Ion)	SWATH (Sequential Window Acquisition of all Theoretical Mass Spectra)	bbCID (broad band Collision Induced Dissociation)



10,66

dd-MS²
(two fragment ions with mass error < 5 ppm)

Ethion
Full scan
384.9949 ± 5 ppm

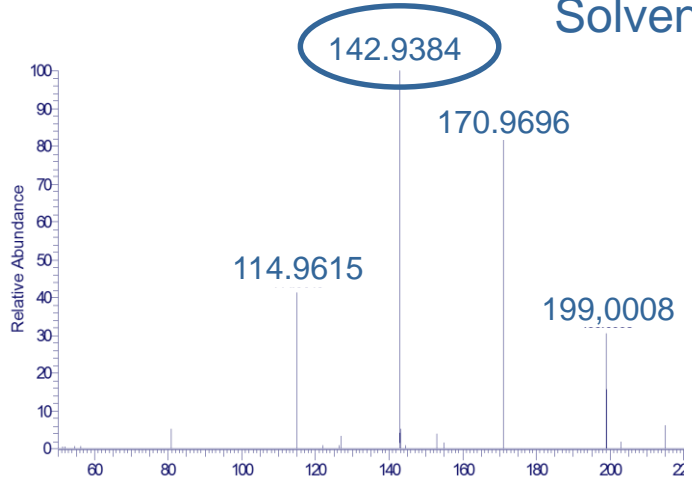


10,66

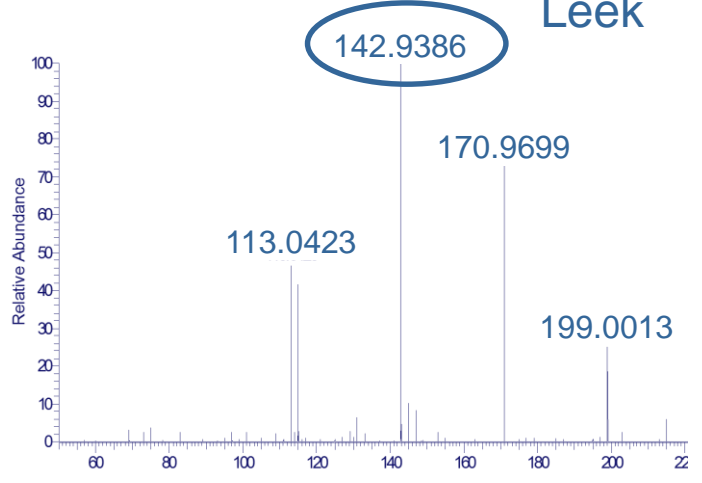


Solvent

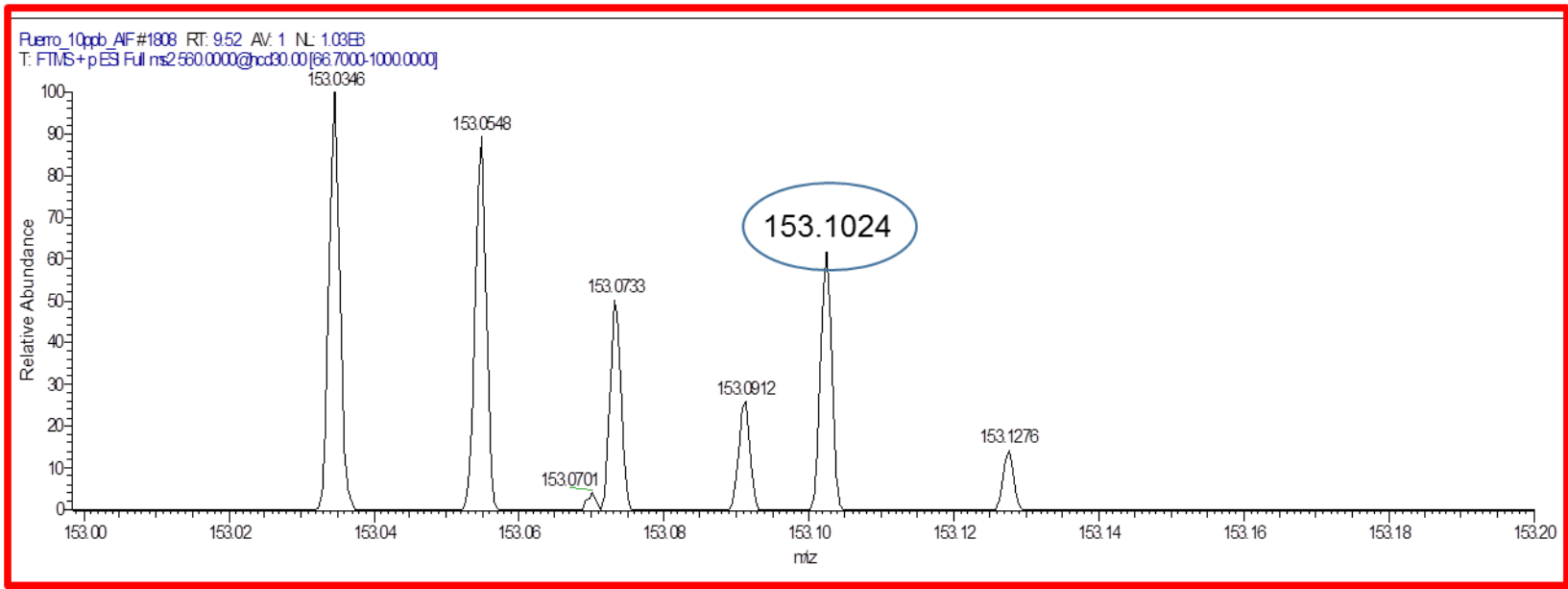
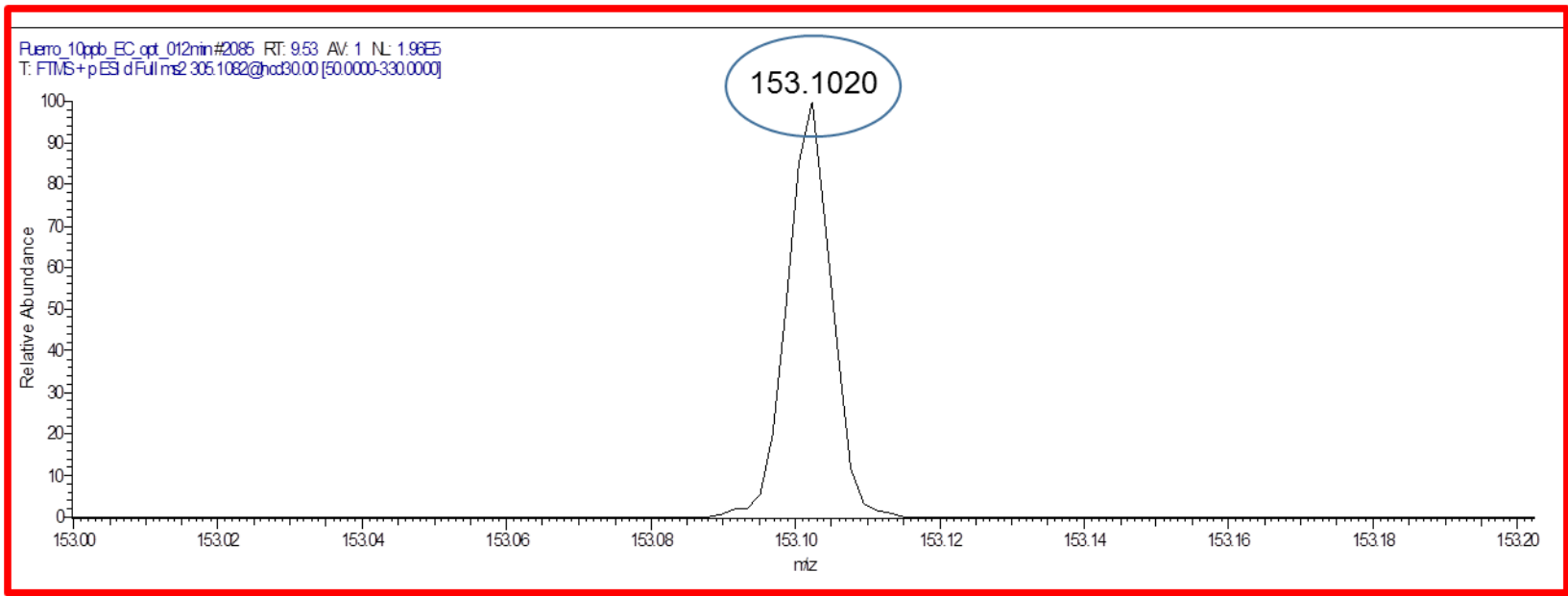
Leek

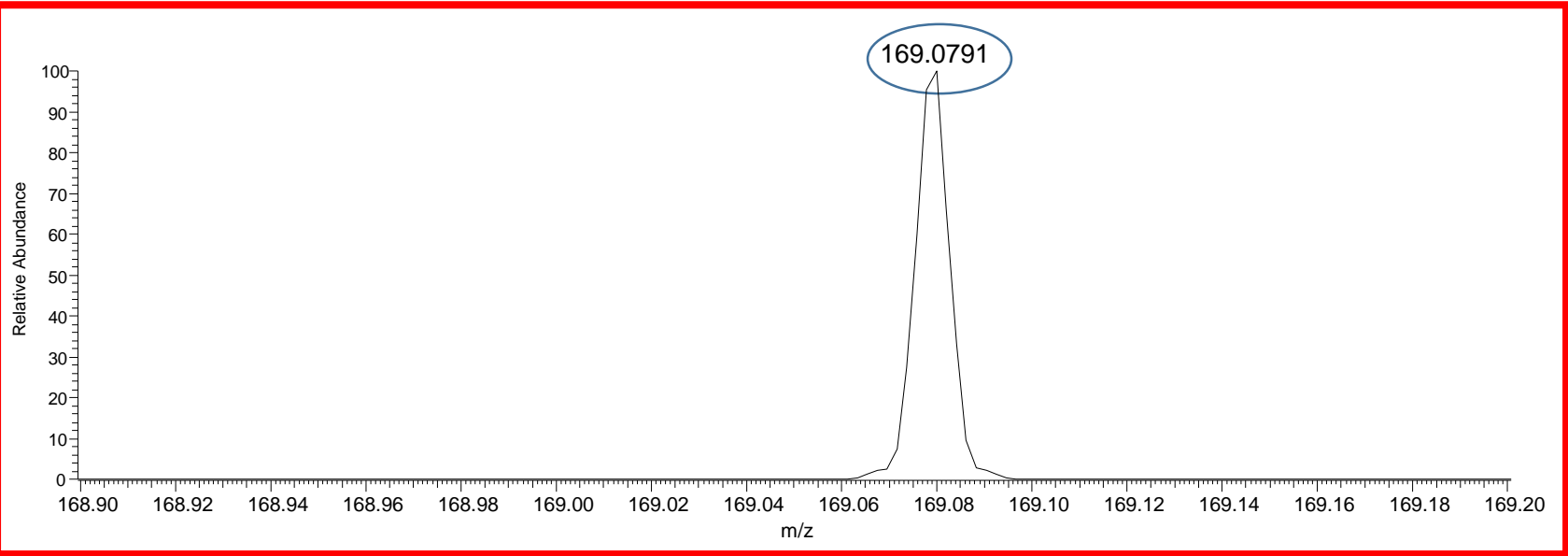
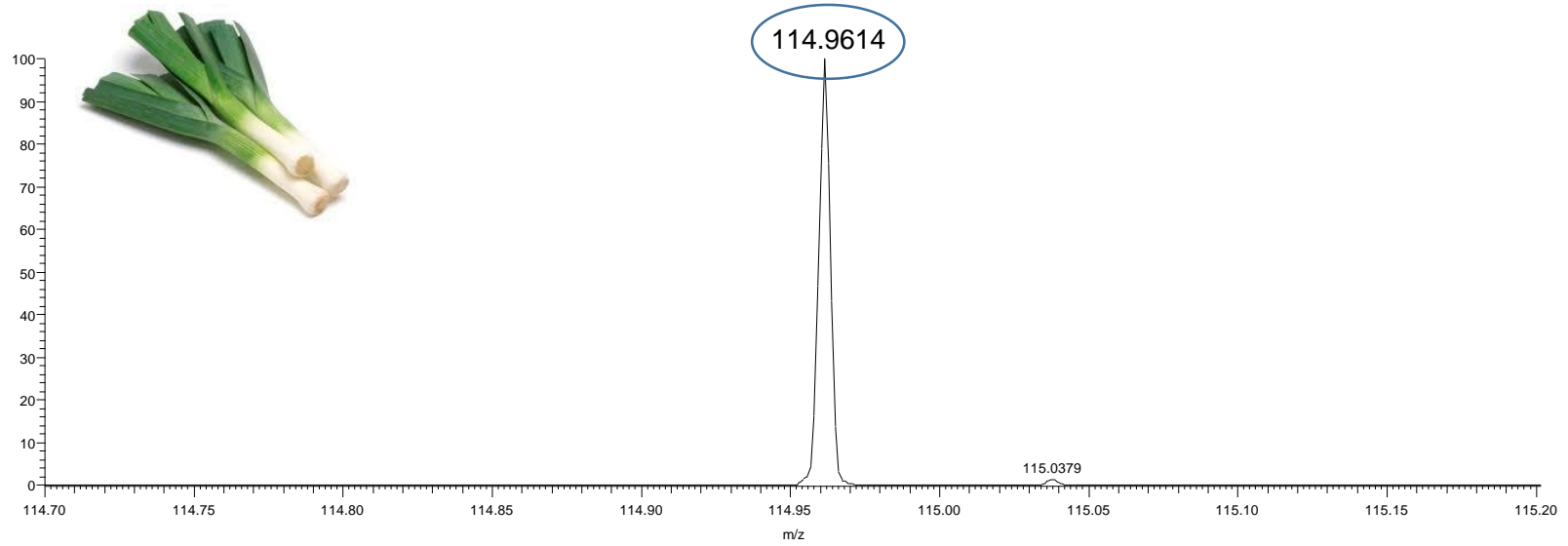


dd-MS²
384.9949 ± 0.5 Da



Fragment ion	Abundance in solvent	Abundance in leek
114.9615	39%	43%
142.9386	100%	100%
170.9699	82%	74%
199.0013	29%	26%

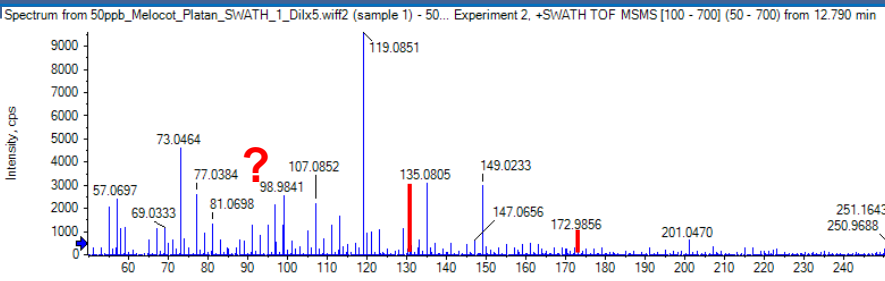




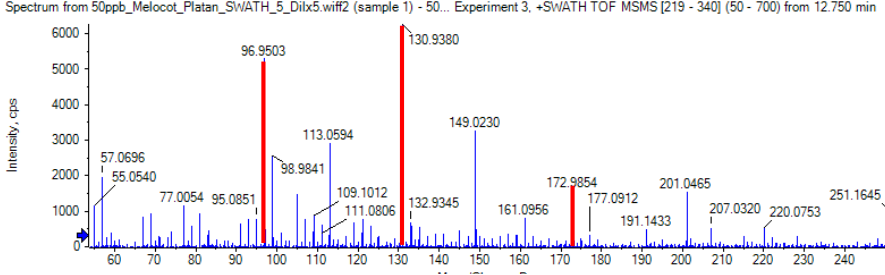
ADVANTAGES IN USING MASS WINDOWS (MS²) IN NON-TARGET DIA

Ethoprophos 50µg/Kg dilx5

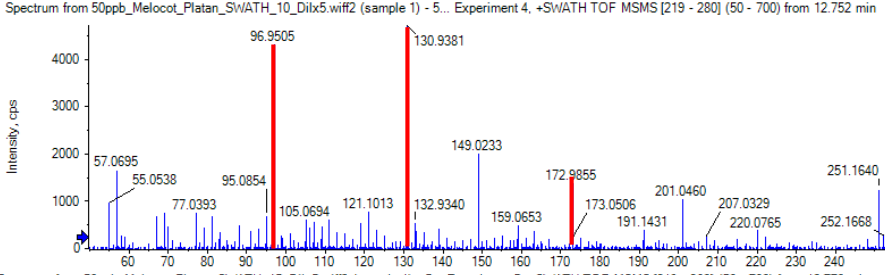
1 Mass Window
650 Da per window



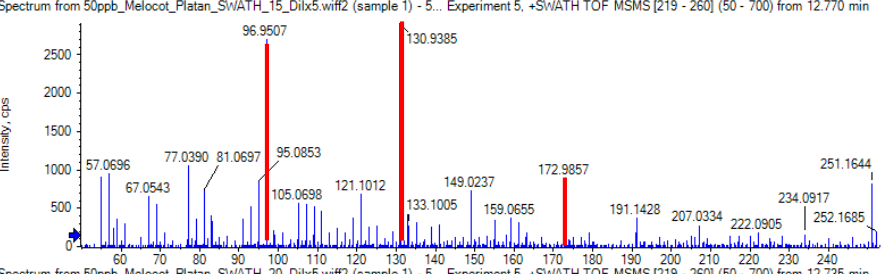
5 Mass Windows
130 Da per window



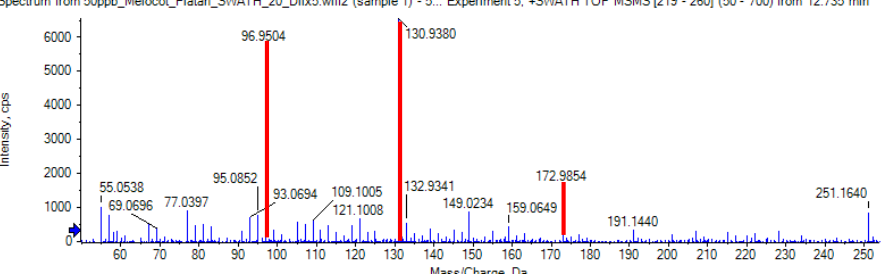
10 Mass Windows
65 Da per window



15 Mass Windows
43 Da per window



20 Mass Windows
32 Da per window



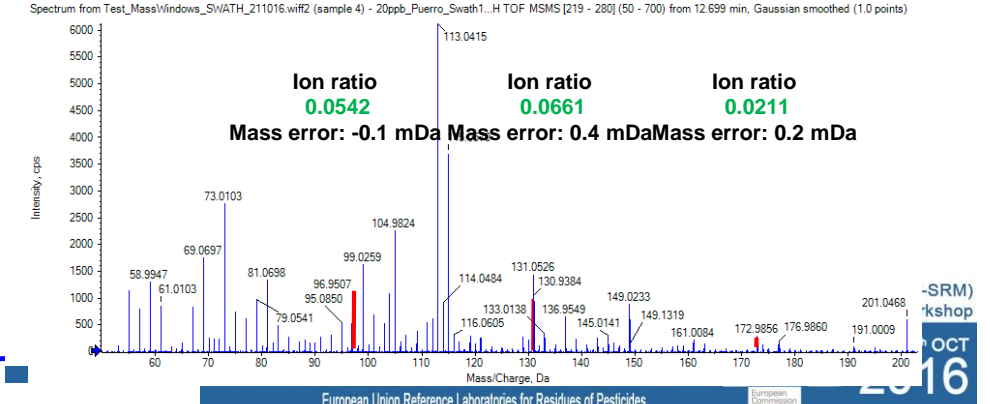
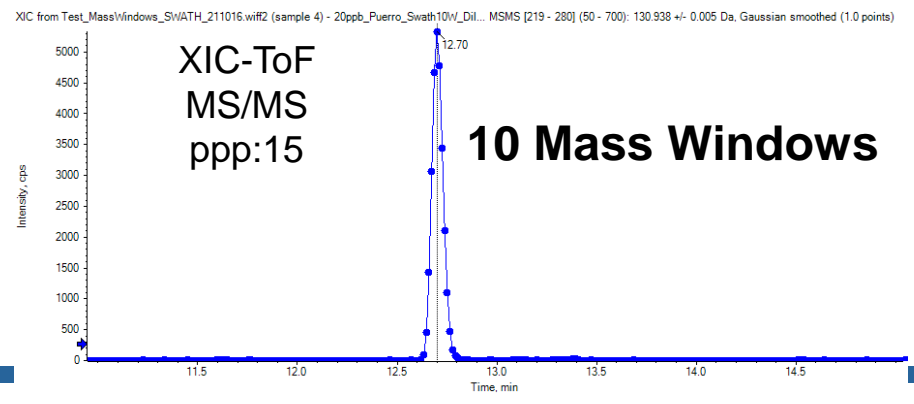
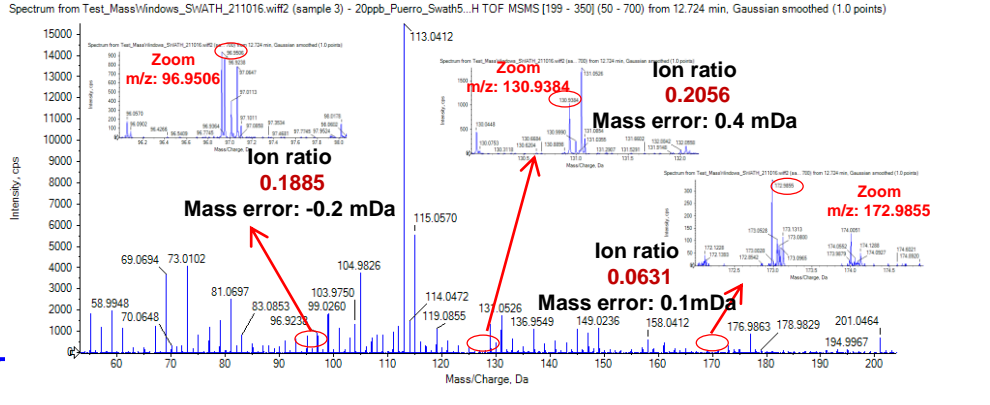
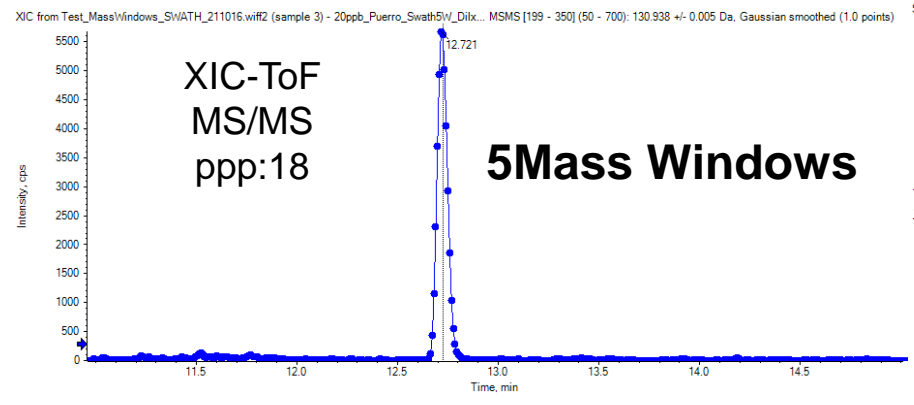
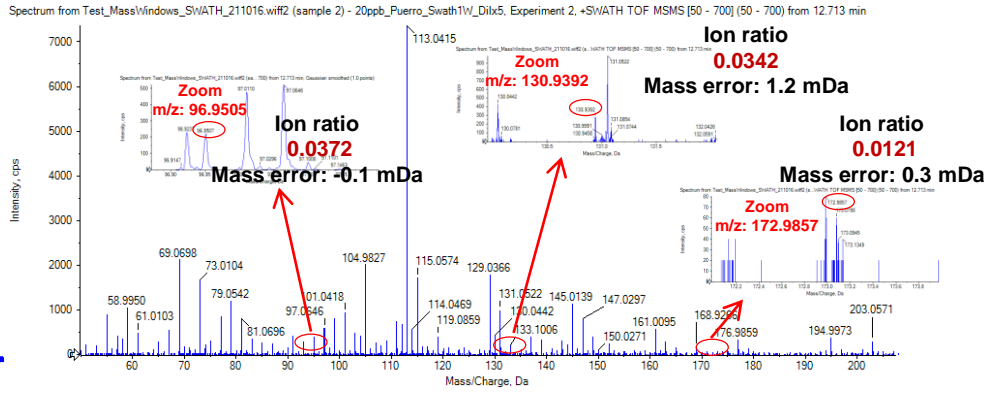
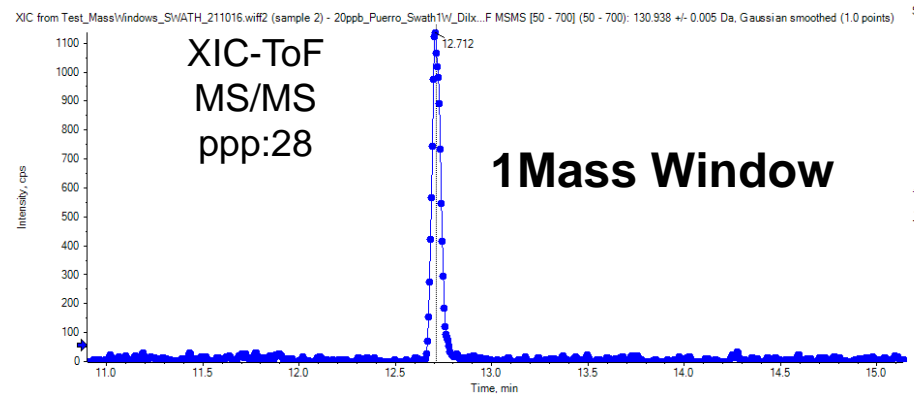
Product ions:

172.9854

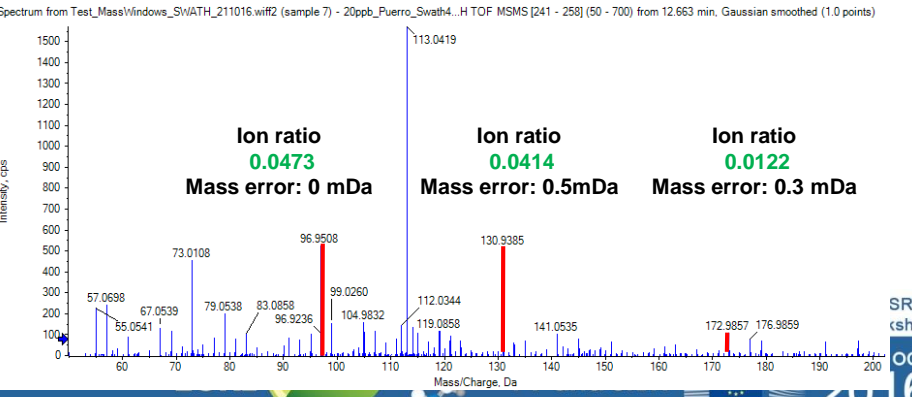
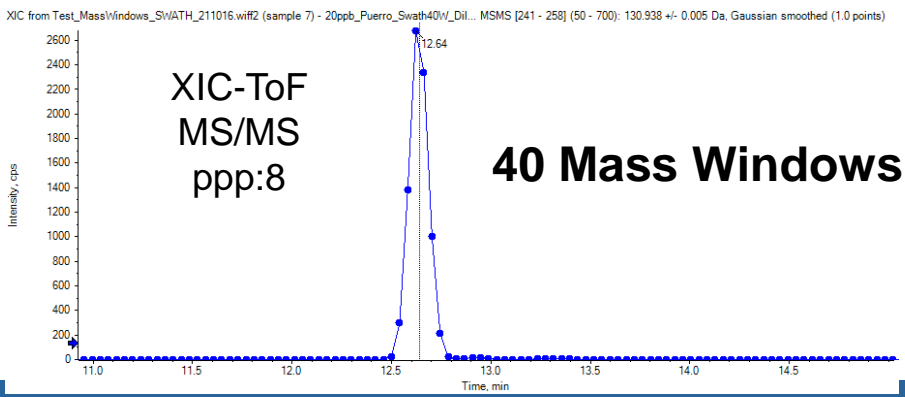
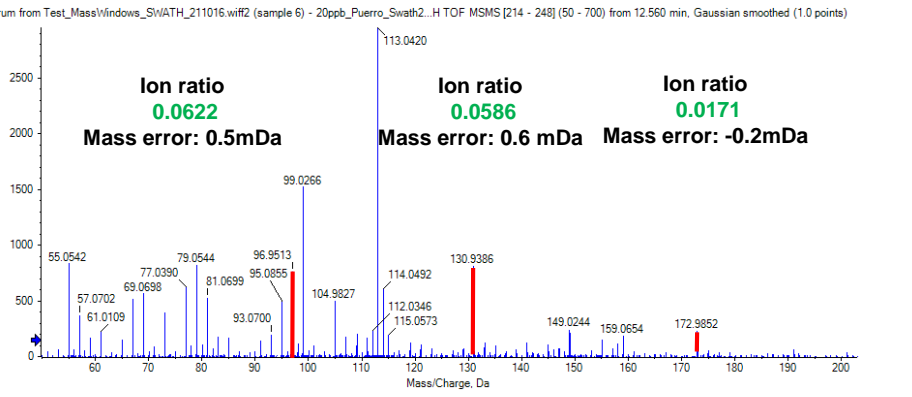
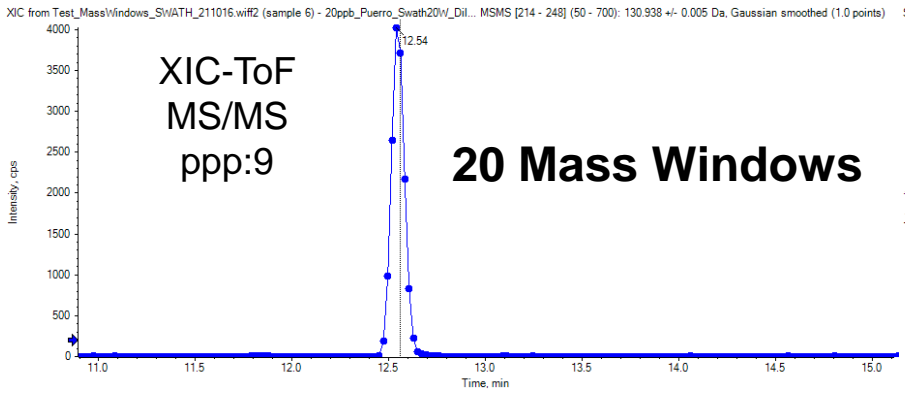
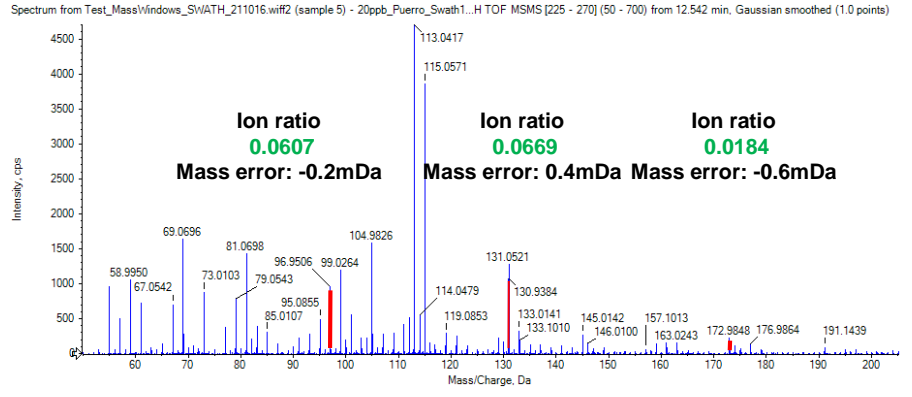
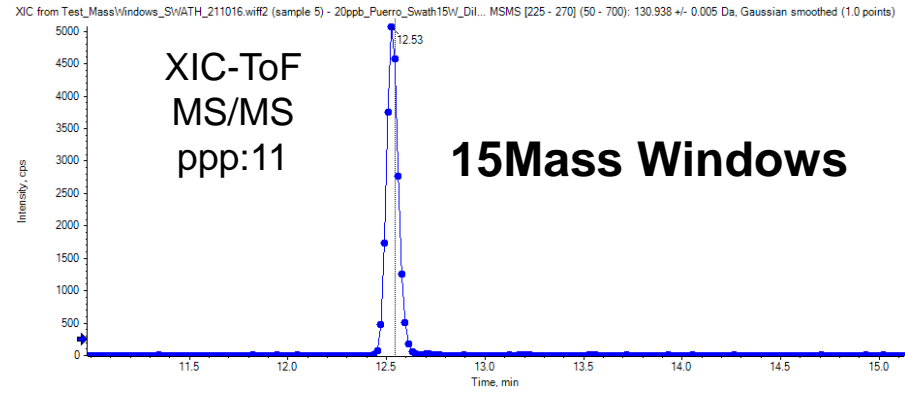
130.9380

96.9508

Ethoprophos 20µg/Kg dilx5 in Leek



Ethoprophos 20µg/Kg dilx5 in Leek



Ion Ratios FS-MSMS Ethoprophos 20µg/Kg dilx5 in Leek Non Target- Independent data acquisition

ion fragments	Mass Windows					
	1W	5W	10W	15W	20W	40W
130.9380	0.0342	0.2056	0.0661	0.0669	0.0586	0.0414
172.9854	0.0121	0.0631	0.0211	0.0184	0.0171	0.0122
96.9508	0.0372	0.1885	0.0542	0.0607	0.0622	0.0473

>35%
 <30%

Mass errors for ion fragments Ethoprophos 20µg/Kg dilx5 in Leek Non Target- Independent data acquisition

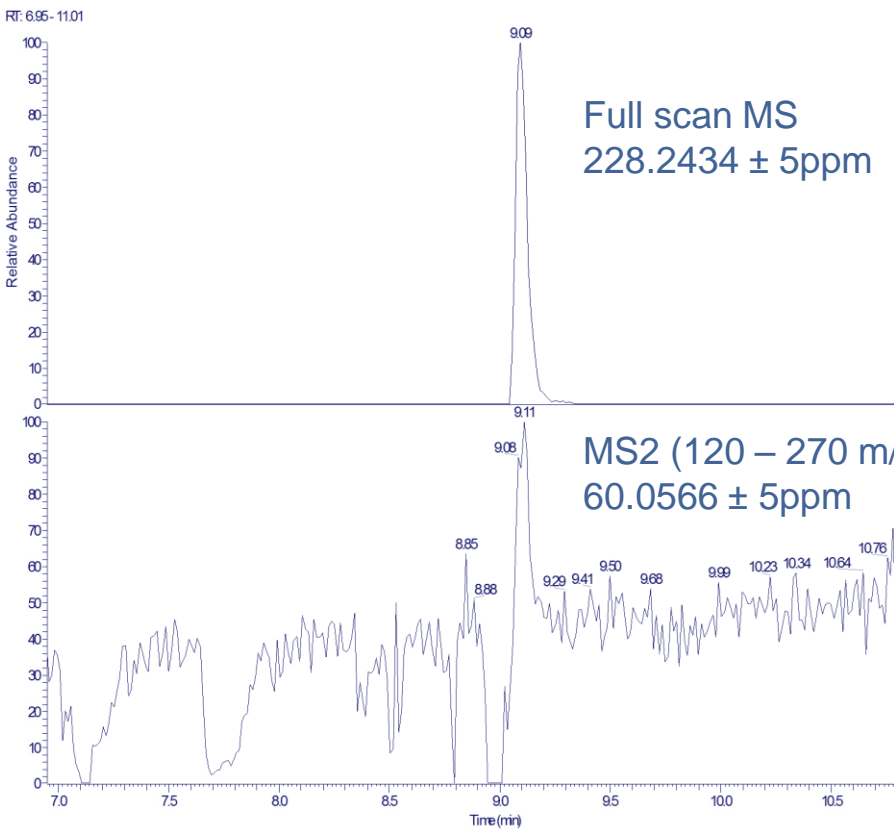
Exact Mass	Mass error (mDa)					
	1W	5W	10W	15W	20W	40W
172.9854	0.3	0.1	0.2	-0.6	-0.2	0.3
130.9380	1.2	0.4	0.4	0.4	0.6	0.5
96.9508	-0.1	-0.2	-0.1	-0.2	0.5	0.0



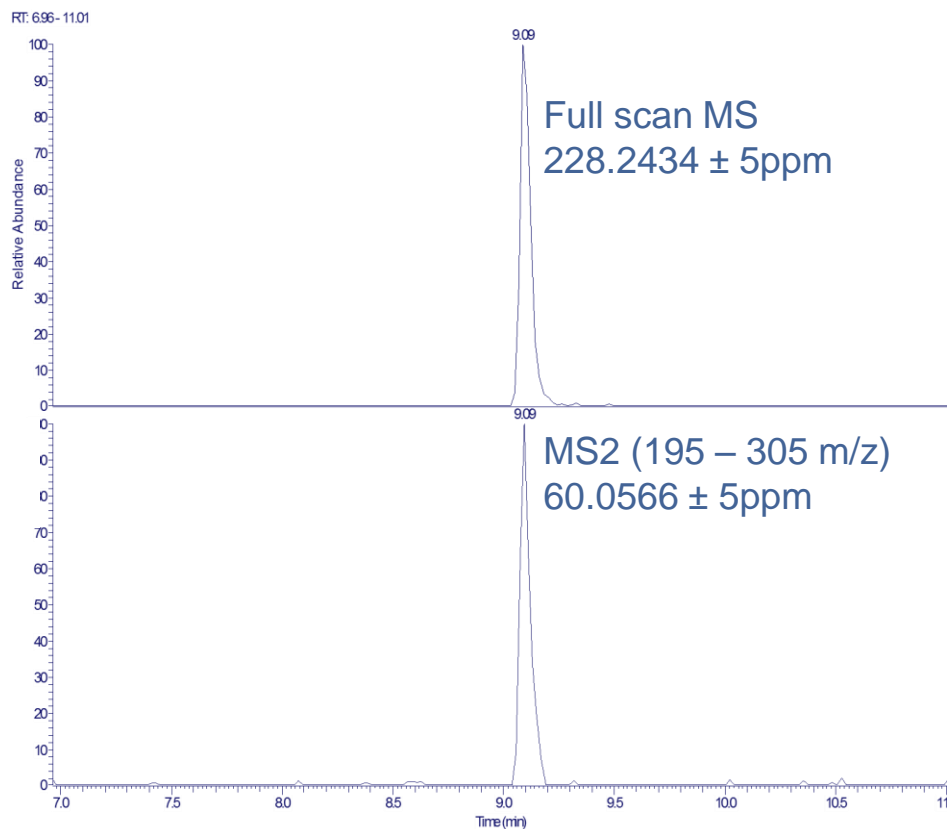
vDIA

causes of no identification

0.01 mg/kg of dodine in orange



3 mass segments



5 mass segments

Resolution 35,000

Conditions for Identification By using Target List (with retention time)

• **Table 4 in Document N° SANTE/2015/11945**. Identification criteria for different MS techniques

MS detector/ characteristics	Typical systems (examples)	Acquisition	Requirements for identification	
			minimum number of ions	other
Unit mass resolution	quadrupole, ion trap, TOF	full scan, limited m/z range, SIM	3 ions	
MS/MS	triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 product ions	<div style="border: 1px solid red; padding: 2px; display: inline-block;">$S/N \geq 3^e)$</div> Analyte peaks in the extracted ion chromatograms must fully overlap.
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	<div style="border: 1px solid red; padding: 2px; display: inline-block;">full scan</div> limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof	<div style="border: 1px solid red; padding: 2px; display: inline-block;">2 ions with mass accuracy $\leq 5 \text{ ppm}^{a,b,c)}$</div>	<div style="border: 1px solid red; padding: 2px; display: inline-block;">Ion ratio within $\pm 30\%$ (relative)</div> of average of calibration standards from same sequence
		<div style="border: 1px solid red; padding: 2px; display: inline-block;">combined</div> single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions: 1 molecular ion, (de)protonated molecule or adduct ion with mass acc. $\leq 5 \text{ ppm}^{a,c}$ plus <div style="border: 1px solid red; padding: 2px; display: inline-block;">1 MS/MS product ion^{d)}</div>	

a) preferably including the molecular ion, (de)protonated molecule or adduct ion

b) including at least one fragment ion

c) $< 1 \text{ mDa}$ for $m/z < 200$

d) **no specific requirement for mass accuracy**

e) in case noise is absent, a signal should be present in at least 5 subsequent scans

FDA Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data

Table 1. Summarized Requirements for Confirmation of Identity

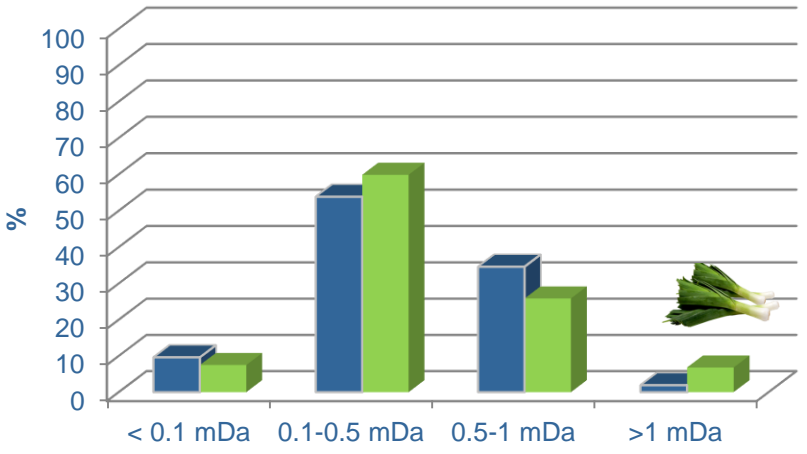
MS mode	MS ¹	MS/MS	MS ¹ and MS/MS
EIC: signal requirement (absolute)	A criterion to be set by one of the following methods: (1) a S/N threshold ≥ 3 ; (2) an intensity ratio relative to the comparison standard equal or above a preset threshold		
EIC: retention time, relative to comparison standard	A criterion to be set by one of the following methods: (1) ≤ 0.2 min, or (2) within $\pm 2.5\%$, not to exceed 0.5 min, or (3) within an established error range, not to exceed 0.5 min		
MS: number of structurally significant ions	Minimum 2	Minimum 2	Minimum 2 combined
MS: mass accuracy	≤ 5 ppm	≤ 10 ppm	MS ¹ : ≤ 5 ppm; MS/MS: ≤ 10 ppm

LC-QToF
X500R

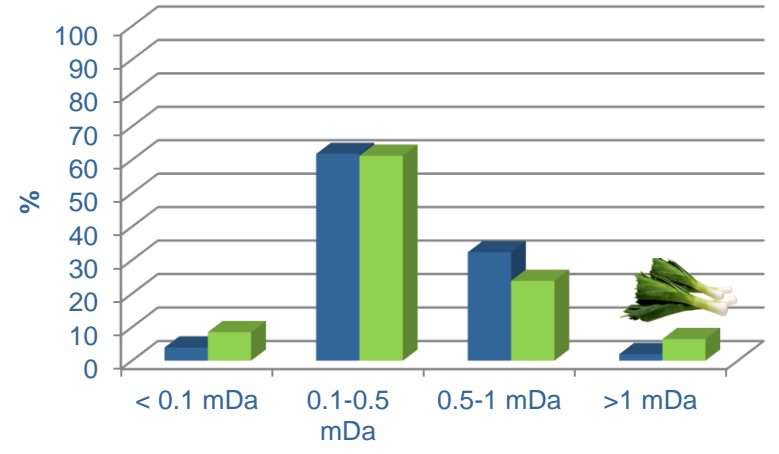
Mass Error

Non Target-Data Independent Acquisition SWATH 10 windows

Full Scan



MS²



Matrices:



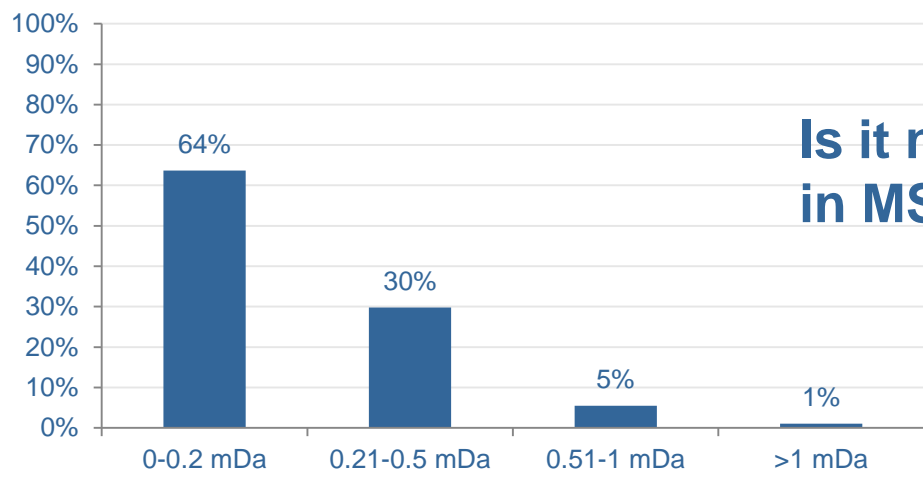
at 0.01, 0.02 and 0.05 mg/kg

Solvent: at 0.01, 0.02 and 0.05 mg/kg

LC-Orbitrap QExactive Focus

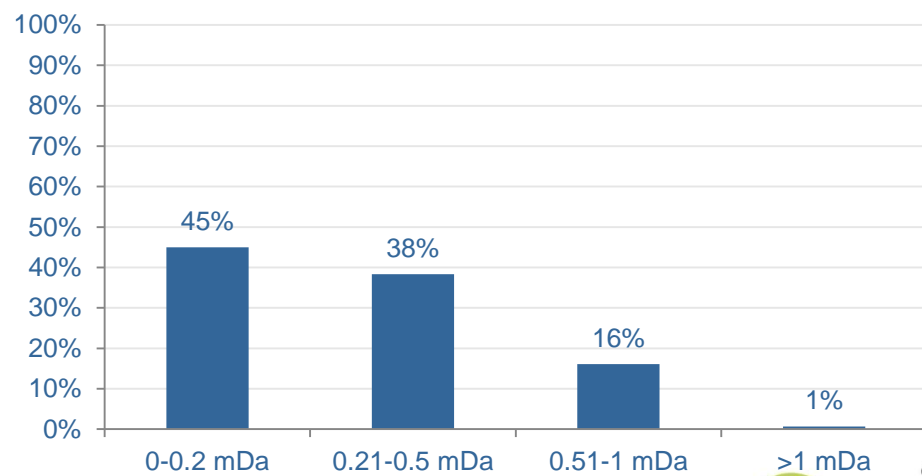


Errors in full scan MS (n = 7444)



Is it necessary 10 ppm
in MS²?

Errors in dd-MS² (n = 30623)



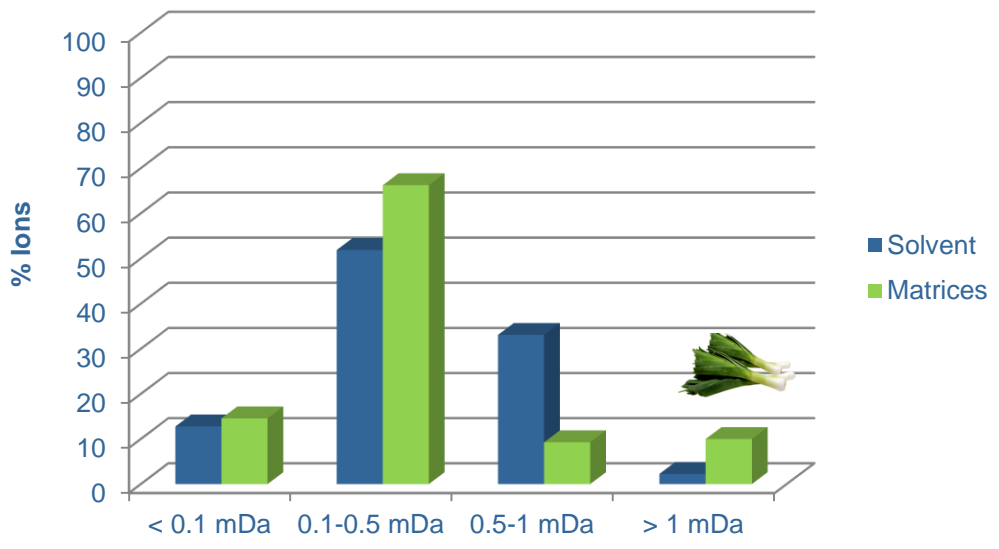
LC-QToF
6550

Mass Error

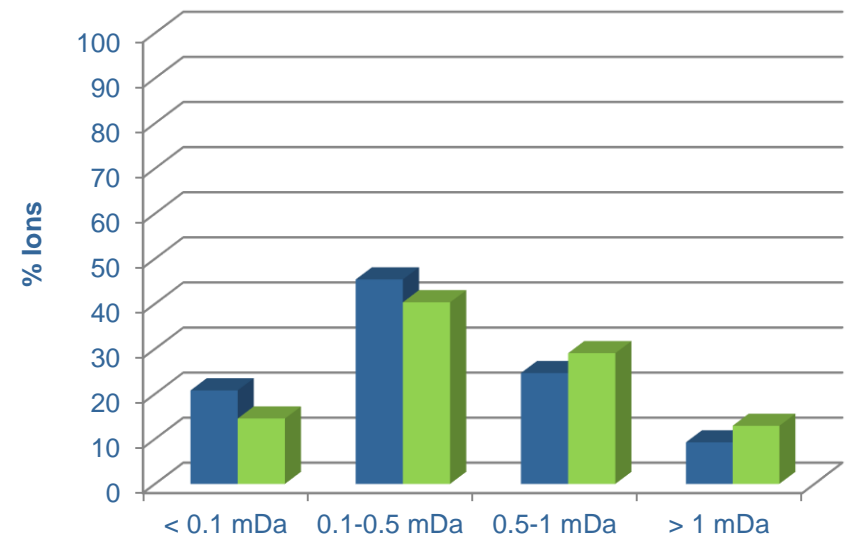
Non Target-Data Independent Acquisition

All Ion

Full Scan



MS²



Matrices:



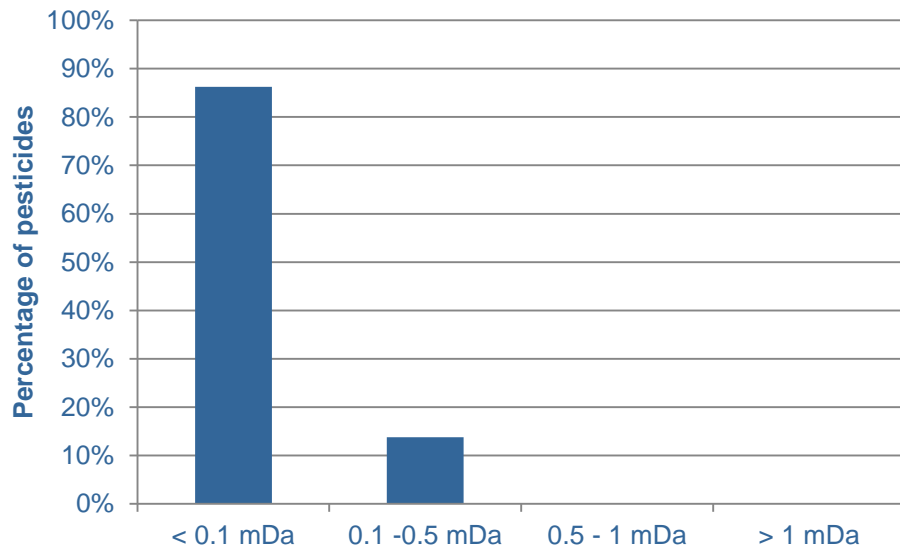
Solvent: at 0.01, 0.02 and 0.05 mg/kg

at 0.01, 0.02 and 0.05 mg/kg

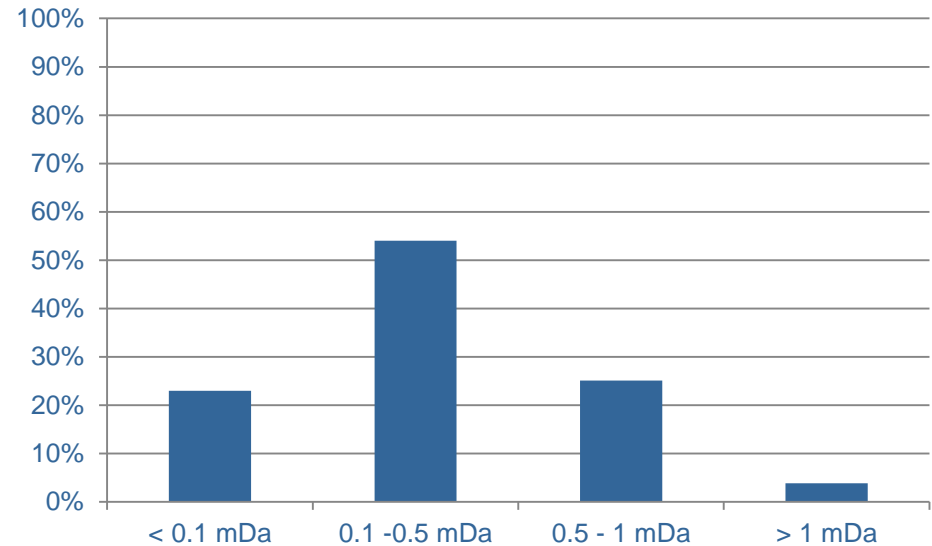
LC-Orbitrap QExactive Focus

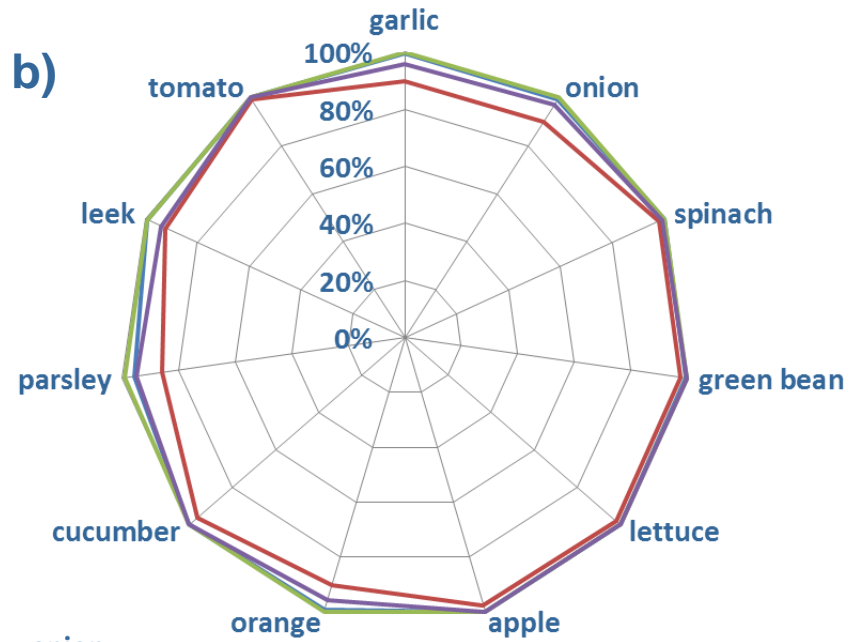
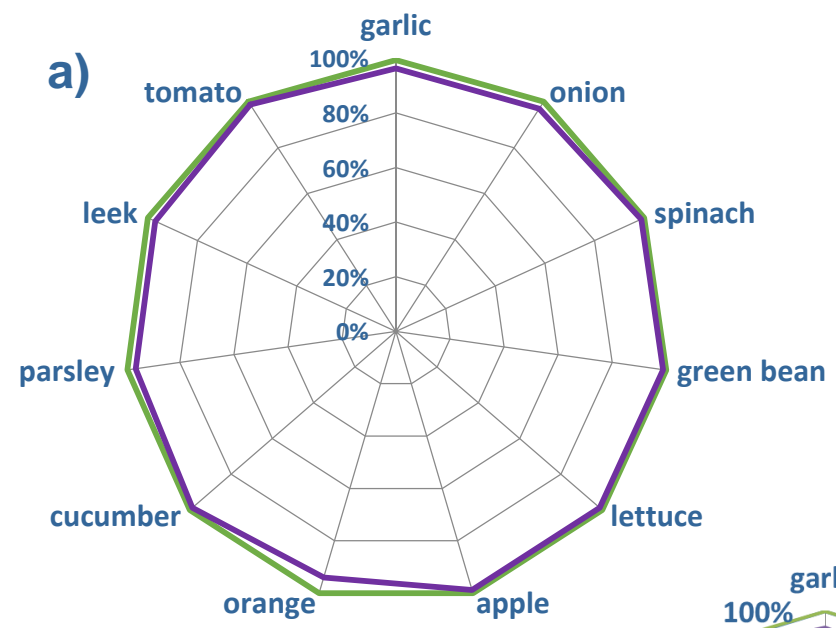
Mass Error Target-Data Dependent Acquisition

Full scan



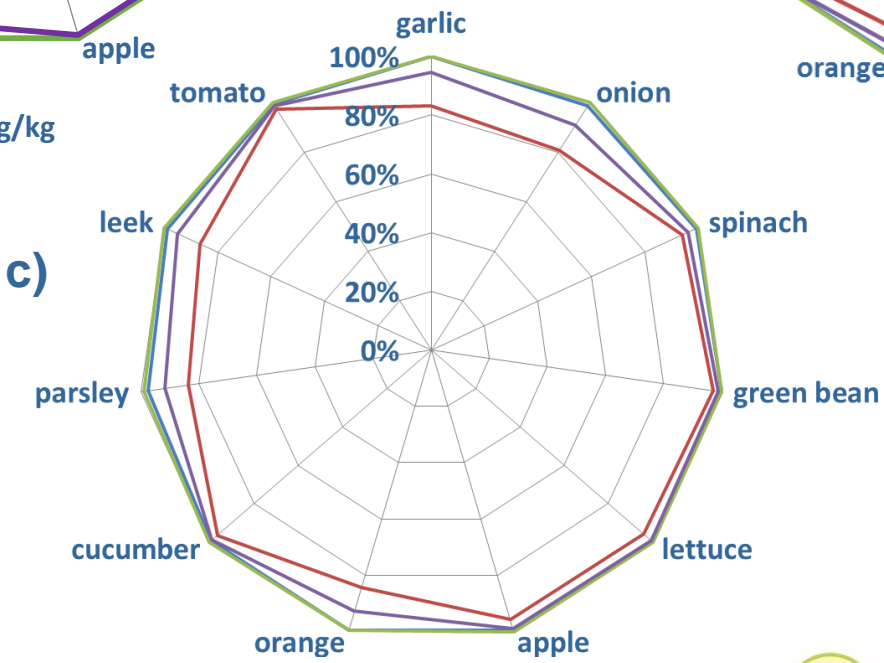
MS²





— 0.1 mg/kg — 0.01 mg/kg
Mass error (two ions)

— 0.1 mg/kg Ion ratio
— 0.01 mg/kg Ion ratio
— 0.1 mg/kg Mass error (two ions)
— 0.01 mg/kg Mass error (two ions)



Scope: 166 compounds

a) dd-MS²
b) vDIA
c) AIF



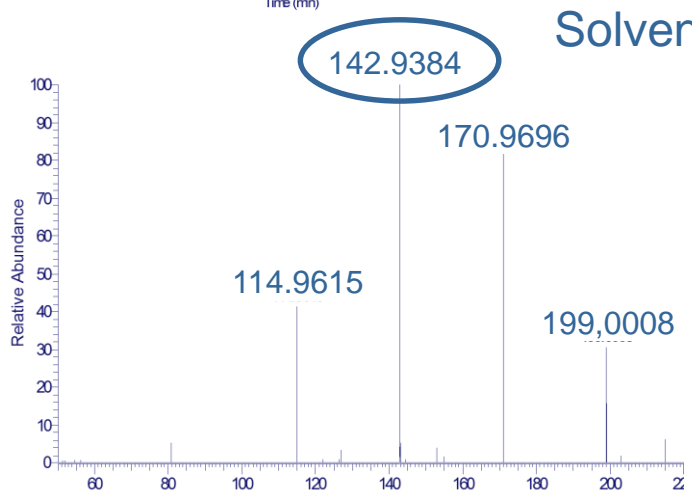
10,66

dd-MS²

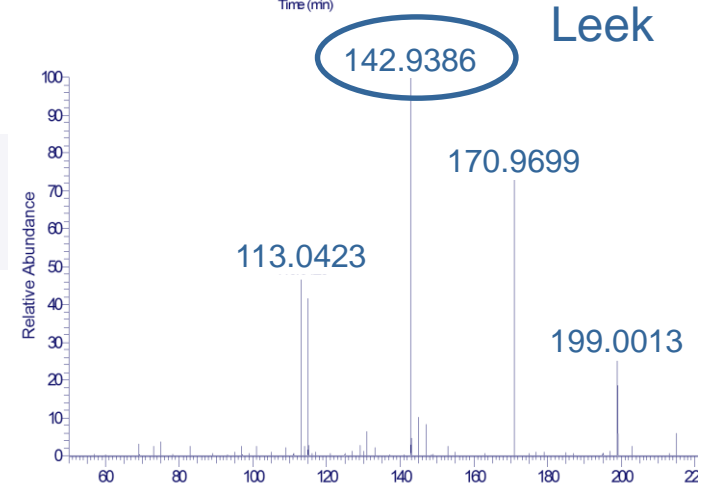
(two fragment ions with mass error < 5 ppm)

Ethion
Full scan
384.9949 ± 5 ppm

10,66

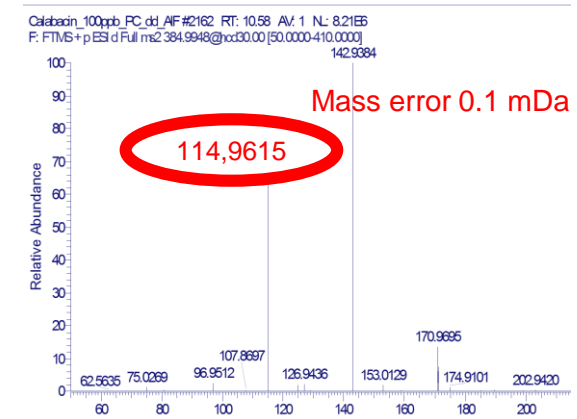
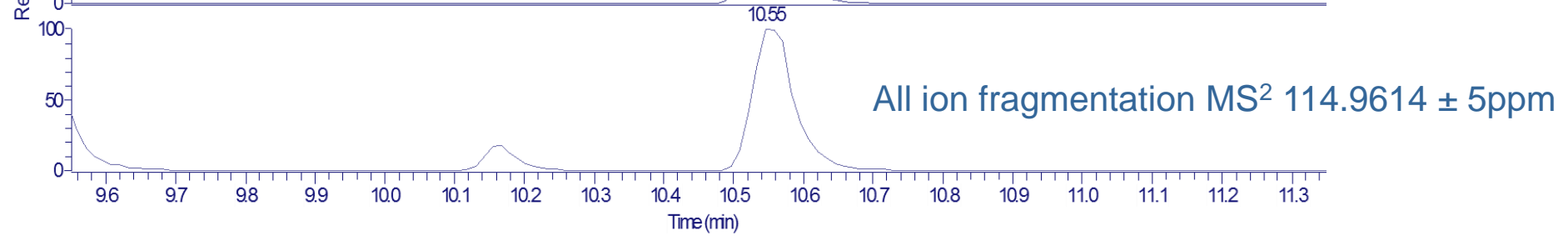
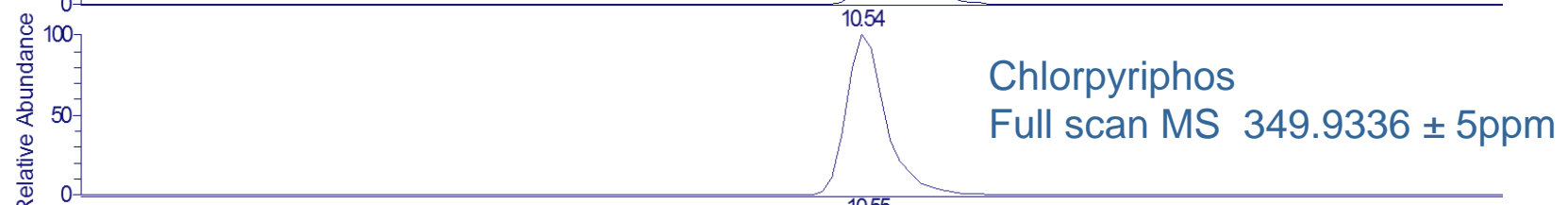
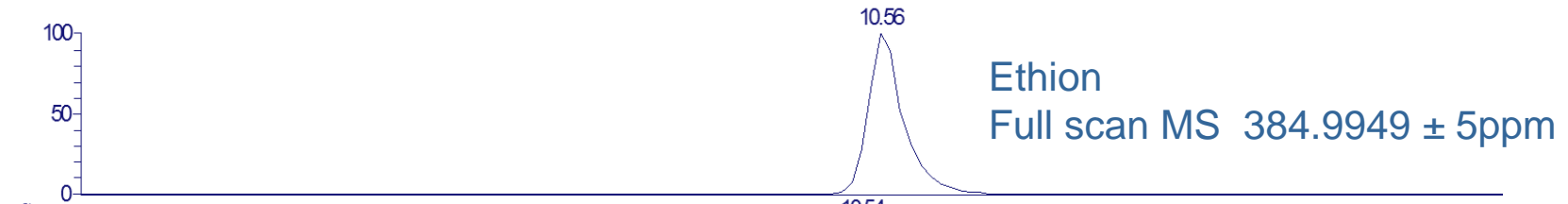


dd-MS²
384.9949 ± 0.5 Da

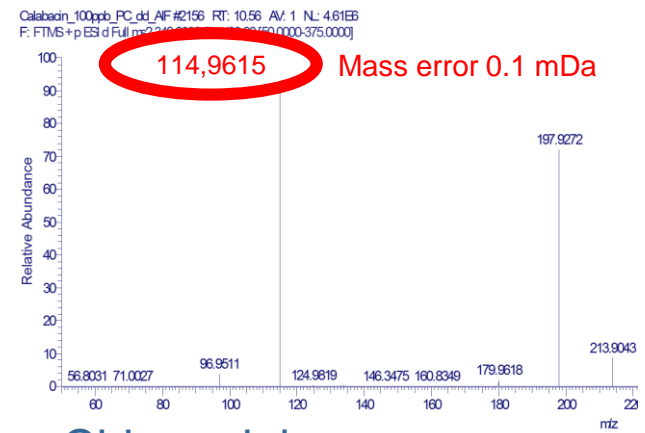


Fragment ion	Abundance in solvent	Abundance in leek	Mass error (mDa)
114.9615	39%	43% ✓	0.3
142.9386	100%	100%	0.2
170.9699	82%	74% ✓	0.3
199.0013	29%	26% ✓	0.5

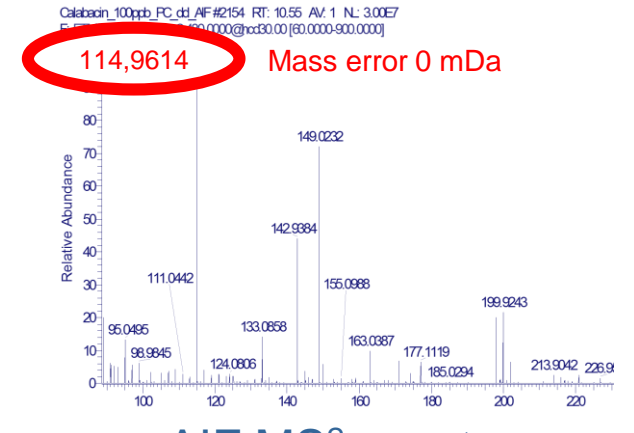
RT: 9.55 - 11.36



Ethion
dd-MS² spectrum



Chlorpyrifos
dd-MS² spectrum



AIF MS² spectrum

Joint EURL/NRLs (FV-SRM)
Pesticide Residue Workshop

TOF

TARGET ANALYSIS (DATA DEPENDENT ACQUISITION)





NON TARGET MS² (DATA INDEPENDENT ACQUISITION)



NON TARGET MS² DIA-VMW



 Full scan MS
 MS²



Orbitrap

- Full scan MS
- MS²

FS/dd-MS²



Target MS² analysis.

Fragmentation is carried out only if precursor ion is detected in full scan MS. List of precursor ions is necessary.

FS/AIF-MS²



Non-target MS² analysis.

Fragmentation is carried out during entire chromatographic run. No precursor selection. No list of precursor ions is present.

FS/vDIA

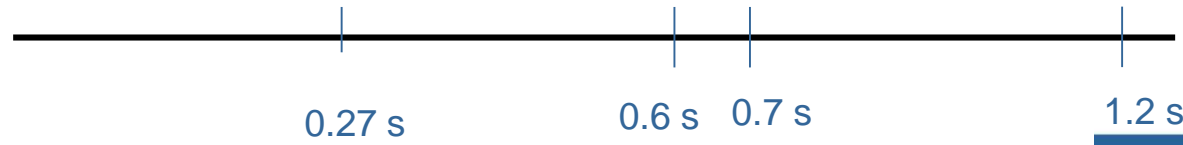


FS/ AIF-MS² / dd-MS²



Combined target and non-target MS² analysis.

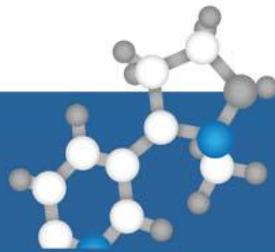
Fragmentation without precursor selection is carried out during entire chromatographic run. List of target precursor ions is present. Fragmentation with precursor selection is carried out only if a precursor ion from the list is detected in full scan MS.



CONCLUSIONS

- GC-HRAMS with Electron ionization source only full scan (no MS^2) was necessary for a correct identification and quantitation of the compounds.
- In case of LC-ESI-HRAMS various MS^2 workflows can be applied depending of the analyzer type (TOF, orbitrap). They can that affect the analytical results especially with difficult matrices and low concentration levels.
- The specific instrument performance for MS^2 for target, non target or a combination of both modes determines their optimum selection for routine analysis.
- It is important to consider how these capabilities and modes can affect to ensure, in practice, the compliance with identification criteria established in the EU SANTE Guideline.

EURL



FV and SRM



25th-26th OCT
2016

European Union Reference Laboratories for Residues of Pesticides

**Thank You
for
Your Attention**



**Ten Years of
Collaboration,
Assistance and
Research**