



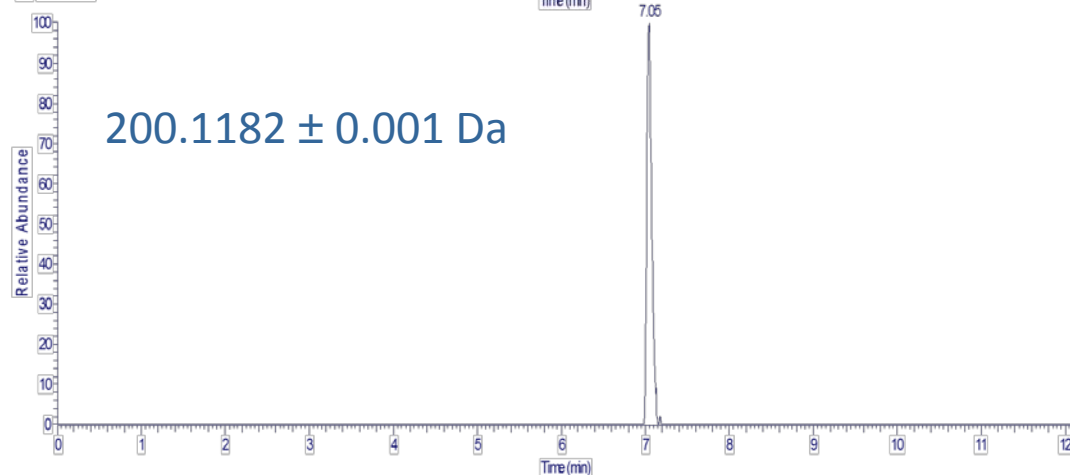
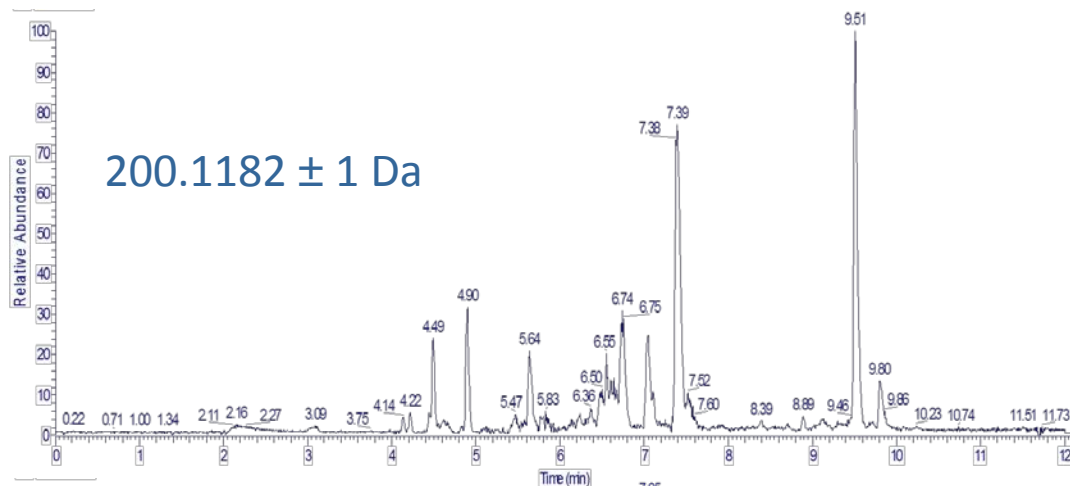
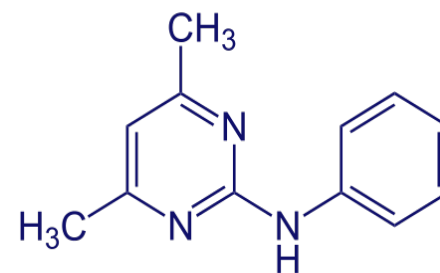
53rd Annual North American Chemical Residue Workshop

EVALUATION OF SIMULTANEOUS MS AND MS2 WORKFLOWS OF GC-LC-HRAMS FOR ANALYSIS OF PESTICIDES IN FRUITS AND VEGETABLES

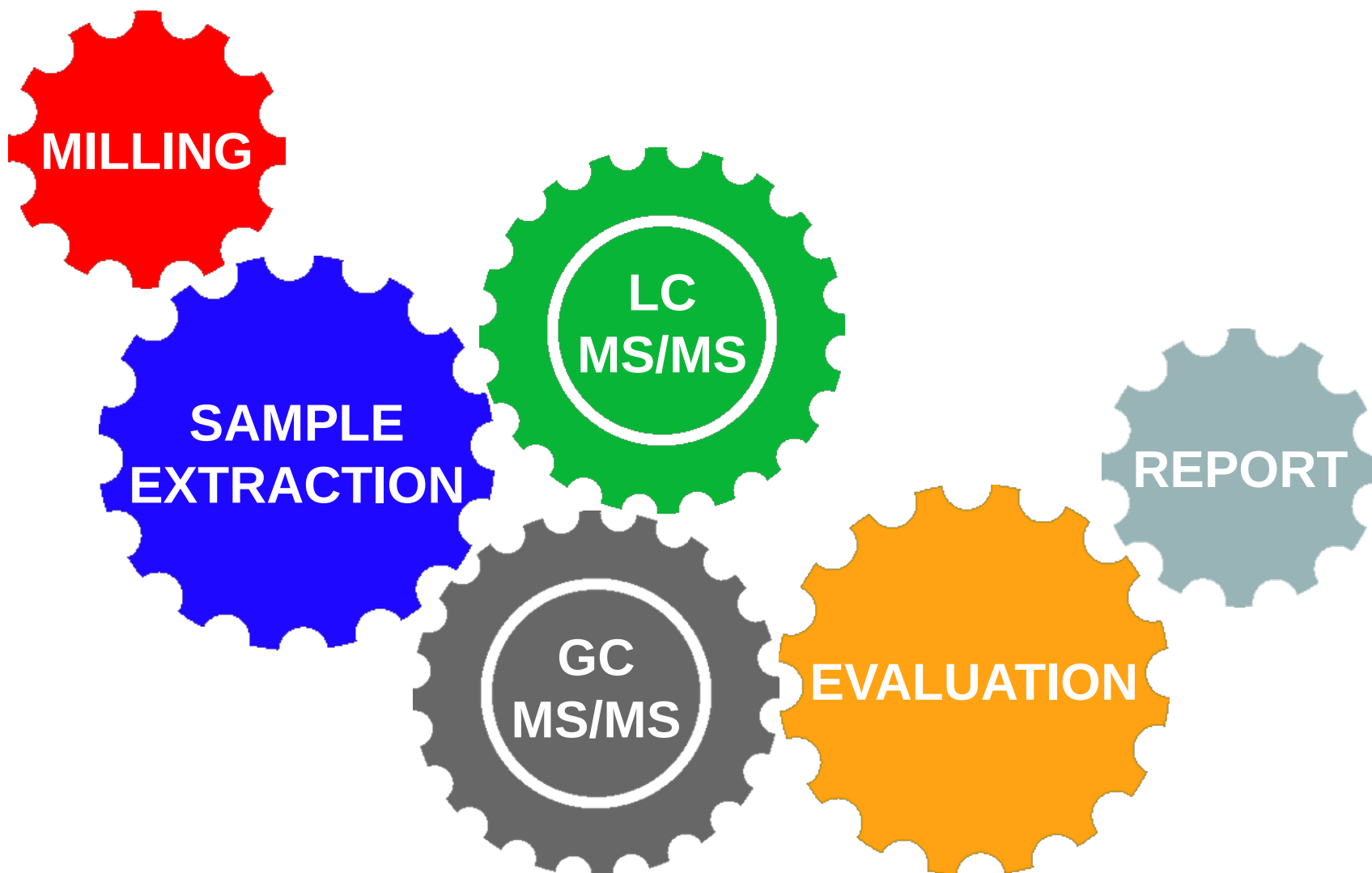
18th July 2016



Pyrimethanil at 0.01 mg/kg



Pyrimethanil
 [M+H⁺] m/z 200.1182
 0.01 mg/ kg in onion
 RT = 7.05 min
 Dilution 5x



Low second transition at 0.01 mg/kg in tomato

LC-QqQ, n=185 (Ratio SRM2/SRM1)		CE (V)
1.	Acephate (10 %)	15
2.	Carbendazim (4 %)	20
3.	Clomazone (2 %)	10
4.	Cyazofamid (3 %)	10
5.	Dichlorvos (14 %)	30
6.	Diniconazole (12 %)	28
7.	Fipronil (12 %)	28
8.	Fludioxonil (12 %)	5
9.	Hexaconazole (10 %)	30
10.	Isofenphos methyl (11 %)	15
11.	Isoxaflutole (8 %)	50
12.	Mepanipyrim (10 %)	20
13.	Metconazole (7 %)	48
14.	Paclobutrazol (6 %)	36
15.	Pencycuron (9 %)	60
16.	Spinosyn A (2 %)	20
17.	Spinosyn D (1 %)	20
18.	Tebuconazole (5 %)	20
19.	Thiacloprid (6 %)	10
20.	Thiobencarb (7 %)	10
21.	Triadimenol (4 %)	5
22.	Triazophos (8 %)	10
23.	Triticonazole (5 %)	41

GC-QqQ, n=210 (Ratio SRM2/SRM1)		CE (V)
1.	2,4'-DDE (12 %)	20
2.	4,4'-DDE (12 %)	20
3.	Bifenthrin (5 %)	40
4.	Bromopropylate (8 %)	20
5.	Cadusafos (11 %)	10
6.	Chlozolinate (11 %)	5
7.	Cypermethrin (11 %)	20
8.	Ethoxyquin (14 %)	30
9.	Fenprovidin (10 %)	3
10.	Heptachlor (10 %)	40
11.	Heptenophos (3 %)	10
12.	Isocarbophos (7 %)	8
13.	Isofenphos-methyl (9 %)	10
14.	Paclobutrazole (13 %)	20
15.	Sebumeton (10 %)	5
16.	Tetrachlorvinphos (14 %)	35

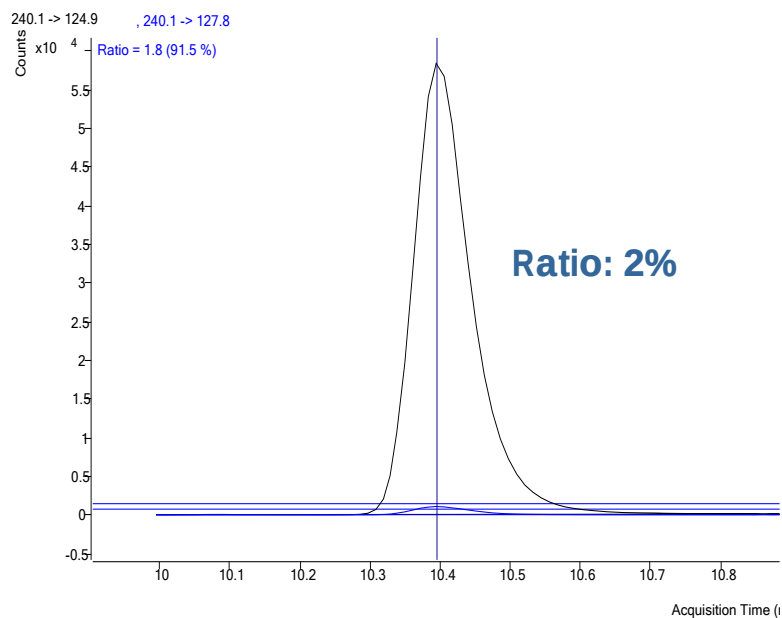
In red, low collision energies

In green, high collision energies

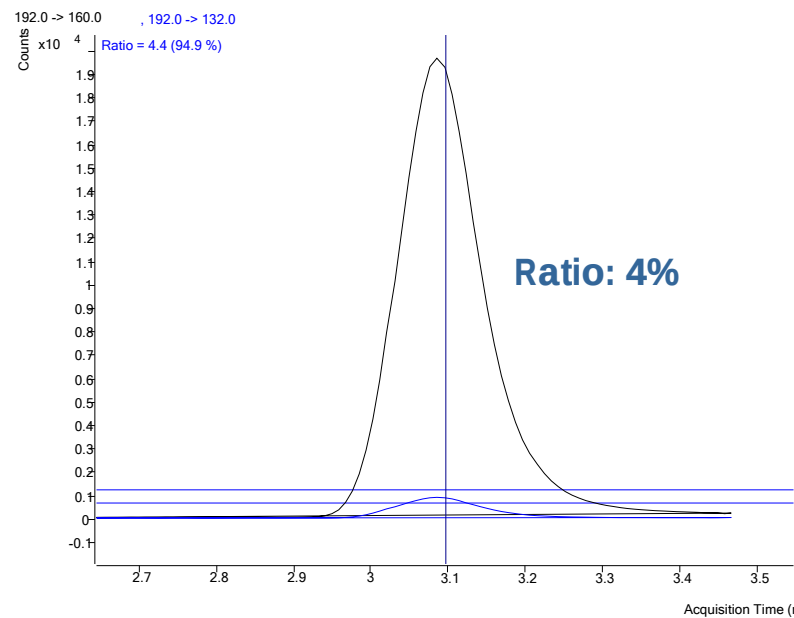
Transitions at 0.05 mg/kg in tomato LC-QQQ-MS/MS



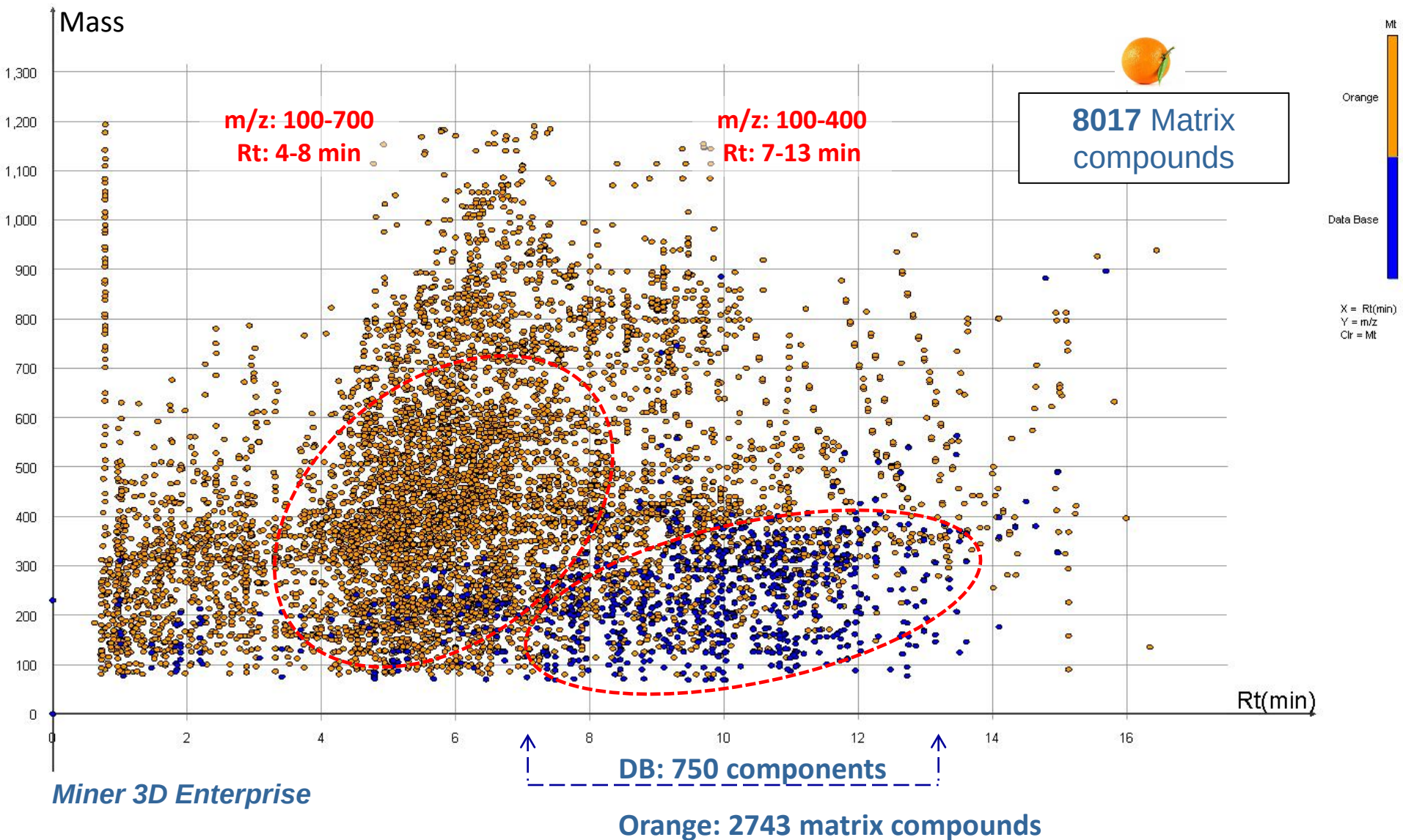
Clomazone



Carbendazim



Data base components- orange matrix compounds (1 g/mL)



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



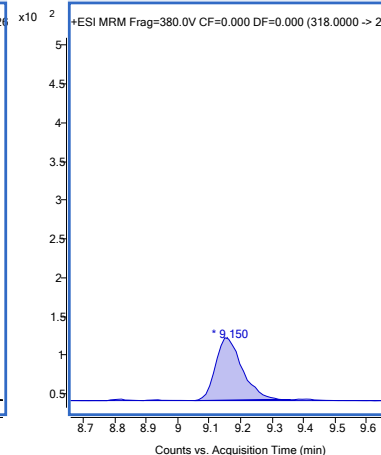
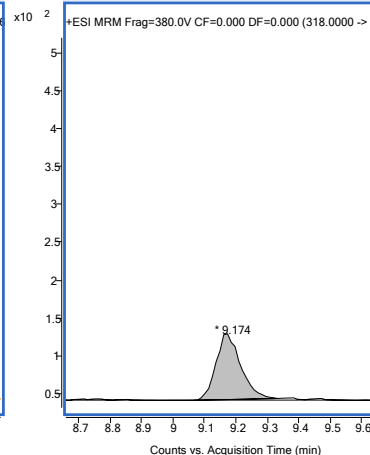
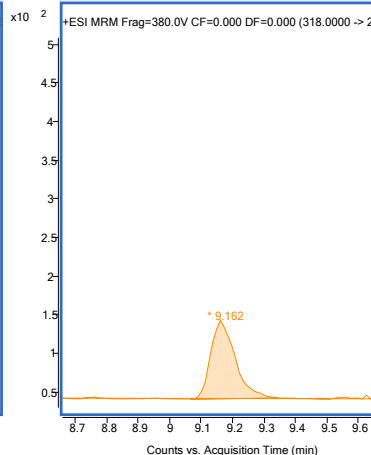
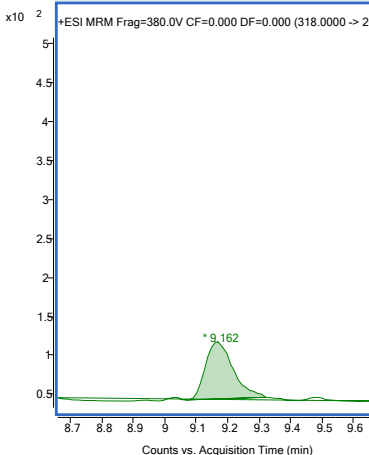
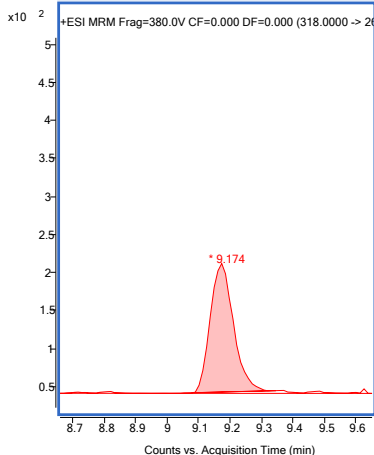
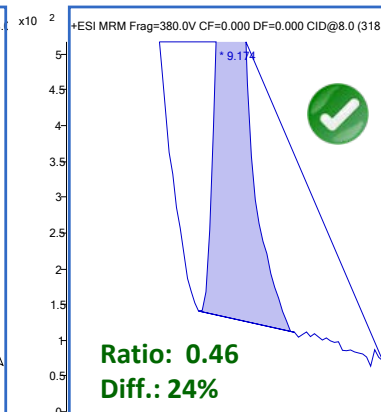
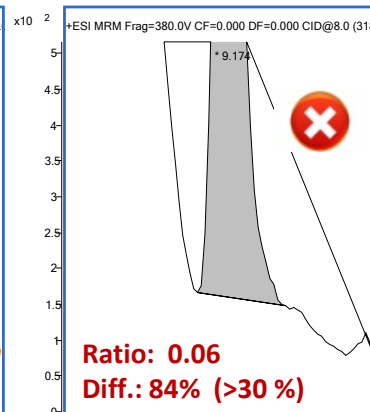
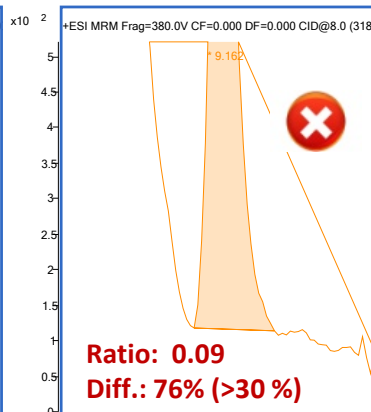
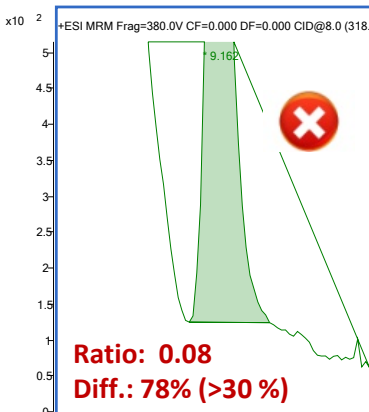
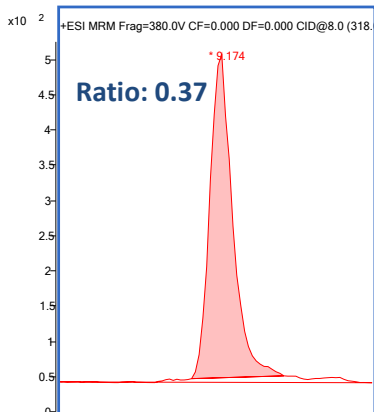
Std in solvent at
10 µg/L

Ethyl Acetate
method

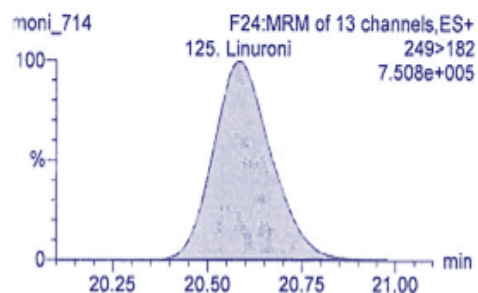
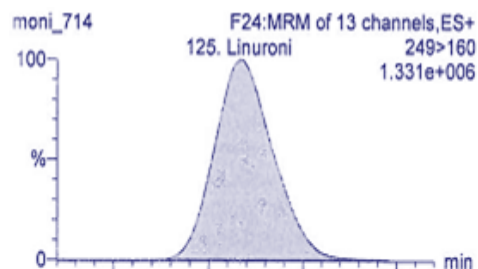
NL method

Citrate QuEChERS without
clean-up method

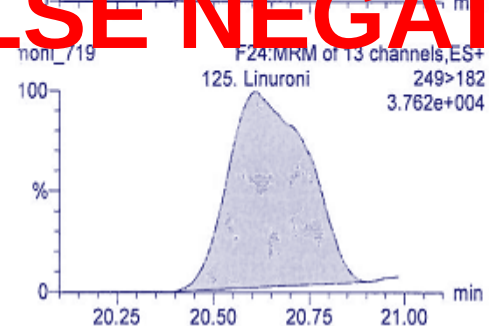
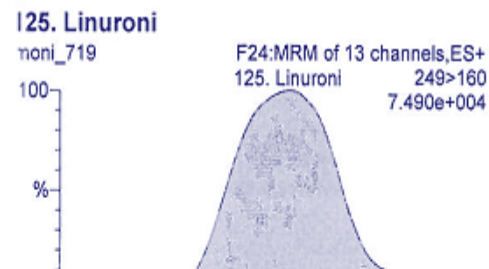
Citrate QuEChERS
method



EUPT Sample: coriander



Linuron
Standard in solvent
Ion ratio: 1.8

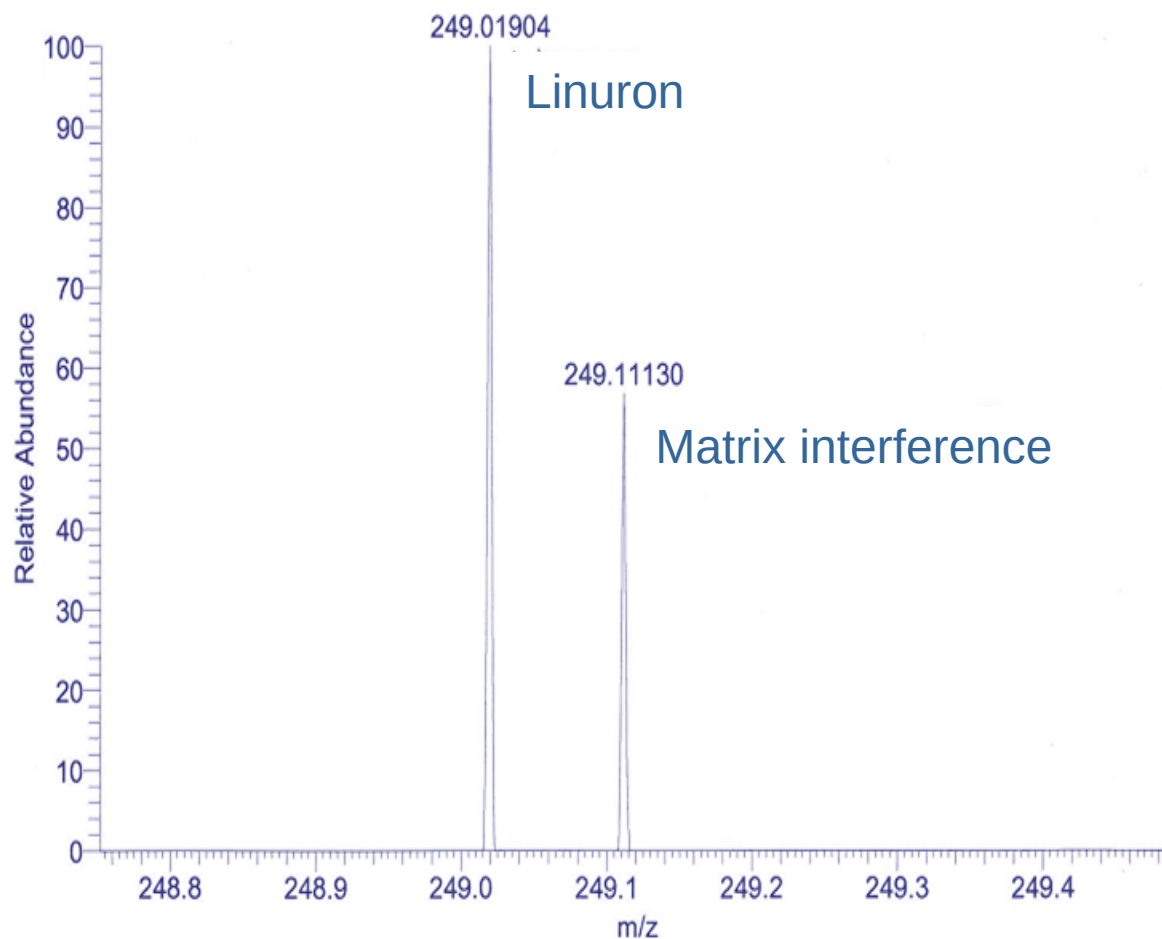


Linuron (0.125 mg/kg)
Real sample of coriander
Ion ratio: 2.4

FALSE NEGATIVE

EUPT Sample of coriander

LC-HRAMS

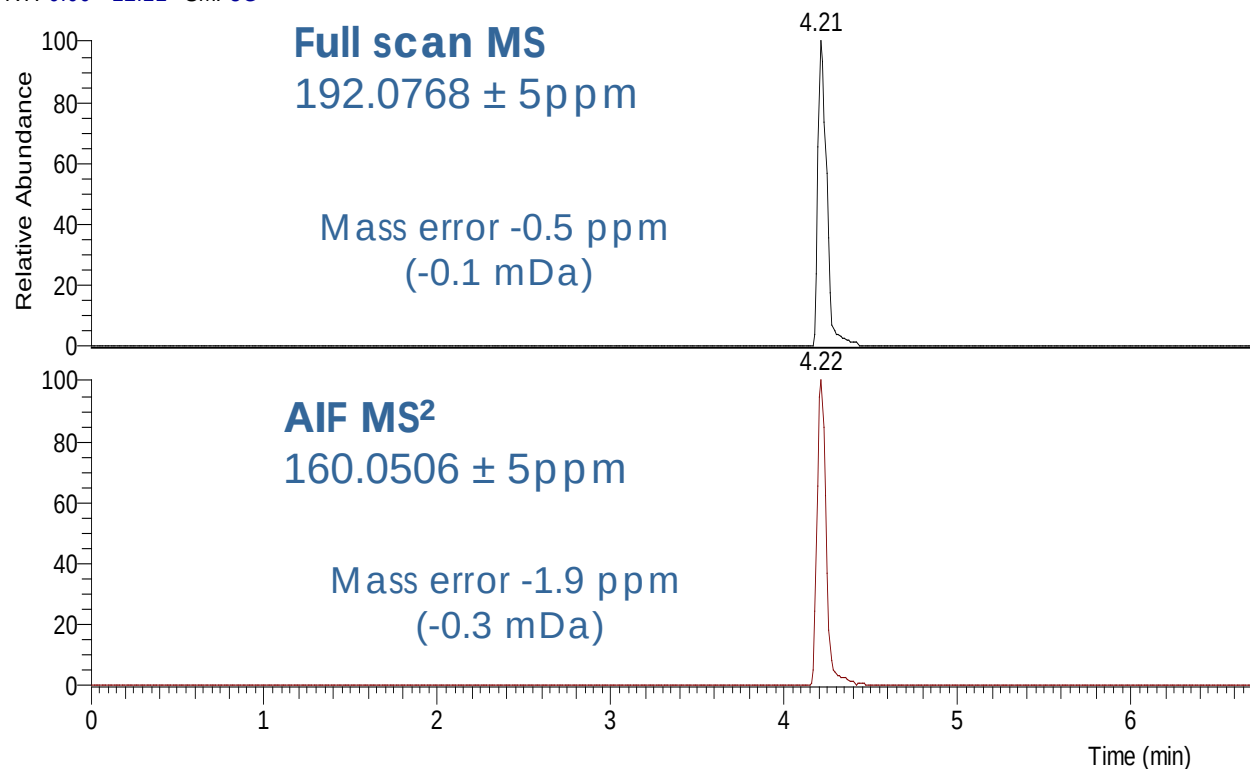




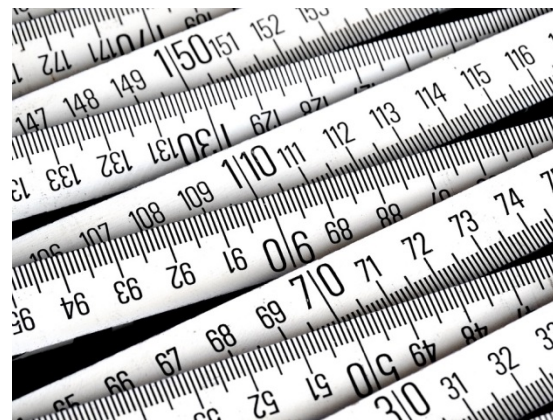
LC-Orbitrap

Carbendazim, 0.01 mg/kg in Tomato

RT: 0.00 - 12.11 SM: 3G

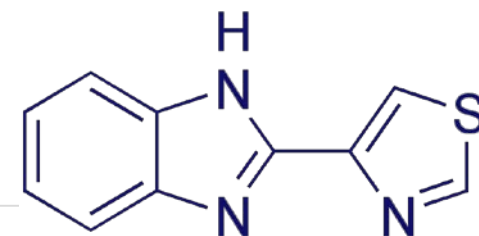


MASS ACCURACY



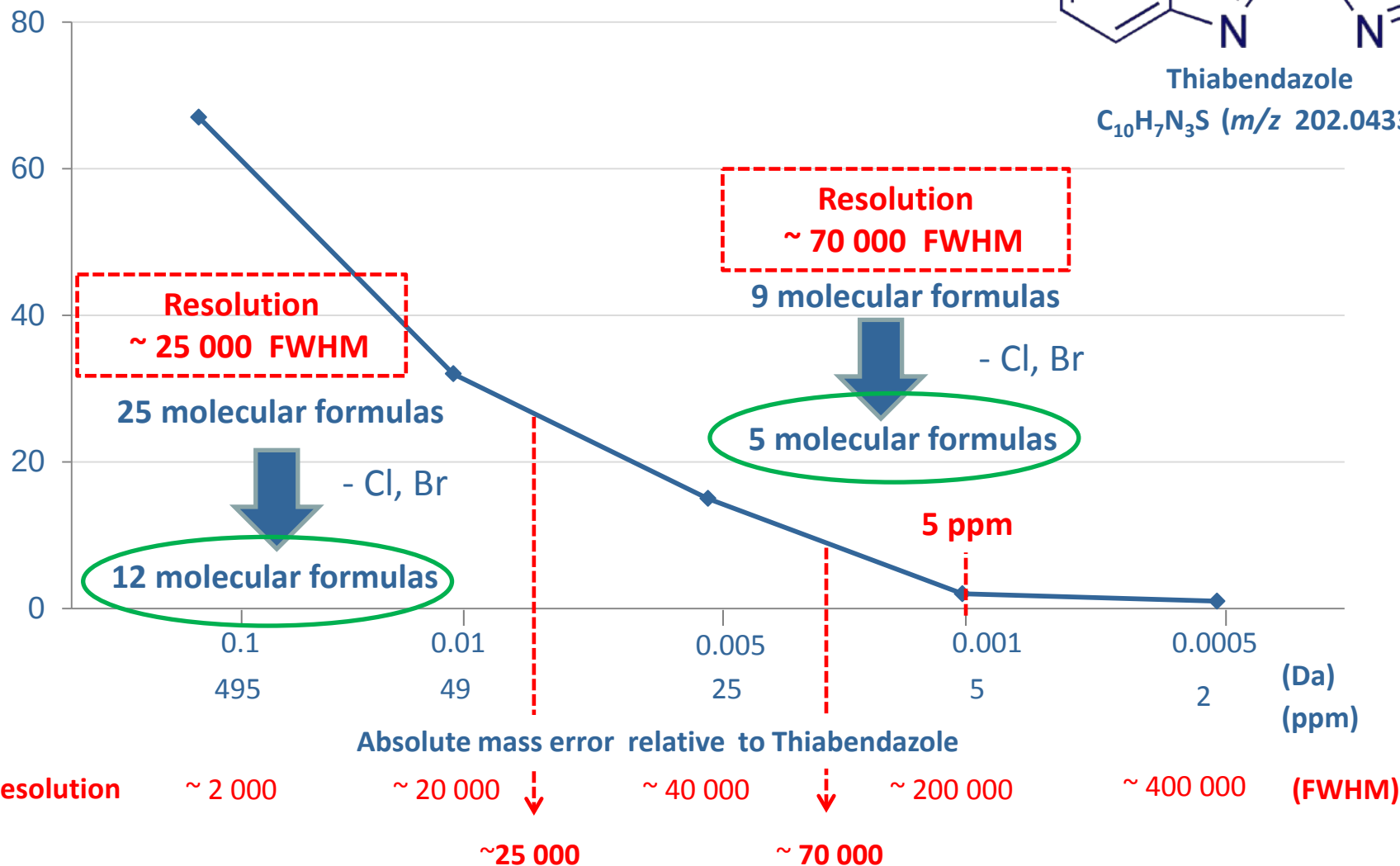
RESOLUTION





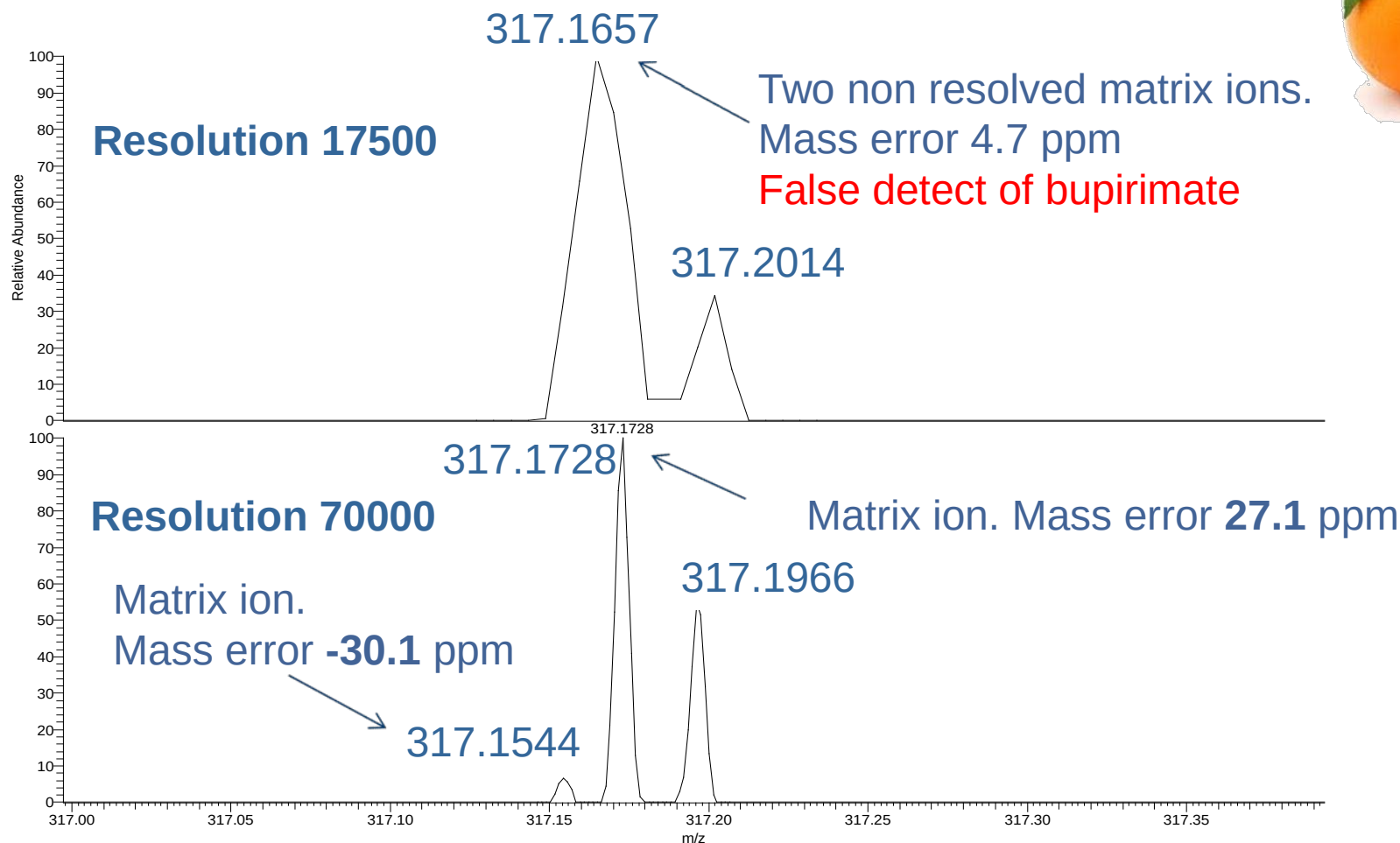
Thiabendazole
 $C_{10}H_7N_3S$ (m/z 202.0433)

Number of possible molecular formulas



Influence of resolution on detection

Exact mass of bupirimate: 317.1642



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	S/N $\geq 3^e)$ lyte peaks in the extracted ion chromatograms must fully overlap. on ratio within 30% (relative) of average of calibration standards from same sequence
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	
Accurate mass measurement	High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS	full scan, limited m/z range fragmentation with or without precursor-ion selection, or combinations thereof combined single stage MS and MS/MS with mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 ions with mass accuracy ≤ 5 ppm^{a,b,c)} 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)}	

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

^{b)} including at least one fragment ion

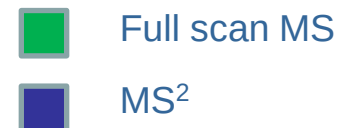
^{c)} < 1 mDa for m/z < 200

^{d)} no specific requirement for mass accuracy

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

Combining Full Scan MS and MS² Mode

Orbitrap



FS/dd-MS²



Target MS² analysis.

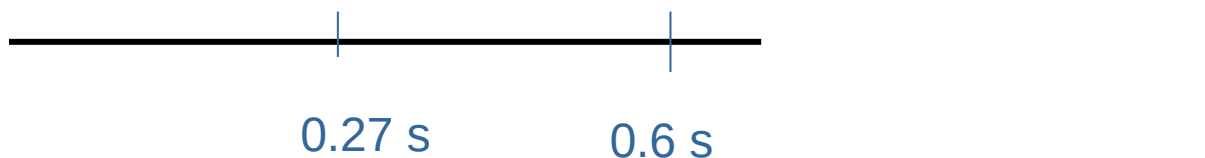
Fragmentation is carried out only if precursor ion is detected in full scan MS. List of precursor ions is necessary.

FS/AIF-MS²



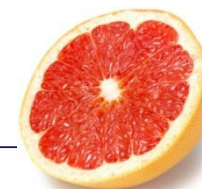
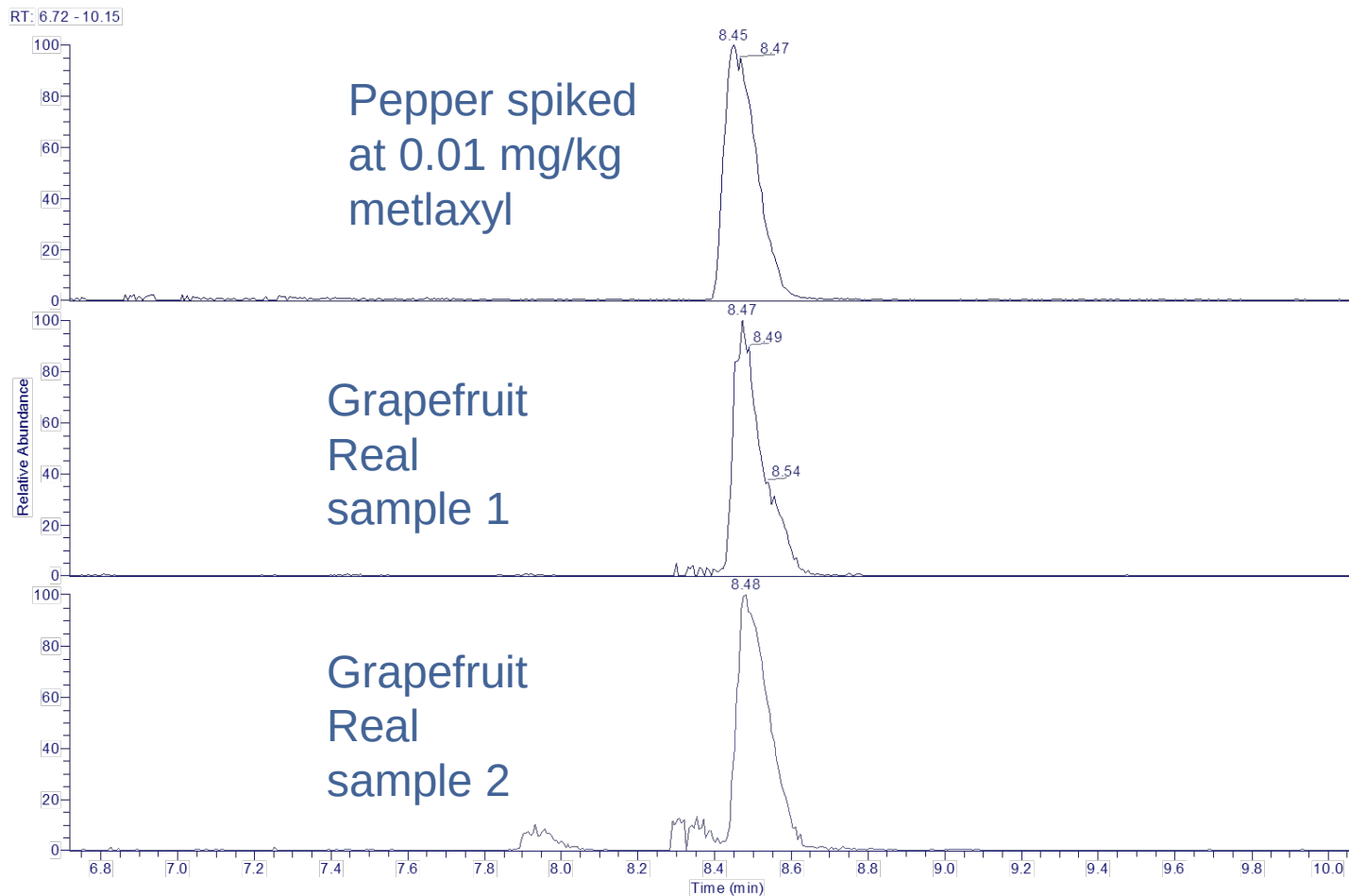
Non-target MS² analysis.

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



Analysis of real samples by LC-HRAMS MS²

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



LC-QOrbitrap (QExactive)

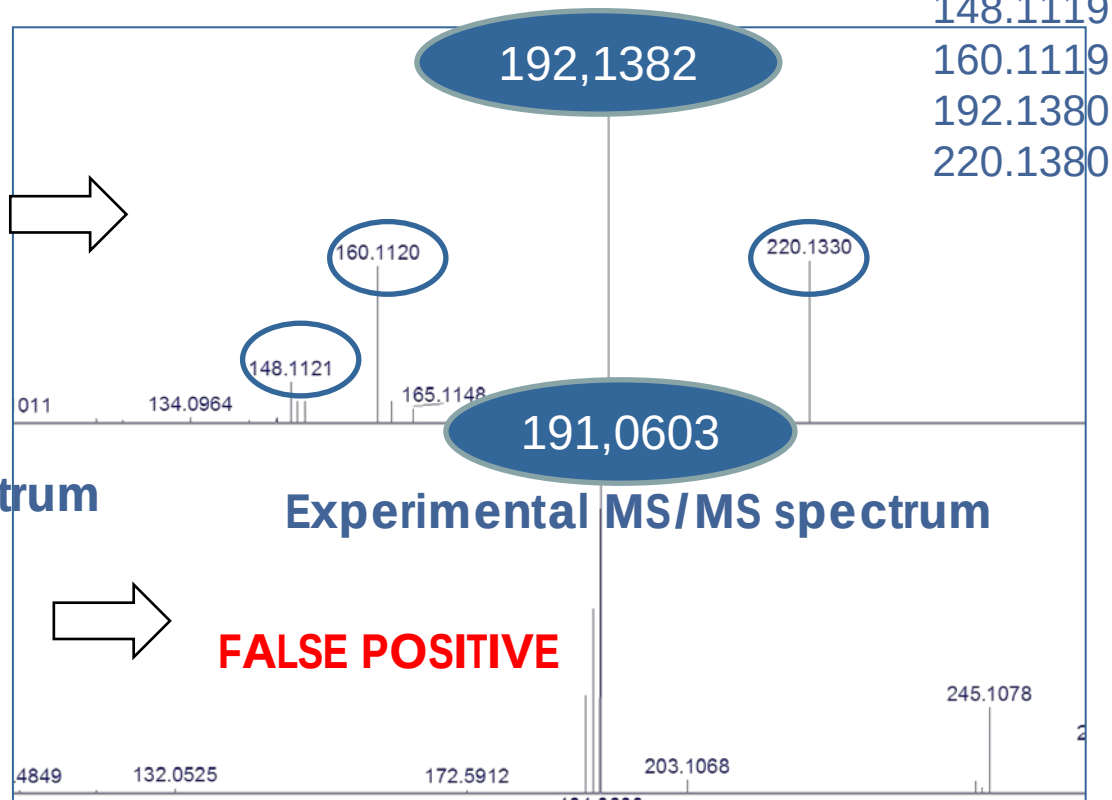
Metalaxyl (XIC m/z 280.1543 ± 5 ppm).

Full scan MS. Resolution 70000

Library MS/MS spectrum

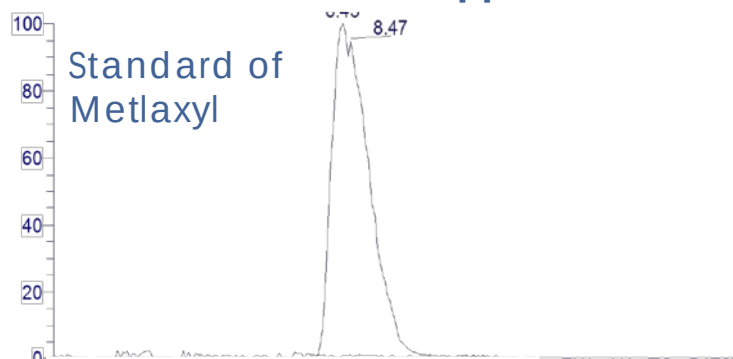
Expected fragments:

148.1119
160.1119
192.1380
220.1380



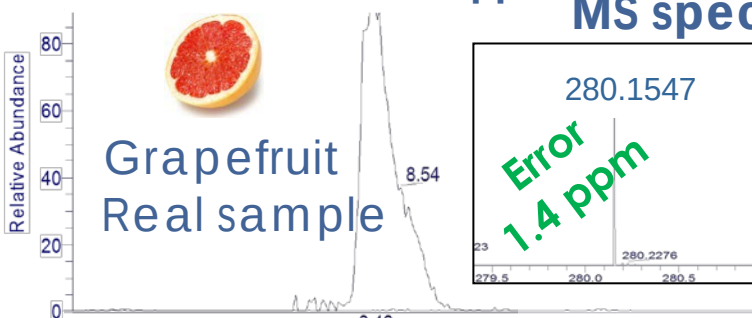
FALSE POSITIVE

RT: 6.72 XIC m/z 280.1543 ± 5 ppm



Standard of Metlaxyl

XIC m/z 280.1543 ± 5 ppm



Grapefruit Real sample

280.1547
Error
1.4 ppm

Tomato 0.01 mg/kg

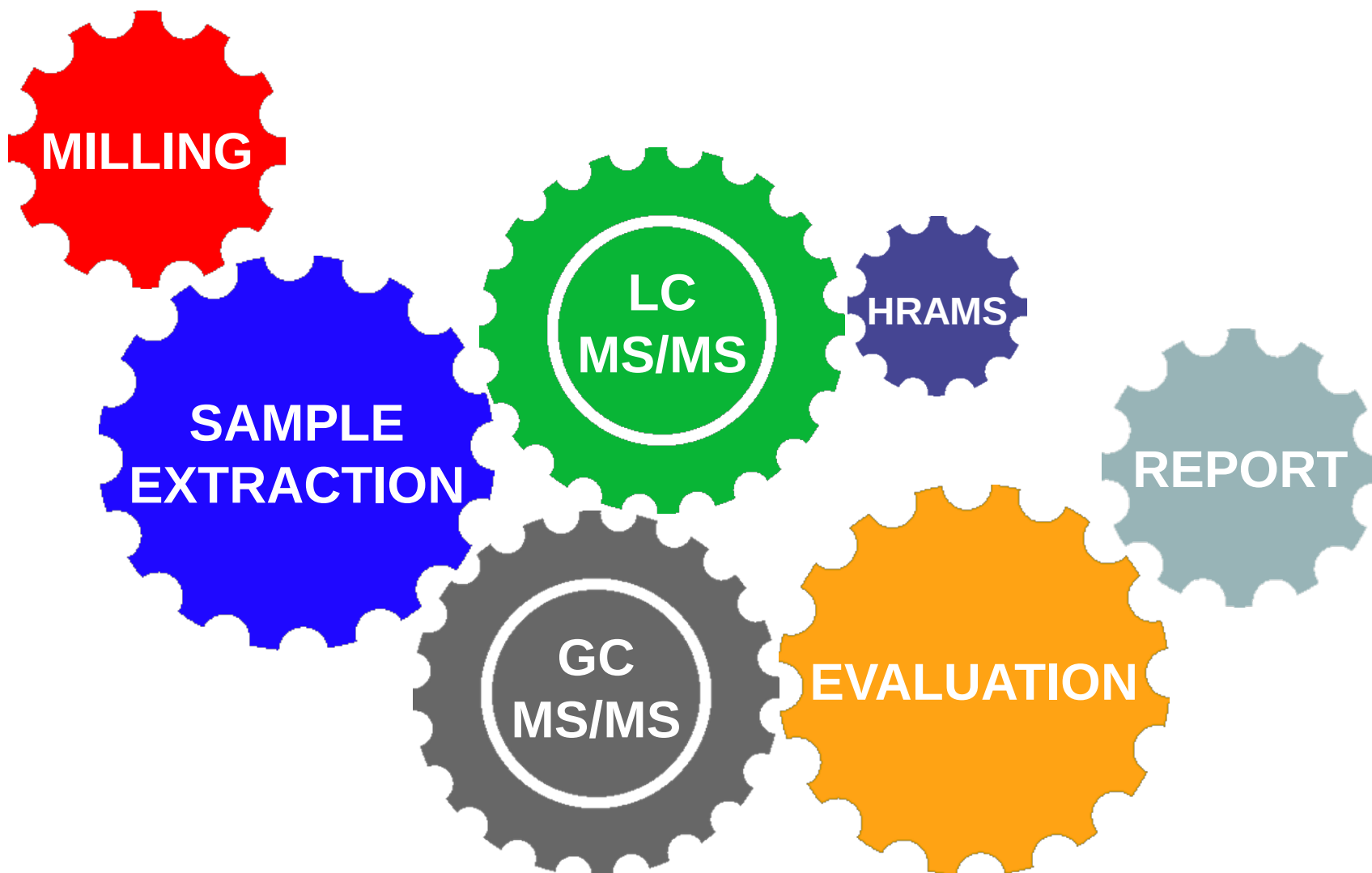


Pesticides	Full scan	QTOF	Orbitrap	MS ²	QTOF	Orbitrap
Azoxystrobin	404.1241	-1.5	0.0	372.0979	0.6	-0.3
Diazinon	305.1083	-1.5	0.3	169.0794	0.9	0.0
Dichlorvos	220.9532	0.5	0.5	109.0047	-1.9	1.6
Fenamiphos - sulfone	336.1029	-0.2	0.6	266.0246	-1.6	0.4
Profenofos	372.9424	1.9	0.5	302.8642	1.0	-0.3

Orange 0.01 mg/kg



Pesticides	Full scan	QTOF	Orbitrap	MS ²	QTOF	Orbitrap
Azoxystrobin	404.1241	0.4	0.0	372.0979	1.4	0.3
Diazinon	305.1083	1.2	-0.3	169.0794	-1.1	-0.6
Dichlorvos	220.9532	1.9	-0.5	109.0047	-2.3	2.4
Fenamiphos - sulfone	336.1029	-0.2	0.0	266.0246	1.5	0.0
Profenofos	372.9424	-0.4	0.0	302.8642	1.1	-1.0



SENSITIVITY

LINEARITY + REPRODUCIBILITY



11 Matrices



Lettuce



Cucumber



Orange



Green bean



Onion



Apple



Parsley



Leek



Tomato



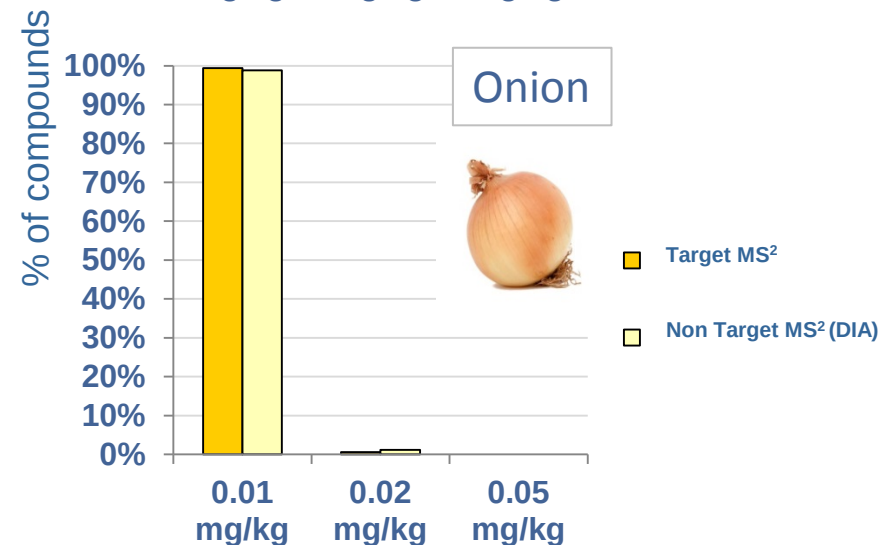
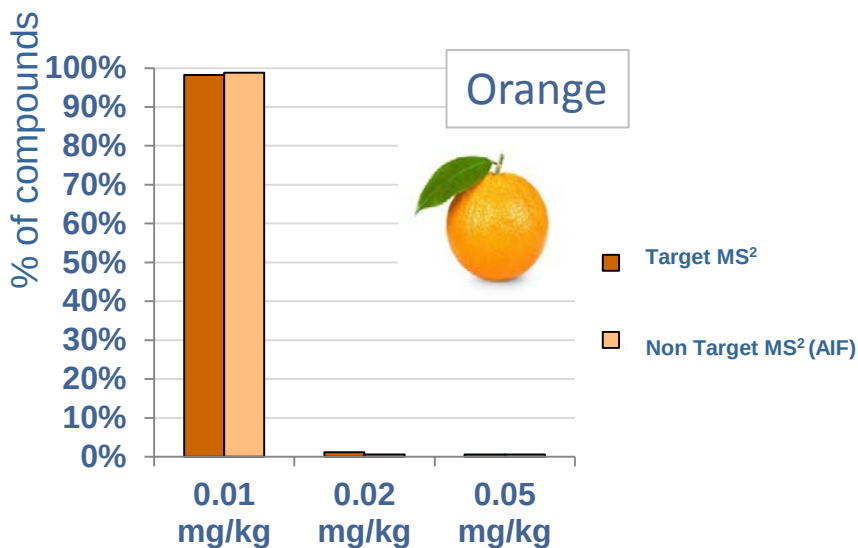
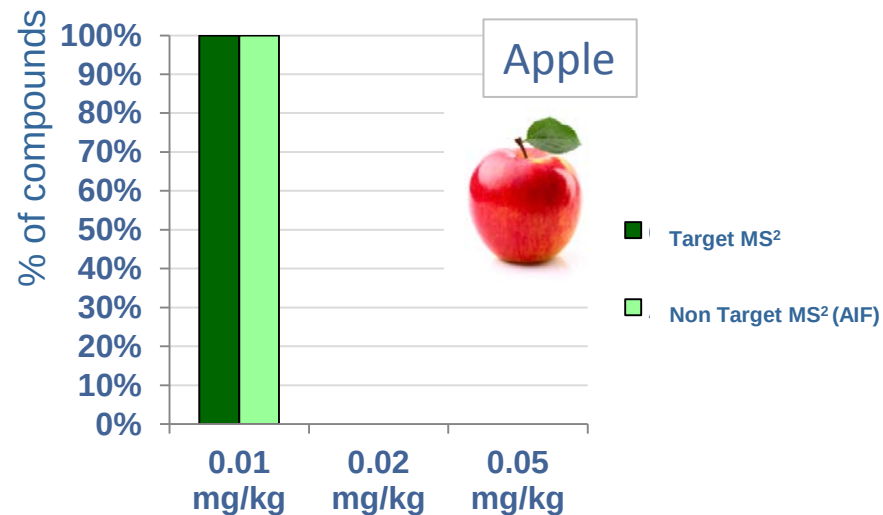
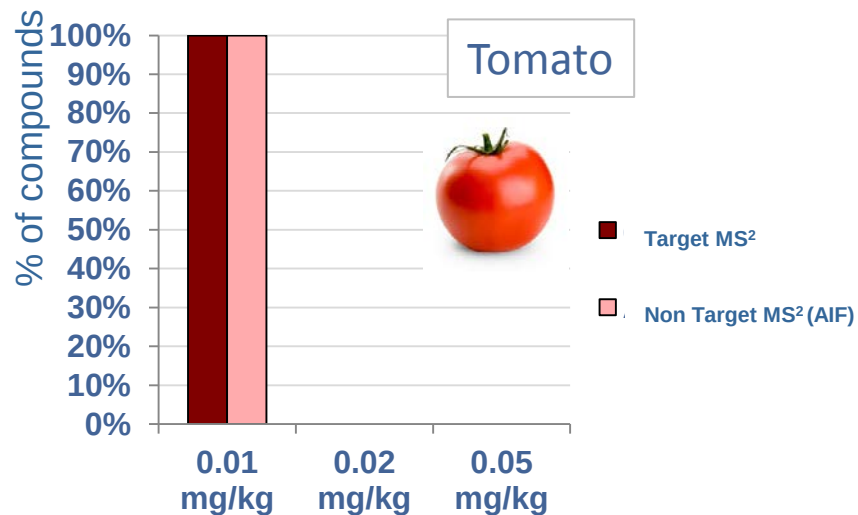
Garlic



Spinach

LC-Orbitrap QExactive Focus

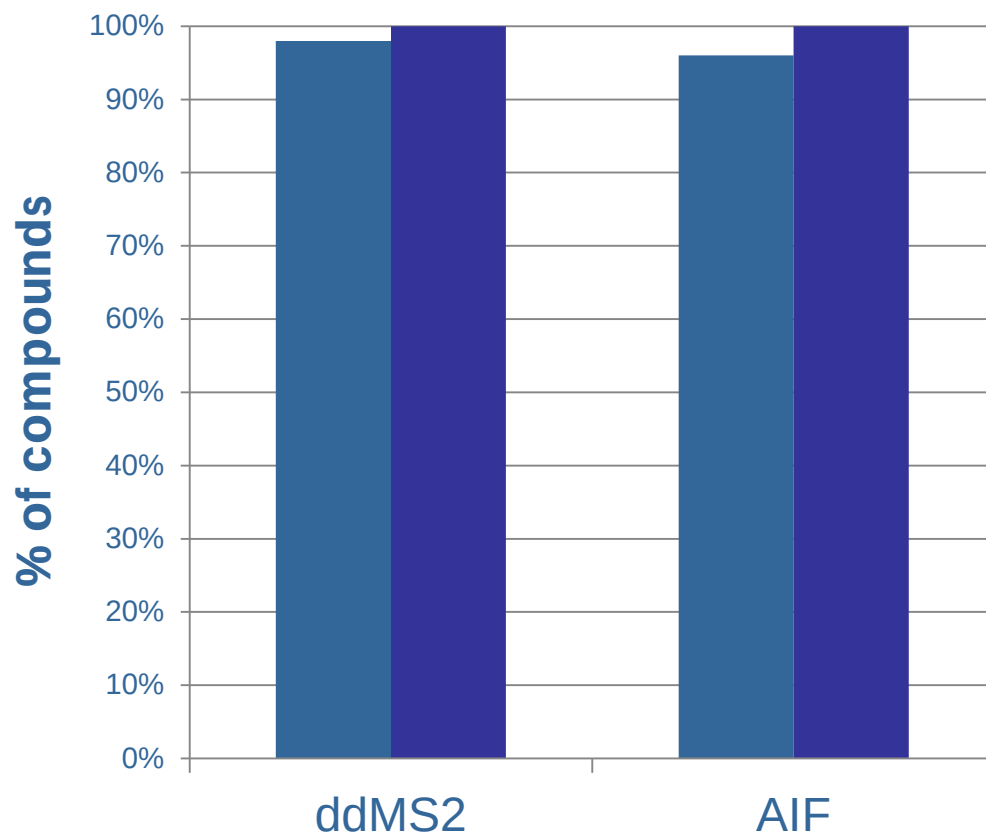
LOQ



LC-Orbitrap
QExactive Focus

Identification

166 pesticides x 11 matrices = 1826 results



■ 0.01 mg/kg

■ 0.1 mg/kg



Lettuce



Cucumber



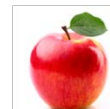
Orange



Green bean



Onion



Apple



Parsley



Leek



Tomato



Garlic

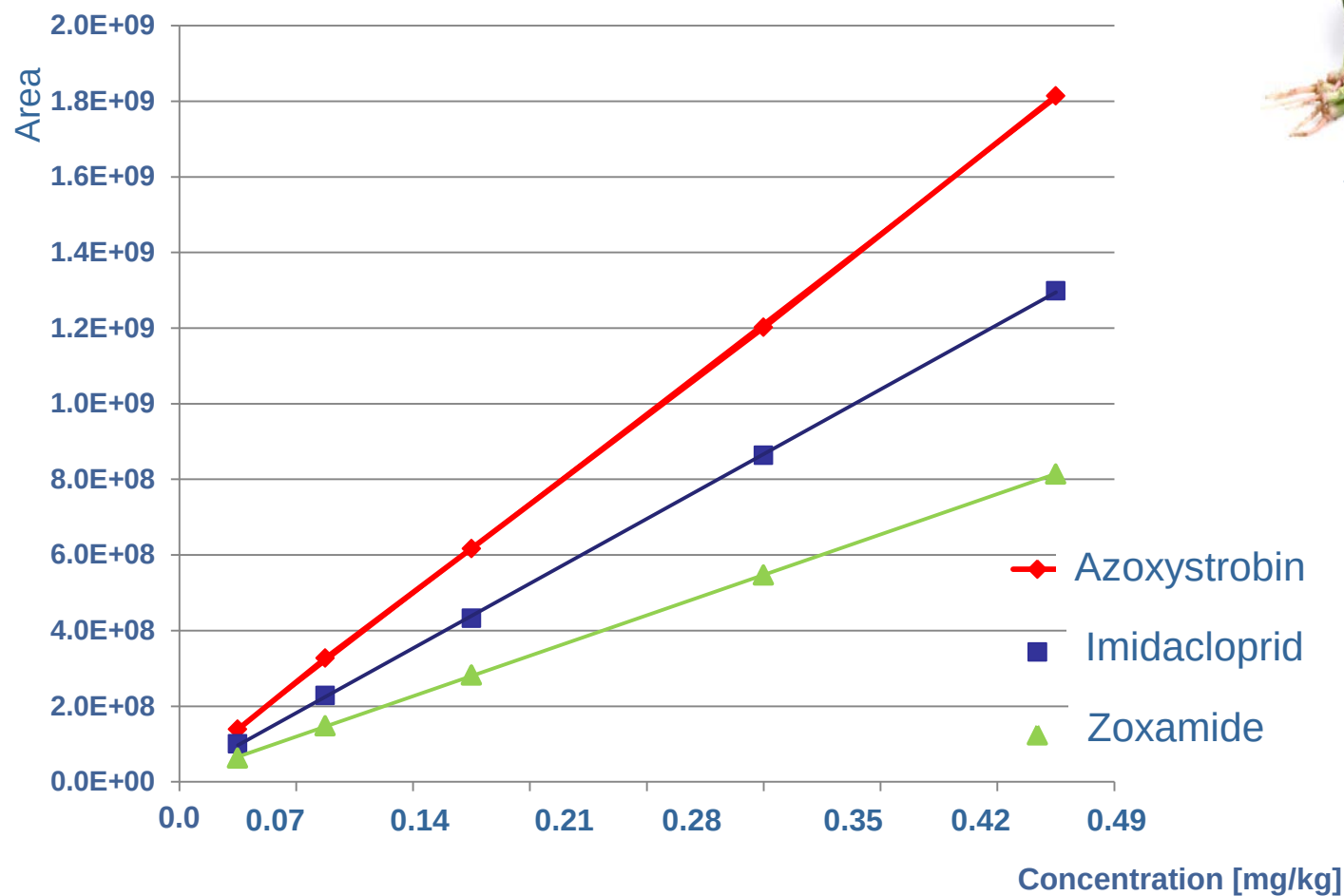


Spinach

Linearity LC-HRAMS

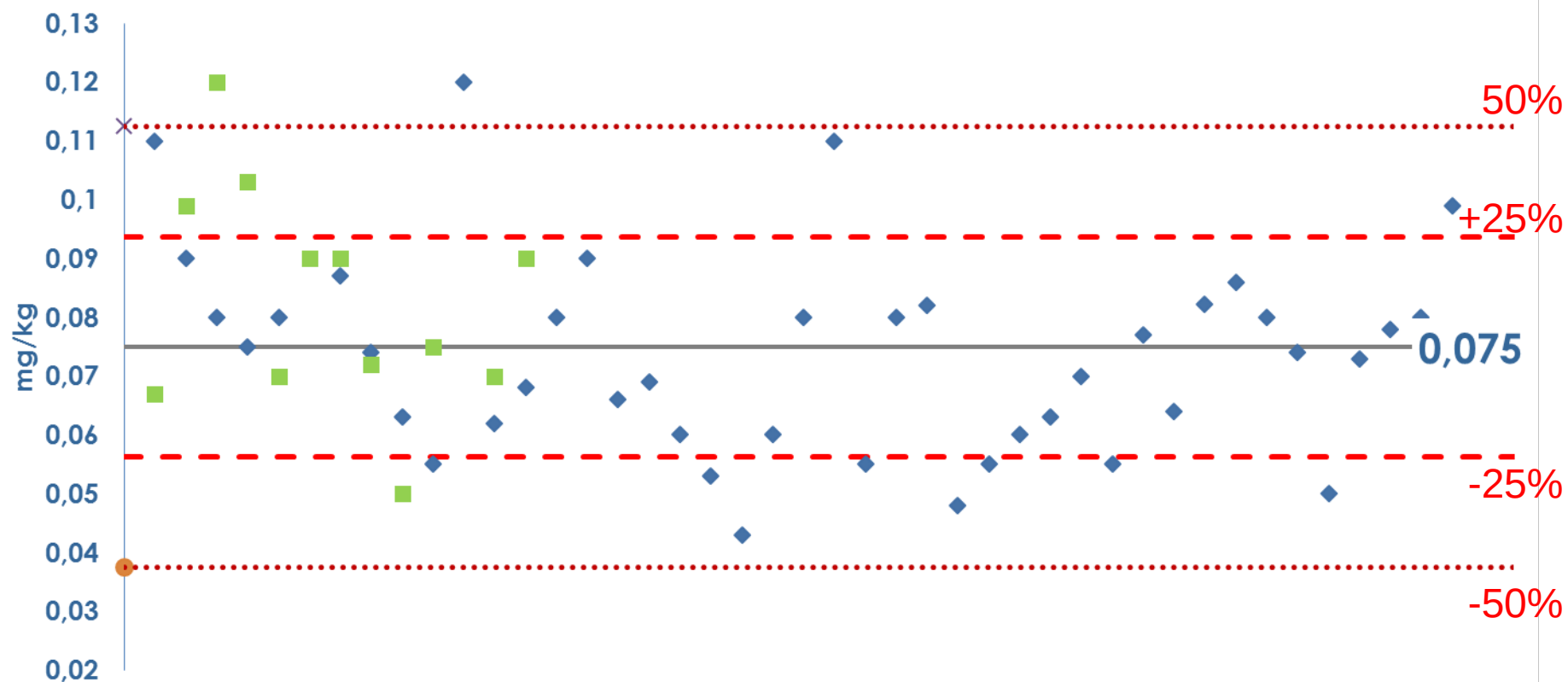


Spinach



EU Proficiency Test

Clomazone



Homogeneity: 0,085 mg/kg

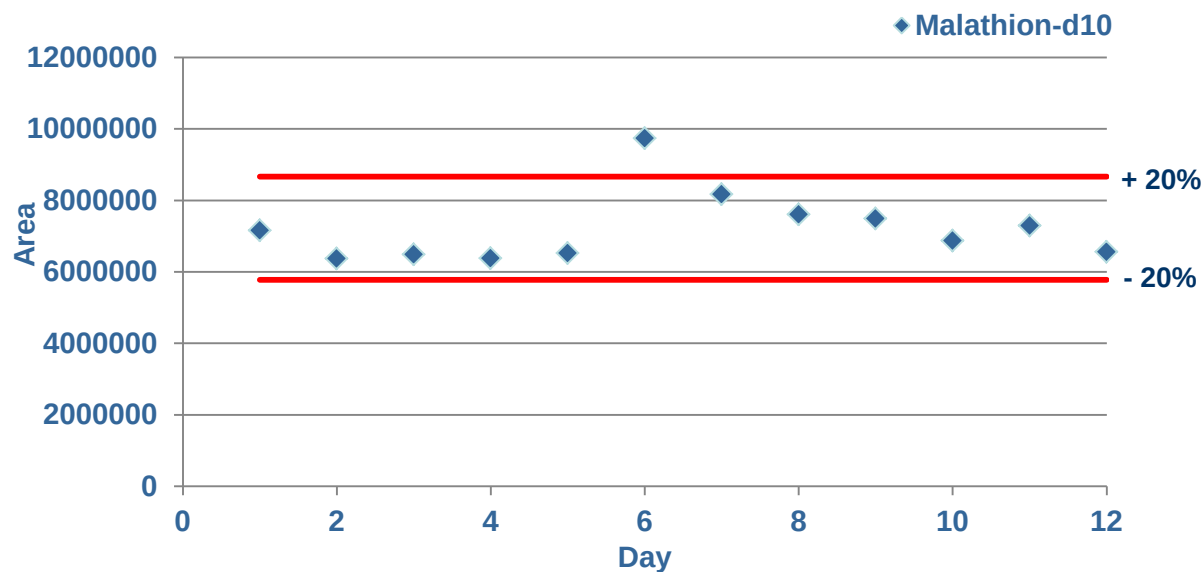
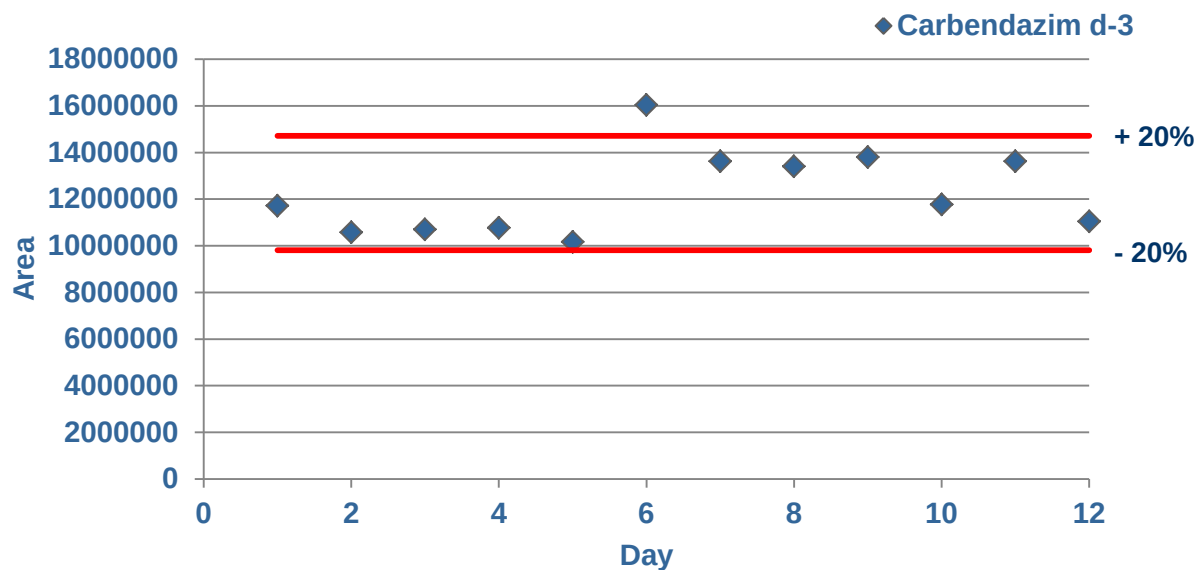
■ HRAMs

■ No HRAMs

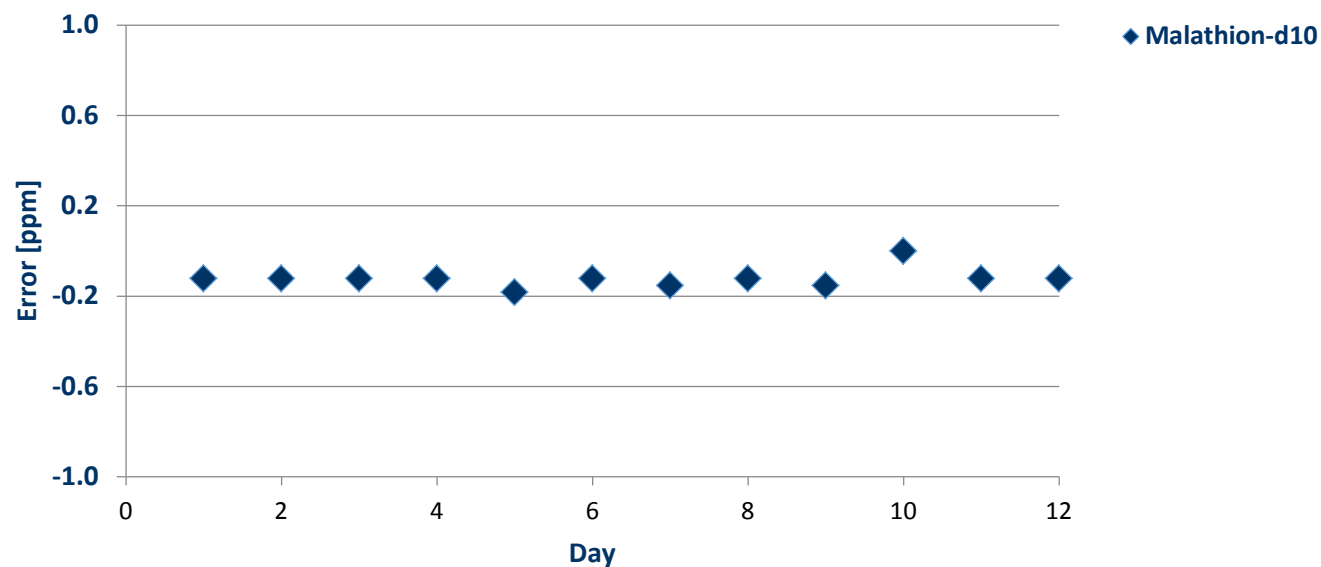
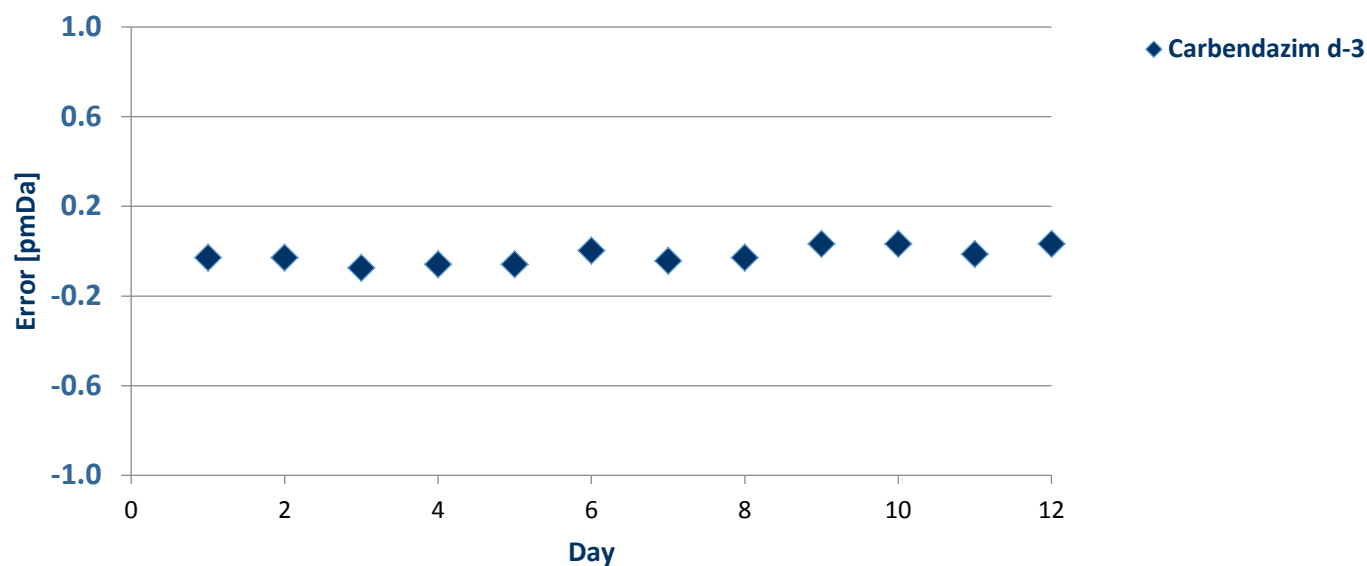
Real Samples Results by LC

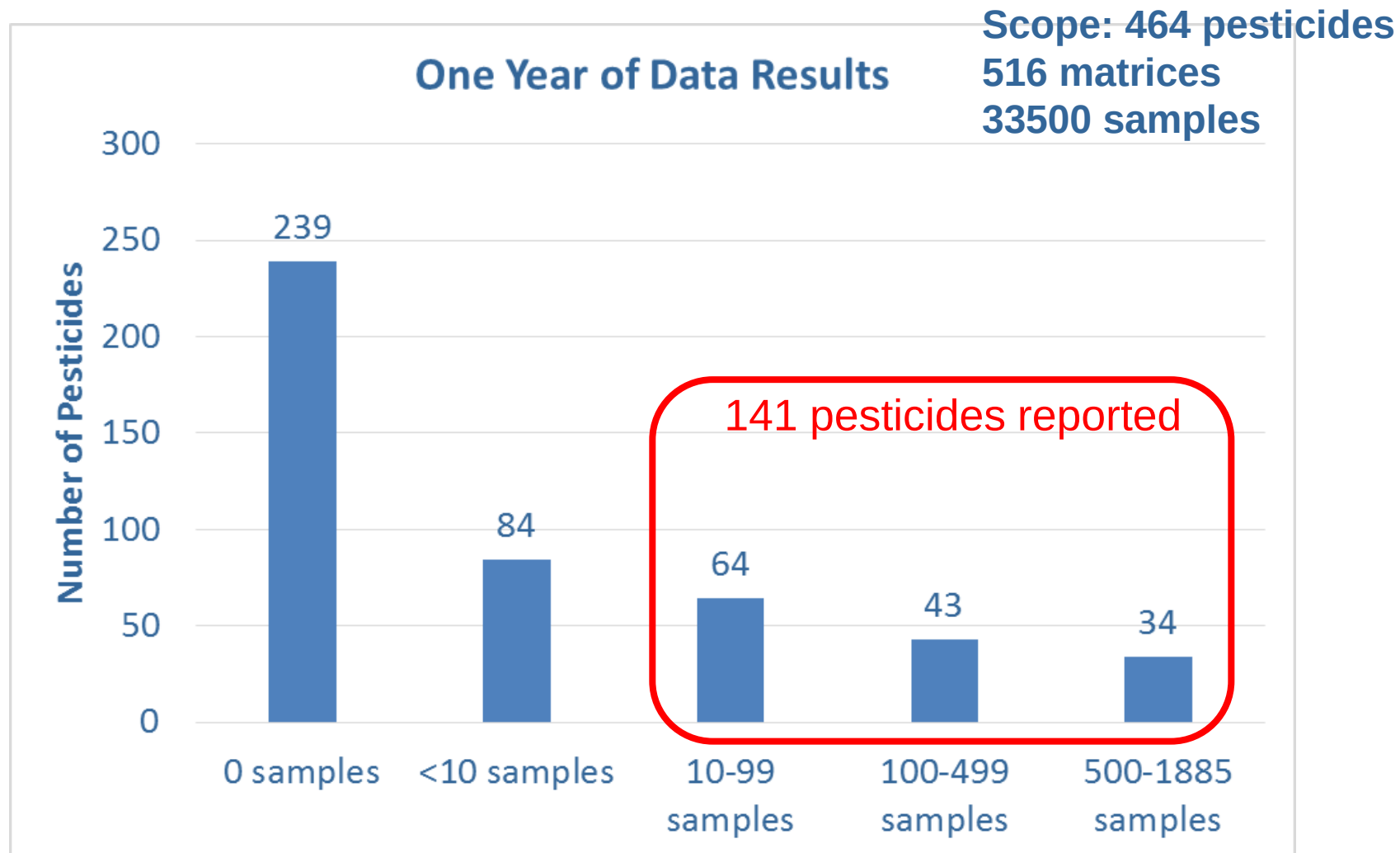
Matrix	Compound	Concentration (mg/kg)		Diff (%)
		LC-QqQ-MS/MS	LC-Orbitrap-MS/MS	
Maniquises Apricot	Tebuconazole	< LOQ	< LOQ	-
Green Celery	Boscalid	< LOQ	< LOQ	-
	Dfenonazole	< LOQ	< LOQ	-
	Linuron	< LOQ	< LOQ	-
	Pirethrin I	< LOQ	< LOQ	-
Broccoli	-	< LOQ	< LOQ	-
Zucchini	Imidacloprid	< LOQ	< LOQ	-
	Myclobutanil	< LOQ	< LOQ	-
	Thiamethoxam	< LOQ	< LOQ	-
Pumpkin	Cyromazine	< LOQ	< LOQ	-
Kiwi	-	< LOQ	< LOQ	-
Peach	Chlorpyrifos	< LOQ	< LOQ	-
Conferencia Pear_E	Boscalid	0.168	0.154	8
	Difenonazole	0.027	0.029	-7
	Diifubenzuron	0.014	0.017	-21
	Imazalil	1.711	1.334	22
	Imidacloprid	0.047	0.052	-11
	Paclobutrazole	< LOQ	< LOQ	-
	Pyraclostrobin	0.114	0.096	16
	Tebuconazole	0.048	0.048	0
	Thiacloprid	0.037	0.036	3
	Trifloxystrobin	0.024	0.021	13
Conferencia Pear_M	Boscalid	0.175	0.177	-1
	Difenonazole	0.012	0.013	-8
	Fluopyram	0.027	0.026	4
	Imazalil	1.359	1.093	20
	Imidacloprid	0.053	0.061	-15
	Kresoxim Methyl	< LOQ	< LOQ	-
	Pyraclostrobin	0.067	0.063	6
	Tebuconazole	0.044	0.047	-7
Red Pepper	Fluopyram	< LOQ	< LOQ	-
	Flutriafof	0.019	0.013	32
Red Chili Pepper	Flutriafof	0.397	0.368	7
	Pirimicarb	0.034	0.034	0
Green Pepper	Flutriafof	< LOQ	< LOQ	-
	Metalaxyl	0.010	0.010	0
Banana	Imazalil	0.915	0.788	14
Carrot_E	-	< LOQ	< LOQ	-
Carrot_M	-	< LOQ	< LOQ	-

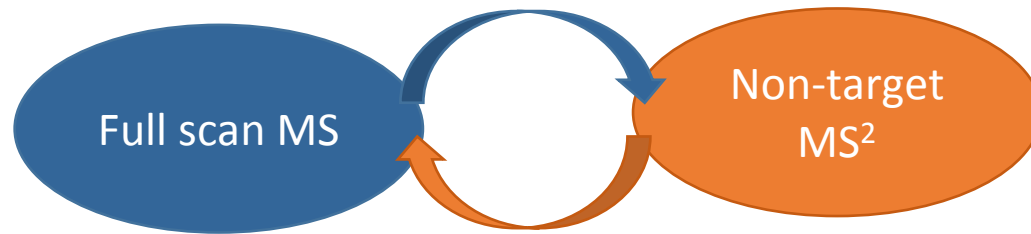
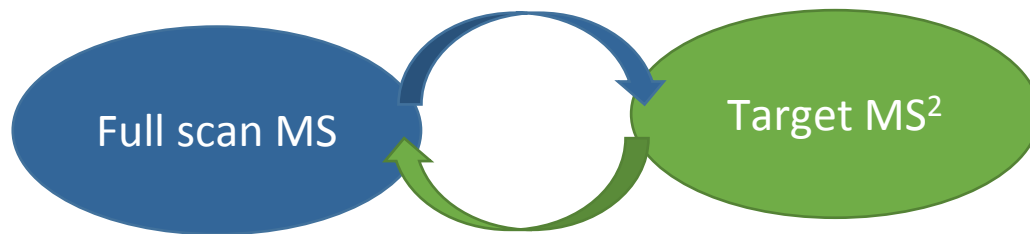
Robustness of peak area LC-Orbitrap



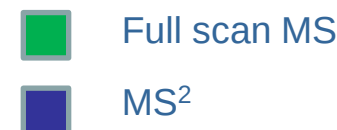
Robustness of mass accuracy LC-Orbitrap







Orbitrap



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/dd-MS²/AIF-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.



LC Orbitrap

Compound list

ddMS²

Target (166 compounds)

Acephate	Emamectin B1a	Isoxaflutole	Propyzamide
Acetamiprid	Epoxiconazole	Kresoxim-methyl	Proquinazid
Aldicarb	Ethion	Linuron	Prothioconazole
Aldicarb-sulfone	Ethirimol	Lufenuron	Pymetrozine
Aldicarb-sulfoxide	Ethoprophos	Malaaxon	Pyraclostrobin
Azinphos-methyl	Etofenprox	Malathion	Pyrethrini
Azoxystrobin	Famoxadone	Mandipropamid	Pyrethrini
Bifenazate	Fenamidone	Mepanipyrim	Pyridaben
Bitertanol	Fenamiphos	Metalaxyl	Pyridate
Boscalid	Fenamiphos-sulfone	Metconazole	Pyrimethanil
Bromuconazole	Fenamiphos-sulfoxide	Methidathion	Pyriproxyfen
Bupirimate	Fenarimol	Methiocarb	Quinoclamine
Buprofezin	Fenazaquin	Methiocarb-sulfone	Quinoxyfen
Carbaryl	Fenbuconazole	Methiocarb-sulfoxide	Rotenone
Carbendazim	Fenhexamid	Methomyl	Spinosyn A
Carbofuran	Fenoxycarb	Methoxyfenozide	Spinosyn D
Chlorantraniliprole	Fenpropathrin	Metobromuron	Spirodiclofen
Chlorfenvinphos, B-	Fenpropimorph	Monocrotophos	Spiromesifen
Chlorpyrifos	Fenpyrazamine	Myclobutanil	Spirotetramat
Chlorpyrifos-methyl	Fenpyroximate	Nitenpyram	Spiroxamine
Clofentezine	Fenthion	Omethoate	Tebuconazole
Clomazone	Fenthion-sulfone	Oxadixyl	Tebufenozide
Clothianidin	Fenthion-sulfoxide	Oxamyl	Tebufenpyrad
Coumaphos	Flonicamid	Paclobutrazol	Terbutylazine
Cyazofamid	Flufenacet	Paraoxon methyl	Tetraconazole
Cymoxanil	Flufenoxuron	Penconazole	Thiabendazole
Cyproconazole	Fluopyram	Pencycuron	Thiacloprid
Cyprodinil	Fluquinconazole	Pendimethalin	Thiamethoxam
Cyromazine	Flusilazole	Phenthoate	Thiobencarb
Demeton-S-Methyl-Sulfone	Flutriafol	Phosalone	Thiodicarb
Demeton-S-methylsulfoxide	Formetanate	Phosmet	Thiophanate-methyl
Diazinon	Fosthiazate	Phoxim	Tolclofos-methyl
Dichlorvos	Haloxifyop	Pirimicarb	Triadimefon
Dicrotophos	Hexaconazole	Pirimicarb, desmethyl-	Triadimenol
Diethofencarb	Hexythiazox	Pirimiphos-methyl	Triazophos
Difenoconazole	Imazalil	Prochloraz	Trichlorfon
Diflubenzuron	Imidacloprid	Profenofos	Trifloxystrobin
Dimethoate	Indoxacarb	Propamocarb	Triflumuron
Dimethomorph	Iprovalicarb	Propaquizafop	Triticonazole
Diniconazole	Isocarboxipos	Propargite	Zoxamide
Diuron	Isofenfos methyl	Propiconazole	
Dodine	Isoprocarb	Propoxur	

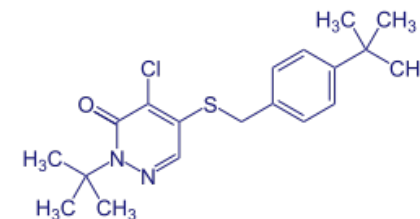
AIF-MS²

Non-target (102 compounds)

Acetochlor	Fenoxaprop-ethyl	Napropamide
Alachlor	Fenpropidin	Nuarimol
Ametryn	Fenuron	Ofurace
Anilofos	Flamprop-methyl	Oxycarboxin
Atrazine	Flazasulfuron	Paraoxon ethyl
Atrazine-Desethyl	Fluacrypyrim	Paraoxon methyl
Azamethiphos	Fluazifop-Butyl	Phosphamidon
Azinphos-ethyl	Fluometuron	Phosphamidon
Bensulfuron-methyl	Fluridone	Picolinafen
Bromacil	Fonofos	Picoxystrobin
Buturon	Forchlorfenuron	Promecarb
Cambendazole	Fuberidazole	Prometryn
Carbetamide	Furathiocarb	Propachlor
Chlorbromuron	Heptenophos	Propaphos
Chloridazon	Imazapyr	Propaquizafop
Chloroxuron	Imazaquin	Propazine
Chromafenozide	Isazofos	Prosulfocarb
Cinosulfuron	Isofenphos	Pyrazophos
Clethodim	Isofenphos-oxon	Pyridaphenthion
Cyanazine	Isoproturon	Pyrifeno
Diafenthiuron	Lenacil	Pyrimidifen
Dialifos	Mebendazole	Quinalphos
Diclobutrazol	Mecarbam	Sulfosulfuron
Difenoxyuron	Metamitron	Sulprofos
Diflufenican	Metazachlor	Terbutylazine
Dimefuron	Metolachlor	Terbutylazine-desethyl
Dimethylvinphos, Z-	Metosulam	Tetrachlorvinphos
Dodemorph	Metoxuron	Thifensulfuron-methyl
Edifenphos	Metribuzin	Thiophanate
Ethiofencarb	Metsulfuron-methyl	Tolfenpyrad
Ethiprole	Mevinphos	Triasulfuron
Etrifos	Monolinuron	Tribenuron-methyl
Fenbendazole	Monuron	Tricyclazole
Fenfuram	Naled	Vamidothion

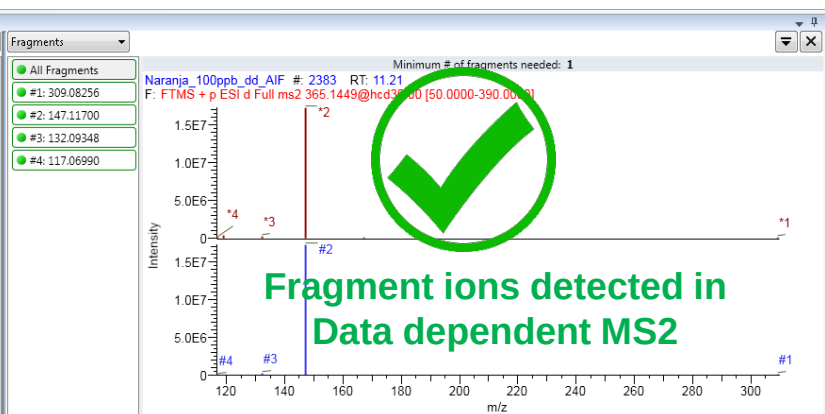
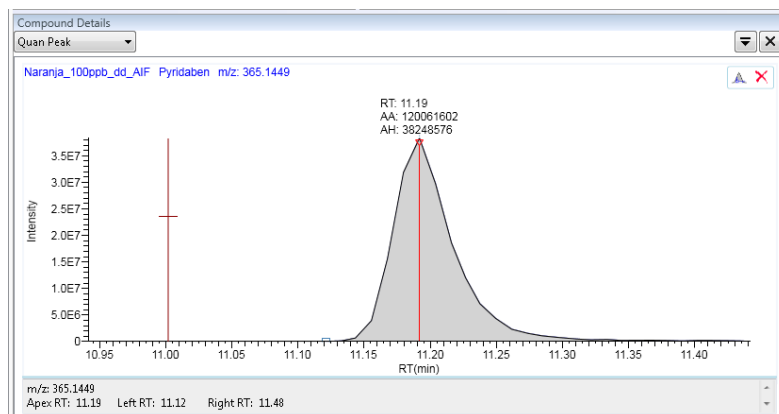
FS/dd-MS²/AIF-MS²

Pyridaben (target compound)
0.1 mg/kg in orange



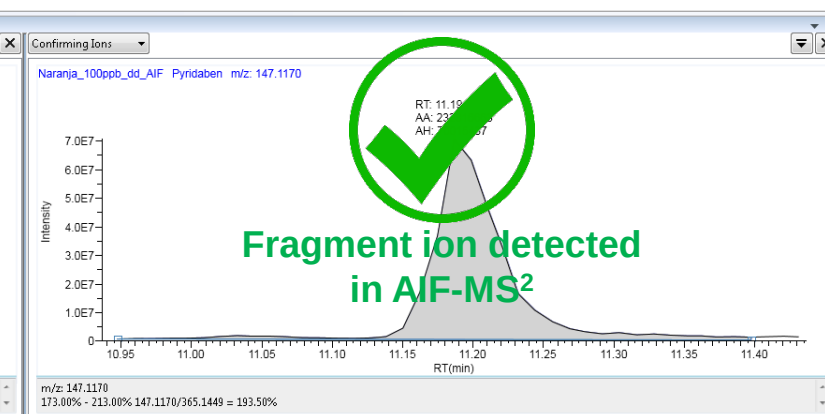
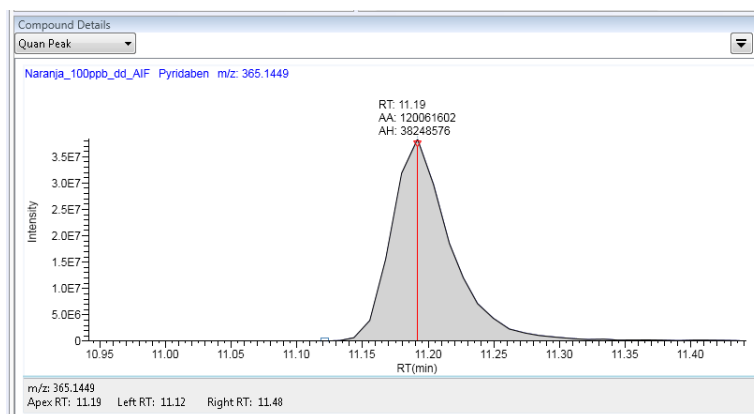
MS

MS²



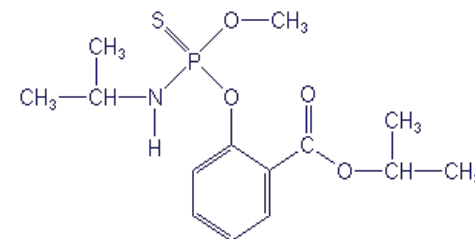
MS

MS²



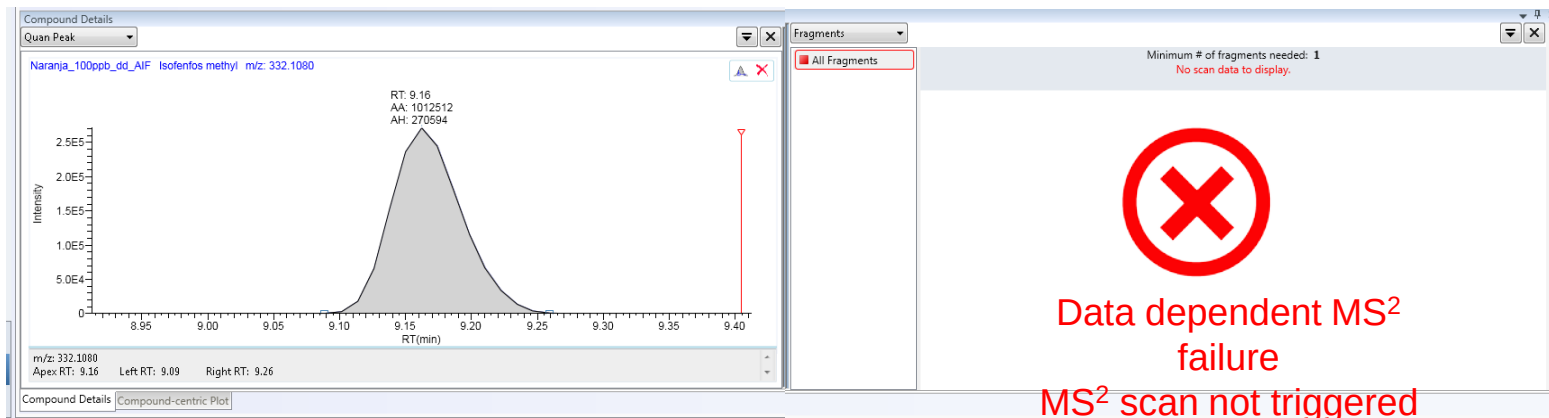
FS/dd-MS2/AIF-MS2

Isofenphos methyl (target compound)
0.1 mg/kg in orange



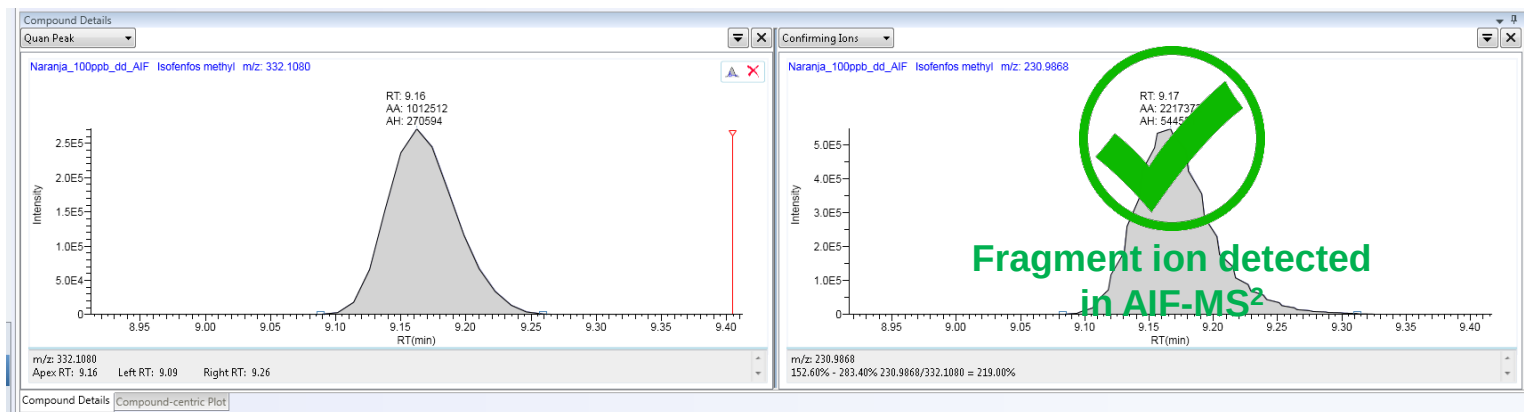
MS

MS²



MS

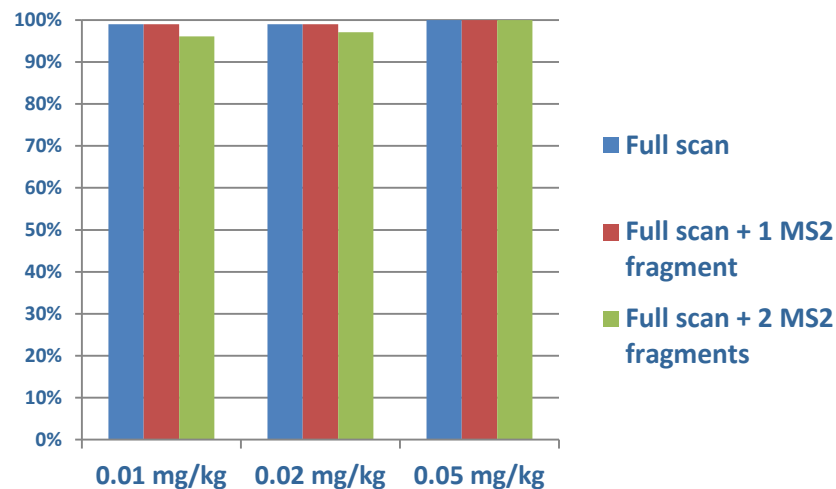
MS²



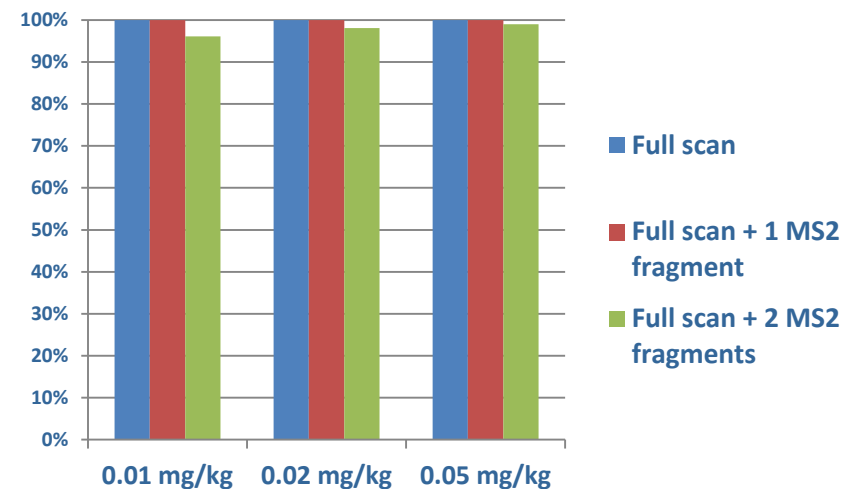
DETECTION OF NON-TARGET PESTICIDES

Number of spiked pesticides 102

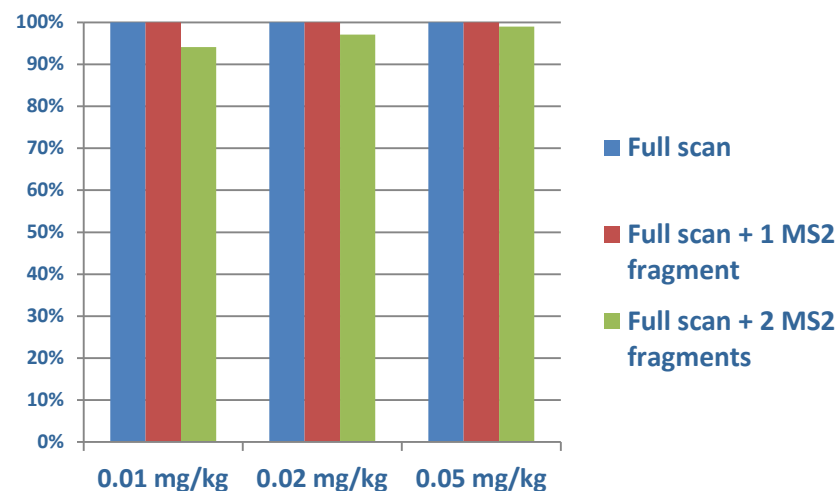
Tomato



Lettuce



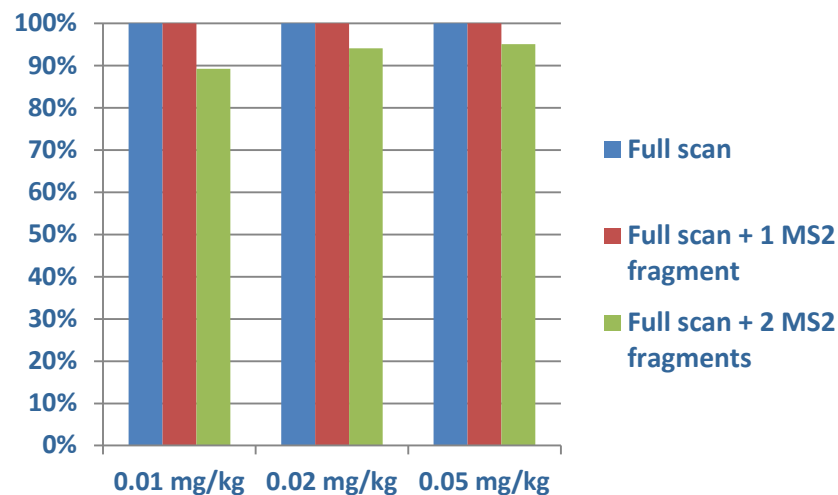
Zucchini



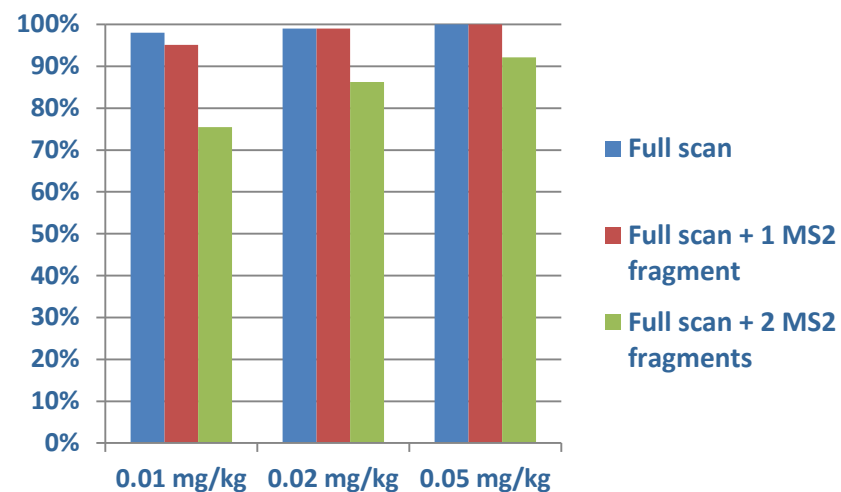
DETECTION OF NON-TARGET PESTICIDES

Number of spiked pesticides 102

Orange

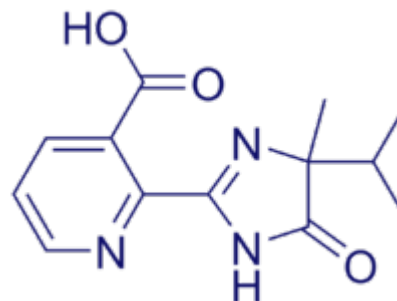


Leek

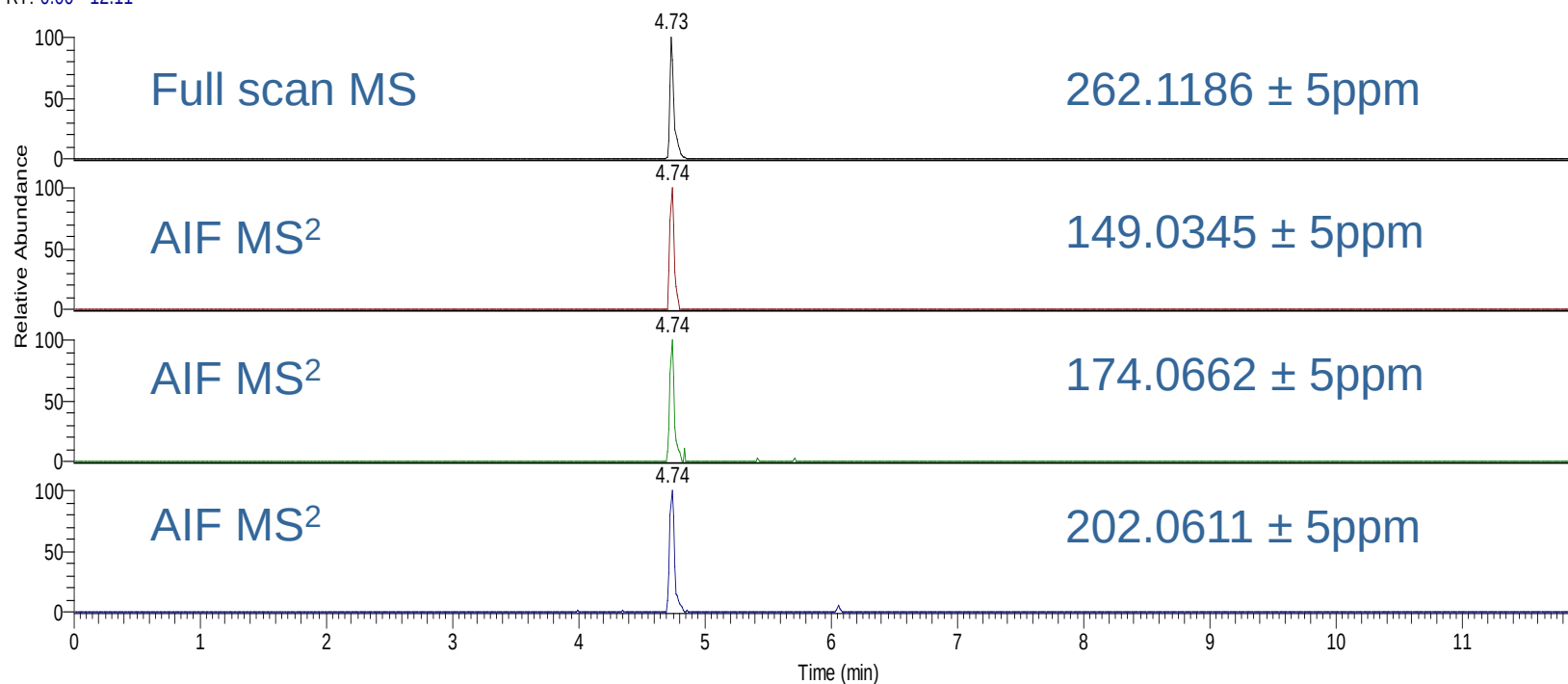


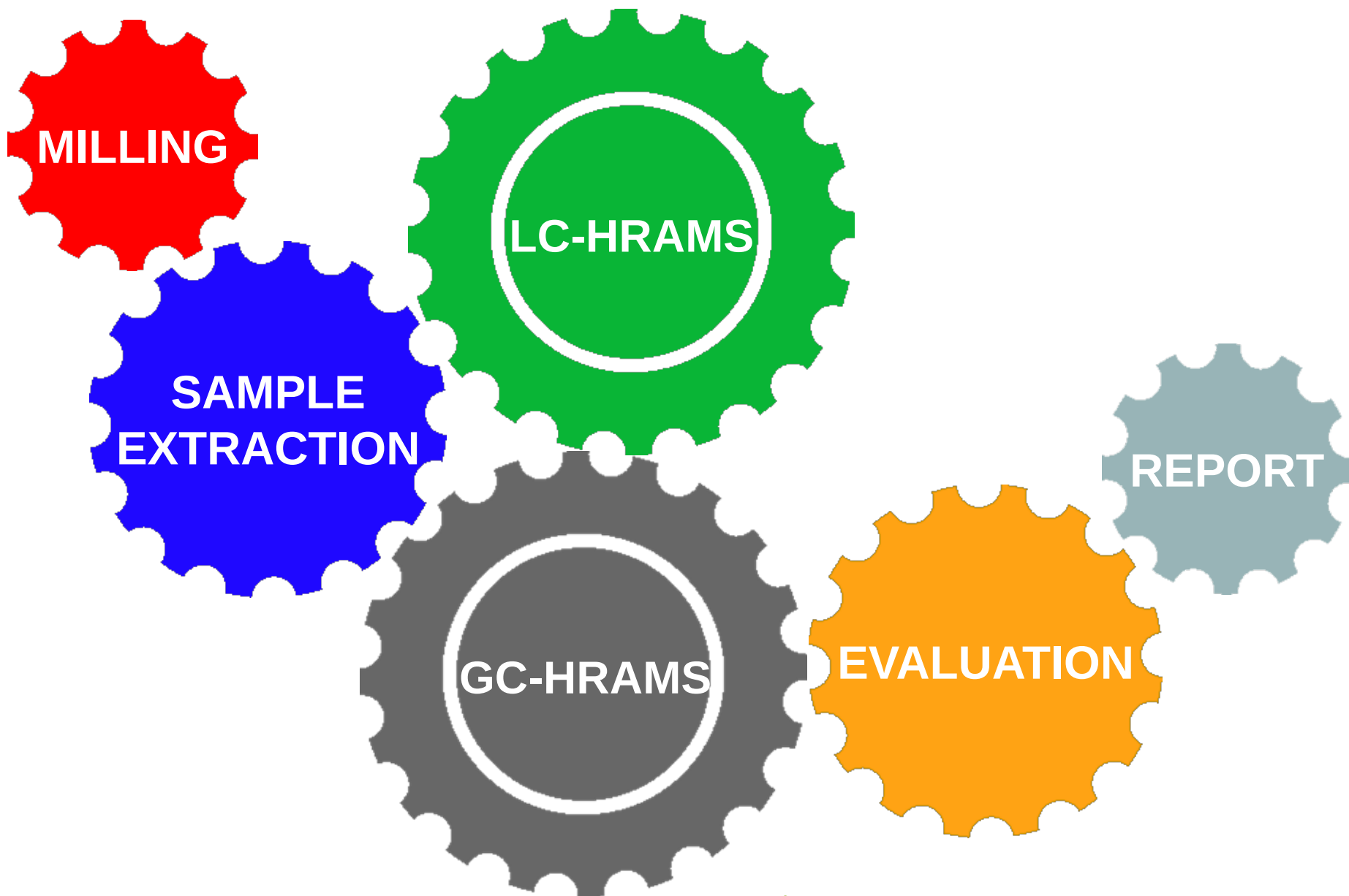
DETECTION OF NON-TARGET PESTICIDES

Imazapyr in pear
(not included in EU Monitoring Program)
(mass of protonated molecule 262.1186)



RT: 0.00 - 12.11





	IDENTIFICATION	QUANTITATION	
Target Analysis	- Retention time - Analytical standard 2 ions (<5 ppm)	- Recoveries - Linearity - Reproducibility - Repeatability - Limit of quantitation	Quantitative validation

	IDENTIFICATION	QUANTITATION	
Target Analysis	- Retention time - Analytical standard 2 ions	- Recoveries - Linearity - Reproducibility - Repeatability - Limit of quantitation	Quantitative validation
Non Target Analysis (low frequent com.)	- Retention time - Analytical standard 1-2 ions(<5 ppm)	Single point calibration - Reproducibility - Repeatability - Limit of detection/quantitation	Qualitative or Semi quantitative validation

	IDENTIFICATION	QUANTITATION	
Target Analysis	- Retention time - Analytical standard 2 ions	- Recoveries - Linearity - Reproducibility - Repeatability - Limit of quantitation	Quantitative validation
Non Target Analysis (low frequent comp.)	- Retention time - Analytical standard 1-2 ions	- Single point calibration - Reproducibility - Repeatability - Limit of detection/quantitation	Qualitative or Semi quantitative validation
	2 -3 ions(<5 ppm) - no analytical standard - Rt?		Informative



Thank You for Your Attention

