Analysis of Polar Ionic Pesticides using High Performance Ion Chromatography coupled with High Resolution Accurate Mass Spectrometry

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CURSO 10
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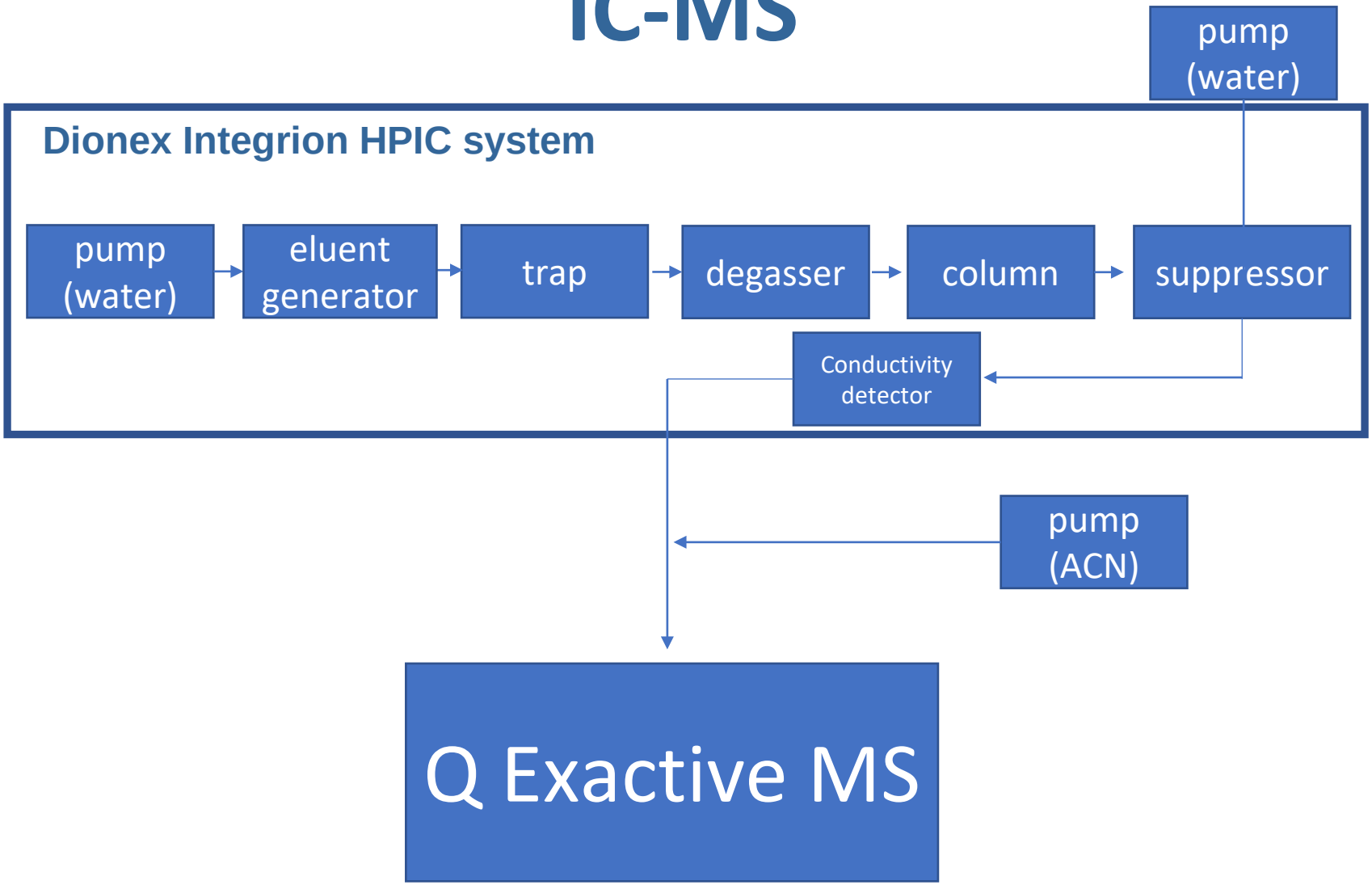
ALMERÍA

Ion chromatography coupled to high resolution mass spectrometry

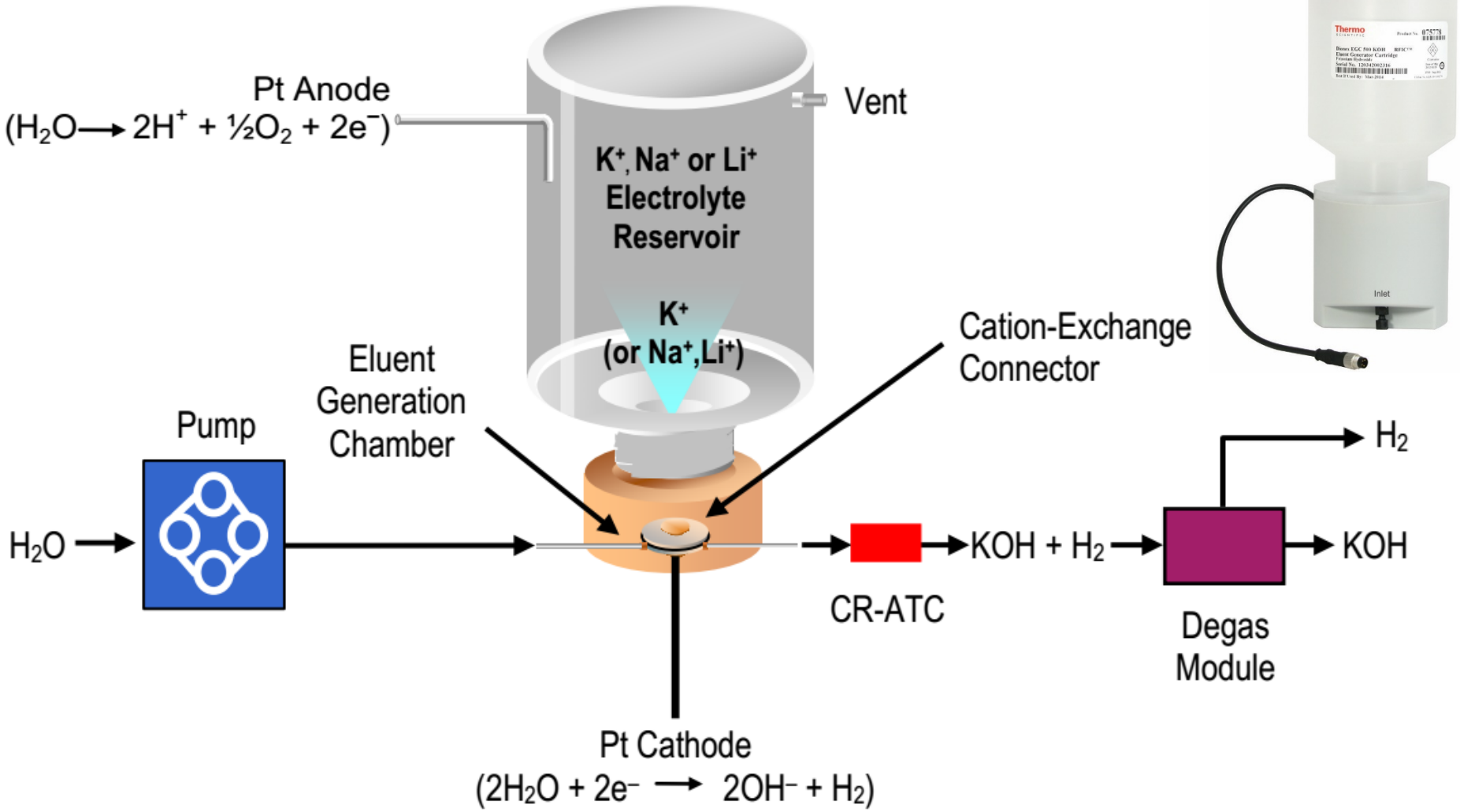
Thermo Scientific
Dionex Integrion
HPIC System

Thermo Scientific
Q Exactive
Focus MS System

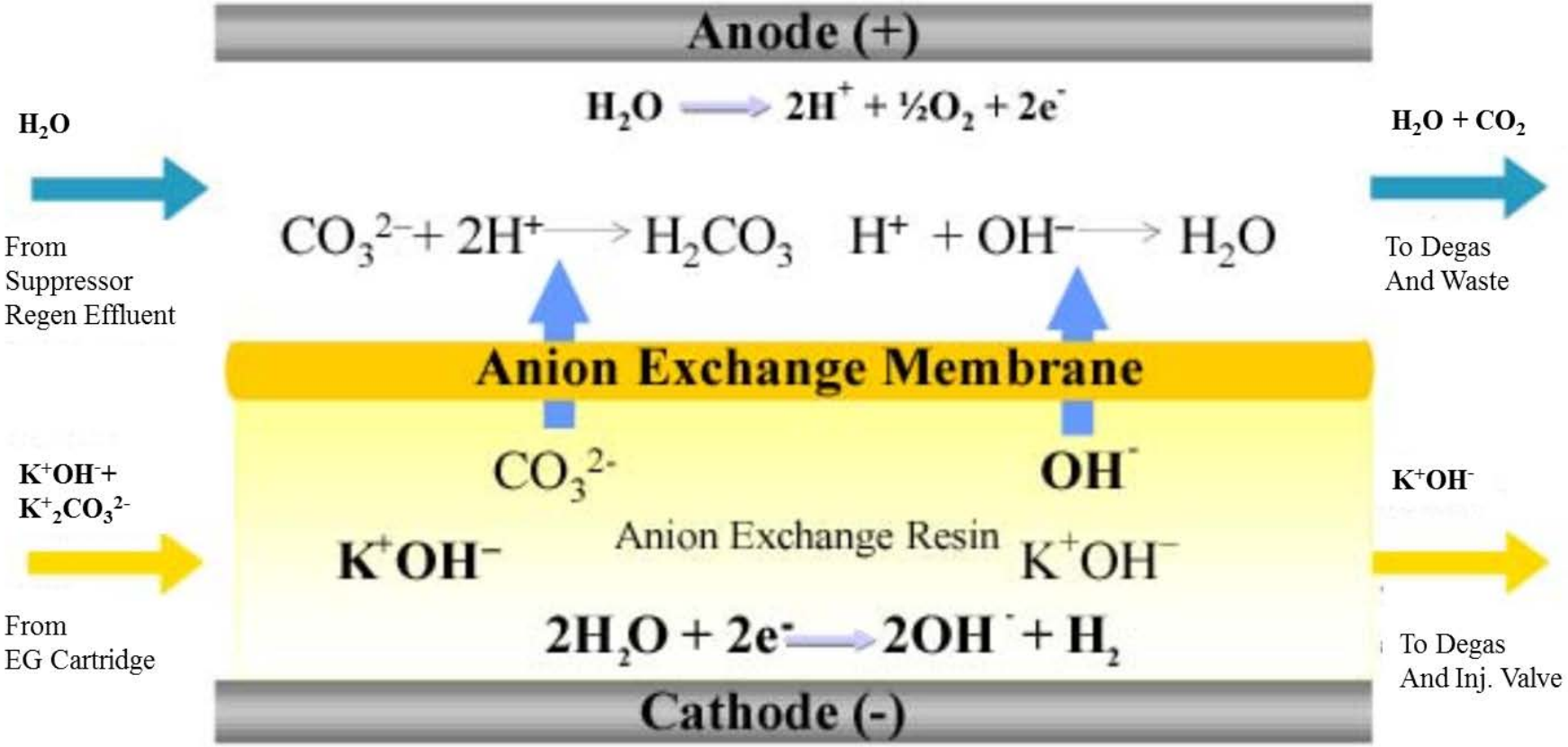
IC-MS


pump (water)
Dionex Integrion HPIC system
pump (water)
eluent generator
trap
degasser
column
suppressor
Conductivity detector
pump (ACN)
Q Exactive MS

Eluent Generator Cartridge



Continuously regenerated trap column (CRTC)

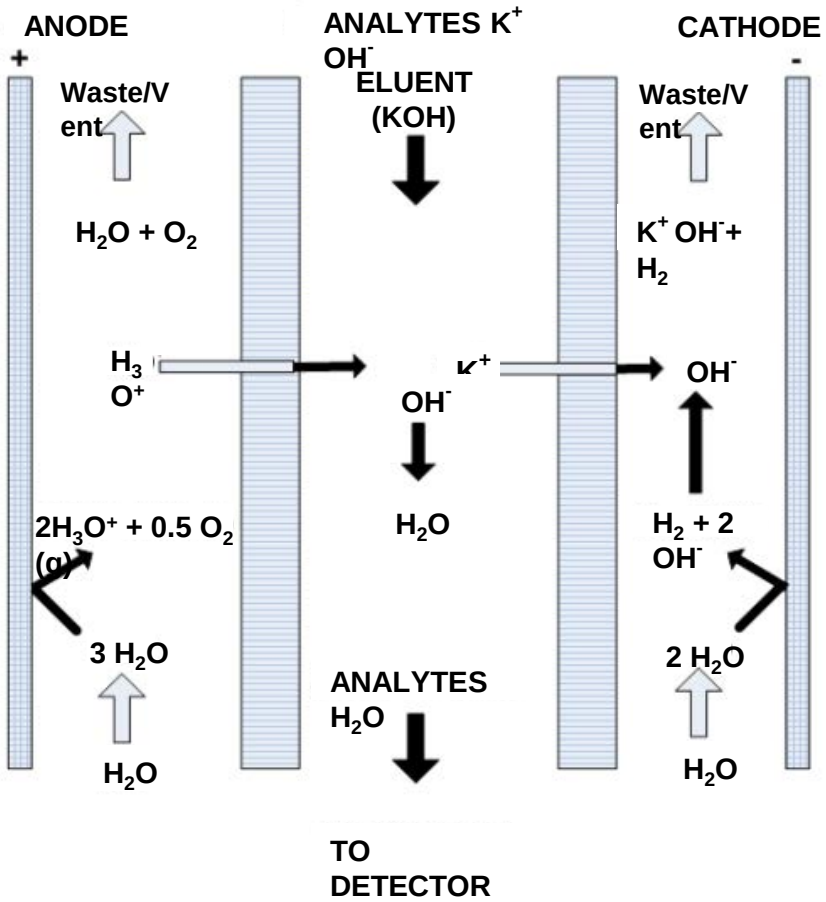


Column

- Guard column Thermo Scientific Dionex IonPac AG19 (50mm x 2mm x 4µm)
- Column Thermo Scientific Dionex IonPac AS19 (250mm x 2mm x 4µm)

	Particle diameter [µm]	Pore Size [Å]	Crosslinking [%DVB]	Resin	Functional Group	Capacity [µeq/column]	Hydrophobicity
AG19	4	< 1	55%	Microporous	Alkanol quaternary ammonium	1.5	Ultralow
AS19	4	2000	55%	Supermacroporous	Alkanol quaternary ammonium	60	Ultralow

Suppressor


ANODE
+
Waste/V ent
$H_2O + O_2$
H_3O^+
$2H_3O^+ + 0.5\ O_2$ (g)
$3\ H_2O$
H_2O
ANALYTES K^+ OH^-
ELUENT (KOH)
K^+
OH^-
H_2O
ANALYTES H_2O
TO DETECTOR
CATHODE
-
Waste/V ent
$K^+\ OH^- + H_2$
OH^-
$H_2 + 2\ OH^-$
$2\ H_2O$
H_2O

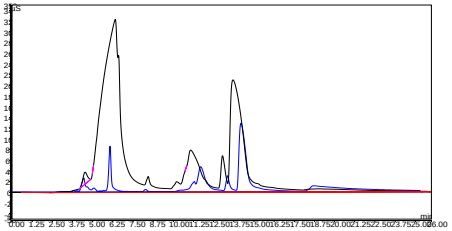
Thermo
SCIENTIFIC
ASRS 300 4 mm

Post-column addition of organic solvent – sensitivity improvement

100% - peak area without organic solvent

	MeOH 0.2 ml/min	ACN 0.2 ml/min	ACN 0.4 ml/min
AMPA	169%	269%	254%
Chlorate	121%	381%	434%
Glyphosate	145%	269%	235%
Perchlorate	132%	365%	454%
Fosetyl-Al	242%	347%	339%
Phosphonic acid	139%	280%	283%
N-acetyl AMPA	147%	254%	250%
N-acetyl glyphosate	138%	268%	255%

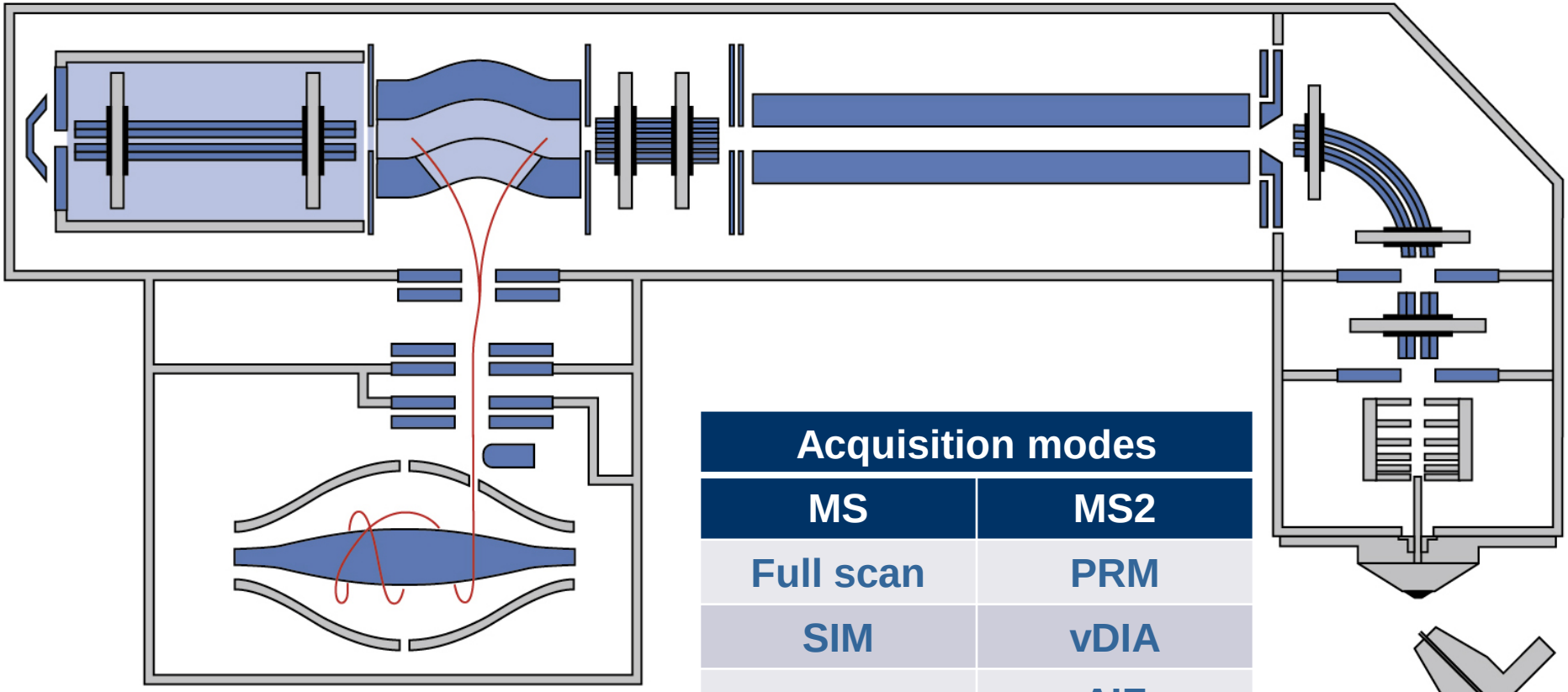
Conductivity detector



- Strawberry, extraction without formic acid
- Strawberry, extraction with formic acid

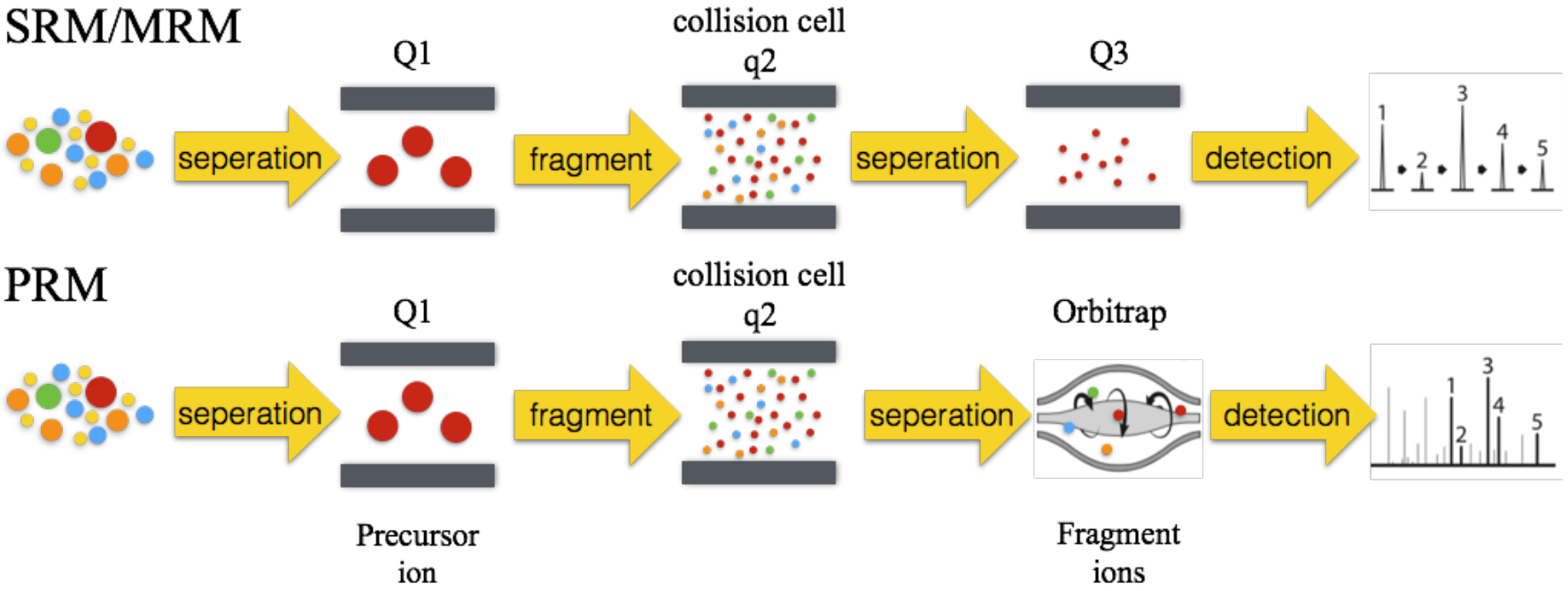
Chromatogram from conductivity detector

Q Exactive MS

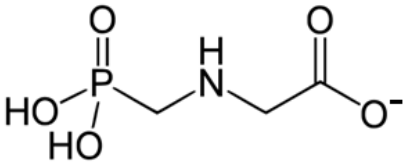


Acquisition modes	
MS	MS2
Full scan	PRM
SIM	vDIA
	AIF
	Data dependent

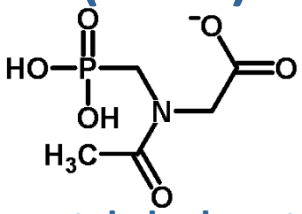
Parallel Reaction Monitoring (PRM)



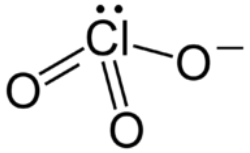
List of Pesticides (n=10)



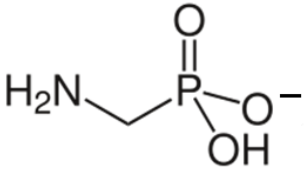
Glyphosate
(*m/z* 168.0067)



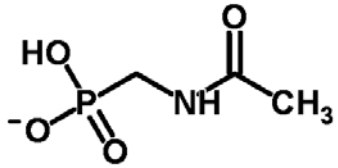
N-acetyl glyphosate
(*m/z* 210.0173)



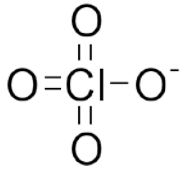
Chlorate
(*m/z* 82.9541)



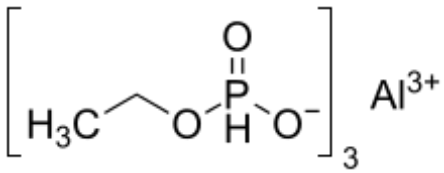
Aminomethylphosphonic acid
(AMPA)
(*m/z* 110.0012)



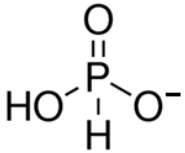
N-acetyl aminomethylphosphonic acid
(N-acetyl AMPA)
(*m/z* 152.0118)



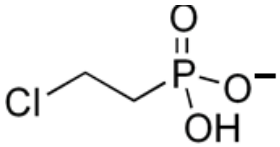
Perchlorate
(*m/z* 98.9491)



Fosetyl-aluminium
(*m/z* 109.0060)



Phosphonic acid
(*m/z* 80.9747)



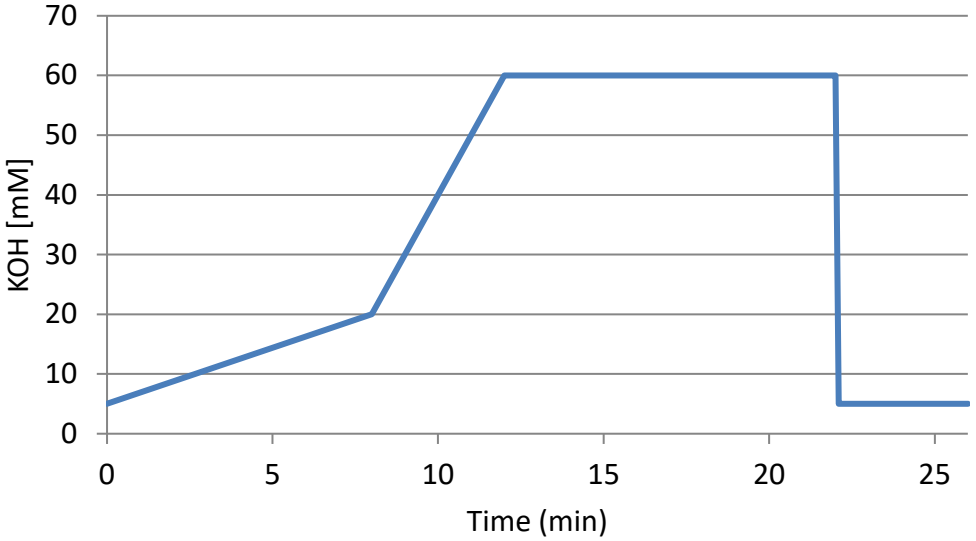
Etephon
(*m/z* 142.9670)

Bromide ion
(*m/z* 78.9189)

IC-MS Conditions

- Column Dionex IonPac AS19 (250mm x 2mm x 4µm)
- Guard column Thermo Scientific ™ Dionex™ IonPac™ AG19 (50mm x 2mm x 4µm)
- Column temperature 40°C
- Mobil phase flow 0.35 ml/min
- Suppressor flow 0.60 ml/min
- Suppressor current 52 mA
- Make-up solvent: acetonitrile
- Make-up solvent flow: 0.40 ml/min
- Injection volume: 50 µL
- Dilution factor: 5

KOH gradient



Workflow: FS-MS + SIM-MS + PRM-MS²

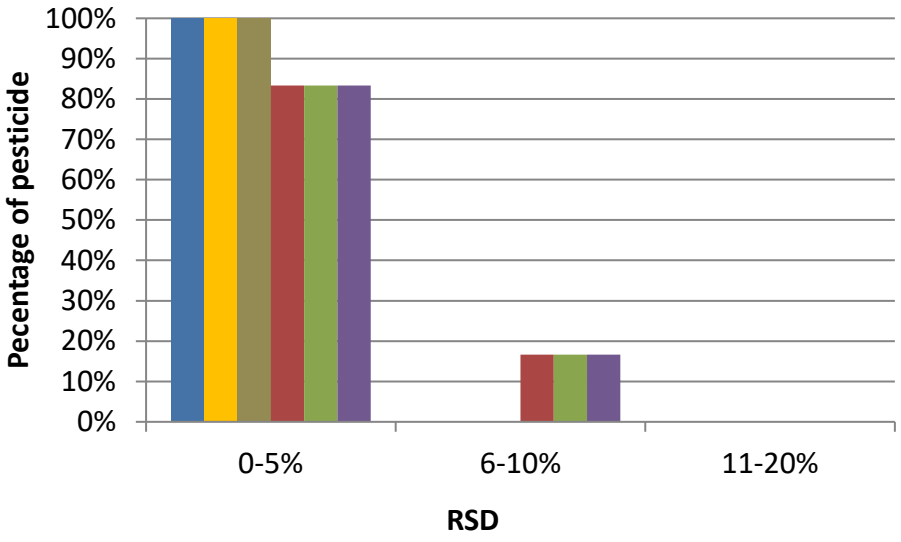
- **MS:**
  - Range 1: *m/z* 78 – 212
  - Range 2: *m/z* 109.5 – 110.5 (for AMPA)
  - Resolution 70,000 (at *m/z* 200)
  - AGC target 1e6
  - Max IT auto

- **MS²:**
  - PRM
  - Resolution 17,500 (at *m/z* 200)
  - Quadrupole isolation window 1Da
  - AGC target 1e6
  - Max IT auto

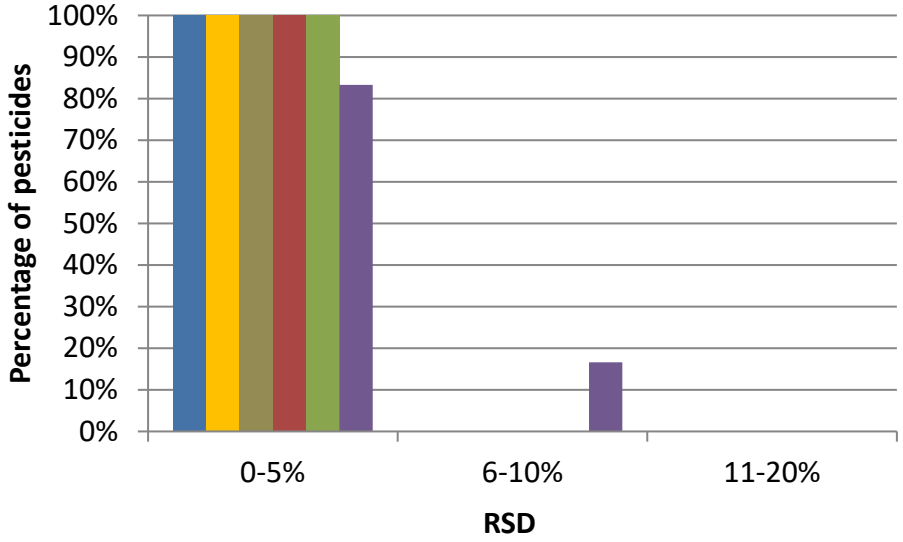
Peak area repeatability

(n = 5)

0.01 mg/kg

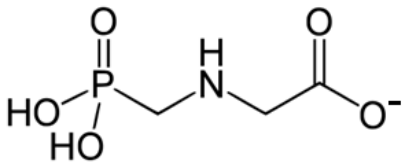


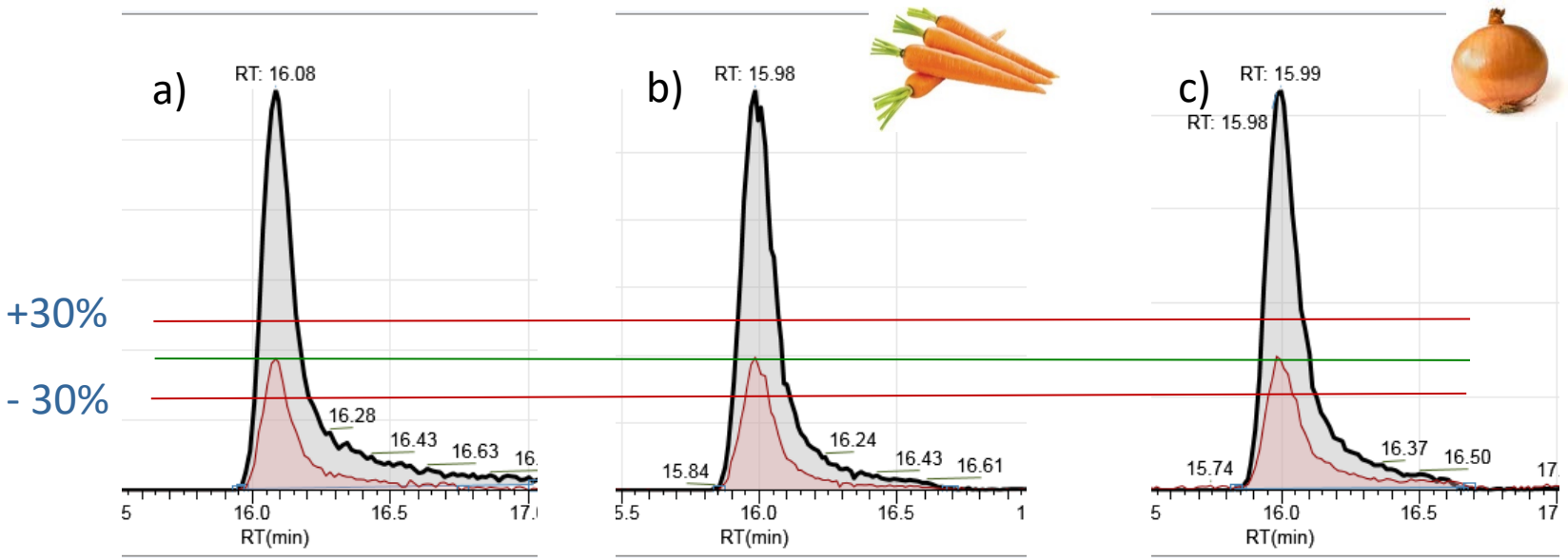
0.05 mg/kg



Ion ratio robustness

0.01 mg/kg of glyphosate





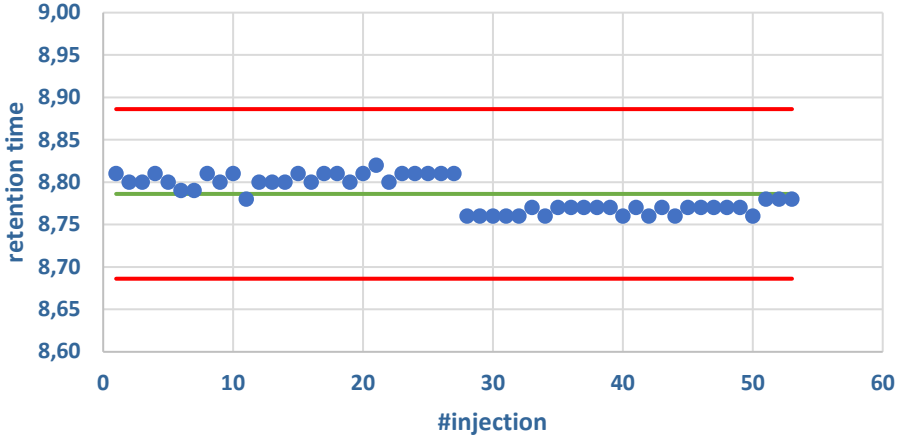
Solvent Carrot Onion

Retention time stability

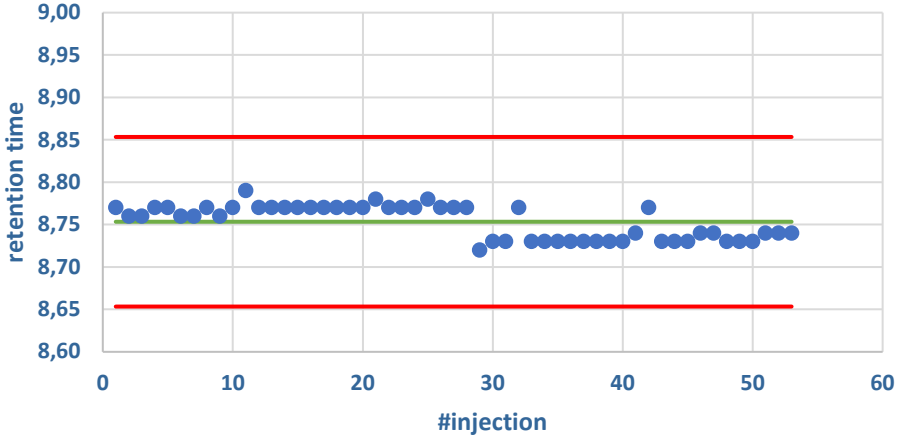
0.05 mg/kg in carrot

(0.005 mg/kg in the vial)

Glyphosate



N-acetyl-glyphosate

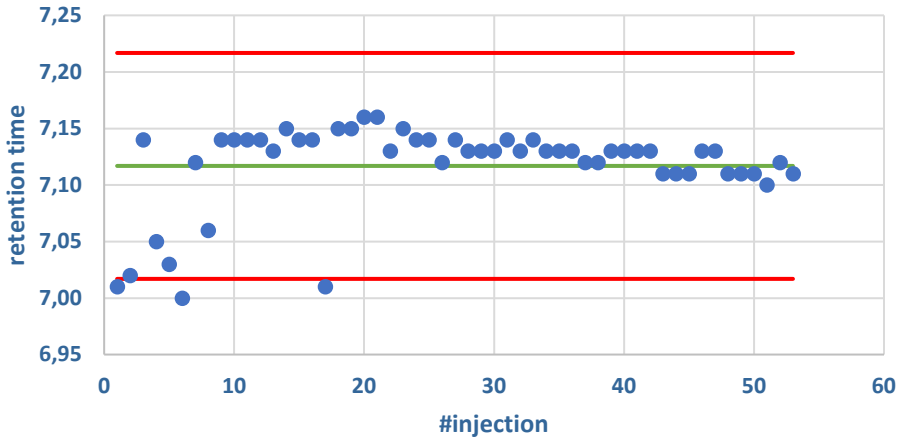


Retention time stability

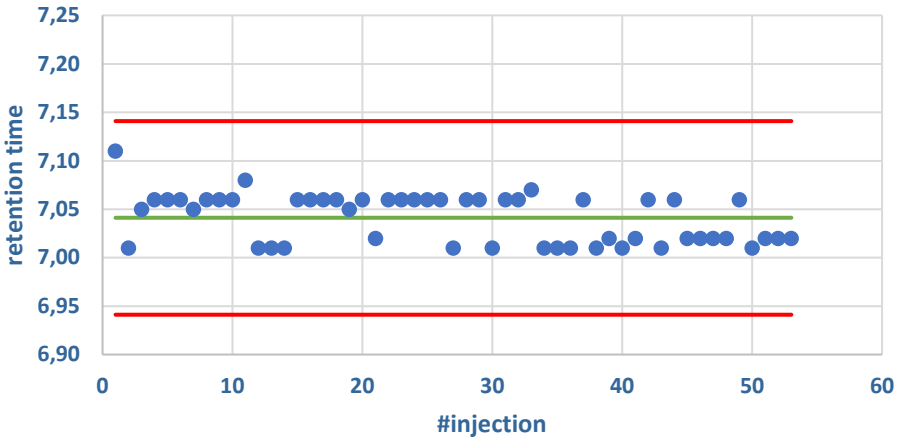
0.05 mg/kg in carrot

(0.005 mg/kg in the vial)

AMPA

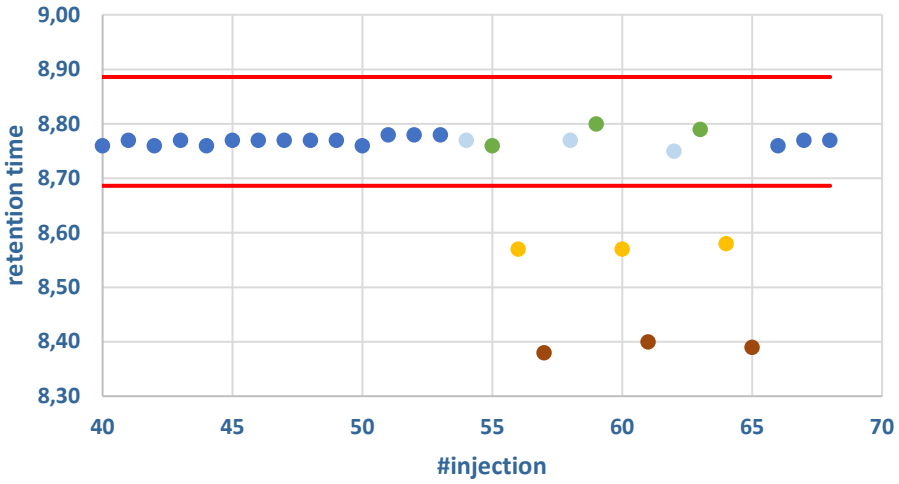


N-acetyl-AMPA

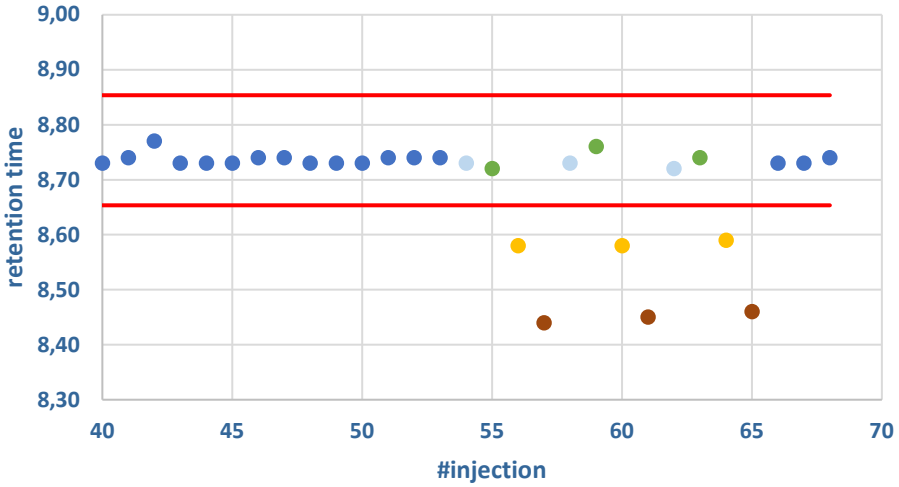


Retention time stability

Glyphosate



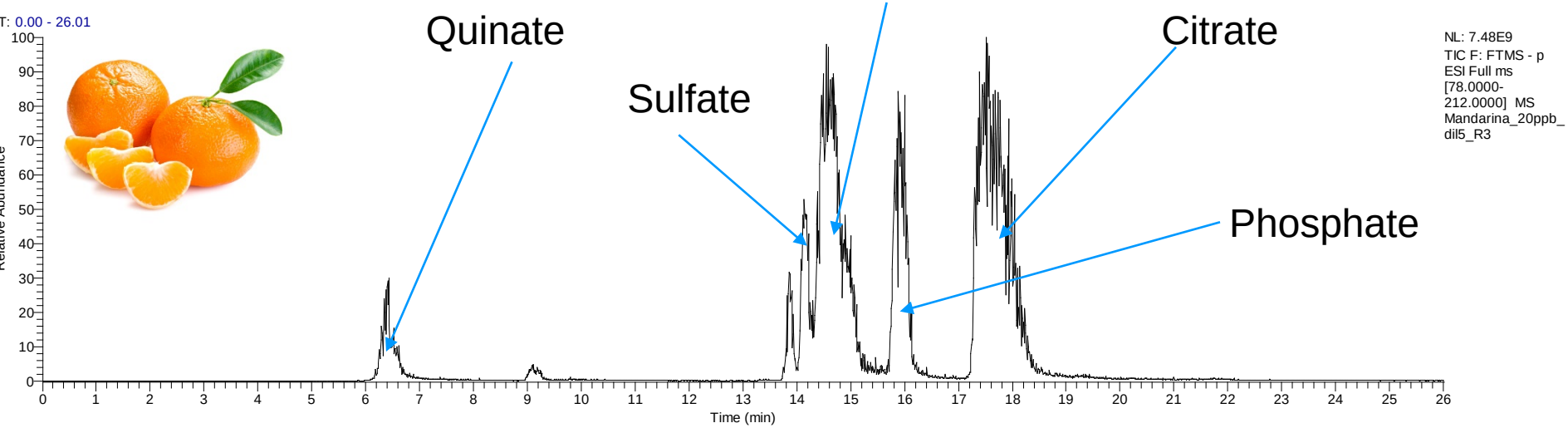
N-acetyl-glyphosate

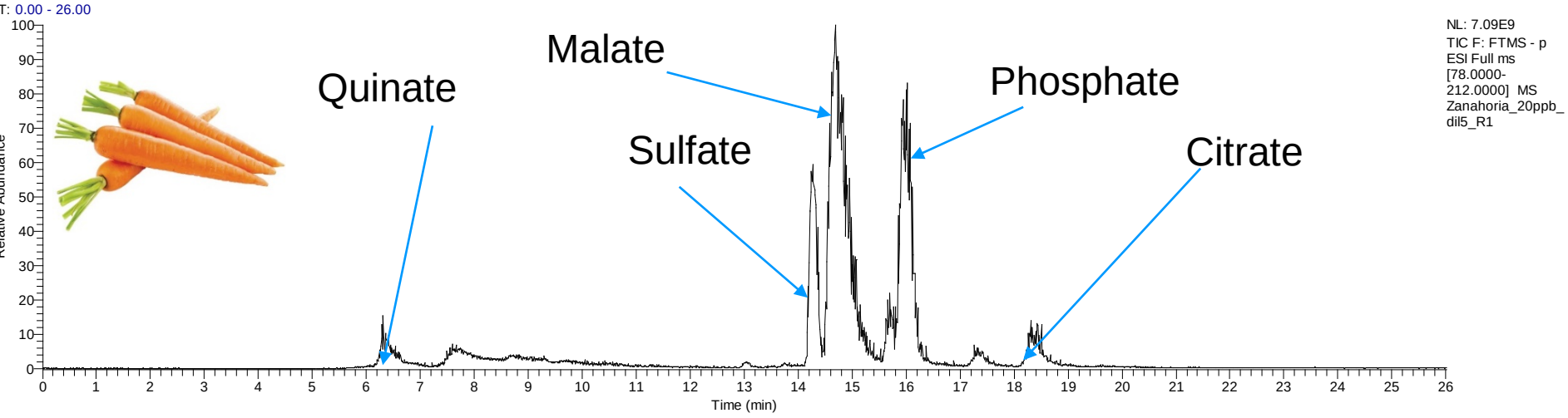


- Carrot 0.05 mg/kg
- Carrot 0.005 mg/kg
- Apple 0.05 mg/kg
- Mandarin 0.05 mg/kg
- Strawberry 0.05 mg/kg

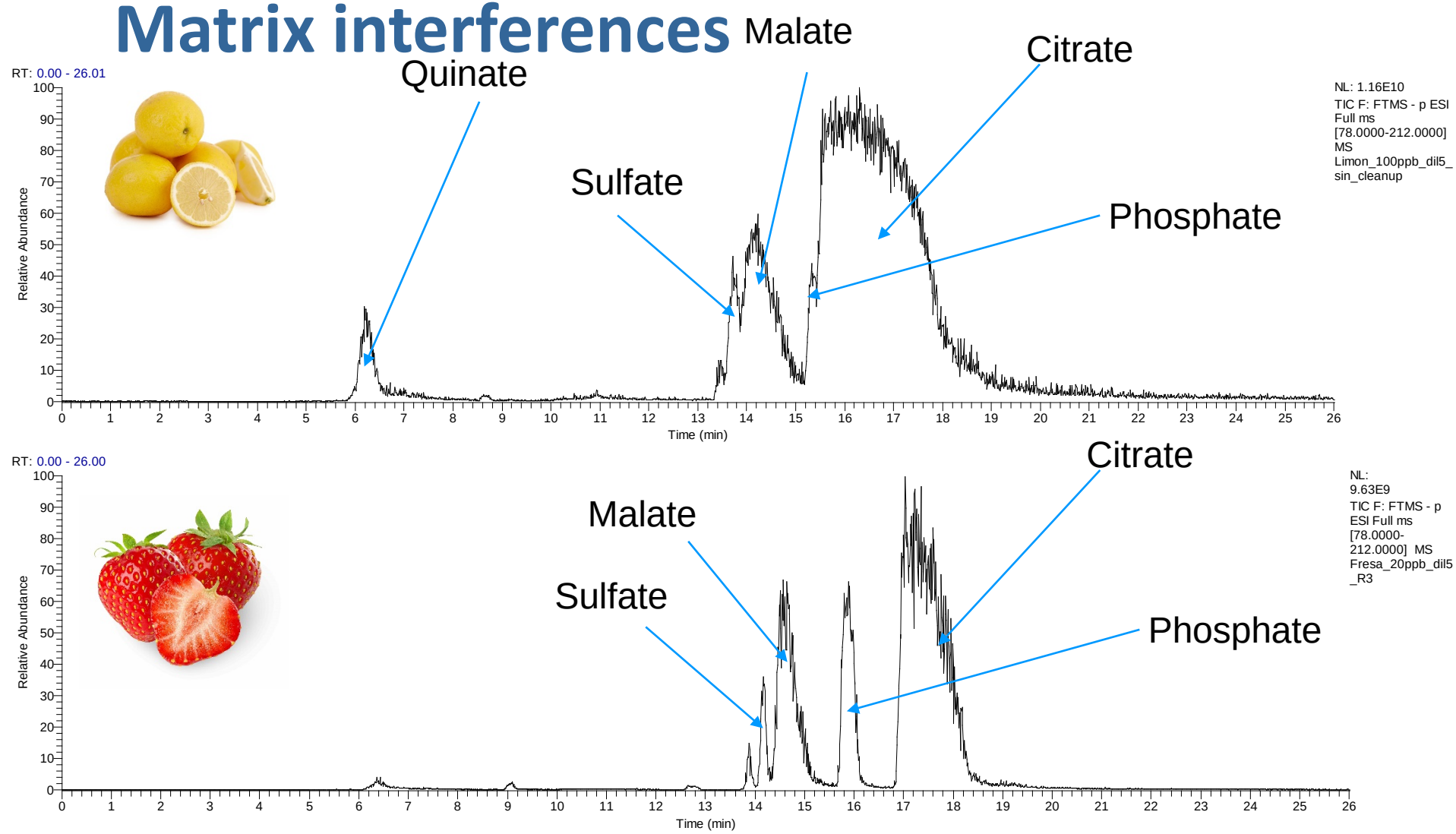
- Carrot 0.05 mg/kg
- Carrot 0.005 mg/kg
- Apple 0.05 mg/kg
- Mandarin 0.05 mg/kg
- Strawberry 0.05 mg/kg

Matrix interferences

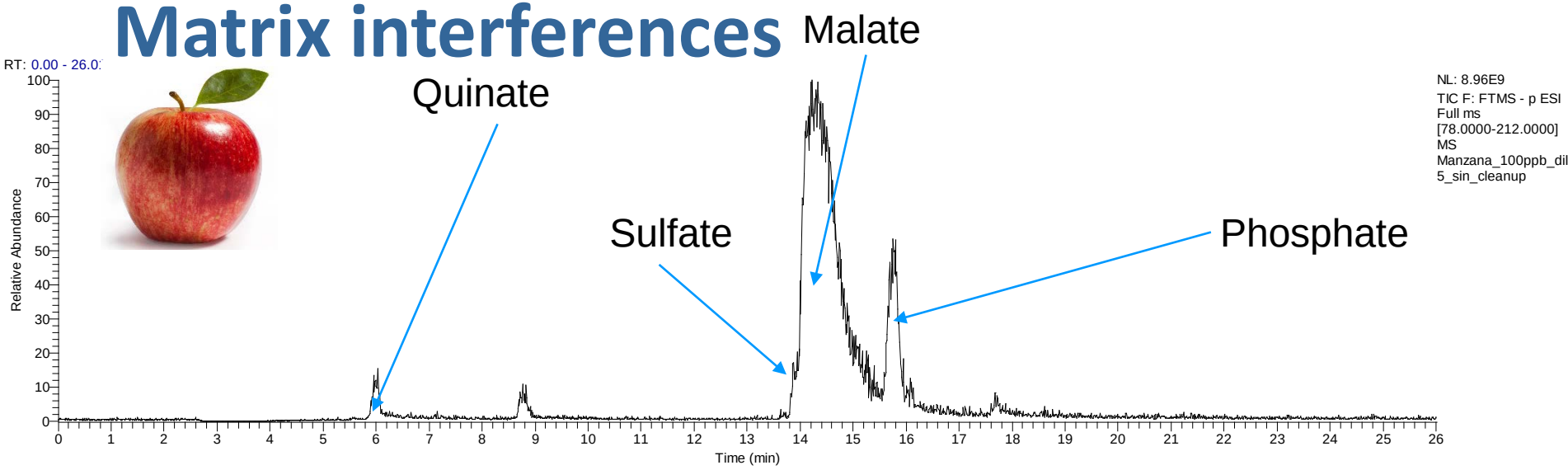


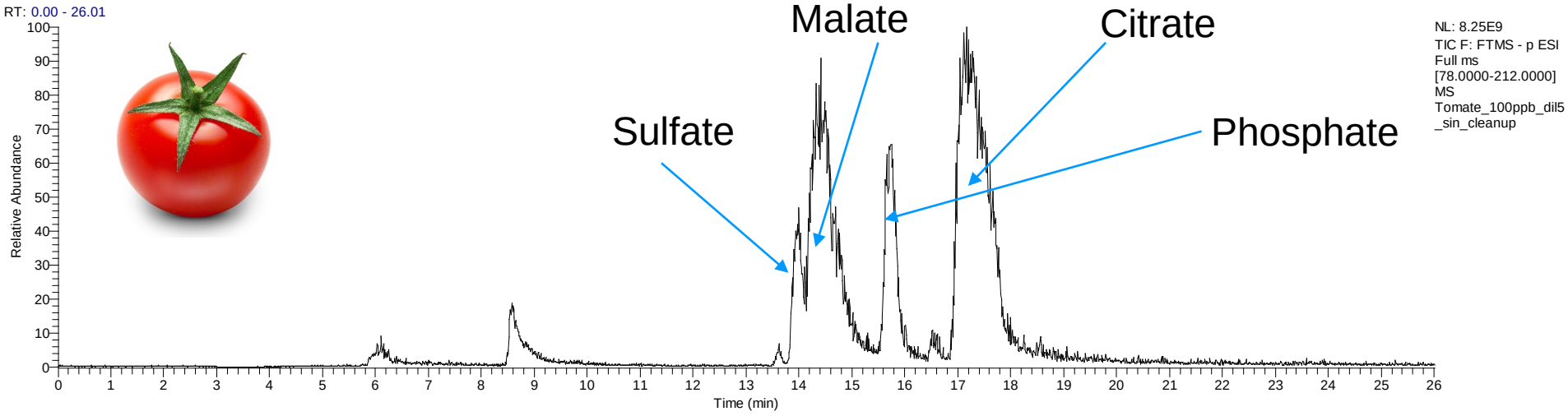


Matrix interferences



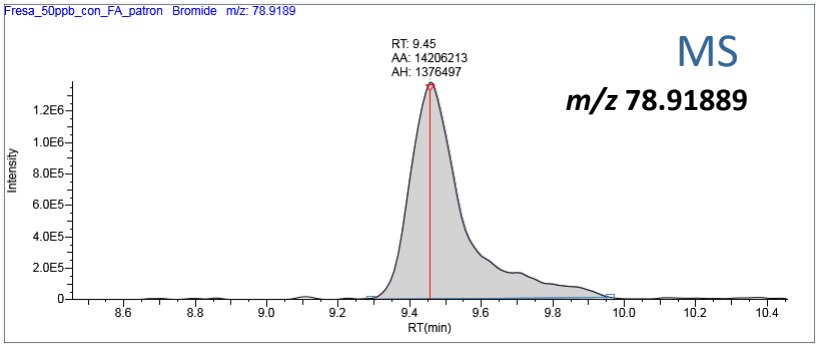
Matrix interferences

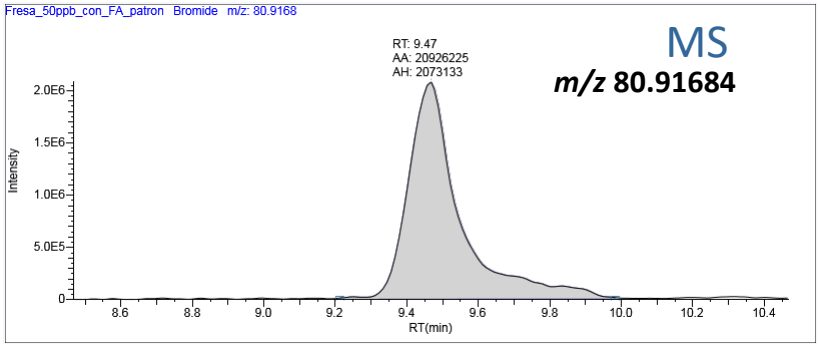




Advantages of high resolution mass spectrometry

Bromide ion





Triple quadrupole:

Transition (?) 1: $^{79}Br^- \rightarrow {}^{79}Br^-$

Transition (?) 2: $^{81}Br^- \rightarrow {}^{81}Br^-$

} No fragmentation

High resolution:

Ion 1: 78.91889

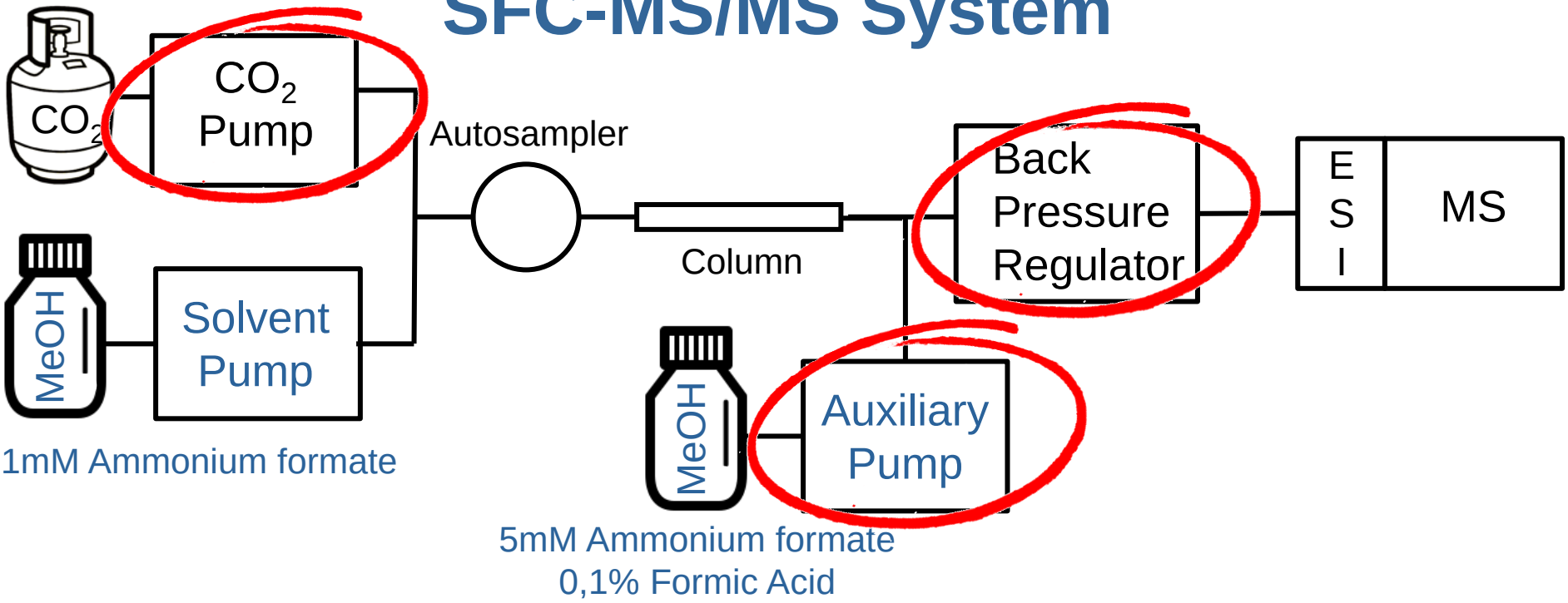
Ion 2: 80.91684

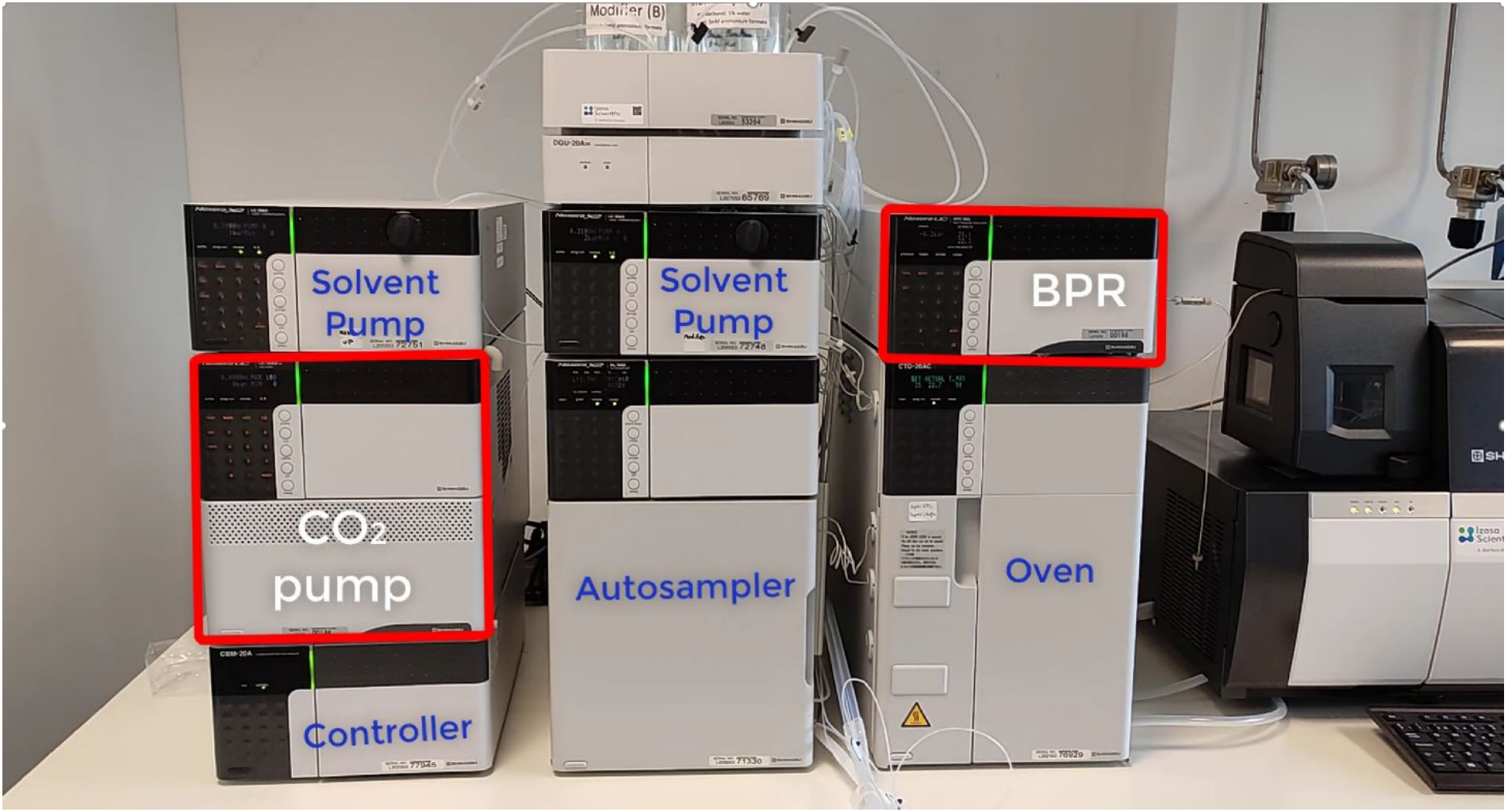
} Very high selectivity

Advantages of SFC-MS/MS

(Nexera UC coupled to Shimadzu LC-MS 8060)

SFC-MS/MS System

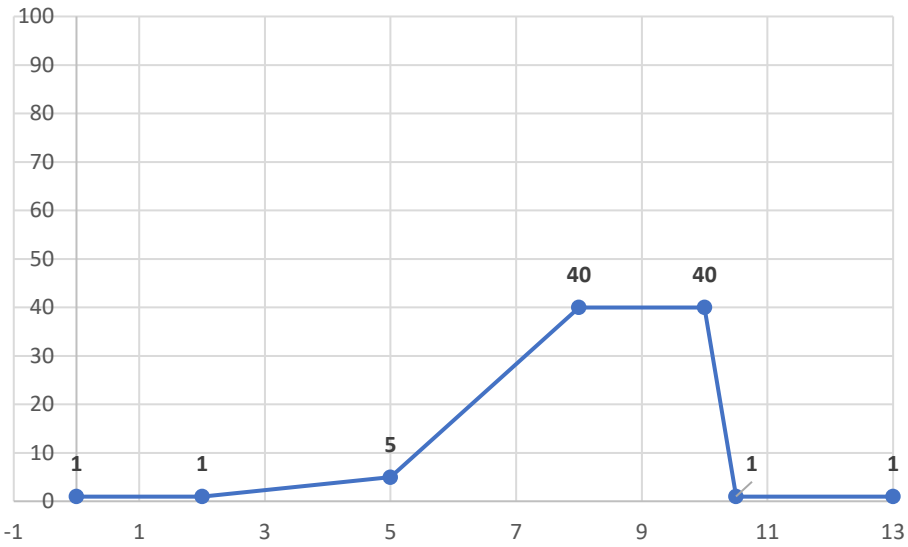



Modifier (B)
Solvent Pump
Solvent Pump
BPR
CO_2 pump
Autosampler
Oven
Controller

SFC

Run time: 13 min
Flow: 1,3mL/min

Make-up Flow: 0,080mL/min

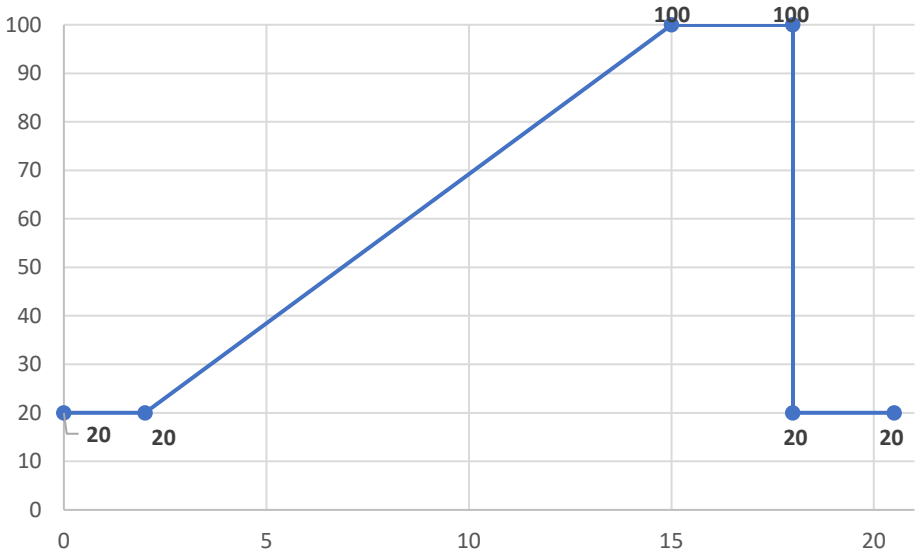


TOTAL RUN CONSUMPTION

MeOH (Gradient) : 2,22mL
MeOH (Make-up): 0,96
} 3,19 mL
CO2 Consumption : 13,7mL
Water: 0mL

LC

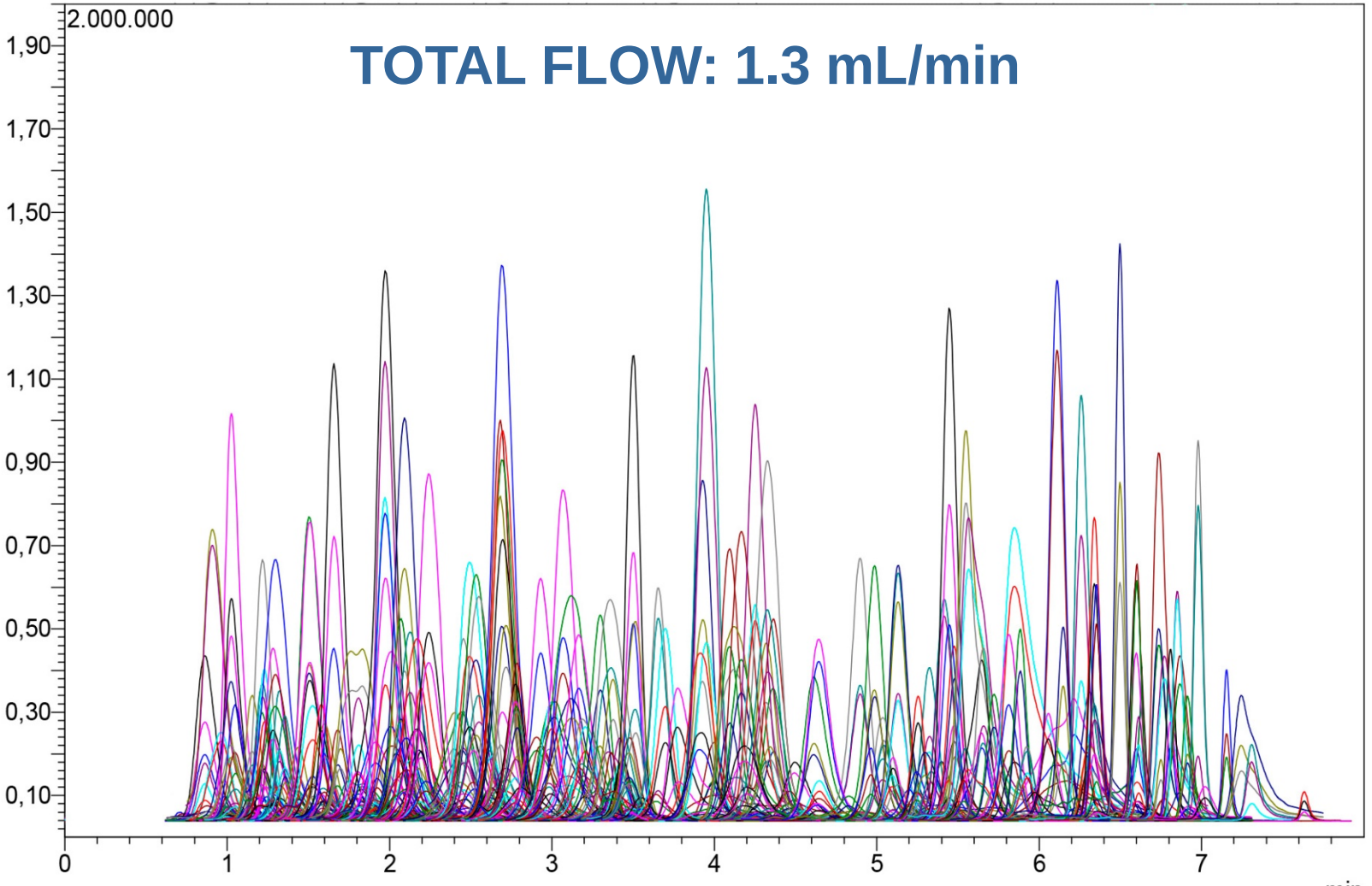
Run time: 20,5min
Flow: 0,3mL/min



TOTAL RUN CONSUMPTION

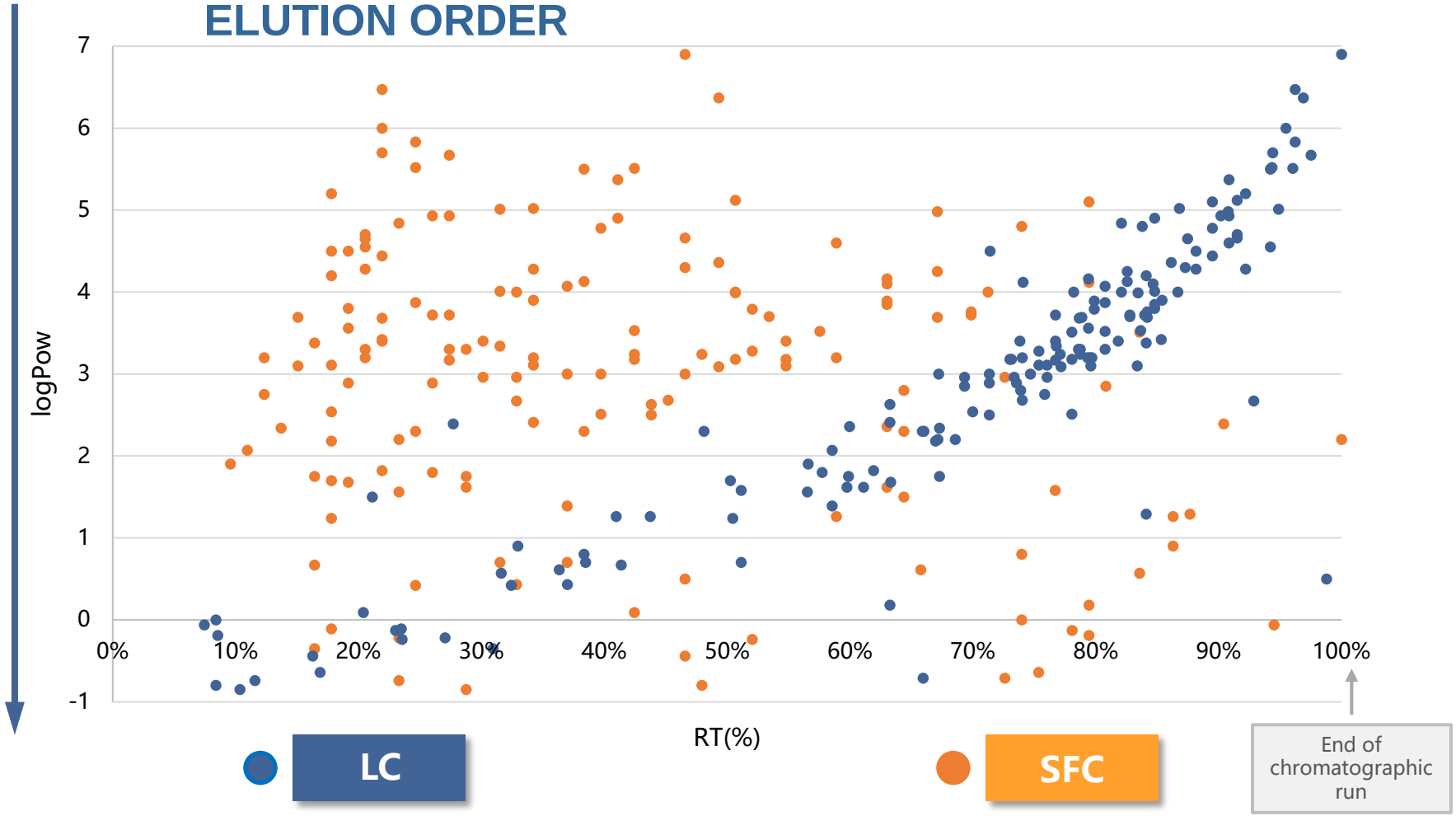
MeOH : 3,51mL
Water : 2,64mL

Pressures: 270 – 350 bar



5 µg/Kg Tomato (218 pesticides)

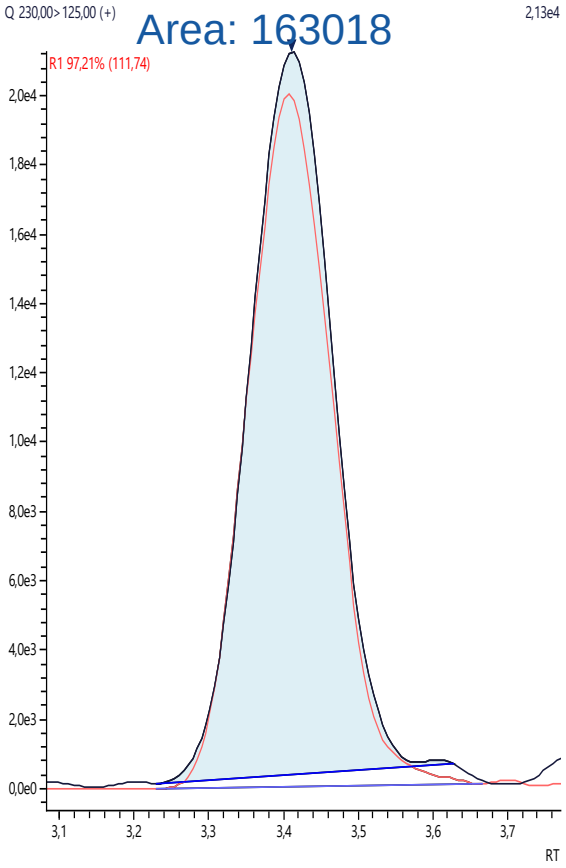
ELUTION ORDER



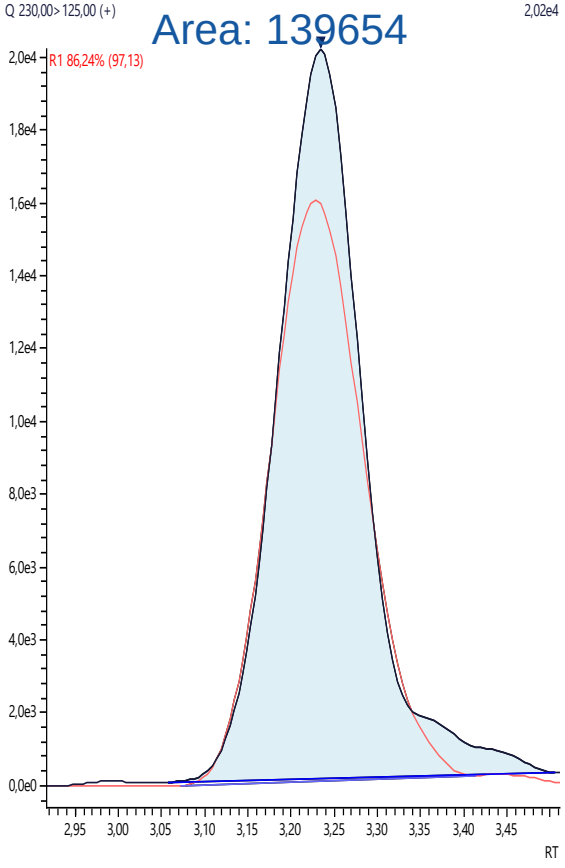
In LC, there is a clear trend; the compounds elute in decreasing order of polarity.
SFC does not follow any polarity criteria for elution.

Dimethoate

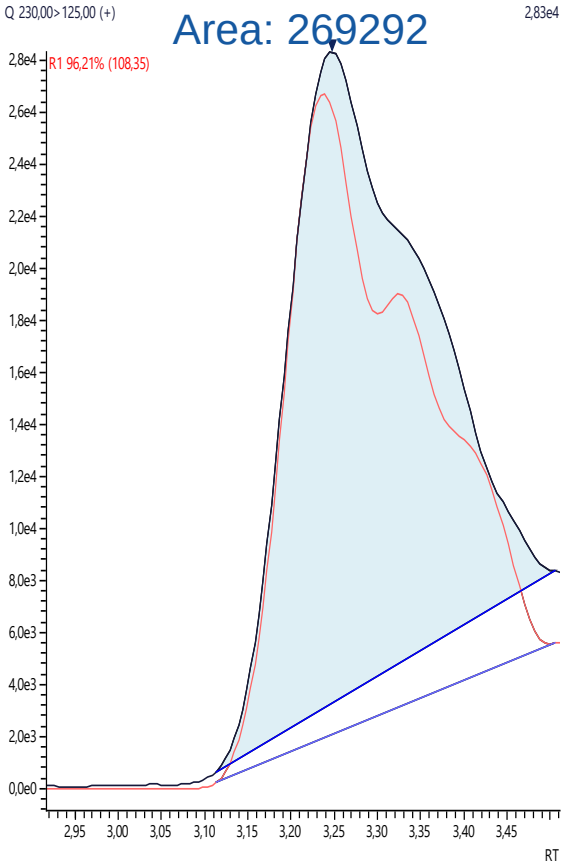
Vial conc: 1 µg/Kg
ACN 1:4 H_2O
Inj.volumen: **2µL**
Area: 163018



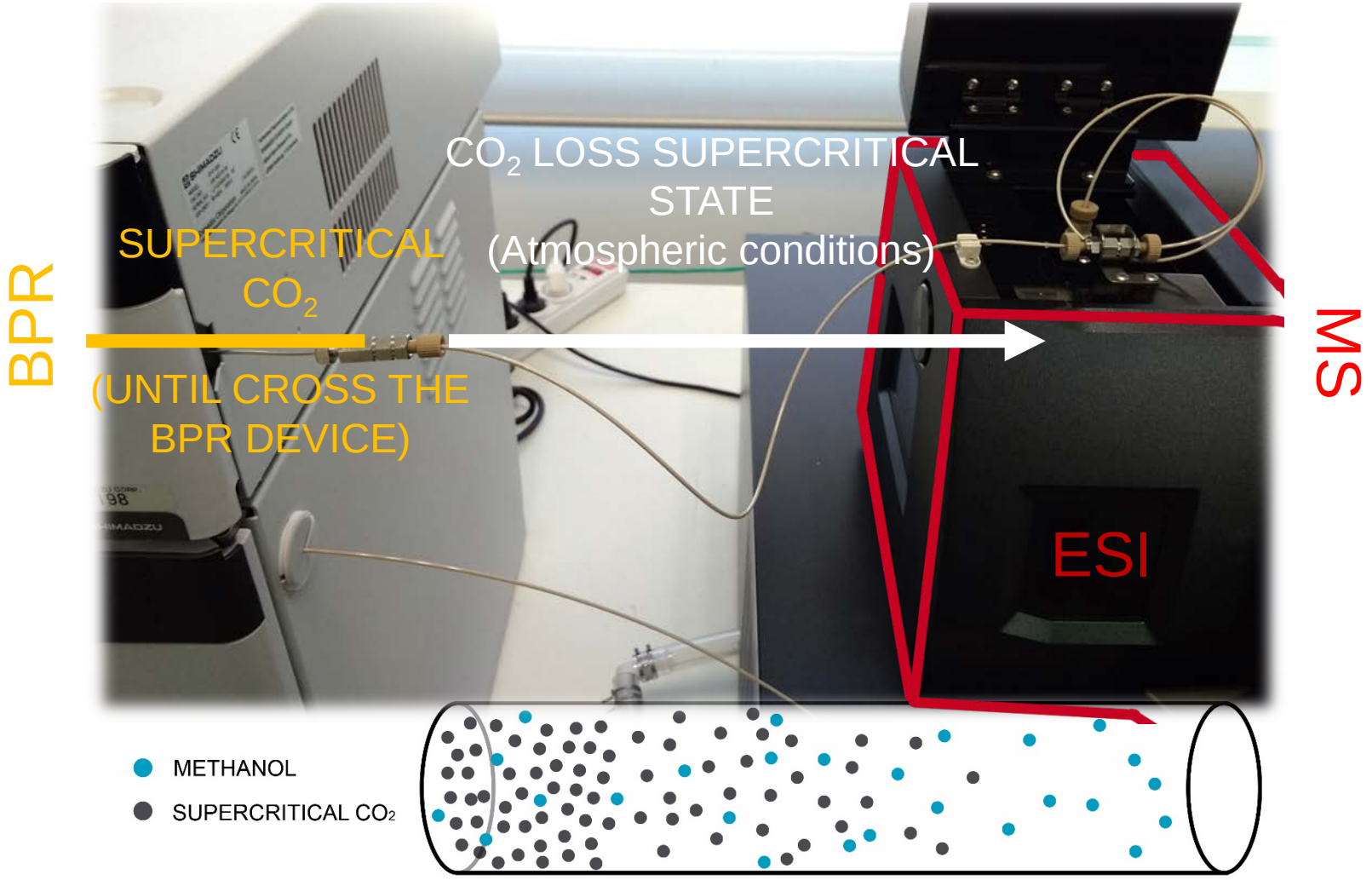
Vial conc: 1 µg/Kg
100% H_2O
Inj.volumen: **2µL**
Area: 139654



Vial conc: 1 µg/Kg
100% H_2O
Inj.volumen: **5µL**
Area: 269292



CO2 loss his supercritical state before ionization

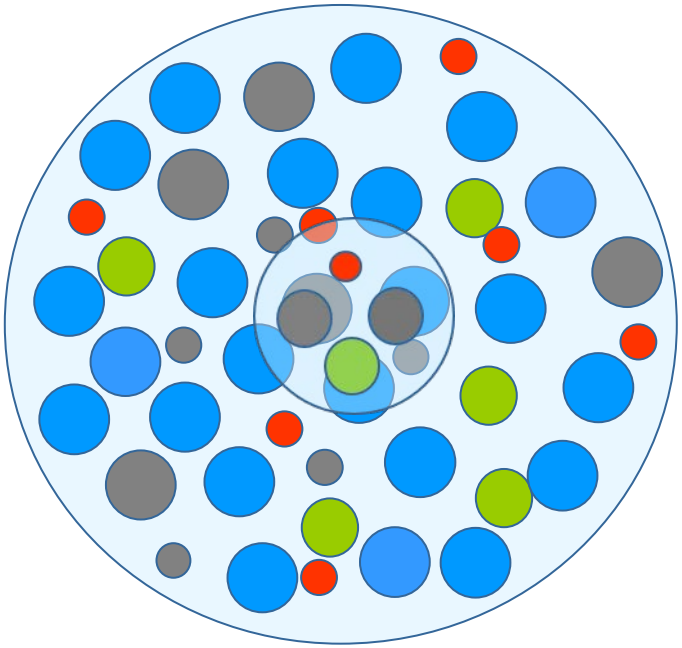


SMALL AMOUNT OF ORGANIC SOLVENT REACHING THE SOURCE

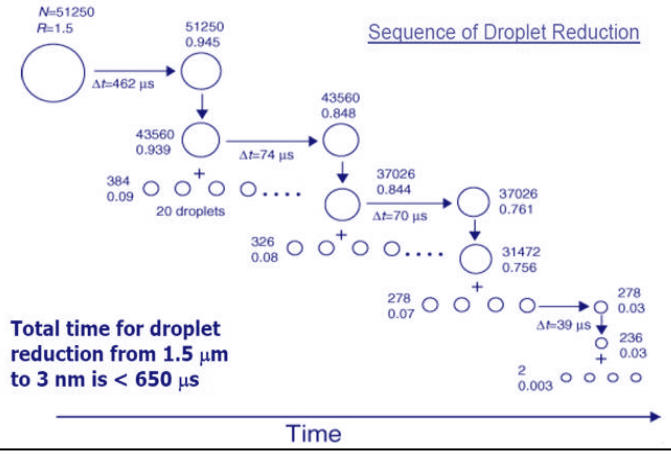
IONIZATION PROCESS

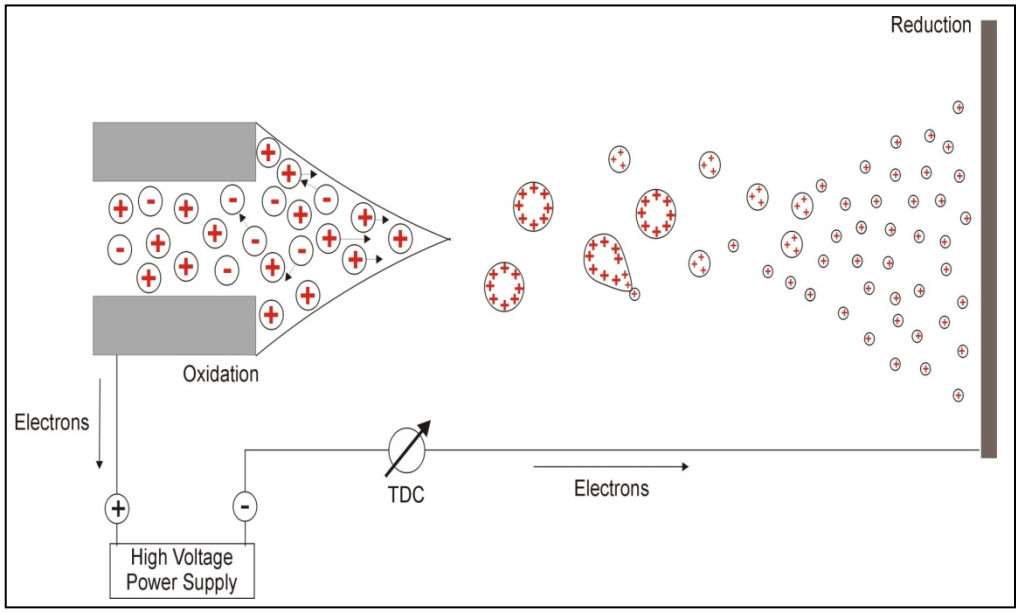
Low methanol flow

70% of compounds: <140 µL/min
(Including make-up solvent)



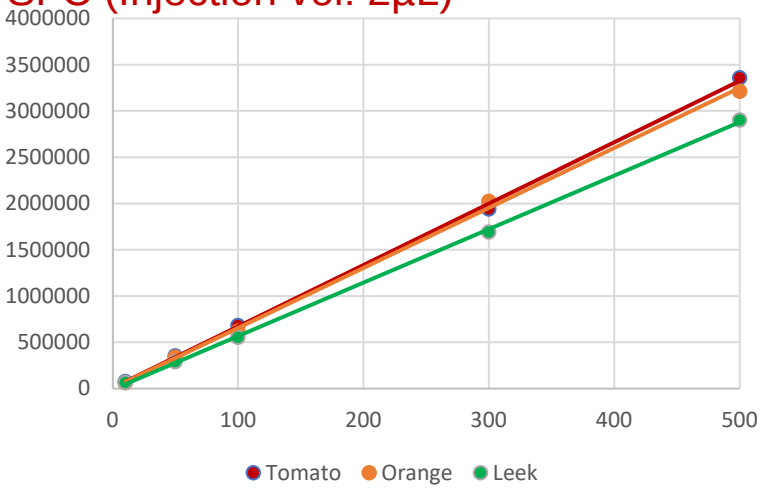
Ion
Water
Matrix
Methanol





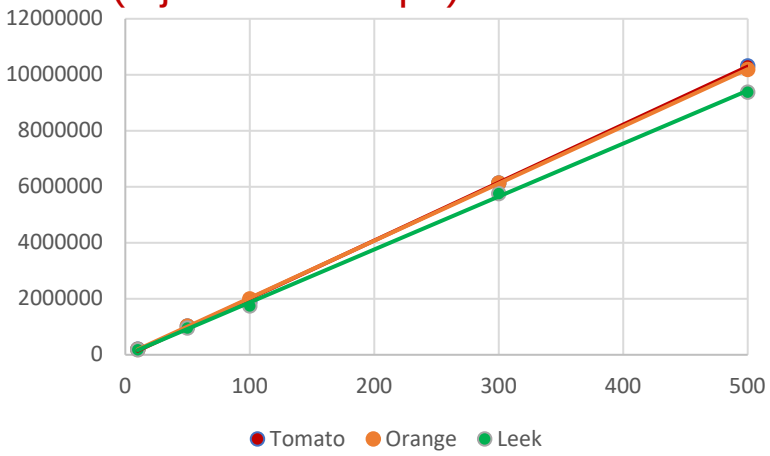
CARBARYL

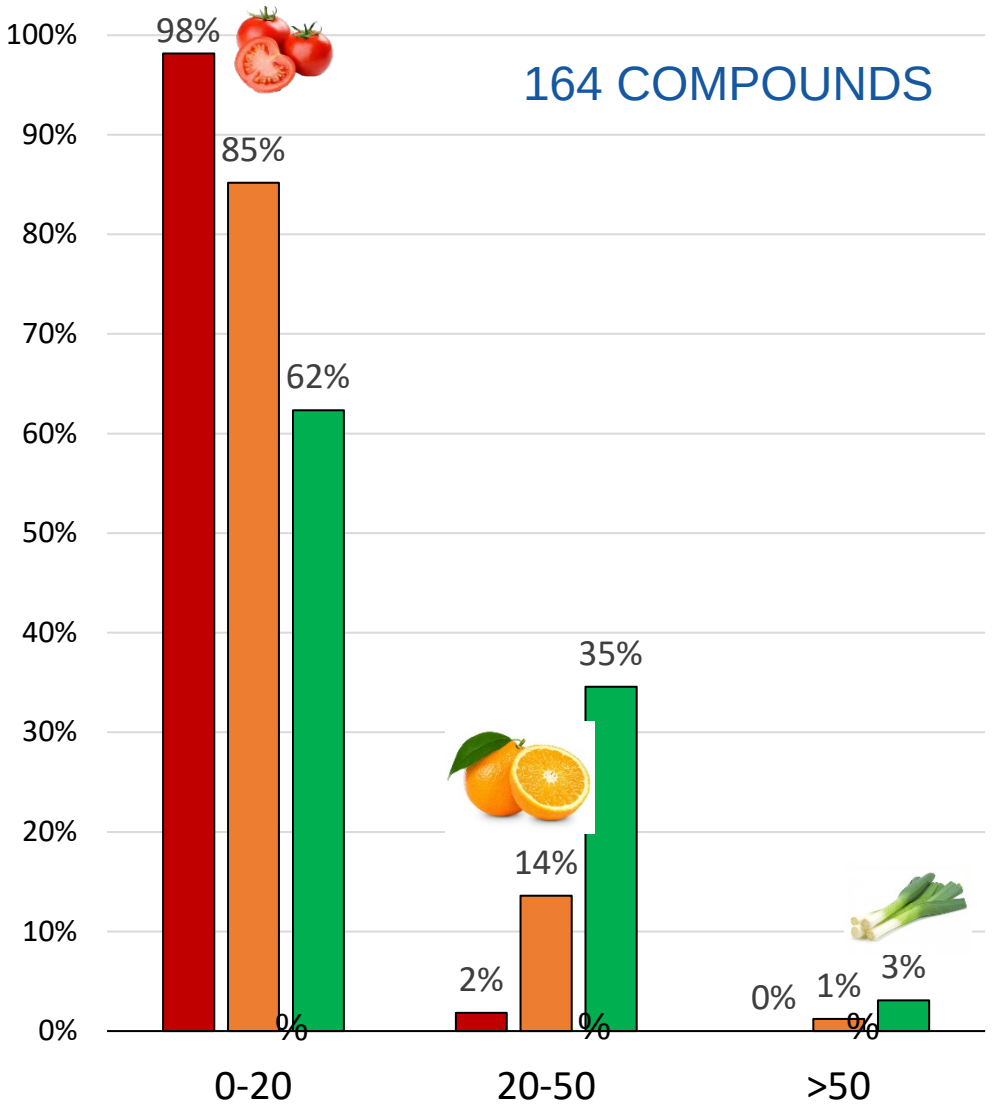
SFC (Injection vol: 2µL)

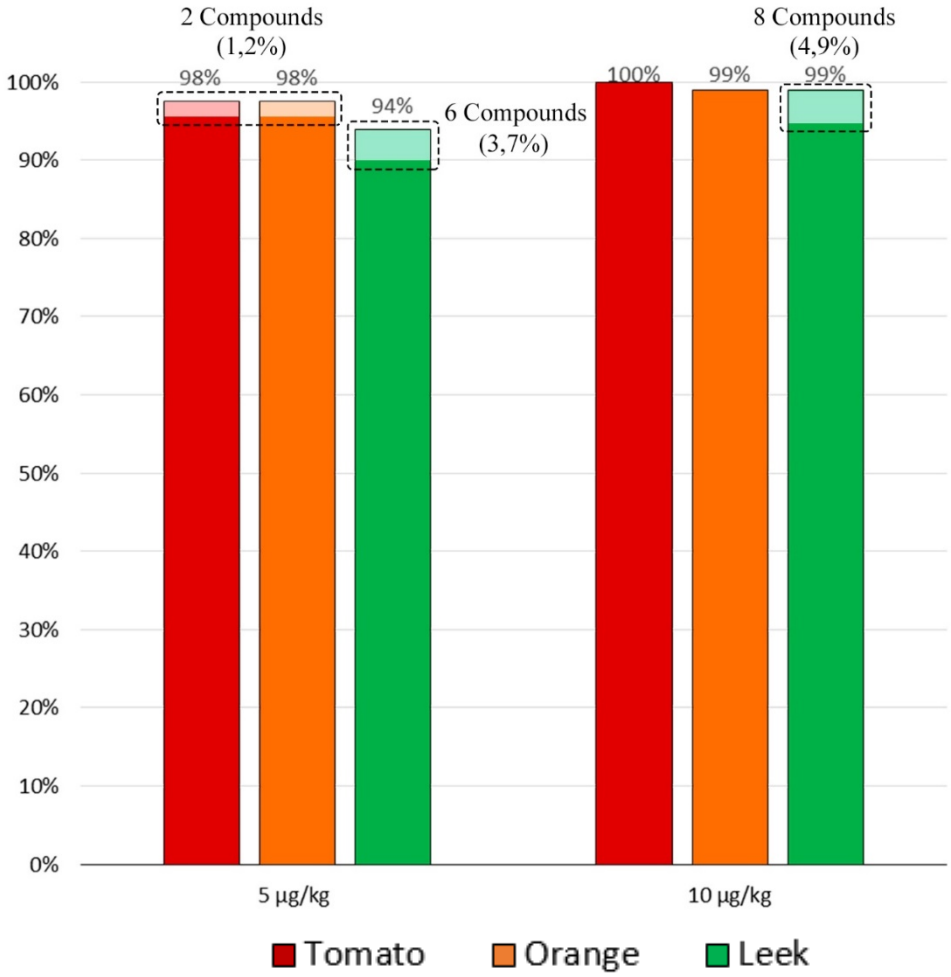


PROQUINAZID

SFC (Injection vol: 2µL)







Sample diluted 5 times: 0,4 µg/Kg in the vial

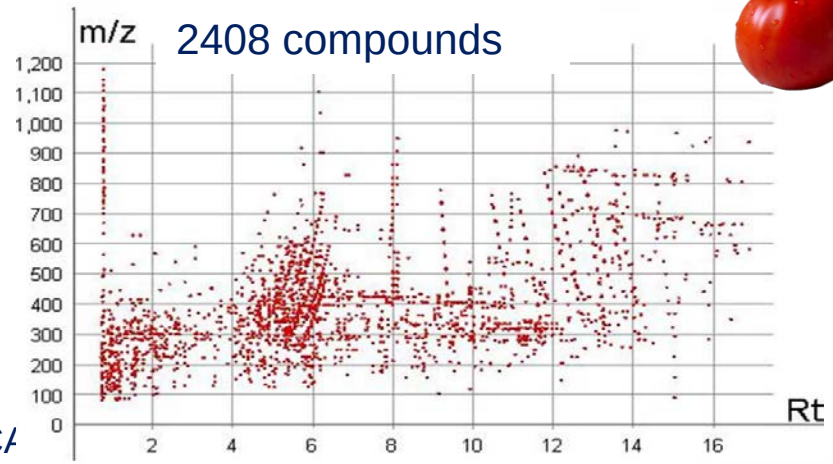
Tomato Orange Leek

IDENTIFIED COMPOUNDS (164)

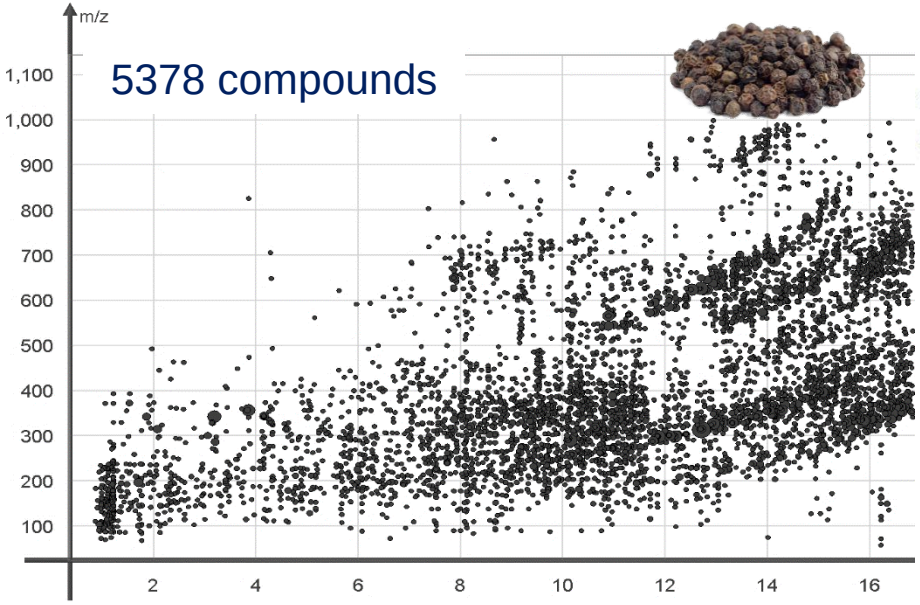
Inj.volumen: 2uL

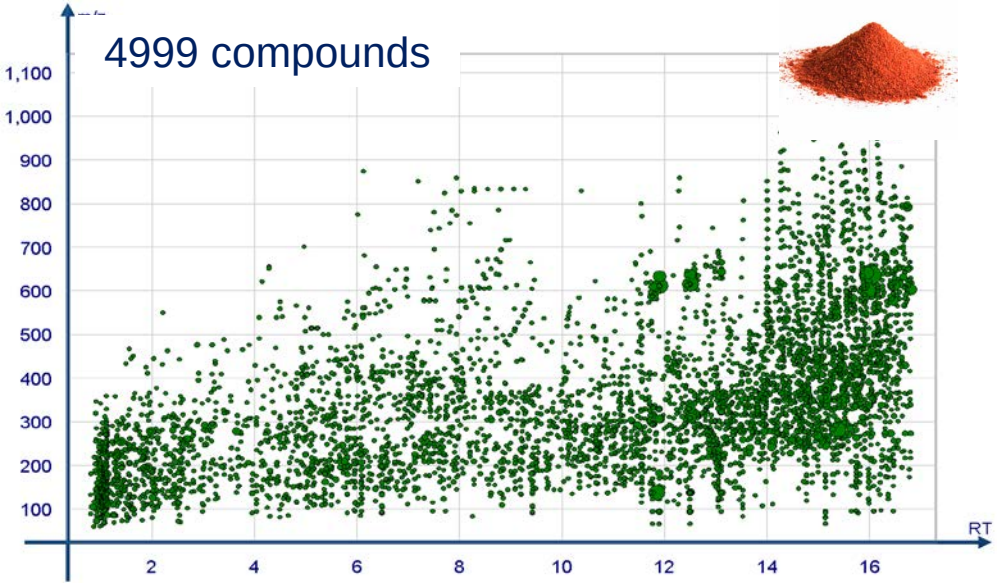
The area of each bar surrounded by a dashed-line box refers to those compounds that presented isobaric interferences and have been identified after adding a third transition.

CO-EXTRACTED MATRIX COMPONENTS (LC-QTOF-MS) Extract: 1g/mL



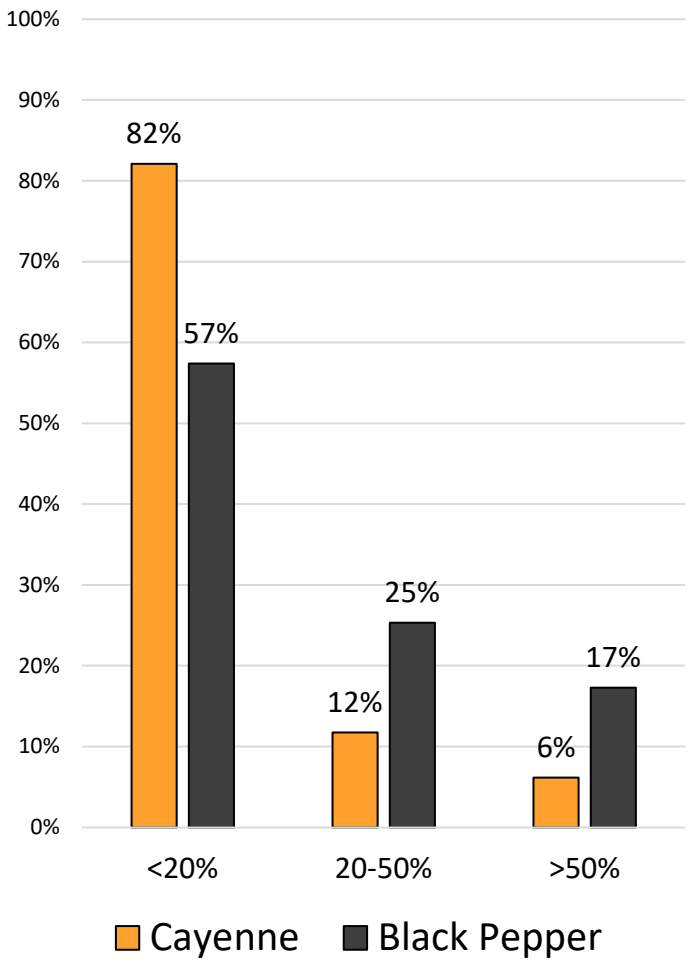
BLACK PEPPER



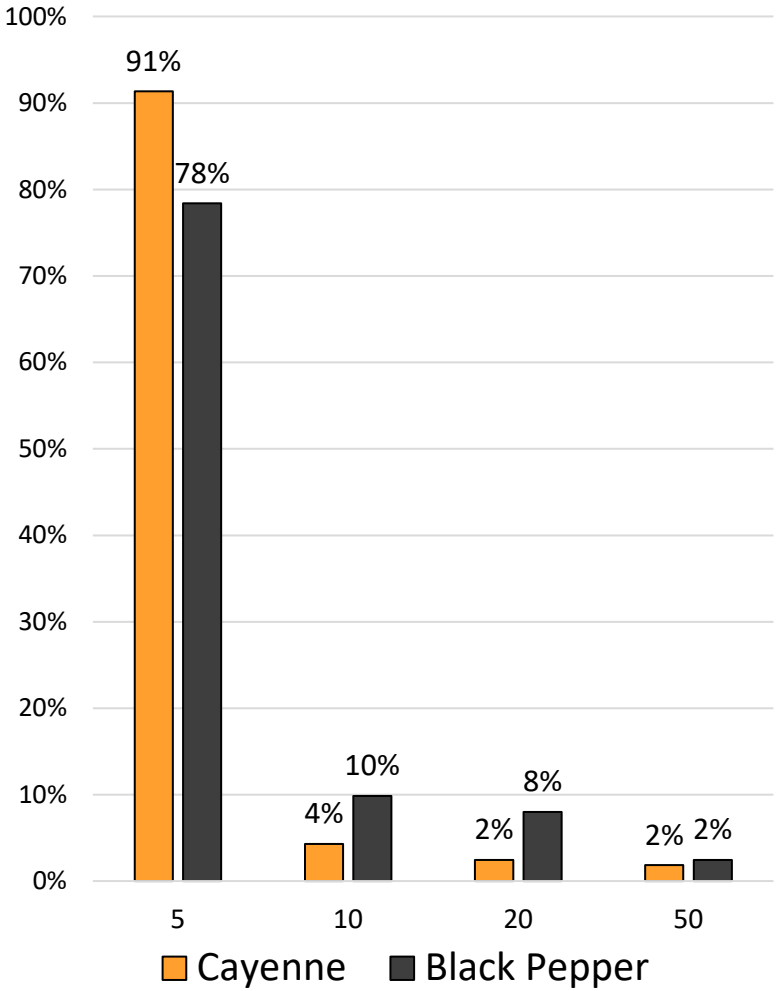


Total amount injected: 0.1 mg

MATRIX EFFECT

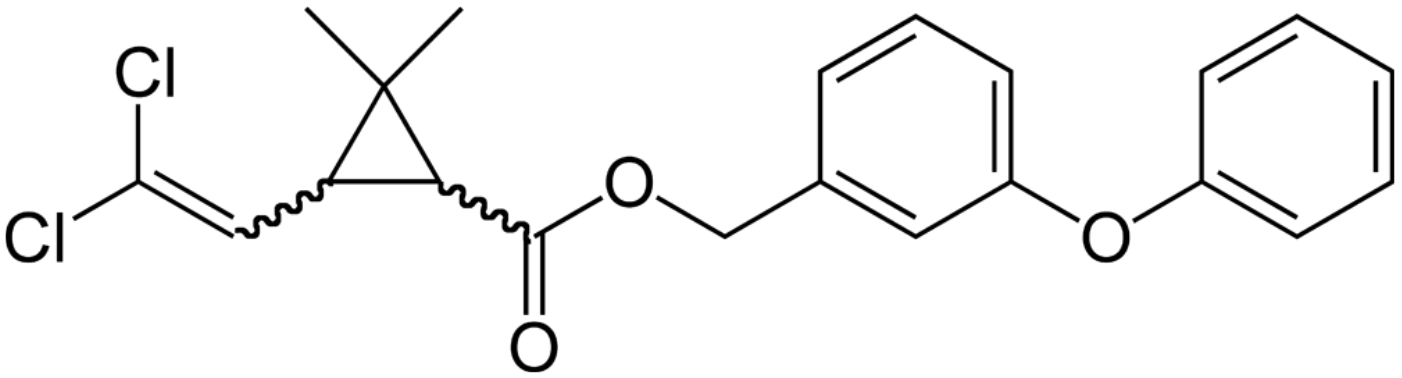


IDENTIFIED COMPOUNDS (162)

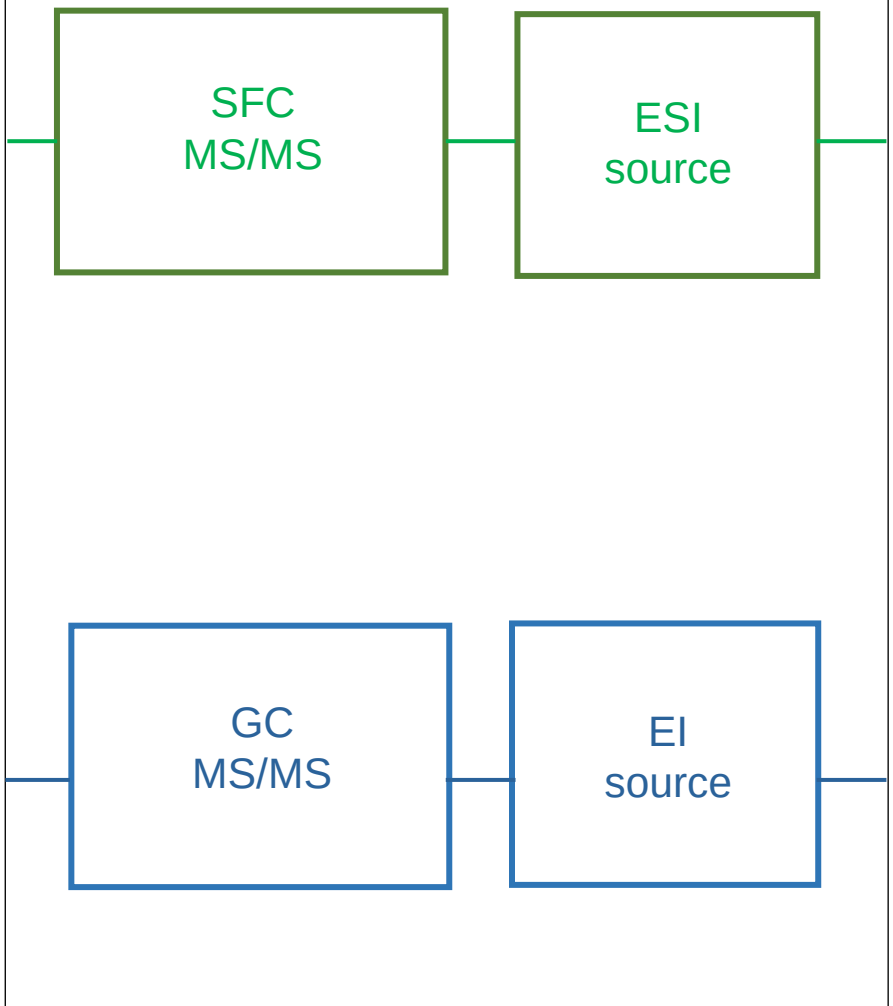


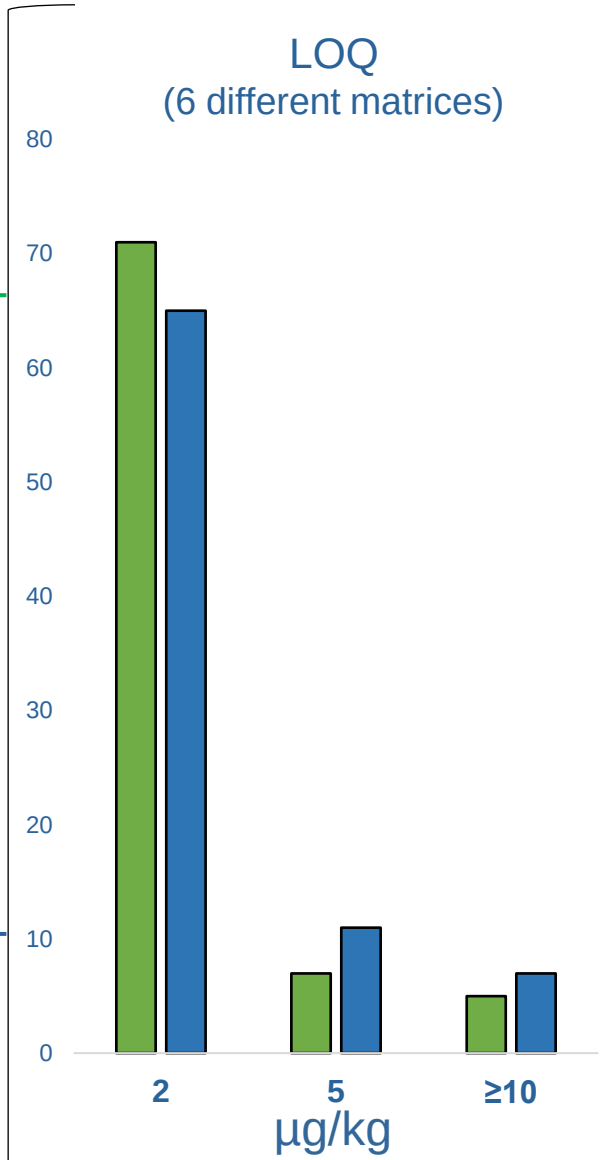
PYRETHROIDS

GC-MS/MS & SFC-MS/MS



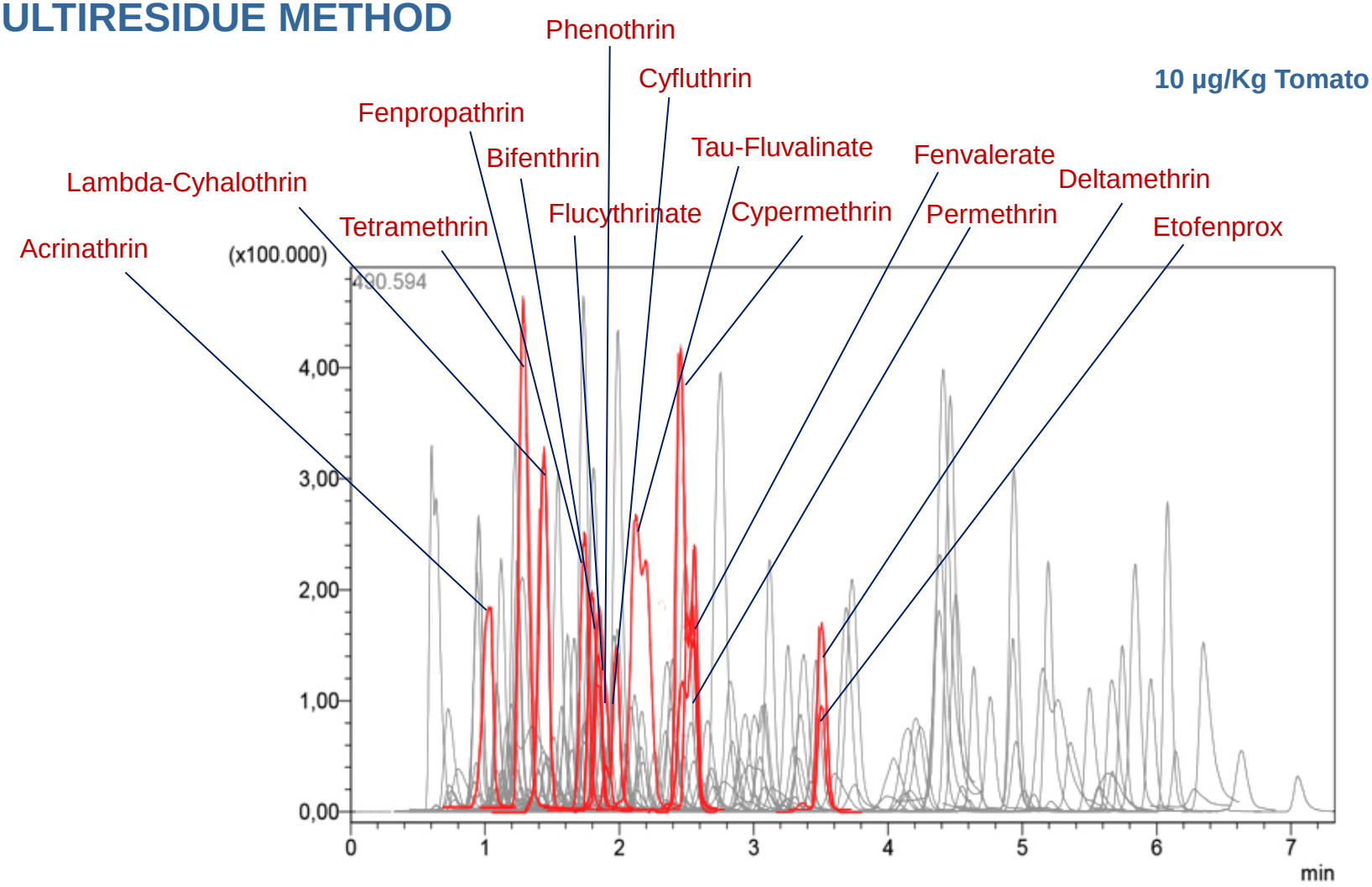
- Achrinathrin
- Bifenthrin
- Cyfluthrin
- Cypermethrin
- Deltamethrin
- Etofenprox
- Fenprotathrin
- Fenvalerate
- Flucythrinate
- Lambda-cyhalothrin
- Permethrin
- Phenothrin
- Tau-Fluvalinate
- Tetramethrin



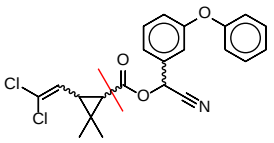


Pyrethroids

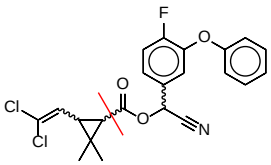
MULTIRESIDUE METHOD



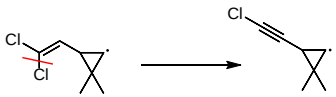
Cypermethrin



Cyfluthrin



GC



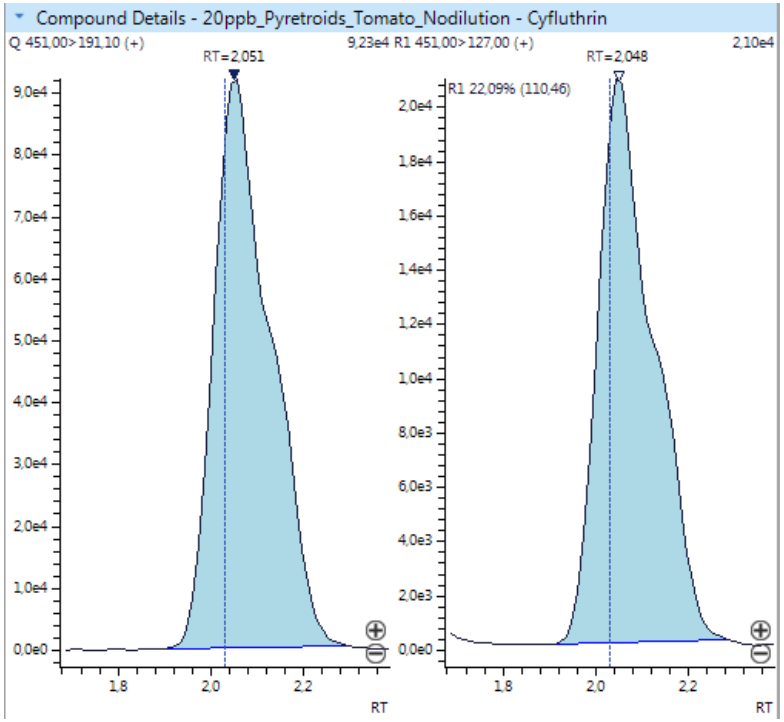
Chemical Formula: $C_7H_9Cl_2^{\bullet}$
Exact Mass: 163,0

Chemical Formula: $C_7H_8Cl^{\bullet}$
Exact Mass: 127,0

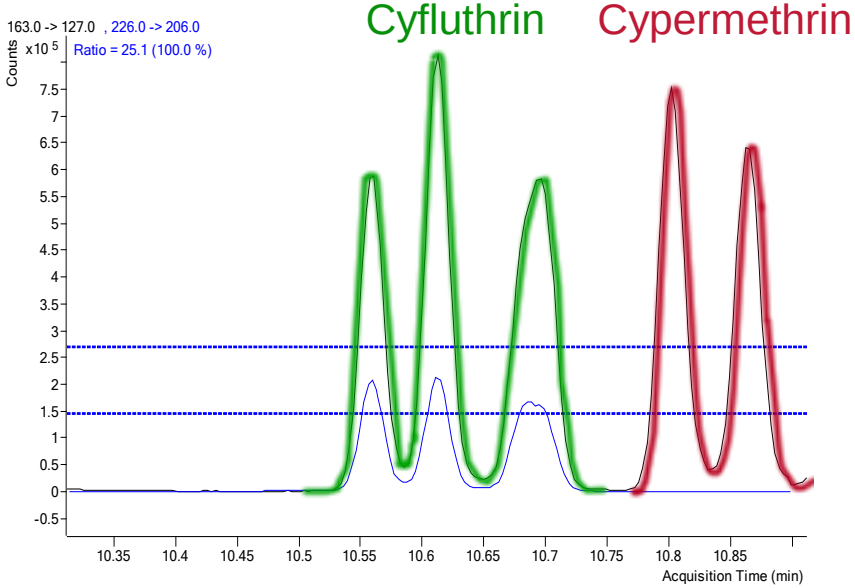
SFC

Cyfluthrin

451>191 451>127

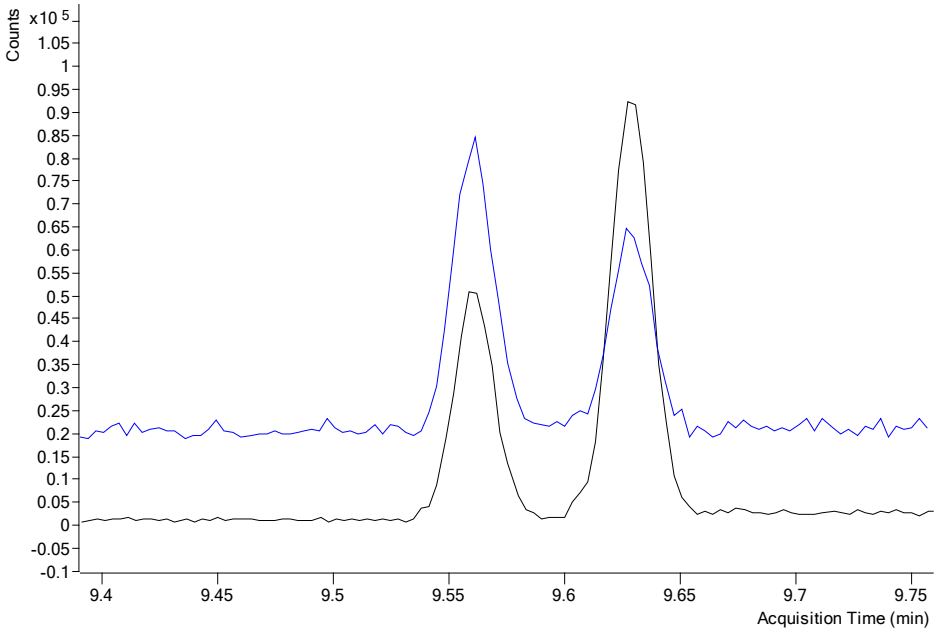


Cyfluthrin Cypermethrin

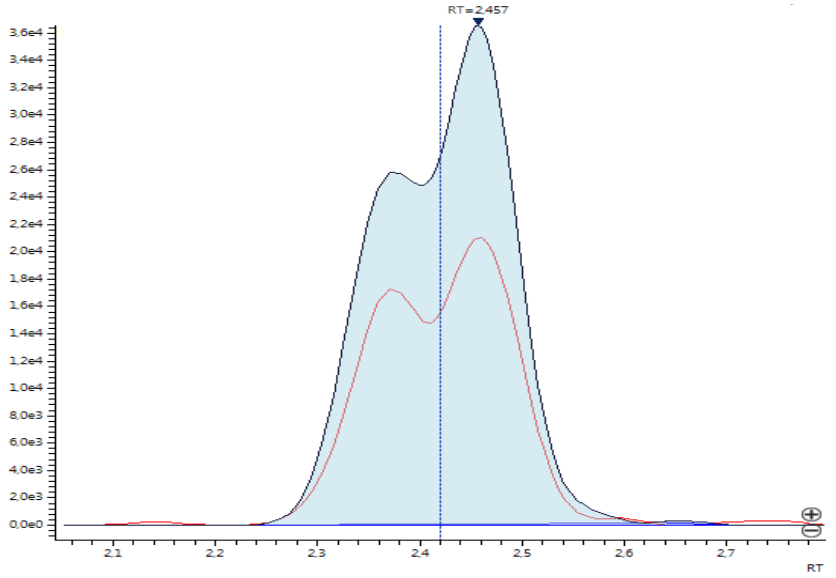


RESOLUTION

PERMETHRIN GC



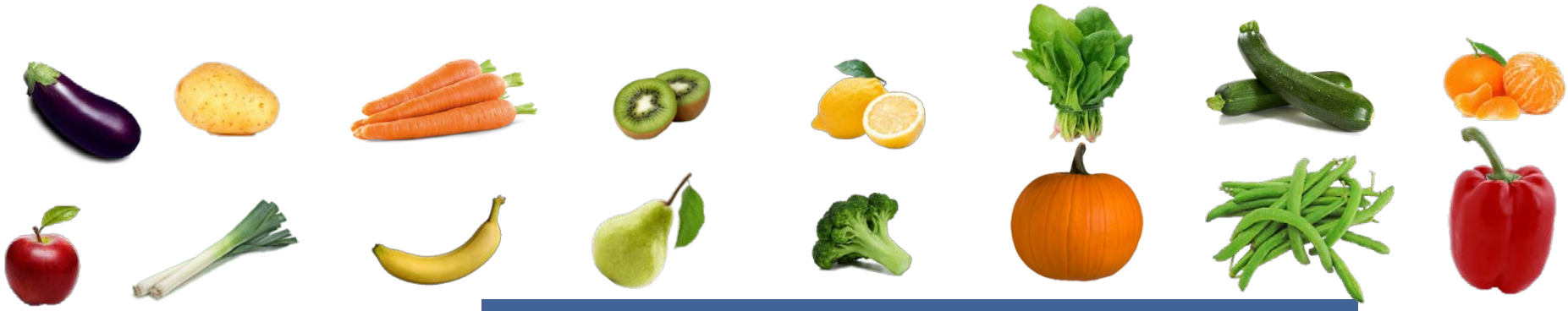
PERMETHRIN SFC



MATRIX EFFECT

	Pear		Zucchini		Orange		Onion		Tea	
	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC
Achrinathrin	5	10	5	7	0	**94**	0	44	-22	**52**
Bifenthrin	10	4	8	8	-7	33	-24	28	-3	5
Cyfluthrin	10	0	15	-1	-4	40	-2	23	**-57**	-5
Cypermethrin	-2	1	4	-2	-8	43	-17	26	-32	3
Deltamethrin	1	11	3	-3	-14	23	**-63**	15	-18	-
Etofenprox	0	1	-1	0	-8	21	**-65**	15	-10	-12
Fenprotathrin	18	5	20	4	-7	41	-25	30	-1	13
Fenvalerate	0	-1	4	10	-6	24	-40	12	-32	1
Flucythrinate	-5	6	-5	1	-8	**52**	-37	34	**-86**	16
Lambda-cyhalothrin	-7	2	-6	2	-9	43	-18	25	-19	18
Permethrin	2	3	8	5	2	**48**	-11	28	-10	2
Phenothrin	6	9	11	12	-8	**53**	-17	41	**-75**	18
Tau-Fluvalinate	6	9	10	-4	-6	**62**	-12	22	**-50**	35
Tetramethrin	-3	4	3	6	-4	**64**	-2	35	-11	-38

REAL SAMPLES



	Matrix	Conc. (µg/kg)	
		SFC-ESI-MS/MS	GC-EI/MS/MS
Acrinathrin	**Pepper**	639	633
Cypermethrin	**Pepper**	397	321
	Potato	135	119
Deltamethrin	**Mandarin**	158	137
Etofenprox	**Green Beans**	502	516
Lambda-Cyhalothrin	**Pepper**	75	80
Permethrin	**Broccoli**	228	184
Tau-Fluvalinate	**Mandarin**	142	137

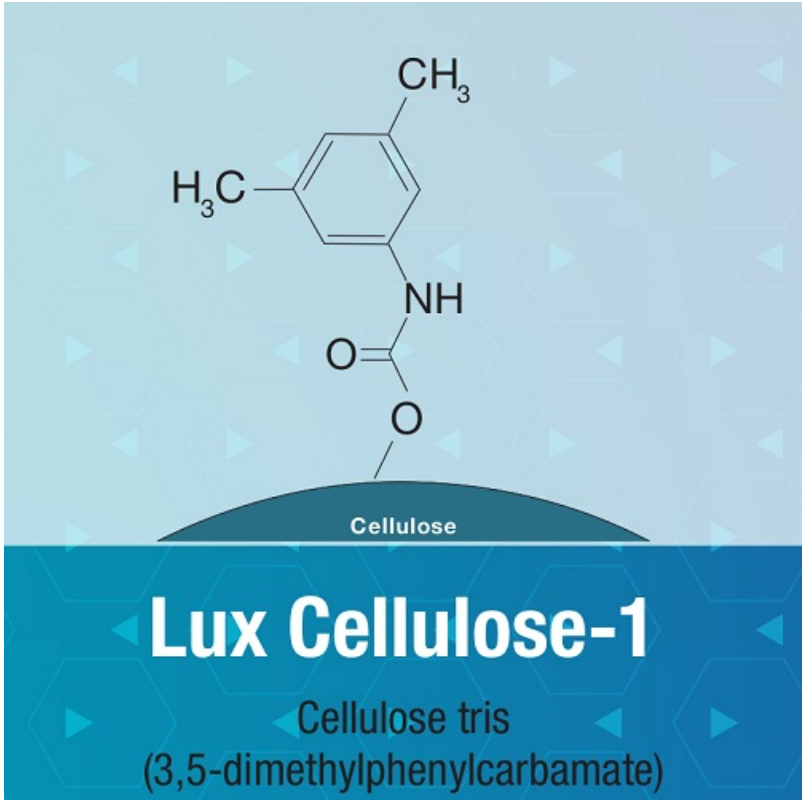
CHIRAL SFC

Column: LUX CELLULOSE-1

Stationary phase: Cellulose tris(3,5-dimethylphenylcarbamate)

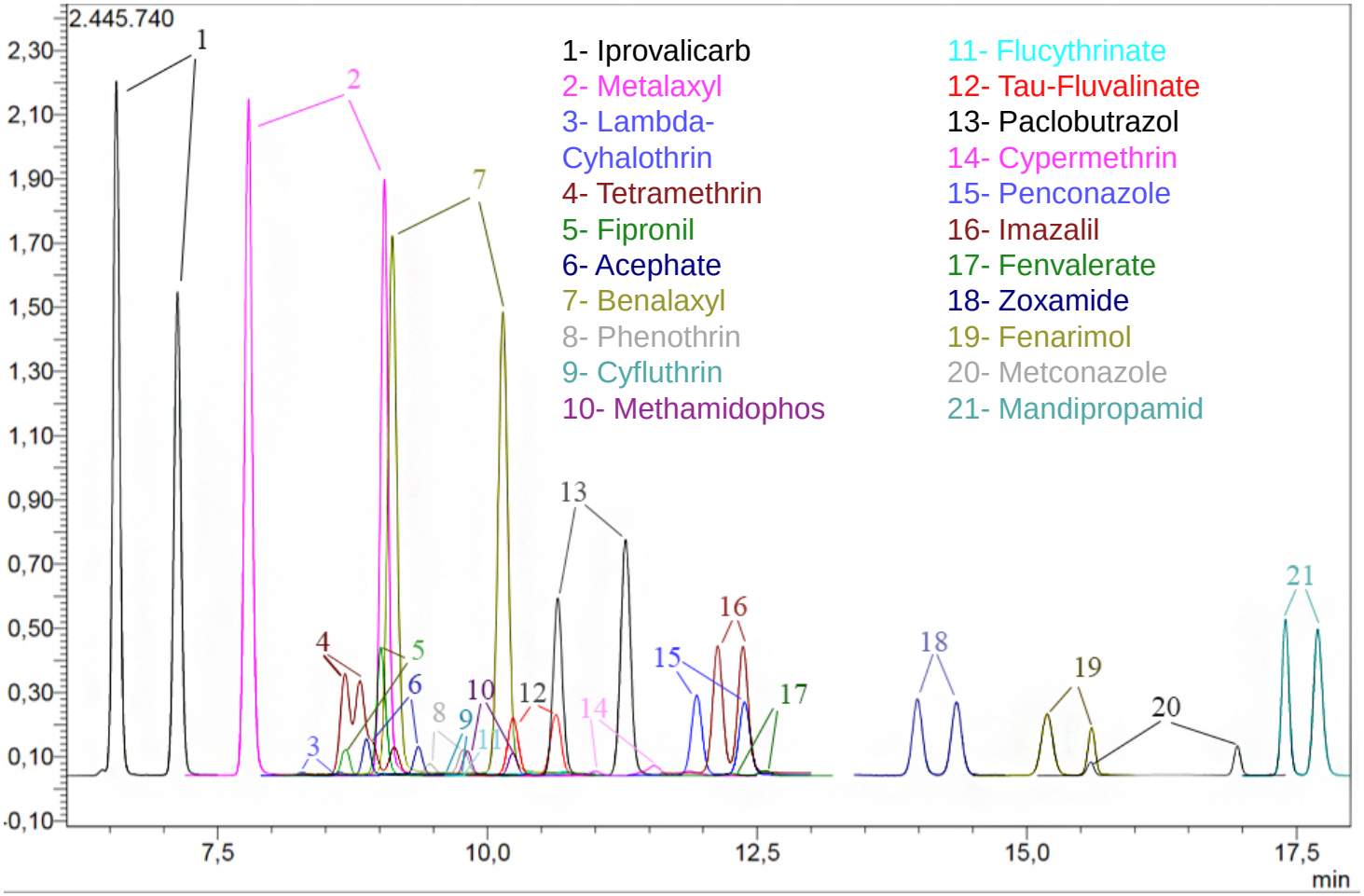
Lenght: 250 x 4.6 mm

Particle size: 5 µm



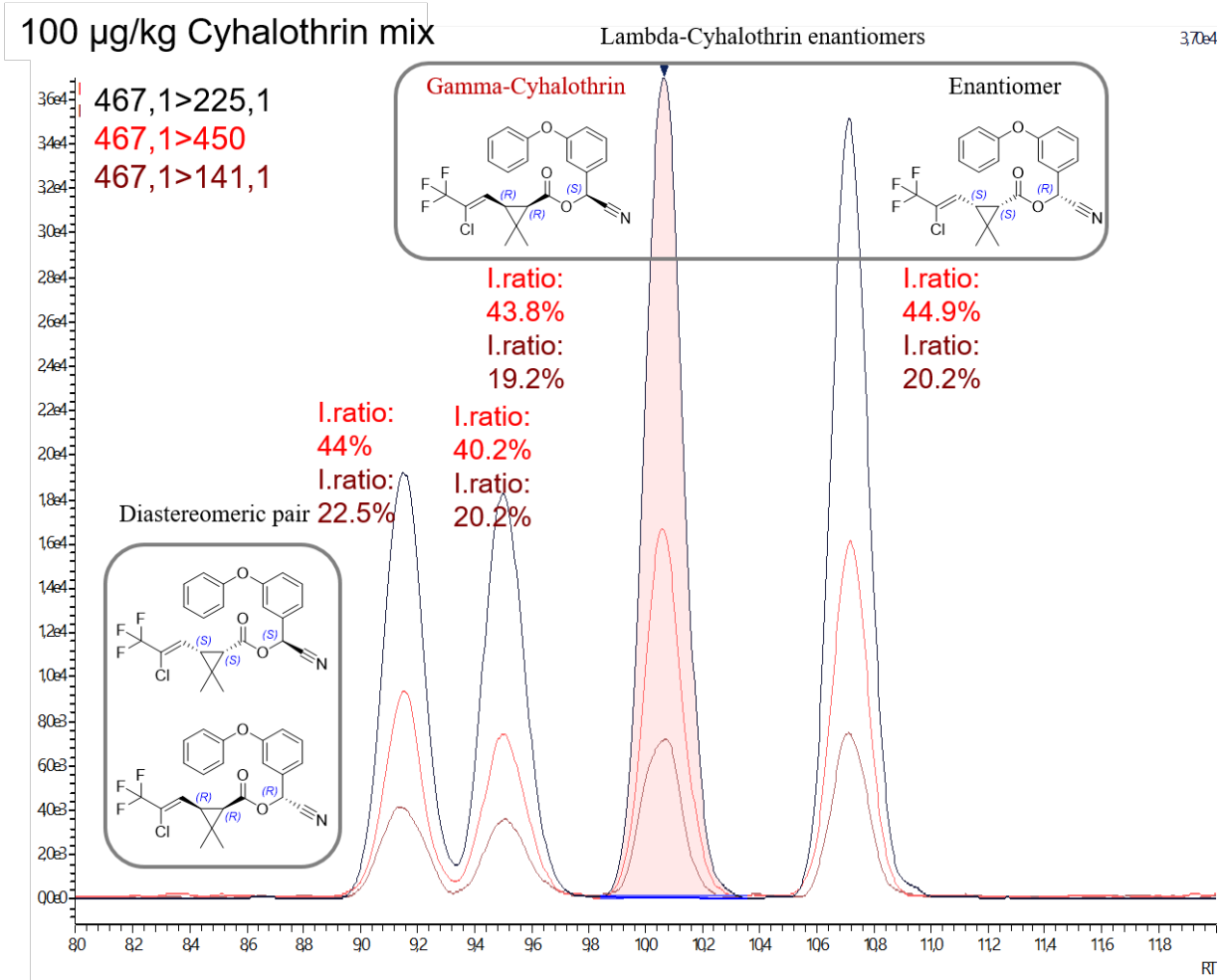
COLUMN: LUX CELLULOSE-1

Cellulose tris(3,5-dimethylphenylcarbamate)



CYHALOTHRIN

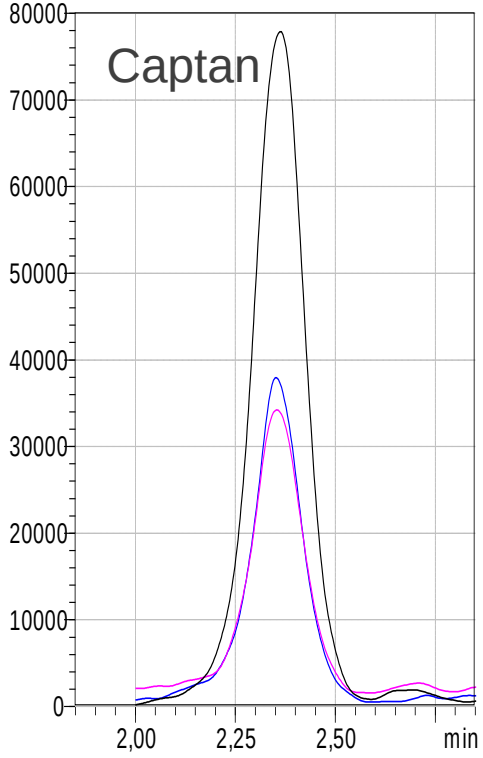
The four isomers of the cyhalothrin mixture can be separated using SFC in combination with the cellulose polysaccharide column.



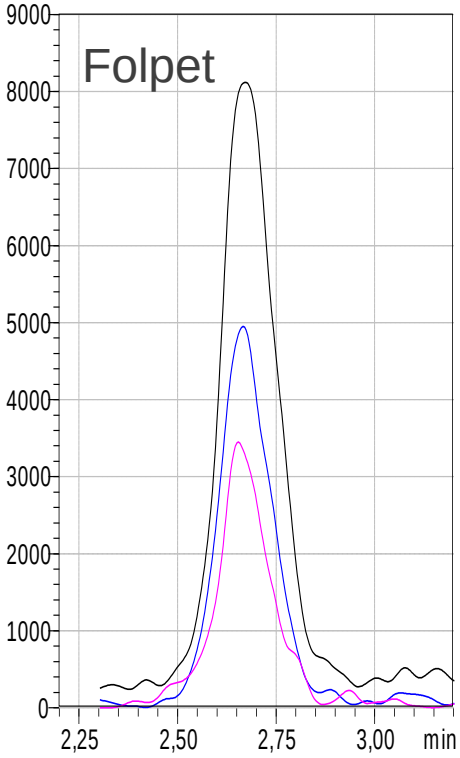
ION SOURCE TEMPERATURE

Same vial: 100 µg/Kg
Same MS Parameters: Ion source 125ºC, DL 125ºC, Heated block 200ºC.

SFC-MS/MS

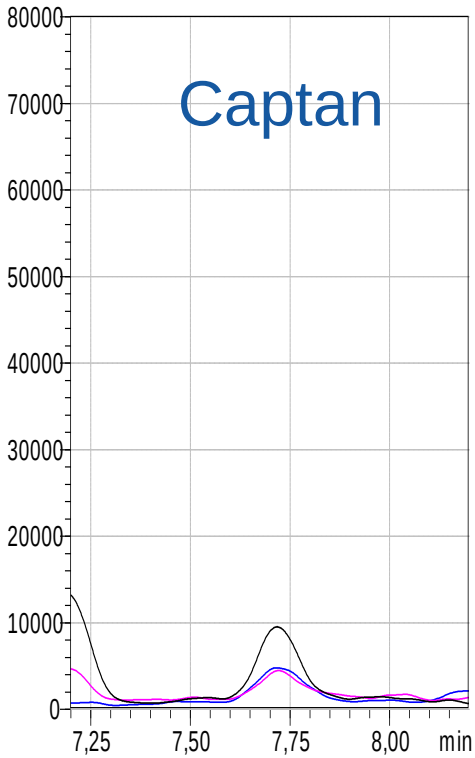


316,7>264,0
316,7>299,9
316,7>79,1

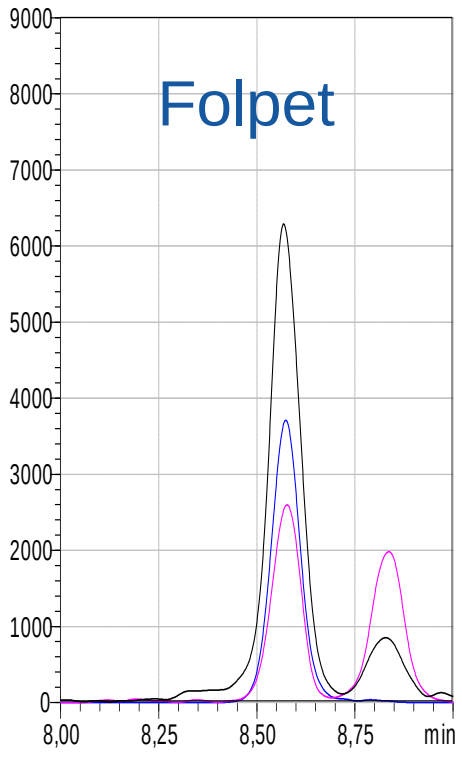


314,6>130,1
314,6>261,8
314,6>102,0

LC-MS/MS



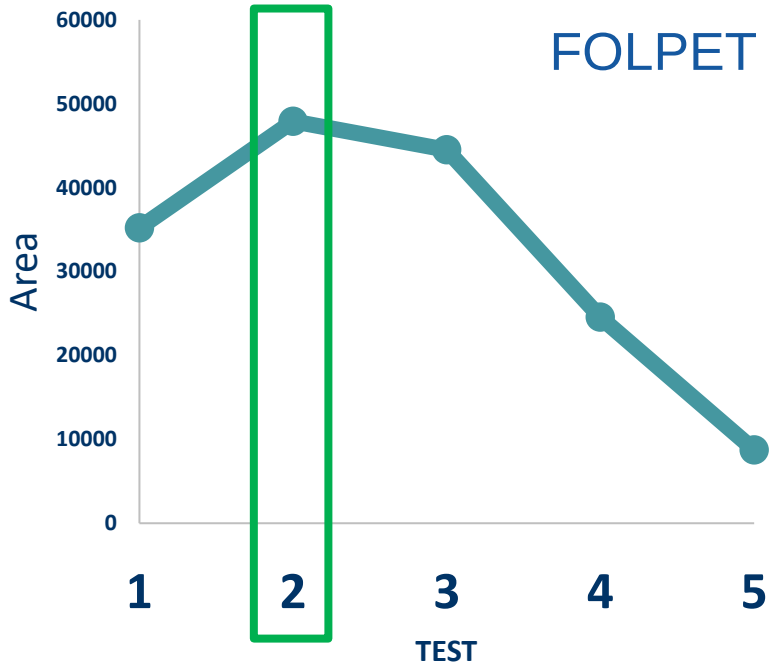
316,7>264,0
316,7>299,9
316,7>79,1

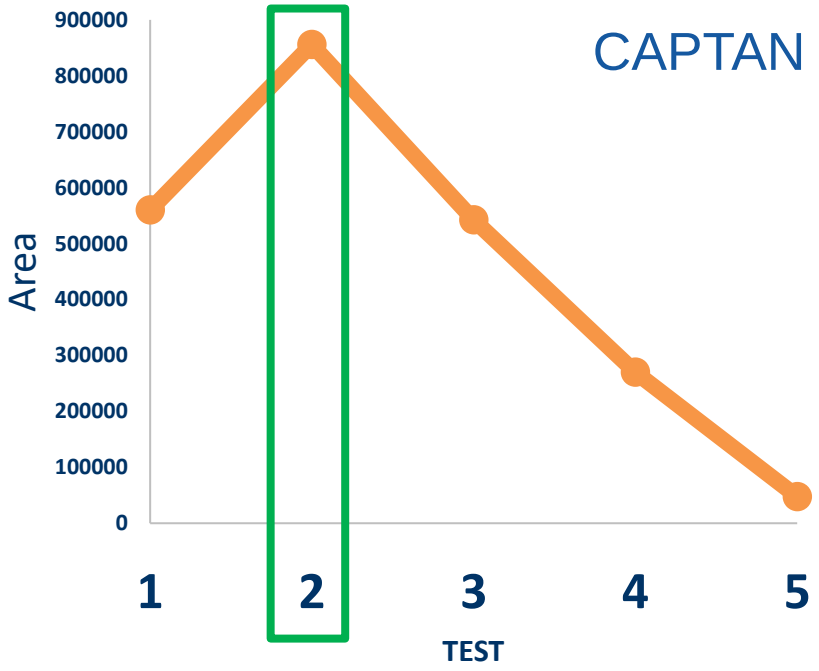


314,6>130,1
314,6>261,8
314,6>102,0

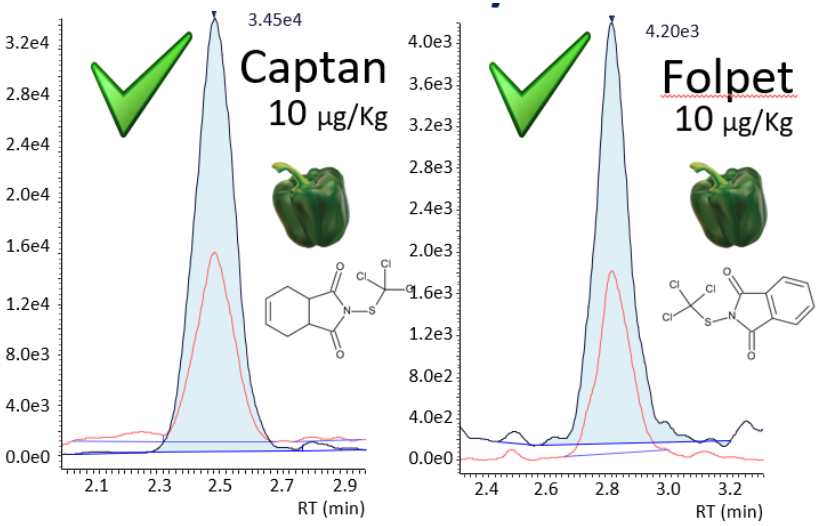
ION SOURCE OPTIMIZATION

Temperature test ESI	Interface (Cº)	DL (Cº)	Heat block (Cº)
T1	100	100	150
T2	125	125	200
T3	150	150	300
T4	200	200	300
T5	300	250	400

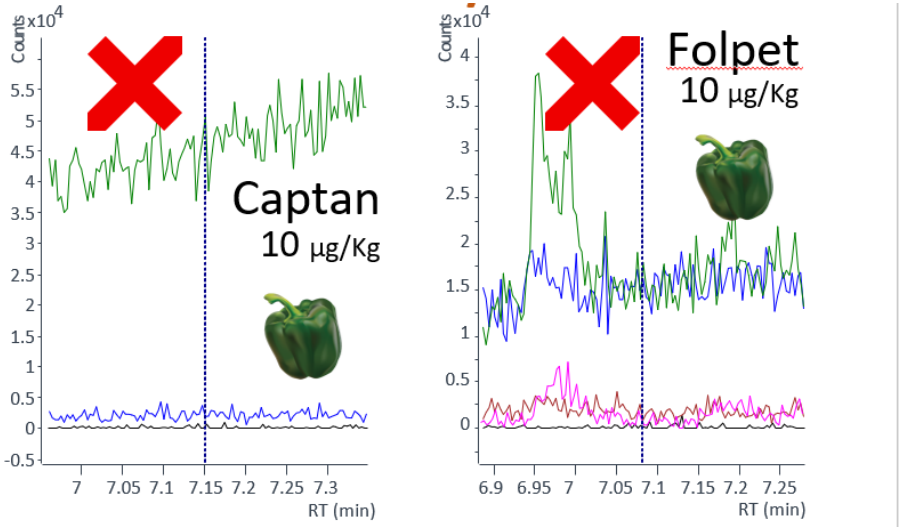




SFC-MS/MS



GC-MS/MS



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Supercritical fluid chromatography coupled to tandem mass spectrometry for the analysis of pesticide residues in dried spices. Benefits and drawbacks

Víctor Cutillas [a], María Murcia-Morales [a], María del Mar Gómez-Ramos [a], Sherif M. Taha [b], Amadeo R. Fernández-Alba [a,*]

[a] European Union Reference Laboratory for Pesticide Residues in Fruits & Vegetables, University of Almería, Agrifood Campus of International Excellence (ceiA3) Department of Hydrogeology and Analytical Chemistry, Ctra. Sacramento S/N°, La Cañada de San Urbano, 04120, Almería, Spain
[b] Agricultural Research Center, Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods, P.O. Box: 12311, Dokki, Giza, Egypt

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Supercritical Fluid Chromatography and Gas Chromatography Coupled to Tandem Mass Spectrometry for the Analysis of Pyrethroids in Vegetable Matrices: A Comparative Study

María Murcia-Morales, Víctor Cutillas, and Amadeo R. Fernández-Alba*

Agrifood Campus of International Excellence (ceiA3), European Union Reference Laboratory for Pesticide Residues in Fruit and Vegetables, Department of Hydrogeology and Analytical Chemistry, University of Almería, Carretera Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain

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Supercritical fluid chromatography separation of chiral pesticides: Unique capabilities to study cyhalothrin and metalaxyl as examples

Víctor Cutillas, Mar García-Valverde, María del Mar Gómez-Ramos, Francisco José Díaz-Galiano, Carmen Ferrer, Amadeo R. Fernández-Alba*

European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, University of Almería, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento S/N°, La Cañada de San Urbano, 04120, Almería, Spain

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Overcoming difficulties in the evaluation of captan and folpet residues by supercritical fluid chromatography coupled to mass spectrometry

Víctor Cutillas [a], Florencia Jesús [b], Carmen Ferrer [a], Amadeo R. Fernández-Alba [a,*]

[a] European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables. University of Almería, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento S/N°, La Cañada de San Urbano, 04120, Almería, Spain
[b] Grupo de Análisis de Compuestos Traza, Polo de Desarrollo Universitario "Abordaje Holístico", CENUR Litoral Norte Sede Paysandú, Universidad de La República, Ruta 3 Km 363, 60000, Paysandú, Uruguay

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