16th Annual Workshop On Emerging High-Resolution Mass Spectrometry (Hrms) And Lc-Ms/Ms Applications In Environmental Analysis And Food Safety

Online, 15-16 October, 2020

Advantages of supercritical fluid chromatography for the determination of pesticide residues in fruits and vegetables.

Víctor Manuel Cutillas Juárez

Amadeo Rodríguez Fernández-Alba

SFC Overview

- Technical and operational parameters
- Efficacy in Multiresidue Methods
- Additional Advantages
    - Lower ion suppression
    - Wider scope
    - Chiral separations
    - Efficiency with a low ion source temperature

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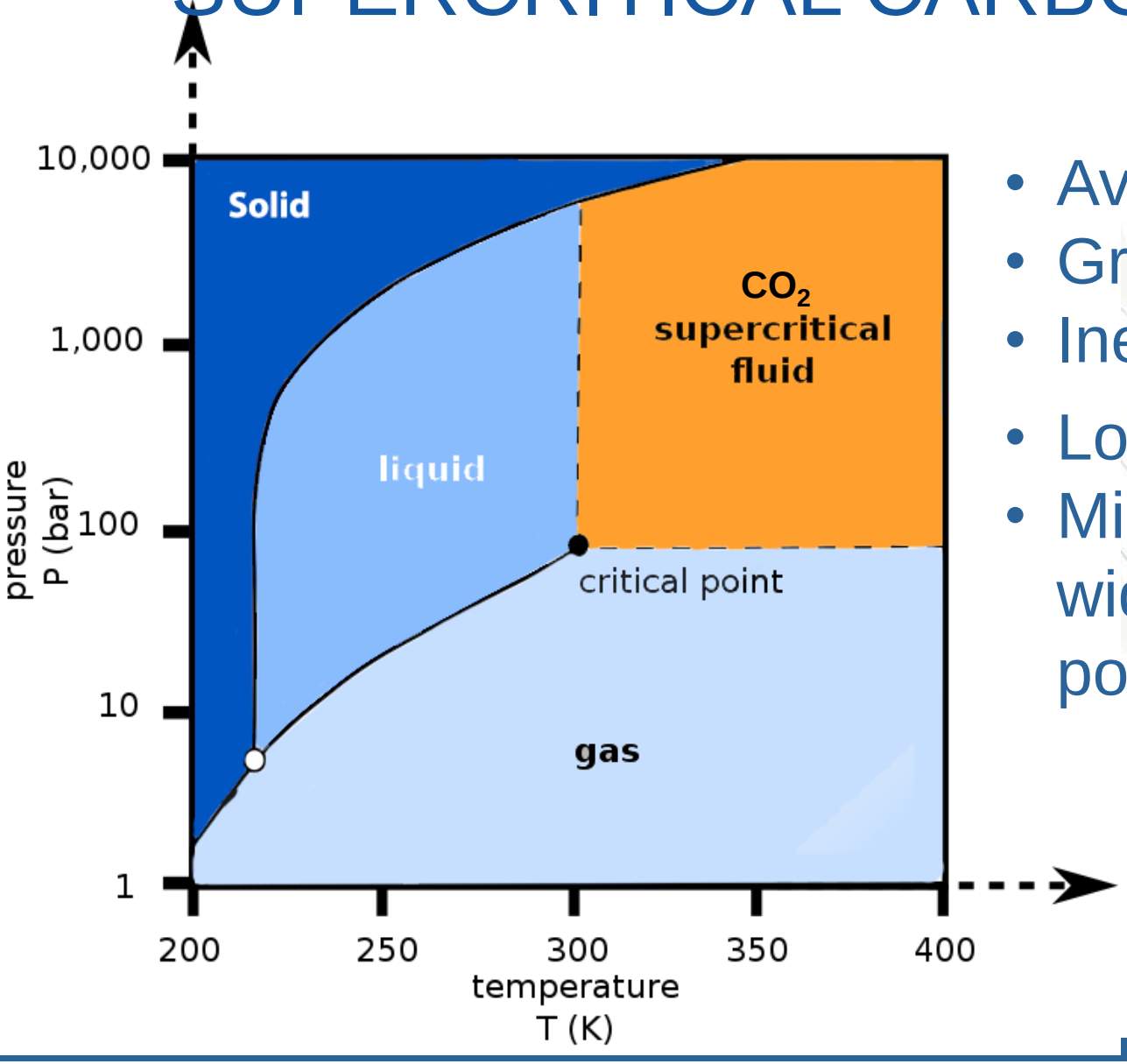
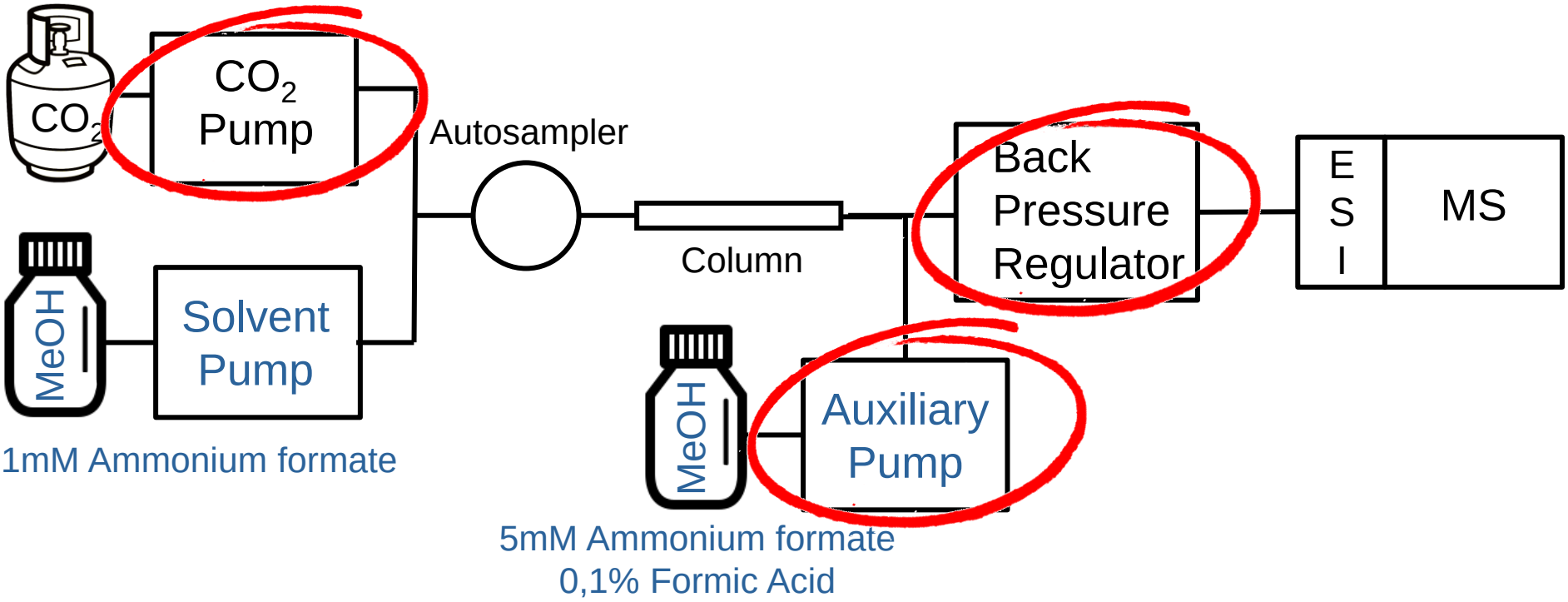
SUPERCRITICAL CARBON DIOXIDE



- Available
- Green
- Inexpensive
- Low critical point
- Miscible with a wide range of polar solvents

SFC-MS/MS System

(Nexera UC coupled to Shimadzu LC-MS 8060)

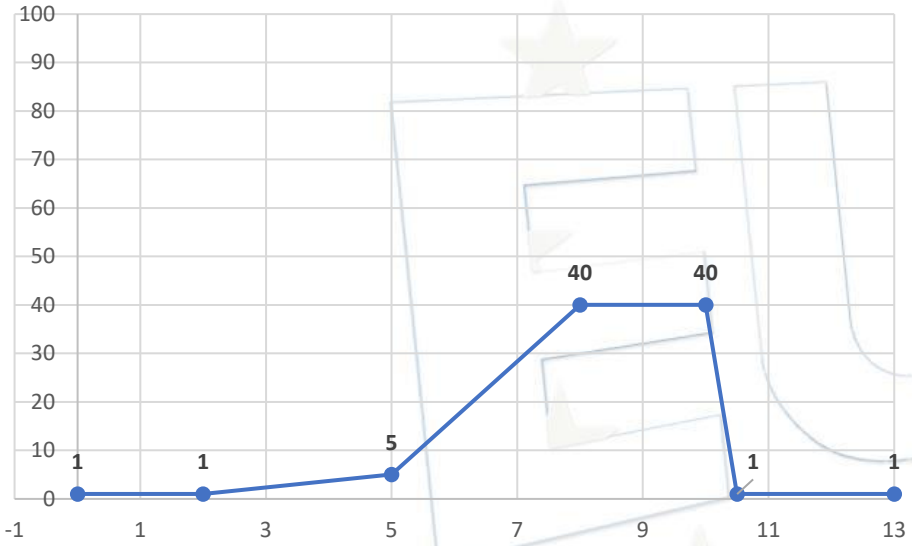


METHANOL AS CO-SOLVENT

SFC

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

Make-up Flow: 0,080mL/min

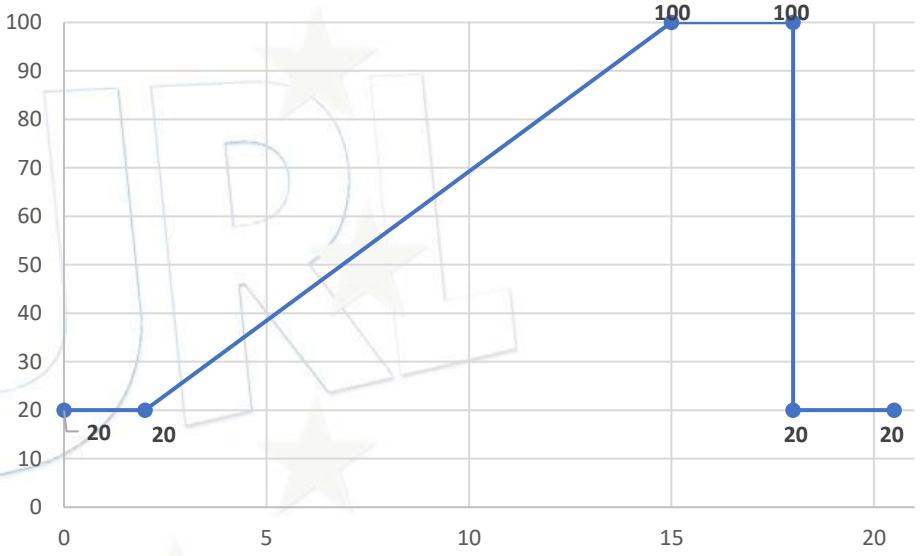


TOTAL RUN CONSUMPTION

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

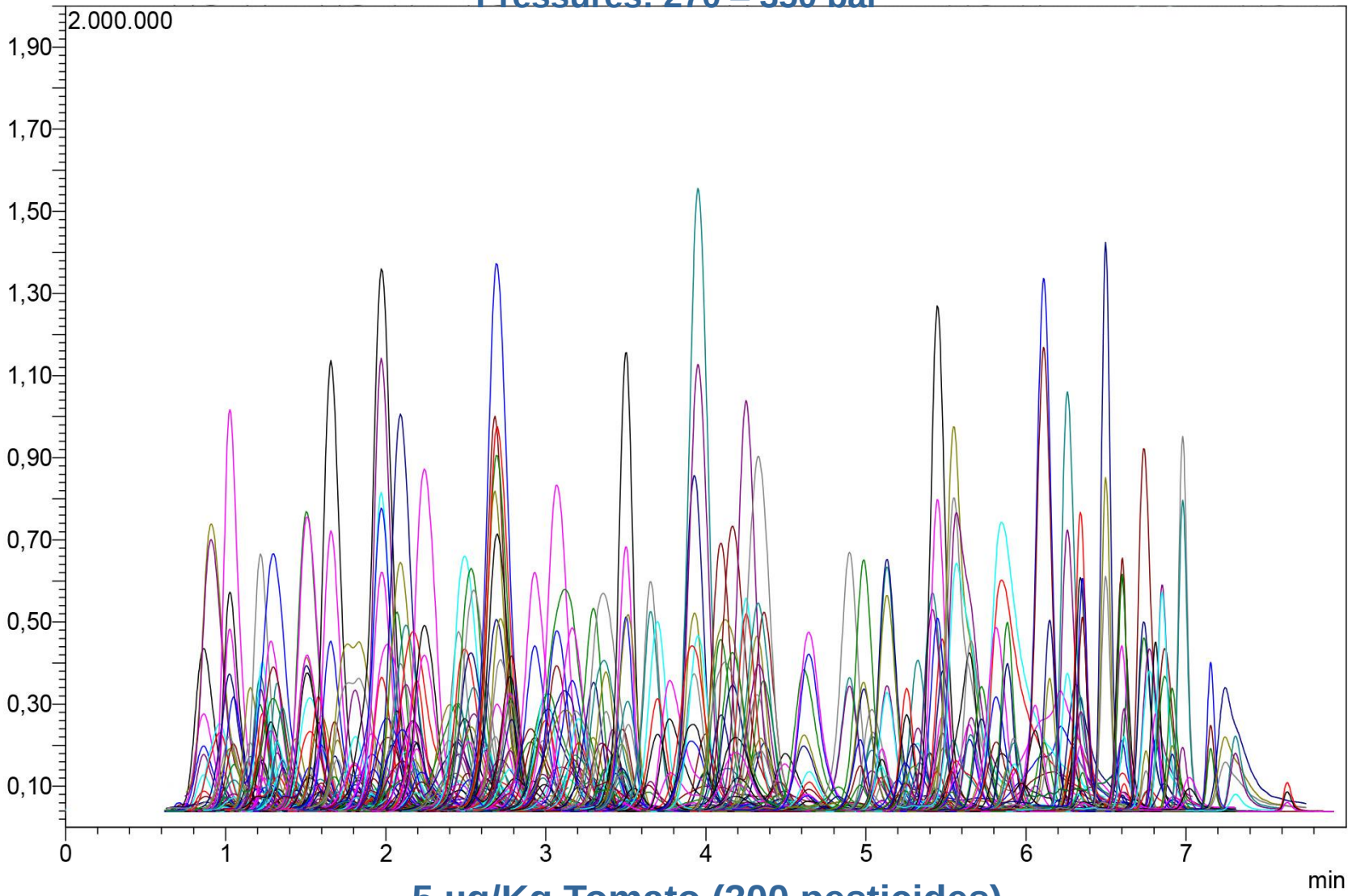


TOTAL RUN CONSUMPTION

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

TOTAL FLOW: 1.3 mL/min

Pressures: 270 – 350 bar



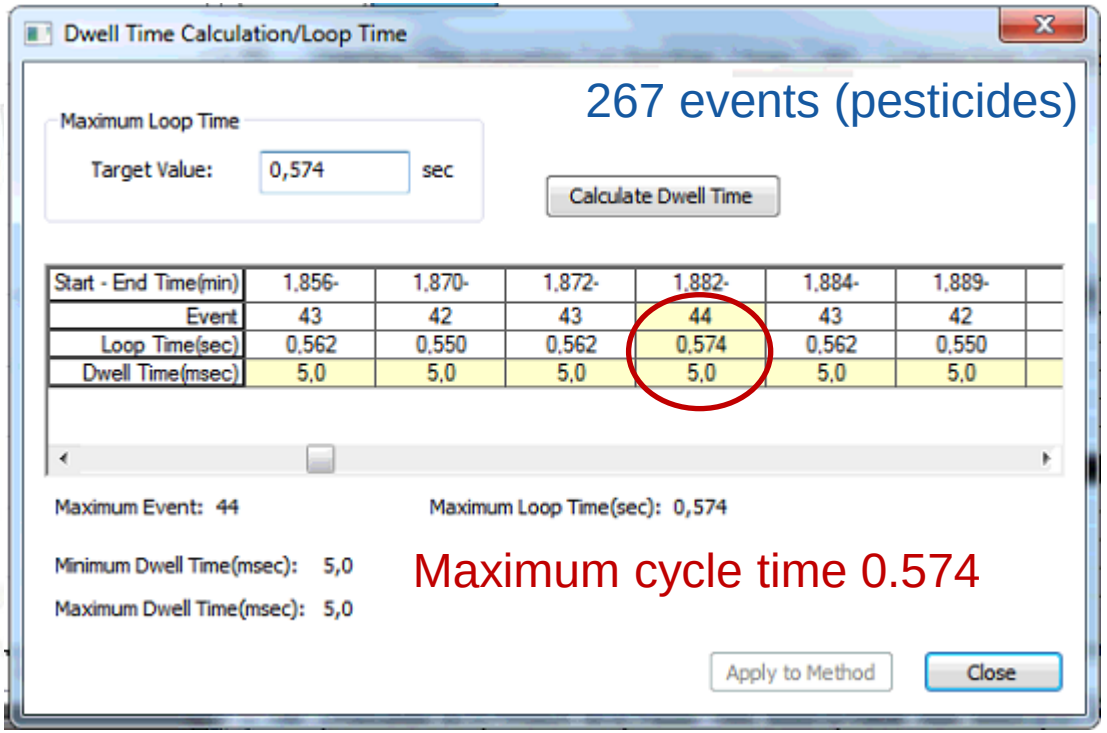
5 µg/Kg Tomato (300 pesticides)

Too much coelution with this short run time?

IS A THIRD TRANSITION NECESSARY?

Acquisition window: +-0,3

MS 8060:
Switching polarity: 5msec
Scan speed: 555 MRM/sec



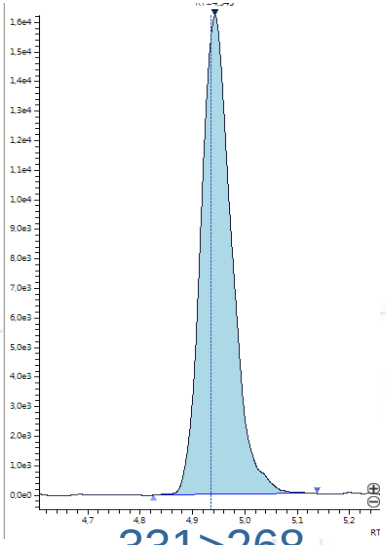
These cycle times allow us to add another transition to compounds without compromising peak shape. Enough points per peak will be obtained.

Some compounds need a more selective transition for their identification at low concentrations in difficult matrices.

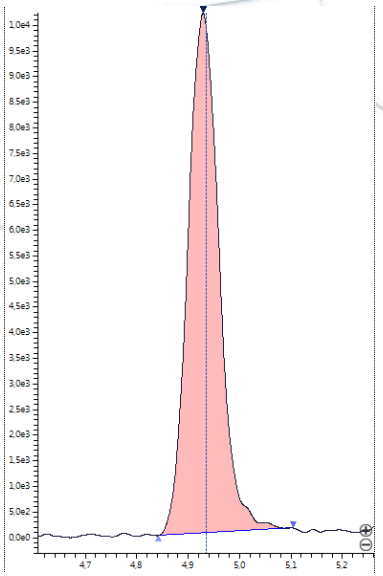
10 µg/kg
Tomato

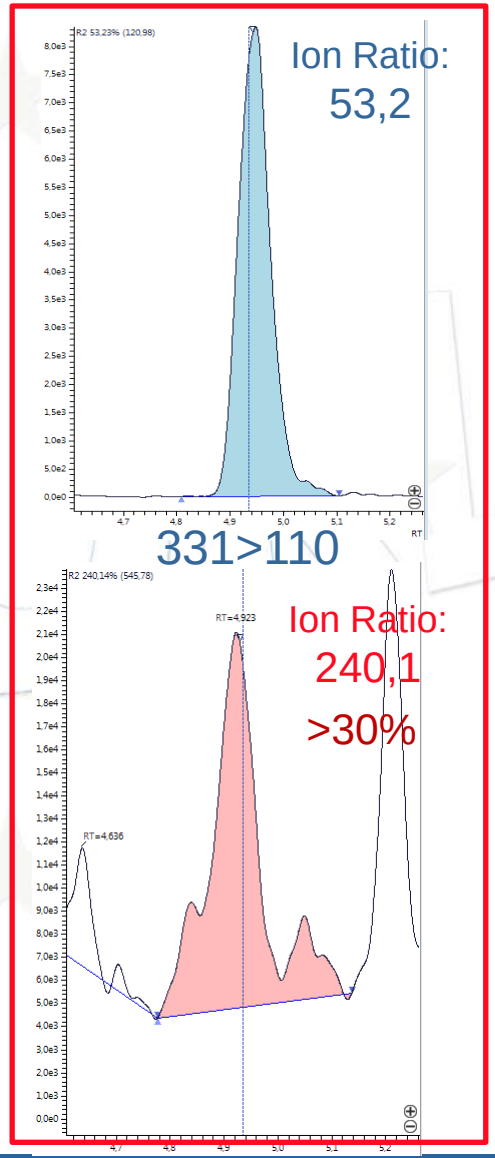
FENARIMOL

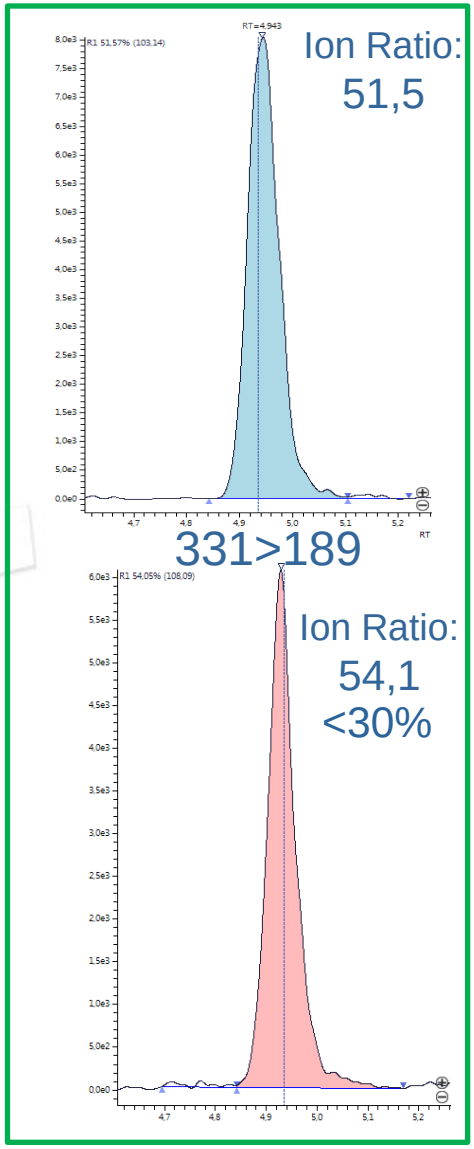
10 µg/kg
Leek



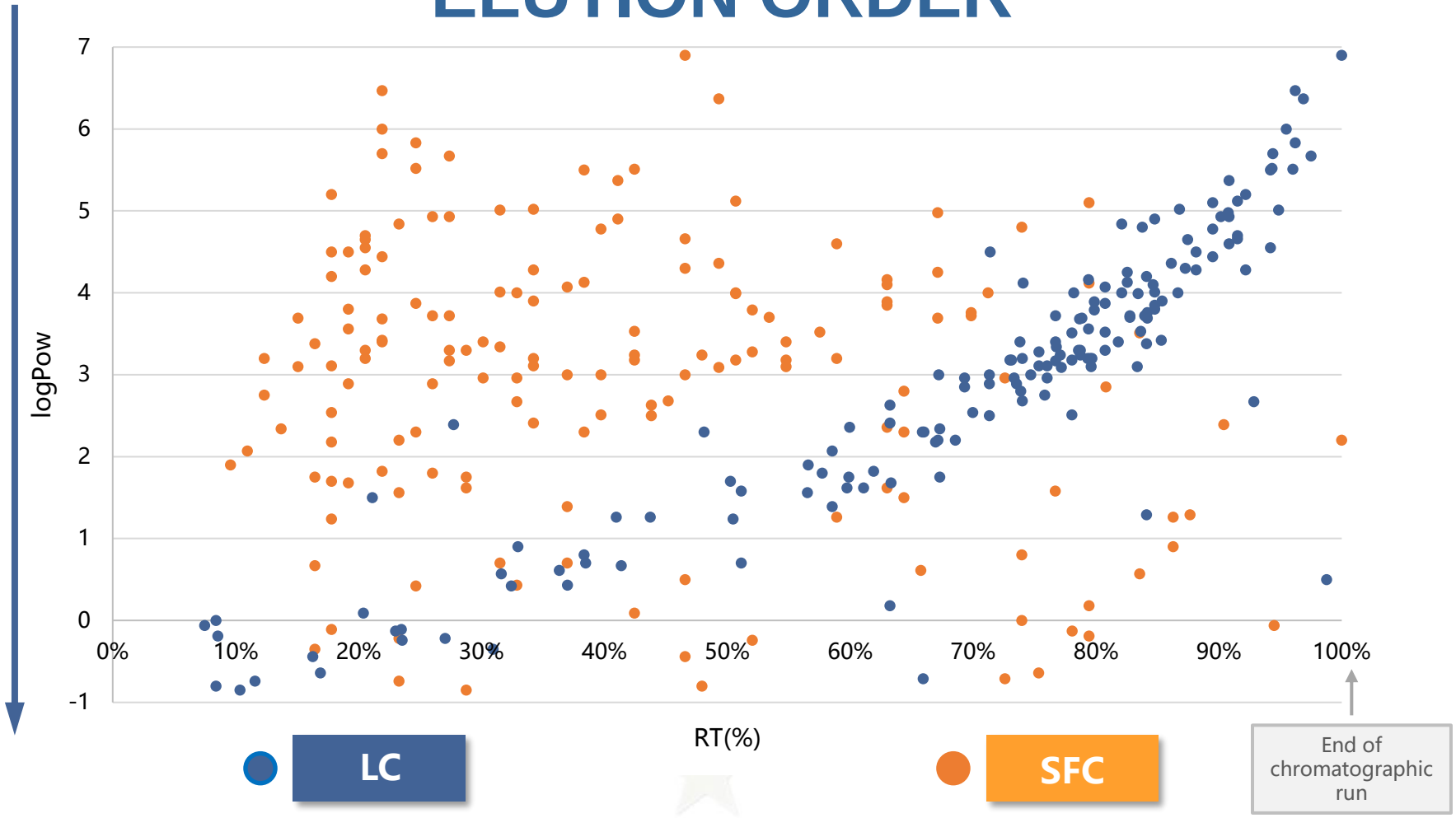
331>268





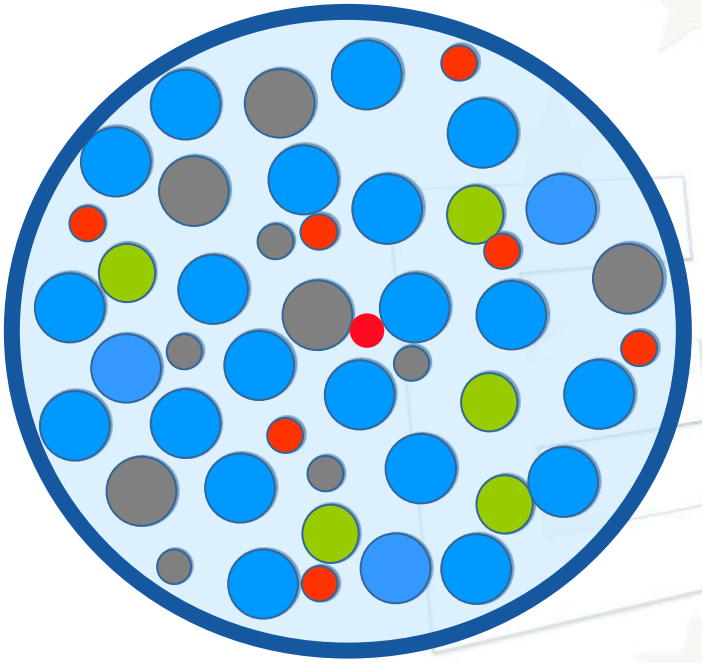


ELUTION ORDER



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

ESI IONIZATION PROCESS



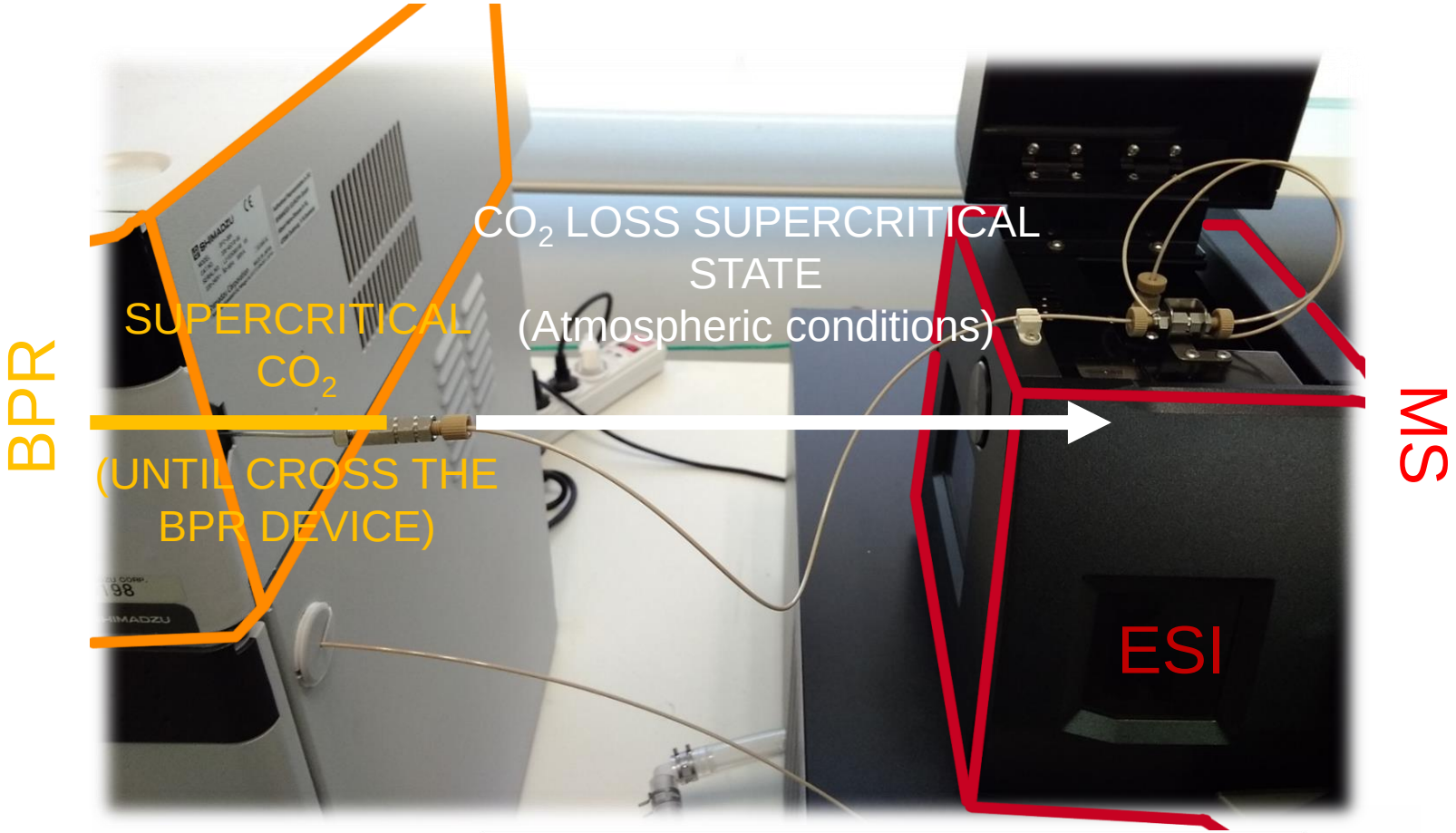
Ion
Water
Matrix
Methanol

SFC MOBILE PHASE:

WATER
Surface tension (20°C): 72.80 mN/m

METHANOL
Surface tension (20°C): 22.70 mN/m

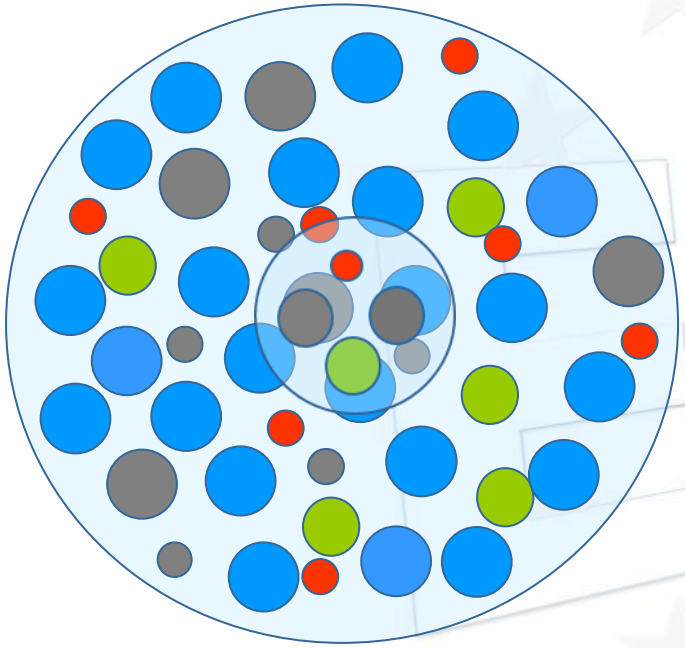
CO2 loss his supercritical state before ionization



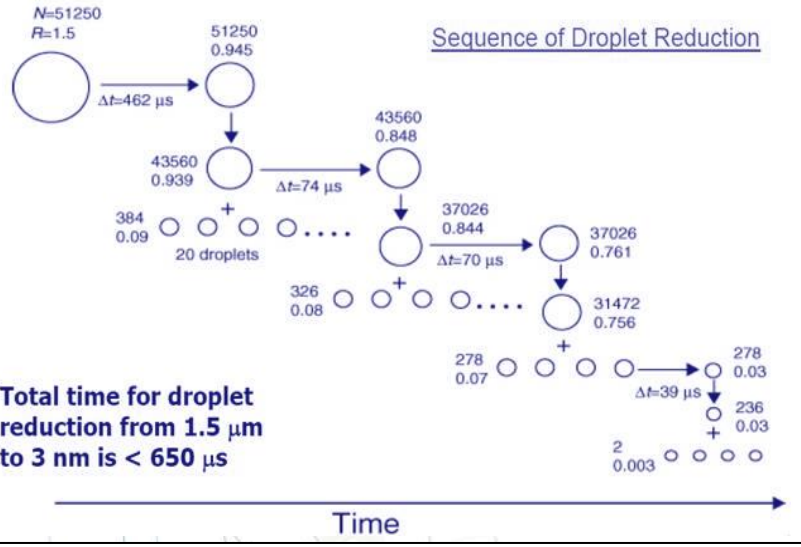
SMALL AMOUNT OF ORGANIC SOLVENT REACHING THE SOURCE

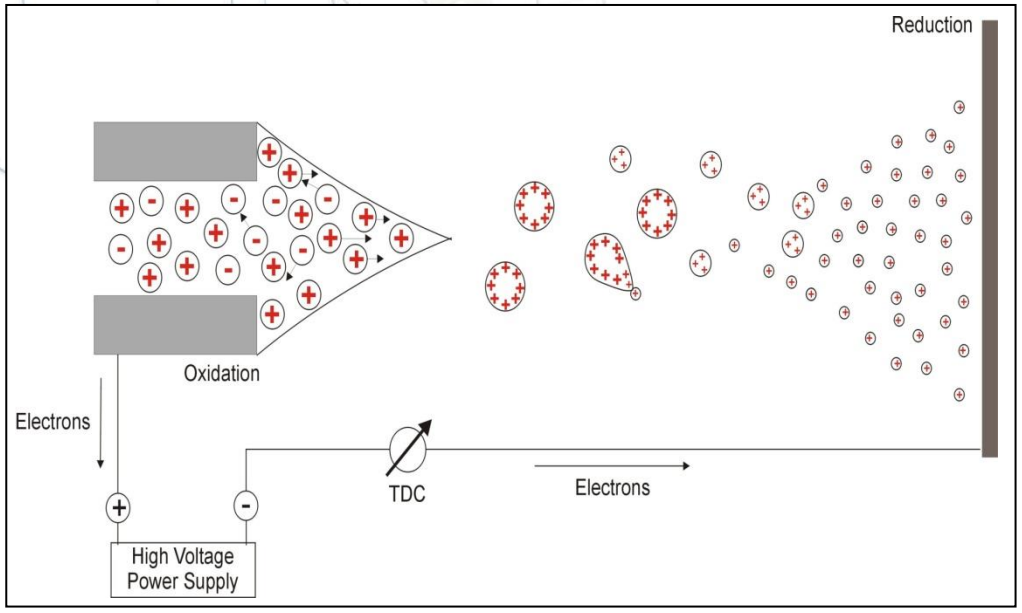
IONIZATION PROCESS

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



Ion
Water
Matrix
Methanol



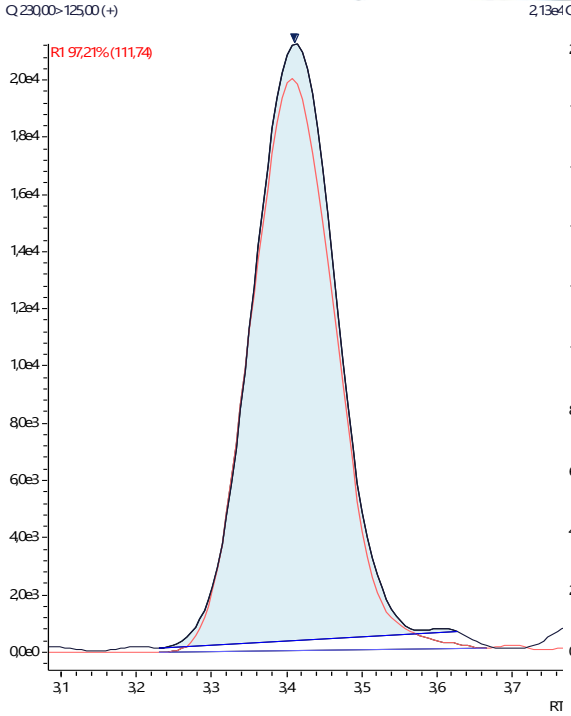


INJECTING 100% WATER VIAL

As SFC mobile phase does not use water as solvent. Injecting high volumes of 100% water samples can disturb the peak shapes of some compounds.

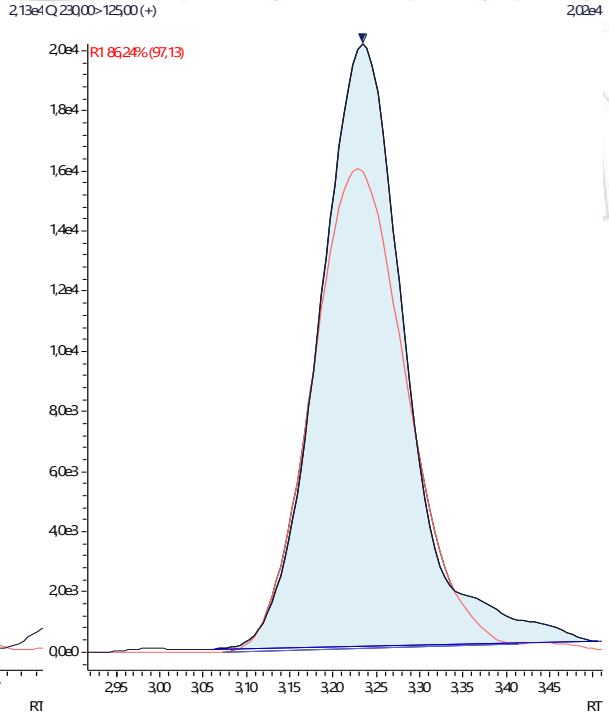
Vial conc: 1 µg/Kg
ACN 1:4 H_2O
Inj.volumen: **2µL**

Area: 163018



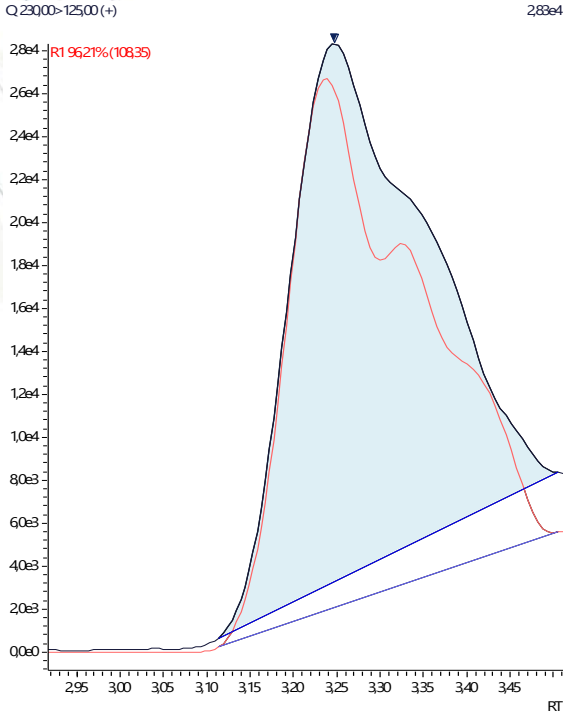
Vial conc: 1 µg/Kg
100% H_2O
Inj.volumen: **2µL**

Area: 139654



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

Area: 269292



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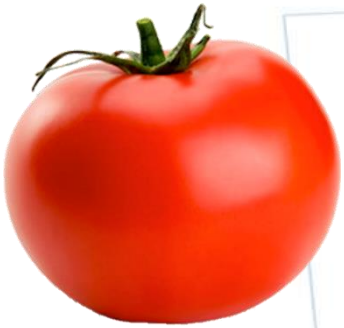
TESTING SFC-MS/MS

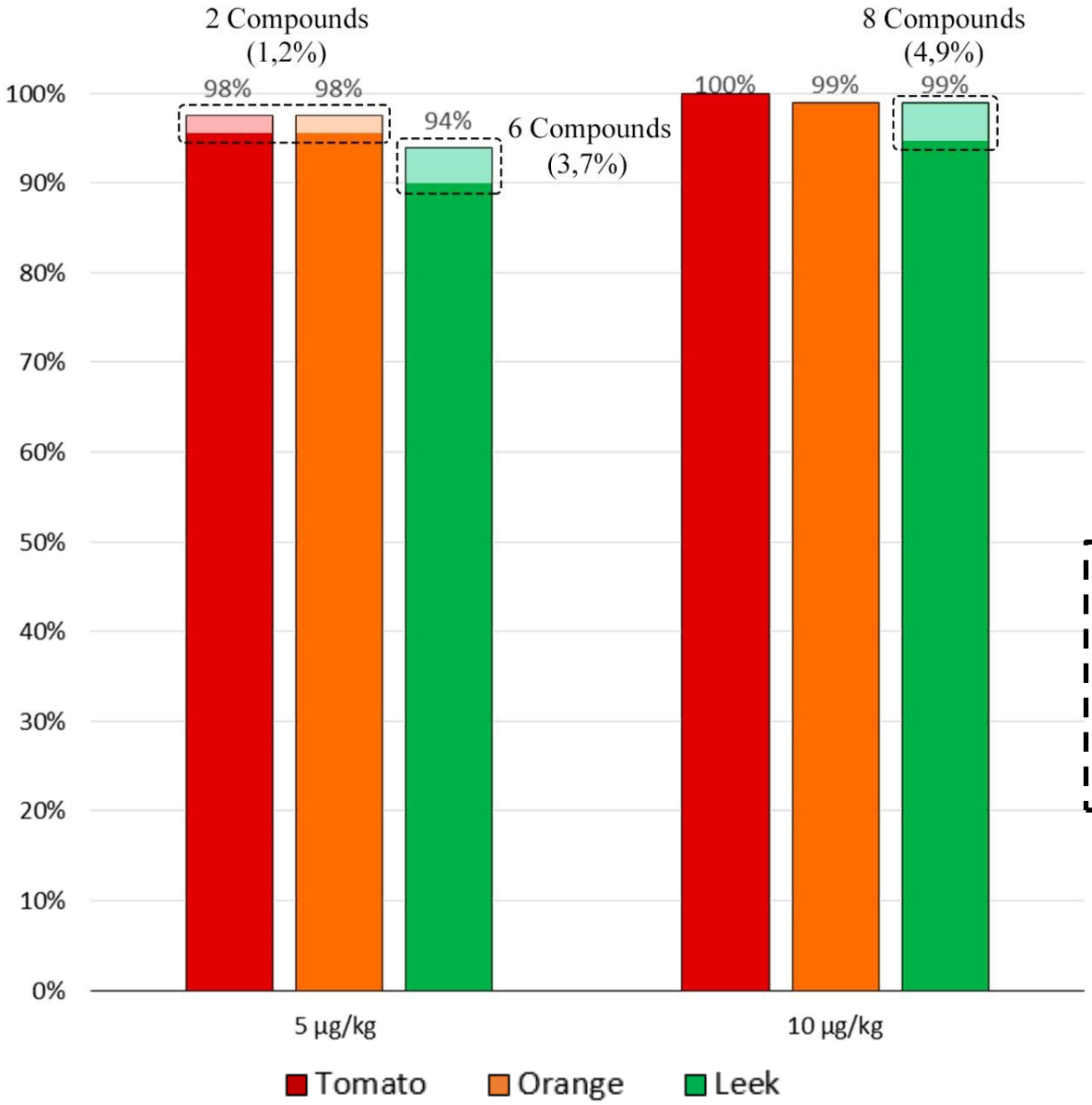
VALIDATION OF THE METHOD



Tomato

Orange

Leek



Tomato Orange Leek



IDENTIFIED COMPOUNDS

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.

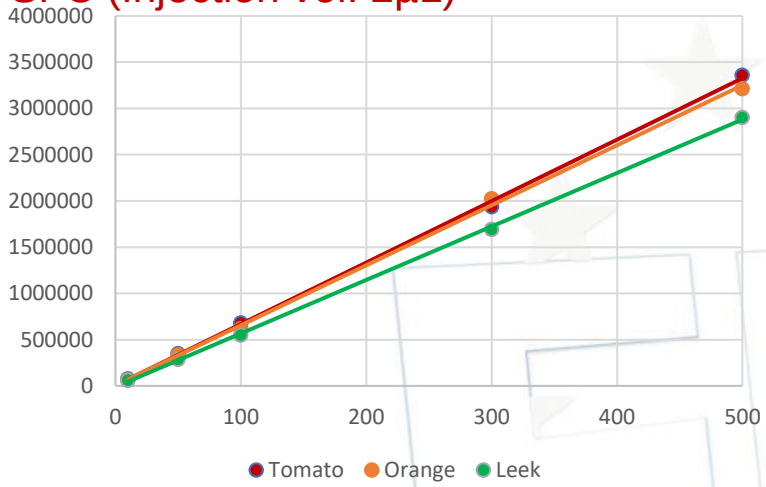
Sample diluted 5 times: 0,4 µg/Kg in the vial

Tomato Orange Leek



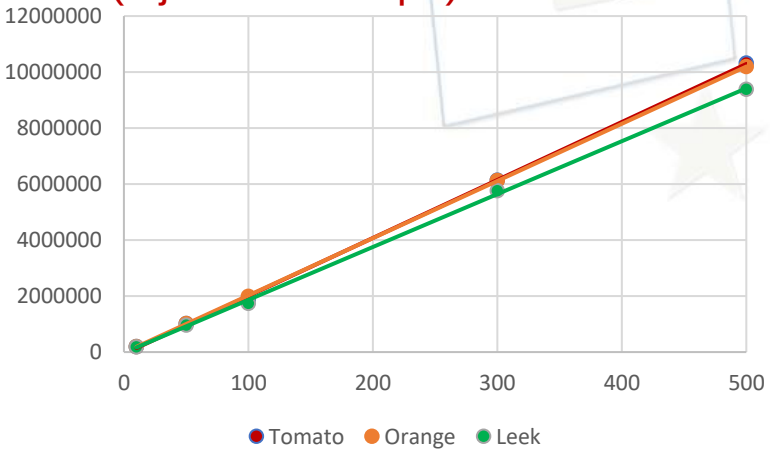
CARBARYL

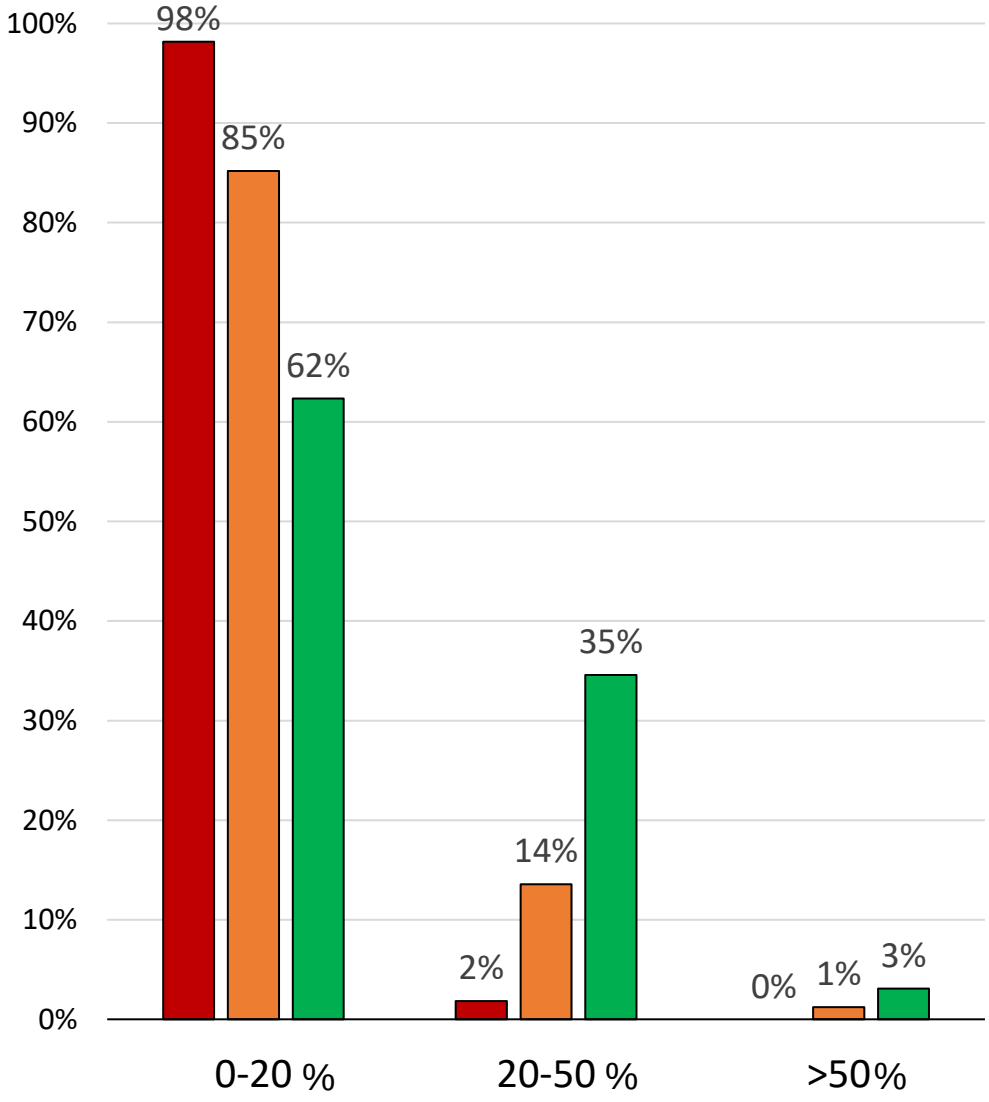
SFC (Injection vol: 2µL)



PROQUINAZID

SFC (Injection vol: 2µL)





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TESTING SFC-MS/MS

SPICES

BLACK PEPPER

CAYENNE

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

TOMATO QuEChERS+PSA

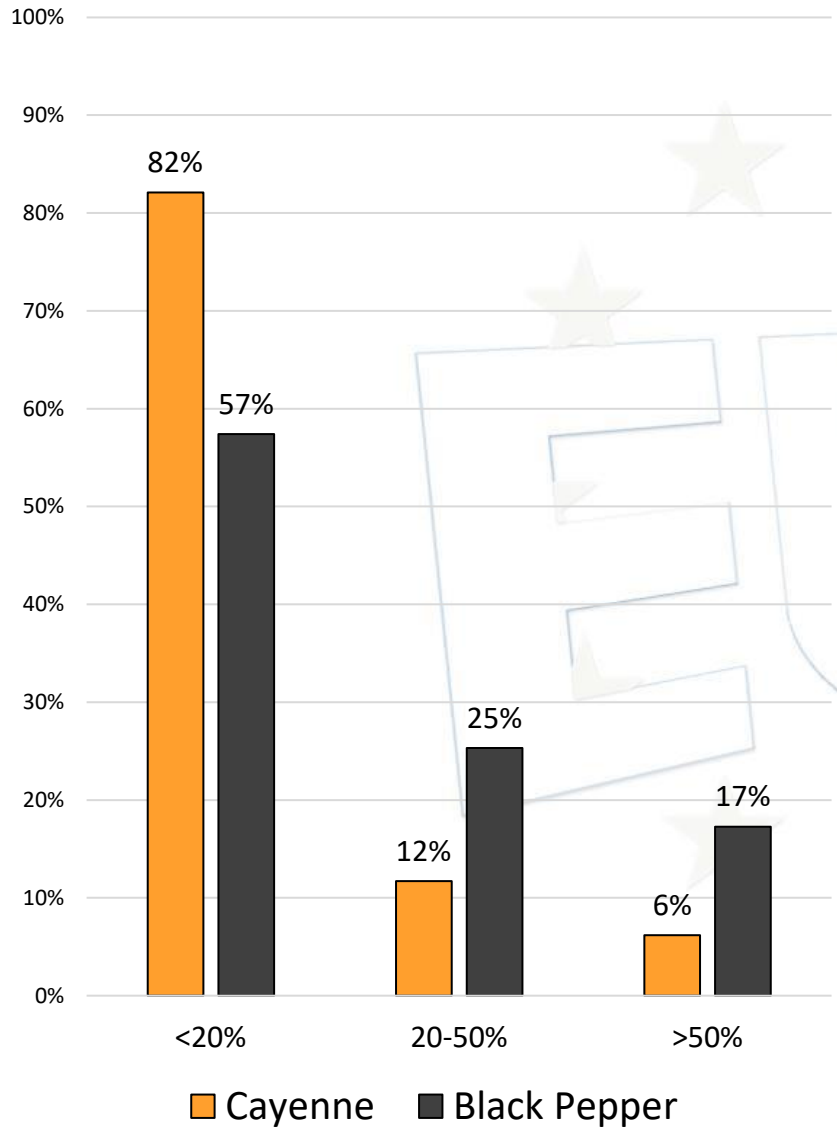
2408 compounds

BLACK PEPPER QuEChERS+PSA

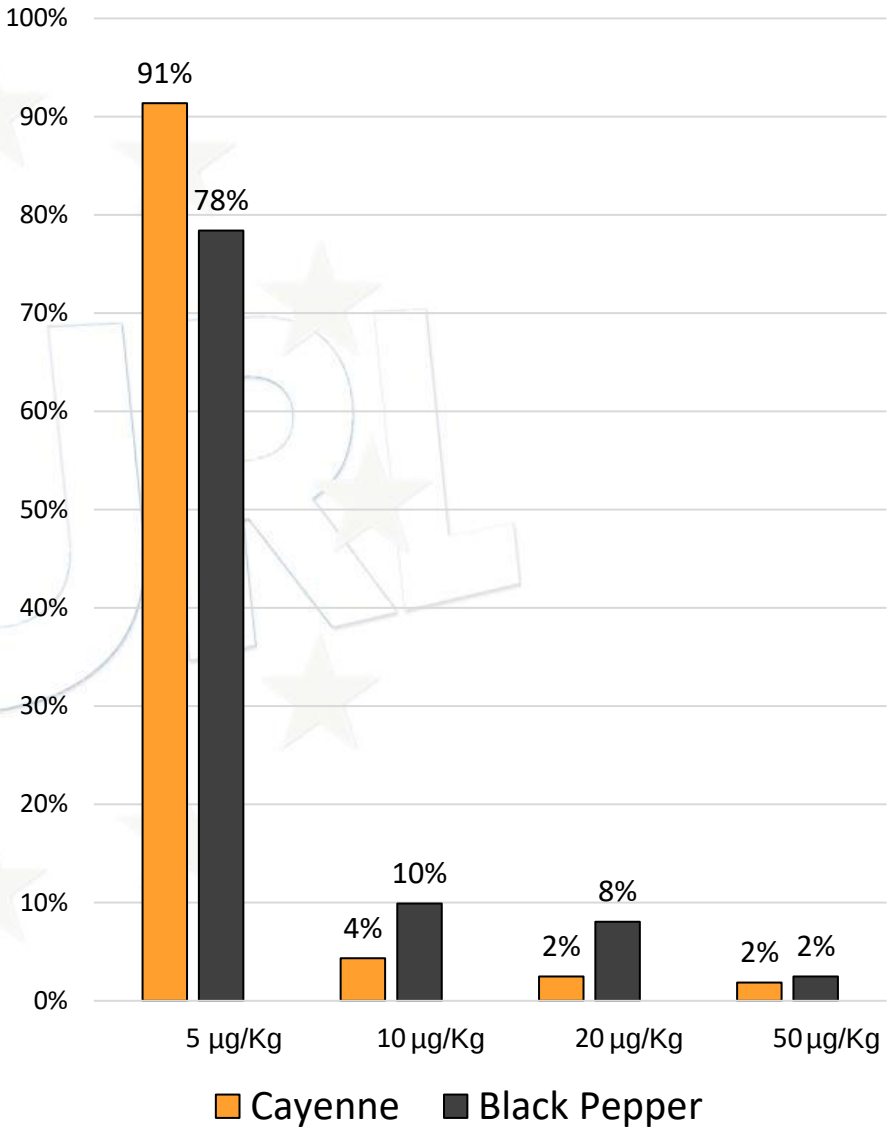
CAYENNE QuEChERS+PSA

Total amount injected: 0.1 mg

MATRIX EFFECT



IDENTIFIED COMPOUNDS (162)



2 gr of sample + 7 ml of Water

Wait 30 min

Add 10ml of AcN

Shake 4 min automatically

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

Shake 4 min automatically

Centrifuge 5 minutes at 3500 rpm

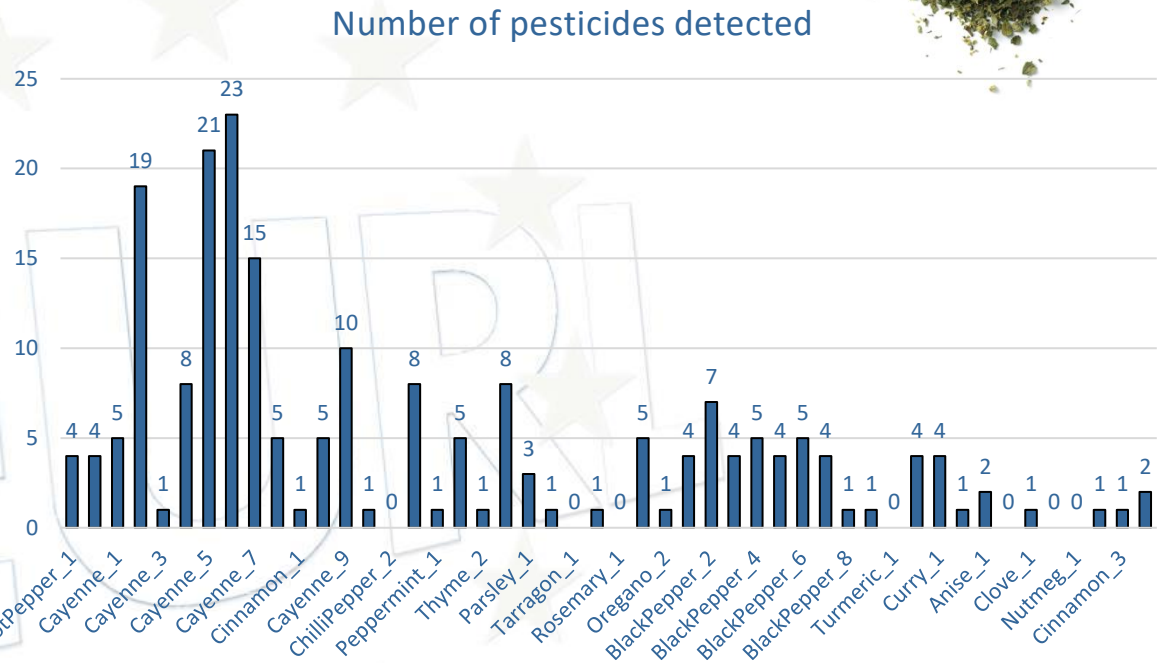
Transfer 5 mL of extract to preaconditioned EMR-Lipid tube, then transfer 5mL to EMR-Polish tube

Shake with vortex 30 s

Centrifuge 5 minutes at 3500 rpm

Analysis (Extract diluted 5 times with H_2O)

REAL SAMPLES (SPICES)



Number of samples analyzed	48
Samples with pesticides	43 (85%)
Range of pesticides detected per sample (LOQ: 5 µg/kg)	0 -23
Samples with pesticides above MRL	25 (52%)
Most detected pesticide	Carbendazim

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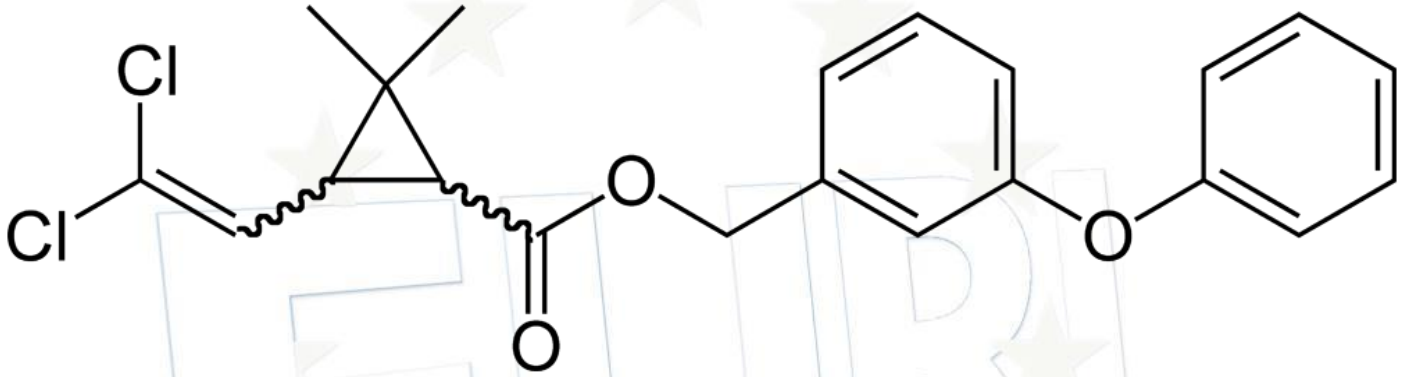
PYRETHROIDS

GC-MS/MS & SFC-MS/MS

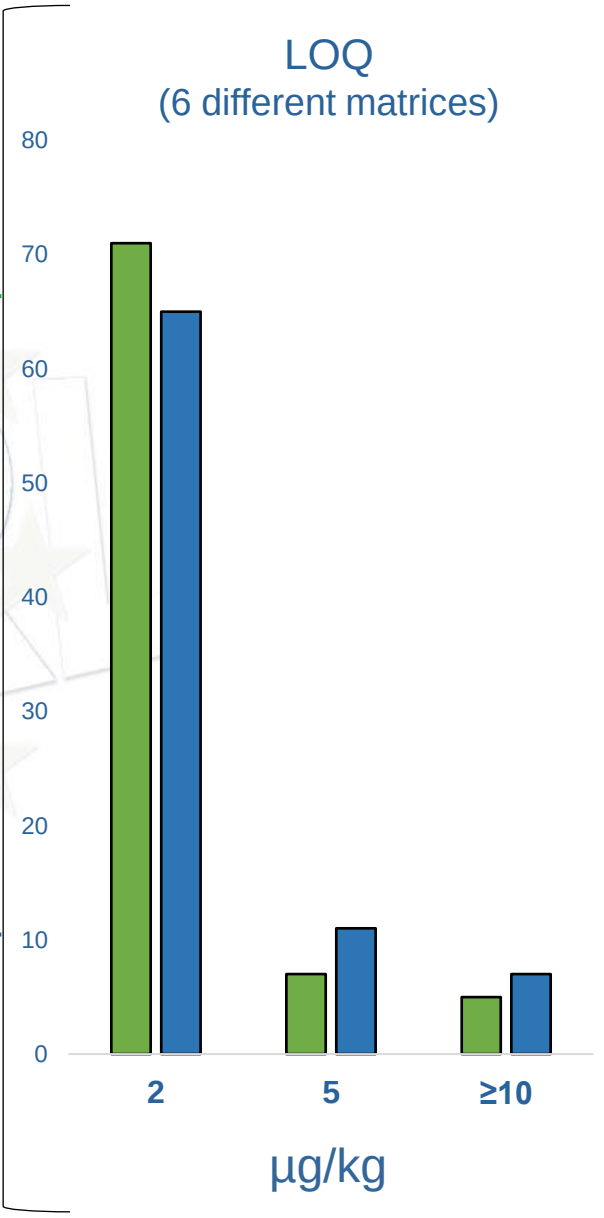


This study describes a comprehensive comparison between supercritical fluid chromatography (SFC) and gas chromatography (GC) coupled to mass spectrometry for the analysis of pyrethroids in vegetable matrices. The ionization process used was electrospray ionization (ESI) in SFC and electron ionization in GC.

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

SFC MS/MS → ESI source

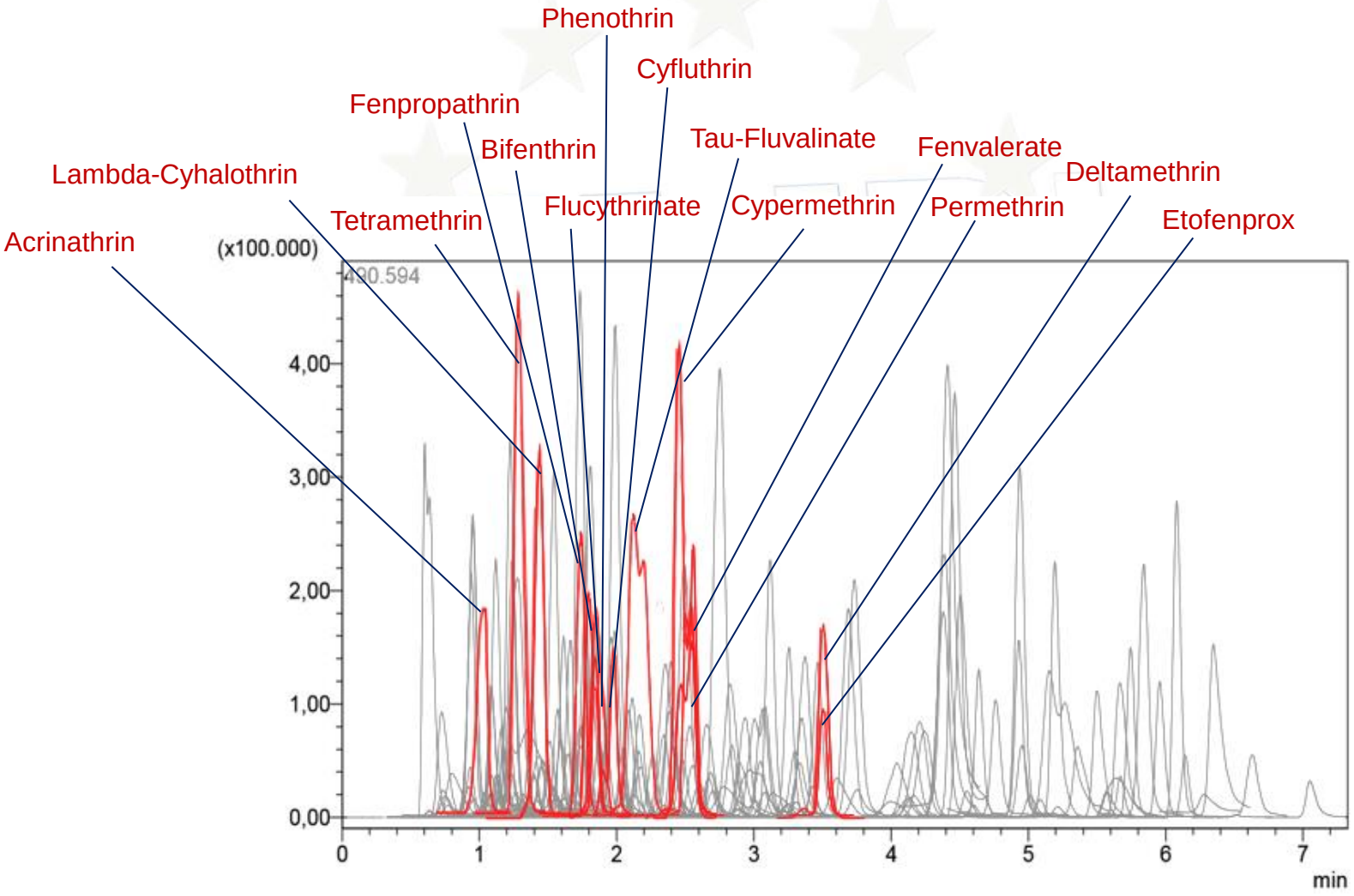
GC MS/MS → EI source



MULTIRESIDUE METHOD

10 µg/Kg Tomato

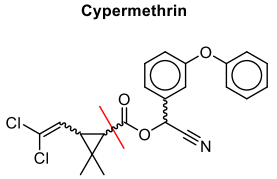
Pyrethroids



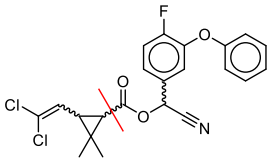
METHOD SELECTIVITY

GC
EI source

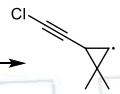
Cypermethrin



Cyfluthrin



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

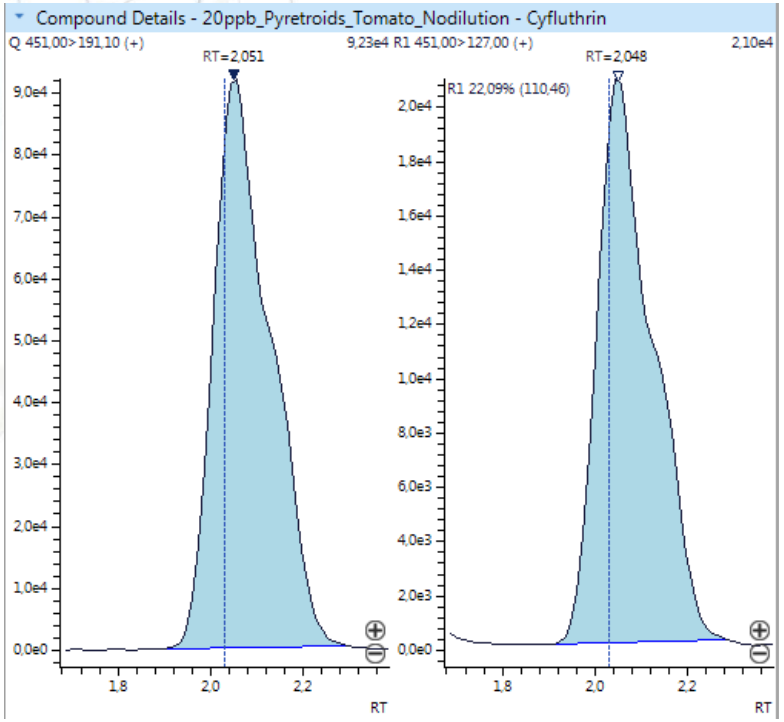


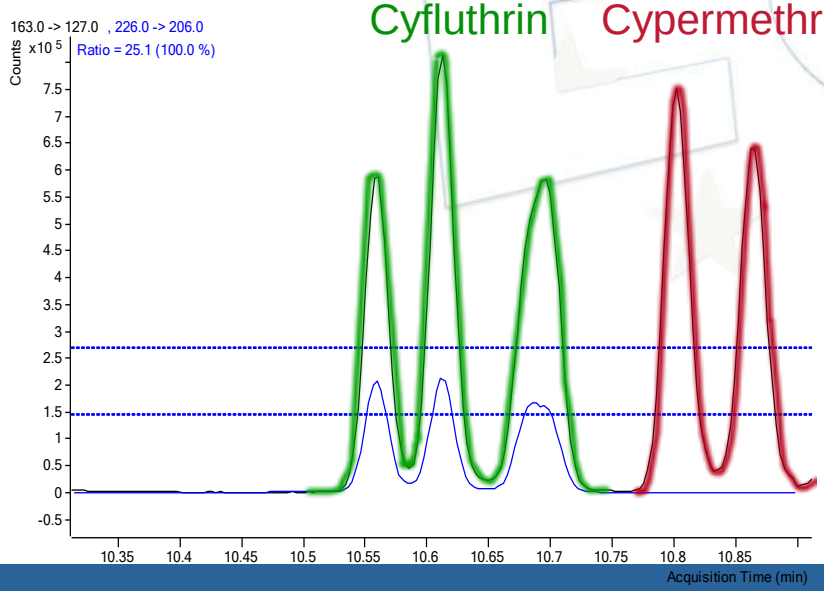
Chemical Formula: $C_7H_8Cl^{\bullet}$
Exact Mass: 127,0

SFC
ESI source

Cyfluthrin

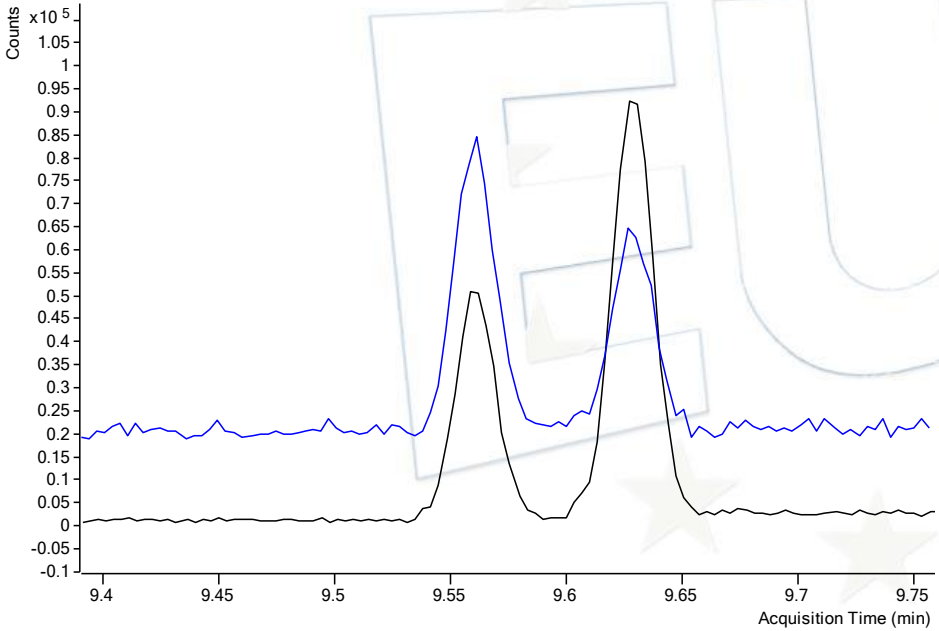
451>191 451>127



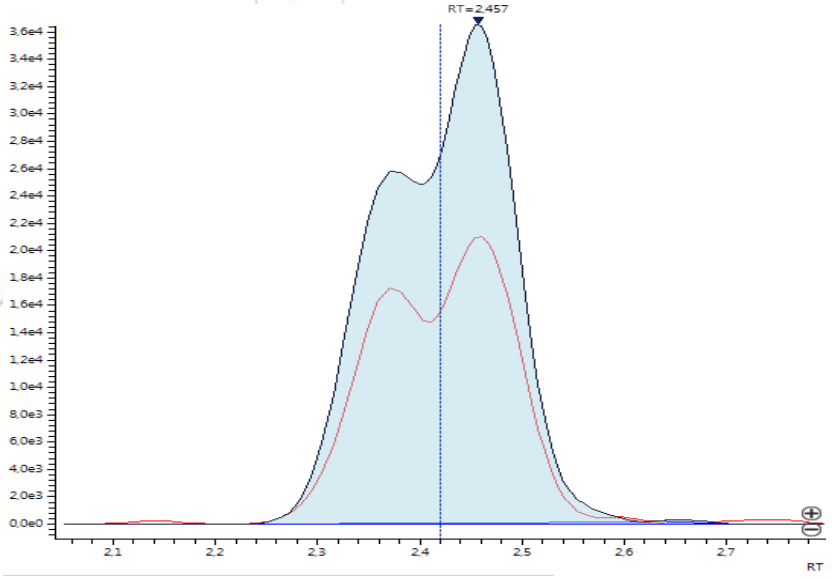


RESOLUTION

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

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CHIRAL SFC

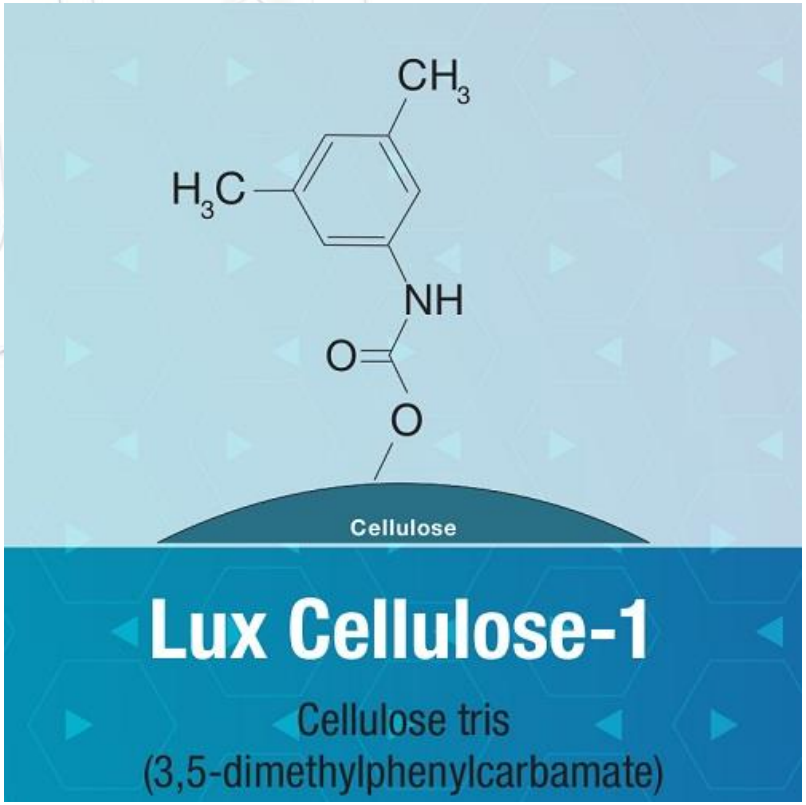
Supercritical fluid chromatography coupled to mass spectrometry (SFC-ESI-MS/MS) has been demonstrated to perform fast and highly efficient separations without the need to change the mobile phase employed in multiresidue pesticide analyses.

Column: LUX CELLULOSE-1

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

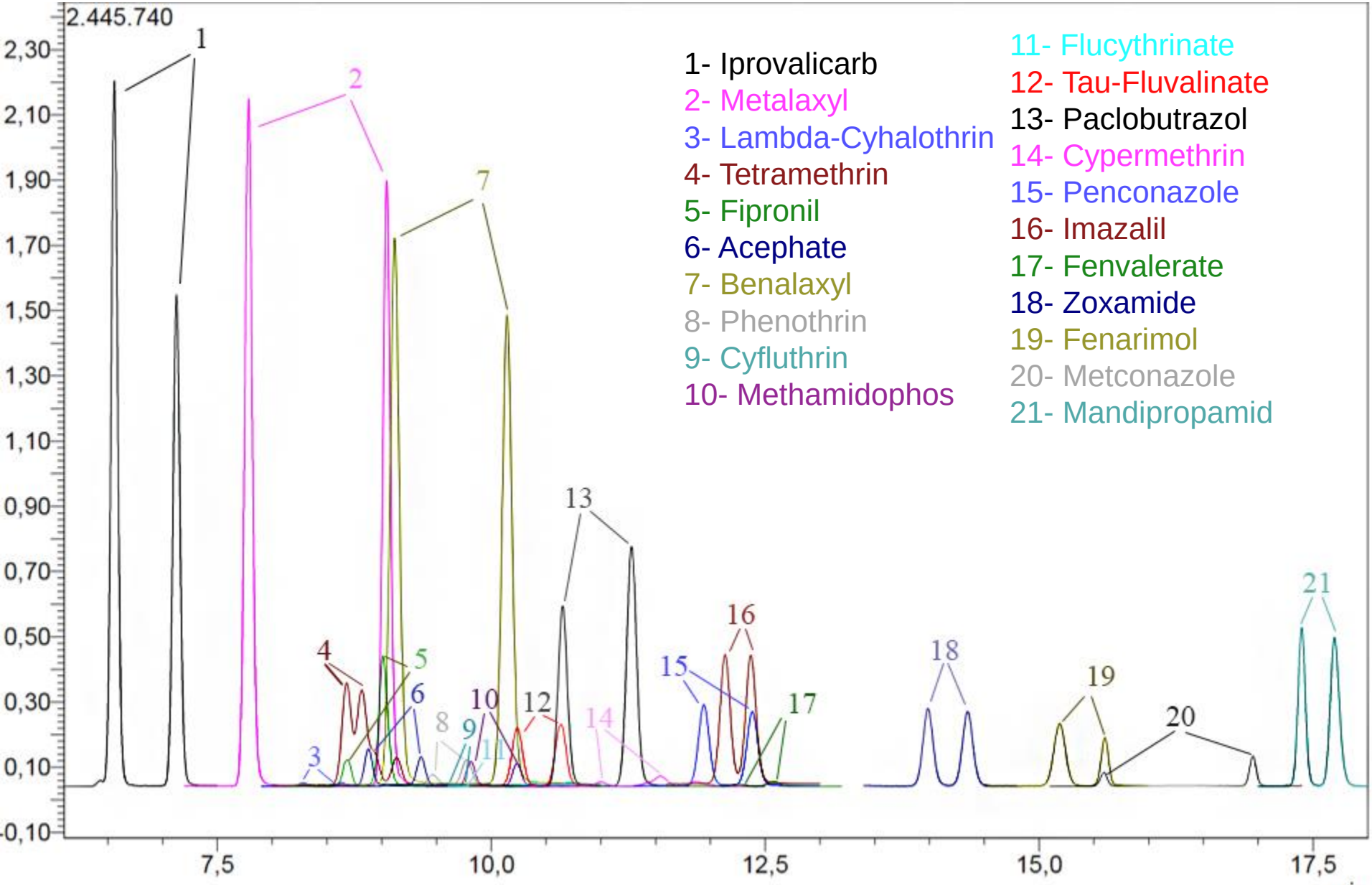
Lenght: 250 x 4.6 mm

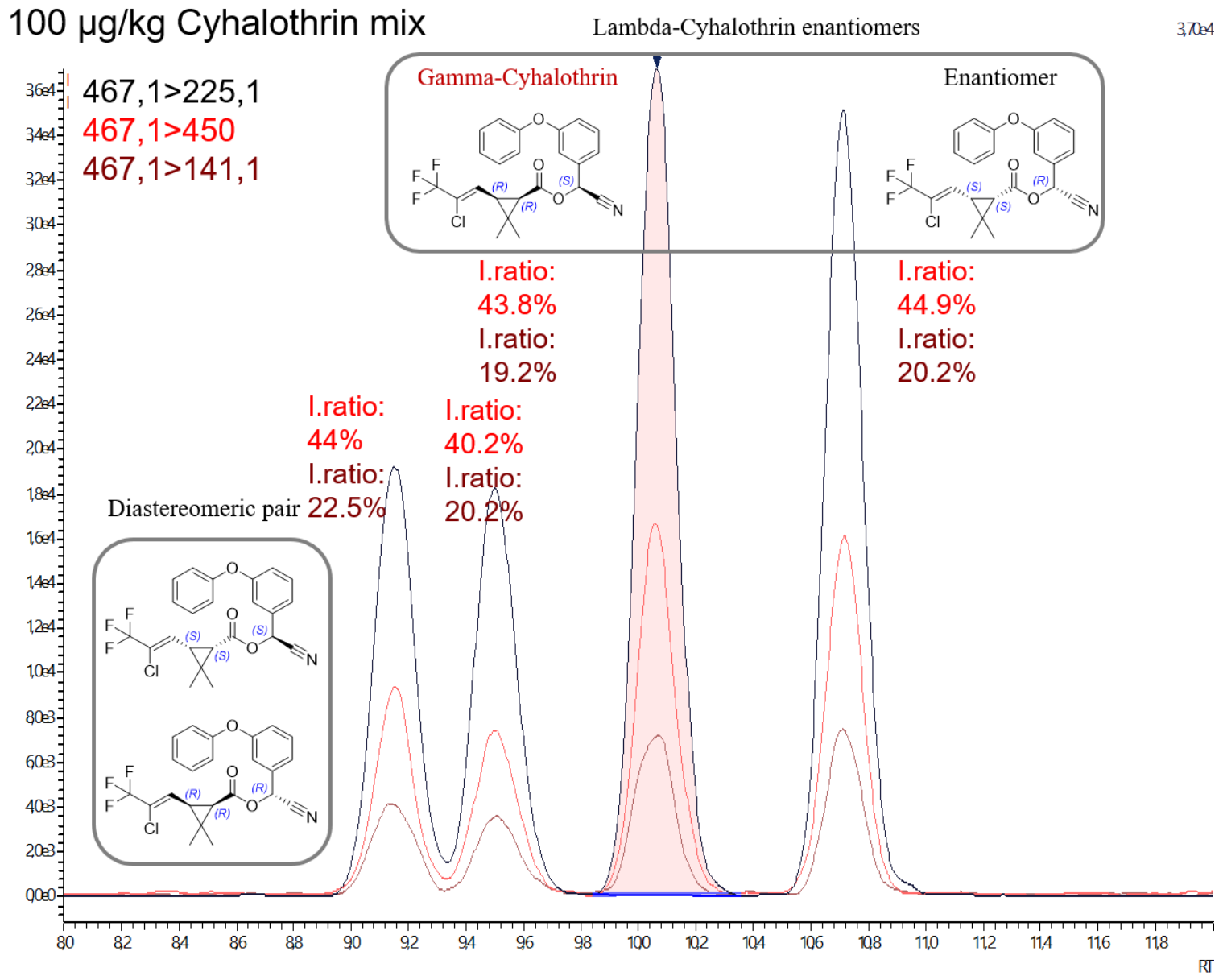
Particle size: 5 µm



COLUMN: LUX CELLULOSE-1

Cellulose tris(3,5-dimethylphenylcarbamate)

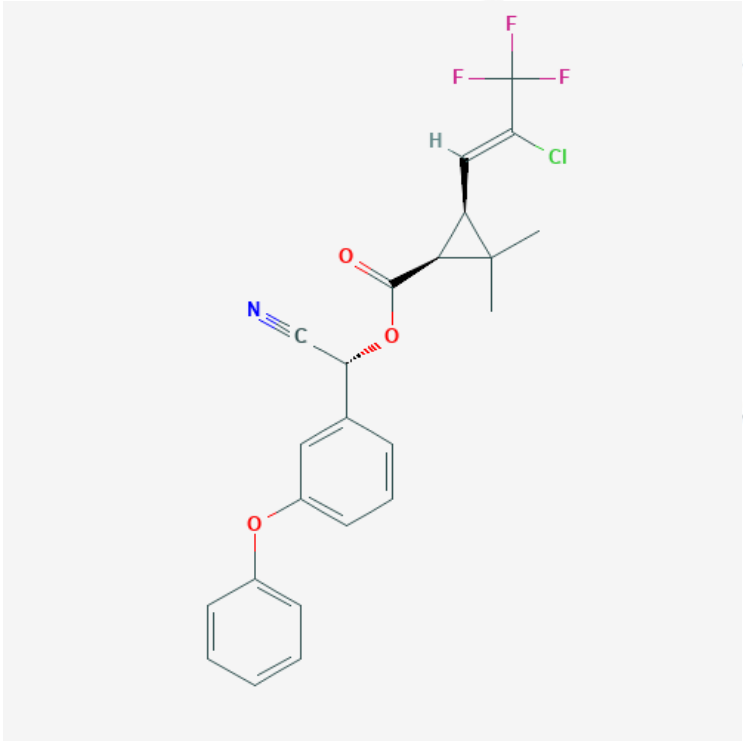



100 µg/kg Cyhalothrin mix
Lambda-Cyhalothrin enantiomers
467,1>225,1
467,1>450
467,1>141,1
Gamma-Cyhalothrin
Enantiomer
I.ratio: 43.8%
I.ratio: 19.2%
I.ratio: 44.9%
I.ratio: 20.2%
I.ratio: 44%
I.ratio: 22.5%
I.ratio: 40.2%
I.ratio: 20.2%
Diastereomeric pair
RT

LAMBDA-CYHALOTHRIN MIXTURE

ENANTIOMER

(R)-α-cyano-3-phenoxybenzyl (1S)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate

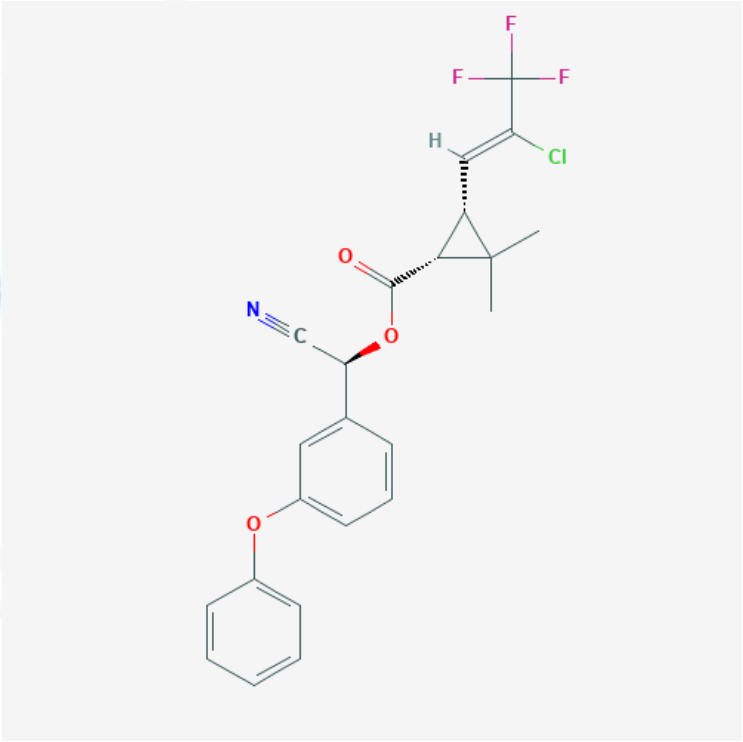


ARfD: 0.005

Source: Reg. (EU) No 1334/2014

GAMMA- CYHALOTHRIN

(S)-α-cyano-3-phenoxybenzyl (1R)-cis-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate



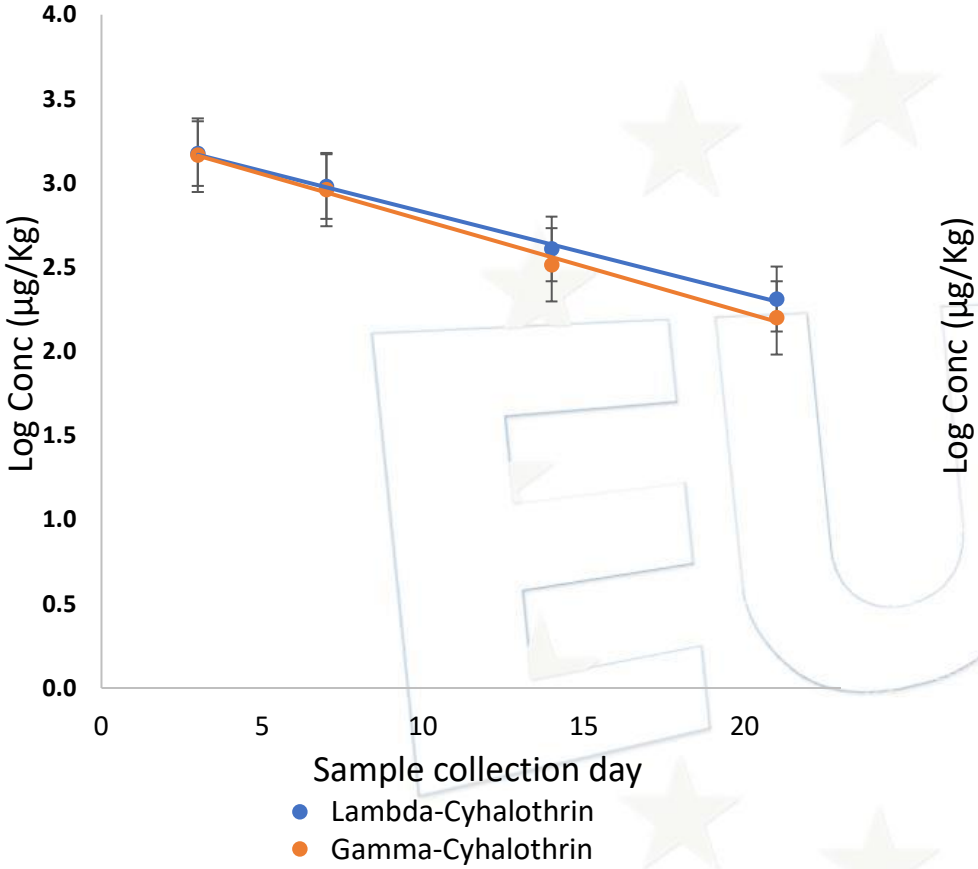
ARfD: 0.0025

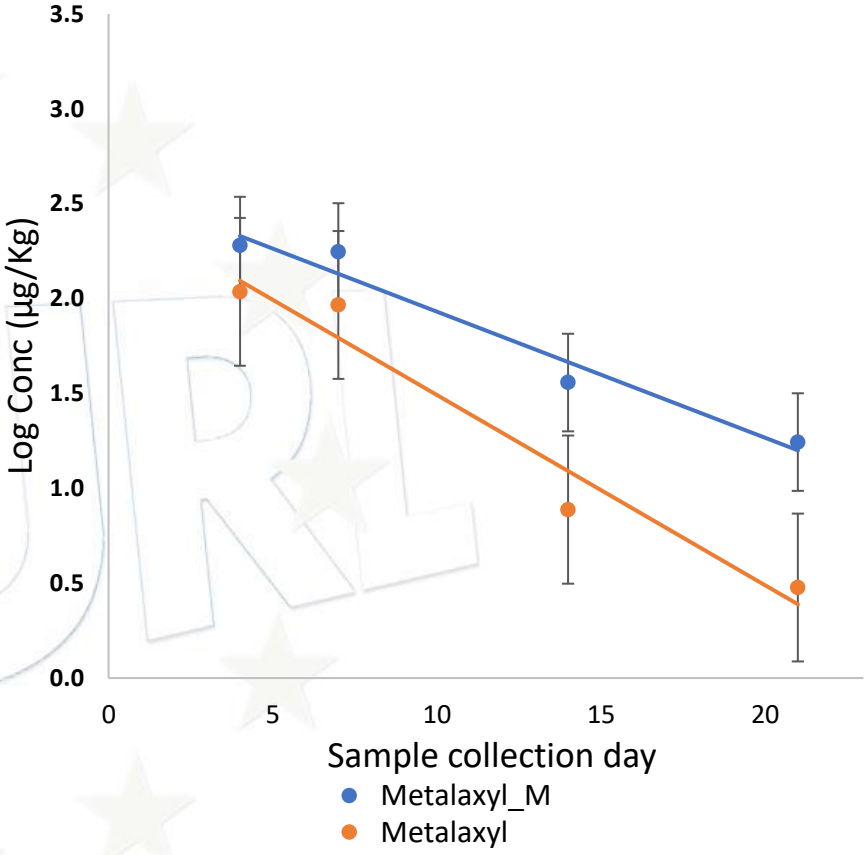
Source: Reg. (EU) No 1334/2014

INCURRED LAMBDA- CYHALOTHRIN/METALAXYL

Two experiments were carried out in a greenhouse located in Almeria. Lettuces were planted in their grown stage and spiked with a commercial formulation containing the mixture of lambda- cyhalothrin enantiomers. The same experiment was repeated with metalaxyl in tomatoes.

DEGRADATION SLOPES OF BOTH ENANTIOMERS





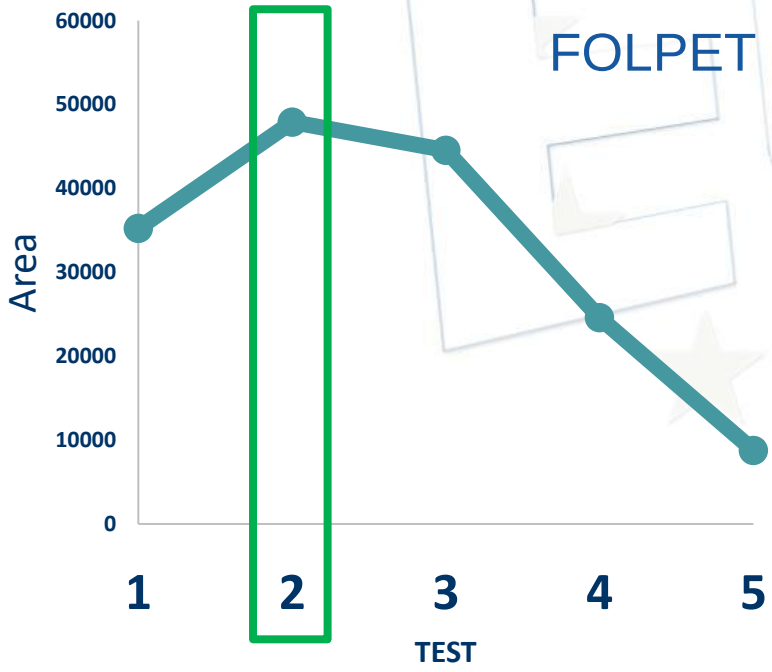
Lambda-cyhalothrin degradation in lettuce was the same for both isomers. However, metalaxyl-M (mefenoxam) degradation in tomato matrix was 2 to 6-times lower than its less active S- enantiomer.

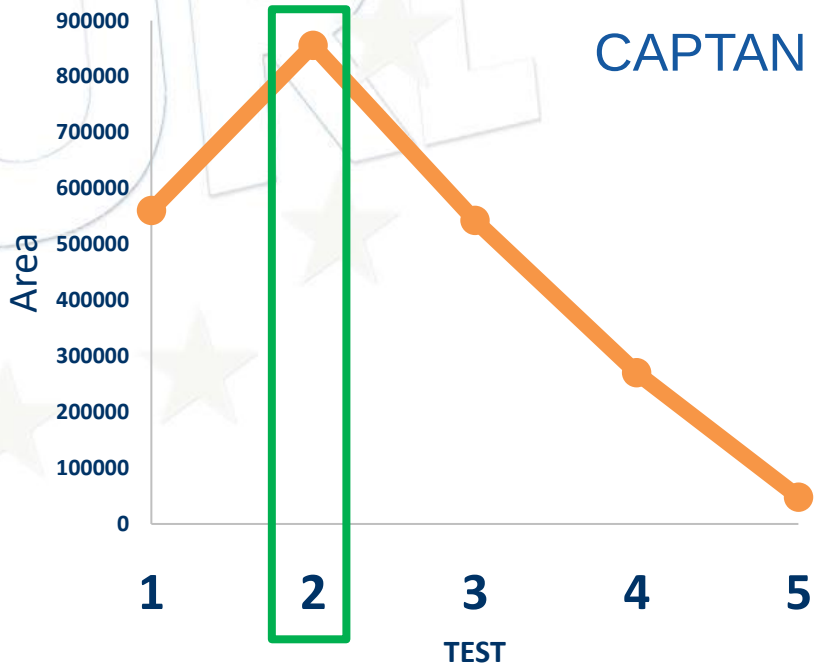
SFC Overview

- Technical and operational parameters
- Efficacy in Multiresidue Methods
- **Additional Advantages**
  - Lower ion suppression
  - Wider scope
  - Chiral separations
  - **Efficiency with a low ion source temperature**

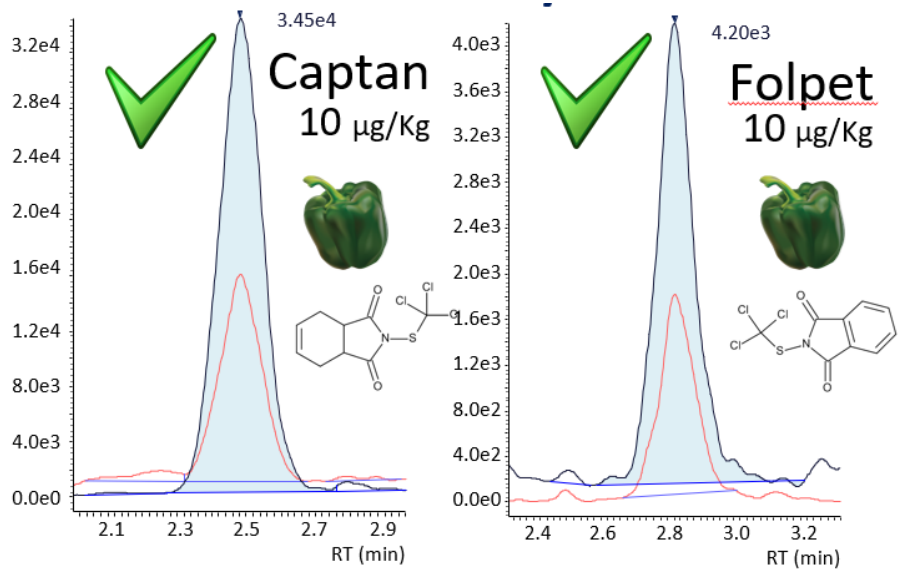
ION SOURCE OPTIMIZATION

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

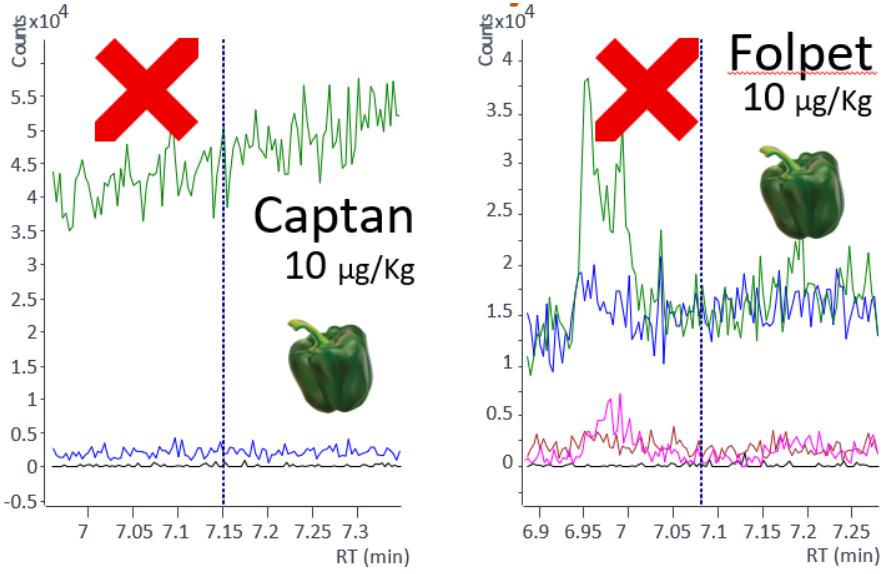




SFC-MS/MS



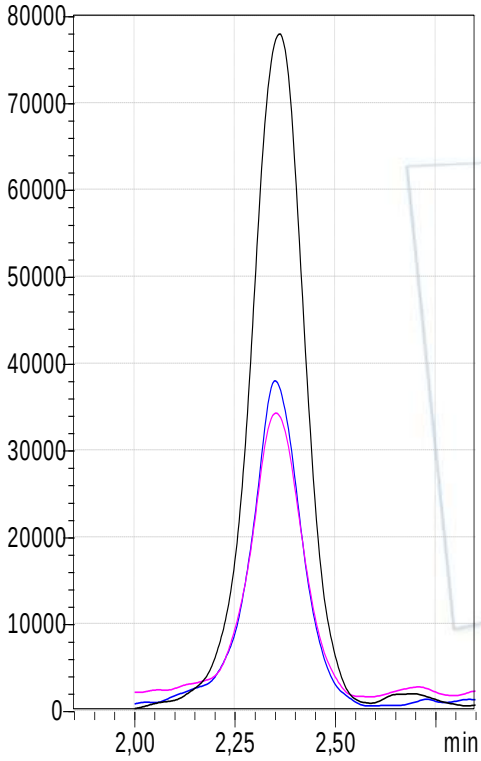
GC-MS/MS



Same vial: 100 µg/Kg

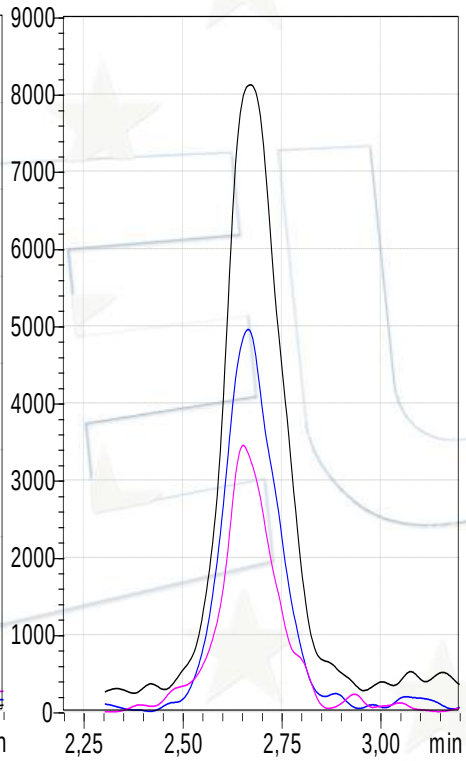
SFC-MS/MS

Captan



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

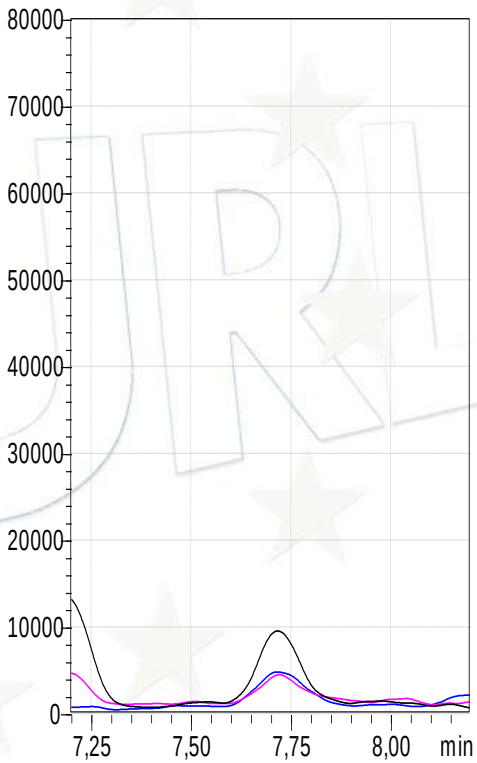
Folpet



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

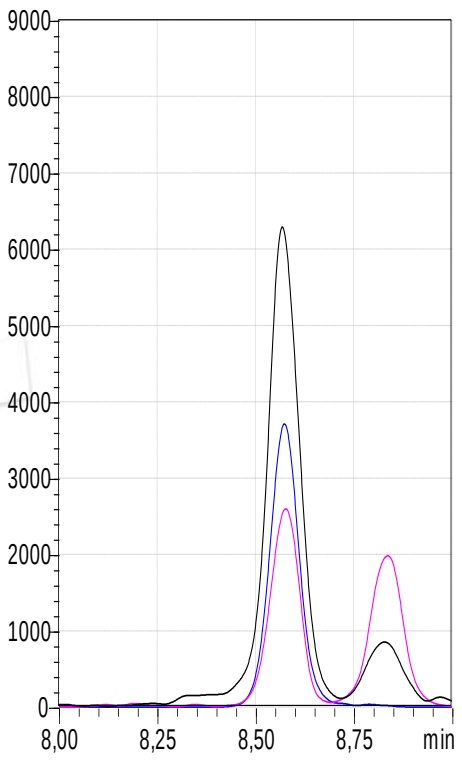
LC-MS/MS

Captan



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

Folpet

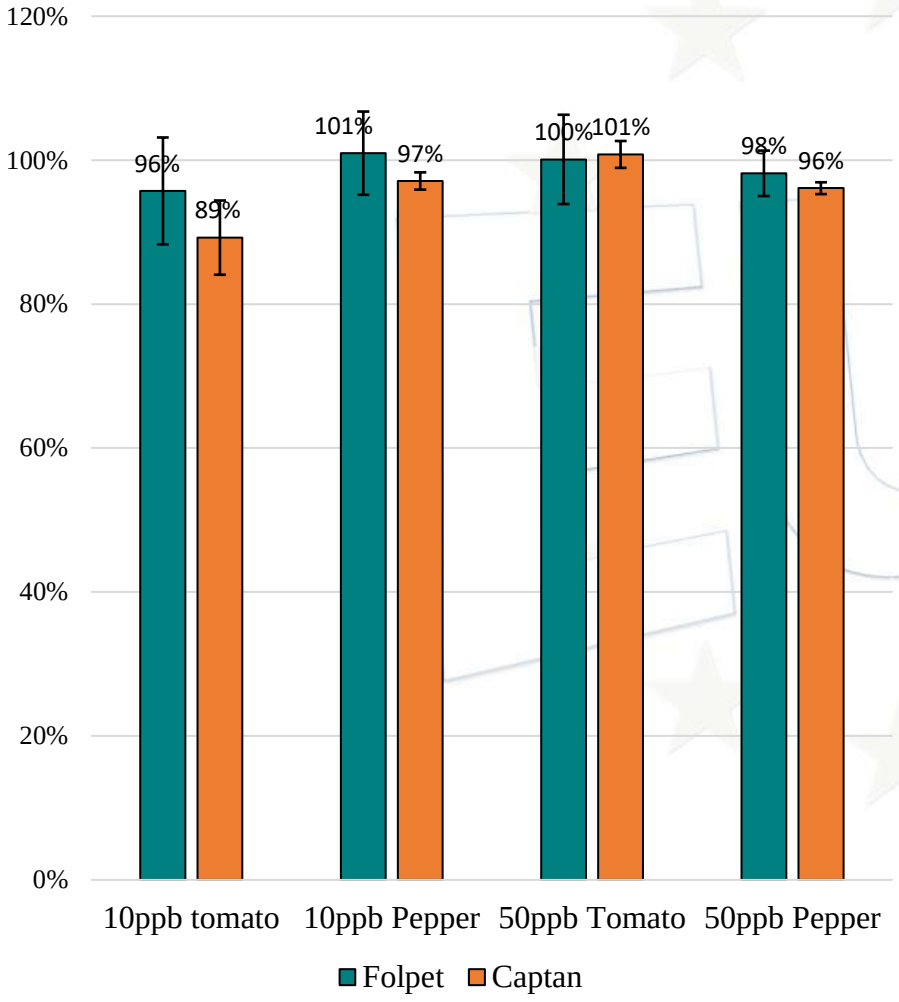


314,6>130,1
314,6>261,8
314,6>102,0

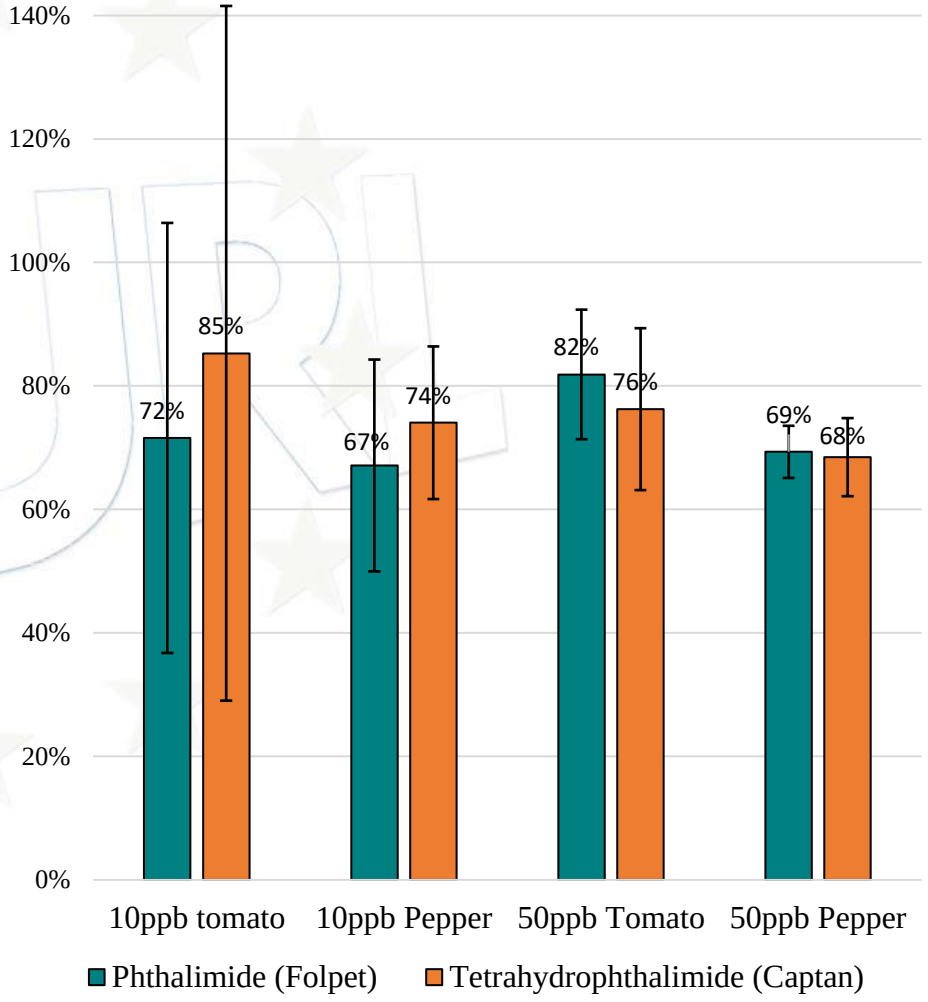
Recoveries

(same extraction)

SFC-MS/MS



GC-MS/MS



FOR FURTHER DATA:

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

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,*]

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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

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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

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

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 Almeria, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento S/N°, La Cañada de San Urbano, 04120, Almería, Spain

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

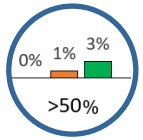
Víctor Cutillas [a], Florencia Jesús [b], Carmen Ferrer [a], Amadeo R. Fernández-Alba [a,*]

[a] European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables. University of Almeria, 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

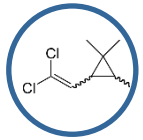
CONCLUSIONS

SHORT RUN TIMES

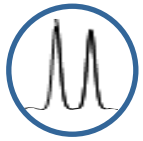
LOWER SOLVENT CONSUMPTION



REDUCTION OF ION SUPPRESSION IN DIFFICULT MATRICES



SCOPE ENLARGEMENT (PYRETHROIDS)



FAST CHIRAL SEPARATIONS

SENSITIVITY WITH A LOW ION SOURCE TEMPERATURE

EURL-FV team

http://www.eurl-pesticides.eu

Thank You for Your Attention