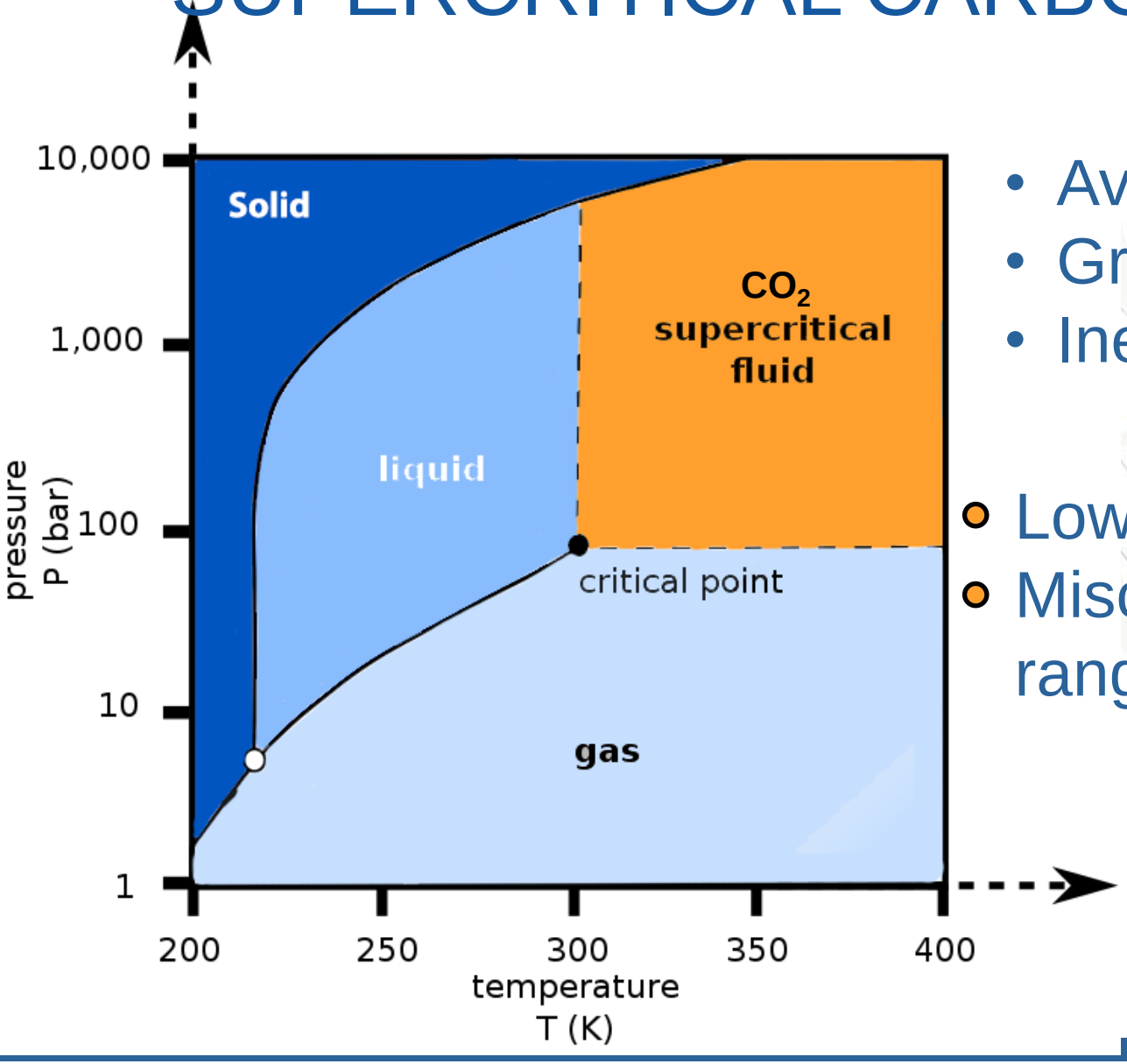
ANALYSIS OF PESTICIDE RESIDUES IN FOOD USING SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Víctor Manuel Cutillas Juárez, María Murcia Morales,
María Martínez Galera, Amadeo Rodríguez Fernández-Alba

SUPERCRITICAL CARBON DIOXIDE

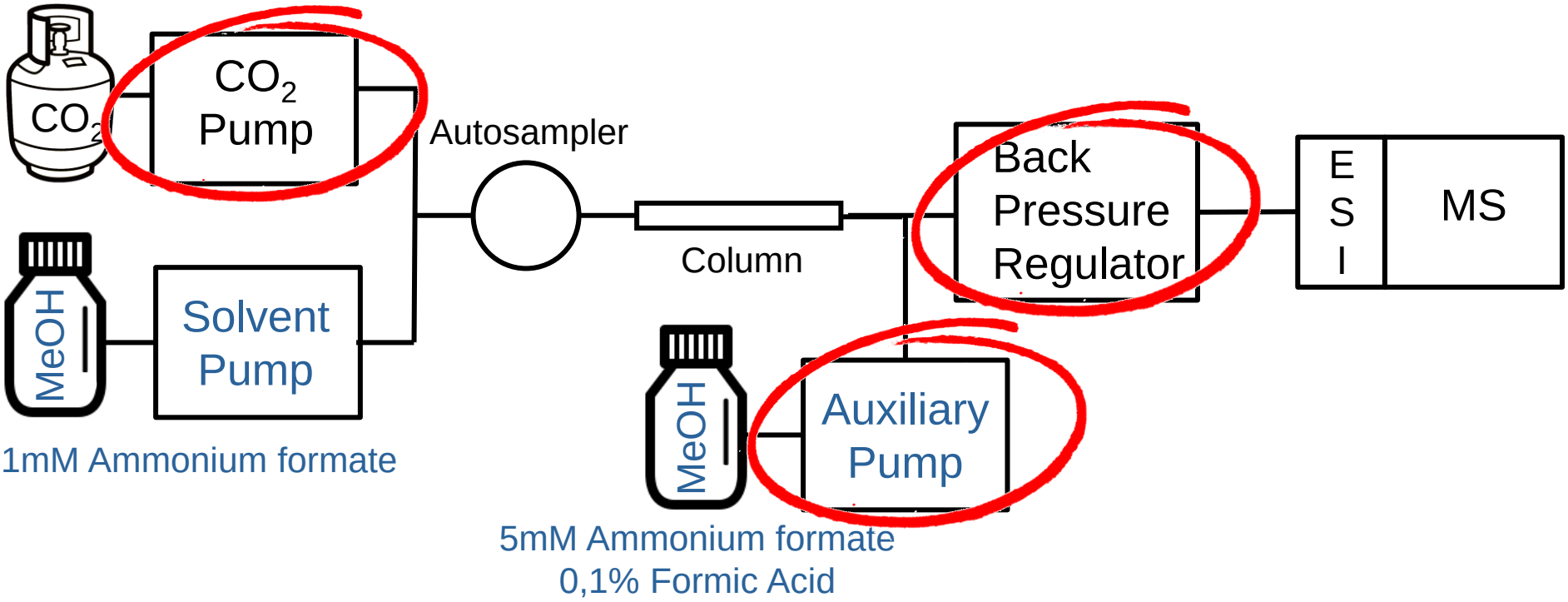


- Available
- Green
- Inexpensive

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

SFC-MS/MS System

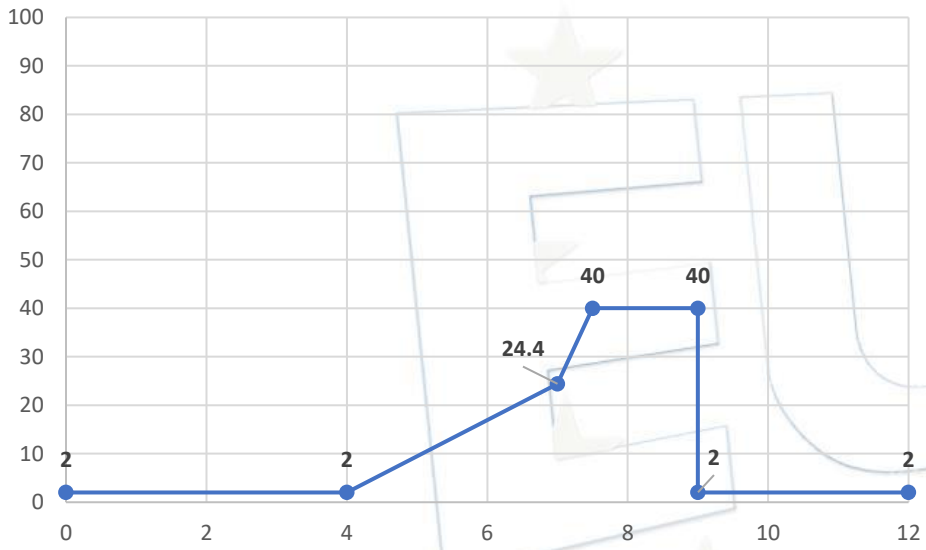
(Nexera UC coupled to Shimadzu LC-MS 8060)



METHANOL AS CO-SOLVENT

SFC

Run time: 12 min
Flow: 1,5mL/min

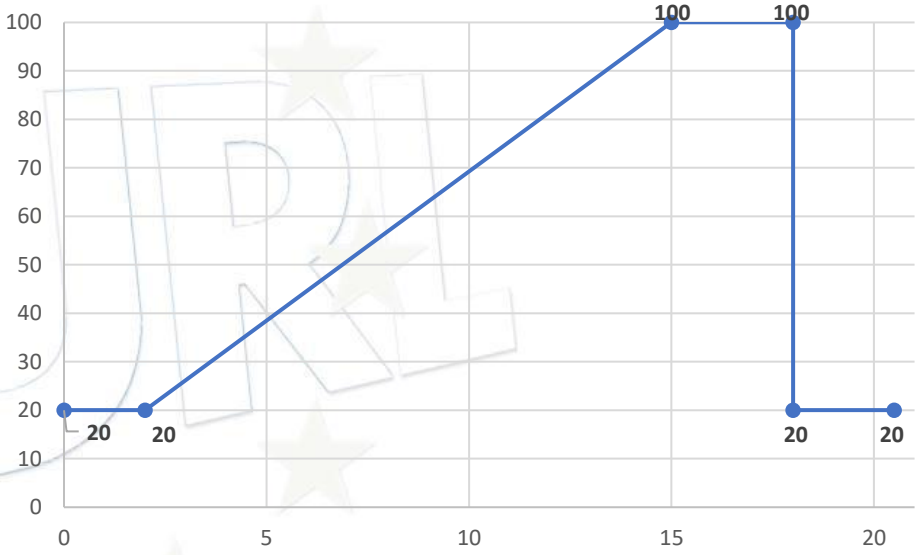


TOTAL RUN CONSUMPTION

MeOH (Gradient) : 1,95mL
MeOH (Post-column): 1,8mL
} 3,75mL
Water: 0mL
CO2 Consumption : 15,32mL

LC

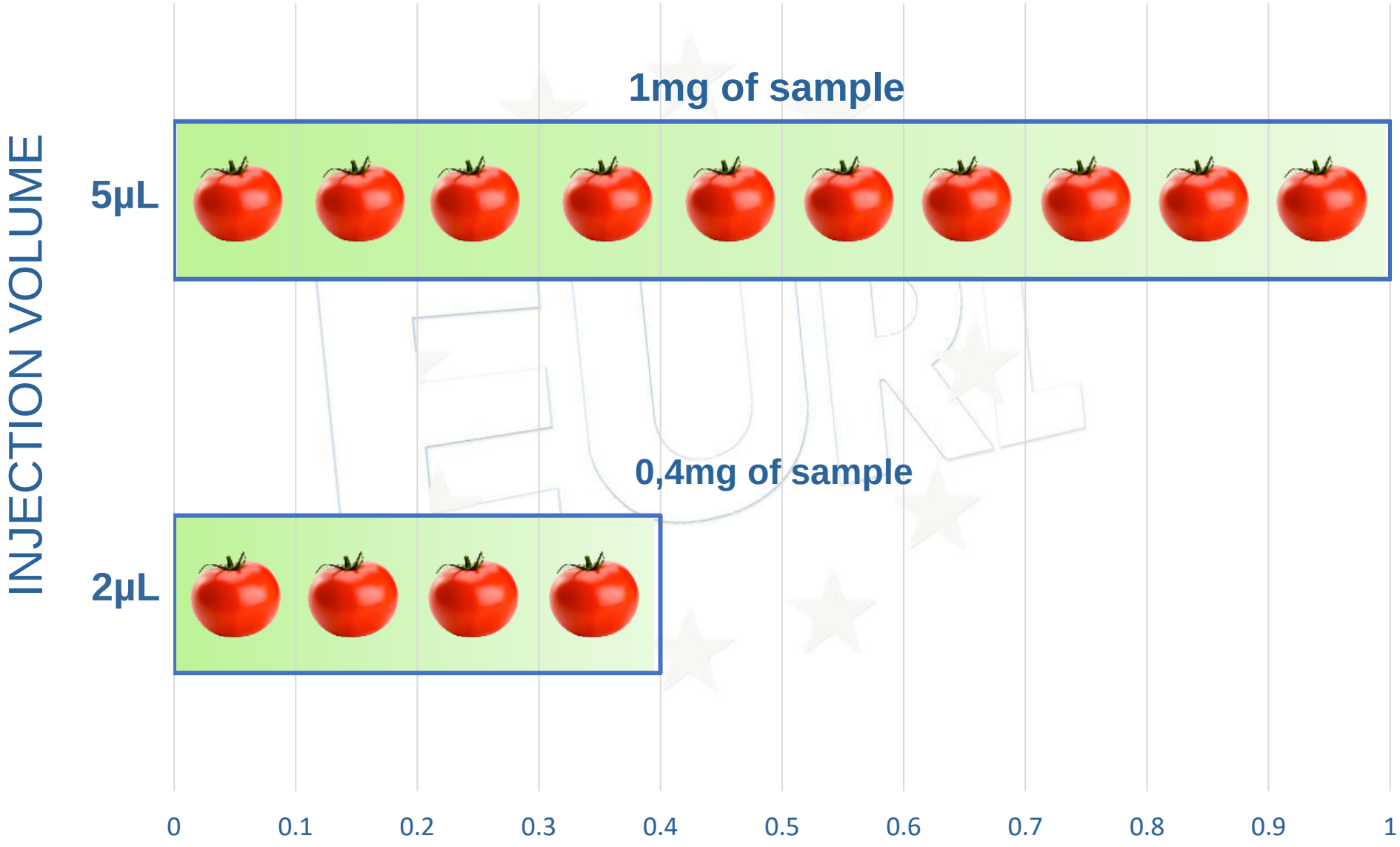
Run time: 20,5min
Flow: 0,3mL/min



TOTAL RUN CONSUMPTION

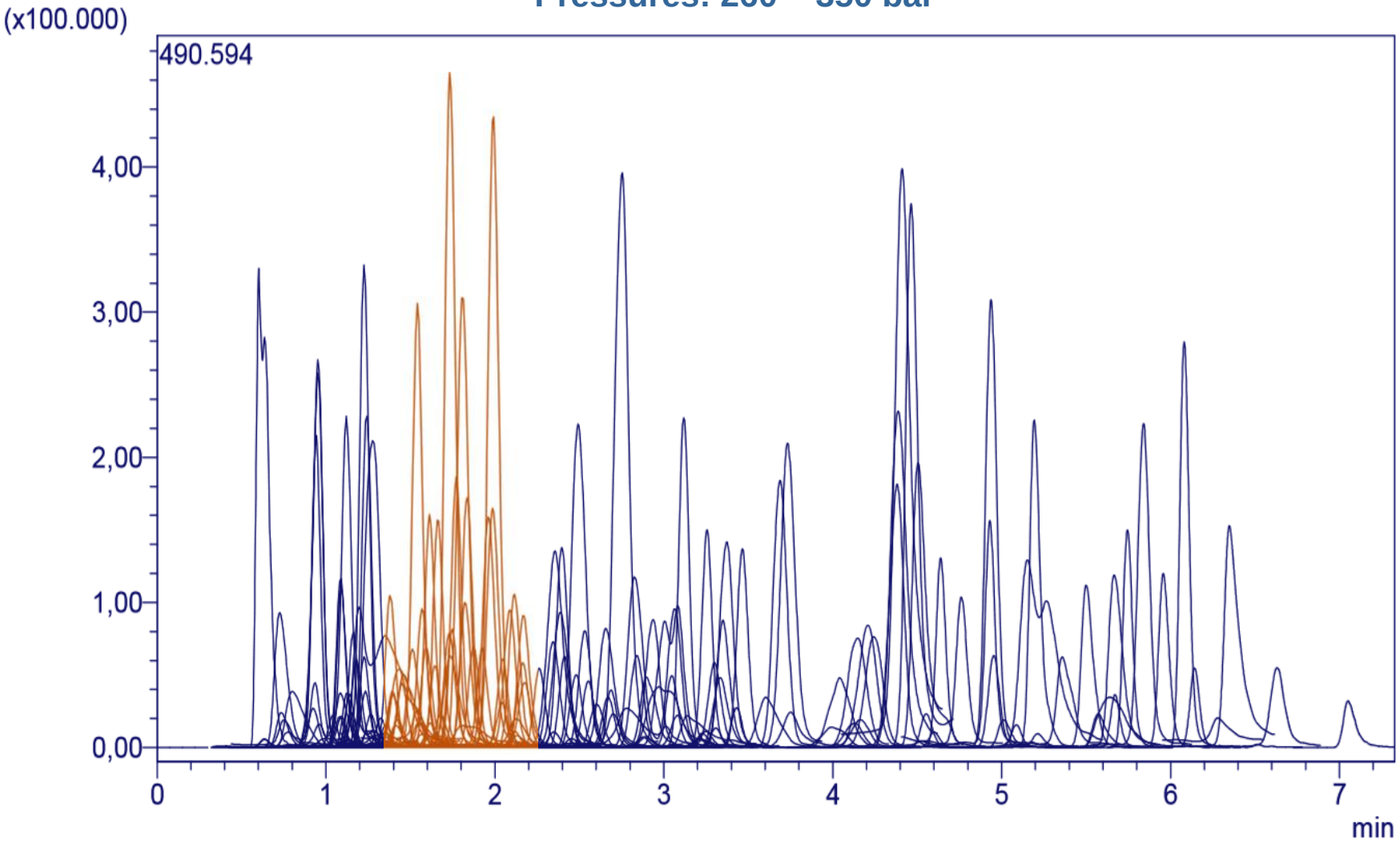
MeOH : 3,51mL
Water: 2,64mL

TOTAL INJECTED SAMPLE



TOTAL FLOW: 1.5 mL/min

Pressures: 260 – 350 bar



10 µg/Kg Tomato (164 pesticides)

A THIRD TRANSITION IS NECESSARY

Too much coelution with this short run time?

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

Acquisition window: +-0,3

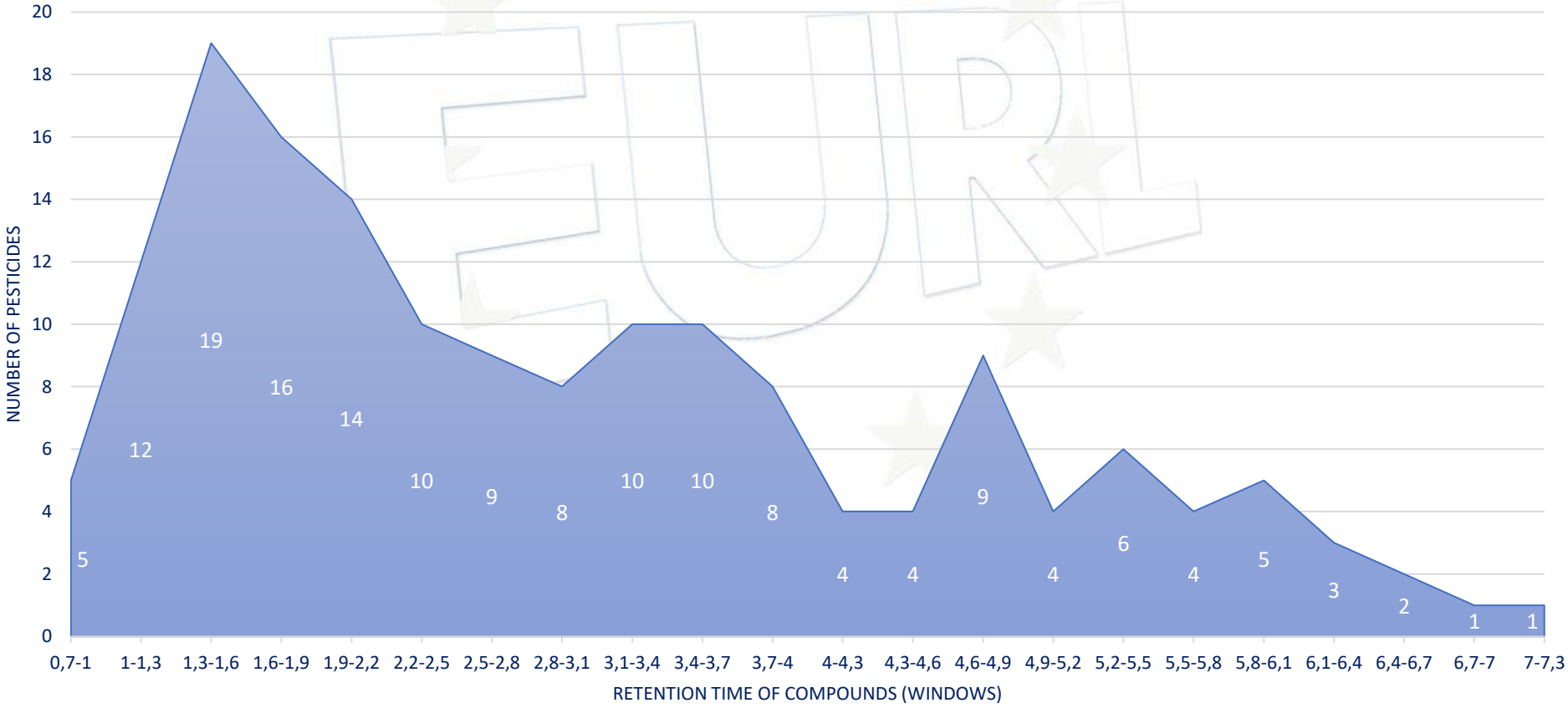
Start - End Time(min)	1,591-	1,596-	1,606-	1,625-	1,641-	1,654-
Event	37	36	39	36	38	37
Loop Time(sec)	0,508	0,496	0,532	0,496	0,520	0,508
Dwell Time(msec)	5,0	5,0	5,0	5,0	5,0	5,0

Maximum cycle time 0.532

Maximum Event: 39 Maximum Loop Time(sec): 0,532

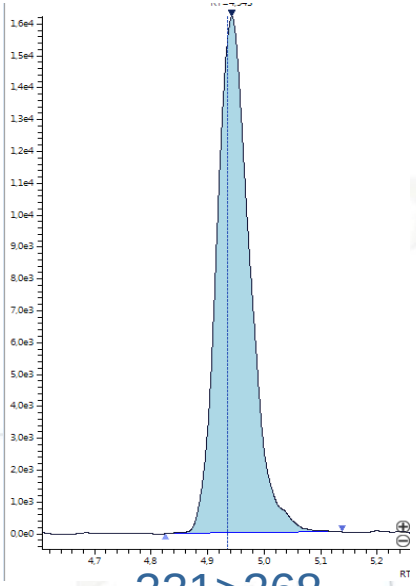
Minimum Dwell Time(msec): 5,0

Maximum Dwell Time(msec): 5,0

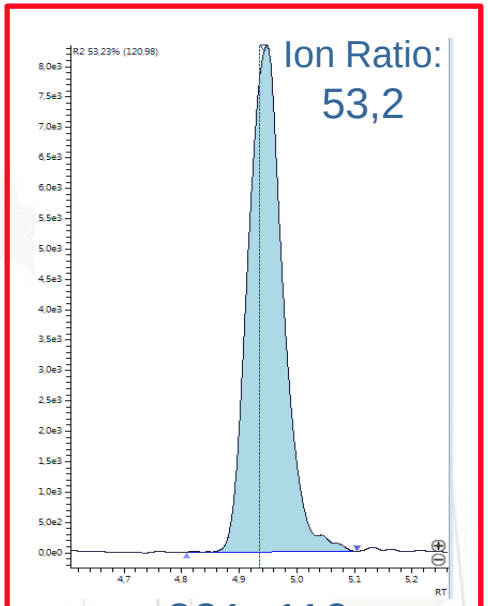


10 µg/kg
Tomato

FENARIMOL



331>268



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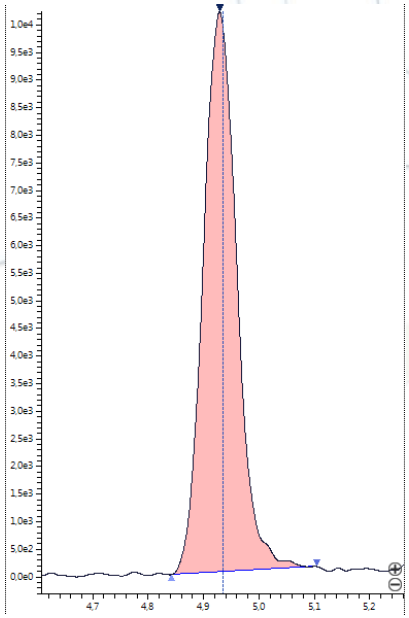

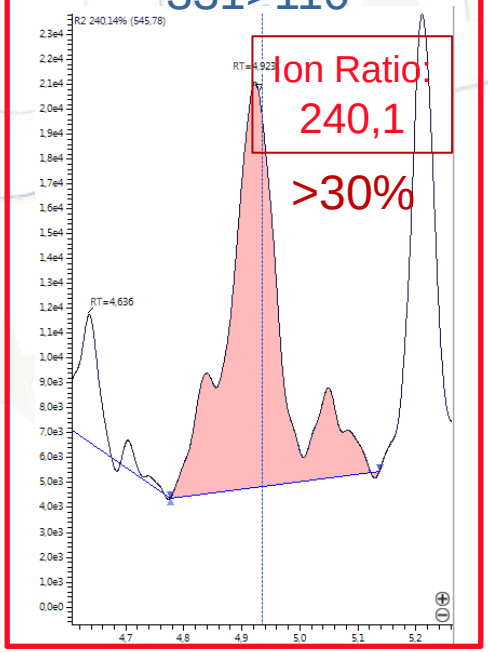

10 µg/kg
Leek

A THIRD TRANSITION IS NECESSARY

Acquisition window: +-0,3

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Event	37	36	39	36	38	37
Loop Time(sec)	0,508	0,496	0,532	0,496	0,520	0,508
Dwell Time(msec)	5,0	5,0	5,0	5,0	5,0	5,0

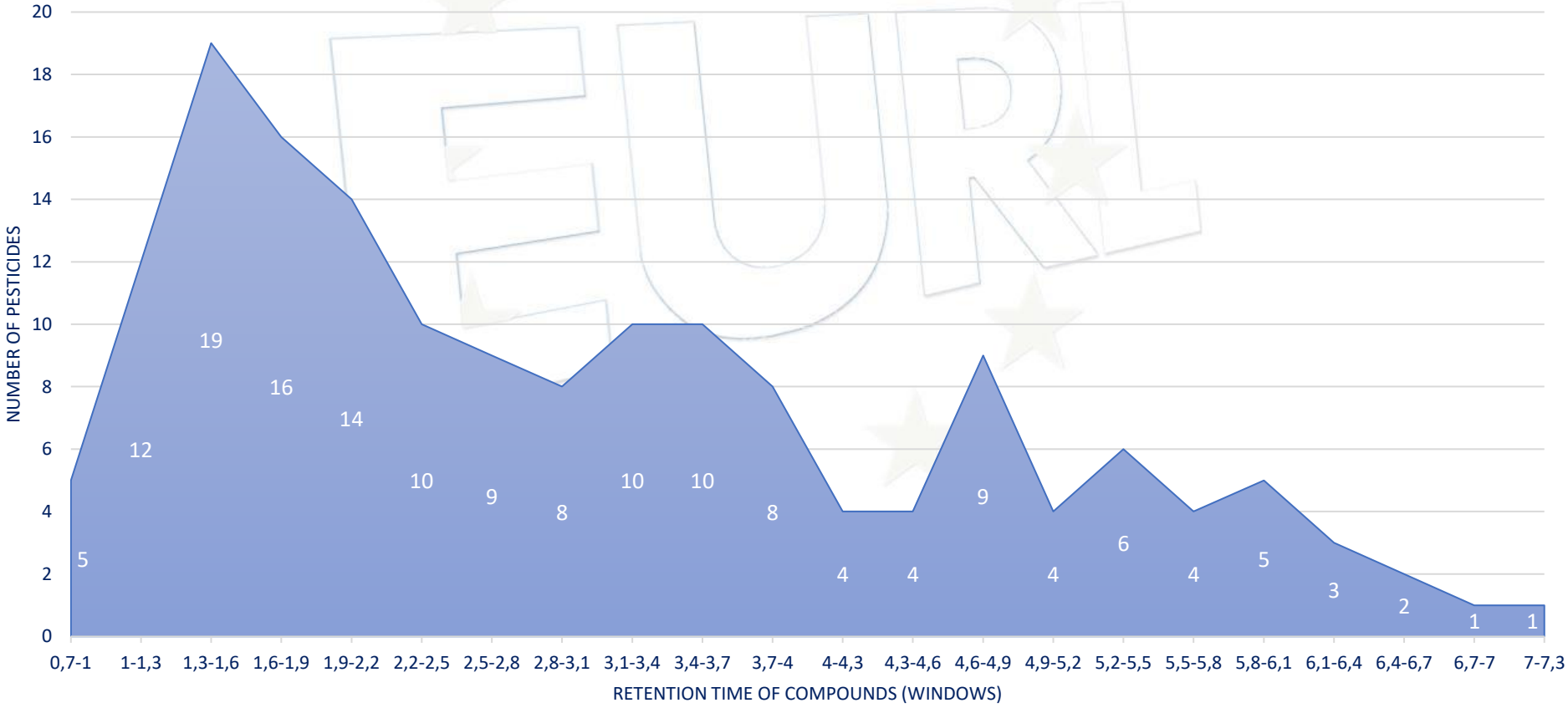
Maximum cycle time 0.532

Maximum Event: 39 Maximum Loop Time(sec): 0,532

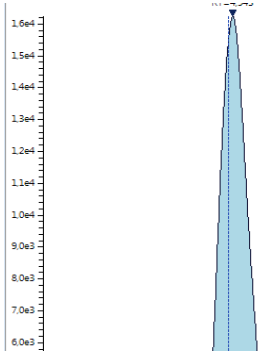
Minimum Dwell Time(msec): 5,0

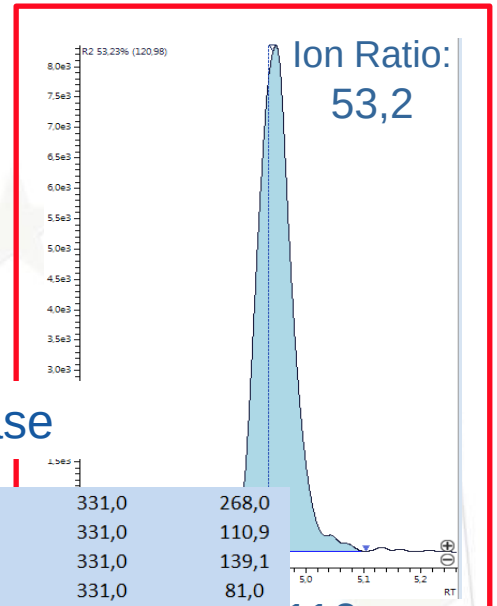
Maximum Dwell Time(msec): 5,0

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

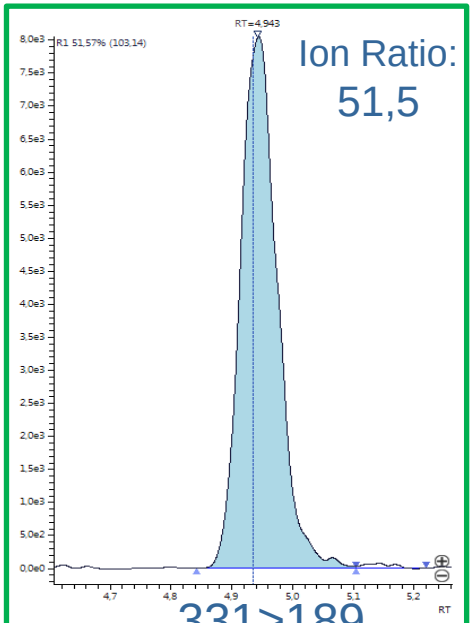


10 µg/kg
Tomato





Ion Ratio:
53,2



Ion Ratio:
51,5

331>189

MRM Shimadzu pesticide database

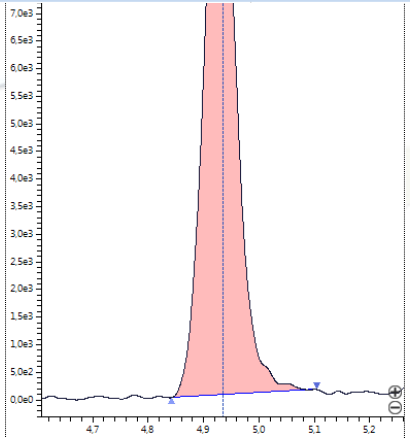
300	☑	Fenarimol	ESI+	[M+H]+	331,0	268,0
300	☑	Fenarimol	ESI+	[M+H]+	331,0	110,9
300	☑	Fenarimol	ESI+	[M+H]+	331,0	139,1
300	☐	Fenarimol	ESI+	[M+H]+	331,0	81,0
300	☐	Fenarimol	ESI+	[M+H]+	331,0	189,0
300	☐	Fenarimol	ESI+	[M+H]+	331,0	259,0
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	270,0
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	268,1
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	81,0
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	189,1
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	261,1
300	☐	Fenarimol	ESI+	[M+2+H]+	333,0	139,0

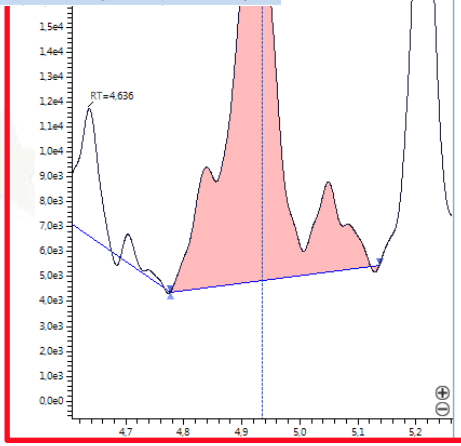
110

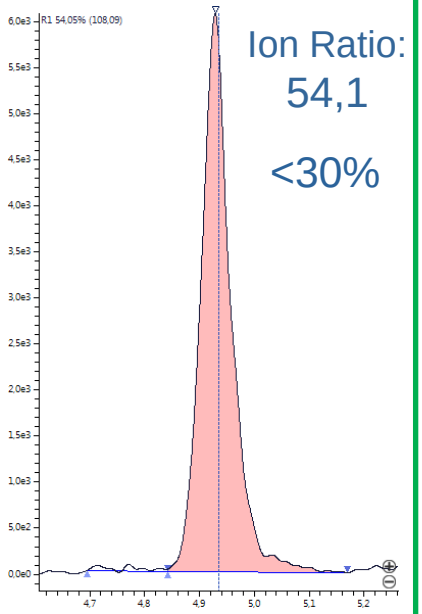
on Ratio:
240,1

>30%

10 µg/kg
Leek



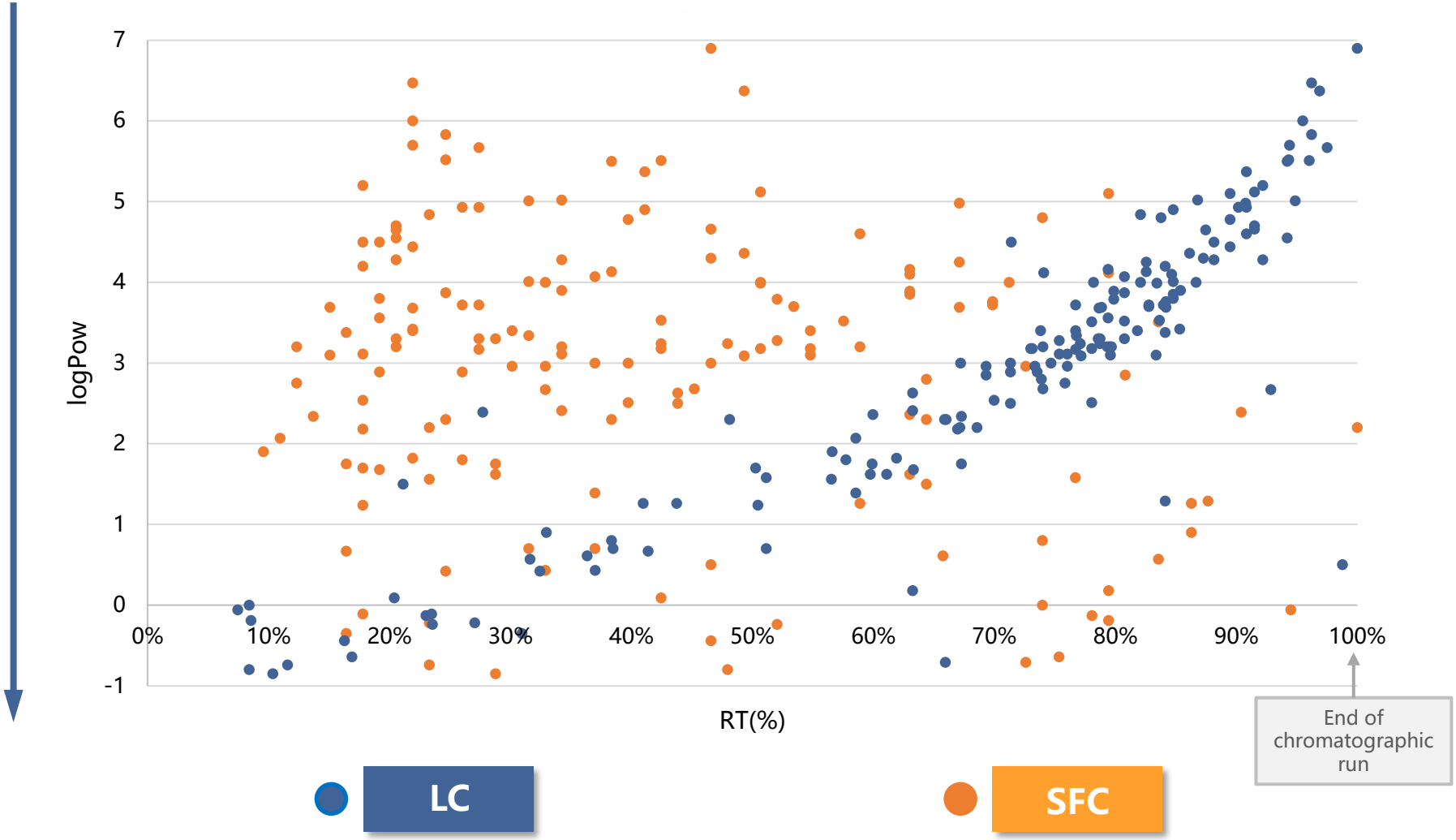




Ion Ratio:
54,1

<30%

ELUTION ORDER

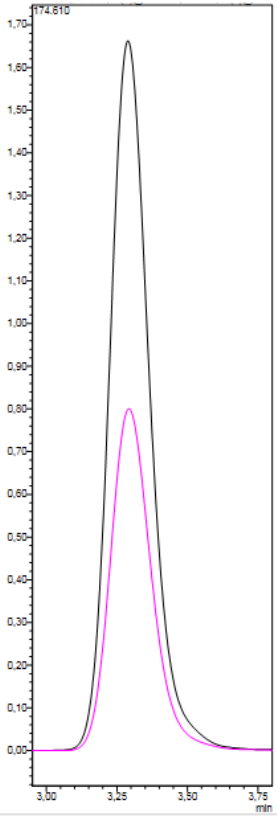


INCREASE OF SENSITIVITY

100 µg/kg Tomato

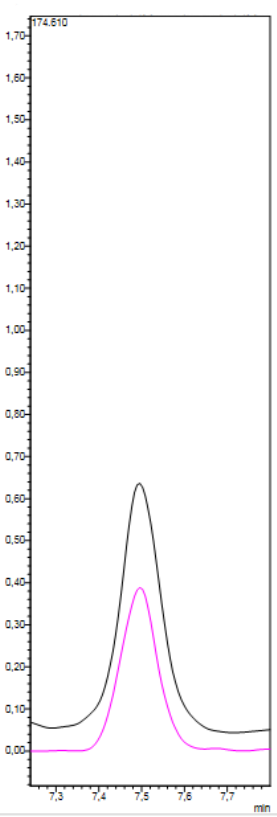
Fluazifop

SFC



RT: 3,741 min

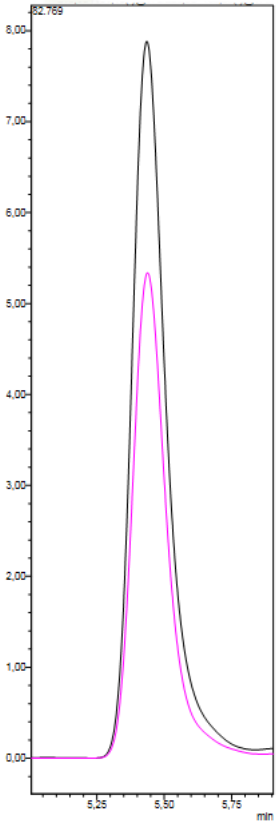
LC



RT: 7,494min

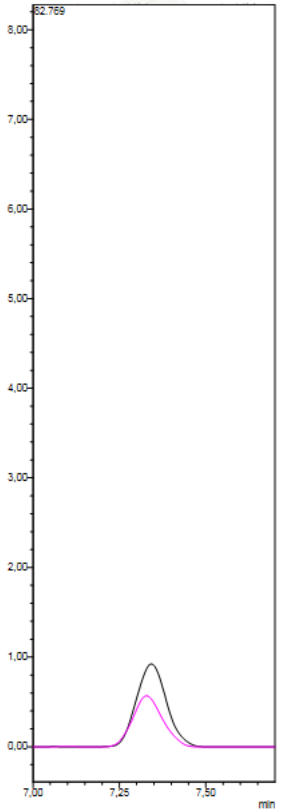
2,4-D

SFC



RT: 5,568 min

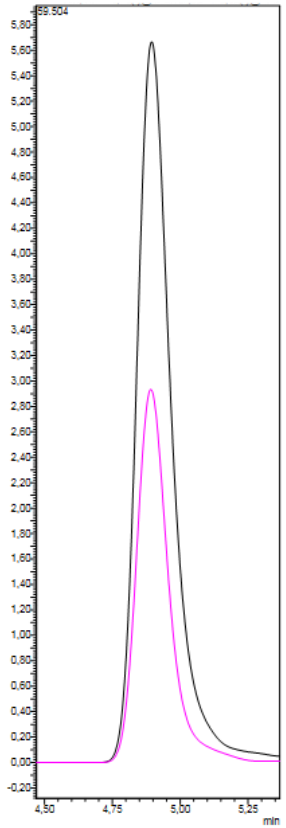
LC



RT: 7,343 min

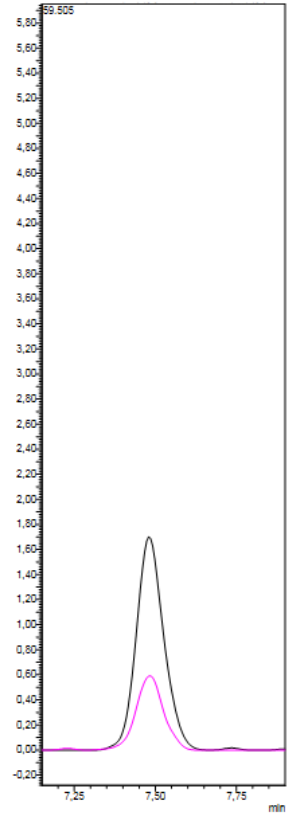
MCPA

SFC



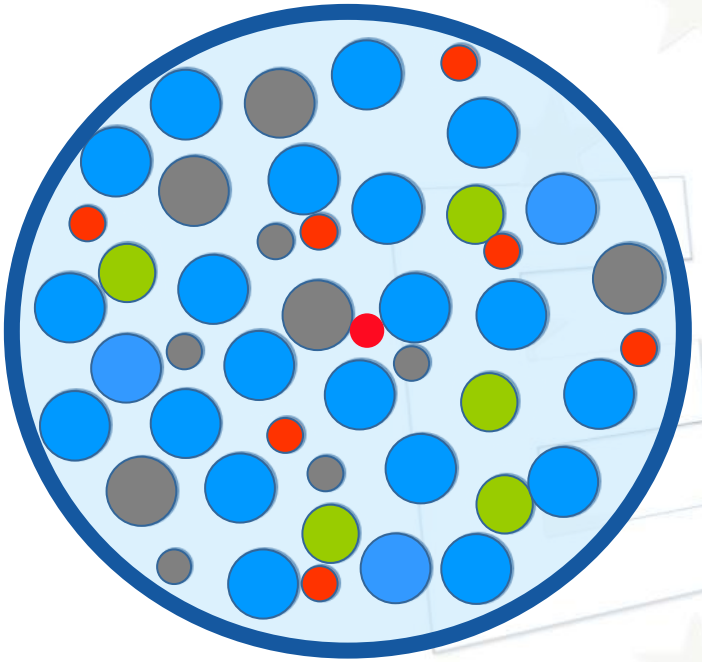
RT: 5,036 min

LC



RT: 7,481 min

ESI IONIZATION PROCESS



Ion
Water
Matrix
Methanol

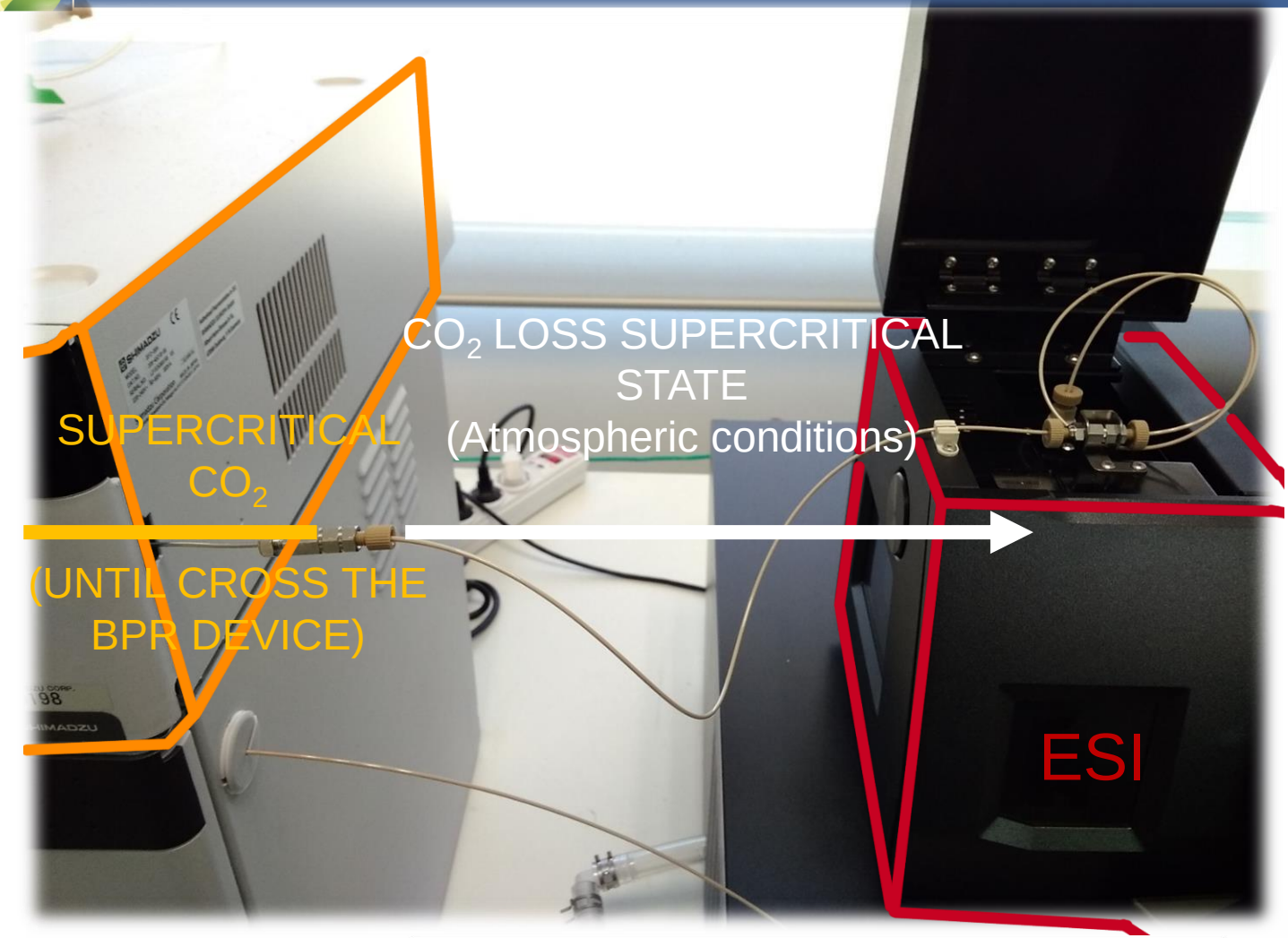
SFC MOBILE PHASE:

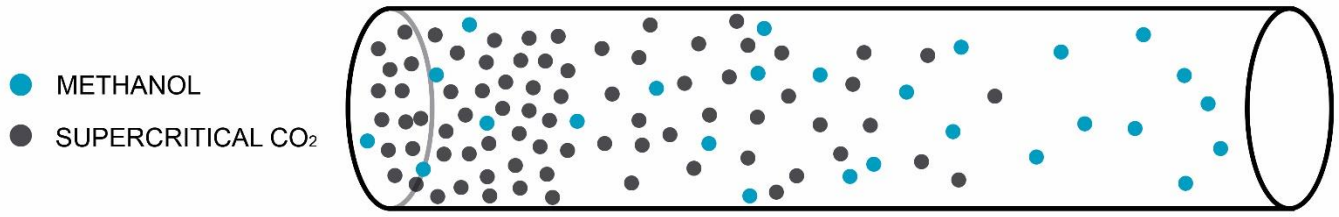
WATER

Surface tension (20°C): 72.80 mN/m

METHANOL

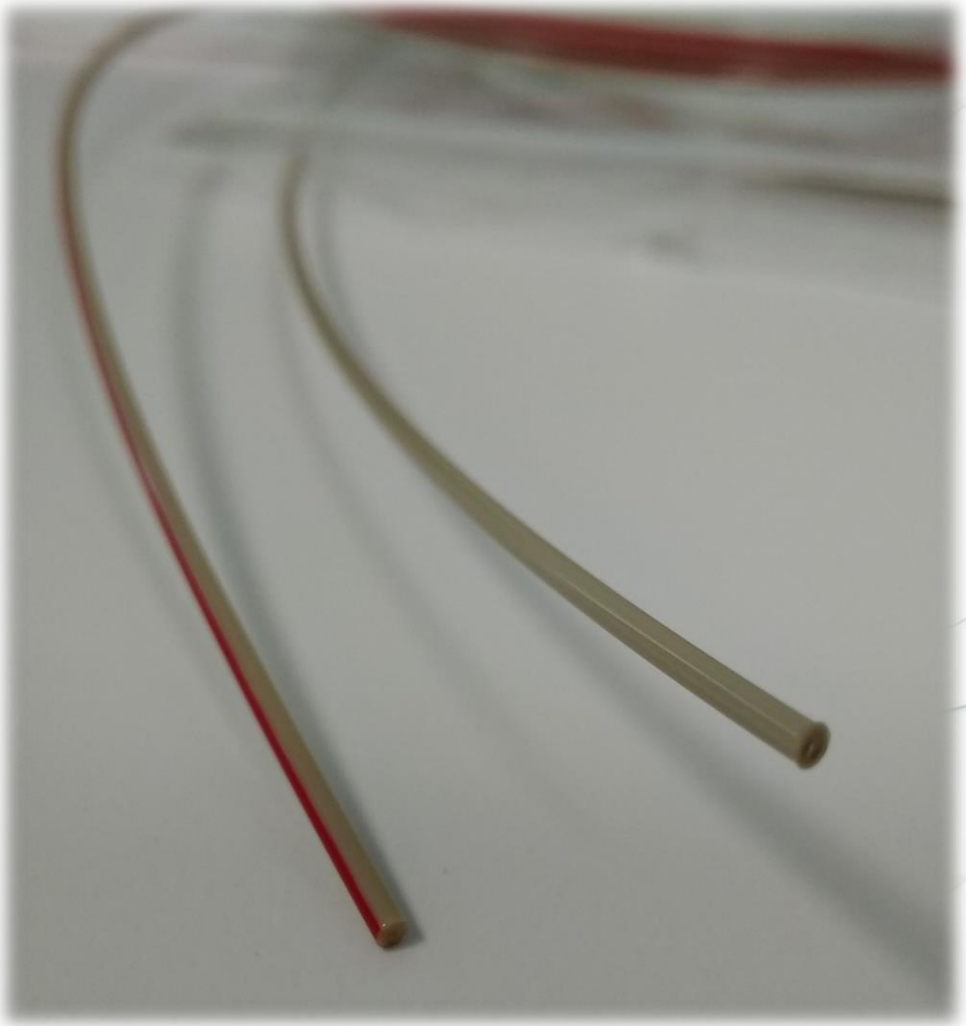
Surface tension (20°C): 22.70 mN/m

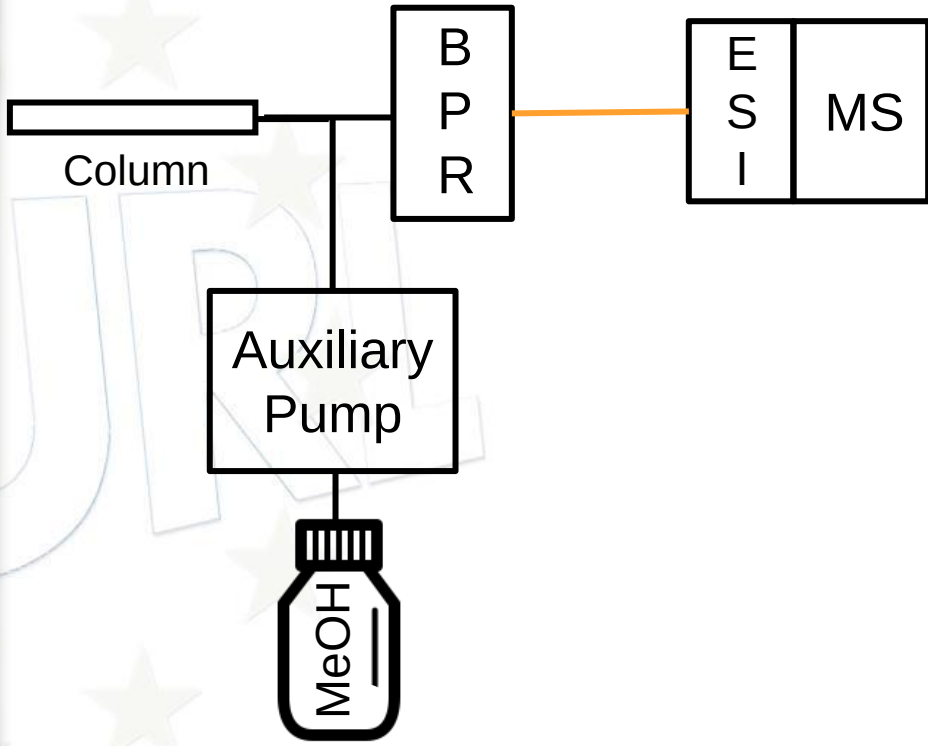




SMALL AMOUNT OF ORGANIC SOLVENT REACHING THE SOURCE

REDUCING POST-COLUMN FLOW





I.D: 127µm

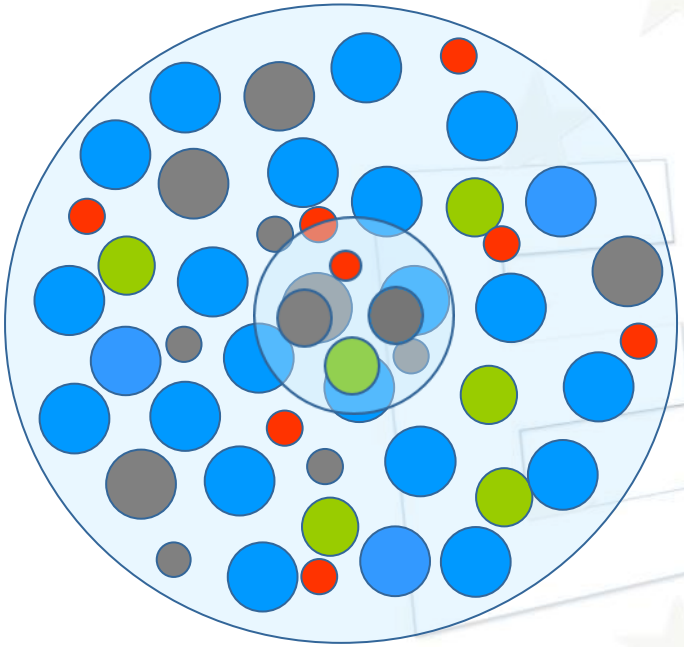
I.D: 64 µm

POST-COLUMN FLOW

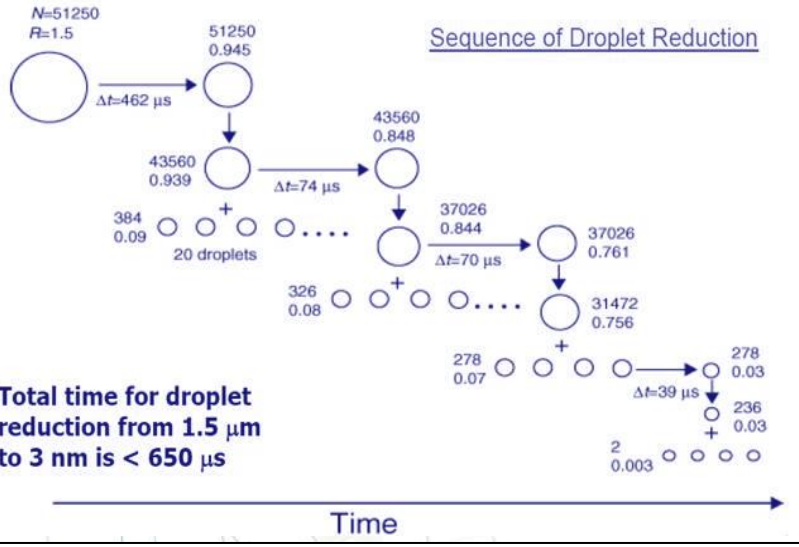
150µL/min → 80µL/min

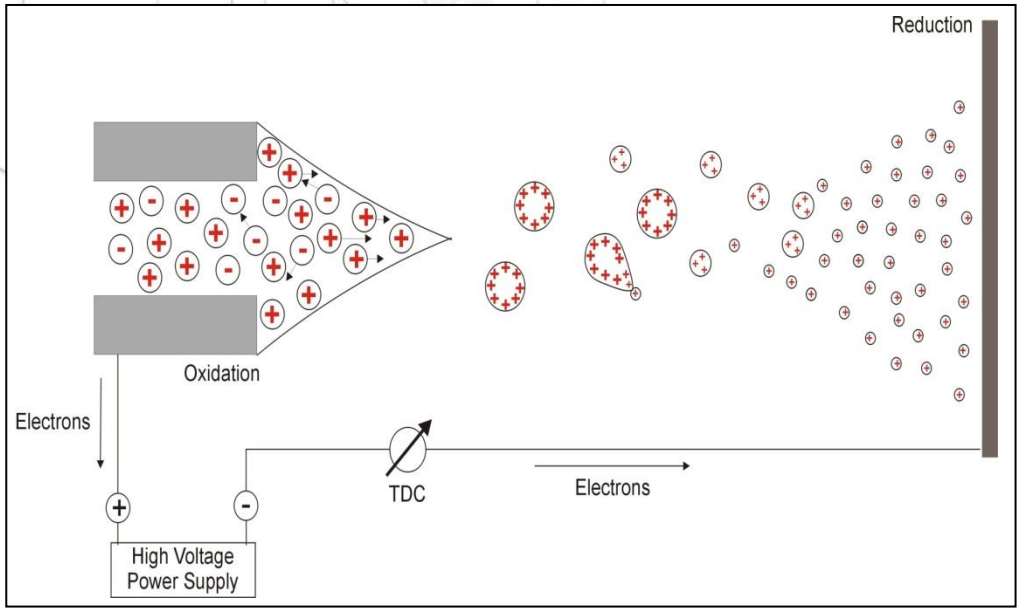
IONIZATION PROCESS

Low methanol flow
56% of compounds: 110 µL/min
(Including post-column solvent)

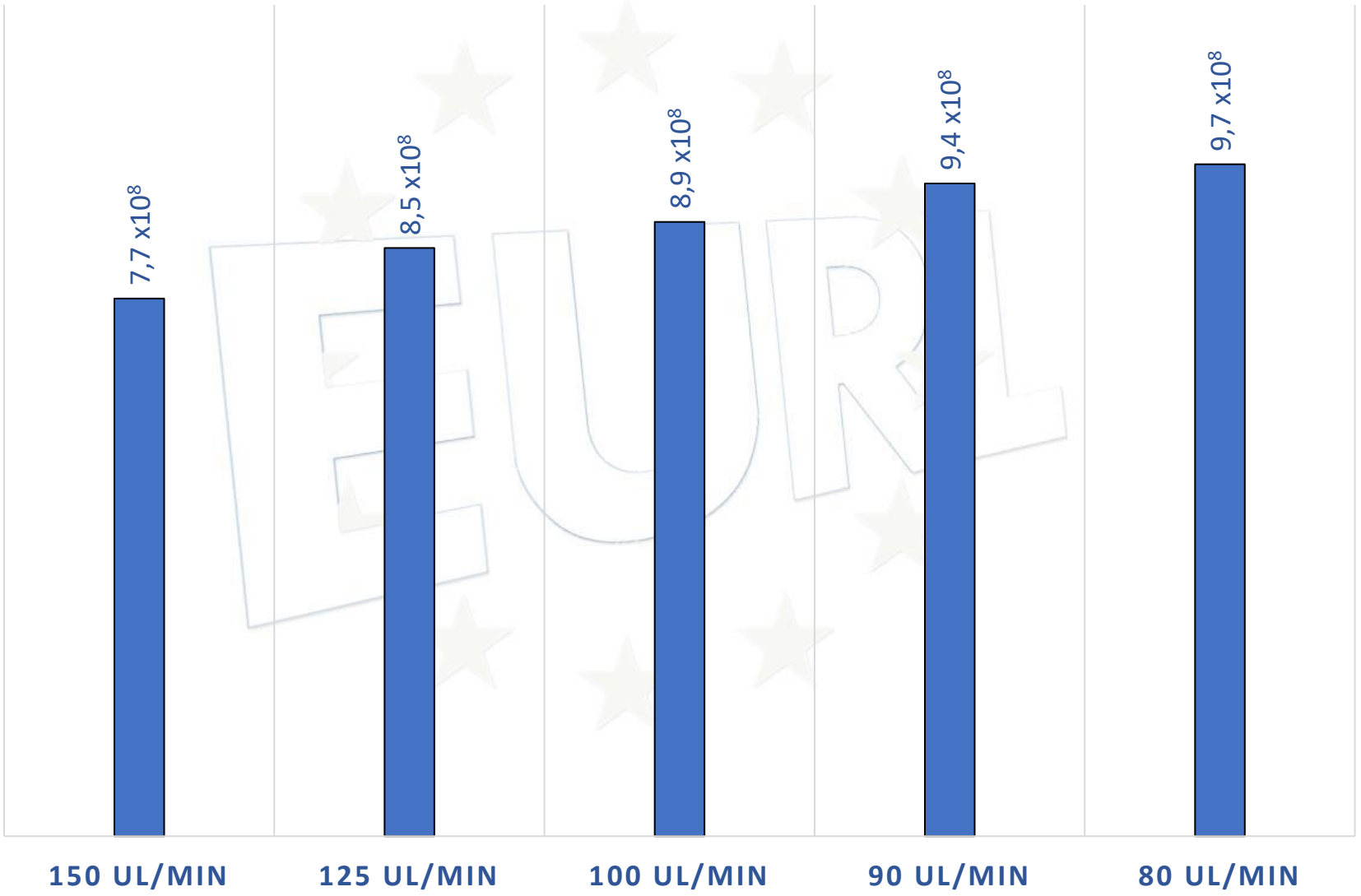


Ion
Water
Matrix
Methanol





REDUCING POST-COLUMN FLOW

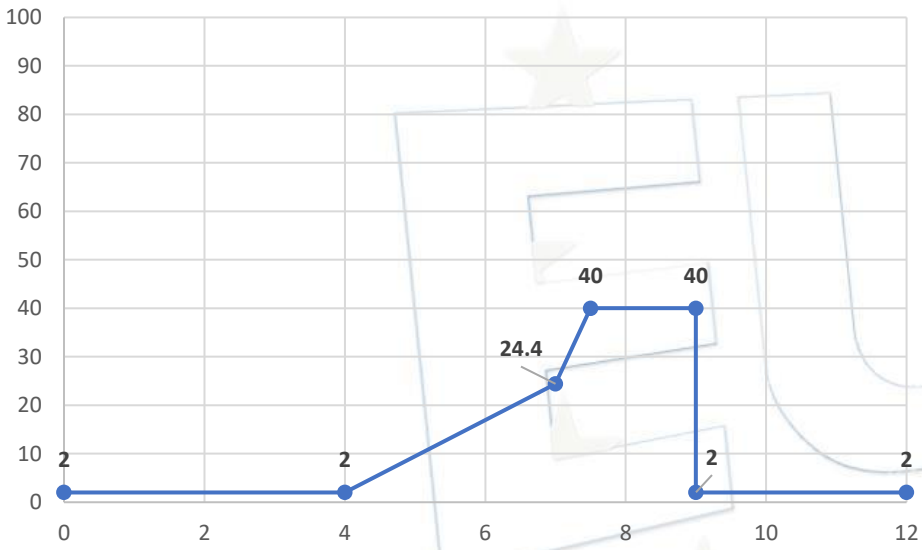


METHANOL AS CO-SOLVENT

SFC

Run time: 12 min
Flow: 1,5mL/min

Post Column Flow: 0,180mL/min

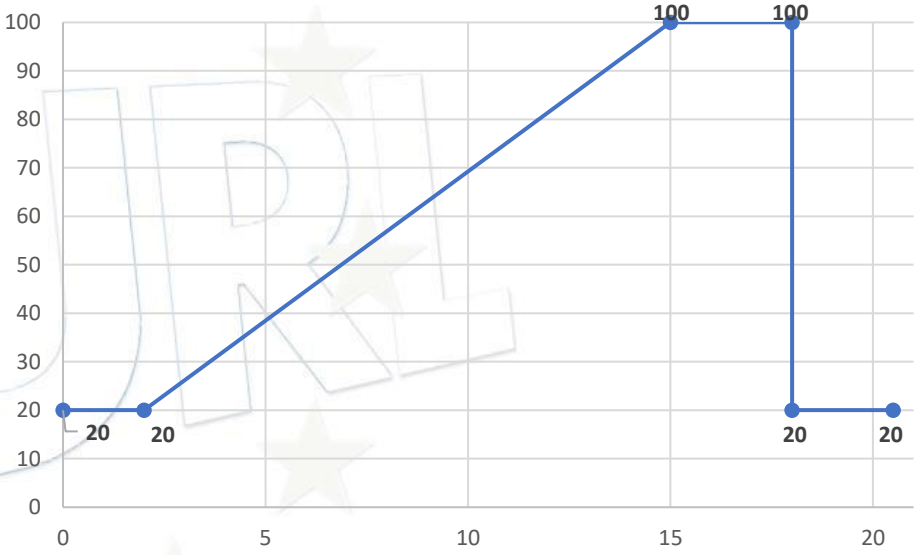


TOTAL RUN CONSUMPTION

MeOH (Gradient) : 1,95mL
MeOH (Post-column): 0,96mL
2,91mL
CO2 Consumption : 15,32mL
Water: 0mL

LC

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



TOTAL RUN CONSUMPTION

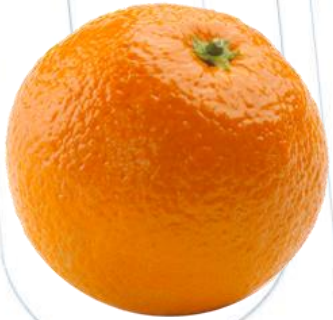
MeOH : 3,51mL
Water : 2,64mL

+20%
Waste of methanol per run

TESTING SFC-MS/MS

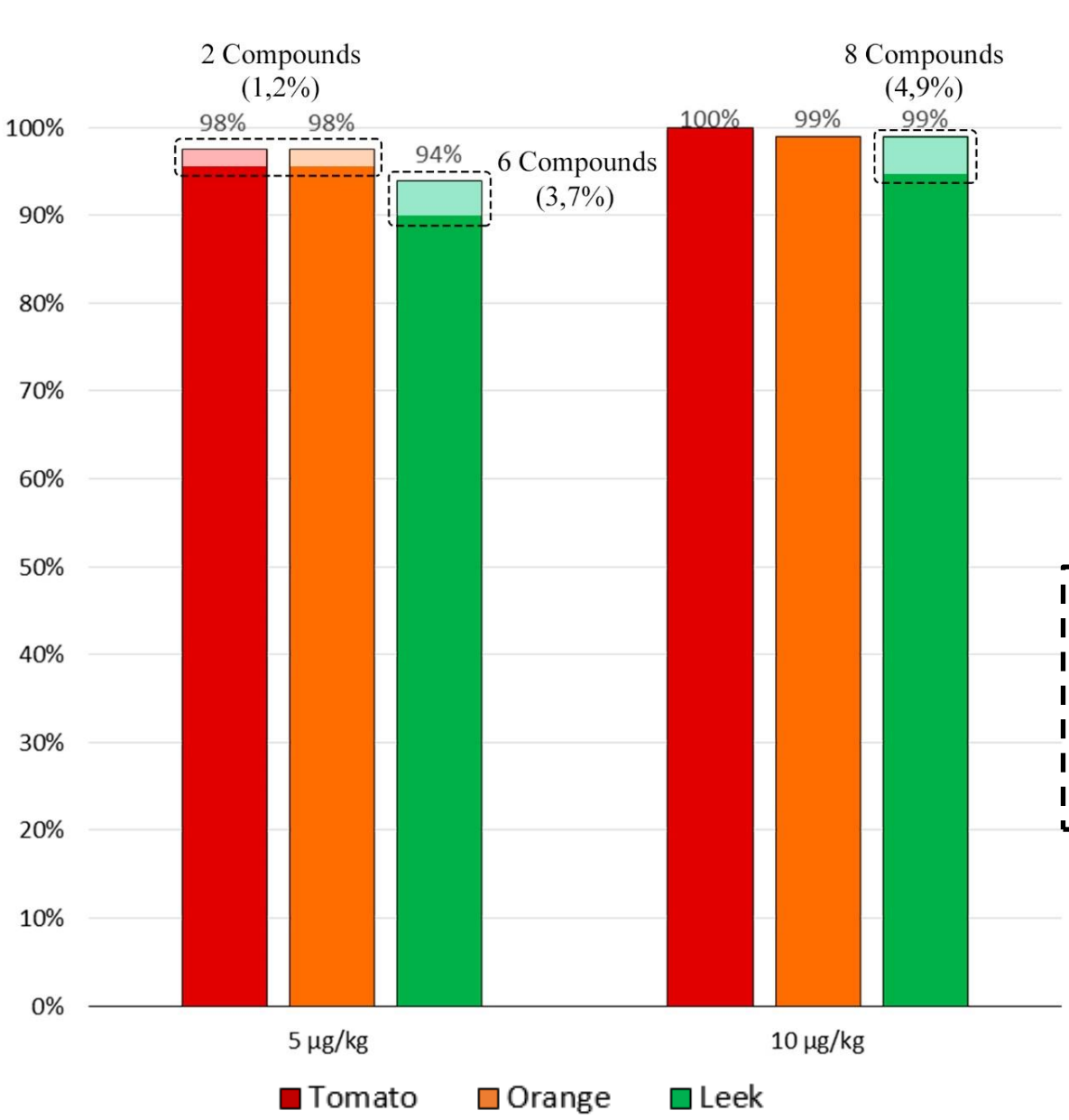
VALIDATION OF THE METHOD

High water content matrix



Acidic matrix

Complex matrix



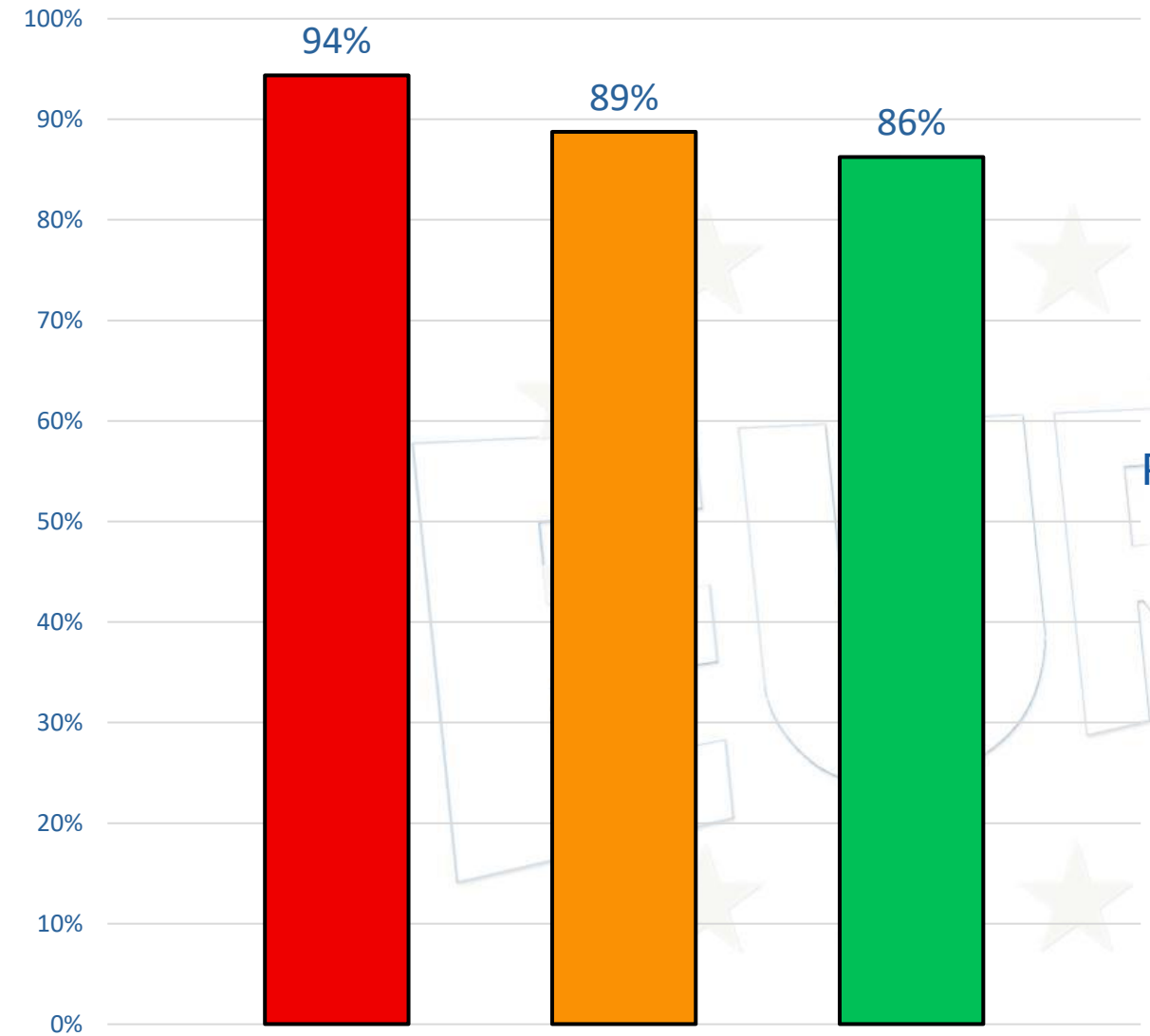
Tomato Orange Leek



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.



Tomato Orange Leek



IDENTIFIED COMPOUNDS (164)

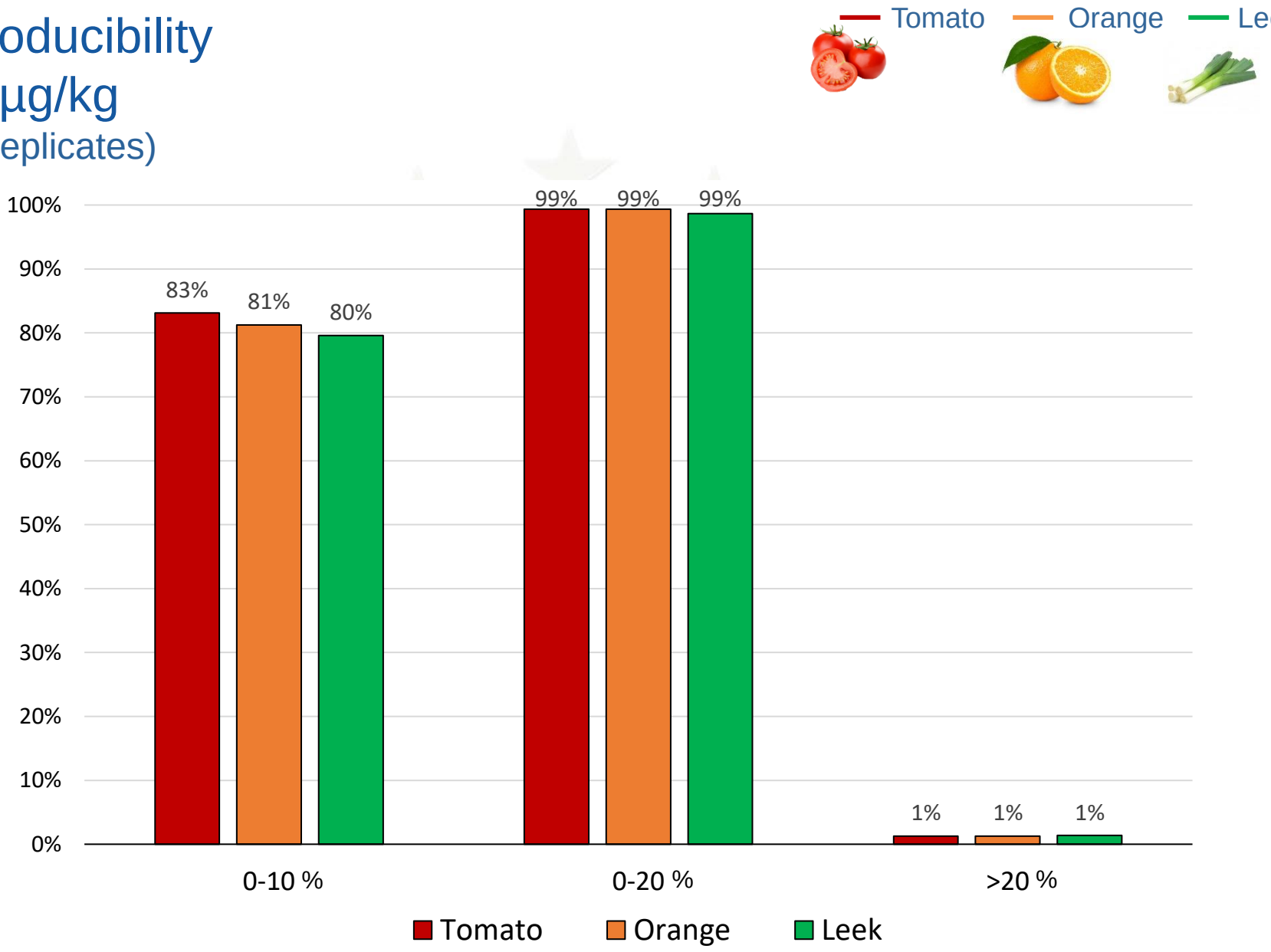
Post-column Flow: 0,080mL/min

Inj.volumen: 2uL

2 µg/Kg

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

Reproducibility 5µg/kg (5 replicates)

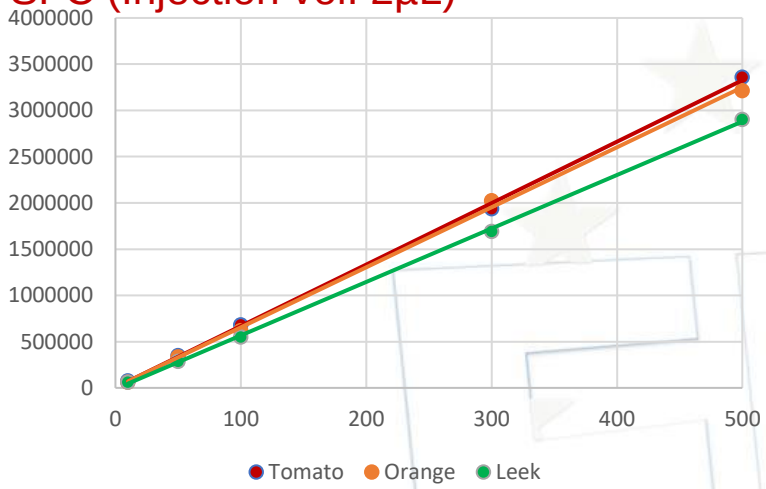


Tomato Orange Leek



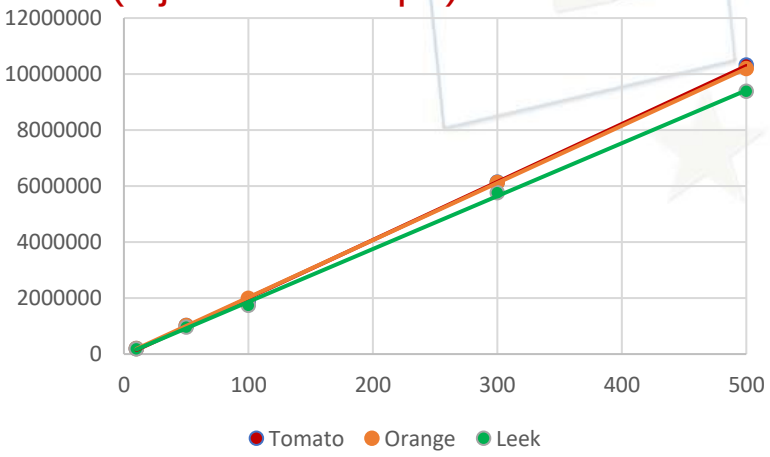
CARBARYL

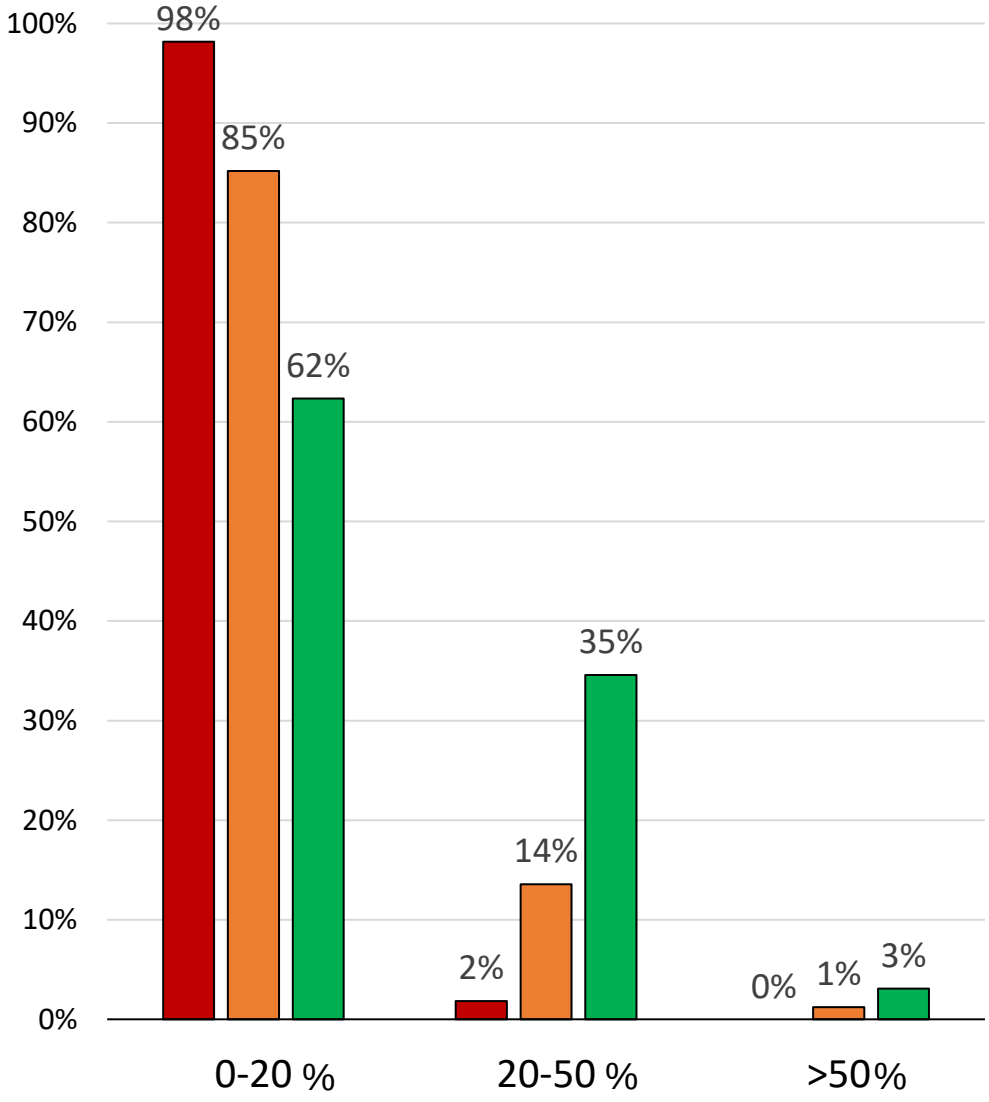
SFC (Injection vol: 2µL)



PROQUINAZID

SFC (Injection vol: 2µL)





REAL SAMPLES (FRUITS AND VEGETABLES)

10gr of sample

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

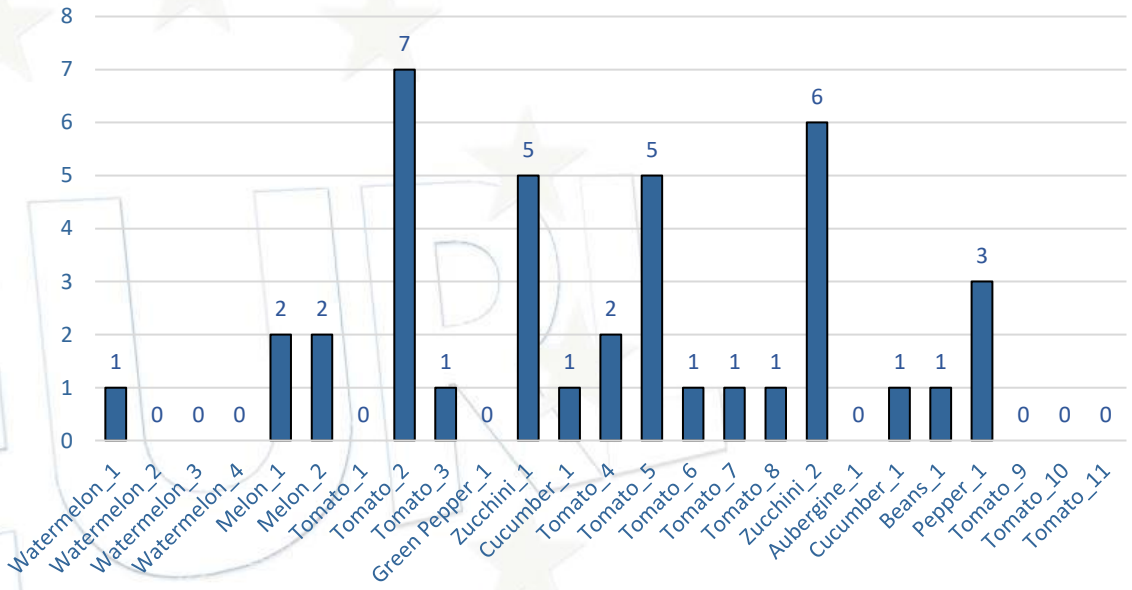
Take 5 mL + 750 mg $MgSO_4$ + 125 mg PSA

Shake with vortex 30 s

Centrifuge 5 minutes at 3500 rpm

Analysis (Extract diluted 5 times with H_2O)

Number of pesticides detected



Number of samples analyzed	25
Samples with pesticides	18 (72%)
Range of pesticides detected per sample (LOQ: 5 µg/kg)	0 -7
Samples with pesticides above MRL	1 (4%)
Most detected pesticides	Chlorantraniliprole (3) Cyprodinil (3) Fenpyroximate (3) Fluopyram (3)

TESTING SFC-MS/MS

SPICES

BLACK PEPPER

CAYENNE

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

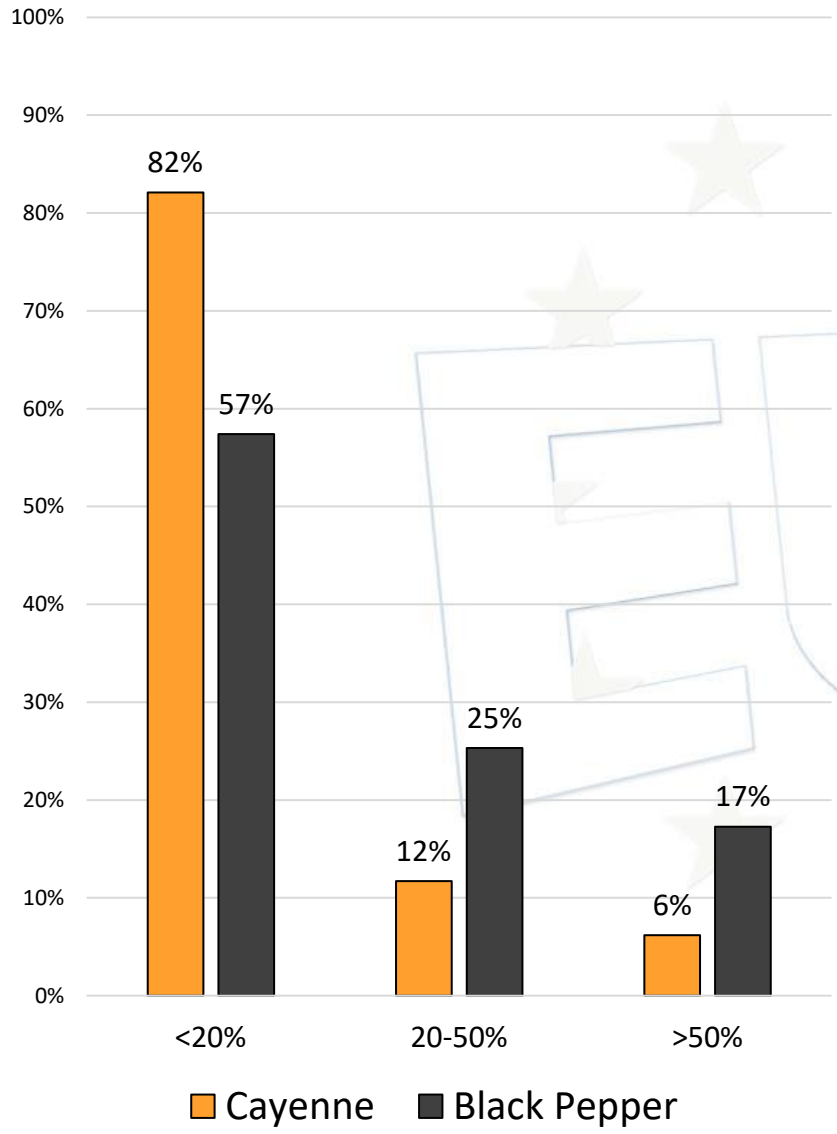
TOMATO QuEChERS+PSA

2408 compounds

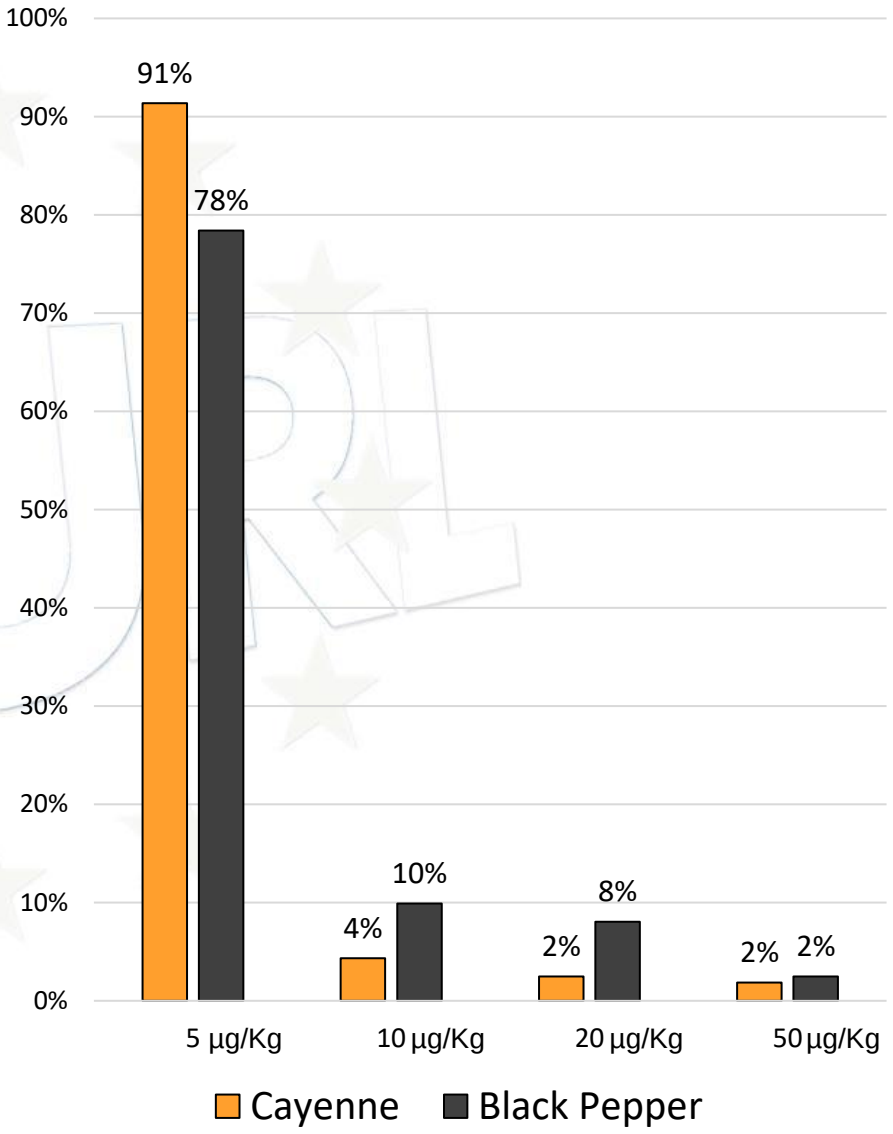
BLACK PEPPER QuEChERS+PSA

CAYENNE QuEChERS+PSA

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

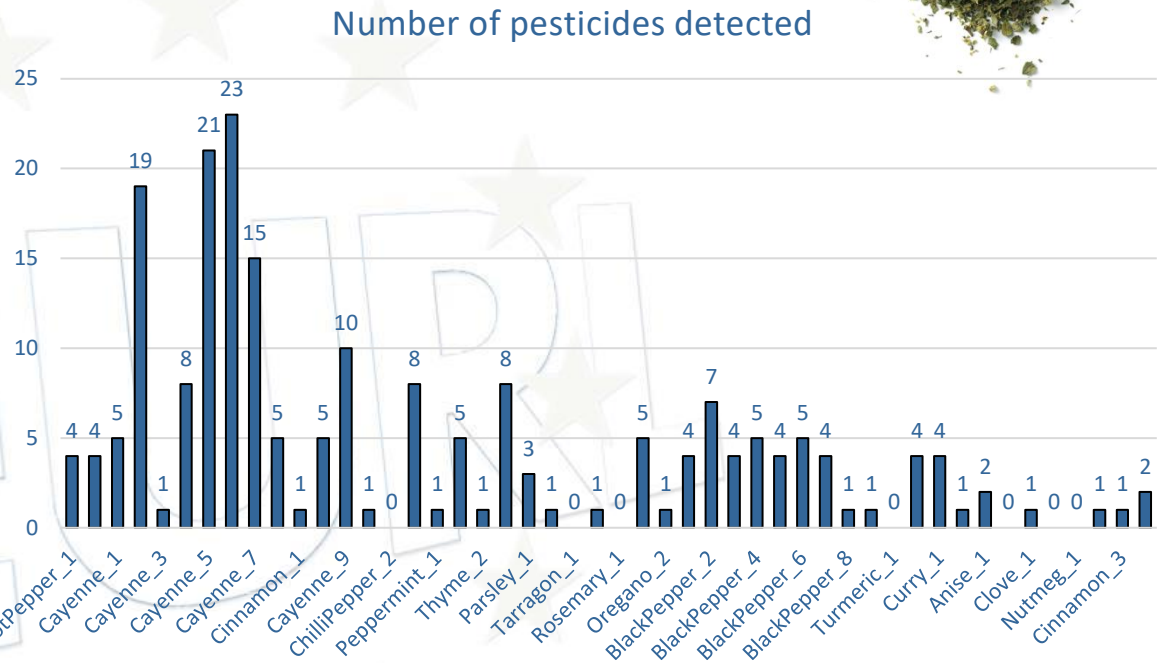
Transfer 5 mL of extract to preaconditioned EMR-Lipid tube, then transfer 5mL to EMR-Polish tube

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

10gr of sample + 10ml of Water

Wait 5 min

Add 10ml of AcN

Shake 4 min automatically at room temperature

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

Shake 5 min automatically at 40 °C

Centrifuge 5 minutes at 4500 rpm

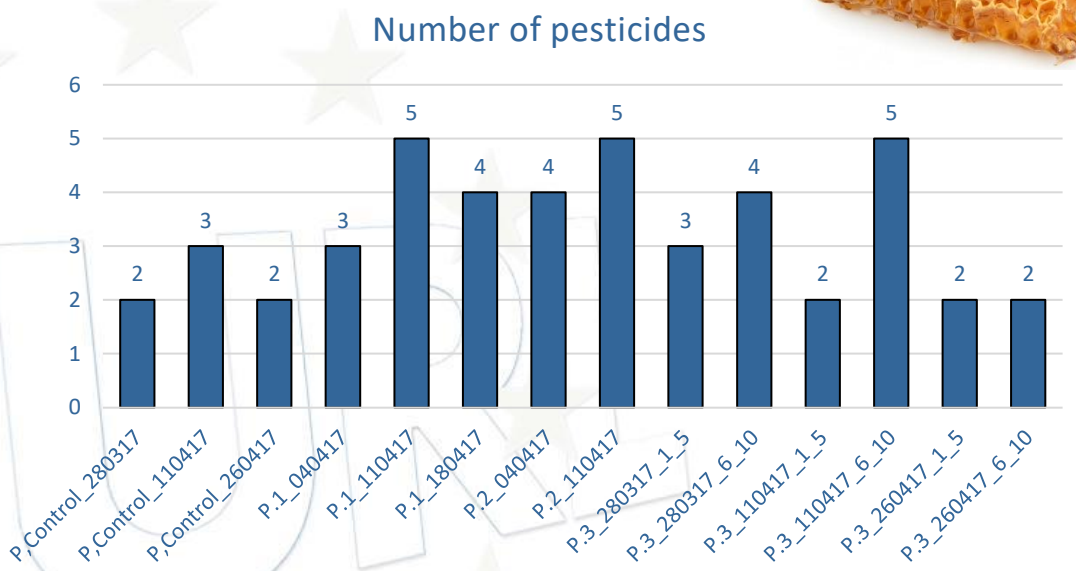
Take 5 mL + 750 mg $MgSO_4$ + 125 mg PSA

Shake with vortex 30 s

Centrifuge 5 minutes at 3500 rpm

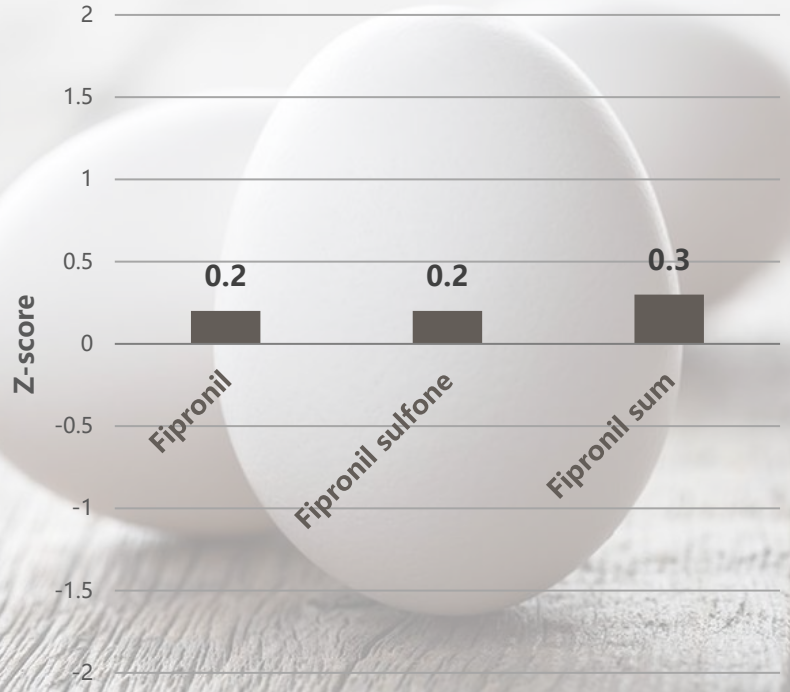
Analysis (Extract diluted 5 times with H_2O)

REAL SAMPLES (Honeywax combs)



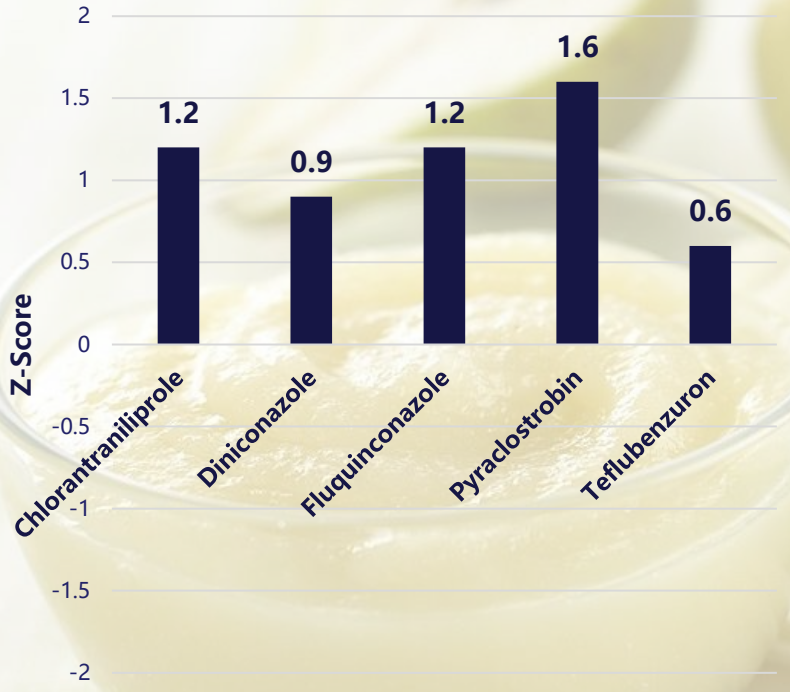
Number of samples analyzed	15
Samples with pesticides	15 (100%)
Range of pesticides detected per sample (LOQ: 5 µg/kg)	2 -5
Total load range (µg/kg)	12 - 667
Most detected pesticide	Flonicamid

PROFICIENCY TESTS



JRC-GEEL (2017)

Fipronil in eggs

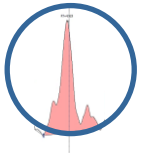


FAPAS (2017)

Pesticide Residues in pear purée

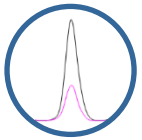
CONCLUSIONS

SHORT RUN TIMES

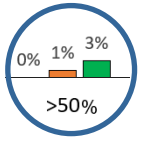


SOME COMPOUNDS AFFECTED BY ISOBARIC INTERFERENCES MAY NEED A MORE SELECTIVE TRANSITION

IMPROVED IONIZATION EFFICIENCY



HIGH SENSITIVITY OF SOME POLAR/ACIDIC COMPOUNDS



REDUCTION OF THE ION SUPRESSION IN DIFFICULT MATRICES

LOWER SOLVENT CONSUMPTION

http://www.eurl-pesticides.eu

Thank You for Your Attention