Amadeo R. Fernández-Alba

NEWS FROM MRM

European Commission

EURL-FV

NEW TECHNOLOGIES

High sensitive LC/GC-TQ-MS/MS

THEORETICAL IMPLEMENTATION

PRACTICAL IMPLEMENTATION

Amadeo R. Fernández-Alba

High sensitive LC/GC-TQ-MS/MS

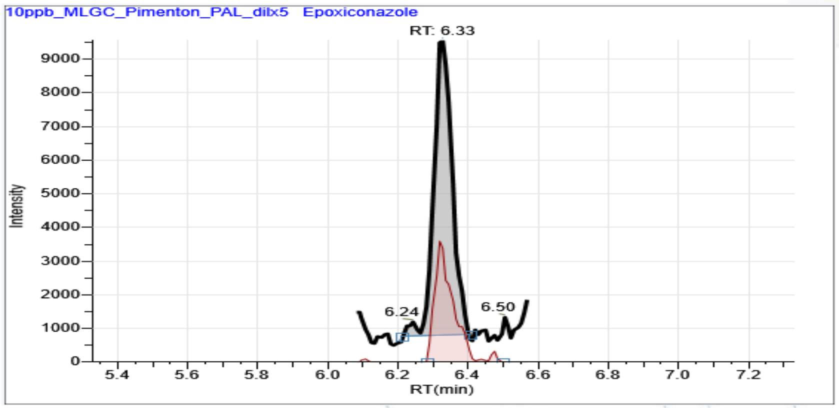
Matrix effect of leek matrix over tomato matrix (250 compounds)

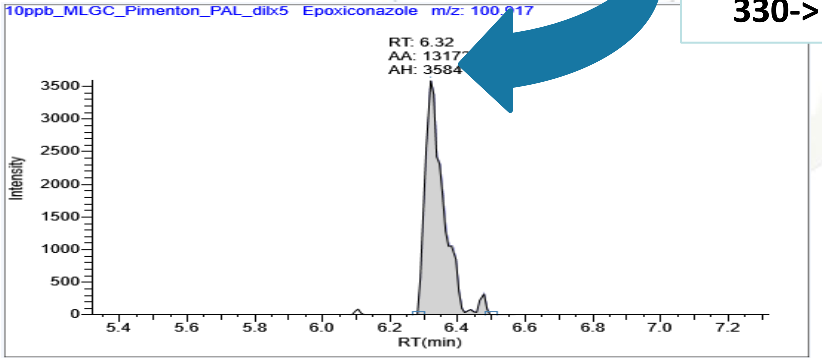
Relative matrix effect

	Leek/Tomato (2.5 µL)	Leek/Tomato (5.0 µL)
Average (%)	-6.6 %	-19.4 %
Median (%)	-7.3 %	-20.9 %
<= |20 %|	168	111
> |20 %| & <= |50 %|	76	111
> |50 %|	6	28
<= |20 %| (%)	67.2 %	44.4 %
> |20 %| & <= |50 %| (%)	30.4 %	44.4 %
> |50 %| (%)	2.4 %	11.2 %
	0.2 mg	0.5 mg

Epoxiconazole

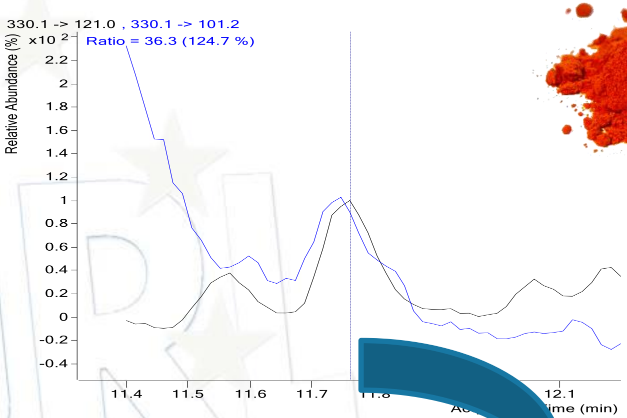
TSQ Altis
Spiked standard 10 µg/kg
Total injected amount: 0.066 mg Paprika

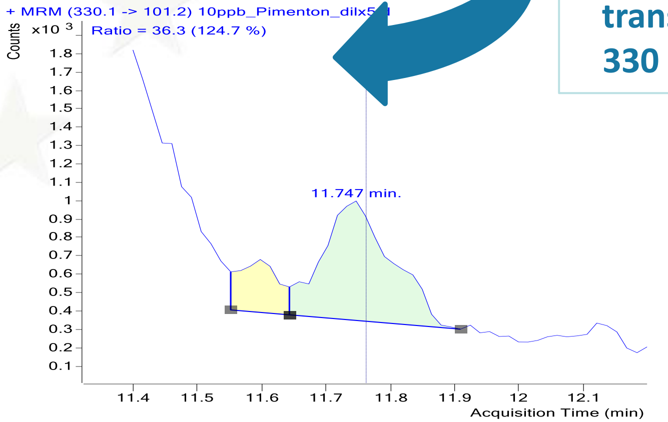




Qualifier transition: 330->101

Regular
Spiked standard 10 µg/kg
Total injected amount: 0.13 mg Paprika





Qualifier transition: 330 -> 101

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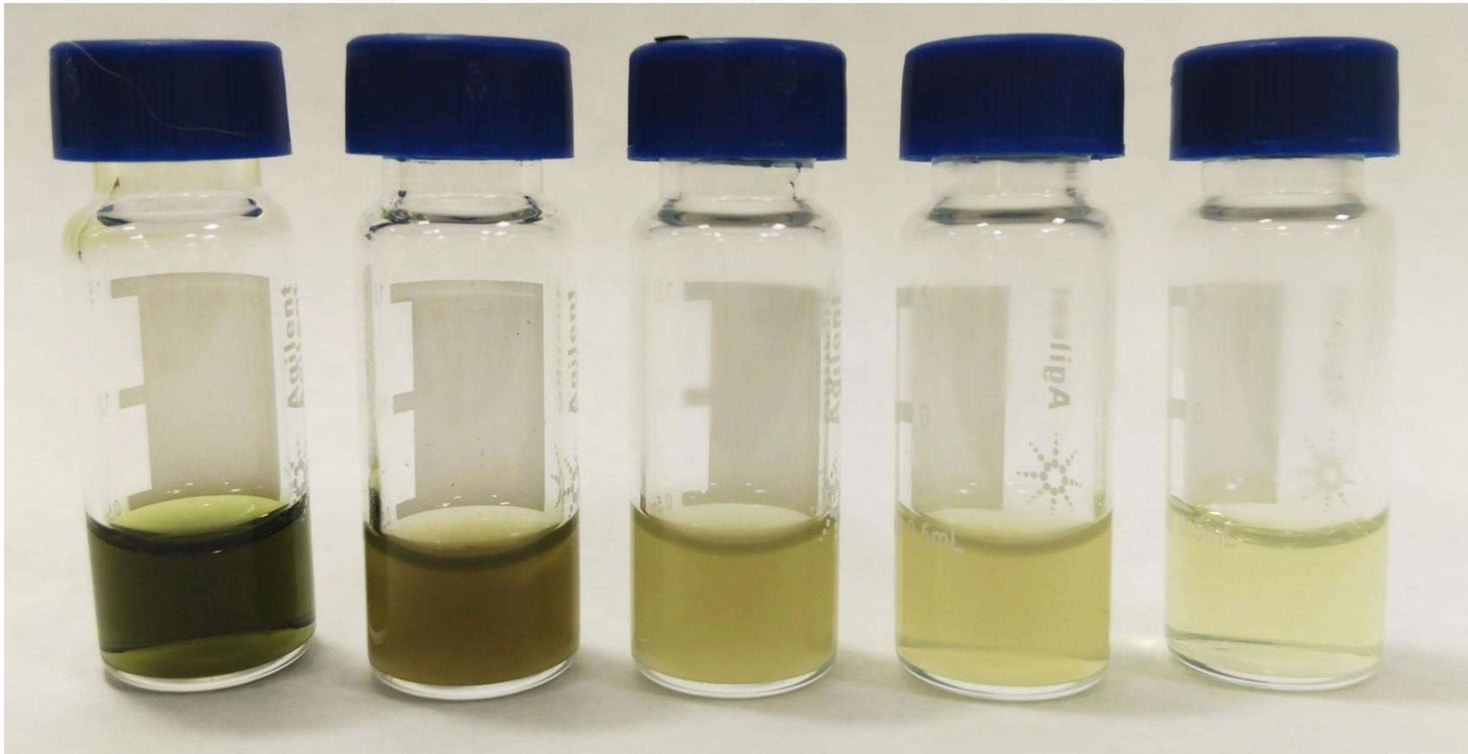
Injection volume	Matrix injected in column				
LC: 5 µL	5 mg	2.5 mg	1 mg	0.5 mg	0.25 mg
GC: 1 µL	1 mg	0.5 mg	0.2 mg	0.1 mg	0.05 mg

Green Tea



Dilx0	Dilx2	Dilx5	Dilx10	Dilx20
1 g/mL	0.5 g/mL	0.2 g/mL	0.1 g/mL	0.05 g/mL

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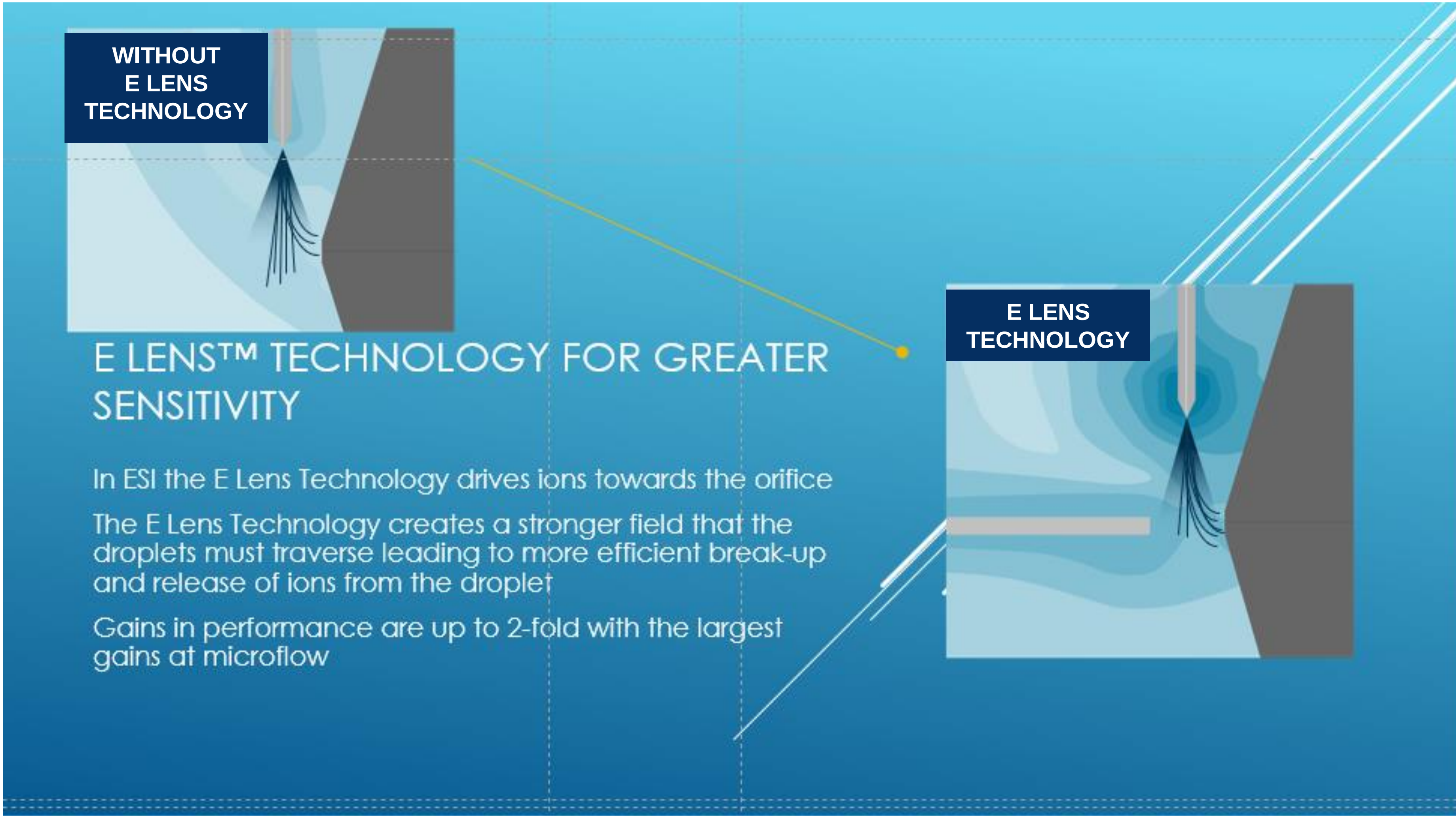
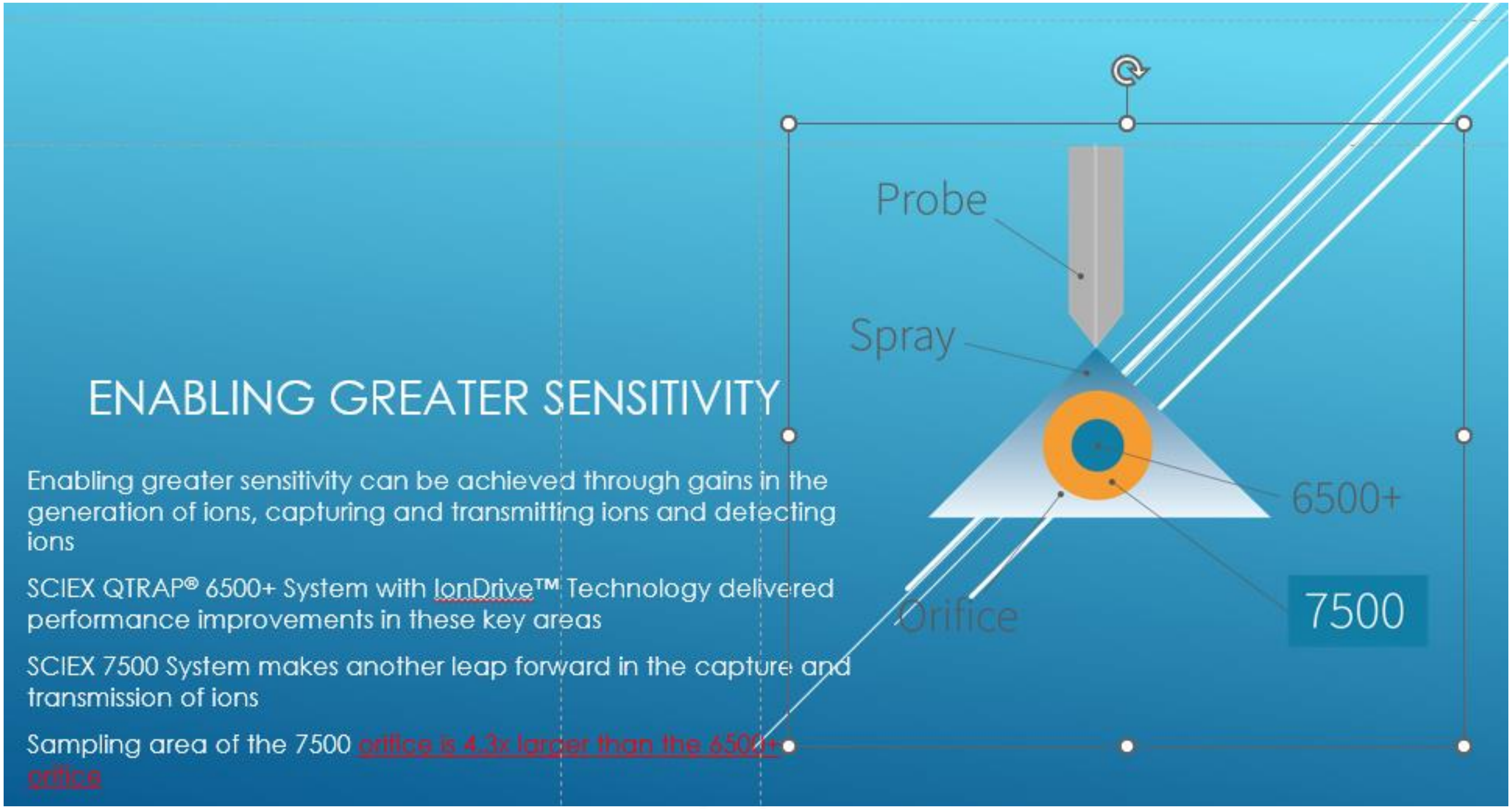
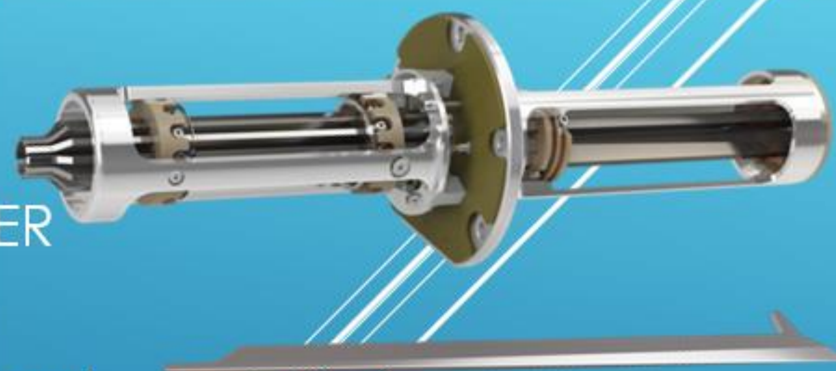

WITHOUT
E LENS
TECHNOLOGY
E LENS
TECHNOLOGY
E LENS™ TECHNOLOGY FOR GREATER SENSITIVITY
In ESI the E Lens Technology drives ions towards the orifice
The E Lens Technology creates a stronger field that the droplets must traverse leading to more efficient break-up and release of ions from the droplet
Gains in performance are up to 2-fold with the largest gains at microflow


ENABLING GREATER SENSITIVITY
Enabling greater sensitivity can be achieved through gains in the generation of ions, capturing and transmitting ions and detecting ions
SCIEX QTRAP® 6500+ System with IonDrive™ Technology delivered performance improvements in these key areas
SCIEX 7500 System makes another leap forward in the capture and transmission of ions
Sampling area of the 7500 orifice is 4.3x larger than the 6500+ orifice
Probe
Spray
6500+
7500
Orifice

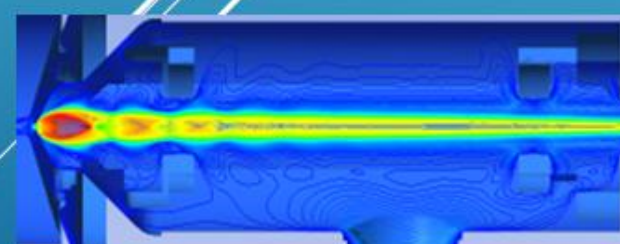
- The d jet Ion guide is a dual stage rf ion guide

D JET™ ION GUIDE FOR GREATER SENSITIVITY



The D Jet Ion Guide efficiently captures and transmits the ions in the high gas flow behind the orifice plate

The tapered dodecapole geometry of the D Jet Ion guide focuses the ions into the second stage QJet® Ion Guide



ADVANTAGES IN USING AN AUTOMATED CLEAN-UP STEP IN PESTICIDE MULTIRESIDUE METHODS BY LC-MS/MS

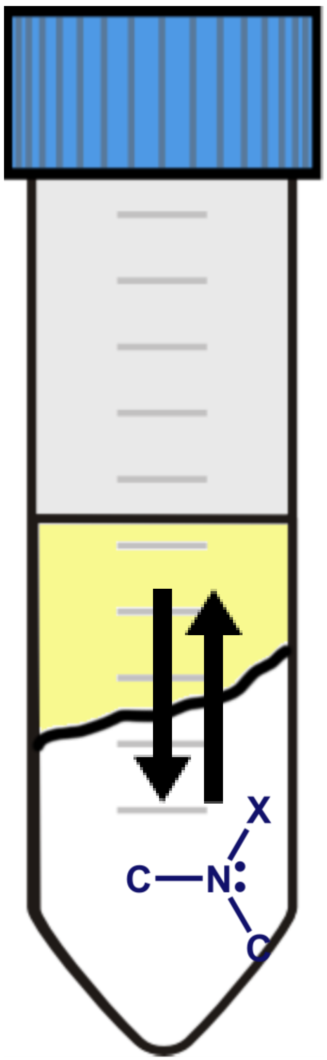
Amadeo R. Fernández-Alba

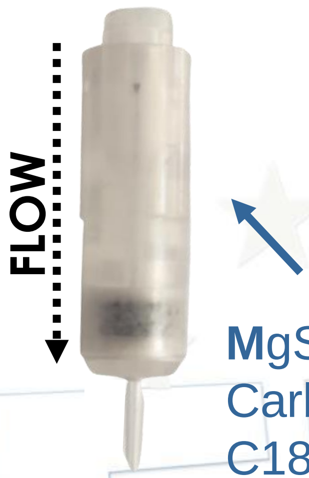
NEW TECHNOLOGIES

Automation

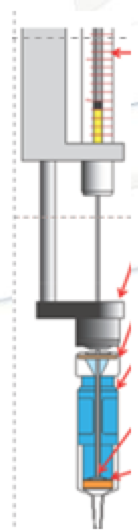
THEORETICAL IMPLEMENTATION

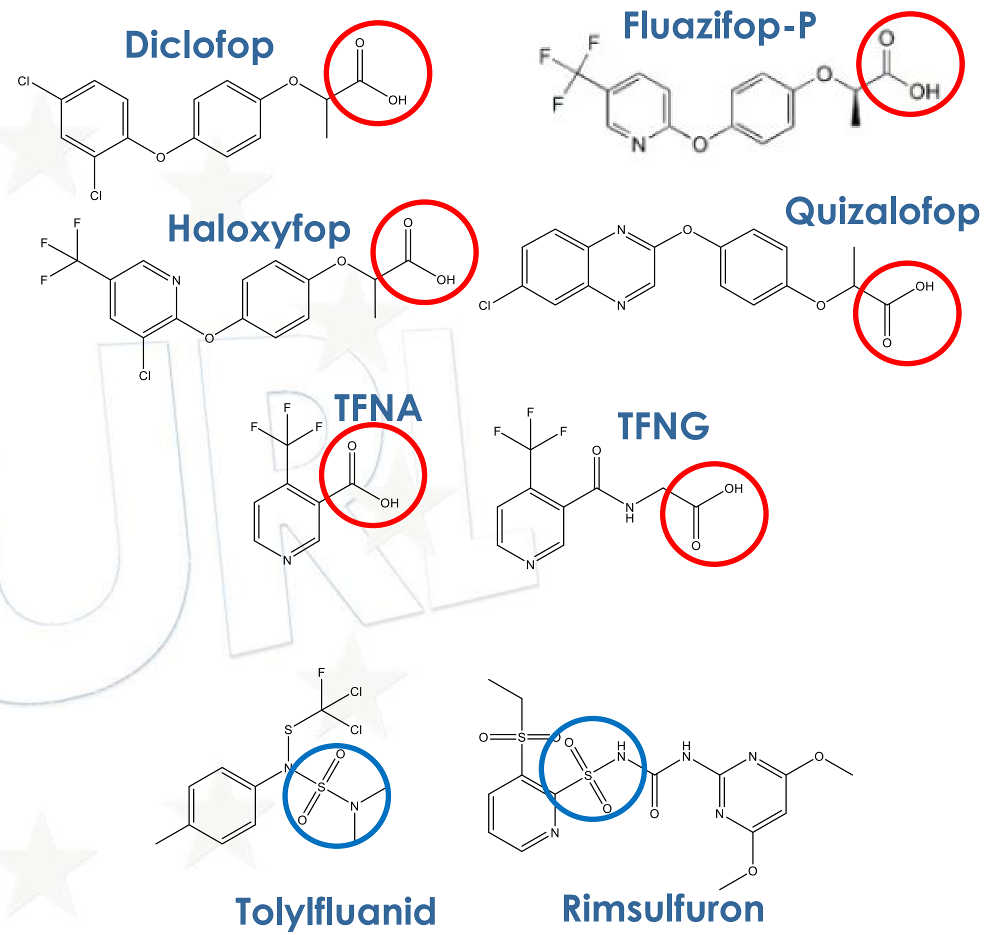
PRACTICAL IMPLEMENTATION



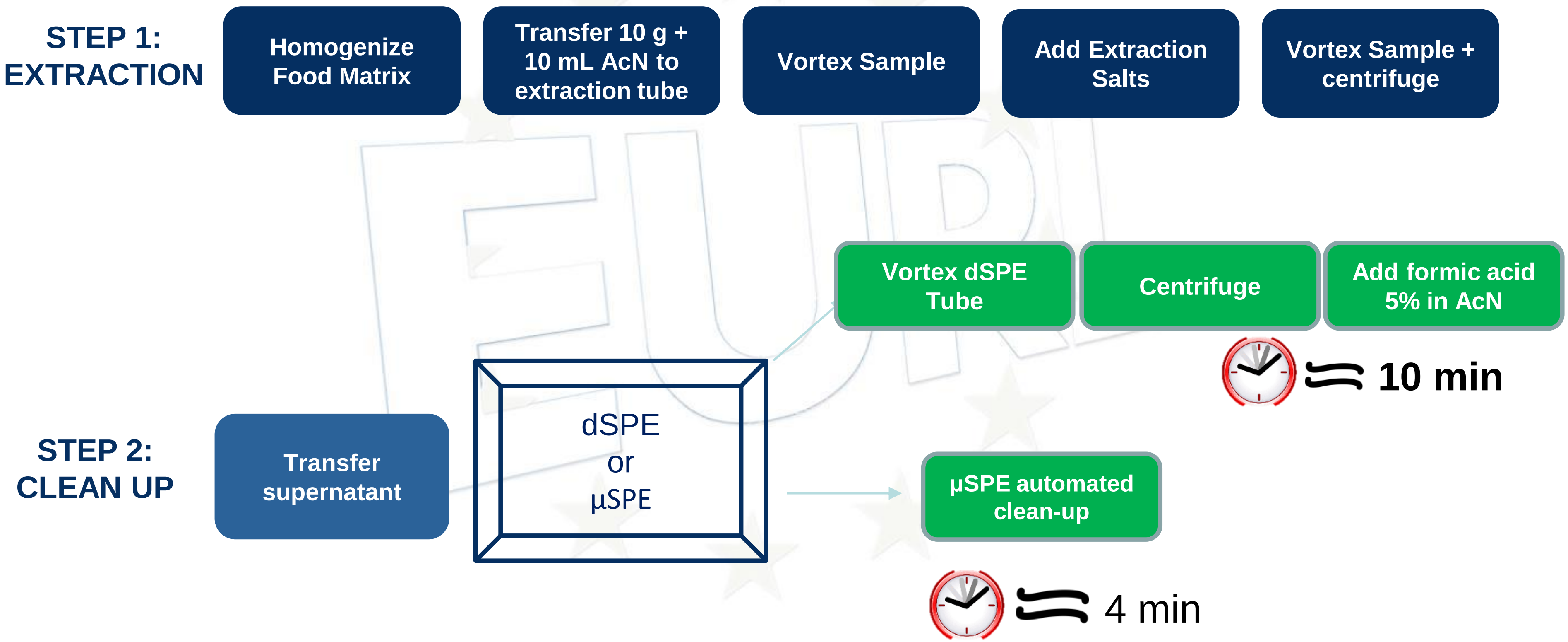


$MgSO_4$
CarbonX
C18
PSA





QuEChERS is the quick, easy, cheap effective, rugged and safe sample preparation method originally developed by M. Anastassiades and S.J. Lehotay in 2003. In the original QuEChERS method, acetonitrile is used as extraction solvent, followed by adding NaCl and buffer salts, vortexing and centrifugation.



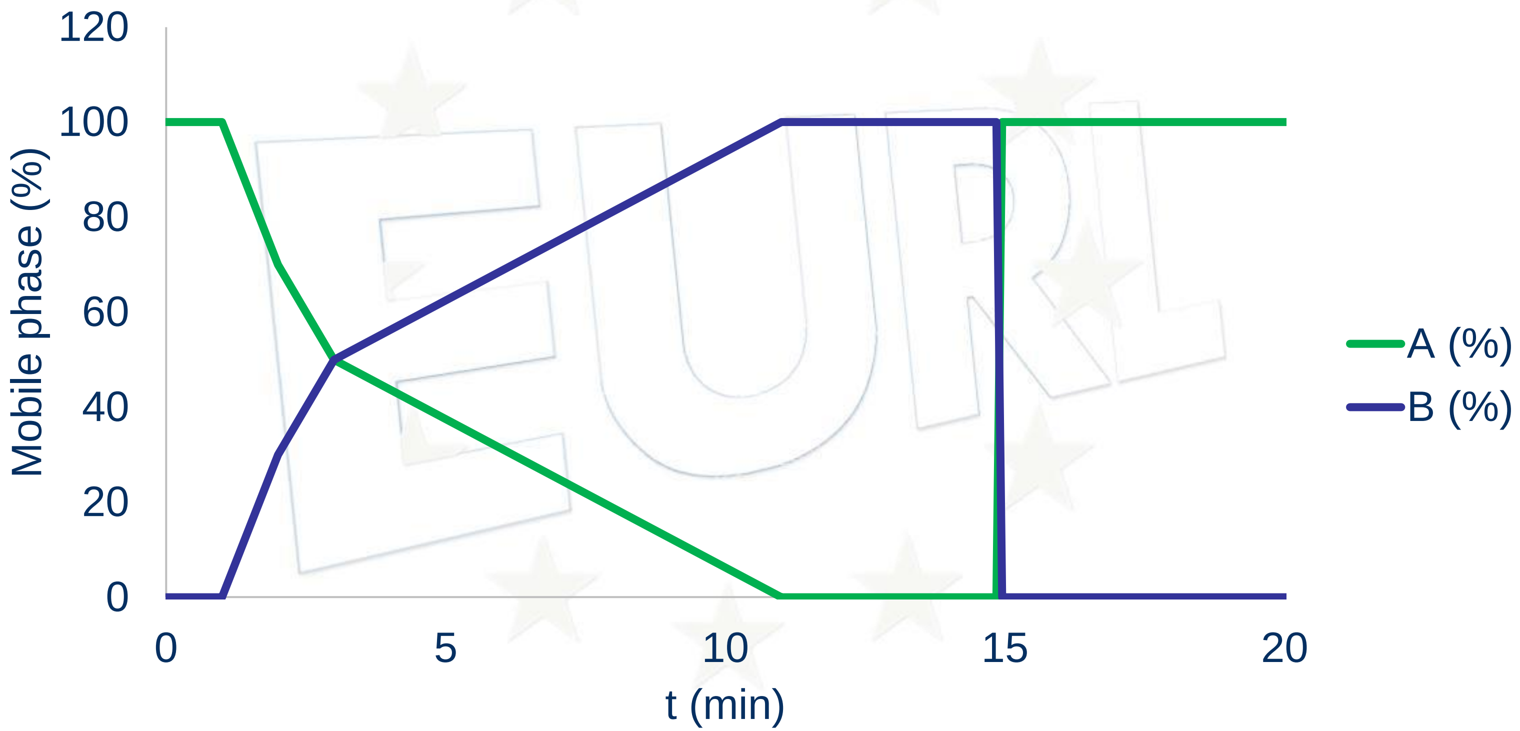
Thermo Scientific™ Transcend™ Duo LX-2 UHPLC System coupled to a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer

Two ThermoFisher Scientific™ Accucore™ C18 columns (100 mm x 2.1 mm x 2.6 mm)

Injection volume: 2.5 µL

Mobile phase A: 98% water 2% methanol, 5 mM ammonium formate 0.1% formic acid

Mobile phase B: 98% methanol 2% water, 5 mM ammonium formate 0.1% formic acid



Data window: 1.10 – 11.55 min


Citrate buffered QuEChERS extraction

Clean-up


	Clean-up salts for the d-SPE and µSPE QC method	
	d-SPE	**µSPE**
Tomate/Orange	$MgSO_4$ + PSA (6:1) Vortex 30 s Centrifuge 4000 rpm 5 min	Mini cartridges containing 45 mg of $MgSO_4$ + PSA + C18 + CarbonX (20:12:12:1)
Avocado	Z-Sep Vortex 30 s Centrifuge 4000 rpm 5 min	
Black Tea	$CaCl_2$ + PSA (2:1) Vortex 30 s Centrifuge 4000 rpm 5 min	

Clean up Step

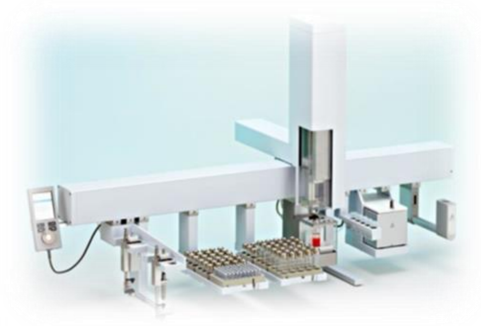
Manual dSPE

5 mL of the supernatant + **750 mg** of **anhydrous magnesium sulphate** and **125 mg of PSA** and vortexed for 30 sec. **Centrifuge** 3700 rpm for 5 min and supernatant was transferred to a 4-mL vial to which **10 µL/ mL extract of formic acid solution in acetonitrile (5% volume).**

µSPE Cartridge:

20 mg Anhydrous $MgSO_4$+ 12mg PSA+ 12 mg C18+ 1 mg CarbonX

Automated µSPE

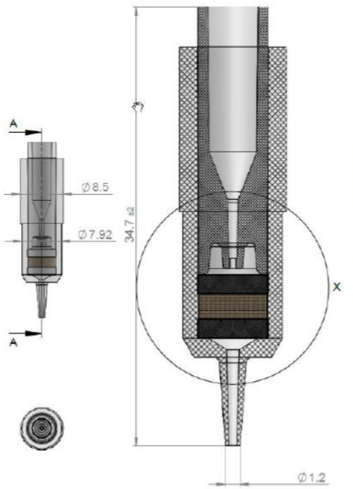


Offline Version

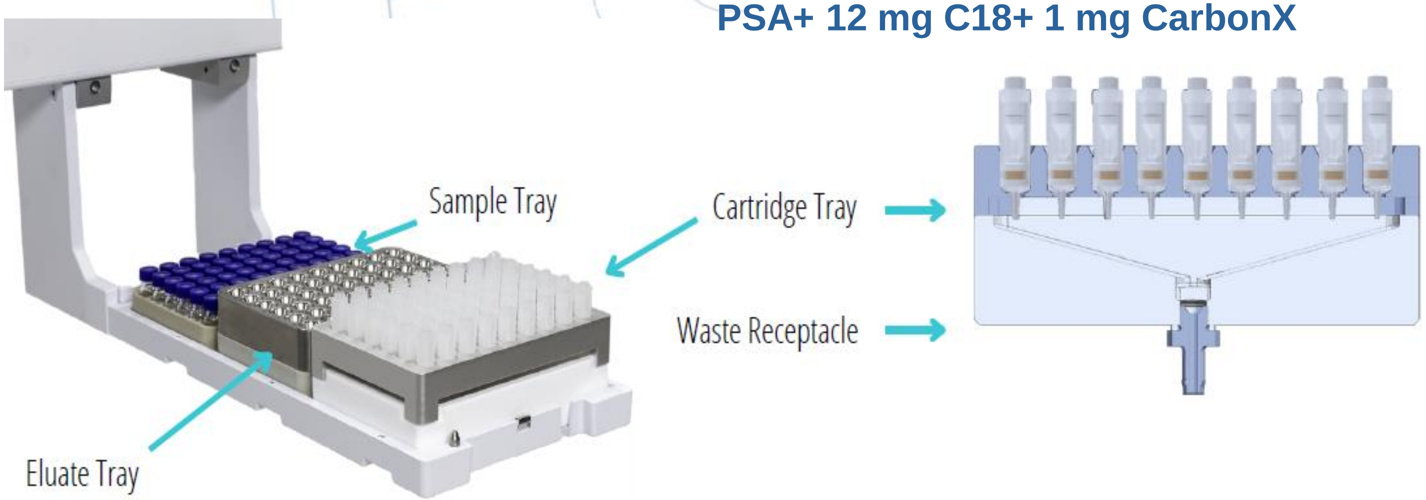
Steps:

1. Condition µSPE cartridge (100 µL ACN)
2. Elution cartridge step with sample (200 µL)
3. Elution cartridge with AcN (5% formic acid) (100 µL)

The PAL µSPE QuEChERS clean-up workflow



- Small dead volumen (< 20µL)
- Pressure tolerance: 15 bar
- Wide range of sorbent masses: 5-150 mg
- Porous filters allow multiple sorbent layers
- Sorbent mass accuracy+/-0,5mg
- **Composition: 20 mg Anhydrous $MgSO_4$+ 12mg PSA+ 12 mg C18+ 1 mg CarbonX**



The PAL µSPE QuEChERS clean-up workflow

Without Elution Step

Setup

Conditioning

Conditioning Solvent Source	Solvent Module1	
Conditioning Solvent Index	1	
Conditioning Solvent Volume	100	µL
Conditioning Solvent Fill Speed	10	µL/s

Sample µSPE

µSPE Sample Load Volume	200	µL
µSPE Sample Fill Speed	10	µL/s

Elution

Elution Solvent Source	none	
Elution Solvent Index	1	
Elution Volume	0	µL
Elution Solvent Fill Speed	10	µL/s

With Elution Step

Setup

Conditioning

Conditioning Solvent Source	Solvent Module1	
Conditioning Solvent Index	1	
Conditioning Solvent Volume	100	µL
Conditioning Solvent Fill Speed	50	µL/s

Sample µSPE

µSPE Sample Load Volume	200	µL
µSPE Sample Fill Speed	100	µL/s

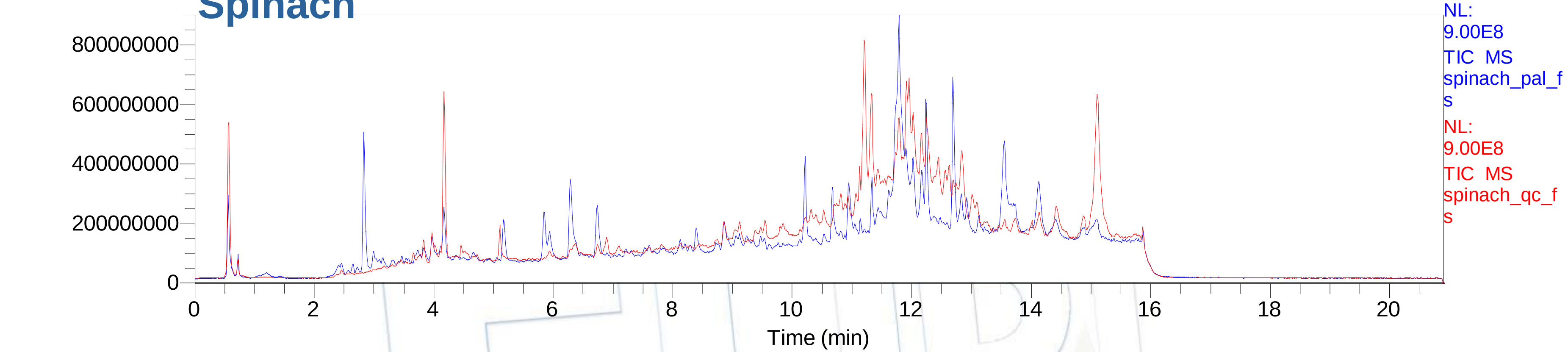
Elution

Elution Solvent Source	Solvent Module1	
Elution Solvent Index	3	
Elution Volume	100	µL
Elution Solvent Fill Speed	100	µL/s

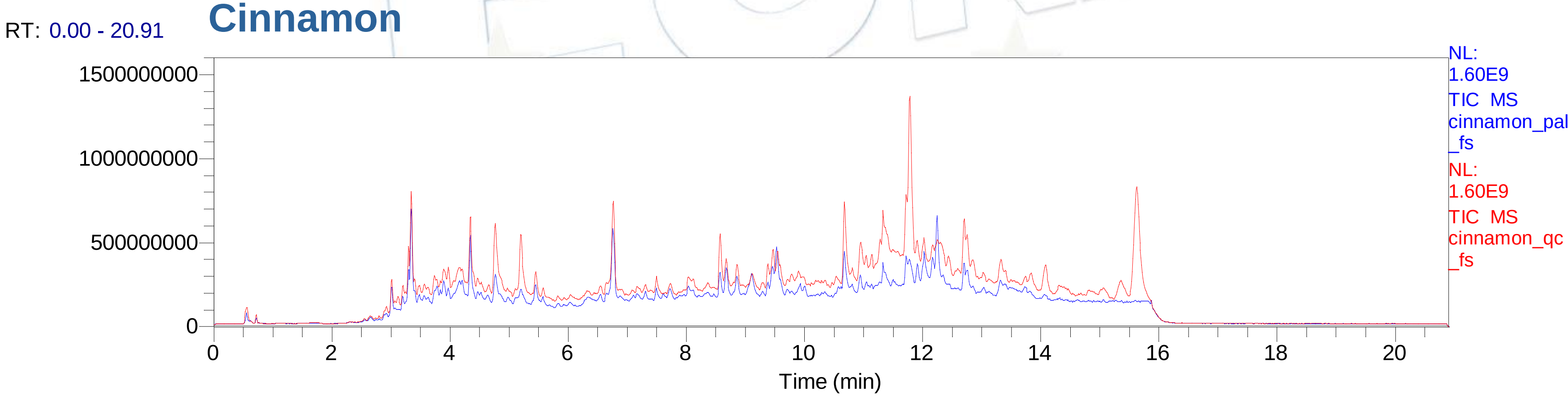
Results

TIC's comparative (dSPE extract vs µSPE)

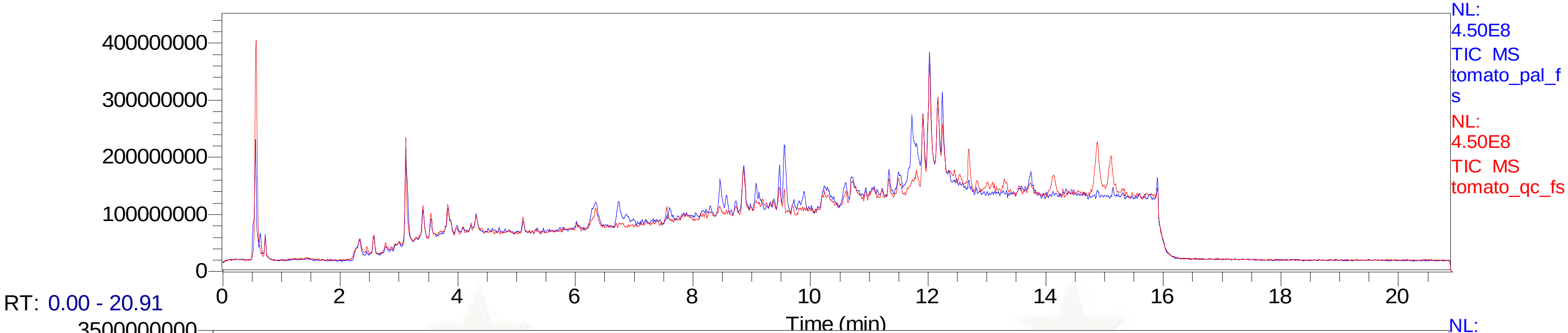
Spinach



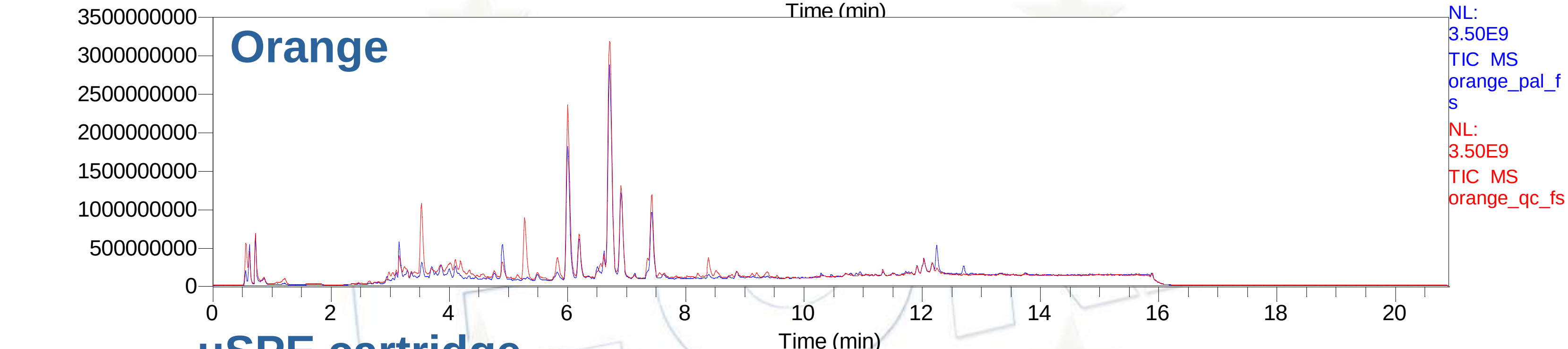
Cinnamon



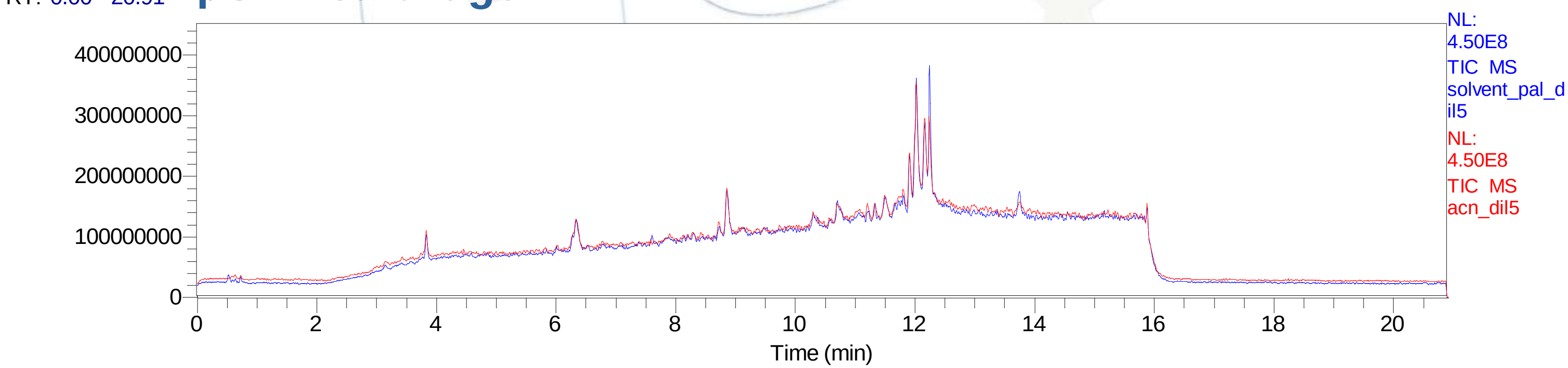
Tomato



Orange



μSPE cartridge

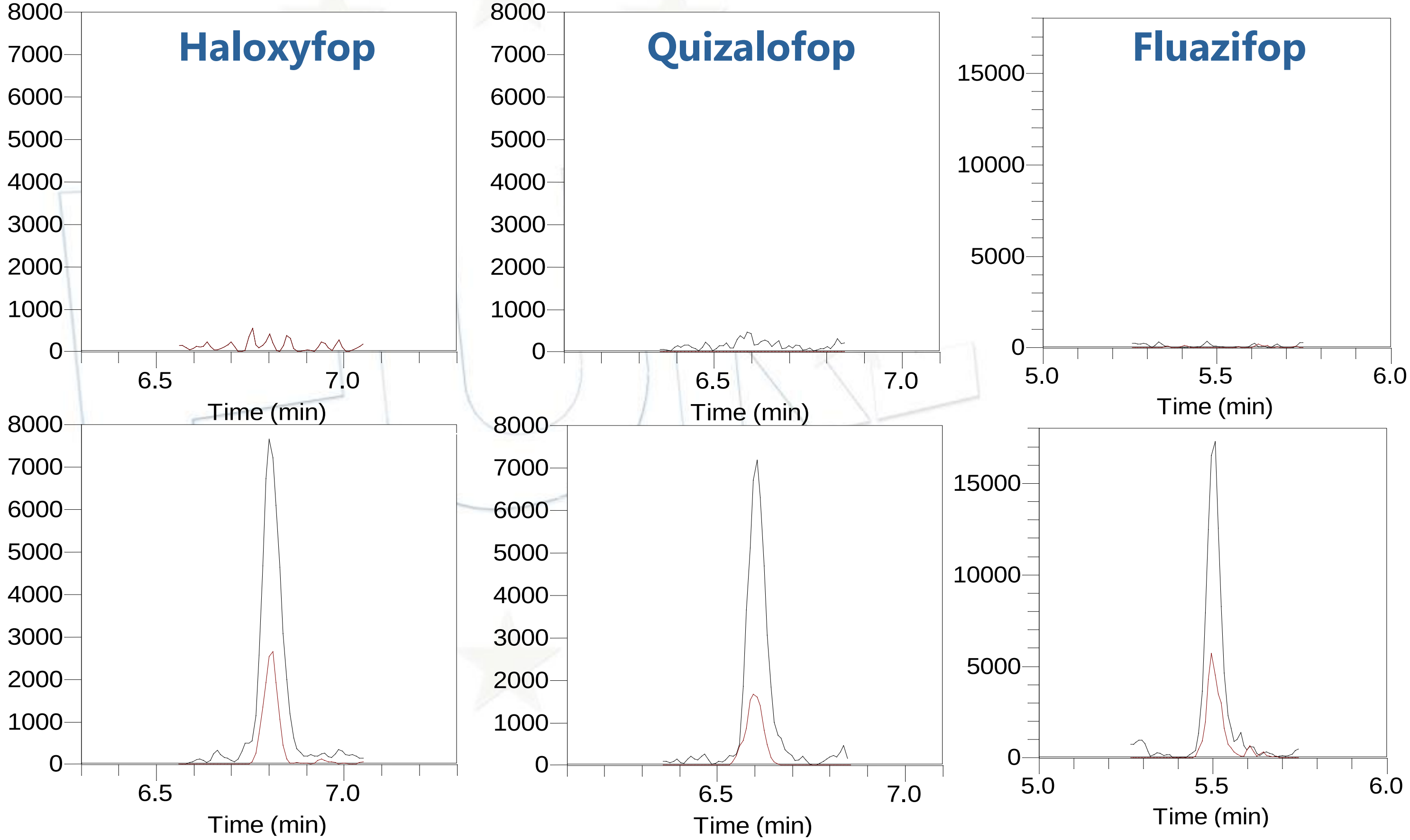


Optimization of PAL µSPE QuEChERS clean-up workflow

Tomato blank extract spiked at 10 ppb

Without elution step

With elution step



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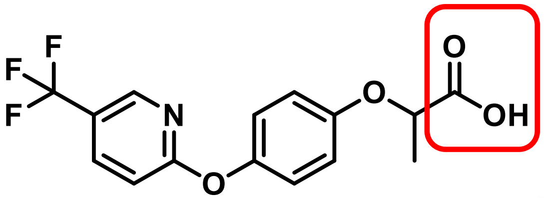
Comparison between dSPE and µSPE of acid compounds

Rice

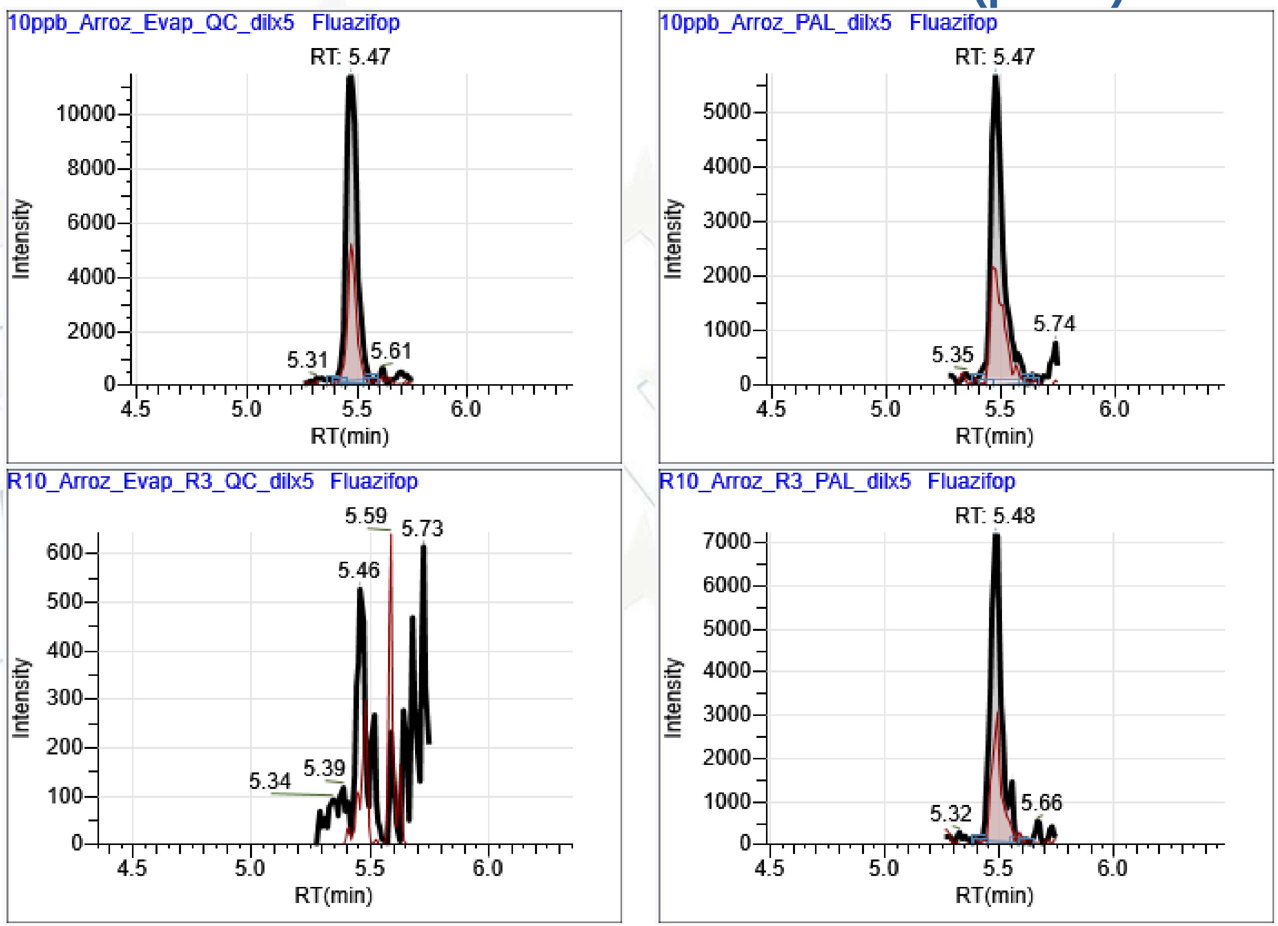


Fluazifop

Standard at 10 µg/kg in extract

Rice spiked at 10 µg/kg

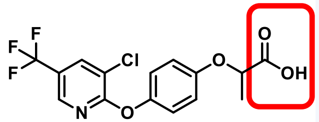
dSPE | PAL (µSPE)



Comparison between dSPE and µSPE of acid compounds in ric

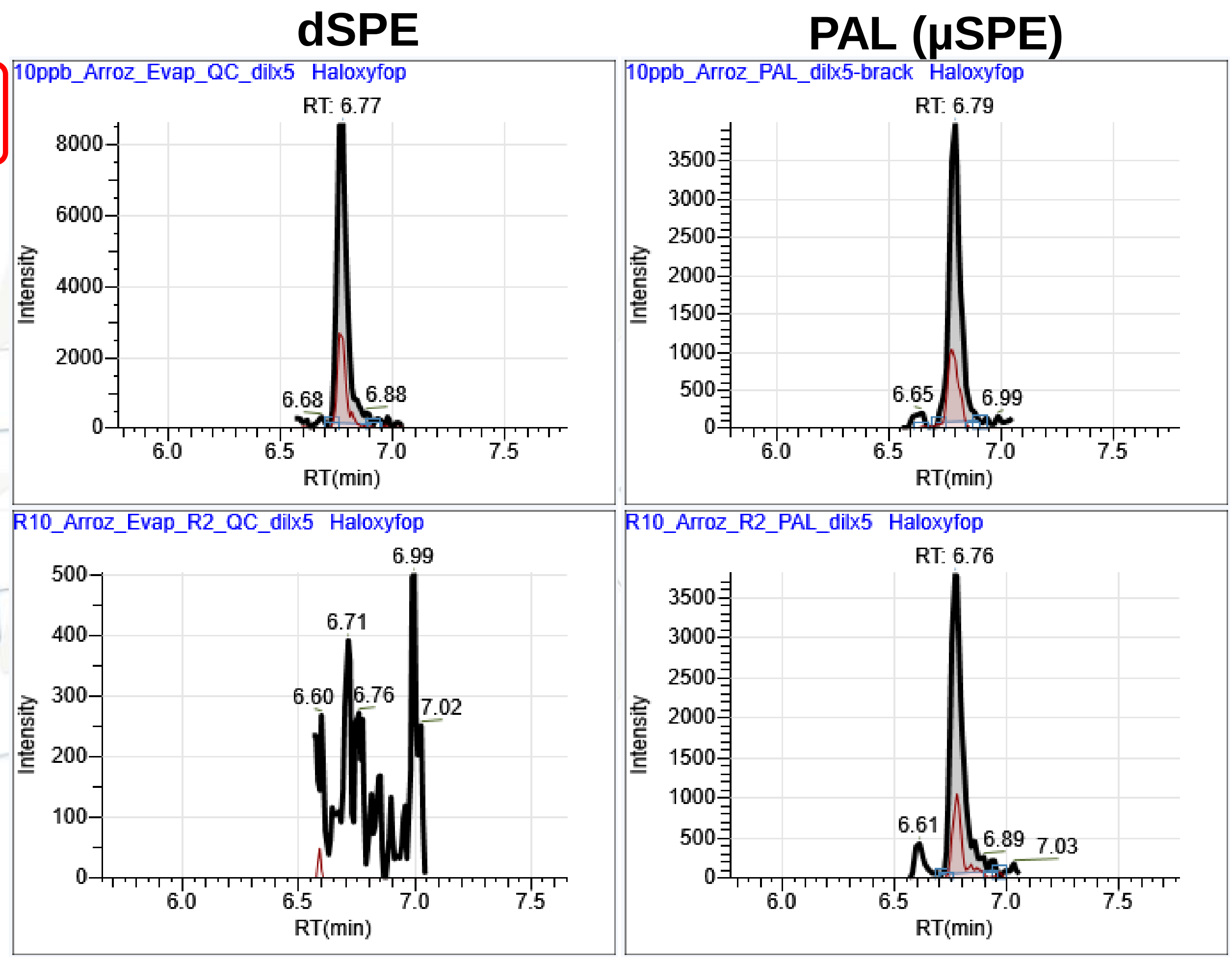
Rice

Haloxyfop



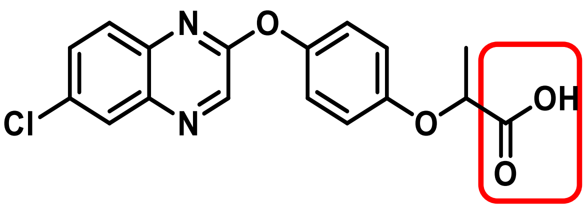
Standard at 10 µg/kg in extract

Rice spiked at 10 µg/kg



Comparison between dSPE and µSPE of acid compounds

Rice



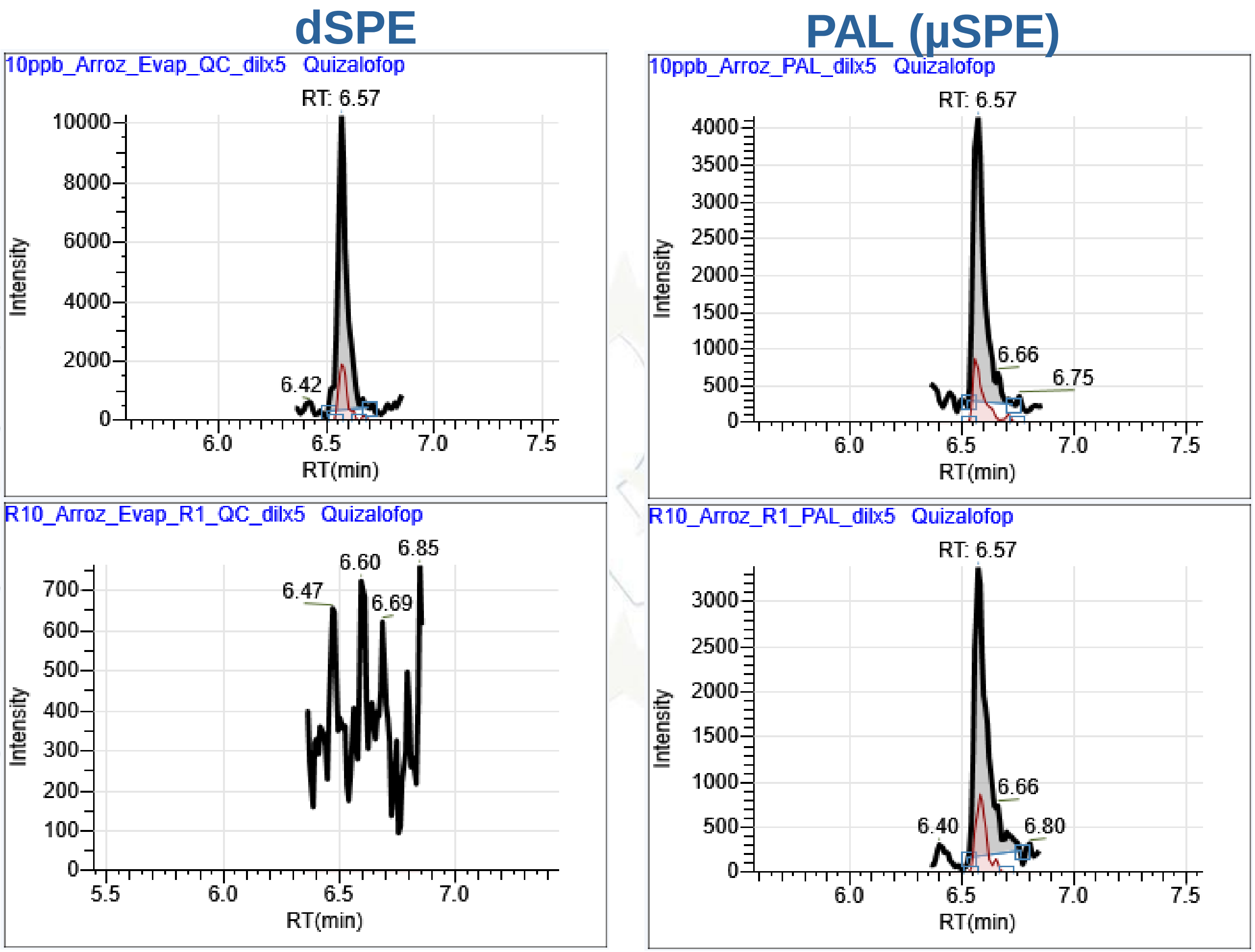
Quizalofop

Standard at 10 µg/kg in extract

Rice spiked at 10 µg/kg

dSPE

PAL (µSPE)



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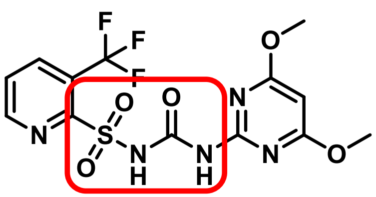
Optimization of PAL µSPE QuEChERS clean-up workflow

TROUBLESOME COMPOUNDS

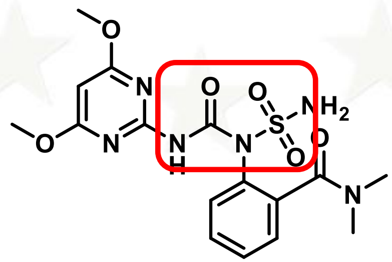
Recovery at 10 ppb of pesticide mix					
Compound	**Without elution**	**Acidified Raw QC extract**	**Elution with 100 µL ACN (5% f.a)**	**Elution with 200 µL ACN (5% f.a)**	**Elution with 600 µL ACN (5% f.a)**
Dodine	ND	ND	115	80	75
Flazasulfuron	ND	ND	104	74	82
Fluazifop	ND	ND	119	80	88
Haloxyfop	ND	ND	112	72	81
Orthosulfamuron	ND	ND	103	76	85
Oxasulfuron	ND	ND	110	80	83
Quizalofop	ND	ND	84	78	94

ND: Not detected

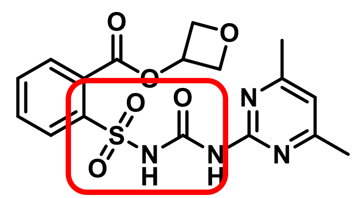
Sulphonylurea group



Flazasulfuron



Orthosulfamuron



Oxasulfuron

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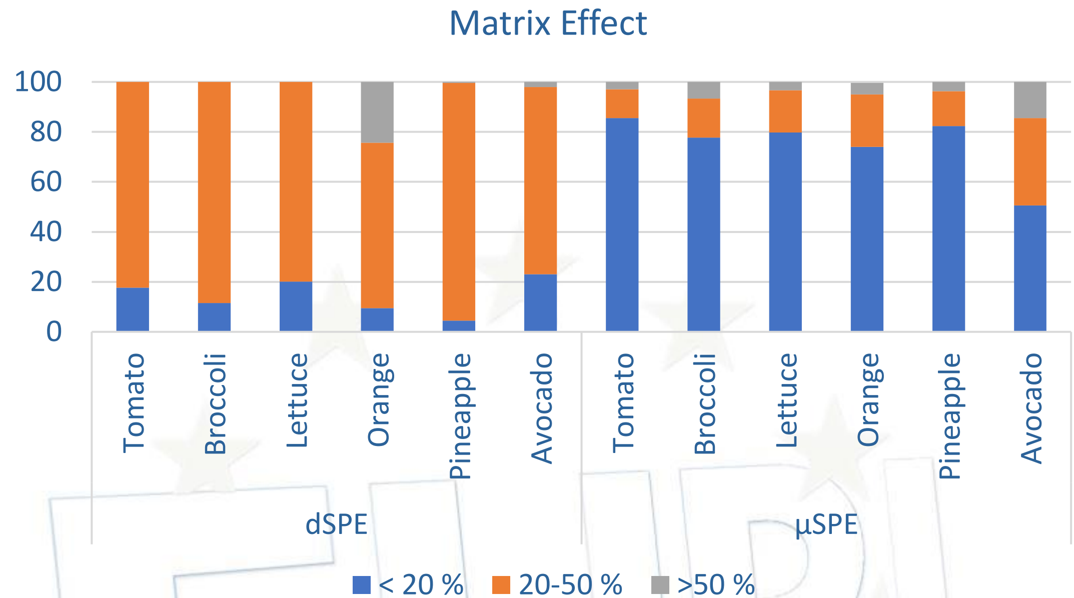
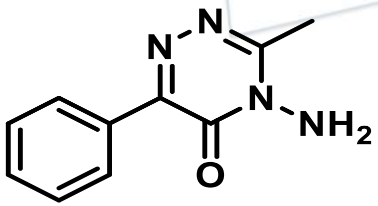
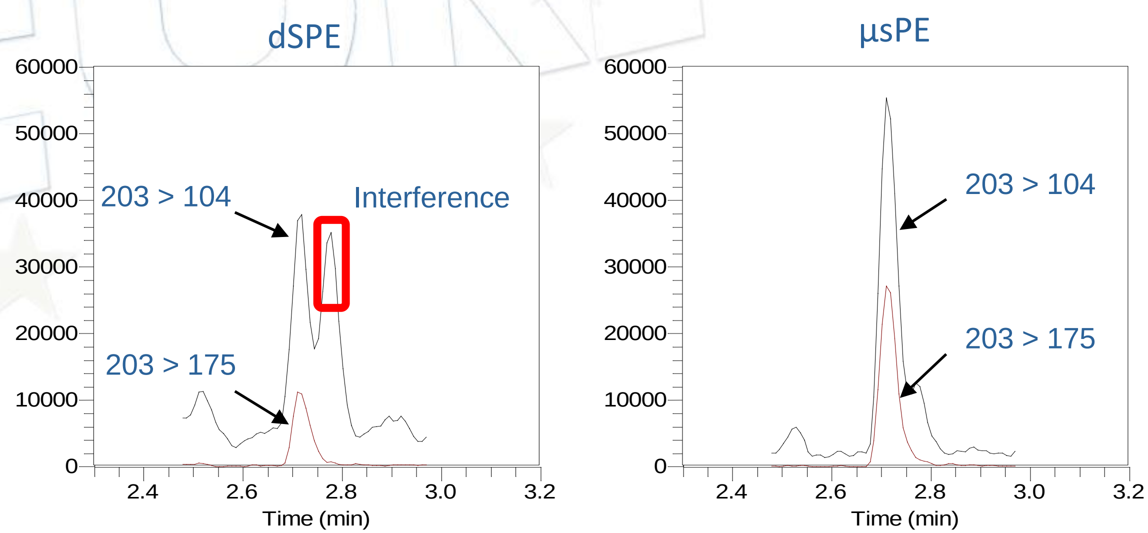
Matrix effect



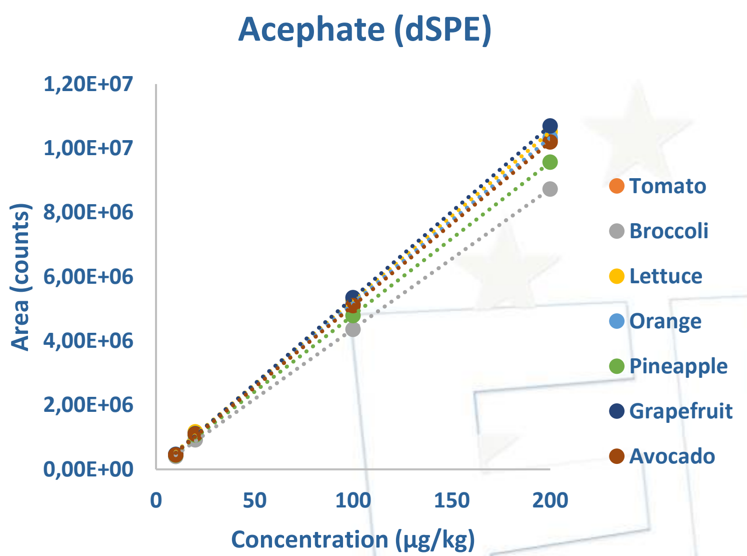
Blank avocado sample spiked at 10 µg L^{-1} with pesticide mix

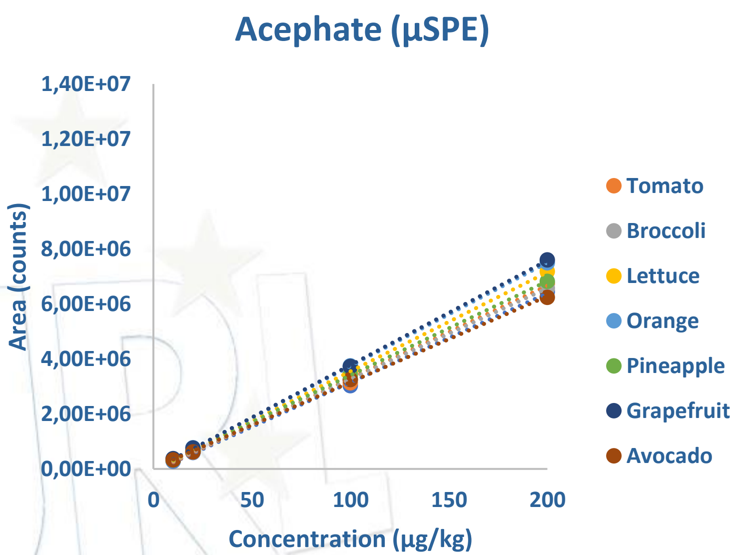


METAMITRON



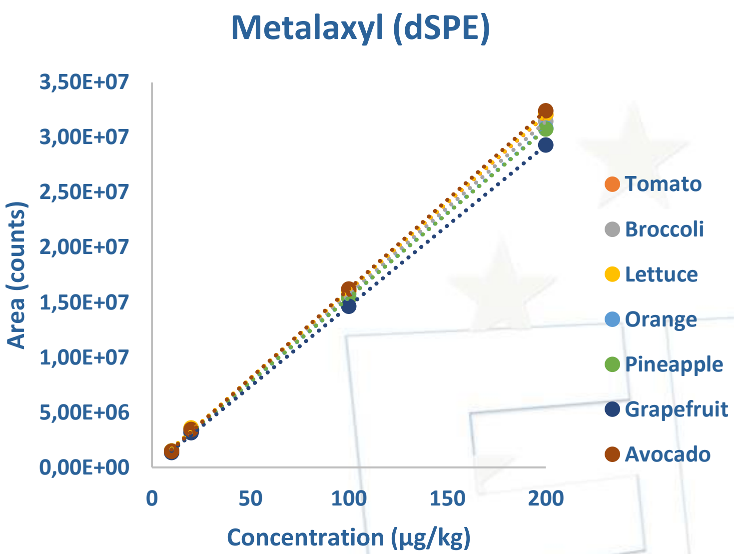
Linearity

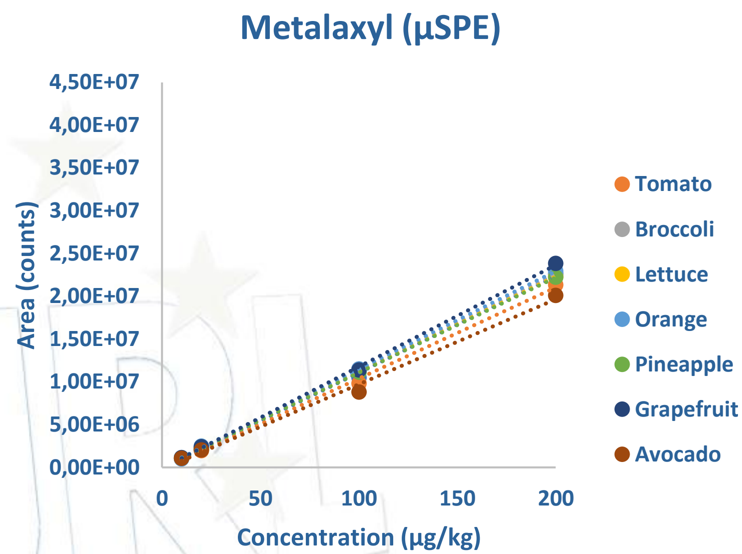




Total injected amount (mg)	
d-SPE	µSPE
0.5	0.33

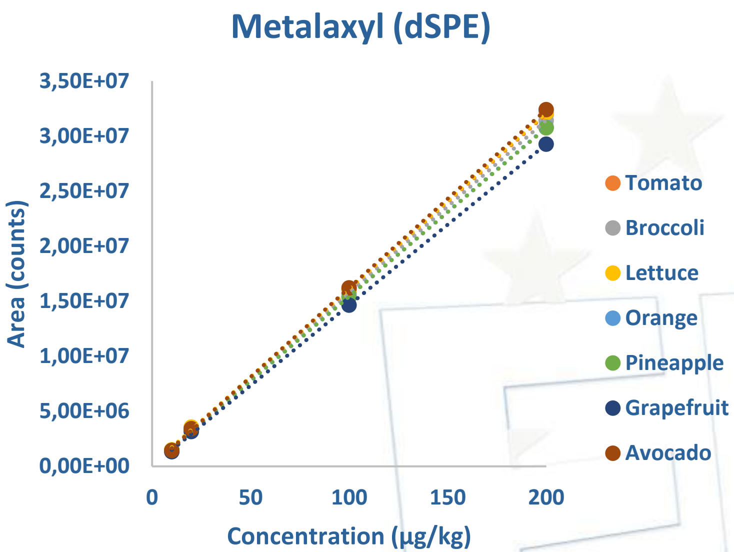
Linearity

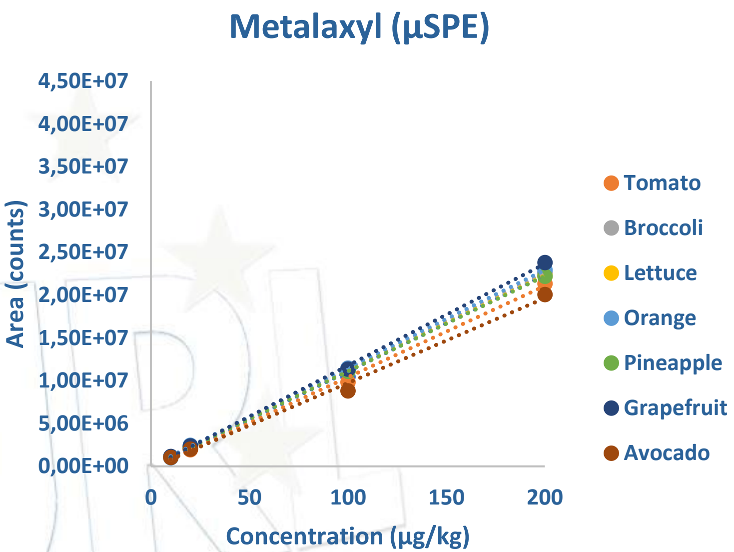




Total injected amount (mg)	
d-SPE	µSPE
0.5	0.33

Linearity

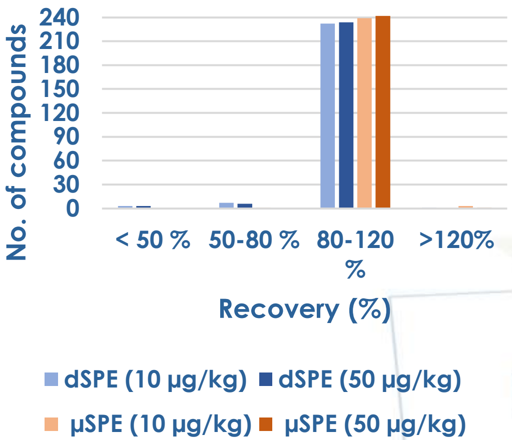




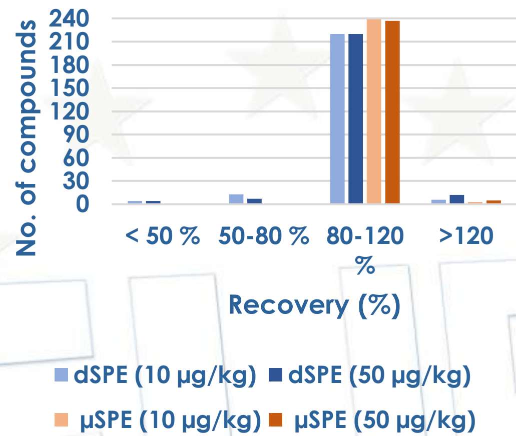
Total injected amount (mg)	
d-SPE	µSPE
0.5	0.33

Recoveries

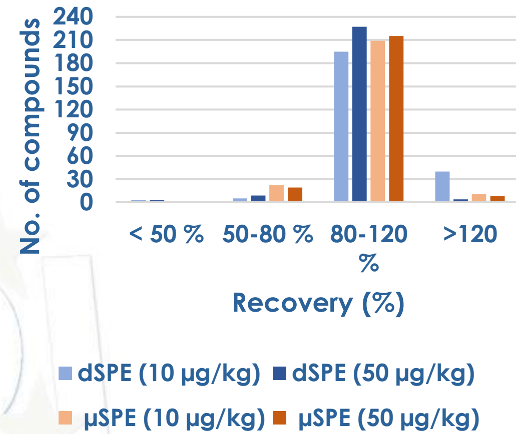
Tomato



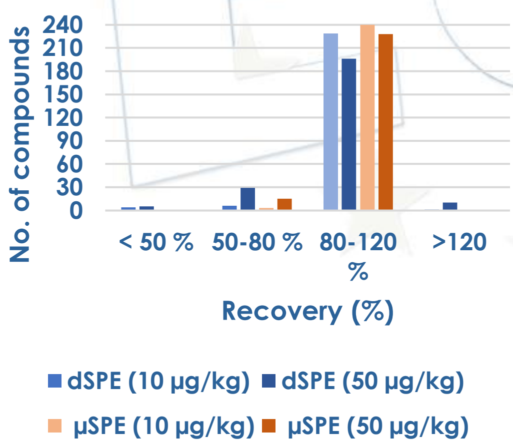
Orange



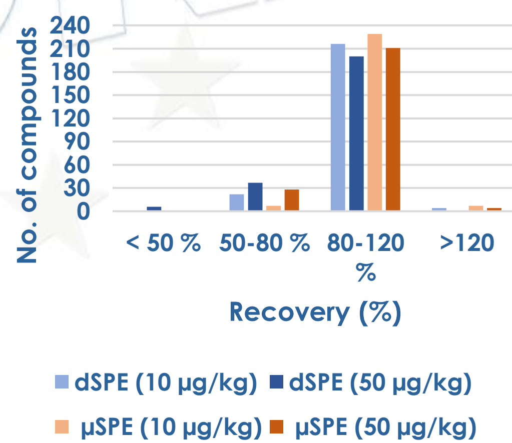
Avocado



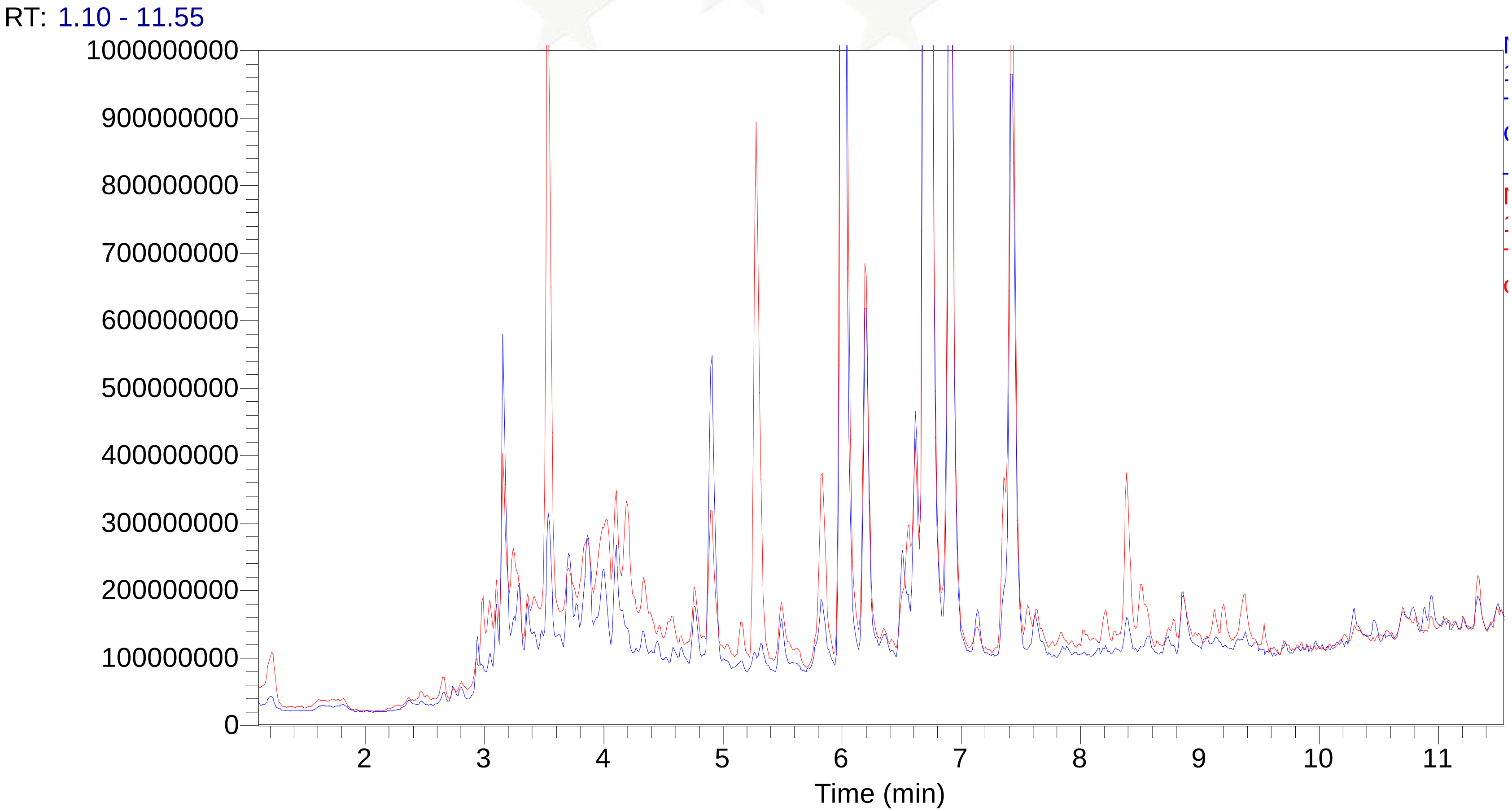
Rice



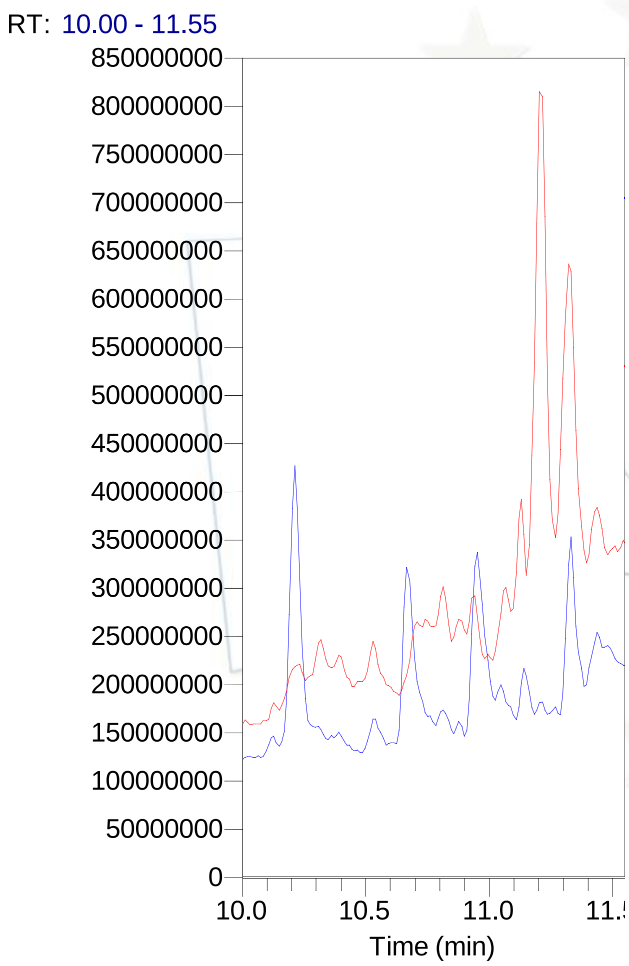
Black Tea



Zoom TIC Orange (data window)

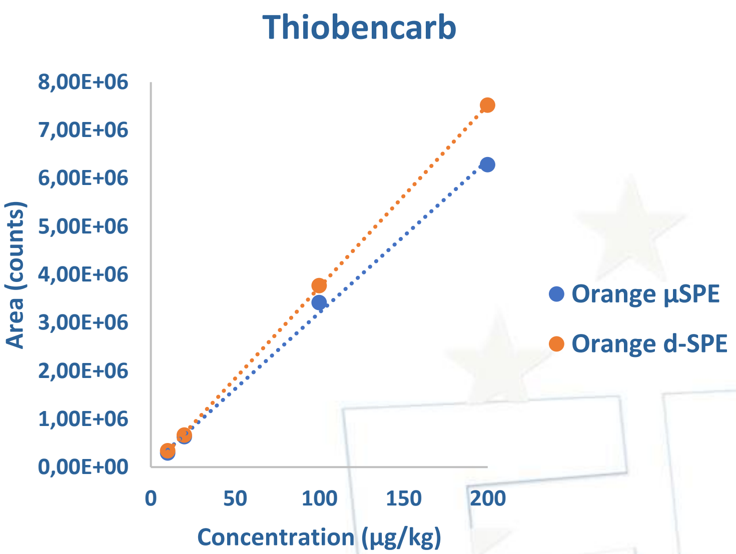


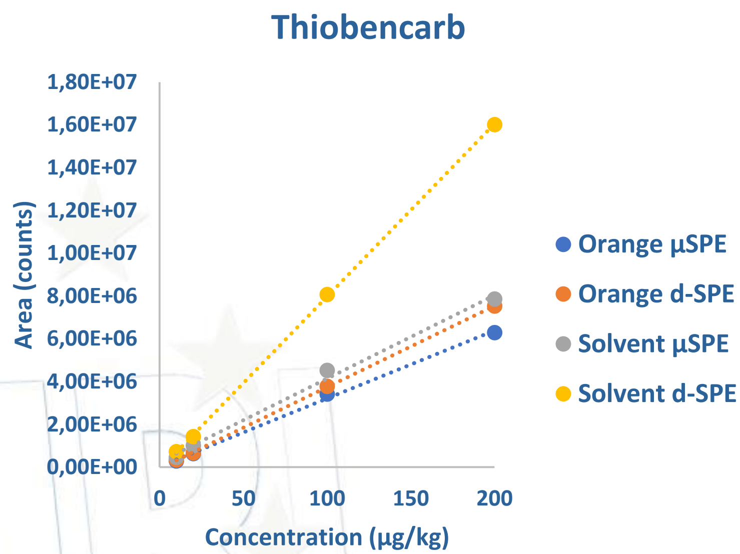
Zoom TIC Spinach (10.00 – 11.55 min)

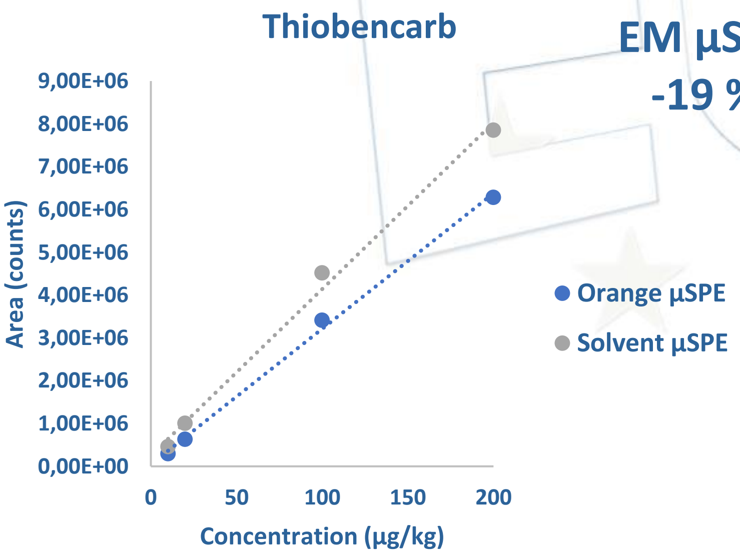


RT (min)	Compound
10.10	Chlorfluazuron
10.05	Proquinazid
10.08	Fenpyroximate
10.21	Pyridaben
10.30	Fenazaquin
10.32	Deltamethrin
10.65	Tau-Fluvalinate
10.90	Etofenprox
10.90	Phenothrin
11.01	Bifenthrin
11.37	Pyridalyl

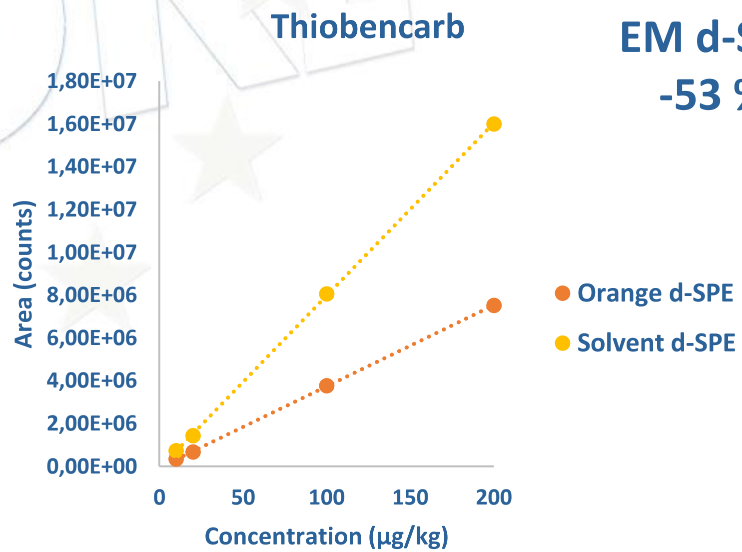
Matrix effect







EM µSPE
-19 %



EM d-SPE
-53 %

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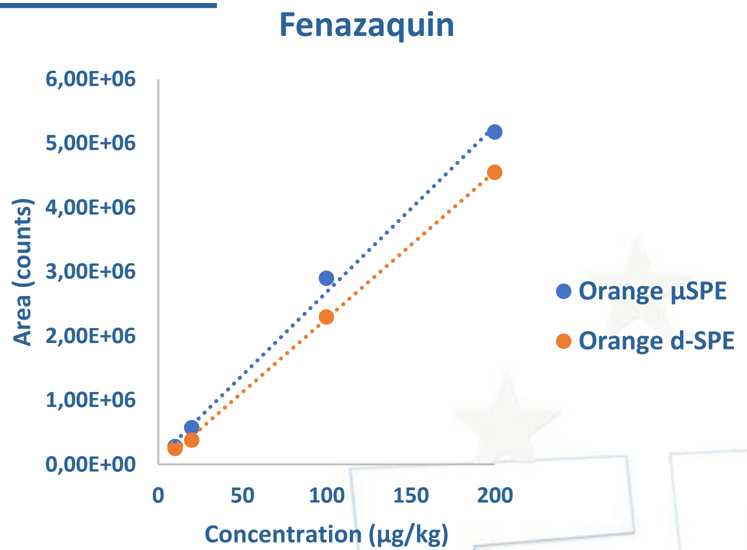
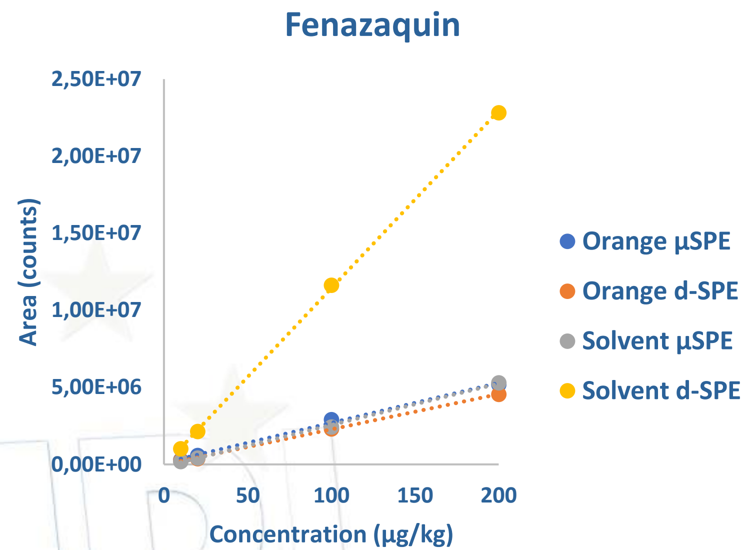
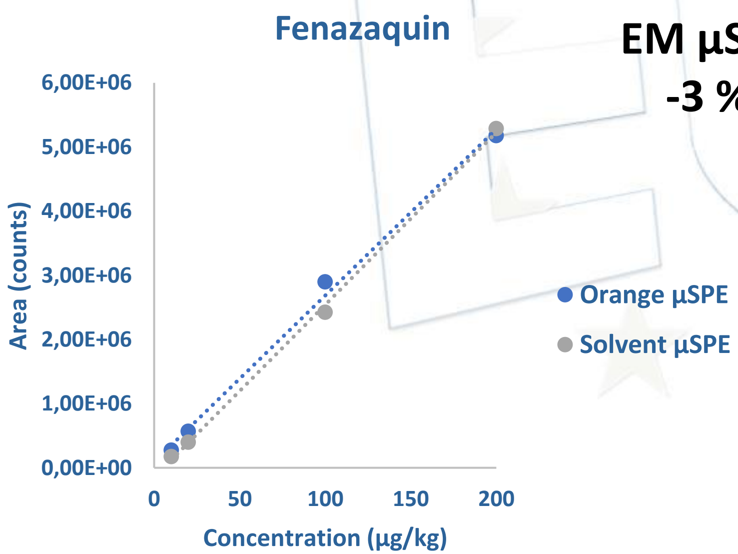
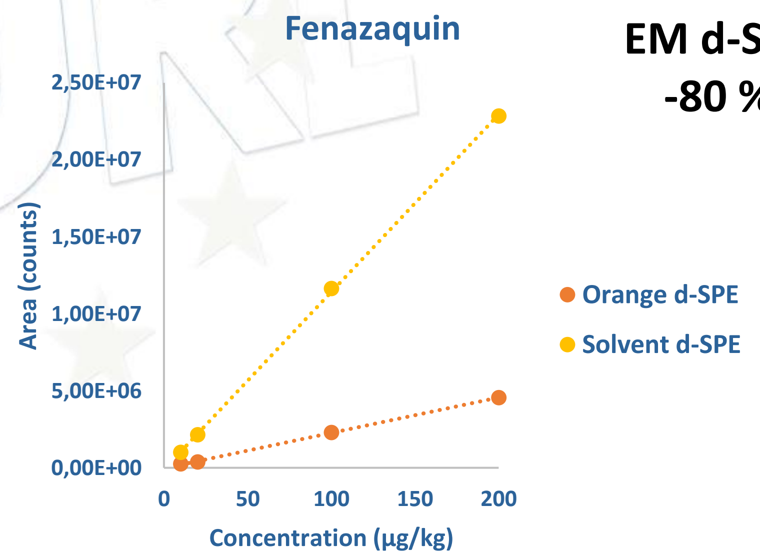
Matrix effect







EM µSPE
-3 %



EM d-SPE
-80 %

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Real samples

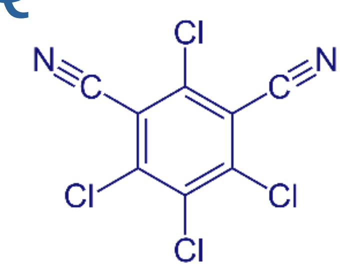
HIGH WATER CONTENT			HIGH ACID CONTENT AND HIGH WATER CONTENT			HIGH PROTEIN CONTENT AND LOW WATER AND FAT CONTENT		
LETTUCE	**µSPE**	**dSPE**	**ORANGE**	**µSPE**	**dSPE**	**BEAN**	**µSPE**	**dSPE**
Acetamiprid	0.036	0.035	Fluazifop	0.069	0.056	Azoxystrobin	0.014	0.013
ZUCCHINI	**µSPE**	**dSPE**	Imazalil	4.871	4.174	Cyproconazole	0.015	0.014
Acetamiprid	0.312	0.321	Phosmet	0.034	0.034	Difenoconazole	0.007	0.006
SPINACH	**µSPE**	**dSPE**	Pyrimethanil	0.350	0.280	Imidacloprid	0.010	0.009
Propamocarb	6.060	5.607	Thiabendazole	4.628	3.824	Tebuconazole	0.192	0.199
APPLE	**µSPE**	**dSPE**	**ORANGE**	**µSPE**	**dSPE**			
Acetamiprid	0.021	0.021	Fluazifop	0.051	0.050			
Boscalid	0.181	0.177	Imazalil	1.496	1.428			
Cyprodinil	0.304	0.300	Methoxyfenozide	0.119	0.121			
Pirimicarb	0.059	0.057	Propiconazole	0.012	0.013			
Pyraclostrobin	0.086	0.084	Thiabendazole	1.121	1.010			
BANANA	**µSPE**	**dSPE**	**GRAPE**	**µSPE**	**dSPE**			
Acetamiprid	0.006	0.006	Boscalid	0.368	0.426			
Indoxacarb	0.020	0.022	Cyazofamid	0.300	0.345			
Thiabendazol	0.337	0.268	Dimethomorph	0.135	0.143			
APPLE	**µSPE**	**dSPE**	Fludioxonil	0.150	0.155			
Boscalid	0.079	0.072	Fluopyram	0.125	0.129			
Pirimicarb	0.020	0.020	**STRAWBERRY**	**µSPE**	**dSPE**			
Pyraclostrobin	0.045	0.043	Azoxystrobin	0.014	0.014			
MELON	**µSPE**	**dSPE**	Difenoconazole	0.018	0.018			
Acetamiprid	0.008	0.009	Fluxapyroxad	0.044	0.045			
Fluopyram	0.047	0.045						
Propamocarb	0.040	0.036						
PEPPER	**µSPE**	**dSPE**						
Fluopyram	0.011	0.011						

Proficiency test on lemon material was analysed using the automated µSPE clean-up method, obtaining Z score values lower than ± 1.2 in all cases

PROFICIENCY TEST FV-19 (MATRIX: LEMON)		
COMPOUND	Calculated Concentracion (mg/kg)	Zscore
BOSCALID	0.4	0.2
CARBENDAZIM	0	0.1
CHLORANTRANILIPROLE	0.166	-0.3
CHLORFENAPYR	NA	NA
CHLORPYRIFOS	0.109	-0.7
DIAZINON	0.118	-1.2
ETHOPROPHOS	0.034	-0.5
FAMOXADONE	0.043	-0.1
FIPRONIL	0.02	0.2
FLUBENDIAMIDE	0.054	-0.2
FLUOPYRAM	0.136	0.3
IMIDACLOPRID	0.134	-0.6
IPRODIONE	NA	NA
LUFENURON	0.43	-1.0
OMETHOATE	0.017	-0.7
PROPAMOCARB	0.104	-0.6
PYRACLOSTROBIN	0.143	-0.9
PENFLUFEN	0.536	0.4
SULFOXAFLOR	0.029	-0.3

NA: Not Analysed.

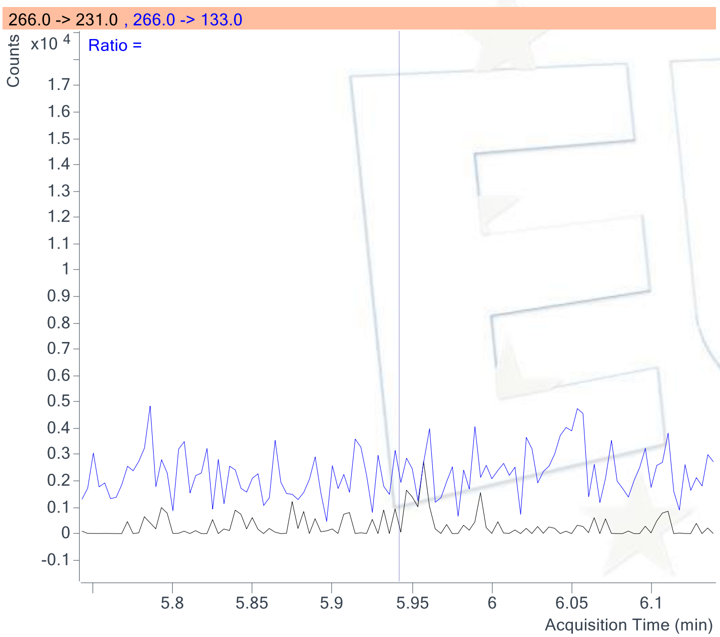
Evaluation of µSPE by GC-QqQ



Chlorthalonil

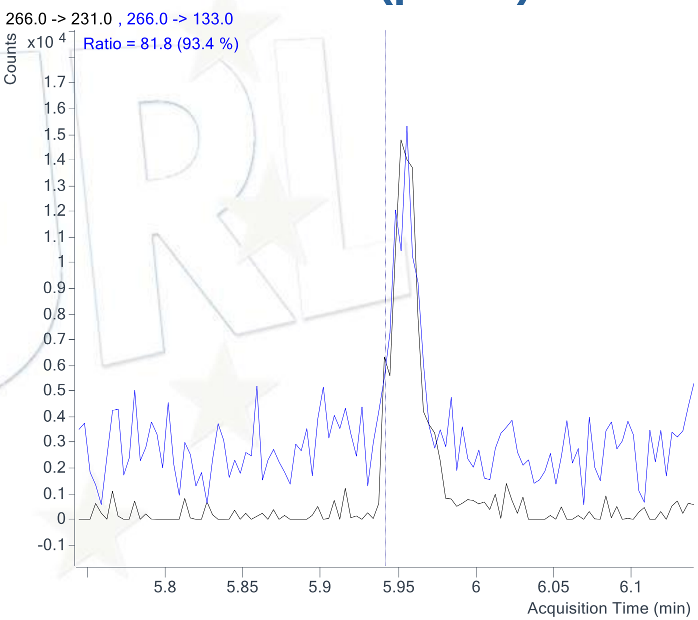
Orange spiked at 10 µg/kg of Chlorthalonil
(Analysis by GC-QqQ)

dSPE



Recovery: 0 %

PAL (µSPE)



Recovery: 96 %

EURL-FV (2022-M44) Automatisation of the clean-up step of multiresidue methods in GC-MS

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Conclusions

Advantages of the PAL µSPE QuEChERS clean up workflow

- One µSPE cartridge for a wide variety of food matrices
- Moreover, as only a single clean-up is employed equally for all commodities, greater homogeneity is typically obtained in the calibration curves
- Analyte-cartridge interaction is compensated for by subjecting the matrix calibration curve to the µSPE cartridges without negative sensitivity effects. Automation means that submitting the calibration curve to the clean-up is not such a tedious and time-consuming step.
- Better clean-up performance compared to dSPE
- Instrument maintenance is also positively affected because, generally, cleaner extracts are obtained and so the lifespan of certain instrument parts (such as the ion source and columns) increase.

Retention µSPE cartridge factor

Blank matrix extract (without clean-up step) was fortified at 10 µg L-1of pesticide mix. An aliquot was passed through the µSPE cartridges, and another aliquot was diluted with ACN to account for µSPE-dilution.

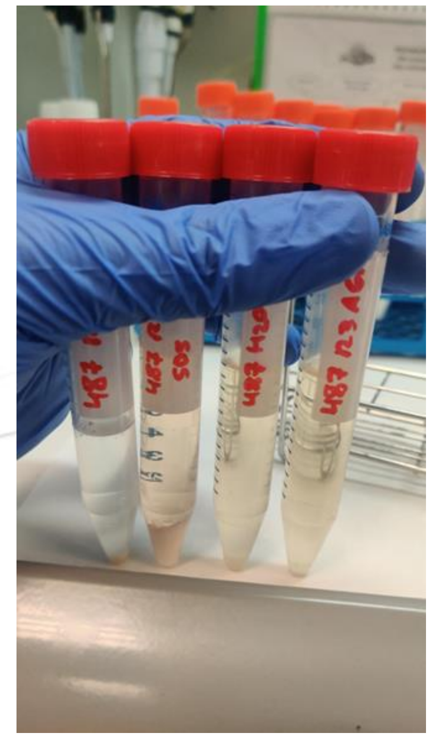
$$Area\ Accuracy\ (\%):\ \frac{\overline{Area\ without\ clean{-}up} - \overline{Area\ with\ \mu SPE}}{\overline{Area\ without\ clean{-}up}}\ x\ 100$$

	Tomato	Orange	Rice
Compound	**Area Accuracy (%)**		
Mebendazole	93	80	89
Albendazole	88	67	86
Fenbendazole	86	73	83
Oxfendazole	83	72	74
Fluazifop	37	37	29
Quizalofop	71	45	63
Haloxyfop	45	22	29
Oxasulfuron	55	37	35
Orthosulfamuron	54	51	24
Flazasulfuron	50	35	29
Chlorfluazuron	75	71	56
Pyridalyl	81	72	66
Pymetrozine	59	32	39
Fenpropidin	56	46	49
Propamocarb	55	49	49
Spiroxamine	49	43	47

N=5. RSD results (%) were lower tan 20 %

Analyte-cartridge interaction is compensated for by **subjecting the matrix calibration curve to the µSPE cartridges without negative sensitivity effects.** Automation means that submitting the calibration curve to the clean-up is not such a tedious and time-consuming step; in contrast, if this were carried out using dSPE, the analysis time would be 30 % longer.

Problems with sample hydration prior to extraction



Different Extraction Approaches For High Protein Content Pulses

- **Sante Document recommends sample hydration prior to extraction**

- **Sample hydration increases extraction of polar compounds**

- **Coextraction of other matrix components can be the source of matrix interferences in the analysis of target analytes**

- **In this case the high content of proteins with amino groups can interact with pesticides *Ex. Dichlorvos***

258 Pesticides					
Carbendazim-d3	Cyflufenamid	Fenbendazole	Isofenphos-methyl	Oxadixyl	Quizalofop-P-ethyl
Dichlorvos-d6	Cyflumetofen	Fenbuconazole	Isofetamid	Oxamyl-NH4	Rotenone
Malathion-d10	Cymoxanil	Fenhexamid	Isoprocarb	Oxasulfuron	Simazine
Acephate	Cyproconazole	Fenobucarb	Isoprothiolane	Oxathiapiprolin	Spinetoram J
Acetamiprid	Cyprodinil	Fenoxycarb	Isoproturon	Oxfendazole	Spinetoram L
Alachlor	Cyromazine	Fenpicoxamid	Isopyrazam	Paclobutrazol	Spinosyn A
Albendazole	Dazomet	Fenpropidin	Isoxaflutole	Penconazole	Spinosyn D
Aldicarb_116	DEET	Fenpropimorph	Kresoxim-methyl	Pencycuron	Spirodiclofen
Ametoctradin	Demeton-S-methyl	Fenpyrazamine	Lenacil	Pendimethalin	Spiromesifen
Anilofos	Demeton-S-methylsulfone	Fenpyroximate_E	Linuron	Penflufen	Spirotetramat
Atrazine	Demeton-S-methylsulfoxide	Fensulfothion	Lufenuron	Penthiopyrad	Spiroxamine
Azinphos-ethyl	Diazinon	Fenthion	Malathion	Permethrin	Sulfoxaflor
Azinphos-methyl	Dichlorvos	Fenthion-sulfone	Mandipropamid	Phenothrin	Tau-Fluvalinate
Azoxystrobin	Dicrotophos	Fenthion-sulfoxide	Matrine	Phenthoate	Tebuconazole
BAC-C10	Diethofencarb	Fenuron	Matrine-N-Oxide	Phosalone	Tebufenozide
BAC-C8	Difenoconazole	Fipronil	Mebendazole	Phosmet	Tebufenpyrad
Benalaxyl	Difenoxuron	Flazasulfuron	Mefentrifluconazole	Phoxim	Teflubenzuron neg
Bendiocarb	Diflubenzuron	Flonicamid	Mepanipyrim	Pirimicarb	Terbuthylazine
Benzovindiflupyr	Dimethoate	Florpyrauxifen-benzyl	Metaflumizone_E	Pirimiphos-methyl	Terbuthylazine-desethyl
Bifenazate	Dimethomorph	Fluacrypyrim	Metaflumizone_Z	Prochloraz	Terbutryn(e)
Bitertanol	Dimethylvinphos	Fluazifop-P-butyl	Metalaxyl	Profenofos	Tetraconazole
Bixafen	Diniconazole	Flubendiamide	Metamitron	Promecarb	Tetramethrin
Boscalid	Dinotefuran	Fludioxonil	Metconazole	Prometryn	Thiabendazole
Bromacil	Diuron	Flufenacet	Methamidophos	Propamocarb	Thiacloprid
Bromuconazole	DMF	Flufenoxuron	Methidathion	Propaquizafop	Thiamethoxam
Bupirimate	DMPF	Fluometuron	Methiocarb	Propazine	Thiobencarb
Buprofezin	Dodine	Fluopicolide	Methiocarb-sulfone	Propiconazole	Tolfenpyrad
Butoxycarboxim	Edifenphos	Fluopyram	Methiocarb-sulfoxide	Propoxur	Triadimefon
Carbaryl	Emamectin B1a	Flupyradifurone	Methomyl	Propyzamide	Triallate
Carbendazim	Epoxiconazole	Fluquinconazole	Methoxyfenozide	Proquinazid	Triazophos
Chlorantraniliprole	Ethiofencarb	Flusilazole	Metobromuron	Prosulfocarb	Trichlorfon
Chlorbromuron	Ethion	Flutriafol	Metolachlor	Pymetrozine	Triclocarban
Chlorfenvinphos	Ethiprole	Fluxapyroxad	Metolcarb	Pyraclostrobin	Tricyclazole
Chlorfluazuron	Ethirimol	Formetanate-hydrochloride	Metrafenone	Pyrethrins1	Trifloxystrobin
Chloridazon	Ethoprophos	Fosthiazate	Monocrotophos	Pyrethrins2	Triflumizole
Chlorotoluron	Etoxazol	Haloxyfop	Monolinuron	Pyridaben	Triflumuron
Chloroxuron	Famoxadone	Hexaconazole	Monuron	Pyridalyl	Trinexapac-ethyl
Chlorpyrifos	Fenamidone	Hexythiazox	Myclobutanil	Pyridaphenthion	Trinexapac-methyl
Chromafenozide	Fenamiphos	Imazalil	Neburon	Pyrifoenone	Triticonazole
Clofentezine	Fenamiphos-sulfone	Imidacloprid	Nitenpyram	Pyrimethanil	Tritosulfuron
Clomazone	Fenamiphos-sulfoxide	Indoxacarb	Novaluron	Pyriproxyfen	Valifenalate
Coumaphos	Fenarimol	Ioxynil	Omethoate	Quinalphos	XMC
Cyantraniliprole		Iprovalicarb	Orthosulfamuron	Quinoclamine	Zoxamide
Cyazofamid	Fenazaquin	Isocarbophos	Oxadiargyl	Quinoxyphen	

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Different Extraction Approaches For High Protein Content Pulses

Analysis

UHPLC (Thermo ScientificTM TranscendTM DUO LX-2 LC)

- Column: Accucore C18 2.1x100 mm and 2.6 µm particle size (Thermo Scientific™)
- Mobile phase A: Water (0.1 % formic acid, 5 mM ammonium formate, 2 % MeOH)
- Mobile phase B: Methanol (0.1 % formic acid, 5 mM ammonium formate, 2 % water)
- Column temperature: 30 °C
- Flow rate: 0.35 ml/min
- Injection volume: 2.5 µL
- Autosampler temperature: 10 °C

Mobile phase gradient for pesticides analysis:

Time [min]	Mobile phase A
0	100 %
1	100 %
2	70 %
3	50 %
11	0 %
14	0 %
14.1	100 %
17	100 %
Data window [min]	1.1-11.55

Triple quadrupole system (Transcend DUO LX-2. A TSQ Altis, Thermo Scientific)

Ion source: Opta Max NG

- Positive ion spray voltage: 3500 V
- Negative ion spray voltage: 2500 V
- Sheath gas: 50
- Aux gas: 10
- Sweep gas: 1
- Ion transfer tube temperature: 25 °C
- Vaporiser temperature: 350 °C

Extraction Method for LC-QqQ-MS/MS analysis

5±0.5 g sample
10 ml H_2O + SDS 0.25g

10 ml AcN+ IS

Shake 6 min

Add 4 g MgSO4 anhidrous + 1 g NaCl + 1 g CitNa3·2H2O + 0.5 g CitNa2H·3/2·H2O

Shake 6 min

Centrifuge 5 min. 3500 r.p.m.

Clean-up step

8 ml extract
Freezing out

5 mL extract
750 mg MgSO4 anh.
+ 125 mg PSA

Centrifuge 5 min. 3500 r.p.m.

LC-MS/MS Analysis (0.1 g sample/mL extract)

Extraction Method for LC-QqQ-MS/MS analysis

5±0.5 g sample

10 ml AcN+ IS

Shake 6 min

Add 4 g $MgSO_4$ anhidrous + 1 g NaCl + 1 g $CitNa_3 \cdot 2H_2O$ + 0.5 g $CitNa_2H \cdot 3/2 \cdot H_2O$

Shake 6 min

Centrifuge 5 min. 3500 r.p.m.

Clean-up step

8 ml extract
Freezing out

5 mL extract
750 mg $MgSO_4$ anh.
+ 125 mg PSA

Centrifuge 5 min. 3500 r.p.m.

LC-MS/MS Analysis (0.1 g sample/mL extract)

Different Extraction Approaches For High Protein Content Pulses

Extraction methods approaches

White bean

- H_2O addition
- 30 min extra shaking
- H_2O+0.25 g SDS
- Only AcN
  No hydration

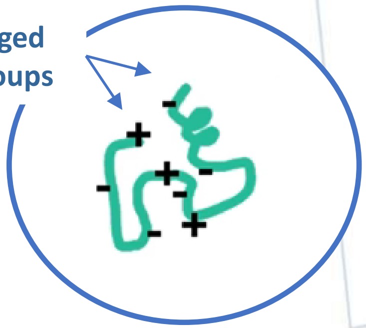
Sodium Dodecyl Sulfate (SDS)

Hydrophobic tail

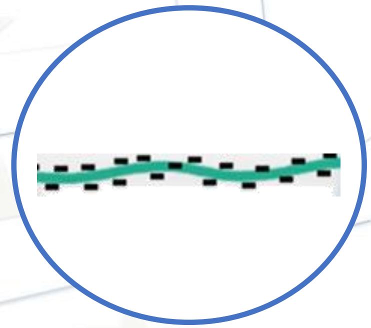
Hydrophilic head

- Anionic Surfactant
- Denaturing agent for proteins
- Bring the folded proteins down to linear molecules with negative charge

Charged
R-groups



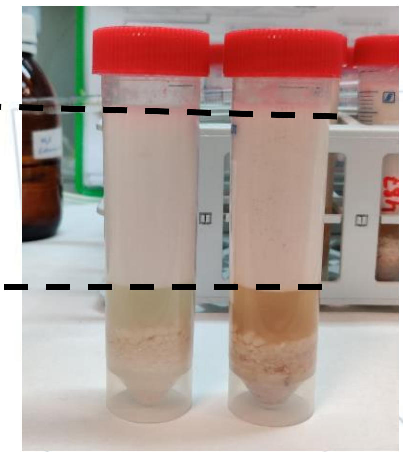
Protein before SDS



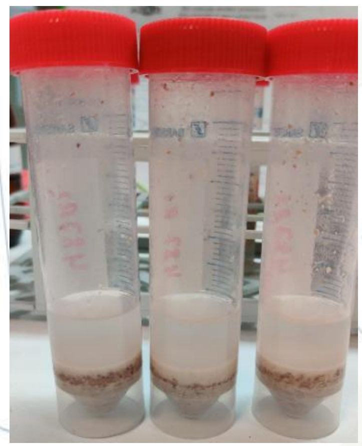
Protein after SDS
(Reduded to its primary structure)

Extraction methods approaches

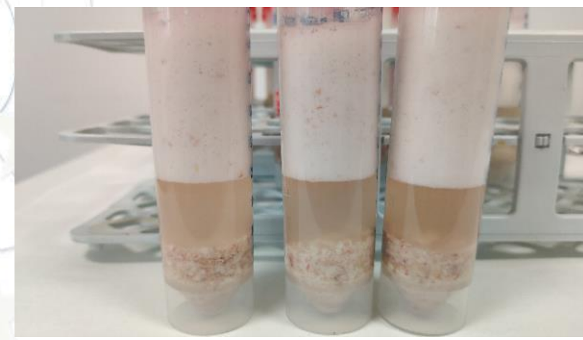
Protein emulsion
Depends on the type of beans



H_2O addition



Only AcN
Non hydration



H_2O+ 0.25 g SDS

Different Extraction Approaches For High Protein Content Pulses

After the freezing out

higher precipitated
in the SDS method.

AcN

H2O + SDS

H2O

30 min
shaking

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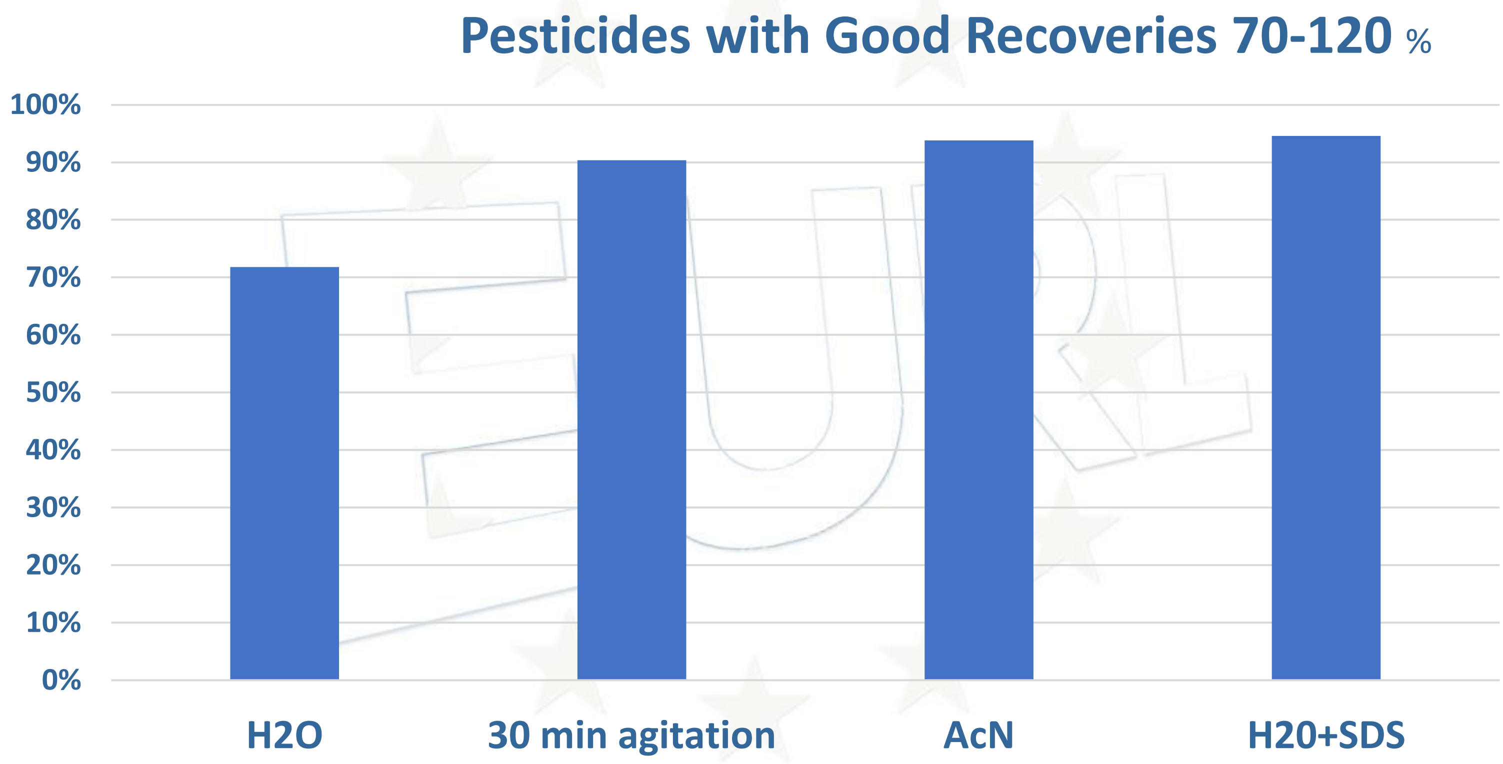
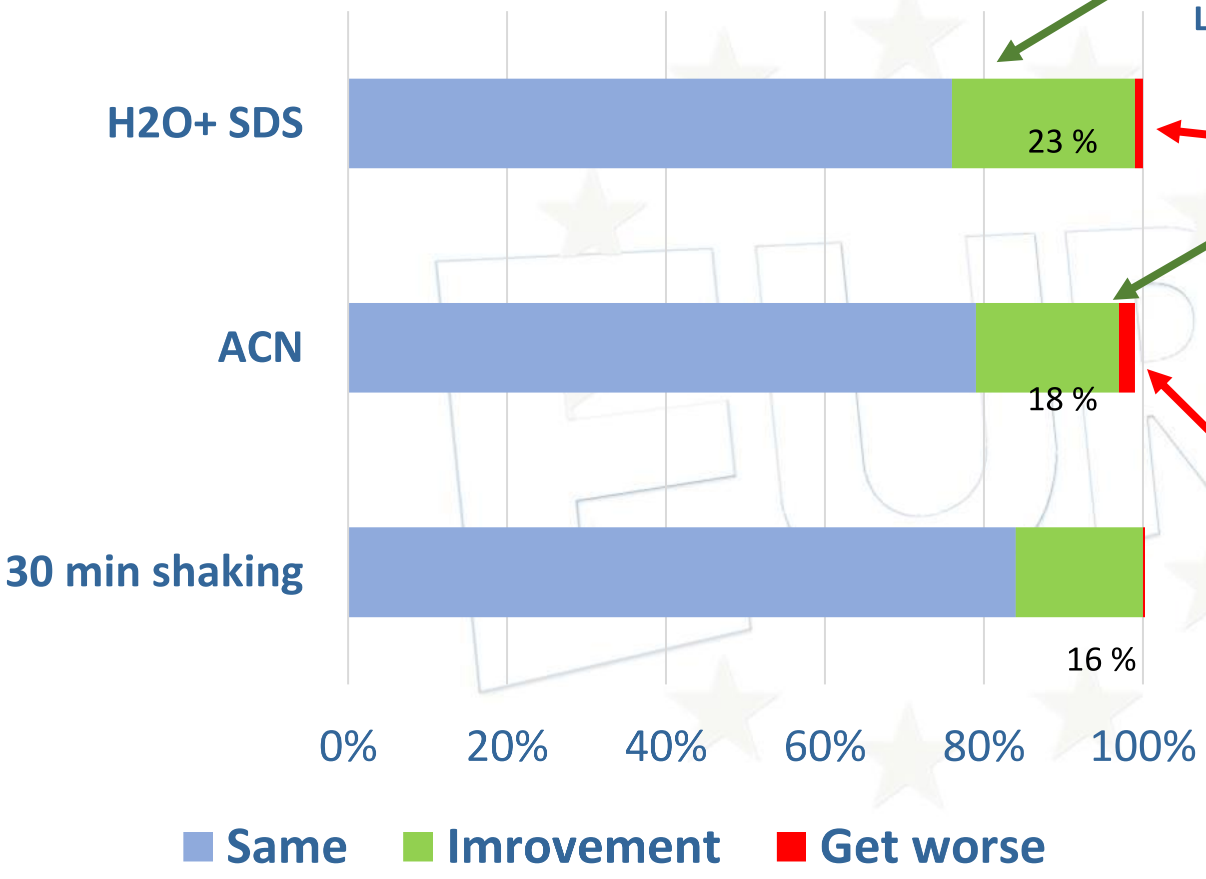
Bean samples spiked at 10 µg/kg (5 Replicates)



Comparision in relation to H_2O method



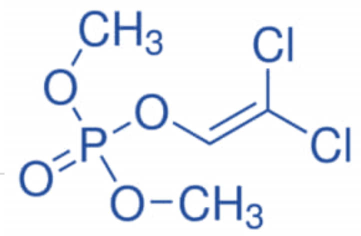
Dichlorvos
Trinexapac-methyl
Trinexapac-ethyl
Matrine
Chlorfuazuron
Lufenuron…

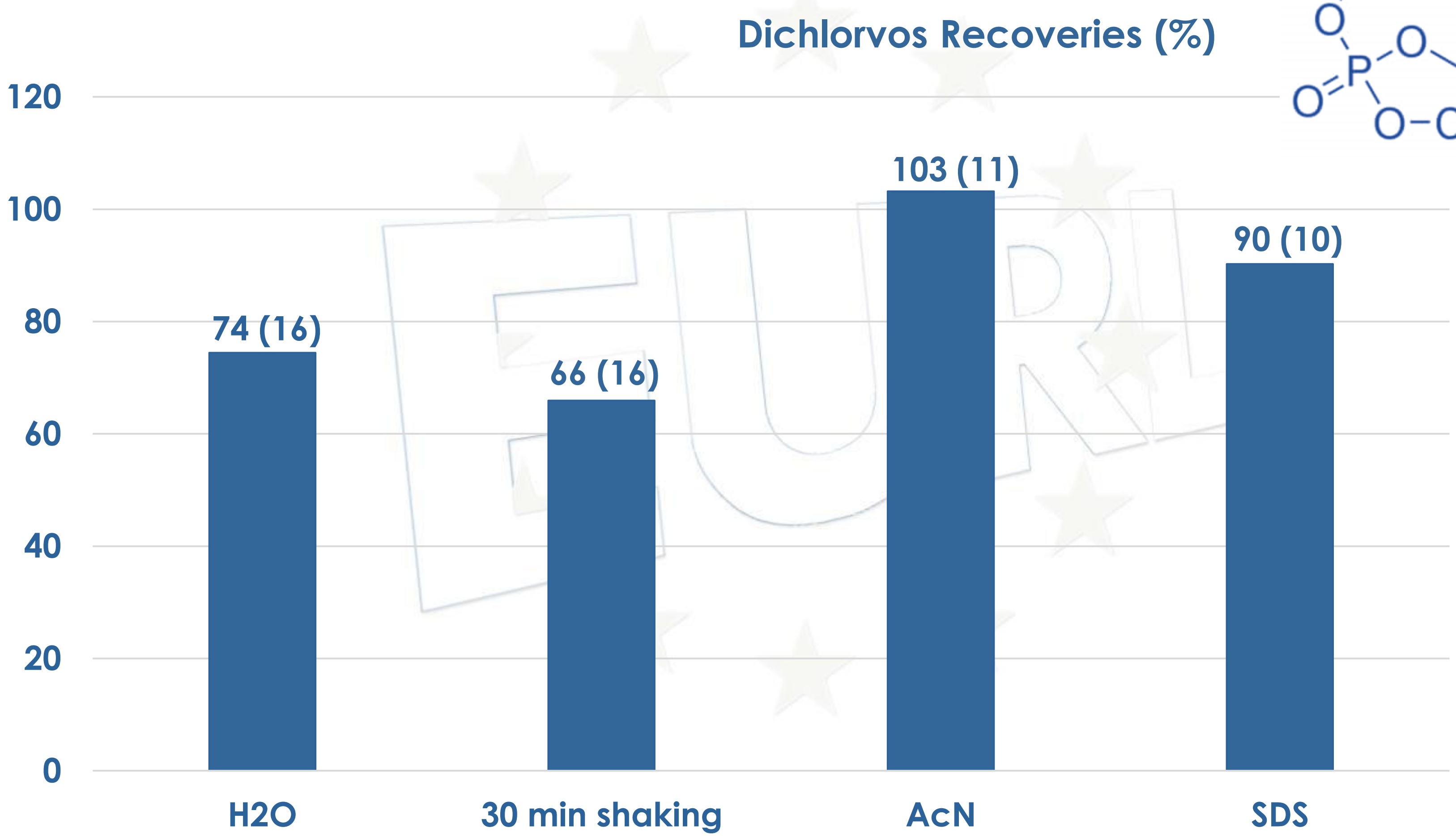
Hexaconazole (Recovery 162 %)
Metconazole (Recovery 158% (RSD 36%))
Nytenpyram (Not Detected)

Dichlorvos
Chlorpyrifos
Pyrethrins
Quinoxyphen….

Dodine (Recovery 20%)
Fenhexamid (Recovery 56%)
Flazasulfuron (Recovery 29%)
Ioxynyl (Recovery 42%)
Orthosulfamuron (Recovery 34%)
Oxasulfuron (Recovery 37%)

Bean samples spiked at 10 µg/kg (5 Replicates)





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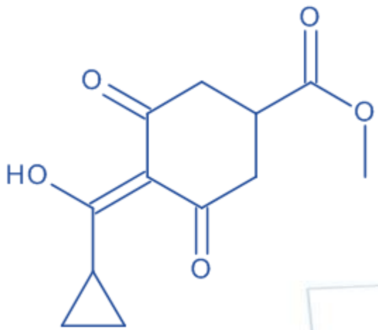
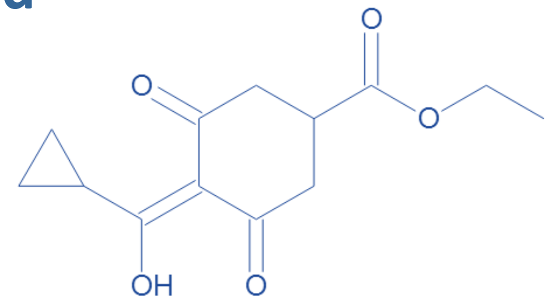
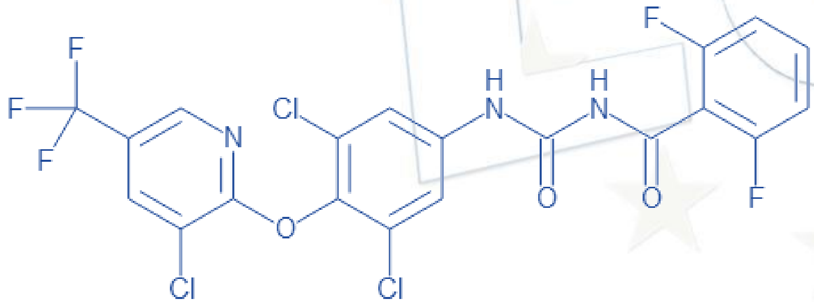
Pesticides only recovered with H_2O+SDS method



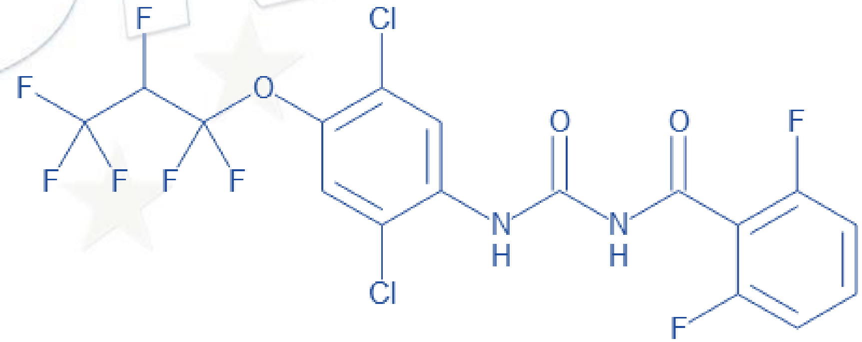
Trinexapac-methyl



Trinexapac-ethyl

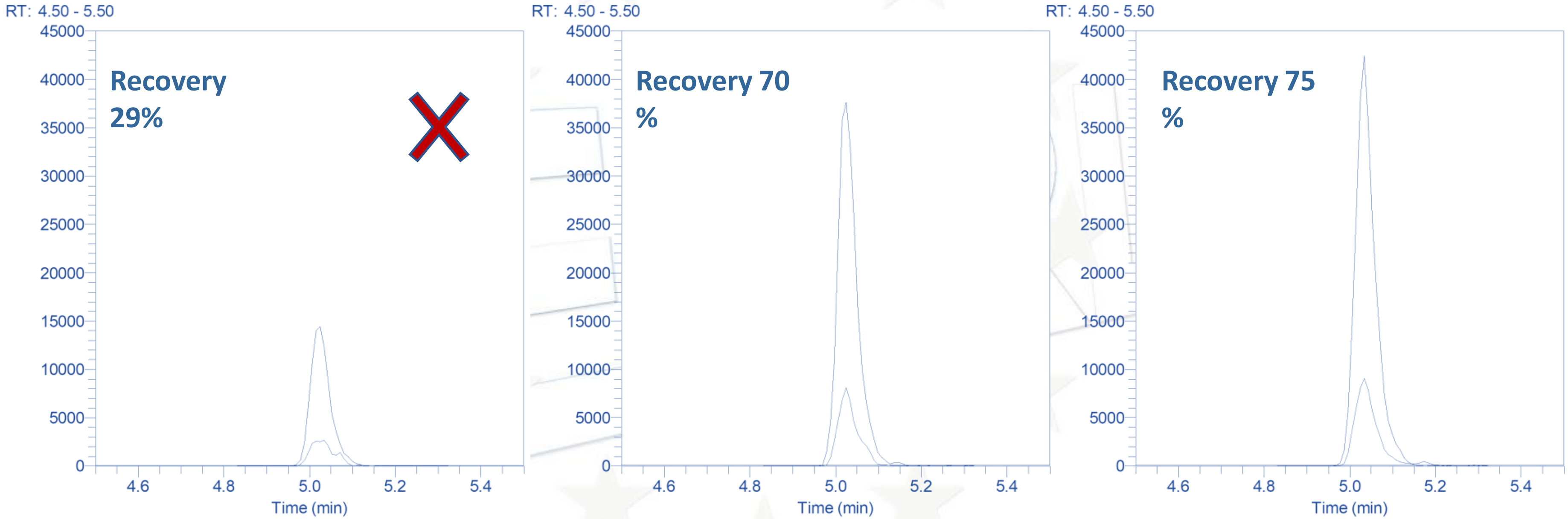


Chlorfluazuron



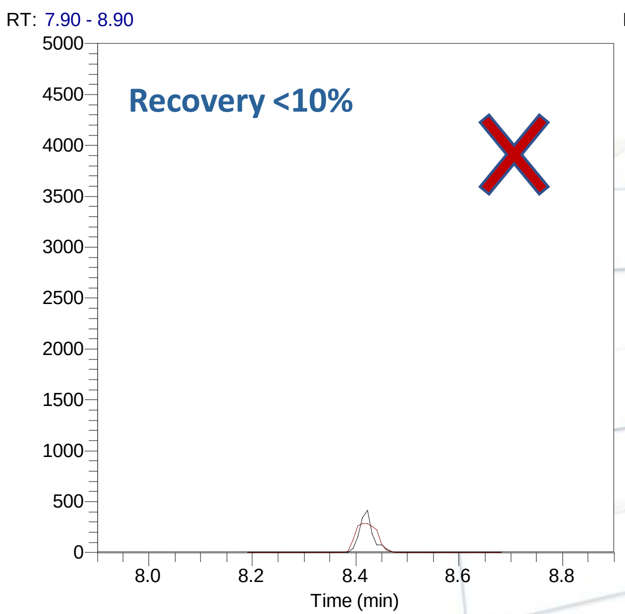
Lufenuron

Flazasulfuron

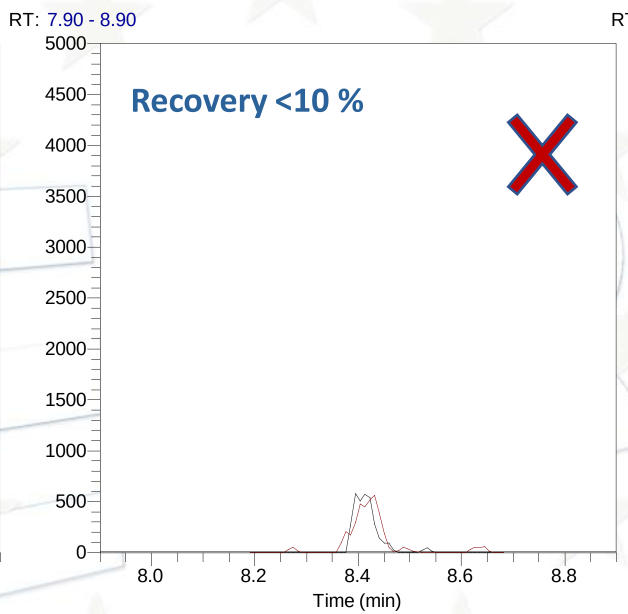


ACN H_2O SDS

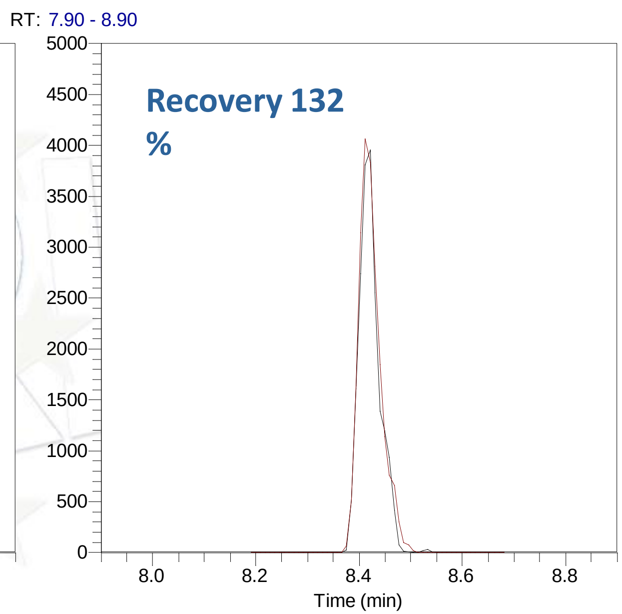
Lufenuron



ACN



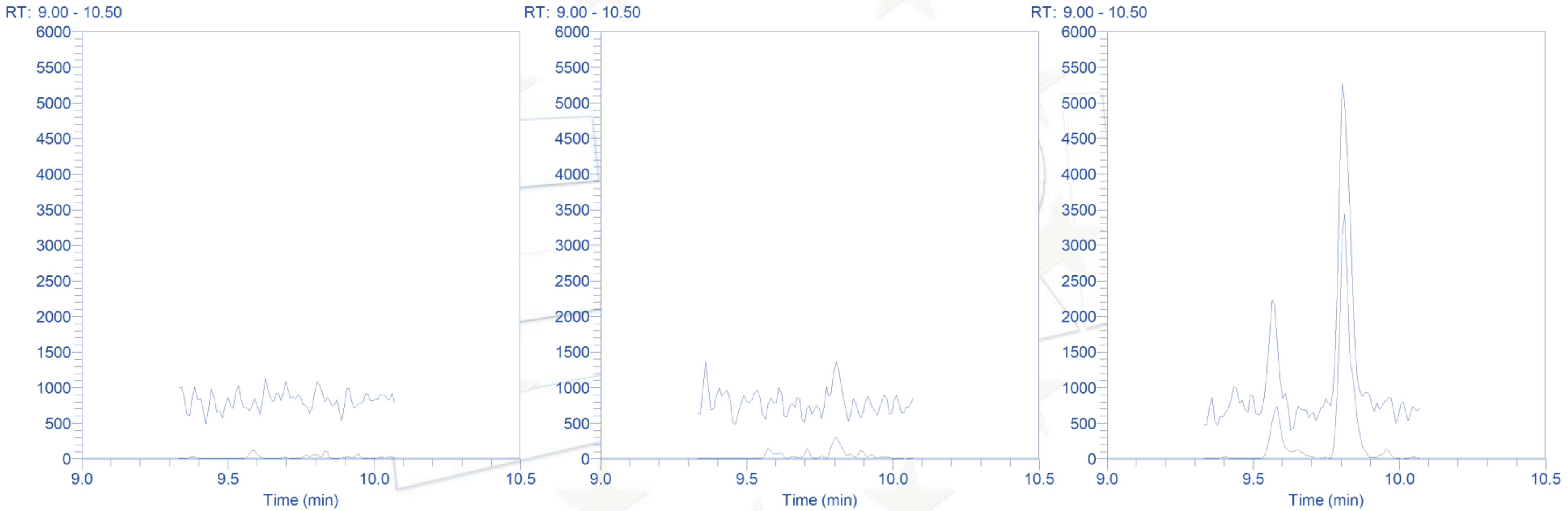
H_2O



SDS

Permethrin

Only detected in LC-QQQ-MS/MS with SDS addition



ACN **H_2O** **SDS**

Chemical Composition of legumes

Legume		Species	Proteín (%)	Lipids (%)	Carbohidrates (%)	Fiber (%)
Soybeans		*Glycine max*	39	19.6	35.5	4.7
Peanuts		*Arachis hypogaea*	24.8	47.9	24.6	3.1
Peas		*Pisum sativum*	25.7	1.6	68.6	1.6
Judías (Beans)		*Phaseolus vulgaris*	24.1 (16-31)	1.8	65.(55-65)	4.5
Chickpeas		*Cicer arietinum*	22.7	5	66.3	3
Haba (Beans)		*Vicia faba*	26.7	2.3	64	7.2
Lentils		*Lens culinaris*	28.6	0.8	67.3	0.8

CONCLUSIONS

- Method approaches with Longer agitation, no water addition and SDS addition improve recoveries for 16-23 % of pesticides

- Best results are obtained for methods with no water addition and with SDS addition, with more than 90 % of pesticides with good recoveries

Thank You
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