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

LORENA MANZANO SÁNCHEZ
UNIVERSITY OF ALMERÍA

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.

Step 1: Extraction

Homogenize Food Matrix → Transfer 10 g + 10 mL AcN to extraction tube → Vortex Sample → Add Extraction Salts → Vortex Sample + centrifuge

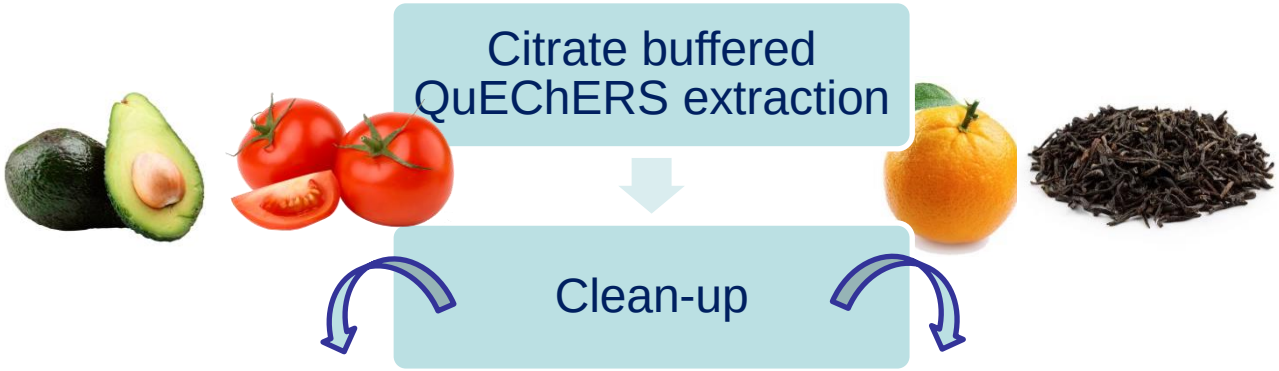
Step 2: Clean up

Transfer supernatant → dSPE or µSPE

dSPE → Vortex dSPE Tube → Centrifuge → Add formic acid 5% in AcN

µSPE → µSPE automated clean-up

How long?

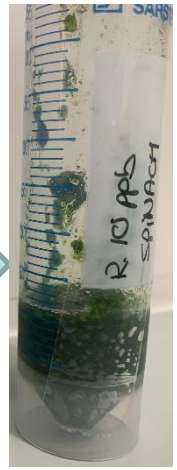


	Clean-up salts for the d-SPE and µSPE QC method	
	d-SPE	**µSPE**
Tomate/Orange	$MgSO_4$ + PSA (6:1) Vortex 30 s Centrifuge 4000 rpm 5 min	Mini cartridges containing 45 mg of $MgSO_4$ + PSA + C18 + CarbonX (20:12:12:1)
Avocado	Z-Sep Vortex 30 s Centrifuge 4000 rpm 5 min	
Black Tea	$CaCl_2$ + PSA (2:1)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

Extraction Step: Citrate QuEChERS

10 g of spinach

10 mL AcN



Agytax

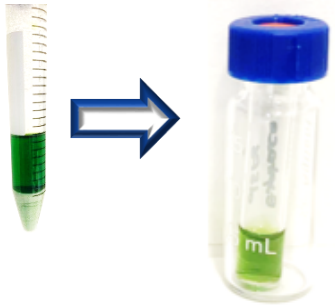
4 g of magnesium sulphate, **1 g** of sodium chloride, **1 g** of trisodium citrate dihydrate and **0.5** g of disodium hydrogen citrate sesquihydrate

Agytax Centrifuge: 3700 rpm (5 min)

Clean up Step

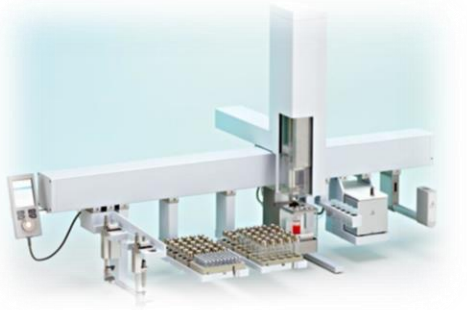
Manual dSPE

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 MgSO4+ 12mg PSA+ 12 mg C18+ 1 mg CarbonX

Automated µSPE

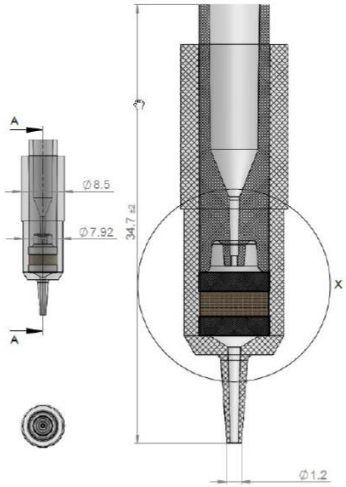


Offline Version

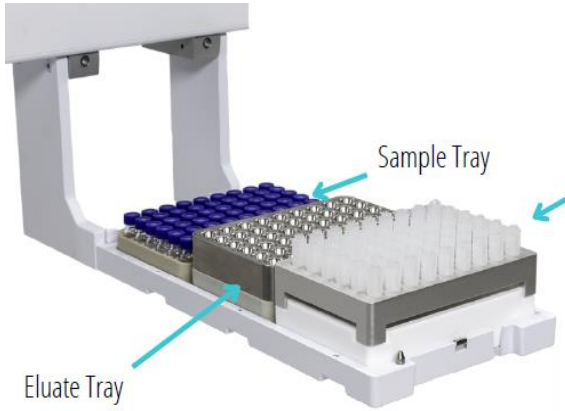
Steps:

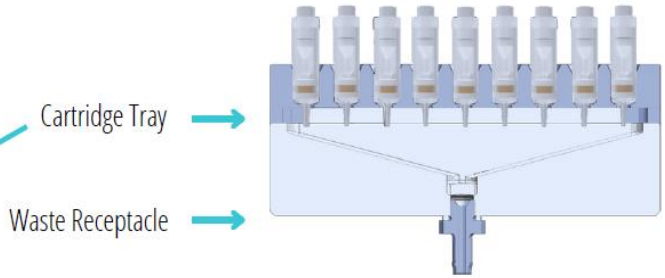
1. Condition µSPE cartridge (100 µL ACN)
2. Elution cartridge step with sample (200 µL)
3. Elution cartridge with AcN (5% formic acid) (100 µL)

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 MgSO4+ 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

PAL vials

Vial with insert

- BM: 200 µL raw blank extract sample + 50 µL AcN
- 10 ppb standard: 200 µL raw blank extract sample + 50 µL AcN (mix 40 ppb)
- Recovery at 10 ppb: 200 µL raw recovery extract sample + 50 µL

Mark a second vial with the same name: it will be the collection vial

Injection vials

- dSPE

BM: 100 µL blank dSPE + 50 µL AcN

10 ppb standard: 100 µL blank + 50 µL AcN (mix 20 ppb)

Recovery at 10 ppb: 100 µL recovery sample + 50 µL AcN

All of them with 400 µL of ultrapure water containing dimethoate-d6 (the injection standard)

Injection vials

- µSPE

BM: 100 µL blank post-PAL + 400 µL ultrapure water (dimethoate-d6)

10 ppb standard: 100 µL standard post-PAL + 400 µL ultrapure water (dimethoate-d6)

Recovery at 10 ppb: 100 µL recovery post-PAL + 400 µL ultrapure water (dimethoate-d6)

With Elution Step

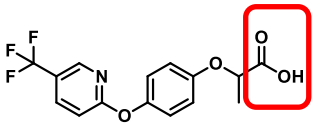
Time (mm:ss)	Steps
0:20	Required tool selected
	Syringe wash (3 cycles)
2:00	**Load 100 µL AcN**
2:30	**Condition µSPE cartridge**
3:30	**Load 200 µL of sample (3 strokes)**
4:00	**Elution cartridge step with sample**
6:00	Syringe wash (3 cycles)
6:30	3 strokes with elution solvent (ACN 5% formic acid)
6:50	**Load 100 µL and elution step**
8:00	Syringe wash (3 cycles)
8:24	Required tool is selected
9:00	Syringe wash (2 cycles)
9:40	Load sample
10:00	1 µL was injected
13:28	Final GC

Results

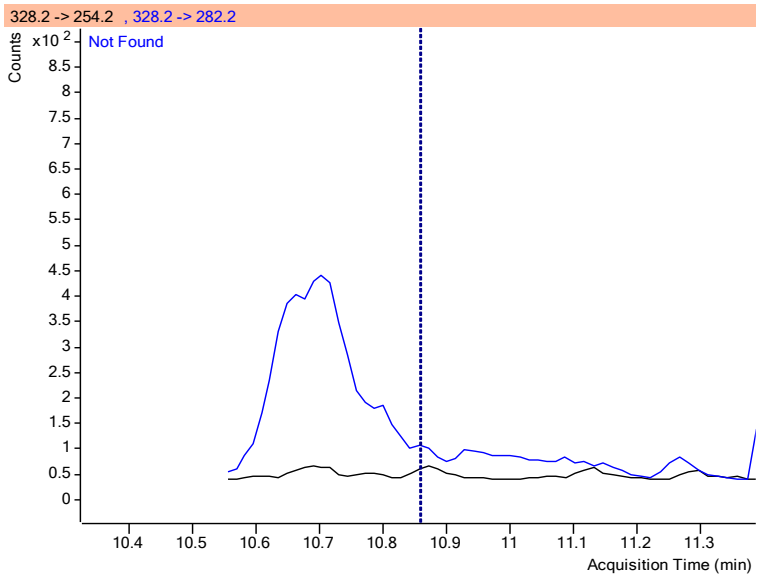
Optimization of PAL µSPE QuEChERS clean-up workflow

Spinach blank extract spiked at 10 ppb

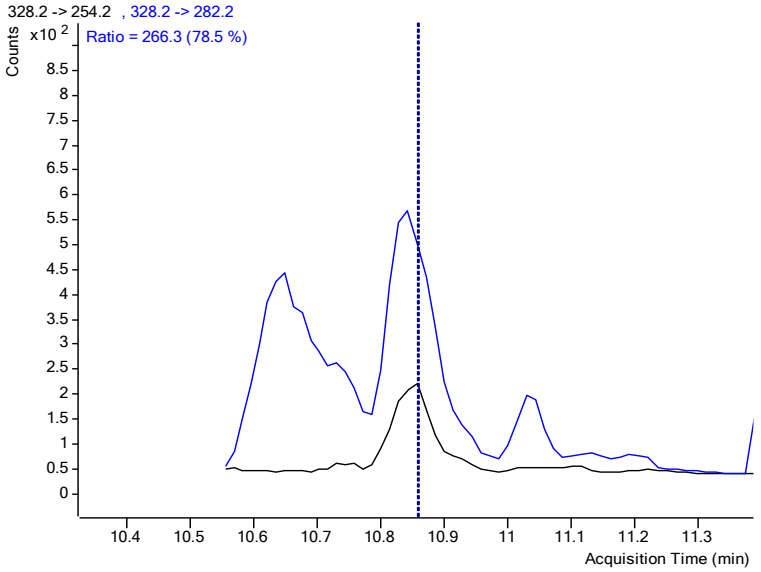
Fluazifop



Without elution step

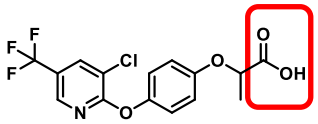


With elution step

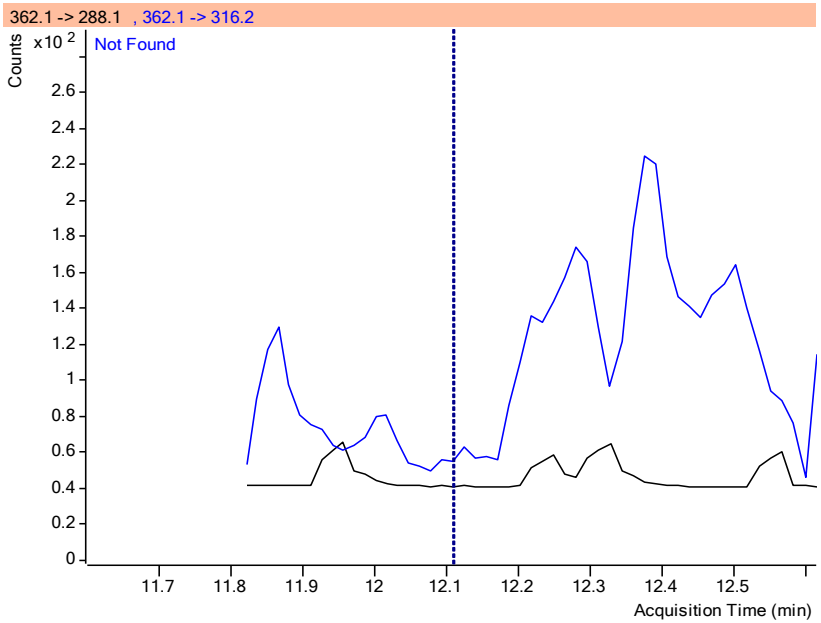


Optimization of PAL µSPE QuEChERS clean-up workflow

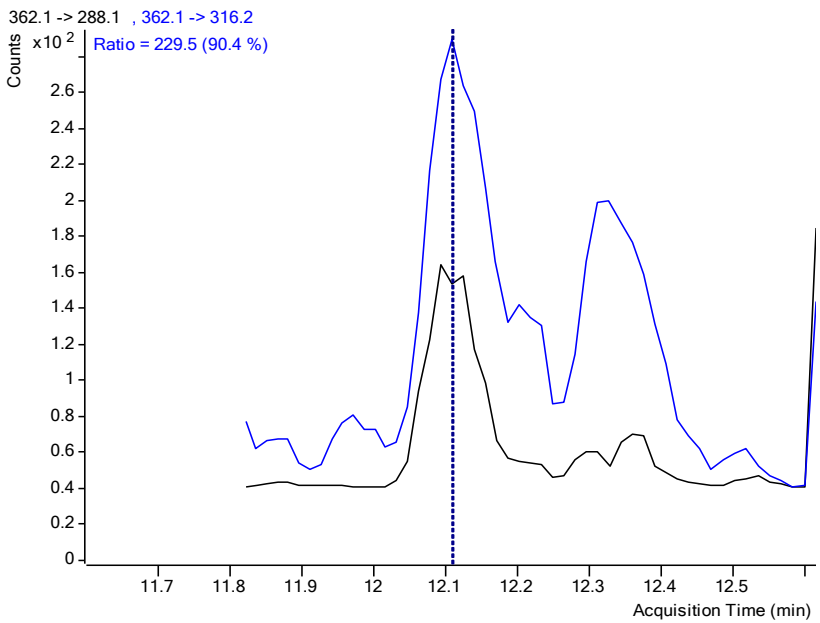
Spinach blank extract spiked at 10 ppb

Haloxyfop 

Without elution step

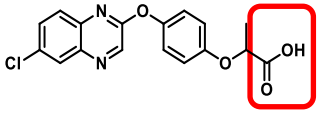


With elution step

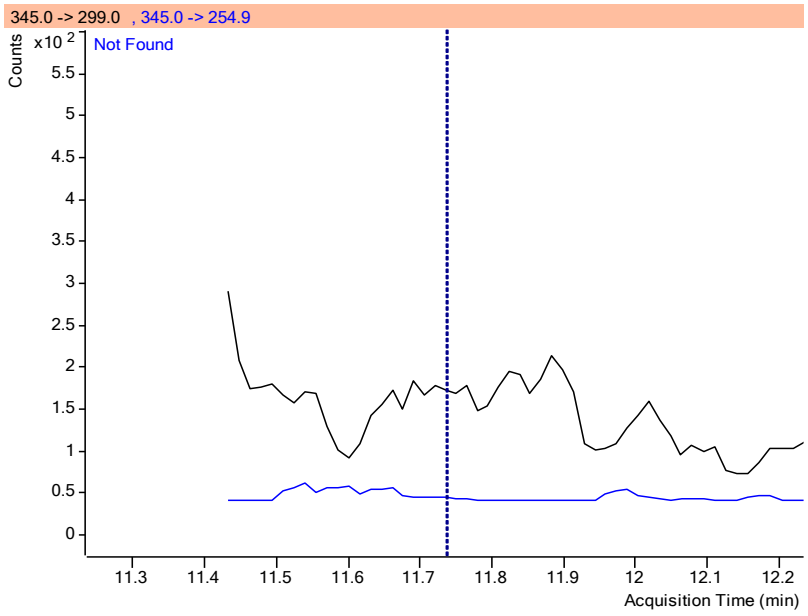


Optimization of PAL µSPE QuEChERS clean-up workflow

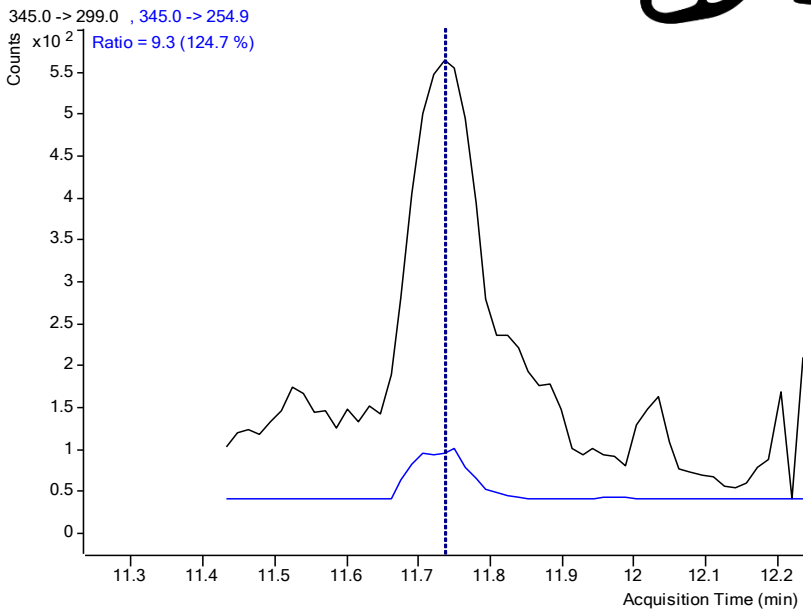
Spinach blank extract spiked at 10 ppb

Quizalofop 

Without elution step



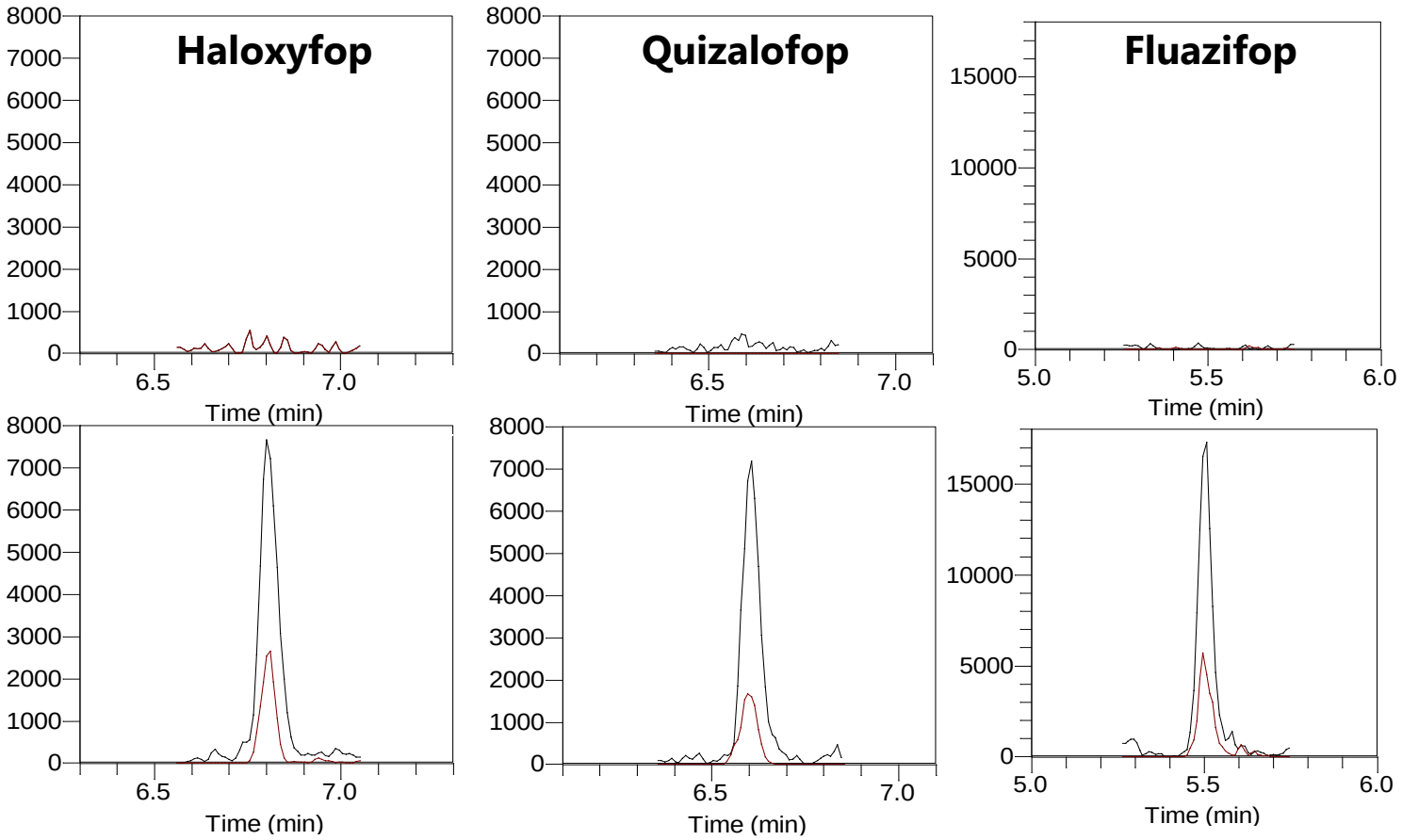
With elution step



Optimization of PAL µSPE QuEChERS clean-up workflow

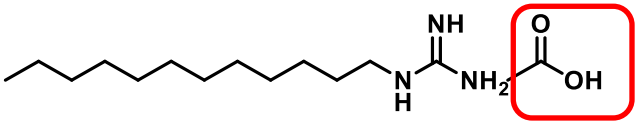
Tomato blank extract spiked at 10 ppb

Triple Quadrupole
TSQ Altis™



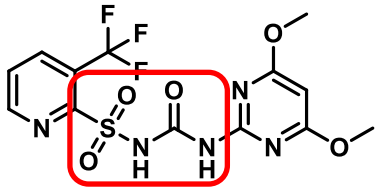
TROUBLESOME COMPOUNDS

Recovery at 10 ppb of pesticide mix					
Compound	**Without elution**	**Acidified Raw QC extract without elution**	**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

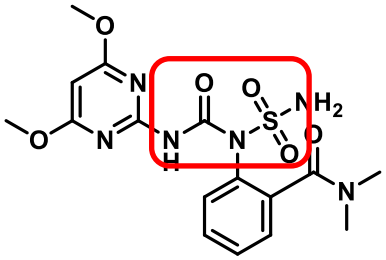


Dodine

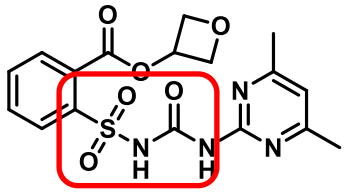
Sulphonylurea group



Flazasulfuron



Orthosulfamuron



Oxasulfuron

Acid Compounds

dSPE clean-up: 750 mg anhydrous $MgSO_4$ + 125 mg PSA

dSPE clean-up: Freezing out step (20 minutes) + 750 mg anhydrous $MgSO_4$ + 125 mg PSA

dSPE clean-up: 125 mg Z-sep

dSPE clean-up: 250 $CaCl_2$ mg + 125 mg PSA

		dSPE				PAL uSPE			
		R 10(%)*	RSD(%)	R 50(%)*	RSD(%)	R 10(%)*	RSD(%)	R 50(%)*	RSD(%)
TOMATO	Quizalofop	14	19	10	10	120	6	112	9
	Fluazifop	16	20	12	4	118	4	105	12
	Haloxyfop	22	11	15	3	119	5	105	10
ORANGE	Quizalofop	28	18	21	6	107	7	107	6
	Fluazifop	31	9	25	6	119	4	106	4
	Haloxyfop	43	18	28	7	109	5	105	4
RICE	Quizalofop	ND	ND	ND	ND	99	3	81	1
	Fluazifop	ND	ND	ND	ND	105	5	90	4
	Haloxyfop	ND	ND	ND	ND	116	4	90	3
AVOCADO	Quizalofop	100	5	81	2	121	5	90	14
	Fluazifop	115	7	90	3	120	4	108	14
	Haloxyfop	120	4	101	5	120	5	111	14
BLACK TEA	Quizalofop	93	5	91	1	116	7	97	2
	Fluazifop	97	3	88	3	105	5	88	2
	Haloxyfop	96	5	89	4	106	4	87	3

*n=3

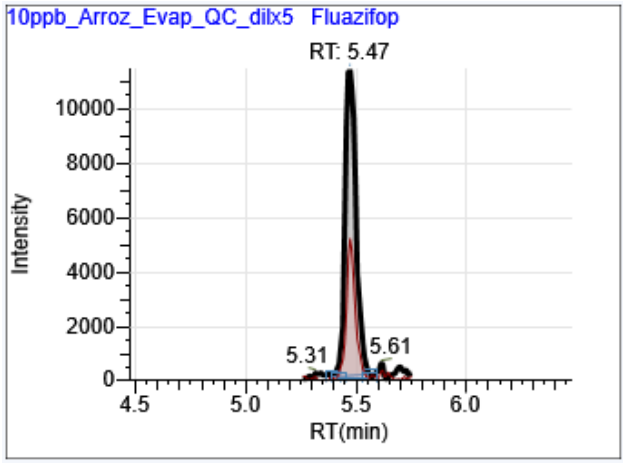
ND: **Not Detected in recovery samples**

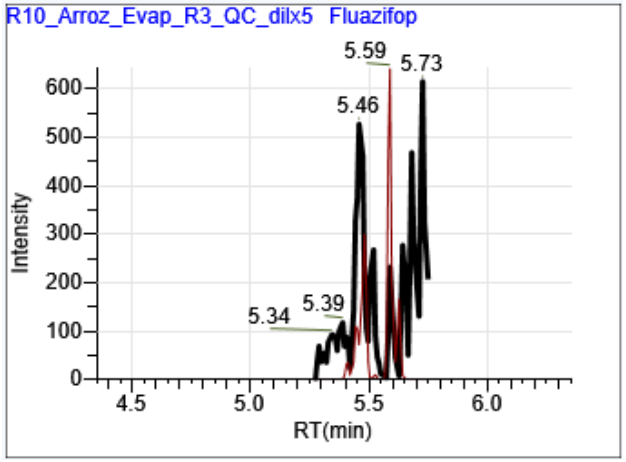
Rice
Fluazifop

Standard at 10 µg/kg in extract

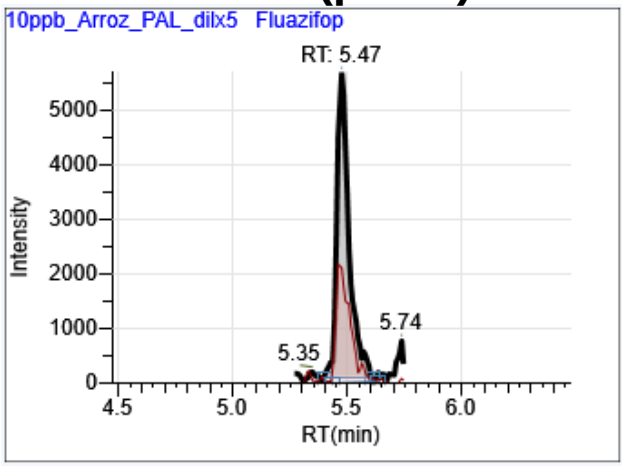
Rice spiked at 10 µg/kg

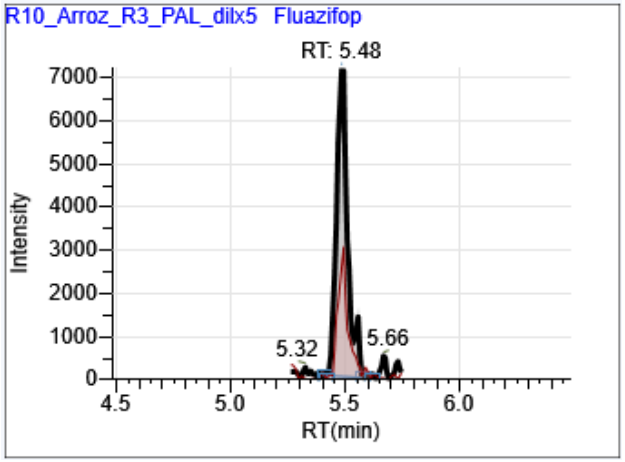
dSPE





PAL (µSPE)



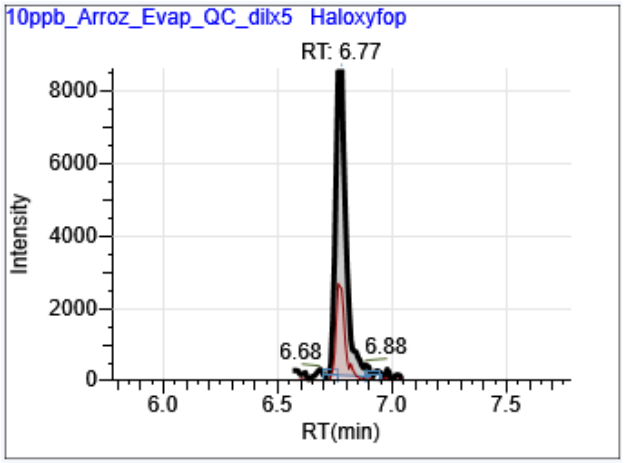


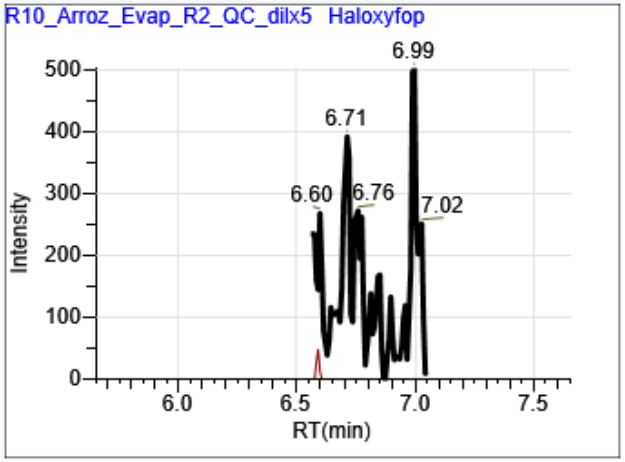
Rice
Haloxyfop

Standard at 10 µg/kg in extract

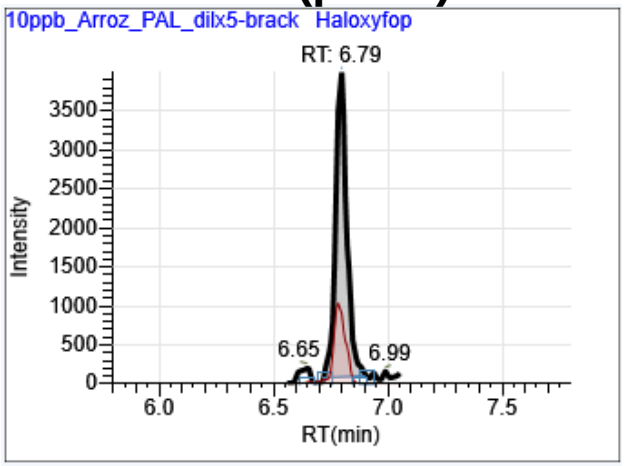
Rice spiked at 10 µg/kg

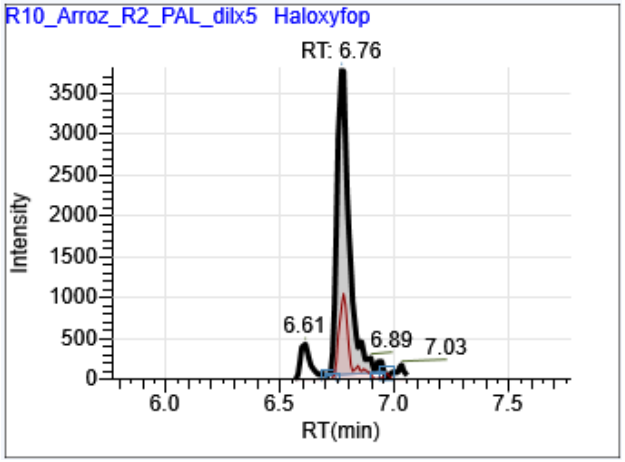
dSPE





PAL (µSPE)



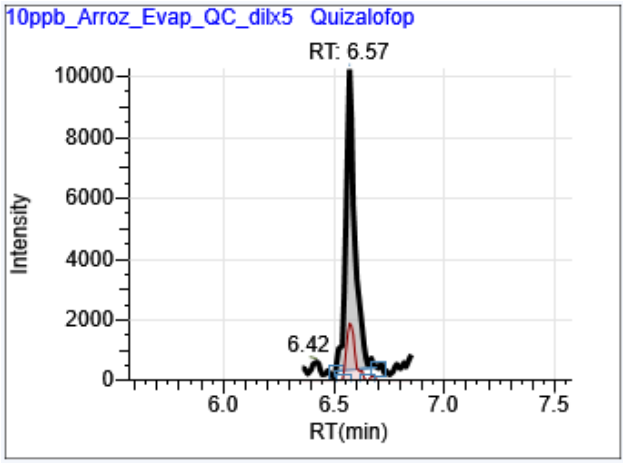


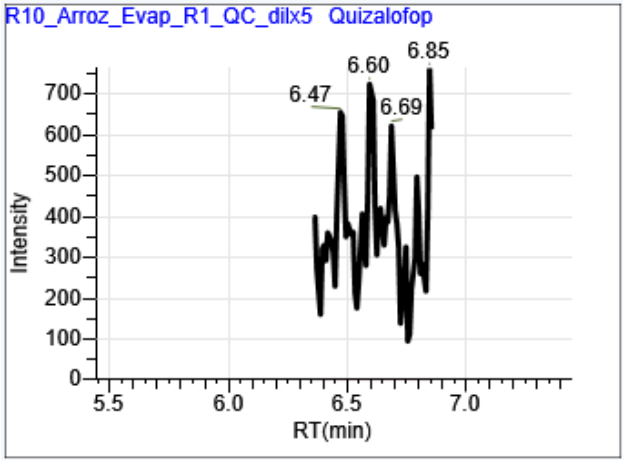
Rice
Quizalofop

Standard at 10 µg/kg in extract

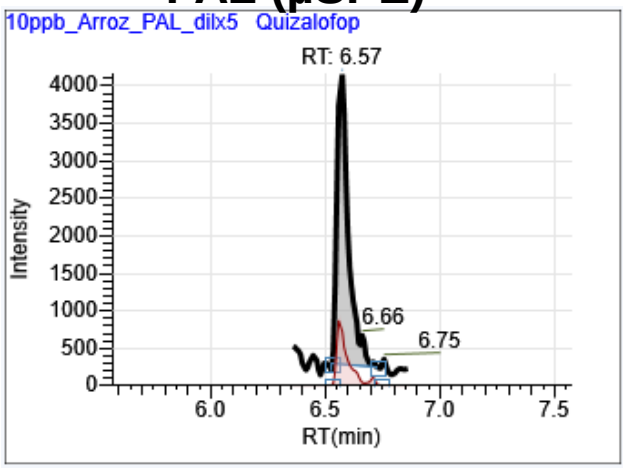
Rice spiked at 10 µg/kg

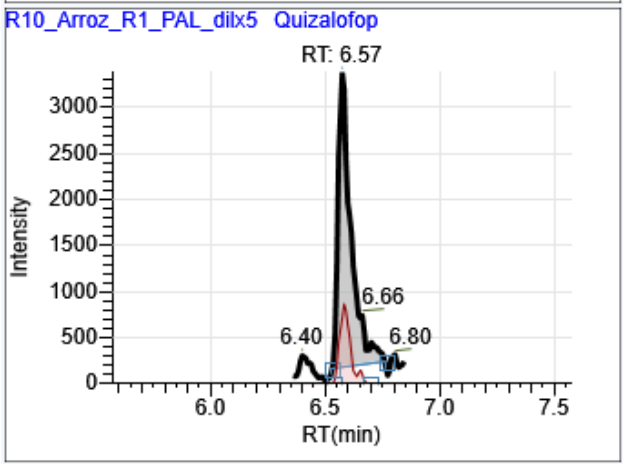
dSPE



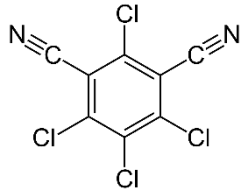


PAL (µSPE)



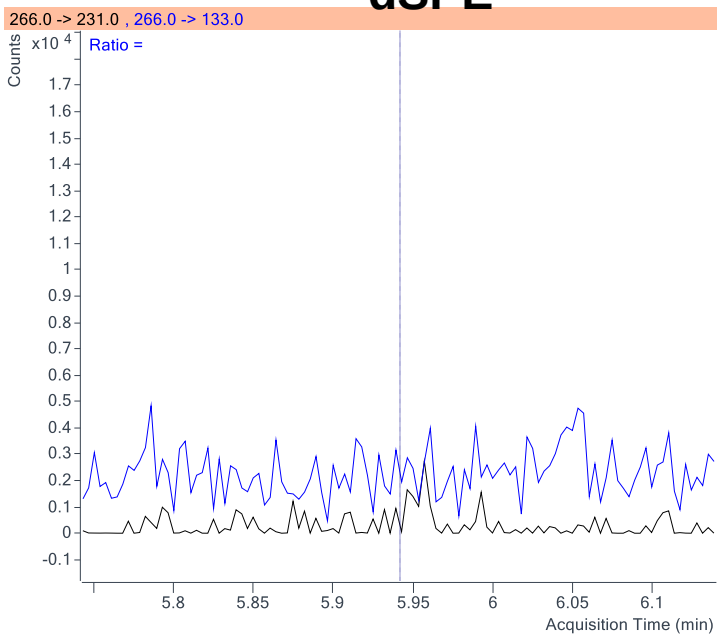


Chlorthalonil



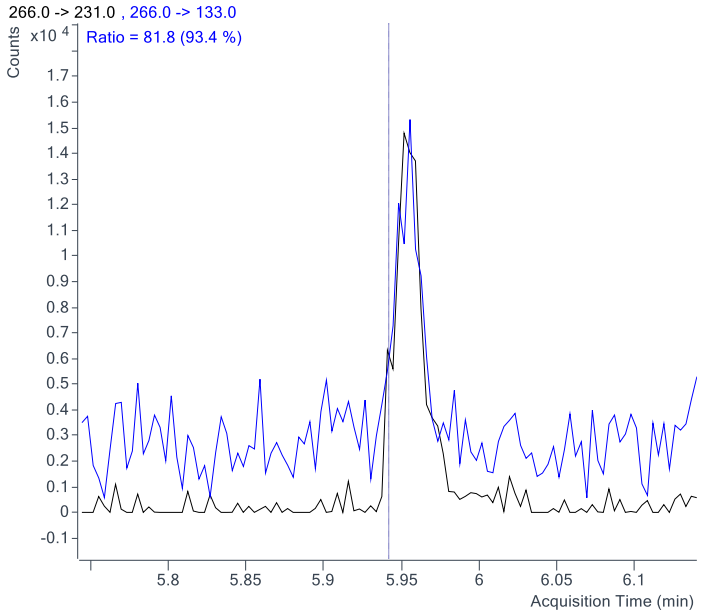
Orange spiked at 10 µg/kg of Chlorthalonil
(Analysis by GC-QqQ)

dSPE



Recovery: 0 %

PAL (µSPE)

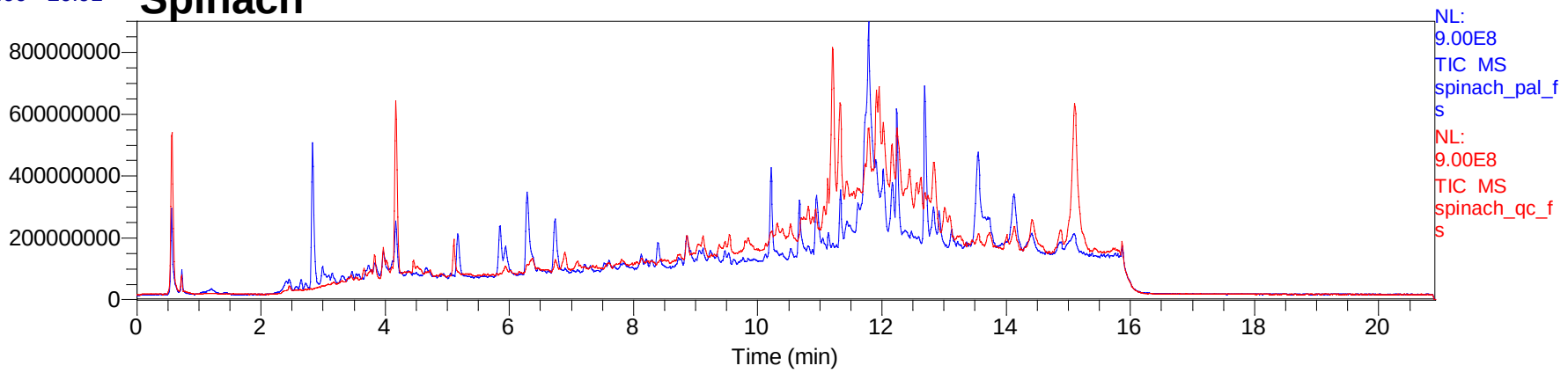


Recovery: 96 %

TIC's comparative (dSPE extract vs µSPE)

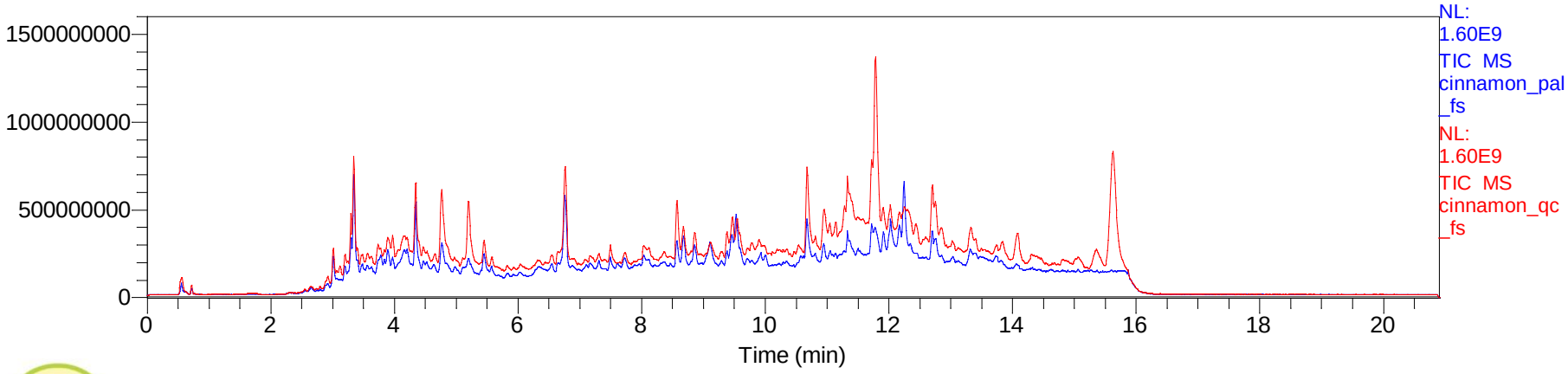
RT: 0.00 - 20.91

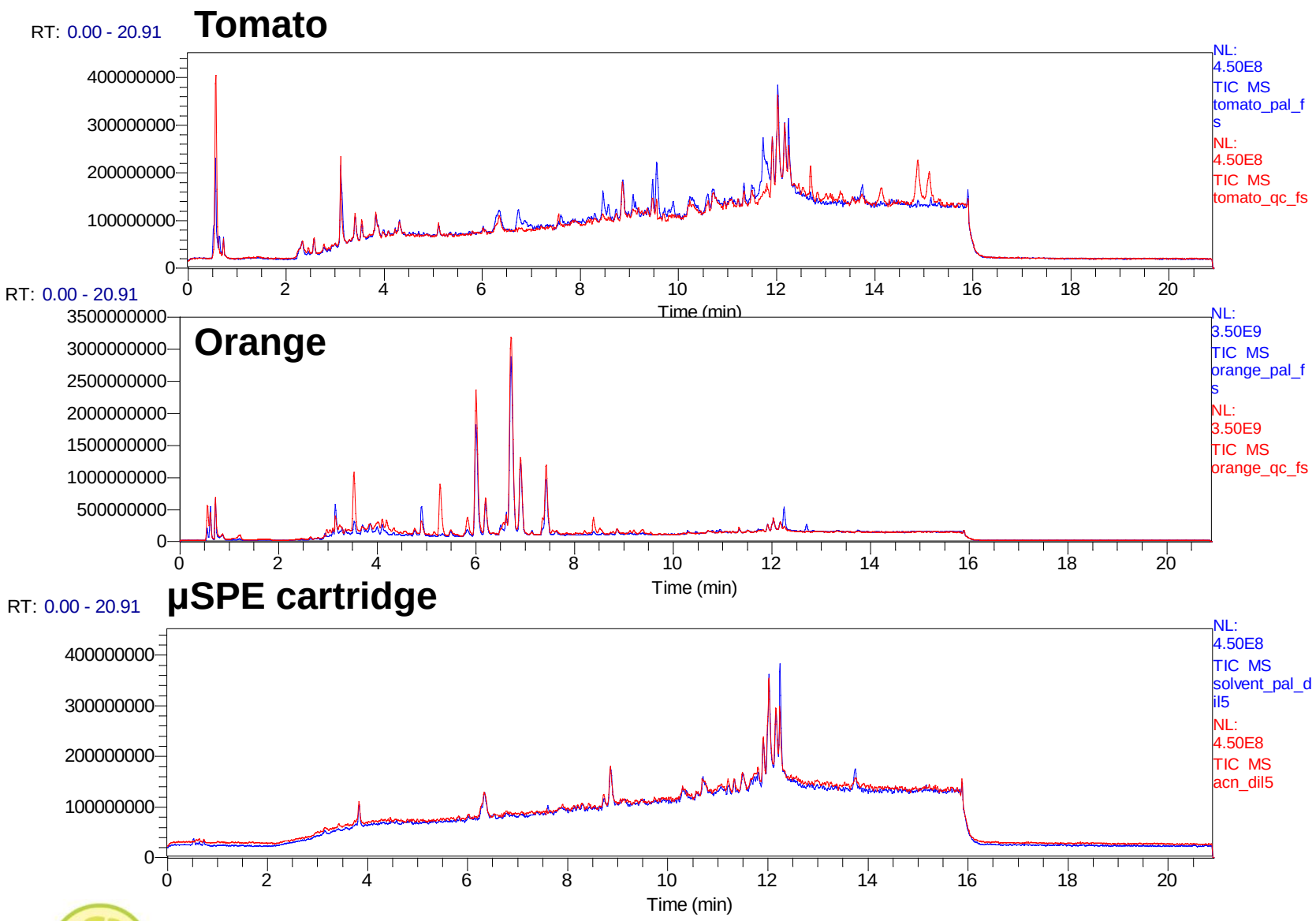
Spinach



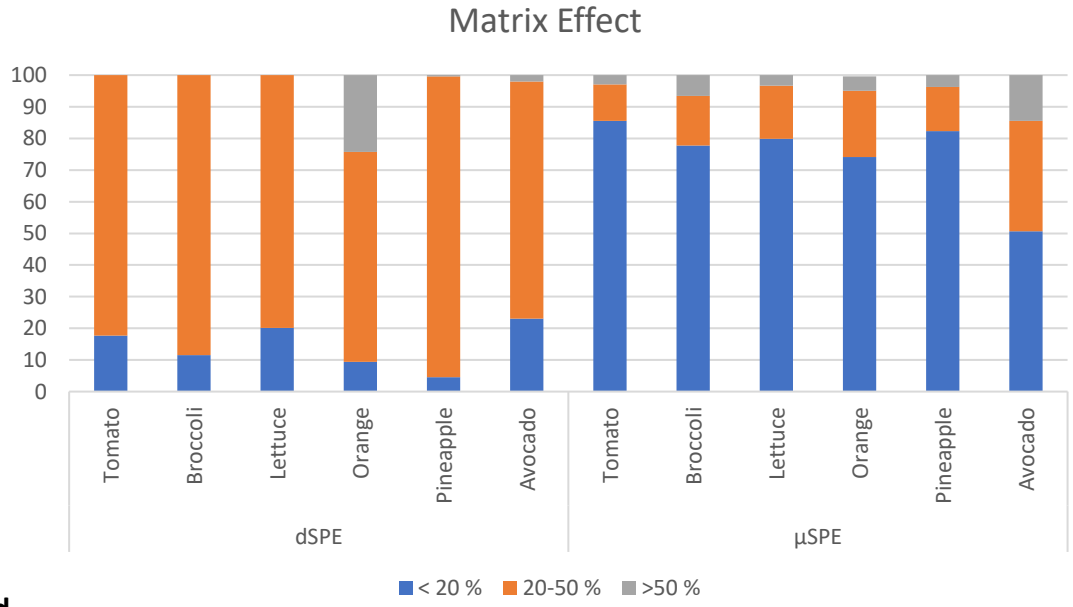
RT: 0.00 - 20.91

Cinnamon



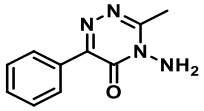

RT: 0.00 - 20.91
Tomato
NL: 4.50E8 TIC MS tomato_pal_fs
NL: 4.50E8 TIC MS tomato_qc_fs
RT: 0.00 - 20.91
Orange
NL: 3.50E9 TIC MS orange_pal_fs
NL: 3.50E9 TIC MS orange_qc_fs
RT: 0.00 - 20.91
µSPE cartridge
NL: 4.50E8 TIC MS solvent_pal_dil5
NL: 4.50E8 TIC MS acn_dil5
Time (min)

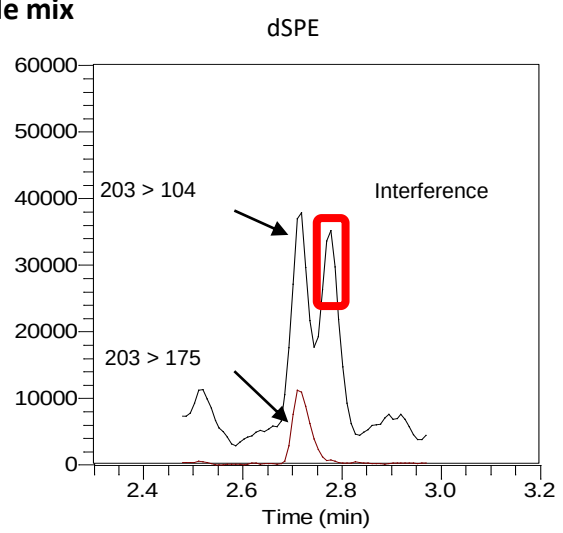
Matrix effect

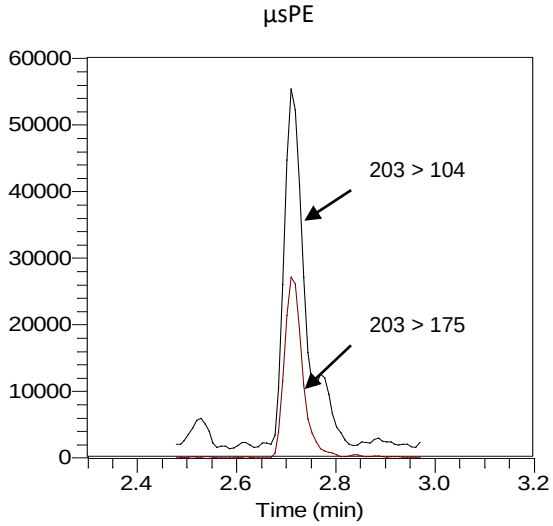


Blank avocado sample spiked at 10 µg L^{-1} with pesticide mix

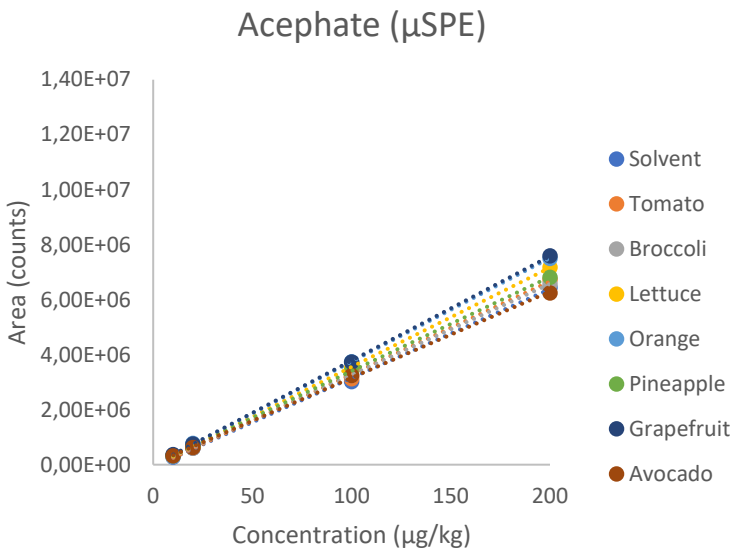
METAMITRON

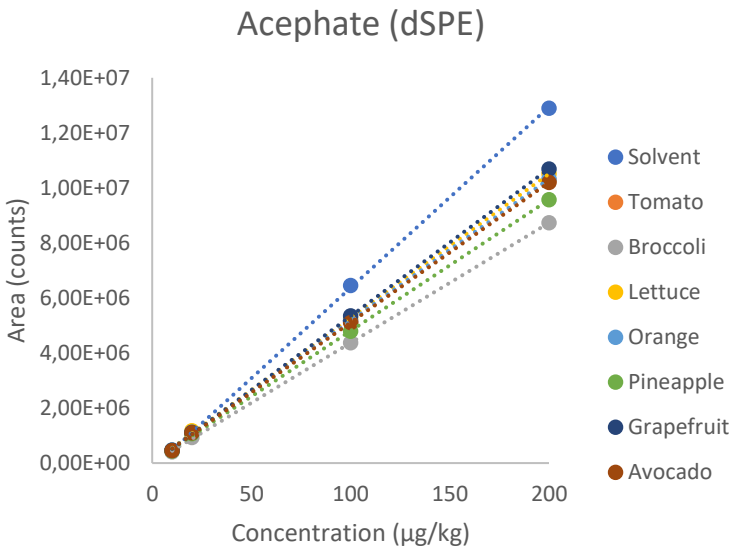




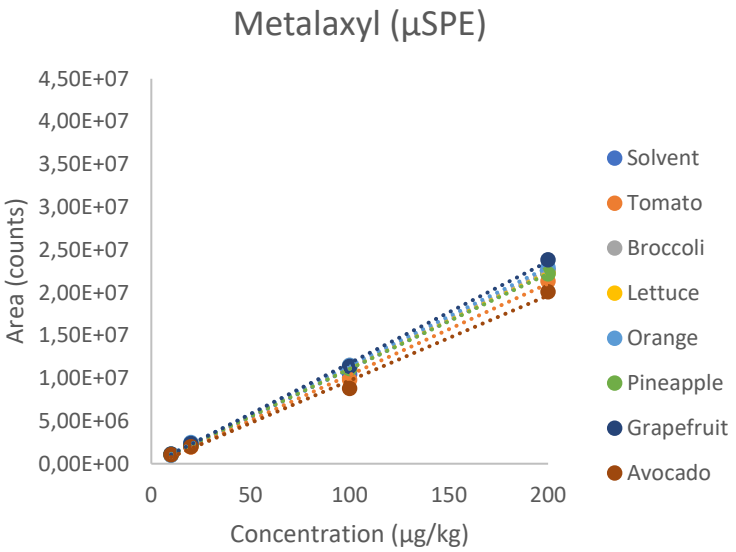


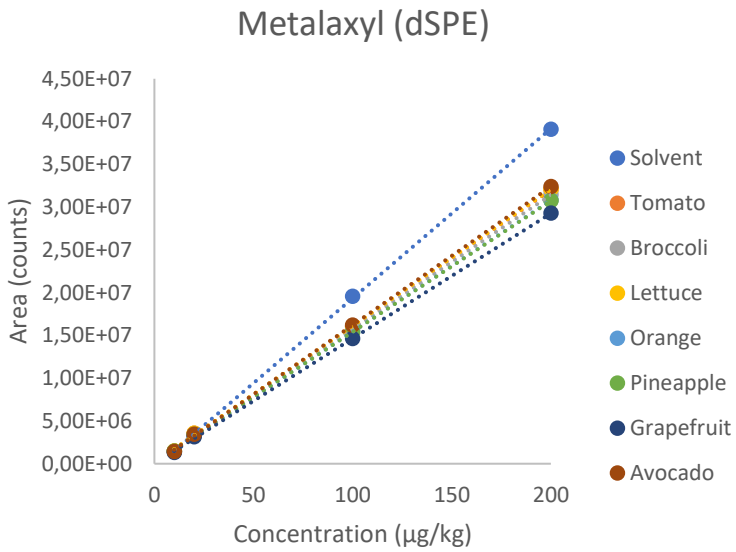
Linearity


Acephate (µSPE)
Area (counts)
1,40E+07
1,20E+07
1,00E+07
8,00E+06
6,00E+06
4,00E+06
2,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

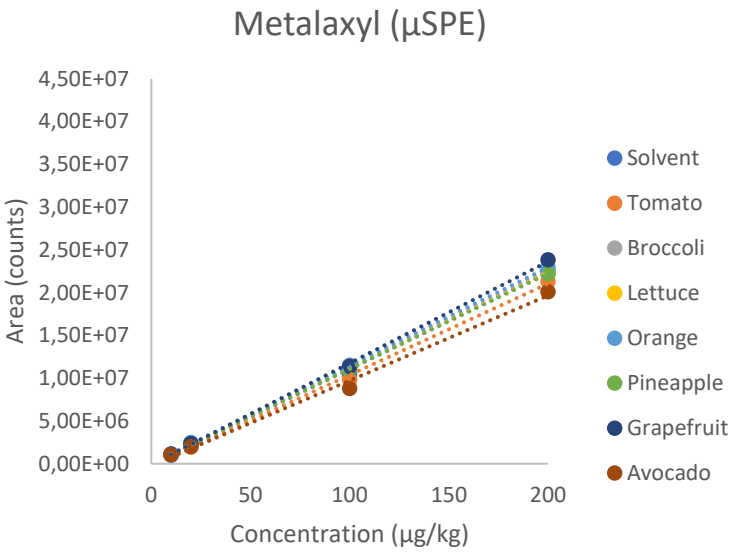

Acephate (dSPE)
Area (counts)
1,40E+07
1,20E+07
1,00E+07
8,00E+06
6,00E+06
4,00E+06
2,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

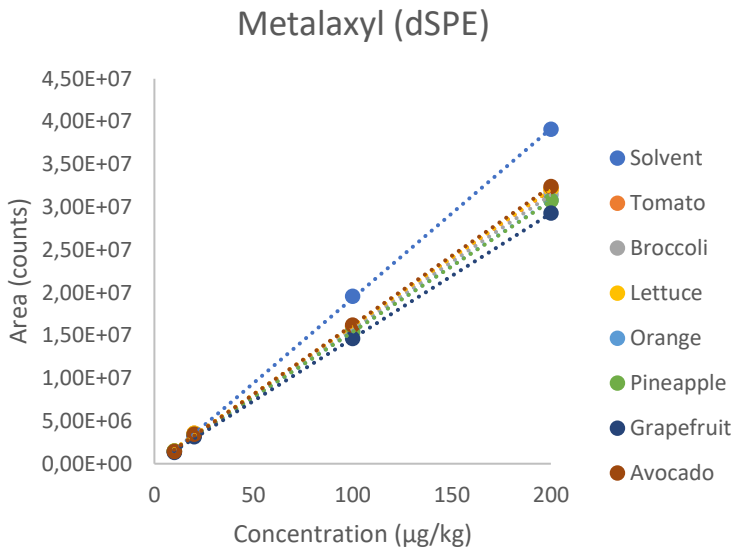
Linearity


Metalaxyl (µSPE)
Area (counts)
4,50E+07
4,00E+07
3,50E+07
3,00E+07
2,50E+07
2,00E+07
1,50E+07
1,00E+07
5,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

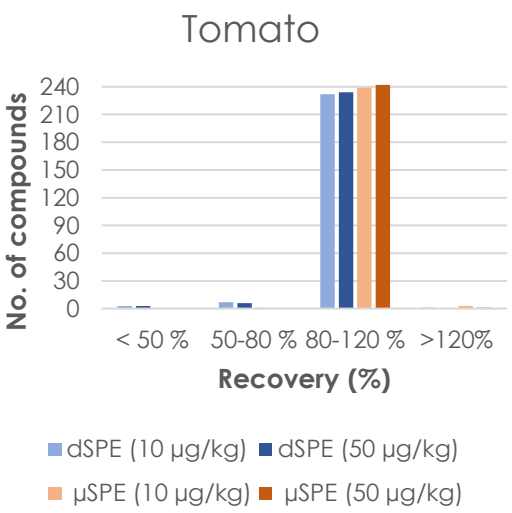

Metalaxyl (dSPE)
Area (counts)
4,50E+07
4,00E+07
3,50E+07
3,00E+07
2,50E+07
2,00E+07
1,50E+07
1,00E+07
5,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

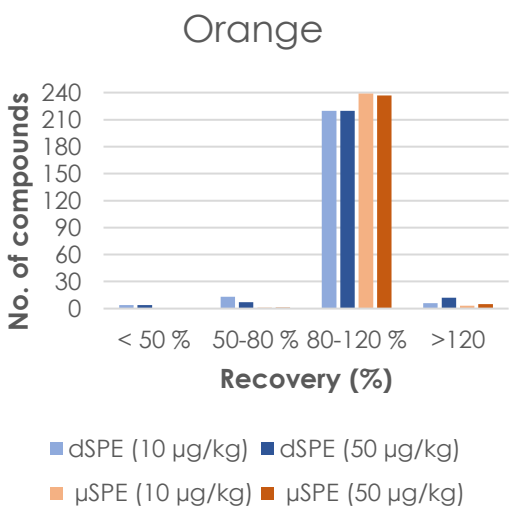
Linearity

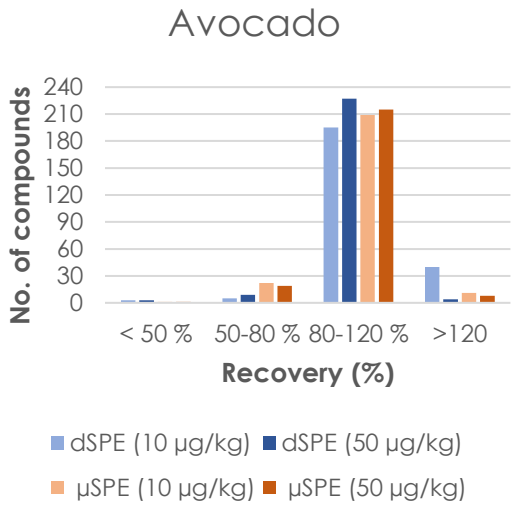

Metalaxyl (µSPE)
Area (counts)
4,50E+07
4,00E+07
3,50E+07
3,00E+07
2,50E+07
2,00E+07
1,50E+07
1,00E+07
5,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

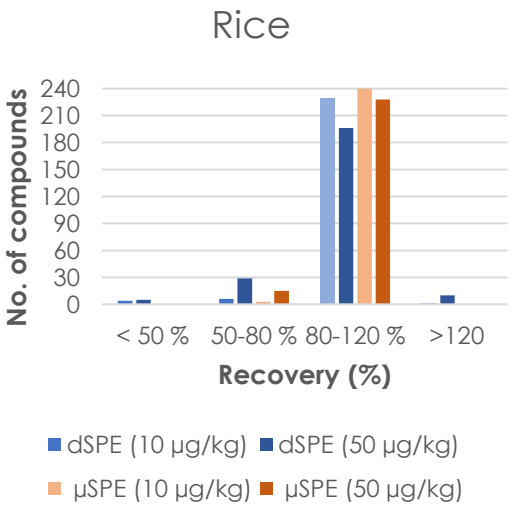

Metalaxyl (dSPE)
Area (counts)
4,50E+07
4,00E+07
3,50E+07
3,00E+07
2,50E+07
2,00E+07
1,50E+07
1,00E+07
5,00E+06
0,00E+00
0
50
100
150
200
Concentration (µg/kg)
Solvent
Tomato
Broccoli
Lettuce
Orange
Pineapple
Grapefruit
Avocado

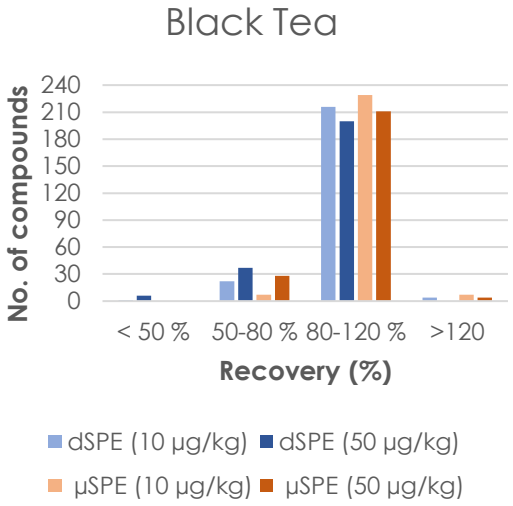
Recoveries


Tomato
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120%
Recovery (%)
dSPE (10 µg/kg)
dSPE (50 µg/kg)
µSPE (10 µg/kg)
µSPE (50 µg/kg)


Orange
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
dSPE (50 µg/kg)
µSPE (10 µg/kg)
µSPE (50 µg/kg)


Avocado
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
dSPE (50 µg/kg)
µSPE (10 µg/kg)
µSPE (50 µg/kg)


Rice
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
dSPE (50 µg/kg)
µSPE (10 µg/kg)
µSPE (50 µg/kg)


Black Tea
No. of compounds
240
210
180
150
120
90
60
30
0
< 50 %
50-80 %
80-120 %
>120
Recovery (%)
dSPE (10 µg/kg)
dSPE (50 µg/kg)
µSPE (10 µg/kg)
µSPE (50 µg/kg)

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.

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.

Thank You
for Your Attention