

TRAINING 2024

ANALYSIS USING AN AUTOMATED μ SPE CLEAN-UP METHOD

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EURL EUROPEAN
UNION
REFERENCE
LABORATORY

PESTICIDES IN FRUITS
AND VEGETABLES

01-10-24 – 02-10-24 ALMERÍA

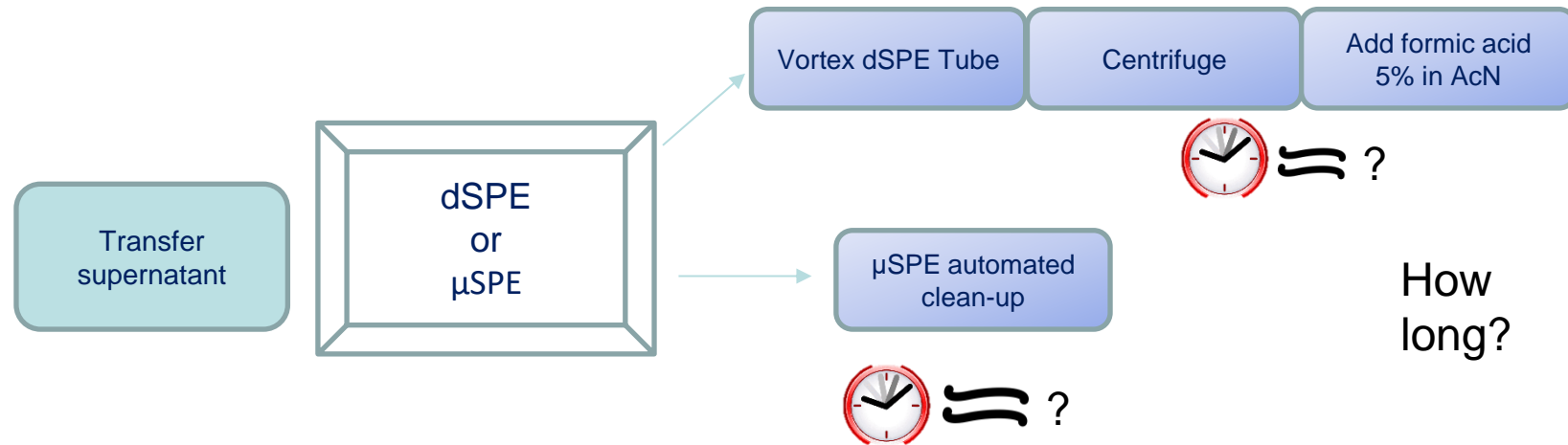
Workflow development for fruit and vegetables

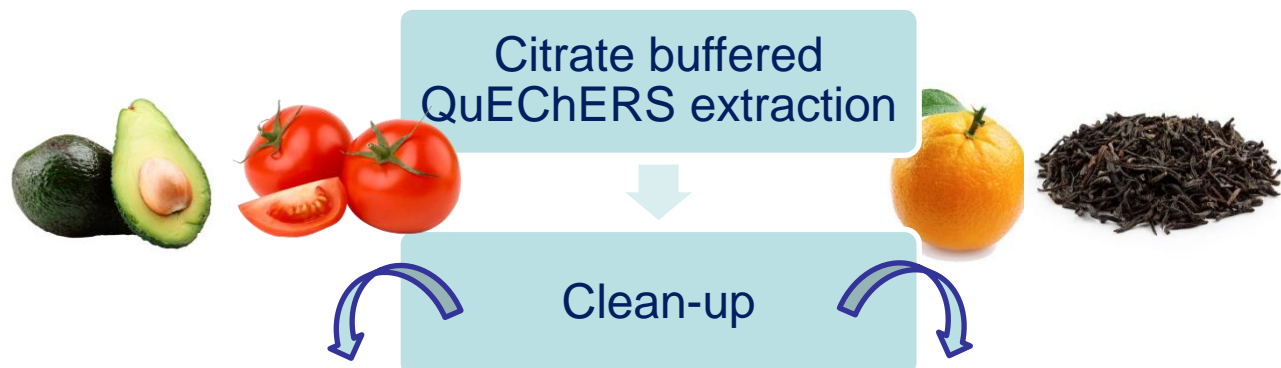
QuEChERS is the quick, easy, cheap effective, rugged and safe sample preparation method originally developed by M. Anastassiades and S.J. Lehotay in 2003. In the original QuEChERS method, acetonitrile is used as extraction solvent, followed by adding NaCl and buffer salts, vortexing and centrifugation.

Step 1: Extraction



Step 2: Clean up

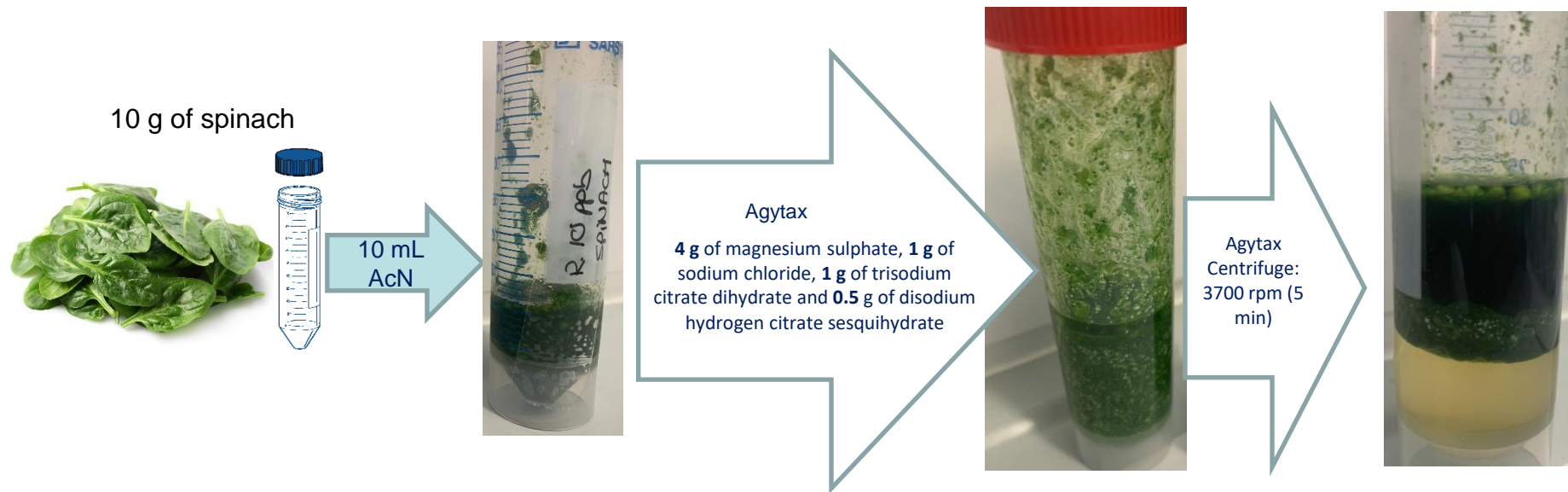




MATRIX	Clean-up salts for the d-SPE and μ SPE QC method	
	d-SPE	μ SPE
Tomato/Orange	<u>750 mg MgSO₄ + 125 mg PSA</u> (6:1) Vortex 30 s Centrifuge 4000 rpm 5 min	Mini cartridges containing 45 mg in total <u>20 mg MgSO₄ + 12 mg PSA + 12 mg C18 + 1 mg CarbonX</u> (20:12:12:1)
Avocado	<u>750 mg MgSO₄ + 175 mg Z-Sep</u> Vortex 30 s Centrifuge 4000 rpm 5 min	
Black Tea	<u>250 mg CaCl₂ + 125 mg PSA</u> (2:1) Vortex 30 s Centrifuge 4000 rpm 5 min	

**Miniaturization
less amount of
sorbents
875 mg ----- 45 mg**

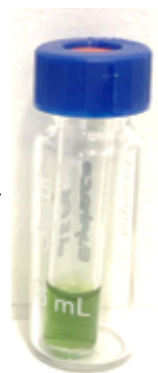
Extraction Step: Citrate QuEChERS



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 5% solution in acetonitrile



µSPE Cartridge:

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

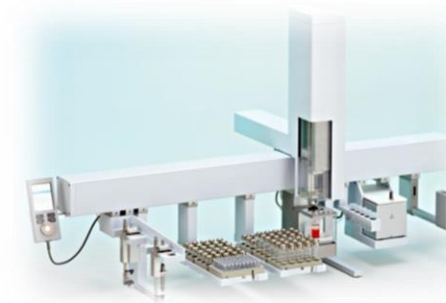
Injection vial:

100 µL of cleaned extract
 +
 400 µL H₂O with dimethoate-d6

Sample
FLOW



Automated µSPE



Off-line Version Steps:

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

Total injected amount: 0.26 mg
 Injection volume 2.5 µL

Total injected amount: 0.5 mg
 Injection volume 2.5 µL

dSPE Clean up

Manual dSPE

Weigh the salts (750 mg of anhydrous magnesium sulphate and 125 mg of PSA) 3:30 min and transfer 5 mL of the supernatant

Vortex for 30 sec.

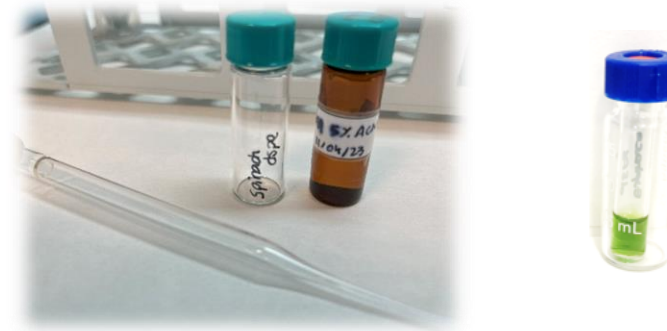
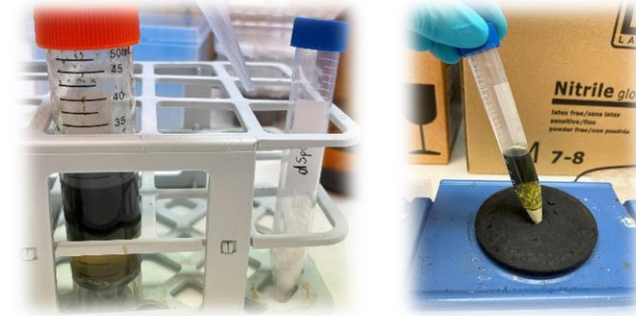
4:00 min

Centrifuge 3700 rpm for 5 min (It actually takes 8 minutes to reach those revolutions and stop.)

12:00 min

supernatant was transferred to a 4-mL vial to which **10 μ L/ mL extract of formic acid solution in acetonitrile (5% volume).**

13:30 min




μ SPE Clean up



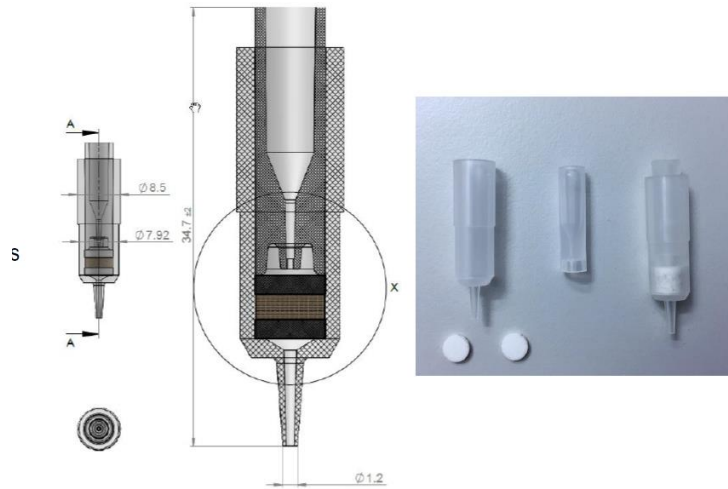
Pre- μ SPE vial

- 200 μ L sample
- 50 μ L ACN
- Calibration curve:
200 μ L blank + 50 μ L of standard mix
at 4 times the level
assessed in the
recovery.



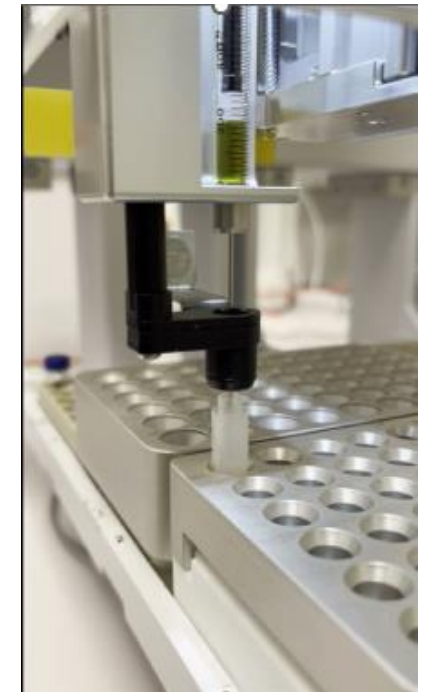
What is the automatic clean up robot like?



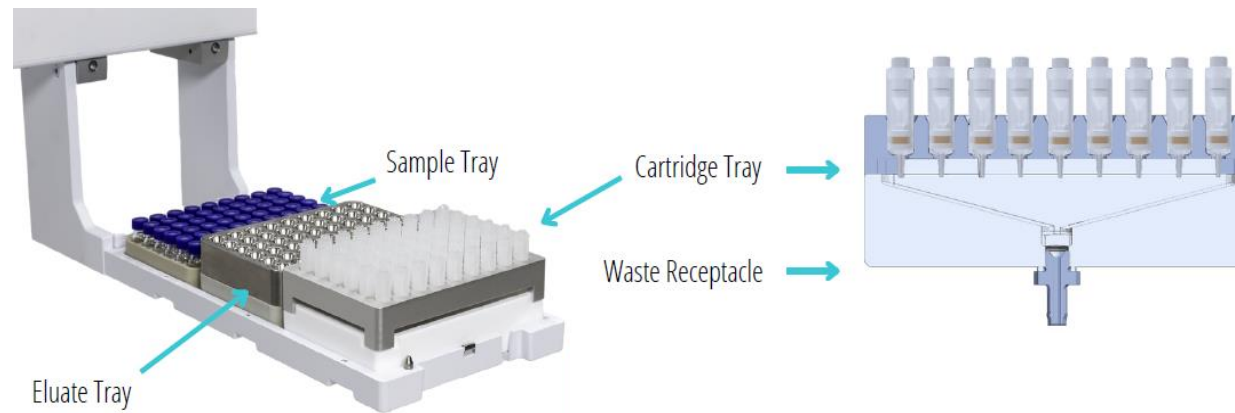


- Small dead volume (< 20µL)
- Wide range of sorbent masses: 5-150 mg
- **Composition: 20 mg Anhydrous MgSO₄+ 12mg PSA+ 12 mg C18+ 1 mg CarbonX**

Syringe passing the sample through the cartridge



Solvent Module



PARAMETERS



Setup

Mode	Offline as Local Script
µSPE Tool	LS 2
AP/Standards Tool	LS 1
Injection Tool	LS 1
Fast Wash Module	HF Fast Wash 1
Use Wash1 during Sample Prep	Yes
Use Wash2 during Sample Prep	No
µSPE Cartridge Tray	Rack 5
Dispose Cartridge	No
Cartridge Waste Container	none
µSPE Elution Tray	Rack 4
Eluate Tray	Rack 2
Bottom Sense	Off

Conditioning

Conditioning Solvent Source	Solvent Module
Conditioning Solvent Index	1
Conditioning Solvent Volume	100 µL
Conditioning Solvent Fill Speed	100 µL/s
Conditioning Solvent Loading Speed	5 µL/s



PARAMETERS

Sample μ SPE

μ SPE Sample Load Location	At Elution Tray
μ SPE Sample Load Volume	200 μ L
μ SPE Sample Fill Speed	100 μ L/s
μ SPE Sample Loading Speed	2 μ L/s

Wash μ SPE

Wash μ SPE Solvent Source	none
Wash μ SPE Solvent Index	2
Wash μ SPE Volume	0 μ L
Wash μ SPE Solvent Fill Speed	10 μ L/s
Wash μ SPE Solvent Loading Speed	2 μ L/s

Elution

Elution Solvent Source	Solvent Module
Elution Solvent Index	2
Elution Volume	100 μ L
Elution Solvent Fill Speed	100 μ L/s
Elution Solvent Loading Speed	2 μ L/s

Transfer Eluate to 2mL Vial

Transfer Tray	none
Transfer Eluate Volume	0 μ L

PARAMETERS



Protectants

Protectant Source	none
Protectant Index	3
Protectant Volume	0 μL
Protectant Fill Speed	5 $\mu\text{L/s}$

Internal Standard

Internal Standard Source	none
Internal Standard Index	1
Internal Standard Volume	0 μL
Internal Standard Fill Speed	1 $\mu\text{L/s}$

Target Standard

Target Standard Source	none
Target Standard Index	2
Target Standard Volume	0 μL
Target Standard Fill Speed	1 $\mu\text{L/s}$

Solvent Addition

Solvent Source	none
Solvent Index	3
Solvent Volume	0 μL
Solvent Fill Speed	5 $\mu\text{L/s}$

PARAMETERS

⌵ Mixing

Enable Mixing Step	No	
Syringe Mixing Cycles	0	
Syringe Mixing Volume	300	μL
Vortex Mixer	none	
Vortex Mixer Speed	2000	rpm
Vortex Mixing Time	15	s

⌵ Sandwich Injection

Sandwich Injection	No	
Sandwich Injection Top Layer Source	none	
Sandwich Injection Top Layer Index	1	
Rear Air Gap Volume	0	μL
Sandwich Injection Top Layer Volume	0	μL
Sandwich Injection Air Gap Volume	0	μL
Sample Volume	1	μL
Sample Air Gap Volume	1	μL
Rinse Cycles	1	
Filling Strokes Count	6	
Sample Vial Penetration Depth	32	mm

⌵ Injection

Injection Mode	Normal	
Injector Penetration Depth	45	mm
Pre-Inject Delay	5	s
Post-Inject Delay	2	s
Syringe Wash Mode	Pre- & PostWash	
Syringe Wash Cycles	3	
Solvent Selection Wash Step 1	Solvent1	
Solvent Selection Wash Step 2	Solvent2	
Analysis Time	50	min

BATCH

The screenshot shows the PALScript Executor software interface. The main window has a menu bar with 'PAL3', 'Method', 'Sample List', 'Job List', and 'Controls'. Below the menu bar is a toolbar with various icons. The main area contains a table with columns: Method, Sample Rack, Index, and Sample Parameter. A 'Vial Selection' dialog box is open, showing three tray holders (Tray Holder 1, 2, and 3). Each tray holder contains a 6x9 grid of vial positions, numbered 1 to 54. Below the grids, there are five 'Injection Order Mode' options: '1:2:3 in Sequence' (selected), 'by Column', 'by Row', 'by shortest X', and 'by shortest Y'. The 'Ok' and 'Cancel' buttons are at the bottom right of the dialog. At the bottom of the main window, there is a status bar with the following information: Connection State: Connected to 192.168.99.230 (GC1) - V3.1.25; Driver State: Ready; Status Message: ; Valve Status: ; PaScriptExecutors's current Lock: None.

Remaining Time: 00:05:57 | Duration: 00:00:00 | Nr Samples: 1

The PAL μ SPE QuEChERS clean-up workflow

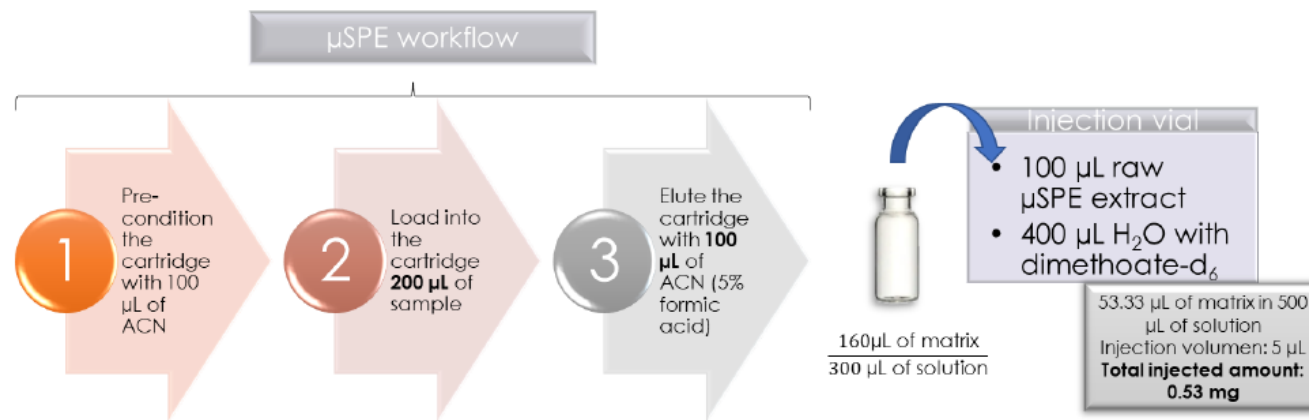
Pre- μ SPE vial

- 200 μ L sample
- 50 μ L ACN
- Calibration curve: 200 μ L blank + 50 μ L of standard mix at 4 times the level assessed in the recovery.

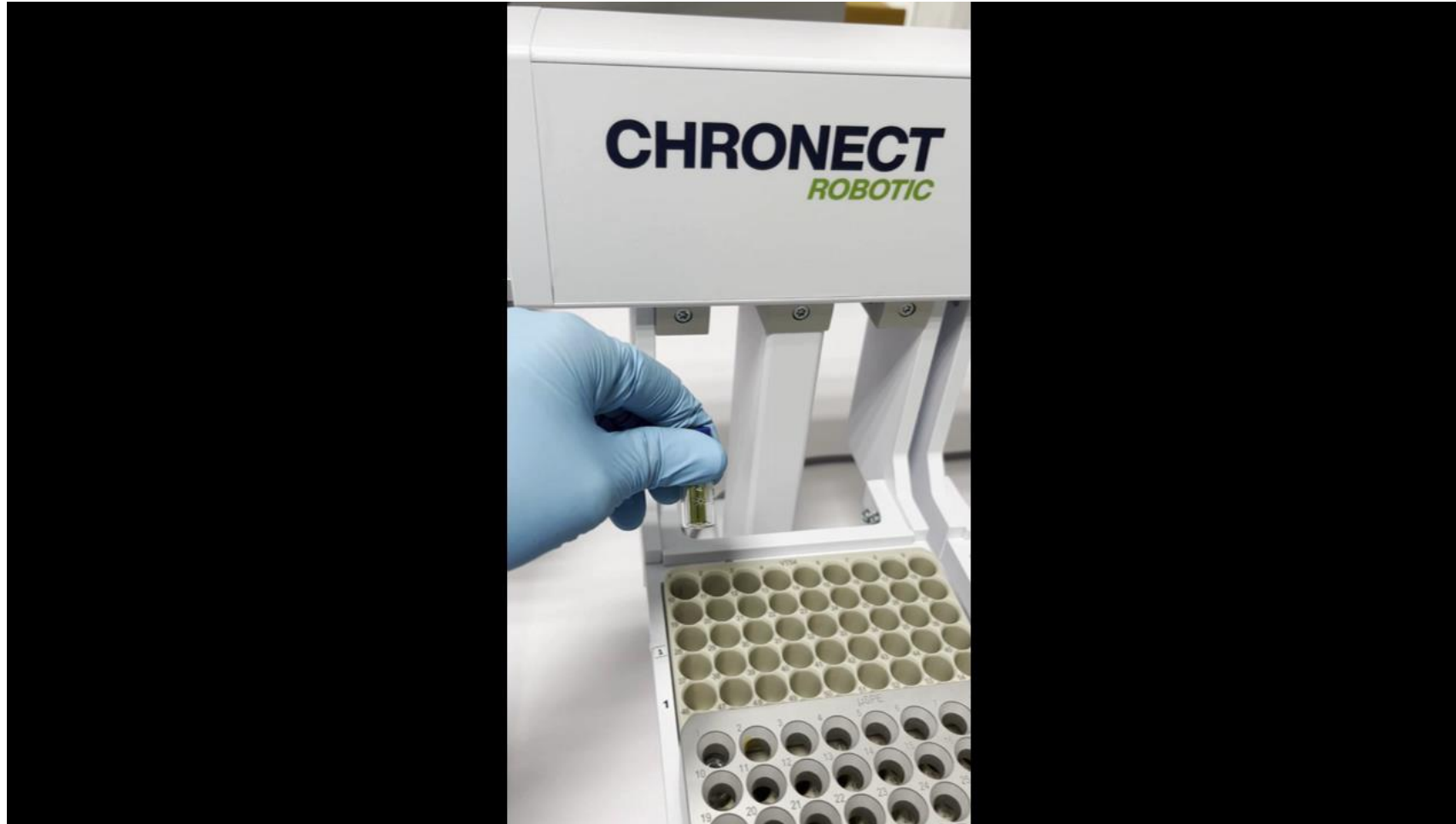
Steps	
Conditioning	Syringe wash (3 cycles) with AcN
	Load 100 μL of AcN
	Condition μ SPE cartridge with ACN (Waste)
Load 200 μL of diluted sample (200 μL raw extract + 50 μL AcN) (Collection Vial)	
Elution	Load 100 μL of AcN (5% formic acid)
	Elution step (Collection Vial)

Pre- μ SPE vial

- 200 μ L sample
- 50 μ L ACN
- Calibration curve: 200 μ L blank + 50 μ L of standard mix at 4 times the level assessed in the recovery.



WORKFLOW



Optional step



LC-QqQ-MS/MS Parameters

Column: Accucore C₁₈
2.1 x 100 mm,
particle size 2.6 µm

Column temperature: 30°C

Flow rate: 0.350 ml/min

Injection volume: 2.5 µL

Autosampler temperature: 10°C



Triple Quadrupole
TSQ Altis™

Ion spray voltage (+): 3500 V

Ion spray voltage (-): 2500 V

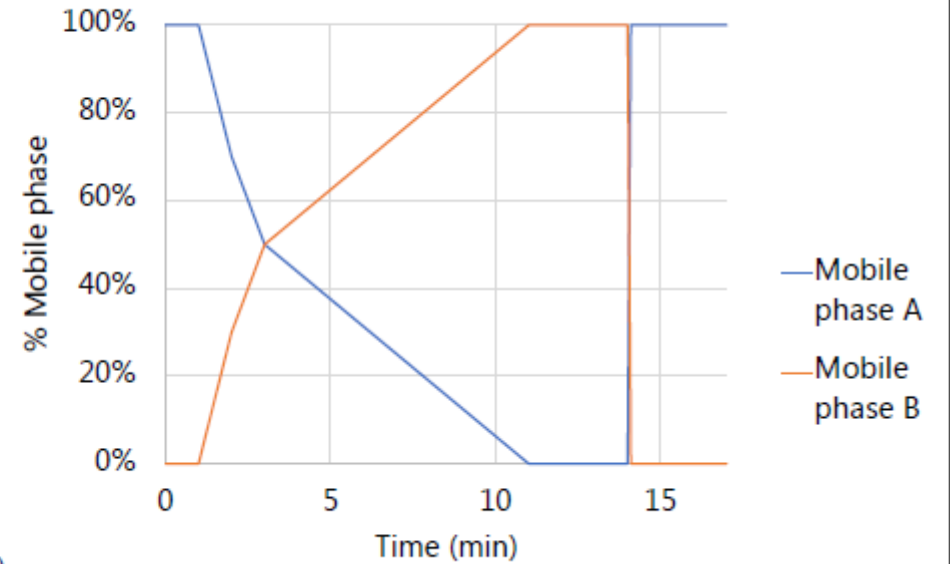
Sweep gas: 1 (arbitrary units)

Ion transfer tube: 325 °C

Vaporiser temp.: 350 °C

Working mode: SRM

- 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)



243 compounds

Results

The PAL μ SPE QuEChERS clean-up workflow

With Elution Step

⊖ **Sample μ SPE**

μ SPE Sample Load Location	At Elution Tray	
μ SPE Sample Load Volume	200	μ L
μ SPE Sample Fill Speed	100	μ L/s
μ SPE Sample Loading Speed	2	μ L/s

⊖ **Wash μ SPE**

Wash μ SPE Solvent Source	none	
Wash μ SPE Solvent Index	2	
Wash μ SPE Volume	0	μ L
Wash μ SPE Solvent Fill Speed	10	μ L/s
Wash μ SPE Solvent Loading Speed	2	μ L/s

⊖ **Elution**

Elution Solvent Source	Solvent Module	
Elution Solvent Index	2	
Elution Volume	100	μ L
Elution Solvent Fill Speed	100	μ L/s
Elution Solvent Loading Speed	2	μ L/s

⊖ **Transfer Eluate to 2mL Vial**

Transfer Tray	none	
Transfer Eluate Volume	0	μ L



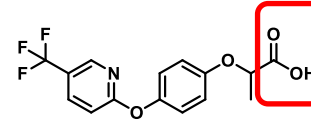
Without Elution Step

Optimization of PAL μ SPE QuEChERS clean-up workflow



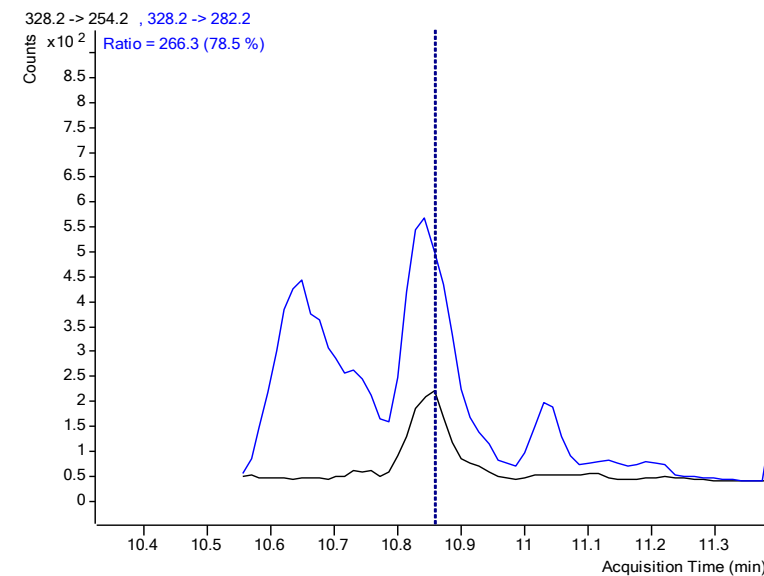
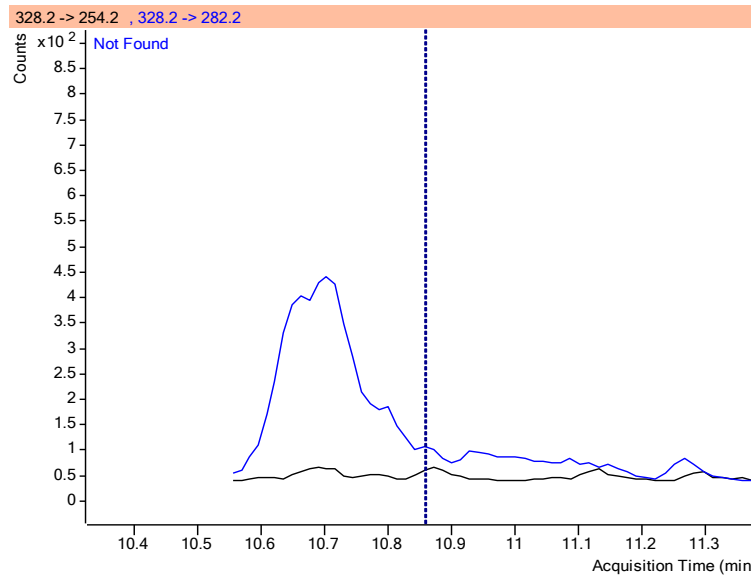
Spinach blank extract spiked at 10 ppb

Fluazifop



Without elution step

With elution step

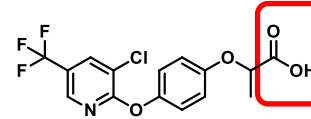


Optimization of PAL μ SPE QuEChERS clean-up workflow

Spinach blank extract spiked at 10 ppb

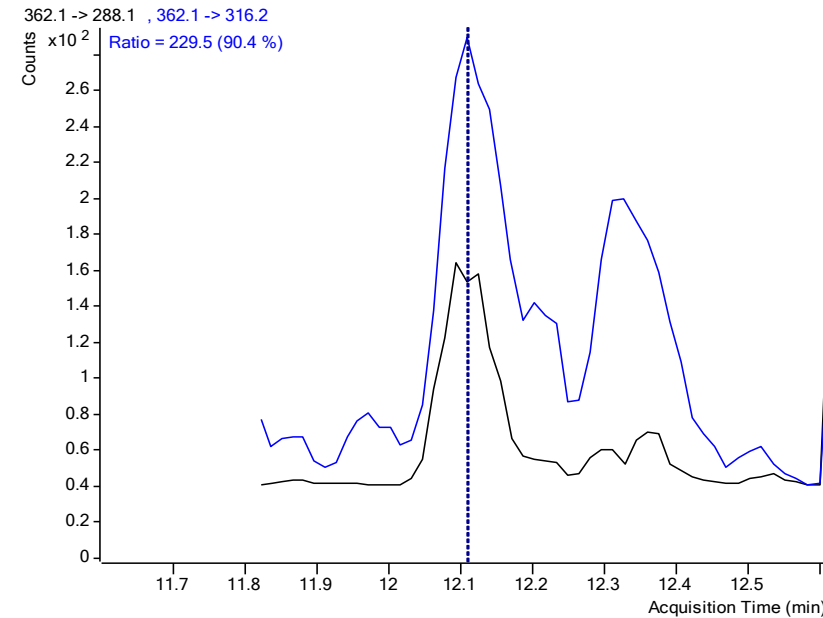
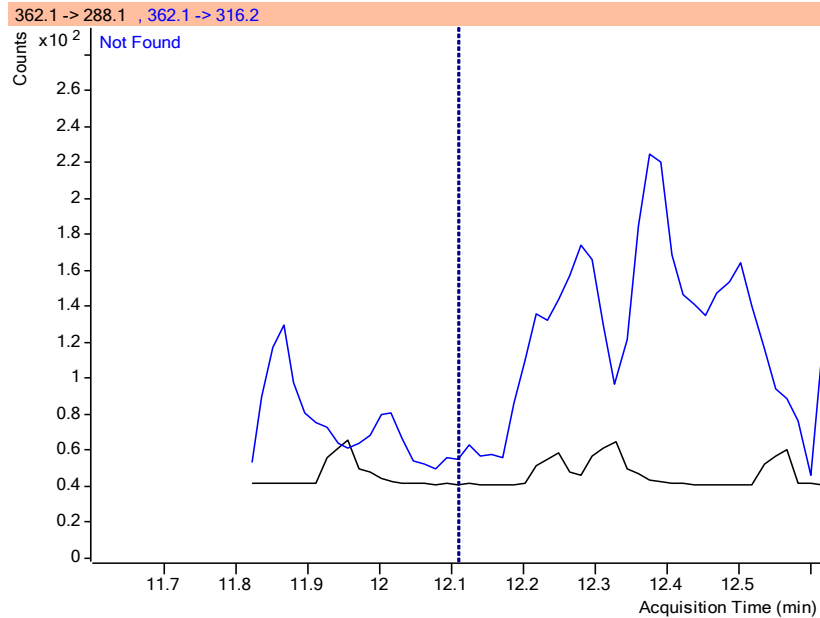


Haloxyfop



Without elution step

With elution step

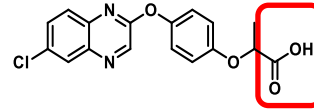


Optimization of PAL μ SPE QuEChERS clean-up workflow



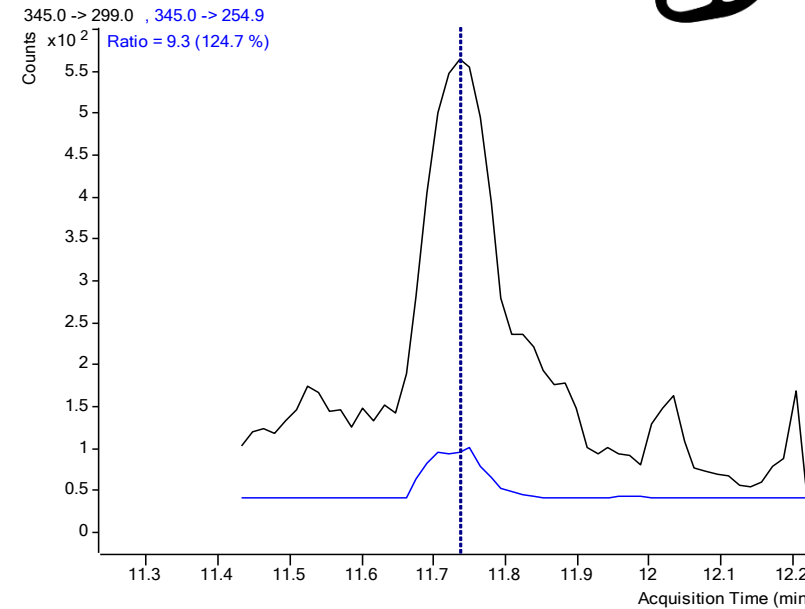
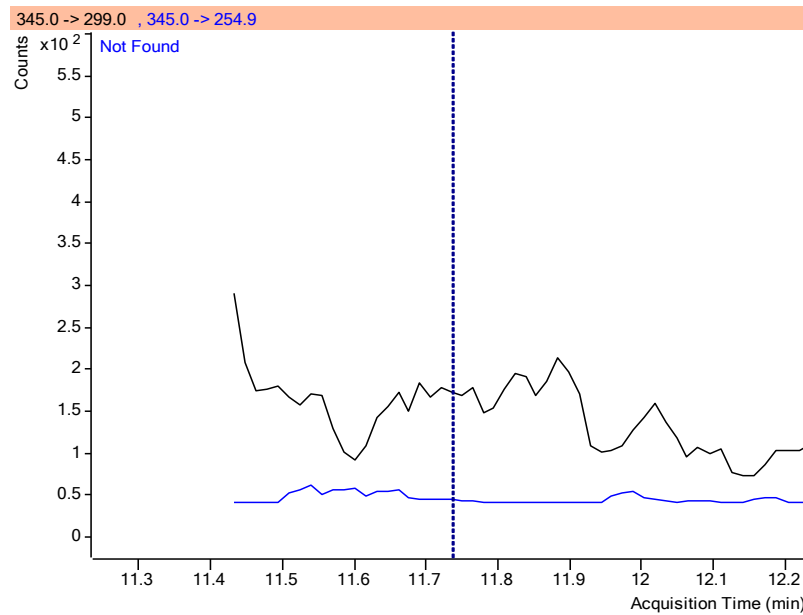
Spinach blank extract spiked at 10 ppb

Quizalofop



Without elution step

With elution step



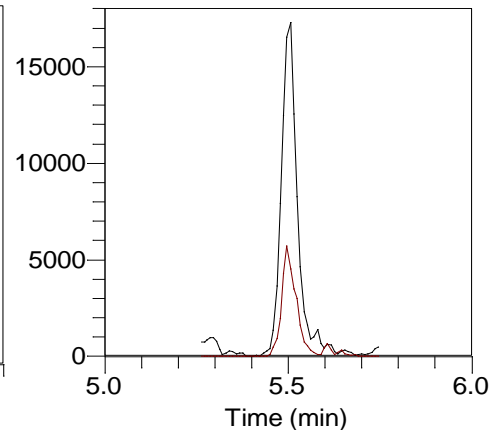
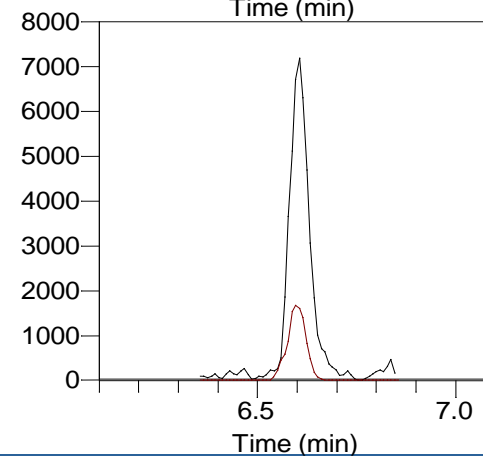
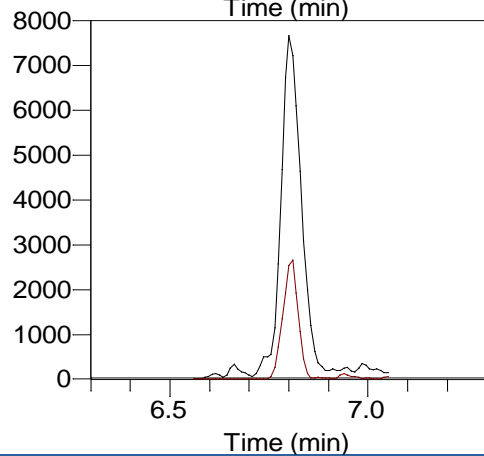
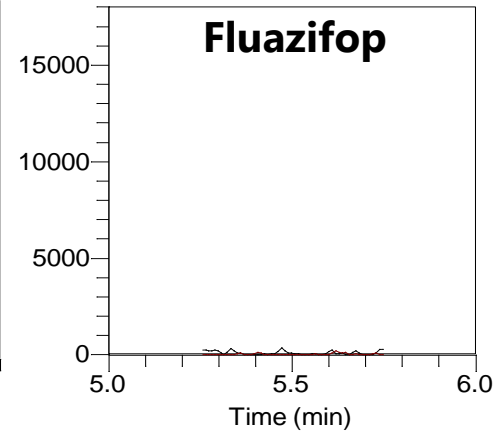
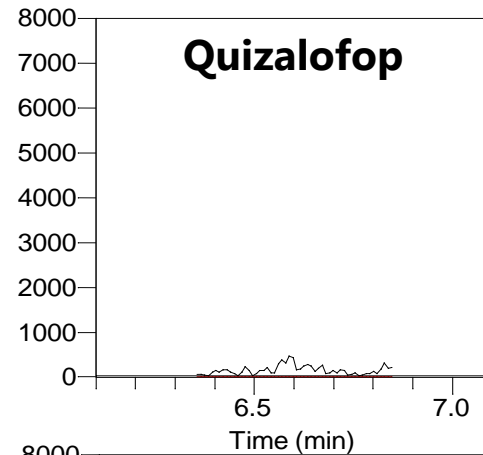
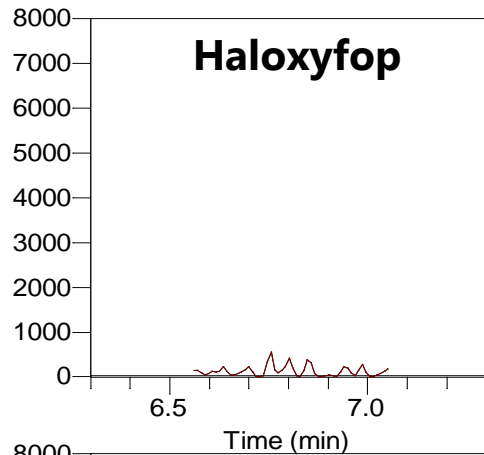
Optimization of PAL μ SPE QuEChERS clean-up workflow



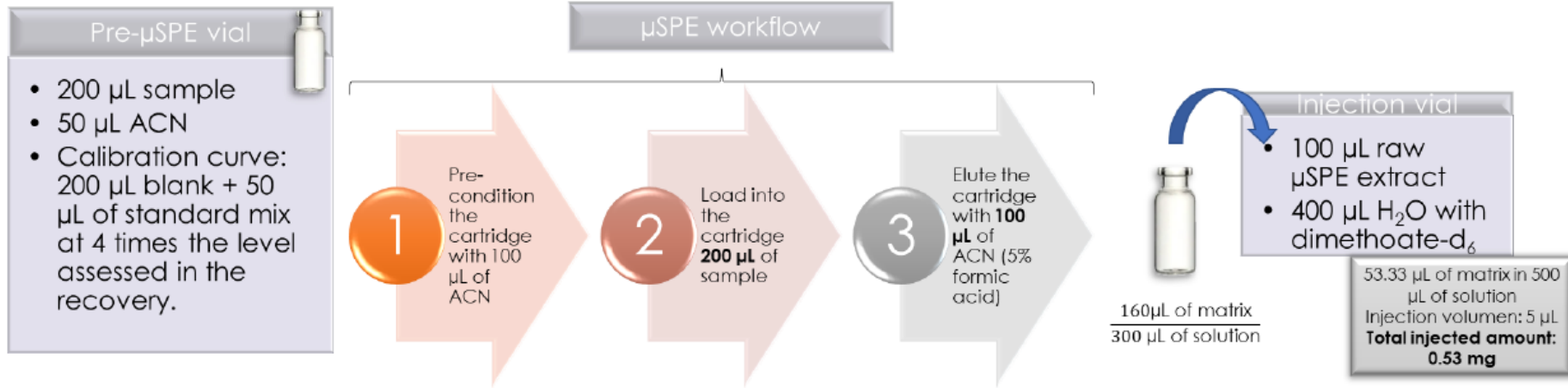
Tomato blank extract spiked at 10 ppb



*Triple
Quadrupole
TSQ Altis™*



Comparison μ SPE and dSPE



dSPE and NOT Clean Up Injection vial

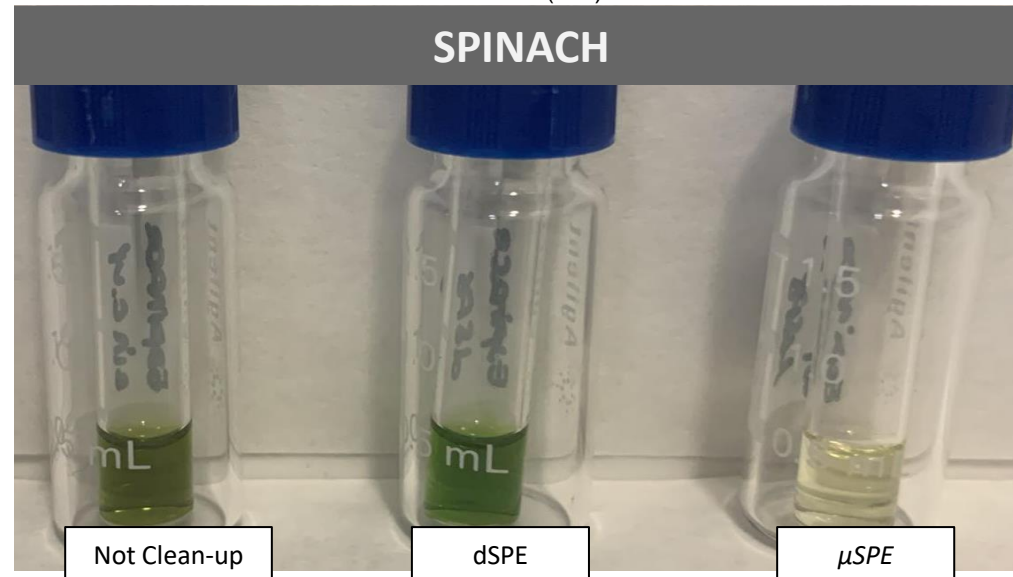
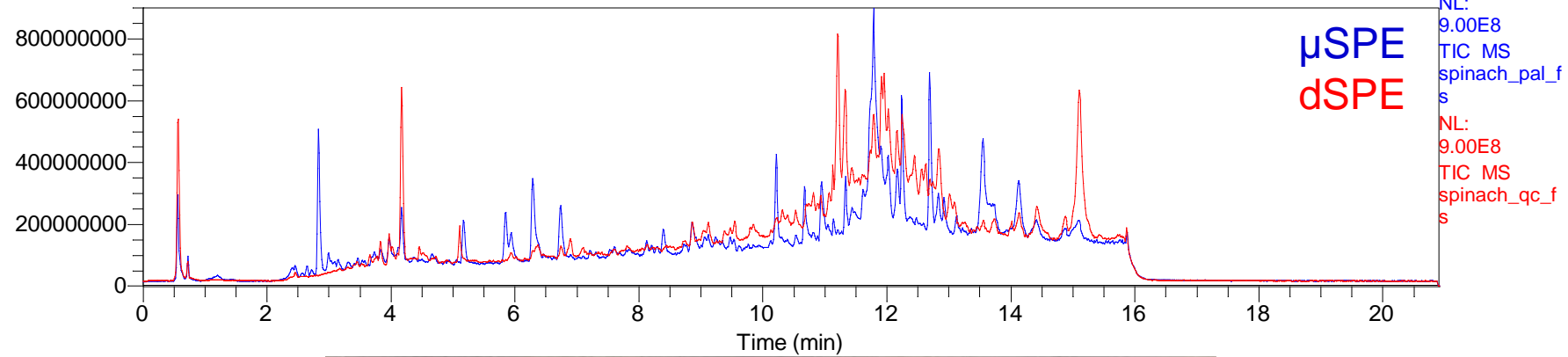
- 440 μ L H₂O with dimethoate-d₆
- 59 μ L of matrix and 51 μ L of ACN
- **Calibration point at 10 ppb:** 440 μ L H₂O with dimethoate-d₆ + 59 μ L blank + 51 μ L of standard mix at 11.57 ppb in ACN

59 μ L of matrix in 550 μ L solution
Injection volumen: 5 μ L
Total injected amount: 0.53 mg

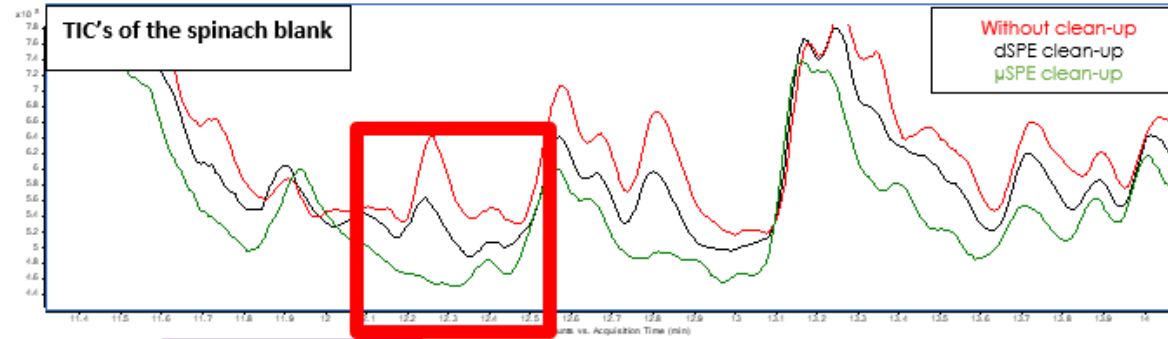
TIC's comparative (dSPE extract vs μ SPE)



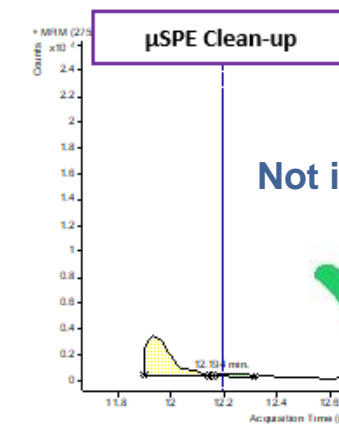
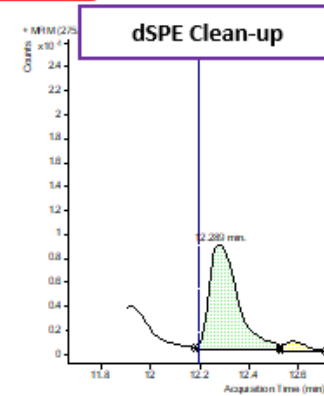
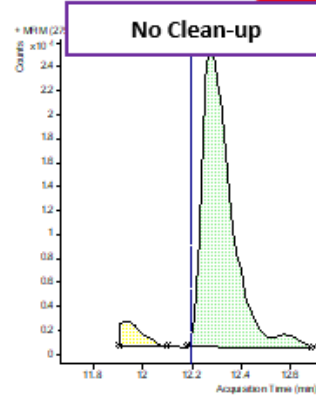
RT: 0.00 - 20.91
Spinach



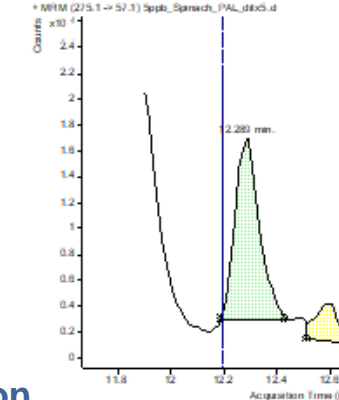
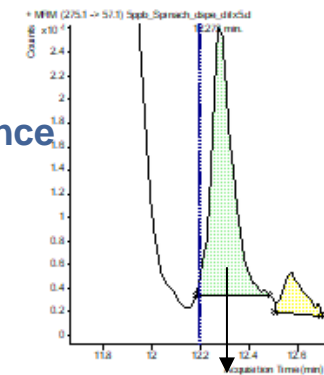
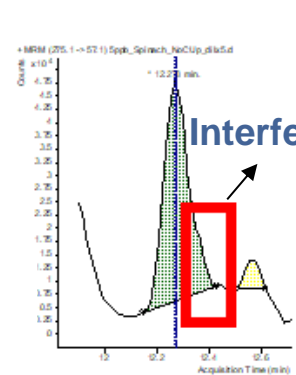
Zone of the retention time of Neburon



Spinach blank
Quantifier transition:
275.1>57.1

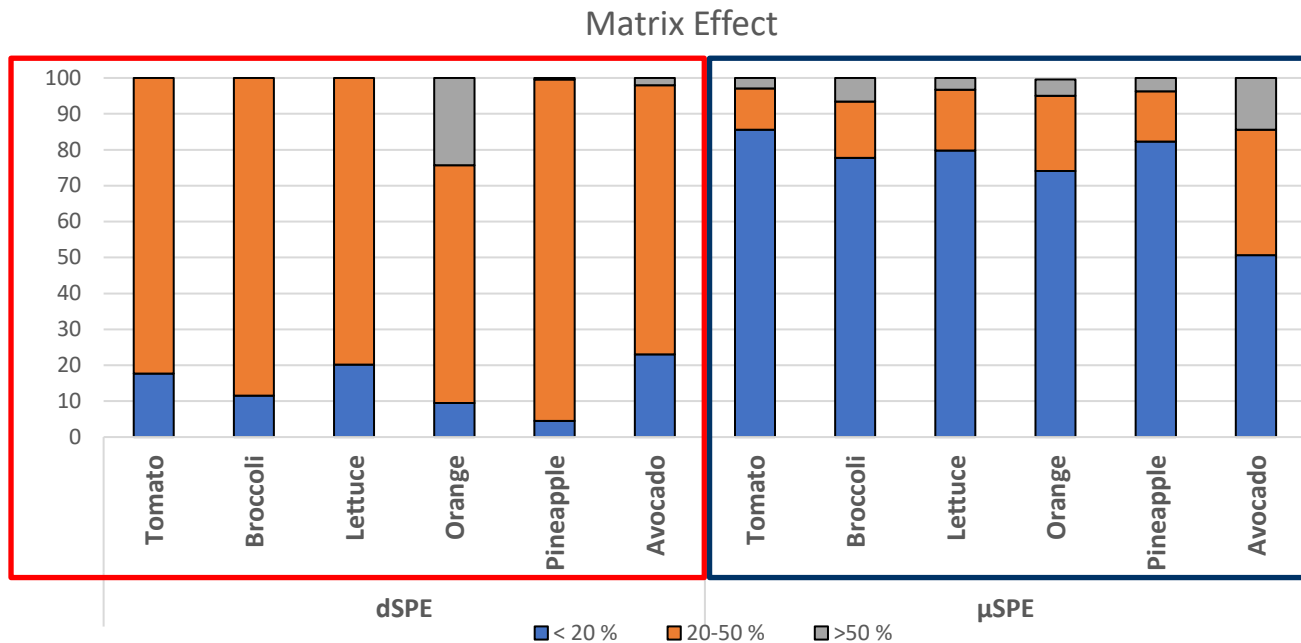


Spinach spiked at
5 ppb
Quantifier transition:
275.1>57.1

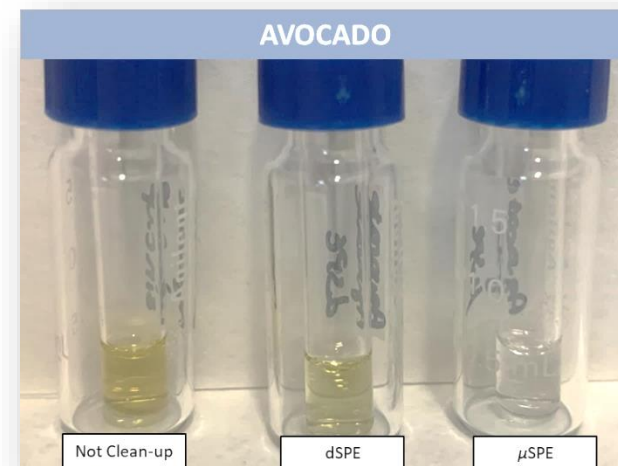
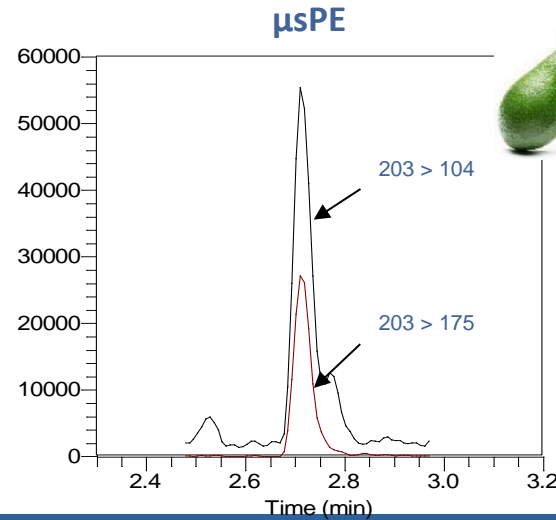
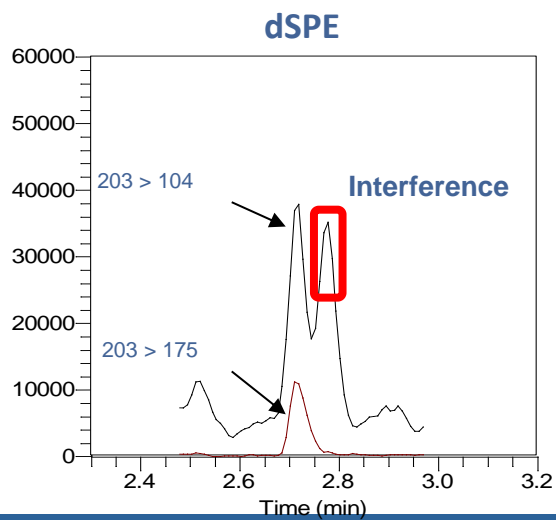
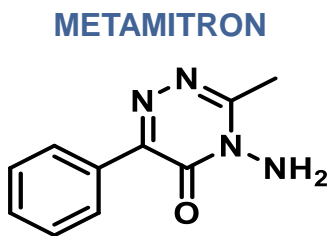


Interference coelution

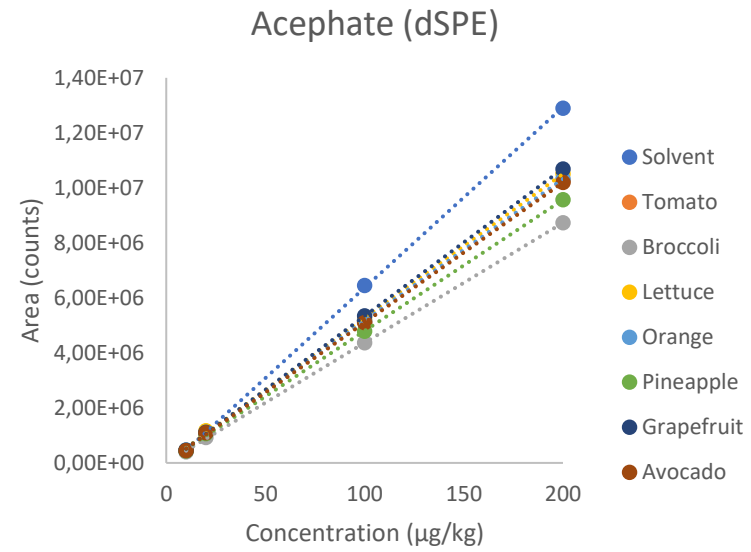
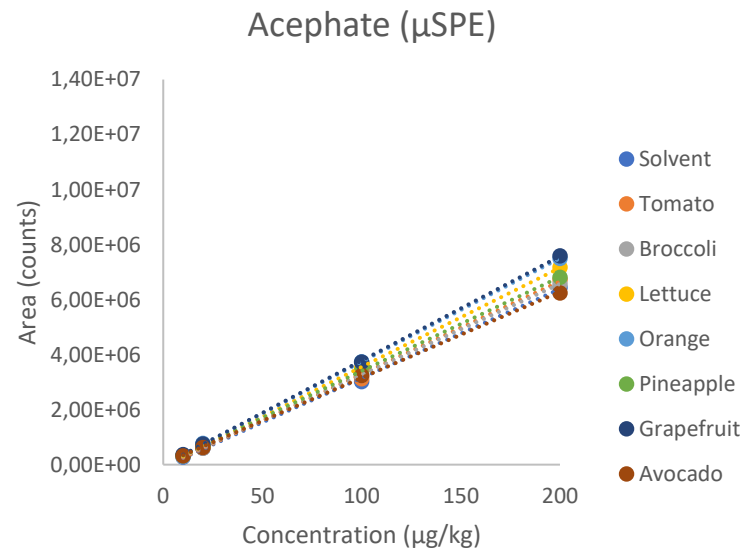
Matrix effect



Blank avocado sample spiked at 10 μg L⁻¹ with pesticide mix



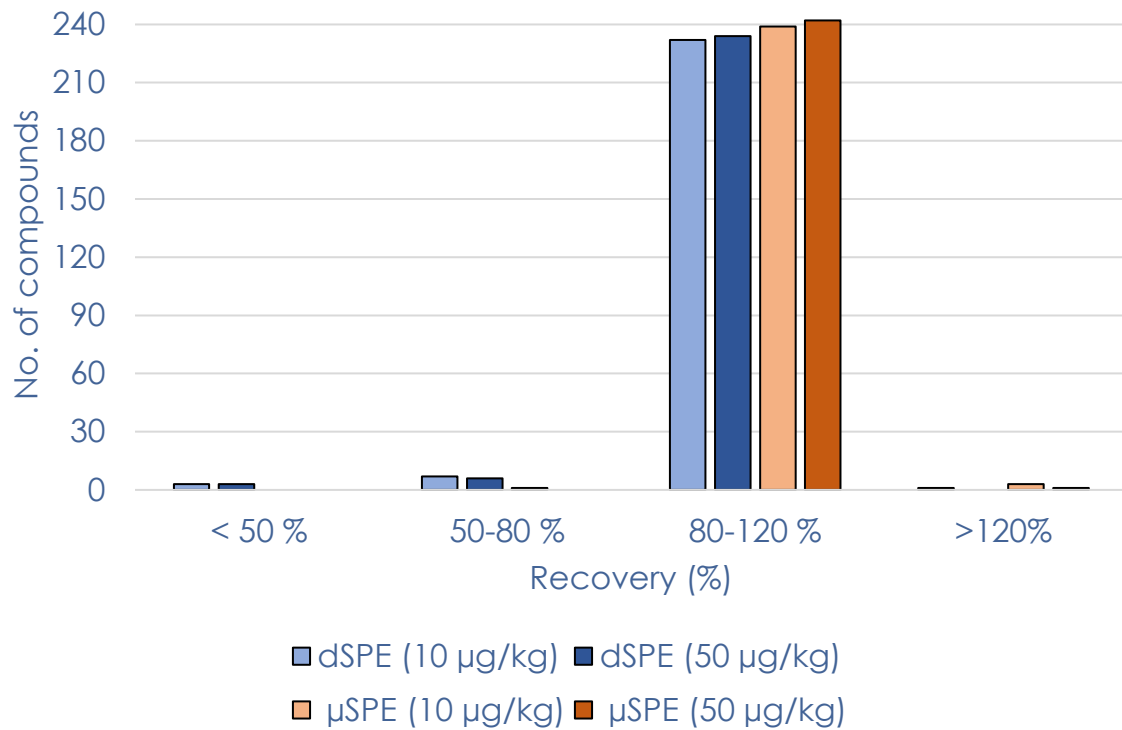
Linearity



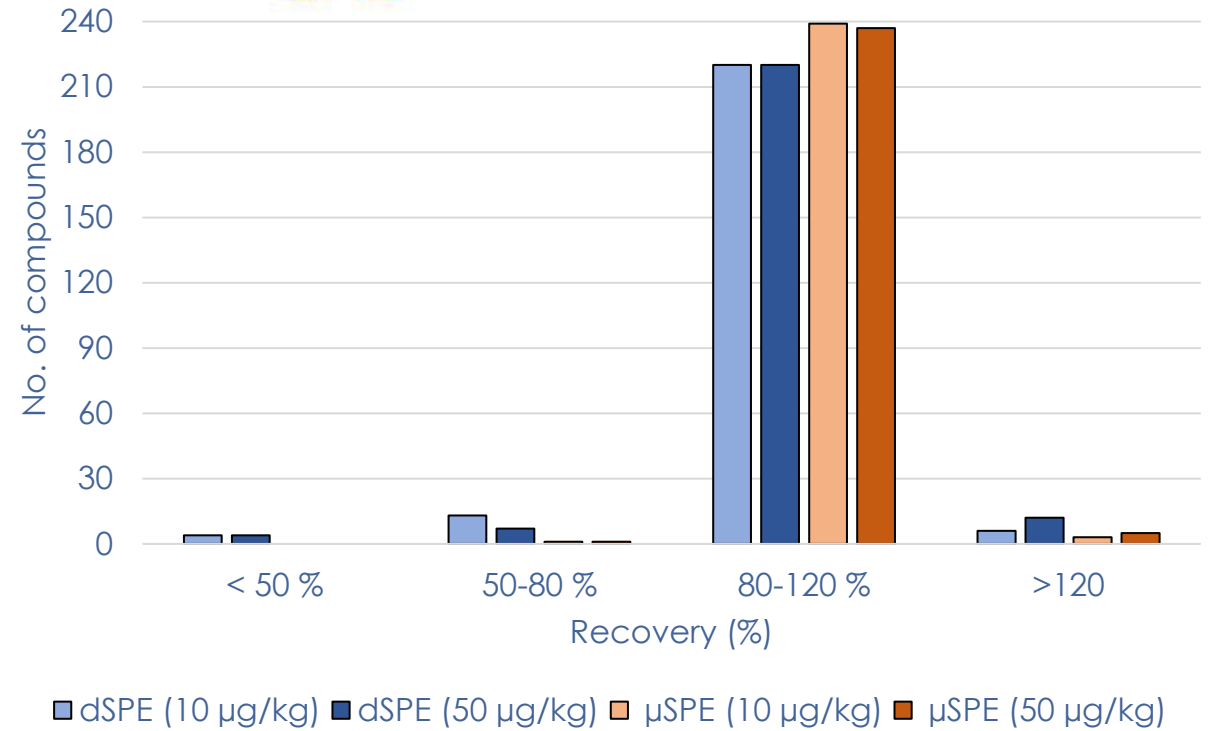
Recoveries



Tomato



Orange



Proficiency test on lemon material was analysed using the automated μ SPE 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
CHLORANTRANILIPROLE	0.166	-0.3
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
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

GC

Módulo PAL RTC (online)



GC-QqQ

Módulo PAL RTC



GC-QqQ-MS/MS Parameters

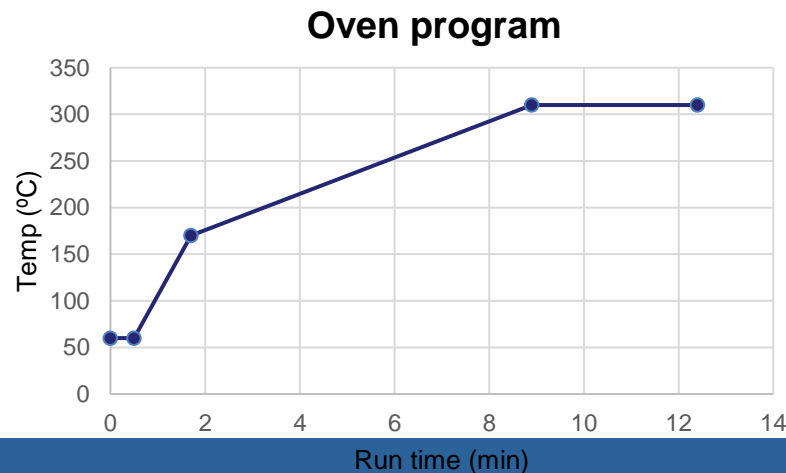
- **Column:** 2 Planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- **Injection mode:** splitless, 1 µL
- Ultra-inert inlet **liner** with glass wool frit
- **Injector temperature:** 70 °C (0.1 min), then up to 325 °C at 800 °C/min (hold for 5 min).
- **Carrier gas:** Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2.
- **Oven temperature:** 60 °C for 0.5 min, up to 170 °C (80 °C/min) and up to 310 °C (20 °C/min).



Intuvo 9000 GC
7010 GC MS/MS
Triple Quadrupole (QqQ)

- **Ionization mode:** electron impact ionization.
- **Temperature of the transfer line:** 280 °C.
- **Temperature of ion source:** 280 °C.
- **Collision gas:** nitrogen.
- **Solvent delay:** 2.6 minutes.

106 GC-amenable compounds



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 5%
solution in acetonitrile



µSPE Cartridge:
20 mg Anhydrous
MgSO₄+ **12mg**
PSA+ **12 mg** C18+
1 mg CarbonX

Injection vial:

dSPE: 50 µL of extract was evaporated under N₂
and reconstituted with 50 µL AcOEt

µSPE: Automatic injection

FLOW



Automated µSPE



Off-line Version

Extract without clean-up were diluted 1:1 with
ACN or with standard mix

Steps:

1. Condition µSPE cartridge (**100 µL** ACN)
2. Elution cartridge step with sample (**200 µL**)

Total injected amount:
0.50 mg

The PAL μ SPE clean-up workflow

STEPS	
Conditioning	Syringe wash (3 cycles) with AcN
	Load 100 μL of AcN
	Condition μ SPE cartridge with ACN (Waste)
Load 200 μL of sample diluted 1:1 with ACN or standard (Automatic injection of 1 μ L of sample)	

1

2:30 min

2

3:30 min

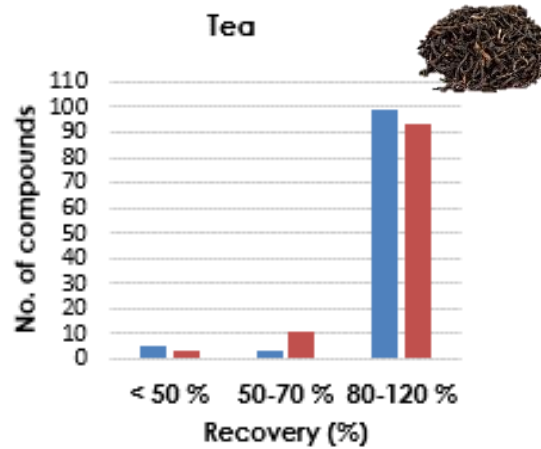
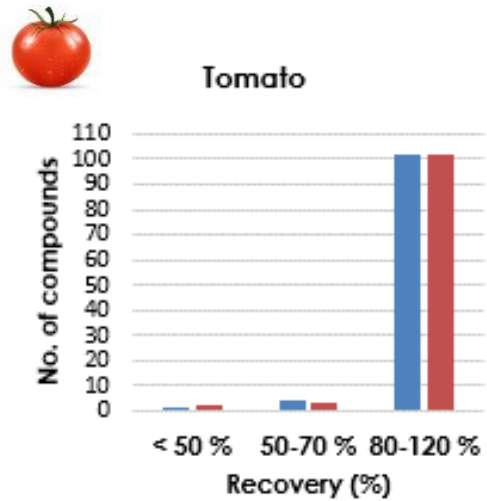
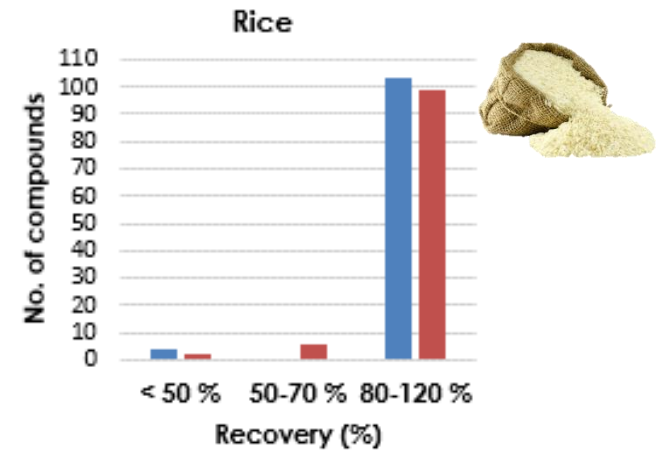
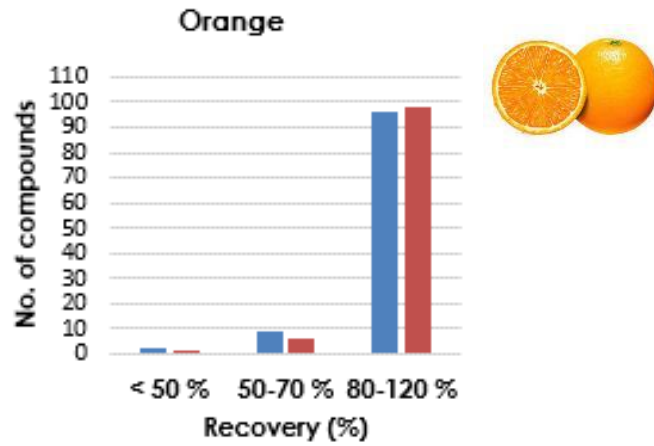
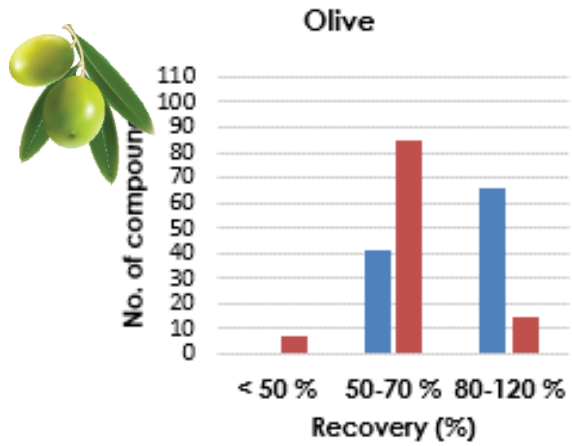


Elution step is not necessary; it is only required for acidic compounds that are LC-amenable

μ SPE workflow




μSPE
dSPE

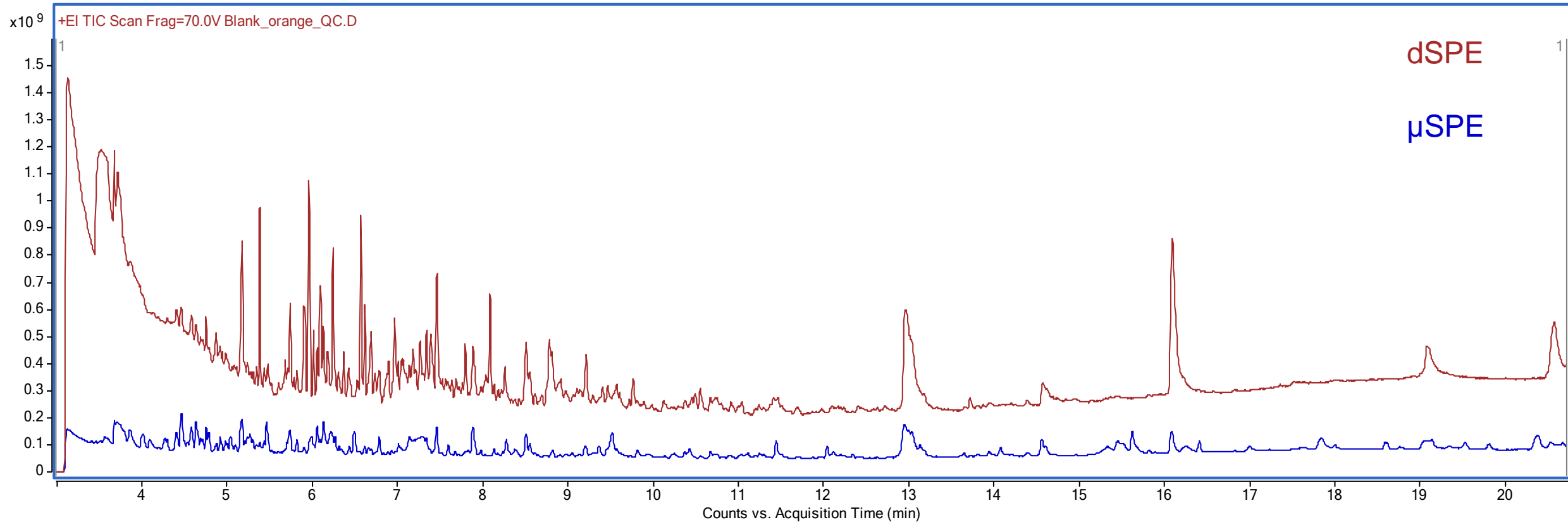


TIC`s comparative



ORANGE

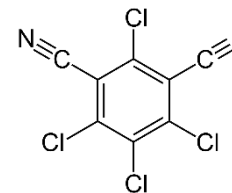
Total injected amount:
 μ SPE = 0.5 mg
dSPE = 0.5 mg



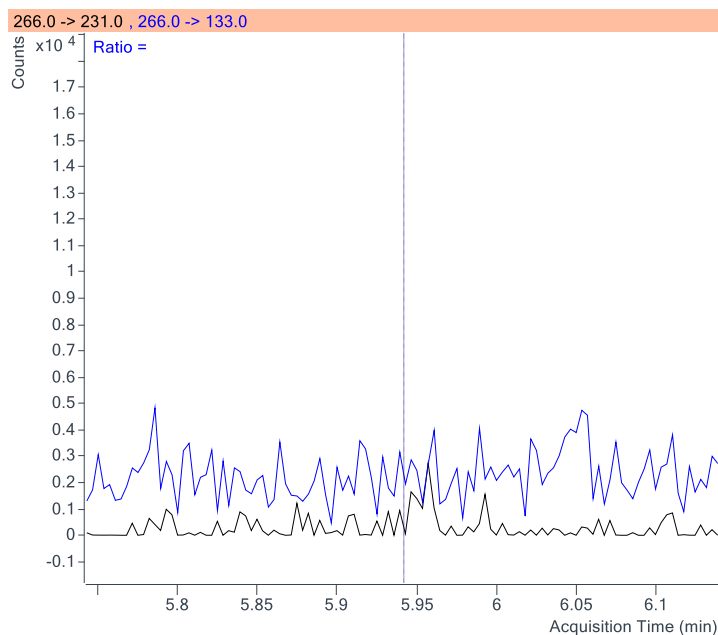


Chlorthalonil

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

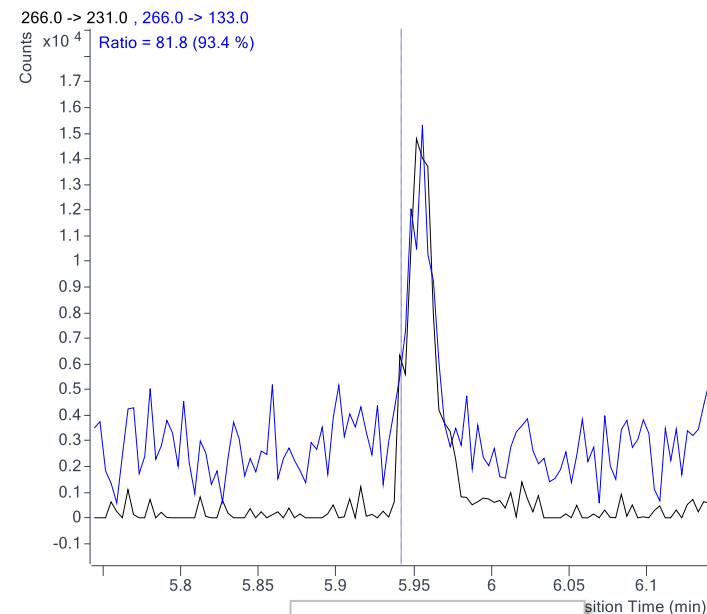


dSPE



Recovery: 0 %

µSPE



Recovery: 96 %

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

Evaluation of the μ SPE for the analysis of spices

Citrate buffered QuEChERS extraction



Step 1: Extraction

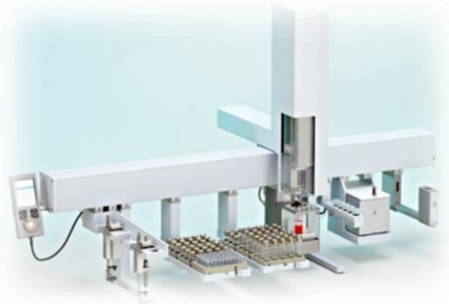
2 g of sample added
7 mL distilled water
(vortex).

10 ml acetonitrile
shaken for 6
minutes.

4 g anhydrous
magnesium sulphate
1 g sodium chloride
1 g trisodium citrate
dihydrate and 0.5 g
disodium hydrogen
citrate sesquihydrate

Axial agitator for 6
min and centrifuge
at 3700 rpm for 5
min.

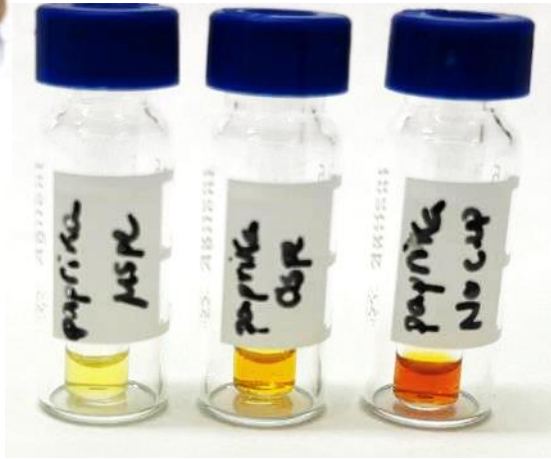
Step 2: Clean-up



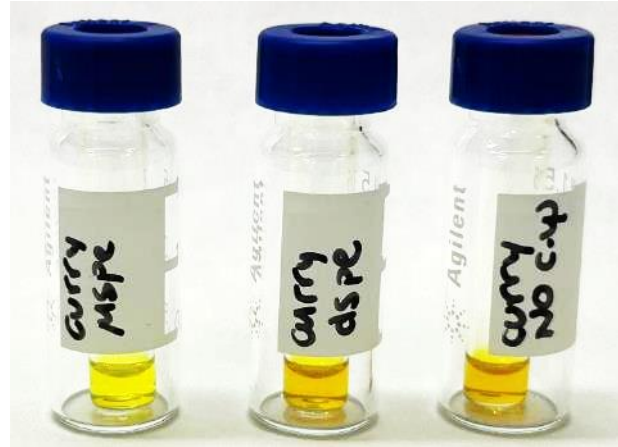
Steps	
Conditioning	Syringe wash (3 cycles) with AcN
	Load 100 µL of AcN
	Condition µSPE cartridge (Waste)
Load 200 µL of diluted sample (200 µL raw extract + 50 µL AcN) (Collection Vial)	
Elution	Load 100 µL of AcN (5% formic acid)
	Elution step (Collection Vial)



Paprika



μSPE dSPE Not clean-up



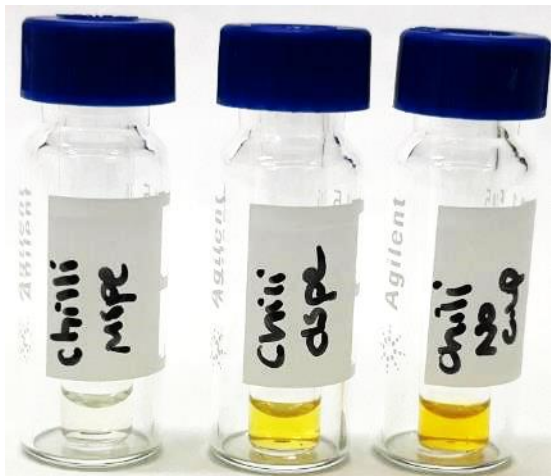
μSPE dSPE Not clean-up



Curry



Dry chili



μSPE dSPE Not clean-up



Turmeric



μSPE dSPE Not clean-up

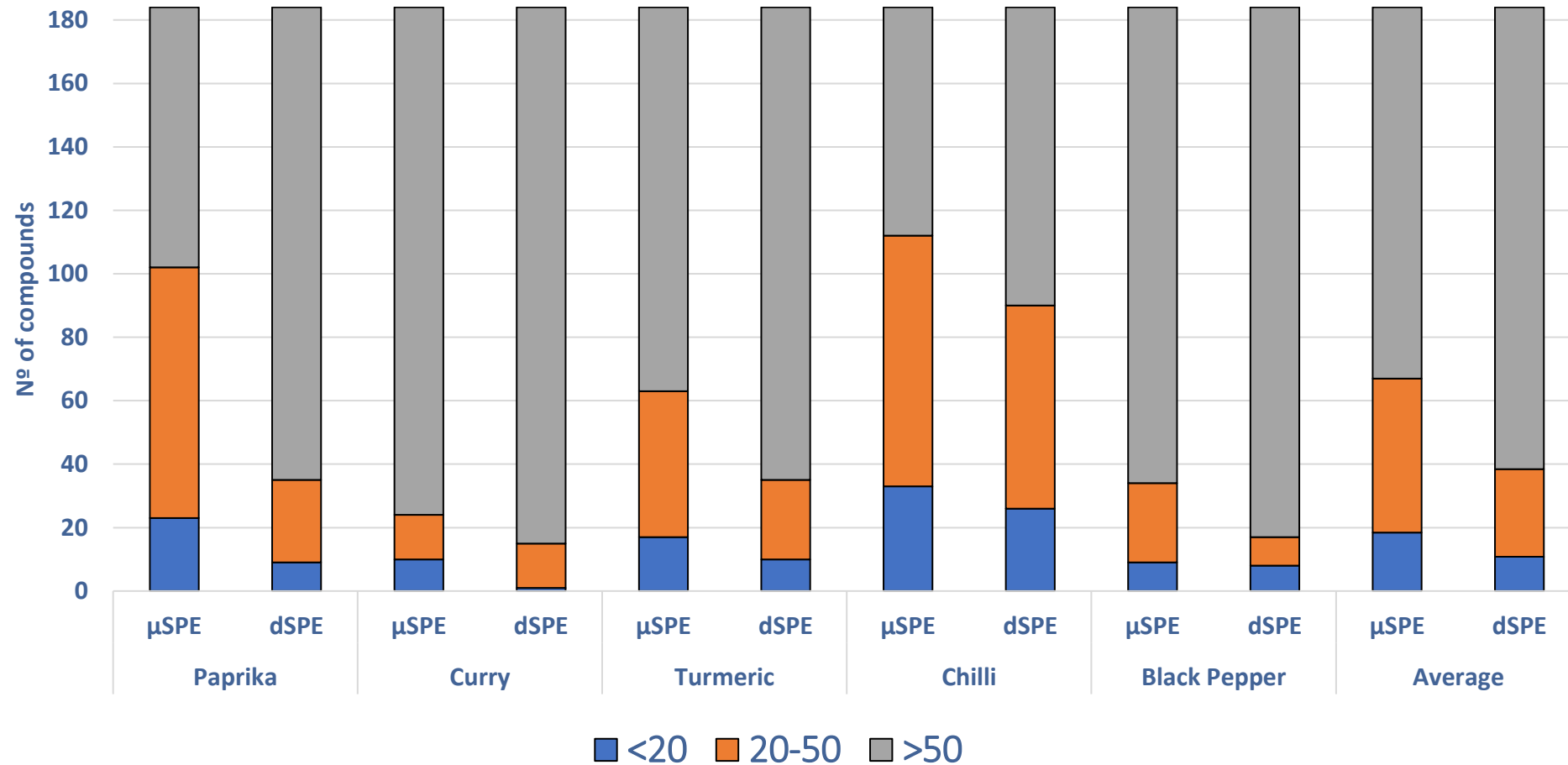


Black pepper

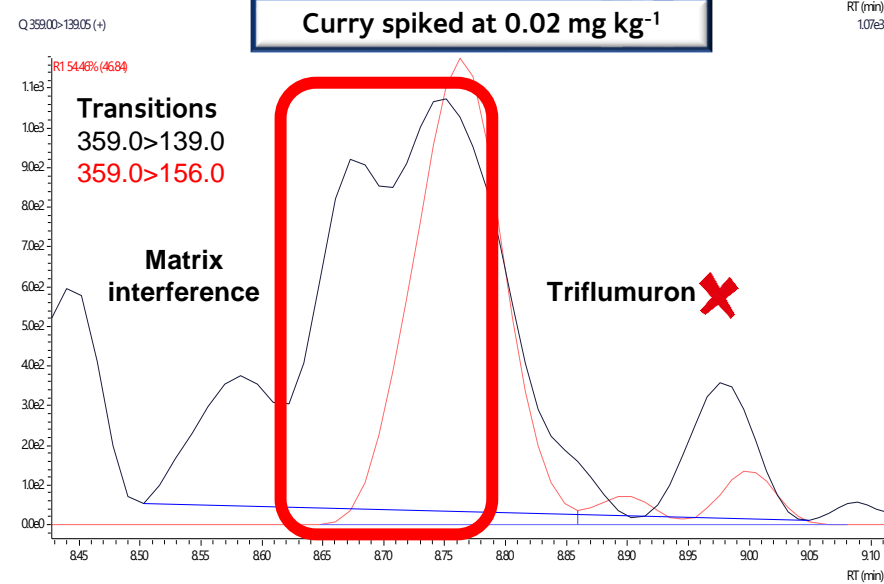
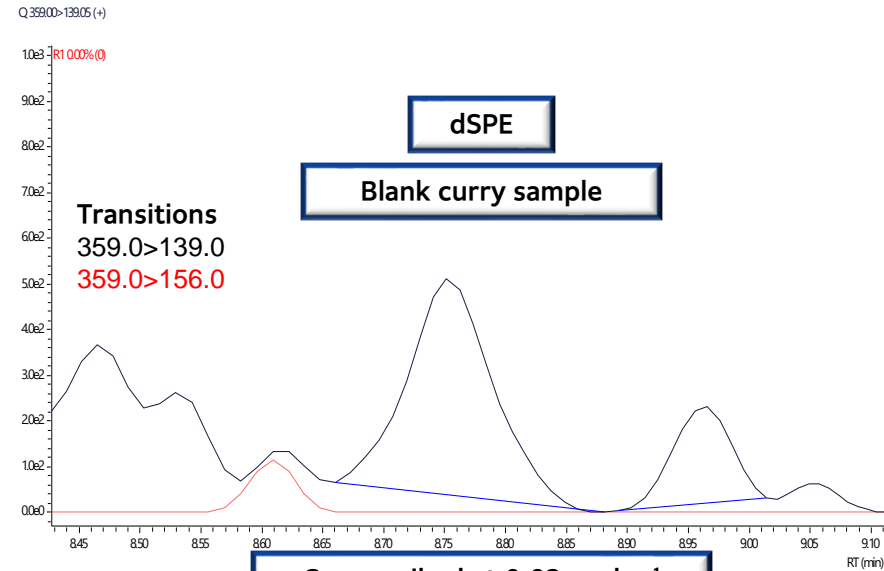
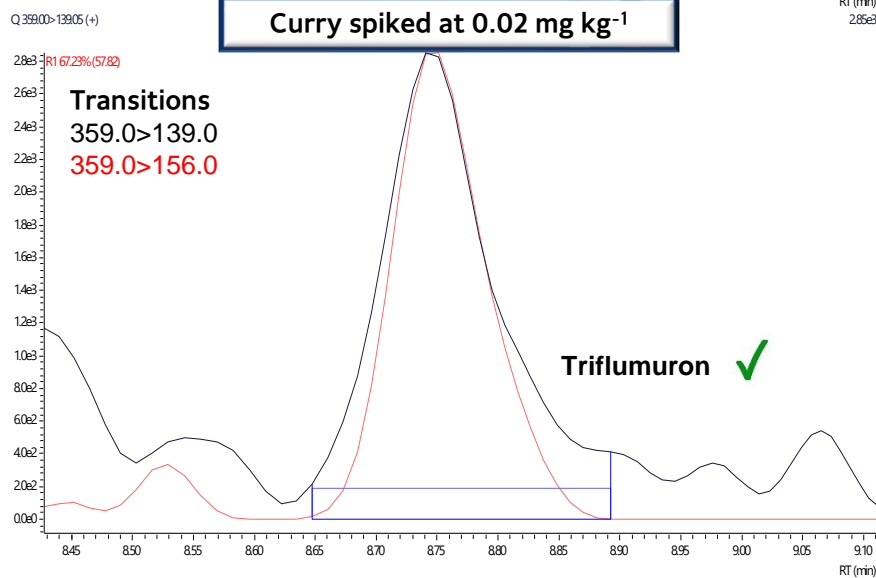
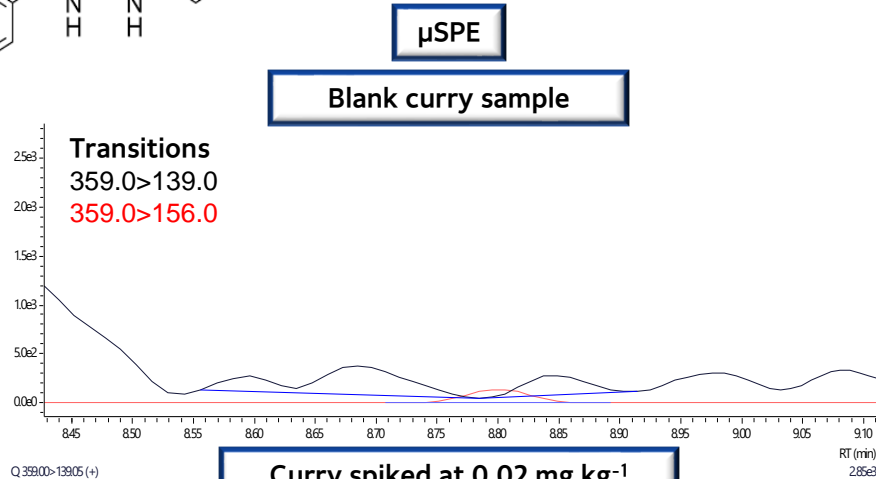
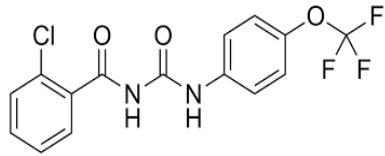


μSPE dSPE Not clean-up

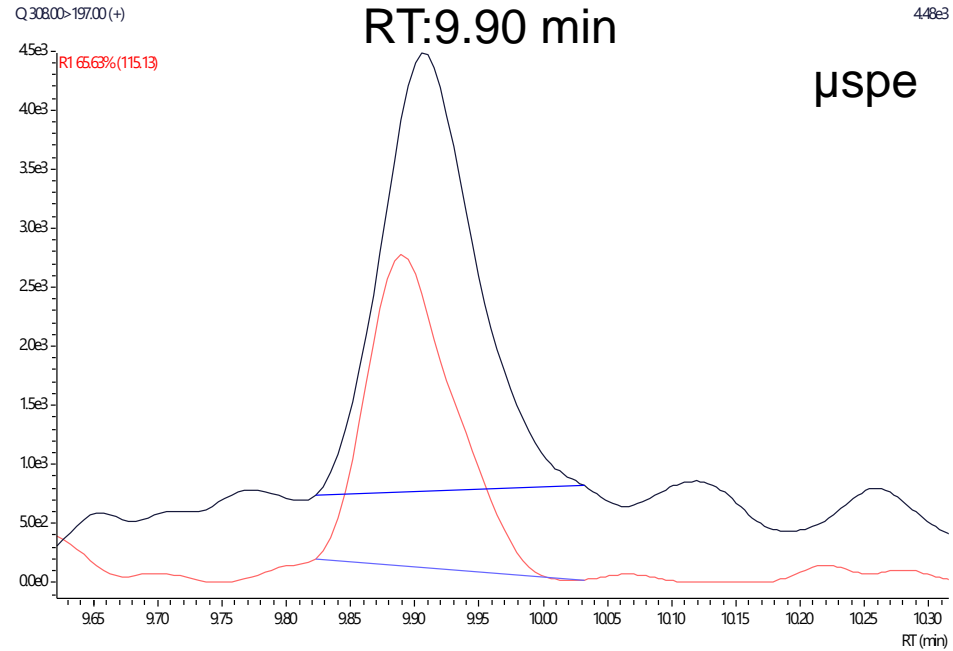
Matrix Effect



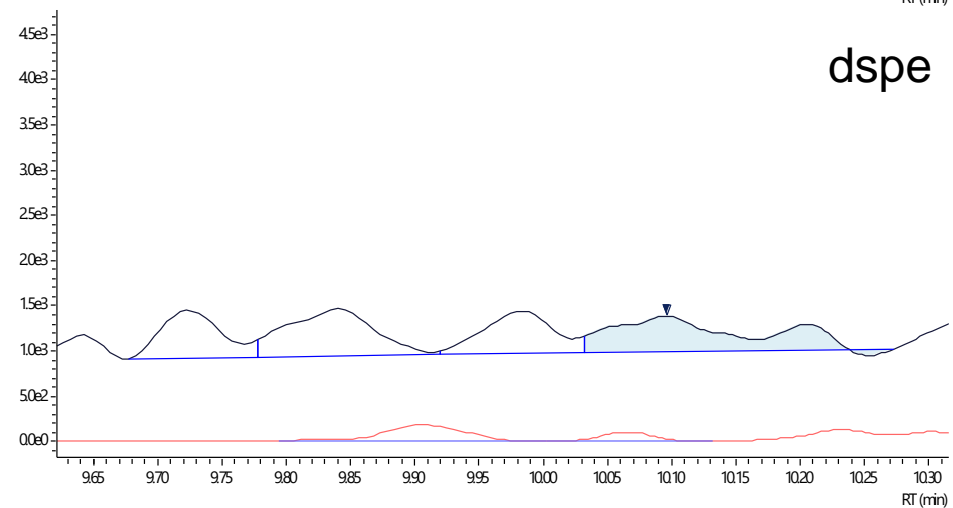
Triflumuron



Curry spiked at 20 ppb Quinoxifen



High ion suppression



Differences in the slopes of the calibration curves

Group 1 Paprika-Chilli

RSD(%)	Paprika-Chilli vs Curry-Turmeric
< 50	131 compounds
> 50	53 compounds

RSD(%)	Paprika-Chilli ✓
< 50	178 compounds
> 50	6 compounds

Bupirimate
Fluopyram
Phosmet
Promecarb
Pyridaphenthion
Triazophos

With specific calibration curve

Not included compounds in MACP in red color

Group 2 Curry-Turmeric ✓

RSD(%)	Curry-Turmeric ✓
< 50	175 compounds
> 50	9 compounds

Bifenazate diazene
Butoxycarboxim
Demeton-S-methyl-sulfone
Dicrotophos
Methomyl
Nitenpyram
Omethoate
Oxamyl
Oxydemeton-methyl

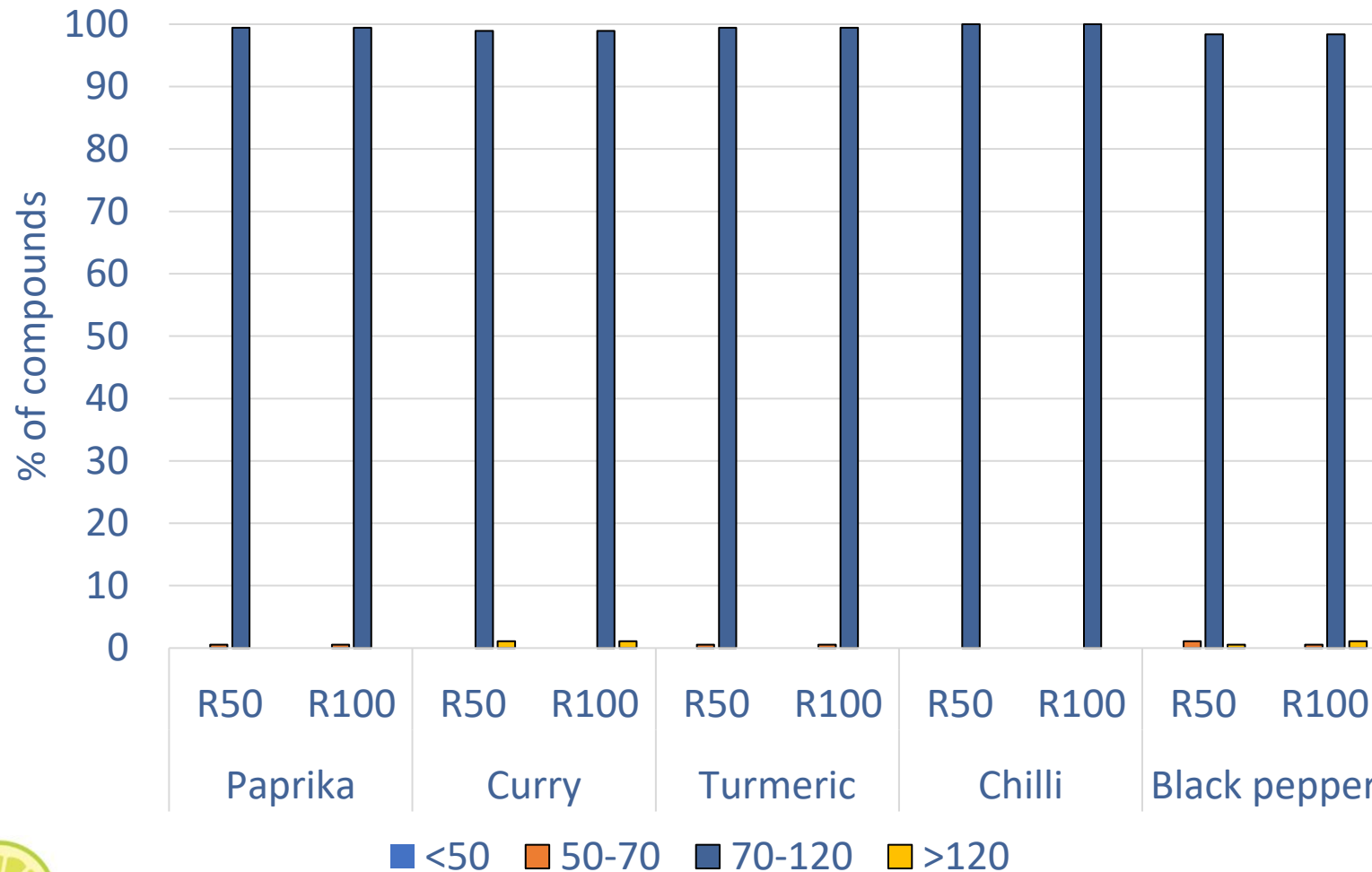
Not included compounds in MACP in red color

Group 3 Black Pepper

RSD(%)	Black pepper vs Paprika-Chilli	Black pepper vs Curry-Turmeric
< 50	100 compounds	118 compounds
> 50	84 compounds	66 compounds

Black pepper needs specific calibration curve

Recoveries using μ spe clean-up



A lowest MRL of 0.05 mg kg⁻¹ or higher for 99% of the analytes

Conclusions

Advantages of the PAL μ SPE QuEChERS clean up workflow

- ✓ One μ SPE cartridge configuration for a wide variety of food matrices
- ✓ Very effective clean-up by μ SPE facilitates the homogeneity in the calibration curves
- ✓ 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.
- ✓ Miniaturisation means less sorbent material is required.
- ✓ Automation allows you to increase the number of samples in a working day by 25%. For example: increase from 20 to 25 samples.
- ✓ But...

**Thank You
for Your Attention**



Advantages of the PAL μ SPE QuEChERS clean up workflow

- Highlight two or three advantages and two or three disadvantages of automated μ SPE.
- What advantage do you see in performing a second elution of the sample?
- Do you think it would be useful for your laboratory?
- To what extent could the introduction of this new instrument impact ISO 17025 compliance?