

TRAINING 2023

ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD

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UNIVERSITY OF ALMERÍA



EURL EUROPEAN
UNION
REFERENCE
LABORATORY

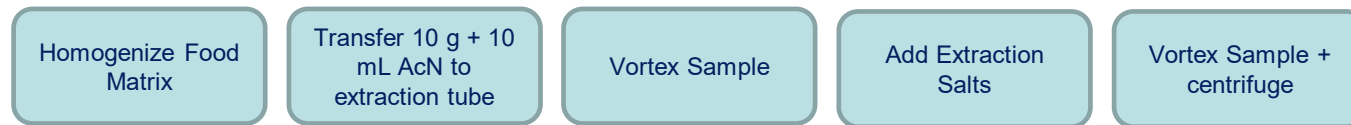
PESTICIDES IN FRUITS
AND VEGETABLES

19/09/2023 ALMERIA

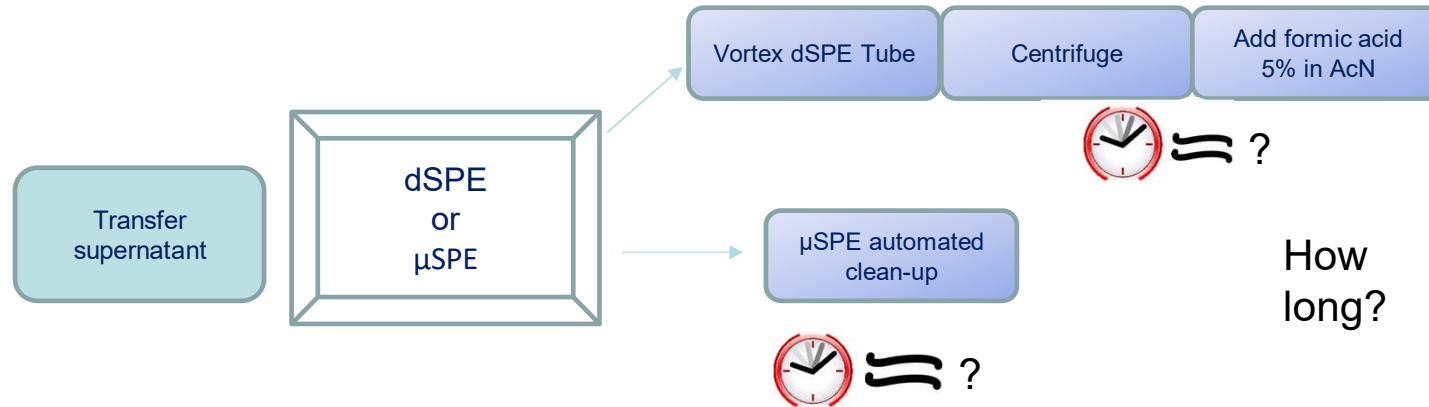
Workflow development for fruit and vegetables

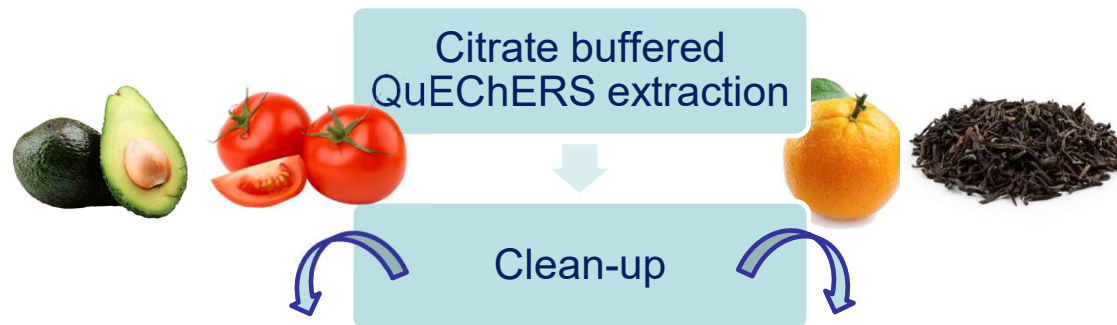
QuEChERS is the quick, easy, cheap effective, rugged and safe sample preparation method originally developed by M. Anastassiades and S.J. Lehotay in 2003. In the original QuEChERS method, acetonitrile is used as extraction solvent, followed by adding NaCl and buffer salts, vortexing and centrifugation.

**Step 1:
Extraction**



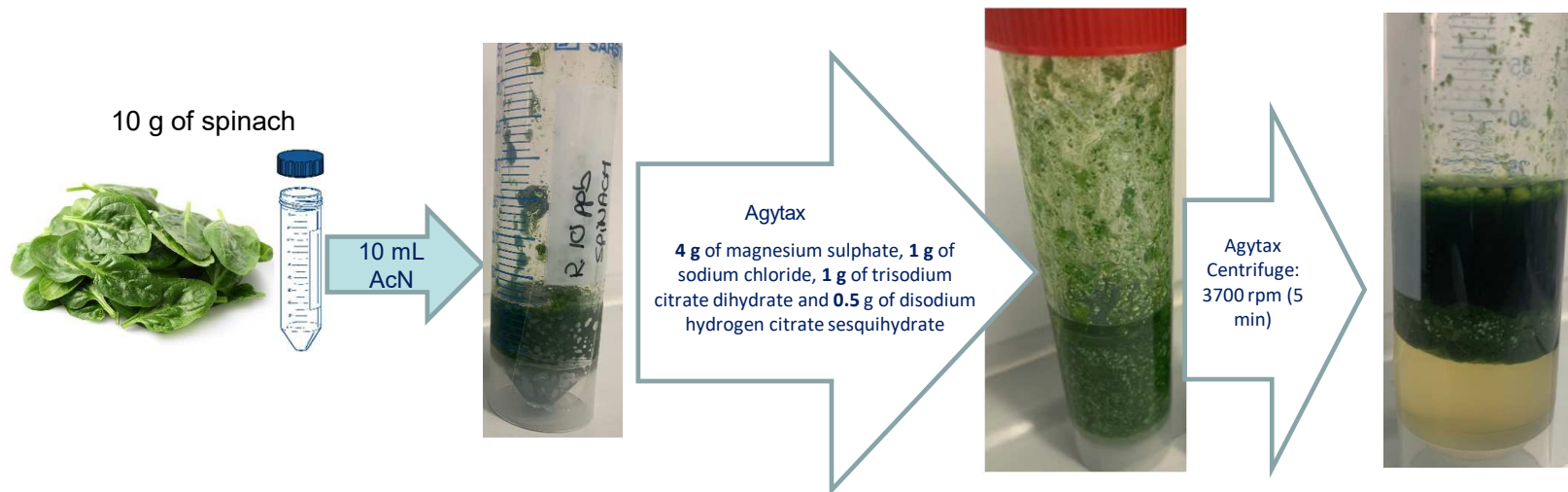
**Step 2:
Clean up**





	Clean-up salts for the d-SPE and μ SPE QC method	
	d-SPE	μ SPE
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



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)

dSPE Clean up

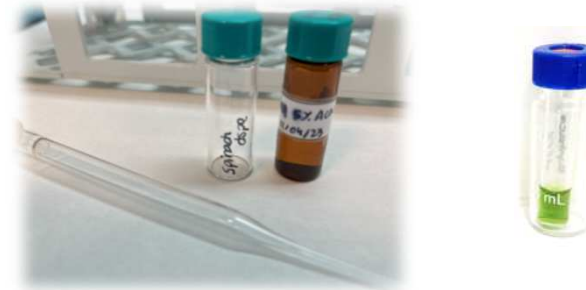
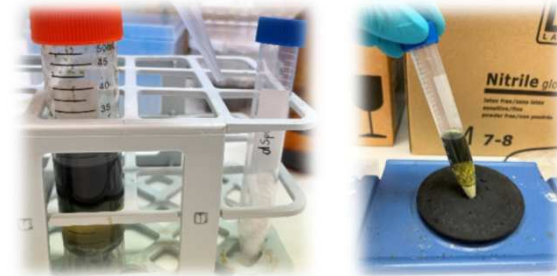
Manual dSPE

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

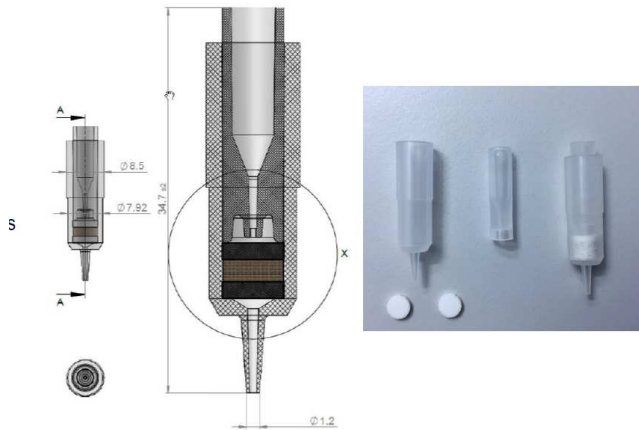
- 200 μ L sample
- 50 μ L ACN
- Calibration curve: 200 μ L blank + 50 μ L of standard mix at 4 times the level assessed in the recovery.



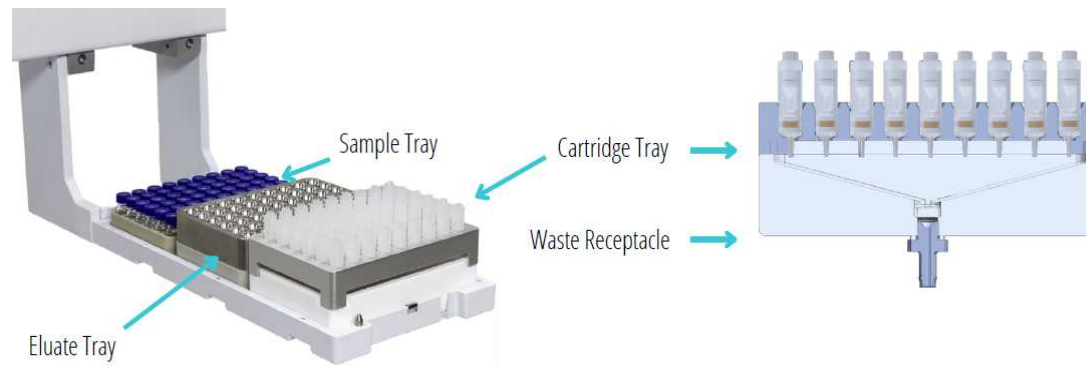
What is the automatic clean up robot like?



ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD



- Small dead volume ($< 20\mu\text{L}$)
- Wide range of sorbent masses: 5-150 mg
- **Composition: 20 mg Anhydrous MgSO_4 + 12mg PSA+ 12 mg C18+ 1 mg CarbonX**



PARAMETERS



Setup

Mode	Offline as Local Script
μ SPE 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
μ SPE Cartridge Tray	Rack 5
Dispose Cartridge	No
Cartridge Waste Container	none
μ SPE 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 μ L
Conditioning Solvent Fill Speed	100 μ L/s
Conditioning Solvent Loading Speed	5 μ L/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 μ L
Protectant Fill Speed	5 μ L/s

Internal Standard

Internal Standard Source	none
Internal Standard Index	1
Internal Standard Volume	0 μ L
Internal Standard Fill Speed	1 μ L/s

Target Standard

Target Standard Source	none
Target Standard Index	2
Target Standard Volume	0 μ L
Target Standard Fill Speed	1 μ L/s

Solvent Addition

Solvent Source	none
Solvent Index	3
Solvent Volume	0 μ L
Solvent Fill Speed	5 μ L/s

PARAMETERS

⊖ Mixing

Enable Mixing Step	No
Syringe Mixing Cycles	0
Syringe Mixing Volume	300 μ L
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 μ L
Sandwich Injection Top Layer Volume	0 μ L
Sandwich Injection Air Gap Volume	0 μ L
Sample Volume	1 μ L
Sample Air Gap Volume	1 μ L
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

ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD

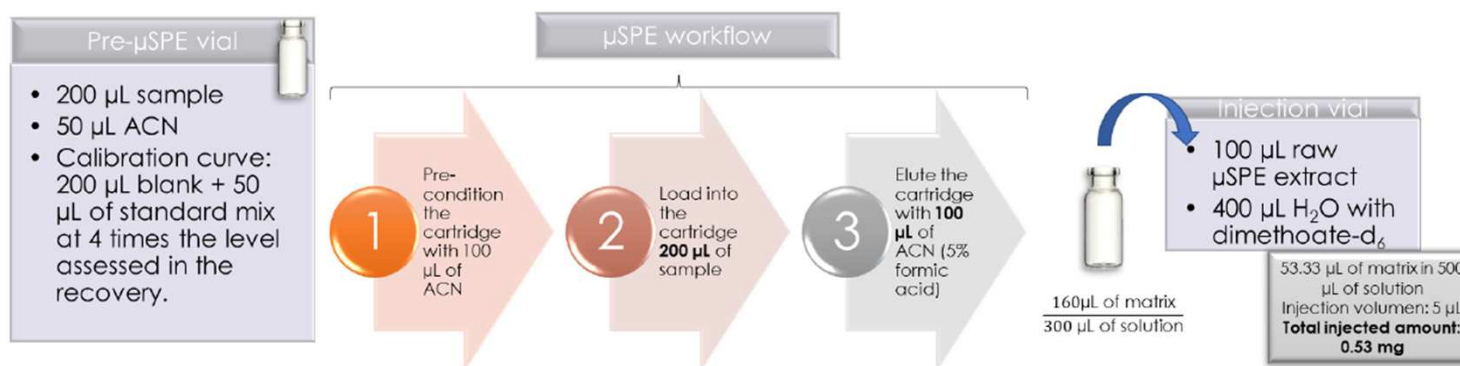
BATCH

The screenshot shows the PALScript Executor interface. A 'Vial Selection' dialog box is open, displaying three 9x9 grids for Tray Holder 1, Tray Holder 2, and Tray Holder 3. The 'Injection Order Mode' is set to 'in Sequence' (1-2-3). The status bar at the bottom shows 'Aborted: False', 'Start: 11.09.2023 08:43', 'Remaining Time: 00:05:26', 'Duration: 00:00:00', and 'Nr Samples: 1'. The 'Remaining Time' value is highlighted with a red box.

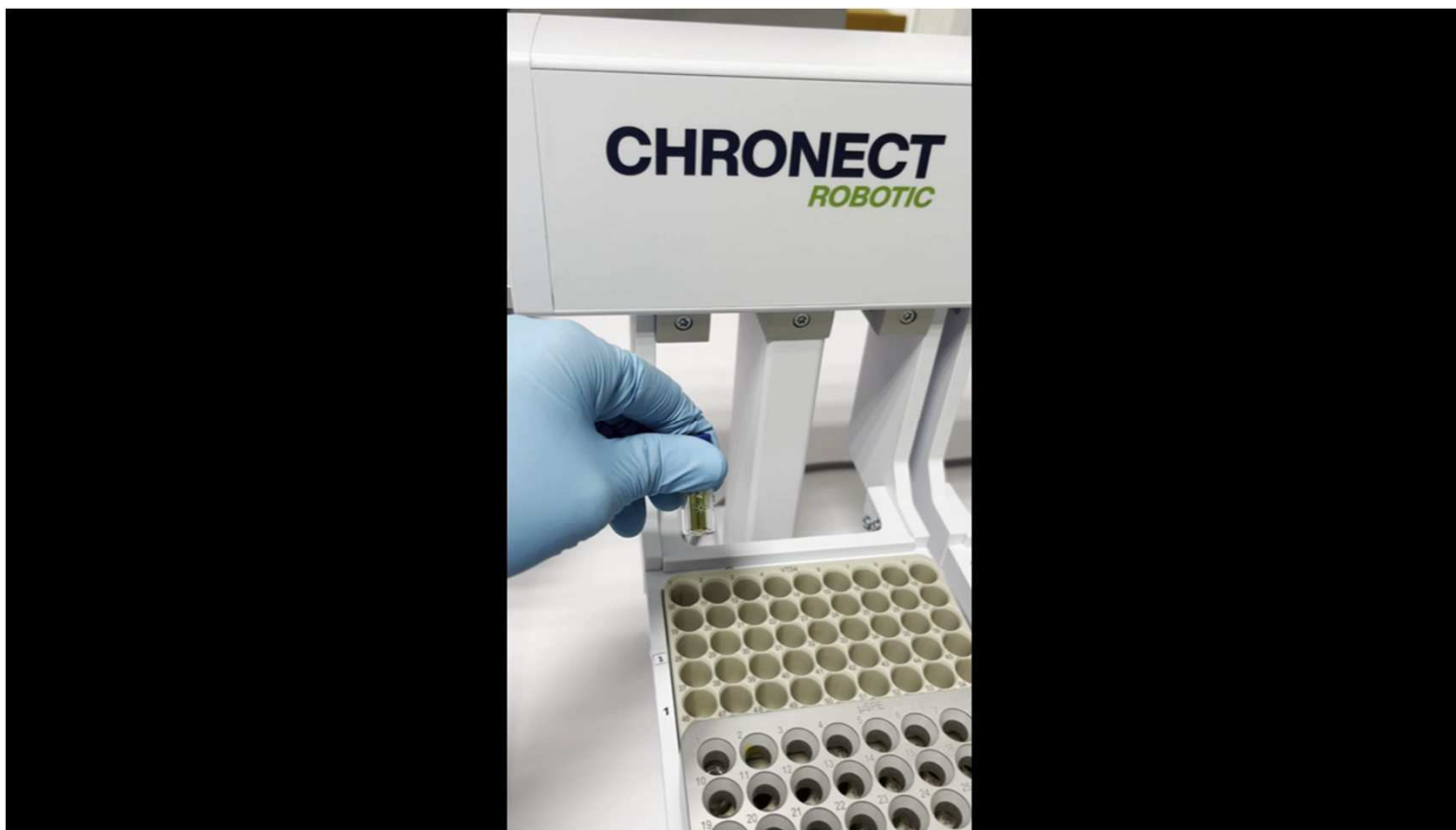
Aborted: False | Start: 11.09.2023 08:43 | Remaining Time: 00:05:26 | Duration: 00:00:00 | Nr Samples: 1

The PAL μ SPE QuEChERS 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 diluted sample (200 μL raw extract + 50 μL AcN) (Collection Vial)	
Elution	Load 100 μL of AcN (5% formic acid)
	Elution step (Collection Vial)



WORKFLOW



Optional step



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

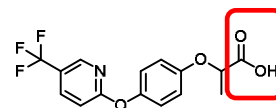
Results

Optimization of PAL μ SPE QuEChERS clean-up workflow

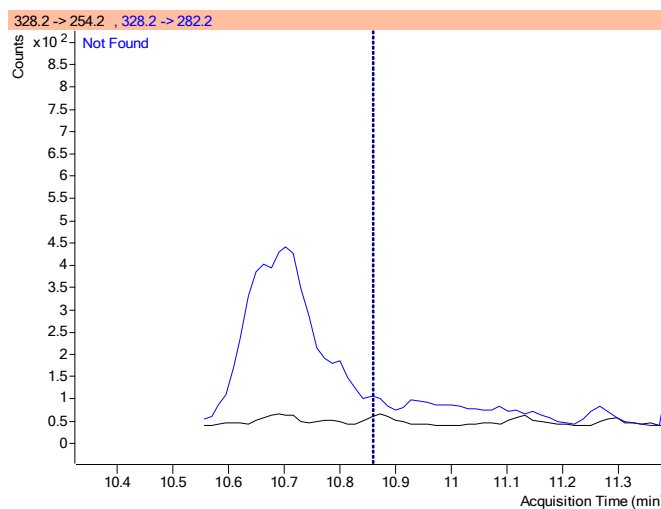


Spinach blank extract spiked at 10 ppb

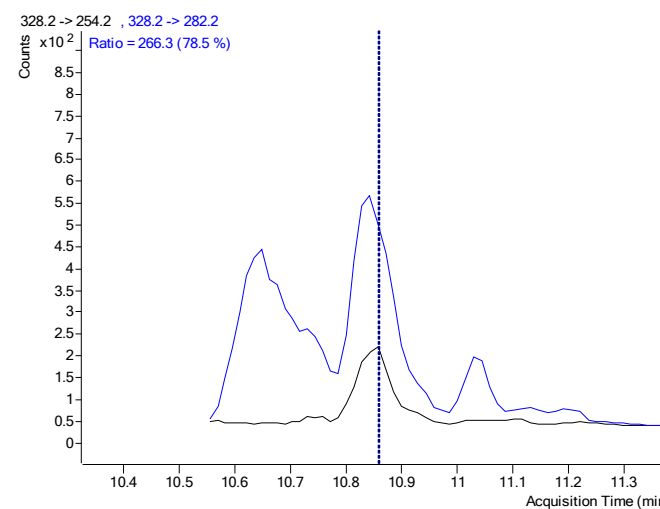
Fluazifop



Without elution step



With elution step

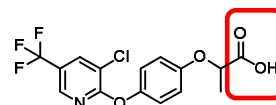


Optimization of PAL μ SPE QuEChERS clean-up workflow



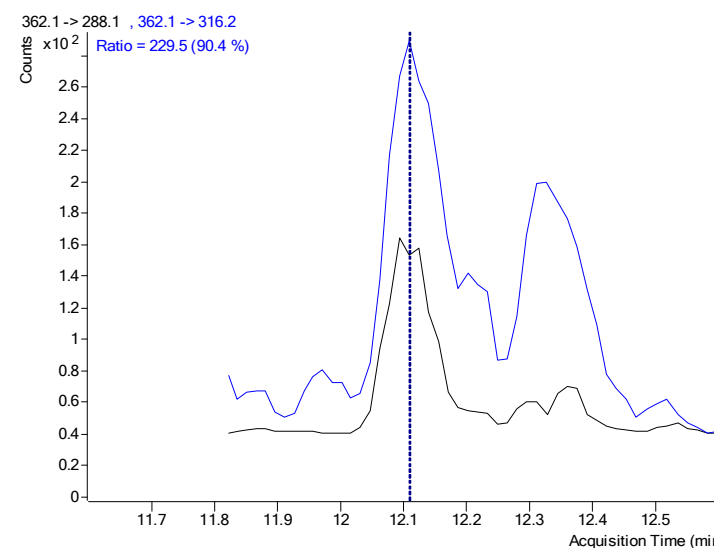
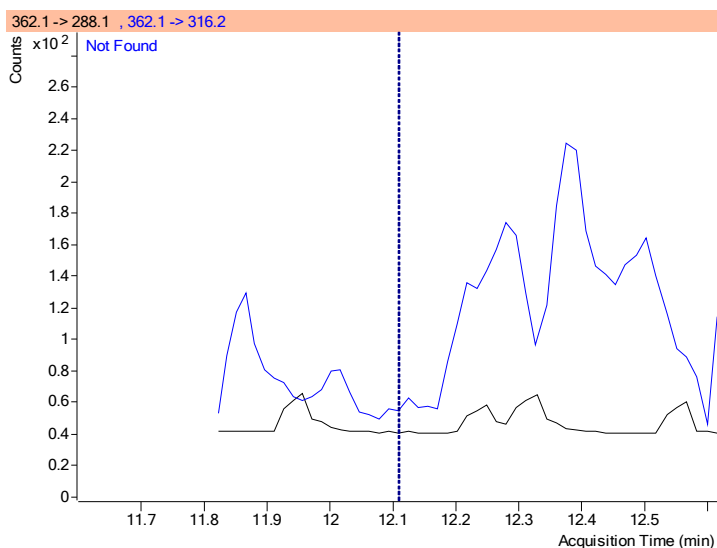
Spinach blank extract spiked at 10 ppb

Haloxyfop



Without elution step

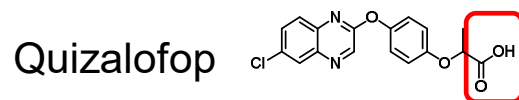
With elution step



Optimization of PAL μ SPE QuEChERS clean-up workflow

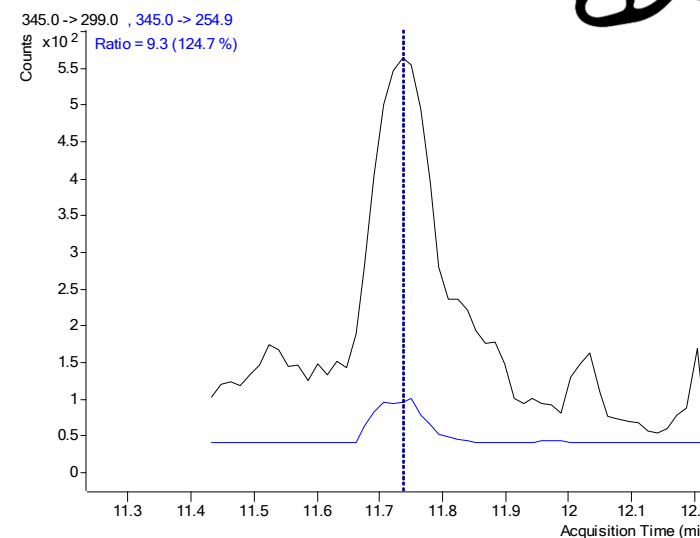
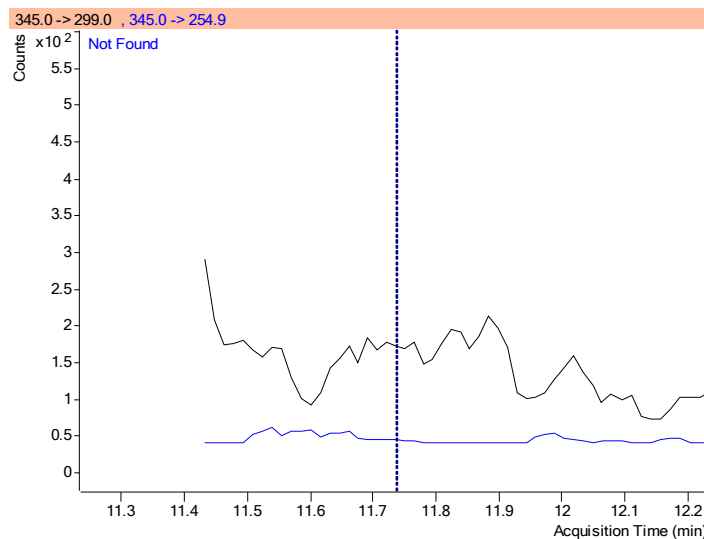


Spinach blank extract spiked at 10 ppb



Without elution step

With elution step



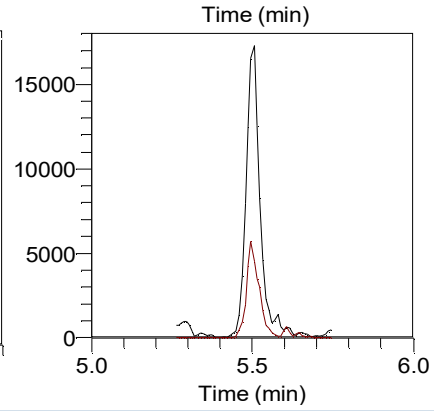
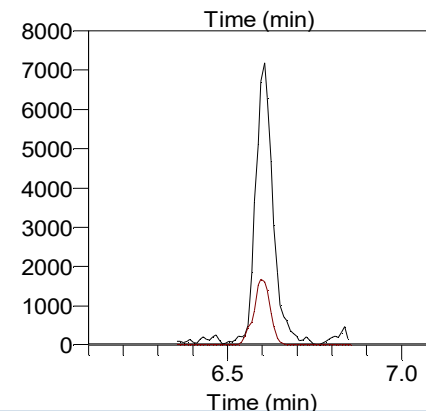
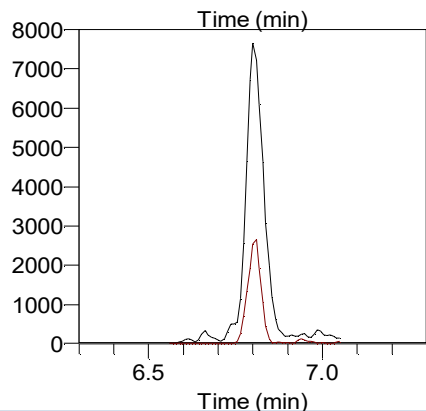
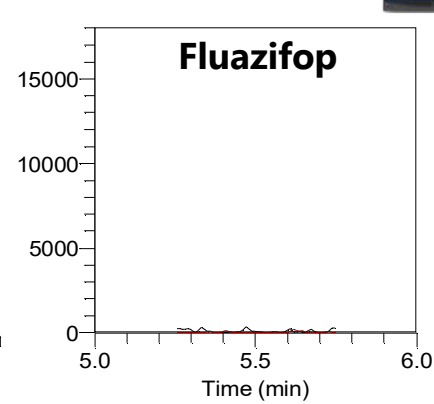
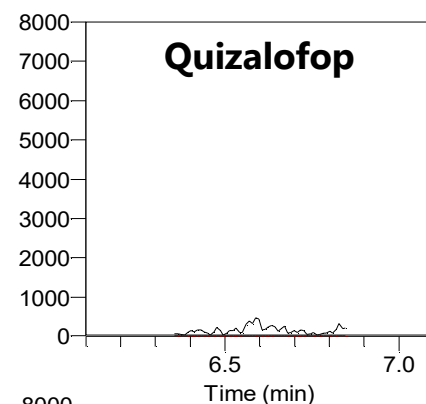
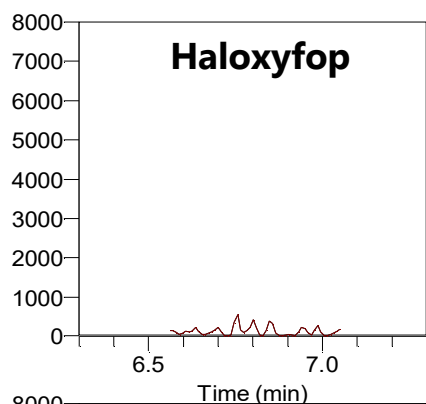
Optimization of PAL μ SPE QuEChERS clean-up workflow



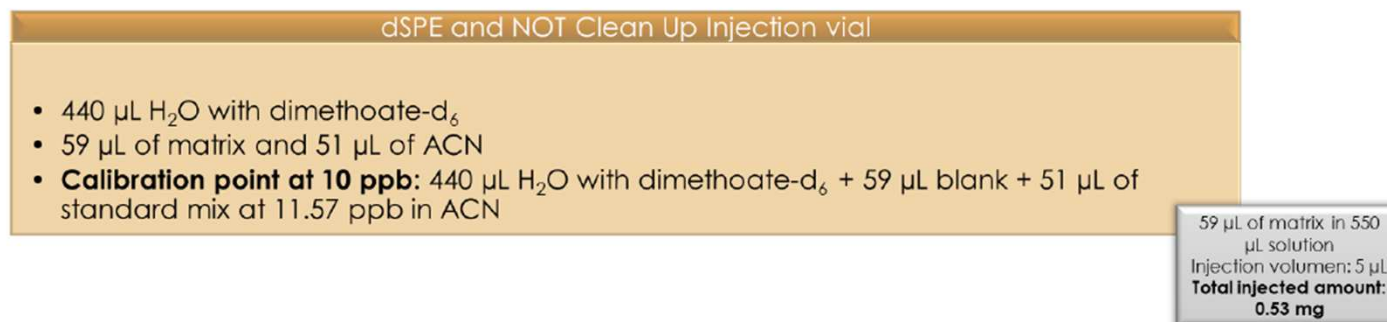
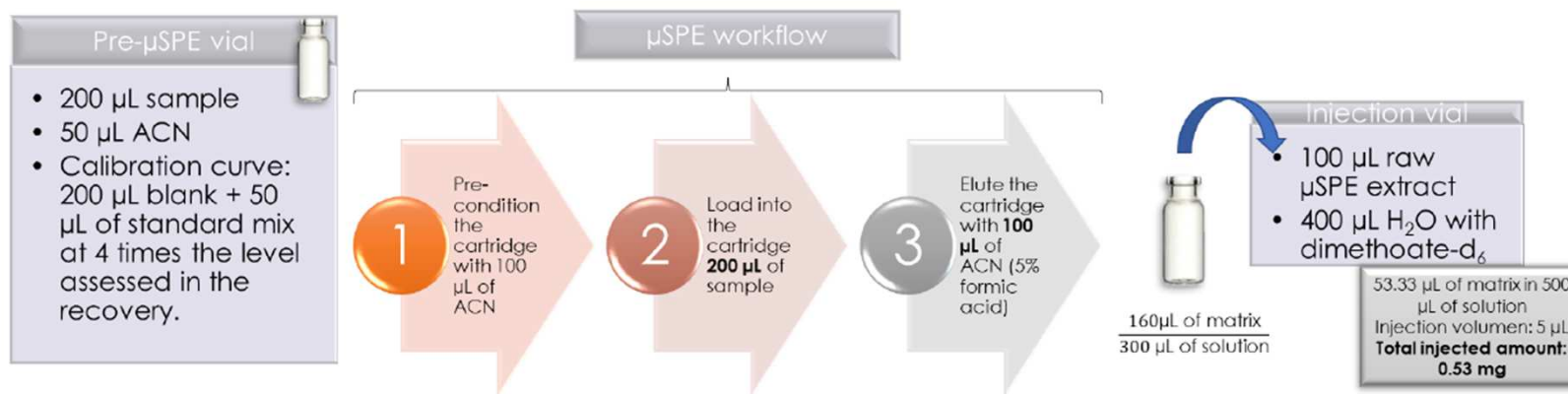
Tomato blank extract spiked at 10 ppb



*Triple
Quadrupole
TSQ Altis™*



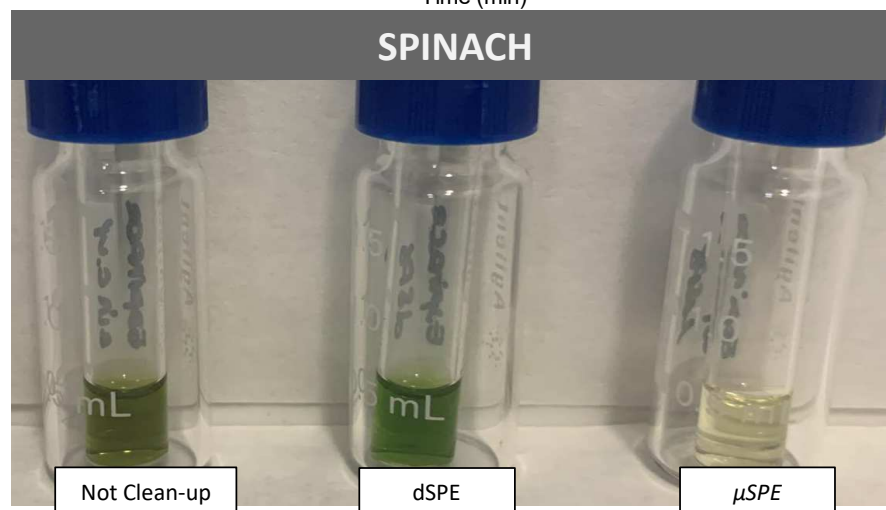
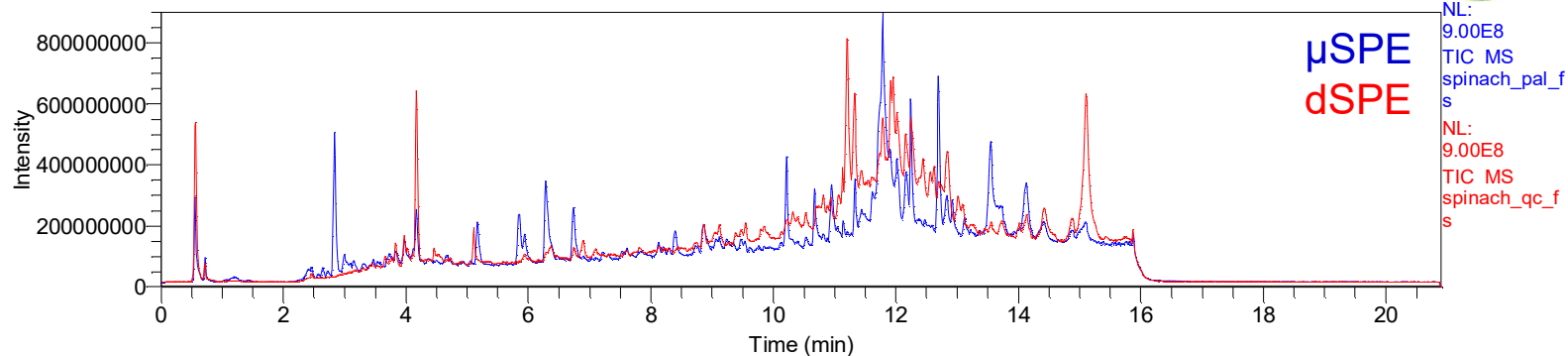
Comparison μ SPE and dSPE



TIC's comparative (dSPE extract vs μ SPE)

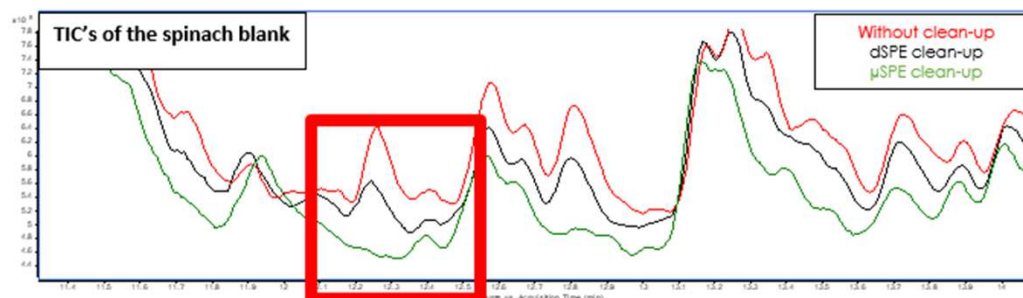


RT: 0.00 - 20.91 **Spinach**

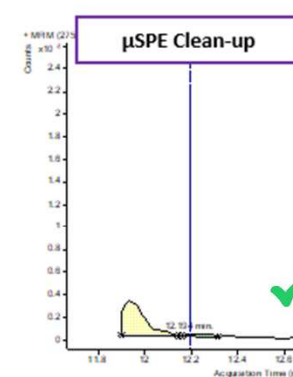
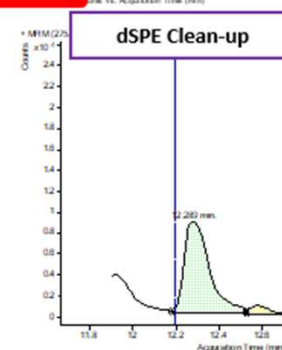
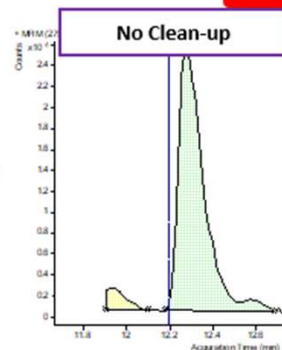


ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD

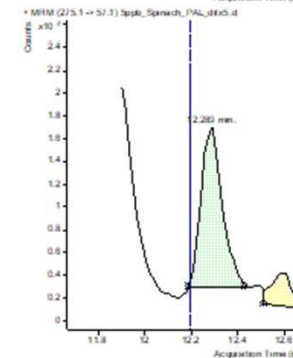
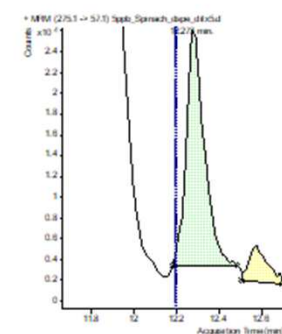
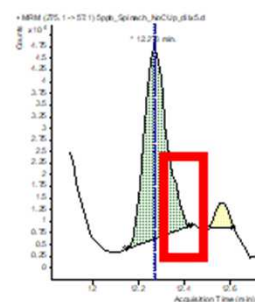
RT of Neburon



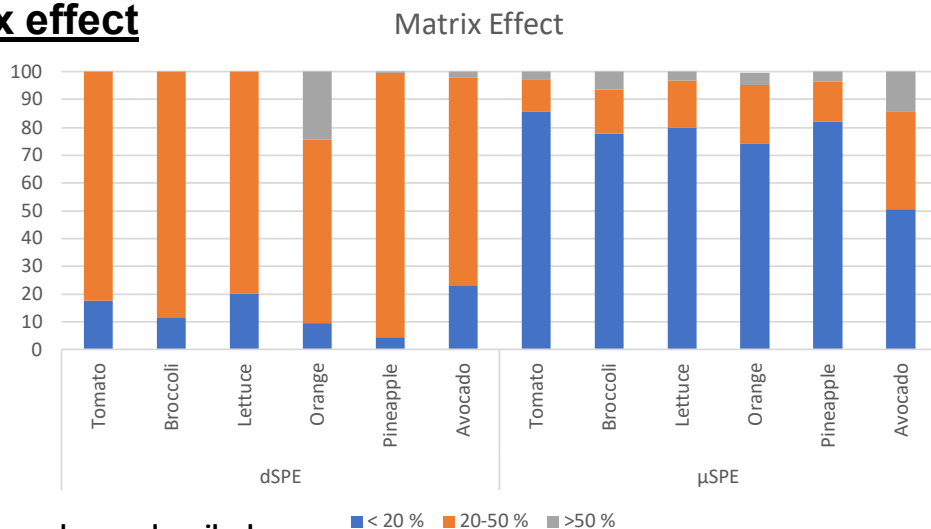
Interference in the spinach blank
275.1 > 57.1



5ppb Neburon (Spinach)
275.1 > 57.1

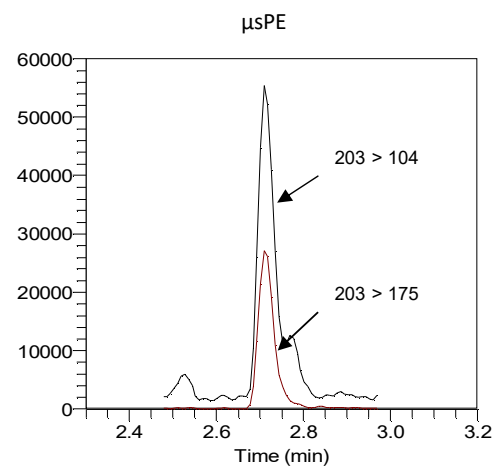
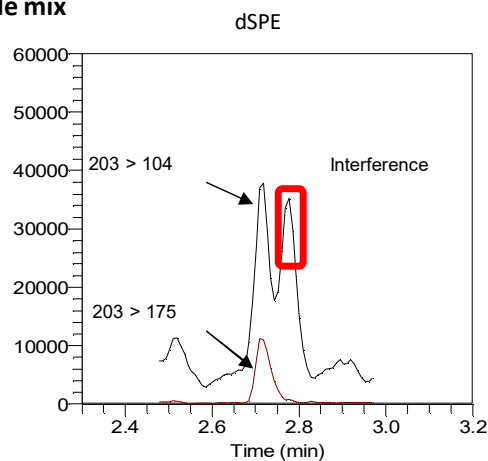
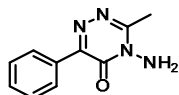


Matrix effect

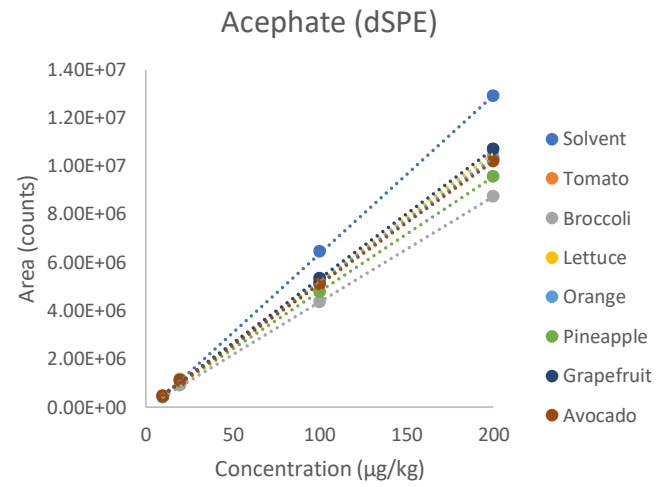
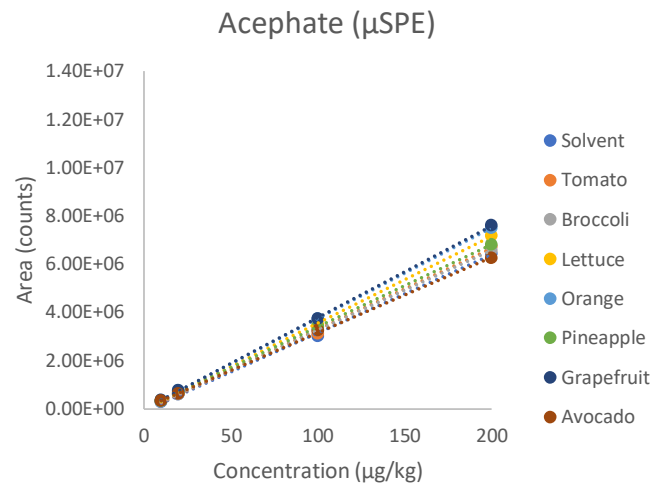


Blank avocado sample spiked at $10 \mu\text{g L}^{-1}$ with pesticide mix

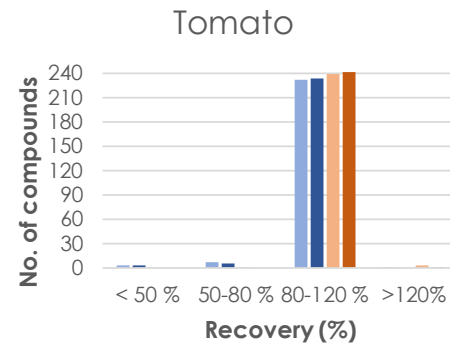
METAMITRON



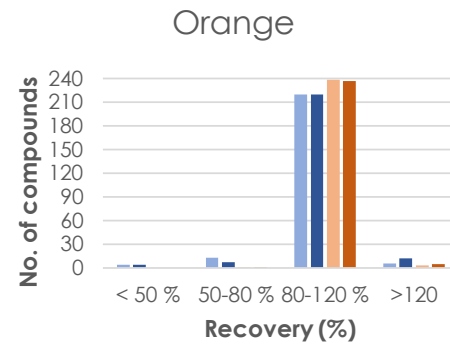
Linearity



Recoveries



■ dSPE (10 μ g/kg) ■ dSPE (50 μ g/kg)
 ■ μ SPE (10 μ g/kg) ■ μ SPE (50 μ g/kg)



■ dSPE (10 μ g/kg) ■ dSPE (50 μ g/kg)
 ■ μ SPE (10 μ g/kg) ■ μ SPE (50 μ g/kg)

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 Concentration (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
SULFOXAFLOL	0.029	-0.3

NA: Not Analysed.

GC

Módulo PAL RTC (online)



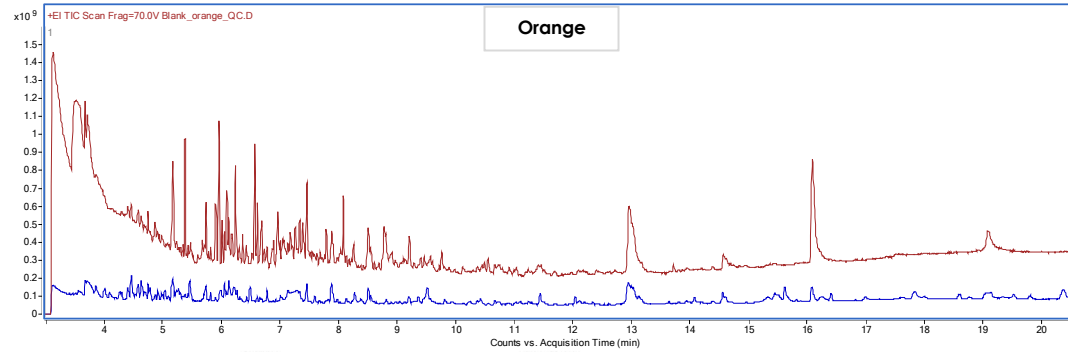
GC-QqQ

Módulo PAL RTC



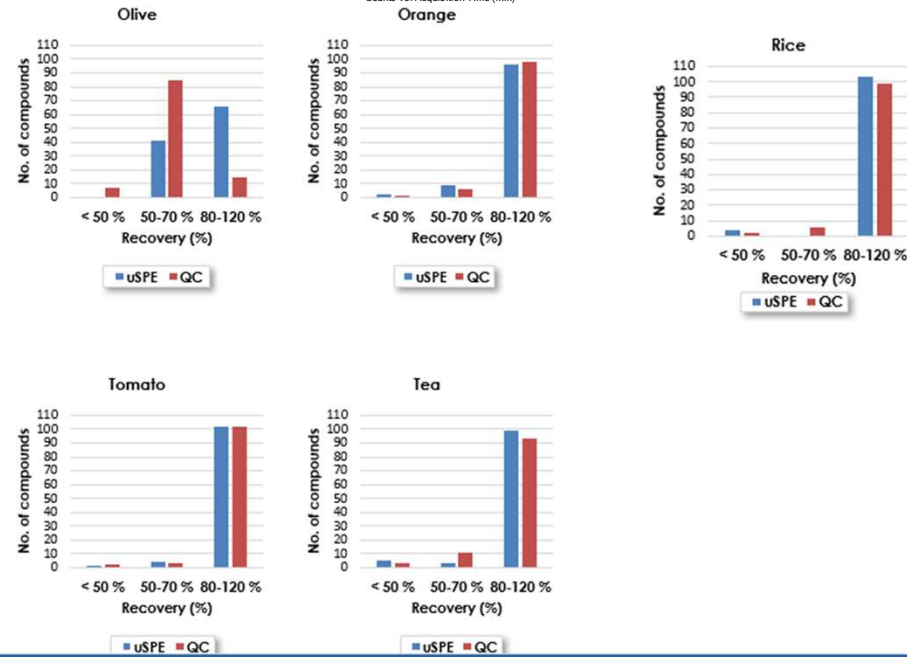
ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD

GC



dSPE

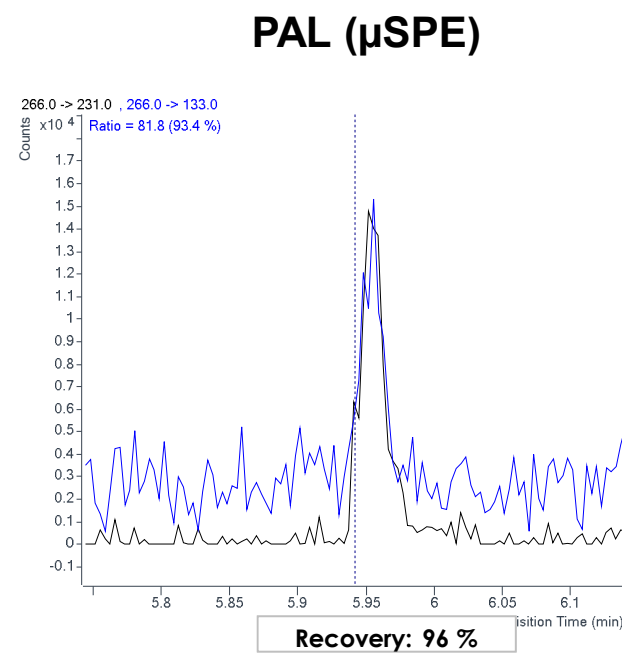
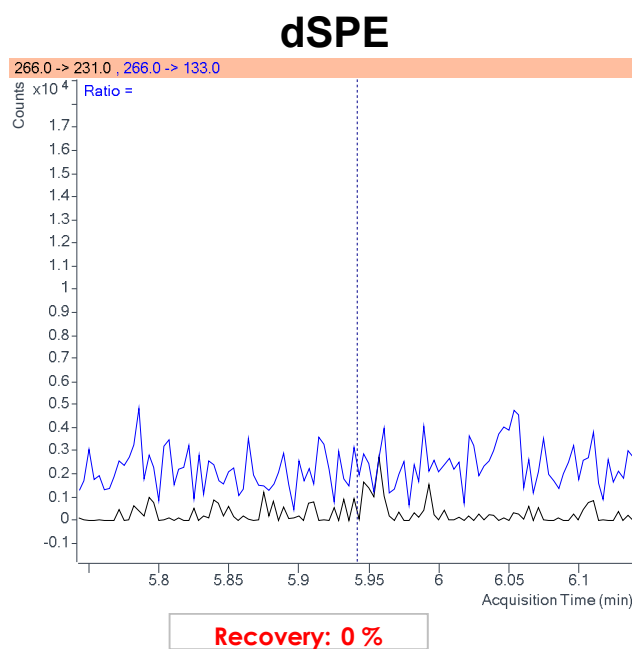
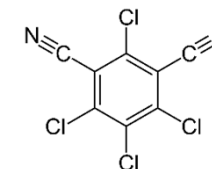
μ SPE





Chlorthalonil

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



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

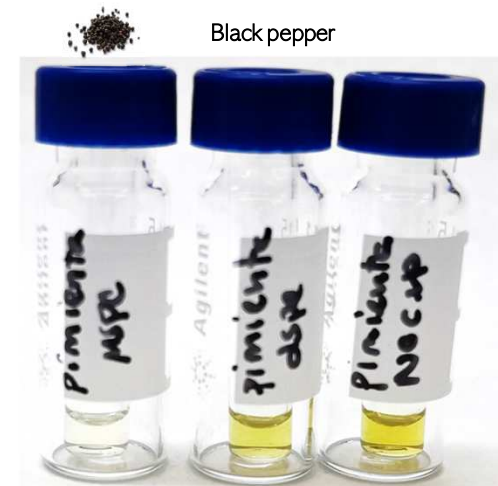
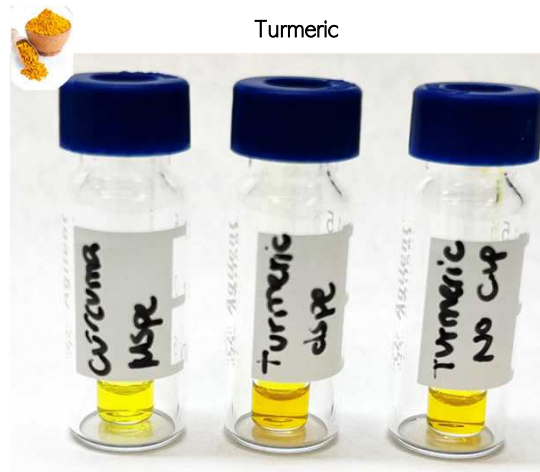
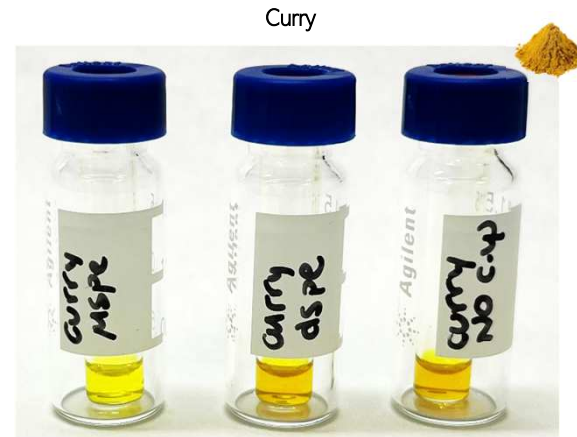
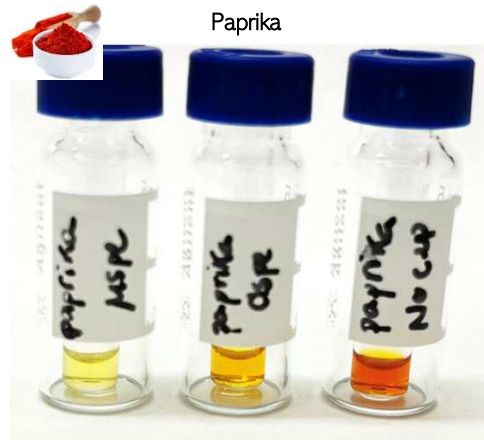
2 g of sample added 7 mL distilled water (vortex).	10 ml acetonitrile shaken for 6 minutes.	4 g anhydrous magnesium sulphate 1 g sodium chloride 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate	Axial agitator for 6 min and centrifuge at 3700 rpm for 5 min.
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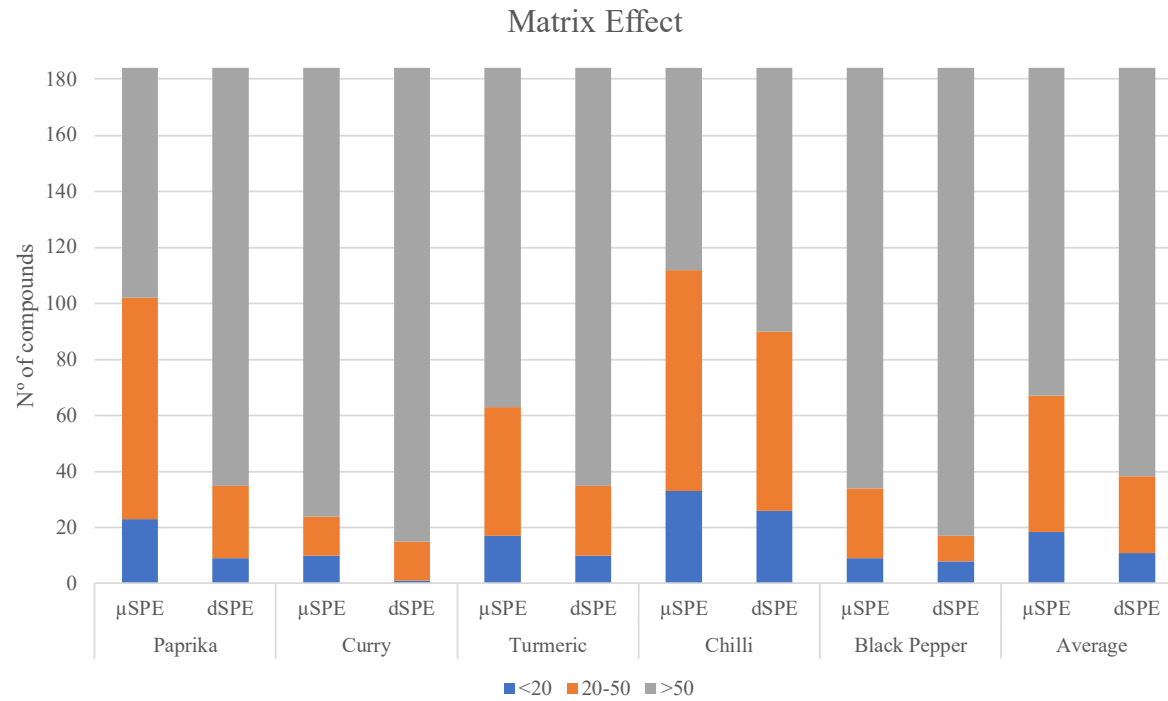
Step 2: Clean-up



Steps	
Conditioning	Syringe wash (3 cycles) with AcN
	Load 100 μL of AcN
	Condition μ SPE 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)

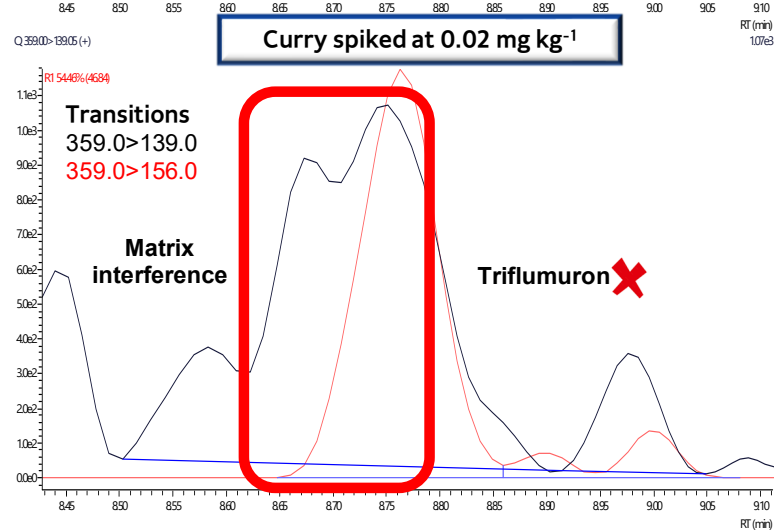
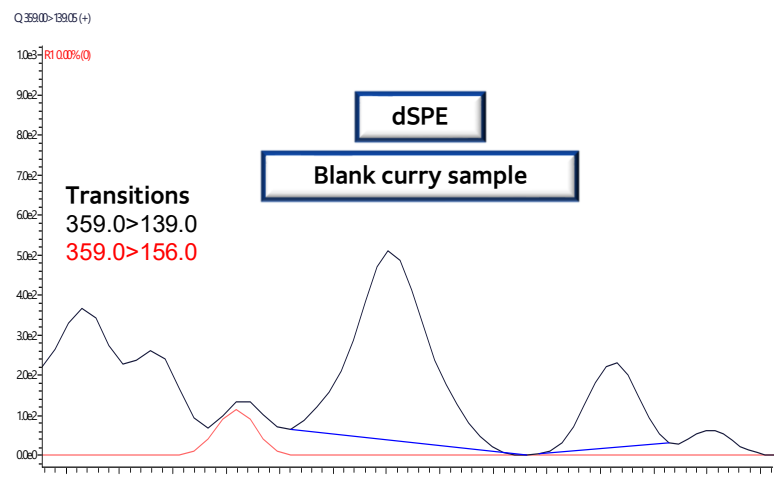
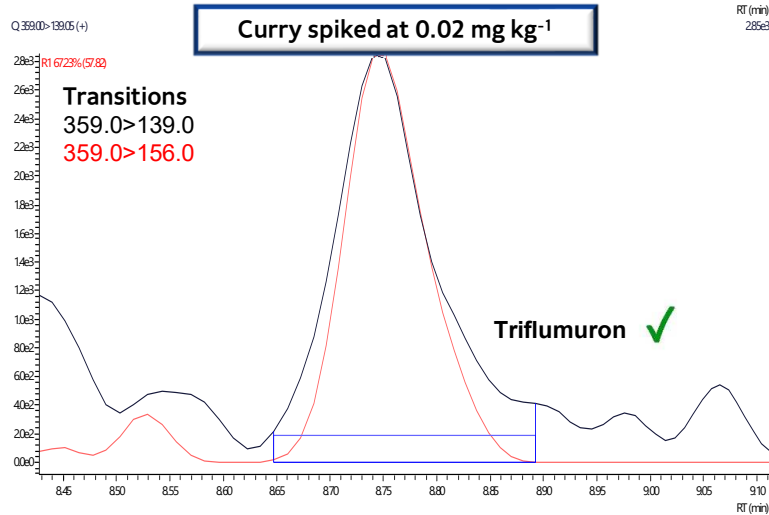
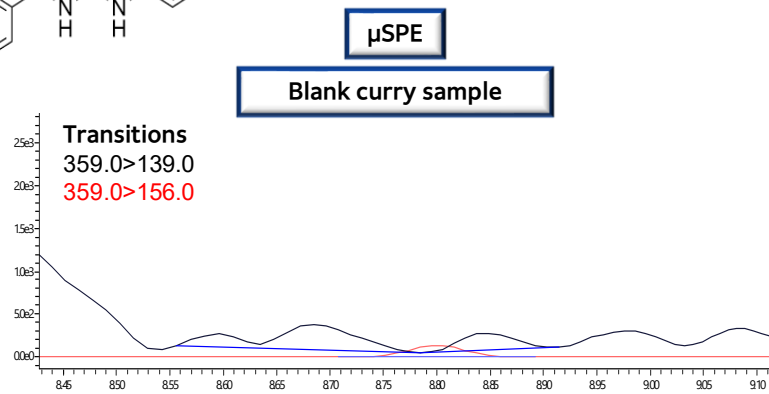
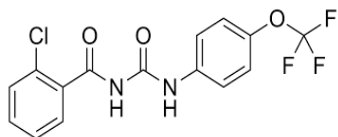
ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD



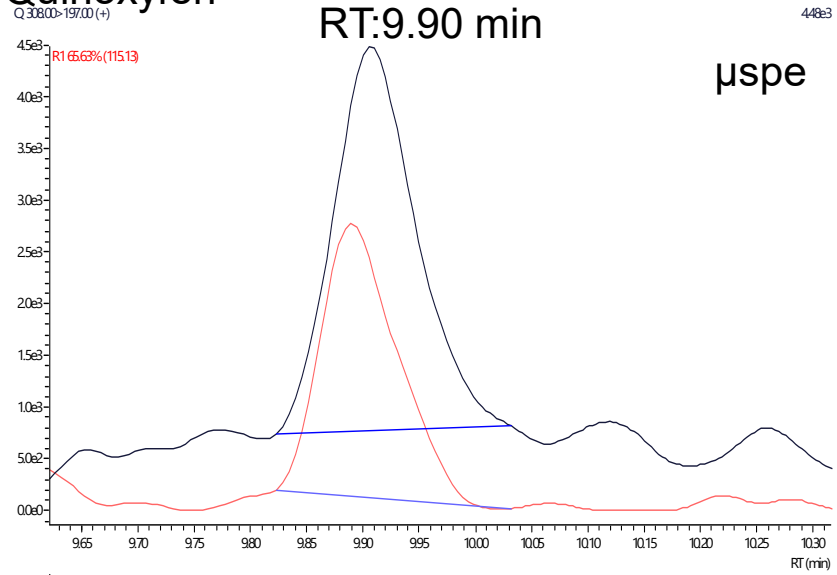


ANALYSIS OF SPICES USING AN AUTOMATED μ SPE CLEAN-UP METHOD

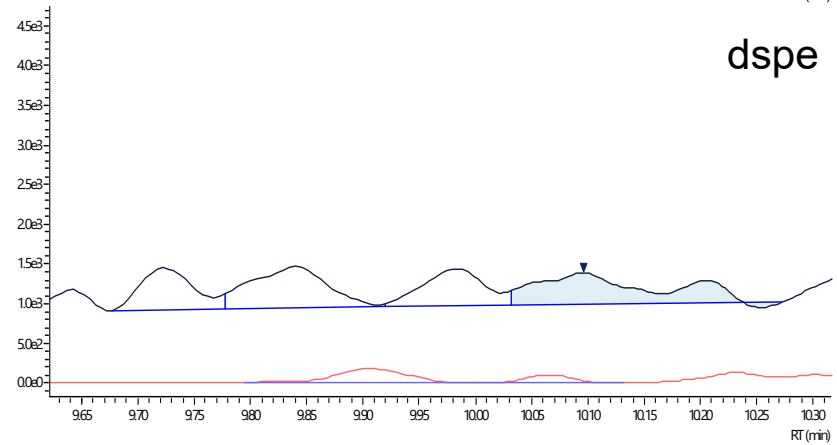
Triflumuron

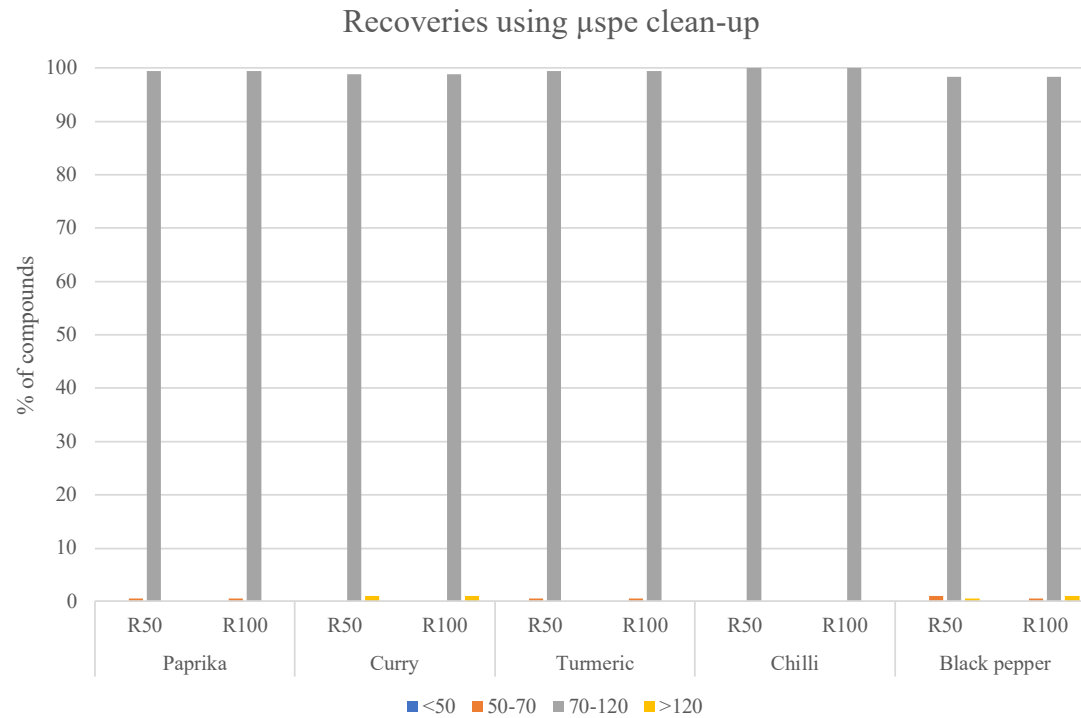


Curry spiked at 20 ppb Quinoxifen



High ion suppression





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.

**Thank You
for Your Attention**

