µSPE

PAL System µSPE GC QuEChERS-45-V Cartridges Performance Qualification Test

Note: This document states the analysis protocol for performance qualification test for µSPE-GCQuE1-45-V cartridges (PAL PN: uSPE-GCQuE1-45-V).

Contents

Chemicals and reagents

- Pesticides standard: AOAC QuEChERS QC Spike Mix (Restek cat# 31999)
- Solvents:
  - Acetonitrile (Sigma-Aldrich product number 34851)
  - Acetic acid (Sigma-Aldrich product number 695092)
  - Water (Sigma-Aldrich product number 270733)
- Analyte protectants
  - 3-ethoxy-1,2-propanediol, 98 % (Sigma-Aldrich product number 260428)
  - D-(+)-Gluconic acid δ-lactone, ≥ 99.0% (Sigma-Aldrich product number G4750)
  - D-Sorbitol, ≥ 98% (Sigma-Aldrich product number S1876)
- QuEChERS Extraction Salt: AOAC 2007.01 Method (Restek cat# 25851)

Solutions and reagents preparation

- Extraction solvent: 1 % acetic acid solution in acetonitrile
- Analyte protectant working solution (AP-Sol) was prepared as stated below.
  - 150 g 3-ethoxy-1,2-propanediol, 15 g D-(+)-Gluconic acid δ-lactone, and 15 g D-Sorbitol were dissolved in 15 ml acetonitrile:acetic acid: water=87:1:12 (v/v/v).
  - AP-Sol contains 10 mg/ml 3-ethoxy-1,2-propanediol, 1 mg/ml D-(+)-Gluconic acid δ-lactone, and 1 mg/ml D-Sorbitol.
- Pesticide QC working standard (QC-STD-1) was prepared either with or without AP-Sol as stated below.
  - Without AP-Sol: 30 µl AOAC QuEChERS QC Spike Mix was diluted with 1470 µl extraction solvent.
  - With AP-Sol: 30 µl AOAC QuEChERS QC Spike Mix and 300 µl AP-Sol was diluted with 1170 µl extraction solvent.
  - QC-STD-1 contains each pesticide analyte at concentration of 800 ng/ml.

Sample matrix

Japanese cucumbers used in this analysis were purchased from Singapore local groceries mart.

QuEChERS extraction

QuEChERS extraction is done manually according to below workflow.

Weigh 15 g of homogenized sample into a 50 ml extraction tube.

↓

Add 15 ml extraction solvent into the extraction tube.

↓

Vortex the extraction tube for 1 minute.

↓

Add extraction salt packet into the extraction tube.

↓

Vortex the extraction tube for 30 seconds.

↓

Shake vigorously by hand the extraction tube for 3 minutes.

↓

Centrifuge the extraction tube, 4500 rpm for 5 minutes.

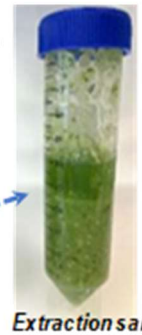
↓

Transfer the extract (supernatant) for µSPE clean-up analysis
or kept refrigerated until analysis.

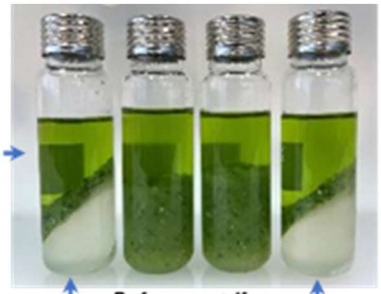
Homogenized cucumber

Sample in extraction tube.

Sample with extraction solvent after vortexed.



Extraction salt added, vortexed, and shaken by hand.



Before centrifuge.

After centrifuged. *After centrifuged.*

GC-MSMS system and chromatography data system

Analysis protocol was developed using an Agilent PAL RTC 120 coupled to Agilent 7890B GC with 7000D Triple Quadrupole GC/MS. Agilent MassHunter Workstation GC/MS Data Acquisition Version 10.1.49 was employed.

Scripts tested

- PAL System µSPE QuEChERS GC Online VAR AGI F31V01 R.xml
  - Agilent PAL Control Software Version 2.6.11.0 with PAL Firmware 3.1.21.1.
- PAL System µSPE QuEChERS GC Online VAR AGI F43V01 R.xml
  - Agilent PAL Control Software Version 2.7.6.0 with PAL Firmware 4.10.

Automated µSPE clean up setup

- µSPE Cartridges:
  - PAL System µSPE Cartridge – GC QuEChERS sorbent mix 1 – 45 mg
  - PAL Part No. µSPE-GCQuE1-45-V
  - Lot No. 617432
  - Expiry date: 13.12.2027

- Extract sample Vial put at sample tray:
  - 1.5 ml amber glass vial (PAL PN: Vial-1.5-ND9-AG-100)
    - Containing 1.2 ml extract sample (spiked/un-spiked)
  - Screw cap with 1.0 mm silicone/PTFE septa (PAL PN: Cap-ND9-PP-SP10-100)

- Collecting Vial put at Eluate Tray:
  - 0.3 ml Clear PP Micro-Vial with conical insert (PAL PN: Vial-0.3-ND9-PP-100)
  - Screw cap with 1.0 mm silicone/PTFE/I-slitted septa (PAL PN: Cap-ND9-PP-SP10S-100)

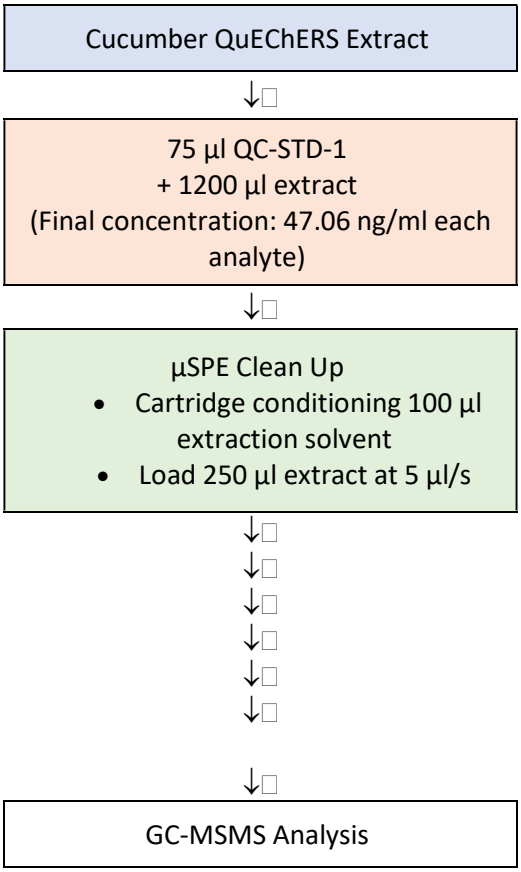
- µSPE Tool with 1000 µl syringe (syringe PAL PN: SF1000-57-T-22-FL)
- Liquid Tool D7/57 with 100 µl syringe (syringe PAL PN: SF100-57-T-23S-CO)
- Liquid Tool D7/57 with 10 µl syringe (syringe PAL PN: SF10-57-M-23S-CO)

Analysis

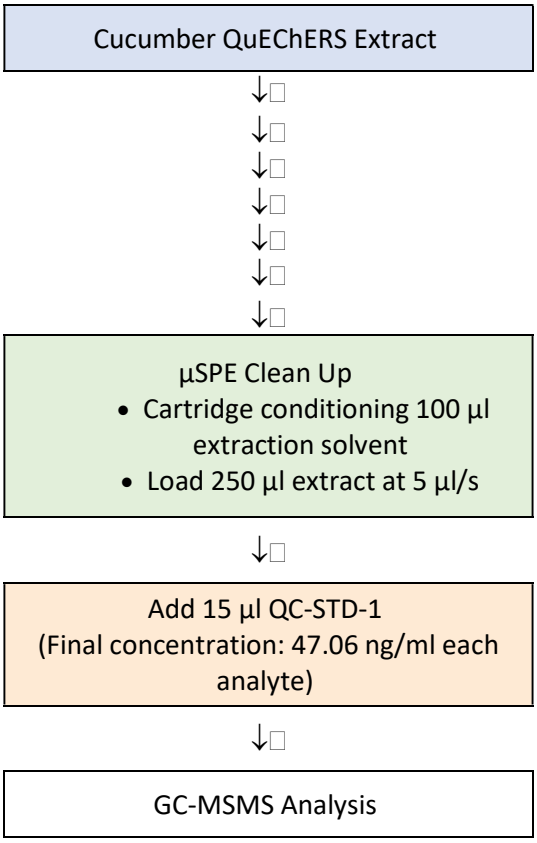
Two analyses are conducted, namely

- **Analysis 1** where QC-STD-1 is spiked into QuEChERS extract manually prior µSPE Clean Up procedure.
- **Analysis 2** where QC-STD-1 is spiked into cleaned-up eluate by PAL System automatically.

Analysis 1:
QC-STD-1 is spiked into QuEChERS extract prior µSPE Clean Up.



Analysis 2:
QC-STD-1 is spiked into cleaned-up eluate.



Recovery is calculated by comparing Quant Peak Area (or Average Quant Peak Area) observed in Analysis 1 versus Quant Peak Area (or Average Quant Peak Area) observed in Analysis 2, denoted in percent, for each analyte. All Quant Peak Area values were used in Recovery calculation as they were, without correction with surrogates or internal standards.

PAL parameters (PAL System µSPE QuEChERS GC Online VAR AGI F31V01 R.xml)

Parameters	Setting for analysis 1	Setting for analysis 2
Setup		
µSPE Tool	µSPE Tool with 1000 µl syringe	
Protectant / Standard tool	Liquid Tool with 25 µl or 100 µl syringe	
Injection tool	Liquid Tool with 10 µl syringe	
Fast wash module	Acetonitrile as wash solvent	
µSPE cartridge tray	Cartridge tray	
Dispose cartridge	No	No
Cartridge waste container	none	none
µSPE elution tray	Elution tray	
Eluate tray	Eluate tray	
Prep ahead	On	On
Bottom sensing	Off	Off
Conditioning		
Conditioning solvent source	Solvent Module / Wash Station	
Conditioning solvent index	Reservoir containing conditioning solvent	
Conditioning volume	100 µl	100 µl
Conditioning solvent fill speed	20 µl/s	20 µl/s
Conditioning speed	5 µl/s	5 µl/s
Conditioning solvent source penetration depth	45 mm	45 mm
Sample µSPE		
µSPE sample load volume	250 µl	250 µl
µSPE sample fill speed	20 µl/s	20 µl/s
µSPE sample load speed	5 µl/s	5 µl/s
µSPE sample vial penetration depth	30 mm	30 mm
Elution		
Elution solvent source	none	
Elution solvent index	1	
Elution volume	0 µl	0 µl
Elution solvent fill speed	20 µl/s	20 µl/s
Elution speed	2 µl/s	2 µl/s
Elution solvent source penetration depth	40 mm	40 mm
Analyte protectants		
Protectant 1 source	none	
Protectant 1 index	1	
Protectant 1 volume	0 µl	0 µl
Protectant 1 fill speed	5 µl/s	5 µl/s
Protectant 1 source penetration depth	30 mm	30 mm

Eluate vial penetration depth (protectant 1 addition)	28 mm	28 mm
Protectant 2 source	none	
Protectant 2 index	1	
Protectant 2 volume	0 µl	0 µl
Protectant 2 fill speed	5 µl/s	5 µl/s
Protectant 2 source penetration depth	30 mm	30 mm
Eluate vial penetration depth (protectant 2 addition)	28 mm	28 mm
Internal standard		
Internal standard source	Wash Station / sample tray	
Internal standard index	Reservoir / vial containing internal standard	
Internal standard volume*	***0 µl***	***15 µl***
Internal standard fill speed	2 µl/s	2 µl/s
Internal standard source penetration depth	30 mm	30 mm
Eluate vial penetration depth	28 mm	28 mm
Mixing		
Mix cycles after protectant / standard addition*	***3***	***5***
Mix speed	20 µl/s	20 µl/s
Mix volume	200 µl	200 µl
Injection		
Injection mode	Normal	Normal
Injection volume	3 µl	3 µl
Air volume	1 µl	1 µl
Rinse cycles with eluate	0	0
Filling strokes count	6	6
Eluate vial penetration depth	30 mm	30 mm
Eluate fill speed	2 µl/s	2 µl/s
Injector penetration depth	45 mm	45 mm
Injection speed	25 µl/s	25 µl/s
Injection signal mode	PlungerDown	PlungerDown
Preinject delay	5 s	5 s
Postinject delay	0 s	0 s
Syringe wash mode	Pre and PostWash	Pre and PostWash
Syringe wash cycles	3	3
Solvent selection wash step 1	Solvent1	Solvent1
Solvent selection wash step 2	Solvent2	Solvent2
Analysis time	GC ready-to-ready cycle time	

**** Parameter with different value for analysis 1 and analysis 2.***

PAL Parameters (PAL System µSPE QuEChERS GC Online VAR AGI F31V01 R.xml)

Parameters	Setting for analysis 1	Setting for analysis 2
Setup		
µSPE tool	µSPE Tool with 1000 µl syringe	
Protectant / standard tool	Liquid Tool with 25 µl or 100 µl syringe	
Injection tool	Liquid Tool with 10 µl syringe	
Fast wash module	Acetonitrile as wash solvent	
µSPE cartridge tray	Cartridge tray	
Dispose cartridge	No	No
Cartridge waste container	none	none
µSPE elution tray	Elution tray	
Bottom sensing	Off	Off
Conditioning		
Conditioning solvent source	Solvent Module / Wash Station	
Conditioning solvent index	Reservoir containing conditioning solvent	
Conditioning volume	100 µl	100 µl
Conditioning solvent fill speed	20 µl/s	20 µl/s
Conditioning speed	5 µl/s	5 µl/s
Conditioning solvent source penetration depth	45 mm	45 mm
Sample µSPE		
µSPE sample load volume	250 µl	250 µl
µSPE sample fill speed	20 µl/s	20 µl/s
µSPE sample load speed	5 µl/s	5 µl/s
µSPE sample vial penetration depth	30 mm	30 mm
Elution		
Elution solvent source	none	
Elution solvent index	1	
Elution volume	0 µl	0 µl
Elution solvent fill speed	20 µl/s	20 µl/s
Elution speed	2 µl/s	2 µl/s
Elution solvent source penetration depth	40 mm	40 mm
Analyte protectants		
Protectant 1 source	none	
Protectant 1 index	1	
Protectant 1 volume	0 µl	0 µl
Protectant 1 fill speed	5 µl/s	5 µl/s
Protectant 1 source penetration depth	30 mm	30 mm
Eluate vial penetration depth (protectant 1 addition)	28 mm	28 mm
Protectant 2 source	none	

Protectant 2 index	1	
Protectant 2 volume	0 µl	0 µl
Protectant 2 fill speed	5 µl/s	5 µl/s
Protectant 2 source penetration depth	30 mm	30 mm
Eluate vial penetration depth (protectant 2 addition)	28 mm	28 mm
Internal standard		
Internal standard source	Wash Station / sample tray	
Internal standard index	Reservoir / vial containing internal standard	
Internal standard volume*	***0 µl***	***15 µl***
Internal standard fill speed	2 µl/s	2 µl/s
Internal standard source penetration depth	30 mm	30 mm
Eluate vial penetration depth	28 mm	28 mm
Mixing		
Mix cycles after protectant / standard addition*	***3***	***5***
Mix speed	20 µl/s	20 µl/s
Mix volume	200 µl	200 µl
Injection		
Injection mode	Normal	Normal
Injection volume	3 µl	3 µl
Air volume	1 µl	1 µl
Rinse cycles with eluate	0	0
Filling strokes count	6	6
Eluate vial penetration depth	30 mm	30 mm
Eluate fill speed	2 µl/s	2 µl/s
Injector penetration depth	45 mm	45 mm
Injection speed	25 µl/s	25 µl/s
Injection signal mode	PlungerDown	PlungerDown
Preinject delay	5 s	5 s
Postinject delay	0 s	0 s
Syringe wash mode	Pre and PostWash	Pre and PostWash
Syringe wash cycles	3	3
Solvent selection wash step 1	Solvent1	Solvent1
Solvent selection wash step 2	Solvent2	Solvent2
Analysis time	GC ready-to-ready cycle time	

GC-MSMS Parameters

The GC-MSMS parameters in this section were employed on Agilent 7890B GC with 7000D Triple Quadrupole GC/MS. Identical parameters should be applied on any brands of GC-MSMS systems.

GC Parameters

- Analytical column: Rxi-5Sil MS (30 m, 0.25 mm ID, 0.25 µm df) (Restek Cat # 13623)
- Inlet:
  - Split-splitless inlet
  - Liner: Ultra inert splitless liner with single taper (Agilent PN 5190-2292)
  - Temperature: 280 °C
  - Pulsed splitless:
    - Injection pulse pressure at 300 kPa until 0.8 minute.
    - Purge flow to split vent at 5 ml/min at 1 minute.
  - Septum purge flow: 3 ml/min
- Column flow
  - Carrier gas: Helium
  - Constant flow at 1.2 ml/min
  - Post run flow at 1.5 ml/min
- Oven program:
  - Equilibration time: 1 minute
  - Initial temperature at 60 °C, hold for 1 minute.
  - Ramp to 170 °C at 50 °C/min.
  - Ramp to 280 °C at 10 °C/min, hold for 2 minutes.
  - Post run at 300 °C for 5 minutes.

MSMS Parameters

- MSD transfer line temperature: 280 °C
- Ion Source:
  - EI with extractor
  - Source temperature: 230 °C
  - Emission: 35 µA
  - Energy: 70 eV
- Quadrupole temperature: 150 °C
- Collision cell
  - Quench gas (helium) flow at 2.25 ml/min
  - Collision Gas (nitrogen) flow at 1.5 ml/min
- Below the MRM transitions for pesticide analytes monitored.

Analyte	Retention time (minutes)	MRM transition	Collision energy (V)	*Dwell (ms)
Methamidophos	4.22	**m/z 141 → 95	5	82.5
Methamidophos	4.22	m/z 95 → 79	10	82.5
Methamidophos	4.22	m/z 95 → 64	10	82.5
Dichlorvos	4.29	m/z 184.9 → 93	10	82.4
Dichlorvos	4.29	m/z 144.9 → 109	10	82.4
Dichlorvos	4.29	**m/z 109 → 79	5	82.4
Carbaryl	5.71	m/z 144 → 116.1	10	110.3
Carbaryl	5.71	**m/z 144 → 115.1	20	110.3
Carbaryl	5.71	m/z 116 → 115.1	10	110.3
Cyprodinil	9.85	m/z 226.2 → 225.3	10	21.9
Cyprodinil	9.85	**m/z 225.2 → 224.3	10	21.9
Cyprodinil	9.85	m/z 224.2 → 208.2	20	21.9
Thiabendazole	10.15	m/z 201.9 → 175	15	24
Thiabendazole	10.15	**m/z 201 → 174	15	24
Thiabendazole	10.15	m/z 173.9 → 65	30	24
Imazalil	10.95	m/z 216.8 → 175	5	25.8
Imazalil	10.95	**m/z 214.9 → 173	5	25.8
Imazalil	10.95	m/z 172.9 → 145	15	25.8
2,4'-DDD	11.23	m/z 237 → 165.2	20	265
2,4'-DDD	11.23	m/z 235 → 200.2	10	26.5
2,4'-DDD	11.23	**m/z 235 → 165.2	20	26.5
Endosulfan sulfate	12.47	m/z 273.8 → 238.9	15	45.4
Endosulfan sulfate	12.47	m/z 273.8 → 236.9	15	45.4
Endosulfan sulfate	12.47	**m/z 271.9 → 237	15	45.4
Tebuconazole	12.70	**m/z 250 → 125	20	45.4
Tebuconazole	12.70	m/z 125 → 99	20	45.4
Tebuconazole	12.70	m/z 125 → 89	15	45.4
Bifenthrin	13.33	m/z 181.2 → 166.2	10	64
Bifenthrin	13.33	**m/z 181.2 → 165.2	25	64
Bifenthrin	13.33	m/z 166.2 → 165.2	20	64
Lambda-Cyhalothrin	14.29	**m/z 208 → 181	5	64
Lambda-Cyhalothrin	14.29	m/z 197 → 141	10	64
Lambda-Cyhalothrin	14.29	m/z 181.1 → 152	25	64

**Dwell time was assigned by MassHunter Dynamic MRM (d-MRM) according to total MRM transitions.*
***Quant MRM transition in the analyses; auto selected based on peak area response.*

Criteria

Analyte	Group	Expected recovery, %
Methamidophos	Organophosphorus	70 % - 90 %
Dichlorvos	Organophosphorus	70 % - 90 %
Carbaryl	N-Methyl Carbamate	65 % - 85 %
Cyprodinil	Pyrimidine	< 60 %
Thiabendazole	Benzimidazole	< 60 %
Imazalil	Conazole	60 % - 80 %
Tebuconazole	Conazole	60 % - 80 %
2,4'-DDD	Organochlorine	65 % - 85 %
Endosulfan Sulfate	Organochlorine	65 % - 85 %
Bifenthrin	Pyrethroid	60 % - 80 %
Lambda-Cyhalothrin	Pyrethroid	60 % - 80 %

Example

Example analysis sequence

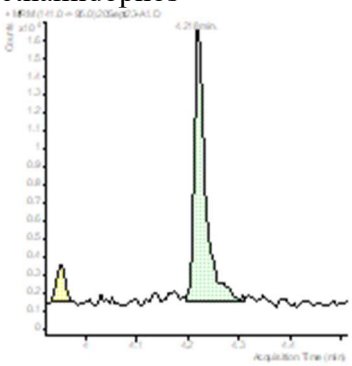
Below the example sequence batch to be executed.

Sequence	Sample
01	Solvent Blank
02	Matrix Blank
03	Analysis 1-Replicate 1
04	Analysis 1-Replicate 2
05	Analysis 1-Replicate 3
06	Solvent Blank
07	Analysis 2-Replicate 1
08	Analysis 2-Replicate 2
09	Analysis 2-Replicate 3

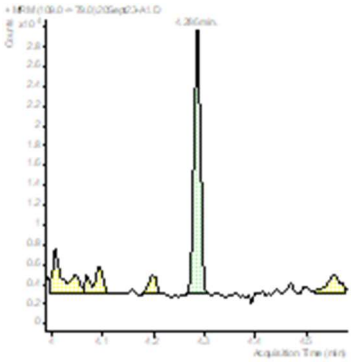
Example chromatograms

The example chromatograms of Quant MRM transition for each analyte were shown as below.

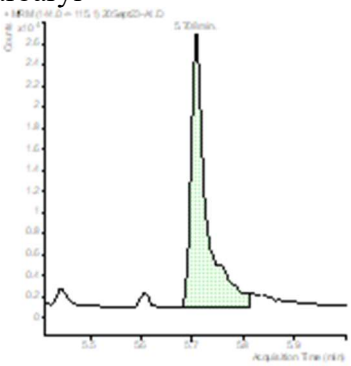
Methamidophos



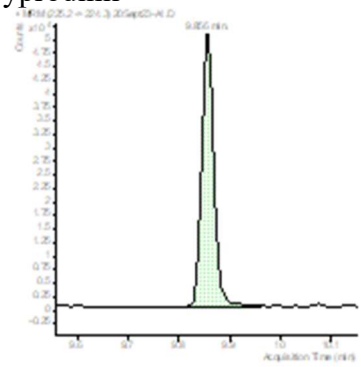
Dichlorvos



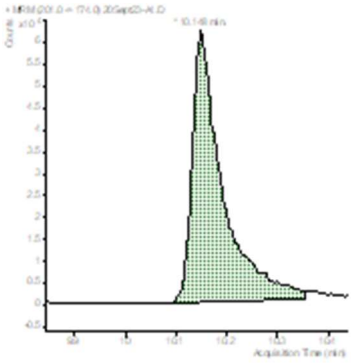
Carbaryl



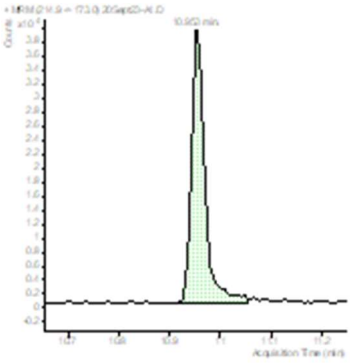
Cyprodinil



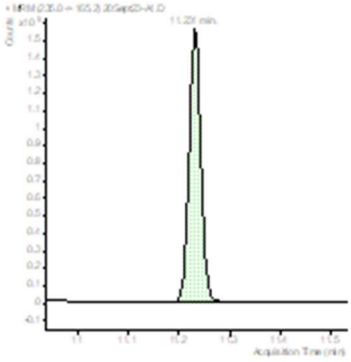
Thiabendazole



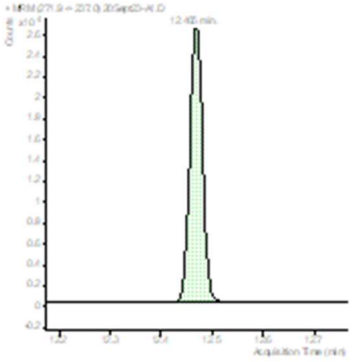
Imazalil



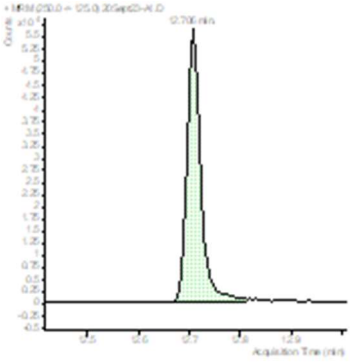
2,4'-DDD



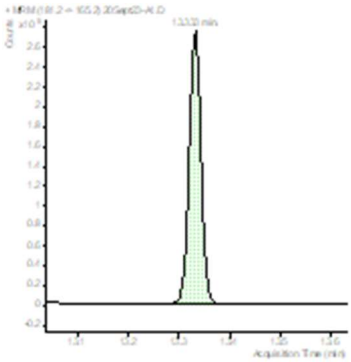
Endosulfan sulfate



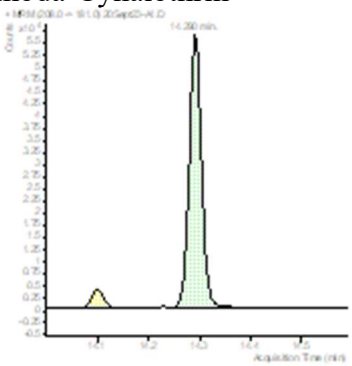
Tebuconazole



Bifenthrin



lambda-Cyhalothrin



Example recovery calculation

One example recovery calculation was shown as below.

The pesticide analyte was Methamidophos, and the expected recovery ranged from 70 % to 90 %. In this batch, there were 6 replicates in analysis 1 and 6 replicates in analysis 2. Thus, average quant peak areas were used for calculating the recovery (%).

	Quant Peak Area	
	Analysis 1	**Analysis 2**
Replicate 1	40312	48186
Replicate 2	39126	51590
Replicate 3	44040	45057
Replicate 4	36845	48999
Replicate 5	38589	42102
Replicate 6	38683	47729
Average	**39599.2**	**47277.2**
Std Dev	**2445.3**	**3295.7**
%RSD	**6.18**	**6.97**
***Recovery, %**	**83.76**	

$$*\text{Recovery (\%)} = \frac{[\text{Average Quant Peak Area Analysis 1}]}{[\text{Average Quant Peak Area Analysis 2}]} \times 100\%$$

Precautions

- The extract from QuEChERS extraction should be kept refrigerated until analysis. However, the refrigerated storage should not be longer than 5 days.
- Pesticide QC Working Standard (QC-STD-1) should be prepared freshly prior analysis. Once spiking of QC-STD-1 into QuEChERS extract (for Analysis 1), the analysis sequence batch should be initiated sooner possible.

Change history / new features

PAL System µSPE GC QuEChERS-45-V Cartridges Performance Qualification Test

Original version.