



μSPE

PAL System
μSPE LCQuE1-30-T Cartridges
Performance Qualification Test

Note: This document states the analysis protocol for performance qualification test for μSPE-LCQuE1-30-T cartridges (PAL PN: μSPE-LCQuE1-30-T).

Performance Qualification Tests for μSPE-LCQuE1-30-T cartridges

This document states the analysis protocol for performance qualification test for μSPE-LCQuE1-30-T Cartridges (PAL PN: μSPE-LCQuE1-30-T).

Chemicals & 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)
- QuEChERS Extraction Salt: AOAC 2007.01 Method (Restek cat# 25851)

Solutions & Reagents Preparation

- Extraction Solvent: 1 % acetic acid solution in acetonitrile
- Pesticide QC Working Standard (QC-STD-1) was prepared as below:
 - 30 μl AOAC QuEChERS QC Spike Mix was diluted with 1470 μl extraction solvent.
 - QC-STD-1 contains each pesticide analyte at concentration of 800 ng/ml.

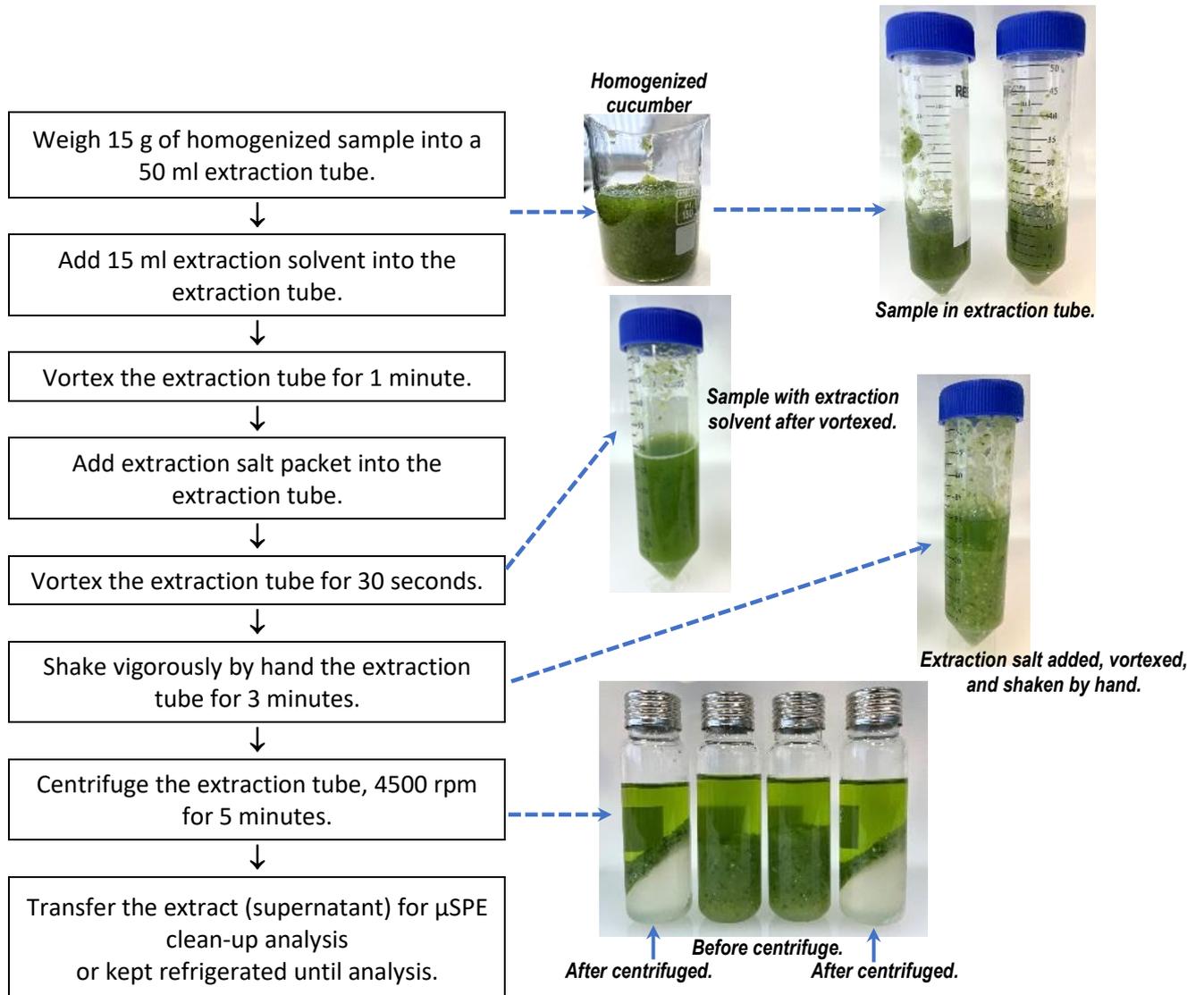
Sample Matrix

Cucumbers used in this analysis were purchased from German local groceries mart.



QuEChERS Extraction

QuEChERS extraction is done manually according to below workflow.



LC/MS/MS System & Chromatography Data System

Analysis protocol was developed using a PAL RTC 120 coupled to Agilent 1290 binary LC pump and SCIEX 6500+ Triple Quadrupole LC/MS. SCIEX Analyst Data Acquisition Version 1.6.3 was employed. The PAL System had firmware version 3.1.22.

Scripts Tested

- PAL System μ SPE QuEChERS LC Online VAR CTC F31V01 R.xml

Automated μ SPE Clean Up Setup

- μ SPE Cartridges:
 - PAL System μ SPE Cartridge – LC QuEChERS sorbent mix 1 – 30 mg
 - PAL Part No. μ SPE-LCQuE1-30-T
 - Lot no. 664713
 - Expiry date: 14.09.2024
- Extract sample vial put at sample tray:
 - 1.5 ml amber glass vial (PAL PN: Vial-1.5-ND9-AG-100)
 - Containing 600 μ l 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:
 - 1.5 ml amber glass vial (PAL PN: Vial-1.5-ND9-AG-100)
 - Screw cap with 1.0 mm silicone/PTFE/l-slitted septa (PAL PN: Cap-ND9-PP-SP10S-100)
- μ SPE Tool with 1000 μ l syringe (syringe PAL PN: SF1000-57-T-22-FL)
- Liquid Tool D7/57 with 100 μ l syringe (syringe PAL PN: SF100-57-T-23S-CO)
- LC/MS Tool with KNF pump and 100 μ l sample loop (PAL PN: PAL3-TH-SLCMS-P-100)
- Valve for LC/MS injection:
 - Cheminert valve 6-Port 0.25 mm 15000 psi, incl. one-piece PEEK needle seal G22, TubeVlvWaste (PAL PN: PAL3-C82VX-1676D-CTC-K)
 - 20 μ l sample loop (PAL PN: CSL20-CTC)

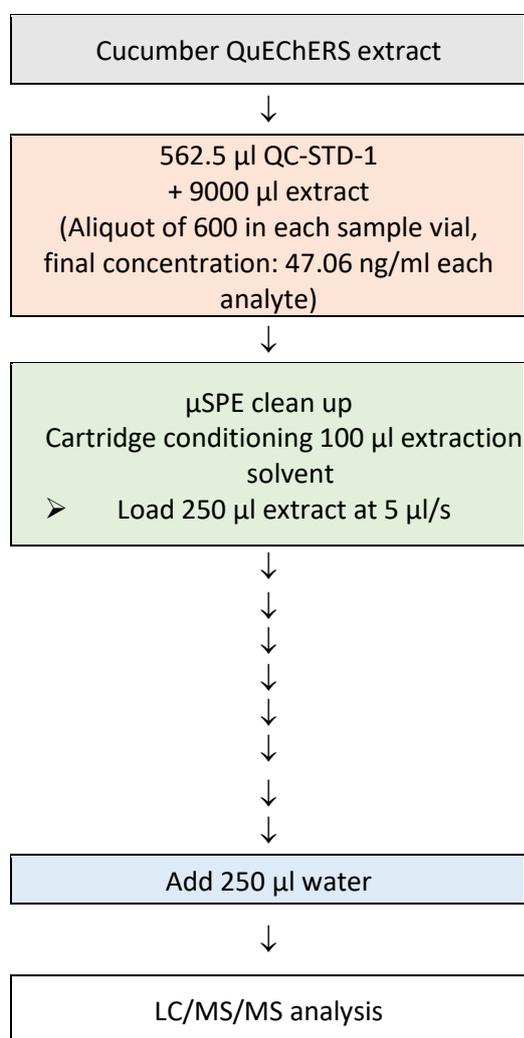
Analysis

Two analyses are conducted, namely

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

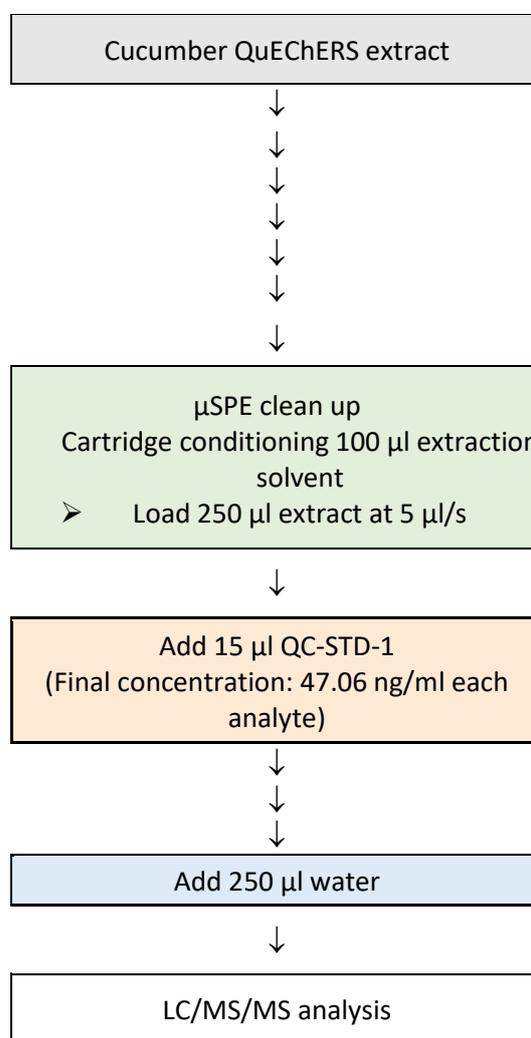
Analysis 1:

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 was used in recovery calculation as it was, without correction with surrogates or internal standards.

Water was added to sample before injection to avoid strong solvent effect.

PAL Parameters (PAL System μ SPE QuEChERS LC Online VAR CTC F31V01 R.xml)

Parameter	Setting for Analysis 1	Setting for Analysis 2
Setup		
μ SPE Tool / water dilution tool	μ SPE Tool with 1000 μ l syringe	
QC Standard tool	Liquid Tool with 25 μ l or 100 μ l syringe (LS9)	
Injection tool	LC/MS Tool with 100 μ l loop (LCP1)	
Fast wash module	Acetonitrile as wash solvent	
μ SPE cartridge tray	Cartridge tray	
Dispose cartridge	No	No
Cartridge waste container	none	none
μ SPE 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 μ l	100 μ l
Conditioning solvent fill speed	20 μ l/s	20 μ l/s
Conditioning speed	5 μ l/s	5 μ l/s
Conditioning solvent source penetration depth	40 mm	40 mm
Sample μSPE		
μ SPE sample load volume	250 μ l	250 μ l
μ SPE sample fill speed	20 μ l/s	20 μ l/s
μ SPE sample load speed	5 μ l/s	5 μ l/s
μ SPE sample vial penetration depth	30 mm	30 mm
Elution		
Elution solvent source	none	
Elution solvent index	1	
Elution volume	0 μ l	0 μ l
Elution solvent fill speed	20 μ l/s	20 μ l/s
Elution speed	2 μ l/s	2 μ l/s
Elution solvent source penetration depth	40 mm	40 mm
Internal standard		
Internal standard source	Wash Station / Sample Tray	
Internal standard index	Reservoir / vial containing internal standard	
Internal standard volume*	0 μ l	15 μ l
Internal standard fill speed	1 μ l/s	1 μ l/s
Internal standard source penetration depth	30 mm	30 mm
Eluate vial penetration depth	28 mm	28 mm
Sample dilution with water		
Water source	Solvent Module / Wash Station	
Water index	Reservoir containing water	

Parameter	Setting for Analysis 1	Setting for Analysis 2
Water volume	250 μ l	250 μ l
Water fill speed	20 μ l/s	20 μ l/s
Water source penetration depth	40 mm	40 mm
Eluate vial penetration depth	28 mm	28 mm
Mixing		
Mix cycles after protectant / standard addition*	3	5
Mix speed	50 μ l/s	50 μ l/s
Mix volume	500 μ l	500 μ l
LC/MS injection		
Front air gap	1 μ l	1 μ l
Front volume	3 μ l	3 μ l
Rear volume	3 μ l	3 μ l
Rear air gap	1 μ l	1 μ l
Sample injection speed	1 μ l/s	1 μ l/s
Check sample pickup	No	No
Bottom sensing	No	No
Activate needle dipping	No	No
Wash performance	1 (Standard)	
Valve clean solvent 1	90 μ l water	90 μ l water
Valve clean solvent 1	180 μ l ACN	180 μ l ACN
Stator wash	No	No
Enable object detection	Yes	Yes
Clogged valve detection	No	No

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

LC/MS/MS Parameters

The LC/MS/MS parameters in this section were employed on Agilent 1290 binary LC pump with SCIEX 6500+ Triple Quadrupole LC/MS. Identical parameters should be applied on any brands of LC/MS/MS systems.

LC Parameters

- Flow rate: 0.4 ml/min
- Mobile phases:
 - Mobile phase A: 98:2 water/methanol + 0.1 % FA
 - Mobile phase B: Methanol + 0.1 % FA
- Gradient: 0 - 0.25 min 5 % B, 7.75 - 8.50 min 100 % B, 9.51 – 12.00 min 5 % B
- Analytical column: Waters ACQUITY BEH C18, 2.1 x 100 mm, 1.7 μ m (Part number 186002352)
- Column temperature: 50 °C
- Injection volume: 1 μ l
- Injection mode: partial loop filling

MS/MS Parameters

- Scan type: MRM
- Polarity: positive
- Curvature: 20.00
- Ion source: ESI
- Temperature: 450.00
- Gas source 1: 60.00
- Gas source 2: 50.00
- CAD: 9.00
- Desolvation parameter: 60.00
- Entrance potential: 10.00
- Ion quard 1: -10.50
- Collision cell exit potential: 10.00

Analyte	Retention time (minutes)	MRM transition	Collision energy (V)	*Dwell (ms)
Atrazine	6.15	m/z 216.1 \rightarrow 174.1	18	20
Azoxystrobin	6.57	m/z 404 \rightarrow 372	15	20
Carbaryl	5.79	m/z 202 \rightarrow 145	22	20
Chlorpyrifos	8.05	m/z 349.9 \rightarrow 97	32	20
Chlorpyrifos-methyl	7.65	m/z 321.8 \rightarrow 125	20	20
Cyprodinil	6.72	m/z 226 \rightarrow 93	33	20
Dichlorvos	5.51	m/z 221 \rightarrow 109	22	20
Ethion	7.96	m/z 384.9 \rightarrow 143	35	20
Imazalil	5.64	m/z 297 \rightarrow 159	22	20
Imidacloprid	4.02	m/z 256.1 \rightarrow 175.1	20	20
Linuron	6.64	m/z 249.1 \rightarrow 160.1	18	20
Methamidophos	1.80	m/z 142 \rightarrow 93.9	13	20
Methomyl	3.32	m/z 163 \rightarrow 88	10	20
Procymidone	7.07	m/z 284.1 \rightarrow 67.1	28	20
Pymetrozine	2.17	m/z 218 \rightarrow 105	20	20
Tebuconazole	7.37	m/z 308 \rightarrow 70.1	22	20
Thiabendazole	3.41	m/z 202 \rightarrow 175	25	20
Tolylfluanid	7.33	m/z 347 \rightarrow 137	28	20

**Different dwell time might be needed for different types of MS instruments.*

Criteria

Analyte	Group	Expected recovery, %
Atrazine	Others	60% - 80%
Azoxystrobin	Others	60% - 80%
Carbaryl	Carbamate	60% - 80%
Chlorpyrifos	Organophosphorus	< 60%
Dichlorvos	Organophosphorus	65% - 85%
Imidacloprid	Neonicotinoid	65% - 85%
Methamidophos	Organophosphorus	65% - 85%
Methomyl	Carbamate	65% - 85%
Pymetrozine	Pyridine	< 60%
Tolyfluanid	Others	60% - 80%

Example

Example Analysis Sequence

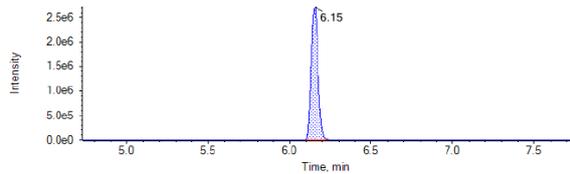
Below the example sequence batch to be executed.

<u>Sequence</u>	<u>Sample</u>
01	Direct injection of diluted standard mix
02	Analysis 2-Replicate 1
03	Analysis 1-Replicate 1
04	Analysis 2-Replicate 2
05	Analysis 1-Replicate 2
06	Analysis 2-Replicate 3
07	Analysis 1-Replicate 3
08	Analysis 2-Replicate 4
09	Analysis 1-Replicate 4
10	Direct injection of diluted standard mix
11	Analysis 2-Replicate 5
12	Analysis 1-Replicate 5
13	Analysis 2-Replicate 6
14	Analysis 1-Replicate 6
...	...

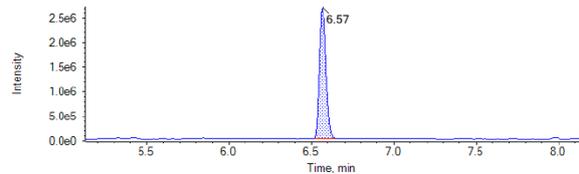
Example Chromatograms

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

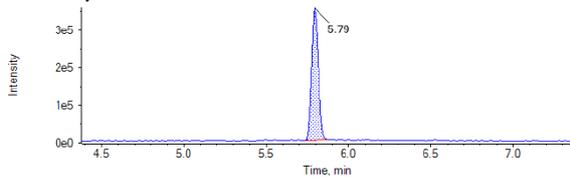
Atrazine



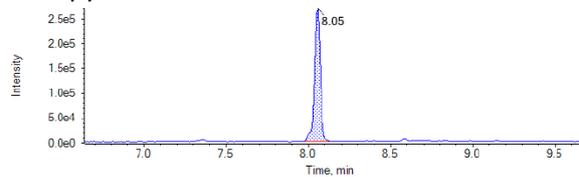
Azoxystrobin



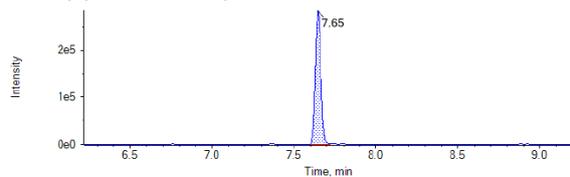
Carbaryl



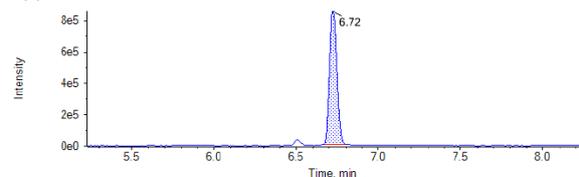
Chlorpyrifos



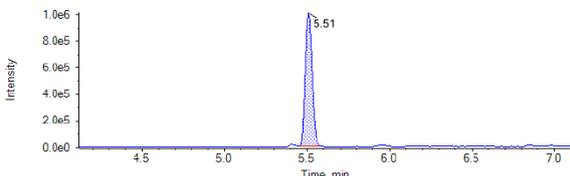
Chlorpyrifos-methyl



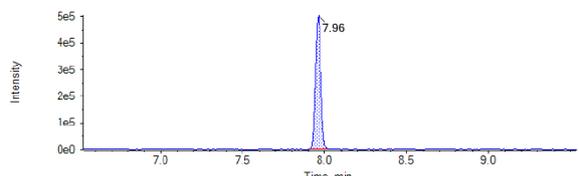
Cyprodinil



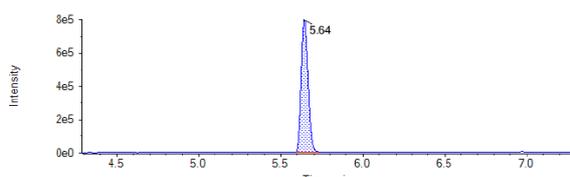
Dichlorvos



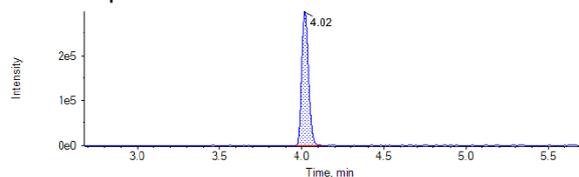
Ethion



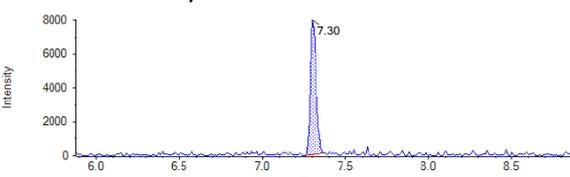
Imazalil



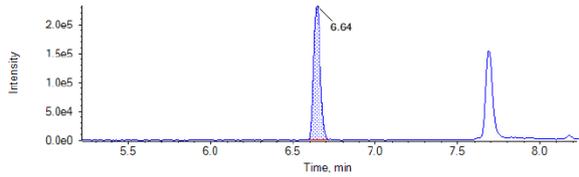
Imidacloprid



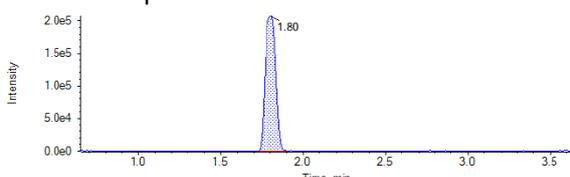
Kresoxim-methyl



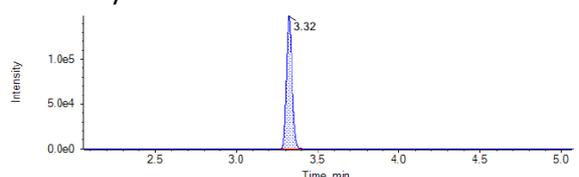
Linuron



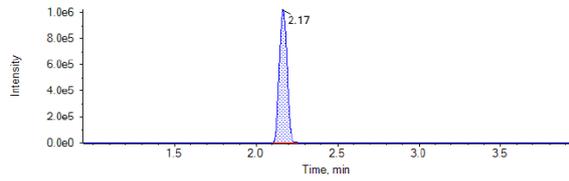
Methamidophos



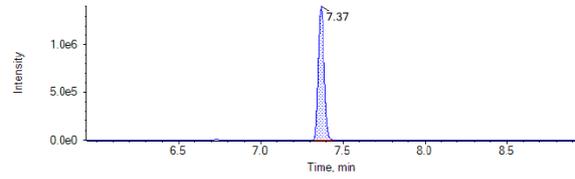
Methomyl



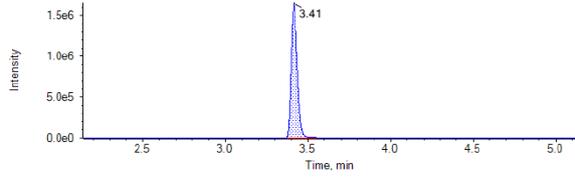
Pymetrozine



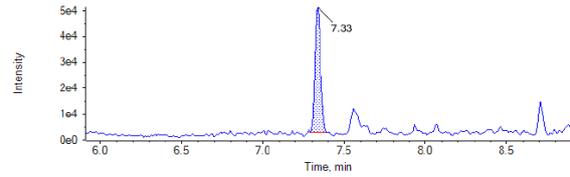
Tebuconazole



Thiabendazole



Tolyfluaniid



Example Recovery Calculation

One example Recovery calculation was shown as below.

The pesticide analyte was Methamidophos, and the expected recovery was from 65 % to 85 %. In this batch, there were 15 replicates in analysis 1 and 15 replicates in analysis 2. Thus, average Quant peak areas were used for calculating the recovery (%).

	Quant Peak Area	
	Analysis 1	Analysis 2
Replicate 1	686676	915011
Replicate 2	683341	890700
Replicate 3	664281	911490
Replicate 4	670050	905545
Replicate 5	650273	877548
Replicate 6	665352	889964
Replicate 7	653278	865279
Replicate 8	660169	892609
Replicate 9	661804	898935
Replicate 10	660120	870213
Replicate 11	645173	874876
Replicate 12	646227	893307
Replicate 13	636326	863357
Replicate 14	626618	837137
Replicate 15	635552	867587
Average	656349	883571
Std Dev	16966	21044
%RSD	2.6	2.4
*Recovery, %	74.3	

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

Precautions

- The extract from QuEChERS extraction should be kept refrigerated until analysis. However, the refrigerated storage must 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 as soon as possible.