



**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 $\mu$ SPE-LCQuE1-30-T cartridges

This document states the analysis protocol for performance qualification test for  $\mu$ SPE-LCQuE1-30-T Cartridges (PAL PN:  $\mu$ SPE-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  $\mu$ l AOAC QuEChERS QC Spike Mix was diluted with 1470  $\mu$ l extraction solvent.
  - QC-STD-1 contains each pesticide analyte at concentration of 800 ng/ml.

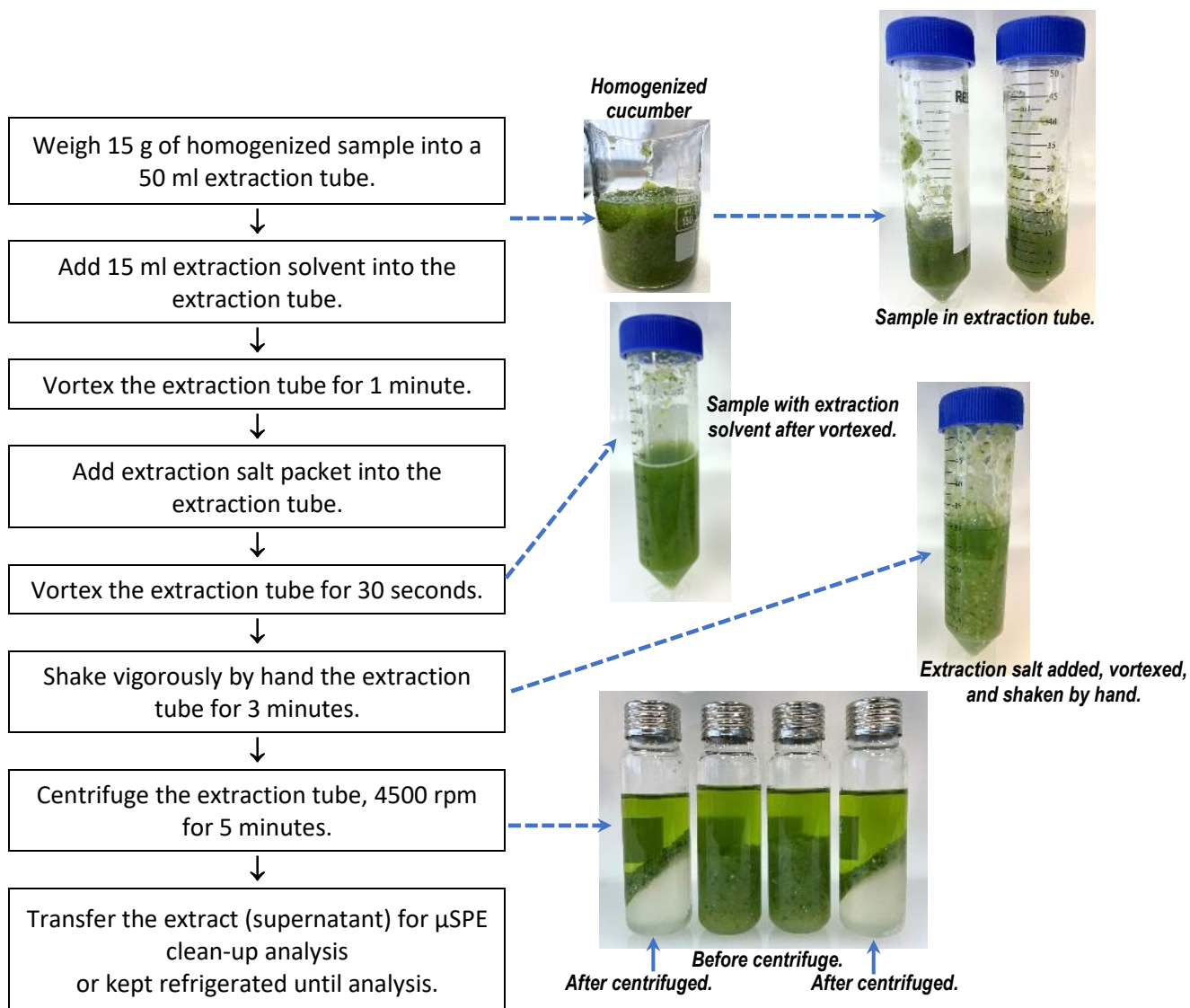
### Sample Matrix

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 μSPE QuEChERS LC Online VAR CTC F31V01 R.xml

## Automated μSPE Clean Up Setup

- μSPE Cartridges:
  - PAL System μSPE Cartridge – LC QuEChERS sorbent mix 1 – 30 mg
  - PAL Part No. μSPE-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 μl 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/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)
- LC/MS Tool with KNF pump and 100 μl 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 μl sample loop (PAL PN: CSL20-CTC)

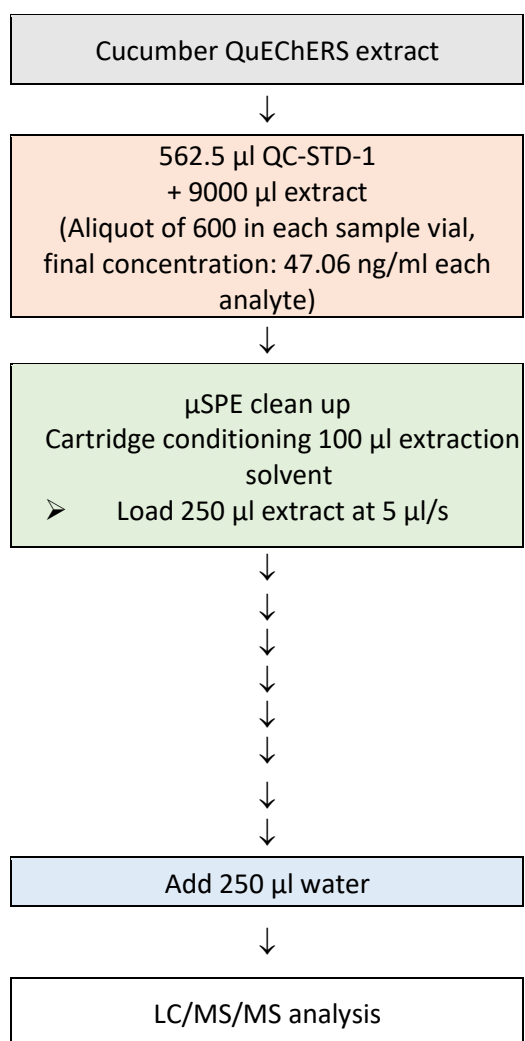
## Analysis

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.

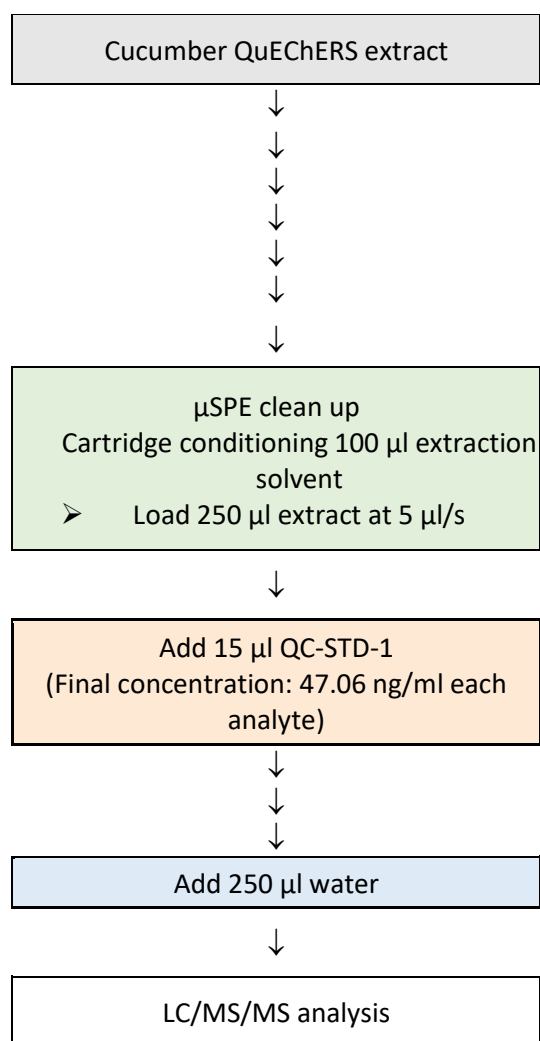
### Analysis 1:

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 were 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 μSPE QuEChERS LC Online VAR CTC F31V01 R.xml)

Parameter	Setting for Analysis 1	Setting for Analysis 2
<b>Setup</b>		
μSPE Tool / water dilution tool	μSPE Tool with 1000 μl syringe	
QC Standard tool	Liquid Tool with 25 μl or 100 μl syringe (LS9)	
Injection tool	LC/MS Tool with 100 μl loop (LCP1)	
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
<b>Conditioning</b>		
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	40 mm	40 mm
<b>Sample μSPE</b>		
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
<b>Elution</b>		
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
<b>Internal standard</b>		
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	1 μl/s	1 μl/s
Internal standard source penetration depth	30 mm	30 mm
Eluate vial penetration depth	28 mm	28 mm
<b>Sample dilution with water</b>		
Water source	Solvent Module / Wash Station	
Water index	Reservoir containing water	

Parameter	Setting for Analysis 1	Setting for Analysis 2
Water volume	250 μl	250 μl
Water fill speed	20 μl/s	20 μl/s
Water source penetration depth	40 mm	40 mm
Elate vial penetration depth	28 mm	28 mm
<b>Mixing</b>		
Mix cycles after protectant / standard addition*	<b>3</b>	<b>5</b>
Mix speed	50 μl/s	50 μl/s
Mix volume	500 μl	500 μl
<b>LC/MS injection</b>		
Front air gap	1 μl	1 μl
Front volume	3 μl	3 μl
Rear volume	3 μl	3 μl
Rear air gap	1 μl	1 μl
Sample injection speed	1 μl/s	1 μl/s
Check sample pickup	No	No
Bottom sensing	No	No
Activate needle dipping	No	No
Wash performance	1 (Standard)	
Valve clean solvent 1	90 μl water	90 μl water
Valve clean solvent 1	180 μl ACN	180 μl 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 μm (Part number 186002352)
- Column temperature: 50 °C
- Injection volume: 1 μl
- 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 → 174.1	18	20
Azoxystrobin	6.57	m/z 404 → 372	15	20
Carbaryl	5.79	m/z 202 → 145	22	20
Chlorpyrifos	8.05	m/z 349.9 → 97	32	20
Chlorpyrifos-methyl	7.65	m/z 321.8 → 125	20	20
Cyprodinil	6.72	m/z 226 → 93	33	20
Dichlorvos	5.51	m/z 221 → 109	22	20
Ethion	7.96	m/z 384.9 → 143	35	20
Imazalil	5.64	m/z 297 → 159	22	20
Imidacloprid	4.02	m/z 256.1 → 175.1	20	20
Linuron	6.64	m/z 249.1 → 160.1	18	20
Methamidophos	1.80	m/z 142 → 93.9	13	20
Methomyl	3.32	m/z 163 → 88	10	20
Procymidone	7.07	m/z 284.1 → 67.1	28	20
Pymetrozine	2.17	m/z 218 → 105	20	20
Tebuconazole	7.37	m/z 308 → 70.1	22	20
Thiabendazole	3.41	m/z 202 → 175	25	20
Tolylfluanid	7.33	m/z 347 → 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

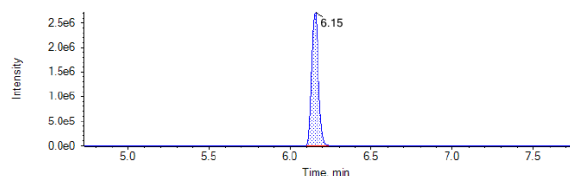
Below the example sequence batch to be executed.

<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
...	...

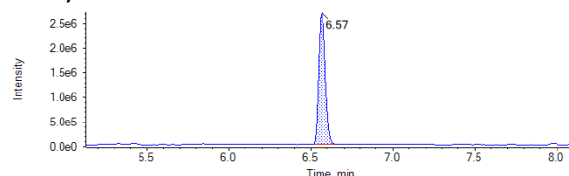
### Example Chromatograms

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

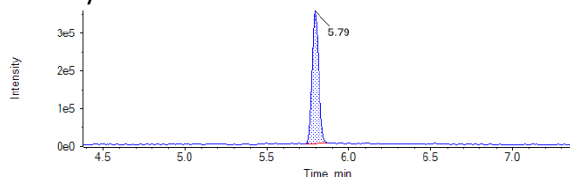
**Atrazine**



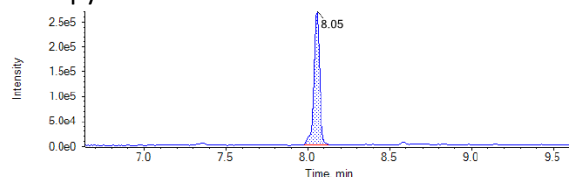
**Azoxystrobin**



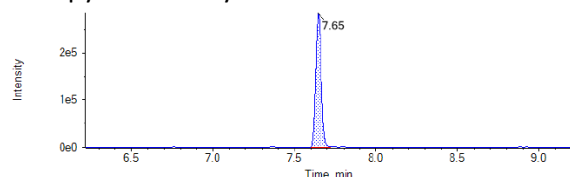
**Carbaryl**



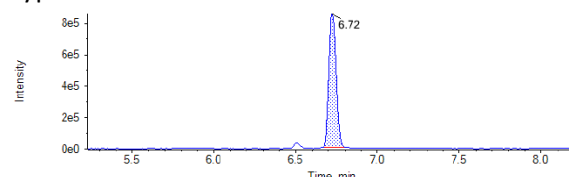
**Chlorpyrifos**



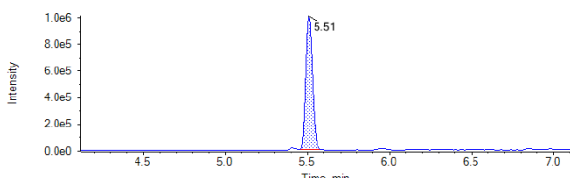
**Chlorpyrifos-methyl**



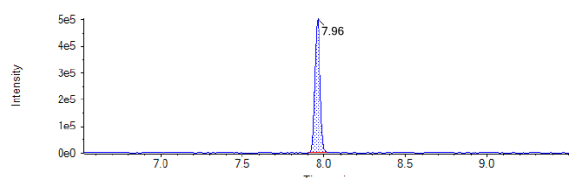
**Cyprodinil**



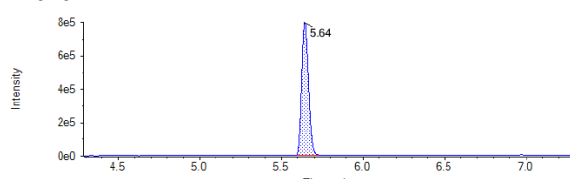
**Dichlorvos**



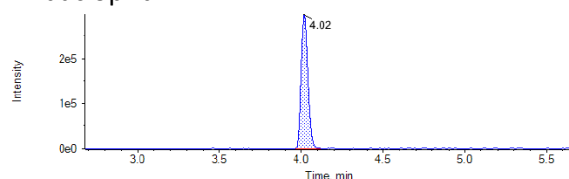
**Ethion**



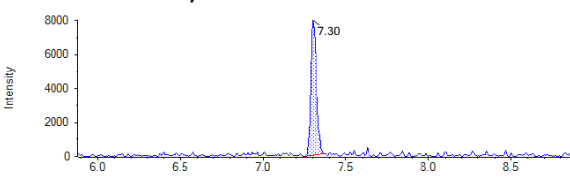
**Imazalil**



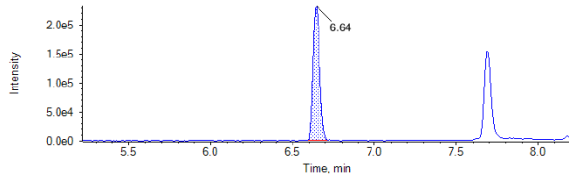
**Imidacloprid**



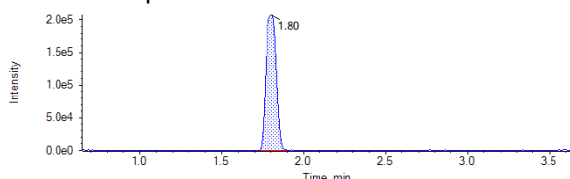
**Kresoxim-methyl**



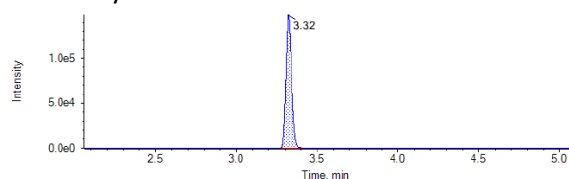
**Linuron**



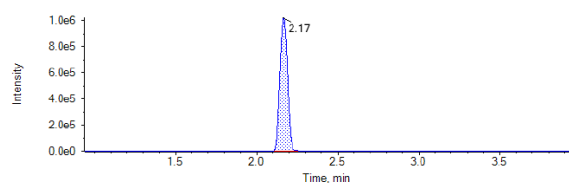
**Methamidophos**



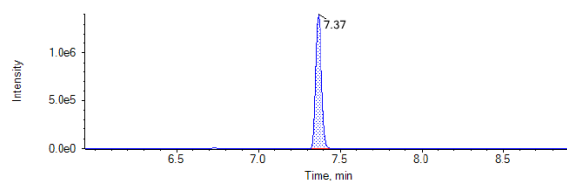
**Methomyl**



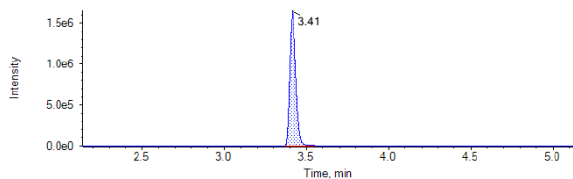
**Pymetrozine**



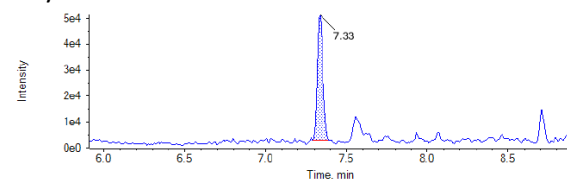
**Tebuconazole**



**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
<b>Average</b>	<b>656349</b>	<b>883571</b>
<b>Std Dev</b>	<b>16966</b>	<b>21044</b>
<b>%RSD</b>	<b>2.6</b>	<b>2.4</b>
<b>*Recovery, %</b>	<b>74.3</b>	

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

## 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.