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Analytical Products

Application Note

Thomas Scientific

Complete Workflow for the Analysis of California List of Pesticides in Cannabis

Detailed protocols for sample preparation and analysis of pesticides by LC-MS/MS and GC-MS/MS.

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1.0 Introduction: Analysis of pesticides in cannabis

Recreational cannabis use has been legalized in 11 states and the District of Columbia with an additional 22 states allowing the use of medical marijuana.

This has led to mandatory testing for growers and processors of cannabis, and its derivative products, to show product safety according to the requirements of individual states. One of the requirements includes the testing of cannabis flowers for the presence of pesticides. This application note demonstrates the use of Supelco® analytical standards, instrument consumables, and reagents to analyze low levels of pesticides in cannabis and, in particular, the 66 pesticides required by the State of California.

Following are the current (April 2020) cannabis pesticide requirements as per the State of California:

There are 21 pesticides listed under category I and which do not have any allowable level. Methods must demonstrate the limit of detection (LOD) for these compounds and their LODs must be at least 0.1 µg/g (or lower limits for inhalable cannabis goods). Any detection of these pesticides causes the rejection of products.

Category II includes 45 pesticides having prescribed action levels. The action levels differ for category II pesticides and depend on whether the product is an inhalable cannabis good or another product form. These pesticides must be below the prescribed action level in order for the product to be accepted.

In this workflow we present:

- Complete details for the analysis of California pesticides by GC-MS/MS and LC-MS/MS
- Step-by-step procedures for calibrator and sample preparation
- Methods for evaluation of suppression and extraction recovery
- Instructions for preparation and use of analyte protectants in GC-MS/MS

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2.0 Preparation of sample and calibration standards

2.1 Pesticide standard working solution (WS) preparation

Calibration curves can be prepared using either a blank matrix extract that has gone through the extraction procedure, or pure solvent. They can also be prepared by "spiking" plant material with a pesticide working solution (WS) in order to best imitate an actual plant sample. It is often desirable to perform each of these types of calibration experiments for different purposes, and the schemes outlined are provided for this purpose. The sets of calibration standards are then used for the evaluation of suppression or enhancement effects and for determining extraction recovery with a set of plant samples spiked prior to extraction.

Step	Standard Working Solution Preparation
1	Prepare a 1 mL solution of a mix of 66 regulated pesticides at 100 (WS1), 10 (WS2) and 1 (WS3) µg/mL in LC-MS grade acetonitrile. Use positive displacement pipettors to ensure accuracy with organic solvents.
2	Ensure that the solution is prepared in an amber volumetric flask as some pesticides are photosensitive and degrade with exposure to light.
3	Ensure that working and stock solutions are stored in a subzero freezer when not in use and with minimal exposure to the environment.

2.2 Sample preparation

Step	Sample Preparation Instructions
1*	Weigh 1.0 ± 0.02 of cannabis sample and transfer into a 50 mL polypropylene centrifuge tube
2	Add homogenization beads, as appropriate for laboratory equipment.
3*	Add 15 mL of LC-MS grade acetonitrile (ACN) and cap
4	Shake the tube for 5-10 min on a vertical shaker at high speed
5	Prepare tube rack with C18 500 mg SPE cartridges (6 mL) and 50 mL centrifuge tubes for collection
6	Add supernatant from step 4 to C18 SPE cartridge and allow it to pass through by gravity (if necessary use vacuum or positive pressure manifold)
7	Add 5 mL of additional ACN to the tube from step 4 and shake for 3-5 min at high speed
8	Transfer supernatant to SPE cartridge
9	Repeat step 7 with 5 mL of ACN and, again, pass through SPE cartridge.
10	Bring final volume of centrifuge tube to 25 mL with ACN

*Note If preparing calibration standards in plant, Working Solutions can be added at step 1 or 3 above and as shown in Scheme I.

2.3 Preparation of plant-based calibration standards (Scheme I)

2.3.1 Pre-extraction spiked calibration standards

Cal Level	µg/g in hemp	µL of Working Solution	Solution
Cal 1	0.02	20	WS3
Cal 2	0.05	50	WS3
Cal 3	0.1	10	WS2
Cal 4	0.2	20	WS2
Cal 5	0.5	50	WS2
Cal 6	1	10	WS1
Cal 7	1.5	15	WS1
Cal 8	3	30	WS1
Cal 9	5	50	WS1

Working solution (WS1): 100 µg/mL combined pesticides in LC-MS grade acetonitrile

Working solution (WS2): 10 µg/mL combined pesticides in LC-MS grade acetonitrile

Working solution (WS3): 1 µg/mL combined pesticides in LC-MS grade acetonitrile

How to prepare calibration standard solution example:

To make Cal 9 add 50 µL of WS1 solution as shown in step 1 (or 3) of sample preparation instructions (shown in 2.2 above)

2.3.2 Post-extraction spiked and solvent calibration standards

(Scheme II)

This scheme is used to prepare a set of calibration standards in either pure solvent or in a matrix extract that has been collected from blank, analyte free plant material

Level	Conc. in Hemp Flower ug/g	Conc. in 25 mL Extract ng/mL	µL	Solution	µL Blank Matrix Extract
Cal 1	0.02	0.8	50	Cal 4	450
Cal 2	0.05	2	10	Cal 9	990
Cal 3	0.1	4	20	Cal 9	980
Cal 4	0.2	8	40	Cal 9	960
Cal 5	0.5	20	50	Cal 9	450
Cal 6	1	40	100	Cal 9	400
Cal 7	1.5	60	6	WS2	994
Cal 8	3	120	12	WS2	988
Cal 9	5	200	20	WS2	980

Working solution (WS2): 10 µg/mL Combined pesticides in LC-MS grade acetonitrile

To make Cal 9 take 20 µL of WS2 solution and add 980 µL of blank matrix extract, or acetonitrile, in a 2 mL amber autosampler vial

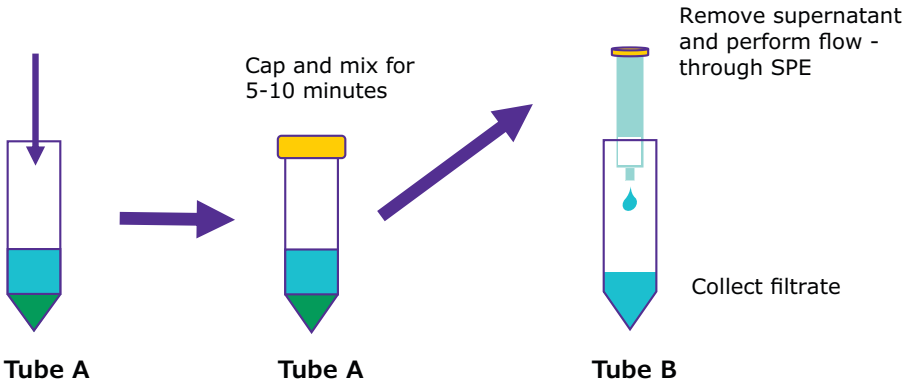
To make Cal 5 take 50 µL of Cal 9 solution and add 450 µL of blank matrix extract, or acetonitrile, in a 2 mL amber autosampler vial

To make Cal 1 take 50 µL of Cal 4 solution and add 450 µL of blank matrix extract, or acetonitrile, in a 2 mL amber autosampler vial

Scheme I

Step 1

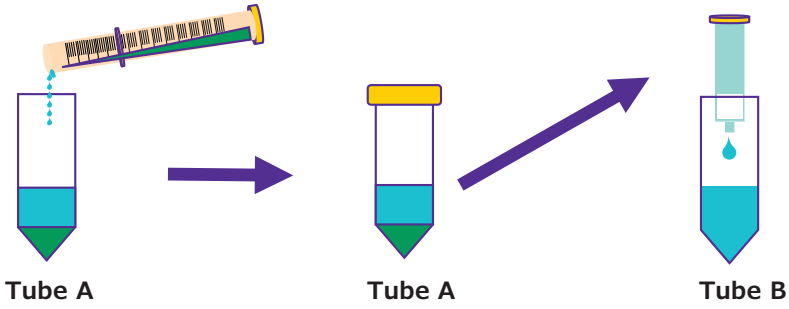
Extraction solvent added to 1g dried, powdered cannabis



Steps 2 and 3

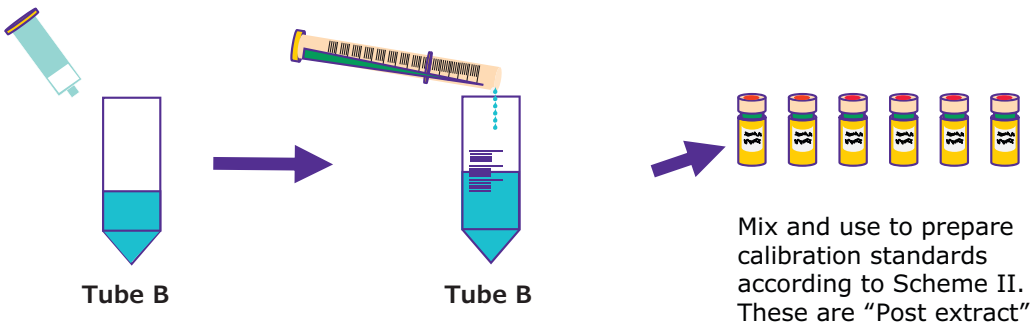
Add additional 5 mL acetonitrile and extract a second time

Remove supernatant and perform flow - through SPE combining with previous collection. Repeat for Step 3.



Step 4

Discard SPE tube and bring volume up to 25 mL with acetonitrile.

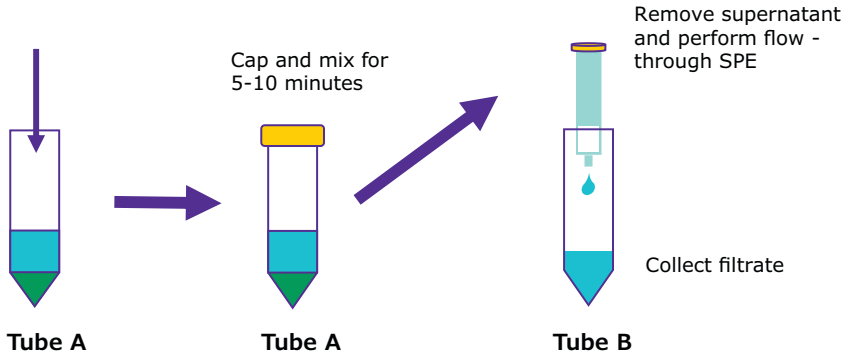


Mix and use to prepare calibration standards according to Scheme II. These are “Post extract” spiked standards

Scheme II

Step 1

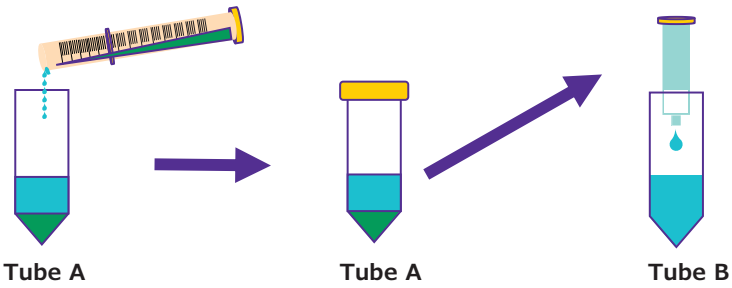
WS added to tube with 1g dried, powdered cannabis as in Scheme I.
One tube per Calibration Standard



Steps 2 and 3

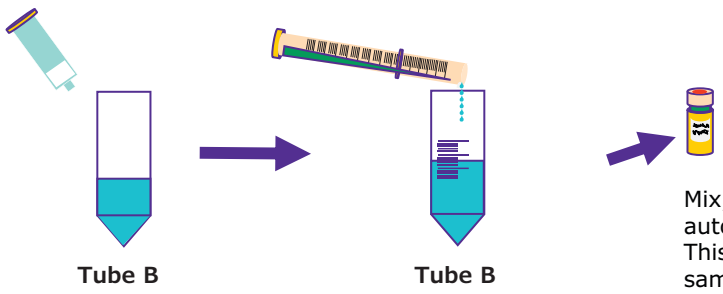
Add additional 5 mL acetonitrile and extract a second time

Remove supernatant and perform flow-through SPE combining with previous collection.
Repeat for Step 3.



Step 4

Discard SPE tube and bring volume up to 25 mL with acetonitrile.



Mix, then transfer 1 mL to autosampler vial.
This 'Pre-Extraction' sample is used to evaluate extraction recovery

3 Evaluation of suppression and extraction recovery using pre-extraction, post-extraction, and solvent standards

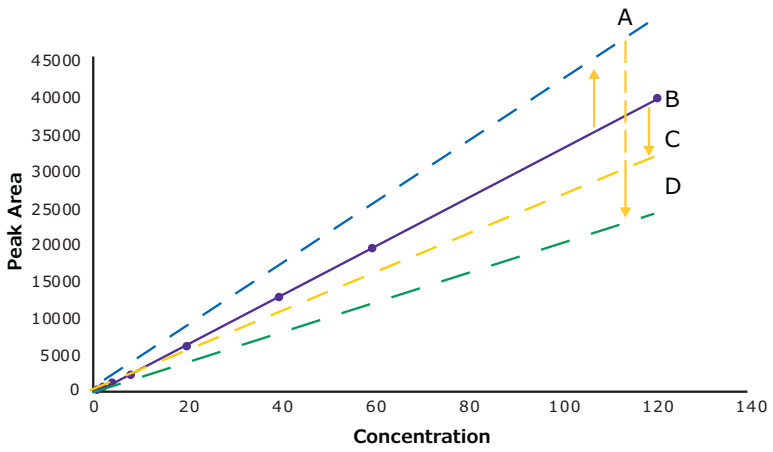
An ideal calibration curve is generated using a set of calibration standards prepared in pure acetonitrile and against which the suppression/enhancement and extraction recovery studies are performed. The calibration standards are prepared as shown in scheme II.

To evaluate suppression and enhancement effects a set of calibrators are prepared from a "post-extraction" sample, as shown in scheme II. For this preparation a "blank" or "control matrix" of analyte free cannabis is taken through the sample preparation procedure before adding the pesticide working solution. A comparison of the "post extract" regression (calibration curve) with the regression performed in pure acetonitrile allows the evaluation of either suppression or enhancement effects.

Note that suppression or enhancement effects can occur with both GC-MS and LC-MS instrumentation although the causes are somewhat different. The study of these effects can provide insight into possible remedies that may be used to improve assay performance.

To evaluate "extraction recovery" a set of plant-based calibration standards is prepared as shown in scheme I. This set of standards is spiked with working solution and then taken through the sample preparation procedure. A comparison of these calibrators with those prepared from the "Post-extraction" set allows for the evaluation of extraction recovery or losses that occur during the extraction procedure.

Matrix Enhancement, Suppression, and Recovery Evaluation



A Post-extract curve showing enhancement
B Curve in pure solvent
C Post-extract curve showing suppression
D Pre-extract curve showing extraction recovery

B to A Enhancement
B to C Suppression
A-C to D Extraction Recovery

(Peak area A/B -1)x(100) = % Enhancement
(1- Peak area C/B)x(100) = % Suppression
(D/C)x(100) or (D/A)x(100) = % Extraction Recovery

4 Complete workflow for LC-MS/MS analysis

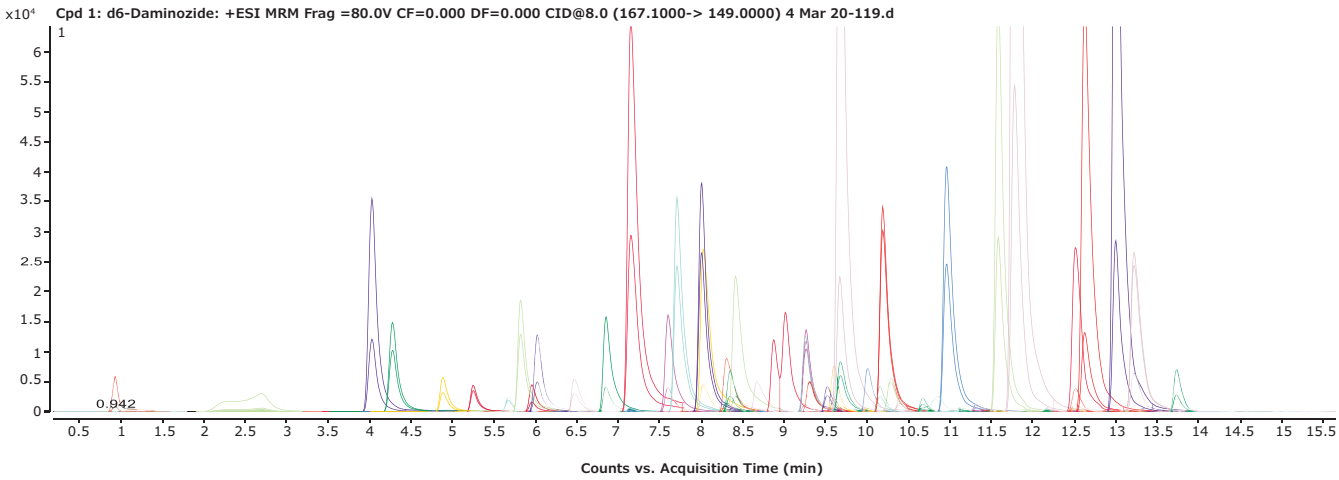
4.1 Mobile phase preparation

Mobile Phases	Instructions
1M ammonium formate	1. Weigh out 630 mg +/- 10 mg on a balance. 2. Transfer to 10 mL of 1:1 methanol:water mixture. Mix thoroughly to dissolve.
2 mM ammonium formate + 0.1% formic acid in 2% methanol	1. Combine 20 mL of methanol with 980 mL of water. 2. Add 1 mL formic acid and 2 mL 1 M ammonium formate. 3. Mix and transfer to HPLC solvent bottle.
2 mM ammonium formate + 0.1% formic acid in 95% acetonitrile	1. Combine 50 mL of water with 950 mL of acetonitrile. 2. Add 1 mL formic acid and 2 mL 1 M ammonium formate. 3. Mix and transfer to HPLC solvent bottle.

4.2 Chromatographic and MS conditions

LC-MS/MS	
Column	Ascentis RP-Amide, 2.1 mm x 10 cm, 3 um particles with guard column
Detection	LC-MS/MS dMRM Acquisition Mode
Instrumentation	Agilent 1290 series HPLC and autosampler with 6460 QQQ
Mobile phase A	2 mm ammonium formate, 0.1% formic acid, 2% methanol in Milli-Q water
Mobile phase B	2 mm ammonium formate, 0.1% formic acid, 5% Milli-Q water in acetonitrile

Standard injection of pesticides in hemp extract



4.3 Acquisition parameters for LC-MS/MS amenable pesticides in the California list:

HPLC gradient

Step	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)	Flow rate (mL/min)
1	0.0	100	0	0.4
2	1.0	100	0	0.4
3	14.0	0	100	0.4
4	17.0	0	100	0.4
5	17.5	100	0	0.4
6	20.0	100	0	0.4

MS parameters

Source Conditions	Value (+)
Gas Temp (°C)	200
Gas Flow (l/min)	10
Nebulizer (psi)	35
Sheath Gas Heat	200
Sheath Gas Flow	10
Capillary Voltage	4000
V Charging	500

4.3 Acquisition parameters for LC-MS/MS amenable pesticides in the California list:

Peaks	Compound	Retention Time (min)	MRM (m/z)	Collision Energy (eV)
1	Acephate	2.70	184.1 -> 143.1	0
2	Acequinocyl	15.27	402.3 -> 343.2	4
3	Acetamiprid	6.01	223.1 -> 126.1	20
4	Aldicarb	6.57	116.1 -> 89.1	4
5	Avermectin B1a	12.52	890.5 -> 305.3	28
6	Azoxystrobin	9.67	404.3 -> 372.2	10
7	Bifenazate	10.19	301.1 -> 198.2	5
8	Boscalid	10.00	343.2 -> 307.2	12
9	Carbaryl	8.34	202.2 -> 145.1	0
10	Carbofuran	7.70	222.2 -> 165.1	5
11	Chlorantraniliprole	9.30	484.0 -> 286.0	10
12	Chlorfenapyr	12.51	424.0 -> 368.0	12
13	Chlorpyrifos	12.46	349.9 -> 97.0	41
14	Clofentezine	11.44	303.2 -> 138.1	10
15	Coumaphos	11.31	363.2 -> 227.0	33
16	Daminozide	0.93	161.1 -> 143.0	8
17	Diazinon	10.96	305.2 -> 169.1	20
18	Dichlorvos	7.14	221.1 -> 109.1	12
19	Dimethoate	5.82	230.2 -> 199.1	0
20	Dimethomorph	9.01	388.1 -> 301.1	15
21	Ethoprophos	9.67	243.2 -> 97.0	30
22	Etofenprox	13.74	394.2 -> 177.2	10
23	Etoxazole	12.63	360.1 -> 141.1	28
24	Fenhexamid	10.64	302.2 -> 97.0	22
25	Fenoxycarb	10.39	302.2 -> 88.1	15
26	Fenpyroximate	12.52	422.3 -> 366.3	15
27	Flonicamid	4.77	230.2 -> 98.0	48
28	Fludioxonil	10.33	229.1 -> 158.1	18
29	Hexythiazox	12.49	353.2 -> 228.2	10
30	Imazalil	6.46	297.2 -> 159.1	20
31	Imidacloprid	5.67	256.2 -> 209.1	16
32	Kresoxim-methyl	10.67	314.2 -> 267.2	0
33	Malathion	10.15	331.2 -> 126.9	5
34	Metalaxyl	8.00	280.2 -> 220.2	10
35	Methiocarb	9.52	226.1 -> 169.1	0
36	Methomyl	4.27	162.9 -> 88.1	0
37	Mevinphos ‡	5.25	225.2 -> 127.1	16
38	Myclobutanil	9.60	289.2 -> 70.0	15
39	Naled	8.71	380.8 -> 127.0	10
40	Oxamyl	4.03	237.2 -> 72.1	15
41	Paclobutrazol	9.25	294.1 -> 70.0	20
42	Phosmet	9.69	317.9 -> 160.0	10
43	Piperonyl butoxide	11.78	356.2 -> 177.1	5
44	Prallethrin II	11.40	301.2 -> 105.1	20
45	Propiconazole	10.29	342.2 -> 159.0	32
46	Propoxur	7.59	210.1 -> 111.1	10
47	Pyrethrins ‡‡	11.45	373.3 -> 161.1	2
48	Pyridaben	13.23	365.2 -> 147.2	20
49	Spinetoram*	8.41	748.5 -> 142.1	26
50	Spinosad **	8.02	732.5 -> 142.1	28
51	Spiromesifen	13.00	388.3 -> 273.3	6
52	Spirotetramat	9.26	374.2 -> 302.2	12
53	Spiroxamine	7.15	298.2 -> 144.1	16
54	Tebuconazole	10.01	308.1 -> 70.0	40
55	Thiacloprid	6.84	253.2 -> 126.1	16
56	Thiamethoxam	4.88	292.2 -> 211.2	8
57	Trifloxystrobin	11.58	409.2 -> 186.1	12

‡ As Mevinphos I

‡‡ as Pyrethrin II

* as Spinetoram J

**as Spinosyn A

4.5 Consistent results

Tabulated results for LC-MS/MS amenable pesticides in the California list:

Peaks	Compound	R2	Recovery (%)	%RSD (n=3)	Category	Minimum Action Level µg/g
1	Acephate	0.998	90	< 5	II	0.1
2	Acequinocyl #	0.991	64	5.3	II	0.1
3	Acetamiprid	0.999	92	< 5	II	0.1
4	Aldicarb	0.999	85	< 5	I	0.1
5	Avermectin B1a	0.998	95	9.8	II	0.1
6	Azoxystrobin	0.996	92	< 5	II	0.1
7	Bifenazate	0.998	96	< 5	II	0.1
8	Boscalid	0.998	82	11.9	II	0.1
9	Carbaryl	0.997	92	< 5	II	0.5
10	Carbofuran	1.000	91	< 5	I	0.1
11	Chlorantraniliprole	0.998	71	6.1	II	10
12	Chlorfenapyr	0.996	88	5.7	I	0.1
13	Chlorpyrifos	0.992	116	30	I	0.1
14	Clofentezine	0.994	91	16	II	0.1
15	Coumaphos	0.998	92	12.8	I	0.1
16	Daminozide	0.997	12	5.4	I	0.1
17	Diazinon	0.998	94	< 5	II	0.1
18	Dichlorvos	0.999	97	12.1	I	0.1
19	Dimethoate	0.993	93	< 5	I	0.1
20	Dimethomorph	1.000	93	< 5	II	2

Peaks	Compound	R2	Recovery (%)	%RSD (n=3)	Category	Minimum Action Level µg/g
21	Ethoprophos	0.998	94	5.8	I	0.1
22	Etofenprox	0.995	97	4.8	I	0.1
23	Etoxazole	0.997	93	< 5	II	0.1
24	Fenhexamid	0.998	110	8.8	II	0.1
25	Fenoxycarb	0.996	92	< 5	I	0.1
26	Fenpyroximate	0.997	92	< 5	II	0.1
27	Flonicamid	0.996	101	14.8	II	0.1
28	Fludioxonil	0.991	100	29.1	II	0.1
29	Hexythiazox	0.995	96	9.3	II	0.1
30	Imazalil	1.000	85	< 5	I	0.1
31	Imidacloprid	0.998	89	6.6	II	5
32	Kresoxim-methyl	0.996	100	< 5	II	0.1
33	Malathion	0.995	88	10	II	0.5
34	Metalaxyl	0.999	93	< 5	II	2
35	Methiocarb	0.997	92	< 5	I	0.1
36	Methomyl	1.000	92	< 5	II	1
37	Mevinphos ‡	0.999	96	6.1	I	0.1
38	Myclobutanil	0.998	93	< 5	II	0.1
39	Naled	0.998	110	13.5	II	0.1
40	Oxamyl	1.000	89	< 5	II	0.5
41	Paclobutrazol	0.998	91	< 5	I	0.1
42	Phosmet	0.998	107	16.8	II	0.1
43	Piperonyl butoxide	0.997	102	< 5	II	3
44	Prallethrin II	0.995	105	9.3	II	0.1
45	Propiconazole	0.998	96	10.5	II	0.1
46	Propoxur	0.999	93	< 5	I	0.1
47	Pyrethrins ‡‡	0.996	92	9.1	II	0.5
48	Pyridaben	0.996	96	< 5	II	0.1
49	Spinetoram *	0.999	82	5.5	II	0.1
50	Spinosad * *	0.999	84	< 5	II	0.1
51	Spiromesifen	0.996	91	< 5	II	0.1
52	Spirotetramat	0.998	87	9	II	0.1
53	Spiroxamine	0.999	81	< 5	I	0.1
54	Tebuconazole	0.998	94	6.7	II	0.1
55	Thiacloprid	0.999	93	< 5	I	0.1
56	Thiamethoxam	0.999	92	< 5	II	5
57	Trifloxystrobin	0.997	94	< 5	II	0.1

‡ As Mevinphos I

‡‡ as Pyrethrin II

* as Spinetoram J

**as Spinosyn A

5 Complete workflow for the GC-MS/MS analysis

5.1 Preparation of analyte protectant solutions

Analyte protectants reduce active sites in the GC inlet and sample-path to ensure reproducible and consistent results when analyzing pesticides at low ppb levels.1

Step	Instructions
1	Weigh ~500 mg of D-Sorbitol into a 10 mL volumetric flask and add 6 mL of LC-MS grade acetonitrile. Make up the volume using Milli-Q water (Solution A).
2	Weigh ~500 mg of L-Gulonic acid γ-lactone into a 10 mL volumetric flask and add 5mL of LC-MS grade acetonitrile. Make up the volume using Milli-Q water (Solution B).
3	Add 2 mL of solution A with 4 mL of solution B in a 10 mL volumetric flask and bring to volume with LC-MS grade acetonitrile
4	Place into the appropriate autosampler vial for making sandwich injection with 0.2 µL of air gap above and 0.2 µL of the analyte protectant solution

5.2 GC-MS/MS instrument conditions

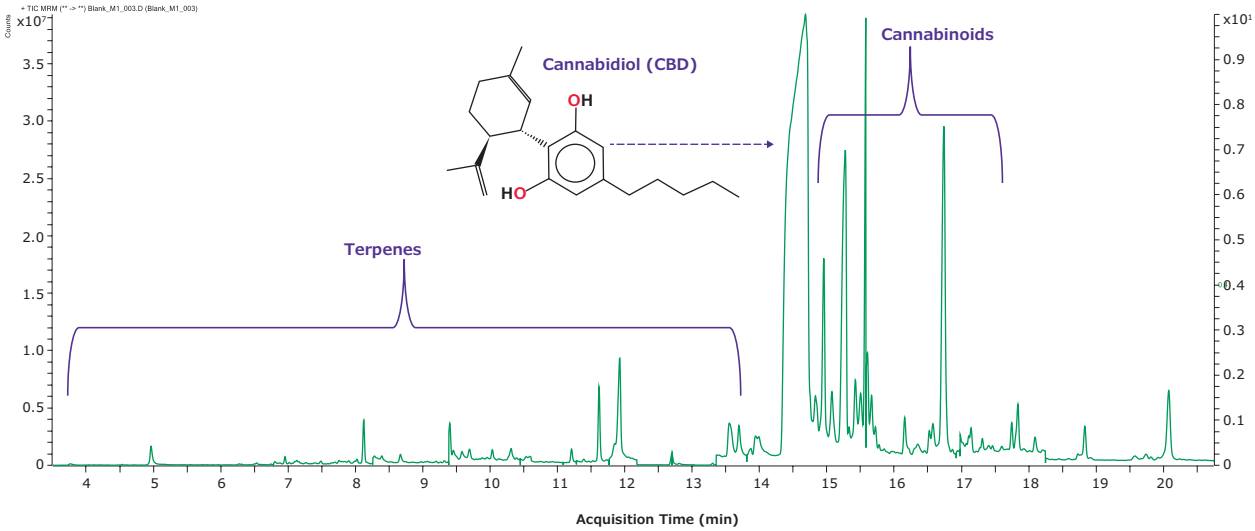
Gas Chromatograph Conditions	
Column	SLB®-5ms L × I.D. 30 m × 0.25 mm, df 0.25 µm
Detector	GC-MS/MS
Inlet	60°C for 0.35 min. and then 600°C/min to reach 300°C; Solvent Vent Mode: 5psi until 0.3 min, split vent flow 50 mL/min at 1.5 min; Air Cooled Pressure and Temperature Programmable Inlet
Column Temperature	60°C for 1 minute, then 40°C/min to reach 170°C. Hold for 0 min. then 10°C/min to reach 310°C. Hold at 310°C for 3 minutes
Flow	1.2 mL/min
Carrier gas	Helium
Liner	2 mm ID
Injection	2 µl – Solvent Vent Splitless injection with 0.2 uL Sandwich of Analyte Protectant Solution
Sample diluent	Acetonitrile
Standard solution	9 Matrix-Matched Calibration Standards of Pesticides Mix in Acetonitrile Extract

MS/MS Conditions	
Tuning	AutoTune
Acquisition	MRM (EI mode)
Collision gas	Nitrogen @ 1.5 mL/min
Quench gas	Helium @ 2.25 mL/min
Solvent delay	3.5 min
MS source temperature	300°C
Quad Temperature	150°C
Electron Energy	70 eV
Dwell time	10 ms
Gain	10

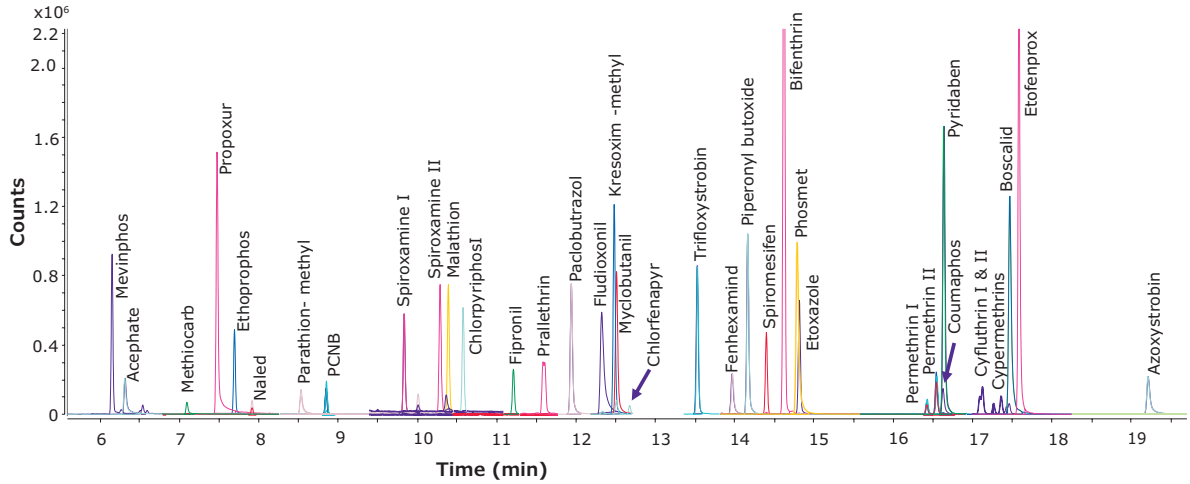
5.3 Acquisition parameters for GC-MS/MS amenable pesticides in California list:

Peaks	Compound	Retention Time (min)	MRM (m/z)	Collision Energy (eV)
1	Dichlorvos	5.178	184.9 -> 109.0	10
2	Mevinphos	6.151	127.0 -> 109.0	10
3	Acephate	6.312	136.0 -> 94.0	10
4	Methiocarb	7.097	168.0 -> 109.1	15
5	Propoxur	7.473	110.0 -> 63.0	25
6	Ethoprop	7.699	157.9 -> 97.0	15
7	Naled	7.915	145.0 -> 109.0	15
8	PCNB	8.847	248.7 -> 213.9	15
9	Parathion-methyl	9.405	125.0 -> 47.0	10
10	Spiroxamine I	9.836	100.0 -> 58.1	10
11	Spiroxamine II	10.288	100.0 -> 58.1	10
12	Malathion	10.393	126.9 -> 99.0	5
13	Chlorpyrifos	10.580	196.9 -> 169.0	15
14	Fipronil	11.213	366.8 -> 212.8	25
15	Prallethrin	11.63	123.0 -> 81.0	10
16	Captan	11.634	116.9 -> 82.0	30
17	Chlordane I	11.907	271.9 -> 236.9	15
18	Paclobutrazol	11.944	236.0 -> 125.1	10
19	Chlordane I & II	12.026	375.0 -> 265.8	25
20	Chlordane II	12.142	375.0 -> 265.8	25
21	Fludioxonil	12.325	248.0 -> 127.1	30
22	Kresoxim-methyl	12.479	116.0 -> 89.0	15
23	Myclobutanil	12.508	179.0 -> 125.1	10
24	Chlorfenapyr	12.672	246.9 -> 227.0	15
25	Trifloxystrobin	13.536	116.0 -> 89.0	15
26	Fenhexamid	13.969	177.0 -> 113.0	15
27	Piperonyl butoxide	14.161	176.1 -> 103.1	25
28	Spiromesifen	14.398	272.0 -> 254.2	5
29	Bifenthrin	14.616	181.2 -> 165.2	25
30	Phosmet	14.789	160.0 -> 77.1	20
31	Etoxazole	14.812	141.0 -> 63.1	30
32	Permethrin I	16.425	162.9 -> 91.1	15
33	Permethrin II	16.546	162.9 -> 91.1	15
34	Coumaphos	16.632	361.9 -> 109.0	15
35	Pyridaben	16.641	147.2 -> 117.1	20
36	Cyfluthrin I & II	17.151	198.9 -> 170.1	25
37	Cypermethrin I II III IV	17.359	181.0 -> 152.1	25
38	Boscalid	17.465	140.0 -> 112.0	10
39	Etofenprox	17.584	163.0 -> 107.1	20
40	Azoxystrobin	19.210	344.1 -> 329.0	15

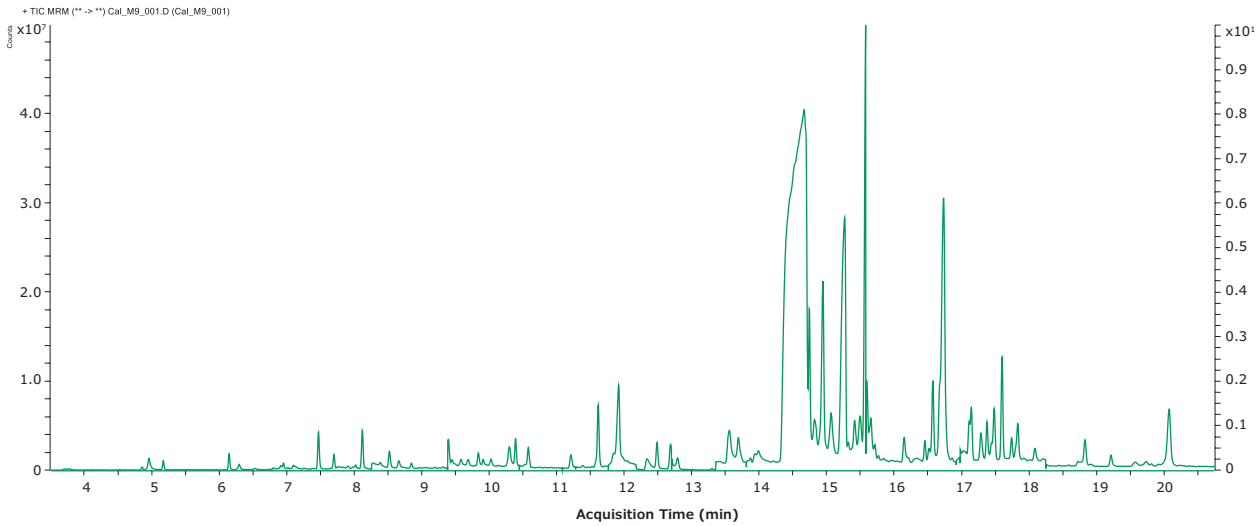
TIC MRM chromatogram of GC-MS/MS amenable pesticides in California list blank sample extract



MRM extracted chromatogram of GC-MS/MS amenable pesticides in the California list - Standard at 200 ppb



TIC MRM chromatogram of GC-MS/MS amenable pesticides in the California list - Standard at 200 ppb



5.5 Consistent results

Tabulated results for GC-MS/MS amenable pesticides in California list:

Peaks	Compound	R2	Recovery (%)	%RSD (n=3)	Cate-gory	Minimum Action Level µg/g
1	Dichlorvos	0.994	96	4.6	I	0.1
2	Mevinphos	0.998	102	4.4	I	0.1
3	Acephate	0.998	103	3.6	II	0.1
4	Methiocarb	0.985	103	3.3	I	0.1
5	Propoxur	0.997	102	4.3	I	0.1
6	Ethoprop	0.997	103	1.3	I	0.1
7	Naled	0.995	99	4.9	II	0.1
8	PCNB	0.999	100	3	II	0.1
9	Parathion-methyl	0.998	105	11.2	I	0.1
10	Spiroxamine I	0.996	105	2.5	I	0.1
11	Spiroxamine II	0.998	104	3.4	I	0.1
12	Malathion	0.999	101	2.4	II	0.5
13	Chlorpyrifos	0.999	102	0.6	I	0.1
14	Fipronil	0.999	99	1.1	I	0.1
15	Prallethrin	0.999	99	6	II	0.1
16	Captan	0.988	100	1	II	0.7
17	Chlordane I	0.995	104	6.8	I	0.1
18	Paclobutrazol	0.999	101	2.6	I	0.1
19	Chlordane I & II	0.998	103	0.6	I	0.1
20	Chlordane II	0.997	101	0.4	I	0.1
21	Fludioxonil	0.999	102	1.9	II	0.1
22	Kresoxim-methyl	0.999	101	1.8	II	0.1
23	Myclobutanil	0.999	102	3.8	II	0.1
24	Chlorfenapyr	0.999	98	5.3	I	0.1
25	Trifloxystrobin	0.999	101	1.8	I	0.1
26	Fenhexamid	0.998	100	1.7	I	0.1
27	Piperonyl butoxide	0.997	102	3.2	II	0.1
28	Spiromesifen	0.999	103	2.2	II	0.1
29	Bifenthrin	0.999	101	2	II	3
30	Phosmet	0.999	100	6	II	0.1
31	Etoxazole	0.999	102	1.7	II	0.1
32	Permethrin I	0.997	102	0.4	II	0.5
33	Permethrin II	0.998	100	2.2	II	0.5
34	Permethrin I & II	0.998	102	1.2	II	0.5
35	Coumaphos	0.998	98	1	I	0.1
36	Pyridaben	0.998	100	2.4	II	0.1
37	Cyfluthrin I & II	0.999	96	2.3	II	2
38	Cypermethrin I II III IV	0.997	99	2.1	II	1
39	Boscalid	0.999	101	3.3	II	0.1
40	Etofenprox	0.998	101	0.2	I	0.1
41	Azoxystrobin	0.998	102	7.5	II	0.1

6 Tabulated results for all the pesticides in the California list:

Analyte	GC-MS/MS MAL Met?	LC-MS/MS MAL Met?
Dichlorvos	YES	YES
Mevinphos	YES	YES
Acephate	YES	YES
Methiocarb	YES	YES
Propoxur	YES	YES
Ethoprop	YES	YES
Naled	YES	YES
PCNB	YES	NO
Parathion-methyl	YES	NO
Spiroxamine I	YES	YES
Spiroxamine II	YES	YES
Malathion	YES	YES
Chlorpyrifos	YES	YES
Fipronil	YES	YES
Prallethrin	YES	YES
Captan	YES	YES
Chlordane I	YES	NO
Paclobutrazol	YES	YES
Chlordane I & II	YES	NO
Chlordane II	YES	NO
Fludioxonil	YES	YES
Tebuconazole	NO	YES
Carbofuran	NO	YES
Clofentezine	NO	YES
Dimethomorph	NO	YES
Flonicamid	NO	YES
Fenoxycarb	NO	YES
Carbaryl	NO	YES
Avermectin	NO	YES
Daminozide	NO	YES
Dimethoate	NO	YES
Hexythiazox	NO	YES
Imazalil	NO	YES
Metalaxyl	NO	YES
Fenproximate	NO	YES
Kresoxim-methyl	YES	YES
Myclobutanil	YES	YES
Chlorfenapyr	YES	YES
Trifloxystrobin	YES	YES
Fenhexamid	YES	YES
Piperonyl butoxide	YES	YES

Analyte	GC-MS/MS MAL Met?	LC-MS/MS MAL Met?
Spiromesifen	YES	YES
Bifenthrin	YES	YES
Phosmet	YES	YES
Etoxazole	YES	YES
Permethrins	YES	YES
Thiacloprid	NO	YES
Pyrethrins	NO	YES
Coumaphos	YES	YES
Pyridaben	YES	YES
Cyfluthrin I & II	YES	NO
Cypermethrin I II III IV	YES	NO
Boscalid	YES	YES
Etofenprox	YES	YES
Azoxystrobin	YES	YES
Acetamiprid	NO	YES
Diazinon	NO	YES
Aldicarb	NO	YES
Spirotetramat	NO	YES
Imidacloprid	NO	YES
Chlorantraniliprole	NO	YES
Bifenazate	NO	YES
Methomyl	NO	YES
Propiconazole	NO	YES
Spinetoram J	NO	YES
Spinoteram L	NO	YES
Spinosyn A	NO	YES
Oxamyl	NO	YES
Thiamethoxam	NO	YES
Acequinocyl	NO	NO

MAL : Minimum Action Level

7. Conclusion

A method has been developed to quantify the California list of pesticides from dried cannabis, as per the state requirements, by using a combination of both LC-MS/MS and GC-MS/MS. A single flowthrough solid-phase extraction is used to prepare the samples for both instrumental techniques. Linearity, recovery, and precision are demonstrated using dried hemp and schemes for performing the calibration, extraction recovery, and suppression/ enhancement studies are provided.

A total of 57 pesticides are reported using LC-MS/MS and 40 using GC-MS/MS. Due to the high levels of interfering CBDA, acequinocyl was not detected at minimum levels with the existing instrumentation. All other pesticides are reported using one or the other analytical technique to meet or exceed the current California regulatory limits for each category.

As seen from the illustrations and results, the use of Supelco® chromatography solvents, consumables, supplies, and analytical reagents in combination with GC-MS/MS and LC-MS/MS instrumentation provides an efficient way to analyze cannabis for the presence of pesticides. The minimum action levels of 0.1 µg/g (100 ppb) for California are easily achievable for most of the compounds. Obtaining consumables and reagents from one supplier ensures that the time is well spent in analyzing samples rather than evaluating multiple sources for analytical supplies.

The GC-MS/MS instrumentation is used here as a supplement to LC-MS/MS in order to detect all California cannabis pesticides at their required minimum action levels. Millipore Sigma does not endorse any particular instrumentation and aims to provide solutions for sample preparations and chromatographic challenges of laboratories regardless of the instrument used.

In this workflow we have presented:

- Complete details for the analysis of the California pesticides by GC-MS/MS and LC-MS/MS
- Step-by-step procedures for calibrator and sample preparation
- Methods for the evaluation of suppression and extraction recovery
- Instructions for the preparation and use of analyte protectants in GC-MS/MS

7.1 Approaches for Further Resolution Gains

This work was a continuation of our earlier studies examining the Oregon list of pesticides. It offers a solution for the issue of poor peak shape of weakly retained, early eluting analytes. However, there are other options available that may permit use of our Ascentis® Express Fused-Core® columns to provide overall better peak shape and resolution.

The issue for early eluting analytes is the high solvent strength of the sample following extraction, versus the solvent strength of the initial mobile phase. The approach taken here was to use a guard column to insert a porous bed into the flow path to provide a space for sample mixing with the solvent. This same approach can be used with our Fused-Core® column technology.

Approaches that can be explored to leverage the Fused-Core® technology:

- Coupling a high-resolution Ascentis Express analytical column with a porous particle guard column of lower hydrophobicity of which there are several options available.
- Sample dilution with a small amount of water. This would entail trying a series of dilutions to determine which affords desirable improvement in the shape of early eluting peaks. In order to keep sample mass constant, the sample volume would need to be increased by the same factor as used in dilution. Additionally, be mindful of issues around analyte solubility as cannabinoids could precipitate out of solution. Utilizing more sensitive LC/MS/MS detection can also permit smaller sample volumes which can ameliorate the problem.

Featured and Related Materials

LC-MS/MS Consumables

	Product Description	Mfr. No.	Thomas No.
Sample Preparation			
	Discovery C18 SPE Cartridges, 500 mg, 6 mL	**52604-U**	**21A00L820**
HPLC Columns			
	Ascentis® RP-Amide 100x2.1 mm, 3 µm	**565301-U**	**---**
	Ascentis Express Guard Cartridge Holder	**53500-U**	**1179R66**
	Ascentis® Express RP-Amide, 2.7 µm Guard Cartridge	**53514-U**	**---**
Accessories			
	50 mL Centrifuge Tubes:	**T2318**	**---**
	15 mL centrifuge tube	**T1818**	**---**
	BenchMixer Shaker/Vortexer	**Z742705**	**---**
	BRAND Seripettor Bottle-top Dispenser	**Z627577**	**---**
	Certified Vial Kit – Amber (Autosampler vials)	**29653-U**	**21A00M258**
	Guard frit with holder	**803410**	**---**
	Replacement frits	**803411**	**---**
	Pipette 2-20 µL	**BR705872-1EA**	**---**
	Pipette 20-200 µL	**BR705878-1EA**	**---**
	Pipette 100-1000 µL	**BR705880-1EA**	**---**
	Pipette tips 2-20 µL box	**Z740102-480EA**	**---**
	Pipette tips 2-200 µL box	**Z740106-480EA**	**---**
	Pipette tips 50-1000 µL box	**Z740108-480EA**	**---**
Water, Solvents and Chemicals			
	Acetonitrile Solution (0.1% formic acid)	**1.59002**	**---**
	Water Solution (0.1% formic acid)	**159013.4**	**---**
	Ammonium Formate, LiChropur	**70221**	**C989U89**
	Formic Acid, LC-MS LiChropur	**5330020050**	**C820P83**
	Methanol	**106035**	**---**
	Ultrapure water from Milli-Q® system or bottled water	**Milli-Q® IQ 7005 or 1.15333**	**--- or ---**

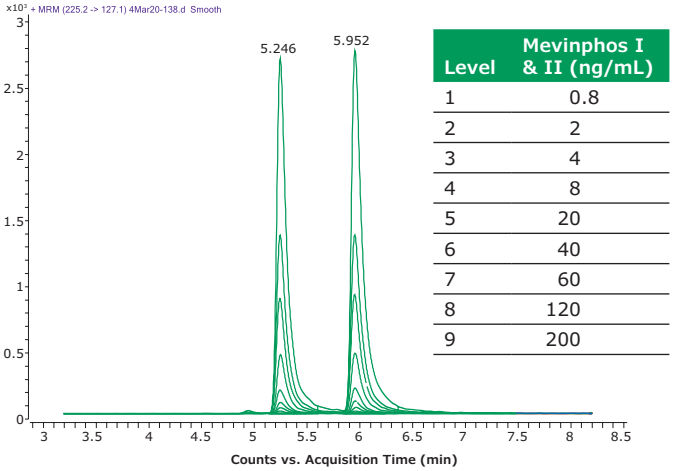
GC-MS/MS Consumables

	Product Description	Mfr. No.	Thomas No.
Sample Preparation			
	Discovery C18 SPE Cartridges, 500 mg, 6 mL	**52604-U**	**21A00L820**
GC Columns			
	SLB®-5ms Capillary GC Column L × I.D. 30 m × 0.25 mm, df 0.25 µm	**28471-U**	**21A00L230**
Accessories			
	Inlet Septa	**28676-U**	**---**
	GC Inlet Liner	**2048505**	**---**
	GC Vials and Caps: 2 mL Amber Vials and PFTE Caps	**29654-U**	**21A00M259**
	GC Syringe: Depending on Autosampler Make and Model		
	BenchMixer Shaker/Vortexer	**Z742705**	**---**
	Pipette 2-20 µL	**BR705872-1EA**	**---**
	Pipette 20-200 µL	**BR705878-1EA**	**---**
	Pipette 100-1000 µL	**BR705880-1EA**	**---**
	Pipette tips 2-20 µL box	**Z740102-480EA**	**---**
	Pipette tips 2-200 µL box	**Z740106-480EA**	**---**
	Pipette tips 50-1000 µL box	**Z740108-480EA**	**---**
	50 mL Centrifuge Tubes	**T2318**	**---**
Water, Solvents and Chemicals			
	Acetonitrile	**1.00017**	**C992G94**
	Ultrapure water from Milli-Q® system	**Milli-Q® IQ 7005**	**---**

Addendum: Detection and Calibration

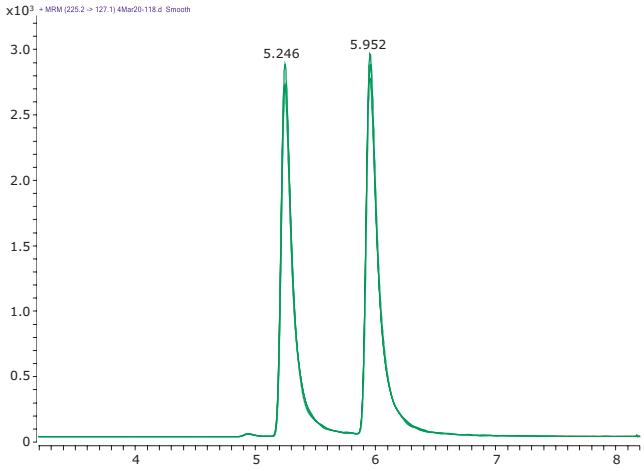
LC-MS/MS Pesticides: Detection and calibration

Mevinphos I & II LC-MS/MS detection and calibration



Level	Mevinphos I & II (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

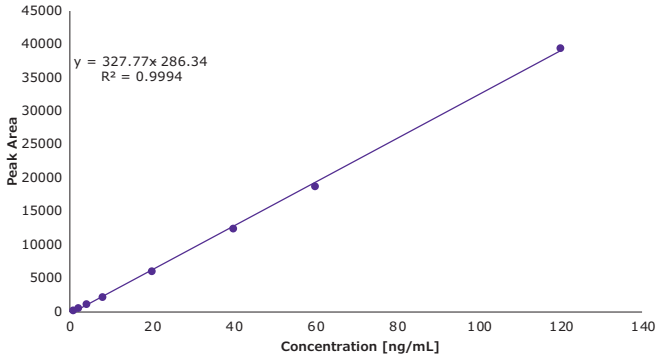
Overlaid MRM chromatograms of nine Mevinphos I and Mevinphos II standards.



Three injections of 120 ng/mL Mevinphos I & II standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
37	Mevinphos I	5.25	225.2->127.1	10 ms	16	10
37	Mevinphos II	5.95	225.2->127.1	10 ms	16	10

Standard Repeatability (120 ng/mL/)	
STD 1	**18954**
STD 2	**19710**
STD 3	**19212**
Mean	**19292**
Standard Deviation	**384.296**
RSD (%)	**1.99**

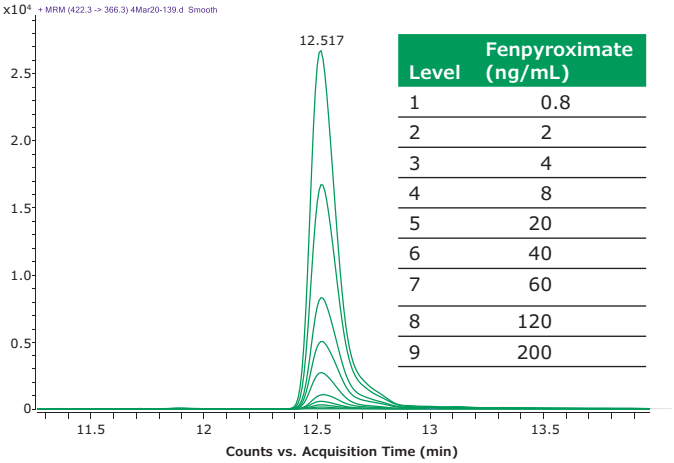


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**268**
2	**607**
4	**1293**
8	**2505**
20	**6335**
40	**12806**
60	**20126**
120	**38160**

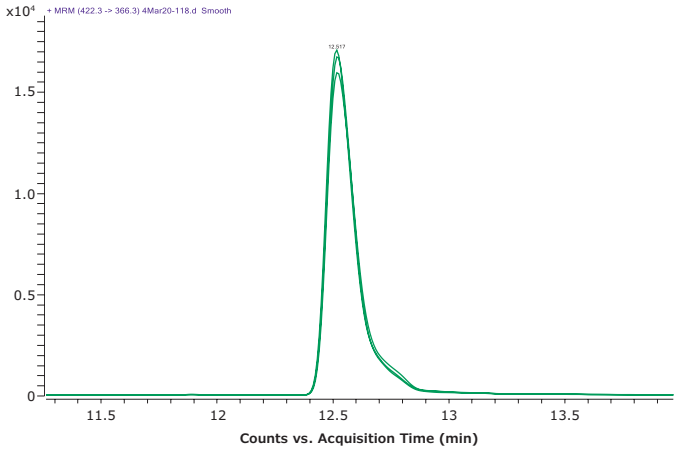
LOD	**0.3**
LOQ	**0.8 ppb**

Fenpyroximate LC-MS/MS detection and calibration



Level	Fenpyroximate (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

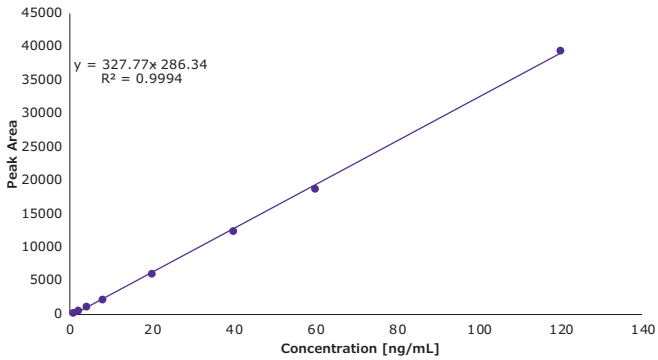
Overlaid MRM chromatograms of nine Fenpyroximate standards.



Three injections of 120 ng/mL Fenpyroximate standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
26	Fenpyroximate	12.52	422.3 -> 366.3	10 ms	15	10

Standard Repeatability (120 ng/mL/)	
STD 1	**148573**
STD 2	**150378**
STD 3	**147517**
Mean	**148822.7**
Standard Deviation	**1446.748**
RSD (%)	**0.97**

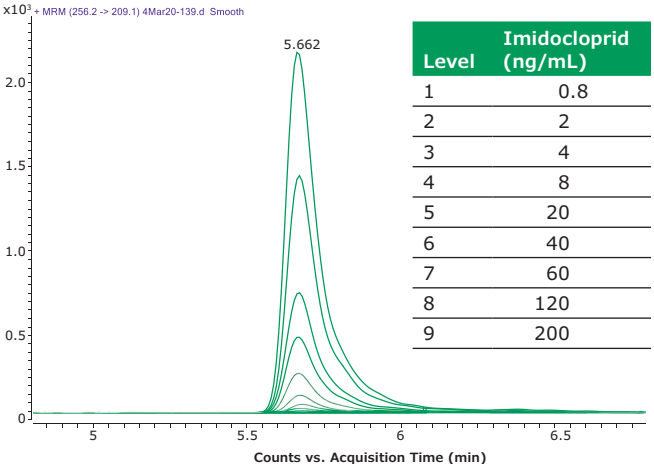


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**1817**
2	**4827**
4	**9932**
8	**19458**
20	**45033**
40	**90386**
60	**140927**
120	**266965**
200	**444136**

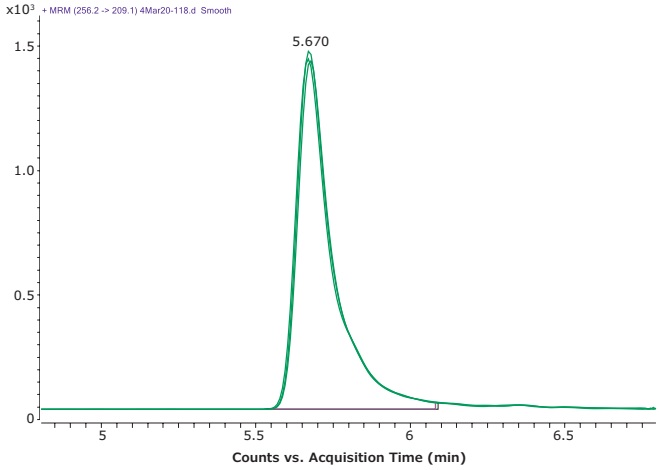
LOD	**0.3**
LOQ	**0.8 ppb**

Imidacloprid LC-MS/MS Detection and Calibration



Level	Imidocloprid (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

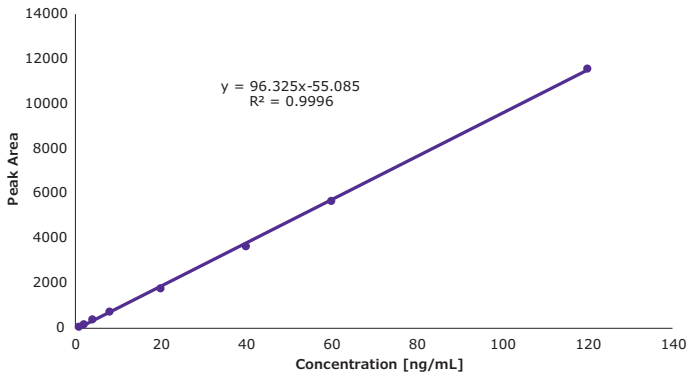
Overlaid MRM chromatograms of nine Imidacloprid standards.



Three injections of 120 ng/mL Imidacloprid standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
31	Imidacloprid	5.67	256.2 -> 209.1	10 ms	16	10

Standard Repeatability (120 ng/mL/)	
STD 1	**11589**
STD 2	**11673**
STD 3	**11396**
Mean	**11552.67**
Standard Deviation	**142.0293**
RSD (%)	**1.23**

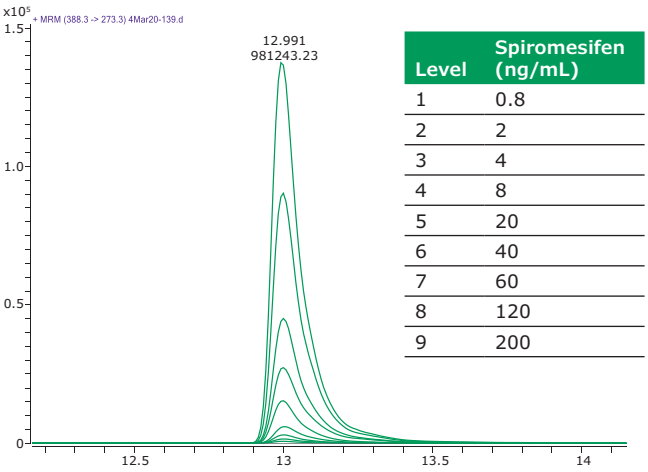


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**76**
2	**179**
4	**397**
8	**742**
20	**1789**
40	**3664**
60	**5673**
120	**11583**

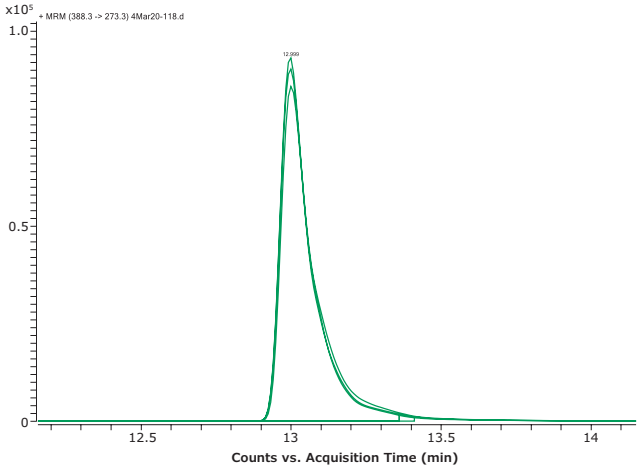
LOD	**0.3**
LOQ	**0.8 ppb**

Spiromesifen LC-MS/MS Detection and Calibration



Level	Spiromesifen (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

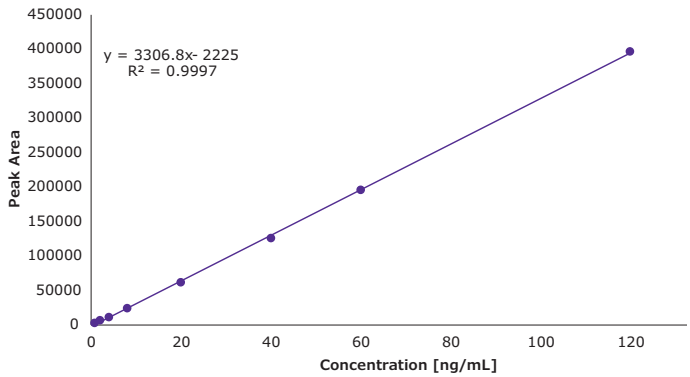
Overlaid MRM chromatograms of nine Spiromesifen standards.



Three injections of 120 ng/mL Spiromesifen standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
51	Spiromexifen	13	388.3 -> 273.3	10 ms	6	10

Standard Repeatability (120 ng/mL/)	
STD 1	**664843**
STD 2	**676135**
STD 3	**665925**
Mean	**668967.7**
Standard Deviation	**6230.624**
RSD (%)	**0.93**

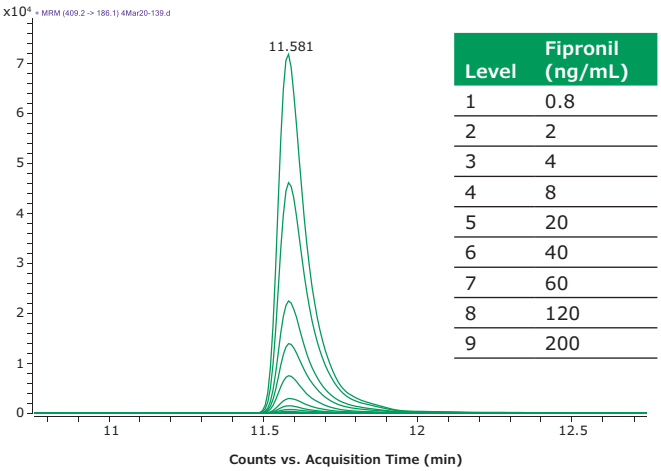


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**2529**
2	**6537**
4	**11422**
8	**24290**
20	**61865**
40	**125957**
60	**195709**
120	**396466**

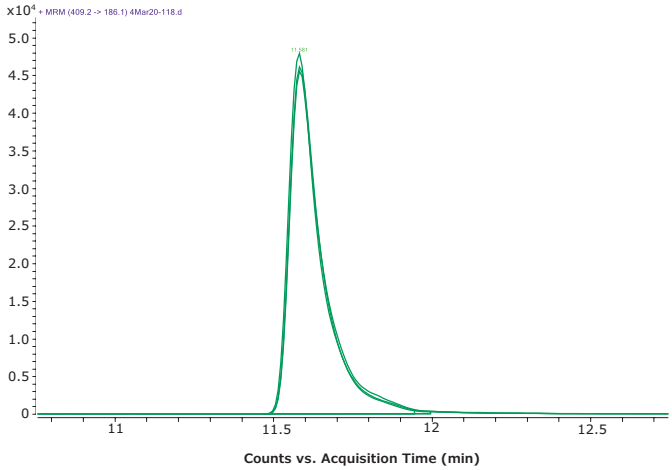
LOD	**0.3**
LOQ	**0.8 ppb**

TrifloXystrobin LC-MS/MS Detection and Calibration



Level	Fipronil (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

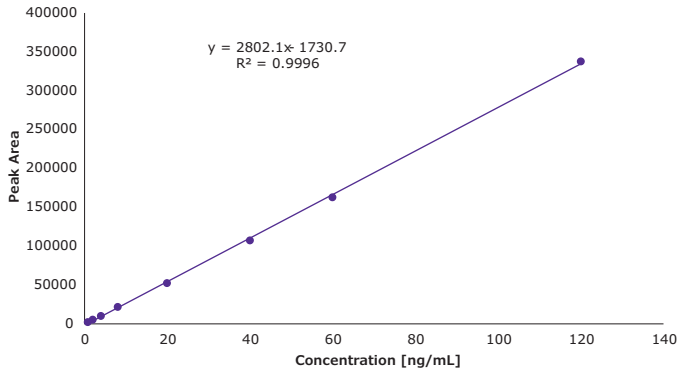
Overlaid MRM chromatograms of nine Trifloxystrobin standards.



Three injections of 120 ng/mL Trifloxystrobin standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
57	Trifloxystrobin	11.58	409.2 -> 186.1	10 ms	12	10

Standard Repeatability (120 ng/mL/)	
STD 1	**329809**
STD 2	**337894**
STD 3	**332280**
Mean	**333327.7**
Standard Deviation	**4143.068**
RSD (%)	**1.24**

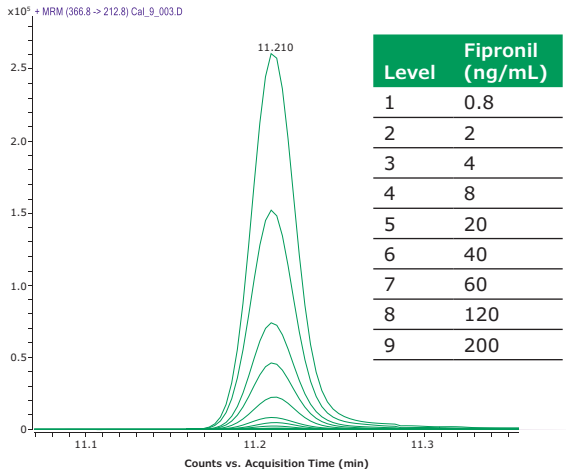


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**2240**
2	**5560**
4	**10348**
8	**21786**
20	**52622**
40	**107386**
60	**162679**
120	**337507**

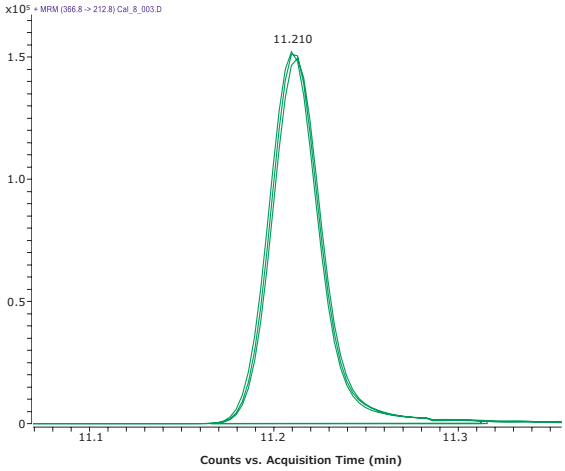
LOD	**0.3**
LOQ	**0.8 ppb**

FIPRONIL GC-MS/MS DETECTION AND CALIBRATION



Level	Fipronil (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine Fipronil standards.



Three injections of 120 ng/mL Fipronil standard solution.

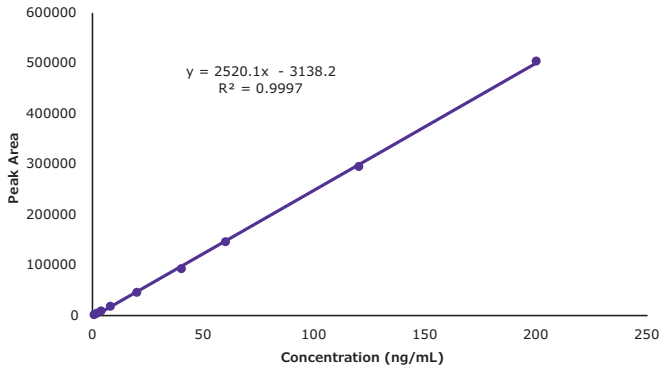
Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
14	Firpronil	11.213	366.8 -> 212.8	10 ms	25	10

Standard Repeatability (120 ng/mL/)	
STD 1	**289996**
STD 2	**295452**
STD 3	**295399**
Mean	**293615.7**
Standard Deviation	**3134.8**
RSD (%)	**1.1**

Linearity, LOD and LOQ

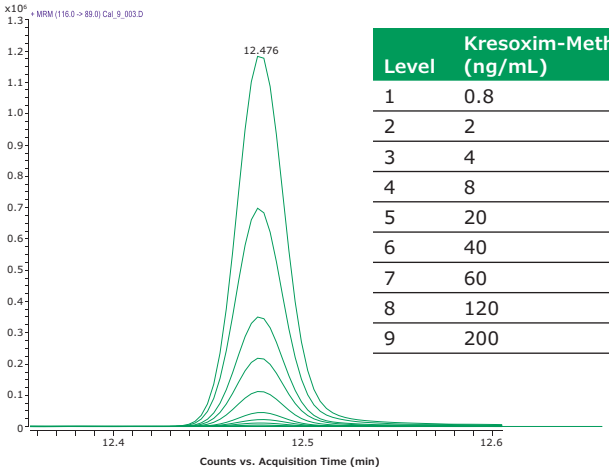
Concentration (ng/mL)	Peak Area
0.8	**1638**
2	**4567**
4	**8905**
8	**17998**
20	**45416**
40	**93201**
60	**145952**
120	**295452**
200	**504777**

LOD	**0.3**
LOQ	**0.8 ppb**



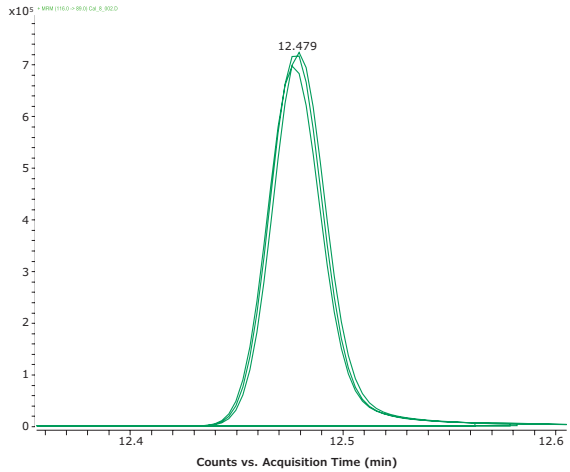
Calibration curve for Fipronil standards from 0.8 ng/mL to 200 ng/mL

Kresoxim-methyl GC-MS/MS DETECTION AND CALIBRATION



Level	Kresoxim-Methyl (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

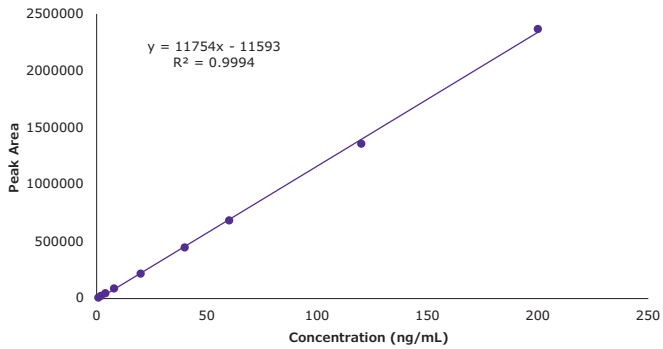
Overlaid MRM chromatograms of nine Kresoxim-Methyl standards.



Three injections of 120 ng/mL Kresoxim-Methyl standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
22	Kresoxim-Methyl	12.479	366.8 -> 212.8	10 ms	25	10

Standard Repeatability (120 ng/mL/)	
STD 1	**1369928**
STD 2	**1357719**
STD 3	**1322181**
Mean	**1349942.7**
Standard Deviation	**24805.2**
RSD (%)	**1.8**

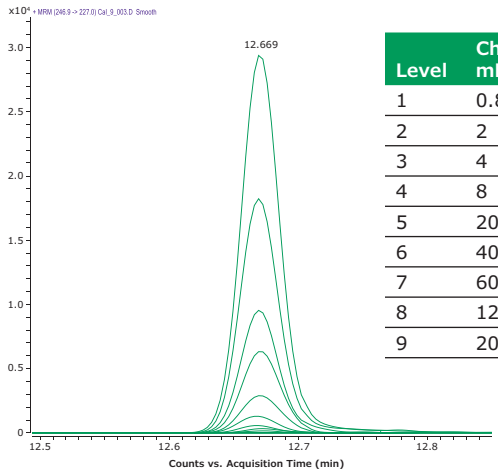


Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**7911**
2	**23306**
4	**46173**
8	**86167**
20	**216345**
40	**449742**
60	**685807**
120	**1357719**
200	**2368236**

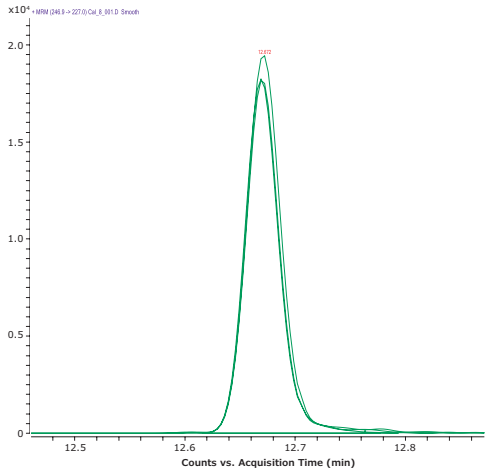
LOD	**0.3**
LOQ	**0.8 ppb**

CHLORFENAPYR GC-MS/MS DETECTION AND CALIBRATION



Level	Chlorfenapyr (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine Chlorfenapyr standards.



Three injections of 120 ng/mL Chlorfenapyr standard solution.

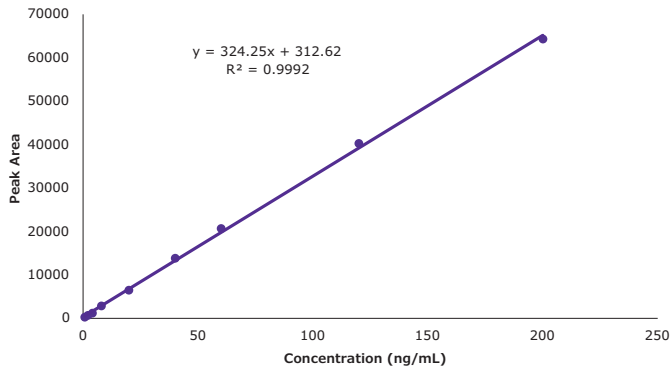
Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
24	Chlorfenapyr	12.672	246.9 -> 227.0	10 ms	15	10

Standard Repeatability (120 ng/mL/)	
STD 1	**43673**
STD 2	**39620**
STD 3	**40201**
Mean	**41164.7**
Standard Deviation	**2191.6**
RSD (%)	**5.3**

Linearity, LOD and LOQ

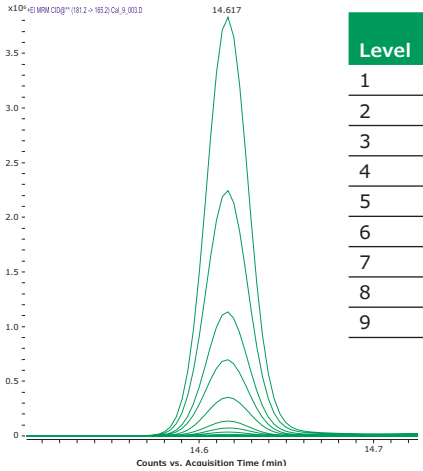
Concentration (ng/mL)	Peak Area
0.8	**292**
2	**663**
4	**1179**
8	**2785**
20	**6458**
40	**13785**
60	**20661**
120	**40201**
200	**64259**

LOD	**0.3**
LOQ	**0.8 ppb**



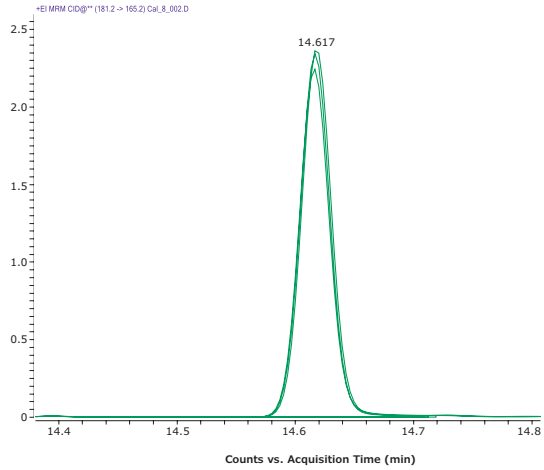
Calibration curve for Chlorfenapyr standards from 0.8 ng/mL to 200 ng/mL

BIFENTHRIN GC-MS/MS DETECTION AND CALIBRATION



Level	Bifenthrin (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine Bifenthrin standards.



Three injections of 120 ng/mL Bifenthrin standard solution.

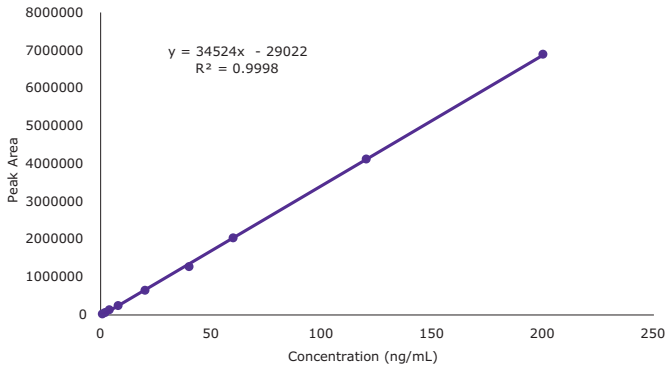
Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
29	Bifenthrin	14.617	181.2 -> 165.2	10 ms	25	10

Standard Repeatability (120 ng/mL/)	
STD 1	**4216546**
STD 2	**4117505**
STD 3	**4285451**
Mean	**4206500.7**
Standard Deviation	**84422.4**
RSD (%)	**2**

Linearity, LOD and LOQ

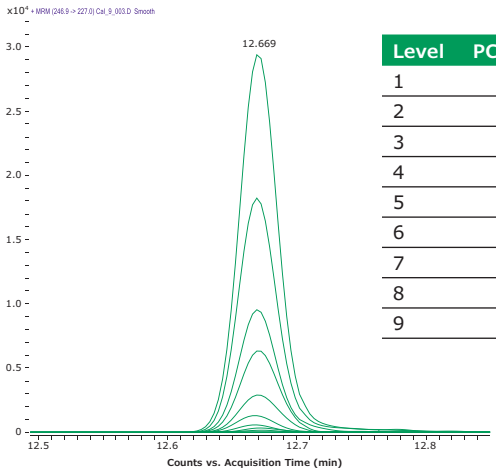
Concentration (ng/mL)	Peak Area
0.8	**24322**
2	**61097**
4	**132198**
8	**250318**
20	**647715**
40	**1278722**
60	**2038589**
120	**4117505**
200	**6889862**

LOD	**0.3**
LOQ	**0.8 ppb**



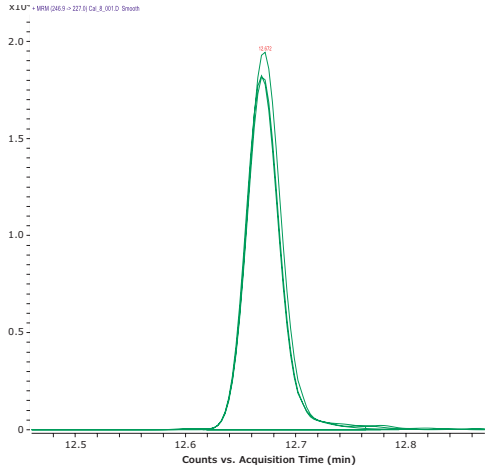
Calibration curve for Bifenthrin standards from 0.8 ng/mL to 200 ng/mL

PCNB GC-MS/MS DETECTION AND CALIBRATION



Level	PCNB (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine PCNB standards.



Three injections of 120 ng/mL PCNB standard solution.

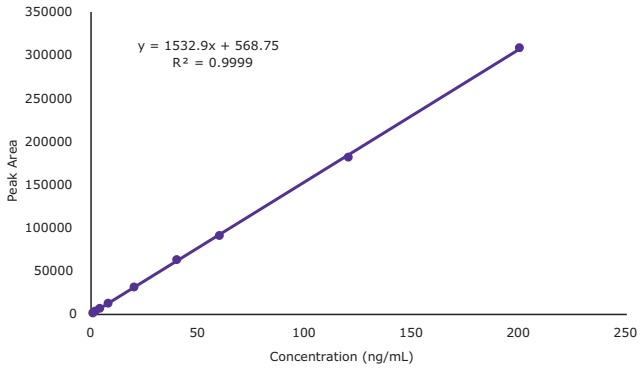
Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
8	PCNB	8.847	248.7 -> 213.9	10 ms	15	10

Standard Repeatability (120 ng/mL/)	
STD 1	**172599**
STD 2	**173017**
STD 3	**182041**
Mean	**175885.7**
Standard Deviation	**5334.8**
RSD (%)	**3**

Linearity, LOD and LOQ

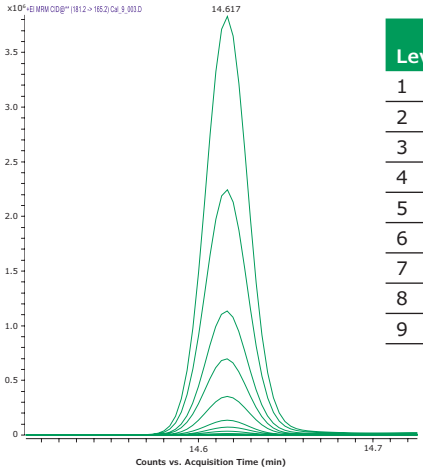
Concentration (ng/mL)	Peak Area
0.8	**1516**
2	**3684**
4	**7022**
8	**13024**
20	**31499**
40	**63399**
60	**91490**
120	**182041**
200	**308606**

LOD	**0.3**
LOQ	**0.8 ppb**



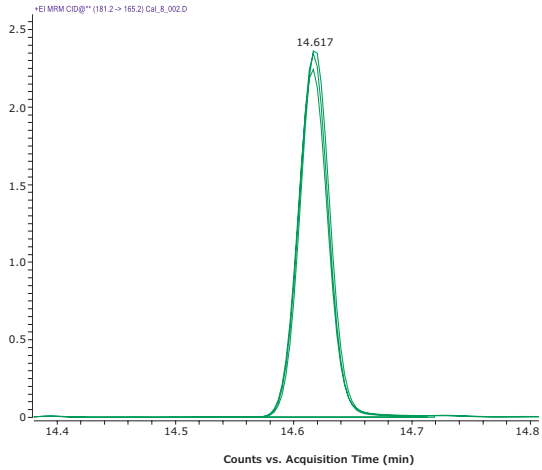
Calibration curve for PCNB standards from 0.8 ng/mL to 200 ng/mL

Cyfluthrin I & II GC-MS/MS DETECTION AND CALIBRATION



Level	Cyfluthrin I & II (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine Cyfluthrin I & II standards.



Three injections of 120 ng/mL Cyfluthrin I & II standard solution.

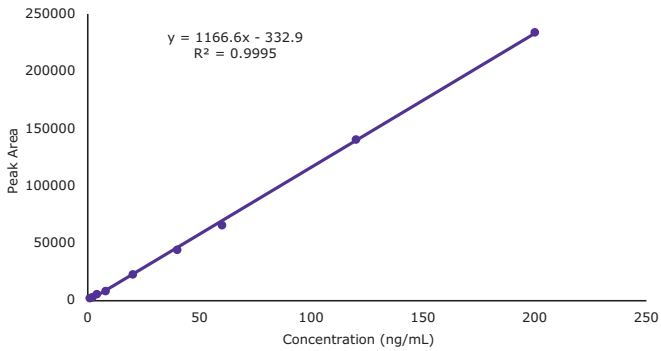
Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
37	Cyfluthrin I	17.086	198.9->170.1	10 ms	25	10
37	Cyflythrin II	17.122	198.9->170.1	10 ms	25	10

Standard Repeatability (120 ng/mL/)	
STD 1	**128772**
STD 2	**130570**
STD 3	**134731**
Mean	**131357.7**
Standard Deviation	**3056.6**
RSD (%)	**2.3**

Linearity, LOD and LOQ

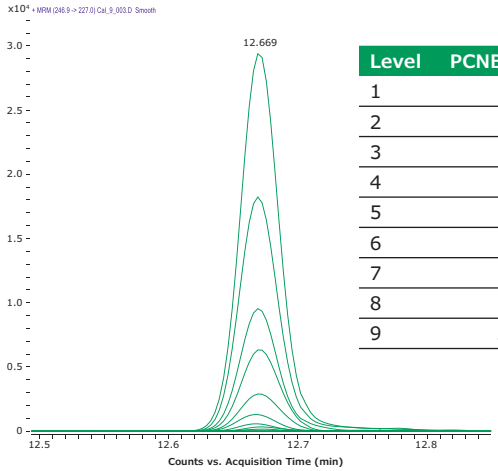
Concentration (ng/mL)	Peak Area
0.8	**2252**
2	**3110**
4	**5650**
8	**8611**
20	**23070**
40	**44494**
60	**65921**
120	**140570**
200	**233894**

LOD	**0.3**
LOQ	**0.8 ppb**



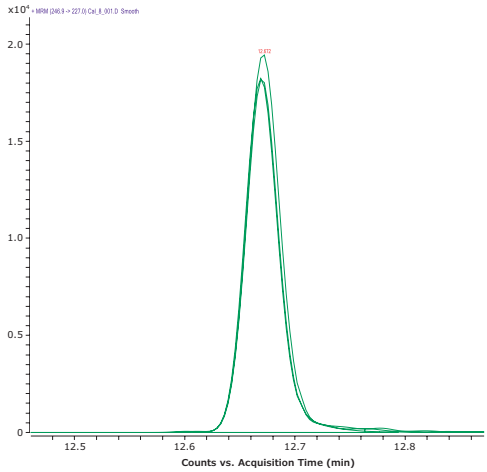
Calibration curve for Cyfluthrin I & II standards from 0.8 ng/mL to 200 ng/mL

PCNB GC-MS/MS DETECTION AND CALIBRATION



Level	PCNB (ng/mL)
1	0.8
2	2
3	4
4	8
5	20
6	40
7	60
8	120
9	200

Overlaid MRM chromatograms of nine PCNB standards.



Three injections of 120 ng/mL PCNB standard solution.

Peaks	Analyte	Retention Time (min)	MRM (m/z)	Dwell time	Collision Energy (eV)	Gain
8	PCNB	8.847	248.7 -> 213.9	10 ms	15	10

Standard Repeatability (120 ng/mL/)	
STD 1	**172599**
STD 2	**173017**
STD 3	**182041**
Mean	**175885.7**
Standard Deviation	**5334.8**
RSD (%)	**3**

Linearity, LOD and LOQ

Concentration (ng/mL)	Peak Area
0.8	**1516**
2	**3684**
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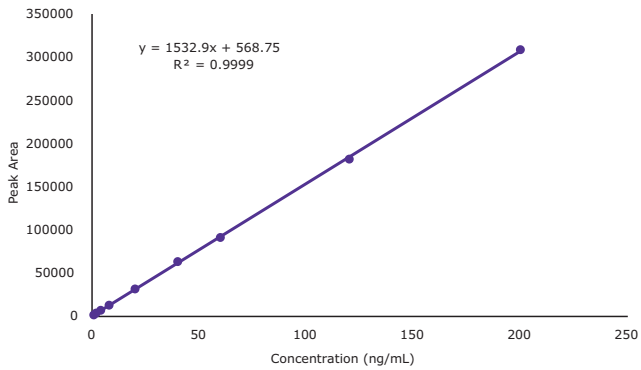
8	**13024**
20	**31499**
40	**63399**
60	**91490**
120	**182041**
200	**308606**

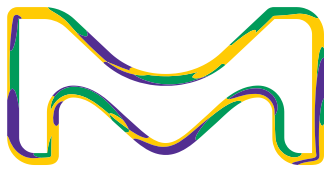
LOD	**0.3**
LOQ	**0.8 ppb**



Calibration curve for PCNB standards from 0.8 ng/mL to 200 ng/mL

Notes:

Notes:



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