Application Note

Cannabis & Hemp Testing

Agilent

Trusted Answers

A Sensitive and Robust Workflow to Measure Residual Pesticides and Mycotoxins from the Canadian Target List in Dry Cannabis Flower

Authors

Jean-Francois Roy[1], Christophe Deckers[1], Ron Honnold[2], Peter Stone[2], Jennifer Hitchcock[2], Anthony Macherone[2,4], Ben Geiling[3], and Scott Young[3]

[1] Agilent Technologies Canada, Inc. 2250 Boul. Alfred Nobel, Montreal, QC, H4S 2C9

[2] Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Santa Clara, CA 95051

[3] Canopy Growth Corporation, 1 Hershey Dr, Smith Falls, Ontario, K7A 3L1

[4] Johns Hopkins University School of Medicine, 725 N. Wolfe Street Baltimore, MD 21205

Abstract

As of October 2018, the recreational use of cannabis is legal at the federal level throughout Canada. Under Canadian law, licensed cannabis producers are obligated to meet strict quality requirements and mandatory testing to ensure consumer safety. The array of mandated testing includes potency determination, heavy metal detection, and microbial screening, amongst others. Of these, the analysis of pesticide residues is the most challenging, and Health Canada mandates a target list of 96 pesticides and five mycotoxins to be tested at limits of quantitation (LOQ) typically lower than any U.S. state. As a result, pesticide residue analysis in cannabis under Canadian regulations require state-of-the-art LC and GC triple quadrupole mass spectrometry (LC/MS/MS and GC/MS/MS, respectively).

Using a standardized sample preparation procedure and both LC/MS/MS and GC/MS/MS platforms, we demonstrate robust, specific, and sensitive quantification of the Canadian pesticide and mycotoxin target lists that meet the required reporting limits as published by Health Canada in dry cannabis. Eighty-eight target pesticides and five mycotoxins were analyzed with the Agilent 6470 LC/MS/MS system and alternatively, the Agilent Ultivo LC/MS/MS system, both coupled to an Agilent 1290 Infinity II UHPLC. Seventeen pesticides were analyzed on the Agilent 7890/7010 GC/MS/MS system.

As in the food and tobacco industries, pesticide testing requirements in cannabis are expected to become more rigorous over time, reinforcing the need for adopting a flexible and sensitive procedure such as the one described here. This multiplatform approach provides a rapid return on investment (ROI) and a stable foundation to meet current and future testing requirements.

Introduction

Many U.S. States have some form of cannabis or cannabinoid legalization. On the U.S. federal level however, cannabis (as defined by a Δ^9-tetrahydrocannabinol concentration >0.3 % wt/wt) is a Schedule 1 controlled substance, thus preventing the creation of clear nation-wide guidelines for cannabis testing. As a result, every state tests for different pesticides and define different limits of quantitation or action levels. The lack of harmonized guidelines results in many disparate methods that do not meet the pesticide testing requirements published by Health Canada in October 2018, where reporting limits are typically 10-fold lower than current requirements in California[1,2].

With respect to the number of target pesticides and action levels, Canada has the most comprehensive list in North America, with action levels for 96 pesticides as low as 20 parts-per-billion (ppb) for dried cannabis, and 10 ppb for fresh (wet) cannabis or cannabis oils. The California list includes 66 target pesticides and action levels down to 100 ppb for inhalable cannabis and other cannabis products. The Canadian list does not completely incorporate the California list, with captan, chlordane, and fenhexamid being unique to California.

Many U.S. state pesticide lists can be analyzed exclusively by LC/MS/MS. Notable exceptions include California, Florida, and Nevada, where GC/MS/MS is also required. This list of exceptions is expected to grow as the states add more compounds and lower the required limits of detection (LODs). Similarly to California, Florida, and Nevada, the extensive Canadian pesticide list presents at least six compounds for which reporting limits cannot be met by LC/MS/MS: endosulfan *alpha* and *beta*, etridiazole, fenthion, kinoprene, and quintozene (pentachloronitrobenzene).

Those compounds and others, such as captan and chlordane, are commonly analyzed through GC/MS/MS using electron ionization.

A brief discussion of sample preparation

Cannabis is a complex plant containing many endogenous chemicals representing numerous chemical classes. Compared to other plants and vegetables, cannabis has higher amounts of potential interferences, and notably high concentrations of terpenes, cannabinoids, flavonoids, phenols, and fatty acids[4]. The complexity of the cannabis matrix makes detection and accurate quantification of trace levels of pesticides more challenging. Interfering compounds can negatively impact ionization in the mass spectrometer, affect signal-to-noise ratios (S/N), and build-up in the instrument source and consumables, thus decreasing productivity and increasing maintenance and operating costs. To overcome this challenge, a combination of optimized sample preparation and state-of-the-art instrumentation is required.

Initially, Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) appeared to be a promising technique to extract pesticides and clean up samples. QuEChERS is a commonly used technique to prepare samples for residual pesticide testing in fruits and vegetables, and is a two-step procedure. The first step is to perform an extraction/partitioning between water and acetonitrile. The resulting acetonitrile layer undergoes a second cleaning step that uses dispersive solid-phase extraction (dSPE) sorbents to capture matrix interferences that would otherwise negatively impact detection by mass spectrometry.

Unfortunately, the QuEChERS approach is not viable for cannabis flower. Cannabis is a unique plant that calls for unique sample preparation. Why?

- QuEChERS requires wetting dry cannabis with water. This procedure increases the pH enough to degrade labile pesticides such as Captan, folpet, and spiroxamine.
- The addition of salts common to the procedure creates an exothermic reaction that also degrades sensitive pesticides.
- Mycotoxins and very polar pesticides such as daminozide are in the water layer in the extraction step.
- Finally, QuEChERS is not a good option to clean up cannabis because plant components such as cannabinoids and terpenes are in such high concentrations that dispersive kits do not have enough capacity to effectively remove matrix interferences.

Additionally, some dispersive compounds used in QuEChERS use a primary secondary amine (PSA) that can potentially capture acidic pesticides and reduce recoveries. Other dispersive reagents contain graphitized carbon black (GCB) that can inadvertently capture planar pesticides without additional solvents and drying steps. For these reasons, an alternative sample preparation approach was developed for simplicity, quick turnaround time, and to provide enough cleanup for improved sensitivity and system uptime.

The information content of this application note, along with ready to run acquisition, quantitation, etc. methods and extensive support information, are available as eMethods Pesticides Residue and Mycotoxin Analysis in Cannabis and Hemp: G5279#030 with the 1290/6470 LC/TQ system and Pesticides Residue Analysis in Cannabis and Hemp G5278#030, with the 7890/7010B GC/TQ MS system.

Experimental

The LC/MS/MS analyses were performed using an Infinity II 1290 UHPLC system coupled to either a 6470 or an Ultivo triple quadrupole mass spectrometer. Both systems used an Agilent JetStream ESI source. The UHPLC system consisted of a binary pump (G7120A), low-carryover multisampler fitted with multiwash and 100-µL loop and metering device options (G7167B), thermostatted column compartment (G7116B), and Agilent MassHunter software.

The GC/MS/MS analyses were performed using an Agilent 7890B GC coupled to a 7010B triple quadrupole mass spectrometer. The GC configuration included a multimode inlet (MMI) and backflush capacity through a Purged Ultimate Union (PUU). The 7010B was equipped with a High Efficiency Source (HES) and the JetClean option, which allows for *in situ* cleaning of the HES source with hydrogen.

Instrumentation

Infinity II UHPLC method conditions	
Column (p/n 695975-312)	Infinity Lab Poroshell 120 Phenyl Hexyl, 3.0 × 100 mm, 2.7, µm
Guard column (p/n 823750-914)	Infinity Lab Poroshell 120 Phenyl Hexyl, 3.0 × 5 mm, 2.7, µm
Column temperature	55 °C
Injection volume	25 µL
Autosampler temperature	4 °C
Multiwash table	Step / Solvent / Time (s) / Seat backflush / Needle wash / Comments 1 / S1 / 10 / Yes / Yes / 0.1 % Formic acid in isopropanol 2 / S2 / 10 / Yes / Yes / 0.1 % Formic acid in acetonitrile 3 / S3 / 20 / Yes / Yes / 50:50 A:B
Mobile phase	A) 5 mM Ammonium formate + 0.1 % formic acid in water B) 0.1 % Formic acid in 90:10 methanol:acetonitrile
Gradient flow rate	0.5 mL/min
Analysis and re-equilibration time	10 minutes, 1.5 minutes
Total run time (sample to sample)	11.5 minutes
Gradient	Time (min) %B 0.00 50 1.00 50 8.00 95 9.00 100 10 100
LC/MS/MS Configuration and parameters	
Configuration	6470 QQQ or Ultivo QQQ Mass Spectrometer, both equipped with Jet Stream (AJS) ESI Source.
MS/MS Parameters	
Acquisition mode	dMRM
Polarity	Positive or Negative (compound-dependent)
Capillary voltage	4,000 V in positive mode, 3,000 V in negative mode
Drying gas flow	10 L/min
Drying gas temperature	200 °C
Nebulizer pressure	35 psi
Sheath gas temperature:	200 °C
Sheath gas flow	10 L/min
Nozzle voltage	300 V (either polarity)
Q1 and Q2 Resolution	Unit (0.7 amu), optimized by autotune
Delta EMV	0 V

Materials and reagents

Pesticide and mycotoxin standards

Pesticides and mycotoxins were obtained either individually or in mixes from various sources. All compounds were mixed to create a stock solution in acetonitrile, with each compound present at 1,000 ppb.

Other reagents

- **LC/MS grade methanol:** Sigma-Aldrich
- **LC/MS grade acetonitrile:** EMD Millipore
- **LC/MS grade water:** Burdick and Jackson
- **Pesticide-grade hexanes:** EMD Millipore
- **Pesticide-grade acetone:** Sigma-Aldrich
- **Formic acid (97+ %):** Sigma-Aldrich
- **Ammonium formate (99+ %):** Fisher Scientific

Sample and calibrator preparation

Several 1-g dried cannabis samples were simultaneously reduced to a fine powder by vertical shaking in clean tubes. Then, pesticides and mycotoxins were extracted from the cannabis powder with acetonitrile, and cleaned up on SampliQ C18 EC SPE cartridges. The resulting cannabis extracts were further diluted and tested by LC/MS/MS and GC/MS/MS (Figures 1 and 2).

Detailed sample preparation common to both LC/MS/MS and GC/MS/MS

1. Weigh 1.0 g of chopped cannabis into a 50-mL polypropylene (PP) centrifuge tube.
2. Add two ceramic homogenizers (p/n 5982-9313) or stainless steel beads to the tube, and cap. The homogenizers will help turn the chopped cannabis into a fine powder.
3. Shake mechanically for 2–5 minutes at high speed, ideally on a vertical shaking device (Geno/Grinder-type machine), to turn the dry cannabis into fine powder.
4. If precleanup spiked matrix samples are to be prepared, pipette the pesticide standard solution(s) and mycotoxin standards into the dry cannabis powder, then vortex for 30 seconds.
5. Add 15 mL of pesticide-grade acetonitrile to the tube from step 3.
6. Shake the tube mechanically for five minutes at high speed, ideally on a vertical shaking device (Geno/Grinder-type machine). This will extract the pesticides and mycotoxins into the acetonitrile.
7. While the tube is shaking, prepare the solid phase extraction (SPE) manifold by placing a SampliQ C18 EC 6 mL 500 mg SPE cartridge (p/n 5982-1365) onto the manifold. Place a collection tube that can hold 25 mL or more. Ideally, use a graduated 50-mL PP centrifuge tube underneath the cartridge in which the eluent will be collected.
8. Decant the supernatant from step 6 into the SampliQ C18 EC SPE cartridge. It will flow by gravity, but might require a small pressure pulse to initiate the flow.

7890B GC Method conditions	
Inlet	MMI
Inlet liner	Ultra Inert, Splitless, 4-mm single taper with deactivated fused silica wool (p/n 5190-2293)
Inlet temperature program	180 °C initial, hold 0 min, 400 °C/min to 280 °C
Injection volume	2 uL
Column 1	Agilent DB-35MS Ultra Inert, 15 m × 0.25 mm, 0.25 µm film thickness (p/n 122-3812UI), connected to MMI and Agilent Purged Ultimate Union
Column 2	Agilent HP-5MS Ultra Inert, 15 m × 0.25 mm, 0.25 µm film thickness (p/n 19091S-431UI), connected to Agilent Purged Ultimate Union and QQQ Transfer Line
Column 1 flow	1.0 mL/min, constant
Column 2 flow	1.4 mL/min, constant
Oven temperature program	Rate (°C/min) / Value (°C) / Hold time (min) / Run time (min) — / 70 / 1 / 1 60 / 240 / 0 / 3.8333 4 / 255 / 0 / 7.5833 30 / 300 / 6.9 / 15.983
Column backflush	Post run, 2.4 min at 2.49 mL/min
Run time	15-minute analysis time, 2.4-minute post run backflush, total sample-to-sample time of 22 minutes
GC/MS/MS Configuration and parameters	
Source	HES
Ionization mode	Electron Impact (EI)
Transfer line temperature	300 °C
Source temperature	280 °C
Quadrupole temperature	150 °C
Acquisition mode	dMRM
Detector gain factor	10
Solvent delay	3.5 minutes
Acquisition rate	7 cycles per second

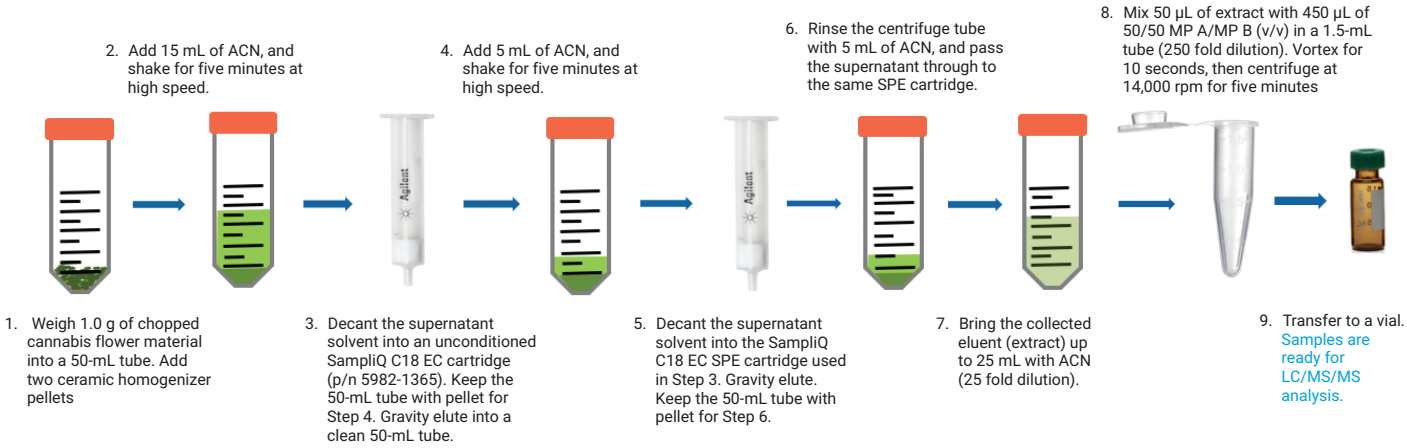

Figure 1. Schematic representation of sample preparation procedure for LC/MS/MS analysis.

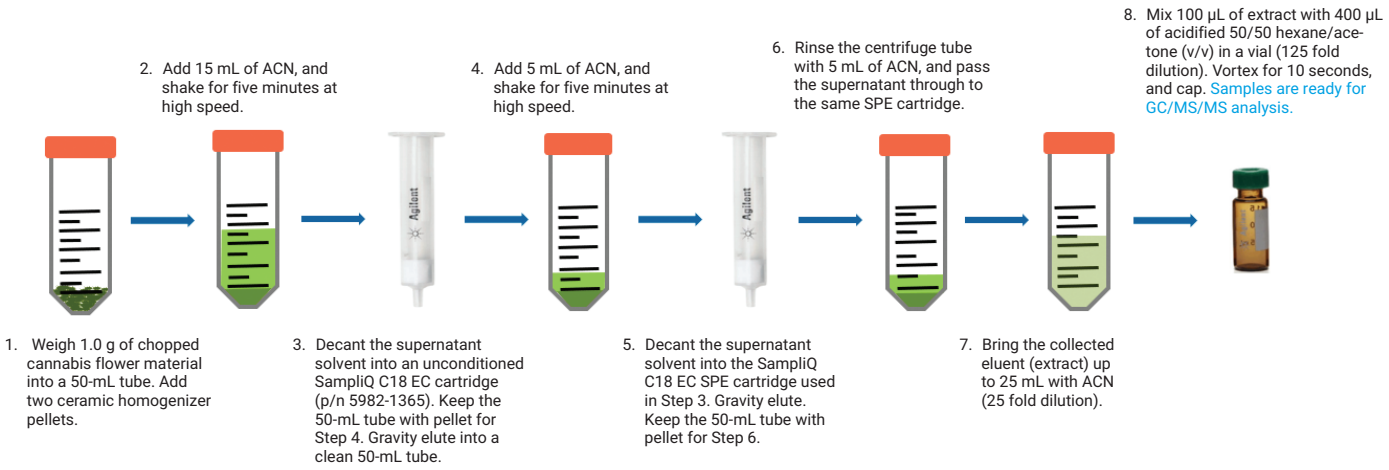

Figure 2. Schematic representation of sample preparation procedure for GC/MS/MS analysis.

9. After the entire solvent has gone through the C18 cartridge and is collected, add 5 mL of acetonitrile to the empty tube from step 6 and shake mechanically for five minutes at high speed. This will extract pesticides and mycotoxins that may still be in the cannabis material.
10. Decant the supernatant from step 9 into the same SampliQ C18 EC SPE cartridge.
11. Rinse the empty tube from step 9 with a final 5 mL of acetonitrile to wash any pesticides that might be retained on the tube wall, then pass this solvent through the same C18 cartridge. A volume of less than 25 mL (three portions of 15, 5, and 5 mL) of acetonitrile extract is collected.
12. Transfer all eluent into a volumetric flask, bring the final volume to 25 mL with acetonitrile or use the 25-mL mark on the graduated 50-mL PP centrifuge tube to adjust to 25 mL total. Vortex. Now the sample has been diluted 25 times.
13. Transfer the cleaned extract (step 12) into a clean tube, cap, and label.

Detailed sample preparation unique to LC/MS/MS

14. In a 1.5-mL centrifuge tube, mix solution 13 with a solution of 50:50 mobile phase A:mobile phase B in a 1-to-9 proportion. A typical scenario would be to mix 100 µL of solution 13 with 900 µL of 50:50 mobile phase A: mobile phase B. Vortex for 10 seconds. The solution might become cloudy. Now the sample has been diluted 250 times.
15. Centrifuge at 14,000 rpm for five minutes. Pellets might be observed at the bottom of the tube after centrifugation. Some cloudiness may be observed.
16. Transfer solution 15 to a 2-mL vial (p/n 5182-0716); avoid pipetting the pellets. Cap using p/n 5190-7021.
17. Inject the solution for LC/MS/MS or spike it with the desired amount of pesticide to obtain a post cleanup spiked matrix.

Matrix-matched calibrators: LC/MS/MS

Pesticide-free, extracted dry cannabis matrix in acetonitrile (1 g in 25 mL of acetonitrile = 25x dilution) further diluted 10x with 50:50 mobile phase A:mobile phase B (referred to as *Extract* in Table 1) was prepared in appropriate volume. Total dilution of matrix was 250x.

Dilutions were done in Eppendorf tubes, then the remaining solution in each tube was transferred into a deactivated glass insert (p/n 5181-8872) placed in a 2-mL vial, which was then capped and injected.

Detailed sample preparation unique to GC/MS/MS

18. Into a 2-mL vial, mix solution 13 with a solution of acidified hexane:acetone solution (0.1 % formic acid in 50:50 hexane:acetone) in a 1-to-4 proportion. A typical scenario would be to mix 200 µL of solution 13 with 800 µL of acidified hexane:acetone solution (0.1% formic acid in 50:50 hexane:acetone). Vortex for 10 seconds. Now the sample has been diluted 125 times.
19. Inject the solution for GC/MS/MS or spike it with the desired amount of pesticide to obtain a post cleanup spiked matrix.

Table 1. Preparation of calibrators for LC/MS/MS analysis.

STD Level (In vial, ppb)	Volume (µL)	Solution		Volume (µL)	Solution
25	12.5	1 ppm pesticide stock	added to	487.5	Extract
10	200	25 ppb	added to	300	Extract
5	250	10 ppb	added to	250	Extract
2.5	250	5 ppb	added to	250	Extract
1	200	2.5 ppb	added to	300	Extract
0.75	375	1 ppb	added to	125	Extract
0.5	333	0.75 ppb	added to	167	Extract
0.25	250	0.5 ppb	added to	250	Extract
0.1	200	0.25 ppb	added to	300	Extract
0.075	375	0.1 ppb	added to	125	Extract
0.05	333	0.075 ppb	added to	167	Extract
0.025	250	0.05 ppb	added to	250	Extract
0.01	200	0.025 ppb	added to	300	Extract
0.0075	375	0.01 ppb	added to	125	Extract
0.005	333	0.0075 ppb	added to	167	Extract

Matrix-matched calibrators: GC/MS/MS

Pesticide-free, extracted dry cannabis matrix in acetonitrile (1 g in 25 mL of acetonitrile = 25x dilution) further diluted 5x with acidified hexane:acetone solution (referred to as *Acidified Extract* in Table 2) was prepared in appropriate volume. Total dilution of matrix was 125x.

Dilutions were done in Eppendorf tubes, then the remaining solution in each tube was transferred into a deactivated glass insert (p/n 5181-8872) placed in a 2-mL vial, which was then capped and injected.

Table 2. Preparation of calibrators for GC/MS/MS analysis.

STD Level (In vial, ppb)	Volume (µL)	Solution		Volume (µL)	Solution
50	50	1 ppm pesticide stock	added to	450	Acidified extract
25	250	50 ppb	added to	250	Acidified extract
10	200	25 ppb	added to	300	Acidified extract
5	250	10 ppb	added to	250	Acidified extract
2.5	250	5 ppb	added to	250	Acidified extract
1	200	2.5 ppb	added to	300	Acidified extract
0.75	375	1 ppb	added to	125	Acidified extract
0.5	333	0.75 ppb	added to	167	Acidified extract
0.25	250	0.5 ppb	added to	250	Acidified extract
0.1	200	0.25 ppb	added to	300	Acidified extract
0.075	375	0.1 ppb	added to	125	Acidified extract
0.05	333	0.075 ppb	added to	167	Acidified extract

Results and discussion

Sample preparation

The use of ceramic homogenizers or stainless steel beads combined with the vertical shaking of multiple samples in individual 50-mL PP tubes eliminates the need for mechanical grinding. Mechanical grinding is typically low-throughput, and requires extra precaution to avoid cross-contamination from sample to sample. Vertical shaking increases lab productivity and reduces labor costs associated with sample handling. Sample size is important, as it must represent a statistically relevant proportion of the cannabis lot to be tested, with a constant pesticide exposure throughout. Therefore, approved sampling methods by Health Canada need to be followed.

Using a simple acetonitrile extraction and SPE cleanup, recoveries of pesticides and mycotoxins were calculated by spiking the acetonitrile extract (25x sample dilution) before the final dilution for either LC/MS/MS or GC/MS/MS analysis. Calculated recoveries (see Table 3) were comparable to those observed in a previous publication[5], although the approach described here was optimized for Canadian reporting limits.

The unique SampliQ C18 EC SPE cartridge used for cleanup displays superior inertness towards pesticides and mycotoxins. This SPE step shows its relevance as extracted samples have a significantly cleaner appearance after going through the cartridge. Cannabis is a unique plant that requires unique sample prep. High amounts of cannabinoids, terpenes, and other interferences can alter proper quantification by LC/MS/MS and GC/MS/MS. Therefore, the combination of SampliQ C18 EC cleanup followed by dilution in optimized solvents provides the best balance between sensitivity and robustness with reduced labor costs.

Table 3. Calculated recoveries from spiking matrix before final dilution.

Compound	Recovery (%)	%RSD (n = 3)
Pesticides		
Abamectin (Avermectin B1a)	102.7	3.3
Acephate	102.3	1.7
Acetamiprid	103.2	1.1
Acequinocyl	37.8	4.2
Aldicarb	101.9	1.5
Allethrin	108.7	2.5
Azadirachtin	92.7	5.5
Azoxystrobin	103.9	1.1
Benzovindiflupyr	106.4	0.9
Bifenazate	101.2	2.6
Bifenthrin	103.9	1.4
Boscalid	91.0	1.8
Buprofezin	103.0	1.3
Carbaryl	101.5	1.7
Carbofuran	102.2	1.1
Chlorantraniliprole	100.2	2.9
Chlorphenapyr	103.7	1.3
Chlorpyrifos	101.0	1.2
Clofentezine	113.0	3.9
Clothianidin	100.4	2.7
Coumaphos	98.9	2.8
Cyantranilipole	99.4	1.3
Cyfluthrin	99.8	2.1
Cypermethrin	92.5	4.8
Cyprodinil	79.8	3.5
Daminozide	102.4	3.2
Deltamethrin	91.9	5.5
Diazinon	103.4	1.6
Dichlorvos	98.9	2.1
Dimethoate	103.5	1.6
Dinotefuran	101.6	2.1
Dodemorph	94.2	2.1
Endosulfan alpha	96.2	3.4
Endosulfan beta	96.3	3.9
Endosulfan sulfate	101.4	3.0

Compound	Recovery (%)	RSD (%, n = 3)
Ethoprophos	102.6	2.3
Etofenprox	66.8	2.2
Etoxazole	86.6	2.0
Etridiazole	102.5	3.3
Fenoxycarb	110.0	1.1
Fenpyroximate	76.4	1.8
Fensulfothion	102.1	2.3
Fenthion	100.7	1.7
Fenvalerate	92.0	1.3
Fipronil	104.3	0.6
Flonicamid	1067.	2.4
Fludioxonil	101.5	1.2
Fluopyram	105.6	2.1
Hexythiazox	93.5	6.0
Imazalil	103.3	1.0
Imidacloprid	100.4	1.1
Kinoprene	96.9	4.0
Kresoxim-methyl	107.6	1.6
Malathion	103.1	1.8
Metalaxyl	101.1	0.5
Methiocarb	105.0	0.3
Methomyl	101.7	4.8
Methoprene	81.5	2.2
Methyl parathion	102.7	1.2
Mevinphos I	96.9	0.2
Mevinphos II	102.1	1.2
MGK-264	102.8	9.9
Myclobutanil	106.1	1.1
Naled	102.0	1.1
Novaluron	98.0	2.2
Oxamyl	103.2	1.9
Paclobutrazol	101.8	3.4
Permethrin	98.2	2.3
Phenothrin	69.7	1.5
Phosmet	104.9	2.7
Piperonyl butoxide	97.3	1.9

Compound	Recovery (%)	RSD (%, n = 3)
Pirimicarb	99.4	1.3
Prallethrin	108.9	0.5
Propiconazole	102.8	0.4
Propoxur	103.2	1.2
Pyraclostrobin	103.7	0.8
Pyrethrin I	88.5	4.1
Pyrethrin II	91.5	5.8
Pyridaben	70.5	0.9
Quintozene (PCNB)	100.2	3.5
Resmethrin	82.5	4.2
Spinetoram J	68.4	2.2
Spinetoram L	58.3	2.9
Spinosyn A	79.0	3.0
Spinosyn D	73.8	1.6
Spirodiclofen	92.1	2.9
Spiromesifen	98.3	1.0
Spirotetramat	100.4	5.5
Spiroxamine	96.3	1.2
Tebuconazole	100.1	5
Tebufenozide	105.0	1.0
Teflubenzuron	98.0	5.4
Tetrachlorvinphos	106.5	1.5
Tetramethrin	101.6	2.9
Thiacloprid	101.8	2.2
Thiamethoxam	103.4	0.4
Thiophanate-methyl	100.7	3.7
Trifloxystrobin	103.8	0.4
Mycotoxins		
Aflatoxin G1	102.8	0.2
Aflatoxin G2	102.7	0.3
Aflatoxin B1	104.8	0.4
Aflatoxin B2	102.3	1.4
Ochratoxin	100.5	2.2

6470 LC/MS/MS results and discussion

The 1290 Infinity II coupled with a 6470 triple quadrupole LC/MS/MS system offers both the level of sensitivity required by Canadian regulations to meet the reporting limits for pesticides in dry cannabis, and the level of robustness required to run this application daily. The combination of sample dilution and mobile phase composition allows one to maintain excellent peak shape for all compounds when injecting 25 µL, but will also allow for larger injection volumes, if necessary. The MRM transitions were optimized using the MassHunter Optimizer program (see Table 5 in Appendix), and the acquisition was performed in dMRM mode, in which the dwell time of each transition was optimized by the MassHunter software based on the retention time of each compound. Linear calibration curves were observed, with a regression fit equal to or greater than 0.99, and the LOQs listed in Table 2 were obtained in spiked matrix.

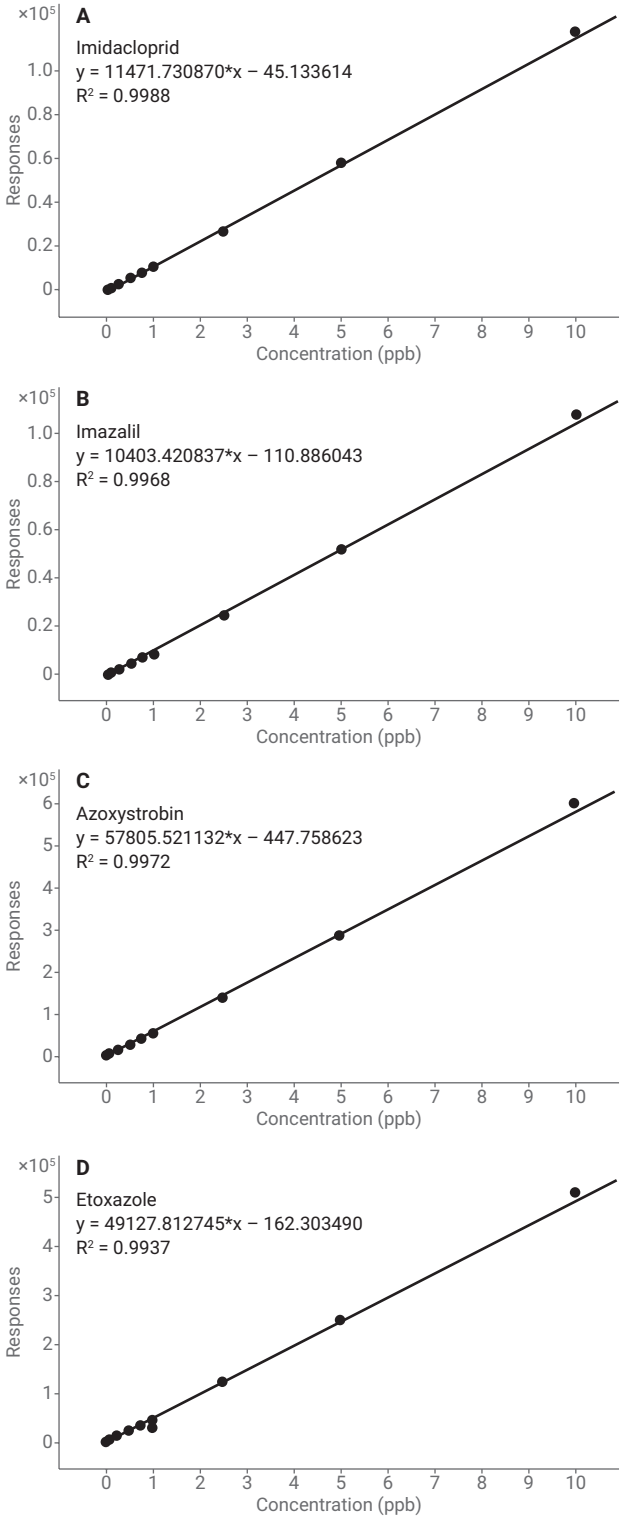

Figure 3. Select 6470 LC/MS/MS calibration curves. A) Imidacloprid, B) Imazalil, C) Azoxystrobin, D) Etoxazole.

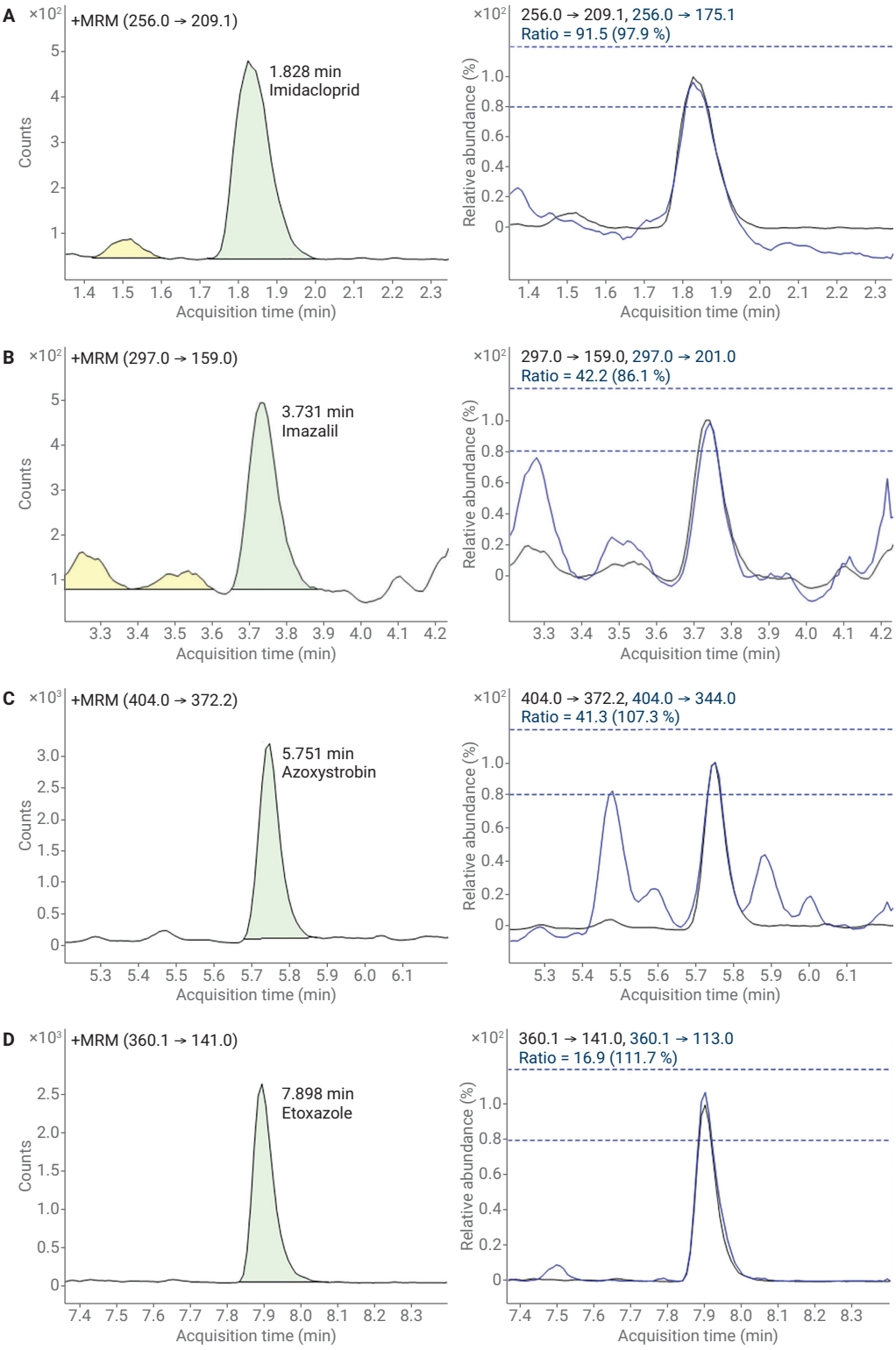

Figure 4. Select 6470 LC/MS/MS chromatograms. A) Imidacloprid, B) Imazalil, C) Azoxystrobin, D) Etoxazole.

Ultivo LC/MS/MS results and discussion

The Ultivo LC/MS/MS was introduced in 2017 as a next-generation LC/MS/MS system, with new optical and electronic components. Its development was based on combining small size, ease of maintenance, and maximum instrument uptime. Given the challenging nature of pesticide residue testing in cannabis, it is of interest to evaluate if the Ultivo can match the 6470 in terms of sensitivity for this application.

The 1290 Infinity II UHPLC stack previously used with the 6470 was connected to the Ultivo, which allowed us to keep retention times identical between the two systems, and thus, identical dwell times for all compounds using the dMRM mode.

The Ultivo equaled the performance of the 6470 in terms of linearity range and LOQ, as illustrated by the equivalent calibration curves in Figure 5 compared to Figure 3.

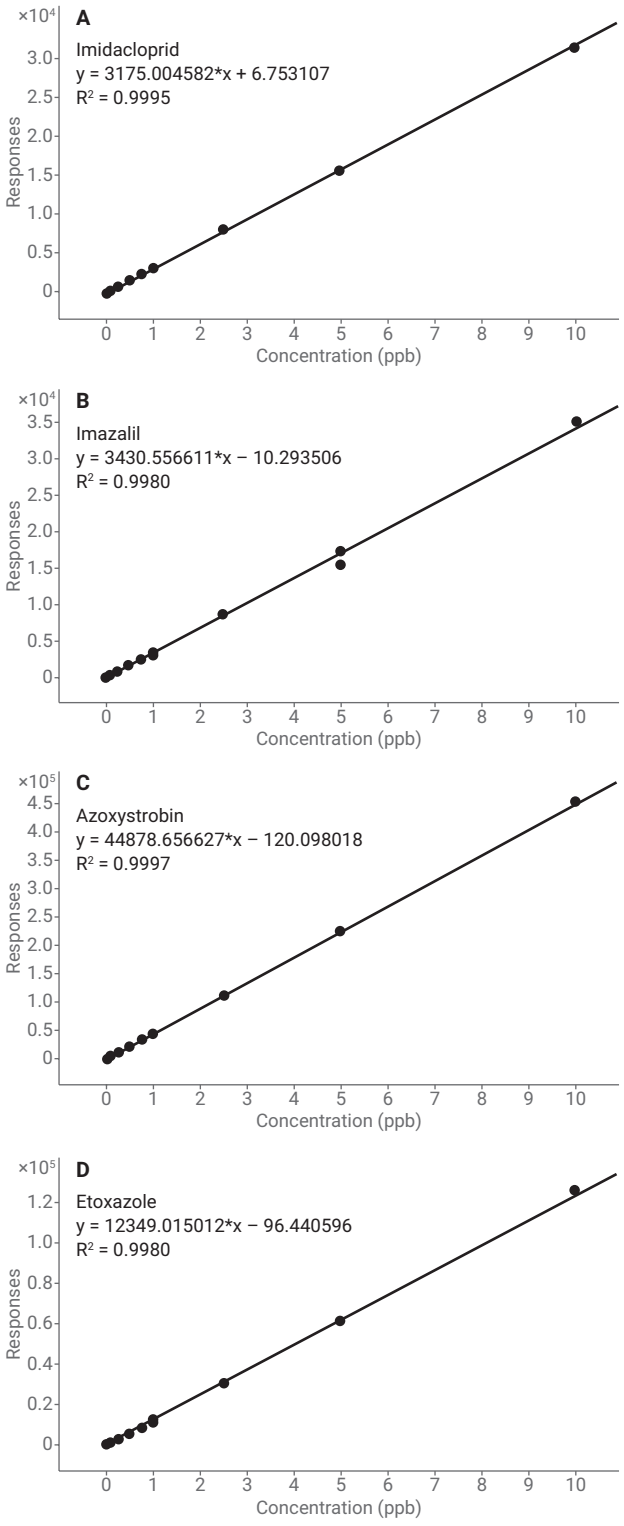

Figure 5. Select Ultivo LC/MS/MS calibration curves. A) Imidacloprid, B) Imazalil, C) Azoxystrobin, D) Etoxazole.

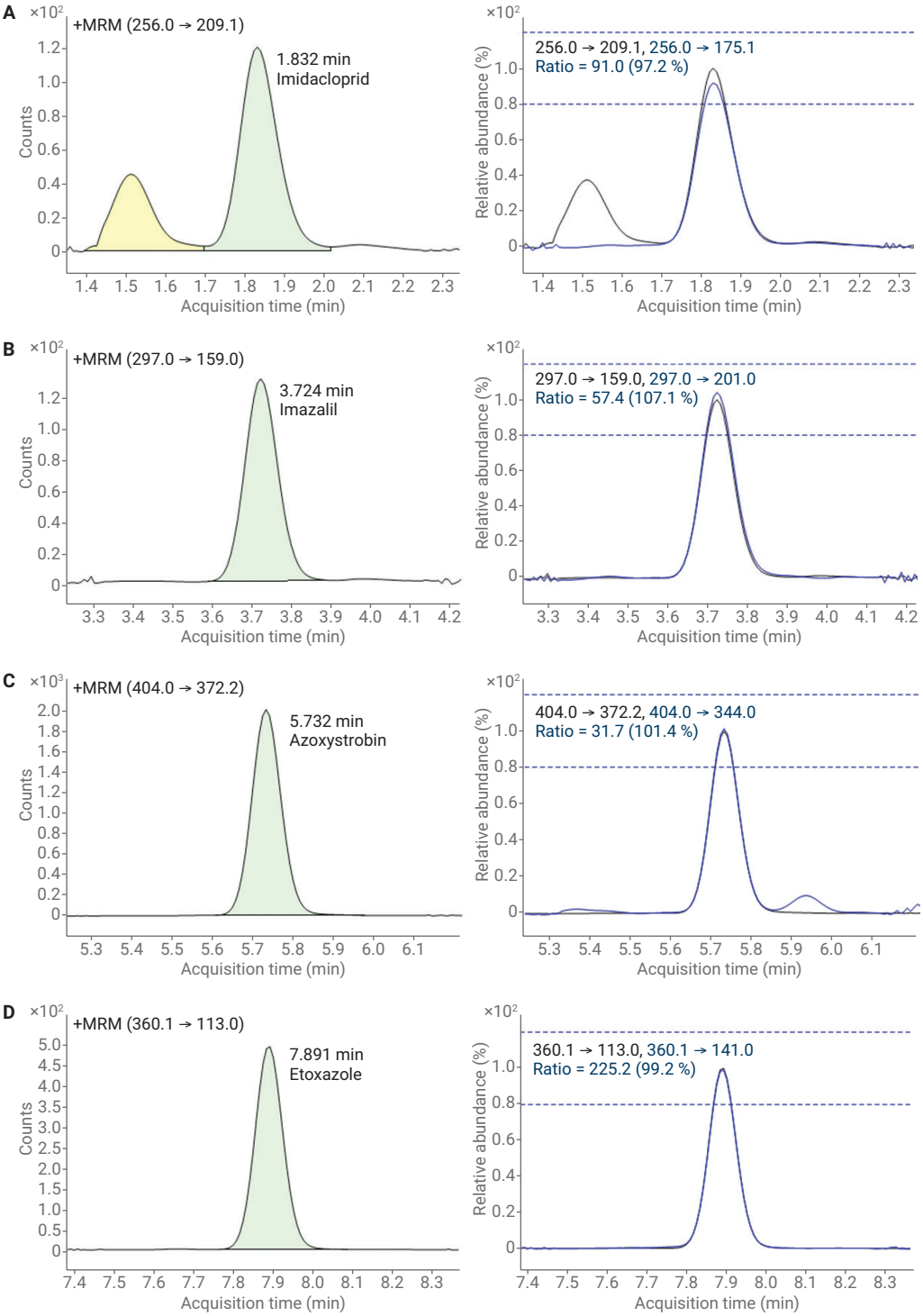

Figure 6. Select Ultivo LC/MS/MS chromatograms. A) Imidacloprid, B) Imazalil, C) Azoxystrobin, D) Etoxazole.

7010 GC/MS/MS results and discussion

A vast majority of the pesticides included in the Health Canada list can be analyzed by LC/MS/MS. However, for some of these pesticides, it is impossible to consistently meet the required reporting limits (RLs) set by Health Canada without very extensive and long sample cleanup, or at the expense of sample throughput through time-consuming instrument optimization. The same is true for some regulated pesticides in California that are not part of the Canadian list, and that are also not amenable to LC/MS/MS. GC/MS/MS is the best choice to complete the coverage of the Canadian and California lists, as it can be used as the primary reporting platform or in a confirmatory approach when matrix could interfere with some compounds in LC/MS/MS.

The first cleanup step in sample preparation is common between LC/MS/MS and GC/MS/MS, but some optimization was required in the second step (dilution) as well as in hardware setup. A mix of acidified acetone:hexane was used for the second 1-in-5 dilution, for a final sample dilution of 125x. To compensate for this smaller dilution factor, and keeping instrument uptime as a primary objective, two hardware options were selected: post run, midcolumn backflush to avoid source contamination by late eluting compounds, and post sequence JetClean source cleaning to restore source conditions from sequence to sequence.

Table 6 in the Appendix lists the 7010 MRM transitions, and Table 4 shows the LOQs.

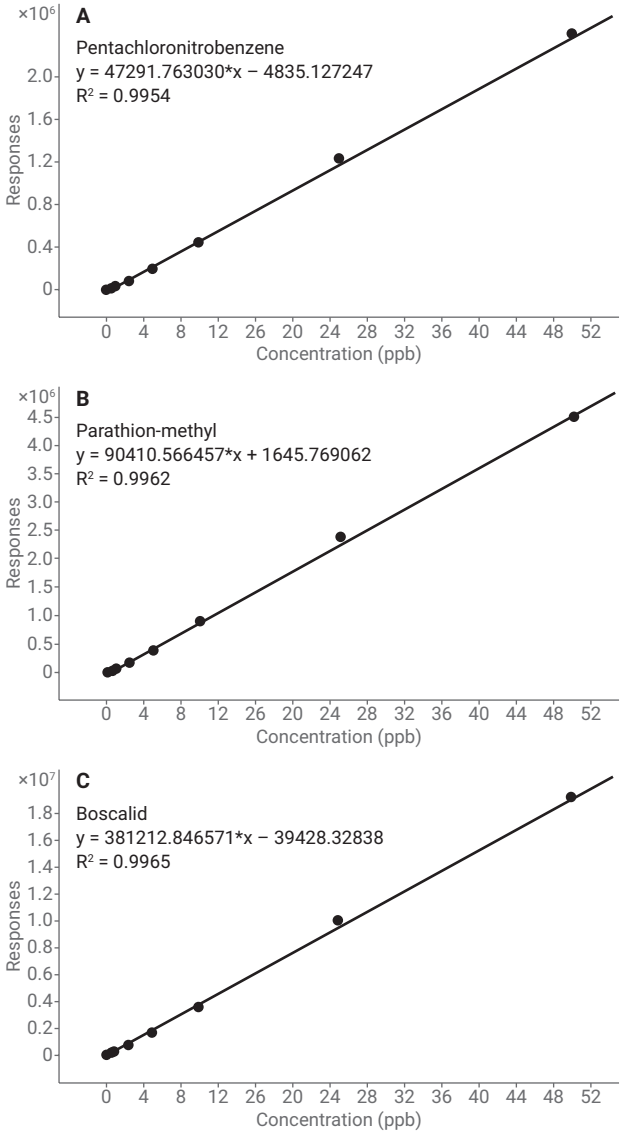

Figure 7. Select 7010 GC/MS/MS calibration curves.
A) Pentachloronitrobenzene (PCNB, Quintozene), B) Parathion-methyl, C) Boscalid.

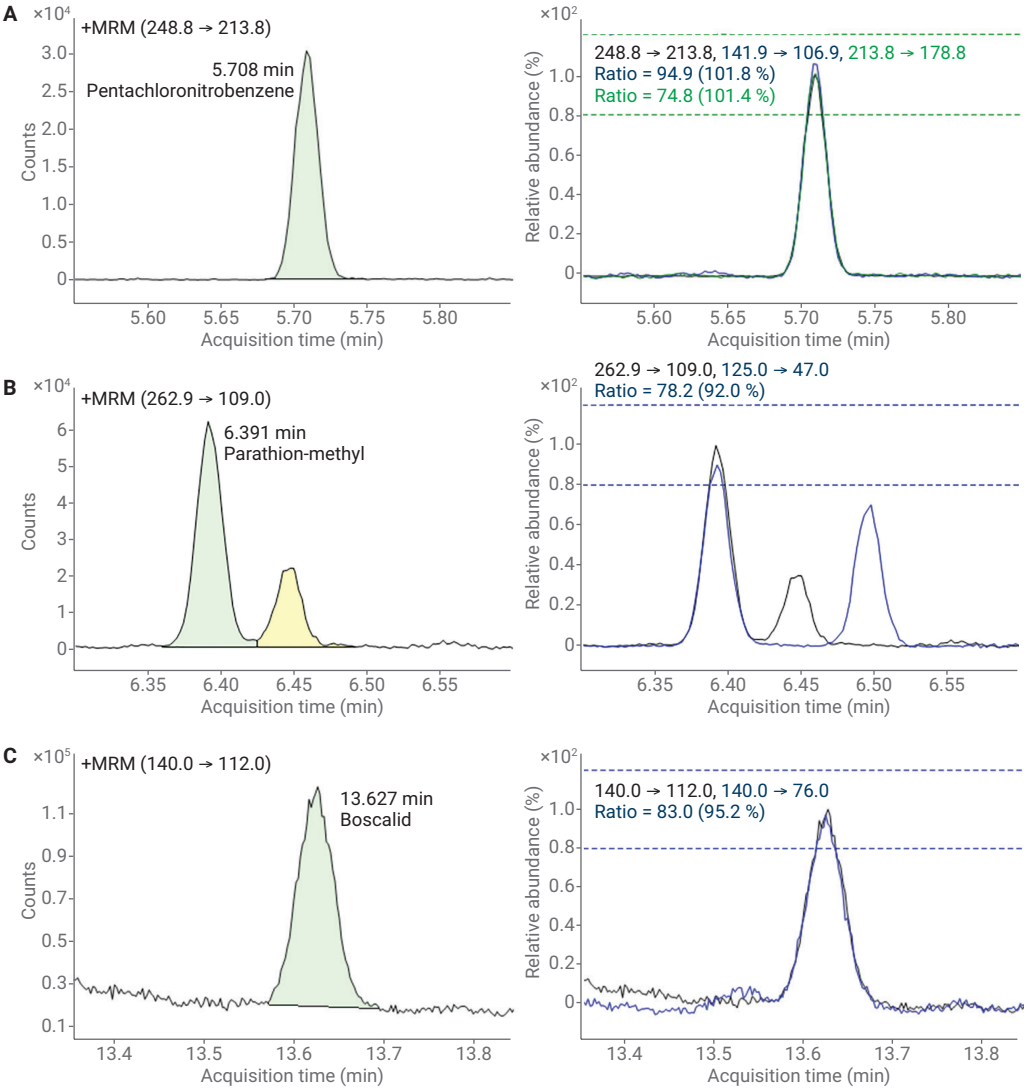

Figure 8. Select 7010 GC/MS/MS chromatograms. A) Pentachloronitrobenzene (PCNB, quintozene), B) Parathion-methyl, C) Boscalid.

Table 4. Calculated LOQs in matrix. A blank cell indicates that no data were collected for a given compound using that specific platform (continued next page).

Compound	Health Canada reporting limit (ppb)	LC/MS/MS LOQ in matrix (ppb)	GC/MS/MS LOQ in matrix (ppb)
Pesticides			
Abamectin (Avermectin B1a)	500	12.5	
Acephate	20	2.5	
Acetamiprid	100	2.5	
Acequinocyl	Under development*	18.75	
Aldicarb	1,000	2.5	
Allethrin	200	125	
Azadirachtin	1,000	12.5	
Azoxystrobin	20	2.5	
Benzovindiflupyr	20	2.5	
Bifenazate	20	2.5	
Bifenthrin	Under development*	62.5	31
Boscalid	20	6.25	12.5
Buprofezin	20	2.5	
Carbaryl	50	2.5	
Carbofuran	20	2.5	
Chlorantraniliprole	Under development*	2.5	
Chlorphenapyr	Under development*	25	
Chlorpyrifos	Under development*	25	6.25
Clofentezine	20	18	
Clothianidin	50	2.5	
Coumaphos	20	6.25	
Cyantranilipole	20	6.25	
Cyfluthrin	Under development*		125
Cypermethrin	Under development*	250	125
Cyprodinil	Under development*	12.5	
Daminozide	Under development*	2.5	
Deltamethrin	Under development*	62.5	62.5
Diazinon	Under development*	2.5	
Dichlorvos	100	6.25	
Dimethoate	20	2.5	
Dinotefuran	100	2.5	
Dodemorph	Under development*	2.5	
Endosulfan *alpha*	Under development*		31
Endosulfan *beta*	Under development*		12.5
Endosulfan sulfate	Under development*	12.5	
Ethoprophos	20	2.5	
Etofenprox	Under development*	6.25	
Etoxazole	20	2.5	
Etridiazole	Under development*		6.25

Compound	Health Canada reporting limit (ppb)	LC/MS/MS LOQ in matrix (ppb)	GC/MS/MS LOQ in matrix (ppb)
Fenoxycarb	20	2.5	
Fenpyroximate	20	2.5	
Fensulfothion	20	2.5	
Fenthion	Under development*		9.4
Fenvalerate	Under development*		31
Fipronil	60	2.5	
Flonicamid	50	12.5	
Fludioxonil	20	2.5	
Fluopyram	20	2.5	
Hexythiazox	Under development*	12.5	
Imazalil	20	2.5	
Imidacloprid	20	2.5	
Iprodione	1000	250	
Kinoprene	Under development*		312.5
Kresoxim-methyl	Under development*	2.5	
Malathion	20	2.5	
Metalaxyl	20	2.5	
Methiocarb	20	2.5	
Methomyl	50	1.25	
Methoprene	Under development*	187.5	
Methyl parathion	Under development*		9.375
Mevinphos I	50	6.25	
Mevinphos II	50	6.25	
MGK-264	Under development*	187.5	31.25
Myclobutanil	20	2.5	
Naled	Under development*	6.25	
Novaluron	50		31.25
Oxamyl	3000	1.25	
Paclobutrazol	20	2.5	
Permethrin	Under development*		125
Phenothrin	50	2.5	
Phosmet	Under development*	2.5	
Piperonyl butoxide	Under development*	2.5	
Pirimicarb	20	2.5	
Prallethrin	Under development*	62.5	
Propiconazole	Under development*	2.5	
Propoxur	20	2.5	
Pyraclostrobin	20	2.5	
Pyrethrin I	50	32.7	
Pyrethrin II	50	32.9	
Pyridaben	50	2.5	

* Under development: The reporting limit in dry cannabis matrix was not established by Health Canada at the time of this Application Note's publication.

A total workflow for pesticide quantitation and reporting

The Agilent workflow for residual pesticides in cannabis flower not only includes a single-stream sample preparation procedure amenable to both LC/MS/MS and GC/MS/MS data acquisition platforms, but also includes unified data analysis and reporting tools. Using the Quant-My-Way features of the MassHunter Quantitative Analysis software package, processing raw data is performed within a graphical environment designed specifically for residual pesticide testing in cannabis and related products. The interface is striated, and controls how a user interacts with the software and the features that are available for specific workflows. For example, the Scientist interface has read/write permissions and offers the abilities to create and edit quantitative procedures and define reporting information. The Analyst interface has read-only permission for use in the daily production environment. Thus, the laboratory can control how data are processed and reported, and capture change-exceptions when necessary.

Compound	Health Canada reporting limit (ppb)	LC/MS/MS LOQ in matrix (ppb)	GC/MS/MS LOQ in matrix (ppb)
Quintozene (PCNB)	Under development*		6.25
Resmethrin	100	6.25	
Spinetoram J	Under development*	6.25	
Spinetoram L	Under development*	12.5	
Spinosyn A	Under development*	6.25	
Spinosyn D	Under development*	6.25	
Spirodiclofen	20	18.75	
Spiromesifen	3000	2.5	
Spirotetramat	20	2.5	
Spiroxamine	Under development*	2.5	
Tebuconazole	Under development*	2.5	
Tebufenozide	20	2.5	
Teflubenzuron	50	18.75	
Tetrachlorvinphos	20	2.5	
Tetramethrin	100	12.5	
Thiacloprid	20	2.5	
Thiamethoxam	20	2.5	
Thiophanate-methyl	50	2.5	
Trifloxystrobin	20	2.5	
Mycotoxins			
Aflatoxin G1	2	1.25	
Aflatoxin G2	2	1.25	
Aflatoxin B1	2	1.25	
Aflatoxin B2	2	1.25	
Ochratoxin	20	2.5	

* Under development: The reporting limit in dry cannabis matrix was not established by Health Canada at the time of this Application Note's publication.

Conclusion

Mandatory reporting limits established by Health Canada for pesticide testing in cannabis are typically lower than those published in various U.S. states, and require both LC/MS and GC/MS for accurate and robust testing. Because dried cannabis leaves and flowers generate many co-extractives that can negatively impact testing results, a simple and cost-effective sample preparation had to be developed to meet the demanding testing requirements in Canada. A combination of acetonitrile extraction, unique SPE on SampliQ C18 EC, and further dilution in optimized solvent is the best approach for accurate pesticide and mycotoxin quantification at levels as low as 20 ppb.

Agilent instrumentation including the 1290 Infinity II LC coupled with a 6470 LC/MS/MS system and Ultivo LC/MS/MS, as well as the 7010 GC/MSMS, provides robust, accurate, and sensitive residual pesticide and mycotoxin testing in challenging matrices such as cannabis.

Acknowledgments

Agilent would like to thank Canopy Growth for providing cannabis extracts. We also need to recognize the many contributions of Rick Jordan from Pacific Agricultural Laboratory in Sherwood, OR USA.

Disclaimer

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

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4. Tuner, C. E.; Elsohly, M. A.; Boeren. For example, *J. Nat. Prod.* **1980**, *43*, 169–234. doi:10.1021/np50008a001.
5. A novel comprehensive strategy for residual pesticide analysis in cannabis flower, *Agilent Technologies Application Note*, publication number 5991-9030EN.

Appendix

Table 5. LC/MS/MS MRM transitions.

Compound	RT (min)	Polarity	LC/MS/MS			
			Precursor (*m/z*)	Product (*m/z*)	Frag	CE
Avermectin B1a	8.48	Positive	890.5	567.1	160	8
Avermectin B1a	8.48	Positive	890.5	567.1	160	8
Avermectin B1a	8.48	Positive	890.5	305.1	160	28
Avermectin B1a	8.48	Positive	890.5	145	160	45
Avermectin B1b	8.29	Positive	876.6	553.2	160	7
Avermectin B1b	8.29	Positive	876.6	291.1	160	15
Acephate	1.26	Positive	184	143	60	5
Acephate	1.26	Positive	184	95	60	20
Acequinocyl	9.58	Positive	402.3	343.2	90	10
Acequinocyl	9.58	Positive	402.3	189.1	90	41
Acetamiprid	2.1	Positive	223	126.1	100	20
Acetamiprid	2.1	Positive	223	90.1	100	35
AflatoxinB1	4.23	Positive	313.1	285.1	160	16
AflatoxinB1	4.23	Positive	313.1	241.1	160	35
AflatoxinB2	3.78	Positive	315.1	287.1	130	17
AflatoxinB2	3.78	Positive	315.1	259.1	130	17
AflatoxinG1	3.46	Positive	329.1	311.1	130	20
AflatoxinG1	3.46	Positive	329.1	243.1	130	17
AflatoxinG2	3.03	Positive	331.1	285.1	150	21
AflatoxinG2	3.03	Positive	331.1	245.1	150	26
Aldicarb	2.22	Positive	116	89.1	50	4
Aldicarb	2.22	Positive	116	70.1	50	4
Allethrin	7.5	Positive	303	169	85	4
Allethrin	7.5	Positive	303	135	85	10
Allethrin	7.5	Positive	303	123	85	16
Azadirachtin	4.36	Positive	703	685	165	8
Azadirachtin	4.36	Positive	703	585	165	12
Azadirachtin	4.36	Positive	703	567	165	12
Azoxystrobin	5.73	Positive	404	372.2	100	10
Azoxystrobin	5.73	Positive	404	344	100	25
Benzovindiflupyr	6.69	Positive	398	378	150	12
Benzovindiflupyr	6.69	Positive	398	342	150	20
Benzovindiflupyr	6.69	Positive	398	322	150	24
Bifenazate	5.83	Positive	301.1	198.2	80	5
Bifenazate	5.83	Positive	301.1	170.1	80	15
Bifenthrin	9.07	Positive	440.1	181.1	90	5
Bifenthrin	9.07	Positive	440.1	166	90	20
Boscalid	5.58	Positive	343	307	140	12
Boscalid	5.58	Positive	343	271	140	28
Buprofezin	7.38	Positive	306	201	105	8
Buprofezin	7.38	Positive	306	116	105	16
Carbaryl	3.33	Positive	202	145	70	0
Carbaryl	3.33	Positive	202	127	70	25
Carbofuran	3.16	Positive	222	165	90	5
Carbofuran	3.16	Positive	222	123	90	20
Chlorantraniliprole	5.04	Positive	483.9	452.9	100	15
Chlorantraniliprole	5.04	Positive	483.9	285.9	100	10
Chlorfenapyr	7.35	Positive	409.2	59	130	20
Chlorfenapyr	7.35	Positive	409.2	31	130	45
Chlorpyrifos	7.95	Positive	349.9	197.9	100	20
Chlorpyrifos	7.95	Positive	349.9	97	100	41
Clofentezine	7.04	Positive	303	138	90	10
Clofentezine	7.04	Positive	303	102.1	90	10
Clothianidin	1.69	Positive	250	169	95	12
Clothianidin	1.69	Positive	250	132	95	16
Coumaphos	7.4	Positive	363	307	125	15
Coumaphos	7.4	Positive	363	226.9	125	33
Cyantranilipole	4.29	Positive	475	444	115	20

Compound	RT (min)	Polarity	LC/MS/MS			
			Precursor (*m/z*)	Product (*m/z*)	Frag	CE
Cyantranilipole	4.29	Positive	475	286	115	12
Cyfluthrin	9.2	Positive	453.3	193	90	13
Cyfluthrin	9.2	Positive	451.3	191	90	13
Cypermethrin	8.72	Positive	435.3	193	90	16
Cypermethrin	8.72	Positive	433.3	191	90	16
Cyprodinil	5.5	Positive	226	133	160	28
Cyprodinil	5.5	Positive	226	93	160	40
Daminozide	1.18	Positive	161	143	80	10
Daminozide	1.18	Positive	161	61.1	80	10
Deltamethrin	9.15	Positive	523	506	100	8
Deltamethrin	9.15	Positive	523	281	100	12
Diazinon	6.43	Positive	305.1	169.1	100	20
Diazinon	6.43	Positive	305.1	153.1	100	20
Dichlorvos	2.72	Positive	221	109	110	12
Dichlorvos	2.72	Positive	221	79	110	24
Dimethoate	1.86	Positive	230	199	80	0
Dimethoate	1.86	Positive	230	125	80	20
Dimethomorph I	7.2	Positive	388.1	301	134	24
Dimethomorph I	7.2	Positive	388.1	165	134	36
Dimethomorph II	7.84	Positive	388.1	301	134	24
Dimethomorph II	7.84	Positive	388.1	165	134	36
Dinotefuran	1.24	Positive	203	157	90	4
Dinotefuran	1.24	Positive	203	129	90	8
Dinotefuran	1.24	Positive	203	87	90	16
Dinotefuran	1.24	Positive	203	73	90	20
Dodemorph	4.1	Positive	282	116	145	24
Dodemorph	4.1	Positive	282	98	145	32
Endosulfan sulfate	7.09	Negative	421	97	130	28
Endosulfan sulfate	7.09	Negative	421	80	130	56
Ethoprophos	5.51	Positive	243	131	90	15
Ethoprophos	5.51	Positive	243	97	90	30
Etofenprox	9	Positive	394.2	177.2	90	10
Etofenprox	9	Positive	394.2	107.1	90	45
Etoxazole	7.87	Positive	360.1	141	140	28
Etoxazole	7.87	Positive	360.1	113	140	50
Fenoxycarb	6.37	Positive	302.1	116.1	100	5
Fenoxycarb	6.37	Positive	302.1	88.1	100	15
Fenpyroximate	8.14	Positive	422.1	366.2	130	15
Fenpyroximate	8.14	Positive	422.1	135.1	130	30
Fensulfothion	4.52	Positive	309	281	125	12
Fensulfothion	4.52	Positive	309	253	125	16

Compound	RT (min)	Polarity	LC/MS/MS			
			Precursor (*m/z*)	Product (*m/z*)	Frag	CE
Fenvalerate	9	Positive	437	167	105	16
Fipronil	6.05	Negative	436.9	332	100	18
Fipronil	6.05	Negative	434.9	330	100	18
Fipronil	6.05	Negative	434.9	250.1	100	30
Flonicamid	1.4	Positive	230.1	203	125	18
Flonicamid	1.4	Positive	230.1	148	125	32
Flonicamid	1.4	Positive	230.1	98	125	48
Fludioxonil	5.01	Negative	247	169	120	36
Fludioxonil	5.01	Negative	247	126	120	40
Fluopyram	5.62	Positive	397	208	150	24
Fluopyram	5.62	Positive	397	173	150	36
Hexythiazox	8.2	Positive	353	228.1	90	10
Hexythiazox	8.2	Positive	353	168.1	90	25
Imazalil	3.73	Positive	297	201	120	15
Imazalil	3.73	Positive	297	159	120	20
Imidacloprid	1.85	Positive	256	209.1	90	16
Imidacloprid	1.85	Positive	256	175.1	90	20
Iprodione	6.77	Positive	332	247	80	16
Iprodione	6.77	Positive	332	56	80	44
Iprodione	6.77	Positive	330	245	80	16
Iprodione	6.77	Positive	330	56	80	50
Kresoxim methyl	6.6	Positive	314.1	267.1	80	0
Kresoxim methyl	6.6	Positive	314.1	222.2	80	10
Malathion	5.7	Positive	331.1	126.9	80	5
Malathion	5.7	Positive	331.1	99	80	10
Metalaxyl	4.06	Positive	280.1	220.2	100	10
Metalaxyl	4.06	Positive	280.1	160.1	100	20
Methiocarb	4.98	Positive	226.1	169.1	70	0
Methiocarb	4.98	Positive	226.1	121.1	70	15
Methomyl	1.38	Positive	162.9	106.1	60	5
Methomyl	1.38	Positive	162.9	88.1	60	0
Methoprene	8.09	Positive	311	151	100	0
Methoprene	8.09	Positive	311	123	100	2
Methoprene	8.09	Positive	311	109	100	4
Methyl-Parathion	5.61	Positive	264	232	140	18
Methyl-Parathion	5.61	Positive	264	125	140	24
Mevinphos I	1.62	Positive	225	193	75	4
Mevinphos I	1.62	Positive	225	127	75	16
Mevinphos II	1.99	Positive	225	193	75	4
Mevinphos II	1.99	Positive	225	127	75	16
MGK-264	6.8	Positive	276.2	210.1	100	12

Compound	RT (min)	Polarity	LC/MS/MS			
			Precursor (*m/z*)	Product (*m/z*)	Frag	CE
MGK-264	6.8	Positive	276.2	98	100	28
Myclobutanil	5.66	Positive	289.1	125	110	35
Myclobutanil	5.66	Positive	289.1	70.1	110	15
Naled (Dibrom)	4.42	Positive	380.8	127	90	8
Naled (Dibrom)	4.42	Positive	378.8	127	90	5
Novaluron	7.19	Positive	493	158	145	20
Novaluron	7.19	Positive	493	141	145	56
Ochratoxin	6.33	Positive	404.1	238.9	120	14
Ochratoxin	6.33	Positive	404.1	220.9	120	32
Oxamyl	1.29	Positive	237	90.1	60	0
Oxamyl	1.29	Positive	237	72.1	60	15
Paclobutrazol	5.05	Positive	294.1	125	110	40
Paclobutrazol	5.05	Positive	294.1	70.1	110	20
Permethrin	7.7	Positive	391.1	355	120	5
Permethrin	7.7	Positive	391.1	183	120	5
Phenothrin	8.73	Positive	351	237	120	8
Phenothrin	8.73	Positive	351	183	120	20
Phenothrin	8.73	Positive	351	168	120	48
Phosmet	5.59	Positive	317.9	160	80	10
Phosmet	5.59	Positive	317.9	133	80	40
Piperonyl butoxide	7.51	Positive	356.2	177.1	90	5
Piperonyl butoxide	7.51	Positive	356.2	119.1	90	35
Pirimicarb	2.7	Positive	239	182	100	16
Pirimicarb	2.7	Positive	239	72	100	24
Prallethrin	7.03	Positive	301.1	169	90	5
Prallethrin	7.03	Positive	301.1	105	90	20
Propiconazole	6.75	Positive	342.1	159	130	32
Propiconazole	6.75	Positive	342.1	69.1	130	16
Propoxur	3	Positive	210	168	60	5
Propoxur	3	Positive	210	111	60	10
Pyraclostrobin	7.18	Positive	388	194	110	8
Pyraclostrobin	7.18	Positive	388	163	110	24
Pyrethrin I	8.3	Positive	329.2	161	90	5
Pyrethrin I	8.3	Positive	329.2	143	90	20
Pyrethrin I	8.3	Positive	329.2	133	90	20
Pyrethrin_II	7.5	Positive	373.2	161	102	2
Pyrethrin_II	7.5	Positive	373.2	133.1	102	24
Pyrethrin_II	7.5	Positive	373.2	77	102	98
Pyridaben	8.53	Positive	365.1	309.1	90	4
Pyridaben	8.53	Positive	365.1	147.2	90	20
Pyridaben	8.53	Positive	365.1	117.1	90	60

Compound	RT (min)	Polarity	LC/MS/MS			
			Precursor (*m/z*)	Product (*m/z*)	Frag	CE
Resmethrin	8.52	Positive	339	171	135	12
Resmethrin	8.52	Positive	339	143	135	28
Spinetoram J	7.81	Positive	748.5	142.1	165	26
Spinetoram J	7.81	Positive	748.5	98.1	165	50
Spinetoram L	7.5	Positive	760.5	142.1	165	26
Spinetoram L	7.5	Positive	760.5	98.1	165	50
Spinosyn A	7.48	Positive	732.5	142.1	160	28
Spinosyn A	7.48	Positive	732.5	98	160	60
Spinosyn D	7.11	Positive	746.5	142.1	160	35
Spinosyn D	7.11	Positive	746.5	98	160	55
Spirodiclofen	8.18	Positive	411	313	140	8
Spirodiclofen	8.18	Positive	411	71	140	16
Spiromesifen	7.85	Positive	388.2	273	80	6
Spiromesifen	7.85	Positive	388.2	255	80	26
Spirotetramat	5.9	Positive	374.2	330.2	110	12
Spirotetramat	5.9	Positive	374.2	302	110	12
Spirotetramat	5.9	Positive	374.2	216.1	110	36
Spiroxamine	4.8	Positive	298.2	144.1	120	16
Spiroxamine	4.8	Positive	298.2	100.1	120	32
Tebuconazole	6.27	Positive	308.1	124.9	120	47
Tebuconazole	6.27	Positive	308.1	70	120	40
Tebufenozide	6	Positive	353.2	297.1	100	4
Tebufenozide	6	Positive	353.2	133	100	20
Tebufenozide	6	Positive	353.2	102.9	100	20
Teflubenzuron	7.87	Negative	379	339	125	8
Teflubenzuron	7.87	Negative	379	196	125	24
Tetrachlorvinphos	6.56	Positive	365	204	125	48
Tetrachlorvinphos	6.56	Positive	365	127	125	12
Tetrachlorvinphos	6.56	Positive	365	109	125	48
Tetramethrin	7.9	Positive	332	314	100	8
Tetramethrin	7.9	Positive	332	286	100	8
Tetramethrin	7.9	Positive	332	164	100	28
Tetramethrin	7.9	Positive	332	135	100	16
Thiacloprid	2.44	Positive	253	126	100	16
Thiacloprid	2.44	Positive	253	90	100	40
Thiamethoxam	1.58	Positive	292	211.1	80	8
Thiamethoxam	1.58	Positive	292	181.1	80	20
Thiophanate-methyl	3.43	Positive	343	311	105	8
Thiophanate-methyl	3.43	Positive	343	151	105	20
Trifloxystrobin	7.35	Positive	409.1	186	100	12
Trifloxystrobin	7.35	Positive	409.1	145	100	52

Table 6. GC/MS/MS MRM transitions (continued next page).

Compound	RT (min)	Precursor (*m/z*)	Product (*m/z*)	CE
Novaluron	3.7	335	167.9	15
Novaluron	3.7	168	139.9	10
Novaluron	3.7	168	75.9	35
Clofentezine	3.88	139	102	16
Clofentezine	3.88	137	102	16
Clofentezine	3.88	102	75	11
Teflubenzuron	4.3	199	162	14
Teflubenzuron	4.3	197	162	14
Teflubenzuron	4.3	157	141	6
Teflubenzuron	4.3	141	113	15
Etridiazole	4.5	213.1	142	25
Etridiazole	4.5	211.1	140	25
Etridiazole	4.5	183	140	15
Pentachloronitroenzene	5.7	248.8	213.8	15
Pentachloronitroenzene	5.7	213.8	178.8	15
Pentachloronitroenzene	5.7	141.9	106.9	30
Kinoprene	5.95	149	93	4
Kinoprene	5.95	149	91	10
Kinoprene	5.95	149	77	14
Parathion-methyl	6.45	262.9	109	10
Parathion-methyl	6.45	125	79	5
Parathion-methyl	6.45	125	47	10
Chlorpyrifos	6.6	313.8	257.8	15
Chlorpyrifos	6.6	198.9	171	15
Chlorpyrifos	6.6	196.9	169	15
Allethrin	6.73	123	81	10
Allethrin	6.73	107	91	10
Allethrin	6.73	91	65	15
MGK-264 I	6.8	164.2	98	10
MGK-264 I	6.8	164.2	67.1	5
MGK-264 I	6.8	111	82	5
Fenthion	6.9	278	169	15
Fenthion	6.9	124.9	47	10
Prallethrin	6.98	123	81	10
Prallethrin	6.98	105	77	20
Prallethrin	6.98	90.9	65	15
MGK-264 II	7.05	164.2	98	10
MGK-264 II	7.05	164.2	67.1	5
MGK-264 II	7.05	111	82	5
Pyrethrin I	7.68	123.1	81	5
Pyrethrin I	7.68	123.1	41.1	30
Pyrethrin I	7.68	91	65	15
Chlordane-*cis*	7.7	372.8	300.9	10
Chlordane-*cis*	7.7	372.8	265.9	25
Chlordane-*cis*	7.7	271.8	236.9	15
Chlordane-*trans*	7.85	374.8	265.8	15
Chlordane-*trans*	7.85	372.8	265.8	15
Chlordane-*trans*	7.85	271.7	236.9	15
Endosulfan-*alpha*	7.95	194.9	160	5
Endosulfan-*alpha*	7.95	194.9	159	5
Endosulfan-*alpha*	7.95	194.9	125	20
Captan	8.1	263.9	79	25
Captan	8.1	148.1	70	15
Captan	8.1	116.9	81.9	20
Pyrethrin II	8.2	123.1	81	5
Pyrethrin II	8.2	123.1	41.1	30
Pyrethrin II	8.2	91	65	15
Endosulfan-*beta*	9.1	276.7	240.9	5
Endosulfan-*beta*	9.1	206.9	172	15
Endosulfan-*beta*	9.1	194.9	158.9	10
Bifenthrin	9.34	181.2	166.2	10
Bifenthrin	9.34	181.2	165.2	25
Bifenthrin	9.34	166.2	165.2	20
Spirodiclofen	11.2	312.1	259	10
Spirodiclofen	11.2	109.1	81.1	10
Spirodiclofen	11.2	109.1	79.1	15
Permethrin, (1R)-*cis*-	11.25	183.1	168.1	10
Permethrin, (1R)-*cis*-	11.25	183.1	153.1	15
Permethrin, (1R)-*cis*-	11.25	182.9	155.1	10
Permethrin, (1R)-*trans*-	11.4	163	127	5
Permethrin, (1R)-*trans*-	11.4	163	91	15
Permethrin, (1R)-*trans*-	11.4	162.9	91.1	15
Cyfluthrin I	11.7	198.9	170.1	25
Cyfluthrin I	11.7	162.9	127	5
Cyfluthrin I	11.7	162.9	90.9	15
Cyfluthrin II	11.8	198.9	170.1	25
Cyfluthrin II	11.8	162.9	127	5
Cyfluthrin II	11.8	162.9	90.9	15
Cyfluthrin III	11.9	198.9	170.1	25
Cyfluthrin III	11.9	162.9	127	5

Compound	RT (min)	Precursor (*m/z*)	Product (*m/z*)	CE
Cyfluthrin III	11.9	162.9	90.9	15
Coumaphos	12.07	362	109	16
Coumaphos	12.07	226	198	10
Coumaphos	12.07	226	163	20
Coumaphos	12.07	210	182	10
Coumaphos	12.07	210	154	18
Acequinocyl	12.09	341.9	187.9	15
Acequinocyl	12.09	189	115	25
Acequinocyl	12.09	187.9	160	5
Cypermethrin	12.5	181	152	25
Cypermethrin	12.5	165	127	5
Cypermethrin	12.5	165	91	15
Cypermethrin	12.5	163	127	5
Cypermethrin	12.5	163	91	15
Boscalid	13.6	140	112	10
Boscalid	13.6	140	76	25
Boscalid	13.6	111.9	76	15
Fenvalerate	13.75	208.9	141.1	15
Fenvalerate	13.75	181	152.1	20
Fenvalerate	13.75	167	125.1	5
Deltamethrin	15	252.9	93	15
Deltamethrin	15	252.9	77	30
Deltamethrin	15	250.7	172	5
Deltamethrin	15	181	152.1	25

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