RESIDUES AND TRACE ELEMENTS

Multiresidue Method of Analysis of Pesticides in Medical Cannabis

JONATHAN R. MOULINS, MICHEL BLAIS, KIM MONTSION, JOSEE TULLY, WILLIAM MOHAN, MATHIEU GAGNON, TYLER MCRITCHIE, KERI KWONG, NEIL SNIDER, and DAVID R. BLAIS[1]
Health Canada, Pest Management Regulatory Agency, Laboratory Services, Ottawa, ON K1A OC6, Canada

Three related analytical methods were developed and validated for the determination of pesticides in cannabis leaves, dried cannabis flowers, and cannabis oil. The methods follow the generic sequence of an acetonitrile extraction, followed by solid-phase extraction cleanup and analysis by HPLC-tandem mass spectrometry (HPLC-MS/MS), GC-MS/MS, and GC-MS. These methods were developed to accommodate sample quantity and lipid content of the different matrices. Validation at a spiking level of 0.01 µg/g was successful for 39 pesticides in cannabis leaves and 40 pesticides in cannabis oil, and at 0.02 µg/g for 32 pesticides in cannabis flowers, with the majority of analytes showing recoveries within the acceptable range of 70–130%. With these methods established, unannounced inspections of Canadian licensed producers of cannabis revealed that out of 144 samples collected, 26 showed the presence of unauthorized pest control products.

Under the Access to Cannabis for Medical Purposes Regulations (ACMPR), licensed producers are permitted to use only pest control products that are currently approved for use on cannabis under the Pest Control Products Act (1, 2) However, unauthorized pesticides have been detected in medical cannabis, prompting voluntary recalls. In an effort to provide added assurance to Canadians that they are receiving quality-controlled cannabis products, Health Canada announced that it would require mandatory testing of all cannabis products for the presence of pesticide active ingredients prior to being sold or provided to any party (2).

To assist in the mandatory testing, a robust analytical method for pesticides in cannabis is required. However, due in part to its status as a controlled substance, multiresidue studies of pesticides in cannabis have been limited. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) extraction has been a well-established sample preparation technique for multiresidue pesticides analysis in fruits and vegetables (3). Recently, QuEChERS has been adapted for the analysis of pesticides in cannabis, which follows the European standard for multiresidue analysis (EN 15662) (4) and the AOAC method (AOAC *Official Method* **2007.01**) (5). Although the use of QuEChERS has been effective, analytical studies of cannabis have proven challenging because of its complex composition of oils, resins, terpenes, and cannabinoids (6). Spectral data of pesticide residues are often accompanied by significant interferences from coextracted cannabinoids, terpenes, and other lipids present in percent abundance.

Traditional cleanup techniques such as solid-phase extraction (SPE) have proven insufficient in removing residual lipids (7–13). Recently, Enhanced Matrix Removal (EMR)-Lipid technology in combination with a QuEChERS extraction was demonstrated as an effective cleanup technique for high-lipid-content matrices (14). The nanoparticles that comprise EMR-Lipid, when dissolved in extract solution, isolate the aliphatic chains of fatty acid molecules from the matrix through hydrophobic interactions and size exclusion. Precipitation and subsequent salting-out of the particle-bound lipids results in a significantly cleaner extract, without impact on analyte recovery.

The present work describes the adaptation of a previously published multiresidue method (15) to render the determination of pesticides in medical cannabis possible. An EMR-Lipid cleanup step has been incorporated in order to expand the method scope to lipid-rich cannabis matrices. These new methods are amenable and meet all the requirements for the determination of pesticides listed in the "Requirements for Mandatory Testing of Pesticide Active Ingredients in Cannabis Products" for cannabis leaves, dried cannabis flowers, and cannabis oil by HPLC-tandem mass spectrometry (HPLC-MS/MS), GC-MS/MS, and GC-MS (16). Recent compliance and enforcement samples from unannounced inspections were analyzed to demonstrate the applicability of these methods.

Experimental

Standards and Reagents

Pesticide analytical standards were purchased from Chem Service (West Chester, PA), Sigma-Aldrich Canada (Oakville, ON), and BASF Canada Incorporated (Mississauga, ON). Analytical grade acetone, toluene, acetic acid, and NaCl were purchased from EMD Millipore (Darmstadt, Germany). Analytical grade acetonitrile and Na_2SO_4 were purchased from Fisher Scientific (Fairlawn, NJ). Water was obtained from a Milli-Q® Plus Ultra Pure Water system (Millipore Corp., Burlington, MA). QuEChERS EMR-Lipid and NaCl/$MgSO_4$ polish tubes were obtained from Agilent Technologies, Inc. (Folsom, CA). RoQ QuEChERS Extraction Kits and Strata™ C18-E SPE columns were obtained from Phenomenex (Torrance, CA). Supelclean™ ENVI™-Carb SPE Tubes were obtained from Supelco (Bellefonte, PA). Sep-Pak® Classic NH_2 Cartridges were obtained from Waters Corp. (Milford, MA).

Received December 11, 2017. Accepted by AK February 26, 2018.
[1]Corresponding author's e-mail: david.blais@canada.ca
DOI: https://doi.org/10.5740/jaoacint.17-0495

Apparatus

For sample preparation, a food processor (Robot Coupe R300; Robot Coupe, Ridgeland, MS), a mixer (Omni-mixer Homogenizer; VWR Canlab, Mississauga, ON), a high-speed shaker (Spex Sample Prep Geno-Grinder; Fisher Scientific, Fairlawn, NJ), a centrifuge (Allegra X15R 208v; Beckman Coulter Inc., Brea, CA), and a solvent evaporator (Xcelvap; Horizon Technologies, Salem, NH) were used. Sample analysis was carried out by GC-MS (Agilent Technologies 7890A gas chromatograph, 5973N mass spectrometer, 7683B autosampler), GC-MS/MS (ThermoFisher Trace GC Ultra with a TSQ Quantum XLS mass spectrometer, TriPlus RSH autosampler), and HPLC-MS/MS (AB Sciex Exion HPLC with an AB Sciex 6500 Q-Trap triple-quadrupole mass spectrometer).

Standard Solution Preparation

High-concentration stock standard solutions were prepared from the purest analytical material available from commercial suppliers, typically ≥95%. In general, stock standard solutions were prepared in the range of 1000–2000 µg/mL in acetone, and in acetonitrile and methanol for HPLC-MS/MS compounds. From these, intermediate standard solutions were prepared at 100 µg/mL. Calibration standards were prepared with each sample set at concentrations of 1×, 2×, 3×, 5×, and 8× the lowest calibrated level (LCL) in pesticide-free cannabis matrix extract to compensate for ion suppression/enhancement effects.

Sample Preparation

Flow diagrams of all three methods described below can be found in Figure 1.

(**a**) *Cannabis leaves (method 1).*—To a 20 g sample, 50 mL water was added and mixed. After standing for 30 min, the leaves were extracted with 100 mL acetonitrile by Omni-Mixer for 5 min; 20 g NaCl was then added before mixing an additional 5 min. The sample was transferred to a 250 mL Nalgene bottle and centrifuged at 4000 rpm for 5 min. The supernatant acetonitrile layer was transferred to a 50 mL centrifuge tube. After preconditioning with acetonitrile, a C18-E column was rinsed with 2 mL sample. The eluent was discarded, and an additional 13 mL sample was eluted by gravity into a 15 mL centrifuge tube. To an EMR-Lipid tube, 5 mL water was added. The tube was vortexed immediately to disperse the sorbent, and 6 mL of the sample extract was transferred to the EMR-Lipid tube and vortexed immediately for 1 min. The tube was centrifuged at 4500 rpm for 3 min, and the supernatant mixture of acetonitrile and water was transferred to an NaCl/$MgSO_4$ polish tube. The tube was vortexed for 1 min and then centrifuged at 4500 rpm for 3 min. A volume of 5 mL of the acetonitrile layer was transferred to a 5 mL centrifuge tube and evaporated to 1 mL under blowing nitrogen. An ENVI-Carb column was topped with 1 g Na_2SO_4 and fitted with a Sep-Pak NH_2 cartridge. After preconditioning with acetonitrile–toluene (3+1, v/v), the sample was transferred quantitatively to the column and eluted by gravity with 10 mL acetonitrile–toluene (3+1, v/v) into a 15 mL centrifuge tube. The sample was concentrated to low volume under blowing nitrogen and solvent exchanged to 1 mL acetone. To 0.5 mL of the extract, 10 µL of 50 µg/mL internal standard 2,4,6-tribromobiphenyl was added for GC-MS and GC-MS/MS analysis. The remaining 0.5 mL extract was solvent exchanged to 0.5 mL acetonitrile. After adding 20 µL of 50 µg/mL internal standard isoprocarb, the sample was diluted to 1 mL with water and passed through a 0.2 µm nylon filter for HPLC-MS/MS analysis.

(**b**) *Dried cannabis flowers (method 2).*—To a 1 g sample, 10 mL water and 10 mL 1% acetic acid in acetonitrile were added. The mixture was shaken by Geno-Grinder at 1500 rpm for 1 min. A QuEChERS salt packet was added and the mixture was shaken again at 1500 rpm for 1 min. The tube was centrifuged at 4500 rpm for 10 min and a 1 mL extract was then diluted to 10 mL with acetonitrile. After preconditioning with acetonitrile, a C18-E column was rinsed with 2 mL sample. The eluent was discarded, and an additional 6 mL sample was eluted by gravity into a 15 mL centrifuge tube. The sample was carried through the EMR-Lipid and ENVI-Carb cleanups as described in method 1. After the sample was solvent exchanged to 0.5 mL acetonitrile, 10 µL of 50 µg/mL isoprocarb was added. The sample was diluted to 1 mL with water and passed through a 0.2 µm nylon filter for HPLC-MS/MS analysis.

(**c**) *Cannabis oil (method 3).*—To 1 g of sample, 5 mL 1:1 (v/v) acetone–acetonitrile (1+1, v/v) was added. The mixture was shaken by Geno-Grinder at 1500 rpm for 2 min and centrifuged at 4500 rpm for 5 min. The supernatant acetone–acetonitrile layer was then transferred to a 5 mL centrifuge tube. To an EMR-Lipid tube, 5 mL water was added. The tube was vortexed immediately to disperse the sorbent. The sample extract was transferred to the EMR-Lipid tube and vortexed immediately for 1 min. The tube was centrifuged at 4500 rpm for 3 min, and the supernatant aqueous acetone–acetonitrile layer was transferred to an NaCl–$MgSO_4$ polish tube. The tube was vortexed for 1 min and then centrifuged at 4500 rpm for 3 min. A 1 mL aliquot of the supernatant acetone–acetonitrile layer was solvent exchanged to 1 mL acetone. Then, 20 µL of 50 µg/mL 2,4,6-tribromobiphenyl was added for GC-MS analysis. An additional 1 mL aliquot was solvent exchanged to 0.5 mL acetonitrile. After adding 20 µL of 50 µg/mL isoprocarb, the sample was diluted to 1 mL with water and passed through a 0.2 µm nylon filter for HPLC-MS/MS analysis.

Instrument Conditions

(**a**) *HPLC-MS/MS.*—Sample analysis was carried out using an Exion HPLC coupled to a 6500 Q-Trap triple-quadrupole mass spectrometer (AB Sciex). Analyst version 1.6.3 and MultiQuant version 3.0.2 software were used for instrument control and data analysis, respectively. A Kinetex C18 column (2.1 × 50 mm, 2.6 µm) was used and maintained at 30°C. The source was maintained at 550°C. The following gas parameters were used: curtain gas, 35 psi; collision gas, 9 psi; ion spray voltage, 5500 V; ion source gas 1, 50 psi; ion source gas 2, 55 psi. The injection volume was 1 µL. The mobile phases were water-methanol (95+5) + 10 mM formic acid + 10 mM ammonium formate (A) and water-methanol (5+95) + 10 mM formic acid + 10 mM ammonium formate (B). The flow rate was 0.7 mL/min. The following elution gradient was used: 0–10 min, 0% B increasing to 100% B; 10–15 min, 100% B. Analysis was carried out by positive electrospray ionization using retention time-scheduled multiple reaction monitoring (MRM) to acquire two transitions (quantitative and qualitative) for each analyte (Table 1).

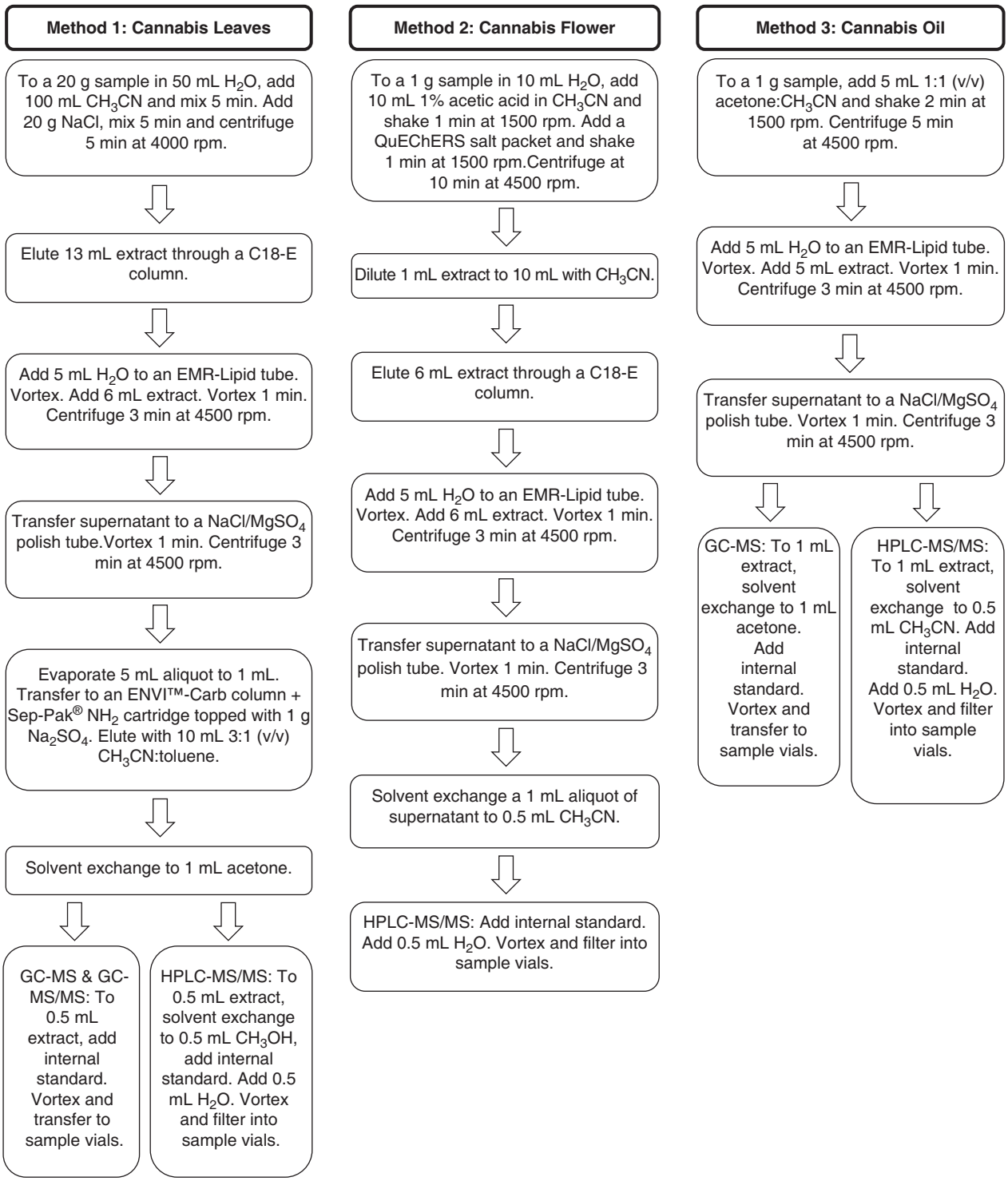

Figure 1. Flow chart of analytical methods developed for cannabis leaves, flowers, and oil.

(**b**) *GC-MS/MS.*—Sample analysis was carried out using a Trace GC Ultra gas chromatograph with a TriPlus RSH Autosampler and coupled to a TSQ Quantum XLS mass spectrometer (Thermo Fisher Scientific). Xcalibur version 3.1.66.10 software (Thermo Fisher Scientific) was used for instrument control and data analysis. The injection port was fitted with a PTV injector. The inlet temperature was maintained at 45°C for 0.15 min, ramped to 66°C at 10°C/s with a 0.3 min hold, ramped to 285°C at 14.5°C/s with a 1.5 min hold, and then finally ramped to 400°C at 14.5°C/min with a 40.45 min hold. The liner was a Siltek deactivated baffle (Thermo Fisher Scientific). The injection volume was 1 µL in splitless mode. Helium carrier gas was maintained at a constant flow of 1.2 mL/min. The TR-pesticide II capillary column (30 m × 0.25 mm × 0.25 µm)

Table 1. Retention times and MRM transitions for compounds determined by HPLC-MS/MS

			MRM transitions			
			Quantitative		Qualitative	
Compound[a]	Method	Rt, min[b]	Precursor	Product	Precursor	Product
Acephate	2, 3	0.74	183.9	143.0	183.9	125.0
Dinotefuran	1–3	1.53	203.1	129.1	203.1	113.1
Flonicamid	1–3	1.76	230.0	203.1	230.0	174.0
Oxamyl	1–3	1.79	237.1	71.9	238.1	220.1
Methomyl	1–3	1.85	163.0	88.0	163.0	106.0
Thiamethoxam	1–3	2.09	292.0	211.1	292.0	181.0
Clothianidin	1–3	2.72	250.0	169.0	250.0	131.9
Imidacloprid	1–3	2.75	256.0	209.0	256.0	175.1
Dimethoate	1–3	2.82	230.0	199.0	230.0	125.0
Mevinphos 1	1–3	3.02	225.1	193.0	225.1	127.0
Acetamiprid	1–3	3.15	223.0	126.0	223.0	90.0
Mevinphos 2	1–3	3.61	225.1	193.0	225.1	127.0
Aldicarb	1–3	3.63	208.1	116.0	208.1	89.0
Thiacloprid	1–3	3.68	253.0	126.0	253.0	90.0
Dichlorvos	1–3	4.28	220.9	109.0	220.9	79.0
Propoxur	1–3	4.44	210.1	111.1	210.1	168.1
Pirimicarb	1–3	4.48	238.9	72.0	238.9	182.1
Carbofuran	1–3	4.57	222.1	165.1	222.1	123.0
Thiophanate-methyl	2, 3	4.72	343.0	151.0	343.0	93.0
Carbaryl	1–3	4.92	202.1	145.1	202.1	127.1
Isoprocarb[c]	1–3	5.34	194.1	95.0	NA[d]	NA
Cyantraniliprole	1–3	5.42	475.0	285.7	475.0	443.9
Imazalil	1–3	5.59	297.0	159.0	297.0	201.0
Azadirachtin	1–3	5.68	738.3	703.2	738.3	685.2
Metalaxyl	1–3	5.69	280.1	220.2	280.1	192.1
Fensulfothion	1–3	5.80	308.9	235.0	308.9	157.0
Methiocarb	1–3	6.31	226.0	169.0	226.0	121.0
Azoxystrobin	1–3	6.51	404.0	372.0	404.0	343.9
Boscalid	1–3	6.54	343.0	307.0	343.0	271.0
Fludioxonil	1–3	6.56	266.0	229.1	266.0	158.0
Paclobutrazol	1–3	6.57	294.1	70.0	294.1	125.0
Malathion	1–3	6.67	331.0	127.0	331.0	284.9
Myclobutanil	1–3	6.80	289.0	70.0	289.0	125.0
Bifenazate	1–3	6.89	301.0	198.1	301.0	170.1
Fluopyram	1–3	6.91	397.0	208.0	397.0	173.0
Ethoprophos	1–3	6.92	243.1	173.0	243.1	215.0
Spirotetramat	1–3	7.06	374.1	302.1	374.1	216.1
Cyprodinil	3	7.13	225.9	93.1	225.9	92.0
Iprodione	1–3	7.14	330.0	245.0	330.0	162.0
Tetrachlorvinphos	1–3	7.30	366.9	127.0	366.9	205.9
Fenoxycarb	1–3	7.32	302.1	116.1	302.1	88.0
Tebufenozide	1–3	7.35	353.1	297.0	353.1	133.1
Fipronil	1–3	7.39	453.9	367.9	453.9	289.8
Fenthion	3	7.44	279.0	169.0	279.0	247.0
Tebuconazole	3	7.48	308.1	70.0	308.1	125.1
Propiconazole	1	7.53	342.0	159.0	342.0	123.0

Table 1. (*continued*)

			MRM transitions			
			Quantitative		Qualitative	
Compound[a]	Method	Rt, min[b]	Precursor	Product	Precursor	Product
Benzovindiflupyr	1–3	7.58	398.0	342.0	398.0	321.9
Coumaphos	1–3	7.64	363.0	227.0	363.0	211.0
Clofentezine	1, 3	7.66	303.0	138.0	303.0	102.0
Pyraclostrobin	1, 3	7.75	388.0	194.1	388.0	163.1
Spinosad A	3	8.05	732.4	142.1	732.4	98.1
Trifloxystrobin	1, 3	8.08	409.0	186.1	409.0	145.1
Pyrethrin II	1–3	8.10	373.1	128.0	373.1	105.0
Tetramethrin 1	1, 3	8.23	332.2	164.0	332.2	135.1
Spinosad D	3	8.32	746.4	142.2	746.4	98.1
Buprofezin	1–3	8.36	306.0	201.1	306.0	116.0
Tetramethrin 2	1, 3	8.37	332.2	164.0	332.2	135.1
Novaluron	1, 3	8.39	492.9	158.0	492.9	141.0
Spinetoram J	3	8.39	748.4	142.1	748.4	98.1
Allethrin	1, 3	8.48	303.1	135.0	303.1	91.0
Teflubenzuron	1, 3	8.49	380.9	158.0	380.9	141.0
Spinetoram L	3	8.64	760.4	142.1	760.4	98.1
Spiromesifen	1, 3	8.84	371.1	273.1	371.1	255.1
Etoxazole	1–3	8.86	360.1	141.0	360.1	177.1
Pyrethrin I	1–3	8.93	329.1	161.1	329.1	143.2
Fenpyroximate	2, 3	9.01	422.2	135.2	422.2	107.0
Spirodiclofen	1–3	9.04	411.0	312.9	411.0	212.9
Pyridaben	1–3	9.21	365.1	308.9	365.1	147.1
Resmethrin 1	1–3	9.27	339.2	171.1	339.2	128.0
Resmethrin 2	1–3	9.34	339.2	171.1	339.2	128.0
Phenothrin 1	1–3	9.46	351.2	183.0	351.2	128.0
Phenothrin 2	1–3	9.52	351.2	183.0	351.2	128.0
Avermectin B1a	1–3	9.61	890.5	567.3	890.5	305.1

[a] A compound name followed by a number indicates that this compound's multiple isomers were detected and identified separately but quantified together. They are numbered according to their order of elution.

[b] Rt = Retention time.

[c] Internal standard.

[d] NA = Not applicable.

was fitted with a 1 m retention gap of the same stationary phase (Thermo Fisher Scientific). The oven temperature was maintained at 70°C for 5 min, ramped to 180°C at 25°C/min, ramped to 280°C at 5°C/min with a 1 min hold, and then ramped to 300°C at 10°C/min with a 10 min hold (total run time: 42.4 min). The temperature of the MS source was maintained at 250°C and the transfer line at 305°C. Argon was used as the collision gas at a pressure of 1.0 mTorr. Analysis was carried out by electron impact ionization using retention time-scheduled MRM to acquire two transitions (quantitative and qualitative) for each analyte (Table 2).

(**c**) *GC-MS.*—Sample analysis was carried out using a gas chromatograph (Agilent Technologies 7890A) equipped with a 7683B autosampler and coupled to a 5973N mass spectrometer. Chemstation version G1701EA E.02.01 software (Agilent Technologies) was used for instrument control and data analysis. The injection port was fitted with a split/splitless injector and maintained at 250°C. The liner was a splitless double taper (Agilent Technologies). The injection volume was 1 µL in splitless mode. Helium carrier gas was held at an initial pressure of 8.7 psi (1 mL/min flow), pulsed to 40 psi for 2 min, followed by 8.7 psi, and then ramped to maintain a 1 mL/min flow for the duration of the run. The ZB-Multiresidue-1 column (30 m × 0.25 mm × 0.25 µm) was fitted with a 1 m retention gap of the same stationary phase (Phenomenex). The oven temperature was maintained at 70°C for 1.5 min, ramped to 150°C at 8°C/min, ramped to 240°C at 2°C/min, and then ramped to 300°C at 15°C/min with a 10.5 min hold (total run time: 71 min). The temperature of the MS source was maintained at 230°C, the quadrupole at 150°C, and the transfer line at 305°C. Analysis was carried out by electron impact ionization using retention time-scheduled selected-ion monitoring to acquire three ions (one quantitative and two qualitative) for each analyte (Table 3).

Table 2. Retention times and MRM transitions for compounds determined by GC-MS/MS

			MRM transitions			
			Quantitative		Qualitative	
Compound[a]	Method	Rt, min[b]	Precursor	Product	Precursor	Product
Etridiazol	1	10.02	210.9	140.0	212.9	184.9
Quintozene	1	12.61	248.9	213.9	294.8	236.9
Diazinon	1	12.80	137.1	84.0	304.1	179.1
Chlorpyrifos	1	15.09	199.0	171.0	313.9	258.0
Kinoprene	1	15.12	148.9	76.9	148.9	90.9
Fenthion	1	15.20	278.0	109.0	278.0	169.0
Dodemorph 1	1	15.70	154.2	97.1	154.2	136.1
S-Methoprene 1	1	15.93	235.1	105.1	235.1	147.3
2,4,6-Tribromobiphenyl[c]	1	16.07	390.0	230.0	NA[d]	NA
Dodemorph 2	1	16.21	154.2	97.1	154.2	136.1
S-Methoprene 2	1	16.72	235.1	105.1	235.1	147.3
Endosulfan-alpha	1	17.31	240.9	205.9	264.9	192.9
Kresoxim-methyl	1	18.30	206.1	116.1	206.1	131.1
Chlorfenapyr	1	18.56	247.0	227.0	328.1	247.2
Endosulfan-beta	1	19.12	240.9	205.9	271.9	236.9
Endosulfan sulfate	1	20.36	269.9	234.9	273.9	238.9
Piperonyl butoxide	1	21.35	149.1	65.0	176.1	131.1
Bifenthrin	1	22.30	181.1	141.0	181.1	166.1
Permethrin	1	25.97	183.0	153.0	183.0	168.0

[a] A compound name followed by a number indicates that this compound's multiple isomers were detected and identified separately but quantified together. They are numbered according to their order of elution.

[b] Rt = Retention time.

[c] Internal standard.

[d] NA = Not applicable.

Quality Control

Each method had a blank and two spiked samples for quality control. For methods 1 and 3, a blank sample was spiked with 40 µL of 0.5 µg/mL GC-MS/MS and HPLC-MS/MS spiking solutions. A second blank sample was spiked with 50 µL of 3 µg/mL GC-MS spiking solution. For method 2, a blank sample was spiked with 40 µL of 0.5 µg/mL HPLC-MS/MS spiking solution. A second blank sample was spiked with 120 µL of 0.5 µg/mL HPLC-MS/MS spiking solution. All spikes were allowed to stand for a minimum of 30 min. The blank and spikes were then processed the same way as the samples.

Results and Discussion

To cover the broadest spectrum possible of different pesticides in cannabis, a combination of GC-MS, GC-MS/MS, and HPLC-MS/MS instrumentation technologies were used in the development and validation of the three methods described. For example, in the case of cannabis oil in method 3, enhanced chromatographic resolution by GC-MS allowed for improved identification and quantification over GC-MS/MS, despite lower selectivity. These discrepancies between GC-MS and GC-MS/MS could be attributed to factors such as differences in ion source technologies, as both instruments are from different manufacturers, different instrument parameters, and the use of different capillary columns, ZB-Multiresidue-1 and TR-Pesticide II, for GC-MS and GC-MS/MS, respectively. Alternatively, it was observed during the analysis of cannabis flowers in method 2 that high matrix interferences did not allow for selective detection, nor reliable quantitation of pesticides by GC, and were therefore not included in method 2. All three methods were validated based on the LCL, accuracy, precision, and linearity. Matrix effects for pesticides in plant material are a well-known issue, and to compensate for it, all standards prepared were matrix matched. Matrix-matched standards were prepared by spiking at the various concentration levels to pesticide-free cannabis extract.

LCL

The LCL for each pesticide was determined by an injection of a series of matrix-matched standards. The LCL was deemed acceptable if the signal of the LCL peak height to the height of the surrounding noise was at a minimum of 5:1 ratio for two ions for the GC/MSD and two transitions for the GC-MS/MS and HPLC-MS/MS. This ratio is calculated by the instrument. In addition, five replicate spikes at the LCL must meet the following method performance criteria: mean recoveries in the range of 70–130% with an RSD ≤30%; exceptionally, a mean recovery below 70% may be acceptable if the recovery is consistent with an RSD ≤20%.

Table 3. Retention times and selected-ion monitoring quantitative and qualitative ions for compounds determined by GC-MS

Compound[a]	Method	Rt, min[b]	SIM ions[c]		
			Quantitative	Qualitative 1	Qualitative 2
Etridiazol	3	14.40	211	183	213
Diazinon	3	24.97	304	276	137
Quintozene	3	24.97	295	265	237
Kinoprene	3	32.65	149	221	125
Chlorpyrifos	3	33.08	314	286	316
S-Methoprene 1	3	34.92	73	153	191
2,4,6-Tribromobiphenyl[d]	3	36.19	390	392	NA[e]
S-Methoprene 2	3	37.34	73	153	191
Endosulfan-alpha	3	39.67	339	277	241
Kresoxim-methyl	3	43.44	116	131	206
Chlorfenapyr	3	44.50	328	363	247
Endosulfan-beta	3	46.64	241	237	239
Endosulfan sulfate	3	50.16	387	272	237
Piperonyl butoxide	3	51.20	176	177	193
Bifenthrin	3	53.20	181	166	165
Permethrin 1	3	59.69	183	163	165
Permethrin 2	3	59.96	183	163	165
Cyfluthrin	3	60.85	206	163	165
Cypermethrin	3	61.11	181	163	165
Fenvalerate 1	3	62.62	167	419	181
Fenvalerate 2	3	62.97	167	419	181
Deltamethrin 1	3	63.70	181	251	253
Deltamethrin 2	3	64.04	181	251	253

[a] A compound name followed by a number indicates that this compound's multiple isomers were detected and identified separately but quantified together. They are numbered according to their order of elution.

[b] Rt = Retention time.

[c] SIM = Selected-ion monitoring.

[d] Internal standard.

[e] NA = Not applicable.

The pesticides in cannabis had a targeted LCL of 0.010 µg/g. However, not all pesticides met the target. The LCL achieved for each pesticide are found in Tables 4–6.

Accuracy and Precision

The accuracy and precision of the pesticide recoveries were measured by spiking each cannabis matrix (leaves, dried flowers, and oil) at the LCL ($n = 5$), 3× LCL ($n = 3$), and 5× LCL ($n = 2$). Tables 4–6 show the recoveries of the pesticides in cannabis leaves, dried cannabis flowers, and cannabis oil, respectively.

In general, all the pesticides analyzed in Tables 4–6 had an average recovery and precision at their respective LCL ranging from 70 to 130%, with RSD ≤30%. In addition, all fortified samples had an average recovery and precision range of 70–130%, with RSD ≤30%. There were some pesticides that had a recovery at the LCL below 70%, but this was deemed acceptable for the analysis as the RSD was ≤20%.

Linearity

Linearity was established based on matrix-matched standards in the concentration range of 0.005–0.04 ng/µL for HPLC-MS/MS, 0.010–0.080 ng/µL for GC-MS/MS, and 0.10–0.80 ng/µL for GC-MS. All three methods demonstrated good linearity as the calibration curves had a correlation coefficient of at least 0.99.

Application of Methods to Compliance and Enforcement Samples

A Canadian licensed producer for cannabis products must adhere to section 66 of the ACMPR, which states that "fresh or dried marihuana or marihuana plants or seeds must not be treated with a pest control product unless the product is registered for use on marihuana under the Pest Control Products Act or is otherwise authorized for use under the Act" (1, 2). Between March 2017 and January 2018, Health Canada conducted unannounced inspections and testing of cannabis products from licensed producers across the country. During these inspections, a total of 144 samples, comprising cannabis leaves, dried cannabis flowers, and cannabis oils, were collected for pesticide analysis. These samples were analyzed for pesticides using methods 1–3 as described above. Of these samples, 26 samples contained unregistered pesticides. Table 7 shows the identity and concentration range of pesticides detected.

Table 4. Recovery data for compounds determined in cannabis leaves (method 1)

		LCL (n = 5)		3 × LCL (n = 3)		5 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Acetamiprid	0.05	104	9	107	4	102	4
Aldicarb	0.5	71	16	89	2	82	3
Allethrin	0.1	82	12	87	1	75	9
Avermectin B1a	0.25	77	12	78	13	66	6
Azadirachtin	0.5	93	14	101	3	89	6
Azoxystrobin	0.01	97	10	102	4	94	4
Benzovindiflupyr	0.01	98	7	105	3	92	0
Bifenazate[a]	0.01	25	10	20	26	13	6
Bifenthrin	0.1	82	3	75	2	71	4
Boscalid	0.01	93	6	95	4	88	5
Buprofezin	0.01	85	9	88	2	77	1
Carbaryl	0.025	111	11	120	1	96	4
Carbofuran	0.01	98	10	102	5	94	2
Chlorfenapyr	0.1	96	4	88	3	87	2
Chlorpyrifos	0.01	63	10	59	6	63	3
Clofentezine	0.01	71	7	71	5	57	4
Clothianidin	0.025	92	9	105	3	101	7
Coumaphos	0.01	67	13	70	7	63	1
Cyantraniliprole	0.01	67	33	74	4	59	9
Diazinon	0.01	97	4	89	4	85	2
Dichlorvos	0.05	22	60	32	20	28	0
Dimethoate	0.01	99	10	103	9	91	6
Dinotefuran	0.05	82	5	80	4	82	9
Dodemorph[a]	0.05	20	57	16	17	15	34
Endosulfan-alpha	0.1	89	2	82	2	77	4
Endosulfan-beta	0.1	85	4	72	4	65	3
Endosulfan sulfate[a]	0.1	82	8	44	4	34	15
Ethoprophos	0.01	77	10	89	5	80	2
Etoxazole	0.01	79	12	90	5	81	5
Etridiazole	0.01	53	5	45	6	35	4
Fenoxycarb	0.01	97	9	101	3	91	3
Fensulfothion	0.01	98	9	103	3	93	4
Fenthion	0.01	71	6	71	4	67	1
Fipronil	0.01	125	14	138	6	105	10
Flonicamid	0.025	95	10	108	6	105	7
Fludioxonil	0.01	77	5	85	3	78	8
Fluopyram	0.01	96	10	105	5	96	3
Imazalil	0.01	36	14	42	6	40	10
Imidacloprid	0.01	99	7	101	4	95	3
Iprodione	0.5	139	19	150	2	97	1
Kinoprene	0.05	70	4	57	5	54	1
Kresoxim-methyl	0.01	102	6	101	2	98	2
Malathion	0.01	55	13	68	4	64	7
Metalaxyl	0.01	97	10	108	3	96	3
Methiocarb	0.01	112	13	123	1	96	1
Methomyl[a]	0.025	33	63	48	26	69	8
S-Methoprene	1	66	3	60	4	57	4
Mevinphos	0.025	95	10	87	6	78	1
Myclobutanil	0.01	88	6	101	3	93	3
Novaluron	0.025	85	9	80	0	66	4
Oxamyl[b]	1.5	78	12	82	4	118	NA[c]
Paclobutrazol	0.01	83	7	104	2	93	1

Table 4. (*continued*)

		LCL (n = 5)		3 × LCL (n = 3)		5 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Permethrin	0.5	83	4	76	2	73	6
Phenothrin	0.025	77	13	76	4	65	4
Piperonyl butoxide	0.25	89	3	85	1	86	3
Pirimicarb	0.01	74	10	86	4	81	1
Propiconazole	0.01	80	16	103	4	93	2
Propoxur	0.01	95	10	85	37	93	5
Pyraclostrobin	0.01	90	8	96	2	88	5
Pyrethrins I and II	0.025	86	7	94	4	85	6
Pyridaben	0.025	73	11	81	4	71	21
Quintozene	0.01	64	4	64	6	60	2
Resmethrin[a]	0.05	2	116	1	43	0	NA
Spirodiclofen	0.01	63	92	70	79	30	47
Spiromesifen[a]	1.5	25	65	48	10	34	25
Spirotetramat[a]	0.01	0	NA	1	0	1	141
Tebufenozide	0.01	100	11	113	8	92	6
Teflubenzuron	0.025	85	10	77	5	65	0
Tetrachlorvinphos	0.01	90	9	97	4	87	2
Tetramethrin	0.05	104	11	107	6	83	5
Thiacloprid	0.01	99	9	101	3	94	2
Thiamethoxam	0.01	92	8	90	1	86	3
Trifloxystrobin	0.01	101	9	108	5	97	2

[a] Compound did not pass validation and was thus monitored qualitatively.

[b] Only one replicate could be calculated at 5 × LCL. The second replicate exceeded the maximum of the quadratic calibration curve.

[c] NA = Not applicable.

Table 5. Recovery data for compounds determined in dried cannabis flowers (method 2)

		LCL (n = 5)		3 × LCL (n = 3)		5 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Acephate	0.02	49	11	45	11	71	1
Acetamiprid	0.1	83	6	78	1	89	2
Aldicarb	1	72	6	66	5	78	5
Allethrin	0.2	71	12	63	9	84	17
Avermectin B1a[a]	0.5	18	10	17	12	36	6
Azadirachtin	1	87	3	75	8	88	3
Azoxystrobin	0.02	83	5	79	1	90	3
Benzovindiflupyr	0.02	78	3	71	2	88	2
Bifenazate	0.02	79	3	74	2	88	4
Boscalid	0.02	78	11	72	6	86	4
Buprofezin	0.02	55	8	48	2	68	3
Carbaryl	0.05	90	6	83	3	97	1
Carbofuran	0.02	82	4	78	1	91	2
Clofentezine	0.02	71	4	67	3	85	5
Clothianidin	0.05	82	4	73	3	87	3
Coumaphos	0.02	41	13	35	6	48	10
Cyantraniliprole[a]	0.02	16	43	13	4	24	3
Dichlorvos	0.1	44	23	42	5	61	1
Dimethoate	0.02	83	4	78	1	90	1

Table 5. (*continued*)

		LCL (n = 5)		3 × LCL (n = 3)		5 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Dinotefuran	0.1	79	4	75	2	87	2
Ethoprophos	0.02	62	12	60	3	80	4
Etoxazole	0.02	55	6	53	1	72	3
Fenoxycarb	0.02	77	6	71	1	75	3
Fenpyroximate	0.02	61	6	48	6	65	3
Fensulfothion	0.02	72	2	66	2	87	2
Fipronil	0.06	62	33	79	14	84	7
Flonicamid	0.05	80	2	84	2	93	1
Fludioxonil	0.02	64	20	74	18	88	1
Fluopyram	0.02	82	6	78	1	90	1
Imazalil[a]	0.02	21	44	16	7	28	5
Imidacloprid	0.02	89	16	80	5	90	0
Iprodione	1	84	10	74	6	85	5
Malathion	0.02	80	5	77	4	89	3
Metalaxyl	0.02	79	9	75	3	91	1
Methiocarb	0.02	80	2	78	1	92	1
Methomyl	0.05	79	2	75	2	89	0
Mevinphos	0.05	62	7	61	3	77	2
Myclobutanil	0.02	67	8	67	2	84	2
Novaluron	0.05	81	6	76	7	92	2
Oxamyl	3	79	5	83	1	93	2
Paclobutrazol	0.02	66	10	67	5	82	7
Phenothrin	0.05	55	5	52	2	70	5
Pirimicarb	0.02	52	3	49	3	74	3
Propoxur	0.02	73	12	75	3	84	2
Pyraclostrobin	0.02	72	4	71	1	85	1
Pyrethrins I and II	0.05	65	10	62	1	76	8
Pyridaben	0.05	58	8	51	2	67	4
Resmethrin	0.1	52	9	43	5	47	2
Spirodiclofen[a]	0.02	57	54	48	4	70	5
Spiromesifen	3	54	16	64	7	93	2
Spirotetramat	0.02	63	8	59	4	76	7
Tebufenozide	0.02	88	12	74	4	94	0
Teflubenzuron	0.05	55	17	57	8	49	4
Tetrachlorvinphos	0.02	74	5	71	4	87	1
Tetramethrin	0.1	47	7	44	2	56	3
Thiacloprid	0.02	81	4	76	1	88	0
Thiamethoxam	0.02	81	3	74	1	86	1
Thiophanate-methyl	0.05	41	18	46	1	58	2
Trifloxystrobin	0.02	79	3	76	2	89	4

[a] Compound did not pass validation and was thus monitored qualitatively.

Table 6. Recovery data for compounds determined in cannabis oil (method 3)

		LCL (n = 5)		3 × LCL (n = 3)		8 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Acephate	0.05	80	8	81	4	78	9
Acetamiprid	0.05	87	7	89	3	85	9
Aldicarb	0.5	74	9	90	5	78	12
Allethrin	0.1	65	20	63	10	55	1
Avermectin B1a	0.25	82	16	86	16	86	0
Azadirachtin	0.5	99	6	98	5	92	7
Azoxystrobin	0.01	86	9	94	4	90	9
Benzovindiflupyr	0.01	79	15	90	6	84	11
Bifenazate	0.01	77	12	83	4	78	10
Bifenthrin	0.5	49	20	19	19	27	29
Boscalid	0.01	71	11	82	4	81	10
Buprofezin	0.01	36	47	56	8	47	2
Carbaryl	0.025	88	9	89	3	86	9
Carbofuran	0.01	90	8	92	2	89	9
Chlorfenapyr	1.5	70	10	47	11	49	7
Chlorpyrifos	0.5	61	8	36	7	40	13
Clofentezine	0.01	53	27	63	4	56	1
Clothianidin	0.025	89	7	89	4	85	9
Coumaphos	0.01	62	21	75	4	67	8
Cyantraniliprole	0.01	92	7	91	4	84	8
Cyfluthrin	3.75	52	17	29	10	33	26
Cypermethrin[a]	3.75	37	61	38	9	32	7
Cyprodinil	0.01	51	25	60	6	56	9
Deltamethrin	12.5	84	21	24	19	30	28
Diazinon[a]	0.5	7	7	7	9	8	9
Dichlorvos	0.05	59	10	60	2	54	7
Dimethoate	0.01	93	9	93	4	89	9
Dinotefuran	0.05	89	8	92	3	88	9
Endosulfan-alpha	2.5	52	10	28	4	34	11
Endosulfan-beta	2.5	61	12	38	10	40	13
Endosulfan sulfate	2.5	80	10	58	3	54	10
Ethoprophos	0.01	71	12	78	4	73	8
Etoxazole	0.01	45	43	53	8	43	2
Etridiazole	0.15	49	15	33	13	40	7
Fenoxycarb	0.01	73	17	82	3	74	8
Fenpyroximate	0.01	45	43	61	7	50	3
Fensulfothion	0.01	87	8	92	4	88	9
Fenthion	0.01	56	17	73	2	66	9
Fenvalerate	3.75	62	14	25	14	32	27
Fipronil	0.01	76	20	91	9	94	5
Flonicamid	0.025	90	9	101	5	92	9
Fludioxonil	0.01	91	7	92	10	87	15
Fluopyram	0.01	84	10	91	3	85	9
Imazalil	0.01	65	24	76	5	69	6
Imidacloprid	0.01	87	7	88	4	84	9

Table 6. (*continued*)

		LCL (n = 5)		3 × LCL (n = 3)		8 × LCL (n = 2)	
Compound	LCL, µg/g	Mean rec., %	RSD, %	Mean rec., %	RSD, %	Mean rec., %	RSD, %
Iprodione	0.5	103	9	103	4	92	9
Kinoprene	1.25	77	4	25	4	23	22
Kresoxim-methyl	0.15	109	13	82	12	79	6
Malathion	0.01	84	10	88	5	85	10
Metalaxyl	0.01	88	9	92	5	86	8
Methiocarb	0.01	80	10	85	4	80	10
Methomyl	0.025	90	9	93	3	88	10
S-Methoprene	5	53	9	29	10	29	15
Mevinphos	0.025	74	17	79	4	75	12
Myclobutanil	0.01	78	18	85	2	82	8
Novaluron	0.025	66	24	82	7	73	6
Oxamyl	1.5	95	8	97	2	91	20
Paclobutrazol	0.01	92	19	88	3	82	10
Permethrin	2.5	53	40	28	24	28	35
Phenothrin	0.25	42	40	33	10	24	0
Piperonyl butoxide	1.25	77	7	53	8	52	8
Pirimicarb	0.01	86	10	86	4	83	9
Propoxur	0.01	91	6	89	3	86	11
Pyraclostrobin	0.01	63	23	77	6	70	6
Pyrethrins I and II	0.025	37	40	54	6	47	8
Pyridaben	0.025	27	68	40	13	32	4
Quintozene	0.5	55	11	28	12	28	15
Resmethrin	0.05	38	20	28	9	20	0
Spinetoram J and L	0.01	66	38	83	5	74	7
Spinosad A and D	0.01	72	26	85	4	79	9
Spirodiclofen	0.01	31	66	53	8	42	5
Spiromesifen[a]	1.5	20	112	66	17	61	30
Spirotetramat	0.01	91	6	92	5	86	10
Tebuconazole	0.01	81	9	84	5	77	6
Tebufenozide	0.01	75	17	90	8	81	14
Teflubenzuron	0.025	58	24	70	9	63	2
Tetrachlorvinphos	0.01	73	16	83	6	76	7
Tetramethrin	0.05	45	35	65	5	60	6
Thiacloprid	0.01	85	9	88	3	82	9
Thiamethoxam	0.01	85	8	86	2	82	10
Thiophanate-methyl	0.025	45	12	35	13	30	14
Trifloxystrobin	0.01	70	21	85	6	77	6

[a] Compound did not pass validation and was thus monitored qualitatively.

Table 7. Pesticides detected in cannabis products from Canadian licensed producers between March 2017 and January 2018

Matrix	Samples analyzed	Samples with pesticides detected	Pesticides detected	Pesticide detection frequency	Pesticide concn range, µg/g
Cannabis leaves	45	9	Myclobutanil	7	0.01–0.03
			Pyraclostrobin	1	0.01
			Boscalid	1	0.01
			Piperonyl butoxide	1	0.8
Dried cannabis flowers	63	8	Myclobutanil	8	0.03–20
			Bifenazate	7	0.03–6
Cannabis oil	36	9	Myclobutanil	5	0.01–6
			Boscalid	3	0.02–1
			Bifenazate	2	0.02–2
			Fludioxonil	2	0.01–0.02
			Fluopyram	1	0.02
			Tebuconazole	1	0.01

Of the 26 samples that contained pesticides, myclobutanil was detected in 20 samples. The median concentration of myclobutanil by matrix was found to be 0.017, 0.1, and 0.023 µg/g for cannabis leaves, dried cannabis flowers, and cannabis oil, respectively. Bifenazate was the next most common pesticide detected. It was found in 9 of the 26 samples. The other pesticides detected were pyraclostrobin, fludioxonil, fluopyram, tebuconazole, piperonyl butoxide, and bocsalid, present in 1–3 samples each.

The application of the methods described above was essential for the quantification of pesticide residues in cannabis leaves, dried cannabis flowers, and cannabis oil and meet the criteria listed in the "Requirements for Mandatory Testing of Pesticide Active Ingredients in Cannabis Products" (16).

Conclusions

Robust methods of analysis for the quantification of pesticides in cannabis were developed using a modified QuEChERS sample extraction with an EMR-Lipid cleanup step. The methods were successfully validated for 66 pesticides in cannabis leaves, 55 in dried cannabis flowers, and 79 in cannabis oil using HPLC-MS/MS, GC-MS/MS, and GC-MS. These methods were successfully applied to compliance and enforcement samples in Canada, as defined in the "Requirements for Mandatory Testing of Pesticide Active Ingredients in Cannabis Products" (16).

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