Analysis of Pesticides in a Cannabis sativa Matrix

INTRODUCTION

Cannabis sativa is well-known for its use as a recreational drug due the presence of the psychoactive compound tetrahydrocannabinol (THC), and has been listed as a Schedule 1 drug in the United States since the passage of the Controlled Substances Act of 1970.[1] With the recent surge in legalization of recreational and medicinal use, there is a need for reliable analytical tools to meet the regulatory requirements for pesticide testing in Cannabis. Action limits for each pesticide vary between jurisdictions, but can be as low as 10 ppb.[2,3]

The JEOL JMS-TQ4000GC triple-quadrupole GC-MS/MS system offers high speed and high sensitivity for quantitation of trace or residual pesticides. The TQ4000 combines a unique short collision cell with JEOL's patented ion accumulation and timed detection technology to provide high sensitivity and selectivity, as well as the fastest selected reaction monitoring (SRM) switching speed available (up to 1000 transitions per second). JEOL msPrimo and Escrime software provide all of the tools needed to develop optimized methods for target compound quantitation and report generation. In this application note, we describe a sensitive method for analyzing pesticides in *Cannabis sativa* matrix using the SRM capabilities of our triple quadrupole system.

EXPERIMENTAL

Dried *Cannabis sativa* flower buds for recreational use were purchased from a local dispensary. Approximately 1 gram of flower was extracted into 10 mL of 90:10 acetonitrile:dimethylacetamide by sonication for 15 minutes. The extract was centrifuged at approximately 2500 rpm for 10 minutes, followed by 10X dilution. One mL of the diluted extract was put through a dSPE cleanup step using Restek Q-sep QuEChERS dSPE Tubes (AOAC 2007.01 method[4], PN# 26125) and following the provided dSPE instructions. The supernatant was used as the matrix for each sample. Each spiked sample was created by adding 10 µL of prepared pesticide standard to 90 µL of the matrix. Samples were analyzed on the JMS-TQ4000GC using the parameters and SRM channels outlined in Tables 1 - 3 below. Optimal product- and precursor-ion pairs and optimized collision energies for each pesticide were determined using built-in SRM optimization tools. Each sample was run in triplicate with the exception of the 1 ppb samples for which 8 replicates were done to calculate the instrument detection limit (IDL) where possible.

RESULTS

Figure 1 shows the total ion current chromatogram (TICC) with labeled peaks, and Figure 2 shows several selected SRM chromatograms. Table 4 lists the data acquired for 46 pesticides analyzed by GC-MS/MS analysis. There were 41 pesticides observed at 1 ppb or less, which translates to 10 ppb on the plant. The IDL and %CV were not calculated for samples that could not be observed at 1 ppb. For samples with isomers (e.g., chlordane), the best performing isomer was used for reporting. All samples below showed good linearity, even up to 100 ppb. Example calibration curves are shown in Figure 3. Although some matrix effects were observed, system performance was generally good with very few pesticides affected by matrix interference.

CONCLUSIONS

The JMS-TQ4000GC is an excellent platform for fast, sensitive analysis of a wide range of pesticides in *Cannabis* matrix. Using built-in SRM optimization tools, optimal ion transitions and collision energies for each pesticide were determined in the presence of the matrix. The SRM method provided high sensitivity and selectivity, and reduced matrix effects without a complicated extraction method. Forty-one pesticides were observed at one ppb or lower with good linearity, which translates to ten ppb on the flower and is sufficient to meet the action limits of jurisdictions of interest.

REFERENCES

(1) United States Drug Enforcement Administration. The Controlled Substances Act https://www.dea.gov/controlled-substances-act (accessed Mar 19, 2020).
(2) Health Canada. Mandatory cannabis testing for pesticide active ingredients - List and limits https://www.canada.ca/en/public-health/services/publications/drugs-health-products/cannabis-testing-pesticide-list-limits.html (accessed Mar 19, 2020).
(3) Dodson, L.; Laprade, N. M. *The Natalie M. Laprade Maryland Medical Cannabis Commission's (MMCC) Technical Authority for Medical Cannabis Testing*; 2019.
(4) Official Methods of Analysis. Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Association of Official Agricultural Chemists: 2007.01.

Table 1. Gas Chromatograph parameters.

GC	7890B (Agilent)
Column	ZB-5MSPlus, 30.0 m, 0.25 mm i.d., 0.25 µm (Phenomenex, Cat#:7HG-G030-11)
Inlet liner	Zebron Plus 4 mm Single Taper w/Wool on bottom (Phenomenex Cat#: AG2-0A11-05)
Inlet Temp.	260 °C
Carrier Gas Type, Flow	He, 1.000 mL/min constant flow
Mode	Pulsed Splitless
Pulsed Pressure, Time	206.84 kPa, 0.550 min
Purge Flow	30 mL/min, 1.0 min
Septum Purge Flow	3.0 mL/min
Saver flow, Time	15 mL/min, 5.0 min
Injection Volume	1.0 µL
Oven Program	80 °C (0.75 min) → 35 °C/min → 190 °C → 5 °C/min → 240 °C → 20 °C/min → 300 °C (6 min)

Table 2. Mass Spectrometer parameters.

JMS-TQ4000GC	
Ion Source Temp.	250 °C
Interface Temp.	300 °C
Ionization Mode	EI+, 70 eV, 100 µA
Measurement Mode	SRM, High Sensitivity
Target Cycle Time	Approx. 330 ms
Acquisition Rate	2.778 Hz
Channel Time	20 – 100 ms
Relative EM Voltage	900 V
Collision Gas	N_2, 10%

Table 3. SRM channel data.

Compound	Quantitative ion		Referenced ion 1		Referenced ion 2		Collision Energy		
	Precursor ion m/z	Product ion m/z	Precursor ion m/z	Product ion m/z	Precursor ion m/z	Product ion m/z	Quantitative ion	Referenced ion 1	Referenced ion 2
Acephate	136	94	136	42	77	51	10	15	15
Azoxystrobin	344	156	388	345	388	360	30	20	10
Bifenazate	258	196	258	199	300	196	15	10	25
Bifenthrin	181	165	181	166	181	164	30	20	30
Boscalid	140	112	140	76	342	140	10	25	20
Carbaril (decomp)	144	115	144	116	89	63	25	15	15
Carbaril (intact)	144	115	144	116	89	63	25	15	15
Carbofuran	164	149	164	103	149	103	15	25	20
Chlordane (cis)	375	266	373	266	373	264	20	25	25
Chlordane (trans)	373	266	373	264	375	266	25	20	20
Chlorfenapyr	59	31	247	227	59	41	5	15	5
Chlorpyrifos	197	169	199	171	197	134	15	15	25
Chlorpyrifos-d10	200	172	260	167	260	139	20	25	30
Cinerin I	150	108	123	79	123	81	10	20	10
Cinerin II	107	91	121	93	121	77	10	5	25
Clofentezine	137	102	137	75	139	102	10	25	15
Cyfluthrin I	226	206	206	151	206	150	15	25	25
Cyfluthrin II	226	206	163	127	163	91	20	10	15
Cyfluthrin III	226	206	163	127	163	91	20	10	15
Cyfluthrin IV	226	206	163	127	163	91	15	10	15
Cypermethrin I	163	127	181	152	163	91	10	25	20
Cypermethrin II	163	127	181	152	163	91	10	25	15
Cypermethrin III	163	127	181	152	163	91	10	25	15
Cypermethrin IV	163	127	181	152	163	91	10	25	15
Diazinone	137	84	199	135	199	93	15	10	15
Dichlorvos	109	79	185	93	79	47	10	15	10
Dimethoate	93	63	87	42	87	46	10	10	20
Ethoprophos	158	97	158	114	97	79	15	10	20
Etofenprox	163	107	163	135	135	107	20	10	10
Etoxazole	141	113	300	270	204	176	15	30	10
Fenoxycarb	116	88	186	157	186	158	10	15	10
Fipronil	213	143	367	213	213	178	25	30	20

Compound	Quantitative ion		Referenced ion 1		Referenced ion 2		Collision Energy		
	Precursor ion m/z	Product ion m/z	Precursor ion m/z	Product ion m/z	Precursor ion m/z	Product ion m/z	Quantitative ion	Referenced ion 1	Referenced ion 2
Fludioxonil	248	127	248	154	248	182	30	25	20
Imazalil	173	145	215	173	173	109	20	10	25
Jasmolin I	164	109	123	79	123	81	10	20	10
Jasmolin II	121	93	121	77	121	91	10	20	20
Kresoxim-methyl	116	89	206	116	206	131	20	10	10
Malathion	127	99	93	63	125	79	10	10	15
Metalaxyl	206	132	132	117	206	105	20	15	20
Methiocarb	168	153	168	109	153	109	10	15	10
Methomyl	105	88	58	31	105	58	5	5	10
Methyl parathion	263	109	125	79	125	47	15	10	15
MGK 264 I	164	93	164	121	164	77	15	10	30
MGK 264 II	164	67	164	80	164	98	10	25	15
Myclobutanil	179	125	150	123	179	90	20	20	30
Naled	145	109	185	93	145	113	10	15	20
Oxamyl	98	58	98	69	72	56	10	5	10
Paclobutrazol	236	125	125	89	236	132	20	25	20
Permethrin (cis)	183	153	183	168	183	165	20	20	20
Permethrin (trans)	183	153	183	168	163	91	20	20	15
Phosmet	160	133	160	105	160	77	15	20	20
Piperonyl butoxide	176	117	176	103	176	131	20	25	15
Prallethrin	123	81	123	79	105	77	10	20	15
Propiconazole I	173	109	173	145	259	191	25	15	10
Propiconazole II	173	109	173	145	259	191	25	15	10
Propoxur	110	63	152	110	110	64	25	10	20
Pyrethrin II	133	105	91	65	107	91	10	15	10
Pyridaben	147	117	147	105	147	132	20	10	15
Spiromesifen	272	254	272	209	272	226	5	15	10
Spiroxamine I	100	72	100	58	100	41	10	10	20
Spiroxamine II	100	72	100	58	100	41	10	10	20
Tebuconazole	250	125	125	89	125	99	25	20	20
Thiamethoxam	132	71	212	139	212	182	10	15	5
Trifloxystrobin	116	89	172	145	131	89	20	20	25

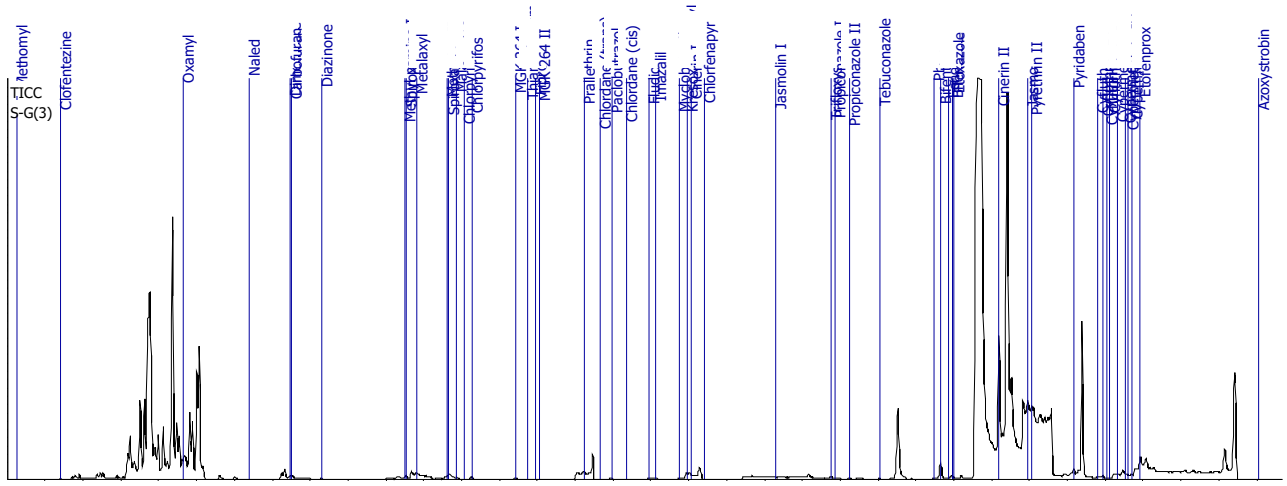

Figure 1. TIC chromatogram.

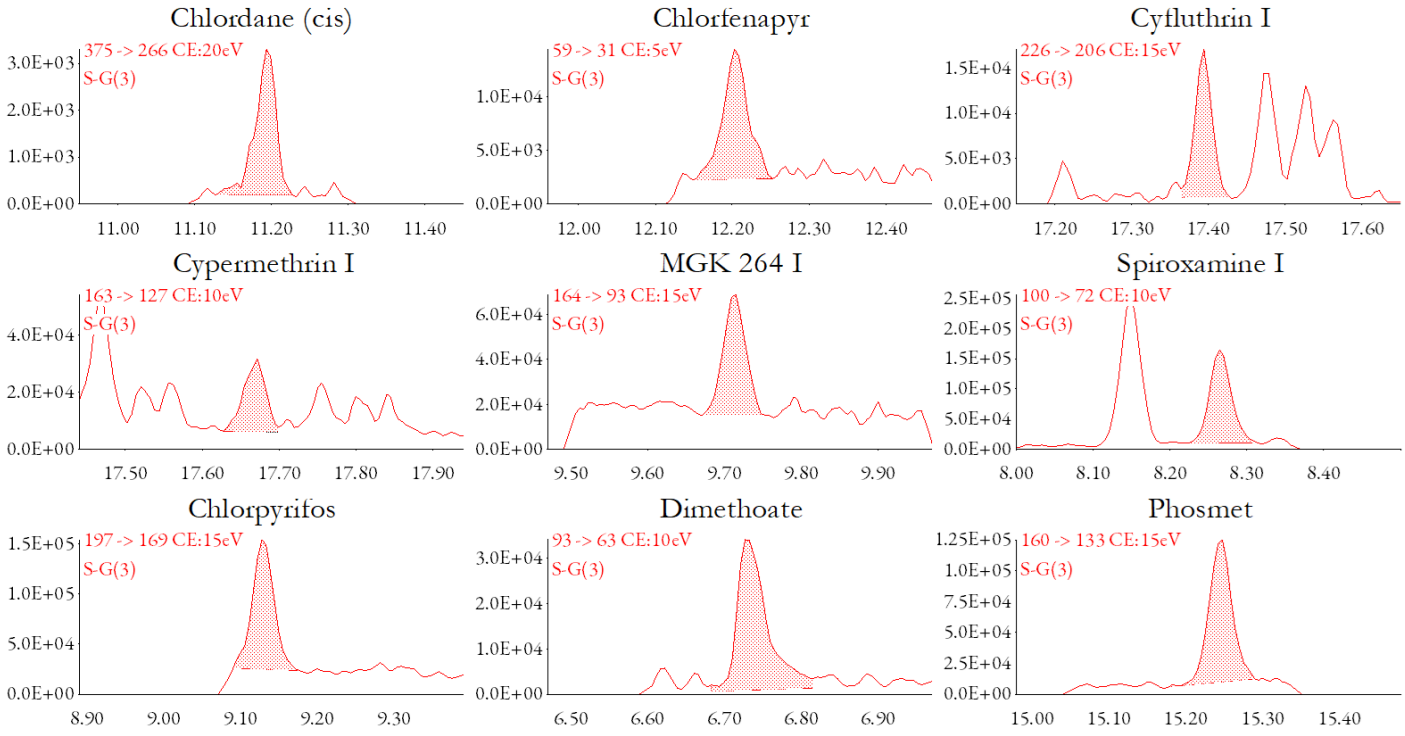

Figure 2. Selected SRM chromatograms.

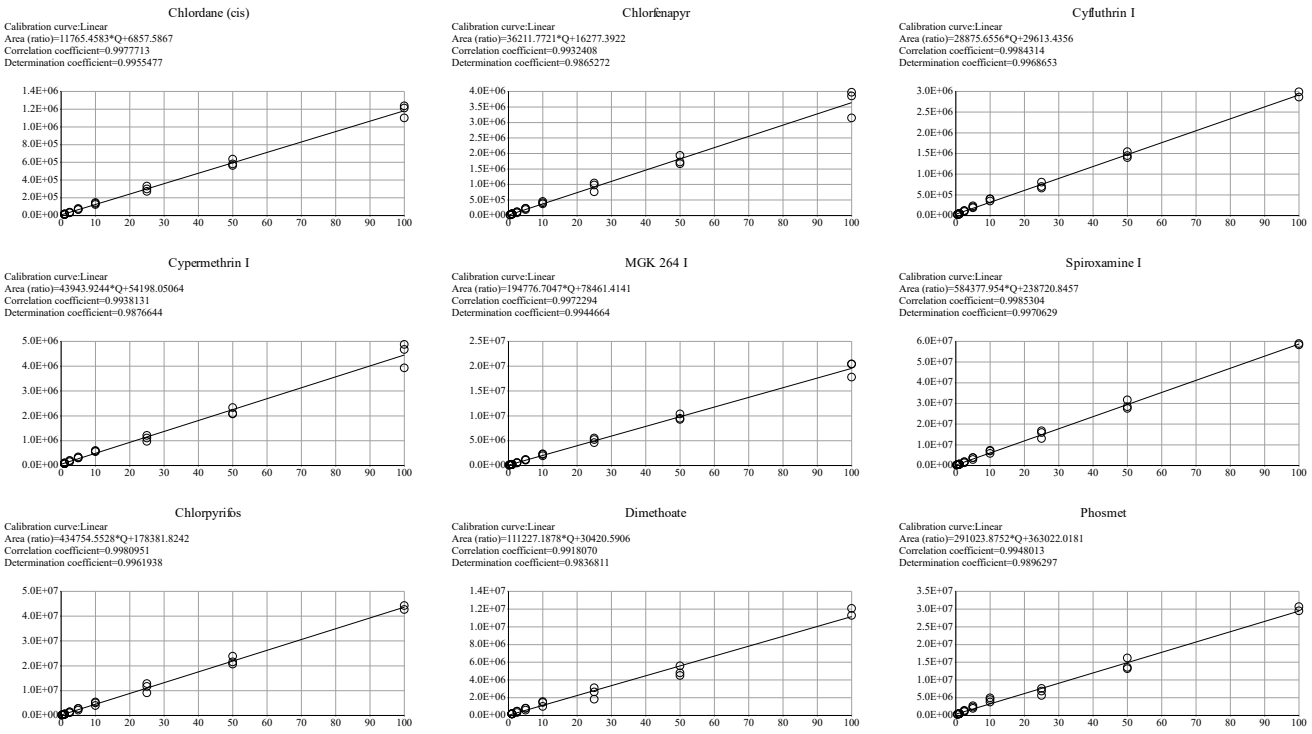

Figure 3. Selected calibrations curves.

Table 4. Performance data for tested pesticides.

Compound	Range (ppb)	Linearity (R^2)	% CV	LOQ (ppb)	IDL (ppb)
Azoxystrobin	0.5 - 100	0.9809	12.50	1	0.37
Bifenazate	0.5 - 100	0.9815	12.18	1	0.37
Bifenthrin	0.25 - 100	0.9928	8.37	0.5	0.25
Boscalid	0.25 - 100	0.9902	4.77	0.5	0.14
Carbaril	0.5 - 100	0.9952	8.10	10	0.24
Carbofuran	0.5 - 100	0.9953	8.57	0.5	0.26
Chlordane	1 - 100	0.9974	18.13	1	0.54
Chlorfenapyr	0.5 - 100	0.9932	13.87	1	0.42
Chlorpyrifos	0.25 - 100	0.9981	8.72	0.5	0.26
Chlorpyrifos-d10	1 - 100	0.9967	16.81	2.5	0.5
Cinerin I	25 - 100	0.9844	N/A	25	N/A
Clofentezine	0.5 - 100	0.9992	6.46	1	0.19
Cyfluthrin	0.5 - 100	0.9963	6.76	0.5	0.2
Cypermethrin	1 - 100	0.9954	6.68	2.5	0.2
Diazinone	0.25 - 100	0.9978	9.22	0.5	0.28
Dichlorvos	0.25 - 100	0.9929	9.28	0.5	0.28
Dimethoate	1 - 100	0.9918	8.10	2.5	0.24

Compound	Range (ppb)	Linearity (R^2)	% CV	LOQ (ppb)	IDL (ppb)
Ethoprophos	0.25 - 100	0.9921	8.77	1	0.26
Etofenprox	0.25 - 100	0.9901	5.18	2.5	0.16
Etoxazole	0.5 - 100	0.9957	7.74	0.5	0.23
Fenoxycarb	0.5 - 100	0.9944	10.05	5	0.3
Fipronil	0.5 - 100	0.9929	10.20	1	0.31
Fludioxonil	0.25 - 100	0.9936	10.46	0.5	0.31
Imazalil	1 - 100	0.9787	20.72	1	0.62
Jasmolin I	2.5 - 100	0.9937	N/A	25	N/A
Kresoxim-methyl	0.5 - 100	0.9975	9.49	0.5	0.28
Malathion	0.25 - 100	0.9961	8.78	2.5	0.26
Metalaxyl	0.5 - 100	0.9975	7.90	2.5	0.24
Methiocarb	1 - 100	0.9957	12.35	1	0.37
Methomyl	0.25 - 100	0.9763	24.74	0.5	0.74
Methyl parathion	0.5 - 100	0.9948	10.82	2.5	0.32
MGK 264	0.5 - 100	0.9972	5.21	0.5	0.16
Myclobutanil	0.25 - 100	0.9923	9.30	0.5	0.28
Naled	25 - 100	N/A	N/A	50	N/A
Paclobutrazol	0.25 - 100	0.9953	14.15	0.5	0.42
Permethrin	2.5 - 100	0.9946	N/A	2.5	N/A
Phosmet	0.5 - 100	0.9948	8.49	0.5	0.25
Prallethrin	10 -100	0.9952	N/A	25	N/A
Propiconazole	0.5 - 100	0.9944	8.70	0.5	0.26
Propoxur	0.25 - 100	0.9955	9.67	0.5	0.29
Pyridaben	0.5 - 100	0.9952	6.66	1	0.2
Spiromesifen	0.25 - 100	0.9934	4.98	0.5	0.15
Spiroxamine	0.25 - 100	0.9985	7.07	2.5	0.21
Tebuconazole	0.5 - 100	0.9934	9.93	0.5	0.3
Thiamethoxam	1 - 100	0.9951	9.59	1	0.29
Trifloxystrobin	0.5 - 100	0.9972	6.59	1	0.2