Measurement of Pesticides in *Cannabis sativa* and Hemp Matrices Using a Gas Chromatograph-Triple Quadrupole Mass Spectrometer

Kirk R. Jensen, A. John Dane, Robert B. Cody, and Koji Okuda*
**Corresponding author: kjensen@jeol.com*
JEOL USA, Inc, Peabody, MA

An increase in legalisation of *Cannabis sativa* for recreational use and the advent of federal guidelines on hemp has created a need for sensitive analytical tools for pesticide measurement. The JEOL JMS-TQ4000GC triple-quadrupole mass spectrometer contains a unique short collision cell that provides sensitive and selective analysis of trace pesticides. To test the capabilities of the instrument for analysing pesticides in *Cannabis* and hemp matrices, dried flower buds from *Cannabis sativa* and hemp plants were extracted, spiked with pesticides, and then measured using selected reaction monitoring. Out of the 51 pesticides spiked into the samples, at least 45 were detected at 100 ppb or less and at least 35 of those at 1 ppb or less.

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]. Hemp is a strain of *Cannabis sativa* that has multiple industrial uses including paper, plastics, woven goods, and even food. Hemp strains are defined by the US federal government as those that contain less than 0.3% THC [2]. Additionally, hemp strains typically contain more cannabidiol (CBD) [3], which was recently approved by the US Food and Drug Administration (FDA) to treat certain types of epilepsy [4], and is currently being investigated as a medical treatment for other afflictions. With the recent surge in legalisation of recreational and medicinal use, and the advent of federal guidelines on the definition of hemp, there is a need for reliable analytical tools to meet the regulatory requirements for pesticide testing in *Cannabis sativa*. The US Environmental Protection Agency (EPA) sets tolerance limits for residual pesticides, and the FDA is responsible for enforcing those tolerances in agricultural products. Many pesticide limits are in the low ppb level, but the acceptable limits are compound dependent. A good example is permethrin in spinach, which has 20 ppm detection limits due to its low toxicity [5]. Because THC is still listed as a Schedule 1 drug, the FDA has not needed to set any pesticide requirements. As such, any limits have been left for individual jurisdictions (typically US state) to decide on which pesticides to regulate for *Cannabis* and to what level. Action limits for each pesticide vary between jurisdictions, but can be as low as 10 ppb [6,7,8].

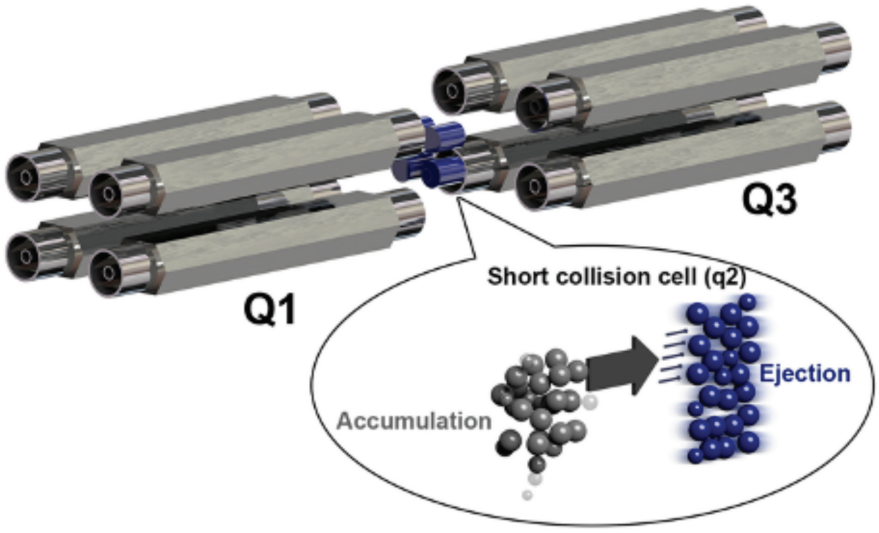

Figure 1: A diagram of the JMS-TQ4000GC triple quadrupole with its unique short collision cell.

Current methods for pesticide analysis in *Cannabis* are entirely based on LC-MS/MS and GC-MS/MS methods, with neither technology able to detect the full range of pesticides at regulated levels. The sample preparation methods and specific chromatography-MS methods vary from laboratory to laboratory and state to state depending on local regulatory requirements, though there does seem to be a general trend of using QuEChERS or QuEChERS-like methods (e.g., extraction into acetonitrile)

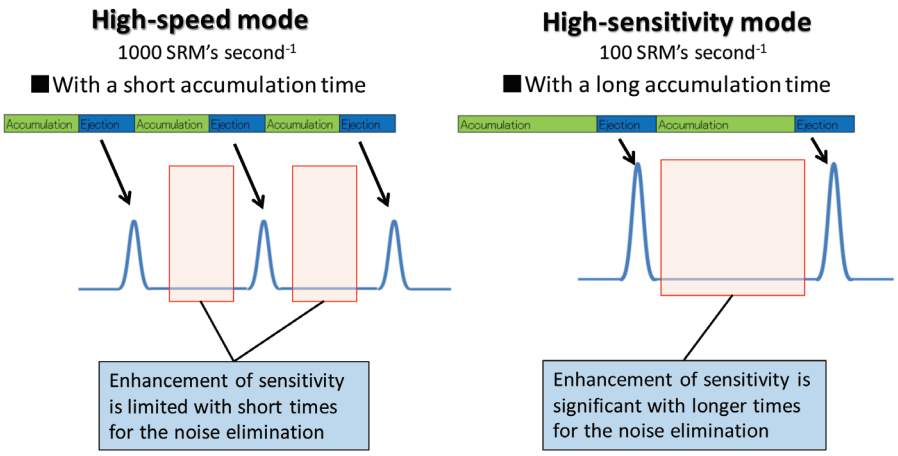

Figure 2: Diagram showing how different ion accumulation times affect sensitivity in high-speed and high-sensitivity modes.

and/or solid phase extraction (SPE) techniques. The AOAC Official Method of Analysis 2007.01 has also been adopted by some laboratories. No specific LC-MS/MS or GC-MS/MS methods have been adopted by government regulatory agencies for *Cannabis* testing, however, the FDA has guidelines for both methods in the Pesticide Analytical Manual for testing other agricultural products [9]. Other reports also mention that both GC-MS and LC-MS are required to analyse a full range of pesticides [7].

The JEOL JMS-TQ4000GC triple-quadrupole gas chromatograph-tandem mass spectrometer (GC-MS/MS) system offers high speed and high sensitivity for quantitation of trace or residual pesticides. The JMS-TQ4000GC combines a unique short collision cell with JEOL's patented ion accumulation and timed detection technology to provide high sensitivity and selectivity (Figure 1), as well as the fastest selected reaction monitoring (SRM) switching speed available (up to 1000 transitions per second). The short collision cell minimises the time that ions reside in q2, making it possible to carry out more SRM's in a given timeframe (high-speed mode, Figure 2), with a maximum switching rate of 1000 SRMs per second. Ion accumulation in q2 combined with rapid ejection reduces interference ions and minimises ion loss when switching precursor/product ion pairs, thus increasing sensitivity. After the fragment ion packet is ejected from q2, the offset of Q3 is adjusted so that all product ions transit through Q3 to the detector with the same timing, independent of *m/z*. Detection is only turned on as the ion packet reaches the detector, further increasing sensitivity. To maximise sensitivity at the cost of SRMs/s (high-sensitivity mode, Figure 2), the accumulation time in q2 can be increased, which results in more analyte ions for detection. Additionally, longer accumulation time also results in longer intervals where the detector is turned off, which results in even less noise and fewer interfering ions, even further increasing sensitivity. JEOL msPrimo and Escrime software provide all of the tools needed to develop optimised methods for target compound measurement and quantitation. Here, we describe a sensitive method for analysing pesticides in *Cannabis sativa* and hemp matrices using the SRM capabilities of our triple quadrupole system. For clarity in this text, samples of the high-THC strain of *Cannabis sativa* will be referred to as *Cannabis* samples, and hemp strains as hemp samples, even though they are the same genus and species of plant.

Table 1: Gas chromatograph and mass spectrometric measurement conditions.

Agilent 7890B GC		JMS-TQ4000GC MS	
Column	ZB-5MSPlus	Ion Source Temp.	250°C
	30.0 m, 0.25 mm i.d., 0.25 µm	Interface Temp.	300°C
	(Phenomenex, Torrence, CA)	Ionisation Mode	EI+, 70 eV, 100 µA
Inlet liner	4 mm Single Taper	Measurement Mode	p.d. SRM, High Sensitivity
	w/Wool on bottom	Target Cycle Time	Approx. 330 ms
	(Phenomenex, Torrence, CA)	Channel Time	20 – 100 ms
Inlet Temp.	260°C	Relative EM Voltage	900 V
Carrier Gas	He, 1.000 mL/min	Collision Gas	N_2, 10%
Mode	Pulsed Splitless	**Oven Program**	
Pulsed Press., Time	206.84 kPa, 0.550 min	80 °C (0.75 min) →	
Purge Flow	30 mL/min, 1.0 min	35 °C/min →	190°C →
Septum Purge Flow	3.0 mL/min	5 °C/min →	240°C →
Injection Volume	1.0 µL	20 °C/min →	300°C (6 min)

Experimental

Oregon pesticide standard mixtures 1-6 were purchased from Restek (PNs 32586 - 32591), as well as a chlordane standard (Restek, PN# 32021) and chlorpyrifos-d10 (Cambridge Isotope Labs, PN# DLM-4360-1.2). Fifty-one of these standards were found to be suitable for GC/MS analysis, and were the focus of this study. A single standard combining the 51 pesticides listed in Table 2 was prepared for spiking purposes.

Dried *Cannabis sativa* flower buds for recreational use were purchased from a local dispensary, and dried hemp flower buds were provided by a collaborator. Approximately 1 gram of flower was extracted into 10 mL of 90:10 acetonitrile:dimethylacetamide for *Cannabis*, and into 10 mL of pure acetonitrile for hemp. Mixtures were sonicated for 15 minutes, centrifuged at approximately 2500 rpm for 10 minutes, and then diluted 1:10 with acetonitrile. One mL of the diluted extract was put through a dSPE cleanup step using Restek Q-sep QuEChERS dSPE Tubes (AOAC 2007.01 method [10], PN# 26125) and following the dSPE instructions provided with the kit. The final 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 in the following

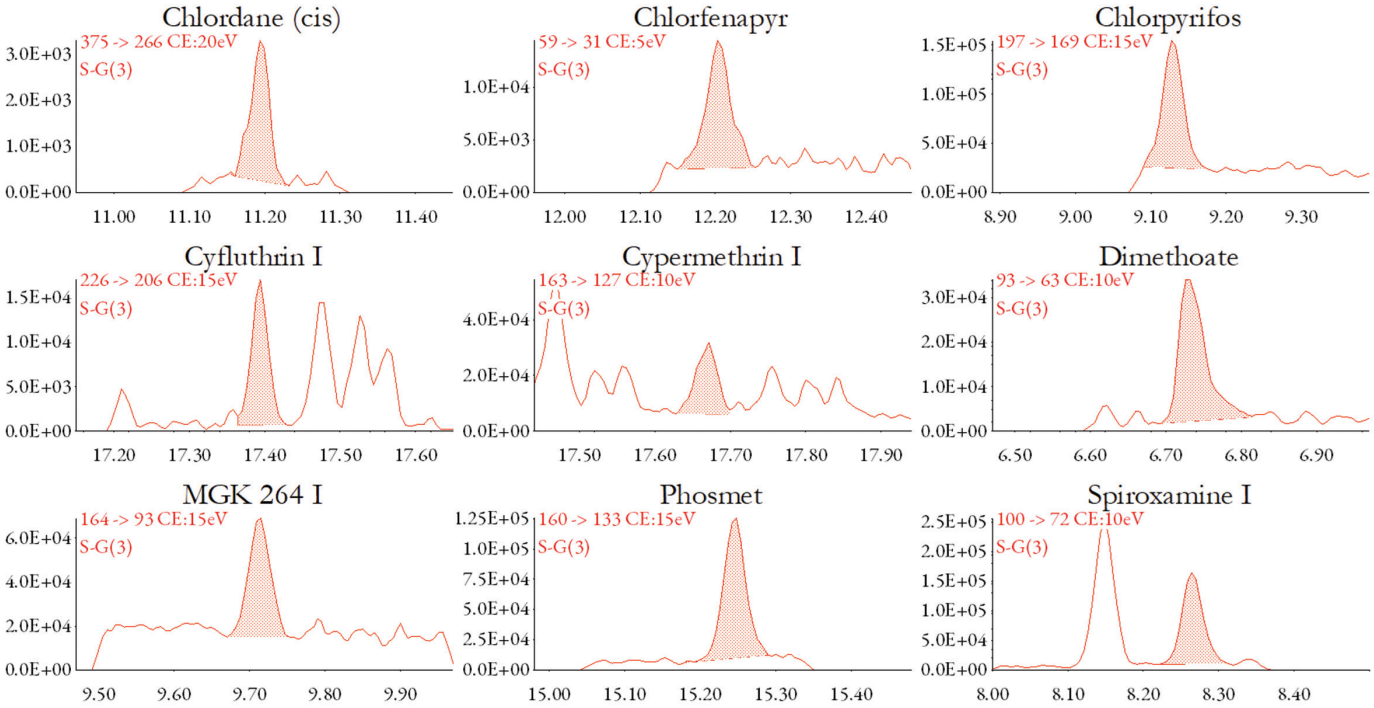

Figure 3: SRM chromatograms for 1 ppb pesticide concentration in Cannabis matrix. Shaded areas denote area calculation for the indicated pesticide.

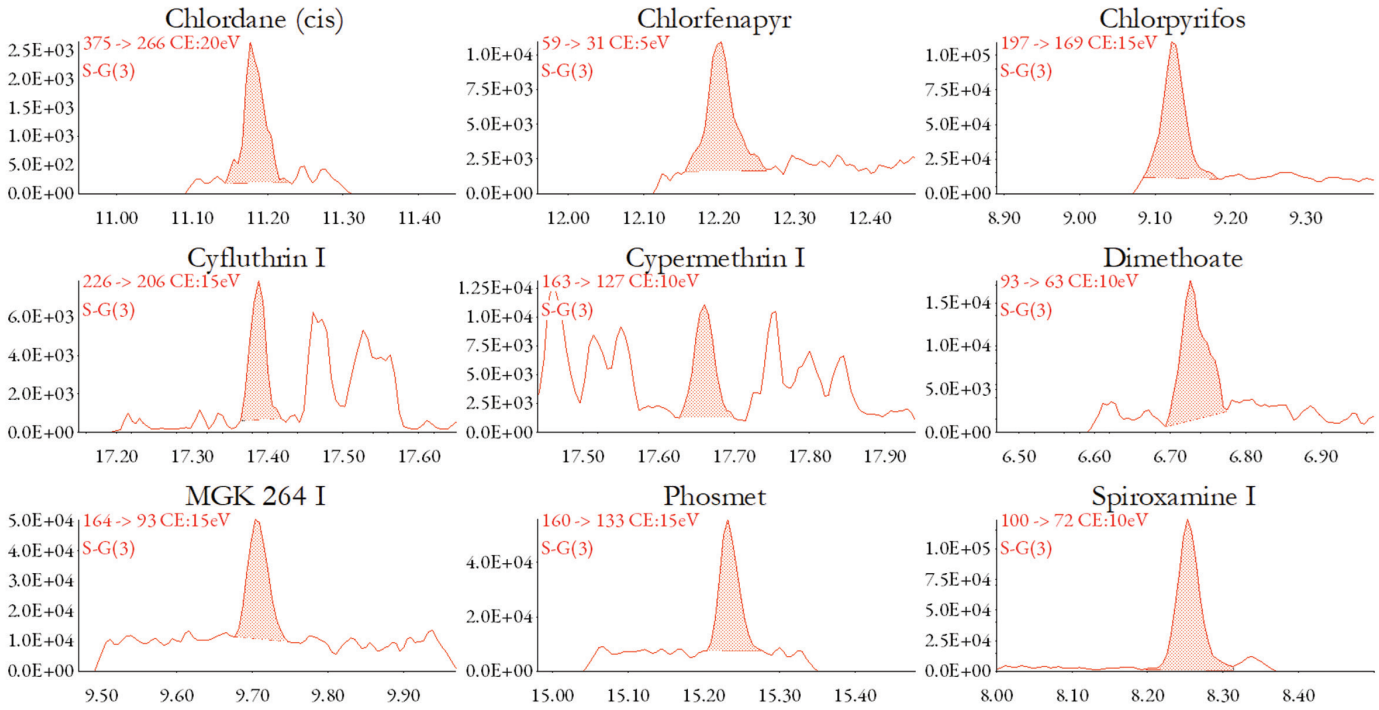

Figure 4: SRM chromatograms for 1 ppb pesticide concentration in hemp matrix. Shaded areas denote area calculation for the indicated pesticide.

concentrations (ppb): 0.10, 0.25, 0.50, 1.0, 2.5, 5.0, 10, 25, 50, and 100. Samples were analysed on the JMS-TQ4000GC using the parameters outlined in Table 1. All 51 pesticides were monitored during a sample run using peak-dependent SRM (p.d. SRM), where individual SRM channel time is determined by the elution time of the analyte into the mass analyser. Optimal product- and precursor-ion pairs and optimised collision energies for each pesticide were determined using built-in SRM optimisation tools and then adjusted for retention time shift by analysing a standard solution in *Cannabis* matrix. Each sample was run in triplicate with the exception of the 1 ppb samples, where 8 replicates were done to calculate the instrument detection limit (IDL) and coefficient of variation (%CV) where possible. In the cases of pesticides with more than one isomer (e.g., cypermethrin), the best performing isomer was reported.

Results and Discussion

Figures 3 and 4 show a few example SRM chromatograms for 1 ppb pesticides in *Cannabis* and hemp matrices, respectively. Of the 51 pesticides monitored, 46 and 45 were detected at 100 pbb or less for *Cannabis* and hemp matrices, respectively. Of the detected compounds, 36 were observed at 1 ppb or less in the *Cannabis* matrix, and 35 for hemp matrix. This is important because 1 ppb corresponds

Table 2: Performance data calculated for pesticides in Cannabis and hemp matrices. Pesticides that could not be detected at all are denoted with a X. Those that could not be detected at 1 ppb have N/A for values that could not be calculated.

	Cannabis				Hemp			
Compound	Linearity (R^2)	CV (%)	LOQ (ppb)	IDL (ppb)	Linearity (R^2)	CV (%)	LOQ (ppb)	IDL (ppb)
Acephate		X				X		
Acetamiprid		X				X		
Azoxystrobin	0.9809	12.50	1.0	0.37	0.9886	N/A	2.5	N/A
Bifenazate	0.9815	12.18	1.0	0.37	0.9943	17.23	2.5	0.52
Bifenthrin	0.9928	8.37	0.5	0.25	0.9928	3.36	0.5	0.10
Boscalid	0.9902	4.77	0.5	0.14	0.9926	8.65	0.5	0.26
Carbaril	0.9952	8.10	10	0.24	0.9893	N/A	25	N/A
Carbofuran	0.9953	8.57	0.5	0.26	0.9902	12.52	2.5	0.38
Chlordane	0.9974	18.13	1.0	0.54	0.9975	11.73	1.0	0.35
Chlorfenapyr	0.9932	13.87	1.0	0.42	0.9959	11.85	1.0	0.36
Chlorpyrifos	0.9981	8.72	0.5	0.26	0.9944	4.12	0.5	0.12
Chlorpyrifos-d10	0.9967	16.81	2.5	0.5	0.9944	N/A	2.5	N/A
Cinerin	0.9844	N/A	25	N/A	0.9749	N/A	25	N/A
Clofentezine	0.9992	6.46	1.0	0.19	0.9974	5.20	1.0	0.16
Cyfluthrin	0.9963	6.76	0.5	0.2	0.9808	5.87	5.0	0.18
Cypermethrin	0.9954	6.68	2.5	0.2	0.9933	9.93	10	0.30
Diazinone	0.9978	9.22	0.5	0.28	0.9954	6.98	0.5	0.21
Dichlorvos	0.9929	9.28	0.5	0.28	0.9964	7.40	0.5	0.22
Dimethoate	0.9918	8.10	2.5	0.24	0.9920	13.25	2.5	0.40
Ethoprophos	0.9921	8.77	1.0	0.26	0.9947	6.38	2.5	0.19
Etofenprox	0.9901	5.18	2.5	0.16	0.9961	6.60	2.5	0.20
Etoxazole	0.9957	7.74	0.5	0.23	0.9947	N/A	2.5	N/A
Fenoxycarb	0.9944	10.05	5.0	0.3	0.9947	5.77	10	0.17
Fipronil	0.9929	10.20	1.0	0.31	0.9966	10.11	1.0	0.30
Fludioxonil	0.9936	10.46	0.5	0.31	0.9935	9.75	0.5	0.29
Imazalil	0.9787	20.72	1.0	0.62		X		
Jasmolin	0.9937	N/A	25	N/A	0.9904	N/A	25	N/A
Kresoxim-methyl	0.9975	9.49	0.5	0.28	0.9958	9.13	0.5	0.27
Malathion	0.9961	8.78	2.5	0.26	0.9909	11.89	5.0	0.36
Metalaxyl	0.9975	7.90	2.5	0.24	0.9978	6.39	2.5	0.19
Methiocarb	0.9957	12.35	1.0	0.37	0.9925	15.18	2.5	0.46
Methomyl	0.9763	24.74	0.5	0.74	0.9895	N/A	50	N/A
Methyl parathion	0.9948	10.82	2.5	0.32	0.9960	12.10	5.0	0.36
MGK 264	0.9972	5.21	0.5	0.16	0.9983	7.06	0.5	0.21
Myclobutanil	0.9923	9.30	0.5	0.28	0.9954	11.00	0.5	0.33
Naled	N/A	N/A	50	N/A	0.9899	N/A	25	N/A
Oxamyl		X				X		
Paclobutrazol	0.9953	14.15	0.5	0.42	0.9942	9.09	0.5	0.27
Permethrin	0.9946	N/A	2.5	N/A	0.9957	16.45	2.5	0.49
Phosmet	0.9948	8.49	0.5	0.25	0.9914	13.01	1.0	0.39
Piperonyl butoxide		X				X		
Prallethrin	0.9952	N/A	25	N/A	0.9929	N/A	25	N/A
Propiconazole	0.9944	8.70	0.5	0.26	0.9948	12.41	1.0	0.39
Propoxur	0.9955	9.67	0.5	0.29	0.9893	7.76	0.5	0.23
Pyrethrin		X				X		
Pyridaben	0.9952	6.66	1.0	0.2	0.9922	5.22	0.5	0.16
Spiromesifen	0.9934	4.98	0.5	0.15	0.9857	8.88	1.0	0.27
Spiroxamine	0.9985	7.07	2.5	0.21	0.9955	5.03	1.0	0.15
Tebuconazole	0.9934	9.93	0.5	0.3	0.9951	9.67	1.0	0.29
Thiamethoxam	0.9951	9.59	1.0	0.29	0.9927	N/A	5.0	N/A
Trifloxystrobin	0.9972	6.59	1.0	0.2	0.9952	9.32	1.0	0.28

to 10 ppb on the flower, which is the action limit for some pesticides in some jurisdictions. *Cannabis* matrix seems to suppress ion signal slightly more than the hemp matrix, which can be observed by comparing cypermethrin I peak quality in Figures 3 and 4. Of the pesticides that were not detected, pyrethrin compounds, in particular, performed poorly, as well as acetamiprid and acephate (compounds that traditionally do well in LC-MS analysis). However, chlorinated compounds that are difficult to analyse by LC-MS, such as chlordane, cyfluthrin, and cypermethrin, were all detectable at 1 ppb or less, demonstrating that GC-MS and LC-MS could be complementary techniques for residual pesticide analysis. Additionally, compounds that have multiple isomers, such as cypermethrin and cyfluthrin, benefit from the short collision cell design, because even in high-sensitivity mode (less SRMs/s), the short ejection time and timed-ion detection still increase sensitivity by reducing noise and interfering ions.

Table 2 lists performance data calculated for each pesticide measured. For pesticides detected at 1 ppb, %CV was less than 10% for most compounds and less than 20% for all compounds except imazalil and methomyl in *Cannabis* matrix. All %CVs were less than 20% in the hemp matrix, which may be attributed to less overall ion signal suppression compared to *Cannabis* matrix. Calculated IDLs were less than 1 ppb for all compounds detected at 1 ppb or less in both matrices. Linearity for most compounds was excellent (R^2 >0.99) from the lowest detected concentration up to 100 ppb in both matrices. Only 5 compounds in *Cannabis* matrix and 11 compounds in hemp matrix had R^2 values of $0.97 < R^2 < 0.99$. No compounds had R^2 values less than 0.97. System performance was generally good, despite some ion suppression from the complex matrices. The SRM method was crucial in reducing interfering ions; however, a more robust cleanup method could vastly improve overall sensitivity.

Conclusions

The JMS-TQ4000GC is an excellent platform for fast, sensitive analysis of a wide range of pesticides in *Cannabis* and hemp matrices. The unique short collision cell, along with ion accumulation and timed ion detection technologies provide increased sensitivity and selectivity, especially when using the high-sensitivity mode. Using built-in SRM optimisation 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. For *Cannabis,* 41 pesticides were observed at one ppb or lower with good linearity, and likewise for 35 pesticides in hemp matrix. This translates to ten ppb on the flower and is sufficient to meet the action limits of some jurisdictions of interest. Even though good performance was observed, better sensitivity could be attained with a more robust cleanup method. A few pesticides that perform well in LC-MS could not be detected or detected only at high concentrations in this study. Conversely, chlorinated pesticides that are traditionally difficult to analyse with LC-MS were detected effectively at 1 ppb or less. These results suggest that GC-MS and LC-MS could be complementary techniques for a complete pesticide analysis platform.

References

1. United States Drug Enforcement Administration. The Controlled Substances Act https://www.dea.gov/controlled-substances-act (accessed Mar 19, 2020).
2. United States Department of Agriculture. Establishment of a Domestic Hemp Production Program; United States of America, 2019.
3. T.E. Swanson, Controlled Substances Chaos: The Department of Justice's New Policy Position on Marijuana and What It Means for Industrial Hemp Farming in North Dakota. North Dekota Law Rev. 90 (3) (2014) 599.
4. E. Stockings, D. Zagic, G. Campbell, M. Weier, W.D. Hall, S. Nielsen, G.K. Herkes, M. Farrell, L. Degenhardt, J. Neurol. Neurosurg. Psychiatry 89 (7) (2014) 741 LP. https://doi.org/10.1136/jnnp-2017-317168.
5. Tolerances and Exemptions for Pesticide Chemical Residues in Foods. Fed. Code Regul. (2014) Part 180 (Subpart C), §180.378.
6. 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).
7. L. Dodson, N.M. Laprade, The Natalie M. Laprade Maryland Medical Cannabis Commission's (MMCC) Technical Authority for Medical Cannabis Testing (2019).
8. Oregon Health Authority. Standards for Pesticides Compliance Testing; https://secure.sos.state.or.us/oard/viewSingleRule.action?ruleVrsnRsn=253953, (2019).
9. United States Food and Drug Administration. The Pesticide Analytical Manual (1999).
10. AOAC Official Methods of Analysis. Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Association of Official Agricultural Chemists: 2007.01.