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Quantitative Analysis of Residual Pesticides in Hemp Oil Extract by Direct Liquid Injection Gas Chromatography/ Mass Spectrometry

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Abstract

The number of cannabis containing products, such as extracts, tinctures, edibles, waxes and oils, available in the United States have increased significantly due to changes in state law and the 2018 Farm Bill. Cannabis concentrates are legally manufactured for both medicinal and recreational use and are quickly becoming the most commonly used products by consumers in comparison to the cannabis sativa flower. The concentrates containing cannabinoids and terpenes are typically extracted from plant material using a variety of solvents. The pesticides, antifungals and performance enhancement reagents that may have been applied to cannabis to increase crop yields may be present in the extracted material and are a concern for consumer safety. There is a need for a highly sensitive and selective analytical methodology to determine the amount of pesticides present in these concentrates to ensure safety and quality for consumers and reduce the risk of human exposure. This study describes the use of the GERSTEL MPS robotic with automated liquid option for the analysis of pesticide residues in hemp oil samples by direct liquid injection gas chromatography/mass spectrometry (GC/MS). This technique is sensitive, accurate and precise and allows the quantitation of pesticides of interest well below the established limits of quantification for the State of California.

Introduction

Currently, there are no federal regulations in the United States on the allowable concentration of pesticide residues present in cannabis or cannabis concentrates. The limits for each pesticide are defined by the individual state in which the cannabis is grown. The Category I residual pesticides as defined by the Bureau of Cannabis Control [1] for the State of California included in this study are shown below in Table 1. For the Category I pesticides, the testing laboratory is required to report whether any Category I pesticides are detected above the limit of detection and must establish a limit of quantification of 0.10 µg/g or lower for all Category I residual pesticides.

Table 1. State of California Category I pesticides tested in cannabis.

Category I Pesticide	CAS No.
DDVP (Dichlorvos)	62-73-7
Mevinphos	7786-34-7
Propoxur	114-26-1
Ethoprophos	13194-48-4
Dimethoate	60-51-5
Carbofuran	1563-66-2
Spiroxamine	118134-30-8
Methyl parathion	298-00-0
Spiroxamine	118134-30-8
Methiocarb	2032-65-7
Chlorpyrifos	2921-88-2
Fipronil	120068-37-3
Paclobutrazol	76738-62-0
Imazalil	35554-44-0
Chlorfenapyr	122453-73-0
Fenoxycarb	72490-01-8
Coumaphos	56-72-4
Etofenprox	80844-07-1

The Category II residual pesticides as defined by the Bureau of Cannabis Control [1] for the State of California are listed below in Table 2, along with the corresponding action levels for both inhaled and other cannabis goods. Only analytes amenable to analysis by gas chromatography-mass spectrometry were included in this study.

Table 2. State of California Category II pesticides tested in cannabis.

		Action Levels (µg/g)	
Category II Pesticide	CAS No.	Inhalable Cannabis Goods	Other Cannabis Goods
Captan	113-06-2	0.7	5
Pentachloro-nitrobenzene	82-68-8	0.1	0.2

The organohalide pesticides included in this study are listed in Table 3. Action levels for these pesticides are not yet defined by the Bureau of Cannabis Control for the State of California. However, these compounds are known for their high toxicity, slow degradation and bioaccumulation, and are a concern for consumer safety.

Table 3. Organohalid pesticides tested in cannabis.

Organohalid Pesticide	CAS No.
Hexachlorocyclopentadiene	77-47-4
Hexachlorobenzene	118-74-1
Lindane	58-89-9
Alachlor	15972-60-8
Heptachlor	76-44-8
Aldrin	309-00-2
Heptachlor epoxide (Isomer B)	1024-57-3
α-Chlordane	5103-71-9
γ-Chlordane	5103-74-2
cis-Nonachlor	5103-73-1
Endrin	72-20-8
Dieldrin	60-57-1
trans-Nonachlor	39765-80-5
Methoxychlor	72-43-5
Etofenprox	80844-07-1

The pyrethrins included in this study are listed in Table 4. Action levels for these pesticides are not yet defined by the Bureau of Cannabis Control for the State of California.

Table 4. Pyrethrins tested in cannabis.

Pyrethrin Isomer	CAS No.
Cinerin I	25402-06-6
Jasmolin I	4466-14-2
Pyrethrin I	121-21-1
Jasmolin II	1172-63-0

The GERSTEL MultiPurpose Sampler (MPS) robotic in combination with the Universal Syringe Module provides the user with a multitude of tools for sample introduction. This study describes the use of the GERSTEL MPS robotic for the direct liquid injection GC/MS technique for quantitative analysis of pesticide residues in hemp oil extract. The GC/MS system used was fitted with a GERSTEL Cooled Injection System (CIS 4) PTV-type GC inlet. The CIS enables highly controlled temperature programmed evaporation of the introduced liquid sample for virtually discrimination free analyte transfer to the GC column. The high quality results achieved show that this technique may be applied to cannabinoid containing products for evaluation of consumer safety. Many of these pesticides and their corresponding byproducts are highly toxic. The automation provided by the GERSTEL MPS robotic and the highly accurate and efficient analyte transfer through the CIS 4 under Maestro software

control enables this technique to be sensitive, accurate and precise. The use of the MSD in single ion monitoring (SIM) mode enables quantification of analytes at very low levels. Table 5 lists the SIM groups for the residual pesticides in hemp oil extract. Table 6 lists the SIM groups for pyrethrins in hemp oil extract.

Experimental

Instrumentation

Agilent 7890 GC / 5977B MSD, GERSTEL MPS robotic with Liquid Option and Cooled Injection System (CIS 4) GC Inlet

Table 5. SIM groups for residual pesticides.

Category I Pesticide	Quant Ion [m/z]	Qual Ion [m/z]	SIM Group Start [min]
DDVP (Dichlorvos)	109	185, 79	7.04
Hexachlorocyclopentadiene	237	239, 235	8.2
Mevinphos	127	109, 192	9.4
Propoxur	110	152, 81	11.7
Ethoprophos	158	97, 139	
Hexachlorobenzene	284	286, 282	12.8
Dimethoate	87	93, 125	13.1
Carbofuran	164	149, 122	
Simazine	201	186, 173	13.34
Atrazine	200	215, 173	
Pentachloronitrobenzene	237	295, 249	
Lindane	181	183, 219	
Spiroxamine I	100	126, 198	14.8
Alachlor	160	188, 45	
Methyl parathion	263	109, 125	
Heptachlor	272	100, 274	
Spiroxamine	100	126, 198	15.3
Methiocarb	168	153, 109	
Chlorpyrifos	197	199, 314	15.6
Aldrin	263	265, 66	
Fipronil	367	369, 213	16.3
Heptachlor epoxide	353	81, 355	
Captan	79	77, 149	
α-Chlordane	373	375, 377	16.9
Paclobutrazol	236	125, 167	
γ-Chlordane	373	375, 377	
cis-Nonachlor	409	408, 411	
Imazalil	215	173, 217	17.4
Dieldrin	79	81, 82	
Chlorfenapyr	59	137, 247	
Endrin	81	79, 263	18.1
trans-Nonachlor	409	408, 410	
Fenoxycarb	116	88, 186	19.9
Methoxychlor	227	228, 212	
Coumaphos	362	226, 109	21.8
Etofenprox	163	135, 107	

Table 6. SIM groups for residual pyrethrins.

Category I Pesticide	Quant Ion [m/z]	Qual Ion [m/z]	SIM Group Start [min]
Cinerin I	123	124, 150	24.75
Jasmolin I	123	214, 164	26.75
Pyrethrin I	167	124, 164	
Jasmolin II	167	93, 107	33.75

Analysis conditions

CIS: baffled liner
splitless mode
40°C; 12°C/s; 280°C (3 min)

Pneumatics: He, constant flow, 1 mL/min

Column: 30 m DB-5MS UI (Agilent)
d_i = 0.25 mm d_f = 0.25 µm

Oven: 80°C (1 min); 10°C/min; 310°C (5 min)
for pyrethrins:
80°C; 20°C/min; 150°C; 3°C/min; 300°C

Sample Preparation. Cold-pressed hemp oil was purchased at a local store. To generate the hemp oil QuEChERS [2] extract, liquid-liquid extraction was performed, and dispersive solid phase extraction (dSPE) was used for cleanup. A 1.5 mL aliquot of hemp oil was directly weighed into a 10 mL screw-cap vial and diluted with 1.5 mL hexane. A 6 mL aliquot of acetonitrile was added to the vial and agitated using the GERSTEL quickMix at 500 rpm for 30 minutes. The layers were allowed to separate for 10 minutes, and 1 mL aliquots of the top layer were transferred to 2 mL dispersive SPE vials containing 150 mg magnesium sulfate and 50 mg PSA (for Fatty samples, AOAC, Agilent #5982-5122). Each vial was vortexed for 60 seconds and centrifuged at 3,000 RPM for 5 minutes. The top layer in the dSPE vials were transferred into a 10 mL screw-cap vial and recombined.

Residual pesticide standards were spiked directly into hemp oil extract in acetonitrile for quantification. The standards were spiked into 2 mL vials containing the hemp oil extract, which were then capped. Eight-point calibration curves were generated with each level prepared in triplicate. Precision data was obtained from n=3 replicates at the median concentration level of each calibration curve. Residual pesticide standards were obtained from AccuStandard (California Category I Residual Pesticides, cat. no. CP-CA-01; Pentachloronitrobenzene, cat. no. AS-E0156; Organohalide Pesticides, cat. no. M-505R-2; Captan, cat. no. P-182S) and Restek (Pyrethrins standard, cat. no. 32578).

Sample Introduction. The 2 mL vials were placed in a VT-54 tray on the MPS robotic. One microliter of sample was introduced into the CIS 4 at 40°C in splitless mode. The CIS 4 was heated to a final temperature of 280°C at a rate of 12°C/s.

Results and Discussion

Table 7 lists the linearity and precision for all analytes included in this study. Excellent linearity and precision were observed for all compounds, with an average percent relative standard deviation (% RSD) of 1.73 % and an average correlation coefficient (r^2) value of 0.995.

Table 7. LODs and LOQs for Category I and II residual pesticides in hemp oil extract.

Compound	Correlation Coefficient	Precision (n=3)
DDVP	0.9971	1.9
Mevinphos	0.9937	5.2
Propoxur	0.9971	0.9
Ethoprop(hos)	0.9980	1.1
Dimethoate	0.9833	2.8
Carbofuran	0.9966	1.6
Spiroxamine I	0.9982	0.4
Methyl parathion	0.9920	1.8
Spiroxamine II	0.9983	1.6
Methiocarb	0.9973	3.0
Chlorpyrifos	0.9985	1.4
Fipronil	0.9978	1.1
Paclobutrazol	0.9501	2.1
Imazalil	0.9917	3.4
Chlorfenapyr	0.9980	2.3
Fenoxycarb	0.9954	3.2

Table 7. LODs and LOQs for Category I and II residual pesticides in hemp oil extract (contd.).

Compound	Correlation Coefficient	Precision (n=3)
Coumaphos	0.9962	2.4
Etofenprox	0.9985	2.0
Pentachloronitrobenzene	0.9986	2.4
Simazine	0.9966	1.5
Atrazine	0.9963	2.0
Hexachlorocyclopentadiene	0.9990	5.1
Hexachlorobenzene	0.9999	0.5
Lindane	0.9999	0.9
Alachlor	1.0000	0.5
Heptachlor	1.0000	1.6
Aldrin	0.9999	0.7
Heptachlor epoxide (Isomer B)	1.0000	0.5
α-Chlordane	0.9999	1.0
γ-Chlordane	0.9999	1.0
cis-Nonachlor	0.9996	0.8
Endrin	0.9972	1.7
Dieldrin	0.9738	0.7
trans-Nonachlor	0.9998	1.7
Methoxychlor	0.9999	0.5
Captan	0.9628	1.2
Cinerin I	0.9981	0.57
Jasmolin I	0.9959	0.53
Pyrethrin I	0.9919	2.76
Jasmolin II	0.9985	2.95

A representative calibration curve for the residual pesticides is shown in Figure 1, which shows the calibration curve for heptachlor. Excellent linearity is observed.

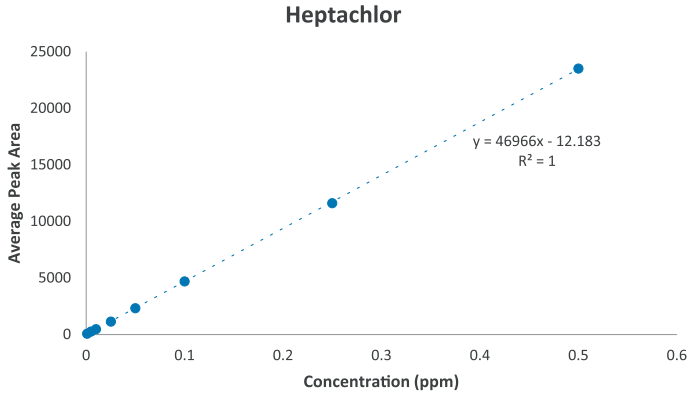

Figure 1. Calibration curve for 0.001 – 0.5 ppm heptachlor standard in hemp oil extract.

For the quantification of these compounds, a single ion monitoring (SIM) mode method was developed. Table 8 shows the limit of detection (LOD) and limit of quantification (LOQ) for the Category I and II residual pesticides in this study. The LOD and LOQ were determined by extracting the quant ion for each analyte to generate the signal to noise value of each peak. The LOD value was determined by multiplying three times the concentration and dividing by the peak to peak noise value (defined as max noise/min noise) generated by the Agilent MSD ChemStation software (version F.01.01.2317). The LOQ value was determined by multiplying ten times the concentration and dividing by the peak to peak noise value generated by the Agilent MSD ChemStation software. For all Category I residual pesticide compounds, the limit of quantification was well below the required limit of 0.1 µg/g. The limit of quantification for the Category II pesticides included in this study was below 0.7 µg/g and 0.1 µg/g for captan and pentachloronitrobenzene respectively, as defined by the Bureau of Cannabis Control for the State of California.

Table 8. LODs and LOQs for Category I and II residual pesticides in hemp oil extract.

Category I or II Pesticide	LOD (ppm)	LOQ (ppm)
DDVP (Dichlorvos)	0.005	0.016
Mevinphos	0.004	0.015
Propoxur	0.004	0.013
Ethoprophos	0.012	0.040
Dimethoate	0.011	0.038
Carbofuran	0.011	0.038
Spiroxamine	0.002	0.005
Methyl parathion	0.017	0.058
Spiroxamine	0.002	0.007
Methiocarb	0.008	0.026
Chlorpyrifos	0.007	0.022
Fipronil	0.015	0.050
Paclobutrazol	0.006	0.019
Imazalil	0.027	0.091
Chlorfenapyr	0.006	0.018
Fenoxycarb	0.003	0.010
Coumaphos	0.022	0.072
Etofenprox	0.003	0.008
Captan	0.005	0.016
Pentachloronitrobenzene	0.010	0.032

Figure 2 shows a representative total ion chromatogram of a 12.5 ppm residual pesticide category I and II standards in hemp oil extract. All compounds in the residual pesticide category I and II included in this study were identified and labeled in the figure. Good chromatographic separation is observed in the figure.

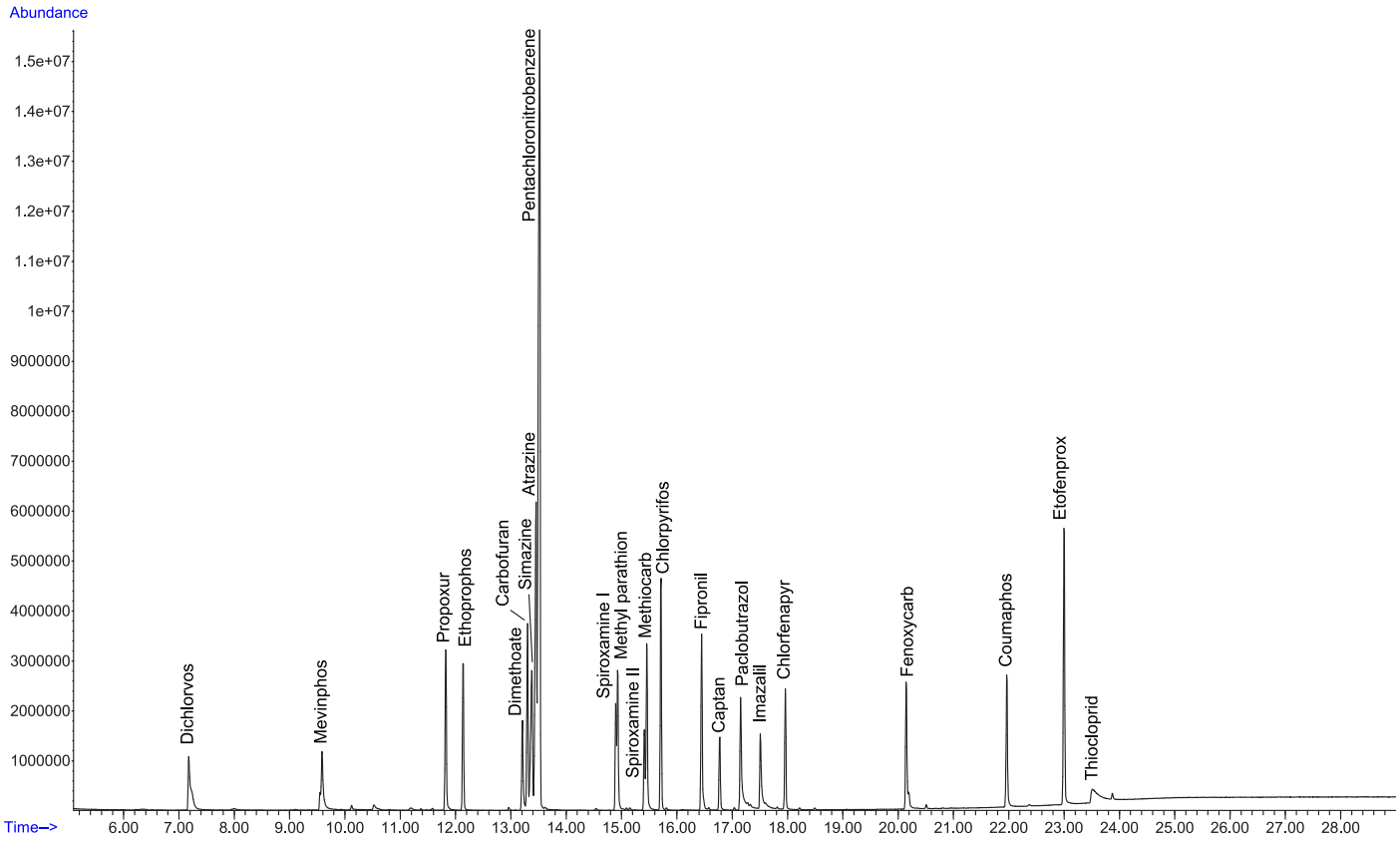

Figure 2. Total ion chromatogram of 12.5 ppm residual pesticide mix in hemp oil extract by direct liquid injection.

Table 9 lists the LODs and LOQs for the organohalide residual pesticides in this study. The LOQs are well below the required limit of quantification of 0.10 µg/g defined for the category I and II residual pesticides.

Table 9. LODs and LOQs for organohalide residual pesticides in hemp oil extract

Compound	LOD (ppm)	LOQ (ppm)
Hexachlorobenzene	0.005	0.016
Lindane	0.001	0.002
Alachlor	0.002	0.006
Heptachlor	0.004	0.013
Aldrin	0.005	0.016
Heptachlor epoxide (Isomer B)	0.005	0.018
α-Chlordane	0.006	0.021
γ-Chlordane	0.006	0.019
cis-Nonachlor	0.007	0.023
Endrin	0.002	0.007
Dieldrin	0.002	0.007
trans-Nonachlor	0.005	0.018
Methoxychlor	0.000	0.001

Figures 3 and 4 show representative extracted ion chromatograms of a 0.5 ppm organohalide pesticide standard in hemp oil extract. The quant ion for each compound was extracted and overlaid. All compounds in the residual organohalide pesticide standard were identified and labeled in the figures.

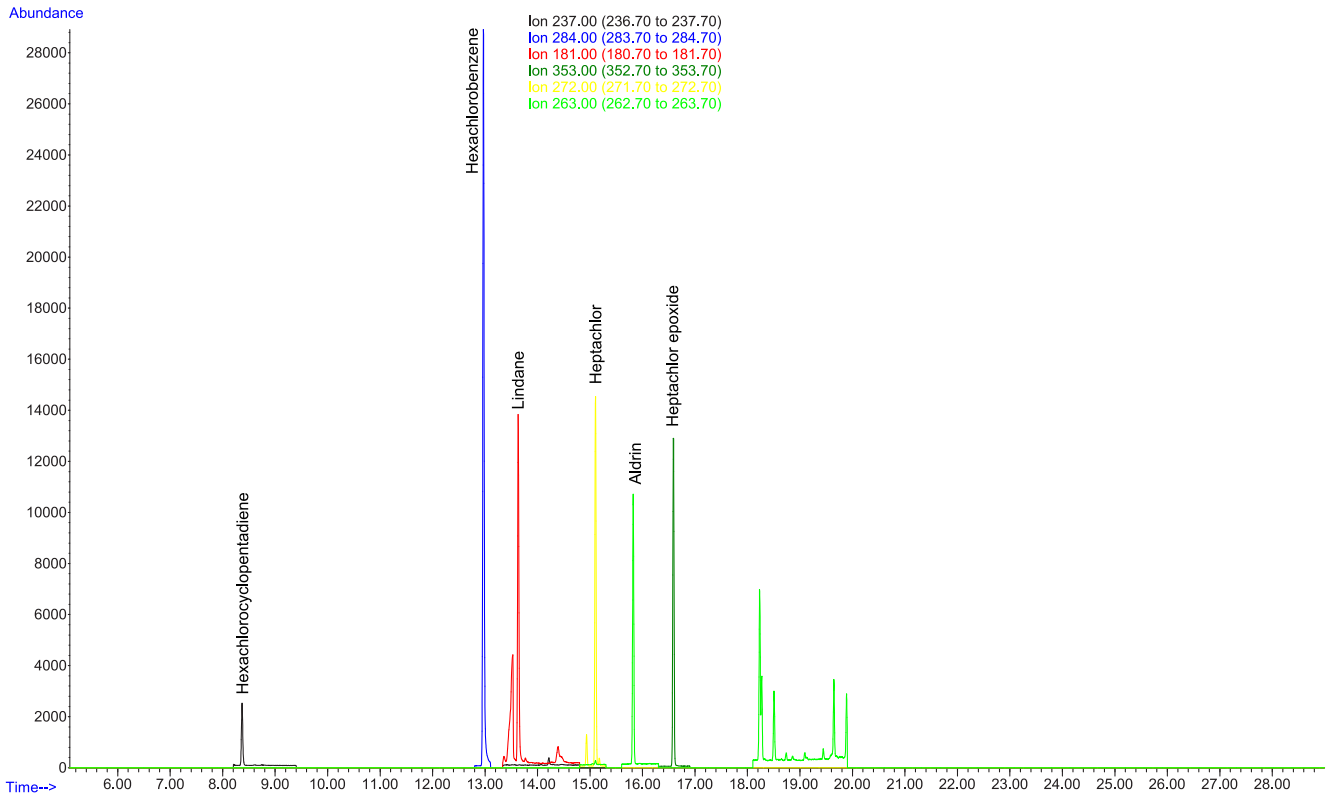

Figure 3. Extracted ion chromatogram of 0.5 ppm hexachlorcyclopentadiene, hexachlorobenzene, lindane, heptachlor, aldrin and heptachlor in hemp oil extract.

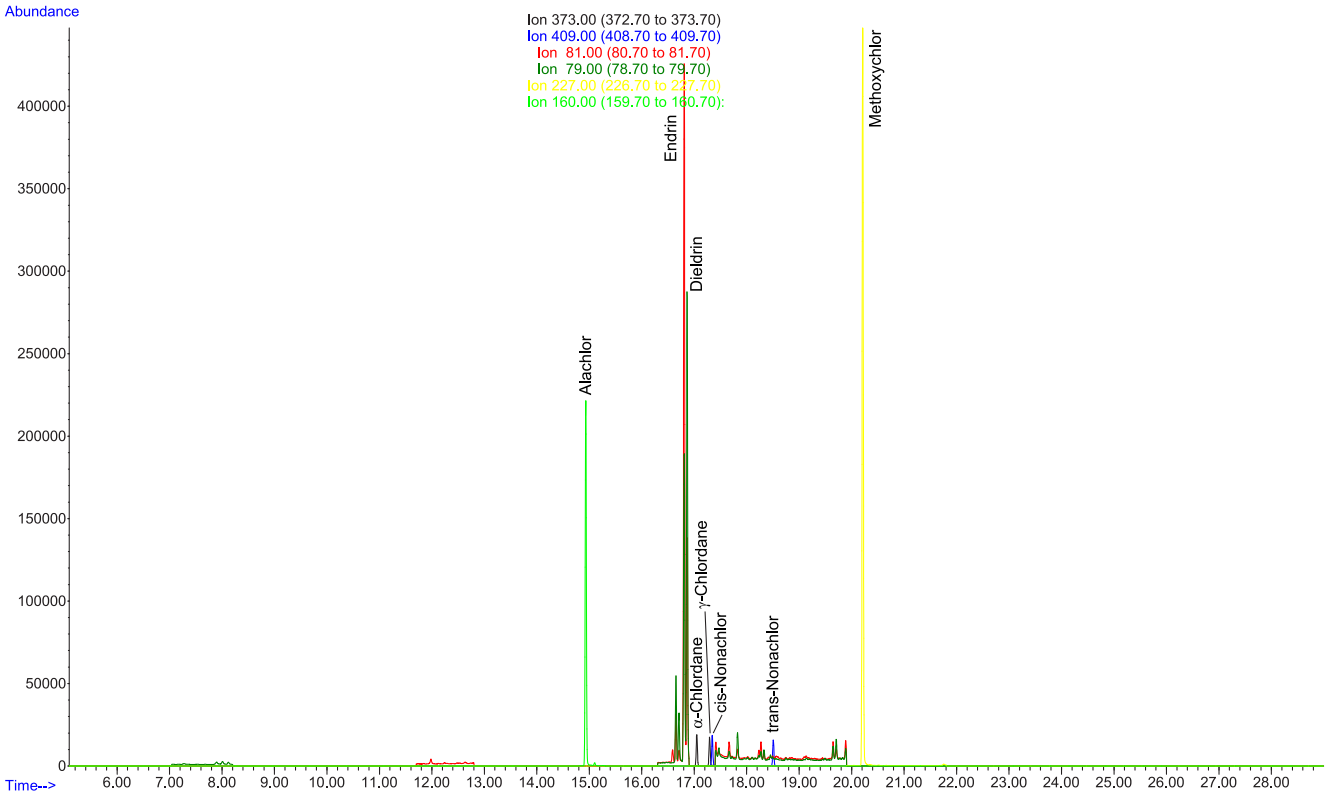

Figure 4. Extracted ion chromatogram of 0.5 ppm alachlor, endrin, dieldrin, α-chlordane, γ-chlordane, cis-nonachlor, trans-nonachlor and methoxychlor in hemp oil extract.

Table 10 lists the LODs and LOQs for the pyrethrin residual pesticides in this study. The LOQs are well below the required limit of quantification of 0.10 µg/g defined for the category I and II residual pesticides.

Table 10. LODs and LOQs for pyrethrin residual pesticides in hemp oil extract.

Compound	LOD (ppm)	LOQ (ppm)
Cinerin I	0.002	0.008
Jasmolin I	0.001	0.002
Pyrethrin I	0.014	0.047
Jasmolin II	0.002	0.008

Figure 5 shows a representative extracted ion chromatogram of a 1 ppm pyrethrin pesticide standard in hemp oil extract. The quant ion for each compound was extracted and overlaid. All compounds in the pyrethrin residual pesticide standard included in this study were identified and labeled in the figure.

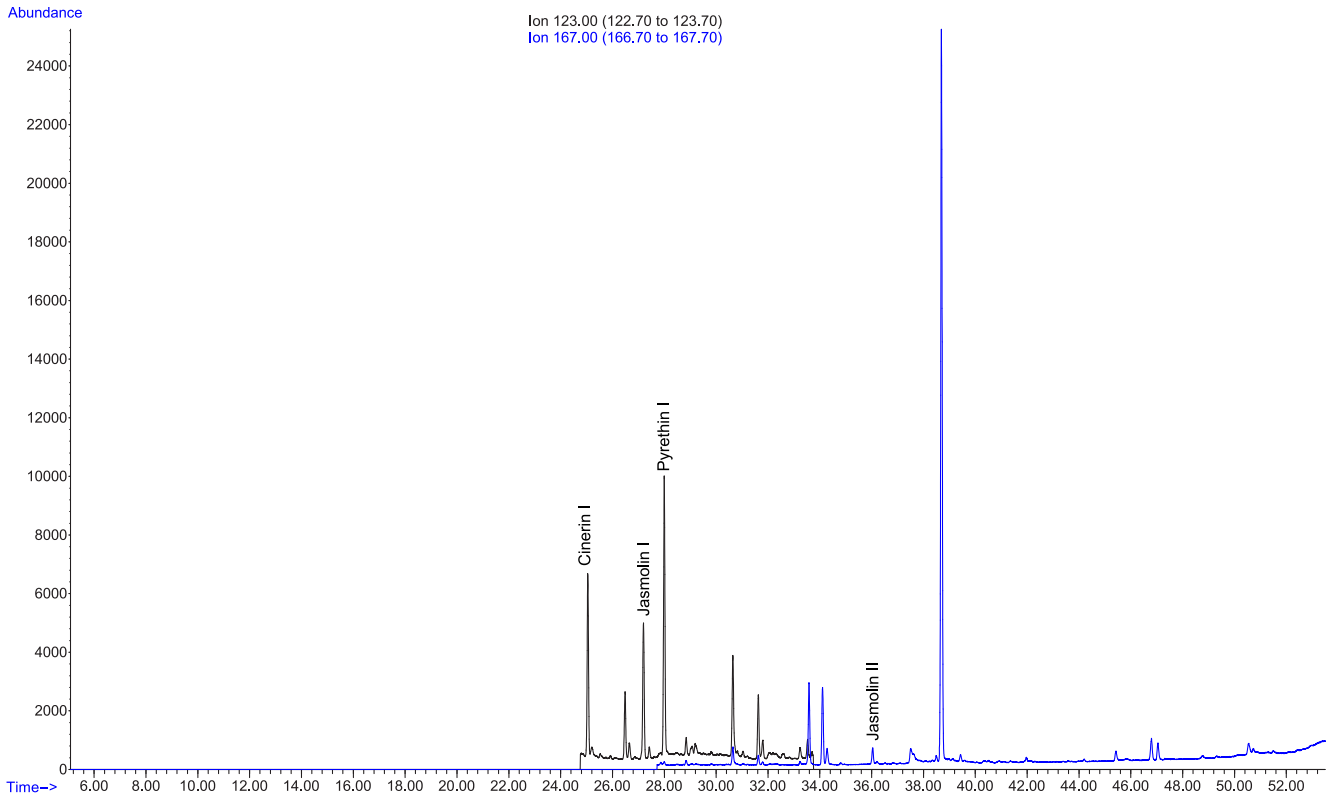

Figure 5. Extracted ion chromatogram of 1 ppm pyrethrin standard in hemp oil extract.

As demonstrated in Table 5, excellent linearity is observed for all compounds with an average correlation coefficient of 0.995. The technique is very precise, with an average relative standard deviation of 1.73 % for all compounds. Low detection levels were demonstrated using the SIM mode for quantification. As demonstrated in Table 8, all LOQs determined in this study were well below the limit of quantification for pesticides established by the Bureau of Cannabis Control for the State of California, from 1.1 to 43.6-fold lower. Although the limit of quantification for organohalide pesticides and pyrethrins has not yet been defined by the State of California, all organohalide pesticides and pyrethrins included in this study have limits of quantification 2.1 to 76.1-fold lower than the 0.1 µg/g LOQ required of the Category I and II pesticides.

Conclusion

This study has demonstrated the efficacy of direct liquid injection GC/MS analysis for the quantification of residual pesticides in hemp oil extract and other cannabis concentrates. The automation provided by the GERSTEL MPS robotic and the accurate and efficient analyte transfer through the CIS 4 GC inlet under Maestro software control enables the sensitive, accurate and precise determination of the pesticides. The use of the MSD in SIM mode allows the operator to achieve low limits of detection for analytes, LOQs were below 0.1 µg/g.

References

[1] California Bureau of Cannabis Control – Proposed Text of Regulations, July 2018; https://cannabis.ca.gov/wp-content/uploads/sites/13/2018/07/Bureau-of-Cannabis-Control-Proposed-Text-of-Regulations.pdf.

[2] S. Cunha, S. Lehotay, K. Mastovska, *J. Sep Sci*, 30 (**2007**) 620.

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