Application Note

Cannabis and Hemp Testing

Analysis of Twenty-Seven GC-Amenable Pesticides Regulated in the Cannabis Industry in North America with the Agilent 8890/7010B Triple Quadrupole GC/MS System

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Abstract

Twenty-seven GC-amenable pesticides and their isomers regulated in the cannabis industry in North America stand out as challenging to analyze using liquid chromatography-triple quadrupole mass spectrometry (TQ LC/MS) with electrospray ionization (ESI). This list includes pentachloronitrobenzene (PCNB, also known as quintozene), kinoprene, captan, methyl parathion, chlorfenapyr, and chlordanes. This application note defines a complete workflow that achieves and exceeds required limits of detection (LOD), limits of quantitation (LOQ), accuracy, and precision defined by the California Bureau of Cannabis Control (BCC) and Health Canada in dry cannabis flower.

Introduction

In the United States, Canada, and other regions where medicinal or adult recreational cannabis use has been legalized, regulatory agencies require chemical and biological testing of the products to ensure compliance and safety. The global movement for cannabis legalization drives the demand for cannabis analytical testing methods, including potency determination, trace metals analysis, residual solvents and terpenes analysis, microbial screening, and quantitation of micotoxins. Of these assays, residual pesticide analysis is particularly challenging due to the very low LOQs required by regulatory entities.

By the beginning of 2020, the list of pesticides regulated by U.S. state legislation and by Health Canada comprised approximately 100 pesticides, with California currently having the largest target list of pesticides tested in recreational cannabis in the U.S.[1] Meanwhile, the Canadian target list mandated by Health Canada generally exhibits lower required LOQs than any U.S. state.[2] Of all the pesticides currently regulated in the cannabis industry in North America, at least 27 compounds and their isomers present a challenge for electrospray TQ LC/MS.

A well-defined sample preparation procedure[3] and state-of-the-art GC[4-6] and LC[7-10] triple quadrupole mass spectrometry is required to enable success in meeting diverse regulatory requirements. This application note focuses on gas chromatography-triple quadrupole mass spectrometry (TQ GC/MS) analysis of 27 GC-amenable pesticides regulated in cannabis in California by the Bureau of Cannabis Control (BCC) and in Canada by Health Canada that commonly stand out as challenging to analyze using electrospray TQ LC/MS. The California and Canadian required limits of quantitation (LOQs) were successfully met for the 27 pesticides. Excellent quantitative accuracy was achieved at action levels established in both California and Canada.The rest of the pesticides regulated in California and Canada are analyzed at the action level by TQ LC/MS as reported in application notes 5994-1743EN,[7] 5994-0648EN,[8] and 5994-0429EN.[9]

The information content of this application note, along with ready to run acquisition and quantitation methods, are available as eMethod G5278AA#010, Pesticides Residue Analysis in Cannabis and Hemp with the 8890/7010B GC/TQ MS system.

Materials and methods

An Agilent 8890/7010B TQ GC/MS system (Figure 1A) configured to achieve the highest sensitivity and minimize common pitfalls with pesticide analyses in high-matrix cannabis samples was used. The GC was configured with the 7693 autosampler and 150-position tray, a MultiMode inlet (MMI) operated in cold solvent vent mode. Mid-column backflush was employed using the Agilent Purged Ultimate Union (PUU) installed between two identical 15 m columns. The 8890 pneumatic switching device (PSD) (Figure 1B) supplied helium to the backflush system. The triple quadrupole mass spectrometer was equipped with the High Efficiency Source (HES) operated in electron ionization (EI) mode at 300 °C. Data were acquired in dynamic MRM (dMRM) mode. dMRM optimizes dwell time distributions to accurately identify and quantify large multi-analyte assays. The acquisition method was retention time-locked to match retention times in the Agilent MassHunter Pesticide & Environmental Pollutant MRM Database (P&EP 4) that allowed for seamless development of the acquisition method. The instrument operating parameters are listed in Table 1. Agilent MassHunter Workstation revision 10, including MassHunter Acquisition 10 SR1, MassHunter Qualitative 10, and MassHunter Quantitative 10.1 packages were used in this work.

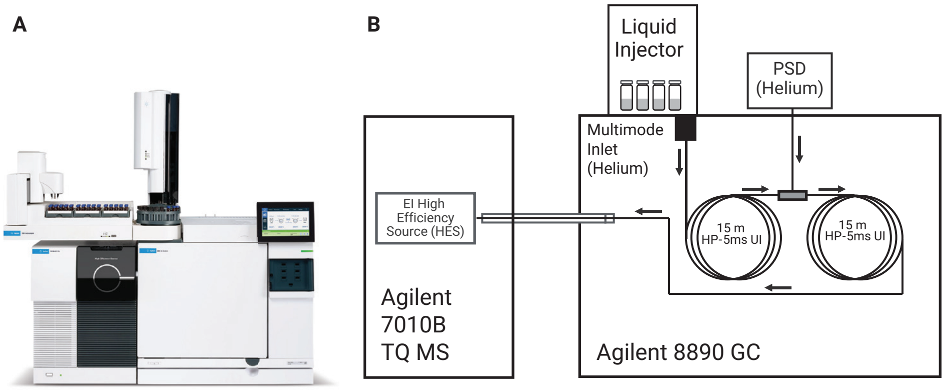


Figure 1. The Agilent 8890/7010B TQ GC/MS system (A) and system configuration (B).

Table 1. Agilent 8890/7010B gas chromatograph and mass spectrometer conditions for pesticide analysis.

Parameter	Value
GC	Agilent 8890 with fast oven, autoinjector, and tray
Inlet	Multimode Inlet (MMI)
Mode	Solvent Vent
Vent Flow and Pressure	25 mL/min, 5 psi until 0.3 min
Purge Flow to Split Vent	50 mL/min at 1.5 min
Septum Purge Flow Mode	Switched
Septum Purge Flow	3 mL/min
Injection Volume	2.0 µL
Sample Depth	1 mm
Plunger Speed	Fast
Injection Type	2-Layer Sandwich (L1, L2)
L1 Airgap	0.2 µL
L2 Injection Volume	0.2 µL
L2 Airgap	0.2 µL
Solvent Wash Mode	A-A6, B-B4 Wash solvent A, 50:50 isopropanol:acetonitrile; Wash solvent B, 100% acetonitrile
Inlet Temperature Program	60 °C for 0.35 minute, then 600 °C/min to 280 °C (14.8 minute hold time), then 600 °C/min to 300 °C (0 minute hold time)
Post Run Inlet Temperature	310 °C
Post Run Total Flow	25 mL/min
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert, splitless, dimpled, 2 mm id
Inlet Liner Part number	5190-2297
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temp 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C/min
Final Temp 2	310 °C
Final Hold 2	1.25 min
Total Run Time	19 min
Post Run Temperature	280 °C
Post Run Time	1.5 min
Equilibration Time	0.5 min

Parameter	Value
Column 1	
Type	Agilent HP-5MS UI (p/n 19091S-431UI)
Length	15 m
Diameter	0.25 mm
Film thickness	0.25 µm
Control Mode	Constant Flow
Flow	1.19 mL/min
Inlet Connection	Multimode Inlet (MMI)
Outlet Connection	PSD (PUU)
Post Run Flow (Backflushing)	-8.60 mL/min
Column 2	
Type	Agilent HP-5MS UI (p/n 19091S-431UI)
Length	15 m
Diameter	0.25 mm
Film thickness	0.25 µm
Control Mode	Constant Flow
Flow	1.39 mL/min
Inlet Connection	PSD (PUU)
Outlet Connection	MSD
Post Run Flow (Backflushing)	9.02 mL/min
PSD Purge Flow	5 mL/min
MSD	
Model	Agilent 7010B
Source	High-Efficiency Source
Vacuum Pump	Performance Turbo
Tune File	Atunes.eihs.tune.xml
Mode	Dynamic MRM (dMRM)
Solvent delay	3 min
EM voltage Gain mode	20
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	300 °C
Transfer line Temperature	280 °C
He Quench Gas	2.25 mL/min
N_2 Collision Gas	1.5 mL/min

Chemicals

CHROMASOLV acetonitrile for pesticide residue analysis, ≥99.9%, was obtained from Honeywell. D-sorbitol, 3-ethoxy-1,2-propanediol, L-gulonic acid γ-lactone (L-gulonolactone), and captan-d6 were obtained from Sigma-Aldrich. Triphenyl phosphate (TPP) and AOAC Method 2007.1 QuEChERS internal standard (IS) solution containing isotopically labeled α-BHC-d6 (α-HCH-d6) and parathion-d10 were obtained from Restek. A custom pesticide standard containing 27 target pesticides was purchased from AccuStandard.

Analyte protectants (APs) were used as described in application notes 5991-1054EN[11] and 5990-1604EN,[4] and the GC/MS/MS Pesticide Residue Analysis Guide.[12] The stock AP solution contained 3-ethoxy-1,2-propanediol at 10 mg/mL, D-sorbitol at 1 mg/mL, and L-gulonolactone at 1 mg/mL dissolved in acetonitrile with 1% acetic acid and 12% water. A 10-fold diluted AP solution was used with each injection of calibrators and samples in two-layer sandwich injection mode.

Data collection

Each batch was comprised of solvent blanks, matrix blanks, eight levels of calibrators and LLOQ ranging from 0.004 to 64.00 ppb in vial (0.5 through 8,000 ppb in matrix). Parathion-d10 at a final concentration of 4 ppb in-vial was used as IS for quantitation of cyfluthrins (I-IV), cypermethrins (I-IV), methyl parathion and captan. α-BHC-d6 at a final concentration of 4 ppb in-vial was used as the IS for quantitation of the remaining compounds. As an alternative to parathion-d10, captan-d6 at a final concentration of 40 ppb in-vial was evaluated as an IS for captan quantitation. TPP was added to each calibration standard at constant concentration of 5 ppb in-vial as a surrogate to monitor method performance over time. Also, TPP was added to the cannabis material used for preparing QCs prior to the extraction, and served as a recovery standard.

Quintuplicate injections were made for the calibration set and QCs to evaluate method precision and calculate LODs and LOQs. Additionally, a calibration curve with one injection per level, followed by the QCs and another bracketing calibration was acquired to simulate a typical workflow in a high-throughput laboratory.

Statistics

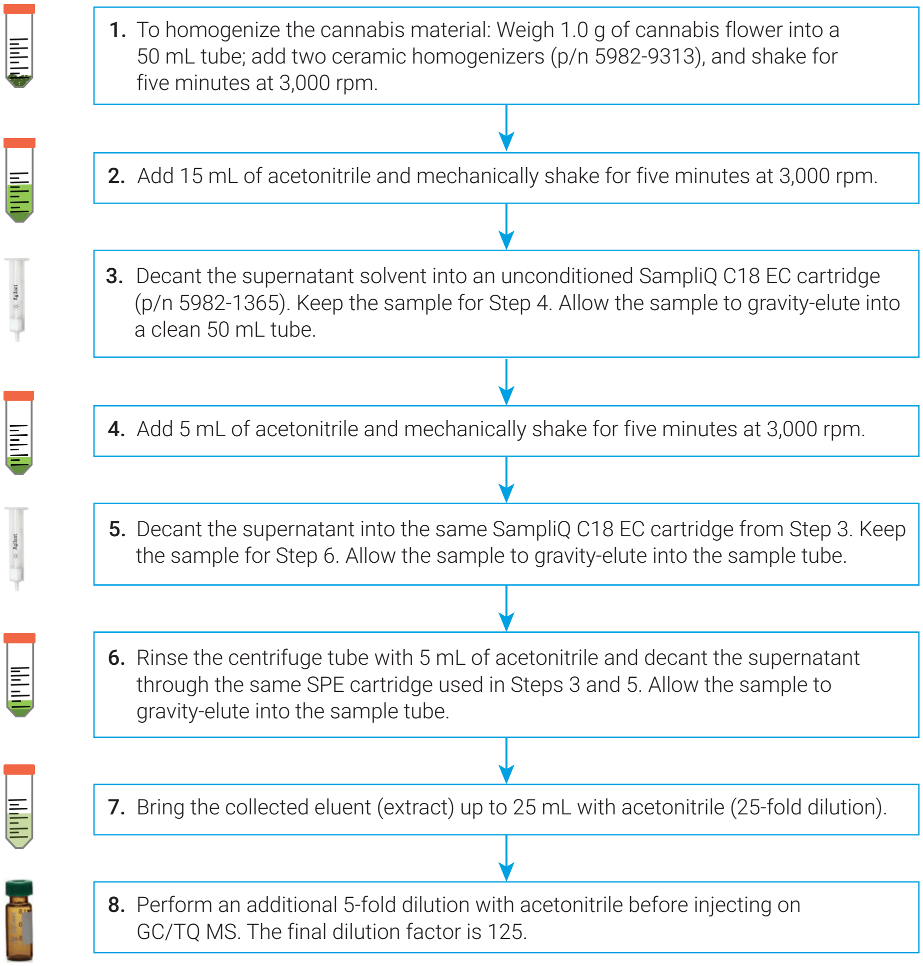
Five replicate injections at each calibration level and LLOQ permitted statistical calculations of MDL, LOD, and, LOQ. This study used these primary equations:

- Average = $\Sigma x_i/n$
- Standard deviation, (s) = $\left[\frac{\Sigma(x - \overline{x})^2}{n - 1}\right]^{1/2}$
- MDL = (s) × (Student t-value, n – 1, 99% Confidence)
- LOD = 3 × (s)
- LOQ = 10 × (s)
- Calculated MDL <Spike Level <10 × Calculated MDL
- Average Percent Recoveries = (calculated average concentration in QC/spiked concentration) × 100

The Calculated MDL <Spike Level <10 × Calculated MDL equation was used to evaluate the empirically determined MDL and ensure its validity.

Sample preparation

The Agilent recommended sample prep procedure for cannabis that allows for obtaining the extracts for simultaneous analysis by TQ GC/MS and TQ LC/MS is shown in Figure 2. The optimized sample preparation workflow is described in detail in application note 5994-0973EN.[3]



1. To homogenize the cannabis material: Weigh 1.0 g of cannabis flower into a 50 mL tube; add two ceramic homogenizers (p/n 5982-9313), and shake for five minutes at 3,000 rpm.
2. Add 15 mL of acetonitrile and mechanically shake for five minutes at 3,000 rpm.
3. Decant the supernatant solvent into an unconditioned SampliQ C18 EC cartridge (p/n 5982-1365). Keep the sample for Step 4. Allow the sample to gravity-elute into a clean 50 mL tube.
4. Add 5 mL of acetonitrile and mechanically shake for five minutes at 3,000 rpm.
5. Decant the supernatant into the same SampliQ C18 EC cartridge from Step 3. Keep the sample for Step 6. Allow the sample to gravity-elute into the sample tube.
6. Rinse the centrifuge tube with 5 mL of acetonitrile and decant the supernatant through the same SPE cartridge used in Steps 3 and 5. Allow the sample to gravity-elute into the sample tube.
7. Bring the collected eluent (extract) up to 25 mL with acetonitrile (25-fold dilution).
8. Perform an additional 5-fold dilution with acetonitrile before injecting on GC/TQ MS. The final dilution factor is 125.

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

Results and discussion

Twenty-seven GC-amenable pesticides met LOQs established in California and Canada

Table 2 summarizes the established reporting limits for the pesticides that often present a challenge for electrospray TQ LC/MS analysis in inhalable cannabis and cannabis products in California and in dried cannabis for Canada. The empirical LOQs achieved in this work were consistently below the established reporting limits.

Five replicate injections at each calibration level and LLOQ permitted statistical calculations of LOD and LOQ. The resulting calibration curve for methyl parathion performed with five injections per level is shown in Figure 3. Figure 3B highlights that high accuracy was maintained throughout the calibration, including low concentrations, under 1 ppb.

Figure 4 illustrates the TQ GC/MS chromatograms at or near the LOQ and the overlaid bracketing calibrations for selected pesticides, where black circles and blue diamonds represent the calibrations performed at the beginning and at the end of the analysis, respectively. Zero or minimal calibration drift was observed in bracketing calibrations for the target 27 pesticides.

Table 2. In-vial and in-matrix LOD and LOQ values for the 27 GC-amenable pesticides from the California and Canada lists.

Name	CA Reporting Limit (ppb)[a]	Canadian Reporting Limit (ppb)[b]	Empirical LOD In-Vial (ppb)	Empirical LOD In-Matrix (ppb)	Empirical LOQ In-Vial (ppb)	Empirical LOQ In-Matrix (ppb)
Bifenthrin	3,000	1,000	0.120	15	0.399	50
Boscalid	100	20	0.003	0.36	0.009	1.2
Captan	700	–	0.446	56	1.488	186
Chlordanes Total	>LOD	–	0.006	0.81	0.022	2.7
Chlorfenapyr	>LOD	50	0.002	0.25	0.007	0.82
Chlorpyrifos	>LOD	40	0.019	2.4	0.064	8.1
Cyfluthrins (I-IV)	2,000	200	0.251	31	0.837	105
Cypermethrins (I-IV)	1,000	300	0.052	6.5	0.175	22
Diazinon	100	20	0.003	0.33	0.009	1.1
Dimethomorph Total	2,000	50	0.069	8.7	0.232	29
Endosulfan I (alpha Isomer)	–	200	0.012	1.5	0.039	4.9
Endosulfan II (beta Isomer)	–	50	0.009	1.1	0.031	3.8
Ethoprophos	>LOD	20	0.004	0.46	0.012	1.5
Etridiazole	–	30	0.029	3.6	0.097	12
Fenthion	–	20	0.001	0.11	0.003	0.38
Fenvalerate Total	–	100	0.007	0.90	0.024	3.0
Fipronil	>LOD	60	0.001	0.15	0.004	0.49
Kinoprene	–	500	0.260	33	0.868	108
Kresoxim-Methyl	100	20	0.002	0.21	0.006	0.69
Metalaxyl	2,000	20	0.005	0.60	0.016	2.0
Methyl Parathion	>LOD	50	0.003	0.38	0.010	1.3
MGK-264	–	50	0.002	0.22	0.006	0.74
Novaluron	–	50	0.007	0.82	0.022	2.7
Pentachloronitrobenzene	100	20	0.001	0.17	0.004	0.56
Permethrins Total	500	500	0.040	5.0	0.134	17
Piperonyl Butoxide	3,000	200	0.225	28	0.752	94
Pyridaben	100	50	0.014	1.7	0.045	5.6

[a] In inhalable cannabis and cannabis products

[b] In dried cannabis flower

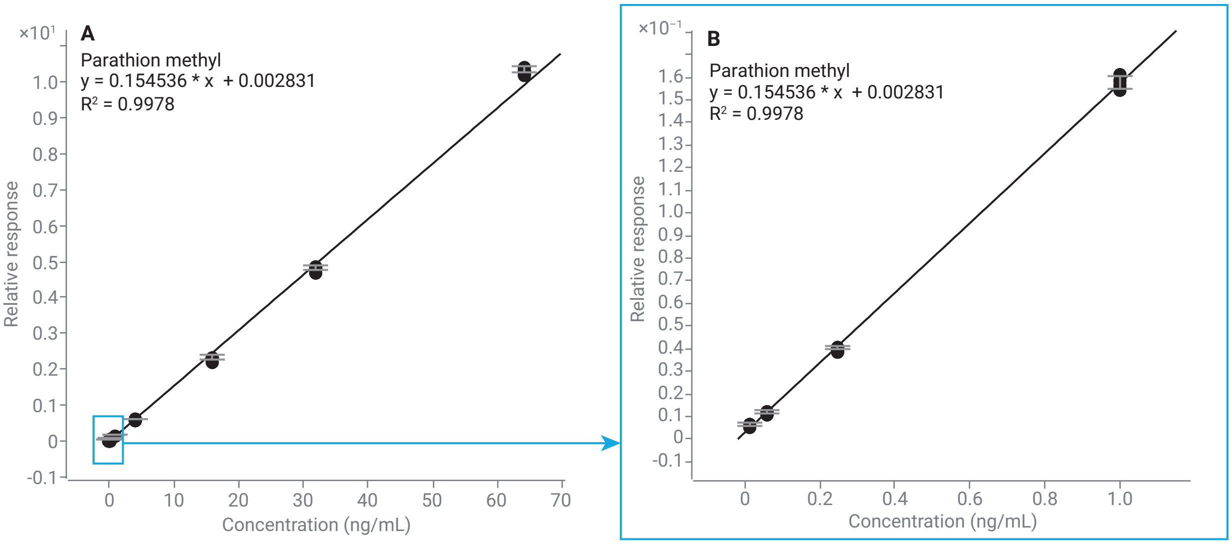


Figure 3. Example calibration curve for methyl parathion performed with five injections per level (A, B).

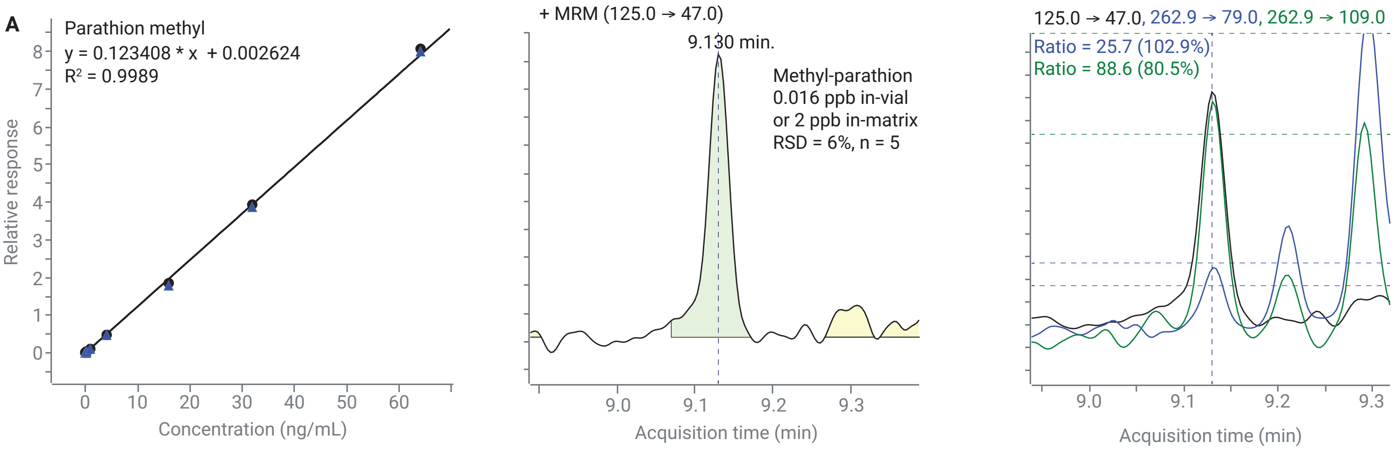


Figure 4A. Example calibration curve and quantitative results for methyl parathion at its estimated LOQ, showing %RSD and number of replicates.

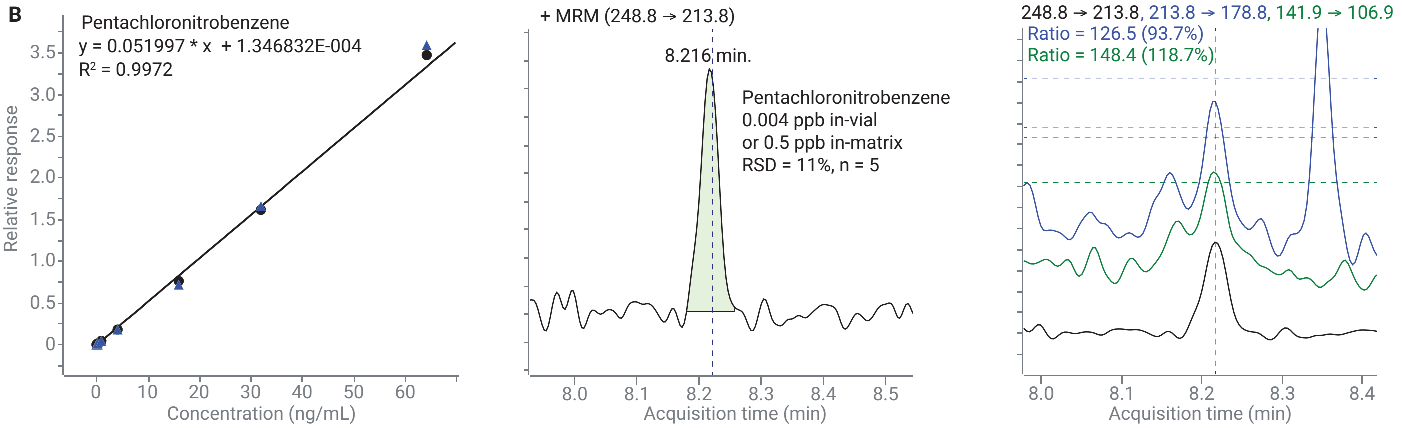


Figure 4B. Example calibration curve and quantitative results for pentachloronitrobenzene at its estimated LOQ, showing %RSD and number of replicates.

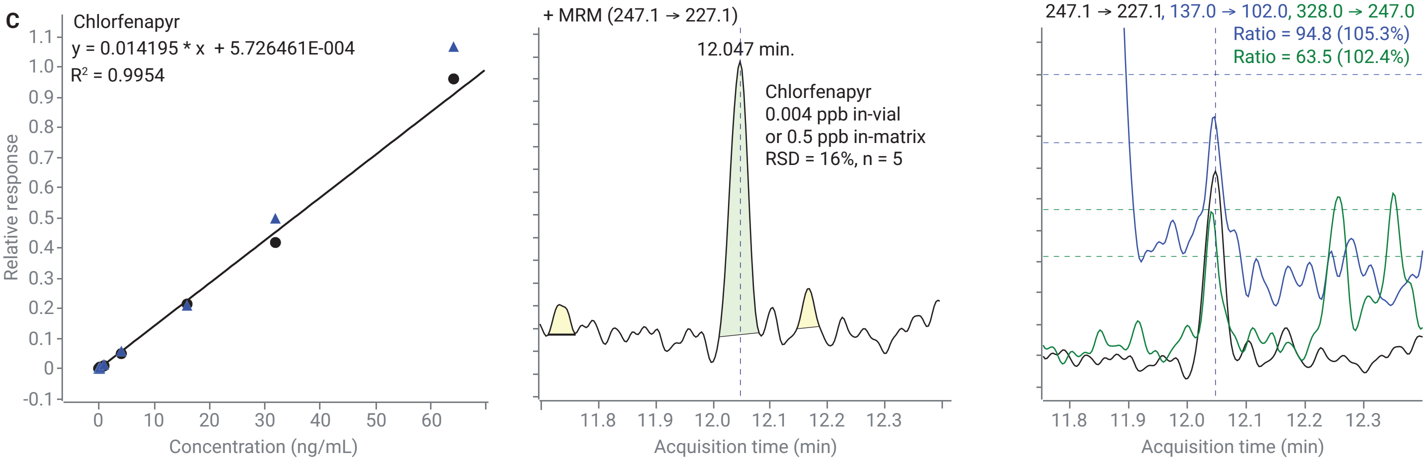


Figure 4C. Example calibration curve and quantitative results for chlorfenapyr at its estimated LOQ, showing %RSD and number of replicates.

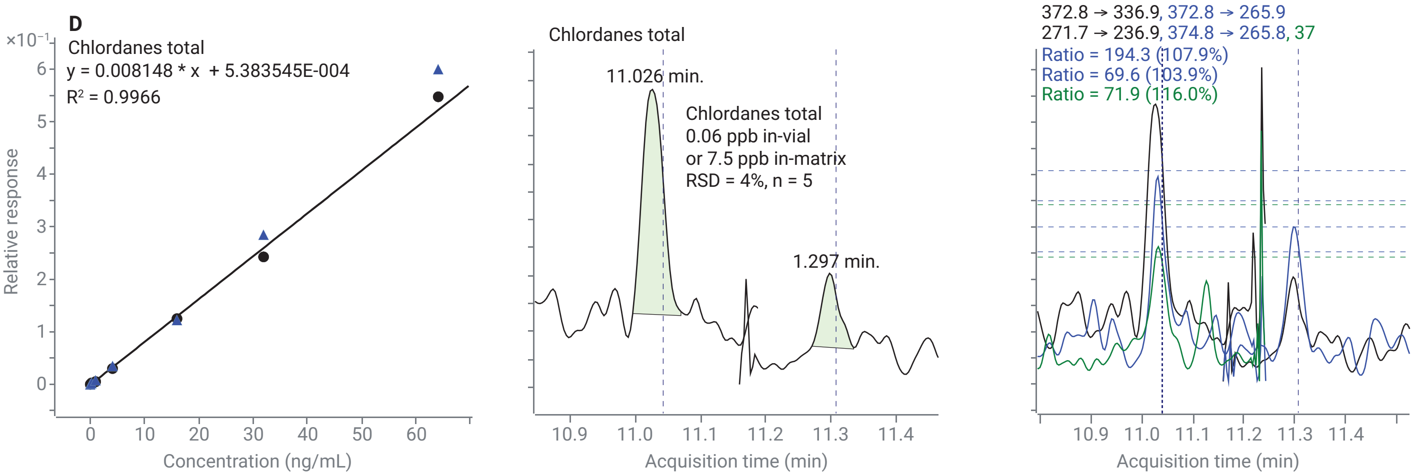


Figure 4D. Example calibration curve and quantitative results for total chlordanes, including *trans*-chlordane and *cis*-chlordane, at its estimated LOQ, showing %RSD and number of replicates.

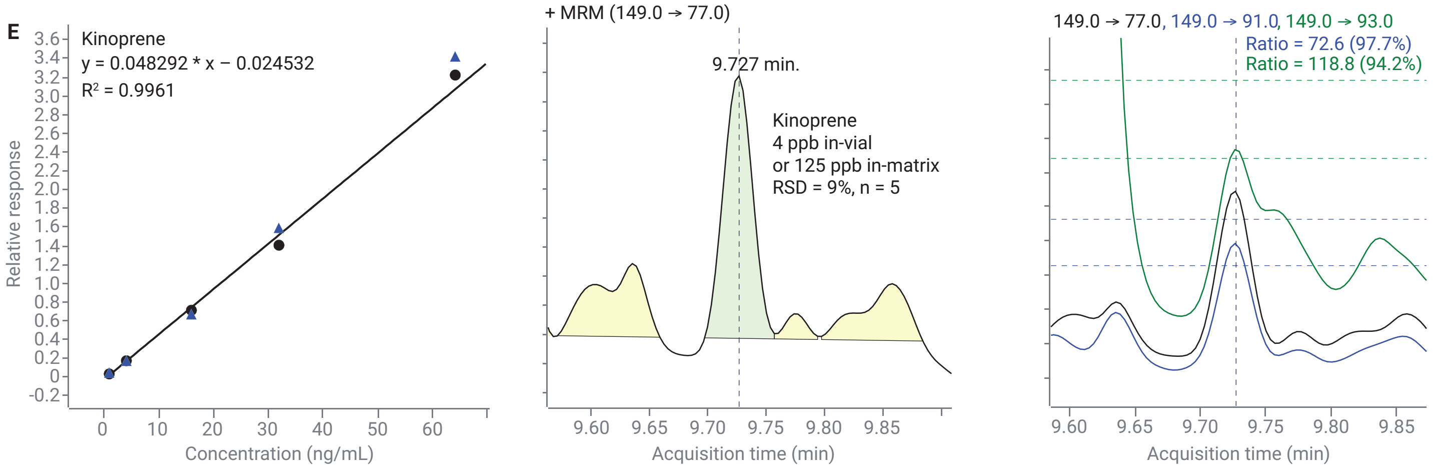


Figure 4E. Example calibration curve and quantitative results for kinoprene at its estimated LOQ, showing %RSD and number of replicates.

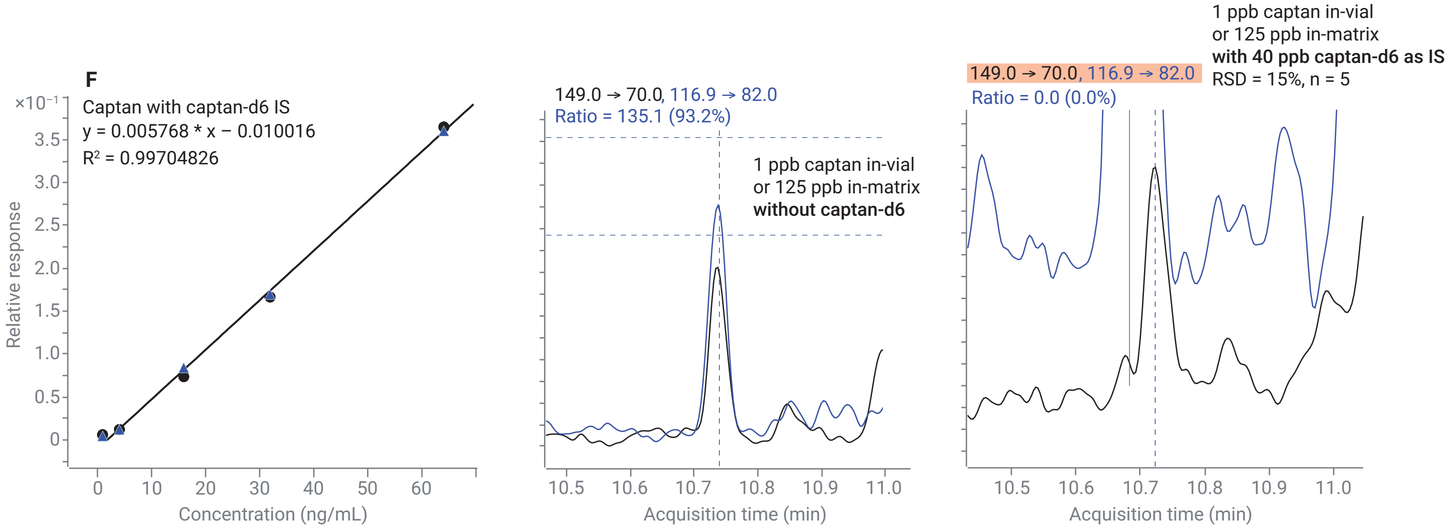


Figure 4F. Example calibration curve and quantitative results for captan at its estimated LOQ, showing %RSD and number of replicates.

Calibration ranges for the 27 pesticides are shown in Table 3. The calibration range for each pesticide was selected so that the quantitation accuracy within ±20% was observed at or near the concentration corresponding to the reporting limit for both California and Canada. Linear calibration with 1/x weighing yielded coefficient of correlation values (R^2) that were >0.99 in all cases.

Captan is known to be a challenging pesticide in both GC/MS and LC/MS analyses. In this work, comparable calibration performance with R^2 values of 0.992 and 0.997 were achieved for captan when using parathion-d10 or captan-d6 as IS for captan, respectively. However, when using captan-d6 as IS, less calibration drift was observed when performing bracketing calibration. On the other hand, use of captan-d6 yields an interference with the qualifier, as shown in Figure 4F.

Table 3. Calibration ranges for the 27 GC-amenable pesticides from the California and Canada lists.

Name	RT (min)	Empirical LOD In-Vial (ppb)	Empirical LOQ In-Vial (ppb)	In-Vial Calibration Range (ppb)	CF R^2	IS	Number of Calibration Levels
Bifenthrin	13.959	0.120	0.399	1 to 64	0.9937	alpha-BHC-d6	5
Boscalid	16.618	0.003	0.009	0.016 to 64	0.9969	alpha-BHC-d6	8
Captan (Parathion-d10 IS)	10.740	0.446	1.488	1 to 64	0.9920	Parathion-d10	5
Captan (Captan-d6 IS)	10.740	0.446	1.488	1 to 64	0.9970	Captan-d6	5
Chlordanes Total	11.043	0.006	0.022	0.06 to 64	0.9966	alpha-BHC-d6	7
Chlorfenapyr	12.058	0.002	0.007	0.016 to 64	0.9954	alpha-BHC-d6	8
Chlorpyrifos	9.962	0.019	0.064	0.06 to 64	0.9966	alpha-BHC-d6	7
Cyfluthrins (I-IV)	16.289	0.251	0.837	1 to 64	0.9912	Parathion-d10	5
Cypermethrins (I-IV)	16.611	0.052	0.175	0.25 to 64	0.9922	Parathion-d10	6
Diazinon	8.289	0.003	0.009	0.016 to 64	0.9973	alpha-BHC-d6	8
Dimethomorph total	18.810	0.069	0.232	0.25 to 64	0.9949	alpha-BHC-d6	6
Endosulfan I (alpha Isomer)	11.277	0.012	0.039	0.06 to 64	0.9968	alpha-BHC-d6	7
Endosulfan II (beta Isomer)	12.288	0.009	0.031	0.06 to 64	0.9974	alpha-BHC-d6	7
Ethoprophos	7.021	0.004	0.012	0.016 to 64	0.9976	alpha-BHC-d6	8
Etridiazole	5.838	0.029	0.097	0.06 to 64	0.9935	alpha-BHC-d6	7
Fenthion	9.924	0.001	0.003	0.016 to 64	0.9958	alpha-BHC-d6	8
Fenvalerate total	17.639	0.007	0.024	0.06 to 64	0.9948	alpha-BHC-d6	7
Fipronil	10.648	0.001	0.004	0.016 to 64	0.9963	alpha-BHC-d6	8
Kinoprene	9.737	0.260	0.868	1 to 64	0.9961	alpha-BHC-d6	5
Kresoxim-Methyl	11.828	0.002	0.006	0.016 to 64	0.9965	alpha-BHC-d6	8
Metalaxyl	9.336	0.005	0.016	0.016 to 64	0.9967	alpha-BHC-d6	8
Methyl Parathion	9.144	0.003	0.010	0.016 to 64	0.9989	Parathion-d10	8
MGK-264	10.444	0.002	0.006	0.016 to 64	0.9969	alpha-BHC-d6	8
Novaluron	4.756	0.007	0.022	0.016 to 64	0.9957	alpha-BHC-d6	8
Pentachloronitrobenzene	8.228	0.001	0.004	0.016 to 64	0.9972	alpha-BHC-d6	8
Permethrins Total	15.754	0.040	0.134	0.25 to 64	0.9981	alpha-BHC-d6	6
Piperonyl Butoxide	13.392	0.225	0.752	1 to 64	0.9982	alpha-BHC-d6	5
Pyridaben	15.807	0.014	0.045	0.25 to 64	0.9978	alpha-BHC-d6	6

Recoveries

Recoveries for all 27 pesticides were determined by comparing calculated average concentration in QC to the spiked concentration. To prepare each QC, cannabis material was prespiked with pesticide and TPP before solvent extraction. The concentration of QC was calculated using matrix-matched calibration with the ranges shown in Table 3. Most of the 27 pesticides had recoveries between 70 and 130%, as shown in Figure 5.

Stability of response

Cannabis is a known challenging matrix. Multiple injections from within a batch sequence degrade a system's performance over time, mostly due to matrix build-up in the GC inlet liner. Figure 6 shows a TIC scan of the 125-fold diluted cannabis extract, and that of the 100 ppb (in-vial) pesticide standard in solvent (acetonitrile) for comparison.

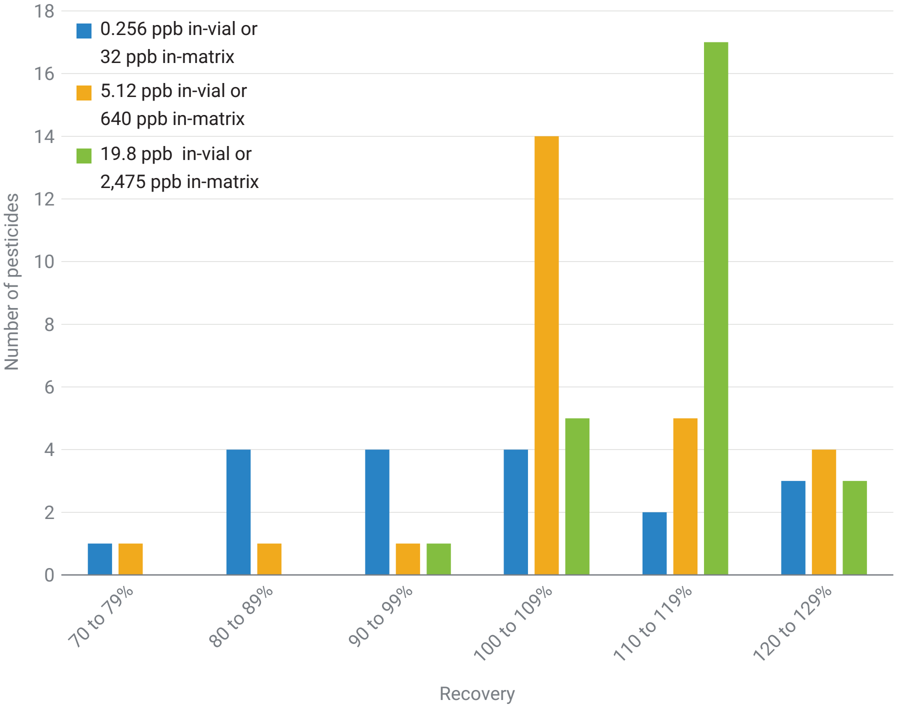


Figure 5. Recoveries for the 27 pesticides prespiked into cannabis and quantified against the matrix-matched calibration.

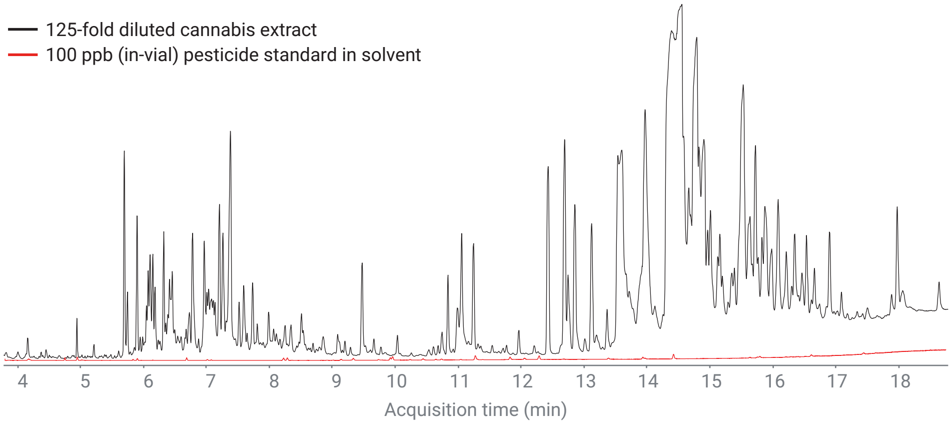


Figure 6. Scan TIC of the 125-fold diluted cannabis extract (in black), and that of the 100 ppb (in-vial) pesticide standard in solvent (in red).

To test method robustness, a matrix-matched calibration standard postspiked into 125-fold diluted cannabis extract containing pesticides and IS at a concentration of 4 ppb in-vial was sequentially injected 114 times. The pesticides were quantified against the matrix-matched calibration curve for each run, and the resulting calculated concentrations were plotted. Figure 7 shows the calculated concentrations and RSD values for 114 injections for several of the analytes.

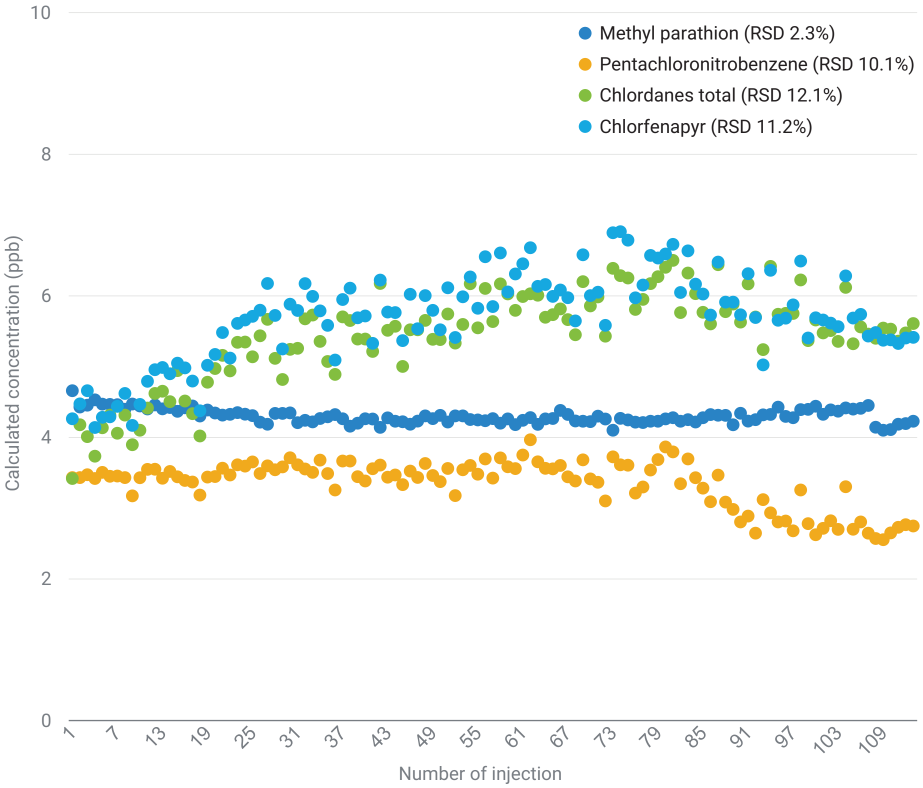


Figure 7. Precision in calculated concentration for the four pesticides postspiked in cannabis matrix at 4 ppb over 114 continuous injections.

Conclusion

Using the Agilent 8890/7010B TQ GC/MS system and abiding by best practices in sample preparation and TQ GC/MS analysis allowed for meeting or exceeding the reporting limits established in California and Canada for 27 pesticides that stand out as challenging to analyze using electrospray TQ LC/MS.

The calibration ranges allowed for accurate quantitation of both Inhalable and Other Cannabis Products as defined by the California Bureau of Cannabis Control, and Dry Cannabis defined by Health Canada. The rest of the pesticides regulated in California and Canada are analyzed by TQ LC/MS as described in application notes 5994-1743EN,[7] 5994-0648EN,[8] and 5994-0429EN.[9]

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DE.5699305556

Printed in the USA, June 17, 2020
5994-1786EN

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