

Analysis of Challenging Pesticides Regulated in the Cannabis and Hemp Industry with the Agilent Intuvo 9000-7010 GC/MS/MS system: The Fast-5

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Abstract

A brief review of the United States individual state and Health Canada pesticides lists, which are regulated in the cannabis and hemp industries, reveals approximately 100 compounds that require identification and quantification. Of these, at least 29 compounds stand out as challenging to analyze using electrospray LC/MS/MS especially in the myriad of sample types that require analysis, for example, dry flowers, concentrates, oils, gummies, etc. This list includes pentachloronitrobenzene (PCNB, also known as quintozone), captan, chlordanes, chlorfenapyr, and methyl parathion. This study developed a novel GC/MS/MS method for the analysis of the five pesticides, known as the Fast-5, and demonstrated excellent accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), range, and linearity.

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. These tests generally include potency determination, trace metals analysis, residual solvents and terpenes analysis, microbial screening, and mycotoxins. Of these assays, residual pesticide analysis is particularly challenging due to the very low LODs required by regulatory entities. This is in part due to the extremely heavy matrix, which includes hundreds of milligrams per gram of cannabinoids, terpenoids, and other endogenous compounds synthesized by *Cannabis spp.* Further complicating residual pesticide analysis is that many state and country target lists include pesticides that are not amenable to electrospray ionization (ESI), the most commonly used analytical tool for pesticide analysis in hemp, cannabis, and cannabinoid matrices when using LC/MS/MS.

In cases where a laboratory may only have access to LC/MS/MS systems, atmospheric pressure chemical ionization (APCI) in negative ionization mode has been suggested for the analysis of pesticides such as PCNB and chlordane. Curtis, *et al.*, have shown that, especially in the case of PCNB, negative ionization APCI tandem mass spectrometry results in nonselective, nonlinear precursor-product ion pairs with regression coefficients not acceptable in states such as California.¹ Previous studies demonstrated GC/MS/MS as extremely selective, sensitive, linear, and robust.² This study

challenges these assertions for the analysis of the Fast-5 pesticides in cannabis flower extracts and proves them to be correct. This investigation demonstrated LODs, LOQs, accuracy, precision, range, linearity, and interday and intraday percent quantitative accuracy using the Agilent Intuvo 9000-7010B GC/MS/MS system.

Experimental

Hardware and software

An Agilent Intuvo 9000-7010B GC/MS/MS system was used for all analyses. The GC was configured with the 7693 autosampler, a 0.791 m length Guard Chip, a MultiMode inlet (MMI), a mid-column backflush flow chip, and two Agilent 15 m × 0.25 mm, 0.25 μm HP-5MS UI columns. The tandem mass spectrometer was configured with the High Efficiency Source (HES) operated in electron ionization (EI) mode at 300 °C. The MRM data were collected in explicit time-segments. Tables 1 and 2 list the GC and MS parameters, respectively. Agilent MassHunter Workstation revision B.10 including MassHunter Acquisition, MassHunter Qualitative, and MassHunter Quantitative packages was used in this work. The Quant-My-Way package was used for data analysis. This utility simplifies data analysis within a cannabis-specific graphical interface.

There are four “Flavors” for the Quant-My-Way Cannabis UI:

- Cannabis by GC/MS/MS for Scientist
- Cannabis by LC/MS/MS for Scientist
- Cannabis by GC/MS/MS for Analyst
- Cannabis by LC/MS/MS for Analyst

The Scientist Flavors have full read-write capabilities for method development and troubleshooting, while the Analyst Flavors have a reduced set of features that allows routine analysis, data review, and reporting.

Table 1. Agilent Intuvo 9000 parameters.

Parameter	Value
Cycle Time Optimization	Column Autoclean
Oven Program	60 °C for 1 minute, then 40 °C/min to 170 °C (0 minute hold time), then 10 °C/min to 280 °C (0.25 minute hold time)
He Quench Gas	2.25 mL/min
N ₂ Collision Gas	1.5 mL/min
Injection Volume	2 μL
Injection Type	Two-layer sandwich (L1,L2)
L1 Airgap	0.2 μL
L2 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
MMI Program	Solvent vent
Septum Purge Flow	3 mL/minute in switched mode with a total flow of 54.3 mL/minute
Gas Saver	20 mL/min after 3 minutes
Purge Flow to Split Vent	50 mL/min at 1.5 minutes
Vent Flow	25 mL/min
Vent Pressure	5 psi for 0.3 minutes
Cryo Type	Air
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 (0.25 minute hold time)
Column 1	Agilent 19091S-431UI-HNT Constant flow: 1.3 mL/min Post run: -5.1052 mL/min In: MM Inlet He Out: PSD 1
Column 2	Agilent 19091S-431UI-HNT Constant flow: 1.5 mL/min Post run: 5.4194 mL/min In: PSD 1 Out: MSD
Guard Chip	791 mm Length × 0.547 mm id
Track Oven	On
BUS Temperature	280 °C
MSD Connector	310 °C
MSD Transfer Line	280 °C
PSD Purge	5 mL/min, Supplies Column 2

Table 2. Agilent 7010B parameters.

Time Segment	Start Time	Compound Name	ISTD	Precursor Ion	MS1 Resolution	Product Ion	MS2 Resolution	Dwell	Collision Energy	Gain
1	6.5	<i>alpha</i> -BHC-d ₆	Yes	224.0	Wide	187.0	Wide	25	15	20
1	6.5	<i>alpha</i> -BHC-d ₆	Yes	224.0	Wide	150.0	Wide	25	15	20
2	8	Pentachloronitrobenzene		248.7	Wide	213.9	Wide	12	15	20
2	8	Pentachloronitrobenzene		236.8	Wide	143.0	Wide	12	30	20
2	8	Pentachloronitrobenzene		213.7	Wide	178.9	Wide	12	15	20
2	8	Pentachloronitrobenzene		141.8	Wide	107.0	Wide	12	28	20
3	8.6	Parathion-methyl		262.9	Wide	79.0	Wide	9	30	20
3	8.6	Parathion-methyl		262.9	Wide	109.0	Wide	9	10	20
3	8.6	Parathion-methyl		125.0	Wide	79.0	Wide	9	30	20
3	8.6	Parathion-methyl		125.0	Wide	47.0	Wide	9	10	20
3	8.6	Parathion-methyl		109.0	Wide	79.0	Wide	9	30	20
4	9.3	Parathion-d ₁₀	Yes	301.0	Wide	83.0	Wide	25	35	20
4	9.3	Parathion-d ₁₀	Yes	301.0	Wide	115.0	Wide	25	15	20
5	10.4	Captan		149.0	Wide	70.0	Wide	15	15	20
5	10.4	Captan		149.0	Wide	79.1	Wide	15	10	20
5	10.4	Captan		116.9	Wide	82.0	Wide	15	30	20
6	10.85	Chlordane - I		377.0	Wide	267.8	Wide	7.5	25	20
6	10.85	Chlordane - I		375.0	Wide	265.8	Wide	7.5	25	20
6	10.85	Chlordane - I		372.8	Wide	265.8	Wide	7.5	25	20
6	10.85	Chlordane - I		372.8	Wide	263.8	Wide	7.5	25	20
6	10.85	Chlordane - I		371.0	Wide	263.8	Wide	7.5	25	20
6	10.85	Chlordane - I		271.9	Wide	236.9	Wide	7.5	15	20
7	11.15	Chlordane - II		377.0	Wide	267.8	Wide	7.5	25	20
7	11.15	Chlordane - II		375.0	Wide	265.8	Wide	7.5	25	20
7	11.15	Chlordane - II		372.8	Wide	265.8	Wide	7.5	25	20
7	11.15	Chlordane - II		372.8	Wide	263.8	Wide	7.5	25	20
7	11.15	Chlordane - II		371.0	Wide	263.8	Wide	7.5	25	20
7	11.15	Chlordane - II		271.9	Wide	236.9	Wide	7.5	15	20
8	11.8	Chlorfenapyr		362.8	Wide	246.8	Wide	9	25	20
8	11.8	Chlorfenapyr		327.8	Wide	246.8	Wide	9	15	20
8	11.8	Chlorfenapyr		249.0	Wide	112.0	Wide	9	30	20
8	11.8	Chlorfenapyr		246.9	Wide	227.0	Wide	9	15	20
8	11.8	Chlorfenapyr		137.0	Wide	102.0	Wide	9	15	20
9	13	Triphenyl Phosphate (SS)		326.1	Wide	233.0	Wide	16	20	20
9	13	Triphenyl Phosphate (SS)		326.1	Wide	215.1	Wide	16	20	20
9	13	Triphenyl Phosphate (SS)		325.0	Wide	169.0	Wide	16	20	20

The package also includes 12 Cannabis Analysis Reports (four Reports with three Versions):

- Compound Results Summary Report
- Calibration Report
- Sample Report with region specific “Out of Specification”, “Action Needed”, and “Fail” flags
- Complete Report with all three above Reports together

The regions include Canada and two regions for the United States.

Chemicals

SupraSolv acetonitrile, PCNB, methyl parathion, captan, *cis/trans*-chlordane, chlorfenapyr, L-gulonic acid γ -lactone (L-gulonolactone), and D-sorbitol, were obtained from Sigma-Aldrich. AOAC Method 2007.1 QuEChERS IS Solution containing isotopically labeled α -BHC- d_6 (α -HCH- d_6) and parathion- d_{10} , and triphenylphosphate (TPP) were obtained from Restek.³

Analyte protectants (APs)

As described in the Appendix, APs were prepared and used with each injection of calibrators, etc. in sandwich injection mode.

Data collection

Each batch was comprised of solvent blanks, matrix blanks, and eight levels of calibrators ranging from 0.016 through 64.00 ppb in vial (2.00 through 8,000.00 ppb in matrix). The internal standard used for quantitation of PCNB and methyl parathion was α -BHC- d_6 . Parathion- d_{10} was used as the IS for quantitation of the remaining compounds. TPP was added to each sample at constant concentration as a surrogate to monitor method performance over time. Quintuplicate injections were made for each. Three independent datasets were collected.

Statistics

The array of calibrators facilitated linearity and range determinations. Five replicate injections at each level permitted statistical calculations of MDL, LOQ, accuracy, precision, and average quantitative accuracy. This study used these primary equations:

- Average = $\sum X_i/n$
- Standard deviation, (s) = $[\frac{\sum(X-X)^2}{n-1}]^{1/2}$
- MDL = (s) \times (Student t-value, $n - 1$, 99% Confidence)
- LOQ = 10 \times (s)
- Calculated MDL < Spike Level < 10 \times Calculated MDL
- Percent Accuracy = 100 – [(spiked concentration – calculated average concentration/spiked concentration)] \times 100

- Precision, (%RSD) = [(s)/Average] \times 100
- Average Quantitative Accuracy = (calculated average concentration/spiked concentration) \times 100

The $n - 1$ degrees of freedom Student t-statistic was 3.747 at the 99% confidence level. The Calculated MDL < Spike Level < 10 \times Calculated MDL equation was used to evaluate the empirically determined MDL and ensure its validity.

Agilent consumables

Table 3 provides the consumables list for sample preparation, sample containment, and GC/MS supplies.

Table 3. Consumables list for sample preparation, sample containment, and GC/MS supplies.

Sample Preparation	Part Number
C18 Endcapped box, 30 \times 6 mL tubes, 500 mg SampliQ Solid Phase Extraction (SPE)	5982-1365
Ceramic homogenizers, 50 mL tubes, 100/pk	5982-9313
Corning tubes, 50 mL centrifuge tubes, 25/pk	5610-2049
Sample Containment	
Cap, screw, green, PTFE/red silicone septa, 100/pk	5182-0718
Vial, screw top, amber, write-on spot, deactivated (silanized), certified, 2 mL, 100/pk	5183-2072
Instrument Supplies	
ALS syringe, Blue Line, 10 μ L, fixed needle, 23/42/cone, PTFE-tip plunger	G4513-80220
Inlet septa, Advanced Green, nonstick, 11 mm, 50/pk	5183-4759
Inlet liner, Ultra Inert, splitless, dimpled, 2 mm id, 5/pk	5190-4006
6 compression bolts, Intuvo	G4581-60260
Intuvo polyimide gasket 5/pk	5190-9072
Guard Chip, Intuvo, multimode inlet, 2/pk	G4587-60665
Flow Chip, Intuvo, D2-MS midcolumn backflush	G4588-60322
Detector Tail, Intuvo, HES MS	G4590-60109
Separation	
2 Agilent J&W HP-5ms Ultra Inert Intuvo GC column modules, 15 m \times 0.25 mm, 0.25 μ m	19091S-431UI-INT

Sample preparation

1. Weigh 1.0 g of homogenized pesticide-free cannabis flower and unknown samples into a 50 mL polypropylene (PP) centrifuge tubes.
2. Add two ceramic homogenizers, and cap.
3. Add 15 mL of pesticide-grade acetonitrile to each tube from step 2.
4. Pipette the pesticide standard solutions for each calibrator level, and vortex for 30 seconds.
5. Mechanically shake the tubes for three to five minutes at high speed, ideally on a vertical shaking device (Geno/Grinder-type machine). This will extract the pesticides and mycotoxins into the acetonitrile.
6. While the tubes are shaking, prepare the SPE manifold by placing a SampliQ C18 EC 6 mL 500 mg SPE cartridge onto the manifold. Use collection tubes that can hold 25 mL or more. Ideally, use a graduated 50 mL polypropylene centrifuge tubes underneath each cartridge in which the eluent will be collected.
7. Decant the supernatant from step 5 into the SampliQ C18 EC SPE cartridge. It will flow by gravity.
8. After the entire solvent has gone through the C18 cartridge and is collected, add 5 mL of acetonitrile to the empty tube from step 5, and shake mechanically for three to five minutes at high speed. This will extract pesticides and mycotoxins that may still be in the cannabis material.
9. Decant the supernatant from step 8 into the same SampliQ C18 EC SPE cartridge.
10. Rinse the empty tube from step 8 with a final 5 mL of acetonitrile to wash any pesticides that might be retained on the tube wall, then pass this solvent through the same C18 cartridge. Bring the final volume to 25 mL using the 25 mL mark on the graduated 50 mL polypropylene centrifuge tube. This results in a 25-fold dilution factor.
11. Into a 2 mL vial, mix 25-fold diluted extract with 100% high purity, pesticide-grade acetonitrile in a 1-to-5 proportion, resulting in a 125-fold dilution factor. Vortex for 10 seconds. The sample is now ready for injection on the GC/MS/MS system.

Results and discussion

LOD and LOQ

Figure 1 illustrates the GC/MS/MS MRM chromatograms at or near the LOD for the compounds in the Fast-5 analytical method, and Figure 2 shows the calibration curves. Table 4 illustrates the empirical LOD and LOQ for each of the five analytes, and Table 5 shows the intraday and interday average quantitative accuracy.

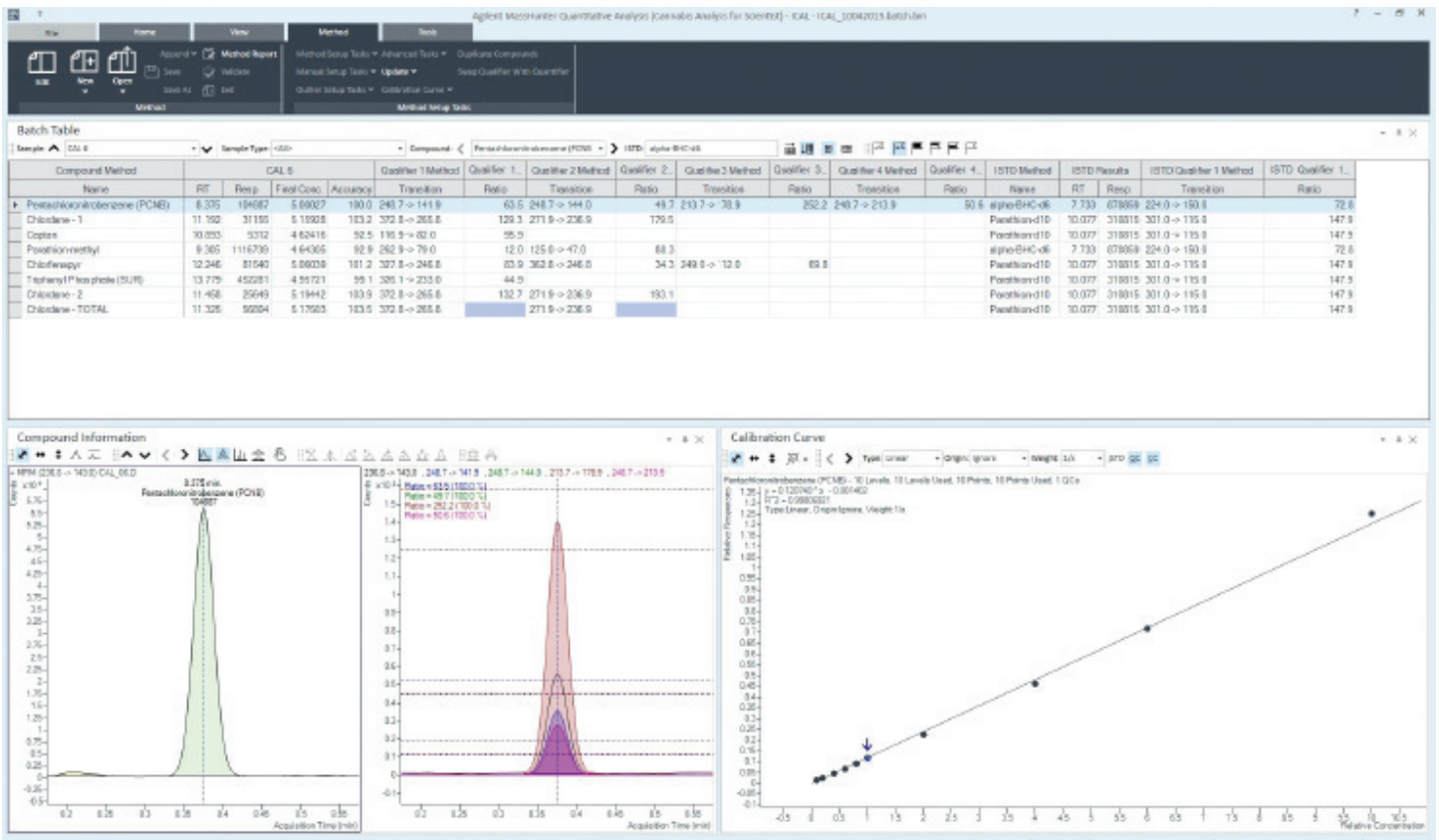


Figure 1. Quant-My-Way Cannabis GC/MS/MS Scientist Flavor.

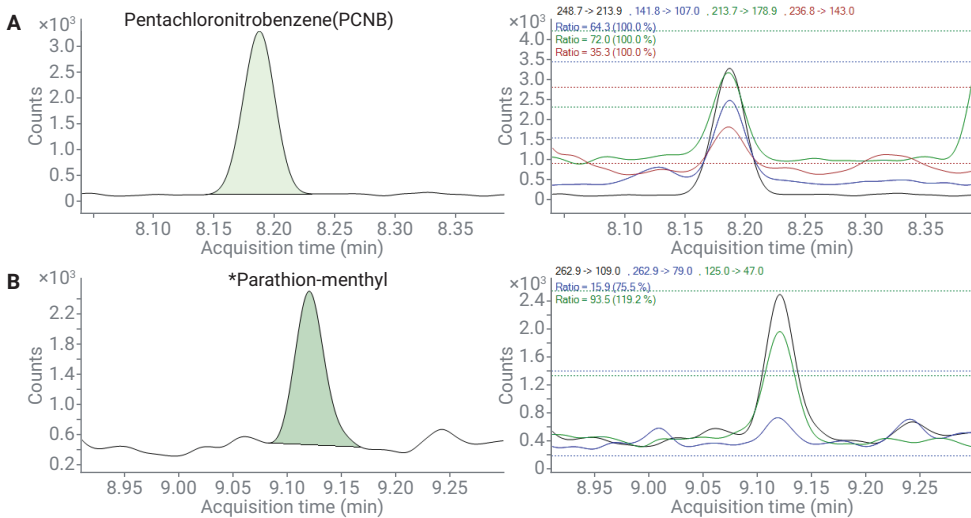


Figure 2. A) PCNB at 0.0625 ppb; B) methyl parathion at 0.016 ppb; C) captan at 1.00 ppb; D) chlordane-1 at 0.25 ppb; E) chlordane-2 at 0.25 ppb; F and G) chlorfenapyr at 0.25 ppb (continued on next page).

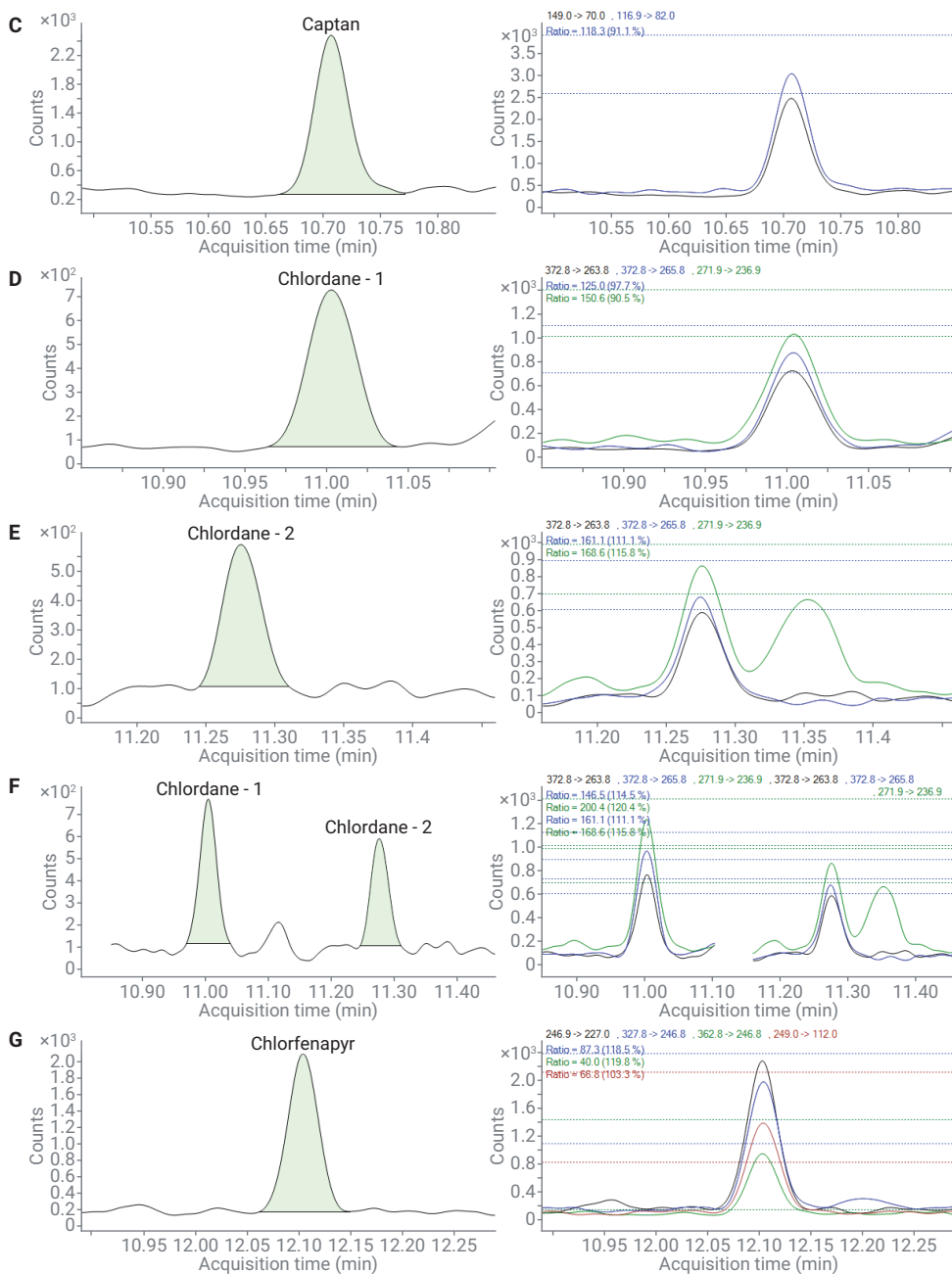


Figure 2. A) PCNB at 0.0625 ppb; B) methyl parathion at 0.016 ppb; C) captan at 1.00 ppb; D) chlordane-1 at 0.25 ppb; E) chlordane-2 at 0.25 ppb; F and G) chlorfenapyr at 0.25 ppb.

Accuracy and precision

Table 6 gives the interday accuracy and precision for each compound and total chlordanes.

Range and linearity

The range and linearity for each compound was determined by making five replicate injections at each of eight levels over the in-vial range of 0.016 to 64 ppb. Taking into consideration the empirical LOD, only captan required a truncated calibrator range, but is more than acceptable based on the current California LOQ of 700 ppb in matrix. Table 7 summarizes the results.

TPP Surrogate

TPP was used to monitor method performance in 120 samples. Its average area response was 27,745, with a standard deviation of 3701. The %RSD was 12.4.

Best practices

This study was designed to build upon previous pesticide work for cannabis flower performed on the Agilent 7890B-7010B GC/MS/MS system.² A significant difference was the use of the Intuvo 9000 GC. The added benefits of the Intuvo GC include a Guard Chip to help keep the system clean and extend column lifetime, and its many ease-of-use and eco-friendly features. Other differences from the previous work included the addition of chlorfenapyr, isotopically labeled internal standards, the inclusion of a surrogate, and changing the inlet injection modality from cold pulsed splitless to cold solvent vent.

Table 4. In-vial and in-matrix MDL and LOQ values.

Compound	CA Category	CA LOQ (ppb)	Empirical LOD In-Vial (ppb)	Empirical LOD In-Matrix (ppb)	Empirical LOQ In-Vial (ppb)	Empirical LOQ In-Matrix (ppb)
PCNB	II	100.00	0.061	7.59	0.16	20.25
Methyl Parathion	I	> LOD	0.031	3.88	0.084	10.50
Captan	II	700.00	1.64	204.75	4.37	546.38
Chlordane 1	I	> LOD	0.23	29.00	0.62	77.38
Chlordane 2	I	> LOD	0.26	32.75	0.70	87.38
Chlorfenapyr	I	> LOD	0.19	23.88	0.51	63.63

Table 5. Intraday and interday quantitative accuracy.

Average Quantitative Accuracy (%). Daily N = 5; Average N = 15				
Compound	Day 1	Day 2	Day 3	3-day Average
PCNB	74.18	92.07	105.95	90.73
Methyl Parathion	109.08	113.18	86.72	102.99
Captan	118.19	117.01	105.3	113.50
Chlordane 1	93.74	92.21	72.15	86.03
Chlordane 2	96.86	96.6	104.68	99.38
Chlorfenapyr	99.99	96.96	112.39	103.11

Table 6. Inter-day accuracy and precision. N = 15.

Compound	Target Calibration level (ppb)	Empirical Average and 99% confidence interval	Percent Accuracy	Precision (%RSD)
PCNB	0.25	0.23 ±0.019	91	6.96
Methyl Parathion	0.25	0.28 ±0.013	111	4.71
Captan	4.00	4.31 ±0.50	108	10.00
Chlordane 1	1.00	0.86 ±0.071	86	7.61
Chlordane 2	1.00	0.99 ±0.081	99	7.02
Total Chlordanes	1.00	0.93 ±0.076	93	7.32
Chlorfenapyr	1.00	1.02 ±0.059	102	5.08

Table 7. Range curve type and weighting for each of the analytes. All R² values were > 0.998.

Compound	In-Vial Range (ppb)	Number of calibrator levels	Curve	Weighting
PCNB	0.016 to 64.00	8	Linear	1/x
Methyl Parathion	0.016 to 64.00	8	Linear	1/x
Captan	1.00 to 64.00	5	Linear	1/x
Chlordane 1	0.016 to 64.00	8	Linear	1/x
Chlordane 2	0.016 to 64.00	8	Linear	1/x
Chlorfenapyr	0.016 to 64.00	8	Linear	1/x

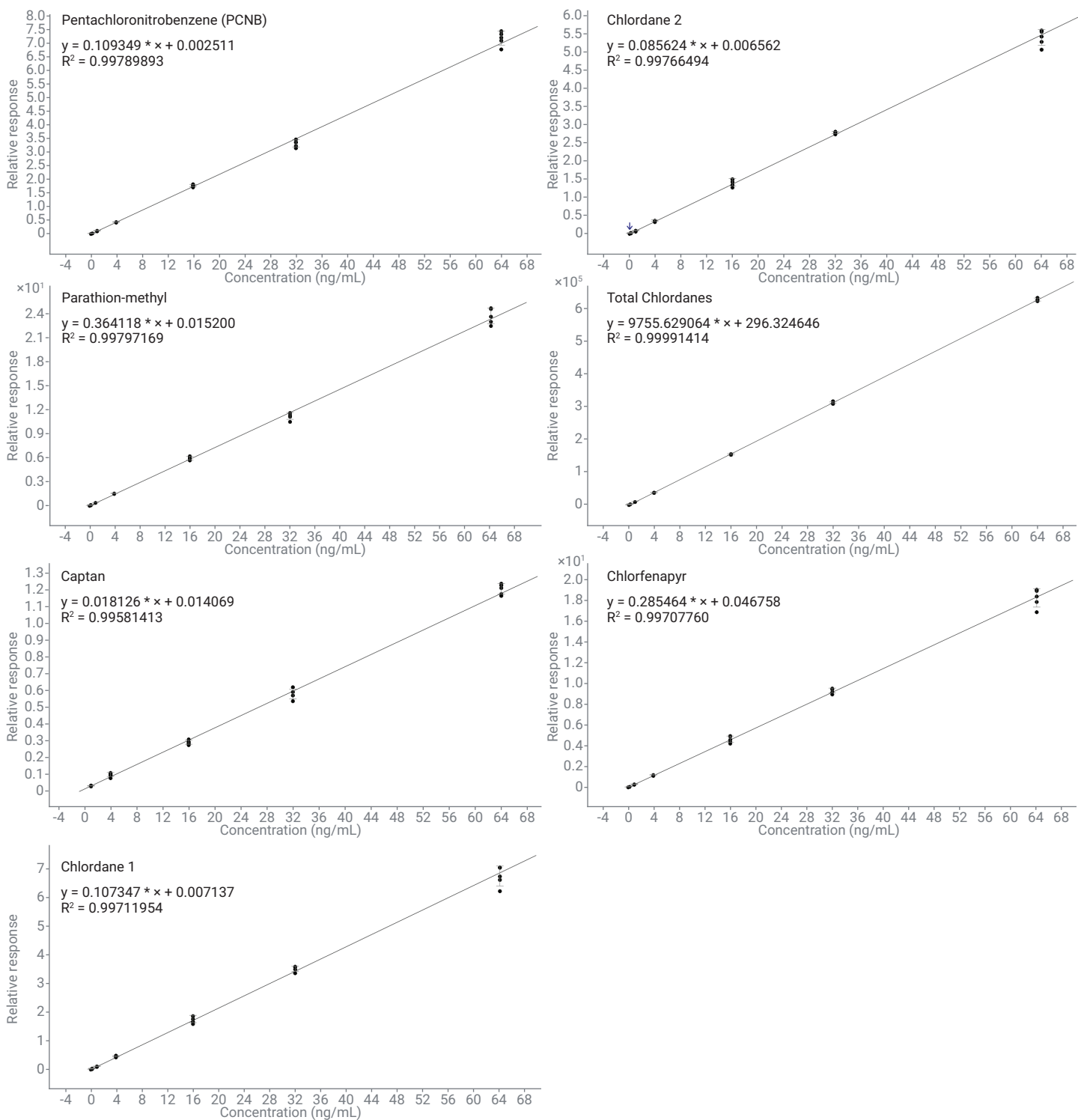


Figure 3. Calibration curves. Linear regression is fit to the 5 replicates at each of the calibration levels. The total chlordanes curve represents the sum of the five replicate injections at each of the eight calibrator levels for a total of 40 data points.

Conclusion

This study demonstrated the accuracy, precision, LODs, LOQs, range, and linearity for the Fast-5 cannabis pesticides using the Agilent Intuvo 9000-7010B GC/MS/MS system. The proven selectivity, sensitivity, and linearity far exceeds that reported for compounds such as PCNB when analyzed using LC/MS/MS in negative ionization APCI mode. The calibrator range of 2 to 8,000 ppb (125 to 8,000 ppb for captan) in matrix allows the quantification of both inhalable and other cannabis products as defined by the California Bureau of Cannabis Control⁴ in a single method. The best practices that have been added have greatly enhanced method reliability and robustness, leading to improved day-to-day production in cannabis laboratories. This method is to be used in conjunction with Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations by Stone *et al.*⁵

References

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Appendix

Analyte protectant preparation

- L-Gulonic acid γ -lactone (L-gulonolactone), CAS no.1128-23-0: >95% purity; Sigma-Aldrich
- (g) D-Sorbitol, CAS no. 50-70-4: >95%purity; Sigma-Aldrich

L-Gulonolactone stock solution

Weigh approximately 500 mg of L-gulonolactone in a 10 mL volumetric flask. Add 4 mL of water, then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(k) D-Sorbitol stock solution

Weigh approximately 500 mg of D-sorbitol into a 10 mL volumetric flask. Add 5 mL of water, then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(l) Analyte protectant (AP) solution (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol composite solution)

Add 4 mL of the L-gulonolactone stock solution and 2 mL of the D-sorbitol stock solution into a 10 mL volumetric flask, and bring to volume with acetonitrile.

For use with the GC/MS/MS system, dilute this mix 1:10 in acetonitrile, and put in position 2 on the autosampler rotating tray. Use the standard sandwich technique: 0.2 μ L air plug above and below, and 0.2 μ L of the protectants.

Keep refrigerated until use. Stored in the refrigerator, it lasts a month. On the rotator tray, it breaks down over time, and fresh solution will need to be replaced each three days on the tray or a loss of sensitivity or tailing will occur.

Setting up a sandwich injection

Place the vial containing analyte protectants in position L2 in the autosampler (2 mL in the vial) at the above concentration. Make a 2 μ L injection of sample, and a 0.2 μ L injection of analyte protectants. This acts as a type of matrix-matched standard. Use this to inject your standards and samples as well.

Alternatively, one can spike each vial with the analyte protectants; however, this increases sample preparation (it is recommended to let the autosampler do the work for you).

Injector

Injection
Syringe Size: 10 μ L

Injection Volume: 2 μ L

Washes and Pumps

	PreInj	PostInj	Volume (μ L)
Solvent A Washes:	0	3	Max (8)
Solvent B Washes:	0	3	Max (8)
Sample Washes:	3	3	
Sample Pumps:	3		

Dwell Time
Pre-Injection: 0 min
Post-Injection: 0 min

Sample Depth
 Enable 0 mm

Plunger Speed (Variable)
 Fast Slow Variable

	Draw	Dispense
Solvent Wash	300 μ L/min	3000 μ L/min
Sample Wash	75 μ L/min	300 μ L/min
Inject		3000 μ L/min

Viscosity Delay: 3 sec

Injection Type
2-layer Sandwich (L1,L2)

L1 air gap:	0.2 μ L
L2 volume:	0.2 μ L
L2 air gap:	0.2 μ L
L3 volume:	1 μ L
L3 air gap:	0.2 μ L

Total syringe volume used: 2.6 μ L




Figure A1. Two-layer sandwich injection setup.

Disclaimer

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