

Determination of Pesticides and Mycotoxins in Cannabis Flower as Defined by Legalized U.S. State Recreational Cannabis Regulations

(California, Michigan, Nevada, Oregon, and Washington)

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Introduction

Since the sanctioning of recreational cannabis use in various U.S. States in recent years, respective lawmakers have introduced their own unique State legislation. These State legislations detail minimum acceptable levels of specified pesticide and mycotoxin content allowed in potential retail material. Table 1 summarizes the specific requirements for pesticide and mycotoxin limits in cannabis flower in legalized U.S. States.

This application note details an LC/MS/MS analytical workflow developed by Agilent for the accurate measurement of combined pesticide and mycotoxin action lists according to the requirements of U.S. States that have legalized recreational marijuana. Not all legalized States have yet published analytical requirements, therefore this application note aims to cover the few that at time of writing describe specific legislation. The workflow illustrates sample preparation and analysis techniques uniquely applied to cannabis flower through to data review and reporting.

The information content of this application note, along with ready to run acquisition, quantitation, etc. methods and extensive support information are available as eMethods: Pesticides Residue and Mycotoxin Analysis in Cannabis and Hemp G5279#010 with the 1260/Ultivo LC/TQ system and G5279#020 with the 1260/6470 LC/TQ system.

Table 1. The Legalized list of pesticides and mycotoxins per Recreational U.S. State, and the defined action (not to exceed) levels for inhalable cannabis products. For California, specifically, Category I pesticide cannot be present at a concentration greater than the empirically determined instrumental limit of detection (LOD). CA Category I pesticides are denoted in Table 1 as >LOD and must be equal to or below 100 ppb in concentration. Not applicable (N/A) denotes when a State does not require the analyte to be tested.

Pesticide List	California (ppb)	Michigan (ppb)	Nevada (ppb)	Oregon (ppb)	Washington (ppb)
Avamectin B1a	100	500	200	500	500
Avamectin B1b	100	500	200	500	500
Acephate	100	400	N/A	400	400
Acequinocyl	100	2000	4000	2000	2000
Acetamiprid	100	200	N/A	200	200
Aldicarb	>LOD	400	N/A	400	400
Azoxystrobin	100	200	N/A	200	200
Bifenazate	100	200	400	200	200
Bifenthrin	3000	200	100	200	200
Boscalid	100	400	N/A	400	400
Captan	700	N/A	N/A	N/A	N/A
Carbaryl	500	200	N/A	200	200
Carbofuran	>LOD	200	N/A	200	200
Chlorantranilprole	10000	200	N/A	200	200
Chlordane	>LOD	N/A	N/A	N/A	N/A
Chlorfenapyr	>LOD	1000	N/A	1000	1000
Chlorpyrifos	>LOD	200	N/A	200	200
Clofentezine	100	200	N/A	200	200
Coumaphos	>LOD	N/A	N/A	N/A	N/A
Cyfluthrin	2000	1000	2000	1000	1000
Cypermethrin	1000	1000	1000	1000	1000
Daminozide	>LOD	1000	800	1000	1000
Diazinon	100	200	N/A	200	200
Dichlorvos (DDVP)	>LOD	1000	N/A	1000	100
Dimethoate	>LOD	200	N/A	200	200
Dimethomorph 1	2000	N/A	2000	N/A	N/A
Dimethomorph 2	2000	N/A	2000	N/A	N/A
Ethoprophos (Ethoprop)	>LOD	200	N/A	200	200
Etofenprox	>LOD	400	N/A	400	400
Etoxazole	100	200	400	200	200
Fenhexamid	100	N/A	1000	N/A	N/A
Fenoxycarb	>LOD	200	N/A	200	200
Fenpyroximate	100	400	N/A	400	400
Fipronil	>LOD	400	N/A	400	400
Flonicamid	100	1000	1000	1000	1000
Fludioxonil	100	400	500	400	400
Hexythiazox	100	1000	N/A	1000	1000
Imazalil	>LOD	200	N/A	200	200
Imidacloprid	5000	400	500	400	400
Kresoxim-methyl	100	400	N/A	400	400
Malathion	500	200	N/A	200	200
Metalaxyl	100	200	N/A	200	200
Methiocarb	>LOD	200	N/A	200	200

Pesticide List	California (ppb)	Michigan (ppb)	Nevada (ppb)	Oregon (ppb)	Washington (ppb)
Methomyl	1000	400	N/A	400	400
Methyl parathion	>LOD	200	N/A	200	200
Mevinphos	>LOD	N/A	N/A	N/A	N/A
MGK-264	N/A	200	N/A	200	200
Myclobutanil	100	200	400	200	200
Naled	100	500	N/A	500	500
Oxamyl	500	1000	N/A	1000	1000
Paclobutrazol	>LOD	400	400	400	400
Pentachloronitrobenzene	100	N/A	800	N/A	N/A
Permethrin	500	200	N/A	200	200
Phosmet	100	200	N/A	200	200
Piperonyl butoxide	3000	2000	3000	2000	2000
Prallethrin	100	200	N/A	200	200
Propiconazole	100	400	N/A	400	400
Propoxur	>LOD	200	N/A	200	200
Pyrethrin I	500	1000	2000	1000	1000
Pyrethrin II	500	1000	2000	1000	1000
Pyridaben	100	200	N/A	200	200
Spinetoram J	100	N/A	1000	N/A	N/A
Spinetoram L	100	N/A	1000	N/A	N/A
Spinosin A	100	200	1000	200	200
Spinosin D	100	200	1000	200	200
Spiromesifen	100	200	N/A	200	200
Spirotetramat	100	200	1000	200	200
Spiroxamine	>LOD	400	N/A	400	400
Tebuconazole	100	400	N/A	400	400
Thiacloprid	>LOD	200	N/A	200	200
Thiamethoxam	5000	200	400	200	200
Trifloxystrobin	100	200	1000	200	200

Experimental

Materials and reagents:

Pesticide and mycotoxin standards: Pesticide mixes representative of respective U. S. States were obtained from LGC USA at a concentration of 100 µg/mL, as were the mixed aflatoxin standards (B1, B2, G1, and G2). The ochratoxin A standard was obtained at a concentration of 2 µg/mL.

Other reagents

- Agilent InfinityLab Ultrapure LC/MS grade methanol (p/n 5191-4497)
- Millipore deionized water >18.2 mOhm, MilliporeSigma (Burlington, Massachusetts, USA)
- Formic acid (97+%)
- Ammonium formate (99+%)

Instrumentation

HPLC: Although most Agilent HPLC configurations can be used for this analysis, the following instruments were used:

- Agilent 1260 Infinity II binary pump (G7112B)
- Agilent 1260 Infinity II multisampler, thermostatted, with 100 µL loop and multiwash options (G7167A)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A with 6-port/2-position valve option #058)

It is imperative that vials 1 and 2 are replenished with 1 to 2 mL of mobile phase A every time prior to every batch run when using the 1260 Infinity II Multisampler.

To offset any extra time required for the injection program, it is recommended for high-throughput environments to perform overlapped injections. These injections should be initiated specifically at 12.0 minutes and started from the Agilent MassHunter worklist run parameters settings by checking the overlapped injection radio button. For this process to operate optimally, it is important to set the autosampler configuration for a 100 µL loop and 100 µL metering device.

All MRM parameters are detailed in Appendix A for Agilent 6470 (G6470AA) and Agilent Ultivo (G6465BA) units. All fragmentor voltage (Frag) settings, respective collision energies (CE), and most abundant/appropriate MS/MS product ions per analyte were determined and obtained using the Agilent MassHunter Optimizer software.

Sample preparation protocol for LC/MS triple quadrupole analysis

- One gram of chopped organic cannabis flower was transferred to a 50 mL polypropylene centrifuge tube.
- Two ceramic homogenizers (p/n 5982-9313) or stainless-steel beads were also placed in the tube, which was then capped.
- The tube was shaken mechanically for 5 minutes at high speed (vertical shaking on a Geno/Grinder-type machine) turning the plant content into fine powder.
- (For prespiked samples only and recovery studies, the pesticide standard solutions were added to the 15 mL used in step 5 at the appropriate concentrations).

HPLC method conditions

Parameter	Value																
Column	Agilent Poroshell 120 phenylhexyl, 3 × 100 mm, 2.7 µm (p/n 695975-312)																
Guard Column	Agilent Poroshell 120 phenylhexyl, 3 × 5 mm, 2.7 µm (p/n 821725-914)																
Column Temperature	55 °C																
Injection Volume	10 µL (with injector program/pretreatment, see Table 2)																
Autosampler Temperature	4 °C																
Needle Wash	Flushport (100% methanol), 10 seconds																
Mobile Phase	A) 5 mM ammonium formate/0.1% formic acid in water B) 0.1% formic acid in methanol																
Gradient Flow Rate	0.5 mL/min																
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>30</td> </tr> <tr> <td>1.00</td> <td>30</td> </tr> <tr> <td>2.00</td> <td>75</td> </tr> <tr> <td>8.00</td> <td>96</td> </tr> <tr> <td>9.00</td> <td>100</td> </tr> <tr> <td>9.50</td> <td>100</td> </tr> <tr> <td>9.51</td> <td>30</td> </tr> </tbody> </table>	Time (min)	%B	0.00	30	1.00	30	2.00	75	8.00	96	9.00	100	9.50	100	9.51	30
Time (min)	%B																
0.00	30																
1.00	30																
2.00	75																
8.00	96																
9.00	100																
9.50	100																
9.51	30																
Analysis And Re-equilibration Time	13 minutes																
Total Run Time (Sample to Sample)	13 to 14 minutes																

Table 2. Injector program/pretreatment.

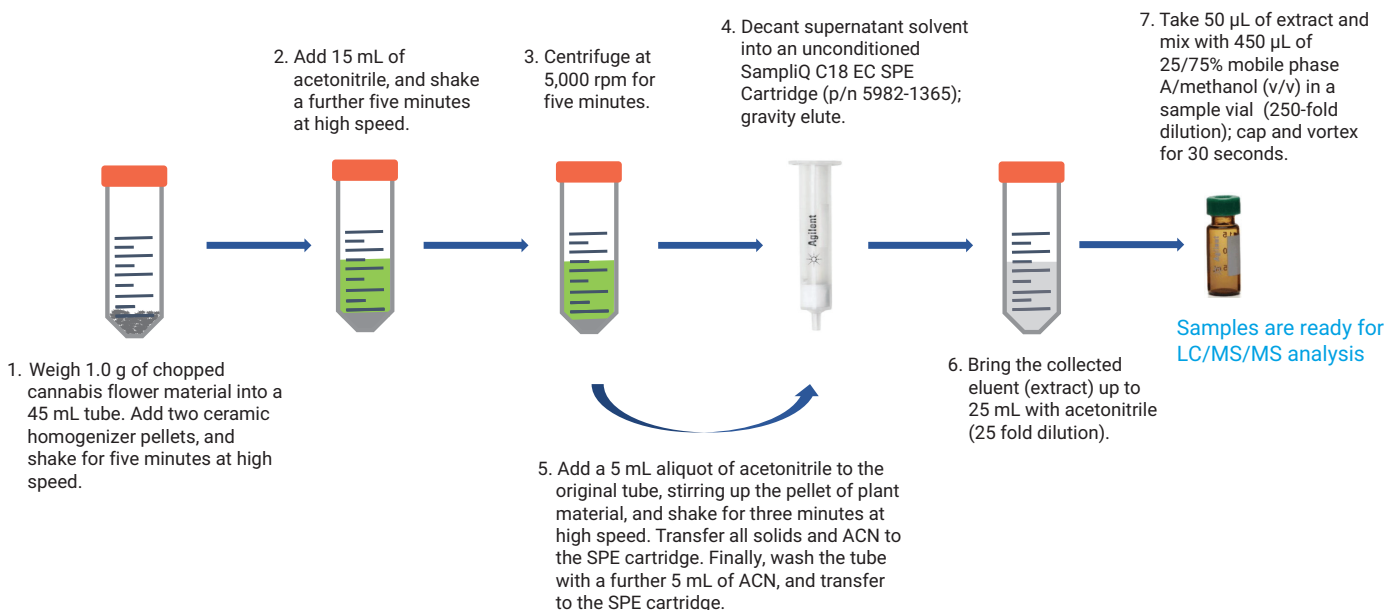
Step	Action	Description
1	Draw	Draw 10 µL from location 1 with default speed using default offset (100% mobile phase A)
2	Draw	Draw default volume from sample with default speed using default offset
3	Wash	Wash needle in flushport for 10 seconds (100% methanol)
4	Draw	Draw 10 µL from location 2 with default speed using default offset (100% mobile phase A)
5	Mix	Mix max volume from air with Max speed for five times
6	Inject	Inject

Mass spectrometer configuration and conditions

Configuration	
Agilent 6470 (G6470AA) or Agilent Ultivo (6465BA) QQQ Mass Spectrometer equipped with Agilent Jet Stream (AJS) ESI Source ²	
Ion Source Conditions	
Ion Mode	AJS ESI, positive and negative polarities
Capillary Voltage	5000 V (Positive ion mode) 3500 V (Negative ion mode)
Drying Gas (Nitrogen)	13 L/min
Drying Gas Temperature	200 °C
Nebulizer Gas (Nitrogen)	55 psi
Sheath Gas Temperature	200 °C
Sheath Gas Flow	10 L/min
Nozzle Voltage	500 V
Q1 and Q2 Resolution	0.7 amu [Unit, autotune]
Delta EMV	0 V (Not applicable to Ultivo 6465BA)

- Fifteen milliliters of LC/MS-grade acetonitrile was added to the tube from step 3.
- The tube and its contents were once more shaken mechanically for five minutes at high speed (vertical shaking on a Geno/Grinder). This shaking was for the extraction of pesticides and aflatoxins into the acetonitrile.
- The tube was then centrifuged at 5,000 rpm for 5 minutes.
- While the tube was being centrifuged, SampliQ C18 EC 6 mL, 500 mg solid phase extraction (SPE) cartridges (p/n 5982-1365) were placed on an SPE manifold or rack (p/n 5191-4104 top and p/n 5191-4112 bottom). To collect the cleaned-up eluent, a collection tube of 25 mL or more capacity was placed underneath the cartridge.
- The supernatant from step 7 was decanted into the SampliQ C18 SPE cartridge. Flow through the cartridge was by gravity. When all solvent had completely passed through the C18 cartridge, the tube and plant pellet from step 7 was mixed with 5 mL of acetonitrile. The plant pellet was then agitated to bring it into a suspension once again and was shaken for three minutes. The contents of the tube were then poured into the same C18 SPE cartridge, and the cleaned eluent collected. Care must be taken before each addition of 5 mL acetonitrile that the surface of the SPE cartridge has become dry. A further 5 mL of acetonitrile was added to the empty tube, vortexed for 30 seconds, and added to the SPE cartridge. This resulted in just under 25 mL volume of cleaned acetonitrile extract, which was made up to 25 mL using the graduations on the outside of the tube by directly adding acetonitrile to the collection tube and not passing it through the SPE cartridge.
- Fifty microliters of eluent from step 9 were added to 450 μ L of mobile phase A/methanol (25%/75% v/v) in a 2 mL sample vial, and capped.
- This 10 \times dilution was vortexed for 20 seconds and was then ready for LC/MS injection.
- For samples, this solution was injected directly into the LC/TQ. For matrix calibrations or postextraction recovery studies, the desired amounts of pesticide and mycotoxins were spiked into the solution at this point.

The sample preparation steps outlined constitute a resultant 1/250 total dilution and are outlined schematically in Figure 1.



These sample preparation steps constitute a resultant 1/250 total dilution.

Figure 1. LC/MS sample preparation—cannabis flower SPE cleanup and dilution (All States).

Results and discussion

Matrix extract calibration standards were prepared down to low part per trillion (ppt) actual levels. This was so the lower limits of quantitation (LLOQ) could be determined and related back to the legislative requirements for all Recreationally legal States. This was

necessary since the outlined sample preparation routine effectively dilutes the original plant material by effectively 250x. Given that most stringent limits are effectively 100 ppb and higher, depending on the analyte concerned, the instrumental detection lower limits would need to be lower than 200 ppt for the most challenging analytes.

Figure 2 illustrates the pesticide mix spiked into matrix, and each analyte overlaid together with aflatoxins B1, B2, G1, G2, and ochratoxin A at an actual concentration of 500 ppt, relating to an original pre-extraction concentration of 125 ppb.

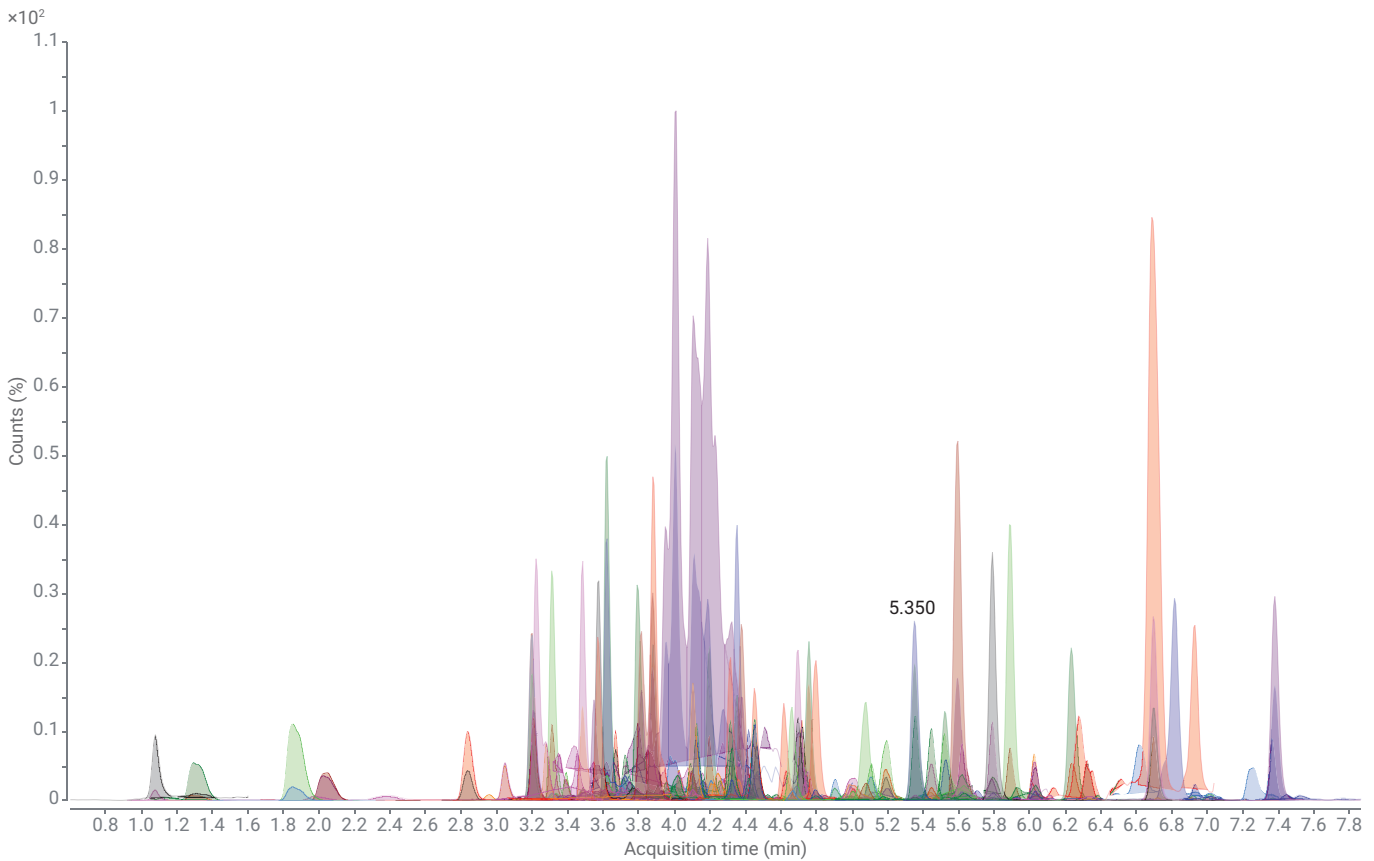


Figure 2. Overlaid chromatograms of typical pesticides list and mycotoxins in extracted flower matrix, actual concentration 500 ppt (pre-extraction concentration = 125 ppb.)

Typical matrix calibration curves and LLOQ chromatography observed and obtained through this sample preparation routine are illustrated in Figures 3 and 4 (A, B, and C), respectively. Linear correlation values (R^2) for the spiked pesticides and mycotoxins were 0.990 or higher.

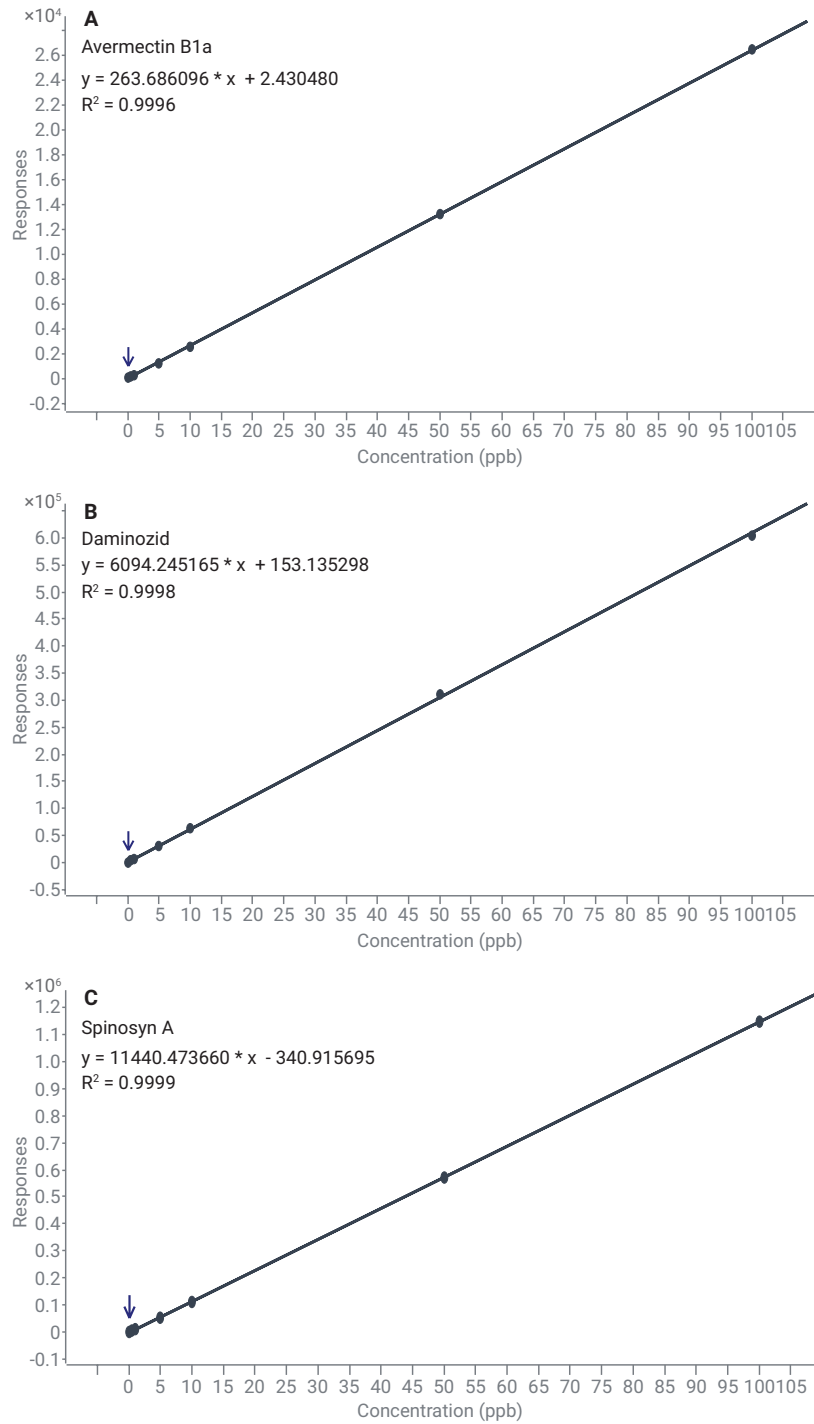


Figure 3. Example calibration curves.

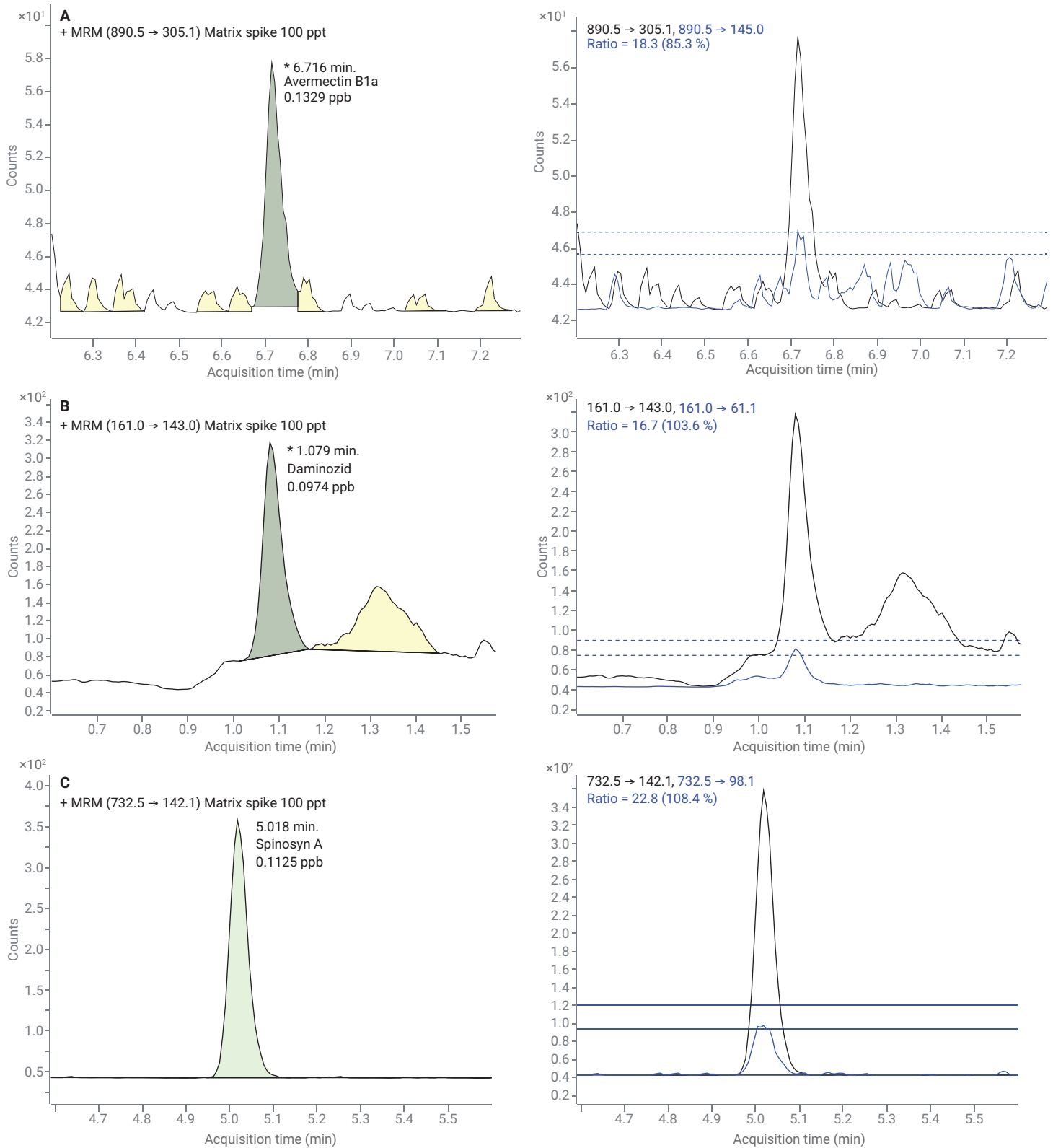


Figure 4. Examples of chromatography near LLOQ. A) Avermectin B1a, 0.1 ppb (ng/mL). B) Daminozide, 0.1 ppb (ng/mL). C) Spinosyn A, 0.1 ppb (ng/mL).

Table 3 outlines observed LLOQ results for pesticides obtained from multiple batches of cannabis flower prepared as outlined in the sample preparation section of this Application Note. The table for some U.S. State action lists

contains four analytes, which need to be analyzed using GC/MS/MS techniques due to a lack of functional groups required to invoke a true molecular ion adduct through LC/MS/MS. These are labeled in the LLOQ column as GC/MS,

and References 1 and 2 outline the techniques and methods required to analyze them successfully. Table 4 summarizes the typical LLOQ values obtained for mycotoxins listed with current U.S. State action levels.

Table 3. Typical pesticide LLOQ results obtained as a mean from multiple (n = 5) batches of cannabis flower and prespiked into the sample extract before the SPE extraction and dilution routine described previously. Analytes typically responding more reliably through GC/MS are denoted in the LLOQ column as GC/MS.

Pesticide List	California (ppb)	Michigan (ppb)	Nevada (ppb)	Oregon (ppb)	Washington (ppb)	LLOQ with 10 μ L injection Volume (Original Plant Concentration) (ppb)
Avamectin B1a	100	500	200	500	500	50
Avamectin B1b	100	500	200	500	500	50
Acephate	100	400	N/A	400	400	25
Acequinocyl	100	2000	4000	2000	2000	2.5
Acetamiprid	100	200	N/A	200	200	2.5
Aldicarb	>LOD	400	N/A	400	400	5
Azoxystrobin	100	200	N/A	200	200	5
Bifenazate	100	200	400	200	200	50
Bifenthrin	3000	200	100	200	200	5
Boscalid	100	400	N/A	400	400	50
Captan	700	N/A	N/A	N/A	N/A	(GC/MS)
Carbaryl	500	200	N/A	200	200	25
Carbofuran	>LOD	200	N/A	200	200	25
Chlorantraniliprole	10000	200	N/A	200	200	25
Chlordane	>LOD	N/A	N/A	N/A	N/A	(GC/MS)
Chlorfenapyr	>LOD	1000	N/A	1000	1000	250 (GC/MS)
Chlorpyrifos	>LOD	200	N/A	200	200	25
Clofentezine	100	200	N/A	200	200	2.5
Coumaphos	>LOD	N/A	N/A	N/A	N/A	5
Cyfluthrin	2000	1000	2000	1000	1000	50
Cypermethrin	1000	1000	1000	1000	1000	50
Daminozide	>LOD	1000	800	1000	1000	25
Diazinon	100	200	N/A	200	200	2.5
Dichlorvos (DDPV)	>LOD	1000	N/A	1000	100	50
Dimethoate	>LOD	200	N/A	200	200	25
Dimethomorph 1	2000	N/A	2000	N/A	N/A	25
Dimethomorph 2	2000	N/A	2000	N/A	N/A	25
Ethoprophos (Ethoprop)	>LOD	200	N/A	200	200	25
Etofenprox	>LOD	400	N/A	400	400	5
Etoxazole	100	200	400	200	200	25
Fenhexamid	100	N/A	1000	N/A	N/A	50
Fenoxycarb	>LOD	200	N/A	200	200	5
Fenpyroximate	100	400	N/A	400	400	25
Fipronil	>LOD	400	N/A	400	400	5
Fonicamid	100	1000	1000	1000	1000	25
Fludioxonil	100	400	500	400	400	25

Pesticide List	California (ppb)	Michigan (ppb)	Nevada (ppb)	Oregon (ppb)	Washington (ppb)	LLOQ with 10 µL injection Volume (Original Plant Concentration) (ppb)
Hexythiazox	100	1000	N/A	1000	1000	5
Imazalil	>LOD	200	N/A	200	200	50
Imidacloprid	5000	400	500	400	400	2.5
Kresoxim-methyl	100	400	N/A	400	400	5
Malathion	500	200	N/A	200	200	100
Metalaxyl	100	200	N/A	200	200	25
Methiocarb	>LOD	200	N/A	200	200	50
Methomyl	1000	400	N/A	400	400	25
Methyl parathion	>LOD	200	N/A	200	200	250 (GC/MS)
Mevinphos	>LOD	N/A	N/A	N/A	N/A	50
MGK-264	N/A	200	N/A	200	200	25
Myclobutanil	100	200	400	200	200	50
Naled	100	500	N/A	500	500	50
Oxamyl	500	1000	N/A	1000	1000	0.5
Paclobutrazol	>LOD	400	400	400	400	25
Pentachloronitrobenzene	100	N/A	800	N/A	N/A	(GC/MS)
Permethrin	500	200	N/A	200	200	50
Phosmet	100	200	N/A	200	200	5
Piperonyl butoxide	3000	2000	3000	2000	2000	5
Prallethrin	100	200	N/A	200	200	25
Propiconazole	100	400	N/A	400	400	25
Propoxur	>LOD	200	N/A	200	200	25
Pyrethrin I	500	1000	2000	1000	1000	50
Pyrethrin II	500	1000	2000	1000	1000	50
Pyridaben	100	200	N/A	200	200	5
Spinetoram J	100	N/A	1000	N/A	N/A	25
Spinetoram L	100	N/A	1000	N/A	N/A	25
Spinosin A	100	200	1000	200	200	5
Spinosin D	100	200	1000	200	200	50
Spiromesifen	100	200	N/A	200	200	25
Spirotetramat	100	200	1000	200	200	25
Spiroxamine	>LOD	400	N/A	400	400	25
Tebuconazole	100	400	N/A	400	400	25
Thiacloprid	>LOD	200	N/A	200	200	25
Thiamethoxam	5000	200	400	200	200	25
Trifloxystrobin	100	200	1000	200	200	2.5

Table 4. Typical mycotoxin LLOQ results obtained as a mean from multiple batches (n = 5) of cannabis flower and prespiked into the sample extract before the described SPE extraction and dilution routine. These are displayed against each respective State regulation

Mycotoxin List	California (ppb)	Michigan (ppb)	Nevada (ppb)	Oregon (ppb)	Washington (ppb)	LLOQ with 10 µL Injection of Original Plant Concentration (ppb)
Aflatoxin G1	Total amount of Aflatoxins not to exceed 20 ppb	Total amount of Aflatoxins not to exceed 20 ppb	Total amount of Aflatoxins not to exceed 20 ppb	N/A	Total amount of Aflatoxins not to exceed 20 ppb	3
Aflatoxin G2				N/A		3.5
Aflatoxin B1				N/A		3
Aflatoxin B2				N/A		3
Ochratoxin A	20	20	20	N/A	20	7

Sample preparation and autosampler pretreatment discussion

Recovery data were gathered for the sample preparation routine outlined in this application note and displayed in Table 5. Prespiked negative cannabis flower and nonspiked negative flower were ground, solvent-extracted, and cleaned up using SPE as outlined in the sample preparation experimental section. The nonspiked extracts from this routine were then spiked at set levels, and the percentage recovery of the pre-and post-spiked matrix-matched samples was calculated for every analyte with the following equation using single point calibrations:

$$\% \text{ Recovery} = \frac{\text{Pre} - \text{SPE spiked sample}}{\text{Post} - \text{SPE spiked sample}} \times 100$$

An important aspect of the sample preparation routine to note is the nature and composition of the diluent used in the final dilution step outlined in the experimental section stage 7 of Figure 1.

Many of the analytes in State action lists are highly nonpolar and can precipitate out of solution when the aqueous content of the diluent is sufficiently high, yielding extremely poor recoveries for these analytes. For this reason, the composition of the final diluent was investigated from a recovery point of view. This investigation determined that the aqueous content of that diluent could be no higher than 25% v/v for the final dilution.

Table 5. Sample preparation percent recoveries observed for each actioned pesticide from five separate batches (n = 5).

Pesticide List	Percent Recovery at 60 ppb
Abamectin B1a	92.5
Abamectin B1b	113.4
Acephate	91.9
Acequinocyl	94.2
Acetamiprid	94.9
Aldicarb	93.7
Azoxystrobin	95.2
Bifenazate	98.8
Bifenthrin	98.0
Boscalid	104.2
Carbaryl	95.7
Carbofuran	94.9
Chlorantraniliprole	98.2
Chlorfenapyr	102.4
Chlorpyrifos	96.4
Clofentezine	100.8
Coumaphos	106.8
Cyfluthrin	97.7
Cypermethrin	96.3
Daminozid	88.4
DDVP (Dichlorvos)	97.3
Diazinon	97.1
Dimethomorph I	107.6
Dimethomorph II	108.2
Dimethoate	97.9
Ethoprophos	103.0
Etofenprox	101.1
Etoxazole	98.6
Fenhexamid	131.5
Fenoxycarb	102.4
Fenpyroximate	103.2
Fipronil	90.6
Fonicamid	97.9
Fludioxonil	107.5

Pesticide List	Percent Recovery at 60 ppb
Hexythiazox	106.3
Imazalil	99.1
Imidacloprid	97.8
Kresoxim-methyl	103.7
Malathion	100.5
Metalaxyl	98.2
Methiocarb	102.7
Methomyl	96.5
Methyl parathion	110.4
Mevinphos	103.9
MGK-264	109.4
Myclobutanil	104.9
Oxamyl	97.0
Paclobutrazol	106.9
Permethrins*	96.8
Phosmet	101.9
Piperonylbutoxide	100.5
Prallethrin	98.1
Propiconazole	104.7
Propoxur	99.2
Pyrethrins†	70.8
Pyridaben	101.0
Spinetoram L	108.6
Spinetoram J	102.4
Spinosin A	101.2
Spinosin D	96.5
Spiromesifen	99.0
Spirotetramat	99.3
Spiroxamine	97.4
Tebuconazole	105.5
Thiacloprid	100.4
Thiamethoxam	97.2
Trifloxystrobin	100.8

Table 6. Average percent recoveries for each mycotoxin (n = 5).

Mycotoxin List	% Recovery at 4 ppb
Aflatoxin G1	102.8
Aflatoxin G2	102.7
Aflatoxin B1	104.8
Aflatoxin B2	102.3
Ochratoxin A	100.5

Injector pretreatment

For reversed-phase chromatography, such a high composition of organic solvent in the sample to be injected (in this case methanol at 75% v/v) can and will result in splitting or smearing the early-eluting analyte peaks upon normal injection. To counter this effect and to keep peak shape and symmetry acceptable for all analytes in this method, an injector pretreatment routine is required, and is outlined in Table 2.

This pretreatment routine effectively dilutes the 75% methanol in the sample when injected by sandwiching it between two equal 10 µL volumes of mobile phase A, mixing this together and effectively diluting it *in situ* to approximately 75/25% v/v aqueous/methanol. The chromatography

gradient composition starts at 30% methanol composition, and peak smearing/splitting is avoided, thus, acceptable peak shapes and symmetry is maintained across the complete chromatographic analysis.

Overlapped injections

To avoid the extra time needed for sample pretreatment in this manner, it is possible to preload samples at the re-equilibration period at the end of the chromatographic gradient by selecting the overlapped injection option. This option is available on all Agilent LC/MS/MS systems and configurations. For this methodology, it is recommended to invoke this function at or after 12.0 minutes to ensure that no retention time shift occurs in subsequent samples injected.

Review and reporting

Agilent LC/MS/MS and GC/MS/MS instruments use the same MassHunter Quantitation software for data review and reporting. This optimizes lab productivity and operators' ease-of-use. To allow for review by exception, MassHunter Quantitation software enables quick and efficient batch processing using outlier settings per analyte. This approach automatically flags any sample or individual analyte and draws the reviewer's attention to anything that may not be within designated limits. Figure 5 illustrates these outlier flags. The red color designates a value above accepted outlier limits, while blue denotes results below the required outlier limits. The batch shown in Figure 5 is for illustrative purposes only and not representative of the results outlined in this application.

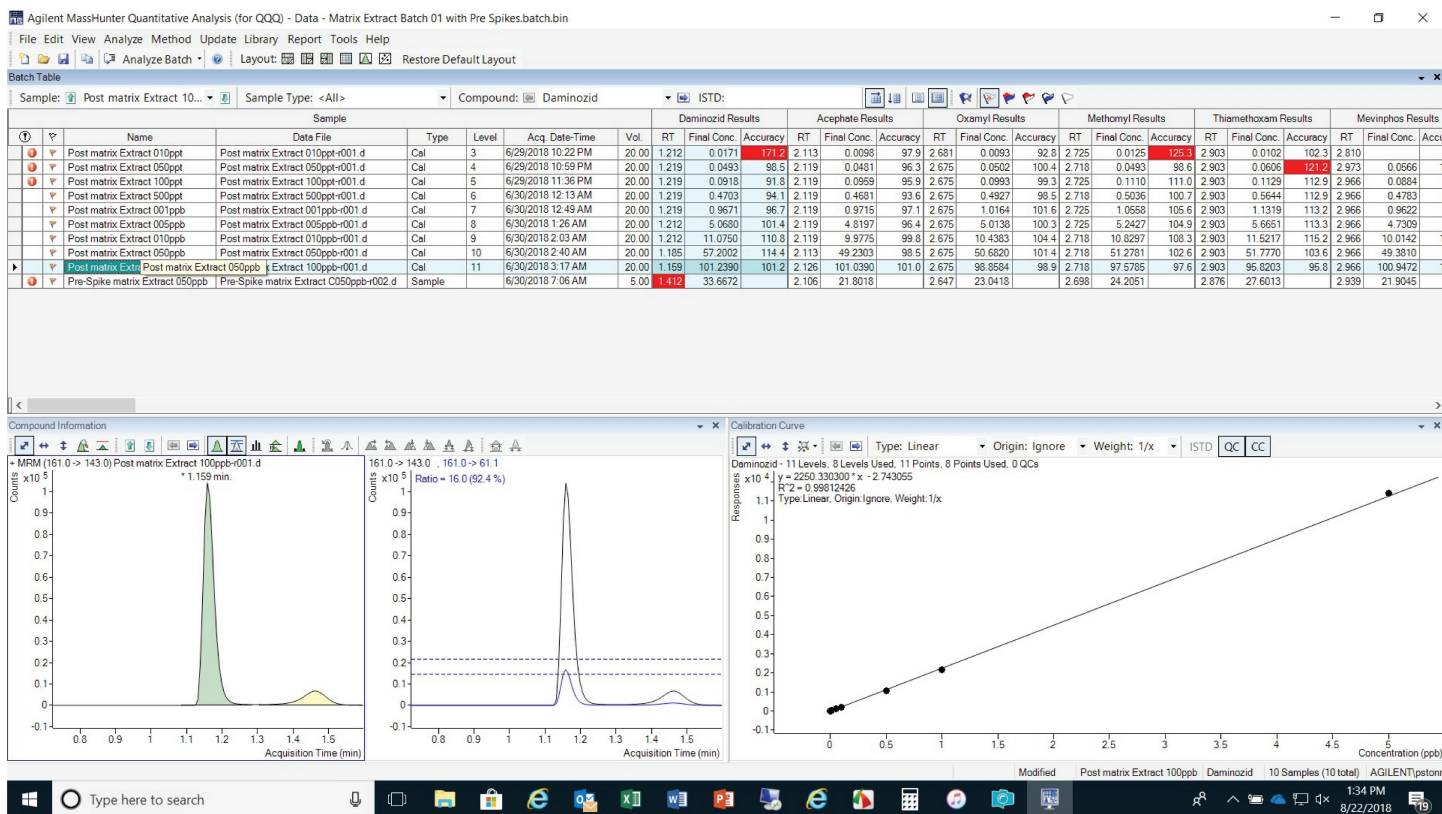


Figure 5. Agilent MassHunter Quantitative Analysis, review by exception batch review.

MassHunter offers the ability to tailor the analysis interface to the application with the Quant-My-Way functionality. Two preset configurations, or flavors, have been developed to meet the needs of cannabis method development, data processing and review, as well as reporting for LC/MS or GC/MS.

First, the Scientist level has complete method setup, batch review, and reporting capabilities for each instrument technique (gas phase or liquid phase.)

Second, the Analyst level has a simplified and uncluttered graphical user interface (GUI), for use in the daily production environment. In this level, batch review and report generation are only allowed from predefined data review criteria, methods, and templates, which are set by the Scientist-designated personnel. Using these different GUI choices, a laboratory can more easily control how data are processed and reported in a more controlled environment.

Custom report templates that have been specifically designed for the cannabis analysis requirements of each geographic region are also available as an integral element of the MassHunter Quantitation software. Figure 6 shows an example of this.

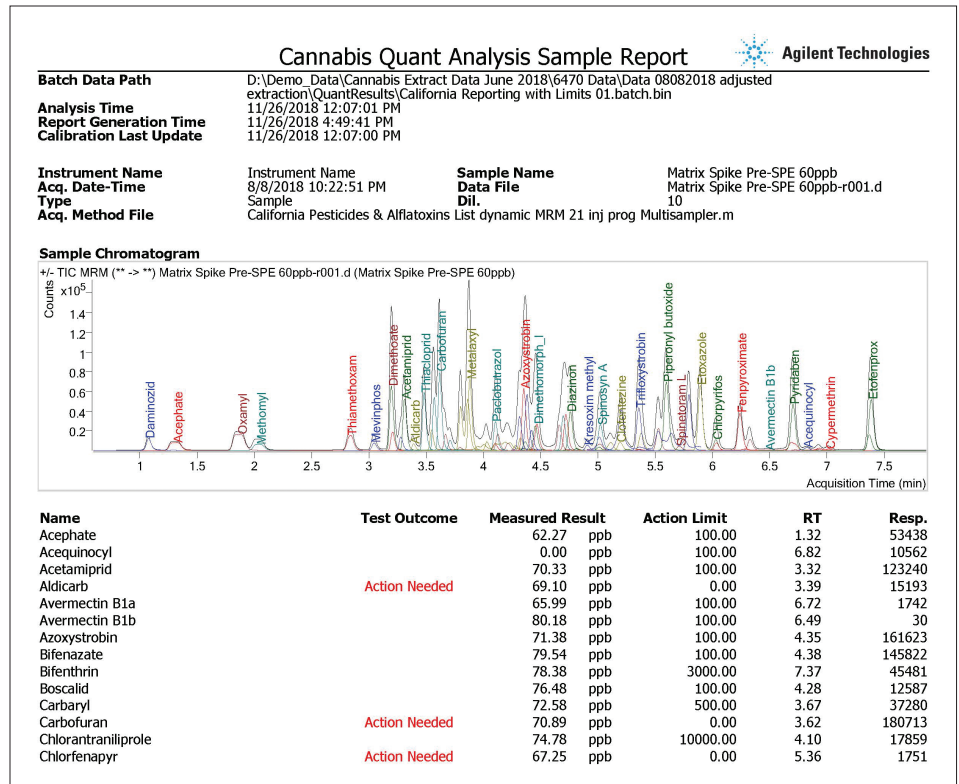


Figure 6. Example of cannabis reporting templates.

Conclusion

This application note describes a robust LC/MS/MS method and sample preparation workflow that reliably meets down to 50% of the respective States' legalized recreational legislative action limits for pesticide and mycotoxin content for cannabis dried flower samples. It uses either the Agilent 6470 (G6470AA) or the Agilent Ultivo (G6465BA) LC-mass spectrometers, which yield similar results. This methodology complements other techniques, which are necessary for a handful of the action list items listed for some U.S. States (captan, chlordane, PCNB, and methyl parathion). These analytes are more reliably analyzed using GC/MS/MS techniques such as that outlined in Agilent application notes 5994-1019EN¹ and 5994-1604EN.²

Sample preparation used a simple gravity-fed SPE filtration approach, recoveries from which were all between 70 to 130%, as required by most State legislation. In addition, most recoveries were close to 100% using the unique SampliQ C18 EC SPE cartridges and routines outlined in the experimental section. The unique ability to use an injector pretreatment routine for injection handling and final preparation of samples adds to the high percent recoveries while allowing for excellent chromatographic peak shapes across the entire analysis gradient.

A two-level graphical user interface approach has been created (if required), consisting of the Scientist and Analyst levels, for seamless data review and method creation using MassHunter Quantitation and batch processing software. This specifically allows a quality testing laboratory to assign access roles and simplify workflows for data review and reporting within its workforce based on access level to methodology and ability levels.

Custom reporting templates are available as standard with MassHunter software, and are focused on regions or states, depending on local requirements.

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Disclaimer

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state and country law.

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1. Honnold, R.; *et al.* A Fast Analysis of the GC/MS/MS Amenable Pesticides Regulated by the California Bureau of Cannabis Control, *Agilent Technologies application note*, publication number 5994-1019EN, **2019**.
2. Hollis, J. S.; *et al.* Analysis of Challenging Pesticides Regulated in the Cannabis and Hemp Industry with the Agilent Intuvo 9000-7010 GC/MS/MS system: The Fast-5, *Agilent Technologies application note*, publication number 5994-1604EN, **2019**.

Appendix A: Agilent 6470 (G6470AA) and Agilent Ultivo (G6465BA) transitions for pesticides and mycotoxins, with an Agilent 1260 Infinity II HPLC

Cell acceleration voltage (CAV) is not required for Ultivo LC/MS instruments

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Retention Time (min)	Retention Time Window (min)	Polarity
Acephate	184	143	60	5	4	1.25	1	Positive
Acephate	184	95	60	20	4	1.25	1	Positive
Acequinocyl	402.3	343.2	90	10	4	8.45	1	Positive
Acequinocyl	402.3	189.1	90	41	4	8.45	1	Positive
Acequinocyl	385.3	189.1	90	41	4	8.45	1	Positive
Acetamiprid	223	126.1	100	20	3	3.48	1	Positive
Acetamiprid	223	90.1	100	35	3	3.48	1	Positive
Aflatoxin B1	313.1	285.1	130	20	3	3.60	2	Positive
Aflatoxin B1	313.1	241.1	130	35	3	3.60	2	Positive
Aflatoxin B2	315.1	287.1	130	25	3	3.40	2	Positive
Aflatoxin B2	315.1	259.1	130	25	3	3.40	2	Positive
Aflatoxin G1	329.1	311.1	130	20	3	3.35	2	Positive
Aflatoxin G1	329.1	243.1	130	25	3	3.35	2	Positive
Aflatoxin G2	331.1	285.1	130	25	3	3.25	2	Positive
Aflatoxin G2	331.1	245.1	130	30	3	3.25	2	Positive
Aldicarb	116	89.1	50	4	3	3.57	1	Positive
Aldicarb	116	70.1	50	4	3	3.57	1	Positive
Avermectin B1a	890.5	567.1	160	8	4	7.01	1	Positive
Avermectin B1a	890.5	305.1	160	28	4	7.01	1	Positive
Avermectin B1a	890.5	145	160	45	4	7.01	1	Positive
Avermectin B1b	876.6	553.2	160	7	4	6.7	4	Positive
Avermectin B1b	876.6	291.1	160	15	4	6.7	4	Positive
Azoxystrobin	404	372.2	100	10	3	4.66	1	Positive
Azoxystrobin	404	344	100	25	3	4.66	1	Positive
Bifenazate	301.1	198.2	80	5	3	4.7	1	Positive
Bifenazate	301.1	170.1	80	15	3	4.7	1	Positive
Bifenthrin	440.1599	181.1012	90	5	5	7.65	1	Positive
Bifenthrin	440.1599	166	90	20	5	7.65	1	Positive
Boscalid	343.0399	307.0633	140	12	5	4.59	1	Positive
Boscalid	343.0399	271	140	28	5	4.59	1	Positive
Carbaryl	202	145	70	0	3	3.92	1	Positive
Carbaryl	202	127.1	70	25	3	3.92	1	Positive
Carbofuran	222.1	165.1	90	5	3	3.85	1	Positive
Carbofuran	222.1	123.1	90	20	3	3.85	1	Positive
Chlorantraniliprole	483.9	452.9	100	15	3	4.4	1	Positive
Chlorantraniliprole	483.9	285.9	100	10	3	4.4	1	Positive
Chlorfenapyr	409.2	59	130	20	3	5.95	2	Positive
Chlorfenapyr	409.2	31	130	45	3	5.95	2	Positive
Chlorpyrifos	349.9336	197.9275	100	20	5	6.32	1	Positive
Chlorpyrifos	349.9336	96.9508	100	41	5	6.32	1	Positive

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Retention Time (min)	Retention Time Window (min)	Polarity
Cinerin I	317.2	107.1	90	16	4	6.18	1	Positive
Cinerin I	317.2	77	90	60	4	6.18	1	Positive
Cinerin I	317.2	149.1	90	4	4	6.18	1	Positive
Cinerin II	361.2	76.8	92	70	4	5.8	2	Positive
Cinerin II	361.2	106.9	92	16	4	5.8	2	Positive
Cinerin II	361.2	149.1	92	4	4	5.8	2	Positive
Clofentezine	303	138	90	10	3	5.51	1	Positive
Clofentezine	303	102.1	90	40	3	5.51	1	Positive
Coumaphos	363	307	125	15	4	5.73	2	Positive
Coumaphos	363	226.9	125	33	4	5.73	2	Positive
Cyfluthrin	453.3	193	90	13	2	7.15	1	Positive
Cyfluthrin	451.3	191	90	13	2	7.15	1	Positive
Cypermethrin	435.3	193	90	16	2	7.2	2	Positive
Cypermethrin	433.3	416.3	90	7	2	7.2	2	Positive
Cypermethrin	433.3	191	90	16	2	7.2	2	Positive
Daminozid	161	143	80	10	2	1.1	2	Positive
Daminozid	161	61.1	80	10	2	1.1	2	Positive
Diazinon	305.1083	169.0794	100	20	5	5.07	1	Positive
Diazinon	305.1083	153.1022	100	20	5	5.07	1	Positive
Dibrom Naled	380.8	127	90	8	3	4.22	1	Positive
Dibrom Naled	378.8	127	90	5	5	4.22	1	Positive
Dichlorvos	220.96	109	110	12	3	3.77	2	Positive
Dichlorvos	220.96	79	110	24	3	3.77	2	Positive
Dimetamorph I	388.1	301.1	145	20	4	4.62	2	Positive
Dimetamorph I	388.1	165	145	32	4	4.62	2	Positive
Dimetamorph II	388.1	301.1	145	20	4	4.80	2	Positive
Dimetamorph II	388.1	165	145	32	4	4.80	2	Positive
Dimethoate	230	199	70	5	3	3.33	1	Positive
Dimethoate	230	125	70	21	3	3.33	1	Positive
Ethoprophos	243	131	90	15	3	4.63	1	Positive
Ethoprophos	243	97	90	30	3	4.63	1	Positive
Etofenprox	394.2	177.2	90	10	3	7.66	1	Positive
Etofenprox	394.2	107.1	90	45	3	7.66	1	Positive
Etoxazole	360.177	141.0146	140	28	5	6.21	1	Positive
Etoxazole	360.177	113.0197	140	50	5	6.21	1	Positive
Fenhexamid	302.1	97.2	145	25	4	4.31	1	Positive
Fenhexamid	302.1	55.1	145	45	4	4.31	1	Positive
Fenoxycarb	302.1	116.1	100	5	3	5.03	1	Positive
Fenoxycarb	302.1	88.1	100	15	3	5.03	1	Positive
Fenpyroximate	422.1	366.2	130	15	3	6.56	1	Positive
Fenpyroximate	422.1	135.1	130	30	3	6.56	1	Positive
Fipronil	436.9	332	100	18	2	4.69	1	Negative
Fipronil	434.9	330	100	18	2	4.69	1	Negative
Fipronil	434.9	250.1	100	30	2	4.69	1	Negative
Fonicamid	230	203	120	15	2	2.16	1	Positive
Fonicamid	230	174	120	20	3	2.16	1	Positive
Fludioxonil	229	185	144	13	3	4.35	1	Positive

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Retention Time (min)	Retention Time Window (min)	Polarity
Fludioxonil	229	158	144	25	3	4.35	1	Positive
Hexythiazox	353	228.1	90	10	3	6.63	1	Positive
Hexythiazox	353	168.1	90	25	3	6.63	1	Positive
Imazalil	297	201	120	15	3	4.01	2	Positive
Imazalil	297	159	120	20	7	4.01	2	Positive
Imidacloprid	256	209.1	90	16	2	3.35	1	Positive
Imidacloprid	256	175.1	90	20	2	3.35	1	Positive
Jasmolin I	331.2	76.9	90	60	3	6	4	Positive
Jasmolin I	331.2	106.9	90	20	3	6	4	Positive
Jasmolin I	331.2	163.0	90	4	3	6	4	Positive
Jasmolin II	375.2	76.8	95	76	3	6	4	Positive
Jasmolin II	375.2	106.9	95	24	3	6	4	Positive
Jasmolin II	375.2	163.2	95	4	3	6	4	Positive
Kresoxim methyl	314.1	267.1	80	0	3	5.22	1	Positive
Kresoxim methyl	314.1	222.2	80	10	3	5.22	1	Positive
Malathion	331.0433	126.9	80	5	5	4.68	1	Positive
Malathion	331.0433	99	80	10	5	4.68	1	Positive
Metalaxyl	280.1	220.2	100	10	3	4.15	1	Positive
Metalaxyl	280.1	160.1	100	20	3	4.15	1	Positive
Methiocarb	226.1	169.1	70	0	7	4.43	1	Positive
Methiocarb	226.1	121.1	70	15	3	4.43	1	Positive
Methomyl	162.9	106.1	60	5	3	1.97	1	Positive
Methomyl	162.9	88.1	60	0	3	1.97	1	Positive
Methyl-Parathion	264	232	140	18	2	4.70	1	Positive
Methyl-Parathion	264	125	140	24	2	4.70	1	Positive
Mevinphos	225	192.9	60	5	4	3.2	2	Positive
Mevinphos	225	126.9	60	17	4	3.2	2	Positive
MGK-264	276.2	210.1	100	12	4	5.4	2	Positive
MGK-264	276.2	98	100	28	4	5.4	2	Positive
Myclobutanil	289.1	125	110	35	3	4.64	1	Positive
Myclobutanil	289.1	70.1	110	15	7	4.64	1	Positive
Ochratoxin A	404.1	238.9	130	26	3	4.14	2	Positive
Ochratoxin A	404.1	220.9	130	32	3	4.14	2	Positive
Oxamyl	237	90.1	60	0	3	1.79	1	Positive
Oxamyl	237	72.1	60	15	3	1.79	1	Positive
Paclobutrazol	294.1	125	110	40	3	4.41	1	Positive
Paclobutrazol	294.1	70.1	110	20	7	4.41	1	Positive
Permethrin	391.09	355	120	5	3	7.55	2	Positive
Permethrin	391.09	183	120	5	3	7.55	2	Positive
Permethrin [M+NH4] ⁺	408.1	183	72	20	3	7.55	2	Positive
Permethrin [M+NH4] ⁺	408.1	355.1	72	4	3	7.55	2	Positive
Phosmet	317.9	160	80	10	3	4.71	1	Positive
Phosmet	317.9	133	80	40	3	4.71	1	Positive
Piperonyl butoxide	356.2	177.1	90	5	3	5.92	1	Positive
Piperonyl butoxide	356.2	119.1	90	35	3	5.92	1	Positive
Prallethrin	301.18	169	90	5	3	5.45	2	Positive
Prallethrin	301.18	105	90	20	3	5.45	2	Positive

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Acceleration (V)	Retention Time (min)	Retention Time Window (min)	Polarity
Propiconazole	342.1	159	130	32	2	5.34	1	Positive
Propiconazole	342.1	69.1	130	16	2	5.34	1	Positive
Propoxur	210	168	60	5	3	3.63	1	Positive
Propoxur	210	111	60	10	3	3.63	1	Positive
Pyrethrin I	329.21	161	90	5	3	6.36	1	Positive
Pyrethrin I	329.21	143	90	20	3	6.36	1	Positive
Pyrethrin I	329.21	133	90	20	3	6.36	1	Positive
Pyrethrin II	373.2	161	102	2	3	5.8	3	Positive
Pyrethrin II	373.2	133.1	102	24	3	5.8	3	Positive
Pyrethrin II	373.2	77	102	98	3	5.8	3	Positive
Pyridaben	365.1	309.1	90	4	2	7.01	1	Positive
Pyridaben	365.1	147.2	90	20	2	7.01	1	Positive
Pyridaben	365.1	117.1	90	60	2	7.01	1	Positive
Spinetoram J	748.5	142.1	165	26	3	5.45	2	Positive
Spinetoram J	748.5	98.1	165	50	3	5.45	2	Positive
Spinetoram L	760.5	142.1	165	26	3	5.90	2	Positive
Spinetoram L	760.5	98.1	165	50	3	5.90	2	Positive
Spinosyn A	732.5	142.1	160	28	2	5.55	1	Positive
Spinosyn A	732.5	98.1	160	60	2	5.55	1	Positive
Spinosyn D	746.5	142.1	160	35	2	5.92	1	Positive
Spinosyn D	746.5	98	160	55	2	5.92	1	Positive
Spiromesifen	388.2	273	80	6	2	6.12	1	Positive
Spiromesifen	388.2	255	80	26	2	6.12	1	Positive
Spirotetramat	374.2	330.2	110	12	5	4.78	1	Positive
Spirotetramat	374.2	302.2	110	12	5	4.78	1	Positive
Spirotetramat	374.2	216.1	110	36	5	4.78	1	Positive
Spiroxamine	298.28	144.1	120	16	4	4.2	2	Positive
Spiroxamine	298.28	100.1	120	32	4	4.2	2	Positive
Tebuconazole	308.1	124.9	120	47	2	5	1	Positive
Tebuconazole	308.1	70	120	40	2	5	1	Positive
Thiacloprid	253	126	100	16	2	3.68	1	Positive
Thiacloprid	253	90	100	40	2	3.68	1	Positive
Thiamethoxam	292.03	211.1	80	8	2	2.81	1	Positive
Thiamethoxam	292.03	181.1	80	20	2	2.81	1	Positive
Trifloxystrobin	409.1	186	100	12	2	5.69	1	Positive
Trifloxystrobin	409.1	145	100	52	2	5.69	1	Positive

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