APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Avinash Dalmia[1], Erasmus Cudjoe[2], Toby Astill[2], Jacob Jalali[3], Feng Qin[2], Molly Murphy[4], Travis Ruthenberg[5]

[1]PerkinElmer, Inc., Shelton, CT

[2]PerkinElmer, Inc., Woodbridge, ON, Canada

[3]PerkinElmer, Inc., San Jose, CA

[4]SC Labs, Tigard, OR

[5]SC Labs, Santa Cruz, CA

A Single Cannabis LC/MS/MS Method to Meet California Pesticide and Mycotoxin Residues Regulatory Requirements

Introduction

Over half of the U.S. has legalized the use of medical cannabis due to its therapeutic benefits for ailments such as cancer, multiple sclerosis, and ALS.[1-3] Like traditional agriculture crops, pesticides are sometimes used in cannabis cultivation to protect plants from pests and improve growth yield. Chronic exposure to pesticides can pose serious health risks; therefore, pesticide analysis in cannabis is an important consumer safety topic. Recent news has reported an alarming percentage of cannabis products to be tainted by high levels of pesticide residue, prompting recalls and public-safety alerts. Banned pesticides like myclobutanil, imidacloprid, abamectin, etoxazole and spiromesifen, have been detected as residue on cannabis flowers and concentrated further in extracts and edibles. A case in Colorado recalled 20,000 packages of cannabis flowers in October 2015 due to pesticide contamination, and in November 2016, Oregon officials issued a health alert for specific batches of cannabis. Moreover, many of today's cannabis products are inhaled after combusting them, so there is growing concern among consumers and regulators due to the unknown effects of pesticide compounds when inhaled.[4-5] In addition to pesticides, the growing conditions for cannabis are also conducive to the growth of molds and fungi which can produce carcinogenic mycotoxins including ochratoxin A and aflatoxins. As a result, testing for the levels of pesticide and mycotoxins in cannabis is important to ensure consumer safety and quality control.

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High performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as method of choice for pesticide and mycotoxin analysis because it offers superior selectivity, sensitivity, ruggedness, and does not require extensive sample preparation before analysis. Although gas chromatography-mass spectrometry (GC-MS/MS) methods have been developed for pesticide analysis in cannabis samples, they are only applicable to a smaller subset number of analytes. Compounds such as daminozide, a highly polar compound, and abamectin, a high molecular weight compound, are not amenable to analysis by GC-MS/MS because they are heat labile and degrade in either the GC injection port or the column at high temperature. GC-MS/MS methods are not as robust as LC-MS/MS methods for pesticide analysis in complex matrices since they require extensive sample preparation to prevent GC injection port contamination from complex matrices.[6,7]

Since there is no federal guidance for the analysis of pesticides analysis in cannabis samples, different States in the U.S. have developed their own testing guidelines. Oregon was the first state in the U.S. to develop comprehensive guidelines for pesticide residues analysis in cannabis and set regulatory limits for 59 pesticides in cannabis.[8] California has however issued more stringent action limits for 66 pesticides (including all but one of those found on Oregon state list, and eight more) and five mycotoxins residues in cannabis flower and edibles.[9] Numerous reports for pesticide analysis in cannabis have been published but these studies have certain deficiencies.[10-12] Most of these studies either do not achieve detection limits to meet the state of California's action limits; or, use time-consuming sample preparation methods (e.g. QuEChERS with dSPE) with poor recoveries for some of the pesticides, which require use of both LC-MS/MS and GC-MS/MS based instruments for analysis of all the pesticides. This increases cost, complexity, and turnaround time of analysis substantially. In this work, the PerkinElmer application development team analyzed all 66 pesticides (including very hydrophobic and chlorinated pesticides typically analyzed by GC-MS/MS) and five mycotoxins spiked in cannabis flower extracts well below the action limits specified by the state of California. A LC-MS/MS instrument was used with ESI and APCI sources and a simple solvent extraction method with excellent recoveries for all analytes in acceptable range of 70-120%.

Experimental

Hardware/Software

Chromatographic separation was conducted on a PerkinElmer LC-MS/MS QSight® LX50 UHPLC system, while detection was achieved using a PerkinElmer QSight 220 MS/MS detector with a dual ionization ESI and APCI source, which operates independently with two separate inlets. All instrument control, data acquisition and data processing was performed using the Simplicity™ 3Q software platform.

Sample Preparation Method

Below is the step by step sample preparation procedure with 10-fold dilution:

- Take approximately 5 grams of cannabis flower as a representative of each sample batch and grind it finely using a grinder.
- Measure 1 gram of sample and place it into 50 mL centrifuge tube.
- Spike 10 µL of internal standard solution.
- Add 3 steel balls (10 mm in diameter) to the tube for efficient extraction during vortex mixing.
- Add 5 mL of LC/MS grade acetonitrile to the tube and cap it.
- Place the tube on multi-tube vortex mixer and allow it to vortex for 10 minutes.
- Centrifuge extract in tube for 10 minutes at 3000 rpm.
- Filter the solvent into a 5 mL glass amber vial using 0.22 micron nylon syringe-filter and cap it.
- Label the bottle with the sample ID.
- Transfer 0.5 mL of extracted sample into a 2 mL HPLC vial and dilute it with 0.5 mL of LC/MS grade acetonitrile and mix it.
- Inject 3 µL of sample for LC-MS/MS analysis, using pesticide methods.

LC Method And MS Source Conditions

The LC method and MS source parameters are shown in Table 1.

Table 1. LC Method and MS Source Conditions.

LC Conditions	
LC Column	PerkinElmer Quasar Pesticide Column (4.6 × 100 mm, 2.7 µm) Part Number: N9306880
Mobile Phase A (ESI method)	2 mM ammonium formate + 0.1% formic acid (in water)
Mobile Phase B (ESI method)	2 mM ammonium formate + 0.1% formic acid (in methanol)
Mobile Phase Gradient	A 18.5 min. (this time includes both analysis time and column equilibration time) LC-MS/MS method with optimized gradient using ESI source was used for separation and analysis of 63 out of 66 pesticides and five mycotoxins residues at low levels in cannabis matrix with minimal matrix interference. A fast 6 min. LC-MS/MS method with short gradient, optimum mobile phase composition and APCI source was used for measurement of remaining three pesticides.
Column Oven Temperature	30 °C
Auto sampler Temperature	10 °C
Injection Volume	3.0 µL for LC-MS/MS method with ESI source. 10 µL for LC-MS/MS method with APCI source.
MS Source Conditions for ESI Source and APCI Source	
ESI Voltage (Positive)	+5500 V
ESI Voltage (Negative)	-4200V
APCI Corona Discharge	-5 µA
Drying Gas	120 arbitrary units
Nebulizer Gas	350 arbitrary units
Source Temperature	315 °C
HSID Temperature	200 °C
Detection mode	Time-managed MRM™

Results and Discussion

Analytical Challenges for Testing Pesticide Residues in Cannabis Samples

Since the pesticides tested in this study include both polar and non-polar compounds, 100% acetonitrile was used to extract all the analytes from the samples. Due to the cannabis matrix's hydrophobicity, further dilution of the extract was performed with the aqueous mobile phase to make it compatible with reverse phase column. This protocol resulted in lower recoveries of some of pesticides due to precipitation. To achieve a higher performing method, cannabis extracts are diluted with acetonitrile by overall factor of 10 to achieve high recovery of pesticides and reduce matrix effects. However, the reverse phase LC method uses aqueous mobile phase at the beginning of the LC run to help better retain the polar compounds on the column. Injecting an organic solvent such as an acetonitrile sample on the LC leads to poor chromatographic peaks for early eluting polar compounds. To overcome this problem, a small sample injection volume of three microliters was used in this study.

Pesticide analysis in cannabis is very challenging since its matrix composition is very complex and contains compounds from different classes such as cannabinoids, terpenes, hydrocarbons, sugars, fatty acids, flavonoids and others. Sample matrix effect remains the main concern for LC-MS/MS, and leads to variable signal ion suppression and matrix interference. Moreover, quantification of pesticide residues in cannabis is a difficult task due to great disparity in high concentration levels of naturally occurring cannabinoids as well as high terpene content. In this work, we used a generic extraction method with dilution, selected the best MRM transitions and optimized the LC gradient to allow low level analysis of pesticides with good recovery in a complex cannabis matrix.

Normally, analysis of pesticides in cannabis and other food matrices is done by both GC-MS/MS and LC-MS/MS since some non-polar and chlorinated pesticides are difficult to ionize with an electrospray ion source.[13-14] To demonstrate the convenience of a single method, the application team developed a LC-MS/MS method using both APCI and ESI techniques to analyze all the pesticides (California regulated pesticide list) with the additional benefits of improved throughput, reduced complexity and lower cost of analysis. Typically, the dirty matrix found with cannabis samples causes build-up on the sampling interface of a GC-MS/MS and LC-MS/MS systems and this would increase the maintenance costs and downtime resulting in a loss of productivity. It showed that the LC-MS/MS method we developed would be more immune to contamination from the dirty cannabis matrix.

Detectability and Reproducibility

Figure 1 shows MRM chromatograms with excellent signal to noise for a representative set of pesticides spiked at low level of 0.01 µg/g in the cannabis flower. The limits of quantification (LOQs) and response reproducibility at LOQ level for each of the pesticides (category II and I) and mycotoxins in cannabis extract are summarized in Table 2, 3 and 4. The LOQs were determined by considering both the signals of the quantifier and qualifier ions (S/N > 10 for both) and ensuring that the product ion ratios were within the 20% tolerance windows of the expected ratio. As demonstrated in Table 2 and 3, the LOQs determined in this study are well below the California action limit by a factor of 2 to 600 for all category II pesticides and mycotoxins listed. The response RSD for each pesticide and mycotoxin at its LOQ level in the cannabis matrix was less than 20 %. The retention time for each analyte was reproducible within ± 0.1 minute over a 24-hour period. This demonstrates that the method is more than adequately sensitive and reproducible for pesticides and mycotoxins analysis in cannabis at the regulatory limit specified by the state of California.

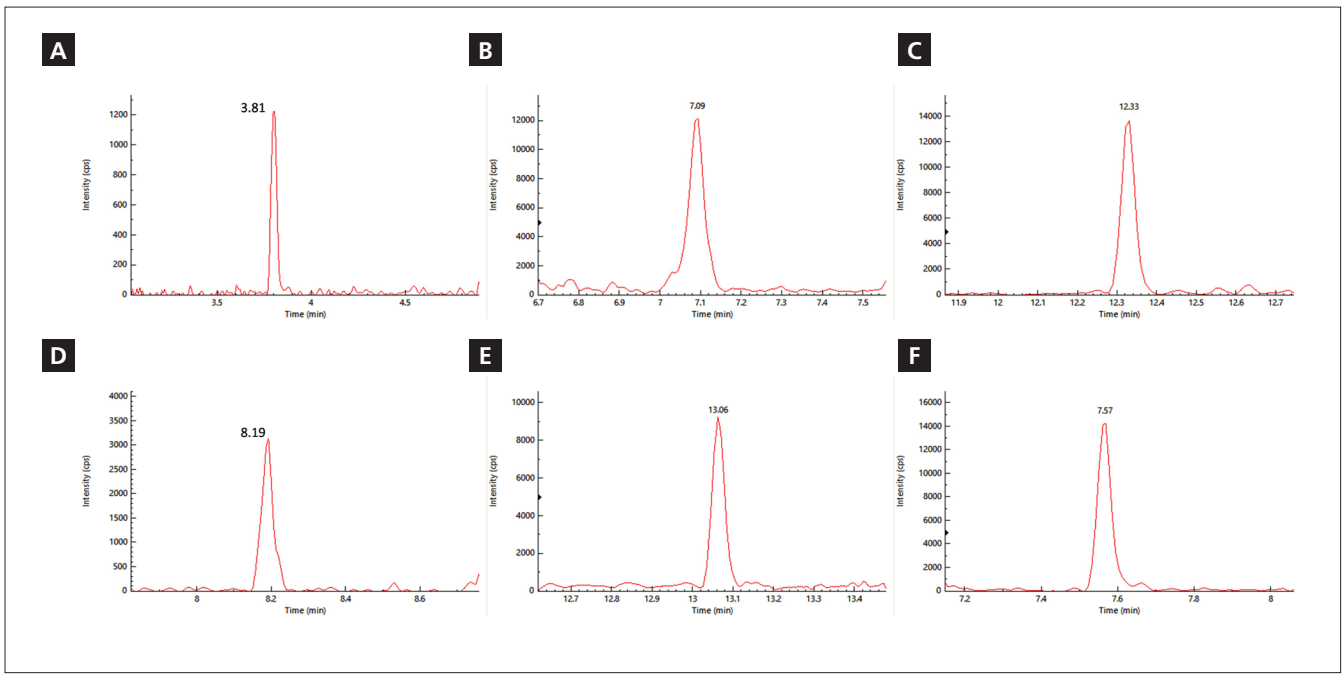

Figure 1. MRM chromatogram of representative set of pesticides-:(a) oxamyl, (b) metalaxyl, (c) fenpyroximate, (d) mycyclobutanil, (e) Etofenprox and (f) Azoxystrobin spiked at level of 0.01 µg/g in cannabis matrix.

Table 2. LOQs for California category II Pesticides with LC-MS/MS in Cannabis. Red/Green: Pesticides typically analyzed by GC-MS/MS, Red: Pesticides analyzed on LC-MS/MS by ESI, Green: Pesticides analyzed on Q-Sight by APCI

S. No.	Category II Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/QSight LOQ
		QSight (µg/g)	%CV (n=7)		
1	Abamectin	0.025	10.6	0.1	4
2	Acephate	0.010	3.1	0.1	10
3	Acequinocyl	0.025	13.3	0.1	10
4	Acetamiprid	0.010	13.1	0.1	10
5	Azoxystrobin	0.005	5.0	0.1	20
6	Bifenazate	0.010	10.8	0.1	10
7	Bifenthrin	0.010	14.4	0.5	50
8	Boscalid	0.025	12.2	0.1	4
9	Captan	0.25	7.0	0.7	2.8
10	Carbaryl	0.010	9.5	0.5	50
11	Chlorantraniliprole	0.025	5.6	10.0	400
12	Clofentezine	0.010	11.3	0.1	10
13	Cyfluthrin	0.25	19.1	1.0	4
14	Cypermethrin	0.100	20.0	1.0	10
15	Diazinon	0.005	3.8	0.2	40
16	Dimethomorph	0.005	1.4	2.0	400
17	Etoxazole	0.005	13.5	0.1	20
18	Fenhexamid	0.010	12.5	0.1	10
19	Fenpyroximate	0.005	6.9	0.1	20
20	Flonicamid	0.010	10.2	0.1	10
21	Fludioxonil	0.050	9.5	0.1	2
22	Hexythiazox	0.005	8.4	0.1	20
23	Imidacloprid	0.010	10.3	3.0	300
24	Kresoxim-methyl	0.025	8.1	0.1	4
25	Malathion	0.010	14.7	0.5	50
26	Metalaxyl	0.010	8.0	2.0	200
27	Methomyl	0.010	8.5	0.1	10
28	Myclobutanil	0.010	10.4	0.1	10
29	Naled	0.010	8.4	0.1	10
30	Oxamyl	0.010	6.7	0.2	20
31	Pentachloronitrobenzene	0.010	13.0	0.1	10
32	Permethrin	0.010	16.0	0.5	50
33	Phosmet	0.005	13.3	0.1	20
34	Piperonylbutoxide	0.005	3.5	3.0	600
35	Prallethrin	0.025	7.4	0.1	4
36	Propiconazole	0.015	8.9	0.1	6.7
37	Pyrethrins	0.1	1.4	0.5	5
38	Pyridaben	0.010	7.9	0.1	10
39	Spinetoram	0.005	13.8	0.1	20
40	Spinosad	0.005	9.3	0.1	20
41	Spiromesifen	0.010	9.4	0.1	10
42	Spirotetramat	0.010	8.4	0.1	10
43	Tebuconazole	0.005	11.0	0.1	20
44	Thiamethoxam	0.010	3.6	4.5	450
45	Trifloxystrobin	0.005	8.4	0.1	20

Table 3. LOQs for California Category II Mycotoxins with LC-MS/MS in Cannabis.

S. No.	Category II Mycotoxin	LOQ		Action Level (µg/g)	Action Level/QSight LOQ
		QSight (µg/g)	%CV (n=7)		
1	Ochratoxin A	0.010	18	0.020	2.0
2	Aflatoxin B1	0.001	18	NA	NA
3	Aflatoxin B2	0.0015	14	NA	NA
4	Aflatoxin G1	0.010	18	NA	NA
5	Aflatoxin G2	0.0015	19	NA	NA
6	Aflatoxin (B1+B2+G1+G2)	0.005	NA	0.020	4.0

Table 4. LOQs for California category I Pesticides with LC-MS/MS in cannabis. Red/Green : Pesticides typically analyzed by GC-MS/MS, Red: Pesticides Analyzed on LC-MS/MS by ESI Green: Pesticides Analyzed on LC-MS/MS by APCI

S. No.	Category I Residual Pesticide	LC-MS/MS LOQ		Action Level (µg/g)	Action Level/LOQ
		(µg/g)	%CV (n=7)		
1	Aldicarb	0.010	10.6	0.1	10
2	Carbofuran	0.010	3.1	0.1	10
3	Chlordane	0.05	13.3	0.1	2
4	Chlorfenpyr	0.05	6.0	0.1	2
5	Chlorpyrifos	0.010	5.0	0.1	10
6	Coumaphos	0.010	10.8	0.1	10
7	Daminozide	0.015	14.4	0.1	6.67
8	DDVP (Dichlorvos)	0.025	12.2	0.1	4
9	Dimethoate	0.010	3.8	0.1	10
10	Ethoprophos	0.010	9.5	0.1	10
11	Etofenprox	0.010	5.6	0.1	10
12	Fenoxycarb	0.010	11.3	0.1	10
13	Fipronil	0.010	19.1	0.1	10
14	Imazalil	0.010	23.1	0.1	10
15	Methiocarb	0.010	3.8	0.1	10
16	Methyl parathion	0.040	1.4	0.1	2.5
17	Mevinphos	0.025	13.5	0.1	4
18	Paclobutrazol	0.010	12.5	0.1	10
19	Propoxur	0.010	6.9	0.1	10
20	Spiroxamine	0.010	10.2	0.1	10
21	Thiacloprid	0.010	9.5	0.1	10

Sample Matrix-Matched Calibration Standards

Matrix matched calibration is the preferred analytical procedure for quantitation because it compensates for matrix effects that are prevalent in cannabis samples. The decrease or increase in response is attributed to ion suppression of the analytes during ionization by the presence of co-eluted matrix compounds. Due to sample matrix effects, a matrix matched calibration curve was used for quantitation and generated by injecting blank cannabis flower extracts and blank cannabis flower extract samples spiked with varying concentrations of pesticides and mycotoxins over a range of 0.1-1000 ng/mL. The calibration curves for all pesticides and mycotoxins were linear with calibration fit of R^2 greater than 0.99 for all the analytes.

Recovery Studies with Solvent Extraction

Utilizing the QuEChERS extraction technique is a common method for extraction of low levels of contaminants such as pesticides from fruit and vegetable matrices with higher water content.[15] The method includes extraction of a broad range of pesticides and removal of sugars, organic acids and other compounds commonly found in fruits and vegetables.[16-20] It is not a suitable method for very polar pesticides, such as Daminozide, which are included in both the California and other states regulatory framework. Since Daminozide is too polar to be extracted efficiently with QuEChERS, it remains in the aqueous phase and does not partition into the organic solvent during salting out step. The recovery of Daminozide from a cannabis matrix with QuEChERS extraction has been reported to be less than 10%.[10] Moreover, a typical cannabis matrix contains mostly hydrophobic compounds such as cannabinoids and terpenes, and therefore the QuEChERS extraction method does not remove the matrix interfering compounds during the salting out step. Different groups have tried to develop an advanced QuEChERS method with d-SPE

step which utilizes PSA and other adsorbents to remove matrix from cannabis extract. However, the addition of the d-SPE step to the QuEChERS method not only makes this method more laborious and expensive, but also leads to low recoveries of compounds such as spinosad, spirotetramat, spioroxamine, ochratoxin A and a few others.[11-12] This is a result of these compounds binding to the PSA adsorbent in the d-SPE step, and resulting in poor recoveries. Due to above shortcomings of the QuEChERS method for extraction of pesticides from a cannabis matrix, the application team used a simple acetonitrile based solvent extraction method for extraction. To confirm this method, fortified cannabis flower samples were used to determine pesticides and mycotoxin recovery. The cannabis flower samples were tested to confirm the absence of pesticides before they were spiked. Five cannabis flower samples were spiked at two levels (low and high) of all pesticides (0.1 and 1 µg/g) and mycotoxins (0.02 and 0.1 µg/g) standard. These two levels were chosen based on regulatory limits, for pesticides and mycotoxins in cannabis, from California and other states. Tables 5-7 show that absolute recoveries of all 66 pesticides and five mycotoxins at two different levels was within acceptable range of 70-120 % with RSD less than 20% for five cannabis flower samples. For two pesticides, the recovery values were not reported at low spiked value since it was below their LOQ value.

LC-MS/MS Method with Optimum MRM Transitions for Challenging Analytes in Cannabis Matrices

As stated, cannabis is a challenging matrix to test, and this is compounded by the low concentration level of the pesticides. To ensure the highest analytical confidence, multiple MRM transitions for a number of pesticides with minimal matrix interference in the cannabis matrix were determined for low level detection. For example, acequinocyl is an insecticide and can be ionized easily as a protonated molecular ion in a standard, but the MRM transitions, based on protonated molecular ion in the cannabis matrix, showed poor LOQ of 0.5 to 1 µg/g about five to 10 times higher than its action limit for the state of California. Therefore, MRM transitions based on alternative modes of ionization, such as adduct formation, were determined to reduce matrix interference and achieve LOQ of 0.025 µg/g (fourtimes below action limits) for acequinocyl in the cannabis matrix. Figure 2 shows the signal overlay of blank cannabis matrix and acequinocyl spiked at level of 0.1 µg/g in cannabis with MRM transitions based on protonated molecular ion and adduct ion of acequinocyl. This figure displays that optimum acequinocyl MRM transitions helped in achieving lower detection limits due to minimal matrix interference.

High molecular weight compounds such as abamectin, and some early eluting polar compounds, such as daminozide, are difficult to measure at low levels using GC-MS/MS since they decompose either in a high temperature GC injector or a GC oven. Although, high molecular weight compounds such as abamectin, and polar compounds such as daminozide, can be ionized with the ESI source, they are also prone to decomposition at high temperatures. Figure 3 shows abamectin response as a function of HSID and source temperature. Based on these results, the optimum temperature values for the ESI source and HSID temperature were set to maximize signals for high molecular weight and polar pesticides. Abamectin is also prone to sodium and potassium adduct formation from the sodium and potassium ions leached into mobile phase from glassware. Since it is difficult to control amount of sodium and potassium ions leached from glassware, the use of the sodium adduct for abamectin as Q1 (parent ion) mass for analysis would lead to response variation. To reduce sodium or potassium adduct formation, a controlled amount of ammonium salt was added to the mobile phase. The combination of ammonium salt in mobile phase and optimum temperature conditions resulted in good and reproducible signals for abamectin.

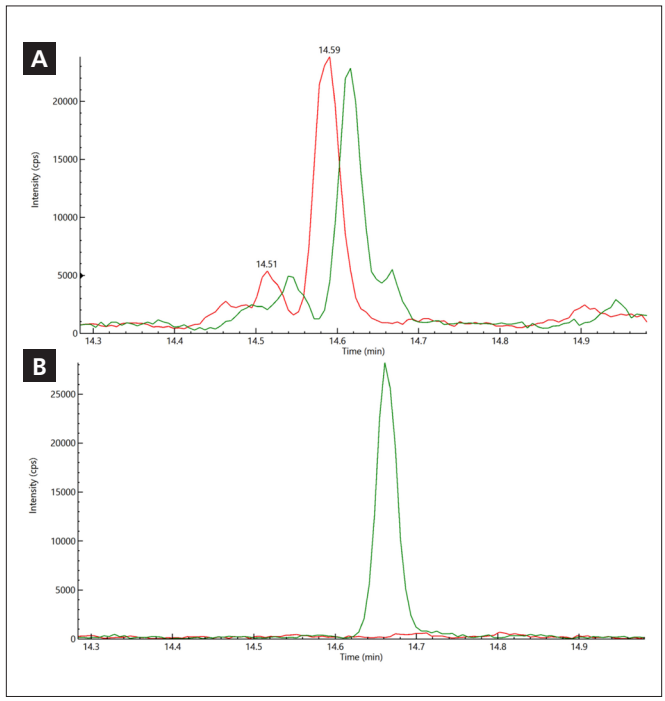

Figure 2. (a) Overlay of response of cannabis matrix (Red) and acequinocyl (Green) spiked at level of 0.1 µg/g in cannabis matrix with MRM transition based on protonated molecular ion and (b)Overlay of response of cannabis matrix (Red) and acequinocyl (Green) spiked at level of 0.1 µg/g in cannabis matrix with MRM transition based on adduct ion.

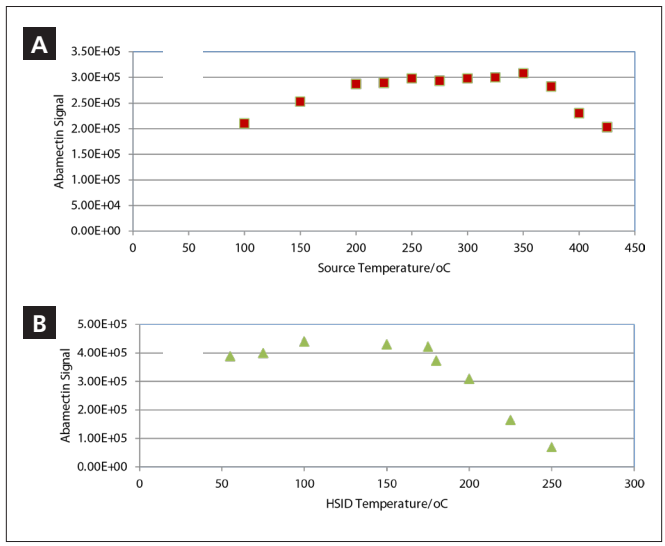

Figure 3. Abamectin signal as a function of ESI source (a) and HSID temperature (b).

Table 5. Recovery of Category II pesticides at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category II Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS% (n=5)
1	Abamectin	85	10	89	9
2	Acephate	93	16	91	9
3	Acequinocyl	90	11	86	6
4	Acetamiprid	87	13	95	9
5	Azoxystrobin	87	12	92	8
6	Bifenazate	88	8	88	7
7	Bifenthrin	84	13	94	7
8	Boscalid	87	10	89	5
9	Captan	NA	NA	70	15
10	Carbaryl	84	12	92	10
11	Chlorantraniliprole	88	13	90	8
12	Clofentezine	87	13	91	12
13	Cyfluthrin	NA	NA	97	17
14	Cypermethrin	98	18	85	13
15	Diazinon	96	10	95	10
16	Dimethomorph	87	15	90	7
17	Etoxazole	89	10	92	10
18	Fenhexamid	87	12	87	7
19	Fenpyroximate	87	9	93	8
20	Flonicamid	93	15	92	12
21	Fludioxonil	94	13	93	8
22	Hexythiazox	86	11	93	7
23	Imidacloprid	89	11	91	9
24	Kresoxim-methyl	91	10	95	8
25	Malathion	90	12	91	7
26	Metalaxyl	86	10	92	8
27	Methomyl	89	10	90	9
28	Myclobutanil	84	10	93	7
29	Naled	87	10	91	7
30	Oxamyl	93	16	94	9
31	Pentachloronitrobenzene	80	16	88	8
32	Permethrin	87	17	92	9
33	Phosmet	86	11	91	7
34	Piperonylbutoxide	91	8	94	8
35	Prallethrin	88	15	94	8
36	Propiconazole	90	14	95	11
37	Pyrethrins	89	12	93	9
38	Pyridaben	84	13	92	9
39	Spinetoram	93	13	94	9
40	Spinosad	88	14	90	10
41	Spiromesifen	90	8	92	5
42	Spirotetramat	97	10	90	7
43	Tebuconazole	94	12	91	7
44	Thiamethoxam	90	10	95	10
45	Trifloxystrobin	86	12	93	9

Table 6. Recovery of Category II mycotoxins at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category II Mycotoxin	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS%(n=5)
1	Aflatoxin 81	75	15	84	9
2	Aflatoxin 82	78	14	82	9
3	Aflatoxin G1	76	12	85	7
4	Aflatoxin G2	79	12	84	6
5	Ochratoxin A	78	20	83	7

Table 7. Recovery of Category I pesticides at two different levels from cannabis with acetonitrile solvent extraction method.

S.No.	Category I Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery%	RSD % (n=5)	Recovery %	RS% (n=5)
1	Aldicarb	87	11	94	11
2	Carbofuran	86	11	91	9
3	Chlordane	87	19	92	10
4	Chlorfenapyr	95	15	99	10
5	Chlorpyrifos	94	8	92	8
6	Coumaphos	90	12	95	10
7	Daminozide	82	15	80	14
8	DDVP (Dichlorvos)	94	14	91	11
9	Dimethoate	89	11	96	9
10	Ethoprop(hos)	92	9	94	7
11	Etofenprox	88	13	93	8
12	F enoxycarb	91	11	93	7
13	Fipronil	89	9	95	8
14	Imazalil	86	10	89	10
15	Methiocarb	81	9	93	6
16	Methyl parathion	89	14	96	11
17	Mevinphos	86	10	95	10
18	Paclobutrazol	79	13	90	6
19	Propoxur	91	13	93	9
20	Spiroxamine	88	9	89	9
21	Thiacloprid	89	13	95	10

Analysis of Pesticides, Typically Analyzed by GC-MS/MS, by LC-MS/MS

A number of pesticides in cannabis, regulated by California and other states, are analyzed traditionally using GC-MS/MS with an EI source since these pesticides have low proton affinity, which results in low ionization efficiency with the ESI source. Some examples of these pesticides analyzed normally with GC/MS are cypermethrin, cyfluthrin, captan, naled, permethrin and pyrethrins. To achieve the required sensitivity, the selected MRM's were optimized with a heated electrospray source. LOQ for these analytes were in the range of 0.01 to 0.25 µg/g, well below the California action limits.

Analysis of Pyrethrin Isomers in Cannabis

The pyrethrins are a class of organic compounds normally derived from chrysanthemum cinerariifolium that have potent insecticidal activity by targeting the nervous systems of insects. Pyrethrins are a group of six isomers and their structures are displayed in Figure 4. The naturally-occurring pyrethrins, extracted from chrysanthemum flowers, are esters of chrysanthemic acid (pyrethrin I, cinerin I, and jasmolin I) and esters of pyrethric acid (pyrethrin II, cinerin II, and jasmolin II). In the U.S., the pyrethrum extract is standardized as 45–55% w/w total pyrethrins and in a commercially available pyrethrin standard, the percentage of pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II is about 56.1, 27.8, 5.7, 3.8, 4 and 2.6%, respectively. A number of compounds in cannabis mimic the structure of pyrethrins, and therefore the analysis of pyrethrins in cannabis is very difficult due to matrix interference. The optimum MRM transitions and LC gradient were developed to analyze the six pyrethrins at low levels in the cannabis matrix with minimal matrix interference. The LOQs, with LC-MS/MS method utilizing optimum MRM transitions and LC gradient, for six pyrethrins -: pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin 1 and jasmolin II were 0.1, 0.1, 0.01, 0.03, 0.025 and 0.01 µg/g, respectively in cannabis flowers.

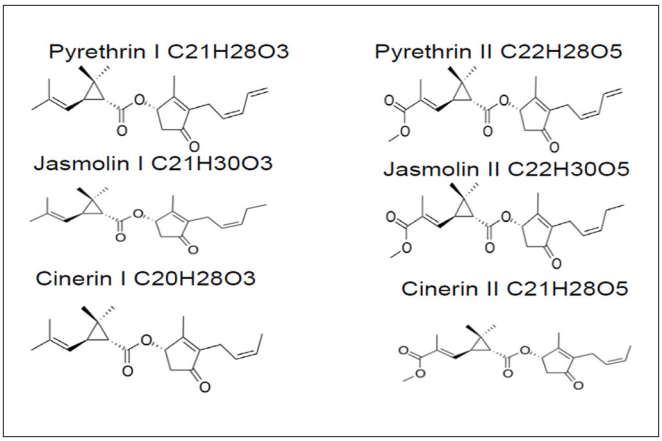

Figure 4. Structure of 6 isomers of pyrethrins.

Pesticides, Which Do Not Ionize Effectively with ESI, Analyzed with APCI

Hydrophobic and halogenated pesticides (eg. pentachloronitrobenzene and chlordane) are traditionally analyzed by GC-MS/MS since they do not ionize effectively by LC-MS/MS with an ESI source. For reference, the structure of the chlorinated pesticides is shown in Figure 5. Since Pentachloronitrobenzene (PCNB) does not contain either hydrogen atoms, for loss of protons, or functional groups with either high proton affinity or which can form ammonia or sodium adducts, it cannot be ionized with the ESI source. Similarly, chlordane is highly chlorinated and has very low proton affinity and therefore difficult to ionize efficiently with an ESI source. Since an APCI ion source is better suited for ionization of very hydrophobic and non-polar analytes, APCI was used to determine the detection limits of pentachloronitrobenzene and chlordane in cannabis. Also, the APCI ion source was used for low level analysis of chlorfenapyr in cannabis, since limits of detection for chlorfenapyr were improved by a factor of two with APCI source in comparison to ESI source due to less ion suppression. Figure 6 shows excellent signal to noise (S/N >=100) for pentachloronitrobenzene (PCNB) spiked at level of 0.1 µg/g in the cannabis matrix using a LC-MS/MS system with an APCI source. Using a fast six minute LC-MS/MS method with short LC gradient and APCI source, LOQ of pentachloronitrobenzene, chlordane and chlorfenapyr in cannabis was 0.01, 0.05 and 0.05 µg/g, respectively.

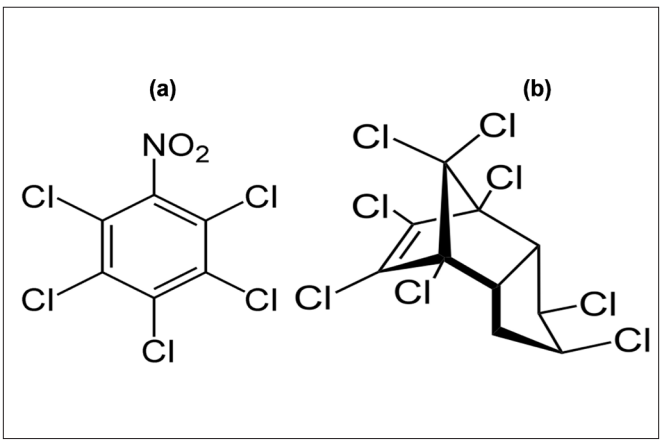

Figure 5. Structure of pentachloronitrobenzene (a) and chlordane (b).

Long Term Stability Data with StayClean™ Source in LC-MS/MS

Long term stability data for pesticide and mycotoxin analysis in cannabis samples was collected using a LC-MS/MS system, fitted with dual ESI and APCI sources, and combined with a heated and self-cleaning StayClean source with a laminar flow interface. Figure 7 shows long term response and stability of the method for 100 ng/ml of Diazinon spiked in cannabis extract over one week. Long term stability data for pesticide analysis in cannabis showed that response RSD over one week for most of pesticides and mycotoxins was between 1.5 to 20%. These results demonstrated that the heated self-cleaning source in the LC-MS/MS system would reduce maintenance needs that are usually prevalent with this matrix. Most published LC-MS/MS methods do not show long term stability data or state that they have to clean the electrospray source frequently to maintain the sensitivity of mass spectrometer.[21] Also, they divert the LC flow to waste for the first few minutes, and after the last peak elutes out to reduce contamination from unretained and late eluting matrix compounds. In this study, excellent long-term stability data was obtained without diverting the LC flow from the MS in the first few minutes, at the end of run, and without periodical cleaning of ion sources.

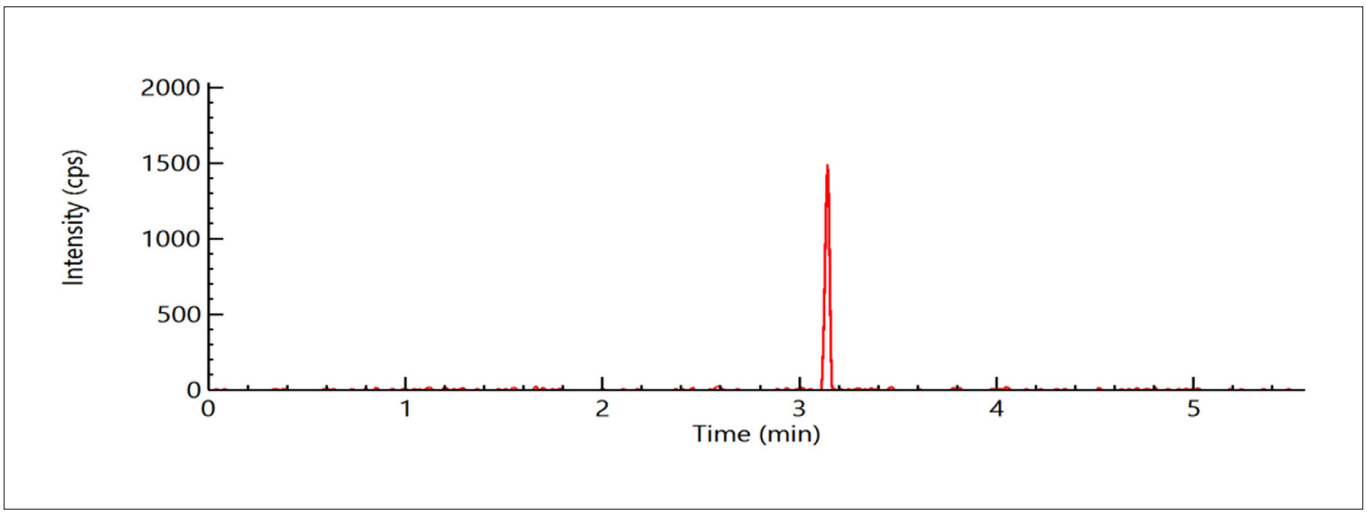

Figure 6. Sample chromatogram of pentachloronitrobenzene (PCNB) spiked at level of 0.1 µg/g in a cannabis matrix using LC-MS/MS system with APCI source.

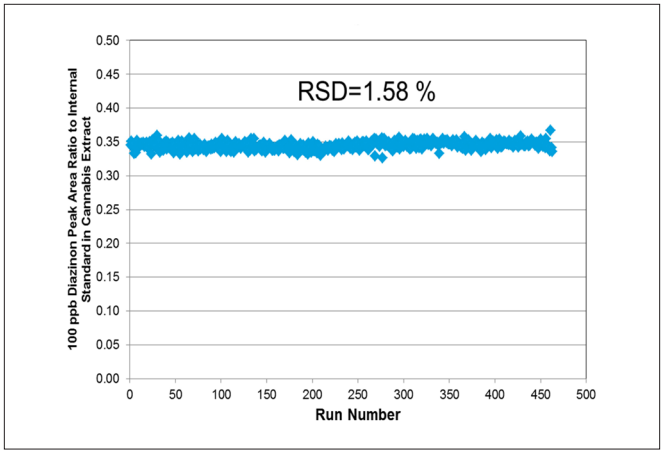

Figure 7. Long term stability data over one week of injections of diazinon at a level of 100 ng/mL spiked in cannabis flower matrix extract.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC-MS/MS method for analysis of different cannabis pesticides and mycotoxins residues in cannabis samples. The proposed solvent extraction method is suitable for labs wanting to comply with the state of California regulations, as the recovery of all pesticides and mycotoxins from a cannabis matrix was in the acceptable range of 70-120% with RSD less than 20%. This method allowed identification and quantification of all 66 pesticides and five mycotoxins at low levels (0.005 to 0.25 µg/g), which is well below the actions limits set by the state of California with good precision. The ability to screen and quantitate all 66 pesticides, including the very hydrophobic and chlorinated compounds normally analyzed on a GC-MS/MS amenable, and the five mycotoxins, makes this method a novel way to screen and quantitate pesticides and mycotoxins in cannabis with a single instrument.

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
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