APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Novel ESI and APCI LC/MS/MS Analytical Method for Testing Cannabis and Hemp Concentrate Sample Types

Introduction

As new adult-use and medicinal cannabis markets emerge in the US and Canada, the use of concentrate cannabis and CBD products (e.g. edibles, beverages, vape products, isolates, topicals, and waxes) continues to increase in popularity. According to market research, concentrates and their derivative products are expected to represent 50% of the consumer market by 2022.[1] This growth, and the diversity in sample type, presents an analytical challenge for testing laboratories. The concentrate matrix has a significant effect on the analytical method, owing to higher sample matrix effects caused by the increased concentration levels (up to 95%/wt) of cannabinoids in the sample. This effect influences the response of certain pesticide molecules, requiring laboratories to validate a pesticide method specific to the sample matrix type.

In this work, an LC/MS/MS method is presented for the analysis of 66 pesticides, including hydrophobic and chlorinated pesticides typically analyzed by GC/MS/MS, and five mycotoxins. Utilizing a cannabis concentrate matrix, the method features a simple solvent extraction, followed by analysis using an LC/MS/MS instrument with dual ESI and APCI sources. The analysis yielded excellent recoveries and detection limits, well below those specified by the State of California cannabis regulations, for all analytes.

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Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer QSight® LX50 UHPLC system. Subsequent detection was achieved using a PerkinElmer QSight 420 MS/MS detector with dual ESI and APCI ionization sources, which operate independently with two separate inlets. All instrument control, data acquisition and data processing were performed using the Simplicity 3Q™ software platform.

Sample Preparation

Below is the step-by-step sample preparation procedure with a 50-fold dilution for the ESI source, and a 25-fold dilution for the APCI source.

1. Measure out approximately five grams of cannabis concentrate as a representative sample for each sample batch.
2. Measure out one gram of sample, and place it into a 50 mL centrifuge tube.
3. Add 10 mL of LC/MS grade acetonitrile with 0.1 % formic acid to the tube, and cap it.
4. Place the tube in a multi-tube vortex mixer, and allow it to vortex for 10 minutes.
5. Centrifuge the extract in the tube for ten minutes at 3000 rpm.
6. Transfer the solvent into a 10 mL glass amber vial and cap it.
7. Label the bottle with the sample ID.
8. For the APCI method, transfer 400 µL of extracted sample (from Step 7) into a 2 mL HPLC vial. Spike with 10 µL of internal standard, and then dilute with 590 µL of LC/MS grade acetonitrile with 0.1 % formic acid and mix it.
9. For the ESI method, transfer 200 µL of extracted sample (from Step 7) into a 2 mL HPLC vial. Spike with 10 µL of internal standard, and then dilute with 790 µL of LC/MS grade acetonitrile with 0.1 % formic acid and mix it.
10. Inject sample for LC/MS/MS analysis, using pesticide methods.

Results and Discussion

Detectability and Reproducibility

Currently, most laboratories deploy multiple analytical instruments (LC/MS/MS and GC/MS/MS) and tedious sample preparation methods (such as QuEChERS) to meet the low pesticide regulatory limits in various food matrices. Herein, a validated LC/MS/MS analytical method with a fast solvent extraction is presented. Utilizing a PerkinElmer liquid chromatograph coupled to a tandem mass spectrometer, the complete analysis of all 66 pesticides and five mycotoxins outlined in the California regulations for cannabis concentrates is detailed. All compounds of concern were analyzed with a QSight 420 dual source mass spectrometer, equipped with both APCI and ESI ionization probes. Pesticides conventionally analyzed by gas chromatography, such as methyl parathion, cypermethrin and pentochloronitrobenzene (quintozene) among others, were all detected utilizing this single platform LC/MS/MS system.

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™ SPP Pesticides (4.6 × 100 mm, 2.7 µm)
Mobile Phase A (ESI method)	2 mM ammonium formate + 0.1% formic acid (in LC/MS grade water)
Mobile Phase B (ESI method)	2 mM ammonium formate + 0.1% formic acid (in LC/MS grade methanol)
Mobile Phase A (APCI method)	LC/MS grade water
Mobile Phase B (APCI method)	LC/MS grade methanol
Mobile Phase Gradient	The run time for the optimized gradient elution method, including analytical column re-conditioning, was 18 minutes for the ESI method, and 12 minutes for the APCI method. The final method ensured separation of the bulk cannabis matrix from the analytes for improved quantitation.
Column Oven Temperature	30 °C
Auto sampler Temperature	20 °C
Injection Volume	3 µL and 10 µL for LC/MS/MS method with ESI and APCI source, respectively.

MS Source Conditions for ESI Source and APCI Source	
ESI Voltage (Positive)	+5100 V
ESI Voltage (Negative)	-4200V
APCI Corona Discharge	-3 µA
Drying Gas	150 arbitrary units
Nebulizer Gas	350 arbitrary units
Source Temperature (ESI Method))	315 °C
Source Temperature (APCI Method)	250 °C
HSID Temperature (ESI Method)	200 °C
HSID Temperature (APCI Method)	180 °C
Detection Mode	Time-managed MRM™

The limit of quantification (LOQ) and response reproducibility at the LOQ for each of the pesticides (Category II and I) and mycotoxins in the cannabis concentrate sample are summarized in Tables 2, 3 and 4. The LOQs were determined by considering the signal of the quantifier ion (S/N > 10), and ensuring that the product ion ratios were within the 30 % tolerance windows of the expected ion ratio. The response RSD for each pesticide and mycotoxin at its LOQ level in the cannabis matrix were less than 20%. As demonstrated in Tables 2, 3 and 4, the LOQs determined in this study are well below the California action limit by a factor of 1.2 to 1,000 for all pesticides and mycotoxins listed. This demonstrates the sensitivity and reproducibility of the method in the analysis of pesticides and mycotoxins in cannabis concentrate samples, in support of California state regulatory program adherence.

Table 2. LOQs for California Category II Pesticides with LC/MS/MS in Cannabis Concentrate. **Red**/**Green**: Pesticides typically analyzed by GC/MS/MS. Of those, analytes highlighted in **red** were analyzed on the QSight by ESI, and those in **green** were analyzed on the QSight by APCI. Pesticides in black were analyzed on the QSight by ESI.

S. No.	Category II Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Abamectin	0.08	14.0	0.1	1.2
2	Acephate	0.01	4.5	0.1	10
3	Acequinocyl	0.05	10.8	0.1	2
4	Acetamiprid	0.01	3.3	0.1	10
5	Azoxystrobin	0.005	11.9	0.1	20
6	Bifenazate	0.005	15.2	0.1	20
7	Bifenthrin	0.005	5.3	0.5	100
8	Boscalid	0.005	14.2	0.1	20
9	**Captan**	0.5	13.0	0.7	1.4
10	Carbaryl	0.005	7.4	0.5	100
11	Chlorantraniliprole	0.01	10.0	10.0	1000
12	Clofentezine	0.01	14.4	0.1	10
13	**Cyfluthrin**	0.9	16.0	2.0	2.2
14	**Cypermethrin**	0.15	7.8	1.0	6.66
15	Diazinon	0.01	7.5	0.2	20
16	Dimethomorph	0.005	17.4	2.0	400
17	Etoxazole	0.01	5.4	0.1	10
18	Fenhexamid	0.05	8.3	0.1	2
19	Fenpyroximate	0.01	6.9	0.1	10
20	Flonicamid	0.01	6.4	0.1	10
21	Fludioxonil	0.005	11.9	0.1	20
22	Hexythiazox	0.005	10.1	0.1	20
23	Imidacloprid	0.01	9.9	3.0	300
24	Kresoxim-methyl	0.05	4.8	0.1	2
25	Malathion	0.005	6.1	0.5	100
26	Metalaxyl	0.005	3.3	2.0	400
27	Methomyl	0.01	12.3	0.1	10
28	Myclobutanil	0.005	5.4	0.1	20
29	**Naled**	0.05	13.0	0.1	2
30	Oxamyl	0.01	4.1	0.2	20
31	**Pentachloronitrobenzene**	0.025	10.2	0.1	4
32	**Permethrin**	0.05	6.8	0.5	10
33	Phosmet	0.01	12.0	0.1	10
34	Piperonylbutoxide	0.15	4.0	3.0	20
35	**Prallethrin**	0.08	14.4	0.1	1.2
36	Propiconazole	0.005	4.5	0.1	20
37	**Pyrethrins**	0.37	5.1	0.5	1.3
38	Pyridaben	0.01	10.4	0.1	10
39	Spinetoram	0.008	9.0	0.1	12.5
40	Spinosad	0.01	10.7	0.1	10
41	Spiromesifen	0.05	5.2	0.1	2
42	Spirotetramat	0.005	12.5	0.1	20
43	Tebuconazole	0.01	13.8	0.1	10
44	Thiamethoxam	0.005	8.5	4.5	900
45	Trifloxystrobin	0.005	4.9	0.1	20

Table 3. LOQs for California Category I Pesticides with LC/MS/MS in Cannabis Concentrate. **Red**/**Green**: Pesticides typically analyzed by GC/MS/MS. Of those, analytes highlighted in **red** were analyzed on the QSight by ESI, and those in **green** were analyzed on the QSight by APCI. Pesticides in black were analyzed on the QSight by ESI.

S. No.	Category I Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Aldicarb	0.025	9.5	0.1	4
2	Carbofuran	0.005	8.5	0.1	20
3	**Chlordane**	0.08	15.3	0.1	1.2
4	**Chlorfenpyr**	0.05	18.0	0.1	2
5	**Chlorpyrifos**	0.05	8.5	0.1	2
6	**Coumaphos**	0.01	15.7	0.1	10
7	Daminozide	0.05	11.3	0.1	2
8	DDVP (Dichlorvos)	0.025	4.2	0.1	4
9	Dimethoate	0.005	5.1	0.1	20
10	Ethoprop(hos)	0.01	12.5	0.1	10
11	Etofenprox	0.01	8.6	0.1	10
12	Fenoxycarb	0.005	5.5	0.1	20
13	Fipronil	0.005	9.8	0.1	20
14	Imazalil	0.005	19.3	0.1	20
15	Methiocarb	0.005	10.9	0.1	20
16	**Methyl Parathion**	0.05	3.0	0.1	2
17	Mevinphos	0.01	8.1	0.1	10
18	Paclobutrazol	0.01	10.2	0.1	10
19	Propoxur	0.01	11.8	0.1	10
20	Spiroxamine	0.01	6.3	0.1	10
21	Thiacloprid	0.005	6.5	0.1	20

Table 4. LOQs for Mycotoxins with LC/MS/MS in the Cannabis Concentrate.

S. No.	Category II Mycotoxin	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=7)		
1	Ochratoxin A	0.0125	12.6	0.020	1.6
2	Aflatoxin B1	0.003	12.4	NA	NA
3	Aflatoxin B2	0.003	13.0	NA	NA
4	Aflatoxin G1	0.004	8.2	NA	NA
5	Aflatoxin G2	0.005	10.5	NA	NA
6	Aflatoxin (B1+B2+G1+G2)	0.015	NA	0.020	1.33

Recovery Studies with Solvent Extraction

In cannabis concentrate testing, sample preparation is often identified as the main bottleneck associated with the analysis of pesticides and mycotoxins. Techniques such as solid phase multiple steps and large amounts of expensive sorbent materials compounds.[3] Solvent extraction, in comparison, offers an efficient, easy and high throughput means of achieving high extraction recovery. As such, a solvent extraction method was utilized in this study for the extraction of pesticides and mycotoxins.

To confirm the recovery performance of the method, spiked cannabis concentrate samples were utilized. The cannabis concentrate samples were analyzed to confirm the absence of pesticides and mycotoxins prior to spiking. Cannabis concentrate samples were then spiked at two levels for each contaminant of concern; 0.1 µg/g (low) and 1.0 µg/g (high) for pesticides, and 0.02 µg/g (low) and 0.2 µg/g (high) for mycotoxins. Tables 5, 6 and 7 show that the absolute recoveries at both spiking levels for all mycotoxins and pesticides were within the acceptable range of 70-120%, with RSD values less than 20%. No recovery data could be obtained for pesticides captan, cyfluthrin and cypermethrin at the lower level of 0.1 µg/g, since their LOQ is higher than 0.1 µg/g.

Table 5. Recoveries of Category II pesticides in cannabis concentrate matrix at two different levels with solvent extraction.

	Category II Residual Pesticide	Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Abamectin	81.6	4.7	83.7	15.5
2	Acephate	98.3	2.0	93.4	1.1
3	Acequinocyl	99.2	5.3	84.7	1.6
4	Acetamiprid	94.7	1.0	94.4	0.7
5	Azoxystrobin	93.0	2.2	98.5	5.3
6	Bifenazate	91.9	3.2	91.6	0.9
7	Bifenthrin	94.5	3.4	93.7	0.3
8	Boscalid	82.0	3.1	98.7	10.5
9	Captan*	-	-	96.4	18.9
10	Carbaryl	93.6	6.1	93.9	4.3
11	Chlorantraniliprole	87.8	5.1	98.1	8.8
12	Clofentezine	71.9	3.3	87.1	16.4
13	Cyfluthrin*	-	-	95.4	5.5
14	Cypermethrin*	-	-	93.4	5.5
15	Diazinon	89.1	1.1	94.5	4.1
16	Dimethomorph	83.7	2.6	93.8	4.0
17	Etoxazole	97.6	1.9	96.9	3.1
18	Fenhexamid	102.8	10.6	103.0	13.5
19	Fenpyroximate	91.1	1.7	95.7	1.2
20	Flonicamid	102.6	5.7	97.8	0.9
21	Fludioxonil	103.3	3.9	96.1	1.6
22	Hexythiazox	79.8	2.7	96.6	11.7
23	Imidacloprid	95.9	2.4	95.4	1.2
24	Kresoxim-methyl	93.4	3.0	96.1	2.5
25	Malathion	95.5	5.2	93.6	3.4
26	Metalaxyl	93.2	2.8	95.1	3.7
27	Methomyl	97.4	2.7	97.4	2.1
28	Myclobutanil	85.7	3.2	94.9	1.6
29	Naled	100.0	8.2	96.9	5.0
30	Oxamyl	98.9	1.7	95.1	0.9
31	Pentachloronitrobenzene	92.8	4.3	96.0	3.5
32	Permethrin	92.8	13.1	98.9	3.0
33	Phosmet	80.2	3.9	94.3	3.3
34	Piperonylbutoxide	90.3	2.0	95.2	2.1
35	Prallethrin	90.5	14.4	101.7	8.3
36	Propiconazole	81.3	1.8	93.9	12.0
37	Pyrethrins	109	16.9	101.0	14.4
38	Pyridaben	91.9	3.5	95.2	2.8
39	Spinetoram	92.1	1.6	93.4	1.8
40	Spinosad	95.1	8.7	97.7	3.4
41	Spiromesifen	99.8	5.0	99.0	5.6
42	Spirotetramat	95.8	2.6	94.7	1.8
43	Tebuconazole	96.4	2.7	94.9	1.7
44	Thiamethoxam	97.6	2.4	96.7	1.7
45	Trifloxystrobin	92.7	3.5	97.0	0.9

Table 6. Recoveries of Category I pesticides in cannabis concentrate matrix at two different levels with solvent extraction.

S. No.	Category I Residual Pesticide	Low Level 0.1 µg/g		High Level 1 µg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Aldicarb	88.9	14.2	95.5	4.1
2	Carbofuran	91.9	1.5	93.8	3.9
3	Chlordane	102.3	15.3	105.2	4.4
4	Chlorfenapyr	94.8	3.0	94.7	4.8
5	Chlorpyrifos	108.6	4.9	97.9	13.4
6	Coumaphos	73.6	5.1	93.5	13.8
7	Daminozide	95.1	6.0	95.2	1.6
8	DDVP (Dichlorvos)	92.6	3.5	95.5	1.2
9	Dimethoate	94.2	0.9	96.9	1.1
10	Ethoprop(hos)	88.0	5.7	95.7	2.7
11	Etofenprox	101.3	4.4	97.4	3.7
12	Fenoxycarb	97.1	3.5	96.9	1.5
13	Fipronil	98.1	3.6	95.8	3.7
14	Imazalil	88.5	9.8	98.0	4.5
15	Methiocarb	94.8	4.4	101.9	1.3
16	Methyl parathion	94.4	4.8	95.6	6.3
17	Mevinphos	93.4	4.0	96.4	1.7
18	Paclobutrazol	94.2	2.6	97.8	1.7
19	Propoxur	90.9	2.7	94.3	3.8
20	Spiroxamine	97.3	0.9	95.9	1.9
21	Thiacloprid	92.9	2.5	93.6	3.0

Table 7. Recoveries of mycotoxins in cannabis concentrate matrix at 2 different levels with solvent extraction.

S. No.	Category II Mycotoxin	Low Level 0.02 µg/g		High Level 0.2 µg/g	
		Recovery/%	RSD/% (n=3)	Recovery/%	RSD/% (n=3)
1	Aflatoxin B1	92	8	93	5
2	Aflatoxin B2	94	9	92	6
3	Aflatoxin G1	81	18	98	9
4	Aflatoxin G2	96	17	91	10
5	Ochratoxin A	87	12	85	3

Internal Standards

As cannabis concentrate samples exhibit a significant matrix effect, owing to the large amount of cannabinoids present (50-95%) in them, 30 internal standards were utilized to improve the quantitative analysis and overall recovery. The use of internal standards compensated for matrix ion suppression effects, and corrected for any analyte loss during sample preparation. According to experimental results shown in Figure 1, use of internals standards significantly increased the overall recovery of coumaphos, calculated based on extracted concentration of pre-spiked analyte versus neat solution (unextracted) concentration, from 56% to 86% owing to correction of matrix effects and analyte loss during extraction step. Finally, the overall recoveries of 70-130 % were achieved for all of 66 pesticides and five mycotoxins with addition of 30 internal standards to cannabis concentrate matrix.

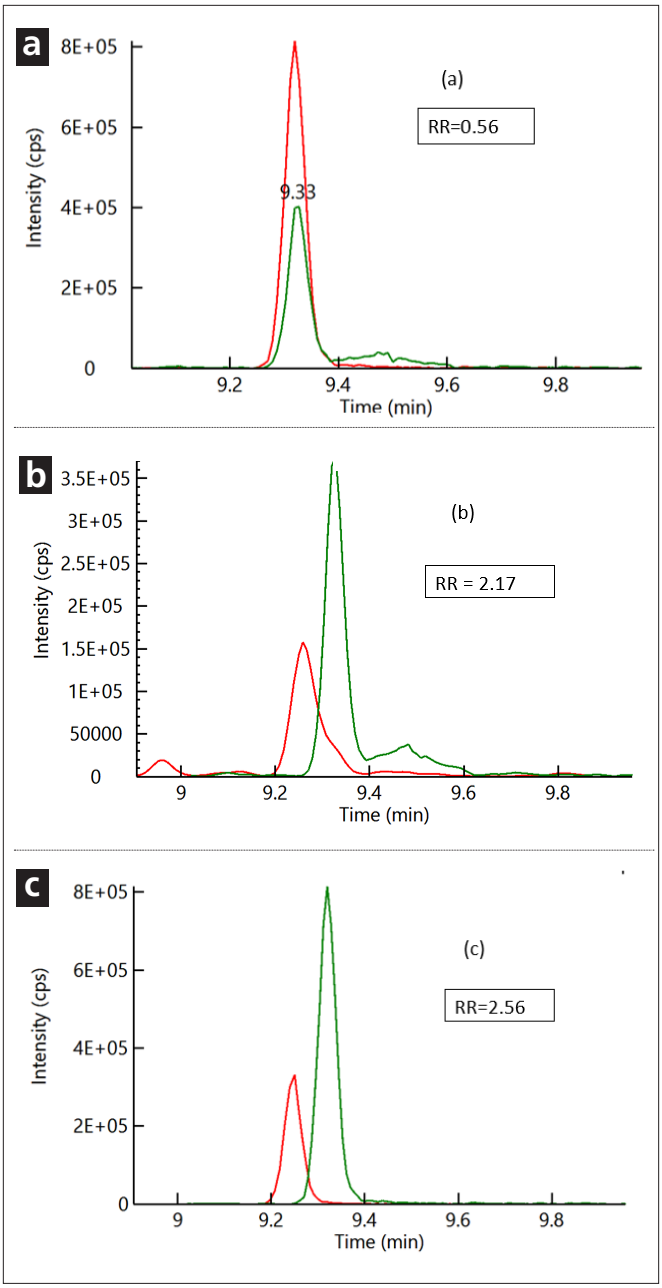

Figure 1. (a) Overlay of the response of coumaphos in solvent (**red**) and coumaphos (**green**) pre-spiked in the cannabis concentrate matrix, without an internal standard. The response ratio (RR) of coumaphos in cannabis extract to solvent standard was 0.56. (b) Overlay of the response of coumaphos (**green**) and coumaphos-D10 internal standard (**red**) in the pre-spiked cannabis concentrate matrix with a response ratio (RR) of 2.17 for the analyte to internal standard. (c) Overlay of the response of coumaphos (**green**) and coumaphos-D10 internal standard (red) in the solvent with a response ratio (RR) of 2.56 for the analyte to internal standard.

Analysis of Pesticides, Typically Analyzed by GC-MS/MS, Using LC/MS/MS With Dual ESI and APCI Ion Source

A number of pesticides, regulated in cannabis by California and other states, are traditionally analyzed using GC/MS/MS with an EI source, as these pesticides exhibit either low proton affinity (which results in low ionization efficiency with the ESI source), or they cannot be ionized by the ESI ion source used in conventional LC/MS/MS systems. Examples of such pesticides, typically analyzed by GC/MS/MS are cypermethrin, cyfluthrin, captan, naled, permethrin, pentachlornitrobenzene, chlorfenapyr, chlordane, methyl parathion, pyrethrins and others.

To achieve the required sensitivity for a number of these pesticides (cypermethrin, cyfluthrin, captan, naled, permethrin, prallethrin, chlorpyrifos, coumaphos, and pyrethrins), the selected MRMs and source conditions (temperature and flow) were optimized with a heated electrospray source to get low detection limits. The other pesticides (pentachlornitrobenzene, chlorfenapyr, chlordane and methyl parathion) were measured at low limits in the cannabis concentrate matrix using the APCI source in the LC/MS/MS instrument. The LOQs for these analytes were in the range of 0.05 to 0.9 µg/g, well below the California action limits. Figure 2 presents a sample chromatogram of cannabis concentrate spiked at a level of 0.1 µg/g with pesticides naled and chlorfenapyr, which are analyzed by LC/MS/MS with ESI and APCI source, respectively.

Method Optimization to Overcome Matrix Ion Suppression Effects from a Challenging Cannabis Concentrate Matrix

As cannabis concentrates are prepared by the extraction of cannabis flowers, they typically exhibit 3-5 times higher levels of cannabinoids (THC and CBD) than cannabis flower raw materials. The higher concentration of cannabinoids (50-95%) in cannabis concentrate matrices can result in a considerably more challenging matrix when compared to cannabis flower samples. This complexity is further compounded by the low concentration levels of the pesticides and mycotoxins in the samples. For pesticide analysis in a cannabis flower sample, the matrix ion suppression effects are minimized by using an overall dilution factor with solvent in the range of 10-20x.

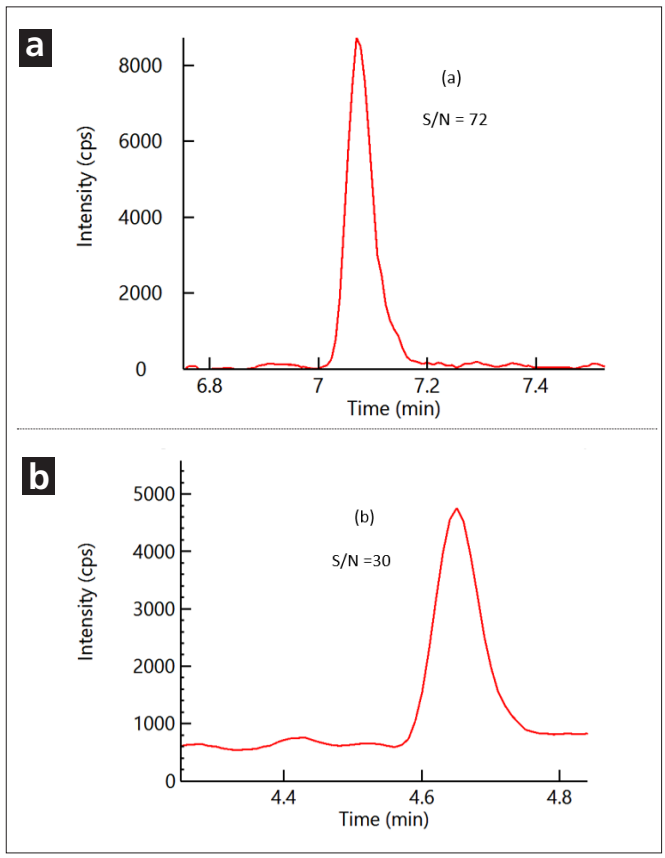

Figure 2. Sample chromatogram of (a) naled and (b) chlorfenapyr spiked at a level of 0.1 µg/g in a cannabis concentrate matrix using an LC/MS/MS system with an ESI and APCI source, respectively.

However, in the case of pesticide analysis in cannabis concentrate with our LC/MS/MS method, a considerably higher overall dilution factor of 25x for the APCI source and 50x for the ESI source was utilized to minimize matrix effects. In a previous study, a fast six-minute LC gradient with the APCI source for the analysis of four pesticides in a cannabis flower matrix was evaluated.[4] When the same six-minute LC gradient method was utilized for the analysis of the same four pesticides in a cannabis concentrate matrix, a significant ion suppression matrix effect was observed, which resulted in a much lower signal and reduced sensitivity. Apart from the higher dilution factor for the cannabis concentrate matrix, a 12-minute slower LC gradient method with an APCI source was developed to separate pesticides and cannabinoids on the LC column and reduce the ion signal suppression effects. Figure 3 illustrates that, when compared to the six-minute fast LC gradient method, the signal-to-noise for PCNB in the cannabis concentrate matrix was improved by a factor of 60 using the 12-minute optimized LC gradient method.

Selectivity of PCNB Analysis and Mechanism of PCNB Ionization With APCI Source

As PCNB does not have a hydrogen atom to lose, it cannot be ionized using an ESI source in negative ion mode. The nonpolar nature of the compound, the low proton affinity, and the inability to form adducts with ammonia and other metal ions further precludes PCNB from forming ions utilizing an ESI source in positive ion mode. Owing to these impediments, the ESI source could not be used for the detection of PCNB. Thus, the APCI source, in negative ion mode, was utilized for selective analysis of PCNB in different cannabis matrices.

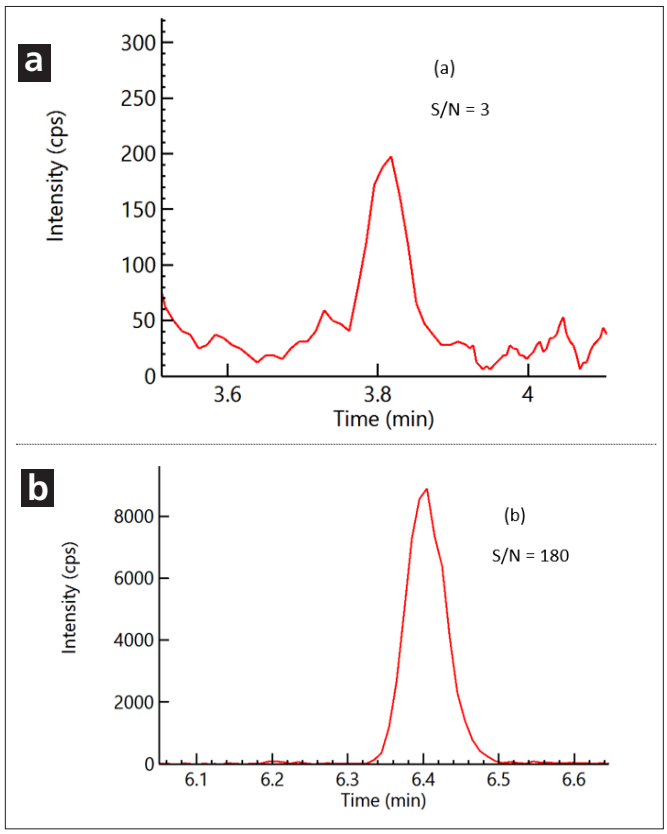

Figure 3. (a) Response for PCNB spiked at level of 1 µg/g in a cannabis concentrate matrix using a fast 6-min LC gradient method, coupled with the QSight MS/MS system with APCI source. (b) Response for PCNB spiked at a level of 1 µg/g in a cannabis concentrate matrix using an optimized 12-minute LC gradient method, coupled with a QSight MS/MS system with APCI source.

Figure 4 illustrates the response for PCNB in a blank cannabis concentrate matrix, and in a cannabis concentrate matrix spiked with 0.1 µg/g of PCNB. FDA method validation guidelines concerning the selectivity of an analysis specify that matrix blanks should be free of any matrix interference peaks at the retention time of an analyte.[5] As shown in figure 4a, the matrix response for PCNB in the blank cannabis concentrate shows low background signal with no matrix interference peak at the retention time of PCNB, thus demonstrating that the measurement of PCNB in the cannabis concentrate matrix is very selective. Further, a good signal-to-noise ratio for PCNB spiked at the California action limit of 0.1 ug/g in the cannabis concentrate matrix demonstrates that PCNB can be determined using an APCI source in LC/MS/MS systems with good selectivity and sensitivity.

Figure 5 details the excellent linearity of the PCNB response over a concentration range of 1-3000 ppb (corresponds to 25-75000 ppb in cannabis concentrate) in the 25x diluted cannabis concentrate extract, with a regression fit (R^2) of 0.9999. As the regression fit value for PCNB is greater than 0.99, the result meets the requirement outlined by the California Bureau of Cannabis Control, which stipulates that regression fits be higher than 0.99.[6] The accuracy of the calibration curve was checked by comparing back-calculated concentrations from the calibration curve with known concentrations of PCNB, ensuring that the strict criterion of a maximum deviation of 10% was met for all concentration levels. The literature claims that analysis of PCNB with an APCI LC/MS source is not selective and may require a quadratic calibration curve susceptible to a poor correlation coefficient, however, this experimental work outlines a robust APCI method that exhibits excellent sensitivity, selectivity and linearity of PCNB analysis in a cannabis sample.[7]

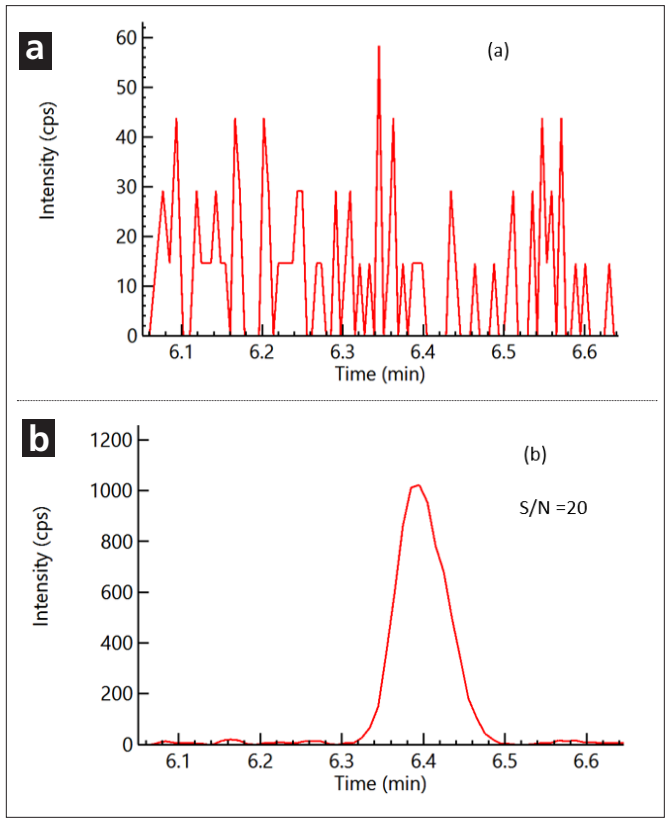

Figure 4. PCNB response in a blank cannabis concentrate matrix (a), and from spiked level of 0.1 µg/g in cannabis concentrate matrix (b).

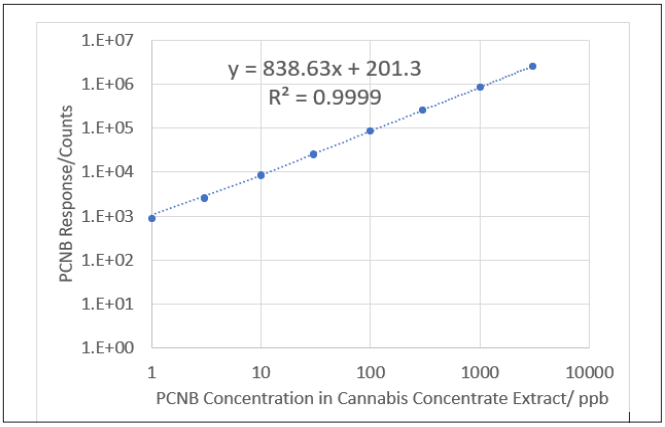

Figure 5. Linearity of PCNB response over 3.5 orders of magnitude in 25 times diluted cannabis concentrate concentrate.

In past studies, different mechanisms for negative APCI ionization, such as proton abstraction, anion adduction, electron capture and dissociative electron capture have been proposed.[8] It has been demonstrated that chlorinated nitrobenzene compounds can form phenoxide ions under negative APCI conditions.[9] Similarly, the following mechanism for ionization of PCNB was proposed in a previous publication, with the APCI source in negative ion mode (where M is PCNB):[10]

$$O_2 + e^- \rightarrow O_2^-$$

$$M + O_2^- \rightarrow [M - Cl + O]^- + ClO$$

Herein, the formation of $[M\text{-}Cl+O]^-$ can be attributed to the formation of the superoxide ion (O_2^-) by electron capture, followed by its chemical reaction with PCNB. This mechanism can be explained further by analyzing Q1 scan data for PCNB infusion into the APCI source. The Q1 scan data showed a monoisotopic base peak at a nominal mass of 274 dalton. The nominal monoisotopic mass of PCNB is 293 dalton, and therefore the mass loss of 19 dalton from an intact molecule of PCNB can be explained by the loss of chlorine (nominal monoisotopic mass of 35) and the addition of an oxygen (nominal monoisotopic mass of 16) atom to the PCNB molecule to form a negatively charged ion. Further, an experimentally observed isotope pattern or ratio of the PCNB ion matched very closely to the theoretical isotope pattern of an ion with four chlorine atoms, and this proved further that PCNB loses one chlorine atom in the APCI ion source. The low mass spectra of the APCI ion source was checked to confirm the formation of the superoxide reagent ion species, which could interact with PCNB to ionize it. It was observed that both the superoxide ion (O_2^-) and the PCNB signal increased roughly by a factor of 300 and 30, respectively, when the mobile phase was changed from 75:25 methanol:water with 0.1% formic acid and 2 mM ammonium formate to just 75:25 methanol:water. This further established that the superoxide ion plays an important role in the ionization of PCNB in the APCI source.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC/MS/MS method, with dual ESI and APCI sources, for the analysis of various pesticides and mycotoxin residues in cannabis concentrates. In the proposed method, 62 pesticides and five mycotoxins were analyzed with an ESI source and run time of 18 minutes, with an additional four pesticides analyzed with an APCI source and run time of 12 minutes.

The proposed solvent extraction method with 30 internal standards is suitable for labs analyzing samples in accordance with California regulations, as the overall recovery of all pesticides and mycotoxins from the cannabis concentrate matrix was in the acceptable range of 70-130%, with an RSD less than 20%. The method allowed for the identification and quantification of all 66 pesticides and five mycotoxins in cannabis concentrate samples at levels (0.005 to 0.9 µg/g) below State of California action limits.

It was further demonstrated that the analysis of PCNB (a pesticide normally analyzed by GC/MSMS with an EI source) utilizing an APCI source is both selective and sensitive, with excellent linearity. The ability to screen and quantitate all 66 pesticides and five mycotoxins, including the hydrophobic and chlorinated compounds normally analyzed by GC/MSMS, eliminates the requirement of using both an LC/MS/MS and GC/MS/MS instrument for this analysis. This method illustrates the use of LC/MS/MS as a novel, cost effective and efficient way to screen and quantitate pesticides and mycotoxins in a cannabis concentrate matrix with a single LC/MS/MS instrument.

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