APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Efficient LC/MS/MS Method for Testing Hemp Samples for Pesticide Residues

To further validate the performance of this method for the industry, The Emerald Test ProficiencyTest (PT) for Pesticides was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test badge of approval seen on the right. https://pt.emeraldscientific.com/

Introduction

The 2018 Agriculture Improvement Act included a provision legalizing the growth of industrial hemp in the United States for the purposes of cannabidiol (CBD) extraction[1]. As a result, increasing concerns over the use of pesticides in hemp production have arisen. Pesticide application is common in the growing of many agricultural crops, and is utilized to protect plants from pests, thus improving crop growth yield.

Owing to the potential detrimental health effects associated with pesticides, quantification of pesticide residue levels in hemp is important for both consumer safety and quality control. However, no federal guidance is currently available for the analysis of pesticide residues in hemp. As such, laboratories and manufacturers of hemp products have had to develop their own testing guidelines, or adopt existing cannabis testing requirements from states such as California, as they build out a QA/QC program for their products.

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In this work, an LC/MS/MS method for the determination of pesticide residues in hemp samples is presented. 66 pesticides, including hydrophobic and chlorinated pesticides typically analyzed by GC/MS/MS, were spiked into hemp samples and subsequently analyzed, with action limits well below those specified by the state of California cannabis regulations. An LC/MS/MS instrument with dual ESI and APCI sources was used in the study, and a simple solvent extraction method was followed, yielding excellent recoveries for all analytes in the acceptable range of 70-120%. To assess and validate the performance of the method, an Emerald Scientific blind proficiency test for 66 pesticides in a hemp sample was conducted. According to the results of the proficiency test, the method described herein produced acceptable results for all 66 pesticides, with no false positives or false negatives reported.

Experimental

Hardware/Software

Chromatographic separation was conducted utilizing a PerkinElmer QSight® LX50 UHPLC system, with detection achieved using a PerkinElmer QSight 420 MS/MS detector with a dual ionization ESI and APCI source, 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 Method

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

- Take approximately 5 g of hemp as a representative of each sample batch, and grind it finely using a grinder.
- Weigh accurately 1 g of sample, and place it into a 50 mL centrifuge tube.
- Spike the sample with 10 µL of internal standard solution. 20 internal standards were selected to compensate for ion suppression effects and improve the quantitative analysis as well as overall recovery, and to correct for any analyte loss during sample preparation.
- 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 a multi-tube vortex mixer, and allow it to vortex for 10 minutes.
- Centrifuge the extract in the tube for 10 minutes at 3000 rpm.
- Filter the solvent into a 5 mL glass amber vial using a 0.22 micron nylon syringe-filter, and then 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.

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) (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 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 ESI method and 6 minutes for APCI. The final method ensured separation of the bulk hemp matrix from the analytes for improved quantitation.
Column Oven Temperature	30 °C
Auto sampler Temperature	20 °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)	+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™

Results and Discussion

Detectability and Reproducibility

Currently, many laboratories performing pesticide analyses deploy both LC/MS/MS and GC/MS/MS instruments, along with tedious sample preparation methods, to meet the low pesticide limits imposed by the state of California. Herein, we present a single LC/MS/MS method, utilizing a PerkinElmer QSight LX50 liquid chromatograph coupled to a QSight 420 tandem mass spectrometer, for the complete analysis of all 66 pesticides outlined in the California state regulations for hemp related products. Pesticides such as methyl parathion, captan, cypermethrin, cyfluthrin, chlorfenapyr, chlordane and pentochloronitrobenzene (quintozene) among others, which are conventionally analyzed by GC/MS/MS, were all detected on this single platform.

The limits of quantification (LOQs) and response reproducibility at the LOQ level for each of the California Category I and Category II pesticides in the hemp extract sample are summarized in Tables 2 and 3. The LOQs were determined by taking into account both the signals of the quantifier and qualifier ions (S/N > 10 for both), and ensuring that the product ion ratios were within the 30% tolerance windows of the expected ratio. The response RSD for each pesticide at its LOQ level was less than 20%, and the retention times for each analyte were reproducible to within ± 0.1 minute over a 24-hour period. This demonstrates that the method is more than adequately sensitive and reproducible for pesticide analysis in hemp at the regulatory limits specified by the state of California.

Table 2. LOQs for California Category II Pesticides by LC/MS/MS in Hemp. 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=3)		
1	Abamectin	0.030	18.5	0.1	3.3
2	Acephate	0.0025	7.1	0.1	40
3	Acequinocyl	0.010	13.3	0.1	10
4	Acetamiprid	0.0025	7.2	0.1	40
5	Azoxystrobin	0.001	12	0.1	100
6	Bifenazate	0.0025	12	0.1	40
7	Bifenthrin	0.0025	9.5	0.5	200
8	Boscalid	0.010	9.3	0.1	10
9	Captan	0.10	9.4	0.7	7
10	Carbaryl	0.0025	4.6	0.5	200
11	Chlorantraniliprole	0.010	9.1	10.0	1000
12	Clofentezine	0.0025	8.6	0.1	40
13	Cyfluthrin	0.10	2.8	1.0	10
14	Cypermethrin	0.10	6.1	1.0	10
15	Diazinon	0.0025	8.6	0.2	80
16	Dimethomorph	0.010	8.9	2.0	200
17	Etoxazole	0.0025	11.7	0.1	40
18	Fenhexamid	0.005	11.9	0.1	20
19	Fenpyroximate	0.0025	6.8	0.1	40
20	Flonicamid	0.0025	10.6	0.1	40
21	Fludioxonil	0.001	12.3	0.1	100
22	Hexythiazox	0.0025	5.4	0.1	40
23	Imidacloprid	0.003	6.3	3.0	1000
24	Kresoxim-methyl	0.010	10.9	0.1	10
25	Malathion	0.0025	18	0.5	200
26	Metalaxyl	0.001	9	2.0	2000
27	Methomyl	0.0025	7.4	0.1	40
28	Myclobutanil	0.010	6.3	0.1	10
29	Naled	0.010	12.2	0.1	10
30	Oxamyl	0.0025	7.9	0.2	80
31	Pentachloronitrobenzene	0.010	5	0.1	10
32	Permethrin	0.010	10.3	0.5	50
33	Phosmet	0.0025	9.4	0.1	40
34	Piperonylbutoxide	0.0025	6.5	3.0	1200
35	Prallethrin	0.010	11.2	0.1	10

Table 2. Continued.

S. No.	Category II Residual Pesticide	LOQ		Action Level (µg/g)	Action Level/LOQ
		LC/MS/MS (µg/g)	%CV (n=3)		
36	Propiconazole	0.020	7.3	0.1	5
37	Pyrethrins	0.010	15.6	0.5	50
38	Pyridaben	0.0025	5.7	0.1	40
39	Spinetoram	0.010	3.6	0.1	10
40	Spinosad	0.005	10.6	0.1	20
41	Spiromesifen	0.010	9.7	0.1	10
42	Spirotetramat	0.0025	8.7	0.1	40
43	Tebuconazole	0.005	16.5	0.1	20
44	Thiamethoxam	0.003	9.8	4.5	1500
45	Trifloxystrobin	0.001	6.4	0.1	100

Table 3. LOQs for California Category I Pesticides by LC/MS/MS in Hemp. 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=3)		
1	Aldicarb	0.0025	10.4	0.1	40
2	Carbofuran	0.001	10.6	0.1	100
3	Chlordane	0.050	7.8	0.1	2
4	Chlorfenpyr	0.010	15.6	0.1	10
5	Chlorpyrifos	0.0025	8.6	0.1	40
6	Coumaphos	0.0025	6.6	0.1	40
7	Daminozide	0.010	13	0.1	10
8	DDVP (Dichlorvos)	0.010	8.5	0.1	10
9	Dimethoate	0.001	5.8	0.1	100
10	Ethoprop(hos)	0.0025	12.6	0.1	40
11	Etofenprox	0.0025	6.4	0.1	40
12	Fenoxycarb	0.005	13	0.1	20
13	Fipronil	0.0025	4.5	0.1	40
14	Imazalil	0.0025	16.5	0.1	40
15	Methiocarb	0.0025	11.6	0.1	40
16	Methyl parathion	0.020	14.6	0.1	5
17	Mevinphos	0.0025	4.8	0.1	40
18	Paclobutrazol	0.0025	11.1	0.1	10
19	Propoxur	0.0025	6.9	0.1	40
20	Spiroxamine	0.0025	6.7	0.1	40
21	Thiacloprid	0.0025	5.8	0.1	40

Recovery Studies with Solvent Extraction

A simple acetonitrile based solvent extraction method was used for extraction of the pesticides from the hemp matrix. Solvent extraction is a quick, high-throughput and easy way to achieve high extraction recovery when compared to other time-consuming sample preparation techniques, such as solid phase extraction (SPE) and QuEChERS, which require multiple steps and large sample and solvent volumes. To confirm this method, fortified hemp samples were used to determine pesticides recovery. Three hemp samples were spiked at a low level of 0.1 µg/g for all 66 Category I and Category II pesticides. This level was chosen based on the lowest regulatory limits mandated for pesticides in hemp related products from California and other states. Tables 4-5 illustrate that the absolute recoveries of all 66 pesticides at a low level of 0.1 µg/g were within the acceptable range of 70-120%, with RSDs less than 20% for the three hemp samples analyzed.

Table 4. Recovery of Category II Pesticides in Hemp with Solvent Extraction .

S. No.	Category II Residual Pesticide	Level 0.1 µg/g	
		Recovery/%	RSD/% (n=3)
1	Abamectin	100	16
2	Acephate	101	3
3	Acequinocyl	95	6
4	Acetamiprid	101	4
5	Azoxystrobin	104	4
6	Bifenazate	98	5
7	Bifenthrin	101	7
8	Boscalid	102	3
9	Captan	90	10
10	Carbaryl	99	4
11	Chlorantraniliprole	102	5
12	Clofentezine	94	6
13	Cyfluthrin	87	10
14	Cypermethrin	90	8
15	Diazinon	101	5
16	Dimethomorph	93	5
17	Etoxazole	98	5
18	Fenhexamid	92	5
19	Fenpyroximate	100	6
20	Flonicamid	100	3
21	Fludioxonil	102	5
22	Hexythiazox	96	6
23	Imidacloprid	97	4
24	Kresoxim-methyl	98	6
25	Malathion	99	5
26	Metalaxyl	103	5
27	Methomyl	99	3
28	Myclobutanil	100	5
29	Naled	100	5
30	Oxamyl	99	4
31	Pentachloronitrobenzene	90	6
32	Permethrin	97	7
33	Phosmet	102	5
34	Piperonylbutoxide	96	6
35	Prallethrin	88	7
36	Propiconazole	96	6
37	Pyrethrins	97	6
38	Pyridaben	99	6
39	Spinetoram	99	8
40	Spinosad	91	10
41	Spiromesifen	100	5
42	Spirotetramat	98	5
43	Tebuconazole	100	5
44	Thiamethoxam	97	3
45	Trifloxystrobin	99	6

Table 5. Recovery of Category I Pesticides in Hemp with Solvent Extraction .

S. No.	Category I Residual Pesticide	Level 0.1 µg/g	
		Recovery/%	RSD/% (n=3)
1	Aldicarb	99	3
2	Carbofuran	100	4
3	Chlordane	91	7
4	Chlorfenapyr	84	8
5	Chlorpyrifos	99	6
6	Coumaphos	99	5
7	Daminozide	74	3
8	DDVP (Dichlorvos)	95	5
9	Dimethoate	98	4
10	Ethoprop(hos)	99	5
11	Etofenprox	98	6
12	Fenoxycarb	100	6
13	Fipronil	102	6
14	Imazalil	95	6
15	Methiocarb	101	3
16	Methyl parathion	99	6
17	Mevinphos	100	4
18	Paclobutrazol	98	5
19	Propoxur	98	4
20	Spiroxamine	99	4
21	Thiacloprid	101	4

LC/MS/MS Method with Optimum MRM Transitions for Challenging Analytes in Hemp

Hemp is a complex and difficult matrix to evaluate in the analysis of low-level pesticides, as the presence of isobaric compounds causes substantial matrix interference in the signal of some pesticides. To improve selectivity, MRM transitions for several pesticides with minimal matrix interference were determined for low level detection. For example, propiconazole can be ionized easily as a protonated molecular ion in a standard. However, the MRM transitions, based on the monoisotopic mass ion in the hemp matrix, resulted in a poor LOQ of 0.5 µg/g. Therefore, MRM transitions based on other masses were determined in order to reduce matrix interference and achieve an LOQ of 0.02 µg/g for propiconazole. Figure 1 shows the signal overlay of a blank hemp matrix and propiconazole spiked at 0.1 µg/g using MRM transitions with (figure 1A) and without (figure 1B) matrix interference. This figure demonstrates that optimum propiconazole MRM transitions helped in achieving lower detection limits owing to minimal matrix interference from hemp.

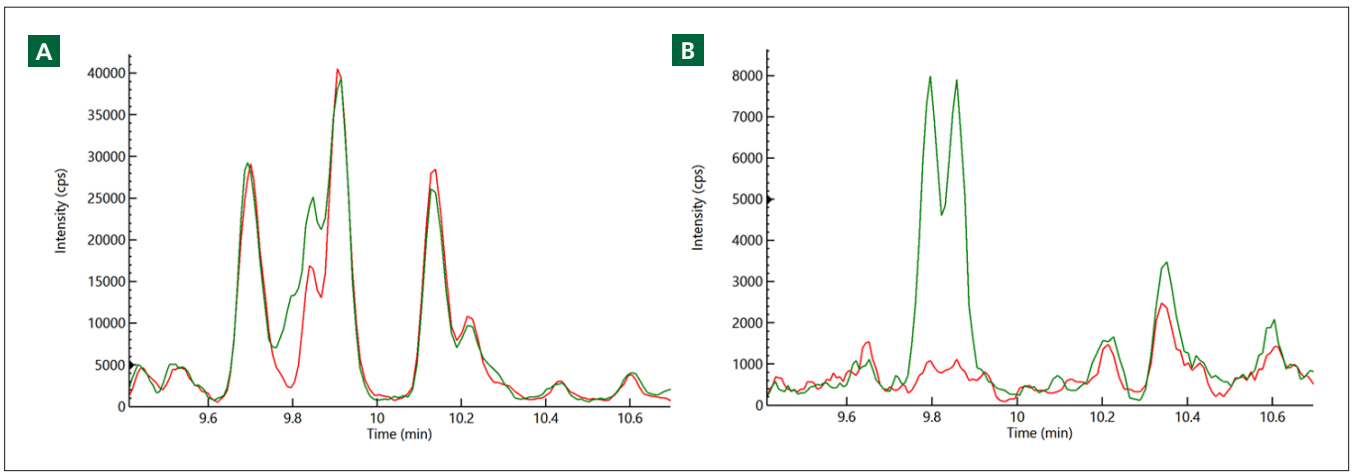

Figure 1. (A) Overlay of response of hemp matrix (Red) and propiconazole (Green) spiked at level of 0.1 µg/g in hemp matrix using MRM transition with matrix interference and (B) Overlay of response of hemp matrix (Red) and propiconazole (Green) spiked at level of 0.1 µg/g in hemp matrix using MRM transition without matrix interference.

Hydrophobic and Nonpolar Pesticides Analyzed with APCI

Hydrophobic and non-polar pesticides (e.g. pentachloronitrobenzene, methyl parathion, chlordane and chlorfenapyr) are traditionally analyzed by GC/MS/MS, as they do not ionize effectively by LC/MS/MS when solely utilizing an ESI source. An atmospheric pressure chemical ionization (APCI) source is an ionization method that complements ESI, excelling in the analysis of non-polar and medium-polar analytes. Since an APCI ion source is better suited for ionization of highly hydrophobic and non-polar analytes, APCI was used to determine the detection limits of chlorfenapyr, pentachloronitrobenzene, methyl parathion and chlordane in hemp. Figure 2 shows excellent signal-to-noise (S/N> = 100) for chlorfenapyr spiked at a level of 0.1 µg/g in the hemp matrix using an LC/MS/MS system with an APCI source and a fast six-minute short LC gradient.

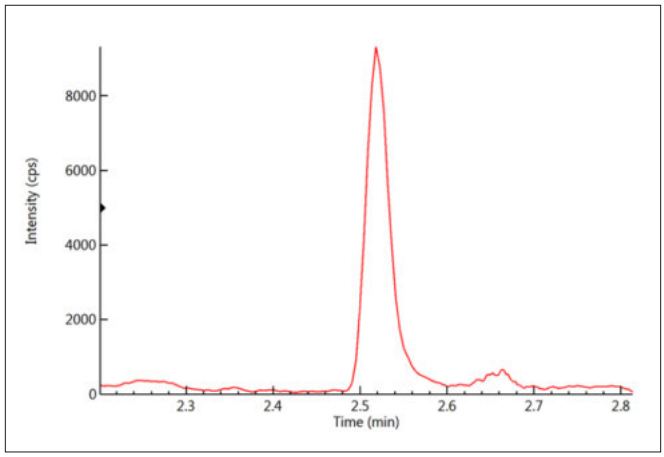

Figure 2. Sample chromatogram of chlorfenapyr spiked at level of 0.1 µg/g in a hemp matrix using LC/MS/MS system with APCI source.

Proficiency Testing

A proficiency test is an interlaboratory test that allows for the evaluation of method performance. To demonstrate the accuracy and validation of the pesticide method described herein, PerkinElmer participated in an Emerald Scientific blind proficiency test for pesticides regulated in a hemp matrix by the state of California[2].

Approximately 50 laboratories participated in this proficiency test for pesticides analysis in a hemp matrix. Each laboratory was provided with a one gram sample of hemp which had been spiked with various amounts of pesticides, as well as one gram of blank hemp material. The labs were asked to report the concentration of pesticide residues found in the provided samples with their methods. After submission of the proficiency test results, Emerald Scientific calculated the average and standard deviation of results obtained from all of laboratories. Conventional statistical methods were used to identify outliers in the submitted data, which were eliminated from this calculation.

According to the *International Harmonized Protocol of Proficiency Testing of Analytical Chemistry Laboratories*[3] (2006), a z-score was used as the quantitative criterion for the evaluation of the performance of laboratory methods. The z-score for each pesticide in the spiked sample was determined by calculating the absolute difference between a given lab result and the mean of all results, and dividing this result by the standard deviation of all laboratory results. The following internationally accepted classification was used [3,4,5].

- $z \leq 2$, satisfactory result;
- $2 < z < 3$, doubtful result;
- $z > 3$, unsatisfactory result.

Using these criteria, the method presented herein generated satisfactory results, as the proficiency test report indicated z-scores of less than two for all pesticides analyzed. Figure 3 illustrates the distribution of z-scores for pesticides quantified in the hemp matrix utilizing the method described in this work. This figure shows that all z-scores were less than the acceptable value of two, and that the majority (approximately 87%) of z-scores were less than 0.5, demonstrating excellent accuracy in the quantification of all 66 pesticides in hemp. The proficiency test data did not report any false positive or false negative results for any of the 66 pesticides regulated in hemp by the state of California.

Stability Studies

Figure 4 illustrates the signal stability for 330 sample injections for six analytes over a period of five days utilizing the method in this work. The % RSDs of the signal for all 66 analytes were less than 25%. These results demonstrate that the heated self-cleaning dual ESI/APCI ion source with laminar flow in the QSight LC/MS/MS system reduces the need for maintenance typically required with this complex and challenging matrix.

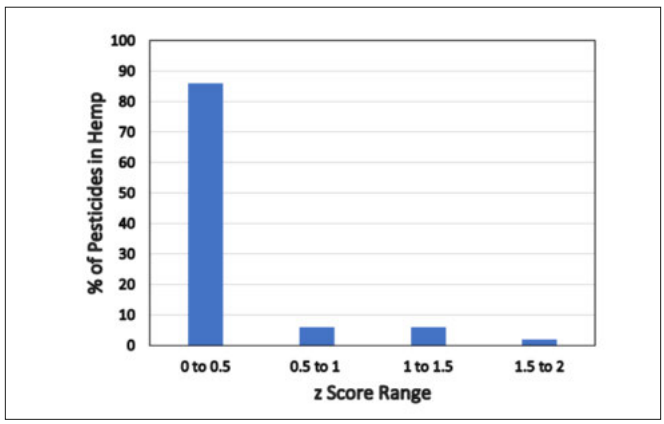

Figure 3. The distribution of z-scores for pesticides quantified in the hemp sample received from Emerald Scientific for proficiency testing of the LC/MS/MS method described herein.

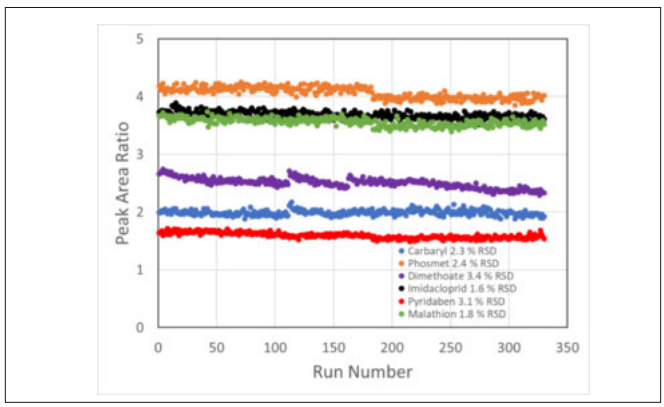

Figure 4. Long term stability data over five days of 330 sample injections of pesticides spiked in hemp extract using LC/MS/MS method.

Conclusions

This study demonstrates a unique, quantitative, rapid, and reliable LC/MS/MS method for the analysis of 66 pesticide residues in hemp samples. The proposed solvent extraction method is suitable for labs seeking compliance with California regulations, as the recovery of all pesticides from the hemp matrix were in the acceptable range of 70-120%, with RSDs less than 20%. This method allowed for the identification and quantification of all 66 pesticides at low levels (0.001 to 0.1 µg/g). The ability to screen and quantitate all 66 pesticides, including the highly hydrophobic and chlorinated compounds typically analyzed on a GC/MS/MS system, makes this method a novel and efficient option for analysis with a single instrument. The proficiency test data illustrated excellent accuracy of the method in the quantification of all 66 pesticide residues in the hemp matrix provided with single LC/MS/MS platform with dual ESI and APCI ion sources.

References

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