Achieving the California Pesticide Regulations in *Cannabis* Using Optimized APCI and ESI Techniques

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Cannabis testing regulations in the USA are currently defined at the state level, with each state outlining which pesticides to monitor and the acceptable maximum residue limits (MRL) for each pesticide. California legalized adult usage of *Cannabis* in 2018 and its state-specific regulations for cannabis testing are still developing. Prior to California legalization, Oregon had one of the most comprehensive pesticide testing panels in the United States. The adoption of the current California testing regulations, however, make it the largest pesticide panel for cannabis-specific testing in the United States, with generally lower MRL's than Oregon.

Currently, the California List is divided in two categories. The Category I pesticides contain 21 residues that must be reported as "Pass" or "Fail," dependent on whether the residue exceeds a limit of detection (LOD) of 0.1 ppm in all Cannabis products. The Category II residues list 45 compounds with MRL's in "Inhalable *Cannabis* Goods" or "Other *Cannabis* Products." The Category II pesticides also have limits of quantitation (LOQ) at variable MRL's for inhalables or "Other *Cannabis* Goods." Generally, inhalables have the lowest action limits at 0.1 ppm. Of the six California List compounds not currently on the Oregon List, three are considered extremely difficult to analyze by LC-MS/MS: (1) Captan, (2) Chlordane and (3) Pentachloronitrobeneze (PCNB). Historically, these have been analyzed by GC-MS. Captan, however, is challenging to analyze by GC-MS due to its temperature sensitive nature and tendency to degrade during analysis.

The variability and diversity of tested matrices make high throughput pesticide residue testing for cannabis particularly difficult. Additionally, the abundance of cannabinoids and terpenes often suppress chemical response in electrospray ionization (ESI) analysis. This suppression can lead to inaccuracies in quantitation and potentially cause reported pesticide values to be lower than actual concentrations. The method presented here was created by SCIEX to optimize pesticide residue testing and to meet the entire California List regulatory requirements. This method uses atmospheric pressure chemical ionization (APCI) for the majority of the panel, as it is less prone to both ion source saturation and ion suppression. While a smaller subsect of the panel is analyzed using ESI.

This two-injection method, utilizing ESI and APCI, allows for the entire pesticide suite on the California List to be analyzed by LC-MS/MS.

Key Advantages of APCI and ESI Ionization

- The entire California pesticide suite can be accomplished using LC-MS/MS on a single instrument
- Analytes analyzed in APCI are less prone to ion suppression, therefore a smaller variety of internal standards are needed to correct for matrix effects
- Noise enhancement of the baseline in dirty matrices, such as *Cannabis*, is highly mitigated in APCI when compared to traditional ESI
- Greater sensitivity for Chlorfenapyr and Methyl Parathion in APCI compared to ESI
- Matrix data at the action limits and recovery against a solvent calibration curve was collected on a SCIEX QTRAP® 6500+ system.

Experimental

Sample Preparation: Analytical standards were purchased from RESTEK (State College, PA) and Sigma Aldrich (St. Louis, MO). Chlordane analysis was spiked with purified cis-chlordane purchased from Supelco. During analysis, it was discovered that technical Chlordane standards from multiple vendors showed varying concentrations of cis- or trans-chlordane at 8-10% purity compared to a purified cis-chlordane analytical standard. Extreme variability was also observed from commercial mixes that contained Chlordane and Captan. Due to concerns about standard stability and purity of cis or trans chlordane, individual purified standards were purchased, and a spiking pesticide mix was created in house.

Samples were extracted into acetonitrile according to the modified vMethod protocol.

- 1 gram of homogenized flower was extracted in 10 mL of acetonitrile
- Sample was vortexed for 30 seconds
- Sonicated for 15 minutes
- Extracts were winterized for at least 2 hours in a -20ºC freezer or colder
- Supernatant was transferred to another vial and winterized again for 2 hours
- Centrifuged at 4000 rpm and passed through a 0.2 µm nylon syringe filter
- Injected 2 µL for ESI analysis and 5 µL for APCI analysis

HPLC Conditions***:*** Analytes from all compound classes were separated on a Phenomenex Luna Omega Polar C18, 3 µm LC Column (150 x 4.6 mm) using a SCIEX ExionLC™ AD system

Figure 1: Variability in Matrix. Flower extract after winterization (left). Flower extract after two rounds of winterization at -20ºC (right).

Table 1. LC Gradient Conditions for ESI Pesticide Panel.

Time	% B Concentration
1.5	*70*
2.0	*80*
6.0	*100*
8.0	*100*
8.1	*70*

Mobile Phase A: 0.1 % Formic Acid (5mM Ammonium Formate in H_2O)
Mobile Phase B: 0.1 % Formic Acid (5mM Ammonium Formate in MeOH)
Column Oven: 30ºC
Flow Rate: 0.8 mL/min

with a 20 µL solvent mixer. Any changes to the LC hardware have been observed to change analyte elution profile and areas of ion suppression in flower samples.

Mass Spectrometry Conditions: All compounds were analyzed using a QTRAP 6500+ system with *Scheduled* MRM™ Pro Algorithm (SCIEX). The Target Scan Time for both positive and negative polarity experiments were optimized to obtain at least 10 scans across each peak. Pesticides analyzed in positive polarity with the following source settings: NC = 5 V, TEM = 350ºC, CUR = 50 psi, CAD = 11, GS1 = 80 psi, GS2 = 60 psi. Pesticides analyzed in negative polarity with the following source settings: NC = -5 V, TEM = 700ºC, CUR = 50 psi, CAD = 11, GS1 = 40 psi.

Table 2. LC Gradient Conditions for APCI Pesticide Panel.

Time	% B Concentration
1.5	*5*
2.75	*65*
3	*65*
7	*70*
9	*85*
15	*95*
16.5	*100*
18	*100*
18.1	*5*

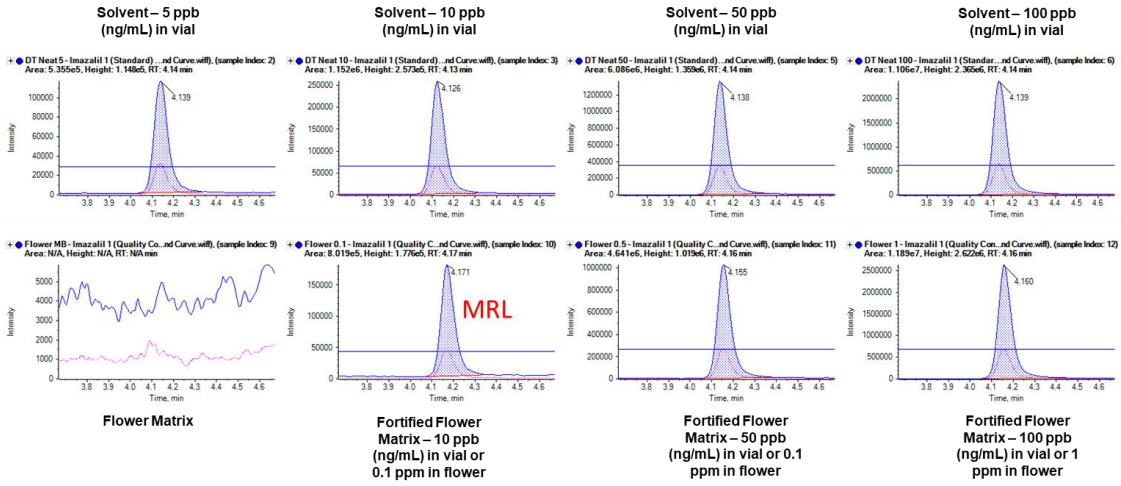
Mobile Phase A: Water
Mobile Phase B: Methanol
Column Oven: 30ºC
Flow Rate: 0.8 mL/min

This method completes the entire California pesticide panel by two separate injections in the same instrument platform. The first injection is analyzed by ESI on the IonDrive™ Turbo V source and the second injection is by APCI. Example data is shown in *Cannabis* flower extract fortified with pesticide standards at the state designated limits for inhalable product, as well as solvent blank for the ESI method (Figure 2) and the APCI method (Figure 3). Example compounds are shown for the unspiked flower matrix and flower matrix spiked with increasing pesticide concentrations. Each increasing spike concentration is shown as two values: the concentration "in-vial," which is calculated by external calibration regression, and the concentration of the original flower sample.

Table 3. Pesticides Analyzed by ESI Method.

Abamectin	*Permethrin*
Acequinocyl	*Phosmet*
Aldicarb	*Piperonyl Butoxide*
Bifenthrin	*Spinetoram*
Captan	*Spinosad*
Cyfluthrin	*Spiromesifen*
Cypermethrin	*Spiroxamine*
Imazalil	*Thiamethoxam*
Methomyl	

Imazalil



Spinosad

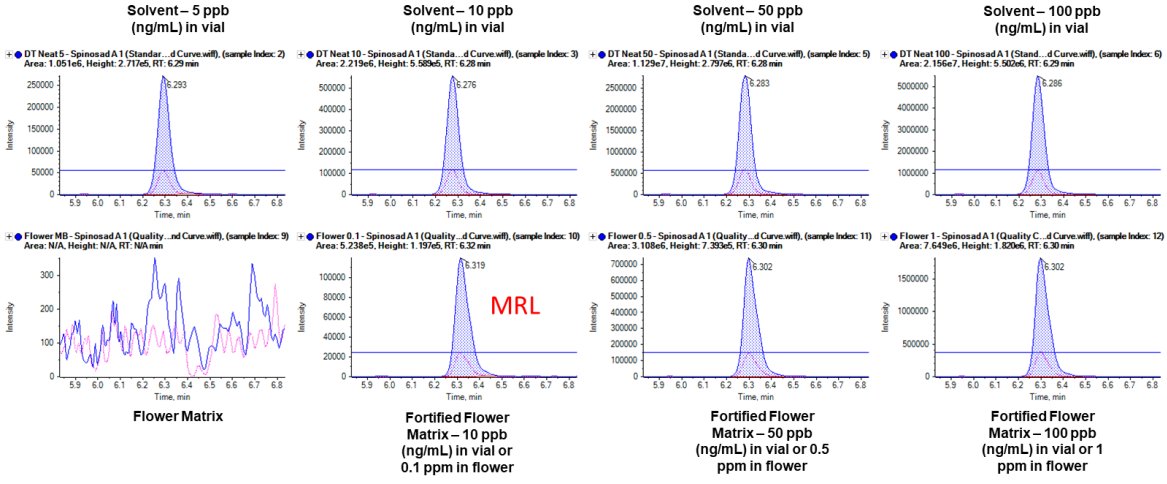
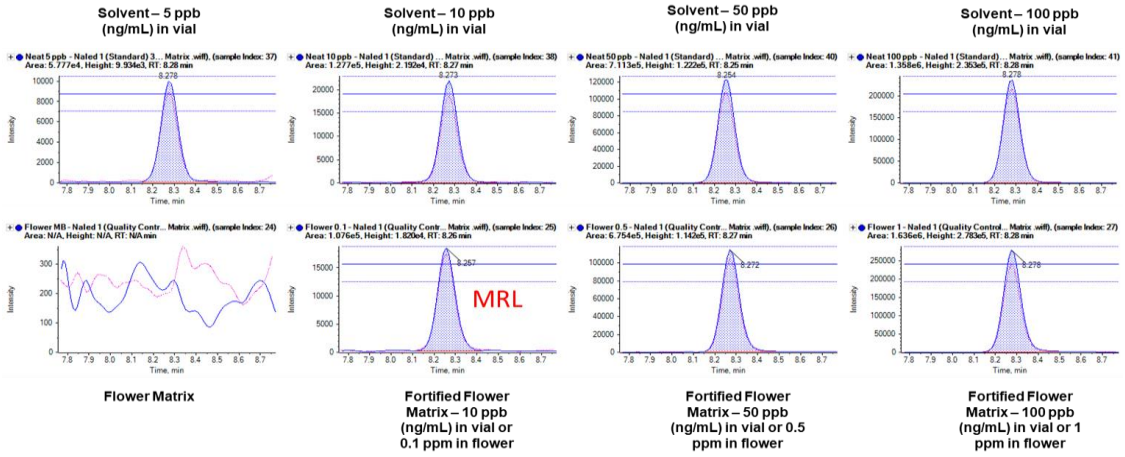
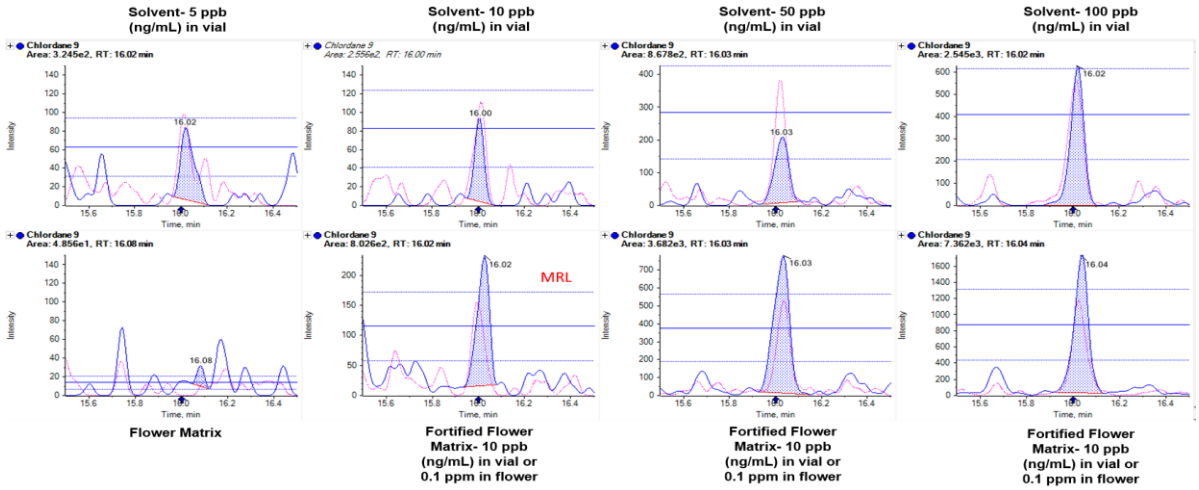


Figure 2: Example Data from Pesticides Monitored in ESI Method. (Top) Imazalil data in solvent and in cannabis flower extract. (Bottom) Spinosad data in solvent and in cannabis flower extract.

Naled



Chlordane



Quintozine (PCNB)

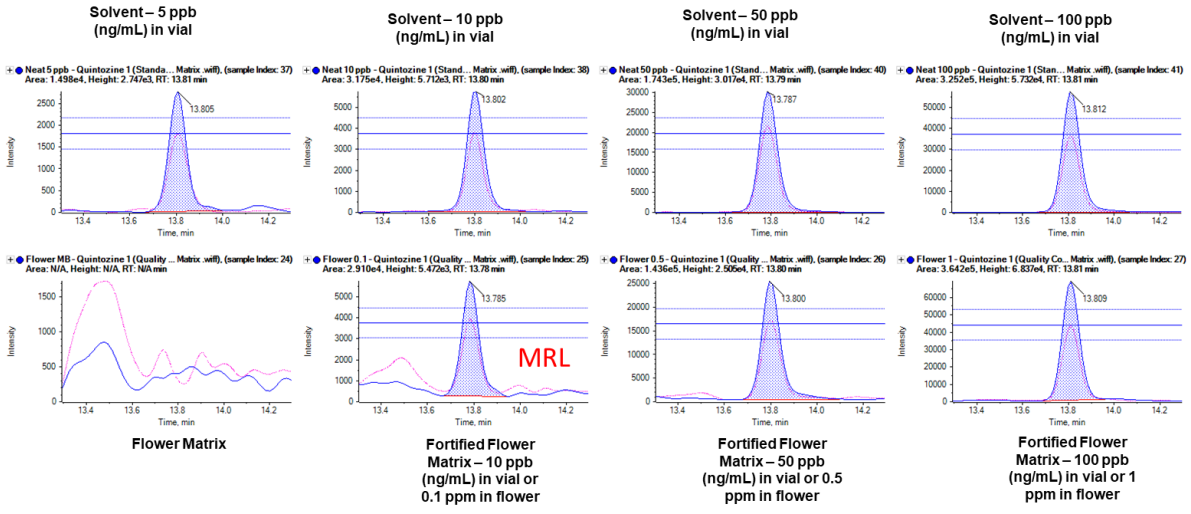


Figure 3: Example Data from Pesticides Monitored in APCI Method. (Top) Naled data in solvent and in cannabis flower extract. (Middle) cis-Chlordane data in solvent and in cannabis flower extract. (Bottom) Pentachloronitrobenzene (PCNB, Quintozine) data in solvent and in cannabis flower extract.

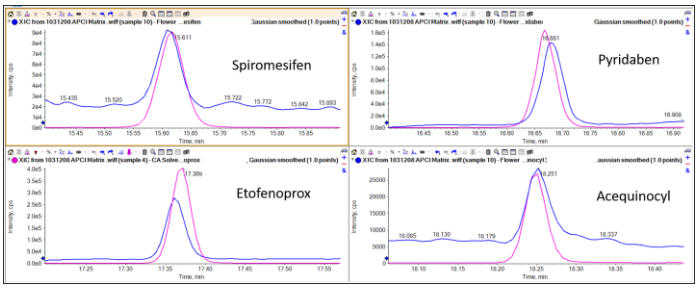


Figure 4: Extracted Ion Chromatogram (XICs) of 4 of the Most Hydrophobic Pesticides. The latest eluting pesticides on a reverse phase column chemistry showing a pesticide solvent standard (pink trace) overlaid with pesticide spiked into cannabis flower extract (blue trace) at the same concentration in vial. Spiromesifen, Pyridaben, and Acequinocyl shows recovery 80-120% as allowed by California. Etofenoprox shows 1.7-fold suppression in cannabis flower extract and will need correction with a deuterated internal standard.

Decreased ion suppression is observed in APCI when compared to ESI due to the differences in ionization mechanism. Therefore, a smaller variety of internal standards is needed to correct for matrix effects (Figure 4). The difference in ionization is key for analysis of complex matrices, such as *Cannabis*, because the abundant cannabinoid (~mg/g) concentrations are not out-competing pesticides for ionization.

Cannabis flower extract was fortified with pesticide analytical standards and back-calculated against a solvent calibration curve (Figure 5) to show matrix spike and recovery. The solvent standards were set as “standards,” while the pesticide-fortified flower extracts were designated as “quality controls” to analyze for %recovery.

Component Name	Sample Name	Num. Values	Mean	Standard De...	Percent CV	Value #1	Value #2	Value #3
Dichlorvos 1	Flower ESI 0.1 ppm	3 of 3	0.10	0.00	4.75	0.09	0.10	0.10
Dichlorvos 1	Flower ESI 0.5 ppm	3 of 3	0.38	0.02	5.75	0.37	0.41	0.37
Dichlorvos 1	Flower ESI 1 ppm	3 of 3	0.99	0.03	2.70	1.00	0.96	1.02

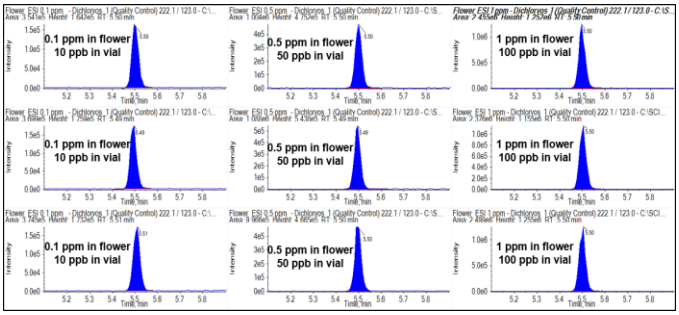


Figure 5: Quantitation of Dichlorvos. (Top) Statistics of back-calculated pesticide spiked cannabis flower against a solvent calibration curve without internal standard. Dichlorvos spiked at 3 different calibration levels and showed % Recovery of 76-100% and %CV of 4.75% at the MRL of 0.1 ppm. (Bottom) XIC’s of Dichlorvos spiked at 3 different calibration levels and showed % Recovery of 76-100% and %CV of 4.75% at the MRL of 0.1 ppm (n=3).

Table 4: Category I Pesticides. This table highlights the ability to analyze in matrix at the MRL on a QTRAP 6500+ system.

Category I Residual Pesticide	Maximum Residue Limit (ppm)	MRL in Matrix
Aldicarb	*0.1*	√
Carbofuran	*0.1*	√
Chlordane	*0.1*	√
Chlorfenapyr	*0.1*	√
Chlorpyrifos	*0.1*	√
Coumaphos	*0.1*	√
Daminozide	*0.1*	√
Dichlorvos	*0.1*	√
Dimethoate	*0.1*	√
Ethoprophos	*0.1*	√
Etofenoprox	*0.1*	√
Fenoxycarb	*0.1*	√
Fipronil	*0.1*	√
Imazalil	*0.1*	√
Methiocarb	*0.1*	√
Methyl Parathion	*0.1*	√
Mevinphos	*0.1*	√
Paclobutrazol	*0.1*	√
Propoxur	*0.1*	√
Spiroxamine	*0.1*	√
Thiacloprid	*0.1*	√

Summary

The two-injection application for the California List is an expansion on the SCIEX vMethod™ Application[2] for Quantitation of Pesticide Residues in *Cannabis* Matrices. Ongoing testing will be conducted in more flower strains and *Cannabis* products to fully address the needs for routine commercial analysis.

All 66 pesticides were ionized using the IonDrive Ion Source, including pesticides that were historically analyzed via GC-MS. The data presented indicates that this method, coupled with the SCIEX 6500+ QTRAP, meets and exceeds the MRLs for *Cannabis* flower defined by the California List (Table 4 and 5).

Table 5. Category I Pesticides. This table highlights the ability to analyze in matrix at the MRL on a QTRAP 6500+ system.

Category II Residual Pesticide	MRL (ppm) Inhalable Goods	MRL in Matrix	Category II Residual Pesticide	MRL (ppm) Inhalable Goods	MRL in Matrix
Abamectin	0.1	√	*Krexosim-methyl*	0.1	√
Acephate	0.1	√	*Malathion*	0.5	√
Acequinocyl	0.1	√	*Metalaxyl*	2	√
Acetamiprid	0.1	√	*Methomyl*	1	√
Azoxystrobin	0.1	√	*Myclobutanil*	0.1	√
Bifenazate	0.1	√	*Naled*	0.1	√
Bifenthrin	3	√	*Oxamyl*	0.5	√
Boscalid	0.1	√	*PCNB*	0.1	√
Captan	0.7	√	*Permethrin*	0.5	√
Carbaryl	0.5	√	*Phosmet*	0.1	√
Chlorantraniliprole	10	√	*Piperonyl Butoxide*	3	√
Clofentezine	0.1	√	*Prallethrin*	0.1	√
Cyfluthrin	2	√	*Propiconazole*	0.1	√
Cypermethrin	1	√	*Pyrethrins*	0.5	√
Diazinon	0.1	√	*Pyridaben*	0.1	√
Dimethomorph	2	√	*Spinetoram*	0.1	√
Etoxazole	0.1	√	*Spinosad*	0.1	√
Fenhexamid	0.1	√	*Spiromesifen*	0.1	√
Fenpyroximate	0.1	√	*Spiroteramat*	0.1	√
Flonicamid	0.1	√	*Tebuconazole*	0.1	√
Fludioxonil	0.1	√	*Thiamethoxam*	5	√
Hexythiazox	0.1	√	*Trifloxystrobin*	0.1	√
Imidacloprid	5	√			

References

1. Farrer DG. Technical report: Oregon Health Authority's process to decide which types of contaminants to test for in cannabis. Oregon Health Authority (2015).
2. Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS. SCIEX Technical Note RUO-MKT-02-6729-B.
3. United States. BUREAU OF CANNABIS CONTROL PROPOSED TEXT OF REGULATIONS. *California Code of Regulations Title 16. Division 42*. *Bureau of Cannabis Control Emergency Regulation Text*. Web. 2 November 2017.http://www.bcc.ca.gov/law_regs/bcc_prop_text_reg.pdf