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Application News

SSI-LCMS-105

Liquid Chromatography Mass Spectrometry

California and Oregon's Complete Residual Pesticide Analysis using a Shimadzu LCMS-8060

Summary: California and Oregon's residual pesticide list for cannabis traditionally have been analyzed using both LCMS and GCMS because certain compounds do not ionize well by ESI-LCMS. This study demonstrates the use of APCI-LCMS and explores the utility of a complete LCMS solution for the analysis of the California and Oregon pesticides lists for cannabis. APCI-LCMS optimization was completed for ten pesticides and the rest were completed using ESI-LCMS. The resulting APCI-LCMS and ESI-LCMS MRM methods was tested in cannabis flower extract on a Shimadzu LCMS-8060. The LOQ determined for each pesticide was below the regulatory action level (Table 1).

Introduction: With the increase in medicinal and recreational cannabis legislation throughout the United States there is an emerging demand for pesticide testing on cannabis products. Currently each state is setting individual regulatory guidelines. This results in variation between the number of analytes tested and their required action levels; currently California regulates a total of 66 pesticides and Oregon regulates a total of 59 pesticides. Other states, such as Michigan, have adopted one of these lists. To analyze these complete lists, laboratories commonly use both LCMS and GCMS. This study evaluates using both ESI and APCI ionization techniques to quantitate the complete California list using only LCMS.

LCMS Instrumentation: A Shimadzu LCMS-8060 triple quadrupole mass spectrometer coupled with a Shimadzu Nexera X2 UHPLC system was employed for this evaluation. The LCMS-8060 was equipped with either an atmospheric pressure chemical ionization (APCI) ionization source or an electrospray (ESI) ionization source. Rapid polarity switching (5 msec) and fast Multiple Reaction Monitoring (MRM) enabled the acquisition of sufficient points across each peak.

LC/MS/MS Method development: Ten pesticides were analyzed by atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI-LCMS). All other pesticides were analyzed by electro spray ionization liquid chromatography mass spectrometry (ESI-LCMS). Each compound was purchased commercially as a certified neat standard or a mixture of like compounds and dissolved in acetonitrile or methanol to 1 mg/mL. The 1 mg/mL stock solutions were used for any necessary dilutions during method development.

Flow injection analysis (FIA) was used for the initial ionization testing and MRM optimization. Ionization evaluation consisted of Q1 and Q3 scans in both positive and negative polarity. Any viable precursors observed were further analyzed using MSMS scans and a range of collision energies.

For each pesticide one to five MRM transitions were acquired and tested with multiple columns. Final column selection was based on the best overall chromatographic separation and peak shape. On column testing was completed using a 1 µL injection. Established MRM transitions and final method parameters were tested in cannabis flower matrix.

Table 1. LOQ determined for each pesticide and the mode of ionization used.

Residual Pesticide	LOQ (ng/g)	Method of Ionization	Residual Pesticide	LOQ (ng/g)	Method of Ionization
Abamectin (-)	31	ESI or APCI	Fluopyram (+)	2	ESI
Acephate (+)	20	ESI	Hexythiazox (+)	15	ESI
Acequinocyl (+/-)	15	ESI or APCI	Imazalil (+)	10	ESI
Acetamiprid (+)	<2	ESI	Imidacloprid (+)	4	ESI
Aldicarb (+)	<2	ESI	Kresoxim-methyl (+)	4	ESI
Allethrin (+)	50	ESI	Malathion (+)	2	ESI
Azoxystrobin (+)	4	ESI	Metalaxyl (+)	2	ESI
Bifenazate (+)	2	ESI	Methiocarb (+)	4	ESI
Bifenthrin (+)	4	ESI	Methomyl (+)	<2	ESI
Boscalid (+)	4	ESI	Methoprene (+)	50	ESI
Buprofezin (+)	<2	ESI	Methyl-parathion (-)	8	APCI
Captan (-)	8	APCI	Mevinphos (+)	4	ESI
Carbaryl (+)	10	ESI	MGK 264 (+)	15	APCI
Carbofuran (+)	<2	ESI	Myclobutanil (+)	10	ESI
Chlorantraniliprole (+)	2	ESI	Naled (+)	2	ESI
Chlordane (-)	15	APCI	Novaluron (-)	15	ESI
Chlorfenapyr (-)	8	APCI	Oxamyl (+)	2	ESI
Chlorpyrifos (+)	10	ESI	Paclobutrazol (+)	2	ESI
Clofentazine (+)	4	ESI	Pentachloronitrobenzene (PCNB) (-)	63	APCI
Clothianidin (+)	4	ESI	Permethrin (+)	10	ESI
Coumaphos (+)	4	ESI	Phenothrin (+)	10	ESI
Cyantraniliprole (-)	2	ESI	Phosmet (+)	10	ESI
Cyfluthrin (-)	15	APCI	Piperonyl butoxide (+)	5	ESI
Cyfluthrin (+)	500	ESI	Pirimicarb (+)	2	ESI
Cypermethrin (+)	60	ESI	Prallethrin (+)	10	ESI
Cyprodinil (+)	10	ESI	Propiconazole (+)	60	ESI
Daminozide (+)	15	ESI	Propoxur (+)	2	ESI
Deltamethrin (+)	30	ESI	Pyraclostrobin (+)	10	ESI
Diazinon (+)	<2	ESI	Pyrethrins (+)	100	ESI
Dichlorvos (DDVP) (+)	15	ESI or APCI	Pyridaben (+)	2	ESI
Dimethoate (+)	<2	ESI	Resmethrin (+)	35	ESI
Dimethomorph (+)	5	ESI	Spinetoram (+)	2	ESI
Dinotefuran (+)	2	ESI	Spinosad (+)	<2	ESI
Dodemorph (+)	4	ESI	Spirodiclofen (+)	10	ESI
Endosulfan-sulfate (-)	4	ESI	Spiromesifen (+)	20	ESI
Ethoprophos (+)	2	ESI	Spirotetramat (+)	2	ESI
Etofenprox (+)	4	ESI	Spiroxamine (+)	2	ESI
Etoxazole (+)	<2	ESI	Tebuconazole (+)	2	ESI
Fenhexamid (+)	20	ESI	Tebufenozide (+)	5	ESI
Fenoxycarb (+)	2	ESI	Teflubenzuron (-)	15	ESI
Fenpyroximate (+)	10	ESI	Tetrachlorvinphos (+)	4	ESI
Fensulfothion (+)	5	ESI	Tetramethrin (+)	4	ESI
Fenthion (+)	100	ESI	Thiacloprid (+)	<2	ESI
Fenvalerate (+)	100	ESI	Thiamethoxam (+)	<2	ESI
Fipronil (-)	2	ESI	Thiophanate-methyl (+)	5	ESI
Flonicamid (-)	25	ESI	Trifloxystrobin (+)	<2	ESI
Fludioxonil (-)	2	ESI			

Final APCI-LCMS Method: Separation was accomplished and retention times determined on a Restek Raptor ARC-18 column (100mm x 2.1mm, 2.7um) using neat standards prior to in-matrix evaluation. A total run time of 15 mins was used with Mobile phase A as water, and mobile phase B as methanol with no additives. The gradient is shown in Figure 1 and the LCMS method parameters are shown in Table 2.

Time	%B
0	3
1	10
3	55
10.5	100
12	100
12.01	3

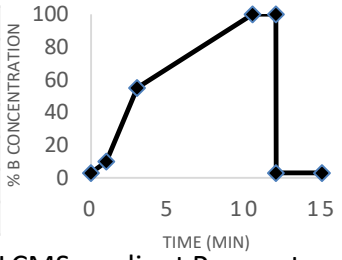

Figure 1: APCI-LCMS gradient Parameters

Table 2: APCI-LCMS Method parameters

Drying Gas	10.0 L/min
Heating Gas	10.0 L/min
Interface Temperature	350°C
DL Temperature	200°C
Heat Block Temperature	300°C
Flow rate	0.4 mL/min
Injection Volume	1µL
Column Oven Temperature	30°C
Sample Tray Temperature	10°C

Final ESI-LCMS Method: Separation was accomplished and retention times determined on a Shim-pack Velox C18 column (150mm x 2.1mm, 2.7um) using neat standards prior to in-matrix evaluation. A total run time of 15 mins was used with Mobile phase A as water with 0.1%Formic acid and 5mM Ammonium formate, and mobile phase B as methanol with no additives. The gradient is shown in Figure 2 and the LCMS method parameters are shown in Table 3.

Time	%B
0	3
1	10
3	55
10.5	100
12	100
12.01	3

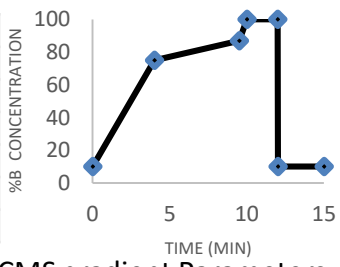

Figure 2: ESI-LCMS gradient Parameters

Table 3: ESI-LCMS Method parameters

Drying Gas	15.0 L/min
Heating Gas	15.0 L/min
Interface Temperature	100°C
DL Temperature	200°C
Heat Block Temperature	100°C
Flow rate	0.5 mL/min
Injection Volume	1µL
Column Oven Temperature	40°C
Sample Tray Temperature	10°C

Sample Extraction: Dried cannabis flower samples, spiked and unspiked (blank), were extracted in the following manner. One gram of dried cannabis flower was weighed. Spiking of pesticide compounds was performed by adding 50 µL of a 40 µg/mL stock solution containing all pesticides. This spiking level is equal to 2 µg/g in cannabis flower. Acetonitrile, 10 mL, was added to each sample. Three steel commercial grinder balls were placed in each sample and the samples were subjected to 5 min of grinding at 1500 RPM. Centrifugation was then performed for 5 min at 2800 RPM and the supernatants transferred to vials. The spiked flower extract was diluted serially with blank flower extract to produce an in-matrix calibration curve.

Calibration: Matrix-matched calibration curves were prepared by serial dilution of spiked flower extract with blank flower extract and evaluated for each pesticide. The calibration set included multiple different concentrations, ranging from 1.0 ng/g to 2000 ng/g. The final concentration range utilized for each pesticide varied depending on the individual detection limit. Pesticide calibration curves were analyzed in order of high to low, and each curve was followed by a QC sample and a blank for performance and carryover assessment. Internal standards were tested with the ESI-LCMS method.

Precision and Accuracy: Method precision and accuracy were determined by measuring the calibration curve levels in triplicate. Accuracy was calculated utilizing LabSolutions software by comparing the measured concentration against the theoretical concentration for each calibration point. Limits of Quantitation (LOQ) were determined from the calibration curve data. The LOQ reported for each pesticide had a signal-to-noise ratio greater than 10, and had a %RSD value less than 20%.

Results and Discussion: Using the same sample, the entire California and Oregon residual pesticide list was analyzed. **T**he APCI-LCMS method demonstrated accurate and precise trace-level quantitation in cannabis flower for 10 pesticides that are traditionally analyzed by GCMS and the ESI-LCMS method demonstrated the same robust and reproducibility for the remaining 84 pesticides. Both gradient methods were successfully used for chromatographic separation and identification of all 94 pesticides (Figure 3 and 4). The LOQ for each pesticide was below the California and Oregon action levels in cannabis, and precision and accuracy results were excellent. LOQs were determined for each pesticide using their corresponding retention time and a S/N calculation above 10:1.

Linear calibration curves were prepared using spiked standards in homogenized cannabis flower. All calibration curves demonstrated linearity with a range from 1 ng/g to 2000 ng/g on flower concentrations. A 1/C weighting factor was used for statistical calculations and resulted in R2 >0.99 for all pesticides. Representative chromatograms and calibration curves can be found in Figure 5 and 6.

Representative data is shown for the APCI-LCMS and ESI-LCMS methods. Chromatographic separation of analytes from matrix interferences results in low signal suppression. Optimized spray voltage and low-temperature interface conditions yield good signal intensity for several challenging analytes.

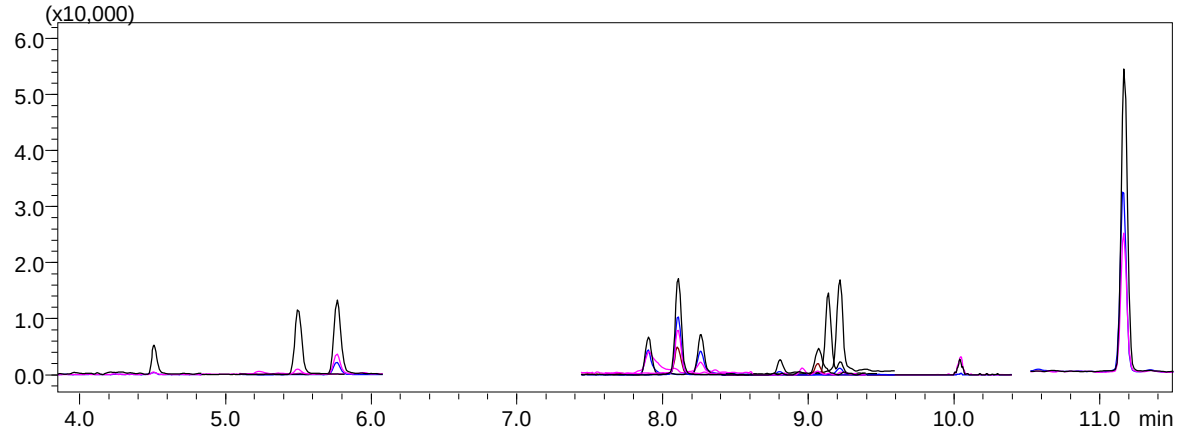

Figure 3. Representative Chromatogram for 10 pesticides using APCI-LCMS

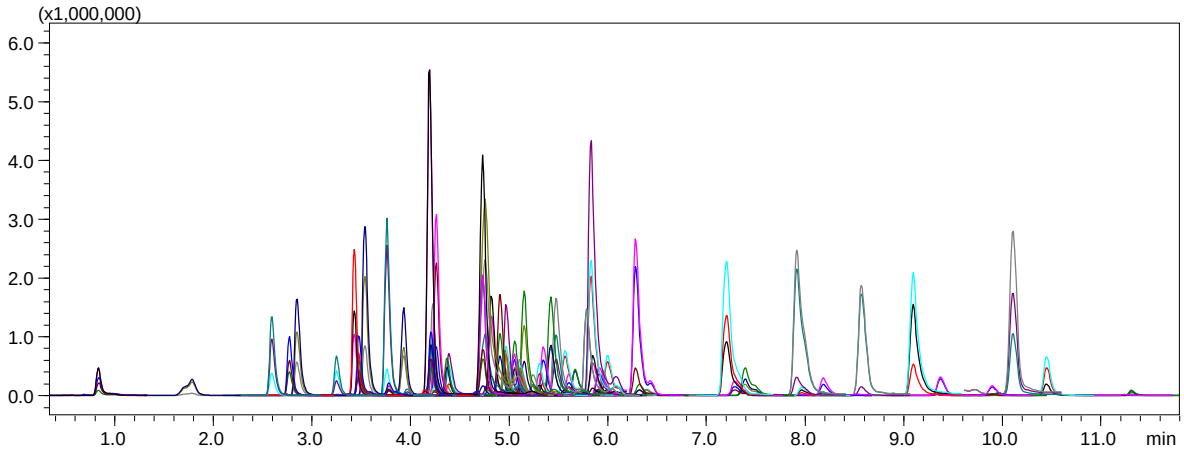

Figure 4. Representative Chromatogram for 84 pesticides using ESI-LCMS

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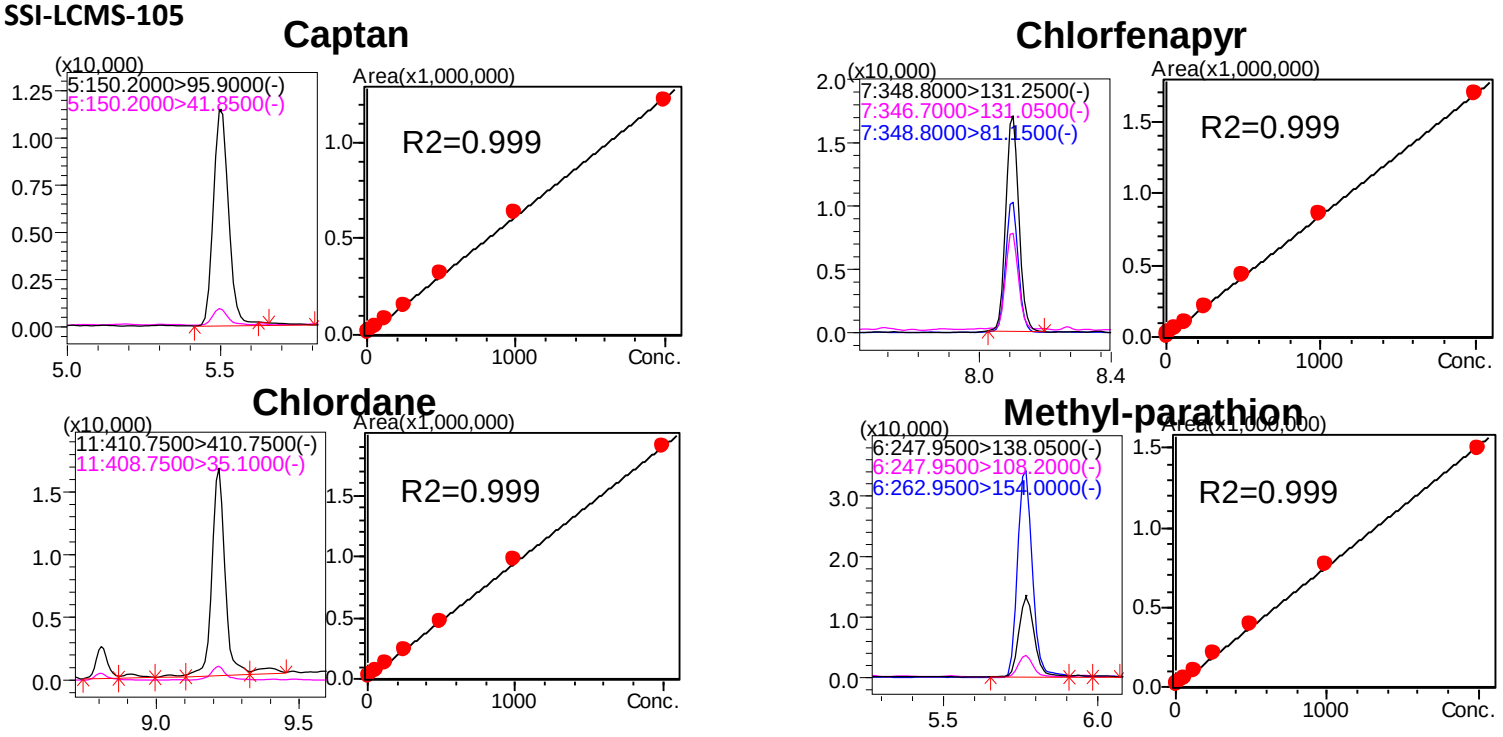

Figure 5. Calibration Curves and MS Chromatograms at 62 ng/g in Cannabis Matrix for APCI-LCMS Pesticides

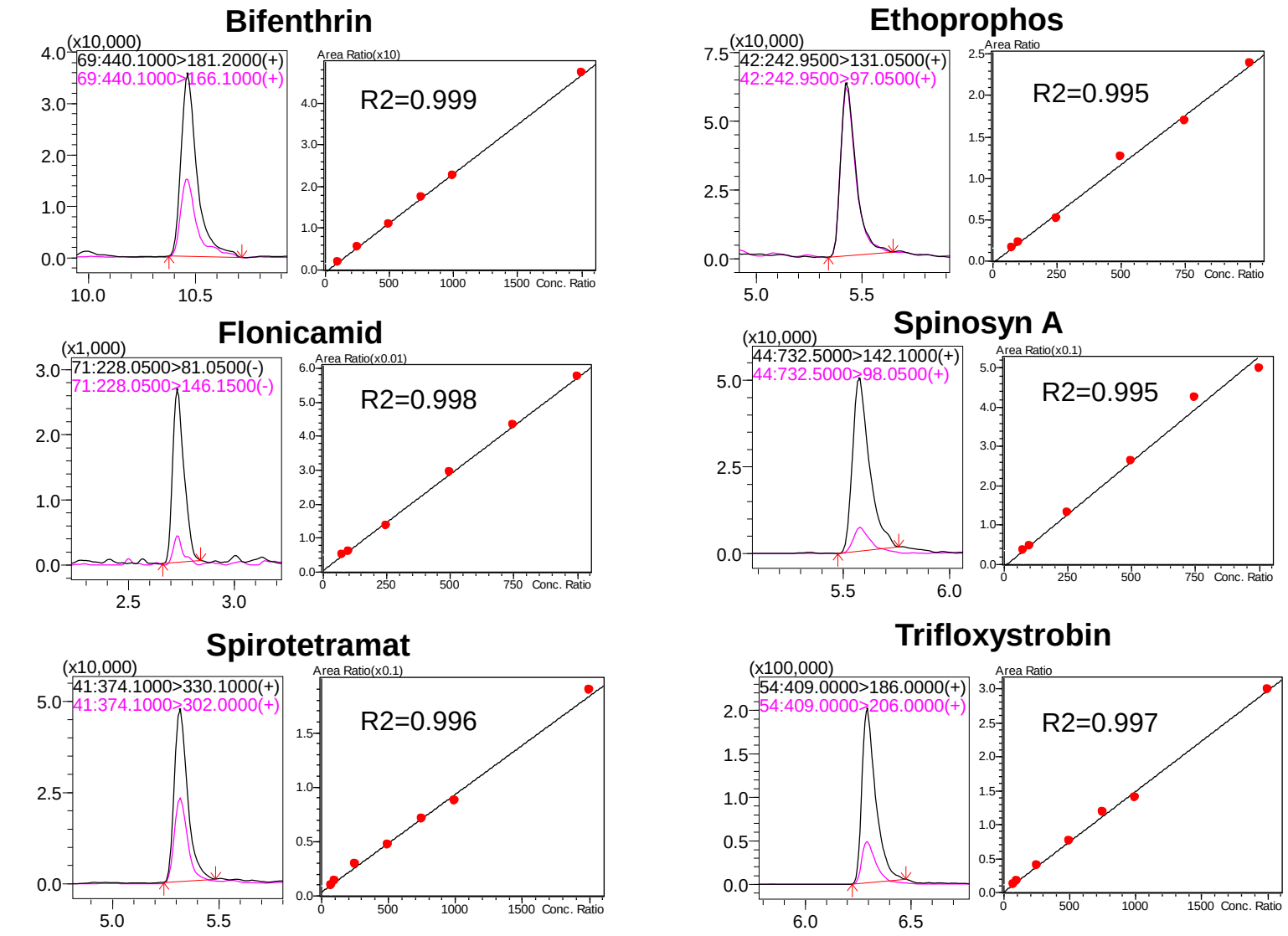

Figure 6. Calibration Curves and MS Chromatograms at 75 ng/g in Cannabis Matrix for ESI-LCMS Pesticides

Conclusion: A complete LCMS solution was developed for residual pesticide testing in cannabis matrix utilizing both APCI and ESI ionization techniques coupled with a single Shimadzu LCMS-8060. The APCI-LCMS method was developed and tested in cannabis flower matrix for the analysis of 10 California and Oregon regulated pesticides that have been traditionally analyzed by GCMS. An ESI-LCMS was further optimized and tested in cannabis flower matrix for the analysis of 84 total pesticides. The LOQs determined in this method were well below the action limits required by California and Oregon, demonstrating the viability of an LCMS total solution for cannabis testing in these two programs. The use of the ultrafast polarity switching capability of the LCMS-8060 allowed for accurate and sensitive quantitation of all 94 pesticides currently being regulated by California or Oregon.

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