Application News

SSI-LCMS-104

Liquid Chromatography Mass Spectrometry

California and Oregon Residual Pesticide Analysis using APCI with LC-MS/MS

■ Summary

California and Oregon both have lists of pesticides in cannabis to be analyzed. These pesticides are typically 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 LCMS for the analysis of the complete California and Oregon pesticides lists for cannabis. APCI-LCMS optimization was completed for ten pesticides. The resulting APCI-LCMS MRM method 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 an APCI-LCMS method for the quantitation of compounds frequently analyzed by GCMS.

■ 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 an atmospheric pressure chemical ionization (APCI) ionization source. Rapid polarity switching (5 msec) and fast Multiple Reaction Monitoring (MRM) enabled the acquisition of sufficient points across each peak.

Table 1: LOQ determined using APCI-LCMS and Required Action Levels for the State of CA and OR

Residual Pesticide	California Action Level (µg/g)	Oregon Action Level (µg/g)	APCI-LCMS LOQ (µg/g)	LOQ %RSD (n=3)
Abamectin (-)	0.1	0.1	0.0313	13.4
Acequinocyl (-)	0.1	1	0.0156	5.9
Captan (-)	0.7	NA	0.0078	3.8
Chlorfenapyr (-)	0.1	1	0.0078	3.7
Chlordane (-)	0.1	NA	0.0156	5.8
Cyfluthrin (-)	2	1	0.0156	7.8
Dichlorvos (DDVP) (+)	0.1	0.1	0.0156	7.2
Methyl-parathion (-)	0.1	0.2	0.0078	11.6
MGK 264 (+)	NA	0.2	0.0156	6.2
Pentachloronitrobenzene (PCNB) (-)	0.1	NA	0.0625	11.4

■ LC/MS/MS Method Development

A total of 10 pesticides were analyzed by atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI-LCMS). Each compound was purchased commercially as a certified neat standard 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. 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. On column testing was completed using a 1 µL injection with a total run time of 15 mins. Mobile phase A was water, and mobile phase B was methanol with no additives. The gradient is shown in Figure 1 and the LCMS method parameters are shown in Table 2. Established MRM transitions and final method parameters were tested in cannabis flower matrix (Table 3).

■ Sample Extration

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 10 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 ranging from 0.00781 µg/g to 2 µg/g.

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

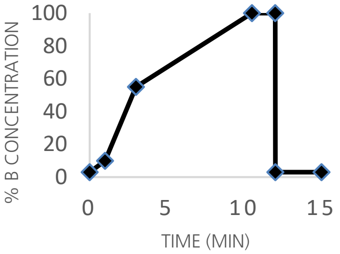

Figure 1: LC gradient Parameters

Table 2: LCMS-8060 Method parameters

Drying 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

■ 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 nine different concentrations, ranging from 0.00781 µg/g to 2 µg/g. The final concentration range utilized for each pesticide varied depending on the individual detection limit. Calibration curves were plotted using peak area versus concentration with a 1/C weighting factor. 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. No internal standards were used.

■ 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%.

Table 3: MRM Transitions used for APCI-LCMS Pesticide Testing

Compound Name	Transition 1	CE 1	Transition 2	CE 2	Transition 3	CE 3
Abamectin (-)	871.35>229.15	34	871.35>565.3	30	871.35>835.3	19
Acequinocyl (-)	384.3>342.15	16	384.3>187.1	35	384.3>159.1	55
Captan (-)	150.2>95.9	21	150.2>41.85	40	NA	
Chlorfenapyr (-)	348.8>131.25	38	346.7>131.05	37	348.8>81.15	34
Chlordane (-)	410.75>410.75	6	408.75>35.1	9	444.75>444.75	5
Cyfluthrin (-)	207.05>35.1	12	NA		NA	
Dichlorvos (DDVP) (+)	220.9>109.1	-18	220.9>78.8	-26	220.9>95.15	-47
Methyl-parathion (-)	247.95>138.05	15	247.95>108.2	35	262.95>154	15
MGK 264 (+)	276.15>210.1	-14	276.15>79.95	-40	276.15>98.05	-24
Pentachloronitrobenzene (PCNB) (-)	275.85>201.9	26	275.85>245.9	14	264.8>35.0	40

■ Results and Discussion

The Shimadzu LCMS-8060 APCI-LCMS method demonstrated accurate and precise trace-level quantitation in cannabis flower for 10 pesticides that are commonly analyzed by GCMS. The gradient method was successfully used for chromatographic separation and identification of all 10 pesticides (Figure 2). 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 7.8ng/g to 2ug/g on flower concentrations. A 1/C weighting factor was used for statistical calculations and resulted in R2=0.996 or higher for all pesticides. Representative chromatograms and calibration curves can be found in Figure 3.

The representative data shown in this application was completed with little chromatographic optimization. Further optimization could be done to allow for a shorter overall run time and better chromatographic separation for the late eluting compounds. Additionally, the sample extraction method used for the acquired data was only a dilute and shoot style method. Further sample preparation studies could be done to increase signal intensity or reduce matrix interference seen with the more difficult pesticides.

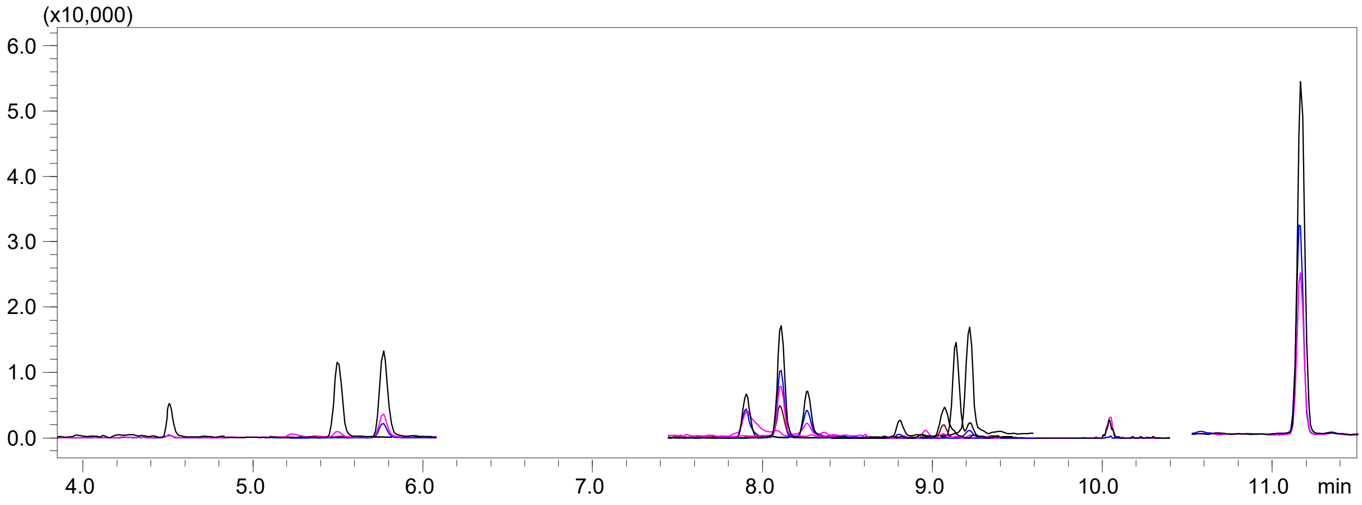

Figure 2: Representative Chromatogram for 10 pesticides using APCI-LCMS

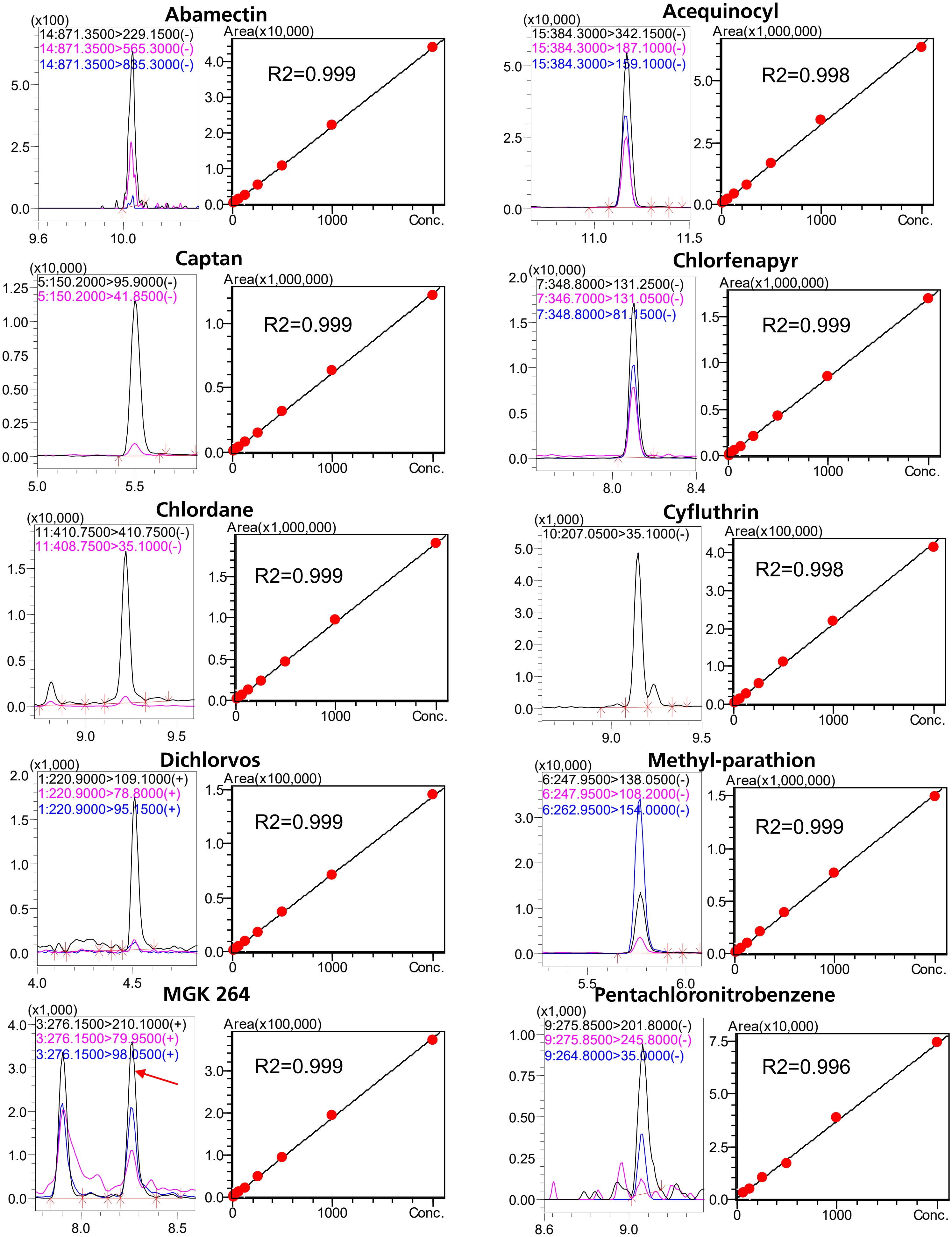

Figure 3: Calibration Curves and MS Chromatograms at 0.062ug/g in Cannabis Matrix for Ten Pesticides

■ Conclusion

An 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. 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.

Utilizing LCMS for these compounds does, however, require the use of certain nonspecific transitions, including fragmentary precursor ions and single chlorine atom product ions and/or SIM mode analysis.

Careful assessment and verification of retention times is required. Accurate retention time windows have been a mainstay of chromatographic analysis for decades, so reliance on retention time as a key feature of positive identification is not unusual, particularly in LCMS triple quadrupole analysis where segmented MRMs are the norm. Nevertheless, the testing laboratory should carefully consider the use of nonspecific transitions and evaluate the risk of false positive results prior to implementation of an LCMS method for these compounds.

UFMS
ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

Founded in 1875, Shimadzu Corporation, a leader in the development of advanced technologies, has a distinguished history of innovation built on the foundation of contributing to society through science and technology. Established in 1975, Shimadzu Scientific Instruments (SSI), the American subsidiary of Shimadzu Corporation, provides a comprehensive range of analytical solutions to laboratories throughout North, Central, and parts of South America. SSI maintains a network of nine regional offices strategically located across the United States, with experienced technical specialists, service and sales engineers situated throughout the country, as well as applications laboratories on both coasts.

For information about Shimadzu Scientific Instruments and to contact your local office, please visit our Web site at www.ssi.shimadzu.com

Shimadzu Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS, INC.
Applications Laboratory
7102 Riverwood Drive, Columbia, MD 21045
Phone: 800-477-1227 Fax: 410-381-1222
URL http://www.ssi.shimadzu.com

For Research Use Only. Not for use in diagnostic procedures. The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to is accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

©Shimadzu Scientific Instruments, 2019

First Edition: December 2019