SHIMADZU

MP070

Detection of typical GCMS Pesticides in Cannabis Matrix utilizing APCI-LCMS

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1. Overview

Residual pesticides in cannabis 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 list for California and Oregon residual pesticide analysis in 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).

2. 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.

Table 1 LOQ determined using APCI-LCMS

Residual Pesticide (Polarity)	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

3. Methods

A Shimadzu LCMS-8060 triple quadrupole mass spectrometer coupled with a Shimadzu Nexera X2 UHPLC system was employed for this evaluation. A total of 10 pesticides were analyzed by atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI-LCMS). For each pesticide one to five MRM transitions were acquired. Separation was accomplished and retention times determined on column using neat standards prior to in-matrix evaluation. 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.

UHPLC conditions (Nexera system)

Column: Restek Raptor ARC-18 (100mm x 2.1mm, 2.7µm)

Mobile phase A: Water

B: Methanol

Flow rate: 0.4 mL/min

Time program: B conc. 3%(0 min) - 10%(1min) - 55%(3min) - 100%(10.5-12min)

Injection vol.: 1 uL

Column temperature: 40 C

MS conditions (LCMS-8060)

Ionization: APCI, Positive/Negative MRM mode

Table 2. MRM transitions used for APCI-LCMS

Compound Name (Polarity)	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

4. Results

4-1. Method development for Residual Pesticides

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 to determine the optimal product ions. Figures 1 through 4 demonstrate the FIA for Chlorfenapyr and Methyl Parathion. Numerous transitions were evaluated in both neat standards and matrix. The transitions used for quantitative results are shown in Table 2.

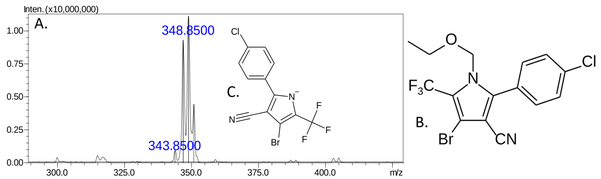

Figure 1 Chlorfenapyr A. Q3 scan, B. Structure, C. Proposed structure of Precursor *m/z* 348.8

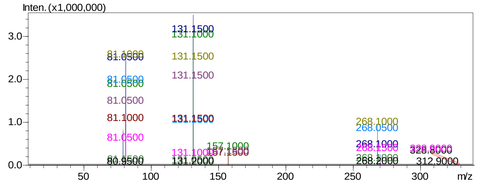

Figure 2 MSMS Spectra for Chlorfenapyr *m/z* 348.8

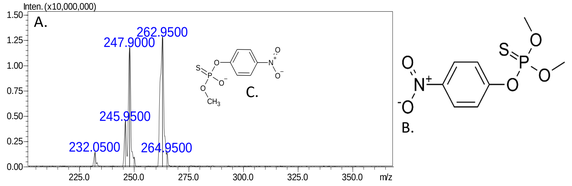

Figure 3 Methyl parathion A. Q3 scan, B. Structure, C. Proposed structure of Precursor *m/z* 247.95

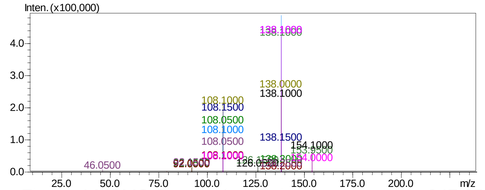

Figure 4 MSMS Spectra for Methyl Parathion *m/z* 247.95

4-2. Sample Preparation for Cannabis Matrix

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.

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 R^2=0.996 or higher for all pesticides. Representative chromatograms and calibration curves can be found in Figure 6. 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%.

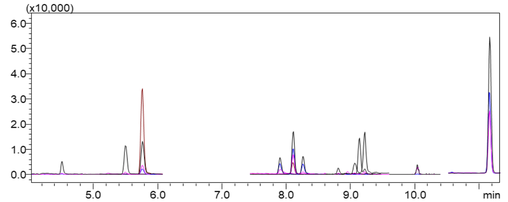

Figure 5 Representative Chromatogram for 10 pesticides using APCI-LCMS

4-3. Quantitative Analysis in Cannabis Matrix

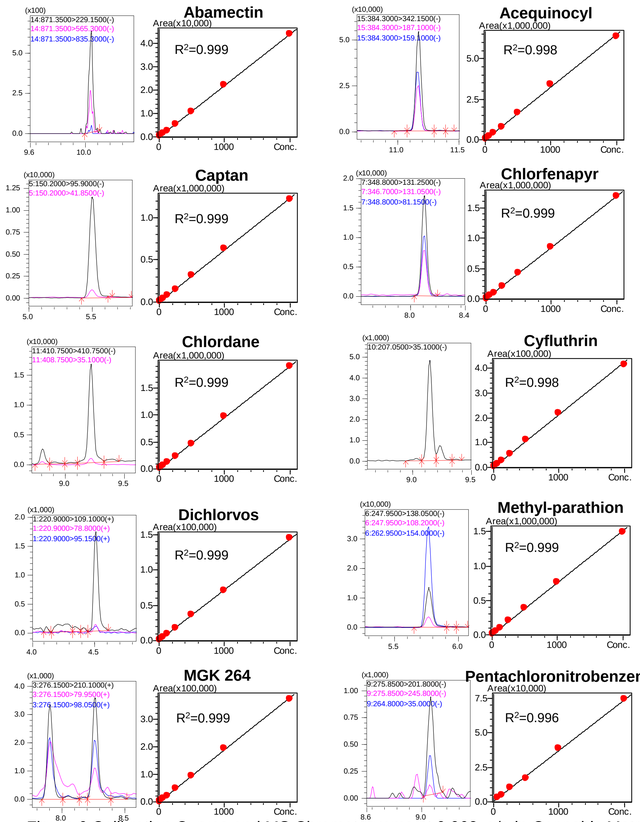

Figure 6 Calibration Curves and MS Chromatograms at 0.062ug/g in Cannabis Matrix

5. Conclusions

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. The use of the ultrafast polarity switching capability of the LCMS-8060 allowed for accurate and sensitive quantitation of all 10 pesticides.