A MULTIRESIDUE APPROACH TO PESTICIDE SCREENING IN CANNABIS USING GC-MS/MS

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INTRODUCTION

With the recent legalization of cannabis for medical and recreational use in many US states and Canada, the need for a reliable and robust method for screening pesticide residues in cannabis and cannabis products has drastically increased. In the United States there are no federal regulations in place for testing cannabis products. Therefore, each state has created its own guidelines for the safety and quality standards. The list of pesticide residues and action limits varies widely from state to state. Therefore, creating a multiresidue approach encompassing hundreds of pesticides is the most practical approach for meeting all regulations. While Gas (GC) and Liquid (LC) chromatography coupled to tandem quadrupole mass spectrometry (MS/MS) are both required for comprehensive analysis of pesticide residues, the focus of this presentation will be on a multiresidue analysis of GC amenable pesticides.

Cannabis is a very complex matrix to work with due to high levels of resins, cannabinoids, and other constituents in the plant material. Pesticides are present at concentrations in the ppb to ppm range while matrix components like THC are present at percent levels. Therefore, the analysis technique must be highly selective to be able to detect trace levels of pesticide residues. Tandem quadrupole mass spectrometry (MS/MS) provides the selectivity and sensitivity required. During the method development for this multiresidue approach, column selectivity and ionization mechanism were investigated to determine the optimal choices for each. Electron Ionization (EI) and Atmospheric Pressure Gas Chromatography (APGC) were considered as ionization options for GC-MS/MS analysis.

For information on the multiresidue analysis of LC-MS/MS pesticides in cannabis, please see Wednesday Poster 156.

METHODS

Sample Preparation

Cannabis flower was homogenized prior to extraction. 0.5 g of flower was spiked with a mix of pesticides and extracted in 5 mL of acetonitrile using a Geno Grinder. After centrifugation the extract was cleaned using dispersive solid phase extraction (dSPE) containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18, and 7.5 mg graphitized carbon black (GCB).

GC Columns Evaluated

Rxi-5MS 20m x 0.18 mm x 0.18 µm
Rxi-5MS 30m x 0.25 mm x 0.25 µm
Rtx-200 30m x 0.25 mm x 0.25 µm
Rtx-440 30m x 0.25 mm x 0.25 µm
Rxi-17SilMS 30m x 0.25 mm x 0.25 µm
Rtx-200 30m x 0.25 mm x 0.25 µm
Rtx 1301 30m x 0.25 mm x 1.0 µm *
*Maximum temperature is 320ºC - oven program adjusted accordingly

GC Conditions:

Injection Type: Pulsed Splitless (45 psi)
Inlet Temperature: 250 ºC
Flow Rate: 2.0 mL/min
Oven Program:

Rate (ºC/min)	Final Temperature (ºC)	Hold Time (min)	Total Time (min)
-	90	1	1
25	180	0	4.6
8	240	1	13.1
30	330	5	21.1

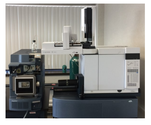
Electron Ionization (EI) Conditions

System: Xevo™ TQ-GC
Ionization Mode: EI+
Electron Energy: 70 eV
Emission: 400 µA
Source Voltage: 4.5 V
Repeller Voltage: 39 V
Extraction Voltage: 52 V
Focus 1: 13 V
Focus 2: 153 V
Transfer Line Temperature: 300 °C
Source Temperature: 250 °C
Injection Volume: 1 µl


Xevo TQ-GC

Atmospheric Pressure Gas Chromatography (APGC) Conditions

MS System: Xevo™ TQ-S micro with APGC source
Ionization Mode: APGC+
Corona Voltage: 3.0 µA
Auxillary Flow: 250 L/hr
Cone Gas Flow: 50 L/hr
Transfer Line Temperature: 300 °C
Source Temperature: 150 °C
Injection Volume: 1 µl



One vial of uncapped water in source to promote mixed mode ionization

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RESULTS AND DISCUSSION

Column Selection

Cannabis is a uniquely challenging matrix to work with when screening for pesticides present at concentrations that are orders of magnitude lower than the matrix interferences. A multiresidue sample extraction and clean up approach must be employed; these approaches are only moderately effective and can only remove some of the interfering cannabinoids. In GC-MS/MS analysis, THC and other cannabinoids are major interfering matrix compounds that have very similar properties to many GC amenable pesticides. Figure 1A demonstrates the region of the chromatogram that is impacted by cannabinoid interference. Figure 1B demonstrates an example of the peak shape distortion exhibited by bifenthrin when in matrix.

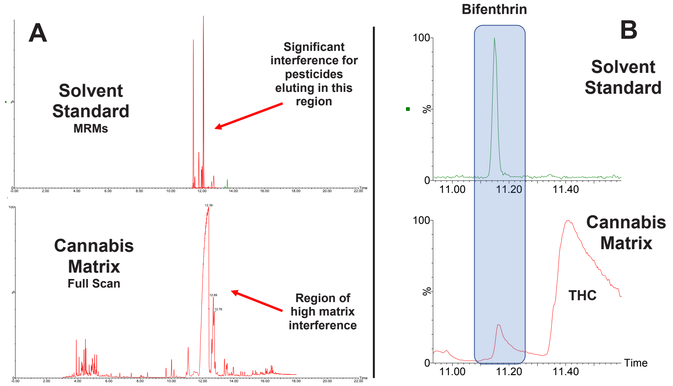

Figure 1. (A) Demonstration of region of high matrix interference and the pesticides affected. (B) Comparison of normal peak shape of bifenthrin in solvent standard and extremely distorted peak shape in cannabis matrix.

Absolute chromatographic resolution of THC from all pesticides is not possible, but chromatography can be optimized by careful column selection. Figure 2 demonstrates the resolution that can be achieved using different column selectivity, using the pesticide bifenthrin as an example. Many GC pesticide methods rely on a –5-type column which provides poor resolution between THC and bifenthrin and does a poor job at chromatographing THC. Both the 440 and 1301 phases provided more than baseline resolution between bifenthrin and THC/other matrix interferences, restoring the peak shape and more importantly allowing accurate quantitation. Additionally, THC chromatographed as a narrower, more defined peak (although still very wide) than the large smear experienced on the –5 column. Although this doesn't solve the THC interference, it makes it much more manageable. The –1301 column provided increased resolution but was not chosen as the final column as it has a maximum temperature of 320 ºC, increasing the GC run time. The 440 column provided enhanced resolution and can be used at the preferred oven temperatures.

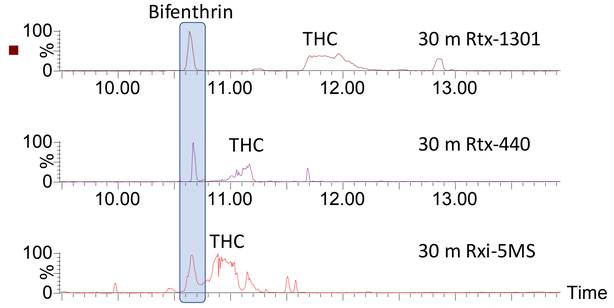

Figure 2. Comparison of three different columns evaluating the resolution between bifenthrin and THC. (top) 30 m Rtx-1301 (middle) 30 m Rtx-440 (bottom) 30 m Rxi-5MS.

Electron Ionization (EI) vs Atmospheric Pressure Gas Chromatography (APGC)

EI is widely used for GC-MS/MS analysis as it has been around for decades and many mass spectral reference libraries are built upon EI data. EI is a very hard ionization technique that causes major fragmentation of many compounds during the ionization process. APGC is a much softer ionization technique that significantly reduces fragmentation during the ionization process. The softer ionization of APGC produces higher abundances of molecular ions that can be used in higher specificity MRM transitions. Figure 3 shows a comparison of the mass spectrum of dichlorvos using both EI and APGC and also highlights the benefits of both techniques. Figure 4 demonstrates the increase in sensitivity that can be observed when using higher specificity (higher mass) transitions. In this example, the EI instrument can only detect chorfenapyr at 50 ng/ml (ppb) in matrix due to the low mass transition (59 > 31), while the detection limit for APGC would be well below the 5 ng/ml (ppb) shown due to the higher specificity MRM transition.

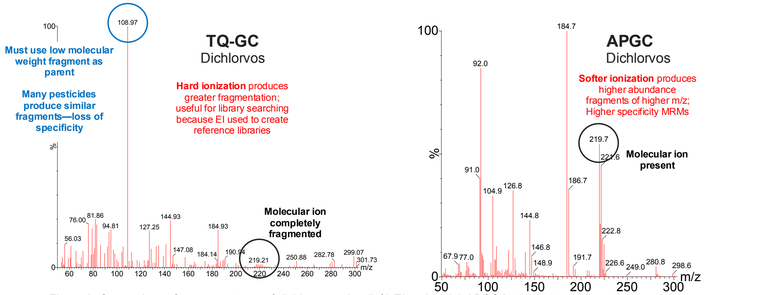

Figure 3. Comparison of mass spectrum of dichlorvos using (left) EI and (right) APGC ionization techniques.

Both EI and APGC are suitable techniques for the analysis of trace levels of pesticide residues in various matrices and produce similar quantitative results. This is demonstrated in Table 1 showing the quantitation of a selection of GC-amenable pesticides spiked into cannabis matrix.

The complexities of cannabis analysis for trace pesticide residues requires the analysis technique to overcome extreme matrix interferences. A combination of sufficient sample extraction and clean up, optimal chromatography, and sensitive and highly selective mass spectrometry is required for a successful and confident analysis method.

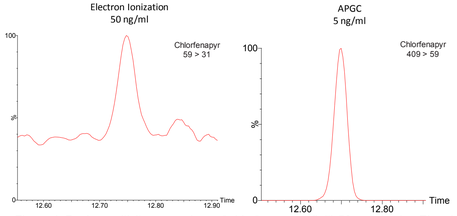

Figure 4. Peak sensitivity comparison of chlorfenapyr at (left) 50 ng/ml using EI and (right) 5 ng/ml using APGC.

Pesticide	APGC (ng/ml)	TQ-GC (ng/ml)
Bifenthrin	198	184
Chlordane	128	140
Coumaphos	159	159
Cypermethrin	155	141
Kresoxim-methyl	431	459
Pentachloronitro-benzene	338	407

Table 1. Quantitation of a selection of GC-amenable pesticides spiked into a cannabis matrix.

CONCLUSIONS

- Cannabis is an extremely difficult matrix to analyze trace residues of pesticides in considering matrix interferences are present at orders of magnitude greater concentrations.
- A multiresidue approach is being generated for GC-MS/MS analysis of pesticides in cannabis to cover all current US and Canadian regulations.
- The Rtx-440 column provides enhanced resolution of pesticides and interfering cannabinoid constituents that can not be fully removed from the extract, like THC.
- EI and APGC techniques are both suitable techniques for the analysis of pesticide residues.
- APGC provides enhanced selectivity that is extremely powerful for a matrix as complex as cannabis. This technique provides greater sensitivity in cannabis which makes it easier to reach lower residue limits.