Comprehensive Untargeted Screening & Quantitation of Pesticides in Cannabis Using GCxGC and High Performance Time-of-Flight Mass Spectrometry

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Introduction

As states continue to institute laws for the medical and recreational use of cannabis, ensuring available products are safe for public consumption has become a primary focus of various state agencies. While potency testing garners the most public attention, pesticide quantitation remains one of the most difficult hurdles for laboratories to overcome. The complexity of varietal and product matrices are the greatest obstacle to efficient, accurate detection and quantitation of pesticides in cannabis. Additionally, regulated pesticide lists are short, creating an incentive for less scrupulous growers to switch to compounds outside the regulatory scope. Untargeted screening approaches could be used to expand current pesticide lists in a meaningful, data-directed way, or in the case of a health issue, to identify possible contaminants. This presentation describes the quantitation and untargeted peak detection methodologies.

LECO Pegasus® BT 4D

- Thermally modulated GCxGC dramatically improves chromatographic resolution and peak detection.
- StayClean® Ion Source radically reduces system maintenance and downtime.
- ChromaTOF® brand software – A single software platform for hardware control and data processing with industry leading deconvolution and non-target detection.

Cannabis Legal Status by State

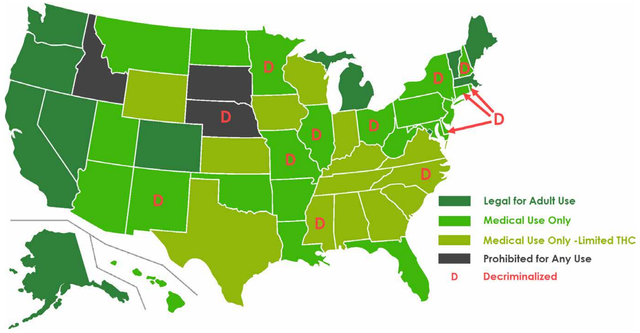


Figure 1. Ironically, as more states move towards legal status, the regulatory map becomes more fractured as each state implements its own set of regulations. Adding to the confusion, neighboring states may have radically different policies and enforcement within a state is not always consistent. Note: In some states the status depicted has not yet gone into effect.

Sample Extraction

Previous work preformed by other groups has shown that a simple solvent extraction and solid phase extraction (SPE) cleanup is an effective method for dealing with dried cannabis and hemp. This approach obviates some of the problematic steps in typical QuEChERS extraction (e.g. hydration, heating of the extract when the salts are added). Additionally the SPE cartridge has higher capacity for removing unwanted matrix constituents.

- 1.0 g of ground sample was combined with 15 mL ACN and shaken for 5 minutes.
- Rinsed a 6 mL SPE cartridge (Agilent SampliQ™ C18 endcapped) with 5 mL ACN, twice.
- Decanted the 15 mL of ACN into the SPE cartridge, collected all effluent under very low vacuum ~1 drop/s.
- Rinsed the sample twice with 5 mL ACN, decanted into SPE, and collected effluent.
- Brought the final volume to 25 mL; final dilution = 25:1.

Data Collection Conditions

Open sales of cannabis in Michigan will not become legal until late 2019 so CBD hemp, purchased at a local retailer, was used as a stand-in matrix. Separate extractions were pooled and spiked with commercially available standard mixes. The 8 standard levels were between 2 and 60 ppb. Accounting for the 25x sample dilution they are the equivalent to sample concentrations of 50 to 1500 ppb. A matrix blank and the standards were injected into the system using the parameters listed in Table 1.

Table 1. Pegasus BT 4D Instrument Conditions

Mass Spectrometer	**LECO Pegasus BT 4D**
Ion Source Temperature	250 °C
Mass Range (m/z)	m/z 45-520
Acquisition Rate	250 spectra/s
Gas Chromatograph	**LECO GCxGC Thermal Modulator and 2nd Oven in 7890 GC**
Injection Volume	1uL MMI (170-280 °C @ 400 °C/min) splitless
Carrier Gas	He, 1.4 mL/min
Guard Column	1 m 0.25 mm Phenomenex Zebron HT (Torrance CA, USA)
Column 1	30 m 0.25 mm x 0.25 µm Rxi-5MS (Restek, Bellefonte, PA, USA)
Column 2	0.7 m 0.25 mm x 0.25 µm Rtx-200 (Restek, Bellefonte, PA, USA)
Temperature Program	70 °C hold 1.5 min, 20 °C/m to 200 °C, 10 °C/min to 300 hold 10 min; Secondary Oven held +5 °C relative to primary oven
Thermal Modulator	10 °C relative to secondary oven
Modulation Period	3 s

Example Quantitation Curves

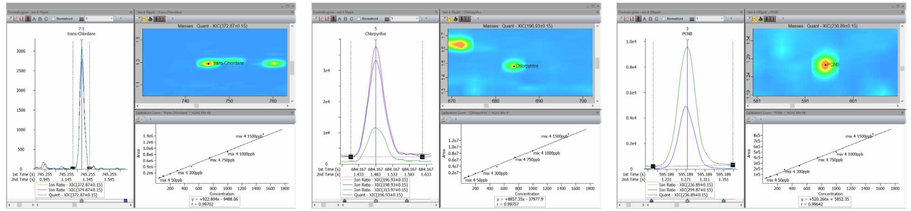


Figure 2. Example quantitation curves, chromatograms, and contour plots for selected analytes. GCxGC allowed for increased separation of the target compound from the ubiquitous matrix interferences. Chlorpyrifos and trans-Chlordane are shown at the lowest concentration, 2 ppb on column. Pentachloronitrobenzene (PCNB) is an example of a compound particularly difficult to detect with electrospray ionization.

Matrix with Spiked Pesticides

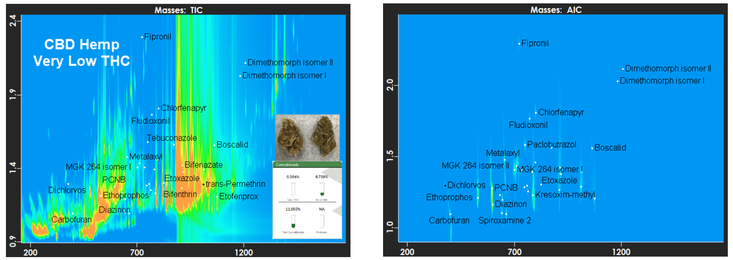


Figure 3. Comparisons of both CBD hemp Total Ion (TIC) and Analytical Ion (AIC) chromatograms. Even with the heavy matrix, a large percentage of the analytes were separated thanks to the chromatic resolution available with GCxGC. The large saturation around 850 to 1100 seconds are primarily CBD and THC related compounds. The AIC displays the analyte-related signals in the target areas.

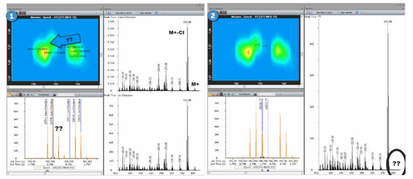
Example LOQs in CBD Hemp

Table 2. Limit of Quantitation for selected analytes compared to the California and Michigan reporting limits. LOQ was determined as the lowest standard within 20% of expected concentration, ion ratio(s) within 30% of the standard average and $R^2 \geq 0.99$. "^" indicates that the spectrum is basically a single ion and an ion ratio was not calculated.

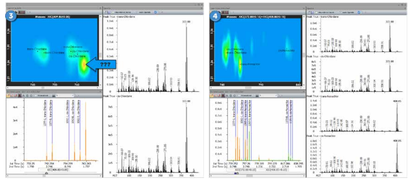
Analyte	BT LOQ (ppb)	CA Action Level (ppb)	MI Action Level
Ancymidol	200	N/A	N/A
Azoxystrobin	100	100	200
Basaloid	50	100	400
Carbaryl	50	500	200
Carbofuran	50	<LOD	200
Chlordane-cis	50	<LOD	N/A
Chlordane-trans	50	<LOD	N/A
Chlorfenapyr ^	50	<LOD	1000
Chlorpyrifos	50	<LOD	200
Coumaphos	50	<LOD	N/A
Dichlorvos	50	<LOD	1000
Dimethomorph I	100	2000	N/A
Dimethomorph II	100	2000	N/A
Etofenprox	200	<LOD	400
Fenhexamide	50	100	N/A
Fipronil	50	<LOD	400
Fludioxonil	50	100	400
Imazalil	50	<LOD	200
Kresoxim-methyl	100	100	400

Analyte	BT LOQ (ppb)	CA Action Level (ppb)	MI Action Level (ppb)
Malathion	200	500	200
Metalaxyl	50	100	200
Mevinphos	100	<LOD	N/A
MGK 264 isomer I	50	N/A	200
MGK 264 isomer II	200	N/A	200
Myclobutanil	50	100	200
Nonachlor-cis	*50***	*N/A*	*N/A*
Nonachlor-trans	*50***	*N/A*	*N/A*
Paclobutrazol	50	<LOD	400
Parathion-methyl	50	<LOD	200
Permethrins	100	500	200
PCNB	50	100	N/A
Prophos	50	N/A	N/A
Propiconazole	50	100	400
Propoxur	100	<LOD	200
Spiroxamine I ^	50	<LOD	400
Spiroxamine II ^	50	<LOD	400
Trifloxystrobin	100	100	200

Non-Target Peak Detection – Issues with Standards You Don't Expect



The images above show a strange set of potential interferences from the standard mix. In these examples, a standard dilution was made in ACN to ensure matrix incurred signal is not an issue. 1) While reviewing the expected cis/trans chlordane ($C_{10}H_6Cl_8$) peaks, an unexpected hotspot was noticed on the contour plot. For both chlordanes the typical down-and-to-the-right slice pattern was observed, so while this hotspot appears to be part of the chlordane peak, its 2nd dimension elution time makes this impossible. 2) Evaluation of the deconvoluted Peak True spectrum of the mystery peak showed no sign of the typical chlordane M+ ion cluster. Based on this spectrum, it appears that the mystery peak is either an in situ degradation (M-Cl) of the chlordane analyte, or possibly an impurity in the original compound used to make the standard.



3) When we plot the one ions from the chlordane M+ cluster (409.85) the mystery peak vanishes, but a new, stronger hotspot appears. 4) The peak deconvolution and library matching algorithms in *ChomaTOF* identified this new peak as trans-nonachlor ($C_{10}H_5Cl_9$) and further supported by the detection and identification of the cis-nonachlor isomer. Neither nonachlor isomer is listed in the certificates of analysis for these standard mixes.

Conclusions

- Continuing evolution of state laws and testing regulations will require labs to adapt to an ever-changing landscape.
- GCxGC dramatically improved chromatographic peak resolution, leading to superior deconvolution and cleaner spectra for identification of non-target compounds in complex standard mixtures.
- Full scan spectral acquisition at SIM-like detection limits enables target and non-target analysis on the same analytical platform.