Developing Cannabis Chemovar Maps Using Comprehensive Two-Dimensional Gas Chromatography with High Performance Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

David E. Alonso*, Elizabeth Humston-Fulmer, Christina Kelly and Joe Binkley | LECO Corporation, St. Joseph, MI U.S.A.

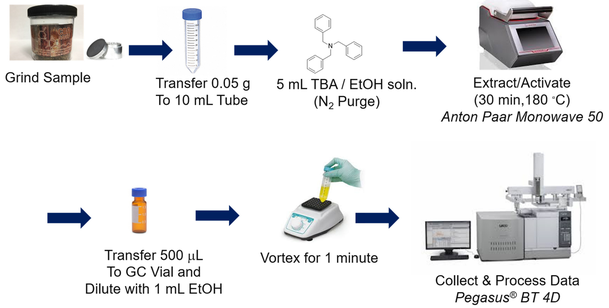
Introduction

- Cannabis is a complex mixture: Terpenes, cannabinoids, fatty acids, sterols, aldehydes, ketones, etc.
- Medicinal uses include treatment of chronic pain, multiple sclerosis, epilepsy, anxiety, and cancer
- Therapeutic benefits extend well beyond cannabinoids to include terpenes and terpenoids
- Comprehensive cannabis analysis and classification is important for determining its risks and benefits

Objectives

- Use comprehensive data for cannabis characterization and comparison
- Maximize the information obtained per analysis
- Implement GCxGC-TOFMS for potency determination and cannabis profiling
- Identify and Classify cannabis constituents with novel processing software

Sample Preparation



Acquisition Parameters

Gas Chromatograph	**LECO GCxGC Quad Jet Thermal Modulator & L-PAL 3 Autosampler**
Injection	1.0 µL (Split 100:1; 250 °C)
Carrier Gas	He @ 1.5 mL/min, Constant Flow
Columns (1st Dimension) (2nd Dimension)	Rxi-5 ms, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA) Rxi-17 sil ms 0.6 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA
Temperature Program	40 °C (1 min), ramped 10 °C/min.to 300 °C (3 min) Secondary oven maintained +5 °C relative to primary oven
Modulation	3 s with temperature maintained +15 °C relative to secondary oven
Mass Spectrometer	**LECO Pegasus BT 4D**
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-510
Acquisition Rate	200 spectra/s

GCxGC-TOFMS Advantages

Removal of Interferences, Group Clustering

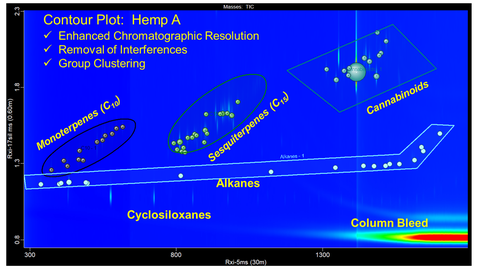


Figure 1: Contour plot demonstrating the advantages of GCxGC-TOFMS: 1) Enhanced chromatographic resolution; 2) Removal of column/septa bleed; and 3) Functional group clustering.

More Compounds Identified

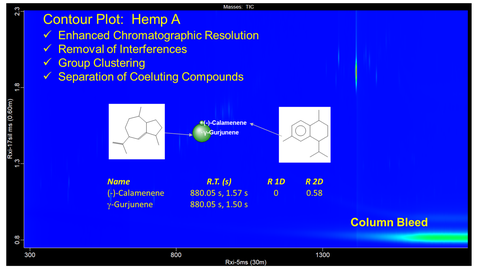


Figure 2: Contour plot displaying two sesquiterpenes that coelute in the 1st dimension (x-axis), but are partially separated in the 2nd dimension. The extra dimension of separation, together with software deconvolution produces rich data for comparisons to large, well-established databases (see below).

Mass Spectral Data

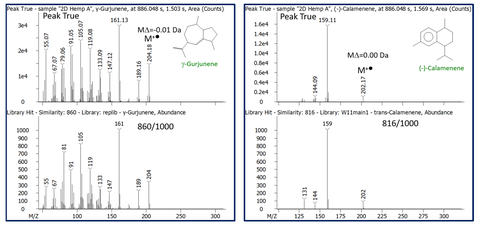


Figure 3: Peak True (deconvoluted) and library mass spectra for γ-gurjunene and (-)-calamenene. The spectral similarity values were 860 and 816 respectively. The mass Δ values were -0.01 and 0.00 Da.

Visualization and Information

Untargeted Processing, Cannabis Screening

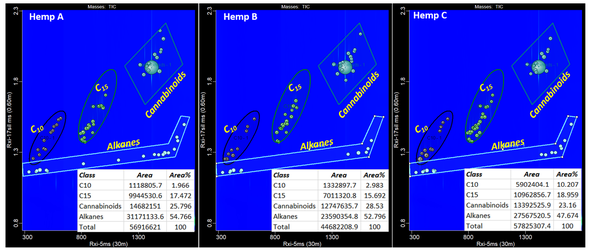


Figure 4: Untargeted processing computes the relative area and area percentages for classified groups in hemp: Monoterpenes (C10), sesquiterpenes (C15), cannabinoids, and alkanes.

Quantitation: Calibration Curves

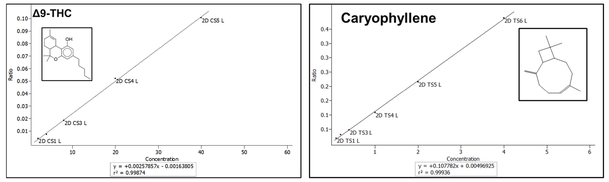


Figure 5: Calibration curves for Δ9-THC and caryophyllene with r^2 values greater than 0.998.

Targeted Processing: Cannabinoids and Terpenes

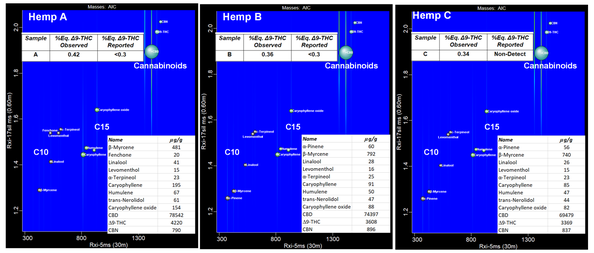


Figure 6: % Eq. Δ9-THC and concentration of select terpenes and cannabinoids in hemp samples A-C.

Cannabis Comparisons

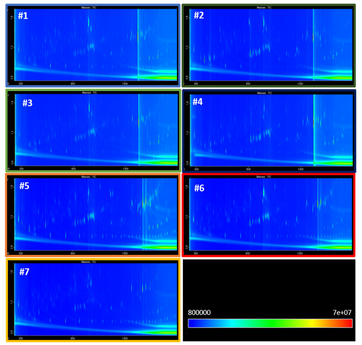


Figure 7: Representative contour plots for seven different cannabis samples of different types (log color scale). These structured plots or maps are backed by underlying high performance TOFMS data facilitating preliminary visual review and differentiation.

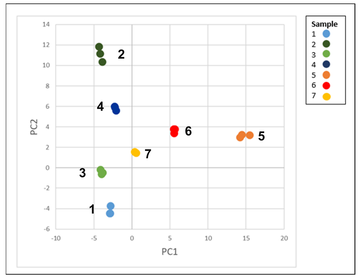


Figure 8: PCA summary of cannabis samples based on the cannabinoid collective and extended terpene content (98 compounds). This PCA is a comprehensive view with three cannabis sample groupings: 1) Fiber-type samples (1,3 and 2,4), and drug-type samples (5-7).

Summary

- GCxGC-TOFMS facilitates cannabis characterization: 1) Similarity searches, and 2) Mass Δ Calculations
- GCxGC-TOFMS produces maps for quantitative analysis and cannabis classification
- Different types of cannabis samples were characterized (e.g., fiber-type, drug-type)
- Downstream statistical processing was used for extended cannabis classification