LC-Time of Flight Mass Spectrometry for Cannabinoid Profiling and Quantitation in Hemp Oil Extracts

WWW.CWCLABS.com
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Chemical Structures of Top 10 Cannabinoids

CBC

CBD

CBG

CBDA

CBDV

CBGA

THC

CBDA

CBG

THCV

THCA

CBN

http://herb.co/2016/02/06/top-10-cannabinoids/
What is Hemp Oil?

- CBD hemp oil is a natural botanical extract of the common hemp plant.
- CBD hemp oil is derived from the seeds and stem of the *Cannabis sativa*.
- CBD hemp oil is high in CBD and very low in THC (below 0.3% delta 9 THC).
- CBD hemp oil is not psychoactive, it does not activate the CB1 receptor.
Our goal

To develop a method which allows quantitation of high levels of CBD and CBDA in the same analytical run as low levels of THC.
UV Spectra of the Chromatographic Peaks

UV spectra only show class of compounds
The 20 minute method

Tof adds specificity to the analysis
10 compound mix with ToF data on Bonus RP 2.1 x 50, 1.8u
5 minute run time
Confirmation of Compounds

We built a library
CBD at high level
THC at high level

**Graph:**
- Linear regression equation: \( y = 3.625904 \times -5.601296 \)
- R² = 0.99999542
- Type: Linear, Origin: Ignore, Weight: None

**Table: THC Results**

<table>
<thead>
<tr>
<th>Name</th>
<th>Data File</th>
<th>Type</th>
<th>Level</th>
<th>Exp. Conc.</th>
<th>RT</th>
<th>Resp.</th>
<th>Calc. Conc.</th>
<th>Final Conc.</th>
<th>Accuracy</th>
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</thead>
<tbody>
<tr>
<td>S peak mix level 1 d</td>
<td>Cal</td>
<td>1</td>
<td>1</td>
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<td>8.408</td>
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<td>2</td>
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<td>68</td>
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</tbody>
</table>

**Image:**
- THC molecule structure

**Logo:**
- CWC Labs
**THC at low level**

12.5 ppb is the limit of quantitation

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### THC Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>THC Method</th>
<th>THC Results</th>
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</thead>
<tbody>
<tr>
<td>THC 12.5 ppb</td>
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<td>THC 125 ppb</td>
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<td>THC 1000 ppb</td>
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<td>THC 500 ppb</td>
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<tr>
<td>THC 250 ppb</td>
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</table>

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12.5 ppb is the limit of quantitation
Validation of our method showing replicates

Note the 25 ppm standard was spiked in Virgin Hemp oil
Carryover with the Agilent Multisampler

1000 ppm standard
Molecular Mass

Measuring the mass-to-charge ratio of atomic and molecular ions

Time of Flight
Gives ions a pulse and measures their flight time

Quadrupole
Passes only ions with the correct mass

Tandem Mass Spectroscopy (MS-MS)
Selects a range of masses → Fragments by collision → Analyzes the fragments

IONS

QTOF

QQQ

CWC LABS
Fragment Ions with High Fragmentor Voltage

Occurs between the capillary and the skimmer

Fragmentor voltage drives “ions” into gas molecules

Higher voltage increases collision energetics producing more fragments
All Ions MS/MS - What is it?

- **Step 1: Fragmentation on TOF without precursor isolation**
  - a) First Scan: Low fragmentation energy to analyze precursors
  - b) Second Scan: High fragmentation energy to analyze fragment ions
Create a library from the standards data with spectra for the cannabinoids
All Ions MS/MS—What is it?

• Step 2: Software Extracts, Correlates, and Confirms
  a) Find by Formula uses library to extract precursors from low energy MS channel
  b) All Ions MS/MS uses library to extract fragments from high energy channel, gives coelution score

Software automatically matches precursors with fragment ions:
Coelution Plot with Coelution Score
We can add a C18 column and use the Agilent Pesticides PCDL kit to ........
PCDL subset of targeted pesticides

Based on the Oregon and Colorado pesticides list
CBD Candy Bar screen for pesticides with All Ions

Extracted with QuEChERS and cleanup with Dispersive EMR(Enhanced Matrix Removal)
We can add a C18 column and use the Agilent Forensic/Tox PCDL kit to
A standard of 96 drugs of abuse was spiked into natural hemp oil without CBD. The sample was then run on the TOF using the All Ions technique.

All of the 96 compounds had calibration curves created with correlation coefficients of 0.995 to 0.999, figure 11 is an example of a curve. The randomly spiked sample was quantitated against this calibration curve. The methodology used has the same mobile phases as the potency assay only a different column.
Any Questions?

Thank You

See our poster WP-184

Cannabinoid Profiling and Quantitation in Hemp Extracts using the Agilent 1290 Infinity II / 6230B LC/TOF system