Chemistry and Testing of Metabolite Hemp

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Specialized Metabolism

Also called:

- Natural Products
- Secondary Metabolite

Compounds which are non-essential for an organism’s growth or development but helps improves its ability to survive.
Specialized Metabolites Roles

Plants synthesize specialized metabolites to:

▪ Deter or kill herbivore pests
▪ Deter or kill pathogens
▪ Deter nearby plants
▪ Attract pollinators or seed dispersers
▪ Attract predators of herbivores
▪ Protect against environmental stresses
Cannabis is an amazing chemical biosynthetic factory, both in quality and quantity of specialized metabolites.
Defining Hemp

- Hemp is *Cannabis sativa* and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a $\Delta^9$-tetrahydrocannabinol (THC) concentration of not more than **0.3%** on a dry weight basis.

  -- Agriculture Improvement Act of 2018
Cannabinoid Production

- Cannabinoids are produced in trichomes
- Primarily on bracts of female flowers
Biosynthesis of Cannabinoids

Hexanoate

\[
\text{H}_3\text{C} - \text{CH}_{11} - \text{COOH} \quad \text{AAE1} \quad \text{H}_3\text{C} - \text{CH}_{11} - \text{COA}
\]

\[
3 \times \text{Malonyl-CoA}
\]

\[
\text{Olivetolic Acid}
\]

\[
\text{O}
\]

\[
\text{Geranyl diphosphate}
\]

\[
\text{OH}
\]

\[
\text{Geranyl diphosphate}
\]

\[
\text{Δ}^9\text{-Tetrahydrocannabinolic Acid (Δ}^9\text{-THCA)}
\]

\[
\text{Cannabidiolic Acid (CBDA)}
\]

\[
\text{CBDAS}
\]

\[
\text{Δ}^9\text{-Tetrahydrocannabinolic Acid (Δ}^9\text{-THCA)}
\]

\[
\text{Cannabigerolic Acid (CBGA)}
\]

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Cannabis Chemistry

Acidic form (produced by the plant)

Neutral form (typically very little in the plant)
Other Cannabinoids

- Cannabis produces >120 cannabinoids

Cannabichromenic Acid (CBCA)

Cannabidivarinic Acid (CBDVA)

Cannabinol (CBN)

$\Delta^8$-Tetrahydrocannabinolic Acid ($\Delta^8$-THCA)
Quantification Methods

Liquid Chromatography

Gas Chromatography

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Gas Chromatography

- Heats samples until they volatilize into a gas
- Inherently decarboxylates cannabinoids
- Only quantifies total neutral cannabinoids
LC Systems

Liquid Chromatography

- High-pressure (HPLC) and Ultrahigh-pressure (UPLC) variants
- Uses a liquid flowing through a column to separate compounds
- Does not employ heat that decarboxylates cannabinoids
- Quantifies acidic and neutral cannabinoids separately
Detectors

GC Systems
- Flame Ionization Detector (FID)
- Mass Spectrometer (MS)
- Others

HPLC Systems
- UV Detector (UV)
- Mass Spectrometer (MS)
- Others

Sophisticated physics to quantify the compound
$\Delta^9$-THC versus Total $\Delta^9$-THC

1. $\Delta^9$-THC Method = $\Delta^9$-THC
2. Total $\Delta^9$-THC = $\Delta^9$-THC + 0.877*($\Delta^9$-THCA)
Understanding Total-THC

- Some people argue THCA and THC are different molecules
  - technically true
  - closely related
- THCA easily converts to THC (the regulated intoxicant)

**Hypothetical Example**

**Hemp?**

<table>
<thead>
<tr>
<th>Initial test results:</th>
<th>New test results:</th>
<th>Hemp? NO!</th>
<th>Intoxicating? YES!</th>
</tr>
</thead>
<tbody>
<tr>
<td>THCA: 6.2%</td>
<td>THCA: 0.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THC: 0.3%</td>
<td>THC: 5.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Heat or Smoke**
Ratio of THCA to THC

Total $\Delta^9$-THC = $\Delta^9$-THC + 0.877*($\Delta^9$-THCA)

1 THCA $\rightarrow$ 1 THC + 1 CO$_2$

Molecular Weight:
THCA = 358 g/mol
THC = 314 g/mol

THC (g) = 1 g THCA \times \frac{1 \text{ mole THCA}}{358 \text{ g THCA}} \times \frac{1 \text{ mole THC}}{1 \text{ mole THCA}} \times \frac{314 \text{ g THC}}{1 \text{ mole THC}}

= 0.877 g THC
Regulatory Compliance

- USDA and Ohio both use the Total THC measurement system
- When quantifying compounds there is a level of uncertainty associated with the metabolite quantification process ("Measurement of Uncertainty", MU)
- "Acceptable THC level" means a measurement of THC where the MU spans the 0.3% level or less

Provides Flexibility for Growers
“Acceptable THC” Examples

Example 1. Your crop comes back testing 0.25% THC

Example 2. Your crop comes back testing 0.33% THC

0.33% - 0.04% = 0.29%
(Spans 0.3% value)

Example 3. Your crop comes back testing 0.36% THC

0.36% - 0.04% = 0.32%
(Does NOT spans 0.3% value)

Margin of error for the testing process is 0.04%
Additional Testing

Only THC testing is mandatory for hemp

Optional tests include:

- Other cannabinoids
- Terpenes
- Moisture Content
- Foreign Matter
- Microbial Contaminants
- Mycotoxins
- Pesticide Residues
- Fertilizer Residue
- Heavy Metals
- Residual Solvents
Terpenes

- Primary flavoring/fragrance compounds of Cannabis
- Commonly analyzed in reports
Certificate of Analysis

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOQ (mg/g)</th>
<th>Mass (%)</th>
<th>Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THCa</td>
<td>0.51</td>
<td>5.1</td>
<td>0.31</td>
</tr>
<tr>
<td>Δ9-THC</td>
<td>0.19</td>
<td>1.9</td>
<td>0.37</td>
</tr>
<tr>
<td>THCV</td>
<td>0.02</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>CBDа</td>
<td>16.94</td>
<td>169.4</td>
<td>115.6</td>
</tr>
<tr>
<td>CBD</td>
<td>2.03</td>
<td>20.3</td>
<td>14.2</td>
</tr>
<tr>
<td>CBDV</td>
<td>0.05</td>
<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
<td>CBN</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
<td>0.00</td>
</tr>
<tr>
<td>CBGa</td>
<td>1.06</td>
<td>10.6</td>
<td>7.0</td>
</tr>
<tr>
<td>CBG</td>
<td>0.23</td>
<td>2.3</td>
<td>1.6</td>
</tr>
<tr>
<td>CBC</td>
<td>0.20</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>21.24</td>
<td>212.4</td>
<td>148.6</td>
</tr>
</tbody>
</table>

Total Δ⁹-THC = 0.19 + 0.877*(0.51) = 0.63
The Ohio Department of Agriculture (ODA) is the only official lab for testing THC for crop compliance.

ODA may contract with other labs for testing on their behalf if necessary.

Compliance testing only covers Total THC.

Growers should utilize private labs to monitor THC levels in their crops, especially important for metabolite crops.
Growing Locations

- Growing location is a contiguous area (not broken by fences, waterways, tree lines, building walls, etc.)
- Each variety/strain at growing location requires separate testing
Defining a Sample

- Fees cover one test sample per growing location
- Additional tests are $150 for preharvest samples

1 + $150
2 + $150

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## Testing Process

If crop is not harvested within 15 days of sampling another test for THC is required.
Questions