Development of Medicinal Marijuana Testing in New Jersey

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NJ Medicinal Marijuana Program (MMP)

- The New Jersey Compassionate Use Medical Marijuana Act (Senate Bill, No. 119, the Act, N.J.S.A. 26:6I-1 et seq) was signed into law on January 18, 2010.
- The NJ Department of Health (NJDOH) Medicinal Marijuana Program was established to implement the law, develop and enforce the regulations.
- NJ PHEL-ECLS started the Marijuana Testing Project in June 2012.
Project Goals

To comply with the Law
• Potency compliance of < 10 % Delta-9-Tetrahydrocannabinol (Δ-9-THC)
  • Free of pesticides, toxic heavy metals, and mycotoxins

To establish reliable test methods
• Establish an accurate, sensitive, cost effective, rugged, defensible test method for medicinal marijuana with a shortest specimen turn around time.
Implementation Protocols for a Test Method

- Select target analytes
- Select analytical instrument
  - HPLC-DAD, ICPMS, and HPLC-Fluorescence Detection
- Provide personnel training
- Develop the test method
- Validate the test method
  - Selectivity, Precision Accuracy, Representation (sample size), Completeness, Comparability, Sensitivity
- Establish SOPs
- Establish reporting system
- Implement the test method

State of New Jersey
Department of Agriculture

NJ Health
New Jersey Department of Health
Analysis of Marijuana for Cannabinoids Using HPLC-DAD* Method

Target Analytes

- Cannabidiol (CBD)
- Cannabidiolic acid (CBDA)
- Cannabigerollic acid (CBGA)
- Cannabigerol (CBG)
- Cannabinol (CBN)
- Delta-8-terahydrocannabinol (Δ-8-THC)
- Delta-9-Tetrahydrocannabinol (Δ-9-THC)
- Tetrahydocannabinolic acid (THCA)

HEAT

Note: THCA -----> Δ-9-THC

*High Pressure Liquid Chromatography – Diode Array Detector
Sample Processing and Analysis Method for Cannabinoids

0.2 gram MM Ground individual or a composite cultivar

Extraction and centrifugation

Filtration and preparation of appropriate dilutions

Evaporation, reconstitution, addition of internal standard, mix and inject

Analysis by High Pressure Liquid Chromatography – Diode Array Detector

Note: % of batch weight per cultivar: 1-5%; ~2.5 g/sample
Summary of Method Evaluation Results for Cannabinoids Analysis

- **Calibration range**: 0.25 – 50 µg/mL (v) (0.005 – 1% by sample wt)
- **Coefficient of Determination (R²)**: ≥ 0.995
- **Method Precision (%RSD)**: 5-12%
- **Accuracy (%Recovery for % Δ-9-THC)**: 105 ± 12.5% (spiked Medical Marijuana samples)
  
  Note: Allowable RSDs on sample duplicates: <30%
  
  Allowable Matrix Spikes Recoveries: 70% - 130%

- **Detection Limit**: 0.25 µg/mL (v) (50 µg/g by wt)
# Elemental Analysis on Medicinal Marijuana

## Target Analytes

<table>
<thead>
<tr>
<th>Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
</tr>
<tr>
<td>Cadmium</td>
</tr>
<tr>
<td>Chromium</td>
</tr>
<tr>
<td>Iron</td>
</tr>
<tr>
<td>Lead</td>
</tr>
<tr>
<td>Manganese</td>
</tr>
<tr>
<td>Mercury</td>
</tr>
<tr>
<td>Nickel</td>
</tr>
<tr>
<td>Selenium</td>
</tr>
<tr>
<td>Zinc</td>
</tr>
</tbody>
</table>
Sample Processing and Analysis of Metals in MM Using ICP-MS

- 0.1 gram MM Cultivar Composite
- Addition of Nitric Acid and Hydrogen Peroxide
- Hot Block Digestion
- Decant or Filter Digestate Supernatant
- Analysis by ICP-MS in Standard Mode

QA/QC
- Internal Standards
- Laboratory Reagent Blank
- Calibration Verification at 3 levels
- Sample Duplicate
- Matrix Spike/Matrix Spike Duplicate
Summary of Elemental Method Evaluations

• **Method Precision (%RSD)**:
  - 1.2% - 7.2% (< ±30%)

• **Accuracy (%Recovery on Matrix Spikes)**:
  - 85% - 114% (< ±30%)

• **Linearity**
  - Concentration Range: 0.2 - 5 mg/g (± 25%)

• **Detection Limits**: 0.2 – 5.0 mg/g
## Analysis of Pesticides in MM by GC-MS

<table>
<thead>
<tr>
<th>Chemical Class</th>
<th>Example Pesticides</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates</td>
<td>Diazinon,</td>
<td>Insecticide</td>
</tr>
<tr>
<td></td>
<td>Phosmet</td>
<td>Insecticide</td>
</tr>
<tr>
<td></td>
<td>Dichlorovos</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>Resmethrin</td>
<td>Insecticide, Repellent</td>
</tr>
<tr>
<td></td>
<td>Pyrethrin</td>
<td>Insecticide, Repellent</td>
</tr>
<tr>
<td></td>
<td>Allethrin</td>
<td>Insecticide, Repellent</td>
</tr>
<tr>
<td>Carbamates</td>
<td>Terbutarcb</td>
<td>Insecticide</td>
</tr>
<tr>
<td></td>
<td>Fenunucarb</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Avermectins</td>
<td>Abamectin (Avid )</td>
<td>Insecticide, Anthelmintic</td>
</tr>
<tr>
<td>Thiabendazoles</td>
<td>Thiabendazole</td>
<td>Parasiticide</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Atrazine</td>
<td>Herbicide</td>
</tr>
<tr>
<td></td>
<td>Bifenazate</td>
<td>Acaricide</td>
</tr>
<tr>
<td></td>
<td>Bromocil</td>
<td>Herbicide</td>
</tr>
<tr>
<td></td>
<td>Chloropropham</td>
<td>Growth Regulator</td>
</tr>
<tr>
<td></td>
<td>Fenarimol</td>
<td>Fungicide</td>
</tr>
<tr>
<td></td>
<td>Pyrimethanil</td>
<td>Fungicide</td>
</tr>
<tr>
<td></td>
<td>Metribuzin</td>
<td>Fungicide</td>
</tr>
</tbody>
</table>

*Note: A total of 83 commonly used pesticides were tested but currently 56 are incorporated into method.*
Sample Processing and Analysis of Pesticides in MM Using GC-MS

0.5 gram MM Cultivar Composite

QuEChERS

Extract and Centrifuge

Extract Cleanup

Dilution and addition of Internal Standard

Inject, PTV-GC-MS (SCAN) Analysis

QA/QC Parameters

• Precision
• Accuracy
• Sensitivity

State of New Jersey
Department of Agriculture

New Jersey Department of Health
Summary of Method Evaluation Results for Pesticides*

- **Method Precision (%RSD):** < 25%
- **Accuracy (%Recovery):** 80-115%
- **Linearity (Concentration Range 50-2000 ppb):**
  Coefficient of Determination (R²) > 0.995
- **Method Detection Limit:** 2.5 µg/g

*Number of Pesticides (n = 56)
Mycotoxin Analysis in MM by HPLC-Fluorescence Detection

- NJDA cooperates with NJ Department of Health to perform Mycotoxins analyses for medical marijuana plant materials
- The tests are aflatoxins (B1, B2, G1, and G2) and Ochratoxin A
- High Performance Liquid Chromatograph with fluorescence detection
Mycotoxins Determination Method for Medical Marijuana

- Weigh 0.25 g
- Add extraction solution
- Vortex for 3 minutes
- Centrifuge for 10 minutes
- Transfer supernant into sample reservoir
- Drive out at the end
- Vortex the elution
- Diluted with MQ H₂O
- Vortex the diluted elution
- Inject on HPLC - fluorescence detection for analysis
Summary of Method Evaluation
Results for Mycotoxin

- Coefficient of Determination \((R^2) \geq 0.995\)
- %Recovery (spiked on matrix): 82-95%
- Precision (RSD %) \(\leq 5\%\)
- Detection Limit: 0.143 – 0.357 ng/g
Summary Results of Cannabinoids Analysis (%wt) (October 2012 – April 2015)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>%Detected</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBD</td>
<td>283</td>
<td>ND</td>
<td>ND</td>
<td>2.130</td>
<td>27.9</td>
<td>0.012</td>
</tr>
<tr>
<td>CBDA</td>
<td>283</td>
<td>0.025</td>
<td>ND</td>
<td>17.59</td>
<td>79.5</td>
<td>0.012</td>
</tr>
<tr>
<td>CBG</td>
<td>283</td>
<td>0.050</td>
<td>ND</td>
<td>0.303</td>
<td>77.7</td>
<td>0.012</td>
</tr>
<tr>
<td>CBGA</td>
<td>283</td>
<td>0.299</td>
<td>ND</td>
<td>1.856</td>
<td>98.2</td>
<td>0.012</td>
</tr>
<tr>
<td>CBN</td>
<td>283</td>
<td>ND</td>
<td>ND</td>
<td>0.125</td>
<td>24.4</td>
<td>0.012</td>
</tr>
<tr>
<td>Δ-8-THC</td>
<td>283</td>
<td>ND</td>
<td>ND</td>
<td>0.282</td>
<td>12.7</td>
<td>0.012</td>
</tr>
<tr>
<td>Δ-9-THC</td>
<td>283</td>
<td>0.446</td>
<td>ND</td>
<td>3.789</td>
<td>99.6</td>
<td>0.012</td>
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<tr>
<td>THCA</td>
<td>283</td>
<td>14.7</td>
<td>0.396</td>
<td>34.2</td>
<td>100</td>
<td>0.012</td>
</tr>
</tbody>
</table>
## Summary Results of Metals Analysis (µg/g)  
(October 2012 – April 2015)

<table>
<thead>
<tr>
<th>Analytes</th>
<th>N</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>%Detected</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>49</td>
<td>ND</td>
<td>ND</td>
<td>1.00</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cadmium</td>
<td>49</td>
<td>ND</td>
<td>ND</td>
<td>0.89</td>
<td>26.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Chromium</td>
<td>49</td>
<td>2.0</td>
<td>ND</td>
<td>2.1</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>Iron</td>
<td>49</td>
<td>211</td>
<td>94.9</td>
<td>493</td>
<td>100</td>
<td>5.0</td>
</tr>
<tr>
<td>Lead</td>
<td>49</td>
<td>0.10</td>
<td>ND</td>
<td>0.29</td>
<td>14.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Manganese</td>
<td>49</td>
<td>228</td>
<td>70.3</td>
<td>443</td>
<td>100</td>
<td>0.2</td>
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<tr>
<td>Mercury</td>
<td>49</td>
<td>ND</td>
<td>ND</td>
<td>0.43</td>
<td>6.1</td>
<td>0.5</td>
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<tr>
<td>Nickel</td>
<td>49</td>
<td>ND</td>
<td>ND</td>
<td>1.3</td>
<td>26.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Selenium</td>
<td>49</td>
<td>ND</td>
<td>ND</td>
<td>2.0</td>
<td>10.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Zinc</td>
<td>49</td>
<td>113</td>
<td>52.6</td>
<td>239</td>
<td>100</td>
<td>5.0</td>
</tr>
</tbody>
</table>
### Summary Results of Mycotoxins Analysis (ng/g) (October 2012 – April 2015)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>% Detected</th>
<th>DL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>47</td>
<td>ND</td>
<td>ND</td>
<td>2.3</td>
<td>2</td>
<td>2.0</td>
</tr>
<tr>
<td>Ochratoxin</td>
<td>47</td>
<td>ND</td>
<td>ND</td>
<td>0.21</td>
<td>2</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Major Challenges

Administrative and Regulatory Perspective

• Staff
• Funding
• Time line
• Supply - DEA license, standards availability, sample collection, submission and receipt procedures
• No official reference values for pesticides, heavy metals or mycotoxins acceptance levels for the MM products in US.
Technical Perspective

- Complex marijuana matrix
  - Interference in pesticide and metal analyses
  - Contamination of the analytical system
- Limited testing materials
- No SRM or PT samples
- Lack of partnership on MM analysis
- Difficulty in comparison among the results obtained from different analytical methods
Future Plan

• Development of a test method for pesticides analysis using Mass Quadrupole Time of Flight (TOF) Liquid Chromatography Mass Spectrometry (Q-TOF LC/MS).
  - increase sensitivity; increase capacity & capability
• Develop a test method for the measurement of microbial contaminants in MM.
• Develop a test method for MM edible forms.
• Establishment of national standardized testing procedures for the analysis of MM.
• Establishment of national acceptable limits for target analytes
• Transition from a routine testing laboratory to a regulatory laboratory
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