

California and Cannabis



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Agenda

- Introduction to CA Cannabis Law, State Licensing Authorities and CDPH role and responsibilities
- Introduction to Cannabis Testing Section (CTS)
- Cannabis testing developments in response to CA regulations
- Overview of microbiologic testing plan in CTS, method validation data
- Overview of chemical testing plan in CTS, method validation data
- ISO 17025 Accreditation



Progression of CA Cannabis Law

1996

- Proposition 215, Compassionate Use Act

2003

- Senate Bill 420, Medical Marijuana Program (MM ID Card)

2015

- Medical Cannabis Regulation & Safety Act (MCRSA)

2016

- Adult Use of Marijuana Act (Prop. 64)

2017

- Medicinal and Adult-Use Cannabis Regulation and Safety Act (MAUCRSA) - SB 94 and AB 133



State Licensing Authorities



CA Department of
Food & Agriculture

*CalCannabis
Cultivation Licensing*

Cultivators
Track-and-Trace



CA Department of
Public Health

*Manufactured
Cannabis Safety
Branch (MCSB)*

Manufacturers



Bureau of
Cannabis Control
(Bureau)

Retailers
Distributors
Testing Labs
Microbusinesses



CDPH – Role and Responsibilities

Role: Protect public health by promoting product and workplace safety

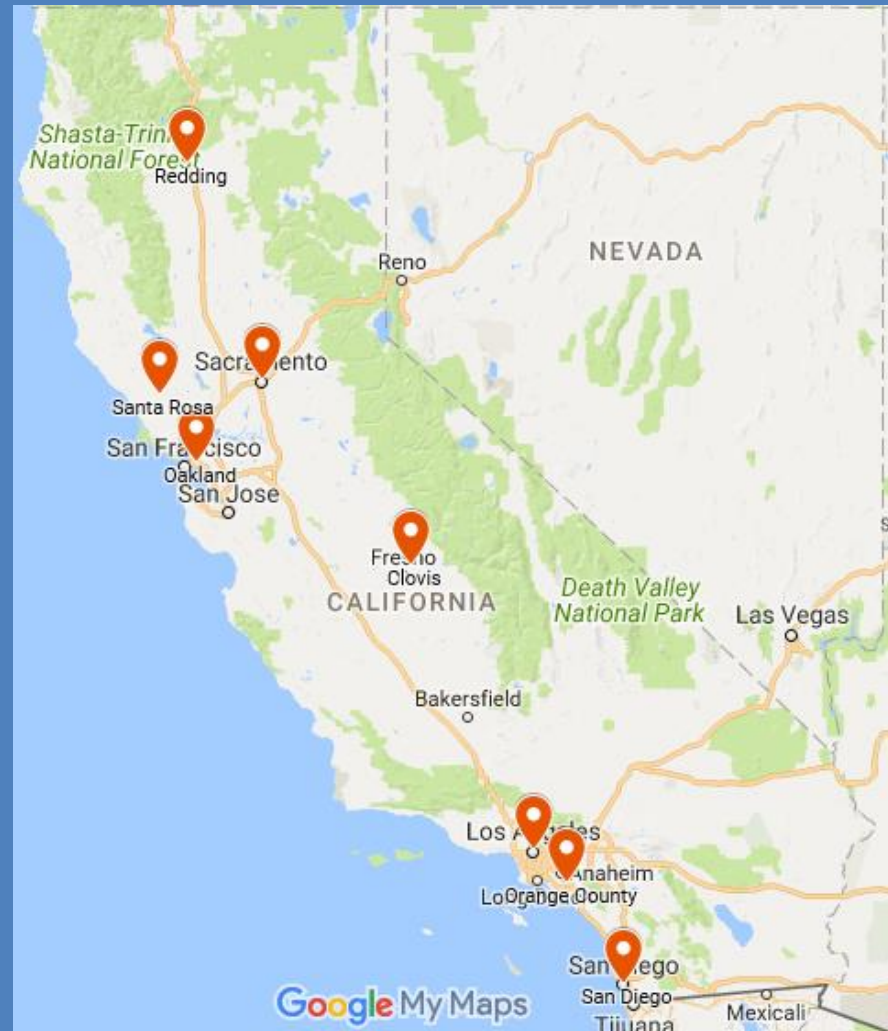
Manufactured Cannabis Safety Branch

- Regulations
- Licensing
- Compliance



CDPH - Cannabis Regulations Evolution

- Starting in September of 2016, pre-regulatory workshops were held throughout CA (Redding, Sacramento, Santa Rosa, Oakland, Fresno, Los Angeles, Orange County, San Diego) to gain feedback on regulatory concepts, and to help inform public, industry and local communities on the regulations.
- Advisory Committee formed to advise licensing authorities (CDPH, BCC, CDFA) on the development of regulations that help protect public health and safety while reducing the illegal market for cannabis.



Cannabis Testing Section (CTS)

- Established in May of 2016, part of Food and Drug Laboratory Branch
- Staff were hired to assist the Bureau of Medical Cannabis Regulation (DCA) in development of regulations for licensing of 3rd party testing laboratories, and to test medical cannabis safety through microbiological and chemical analyses
- CTS's main function is to support CDPH's Manufactured Cannabis Safety Branch (MCSB). This includes analytical support of routine inspections, investigations and response to public health outbreaks.
- Purchasing equipment and supplies, training staff, developing and validating methods, planning for ISO 17025 accreditation
- Still in process of remodeling laboratory space ~2,000 sq ft
- Equipment (Chemistry, Filth and Microbiological testing) purchased and installed.
- Training – ongoing, Method Development in progress, Method Validations for critical methods scheduled to be completed by July 2018



§ 5714. Required Testing

CALIFORNIA CODE OF REGULATIONS TITLE 16 DIVISION 42. BUREAU OF CANNABIS CONTROL



- **Cannabinoids** - Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), Cannabidiol (CBD), Cannabidiolic Acid (CBDA), Cannabigerol (CBG), Cannabinol (CBN).
- Foreign material
- Heavy metals
- Microbial impurities
- Mycotoxins
- Moisture content and water activity
- Residual pesticides
- Residual solvents and processing chemicals
- Terpenes
- Homogeneity





BUREAU OF CANNABIS CONTROL

CALIFORNIA

ALL CANNABIS HARVESTED ON OR AFTER 1/1/2018 AND ALL CANNABIS PRODUCTS MANUFACTURED ON OR AFTER 1/1/2018, SHALL BE TESTED ACCORDING TO TITLE 16 OF THE CALIFORNIA CODE OF REGULATIONS, SECTION 5715, AND THE REGULATIONS THAT FOLLOW.

| PHASE-IN OF REQUIRED LABORATORY TESTING | INHALABLE CANNABIS | INHALABLE CANNABIS PRODUCTS | OTHER CANNABIS & CANNABIS PRODUCTS |
|--|--------------------|-----------------------------|------------------------------------|
| JANUARY 1, 2018 | | | |
| Cannabinoids Testing | ✓ | ✓ | ✓ |
| Moisture Content Testing | ✓ | | |
| Category II Residual Solvents and Processing Chemicals Testing | | ✓ | ✓ |
| Category I Residual Pesticides Testing | ✓ | ✓ | ✓ |
| Microbial Impurities Testing (A. fumigatus, A. flavus, A. niger, A. terreus) | ✓ | ✓ | |
| Microbial Impurities Testing (Escherichia coli and Salmonella spp.) | ✓ | ✓ | ✓ |
| Homogeneity Testing of Edible Cannabis Products | | | ✓ |
| JULY 1, 2018 | | | |
| Category I Residual Solvents and Processing Chemicals Testing | | ✓ | ✓ |
| Category II Residual Pesticides Testing | ✓ | ✓ | ✓ |
| Foreign Material Testing | ✓ | ✓ | ✓ |
| DECEMBER 31, 2018 | | | |
| Terpenoids Testing | ✓ | ✓ | ✓ |
| Mycotoxins Testing | ✓ | ✓ | ✓ |
| Heavy Metals Testing | ✓ | ✓ | ✓ |
| Water Activity Testing of Solid or Semi-Solid Edibles | ✓ | | ✓ |



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For the latest updates, follow the Bureau on social media



Current Microbiology Work

- Completed validations:
 - Detection of *Salmonella* in environmental swabs using a real-time PCR and culture based method (modified FDA BAM method)
 - Detection of *Salmonella* in chocolate using a real-time PCR and culture based method (modified FDA BAM method)
 - Detection of Shiga-toxin producing *E.coli* (STEC) in environmental swabs using a real-time PCR and culture based method (modified FDA BAM method).
- Developing methods or planned validations:
 - Detecting of *Salmonella* in plant material, various edibles, oils, etc.
 - Detecting Shiga-toxin producing *E. coli* (STEC) in plant material, various edibles, etc.
 - Detecting *Aspergillus niger*, *fumigatus*, *flavus*, and *terreus* in inhalable cannabis and cannabis products by real-time PCR
 - Bacterial and fungal strain identification by MALDI-TOF
 - Developing fungal culturing and detection methods by microscopy



Current Microbiology Work

- Methods Training/Evaluation:
 - Modified FDA BAM methods for detecting *Salmonella* and Shiga-toxin producing *E. coli* (STEC)
 - MALDI-TOF method for bacterial and fungal cell preparation for sample identification analysis
 - qPCR based kits for detection of pathogenic *Aspergillus* from cannabis and cannabis containing products
- Equipment training:
 - Real time qPCR instruments
 - MALDI-TOF
 - Commercial platforms for pathogen detection and confirmation
- Purchasing of equipment, supplies, reagents/kits in anticipation of meeting the sample workload requirements
- Hiring new staff
- ISO 17025 training and implementation



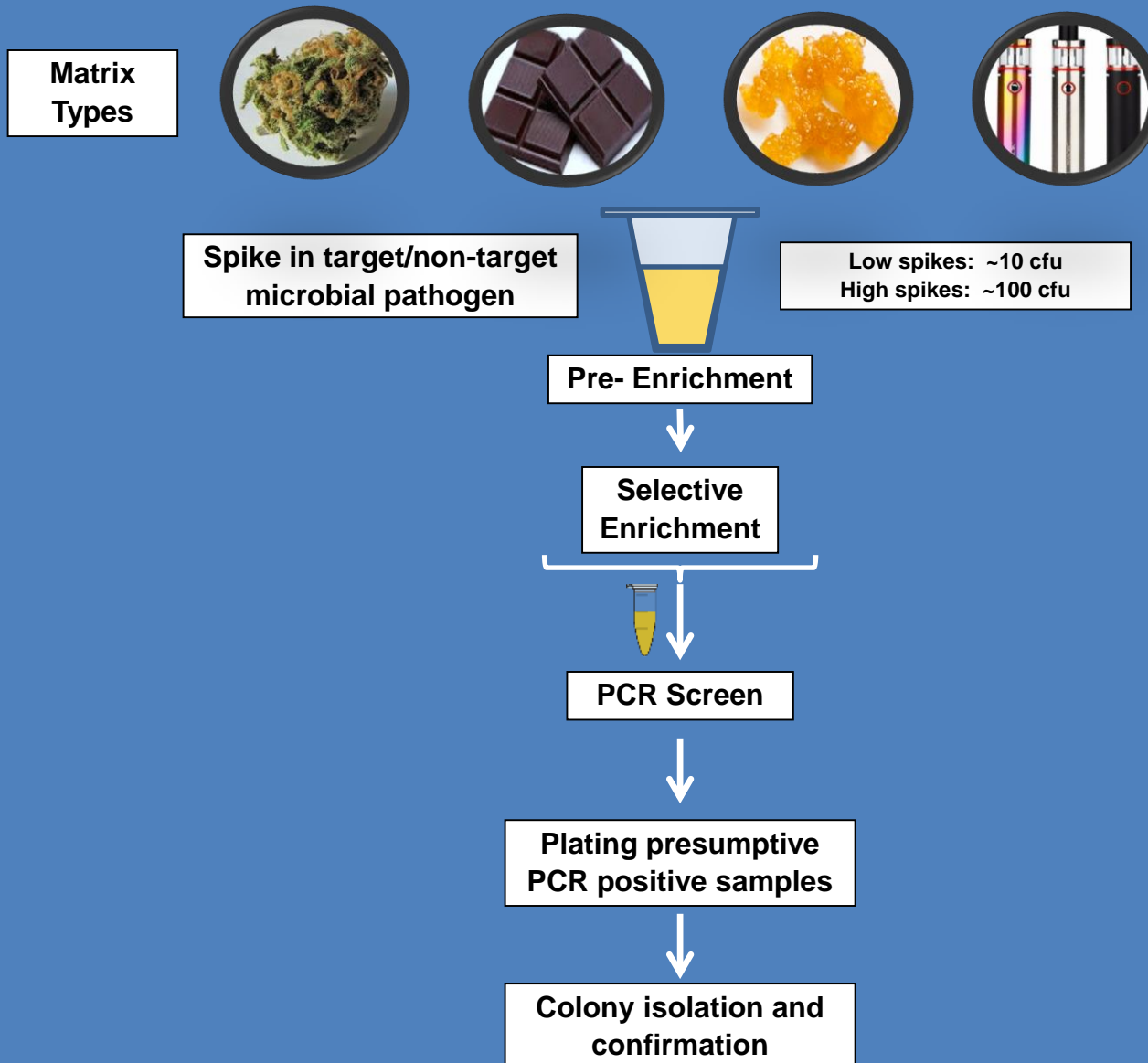
Microbiology Method Validation Requirements

- **§ 5713. Validation of Test Methods**
- (a) The laboratory may use a nonstandard, amplified, or modified test method or a method that is designed or developed by the laboratory to validate the methods for analyses of samples.
- (b) The laboratory shall follow the guidelines set forth in the US Food and Drug Administration's Guidelines for the Validation of Methods for the Detection of Microbial Pathogens in Foods and Feeds, 2nd Edition, 2015, incorporated herein by reference, to validate test methods for the microbial analysis.

| Criteria | Requirement |
|---|---|
| Number of target organisms; inclusivity | 5 |
| Number of non-target organisms; exclusivity | 5 |
| Number of analyte levels per matrix: Qualitative methods | 3 levels: high and low inoculum levels and 1 uninoculated level |
| Number of analyte levels per matrix: Quantitative methods | 4 levels: low, medium and high inoculum levels and 1 uninoculated level |
| Replicates per food at each level tested | 2 or more replicates per level |



Microbial Pathogen Validation Workflow



Salmonella Validation Data for Swabs and Chocolate

| | Organism | Strain | PCR Screen (%) ¹ | Confirmed (%) ² |
|-------------|-------------------------------|-------------------|-----------------------------|----------------------------|
| Targets | Salmonella enterica | ATCC 8324 (low) | 100 | 100 |
| | Salmonella enterica | ATCC 8324 (high) | 100 | 100 |
| | Salmonella enterica | ATCC 13311 (low) | 100 | 100 |
| | Salmonella enterica | ATCC 13311 (high) | 100 | 100 |
| | Salmonella enterica | ATCC 13314 (low) | 100 | 100 |
| | Salmonella enterica | ATCC 13314 (high) | 100 | 100 |
| | Salmonella enterica | FDA-A49 (low) | 100 | 100 |
| | Salmonella enterica | FDA-A49 (high) | 100 | 100 |
| | Salmonella enterica | F15M02184 (low) | 100 | 100 |
| | Salmonella enterica | F15M02184 (high) | 100 | 100 |
| Non-Targets | Escherichia coli | ATCC 25922 | 0 | - |
| | Klebsiella pneumoniae | ATCC 13882 | 0 | - |
| | Pseudomonas aeruginosa | ATCC 27853 | 0 | - |
| | Staphylococcus aureus | ATCC 33591 | 0 | - |
| | Serratia marcescens | ATCC 14756 | 0 | - |
| | Enterobacter aerogenes | ATCC 13048 | 0 | - |
| | Media Control | | 0 | - |

¹Percent positive out of two replicates. ²Only samples testing positive in the screen were plated.

Salmonella was detected, recovered and confirmed in 100% of all samples spiked with Salmonella



STEC Validation Data for Swabs

| | Organism | Strain | PCR Screen (%) ¹ | Confirmed (%) ² |
|----------------------------------|----------------------------------|---------------------|-----------------------------|----------------------------|
| Targets | Escherichia coli O157:H7 | ATCC 43888 (low) | 100 | 100 |
| | Escherichia coli O157:H7 | F1701010-004 (low) | 100 | 100 |
| | Escherichia coli O157:H7 | ATCC 43894 (low) | 100 | 100 |
| | Escherichia coli O157:H7 | F06M00948-10 (low) | 100 | 100 |
| | Escherichia coli O157:NM | F1703020-001 (low) | 100 | 100 |
| | Escherichia coli O26:H11 | CDC-272 (low) | 100 | 100 |
| | Escherichia coli O45:H2 | CDC-267 (low) | 100 | 100 |
| | Escherichia coli O103:H2 | CDC-269 (low) | 100 | 100 |
| | Escherichia coli O111:NM | F1702010-001 (low) | 100 | 100 |
| | Escherichia coli O121:H19 | CDC-268 (low) | 100 | 100 |
| | Escherichia coli O157:H7 | ATCC 43888 (high) | 100 | 100 |
| | Escherichia coli O157:H7 | F1701010-004 (high) | 100 | 100 |
| | Escherichia coli O157:H7 | ATCC 43894 (high) | 100 | 100 |
| | Escherichia coli O157:H7 | F06M00948-10 (high) | 100 | 100 |
| | Escherichia coli O157:NM | F1703020-001 (high) | 100 | 100 |
| | Escherichia coli O26:H11 | CDC-272 (high) | 100 | 100 |
| | Escherichia coli O45:H2 | CDC-267 (high) | 100 | 100 |
| | Escherichia coli O103:H2 | CDC-269 (high) | 100 | 100 |
| Escherichia coli O111:NM | F1702010-001 (high) | 100 | 100 | |
| Escherichia coli O121:H19 | CDC-268 (high) | 100 | 100 | |
| Non-Targets | Escherichia coli | ATCC 25922 | 0 | -- |
| | Salmonella enterica | ATCC 8324 | 0 | -- |
| | Enterobacter aerogenes | ATCC 13048 | 0 | -- |
| | Pseudomonas aeruginosa | ATCC 27853 | 0 | -- |
| | Staphylococcus aureus | ATCC 33591 | 0 | -- |
| | Media Control | | 0 | -- |

¹Percent positive out of two replicates.
²Only samples testing positive in the screen were plated.

STEC was detected, recovered and confirmed in 100% of all samples spiked with STEC



Current Chemistry Work

- Cannabinoid profile determination by UHPLC-DAD
 - THC, THCA, CBD, CBDA, CBG, CBN, THCV, CBC
 - Validation completed for Hemp Oil
 - Planning validation for cannabis concentrates and edibles
- Residual solvents and Terpenes tests using Headspace GC-MS/FID
 - Method developed for 20 residual solvent compounds listed in CA regulation and currently undergoing method validation
 - Method developed for 21 terpenes and currently undergoing method validation
- Contaminants Screening test using LC-MS
 - Method for synthetic cannabis screening from natural cannabis products developed and currently undergoing method validation
- Equipment Installation/Training
 - ICP-MS for Cadmium, Lead, Arsenic, and Mercury listed in CA regulation;
 - ELISA reader for Mycotoxin detection (total of aflatoxin B1, B2, G1, and G2, and ochratoxin A)



Chemistry Method Validation Requirements

- **§ 5713. Validation of Test Methods**
- (c) The laboratory shall follow the guidelines set forth in the US Food and Drug Administration's *Guidelines for the Validation of Chemical Methods for the FDA FVM Program*, 2nd Edition, 2015, incorporated herein by reference, to validate test methods for chemical analysis of samples.
- (1)The laboratory shall include and address the criteria listed below to validate test methods for chemical analyses of samples.
- (A) Accuracy; (B) Precision; (C) Linearity and range; (D) Calibration standard; (E) Sensitivity and selectivity; (F) Limit of detection and limit of quantitation; (G) Recovery; (H) Reproducibility; and (I) Robustness.
- (d) If available, the laboratory shall use cannabis reference materials or certified reference materials to validate test methods.



Cannabinoids Concentration Test using UHPLC-DAD

- Modified method based on Elizabeth Mudge, et al. “**Leaner and greener analysis of cannabinoids**” (Analytical and Bioanalytical Chemistry (2017) 409(12):3153-3163) - <https://www.ncbi.nlm.nih.gov/pubmed/28233028#>
- Column: Phenomenex Kinetex C18 100mm x 3.0 mm, 1.7 um with guard cartridge
- Mobile phase A: 10 mM ammonium formate, pH 3.6
- Mobile phase B: Acetonitrile

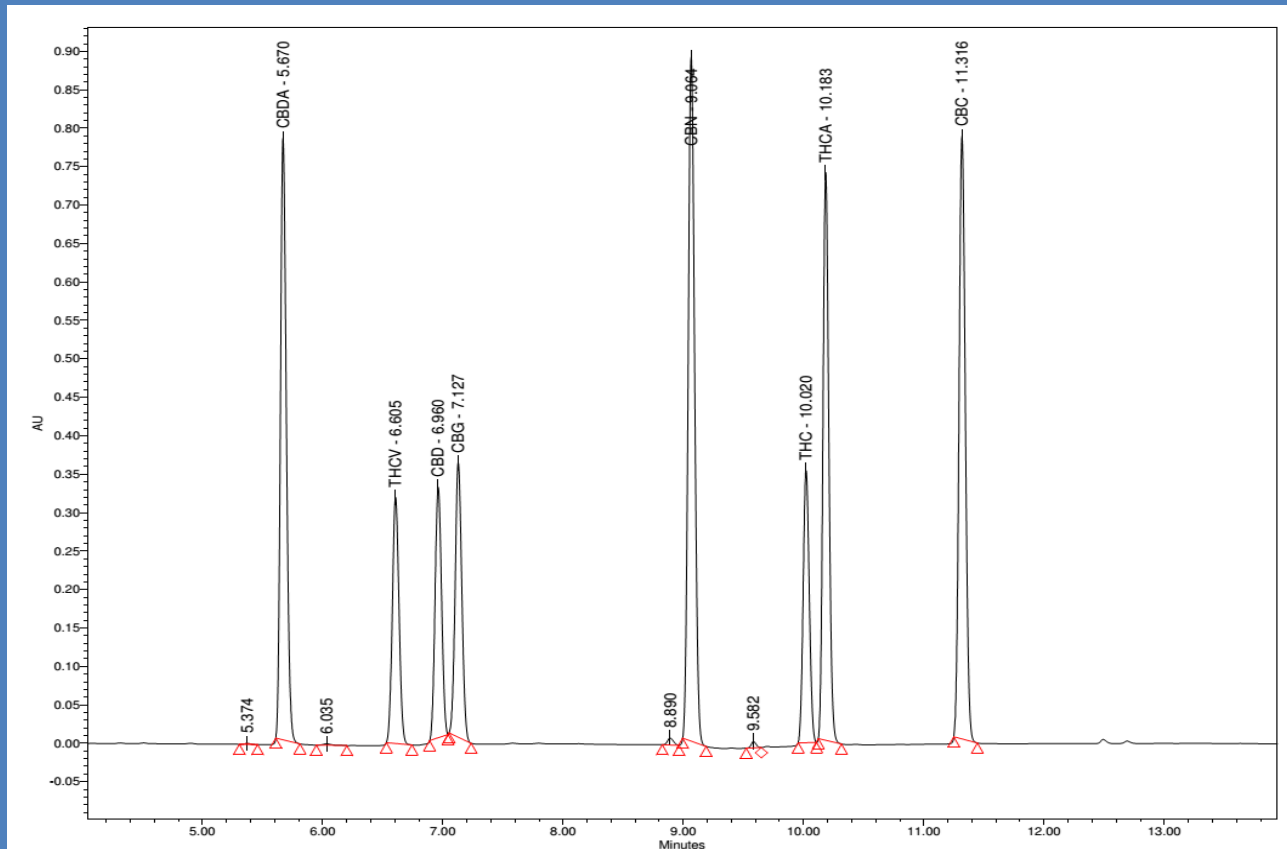
Gradient Program:

| Time (min) | Flow rate (mL/min) | % Mobile Phase A | % Mobile Phase B |
|------------|--------------------|------------------|------------------|
| 0 | 0.8 | 48 | 52 |
| 8.0 | 0.8 | 34 | 66 |
| 8.5 | 0.8 | 30 | 70 |
| 13 | 0.8 | 20 | 80 |
| 15 | 0.8 | 20 | 80 |
| 15.1 | 0.8 | 48 | 52 |
| 22 | 0.8 | 48 | 52 |

- Flow Rate: 0.8 mL/min
- Detection: 228 nm
- Run time: total 22 min: 15 min + 7 min column re-equilibration
- Column Temperature: 25°C
- Autosampler Temperature: 4°C



Cannabinoids Concentration Test using UHPLC-DAD



Chromatogram of 100 ppm cannabinoids mix standards



Method Validation on Hemp Oil

Cannabinoids concentration in three different hemp oil samples

| | Concentration in sample (ug/g) | | | | | | | |
|-------------|--------------------------------|------|------|-----|-----|-----|------|-----|
| | CBDA | THCV | CBD | CBG | CBN | THC | THCA | CBC |
| Hemp Oil #1 | 42.7 | 10.1 | 7.87 | ND | ND | ND | 3.24 | ND |
| Hemp Oil #2 | ND | ND | 11.1 | ND | ND | ND | ND | ND |
| Hemp Oil #3 | 29.4 | 5.98 | 9.38 | ND | ND | ND | 2.05 | ND |

Inter-day precision (3-day RSD)

| | RSD of Concentration in sample (ug/g) | | | | | | | |
|-------------|---------------------------------------|------|------|-----|-----|-----|------|-----|
| | CBDA | THCV | CBD | CBG | CBN | THC | THCA | CBC |
| Hemp Oil #1 | 2.8% | 7.6% | 2.8% | N/A | N/A | N/A | 2.9% | N/A |
| Hemp Oil #2 | N/A | N/A | 6.3% | N/A | N/A | N/A | N/A | N/A |
| Hemp Oil #3 | 4.6% | 7.6% | 4.3% | N/A | N/A | N/A | 2.6% | N/A |



Method Validation on Hemp Oil – Spike Recovery

- Spike 1: spike 30 ug of CBD to hemp oil #2

| Hemp Oil #2 | Recovery |
|-------------|-----------|
| Spike 1 | 30 ug CBD |
| Day 1 | 80.0% |
| Day 2 | 78.9% |
| Day 3 | 74.6% |

- Spike 2: spike 20 ug of each cannabinoids to hemp oil #2

| Hemp Oil #2 | Recovery | | | | | | | |
|-------------|----------|--------|-------|-------|-------|--------|-------|-------|
| Spike 2 | CBDA | THCV | CBD | CBG | CBN | THC | THCA* | CBC |
| Day 1 | 93.0% | 104.9% | 86.8% | 89.8% | 93.7% | 108.9% | 67.7% | 90.5% |
| Day 2 | 91.7% | 104.2% | 87.1% | 89.2% | 94.0% | 110.0% | 68.4% | 90.4% |
| Day 3 | 86.4% | 97.3% | 79.9% | 83.8% | 87.3% | 110.0% | 64.1% | 84.4% |

* THCA recovery low may be due to matrix effect



Path to ISO 17025 Accreditation

- SOP writing
- Forms, recordkeeping
- Quality manual
- ISO 17025 compliance implementation in laboratory operations
- ISO 17025 standard training



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Thank you

Resources:

Manufactured Cannabis Safety Branch

www.cdph.ca.gov/mcsb

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Cannabis Portal

www.cannabis.ca.gov

