Hemp Processing and Analysis:
Extraction, refining, and testing for consumer potency and safety

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Essex, Vermont

Overview

- Definition
- Phytocompounds of interest
- Extraction methods
- Analytical testing
- Future directions

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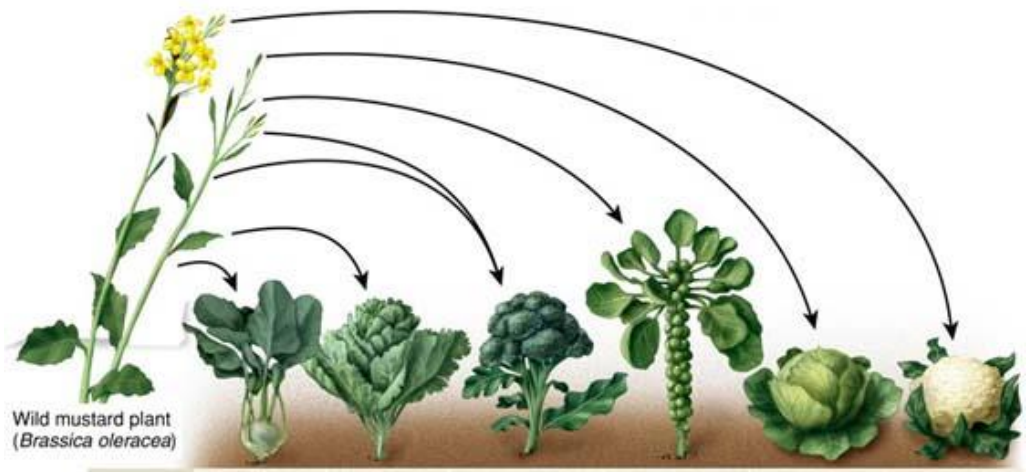
The same plant, but different…

Brassica oleracea (wild mustard)

- Artificial selection (traits)
- Natural selection (resistance)



Strain	Kohlrabi	Kale	Broccoli	Brussels sprouts	Cabbage	Cauliflower
Modified trait	Stem	Leaves	Flower buds and stem	Lateral leaf buds	Terminal leaf bud	Flower buds

Same plant, different **phenotypes**.

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Cannabis sativa L.

Taxonomy is complex

- *Cannabis sativa*
- *C. indica*
- *C. ruderalis*

Used for millennia

- Grain
- Oil
- Fiber
- Fuel
- Medicine

Hemp

- <0.3% Δ9-THC by dry weight (3 mg THC/g)
- Fact: Arbitrary designation (data collected from new fan leaves).
- Opinion: Inappropriate standard.

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Cannabis sativa L.

Cannabis today!

- Seed oil & nutritional content
- Fiber production
- Biopolymers and bioplastics
- Phytoremediation
- Fuel biomass
- **Phytochemicals**

Plants are “chemical factories”

Genetics >
enzymes >
biochemical ratios (CBD:THC)

Genetics largely determine molecules produced.

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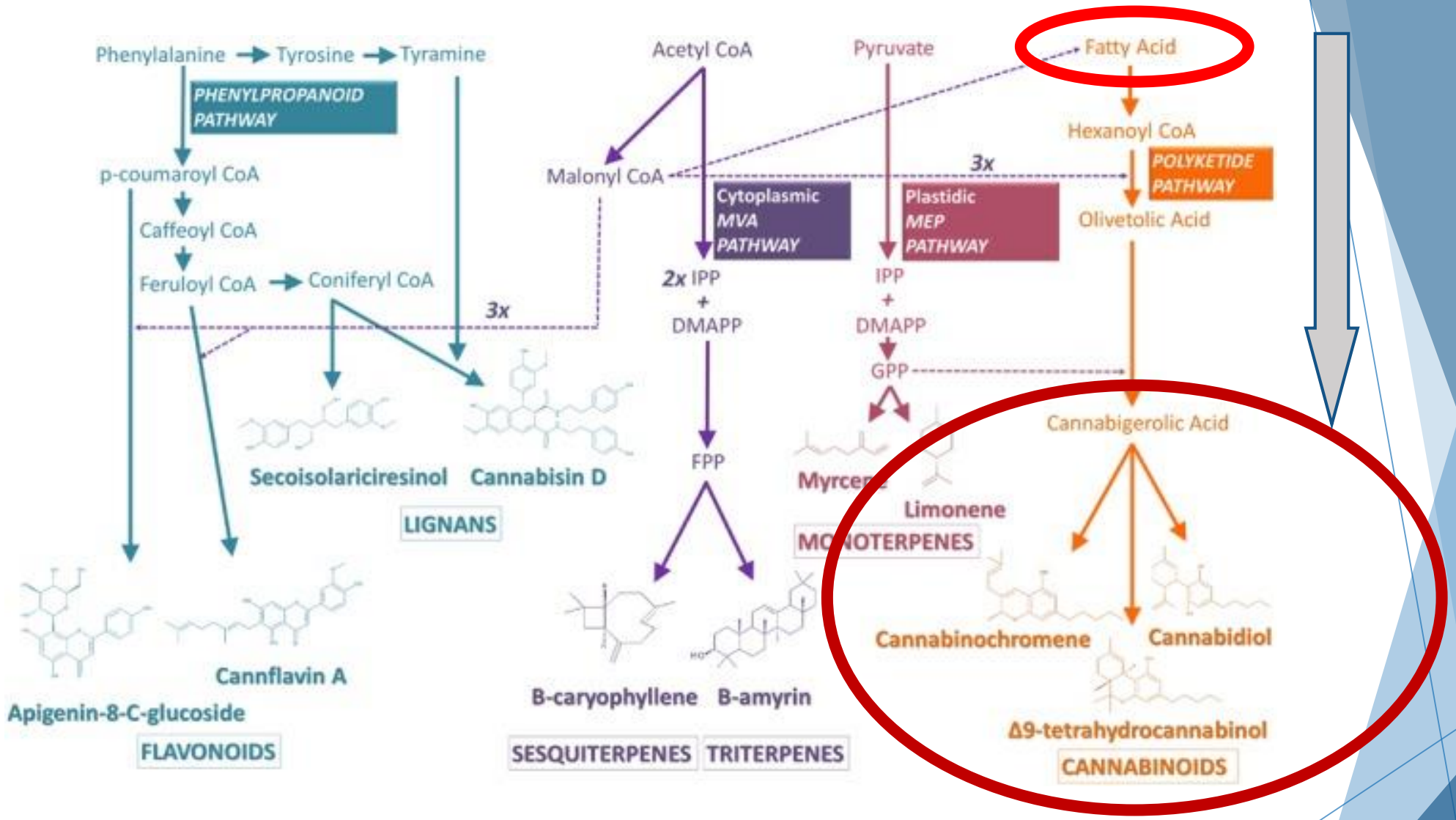

Schematic view of the biosynthetic pathways leading to the *Cannabis* secondary metabolites discussed in this review. Transport of precursors is represented by dashed arrows, while direct catalytic reactions are depicted by bold arrows. See text for detailed pathways. Abbreviations used: IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; MVA, mevalonate; MEP, methylerythritol phosphate.

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Source: Andre, C. M., Hausman, J. F., & Guerriero, G. (2016). Cannabis sativa: The Plant of the Thousand and One Molecules. *Frontiers in plant science*, *7*, 19. doi:10.3389/fpls.2016.00019

Hemp Oil Extraction and Refining

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Processing, Extraction & Refining

Producing botanical oils from hemp

Goal: Separate and collect molecules of interest from plant material.

Solvent – dissolves molecules to make a solution.
Solventless – non-chemical separation (temperature, pressure)

Common solvents used for hemp oil extraction:

- Alcohols
- Oils and fats
- Carbon dioxide (supercritical)
- Hydrocarbons (e.g. butane, propane)

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Producing botanical oils from hemp

When selecting and using a solvent consider…

- End goal (product & application)
- Expense
- Source & quality
- Safety & PPE
- Handling & storage
- Extraction system

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Processing, Extraction & Refining

Producing botanical oils from hemp

“Like dissolves like”

- your chemistry teacher probably

Solvents can be considered:

- **polar** (water)
- **non-polar** (fats, oils and CO2)
- **both** (alcohol)

Cannabinoids are **non-polar** molecules and readily dissolve in lipids and alcohol.

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Producing botanical oils from hemp

Lipid Extraction

- Easy and relatively inexpensive.
- Naturally incorporated into diet.
- Nutritional value can be added.
- Lipids promote absorption of cannabinoids in intestinal tract.

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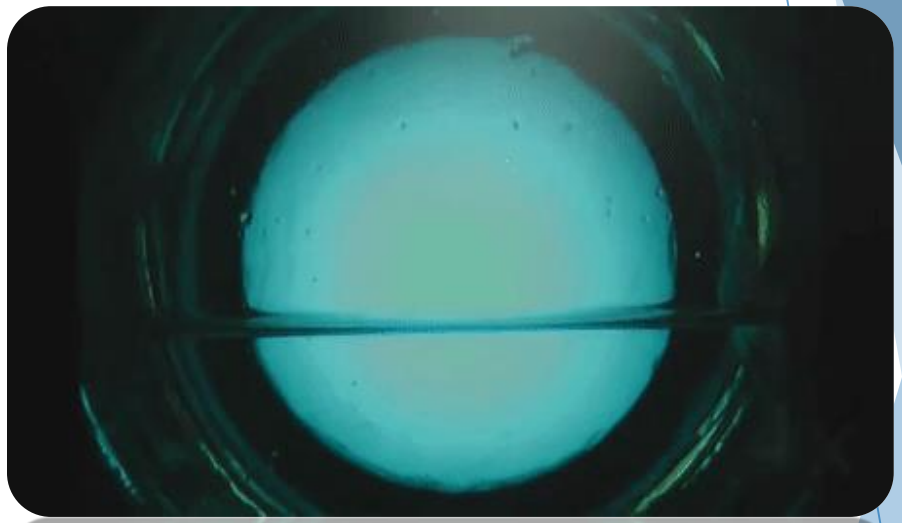
Processing, Extraction & Refining

Producing botanical oils from hemp



Carbon dioxide (CO_2)

- Solid when cold (dry ice)
- Gas at room temp & pressure
- Liquid under pressure

- **Supercritical fluid** when under heat and pressure
    - Penetrates feed material like a gas
    - Has solvent properties of a liquid
    - Closed-loop system
    - Used to decaffeinate coffee and for dry cleaning

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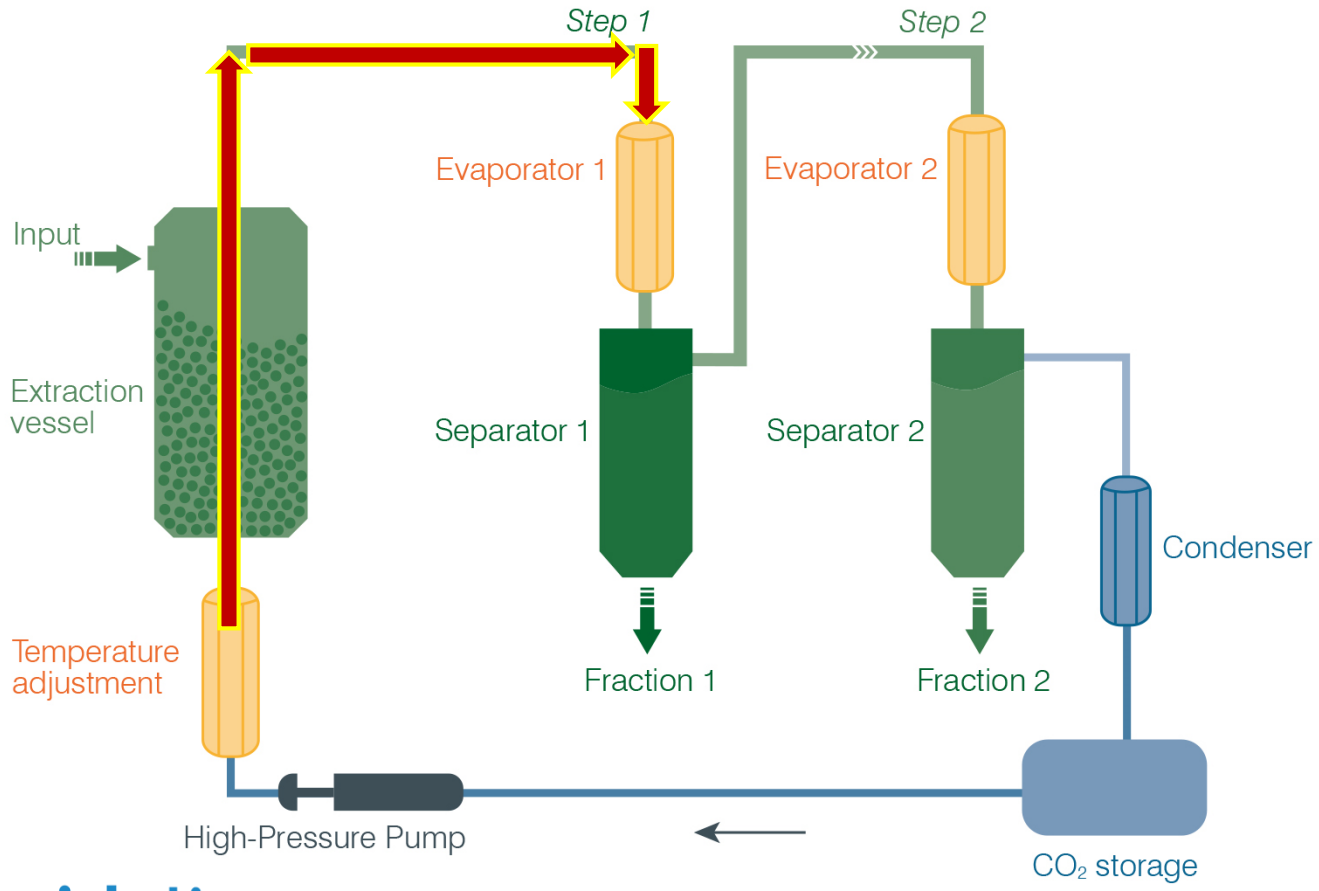
Producing botanical oils from hemp

Supercritical CO_2 System Diagram



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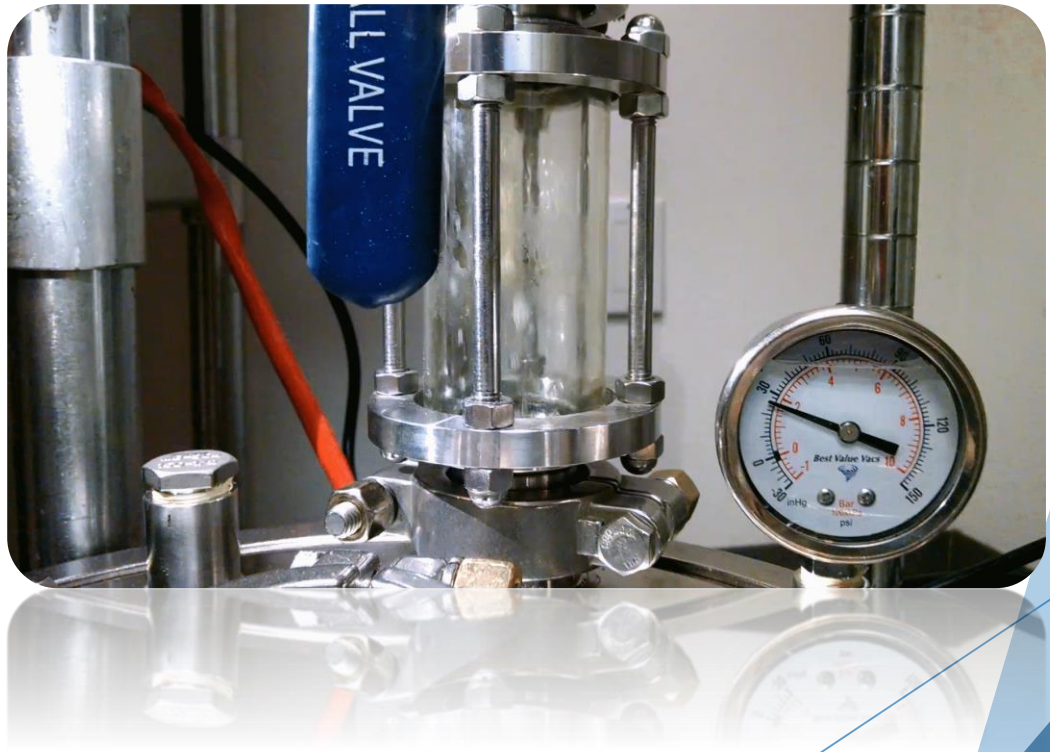
Producing botanical oils from hemp

Hydrocarbon extraction

- **<u>Not permitted in Vermont!</u>**
- Non-polar solvent
- Efficient and selective

- Solvent purity is low
- Highly volatile!
- Closed-loop systems only!
- Do <u>not</u> try this at home!



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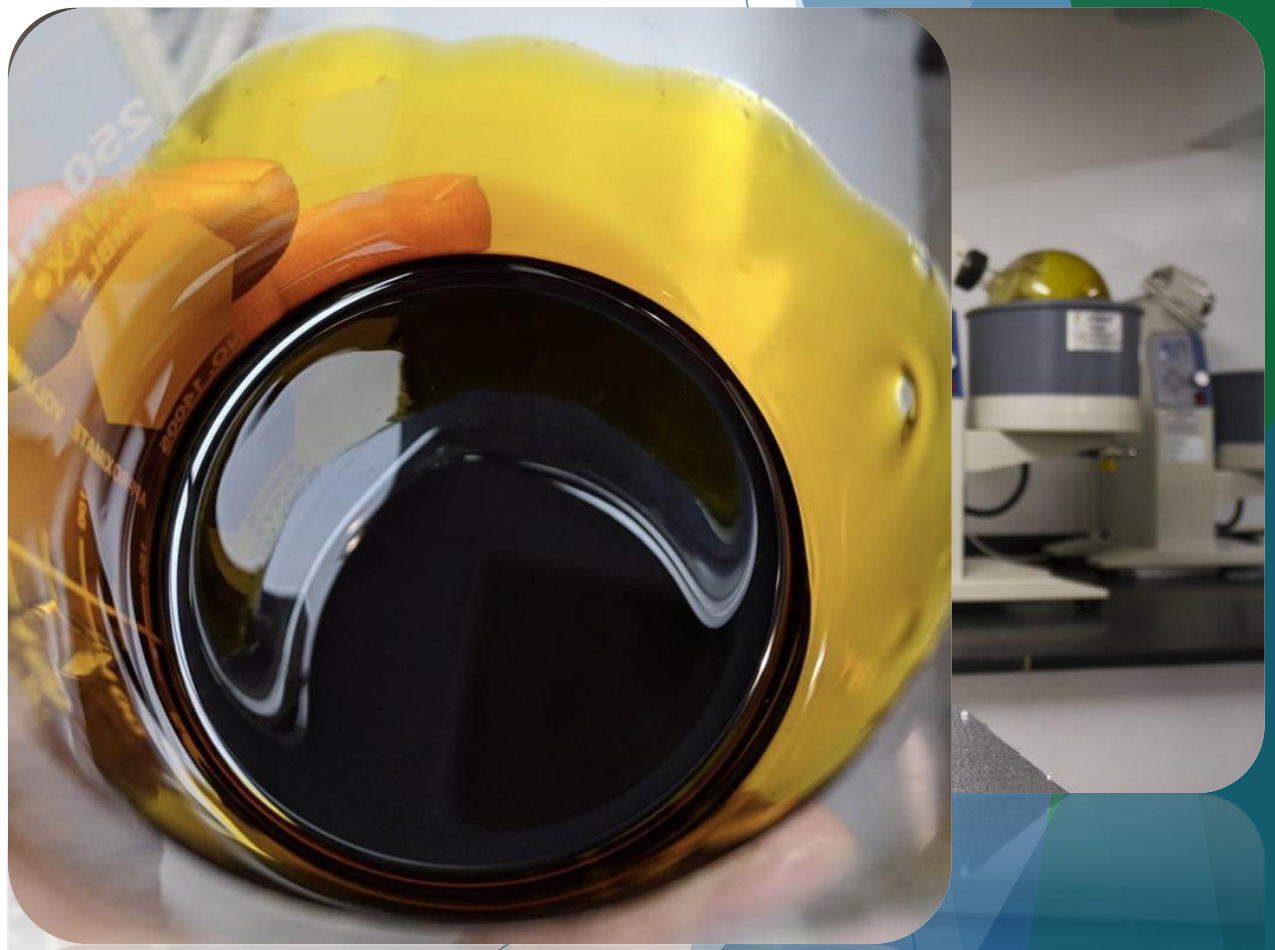
Processing, Extraction & Refining

Producing botanical oils from hemp

Alcohol Extraction

- Ethanol (ETOH) is alcohol of choice.
- Aggressive solvent.
- Will pull out polar molecules too (sugars and chlorophyll).
- QWET (quick wash ethanol)
- ETOH can be reclaimed and reused and/or evaporated off.



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Processing, Extraction & Refining

Producing botanical oils from hemp

Refining

Winterization – adding ETOH to extract, freezing, and cold filtering solution in order to remove polar molecules, fats, and waxes.

Fractional distillation – temperature gradients used to separate hemp oil into constituent molecules (**distillate**).

Decarboxylation – applying heat to convert the acidic compounds in plant material or oils into neutral compounds (e.g. CBDa>CBD, CBGa>CBG).

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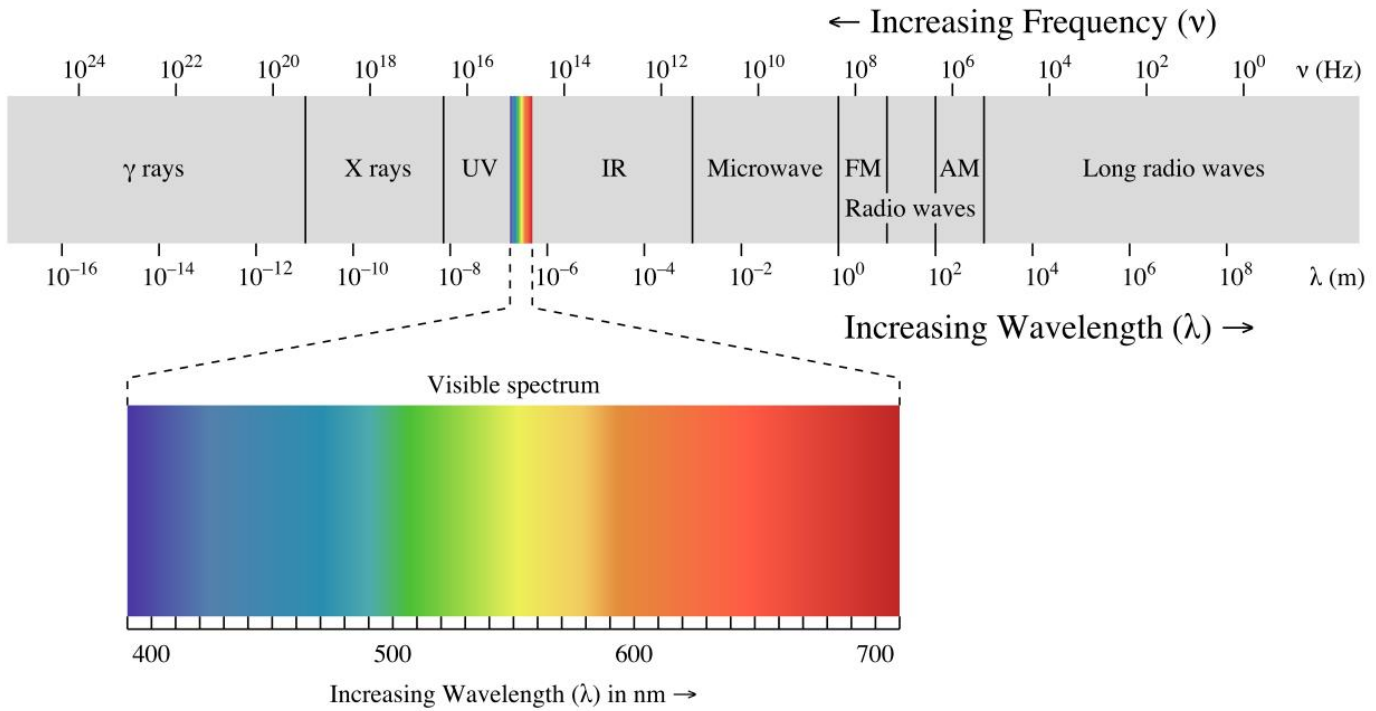
Processing, Extraction & Refining

Final products

Crude – Oil that is first extracted. May be winterized or not (20-60%).

Distillate – Oil that has been separated using heating and condensation to collect specific groups of molecules (70-90%).

Isolate – A “pure” form of a particular cannabinoid (~99.9%).



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Hemp Analytical Testing

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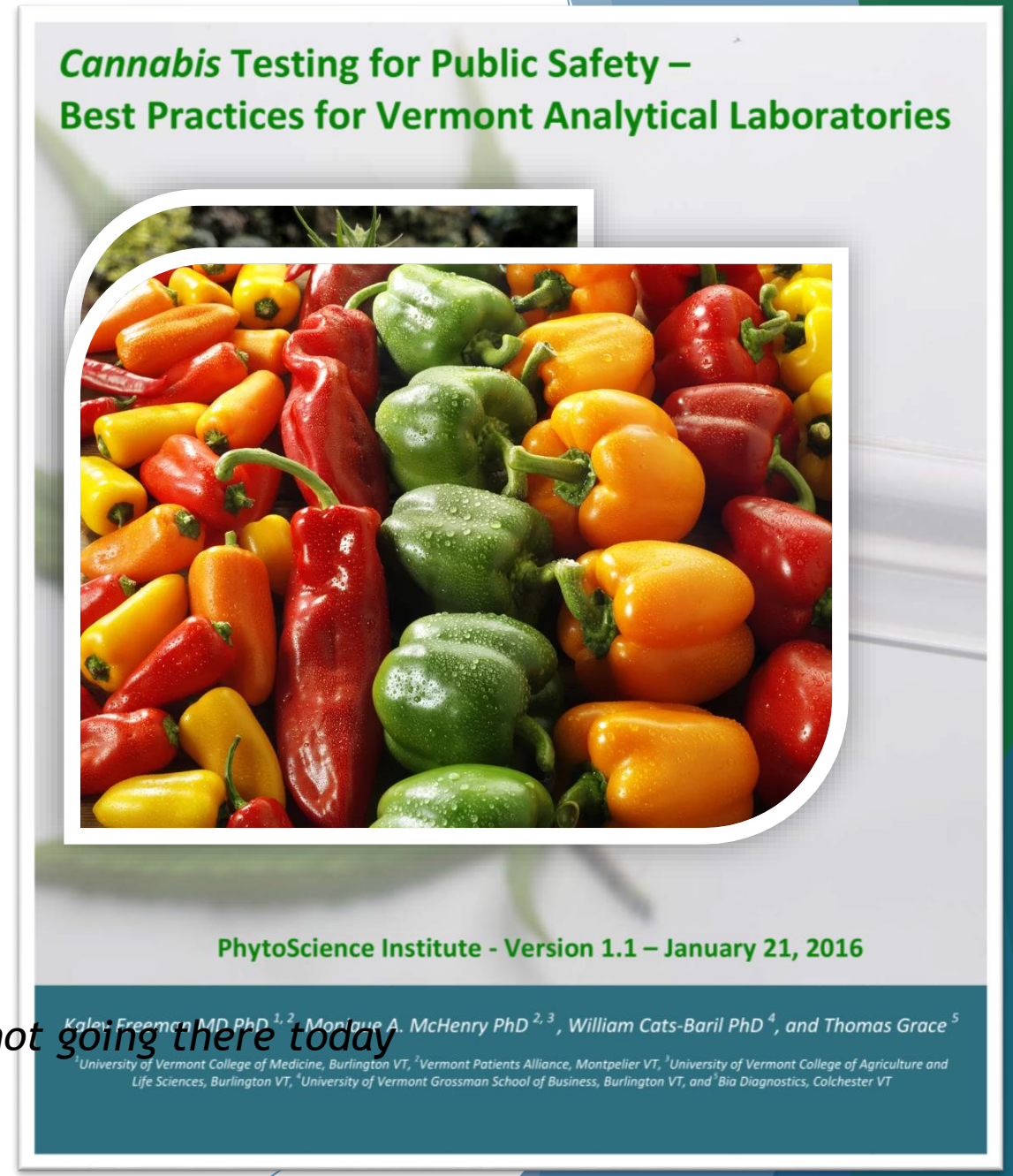
Hemp Analytical Testing

What is an appropriate model for testing hemp?

- Other agricultural commodities.
- **Goals**: assessment of phytochemical composition & abundance (**potency**) and screening for contaminants (**safety**).
- Distinction: combustion & inhalation.*
  - ➢ myclobutanil → HCN, HCl…



** not going there today*

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Analytical Testing

Cannabinoid ratios matter.

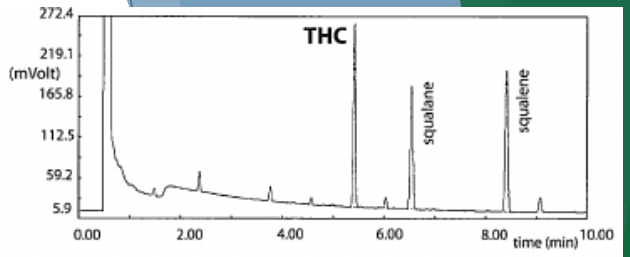
A *Cannabis sativa* chemovar is **NOT** considered to be hemp if it is:

- **Chemotype I:** THC dominant
- **Chemotype II:** THC/CBD co-dominant

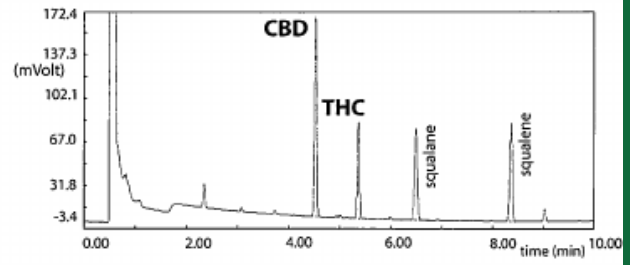
C. sativa chemovars considered "**HEMP**"

- **Chemotype III:** CBD dominant
- **Chemotype IV:** CBD/CBG co-dominant
- **Chemotype IV:** no cannabinoids

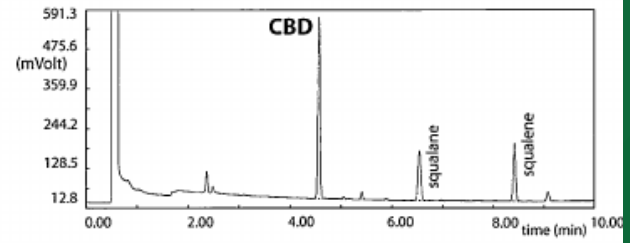
chemotype I:
prevalent THC
THC > 0.3% d.w.
CBD < 0.5% d.w.



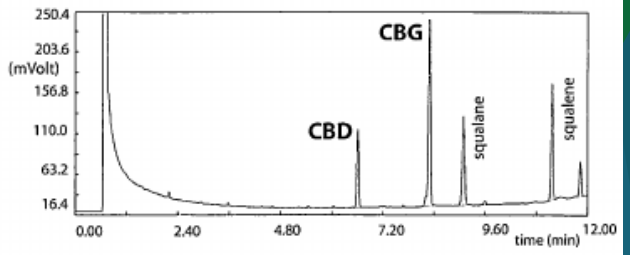
chemotype II:
intermediate
THC ≥ 0.3% d.w.
CBD > 0.5% d.w.



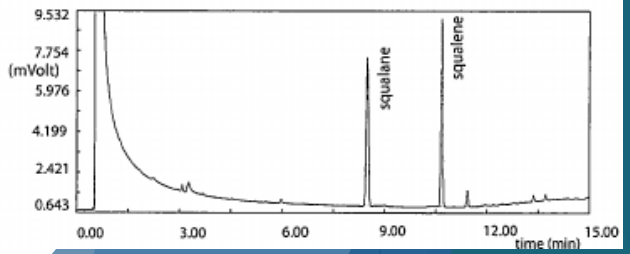
chemotype III:
prevalent CBD
THC < 0.3% d.w.
CBD > 0.5% d.w.



chemotype IV:
prevalent CBG
CBG > 0.3% d.w.
CBD < 0.5% d.w.



chemotype V:
zero cannabinoids
total cannabinoids
content < 0.2% d.w.



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Analytical Testing

What do you want to know?

1. *What is it?*

Hemp: Δ9-THC ≤ 0.3% by mass
Not Hemp: Δ9-THC > 0.3% by mass

How is this determined?

Potency using chromatography

- Liquid (HPLC)
- Gas (GC)

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Analytical Testing

ISO Standard

- Testing Laboratories **ISO 17025**
- Traceability
- Documentation
  - Solid record keeping
  - Corrective Actions

Cannabis testing certification

The Emerald Test

Emerald Scientific

ISO 17025

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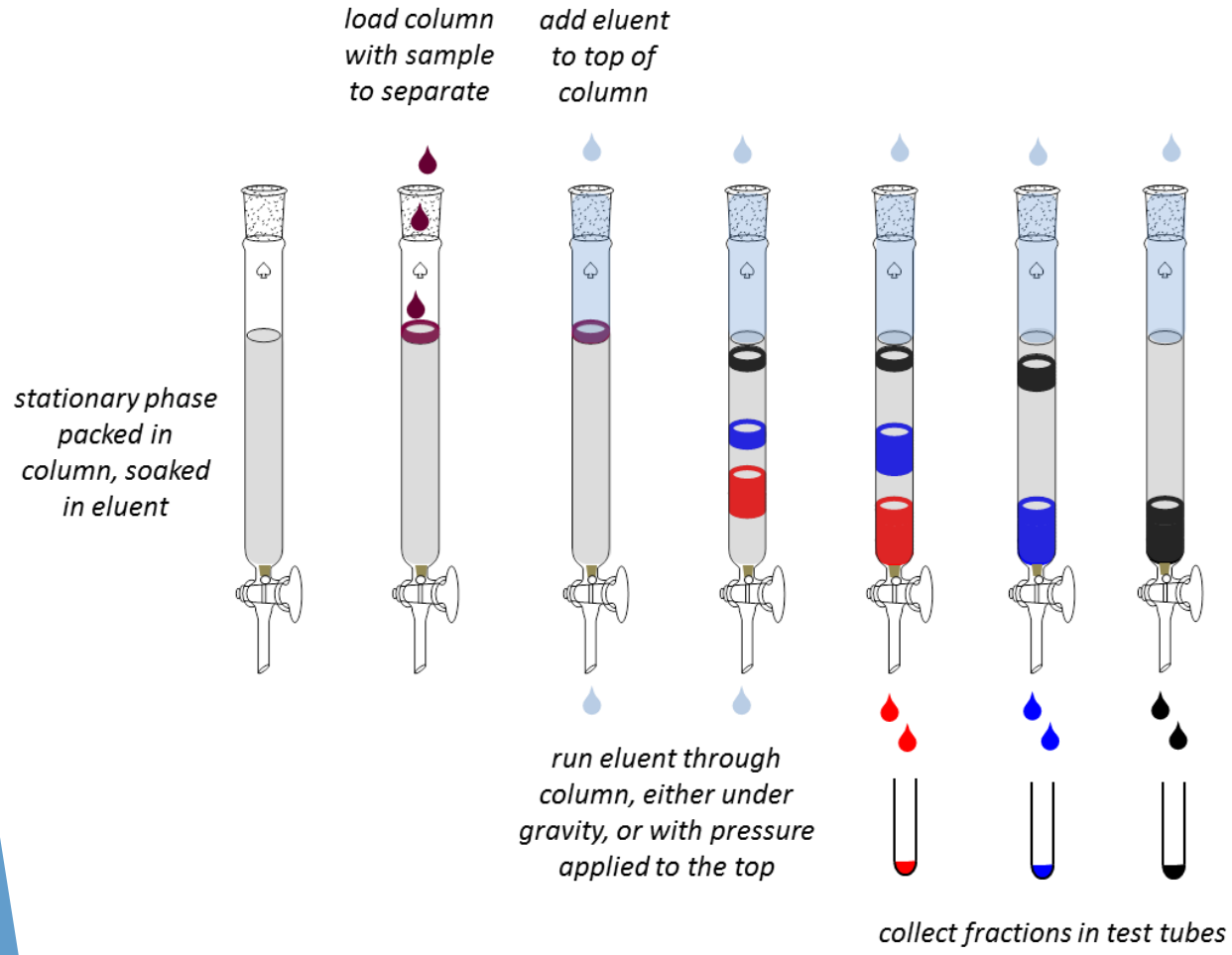
Analytical Testing



Chromatography

"to write color"

Solution is separated into different fractions based on molecule size and chemical properties

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Analytical Testing

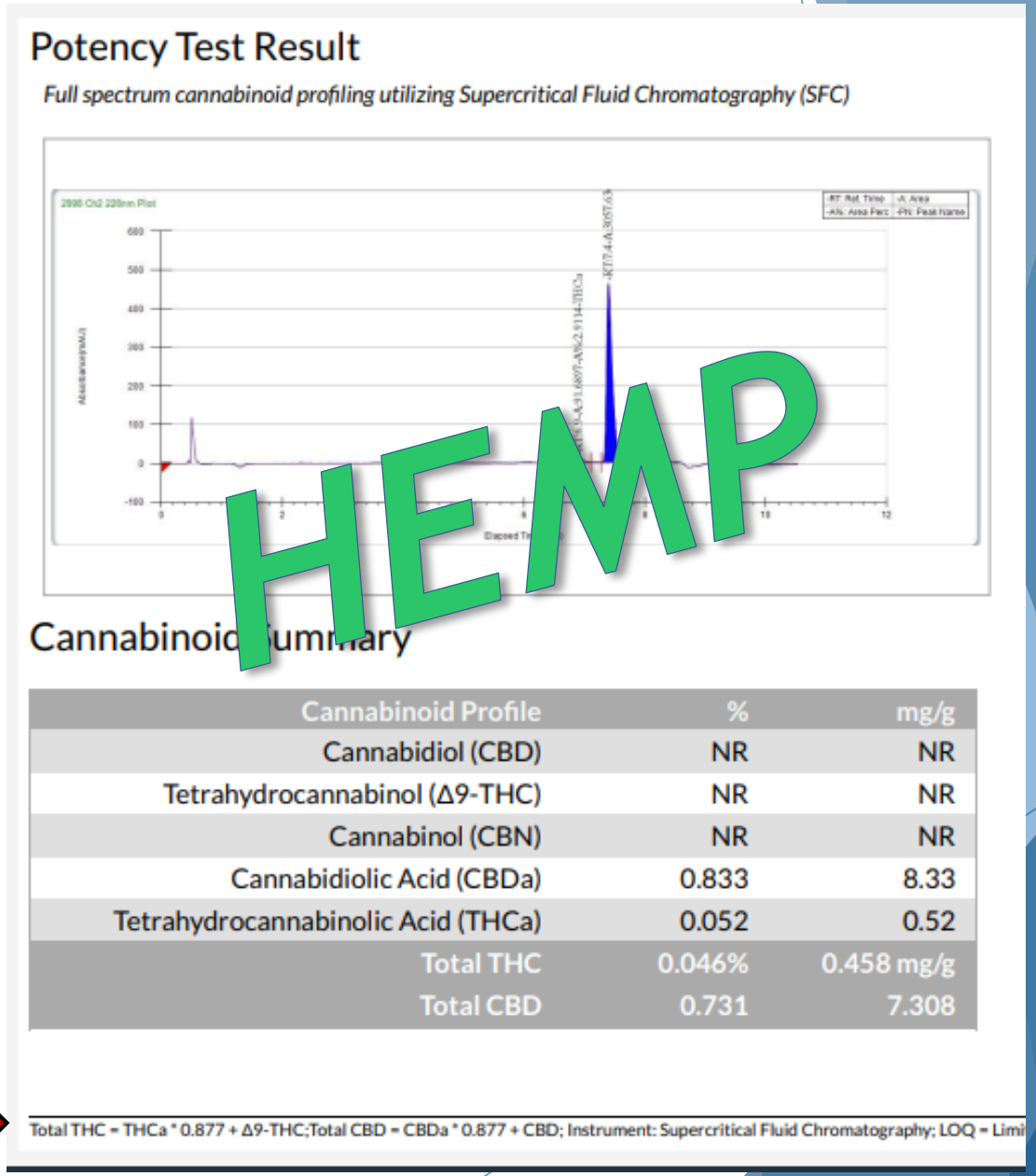
What will the results look like?

HPLC
High Performance Liquid Chromatography
major cannabinoids

Δ9-THC	(low in plant)
CBD	(low in plant)
THCa	(dominant)
CBDa	(dominant)

Analysis:

- Δ9-THC is effectively absent
- 'Total THC' is 0.05%
- CBD:THC ratio is 16:1
- Results:



Potency Test Result

Full spectrum cannabinoid profiling utilizing Supercritical Fluid Chromatography (SFC)

HEMP

Cannabinoid Summary

Cannabinoid Profile	%	mg/g
Cannabidiol (CBD)	NR	NR
Tetrahydrocannabinol (Δ9-THC)	NR	NR
Cannabinol (CBN)	NR	NR
Cannabidiolic Acid (CBDa)	0.833	8.33
Tetrahydrocannabinolic Acid (THCa)	0.052	0.52
Total THC	0.046%	0.458 mg/g
Total CBD	0.731	7.308

Total THC = THCa * 0.877 + Δ9-THC;Total CBD = CBDa * 0.877 + CBD; Instrument: Supercritical Fluid Chromatography; LOQ = Limi

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Analytical Testing

What will the results look like?

HPLC
High Performance Liquid Chromatography
major cannabinoids

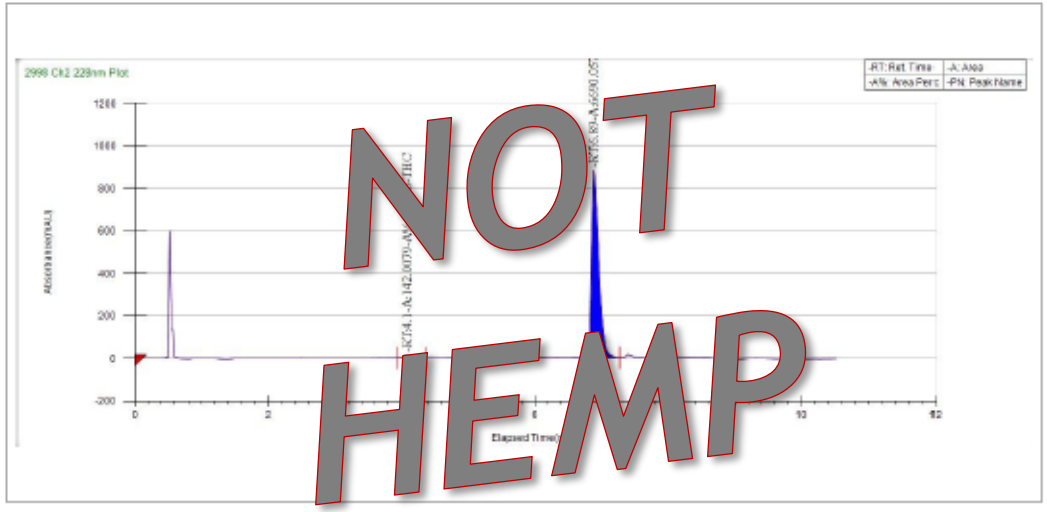
Δ9-THC	(low in plant)
CBD	(low in plant)
THCa	(dominant)
CBDa	(dominant)

Analysis:

- Δ9-THC is below 0.3%
- 'Total THC' = 5.73%*
- Results:

Potency Test Result

Full spectrum cannabinoid profiling utilizing Supercritical Fluid Chromatography (SFC)



Cannabinoid Summary

Cannabinoid Profile	%	mg/g
Cannabidiol (CBD)	NR	NR
Tetrahydrocannabinol (Δ9-THC)	0.259	2.59
Cannabinol (CBN)	NR	NR
Cannabidiolic Acid (CBDa)	NR	NR
Tetrahydrocannabinolic Acid (THCa)	6.236	62.36
Total THC	**5.727%**	**57.275 mg/g**
Total CBD		

Total THC = THCa * 0.877 + Δ9-THC;Total CBD = CBDa * 0.877 + CBD; Instrument: Supercritical Fluid Chromatography; LOQ = Li

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Analytical Testing

What will the results look like?

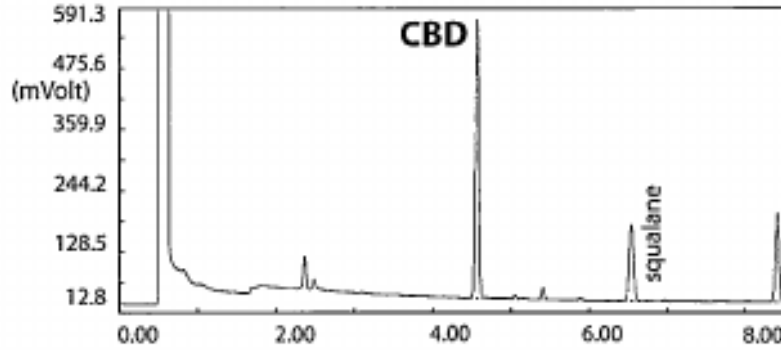
Gas Chromatography (GC)
neutral cannabinoids only

- Acidic cannabinoids (THCa, CBDa, CBGa) are decarboxylated (THC, CBD, CBG) during prep and volatilized into a gas.
- GC values roughly equal “total theoretical” values on HPLC reports.
- GC can also detect and quantify dominant terpenes.

chemotype III:
prevalent CBD

THC < 0.3% d.w.

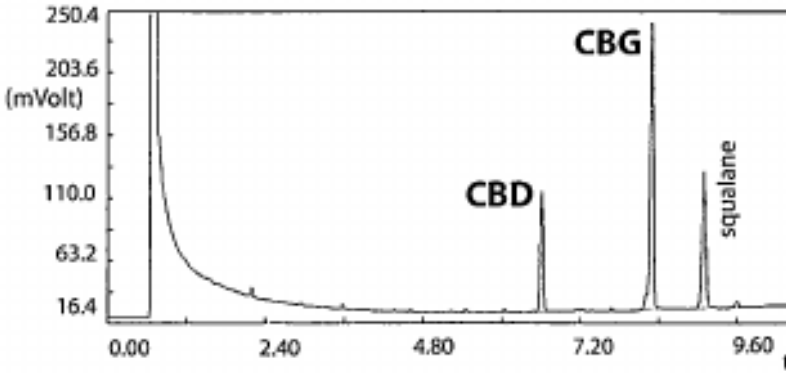
CBD > 0.5% d.w.



chemotype IV:
prevalent CBG

CBG > 0.3% d.w.

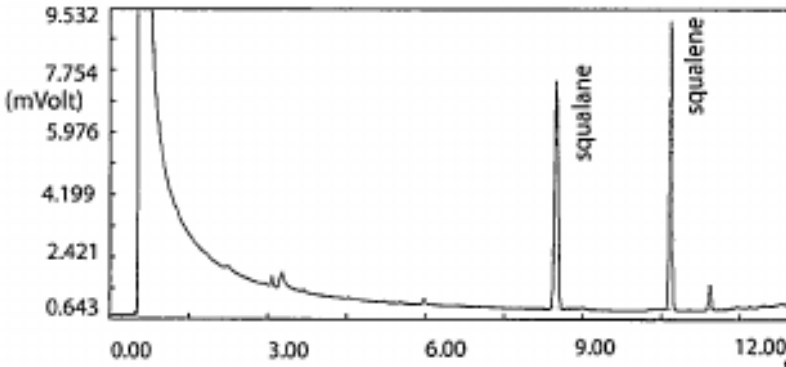
CBD < 0.5% d.w.



chemotype V:
zero cannabinoids

total cannabinoids

content < 0.2% d.w.



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Analytical Testing

What do you want to know?

1. *What is it?*

Answer: HEMP

We also now know the relative abundance of selected **terpenes** and **cannabinoids** in the sampled material (potency).

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Analytical Testing

What do you want to know?

2. *Is it safe to consume?*

- **Pesticides**, like myclobutanil, diazinon, and permethrin
  GC-MS (gas chromatography + mass spectrometry)
- **Heavy metals**, like lead, cadmium, and chromium
  ICP-MS (inductively coupled plasma mass spectrometry)
- **Microbes**, like *Aspergillus* spp. and *E. coli*
  qPCR (quantitative polymerase chain reaction)

Analytical Testing

Comparing qPCR to traditional agar plating

- Microbes culture broadly; beneficial and commensal included.
- Initial results in 48 to 72 hours after prep.
- Not all pathogenic microbes culture on agar.
- Does not indicate the presence of mycotoxic fungal DNA.
- Composition of microbes is “ballparked” and changes over days.
- What grows on plate is the result; # of CFUs counted as proxy.
- False positives.

- Amplifies targeted microbes only.
- Final results as soon as **2 hours** after prep.
- Identifies “hit list” pathogens with high fidelity.
- Detects DNA of mycotoxic fungi (dead or alive).
- Pathogen quantification is in real time and precise.
- Ability to multiplex (multiple pathogens) in same tube with high level of precision.
- No false positives.

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Analytical Testing

Comparing qPCR to traditional agar plating

genomeweb

My GenomeWeb

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Home » Business, Policy & Funding » Business News » Bio-Rad Wins USDA Food Safety Testing Contr

Bio-Rad Wins USDA Food Safety Testing Contract

Nov 13, 2018 | staff reporter

NEW YORK (GenomeWeb) — Bio-Rad said today that it has received a US Department of Agriculture contract for its iQ-Check RT-PCR pathogen detection test kits and related iQ-Check sample prep automation system.

The food safety testing kits use DNA probes to detect pathogens including *Salmonella spp.*, *Listeria monocytogenes*, *Campylobacter*, *Escherichia coli* O157:H7, and Shiga toxin-producing *E. coli*. The iQ-Check prep system is a robotic liquid handling platform for performing DNA extraction and PCR plate set-up.

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Analytical Testing

What do you want to know?

2. *Is it safe to consume?*

- **Pesticides**, like myclobutanil, diazinon, and permethrin
  GC-MS (gas chromatography + mass spectrometry)
- **Heavy metals**, like lead, cadmium, and chromium
  ICP-MS (inductively coupled plasma mass spectrometry)
- **Microbes**, like *Aspergillus* spp. and *E. coli*
  qPCR (quantitative polymerase chain reaction)
- **Mycotoxins**, toxic fungal metabolites like aflatoxin and ergot
  qPCR or ELISA (enzyme-linked immunosorbent assay)

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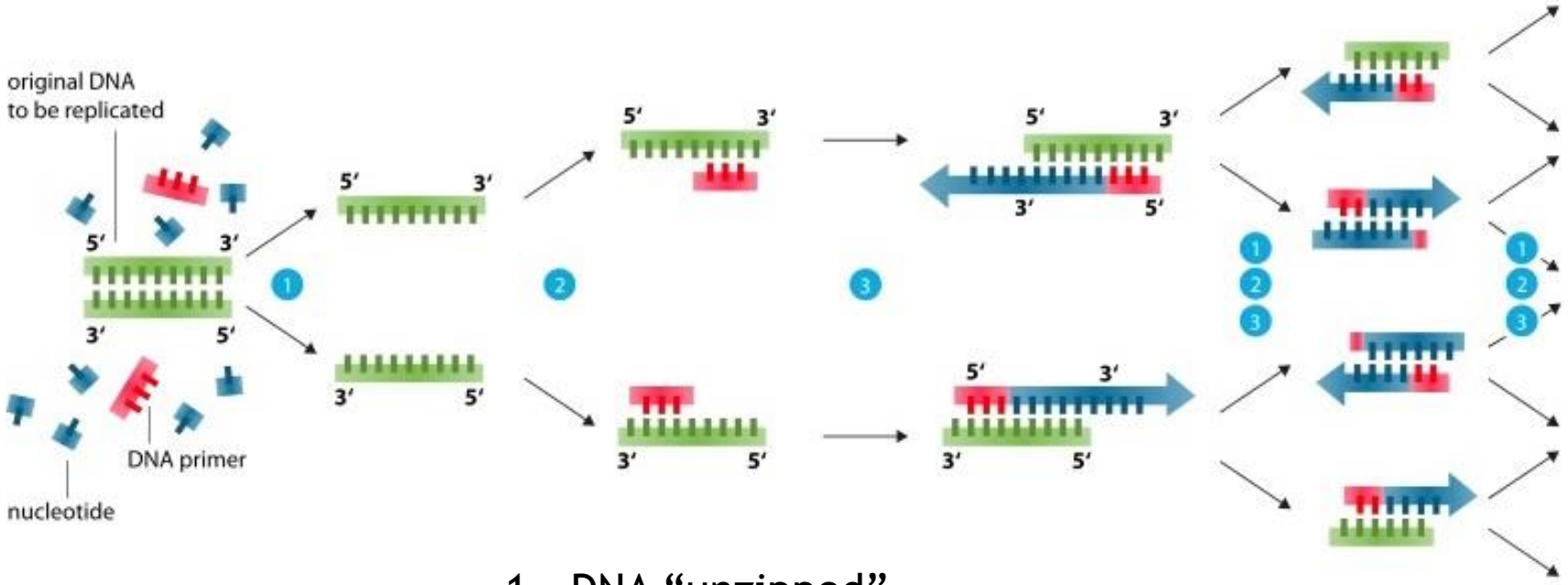
Analytical Testing

How qPCR works



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

1. DNA “unzipped”.
2. Taxon-specific **primers** adhere to ssDNA.
3. Free-floating **nucleotides** in solution.
4. **Polymerase** builds complementary strand.
5. Process repeats several times and amplifies target sequences.

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Analytical Testing

qPCR

Potential “hit list” of pathogenic microbes on *Cannabis:*

- *Aspergillus* spp.
- *Coliform* spp.
- Enterobacteriaceae
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Salmonella* spp.
- mycotoxins

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Analytical Testing

Small scale

- DNA-based tests
- colormetric results: +/- (red/yellow)
    - male/female
    - +/- THCa synthase
    - +/- CBDa synthase
    - +/- powdery mildew
    - +/- *Botrytis*
    - +/- russet mites

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MEDICINAL GENOMICS

Appreciation

Dr. Heather Darby

The University of Vermont Extension

Cary Giguere
Stephanie Smith
Erica Cummings

Vermont Agency of Agriculture, Foods & Markets

Kelly McDowell, MFA

eLucidation

Tim Fair, Esq. and **Andrew Subin**, Esq.
Vermont Cannabis Solutions

VCS

Zac Eaton and **Kevin McKernan**

Medicinal Genomics