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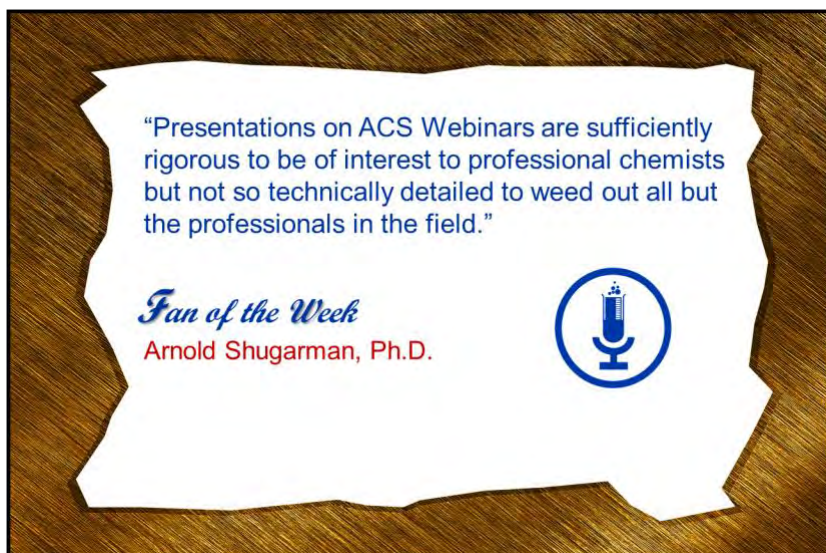
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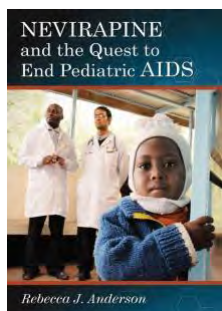


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## Cannabis Chemistry 201



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## Cannabis Chemistry 201

**ACS Webinar**  
**November 6, 2014**

Christopher J. Hudalla, Ph. D.

## Cannabis Chemistry 101: Review

- ✦ **History of Cannabis**
  - ✦ Legal status in the US
- ✦ **Endocannabinoid System**
  - ✦ Therapeutic benefits
- ✦ **Chemical Complexity**
  - ✦ Phytochemical constituents
  - ✦ Potential contaminants
  - ✦ Matrix complexity
- ✦ **Biosynthetic Pathways**
  - ✦ Formation of cannabinoids
- ✦ **Cannabinoid Reactions**
  - ✦ Degradation/Decarboxylation
- ✦ **Opportunities for Analytical Chemistry**
  - ✦ Application of modern technologies
  - ✦ Ensure consumer safety
  - ✦ Research opportunities



## Role of Analytical Chemistry

- ✦ **Ensuring Consumer Safety**
  - ✦ Confirm products are free from contamination
  - ✦ Assist in determining proper dosage
- ✦ **Optimization of Cultivation Practices**
  - ✦ Monitoring nutrient uptake
  - ✦ Early identification of phenotypes
- ✦ **Design and Development of Marijuana Infused Products (MIPs)**
  - ✦ Optimization of extractions and processes
  - ✦ Quantitation required for product labeling



## Audience Poll

What is typically the most abundant cannabinoid found in cannabis?

- THC (Tetrahydrocannabinol)**
- CBD (Cannabidiol)**
- THCA (Tetrahydrocannabinolic Acid)**
- CBDA (Cannabidiolic Acid)**



## Analytes and Analytical Techniques

|                | Analyte                            | Analytical Technique                 |
|----------------|------------------------------------|--------------------------------------|
| Phytochemicals | Cannabinoids                       | TLC, GC, HPLC, UPLC, <b>CC (SFC)</b> |
|                | Terpenes                           | <b>GC</b>                            |
|                | Water (Residual Moisture)          | Gravimetric, Water Activity          |
| Contaminants   | Heavy Metals                       | AA, ICP, ICP-MS, <b>TXRF</b>         |
|                | Volatile Organic Compounds (VOCs)  | <b>Headspace GC</b> , GC/MS          |
|                | Mycotoxins                         | <b>ImmunoAffinity (IA) Assays</b>    |
|                | Microbiological Contaminants       | Cultures, qPCR                       |
|                | Pesticides/Plant Growth Regulators | LC/MS, GC/MS                         |

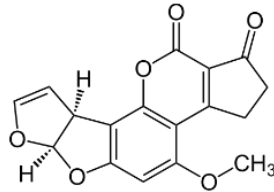




## Mycotoxins

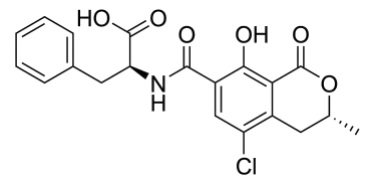
### ✦ Four Key Aflatoxins: B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>

- ✦ Produced by some Aspergillus molds
- ✦ Results in liver damage



### ✦ Ochratoxin A

- ✦ Produced by some Aspergillus and Penicillium molds
- ✦ Results in kidney damage and immune suppression



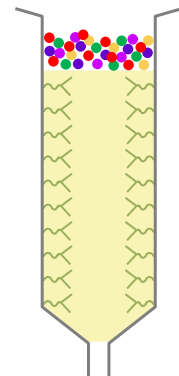
### ✦ Found in many commodities



## ImmunoAffinity (IA) Assays

### ✦ Monoclonal Antibody Based Affinity Chromatography

- ✦ Extracted sample is loaded on to the column
- ✦ Sample is flushed through the column
- ✦ Mycotoxins are selectively bound to the antibodies
- ✦ Additional constituents are passed through to waste
- ✦ Mycotoxins are collected selectively for analysis



## Mycotoxin Testing

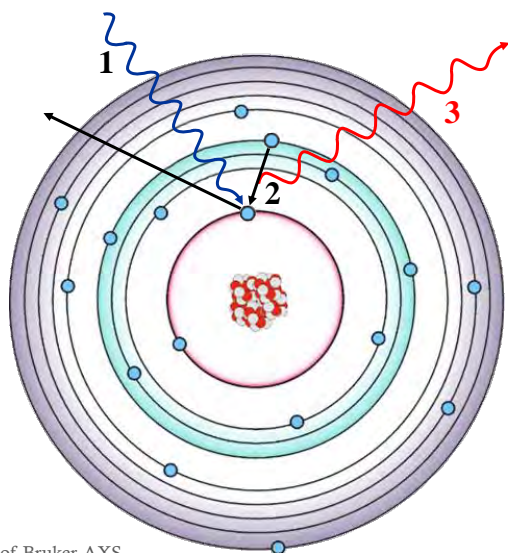
Mycotoxin concentrations can be measured with a digital fluorometer. Provides total aflatoxin and total ochratoxin concentrations.



Analysis with LC or LC/MS/MS delivers additional sensitivity and specificity, providing separation and quantitation of the individual mycotoxins.



## X-ray Fluorescence (XRF) Spectroscopy



- An X-ray quantum hits an inner shell electron in a (sample) atom. The electron is removed leaving the atom in an excited state (1)
- A electron from a higher orbital will drop down to fill the space. (2)
- The energy difference between the inner and outer shell is balanced by the emission of a photon quantum (fluorescence radiation, 3)
- These transitions are instantaneous. Fluorescence emissions are specific to individual elements, with intensities proportional the concentration of those elements.

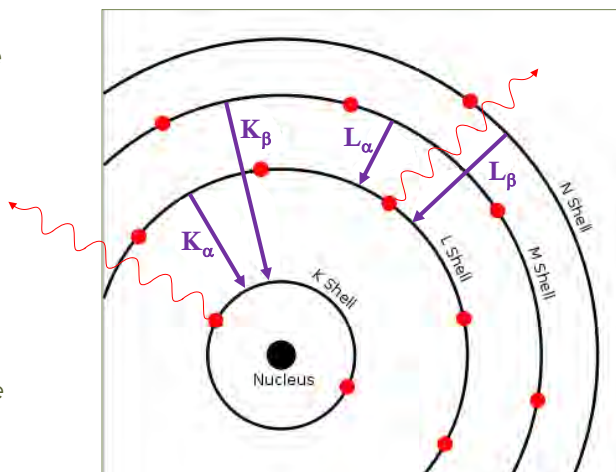
Courtesy of Bruker-AXS

## X-ray Fluorescence (XRF) Spectroscopy

✦ Each element shows a specific line pattern in a spectrum depending on the orbitals involved

- ✦ L→K transition =  $K\alpha$  line
- ✦ M→K transition =  $K\beta$  line
- ✦ M→L transition =  $L\alpha$  line
- ✦ N→L transition =  $L\beta$  line

✦ The higher the atomic number, the more "shells" (generally speaking)



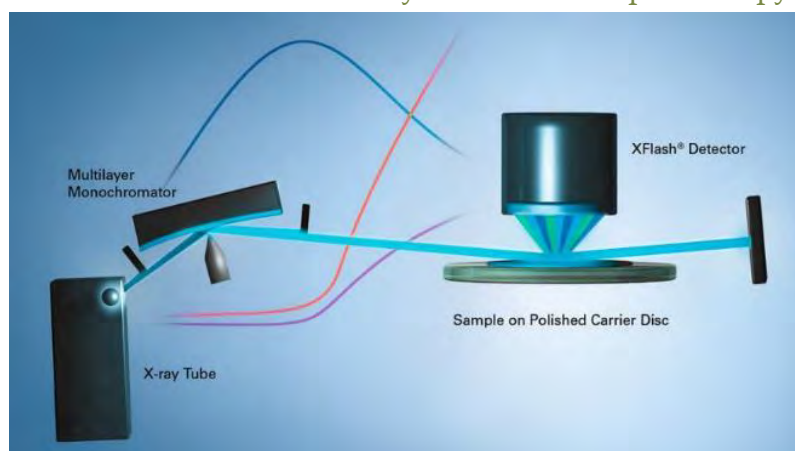
Each element has its own set of "fingerprints"

Courtesy of Bruker-AXS



## Quantitative Elemental Analysis

### TXRF - Total reflection X-ray Fluorescence Spectroscopy

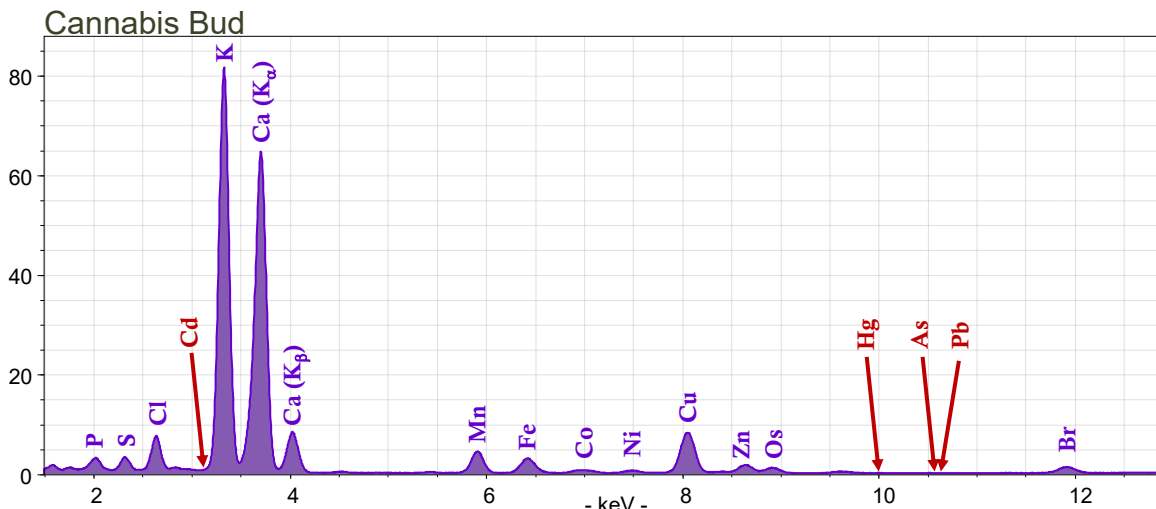


- Provides quantitative multi-element microanalysis
- Lower limits of detection in ppb range
- Meets USP requirements established for pharmaceutical products (USP 232/233)

Courtesy of Bruker-AXS



# Quantitative Elemental Analysis



# Quantitative Elemental Analysis

- + QC Testing for Soil, Fertilizers and Water During Cultivation
- + Better Understand Nutrient Uptake by Plants
- + Identify of Heavy Metal Contamination

**Hemp Extract Based Product  
– Sourced out of China**

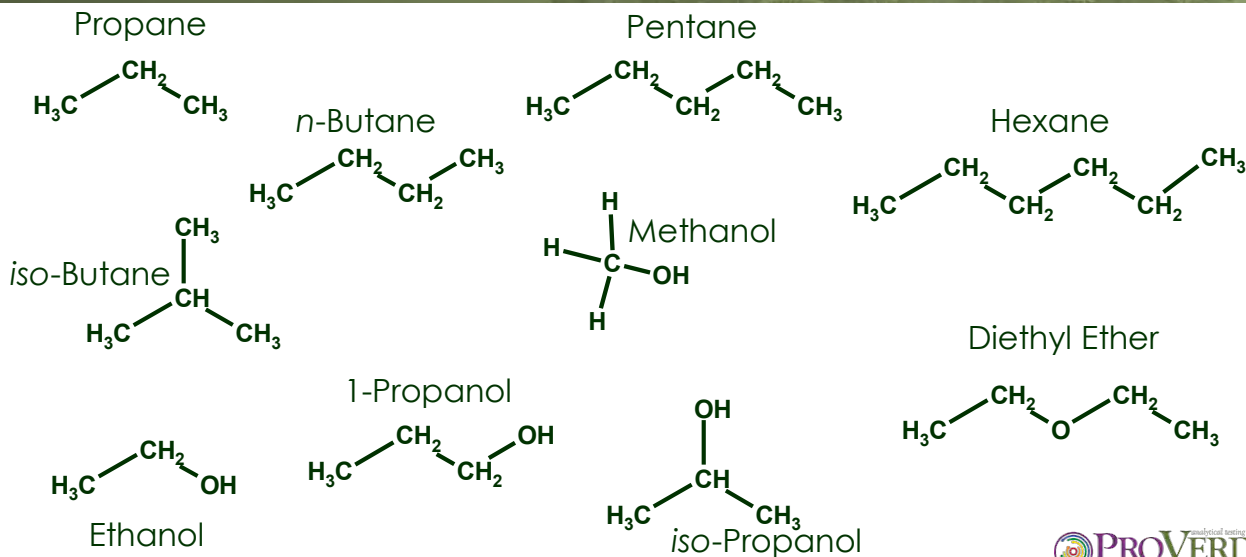
| Element             | USP Limits (ppm)* | Measured Conc.(ppm) | Limits of Detection (ppm) |
|---------------------|-------------------|---------------------|---------------------------|
| Cr (Chromium)       | 2.5               | ND                  | 0.013                     |
| Mn                  |                   | 0.191               | 0.011                     |
| Fe (Iron)           |                   | 0.169               | 0.009                     |
| Ni (Nickel)         | 0.15              | ND                  | 0.006                     |
| Cu (Copper)         | 10                | 0.123               | 0.005                     |
| Zn                  |                   | 0.31                | 0.005                     |
| Ga                  |                   | 5                   | 0.004                     |
| As (Arsenic)        | 0.15              | ND                  | 0.003                     |
| Rb                  |                   | 0.907               | 0.004                     |
| Sr                  |                   | 0.41                | 0.004                     |
| <b>Hg (Mercury)</b> | <b>0.15</b>       | <b>0.203</b>        | 0.005                     |
| Pb (Lead)           | 0.5               | ND                  | 0.004                     |

Mercury concentration higher than acceptable limits

\* Limits established for administration of pharmaceuticals by inhalation – United States Pharmacopeia USP 232/233



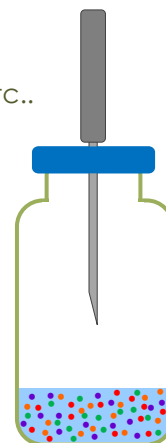
## Volatile Organic Compounds (VOCs)



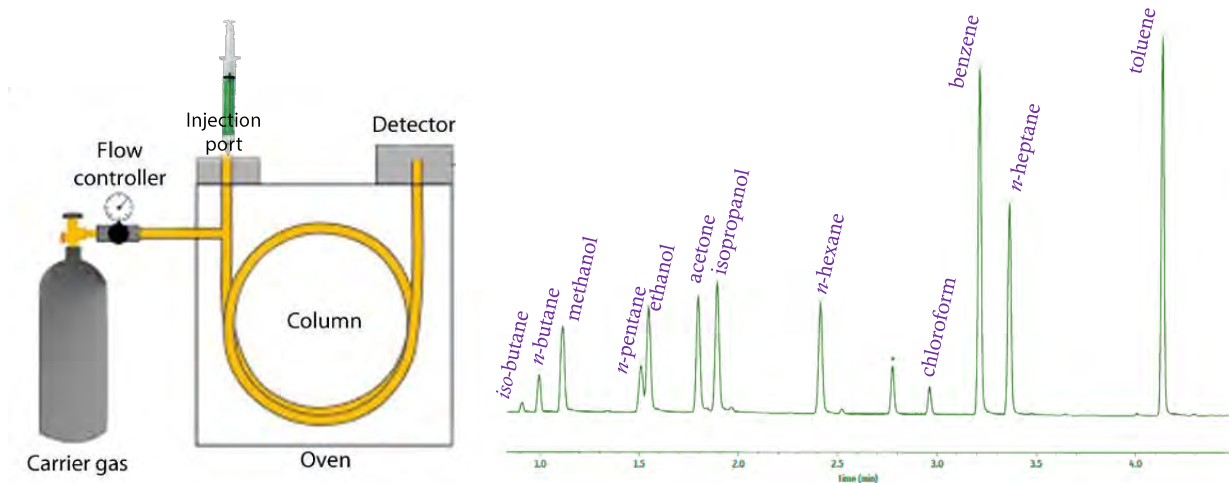
## Headspace Gas Chromatography

### ✦ Measurement of Volatile Organic Compounds (VOCs)

- ✦ Measure residual solvents from cannabis extractions
  - ✦ Includes common extraction solvents: butane, propane, ethanol, etc..
- ✦ Vial is heated to volatilize organic compounds
- ✦ Sample is collected from the headspace above the bulk
- ✦ Sample is injected for Gas Chromatographic (GC) analysis



## Headspace Gas Chromatography: VOCs

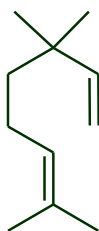


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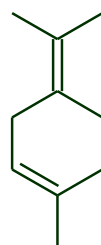
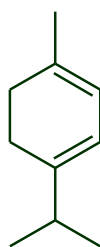


## Terpenes

Linalool

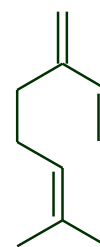


Terpinene

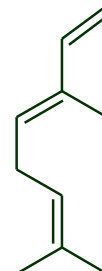


Terpinolene

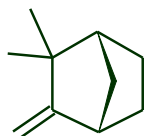
Myrcene



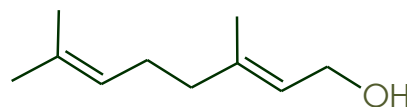
*trans*-Ocimene



$\alpha$ -Pinene



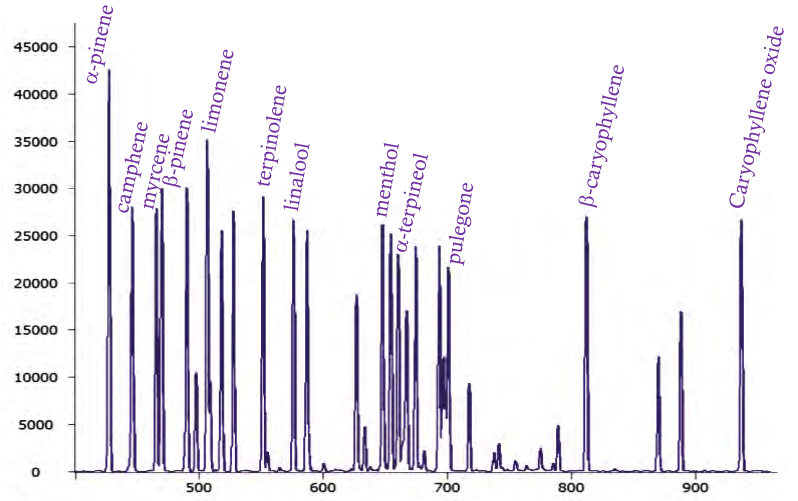
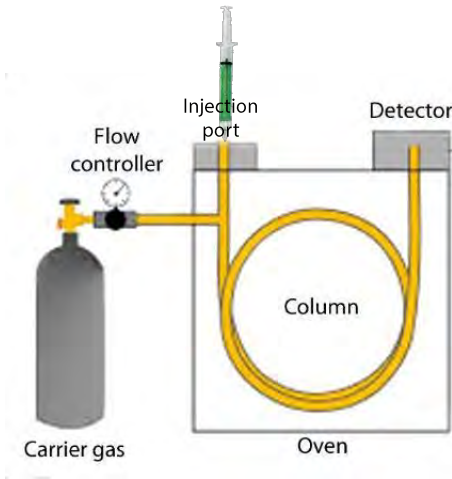
Camphene



Geraniol



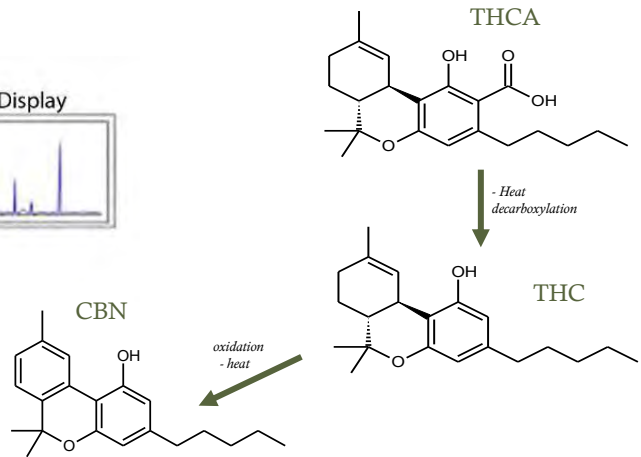
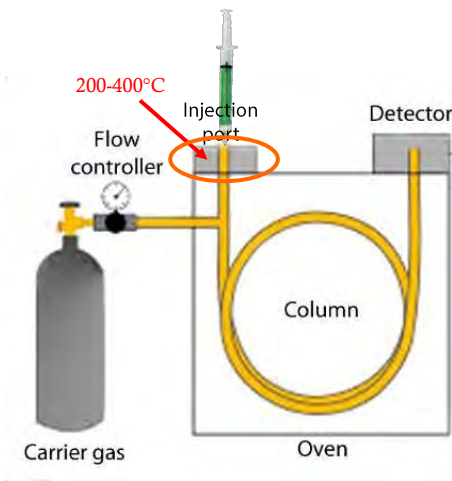
# Gas Chromatography: Terpenes



From Restek application note



# Gas Chromatography: Cannabinoids



## Liquid Chromatography



### ✦ High Performance Liquid Chromatography (HPLC)

- ✦ Maintains the quantitative information of the acid and neutral cannabinoids



### ✦ UltraPerformance Liquid Chromatography (UPLC)

- ✦ Ultra High Pressure Liquid Chromatography (UHPLC)
- ✦ Faster and more efficient than HPLC

Both methodologies use organic solvents to achieve the separation and quantitation of analytes



## Convergence Chromatography



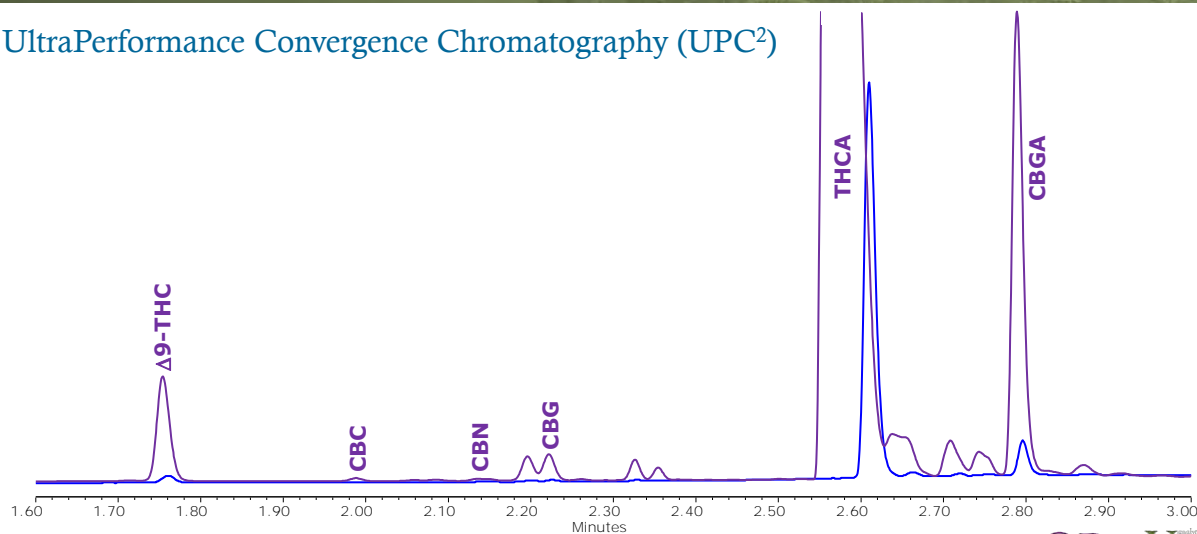
- ✦ UltraPerformance Convergence Chromatography System (UPC<sup>2</sup>)
- ✦ Based on the theory of Supercritical Fluid Chromatography (SFC)
  - ✦ Uses liquid CO<sub>2</sub> as the primary mobile phase
- ✦ Reduces the hazardous waste generated relative to conventional liquid chromatography
- ✦ Captures quantitative information on both acid and neutral (decarboxylated) form of the cannabinoids
- ✦ Amenable to non-polar solvents, ideal for analysis of analytes in lipid-rich matrices





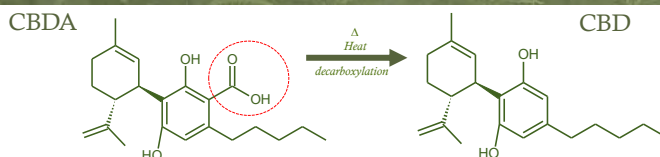
# Analysis of Cannabis Flower

UltraPerformance Convergence Chromatography (UPC<sup>2</sup>)

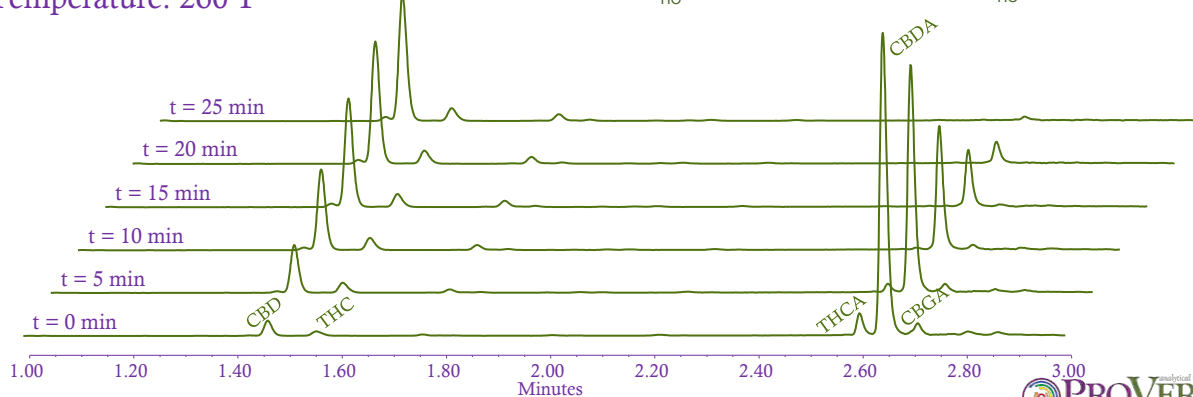


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# Decarboxylation

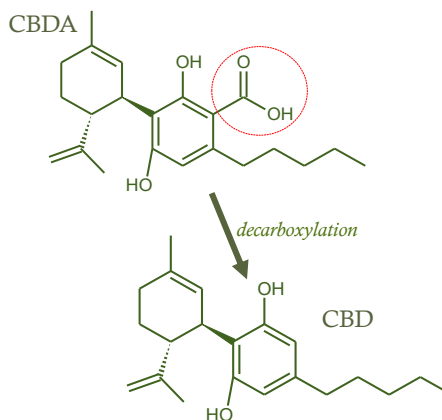
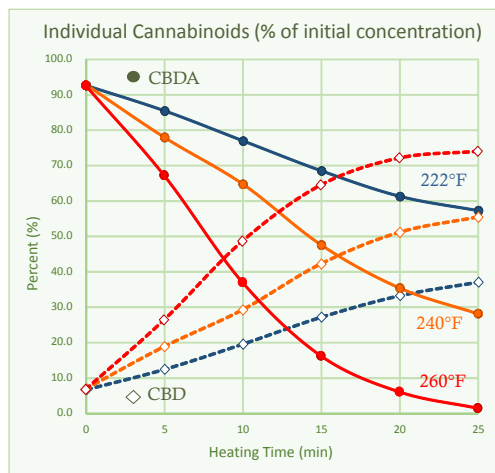


Temperature: 260°F



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## Decarboxylation



## Audience Poll

Which United States President was NOT a hemp farmer?

- George Washington
- Thomas Jefferson
- James Buchanan
- James Madison



## Marijuana Infused Products

### ✦ Marijuana Infused Products (MIPs)

- ✦ Becoming increasingly important to the industry
- ✦ Provides delivery formats for patients that do not want to smoke cannabis
- ✦ Some states allow, by regulation, only derivative products to be available



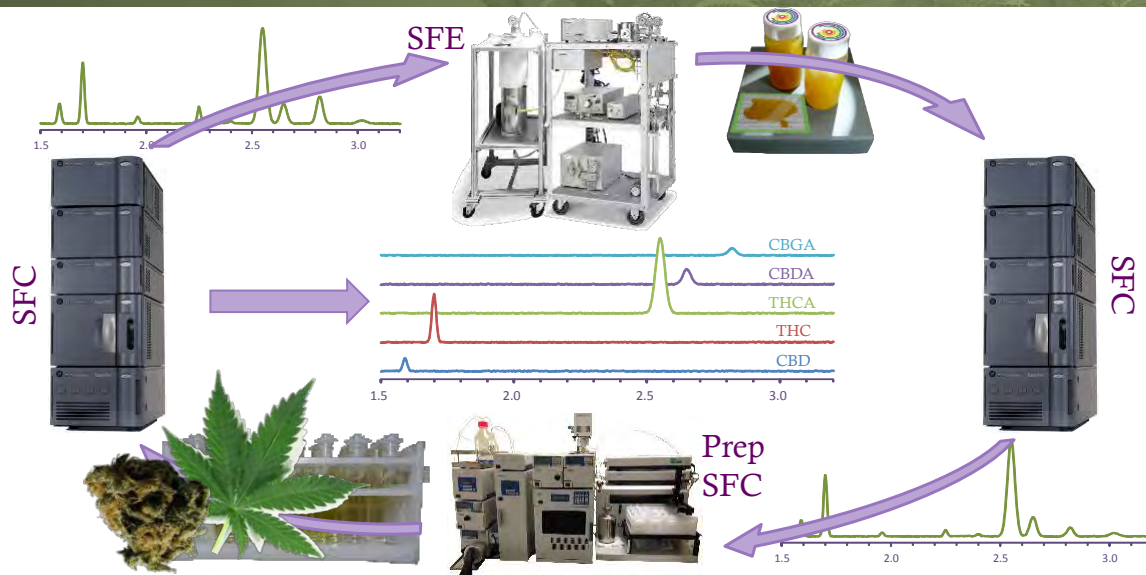
## Cannabis Extractions

### ✦ Supercritical Fluid Extractions (SFE) using liquid CO<sub>2</sub>

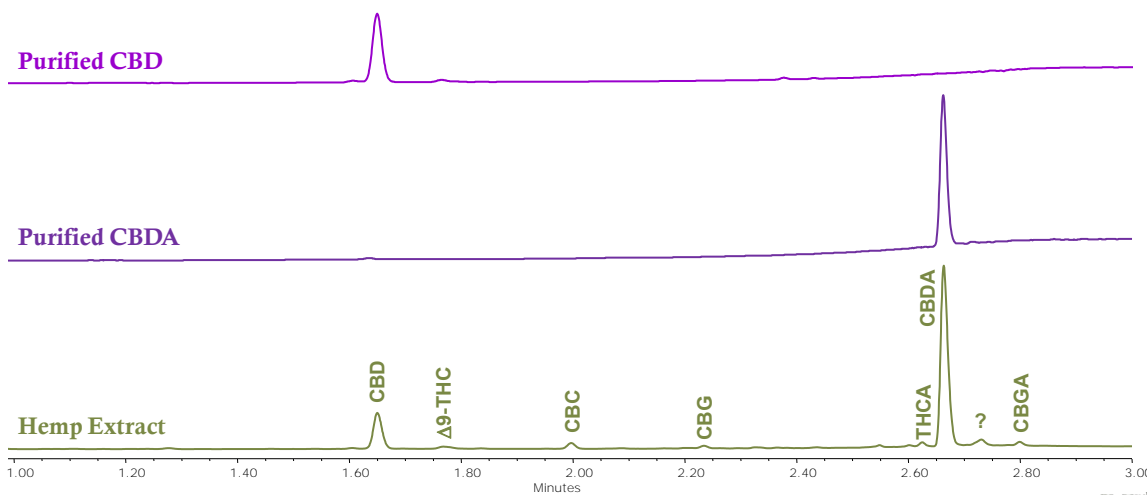
- ✦ Safest Extraction Option: Non-toxic, non-flammable, environmentally neutral
- ✦ Maintains terpene-rich extract profile
- ✦ Extract can be used as the basis for many products



# SFX Work Flow



# Purification of Individual Cannabinoids



## Summary

- ✦ Analytical chemistry will play a key role in the expanding cannabis industry, with a primary focus on ensuring patient/consumer safety
- ✦ The complexity of cannabis, as a natural product, with the potential for a variety of contaminants provides a number of analytical challenges that requires multiple analytical techniques to understand more fully
- ✦ The current trends for increased acceptance of cannabis as a legitimate industry provides the opportunity for the application of current analytical technologies to address these challenges



## References for Additional Information

- ✦ ***Chemistry and Analysis of Phytocannabinoids and Other Cannabis Constituents***, Rudolf Brenneisen, Marijuana and the Cannabinoids (Chapter 2), **2007**, pp 17-49, ISBN 978-1-58829-456-2.
- ✦ ***Taming THC: Potential Cannabis Synergy and Phytocannabinoid-Terpenoid Entourage Effects***, Ethan Russo, British Journal of Pharmacology, 2011, **163**, 1344-1364.
- ✦ ***Non-Psychotropic Plant Cannabinoids: New Therapeutic Opportunities from an Ancient Herb***, Angelo Izzo, et al., Trends in Pharmacological Sciences, **2009**, 30(10), 515-527.
- ✦ ***Naturally Occurring and Related Synthetic Cannabinoids and their Potential Therapeutic Applications***, Mahmoud Elsohly, et al., Recent Patents on CNS Drug Discovery, **2009**, 4, 112-136.
- ✦ ***A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates***, Corby Hilliard, et al., Restek Application Note, [www.restek.com](http://www.restek.com).





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## Cannabis Chemistry 201



**Dr. Jeff Kiplinger**  
President and Founder,  
Averca Discovery Services

**Dr. Christopher Hudalla**  
Chief Scientific Officer,  
ProVerde Laboratories

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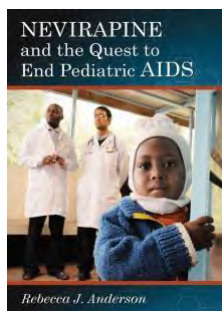


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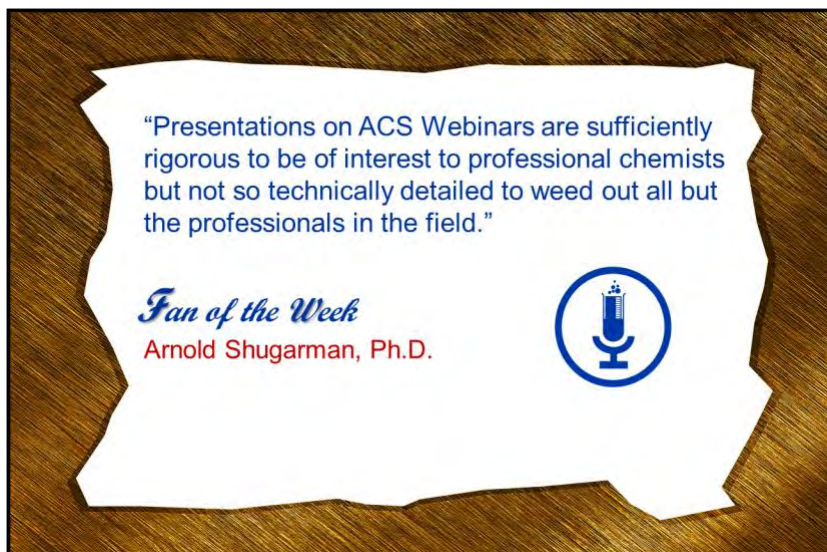
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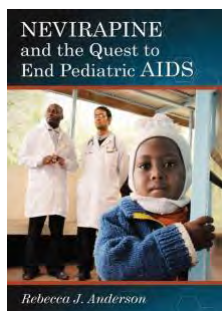


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