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Module 3

Testing Methodologies

Dr. Ariane Vasilatis

- **Components of a testing lab**
- **Cannabinoid and terpene testing**

Rutgers Hemp Extension Lab

Hemp analytics lab

- Farm bill passed December 20, 2018
- Took a year for Rutgers to apply, and receive permits to grow and handle hemp
- March 2020, plants were in the ground and the analytical lab was underway
- 5 people: 3 students, 2 advisors, and a whole lot of questions

Left to Right: Dr. Qing-Li Wu (Director), Anthony Lockhart (Ph.D. Student), Dr. James Simon(Director), Ariane Vasilatis (Ph.D. Candidate, ABD), Harna Patel (Ph.D. Candidate)

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Lab – Personnel

- Processing
  - Fresh samples→ dried
  - Dried samples → homogenized
- Analysis
  - Extraction of cannabinoids
  - Identification – which cannabinoids?
  - Quantification – how much?
- Client relations
  - Report output, what does your client want?
  - Scheduling: when, and how, will your samples arrive?

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Lab – Equipment

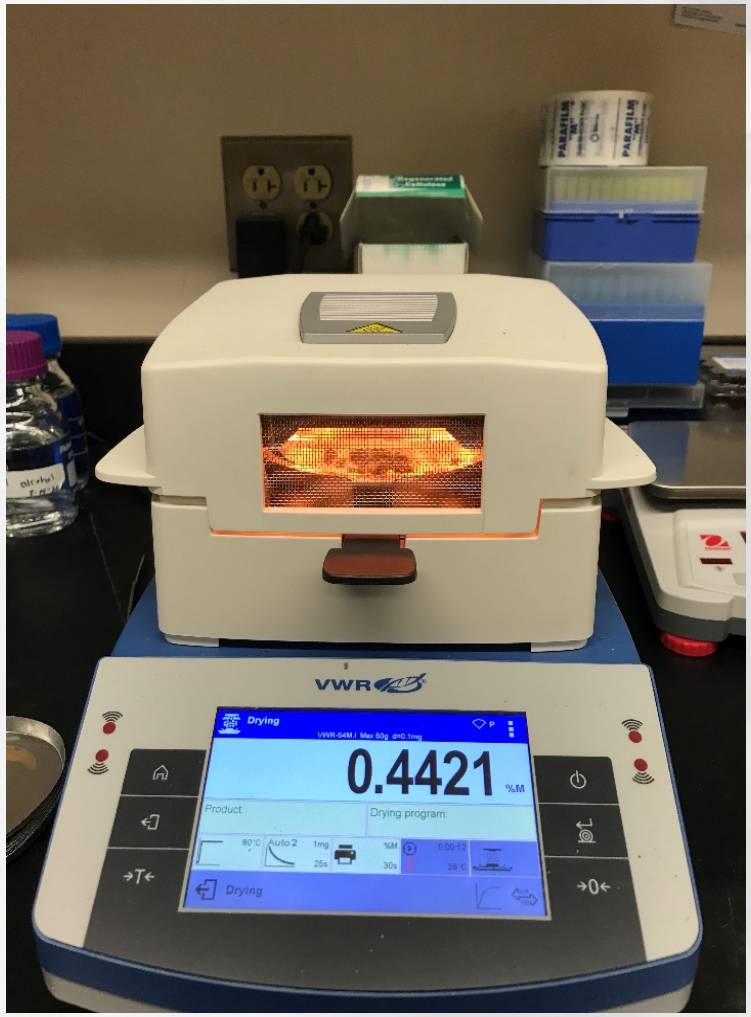
- Equipment
- Personnel
- Methods of analysis

Binder Dryer

- Hemp samples come to us fresh from the plot

Moisture Analyzer

- Hemp needs to have between **5-12% moisture** after drying for accurate analysis



VWR Moisture Analyzer

Binder Oven: Model FDS-15

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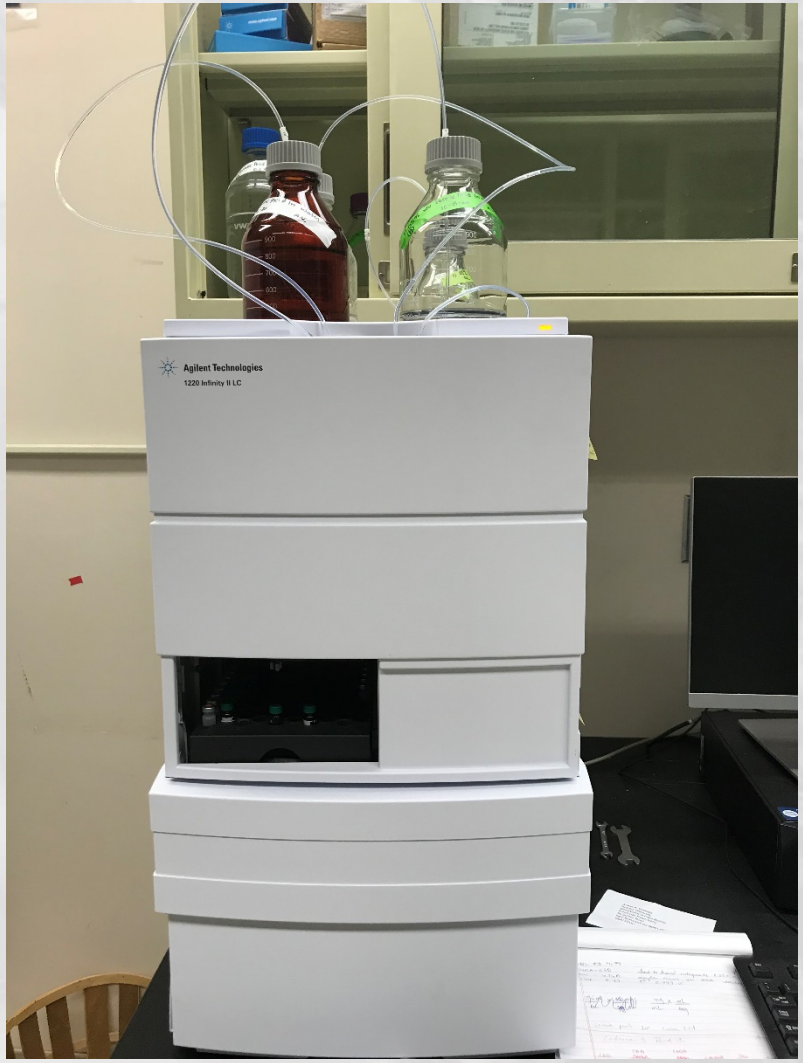
Lab – Equipment

High Performance Liquid Chromatograph with Diode Array Detector (HPLC-DAD)

Identification and quantitation of cannabinoids

Can also be used for terpene analysis but there are some drawbacks

- Co-elution
- Weak signal for DAD



Agilent 1220 Infinity II LC- DAD

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Lab – Equipment

Grinder

- Aids in creating homogenous samples
- High surface area samples yield the most effective extractions of compounds

Geno Grinder 2010

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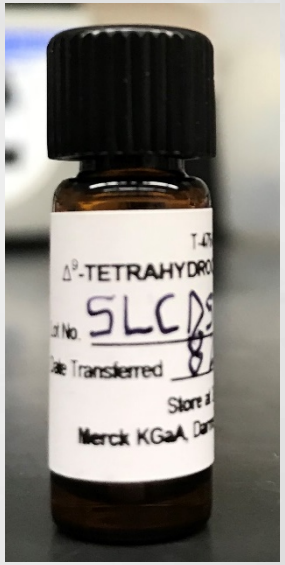
Lab – Equipment

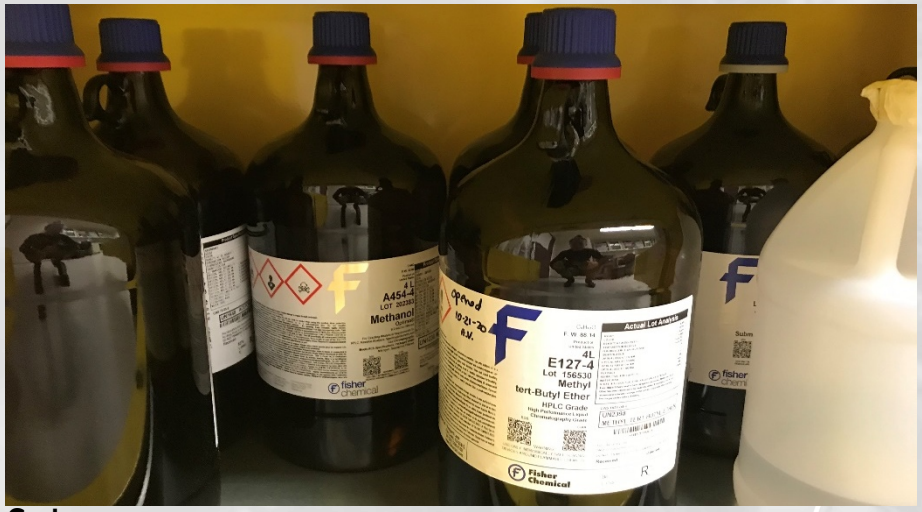
Consumables

- Solvents
  - HPLC
  - Extraction
- Standards- Cannabinoids
  - Quantify
  - Identify
- Vials/Caps
  - House samples for analysis



THC Standard

Caps, Pipette Tips, Vials



Solvents

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Lab – Methods of Analysis

Analyses need to be accurate and reproducible

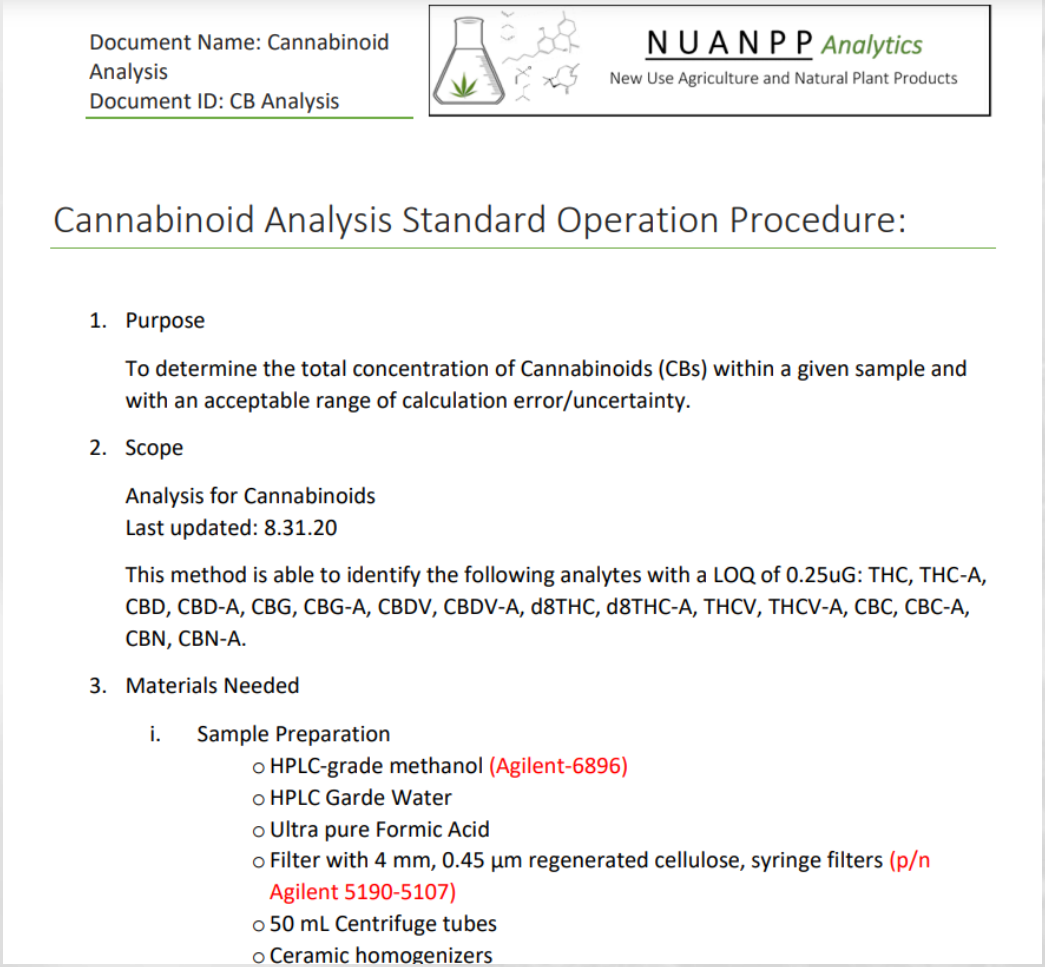
- A more elaborate manual

Standard operating procedures (SOPs)

- Maintenance of instruments
- How to process and extract samples

Analytical methods

- How to quantify and identify compounds



Document Name: Cannabinoid Analysis
Document ID: CB Analysis

NUANPP *Analytics*
New Use Agriculture and Natural Plant Products

Cannabinoid Analysis Standard Operation Procedure:

1. Purpose

   To determine the total concentration of Cannabinoids (CBs) within a given sample and with an acceptable range of calculation error/uncertainty.

2. Scope

   Analysis for Cannabinoids
   Last updated: 8.31.20

   This method is able to identify the following analytes with a LOQ of 0.25uG: THC, THC-A, CBD, CBD-A, CBG, CBG-A, CBDV, CBDV-A, d8THC, d8THC-A, THCV, THCV-A, CBC, CBC-A, CBN, CBN-A.

3. Materials Needed

   i. Sample Preparation
      - HPLC-grade methanol (Agilent-6896)
      - HPLC Garde Water
      - Ultra pure Formic Acid
      - Filter with 4 mm, 0.45 µm regenerated cellulose, syringe filters (p/n Agilent 5190-5107)
      - 50 mL Centrifuge tubes
      - Ceramic homogenizers

SOP for Cannabinoid Analysis from NUANPP Analytics

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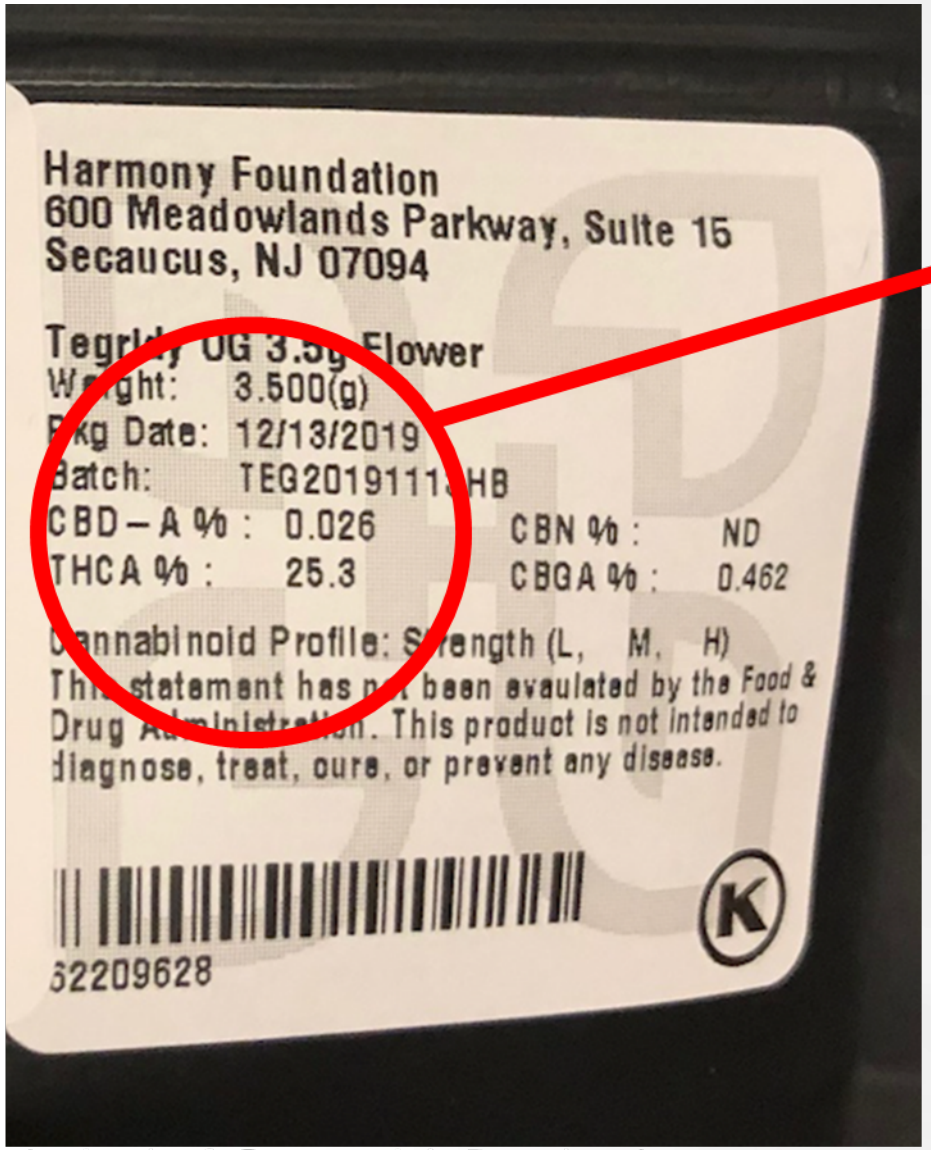
What We Offer

- Rutgers Hemp Analytics lab offers cannabinoid and terpenes analysis
- We can identify and quantify 16 cannabinoids: CBD, CBD-A, d9THC, d9THC-A, d8THC, CBN, CBN-A, CBG, CBG-A, THCV, THCV-A, CBDV, CBDV-A, CBC, CBC-A, CBL.
- We are able to quantify an unlimited variety of terpenes, and other volatile organic compounds (VOCs) by way of Mass Spectrometry.
- In the future, we are looking to include pesticide and mycotoxin screening as well.
- Chemical analysis is not easily translated, pesticide residue (exterior), plant metabolites (interior).

What Goes Into A Label?



Label of Cannabis Product

What do you see?

- CBD-A - Cannabidiolic Acid
- THC-C - Delta 9-Tetrahydrocannabinolic Acid
- CBG-A - Cannabigerolic Acid

% Refers to dry weight of product

- 25.3% of 3.5 g is 88mg of THC-A

Obtaining cannabinoid concentration requires the initial dry weight of sample prior to extraction

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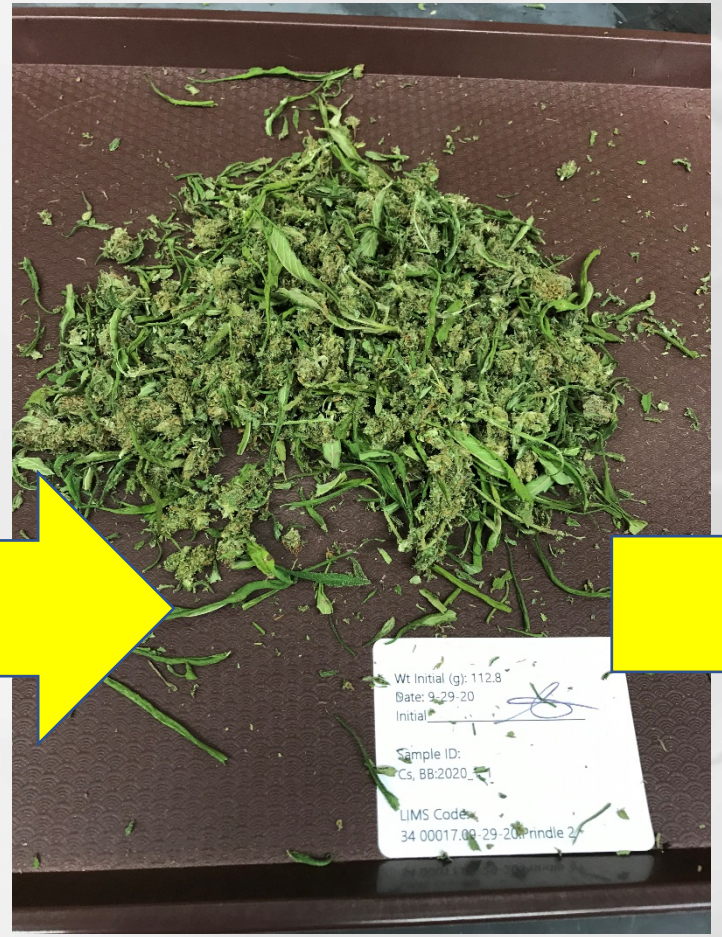
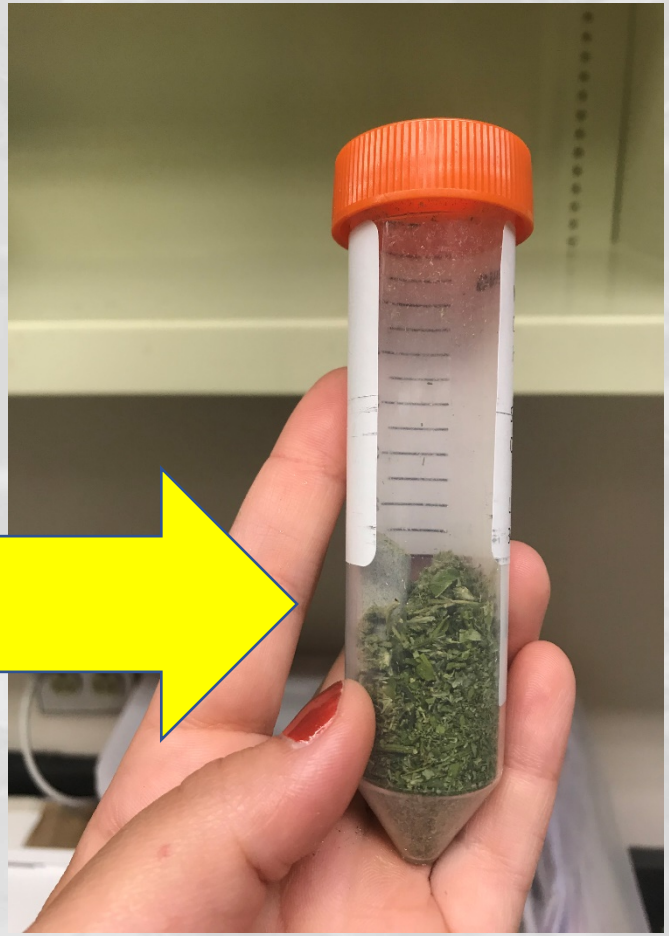
Processing for a Homogenous Sample

A **homogenous** sample is one that is uniform in texture and contents

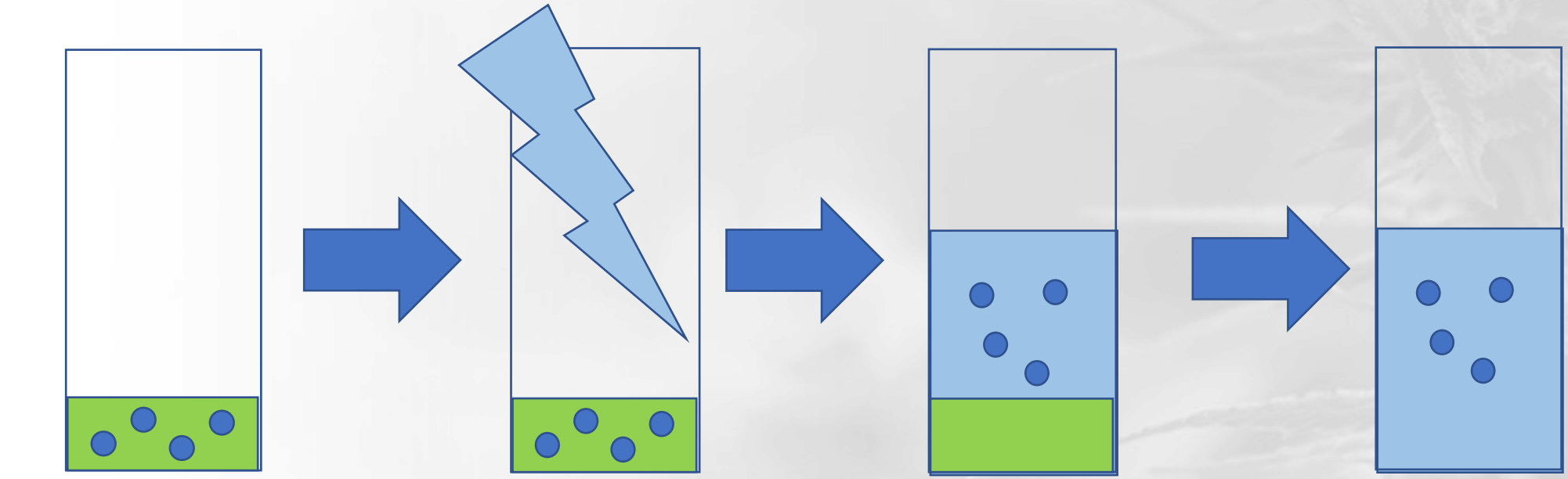
USDA Guidelines - Key is to get a **representative** sample of a plot, field, or cultivar*





Extraction

- Further pulverizing samples make for a better extraction
- Extraction of cannabinoids from hemp is usually completed using **methanol**
  - Methanol is a polar solvent that and can extract a wide array of compounds
  - Cannabinoids are special because they have polar and non-polar regions



Homogenized sample

Addition of methanol

Methanol extracting Cannabinoids from sample

Removal of sample, to generate extract

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Chromatography

The separation of compounds from a mixture by passing it through a medium that moves the components at different rates based on their chemical characteristics (polarity, size).

1) Cannabinoid Analysis –
   - High Pressure Liquid Chromatography
   - Utilizes solvents as its mobile phase and
   - A selective column as its stationary phase

2) Terpene Analysis –
   - Gas Chromatography
   - Utilizes gas (Helium) as its mobile phase
   - A heated selective column as its stationary phase

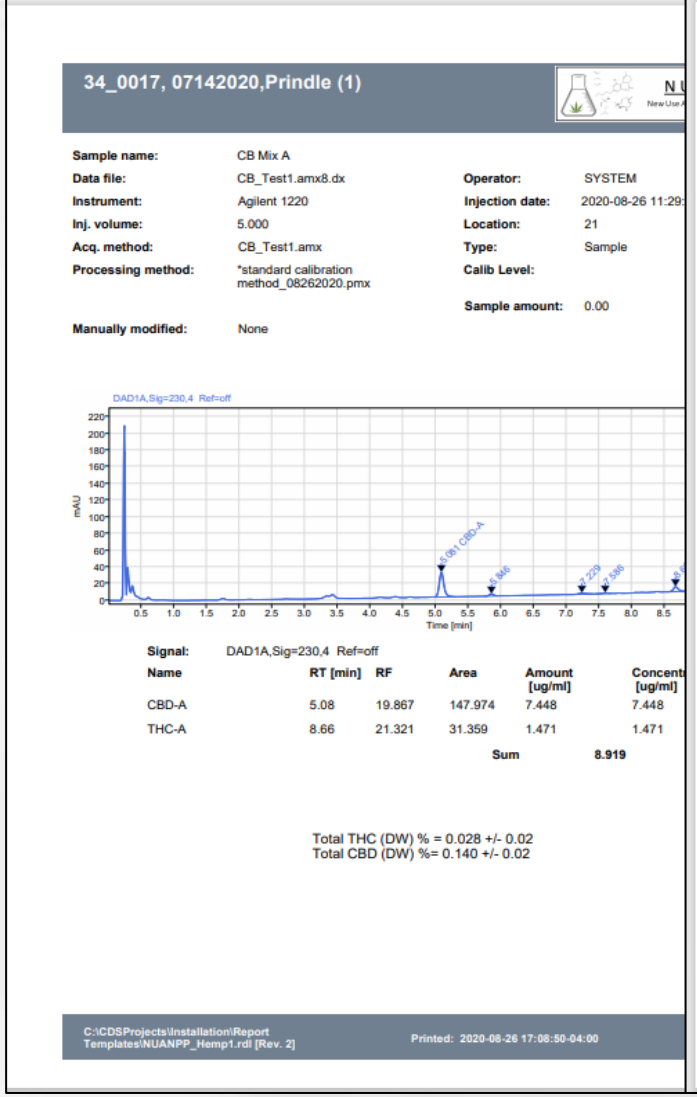
Obtaining Cannabinoid Concentration

1. Requires initial dry weight of sample prior to extraction.
2. Standard curve of pure cannabinoids at known concentrations.
3. Total cannabinoid equations which consider the free and acidified form of the compound.

Total THC = THC + 0.877*THC-A

Total CBG = CBG + 0.878*CBG-A

Generates the %THC of dry weight, as shown on label



34_0017, 07142020,Prindle (1)

Sample name:	CB Mix A		
Data file:	CB_Test1.amx8.dx	Operator:	SYSTEM
Instrument:	Agilent 1220	Injection date:	2020-08-26 11:29:
Inj. volume:	5.000	Location:	21
Acq. method:	CB_Test1.amx	Type:	Sample
Processing method:	*standard calibration method_08262020.pmx	Calib Level:	
		Sample amount:	0.00
Manually modified:	None		

DAD1A,Sig=230,4 Ref=off

mAU

Time [min]

Signal: DAD1A,Sig=230,4 Ref=off

Name	RT [min]	RF	Area	Amount [ug/ml]	Concent [ug/ml]
CBD-A	5.08	19.867	147.974	7.448	7.448
THC-A	8.66	21.321	31.359	1.471	1.471
			Sum	8.919	

Total THC (DW) % = 0.028 +/- 0.02
Total CBD (DW) %= 0.140 +/- 0.02

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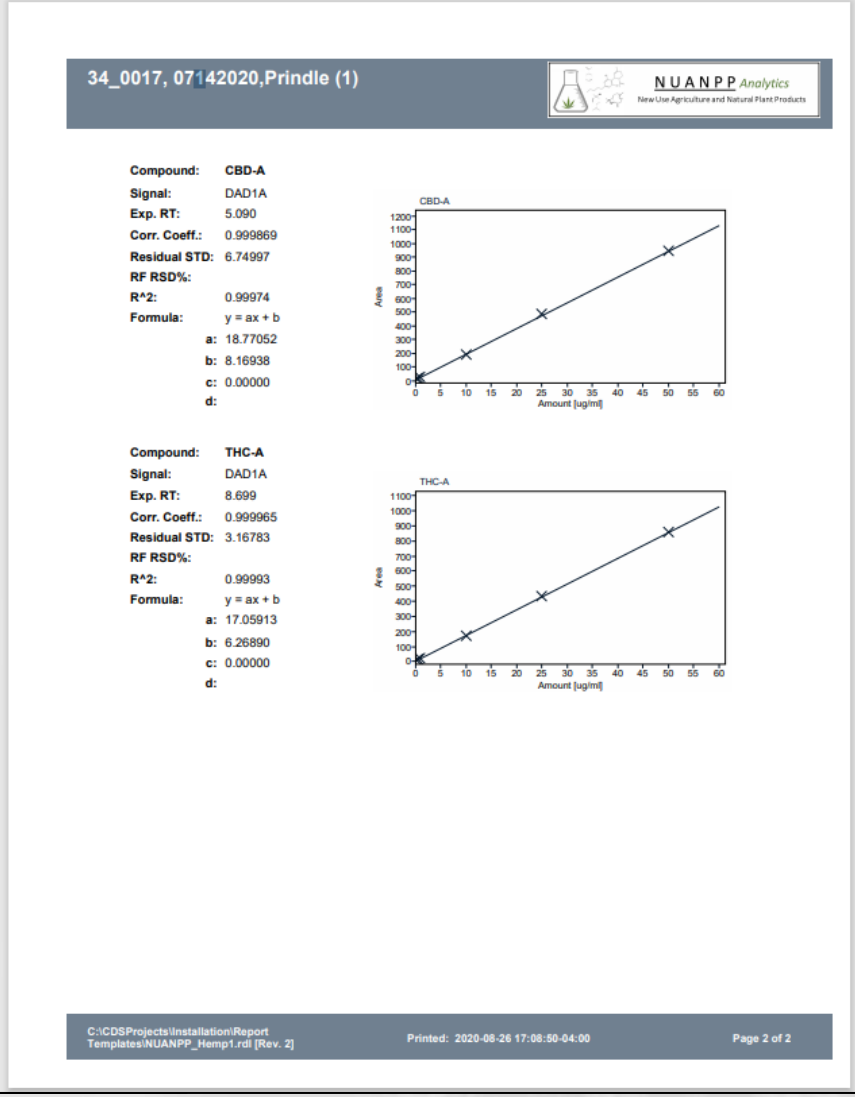

34_0017, 07142020,Prindle (1)

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Compound:	CBD-A
Signal:	DAD1A
Exp. RT:	5.090
Corr. Coeff.:	0.999869
Residual STD:	6.74997
RF RSD%:	
R^2:	0.99974
Formula:	y = ax + b
a:	18.77052
b:	8.16938
c:	0.00000
d:	

CBD-A

Area

Amount [ug/ml]

Compound:	THC-A
Signal:	DAD1A
Exp. RT:	8.699
Corr. Coeff.:	0.999965
Residual STD:	3.16783
RF RSD%:	
R^2:	0.99993
Formula:	y = ax + b
a:	17.05913
b:	6.26890
c:	0.00000
d:	

THC-A

Area

Amount [ug/ml]

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- *Known concentrations varying from low to high to generate a linear curve*
- *Use standard curve to generate an association between peak area and concentration.*
- *Utilize standards of known concentrations to generate a coefficient we use to calculate concentrations in samples.*

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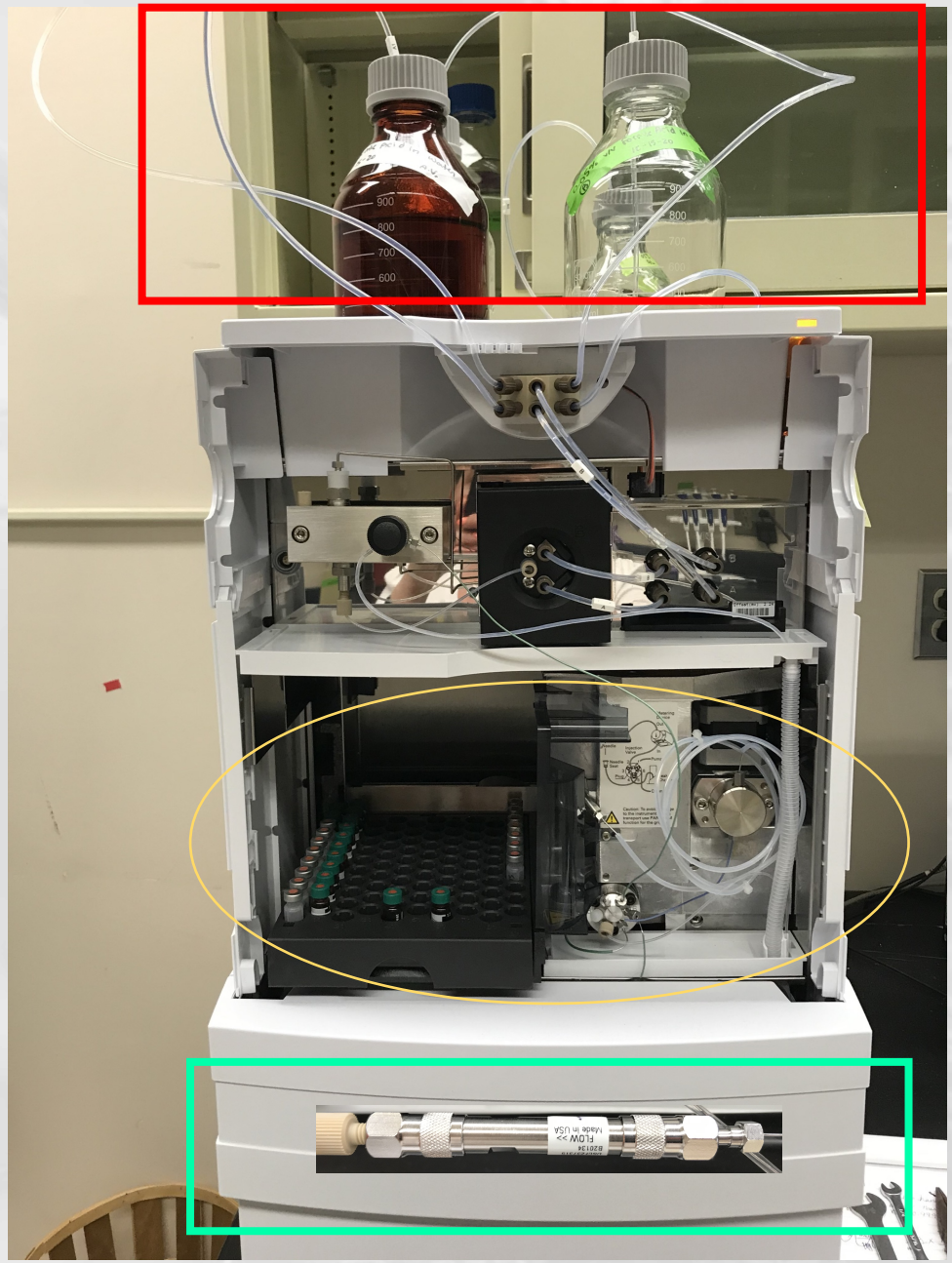
Definitions and HPLC parts

- **Mobile Phase:** Interacts with molecules of interest from an extract so that they are sufficiently dissolved and carried through instrument to the stationary phase.
- **Stationary Phase:** A tube packed with selective chemical compounds (typically porous) that interact with the compounds of interest, chemically, to separate them from on another (polarity, weight).
- **Autosampler:** Motor and syringe of an instrument that takes up the extract of a sample and runs it through the machine to identify and quantify.
- **Diode Array Detector (DAD):** Sample is passed through light of known wavelength (nm) and a measurement the absorbance of the light by the sample is recorded. Wavelength is determined by analyte's chemical composition and can vary. Can measure multiple wavelengths at a time.
- **Chromatogram:** Out put of compound absorbances of UV light from DAD .



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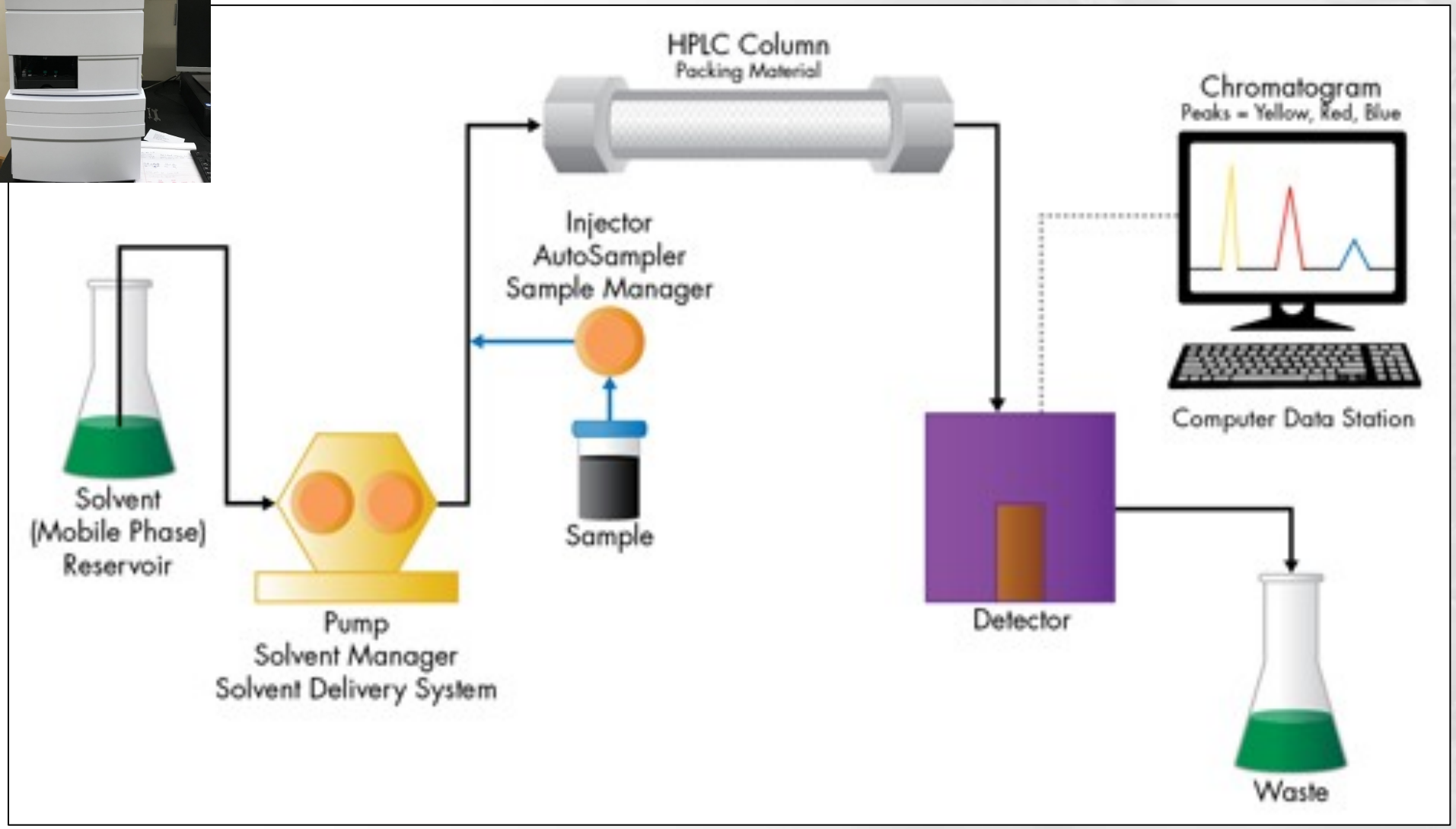
Flow of HPLC System



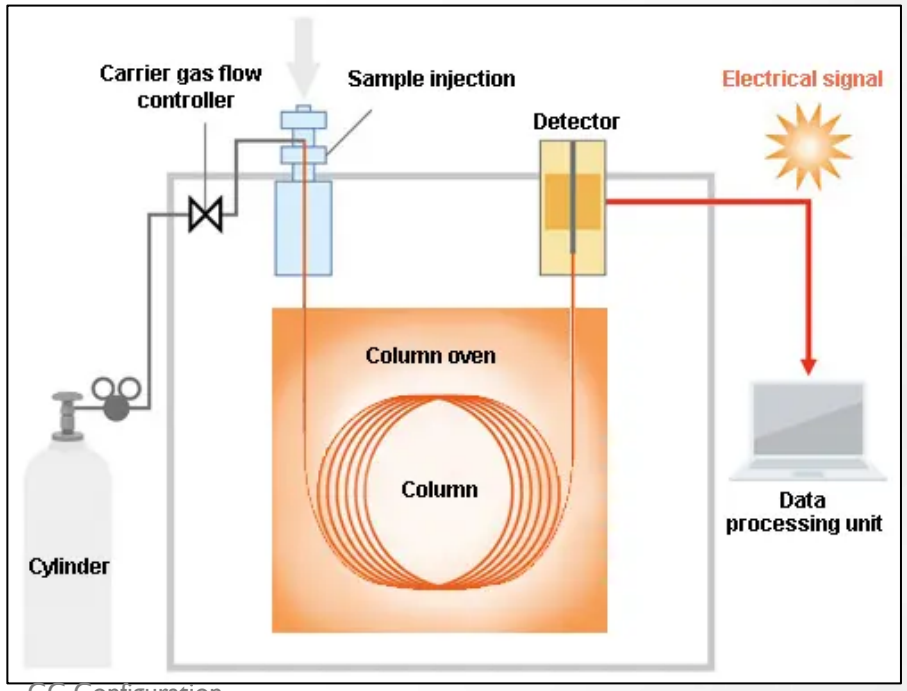
- Extracts are diluted then analyzed using an HPLC
- HPLC utilizes the chemical composition of its column and solvent stream to separate compounds out of the extract
- We see peaks using a Diode Array Detector which puts out a chromatogram
- Uses specific light spectrum to excite molecules within cannabinoid structure (280 nm)

Representation of an HPLC system https://www.waters.com/waters/en_GB/Chromatographic-Bands%2C-Peaks-and-Band-Spreading/nav.htm?locale=en_GB&cid=134803614

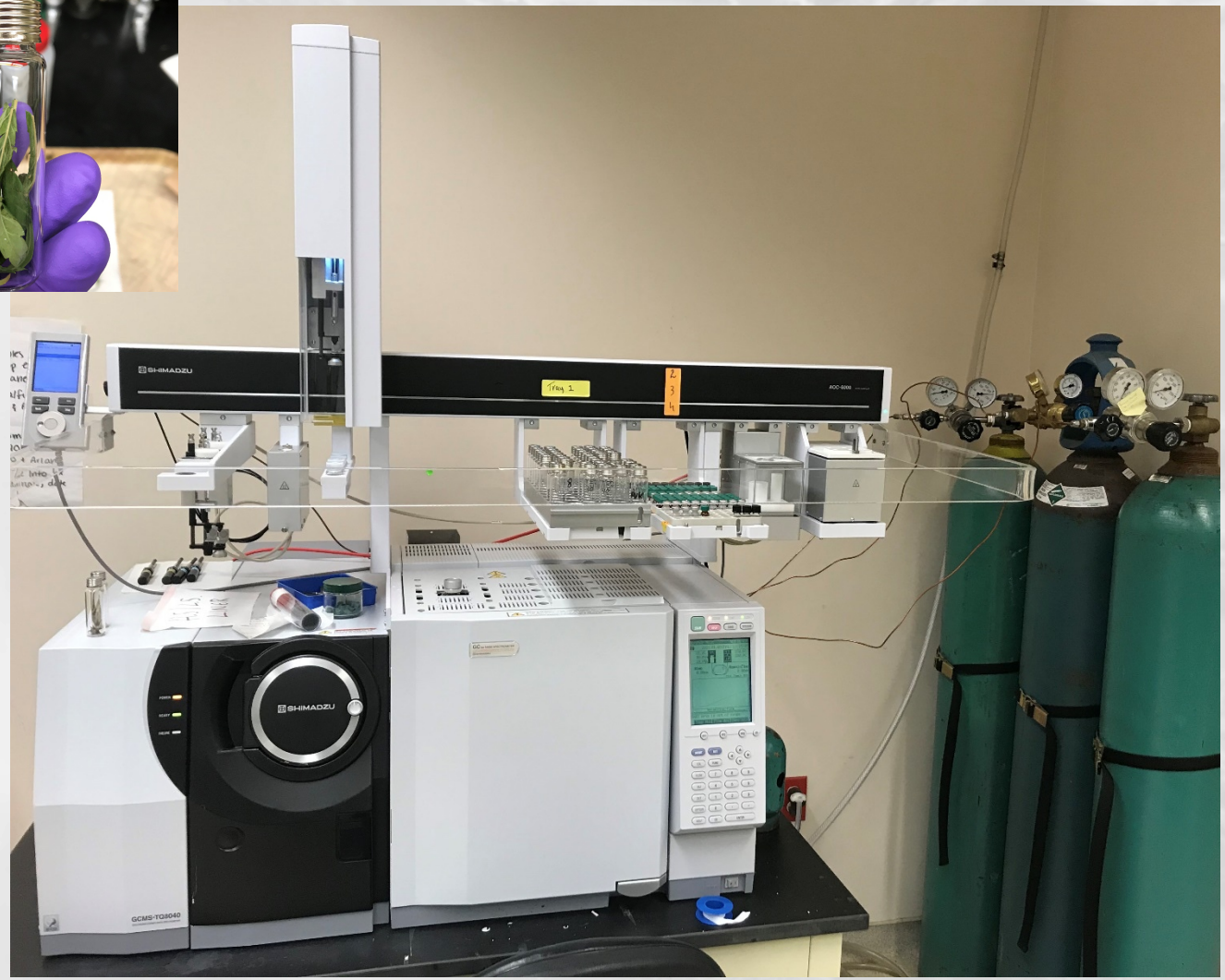
Terpene Analysis: Gas Chromatogrpahy

Common analysis method is Gas Chromatography - GC.

GCs are preferred instruments for terpene analysis because terpenes are volatile compounds that readily vaporize.



GC Configuration
https://www.ssi.shimadzu.com/products/gas-chromatography/fundamental-guide-to-gas-chromatography/what-is-gas-chromatography.html



Shimadzu GC 2010 Plus Gas Chromatograph with TQ8040 Mass Spectrometer

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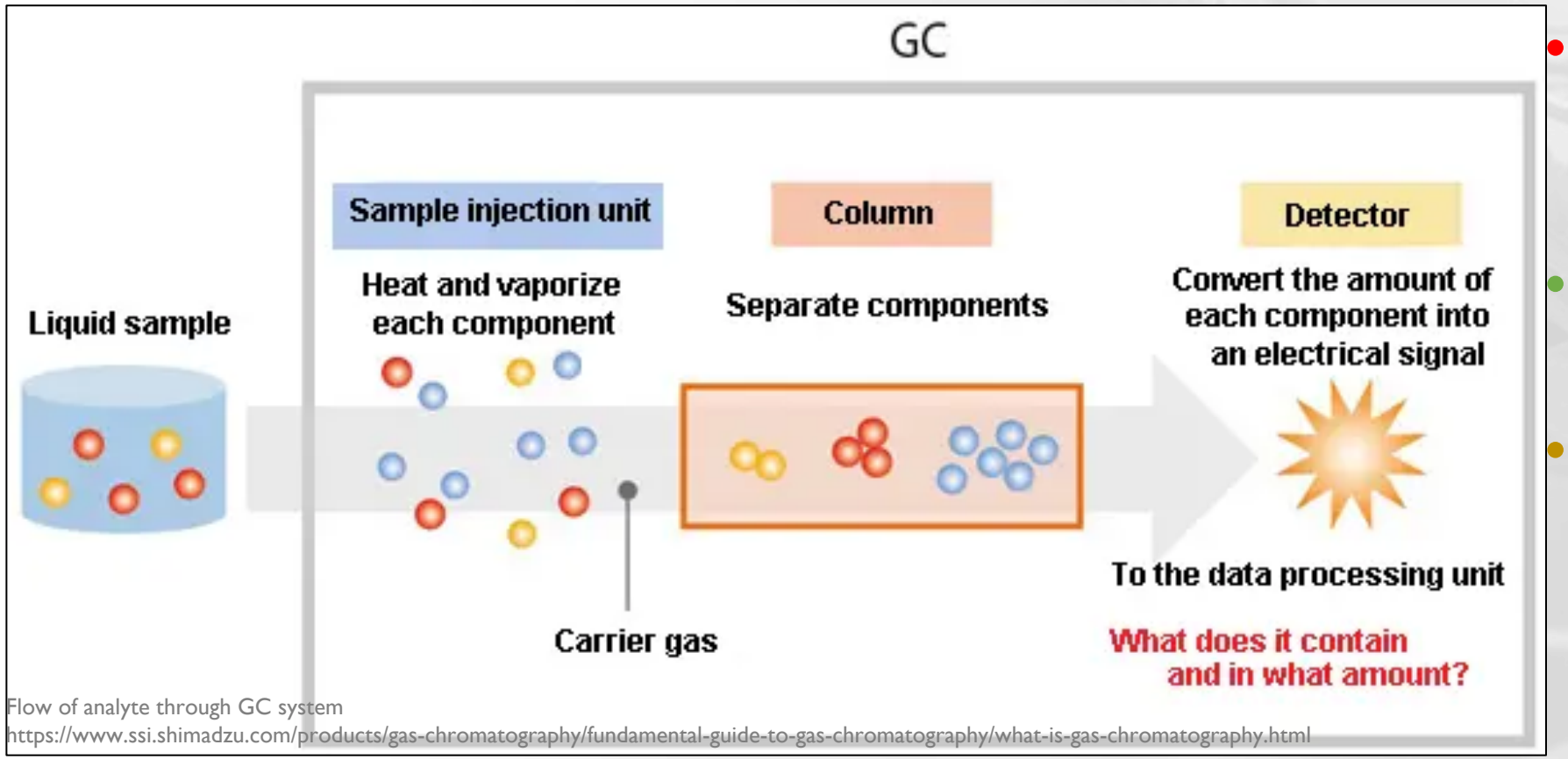
Definitions and Components of GC

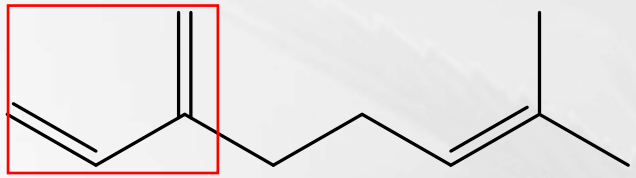


Flow of analyte through GC system
https://www.ssi.shimadzu.com/products/gas-chromatography/fundamental-guide-to-gas-chromatography/what-is-gas-chromatography.html

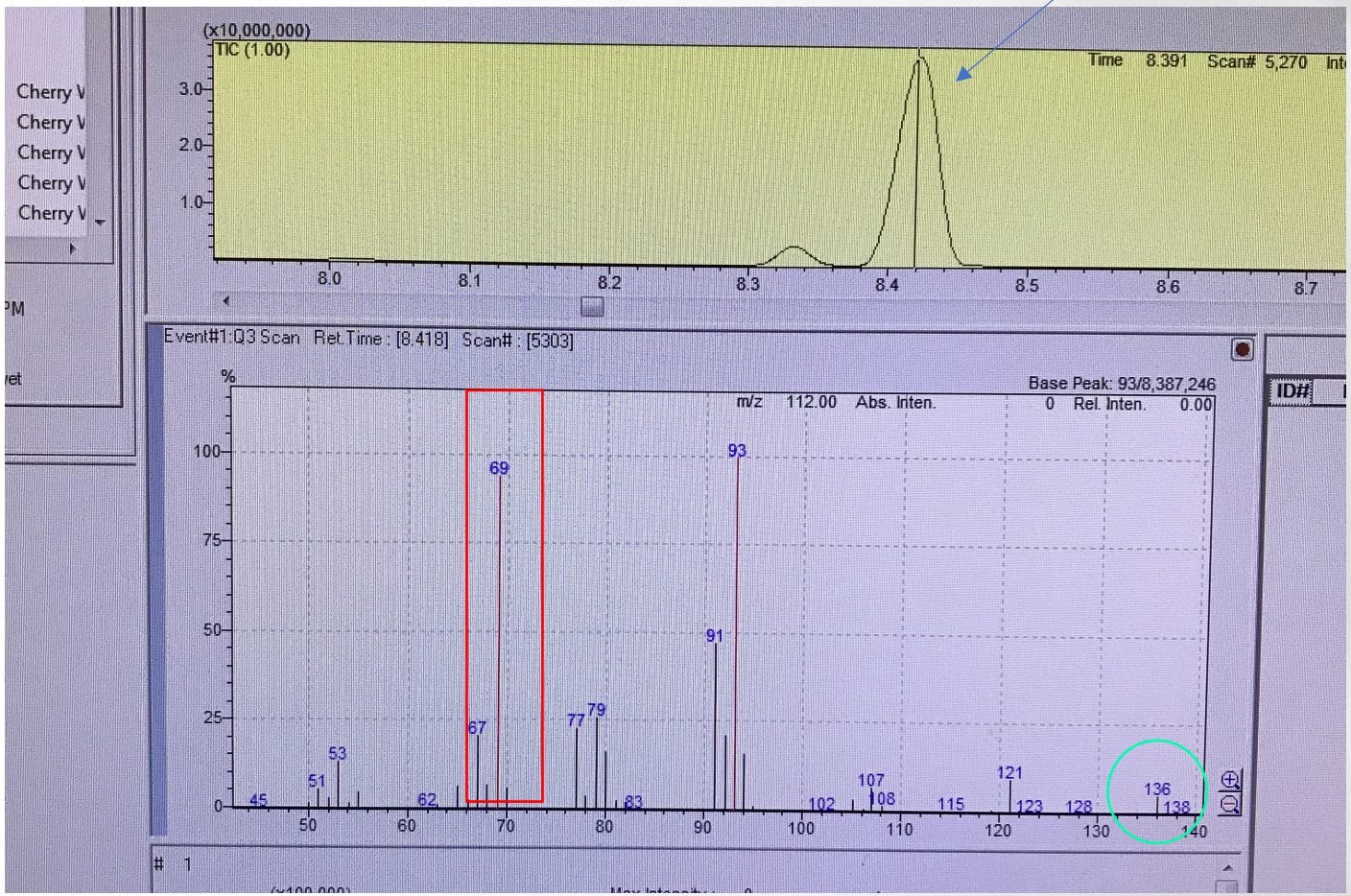
- Gas Chromatography (GC) utilizes a heated column and a flowing gas to separate compounds.
- Use a Mass Spectrometer to identify compounds which outputs a chromatogram.

- **Mobile Phase/Carrier Gas**
  - Helium Gas
- **Stationary Phase**
  - Silica based column
- **Detector** (Mass Spectrometry)
  - Detects fragments of compounds after bombardment with electric energy

Mass Spectra



beta-Myrcene



- As the compound is bombarded by electrical energy it generates ions.
- These ions/fragments are passed trough a magnetic field and sorted based on mass to charge ratio (m/z).
- Height is related to stability of peak.
- Ex: m/z 69 equates to the molecular weight of 4 carbons (16) and 7 hydrogens (1).

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Summary - Testing

- *Homogeneity of a sample will lead to a more effective extraction process and aids in accurate representation of compound concentration in samples.*

- *High performance liquid chromatography and gas chromatography are leading instruments in cannabinoid and terpene analysis.*

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Thank You!