

# Laboratory Analysis for the Differentiation between Marijuana and Hemp

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# Cannabis, Marijuana, Hemp

- Cannabis Sativa L
  - Aka: Marihuana, Marijuana, Hemp
- Green leafy plant with palmate leaves
- Contain various compounds (Cannabinoids) with pharmacological properties
- Grown for recreational, medical and industrial use
- Genetically engineered to target specific cannabinoid levels to suit target product needs
  - Higher THC/Lower CBD levels for recreational uses
  - Lower THC levels/Higher CBD levels for industrial (hemp) uses

# Cannabinoids

## Delta 9 THC

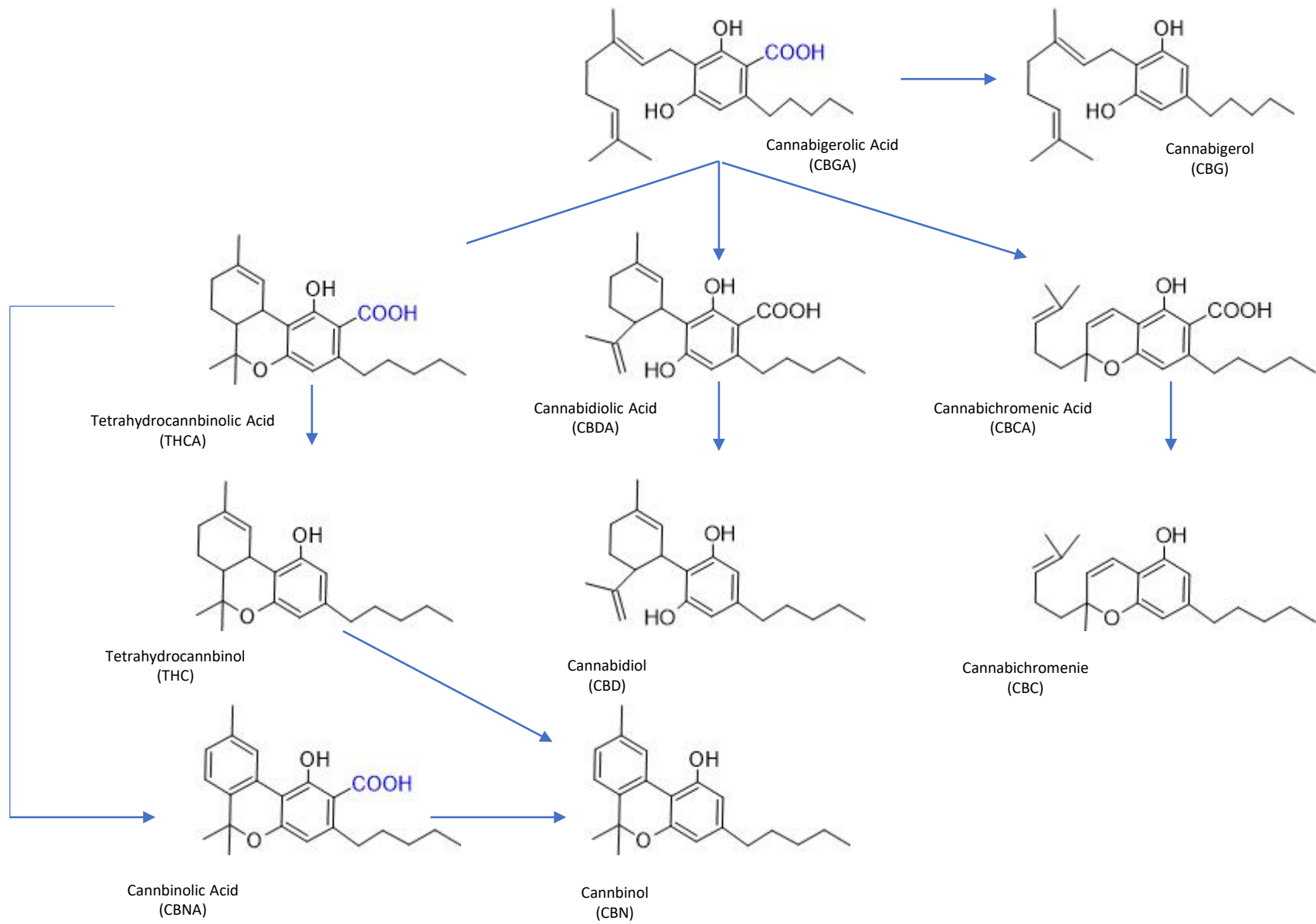
### (tetrahydrocannabinol)

- Psychoactive
- Primarily responsible for “high”
- Most abundant cannabinoid in recreational marijuana
- Can be extracted/concentrated for use in various products including vape oils, infused foods, etc.

## CBD

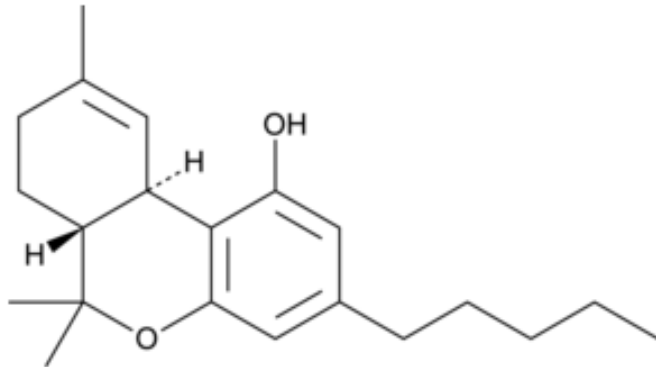
### (cannabidiol)

- Pharmacologically active
  - Approved for use in treatment of forms epilepsy in children
  - Anecdotally attributed to other health benefits
- Does not result in “high”
- Most abundant cannabinoid in hemp
- Least abundant cannabinoid in recreational marijuana
- Can be extracted/concentrated/processed for use in various products including oils and infused foods



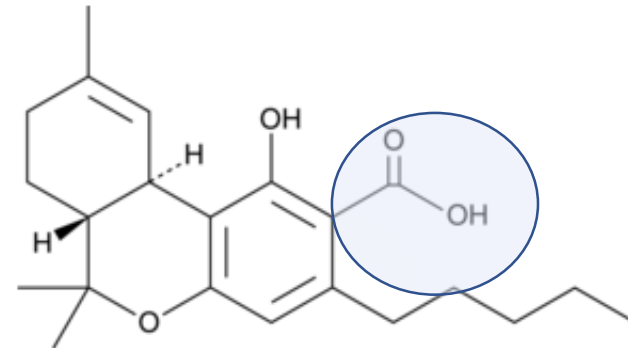
# TOTAL d9-THC

- THC (delta-9 tetrahydrocannabinol)



# d9-THC + d9-THCA

- THCA (tetrahydrocannabinolic acid)



As Cannabis (in any form) is dried or smoked, the acid forms of the plant convert to the psychoactive compound d9-THC. The same occurs with CBDA/CBD and most other cannabinoids.

From a chemistry standpoint – d9-THC is very different than TOTAL d-9THC

There is very little d9-THC in growing plant.

# 2018 Farm Bill

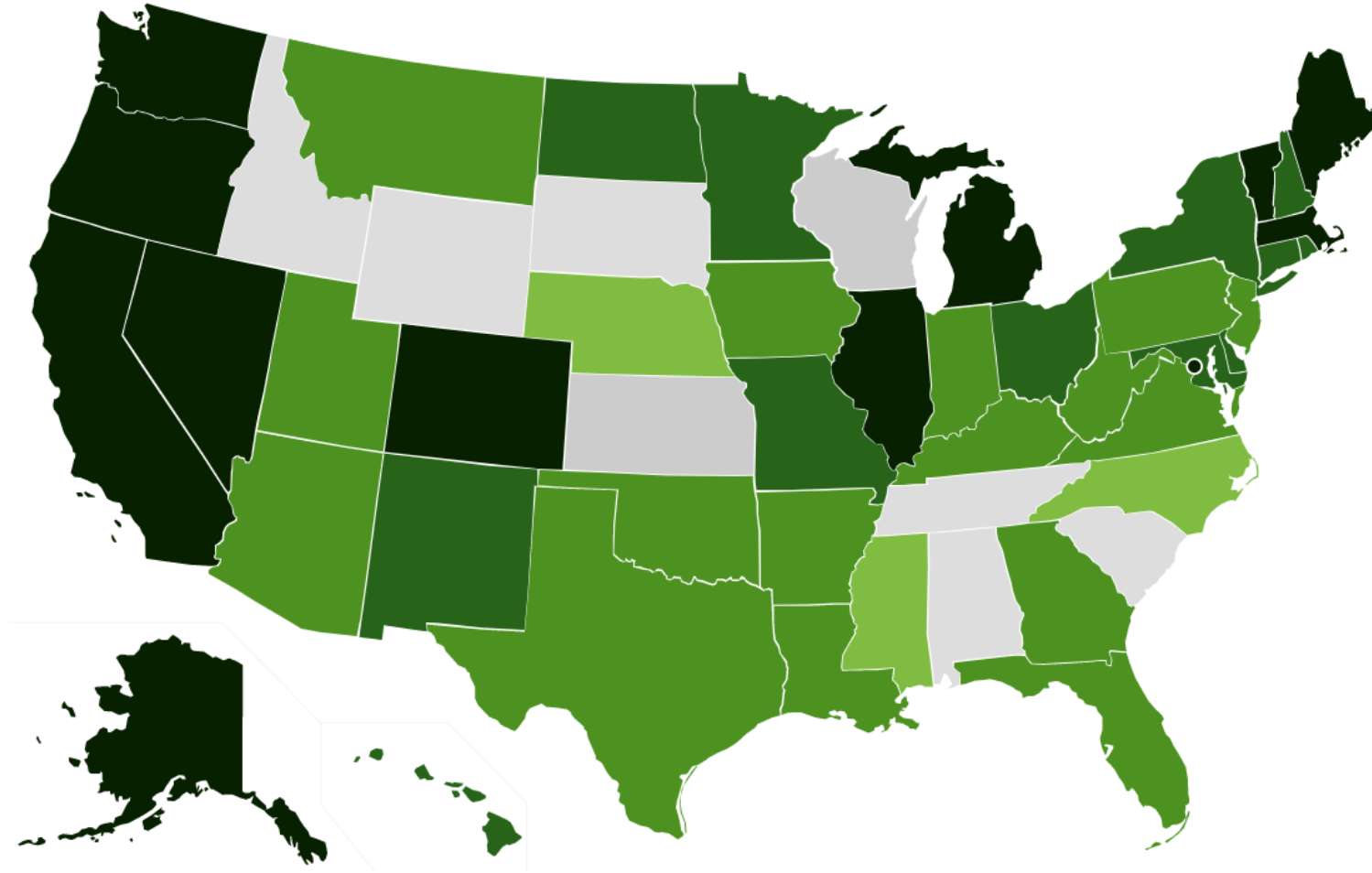
- Legalized hemp version of Cannabis as an agricultural product
- Hemp defined as Cannabis plant with less than 0.3% THC by dry weight
- Allowed hemp cultivation for the production of hemp derived products
- Removed restrictions on sale, transportation, and possession of hemp products
- Regulated who can grow hemp (does not allow to be grown for personal use)
- Made hemp-derived products legal
  - But oddly kept CBD listed as a schedule I substance

# Legal Status: States

- State law regarding Marijuana (excluding hemp)
  - Varies widely
  - Recreational use approved in 13 states
    - Restrictions regarding age and %THC content vary
  - Medical use approved in 34 states
    - Restriction regarding age, medical conditions, process, and % THC content varies

# Legal Status Varies by State

■ Legalized ■ Medical and Decriminalized ■ Medical ■ Decriminalized ■ Fully Illegal



<https://disa.com/map-of-marijuana-legality-by-state>



# Florida laws

## FSS 893

- Schedule I:
  - “Cannabis means all parts of any plant of the genus Cannabis, whether growing or not; the seeds thereof; the resin extracted from any part of the plant; and every compound, manufacture, salt, derivative, mixture, or preparation of the plant or its seeds or resin....”
- Schedule V:
  - The term does not include “low-THC cannabis” as a drug product in finished dosage formulation that has been approved by the United States Food and Drug Administration that contains cannabidiol (2-[1R-3-methyl-6R-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol) derived from cannabis and no more than 0.1 percent (w/w) residual tetrahydrocannabinols

## FSS 581.217

- Hemp means the plant Cannabis sativa L and any part of that plant, including the seeds thereof, and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers thereof, whether growing or not, that has a **total delta nine-tetrahydrocannabinol** concentration that does not exceed 0.3% on a dry weight basis.
- Hemp-derived cannabinoids, including, but not limited to cannabidiol are not controlled substances.
- Hemp extract means a substance or compound intended for ingestion that is derived from or contains hemp and does not contain other controlled substances.
  - Note: there is no defined THC content limit for hemp extracts

# THC Content

- Varies from plant to plant and varies within a plant
  - Most concentrated in the resin glands of the buds (flowering tops) of female plants
  - Leaves have some THC but are less potent than the buds
  - Seeds, twigs, stalks, and root have very small amounts
- THC/CBD ratios can be optimized based on use

	Chemotype I (Recreational)	Chemotype II	Chemotype III (Hemp)
Average THC/CBD Ratio	205	0.63	0.05
Average %THC	6.3	2.3	0.24
Average %CBD	0.04	3.8	4.6

# Analysis Process

Identification of Cannabis

```
graph TD; A[Identification of Cannabis] --> B[Screening for THC/CBD Content]; B --> C[Assessment of % THC];
```

Screening for THC/CBD Content

Assessment of % THC

# Identification of Cannabis



- SWGDRUG Recommendations – Version 8, June, 2019
- ASTM E2329-17: Standard Practice for Identification of Seized Drugs  
(OSAC Registry Document)
- Requires at a minimum an assessment of morphological features and a chemical color test
  - Prior to legalization – often allowed at the officer level
  - Does NOT differentiate type (cannot determine industrial hemp from this testing protocol)

# Identification of Cannabis

- Did not require forensic laboratory analysis in some jurisdictions
- Combination of assessment of morphological features and color tests often used by LE officers
- Laboratories used combination of morphological features, color tests, and chromatography (to visualize cannabinoids) for identification
- Neither protocol will differentiate hemp from other forms of Cannabis



Virginia Department of Law Enforcement

# Screening for THC/CBD Content

- Hemp is high in CBD, but low in THC
- Marijuana is high in THC, but low in CBD
- Other cannabinoids are present
- Two common screening test for initial assessment of THC content
  - Color test “Swiss Test”
  - Chromatographic Testing



# 4-AP (Swiss Test)

- 4-aminophenol
- Cannabis “Typification” Color Test
- Blue result with “Drug Hemp”
  - Recreational, medicinal
- Pink results with “Industrial Hemp”
- Results based upon CBD:THC ratio
  - Tiny amount of sample
  - Overloading can lead to false results
  - False negatives with some other material including oregano
- NOT suitable for determination of %THC, simply indicates extremes of THC/CBD ratios





# 4-AP (Swiss Test)





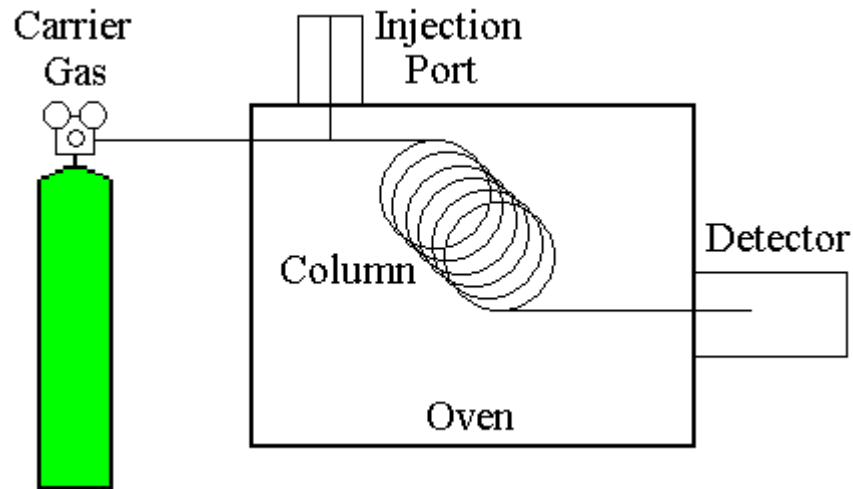
# Assessment of % THC

- Full quantitation by chromatographic methods to determine the actual concentration (within a reported margin of error) of target cannabinoids
- Threshold testing by chromatographic methods to determine if the amount of total delta nine THC exceeds a specified value
  - The actual concentration is not determined

# Quantitation of Cannabinoids

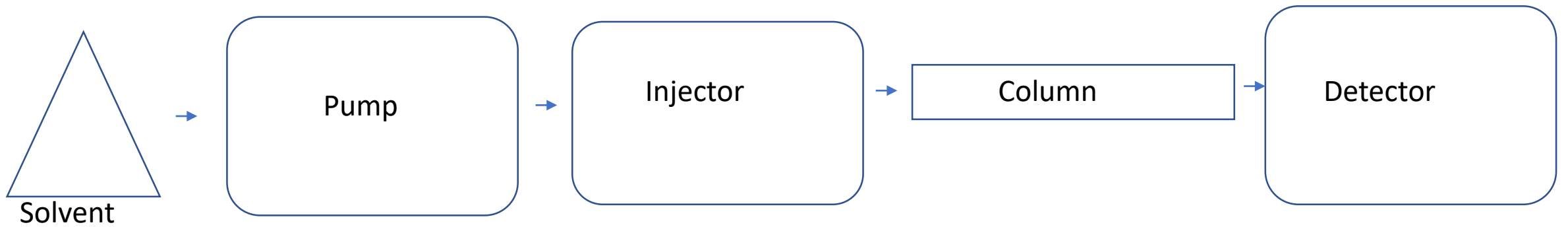
- Laboratory methods using liquid or gas chromatography to determine % concentration (based on weight) of targeted cannabinoids in a given sample
- Uses a calibration curve made from standard of known concentrations ratioed against an internal standard
- Response of unknown compared to known is used to determine concentration

# Gas Chromatography



- Separation based on chemical and physical properties
- Sample is volatilized/vaporized in the injection port
- Uses a gas carrier to push sample through coated column where separation occurs
- Detector response is associated with the abundance/ concentration of the analyte.
- Comparative analysis to known concentrations allows for quantitative analysis

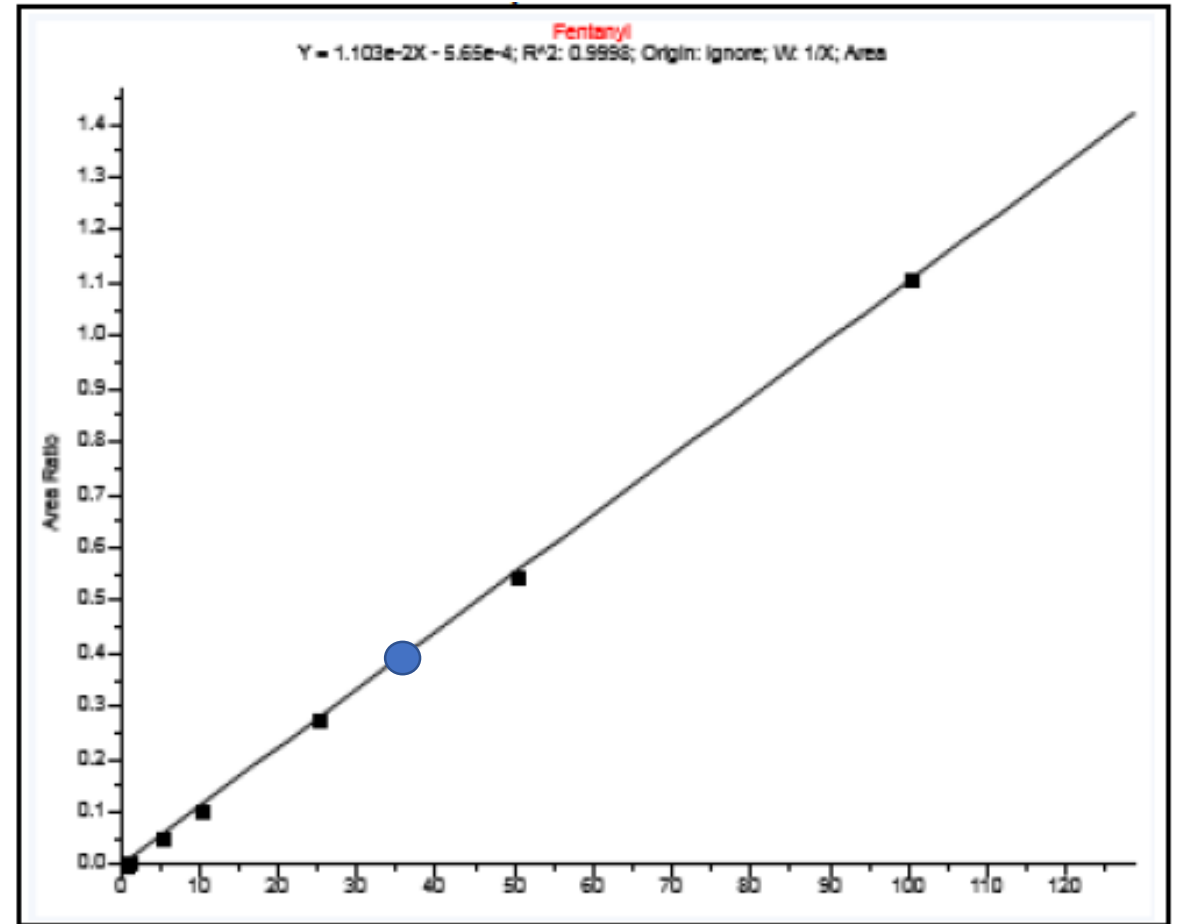
# Liquid Chromatography



- Separation based on chemical properties
- Sample is pushed through, relatively unchanged, using a solvent
- Detector response is associated with the abundance/concentration of the analyte
- Comparative analysis to known concentrations allows for quantitative analysis
- There is no thermal breakdown of the compounds

# Calibration Curve

- Known concentrations of standards analyzed
- Internal Standard used to control for extraction, injection, and other analytical variances
- Ratio of standard to internal standard plotted to create response vs concentration curve
- Responses ratio of unknown compared to determine concentration



# Chromatographic Methods

## Gas Chromatography

- Not ideal for use to differentiate THC from THCA
  - High heat in injection port will convert THCA to THC
- Has been validated for use in determining total THC, total CBD, etc.

## Liquid Chromatography

- Does not convert carboxylic acid form of cannabinoids to parent forms
- Allows for differentiation and quantitation of THC and THCA
- Calculations then used to determine TOTAL THC

# Threshold Testing

- Gas chromatographic method used to determine if the concentration of the unknown is **at least** a designated value
- Does not provide for actual concentrations of cannabinoids
- Developed as a more efficient way to differentiate hemp
- Threshold concentrations varies among laboratories
  - 1-3% most common
- Inaccuracy trends toward underestimation of total THC content

# Typical Threshold Analysis Process

- Small sample of plant material (~50mg) is extracted in a solvent that contains an internal standard
  - Of note: sample is not homogenized, sample is not dried
    - This benefits the defendant as a fully dried sample will have a higher concentration of THC
- Extracted sample is analyzed by GC-MS
  - THCA is converted to THC resulting in a total THC evaluation
- Standards of known concentration (threshold concentration) are analyzed
- If the unknown sample has a response ratio higher than the standard threshold sample – then it is concluded to be marijuana (not hemp)



# Threshold Analysis

## Advantages

- Faster/more efficient analysis process than full quantitation
- Inherently trends toward lower than actual total THC values
  - 1-3% threshold level
  - Non dried sample
  - Inefficiencies of extraction
- False positives are less likely

## Disadvantages

- Does not provide for actual concentrations
  - Concentrations cannot be inferred based on response
- Lower THC Cannabis varieties can result in false negative or inconclusive results in samples with greater than 0.3% total THC

# Analysis Summary

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Screening for THC/CBD Content

Assessment of % THC

# Edibles, Waxes, Concentrates

- Similar analytical methods exist
  - Instrumental limitations based on matrix
    - Gummy bears, food products
  - Infused vs. coated products
- Legal status varies by state/jurisdiction
- Lack of clear legal verbiage regarding acceptable concentration is a concern
  - “derived from hemp”
  - “extracted from hemp”

# Testing and Laboratory Integrity

- Laboratory Structure
  - Forensic Labs vs. Compliance Labs
  - Accreditation
    - ISO 17025
  - Validation
    - Fully documented validation
  - Standardized Methods
  - DEA 222
  - Chain of Custody
  - Certifying Scientists vs. Performing Scientists

# Summary

- Cannabis/Marijuana/Hemp analysis must now include element beyond simple identification
- Laboratory methodology that allows for assessment of %THC concentration is a necessity
- Differentiation between hemp and other types of Cannabis can no longer be done in the field
  - Screening of probable hemp by use of color tests to exclude illicit Cannabis excepted
- Laboratories performing analysis must be cognizant/trained in the legal aspects including chain of custody, testifying analyst (vs certifying analyst), controlled substances laws, rules of evidence, and DEA controlled substances registration requirements.
- ASTM has committee dedicated to developing and maintaining standards including analytical methods for the analysis of Cannabis
  - ASTM D37 Committee: Cannabis
  - ASTE E30 Committee: Forensic Science
  - Additionally, OSAC is working with NIST to standardize the analysis of THC in the laboratory and the field

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