Application of headspace solid phase micro extraction in chemical forensics

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Disclaimer

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Our Research Interests

General process of Physical Evidence

- Observation
- Recognition
- Collection
- Preservation
- Identification → Individualization
- Interpretation (Reconstruction)

- as **investigative clues** during investigation.
- as **forensic evidence** in the court room.

Our Research Interests

- Physical (Impression)
  - Fingerprints, firearms, handwritings, number restoration, footprints and tire marks, typewriting,
- Biological
  - Blood, semen, saliva, other body fluids, hair, botanical, pathological
- Chemical
  - Fibers, chemicals, glass, soil, gun powder, metallurgical, mineralogical, narcotics, paper, pharmacological, toxicological
- Others
  - Voiceprint, photograph, etc.

Chemical Forensics

Headspace Chemical Analysis

Chemical Attribution Signatures

Chemical Forensics

Chemical forensics is a scientific discipline that aims to attribute a chemical (or mixture) to it's source by the analysis of the chemical itself or associated materials to address investigative, legal and intelligence questions.

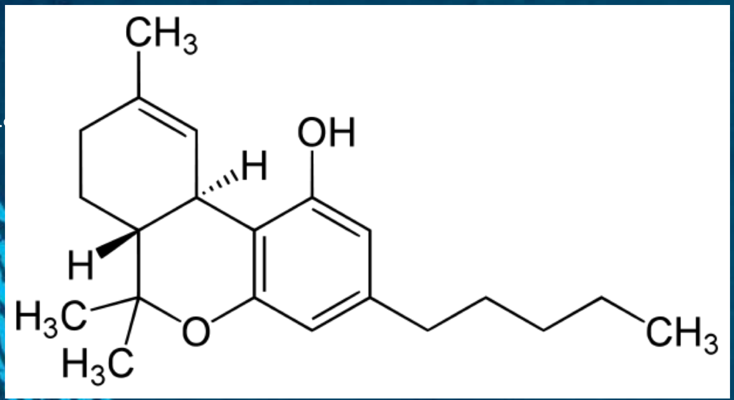
- Chemicals: Chemical Warfare Agents, explosive, toxic substances, etc.
- Source: synthetic route, manufacturer, geographic origin, reagent or precursor stock.

Headspace Chemical Forensics

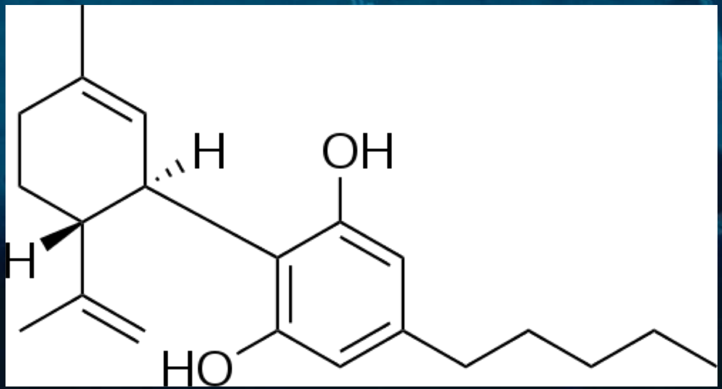
- Hypothesis: The chemical attributes (signatures) extracted from sample headspace will be **sufficient** for the purpose of crime investigation and forensics.
- The chemical signatures can be **non-destructively** collected from evidence.
- The headspace chemical signature might provide a potential for the establishment of **database**.
- Easy for automation, therefore reduce the threat to analyst when toxic substance is involved, and increase **throughput**.

MARIJUANA

- Dried plant material from *Cannabis sativa*
- Often smoked or added to baked goods
- Delta 9-tetrahydrocannabinol (THC)
- Cannabidiol (CBD)



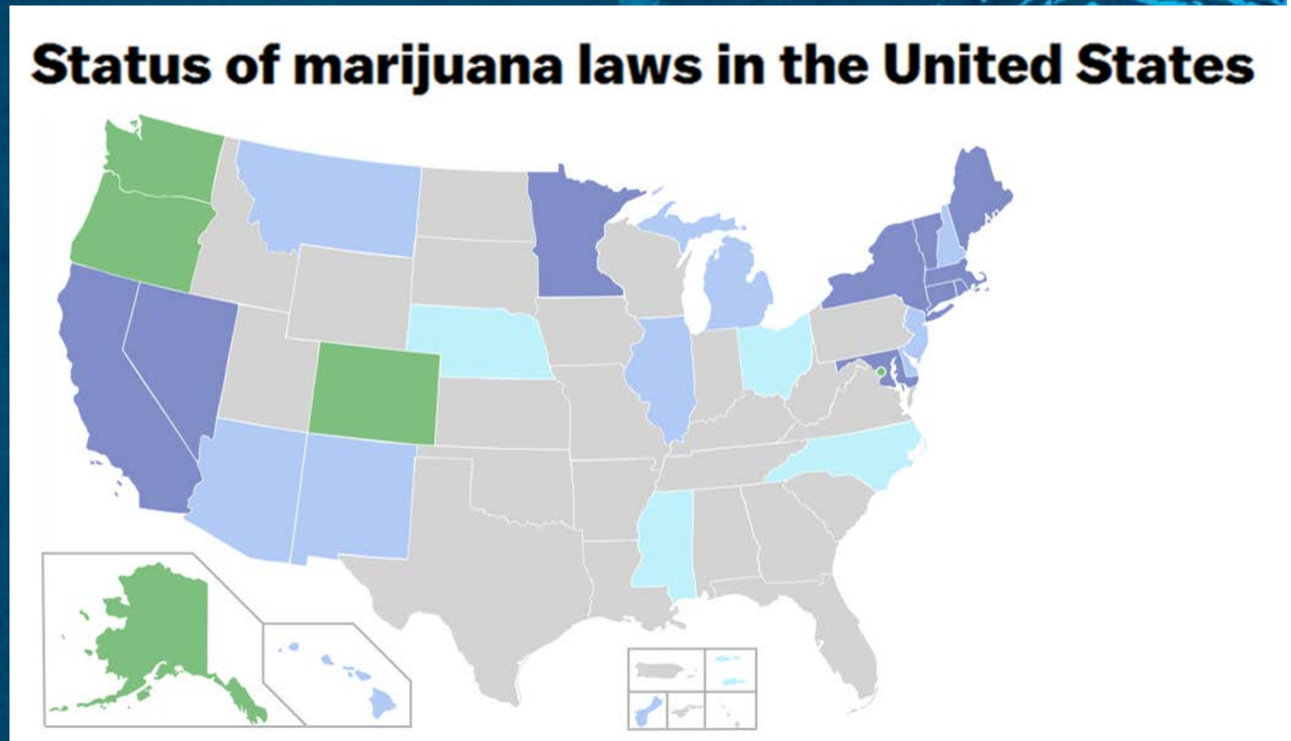
THC

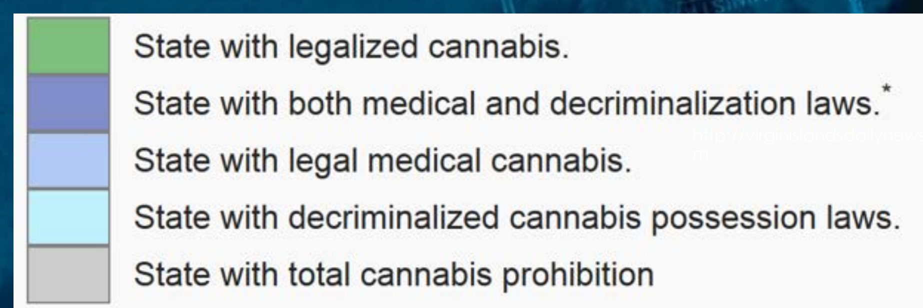


CBD

THE CHANGE OF LEGAL LANDSCAPE

- Federally classified as a Schedule I drug under the CSA
- Some states approved medicinal and/or recreational use





Law Enforcement Concerns

- Investigation questions:
  - Grown illegally.
  - Smuggled into the United States.
  - Sophisticated growing operation.
  - Diverted from states where marijuana is legal.
  - Black market ←→ legal market.
  - Medical vs recreational.

An efficient, affordable analytical platform is desirable.

Headspace Chemical Forensics

- Collect headspace chemical signature to link marijuana seizures by their common origin/growing condition/

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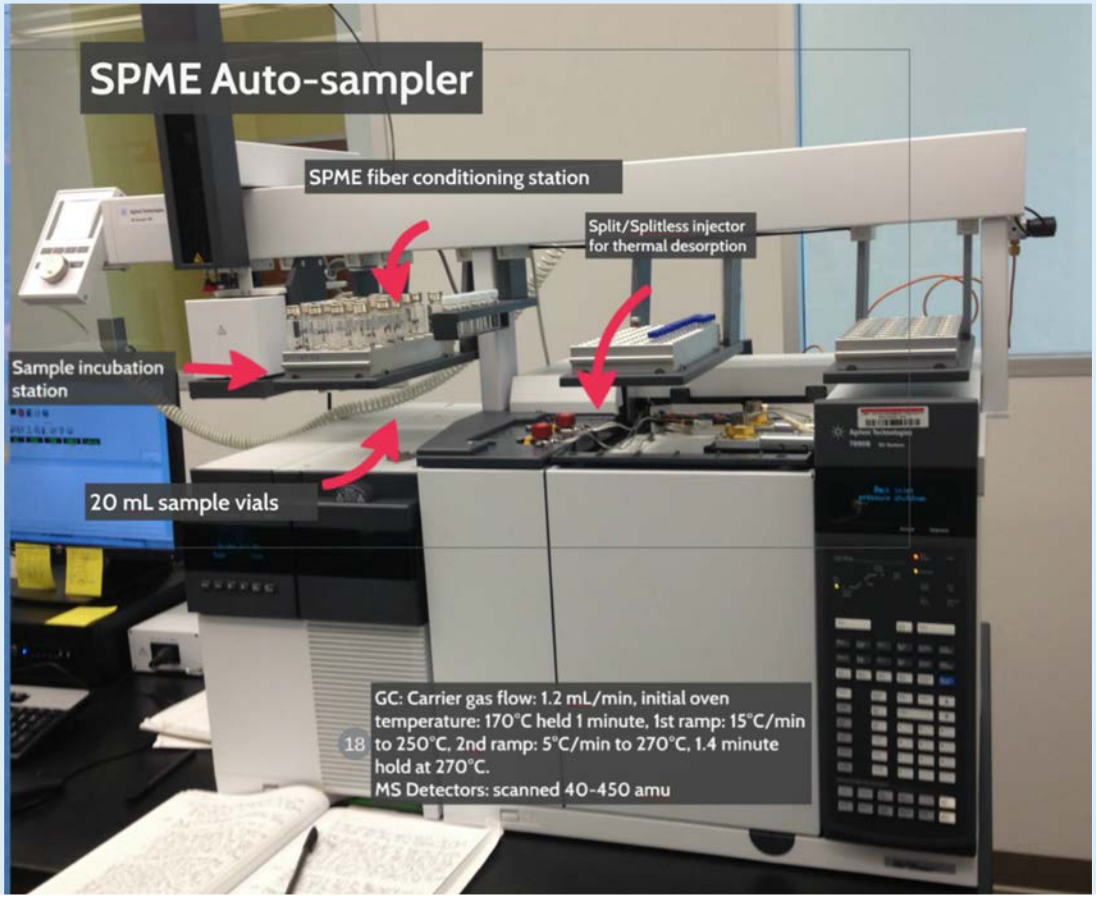
Headspace chemical analysis:

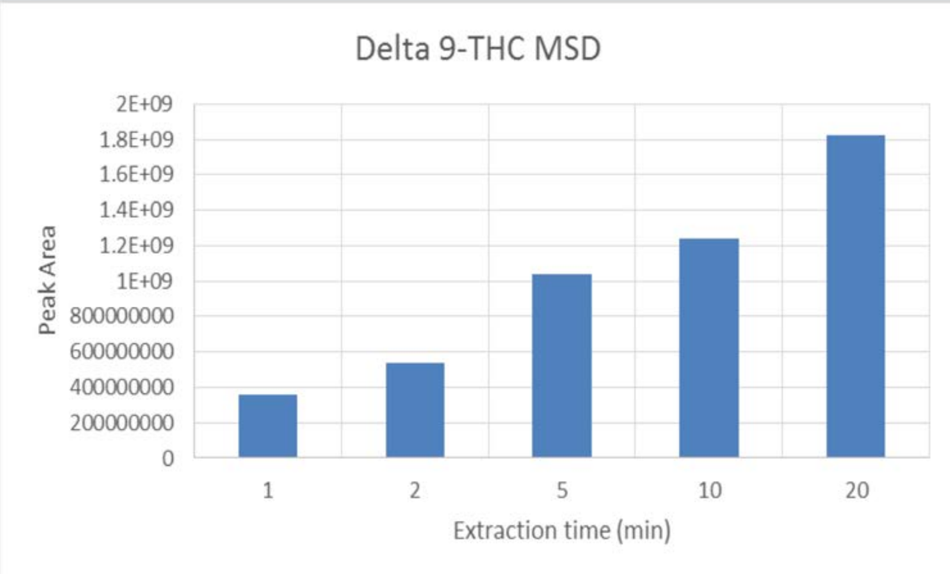
- Cleaner extract compared to liquid extract.
- Easy automation.
- Readily adopted by any crime laboratory with a GC/MS.

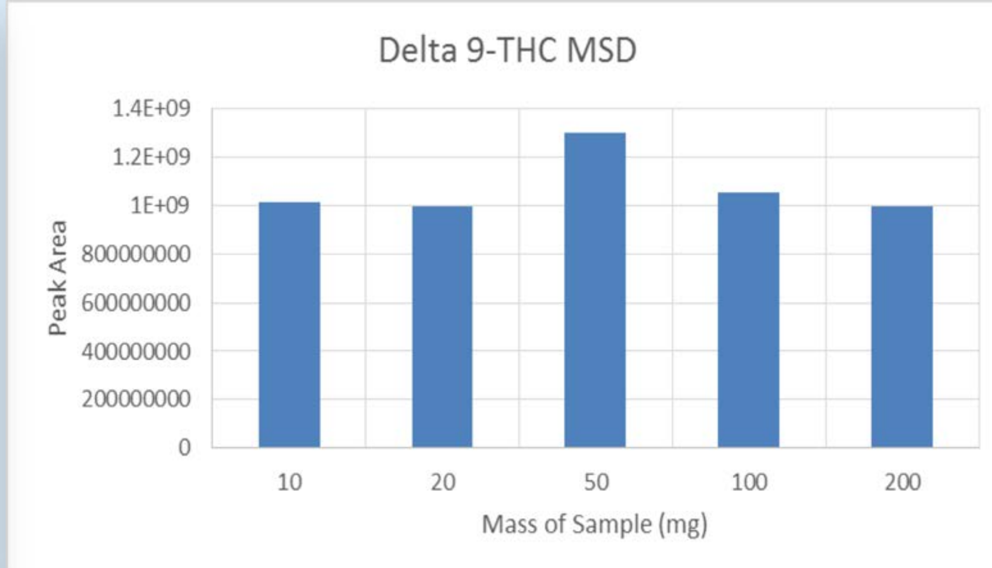
Heated Headspace Solid Phase Microextraction (HHS-SPME)

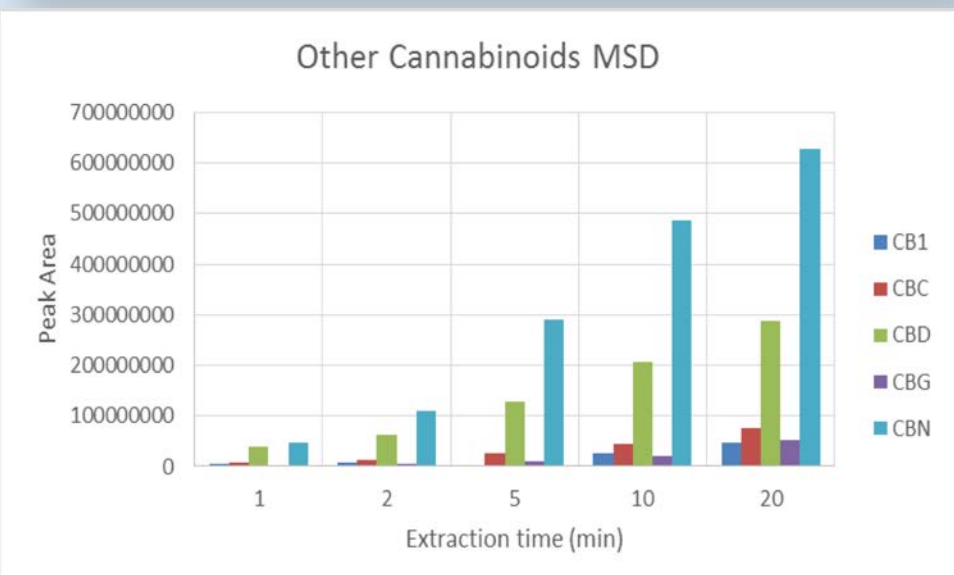
- Automated heated headspace solid phase microextraction (HHS-SPME)
  - Agilent GC Sampler 120 autosampler and Polydimethylsiloxane (PDMS) fiber
  - Sample weighed and sealed in headspace vial

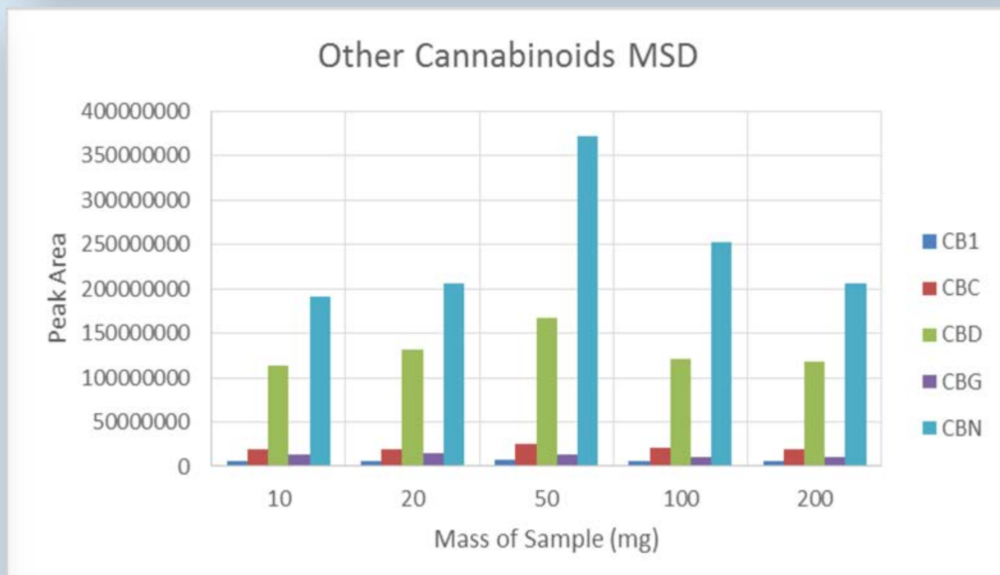


HHS-SPME Optimization

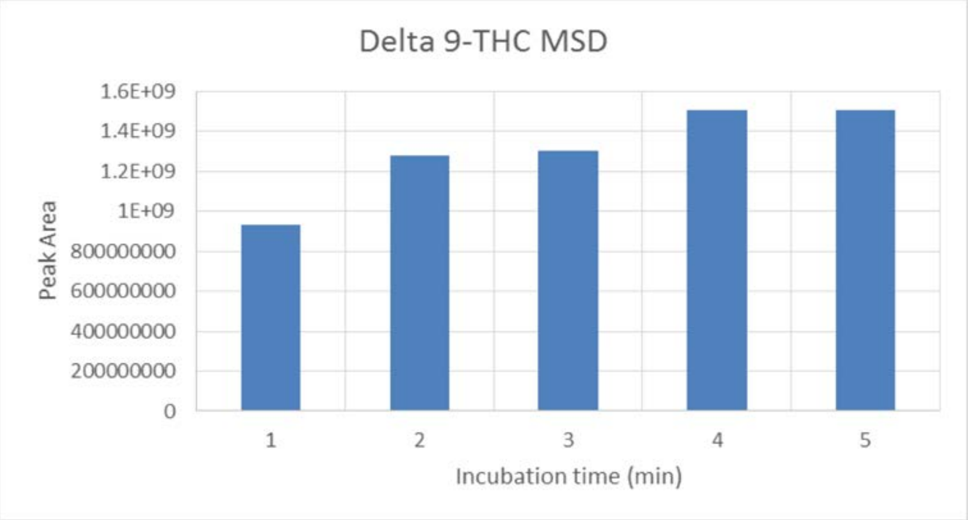


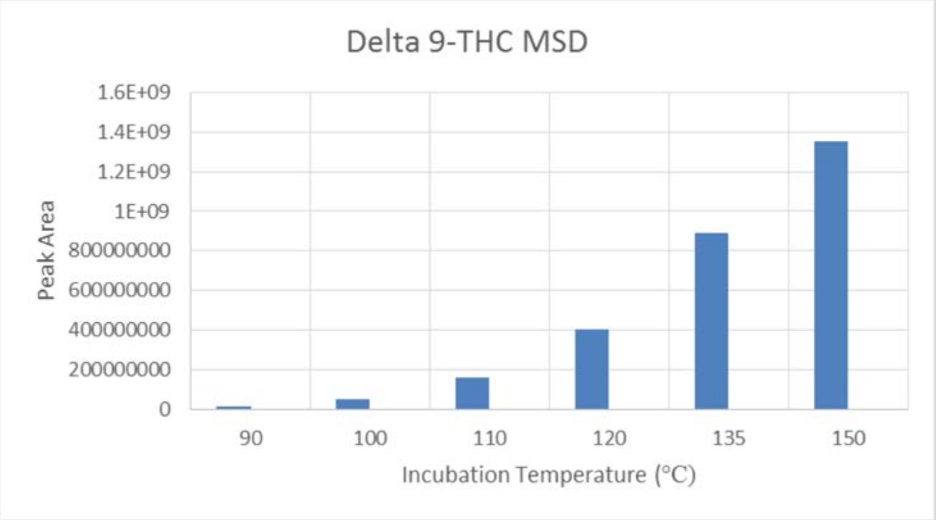


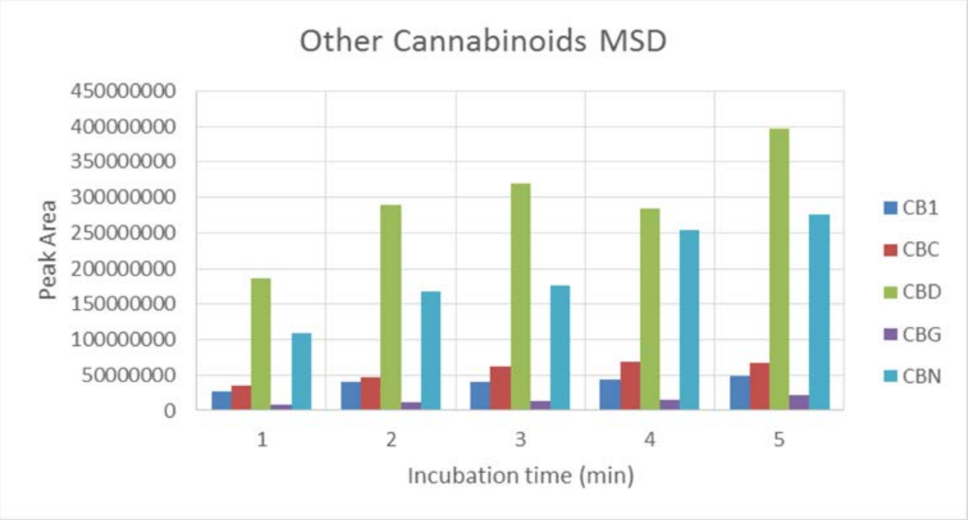


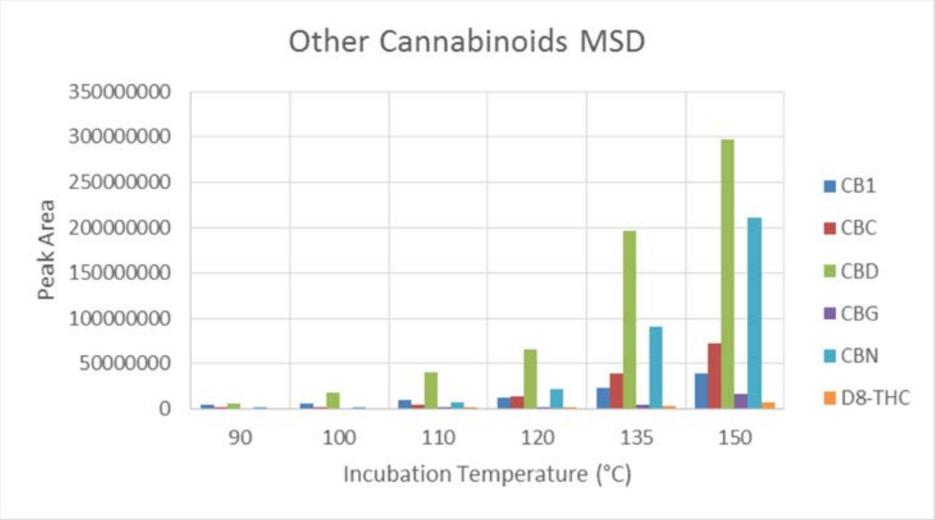


HHS-SPME Optimization





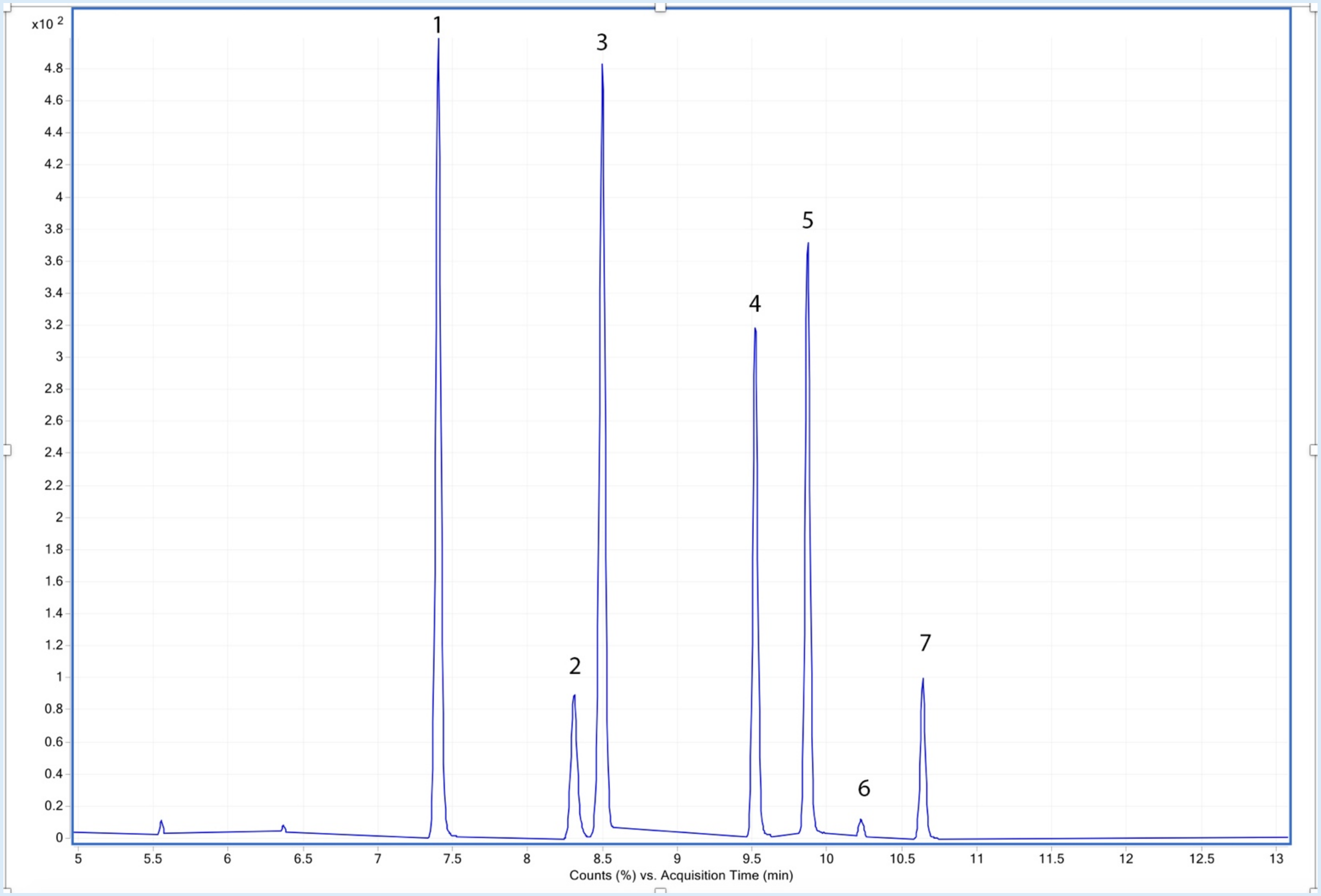




Current HHS-SPME for Marijuana Analysis

HHS-SPME Steps	Condition
Pre-Fiber Conditioning Temperature (°C)	250
Pre-Fiber Conditioning Time (s)	0
Pre-Incubation Time (s)	300
Incubation Temperature (°C)	140
Pre-Incubation Agitator Speed (rpm)	250
Agitator On Time (s)	2
Agitator Off Time (s)	10
Vial Needle Penetration (mm)	11
Vial Fiber Exposure (µl)	12
Extraction Time (s)	150
Desorb to	GC Injection port
Injection Needle Penetration (mm)	32
Injection Fiber Exposure (µl)	12
Desorption Time (s)	30
Post-Fiber Conditioning Temperature (°C)	250
Post-Fiber Conditioning Time (s)	1200
GC Runtime (s)	300

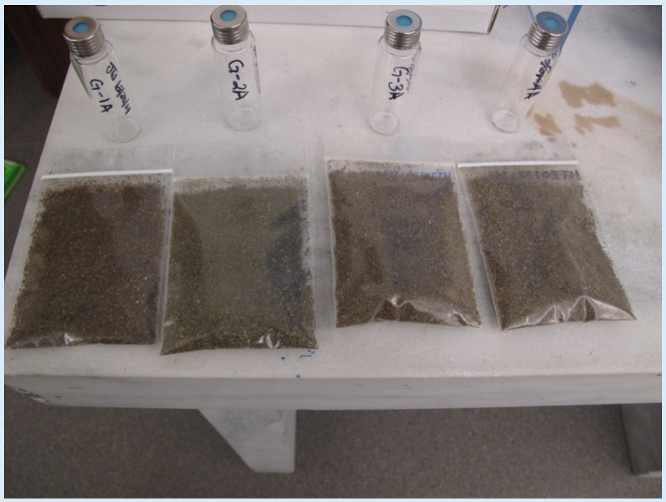
HHS-SPME-GC/MS of Reference Phytocannabinoids



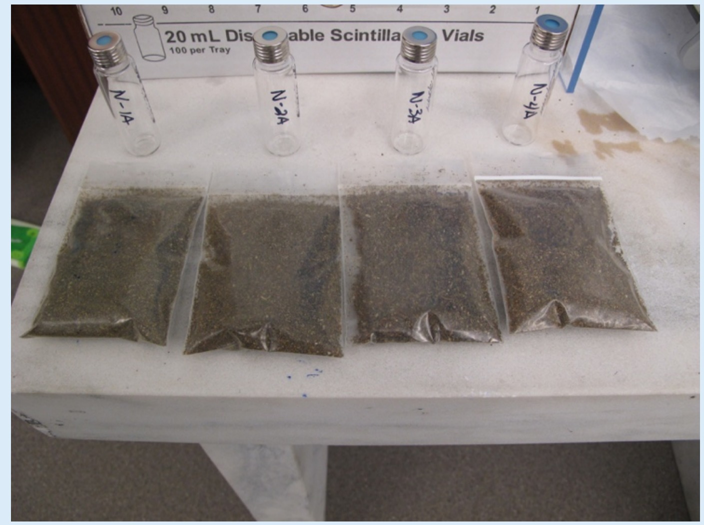
1) THCV (tetrahydrocannabivarin), 2) CBC (cannabichromene), 3) CBD (Cannabidiol), 4) Δ8-THC (tetrahydrocannabinol), 5) Δ9-THC, 6) CBG (cannabigerol), and 7) CBN (cannabinol). 400 ng each in a 20 mL headspace vial.

HHS-SPME-GC/MS of Seized Marijuana Samples

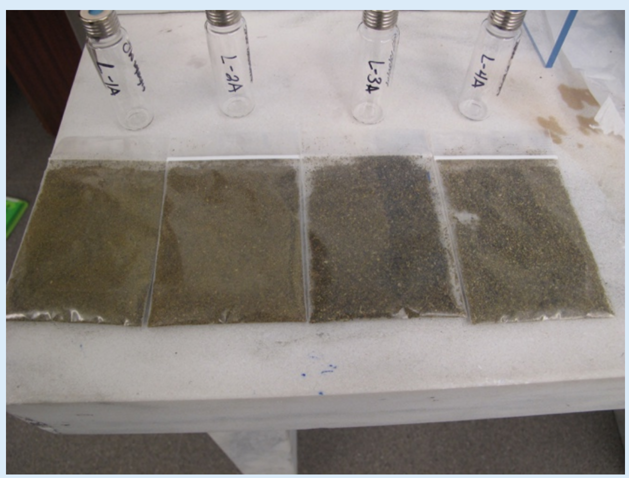
Sample G



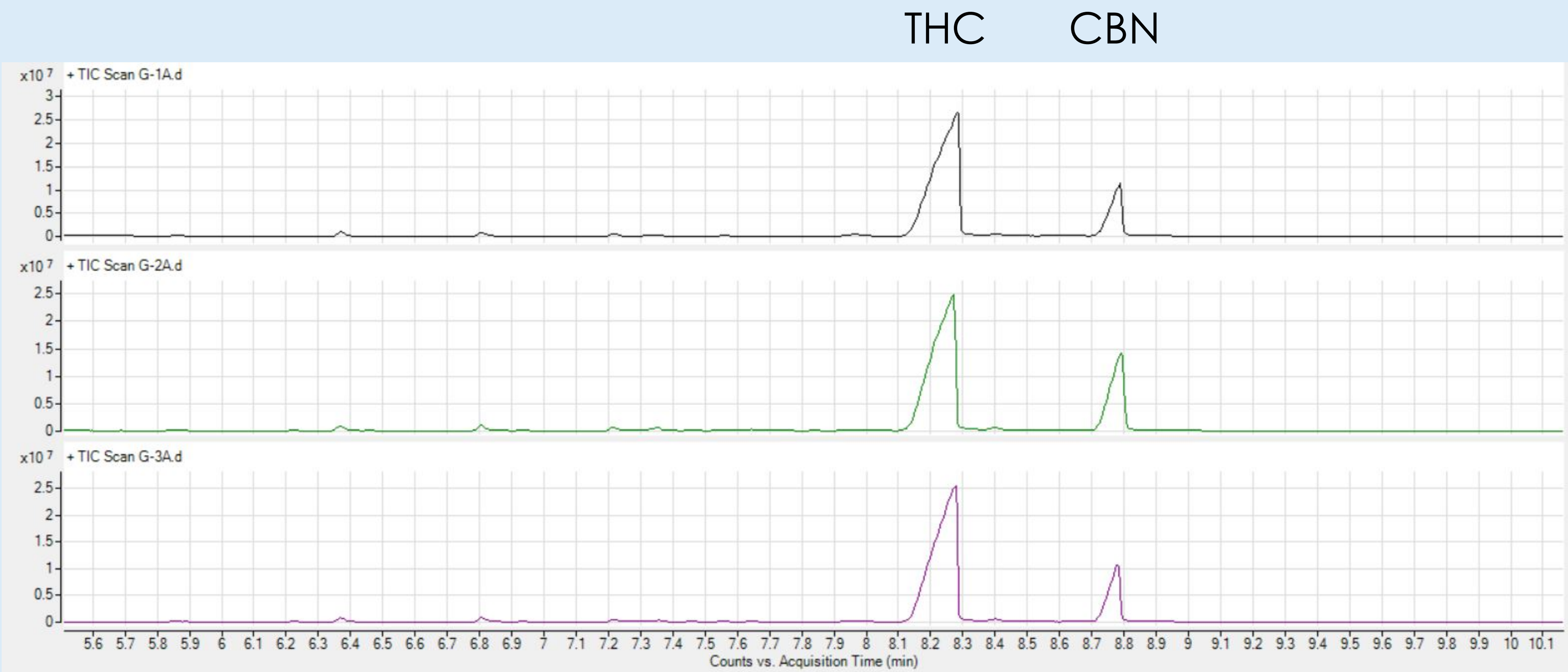
Sample N



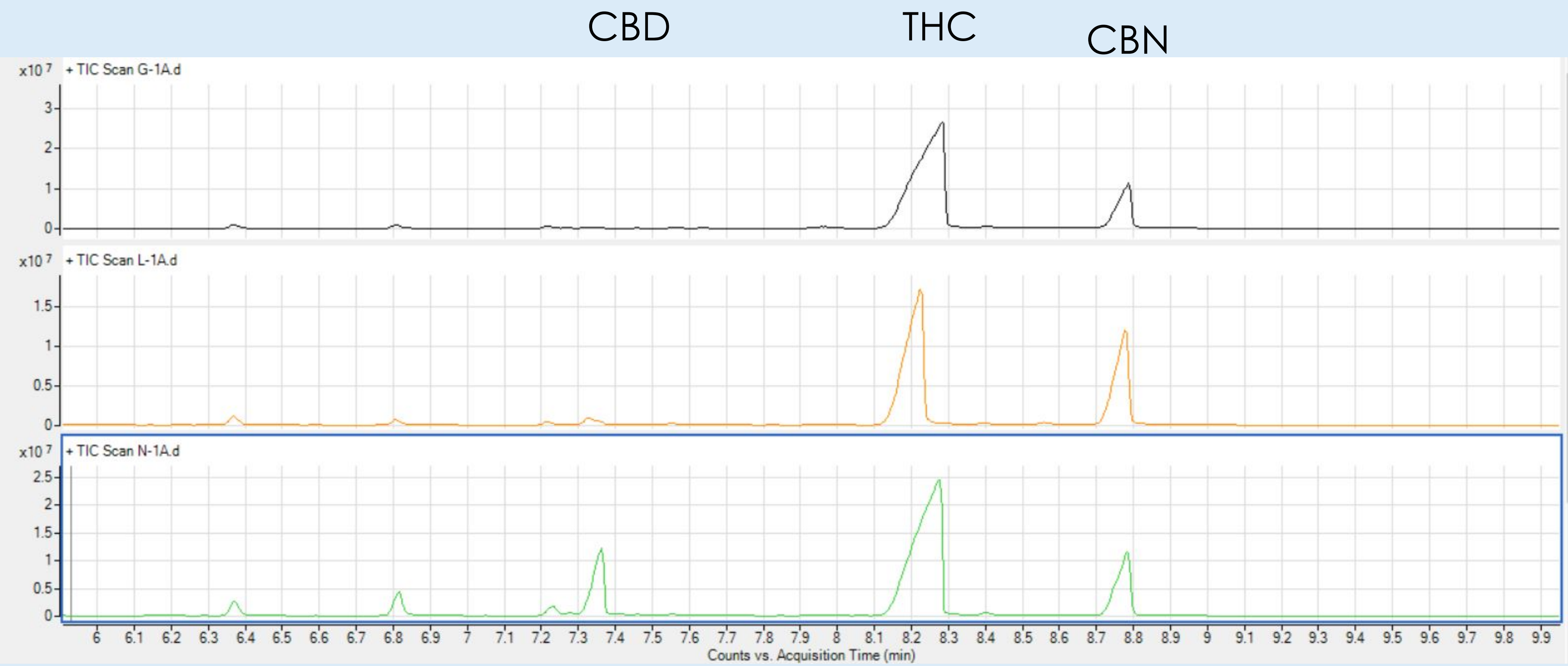
Sample L



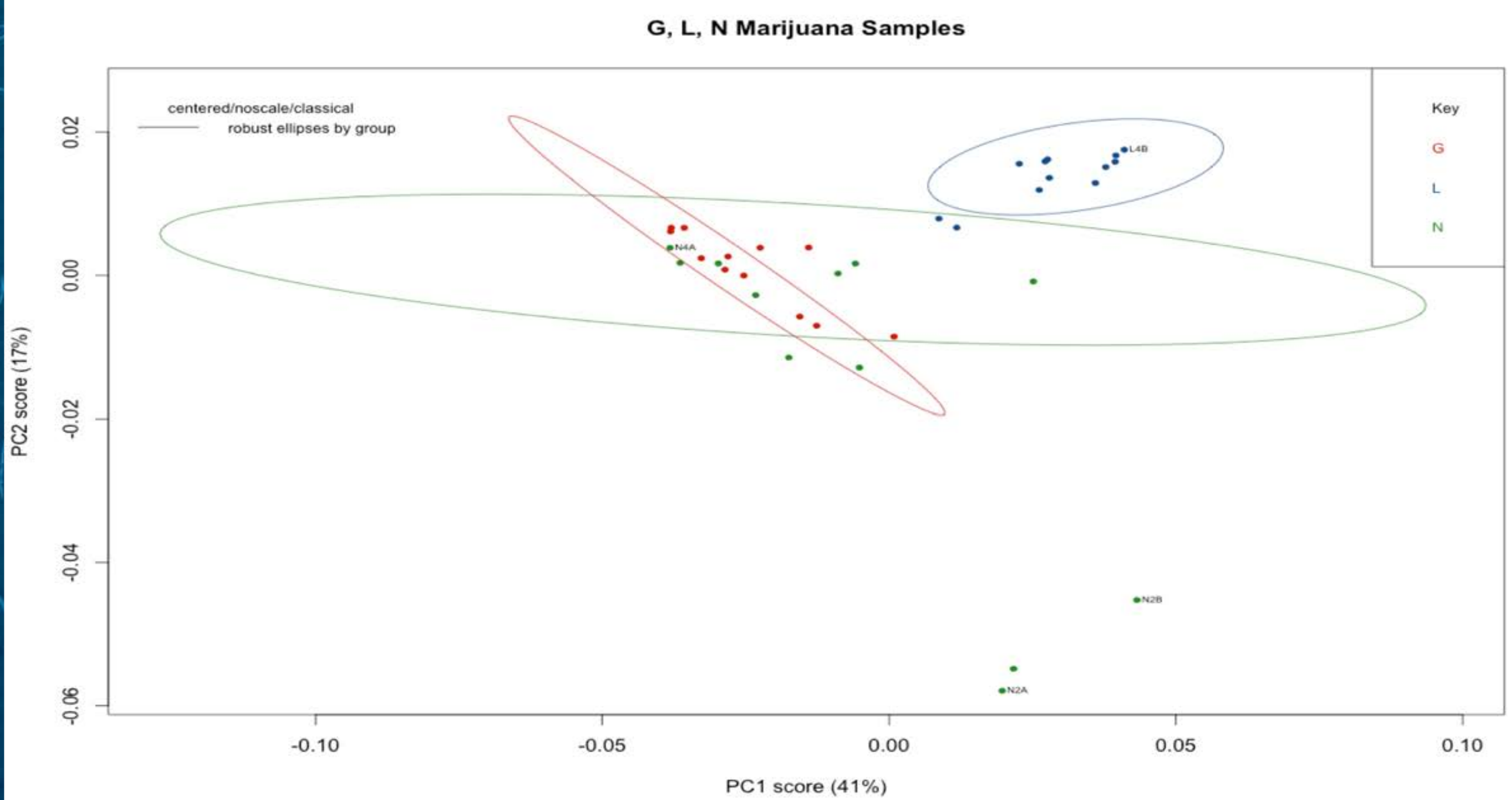
Within Group Results



Between Group Results



Principal Component Analysis of HHS-SPME-GC/MS Data for Seized Marijuana Samples



Principal component analysis (PCA) was performed using the statistical program 'R'. The ChemoSpec package was installed within the R program and was used to perform PCA analysis.

R: http://www.R-project.org/.

Chemical Attribution Signatures for marijuana could be extracted from the sample headspace

Standard Marijuana (Ground Truth Samples)

Table 1. Standard marijuana samples with known levels of THC and CBD

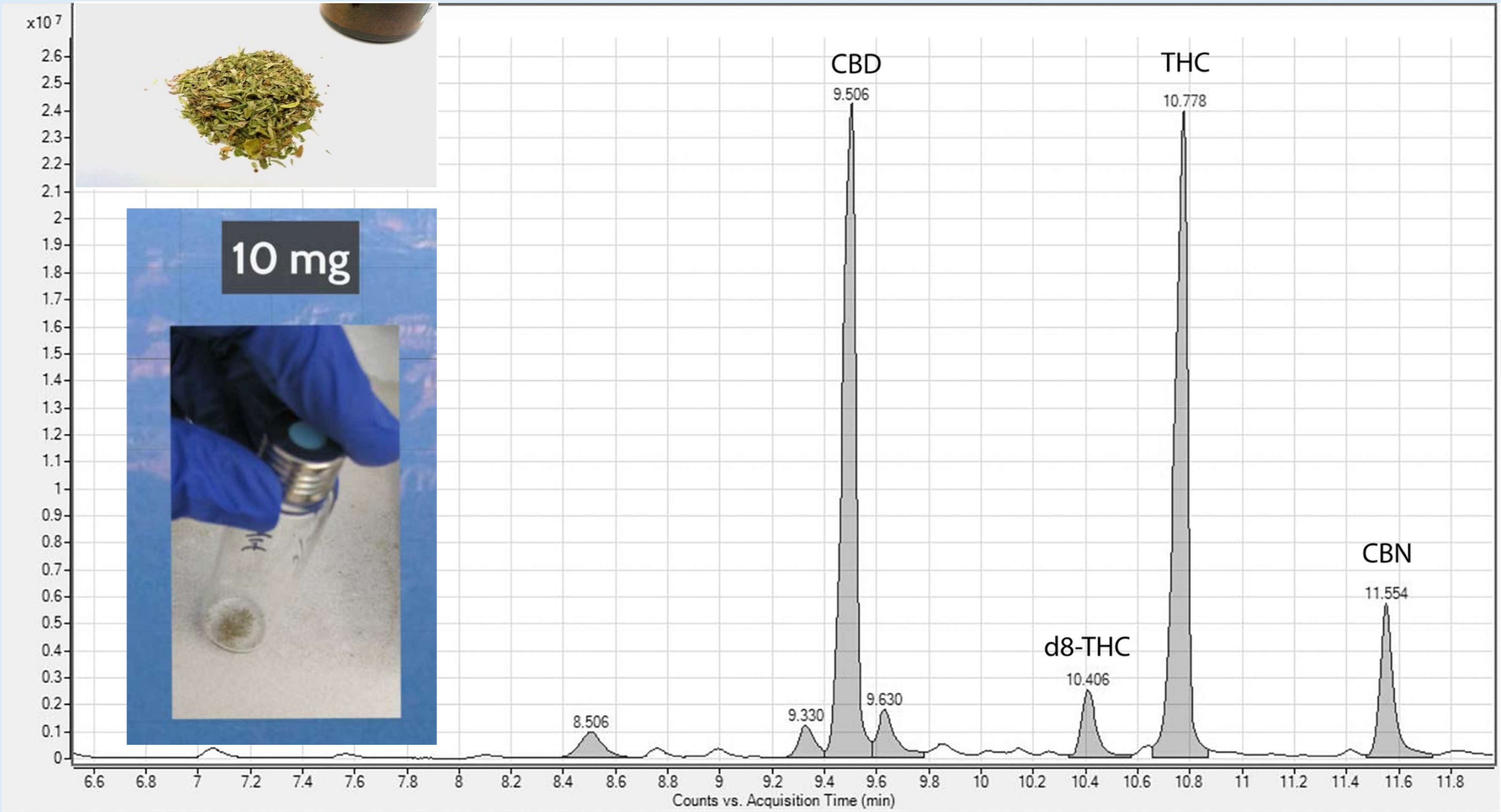
Marijuana samples	Known THC% (w/w)	Known CBD% (w/w)
1 (marijuana placebo)	Not Detected	Not Detected
2	0.08	3.4
3	1	0.01
4	2	0.16
5	3.1	0.01
6	3.8	6.5
7	4.7	0.01
8	7	0.03
9	7.5	13.9
10	7.9	0.05
11	8.9	9.3
12	10.4	0.03
13	10.6	0.03
14	13.4	0.03

Obtained from NIDA (National Institute on Drug Abuse)

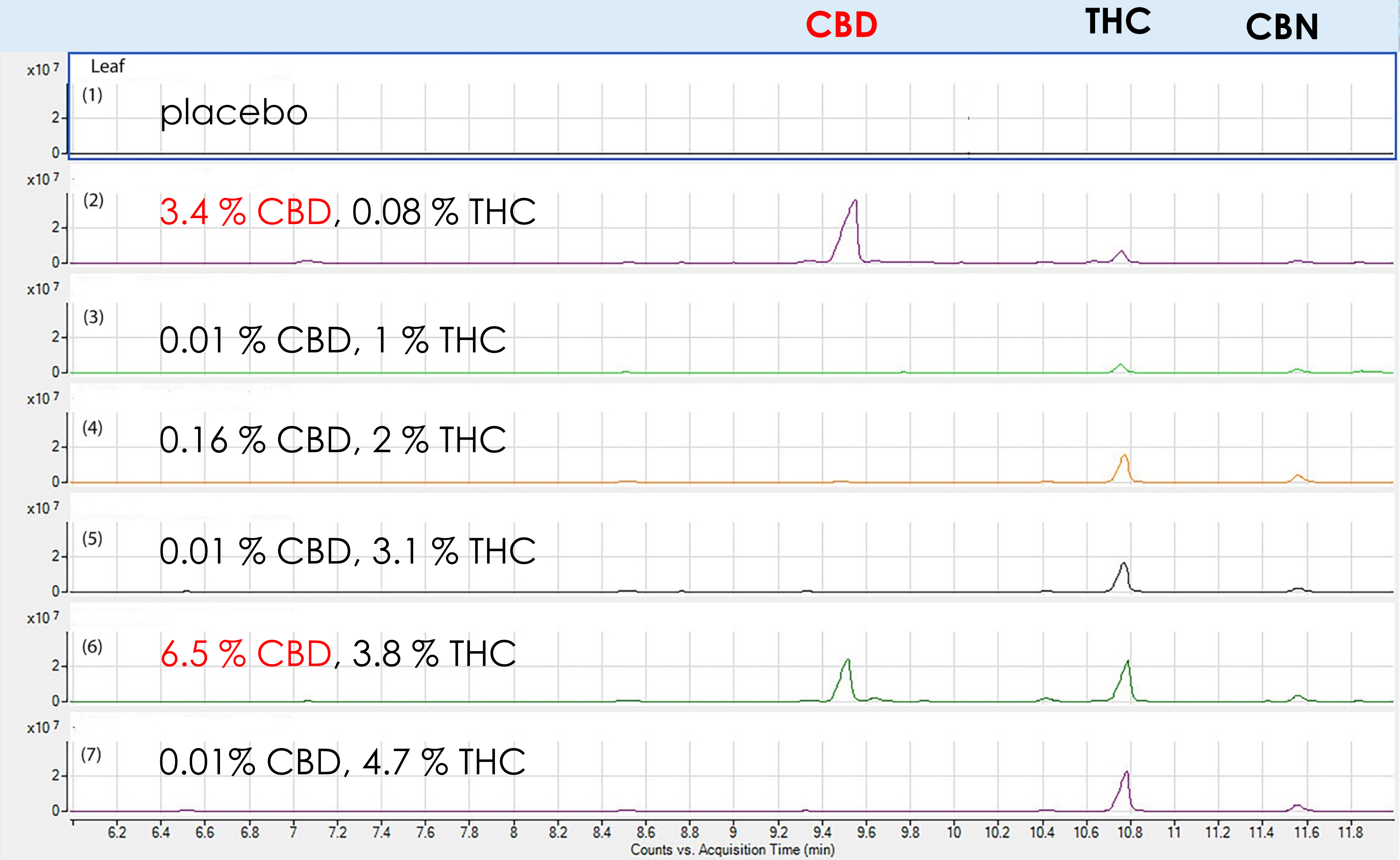
Preparation of Samples

a. Different botanical structures observed in a typical marijuana sample. b. Typical floral structures (Calyx). c. typical stem structures. d. typical leave structures. All images were taken under x20 magnification.

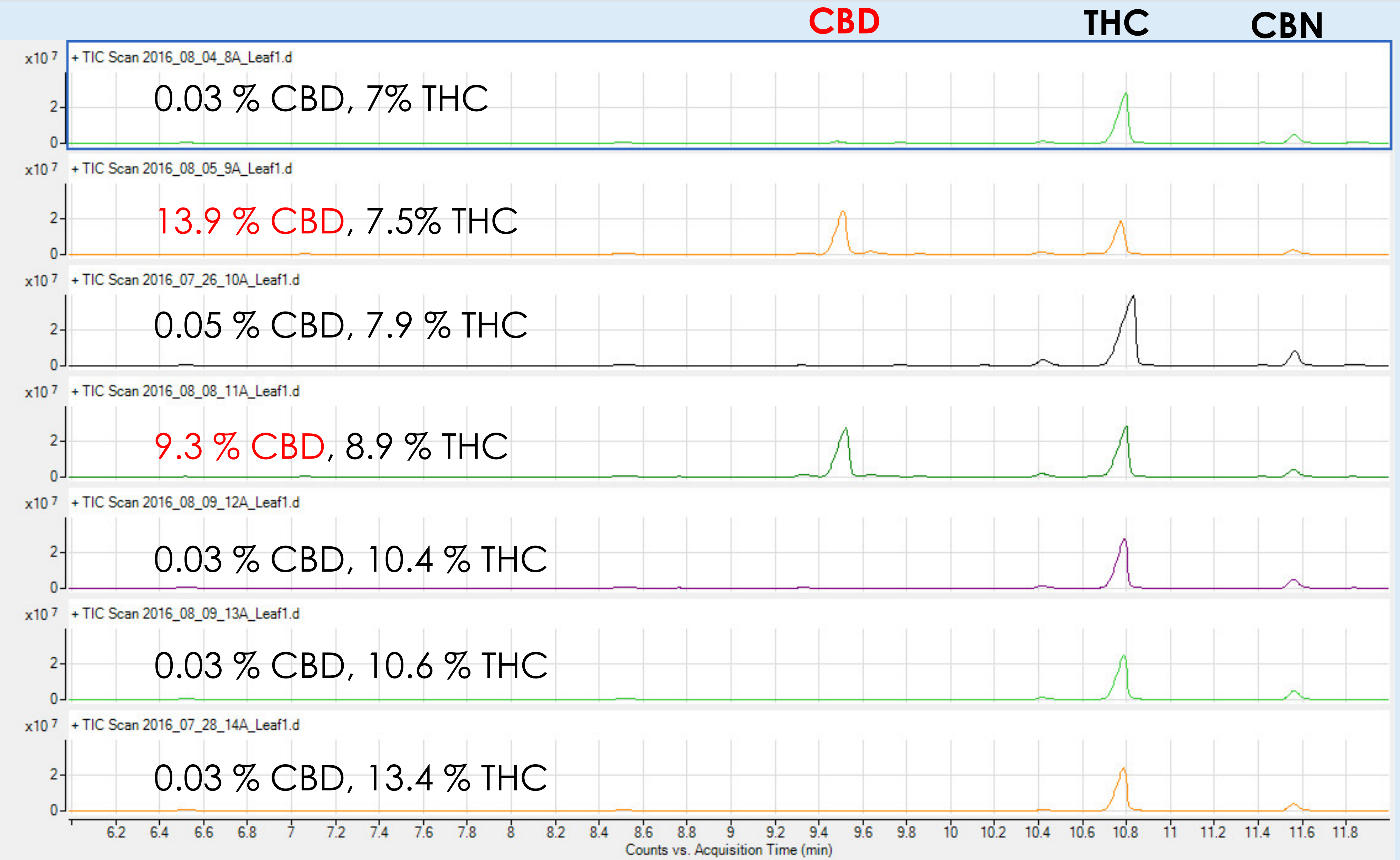
Sample #6 (3.8% THC, 6.5% CBD)



Headspace Phytocannabinoids Profiles



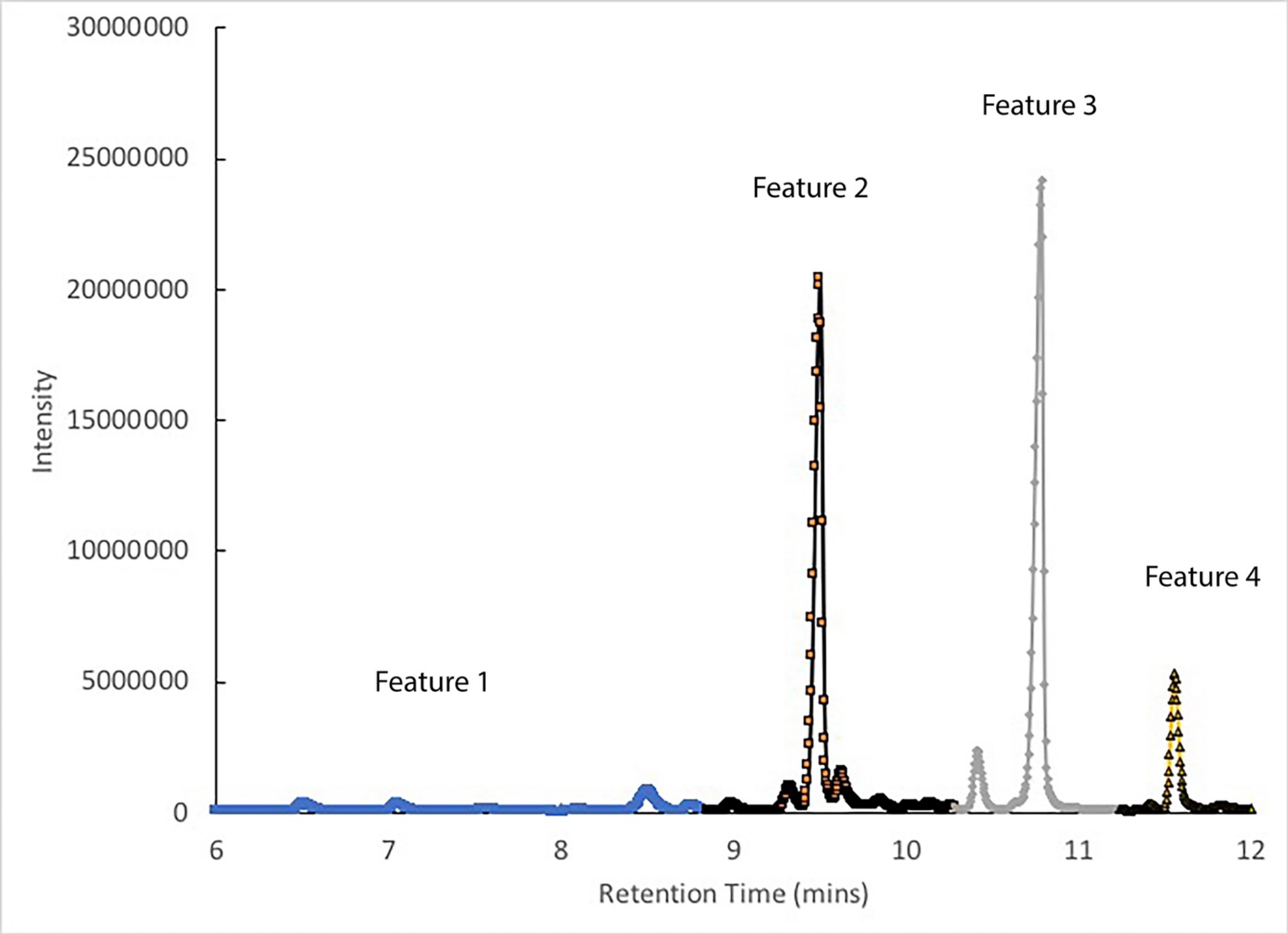
Headspace Phytocannabinoids Profiles



Machine Learning

- Many daily life and industrial applications.
- The new development of learning algorithm in drug discovery.
- Numerous applications:
  - A supervised hierarchical machine learning algorithm was developed for the detection of chemical signatures in breath in order to detect disease and other conditions that cause homeostatic imbalance.
  - Application to some extent of artificial intelligence techniques or statistical science in data analytics is an important process in chemical forensics.
  - Machine learning technique has been adopted to assure the authenticity of white wine varieties.

Feature Selection



Dataset - 14 marijuana varieties 198 TICs

As it is

Stem

Leaf

Buds (calyx)

Grinded

10 (varieties) x 5 (botanical structure) x 3 (triplicates) = 150
4 (varieties) x 4 (botanical structure) x 3 (triplicates) = 48

There were only 12 HHS-SPME-GC/MS data randomly collected for sample 1, 3, 4, and 9 due to either missing buds structure or stem structure.

- Machine learning experiments were carried out 100 times on this dataset for supervised learning. For each learning process, from each variety, 80% of the dataset were randomly selected for supervised training to build classification models, the remaining 20% of the data were used as unknown in order to test the accuracy of the model. Support Vector Machines (SVM) and ensemble learning were used for supervised learning and testing in the study.
- Machine learning experiment was carried out by Dr. Frank Liu with the Department of Computer Science using Matlab platform.

Placebo Marijuana Determination

Mean testing accuracy (100 time of learning model development for distinguishing placebo marijuana and other marijuana varieties.

		Prediction accuracy (%, six features)		Prediction accuracy (%, four features)	
		Placebo	Other types	Placebo	Other types
Truth	Placebo	100	0	100	0
	Other types	0.2	99.8	0	100

Marijuana with CBD

Mean testing accuracy (n=3974) for distinguishing marijuana varieties with CBD (Group 1) and marijuana varieties without CBD (Group 2).

		Prediction accuracy (%, six features)		Prediction accuracy (%, four features)	
		Group 1	Group 2	Group 1	Group 2
Truth	Group 1	97.5	2.5	100	0
	Group 2	2.7	97.3	10.4	89.6

Group 1: Sample 2, 6, 9, 11
Group 2: Sample 3, 4, 5, 7, 8, 10, 12, 13, 14

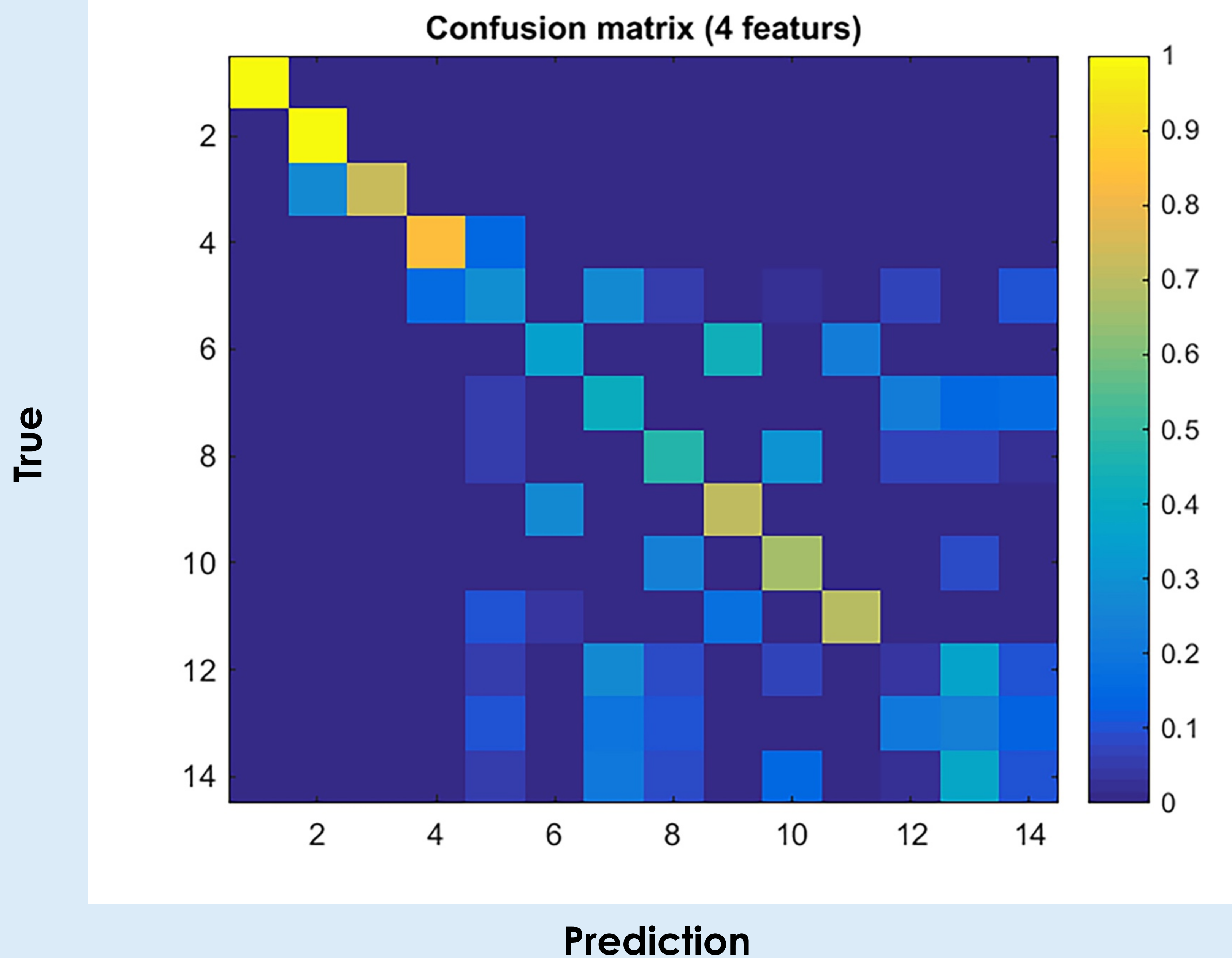
Marijuana with high CBD and low THC

Mean testing accuracy for distinguishing low THC/medium CBD (Group 1) and other three types (Group 2)

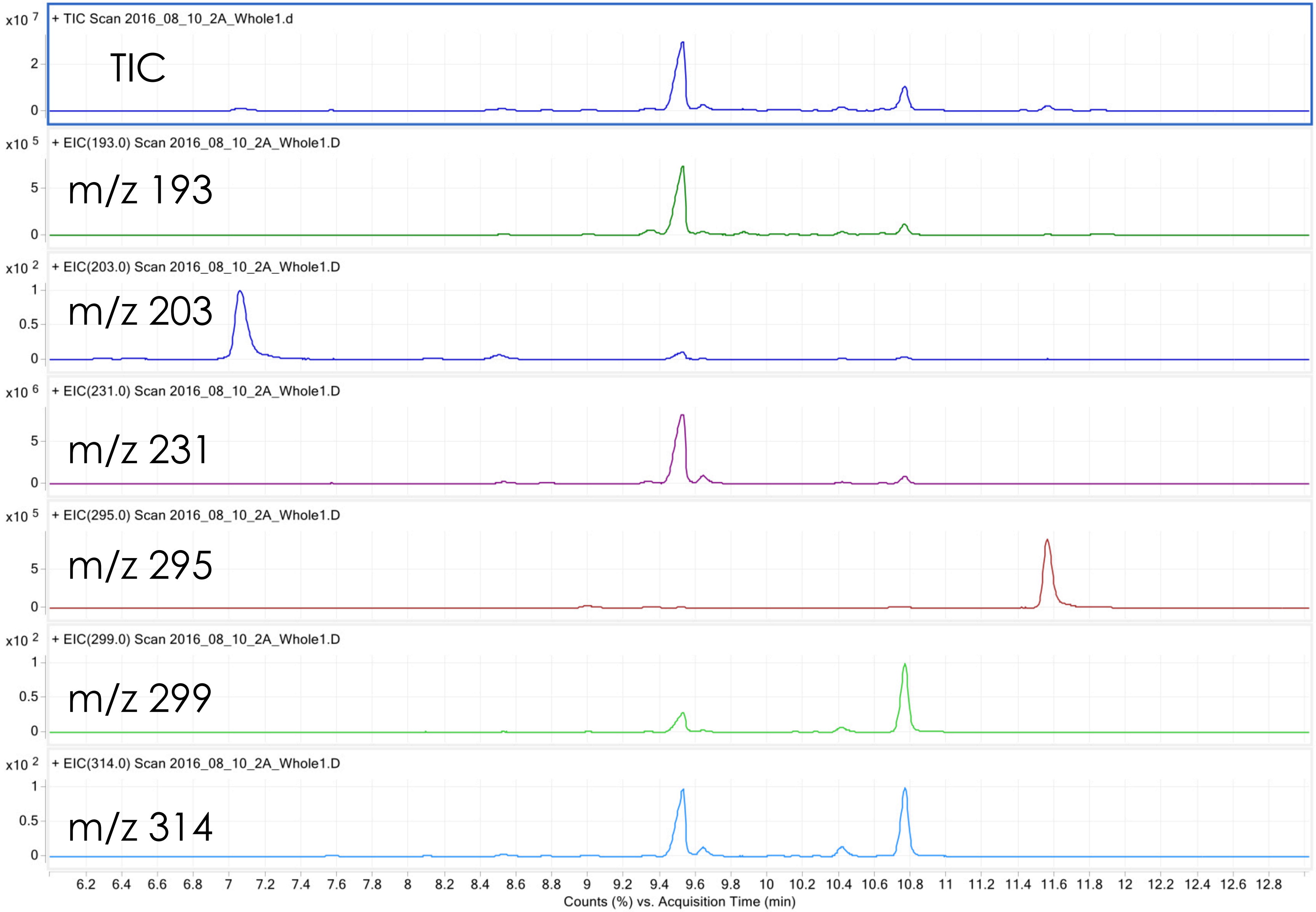
		Prediction accuracy (%, six features)		Prediction accuracy (%, four features)	
		Group 1	Group 2	Group 1	Group 2
Truth	Group 1	98.0	2.0	100	0
	Group 2	0	100	0	100

Group 1: Sample 2
Group 2: Sample 6, 9, 11

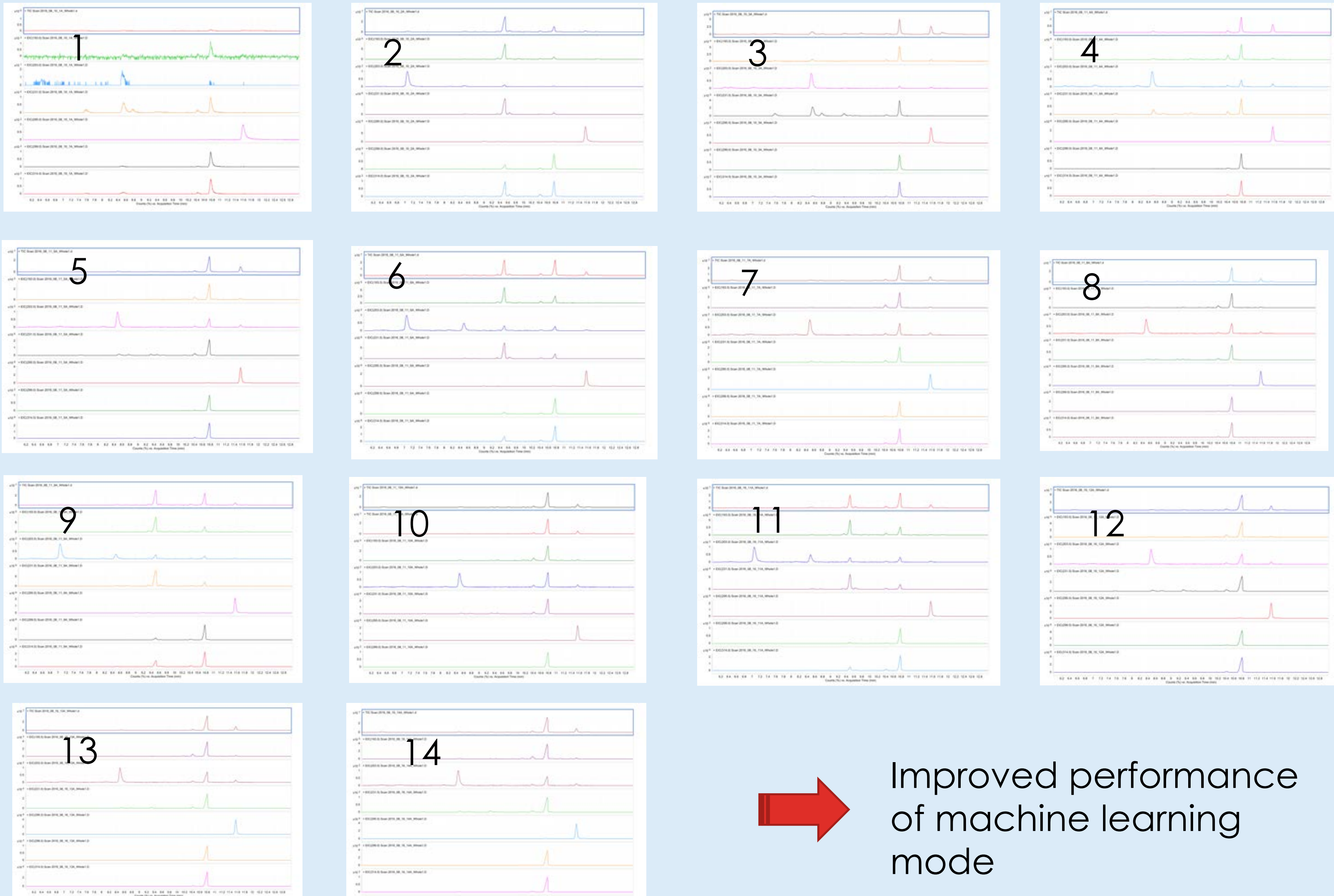
Determination of Marijuana Varieties



Extract Ion Profiles



Extraction Ion Profiles

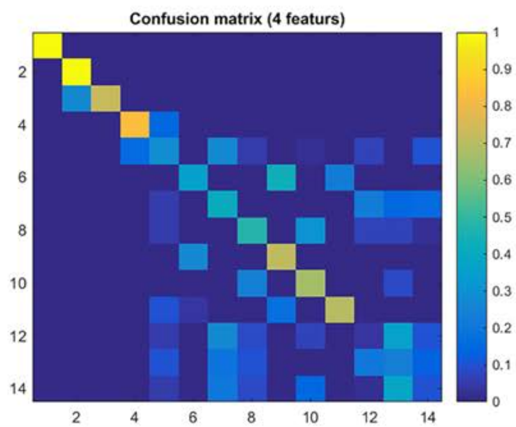


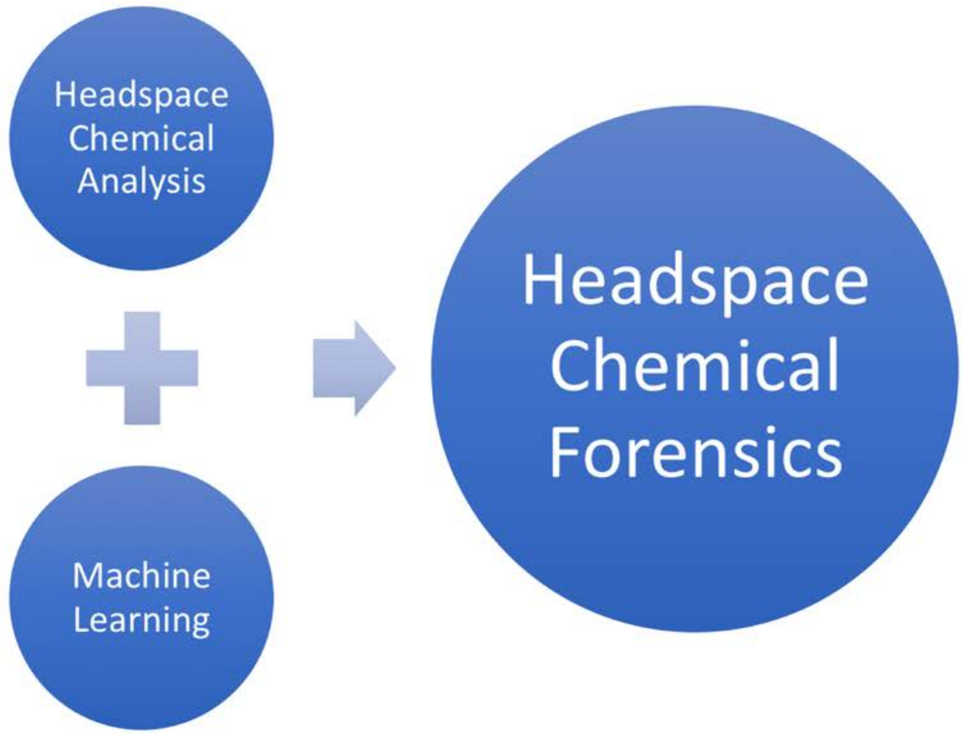
Improved performance of machine learning mode

Conclusion

- This new extraction and data analysis procedure for marijuana samples is solvent-free and can nearly non-destructively capture chemical attribution signatures from 10 mg of marijuana sample.
- No sample preparation is required and the entire marijuana intelligence production can be automated without human intervention.
- The HHS-SPME-GC/MS headspace chemical analysis testing platform combining with machine learning technology potentially offer a new way for chemical forensics.

New Platform for Chemical Forensics





This analytical platform is versatile and can be easily adopted by any crime labs with a

Future Development

- Collect ground truth marijuana samples with known source/attributes for headspace chemical analysis.
- Controlled substance analysis
  - Chemical forensics for Fentalogs.
  - Synthetic routes for designer drugs.
  - Toxins, botanicals, heroin, cocaine, etc.
- Trace evidence analysis
  - Residual VOCs in 3D printed materials to source the origin.
  - Residual drug detection and source prediction.

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Questions?

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