

# An Evaluation of Growing Environment on the Production of Secondary Metabolites in Cannabis

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# Introduction



# Background

- Cannabis cultivation options
- Environmental impact
- Plant compound production?



# Variables

- Plant genetics
- Nutrition/irrigation
- Lighting due to plant orientation





# Hypothesis

- Clones sharing the same genetic information will express different secondary metabolites as a function of the environmental conditions that they are grown in



# Design

- Three cultivators
- Five cultivars
- Indoor and outdoor growing environments
- Samples were provided to two labs for analysis



# Methodology

- Targeted quantitative analysis
- Untargeted broad range scan





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# Results





# Overview of Findings

- Targeted approach
  - Trends show higher secondary metabolites in samples grown outdoors
- Untargeted approach
  - Greater number of larger oxygenated sugar conjugated species vs lighter oxygenated species such as linalool, terpinolene, and borneol
- Strain specific trends
- Heavy metal observation

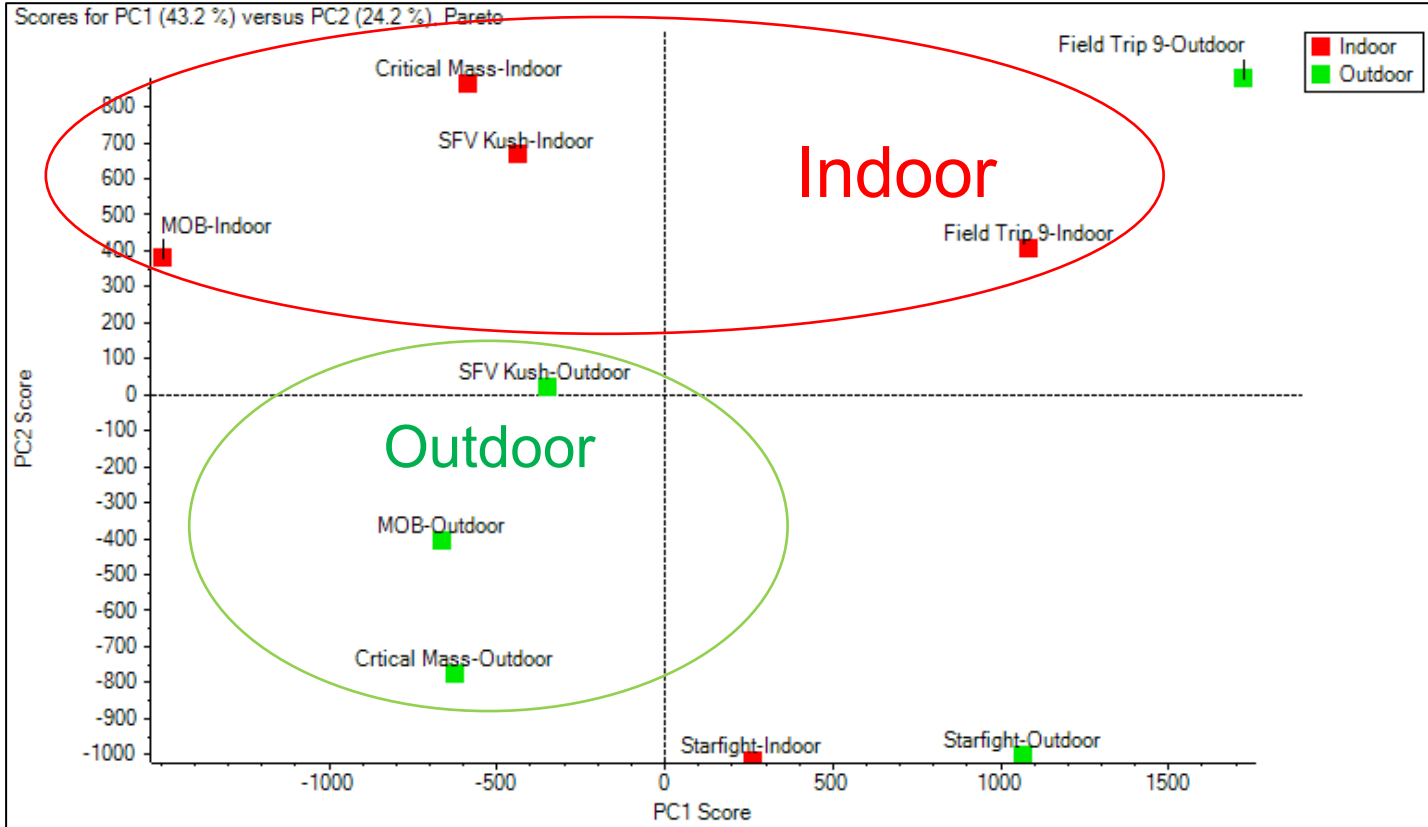
# Targeted Data Tables

Sample Name/ Target Analyte WT%	Field Trip 9 Indoor	Field Trip 9 Outdoor	Starfighter Indoor	Starfighter Outdoor	Critical Mass Indoor	Critical Mass Outdoor	SFV Kush Indoor	SFV Kush Outdoor	MOB Indoor	MOB Outdoor
D9 THC	0.5	<b>0.4</b>	0.3	<b>0.5</b>	3.5	<b>0.6</b>	2.1	<b>1.0</b>	3.2	<b>1.0</b>
THCa	25.0	<b>27.8</b>	16.1	<b>20.4</b>	16.4	<b>15.4</b>	17.0	<b>18.4</b>	12.9	<b>11.4</b>
CBGa	0.8	<b>0.9</b>	0.9	<b>2.1</b>	0.5	<b>0.6</b>	0.2	<b>0.4</b>	0.2	<b>0.5</b>
<b>Total Cannabinoids</b>	27.0	<b>30.1</b>	17.5	<b>24.0</b>	20.8	<b>17.0</b>	19.6	<b>20.8</b>	17.1	<b>13.5</b>
<b>Total Terpenes</b>	1.8	<b>2.2</b>	0.9	<b>1.3</b>	0.5	<b>0.9</b>	0.8	<b>1.2</b>	0.9	<b>1.4</b>
<b>Total Measured Metabolites</b>	28.8	<b>32.3</b>	18.4	<b>25.2</b>	21.3	<b>17.9</b>	20.4	<b>21.9</b>	17.9	<b>14.9</b>

# Targeted Data Tables

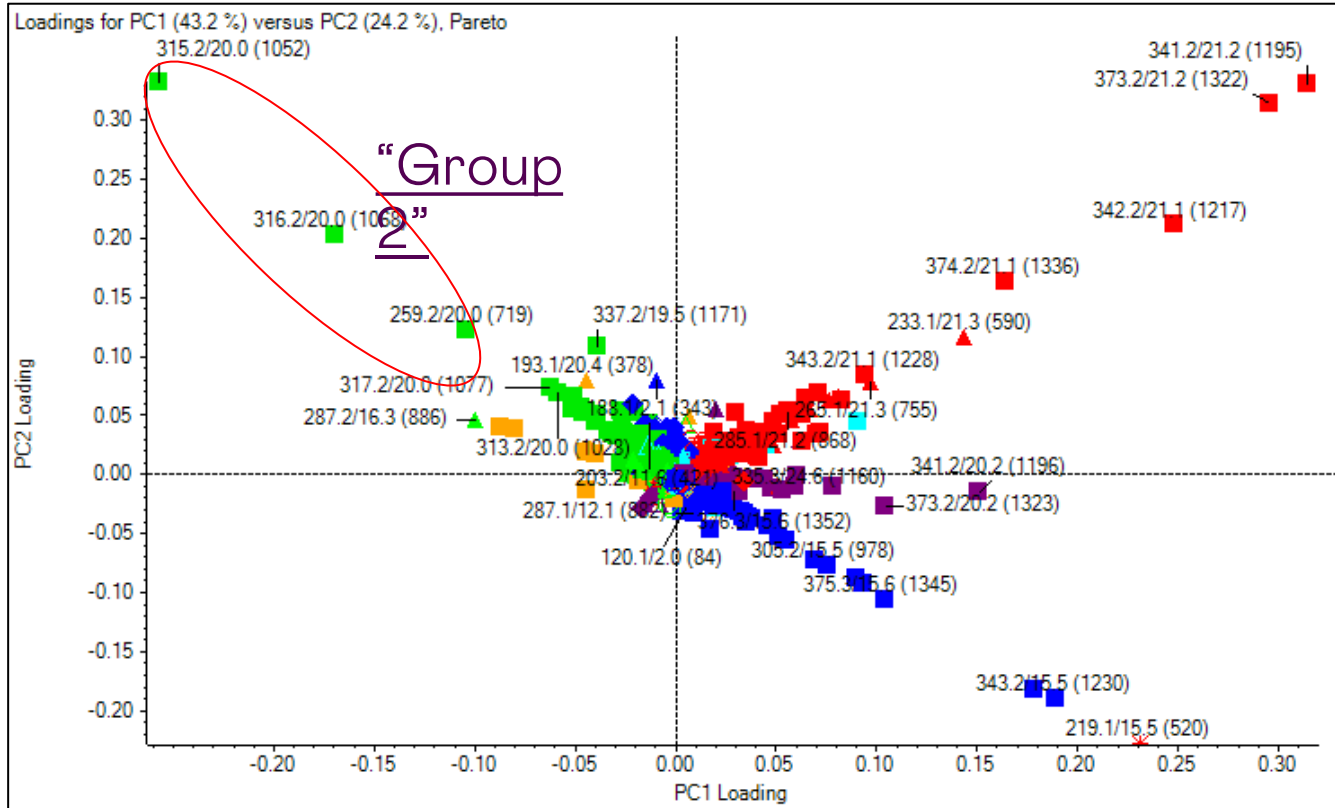
Sample Name/ Target Analyte PPB	Field Trip 9 Indoor	Field Trip 9 Outdoor	Starfighter Indoor	Starfighter Outdoor
<b>Arsenic</b>	BQL	ND	ND	ND
<b>Cadmium</b>	125.5	ND	181.9	BQL
<b>Mercury</b>	BQL	ND	BQL	ND
<b>Lead</b>	BQL	BQL	BQL	BQL

## Score Plot – Indoor/Outdoor Differences

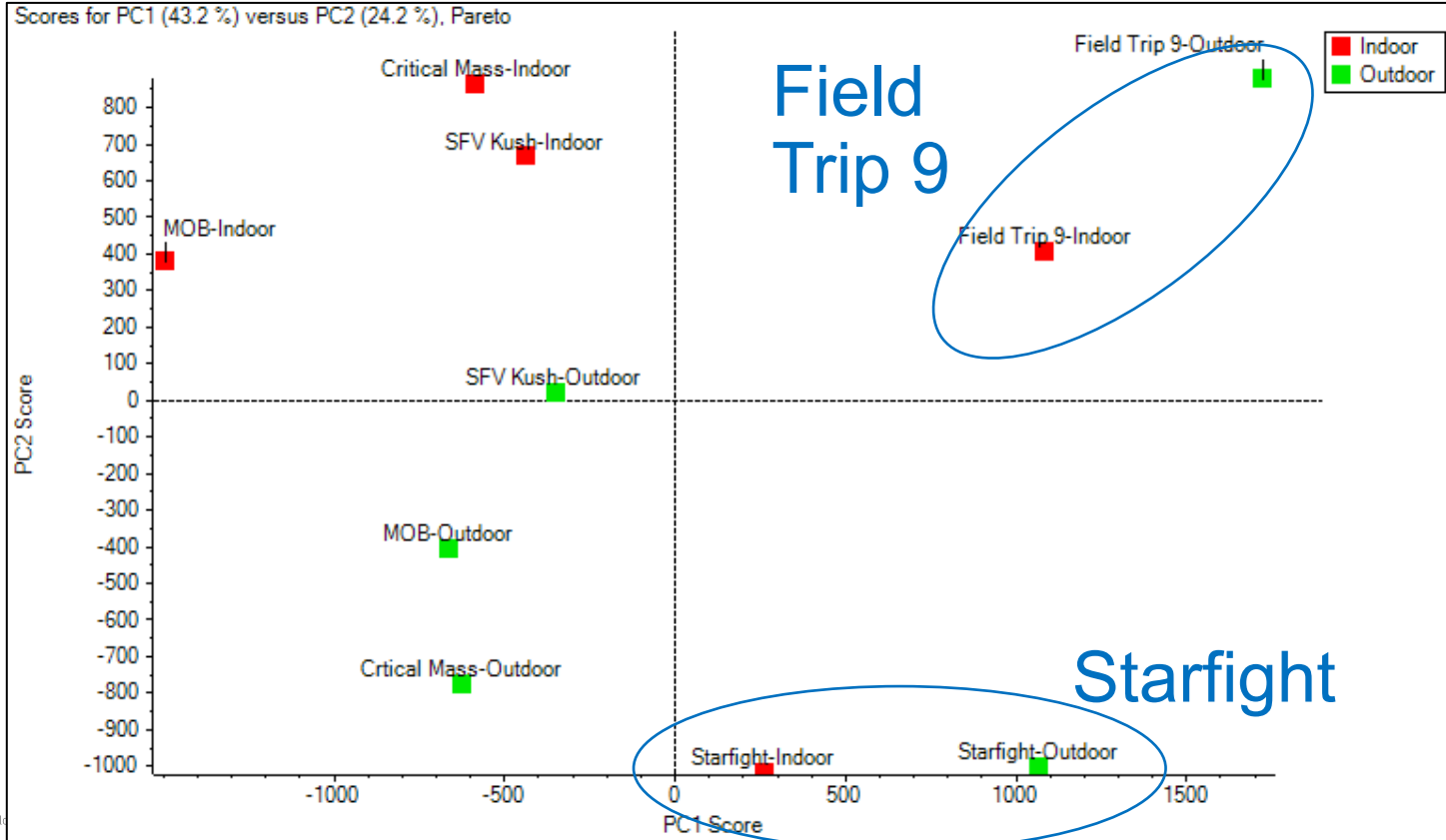




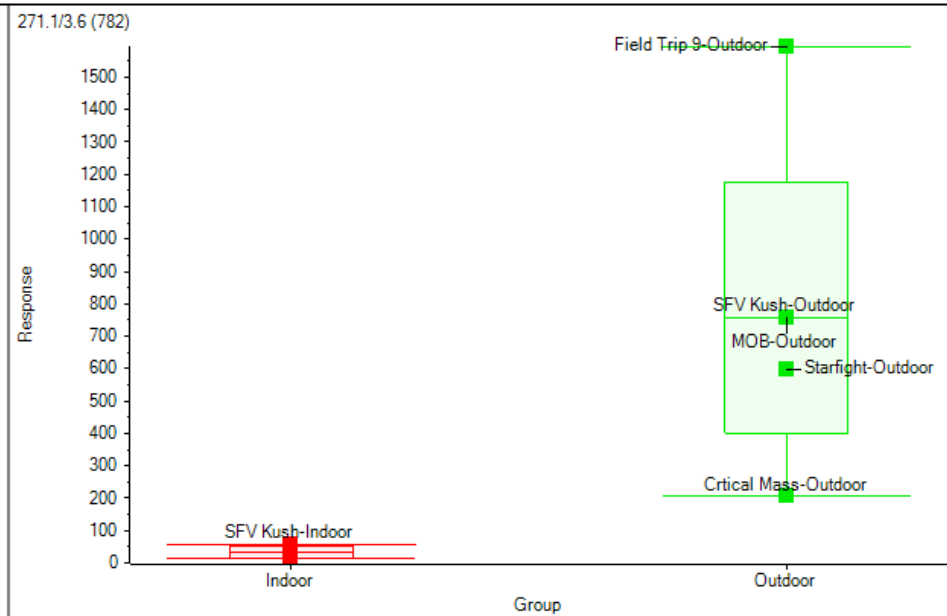
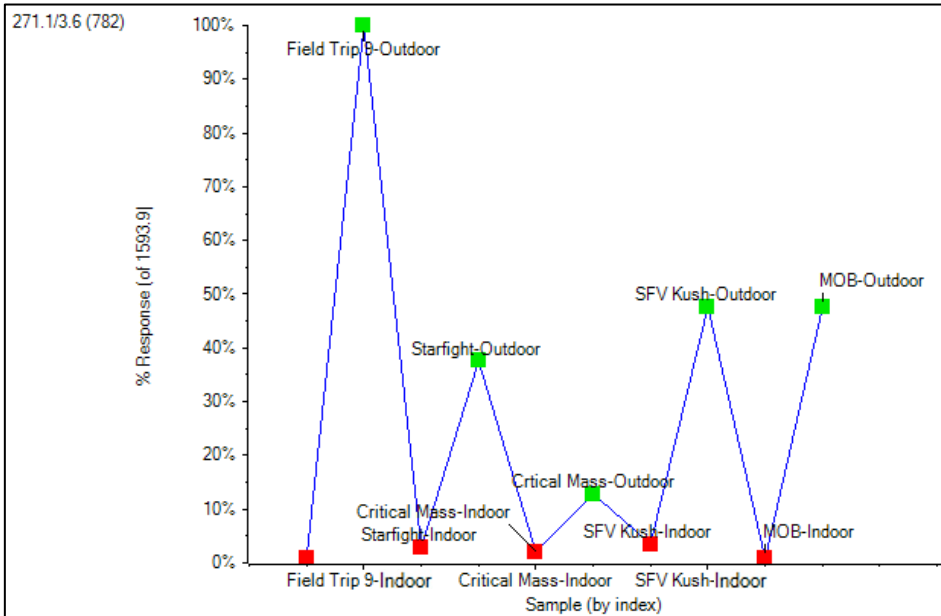
# PCA: PC1 vs PC2 – Loadings Plot – Group 2



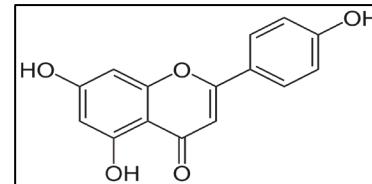
## Score Plot – Unique Strains



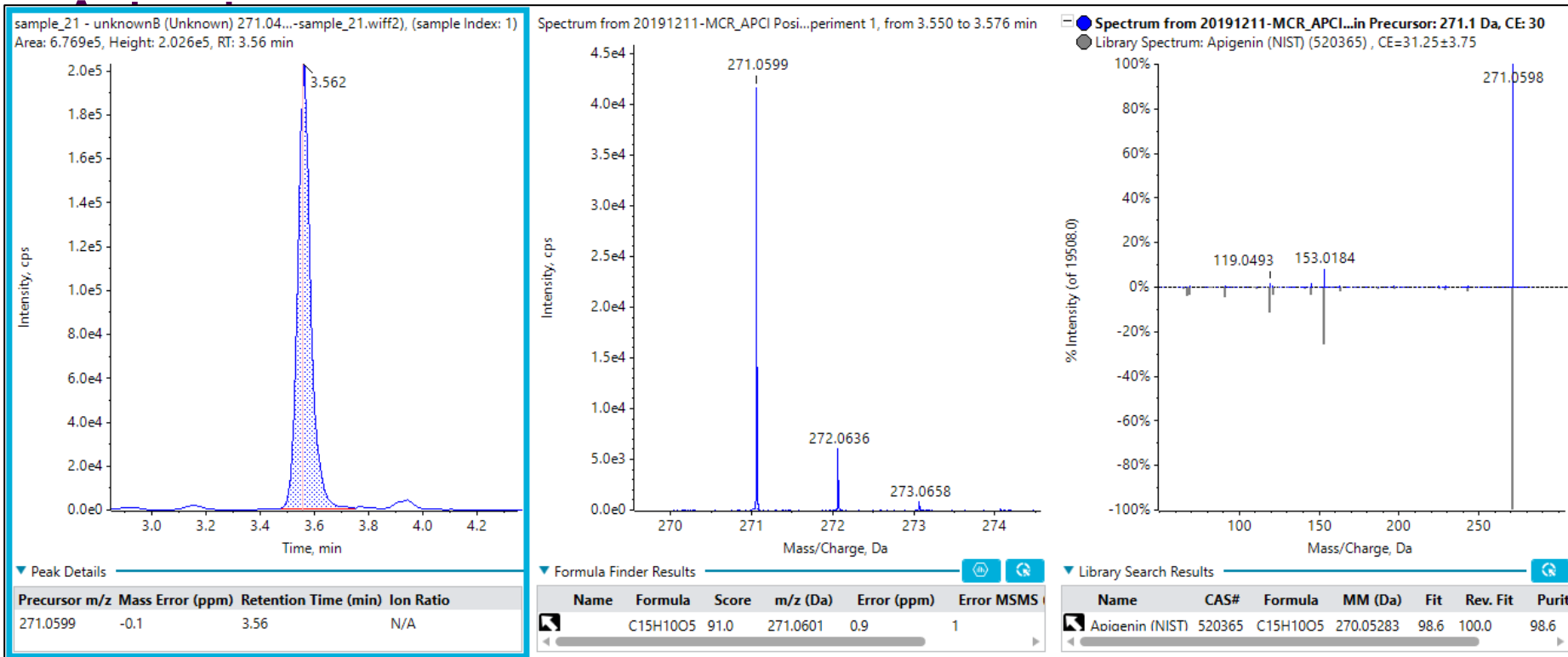
# Unknown A: m/z 271.060, RT = 3.6 min



## Unknown A: m/z 271.060, RT = 3.6 min

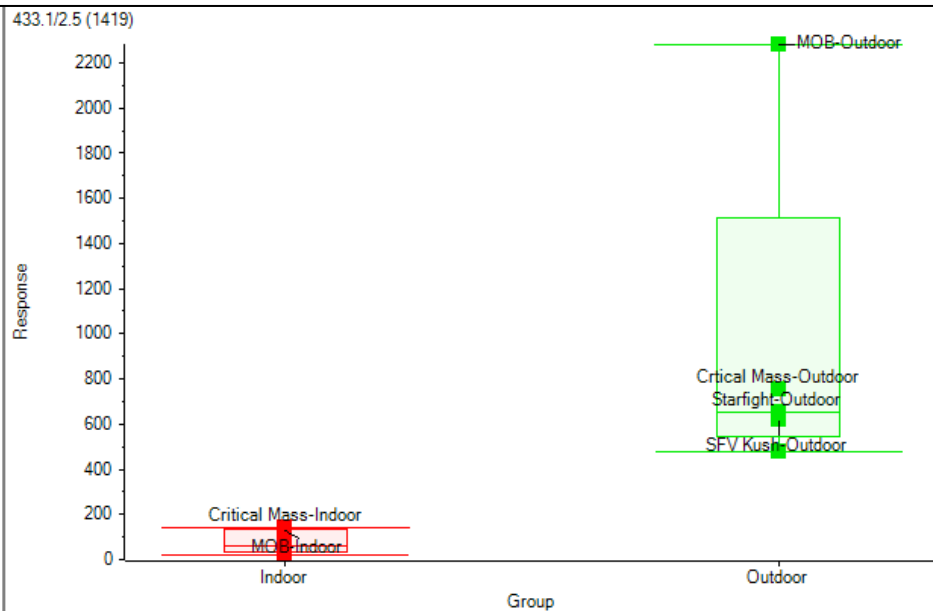
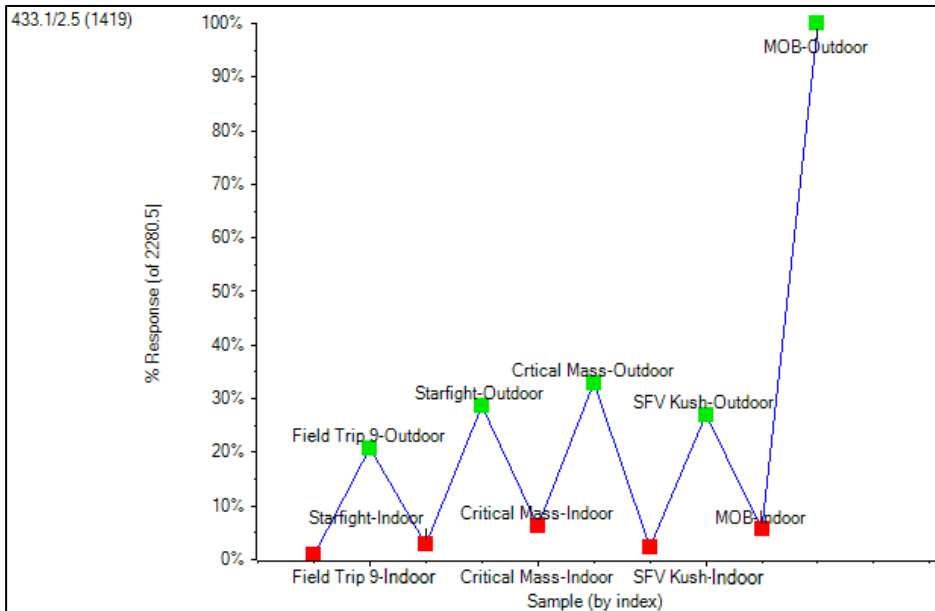


### • NIST Library Match =

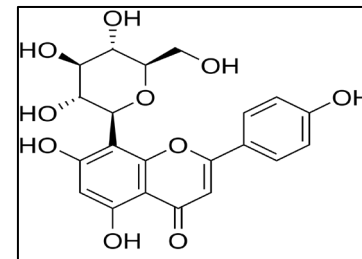




## Unknown B: m/z 433.1122, RT = 2.5 min

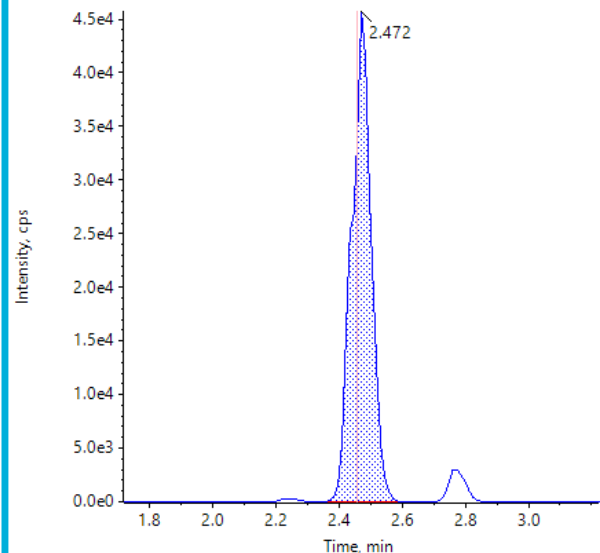


## Unknown B: m/z 433.1122, RT = 2.5 min



### • NIST Library Match =

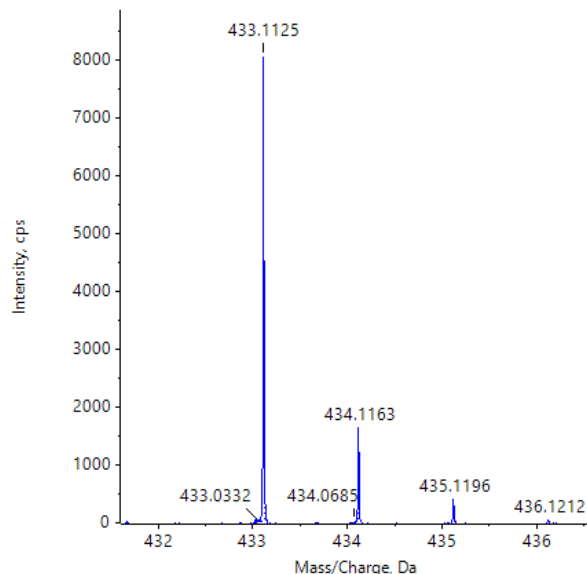
sample\_23 - unknownBB (Unknown) 433....-sample\_23.wiff2), (sample Index: 1)  
Area: 1.977e5, Height: 4.571e4, RT: 2.47 min



▼ Peak Details

Precursor m/z	Mass Error (ppm)	Retention Time (min)	Ion Ratio
433.1122	0.7	2.47	N/A

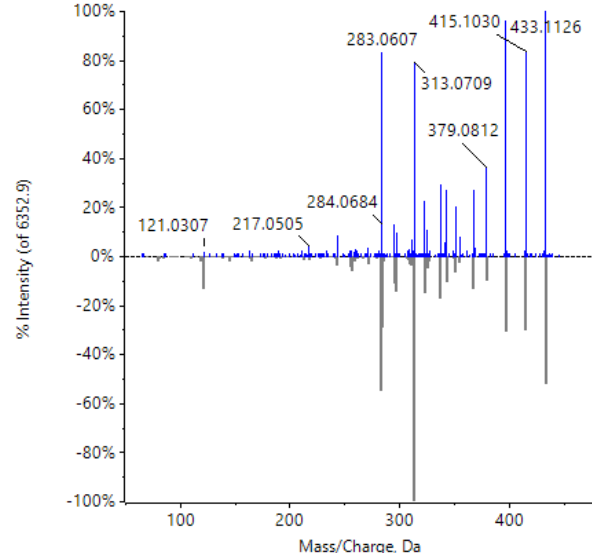
Spectrum from 20191211-MCR\_APCI Posi...periment 1, from 2.458 to 2.483 min



▼ Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MSMS
	C21H20O10	90.1	433.11292	1	1

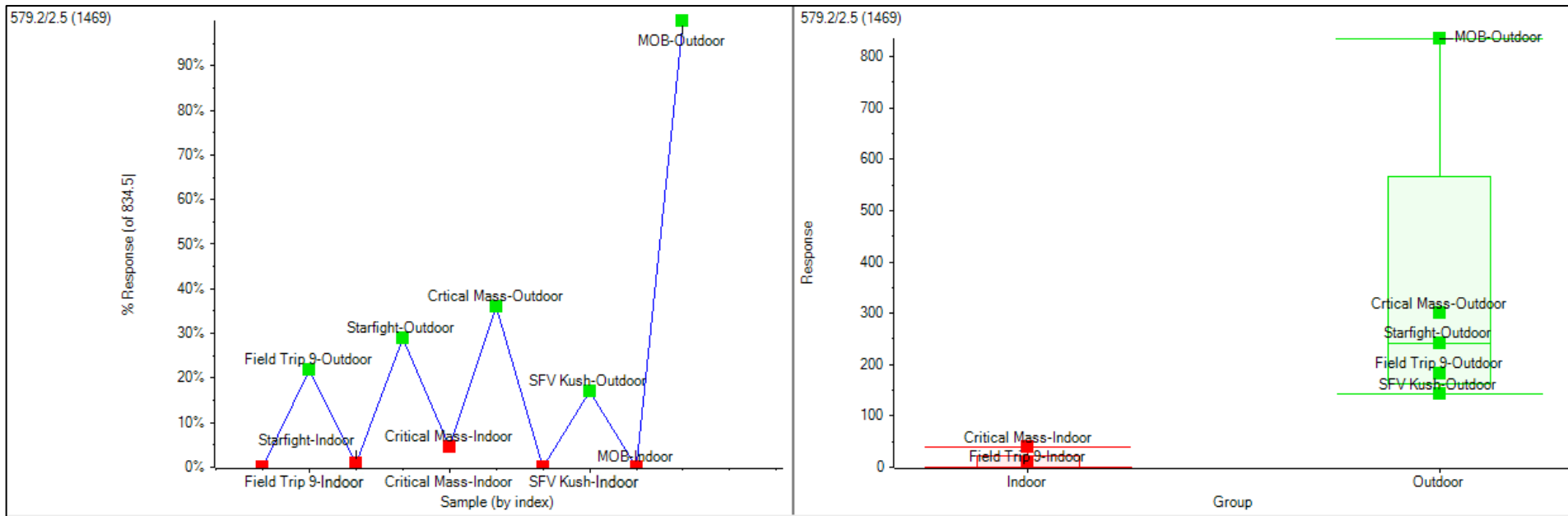
● Spectrum from 20191211-MCR\_APCI...in Precursor: 433.1 Da, CE: 30  
● Library Spectrum: Vitexin (NIST) (3681934), CE=49.140625±8.859375



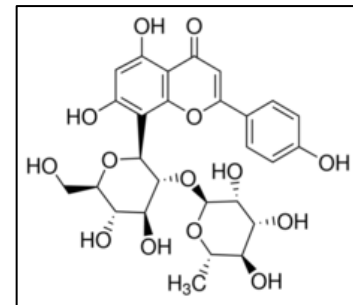
▼ Library Search Results

Name	CAS#	Formula	MM (Da)	Fit	Rev. Fit	Purity
	Vitexin (NIST) 3681934	C21H20O10	432.10565	99.6	99.7	99.3

## Unknown B Conjugate: m/z 579.1703, RT = 2.5 min

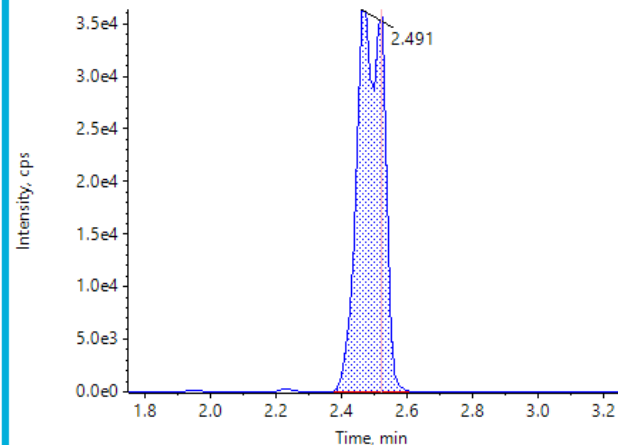


## Unknown B Conjugate: m/z 579.1703, RT = 2.5 min



- NIST Library Match = Vitexin-2'-O-Rhamnoside

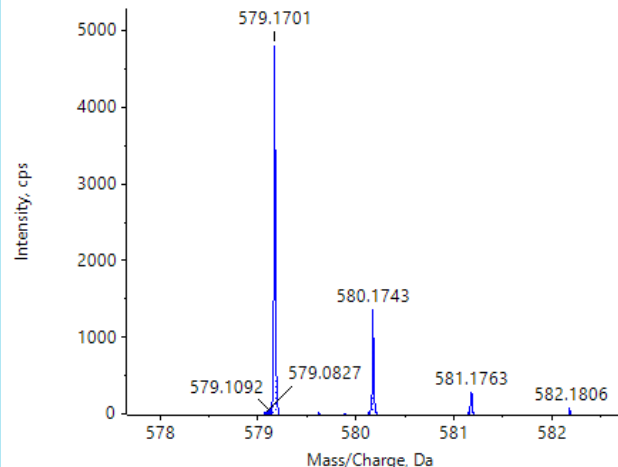
sample\_29 - unknownCC (Unknown) 579....-sample\_29.wiff2), (sample Index: 1)  
Area: 2.138e5, Height: 3.631e4, RT: 2.49 min



▼ Peak Details

Precursor m/z	Mass Error (ppm)	Retention Time (min)	Ion Ratio
579.1703	-0.4	2.49	N/A

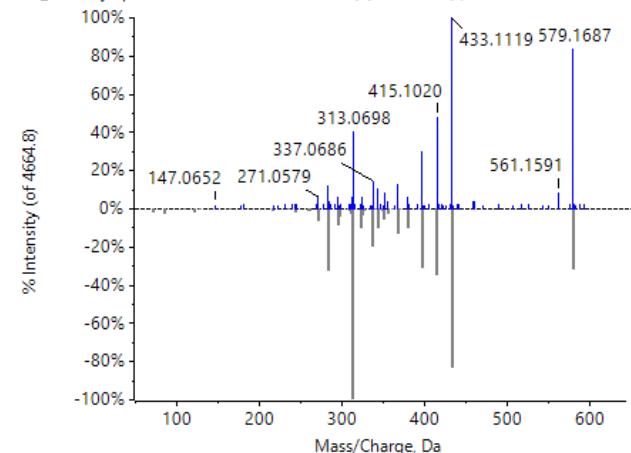
Spectrum from 20191211-MCR\_APCI Posi...periment 1, from 2.475 to 2.500 min



▼ Formula Finder Results

Name	Formula	Score	m/z (Da)	Error (ppm)	Error MS1
	C32H26N4O5S	89.4	579.16967	0.7	1.7

● Spectrum from 20191211-MCR\_APCI...in Precursor: 579.2 Da, CE: 30  
● Library Spectrum: Vitexin-2'-O-rha...IST) (64820991) , CE=51.5625±5.4375

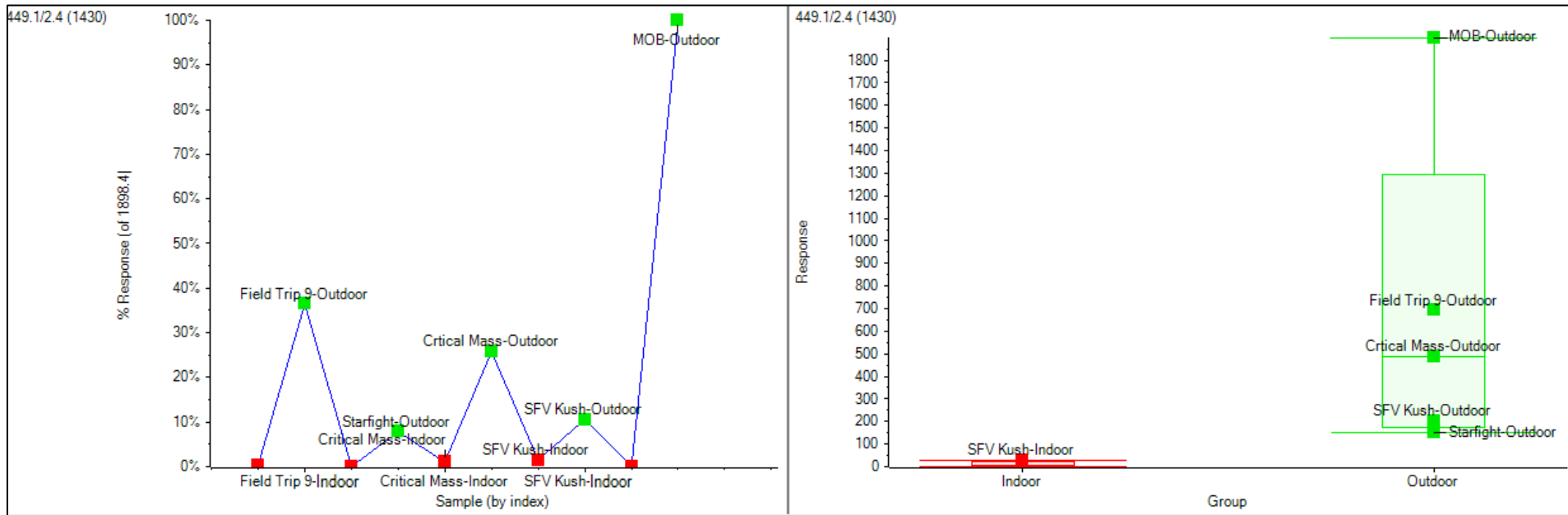


▼ Library Search Results

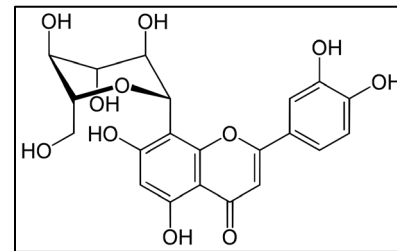
Name	CAS#	Formula	MM (Da)	Fit
Vitexin-2'-O-rhamnoside (NIST)	64820991	C27H30O14	578.16357	93.2



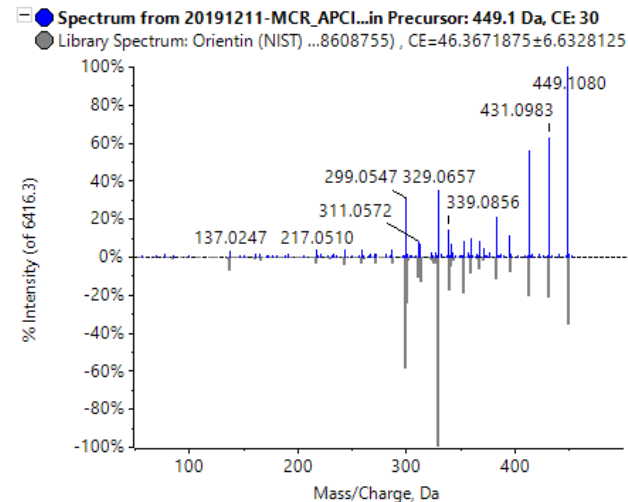
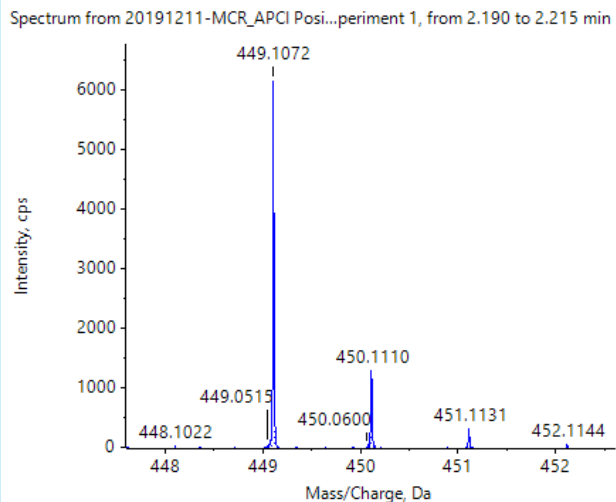
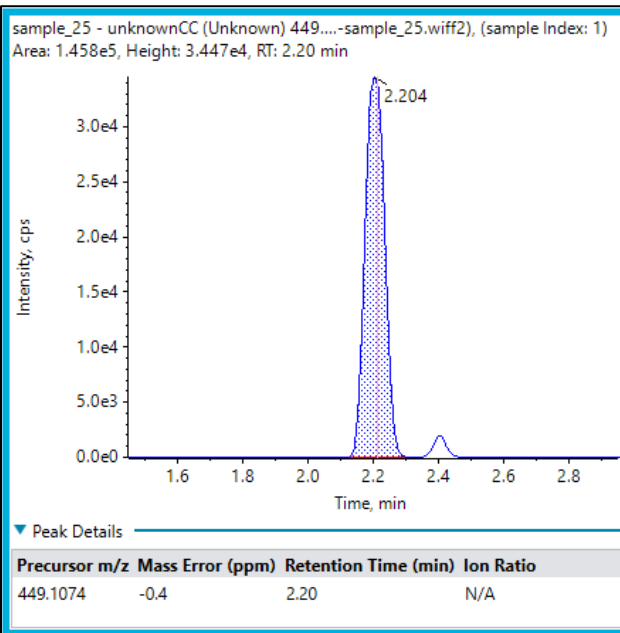
## Unknown C: m/z 449.1074, RT = 2.4 min



## Unknown C: m/z 449.1074, RT = 2.4 min



- NIST Library Match = Orientin



# Targeted Approach Findings

- Levels of secondary metabolites (cannabinoids, terpenes, etc.) were elevated in outdoors samples vs indoor samples overall
- Limited data for heavy metals shows that the two strains evaluated showed cadmium detected in the indoor plants but not outdoor plants

# Untargeted Approach Findings

- Indoor & outdoor samples are mostly separated by PC2
- Some strains are unique, and their indoor vs outdoor profiles are similar
- The types of natural products that are elevated are different depending on if it is grown indoors or outdoors

# Conclusion

- Cultivation method impacts both amount and type of secondary metabolites
- Implications for recreational and medical cultivation
- Future studies



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# Q&A

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