Application News

Gas Chromatograph Mass Spectrometer

Simplified Cannabis Terpene Profiling by GCMS

No. GCMS-1604

■ Introduction

Terpene and terpenoid compounds are naturally occurring aromatic compounds that give cannabis its unique flavor and fragrance. Aside from their aromatic properties, terpenes have advantageous health benefits. They also have a synergistic relationship with cannabinoids, which further enhance the therapeutic effect of THC.

Cannabis has over 140 terpene components, many of which are of medicinal interest.[1] Predominant terpenes in cannabis include β-myrcene, which has antibiotic properties and enhances the THC muscle relaxant effect; α-pinene, which has anti-inflammatory properties and enhances the THC bronchodilator effect; and β-caryophyllene, which also has anti-inflammatory properties and enhances the THC gastric cytoprotective effect amongst other health benefits. [2, 3] The concentration of individual terpenes varies by strain, can be anywhere from 0.1 to 1.5% of its total dry weight, and can vary depending on harvest time, drying and storage conditions.[1, 4] Terpene levels can decrease over time, and after three months of storage, can reduce terpene levels by more than half.[4] The decrease in terpene amount over time varies for different terpenes.

Recent proliferation of new terpene profiling methods can be attributed to the ever-increasing state legalization of cannabis use. The state of Nevada added the testing of ten terpenes in accordance with NRS 453A.368 in May of 2015.[5] Due to the uniqueness of terpene profiles, they can be used by cultivators as a "fingerprint" to partially ID the specific strain in question. This application note describes the analysis of several strains of cannabis for 41 terpenes on Gas Chromatography Mass Spectrometry (GCMS) with headspace injection.

■ Experimental

Terpene analyses were conducted using a Shimadzu GCMS-QP2010SE single quadrupole mass spectrometer with the HS-20 headspace autosampler for sample introduction. As terpene concentration can be in the percent level, effluent from the HS-20 was split 50-to-1 to allow for analyzing high concentration standards. The MS was run using the FAAST (Fast Automated SCAN/SIM Type) where in the SCAN mode was used for identification and the SIM mode was used for greater quantitation. The instrument and operating conditions are shown in Table 1.

Shimadzu GCMS-QP2010SE

Table 1: Instrument Operating Conditions and Method Parameters

Head Space	**HS-20 Loop Model**
Operation Mode	Static headspace with loop
Sample	10uL sample volume 10-mL headspace vial
Equilibration	30 minutes at 150ºC
Sample Loop	1-mL loop Vial pressurization 1.00 min, equilibration 0.20 min Loop load time 1.00 min, equilibration 0.20 min Injection time 1.0 min
Sample Pathway Temperature	150ºC
Transfer Line Temperature	150ºC

Gas Chromatogram	**GC-2010 Plus**
Injection	Split injection from HS-20, with 50:1 split ratio
Column	Rxi-624 Sil MS 30.0m x 0.25 mm x 1.40 µm Helium carrier gas Constant linear velocity, 47.2 cm/sec Column Flow 1.64 mL/min Purge Flow 3.0 ml/min
Oven Program	80 ºC, hold 1.0 min 12 ºC /min to 150 ºC, hold 1.0 min 9 ºC /min to 250 ºC, hold 1 min Total GC run time 19.94 min Total cycle time 24 min

Detector	**GCMS-QP2010 SE**
Operating mode	Selected Ion Monitoring (SIM) and SCAN
Ion Source	200 ºC, EI mode, 70eV
Solvent Cut Time	2 min
MS Interface	300 ºC

Sample Preparation

In order to obtain a more complete terpene profile, two sets of standards were used. Standard one, purchased from SPEX (Metuchen, NJ), included 38 terpenes in a 100µg/mL stock solution. Standard two, purchased from Restek (Bellefonte, PA), included 23 terpenes in a 2500 µg/mL stock solution.

As plant material does not dissolve in solvent, the full evaporation headspace technique (FET) is used for quantitation. Using FET, a small amount of standard and sample is used to create a single phase gas system, as compared to a two phase liquid-gas system as in traditional headspace techniques.[6] A five-point calibration curve was created from the SPEX terpene standard with concentrations ranging from 12.5-100 µg/mL. An aliquot of 10µL of the standard was placed in a 10mL headspace vial and capped. A seven-point calibration curve was created from the Restek standard in a similar fashion as described above, with the concentration range from 78.125 µg/mL to 2500 µg/mL. The calculated amount of terpene injected on column is shown in Table 2.

Table 2: Calculated amount of terpene standard on column

Standard	**Concentration (µg/mL)**	**Injection Volume (mL)**	**Amount On-Column (µg)**
SPEX	12.5	0.01	0.125
SPEX	25	0.01	0.25
SPEX	50	0.01	0.50
SPEX	75	0.01	0.75
SPEX	100	0.01	1.0
Restek	78.125	0.01	0.78
Restek	156.25	0.01	1.25
Restek	312.5	0.01	3.25
Restek	625	0.01	6.25
Restek	1250	0.01	12.5
Restek	2000	0.01	20.0
Restek	2500	0.01	25.0

A part of the flower weighing 1.0 gram was frozen, followed by grinding to ensure a representative sample. Ten to 30mg of the flower were then weighed into a headspace vial and capped. The final result was calculated to give wt %.

■ Results and Discussion

Chromatography

Figures 1a and 1b show the TIC chromatographic separation using the SPEX and Restek standards.

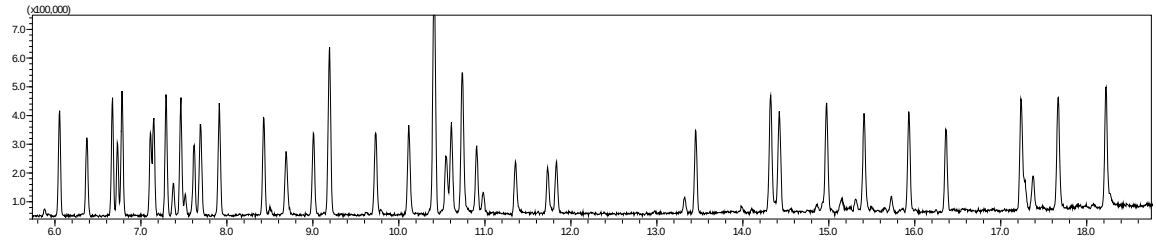


Figure 1a: TIC from SPEX standard

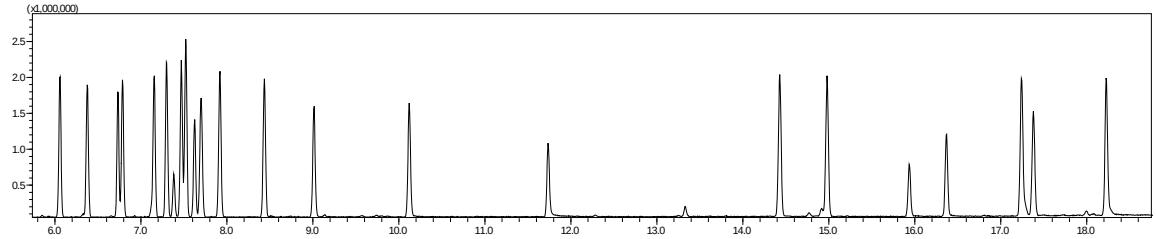
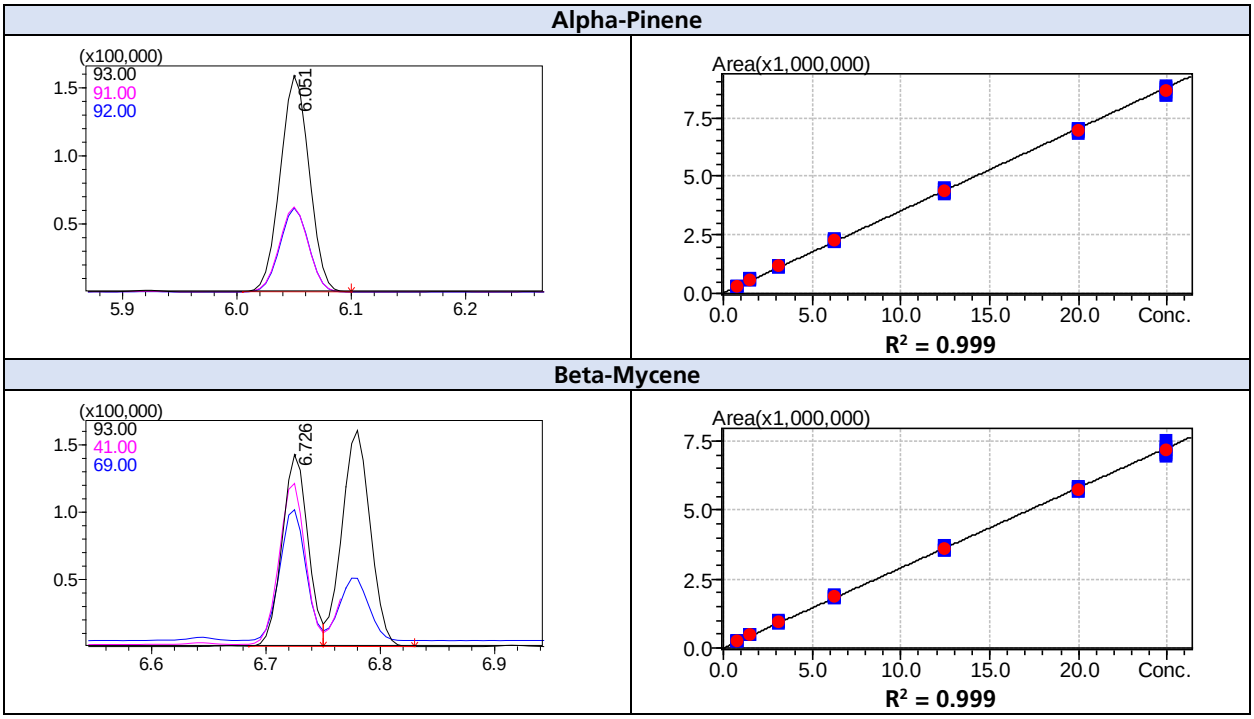


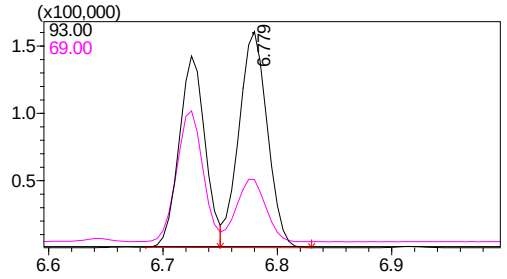
Figure 1b: TIC chromatogram from Restek standard

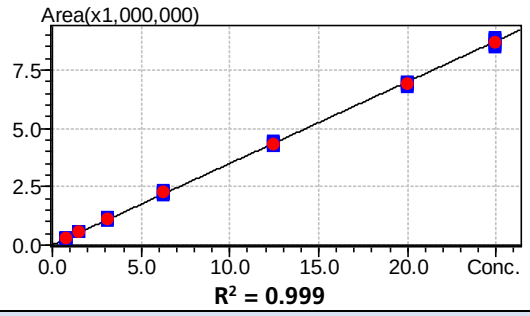
Calibration

Calibration standards were prepared and analyzed using the optimized HS-20 and GC-MS parameters with concentrations ranging from 0.125µg to 0.1µg using the SPEX terpene standard, and 0.78µg to 25µg using the Restek standard. All points on the calibration curve were run in replicates of six. Figure 2 shows the terpene calibration curves required by the state of Nevada with corresponding correlation coefficients illustrating linearity. The SIM chromatograms represent the lowest calibration point (0.78µg) for each terpene. All of the chromatographic peaks were easily integrated at each level. Full statistics on all calibration points are listed in Table 3a and 3b.



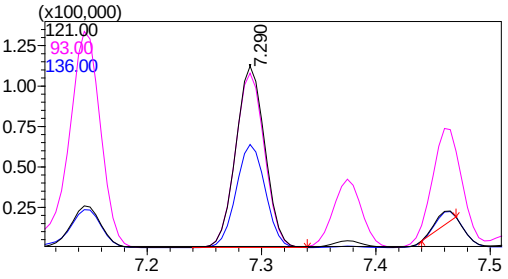
Beta-Pinene

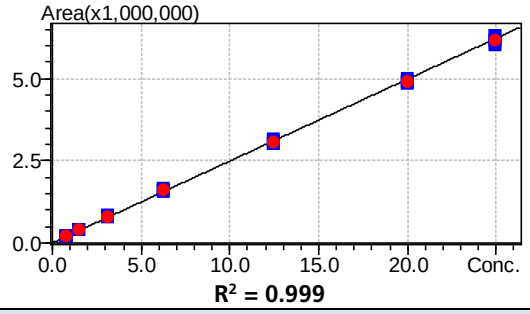




$R^2 = 0.999$

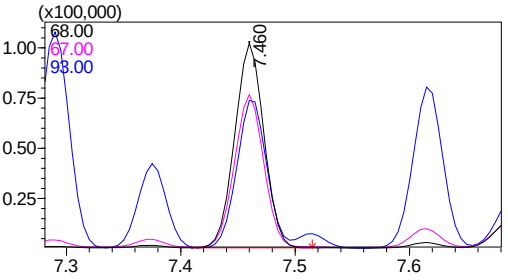
alpha.-Terpinene

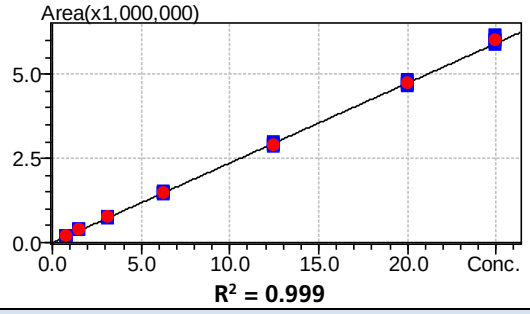




$R^2 = 0.999$

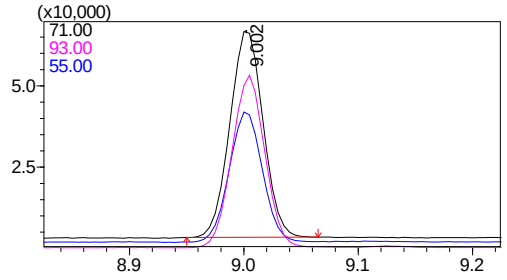
(R)-(+)-Limonene

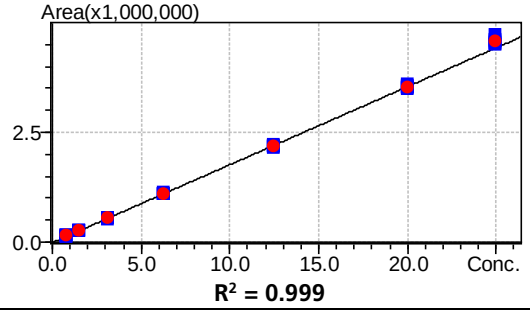




$R^2 = 0.999$

Linolool





$R^2 = 0.999$

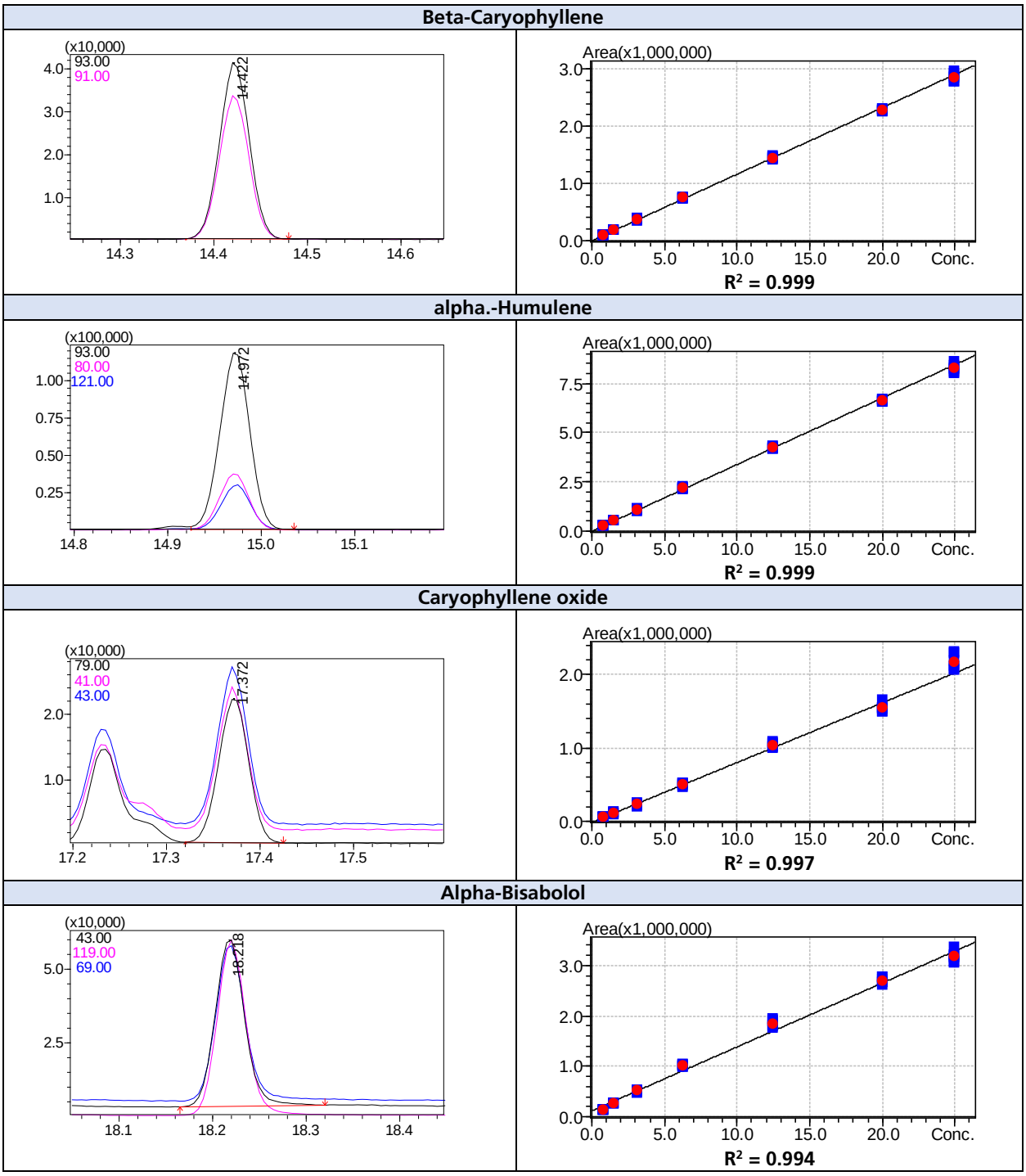


Figure 2: Calibration curves for Restek terpene testing required by the state of Nevada; ranges shown are 1.25µg to 25µg on column

Table 3a: 5-point calibration and precision results for analysis of terpenes (SPEX standards)

Terpene	Calibration Results		Precision at 12.5ppm (n=6)		Precision at 100 ppm (n=6)	
	RF RSD (%)	R^2	Mean Conc	RSD (%)	Mean Conc	RSD (%)
Alpha-Pinene	5.549	0.998	0.123	0.796	0.948	2.980
Camphene	3.642	0.999	0.124	1.164	0.964	3.734
Sabinene	3.440	0.999	0.121	1.447	0.972	2.005
beta.-Myrcene	3.732	0.997	0.126	1.317	0.994	3.038
beta.-Pinene	3.998	0.998	0.124	1.180	0.963	2.294
.alpha.-Phellandrene	4.367	0.999	0.124	3.533	0.959	2.602
(1S)-3-Carene	5.247	0.999	0.124	3.386	0.978	2.841
alpha.-Terpinene	5.916	0.996	0.107	1.269	0.935	1.982
trans-.beta.-Ocimene	3.757	0.998	0.125	1.041	0.957	2.857
(R)-(+)-Limonene	5.817	0.997	0.123	0.887	0.950	1.914
Ocimene (mixture of isomers)	4.109	0.997	0.124	1.334	0.950	2.776
Eucalyptol	3.675	0.998	0.124	0.766	0.952	2.720
gamma.-Terpinene	3.695	0.998	0.124	0.908	0.947	1.954
Terpinolene	4.095	0.998	0.124	1.024	0.948	1.426
Sabedine Hydrate	9.252	0.995	0.122	2.926	0.950	5.300
Linolool	2.863	0.999	0.126	1.763	0.989	2.055
Fenchone (mix of isomers)*	0.607	0.999	0.125	1.011	-	-
(R)-Endo-(+)-Fenchyl Alcohol	5.338	0.999	0.125	4.560	0.980	0.979
Isopulegol	2.433	0.999	0.126	2.210	0.981	2.109
Camphor (mix of isomers)*	0.785	0.999	0.125	0.952	-	-
Isoborneol	8.659	0.992	0.130	6.949	1.050	2.745
Hexahydrothymol	4.979	0.998	0.128	3.287	1.005	1.939
Borneol (isomers)	4.774	0.997	0.125	5.422	0.983	2.094
Terpineol (mix of isomers)	1.376	0.999	0.125	1.909	0.981	1.242
gamma.-Terpineol	2.161	0.999	0.126	1.657	0.985	1.359
Nerol	11.23	0.998	0.127	6.418	0.997	3.828
Geraniol	14.27	0.999	0.125	8.386	0.985	2.858
Pulegone	1.859	0.999	0.124	2.093	0.973	1.841
Geraniol acetate	3.574	0.998	0.126	0.922	0.979	2.142
alpha.-Cedrene	3.058	0.999	0.124	1.087	0.958	1.630
Trans-Caryophyllene	2.899	0.999	0.126	1.899	1.021	4.524
alpha.-Humulene	2.146	0.999	0.125	1.180	0.969	1.652
Valencene	2.382	0.999	0.126	1.427	0.979	2.107
cis-Nerolidol	3.688	0.999	0.126	1.141	0.993	2.531
trans-Nerolidol	1.577	0.999	0.125	5.449	0.988	1.360
Guaiol	1.565	0.999	0.125	1.246	0.976	1.237
(+)-Cedrol	3.077	0.999	0.126	1.211	0.984	1.750
.alpha.-Bisabolol	2.911	0.999	0.126	1.862	0.986	1.536

* Data at the high level is not applicable

Table 3b: 7-point calibration and precision results for analysis of terpenes (SPEX standards)

Terpene	Calibration Results		Precision at 78.125 ppm (n=6)		Precision at 2500 ppm (n=6)	
	RF RSD (%)	R^2	Mean Conc	RSD (%)	Mean Conc	RSD (%)
Alpha-Pinene	2.545	0.999	0.764	1.478	24.614	2.12
Camphene	2.095	0.999	0.766	1.674	24.501	2.195
beta.-Myrcene	1.833	0.999	0.768	1.599	24.604	3.013
beta.-Pinene	1.973	0.999	0.767	1.386	24.725	1.948
(1S)-3-Carene	2.029	0.999	0.767	1.412	24.690	2.390
alpha.-Terpinene	2.150	0.999	0.766	1.611	24.706	1.940
trans-.beta.-Ocimene	1.746	0.999	0.777	1.349	25.381	2.525
(R)-(+)-Limonene	1.611	0.999	0.777	1.630	25.395	1.914
p-cymene	2.777	0.999	0.767	1.780	24.712	2.390
Ocimene (mixture of isomers)	1.452	0.999	0.772	1.395	25.011	2.494
Eucalyptol	3.597	0.998	0.768	1.498	25.664	4.007
gamma.-Terpinene	2.231	0.999	0.766	1.562	24.578	2.489
Terpinolene	1.831	0.999	0.768	1.553	24.726	2.380
Linolool	3.545	0.999	0.784	1.156	25.783	1.876
Isopulegol	2.816	0.999	0.780	1.773	25.432	1.992
Geraniol	10.282	0.999	0.796	1.273	25.944	2.812
Trans-Caryophyllene	2.077	0.999	0.768	1.493	24.565	2.487
alpha.-Humulene	2.213	0.999	0.768	1.430	24.436	2.553
cis-Nerolidol	2.532	0.999	0.772	1.640	24.095	3.597
trans-Nerolidol	3.043	0.999	0.769	1.699	23.772	3.692
Guaiol	5.176	0.996	0.757	1.535	22.852	3.800
Caryophyllene oxide	9.397	0.997	0.804	7.127	26.803	4.869
.alpha.-Bisabolol	9.873	0.993	0.046	28.412	24.053	3.741

Cannabis Results

Table 4 shows the wt% of terpenes in three different strains of cannabis: CB Diesel, Blue Dream, and Haze Wreck.

Figure 5 shows the chromatograms of the required terpenes by the state of Nevada.

Table 4: wt% of terpenes in three different strains of cannabis

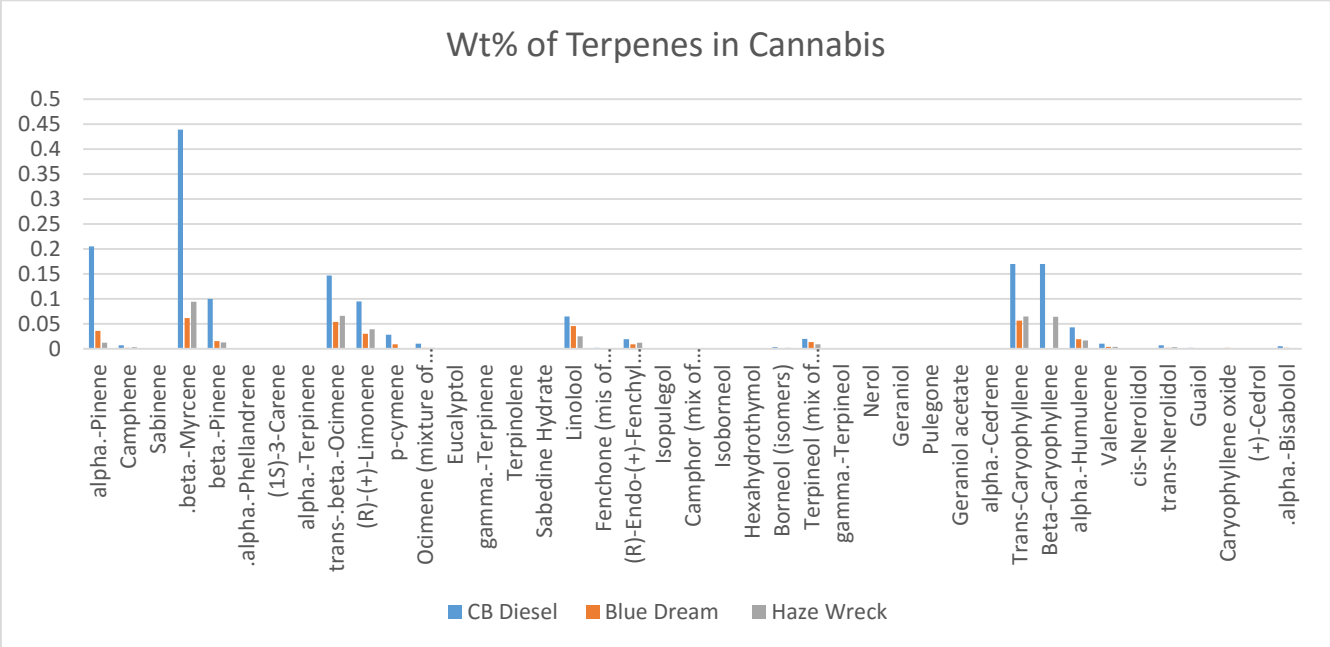
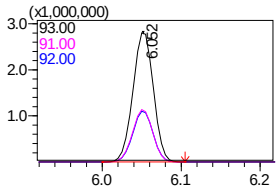
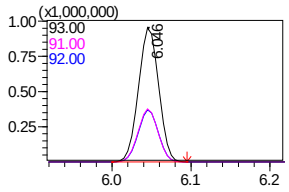
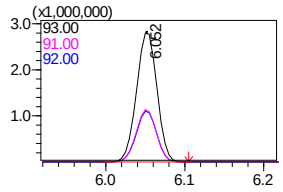
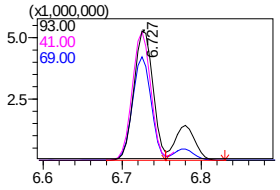
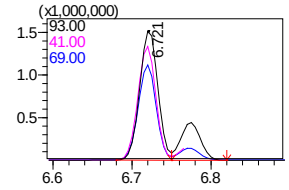
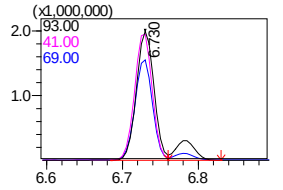
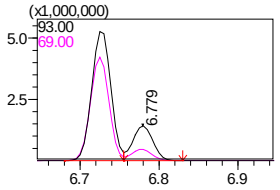
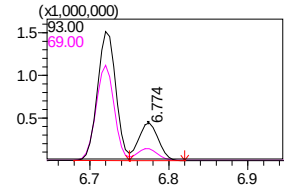
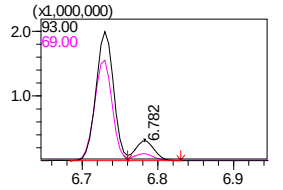
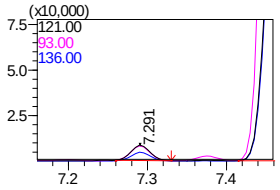
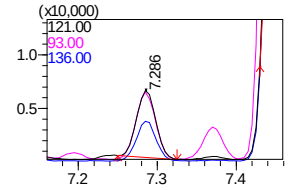
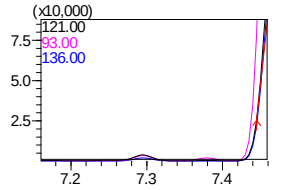
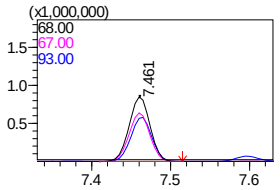
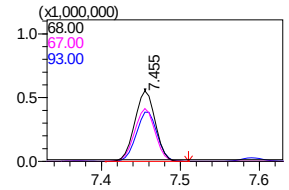
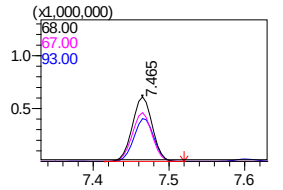
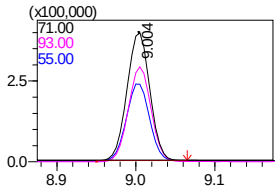
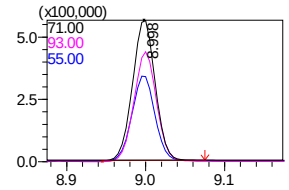
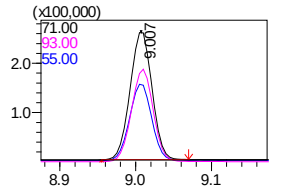
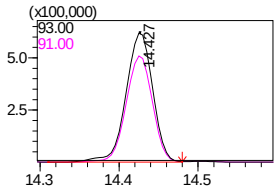
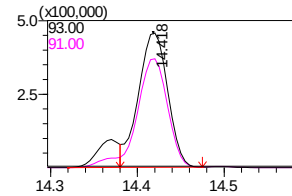
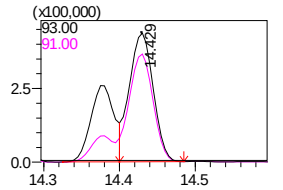
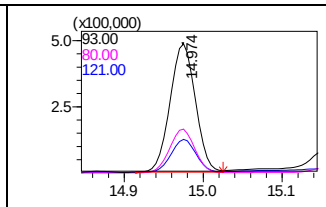
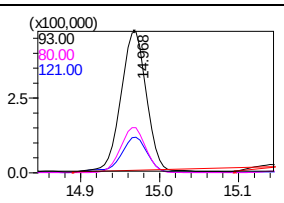
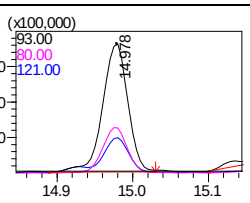
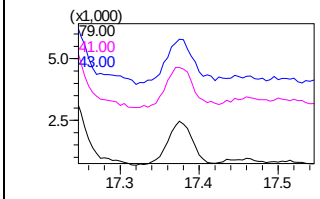
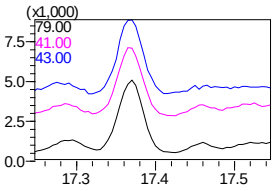
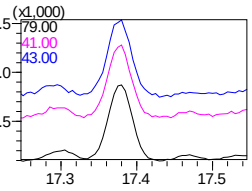
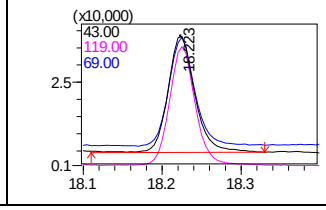
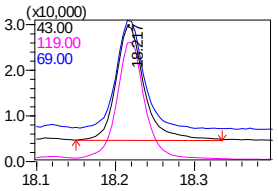
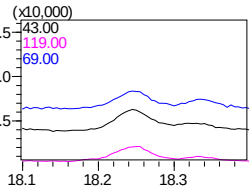


Figure 5: Chromatograms for terpenes required by the state of Nevada for three strains of cannabis

Terpene	CB Diesel	Blue Dream	Haze Wreck
Alpha-Pinene			
Beta-Mycene			
Beta-Pinene			
alpha.-Terpinene			
(R)-(+)-Limonene			
Linolool			
Beta-Caryophyllene			

alpha.-Humulene			
Caryophyllene oxide			
Alpha-Bisabolol			

■ Conclusion

The first sample of cannabis analyzed, CB Diesel, was analyzed shortly after harvest. The resulting wt% of terpenes is similar to that in current literature.[1] The other two samples, Blue Dream and Haze Wreck, were stored at ambient temperature and exposed to light for one month prior to analyzing. It has been demonstrated that different storage conditions can change terpene results over time,[4] and this should be taken into consideration when analyzing cannabis samples as the results show less than expected results. Varying storage conditions and degradation experiments should be the next study in the ever-changing world of regulatory cannabis testing.

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