Robust Terpene Analysis in Cannabis by Using Liquid Injection

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1 Introduction

Terpenes are a classification of aromatic compounds that are nearly ubiquitous throughout nature. Terpenes are primarily found in plants, but can also be observed in marine organisms, insects, and, to a lesser extent, higher-order animals. They are present in cannabis in significant concentrations and are one of the most interesting and diverse aspects of cannabis. They provide the unique aroma of the plant and are critical to the cannabis experience; however, understanding the role that they play in the psychoactive experience of cannabis consumption is still not well understood. Terpenes themselves are built from repeating five-carbon units called isoprene. Their classification as a monoterpene, diterpene, etc., is dependent on the number of isoprene units in their structure. Terpene content in cannabis is typically not regulated, but can provide unique insights into the "flavor profile" of the cannabis flower. Demand for terpene testing has increased significantly in the past few years as consumers become increasingly interested in the terpene profiles of the cannabis strains they consume. Cannabis has a high abundance of mono- and sesquiterpenes, and the majority of terpenes present in the flower fall into these classifications.

This study presents a comprehensive gas chromatography-mass spectrometry (GC-MS) method for the analysis of 22 terpenes in cannabis flower, with a focus on developing a rapid and robust method for the analysis of terpenes in a commercial laboratory.

2 Experimental

2.1 Instrumentation and Materials

An Agilent 7890B Gas Chromatograph coupled with a JEOL JMS-TQ4000GC Mass Spectometer was used in this study. Cannabis terpene standard solutions (Catalog# 34095 and 34096) were purchased from Restek (Bellefonte, PA) and prepared by serial dilution in HPLC-grade methanol purchased from Filtrous (Poway, CA). The GC-MS parameters used in this method are listed in Table 1. Liquid injection was used during method development, but headspace analysis is also applicable using the same method parameters. The 22 terpenes measured in this study are listed in Table 2, along with their associated retention times and ions used for quantification.

Table 1: GC-MS parameters.

Agilent 7890B GC	
Column	Rxi-5ms
	30.0 m, 0.25 mm i.d., 0.25 μm
	(Restek, Cat# 13423)
Inlet Liner	Zebron 4 mm single taper
	w/wool on bottom
	(Phenomenex, Cat# AG2-0A11-05)
Inlet Temp.	250 ℃
Carrier Gas, flow	He, 1.000 mL/min
Mode	Split (10:1)
Pulsed Press., Time	206.84 kP, 0.55 min
Purge Flow	30 mL/min, 1.0 min
Septum Purge Flow	3.0 mL/min
Injection Volume	1.0 μL
Oven Program	75 ℃ (0.75 min) $\rightarrow$
	8 ℃/min $\rightarrow$ 150 ℃ $\rightarrow$
	25 ℃/min $\rightarrow$ 250 ℃ (2.0 min)
JMS-TQ4000GC	
Ion Source Temp.	210 ℃
Interface Temp.	210 ℃
Ionization Mode	EI+, 70 eV, 100 μA
Measurement Mode	Single QMS
Aquisition Rate	2.778 Hz
Cycle Time	23.3 ms
Channel Time	25 ms
Relative EM Voltage	900 V

Table 2: The names, quantifier ions, and retention times for the 22 terpenes measured in this study.

Compound	Quant. Ion (*m/z*)	Ret. Time (min)
α-pinene	93	3.85
Camphene	93	4.08
β-Myrcene	77	4.55
(-)-β-pinene	41	4.44
δ-3-Carene	93	4.89
α-Terpinene	121	4.95
p-Cymene	119	5.08
d-Limonene	68	5.12
Eucalyptol	154	5.24
Ocimene	93	5.38
γ-Terpinene	93	5.61
Terpinolene	136	6.07
Linalool	71	6.20
(-)-Isopulegol	67	7.05
Geranol	69	8.72
Caryophyllene	69	11.33
Humelene	93	11.71
trans-Nerolidiol	69	12.28
cis-Nerolidiol	69	12.54
Caryophyllene Oxide	79	12.80
(-)-Guiaol	161	12.87
(-)-α-Bisabolol	69	13.38

2.2 Sample Preparation

First, cannabis flower was ground up, followed by extraction of 0.5 g of the ground material using 10 mL of HPLC-grade methanol. The extract was filtered using a 0.22 μm syringe filter prior to injection on the instrument. A 20:1 dilution of the sample allows for the majority of the terpenes to fall within the calibration range provided in this method. When running liquid injection, an ideal dilution ratio minimizes the amount of non-volatile compounds that enter the injection port.

3 Results

A total ion current chromatogram of the 22-terpene standard sample is shown in Figure 1, and peak labels are shown in Figure 2. Co-elution was observed in some of the early-eluting compounds, however, all 22 terpenes were detected and baseline separation was observed for most compounds. Peak identities were confirmed using extracted-ion chromatograms (EIC) to look at qualifying ions (Figure 3), as well as matching mass spectra against a database search.

An eight-point calibration curve was determined from 4.88 to 625 ppm for each compound except for nerolidol, which ranged from 1.90 to 244 pmm for the *cis* isomer and 2.98 to 381 ppm for the *trans* isomer (based on the isomer mixture reported by Restek on the standard sample certificate of analysis). The standard samples used for calibration were made by serial diluting the commercial standards in HPLC-grade methanol. An example calibration curve of α-bisabolol is shown in Figure 4. Please note that the detection limits of the JMS-TQ4000 are much lower than what is presented in this method.

4 Discussion

This method is appropriate for commercial laboratories conducting routine terpene analysis on cannabis flower. The total terpene concentration in flower can range from $<$0.5% to $>$2% by weight. Several factors can influence the terpene concentrations in cannabis flower. The age of the flower itself is an important consideration as terpenes are volatile compounds and may volatize as the flower ages. Fresh flower typically has a much different terpene profile than cured flower. This method covers the most common terpenes present in cannabis, but there is an abundance of other terpenes present in cannabis. Mass spectral library searching can assist in the identification of untargeted terpenes (Figure 5), and can give further insight into the terpene profile of the flower.

5 Conclusions

A robust method has been developed for the analysis of terpenes in cannabis flower. Baseline separation was observed for most compounds measured in this study, and all compounds were successfully matched against a mass spectral database search. The method described above is appropriate for commercial production laboratories as well as research laboratories, and it provides a balance of speed and comprehensive terpene analysis.

Terpenes in cannabis can be particularly difficult to separate and analyze. Due to similar chemical properties between various terpenes, complete chromatographic separation may not be possible with short GC runtimes or complex samples; however, the information provided by mass spectrometry can be used to identify unknown or poorly separated terpenes.

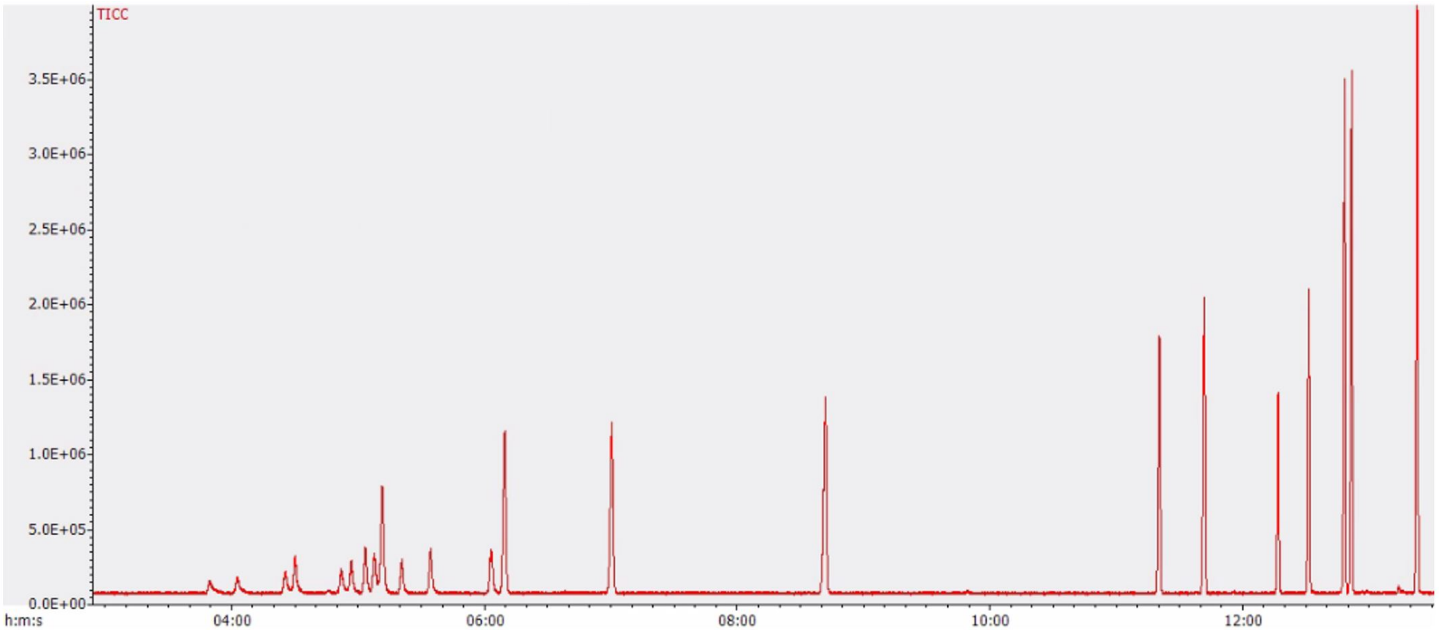

Figure 1: The chromatographic separation of 22 terpenes.

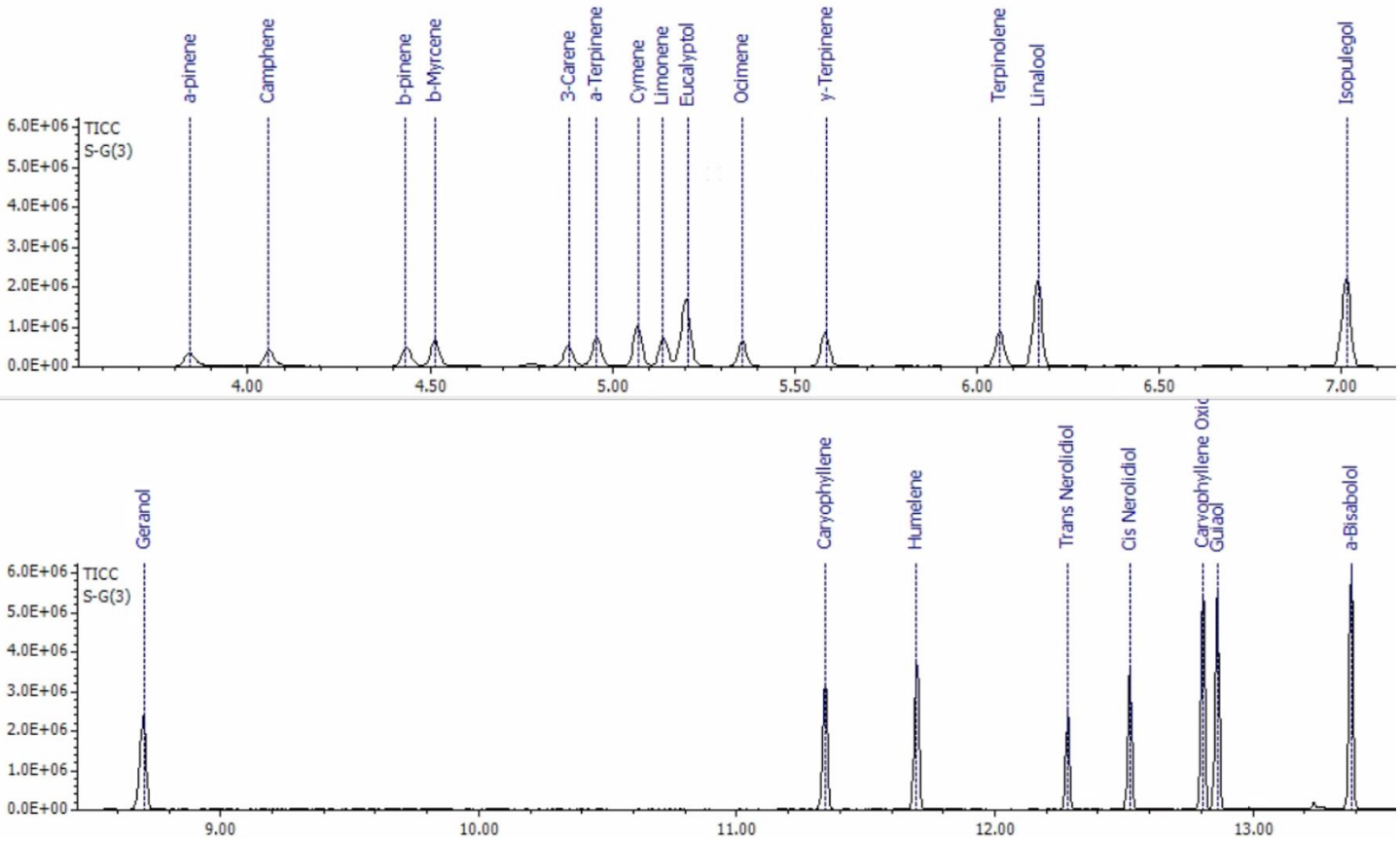

Figure 2: Labeled terpenes from Figure 1.

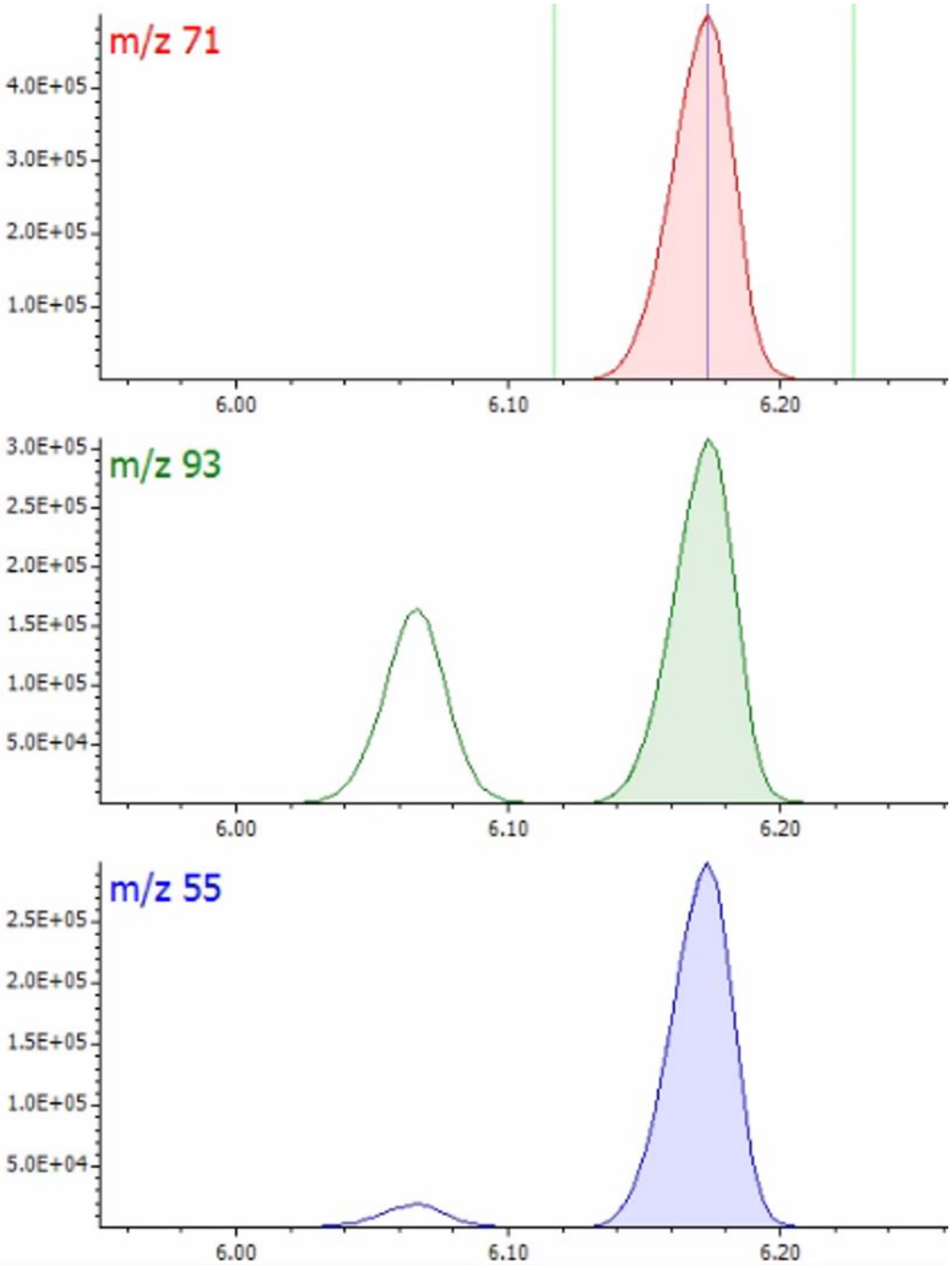

Figure 3: Peak identification of linalool at mass 71.

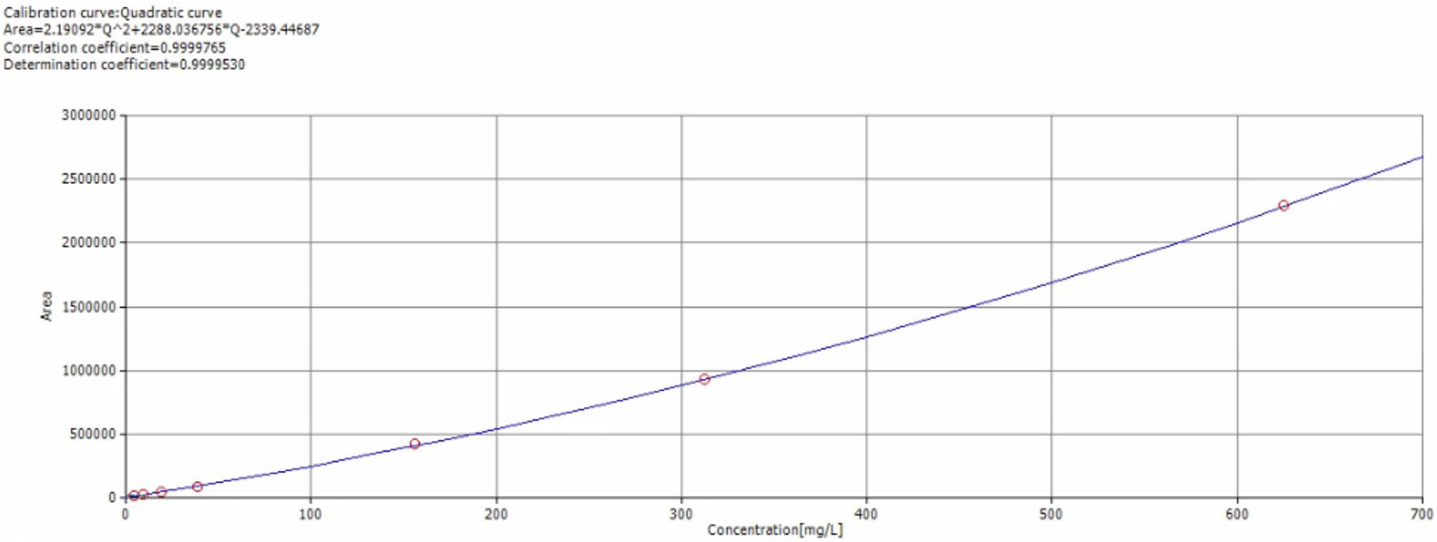

Figure 4: Example calibration curve of α-bisbolol from 4.88 to 625 ppm.

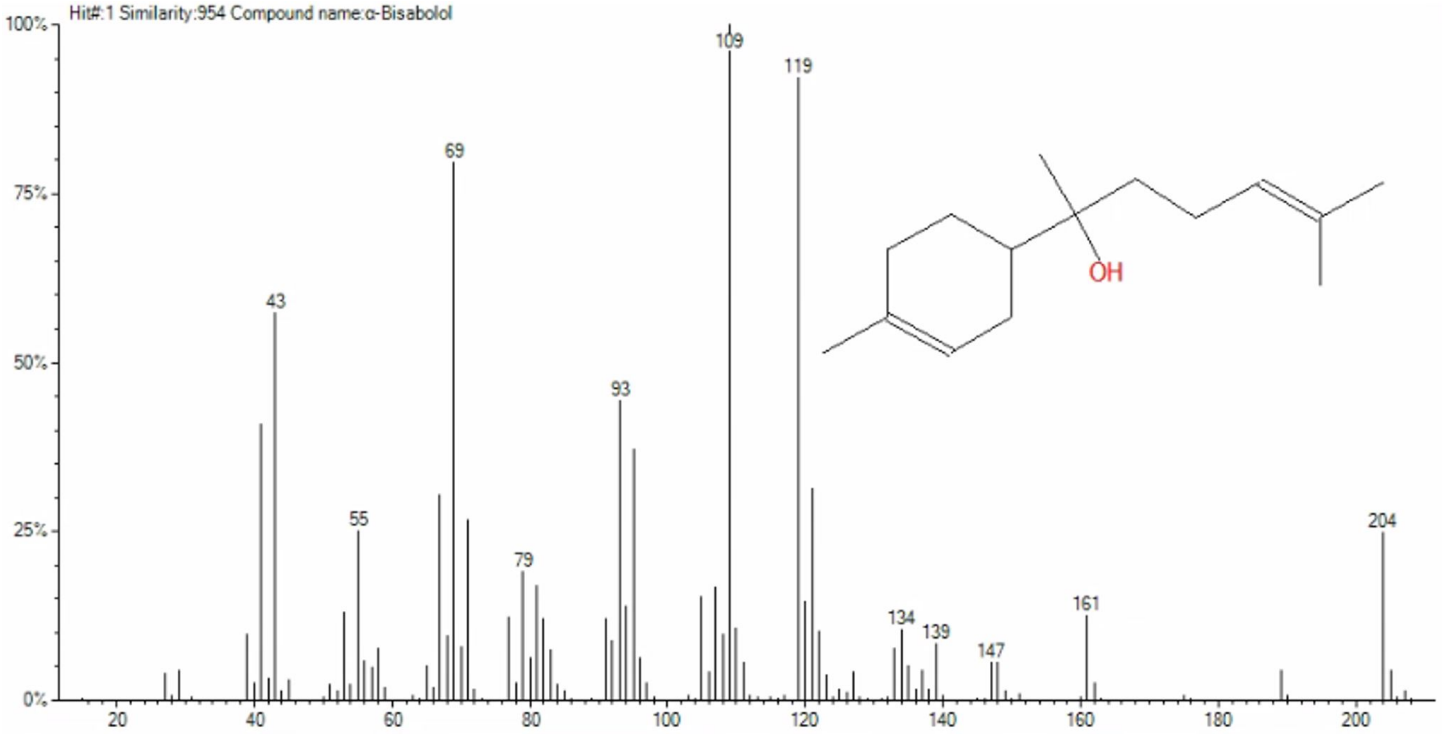

Figure 5: Unknown searches can be an incredibly useful tool in terpene analysis.