Application Note

Food

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SPME-GC/MS of Selected Terpenes Using Agilent DVB/CAR-WR/PDMS SPME Fiber

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Introduction

The increased legalization of cannabis in the U.S. States and in countries throughout the world have pushed for qualifying and quantifying terpene concentrations to the forefront of the analytical industry. The development of a faster, more efficient method to produce rapid and accurate results at a low cost is needed. The high vapor pressures and volatility of terpenes make them excellent candidates for static headspace (HS) gas chromatography/mass spectrometry (GC/MS) analysis. This approach offers several advantages compared to solvent extraction and GC/FID. It:

- Does not require the use of organic solvents
- Does not coextract matrix interferences
- Provides additional means of peak identification and purity using spectral data

Please note that this application of cannabis flower terpene analysis by SPME is meant to be a proof of principal study illustrating the capabilities of SPME extraction for a variety of complex matrices. This methodology is not recommended for routine terpene testing in cannabis and hemp matrices without further method development and verification.

Experimental

DVB/CAR-WR/PDMS Fiber reproducibility

A 10 µL amount of a 5 ppm terpene mixture (part numbers TPM-100-1 and TPM-105-1) was added to 8 mL of Milli-Q 18.2 Ω water in a 20 mL headspace vial.

Samples

Cannabis flower samples were obtained from the University of Mississippi Marijuana Project with permission from the U.S. Drug Enforcement Administration (DEA).

Terpene profiling of various cannabis samples

An 8 mL volume of Milli-Q 18.2 Ω water was added to approximately 0.1 g of homogenized cannabis plant material in a 20 mL headspace vial.

Solid phase microextraction (SPME)

In SPME, analytes establish equilibria within the sample matrix, the headspace above the sample, and a polymer coating on a fused silica fiber. Extracted analytes are thermally desorbed from the fiber to a capillary GC column. Table 1 shows the SPME headspace parameters.

GC/MS analysis

Selected terpenes were analyzed using SPME headspace with a PAL RTC rail system. This was combined with an Agilent 7890B GC system coupled with an Agilent 5977B high efficiency source GC/MSD (Figure 2).

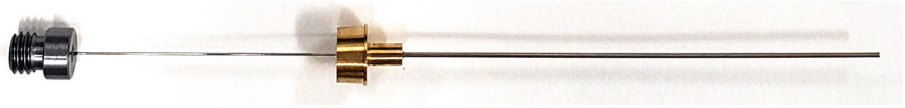

Figure 1. DVB/CAR-WR/PDMS SPME Fiber (part number 5191-5874).

Table 1. SPME headspace parameters.

Parameter	Value
Script Name	ARROW-STD-V2.0
Tool	SPME 1
SPME Fiber Phase	80/10 µm DVB/CAR-WR/PDMS (p/n 5191-5874)
Incubation Time	5 minutes
Stirrer	Heatex Stirrer 1
Heatex Stirrer Speed (Agitation)	1,000 rpm
Heatex Stirrer Temperature (Extraction Temp)	40 °C
Agitator	None
Sample Extract Time	20 minutes
Extraction Temperature	40 °C
Sample Vial Penetration Depth	40 mm
Sample Vial Penetration Speed	20 mm/s
Inlet Penetration Depth	40 mm
Inlet Penetration Speed	100 mm/s
Injection Signal Mode	Before fiber expose
Sample Desorption Time	3 minutes
Conditioning Port	SPMEArrowCond 1
Pre-Desorption Conditioning Time	5 minutes (analytical run)/60 minutes (precondition)
Fiber Conditioning Station Temperature	270 °C
Post Desorption Conditioning Time	0 minutes
GC Cycle Time	5 minutes (set for sequence overlap)

Figure 2. The PAL RTC rail system combined with an Agilent 7890B GC and an Agilent 5977B GC/MSD.

Results and discussion

DVB/CAR-WR/PDMS Fiber reproducibility

Twelve replicate injections of a 5 ppm standard were performed on three different DVB/CAR-WR/PDMS 100 µm Fibers within a single batch. Percent RSDs were calculated for each Fiber, then averaged together. Each set of replications maintained a percentage of RSDs lower than 20%. Table 3 shows the average results.

Table 2. Agilent 7890B GC settings.

Parameter	Value
Inlet Liner	Inlet liner, Ultra Inert, splitless, straight, 0.75 mm id (p/n 5190-4048)
Injection Mode/Temperature	Splitless/270 °C
Oven Program	60 °C (hold 2 minutes), 5 °C/min to 140 °C (hold 1 minute), 15 °C/min to 250 °C (hold 4 minutes)
Equilibration Time	0.5 minutes
Control Mode	Constant flow (1 mL/min)
Column	Agilent J&W DB-1ms, 60 m, 0.25 mm, 0.25 µm GC column (p/n 122-0162)
Septum Purge Flow Mode	Standard at 3 mL/min
Purge Flow to Split Vent	15 mL/min at 0.35 minutes
Agilent 5977B GC/MS Conditions	
Transfer Line	280 °C
Acquisition Mode	SCAN
Solvent Delay	8 minutes
Tune File	atune.u
Gain	1
MS Source Temperature	280 °C
MS Quad Temperature	150 °C

Table 3. Compound %RSD results per DVB/CAR-WR/PDMS Fiber.

Compound	Fiber 01	Fiber 02	Fiber 03	Average
Camphene	4.77	3.54	1.79	3.37
Sabinene	7.99	5.04	3.41	5.48
b-Pinene	5.94	3.77	2.93	4.21
3-Carene	7.09	2.41	3.17	4.22
(R)-(+)-Limonene	4.64	1.81	3.54	3.33
Ocimene	16.60	5.57	4.84	9.00
γ-Terpinene	9.54	8.00	5.49	7.68
Fenchone	20.07	20.11	5.27	15.15
(−)-Camphor	9.73	4.83	5.18	6.58
(−)-Isopulegol	8.96	7.18	4.68	6.94
Isoborneol	16.46	10.55	6.93	11.31
Terpineol	9.34	18.86	12.17	13.46
Pulegone	15.44	8.29	10.18	11.30
b-Caryophyllene	19.65	5.26	5.36	10.09
(−)-Guaiol	24.43	18.32	14.55	19.10
(+)-Cedrol	17.85	12.90	10.69	13.81
(−)-*alpha*-Bisabolol	22.31	18.24	13.84	18.13

Terpene profiling of various cannabis samples

Terpenes account for the vast array of smells and flavors in cannabis flowers and extracts, and provide the notable aromas of cannabis varieties.

Several cannabis flower samples were profiled with the use of the DVB/CAR-WR/PDMS SPME Fiber (part number 5191-5874). Selected identified terpenes are reported in Table 4, and representative chromatograms are shown in Figures 3 to 13.

Limonene[2]

Limonene is the second most abundant terpene in all cannabis strains, but not all strains necessarily have it. Limonene gives strains a citrusy smell that resembles lemons.

Table 4. Qualitative determination of terpenes in samples (concentration >5 ppm).

Name	1367	1330G	THC000A	3486	3653	1103	2524	3401	3535	3648	3658	3812
Camphene	x		x									
Sabinene	x											
b-Pinene	x	x				x	x		x	x		x
3-Carene												
(R)-(+)-Limonene	x	x				x	x	x	x	x		x
Ocimene	x											
g-Terpinene	x		x		x							
Fenchone	x				x		x					x
(−)-Camphor	x	x			x	x		x	x	x		x
(−)-Isopulegol	x	x	x		x	x	x	x	x	x	x	x
Isoborneol	x		x									
Terpineol	x	x	x	x	x	x	x	x	x	x	x	x
Pulegone												
b-Caryophyllene	x	x	x	x	x	x	x	x	x	x	x	x
(−)-Guaiol			x		x		x					
(+)-Cedrol	x		x	x		x	x	x	x	x	x	x
(−)-*alpha*-Bisabolol	x	x	x		x	x	x	x	x	x	x	x

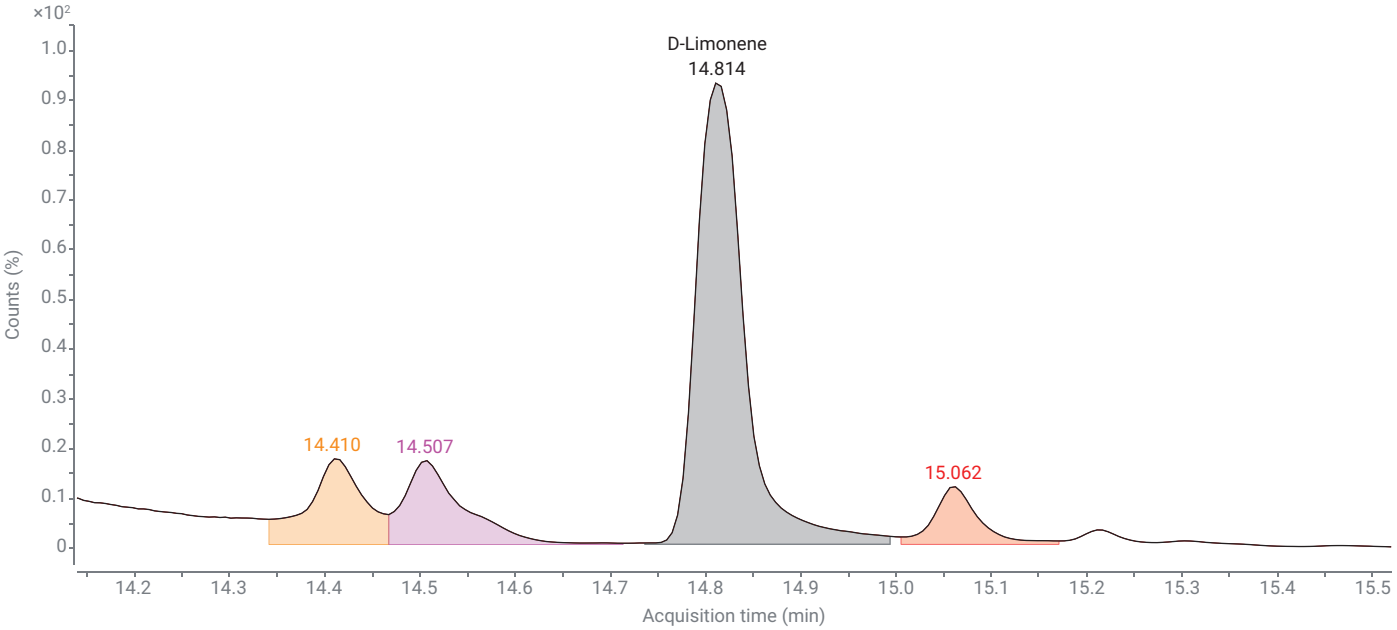

Figure 3. (R)-(+)-Limonene identified in cannabis flower sample 1103.

gamma-Terpinene (*g*-Terpinene or γ-Terpinene)[2]

g-Terpinene has an herbaceous, citrusy sweet aroma. It is commercially extracted from tea tree oil.

Isopulegol[3,4]

Isopulegol, the chemical precursor to menthol, is a terpene known for its minty smell. It is commonly used as a flavoring agent in foods and fragrance in cosmetics.

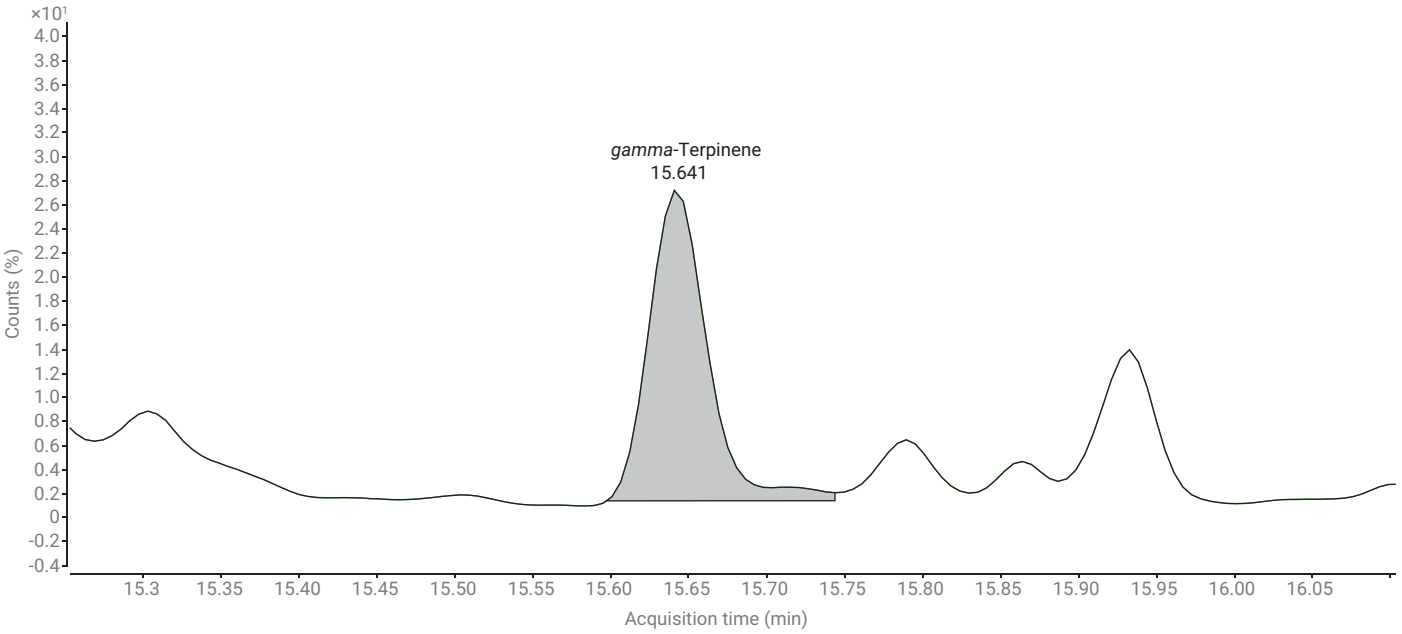

Figure 4. *g*-Terpinene identified in cannabis flower sample 3653.

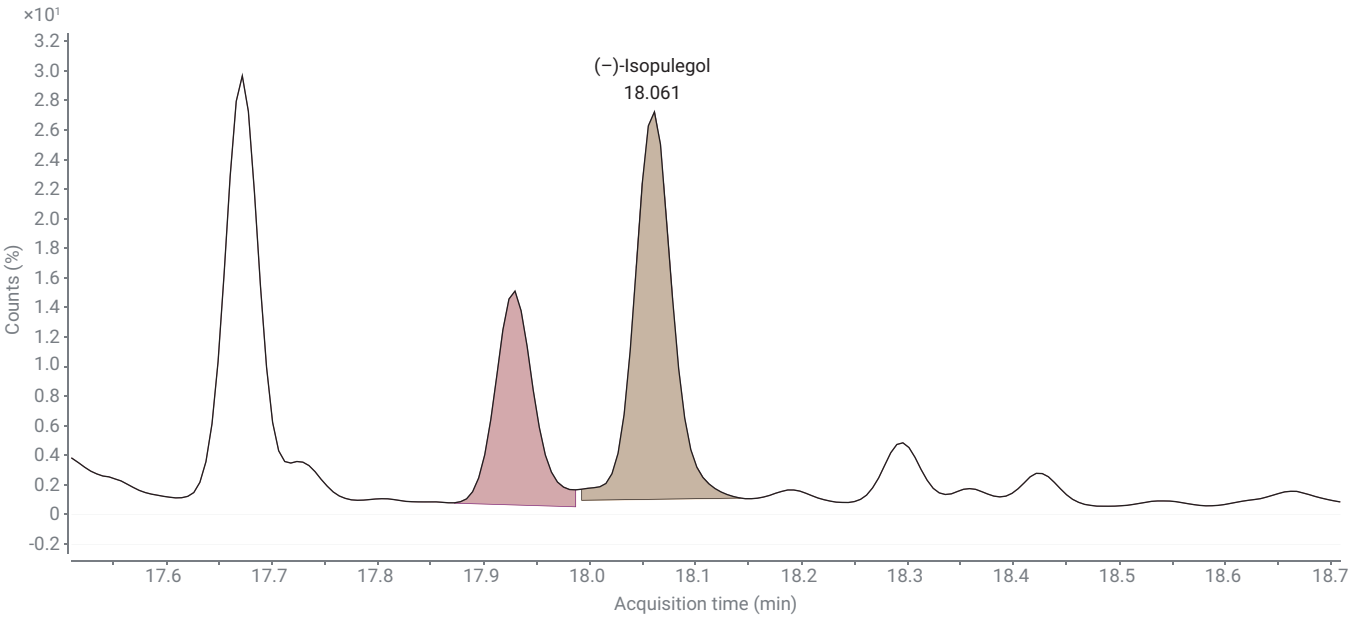

Figure 5. (−)-Isopulegol identified in cannabis flower sample 3535.

Terpineol[3]

Terpineol occurs naturally in lilacs, pine trees, lime blossoms, and eucalyptus sap. It is frequently used to create pleasant aromatic profiles in products such as soap, lotion, and perfume. Terpineol is characterized by its ability to relax the consumer.

Caryophyllene[2]

Caryophyllene is the only terpene that binds to cannabinoid receptors. It is best known for its spicy and peppery notes. Caryophyllene is found in black pepper, cinnamon, cloves, and spices such as oregano, basil, and rosemary.

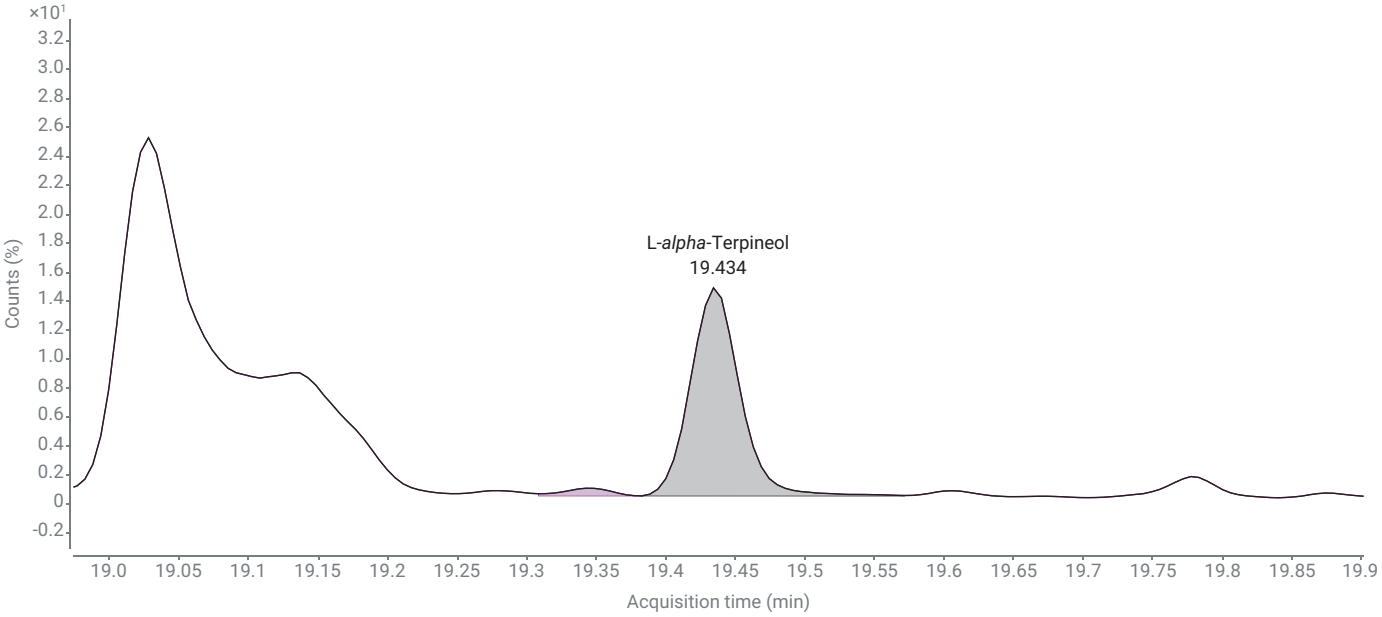

Figure 6. Terpineol identified in cannabis flower sample 3658.

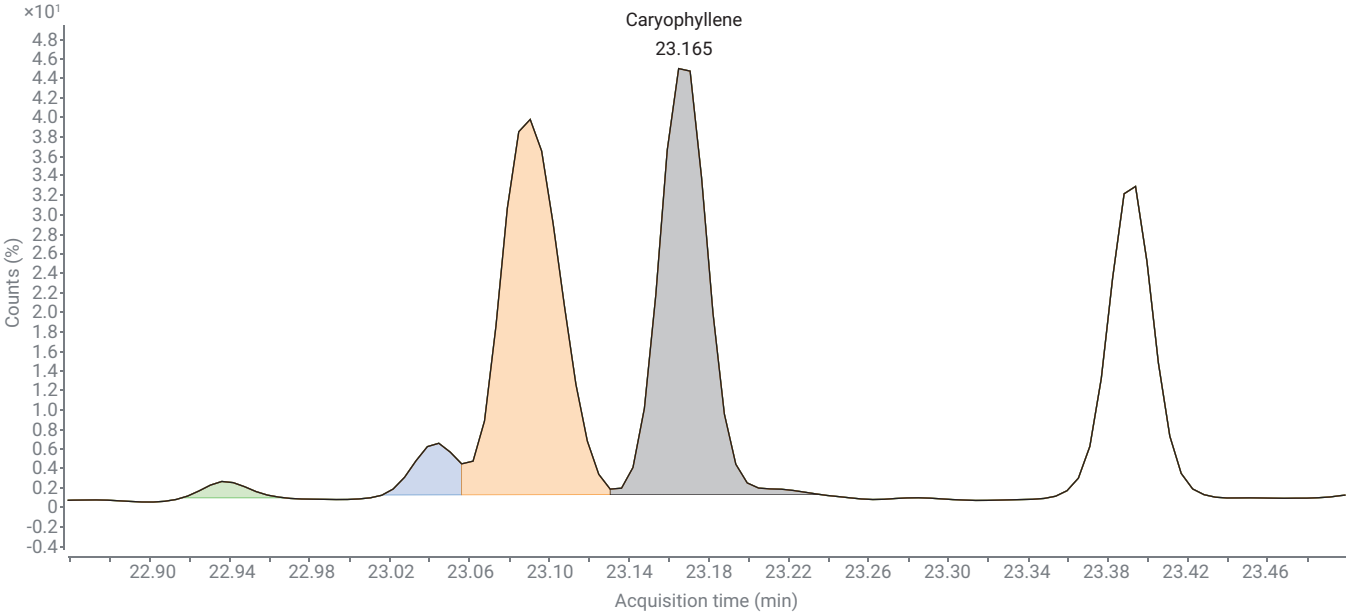

Figure 7. *b*-Caryophyllene identified in cannabis flower sample 2524.

Cedrol[5]

Cedrol is a sesquiterpene alcohol found in the essential oil of conifers (cedar oil), especially in cypress and juniper. Studies have shown cedrol to have deeply sedative effects when inhaled.

alpha-Bisabolol[2]

alpha-Bisabolol has been primarily used in the cosmetics industry, but lately has been seen to be effective in treating bacterial infections and wounds. Its pleasant floral aroma can also be found in the chamomile flower, and is an antioxidant with anti-irritation and analgesic properties.

Different cannabis strains have been developed containing distinct aromas and flavors that make up its specified terpene profile.

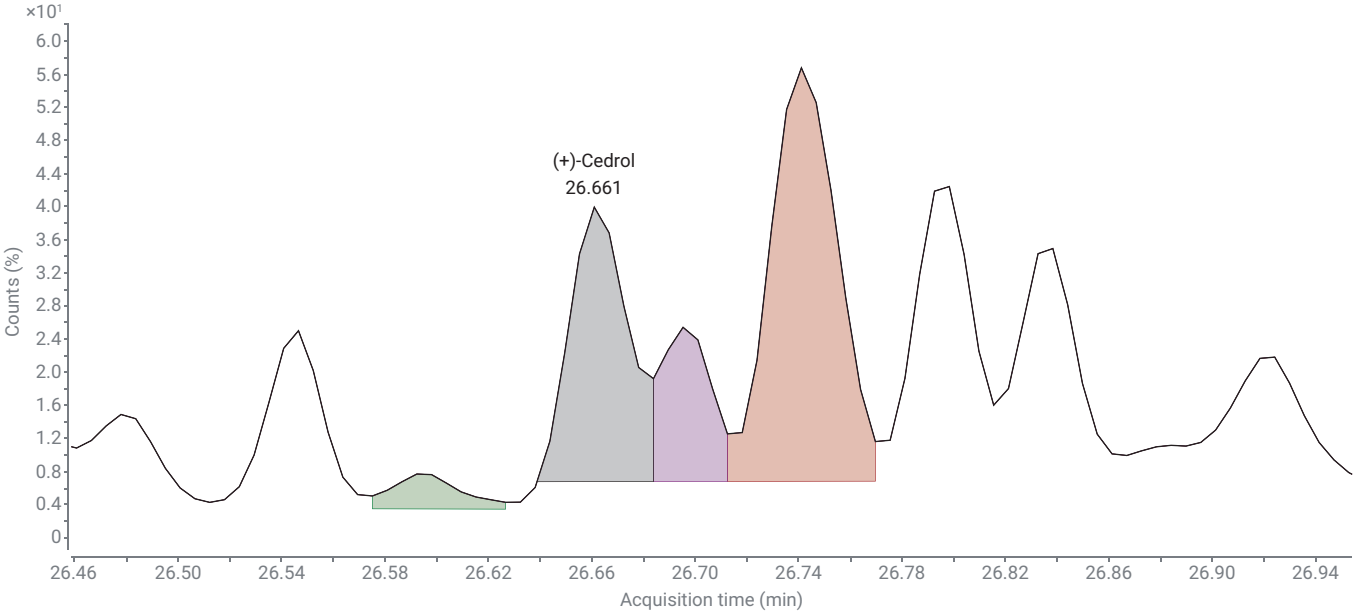

Figure 8. (+)-Cedrol identified in cannabis flower sample 3401.

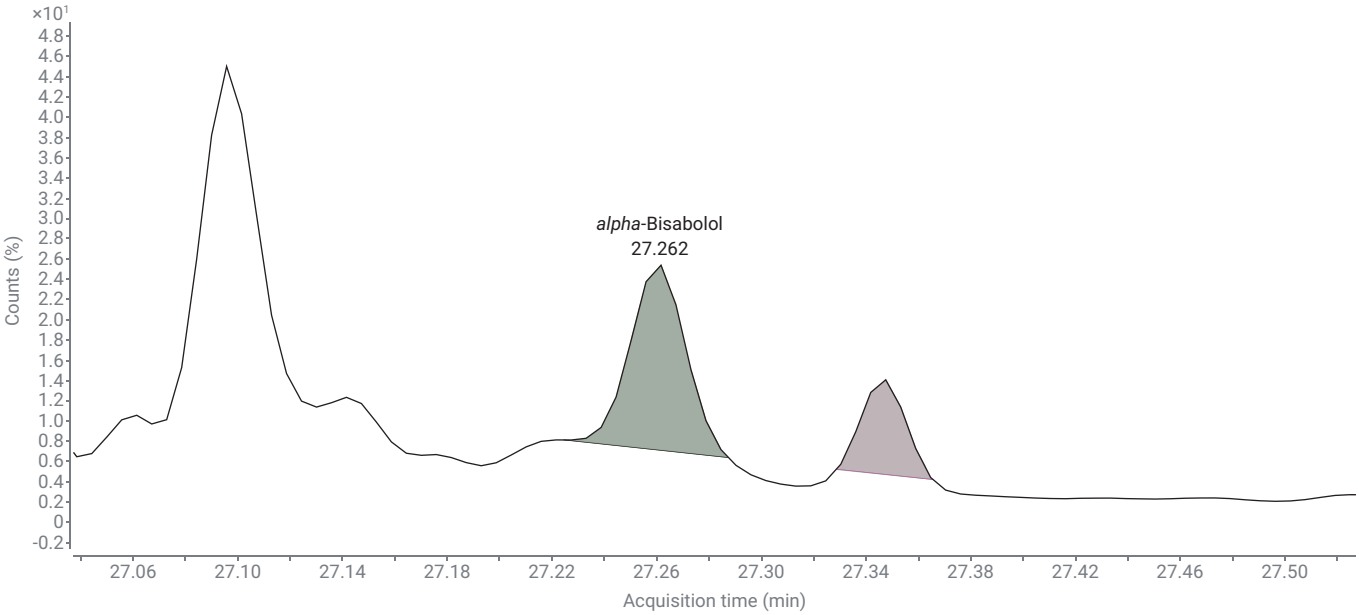

Figure 9. (–)-*alpha*-Bisabolol identified in cannabis flower sample 3812.

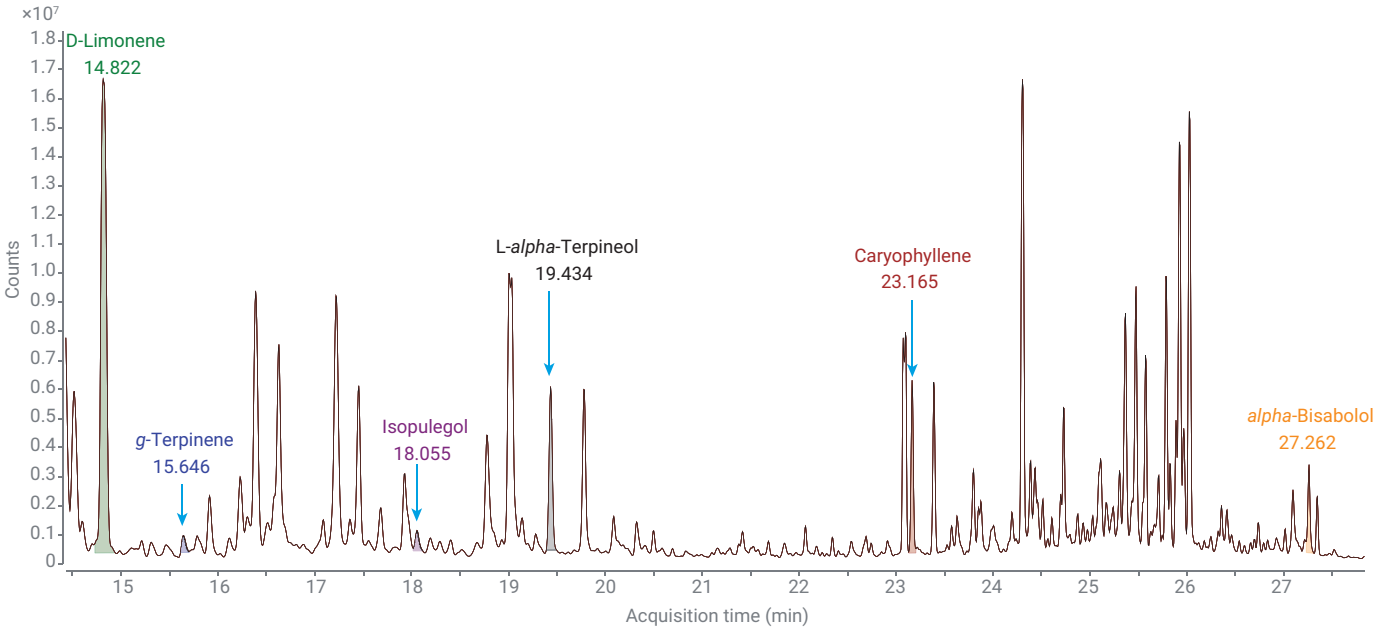

Figure 10. Selected terpenes in cannabis flower sample 1367: (R)-(+)-limonene, *g*-terpinene, (–)-isopulegol, terpineol, *b*-caryophyllene, and (–)-*alpha*-bisabolol.

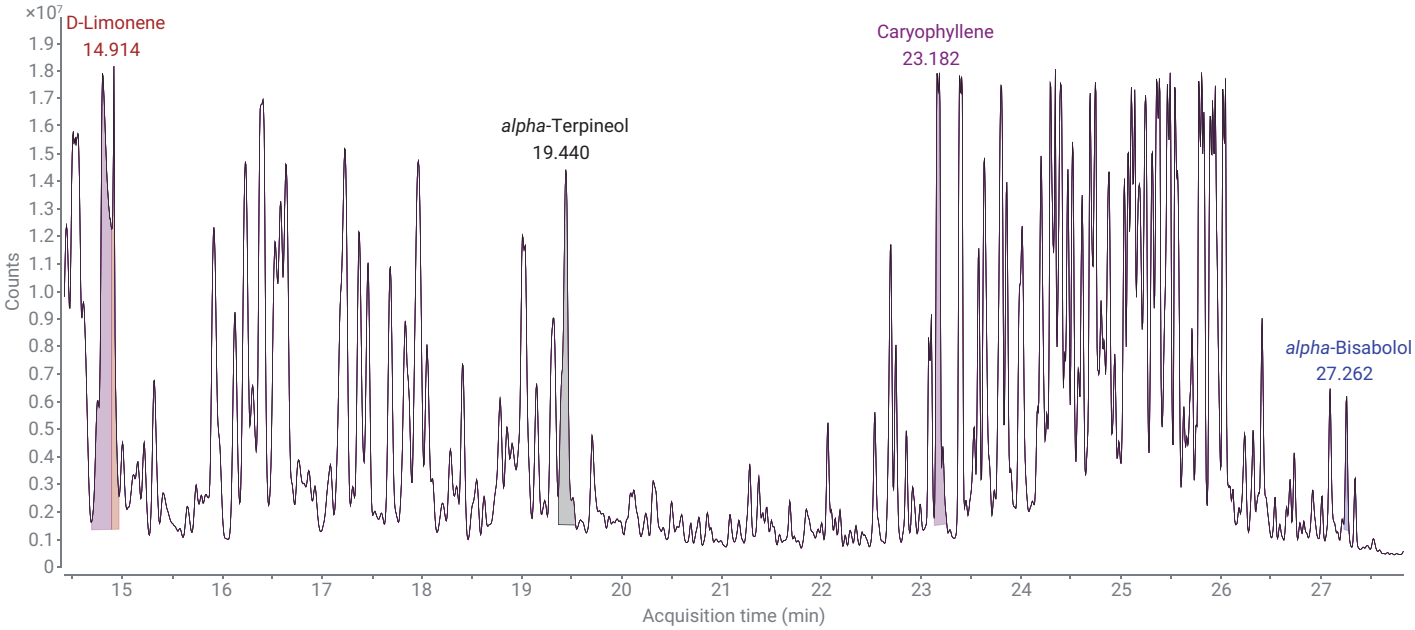

Figure 11. Selected terpenes in cannabis flower sample 1330G: (R)-(+)-limonene, terpineol, *b*-caryophyllene, and (–)-*alpha*-bisabolol.

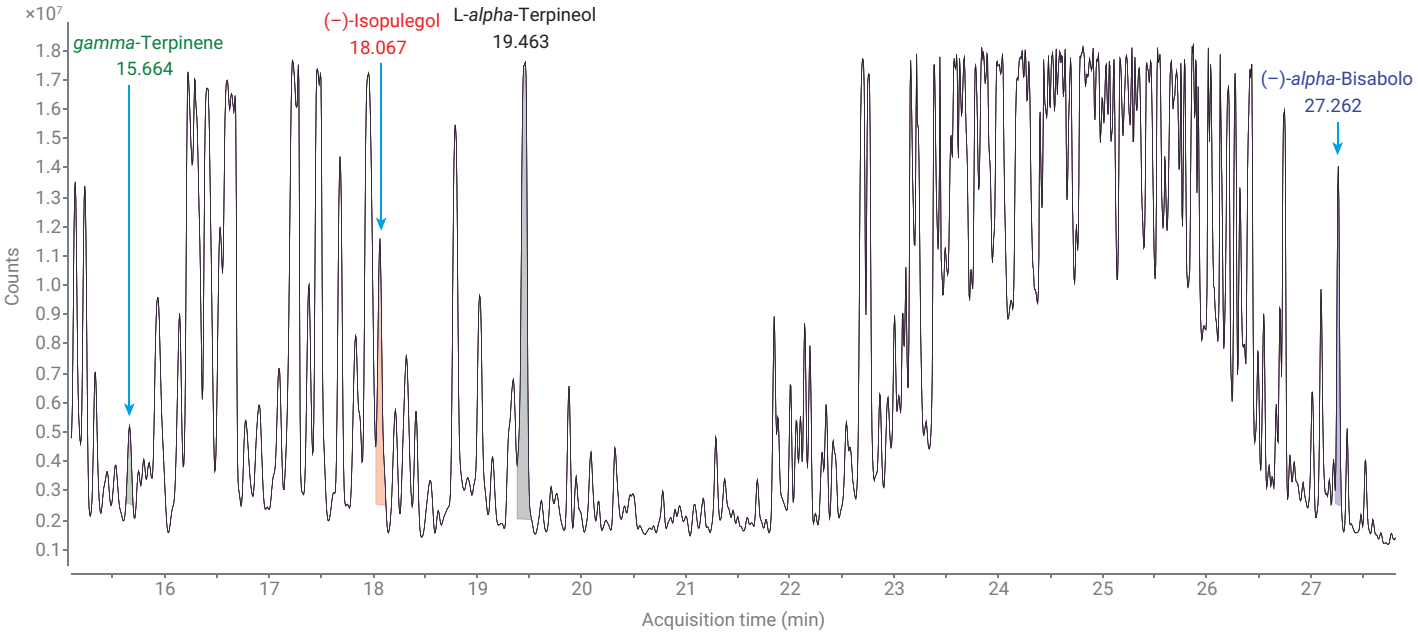

Figure 12. Selected terpenes in cannabis flower sample THC001A: *g*-terpinene, (–)-isopulegol, terpineol, and (–)-*alpha*-bisabolol.

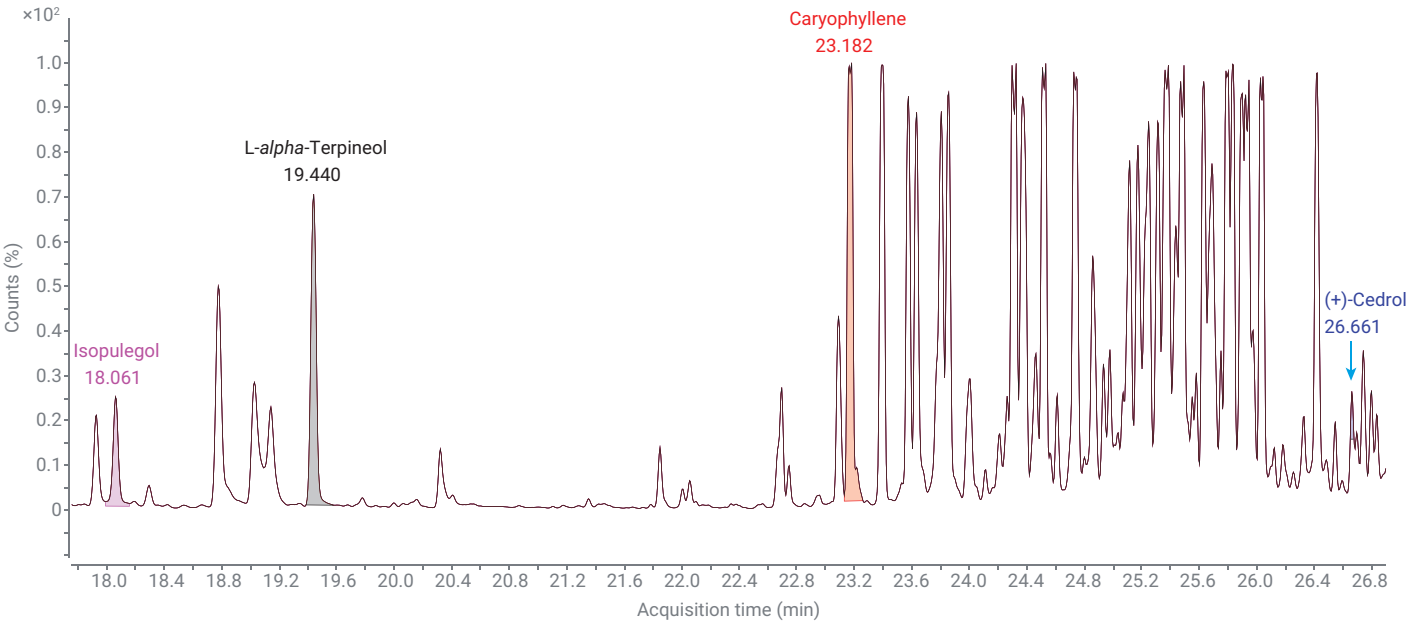

Figure 13. Selected terpenes in cannabis flower sample 3648: (–)-isopulegol, terpineol, *b*-caryophyllene, and (+)-cedrol.

Conclusion

Since terpenes have high vapor pressures, and are extremely volatile, they are excellent candidates for static headspace (HS) gas chromatography/mass spectrometry (GC/MS) analysis. This application of cannabis flower terpene analysis by SPME is meant to be a proof of principal study illustrating the capabilities of SPME extraction for a variety of complex matrices. Similar SPME extraction methodology may be useful in the measurement of volatile phytochemicals found in fruit, vegetables and other plant-based matrices.

References

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Printed in the USA, October 8, 2019
5994-1411EN

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