Application Note

Sample Preparation

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SPME Arrow Sampling of Terpenes in Cannabis Plant Material

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Introduction

With the growing legalization of medicinal cannabis worldwide, methods for qualifying and quantifying terpene concentrations has risen to the forefront in the analytical industry. The development of a faster, more efficient method that will produce rapid, accurate results at a low cost is highly desirable. Since terpenes have high vapor pressures, and are extremely volatile, they are excellent candidates for static headspace (HS) gas chromatography (GC) analysis. PAL SPME Arrows can be used for qualitative and quantitative determination of terpenes in plant material by HS sampling combined with GC/MS. 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 (which could potentially interfere with the chromatographic analysis or contaminate the GC system), and provides additional means of peak identification and purity using spectral data. PAL SPME Arrows provided the sensitivity and robustness needed to profile the predominant terpenes in an unknown variety of cannabis plant samples.

Terpenes

Cannabis contains more than 100 different terpenes and terpenoids, as well as miscellaneous compounds of terpenoid origin. Different cannabis strains have been developed containing distinct aromas and flavors. This is a result of the differing amounts of specific terpenes present. Terpenes are the naturally occurring combination of carbon and hydrogen, whereas terpenoids are terpenes that have been modified through a drying and curing process (chemical modification), altering the oxygen content of the compound.

Methodology

Terpene profiling (qualitative):

- **Sample:** 0.02 to 0.03 g of homogenized cannabis plant material was weighed into a 20 mL headspace vial
- **SPME Arrow:** 1.1 mm, 120 µm DVB/CAR WR/PDMS (p/n 5191-5861)(Figure 1)

Target terpene analysis (quantitative):

- **Sample:** 0.1g of homogenized cannabis plant material was weighed into a 20 mL headspace vial
- **Calibration:** 10 µL of prepared calibration standard (2 to 50 ppm) was added to each sample. Samples were capped, and after a 10 minute equilibration at room temperature, 8 mL of Milli-Q H_2O was added to each sample.
- **SPME Arrow:** 1.1 mm; 100 µm PDMS (p/n 5191-5862) (Figure 2). PDMS is less prone to overload than the DVB/CAR WR/PDMS phase.

Note: The homogenization of the sample and the addition of water increases reproducibility.

SPME-GC/MSD

The analysis of BTEX in water was extracted using SPME headspace with a PAL RTC rail system combined with an Agilent 7890B GC system coupled with the Agilent 5977B High Efficiency Source GC/MSD (Figure 3).

Figure 1. SPME Arrow, 1.1 mm, 120 µm DVB/CAR WR/PDMS.

Figure 2. SPME Arrow, 1.1 mm, 100 µm PDMS.

Instrument conditions

Agilent 7890B GC Settings	
Turn Top Assembly	Agilent 7890 Turn Top Assy Enlarged id – Inert (p/n G3452-60930)
Inlet Liner	Inlet liner, Ultra Inert, straight, 2 mm id (p/n 5190-6168)
Injector Temperature	270 °C
Injection Mode	100:1 split
Control Mode	Constant flow (1 mL/min; 1.4 mL/min into MSD)
Column	J&W DB-1ms GC column, 60 m × 0.25 mm, 0.25 µm (p/n 122-0162)
Oven Program	60 °C (hold two minutes); 5 °C/min to 140 °C (hold one minute); 15 °C/min to 250 °C (hold four minutes)

SPME Headspace Parameters	
Incubation Time	5 minutes
Heatex Stirrer Speed (Agitation)	1,000 rpm
Heatex Stirrer Temperature (Extraction Temperature)	40 °C
Sample Extract Time	5 minutes
Sample Desorption Time	3 minutes
Conditioning Time	5 minutes
Conditioning Temperature	270 °C

Agilent 5977B MS Conditions	
Transfer Line	300 °C
Acquisition Mode	Scan
Solvent Delay	7 minutes
Tune File	atune.u
Gain	1
MS Source Temperature	280 °C
MS Quad Temperature	150 °C

Figure 3. PAL RTC rail system combined with an Agilent 7890B GC + 5977B High GC/MSD.

Results and discussion

Terpene profiling

Flower Samples were profiled with the use of the 120 µm DVB/CAR WR/PDMS (p/n 5191-5861) Arrow (Figure 4).

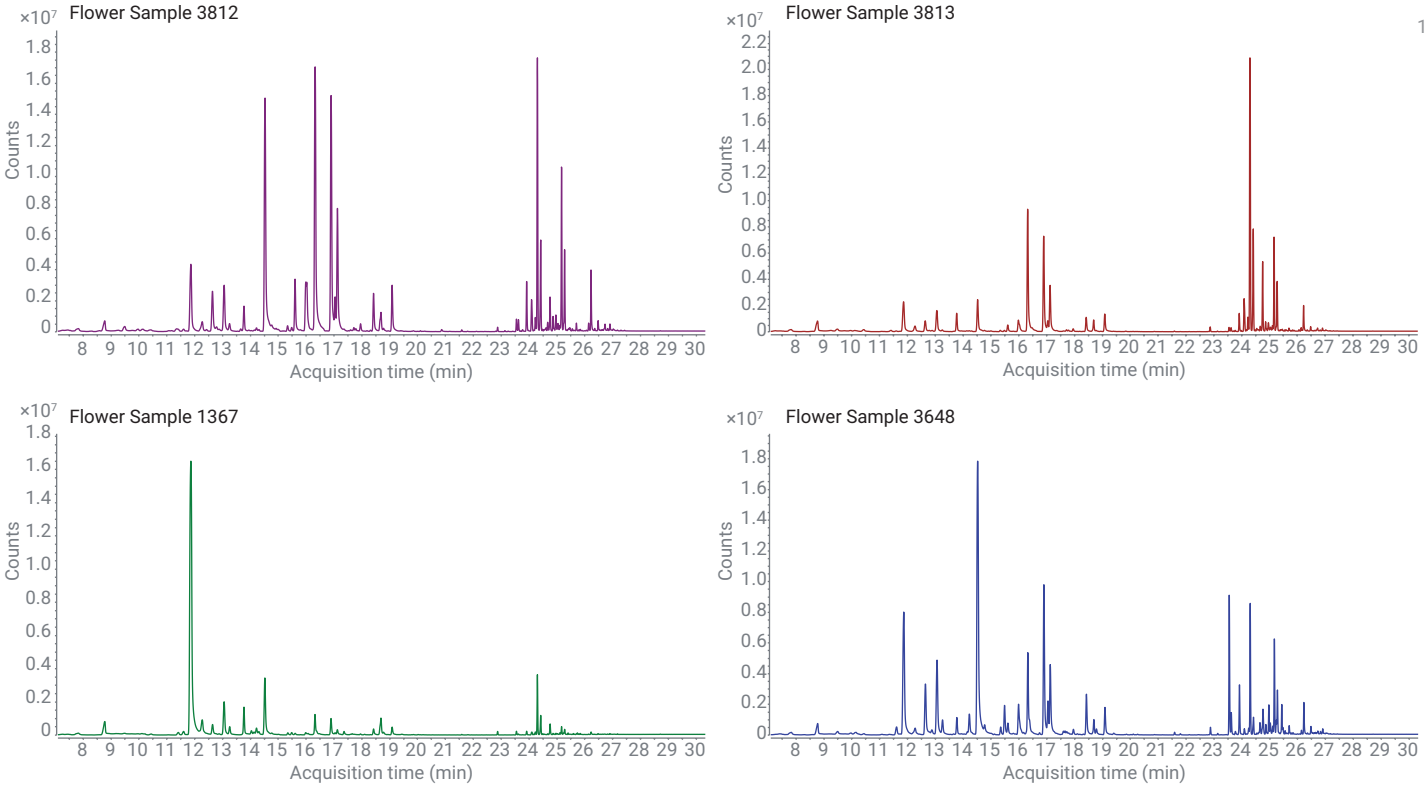

Figure 4. Flower samples profiled with the use of the 120 µm DVB/CAR WR/PDMS (p/n 5191-5861) Arrow.

Targeted terpene analysis

Flower samples were extracted with the use of the 100 µm PDMS (p/n 5191-5862) Arrow.

Table 1. Retention times and linearity of selected terpenes (100 µm PDMS Arrow calibration curves; 2 to 50 ppm).

Terpene	RT	R^2
alpha-Pinene	11.865	0.9956
Camphene	12.274	0.9979
Sabinene	12.876	0.9979
Myrcene (tech)	13.061	0.9974
beta-Pinene	13.267	0.9972
a-Phellandrene	13.782	0.9970
3-Carene	14.039	0.9975
(R)-(+)-Limonene	14.526	0.9969
Ocimene	14.926	0.9963
Fenchone	15.985	0.9984
Terpinolene (tech grade)	16.26	0.9979
(+)-Fenchol	16.895	0.9981
Camphor	17.598	0.9991
Isoborneol	18.188	0.9964
Menthol	18.606	0.9988
b-Caryophyllene	24.35	0.9979
a-Humulene	24.76	0.9974
Caryophyllene oxide	26.222	0.9991
(−)-Guaiol	26.307	0.9961
(+)-Cedrol	26.486	0.9968

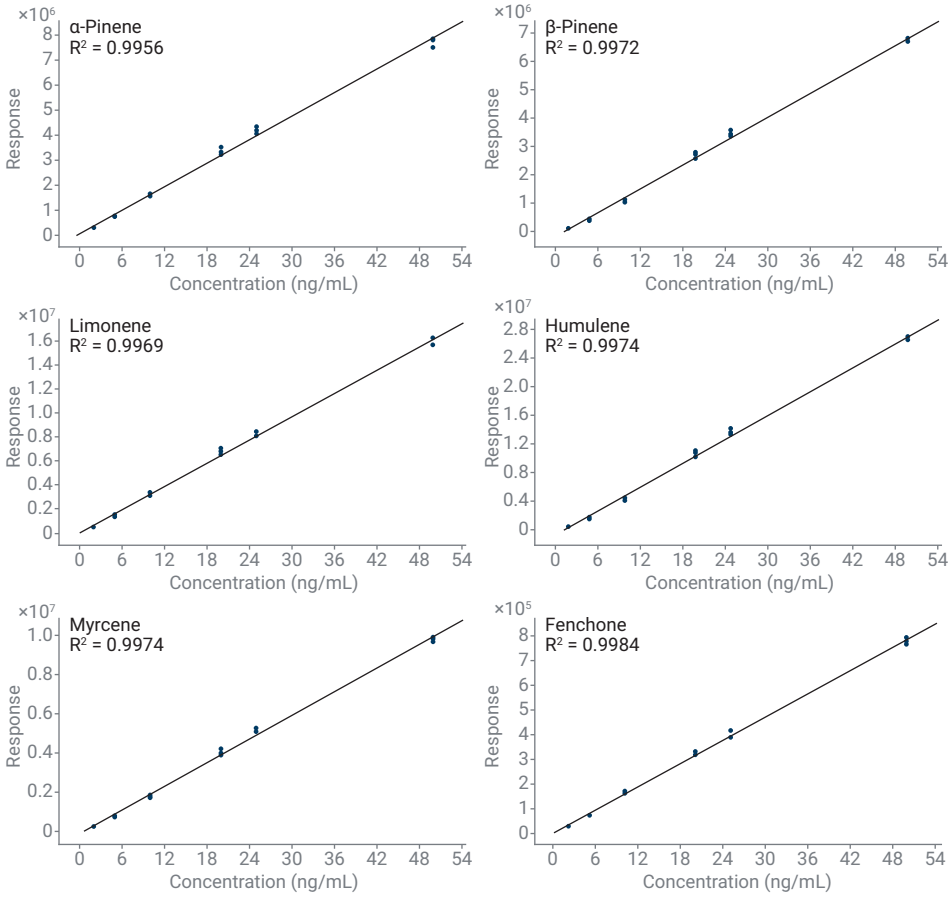

Figure 5. Calibration curves for 100 µm PDMS Arrow (2 to 50 ppm) of selected terpenes.

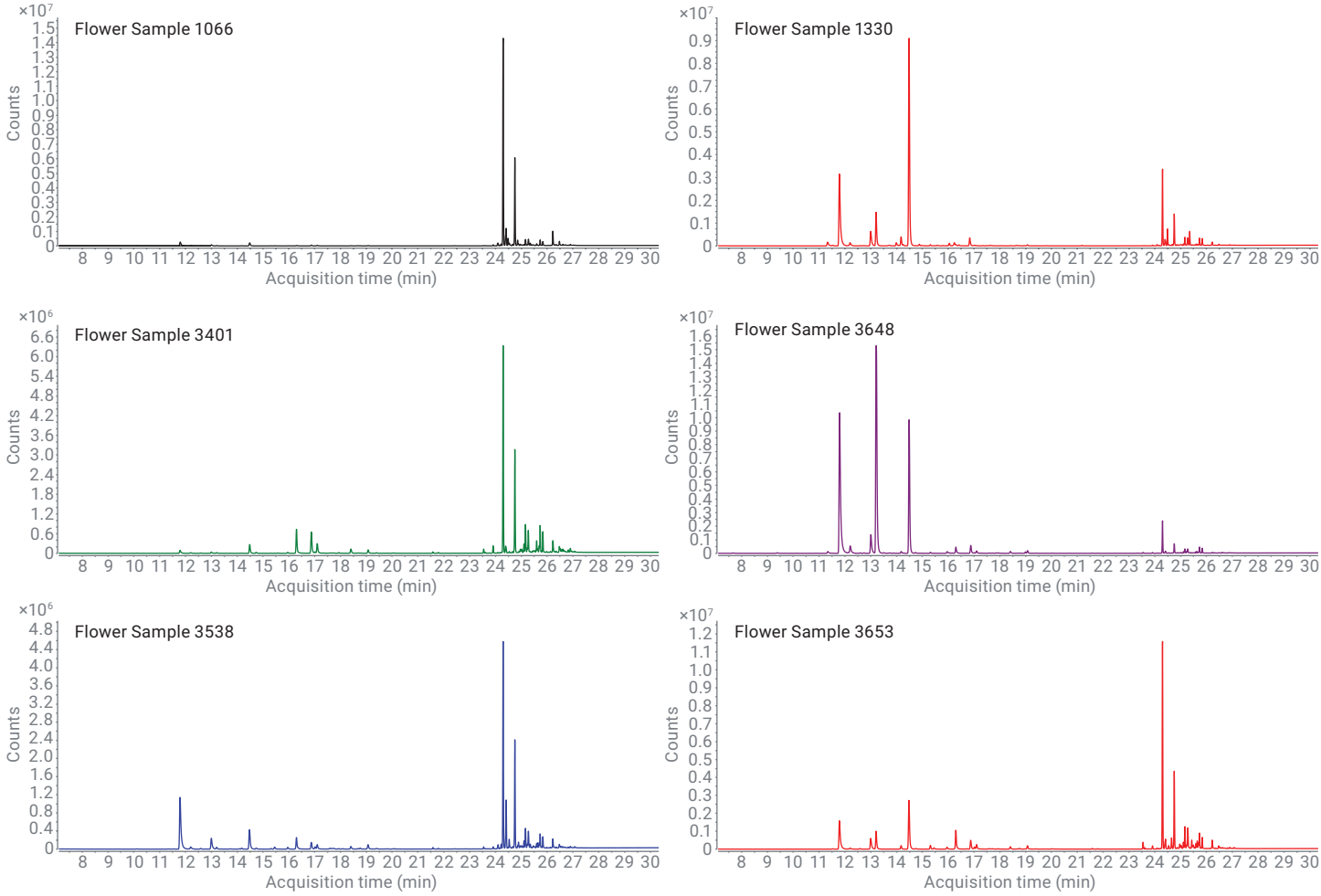

Figure 6. 100 µm PDMS Arrow GC/MS chromatograms (scan) of selected flower samples.

Table 2. Identification and quantitation of terpenes in selected flower samples (100 µm PDMS Arrow).

Terpene	Flower 1066	Flower 1330	Flower 3401	Flower 3538	Flower 3648	Flower 3653
alpha-Pinene	6.22	77.70	1.76	28.44	259.56	38.66
Camphene	0.72	3.96	0.73	1.48	14.19	1.88
Sabinene	0.82					0.89
Myrcene (tech)	1.91	12.09	1.25	4.90	24.75	11.43
b-Pinene	1.99	30.79	1.84	2.35	376.83	21.38
a-Phellandrene	0.16	0.65	0.14	0.17	0.45	0.20
3-Carene	0.09	1.79	0.10			
(R)-(+)-Limonene		78.93		4.66	85.49	26.11
Ocimene					1.07	1.05
Fenchone	1.48	1.36	5.31	7.37	24.31	19.53
Terpinolene (tech grade)	0.64	0.93	7.42	2.78	4.06	9.58
(+)-Fenchol			597.15	137.83	536.99	455.93
Camphor		7.62	4.95	5.37	7.15	7.48
Isoborneol			3.69		3.56	2.89
Menthol		19.23				
b-Caryophyllene	51.42	13.08	23.19	17.46	10.20	41.23
a-Humulene	17.48	4.87	10.04	7.74	3.04	13.21
Caryophyllene oxide	142.20	21.20	63.99	33.62	9.51	71.22
(−)-Guaiol	8.53			1.01	6.88	
(+)-Cedrol	34.87					

Conclusion

PAL SPME Arrows provided the sensitivity and robustness needed to profile the predominant terpenes in an unknown variety of cannabis plant samples thus showing that the SPME Arrows can be used for qualitative and quantitative determination of terpenes in plant material by HS sampling combined with GC/MS.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

References

1. Analysis of Terpenes in Cannabis Using the Agilent 7697A/7890B/5977B Headspace GC-MSD System Faster Analysis Time = Greater Productivity, *Agilent Technologies Application Note*, publication number 5991-8499EN. September **2017**.
2. Stenerson, K.; Halpenny, M. Analysis of Terpenes in Cannabis Using Headspace Solid-Phase Microextraction and GC–MS. *LCGC*, 01 May **2019**. http://www.chromatographyonline.com/analysis-terpenes-cannabis-using-headspace-solid-phase-microextraction-and-gc-ms.

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