



APPLICATION NOTE

Gas Chromatography/ Mass Spectrometry

Authors:

Lee Marotta

David Scott

Adam Floyd

Toby Astill

PerkinElmer Inc.
Shelton, CT

Cassandra (Cassie) Eremán

Ben Armstrong

Juniper Analytics LLC

Fast, Accurate, and Precise Terpene Testing of Cannabis Samples

Introduction

Like all botanicals and plants found in nature, cannabis also contains terpenes, which are the aromatic oils that give rise to the distinctive flavors and aromas found in cannabis varieties. There have been up to 140 different types of terpenes reported in cannabis, but multiple studies suggest that approximately 17 are the most common and can be used for examining their chemotype (chemotype: those strains that have chemical properties that differ from each other's).¹ Among them are monoterpenes, diterpenes, and sesquiterpenes, which are characterized by the number of repeating units of a five-carbon molecule, called isoprene, the structural hallmark of all terpenoid compounds.

The diverse palate of cannabis terpenes is impressive enough, but arguably their most fascinating characteristic is their ability to interact synergistically with other compounds in the plant, like cannabinoids. In the past few decades, a significant amount of work has been performed to understand the 'entourage effect', which scientists refer to as synergistic interaction between terpenes and cannabinoids in the human body. This effect is believed to magnify the therapeutic benefits of the plant's individual components — so that the medicinal impact of the whole plant is greater than the sum of its parts quantifying which terpenes are present is an important aspect of understanding the unique effects of cannabis for both medicinal and recreational users.

To further validate the performance of this method for the industry, The Emerald Test Proficiency Test (PT) for Terpenes was conducted. The Emerald Test™ is an Inter-Laboratory Comparison and Proficiency Test (ILC/PT) program for cannabis testing labs. The results from the PT inter-laboratory samples passed; therefore, the method meets inter-laboratory reproducibility and accuracy. The method was awarded the Emerald Test badge of approval seen on the right.
<https://pt.emeraldscientific.com/>



This paper will demonstrate a turnkey solution for the analysis of terpenes in cannabis samples by pressure-balanced headspace (HS) sample introduction and gas chromatography-mass spectrometry (GC/MS). In addition to a discussion of the instrumental parameters, optimization of the method to allow the highest sample throughput will be presented.

Headspace Sample Introduction Technique

One of the many benefits of this approach is that headspace using the pressure-balanced injection, is a fast, simple, accurate and precise solution, which allows the components of interest (e.g. terpenes and residual solvents) to be introduced into the analytical system. The non-volatile matrix components remain in the sample vial and do not enter the GC, which results in a mostly maintenance-free system, and faster analysis time.² In addition, the technique is mature and has already been accepted for quantitation in several regulatory industries including pharmaceutical (FDA), forensics, and environmental.³⁻⁵ It is routinely used for the characterization of flavors and fragrances in several matrices.⁶

Instrumentation

The TurboMatrix™ HS sampler and a Clarus® SQ 8 GC/MS (PerkinElmer Inc., Shelton, CT) was used for this study. It's worth emphasizing that MS detection was chosen so that the targeted compounds may be identified by their spectra, which ensures that any unknown components found in the sample that are not included in the standard, can be identified if present in the mass spectral library being used. In addition, the human taste threshold is very sensitive; therefore, a flavor can be present at very low levels, but still have high potency, which requires the high sensitivity of MS detection (compared to the flame ionization detector). Identifying and quantifying all the terpene and other flavor components using this turnkey solution, results in a faster analysis, enhanced productivity, quicker release of product, and maximized system uptime for high throughput cannabis testing labs.

Experimental

Fast chromatography was achieved using a commercially-available standard containing 42 terpenes (CAN-TERP-MIX1H and CAN-TERP-MIX2H, SPEX CertiPrep®, Metuchen, NJ) at 1000 µg/mL stock solution. Figure 1 represents the fast chromatogram at 20 ppm of both MIX 1 and MIX 2, in less than 12.5 minutes.

Alternatively, a faster time of under 8.5 minutes may be achieved which is seen in Figure 2. The difference between these two chromatograms is that there is one co-elution with an uncommon terpene.

For samples whose terpene concentrations exceeded the 500 ppm calibration using the SPEX CertiPrep® stock standard solution, a higher concentration commercially available standard (Restek® Corporation, Bellefonte, PA - catalog number 34095), was analyzed.

Figure 3 displays the chromatography of the Restek® standard using the fast 8.5 minute method.

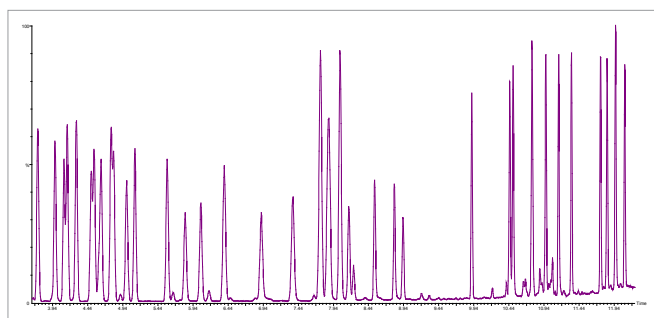


Figure 1. Fast total ion chromatogram (TIC) at standard concentration of 20 ppm of 42 terpenes in less than 12.5 minutes.

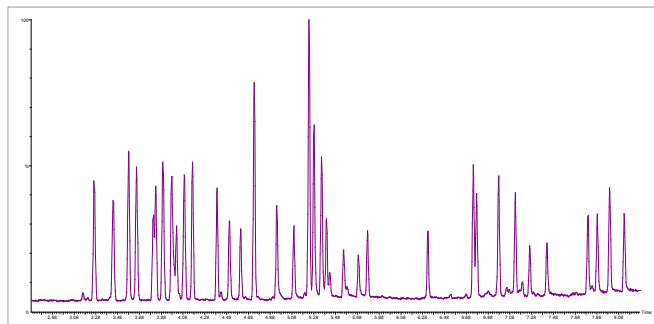


Figure 2. Fast total ion chromatogram (TIC) of a standard mixture of 42 terpenes from SPEX CertiPrep® in less than 8.5 minutes at a concentration of 20 ppm.

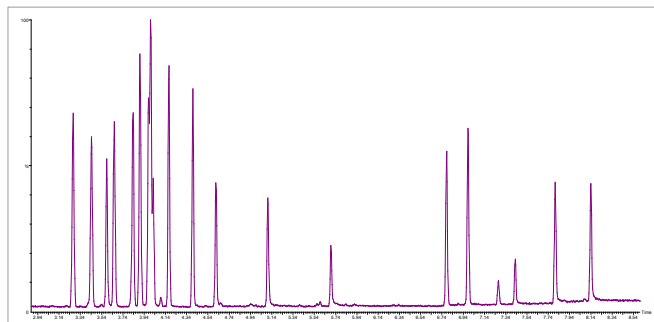


Figure 3. Fast total ion chromatogram (TIC) at standard concentration of 20 ppm of 42 terpenes in less than 8.5 minutes.

Validation

The experiments performed for validation of the method and analytical performance included dynamic range (linearity) and precision (repeatability). In addition, a cannabidiol (CBD) oil was spiked with a known standard concentration to determine compound matrix recoveries from a real sample, and proficiency test (PT)⁷ samples were analyzed for inter-laboratory reproducibility. Both the matrix spike and the PT results will demonstrate method accuracy.

Calibration

A seven (7) point calibration standard of 1.9 to 500 ppm, using the SPEX CertiPrep® standard was generated for all the terpenes under investigation. An example of the 1st order calibration plot of Linalool, a naturally-occurring terpene, is found in Figure 4.

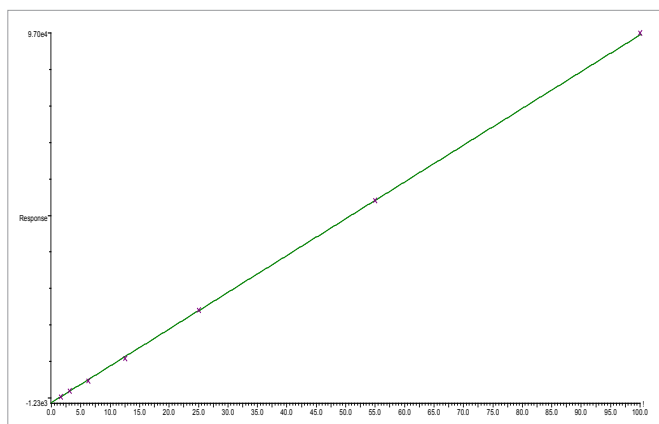


Figure 4. Graphical representation of the calibration for linalool from 1.9 to 500 ppm.

In addition, a seven-point calibration range of 2.5 to 2500 ppm was generated using the Restek® standard. An example of the 1st order calibration plot of β -myrcene is found in Figure 5.

Note: It's also important to emphasize that lower detection limits of terpenes can be achieved by this method if required.

Repeatability

Repeatability (precision) was determined using a 20 ppm SPEX CertiPrep® standard and a 50 ppm Restek® standard, inserting 20 μ L into eight separate headspace vials and analyzing them consecutively.

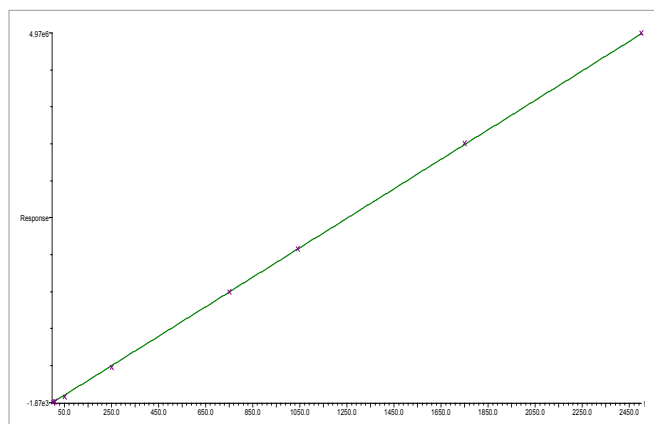


Figure 5. Calibration plot for β -myrcene from 2.5 to 2500 ppm.

Spike Recovery

A cannabidiol (CBD) oil was spiked at a concentration of 125 ppm using the SPEX CertiPrep® standard to test for recoveries, and 20 μ L was inserted into a headspace vial and analyzed.

Results

The results for calibration were demonstrated by calculating the correlation coefficients (r^2) for both standards. These data are exemplified in Table 1, which shows the results for the dynamic range, precision testing protocol and matrix spike recoveries, thus validating the terpene turnkey solution using the 12.5 minute methodology.

Table 1. The results for dynamic range (linearity), precision and recovery testing protocol.

Standard	SPEX CertiPrep Standard		Restek Standard		Matrix Spike
Compound	Calibration 1.9 to 500 ppm	Precision @ 20 ppm (n=8)	Calibration 2.5 to 2500 ppm	Precision @ 50 ppm (n=8)	% Recovery
α -Pinene	0.9997	1.99	0.9997	2.24	95.6%
Camphene	0.9998	1.60	0.9996	3.10	96.9%
β -Myrcene	0.9999	1.57	0.9998	1.87	99.8%
Sabinene	0.9999	1.57	np*	np	86.4%
β -Pinene	0.9998	1.98	0.9995	1.19	99.9%
α -Phellandrene	0.9997	1.77	np	np	92.1%
3-Carene	0.9999	0.98	0.9998	2.78	96.0%
α -Terpinene	0.9999	1.21	0.9990	2.48	102.9%
Limonene	0.9997	1.88	0.9994	1.49	92.6%
p-Cymene	np	np	0.9992	1.98	np
Ocimene (Isomers)	0.9995	2.47	0.9992	2.96	84.4%
Eucalyptol	0.9999	0.79	np	np	99.4%
γ -Terpinene	0.9998	1.10	0.9995	2.40	96.9%
Terpinolene	0.9997	1.89	0.9978	2.11	101.0%
Sabinene Hydrate	0.9996	2.10	np	np	88.6%
Linalool	0.9999	0.98	0.9982	2.67	88.8%
Fenchone (Isomers)	0.9996	1.55	0.9997	2.41	101.0%
Fenchol	0.9995	2.40	0.9992	3.19	89.3%
Isopulegol	0.9999	1.99	0.9995	2.50	107.0%
Camphor (Isomers)	0.9997	1.50	np	np	98.7%
Isoborneol	0.9996	1.70	np	np	99.2%

Table 1. CONTINUED

Standard	SPEX CertiPrep Standard		Restek Standard		Matrix Spike
Compound	Calibration 1.9 to 500 ppm	Precision @ 20 ppm (n=8)	Calibration 2.5 to 2500 ppm	Precision @ 50 ppm (n=8)	% Recovery
Hexahydrothymol (Menthol)	0.9996	1.70	np	np	101.3%
Borneol (+) and (-)	0.9996	1.99	np	np	99.0%
α -Terpineol	0.9991	2.87	np	np	84.9%
γ -Terpineol	0.9990	2.99	np	np	92.1%
Nerol	0.9999	1.50	np	np	106.3%
Geraniol	0.9997	1.61	0.9999	1.95	103.0%
Pulegone	0.9996	1.55	np	np	98.7%
Geranyl acetate	0.9995	1.43	np	np	112.0%
α -Cedrene	0.9999	1.11	np	np	95.1%
trans- β -Caryophyllene	0.9998	1.85	0.9997	2.95	97.7%
Farnesene (Isomers)	0.9995**	3.56	np	np	101.1%
α -Humulene	0.9997	2.85	0.9990	3.22	97.7%
Valencene	0.9999	2.10	np	np	100.3%
cis-Nerolidol	0.9990	3.10	0.9990	4.50	100.4%
trans-Nerolidol	0.9991	2.99	0.9993	4.35	97.3%
Guaiol	0.9999	2.77	0.9990	3.79	99.1%
Caryophyllene Oxide	0.9991	3.60	np	np	106.5%
Cedrol	0.9990	3.89	np	np	106.1%
α -Bisabolol	0.9990	3.85	0.9976	4.06	98.3%

*Component not present in this commercial stock standard

** Data from one isomer

Table 2 displays the results of terpenes found from an extracted flower analyzed on the TurboMatrix HS sampler and a Clarus SQ 8 GC/MS by Cassandra (Cassie) Eremán, Juniper Analytics, Bend, Oregon.

Table 2. Terpene results from an extracted flower courtesy of Cassandra (Cassie) Eremán.

Terpene Profile					
Compound	$\mu\text{g/g}$	%	Compound	$\mu\text{g/g}$	%
α -Pinene	1327.33	0.133	Isopulegol	nd	0.000
Camphene	125.78	0.013	Camphor	nd	0.000
Sabinene	80.96	0.008	Isoborneol	nd	0.000
β -Myrcene	3997.07	0.400	Borneol	78.67	0.008
β -Pinene	1711.53	0.171	Terpineol	1030.94	0.103
α -Phellandrene	665.95	0.067	γ -Terpineol	nd	0.000
3-Carene	481.17	0.048	Nerol	nd	0.000
α -Terpinene	252.93	0.025	Geraniol	nd	0.000
trans- β -Ocimene	nd*	0.000	(+)-Pulegone	nd	0.000
Limonene	4496.99	0.450	Geranyl acetate	nd	0.000
p-Cymene	nd	0.000	α -Cedrene	nd	0.000
cis- β -Ocimene	nd	0.000	trans- β -Caryophyllene	2882.43	0.288
Eucalyptol	nd	0.000	α -Humulene	1014.02	0.101
γ -Terpinene	309.19	0.031	Valencene	nd	0.000
Terpinolene	9445.88	0.945	cis-Nerolidol	nd	0.000
Sabinene Hydrate	nd	0.000	trans-Nerolidol	nd	0.000
Linalool	733.19	0.073	Guaiol	nd	0.000
allo-Ocimene	nd	0.000	Caryophyllene oxide	nd	0.000
Fenchone	50.77	0.005	Cedrol	nd	0.000
Fenchol	474.76	0.047	α -Bisabolol	nd	0.000
Total				29159.57	2.916

*not detected compound in this sample

Figure 6 is a chart displaying the terpene profile of this extract.

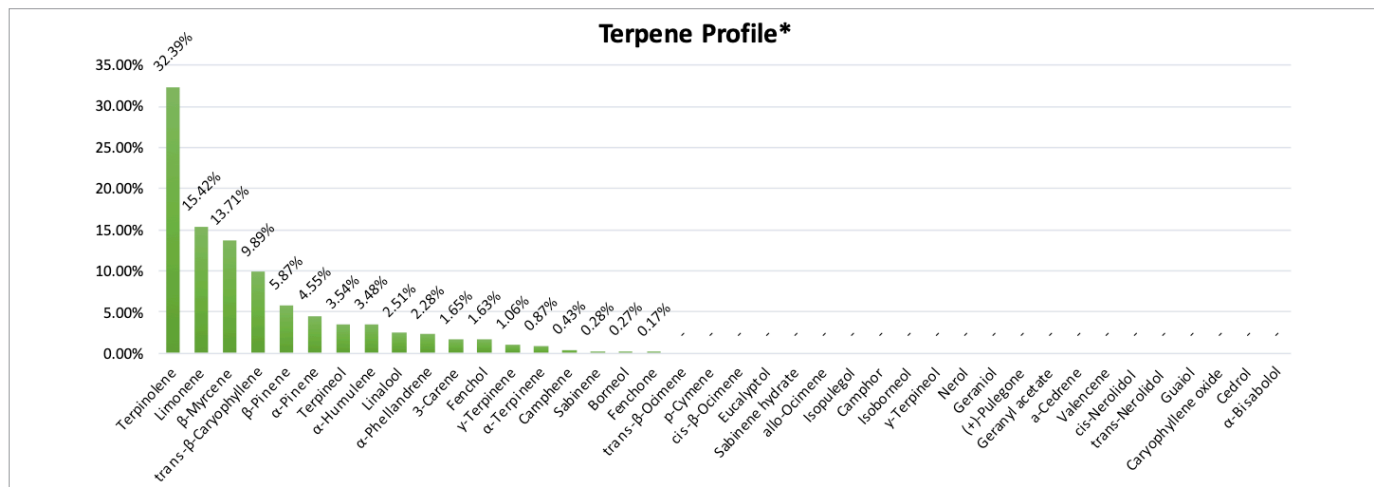


Figure 6. Terpene profile of the results from an extracted flower.

Discussion of Results

The objective of this study was to create a method to determine the concentration of terpenes in cannabis and cannabis products that is fast, linear, precise and accurate, which has clearly been achieved. In addition, since the Clarus 690 GC has rapid cooling returning to initial temperature only takes 1.6 minutes, so as a result the sample throughput is 10.5 or 14.0 minutes, depending on which of the two methods the laboratory chooses.

The correlation coefficient for all compounds is at or better than 0.999, most being greater than 0.9996, demonstrating excellent linearity. Precision and spiked recovery values are better than the criteria of regulatory requirements.

Conclusion

In conclusion, the evidence strongly suggests that the PerkinElmer HS-GC/MS solution for the determination of terpenes is fast and robust providing the laboratory with greater sample throughput, instrument uptime, essentially a maintenance free system, and enhanced profits.

Additionally, this procedure is a turnkey solution; therefore all acquisition and processing methods and standard operating procedures (SOP) will be provided upon installation of the system.

Note: For a turnkey solution of residual solvents in cannabis concentrates using headspace (HS) sampling coupled with GC/MS, please refer the following citation.⁸

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com



For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

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