GERSTEL Application Note No. **189**, *2017*

Quantitative Determination of Terpenes in Cannabis Using Headspace Solid Phase Microextraction and GC/MS

Michael Halpenny, Katherine K. Stenerson

MilliporeSigma, 595 North Harrison Road, Bellefonte, PA 16823, USA

Introduction

Well known for their characteristic flavor and fragrance characteristics, terpenes are contained in the derived essential oils of cannabis. Analysis of cannabis for terpene concentrations can be applied to strain identification, referred to as fingerprinting, and for concentration accuracy when applied to medicinal treatments. Terpenes have high vapor pressures, are extremely volatile and thus are an excellent candidate for static headspace GC analysis. In this work, headspace SPME (HS-SPME) was combined with GC/MS for the quantitative analysis of several selected terpenes in cannabis. The conventional approach for terpene analysis in cannabis involves a solvent extraction followed by GC/FID analysis. HS-SPME offers several advantages over the solvent extraction method in that it is non-destructive to the sample, requires a very small sample size, produces minimal interference from co-extracted matrix, protects the GC instrument from contamination, and can be easily automated.

Experimental

Three terpenes were selected for quantitative measurement by HS-SPME: α-Pinene, R-(+)-Limonene, and Linalool. SPME was performed using an XYZ autosampler as shown in Figure 1. This enhanced method reproducibility and allowed for minimal “hands-on” time during sample preparation. The cannabis used for the study was provided courtesy of Dr. Hari H. Singh, program Director at the Chemistry & Physiological Systems Research Branch of the National Institute on Drug Abuse at the National Institute of Health. The strain of the cannabis sample was unknown, and HS-SPME analysis of this sample showed it to have much lower terpene content than what is typically reported for many strains sold for medical and recreational use [1]. Thus, in order to evaluate method accuracy in relevant concentration ranges, the sample had to be spiked with additional amounts of terpenes. Spiking was done by weight using a solution of terpenes in Hexane. Samples were allowed to equilibrate for 10 minutes after the spike was added and prior to proceeding with HS-SPME analysis. Quantitation of the spiked samples was done by external standard analysis, against a 5-point matrix matched calibration curve in cannabis.

Figure 1. MultiPurpose Sampler (MPS) in use during terpene analysis.

Instrumentation. Analyses were performed on a 6890 GC equipped with a 5973N MSD (Agilent Technologies) and a MPS Sampler with SPME Option (GERSTEL), controlled by MAESTRO software, integrated with the Agilent software, using one method and one integrated sequence table for the complete system.

Analysis conditions.

MPS:	SPME (PDMS fused silica)
	d_f = 100 µm d_o=24 ga
Equilibration:	40°C (5 min), at 60 rpm
Extraction:	40°C (10 min), at 250 rpm (headspace)
Desorption:	270°C (3 min)
Postbake:	270°C (5 min)
S/SL:	270°C (isothermal)
	SPME liner, d_i = 0.75 mm
Pneumatics:	constant flow
Column:	60 m Equity-1 (Supelco),
	d_i = 0.25 mm d_f = 0.25 µm
Oven:	60°C (2 min); 5°C/min; 140°C; 15°C/min; 250°C (0 min)
MSD:	full scan, 50-500 amu
	300°C transfer line temp.

Sample preparation. 0.1 g ground cannabis weighed into 20 mL clear glass, screw top vial with 8 mL deionized water added.

Results & Discussion

The results of analyses of the five point cannabis matrix-matched calibration curves for each terpene are shown in Figure 2. The unspiked cannabis was used as a zero point in each calibration. Linearity was good for all three terpenes across the indicated ranges. The high sensitivity of the HS-SPME method made it necessary to reduce the sample size to 100 mg and to open the splitter in the GC inlet during desorption. These measures were necessary to prevent overloading the GC/MS system.

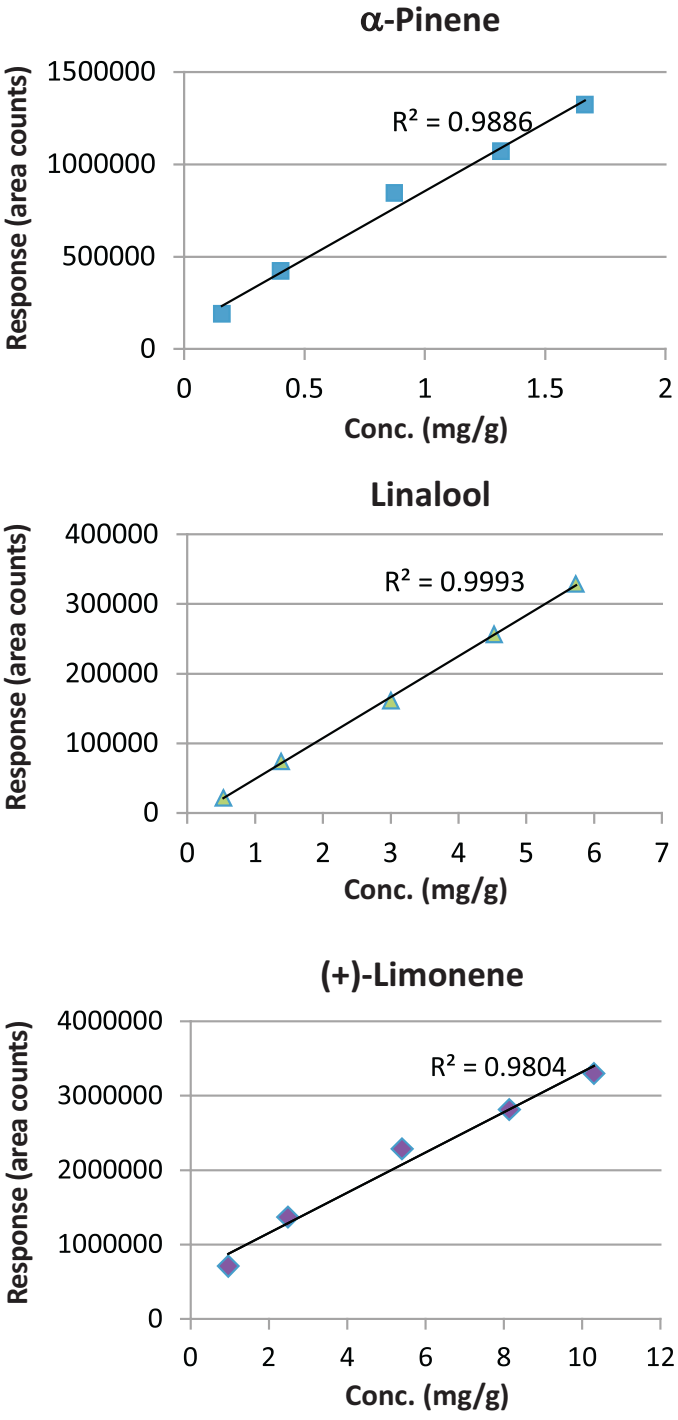

Figure 2. Head-space SPME extracted calibration curves of cannabis spiked with terpenes.

An example of a chromatogram of a terpene-spiked cannabis sample is shown in Figure 3. For comparison, an unspiked sample of the same cannabis is shown in Figure 4. As indicated by the response scales on the Y-axis for the chromatograms, the terpenes targeted in the spiking study (α-Pinene, (R)-(+) Limonene and Linalool) were present in the unspiked cannabis, but at levels significantly lower than the spiked samples. In the lower scale chromatogram shown in Figure 4, heavier terpenes are also visible eluting towards the end of the run. Several of these were previously identified, and include Caryophyllene, Bergamotene, Farnesene, and other sesquiterpenes [2]. Table 1 shows a summary of method accuracy and reproducibility from analysis of spiked replicates. Accuracies for the targeted terpenes were > 90 % with RSD values of < 5 %.

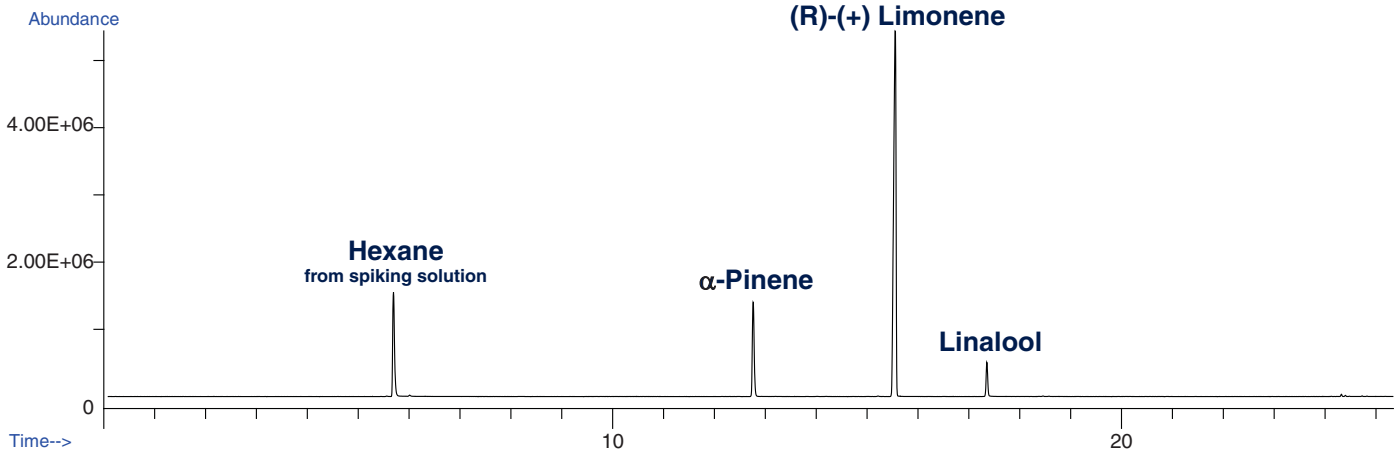

Figure 3. HS-SPME GC/MS analysis of cannabis spiked with terpenes: 3-7 mg/g.

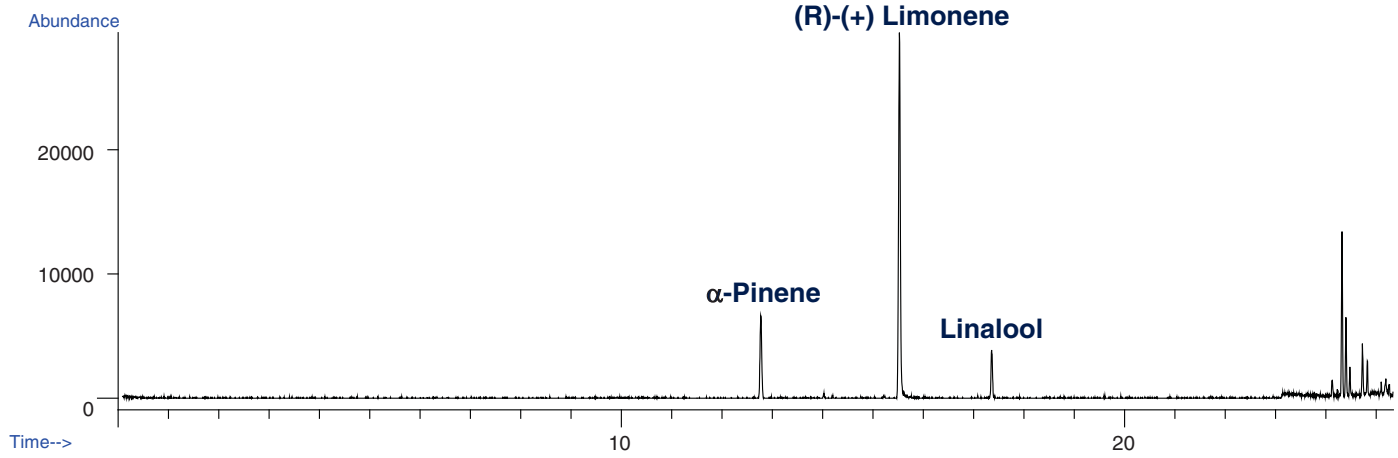

Figure 4. HS-SPME GC/MS analysis of unspiked cannabis, unknown variety.

Table 1. Results of analysis of spiked cannabis sample using HS-SPME method.

Compound	Spike Conc. [mg/g]	Avg. amount measured [mg/g]	Accuracy [%]	RSD (n=3) [%]
α-Pinene	1.08	1.11	103	0.9
(R)-(+)-Limonene	6.69	6.11	91	2.7
Linalool	3.72	3.62	97	3.0

Conclusions

An HS-SPME method was developed which allows for an easy and accurate determination of terpene content in cannabis. The method was shown for three important terpenes found in cannabis: α-Pinene, (R)-(+) Limonene and Linalool, however it could also be used for other terpenes as well. The method does not require organic solvents, and with the use of an autosampler, is highly reproducible and requires little "hands-on" sample preparation time. In addition, due to the high sensitivity of the SPME technique, very little sample is required. Using headspace produces a very clean chromatographic analysis with little to no background from co-extracted matrix, which in turn will maintain the cleanliness of the GC system. Using GC/MS offers the added benefit of spectral confirmation of peaks to ensure that identification is accurate with no co-eluting interferences.

References

[1] Williams, K. Cannabinoids and Terpenoids: *The Future of Cannabis Connoisseurship*. **2/21/2014**, www.leafly.com/news/tags/connoisseur (accessed June 2016)

[2] Stenerson, K.K. *Headspace SPME-GC/MS Analysis of Terpenes in Hops and Cannabis*; MilliporeSigma Reporter 34.4: 3-6.

Acknowledgements

The authors would like to thank Bob Shirey and Yong Chen of MilliporeSigma for their many helpful discussions on SPME.

GERSTEL AppNote

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
+41 (41) 9 21 97 25
swiss@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
Level 25, North Tower
One Raffles Quay
Singapore 048583
+65 6622 5486
+65 6622 5999
SEA@gerstel.com
www.gerstel.com

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

CERTIFIED QM-SYSTEM * * * ISO 9001 * ISO 9001:2008 CERTIFICATE NO. 0086 D

ECOPROFIT®
Awarded for the active pursuit of environmental sustainability