Supelco®
Analytical Products

**Application Note**

# Headspace SPME-GC/MS Analysis of Terpenes in Cannabis

A rapid method to identify cannabis terpenes for forensic and organoleptic applications

*Cannabis sativa* (cannabis or marijuana) contains over 100 different terpenes and terpenoids, including mono, sesqui, di and tri, as well as other miscellaneous compounds of terpenoid orgin.[1] Terpenes give the plant distinct organoleptic properties and produce characteristic aromas when the buds are heated or vaporized.[2] Although the terpene profile does not necessarily indicate geographic origin of a cannabis sample, it can be used in forensic applications to determine the common source of different samples.[3] In addition, different cannabis strains have been developed which have distinct aromas and flavors, a result of the differing amounts of specific terpenes present.[4]

## Experimental

Dried cannabis sample was obtained courtesy of Dr. Hari H. Singh, Program Director at the Chemistry and Physiological Systems Research Branch of the United States National Institute on Drug Abuse at the National Institute of Health. Terpenes were isolated using headspace solid phase microextraction (SPME) followed by chromatographic separation on an Equity®-1 capillary GC column. Peak identifications were assigned using MS spectral matching against reference spectra in the Wiley and NIST libraries. Confirmatory identification was done based on retention index, which was calculated for the compounds identified in each sample using an n-alkane standard analyzed under the same GC conditions. This data was compared with published values and peak identifications were assigned.[5,6,7] Final analytical conditions appear in **Figure 1**.

## Results and Discussion

The terpenes identified in the cannabis sample (**Figure 1**) are indicated in **Table 1**. The profile was similar to those found previously in the analysis of dried cannabis.[3,5] Early eluting peaks generally were monoterpenes and monoterpenoids. The later eluting peaks consisted of sequiterpenes and caryophyllene oxide, which is a sequiterpenoid. The most abundant terpene was caryophyllene. The predominance of this compound could be due to the specific strain of cannabis tested, and/

**Figure 1. Headspace SPME-GC/MS Analysis of Dried *Cannabis Sativa***

(See **Table 1** for peak identification)

| | |
|---|---|
| sample/matrix: | 0.5 g dried, ground *cannabis sativa* |
| SPME fiber: | 50/30 µm DVB/CAR/PDMS (57298-U) |
| sample equilibration: | 30 min, 40 °C |
| extraction: | 20 min, headspace, 40 °C |
| desorption process: | 3 min, 270 °C |
| fiber post bake: | 3 min, 270 °C |
| column: | Equity®-1, 60 m × 0.25 mm I.D., 0.25 µm (28047-U) |
| oven: | 60 °C (2 min), 5 °C/min to 275 °C (5 min) |
| inj. temp.: | 270 °C |
| detector: | MSD |
| MSD interface: | 300 °C |
| scan range: | full scan, m/z 50-500 |
| carrier gas: | helium, 1 mL/min constant flow |
| liner: | 0.75 mm ID SPME |
| instrument: | Agilent® 6890/5973N GC/MS |

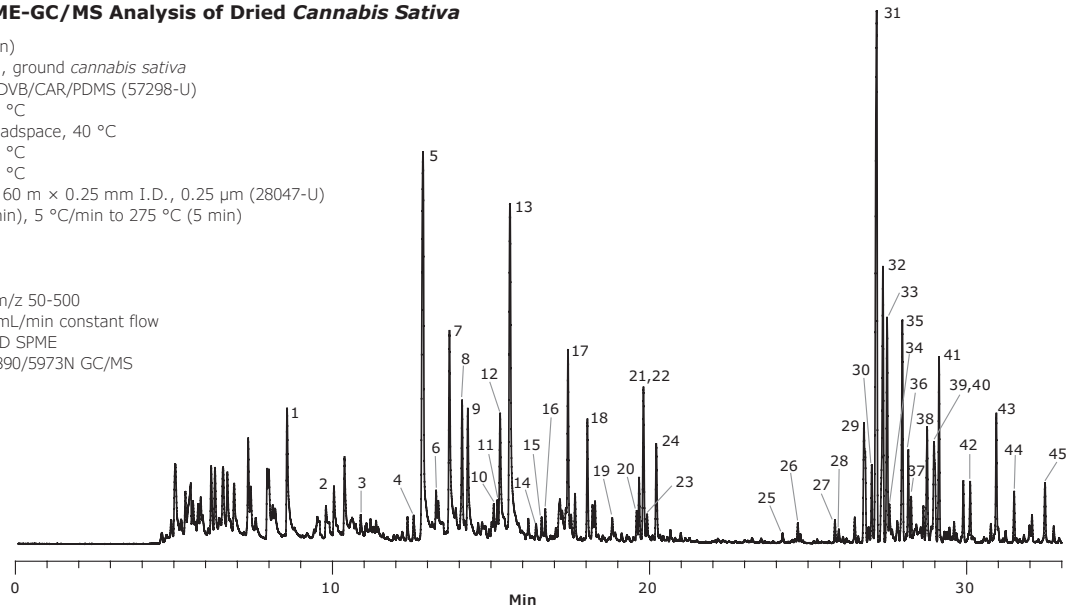

**Table 1. Terpenes in Dried Cannabis Identified by MS Spectral Library Match and Retention Index**

| Peak No. | RT (min) | Name | RI (calculated) | RI (literature) |
|---|---|---|---|---|
| 1 | 8.57 | Hexanal | — | — |
| 2 | 10.05 | Hexene-1-ol | — | — |
| 3 | 10.89 | 2-Heptanone | — | — |
| 4 | 12.56 | α-Thujene | 928 | 938 |
| 5 | 12.86 | α-Pinene + unknown | 939 | 942 |
| 6 | 13.27 | Camphene | 953 | 954 |
| 7 | 13.69 | 6-Methyl-5-hepten-2-one | 966 | 968 |
| 8 | 14.09 | β-Pinene | 979 | 981 |
| 9 | 14.27 | β-Myrcene | 984 | 986 |
| 10 | 15.09 | Δ-3-Carene | 1010 | 1015 |
| 11 | 15.20 | α-Terpinene | 1014 | 1012 |
| 12 | 15.29 | Cymene | 1018 | 1020 |
| 13 | 15.60 | d-Limonene | 1028 | 1030 |
| 14 | 16.42 | γ-Terpinene | 1056 | 1057 |
| 15 | 16.60 | *trans*-Sabinene hydrate | 1062 | 1078 |
| 16 | 16.72 | cis-Linalool oxide | 1066 | 1068 |
| 17 | 17.43 | Linalool | 1087 | 1092 |
| 18 | 18.04 | d-Fenchyl alcohol | 1107 | 1110 |
| 19 | 18.82 | *trans*-Pinocarveol | 1135 | 1134 |
| 20 | 19.59 | Borneol L | 1161 | 1164 |
| 21 | 19.81 | 1,8-Methandien-4-ol | 1168 | 1173 |
| 22 | 19.81 | p-Cymen-8-ol | 1168 | 1172 |
| 23 | 19.92 | Terpinene-4-ol | 1172 | 1185 |
| 24 | 20.22 | α-Terpineol | 1181 | 1185 |
| 25 | 24.20 | Piperitenone | 1322 | 1320 |
| 26 | 24.76 | Piperitenone oxide | 1344 | 1352 |
| 27 | 25.85 | α-Ylangene | 1384 | 1373 |
| 28 | 25.97 | α-Copaene | 1388 | 1398 |
| 29 | 26.76 | γ-Caryophyllene | 1419 | 1403 |
| 30 | 27.01 | α-Santalene | 1429 | 1428 |
| 31 | 27.16 | Caryophyllene | 1435 | 1428 |
| 32 | 27.36 | *trans*-α-Bergamotene + unknown | 1443 | 1443 |
| 33 | 27.49 | α-Guaiene | 1448 | 1441 |
| 34 | 27.56 | *trans*-β-Farnesene | 1451 | 1446 |
| 35 | 27.98 | Humulene | 1467 | 1465 |
| 36 | 28.17 | Alloaromadendrene | 1475 | 1478 |
| 37 | 28.25 | α-Curcumene | 1478 | 1479 |
| 38 | 28.75 | β-Selinene | 1497 | 1487 |
| 39 | 28.97 | α-Selinene | 1507 | 1497 |
| 40 | 28.97 | β-Bisobolene | 1507 | 1506 |
| 41 | 29.13 | α-Bulnesene | 1514 | 1513 |
| 42 | 30.12 | Selina-3,7(11)-diene | 1556 | 1542 |
| 43 | 30.94 | Caryophyllene oxide | 1590 | 1595 |
| 44 | 31.50 | Humulene oxide | 1614 | 1599 |
| 45 | 32.48 | Caryophylla-3,8(13)-dien-5-ol A | 1658 | 1656 |

or the nature of the sample tested, which was dried. Previous studies have shown the level of this compound to increase significantly relative to other terepenes and terpenoids with drying.[3] Consequently, the levels of the more volatile monoterpenes and terpenoids would be expected to be less, and this was observed to some degree. Among the monoterpenes and terpenoids, the most abundant were α-pinene and limonene.

**References**


1. Turner, C.E.; Elsohly, E.G.; Boeren, E.G. Constituents of Cannabis Sativa L., XVIII. A Review of the Natural Constituents. *J. Nat. Prod.*, **1980**, 43, 169-234.
2. Kemsley, J. Cannabis Safety. *CEN,* **2015**, April 20, 27-28.
3. Hood, L.V.S.; Barry, G.T. Headspace Volatiles of Marihuana and Hashish: Gas Chromatographic Analysis of Samples of Different Geographic Origin. *J. Chrom. A.*, **1978**, 166, 499-506.
4. Rahn, B. Terpenes: The Flavors of Cannabis Aromatherapy. 2/12/2014. www.leafly.com/news, accessed September 18, 2015.
5. Marchini, M.; Charvoz, C.; Dujourdy, L.; Baldovini, N. Multidimensional Analysis of Cannabis Volatile Constituents: Identification of 5,5-dimethyl-1-vinylbicyclo[1.1.1]hexane as a Volatile Marker of Hashish, the Resin of Cannabis Sativa L. J. Chrom A., 2014, 1370, 200-215.
6. Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*, Academic Press, 1980.
7. NIST Chemistry WebBook. http://webbook.nist.gov/chemistry.


## Featured and Related Products

| Description | Cat. No. |
|---|---|
| **SPME Fibers and Accessories** | |
| SPME fiber assembly Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS), needle size 23 ga, StableFlex™, for use with autosampler | **57298-U** |
| SPME fiber assembly Polydimethylsiloxane (PDMS), $d_f$ 100 µm (nonbonded phase), needle size 23 ga, for use with autosampler | **57341-U** |
| SPME fiber holder for CTC autosampler | **57347-U** |
| SPME fiber holder for manual sampling | **57330-U** |
| **Capillary GC Column** | |
| Equity®-1, 60 m × 0.25 mm I.D., 0.25 µm | **28047-U** |
| **Certified Reference Materials** | |
| **Explore Terpene Solution CRMs and Mixes at SigmaAldrich.com/cannabis** | |
| **Accessories** | |
| Inlet Liner, Direct (SPME) Type, Straight Design, 0.75 mm I.D. for Agilent GC | **2637501** |
| Molded Thermogreen® LB-2 Septa, with injection hole, 11 mm, pk. of 50 | **28336-U** |
| Headspace Vial, Screw Top, rounded bottom, 10 mL, clear glass, pk of 100 | **SU860099** |
| Magnetic Screw Cap for Headspace Vials, PTFE/silicone septum, pk of 100 | **SU860103** |

Visit our cannabis testing resources at
**SigmaAldrich.com/cannabis**

## To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
Technical Service: **SigmaAldrich.com/techservice**
Safety-related Information: **SigmaAldrich.com/safetycenter**

**SigmaAldrich.com**




Lit. No. RYI 2017-08269 08/2018