FOOD & BEVERAGE

Headspace SPME-GC/MS Analysis of Terpenes in Hops and Cannabis

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In this application, headspace-SPME combined with GC/MS was used to analyze some of the terpenes present in both common hops and cannabis.

Terpenes are small molecules synthesized by some plants. The name terpene is derived from turpentine, which contains high concentrations of these compounds. Terpene molecules are constructed from the joining of isoprene units in a head-to-tail configuration (**Figure 1**). Classification is then done according to the number of these isoprene units in the structure (**Table 1**). The configurations of terpenes can be cyclic or open, and can include double bonds, and hydroxyl, carbonyl or other functional groups. If the terpene contains elements other than C and H, it is referred to as a terpenoid.[1]

Figure 1. Isoprene Unit

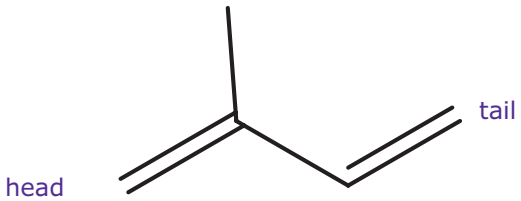

Table 1. Classification of Terpenes

Classification	Number of Isoprene Units
Monoterpene	2
Sesquiterpene	3
Diterpene	4
Triterpene	6
Tetraterpene	8

Terpenes are present in essential oils derived from plants and often impart characteristic aromas to the plant or its oil. For example, d-Limonene, which is found in lemon, orange, caraway and other plant oils, has a lemon-like odor. Essential oils, with their component terpenes and terpenoids, have been applied in therapeutic use known as aromatherapy to aid in the relief of conditions such as anxiety, depression, and insomnia.[2] This has led to the use of plants which contain these compounds in preparations such as oils, teas, and tonics.

Using Terpene Profile for Plant Identification

The *cannabis sativa* (cannabis or marijuana) plant contains over 100 different terpenes and terpenoids, including mono, sesqui, di, and tri, as well as other miscellaneous compounds of terpenoid orgin.[3] 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.[4] In addition, different cannabis strains have been developed which have distinct aromas and flavors; a result of the differing amounts of specific terpenes present.[5] *Humulus lupulus* (common hops) and cannabis are both members of the family Cannabaceae.[6] Consequently, there are similarities in the terpenes each contains. Terpenes give both plant commodities characteristic organoleptic properties and, in the case of cannabis, produce characteristic aromas when the buds are heated or vaporized.[7]

Experimental

Dried cannabis sample was obtained courtesy of Dr. Hari H. Singh, Program Director at the Chemistry & Physiological Systems Research Branch of the United States National Institute on Drug Abuse at the National Institute of Health. The extract strain of the sample was not known. Hop flowers of an unknown variety were purchased from an on-line source. Pelletized of Cascade and US Golding hop varieties were purchased at a local home-brew supply shop. Chromatographic separation was performed on an Equity®-1 capillary GC column, and identification was done using retention indices and spectral library match. Final analytical conditions appear in the figures.

SPME Method Optimization

The SPME method was developed using a sample of dried hops flowers (0.2 g in 10 mL vial). The initial SPME parameters were based on previously published work.[8] The GC/MS results of this analysis are shown in **Figure 2**. This initial set of parameters used the 100 µm PDMS fiber, a 1 g sample size, and 60 minute equilibration at room temperature prior to extraction. The sample size was then scaled down to 0.2 g, and the equilibration temperature increased to 40 °C. This increased temperature allowed the equilibration time to be decreased from 60 to 30 minutes without a loss in sensitivity (**Figures 3 and 4**). The initial extraction

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Figure 2. Headspace SPME-GC/MS Analysis of Dried Hops Flowers (100 µm PDMS Fiber, 1 g Sample)

Sample/matrix:	1 g ground hop flowers
SPME fiber:	100 µm PDMS (57341-U)
Sample equilibration:	60 min, room temperature
Extraction:	20 min, headspace, 40 °C
Desorption process:	3 min, 270 °C
Fiber post bake:	3 min, 270 °C
Column:	Equity®-1, 60 m x 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

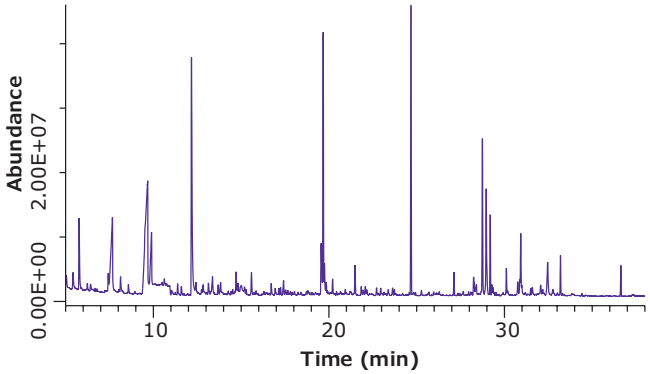
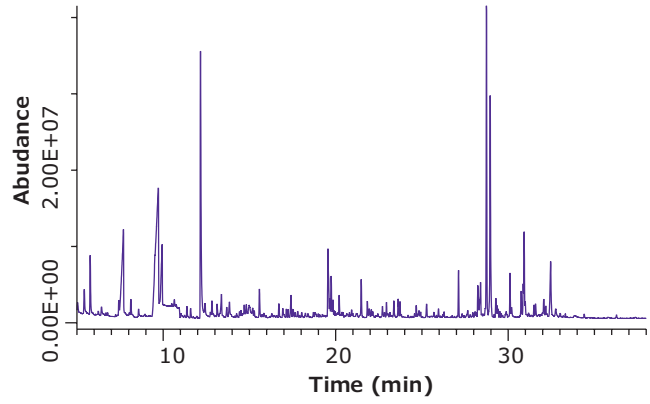


Figure 3. Headspace SPME-GC/MS Analysis of Dried Hops Flowers (100 µm PDMS Fiber, 0.2 g Sample)

Conditions same as Figure 2 except:
sample/matrix: 0.2 g ground hop flowers



time used was 20 min, and a shorter extraction time of 10 minutes was evaluated. However a loss in sensitivity was noted, thus extraction time was maintained at 20 minutes. The DVB/CAR/PDMS fiber was then evaluated (**Figure 5**). As expected, this fiber extracted more of the lighter compounds, which by MS spectral match, were identified as short chain alcohols and acids.

Figure 4. Headspace SPME-GC/MS Analysis of Dried Hops Flowers, Increased Sample Equilibration Temperature (100 µm PDMS Fiber, 0.2 g Sample)

Conditions same as Figure 2 except:	
sample/matrix:	0.2 g ground hop flowers
sample equilibration:	30 min, 40 °C

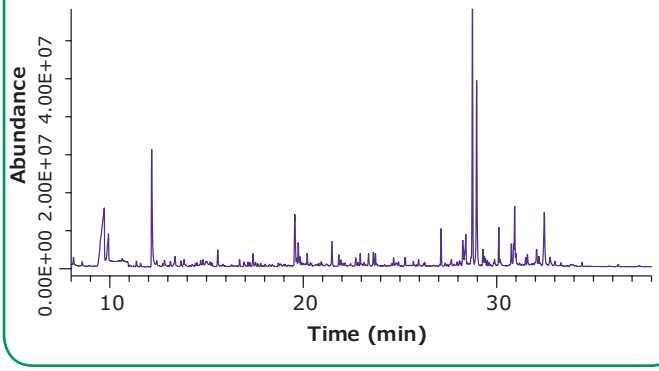
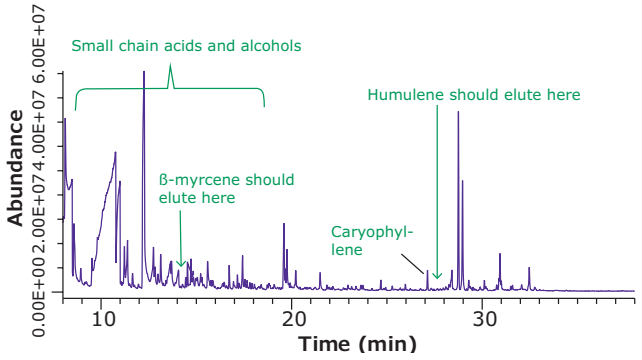


Figure 5. Headspace SPME-GC/MS Analysis of Dried Hops Flowers, Increased Sample Equilibration Temperature (DVB/CAR/PDMS Fiber, 0.2 g Sample)

Conditions same as Figure 2 except:	
sample/matrix:	0.2 g ground hop flowers
SPME fiber:	50/30 µm DVB/CAR/PDMS (57298-U)
sample equilibration:	30 min, 40 °C



Identification of Terpenes Using GC/MS

Using the DVB/CAR/PDMS fiber, samples of hops and cannabis were analyzed using the optimized SPME method. 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. Retention indices were 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 (**Tables 2 and 3**), and final identifications were assigned, as shown in **Figures 6 and 7**.

Terpenes in Hops Samples

For the dried hop flower sample (**Figure 5**), the terpene profile should have shown a predominance of β-myrcene, humulene, and caryophyllene, which are typical aroma compounds in hops and hop oil.[9] While caryophyllene was identified, both β-myrcene and humulene were not present at levels high enough to be detected by a library search. This may be due to the condition of the

Table 2. Terpenes in Hops Pellets Identified by MS Spectral Library Match and Retention Index

Peak No.	RT (min)	Name	RI (calculated)	RI (literature)	Reference
1	8.58	Hexanal	—	780	11
2	12.84	α-Pinene	939	942	11
3	13.28	Camphene	953	954	11
4	13.71	6-Methyl-5-hepten-2-one	966	968	11
5	14.1	β-Pinene	979	981	11
6	14.41	β-Myrcene	988	986	11
7	15.32	Cymene	1018	1020	11
8	15.65	d-Limonene	1030	1030	11
9	15.98	β-Ocimene	1041	1038	11
10	16.72	*cis*-Linalool oxide	1066	1068	11
11	17.49	Linalool	1089	1092	11
12	21.86	Geraniol	1239	1243	11
13	25.28	Geranyl acetate	1363	1364	11
14	25.85	α-Ylangene	1384	1373	8
15	25.97	α-Copaene	1388	1398	11
16	27.22	Caryophyllene	1437	1428	11
17	27.4	*trans*-α-Bergamotene + unknown	1445	1443	12
18	17.63	*trans*-β-Farnesene	1454	1450	8
19	28.11	Humulene	1473	1465	11
20	28.41	γ-Muurolene	1484	1475	11
21	28.45	γ-Selinene	1486	1472	12
22	28.68	Geranyl isobutyrate	1495	1493	11
23	28.79	β-Selinene	1499	1487	8
24	28.94	α-Muurolene	1505	1500	11
25	28.97	α-Selinene	1507	1501	12
26	29.31	γ-Cadinene	1521	1518	11
27	29.37	Calamenene	1524	1518	11
28	29.45	Δ-Cadinene	1527	1524	11
29	30.93	Caryophyllene oxide	1590	1584	8
30	31.5	Humulene oxide	1614	1599	12

Figure 6. Headspace SPME-GC/MS Analysis of Hops Pellets Using Final Optimized Method

The peak elution order is listed in Table 2.	
Conditions same as Figure 2 except:	
sample/matrix:	0.5 g ground hop flowers (hops pellets)
SPME fiber:	50/30 µm DVB/CAR/PDMS (57298-U)
sample equilibration:	30 min, 40 °C

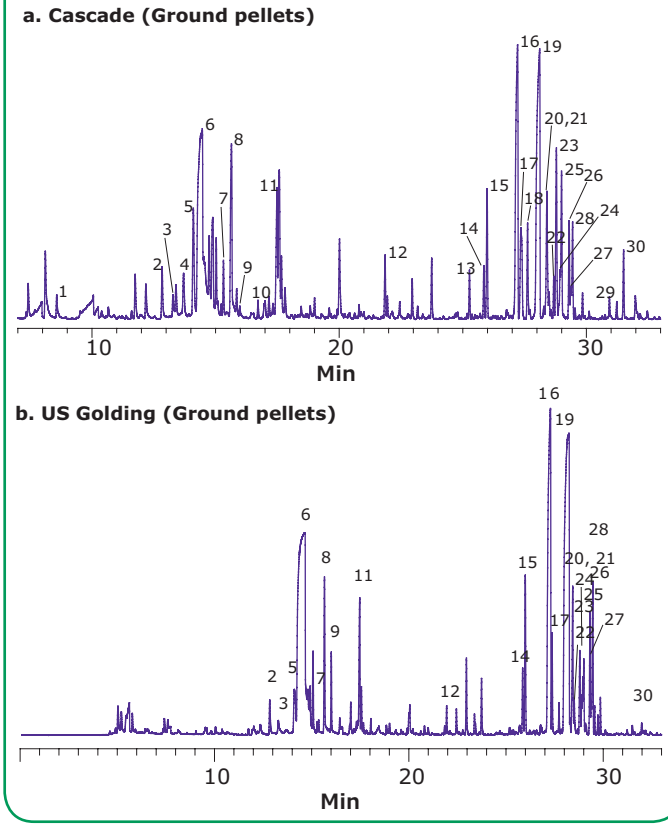
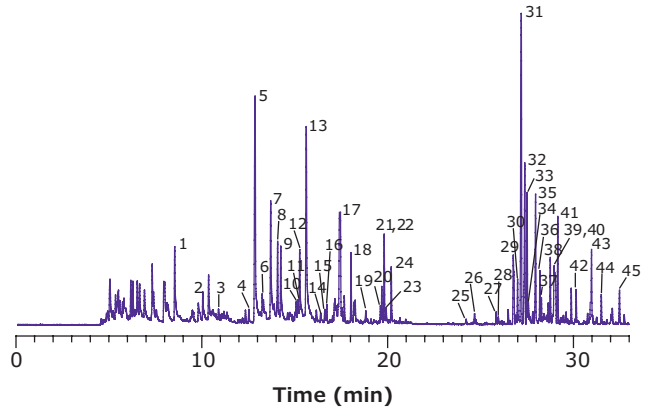


Figure 7. Headspace SPME-GC/MS Analysis of Dried Cannabis Using Final Optimized Method

The peak elution order is listed in Table 3.	
Same as Figure 2 except:	
sample/matrix:	0.5 g dried, ground cannabis
SPME fiber:	50/30 µm DVB/CAR/PDMS (57298-U)
sample equilibration:	30 min, 40 °C



sample or the actual variety of hops analyzed since terpene profiles are known to vary between different hop varieties[10]. The variety of the hop flowers analyzed is unknown, as the identity was not indicated on the packaging. For comparison, samples of two different varieties of pelletized hops were analyzed after grinding. These samples appeared green in color, and had a much more characteristic hops-like odor than the dried flowers. Analysis of these samples showed a characteristic terpene profile, with high levels of β-myrcene, caryophyllene, and humulene present in both (**Figure 6**). The SPME method was able to detect differences in the terpene profiles between the two hops varieties. For example, farnesene (peak 18) was identified in the Cascade hops, but was too low to be confirmed in the US Goldings sample. The level of farnesene in Cascade hops is expected to be 3-7% of total oils, while in US Goldings the level should be <1%.[13]

Terpenes in Cannabis Sample

The terpenes identified in the cannabis sample (**Figure 7**) are indicated in **Table 3**. The profile was similar to those found previously in the analysis of dried cannabis.[4,8] Peaks 1-27 in **Figure 7** (with the exception of peak 7) were monoterpenes and monoterpenoids. The later eluting peaks consisted of sequiterpenes and caryophyllene oxide,

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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/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.[4] 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 d-Limonene.

Table 3. Terpenes in Dried Cannabis Identified by MS Spectral Library Match and Retention Index

Peak No.	RT (min)	Name	RI (calculated)	RI (literature)	Reference
1	8.57	Hexanal	—	—	—
2	10.05	Hexene-1-ol	—	—	—
3	10.89	2-Heptanone	—	—	—
4	12.56	α-Thujene	928	938	11
5	12.86	α-Pinene + unknown	939	942	11
6	13.27	Camphene	953	954	11
7	13.69	6-Methyl-5-hepten-2-one	966	968	11
8	14.09	β-Pinene	979	981	11
9	14.27	β-Myrcene	984	986	11
10	15.09	δ-3-Carene	1010	1015	12
11	15.2	α-Terpinene	1014	1012	12
12	15.29	Cymene	1018	1020	11
13	15.6	d-Limonene	1028	1030	11
14	16.42	γ-Terpinene	1056	1057	11
15	16.6	*trans*-Sabinene hydrate	1062	1078	11
16	16.72	*cis*-Linalool oxide	1066	1068	11
17	17.43	Linalool	1087	1092	11
18	18.04	d-Fenchyl alcohol	1107	1110	11
19	18.82	*trans*-Pinocarveol	1135	1134	12
20	19.59	Borneol L	1161	1164	11
21	19.81	1,8-Methandien-4-ol	1168	1173	8
22	19.81	*p*-Cymen-8-ol	1168	1172	12
23	19.92	Terpinene-4-ol	1172	1185	11
24	20.22	α-Terpineol	1181	1185	11
25	24.2	Piperitenone	1322	1320	12
26	24.76	Piperitenone oxide	1344	1352	12
27	25.85	α-Ylangene	1384	1373	8
28	25.97	α-Copaene	1388	1398	11
29	26.76	γ-Caryophyllene	1419	1403	12
30	27.01	α-Santalene	1429	1428	12
31	27.16	Caryophyllene	1435	1428	11
32	27.36	*trans*-α-Bergamotene + unknown	1443	1443	12
33	27.49	α-Guaiene	1448	1441	8
34	27.56	*trans*-β-Farnesene	1451	1446	12
35	27.98	Humulene	1467	1465	11
36	28.17	Alloaromadendrene	1475	1478	11
37	28.25	α-Curcumene	1478	1479	12
38	28.75	β-Selinene	1497	1487	8
39	28.97	α-Selinene	1507	1497	8
40	28.97	β-Bisobolene	1507	1506	8
41	29.13	α-Bulnesene	1514	1513	12
42	30.12	Selina-3,7(11)-diene	1556	1542	12
43	30.94	Caryophyllene oxide	1590	1595	12
44	31.5	Humulene oxide	1614	1599	12
45	32.48	Caryophylla-3, 8(13)-dien-5-ol A	1658	1656	12

Conclusion

A simple headspace SPME-GC/MS method was used in the analysis of the terpene/terpenoid profiles of both hops and cannabis. The method was able to detect the characteristic terpenes and terpenoids of both, and to distinguish between different hops varieties.

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