Analysis of Terpenes in Cannabis Using the Agilent 7697A/7890B/5977B Headspace GC-MSD System

Faster Analysis Time = Greater Productivity

Application Note

Cannabis, Food

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Abstract

Terpenes and terpenoids are naturally occurring volatile isoprenes constructed from a $(C_5H_8)_n$ building block where n is the number of isoprene units linked to form linear or ring molecules. In marijuana (*Cannabis sativa*), terpenes are biosynthesized in the flower along with other compounds such as psychoactive Δ^9-tetrahydrocannabinol (THC) and other nonpsychoactive cannabinoids [1]. Each strain of marijuana is characterized by its unique terpene profile, that creates the distinctive aroma and flavor [2]. This application note describes the analysis of 22 terpenes common to marijuana in under 6 minutes using the Agilent 7697A/7890B/5977B headspace-gas chromatography-mass selective detection (HSS-GC-MSD) system with concomitant flame ionization detector (FID) detection using integrated Capillary Flow Technology to split the column effluent in a controllable and precise manner to the two detectors.

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Introduction

The concentration of individual terpenes in the *C. sativa* plant varies by strain, harvest time, and drying and storage conditions. Depending on these conditions, the relative terpene concentrations decrease over time – for example, after three months of storage, levels can decrease by more than half [3,4]. Analysis of terpenes in *C. sativa* strains is typically performed using HSS-GC/MSD, using the Agilent Residual Solvent Analyzer hardware configuration to provide an optimum linear range of detection and mass spectral speciation.

Experimental

Hardware and consumables

- Agilent 7697A Headspace Sampler, Agilent 7890B GC with FID, and Agilent 5977B MSD (Residual Solvent Analyzer)

- Split/Splitless Inlet

- Purged Two-Way Splitter: split 3:1 FID:MSD

- Agilent Headspace liner, Ultra Inert, splitless, straight 1 mm (p/n 5190-4047)

- Agilent 10 mL Headspace vials (p/n 5190-2285)

- Agilent Headspace vial caps (p/n 8010-0116)

- Agilent VF-35 column, 30 m × 0.25 mm, 0.25 μm (p/n CP8877)

- MSD Restrictor - Deactivated Fused Silica 1.7 m × 150 μm × 0 μm

- FID Restrictor - Deactivated Fused Silica 0.7 m × 250 μm × 0 μm

Certified terpene reference standards 34095-DGEQ and 34096-DGEQ were obtained from Restek (Bellefonte, PA).

Agilent 7697 Headspace parameters

Parameter	Value
Instrument settings	
Vial pressurization gas	Helium
Loop size	1 mL
Keyboard lock	OFF
Transfer line type	DB-ProSteel
Transfer line diameter	0.53 mm
System configuration	
Carrier control	GC Instrument
Oven temperature	120 °C
Loop temperature	120 °C
Transfer line temperature	140 °C
Vial equilibration	10.00 minutes
Injection duration	0.50 minutes
GC cycle time	10 minutes
Vial size	10 mL
Vial shaking	Level 1
Fill mode	Default
Fill pressure	15 psi
Loop fill mode	Custom
Loop ramp rate	40 psi/min
Loop final pressure	13 psi
Loop equilibration time	0.05 minutes
Carrier control mode	GC controls carrier
Extraction mode	Single extraction
Vent after extraction	ON
Post injection purge	100 mL/min for 3 minutes
Acceptable leak check	Default, 0.2 mL/min
Agilent 7890B GC conditions	
GC oven temperature	60 °C
Hold time	0.5 minutes
Oven program	45 °C/min to 150 °C, hold 0 minutes then 35 °C/min to 250 °C, hold 0.5 minutes
Equilibration time	1 minute
Max temperature	260 °C
Column flow	3.0 mL/min
Front S/SL inlet He mode	Split
Heater	220 °C
Pressure	29.45 psi
Total flow	303 mL/min
Septum purge flow	Off
Gas saver	30 mL/min at 15 minutes
Split ratio	100:1
MSD Transfer line	260 °C

Agilent 5977B MSD conditions

Acquisition mode	Scan (40 m/z–400 m/z))
Solvent delay	1.8 minutes
Tune file	etune.u
EM Setting mode	Gain = 1
Scan speed	Normal
Scan parameters	
Threshold	125
MSD Source temperature	300 °C
MSD Quadrupole temperature	150 °C

FID Conditions

Detector temperature	300 °C
H_2 Flow	40 mL/min
Air flow	400 mL/min
Makeup flow	25 mL/min
Flame and electrometer	On

Aux EPC 3 supplies Column 2 with 1.4 mL/min constant flow to Restrictor 1 going to the MSD, FID/MSD split ratio approximately 3:1.
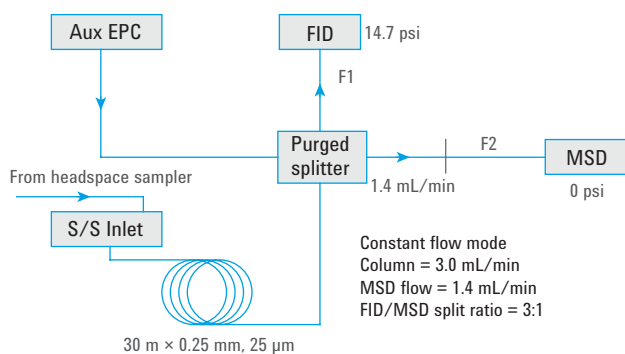

Figure 1. *Capillary flow diagram of the Agilent 7890B configured with a two-way splitter for simultaneous collection of FID and MSD data.*

Standards, sample preparation, and quantitation

Standard calibrator preparation

For quantitation, a seven-point calibration curve from 10 ppm to 1,250 ppm was created from the terpene reference standards by adding 10 µL of each pre-prepared calibration level to a 10-mL headspace vial, and capping the vial. The calibrator levels were: 10, 20, 50, 100, 200, 500, and 1,250 ppm.

Sample preparation

For cannabis plant material, it is recommended that samples be frozen prior to grinding, or that grinding occur under liquid nitrogen. This keeps the samples cold during the grinding process, reducing loss of the more volatile terpenes.

The headspace method uses full evaporation technique (FET) standard preparation. Since cannabis product matrices are extremely varied, and plant material will not dissolve in solvent, sampling involves the use of a very small sample amount (10–50 mg) of plant or wax material weighed into the headspace vial, which is then capped and analyzed. For this study, a challenge sample of lemon grass tea was prepared to demonstrate the power of mass spectrometry to differentiate targeted terpenes from interfering chemical compounds often found in real samples.

Quantitation

The terpene concentrations in the unknown samples were determined in Agilent MassHunter quantitative software through linear regression analysis of the linear calibration curve constructed from the known calibrator levels.

Results and Discussion

The analytical method developed in this study is ultra-fast, robust, and reliable. It leverages the large dynamic range of FID detection and mass spectral confirmation to identify and quantitate 22 targeted terpenes found in cannabis and cannabis wax samples. Figure 2 illustrates the chromatography for both the FID and the MSD at 10, 100, and 1,250 ppm. All terpenes elute in less than 6 minutes, and the total cycle-time (injection to injection) is 10 minutes. Figure 3 shows typical calibration curves for select terpenes,

where the average linear regression coefficient (R^2) was 0.997 for the FID data, and 0.998 for the MSD. Figure 4 shows the extracted ion chromatogram (EIC) for *alpha*-pinene with the FID and MSD calibration curves over the 10–1,250 ppm range. Table 1 outlines the average retention time (RT), average concentration, the standard deviation, and the percent relative standard deviation (%RSD) for the target concentration, the limit of quantitation (LOQ), the limit of detection (LOD), the average response factor, and the %RSD the average response factor of eight replicate injections of a 50 ppm standard.
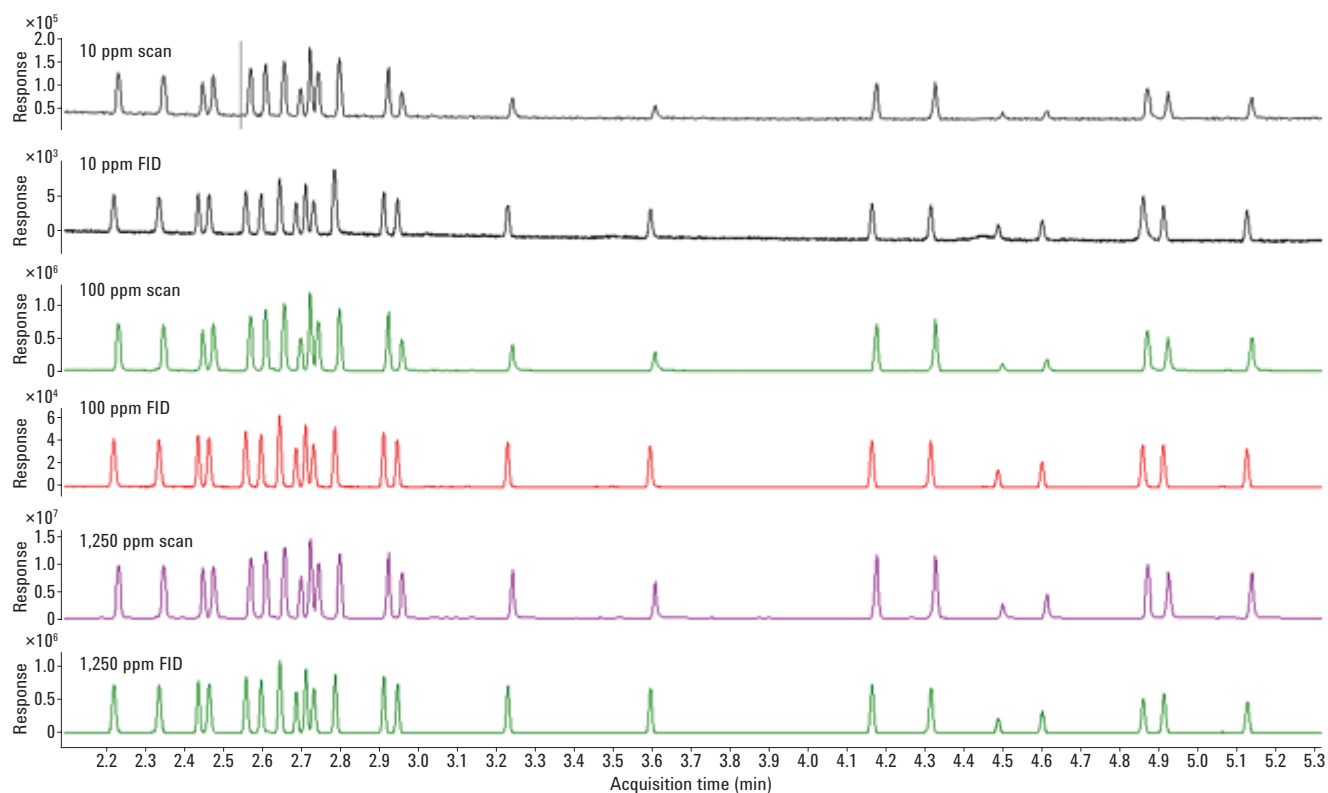

Figure 2.   FID and MSD chromatograms at 10 ppm, 100 ppm, and 1,250 ppm of the 22-compound terpene mix.
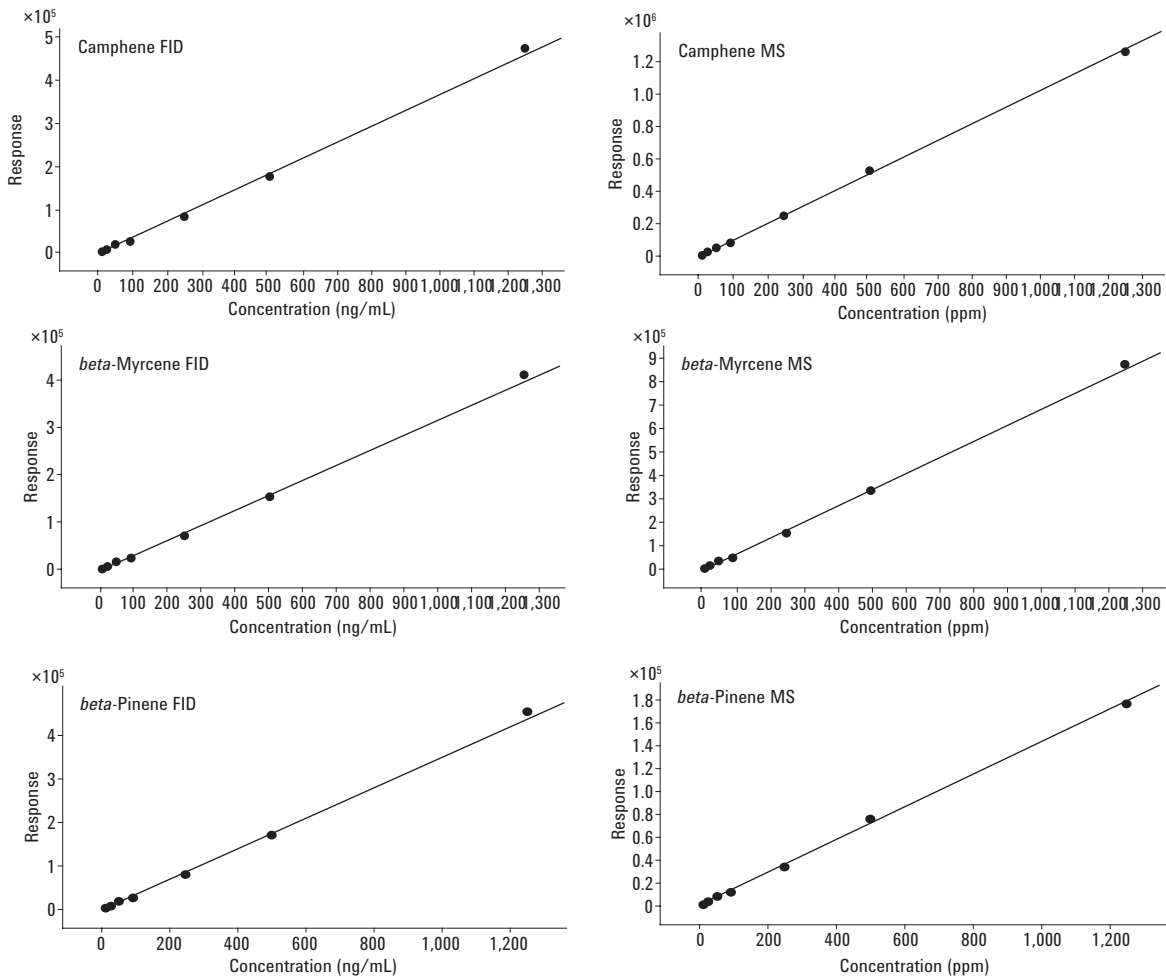
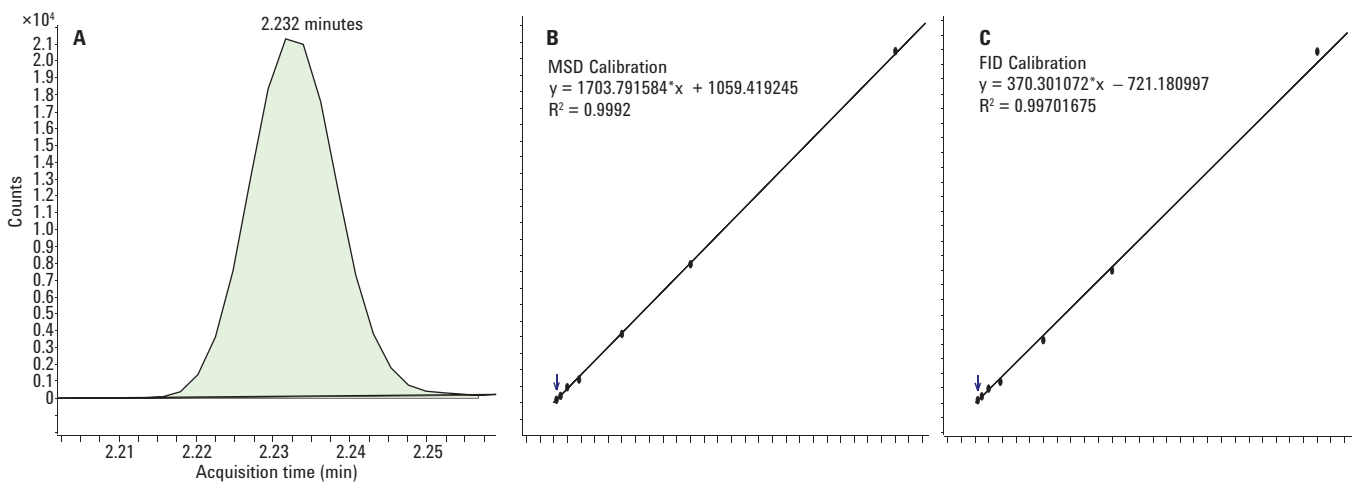Figure 3.   *Typical MSD and FID terpene calibration curves over the 10 ppm to 1,250 ppm calibration levels.*

Figure 4.   *Ten-ppm extracted ion chromatogram (EIC) for alpha-pinene. Calibration levels range from 10 ppm to 1,250 ppm.*

Table 1.   *Analysis of Eight Replicates of 50 ppm Standard*

| Name	RT	Avg. conc.	Std. dev.	Conc. %RSD	LOQ	LOD	Avg. resp.	Resp. %RSD
alpha-Pinene FID	2.221	49.40	0.46	0.9	4.65	1.39	17573	1
alpha-Pinene	2.23	50.70	0.38	0.8	3.81	1.14	87450	0.7
Camphene FID	2.337	48.88	0.54	1.1	5.47	1.64	17390	1.2
Camphene	2.35	50.00	1.30	2.6	13.01	3.90	52233	2.6
beta-Myrcene FID	2.437	49.21	0.43	0.9	4.31	1.29	15002	0.9
beta-Myrcene	2.448	49.35	2.83	5.7	28.36	8.50	32674	5.9
beta-Pinene FID	2.465	49.37	0.45	0.9	4.58	1.37	16699	1
beta-Pinene	2.476	50.53	2.28	4.5	22.84	6.85	73972	4.5
delta-3-Carene FID	2.559	49.75	0.48	1	4.87	1.46	18078	1
delta-3-Carene	2.569	50.76	0.92	1.8	9.26	2.77	75715	1.8
alpha-Terpinene FID	2.598	49.64	0.36	0.7	3.68	1.10	15808	0.8
alpha-Terpinene	2.61	49.41	0.60	1.2	6.07	1.82	63342	1.3
D-Limonene FID	2.645	49.59	0.42	0.9	4.22	1.26	23170	0.9
D-Limonene	2.658	52.29	0.60	1.2	6.02	1.80	49501	1.1
beta-Ocimene FID	2.687	49.54	0.52	1.1	5.25	1.57	10924	1.1
beta-Ocimene	2.699	49.10	1.87	3.8	18.74	5.62	12853	4
p-Cymene FID	2.712	48.99	0.55	1.1	5.55	1.66	18568	1.2
p-Cymene	2.722	53.50	0.69	1.3	6.98	2.09	174393	1.2
Eucalyptol FID	2.732	55.98	0.63	1.1	6.31	1.8	15425	1.2
Eucalyptol	2.743	59.51	1.20	2	12.02	3.60	26624	2
gamma-Terpinene FID	2.787	45.75	3.42	7.5	34.24	10.27	18691	6.7
gamma-Terpinene	2.797	50.81	0.51	1	5.13	1.53	71814	1
Terpinolene FID	2.911	49.43	0.47	1	4.79	1.43	16371	1
Terpinolene	2.923	50.48	0.77	1.5	7.71	2.31	46268	1.5
Linalool FID	2.946	49.00	0.43	0.9	4.30	1.29	14363	0.9
Linalool	2.957	48.00	1.17	2.4	11.72	3.51	11918	2.7
Isopulegol FID	3.228	48.99	0.42	0.9	4.24	1.27	14174	0.9
Isopulegol	3.24	42.82	2.23	5.2	22.31	6.69	7449	6.5
Geraniol FID	3.593	49.68	0.34	0.7	3.49	1.04	13415	0.7
Geraniol	3.605	47.91	1.12	2.3	11.20	3.36	16929	2.6
beta-Caryophyllene FID	4.16	50.16	0.31	0.6	3.10	0.93	16524	0.6
beta-Caryophyllene	4.171	46.59	1.01	2.2	10.15	3.04	19222	2.4
alpha-Humulene FID	4.31	50.12	0.29	0.6	2.95	0.88	16531	0.6
alpha-Humulene	4.324	47.85	0.15	0.3	1.50	0.45	61806	0.3
Nerolidol 1 FID	4.483	54.25	2.72	5	27.24	8.17	5904	5
Nerolidol 1	4.493	41.91	1.59	3.8	15.99	4.79	3914	5.1
Nerolidol 2 FID	4.595	53.06	0.49	0.9	4.92	1.47	8781	0.9
Nerolidol 2	4.607	39.69	1.55	3.9	15.57	4.67	6641	5.5
Guaiol FID	4.853	54.61	0.79	1.5	7.93	2.38	17152	1.2
Guaiol	4.864	40.22	0.66	1.7	6.67	2.00	19534	2.1
Caryophyllene oxide FID	4.906	57.90	0.99	1.7	9.90	2.97	17140	1.7
Caryophyllene oxide	4.919	51.70	1.28	2.5	12.81	3.84	9868	2.6
alpha-Bisabolol FID	5.119	53.73	0.71	1.3	7.19	2.15	13491	1.3
alpha-Bisabolol	5.134	39.81	0.83	2.1	8.31	2.49	11553	2.7
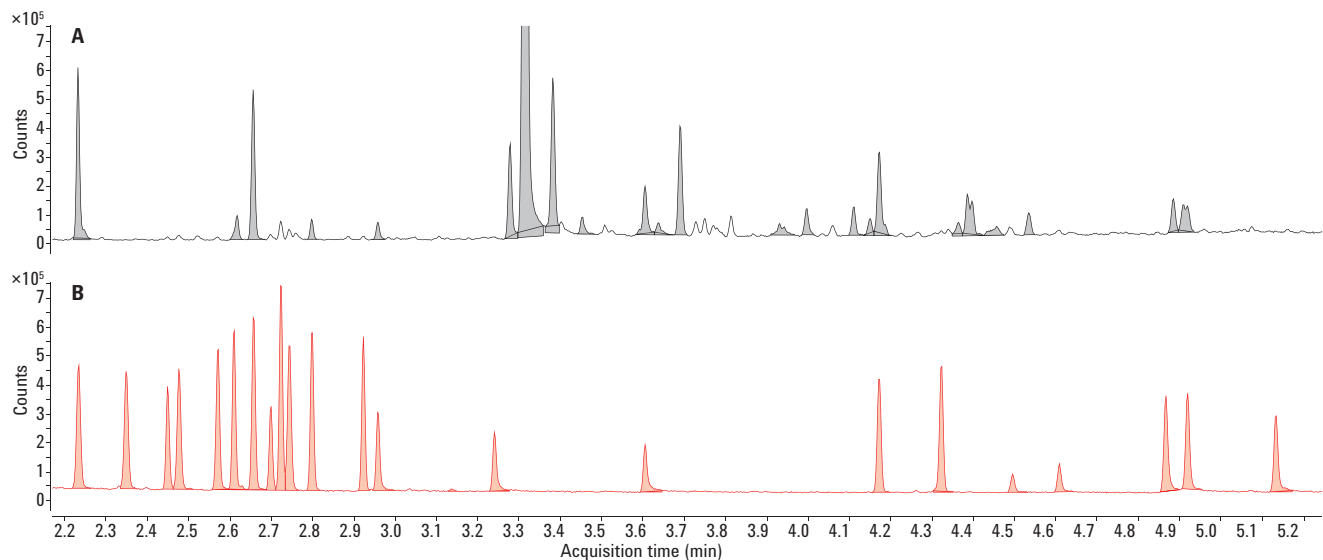
Figure 5.   Total ion chromatogram (TIC) comparison of a 50 ppm terpene reference standard (B) versus a 30 mg challenge sample of lemon grass tea (A) known to contain similar terpenes and other compound that may interfere with proper identification when only using FID detection.
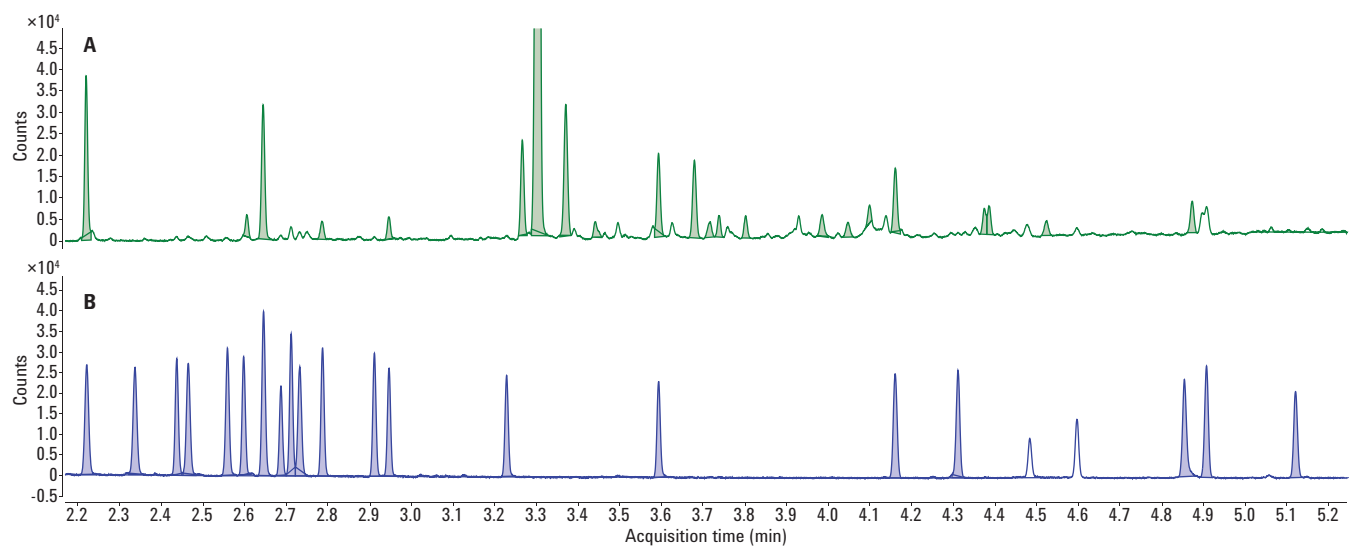

Figure 6.   FID chromatogram comparing a 50 ppm terpene reference standard (B) with a 30 mg sample of lemon grass tea (A). When using FID detection only, the peak at 2.22 minutes may be misidentified as alpha-pinene.
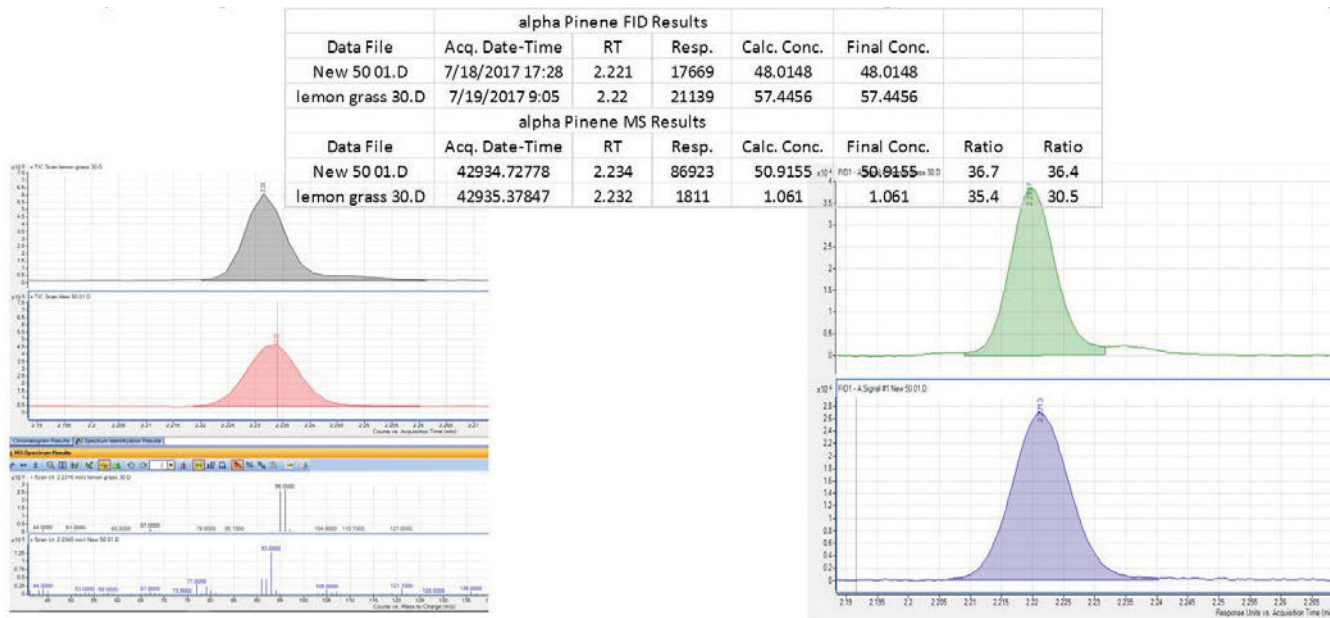
alpha Pinene FID Results							
Data File	Acq. Date-Time	RT	Resp.	Calc. Conc.	Final Conc.		
New 50 01.D	7/18/2017 17:28	2.221	17669	48.0148	48.0148		
lemon grass 30.D	7/19/2017 9:05	2.22	21139	57.4456	57.4456		
alpha Pinene MS Results							
Data File	Acq. Date-Time	RT	Resp.	Calc. Conc.	Final Conc.	Ratio	Ratio
New 50 01.D	42934.72778	2.234	86923	50.9155	50.9155	36.7	36.4
lemon grass 30.D	42935.37847	2.232	1811	1.061	1.061	35.4	30.5

Figure 7.   *The nearly coeluting peak in the challenge sample (lemon grass tea) shown in the top chromatogram and spectrum is clearly different from the alpha-pinene spectrum of the terpene reference standard. This illustrates the advantage of MSD selectivity to rule out interfering species that may otherwise be misidentified as a target analyte when using FID detection alone.*

The use of both FID and MSD allows for more comprehensive data analysis, as terpenes are often in high percent values, which would saturate the MS, whereas the FID will not saturate. Optimized conditions were obtained using the 7697A method development tools and a parameter increment function built into the software. These tools sequentially optimize the headspace oven temperature, vial equilibration time, and vial shaking level by a set amount over a series of analytical runs. By using the Agilent 7697A tools, the final method can rapidly be optimized versus using manual optimization of other headspace systems. For this analysis, a constant incubation temperature and extraction time was used to ensure volatilization of all terpenes and terpenoids in the sample for reproducible, quantitative results. The Prep Ahead feature of the 7697A headspace system allows an optimum workflow for the short sample analysis time.

Conclusion

Using the Agilent Residual Solvent Analyzer with an Agilent VF-35 GC column and appropriate restrictors enables full chromatographic separation of 22 targeted terpenes that naturally occur in *C. sativa* plant material and wax samples and give the plant its distinctive aroma and character. The analysis completed in less than 6 minutes, and uses both FID detection for quantification and extended linear range, and mass selective detection (MSD) for terpene speciation. This ultra-fast methodology almost quadruples laboratory productivity compared to traditional terpene analysis, which takes approximately 30 minutes per sample.

References

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