Application Note

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Analysis of Terpene and Terpenoid Content in Cannabis Sativa Using Headspace with GC/MSD

7967A-8890 GC-5977 MSD

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Introduction

Terpenes and terpenoids are compounds produced by botanical species to flourish in their environment. The compounds often attract pollinators, repel pests, and assist with adaptation throughout a growth cycle.[1] Chemically, terpenes are comprised of carbon and hydrogen atoms, and are built from isoprene (C_5H_8) subunits. Terpenoid describes a larger class of molecules that include oxygen in the chemical structure. Both classes of compounds will be generalized to terpenes for this application note, but they are two distinct classes in the broader scope.

Terpenes have an associated fragrance, and have historically been isolated from various botanical sources for a wide range of commercial or therapeutic uses.[2] D-limonene is a common component of citrus-scented personal care or disinfecting products, eucalyptol contributes to the minty aoma in many therapeutic products, and linalool is largely responsible for the floral fragrance of lavender-scented products. These terpenes, along with others produced by cannabis plants, are of interest as they are commonly marketed to enhance effects in the population consuming cannabis for medicinal or recreational use.

The information obtained in terpene profiling helps determine how the plant might be optimally commercialized, as well as providing valuable feedback to the growers for establishing and maintaining consistent plants. The Agilent 8890 GC system, including an Agilent 5977A series single quadrupole mass selective detector and an Agilent 7697A headspace sampler for sample introduction can easily separate, identify, and quantify the terpenes in a given sample. It also provides an enhanced operating experience, maintenance tracking, and on-board diagnostics, accessed through the touch screen interface or through an integrated browser interface.

Experimental

An 8890 gas chromatograph configured with a multimode inlet (MMI), a flame ionization detector (FID), a 5977 single quadrupole mass selective detector (SQ-MSD) with a 9 mm extractor lens, and a 7697A headspace sampler was used to generate the data. The data acquisition and analysis were done in GC/MS MassHunter, with the NIST 17 reference library used for unknown identification. Although the 8890 GC used is configured for a fast oven (240 V), the enhanced oven performance is not required for this work.

Restek Cannabis Terpene standards were purchased and diluted in isopropanol (Millipore-Sigma, >99.5%). Table 1 lists the consumables used in the method development. Cannabis samples were analyzed following the full evaporative technique (FET)[3], and 20 mL vials were prepared, each containing approximately 25 mg of ground cannabis flower.

Several column chemistries were evaluated for performance, but ultimately the Agilent DB-HeavyWAX was selected for use. WAX columns are regularly used in the flavor and fragrance industry,[4] but are limited by the maximum allowable oven temperature. The HeavyWAX is a new addition to the Agilent WAX portfolio that extends the maximum operating temperature beyond that of previous WAX options.[5] As the FET method of terpenes analysis does not include solvent, ambient air from the headspace vial will be injected with the sample for analysis. The HeavyWAX column showed significantly lower levels of bleed compared to siloxane-based chemistries under similar conditions.

Capillary flow technology (CFT) devices, which add abilities such as backflush and postcolumn splitting, are available on the 8890 GC. The 8890 GC was configured with a pneumatic switching device (PSD) connected to a purged splitter, and the column effluent was split between the FID and MSD at a ratio of 3:1, respectively. The CFT calculator included with the purged splitter is intended to assist with the calculations, but exact restrictor dimensions used in data collection are provided as a graphical representation in Figure 1. Tables 2, 3, and 4 detail extended instrument setpoints.

Table 1. Consumables used for terpenes analysis.

Description	Agilent Part Number	Non-Agilent Part Number
Cannabis Terpene Standards		Restek: 34095, 34096
20 mL Crimp Vials/Caps	5188-2753	
Advanced Green Inlet Septa (Green)	5183-4761	
Ultra Inert Low Pressure Drop Inlet Liner wirh Wool	5190-2295	
Agilent DB-HeavyWAX Column	122-9632	
Extractor Source Large Diameter Lens (9 mm)	G3870-20449	

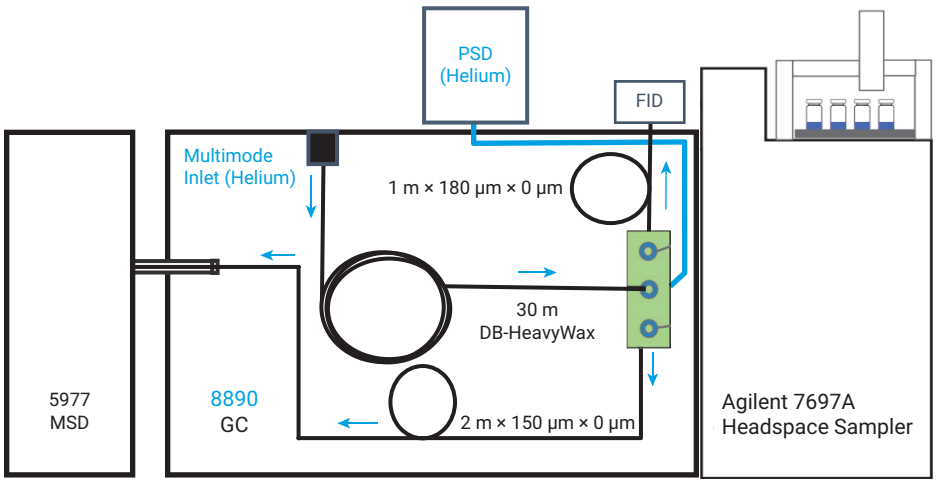

Figure 1. Column configurations for detector split in terpenes analysis.

Table 2. Agilent 8890 gas chromatograph conditions for terpenes analysis.

Agilent 8890A GC Parameters	
Inlet Type	MMI
Inlet Mode	Split, 100:1
Inlet Temperature	175 °C
Inlet Pressure	25.8 psi
Septum Purge	3 mL/min
Gas Saver	20 mL/min after 3 minutes
PSD Pressure	4.85 psi
PSD Purge Flow	5 mL/min
MSD Transfer Line	275 °C
GC Run Time	17.1 minutes
Carrier Gas	Helium
Column 1	Agilent DB-HeavyWAX, 122-7133
Column Dimension	30 m × 250 µm × 0.5 µm
Column Mode	1.95 mL/min, constant flow
Oven Equilibration	2.5 minutes
Oven Program	50 °C, hold 0.75 minute
Ramp at 5 °C/min	80 °C, hold 0 minutes
Ramp at 30 °C/min	240 °C, hold 5 minutes
FID Temperature	300 °C
FID Air	400 mL/min
FID Hydrogen	30 mL/min
FID Make Up (N_2)	15 mL/min

Table 3. Agilent 5977A (Extractor) conditions for analysis.

MSD Conditions	Agilent 5977-SQMSD
Source	Extractor, 9 mm lens
Hi Vacuum Pump	Performance turbo
Mode	Scan
Range	45 to 450 *m/z*
Threshold	50
Tune Algorithm	Etune
Source Temperature	325 °C
Quad Temperature	200 °C

Table 4. Agilent 7697A headspace sampler conditions for analysis.

Headspace Conditions	Agilent 7697A - HSS
Pressurization Gas	Helium
Loop Size	1 mL
Oven Temperature	110 °C
Loop Temperature	120 °C
Transferline Temperature	150 °C
Vial Equilibration	10 minutes
Injection Time	0.5 minutes
Fill Pressure	20 psi
Loop Final Pressure	12 psi
Loop Equilibration Time	0.1 minutes

Calibration standards were prepared by diluting the Restek reference standards in isopropanol, then transferred using a syringe to headspace vials for analysis. To consider the impact of solvent on separation and reproducibility, two methods of preparation were evaluated. The first method involved spiking different volumes (0.1 to 1,000 µL) of the same concentration standard into each vial, and the second involved spiking a fixed volume of different concentration standards into each vial.

The amount of solvent added to the headspace vial did influence the chromatography, exhibiting a pronounced retention time shift with the varied volume preparation technique. When the same calibration range was evaluated using a constant volume of multiple standard concentrations, the retention times were stable. As a result, new calibration standards were prepared to target 10 µL of standard in each headspace vial.

Many terpene lists contain several structural isomers known as monoterpenes. These compounds share an empirical formula of $C_{10}H_{15}$ and contain major fragments of 93, 121, and 136 *m/z*, in addition to others. This similar pattern makes identification by library spectra alone difficult. When using standard nonpolar columns, many of the monoterpenes have been thoroughly characterized and have a retention index (RI) value, which is a comparison of retention time against that of a series of straight-chain alkanes.[6] The DB-HeavyWAX does not yet have established retention indices to reference. In this work, single-component reference standards were purchased and analyzed for identification of elution order. Table 5 provides compound names, vendor, and part numbers.

Table 5. Individual component standards used to identify monoterpenes on DB-HeavyWAX.

Compound Name	Vendor	Part Number
γ-Terpinene	Supelco	CRM40431
Terpinolene	Supelco	CRM40929
β-Pinene	Supelco	CRM40433
+-3-Carene	Supelco	CRM40416
α-Terpinene	Supelco	CRM40443
α-Pinene	Supelco	CRM40339

Results and discussion

To determine a workable linear range, a nine-point calibration curve encompassing 5 to 2,500 ng was prepared and analyzed. The 20 mL vials were prepped with 10 µL of a prepared calibration standard, then capped and loaded onto the headspace autosampler. Calibration masses are 5, 10, 25, 50, 100, 250, 500, 1,000, and 2,500 ng. Figure 2 shows an example chromatogram, and Table 6 presents quantitated range, correlation information, and accuracy of the curves.

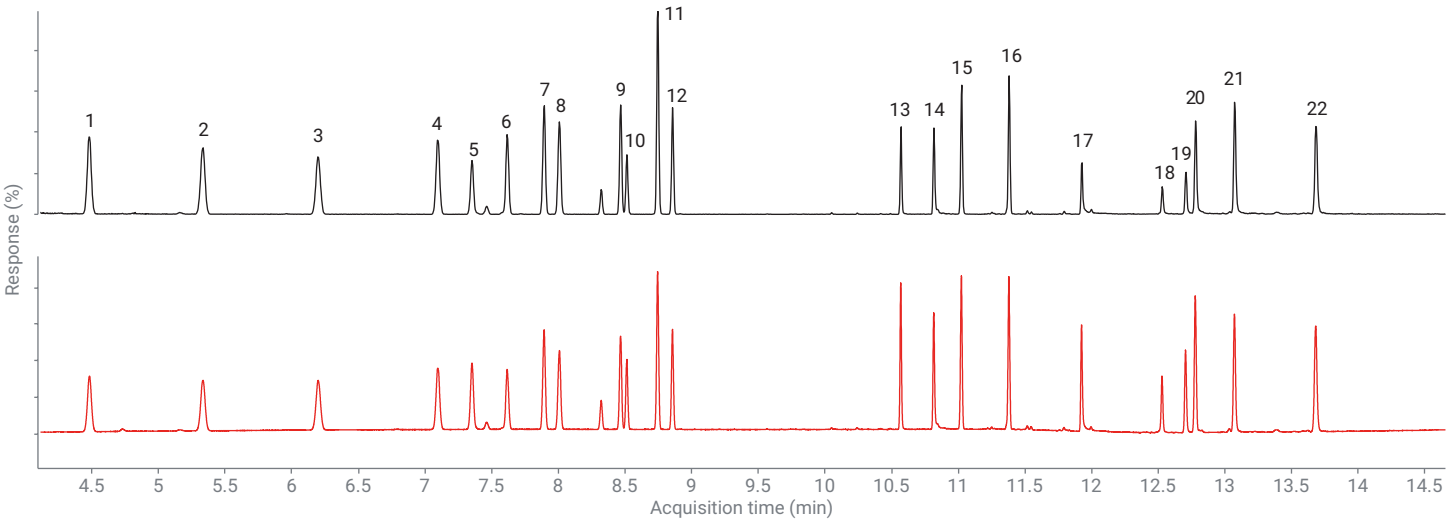

Figure 2. Total ion chromatogram (black) and FID chromatogram (red) of calibration standard.

Table 6. Compound list, retention time, and calibration information for MSD and FID signals.

Peak Index	Compound Name	Retention Time (min)	MSD Lower Calibration Point (ng)	MSD Upper Calibration Point (ng)	MSD Correlation (R^2)	MSD QC accuracy (750 ng)	FID Lower Calibration Point (ng)	FID Upper Calibration Point (ng)	FID Correlation (R^2)	FID QC Accuracy (750 ng)
1	α-Pinene	4.484	5	2,500	0.998	96.5%	5	2,500	0.999	98.9%
2	Camphene	5.330	5	2,500	0.997	97.9%	25	2,500	0.999	96.8%
3	β-Pinene	6.201	5	2,500	0.998	97.8%	25	2,500	0.995	98.3%
4	3-Carene	7.095	5	2,500	0.998	97.4%	25	2,500	0.999	97.3%
5	β-Myrcene	7.352	5	2,500	0.998	93.6%	50	2,500	0.998	95.7%
6	α-Terpinene	7.615	10	2,500	0.998	95.5%	25	2,500	0.997	97.7%
7	D-Limonene	7.895	5	2,500	0.999	93.8%	25	2,500	0.998	97.2%
8	Eucalyptol	8.008	10	2,500	0.999	96.3%	10	2,500	1.000	99.5%
9	Γ-Terpinene	8.467	5	2,500	0.999	97.3%	10	2,500	0.999	96.6%
10	β-Ocimene	8.513	10	2,500	0.998	97.2%	10	2,500	0.998	96.7%
11	P-Cymene	8.747	5	2,500	0.998	96.5%	5	2,500	0.998	96.9%
12	Terpinolene	8.855	10	2,500	0.999	97.1%	10	2,500	0.998	98.2%
13	Linalool	10.571	5	2,500	0.999	100.9%	5	2,500	0.998	97.8%
14	Isopulegol	10.818	50	2,500	1.000	95.2%	25	2,500	0.997	98.4%
15	Caryophyllene	11.022	5	2,500	0.997	95.9%	5	2,500	0.997	99.4%
16	Humulene	11.381	5	2,500	0.997	94.9%	10	2,500	0.997	99.6%
17	Geraniol	11.924	5	2,500	0.996	100.4%	10	2,500	0.999	104.3%
18	Nerolidol 1	12.5347	10	2,500	0.996	94.0%	25	2,500	0.997	100.0%
19	Nerolidol 2	12.705	10	1,000	0.995	97.6%	25	2,500	0.997	99.1%
20	Caryophyllene Oxide	12.784	25	2,500	0.999	91.2%	5	2,500	1.000	102.0%
21	Guaiol	13.072	5	2,500	0.999	93.2%	10	2,500	0.997	100.2%
22	α-Bisabolol	13.682	5	2,500	0.996	93.3%	5	2,500	0.996	103.5%

MSD calibration limits are affected by choice of quantitation method. These data were quantified using a quantifier ion with qualifiers to confirm. Examples of additional quantitation methods might include integration of the total ion chromatogram, or summing peak areas of both quantifier and qualifier ions, or using the total ion chromatogram response.

FID calibration ranges were determined by ensuring the peak has a 3:1 signal-to-noise ratio (S/N). The reported FID values are conservative for expected FID performance, provided the system gases and consumables are clean.

If lower-level detection is needed for some compounds, the inlet split ratio can be reduced with no configuration changes to the system. If this does not improve, the MS/FID split can be modified by either calculating a different detector split or by eliminating the detector split altogether and connecting the column directly to one detector.

Once a suitable calibration range was determined, cannabis samples were processed. To increase confidence in the terpene profile, the sample should be temperature controlled from harvest to analysis, ideally below freezing for dried flower material. In the samples evaluated, the cultivar information was not provided, so the reference is unavailable.

As some cannabis strains are expected to contain over 100 compounds, additional standards might be required to properly characterize the sample. Several standards are available to run against the method to build a larger reference for identification. A 33-compound Supelco terpene standard mix set (p/n CRM40755(A) and CRM40937(B)) was purchased and analyzed for comparison. Figure 3 shows the overlaid total ion chromatograms of the Restek standard, the Supelco standard, and the two cannabis samples. In these two samples, many peaks are present in the 10 to 12 minute range that are not present in the Restek standard, so having the extra information of a second standard may be useful. Figures 4 and 5 show expanded views of the four to eight and 10 to 13 minute overlays. Figure 4 shows that the expected early eluting monoterpenes are present in both strains, but in different amounts. The expanded overlays also show informational peaks around the base of D-limonene (7.8 minutes). This is an example of the improved separations given by the DB-HeavyWAX column. Adequate resolution of the smaller peaks enables a more complete characterization of the sample. The expanded segment in Figure 5 shows another area of the chromatogram that contains peaks not present in either standard. The unknown peaks in each sample were identified using NIST 17 library spectra and MassHunter Qualitative Analysis, most abundant peaks had a match factor exceeding 90.

For a more complete characterization with automated deconvolution, MassHunter Unknowns Analysis was used to process cannabis sample A. Figure 6 shows a screen capture Unknowns Analysis. With this tool, filters for peak identification and deconvolution are built into a method, which will run an automated search program on samples. The two standards combined contained 37 standards, which cover the common terpenes in high abundance. However, using the MassHunter Unknowns Analysis, 41 and 78 compounds were easily identified on cannabis samples A and B. Unknowns Analysis supports scan data collected by an MSD.

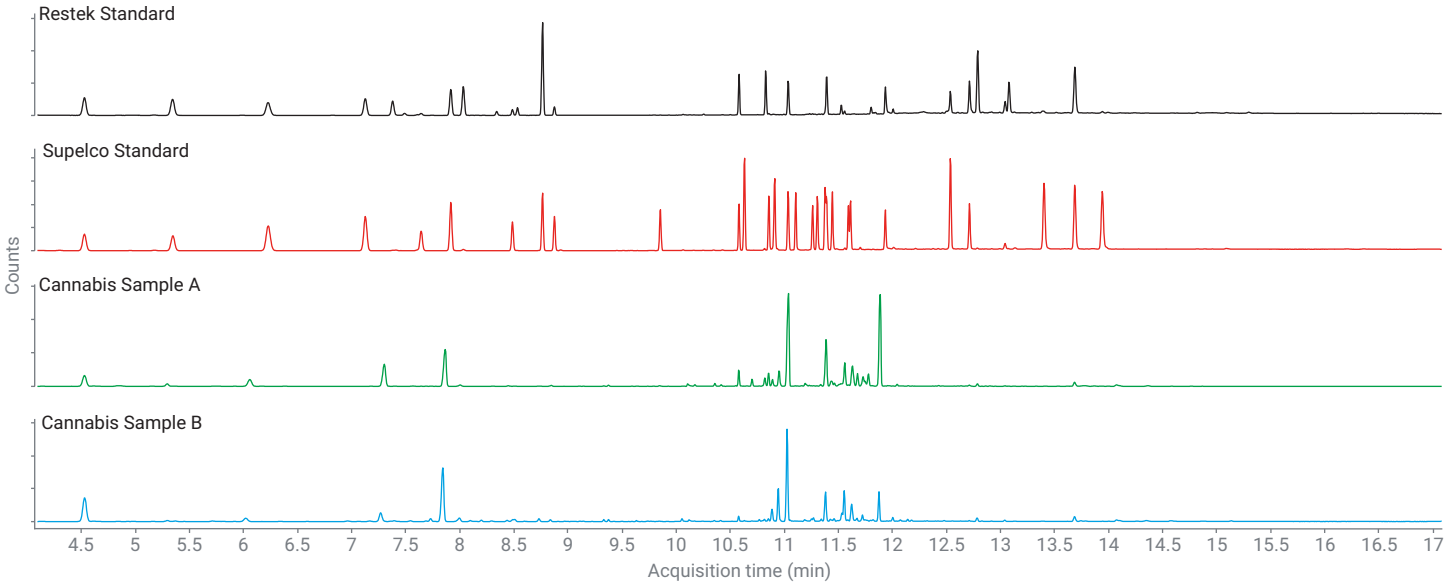

Figure 3. Total Ion Chromatogram of Restek and Supelco standard with two different cannabis.

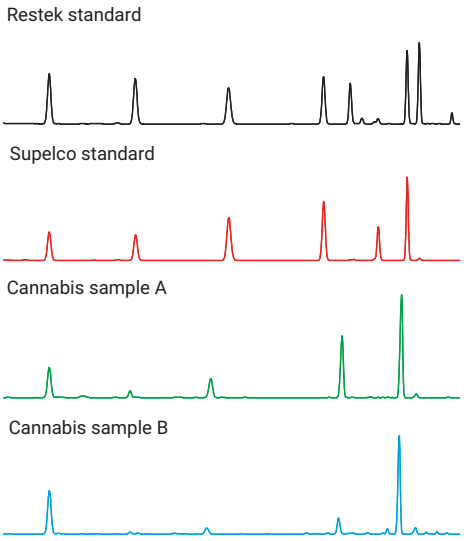

Figure 4. Expanded view of four to eight minute time range. α-Pinene, β-Pinene, 3-carene, and β-Myrcene, and D-limonene are present in both cannabis samples.

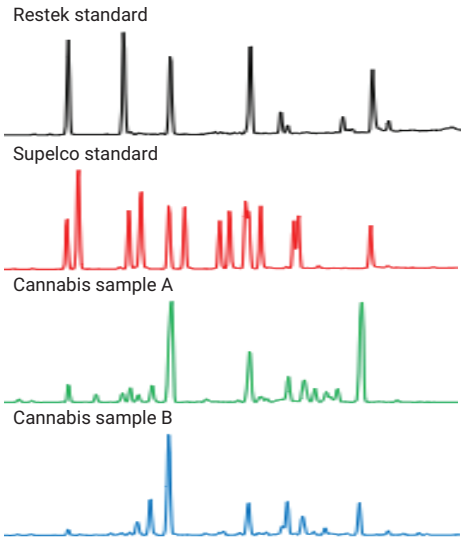

Figure 5. Expanded view of 10 to 13 minute range. The Agient DB-HeavyWAX separation excelled in this region. Several compounds are present in one sample or the other that are not part of either standard.

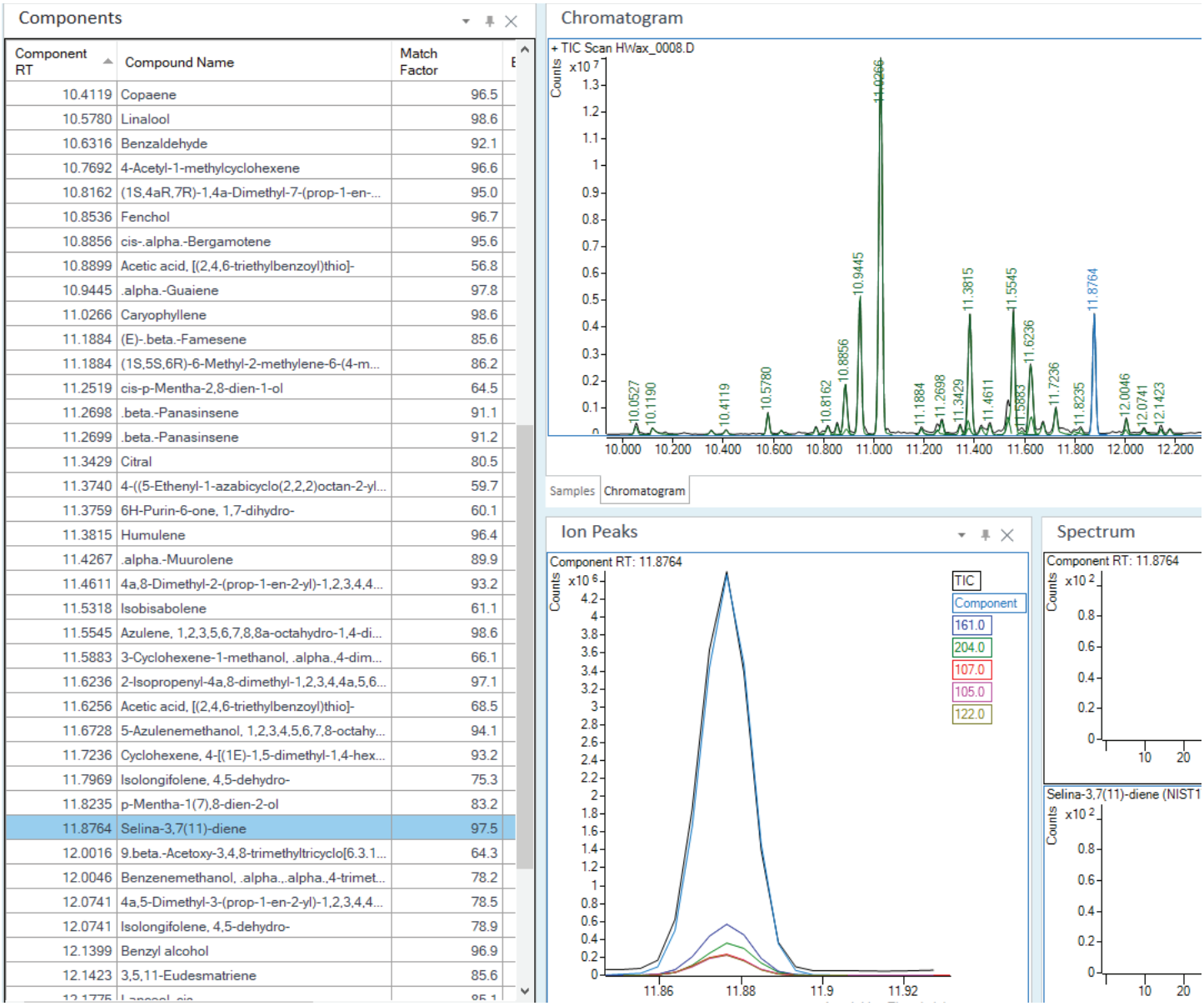

Figure 6. Screen capture of Unknowns Analysis processing of sample A.

Conclusion

Terpene profiles of two different cannabis samples were analyzed using the Agilent 8890 GC with 7697A headspace autosampler introduction, purged CFT splitter with PSD, and combined FID/MSD signal collection. Agilent MassHunter version 10 introduces a new platform, useful for screening workflows with Unknowns Analysis. The Agilent DB-HeavyWAX column is at the core of the results, as the sample is efficiently separated in less than 17 minutes.

When characterization of a sample is important to the workflow, mass spectrometry gains an advantage by incorporating tools such as library matching and deconvolution. When quantitation of known compounds is the goal of the workflow, the FID performs the analysis at a lower cost, and can use tools like retention index matching as a secondary reference to retention time. Regardless of detector selection, optimum separation will improve confidence in the resulting data. Combining both techniques into a method, applies the strengths of both the FID and MSD on a single system.

The 8890A GC enhances the Agilent legacy of robust sample analysis by incorporating easy-to-use maintenance counters, extensive troubleshooting tools, and an embedded browser interface that allows remote access. The innovation of the DB-HeavyWAX column provides a higher temperature alternative to traditional wax chemistries resulting in improved separation of terpenes. Optimizing separations, maintaining sample temperature, and using multiple reference standards are important considerations when profiling terpenes. As research and commercialization of cannabis-based products continue to gain ground, Agilent provides a complete and robust system for terpene analysis.

References

1. Singh, B.; Sharma, R. A. Plant Terpenes: Defense Responses, Phylogenetic Analysis, Regulation and Clinical Applications. *3 Biotech* **2015**, *5(2)*, 129–151. doi:10.1007/s13205-014-0220-2
2. Lockwood, G.B. Techniques for Gas Chromatography of Volatile Terpenoids from a Range of Matrices. *Journal of Chromatography A*. November **2001**, *936(1-2)*, 23–31.
3. Hilliard, C.; Rigdon, A. *et al.* A Fast, Simple FET Headspace GC-FID Technique for Determining Residual Solvents in Cannabis Concentrates. https://www.restekgmbh.de/aktuelles/fast-simple-fet-headspace-gc-fid-technique-determining-residual-solvents-cannabis
4. Zou, Y. Lavender Oil Analysis Using Agilent J&W DB-WAX Ultra Inert Capillary GC Columns. *Agilent Technologies*, publication number 5991-6635EN, **2016**
5. Abercrombie, V.; Provoost, L. Increased Thermal Stability and Maximum Temperature of the Agilent J&W DB-HeavyWax column. *Agilent Technologies*, publication number 5991-9035EN, **2018**
6. The Kováts Retention Index System. *Analytical Chemistry,* **1964**, *36 (8)*, 31A-41A DOI: 10.1021/ac60214a727

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