Application Note

Cannabis

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Fast Analysis of 22 Terpenes in Hemp and Cannabis with the Agilent 9000 Intuvo GC with FID using Hydrogen Carrier Gas

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Abstract

This application note describes the procedure for the analysis of 22 common terpenes in cannabis and hemp using the Agilent Intuvo gas chromatograph (GC) and flame ionization detector (FID), with hydrogen carrier gas, and post column backflush. The GC injection-to-injection cycle time was 10 minutes allowing for six samples per hour. A group of 1,200 hemp samples were analyzed to test the robustness of the method, and to determine a maintenance schedule.

Introduction

Terpenes are the main flavor and aromatic components of cannabis and hemp. Strains can be identified by their specific fingerprint for these terpenes. The abundance of particular terpenes may also indicate the character and usefulness of a particular strain. This identification also allows cultivators to monitor the consistency of their particular strains from one crop to the next, and to maintain better quality control. With ever-increasing varieties of cannabis flowers and the number of individual tests performed, the overall speed of analysis time has become more important.

Helium has been used as the carrier gas for most GC analyses. Recent limitations on its availability have added to the cost of testing. This application focuses on a fast and robust analysis of 22 of the most common terpenes found for strain identification, as well as providing information for labeling purposes. This application utilizes hydrogen carrier gas with flame ionization detection (FID) to help shorten the analysis time and therefore, keep the cost per sample down.

Methods and materials

An Agilent Intuvo 9000 GC fitted with a split/splitless (SSL) inlet, Guard Chip, FID, and post column backflush chip was used. The Agilent 7650A automated liquid sampler (ALS) 50-position autosampler was installed and fitted with a 10 µL syringe. A mid-frit, Agilent Ultra Inert inlet liner (part number 5190-5105) and an Agilent J&W DB-35ms analytical column; 25 m × 0.20 mm, 0.33 µm (part number 128-3822-INT) were used. Method parameters are listed in Table 1. Data were collected using Agilent MassHunter Acquisition software version 10.2. All data analysis was performed using Agilent MassHunter GC Quantitative software version 10.2.

Table 1. GC parameters.

Agilent Intuvo 9000	
Inlet	SSL
Inlet Temperature	280 °C
Mode	Split 40:1
Gas Saver	On at 2 min, 20 mL/min
Carrier Gas	Hydrogen at 80.7 cm/s (constant flow)
Liner	Split, mid-frit (p/n 5190-5105)
Injection Volume	1 µL
Column 1	Agilent DB-35ms, 25 m × 0.20 mm, 0.33 µm (p/n 128-3822-INT)
Column 2 (PSD)	Agilent Backflush Restrictor
Flow	3.3 mL/min (constant flow)
Postrun	23.131 mL/min
Intuvo Flow Path	
Guard Chip	280 °C Postrun 300 °C
Bus Temperature	280 °C
Oven Program	110 °C (2 min) 75 °C/min to 250 °C (0.75 min)
Postrun	275 °C
GC Run Time	4.62 min
GC Cycle Time	10.0 min
Detector FID	
Temperature	280 °C
Air	400 mL/min
Hydrogen	30 mL/min (carrier + fuel = constant)
Makeup (N_2)	25 mL/min
Data Rate	50 Hz
Backflush	
Start Time	4.62 min
Postrun Duration	3.00 min
PSD Pressure	30 psi
SSL Inlet	1 psi
Void Volumes	4.5

Table 2. Agilent consumables.

Part Name	Description	Part Number
Inlet Liner	Inlet liner, universal, Ultra Inert, mid-frit, 870 µL	5190-5105
Inlet Septa	Inlet septa, long-life, nonstick, 11 mm	5183-4761
Autosampler Syringe	ALS syringe, 10 µL, fixed needle, 23-26s/42/cone	5181-1267
Guard Chip	Intuvo, split/splitless Guard Chip	G4587-60565
Inlet Chip	Intuvo inlet chip	G4588-60031
Column	DB-35ms, 25 m × 0.20 mm, 0.33 µm	128-3822-INT
Backflush Chip	D1 Post column backflush	G4588-60302
Vial	Amber, screw top, 2 mL, write-on spot	5182-0716
Vial Cap	Blue, PTFE/red silicone septa	5182-0717
Vial Insert	250 µL, Deactivated with polymer feet	5181-8872
Centrifuge Tube	50 mL, Polypropylene	5610-2049
Ceramic Homogenizers	Ceramic homogenizers, 50 mL tubes	5982-9313
Syringe Filter	PTFE, 0.45 µm, 25 mm	5190-5087
Syringe	2.5 mL, PTFE, Luer Lock	5190-1534

Calibration curve

Certified terpene standards were obtained from Restek (Bellefonte, PA), part numbers 34095 and 34096. Serial dilutions of the mixed standards were made. To these standards, 5 mg of commercially available hemp oil and 10 µL of internal standard (ISTD) were added. Calibration points are listed in Table 3.

Internal standard preparation

- **ISTD 1** (for calibration standard): weigh 0.100 g of 2-fluorobiphenyl into a 10 mL volumetric flask and dilute with isopropyl alcohol. 10 µL of this will be used with all 0.5 mL of calibration standards.
- **ISTD 2** (for samples): weigh 0.200 g of 2-fluorobipheny into a 1.0 L volumetric flask. Dilute with isopropyl alcohol and mix. This standard becomes the ISTD to be used in the sample preparation.

Sample preparation

For this application, 250 mg of ground hemp flower from Absolute Standards, Hamden, CT (part number 54999C) was used. Depending upon individual state requirements, this amount of sample may need to be altered. For natural cannabis or hemp flower, a Geno/Grinder-type machine (high-velocity vertical shaker) should be used to break up the plant material. The ground hemp was placed into a 50 mL centrifuge tube with a ceramic homogenizer. To this, 25 mL of ISTD 2 was placed in the tube and vortexed for 2 minutes. The samples were allowed to sit for 10 minutes to allow most of the plant material to settle out. Then an aliquot was taken and passed through an Agilent Captiva PTFE syringe filter to further remove any plant material. This sample was placed in a 2 mL sample vial and loaded onto the autosampler for testing.

For the robustness study, a continuing calibration verification (CCV) was run after every 10 hemp samples. The liner and septum were changed at an interval of 200 hemp samples. The number of injections was higher as this did not account for CCVs, blanks, or any additional curve points that were also added to check for system robustness. The Guard Chip was changed after 376 injections to observe any analysis changes. A total of 1,200 hemp samples were analyzed, producing a total of 126 CCV check samples.

Results and discussion

The method developed here proved to be fast, reliable, and robust. The GC cycle time was 10 minutes, allowing the analyst to run six samples an hour. The resolution of the 22 compounds is illustrated in Figure 1. The use of the backflush helped to keep the matrix residue from progressing through the flow path. The Guard Chip was changed in this study only to observe any changes in performance such as retention time, resolution, or response. A second Guard Chip was not installed in this study, as it was not needed. Depending upon sample type and concentration of additional matrix compounds such as cannabinoids, the Guard Chip may need to be changed at a different interval for optimum efficiency. Liners were changed proactively after 200 hemp samples. Upon liner removal, it was obvious that there was significant matrix residue present as is seen by the dark material on the frit of the liner shown in Figure 2. It is not recommended to pull the liner out to note any discoloration in the fritted area, only to reinstall it. If a liner is pulled out at all, it is best to replace it. Analyzing other matrices, such as cannabis and concentrates, may change the frequency of the maintenance. Through previous work, it is noted that the loss of response of caryophyllene oxide is a good indicator of Guard Chip contamination.

Figure 2. Dirty liners.

The use of an ISTD is a good way to adjust for potential variation in injection volumes. The internal standard is used to minimize systemic anomalies. A total of 126 CCVs were analyzed to monitor the robustness of the application. The results are listed in Table 4. Outliers were set at ± 20% as a gauge for the need of maintenance in addition to the liner change. Through this study, no additional maintenance was required.

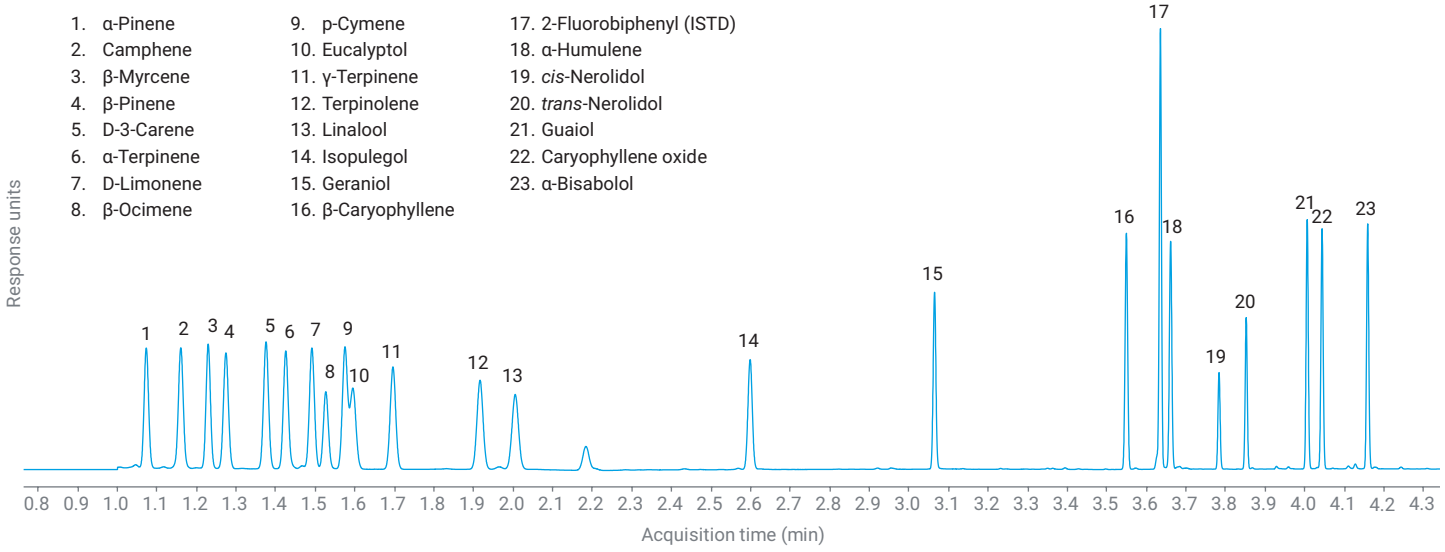

Figure 1. Standard chromatogram of 22 terpenes and ISTD at a concentration of 125 ppm.

Table 3. Calibration table of terpenes.

Name	Cal 1 (ug/mL)	Cal 2 (ug/mL)	Cal 3 (ug/mL)	Cal 4 (ug/mL)	Cal 5 (ug/mL)	Cal 6 (ug/mL)	Cal 7 (ug/mL)	Cal 8 (ug/mL)	Cal 9 (ug/mL)	Cal 10 (ug/mL)	Cal 11 (ug/mL)	Cal 12 (ug/mL)
All Terpenes Except Nerolidol	0.61	1.21	2.43	4.86	9.72	19.43	38.86	77.72	155.44	310.88	621.77	1243.53
cis-Nerolidol	0.24	0.48	0.97	1.93	3.87	7.74	15.48	30.96	61.92	123.83	247.66	495.32
trans-Nerolidol	0.36	0.73	1.45	2.90	5.80	11.61	23.22	46.44	92.87	185.75	371.49	742.98

Table 4. Results from robustness study: data from CCV standard (n = 117).

Name	RT	RT %RSD	Average CCV Accuracy	CCV Accuracy %RSD
α-Pinene	1.073	0.089	98.393	4.789
Camphene	1.160	0.092	98.571	4.455
β-Myrcene	1.229	0.110	98.574	4.444
β-Pinene	1.273	0.103	98.879	4.072
D-3-Carene	1.375	0.112	98.995	4.333
α-Terpinene	1.424	0.113	96.209	10.433
D-Limonene	1.490	0.118	99.313	4.281
β-Ocimene	1.525	0.129	98.559	6.299
p-Cymene	1.574	0.127	102.890	9.819
Eucalyptol	1.593	0.127	101.868	5.006
λ-Terpinene	1.694	0.133	97.673	8.093

Name	RT	RT %RSD	Average CCV Accuracy	CCV Accuracy %RSD
Terpinolene	1.913	0.144	98.798	6.464
Linalool	2.002	0.152	103.171	6.589
Isopulegol	2.594	0.109	103.348	6.620
Geraniol	3.058	0.093	101.938	7.108
β-Caryophyllene	3.541	0.048	101.497	6.374
α-Humulene	3.652	0.046	102.122	6.883
cis-Nerolidol	3.773	0.050	99.733	7.335
trans-Nerolidol	3.842	0.054	99.223	7.013
Guaiol	3.995	0.055	99.827	6.918
Caryophyllene Oxide	4.033	0.053	100.791	7.792
α-Bisabolol	4.147	0.062	98.708	6.917

A total of 12 calibration levels were used to cover the expected range of terpenes. These levels can be altered to best fit the target concentrations and requirements needed for this application. The average linear regression coefficient (R^2) for all compounds was 0.999, as illustrated in Figure 3.

The initial concentration for each terpene varied by 1.2%. For ease of building the calibration curves, all terpene concentrations were "generalized" apart from cis and trans nerolidol as is shown in Table 3.

An overlay of the hemp standard and a calibration point is illustrated in Figure 4. The retention time stability makes it easy to positively identify the peaks corresponding to the 22 terpenes from the calibrant. However, additional compounds are observed in the hemp standard that cannot be identified with the FID by retention time matching.

Logically, this phenomenon is likely to happen fairly often, due to the diversity of compounds and their level of expression in cannabis and hemp. If further clarity is required in terms of identifying those peaks or establishing the purity of a given peak, Agilent recommends using a mass spectrometer as the primary detector. Such detector can be successfully used in conjunction with hydrogen carrier gas, as shown in Agilent application note 5994-6216EN.[2]

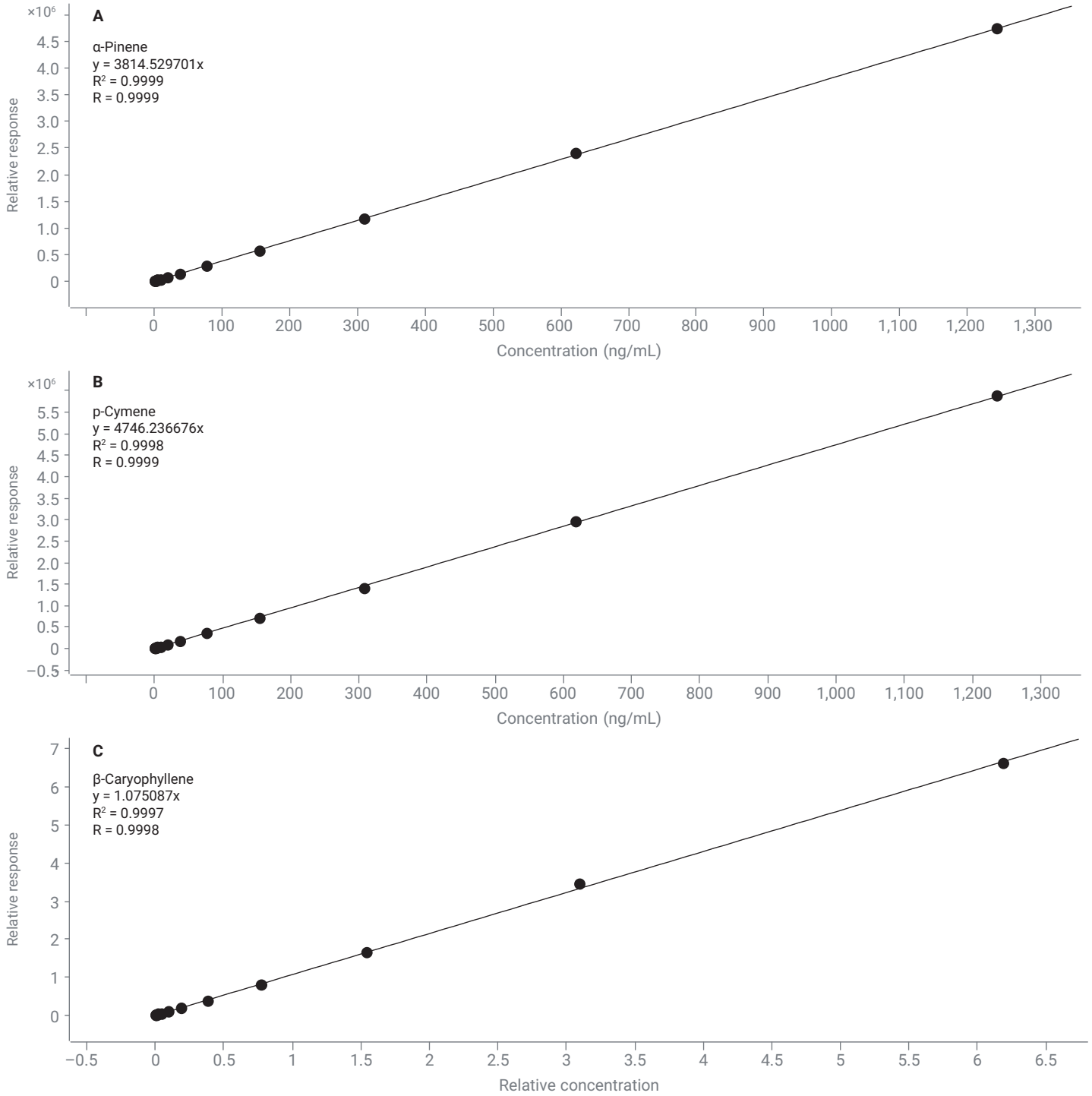

Figure 3 . Examples of calibration curves throughout the chromatogram: (A) α-pinene, (B) p-cymene, and (C) β-caryophyllene.

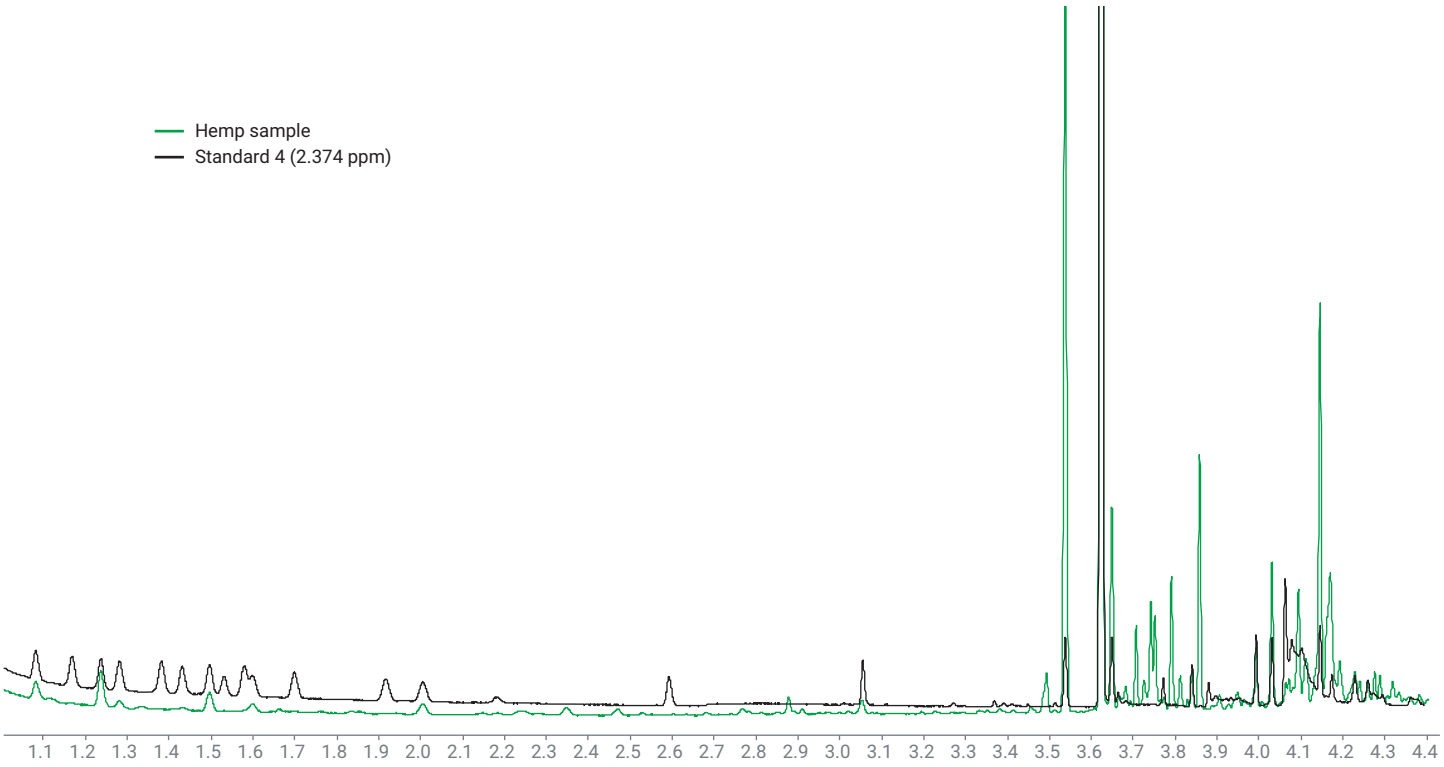

Figure 4. The overlay of a hemp sample is shown in green and standard 4 (2.374 ppm) is shown in black.

Conclusion

This method proved to be a very robust, high throughput method with a GC cycle time of only 10 minutes (6 samples/hour). This study showed that the regular maintenance of changing liners, septa, and Guard Chips when needed, maintained retention time and curve stability. The use of backflushing helped keep the Guard Chip and inlet cleaner and minimized maintenance.

All calibration curves showed great linear response across the concentration range, through all 12 calibration standards, as seen in Figure 3.

Reminders can be set up using counters to alert the analyst when maintenance is needed according to the user's desired sample load. Being careful to install the liner in the same fashion helps to keep the tolerance of the CCV results within the ± 20% limits. It is noted and shown in Figure 4 that there may be additional terpenes present in samples that are not covered by the terpenes in the standards for this application. If this is of concern, a more encompassing standard may be used. Also, using a mass spectral detector may be more beneficial for the application.[2] Other matrices may require a revised maintenance schedule.

References

1. Hollis, J. S.; Harper, T.; Macherone, A. Terpenes Analysis in Cannabis Products by Liquid Injection using the Agilent Intuvo 9000/5977B GC/MS System, *Agilent Technologies application note*, publication number 5994-2032EN, **2020**.
2. Haddad, S. P.; Patel, S. U.; Westland, J. L. Analysis of Terpenes in Cannabis with Hydrogen Carrier Gas and the Agilent HydroInert Source on the Agilent Intuvo 9000/5977C GC/MS, *Agilent Technologies application note*, publication number 5994-6216EN.

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