Analysis of Terpenes in Cannabis with Hydrogen Carrier Gas and the Agilent HydroInert Source on the Agilent 8890/5977C GC/MS

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Abstract

This application note summarizes the analysis of terpenes in cannabis using gas chromatography/mass spectrometry (GC/MS) with hydrogen carrier gas. GC/MS has become an important tool for characterizing the taste and smell of commercially available cannabis strains. The Agilent 8890 GC and 5977C single quadrupole GC/MS system with a novel electron ionization (EI) source—the Agilent HydroInert source—were used for the analysis of 40 chromatographically resolved terpenes. The optimized method provided a similar elution profile to helium and excellent linearity of R^2 ≥0.99 over the respective calibration ranges. All target analyte accuracy values fell between 92.5 and 115.9%, and all precision relative standard deviation (%RSD) values were under 2.7%. For laboratories looking to conduct their terpenes analysis with more sustainable hydrogen carrier gas, the HydroInert source with the 9 mm extractor lens and smaller diameter (0.18 mm) columns enables the transition with similar performance.

Introduction

Terpenes are a class of compounds that are responsible for the unique flavor and aroma in each strain of *Cannabis spp*. Characterizing the flavor and aroma profiles of commercial cannabis strains is often done using GC /MS, with helium as the preferred carrier gas. However, recurring helium shortages and mounting costs have increased demand for applications using hydrogen as the carrier gas. This application note focuses on the analysis of terpenes using liquid injection on a single quadrupole GC/MS in selective ion monitoring (SIM) mode with hydrogen as the GC carrier gas.

When adopting hydrogen for GC/MS analysis, there are several factors to consider. First, hydrogen is a reactive gas, and it may potentially cause chemical reactions in the inlet, column, and sometimes the MS EI source, which can change analysis results. To address potential issues in the MS source, the Agilent HydroInert source was used. Additional information can be found in the Agilent technical overview of the HydroInert source.[1] Second, for GC/MS applications, hardware changes in the gas chromatograph and mass spectrometer may be required when switching to hydrogen carrier gas. The Agilent Helium to Hydrogen Carrier Gas Conversion Guide[2] describes in detail the steps for conversion from helium to hydrogen carrier gas. Lastly, it is recommended that when working with flammable or explosive gases, laboratories maintain proper safety in gas handling and use. Further information on the safe use of hydrogen can be found in the Agilent Hydrogen Safety Manual[3] and Hydrogen Safety for the Agilent GC System Guide.[4]

Experimental

Chemicals and reagents

Terpenes standard mixes CAN-TERP-MIX1H and CAN-TERP-MIX2H, each containing 21 terpenes, were purchased from SPEX CertiPrep (Metuchen, NJ, US). The internal standard (ISTD), 2-fluorobiphenyl, and ethyl acetate (purity 99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, US). The test matrix for calibration, cold pressed hemp seed oil (blank matrix), was obtained from Nutiva (Richmond, CA, US). Various strains of ground cannabis flower were obtained from the University of Mississippi. Eight calibration levels were prepared: 3.83, 7.66, 15.32, 30.64, 61.28, 122.55, 245.10, and 490.20 µg/mL. Borneol, fenchone, and camphor were present in both standard mixtures and created racemic mixtures with the same retention time. For borneol and fenchone, the high-level concentration was 980.40 µg/mL, and camphor was 1,470.60 µg/mL.

Instrumentation

The GC/MS method parameters followed the methodology described in Agilent application note 5994-2032EN[5] with some modifications for hydrogen carrier gas and the use of the Agilent 8890 GC. The GC instrument parameters can be found in Table 1, the MS instrument parameters in Table 2, and the SIM time segment parameters in Table 3. Table 4 contains a list of consumable items used for the current application.

Table 1. GC and backflush conditions for terpenes analysis.

Agilent 8890 GC, Auto Injector, and Tray	
Injection Volume	1.0 µL
Inlet	EPC split/splitless
Mode	Split
Split Ratio	150:1
Septum Purge Flow	Standard, 3 mL/min
Inlet Temperature	250 °C
Oven	Initial: 75 °C (1 min hold)
	Ramp 1: 10 °C/min to 125 °C
	Ramp 2: 3 °C/min to 145 °C
	Ramp 3: 10 °C/min to 165 °C
	Ramp 4: 175 °C/min to 250 (6.2 min hold)
Total Run Time	22.75 min
Column 1	Agilent J&W DB-Select 624, 20 m × 0.18 mm, 1.0 µm
Control Mode, Flow	Constant flow, 0.60 mL/min
Column 2	Agilent J&W DB-Select 624, 20 m × 0.18 mm, 1.0 µm
Control Mode, Flow	Constant flow, 0.75 mL/min
PSD Purge Flow	3 mL/min
Agilent 8890 GC Backflush Parameters	
Inlet Pressure	2 psi
Backflush Pressure	50 psi
Void Volumes	5
Backflush Time	1.47 min

Table 2. MS conditions for terpenes analysis.

Agilent 5977C MSD	
Source	Agilent HydroInert
Extractor Lens	9 mm
Transfer Line Temperature	260 °C
Source Temperature	300 °C
Quadrupole Temperature	200 °C
Mode	SIM
EM Voltage Gain	Variable
Solvent Delay	9 min
Tune File	atune.u

Table 3. Retention time, time segment, EM gain, and SIM ions used for terpenes analysis.

Target Analyte	Retention Time (min)	Time Segment	Segment Start Time	EM Gain Factor	Quantifier Ion	Qualifier Ion 1	Qualifier Ion 2
alpha-Pinene	9.423	1	9.00	10	93	91	77
Camphene	9.998	2	9.70	10	93	107	136
Sabinene	10.561	3	10.20	10	136	93	91
beta-Myrcene	10.673	3			121	69	77
beta-Pinene	10.784	3			93	69	77
alpha-Phellandrene	11.427	4	11.00	10	93	77	136
delta-3-Carene	11.507	4			93	77	121
alpha-Terpinene	11.791	4			121	93	105
E-beta-Ocimene	11.947	4			93	91	79
D-Limonene	12.121	4			68	93	121
Z-beta-Ocimene	12.417	4			93	91	79
Eucalyptol	12.595	4			81	111	139
gamma-Terpinene	12.955	5	12.80	10	93	77	136
Terpinolene	13.743	6	13.20	10	121	93	136
Sabinene Hydrate	14.085	6			71	121	139
Linalool	14.367	6			71	93	55
[±]-Fenchone	14.533	6			81	69	152
Endo-Fenchyl Alcohol	14.905	7	14.80	10	81	80	111
Isopulegol	15.150	7			121	136	154
[+/N/-]-Camphor	15.338	8	15.25	10	152	81	108
Isoborneol	15.410	8			95	110	121
Menthol	15.435	8			71	123	138
[±]-Borneol	15.520	8			95	110	121
alpha-Terpineol	15.600	8			93	121	136
gamma-Terpineol	15.651	8			59	93	121
Nerol	15.844	9	15.75	15	69	93	84
Geraniol	16.046	9			69	93	121
Pulegone	16.149	9			152	81	109
Geranyl Acetate	16.951	10	16.70	15	68	69	121
2-Fluorobiphenyl [ISTD]	17.256	11	17.10	10	172	171	170
Farnesene	17.468	12	17.40	15	69	93	79
alpha-Cedrene	17.631	12			93	119	105
E-Caryophyllene	17.674	12			93	161	105
alpha-Humulene	18.026	13	17.85	15	93	80	121
Valencene	18.302	14	18.20	20	161	189	204
Z-Nerolidol	18.497	15	18.40	20	69	81	121
E-Nerolidol	18.813	16	18.70	20	69	81	121
Guaiol	19.754	17	19.40	20	161	107	91
Caryophyllene Oxide	20.018	18	19.91	20	79	91	109
Cedrol	20.334	19	20.20	20	95	151	150
alpha-Bisabolol	20.650	20	20.52	20	109	119	93

Table 4. Agilent consumables and part numbers used in the method for terpenes analysis. Custom columns can be requisitioned from the Agilent GC custom columns shop.[6]

Consumable	Description	Part Number
Injector Syringe	Blue Line autosampler syringe, 10 µL, fixed needle	G4513-80220
Inlet Septum	Advanced Green septum, nonstick, 11 mm	5183-4759
Inlet Liner	Universal Ultra Inert low pressure drop inlet liner	5190-2295
Sample Vials	Screw top, amber, write-on, certified, 2 mL	5182-0716
Vial Caps	Screw top, certified, PTFE/white silicone septa	5182-0720
Column	J&W DB-Select 624 Ultra Inert, 20 m × 180 µm × 1.0 µm (quantity: 2)	Custom Part Number
Inlet Nut	Column nut, collared, self-tightening	G3440-81011
Ferrules	15% graphite/85% Vespel, 0.1 to 0.25 mm column	5181-3323
MSD Nut	Column nut, collared, self-tightening	G3440-81013
Steel Tubing	Install kit for GCs, stainless steel	19199S
GC/MS Source	HydroInert complete source assembly for 5977C	G7078-67930

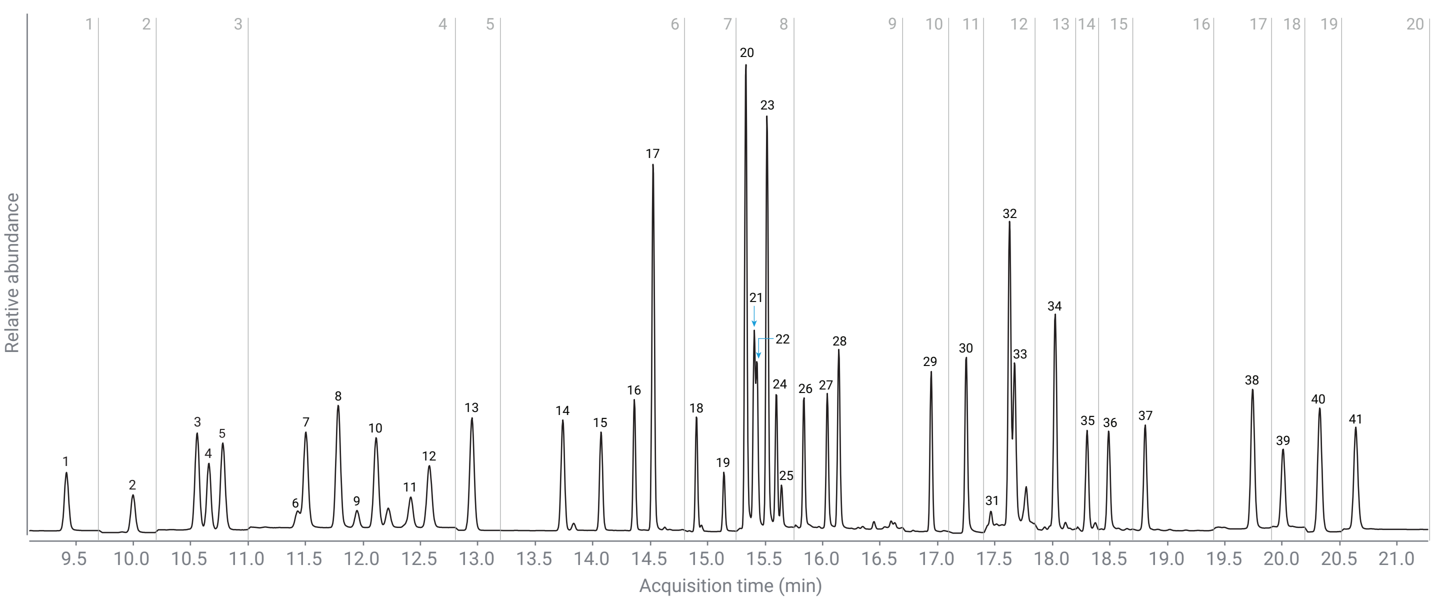
Internal standards, calibration standards, and sample preparation

Preparation of standards and samples followed the methodology described in Agilent application note 5994-2032EN.[5] Optionally, preparation of all standards, calibrators, and samples can be automated using the Agilent PAL3 Series II RTC instrument, as described in Agilent application note 5994-6007EN.[7]

Results and discussion

Chromatography, Calibration, Accuracy, and Precision

Figure 1 shows the SIM time segment chromatogram of calibration level 8 (490.20 µg/mL). Using hydrogen carrier gas, an elution profile similar to previous work in helium[5,7] was observed. Due to the combination of hydrogen carrier and a smaller diameter column, the run time with the current method has been reduced from 30 minutes when using helium to 23 minutes when using hydrogen. Note that the 9 mm extractor lens is recommended for terpenes analysis with helium. The Agilent HydroInert source uses the 9 mm extractor lens as a standard configuration with hydrogen carrier gas. Use of the 9 mm extractor lens remains



1. alpha-Pinene
2. Camphene
3. Sabinene
4. beta-Myrcene
5. beta-Pinene
6. alpha-Phellandrene
7. delta-3-Carene
8. alpha-Terpinene
9. E-beta-Ocimene
10. D-Limonene
11. Z-beta-Ocimene
12. Eucalyptol
13. gamma-Terpinene
14. Terpinolene
15. Sabinene hydrate
16. Linalool
17. [+/−]-Fenchone
18. Endo-fenchyl alcohol
19. Isopulegol
20. [+/N/−]-Camphor
21. Isoborneol
22. Menthol
23. [+/−]-Borneol
24. alpha-Terpineol
25. gamma-Terpineol
26. Nerol
27. Geraniol
28. Pulegone
29. Geranyl acetate
30. 2-Fluorobiphenyl [ISTD]
31. Farnesene
32. alpha-Cedrene
33. E-Caryophyllene
34. alpha-Humulene
35. Valencene
36. Z-Nerolidol
37. E-Nerolidol
38. Guaiol
39. Caryophyllene oxide
40. Cedrol
41. alpha-Bisabolol

Figure 1. SIM time segment chromatogram of calibration level 8 (490.20 µg/mL).

the recommendation for the current application. Table 5 provides the correlation coefficient (R^2) for three independent calibration curves; limit of detection (LOD); and limit of quantitation (LOQ). All R^2 values were >0.99, demonstrating excellent fit of prepared calibration standards.

Table 5. The calibration range, correlation coefficient (R^2) for three independent calibration curves, LOD, and LOQ. All calibration curves were fit with quadratic relationships, and 1/x weighting was used. LOD and LOQ were determined using eight replicates of the first calibration point (3.83 µg/mL) and the Agilent MassHunter Software onboard calculation.

Target Analyte	Calibration Range (µg/mL)	Cal 1 (R^2)	Cal 2 (R^2)	Cal 3 (R^2)	LOD (µg/mL)	LOQ (µg/mL)
alpha-Pinene	3.83 to 490.20	0.9984	0.9973	0.9978	0.10	0.34
Camphene	3.83 to 490.20	0.9989	0.9985	0.9986	0.20	0.66
Sabinene	3.83 to 490.20	0.9993	0.9989	0.9990	0.22	0.72
beta-Myrcene	3.83 to 490.20	0.9992	0.9989	0.9990	0.25	0.82
beta-Pinene	3.83 to 490.20	0.9992	0.9989	0.9990	0.13	0.42
alpha-Phellandrene	3.83 to 490.20	0.9993	0.9990	0.9991	0.23	0.76
delta-3-Carene	3.83 to 490.20	0.9991	0.9989	0.9991	0.15	0.51
alpha-Terpinene	3.83 to 490.20	0.9985	0.9980	0.9984	0.14	0.45
E-beta-Ocimene	3.83 to 490.20	0.9997	0.9992	0.9993	0.17	0.57
D-Limonene	3.83 to 490.20	0.9996	0.9995	0.9992	0.25	0.83
Z-beta-Ocimene	3.83 to 490.20	0.9998	0.9991	0.9995	0.30	0.99
Eucalyptol	3.83 to 490.20	0.9994	0.9994	0.9994	0.27	0.91
gamma-Terpinene	3.83 to 490.20	0.9998	0.9996	0.9995	0.19	0.62
Terpinolene	3.83 to 490.20	0.9995	0.9992	0.9988	0.12	0.40
Sabinene Hydrate	3.83 to 490.20	0.9994	0.9991	0.9992	0.21	0.69
Linalool	3.83 to 490.20	0.9992	0.9987	0.9990	0.18	0.61
[±]-Fenchone	7.66 to 980.40	0.9994	0.9992	0.9992	0.20	0.68
Endo-Fenchyl Alcohol	3.83 to 490.20	0.9993	0.9992	0.9992	0.28	0.92
Isopulegol	3.83 to 490.20	0.9992	0.9990	0.9990	0.29	0.96
[+/N/-]-Camphor	11.49 to 1,470.60	0.9993	0.9993	0.9992	0.25	0.83
Isoborneol	3.83 to 490.20	0.9992	0.9994	0.9995	0.17	0.58
Menthol	3.83 to 490.20	0.9990	0.9991	0.9994	0.31	1.03
[±]-Borneol	7.66 to 980.40	0.9993	0.9993	0.9995	0.22	0.75
alpha-Terpineol	3.83 to 490.20	0.9991	0.9993	0.9994	0.26	0.86
gamma-Terpineol	3.83 to 490.20	0.9994	0.9991	0.9988	0.33	1.10
Nerol	3.83 to 490.20	0.9995	0.9998	0.9993	0.27	0.89
Geraniol	3.83 to 490.20	0.9995	0.9998	0.9991	0.23	0.77
Pulegone	3.83 to 490.20	0.9994	0.9993	0.9993	0.27	0.89
Geranyl Acetate	3.83 to 490.20	0.9994	0.9997	0.9996	0.32	1.05
Farnesene	3.83 to 490.20	0.9991	0.9998	0.9997	0.28	0.93
alpha-Cedrene	3.83 to 490.20	0.9998	0.9994	0.9997	0.24	0.79
E-Caryophyllene	3.83 to 490.20	0.9998	0.9998	0.9997	0.24	0.79
alpha-Humulene	3.83 to 490.20	0.9999	0.9999	0.9999	0.21	0.71
Valencene	3.83 to 490.20	0.9995	0.9997	0.9999	0.33	1.12
Z-Nerolidol	3.83 to 490.20	0.9992	0.9996	0.9997	0.21	0.71
E-Nerolidol	3.83 to 490.20	0.9994	0.9995	0.9996	0.20	0.67
Guaiol	3.83 to 490.20	0.9996	0.9994	0.9995	0.15	0.49
Caryophyllene Oxide	3.83 to 490.20	0.9998	0.9996	0.9997	0.34	1.13
Cedrol	3.83 to 490.20	0.9997	0.9996	0.9997	0.14	0.45
alpha-Bisabolol	3.83 to 490.20	0.9996	0.9996	0.9995	0.28	0.95

Table 6 displays the accuracy and precision data for three different calibration levels. All accuracy values fell between 92.5 and 115.9%, and all precision values were under 2.7%.

Calibration, accuracy, and precision data are similar to, or in some cases better than, previous work[7] using the helium method described in Agilent application note 5994-2032EN.[5]

Table 6. Accuracy as the average percent (n = 3) and precision as %RSD (n = 5) at calibration level 3.83, 30.64, and 245.10 µg/mL.

	Accuracy (Average %, n = 3)			Precision (% RSD, n = 5)		
Target Analyte	**3.83 µg/mL**	**30.64 µg/mL**	**245.10 µg/mL**	**3.83 µg/mL**	**30.64 µg/mL**	**245.10 µg/mL**
alpha-Pinene	109.5	97.3	106.7	1.3	0.9	1.5
Camphene	110.0	98.0	104.7	0.8	0.5	0.9
Sabinene	108.3	96.8	104.2	0.6	0.8	1.1
beta-Myrcene	104.6	96.4	103.8	1.9	0.3	1.0
beta-Pinene	109.8	97.5	104.1	1.0	0.8	0.8
alpha-Phellandrene	109.7	96.1	103.3	1.6	1.8	1.8
delta-3-Carene	104.4	96.5	103.8	2.3	0.8	0.6
alpha-Terpinene	115.9	93.0	103.4	1.1	0.6	0.7
E-beta-Ocimene	106.0	96.8	103.1	1.9	2.1	1.4
D-Limonene	109.5	97.4	103.2	0.9	0.5	0.8
Z-beta-Ocimene	104.7	97.7	103.0	1.2	0.7	1.4
Eucalyptol	109.6	97.0	103.3	1.6	1.3	0.6
gamma-Terpinene	107.8	98.1	102.4	1.4	0.2	0.9
Terpinolene	112.9	95.8	103.6	1.3	0.7	0.8
Sabinene Hydrate	112.1	94.9	103.7	1.4	0.6	1.1
Linalool	114.3	94.5	104.5	2.5	0.7	0.9
[±]-Fenchone	111.1	96.3	104.0	0.4	0.2	1.0
Endo-Fenchyl Alcohol	112.3	96.3	103.9	0.6	0.8	0.6
Isopulegol	114.2	94.1	104.0	0.4	1.0	1.6
[+/N/-]-Camphor	109.1	94.6	103.7	0.4	0.3	0.5
Isoborneol	111.4	95.8	103.5	1.2	1.4	0.6
Menthol	113.2	95.6	104.0	1.0	0.7	0.8
[±]-Borneol	112.1	95.2	103.5	1.6	0.5	0.5
alpha-Terpineol	113.8	95.1	103.6	0.9	0.5	0.5
gamma-Terpineol	110.9	92.5	103.5	1.1	1.7	1.0
Nerol	109.6	94.9	101.5	0.8	0.5	0.9
Geraniol	112.1	95.0	102.0	0.8	0.6	0.6
Pulegone	111.2	95.0	103.5	0.4	0.6	0.8
Geranyl Acetate	106.2	99.0	102.1	0.5	0.6	0.8
Farnesene	106.9	101.2	98.7	0.9	0.9	1.8
alpha-Cedrene	97.7	103.3	98.8	1.2	0.6	2.0
E-Caryophyllene	105.3	96.5	99.8	2.7	1.1	1.6
alpha-Humulene	104.1	99.2	99.8	2.2	0.6	1.0
Valencene	100.6	101.8	98.3	2.4	1.1	0.8
Z-Nerolidol	110.6	95.3	102.8	1.5	1.8	0.8
E-Nerolidol	111.5	95.7	102.9	1.1	1.2	0.7
Guaiol	111.4	95.2	102.8	1.0	0.9	0.8
Caryophyllene Oxide	106.3	96.6	102.1	1.1	1.2	0.8
Cedrol	109.0	95.7	102.4	1.3	1.0	0.6
alpha-Bisabolol	111.4	95.4	102.6	2.7	0.6	0.7

Analysis of various cannabis samples

All LODs and LOQs fell below the lowest calibration point for all compounds. Thus, the reporting limits for the current study were defined as any value greater than the lowest calibration point for the respective compound. The terpene concentrations found in the different cannabis samples are presented in Table 7. Each sample shows a unique terpenes profile. The %RSD for detected terpenes ranged between 1.31 to 5.52% in sample 5 and 1.73 to 4.28% in sample 6. Detected concentration in the six samples presented, as well as %RSDs for sample 5 and 6, are similar to, or in some cases better than previous work[7] where the same samples were run using the helium method described in Agilent application note 5994-2032EN.[5]

Table 7. Concentration (µg/mL) of terpenes found in six different cannabis flower samples. Samples 5 and 6 were run in quadruplicate (n = 4) to determine the standard deviation (SD).

Target Analyte	Sample 1 (µg/mL)	Sample 2 (µg/mL)	Sample 3 (µg/mL)	Sample 4 (µg/mL)	Sample 5 (µg/mL ± SD, n = 4)	Sample 6 (µg/mL ± SD, n = 4)
alpha-Pinene		5.14				
D-Limonene					4.54 ± 0.12	
Eucalyptol	8.60					
Linalool	21.56	4.62	10.96	4.75	11.13 ± 0.51	
Endo-Fenchyl Alcohol	9.59		15.64		9.82 ± 0.15	
[±]-Borneol			14.41			
alpha-Terpineol	17.70		28.32	7.55	9.31 ± 0.12	3.97 ± 0.08
gamma-Terpineol	26.58		42.14	10.96	13.92 ± 0.75	5.77 ± 0.10
Farnesene	136.66	20.27	97.22	139.82	63.34 ± 1.87	19.43 ± 0.37
E-Caryophyllene	86.79	19.79	51.31	56.89	147.27 ± 3.82	20.37 ± 0.83
alpha-Humulene	43.21	8.01	20.18	19.36	43.35 ± 1.20	6.49 ± 0.28
E-Nerolidol	15.74		16.27	5.59	8.91 ± 0.26	
Guaiol			9.94			
Caryophyllene Oxide	33.52	22.46	55.25	46.98	25.04 ± 1.38	10.02 ± 0.26
alpha-Bisabolol	15.33	31.82	54.83	24.29	126.75 ± 3.09	27.09 ± 1.16

Conclusion

The presented application demonstrates that hydrogen carrier gas, when paired with the Agilent HydroInert source, is a viable option for the analysis of terpenes in cannabis. The HydroInert source, with the 9 mm extractor lens, and smaller diameter (0.18 mm) columns, delivers acceptable performance similar to GC/MS analysis using helium carrier gas.

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