Application Note

Cannabis and Hemp Testing

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Fully Automated Sample Preparation for the Analysis of Terpenes in Cannabis Flower

Using the Agilent PAL3 Series II RTC Automation with an Agilent Intuvo 9000/5977C GC/MS

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Abstract

Analysis of terpenes in cannabis by gas chromatography/mass spectrometry (GC/MS) has become an important tool for the characterization of taste and smell profiles of commercially available cannabis strains. This application brief demonstrates the capability of the Agilent PAL3 Series II robotic tool change (RTC) sampler to fully automate the sample preparation process for terpenes in cannabis flower. The use of automated sample preparation helps reduce user errors and the amount of sample and solvent needed for analysis. Coupled with an Agilent Intuvo 9000/5977C GC/MS system, PAL3 Series II RTC automation can save time and cut down on energy consumption, leading to a greener high-throughput laboratory.

Introduction

Terpenes are a class of compounds that are responsible for the unique flavor and aroma in each strain of *Cannabis* spp. Analysis of terpenes in cannabis by gas chromatography/mass spectrometry (GC/MS) has become an important tool for the characterization of taste and smell profiles of commercially available cannabis strains. The manual solvent extraction process, while straightforward, can introduce extraction errors at the bench. This application brief demonstrates the capability of the Agilent PAL3 Series II RTC sampler to fully automate the sample preparation process for terpenes in cannabis flower. The PAL3 Series II RTC, paired with the Agilent Intuvo 9000 GC and 5977C MS detector (MSD), creates an easy and robust solution for any cannabis lab looking to analyze terpenes.

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.

Instrumentation

The GC/MS instrument method parameters followed the methodology described in Agilent application note 5994-2032EN.[1] GC instrument parameters can be found in Table 1, MS instrument parameters in Table 2, and PAL3 Series II RTC instrument configuration in Table 3. Table 4 contains a list of consumable items used for the current application.

Table 1. GC conditions for terpenes analysis.

Agilent Intuvo 9000 GC, Auto Injector, and Tray	
Injection Volume	1.0 µL
Inlet	EPC split/splitless
Mode	Split
Split Ratio	150:1
Septum Purge Flow Mode	Standard, 3 mL/min
Inlet Temperature	250 °C
Guard Chip Temperature	250 °C (Track Oven Off)
Bus Temperature	260 °C
Oven	Initial: 75 °C (1 min hold) Ramp 1: 5 °C/min to 165 °C Ramp 2: 175 °C/min to 250 °C (10.514 min hold)
Column 1	Agilent J&W DB-Select 624, 30 m × 0.25 mm, 1.4 µm
Control Mode, Flow	Constant flow, 2.0 mL/min
Column 2	Agilent J&W DB-Select 624, 30 m × 0.25 mm, 1.4 µm
Control Mode, Flow	Constant flow, 2.2 mL/min
PSD Purge Flow	3 mL/min
Agilent Intuvo 9000 GC Backflush Parameters	
Inlet Pressure (Backflushing)	2 psi
Backflush Pressure	65 psi
Void Volumes	5
Backflush Time	3.236

Table 2. MS conditions for terpenes analysis.

Agilent 5977C MSD	
Source	Agilent Extractor
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	13 min
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Table 3. PAL 3 configuration for terpenes analysis.

PAL3 Series II RTC Rail	
Tools	Liquid handling tools
Syringes	10 µL (part number 8010-1307) 25 µL (part number 8010-1310) 1 mL (part number 8010-1326)
Control System	PAL Method Composer v1.4
PAL Modules	Vortex mixer, tray holder, large wash module
Optional Modules	Fast wash station, dilutor module

Table 4. Agilent consumables and part numbers used in the method for terpenes analysis.

Consumable	Description	Part Number
Inlet Septum	Advanced Green septum, nonstick, 11 mm	5183-4759
Inlet Liner	Universal Ultra Inert low pressure drop inlet liner	5190-2295
Guard Chip	Agilent Intuvo S/SL Guard Chip	G4587-60565
Column	DB-Select 624 30 m × 250 µm, 1.4 µm (quantity: 2)	122-0334UI-INT
Gaskets	Intuvo polyimide gasket	5190-9072
Compression Bolts	Intuvo compression bolts	G4581-60260
Detector Tail	Intuvo MS tail	G4590-60009
Extractor Lens	Extractor EI 9 mm lens	G3870-20449
PAL Vials	Screw top, clear, round bottom, 10 mL	5188-5392
PAL Screw Caps	Steel, magnetic cap, PTFE/silicone septa 18 mm	5188-2759

Automated calibration and sample preparation

All automated preparation steps were scripted using PAL Method Composer (G7388A). Figure 1 details the working instructions for the automated preparation of the calibration curve levels, while Figure 2 details the working instructions for the automated sample preparation.

To evaluate the automated preparation of calibration curves, three separate curves were created using the PAL3 Series II RTC. A total of eight calibration points were used, ranging from 3.83 µg/mL to 490.2 µg/mL. Accuracy and precision were calculated for each terpene (n = 3) using a low (3.83 µg/mL), a mid (30.64 µg/mL), and a high (245.10 µg/mL) calibration level standard.

To evaluate the automated preparation of samples, homogenized cannabis flower was extracted. Two different cannabis flower samples were extracted in quadruplicate (n = 4) to show precision (Figure 3), and six additional cannabis flower samples were extracted (n = 1) to show sample variation (Figure 4).

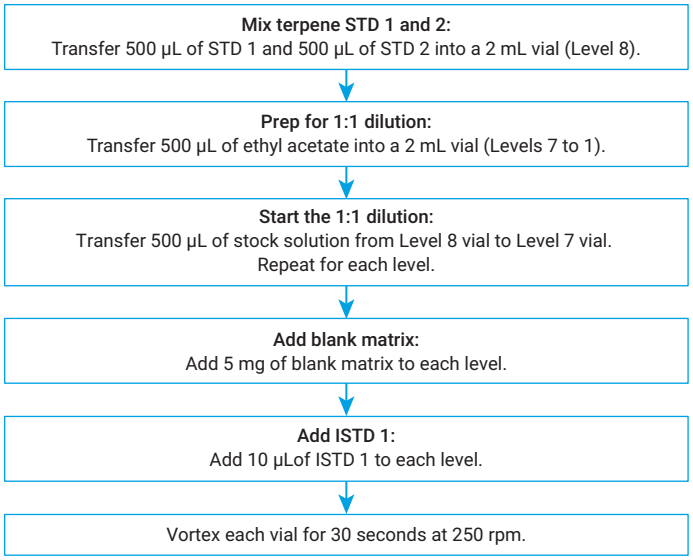

Figure 1. PAL Method Composer working instructions for the automated preparation of terpene calibration curves. The volume needed to accurately transfer 5 mg of hemp seed oil was determined experimentally, while keeping its density and viscosity in mind. Preparation instructions for ISTD 1 (10,000 µg/mL of 2-fluorobiphenyl in ethyl acetate) can be found in Agilent application note 5994-2032EN.[1]

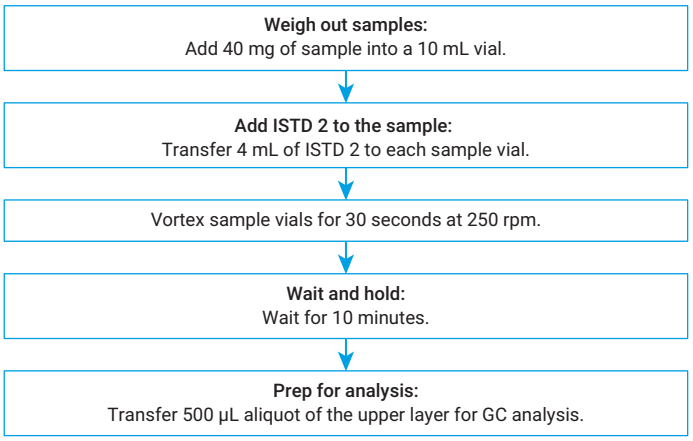

Figure 2. PAL Method Composer working instructions for the automated preparation of terpenes samples. Preparation instruction for ISTD 2 (200 µg/mL of 2-fluorobiphenyl in ethyl acetate) can be found in Agilent application note 5994-2032EN.[1].

Figure 3. Two different cannabis flower samples.

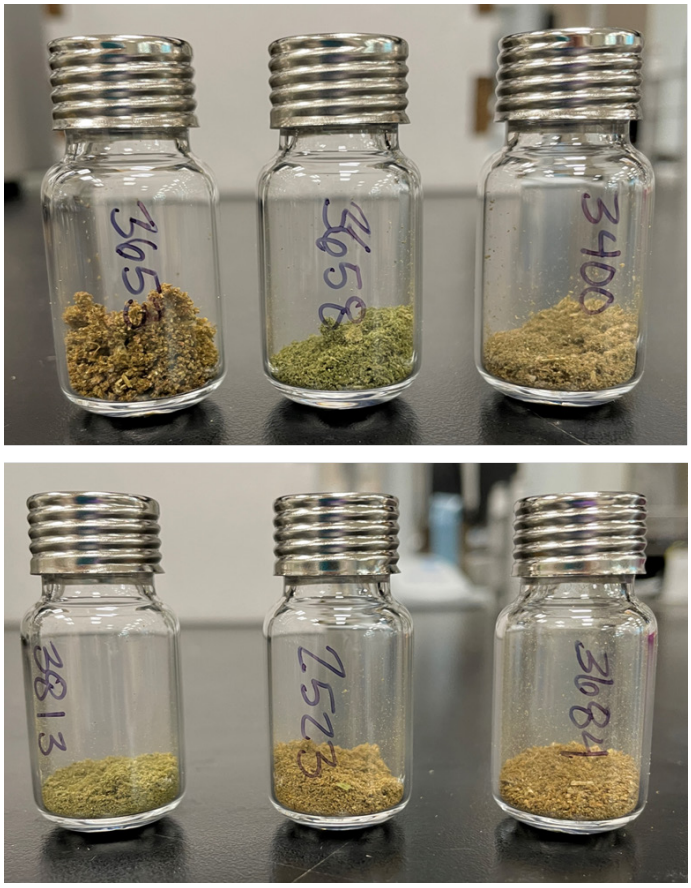

Figure 4. Six different cannabis flower samples.

Results and discussion

Calibration, accuracy, and precision

The main advantage of automated sample preparation is the reduction of errors and resources. The results show a robust process that can be easily implemented and maintained with minimal user intervention. Table 5 provides the retention time and linearity (R^2) for three independent calibration curves prepared using the PAL3 Series II RTC rail. All R^2 values were >0.99, demonstrating excellent fit of prepared calibration standards. Table 6 displays the accuracy and precision data for three different calibration levels. All accuracy values fell between 91.5 to 118.6% and all precision values were under 8.7%.

Table 5. The retention time (RT) and linearity (R^2) for three independent calibration curves generated using the PAL3 Series II RTC rail. All calibration curves were fit with quadratic relationships, and 1/x weighting was used.

Name	RT (min)	Cal 1 (R^2)	Cal 2 (R^2)	Cal 3 (R^2)
alpha-Pinene	13.963	0.9993	0.9980	0.9992
Camphene	14.710	0.9994	0.9984	0.9992
Sabinene	15.399	0.9996	0.9987	0.9989
beta-Myrcene	15.536	0.9989	0.9987	0.9991
beta-Pinene	15.665	0.9993	0.9984	0.9995
alpha-Phellandrene	16.449	0.9992	0.9989	0.9992
delta-3-Carene	16.496	0.9995	0.9984	0.9991
alpha-Terpinene	16.817	0.9982	0.9968	0.9978
E-beta-Ocimene	16.995	0.9996	0.9992	0.9987
D-Limonene	17.177	0.9995	0.9986	0.9988
Z-beta-Ocimene	17.490	0.9996	0.9991	0.9989
Eucalyptol	17.685	0.9998	0.9997	0.9992
gamma-Terpinene	18.072	0.9996	0.9989	0.9989
Terpinolene	19.053	0.9993	0.9984	0.9984
Sabinene Hydrate	19.484	0.9999	0.9998	0.9981
Linalool	19.779	1.0000	0.9998	0.9980
[±]-Fenchone	19.975	0.9998	0.9998	0.9989
Endo-Fenchyl Alcohol	20.446	0.9999	0.9997	0.9983
Isopulegol	20.802	0.9999	0.9998	0.9979
[±/N]-Camphor	21.088	0.9999	0.9998	0.9987
Isoborneol	21.186	0.9991	0.9999	0.9975
Menthol	21.207	0.9994	0.9996	0.9989
[±]-Borneol	21.330	0.9999	0.9997	0.9971
alpha-Terpineol	21.431	0.9999	0.9998	0.9981
gamma-Terpineol	21.431	0.9999	0.9998	0.9977
Nerol	21.729	0.9999	0.9999	0.9975
Geraniol	21.996	0.9999	0.9998	0.9972
Pulegone	22.147	0.9999	0.9998	0.9978
Geranyl Acetate	23.215	0.9999	0.9999	0.9985
Farnesene	23.935	0.9998	0.9989	0.9981
alpha-Cedrene	24.175	0.9999	0.9992	0.9987
E-Caryophyllene	24.227	0.9984	0.9990	0.9992
alpha-Humulene	24.721	0.9997	0.9996	0.9985
Valencene	25.106	0.9994	0.9995	0.9984
Z-Nerolidol	25.357	0.9999	0.9999	0.9991
E-Nerolidol	25.802	0.9999	0.9999	0.9991
Guaiol	27.097	0.9999	0.9999	0.9990
Carophyllene Oxide	27.460	0.9997	0.9998	0.9991
Cedrol	27.918	0.9999	1.0000	0.9992
alpha-Bisabolol	28.359	0.9999	0.9999	0.9990

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

	Accuracy (average %, n = 3)			Precision (% RSD, n = 3)		
Compound Name	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	108.1	95.9	105.3	1.5	2.4	2.0
Camphene	108.0	95.9	104.8	1.4	2.2	1.7
Sabinene	108.5	95.4	104.5	1.0	1.6	1.7
beta-Myrcene	109.6	96.7	104.9	1.5	3.7	0.4
beta-Pinene	109.3	96.2	104.3	1.2	0.4	1.9
alpha-Phellandrene	105.8	97.8	102.0	1.4	4.1	4.0
delta-3-Carene	108.0	95.7	104.6	1.3	2.3	1.9
alpha-Terpinene	118.6	91.5	106.4	3.0	0.6	1.6
E-beta-Ocimene	111.2	95.2	104.0	3.8	1.4	1.2
D-Limonene	109.8	95.2	104.8	1.4	2.0	1.4
Z-beta-Ocimene	109.0	95.2	104.1	2.7	1.7	1.1
Eucalyptol	107.2	97.0	102.8	2.1	3.1	1.2
gamma-Terpinene	108.7	95.6	104.3	2.1	1.9	1.4
Terpinolene	112.5	94.3	105.0	2.4	2.0	1.3
Sabinene Hydrate	106.6	96.8	103.1	5.1	3.3	2.9
Linalool	105.9	96.5	103.2	5.1	3.6	3.0
[±]-Fenchone	106.9	96.7	103.0	3.6	2.5	1.5
Endo-Fenchyl Alcohol	107.7	96.8	103.1	1.5	2.1	2.8
Isopulegol	108.9	95.1	103.0	5.3	2.5	3.2
[±/N]-Camphor	107.3	97.0	102.9	5.3	3.6	1.9
Isoborneol	107.3	97.1	104.1	7.9	4.3	2.9
Menthol	107.5	97.7	103.4	4.0	3.7	1.2
[±]-Borneol	108.9	95.5	104.0	6.6	4.0	3.4
alpha-Terpineol	108.2	96.3	103.2	5.1	4.1	2.7
gamma-Terpineol	107.9	95.6	103.5	6.1	4.0	2.9
Nerol	105.9	98.0	103.2	8.7	5.4	3.5
Geraniol	111.2	95.1	103.4	7.6	5.4	3.4
Pulegone	108.6	96.1	103.4	6.3	4.2	2.8
Geranyl Acetate	106.7	97.1	102.9	3.2	3.5	2.3
Farnesene	109.1	98.6	104.1	7.5	4.4	2.4
alpha-Cedrene	108.6	94.6	103.5	4.8	3.1	1.8
E-Caryophyllene	107.0	97.0	104.9	3.7	1.4	0.9
alpha-Humulene	109.2	95.8	103.7	3.8	2.4	1.7
Valencene	108.2	95.5	104.3	4.3	2.2	1.4
Z-Nerolidol	107.7	96.2	102.1	3.0	2.7	1.8
E-Nerolidol	106.3	96.6	102.1	3.5	2.5	1.8
Guaiol	108.0	96.3	102.1	3.7	2.0	2.0
Carophyllene Oxide	108.8	95.4	102.7	1.2	1.6	1.4
Cedrol	106.1	96.4	101.7	3.6	2.0	2.0
alpha-Bisabolol	106.7	96.3	102.5	4.4	2.3	1.7

Analysis of various cannabis samples

All limits of detection (LOD) and limits of quantitation (LOQ) fell below the lowest calibration point (3.83 µg/mL). Thus, the reporting limit for the current study was defined as any value greater than 3.83 µg/mL. The terpene concentrations found in the different cannabis samples are presented in Table 7.

Each sample shows a unique terpene profile. Concentration of detected terpenes in four replicates are presented for sample 5 (Figure 5) and sample 6 (Figure 6). The % RSD for detected terpenes ranged between 0.69 to 7.92% in sample 5 and 3.47 to 10.16% in sample 6.

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

Compound Name	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	N/A	4.20	N/A	N/A	N/A	N/A
D-Limonene	N/A	N/A	N/A	N/A	4.08 ±0.12	N/A
Eucalyptol	8.02	N/A	N/A	N/A	N/A	N/A
Linalool	19.26	4.45	9.78	4.23	10.16 ±0.44	N/A
Endo-Fenchyl Alcohol	7.46	N/A	11.26	N/A	8.22 ±0.06	N/A
[±]-Borneol	N/A	N/A	7.72	N/A	N/A	N/A
alpha-Terpineol	18.39	N/A	35.21	7.57	9.26 ±0.45	4.02 ±0.25
gamma-Terpineol	15.34	N/A	24.71	6.90	8.18 ±0.47	N/A
Farnesene	139.52	20.34	98.73	130.25	76.83 ±6.08	31.24 ±3.17
E-Caryophyllene	95.40	21.35	57.19	63.06	159.99 ±4.61	21.12 ±0.73
alpha-Humulene	38.52	7.87	20.82	17.61	40.69 ±1.41	6.33 ±0.50
E-Nerolidol	13.92	N/A	14.50	5.38	7.18 ±0.38	N/A
Guaiol	N/A	N/A	9.04	N/A	N/A	N/A
Carophyllene Oxide	26.59	20.78	50.75	44.05	20.97 ±1.08	8.47 ±0.40
alpha-Bisabolol	16.82	29.43	48.34	21.02	117.33 ±5.29	23.23 ±0.94

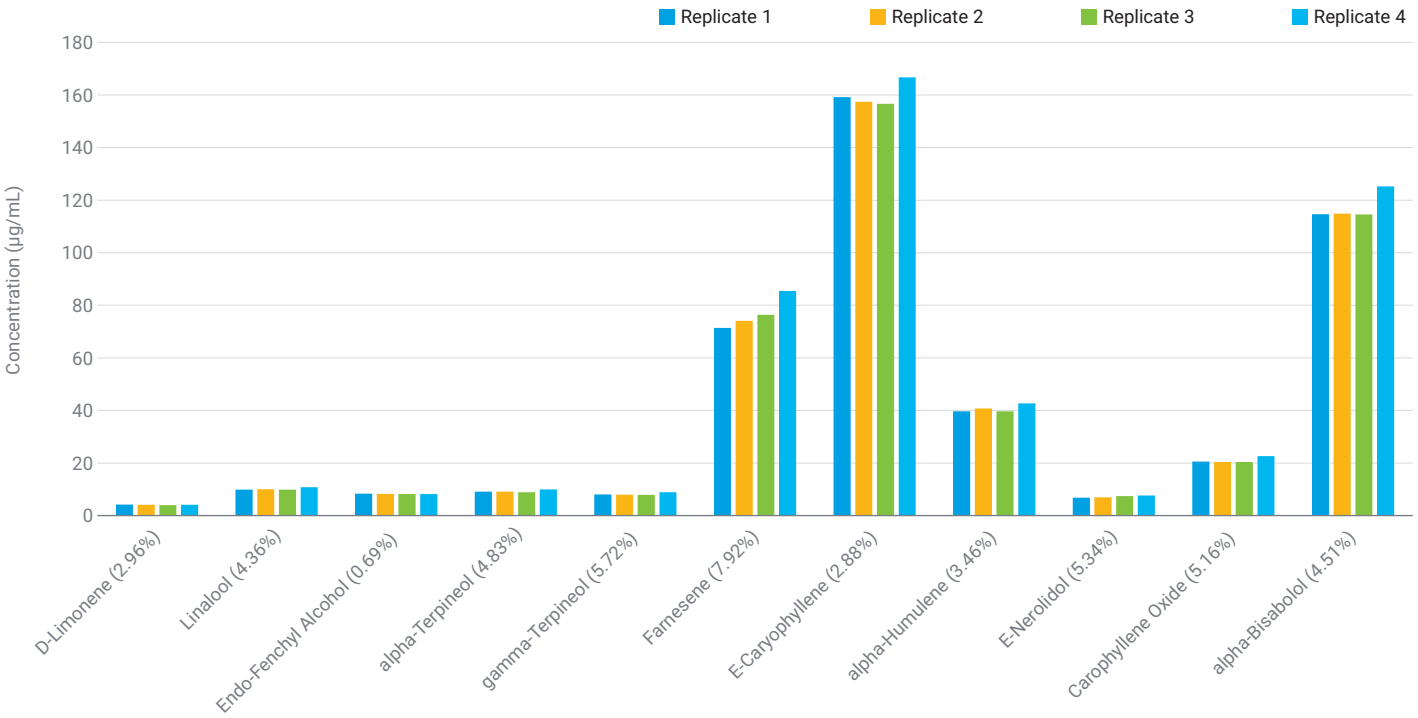

Figure 5. Terpenes concentration (µg/mL) in sample 5 (n = 4). The % RSD among replicates is shown in parenthesis.

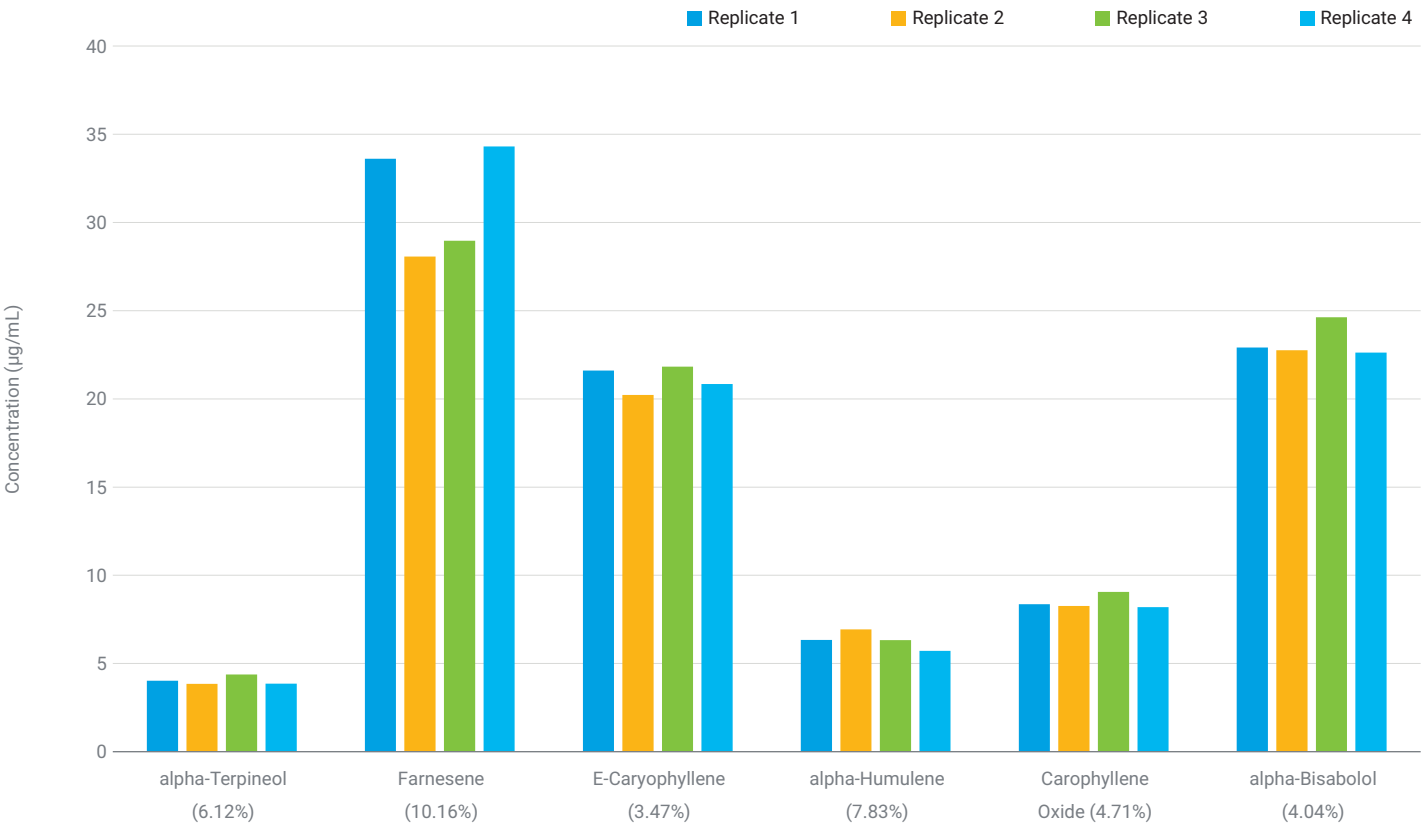

Figure 6. Terpenes concentration (µg/mL) in sample 6 (n = 4). The % RSD among replicates is shown in parenthesis.

Conclusion

The presented application demonstrates that the Agilent PAL3 Series II RTC sampler can accurately and precisely conduct the automated sample preparation for the analysis of terpenes in cannabis flower. The use of automated sample preparation helps reduce user errors and the amount of sample and solvent needed for analysis. Coupled with an Agilent Intuvo 9000/5977C GC/MS system, PAL3 Series II RTC automation can save time and reduce energy consumption, leading to a greener high-throughput laboratory.

Reference

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.

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