Development of a Testing Method for Terpenes in Cannabis Utilizing Headspace Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry

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Introduction

Terpenes are volatile compounds that provide the aromas and flavors that naturally exist within flora and fauna found globally. Research on terpenes has been on a rise as studies have indicated medical properties linked to these active chemical compounds. Primarily in cannabis products, certain states such as New Jersey have regulated terpenes to maximize the entourage effect while preventing adulteration. The New Jersey Department of Health (NJDOH) is developing a method to detect 32 terpenes in cannabis utilizing Headspace Solid Phase Microextraction Gas Chromatography Mass Spectrometer (HS-SPMEGC/MS) by using a published app note as reference[1]. We modified the method conditions including the sample introduction into GC/MS, vial and injection penetration as well as run time, and the addition of 15 terpenes. The optimized method reduces matrix interferences and improves analyte recoveries. The analytical sensitivity for the targeted analytes in the method has an limit of detection (LOD) between 5.00-15.1 ng/mL and a limit of quantitation (LOQ) below 49.0 ng/mL. The linear calibration curve has a range between 49.0-3125 ng/mL with an R^2 value ≥ 0.980. The optimized method will be validated by evaluating the accuracy, precision, analytical specificity, and complex interferences in cannabis. The validated method will be applied to measure terpene concentrations and sample stability in cannabis plant material and then be expanded for other matrices in the future.

Method

Fig 1. HS-SPME GC/MS Instrument

Fig 2. SPME Fiber 80/10 µm DVB/CAR-WR/PDMS

Fig 3. Agilent J&W DB-1ms, 60 m, 0.25 mm, 0.25 µm GC column

Sample Preparation

- Calibrators: Pipette 10 µL of a 5-ppm terpene mixture and 2000 µL of de-ionized water into a 10 mL headspace vial.
- Samples: Place 0.1 g of homogenized cannabis and 2000 µL of de-ionized water into a 10 mL headspace vial.

HS-SPME GC/MS Parameters

Headspace SPME	Value
SPME Fiber Phase	80/10 µm DVB/CAR-WR/PDMS
Incubation Temperature	40 °C
Incubation Time	5 min
Agitator Speed	300 rpm
Agitator ON/ OFF time	5s/ 2s
Vial Penetration App. Note	22 mm (40 mm)
Extraction Time	20 min
Injection Penetration App. Note	54 mm (40 mm)
Desorption Time	180s
Maestro Run Time App. Note	40.33 min (30 min)

Table 1. Parameters of Headspace Solid Phase Microextraction

Agilent GC	Value
Inlet Liner	Inert, splitless, straight 0.75 mm
Injection Mode/ Temp.	Splitless/ 270 °C
Oven Program	1) 60 °C (2-min hold) 2) 5 °C min to 140 °C (1-min hold) 3) 15 °C/min to 250 °C (4-min hold)
Equilibration Time	0.5 min
Control Mode	1 mL/min
Column	Agilent J&W DB-1ms, 60 m, 0.25 mm, 0.25 µm GC column
Septum Purge Flow Mode	3 mL/min
Purge flow to Split Vent	15 mL/min at 0.35 min

Table 2. Parameters of Gas Chromatography

Agilent MS	Value
Transfer Line	280 °C
Acquisition Mode	SCAN
Solvent Delay App. Note	0 min (8 min)
Gain Factor	1
MS Source Temperature	280 °C
MS Quad Temperature	150 °C

Table 3. Parameters of Single Quadrupole Mass Spectrometer

Results

Library Setup

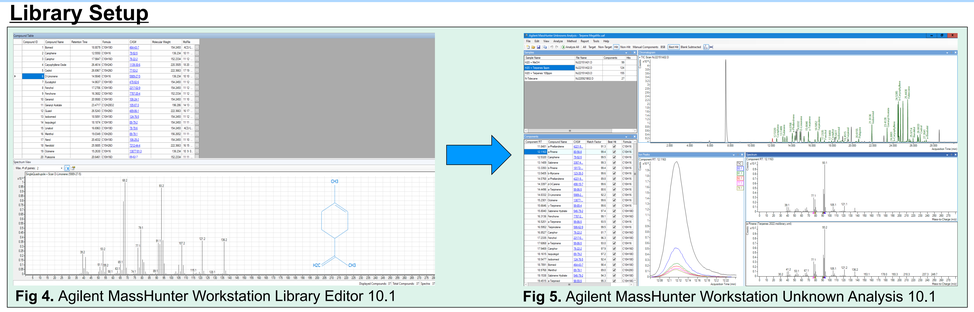

Fig 4. Agilent MassHunter Workstation Library Editor 10.1

Fig 5. Agilent MassHunter Workstation Unknown Analysis 10.1

- Library Editor: Created an in-house terpene mass spectral library
- Cayman Chemicals & Millipore Sigma standards were used
- Included in software:
  - ✓ Molecular Formula and Weight
  - ✓ Chemical Structure
  - ✓ Retention Time (RT)
  - ✓ CAS # and more.
- Unknown Analysis: Created a method to identify 32 terpenes at ng/mL levels
- Restek Terpene Megamix 1 & 2 were used
- Included in software:
  - ✓ Components similar to Library Editor (e.g., Molecular Formula & RT)
  - ✓ Match Factor
  - ✓ TIC Analysis
  - ✓ Best Hit and more.

Calibration Curve

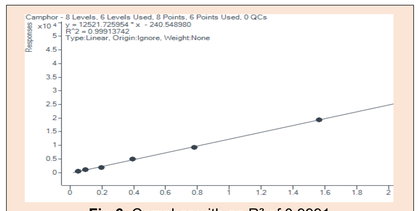

Fig 6. Camphor with an R^2 of 0.9991

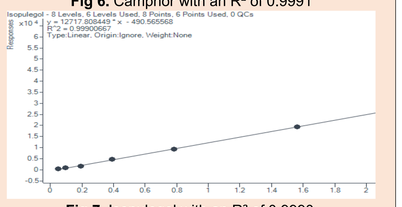

Fig 7. Isopulegol with an R^2 of 0.9990

Blank and Analyte Chromatograms

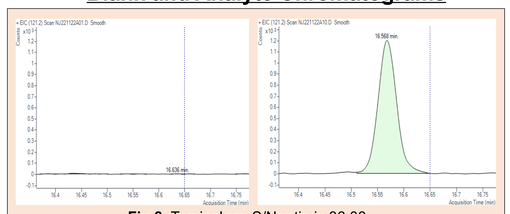

Fig 8. Terpinolene S/N ratio is 36.08

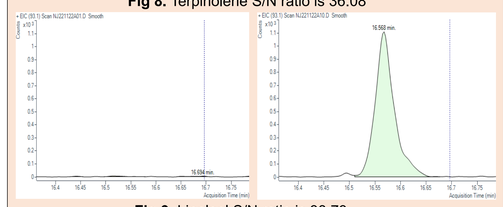

Fig 9. Linalool S/N ratio is 38.73

- The calibration curve shown above represents the linearity of all analytes exceeding the proposed coefficient of determination ≥ 0.980 within the ranges of 49.0 ng/mL to 3125 ng/mL.
- The chromatograms shown above have a baseline (left) and a peak symmetry (right) representative to all analytes for a confident signal detection.

Terpenes in Cannabis	Molecular Formula	RT min.	LOD ng/mL	LOQ ng/mL	R^2	Terpenes in Cannabis	Molecular Formula	RT min.	LOD ng/mL	LOQ ng/mL	R^2
α-Pinene	$C_{10}H_{16}$	12.1	11.6	34.7	0.981	Fenchol	$C_{10}H_{18}O$	17.2	15.1	45.2	0.999
Camphene	$C_{10}H_{16}$	12.5	11.0	33.1	0.983	Camphor	$C_{10}H_{16}O$	18.0	13.1	39.3	0.999
Sabinene	$C_{10}H_{16}$	13.2	12.4	37.2	0.990	Isopulegol	$C_{10}H_{18}O$	18.2	14.0	41.9	0.999
β-Pinene	$C_{10}H_{16}$	13.4	6.40	19.3	0.984	Isoborneol	$C_{10}H_{18}O$	18.6	9.90	29.7	0.998
β-Myrcene	$C_{10}H_{16}$	13.5	11.9	35.7	0.997	Borneol	$C_{10}H_{18}O$	18.8	10.8	32.5	0.996
α-Phellandrene	$C_{10}H_{16}$	14.1	13.4	40.2	0.991	α-Terpineol	$C_{10}H_{18}O$	19.5	13.9	41.8	0.996
Δ 3-Carene	$C_{10}H_{16}$	14.4	8.10	24.4	0.981	Pulegone	$C_{10}H_{16}O$	20.6	7.00	21.0	0.996
α-Terpinene	$C_{10}H_{16}$	14.5	14.1	42.3	0.995	Geranyl acetate	$C_{12}H_{20}O_2$	23.5	10.3	30.8	0.994
Eucalyptol	$C_{10}H_{18}O$	14.8	13.7	41.0	0.999	α-Cedrene	$C_{15}H_{24}$	24.5	5.00	15.0	0.981
d-Limonene	$C_{10}H_{16}$	14.9	8.70	26.2	0.984	β-Caryophyllene	$C_{15}H_{24}$	24.6	13.8	41.5	0.998
Ocimene	$C_{10}H_{16}$	15.2	12.4	37.1	0.994	α-Humulene	$C_{15}H_{24}$	25.0	14.4	43.3	0.997
γ-Terpinene	$C_{10}H_{16}$	15.7	11.1	33.4	0.991	Valencene	$C_{15}H_{24}$	25.5	14.2	42.7	0.995
Sabinene hydrate	$C_{10}H_{18}O$	15.8	14.9	44.8	1.000	Nerolidol	$C_{15}H_{26}O$	26.0	14.2	42.5	0.996
Fenchone	$C_{10}H_{16}O$	16.3	13.8	41.4	0.999	Caryophyllene oxide	$C_{15}H_{24}O$	26.5	10.4	31.2	0.999
Terpinolene	$C_{10}H_{16}$	16.6	11.0	33.0	0.992	Guaiol	$C_{15}H_{26}O$	26.5	14.4	43.2	0.999
Linalool	$C_{10}H_{18}O$	16.6	5.00	14.9	0.982	α-Bisabolol	$C_{15}H_{26}O$	27.3	9.60	28.9	0.985

Table 4. Experimental data of retention time, coefficient of determination & the limits of detection/quantitation values all below the lowest calibrator.

Conclusions

- An HS-SPME GC/MS method was setup to detect 32 terpenes in cannabis based on Agilent's Application note (5994-1411EN).
- The quantitative method was modified by optimizing:
  - ✓ HS-SPME parameters used to efficiently adsorb analytes
  - ✓ GC conditions by using a splitless injection mode
  - ✓ MS parameters by using a SCAN acquisition mode
- An in-house spectral library was created shown in **figure 4** and **5**.
- Reportable range was achieved with an $R^2 \geq 0.980$ for all analytes, examples shown in **figure 6** and **7**.
- Separation of the analytes were achieved as represented in **figure 8** and **9**.
- Excellent analytical sensitivities were achieved as represented in **table 4**.
  - ✓ LOD/LOQ values all below the lowest calibrator of 0.49 ng/mL

Future Plans

- Incorporate five additional terpenes (Menthol, Nerol, Geraniol, Cedrol, and β-Eudesmol) into method and library.
- Continue to improve the method sensitivity and selectivity by optimizing:
  1. HS-SPME GC/MS parameters
  2. Sample preparation procedures
- Validate the method by following ISO/IEC 17025
  - ✓ ISO/IEC 17025 accreditation: General requirements for the competence of testing and calibration laboratories (ISO®. 2017)[2].
- Expand the method to other matrices under cannabis (e.g., concentrates and edibles).

References

1. Westland, Jessica. "SPME-GC/MS of Selected Terpenes Using Agilent DVB/CAR-WR/PDMS SPME Fiber." Agilent Technologies, Inc., 8 Oct. 2019, https://www.agilent.com/cs/library/applications/application-selected-terpenes-by-spme%20fiber-dvbcar-wrpdms 5994-1411en-agilent.pdf.
2. "ISO - International Organization for Standardization." ISO/IEC 17025, ISO, Oct. 2017, https://www.iso.org/files/live/sites/isoorg/files/store/en/PUB100424.pdf

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