Application Note

Cannabis & Hemp Testing

Agilent

Trusted Answers

Terpenes Analysis in Cannabis Products by Liquid Injection using the Agilent Intuvo 9000/5977B GC/MS System

Authors

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Abstract

Terpenes are volatile and semivolatile chemicals that engender flavor and aroma organoleptic properties to cannabis and cannabinoid products. Cannabis growers and producers use terpene profiles to characterize specific strains of cannabis and hemp. To this end, a robust analytical method is necessary to chemically profile terpenes in cannabis and cannabinoid products prior to use in medicinal and recreational marijuana programs. Although regulatory agencies such as the California Bureau of Cannabis Control (BCC) do not regulate terpene content unless there is a specific label claim, terpenes are commonly analyzed in regulatory laboratories.[1] The most common approach to terpenes analyses in these laboratories is headspace gas chromatography (GC) with flame ionization detection (FID), mass spectrometry (MS), or both (FID/MS). Over the past several years, issues such as losses of sesquiterpenoids like α-bisabolol have been observed in high-potency cannabis samples with headspace methodologies. This has led to a need for liquid injection terpenes analysis. In this application note, we demonstrated a selective, sensitive, and robust method for the analysis of 40 chromatographically resolved terpenes common to *Cannabis* spp. using liquid injection GC/MS.

Introduction

Terpenes in cannabis

Terpenes are n-mers of isoprene (C_5H_8). Approximately 35,000 terpenes have been identified, but the biological functions of most have not been determined.[2] Common terpenes found in *Cannabis* spp. include monoterpenes and sesquiterpenes. Monoterpenes have the general empirical formula $C_{10}H_{16}$ (e.g., limonene), and the general empirical formula for sesquiterpenes is $C_{15}H_{24}$ (e.g., farnesene). Terpenoids and sesquiterpenoids are functionalized terpenes that contain other elements such as oxygen (e.g., camphor).
The chemical profile of terpenes is a variable phenotypic trait across *Cannabis* spp. Common terpenes include β-caryophyllene, α-pinene, β-myrcene, α-humulene, (+)-limonene, linalool, α-bisabolol, and (E)-β-farnesene.[3] This application note, along with additional information, and ready-to-run acquisition and quantitation methods, are available as eMethod G5282AA#010, Terpenes analysis in cannabis products using liquid injection with the Intuvo/5977 GC/MS system.

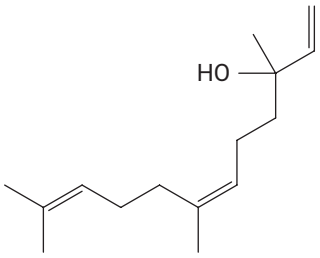
Terpene biosynthesis

Terpenes are primarily synthesized in the trichomes of *Cannabis* spp. inflorescence where acid phytocannabinoids are also synthesized; the latter are sometimes referred to as terpenophenols.[4] The plastidial methylerythritol phosphate (MEP) and the cytosolic mevalonate (MEV) pathways synthesize dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). Geranyl diphosphate synthase (GPPS) and farnesyl diphosphate synthase (FPPS) combine one molecule of IPP and one or two molecules of DMAPP to synthesize the 10-carbon monoterpene precursor geranyl diphosphate (GPP) and the 15-carbon sesquiterpene precursor farnesyl diphosphate (FPP), respectively.[5]

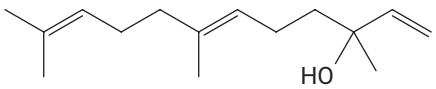
In two separate polyketide pathways, GPP is the substrate for geranylpyrophosphate:olivetolate geranyltransferase that synthesizes cannabigerovarinic acid (CBGVA) and cannabigerolic acid (CBGA). In these pathways, CBGVA and CBGA are the precursors for six acid phytocannabinoids through the action of three acid phytocannabinoid synthases.[6-8]

Stereochemistry of terpenes

Terpenes exist in nature in diverse configurations that give rise to stereoisomers and chemical properties such as optical rotation. Examples of configurational isomerism are the sesquiterpenoids Z-nerolidol and E-nerolidol, shown in Figure 1. Compounds such as these, which are not mirror images of one another, are known as diastereomers. Z and E diastereomers tend to have different chemical properties that allow them to be chromatographically separated.



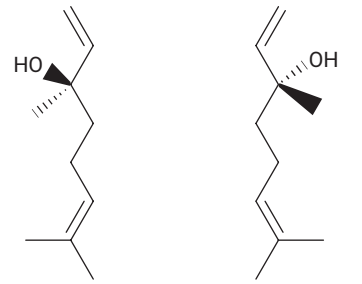
Z-Nerolidol



E-Nerolidol

Figure 1. Z-nerolidol and E-nerolidol. Z (zussammen) means together or on the same side. E (entgegen) means opposite sides. The nomenclature cis and trans also refers to the same or opposite sides, respectively of a molecule but are generally ambiguous identifiers for alkenes.

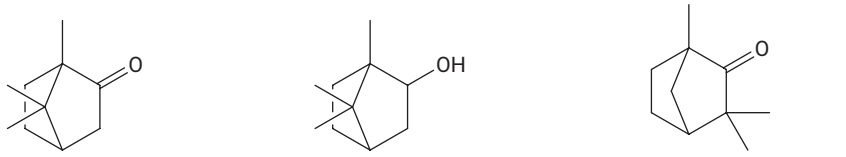
Many terpenes exist as enantiomers: nonsuperimposable, mirror images of one another. These terpenes are comprised of the same atoms with the same connectivity but differ in three-dimensional configuration. Enantiomers have identical physical properties such as chromatographic retention time on a non-chiral column, acidity, and melting point, and are optically active i.e., rotate plane polarized light in either a clockwise or counterclockwise direction.[9] Clockwise rotation is designated (+) and counterclockwise as (−). An obsolete but still used system of optical rotation nomenclature is dextrorotatory (d) and levorotatory (l), but this usage is discouraged by the International Union of Pure and Applied Chemistry (IUPAC).[10] The stereodescriptors d and l are in lower case, but sometimes capital D and L are used, leading to much confusion. The D and L designation arose from the Fischer-Rosanoff convention where (+)-glyceraldehyde was arbitrarily described as D-glyceraldehyde and its enantiomer as L-glyceraldehyde (Moss, 1996). In this case, the D and L usage was referring to absolute configuration of the molecules. The use of D and L is discouraged by IUPAC in favor of R and S stereodescriptors to define absolute configuration. An example of R and S enantiomers are given in Figure 2 for linalool.



(S)-(+)-Linalool (R)-(−)-Linalool

Figure 2. Linalool enantiomers.

Commercially available enantiomeric reference standards may be obtained as a pure enantiomer (+) or (–), or as a racemic mixture (±) of both enantiomers. In this latter case, if the enantiomers or not present in a 50/50 ratio, the enantiomeric excess (EE) of the higher concentration enantiomer should be reported if known. Examples of racemic terpenes are given in Figure 3.



(±)-Camphor
Chemical formula: $C_{10}H_{16}O$
Molecular weight: 152.2370

(±)-Borneol
Chemical formula: $C_{10}H_{18}O$
Molecular weight: 154.2530

(±)-Fenchone
Chemical formula: $C_{10}H_{16}O$
Molecular weight: 152.2370

Figure 3. Racemic mixtures of terpenes.

Materials and methods

Hardware and software

An Agilent Intuvo 9000 gas chromatograph (G3950A) configured with a mid-column backflush Flow-Chip (option 881), a multimode inlet (MMI) and Guard Chip (G4587-60665) was used. Note that a split/splitless inlet (S/SL) can be used as an alternative (the Guard Chip part number for the S/SL is G4587-60565). The Agilent 7650A 50-position automatic liquid sampler (ALS) configured with a 10.0 µL syringe was installed. (G4567A). Optionally, the Agilent XLSI weldment may be used for side mount of the Agilent 7697A headspace autosampler transfer line (G3969A) if the headspace system is attached. A 4 mm Ultra Inert, low pressure drop, glass wool split liner (5190-2295) and two DB-Select 624 Ultra Inert columns (30 m × 0.25 mm id, 1.4 µm film thickness, 122-0334UI-INT) were used for all analyses. The GC system was connected to an Agilent 5977B mass selective detector (MSD) with EI Extractor source (G7077BA EI InertPlus Turbo with 9 mm extractor lens). Data were collected using Agilent MassHunter B.10 GC/MS Acquisition software. All data analyses were performed using MassHunter Quantitative Software B.10.1. Tables 1 to 4 provide the GC/MS parameters.

Table 1. Agilent 7650A autosampler.

Parameter	Value
Syringe Size	10 µL
Injection Volume	1.0 µL
Air Gap	0.2 µL
Solvent A Washes (Ethyl Acetate)	3 times post injection with 3.0 µL
Solvent B Washes (Ethyl Acetate)	3 times post injection with 3.0 µL
Sample Washes	3 times with 3.0 uL

Table 2. Agilent Intuvo 9000 GC.

Parameter	Value
Flow Rate Column 1	2.0 mL/min
Flow Rate Column 2	2.2 mL/min
Inlet Temperature	250 °C
Inlet Mode	Split
Split Ratio	150:1
Gas Saver	On after 3 minutes
Initial Oven Temperature	75 °C
Initial Hold Time	1 minute
Ramp Rate 1	5 °C/min
Final Temperature	165 °C
Hold Time	0 minutes
Ramp Rate 2	175 °C/min
Final Temperature 2	250 °C
Final Hold	10.514 minutes
Total Run Time	30.0 minutes
Post Run Backflush	3.236 minutes

Table 3. Agilent 5977B MS.

Parameter	Value
Solvent Delay	14 minutes
Acquisition Mode	SIM
EM Setting mode Gain	Variable per SIM segment
Source Temperature	300 °C
Quadrupole Temperature	200 °C
Trace Ion Detection	On
MS Tuning	AUTOTUNE [ATUNE.U]
Number of SIM Groups	20
Run Time	30 minutes

Table 4. MS SIM parameters.

SIM Group	(Mass, Dwell)
Group 1	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 14	
Number of Ions: 3	
(Mass, Dwell) In Group	(77.00,75) (91.00,75) (93.00,75)
Group 2	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 15.25	
Number of Ions: 3	
(Mass, Dwell) In Group	(93.00,75) (107.00,75) (136.00,75)
Group 3	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 16	
Number of Ions: 6	
(Mass, Dwell) In Group	(69.00,37) (77.00,37) (91.00,37) (93.00,37) (121.00,37) (136.00,37)
Group 4	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 17	
Number of Ions: 12	
(Mass, Dwell) In Group	(68.00,18) (77.00,18) (79.00,18) (81.00,18) (91.00,18) (93.00,18) (105.00,18) (111.00,18) (121.00,18) (136.00,18) (139.00,18) (154.00,18)
Group 5	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 18.95	
Number of Ions: 4	
(Mass, Dwell) In Group	(77.00,75) (91.00,75) (93.00,75) (136.00,75)
Group 6	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 19.5	
Number of Ions: 10	
(Mass, Dwell) In Group	(55.00,22) (69.00,22) (71.00,22) (81.00,22) (93.00,22) (121.00,22) (136.00,22) (139.00,22) (152.00,22) (154.00,22)

SIM Group	(Mass, Dwell)
Group 7	
Resolution: HIGH	
Gain Factor: 15	
Group Start Time: 20.8	
Number of Ions: 6	
(Mass, Dwell) In Group	(80.00,37) (81.00,37) (111.00,37) (121.00,37) (136.00,37) (154.00,37)
Group 8	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 21.45	
Number of Ions: 12	
(Mass, Dwell) In Group	(59.00,18) (71.00,18) (81.00,18) (93.00,18) (95.00,18) (108.00,18) (110.00,18) (121.00,18) (123.00,18) (136.00,18) (138.00,18) (152.00,18)
Group 9	
Resolution: HIGH	
Gain Factor: 15	
Group Start Time: 22.1	
Number of Ions: 7	
(Mass, Dwell) In Group	(69.00,32) (81.00,32) (84.00,32) (93.00,32) (109.00,32) (123.00,32) (152.00,32)
Group 10	
Resolution: HIGH	
Gain Factor: 15	
Group Start Time: 23.5	
Number of Ions: 4	
(Mass, Dwell) In Group	(68.00,50) (69.00,50) (121.00,50) (136.00,50)
ISTD	
Resolution: HIGH	
Gain Factor: 10	
Group Start Time: 24.1	
Number of Ions: 3	
(Mass, Dwell) In Group	(170.00,75) (171.00,75) (172.00,75)
Group 11	
Resolution: HIGH	
Gain Factor: 15	
Group Start Time: 24.45	
Number of Ions : 6	
(Mass, Dwell) In Group	(69.00,37) (79.00,37) (93.00,37) (105.00,37) (119.00,37) (161.00,37)

SIM Group	(Mass, Dwell)
Group 12	
Resolution: HIGH	
Gain Factor: 15	
Group Start Time: 25.2	
Number of Ions: 3	
(Mass, Dwell) In Group	(80.00,75) (93.00,75) (121.00,75)
Group 13	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 25.7	
Number of Ions: 3	
(Mass, Dwell) In Group	(161.00,75) (189.00,75) (204.00,75)
Group 14	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 26	
Number of Ions: 3	
(Mass, Dwell) In Group	(69.00,75) (81.00,75) (121.00,75)
Group 15	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 26.4	
Number of Ions: 3	
(Mass, Dwell) In Group	(69.00,75) (81.00,75) (121.00,75)
Group 16	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 27.5	
Number of Ions: 3	
(Mass, Dwell) In Group	(91.00,75) (107.00,75) (161.00,75)
Group 17	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 28.4	
Number of Ions: 3	
(Mass, Dwell) In Group	(79.00,75) (91.00,75) (109.00,75)
Group 18	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time : 28.85	
Number of Ions: 3	
(Mass, Dwell) In Group	(95.00,75) (150.00,75) (151.00,75)

SIM Group	(Mass, Dwell)
Group 19	
Resolution: HIGH	
Gain Factor: 20	
Group Start Time: 29.4	
Number of Ions : 3	
(Mass, Dwell) In Group	(93.00,75) (109.00,75) (119.00,75)

Chemicals

- Ethyl acetate (99.9%)
- 2-Fluorobiphenyl
- Terpene standards (SPEX CertiPrep)
  - CAN-TERP-MIX1H
  - CAN-TERP-MIX2H

Table 5 lists the terpenes in the standards used in this work.

Data collection

A total of three independent datasets were collected over multiple days. Each dataset was comprised of quintuplicate injections of solvent blanks, matrix blanks, and eight levels of calibrators ranging from approximately 4 µg/mL through approximately 485 µg/mL. Each sample, calibrator, etc. contained 2-fluorobiphenyl as an internal standard (ISTD). A separate MDL study was performed, which entailed collecting two individual datasets of eight replicate injections at 50% of the low calibrator concentrations for each compound.

Statistics

1. Average = $\Sigma x_i/n$
2. Standard deviation, (SD) = $\left[\frac{\Sigma(x - \bar{x})^2}{n - 1}\right]^{1/2}$
3. Limit of detection (LOD) defined as MDL = (s) × (Student t-value, n – 1, 99% Confidence)
4. Limit of quantitation (LOQ) = 10 × (SD)
5. Average percent accuracy = (average calculated final concentration (n =5)/ spiked concentration) × 100
6. Precision, (%RSD) = [(SD)/Average] × 100

Table 5. Target terpenes.

CAS Number	Preferred Nomenclature	Alternate Nomenclature	Chemical Class
80-56-8	α-Pinene	α-Pinene	Monoterpene
79-92-5	Camphene	Camphene	Monoterpene
3387-41-5	Sabinene	Sabinene	Monoterpene
127-91-3	β-Pinene	β-Pinene	Monoterpene
123-35-3	β-Myrcene	β-Myrcene	Monoterpene
99-83-2	α-Phellandrene	α-Phellandrene	Monoterpene
498-15-7	(+)-δ-3-Carene	(1S)-(+)-3-Carene	Monoterpene
99-86-5	α-Terpinene	*p*-Mentha-1,3-diene	Monoterpene
3779-61-1	E-β-Ocimene	3,7-Dimethyl-1,3,6-octatriene	Monoterpene
5989-27-5	(+)-Limonene	(R)-p-mentha-1,8-diene	Monoterpene
3338-55-4	Z-β-Ocimene	3,7-Dimethyl-1,3,6-octatriene	Monoterpene
470-82-6	Eucalyptol	Eucalyptol	Monoterpenoid
99-85-4	γ-Terpenene	*p*-Mentha-1,4-diene	Monoterpene
586-62-9	Terpinolene	δ-Terpenine	Monoterpene
546-79-2	Sabinene Acetate	4-Thujanol	Monoterpenoid
78-70-6	Linalool	Linalool	Monoterpenoid
4695-62-9 and 7787-20-4	(±)-Fenchone	dl-Fenchone	Monoterpenoid
2217-02-09	(+)-Endo-Fenchyl Alcohol	(1R)-Endo-(+)-Fenchyl Alcohol	Monoterpenoid
89-79-2	Isopulegol	(–)-Isopulegol	Monoterpenoid
464-49-3	(±)-Camphor	dl-Camphor	Monoterpenoid
124-76-5	Isoborneol	Isoborneol	Monoterpenoid
89-78-1	Menthol	Menthol	Monoterpenoid
464-45-9 and 464-43-7	(±)-Borneol	(1S,2R,4S)-(–)-Borneol and (1R,2S,4R)-(+)-Borneol	Monoterpenoid
8000-41-7	α-Terpineol	Terpineol mix of α and γ	Monoterpene
8000-41-7	γ-Terpineol	Terpineol mix of α and γ	Monoterpene
106-25-2	Nerol	Nerol	Monoterpenoid
106-24-1	Geraniol	Geraniol	Monoterpenoid
89-82-7	Pulegone	Pulegone	Monoterpenoid
105-87-3	Geranyl Acetate	Geranyl Acetate	Monoterpenoid
502-61-4	Farnesene	Farnesene	Sesquiterpene
469-61-4	α-Cedrene	*alpha*-Cedrene	Sesquiterpene
87-44-5	E-Caryophyllene	*trans*-Carophyllene	Sesquiterpene
6753-98-6	α-Humulene	α-Humulene	Sesquiterpene
4630-07-03	Valencene	Valencene	Sesquiterpene
3790-78-1	Z-Nerolidol	*cis*-Nerolidol	Sesquiterpenoid
40716-66-3	E-Nerolidol	*trans*-Nerolidol	Sesquiterpenoid
489-86-1	(–)-Guaiol	(–)-Guaiol	Sesquiterpenoid
1139-30-6	Carophyllene Oxide	Caryophyllene oxide	Sesquiterpenoid
77-53-2	Cedrol	Cedrol	Sesquiterpenoid
23089-26-1	(-)-α-Bisabolol	(-)-Alpha-Bisabolol	Sesquiterpenoid

Calibrator, ISTD, and sample preparation

ISTD Preparation 1: 2-fluorobiphenyl in ethyl acetate at 10,000 µg/mL. Accurately weigh 0.100 g of the neat 2-fluorobiphenyl solid into a 10 mL volumetric flask. Dilute to volume with ethyl acetate, mix thoroughly, and store at room temperature. Ten microliters per 0.5 mL will be used in preparation of each calibration standard level.

ISTD Preparation 2: 2-fluorobiphenyl in ethyl acetate at 200 µg/mL. Accurately weigh 0.200 g of the neat 2-fluorobiphenyl solid into a 1.0 L volumetric flask. Dilute to volume with ethyl acetate, mix thoroughly, and store at room temperature. This will be used for dilution in the sample preparation steps.

Preparation of terpene calibration standards

Carefully weigh 0.005 g of blank matrix (we used food grade hempseed oil readily available online) into a liquid scintillation vial and accurately record the weight. Add 0.5 mL of calibration standard mix of terpenes at 500 µg/mL each and 10 µL of ISTD Preparation 1. Several of the terpenes were present in both standard mixtures we purchased. In these cases, the high-level concentration was 1,000 µg/mL, and in one case (camphor), 1,500 µg/mL. Repeat the addition of ISTD Preparation 1 in each of the seven 1 + 1 serial dilutions to create the eight-level calibration curve. Note that 0.005 g of blank matrix in 0.5 mL = 0.010 g/mL matrix, which matches the amount in the samples.

Sample preparation

Carefully weigh 0.500 g of sample into a liquid scintillation vial and accurately record the weight. Add 10 mL of ethyl acetate + ISTD Preparation 2. Cap and vortex until dissolved. Transfer the liquid to a 50 mL volumetric flask. Repeat the procedure twice with 10 mL aliquots of ethyl acetate + ISTD Preparation 2 and transfer to the same 50 mL volumetric flask. After the three aliquots are transferred to the 50 mL volumetric flask, bring to volume with ethyl acetate + ISTD Preparation 2. The prepared solution nominally represents 0.5 g of sample in 50 mL final volume = 0.010 g/mL. For solutions of resin with terpene levels >5.0%, perform an additional 10-fold dilution with ethyl acetate + ISTD solution. Table 6 defines the calibrator levels in ppm, % (wt/wt), and µg/mL.

Agilent consumables

- 1.8 mL glass autosampler vials (part number 5188-6535) with screw caps (part number 5182-0724)

Ancillary equipment

- 1, 4, and 8 mL disposable glass vials with caps and septa
- Disposable 20 mL liquid scintillation vials or equivalent
- 4 mL amber glass vials with screw caps for storing standards
- 10, 25, 50, 100, and 1,000 mL class "A" volumetric flasks
- 10, 50, 100, 500, and 1,000 mL glass beakers
- Gas-tight syringes in the following sizes:
  - 10 µL
  - 25 µL
  - 100 µL
  - 250 µL
  - 1,000 µL
- Manual pump pipettor
- Re-pipettor capable of dispensing 1 to 10 mL
- Analytical balance capable of weighing to 0.001 g
- Laboratory freezer for storing chemical standards

Table 6. Calibration table.

Preferred Nomenclature	Standard Conc. (µg/mL)	Mix Conc. (µg/mL)	Volume Taken (mL)	Amt. (µg)	Sample wt. (g)	Cal_08		Cal_07		Cal_06		Cal_05		Cal_04		Cal_03		Cal_02		Cal_01	
						% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL	% (wt./wt.)	µg/mL
α-Pinene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Camphene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Sabinene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
β-Pinene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
β-Myrcene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
α-Phellandrene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(+)-δ-3-Carene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
α-Terpinene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
E-β-Ocimene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(+)-Limonene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Z-β-Ocimene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Eucalyptol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
γ-Terpenene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Terpinolene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Sabinene Acetate	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Linalool	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(±)-Fenchone	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(+)-Endo-Fenchyl Alcohol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Isopulegol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(±)-Camphor	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Isoborneol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Menthol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(±)-Borneol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
α-Terpineol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
γ-Terpineol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Nerol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Geraniol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Pulegone	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Geranyl Acetate	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Farnesene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
α-Cedrene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
E-Caryophyllene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
α-Humulene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Valencene	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Z-Nerolidol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
E-Nerolidol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(−)-Guaiol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Carophyllene Oxide	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
Cedrol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81
(−)-α-Bisabolol	1000	487.3	1.0	487.3	0.01	4.87	487.30	2.44	243.65	1.22	121.83	0.61	60.91	0.30	30.46	0.15	15.23	0.076	7.61	0.038	3.81

Results and discussion

LOD and LOQ determinations

Two independent datasets were collected using eight replicate injections at 50% of the lowest calibrator concentration to determine LOD (defined as MDL) and LOQ for each target terpene. The intraday and interday LODs were calculated statistically with a Student t-statistic of 2.998 for n – 1 degrees of freedom at the 99% confidence level. LOQ for each analyte were determined statistically from this dataset using (10 × standard deviation) for both the intraday and interday data. Intraday and interday precision were also determined in this dataset as %RSD. Table 7 illustrates these results.

Accuracy and precision

Three independent batches of eight calibrator levels were prepared in hempseed oil matrix. Each batch was collected over the course of 5 days and designated P1, P2, and P3, respectively. Each calibrator level in each batch was injected five times. The intraday and interday accuracy was determined. Percent accuracy acceptability criteria was defined as an average percent accuracy greater than 80% and less than 120%. The intraday batch precision was determined as %RSD. These data are shown in Table 8.

Regression statistics

From the three datasets, the range, regression curve type (linear or quadratic), weighting, and regression statistics of the compounds were determined. These are shown in Table 9. In all cases, eight calibrator levels and 1/x weighting were used. The coefficients of determination (R^2) were >0.99 for all target terpenes. Figure 4 is a typical chromatogram for the high calibrator, and Figure 5 shows calibration curves for four terpenes that eluted over the chromatographic range. In all calibration determinations, five replicates of eight levels (n = 40) were used.

Table 7. LOD as defined by MDL, LOQ, and precision as defined by %RSD.

Compound Information				Intraday (Day 1, n = 8)			Intraday (Day 2, n = 8)			Interday (n = 16)		
Name	RT (min)*	ΔRT (min)	SIM Quant Ion	MDL % (wt/wt)	LOQ % (wt/wt)	Conc. %RSD	MDL % (wt/wt)	LOQ % (wt/wt)	Conc. %RSD	MDL % (wt/wt)	LOQ % (wt/wt)	Average Conc. %RSD
α-Pinene	14.893		93	0.0005	0.0015	1.0	0.0004	0.0015	0.9	0.0005	0.0015	1.0
Camphene	15.662	0.769	93	0.0006	0.0018	1.1	0.0012	0.0039	2.3	0.0009	0.0029	1.7
Sabinene	16.394	0.732	136	0.0020	0.0068	4.2	0.0024	0.0079	4.9	0.0022	0.0074	4.6
β-Pinene	16.535	0.141	93	0.0005	0.0017	16.3	0.0004	0.0013	15.1	0.0005	0.0015	15.7
β-Myrcene	16.659	0.124	121	0.0026	0.0087	15.4	0.0027	0.0089	16.3	0.0027	0.0088	15.9
α-Phellandrene	17.443	0.784	93	0.0013	0.0044	2.3	0.0121	0.0402	23.1	0.0067	0.0223	12.7
(+)-δ-3-Carene	17.532	0.089	93	0.0014	0.0046	6.8	0.0010	0.0034	5.1	0.0012	0.0040	6.0
α-Terpinene	17.858	0.326	121	0.0007	0.0023	0.9	0.0010	0.0034	1.3	0.0009	0.0029	1.1
E-β-Ocimene	18.049	0.191	93	0.0091	0.0305	22.2	0.0067	0.0222	17.0	0.0079	0.0264	19.6
(+)-Limonene	18.226	0.177	68	0.0022	0.0073	11.3	0.0013	0.0044	6.6	0.0018	0.0059	9.0
Z-β-Ocimene	18.561	0.335	93	0.0021	0.0071	5.3	0.0010	0.0032	2.5	0.0016	0.0052	3.9
Eucalyptol	18.721	0.160	81	0.0011	0.0037	2.3	0.0017	0.0056	3.5	0.0014	0.0047	2.9
γ-Terpinene	19.145	0.424	93	0.0006	0.0020	1.2	0.0006	0.0019	1.2	0.0006	0.0020	1.2
Terpinolene	19.782	0.637	121	0.0016	0.0055	6.2	0.0019	0.0062	7.1	0.0018	0.0059	6.7
Sabinene Acetate	20.045	0.263	71	0.0016	0.0054	2.7	0.0012	0.0039	2.0	0.0014	0.0047	2.4
Linalool	20.327	0.282	71	0.0012	0.0040	2.4	0.0010	0.0034	2.1	0.0011	0.0037	2.3
(±)-Fenchone	20.515	0.188	81	0.0015	0.0049	1.7	0.0017	0.0055	1.9	0.0016	0.0052	1.8
(+)-Endo-Fenchyl Alcohol	21.007	0.492	81	0.0028	0.0094	6.4	0.0032	0.0105	7.2	0.0030	0.0100	6.8
Isopulegol	21.303	0.296	121	0.0046	0.0152	8.9	0.0048	0.0161	9.8	0.0047	0.0157	9.4
(±)-Camphor	21.564	0.261	152	0.0023	0.0076	1.8	0.0015	0.0051	1.2	0.0019	0.0064	1.5
Isoborneol	21.661	0.097	95	0.0079	0.0262	14.7	0.0076	0.0253	14.6	0.0078	0.0258	14.7
Menthol	21.674	0.013	71	0.0021	0.0070	4.2	0.0010	0.0035	2.1	0.0016	0.0053	3.2
(±)-Borneol	21.81	0.136	95	0.0022	0.0072	1.7	0.0021	0.0070	1.7	0.0022	0.0071	1.7
α-Terpineol	21.903	0.093	93	0.0022	0.0074	3.4	0.0016	0.0054	2.5	0.0019	0.0064	3.0
γ-Terpineol	21.989	0.086	59	0.0032	0.0107	4.6	0.0027	0.0092	4.0	0.0030	0.0100	4.3
Nerol	22.214	0.225	69	0.0023	0.0076	4.5	0.0027	0.0090	5.3	0.0025	0.0083	4.9
Geraniol	22.489	0.275	69	0.0026	0.0087	4.7	0.0028	0.0093	5.1	0.0027	0.0090	4.9
Pulegone	22.657	0.168	152	0.0023	0.0075	2.7	0.0011	0.0036	1.3	0.0017	0.0056	2.0
Geranyl Acetate	23.768	1.111	68	0.0012	0.0041	2.5	0.0041	0.0137	8.7	0.0027	0.0089	5.6
Farnesene	24.56	0.792	69	0.0042	0.0141	11.9	0.0065	0.0218	15.0	0.0054	0.0180	13.5
α-Cedrene	24.851	0.291	93	0.0028	0.0094	6.4	0.0009	0.0029	2.0	0.0019	0.0062	4.2
E-Caryophyllene	24.915	0.064	93	0.0034	0.0112	6.2	0.0020	0.0067	3.7	0.0027	0.0090	5.0
α-Humulene	25.453	0.538	93	0.0009	0.0030	1.8	0.0013	0.0043	2.6	0.0011	0.0037	2.2
Valencene	25.881	0.428	161	0.0044	0.0147	8.1	0.0058	0.0193	11.2	0.0051	0.0170	9.7
Z-Nerolidol	26.158	0.277	69	0.0023	0.0077	4.8	0.0025	0.0084	5.3	0.0024	0.0081	5.1
E-Nerolidol	26.668	0.510	69	0.0025	0.0084	4.9	0.0018	0.0061	3.6	0.0022	0.0073	4.3
(−)-Guaiol	28.17	1.502	161	0.0036	0.0121	4.9	0.0021	0.0071	2.9	0.0029	0.0096	3.9
Carophyllene Oxide	28.594	0.424	79	0.0035	0.0117	7.0	0.0027	0.0089	5.6	0.0031	0.0103	6.3
Cedrol	29.099	0.505	95	0.0030	0.0100	7.0	0.0021	0.0069	4.8	0.0026	0.0085	5.9
(−)-α-Bisabolol	29.644	0.545	109	0.0039	0.0131	8.1	0.0042	0.0139	8.9	0.0041	0.0135	8.5

* ΔRT is the retention time difference between a compound and the previous eluting compound: e.g., Camphene (RT2 min) − α-Pinene (RT1 min) = ΔRT = 0.769 min.

Table 8. Intraday accuracy and precision at calibrator level 1.

Sample Information	Intraday Average % Accuracy (n = 5)			Interday % Accuracy (n = 15)		
Preferred Nomenclature	Average % P1	Average % P2	Average % P3	80 < Average <120 (n = 15)	SD (n = 15)	%RSD (n = 15)
α-Pinene	81.38	83.38	87.82	84.19	3.30	3.91
Camphene	85.60	87.82	89.76	87.73	2.08	2.37
Sabinene	87.54	89.06	93.22	89.94	2.94	3.27
β-Pinene	89.12	91.46	97.92	92.83	4.56	4.91
β-Myrcene	88.40	90.78	94.54	91.24	3.10	3.39
α-Phellandrene	88.84	90.00	92.30	90.38	1.76	1.95
(+)-δ-3-Carene	86.42	87.60	91.84	88.62	2.85	3.22
α-Terpinene	90.88	91.68	94.26	92.27	1.77	1.91
E-β-Ocimene	97.38	96.66	89.06	94.37	4.61	4.88
(+)-Limonene	87.44	84.08	71.42	80.98	8.45	10.43
Z-β-Ocimene	95.66	95.04	85.96	92.22	5.43	5.89
Eucalyptol	90.98	108.89	107.78	102.55	10.04	9.79
γ-Terpinene	88.42	89.04	92.14	89.87	1.99	2.22
Terpinolene	90.74	91.60	93.76	92.03	1.56	1.69
Sabinene Acetate	99.18	99.68	100.48	99.78	0.66	0.66
Linalool	93.02	94.54	94.78	94.11	0.95	1.01
(±)-Fenchone	97.86	96.92	96.32	97.03	0.78	0.80
(+)-Endo-Fenchyl Alcohol	93.96	94.30	92.94	93.73	0.71	0.76
Isopulegol	95.52	95.56	95.62	95.57	0.05	0.05
(±)-Camphor	103.50	104.22	104.22	103.98	0.42	0.40
Isoborneol	91.14	94.24	94.82	93.40	1.98	2.12
Menthol	92.88	95.94	97.56	95.46	2.38	2.49
(±)-Borneol	97.06	99.06	101.36	99.16	2.15	2.17
α-Terpineol	99.60	101.18	103.12	101.30	1.76	1.74
γ-Terpineol	101.74	105.88	106.68	104.77	2.65	2.53
Nerol	93.52	98.02	97.16	96.23	2.39	2.48
Geraniol	96.42	99.60	98.40	98.14	1.61	1.64
Pulegone	111.22	99.24	115.98	108.81	8.63	7.93
Geranyl Acetate	91.02	94.20	93.44	92.89	1.66	1.79
Farnesene	92.48	101.60	104.54	99.54	6.29	6.32
α-Cedrene	87.94	80.72	73.80	80.82	7.07	8.75
E-Caryophyllene	95.80	95.04	93.82	94.89	1.00	1.05
α-Humulene	90.44	92.00	92.82	91.75	1.21	1.32
Valencene	92.32	94.98	95.08	94.13	1.57	1.66
Z-Nerolidol	90.36	92.88	92.26	91.83	1.31	1.43
E-Nerolidol	93.00	97.60	94.38	94.99	2.36	2.48
(−)-Guaiol	103.44	111.70	110.20	108.45	4.40	4.06
Carophyllene Oxide	90.14	90.10	93.28	91.17	1.82	2.00
Cedrol	90.88	94.40	90.16	91.81	2.27	2.47
(−)-α-Bisabolol	92.82	96.06	90.22	93.03	2.93	3.14

Table 9. Regression statistics.

Preferred Nomenclature	Curve Type	Curve Fit Equation	P1 R2	P2 R2	P3 R2	Average R^2
α-Pinene	Quadratic	y = -0.019745 * x ^ 2 + 0.495249 * x + 0.003294	0.9992	0.9994	0.9990	0.9992
Camphene	Quadratic	y = -0.001479 * x ^ 2 + 0.304899 * x + 0.001644	0.9994	0.9995	0.9989	0.9993
Sabinene	Quadratic	y = -2.254085E-004 * x ^ 2 + 0.050802 * x + 2.450207E-004	0.9996	0.9997	0.9993	0.9995
β-Pinene	Quadratic	y = 0.008549 * x ^ 2 + 0.139747 * x + 6.999597E-004	0.9995	0.9991	0.9970	0.9985
β-Myrcene	Quadratic	y = 3.956276E-005 * x ^ 2 + 0.066651 * x + 2.695459E-004	0.9997	0.9997	0.9991	0.9995
α-Phellandrene	Quadratic	y = -0.044281 * x ^ 2 + 0.395442 * x + 0.001260	0.9984	0.9987	0.9994	0.9988
(+)-δ-3-Carene	Quadratic	y = 0.005042 * x ^ 2 + 0.399393 * x + 0.001870	0.9995	0.9996	0.9991	0.9994
α-Terpinene	Quadratic	y = -0.009905 * x ^ 2 + 0.357167 * x + 4.720536E-004	0.9997	0.9997	0.9992	0.9995
E-β-Ocimene	Linear	y = 0.041441 * x + 3.630411E-005	0.9992	0.9994	0.9990	0.9992
(+)-Limonene	Linear	y = 0.247672 * x + 0.001014	0.9997	0.9993	0.9950	0.9980
Z-β-Ocimene	Linear	y = 0.195580 * x + 2.943487E-004	0.9996	0.9997	0.9986	0.9993
Eucalyptol	Quadratic	y = 0.010438 * x ^ 2 + 0.139275 * x + 2.821867E-004	0.9993	0.9969	0.9969	0.9977
γ-Terpinene	Quadratic	y = -0.011031 * x ^ 2 + 0.432019 * x + 0.001675	0.9997	0.9997	0.9995	0.9996
Terpinolene	Quadratic	y = -0.005568 * x ^ 2 + 0.246553 * x + 5.362260E-004	0.9997	0.9997	0.9995	0.9997
Sabinene Acetate	Linear	y = 0.140973 * x + 1.332651E-004	0.9994	0.9993	0.9992	0.9993
Linalool	Quadratic	y = 0.021704 * x ^ 2 + 0.146058 * x + 4.197153E-004	0.9993	0.9992	0.9993	0.9993
(±)-Fenchone	Linear	y = 0.610942 * x + 0.001657	0.9991	0.9992	0.9992	0.9992
(+)-Endo-Fenchyl Alcohol	Linear	y = 0.433887 * x + 0.001105	0.9997	0.9997	0.9996	0.9997
Isopulegol	Quadratic	y = 0.015742 * x ^ 2 + 0.102431 * x + 3.305958E-004	0.9993	0.9992	0.9993	0.9993
(±)-Camphor	Linear	y = 0.069959 * x - 1.570782E-004	0.9988	0.9988	0.9988	0.9988
Isoborneol	Quadratic	y = 0.080424 * x ^ 2 + 0.613288 * x + 0.001336	0.9992	0.9991	0.9993	0.9992
Menthol	Quadratic	y = 0.032422 * x ^ 2 + 0.207697 * x + 5.710364E-004	0.9992	0.9981	0.9985	0.9986
(±)-Borneol	Linear	y = 0.652118 * x + 0.001195	0.9995	0.9996	0.9995	0.9995
α-Terpineol	Linear	y = 0.121693 * x + 3.974486E-006	0.9996	0.9995	0.9994	0.9995
γ-Terpineol	Linear	y = 0.169806 * x - 1.416370E-006	0.9993	0.9991	0.9991	0.9992
Nerol	Quadratic	y = 0.058877 * x ^ 2 + 0.316738 * x + 8.123609E-004	0.9993	0.9991	0.9991	0.9992
Geraniol	Quadratic	y = 0.029225 * x ^ 2 + 0.396115 * x + 5.218178E-004	0.9997	0.9997	0.9997	0.9997
Pulegone	Linear	y = 0.260509 * x - 8.899333E-004	0.9981	0.9997	0.9976	0.9985
Geranyl Acetate	Quadratic	y = 0.023649 * x ^ 2 + 0.157117 * x + 5.472442E-004	0.9993	0.9993	0.9993	0.9993
Farnesene	Quadratic	y = 0.002349 * x ^ 2 + 0.022238 * x + 1.734340E-004	0.9992	0.9990	0.9991	0.9991
α-Cedrene	Quadratic	y = -0.009891 * x ^ 2 + 0.272395 * x + 0.001164	0.9994	0.9989	0.9980	0.9988
E-Caryophyllene	Quadratic	y = 0.015233 * x ^ 2 + 0.165136 * x + 4.999558E-004	0.9989	0.9994	0.9988	0.9990
α-Humulene	Linear	y = 0.687931 * x + 0.002405	0.9997	0.9997	0.9997	0.9997
Valencene	Quadratic	y = 0.029306 * x ^ 2 + 0.278097 * x + 8.453314E-004	0.9991	0.9991	0.9990	0.9991
Z-Nerolidol	Quadratic	y = 0.068097 * x ^ 2 + 0.349922 * x + 0.001597	0.9994	0.9992	0.9992	0.9993
E-Nerolidol	Quadratic	y = 0.026726 * x ^ 2 + 0.365077 * x + 9.104884E-004	0.9998	0.9997	0.9997	0.9997
(−)-Guaiol	Linear	y = 0.444141 * x + 2.338193E-004	0.9986	0.9977	0.9965	0.9976
Carophyllene Oxide	Quadratic	y = 0.043632 * x ^ 2 + 0.223155 * x + 8.987763E-004	0.9992	0.9990	0.9990	0.9991
Cedrol	Quadratic	y = 0.060148 * x ^ 2 + 0.379565 * x + 0.001679	0.9993	0.9993	0.9991	0.9992
(−)-α-Bisabolol	Quadratic	y = 0.056789 * x ^ 2 + 0.272930 * x + 0.001303	0.9994	0.9993	0.9984	0.9990

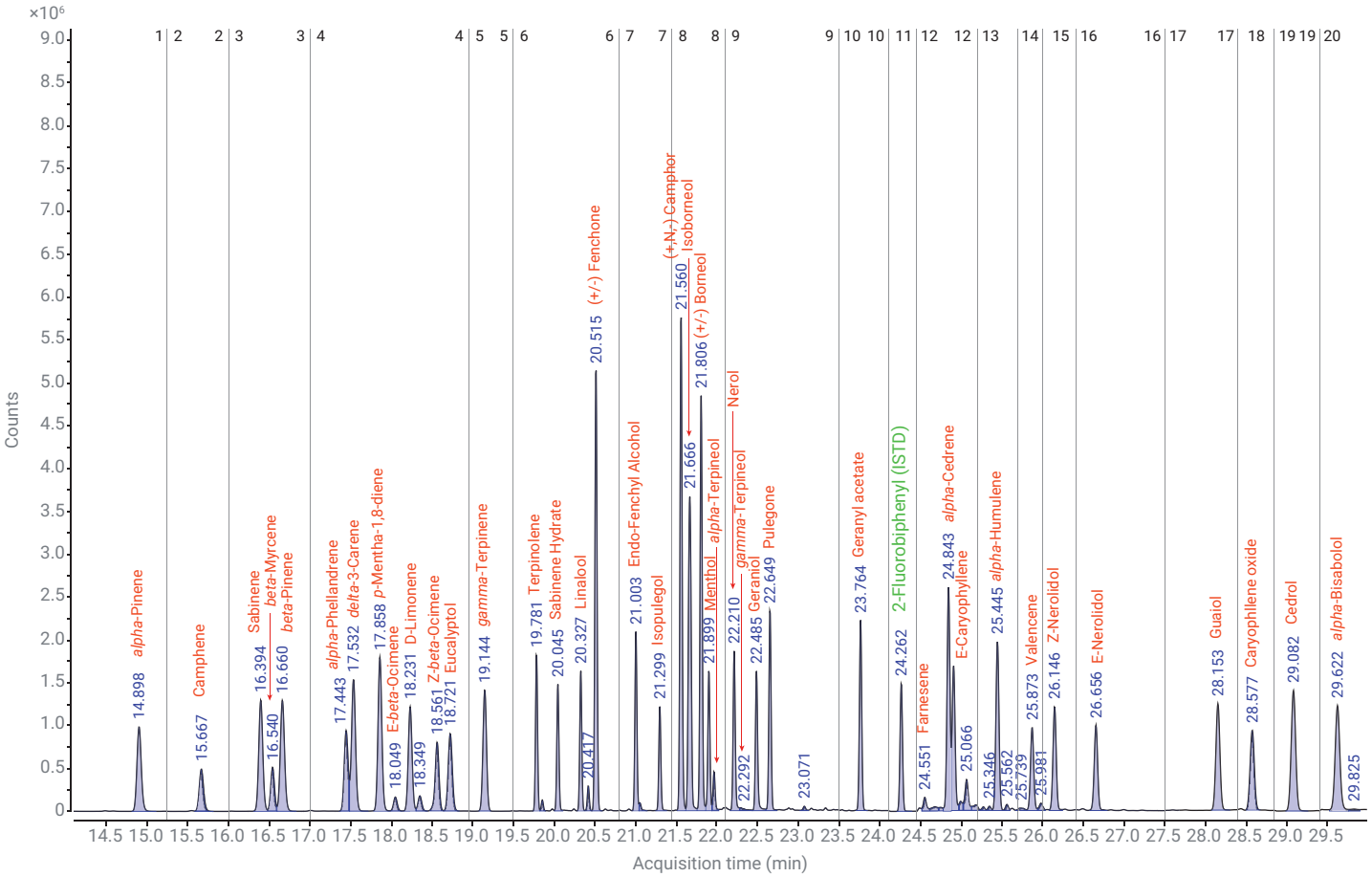


Figure 4. Terpene chromatogram of high calibrator in hempseed oil matrix.

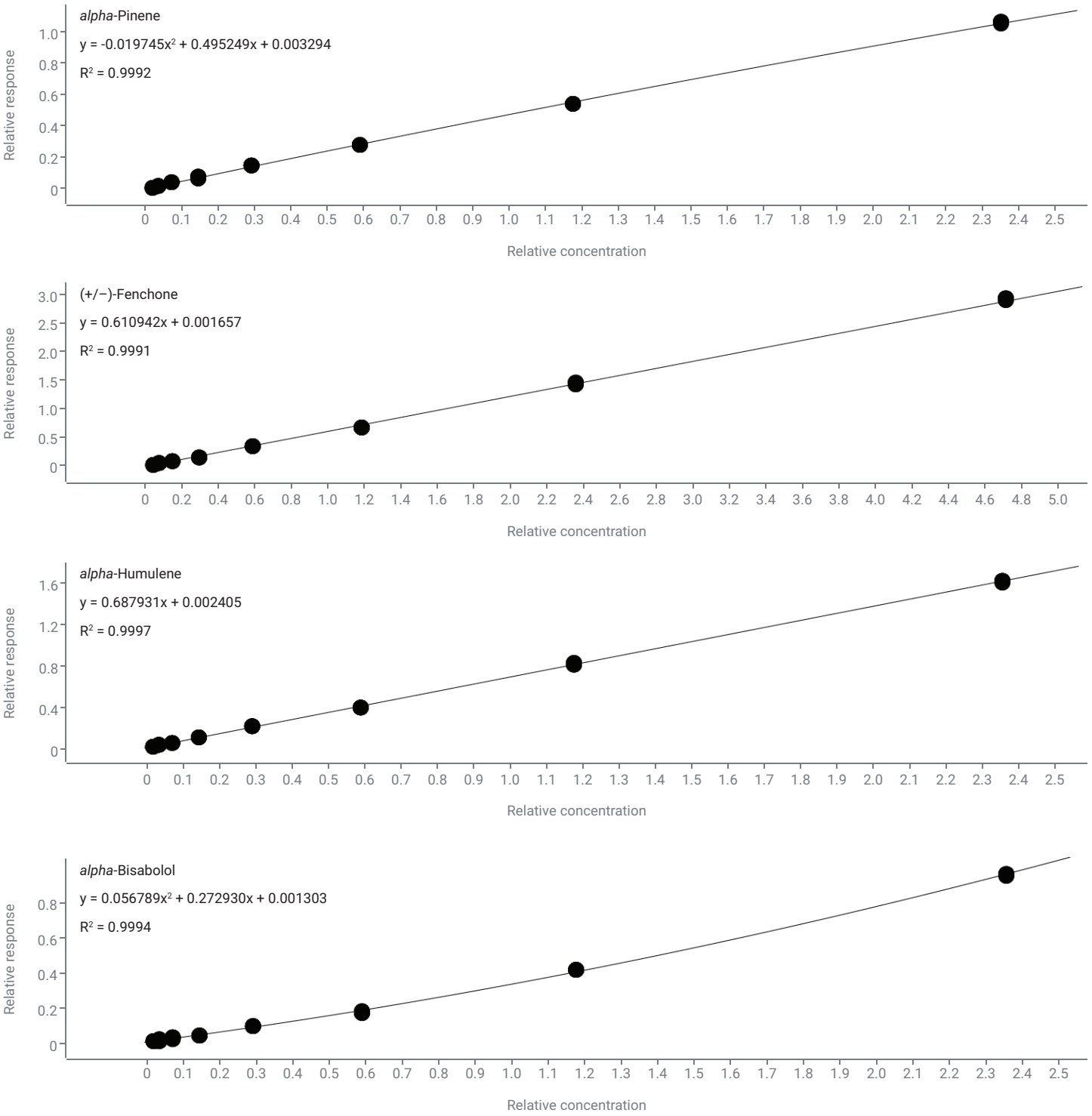


Figure 5. Calibration curves for four terpenes spanning the chromatographic run time. α-Pinene elutes first, and α-bisabolol elutes last.

ISTD stability

As a measure of method performance, we evaluated the stability of the 2-fluorobiphenyl internal standard over the time course of this study. Using a relative response factor calculated by dividing each ISTD response by the average of all ISTD responses (n = 144), we determined the mean, %RSD, and the 95% confidence intervals for the three datasets as:

- P1: 0.98, 95% CI [0.97, 0.99], RSD = 4.00%
- P2: 0.95, 95% CI [0.94, 0.96], RSD = 3.64%
- P3: 1.06, 95% CI [1.04, 1.07], RSD = 6.08%.

Figure 6 is a Box-Whisker plot of the results. The most variability was observed in the P3 dataset, but was still well within an acceptable tolerance.

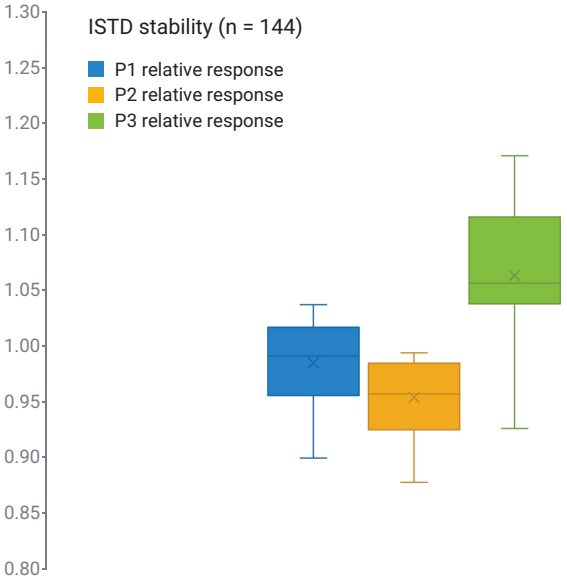


Figure 6. Box-Whisker plot of internal standard relative response over the time-course of the study.

Calculations to determine wt/wt and % (wt/wt) from results determined in µg/mL

The analytical results for the unknowns were determined through regression analysis. As an example of converting the wt/v results into wt/wt and % (wt/wt), consider a cannabis oil sample with a calculated concentration of 250 µg/mL. The original sample was prepared by transferring a nominal volume of 0.6 mL into a tared flask. The mass of the transferred material was determined to be 0.55 g. From the sample preparation section above, the volume (V) of the ethyl acetate + ISTD solution used to dissolve the sample was 50 mL and no additional dilution was made (dilution factor, DF = 1). This example is shown in Equations 1 and 2.

$$\mu g/g\ (ppm) = \frac{(100\ \mu g/mL) \times 50\ mL \times 1}{0.55\ g} = 22{,}727\ \mu g/g$$

Equation 1.

$$\%\ (wt/wt) = \left(\frac{22{,}727\ \mu g/g}{1 \times 10^{6}\ \mu g/g}\right) \times 100 = 2.27\%$$

Equation 2.

Sample results

A commercially available terpene blend was purchased. According to the product insert, approximately 94% of this product was comprised of (+)-limonene, β-caryophyllene, β-myrcene, α-pinene, linalool, β-pinene, α-humulene, terpinolene, and α-bisabolol with (+)-limonene, β-caryophyllene, and β-myrcene amounting to 71% of the total. The product also contained at least 31 other known terpenes with concentrations < or << than 1.0% (wt/wt). The product was not described as a certified reference material by the manufacturer. No lot or analytical description was provided in the product insert. We therefore surmised that the product insert was more of a general description of the terpene content rather than a true certificate of analysis.

In each of the three datasets used to determine method performance, the sample was analyzed twice (n = 6) in SIM/SCAN mode. Each replicate was defined as P1R1, P1R2, P2R1, P2R2, P3R1, and P3R2. The quantitative results for target terpenes in % (wt/wt) were compared to the values provided by the manufacturer. β-caryophyllene, α-pinene, linalool, β-pinene, terpinolene, and α-bisabolol (6/9 of the major terpenes in the product) were determined to be within ±10% of the % (wt/wt) provided in the product insert. Figure 7 is a clustered column graph of the terpene replicate accuracy over the 3 datasets. Figure 8 is the average quantitative result for each terpene and the 95% confidence interval (CI) upper and lower bounds.

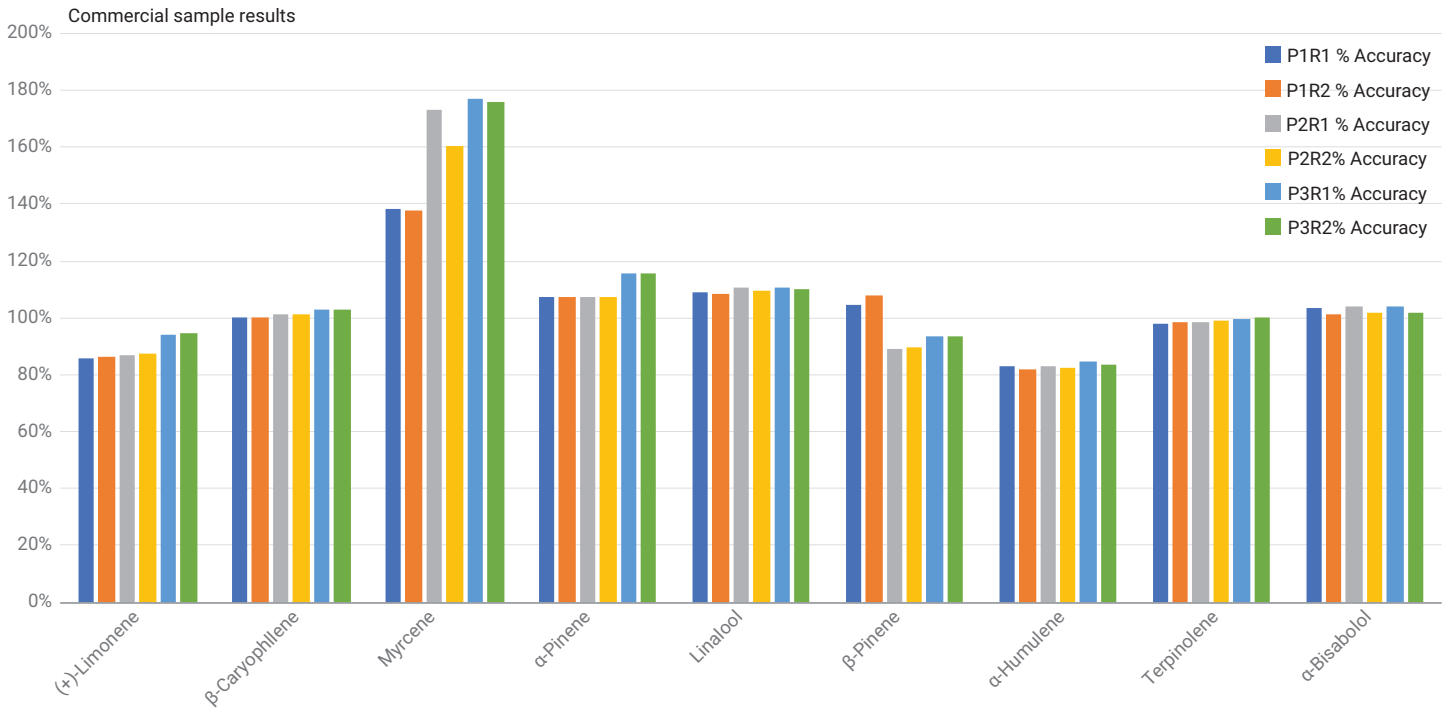


Figure 7. Terpene accuracy. Two replicate injections in each of three analysis.

α-humulene was determined to be consistently low, with an average concentration of 3.70%, 95% CI [3.67%, 3.73%] (wt/wt). These statistical descriptors led us to posit that this analytical result more closely represented the amount in the sample than described in the product insert. The (+)-limonene content in the sample was determined to be on average <11% of the value provided in the product insert. There was excellent intraday precision for (+)-limonene, but the concentration increased by 2% by P3, which was 5 days after the beginning of the study. The RSD was 4.61%, and the average concentration was determined to be 24.81% 95% CI [23.89%, 25.72%] (wt/wt). Given the precision of the intraday studies, and we again posit that this concentration more closely reflects the actual (+)-limonene concentration in the sample.

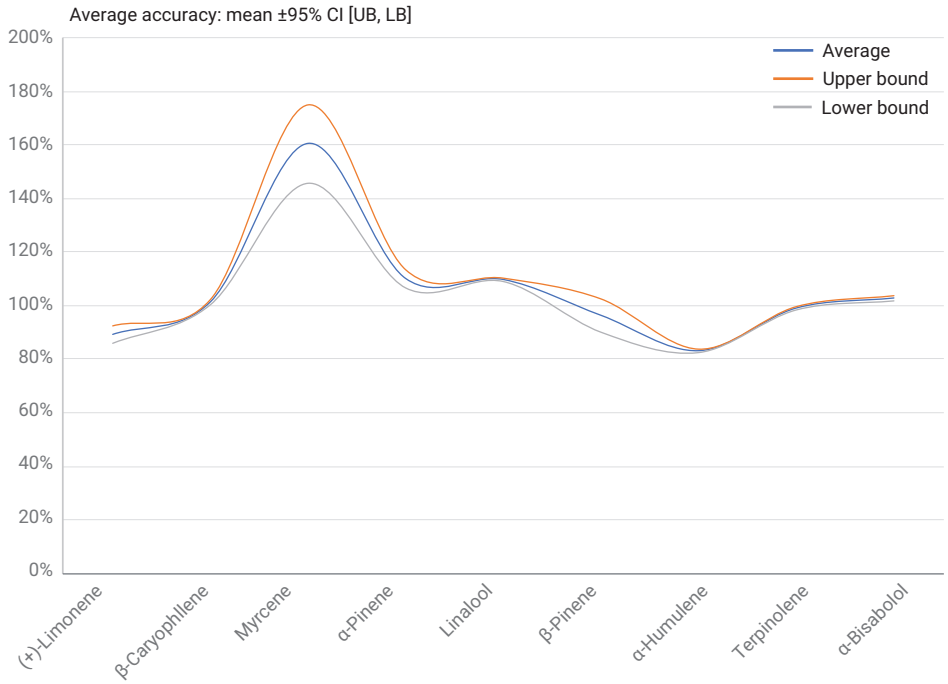


Figure 8. Average accuracy and limits. Larger boundaries reveal variability.

The β-myrcene concentration accuracy determined for the sample was on average 60.5% greater than the concentration provided in the product insert with poor interday precision (RSD = 11.4%, n = 6). However, the intraday precision was <1.0% RSD in 2/3 datasets. The P2 data for myrcene had the most variability between the two replicates, as shown in Table 10. This level of variability was not observed for myrcene in the interday datasets given in Table 8 (RSD = 3.4%, n = 15), the internal standard evaluation (n = 144), or for any of the other target terpenes in the sample. Based on the other results, we surmised the myrcene concentration was higher than provided in the product insert: approximately 28.77%, 95% CI [26.14%, 31.40%] (wt/wt).

Table 10. Intraday average accuracy and precision.

Myrcene			
Intraday	**P1**	**P2**	**P3**
Average	138.03%	166.77%	176.57%
Std. Deviation	0.75%	8.85%	0.92%
%RSD	0.55%	5.30%	0.52%

Unknowns analysis and SIM/Scan

The use of SIM/Scan data collection with the mass spectrometer enabled targeted quantitative analysis and interrogation of the full *m/z* scan spectra to search for nontargeted terpenes and putatively identify them using a known mass spectral database. We used the NIST Mass Spectral Search Program[11] within the MassHunter Unknowns Analysis 10.1 software that is part of the MassHunter Quantitative Analysis package to identify other terpenes in the sample. In addition to the nine major components in the product listed above, the spectral library search identified four trace terpenes listed in the product insert: γ-terpinene 0.04% (wt/wt), bornyl acetate 0.23% (wt/wt), camphene 0.45% (wt/wt), and α-cubebene 0.05% (wt/wt).

Best practices

In MassHunter B.10 GC/MS Acquisition software, values such as the mass of the sample used for the analysis, the volume of the extraction or dissolution solvent, and the dilution factor can be entered specific columns in the acquisition sequence. The user enters the mass of each sample in grams into the "Amt." column, the volume of solvent in mL is entered the "Total Amt." column, and the dilution factor (e.g., a 10,000-fold dilution is entered as 0.0001) entered in the "Dilution" column. By entering these three values into the sequence, MassHunter Quantitative Software B.10 to reports the final concentration in % (wt/wt).

Conclusion

This work developed and verified method parameters and outcomes for the liquid injection analysis of 40 chromatographically resolved terpenes in cannabis and in cannabinoid products using the Agilent Intuvo 9000/5977B GC/MS system. All data were matrix-matched and used an internal standard. This novel method used capillary flow technology to backflush matrix and other unwanted compounds before the next injection. Accuracy, precision, range, linearity, limits of detection (defined as MDL), and limits of quantitation were determined through both intraday and interday studies.

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