Systems and methodologies for rapid and robust Cannabis Terpene Analysis
5 minute analysis time = more productivity

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Introduction

There is a growing need for the analysis of residual solvents and terpenes in cannabis and cannabinoid concentrates. These methods must assure safety and quality and the list of terpenes and residual solvents that are to be analyzed is continuously being increased by state and local governing bodies. A common configuration for these analyses connects headspace sampling techniques with GC or GC-MS systems. Agilent is collaborating with major laboratories to rapidly develop high-end instrument configurations and methodologies that are robust, accurate and precise while still allowing for easy to implement workflows in high productivity laboratories. These methods must assure safety and quality and the list of terpenes and residual solvents that are to be analyzed is continuously being increased by state and local governing bodies. A common configuration for these analyses connects headspace sampling techniques with GC or GC-MS systems. The Agilent headspace-GC-MS systems for the analysis of residual solvents and terpenes includes the 7697A headspace auto-sampler, the 7890B or the Intuvo 9000 GC and the 5977B mass spectrometer operated on MassHunter software. This presentation will discuss the Agilent systems and methodologies for the rapid and robust analysis of terpenes in cannabis and cannabinoid samples.

Experimental

Workflow for samples and standards:

10µL of the a standard is placed in a 10mL headspace vial and capped. A 5 or 10 point calibration curve is created from the standards.

As plant and extract material does not dissolve in solvent, a full evaporation headspace technique (FET) is used for quantitation.

A part of the flower weighing 1.0 gram is frozen, followed by grinding to ensure a representative sample. 10-30 mg of the flower is then weighed into a headspace vial and capped.

For different species of samples:

plant, extracts, etc. Use 5-7 mg +/- 15 mg place into 10 mL headspace vial, evaluate against curve.

Run QC to verify performance

7697A Headspace parameters
Carrier Control: GC
Instrument Conditions
Oven Temperature (°C): 120
Loop Temperature (°C): 120
Transfer Line Temperature (°C): 140
Vial Equilibration (min): 10.00
Injection Duration (min): 0.50
GC Cycle Time (min): 10.00
Vial Size: 10
Vial Shaking: Level 1

Front SS Inlet He
Mode Split
Heater 220 °C
Pressure 29.45 psi
Total Flow 303 mL/min
Septum Purge Flow off
Split Ratio 100 :1

7890B GC Column Summary
Oven Temperature Program
(Initial) 60 °C
Hold Time 0.5 min
#1 Rate 45 °C/min
#1 Value 150 °C
#1 Hold Time 0 min
#2 Rate 35 °C/min
#2 Value 250 °C
#2 Hold Time 0.5 min
Equilibration Time 1 min

Front Detector FID Conditions
Makeup He
Heater 300 °C
H2 Flow 40 mL/min
Air Flow 400 mL/min
Makeup Flow 25 mL/min

Fill Pressure (psi): 15
Loop Fill Mode: Custom
Loop Ramp Rate (psi/min): 40
Loop Final Pressure (psi): 13
Loop Equilibration Time: 0.05
Carrier Control Mode: GC controls
Extraction
Vent After Extraction: ON
Post Injection Purge: 100 mL/min for 3 min
Acceptable Leak Check: Default, 0.2mL/m

Thermal Aux 2 (MSD Transfer Line)
Set point 260 °C

5977 MS Conditions
Acquisition Mode : Scan
Solvent Delay (minutes) : 1.8
Tune file:
D:\MassHunter\GCMS\1\5977\etune.u
EM Setting mode Gain : 1.000000

Normal or Fast Scanning : Normal Scanning

[Scan Parameters]
Start Time 1.8
Low Mass 40
High Mass 400
Threshold 125
A/D Samples: 2

MS Source Temp 300 C
MS Quad Temp 150 C

Experimental

Analytical Column
Agilent CP8877: SN 9352219
VF-35ms
40 °C—340 °C (360 °C): 30 m x 250 µm x 0.25 µm
In Front SS Inlet He
Out Aux EPC 1
(Initial) 60 °C
Pressure 29.45 psi
Flow 3.0 mL/min
Average Velocity 51.103 cm/sec
Holdup Time 0.97842 min

MSD Restrictor
-60 °C—350 °C (350 °C): 1.7 m x 150 µm x 0 µm
Column Deactivated fused silica
In Aux EPC 1 He
Out MSD
(Initial) 60 °C
Pressure 3.1709 psi
Flow 1.4 mL/min
Average Velocity 182.03 cm/sec

FID Restrictor
-60 °C—350 °C (350 °C): 0.7 m x 250 µm x 0 µm
Column Deactivated fused silica
In Aux EPC 1 He
Out Front Detector FID
(Initial) 60 °C
Pressure 3.1709 psi
Flow 8.4855 mL/min
Average Velocity 289.67 cm/sec

The Intuvo USP-437 configuration can also be implemented

Results and Discussion

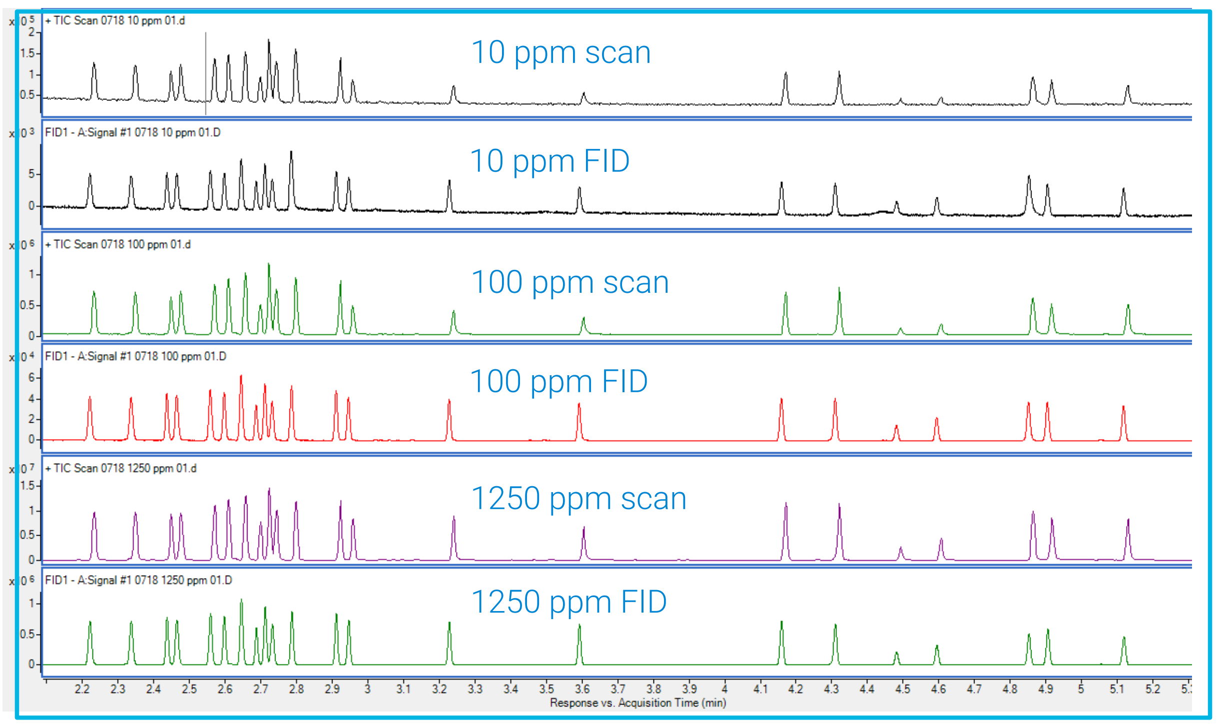

Figure 1. FID and MS Chromatograms 10 ppm, 100 ppm, and 1250 ppm of a 22 compound terpene mix, 10 minute cycle times

Exp. Conc.	RT	Resp.	Calc. Conc.	Accuracy
10	2.232	17999	9.9423	99.4
25	2.232	44230	25.3379	101.4
50	2.234	94928	55.094	110.2
92.6	2.232	143007	83.313	90
250	2.232	422327	247.2533	98.9
500	2.234	847740	496.9391	99.4
1250	2.234	2147360	1259.72	100.8

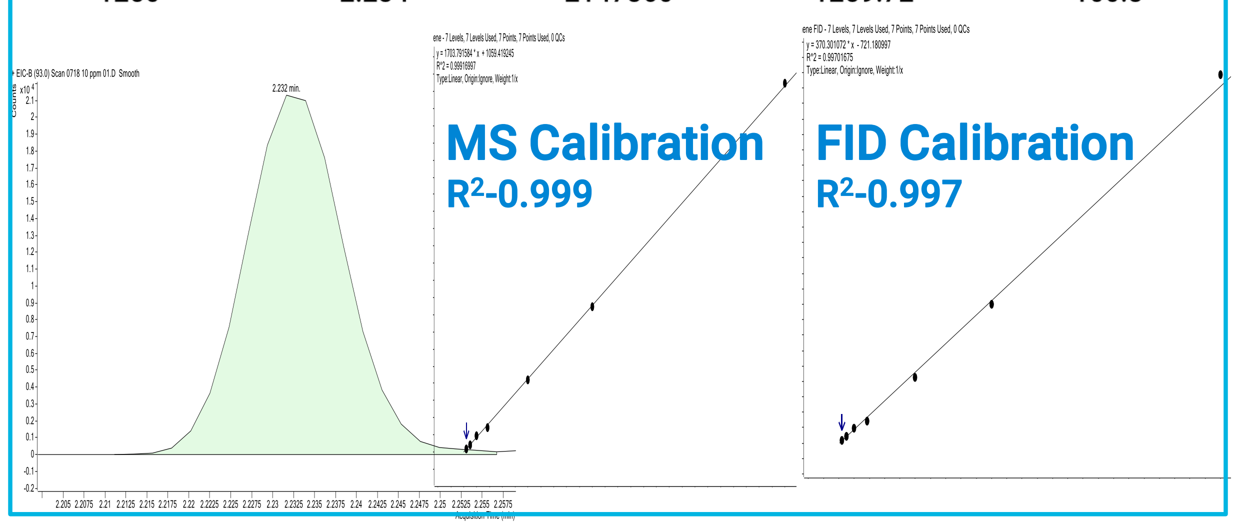

Figure 2. 10 ppm extracted Ion, calibration from 10-1250 ppm alpha-Pinene

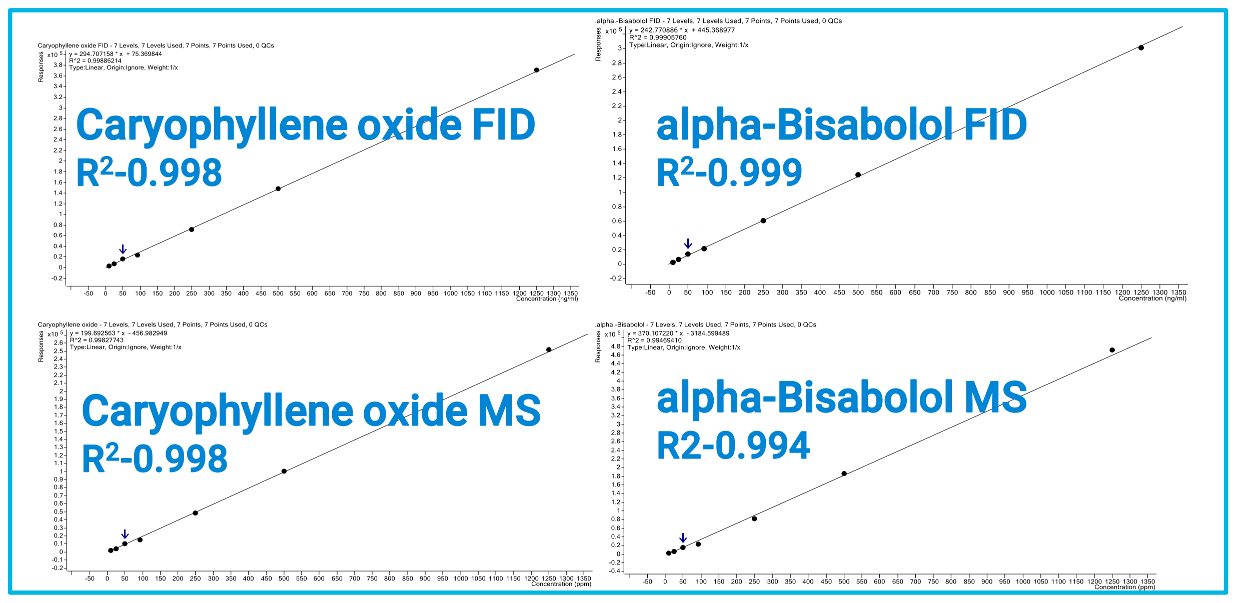

Figure 3. Calibration curves 10 ppm to 1250 ppm

Results and Discussion

Name	RT	Avg Conc.	Std. Dev.	Conc. RSD	LOQ	LOD	Avg. Resp	Resp. RSD
alpha Pinene FID	2.221	49.40	0.46	0.9	4.65	1.39	17573	1
alpha Pinene	2.23	50.70	0.38	0.8	3.81	1.14	87450	0.7
Camphene FID	2.337	48.88	0.54	1.1	5.47	1.64	17390	1.2
Camphene	2.35	50.00	1.30	2.6	13.01	3.90	52233	2.6
beta Myrcene FID	2.437	49.21	0.43	0.9	4.31	1.29	15002	0.9
beta Myrcene	2.448	49.35	2.83	5.7	28.36	8.50	32674	5.9
beta Pinene FID	2.465	49.37	0.45	0.9	4.58	1.37	16699	1
beta Pinene	2.476	50.53	2.28	4.5	22.84	6.85	73972	4.5
delta-3-Carene FID	2.559	49.75	0.48	1	4.87	1.46	18078	1
delta-3-Carene	2.569	50.76	0.92	1.8	9.26	2.77	75715	1.8
alpha terpinene FID	2.598	49.64	0.36	0.7	3.68	1.10	15808	0.8
alpha terpinene	2.61	49.41	0.60	1.2	6.07	1.82	63342	1.3
D-Limonene FID	2.645	49.59	0.42	0.9	4.22	1.26	23170	0.9
D-Limonene	2.658	52.29	0.60	1.2	6.02	1.80	49501	1.1
.beta.-Ocimene FID	2.687	49.54	0.52	1.1	5.25	1.57	10924	1.1
.beta.-Ocimene	2.699	49.10	1.87	3.8	18.74	5.62	12853	4
p-Cymene FID	2.712	48.99	0.55	1.1	5.55	1.66	18568	1.2
p-Cymene	2.722	53.50	0.69	1.3	6.98	2.09	174393	1.2
Eucalyptol FID	2.732	55.98	0.63	1.1	6.31	1.8	15425	1.2
Eucalyptol	2.743	59.51	1.20	2	12.02	3.60	26624	2
.gamma.-Terpinene FID	2.787	45.75	3.42	7.5	34.24	10.27	18691	6.7
.gamma.-Terpinene	2.797	50.81	0.51	1	5.13	1.53	71814	1
Terpinolene FID	2.911	49.43	0.47	1	4.79	1.43	16371	1
Terpinolene	2.923	50.48	0.77	1.5	7.71	2.31	46268	1.5
Linalool FID	2.946	49.00	0.43	0.9	4.30	1.29	14363	0.9
Linalool	2.957	48.00	1.17	2.4	11.72	3.51	11918	2.7
Isopulegol FID	3.228	48.99	0.42	0.9	4.24	1.27	14174	0.9
Isopulegol	3.24	42.82	2.23	5.2	22.31	6.69	7449	6.5
Geraniol FID	3.593	49.68	0.34	0.7	3.49	1.04	13415	0.7
Geraniol	3.605	47.91	1.12	2.3	11.20	3.36	16929	2.6
beta Caryophyllene FID	4.16	50.16	0.31	0.6	3.10	0.93	16524	0.6
beta Caryophyllene	4.171	46.59	1.01	2.2	10.15	3.04	19222	2.4
alpha Humulene FID	4.31	50.12	0.29	0.6	2.95	0.88	16531	0.6
alpha Humulene	4.324	47.85	0.15	0.3	1.50	0.45	61806	0.3
Nerolidol 1 FID	4.483	54.25	2.72	5	27.24	8.17	5904	5
Nerolidol 1	4.493	41.91	1.59	3.8	15.99	4.79	3914	5.1
Nerolidol 2 FID	4.595	53.06	0.49	0.9	4.92	1.47	8781	0.9
Nerolidol 2	4.607	39.69	1.55	3.9	15.57	4.67	6641	5.5
Guaiol FID	4.853	54.61	0.79	1.5	7.93	2.38	17152	1.2
Guaiol	4.864	40.22	0.66	1.7	6.67	2.00	19534	2.1
Caryophyllene oxide FID	4.906	57.90	0.99	1.7	9.90	2.97	17140	1.7
Caryophyllene oxide	4.919	51.70	1.28	2.5	12.81	3.84	9868	2.6
.alpha.-Bisabolol FID	5.119	53.73	0.71	1.3	7.19	2.15	13491	1.3
.alpha.-Bisabolol	5.134	39.81	0.83	2.1	8.31	2.49	11553	2.7

Figure 4. 8 replicates for reproducibility (50 ppm)

Conclusions

Newest methodology enhancements allowing full scan acquisition with a 5 minute run time and great separation using both FID and MS for quantitation.

- All compounds library search giving the proper hit in the top results with the NIST library.
- This allows for approximately 6 runs an hour an increase from the current 2-3 runs an hour by optimizing the GC parameters and column configuration.
- This method will increase lab productivity allowing the dedication of a Terpene system for optimum lab productivity.
- The Intuvo USP-437 configuration can also be implemented

References

www.agilent.com/cs/library/applications/5991-8032EN
www.agilent.com/cs/library/applications/5990-7625EN

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