Method Development for the Analysis of Residual Solvents and Terpenes in one run using headspace GC-MS/FID

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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. The Agilent headspace-GC-MS systems for the analysis of residual solvents and terpenes includes the 7697A headspace auto-sampler, the 7890B GC and the 5977B mass spectrometer operated on MassHunter software. This presentation will discuss the Agilent systems and methodologies for the analysis of terpenes and residual solvents in cannabis and cannabinoid samples.

Experimental

Workflow for samples and standards:

10 µL standard is placed in a 20mL 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 20 mL headspace vial, evaluate against curve.

Run QC to verify performance

7697A Headspace parameters

Carrier Control:	GC
Instrument Conditions	
Oven Temperature (°C):	85
Loop Temperature (°C):	85
Transfer Line Temperature (°C):	100
Vial Equilibration (min):	20.00
Injection Duration (min):	0.50
GC Cycle Time (min):	29.00
Vial Size:	20
Vial Shaking:	Level 2
Fill Pressure (psi):	15
Loop Fill Mode:	Custom
Loop Ramp Rate (psi/min):	40
Loop Final Pressure (psi):	10
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

Front SS Inlet He

Mode	Split
Heater	140 °C
Pressure	19.92 psi
Total Flow	21 mL/min
Septum Purge Flow	off
Split Ratio	20 :1

Thermal Aux 2 (MSD Transfer Line)

Set point	280 °C

7890B GC Column Summary

Oven Temperature Program	
(Initial)	35 °C
Hold Time	10.0 min
#1 Rate	30 °C/min
#1 Value	180 °C
#1 Hold Time	0 min
#2 Rate	40 °C/min
#2 Value	260 °C
#2 Hold Time	5.0 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

5977 MS Conditions

Acquisition Mode	: Scan
Solvent Delay (minutes)	: 0.1
Tune file:	D:\MassHunter\GCMS\1\5977\atune.u
EM Setting mode Gain	: 1.000000
Normal or Fast Scanning	: Normal Scanning
[Scan Parameters]	
Start Time	0.1
Low Mass	30
High Mass	400
Threshold	150
A/D Samples:	4
MS Source Temp	300 C
MS Quad Temp	150 C

Experimental

Analytical Column

Agilent 122-1334UI
DB- 624 Ultra Inert
-20 °C—260 °C (360 °C): 30 m x 250 µm x 1.4 µm

In	Front SS Inlet He
Out	Aux EPC 1
(Initial)	35 °C
Pressure	11.922 psi
Flow	1.0 mL/min
Average Velocity	24.63 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)	35 °C
Pressure	0.47017 psi
Flow	1.15 mL/min
Average Velocity	162.94 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)	35 °C
Pressure	0.18046 psi
Flow	0.5 mL/min
Average Velocity	17.439 cm/sec

Results

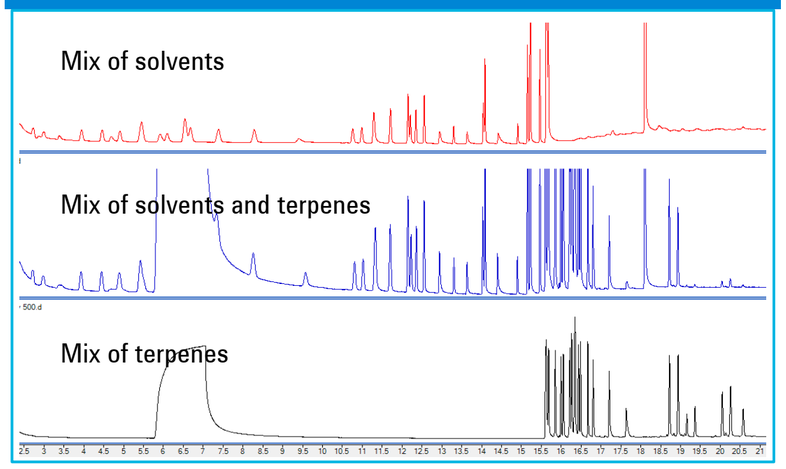
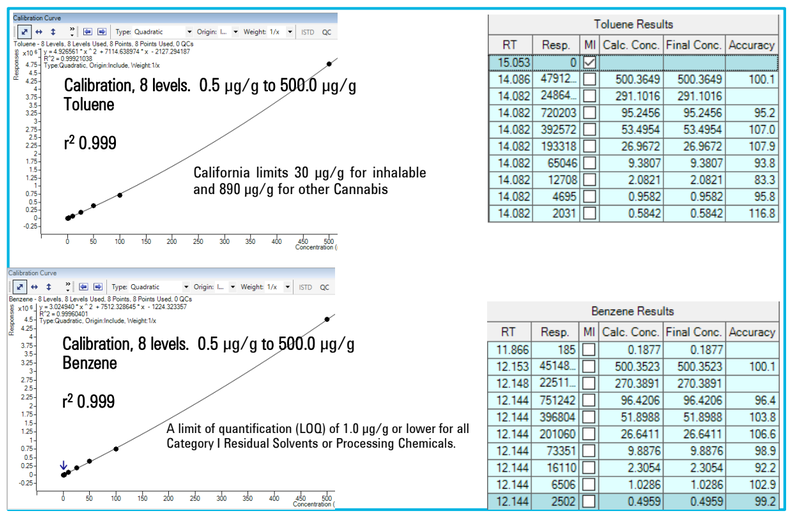


Figure 1. MS Chromatograms residual solvent mix only (top), a mix of both (middle) and terpene only mix(bottom), 29.0 minute cycle times



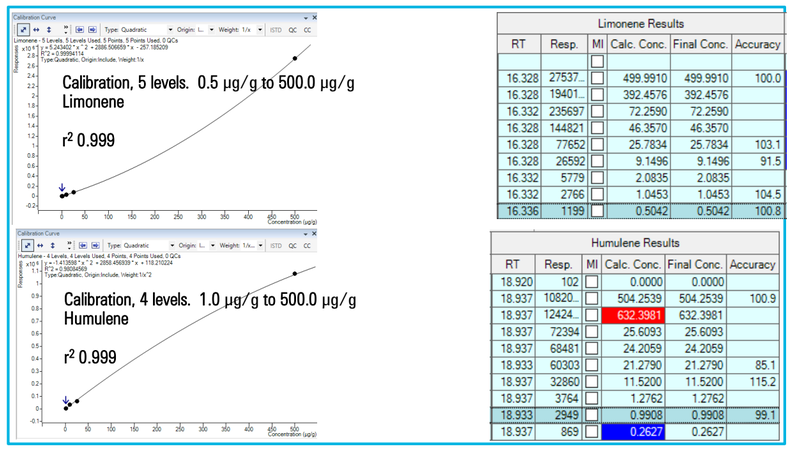
Toluene Results

RT	Resp.	ISTD	Calc. Conc.	Final Conc.	Accuracy
15.053	0	☑			
14.086	47912...	☐	500.3649	500.3649	100.1
14.082	24864...	☐	291.1016	291.1016	
14.082	720203	☐	95.2456	95.2456	95.2
14.082	392572	☐	53.4954	53.4954	107.0
14.082	193318	☐	26.9672	26.9672	107.9
14.082	65046	☐	9.3807	9.3807	93.8
14.082	12708	☐	2.0821	2.0821	83.3
14.082	4695	☐	0.9582	0.9582	95.8
14.082	2031	☐	0.5842	0.5842	116.8

Benzene Results

RT	Resp.	ISTD	Calc. Conc.	Final Conc.	Accuracy
11.866	185	☐	0.1877	0.1877	
12.153	45148...	☐	500.3523	500.3523	100.1
12.148	22511...	☐	270.3891	270.3891	
12.144	751242	☐	96.4206	96.4206	96.4
12.144	396804	☐	51.8988	51.8988	103.8
12.144	201060	☐	26.6411	26.6411	106.6
12.144	73351	☐	9.8876	9.8876	98.9
12.144	16110	☐	2.3054	2.3054	92.2
12.144	6506	☐	1.0286	1.0286	102.9
12.144	2502	☐	0.4959	0.4959	99.2

Figure 2. Example Residual Solvent calibration with California action limits for Benzene and Toluene 0.5 µg/g to 500 µg/g



Limonene Results

RT	Resp.	ISTD	Calc. Conc.	Final Conc.	Accuracy
		☐			
16.328	27537...	☐	499.9910	499.9910	100.0
16.328	19401...	☐	392.4576	392.4576	
16.332	235697	☐	72.2590	72.2590	
16.328	144821	☐	46.3570	46.3570	
16.328	77652	☐	25.7834	25.7834	103.1
16.328	26592	☐	9.1496	9.1496	91.5
16.332	5779	☐	2.0835	2.0835	
16.332	2766	☐	1.0453	1.0453	104.5
16.336	1199	☐	0.5042	0.5042	100.8

Humulene Results

RT	Resp.	ISTD	Calc. Conc.	Final Conc.	Accuracy
18.920	102	☐	0.0000	0.0000	
18.937	10820...	☐	504.2539	504.2539	100.9
18.937	12424...	☐	632.3981	632.3981	
18.937	72394	☐	25.6093	25.6093	
18.937	68481	☐	24.2059	24.2059	
18.933	60303	☐	21.2790	21.2790	85.1
18.937	32860	☐	11.5200	11.5200	115.2
18.937	3764	☐	1.2762	1.2762	
18.933	2949	☐	0.9908	0.9908	99.1
18.937	869	☐	0.2627	0.2627	

Figure 3. Example Terpene Calibration curves 0.5 µg/g to 500 µg/g

Compound list, target ion, and retention times

NAME	m/z	RT
propane	44	2.221
Isobutane	43	2.72
Butane	43	2.988
Acetone	43	3.122
Methanol	31	3.37
Butane, 2-methyl-	57	3.94
Pentane	43	4.46
Ethanol	31	4.67
Ethyl ether	74	4.9
Butane, 2,2-dimethyl-	71	5.437
Isopropyl Alcohol	45	5.88
Acetonitrile	41	6.08
Methylene chloride	84	6.53
Pentane, 2-methyl-	71	6.673
Pentane, 3-methyl-	57	7.38
n-Hexane	57	8.27
1-Propanol	31	9.35
2-Butanone	72	10.75
Ethyl Acetate	43	10.98
Tetrahydrofuran	72	11.27
Cyclohexane	84	11.7
Benzene	78	12.14
Ethane, 1,2-dimethoxy-	45	12.2
Isopropyl acetate	43	12.34
Heptane	71	12.55
1-Butanol	56.1	12.929
1,4-Dioxane	88	13.293
Ethanol, 2-ethoxy-	59	13.62
Pyridine	79	14.03
Toluene	91.1	14.082
1-Pentanol	55	14.4
Formamide, N,N-dimethyl-	73	14.9
Ethylbenzene	91	15.15
m-p-Xylene	91.1	15.218
o-xylene	106	15.46
Dimethyl Sulfoxide	78	15.62
N,N-Dimethylacetamide	87	15.66
Cumene	120	15.67
tetramethylene sulfone	120	15.675
.alpha.-Pinene	93	15.683
Camphene	93	15.851
.beta.-Myrcene	69	15.993
.beta.-Pinene	93	16.055
delta -3 Caren3	93	16.218
alpha terpinene	121	16.276
Limonene	68	16.331
p-Cymene	119	16.347
.beta.-Ocimene	79	16.356
Eucalyptol	81	16.435
.gamma.-Terpinene	93	16.486
terpinolene	136	16.672
Linalool	69	16.803
Isopulegol	81	17.206
Geraniol	93	17.637
Caryophyllene	133	18.722
Humulene	93	18.941
Nerolidol	93	19.159
Nerolidol 2	93	19.364
Guaiol	161	20.049
Caryophyllene oxide	93	20.259
.alpha.-Bisabolol	109	20.576

Conclusions

- Newest methodology enhancements allowing full scan acquisition of residual solvents and terpenes with a 29 minute run time and great separation using both FID and MS for quantitation.
- NIST spectral library search properly identifies all compounds
- At least two samples per hour can be run negating the need for two independent tests.
- Increased lab productivity

References

- USP 467 Analysis of Residual Solvents. (2017) Agilent Technologies Application Note 5991-8032.
- Firor, R. L. Analysis of USP <467> Residual Solvents with Improved Repeatability Using the Agilent 7697A Headspace Sampler. (2012) Agilent Technologies Application Note 5990-7625.
- Honnold, R., Kubas, R., Macherone, A. (2017) Analysis of Terpenes in Cannabis Using the Agilent 7697A/7890B/5977B Headspace GC-MSD System: Faster Analysis Time = Greater Productivity. Agilent Technologies Application Note 5991-8499.