Streamlining Cannabis Testing Using Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry (GCxGC-TOFMS)

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Background

- The composition of cannabis is very important in determining its utility, potency, and medicinal effectiveness
- Cannabis is difficult to analyze because of its chemical diversity, but more importantly, the wide concentration ranges of its constituents
- Analysis often requires: A) Tedious sample preparation, and B) Specialized (targeted) instrumental analysis

Objectives

- Reduce sample manipulation and shorten preparation times
- Maximize the information obtained per analysis
- Implement the use of GC-TOFMS and GCxGC-TOFMS to effectively profile cannabis samples

Sample Extraction

1) 0.10 g sample
2) 2 mL of solvent (e.g., $CHCl_3$, MeOH, or EtOH)
3) Sonicate (5 min) and filter

CBD oils & Botanicals | Medical Marijuana | Recreation Marijuana

Analytical Instrumentation and Acquisition Parameters

Pegasus® BT 4D

Gas Chromatograph	LECO GCxGC (Thermal), in 7890 and L-PAL 3 Autosampler
Injection	1 µL, Split 25:1 (250 °C)
Carrier Gas	He @ 1.0 mL/min, Constant Flow
Columns (1st Dimension) (2nd Dimension)	Rxi-5 MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA) Rxi-17 Sil MS 0.6 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA
Temperature Program	40 °C (5 min), ramped 10 °C/min to 300 °C (2 min) Secondary oven maintained +5 °C relative to primary oven
Modulation	2s with temperature maintained +15 °C relative to secondary oven
Mass Spectrometer	**LECO *Pegasus* BT 4D**
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-600
Acquisition Rate	10 spectra/s (1D); 200 spectra/s (2D)

GC-TOFMS Results: Cannabinoids in a CBD Botanical

Name	*Formula*	*R.T. (s)*	*Mass Delta (Da)*	*Similarity*
Cannabidivarol	$C_{19}H_{26}O_2$	1342	0.01	882
Cannabidiol	$C_{21}H_{30}O_2$	1445	0.07	905
Delta 8-THC	$C_{21}H_{30}O_2$	1483	0.01	837
Delta 9-THC	$C_{21}H_{30}O_2$	1491	-0.01	928
Cannabigerol	$C_{21}H_{32}O_2$	1510	0.01	900
Cannabinol	$C_{21}H_{26}O_2$	1522	0.01	902
Cannabichromene	$C_{21}H_{30}O_2$	1578	N/A	873

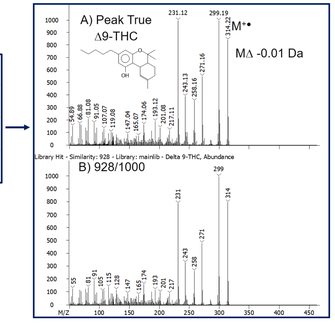
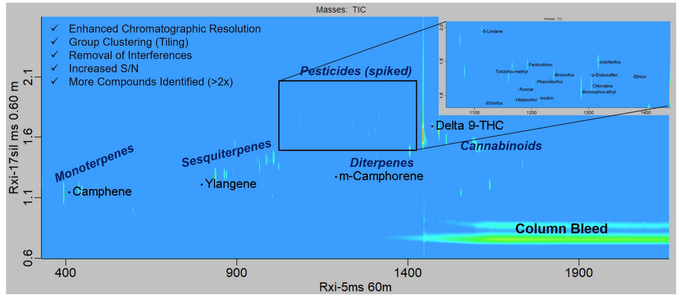
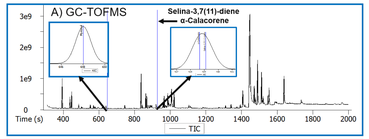


Figure 1. List of representative cannabinoids with Peak True (deconvoluted) and NIST library spectra for Delta 9-THC (A, B).

GCxGC-TOFMS Results: Contour Plot Showing Separation of a Variety of Compound Classes

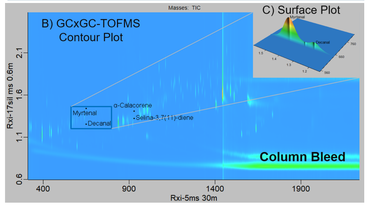


GC→ GCxGC-TOFMS – More than 2x More Compounds Confidently Identified



GC-TOFMS

Name	*Formula*	*R.T. (s)*	*Similarity*
Decanal	$C_{10}H_{20}O$	Not Found	
Myrtenal	$C_{10}H_{14}O$	648	745
Selina-3,7(11)-diene	$C_{15}H_{24}$	929	805
α-Calacorene	$C_{15}H_{20}$	929	878



GCxGC-TOFMS

Name	*R.T. (s)*	*Similarity*
Decanal	648 s, 1.262 s	723
Myrtenal	648 s, 1.445 s	890
Selina-3,7(11)-diene	928 s, 1.323 s	869
α-Calacorene	928 s, 1.414 s	881

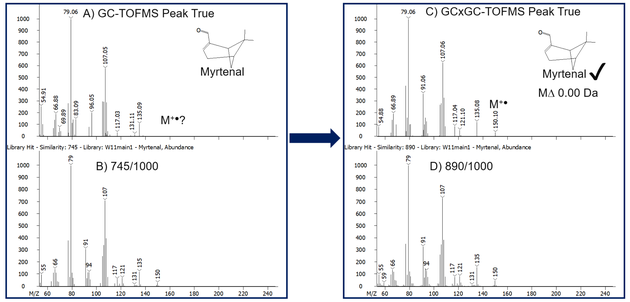


Figure 2. GC (A) vs GCxGC (C) deconvoluted and Wiley library mass spectra (B, D) for myrtenal.

GCxGC-TOFMS – Representative Compounds in a CBD Botanical

Name	*Formula*	*R.T. (s)*	*Similarity*	*Mass Δ (Da)*
2-Heptanone	$C_7H_{14}O$	348 s, 1.240 s	958	0.00
p-Xylene	C_8H_{10}	354 s, 1.250 s	962	0.00
Nonane	C_9H_{20}	356 s, 1.061 s	968	0.00
Heptanal	$C_7H_{14}O$	358 s, 1.233 s	915	0.00
2-Pinene	$C_{10}H_{16}$	394 s, 1.136 s	966	0.00
Camphene	$C_{10}H_{16}$	410 s, 1.150 s	964	0.00
(3E)-3-Hexene-2,5-dione	$C_6H_8O_2$	414 s, 1.553 s	968	0.02
Benzaldehyde	C_7H_6O	420 s, 1.517 s	972	0.00
ß-Pinene	$C_{10}H_{16}$	438 s, 1.170 s	956	0.00
ß-Myrcene	$C_{10}H_{16}$	448 s, 1.154 s	956	0.00
Octanal	$C_8H_{16}O$	458 s, 1.242 s	945	N/A
3-Carene	$C_{10}H_{16}$	470 s, 1.170 s	867	0.00
a-Terpinene	$C_{10}H_{16}$	476 s, 1.184 s	856	0.00
trans-3-Caren-2-ol	$C_{10}H_{16}O$	484 s, 1.222 s	882	N/A
D-Limonene	$C_{10}H_{16}$	488 s, 1.181 s	972	0.00
2-Thujene	$C_{10}H_{16}$	490 s, 1.199 s	895	0.00
Benzenemethanol	C_7H_8O	492 s, 1.523 s	941	0.00
ß-Ocimene	$C_{10}H_{16}$	502 s, 1.176 s	958	0.00
p-Cresol	C_7H_8O	508 s, 1.454 s	830	0.00
Benzene, 2-ethyl-1,4-dimethyl-	$C_{10}H_{14}$	516 s, 1.247 s	825	0.00
o-Xylene, 4-ethyl-	$C_{10}H_{14}$	542 s, 1.272 s	926	0.00
Fenchone	$C_{10}H_{16}O$	546 s, 1.321 s	876	0.00
Nonanal	$C_9H_{18}O$	556 s, 1.245 s	918	N/A
Benzene, 1-ethyl-2,3-dimethyl-	$C_{10}H_{14}$	562 s, 1.307 s	921	0.00
Phenylethyl Alcohol	$C_8H_{10}O$	568 s, 1.547 s	862	0.00
Benzene, 1,2,3,4-tetramethyl-	$C_{10}H_{14}$	572 s, 1.294 s	892	0.00
Terpinen-4-ol	$C_{10}H_{18}O$	628 s, 1.312 s	866	0.00
Naphthalene	$C_{10}H_8$	638 s, 1.571 s	969	0.00
a-Terpineol	$C_{10}H_{18}O$	640 s, 1.340 s	953	-0.03
Decanal	$C_{10}H_{20}O$	648 s, 1.262 s	723	N/A
Myrtenal	$C_{10}H_{14}O$	648 s, 1.445 s	890	0.00

Name	*Formula*	*R.T. (s)*	*Similarity*	*Mass Δ (Da)*
(-)-Verbenone	$C_{10}H_{14}O$	660 s, 1.482 s	919	0.00
Citronellol	$C_{10}H_{20}O$	666 s, 1.276 s	838	-0.01
3-Decen-1-ol, (Z)-	$C_{10}H_{20}O$	692 s, 1.289 s	919	0.05
Nonanoic acid	$C_9H_{18}O_2$	694 s, 1.273 s	898	-0.01
Naphthalene, 2-methyl-	$C_{11}H_{10}$	734 s, 1.556 s	875	0.00
Naphthalene, 1-methyl-	$C_{11}H_{10}$	750 s, 1.584 s	799	0.00
Ylangene	$C_{15}H_{24}$	796 s, 1.216 s	922	0.00
Isocaryophyllene	$C_{15}H_{24}$	838 s, 1.270 s	961	0.00
trans-a-Bergamotene	$C_{15}H_{24}$	844 s, 1.216 s	952	0.00
(E)-ß-Famesene	$C_{15}H_{24}$	854 s, 1.220 s	953	0.00
a-Humulene	$C_{15}H_{24}$	864 s, 1.292 s	961	0.00
Alloaromadendrene	$C_{15}H_{24}$	870 s, 1.284 s	953	0.00
ß-Selinene	$C_{15}H_{24}$	888 s, 1.302 s	943	0.00
7-Cadinene	$C_{15}H_{24}$	906 s, 1.314 s	955	0.00
(E)-a-Bisabolene	$C_{15}H_{24}$	920 s, 1.260 s	953	0.00
Selina-3,7(11)-diene	$C_{15}H_{24}$	928 s, 1.323 s	869	0.00
a-Calacorene	$C_{15}H_{20}$	928 s, 1.414 s	881	0.00
2-Ethyl-3-methylnaphthalene	$C_{13}H_{14}$	932 s, 1.556 s	892	0.00
a-Bisabolol	$C_{15}H_{26}O$	1022 s, 1.357 s	933	0.01
Anthracene	$C_{14}H_{10}$	1096 s, 1.860 s	913	0.00
m-Camphorene	$C_{20}H_{32}$	1188 s, 1.273 s	870	0.00
p-Camphorene	$C_{20}H_{32}$	1208 s, 1.293 s	894	0.00
Bromofos	$C_8H_8BrCl_2O_3PS$	1238 s, 1.721 s	875	0.02
Cannabichromene	$C_{21}H_{30}O_2$	1418 s, 1.587 s	920	0.07
Hexadecanal	$C_{16}H_{32}O$	1434 s, 1.238 s	934	N/A
Behenic alcohol	$C_{22}H_{46}O$	1464 s, 1.238 s	920	N/A
Delta 9-THC	$C_{21}H_{30}O_2$	1470 s, 1.694 s	931	0.02
Cannabigerol	$C_{21}H_{32}O_2$	1512 s, 1.584 s	939	0.02
7-Tocopherol	$C_{28}H_{48}O_2$	1724 s, 1.816 s	831	0.04
ß-Amyrin	$C_{30}H_{50}O$	1964 s, 1.256 s	894	0.05
a-Amyrin	$C_{30}H_{50}O$	2008 s, 1.655 s	899	0.03

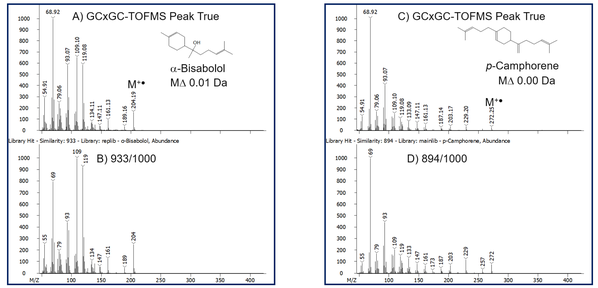


Figure 3. Deconvoluted and NIST library mass spectra for α-bisabolol (A, B) and p-camphorene (C, D) obtained using GCxGC .

Summary

- Simple liquid extraction methods can be used to prepare cannabis products for analysis by gas chromatography techniques.
- Comprehensive profiling of cannabis is an attractive alternative to targeted, panel-based methods.
- Compound identification through increased chromatographic resolution and high performance mass spectrometry (GCxGC-TOFMS) leads to far more compounds confidently identified.
- Identification was accomplished using a combination of MS database searches and mass Δ calculations.