Fingerprinting the Terpene Profiles of Various *Cannabis* Strains using GC and GCxGC with High Performance TOFMS

David E. Alonso[1], Julie Kowalski[2], and Joseph E. Binkley[1] | [1]LECO Corporation, St. Joseph, Michigan; [2]Trace Analytics, Spokane, Washington

Introduction

- *Cannabis* is a complex mixture: terpenes, cannabinoids, flavonoids, etc.
- Medicinal uses include treatment of chronic pain, multiple sclerosis, epilepsy, anxiety, and cancer.
- Its total composition is important in determining potency and medical effectiveness (Entourage Effect).
- In this study, a novel analytical approach was utilized for the effective characterization of terpenes in different *cannabis* strains.

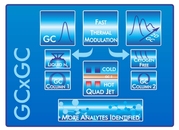
Investigative Objectives

- Implement the use of enhanced, comprehensive two-dimensional gas chromatography (GCxGC) for the separation of *cannabis* terpenes.
- Use a benchtop, high performance time-of-flight mass spectrometer and powerful processing software to quickly and confidently identify terpenes and other *cannabis* compounds.
- Use software tools to compare different *cannabis* strains.

Sample Preparation

1) Distillates from 23 *cannabis* strains and over 40 terpene standards were obtained from a collaborating test facility.
2) Samples were diluted in isopropanol and transferred to 2 mL GC vials for analysis.

Data Acquisition & Processing



LECO Pegasus® BT 4D

Table 1. Instrument acquisition parameters

Gas Chromatograph	Agilent 7890, LECO Dual Stage Quad Jet Modulator and L-PAL 3 Autosampler
Injection	0.5 µL, Split 250:1; 250 °C
Carrier Gas	He @ 1.4 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 (1 min), ramped 10 °C/min to 325 °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)

Terpene Standard Results: GC vs. GCxGC-TOFMS

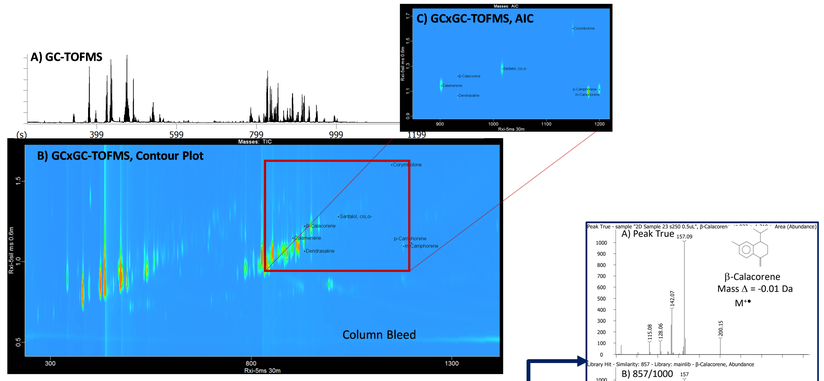


Figure 1. A) GC-TOFMS TIC for *Indica* terpenes B) GCxGC-TOFMS plot & expansion showing separated sesquiterpenes, dendrasline, and β-calacorene (Coelute in GC-TOFMS TIC).

Table 2. Comparison of GC & GCxGC-TOFMS spectral similarity values for *cannabis* terpenes (Unknowns → Knowns)

GC-TOFMS

Name	*Formula*	*R.T. (s)*	*Similarity*
Calamenene	$C_{15}H_{22}$	898.9	734
Dendrasaline	$C_{15}H_{22}O$	936.9	485
β-Calacorene	$C_{15}H_{20}$	Not	Found
Santalol, cis,α-	$C_{15}H_{24}O$	1013.6	551
Corymbolone	$C_{15}H_{24}O_2$	1146.0	738
m-Camphorene	$C_{20}H_{32}$	1175.0	902
p-Camphorene	$C_{20}H_{32}$	1199.4	428

GCxGC-TOFMS

R.T. (s)	*Similarity*
903 s, 1.145 s	901
933 s, 1.064 s	799
933 s, 1.219 s	857
1017 s, 1.281 s	797
1149 s, 1.598 s	849
1179 s, 1.096 s	943
1199 s, 1.113 s	936

Figure 2. GCxGC-TOFMS A) Peak True and B) library mass Spectra for β-calacorene. Not found in GC-TOFMS.

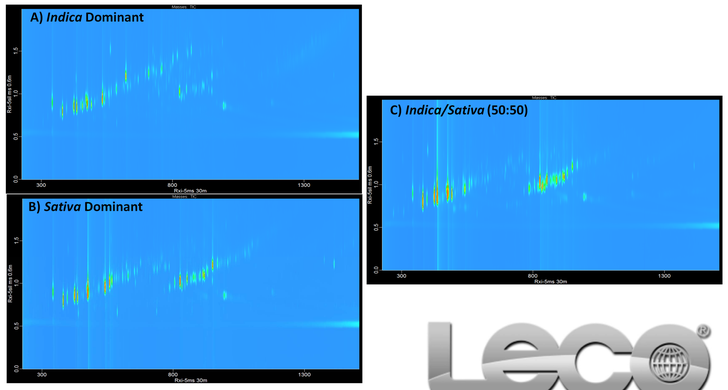


Figure 3. Terpene Contour plots (Fingerprints) for commercially available *cannabis* products: A) *Indica* dominant, B) *Sativa* dominant and C) 50:50 mixture.

GCxGC-TOFMS *Cannabis* Analysis: Representative Compounds

Table 3. Representative list of compounds in *C. indica* with retention times and spectral similarity values

Name	*Formula*	*R.T. (s)*	*Mass Δ (Da)*	*Similarity*
Toluene	C_7H_8	233 s, 0.811 s	-0.01	961
2-Butenal, 3-methyl-	C_5H_8O	237 s, 0.972 s	0.00	980
Octane	C_8H_{18}	249 s, 0.665 s	-0.01	850
Piperidine, 1-butyl-	$C_9H_{19}N$	253 s, 0.903 s	0.12	951
2,4-Dimethyl-1-heptene	C_9H_{18}	287 s, 0.702 s	-0.01	886
1-Octene, 4-methyl-	C_9H_{18}	301 s, 0.693 s	N/A	948
Octane, 4-methyl-		309 s, 0.690 s	-0.01	949
Trans-3-methylpent-3-ene-5-ol	$C_6H_{12}O$	325 s, 0.569 s	0.00	992
2-Heptanone	$C_7H_{14}O$	335 s, 0.915 s	-0.01	977
Heptanal	$C_7H_{14}O$	345 s, 0.921 s	-0.01	926
Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$	359 s, 0.856 s	0.10	961
2-Butenoic acid, 3-methyl-, ethyl ester	$C_7H_{12}O_2$	369 s, 0.945 s	-0.01	970
2(5H)-Furanone, 5,5-dimethyl-	$C_6H_8O_2$	401 s, 1.353 s	-0.03	955
2-Methylthioacetic acid	$C_3H_6O_2S$	409 s, 1.259 s	0.02	963
5-Hepten-2-one, 6-methyl-	$C_8H_{14}O$	431 s, 0.994 s	-0.01	873
5-Norbornen-2-ol	$C_7H_{10}O$	433 s, 1.187 s	-0.01	944
Aniline	C_6H_7N	435 s, 0.954 s	0.01	916
cis-2-(2-Pentenyl)furan	$C_9H_{14}O$	445 s, 0.930 s	0.00	802
Glutaranilic acid	$C_{11}H_{13}NO_3$	459 s, 0.796 s	-0.14	873
1,4-Cyclohex-2-enedione	$C_6H_6O_2$	459 s, 1.707 s	-0.01	947
N-Allyl-N,N-dimethylamine	$C_5H_{11}N$	489 s, 0.725 s	0.00	998
Phenol, 4-(2-methylpropyl)-	$C_{10}H_{14}O$	507 s, 0.932 s	-0.01	835
1-Octanol	$C_8H_{18}O$	511 s, 0.925 s	N/A	863
Pyrimidine, 4,6-dimethyl-	$C_6H_8N_2$	515 s, 1.186 s	-0.03	848
6-Methyl-3,5-heptadiene-2-one	$C_8H_{12}O$	547 s, 1.154 s	-0.01	951
Limona ketone	$C_8H_{12}O$	573 s, 1.159 s	-0.01	901
Isobutyl caproate	$C_{10}H_{20}O_2$	587 s, 0.909 s	N/A	952
γ-Heptalactone	$C_7H_{12}O_2$	591 s, 0.752 s	N/A	887
1H-Pyrazole, 1,3-dimethyl-	$C_5H_8N_2$	593 s, 1.192 s	-0.02	837
1-Hexyl butyrate	$C_{10}H_{20}O_2$	623 s, 0.942 s	N/A	867
3-Methylacetophenone	$C_9H_{10}O$	623 s, 1.331 s	-0.01	948
Crypton	$C_9H_{10}O$	625 s, 1.268 s	0.00	911
Naphthalene	$C_{10}H_8$	625 s, 1.345 s	-0.01	911
Dodecane	$C_{12}H_{26}$	631 s, 0.773 s	-0.01	883
Acetic acid, octyl ester	$C_{10}H_{20}O_2$	641 s, 0.943 s	N/A	963
Benzofuran, 7-methoxy-	$C_9H_8O_2$	639 s, 1.100 s	0.03	859
Benzothiazole	C_7H_5NS	661 s, 1.572 s	-0.01	975
3-Isopropylbenzaldehyde	$C_{10}H_{12}O$	673 s, 1.260 s	0.00	883
Isopentyl hexanoate	$C_{11}H_{22}O_2$	675 s, 0.940 s	N/A	935
3-Ethyl-2-hexene	C_8H_{16}	683 s, 1.721 s	-0.09	915
1H-Pyrazole-4-carbonitrile	$C_4H_3N_3$	699 s, 0.605 s	0.03	980
2,6-Heptanedione	$C_7H_{12}O_2$	783 s, 1.344 s	N/A	843
Oxazole, 2-ethyl-4,5-dihydro-	C_5H_9NO	863 s, 0.976 s	0.00	943
Ethyl 4-ethoxybenzoate	$C_{11}H_{14}O_3$	897 s, 1.339 s	0.00	954
Hexadecane	$C_{16}H_{34}$	927 s, 0.814 s	N/A	788
Furo[3,4-b]pyrazine-5,7-dione	$C_6H_4N_2O_3$	933 s, 0.607 s	0.09	812
Butanal, 3-hydroxy-	$C_4H_8O_2$	1021 s, 0.897 s	N/A	940
Heptadecane	$C_{17}H_{36}$	1023 s, 0.819 s	N/A	806
Octadecane	$C_{18}H_{38}$	1093 s, 0.838 s	N/A	864
(E)-2-Pentenenitrile	C_5H_7N	1201 s, 0.725 s	0.27	996
Pyrene	$C_{16}H_{10}$	1255 s, 1.994 s	0.00	879
Docosane	$C_{22}H_{46}$	1313 s, 0.894 s	N/A	877
Heptacosane	$C_{27}H_{56}$	1549 s, 0.977 s	N/A	877
2-Ethylisovaleraldehyde	$C_7H_{14}O$	1805 s, 1.522 s	N/A	966

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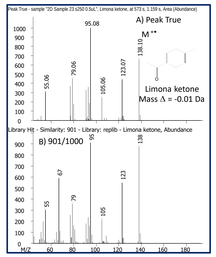
Table 4. Representative list of terpenes in *C. indica* with retention times and spectral similarity values

Name	*Formula*	*R.T. (s)*	*Area*	*Mass Δ (Da)*	*Similarity*
α-Thujene	$C_{10}H_{16}$	373 s, 0.784 s	13388350	-0.01	954
α-Pinene	$C_{10}H_{16}$	383 s, 0.808 s	68418847	0.00	967
Camphene	$C_{10}H_{16}$	397 s, 0.828 s	78914464	-0.01	973
β-Pinene	$C_{10}H_{16}$	425 s, 0.868 s	678661250	0.00	965
β-Myrcene	$C_{10}H_{16}$	435 s, 0.908 s	407419967	0.03	937
α-Phellandrene	$C_{10}H_{16}$	451 s, 0.869 s	12521026	-0.01	916
3-Carene	$C_{10}H_{16}$	457 s, 0.852 s	29705486	-0.01	935
α-Terpinene	$C_{10}H_{16}$	463 s, 0.868 s	3134640	-0.01	812
2-Menthene	$C_{10}H_{18}$	467 s, 0.837 s	475612	0.00	846
p-Cymene	$C_{10}H_{14}$	471 s, 0.939 s	203103101	-0.01	987
D-Limonene	$C_{10}H_{16}$	475 s, 0.924 s	430592997	-0.03	931
β-Phellandrene	$C_{10}H_{16}$	477 s, 0.889 s	96796169	-0.01	937
Eucalyptol	$C_{10}H_{18}O$	479 s, 0.916 s	65402054	0.00	937
β-Ocimene	$C_{10}H_{16}$	481 s, 0.866 s	25381328	0.00	947
m-Cymene	$C_{10}H_{14}$	485 s, 0.911 s	278381	-0.01	834
α-Ocimene	$C_{10}H_{16}$	491 s, 0.887 s	287724336	0.00	967
cis-Sabinene hydrate	$C_{10}H_{18}O$	513 s, 0.937 s	4198026	-0.01	943
Terpinolene	$C_{10}H_{16}$	533 s, 0.923 s	21973373	-0.01	936
Fenchone	$C_{10}H_{16}O$	535 s, 1.048 s	56410861	0.00	986
Linalool	$C_{10}H_{18}O$	541 s, 0.945 s	122086671	0.00	926
Perillene	$C_{10}H_{14}O$	543 s, 0.951 s	49131901	0.00	947
cis-Pinen-3-ol	$C_{10}H_{16}O$	551 s, 1.037 s	2281959	N/A	793
Fenchol	$C_{10}H_{18}O$	557 s, 0.999 s	55025158	0.00	987
Myrcenole	$C_{10}H_{16}O$	579 s, 1.014 s	9528563	0.04	887
trans-Pinocarveol	$C_{10}H_{16}O$	583 s, 1.057 s	7778044	N/A	843
L-camphor	$C_{10}H_{16}O$	589 s, 1.150 s	695704	-0.01	917
Pinocarvone	$C_{10}H_{14}O$	605 s, 1.183 s	1327877	-0.01	917
Borneol	$C_{10}H_{18}O$	607 s, 1.070 s	9611351	-0.01	973
α-Terpineol	$C_{10}H_{18}O$	629 s, 1.081 s	12095260	N/A	945
Coxmen-2-ol	$C_{10}H_{18}O$	639 s, 1.084 s	3643945	-0.01	887
Carveol	$C_{10}H_{16}O$	653 s, 1.136 s	1911682	0.01	849
Carvone	$C_{10}H_{14}O$	675 s, 1.243 s	2065844	0.00	942
Isomyrcenol	$C_{10}H_{18}O$	723 s, 1.188 s	3122238	N/A	843
p-Mentha-1,4-dien-7-ol	$C_{10}H_{16}O$	731 s, 1.199 s	2405894	N/A	831
α-Cubebene	$C_{15}H_{24}$	765 s, 0.925 s	7336729	0.00	935
Copaene	$C_{15}H_{24}$	789 s, 0.955 s	32568210	0.00	939
(+)-Sativen	$C_{15}H_{24}$	803 s, 0.978 s	1107397	0.00	888
(±)-β-Isocomene	$C_{15}H_{24}$	805 s, 1.014 s	16996864	0.00	850
trans-α-Bergamotene	$C_{15}H_{24}$	817 s, 0.974 s	26143508	0.00	957
α-Santalene	$C_{15}H_{24}$	821 s, 0.966 s	28314886	0.00	956
Caryophyllene	$C_{15}H_{24}$	827 s, 1.026 s	183078148	0.00	976
α-Guaiene	$C_{15}H_{24}$	837 s, 0.989 s	67059903	0.00	949
(E)-β-Farnesene	$C_{15}H_{24}$	843 s, 0.967 s	107387589	0.01	940
Humulene	$C_{15}H_{24}$	853 s, 1.055 s	30692582	0.00	989
Alloaromadendrene	$C_{15}H_{24}$	859 s, 1.050 s	2716555	0.00	928
γ-Muurolene	$C_{15}H_{24}$	867 s, 1.050 s	3004124	0.00	950
β-Selinene	$C_{15}H_{24}$	877 s, 1.075 s	25274135	0.00	967
β-Bisabolene	$C_{15}H_{24}$	887 s, 1.008 s	19223322	0.00	947
α-Bulnesene	$C_{15}H_{24}$	889 s, 1.056 s	58925547	0.00	937
γ-Cadinene	$C_{15}H_{24}$	895 s, 1.085 s	6074040	0.00	921
Guaia-3,9-diene	$C_{15}H_{24}$	903 s, 1.081 s	33816955	0.00	870
Calamenene	$C_{15}H_{22}$	903 s, 1.145 s	142666609	N/A	901
Selina-3,7(11)-diene	$C_{15}H_{24}$	919 s, 1.089 s	90112337	0.00	927
Germacrene B	$C_{15}H_{24}$	931 s, 1.136 s	37709215	0.00	944
Dendrasaline	$C_{15}H_{22}O$	933 s, 1.064 s	750552	-0.05	799
β-Calacorene	$C_{15}H_{20}$	933 s, 1.219 s	284816	-0.01	857
Caryophyllene oxide	$C_{15}H_{24}O$	949 s, 1.212 s	39083444	0.01	975
cis-2-α-Bisabolene epoxide	$C_{15}H_{24}O$	983 s, 1.239 s	2262152	0.00	799
Santalol, cis,α-	$C_{15}H_{24}O$	1017 s, 1.281 s	992953	N/A	797
Corymbolone	$C_{15}H_{24}O_2$	1149 s, 1.598 s	210905	0.00	849
m-Camphorene	$C_{20}H_{32}$	1179 s, 1.096 s	7314409	0.01	943
p-Camphorene	$C_{20}H_{32}$	1199 s, 1.113 s	2581166	0.02	936



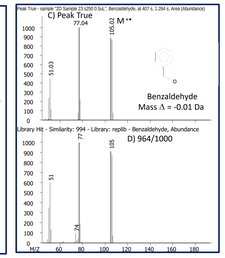
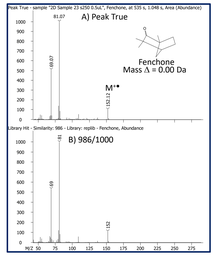


Figure 4. *C. indica* GCxGC-TOFMS Peak True spectra, library mass spectra for Limona ketone (A/B) and benzaldehyde (C/D).



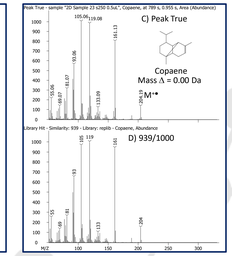


Figure 5. *C. indica* GCxGC-TOFMS Peak True (deconvoluted) and library mass spectra for fenchone (A/B) and copaene (C/D).

Statistical Analysis of Terpene GCxGC-TOFMS Data

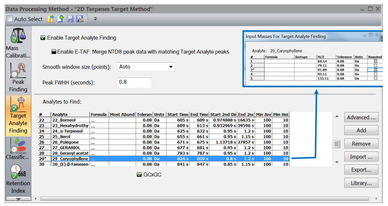
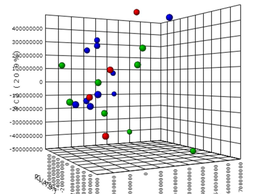


Figure 6. Two-Dimensional Target Analyte Finding (TAF) processing method for rapid and robust identification of terpenes in *cannabis* contour plots.

A) PCA Plot



B) Heat Map

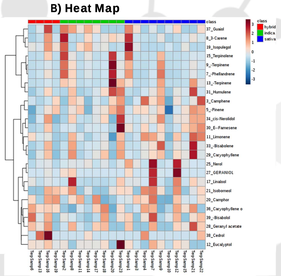


Fig 7. Statistical processing results for 23 samples, 39 terpenes (m/z/rt) data matrix: A) PCA plot illustrates the lack of correlation between *cannabis* strain designations and terpene composition. B) A heat map displaying terpene variability in *indica* (green), *sativa* (blue), and hybrid strains (red).

Summary

- The Pegasus BT 4D facilitates fast and confident *cannabis* product "fingerprinting" through enhanced two-dimensional chromatographic resolution and high performance TOFMS.
- Robust compound identification was achieved through spectral similarity searches of large, well-established databases, mass Δ determinations, and retention index filtering.
- Statistical processing of *cannabis* distillates resulted in no group clustering, suggesting that different products contained similar types and concentrations of terpenes.
- Alternative sample preparation techniques will be explored to increase extraction yields and include a majority of *cannabis* components to more effectively study the entourage effect in medical marijuana.

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