Application Note

Instrument: Pegasus® BT 4D

LECO EMPOWERING RESULTS

Determining Terpene Profiles of Cannabis Strains Using GC and GCxGC with High Performance TOFMS

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Key Words: Pegasus BT, Pegasus BT-4D, Source Stability, Reproducibility, High Matrix

Introduction

Cannabis is a complex mixture of compounds (>500) including cannabinoids, terpenes, terpenoids, non-cannabinoid phenols, nitrogenous compounds, flavonoids, and contaminants such as residual solvents and pesticides. It is the total composition of cannabis that is important in determining its potency and medicinal effectiveness. This "entourage effect" is a synergistic relationship that exists between terpenes, cannabinoids, and potentially other cannabis components. Identification of cannabis components is critical for the "chemical categorization" of different cannabis strains. In this study, a novel analytical approach was utilized for the effective characterization of terpenes in different cannabis strains. The preliminary results of this "terpene fingerprinting" (Fig. 1) are the basis for larger studies that will comprehensively profile cannabis plants and lead to a shift from cultivar to chemical classification of marijuana.

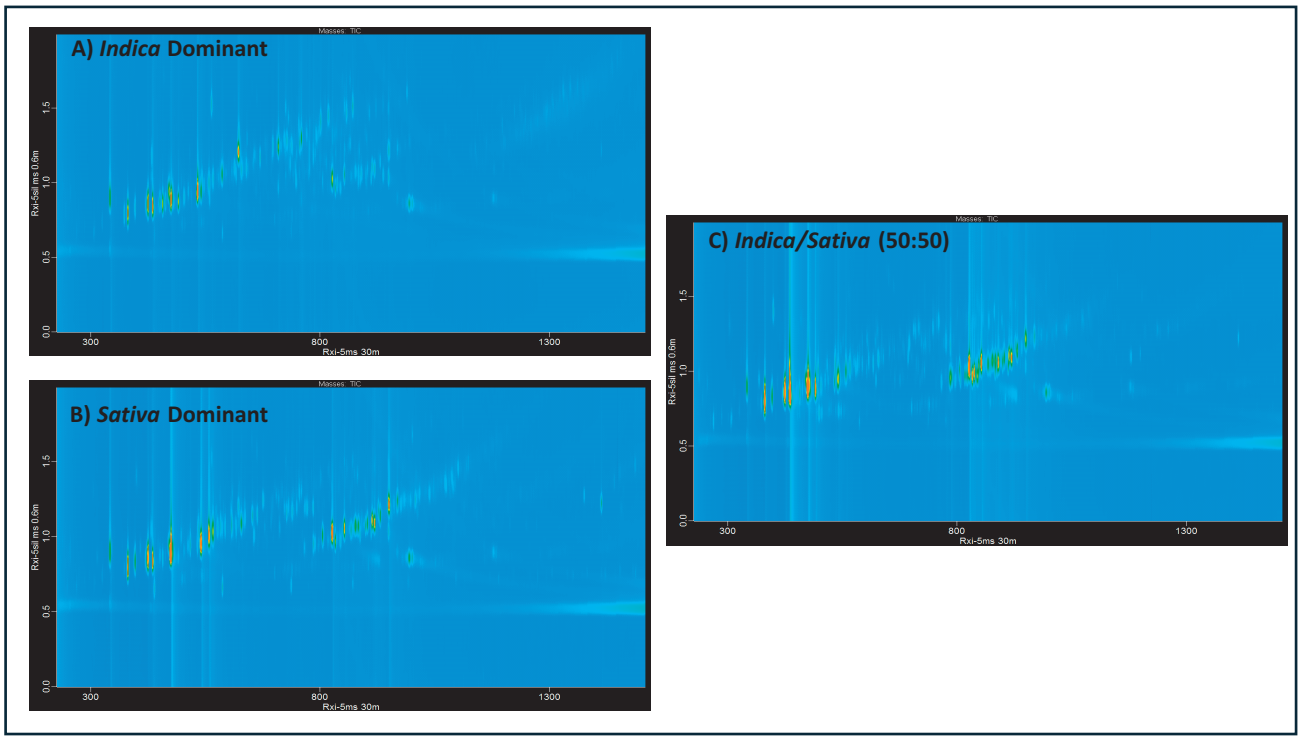

Figure 1. Terpene contour plots (Fingerprints) for commercially available Cannabis products: A) Indica dominant, B) sativa dominant, and C) a 50:50 mixture of indica/sativa.

Experimental

Distillates from 23 cannabis strains and over 40 terpene standards were obtained from a collaborating test facility. These distillates and standards were diluted in isopropanol and transferred to 2mL GC vials for analysis under conditions shown in Table 1. Gas chromatography and comprehensive two-dimensional gas chromatography (GCxGC) with high-performance TOFMS were employed to profile various cannabis strains. Sample analyses were relatively fast, reproducible, and provided excellent chromatographic resolution. Automated peak finding with NonTarget Deconvolution® (NTD®) provided mass spectra which were searched against large, well-established spectral libraries (e.g., NIST 17, Wiley 11) to facilitate confident identification of individual sample components. In addition, terpene standards were analyzed and retention times along with spectral information used for the development of an internal library and 2D target analyte finding method. Statistical analyses of the resulting data were performed to identify markers for potential differentiation of cannabis strains.

Table 1. GC-High Performance TOFMS *(Pegasus* BT 4D) Conditions

Gas Chromatograph	**LECO GCxGC Quad Jet Thermal Modulator & L-PAL 3 Autosampler**
Injection	0.5 µL, Split 250:1 @ 250°C
Carrier Gas	He @ 1.4 mL/min, Constant Flow
Column 1	Rxi-5 Sil MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Column 2	Rxi-17 Sil MS, 0.6 m x 0.25 mm i.d., x 0.25µm(Restek, Bellefonte, PA, USA)
Temperature Program	40 °C (1 min), to 325 °C @ 10°C/min (2 min) Secondary oven maintained +5°C relative to primary oven
Modulation	2s with temperature maintained +15°C relative to primary oven
Mass Spectrometer	**LECO (*Pegasus* BT 4D)**
Transfer Line	300 °C
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-600
Acquisition Rate	10 spectra/s (1D); 200 spectra/s (2D)

Results and Discussion

GC-TOFMS and GCxGC-TOFMS were used to analyze terpenes from different strains of cannabis. GCxGC-TOFMS analysis resulted in a >4x increase in peaks detected and >50% increase in terpenes identified over those found with single dimension GC-MS. This additional information was a direct result of coupling enhanced chromatography with high performance TOFMS (Figure 2). For example, sesquiterpenes dendrasaline ($t_{R1,2}$ = 933, 1.06s) and β-calacorene ($t_{R1,2}$ = 933, 1.22s) coeluted perfectly in the 1st dimension, but were separated in the 2nd dimension allowing for robust identification of these cannabis terpenes using GCxGC-TOFMS. The overall result is a significant increase in spectral similarity values for terpenes identified using GCxGC-TOFMS (Ave. Similarity = 869/1000) versus GC-TOFMS data (Ave. Similarity = 548/1000, Table 2).

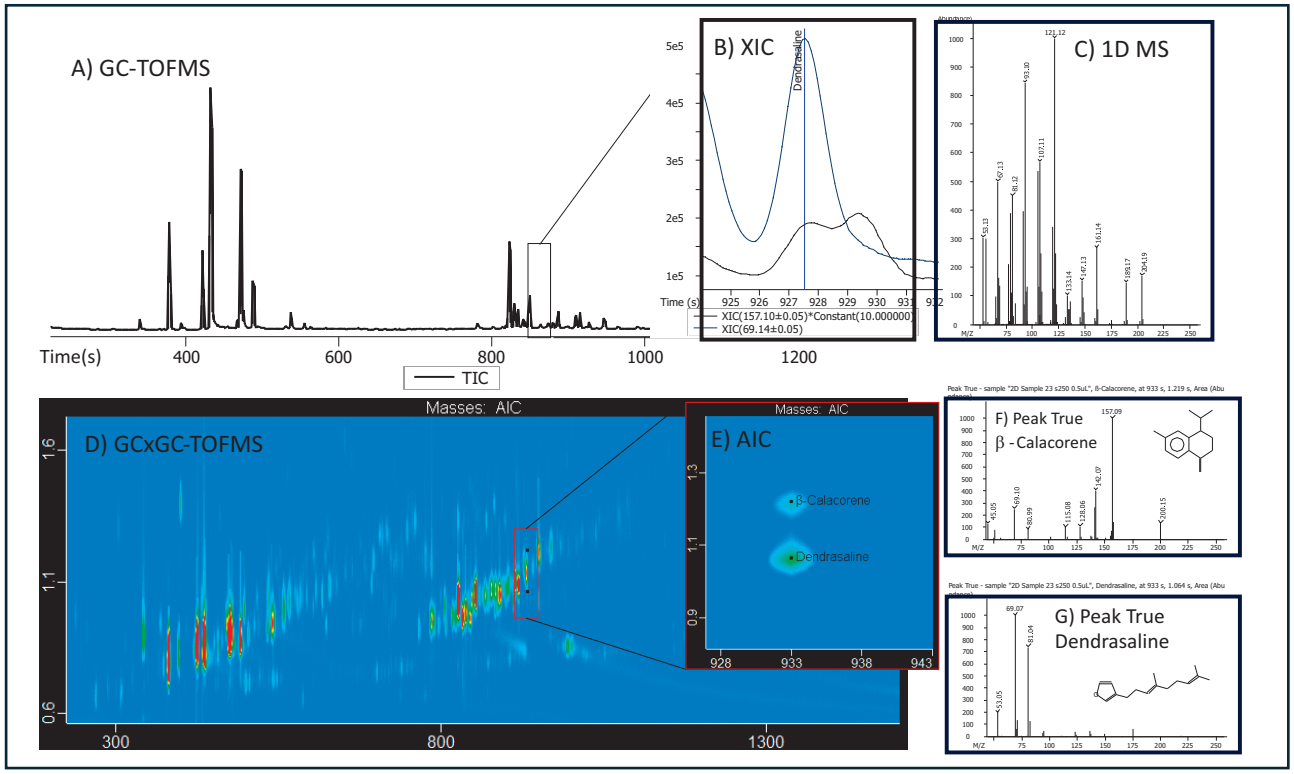

Figure 2. A) GC-TOFMS TIC for indica terpenes; B) Extracted ion expansion showing coelution in 1D GC-MS; C) Spectrum derived from 1D GC-MS coelution; D) GCxGC-TOFMS contour plot; E) Expansion showing GCxGC separated sesquiterpenes dendrasaline and β-calacorene which coeluted in 1D GC-TOFMS; F,G) Clean spectra for chromatographically separated β-calacorene and dendrasaline.

Table 2. Comparison of GC and GCxGC-TOFMS spectral similarity values for Cannabis terpenes. This demonstrates how GCxGC can convert unknowns from 1D GC-MS into knowns by providing cleaner, more searchable spectra.

	GC-TOFMS			GCxGC-TOFMS	
Name	***Formula***	***R.T. (s)***	***Similarity***	***R.T. (s)***	***Similarity***
Calamenene	$C_{15}H_{22}$	898.9	734	903 s, 1.145 s	901
Dendrasaline	$C_{15}H_{22}O$	936.9	485	933 s, 1.064 s	799
β-Calacorene	$C_{15}H_{20}$	Not	Found	933 s, 1.219 s	857
Santalol, cis,α-	$C_{15}H_{24}O$	1013.6	551	1017 s, 1.281 s	797
Corymbolone	$C_{15}H_{24}O_2$	1146.0	738	1149 s, 1.598 s	849
m-Camphorene	$C_{20}H_{32}$	1175.0	902	1179 s, 1.096 s	943
p-Camphorene	$C_{20}H_{32}$	1199.4	428	1199 s, 1.113 s	936

Representative compounds in an indica dominant sample not only consisted of terpenes and terpenoids, but also included alkanes, alkenes, aldehydes, esters, heterocyclic compounds, and polyaromatic hydrocarbons with average spectral similarity values of >900/1000 respectively for the sample (Tables 3 and 4). Compounds were confidently identified using automated peak find with NTD, spectral similarity, and mass Δ values as shown for fenchone and copaene in Figure 3. GCxGC-TOFMS profiling (fingerprinting) of cannabis plant distillates was an effective way to determine the presence and relative amounts of volatile and semi-volatile cannabis constituents to provide unique component maps for differentiation of cannabis strains.

Table 3. Representative list of terpenes in an indica dominant sample based on spectral similarity which averaged 916 out of 1000

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	$C_{10}H_{16}$	381 s, 0.808 s	684188347	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	407411967	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	29705496	-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	203310101	-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	65403054	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
Myroxide	$C_{10}H_{16}O$	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	9611931	-0.01	973
α-Terpineol	$C_{10}H_{18}O$	629 s, 1.081 s	12095260	N/A	945
Cosmen-2-ol	$C_{10}H_{16}O$	639 s, 1.084 s	2643945	-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	$C_{10}H_{16}O$	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	12568210	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	182078148	0.00	976
α-Guaiene	$C_{15}H_{24}$	837 s, 0.989 s	67059903	0.00	949
(E)-β-Famesene	$C_{15}H_{24}$	843 s, 0.967 s	107387589	0.01	940
Humulene	$C_{15}H_{24}$	853 s, 1.055 s	306927182	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	30024124	0.00	910
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	58923547	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, 1.085 s	32816955	0.00	870
Calamenene	$C_{15}H_{22}$	903 s, 1.145 s	14266609	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-Z-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	950918	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

Average: |Mass Δ|= 0.01, Spectral Similarity = 916/1000

Table 4. General list of compounds in an indica dominant sample based on spectral similarity which averaged 911 out of 1000

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-	C_9H_{20}	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	$C_9H_{12}O$	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	$C_9H_{14}O$	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	$C_9H_{14}O$	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-	$C_9H_8O_2$	659 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	$C_7H_{12}O_2$	781 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	$C_6H_2N_2O_3$	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	$C_{18}H_{38}$	1063 s, 0.838 s	N/A	873
Octadecane	$C_{18}H_{38}$	1077 s, 0.835 s	N/A	881
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

Average: |Mass Δ|= 0.01, Spectral Similarity = 911/1000

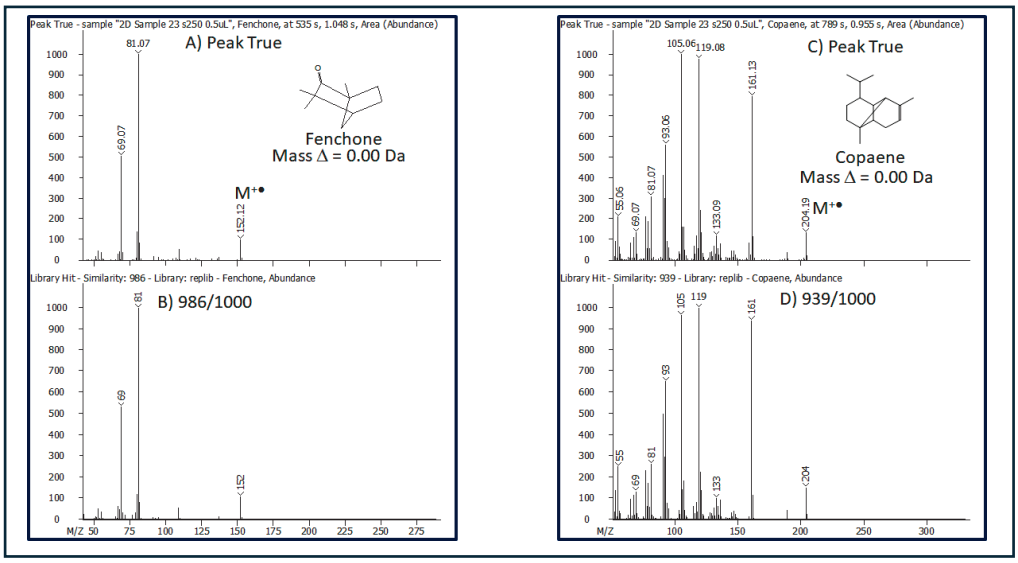

Figure 3. A) GC-TOFMS Peak True and Library Mass Spectra for fenchone (A/B) and copaene (C/D).

Specific terpenes were targeted in the comprehensive GCxGC-TOFMS data using two-dimensional Target Analyte Find processing (Fig. 4). Data for 23 samples (retention times, m/z values, and peak areas) were exported and processed using statistical software; unfortunately, this data did not display group clustering of related strains (Fig. 5). This implied that there is no correlation between product names, listed strain percentages, and terpene content.

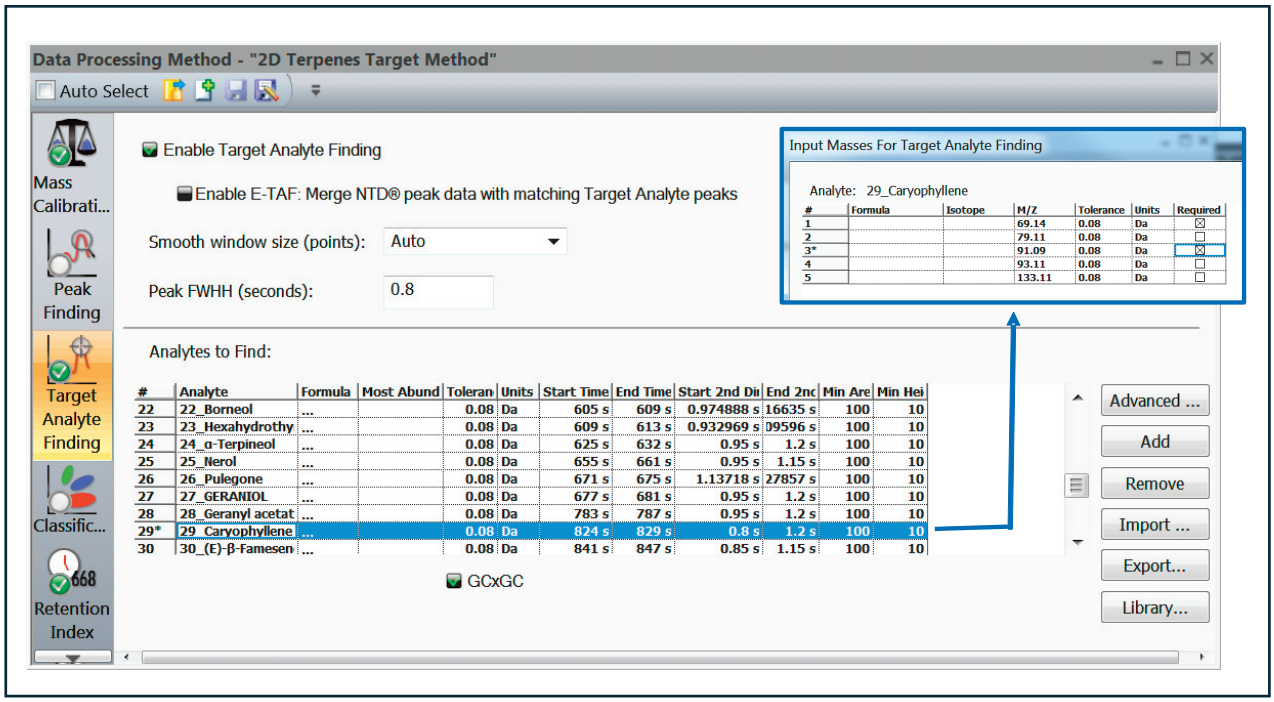

Figure 4. Two-dimensional Target Analyte Find (TAF) processing method for rapid and robust identification of terpenes in Cannabis contour plots.

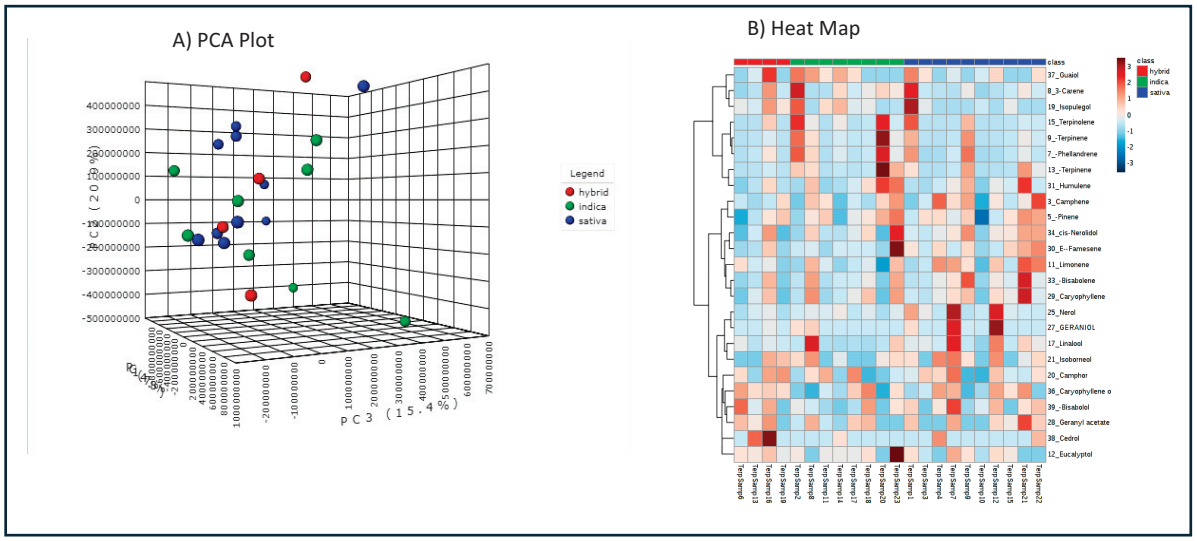

Figure 5. A) PCA plot illustrating the lack of correlation between Cannabis strain designations and terpene composition, B) A heat map displaying terpene variability in indica (green), sativa (blue), and mixed hybrid strains (red).

Conclusion

The *Pegasus* BT 4D facilitated fast and confident Cannabis product "fingerprinting" through enhanced two-dimensional chromatographic resolution and high performance TOFMS. Robust compound characterization was achieved through spectral similarity searches of large, well-established databases, and mass Δ values increased confidence in these determinations. Statistical processing of Cannabis strain distillates did not result in specific group clustering, suggesting that differently labeled products actually contained similar types and concentrations of terpenes. Alternative sample preparation techniques may be explored in future studies to increase extraction yields and include a greater portion of Cannabis components to more effectively study the entourage effect in medical marijuana.

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