Application Note

Instrument: Pegasus® BT 4D

Characterization and Comparison of Flavored CBD Beverages

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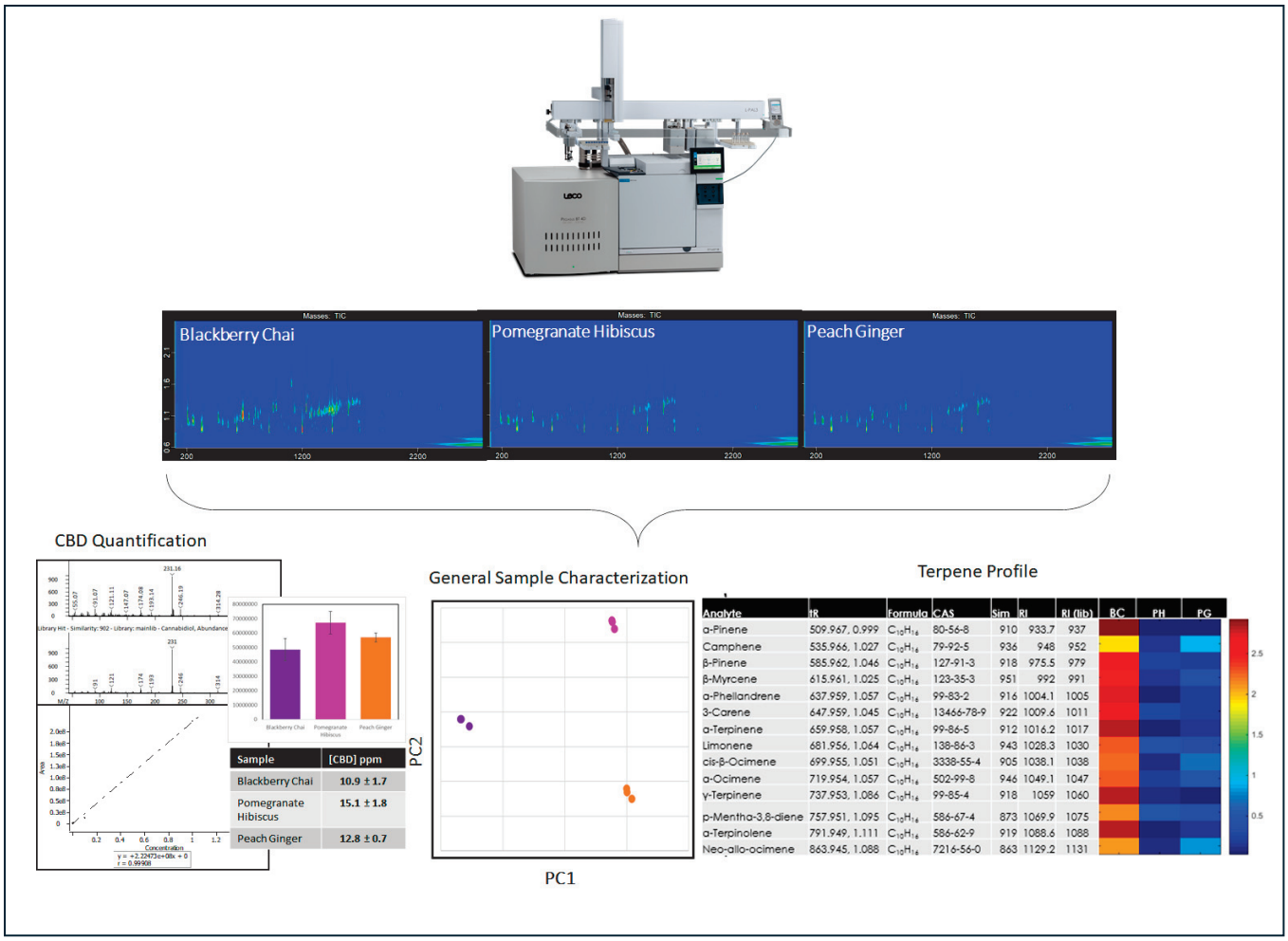
Key Words: CBD, Cannabis, Food, Beverages, Aroma Profile, Characterization, Comparison, Retention Index, GC, GCxGC, MS, TOFMS, Deconvolution

Introduction

There is a large and growing market around consumer products containing cannabidiol (CBD). CBD is a non-psychoactive cannabinoid extracted from cannabis that has been sold as an extract, infused into a variety of consumer products, and marketed for a range of uses. In recent years a wide variety of consumable CBD products have been developed and many analytical questions around these products remain. In particular, there are ongoing questions to the therapeutic potential of these types of products, which can relate to both the level of CBD and to the possible impact of other chemical constituents (for example, the terpenes) coextracted with the CBD. There are also ongoing flavor and aroma challenges with determining the most effective ways to mask or complement the distinct flavors of CBD extracts. Uncovering a more complete picture of the chemical profile is beneficial for addressing many of these analytical questions. Gas chromatography (GC) is well-suited for these objectives and this application note explores the benefits of extending the GC separation to a second dimension with comprehensive two-dimensional GC (GCxGC). Pairing this GCxGC separation with time-of-flight mass spectrometry (TOFMS) extends the benefits even further and allows for non-target analyte discovery, identification through library searching of full m/z range data, and deconvolution of the non-skewed spectra. In this application note, we demonstrate how a single GCxGC-TOFMS method can uncover a broad chemical profile that may be relevant to many of these analytical questions.



Analyte	tR	Formula	CAS	Sim	RI	RI (lib)	BC	PH	PG
α-Pinene	509.967, 0.999	$C_{10}H_{16}$	80-56-8	910	933.7	937			
Camphene	535.966, 1.027	$C_{10}H_{16}$	79-92-5	936	948	952			
β-Pinene	585.962, 1.046	$C_{10}H_{16}$	127-91-3	918	975.5	979			
β-Myrcene	615.961, 1.025	$C_{10}H_{16}$	123-35-3	951	992	991			
α-Phellandrene	637.959, 1.057	$C_{10}H_{16}$	99-83-2	916	1004.1	1005			
3-Carene	647.959, 1.045	$C_{10}H_{16}$	13466-78-9	922	1009.6	1011			
α-Terpinene	659.958, 1.057	$C_{10}H_{16}$	99-86-5	912	1016.2	1017			
Limonene	681.956, 1.064	$C_{10}H_{16}$	138-86-3	943	1028.3	1030			
cis-β-Ocimene	699.955, 1.051	$C_{10}H_{16}$	3338-55-4	905	1038.1	1038			
α-Ocimene	719.954, 1.057	$C_{10}H_{16}$	502-99-8	946	1049.1	1047			
γ-Terpinene	737.953, 1.086	$C_{10}H_{16}$	99-85-4	918	1059	1060			
p-Mentha-3,8-diene	757.951, 1.095	$C_{10}H_{16}$	586-67-4	873	1069.9	1075			
α-Terpinolene	791.949, 1.111	$C_{10}H_{16}$	586-62-9	919	1088.6	1088			
Neo-allo-ocimene	863.945, 1.088	$C_{10}H_{16}$	7216-56-0	863	1129.2	1131			

Figure 1. GCxGC-TOFMS from a single analysis provides broad analyte coverage. This information can be used to address multiple analytical questions including target quantification (CBD spectra, calibration, and calculated concentrations are shown), general characterization (PCA scores plot is shown), and non-target compound class profiling (monoterpene identification and relative trends are shown).

Experimental

A variety of CBD beverages were analyzed with GC-TOFMS and GCxGC-TOFMS, as described in Table 1. An aliquot of each beverage was placed in a 20 mL vial. The samples were incubated at 80 °C for 5 min and extracted at the same temperature with a triphase SPME fiber (PDMS/DVB/Carboxen, Supelco) for 10 min. The SPME fiber was conditioned for 5 min at 250 °C between injections. An alkane standard (C6 through C24) was also collected with the same methods for Retention Index (RI) determinations and a CBD standard was analyzed for calibration and quantitation.

Table 1. GC-TOFMS (Pegasus® BT) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	SPME injection, splitless
Gas Chromatograph	**LECO GCxGC QuadJet™ Thermal Modulator**
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min, corrected constant flow
Columns	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek) Rxi-17SilMS, 0.45 m x 0.25 mm x 0.25 µm coating (Restek)
Temperature Program	Hold 2 min at 40 °C, ramp 5 °C/min to 200, ramp 10 °C/min to 300, hold 2 min Secondary oven: +20 °C relative to primary oven
Modulation	2 s with temperature maintained +15 °C relative to 2nd oven
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	33-500 m/z
Acquisition Rate	10 spectra/s (GC) and 200 spectra/s (GCxGC)

Results and Discussion

As shown in Figure 1, a single GCxGC-TOFMS analysis can provide data to address a wide range of analytical questions. Target quantification of CBD, general sample comparison, and non-target profiling of specific compound classes in the CBD beverages were all determined from this single analysis. Isolating more chemical compounds from each other led to the broad analyte coverage that allowed for addressing these multiple analytical questions. The *Pegasus* BT 4D GCxGC-TOFMS is an excellent tool for determining individual analyte components within a complex mixture because the primary column separation is supplemented with both a second dimension of separation and TOFMS detection. GCxGC adds to the chromatographic resolution by coupling two columns with complementary stationary phases so that a sample is separated by both mechanisms in a single analysis. This spreads analytes out into two-dimensional space. TOFMS provides non-skewed spectral data that can often be deconvoluted to mathematically separate overlapping analytes in instances of coelutions that remain. The combination of these tools leads to the separation and identification of more individual analytes in a complex mixture as shown in Figures 2-5.

A representative GC and GCxGC separation for a blackberry chai flavored beverage is shown in Figure 2. The GCxGC separation is shown from a top-down view as a contour plot with the primary separation along the x-axis and the secondary separation along the y-axis. Some analytes are chromatographically separated and reliably determined with either GC or GCxGC. For example, CBD, shown in Figure 3, is well resolved in the first dimension. The high-quality spectral data can be matched to library databases and retention time confirmation to a CBD standard supports this identification. Target screening of CBD could likely be done with either GC or GCxGC.

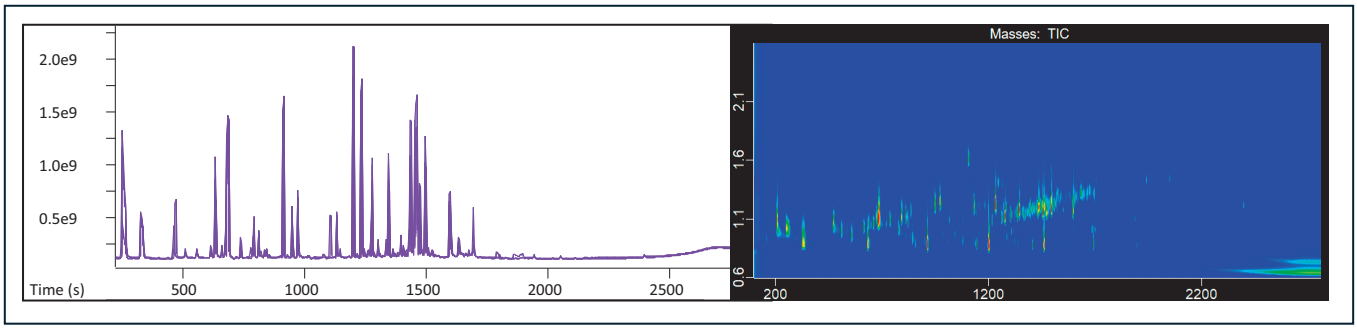


Figure 2. GC and GCxGC separation of blackberry chai CBD beverage. The first-dimension separation is matched with both methods, but the GCxGC separation spreads analytes into the second dimension, as shown in the contour plot.

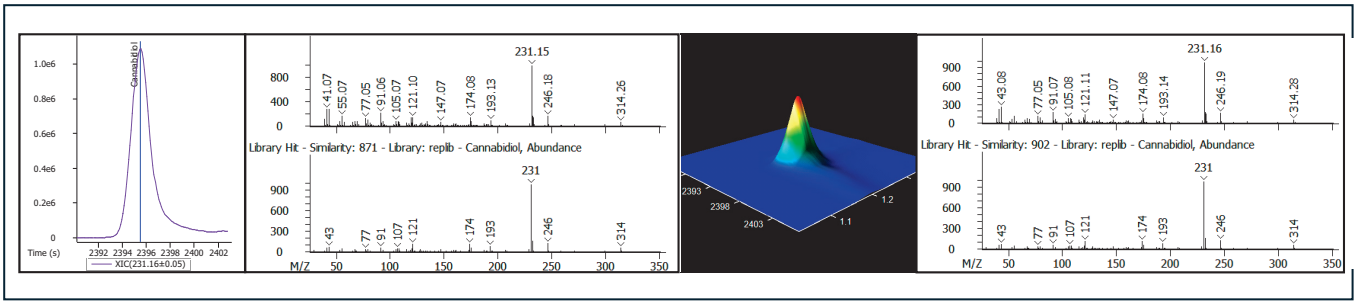


Figure 3. CBD is chromatographically isolated with GC and GCxGC.

With non-target analytical goals, however, the additional capabilities of GCxGC and TOFMS are beneficial. In particular, coelution can be common with complex samples, like these beverages, and techniques to better separate these instances of chromatographic overlap are useful. In many cases, the TOFMS data can be mathematically separated with deconvolution to provide pure information for each analyte. Deconvolution yields clean spectral data for the chromatographically overlapped analytes that can be compared to mass spectral databases for library matching. As shown in Figure 4, what appears as a single peak in the TIC of the GC separation can be mathematically separated into coumarin and alpha-guaiene with spectral similarity scores of 872 and 922, respectively. Retention time information for each analyte is also converted to RI and verified against library RI database information. Coumarin and alpha-guaiene have RI of 1440 and 1439 (on a semi-standard nonpolar column), respectively, which is consistent with the observed RI values of 1440 and 1441. This supports the identifications and also suggests that this coelution is expected. While GC was able to deconvolute these analytes, GCxGC was able to chromatographically resolve the analytes in the second dimension, as also shown in Figure 4. These analytes overlap on the primary column, a semi-standard nonpolar column (with RI values of 1440 and 1439), but they are well-separated on the secondary column, a polar column (with RI values of 1598 and 2454). While these are tentative, matching with both spectral and RI information adds confidence to the identifications. Both analytes have potentially interesting aroma properties (sweet, hay, and tonka for coumarin; and sweet, woody, balsam, and peppery for alpha-guaiene) and would be difficult to determine without these instrument capabilities.

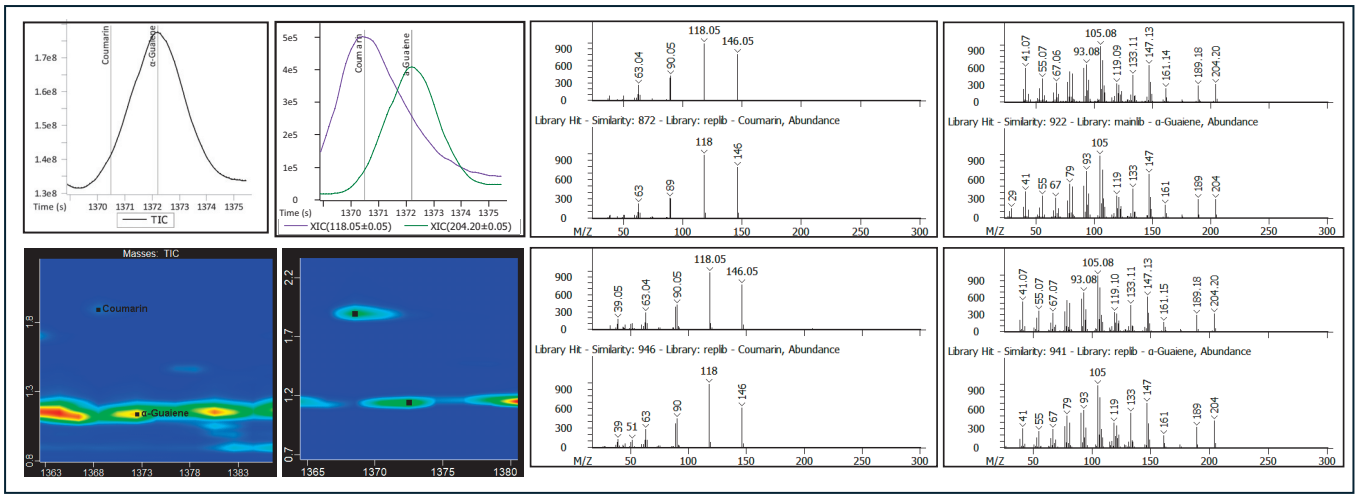


Figure 4. A first dimension coelution was deconvoluted in the GC data and chromatographically separated in the second dimension in the GCxGC data.

In other instances, the coelution with a GC separation is so complete that it exceeds deconvolution capabilities. An example of this is shown in Figure 5. In this case, there is one apparent peak in the TIC, but there is not a reliable identification. The observed spectral data has many extra fragments compared to the library match. With GCxGC, though, this one unknown is revealed to be two analytes that were merged together in the first dimension. The additional chromatographic separation in the second dimension led to clean spectral information that was matched to library databases. Fenchyl acetate and cis-cinnamaldehyde matched with similarity scores of 805 and 917, respectively. The RI information supports the identifications and explains the coelution. The semi-standard nonpolar RI information was 1224 and 1219 for fenchyl acetate and cis-cinnamaldehyde, respectively. This is consistent with the observed RI value of 1218. The polar RI values, however, are 1466 and 1884, respectively. While these features are obscured by coelution in the first dimension, they are chromatographically resolved with the second dimension. Fenchyl acetate has fresh, sweet, pine, fir, herbal, and citrus aroma properties. Cis-cinnamaldehyde has spicy and cinnamon aroma properties. These are likely important contributors to the flavor and were hidden without GCxGC.

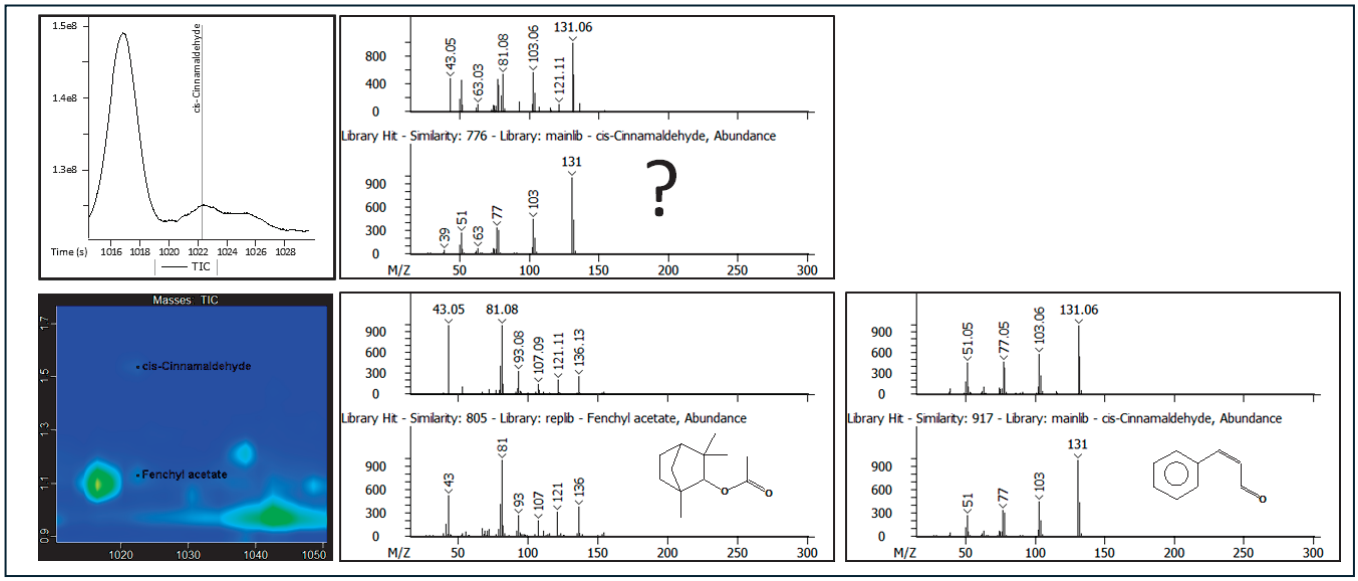


Figure 5. A perfect coelution in the GC data exceeded deconvolution capabilities. GCxGC separated this coelution on the second dimension and turned one unknown analyte into two tentatively identified features.

GCxGC was useful for uncovering some of the important analytes in this work. Figures 4 and 5 showed examples of analytes that were likely important for understanding the aroma characteristics (esters, etc.) and potentially of interest when studying the therapeutic potential (terpenes, etc.) that would have been difficult to determine without GCxGC. As screening for these was part of the non-targeted analysis objectives, GCxGC was a good choice for analyzing the CBD beverage samples. A variety of flavors were compared, and representative chromatograms were shown in Figure 1. The same set of data was analyzed for a variety of goals. One of the objectives was to calibrate and quantify CBD. A CBD calibration curve, shown in Figure 1, was determined and applied to the beverage samples. This provided concentration or dose level information for CBD in each beverage, also shown in Figure 1.

A non-targeted review of the data was also done to understand the aroma characterizations of the samples and to address more general therapeutic questions. Peak areas for a collection of over 380 analytes were compiled and compared across the sample set. For a general characterization and comparison, PCA was performed using these analytes as variables. The scores plot is shown in Figure 1 and three distinct clusters of samples, corresponding to the different flavors of beverages, can be observed. The associated loadings provided information on the analytes that were most responsible for these distinctions.

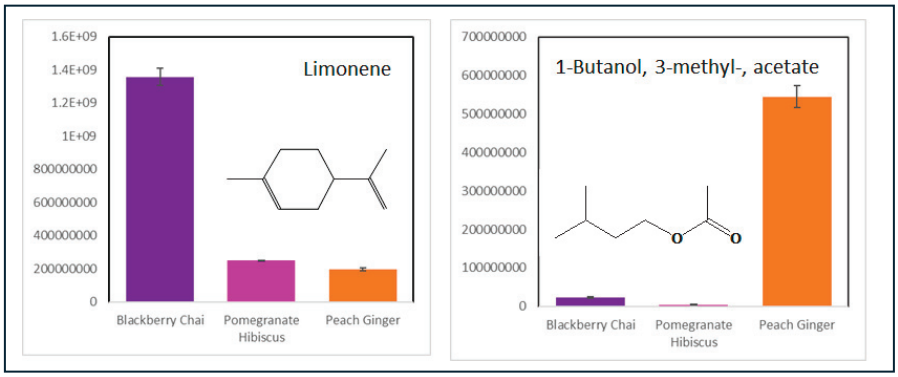


Figure 6. Highly loaded variables associated with the PCA scores shown in Figure 1.

Two of the highest loaded variables are shown in Figure 6. One is a terpene that is elevated in the blackberry chai sample and the other is an ester that is elevated in the Peach Ginger sample. These variables suggest that terpenes and esters, both compound classes of particular interest, are differentiating features of these samples. Terpenes are hypothesized to be involved in therapeutic properties and esters lend important aroma and flavor contributions. A closer look at these compound classes was done by compiling the features in each of these compound classes. The monoterpene profile is shown in Figure 1, the sesquiterpene profile is shown in Table 2, and the ester profile is shown in Table 3. Identifications are supported by both mass spectral and RI matching to library information. The terpene levels are consistently elevated in the blackberry chai sample while there appears to be a unique ester profile per flavor. Many of the esters that are elevated in a particular flavor have aroma characteristics that connect to that sample flavor.

Table 2. Sesquiterpene profile.

Analyte	tR	Formula	CAS	Sim	RI	RI (lib)	BC	PH	PG
δ-Elemene	1215.92, 1.084	$C_{15}H_{24}$	20307-84-0	939	1341.1	1338			
Cyclosativene	1259.92, 1.110	$C_{15}H_{24}$	22469-52-9	930	1368.4	1368			
Ylangene	1269.92, 1.105	$C_{15}H_{24}$	14912-44-8	909	1374.6	1372			
Copaene	1275.92, 1.121	$C_{15}H_{24}$	3856-25-5	941	1378.3	1376			
β-Patchoulene	1285.92, 1.131	$C_{15}H_{24}$	514-51-2	848	1384.5	1381			
β-Elemene	1299.92, 1.134	$C_{15}H_{24}$	515-13-9	945	1393.1	1391			
Sativene, (+)-	1307.92, 1.141	$C_{15}H_{24}$	3650-28-0	923	1398.1	1396			
Sesquithujene	1319.92, 1.095	$C_{15}H_{24}$	58319-06-5	807	1406	1402			
Isocaryophyllene	1323.92, 1.151	$C_{15}H_{24}$	118-65-0	922	1408.7	1406			
cis-α-Bergamotene	1335.91, 1.128	$C_{15}H_{24}$	18252-46-5	935	1416.8	1415			
Caryophyllene	1343.91, 1.174	$C_{15}H_{24}$	87-44-5	954	1422.2	1419			
10,10-Dimethyl-2,6-dimethyl....	1355.91, 1.169	$C_{15}H_{24}$	357414-37-0	907	1430.3	1440			
β-Copaene	1357.91, 1.164	$C_{15}H_{24}$	18252-44-3	902	1431.7	1432			
γ-Elemene	1361.91, 1.152	$C_{15}H_{24}$	29873-99-2	942	1434.4	1434			
α-Bergamotene	1365.91, 1.119	$C_{15}H_{24}$	17699-05-7	948	1437.1	1435			
α-Guaiene	1371.91, 1.138	$C_{15}H_{24}$	3691-12-1	940	1441.1	1439			
4,11,11-trimethyl-8-methylene...	1387.91, 1.168	$C_{15}H_{24}$	889360-49-0	854	1451.9	1460			
Humulene	1393.91, 1.197	$C_{15}H_{24}$	6753-98-6	945	1456	1454			
Amorpha-4,11-diene	1403.91, 1.178	$C_{15}H_{24}$	92692-39-2	896	1462.7	1458			
(-)-Aristolene	1409.91, 1.173	$C_{15}H_{24}$	6831-16-9	814	1466.8	1453			
γ-Selinene	1425.91, 1.178	$C_{15}H_{24}$	515-17-3	849	1477.6	1479			
γ-Muurolene	1427.91, 1.189	$C_{15}H_{24}$	30021-74-0	846	1479	1477			
α-Amorphene	1433.91, 1.191	$C_{15}H_{24}$	20085-19-2	885	1483	1482			
4a,8-Dimethyl-2-(prop-1-en-2....	1439.91, 1.212	$C_{15}H_{24}$	103827-22-1	886	1487.1	1492			
β-Selinene	1443.91, 1.210	$C_{15}H_{24}$	17066-67-0	945	1489.8	1486			
α-Zingiberene	1451.91, 1.158	$C_{15}H_{24}$	495-60-3	906	1495.2	1495			
Ledene	1455.91, 1.194	$C_{15}H_{24}$	21747-46-6	917	1497.9	1493			
γ-Cadinene	1459.91, 1.208	$C_{15}H_{24}$	39029-41-9	903	1500.6	1513			
β-Bisabolene	1471.91, 1.153	$C_{15}H_{24}$	495-61-4	916	1509.1	1509			
β-Curcumene	1475.91, 1.167	$C_{15}H_{24}$	28976-67-2	883	1512	1514			
γ-Cadinene	1481.91, 1.221	$C_{15}H_{24}$	39029-41-9	894	1516.3	1513			
(-)-α-Panasinsen	1489.9, 1.237	$C_{15}H_{24}$	56633-28-4	905	1522	1527			
Zonarene	1497.9, 1.221	$C_{15}H_{24}$	41929-05-9	807	1527.7	1527			
Naphthalene, 1,2,3,4,4a,7-hex...	1507.9, 1.224	$C_{15}H_{24}$	16728-99-7	913	1534.8	1533)			
α-Cadinene	1515.9, 1.215	$C_{15}H_{24}$	24406-05-1	875	1540.5	1538			
Selina-3,7(11)-diene	1521.9, 1.222	$C_{15}H_{24}$	6813-21-4	917	1544.8	1542			

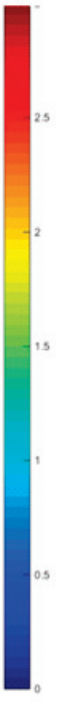


Table 3. Ester profile.

Name	tR	Formula	CAS	Similarity	RI	RI (lib)	BC	PH	PG
Ethyl butyrate	297.981, 1.013	$C_6H_{12}O_2$	105-54-4	940	806.2	802			
Acetic acid, butyl ester	317.98, 1.032	$C_6H_{12}O_2$	123-86-4	870	818.6	812			
Butanoic acid, 2-methyl-, ethyl ester	371.976, 1.017	$C_7H_{14}O_2$	7452-79-1	952	852.2	849			
Butanoic acid, 3-methyl-, ethyl ester	383.975, 1.020	$C_7H_{14}O_2$	108-64-5	920	859.6	854			
1-Butanol, 3-methyl-, acetate	415.973, 1.042	$C_7H_{14}O_2$	123-92-2	961	879.5	876			
Propyl butyrate	451.971, 1.055	$C_7H_{14}O_2$	105-66-8	835	901.7	896			
Hexanoic acid, ethyl ester	631.96, 1.076	$C_8H_{16}O_2$	123-66-0	895	1000.8	1000			
3-Hexen-1-ol, acetate, (Z)-	645.959, 1.107	$C_8H_{14}O_2$	3681-71-8	871	1008.5	1005			
2-Heptanol, acetate	709.955, 1.050	$C_9H_{18}O_2$	5921-82-4	876	1043.6	1045			
Hexanoic acid, 2-propenyl ester	779.95, 1.112	$C_9H_{16}O_2$	123-68-2	811	1082	1080			
Isoamyl isovalerate	821.947, 1.062	$C_{10}H_{20}O_2$	659-70-1	959	1105.2	1104			
Ethyl benzoate	937.94, 1.349	$C_9H_{10}O_2$	93-89-0	915	1171.4	1171			
Octanoic acid, ethyl ester	981.937, 1.121	$C_{10}H_{20}O_2$	106-32-1	860	1196.5	1196			
Acetic acid, octyl ester	1005.94, 1.115	$C_{10}H_{20}O_2$	112-14-1	913	1211	1210			
Fenchyl acetate	1021.93, 1.131	$C_{12}H_{20}O_2$	13851-11-1	805	1220.9	1223			
Hexyl 2-methylbutyrate	1047.93, 1.090	$C_{11}H_{22}O_2$	10032-15-2	815	1237	1236			
Bornyl acetate	1131.93, 1.212	$C_{12}H_{20}O_2$	76-49-3	943	1289.1	1285			
trans-Sabinyl acetate	1141.93, 1.209	$C_{12}H_{18}O_2$	139757-62-3	868	1295.3	1297			
2-Cyclohexen-1-ol, 3-methyl-6-(1-meth...	1153.93, 1.201	$C_{12}H_{20}O_2$	1204-30-4	869	1302.7	1303			
δ-Terpineol, acetate	1181.92, 1.212	$C_{12}H_{20}O_2$	93836-50-1	891	1320	1315			
Methyl geranate	1189.92, 1.226	$C_{11}H_{18}O_2$	2349-14-6	916	1325	1323			
Myrtenyl acetate	1195.92, 1.242	$C_{12}H_{18}O_2$	1079-01-2	826	1328.7	1327			
α-Terpinyl acetate	1231.92, 1.220	$C_{12}H_{20}O_2$	80-26-2	910	1351	1350			
Neryl acetate	1253.92, 1.195	$C_{12}H_{20}O_2$	141-12-8	903	1364.6	1364			
4-tert-Butylcyclohexyl acetate	1263.92, 1.205	$C_{12}H_{22}O_2$	32210-23-4	887	1370.8	1368			
3-Phenyl-1-propanol, acetate	1263.92, 1.394	$C_{11}H_{14}O_2$	122-72-5	936	1370.8	1373			
Propanoic acid, 2-methyl-, 2-ethyl-3-hy...	1267.92, 1.211	$C_{12}H_{24}O_3$	74367-31-0	838	1373.3	1373			
Butyl benzoate	1267.92, 1.340	$C_{11}H_{14}O_2$	136-60-7	804	1373.3	1376			
cis-3-Hexenyl caproate	1279.92, 1.150	$C_{12}H_{22}O_2$	31501-11-8	849	1380.7	1380			
Geranyl acetate	1283.92, 1.204	$C_{12}H_{20}O_2$	105-87-3	887	1383.2	1382			
Methyl cinnamate	1285.92, 1.496	$C_{10}H_{10}O_2$	103-26-4	791	1384.5	1380			
Cinnamyl acetate	1377.91, 1.459	$C_{11}H_{12}O_2$	103-54-8	914	1445.2	1446			
Ethyl cinnamate	1407.91, 1.450	$C_{11}H_{12}O_2$	103-36-6	924	1465.4	1464			
Dodecanoic acid, 1-methylethyl ester	1631.9, 1.099	$C_{15}H_{30}O_2$	10233-13-3	889	1624.5	1618			
Benzoic acid, 2-ethylhexyl ester	1743.89, 1.290	$C_{15}H_{22}O_2$	5444-75-7	907	1709.1	1735			
n-Dodecyl methacrylate	1821.88, 1.129	$C_{16}H_{30}O_2$	142-90-5	866	1770.8	1775			
Isopropyl myristate	1885.88, 1.097	$C_{17}H_{34}O_2$	110-27-0	926	1823.4	1827			
Ethyl propanoate	195.987, 0.963	$C_5H_{10}O_2$	105-37-3	821	718.5	710			
Hexadecanoic acid, methyl ester	2003.87, 1.137	$C_{17}H_{34}O_2$	112-39-0	842	1924.9	1926			
Isopropyl palmitate	2109.86, 1.042	$C_{19}H_{38}O_2$	142-91-6	905	2026.2	2023			
Hexadecanoic acid, butyl ester	2241.86, 1.003	$C_{20}H_{40}O_2$	111-06-8	849	2210.6	2188			

This single GCxGC-TOFMS analysis uncovered a large amount of information about the CBD beverages and was able to support investigating a variety of analytical questions. Many analytes would have been difficult to determine without the instrument capabilities highlighted in this application note.

Conclusion

In this application note, we have demonstrated the use of GCxGC-TOFMS for the analysis of CBD infused beverages and the broad versatility of the associated data. The same data was used for a variety of analytical objectives, including CBD quantification, non-targeted characterization, determination of the mono and sesquiterpene profiles, and determination of the ester profiles. The data could also be further probed for additional objectives. The chromatographic separation provided by GCxGC helped isolate analyte coelutions that were difficult to separate with a 1D separation. TOFMS added another level of separation with the mathematical deconvolution capabilities. This analytical platform is a powerful tool for both your targeted and non-targeted analytical research.

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