Application Note

Instrument: Pegasus® BT and ChromaTOF® Sync

Characterization and Comparison of Whiskey* Aroma Profiles with GC-TOFMS and *ChromaTOF* Sync

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Key Words: Whiskey, GC, TOFMS, *ChromaTOF* Sync, Alignment, PCA, Aroma Profile, Deconvolution

Introduction

Characterizing and comparing the aroma profiles of food and beverage products, like whiskeys, can be very interesting for better understanding the products and the specific chemicals that may contribute to the sensory observations of the products. Gas chromatography with mass spectrometry (GC-MS) is an excellent analytical technique for probing the aroma profile of these types of samples. The aroma contributing analytes tend to be volatile and semi-volatile and are well-suited to GC analysis. Chromatography effectively separates individual analytes in these complex samples, and MS detection then provides spectral information and good tentative identifications. With full m/z range acquisition and sensitive detection, LECO's *Pegasus* BT Time-of-Flight (TOF) MS produces rich data that describes these complex samples. Analytical software tools, like *ChromaTOF* Sync, are powerful for then probing the data and uncovering similarities, differences, and trends between the samples. *ChromaTOF* Sync performs sample set peak finding and incorporates deconvolution to produce a combined peak table that compiles and aligns analyte information across the entire sample set. Combining sample set information facilitates data review and comparing features through the set of samples. *ChromaTOF* Sync also includes additional comparative tools, like Principal Component Analysis (PCA) for general characterization and exploring trends.

In this application note, six different whiskeys were analyzed with LECO's *Pegasus* BT GC-TOFMS. The associated data was compared with *ChromaTOF* Sync to uncover interesting analytes and trends in the whiskey samples. Several examples are highlighted and discussed here.

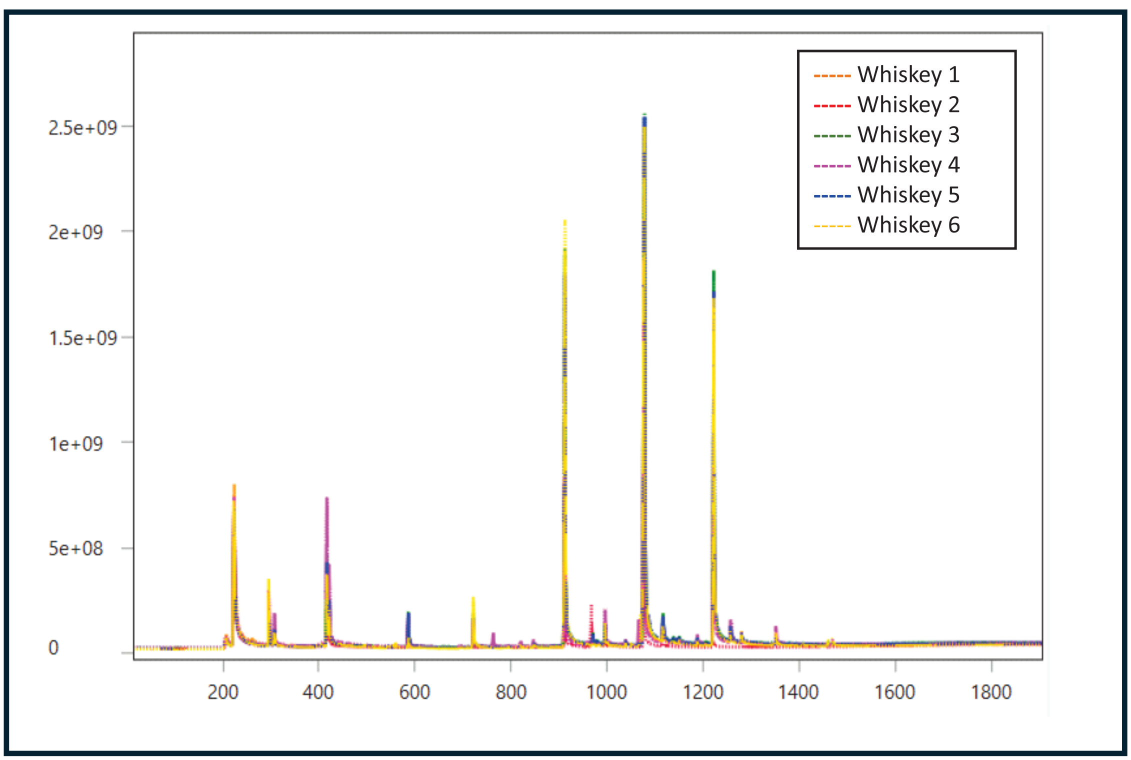


Figure 1. Six different whiskey samples were analyzed (in duplicate). Overlaid Total Ion Chromatograms (TIC) are shown.

**Please note that all samples are referred to as "whiskey" and "whiskeys," throughout this application note for simplicity and consistency, because the samples were sourced from three geographic origins—US, Scotland, and Ireland. It is important to acknowledge that in Scotland (and Canada), the product "Scotch," is referred to as "Whisky" or "Whiskies," whereas in the US and Ireland the products are referred to as "Whiskey," and "Whiskeys."*

Experimental

A variety of whiskey samples were analyzed with HS-SPME and GC-TOFMS. The samples were incubated for 10 min at 50 °C in the agitator and then extracted for 10 min at the same temperature with a tri-phase SPME fiber (PDMS, DVB, Car). GC and MS conditions are described in Table 1.

Table 1. Instrument *(Pegasus* BT) Conditions

Auto Sampler	**LECO L-PAL 3 Autosampler**
Injection	Desorb for 5 min in GC inlet, split 50:1
Gas Chromatograph	
Inlet	250 °C
Carrier Gas	He @ 1.2 mL/min
Column	Rxi-5Sil ms, 30 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	40 °C (hold 3 min), ramp 10 °C/min to 280 °C (hold 5 min)
Transfer Line	300 °C
Mass Spectrometer	**LECO *Pegasus* BT**
Ion Source Temperature	250 °C
Mass Range	34-600 m/z
Acquisition Rate	10 spectra/s

Results and Discussion

Overlaid chromatograms for the six different whiskey samples (run in duplicate) are shown in Figure 1. Many chromatographic peaks are observed, and some similarities and differences can be noted. *ChromaTOF* Sync software was used to better understand the chemical similarities and differences and to characterize these whiskey samples. The software tool provides sample set peak finding and deconvolution to produce a composite peak table for the sample set. Individual analytes can be determined and compared across the different whiskeys. For example, one of the larger peaks in the chromatogram, the ethyl ester of octanoic acid, is shown in Figure 2. Spectral information, overlaid chromatograms, and bar graphs of relative trends across the sample set from peak areas are shown. The observed spectrum was matched to the ethyl ester octanoic acid in the NIST library database with a similarity score of 921. The aroma of this compound is fruity with descriptors of fruity, wine, waxy, sweet, apricot, banana, brandy, and pear.[1] These aroma notes connect with some of the typical descriptors of whiskeys. It is interesting to note that this analyte is observed in every whiskey, but at different levels. Whiskey 2 and 4 have notably less than the other whiskeys. Understanding the relative amounts as well as the potential aroma contribution can be important for better understanding these samples.

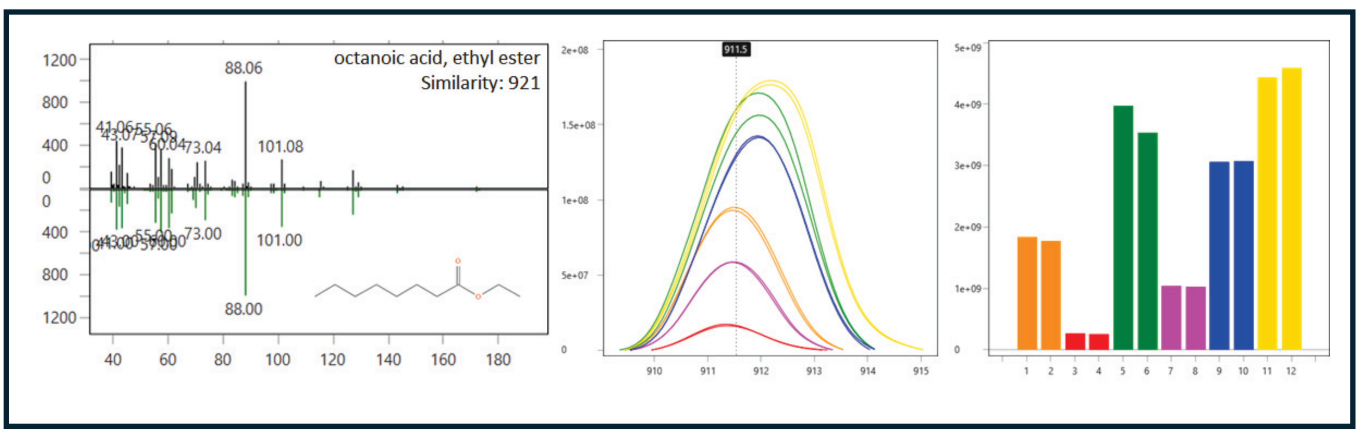
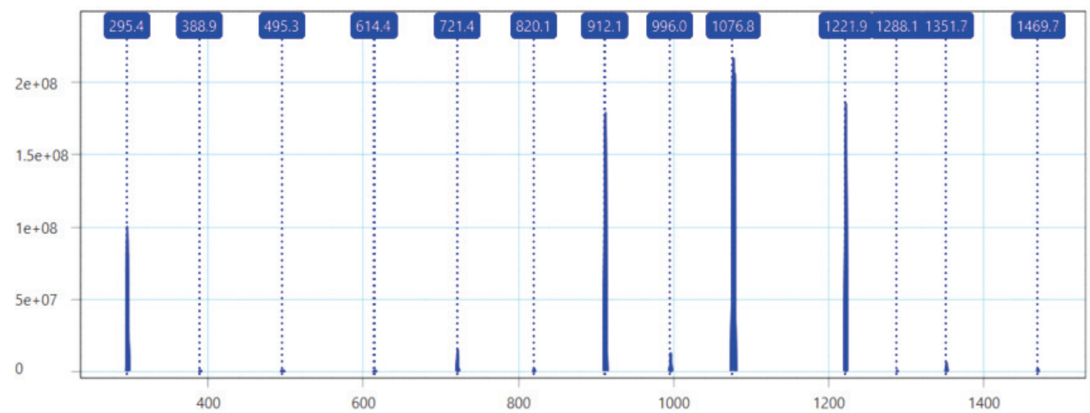


Figure 2. The ethyl ester of octanoic acid is one of the major peaks in the chromatogram. The spectral information, overlaid chromatographic profiles, and relative trends (as a bar graph) are shown.

Esters are an important compound class in whiskeys as they are formed during fermentation and tend to have important aroma contributions. Many other esters were observed in these samples, and the trends across the sample set for some of these can also be observed in *ChromaTOF* Sync. For example, the series of ethyl esters is shown in Figure 3. The associated table lists the identification metrics (similarity to library, etc.), the associated aroma notes, as well as the relative trends. The heat map indicates the trends of the esters across the samples set, with red indicating higher amounts and blue indicating lower amounts. The trends depend on the specific ester, but there are some general trends that are consistent. For example, whiskey 1 and 2 tend to have lower amounts of these esters and whiskeys 4 and 6 tend to have higher amounts of the esters.



Name	Formula	Similarity	CAS	Quant mass	R.I. lib	S/N	aroma	descriptor	Med RT	1	2	3	4	5	6	7	8	9	10	11	12
Ethyl Acetate	$C_4H_8O_2$	959	141-78-6	43.04	612	23459.91	ethereal	ethereal fruity sweet weed green	295.8	3.03	5.31	1.85	60.00	39.13	3.15	3.28	1.91	5.26	023.02	55.85	86.34
Propanoic acid, ethyl ester	$C_5H_{10}O_2$	924	105-37-3	57.05	710	1590.53	fruity	sweet fruity rum juicy fruit grape pineapple	389.1	5.69	1.77	7.19	20.86	08.14	1.90	0.09	2.93	6.00	547.78	30.76	21.21
Butanoic acid, ethyl ester	$C_6H_{12}O_2$	927	105-54-4	71.06	802	1623.17	fruity	fruity juicy fruit pineapple cognac	495.5	4.52	2.35	4.60	37.63	73.33	3.25	2.11	8.75	7.94	224.41	87.56	54.10
Pentanoic acid, ethyl ester	$C_7H_{14}O_2$	907	539-82-2	85.09	900	477.24	fruity	sweet fruity apple pineapple green tropical	614.5	1.74	3.22	0.57	90.72	98.29	5.91	7.80	1.67	8.38	274.77	23.05	31.94
Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	925	123-66-0	88.07	999	8797.95	fruity	sweet fruity pineapple waxy green banana	721.5	0.13	5.39	2.03	19.68	33.03	8.36	5.19	7.95	1.99	454.06	59.65	08.38
Heptanoic acid, ethyl ester	$C_9H_{18}O_2$	886	106-30-9	88.08	1098	2483.23	fruity	fruity pineapple cognac rum wine	820.2	2.65	9.09	3.62	46.35	07.66	0.16	3.88	2.02	0.05	150.12	49.23	42.21
Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	921	106-32-1	88.06	1196	53837.59	waxy	fruity wine waxy sweet apricot banana brandy pear	911.5	7.36	8.76	3.91	99.23	75.22	5.07	4.59	5.05	7.45	779.14	62.21	62.53
Nonanoic acid, ethyl ester	$C_{11}H_{22}O_2$	903	123-29-5	88.08	1295	7337.57	waxy	fruity rose waxy rum wine natural tropical	996.1	3.01	1.44	0.68	97.20	66.56	0.27	7.12	3.72	4.03	470.05	19.08	77.87
Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	920	110-38-3	88.10	1396	66485.57	waxy	sweet waxy fruity apple grape oily brandy	1076.8	0.88	4.55	2.26	72.55	56.07	7.47	4.80	1.17	0.41	168.17	56.24	03.85
Dodecanoic acid, ethyl ester	$C_{14}H_{28}O_2$	947	106-33-2	88.07	1594	38762.44	waxy	sweet waxy floral soapy clean	1222.0	4.14	4.92	1.41	74.32	29.51	2.92	5.06	1.98	0.41	488.95	14.77	33.42
Tridecanoic acid ethyl ester	$C_{15}H_{30}O_2$	815	28267-29-0	88.08	1687	282.96	none	none	1288.4	8.65	3.17	0.00	0.00	32.83	3.85	5.82	6.34	3.63	834.09	42.16	73.11
Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	921	124-06-1	88.08	1793	4127.35	waxy	sweet waxy violet orris	1352.0	9.30	8.88	0.00	0.00	22.87	2.22	6.26	3.72	4.50	332.71	14.54	36.43
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	894	628-97-7	88.07	1993	2320.10	waxy	mild waxy fruity creamy milky balsamic greasy oily	1469.9	0.87	6.56	0.00	0.00	05.32	3.69	9.02	9.15	1.81	337.47	93.16	12.98

Figure 3. Many ethyl esters are observed in the samples and details about this compound class are compiled.

Reviewing this compound class provides interesting context about the whiskey samples in terms of their aroma attributes, and it can also provide additional context for the identifications of the other analytes in the samples. While this series is tentatively identified, the consistency across the series and the very good similarity scores makes it a good option for retention index calculations. Essentially, the ethyl ester series was used as a naturally occurring series to calculate retention index values for the sample overall. Thus, the observed retention times and known retention index values for this series were used with *ChromaTOF* Sync for calculating retention index and supporting identifications of other analytes in the samples.

For example, 2-methyl 1-butanol is shown in Figure 4. This analyte had a very good similarity to the library with a score of 897. Retention index further supports this identification with an observed RI value of 738 compared to the library RI value of 739. The trends across the sample set can also be observed in Figure 4 with highest levels of this analyte in whiskey 4. This analyte has an aroma type of ethereal with descriptors of ethereal, whiskey, fusel, alcoholic, fatty, greasy, winey, leathery, and cocoa.[1] This identification and the relative trends can provide additional insight to these whiskey samples.

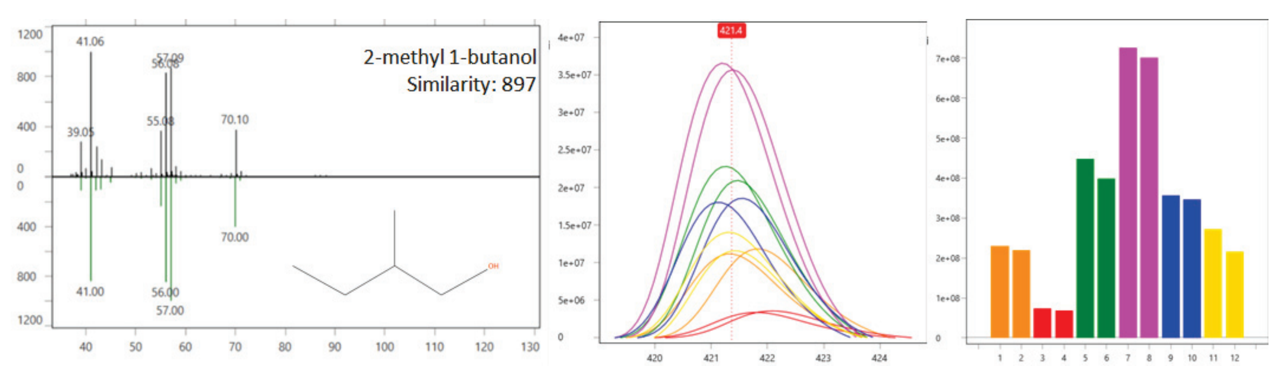


Figure 4. 2-methyl 1-butanol. Spectral information, overlaid chromatographic profiles, and relative trends (as a bar graph) are shown.

ChromaTOF Sync also incorporates deconvolution into the peak finding, allowing for additional separation and mathematical resolution in instances of chromatographic overlap. An example is shown in Figure 5. A TIC chromatogram is shown in the top left corner of this figure. Two peak markers are indicated, but it is not readily apparent that two peaks are eluting in this section of the chromatogram when viewing the TIC. Deconvolution effectively resolves these coeluting features from each other, and from other analytes and background, and provides pure spectra for each as well as pure chromatographic profiles for each by indicating unique masses per feature. In this case, benzaldehyde and 1-heptanol coelute and are resolved with deconvolution. Benzaldehyde has a similarity score of 917 while 1-heptanol has a similarity score of 838. Both identifications are supported with retention index with observed RI values of 973 and 975 and library RI values of 970 and 975 for benzaldehyde and 1-heptanol, respectively. Neither of these analytes are very clear in the TIC, but their good identifications and relative trends across the sample set are readily determined with deconvolution. Both of these vary between the whiskey types and have potentially interesting aroma contributions. The alcohol has a green aroma type with descriptors of musty, leafy, violet, herbal, green, sweet, woody, and peony, and benzaldehyde has a fruity aroma type with descriptors of strong, sharp, sweet, bitter, almond, and cherry.[1]

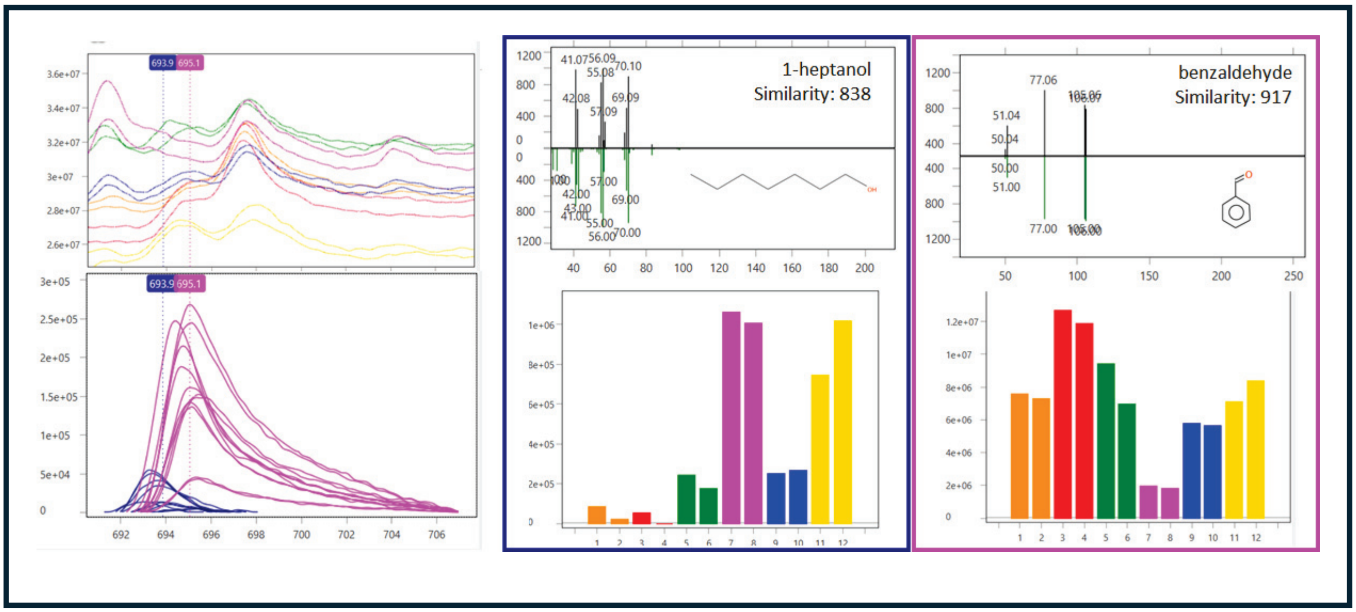


Figure 5. Deconvolution example. Benzaldehyde and 1-heptanol coelute and are mathematically isolated with ChromaTOF Sync deconvolution.

Benzaldehyde has fruity aroma notes and interesting trends across the sample set. Fruity tends to be an important descriptor of many whiskeys and reviewing other analytes that have a fruity aroma type may also be interesting. Several analytes with "fruity" aroma types are tabulated in Figure 6. Identification metrics (similarity scores, retention index, etc.) and aroma notes can be observed in the table and trends across sample set can be observed in the heat map.

Name	Formula	Similarity	CAS	Quant mass	R.I. calc	R.I. lib	aroma	descriptor	Med RT
Propanoic acid, ethyl ester	$C_5H_{10}O_2$	924	105-37-3	57.05	710	710	fruity	sweet fruity rum juicy fruit grape pineapple	389.1
Propanoic acid, 2-methyl-, ethyl ester	$C_6H_{12}O_2$	911	97-62-1	43.08	758	756	fruity	sweet ethereal fruity alcoholic fusel rummy	444.6
Isobutyl acetate	$C_6H_{12}O_2$	897	110-19-0	43.04	774	772	fruity	sweet fruity ethereal banana tropical	462.8
Butanoic acid, ethyl ester	$C_6H_{12}O_2$	927	105-54-4	71.06	802	802	fruity	fruity juicy fruit pineapple cognac	495.5
Butanoic acid, 2-methyl-, ethyl ester	$C_7H_{14}O_2$	914	7452-79-1	57.09	851	849	fruity	sharp sweet green appe fruity	554.9
Butanoic acid, 3-methyl-, ethyl ester	$C_7H_{14}O_2$	887	108-64-5	88.07	855	853	fruity	fruity sweet apple pineapple tutti frutti	559.9
1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	902	123-92-2	70.10	877	876	fruity	sweet fruity banana solvent	586.3
Pentanoic acid, ethyl ester	$C_7H_{14}O_2$	907	539-82-2	85.09	900	900	fruity	sweet fruity apple pineapple green tropical	614.5
Ethyl tiglate	$C_7H_{12}O_2$	950	5837-78-5	83.07	941	939	fruity	sweet fruity tutti frutti tropical berry floral caramel	659.1
Benzaldehyde	C_7H_6O	917	100-52-7	106.07	975	962	fruity	strong sharp sweet bitter almond cherry	695.1
Furan, 2-pentyl-	$C_9H_{14}O$	807	3777-69-3	81.06	995	993	fruity	fruity green earthy beany vegetable metallic	717.2
Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	925	123-66-0	88.07	999	999	fruity	sweet fruity pineapple waxy green banana	721.5
Acetic acid, hexyl ester	$C_8H_{16}O_2$	875	142-92-7	43.05	1013	1011	fruity	fruit green apple banana sweet	735.9
2-Nonanone	$C_9H_{18}O$	845	821-55-6	58.06	1095	1092	fruity	fresh sweet green weedy earthy herbal	817.1
Heptanoic acid, ethyl ester	$C_9H_{18}O_2$	886	106-30-9	88.08	1098	1098	fruity	fruity pineapple cognac rum wine	820.2
Hexanoic acid, 2-methylpropyl ester	$C_{10}H_{20}O_2$	877	105-79-3	99.11	1152	1149	fruity	fruity pineapple green apple skin green apple sour tropical peach	870.6
Butanedioic acid, diethyl ester	$C_8H_{14}O_4$	887	123-25-1	101.05	1178	1181	fruity	mild fruity cooked apple ylang	894.6
Ethyl (E)-2-octenoate	$C_{10}H_{18}O_2$	877	7367-82-0	125.14	1248	1249	fruity	fruity pear skin green waxy tropical plum skin fatty	956.3
Isopentyl hexanoate	$C_{11}H_{22}O_2$	853	2198-61-0	43.08	1251	1250	fruity	fruity banana apple pineapple green	958.8
n-Caprylic acid isobutyl ester	$C_{12}H_{24}O_2$	885	5461-06-3	57.08	1348	1348	fruity	fruity green oily floral	1038.6
Ethyl 9-decenoate	$C_{12}H_{22}O_2$	852	67233-91-4	88.07	1388	1388	fruity	fruity fatty	1069.9
Octanoic acid, 3-methylbutyl ester	$C_{13}H_{26}O_2$	903	2035-99-6	70.09	1449	1446	fruity	sweet oily fruity green soapy pineapple coconut	1116.1
Limonene	$C_{10}H_{16}$	887	138-86-3	68.08	1040	1030	fruity	citrus herbal terpene camphor	762.8
Propyl octanoate	$C_{11}H_{22}O_2$	852	624-13-5	145.15	1292	1290	fruity	coconut cacoa gin	993.4

Name	1	2	3	4	5	6	7	8	9	10	11	12
Propanoic acid, ethyl ester	5.69	1.77	7.19	0.86	8.14	1.90	0.09	2.93	6.00	47.78	30.76	1.21
Propanoic acid, 2-methyl-, ethyl ester	4.97	6.92	9.76	4.43	9.12	4.40	6.98	2.01	36.15	59.34	35.00	5.19
Isobutyl acetate	8.94	3.93	9.66	9.18	3.84	4.58	8.33	5.91	27.52	05.11	40.10	0.16
Butanoic acid, ethyl ester	4.52	2.35	4.60	7.63	3.33	3.25	2.11	8.75	7.94	24.41	37.56	4.10
Butanoic acid, 2-methyl-, ethyl ester	4.35	9.25	1.05	4.28	0.28	3.63	9.02	0.76	35.05	09.18	32.70	8.94
Butanoic acid, 3-methyl-, ethyl ester	1.93	2.07	1.04	9.03	2.64	0.98	3.26	0.54	10.26	54.27	21.16	15.96
1-Butanol, 3-methyl-, acetate	0.66	1.02	6.22	6.74	2.26	0.09	8.65	5.44	14.14	41.31	25.54	7.21
Pentanoic acid, ethyl ester	1.74	3.22	0.57	0.72	8.29	5.91	7.80	1.67	8.38	74.77	23.05	1.94
Ethyl tiglate	8.84	8.99	2.79	3.97	0.72	6.92	8.82	4.12	35.00	47.74	40.37	2.61
Benzaldehyde	8.97	3.25	6.53	0.28	8.91	5.43	0.66	5.84	40.39	72.52	44.39	39.52
Furan, 2-pentyl-	3.44	6.00	1.69	8.82	13.03	3.90	4.31	3.44	71.68	75.59	37.86	36.78
Hexanoic acid, ethyl ester	0.13	5.39	2.03	9.68	13.03	8.36	5.19	7.95	51.99	54.06	59.65	8.38
Acetic acid, hexyl ester	3.44	0.38	0.00	0.00	13.51	9.36	7.01	2.58	74.75	74.47	95.63	2.01
2-Nonanone	9.68	1.71	17.75	1.24	9.94	3.70	8.39	0.54	37.58	18.96	75.47	13.59
Heptanoic acid, ethyl ester	2.65	9.09	3.62	16.35	7.66	0.16	13.88	2.02	30.05	50.12	49.23	12.21
Hexanoic acid, 2-methylpropyl ester	3.48	0.12	0.00	0.00	5.51	9.28	8.89	8.69	4.96	45.10	26.61	8.97
Butanedioic acid, diethyl ester	1.07	5.96	5.97	5.21	2.30	8.18	9.15	8.56	14.91	63.86	71.27	6.20
Ethyl (E)-2-octenoate	4.16	3.25	0.00	0.00	2.37	0.98	1.21	3.81	7.49	76.62	10.87	7.20
Isopentyl hexanoate	1.16	9.82	1.09	2.52	6.90	8.40	7.83	2.53	92.02	86.61	5.48	2.07
n-Caprylic acid isobutyl ester	7.53	6.56	6.66	9.78	1.85	7.55	7.05	6.82	45.79	79.85	33.79	18.65
Ethyl 9-decenoate	2.81	9.98	0.00	0.00	9.91	5.80	0.00	0.00	9.24	94.63	36.25	5.50
Octanoic acid, 3-methylbutyl ester	4.08	6.50	1.07	7.79	4.35	0.70	2.89	7.04	30.01	07.28	92.28	4.40
Limonene	3.24	8.48	17.66	1.42	7.79	1.44	4.08	5.87	16.84	63.76	5.06	1.28
Propyl octanoate	4.52	7.66	8.29	0.70	9.69	4.77	9.47	1.60	9.06	00.38	2.97	3.86

Figure 6. Analytes with "fruity" aroma types.

While it is interesting to look at specific compound classes (like the ethyl esters), individual analytes (like benzaldehyde), and analytes with similar aroma attributes (like those with fruity notes), *ChromaTOF* Sync also supports overall unsupervised sample comparisons such as Principal Component Analysis (PCA). Features in the compiled peak table can be used as variables to explore general trends in the samples. In this case, tentatively identified analytes (similarity > 800 and library RI within 30 units) were used for PCA. The scores and loadings plots are shown in Figure 7. The scores suggest that the whiskeys 1, 3, 5, and 6 were more similar to each other while whiskey 2 (red) and whiskey 4 (pink) were most distinct. It is potentially interesting to note that sample 2 is from a distillery in Ireland, sample 4 is from a distillery in the United States, and the other whiskeys are all from Scotland.

Figure 7. PCA Scores (left) and Loadings (right) for the 6 whiskeys.

The scores plot can indicate which samples are most similar or different from each other and the associated loadings can help provide insight to those differences. For example, whiskey 2 (red) has the lowest PC1 scores, and 4 analytes with the lowest loadings on PC1 are also indicated in Figure 7. These analytes are distinct to whiskey 2 and are listed in Figure 8. These analytes are particularly interesting as they all have aroma types of herbal with additional descriptors of camphor, eucalyptol, and other spicy notes.[1] Relative to the other whiskeys, whiskey 2 also had some distinct sensory descriptors with more spicy and clove notes than the other whiskeys. It is likely that some of these distinct analytes are connected to those distinct sensory notes.

Name	Formula	Similarity	CAS	Quant mass	R.I. calc	R.I. lib	aroma	descriptor	Med RT	1	2	3	4	5	6	7	8	9	10	11	12
cis-Dihydrocarvone	$C_{10}H_{16}O$	864	3792-53-8	95.08	1213	1195	herbal	herbal warm	926.4	0.00	0.00	5.95	2.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Isocineole	$C_{10}H_{18}O$	826	470-67-7	111.11	1025	1016	herbal	cooling pine minty camphor terpene green	747.2	0.00	0.00	4.55	7.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bicyclo[3.1.0]hexan-3-one, 4-methy	$C_{10}H_{16}O$	861	471-15-8	110.13	1132	1114	herbal	herbal warm	852.2	0.00	0.00	2.31	8.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eucalyptol	$C_{10}H_{18}O$	858	470-82-6	111.11	1046	1032	herbal	eucalyptus herbal camphor medicinal	768.0	0.00	0.00	3.05	4.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 8. Four analytes distinct in whiskey 2.

Whiskey samples are very complex with many chemical components. *ChromaTOF* Sync helps to uncover specific analytes and facilitates exploring these interesting analyte trends in the data.

Conclusion

In this application note, LECO's *Pegasus* BT and *ChromaTOF* Sync were used to compare and characterize six different whiskey samples. Aroma profile information was determined, and the software tools helped to uncover interesting analytes and trends within the data.

References

[1]Good Scents Company database (www.thegoodscentscompany.com)

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