

Instrument: Pegasus[®] BTX 4D and ChromaTOF[®] Tile

Determining Differences in Control vs Off-Odor Water Samples Using GCxGC-TOFMS and ChromaTOF[®] Tile

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Introduction

Across many markets and types of samples, a common analytical goal can be to distinguish one sample from another and to determine the specific chemical differences between the samples. This type of information can be relevant for a wide range of purposes and can help with characterization of the samples as well as many different problem-solving tasks. Generally, a combination of analytical hardware and software tools are useful to achieve this type of non-targeted comparison.

GCxGC-TOFMS is a great choice for the hardware as it provides exceptional analyte coverage for the volatile and semi-volatile components of a sample of interest. Using two dimensions of separation with GCxGC improves the peak capacity and, therefore, more chemical detail can often be uncovered than with a standard GC separation. TOFMS provides sensitive detection with full m/z range non-skewed spectral data that can be readily searched against mass spectral databases. This combination leads to detailed chemical data about a sample. *ChromaTOF* Tile is a useful software tool for quickly highlighting differences within this detailed GCxGC-TOFMS data. With *ChromaTOF* Tile, all m/z of the raw data are compared with chromatographic tile grids to highlight the retention windows and masses of sample differences. This rapidly points to the most interesting regions of the data, which can then be explored to determine the associated chemical features related to these differences.

In this work, GCxGC-TOFMS hardware and *ChromaTOF* Tile software were used to investigate an off-odor water sample compared to a control water sample. Both samples were analyzed with HS-SPME and GCxGC-TOFMS, and the associated data were compared with the fold-change option in *ChromaTOF* Tile. Analytes higher in the off-odor sample were efficiently determined, leading to a list of analytes that may be associated with the off-odor.



Figure 1. The Pegasus BTX 4D and ChromaTOF Tile were used to compare two water samples – one with an off-odor and a control water without the odor.

Experimental

Two water samples, one control and one with an odor, were analyzed with HS-SPME and GCxGC-TOFMS. For each sample, 2.0 mL were added to a 20 mL SPME vial. Each vial was incubated for 2 min at 40 °C and extracted with the tri-phase fiber (PDMS, DVB, C-WR) for 5 min at the same temperature. The samples were then analyzed by GCxGC-TOFMS with LECO's Pegasus BTX 4D, as described in Table 1. An alkane standard was also analyzed with the same methods for retention index (RI) determinations. The samples were compared with *ChromaTOF* Tile using the fold-change comparison mode.

Table 1. Instrument (Pegasus BTX 4D) Conditions

Auto Sampler	LECO L-PAL 3 Autosampler
Injection	SPME, Desorb for 2 min in GC inlet, split 10:1
Gas Chromatograph	LECO QuadJet™ GCxGC
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min
Column	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating Rxi-17Sil ms, 0.6 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	40 °C (hold 2 min), ramp 6 °C/min to 200 °C, ramp 24 °C/min to 250 °C Secondary Oven +10 °C
Modulation	QuadJet thermal modulator, 2.5 s
Transfer Line	250 °C
Mass Spectrometer	LECO Pegasus BTX
Ion Source Temperature	250 °C
Mass Range	35-500 m/z
Acquisition Rate	100 spectra/s

Results and Discussion

GCxGC contour plot chromatograms for each of the water samples are shown in Figure 2. The primary objective was to determine the analytes that were elevated in the off-odor sample relative to the control sample, as these are most likely the causes of the off-odor. With a few exceptions, major differences are not obvious by initial visual comparison of these samples. The most apparent peaks in the chromatograms are siloxanes, which don't differ between the samples and are likely related to the sampling procedures.

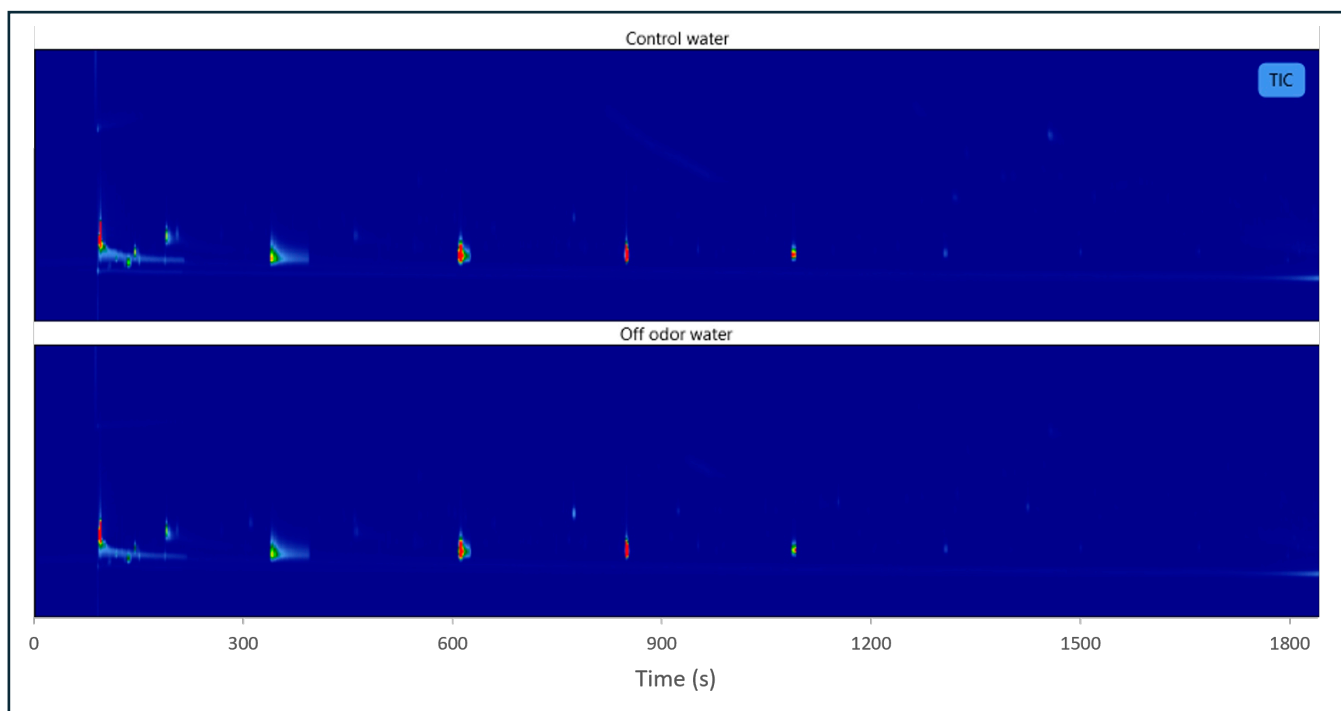


Figure 2. GCxGC contour plot chromatograms for the control water (top) and off-odor water (bottom).

Each sample could be explored with full non-target peak finding with *ChromaTOF* software that produces a peak table for each sample, but *ChromaTOF* Tile can streamline the analysis when finding differences between the samples is the primary objective, as it is here. Instead of peak finding, *ChromaTOF* Tile compares raw data to find chromatographic windows and m/z that differ, which both focuses the analysis and reduces processing time. In this case, the fold-change option was used to find retention windows in the data with large relative intensity ratio differences between the two samples.

The chemical features associated with these retention windows were further explored. Those that were higher in the off-odor sample are summarized in Table 2. Tentative identifications were determined with automated spectral matching of background subtracted caliper spectra to library databases with similarity scores listed. Identifications were further supported by comparing observed RI information to the library database as listed. Many of the analytes were aldehydes. In addition to the tentative identifications, the table summarizes other details about the sample and the relative intensity trends. These trends are shown in the last two columns, with the control sample in column 1 and the off-odor sample in column 2. The heat map indicates relative intensity with red being higher and blue being lower. The aroma descriptions were also added to the table to provide additional context on these differentiating features.

Table 2. Chemical features observed at higher levels in the off-odor sample

Name	Formula	Similarity	CAS	Quant	R.I. calc	R.I. lib	LG2FC	Aroma	Description	Med RT1	Med RT2	1	2
Geranyl acetone	C ₁₅ H ₂₂ O	879	3796-70-1	151	1455	1453	5.21	floral	fresh green fruity waxy rose woody magnolia tropical	1249.9	1.08	2110.31	77856.98
5-Hepten-2-one, 6-methyl	C ₈ H ₁₄ O	847	110-93-0	108	991	986	4.42	citrus	citrus green musty lemongrass apple	592.5	0.98	20023.14	429332.50
Hexanal	C ₆ H ₁₂ O	849	66-25-1	56	804	801	3.34	green	fresh green fatty aldehydic grass leafy fruity sweaty	307.5	0.86	364981.78	3706640.25
Kharismal	C ₁₃ H ₂₂ O ₂	748	24851-98-7	83	1661	1656	2.70	floral	floral oily jasmin green lactonic tropical natural	1489.9	1.30	31701.69	206391.92
Decanal	C ₁₀ H ₂₀ O	868	112-31-2	56	1208	1206	2.12	aldehydic	sweet aldehydic waxy orange peel citrus floral	922.4	0.97	140347.03	608244.94
4-Nonenal, (E)-	C ₉ H ₁₆ O	853	2277-16-9	55	1097	1105	1.97	fruity	fruity	757.5	0.98	66546.03	261239.61
Heptanal	C ₇ H ₁₄ O	864	111-71-7	70	905	901	1.93	green	fresh aldehydic fatty green herbal wine-lee ozone	457.5	0.89	218117.41	833146.69
Isopropyl myristate	C ₁₇ H ₃₄ O ₂	753	110-27-0	60	1831	1825	1.79	mild	faint oily fatty	1664.9	0.94	23856.66	82697.40
Nonanal	C ₉ H ₁₈ O	877	124-19-6	98	1107	1104	1.78	aldehydic	waxy aldehydic rose fresh orris orange peel fatty peely	772.5	0.94	724782.31	2497595.50
Octanal	C ₈ H ₁₆ O	832	124-13-0	84	1006	1003	1.60	aldehydic	aldehydic waxy citrus orange peel green herbal fresh fatty	617.5	0.91	190737.05	577003.25
Dodecanal	C ₁₂ H ₂₄ O	826	112-54-9	56	1411	1409	1.29	aldehydic	soapy waxy aldehydic citrus green floral	1194.9	0.98	68259.59	166689.52
Undecanal	C ₁₁ H ₂₂ O	842	112-44-7	57	1310	1307	1.04	aldehydic	waxy soapy floral aldehydic citrus green fatty fresh laundry	1062.4	0.97	70643.02	145227.70

ChromaTOF Tile was able to efficiently determine this list of chemical information. This is a faster approach than data processing each sample independently and also provides more information than a visual comparison of the chromatograms could have done. It is true that some of these features likely would have been found with a visual comparison of the chromatograms. For example, nonanal, with a first dimension retention time of 772 s, can be noted in the contour plots shown in Figure 2. A zoom-in of the chromatogram for nonanal is shown in Figure 3, and the difference is apparent in the TIC chromatograms and also the XIC for m/z 98. Hexanal at tR=307 may also be noted visually in Figure 2.

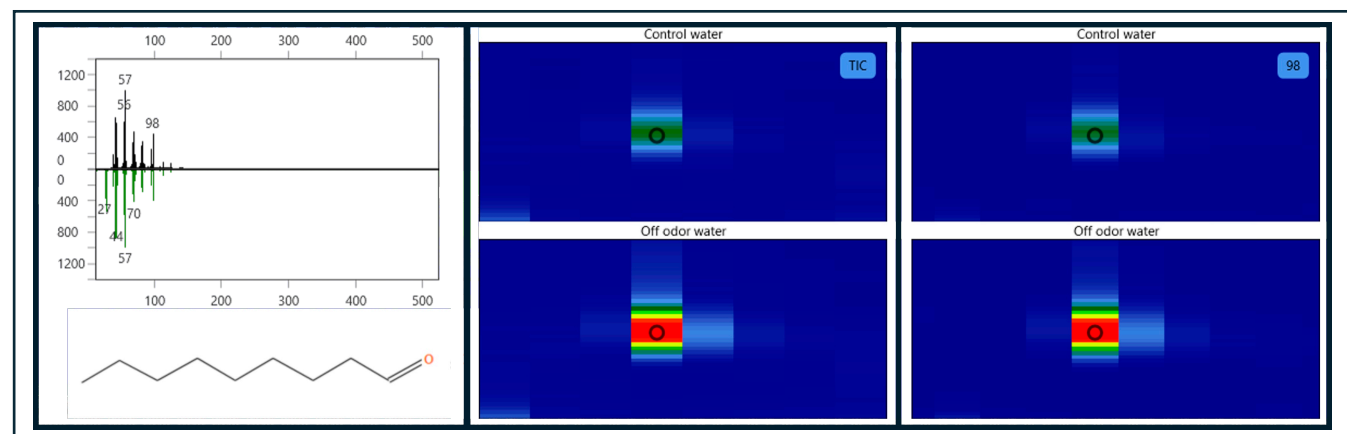


Figure 3. Nonanal is elevated in the off-odor sample compared to the control, which is apparent in the TIC (middle panel) and in the XIC for m/z 98 (right panel).

However, other analyte differences are difficult to find with visual review and may be overlooked without data comparison software tools. For example, octanal is listed in Table 2 and shown in Figure 4. This analyte was present at lower S/N and not apparent in the TIC with standard color scales. The difference is clear, though, when plotting the XIC contour plot for m/z 84. Because *ChromaTOF* Tile compares all m/z in the data when checking for differences, analytes with lower S/N that are difficult to see in the TIC can still be uncovered with the software. A closely eluting siloxane peak is visible in the contour plot. The benefits of GCxGC can also be noted here as octanal was chromatographically separated in the second dimension from the tail of the siloxane.

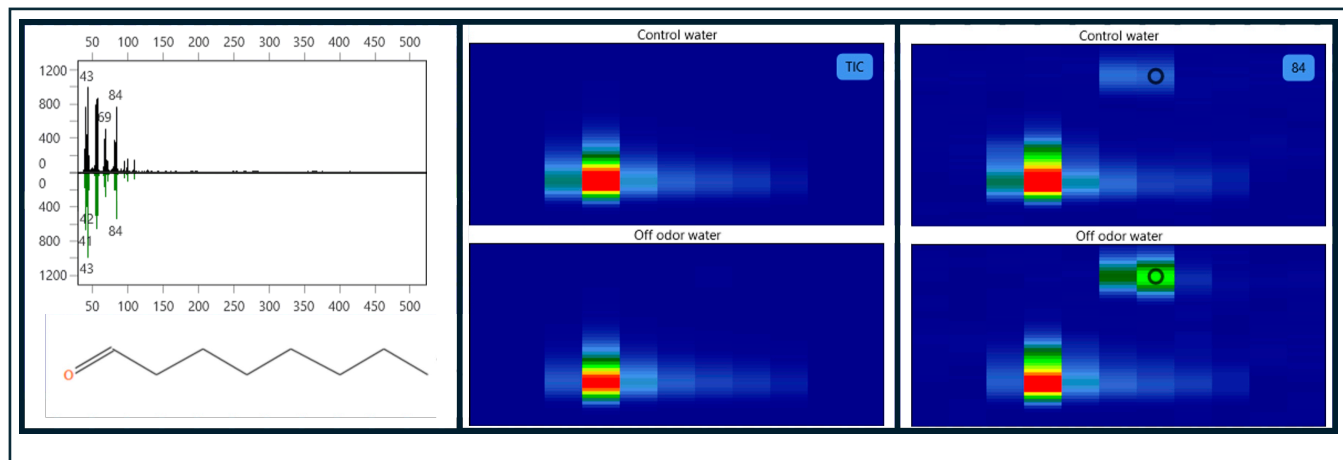


Figure 4. Octanal was observed at higher levels in the off-odor sample compared to the control. This difference was not apparent in the TIC (middle panel) because of the lower S/N, but could be observed with XIC contour plots (right panel).

Other analyte differences may also be difficult to find with visual review if they are obscured by closely eluting features. For example, heptanal is listed in Table 2 and shown Figure 5. Here, a coeluting background interference dominates the TIC contour plot and buried heptanal. The *ChromaTOF* Tile software uncovered heptanal, though, and when an XIC unique to heptanal is reviewed, the difference is clear.

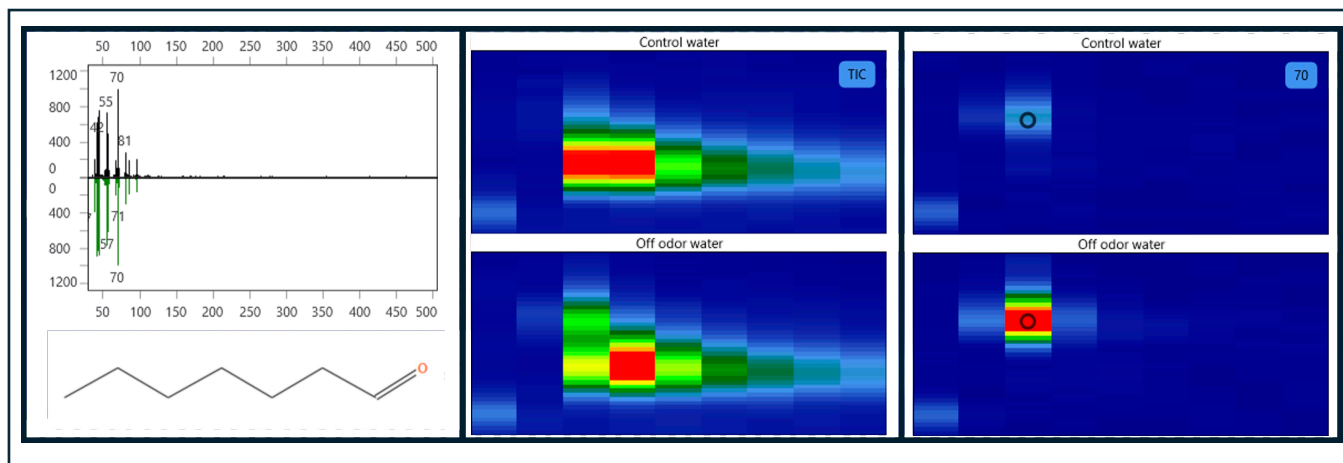


Figure 5. Heptanal was observed at higher levels in the off-odor sample compared to the control. This difference was difficult to note in the TIC (middle panel) because of a coeluting background peak, but it could be observed with XIC contour plots (right panel).

ChromaTOF Tile efficiently found chemical features that were higher in the off-odor sample. This was done faster than with standard peak finding and more thoroughly than with visual review.

Conclusion

In this work, LECO's *Pegasus* BTX 4D GCxGC-TOFMS and *ChromaTOF* Tile software were used to efficiently find chemical differences between a control water and off-odor water sample. GCxGC-TOFMS provided an increased peak capacity and sensitive detection for the volatile and semi-volatile components of the water samples. *ChromaTOF* Tile software streamlined the comparison of the data to quickly highlight differences. Several specific analytes were determined and tentatively identified. While this application specifically focused on comparing a control and off-odor sample, the analytical tools and workflow are applicable for the direct comparison of any two samples for other types of non-targeted comparison work.