Application Note

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Instrument: Pegasus® BTX 4D, ChromaTOF® Tile

Investigation of Musty Malodor in Consumer Bath Towels with GCxGC and TOFMS

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Key Words: Fabric, Towel, Malodor, Off-odor, Aroma Profile, Solid Phase Microextraction, SPME, GC, GCxGC, MS, TOFMS, Deconvolution, Retention Index, RI, Sample Differentiation, *ChromaTOF, ChromaTOF* Tile

Introduction

Malodor aromas can be an issue for many different types of samples, including consumer fabrics like clothing and bath towels. With these fabrics, a musty malodor can sometimes arise after extended use. These odors can be challenging to remove with standard laundry and washing cycles, so they are a concern for consumers. Determining the source of these aromas with analytical tools can also be a challenge because the fabric materials can be quite complex and the specific analytes contributing to the aroma may be present at very low levels.

Comprehensive two-dimensional gas chromatography (GCxGC) with time-of-flight mass spectrometry (TOFMS) is a powerful tool for investigating these types of malodors. GCxGC helps to address the sample complexity by improving the peak capacity with two dimensions of complementary separations. This can uncover more of the chemical information about complex samples that may be challenging to determine with a traditional single-dimension GC separation. The *Pegasus* BTX TOFMS helps to address the sensitivity challenges by providing low-level detection with full m/z range non-skewed spectral data that can be readily searched against mass spectral databases to provide identifications for analytes, even at very low levels. This combination leads to detailed chemical data about challenging samples that can be screened for target analytes and investigated to determine non-targeted analytes of importance.

In this work, we investigated the volatile and semi-volatile chemical components of used consumer towels with a goal toward exploring the musty malodors. A collection of towel swatches, some with the malodor and some without, were sampled with headspace solid phase microextraction (HS-SPME) and analyzed with GCxGC-TOFMS. There were some analytes of interest with known aroma properties similar to what was observed with the sensory analysis of the towels. An additional non-targeted review of the data was also performed to determine other analytes of potential interest. Various software tools facilitated these data analysis tasks and are also discussed.

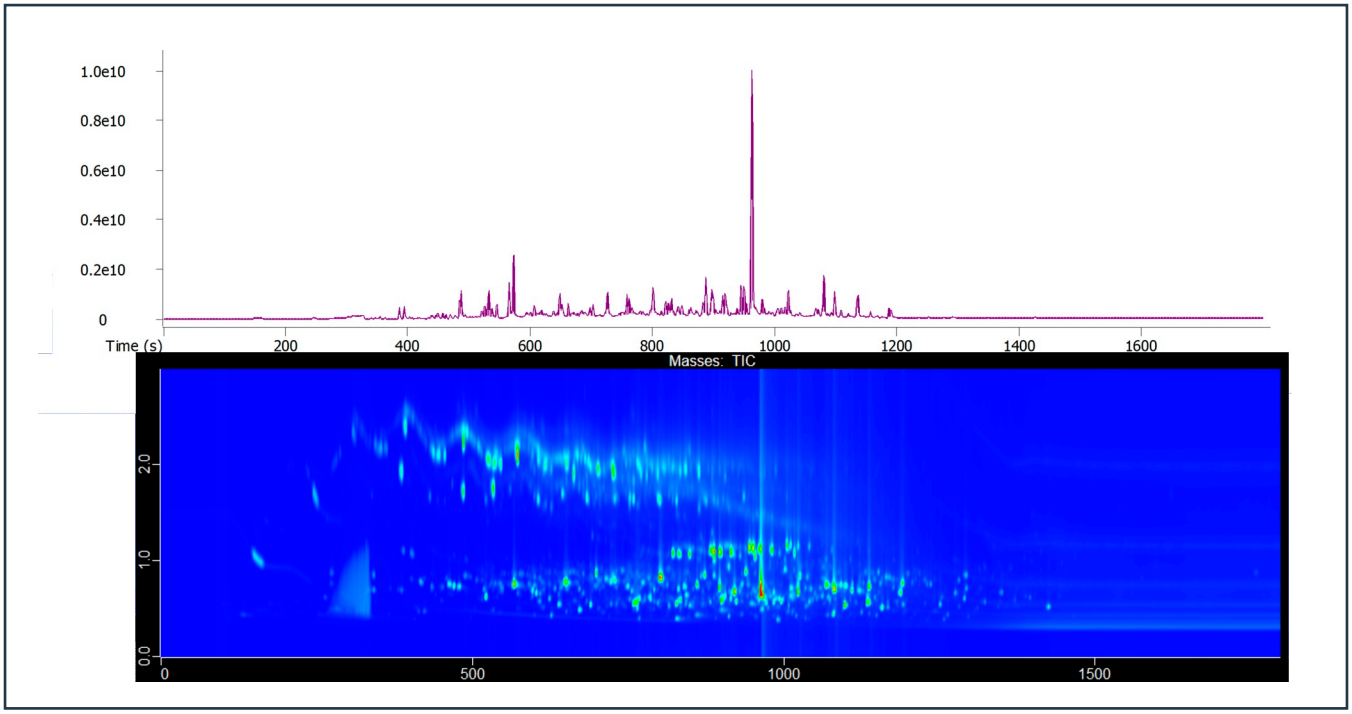


Figure 1. Representative GC chromatogram (top) and GCxGC contour plot (bottom) for the control towel sample, 004. The samples are very complex.

Experimental

Four post-use consumer towels were analyzed with GCxGC-TOFMS for their volatile and semi-volatile chemical profile. Sensory analysis had determined that three of the towels (001, 002, and 003) had some presence of a musty malodor, and one towel (004) was a control with a different dominant odor character. Prior to GCxGC-TOFMS analysis, each towel swatch (approximately 2x1.25 inches) was moistened with 300 µL of DI water and placed in a 20 mL HS-SPME vial. The vial was heated at 40 °C for 60 min in an LPAL agitator and then sampled with HS-SPME at a time between 0.5 and 24 h after heating. For HS-SPME, each vial was incubated for 10 min at 65 °C and extracted with a tri-phase fiber (PDMS, DVB, C-WR) for 30 min at the same temperature. The samples were then analyzed 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. Data were analyzed with *ChromaTOF* for peak finding and *ChromaTOF* Tile for automated differentiation.

Table 1. Instrument (*Pegasus* BTX 4D) Conditions

Autosampler	**LECO L-PAL 3 Autosampler**
Injection	SPME, Desorb for 2 min in GC inlet, splitless
Gas Chromatograph	**Agilent 8890 w/LECO QuadJet™ Thermal Modulator**
Inlet	250 °C
Carrier Gas	He @ 1.4 mL/min
Column	Stabilwax, 30 m x 0.25 mm i.d. x 0.25 µm coating Rxi-5ms, 0.6 m x 0.25 mm i.d. x 0.25 µm coating
Temperature Program	Hold 2 min at 40 °C, ramp 10 °C/min to 250 °C, hold 7 min Secondary Oven + 10 °C
Modulation	*QuadJet* thermal modulator, 3 s
Transfer Line	250 °C
Mass Spectrometer	**LECO *Pegasus* BTX**
Ion Source Temperature	250 °C
Mass Range	35-500 m/z
Acquisition Rate	10 spectra/s (GC); 100 spectra/s (GCxGC)

Results and Discussion

Uncovering analytes associated with aromas or odors can be challenging. The samples are often quite complex, which can make it difficult to distinguish individual analytes. Sensitivity can also be an issue as some analytes have very impactful aromas even at ultra-trace levels. The *Pegasus* BTX 4D GCxGC-TOFMS is well suited to address both of these challenges and, in this work, provided rich data to help characterize the towel samples.

For example, representative GC and GCxGC chromatograms for the control towel 004 are shown in Figure 1. The complexity of the sample is quite apparent, and GCxGC provided important benefits to better characterize these towels. To start investigating the individual analytes in these samples, LECO's non-target data processing was applied to automatically perform peak finding, deconvolution, peak integration, retention index (RI) calculations, and library searching for both the GC and GCxGC separations. This generated thorough peak table information for each sample with many analytes spanning from high S/N to low S/N.

Many analytes were well characterized with GC or GCxGC. For example, the peak with the highest S/N in Figure 1 was 1-dodecanol, which is shown in Figure 2. 1-dodecanol was determined with a good similarity score (926 and 930 in the GC and GCxGC data, respectively) and RI agreement to database values (1960 observed and 1969 library) in both the GC and GCxGC analyses. This compound is a surfactant and emulsifying agent that is used in detergents and other personal care products, so its presence on a consumer towel is not unexpected. It is likely not associated with the malodor, but it does have aroma properties that may be of interest. It has a "waxy" odor type with soapy, earthy, waxy, fatty, honey, and coconut descriptors.[1]

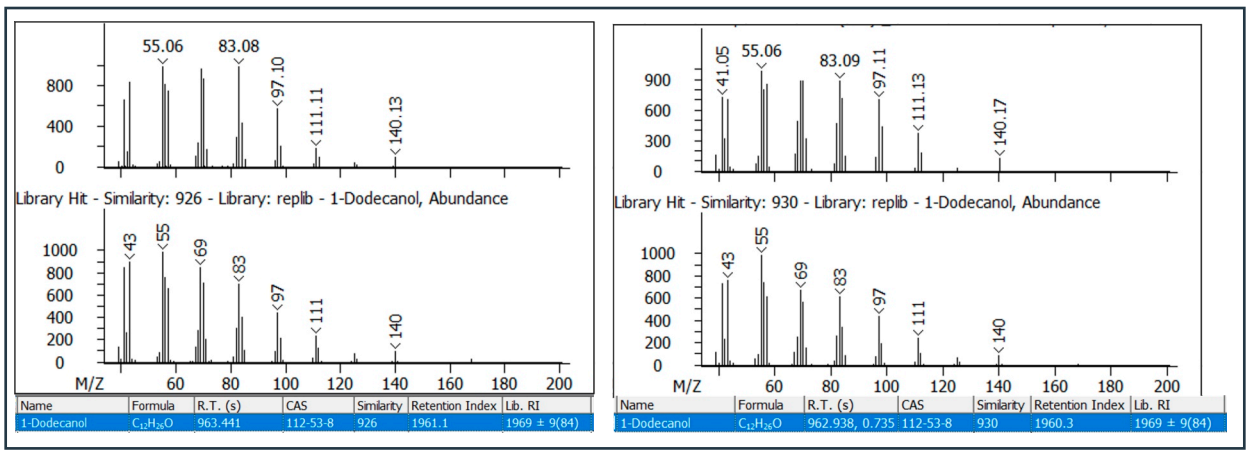
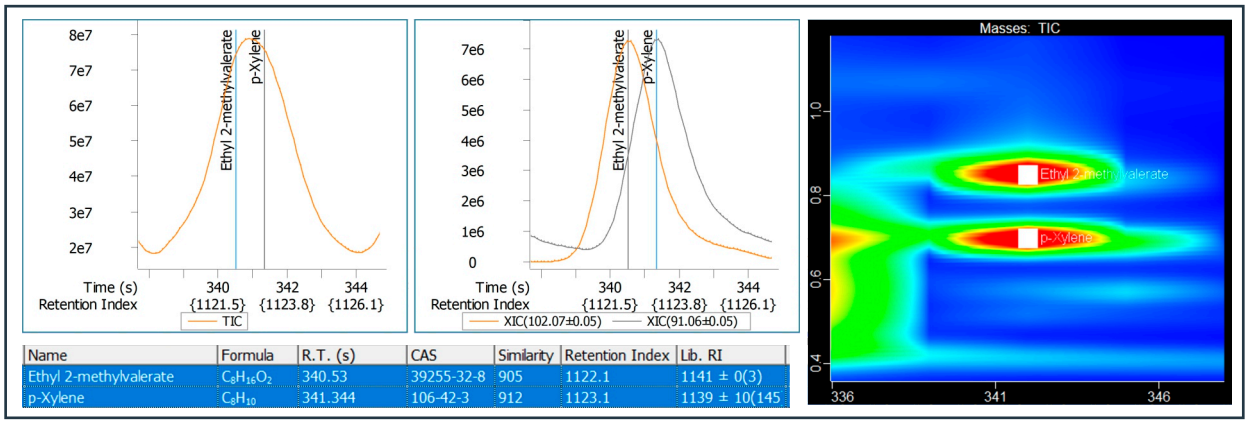


Figure 2. 1-dodecanol is the most intense peak in the GC and GCxGC chromatograms and was determined with GC and GCxGC analyses.

Other lower-level features were also well determined with both GC and GCxGC, including some that had coelutions which are quite common in samples with this type of complexity. In many cases, these primary column coelutions were mathematically deconvoluted in GC data, for example as shown in Figure 3. In this case, deconvolution of the GC data determined ethyl 2-methyl valerate and p-xylene with good similarity scores (905 and 912, respectively) and RI agreement (1122 to 1141 and 1123 to 1139, respectively). With GCxGC, these two analytes were chromatographically separated in the second dimension, also shown in Figure 3. This is another pair of analytes that was well-determined with GC or with GCxGC, even with their lower S/N and coelution.

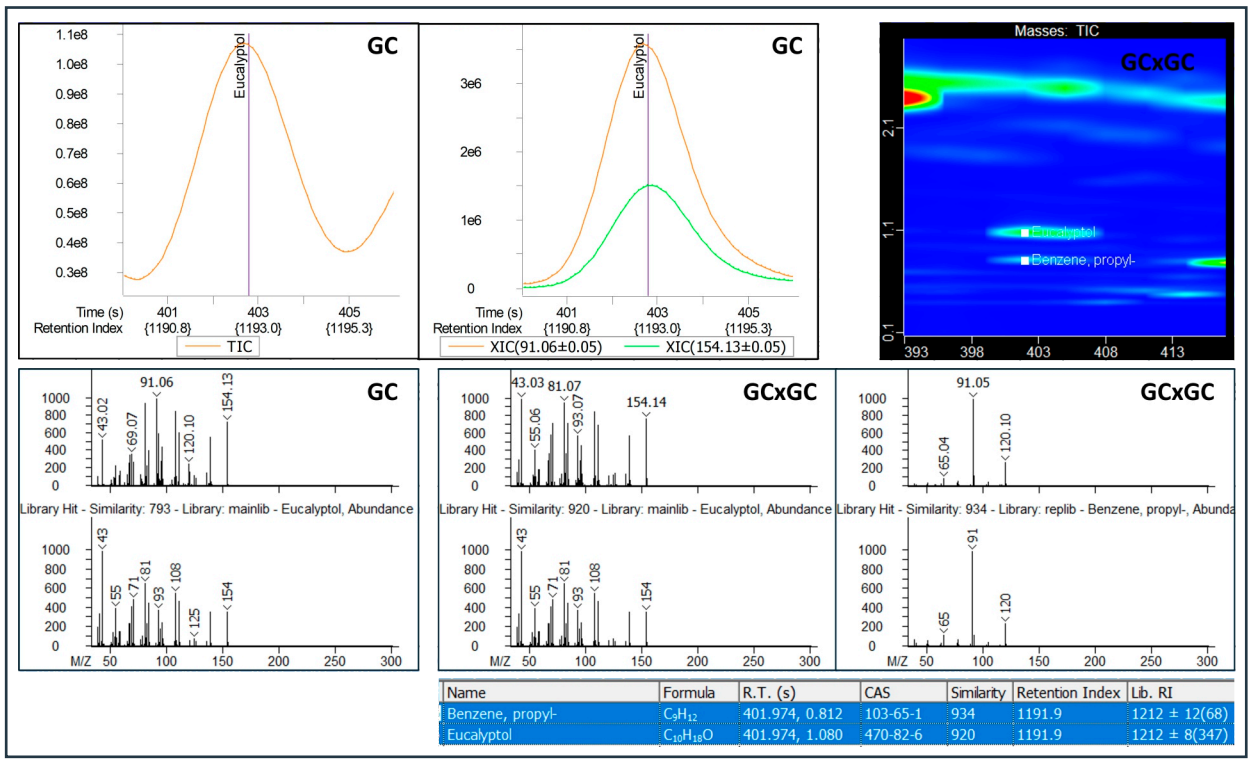


Name	Formula	R.T. (s)	CAS	Similarity	Retention Index	Lib. RI
Ethyl 2-methylvalerate	$C_8H_{16}O_2$	340.53	39255-32-8	905	1122.1	1141 ± 0(3)
p-Xylene	C_8H_{10}	341.344	106-42-3	912	1123.1	1139 ± 10(145

Figure 3. A coelution in the primary separation dimension is deconvoluted in the GC data and chromatographically separated in the GCxGC data.

However, there were other primary column coelutions that exceed deconvolution, as shown in Figures 4 and 5. In many of these instances, GCxGC chromatographically resolved these analytes in the second dimension, uncovering additional information about the sample that was obscured in the GC data.

For example, in Figure 4, the GC data indicated one analyte, eucalyptol, with a moderate similarity score of 793. A close look at the spectral information indicated some extra m/z (91 and 120, for example) contaminating the observed spectrum. In the GCxGC data, a second analyte, propyl benzene, was observed eluting with eucalyptol. This analyte still coelutes in the primary separation, but it is chromatographically resolved in the second dimension. The extra m/z in the 1D spectrum, 91 and 120, are associated with propyl benzene, indicating that it was completely merged with eucalyptol in the GC data, as can also be noted in XIC chromatogram in Figure 4. With the improved peak capacity, the similarity score for eucalyptol improved to 920 and propyl benzene was uncovered with a similarity score of 934. Both identifications were also supported by RI, as indicated in Figure 4. In this case, one moderately identified feature in the GC data became two well-identified features in the GCxGC data. The situation is the same for another coeluting pair, benzaldehyde and tetralin, shown in Figure 5, and for many other analytes in the data.



Name	Formula	R.T. (s)	CAS	Similarity	Retention Index	Lib. RI
Benzene, propyl-	C_9H_{12}	401.974, 0.812	103-65-1	934	1191.9	1212 ± 12(68)
Eucalyptol	$C_{10}H_{18}O$	401.974, 1.080	470-82-6	920	1191.9	1212 ± 8(347)

Figure 4. A coelution in the primary separation dimension exceeds deconvolution in GC data, but is chromatographically resolved in the GCxGC data.

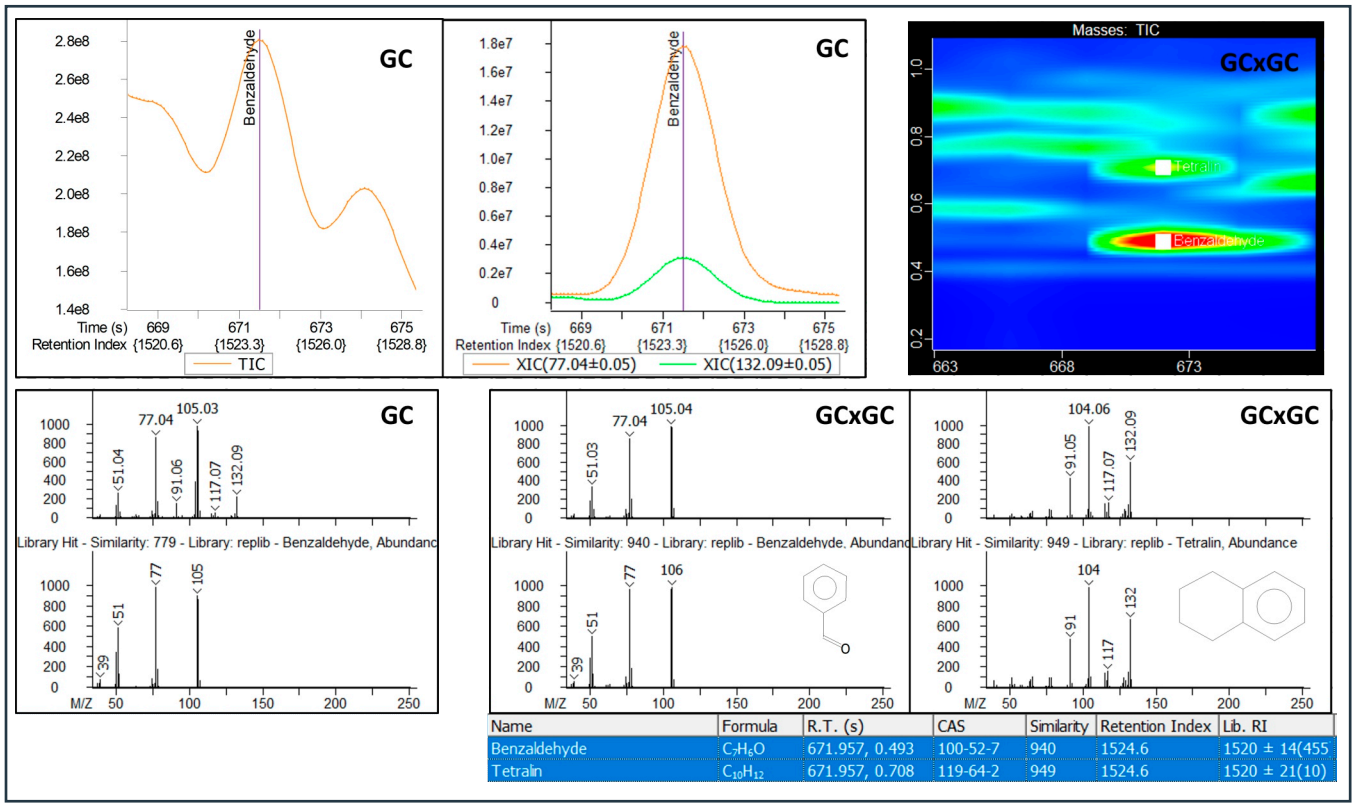


Figure 5. A coelution in the primary separation dimension exceeds deconvolution in GC data and chromatographically resolved in the GCxGC data.

These examples demonstrate why GCxGC is a good choice for investigating the malodor towels. More chemical information is uncovered, which is beneficial for non-targeted investigations where the analytes of interest are unknown. Additionally, this level of sample complexity can be challenging even for a targeted analysis as those analytes may be obscured. GCxGC often reveals buried analytes. Adding the excellent sensitivity of the BTX also helps uncover low-level analytes, providing even more chemical information about these samples, whether targeted or non-targeted. Thus, replicates of all four towel samples were acquired with GCxGC-TOFMS, and representative chromatograms for each are shown in Figure 6.

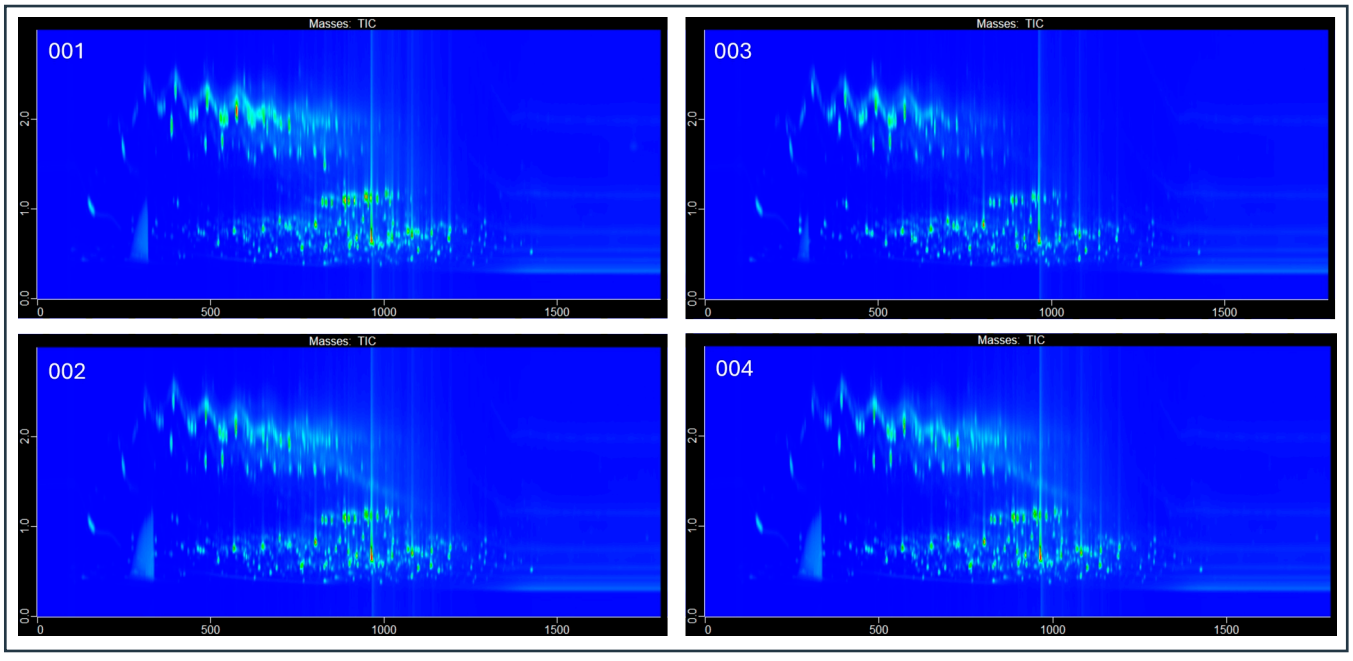


Figure 6. Representative GCxGC contour plots for all 4 towel samples.

While the complexity of the samples is quite apparent, obvious differences are not, so data analysis tools are useful to explore the data. A non-target comparison can help reveal unknown analytes of importance, but we can also start by exploring the rich data for analytes that were anticipated to be of interest. For example, several compounds with musty aroma notes that were consistent with the observed sensory analysis were noted from literature references.[2-4] These specific analytes are often challenging to detect because they can be impactful even at trace levels or can be buried with other analytes due to the sample complexity. With the *Pegasus* BTX 4D, four of the analytes hypothesized to be associated with the malodor were tentatively determined, as shown in Figure 7. All of these elute in complex regions of the chromatogram with significant coelution in the first dimension. GCxGC effectively separated them in the second dimension. The identifications were determined by matching the mass spectral data and most were supported by RI information, as indicated. The second-dimension elution position also supported the identifications, as the analytes elute in the region of the GCxGC structured chromatographic space as expected. The trends in the various towel samples are also shown in the bar charts. While some variability is observed and expected, as there was also heterogeneity between towel swatches in the malodor notes in the sensory data, the analytes were generally observed at higher levels in the malodor samples. These may be reasonable candidates for contributing to the musty aromas.

Comı	Name	Formula	R.T. (s)	CAS	Similarity	Retention Index	Lib. RI
1	6-Heptenoic acid	$C_7H_{12}O_2$	995.936, 0.404	1119-60-4	780	2015.4	
2	4-Ethyloctanoic acid	$C_{10}H_{20}O_2$	1118.93, 0.449	16493-80-4	655	2233.1	2198 ± 13(4)
3	1-Octen-3-ol	$C_8H_{16}O$	608.961, 0.527	3391-86-4	816	1441.7	1450 ± 7(304)
4	1-Octen-3-one	$C_8H_{14}O$	488.969, 0.673	4312-99-6	883	1292.9	1301 ± 9(202)

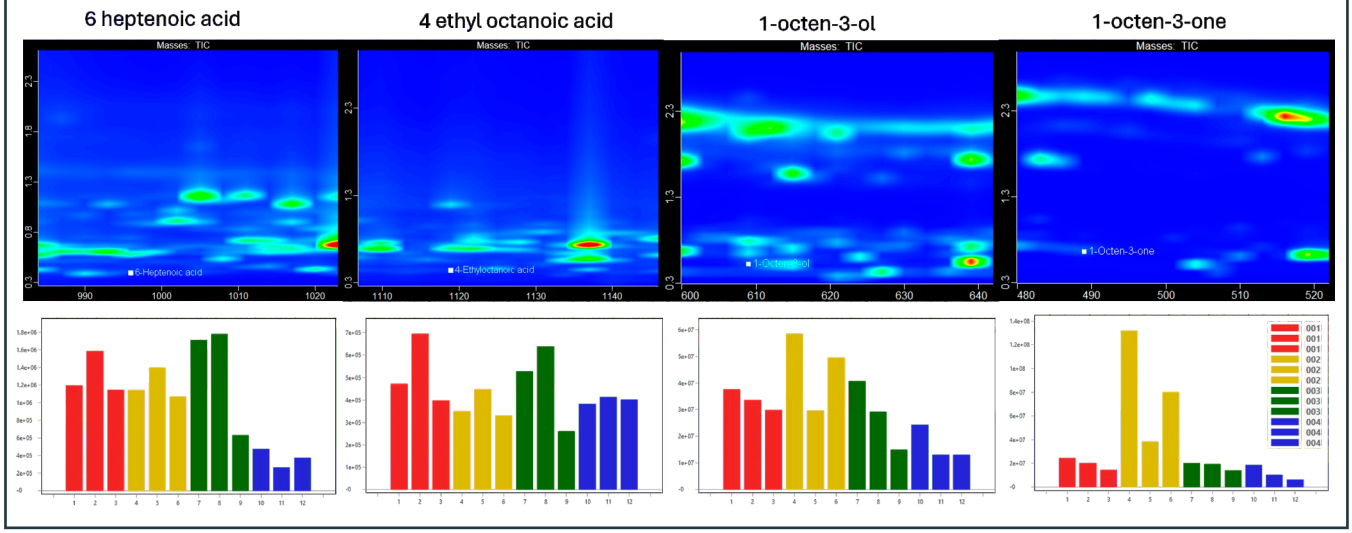


Figure 7. Several targeted musty aroma compounds were determined in the GCxGC-TOFMS data.

Other analytes that are often associated with strong odors can also be explored in a semi-targeted way. For example, some representative Sulfur-containing compounds are shown in Figure 8, and some carboxylic acids are shown in Figure 9. *ChromaTOF* software tools, like peak table filtering, were helpful for focusing on these types of specific analytes from the peak table results. These analytes were determined by matching the observed spectra and RI to library values, as indicated in the tables. The relative trends are also shown in the associated bar charts. Some of these analytes are generally higher in the musty towels and some are not.

Com	Name	Formula	R.T. (s)	CAS	Similarity	Retention Index	Lib. RI
1	Methanethiol	CH_4S	110.993, 0.979	74-93-1	831	673.5	690 ± 12(17)
2	Dimethyl disulfide	$C_2H_6S_2$	290.981, 0.559	624-92-0	922	1064.4	1077 ± 8(141)
3	Dimethyl trisulfide	$C_2H_6S_3$	557.964, 0.589	3658-80-8	921	1376.9	1377 ± 11(157)
4	Methional	C_4H_8OS	620.96, 0.472	3268-49-3	701	1457.2	1454 ± 8(199)
5	2-Thiophenecarboxaldehyde	C_5H_4OS	785.95, 0.450	98-03-3	826	1684.6	1684 ± 15(32)
6	Benzothiazole	C_7H_5NS	962.938, 0.499	95-16-9	939	1960.3	1958 ± 12(53)
7	2-(Methylmercapto)benzothiazole	$C_8H_7NS_2$	1226.92, 0.558	615-22-5	803	2427.5	2422 ± 0(4)
8	Dibenzothiophene	$C_{12}H_8S$	1343.91, 0.583	132-65-0	915	2638.0	

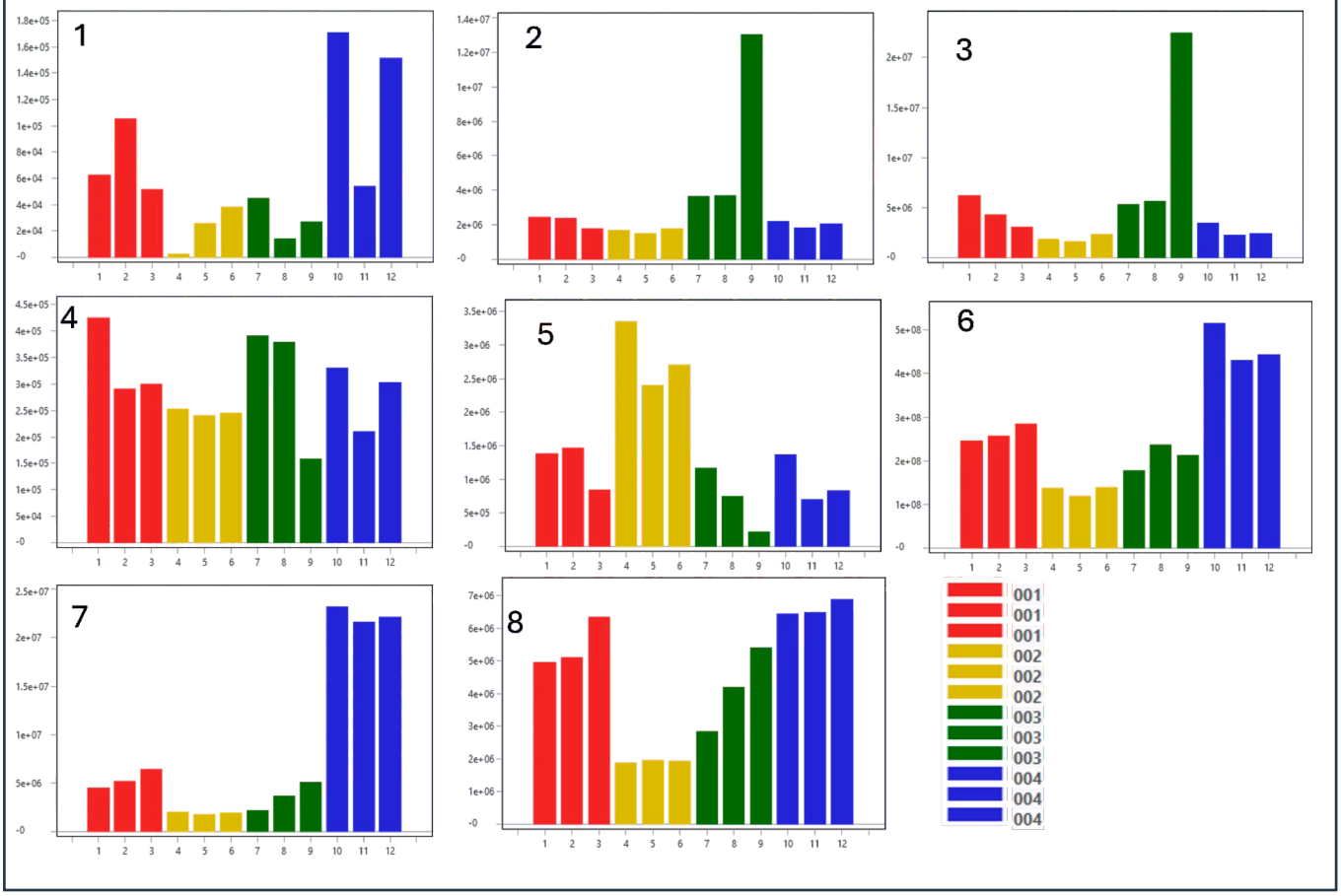


Figure 8. Representative Sulfur-containing compounds.

Comm	Name	Formula	R.T. (s)	CAS	Similarity	Retention Inc	Lib. RI
1	Acetic acid	$C_2H_4O_2$	620.96, 0.373	64-19-7	941	1457.2	1449 ± 13(358)
2	Hexanoic acid	$C_6H_{12}O_2$	890.943, 0.404	142-62-1	835	1844.0	1846 ± 12(320)
3	Heptanoic acid	$C_7H_{14}O_2$	956.939, 0.421	111-14-8	877	1950.4	1950 ± 15(89)
4	Octanoic acid	$C_8H_{16}O_2$	1019.93, 0.430	124-07-2	894	2056.8	2060 ± 15(259)
5	Nonanoic acid	$C_9H_{18}O_2$	1079.93, 0.453	112-05-0	894	2162.9	2171 ± 18(142)
6	Decanoic acid	$C_{10}H_{20}O_2$	1136.93, 0.454	334-48-5	888	2265.5	2276 ± 15(176)

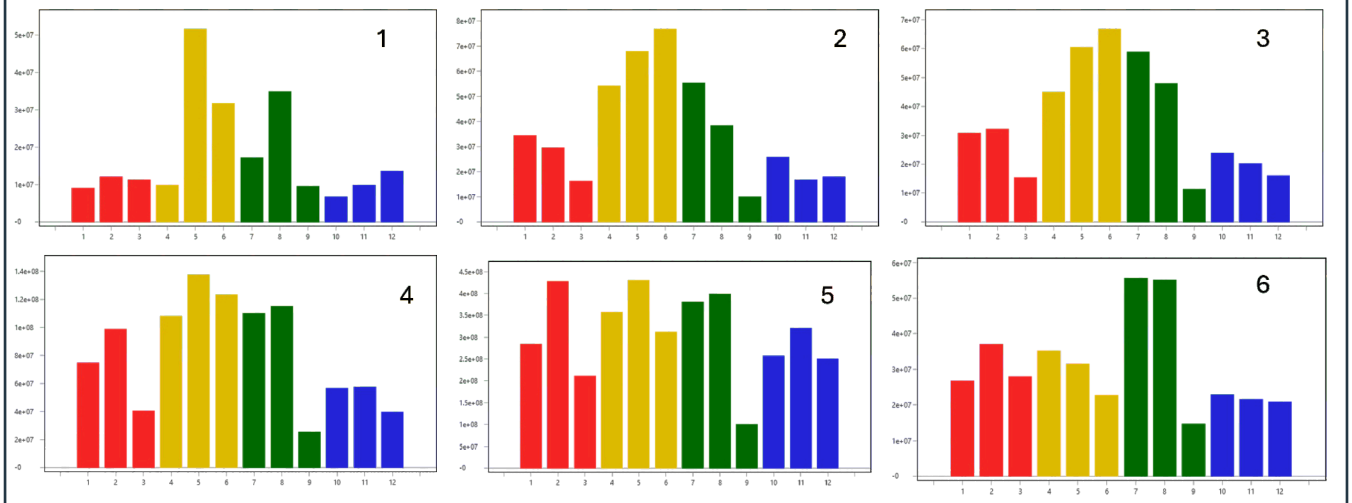
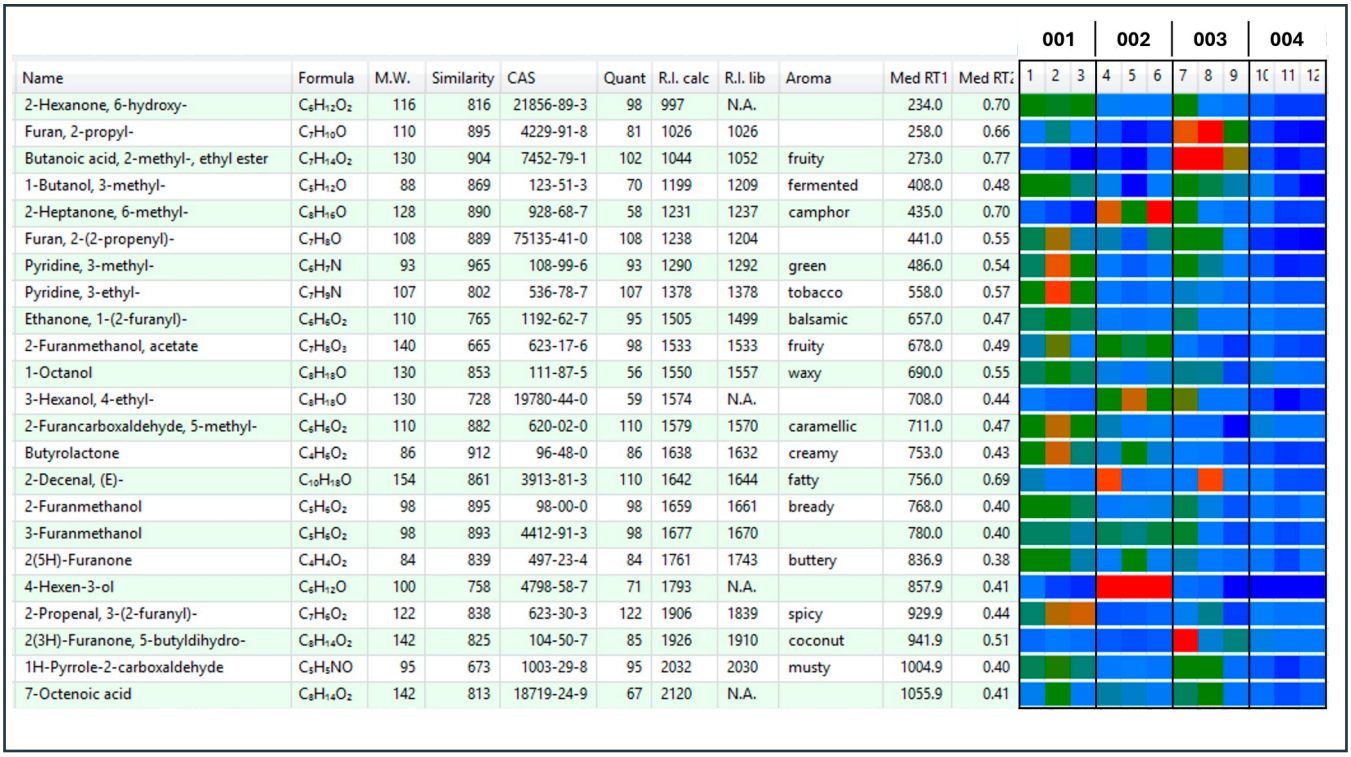


Figure 9. Representative carboxylic acids compounds.

In addition to reviewing the data for targeted analytes, a non-targeted investigation can also reveal additional interesting information about the samples. This can be done with *ChromaTOF* peak find results, but software that analyzes the sample set together, like *ChromaTOF* Tile, can also be particularly helpful for this type of data review. *ChromaTOF* Tile connects the samples and rapidly compares the raw data to highlight differences across the sample set. This is an efficient way to focus data review on the regions of the chromatogram where the samples differ, streamlining the non-targeted investigation and making it possible to efficiently find features with specific trends. For example, we can review the feature trends and focus on those that were generally observed at higher levels in towels 001, 002, and/or 003 relative to 004. As noted previously, variability between the towel swatches is anticipated and observed, but some representative examples with these general trends are shown in Figure 10. Tentative identifications were determined in Tile with automated spectral matching of background subtracted caliper spectra to library databases with similarity scores listed. In many cases, the identifications were further supported by comparing observed RI information to the library database, as indicated. The relative intensity trends across the sample set are summarized in the heatmap columns of the table. The first three columns are the replicates of towel 001, the next 3 columns are towel 002, etc. Red in the heatmap indicates higher levels and blue indicates lower levels. These tools can help to reveal analyte trends and uncover additional analytes of interest that may not have been known to target.



Name	Formula	M.W.	Similarity	CAS	Quant	R.I. calc	R.I. lib	Aroma	Med RT1	Med RT2
2-Hexanone, 6-hydroxy-	$C_6H_{12}O_2$	116	816	21856-89-3	98	997	N.A.		234.0	0.70
Furan, 2-propyl-	$C_7H_{10}O$	110	895	4229-91-8	81	1026	1026		258.0	0.66
Butanoic acid, 2-methyl-, ethyl ester	$C_7H_{14}O_2$	130	904	7452-79-1	102	1044	1052	fruity	273.0	0.77
1-Butanol, 3-methyl-	$C_5H_{12}O$	88	869	123-51-3	70	1199	1209	fermented	408.0	0.48
2-Heptanone, 6-methyl-	$C_8H_{16}O$	128	890	928-68-7	58	1231	1237	camphor	435.0	0.70
Furan, 2-(2-propenyl)-	C_7H_8O	108	889	75135-41-0	108	1238	1204		441.0	0.55
Pyridine, 3-methyl-	C_6H_7N	93	965	108-99-6	93	1290	1292	green	486.0	0.54
Pyridine, 3-ethyl-	C_7H_9N	107	802	536-78-7	107	1378	1378	tobacco	558.0	0.57
Ethanone, 1-(2-furanyl)-	$C_6H_6O_2$	110	765	1192-62-7	95	1505	1499	balsamic	657.0	0.47
2-Furanmethanol, acetate	$C_7H_8O_3$	140	665	623-17-6	98	1533	1533	fruity	678.0	0.49
1-Octanol	$C_8H_{18}O$	130	853	111-87-5	56	1550	1557	waxy	690.0	0.55
3-Hexanol, 4-ethyl-	$C_8H_{18}O$	130	728	19780-44-0	59	1574	N.A.		708.0	0.44
2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	110	882	620-02-0	110	1579	1570	caramellic	711.0	0.47
Butyrolactone	$C_4H_6O_2$	86	912	96-48-0	86	1638	1632	creamy	753.0	0.43
2-Decenal, (E)-	$C_{10}H_{18}O$	154	861	3913-81-3	110	1642	1644	fatty	756.0	0.69
2-Furanmethanol	$C_5H_6O_2$	98	895	98-00-0	98	1659	1661	bready	768.0	0.40
3-Furanmethanol	$C_5H_6O_2$	98	893	4412-91-3	98	1677	1670		780.0	0.40
2(5H)-Furanone	$C_4H_4O_2$	84	839	497-23-4	84	1761	1743	buttery	836.9	0.38
4-Hexen-3-ol	$C_6H_{12}O$	100	758	4798-58-7	71	1793	N.A.		857.9	0.41
2-Propenal, 3-(2-furanyl)-	$C_7H_6O_2$	122	838	623-30-3	122	1906	1839	spicy	929.9	0.44
2(3H)-Furanone, 5-butyldihydro-	$C_8H_{14}O_2$	142	825	104-50-7	85	1926	1910	coconut	941.9	0.51
1H-Pyrrole-2-carboxaldehyde	C_5H_5NO	95	673	1003-29-8	95	2032	2030	musty	1004.9	0.40
7-Octenoic acid	$C_8H_{14}O_2$	142	813	18719-24-9	67	2120	N.A.		1055.9	0.41

Figure 10. Representative non-targeted features determined with ChromaTOF Tile.

GCxGC, TOFMS, and software tools were all useful for uncovering the compounds shown in Figures 7-10. *ChromaTOF* Tile compiled the sample set data, which was helpful for reviewing the trends shown in Figure 10, and was also useful for compiling trends for other features, for example the bar charts shown in Figures 7-9. Being able to determine these analytes within these complex samples is an important step towards understanding the malodors, and these can be further investigated as candidates.

Conclusion

In this work, LECO's *Pegasus* BTX 4D GCxGC-TOFMS was used to evaluate a set of towel swatches, some with malodors. The samples were very complex with many analytes spanning a large dynamic range. GCxGC effectively separated individual analytes within this complex sample and the BTX TOFMS provided MS detection with excellent sensitivity. This hardware combination helped uncover more analyte information than could be determined with a single GC separation. The data were reviewed for target analytes that were hypothesized to connect with the malodor, and a non-targeted review was performed with *ChromaTOF* Tile to uncover additional analytes that may be of interest. Many analytes with relevant aroma descriptors and sample trends were determined.

References

[1] www.goodscents.com

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