

Instrument: Pegasus® BT 4D**Differentiating Original-Brand and Imitation Perfumes with GCxGC-TOFMS and ChromaTOF® Tile**

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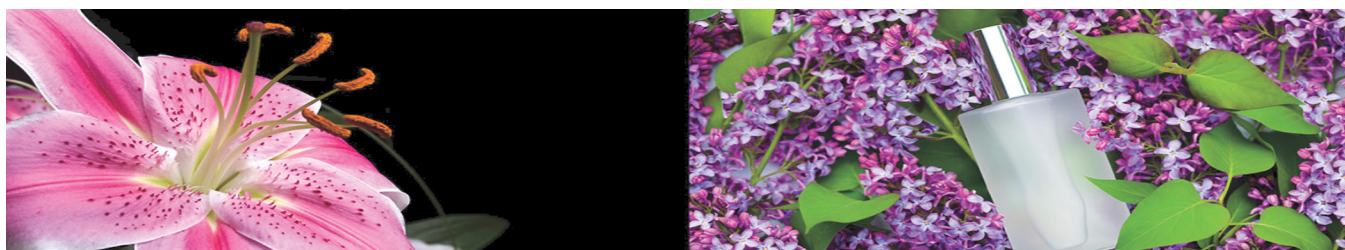
Key Words: Perfume, GCxGC, Differentiation

Introduction

The creation of a perfume can be a significant investment for a company. It is a long process, requiring many trials, and a lot of effort and resources are typically invested to refine the final formula. Additionally, a number of fairly expensive tests are conducted to prove compliance with the current IFRA (International Fragrance Association) regulations and to ensure the products' safety for the consumer. Nonetheless, the global perfume market has demonstrated remarkable growth in the last years with positive forecasts for the coming years. Coinciding with this market growth, the counterfeit perfume industry is also booming. Such illegal counterfeits often copy not only the smell but also the bottle and packaging of a genuine perfume.

In addition to the financial implications for the brand and consumer, these counterfeit perfumes are often made with cheap ingredients, and sometimes contain harmful components that can be damaging to consumer health and the planet. Since the manufacturing process of counterfeit perfumes is often done in an unregulated way, there is little control over the quality of the final product.

To investigate and to protect both the consumer and the original brands from the surge of counterfeits, perfume manufactures are continuously looking for effective methods to analyze and differentiate perfume samples, including authentic perfumes from counterfeit. In this Application Note, we use comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOFMS) and ChromaTOF® Tile, a statistical analysis software for GCxGC data, to differentiate perfume samples. The use of hydrogen as carrier gas for the GC analysis is also demonstrated. This presents a more cost-conscious alternative to helium, not only for high throughput analyses such as those conducted in routine laboratories, but also offers higher sensitivity at significantly lower operating costs.

**Experimental**

One original perfume sample and two imitation products were analyzed for this investigation. The imitation perfumes were used to represent counterfeit samples. Prior to liquid injection, the samples were diluted to 1% (v/v) in hexane. Table 1 summarizes the parameters applied for the injection conditions and the GCxGC-TOFMS analysis. The use of hydrogen as carrier gas resulted in a total run time <22 minutes. An n-Alkane standard (C7–C30) was analyzed for calculation of linear retention indices (RI).

Table 1: GCxGC-TOFMS method parameters.

Gas Chromatograph	LECO GCxGC Quad Jet Thermal Modulator
Injector	0.5 μ L, split (1:100), 250 °C
Carrier Gas	Hydrogen, 1.26 mL/min, constant flow
Columns	Rxi-5ms 20 m x 0.18 mm x 0.18 μ m (Restek) Rxi-17ms 0.6 m x 0.18 mm x 0.18 μ m (Restek)
Oven Program	50 °C (0.1 min), 10 °C/min to 240, 30 °C/min to 280 °C (1 min)
Secondary Oven Temp	+6 °C (relative to the GC oven temperature)
Modulator Temp	+15 °C (relative to the secondary oven temperature)
Modulation Period	2 s
Transfer Line	280 °C
Mass Spectrometer	LECO Pegasus BT 4D
Ion Source Temp	250 °C
Mass Range	40 – 500 m/z
Acquisition Rate	200 spectra/s

Results and Discussion

One of the challenges with the characterization of counterfeit samples is that there can be many different versions with varying compositions. As such, non-target analyses are generally needed since it is not usually apparent prior to analysis which analytes will be of interest during measurement and compared in these complex samples.

GC-MS is a widely used analytical technique for the non-target investigation of perfume samples, and it can be applied to the determination of counterfeit samples as well. An even greater amount of information can be gained by pairing an additional complementary separation, such as that offered by GCxGC. Coupling either of these separations with TOFMS also provides superior information, ultimately resulting in higher confidence in analyte identifications.

The use of GCxGC-TOFMS for the analysis of perfumes provides improved understanding of the samples. GCxGC typically results in a better chromatographic separation, as many analytes that would be coeluting in a single chromatographic dimension can be separated in the second. This led to a greater number of detected analytes, many with important odor characteristics, and high-quality spectral information supported by retention index calculation. These benefits can improve the differentiation and characterization of perfume samples.

Figure 1 demonstrates the power of GCxGC in the brand perfume. Two analytes, benzyl acetate (CAS: 140-11-4) and grapefruit acetal (CAS: 67674-46-8), are highlighted. The two compounds have the same experimental retention time (RT_{exp}) in the first dimension (RT₁). This coelution is not surprising, as their library retention index (RI_{lib}) values are only three RI units apart. However, the two analytes were well separated in the second dimension, allowing for a straightforward identification. Both compounds are odor active and contribute to the smell of the perfume.

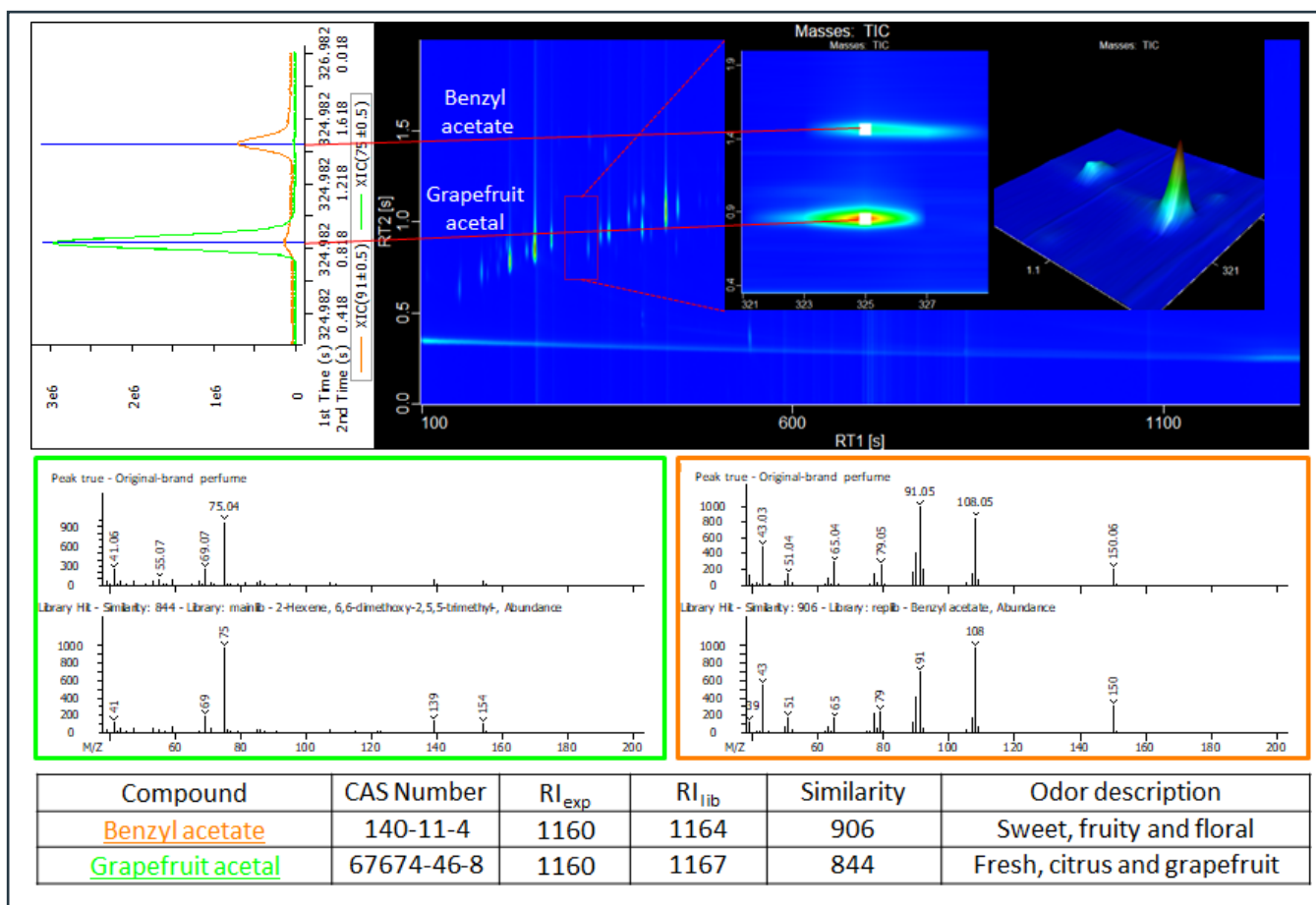


Figure 1: GCxGC separations of a brand perfume sample. GCxGC provides improved detectability, increased peak capacity, and structured chromatograms. Here, benzyl acetate and grapefruit acetal were chromatographically separated in the second dimension and identified.

A comparison of these two analytes in the three perfume samples are shown in Figure 2. The difference in concentration of the individual analytes in the three tested products is apparent and points to some significant differences in the formulations. Therefore, a correct determination of these analytes is essential for a meaningful interpretation of the samples and may have been difficult to achieve with a 1D GC analysis.

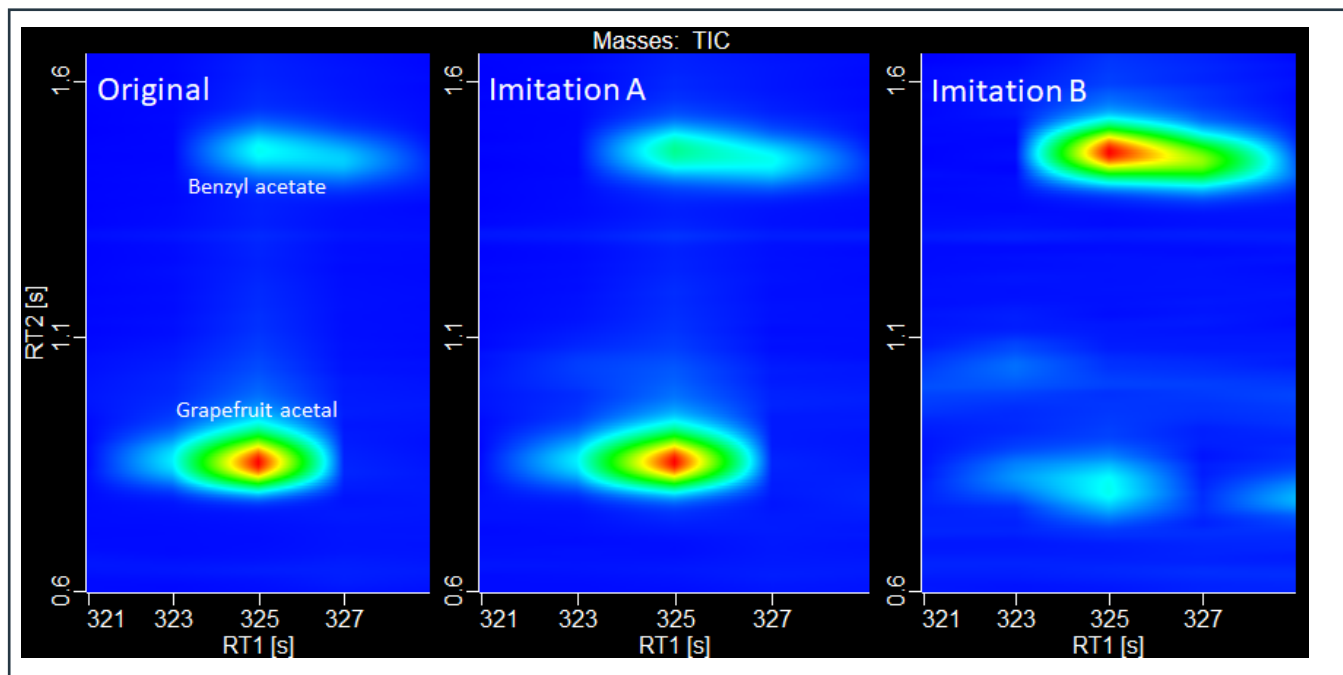


Figure 2: Close up of the total ion chromatogram (TIC) GCxGC-contour plots for benzyl acetate and grapefruit acetal. The analytes were observed at different levels in the original-brand perfume and the imitation products, A and B.

Determining additional differences in the perfumes requires comparing GCxGC data across multiple samples. The amount of data generated by multiple GCxGC runs can be a challenge to manage, and analytical chemists encounter persistent difficulties when performing non-targeted analyses of multiple data. Software tools are crucial for streamlining data analysis and interpretation. *ChromaTOF* Tile is a software tool that employs a Tile-based Fisher-Ratio approach to facilitate the comparison of extensive data sets. Specifically, it generates a list of hits that directs towards compounds with high discriminative power for differentiating sample groups. In other words, compounds which show a different behavior in the compared groups of samples are easily highlighted without getting lost in the volume of data. A subset of these analytes are shown in Figure 3. On the right side of the table, a heat map indicates the relative trends of each analyte in the three different perfumes. For example, 2-ethylhexyl salicylate (CAS: 118-60-5) at the top of the table, having a floral odor, is high in the original-brand perfume (red color) and low in the two imitation products (blue color). Another example is alpha-gurjunene (CAS: 489-40-7) at the bottom of the table, with a woody odor; this component is highly present in the imitation B, and relatively low in the imitation A and the original-brand perfume. These individual features can be explored further and their relative trends e.g., of benzyl acetate and grapefruit acetal, can be displayed using a bar chart, as shown in Figures 4 and 5.

Name	Similarity	CAS	R.I. calc	R.I. lib	R.I. Δ	F-ratio	Med RT1	Med RT2	1	2	3	4	5	6	7	8	9
2-Ethylhexyl salicylate	845	118-60-5	1799	1807	-8	444998.06	791.0	1.20	32	39	12	37	39	30	35	30	37
Grapefruit acetal	842	67674-46-8	1160	1167	-7	13158.26	325.0	0.86	37	18	31	13	39	74	35	34	16
3-Carene	904	13466-78-9	1008	1011	-3	3437.51	203.0	0.75	33	31	16	35	37	39	78	10	31
Lilial	839	80-54-6	1525	1533	-8	49478.57	607.0	1.39	36	33	24	38	33	19	39	35	19
Camphene	937	79-92-5	949	952	-3	323.47	163.0	0.66	30	31	39	13	35	13	28	70	34
Linalyl acetate	864	115-95-7	1249	1257	-8	9854.41	397.0	1.00	38	33	38	73	13	39	31	37	72
Manzanate	935	39255-32-8	934	941	-7	1525.47	153.0	0.72	34	75	39	33	11	76	35	77	32
Caryophyllene	910	87-44-5	1420	1419	1	576.12	531.0	0.98	70	73	37	33	13	24	78	36	31
Oxacyclohexadecen-2-one	895	34902-57-3	1819	1815	4	8400.81	803.0	1.43	34	38	37	21	30	75	37	13	35
Copaene	931	3856-25-5	1376	1376	0	3505.61	497.0	0.90	72	30	39	30	26	34	74	35	17
Isoamyl salicylate	857	87-20-7	1533	1535	-2	4038.93	613.0	1.26	32	13	33	33	32	31	36	32	37
Gamma-gurjunene	914	22567-17-5	1472	1473	-1	3573.50	569.0	1.04	77	37	39	70	15	32	39	36	39
Laevo-camphor	934	464-48-2	1145	1145	0	1662.45	313.0	1.21	38	36	25	34	78	31	35	32	37
Beta-patchoulene	897	514-51-2	1383	1381	2	2728.79	503.0	0.92	39	36	33	27	30	30	31	37	77
Eucalyptol	910	470-82-6	1031	1032	-1	2470.82	221.0	0.84	23	13	34	78	39	39	33	13	16
Benzyl Benzoate	901	120-51-4	1765	1763	2	78195.97	769.0	1.85	31	34	39	74	34	36	34	38	36
α-Guaiene	928	3691-12-1	1436	1439	-3	8175.98	543.0	0.96	11	36	39	78	10	32	35	13	31
Isopropyl palmitate	883	142-91-6	2023	2024	-1	700.30	922.9	0.91	21	37	31	38	11	16	36	35	39
Guaiol	872	489-86-1	1596	1596	0	16161.22	657.0	1.17	35	37	37	70	33	36	36	33	39
Isopropyl myristate	926	110-27-0	1825	1827	-2	5120350.90	807.0	0.91	35	32	36	78	70	37	39	39	33
Alpha-gurjunene	924	489-40-7	1407	1409	-2	69629.44	521.0	0.95	12	39	12	27	30	17	74	38	72
2'-hydroxyacetophenone	930	118-93-4	1158	1163	-5	431.87	323.0	1.56	30	30	30	34	37	36	30	30	30

Figure 3: Peak identification metrics (similarity score, RI information) and relative trends (heat map color scale) for representative analytes from various compound classes. Imitation A: columns 1-3, Imitation B: columns 4-6 and, original-brand perfume: columns 7-9.

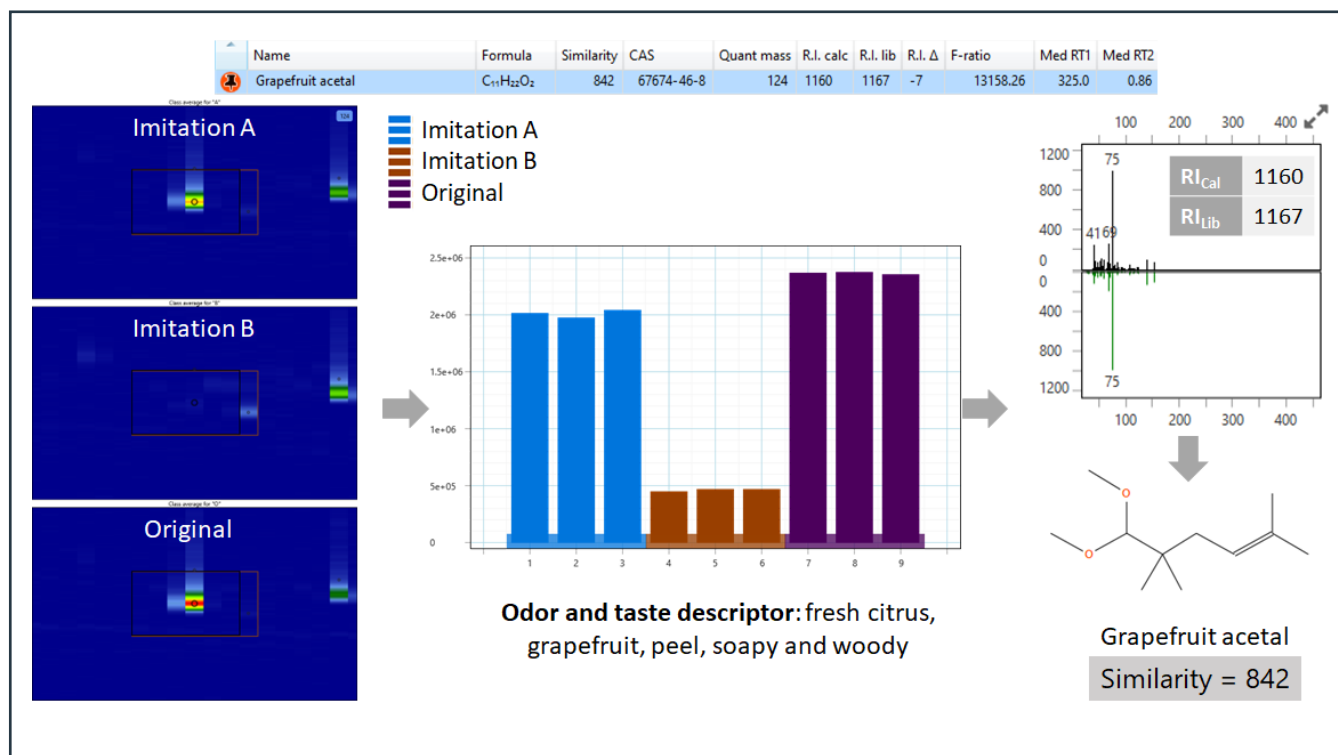


Figure 4: Relative trend of grapefruit acetal (CAS: 67674-46-8) which was determined as a class-distinguishing feature through ChromaTOF Tile. It was identified through spectral and RI matching and is observed at higher levels in the original-brand perfume and imitation product A.

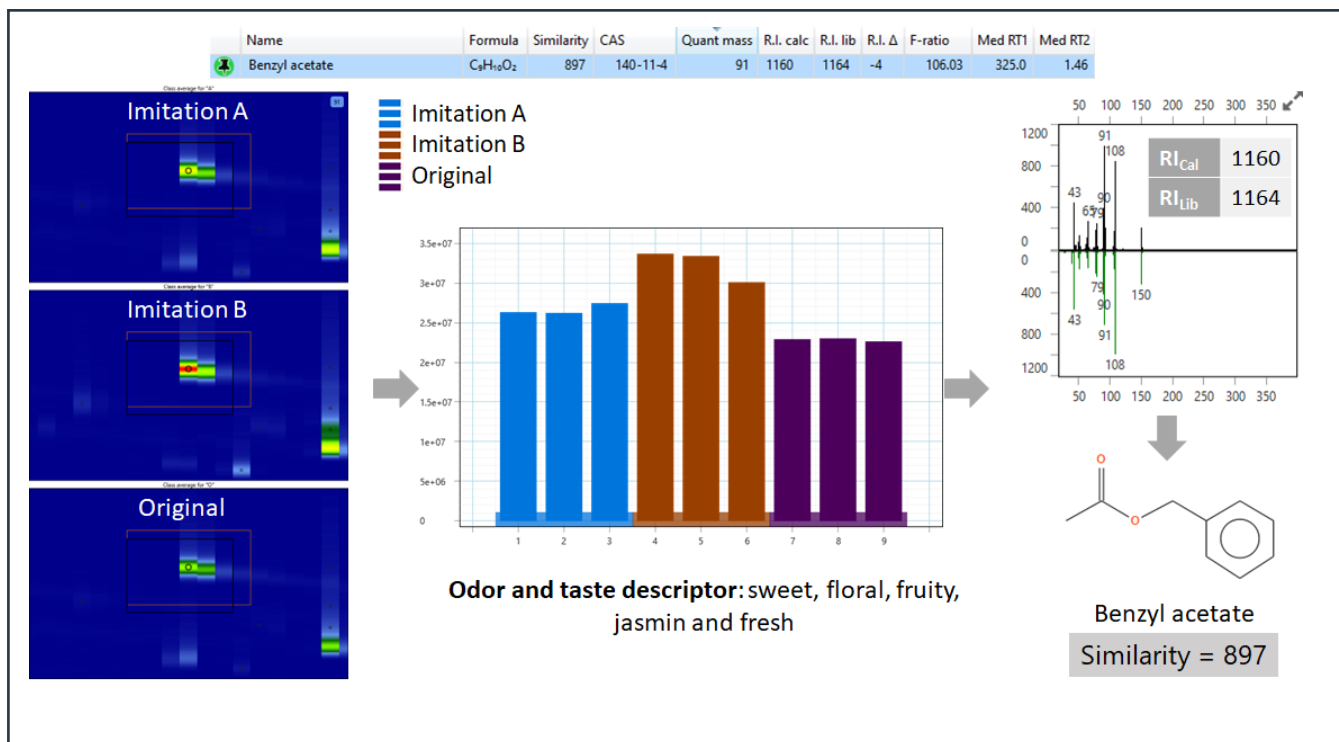


Figure 5: Relative trend of benzyl acetate (CAS: 140-11-4) which was determined as a class-distinguishing feature through ChromaTOF Tile. It was identified through spectral and RI matching and is observed at higher levels in the imitation product B.

Principal Component Analysis (PCA), which is commonly employed for the reduction of high dimensional data, can also be conducted with ChromaTOF Tile to reveal clustering based on chemical similarities and differences in the data. Figure 6 shows the scores plot as a result of the PCA run on the differentiating features of the original perfume and the two imitations. The distinct grouping of the sample shows that the formulations differ to such an extent that the chemical profiles can be distinguished by these means.

Furthermore, the use of hydrogen as carrier gas for this work allowed for a shorter analysis time. The overall analysis time was less than 22 minutes. This was achieved without forfeiting the chromatographic resolution or spectral quality, making such an approach extremely suitable for high-throughput and routine analysis pressured on time and cost savings on each sample analysis.

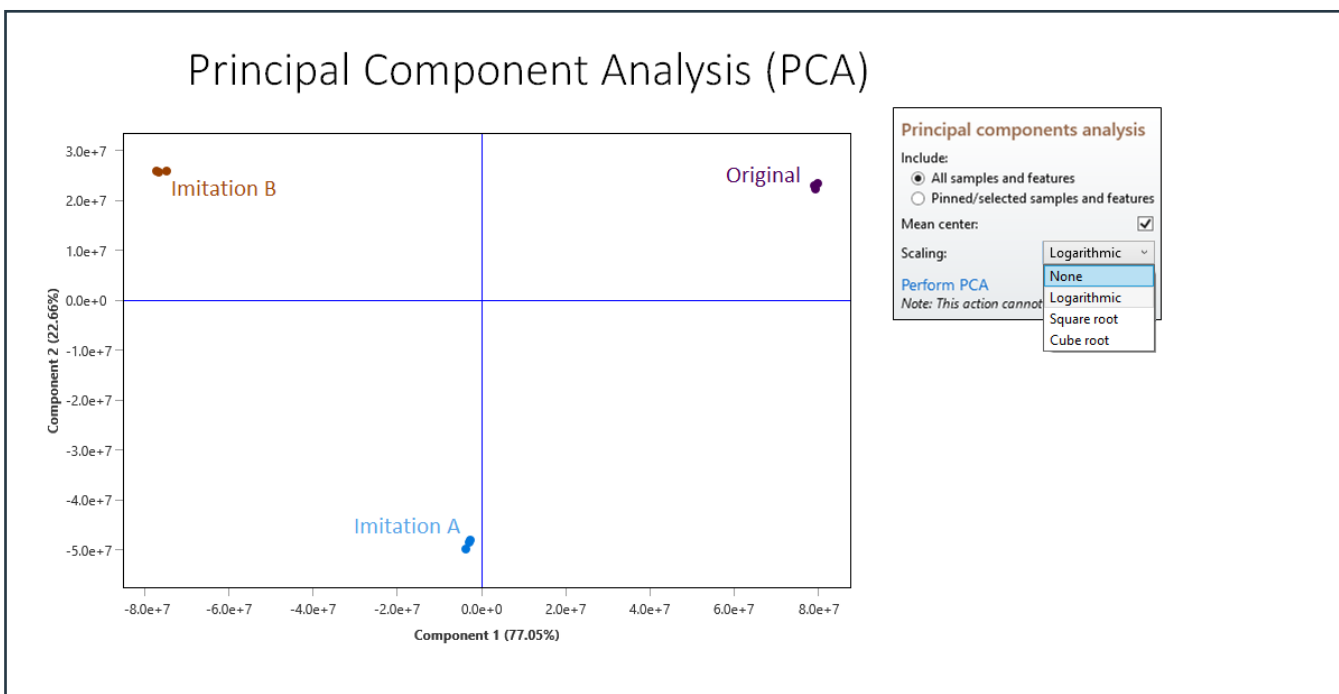


Figure 6: Principal Component Analysis (PCA) Scores plot shows distinct clusters for the original-brand and the imitation perfumes.

Conclusion

This Application Note presents the analysis of original-brand and imitation perfumes by GCxGC-TOFMS in combination with statistical data processing by *ChromaTOF* Tile. The improved separation power provided by GCxGC is preserved when using hydrogen as carrier gas and was crucial for separating first dimension coelutions in these samples. The data processing was supported by *ChromaTOF* Tile, which assisted in the discovery of trends within the data sets. Differences between the original-brand perfume and the two tested imitation products could be explored aiding to differentiate the products.

GCxGC technology together with *ChromaTOF* Tile software are an effective way to draw a comprehensive picture of these samples and the ability to distinguish differentially expressed analytes. Compounds of importance were confidently identified, including many with important odor characteristics. Using *ChromaTOF* Tile, even 100's of samples can be processed simultaneously. Thus, this approach can be considered powerful for differentiation purposes such as the investigation of counterfeit or competitor products, in laboratories where the throughput and the quality of the results are the key point of success, while saving on overall operating costs.

