Application Note

Instrument: Pegasus® BT 4D

Improved Characterization and Differentiation of Perfume Samples with GCxGC

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Key Words: Perfume, Aroma Profile, Sample Differentiation, HS-SPME, GC-TOFMS, GCxGC-TOFMS, Flow Modulation, Deconvolution, Perfect Coelution, Peak Capacity

Introduction

Gas chromatography (GC) is an important tool to separate individual analytes in a complex sample. Coupling GC with time-of-flight mass spectrometry (TOFMS) allows for identification and quantification of the separated analytes. Using GC-TOFMS for aroma profiling can provide insight to a variety of sample types for a wide range of purposes, including general characterization, sample differentiation, process tracking, quality control monitoring, reverse engineering of a product, and overall improved understanding of your sample. In this work, we compare a brand perfume to two drugstore imitations that are intended to mimic the brand. The samples are easily distinguished by scent, and the detailed chemical information provided by GC-TOFMS shows both similarities and differences in the chemical profiles of these perfumes. Many of these chemical similarities and differences can be determined and understood with GC-TOFMS, but some analytes of interest were difficult to confidently identify and reliably quantify. For complex samples like these, it is common for some analytes to coelute and exceed the capability of the first dimension separation. Extending the chromatographic resolution into a second dimension with GCxGC can help separate the coelutions and clarify the information in many cases. GCxGC adds the second dimension of separation by connecting a second column with a complementary stationary phase in series with the primary column through a modulating device that collects primary effluent and reinjects to the second column. Flow-based modulation with LECO's *Pegasus* BT 4D FLUX™ offers a lower-cost, robust, and easy-to-use option for adding GCxGC to your routine analyses. Some key analytes of interest that were difficult to understand with GC were clarified by GCxGC in this work.

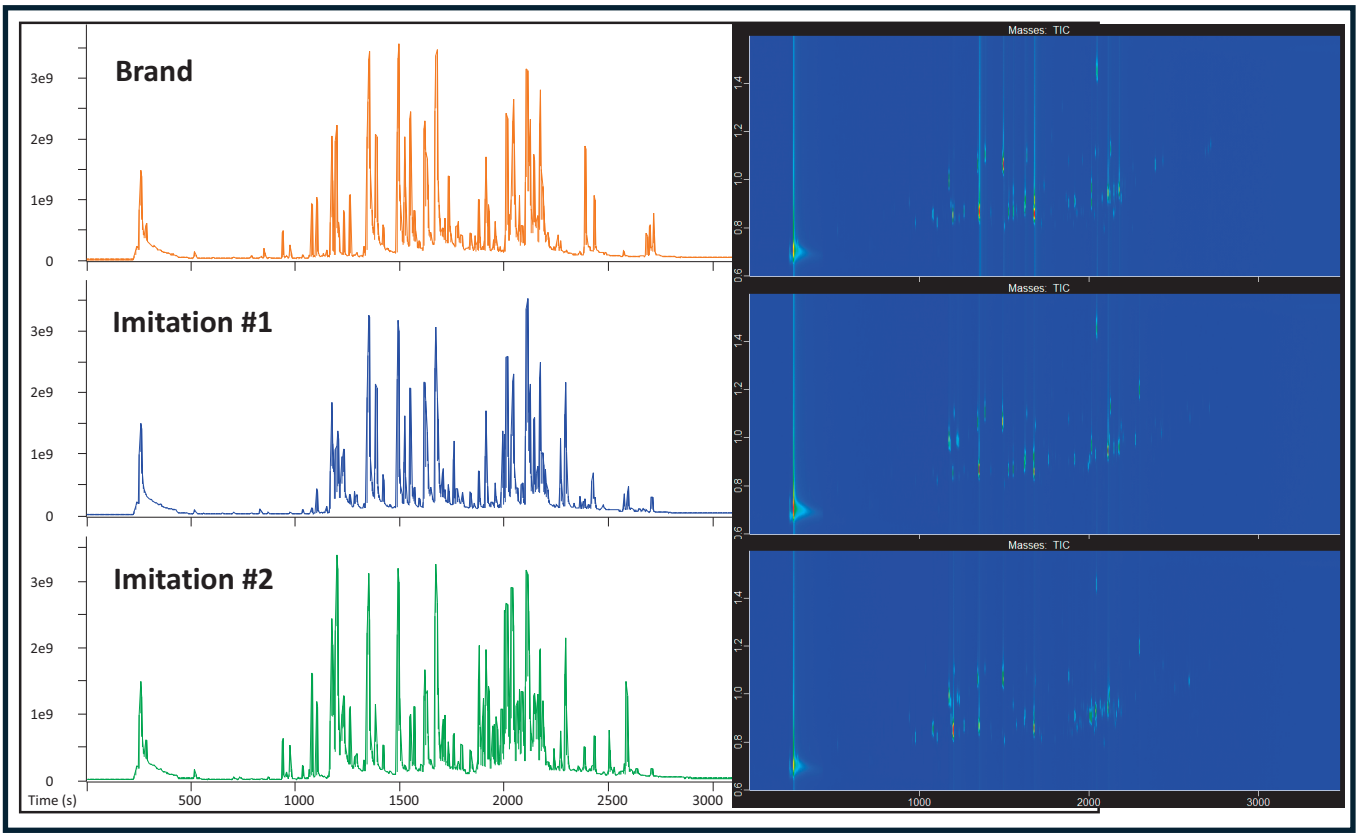

Figure 1. Three perfume samples were analyzed and compared by GC and by GCxGC with LECO's FLUX flow-modulator based system.

Experimental

Three perfume samples, one brand and two drugstore imitations, were analyzed with HS-SPME coupled to GC-TOFMS. For each sample, 10 µL of perfume were pipetted into a 20 mL vial. Each sample was also analyzed with GCxGC-TOFMS on the same instrument by simply turning on the modulator and increasing the mass spectral acquisition rate through software control. The samples were analyzed with the instrument conditions listed in Table 1. An alkane standard was also analyzed with a liquid injection for retention index calculations.

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

Autosampler	**LECO LPAL 3**
SPME fiber	DVB/CAR/PDMS fiber (conditioned 5 min pre and post injection at 250 °C)
Incubation and Extraction	Incubate 5 minutes and Extract 10 minutes at 40 °C
Gas Chromatograph	**LECO FLUX GCxGC**
Injection	SPME, 3 min desorption in 250 °C inlet, split 10:1
Carrier Gas	He @ 0.80 mL/min, corrected constant flow
Column One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek)
Column Two	Rxi-17 Sil MS, 0.91* m x 0.10 mm i.d. x 0.10 µm coating (Restek) *0.60 m coiled in 2nd oven and 0.31 m in transfer line
Temperature Program	3 min at 40 °C, ramped 4.2 °C/min to 250 °C, hold 5 min
Secondary Oven	+20 °C relative to primary oven
2nd Dim Separation Time	1 s, injection duration of 0.05 s
Transfer Line	250 °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

Two drugstore imitation perfumes were characterized and compared to the brand perfume that they were intended to mimic. Representative GC and GCxGC chromatograms for each sample are shown in Figure 1, with many apparent differences and similarities. Differences were explored by comparing relative amounts of individual analytes between the samples. A terpene, β-myrcene, is shown as an example in Figure 2. β-myrcene has odor characteristics described as peppery, terpene, spicy, and balsam. This analyte was identified through automated data processing, based on the mass spectral similarity (similarity score = 950/1000) and retention index matching compared to NIST library databases (observed RI = 993 and Library RI = 991). The relative amounts were determined by integrating the peak areas of specific m/z. Three m/z that were characteristic of the observed spectrum, m/z 69, 93, and 121, were integrated and peak areas were compared. β-myrcene is observed at lower levels in Imitation #1 and at higher levels in Imitation #2 compared to the brand. These relative trends are consistent no matter which mass was used for integration.

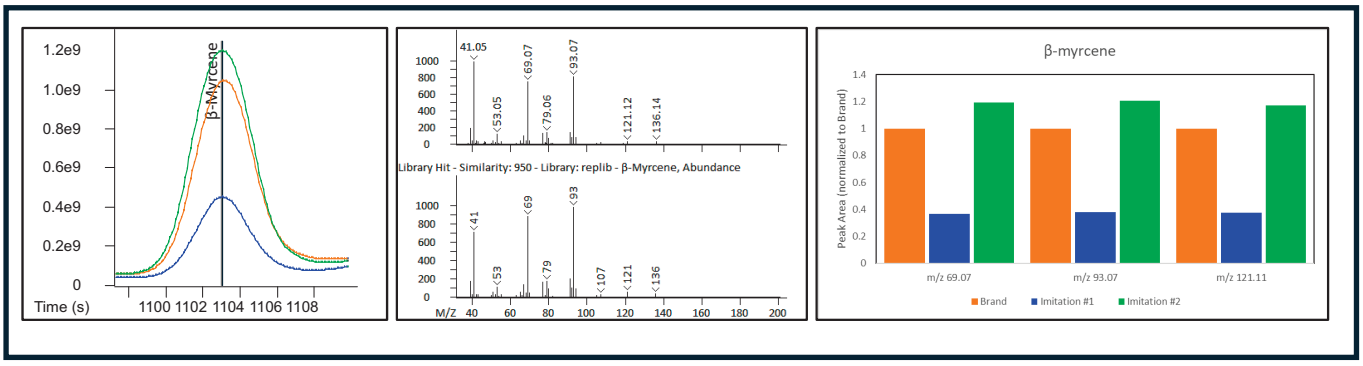

Figure 2. A chromatographically separated analyte, β-myrcene, is shown. The relative trends between the Brand and Imitations is consistent no matter which m/z was integrated for area comparisons.

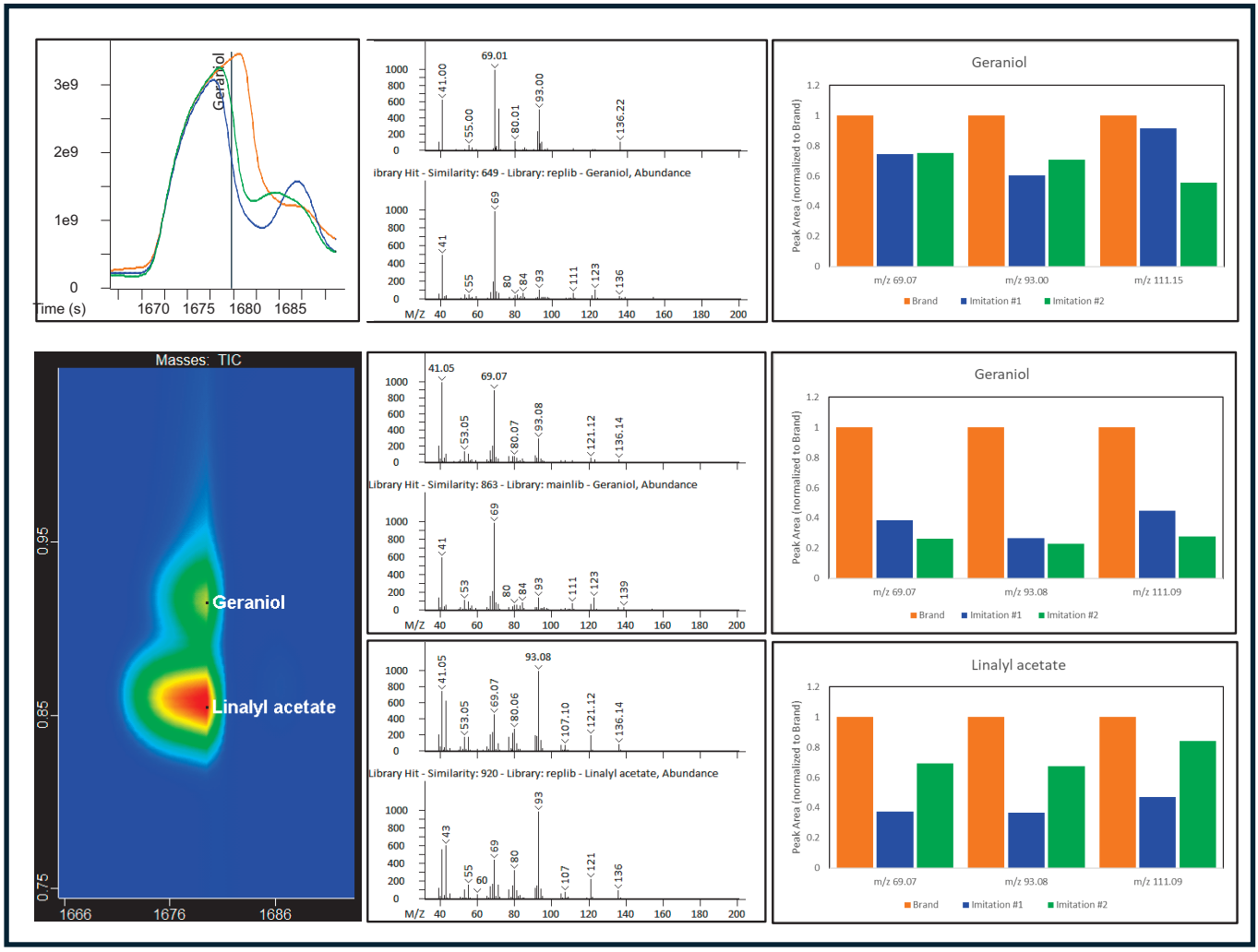

Figure 3. A coelution that exceeds deconvolution is shown on top for geraniol with poor spectral similarity and discrepancy in relative amounts. GCxGC was required to separate the coeluting analytes in the second dimension and to correctly determine the relative amounts of these analytes with confidence.

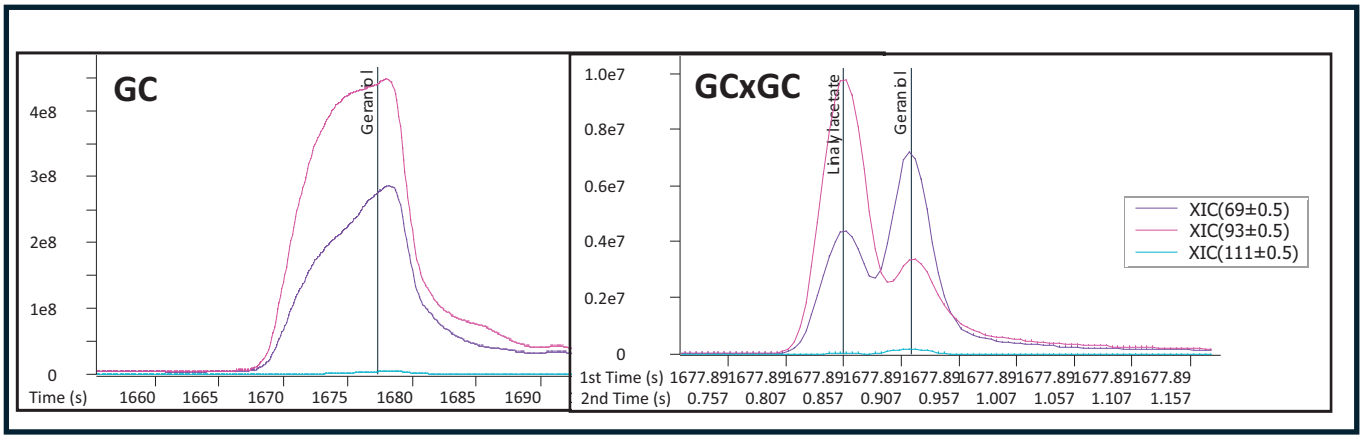

Figure 4. Two analytes with shared m/z perfectly coelute in the GC separation (left), but are chromatographically resolved as shown in the linear view of the GCxGC separation (right).

Another analyte of interest, geraniol, is shown in Figure 3. Geraniol has odor characteristics described as sweet, floral, fruity, waxy, and citrusy. With GC-MS, this analyte was tentatively identified by mass spectral and retention index matching compared to NIST library databases (Spectral similarity score = 649, and the RI observed = 1261 versus RI Library = 1255). To compare the relative amounts of geraniol between the three perfume samples, peak areas were again determined by integrating specific m/z characteristic of the observed spectrum (m/z 69, 93, and 111). Unlike β-myrcene in Figure 2, however, the relative trends for geraniol did differ depending on the mass selected for integration. Geraniol appears to be at highest levels in the brand, but Imitation #1 could be either equal, lower, or higher compared to Imitation #2 depending on the mass used for quantification. It is unclear from the GC data how the levels of geraniol compare between the perfume samples. To try to clarify this, GCxGC data were collected which revealed that geraniol was completely coeluting with linalyl acetate in the GC data (see Figure 3). This coelution, with many shared masses, caused the lower spectral similarity score and was also responsible for the discrepancies in determining the relative amounts of geraniol in each sample. With GCxGC, geraniol and linalyl acetate still coelute in the first dimension (as expected by retention index, linalyl acetate observed RI = 1260 and Library RI = 1257), but they are separated in the second dimension. With this additional chromatographic resolution, the similarity scores improved for each analyte (863 and 920 for geraniol and linalyl acetate, respectively) and the coeluting shared masses chromatographically separate, as shown in Figure 4. With GCxGC, consistent trends were determined for each analyte regardless of the m/z used for quantification. GCxGC was crucial for clarifying geraniol and for detecting linalyl acetate, which also had important odor characteristics (described as sweet, green, citrus, bergamot, lavender, and woody).

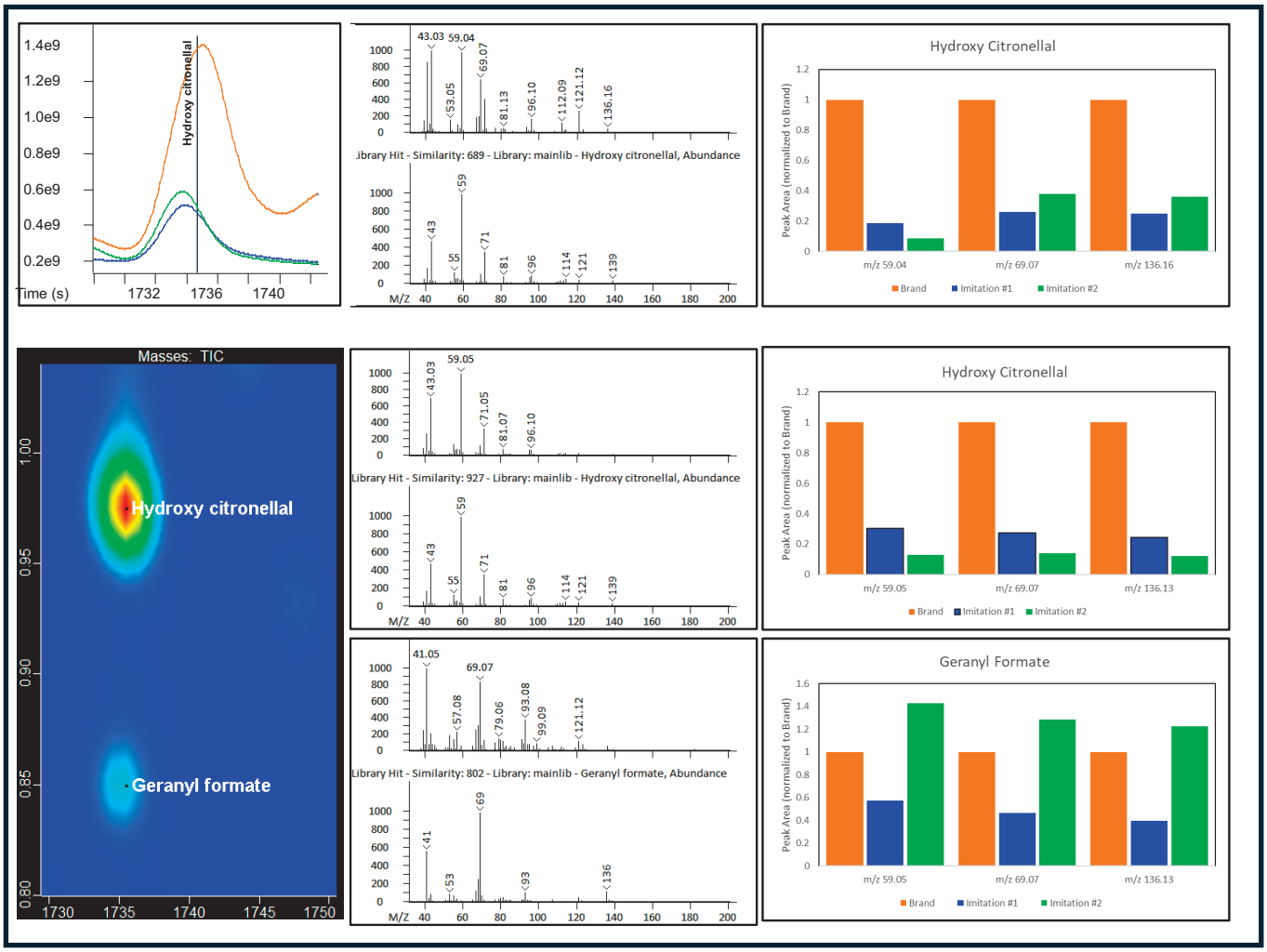

Figure 5. Another coelution that exceeds deconvolution is shown (top). GCxGC was required to separate the coeluting analytes in the second dimension and to correctly determine the relative amounts of these analytes with confidence.

Hydroxy citronellal, with floral, lily, sweet, green, waxy, tropical, and melon descriptors, is another analyte of interest that is shown in Figure 5. With GC-MS, this analyte was tentatively identified by mass spectral and retention index matching compared to NIST library databases (Spectral similarity score = 689, and the RI observed = 1289 versus RI Library = 1300). To compare the relative amounts of hydroxy citronellal between the three perfume samples, peak areas were determined by integrating specific m/z characteristic of the observed spectrum (m/z 59, 69, and 136). The brand perfume is observed at highest levels regardless of the m/z used, but the trends between Imitation #1 and #2 differ depending on which mass was integrated for comparison. GCxGC data were again reviewed which revealed that hydroxy citronellal also perfectly coeluted with another analyte, geranyl formate, that had many shared m/z.

With GCxGC, geranyl formate and hydroxy citronellal still coelute in the first dimension (as expected by retention index, geranyl formate observed RI = 1289 and Library RI = 1300), but they are separated in the second dimension with the additional chromatographic resolution, as shown in Figure 6. The similarity scores for each analyte improved (927 and 802 for hydroxy citronellal and geranyl formate, respectively), and the peaks were reliably integrated without interferences from each other. With GCxGC, trends were consistently determined for each analyte regardless of the m/z used for quantification. Geranyl formate has interesting odor characteristics also (described as fresh, rose, neroli, tea, rose, green) and GCxGC was crucial for discovering this analyte in the data.

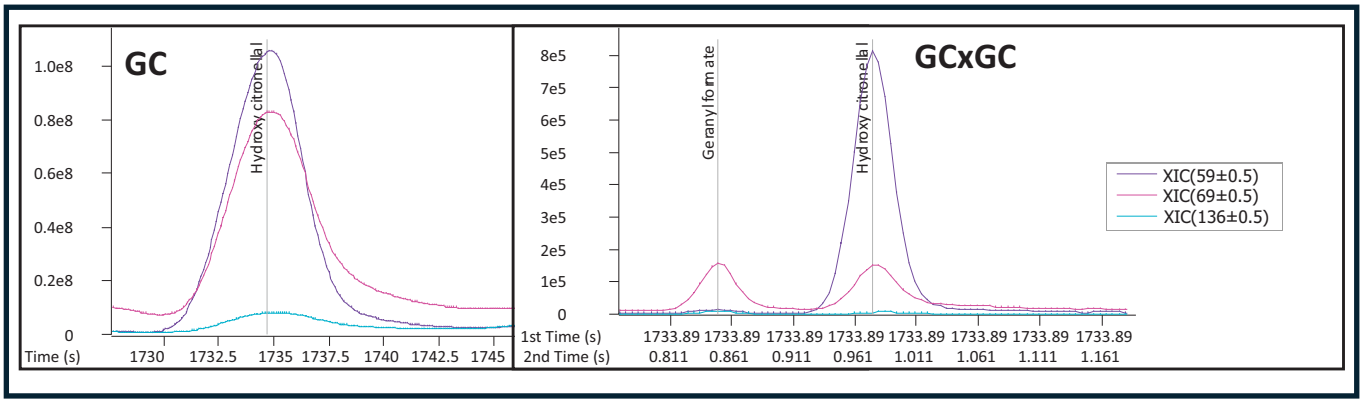

Figure 6. Shared m/z perfectly coelute in the GC separation (left), but are chromatographically resolved as shown in the linear view of the GCxGC separation (right).

Conclusion

In this work, the aroma profiles of three different perfume samples were compared with GC and GCxGC-TOFMS that incorporated a flow-based modulator. Detailed chemical information was provided by GC-TOFMS and uncovered similarities and differences between these perfume samples. Some analytes of interest were difficult to characterize and quantify with GC alone due to their complete coelution with other matrix analytes. Extending the chromatographic resolution with GCxGC separated the coelutions in the second dimension and provided the information required to gain a better understanding of the samples. LECO's *Pegasus* BT 4D with *FLUX* flow-based modulation offers a low-cost, robust, rugged, and easy-to-use option for adding GCxGC to your routine analyses.

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