Application Note

Instrument: Pegasus® GC-HRT 4D

Improved Identification of Brand-Distinguishing Analytes in Perfume Samples with GCxGC and HR-TOFMS

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Key Words: Pegasus HT, Pegasus 4D, Pegasus GC-HRT 4D, Perfume Analysis

Introduction

Sample differentiation is important in the perfume industry, and distinguishing samples and their individual analytes can help maintain quality control, aid process optimization, and drive product development through competitive analysis and brand awareness. These analyses are often done with non-targeted analytical methods, such as gas chromatography with mass spectrometry (GC-MS) because targeted approaches typically yield insufficient analyte coverage to fully understand the samples. GC-TOFMS is a powerful analytical tool for characterization and additional analytical capabilities, such as GCxGC and HR-TOFMS, provide an even greater amount of information for an analyst to determine what they've been missing. GCxGC pairs an additional complementary separation to improve the chromatographic separation of first dimension coelutions. High resolution TOFMS adds another layer of information to the analysis with accurate mass data that are used for definitive formulae determinations and confident analyte identifications. Brand and imitation perfumes were analyzed and compared with GC-TOFMS, GCxGC-TOFMS, and GCxGC-HR-TOFMS. These analytical technologies together offer a comprehensive picture of the perfume samples and the ability to distinguish and confidently identify differentially expressed analytes, including many with important odor characteristics, some that were challenging to separate with a one-dimensional separation, and others that were difficult to identify without HR-TOFMS.

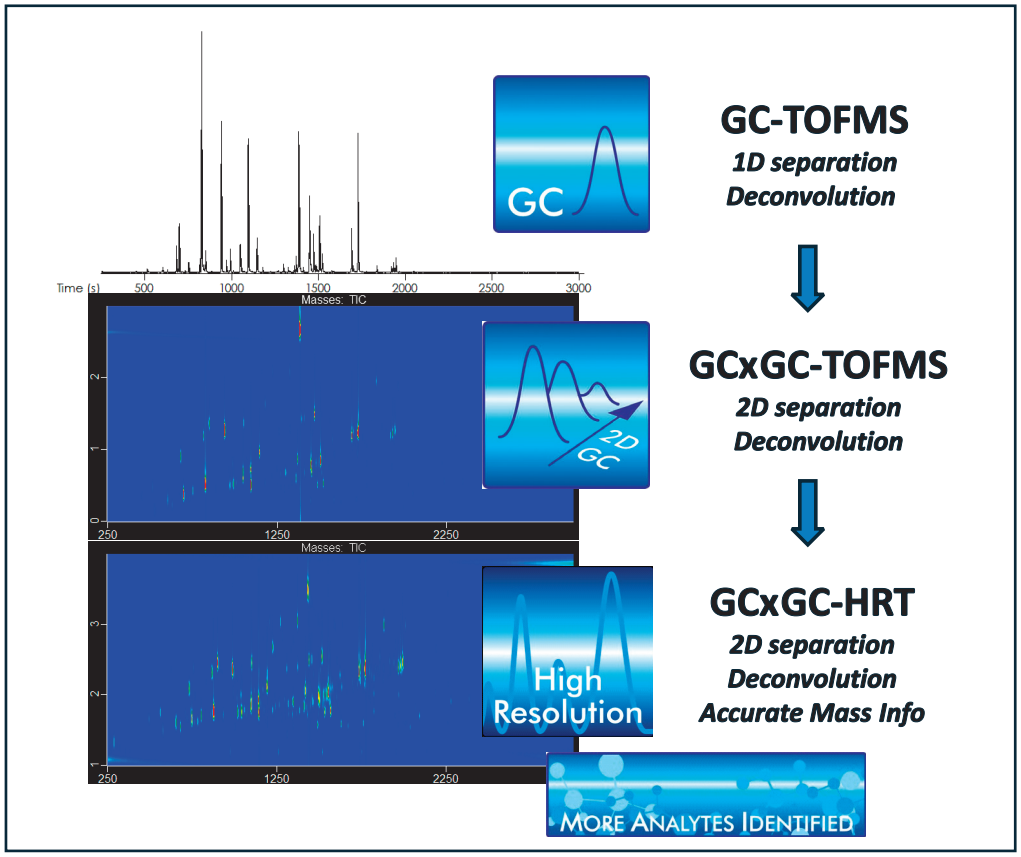


Figure 1. Perfume samples were analyzed by GC-TOFMS, GCxGC-TOFMS, and GCxGC-HR-TOFMS. Each platform added analytical capabilities which led to an overall increase in separated and confidently identified analytes.

Experimental

A brand and two drugstore imitation perfume samples were analyzed by GC and GCxGC coupled to TOFMS, and also with GCxGC coupled to high resolution TOFMS (GCxGC-HR-TOFMS). The samples were diluted in ethanol prior to injection and analyzed by LECO's *Pegasus* HT, 4D, and GC-HRT 4D with the instrument conditions listed in Table 1.

Table 1. Instrument Conditions

GC	**LECO GCxGC Quad Jet Thermal Modulator & MPS2 Autosampler**
Injection	1µL, splitless @ 250°C
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column One	Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm (Restek)
Column Two	Rxi-17SilMS, 1.20 m x 0.25 mm x 0.25 µm coating (Restek)
Temperature Program	40°C (2 min), to 280°C @ 5°C/min (10 min) Secondary oven maintained +15°C relative to primary oven
Modulation	3 s with temperature maintained +15°C relative to secondary oven
Mass Spectrometer	**LECO *Pegasus* HT/4D or *Pegasus* GC-HRT 4D**
Transfer Line	250°C
Ion Source Temperature	250°C
HRT Acquisition Mode	High Resolution, R = 25,000 (FWHM)
Ionization Mode	EI
Mass Range (m/z)	33-500
Acquisition Rate	20 spectra/s (100 spectra/s for GC×GC)

Results and Discussion

Hundreds of analytes were detected and identified within the perfume samples, many with important odor characteristics and differential expression between the brand and imitation samples. Esters, aromatic species, terpenes, oxygenated terpenes, and phthalates were all observed and many had differential expression between the brands. This type of analysis gave insight to the similarities and differences between the brand and imitations, and a great deal of information was gained with the GC-TOFMS data alone. Even greater insight was uncovered with each additional analytical capability. GCxGC offered better sample characterization and detection of more individual analytes with chromatographic separation for first dimension coelutions that exceed deconvolution capabilities. The addition of HR-TOFMS added more confidence in identifications and improved identifications of these separated analytes with accurate mass information. Specific examples are shown in Figure 2-5.

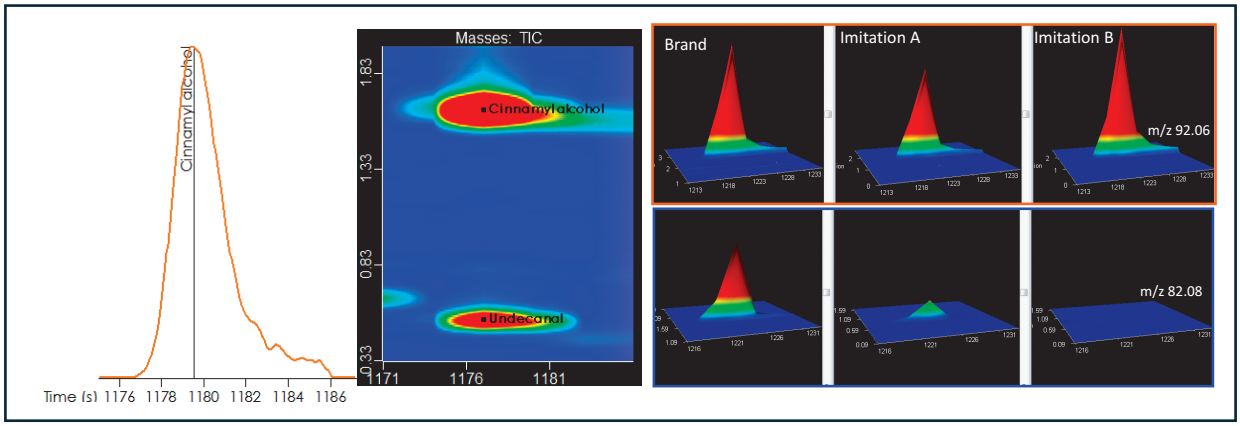
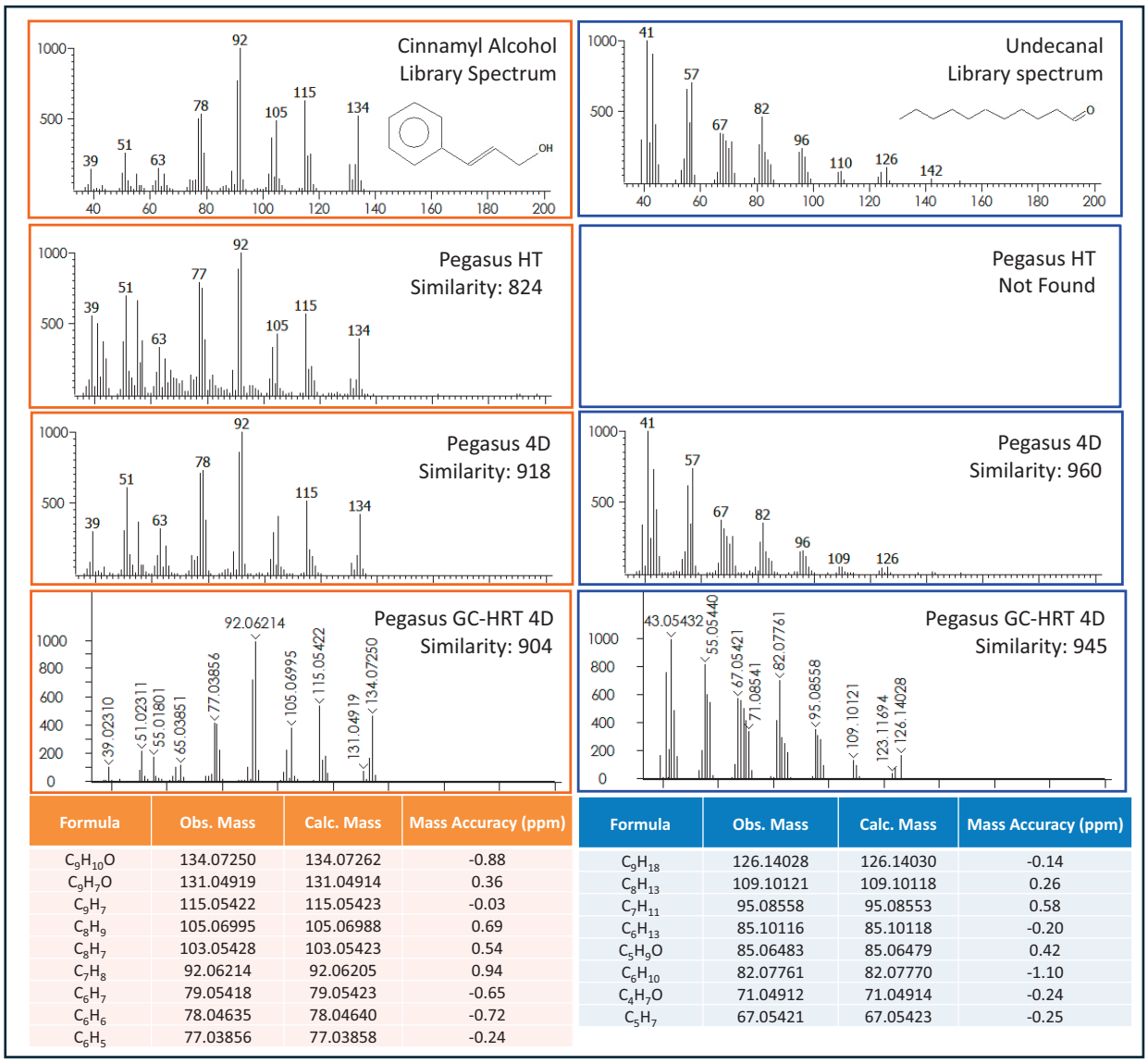


Figure 2. With GC-TOFMS, cinnamyl alcohol perfectly coeluted with one other analyte and only a single peak was determined. This coelution exceeded mathematical deconvolution capabilities and the MS spectrum for the single peak was the combination of both analytes (Figure 3). The improved separation with GCxGC led to the detection and identification of an additional analyte, undecanal. The differential expression between the brand and imitations, demonstrated in the GCxGC-HR-TOFMS data, shows that important information was hidden within the GC-TOFMS data.



Formula	Obs. Mass	Calc. Mass	Mass Accuracy (ppm)
$C_9H_{10}O$	134.07250	134.07262	-0.88
C_9H_7O	131.04919	131.04914	0.36
C_9H_7	115.05422	115.05423	-0.03
C_8H_9	105.06995	105.06988	0.69
C_8H_7	103.05428	103.05423	0.54
C_7H_8	92.06214	92.06205	0.94
C_6H_7	79.05418	79.05423	-0.65
C_6H_6	78.04635	78.04640	-0.72
C_6H_5	77.03856	77.03858	-0.24

Formula	Obs. Mass	Calc. Mass	Mass Accuracy (ppm)
C_9H_{18}	126.14028	126.14030	-0.14
C_8H_{13}	109.10121	109.10118	0.26
C_7H_{11}	95.08558	95.08553	0.58
C_6H_{13}	85.10116	85.10118	-0.20
C_5H_9O	85.06483	85.06479	0.42
C_6H_{10}	82.07761	82.07770	-1.10
C_4H_7O	71.04912	71.04914	-0.24
C_5H_7	67.05421	67.05423	-0.25

Figure 3. Improved separation with GCxGC and accurate mass information with HR-TOFMS gave more confidence in the identification of both cinnamyl alcohol and undecanal. The GC-TOFMS cinnamyl alcohol spectrum contained m/z from the perfect coelution, undecanal. This led to a lower overall similarity score. GCxGC chromatographically separated these two analytes in the second dimension and provided individual peaks and spectra for each. This analytical capability added information for undecanal and improved the similarity for cinnamyl alcohol. The identification was further supported with formulae determinations from accurate mass data generated with HR-TOFMS.

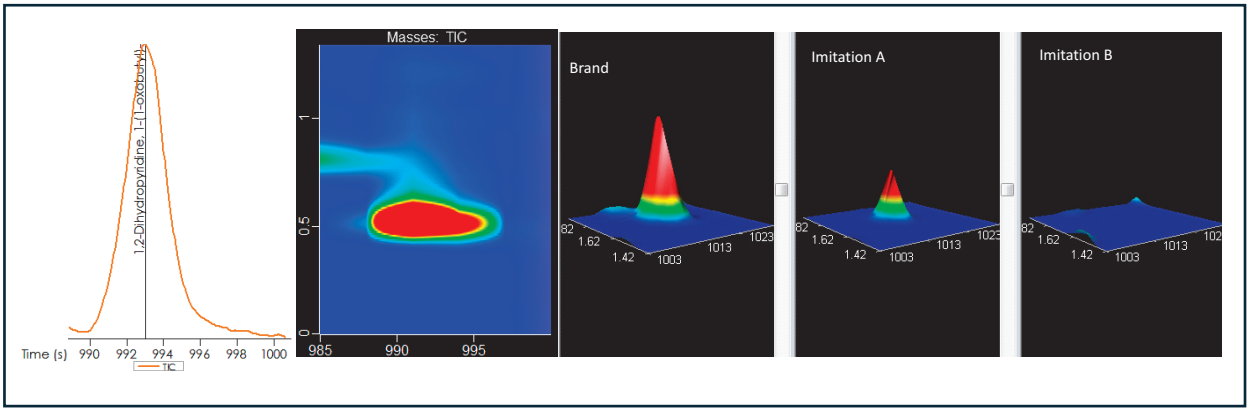
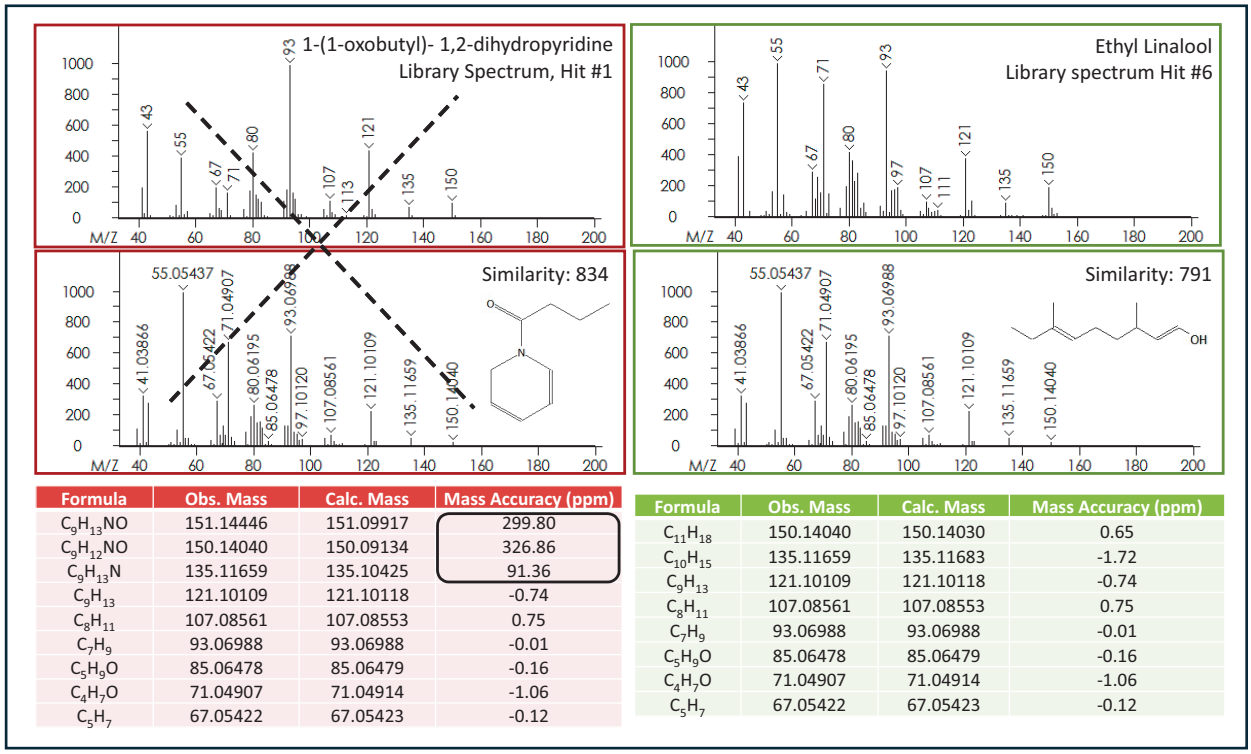


Figure 4. A differentially expressed analyte was found and initially identified as 1-(1-oxobutyl)-1,2-dihydropyridine on all of the analytical platforms. The similarity scores were 801, 815, and 834 on the Pegasus HT, 4D, and GC-HRT 4D data, respectively.



Formula	Obs. Mass	Calc. Mass	Mass Accuracy (ppm)
$C_9H_{13}NO$	151.14446	151.09917	299.80
$C_9H_{12}NO$	150.14040	150.09134	326.86
$C_9H_{13}N$	135.11659	135.10425	91.36
C_9H_{13}	121.10109	121.10118	-0.74
C_8H_{11}	107.08561	107.08553	0.75
C_7H_9	93.06988	93.06988	-0.01
C_5H_9O	85.06478	85.06479	-0.16
C_4H_7O	71.04907	71.04914	-1.06
C_5H_7	67.05422	67.05423	-0.12

Formula	Obs. Mass	Calc. Mass	Mass Accuracy (ppm)
$C_{11}H_{18}$	150.14040	150.14030	0.65
$C_{10}H_{15}$	135.11659	135.11683	-1.72
C_9H_{13}	121.10109	121.10118	-0.74
C_8H_{11}	107.08561	107.08553	0.75
C_7H_9	93.06988	93.06988	-0.01
C_5H_9O	85.06478	85.06479	-0.16
C_4H_7O	71.04907	71.04914	-1.06
C_5H_7	67.05422	67.05423	-0.12

Figure 5. The nominal mass fragments for the first library hit aligned very well with the observed data. This identification was precluded, however, by the accurate mass formula determinations. m/z 151 and 150 had extremely poor mass accuracy values with the initial proposed formulae, $C_9H_{13}NO$ and $C_9H_{12}NO$. A formula calculation determined that these masses were better explained by $C_{11}H_{18}$ and its 13C isotope. This improved formula led to a different identification with a lower library similarity, but far better mass accuracy. The improved identification had different odor characteristics than the preliminary identification, providing a different and better interpretation of this difference between the samples. These identification candidates had retention index of 1231 and 1181, which were not different enough to definitively adjust identifications from retention time alone.

Conclusion

This study demonstrates the benefits of LECO's analytical platforms for characterization and differentiation of perfume samples to see what you are missing. Three commercial perfume samples were analyzed with GC-TOFMS, GCxGC-TOFMS, and GCxGC-HR-TOFMS. The detection and identification of various esters, aromatic species, terpenes, oxygenated terpenes, and phthalates within the GC-TOFMS data provided information on the similarities and differences between the samples. The addition of GCxGC and HR-TOFMS analytical capabilities offered an even better understanding of the samples. A complementary separation dimension with GCxGC led to the detection of more analytes in the sample by chromatographically separating first dimension coelutions in the second dimension. Analytes with important odor properties were detected by GCxGC that were missed with the GC separation. The further addition of HR-TOFMS gave improved confidence in the identifications of the separated analytes. Some initial library matches were confirmed with HR-TOFMS data and others, even with good similarity scores, were ruled out based on the poor mass accuracy with the proposed formulae. Better formulae and identifications were proposed using the accurate mass information leading to a greater understanding of the samples. These tools allow a user to confidently discover even more about their sample.

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