# Forensic Analysis of Brand and Imitation Perfume Samples with GC, GC×GC, and HR-TOFMS

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## Introduction

Characterizing perfumes by determining individual components provides important information to differentiate samples. This type of information can be used to maintain quality control, aid process optimization, drive product development through competitive analysis and brand awareness, and screen for fraud. Non-targeted analytical methods, such as gas chromatography with mass spectrometry (GCMS), are essential as targeted approaches likely do not provide enough analyte coverage to fully understand the samples. An even greater amount of information about a perfume sample can be gained by pairing an additional complementary separation with two-dimensional gas chromatography (GC×GC) to improve the chromatographic separation of coelutions. The addition of a high resolution mass spectrometer also provides more information and confident formula determinations, combining for a powerful analytical method to better understand a sample and confidently separate, discover, and identify more analytes.

## Methods

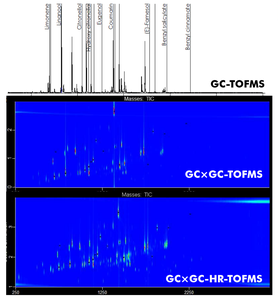
A brand and two drugstore imitation perfume samples were analyzed by GC and GC×GC coupled to TOFMS, and also with GC×GC coupled to high resolution TOFMS (GC×GC-HR-TOFMS). LECO's Pegasus® HT, Pegasus 4D, and Pegasus HRT 4D were used for the analyses. These analytical tools provided characterization and comparison information for the brand and imitation perfume samples. The samples were diluted in ethanol prior to injection and analyzed with the instrument conditions listed in Table 1.

**Table 1. Instrument Conditions. *(GC×GC parameters are in italics and parentheses).***

| | |
|---|---|
| GC(×GC) | Agilent 7890 with Dual Stage Quad Jet Modulator and MPS2 Autosampler |
| Injection | 1 µL splitless with inlet @ 250°C |
| Carrier Gas | He @ 1.0 ml/min, *(Pressure Corrected Constant Flow)* |
| Column One | Rxi-5ms, 30 m x 0.25 mm i.d. x 0.25 µm coating (Restek) |
| *(Column Two* | *Rxi-17SilMS, 1.20 m x 0.25 mm x 0.25 µm coating (Restek))* |
| Temperature Program | 2 min at 40°C, ramped 5°C/min to 280°C, held 10 min *(Secondary oven maintained +15°C relative to primary oven)* |
| *(Modulation* | *3 s with temperature maintained +15°C relative to secondary oven)* |
| Transfer Line | 250°C |
| MS | LECO Pegasus HT/4D or Pegasus GC-HRT 4D |
| Ion Source Temp | 250°C |
| Mass Range | 33-500 m/z |
| Acquisition Rate | 20 spectra/s for GC *(100 spectra/s for GC×GC)* |
| Resolution Mode | High Resolution (Rs = 25,000) |

## Brand Ingredient List

All analytical platforms (GC-TOFMS, GC×GC-TOFMS, and GC×GC-HR-TOFMS) provided the ability to characterize the samples and detect specific analyte differences and similarities. The brand sample had 16 fragrance ingredients listed on the packaging information that could be considered target analytes for comparison. These were also used for analytical platform comparisons.

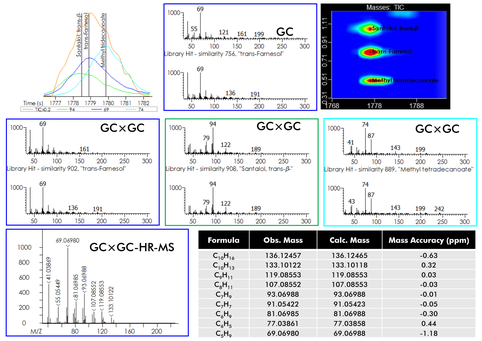



| Ingredient | CAS | Odor |
|---|---|---|
| benzyl alcohol | 100-51-6 | floral |
| benzyl benzoate | 120-51-4 | balsamic |
| benzyl cinnamate | 103-41-3 | balsamic |
| benzyl salicylate | 118-58-1 | balsamic |
| cinnamyl alcohol | 104-54-1 | balsamic |
| citral | 141-27-5 | citrus |
| citronellol | 106-22-9 | floral |
| coumarin | 91-64-5 | tonka |
| eugenol | 97-53-0 | spicy |
| farnesol | 106-28-5 | floral |
| geraniol | 106-24-1 | floral |
| hydroxycitronellal | 107-75-5 | floral |
| isoeugenol | 97-54-1 | spicy |
| limonene | 138-86-3 | citrus |
| linalool | 78-70-6 | floral |
| α-isomethyl ionone | 127-51-5 | floral |

**Figure 1. Representative chromatograms of the brand perfume sample analyzed with each instrument platform. Peak markers for each brand ingredient are shown.**

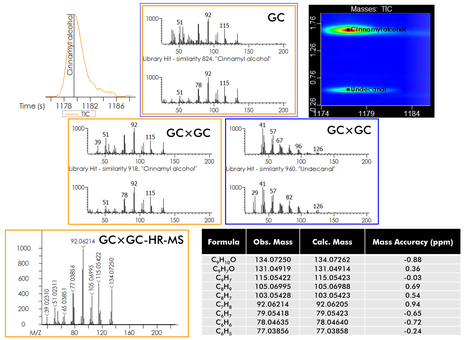
## GC×GC and HR-TOFMS Benefits

The addition of a second dimension separation with GC×GC, and accurate mass information with HR-TOFMS, improved the understanding of the samples. GC×GC provided chromatographic separation for many analytes that were coeluting in the first dimension separation. This led to a greater number of detected analytes, some with important odor characteristics, and improved spectral information in many cases. The additional capability of HR-TOFMS also gave more information. HR-TOFMS provided accurate mass data that were used for definitive formula determinations adding more confidence to analyte identifications.




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{15}H_{24}$ | 136.12457 | 136.12465 | -0.63 |
| $C_{10}H_{13}$ | 133.10122 | 133.10118 | 0.32 |
| $C_9H_{11}$ | 119.08553 | 119.08553 | 0.03 |
| $C_8H_{11}$ | 107.08552 | 107.08553 | -0.03 |
| $C_7H_9$ | 93.06988 | 93.06988 | -0.01 |
| $C_7H_7$ | 91.05422 | 91.05423 | -0.05 |
| $C_6H_9$ | 81.06985 | 81.06988 | -0.30 |
| $C_6H_5$ | 77.03861 | 77.03858 | 0.44 |
| $C_5H_8$ | 69.06980 | 69.06988 | -1.18 |

**Figure 2. With GC-TOFMS, farnesol coeluted with two other analytes. These were mathematically deconvoluted in the GC data and chromatographically separated in the GC×GC data. The improved separation and formula confirmation from accurate mass information gave cleaner spectra and more confidence in the identification of this brand ingredient.**




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_9H_{10}O$ | 134.07250 | 134.07262 | -0.88 |
| $C_9H_8O$ | 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 |
| $C_6H_6$ | 92.06214 | 92.06205 | 0.94 |
| $C_6H_5$ | 79.05418 | 79.05423 | -0.65 |
| $C_6H_4$ | 78.04635 | 78.04640 | -0.72 |
| $C_6H_3$ | 77.03856 | 77.03858 | -0.24 |

**Figure 3. With GC-TOFMS, cinnamyl alcohol perfectly coeluted with one other analyte. This coelution exceeded mathematical deconvolution capabilities, and the GC spectrum is the combination of both analytes. The improved separation and accurate mass information gave more confidence in the identification of this brand ingredient, and provided additional information on undecanal that was not found in the GC data.**

## Added Confidence in Ingredient IDs

These analytical technologies provided important benefits to chromatographically separate and confidently identify the brand ingredients. GC×GC chromatographically separated many coelutions while HR-TOFMS provided accurate mass information for formula calculations. Some of these analytes were challenging to separate and identify without these capabilities. The peak metrics for all 16 brand ingredients are compiled in Table 2.

**Table 2. Brand ingredients analyzed on each analytical platform.**

| Ingredient | tR 1 | GC | GC×GC | HRT | formula | obs. mass | calc. mass | ppm |
|---|---|---|---|---|---|---|---|---|
| benzyl alcohol | 712.8 | 733 | 908 | 935 | $C_7H_8O^*$ | 108.05705 | 108.05697 | 0.81 |
| benzyl benzoate | 1837.4 | 941 | 956 | 952 | $C_{14}H_{12}O_2^*$ | 212.08317 | 212.08318 | -0.05 |
| benzyl cinnamate | 2218.1 | 779 | 906 | 812 | C15H12 | 192.09331 | 192.09335 | -0.20 |
| benzyl salicylate | 1965.6 | 898 | 922 | 932 | $C_{14}H_{12}O_3^*$ | 228.07785 | 228.0781 | -1.09 |
| cinnamyl alcohol | 1179.6 | 824 | 918 | 904 | $C_9H_{10}O^*$ | 134.07250 | 134.07262 | -0.88 |
| citral | 1121.2 | 847 | 914 | 837 | $C_{10}H_{16}$ | 94.07765 | 94.07770 | -0.56 |
| citronellol | 1050.1 | 870 | 927 | 803 | $C_{10}H_{20}O^*$ | 156.15072 | 156.15087 | -0.95 |
| coumarin | 1387.7 | 947 | 966 | 956 | $C_9H_6O_2^*$ | 146.03621 | 146.03623 | -0.17 |
| eugenol | 1262.8 | 889 | 927 | 939 | $C_{10}H_{12}O_2^*$ | 164.08322 | 164.08318 | 0.23 |
| farnesol | 1778.9 | 756 | 902 | 765 | $C_{15}H_{26}$ | 136.12457 | 161.13248 | -0.63 |
| geraniol | 1094.5 | 830 | 896 | 818 | $C_{10}H_{18}O$ | 139.11170 | 139.11174 | -0.31 |
| hydroxycitronellal | 1146.8 | 909 | 918 | 901 | $C_{10}H_{20}O$ | 139.11164 | 139.11174 | -0.75 |
| isoeugenol | 1405 | 438 | 883 | 888 | $C_{10}H_{12}O_2^*$ | 164.08313 | 164.08318 | -0.31 |
| limonene | 698.3 | 915 | 939 | 942 | $C_{10}H_{16}^*$ | 136.12456 | 136.12465 | 0.71 |
| linalool | 827 | 781 | 926 | 959 | $C_{10}H_{18}$ | 136.12468 | 136.12465 | 0.22 |
| α-isomethyl ionone | 1449.8 | 855 | 910 | 927 | $C_{14}H_{22}O^*$ | 206.16660 | 206.16652 | 0.40 |

*molecular ion

**GC → GC×GC**
**Average Similarity: +94**

**HR-TOFMS Mass Accuracy ↓**
**Average (absolute value): 0.52**

## Brand Comparisons

With confident identification of the ingredients, the brand and imitation perfumes could be compared. Even in the known brand ingredients, variations were observed between the perfume samples. Many other non-targeted analytes were also observed and expressed differently including esters, aromatic species, terpenes, oxygenated terpenes, and phthalates.

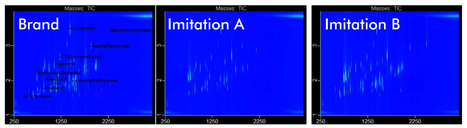
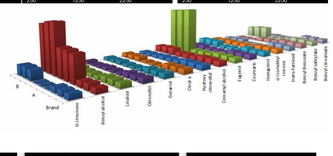
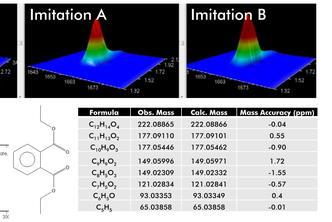



**Figure 4. The brand ingredient analytes were compared between the perfume samples. Each perfume was analyzed in triplicate and relative peak area information is shown. Differences were observed between the brand and imitations in the known brand ingredients.**



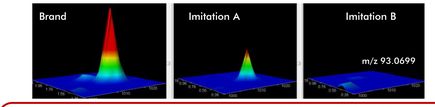



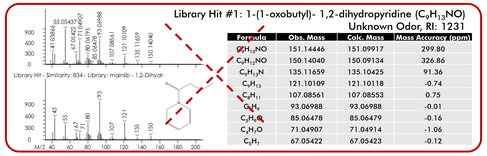

| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{12}H_{14}O_4$ | 222.08865 | 222.08866 | -0.04 |
| $C_{11}H_{13}O_3$ | 177.09110 | 177.09101 | 0.55 |
| $C_{11}H_9O_3$ | 177.05446 | 177.05462 | -0.90 |
| $C_8H_5O_3$ | 149.05996 | 149.05971 | 1.72 |
| $C_8H_5O_2$ | 149.02309 | 149.02332 | -1.55 |
| $C_7H_5O_2$ | 121.02834 | 121.02841 | -0.57 |
| $C_6H_5O$ | 93.03353 | 93.03349 | 0.4 |
| $C_5H_5$ | 65.03858 | 65.03858 | -0.01 |

**Figure 5. Many other non-targeted differences were also observed. Diethyl phthalate was observed in both imitations, but not the brand sample.**

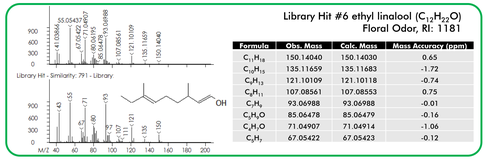
## Improved Identifications

In some examples of differential expression, the accurate mass information provided by HR-TOFMS was crucial for making the correct interpretation of the data.




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_9H_{13}NO$ | 151.14446 | 151.09917 | 299.80 |
| $C_5H_{12}NO$ | 150.14040 | 150.09134 | 326.86 |
| $C_8H_{13}N$ | 135.11659 | 135.10425 | 91.36 |
| $C_8H_{13}$ | 121.10109 | 121.10118 | -0.74 |
| $C_7H_{11}$ | 107.08561 | 107.08553 | 0.75 |
| $C_6H_6N$ | 93.06988 | 93.06988 | -0.01 |
| $C_5H_6O$ | 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) |
|---|---|---|---|
| $C_{11}H_{16}$ | 150.14040 | 150.14030 | 0.65 |
| $C_{10}H_{15}$ | 135.11659 | 135.11683 | -1.72 |
| $C_8H_{13}$ | 121.10109 | 121.10118 | -0.74 |
| $C_7H_{11}$ | 107.08561 | 107.08553 | 0.75 |
| $C_7H_9$ | 93.06988 | 93.06988 | -0.01 |
| $C_5H_6O$ | 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 6. An analyte indicative of the brand perfume sample was found and initially identified with a library similarity score of 834. The nominal mass fragments aligned very well, but were not correct based on accurate mass formula determinations. An identification with lower library similarity, but far improved mass accuracy better explains the data.**

## Musk Odor

Musk is a common base note in perfume samples and can be achieved with a variety of aromatic substances. With GC×GC separation and confident analyte identification, several musk odor analytes were observed in these samples. It can be determined that each manufacturer used different analytes to achieve the musk notes in their perfume which also led to different sensory side effects.

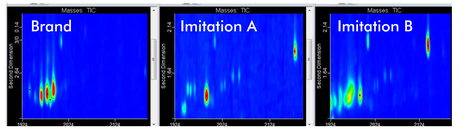



**Figure 7. A variety of analytes were determined with overall musk odor properties. Each analyte was described as musk overall, but also provided other odor notes to the perfume, as described in Table 3.**

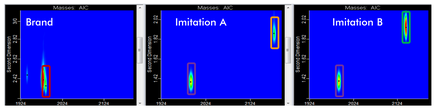
**Table 3. Musk odor analytes and the associated identification information and odor properties.**

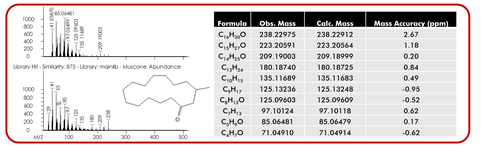
| Analyte (CAS) | Similarity | Formula | Obs. Mass | Calc. Mass | ppm | Musk | Sweet | Animal | Powdery | Fatty | Natural | Grassy | Dry | Fresh | Clean | Strong | Diffuse | Floral | Ambrette | Wood | Soapy | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| muscone (541-91-3) | 875 | $C_{16}H_{30}O^*$ | 238.22975 | 238.22912 | 2.67 | X | X | X | X | X | X | | | | | | | | | | | BRAND |
| normuscone (502-72-2) | 837 | $C_{15}H_{28}$ | 135.11683 | 135.11683 | 0 | X | | X | X | | X | X | | | | | | | | | | BRAND |
| musk ambaral (37609-25-9) | 908 | $C_{15}H_{24}O^*$ | 236.21331 | 236.21347 | -0.65 | X | | | X | | | | | X | X | X | | | | | | BRAND |
| galaxolide (1222-05-5) | 878 | $C_{18}H_{26}O^*$ | 258.1979 | 258.19782 | 0.32 | X | X | X | | | | | | | | | X | X | X | | | A & B |
| ethylene brassylate (105-95-3) | 878 | $C_{17}H_{30}O_4$ | 227.16439 | 227.16417 | 0.98 | X | X | | X | | | | | | | | | | | X | X | A |
| musk ketone (81-14-1) | 928 | $C_{14}H_{18}N_2O_5^*$ | 294.12110 | 294.12102 | 0.25 | X | | | X | X | | | | | X | | | | | | X | B |

*molecular ion

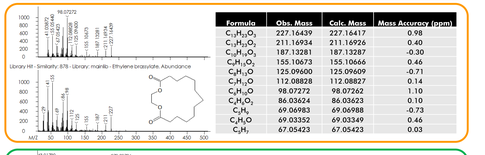
## Musk Odor Analytes

Detailed identification information for representative musk odor analytes from Figure 7 and Table 3 are shown below.

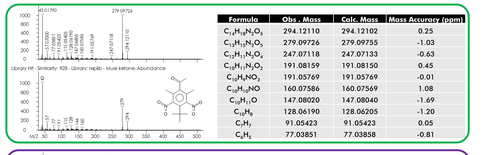


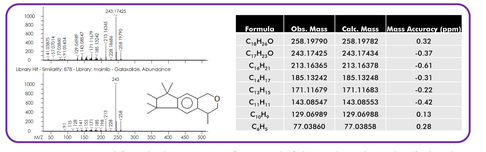

| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{16}H_{30}O$ | 238.22975 | 238.22912 | 2.67 |
| $C_{13}H_{22}O$ | 223.20591 | 223.20564 | 1.18 |
| $C_{14}H_{26}O$ | 209.19003 | 209.18999 | 0.20 |
| $C_{13}H_{25}$ | 180.18740 | 180.18725 | 0.84 |
| $C_{11}H_{15}$ | 135.11689 | 135.11683 | 0.49 |
| $C_8H_{11}$ | 125.13236 | 125.13248 | -0.95 |
| $C_8H_{13}O$ | 125.09603 | 125.09609 | -0.52 |
| $C_5H_{13}$ | 97.10124 | 97.10118 | 0.62 |
| $C_6H_9O$ | 85.06481 | 85.06479 | 0.17 |
| $C_4H_7O$ | 71.04910 | 71.04914 | -0.62 |




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{17}H_{31}O_4$ | 227.16439 | 227.16417 | 0.98 |
| $C_{11}H_{23}O_2$ | 211.16934 | 211.16926 | 0.40 |
| $C_{12}H_{25}O$ | 187.13281 | 187.13287 | -0.30 |
| $C_7H_{15}O_2$ | 155.10673 | 155.10666 | 0.46 |
| $C_8H_{17}O$ | 125.09600 | 125.09609 | -0.71 |
| $C_7H_{13}O$ | 112.08828 | 112.08827 | 0.14 |
| $C_5H_{12}O$ | 98.07272 | 98.07262 | 1.10 |
| $C_5H_{10}O_2$ | 86.03624 | 86.03623 | 0.10 |
| $C_5H_9$ | 69.06983 | 69.06988 | -0.73 |
| $C_5H_9O$ | 69.03352 | 69.03349 | 0.46 |
| $C_5H_5$ | 67.05423 | 67.05423 | 0.03 |




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{13}H_{15}N_2O_5$ | 294.12110 | 294.12102 | 0.25 |
| $C_{12}H_{11}N_2O_5$ | 279.09726 | 279.09755 | -1.03 |
| $C_{11}H_{11}N_2O_4$ | 247.07118 | 247.07133 | -0.63 |
| $C_{12}H_{11}N_2O_3$ | 191.08159 | 191.08150 | 0.45 |
| $C_{12}H_{13}NO_3$ | 191.05769 | 191.05769 | -0.01 |
| $C_{12}H_{14}NO$ | 160.07586 | 160.07569 | 1.08 |
| $C_{10}H_{11}O$ | 147.08020 | 147.08040 | -1.69 |
| $C_{10}H_8$ | 128.06190 | 128.06205 | -1.20 |
| $C_7H_7$ | 91.05423 | 91.05423 | 0.05 |
| $C_6H_5$ | 77.03851 | 77.03858 | -0.81 |




| Formula | Obs. Mass | Calc. Mass | Mass Accuracy (ppm) |
|---|---|---|---|
| $C_{18}H_{26}O$ | 258.19790 | 258.19782 | 0.32 |
| $C_{17}H_{23}O$ | 243.17425 | 243.17434 | -0.37 |
| $C_{14}H_{21}$ | 213.16365 | 213.16378 | -0.61 |
| $C_{14}H_{17}$ | 185.13242 | 185.13248 | -0.31 |
| $C_{11}H_{15}$ | 171.11679 | 171.11683 | -0.22 |
| $C_{11}H_{11}$ | 143.08547 | 143.08553 | -0.42 |
| $C_{10}H_8$ | 129.06989 | 129.06988 | 0.13 |
| $C_6H_5$ | 77.03860 | 77.03858 | 0.28 |

**Figure 8. HR-TOFMS and formula determinations for several of the musk analytes identified in these samples are shown. The library similarity scores are quite good, and the accurate mass information supports the formulae with excellent mass accuracy across the mass range for each fragment. A filtered AIC chromatogram is shown to highlight these specific analytes and their differential expression.**

## Conclusions

The perfume samples were characterized and compared with a combination of analytical instruments including GC-TOFMS, GC×GC-TOFMS, and GC×GC-HR-TOFMS. GC×GC provided chromatographic separations in the second dimension helping in instances of GC coelution, and HR-TOFMS provided better confidence and improved analyte identifications for a variety of analytes. These analytical technologies together provided a comprehensive picture of these samples and the ability to distinguish differentially expressed analytes and confidently identify them, including some that were challenging to separate with a one dimensional separation and many with important odor characteristics.