Application Note

Instrument: Pegasus® BTX 4D

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Improved GCxGC-TOFMS Workflow to More Efficiently Meet Compliance and Confidence Requirements for Fragrance Allergen Analysis Using the LECO *Pegasus* BTX 4D

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Key Words: Allergens; Perfume; Fragrance; Cosmetics; GCxGC; TOFMS; BTX

Introduction

The European Union (EU) has established comprehensive legislation on perfumes and cosmetic products to protect individuals with allergies, ensuring sufficient labeling of fragrance allergens to enable informed purchasing decisions. On 26th July 2023, the EU adopted the Regulation (EU) 2023/1545 to update the labeling of fragrance allergens under Annex III to Regulation (EC) 1223/2009 of the European Parliament and of the Council in regard to labeling of fragrance allergens in cosmetic products.[1] This updated Regulation expands the list of allergenic substances to 80+ compounds including 57 new substances. In practice, any substance present in the new list and whose concentration exceeds 0.001% in leave-on products or 0.01% in rinse-off products must be noted on the labeling of cosmetic products.

Other countries outside of the EU also have legislation for labeling allergens which might differ from country to country. This is important to take into consideration when exporting such products to those countries.

To comply with these regulations, the International Fragrance Association (IFRA) recommends a method based on gas chromatography coupled to mass spectrometry (GC-MS) for quantifying fragrance allergens. However, the proposed workflow employs separate analyses with two columns of different polarities and requires a minimum of four different GC runs to properly quantify the fragrance allergens in complex matrices such as fragrance raw materials, cosmetic products and/or complex natural substances. Moreover, to cover the full dynamic range present in such products, up to 16 analytical runs per sample may be required with different dilutions or split ratios. Ultimately, this places a significant burden on the analyst, who must consolidate and review data from multiple analytical runs to ensure reliable and accurate results.

To meet the need of a fast, robust, and reliable—but also sufficiently flexible—method to quantify fragrance allergens, an analytical workflow based on comprehensive two-dimensional gas chromatography (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) has been developed.[2] This approach reduces the number of required analytical runs per sample. Here, the analysis of 57 fragrance allergens and their relevant isomers is demonstrated with GCxGC-TOFMS using the LECO *Pegasus* BTX 4D system equipped with the QuadJet™ thermal modulator using liquid nitrogen (LN_2) as coolant. Examples of calibration curves and precision and accuracy metrics are provided. In addition, a perfume sample was analyzed under the same conditions the compatibility of this method with other analytical approaches, such as a non-target screening or characterization.

Experimental

The experimental conditions for the GCxGC-TOFMS analysis are summarized in Table 1. Fragrance allergen mixes A1 and A2 from Millipore Sigma (#89131 and #16558), containing the analytes listed in Table 2 (Appendix, page 6), were used to prepare calibration standards ranging from 0.2 to 25 µg/mL in methyl tert-butyl ether (MTBE), following the IFRA guidelines. The following calibration levels were prepared: 0.2, 0.5, 1, 2.5, 5, 10, 20, and 25 µg/mL. Internal standards (ISTDs) 1,4-dibromobenzene and 4,4'-dibromobiphenyl, obtained from Thermo Fisher Scientific (#A10517 and #A14139), were added to each calibration level at equal concentrations of 10 µg/mL. To assess precision and accuracy of the method, two allergen solutions at 1 µg/mL and 10 µg/mL concentration level were prepared in solvent and injected in replicates (n = 8).

Table 1: GC×GC-TOFMS Analysis Parameters

GCxGC Parameters	LECO GCxGC with QuadJet™ Thermal Modulator
Injector	1 µL split (1:100), 280 °C
Liner	Topaz, Low Pressure Drop Precision #23309 (Restek)
Carrier Gas	Helium: 1.30 mL/min ramped to 1.6 mL/min
Columns	VF-35ms 60 m x 0.25 mm x 0.25 µm (Agilent) MEGA-WAX-HT 0.6 m x 0.15 mm x 0.15 µm (Mega)
Oven Program	65 °C (1 min), 1.5 °C/min to 80 °C, 3 °C/min to 220 °C, 4 °C/min to 270 °C (17 min)
Secondary Oven Temp	+15 °C (relative to the GC oven temperature)
Modulator Temp	+15 °C (relative to the secondary oven temperature)
Modulation Period	1.4–2.8 s (Variable)
Transfer Line	270 °C
TOFMS	**LECO *Pegasus* BTX4D**
Ion Source Temp	250 °C
Mass Range	35–400 m/z
Acquisition Rate	150 spectra/s

The calibration curves were established in *ChromaTOF* using the quantifier and qualifier masses for each allergen, as provided in Table 2 (Appendix, page 6). Furthermore, for each target analyte, two ion ratios m/z with a tolerance of 15 % were implemented. The compounds listed in Table 2 (Appendix, page 6)are presented in the order of their sequential elution, in agreement with the 1D column featuring a 35% phenylmethyl stationary phase. For convenience, the correlation coefficients (R^2) from the calibration are also reported in the same table.

Results and Discussion

As suggested by the IFRA Analytical Working Group, most methods for fragrance allergens analysis rely on a GC-MS based method using either scan or selected ion monitoring (SIM) modes.[3] Independently of the MS mode, the number of GC runs per sample imposes considerable use of instrument time and human resources for data recombination, as at least four GC runs using two different column polarities are required to quantify the full allergens list. Additionally, these methods are challenged by complex matrices and partly insufficient chromatographic resolution.

Figure 1 displays a GCxGC-TOFMS contour plot for the full list of allergens in allergens mixes A1 and A2, including ISTDs. The numbers correspond to the target allergens in Table 2 (Appendix, page 6). The specific GCxGC column setup employed here significantly reduces the number of analytical runs, as full chromatographic resolution can be achieved in a single run for all allergens. The approach leverages the superior separation power of the GCxGC technique, allowing even complex samples—such as fine fragrances and cosmetic products—to be analyzed and quantified with ease. This translates directly into a cost- and time-efficient method.

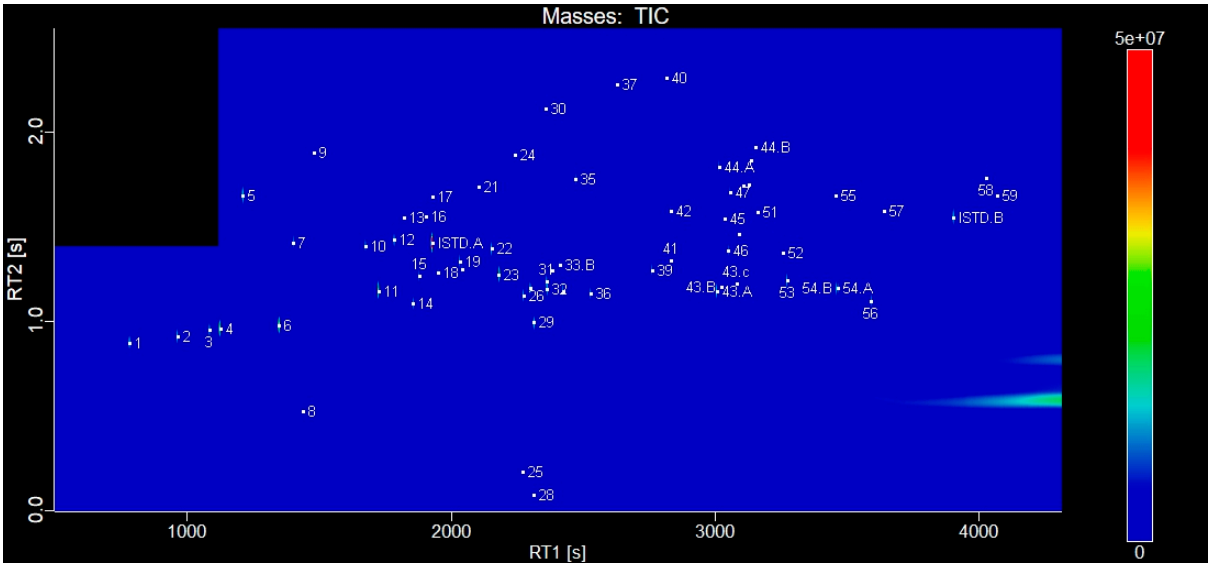

Figure 1: The GCxGC-contour plot demonstrates the excellent separation of the full list of allergens.

In addition, the excellent sensitivity of the *Pegasus* BTX 4D enabled calibration over a range of 0.2 to 25 µg/mL. As a result, samples can be appropriately diluted, reducing the risk of contamination and buildup in the injector port or liner. This is particularly advantageous for matrix-rich samples, as it extends the intervals between required instrument maintenance.

Calibration curve plots, ion ratio masses, contour plots, and quantitation masses for five representative compounds are chosen and displayed in Figure 2. Mass spectra and MS library references are displayed as well.

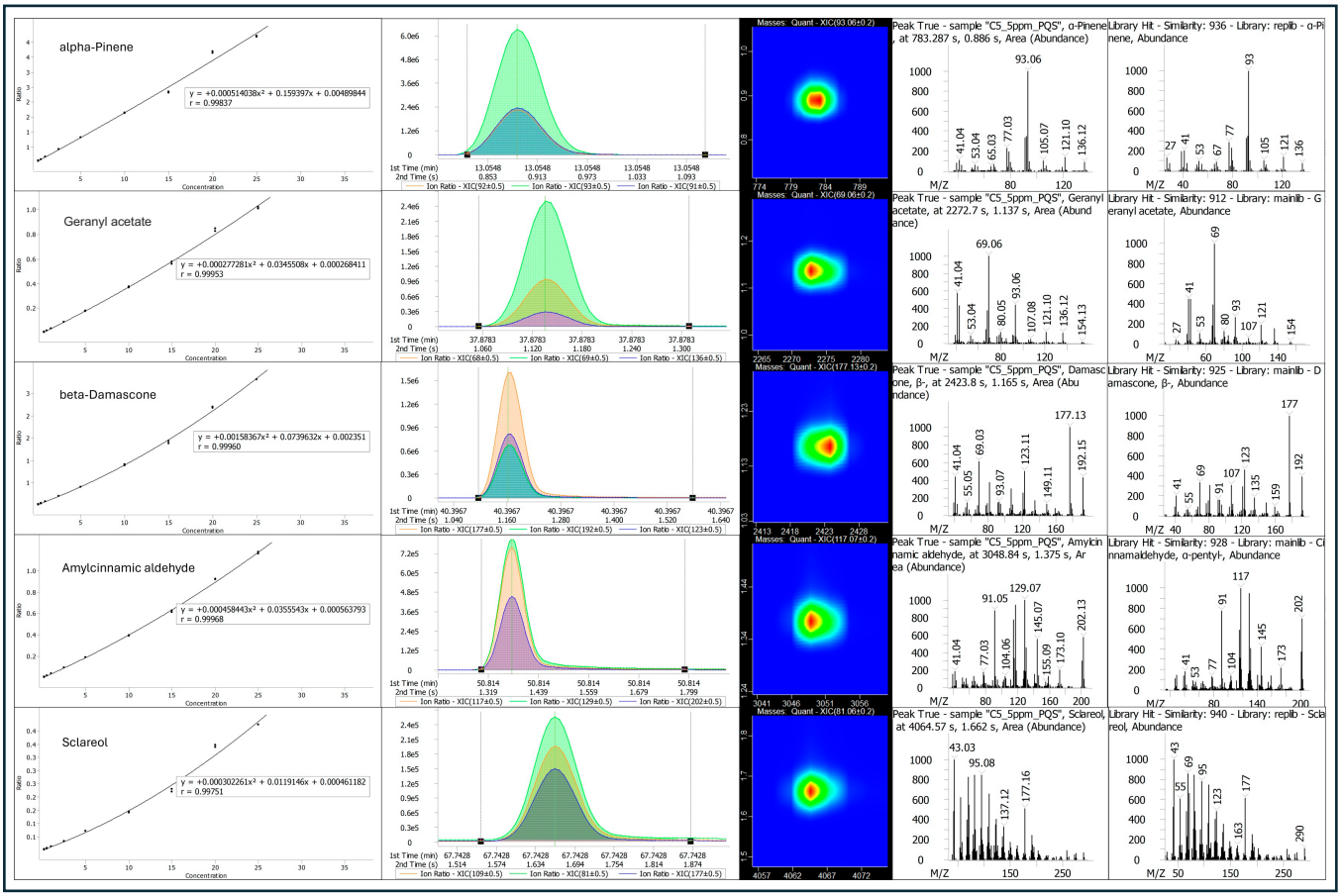

Figure 2: Example of calibration curves, ion ratio masses extracted ion chromatograms, contour plots, and mass spectra (deconvoluted, left and library, right) for a selected list of fragrance allergens.

Correlation coefficients (R^2) for all allergen standards are listed in Table 2 (Appendix, page 6). Overall, calibration curves showed an average correlation coefficient (R^2) of 0.9988 indicating reliable quantitation performances. Randomized replicate injections (n=8) of two allergen mixes at concentration levels 1 and 10 µg/mL were used to determine the method's precision and accuracy. Six compounds belonging to different chemical families were selected for this purpose, namely: alpha-pinene, geraniol, lilial, coumarin, eugenyl acetate, and benzyl cinnamate. Figure 3 displays precision results at both concentration levels for n=8 replicate injections. The figure includes the relative standard deviation (RSD %) values, below 5% for all compounds.

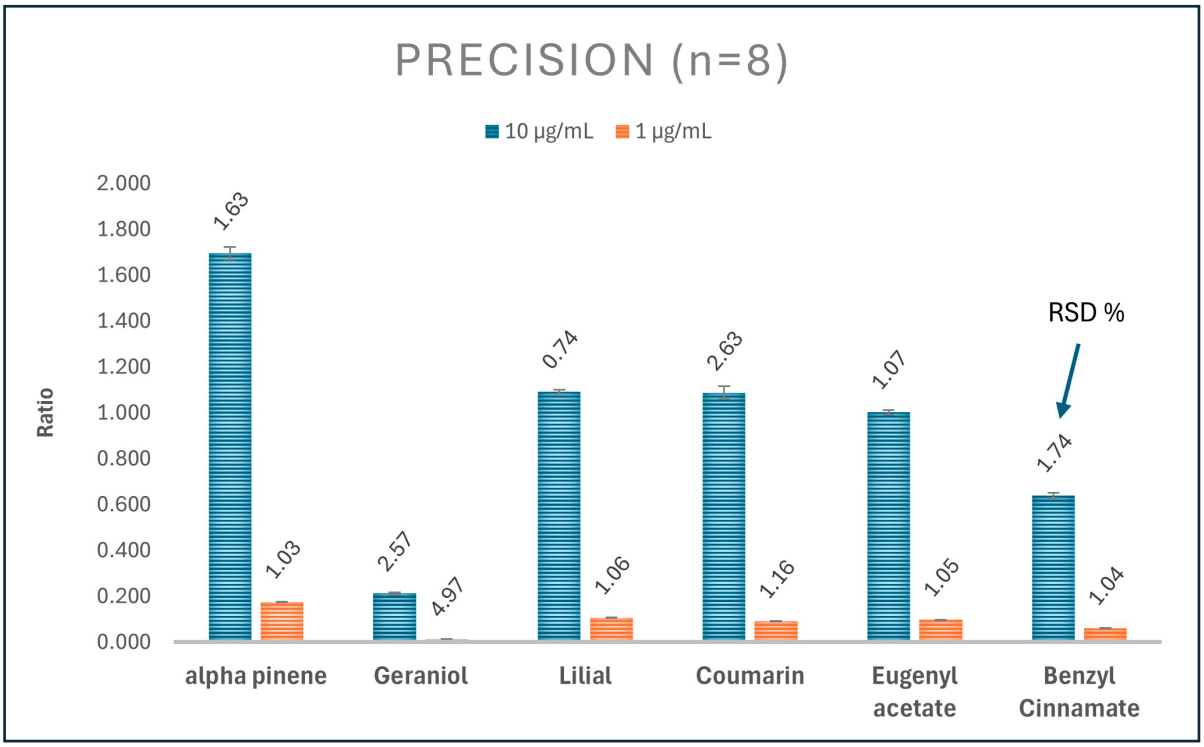

Figure 3: Method's precision demonstrated by performing replicate injections (n=8) at two concentration levels (i.e., 1 and 10 µg/mL).

Accuracy was determined by evaluating the mean repeatability (n=8) for the same components at both calibration levels and displayed in Figure 4a and 4b. Also in this case, the RSD% values were well below 5%.

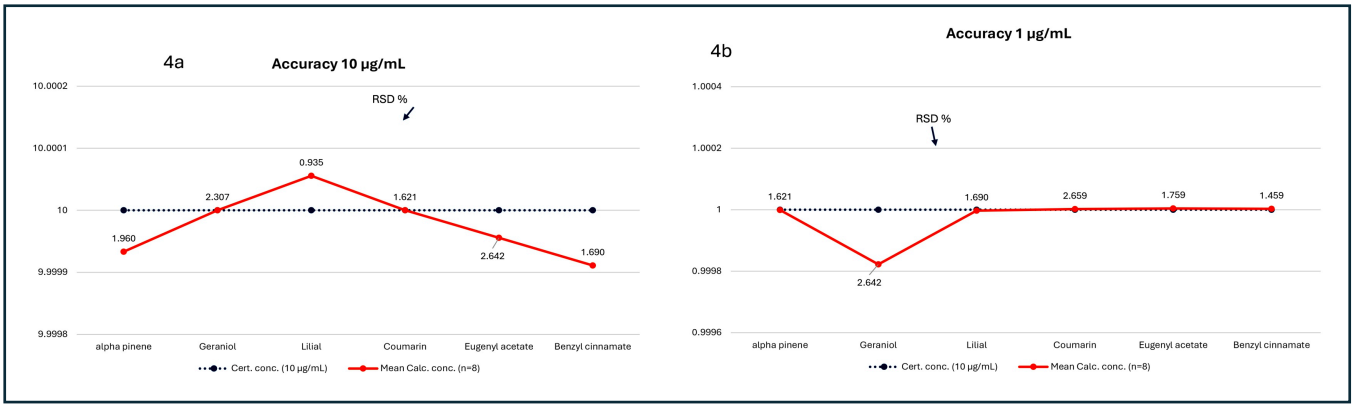

Figure 4a & 4b: Method's accuracy demonstrated by performing replicate injections (n=8) at two concentration levels (i.e., 1 and 10 µg/mL). Excellent accuracy is shown at both levels.

The GCxGC-contour plot of a fine fragrance sample (Figure 5) demonstrates the efficiency of the method for challenging samples. The zoomed-in area highlights a region with many compounds having similar retention times in the first dimension. However, the separation power of GC×GC allows these potential coelutions to be chromatographically resolved, resulting in much cleaner mass spectral data for a targeted fragrance allergen, namely isoeugenol acetate. Additionally, a ubiquitous contaminant (i.e. Diethyl Phthalate, CAS: 84-66-2) was also positively identified. Analytes such as this would likely remain undetected using conventional targeted methods.

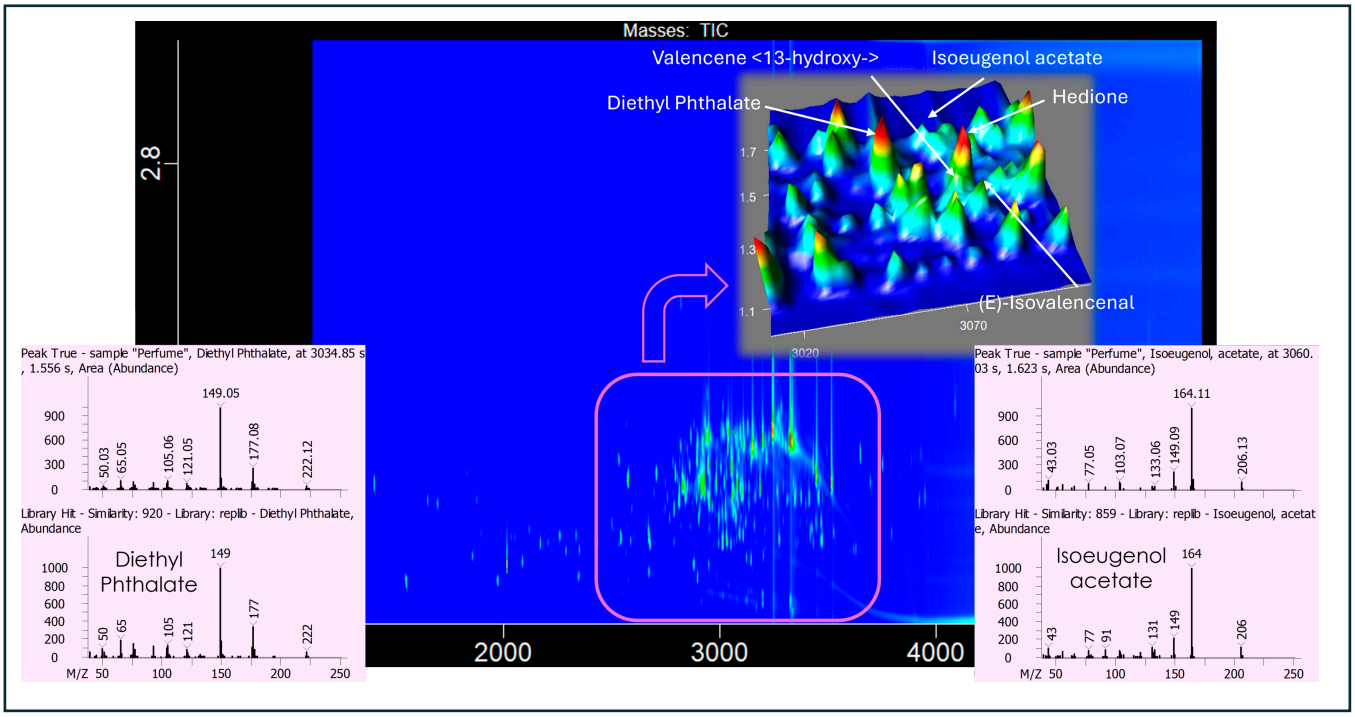

Figure 5: GCxGC-contour plot of a fine fragrance sample. Mass spectral information for a target allergen (isoeugenol acetate) and a contaminant (diethyl phthalate) are provided.

Thanks to the acquisition of full mass spectral data throughout the entire run, the proposed method allows for easy adaptation in terms of target analytes and additional compounds such as phthalates (see Figure 5) which can be monitored in the same run. The method can be tailored depending on the market region and its specific legal regulations ensuring that products conform with local requirements. If desired, TOFMS data from earlier analyzed samples can always be investigated retrospectively, probing for compounds not previously monitored.

Conclusion

The *Pegasus* BTX 4D platform represents a powerful alternative to conventional GC-MS-based methods for the analysis of fragrance allergens. The use of GCxGC-TOFMS significantly enhances chromatographic resolution in a single analytical run respecting nevertheless the IFRA recommended technical features using two different stationary phases. The direct coupling of the columns enables substantial reductions in overall analysis time and eliminates the need for multiple injections and labor-intensive data merging procedures. The high spectral fidelity of TOFMS, combined with the acquisition of full mass range spectra, enables robust and accurate quantification across a wide concentration range, while also supporting non-target screening and retrospective data analysis.

Acknowledgement

Elsa Boudard and Thomas Dutriez from Givaudan, Switzerland

References

[1]EUR-Lex. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. https://eur-lex.europa.eu/eli/reg/2009/1223/oj.

[2]Dutriez et al. Method of resolving allergens in perfume ingredients, WO2017207467A1, 2016.

[3]Analytical Method to Quantify 57 Suspected Allergens (and isomers) in Ready to Inject Fragrance Materials by Gas Chromatography and Mass Spectrometry. The International Fragrance Association, Analytical Working Group, 2016.

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APPENDIX

Table 2: Name, CAS number, Quantifier (Quant Mass), Qualifier Masses (Qual1 and Qual2) and correlation coefficients (R^2) for the target allergens and their isomers.

#	Name	CAS #	Quant Mass	Qual1	Qual2	R^2
1	alpha-Pinene	80-56-8	93.06	92	91	0.99837
2	beta-Pinene	127-91-3	93.06	92	91	0.99840
3	alpha-Terpinene	99-86-5	121.08	93	136	0.99762
4	Limonene	5989-27-5	68.05	93	67	0.99836
5	Benzaldehyde	100-52-7	106.02	105	51	0.99949
6	Terpinolene	586-62-9	121.08	121	136	0.99959
7	Linalool	78-70-6	71.04	93	55	0.99770
8	Benzyl alcohol	100-51-6	79.04	108	77	0.99735
9	Salicylaldehyde	90-02-8	122.02	121	65	0.99922
10	Menthol	89-78-1	71.04	81	95	0.99789
11	Camphor	464-49-3	95.08	81	108	0.99950
12	alpha-Terpineol	98-55-5	59.04	93	121	0.99775
13	Citronellol	106-22-9	69.06	67	81	0.99718
14	Linalyl acetate	115-95-7	93.06	69	80	0.99940
15	Folione®	111-12-6	95.08	55	123	0.99945
16	Methyl Salicylate	119-36-8	120.02	92	152	0.99951
ISTD.A	1,4-Dibromobenzene	106-37-6	235.86	234	238	NA
17	Geraniol	106-24-1	69.06	41	123	0.99755
18	Neral	106-26-3	69.06	94	109	0.99947
19	Carvone	99-49-0	82.03	54	108	0.99943
20	Geranial	141-27-5	69.06	84	152	0.99945
21	Hydroxycitronellal	107-75-5	59.04	71	96	0.99959
22	trans-Anethole	4180-23-8	148.09	147	117	0.99751
23	DMBCA	151-05-3	117.07	132	91	0.99938
24	Cinnamic aldehyde	104-55-2	131.05	132	103	0.99957
25	Anisyl alcohol	105-13-5	138.06	109	77	0.99747
26	Geranyl acetate	105-87-3	69.06	68	136	0.99953
27	delta-Damascone	57378-68-4	69.03	81	123	0.99948
28	Cinnamic alcohol	104-54-1	92.06	134	91	0.99747
29	beta-Caryophyllene	87-44-5	93.06	133	91	0.99736
30	Eugenol	97-53-0	164.08	149	103	0.99783
32	beta-Damascenone	23726-93-4	123.11	190	105	0.99938
31	alpha-Damascone	24720-09-0	121.10	192	81	0.99944
33.A	Ebanol 1	67801-20-1	107.08	108	55	0.99769
33.B	Ebanol 2	67801-20-1	107.08	108	55	0.99801
34	beta-Damascone	23726-91-2	177.13	192	123	0.99960
35	Majantol®	103694-68-4	106.07	105	178	0.99801
36	alpha-Isomethylionone	127-51-5	135.08	107	150	0.99967
37	trans-Isoeugenol E	5932-68-3	164.08	103	131	0.99797
38	Vanillin	121-33-5	151.04	152	81	0.99969
39	Lilial®	80-54-6	189.12	147	131	0.99981
40	Coumarin	91-64-5	118.04	146	90	0.99979
41	Amyl salicylate	2050-08-0	120.01	138	208	0.99971
42	Eugenyl acetate	93-28-7	164.08	149	206	0.99978
43.A	beta-ISO E SUPER® (major)	54464-57-2	191.17	119	219	0.99975
44.A	Propylidene phthalide (major)	56014-72-3	159.04	104	174	0.99979
43.B	gamma-ISO E SUPER®	54464-57-2	191.17	109	69	0.99978
45	alpha-Santalol	115-71-9	94.07	94	121	0.99798
46	Amyl cinnamic aldehyde	122-40-7	117.07	129	202	0.99968
47	Isoeugenyl acetate	93-29-8	164.08	149	131	0.99973
43.C	alpha-ISO E SUPER®	54464-57-2	135.11	191	107	0.99834
48	trans-trans-Farnesol	106-28-5	69.06	81	107	0.99797
49.A	Cyclohexal/Lyral® (minor)	31906-04-4	105.07	59	107	0.99961
49.B	Cyclohexal/Lyral® (major)	31906-04-4	136.09	79	93	0.99978
50	Amyl cinnamic alcohol	101-85-9	133.06	133	115	0.99808
44.B	Propylidene phthalide (minor)	56014-72-3	159.04	174	104	0.99963
51	beta-Santalol	77-42-9	94.07	93	79	0.99794
52	Jasmonal®	101-86-0	129.07	117	216	0.99988
53	Vertofix®	32388-55-9	119.08	119	231	0.99795
55	Benzyl benzoate	1222-05-5	105.03	213	258	0.99976
54.A	Galaxolide 1	120-51-4	243.17	91	212	0.99846
54.B	Galaxolide 2	1222-05-5	243.17	213	258	0.99868
56	Hexadecanolide	109-29-5	55.05	69	236	0.99965
57	Benzyl salicylate	118-58-1	91.05	65	228	0.99952
ISTD.B	1,1'-Biphenyl, 4,4'-dibromo-	92-86-4	152.06	311	76	NA
58	Benzyl cinnamate	103-41-3	91.05	131	192	0.99960
59	Sclareol	515-03-7	81.06	109	177	0.99751