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Analysis of Allergens in Fragrance Samples Using a Comprehensive GCxGC in Combination with a High-Resolution Mass Spectrometry

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Introduction

Fragrances are highly complex mixtures that combine natural and synthetic components. Analytical separation of these samples on a single GC column results in coelution of isomers and structurally similar compounds which produce similar spectral fragmentation patterns. This significantly complicates identification and quantitative analysis of individual components of the fragrances. Here we demonstrate a power of a comprehensive GCxGC approach combined with high resolution MS for identification and quantitative analysis of 64 allergens in fragrances. With added confidence in compound identification provided by accurate mass, this approach also streamlines a non-targeted analysis of the fragrances to determine their composition.

Figure 1. Agilent 7250 GC/Q-TOF

Experimental

The samples were separated on an 8890 GC using a comprehensive GCxGC configuration with a reverse flow modulator (RFM). The non-polar/mid-polar column set was used and comprised of a 20m x 0.1mm x 0.1um DB-1ms column (100% Dimethylpolysiloxane) coupled to a 5m x 0.25mm x 0.15um DB-17ms column (equivalent to (50%-phenyl)-methylpolysiloxane). Optimized instrumental parameters are shown in Table 1.

Table 1. Instrument and method parameters

Parameter	Value
MS	Agilent 7250 GC/Q-TOF
GC	Agilent 8890 GC
Inlet/Liner	MMI, Agilent 5190-2294: 990 µL (Split, straight, wool, Ultra Inert)
Injection Mode	Split; 10:1
Injection Volume	1.5 µL
Inlet Temperature	250 °C
Column 1D & Flow	Agilent DB-1ms, 20 m x 0.1 mm x 0.1 µm; 0.2 mL/min
Column 2D & Flow	Agilent DB-17ms, 5 m x 0.25 mm x 0.15µm; 10 mL/min
Restrictor to Modulator Vent FID	Deactivated fused silica; 0.4 m x 0.05 mm
Purged Splitter Restrictor to Q-TOF & Flow	Deactivated fused silica; 0.6 m x 0.12 mm; 1.3 mL/min
Purged Splitter Restrictor to Front FID	Deactivated fused silica; 1 m x 0.25 mm
Modulation Delay	0.5 min
Modulation Period	6.3 sec
Injection Time	0.185 sec
Carrier Gas	Helium
Oven Temperature Program	50 °C for 4 min; 4 °C/min to 266 °C
Transfer Line Temperature	305 °C
Source Temperature	300 °C
Quadrupole Temperature	150 °C
Collision Cell Gas Flows	N2, 1 mL/min + He, 4 mL/min
Electron Energy	70 eV
Emission Current	5 µA
Spectral Acquisition Rate	50 Hz
Mass Range	*m/z* 45 to 350
FID Temperature	300 °C
FID H2 Flow	30 mL/min
FID Air Flow	400 mL/min
FID Makeup Flow (N2)	15 mL/min (Front); 25 mL/min (Vent)

The data were acquired using the high-resolution 7250 GC/Q-TOF at data rate of 50 Hz. For compound identification the Unknown Analysis tool of MassHunter Quantitative Analysis software version 12.1 and the GC Image software version 2024 R3 were used. The linear retention indices (RIs) were used to increase confidence in compound identification. Statistical analysis was performed in Mass Profiler Professional (MPP) software version 15.1.

GCxGC Configuration

A GCxGC RFM method was developed to achieve an optimal chromatographic separation of all the allergens while also ensuring adequate sensitivity and carrier gas flow to the MS. GCxGC RFM setup included two detectors: the Q-TOF MS and the FID. The configuration provided an optimal flow to the MS of 1.3 mL/min (11.8% of total flow) maintaining a constant split between FID and MS throughout the entire oven temperature ramp. The configuration is shown in Figure 2.

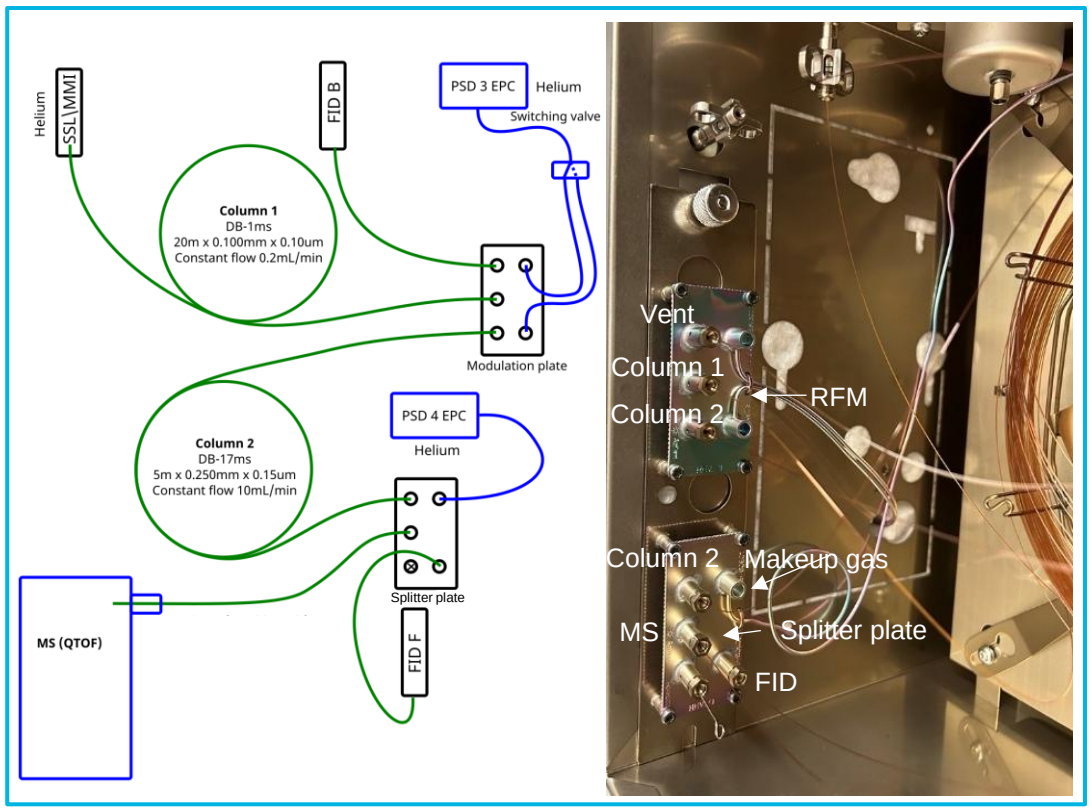

Figure 2. GCxGC setup using a purged splitter.

Allergens Separation and Quantitation

Current method allowed the separation of all allergen standards (Figure 3).

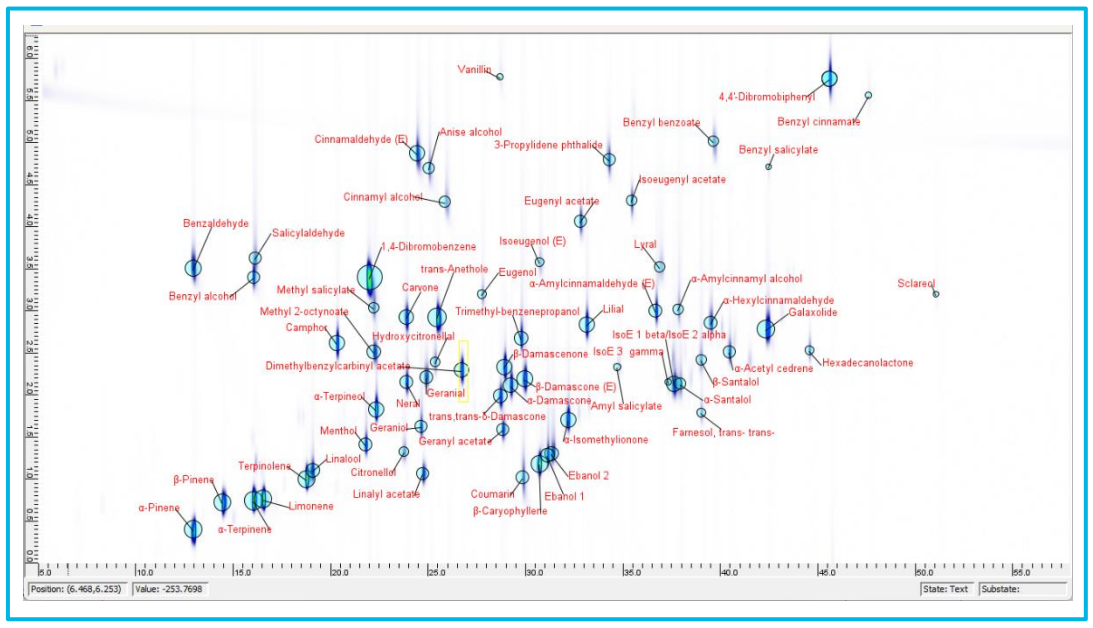

Figure 3. Separation of allergen standards using GCxGC.

Quantitation of allergens was performed in GC Image software. A GC Image template containing allergens spectra and RT1/RT2 information was created and applied to the fragrance images (Figure 4).

Calibration curves were built based on allergen standards and performed in the range of 1 – 100 ppm, (Figure 5). 1,4-dibromobenzene and 4,4'-dibromobiphenyl were used as internal standards.

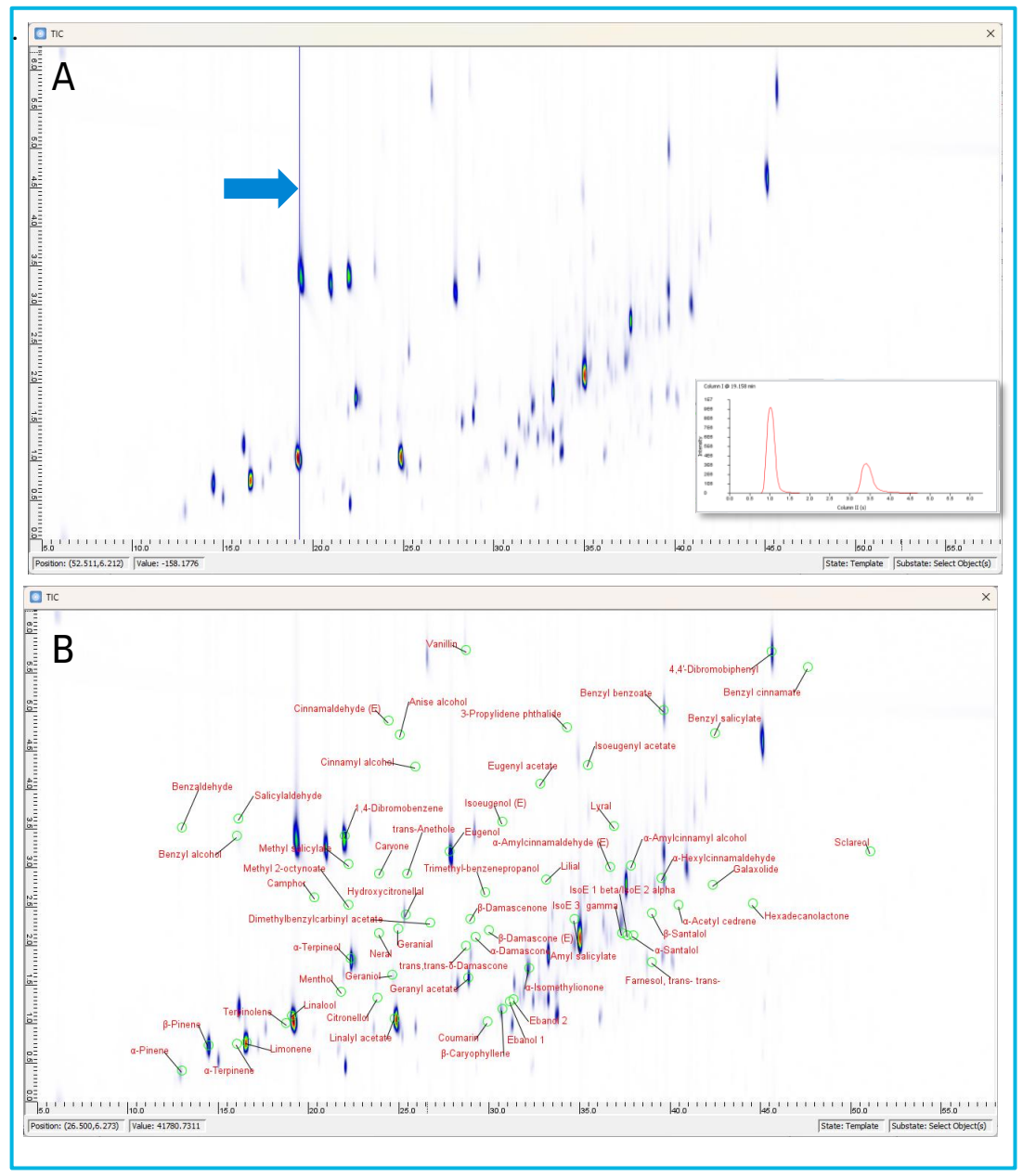

Figure 4. A) Separation of an unspiked fragrance components. The vertical line marks the components co-eluting in 1D. Their separation in the 2nd dimension shown on the right. B) Same image overlaid with the allergens template.

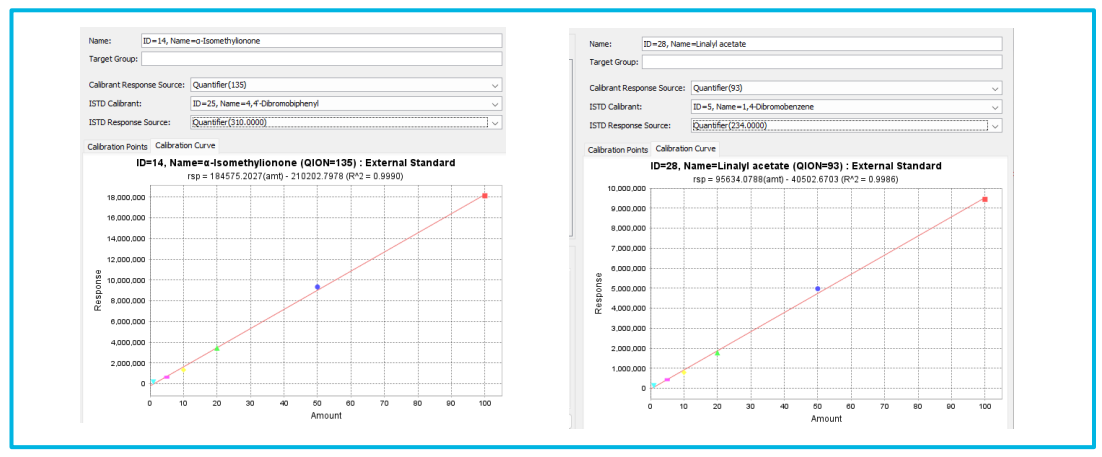

Figure 5. Examples of allergens calibration curves made from neat standards in GC Image.

Results and Discussion

One of the fragrances was spiked with allergens at 10 ppm and 100 ppm concentration analyzed as described above. The results are summarized in Table 2. The results for unspiked fragrances are displayed in Table 3.

Compound Name	Quant ion	RT I (min)	RT II (sec)	G1	G1 + 10 ppm	G1 + 100 ppm	Compound Name	Quant ion	RT I (min)	RT II (sec)	G1	G1 + 10 ppm	G1 + 100 ppm
α-Pinene	121.1012	12.87	0.38	13.0	25.7	117.4	β-Damascenone	121.1012	28.91	2.26	0.1	12.1	78.5
Benzyl alcohol	108.0570	13.08	3.43	5.1	20.8	99.4	α-Damascone	192.1509	29.23	2.10	-	9.8	77.0
Benzaldehyde	105.0335	13.08	3.53	0.2	4.8	79.2	Trimethyl-benzenepropanol	106.0777	29.86	2.64	-	12.1	82.5
β-Pinene	136.1247	14.44	0.71	41.9	57.8	155.7	Coumarin	146.0362	29.96	0.91	*	*	*
α-Terpinene	136.1247	15.80	0.65	3.5	16.4	113.1	ß-Damascone (E)	192.1509	29.96	2.16	-	8.5	69.7
Salicylaldehyde	122.0362	16.22	3.49	-	7.7	101.9	β-Caryophyllene	133.1012	30.59	1.17	*	*	*
Limonene	136.1247	16.54	0.75	*	*	*	Isoeugenol (E)	164.0832	30.80	3.51	-	14.9	91.7
Terpinolene	136.1247	18.74	1.01	1.5	22.5	108.1	Ebanol 1	149.1326	30.91	1.15	4.8	15.4	81.6
Linalool	136.1247	19.16	1.03	*	*	*	Ebanol 2	149.1326	31.01	1.31	-	15.0	78.1
Camphor	152.1196	20.42	2.58	3.5	13.4	85.1	α-Isomethylionone	150.1039	32.27	1.59	*	*	*
Menthol	123.1168	21.78	1.41	-	12.3	94.4	Eugenyl acetate	164.0832	32.90	3.97	0.3	11.9	82.4
Methyl salicylate	120.0206	22.20	2.96	3.8	12.5	94.4	Lilial	189.1274	33.21	2.78	-	10.3	79.3
α-Terpineol	136.1247	22.31	1.82	*	*	*	3-Propylidene phthalide	159.0441	34.26	4.74	-	8.8	72.4
Methyl 2-octynoate	123.0804	22.31	2.42	-	14.4	95.4	Amyl salicylate	120.0206	34.79	2.32	-	-	89.1
Citronellol	138.1403	23.46	1.25	16.6	24.5	102.7	Isoeugenyl acetate	164.0832	35.73	4.20	-	12.2	85.0
Carvone	108.0934	23.88	2.84	5.6	17.8	93.2	Lyral	192.1509	36.46	3.47	0.1	11.2	76.3
Neral	137.0961	23.88	2.10	13.7	27.3	121.5	α-Amylcinnamaldehyde (E)	202.1352	36.67	2.96	-	11.2	80.0
Cinnamaldehyde (E)	131.0492	24.51	4.80	-	7.6	81.3	α-Amylcinnamyl alcohol	133.0648	37.20	2.92	-	9.5	91.7
Geraniol	123.1168	24.72	1.55	87.3	98.3	187.2	IsoE 1 beta/IsoE 2 alpha	191.1794	37.72	2.16	0.2	11.4	72.3
Linalyl acetate	121.1012	24.82	1.03	*	*	*	α-Santalol	121.1012	37.93	2.08	2.0	10.0	89.1
Geranial	137.0961	24.93	2.16	15.2	22.6	83.4	(E,E)-Farnesol	121.1012	38.98	1.77	1.2	14.8	69.0
Anise alcohol	109.0648	25.14	4.60	0.3	8.9	86.4	β-Santalol	94.0777	38.98	2.40	*	*	*
Hydroxycitronellal	121.1012	25.35	2.38	-	6.7	65.9	α-Hexylcinnamaldehyde	129.0699	39.61	2.84	13.1	21.9	81.7
trans-Anethole	148.0883	25.45	2.90	-	11.5	91.6	Benzyl benzoate	212.0832	39.72	4.96	*	*	*
Cinnamyl alcohol	134.0726	25.98	4.22	-	14.9	78.8	α-Acetyl cedrene	246.1978	40.45	2.50	0.2	14.7	73.2
DMBCA	132.0934	26.71	2.30	0.5	9.8	79.4	Benzyl salicylate	91.0542	42.44	4.60	-	10.5	89.8
Eugenol	165.0866	27.86	3.15	*	*	*	Galaxolide	258.1978	42.44	2.76	0.4	7.8	64.2
(E,E)-δ-Damascone	192.1509	28.70	1.96	-	13.2	84.3	Hexadecanolactone	254.2240	44.33	2.46	1.5	10.1	73.4
Vanillin	152.0468	28.81	5.67	*	*	*	Benzyl cinnamate	192.0934	47.58	5.53	18.5	34.9	121.5
Geranyl acetate	154.1352	28.91	1.59	*	*	*	Sclareol	257.2264	51.04	3.19	-	13.6	97.2

* - starting concentration is > 100 ppm

Table 2. Allergens concentrations determined in a fragrance before and after spiking at 10 ppm and 100 ppm levels.

Compound Name	A1	A2	A3	A4	B1	C1	C2	D1	E1	F1	G2	G3	H1	I1	J1
α-Pinene	-	37.6	11.8	198.7	15.1	22.7	73.5	**	55.1	38.2	**	**	-	194.4	6.9
Benzyl alcohol	-	0.3	0.9	66.4	0.6	5.6	2.8	15.4	1.1	19.4	-	0.8	-	6.8	0.2
Benzaldehyde	11.8	-	2.1	15.3	5.1	-	58.5	23.0	1.7	1.3	20.1	1.4	-	6.9	11.4
β-Pinene	-	**	102.7	179.3	33.9	47.1	**	**	54.9	93.2	**	**	**	**	59.2
α-Terpinene	-	2.6	-	-	-	-	-	-	166.7	-	-	-	73.8	-	-
Salicylaldehyde	1.1	-	1.1	-	-	1.1	2.4	1.1	-	1.3	1.6	2.2	-	-	-
Limonene	5.0	**	163.3	142.4	**	50.7	-	-	**	130.3	-	-	2.2	**	181.4
Terpinolene	-	-	1.9	26.2	51.9	-	-	-	-	-	49.2	19.8	0.7	8.9	29.5
Linalool	36.1	**	-	-	**	**	**	52.9	124.0	**	-	**	0.0	**	10.2
Camphor	-	2.2	-	-	0.6	-	-	0.5	-	-	28.1	19.1	-	0.1	-
Menthol	1.3	92.5	-	1.0	7.6	0.8	2.5	2.8	-	1.8	50.8	-	-	2.5	10.0
Methyl salicylate	-	-	-	-	-	-	19.3	-	-	-	-	-	-	-	-
α-Terpineol	**	128.9	1.9	275.3	3.1	20.6	14.8	43.5	-	-	90.1	33.7	15.5	43.6	6.4
Methyl 2-octynoate	2.7	-	-	1.9	1.9	2.2	2.4	-	1.8	1.7	3.1	-	1.7	1.8	-
Citronellol	**	5.8	-	-	**	**	-	**	**	**	144.7	**	**	64.1	-
Carvone	30.3	176.9	0.8	3.7	5.2	1.0	3.3	25.6	0.8	3.0	6.2	9.3	1.2	6.0	1.2
Neral	30.7	91.4	3.3	52.8	3.3	13.6	-	31.3	3.3	16.4	2.7	44.5	10.0	-	2.0
Cinnamaldehyde (E)	-	-	-	-	-	-	-	-	-	-	-	-	2.7	-	-
Geraniol	19.1	60.1	0.7	-	132.3	**	12.0	13.7	0.0	10.8	-	**	1.0	118.0	74.9
Linalyl acetate	**	**	3.0	**	**	53.9	**	**	55.9	**	8.2	**	**	**	**
Geranial	15.2	120.8	4.2	-	2.7	7.4	49.8	27.5	5.2	12.4	2.8	45.7	7.7	7.9	
Anise alcohol	-	-	-	-	-	4.6	2.2	-	-	8.3	-	5.5	2.7	-	1.5
Hydroxycitronellal	84.8	5.8	5.6	2.8	-	**	**	198.3	3.3	**	11.6	2.8	-	-	**
trans-Anethole	4.5	2.8	1.4	1.2	1.2	-	-	-	-	-	27.8	6.6	1.1	-	-
Cinnamyl alcohol	-	-	-	-	-	1.3	-	126.6	-	-	1.5	2.2	-	-	-
DMBCA	-	-	-	-	-	0.5	-	0.8	-	-	-	-	-	-	-
Eugenol	3.8	33.9	12.4	3.7	90.8	69.5	22.7	21.4	4.0	-	**	**	5.3	9.2	-
(E,E)-δ-Damascone	0.2	0.1	0.1	0.1	0.4	0.1	0.3	-	0.1	0.1	-	0.1	0.1	14.8	0.4
Vanillin	-	-	-	-	-	35.1	-	**	-	-	-	-	37.3	**	58.7
Geranyl acetate	**	141.9	15.0	**	32.6	66.8	-	-	-	75.2	**	**	155.0	96.1	118.5
β-Damascenone	2.6	3.3	-	2.9	11.3	2.6	20.0	35.8	-	2.6	2.6	3.3	21.8	2.6	18.0
a-Damascone	44.2	3.0	3.1	3.0	11.4	3.0	-	-	5.1	3.1	-	-	3.0	3.0	18.2
Trimethyl-benzenepropanol	17.4	0.0	3.0	-	-	-	**	-	-	0.5	-	-	3.1	-	-
Coumarin	34.6	-	-	-	-	57.8	-	46.6	3.5	-	-	-	3.4	113.6	-
ß-Damascone (E)	-	-	2.8	-	-	2.9	15.5	-	3.2	-	4.1	-	-	3.5	-
β-Caryophyllene	0.5	3.5	40.2	11.7	2.4	3.2	16.3	-	1.5	11.4	48.9	-	1.8	2.1	1.6
Isoeugenol (E)	-	17.8	31.7	10.8	9.4	7.4	-	-	-	-	61.7	-	-	9.8	9.8
Ebanol 1	3.9	3.9	7.0	4.6	3.4	19.4	7.4	-	4.1	-	3.8	-	3.7	-	111.0
Ebanol 2	1.5	2.2	41.1	6.2	0.9	31.8	23.4	-	3.0	5.1	-	24.5	2.0	-	110.3
α-Isomethylionone	121.4	187.0	11.1	1.6	173.6	**	1.7	169.4	**	**	121.5	**	**	1.9	**
Eugenyl acetate	-	-	10.0	-	-	26.9	3.5	-	-	-	4.0	-	-	1.9	-
Lilial	180.1	3.4	-	3.1	**	-	197.1	**	**	-	3.0	3.1	168.2	**	**
3-Propylidene phthalide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amyl salicylate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isoeugenyl acetate	-	-	-	-	-	-	-	-	9.0	-	197.9	142.4	-	-	-
Lyral	-	8.2	14.1	4.9	17.5	12.8	2.5	4.6	22.2	34.6	5.4	-	9.9	8.1	-
a-Amylcinnamaldehyde (E)	2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Amylcinnamyl alcohol	-	3.2	-	-	-	11.0	-	-	-	-	-	-	-	-	-
IsoE 1 beta/IsoE 2 alpha	3.1	5.4		4.4	**	4.6	**	78.6	30.8	51.3	3.2	20.0	40.6	165.0	185.4
α-Santalol	2.0	2.7	-	1.2	-	9.1	12.5	-	**	**	5.5	-	**	**	**
(E,E)-Farnesol	10.0	5.8	16.5	12.9	4.6	13.2	9.3	-	77.4	4.0	7.6	75.0	7.2	25.3	5.1
β-Santalol	16.1	7.8	-	4.8	6.2	29.2	11.0	-	-	-	19.9	**	-	91.1	17.8
α-Hexylcinnamaldehyde	156.2	**	**	**	**	**	-	**	-	-	3.2	**	19.9	27.2	**
Benzyl benzoate	88.7	-	-	-	**	-	-	-	-	-	**	-	60.6	4.8	22.1
α-Acetyl cedrene	164.6	-	2.9	-	5.5	102.1	-	**	-	3.0	2.9	3.2	-	80.4	-
Benzyl salicylate	**	10.4	-	-	-	51.6	**	-	-	-	-	**	-	67.7	-
Galaxolide	2.6	2.9	-	-	**		16.3	-	**	-	2.5	144.1	13.4		2.6
Hexadecanolactone	-	4.1	-	-	-	-	-	-		7.6	-	7.7	-	-	4.4
Benzyl cinnamate	80.9	-	-	-	-	-	-	-	19.9	-	**	-	-	-	-
Sclareol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

** - concentration is > 200 ppm

Table 3. Allergens concentrations in unspiked fragrances.

Composition and Comparison of Fragrances of Different Aroma Types Using Untargeted Analysis

Compound identification was performed in the Unknowns Analysis using NIST 23 and accurate mass allergens in house libraries (Figure 6), and the results were exported to MPP for further statistical analysis.

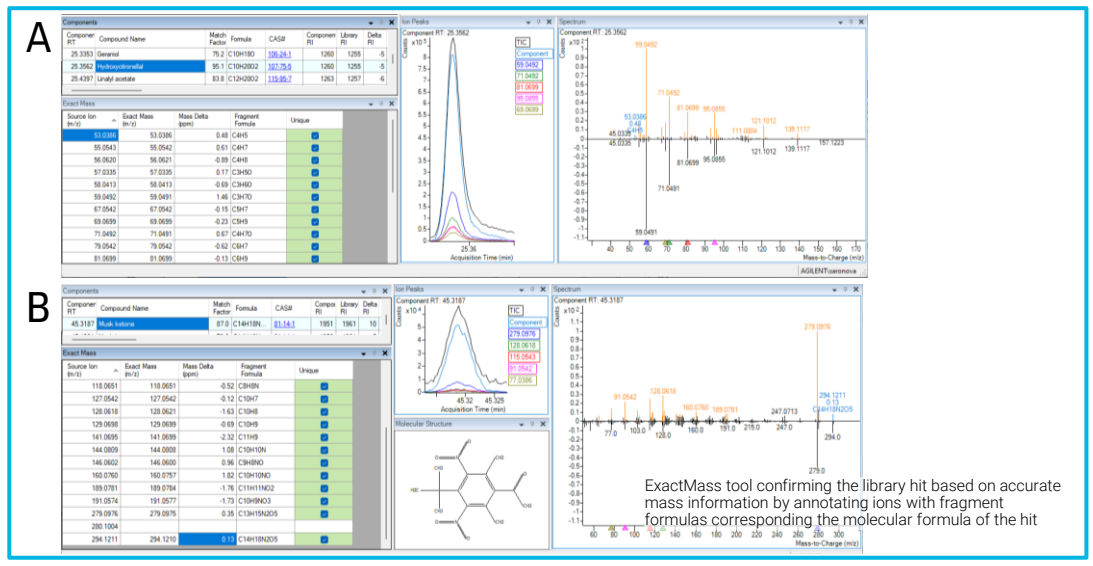

Figure 6. Accurate mass confirmation of the compound ID using accurate mass Allergens PCDL (A) and unit mass NIST23 library (B).

244 identified compounds differed significantly in their levels between floral and woody fragrances, as shown in Figure 7.

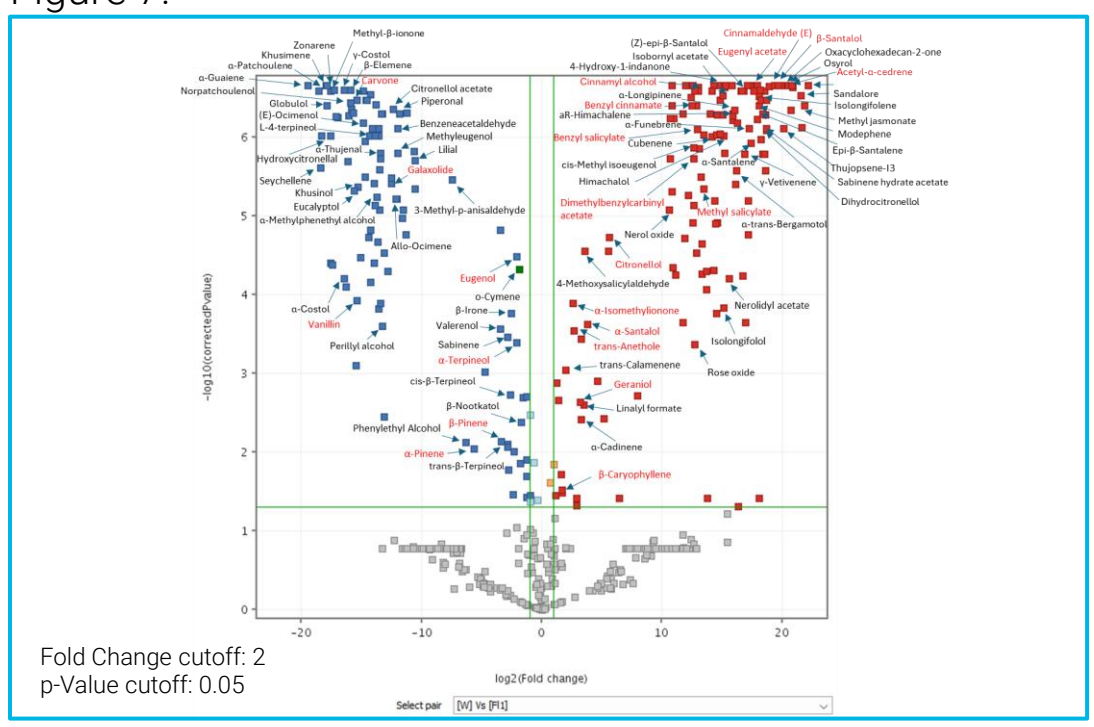

Figure 7. Volcano plot comparing the compositions of a woody and a floral scented fragrances. Known allergens are highlighted in red.

Conclusions

- Allergens in fragrance samples have been analyzed using comprehensive GCxGC combined with the high-resolution GC/Q-TOF and RFM.
- Fragrances with different types of aroma were analyzed using the GCxGC/HRMS combined with statistical analysis to identify the major differentiating compounds that may contribute to the particular type of scent.

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