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IMPROVED IDENTIFICATION OF TARGET AND NON-TARGET ALLERGENS IN PERFUMES BY GC×GC-QTOF.

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The fragrance industry is bound to ever-increasingly strict norms for safety and quality control. A key regulation concerns the presence of known and potential allergens. Their use is restricted and above a certain amount their presence must be indicated on the product label. Therefore, it must be possible to identify and quantify individual allergens accurately and reliably. Since fragrances are often highly complex mixtures and the list of regulated compounds has been expanding in the last years, with prospects to expand even further in the future, the analytical challenge behind this demand is not trivial.

Gas chromatography Mass Spectrometry (GC-MS) is a widely employed technique for the analysis of volatiles. However, separate analyses with columns based on different separation mechanism are required to achieve the target resolution for all allergens.

Comprehensive two-dimensional gas chromatography (GC×GC) benefits from the coupling of two different separation mechanism in a single analysis. The improved separation power and superior peak capacity make GC×GC a very powerful tool to unravel sample complexity. It is thus not surprising that GC×GC-MS is becoming more and more used for the analysis of fragrances, in some instances in combination with tandem FID detection (GC×GC-FID/MS) for more robust quantification. This approach provides good performance for targeted analysis of allergens, as well as the possibility to perform untargeted screening. Nevertheless, sometimes identification can be challenging.

In this work we present the use of GC×GC with thermal modulation coupled to a QTOF detector for improved identification of allergens in perfumes. GC×GC in combination with High Resolution Mass Spectrometry (HRMS) is shown to be a powerful profiling tool capable of providing value added for identity confirmation and untargeted analysis.

Experimental details

Standard: allergens mixture containing 65 allergens with individual concentrations in the range 150 µg/mL (or ppm, w/v) in acetone. Dilutions of this solution are prepared using n-hexane as solvent.
Samples: 6 commercial perfumes diluted to 1% in n-hexane.

All measurements are performed on an Agilent 7890B GC equipped with a Zoex ZX2 cryogen-free thermal modulator and an Agilent 7200B QTOF Detector. The QTOF is operated in Extended Dynamic Range (EDR) acquisition mode and the maximum acquisition speed of 50 Hz. All 2D data are visualized and processed using the GC Image HR software.

Results and discussion

1. *Separation and identification*

Fig. 1 shows the 2D plot for the allergens standard mixture. The two-dimensional separation provides enhanced resolution, several allergens fully co-eluting in the primary column are resolved on the second dimension. The practical advantages for complex real-life samples are an easier identification without need for laborious deconvolution and reduced matrix interference.

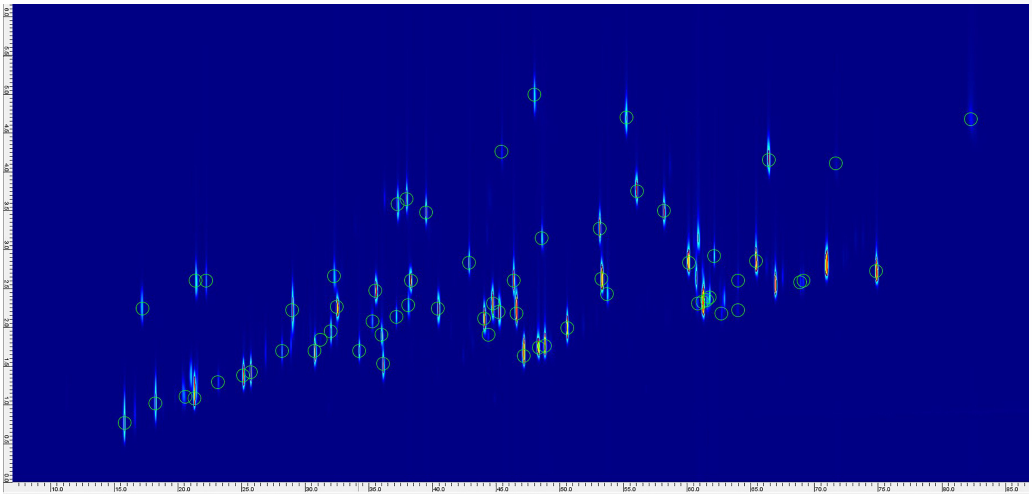


Fig. 1
GC×GC- QTOF 2D chromatogram for the allergens standard mix at 150 ppm.

2. *Repeatability*

The repeatability of retention times for 5 replicate analyses is shown in Table 1 for a dilution of the standard solution at 7 ppm. The RSDs for the retention times in the primary column are mostly <0.01% and in all cases <0.3%. In the second dimension the RSDs are <1.0% for 62 allergens out of 65 and always <1.9%, with an average of <0.6%. This is remarkable if one considers that these retention times are only a few seconds.

These results confirm that GC×GC with thermal modulation is a robust technique and suggest that the position in the 2D separation space can be used, in combination with the MS spectrum, as a reliable feature for the identification of targets.

Table 1
List of allergens and retention time repeatability calculated at a concentration of 7 ppm for 5 replicate analyses.

#	Compound name	Retention time I in minutes (n=5)		Retention time II in seconds (n=5)	
		Average	RSD %	Average	RSD %
1	1-Terpineol	28.12	<0.01	1.68	0.50
2	6-methyl Coumarin	55.18	<0.01	4.64	<0.01
3	alpha-Damascone-E	43.98	<0.01	2.12	0.40
4	alpha-Damascone-Z	45.15	<0.01	2.20	1.04
5	alpha-Isomethyl ionone	50.52	<0.01	2.00	0.79
6	alpha-Pinene	15.75	<0.01	0.73	1.81
7	alpha-Santalol	61.72	<0.01	2.34	0.44
8	alpha-Terpineol	31.97	<0.01	1.94	0.81
9	alpha-Terpinene	20.42	<0.01	1.14	0.74
10	Amyl cinnamic alcohol	62.07	<0.01	2.85	0.59
11	Amyl cinnamic aldehyde	60.08	<0.01	2.80	0.37
12	Amyl salicylate	53.67	<0.01	2.36	0.44
13	Anethole	38.27	<0.01	2.61	0.60
14	Anise alcohol	37.92	<0.01	3.61	0.44
15	Benzaldehyde	17.15	<0.01	2.29	<0.01
16	Benzyl alcohol	21.42	0.27	2.60	0.94

Table 1
Continuing from previous page

#	Compound name	Retention time I in minutes (n=5)		Retention time II in seconds (n=5)	
		Average	RSD %	Average	RSD %
17	Benzyl Benzoate	66.38	<0.01	4.13	0.20
18	Benzyl cinnamate	81.90	<0.01	4.67	0.46
19	Benzyl salicylate	71.49	0.39	3.99	0.61
20	beta-Caryophyllene	47.13	<0.01	1.67	0.62
21	beta-Damascenone	44.68	<0.01	2.31	0.57
22	beta-Damascone	46.55	<0.01	2.23	0.46
23	beta-Pinene	18.20	<0.01	0.99	0.85
24	beta-Santalol	63.93	<0.01	2.56	0.52
25	Camphor	28.93	<0.01	2.24	0.46
26	Carvone	35.47	<0.01	2.49	0.41
27	Cinnamaldehyde	37.22	<0.01	3.58	0.47
28	Cinnamic alcohol	39.43	<0.01	3.44	0.55
29	Citronellol	34.18	<0.01	1.68	0.79
30	Coumarin	47.95	<0.01	4.97	0.32
31	DMBCA	40.37	<0.01	2.25	<0.01
32	Ebanol 2	48.30	<0.01	1.70	0.50
33	Ebanol 3	48.77	<0.01	1.73	0.59
34	Eugenol	42.82	<0.01	2.77	0.48
35	Eugenyl acetate	53.08	<0.01	3.23	0.41
36	Farnesol 1	62.65	<0.01	2.11	0.80
37	Farnesol 2	63.93	<0.01	2.16	0.61
38	gamma-Terpinene	23.10	<0.01	1.28	<0.01
39	Geraniol	35.93	<0.01	1.88	0.89
40	Geranyl acetate	44.33	<0.01	1.88	0.55
41	Geranial	37.10	<0.01	2.15	0.39
42	Hexadecalactone	74.78	<0.01	2.65	0.50
43	Hexyl cinammaldehyde	65.33	<0.01	2.78	0.37
44	Hydroxy citronellal	38.03	<0.01	2.23	0.46
45	ISO E SUPER 2	61.48	<0.01	2.33	0.81
46	ISO E SUPER 4	61.18	0.09	2.31	0.36
47	ISO E SUPER 7	60.78	<0.01	2.28	0.45
48	Isoeugenol	48.53	<0.01	3.07	0.50
49	Isoeugenyl acetate	58.10	<0.01	3.44	0.30
50	Lilial	53.20	<0.01	2.61	0.39
51	Limonene	21.23	<0.01	1.15	0.90
52	Linalool	25.67	<0.01	1.41	0.60
53	Linalyl acetate	36.05	<0.01	1.49	0.56
54	Majantol	46.32	<0.01	2.57	0.40
55	Menthol	30.68	<0.01	1.67	1.28
56	Methyl 2-octynate	32.55	<0.01	2.25	0.70
57	Methyl salicylate	32.20	<0.01	2.60	0.65
58	MUSK G 2	69.07	<0.01	2.55	0.84
59	MUSK G 3	68.83	<0.01	2.53	0.66
60	Neral	35.23	<0.01	2.08	0.40
61	Propyldene phthalide	56.00	<0.01	3.73	0.22
62	Salicylaldehyde	22.17	<0.01	2.57	0.95
63	Terpinen-4-ol	31.15	<0.01	1.81	0.57
64	Terpinolene	25.08	<0.01	1.36	<0.01
65	Vanillin	45.38	<0.01	4.15	0.48

3. *Mass accuracy*

Fig. 2 and Table 2 show the mass accuracy (deviation between measured and theoretical mass, values expressed in ppm) and its stability in time for the molecular ion of few allergens selected from the standard mixture. To ensure consistency we calculate the accuracy always at the blob apex. The experimental masses show very small deviations from the expected values, with errors mostly in the 1-2 ppm range and always <5.2 ppm. Additionally, stability in time shows good performance consistency.

Fig. 2
Molecular ion mass accuracy calculated at the blob apex for allergens at 7 ppm.

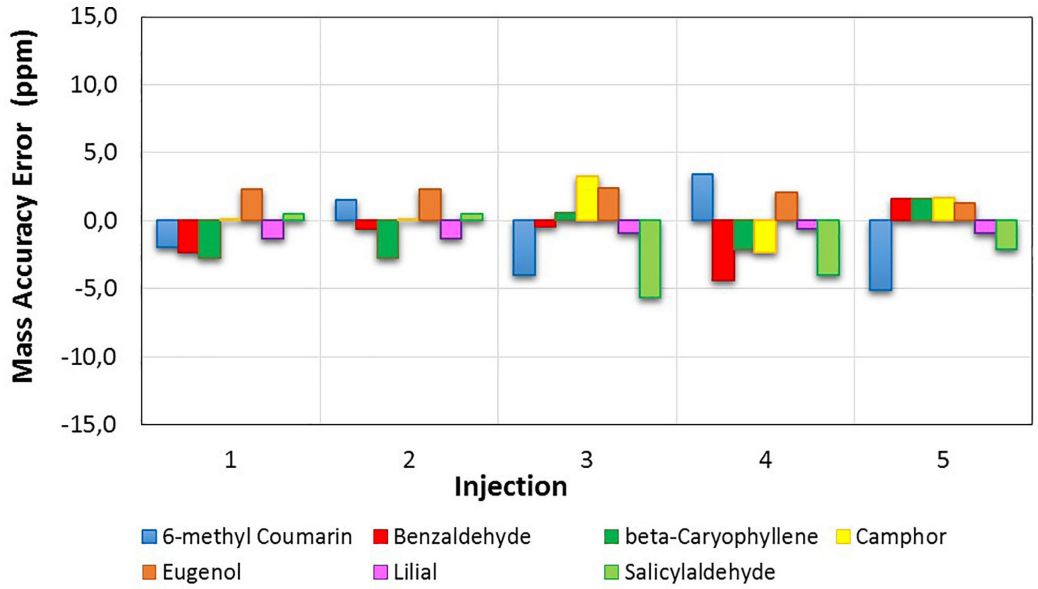


Table 2
Mass accuracy evaluation for selected test allergens at 7 ppm. Experimental mass and mass accuracy values are average of 5 repeated injections. Experimental masses refer to blobs' apex.

Compound name	Molecular fragment	RSD %	Average	RSD %
6-methyl Coumarin	$C_{10}H_8O_2^+$	160.0519	160.0517	3.2
Benzaldehyde	$C_7H_6O^+$	106.0413	106.0412	1.6
beta-Caryophyllene	$C_{15}H_{24}^+$	204.1873	204.1870	1.9
Camphor	$C_{10}H_{16}O^+$	152.1196	152.1197	1.5
Eugenol	$C_{10}H_{12}O_2^+$	164.0832	164.0829	2.1
Lilial	$C_{15}H_{20}O^+$	204.1509	204.1507	1.0
Salicylaldehyde	$C_7H_6O_2^+$	122.0362	122.0360	2.5

4. *Analysis of real-life perfume samples*

Examples of the 2D separations obtained for the real-life samples are shown in Fig. 3. We use a template built on the standard mixture 2D pattern to perform automated identification of the allergens in the perfumes based on retention times in the two-dimension and MS spectral similarity. Several allergens are found at various concentration levels in all samples (Examples in Table 3 and Fig. 4).

Beside the target compounds in the list, several other allergens are identified in the perfumes: BHT, Isoeugenol, Ethyl vanillin, Piperonal, 3-Carene, Ethylene brassylate etc. Accurate mass data can be used both for identity confirmation of targets and for more confident identification of unknowns (Table 4). Moreover, the very small mass windows possible allow improving sensitivity thanks to the better signal-to-noise ratios (Fig. 5).

Fig. 3
Examples of GC×GC- QTOF 2D chromatograms for real-life perfume samples.

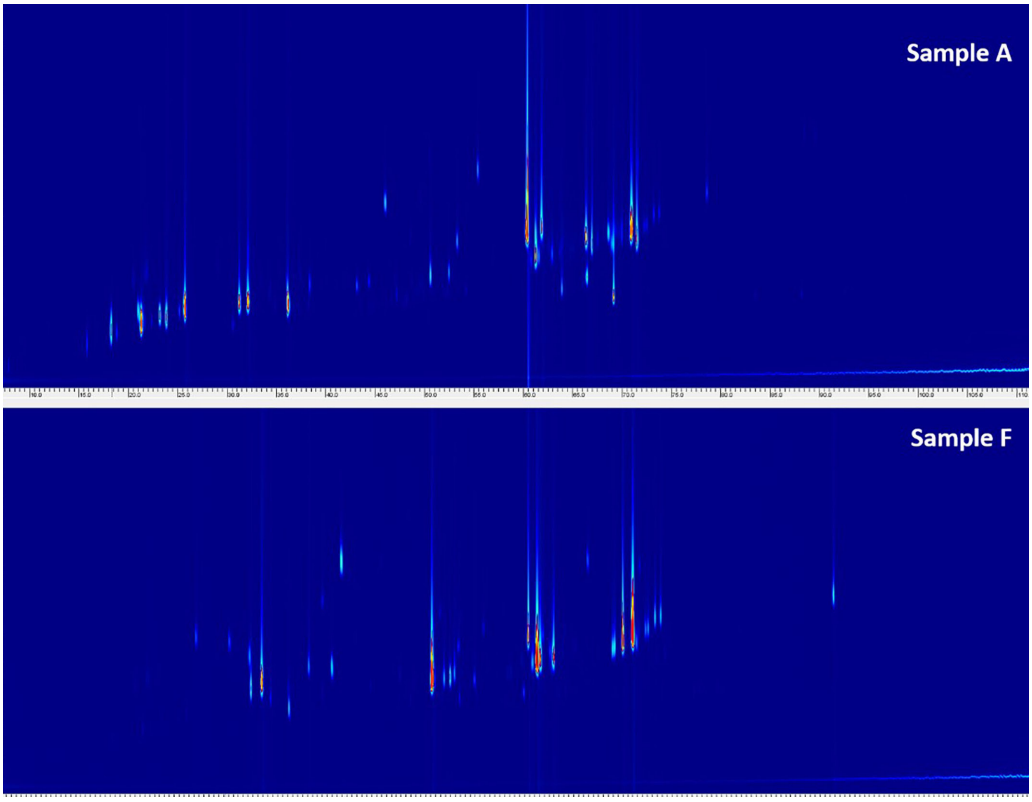


Table 3
Examples of target allergens identification in the perfume samples.

Compound name	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F
alpha-Pinene	+	+	+	-	-	+
Anethole	-	+	-	-	+	-
Benzaldehyde	-	-	-	-	-	-
Benzyl benzoate	-	-	-	-	-	+
beta-Pinene	+	+	+	-	-	+
Cinnamic alcohol	-	-	-	-	-	+
Citronellol	+	-	+	-	-	+
Limonene	+	+	+	+	+	+
Linalool	+	-	+	+	+	+
Menthol	-	+	-	-	-	-
Vanillin	-	+	-	-	+	+

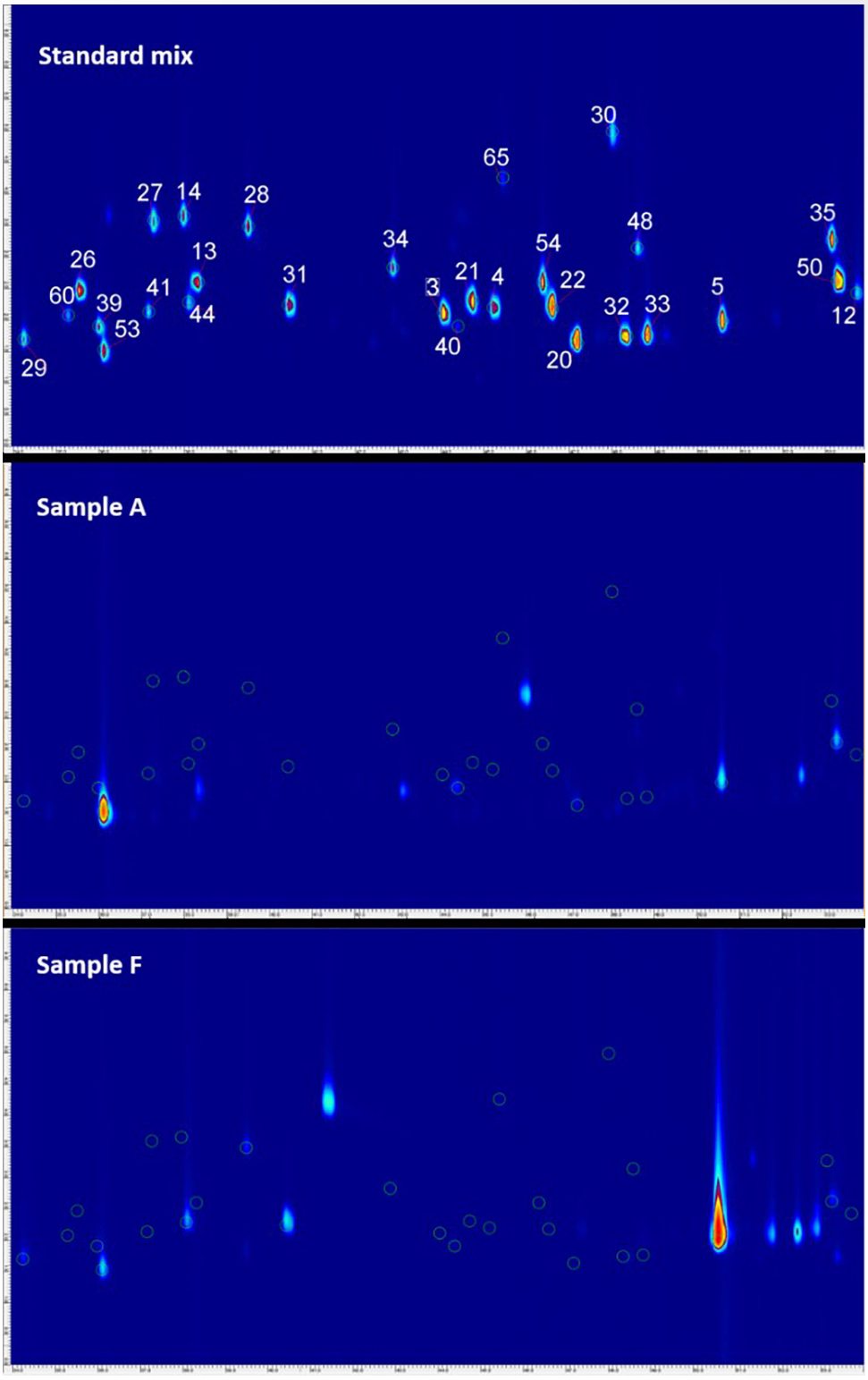
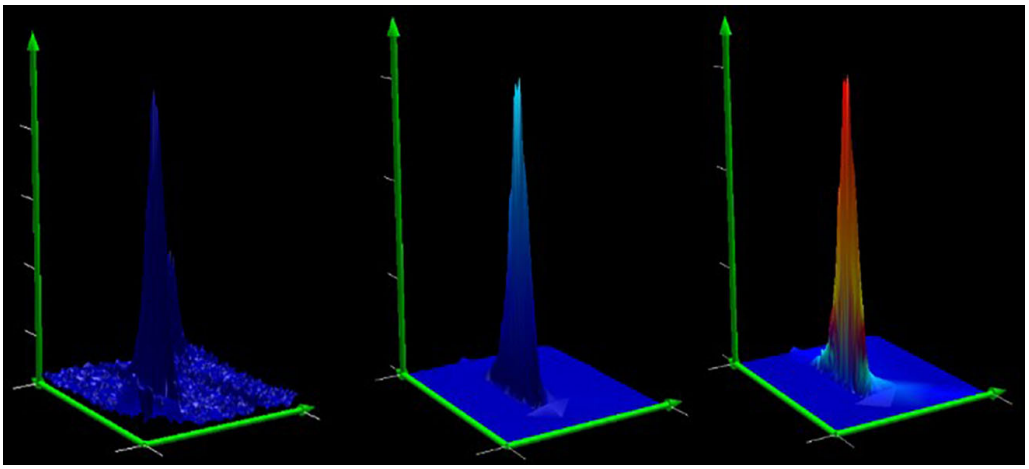


Fig. 4
TIC GC×GC chromatograms in the region 34-54 min for the standard mix at 150 ppm and two samples.

Table 4
Multi-fragment evaluation of mass accuracy in real-life matrix for target (Lilial) and non-target (BHT) allergens. Experimental masses refer to blob apex.

Compound	Measured mass (m/z)	Formula	Theoretical mass (m/z)	Mass difference	Mass accuracy (ppm)
Lilial	204.1500	$C_{14}H_{20}O^+$ (M^+)	204.1509	0.0009	4.1
	189.1274	$C_{13}H_{17}O^+$	189.1274	<0.0001	0.2
	131.0851	$C_{10}H_{11}^+$	131.0856	0.0005	3.3
	91.0540	$C_7H_7^+$	95.0542	0.0002	1.7
BHT	220.1829	$C_{15}H_{20}O^+$ (M^+)	220.1822	0.0007	3.3
	205.1596	$C_{14}H_{21}O^+$	205.1587	0.0009	4.2
	189.1267	$C_{13}H_{17}O^+$	189.1274	0.0007	3.4
	145.1013	$C_{11}H_{13}^+$	145.1012	0.0001	0.7

Fig. 5
3D view of Vanillin at 7 ppm in TIC (left) and in EIC for molecular ion in nominal mass 152±0.5 m/z (middle) and accurate mass 152.0465±0.005 m/z (right).



Accurate mass data can be used to make discrimination between compounds with similar MS spectrum, but different formula and thus accurate mass. An example is shown in Fig. 6. For the MS spectrum under exam the library search proposes as best match 1-butyhyl-1, 2-dihydro-pyridine, but mass accuracy does not support this identification. Ethyl linalool has a similar MS spectrum but a lower match factor. On the other hand, the accurate mass results clearly show that it structure is compatible with the unknown and is therefore a much more likely hit.

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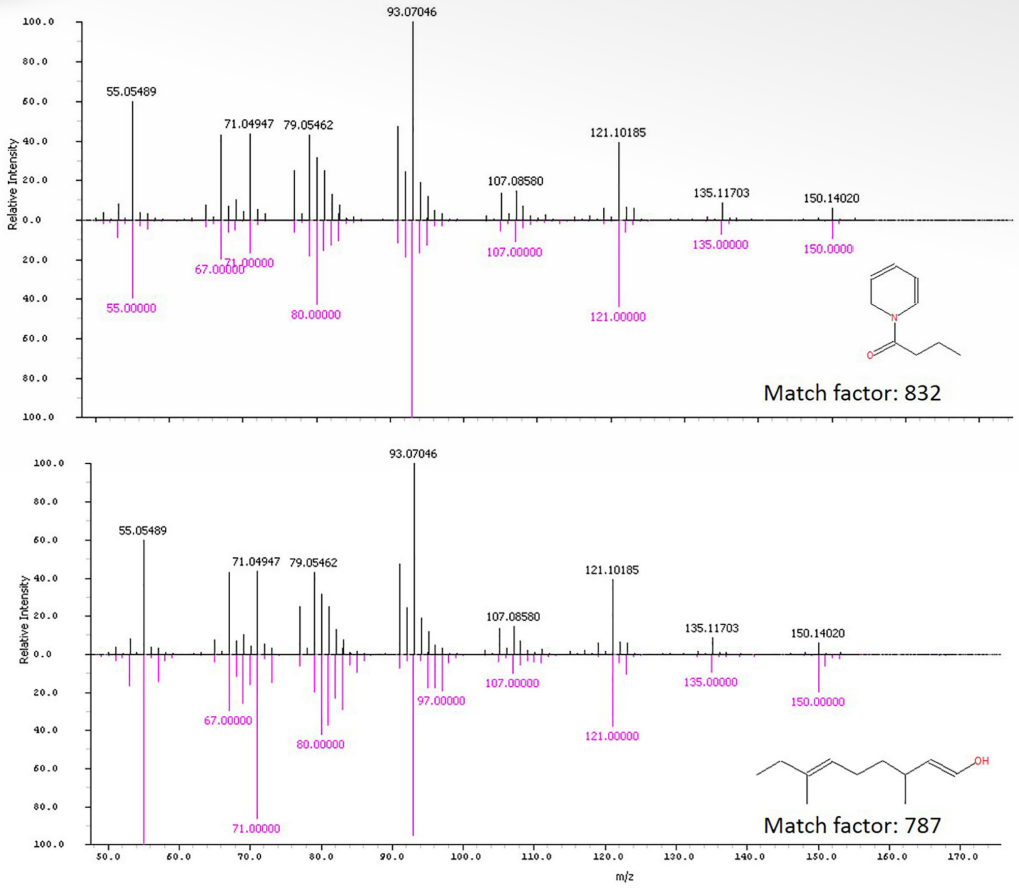

Fig. 6
Example of incorrect library search assignment and improved identification based on multi-fragment accurate mass evaluation.

Measured mass	1-butyl-1,2-dihydropyridine			ethyl linalool		
	Formula	Theoretical mass	Mass accuracy (ppm)	Formula	Theoretical mass	Mass accuracy (ppm)
150.1402	$C_9H_{12}NO^+$	150.0913	325.51	$C_{11}H_{18}^+$	150.1403	0.70
135.1170	$C_8H_9NO^+$	135.0679	364.00	$C_{10}H_{15}^+$	135.1168	1.50
121.1018	$C_7H_7NO^+$	121.0522	410.02	$C_9H_{13}^+$	121.1012	5.55
80.0622	$C_5H_6N^+$	80.0495	159.30	$C_6H_8^+$	80.0621	2.20
95.0852	$C_5H_5NO^+$	95.0366	512.15	$C_7H_{11}^+$	95.0855	3.03
71.0622	$C_4H_7O^+$	71.0491	4.62	$C_4H_7O^+$	71.0494	4.62
67.0546	$C_4H_5N^+$	67.4017	193.54	$C_5H_7^+$	57.0542	5.95

CONCLUSIONS

- GC×GC with thermal modulation provides high resolution power and allows for easier, detailed separation of allergens also in complex matrices.
- The excellent repeatability of the retention times in both dimensions supports that GC×GC with thermal modulation is robust and suitable for reliable identification of target compounds.
- The QTOF's high resolution and mass accuracy provide additional identification confidence, selectivity and sensitivity.
- Mass accuracy evaluation shows that accurate mass measurements are precise and consistent.
- GC×GC-QTOF is a powerful technique for reliable targeted analysis allergens as well as detailed characterization of unknowns in perfumes.

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