White Paper 013
April 2017

Tackling the extended list of fragrance allergens by flow-modulated GC×GC–TOF MS/FID

This preliminary study investigates the use of flow-modulated GC×GC to analyse an 84-component allergen calibration standard, with simultaneous detection by TOF MS for confident identification and FID for robust quantitation. A high degree of linearity and repeatability is demonstrated, showing that flow-modulated GC×GC is a promising approach for high-throughput quality control of fragranced products. The validity of the GC×GC–TOF MS/FID method is then demonstrated with the examples of essential oils and a fragrance mix.

Introduction

In 2003, an EU Directive[1] restricting the use of allergenic compounds in fragrances was released. The Directive named a total of 26 allergens, stating that they should be labelled if present at >100 ppm in 'wash-off' products (such as shower gels), or >10 ppm in 'leave-on' products (such as perfumes).

Compliance with this Directive therefore requires that these compounds are identified and quantified accurately, which is a considerable challenge due to the complex matrix and wide concentration ranges involved.[2]

Currently, this necessitates the use of different stationary phases to achieve the required chromatographic resolution for each target compound, making it a laborious process.[3] Given the proposal to expand the list of monitored allergens to over 80 individual compounds,[4] this process will only become more demanding.

To tackle this issue, the fragrance industry has turned to comprehensive two-dimensional GC coupled with time-of-flight mass spectrometry (GC×GC–TOF MS).[5] The enhanced separation capacity copes with the most complex of matrices, while the commercialisation of simple, consumable-free flow modulation devices has made routine use more feasible.

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This preliminary study evaluates the use of GC×GC with parallel detection by flame ionisation detection (FID) and TOF MS for an extended list of 84 allergens. This enables robust quantitation and confident identification in a single run, making it an ideal system for R&D labs requiring full sample characterisation. Moreover, once method optimisation and validation is complete, the excellent repeatability of flow modulation allows the method to be easily translated across GC×GC–FID systems in multiple quality-control laboratories.

Experimental

Sample preparation: A series of calibration standards ranging from 3.2–400 µg/mL were prepared for a mixture of 84 allergens (plus 1,4-dibromobenzene as an internal standard) in methyl *tert*-butyl ether (MTBE). The essential oils and the perfume mix were diluted in MTBE to 0.5% and 1.5%, respectively.

GC×GC: Injector: Split/splitless; Injection volume: 1.0 µL; Split 25:1. Flow modulator: INSIGHT™ (SepSolve Analytical). A splitter was used to direct the flow to the TOF MS and FID detectors in the ratio 1:4.

TOF MS: Instrument: BenchTOF-Select™ (Markes International).

Software: ChromSpace® GC×GC software (Markes International).

Please contact SepSolve for full analytical parameters.

Results and discussion

1. Separation and identification of allergens

The enhanced separation provided by GC×GC for the fragrance allergens mix (Figure 1) means that only five pairs of co-elutions remain (two of which are between geometric isomers) when using this preliminary method.

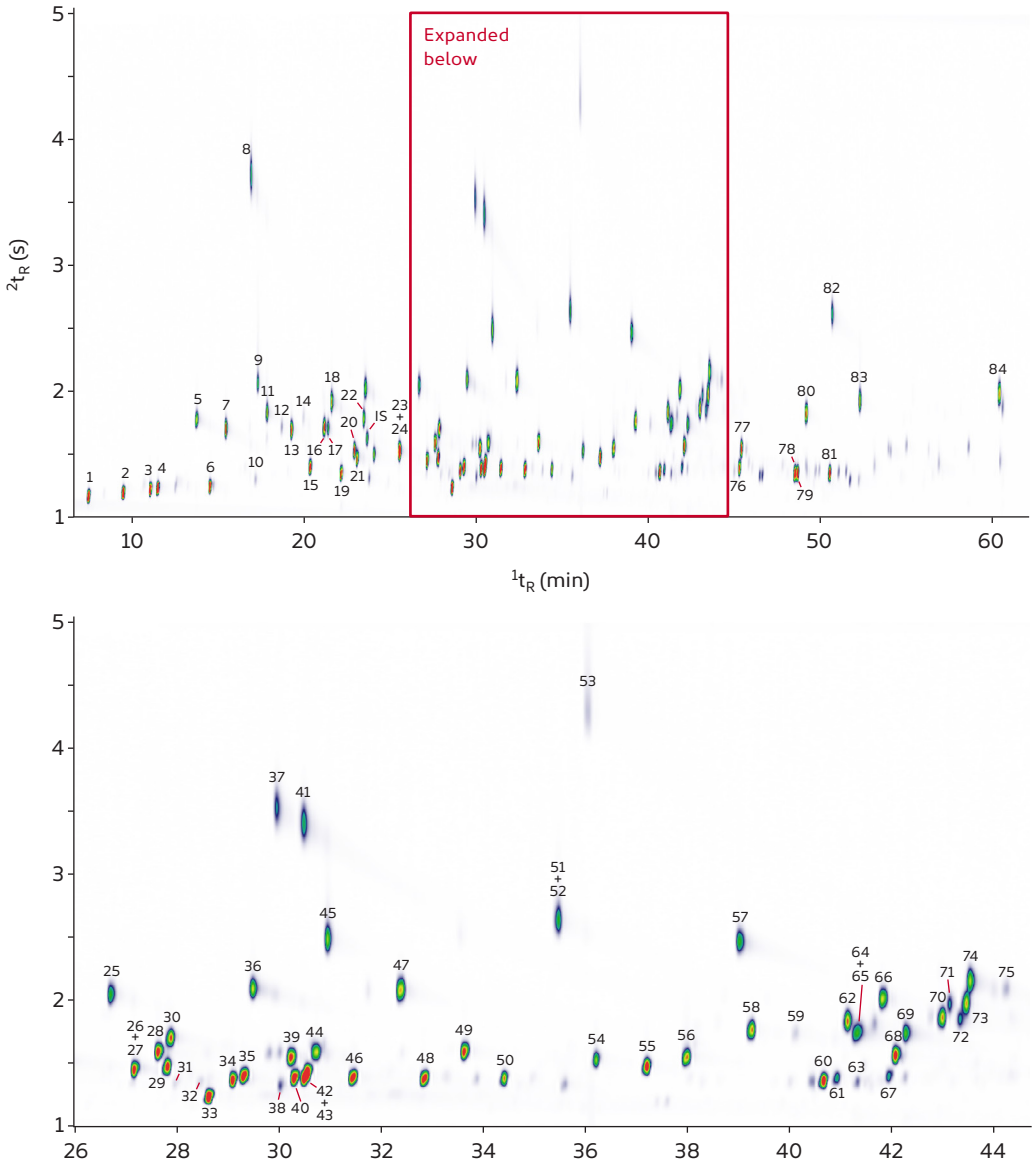

Figure 1

GC×GC colour plot showing the separation achieved for the standard mix of fragrance allergens. Peak identities are listed in Table 1. Note the occurrence of only four co-eluting pairs.

2. Repeatability

A major advantage of flow-modulated GC×GC over thermally-modulated systems (aside from the reduction in running costs) is the superior repeatability that can be achieved. Unlike thermal devices, where small variations in column position can have a large impact on results, the precisely-defined microfluidic design allows identical configurations to be installed across multiple instruments and easily used for large sample batches, making it ideal for routine use across multiple quality-control laboratories.

The repeatability of peak area and retention times in both dimensions for the GC×GC–FID analysis is provided in Table 1 for replicate analysis of a 10.4 µg/mL standard. The relative standard deviations (RSDs) are <5% for all peak areas, and are well below 1% for retention times.

No.	Compound name	^1t_R (min)	^2t_R (s)	RSD (%) (n = 5)			R^2
				Peak area	^1t_R	^2t_R	
1	α-Pinene	7.5345	1.1818	3.67	0.79	0.37	0.9956
2	β-Pinene	9.5336	1.2086	1.25	0.31	0.51	0.9981
3	α-Terpinene	11.0909	1.2286	1.26	0.27	0.22	0.9976
4	Limonene	11.5351	1.2435	0.28	0.26	0.50	0.9983
5	Benzaldehyde	13.8155	1.7851	1.62	0.26	0.24	0.9986
6	Terpinolene	14.5686	1.2484	4.18	<0.01	0.49	0.9989
7	Linalool	15.4839	1.7105	1.12	<0.01	0.38	0.9995
8	Benzyl alcohol	16.9548	3.7079	4.59	<0.01	0.28	0.9990
9	Salicylaldehyde	17.3496	2.0683	1.86	<0.01	0.37	0.9985
10	*cis*-β-Terpineol	17.8630	1.6522	4.02	<0.01	0.34	0.9992
11	Phenyl acetaldehyde	17.8827	1.8397	0.74	<0.01	0.19	0.9976
12	*trans*-β-Terpineol	18.7317	1.7247	1.61	<0.01	0.26	0.9985
13	Menthol	19.2944	1.7143	0.54	<0.01	0.36	0.9995
14	δ-Terpineol	19.9919	1.8075	3.02	0.15	0.50	0.9996
15	Camphor	20.3671	1.4141	0.37	0.15	0.65	0.9996
16	α-Terpineol	21.2026	1.7350	0.19	0.14	0.49	0.9995
17	γ-Terpineol	21.4135	1.7247	0.98	<0.01	0.34	0.9979
18	Citronellol	21.6307	1.9317	2.56	<0.01	0.34	0.9991
19	Linalyl acetate	22.1934	1.3623	0.53	<0.01	0.63	0.9988
20	Estragole	22.9327	1.5487	1.97	<0.01	0.43	0.9987
21	Methyl oct-2-ynoate	23.1114	1.4866	1.39	0.13	0.46	0.9993
22	Methyl salicylate	23.4964	1.7868	0.79	<0.01	0.37	0.9995
IS	1,4-Dibromobenzene	23.6939	1.6419	3.58	<0.01	0.46	–
23	Neral	25.5457	1.5150	0.98	<0.01	0.52	0.9956
24	Carvone	25.5700	1.5383				
25	Hydroxycitronellal	26.7117	2.0595	1.67	<0.01	0.45	0.9973
26	*trans,cis*-δ-Damascone	27.0967	1.4114	1.01	<0.01	0.53	0.9994
27	Methyl non-2-ynoate	27.1658	1.4574				
28	*trans*-Anethole	27.6495	1.5912	0.70	<0.01	0.50	0.9994
29	1,1-Dimethyl-2-phenethyl acetate (DMBCA)	27.8074	1.4741	0.69	0.13	0.61	0.9993
30	Safrole	27.8667	1.6957	0.59	0.13	0.34	0.9996
31	*cis*-Isodamascone	27.9851	1.3528	3.09	<0.01	0.70	0.9961
32	*cis*-α-Damascone	28.4590	1,.357	3.96	0.13	0.67	0.9982
33	β-Caryophyllene	28.6367	1.2441	0.27	<0.01	0.84	0.9990
34	Geranyl acetate	29.0908	1.3695	0.74	<0.01	0.76	0.9991
35	*trans,trans*-δ-Damascone	29.3080	1.4072	0.43	0.10	0.69	0.9993
36	Cinnamic aldehyde	29.4813	2.1077	1.22	<0.01	0.39	0.9996
37	Anisyl alcohol	29.9552	3.5467	4.42	0.30	0.37	0.9951
38	*cis*-β-Damascone	30.0341	1.3313	1.15	<0.01	0.17	0.9970
39	Ebanol (isomer 1)	30.2316	1.5590	0.39	<0.01	0.37	0.9995
40	*trans*-Isodamascone + *cis,trans*-δ-Damascone	30.2908	1.4038	0.63	<0.01	0.70	0.9987
41	Cinnamic alcohol	30.4685	3.4432	3.97	0.12	0.34	0.9994
42	Damascenone	30.4981	1.4245	0.63	<0.01	0.70	0.9987
43	*trans*-α-Damascone	30.5573	1.4348				
44	Ebanol (isomer 2)	30.7054	1.5901	0.49	0.10	0.22	0.9988
45	Eugenol	30.9522	2.5218	1.73	0.10	0.31	0.9995
46	*trans*-β-Damascone	31.4557	1.4038	0.92	0.09	0.65	0.9987
47	Majantol	32.3935	2.0870	0.41	0.09	0.28	0.9994
48	α-Isomethylionone	32.8279	1.3830	0.16	0.09	0.76	0.9994

Table 1

List of allergens in the 84-component standard, with repeatability and linearity results obtained using GC×GC–FID. The co-eluting pairs are shaded in grey, with repeatability/linearity data displayed for the summed responses.
(Continued on next page)

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No.	Compound name	^1t_R (min)	^2t_R (s)	RSD (%) (n = 5)			R^2
				Peak area	^1t_R	^2t_R	
49	Methyl eugenol	33.6472	1.6108	0.83	0.00	0.62	0.9991
50	Butylated hydroxy toluene	34.4074	1.3727	0.25	0.09	0.58	0.9964
51	*cis*-Isoeugenol	35.4735	2.6668	1.00	0.10	0.22	0.9964
52	*trans*-Isoeugenol	35.4735	2.6668				
53	Vanillin	36.0383	4.3426	2.94	0.18	0.44	0.9969
54	Isoamyl salicylate	36.2160	1.5460	0.93	0.08	0.38	0.9994
55	Lilial	37.2130	1.4791	0.62	0.10	0.62	0.9986
56	n-Pentyl salicylate	37.9830	1.5682	0.70	0.08	0.55	0.9996
57	Coumarin	39.0295	2.4930	0.72	<0.01	0.33	0.9995
58	Eugenyl acetate	39.2664	1.7799	0.62	0.08	0.45	0.9994
59	*cis,cis*-Farnesol	40.1351	1.7465	1.76	0.09	0.16	0.9953
60	Isocyclemone E (Iso super E) (isomer 1)	40.6682	1.3788	2.35	0.07	0.58	0.9996
61	Isocyclemone E (Iso super E) (isomer 2)	40.9347	1.3900	0.57	0.09	0.61	0.9994
62	α-Santalol	41.4120	1.8579	1.02	0.07	0.31	0.9979
63	Isocyclemone E (Iso super E) (isomer 3)	41.3395	1.3788	3.11	0.09	0.72	0.9994
64	*trans,cis*-Farnesol	41.3493	1.7799	1.50	0.07	0.29	0.9985
65	*cis,trans*-Farnesol	41.3493	1.7799				
66	3-Propylidene phthalide (minor isomer)	41.8331	2.0362	0.48	0.07	0.35	0.9996
67	Isocyclemone E (Iso super E) (isomer 4)	41.9515	1.4123	0.82	<0.01	0.70	0.9997
68	α-Amylcinnamaldehyde	42.0799	1.5905	0.45	0.07	0.42	0.9993
69	*trans,trans*-Farnesol	42.2872	1.7465	2.96	0.07	0.38	0.9952
70	Isoeugenyl acetate	42.9979	1.8914	2.08	0.08	0.27	0.9994
71	Lyral (isomer 1)	43.1559	1.9916	2.92	0.07	0.29	0.9957
72	β-Santalol	43.3533	1.8691	3.02	0.07	0.23	0.9953
73	Lyral (isomer 2)	43.4718	1.9916	2.57	0.08	0.23	0.9964
74	α-Amylcinnamyl alcohol (*cis*/*trans*)	43.5508	2.1811	1.19	0.07	0.21	0.9966
75	3-Propylidene phthalide (major isomer)	44.2445	2.1253	2.23	0.08	0.13	0.9981
76	Acetyl cedrene	45.2811	1.4011	0.51	<0.01	0.71	0.9997
77	Hexyl cinnamaldehyde	45.3995	1.5794	1.11	0.07	0.49	0.9992
78	Galaxolide (isomer 1)	48.5137	1.3632	2.18	0.06	0.73	0.9997
79	Galaxolide (isomer 2)	48.6632	1.3677	1.69	0.07	0.70	0.9984
80	Benzyl benzoate	49.1550	1.8421	0.24	<0.01	0.41	0.9994
81	Hexadecanolide	50.5292	1.3715	0.75	0.07	0.57	0.9992
82	7-Methoxycoumarin	50.6745	2.6490	0.95	0.06	0.36	0.9995
83	Benzyl salicylate	52.2737	1.9359	0.69	0.06	0.23	0.9993
84	Benzyl cinnamate	60.3649	2.0251	0.61	<0.01	0.49	0.9995

Table 1

List of allergens in the 84-component standard, with repeatability and linearity results obtained using GC×GC–FID. The co-eluting pairs are shaded in grey, with repeatability/linearity data displayed for the summed isomers.
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3. Validation of peak purity

The benefit of parallel detection by TOF MS and FID is that the TOF MS data can be used to confirm peak identity and purity. This is an important factor when optimising and validating methods. Figures 2 and 3 demonstrate excellent retention time correspondence between the FID and MS datasets, enabling simplified data processing.

Despite the enhanced separation offered by GC×GC, a number of co-elutions were detected in this preliminary method. Nevertheless, the excellent spectral quality of the BenchTOF detector used, combined with the powerful deconvolution algorithm of ChromSpace, provide successful identification of co-eluting targets, as seen in Figure 4.

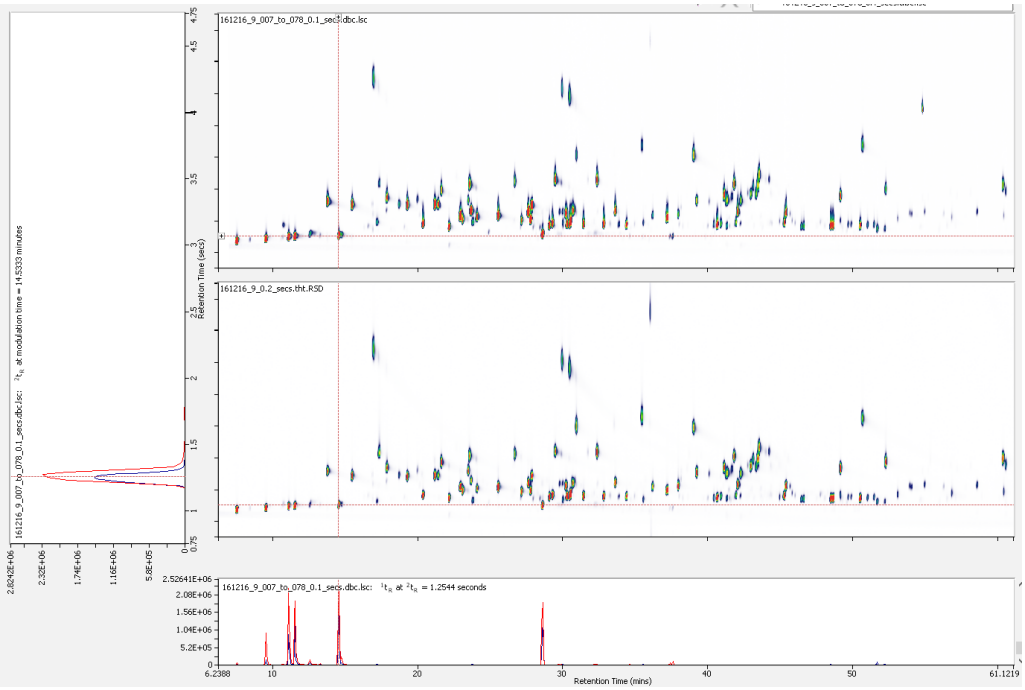

Figure 2

ChromSpace display for the allergen standard. The side panels represent the $^{1}t_{R}$ and $^{2}t_{R}$ projections for TOF MS (red) and FID (blue).

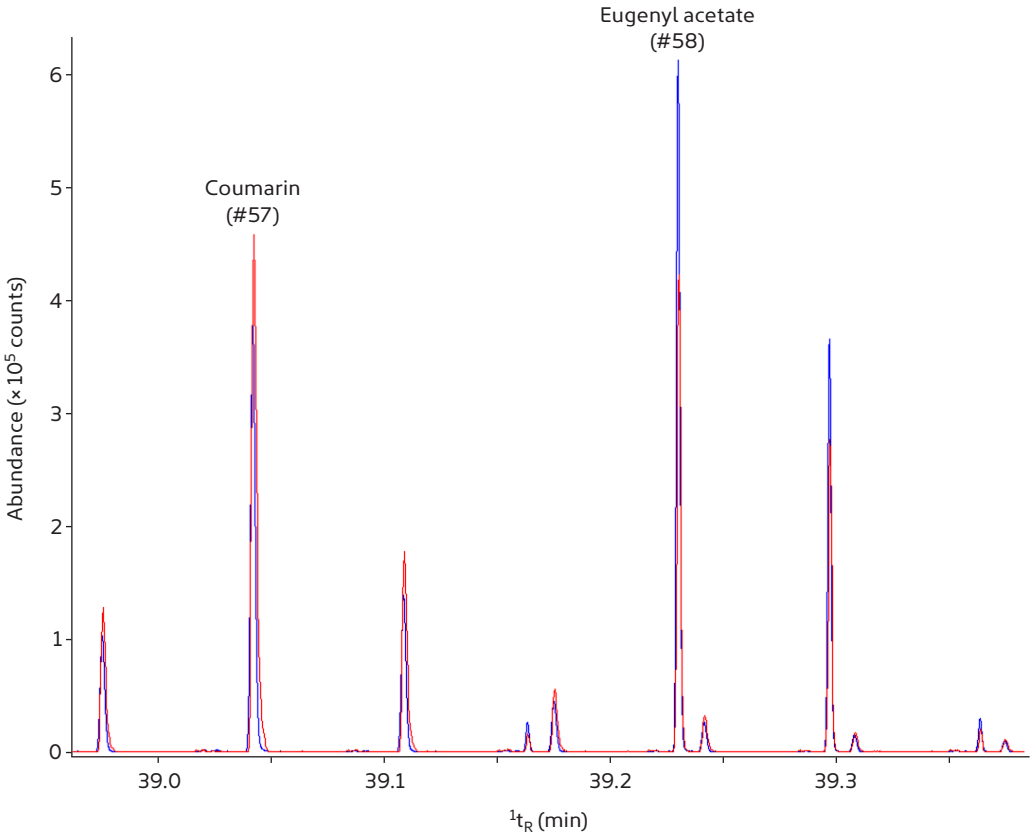

Figure 3

Expansion of the $^{1}t_{R}$ axis of Figure 2, showing the excellent retention time correspondence between TOF MS (red) and FID (blue) datasets for two peaks in the allergen standard.

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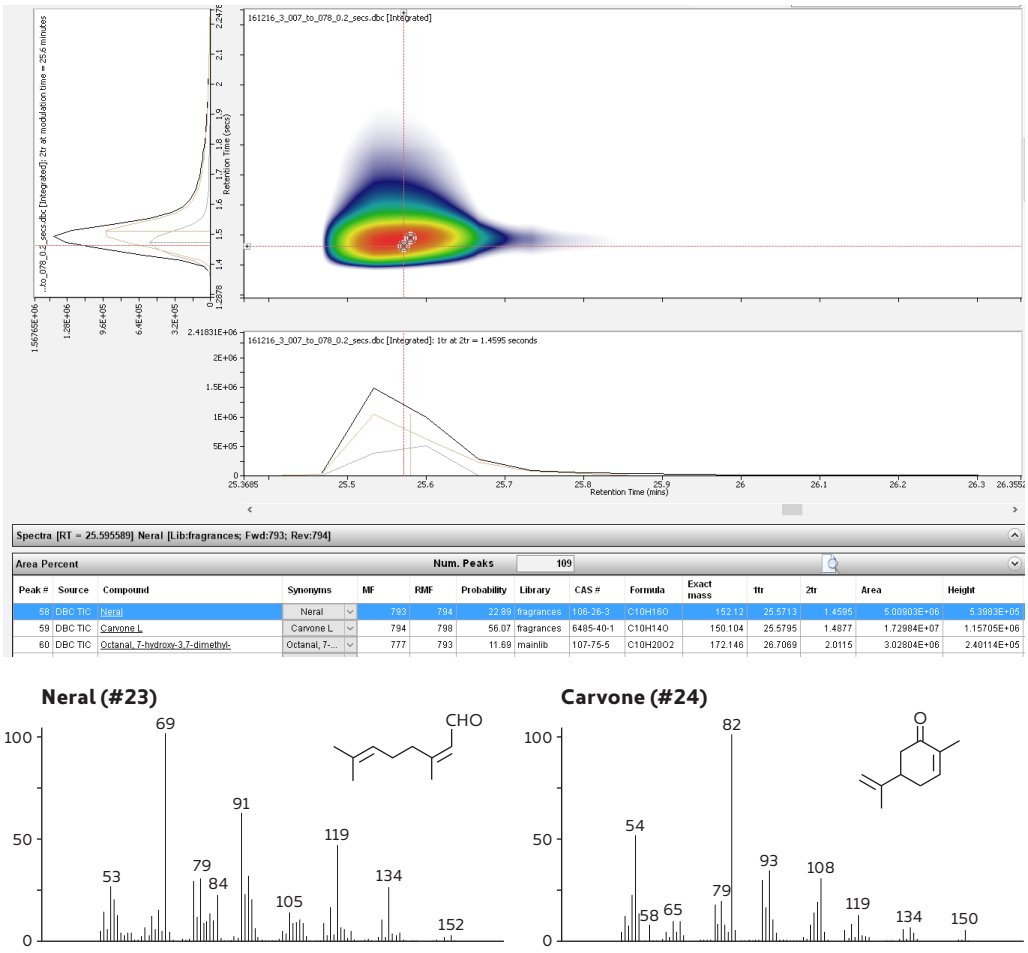


Figure 4

Deconvolution of two co-eluting species in the GC×GC–TOF MS colour plot of the allergen standard using ChromSpace.

4. Linearity

FID is the 'gold standard' for quantitative GC×GC applications in the fragrance industry for many reasons, including wide linear range, fast data capture and mass-dependent response.

The calibration standards were analysed in triplicate at five concentration levels (from 3.2–400 µg/mL), and Table 1 summarises the quantitation results. The R^2 values ranged from 0.9951 to 0.9997, with an average of 0.9985, indicating strong linearity. Figure 5 shows example calibration curves for seven allergens across the analytical run.

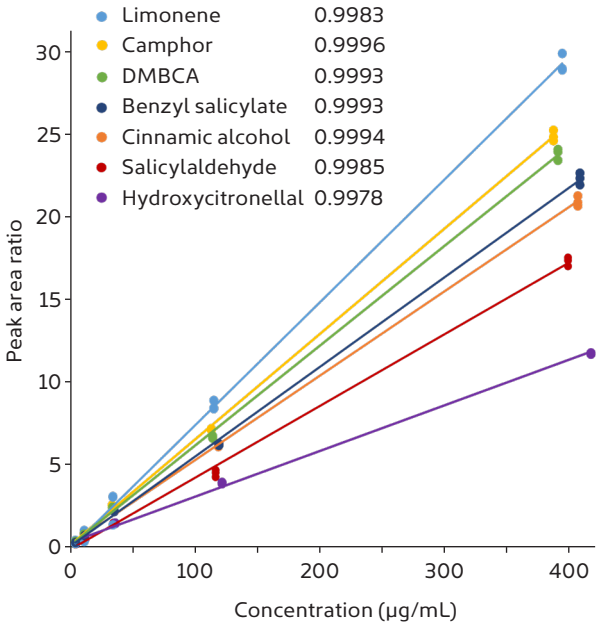


Figure 5

Example GC×GC–FID calibration curves (ranging from 3.2–400 µg/mL) for selected allergens in the standard.

Typically, much of the time associated with analysis of allergens is devoted to data processing and review of results. To reduce this time burden, reviewing sub-peaks in ChromSpace is simplified with the peak slice explorer, which enables users to easily browse the target compound list and verify peak-merging (Figure 6).

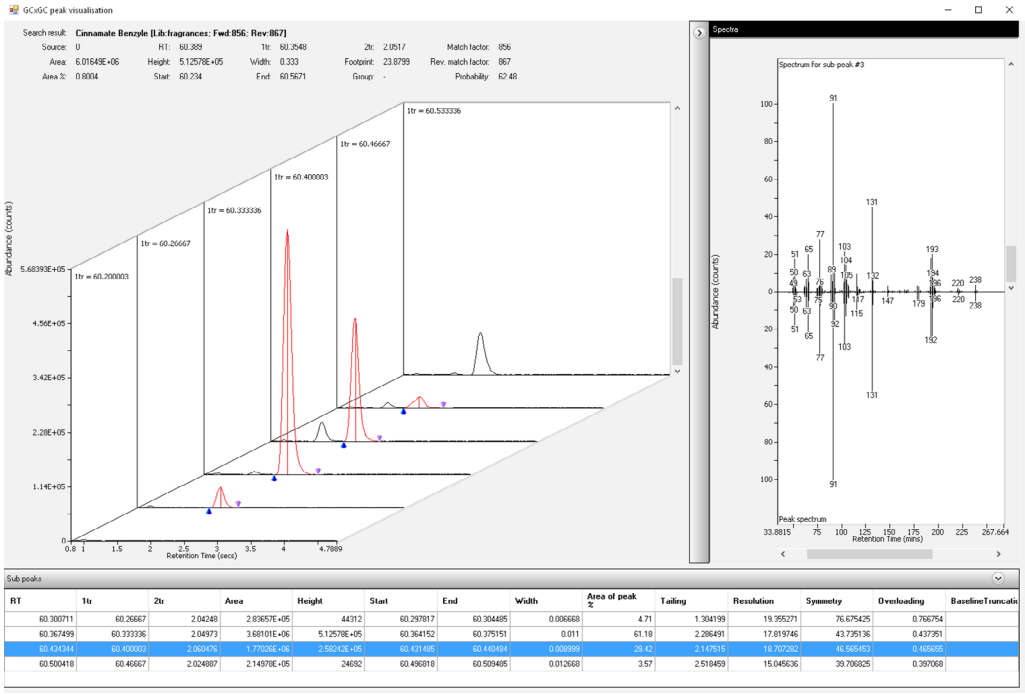


Figure 6

The peak slice explorer in ChromSpace, for simple review of peak merging.

5. Analysis of real-world fragrance samples

Using the GC×GC–FID calibration curves for the 84-component allergen standard, allergen levels were quantified in five essential oils and one perfume mix (Table 2).

The results show that the vetiver oil is allergen-free, while all other analysed samples contained numerous allergens on the extended list. As expected, high levels of limonene were found in lime oil. Surprisingly, however, patchouli oil (which under the current directive is generally considered to be allergen-free) also contained a small amount of limonene, as well as three compounds on the extended list.

Another point worth mentioning is that the proposal to expand the list of monitored allergens actually suggests a complete ban for three components.[4] One of these, Lyral (#71/#73), was identified in the perfume mix.

Complementing the FID results, the TOF MS data was used to carry out a non-target screen, as shown in the examples of ylang oil, lavender oil and the perfume mix in Figure 7. This shows that over a third of the identified components in lavender oil are on the extended list of allergens, with linalool and linalyl acetate the most dominant peaks. In ylang oil, linalool was also prevalent,

No.	Compound name	Concentration (mg/mL)					
		Lime	Ylang	Patchouli	Lavender	Vetiver	Perfume mix
1	α-Pinene	64.70	5.22	6.41	4.29	–	0.98
2	β-Pinene	125.91	0.51	4.80	0.75	–	0.38
3	α-Terpinene	1.87	–	–	–	–	–
4	Limonene	545.44	–	0.64	3.81	–	1.91
6	Terpinolene	5.69	–	–	1.48	–	–
7	Linalool	1.20	112.92	–	322.06	–	1.97
15	Camphor	–	–	–	5.29	–	0.05
16	α-Terpineol	2.46	0.31	–	17.70	–	–
19	Linalyl acetate	–	–	–	528.57	–	2.30
28	*trans*-Anethole	–	0.53	–	–	–	–
33	β-Caryophyllene	4.65	66.83	46.97	34.90	–	0.13
34	Geranyl acetate	–	37.86	–	13.95	–	–
51	*cis*-Isoeugenol	–	0.08	–	–	–	–
57	Coumarin	–	–	–	1.88	–	0.64
60	Isocyclemone E (Iso super E) (isomer 1)	–	–	–	–	–	0.38
61	Isocyclemone E (Iso super E) (isomer 2)	–	–	–	–	–	6.41
63	Isocyclemone E (Iso super E) (isomer 3)	–	–	–	–	–	1.94
67	Isocyclemone E (Iso super E) (isomer 4)	–	–	–	–	–	1.14
69	*trans,trans*-Farnesol	–	7.90	–	–	–	–
71	Lyral (isomer 1)	–	–	–	–	–	0.38
78	Galaxolide (isomer 1)	–	–	–	–	–	3.77
79	Galaxolide (isomer 2)	–	–	–	–	–	2.31
80	Benzyl benzoate	–	73.23	–	0.99	–	0.36
82	7-Methoxycoumarin	2.29	–	–	–	–	–
83	Benzyl salicylate	–	33.42	–	–	–	–

Table 2

Levels of allergens from the extended list in five undiluted essential oils and one perfume mix, quantified using GC×GC–FID.

alongside aromatics, including methyl benzoate, benzyl acetate and the later-eluting benzyl salicylate and benzyl benzoate. Unsurprisingly, the perfume mix contained a wide range of synthetic compounds, including Galaxolide, Vertofix, Verdox and Lyral.

This form of non-targeted screening is important for both R&D and for screening raw materials, as many essential oils have high market value and are often subject to adulteration to increase volume (and thus economic profit).[6]

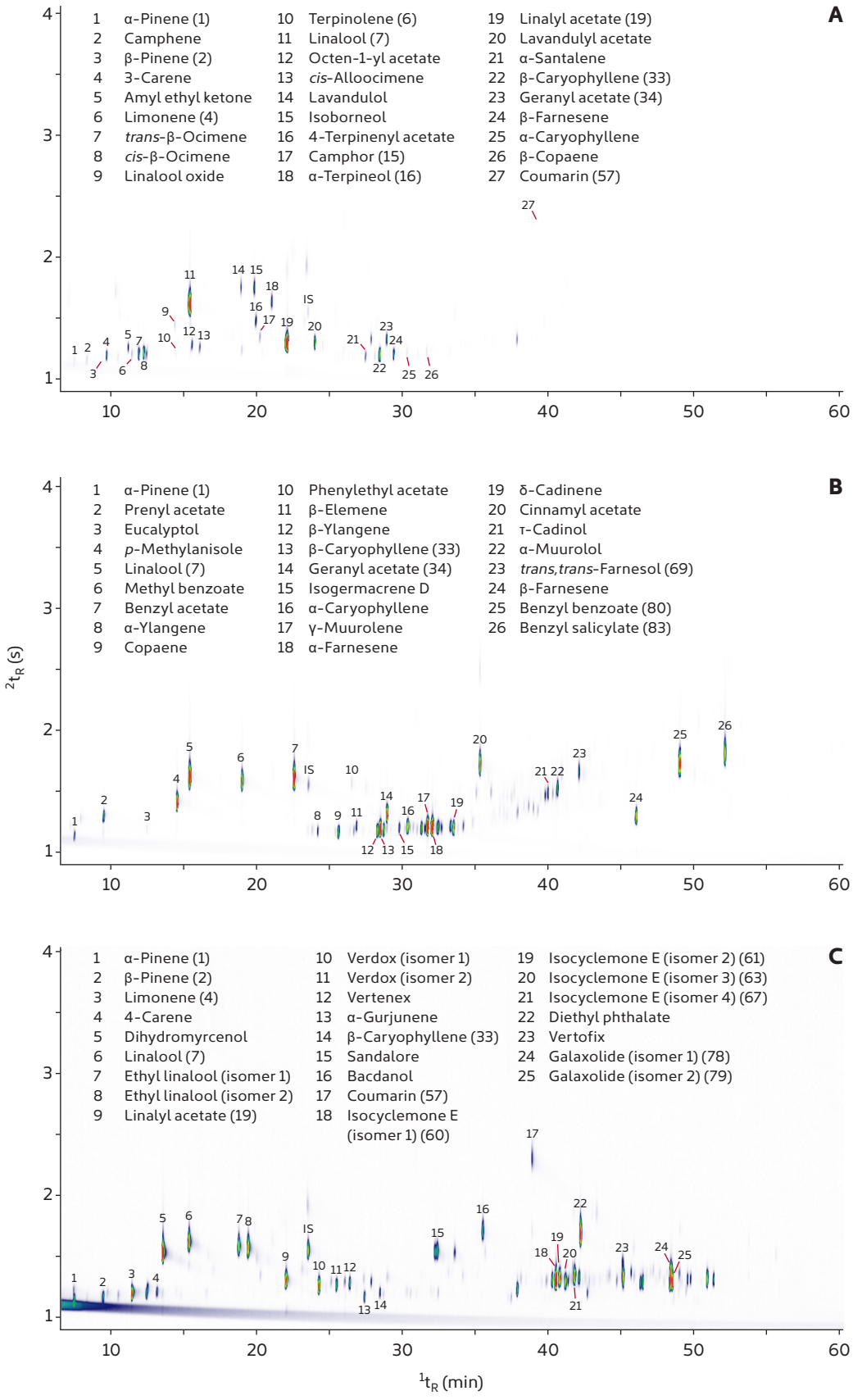


Figure 7

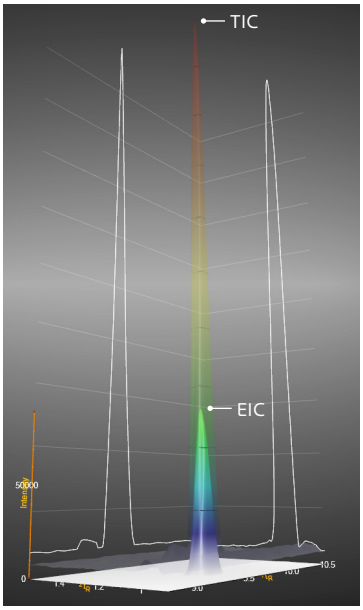
Key components identified in a non-targeted screen of (**A**) lavender and (**B**) ylang essential oils, and in (**C**) a perfume mix, by GC×GC–TOF MS. Compounds present in the allergens list are indicated with numbers in parentheses.

6. Detection limits

This preliminary study focused on a calibration range from 3.2–400 µg/mL. However, due to the split ratio (25:1) and the parallel-detection splitter (to TOF MS and FID in the ratio 1:4) used, the amount of each component directed to the TOF MS in the lowest calibration sample was generally in the range 20–30 pg.

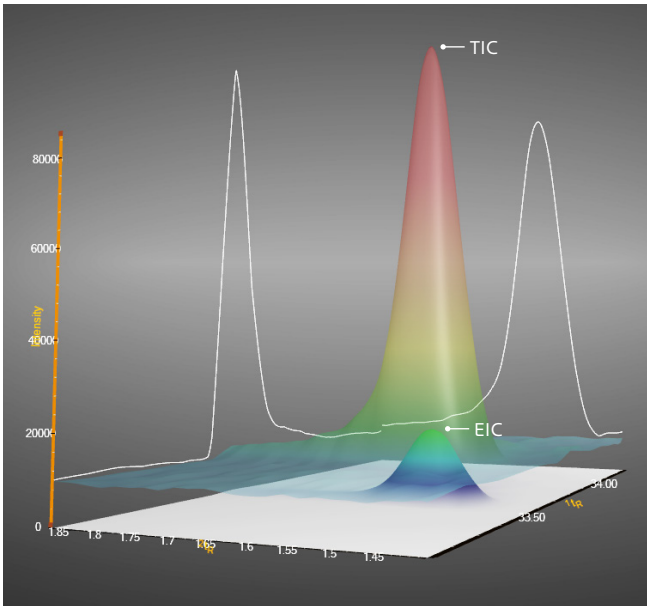
Figure 8 demonstrates that this is not even close to the detection limit of the BenchTOF detector. Based on theoretical detection limits, we can clearly see from the S/N ratios of the extracted-ion chromatograms (EICs) of two selected allergens that these compounds would be comfortably detected at a concentration an order of magnitude lower.

A β-Pinene



RMS S/N (EIC m/z 93) = 5200
RMS S/N (TIC) = 448

B Methyl eugenol



RMS S/N (EIC m/z 178) = 2295
RMS S/N (TIC) = 197

Figure 8

Signal-to-noise (S/N) ratios for the TOF MS TIC (part-transparent overlay) and EIC peaks of (**A**) 31.3 pg β-pinene and (**B**) 32.6 pg methyl eugenol on-column.

Conclusions

In this preliminary study, we have shown that flow-modulated GC×GC can provide enhanced separation and confident quantitation of fragrance allergens in a single run, without the inconvenience or running costs associated with thermal modulation.

Moreover, parallel detection by FID and TOF MS provides both robust quantitation and confident identification of fragrance allergens in a single run, making it an ideal system for R&D laboratories requiring full sample characterisation. As shown in this work, the retention-time correspondence between the parallel-detection FID and TOF MS datasets enables simple

validation of measured peaks, while the excellent repeatability enables fast and confident processing of data from large sample batches.

In addition, once method optimisation and validation is complete, the excellent repeatability of flow modulation allows the method to be easily translated across multiple GC×GC–FID systems for robust and affordable analysis in quality control laboratories.

For more information on this application, or any of the techniques or products used, please contact SepSolve.

References

[1] Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.

[2] A. Rey, E. Corbi, C. Pérès and N. David, Determination of suspected fragrance allergens extended list by two-dimensional gas chromatography-mass spectrometry in ready-to-inject samples, *Journal of Chromatography A*, 2015, 1404: 95–103, http://dx.doi.org/10.1016/j.chroma.2015.05.045.

[3] A. Chaintreau, D. Joulain, C. Marin, C.-O. Schmidt and M. Vey, GC-MS quantitation of fragrance compounds suspected to cause skin reactions. 1, *Journal of Agricultural and Food Chemistry*, 2003, 51: 6398–6403, http://dx.doi.org/10.1021/jf030363t.

[4] Opinion on fragrance allergens in cosmetic products (SCCS/1459/11), European Commission Scientific Committee on Consumer Safety, June 2012, http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_102.pdf.

[5] The value of GC×GC–TOF MS to a leading fragrance manufacturer is summarised in the following article: N. Owen, Mass spectrometry and Givaudan – A positive working relationship, *Mass Matters*, December 2016, pp. 14–15.

[6] K.E. Boren, D.G. Young, C.L. Woolley, B.L. Smith and R.E. Carlson, Detecting essential oil adulteration, *Journal of Environmental Analytical Chemistry*, 2015, 2: 132, http://dx.doi.org/10.4172/2380-2391.1000132.

D0019_3_130418

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