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Comparison of aroma compounds in whisky by SPME–GC×GC–TOF MS/FID

This study demonstrates the high performance of a flow-modulated GC×GC–TOF MS system, coupled with sample preparation by solid-phase micro-extraction (SPME), for the separation and identification of trace volatiles in three brands of whisky.

Introduction

Over 1000 compounds from a wide range of chemical classes are known to contribute to the aroma of whisky, and these include alcohols, phenolics, fatty acids, esters, lactones, aldehydes and nitrogen-containing compounds.[1] It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is ideal for the analysis of such complex samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute.[2]

The key component in the GC×GC system is the modulator – the device that samples and re-injects the first-column effluent on to the second column in narrow bands to ensure that the first-dimension separation is retained and that the short second-dimension column does not become overloaded. Thermal modulation is the most commonly used technique, but this often requires expensive liquid cryogen and may struggle to modulate whisky volatiles that have boiling points similar to, or lower than, pentane.[3]

This study investigates the application of flow-modulated GC×GC–TOF MS using the INSIGHT™ reverse-fill/flush device from SepSolve, which allows separation of volatiles ranging from C_1 to C_{40} (and above), the flexibility to change the loop volume in method optimisation, and additional options including heart-cutting, backflushing, and splitting for simultaneous detection.

The latter feature was employed here to allow simultaneous detection by TOF MS and FID. Simultaneous detection is exceptionally useful for complex aroma samples, as FID has the dynamic range necessary to tackle such diverse concentrations, while TOF MS provides confident identification.

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Experimental

A schematic of the analytical system is shown in Figure 1. Note that both TOF MS and FID datasets were collected, but for the purposes of this study, we will focus on the TOF MS data.

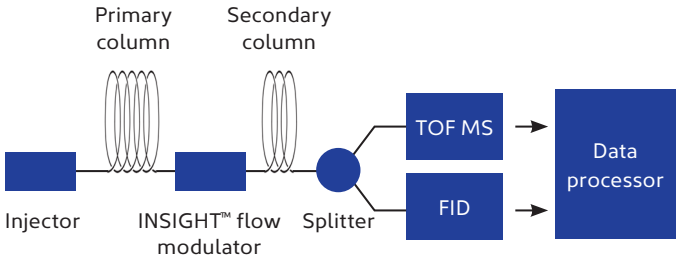

Figure 1
Schematic of the GC×GC–TOF MS/FID analytical system.

Samples: Three commercial whisky samples were studied: Sample A – an Islay single malt; Sample B – a Speyside single malt; Sample C – a blended Irish whiskey. All samples were adjusted to exactly 40% (v/v) alcohol prior to extraction. 1 mL of whisky was placed in a 20 mL headspace vial, diluted 3:1 with water and salt (0.15 g/mL) added. Samples were incubated at 35°C with agitation at 250 rpm for 5 min prior to SPME sampling.

SPME: Headspace sampling was performed by using a 2 cm 50/30 µm DVB/Car/PDMS StableFlex™ SPME fiber (Sigma-Aldrich) at 35 °C for 10 min.

GC×GC: Injector: Split/splitless; Liner: Straight, 7.5 mm (i.d.); Carrier gas: Helium, constant-flow at 0.47 mL/min; Mode: Split 5:1; Temperature: 250°C; Septum purge: On, 3 mL/min. Modulator: INSIGHT flow modulator (SepSolve Analytical). 2D column set: 1st dimension: VF-35™ (Agilent Technologies), 20 m × 0.15 mm × 0.15 µm; 2nd dimension: SolGel-WAX™ (SGE Analytical Science), 5 m × 0.25 mm × 0.25 µm. Temperature program: Main oven: 35°C (2 min), 4.8°C/min to 240°C (hold 15 min). Loop volume: 25 µL; Fill time: 3900 ms; Flush time: 100 ms; P_M: 4.0 s.

TOF MS: Instrument: BenchTOF-HD™ (Markes International); Filament voltage: 1.8 V; Ion source: 350°C; Transfer line: 300°C; Mass range: m/z 40–350; Data rate: 50 Hz.

FID: H_2 flow: 30 mL/min; Air flow: 300 mL/min; Data rate: 200 Hz.

Software: Instrument control and GC×GC data processing was carried out using TOF-DS™ with ChromSpace®.

Results and discussion

The SPME–GC×GC–TOF MS colour plots for the three whisky headspace samples are shown in Figure 2. Each sample contained high levels of 3-methylbutan-1-ol (isoamyl alcohol) and a range of esters (annotated). 3-Methylbutan-1-ol (#2) is a yeast metabolism by-product that is one of the main 'fusel alcohols' found in whisky, and (like the esters) provides a fruity aroma.[4]

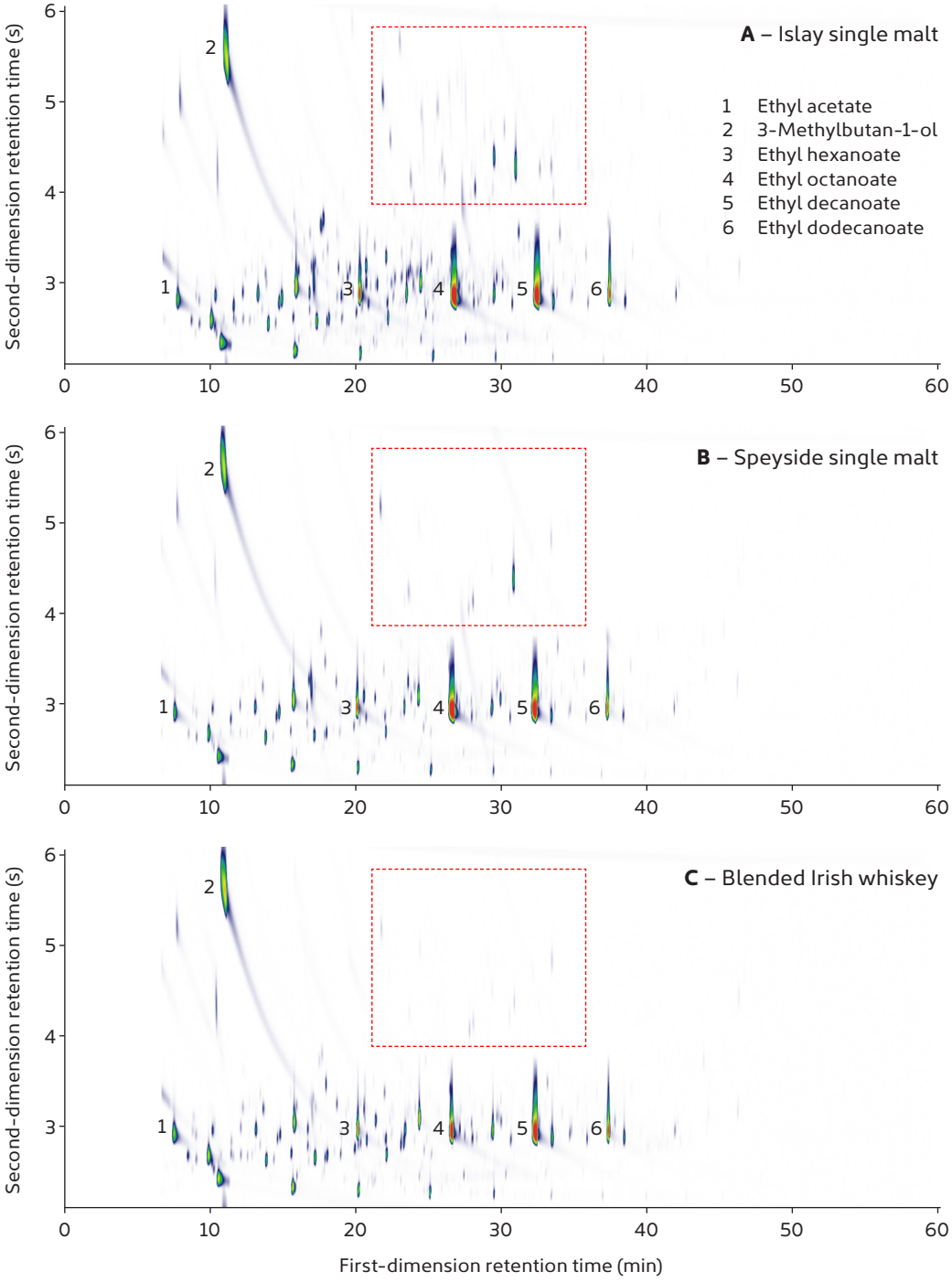

Figure 2

SPME–GC×GC–TOF MS colour plots of the three whisky samples with highest-loading components annotated. The boxes indicate the areas expanded in Figure 3.

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Based on the full-scale colour plots in Figure 2, the compositions of the three whisky samples appear very similar, but when the boxed areas are enlarged (Figure 3) a large number of differences can be seen. Table 1 lists selected aroma compounds detected across the whisky samples.

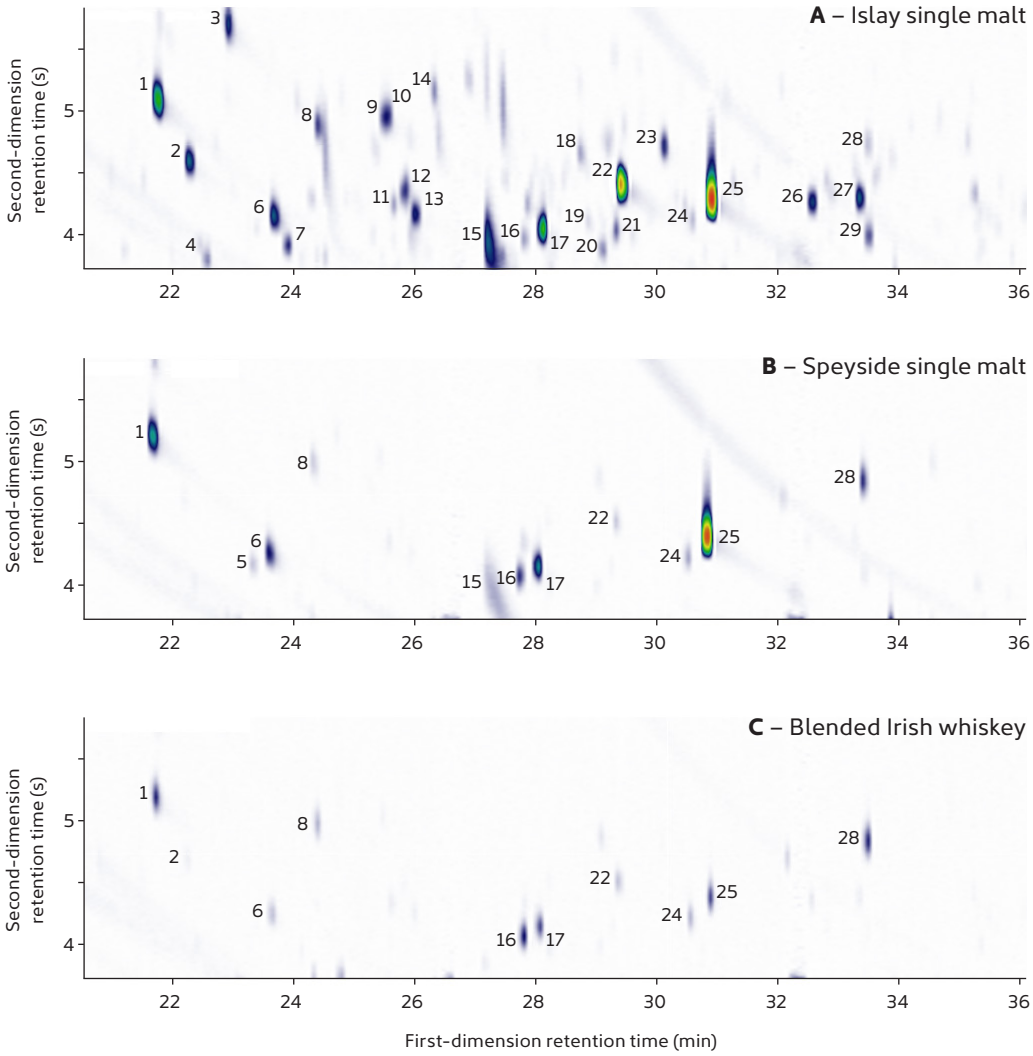

Figure 3

Expansion of the boxed regions of Figure 2, showing clear differences in composition between the three brands of whisky.

1	Benzaldehyde	11	Methylbenzofuran (isomer 1)	21	Dimethylbenzofuran (isomer 2)
2	Benzofuran	12	Methylbenzofuran (isomer 2)	22	Naphthalene
3	Benzonitrile	13	Methylbenzofuran (isomer 3)	23	Benzo[b]thiophene
4	*p*-Methylanisole	14	3-Methylbenzonitrile	24	Ethyl phenylacetate
5	1,3-Dichlorobenzene	15	Phenethyl alcohol	25	Phenethyl acetate
6	β-Pinene	16	Ethyl succinate	26	2-Methylnaphthalene
7	Indene	17	Ethyl benzoate	27	1-Methylnaphthalene
8	Ethyl 2-furoate	18	*m*-Methylacetophenone	28	Whiskey lactone
9	Acetophenone	19	Ethylbenzofuran	29	Ethyl hydrocinnamate
10	4-Methylbenzonitrile	20	Dimethylbenzofuran (isomer 1)		

Compound	^1t_R (min)	^2t_R (s)	Peak area			Reported aroma
			A	B	C	
Aliphatic esters						
Ethyl acetate	7.7717	2.8081	1.69×10^7	1.17×10^7	1.88×10^7	Fruity, solvent[4]
3-Methylbutyl acetate	15.8439	2.9566	3.41×10^7	2.50×10^7	1.65×10^7	Pear, banana[6]
Hexyl acetate	20.6399	2.9969	2.44×10^6	3.54×10^6	3.51×10^4	Green, fruity[7]
Ethyl hexanoate	20.2112	2.8785	5.92×10^7	4.08×10^7	2.58×10^7	Apple, aniseed[4]
Ethyl octanoate	26.6988	2.8510	2.42×10^8	2.08×10^8	9.59×10^7	Apple-like[4]
Ethyl decanoate	32.3729	2.8535	2.18×10^8	1.79×10^8	1.71×10^8	Sweet, fruity, waxy[4]
Ethyl dodecanoate	37.3733	2.8775	5.91×107	4.16×10^7	4.81×10^7	Sweetish, fruity, apple[4]
Aromatic esters						
Ethyl benzoate	28.1859	0.0446	2.15×10^6	9.89×10^5	3.07×10^5	Sweet, medicinal[7]
Methyl salicylate	29.1867	0.7511	4.66×10^4	3.77×10^4	4.71×10^4	Root beer, wintergreen[6]
Ethyl phenylacetate	30.6529	0.1164	1.39×10^5	1.66×10^5	1.15×10^5	Sweet, honey, cocoa-like[7]
Ethyl dihydrocinnamate	33.5455	3.9925	2.37×10^5	4.38×10^4	–	Spicy, fruity[7]
Phenethyl acetate	35.8633	3.8334	2.14×10^4	–	–	Roses, honey[4]
Aldehydes						
Furfural	17.0398	3.0336	8.44×10^6	7.59×10^6	1.55×10^6	Bready, nutty, caramel[7]
Benzaldehyde	21.8577	1.0755	3.07×10^6	1.78×10^6	4.78×10^5	Sweet, almond[7]
Oct-2-enal	35.4748	2.6046	–	–	4.88×10^4	Green, herbal[7]
Ketones						
Heptane-2,3-dione	16.7569	2.7017	7.05×10^5	7.96×10^5	9.02×10^5	Butterscotch[7]
Acetophenone	25.4638	0.8970	1.00×10^6	–	–	Sweet, almond[7]
Alcohols, lactones and ethers						
Anisole	19.0679	3.9563	1.39×10^5	–	–	Anise, ethereal[6]
p-Methylanisole	22.5846	3.8005	2.73×10^5	–	–	Naphthyl, phenolic, woody[7]
Phenethyl alcohol	27.2311	3.9231	2.12×10^6	1.18×10^6	–	Rose, floral[7]
γ-Octalactone	33.5595	0.7211	1.45×10^5	–	4.42×10^5	Coconut[7]
Phenols						
o-Cresol	24.5531	0.5263	4.03×10^5	–	–	Musty, medicinal[5]
2,6-Xylenol	26.3961	0.7906	9.07×10^4	–	–	Sweet, medicinal[5]
4-Ethylphenol	27.1968	1.2274	1.55×10^5	–	–	Smoky[7]
2,4-Xylenol	27.4687	1.0224	4.06×10^5	–	–	Sweet, medicinal[5]
4-Ethylguaiacol	31.5608	2.4057	3.03×10^5	–	–	Smoky, meaty[5]
Nitrogen-containing heterocycles						
2-Methylpyrazine	16.2813	0.0856	1.05×10^5	–	–	Nutty, roasted[7]
2-Ethylpyrazine	19.3344	3.7639	1.06×10^5	–	–	Nutty[7]

Table 1

A selection of aroma compounds identified in the three whisky samples A–C.

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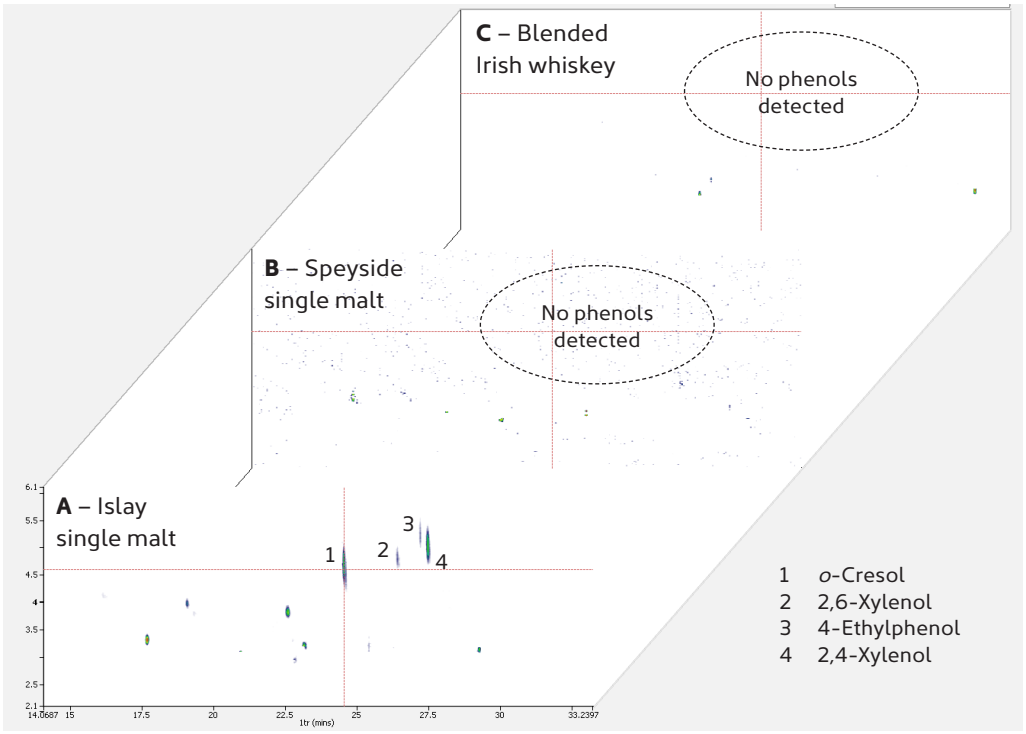
Sample A, the Islay single malt, has a strong peaty aroma that is in accordance with the compounds detected – for example, only this sample contained a range of phenols.[5] Using a simple scripting function within ChromSpace software, the phenols are highlighted in Figure 4. Such scripting expressions speed up data processing by allowing target compounds (or classes) to be readily grouped.



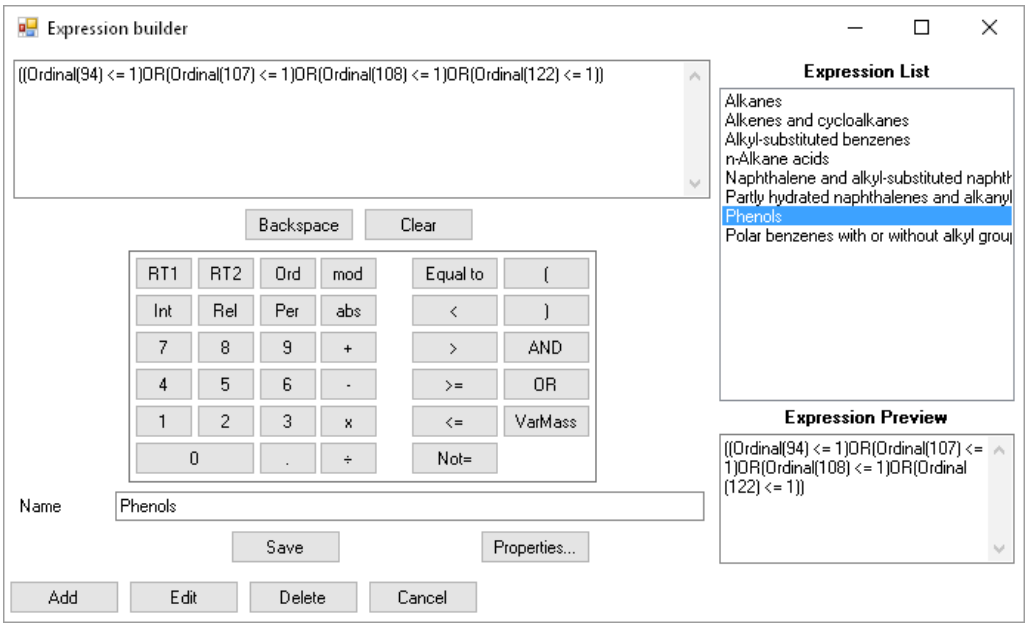
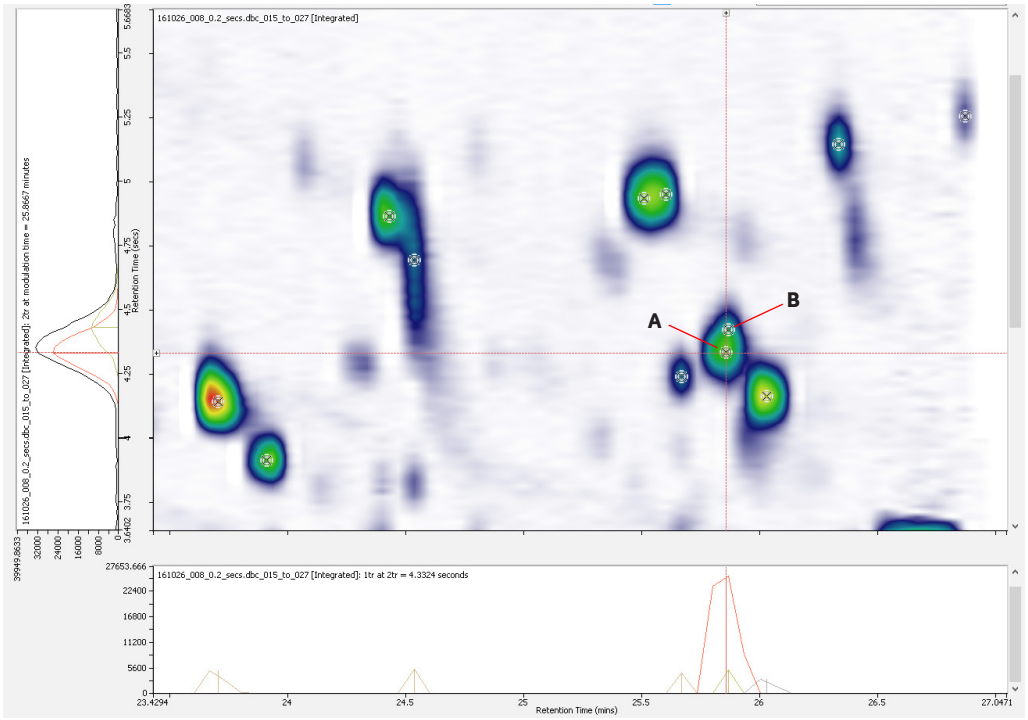


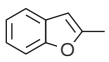
Figure 4

Identification of phenols in sample A using a simple scripting expression in ChromSpace (top), as shown in the expression builder (bottom).

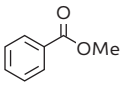
Deconvolution is imperative when food and beverages are concerned, as important flavour or aroma compounds may be masked by higher-loading peaks.[8] In the example shown in Figure 5, two co-eluting species are successfully deconvolved and confidently identified. Methyl benzoate has a 'cherry/phenolic' aroma while 2-methylbenzofuran is described as 'burnt/phenolic'. Such distinctions are extremely important in research & development within the food industry, since even minor changes in chemical composition can greatly affect the perceived aroma.



A: 2-Methylbenzofuran
Forward match 893
Reverse match 903



B: Methyl benzoate
Forward match 920
Reverse match 920



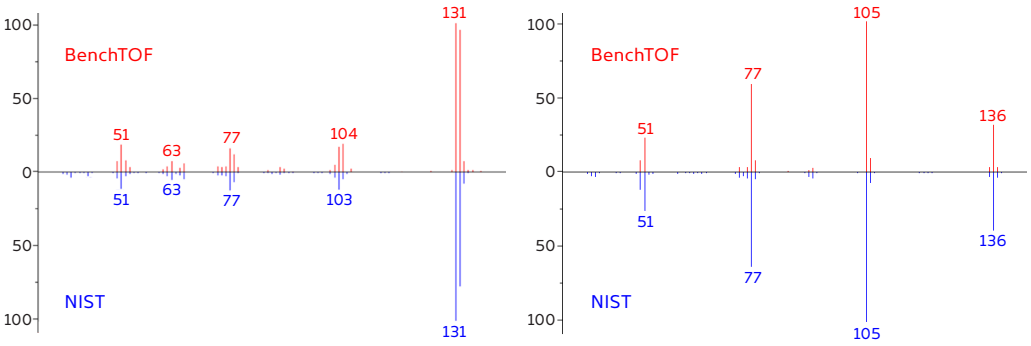


Figure 5

Expansion of whisky sample A, showing deconvolution applied in ChromSpace software. Two co-eluting species, (**A**) 2-methylbenzofuran and (**B**) methyl benzoate, were confidently identified using the NIST 14 library, as shown in the comparisons of BenchTOF spectra (top, red) and NIST 14 spectra (bottom, blue).

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Conclusions

This study has illustrated the power of flow-modulated GC×GC to provide simple, robust and affordable separation of complex aroma profiles. Confident identification through reference-quality TOF MS spectra enables comprehensive characterisation of samples, while ChromSpace software is shown to further enhance analyses, by speeding up data navigation and providing another level of information through global deconvolution.

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

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