SepSolve
Analytical

Enhanced separation of hop oils by flow-modulated GC×GC–TOF MS

This study demonstrates the high performance of a flow-modulated GC×GC–MS system for the evaluation of complex hop oils, which allows effective modulation of even the most volatile components without the need for expensive liquid cryogen. Analyte speciation is also aided by Tandem Ionisation for simultaneous acquisition of hard and soft EI data.

Introduction

Beer contains hundreds of organic ingredients, with concentrations spanning many orders of magnitude, and including monoterpenes (C_{10}) and sesquiterpenes (C_{15}). These aroma-active hydrocarbons are found in the essential oils of various plants, including hops, and provide much of the characteristic 'bitterness' of the finished beer.

Of the greatest importance for beer is the monoterpene β-myrcene, and the sesquiterpenes caryophyllene, β-farnesene and α-humulene. However, there are hundreds of other terpenes that may also be present and have an impact on the final aroma and flavour – and a number of factors can affect levels of these compounds, including seasonal variations, packaging, storage and ageing. This makes robust quality control essential (for an example of the rapid headspace analysis of hop cones using TD–GC–MS, see our separate white paper).

Brewing not only uses hops directly, but can also use the extracted essential oils. These hop oils also have uses in alternative medicine, such as the treatment of anxiety, insomnia and other sleep disorders, making it even more important that their content is fully characterised.

Comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC–MS) has become the technique of choice for the separation of complex oils. The enhanced separation capacity offered by the coupling of two columns of different selectivity, combined with highly sensitive mass spectral identification, provides a high-performance approach to sample characterisation.

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 can make it difficult to achieve precise replication of results across multiple instruments. An alternative approach is flow modulation, which avoids the inconvenience and expense of liquid cryogen, and offers much better between-sample and between-instrument repeatability.

This study investigates the application of flow-modulated GC×GC–MS to enhance the separation of hop oils. The modulator used is the INSIGHT® 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, splitting for simultaneous detection and backflushing.

In addition, we examine the benefit offered by the use of Tandem Ionisation® [1] technology, which simultaneously acquires both conventional 70 eV spectra for library matching *and* low-eV spectra for added confidence in analyte identity.

Experimental

Sample preparation: 10% (v/v) dilutions of two hop oils were prepared in hexane.

GC×GC: Injector: Split/splitless; Liner: Single taper with wool, 4 mm (i.d.); Carrier gas: Helium, constant-flow at 0.6 mL/min; Injection volume: 0.5 µL; Split: 100 : 1; Temperature: 280°C; Septum purge: On, 1 mL/min. 2D column set: 1st dimension: BPX5™, 20 m × 0.18 mm × 0.18 µm; 2nd dimension: DB-1701™, 2 m × 0.25 mm × 0.15 µm. Temperature program: Main oven: 40°C (1 min), 3°C/min to 260°C (10 min). Modulator: INSIGHT® flow modulator (SepSolve Analytical); Loop dimensions: 0.53 mm i.d. × 230 mm (loop volume: 50 µL); Fill time: 3600 ms; Flush time: 200 ms; P_M: 3.8 s.

TOF MS: Instrument: BenchTOF-Select™ (Markes International); Filament voltage: 1.7 V; Ion source: 300°C; Transfer line: 280°C; Mass range: m/z 40–300; Data rate: 50 Hz; Tandem Ionisation®: Simultaneous acquisition of 70 eV and 12 eV data.

Results and discussion

Flow-modulated GC×GC–TOF MS

The chromatograms resulting from flow-modulated GC×GC–MS of two hop oils are shown in Figure 1. As is common in GC×GC, the identification process is simplified because different chemical classes elute together in bands (the 'roof-tiling' effect).
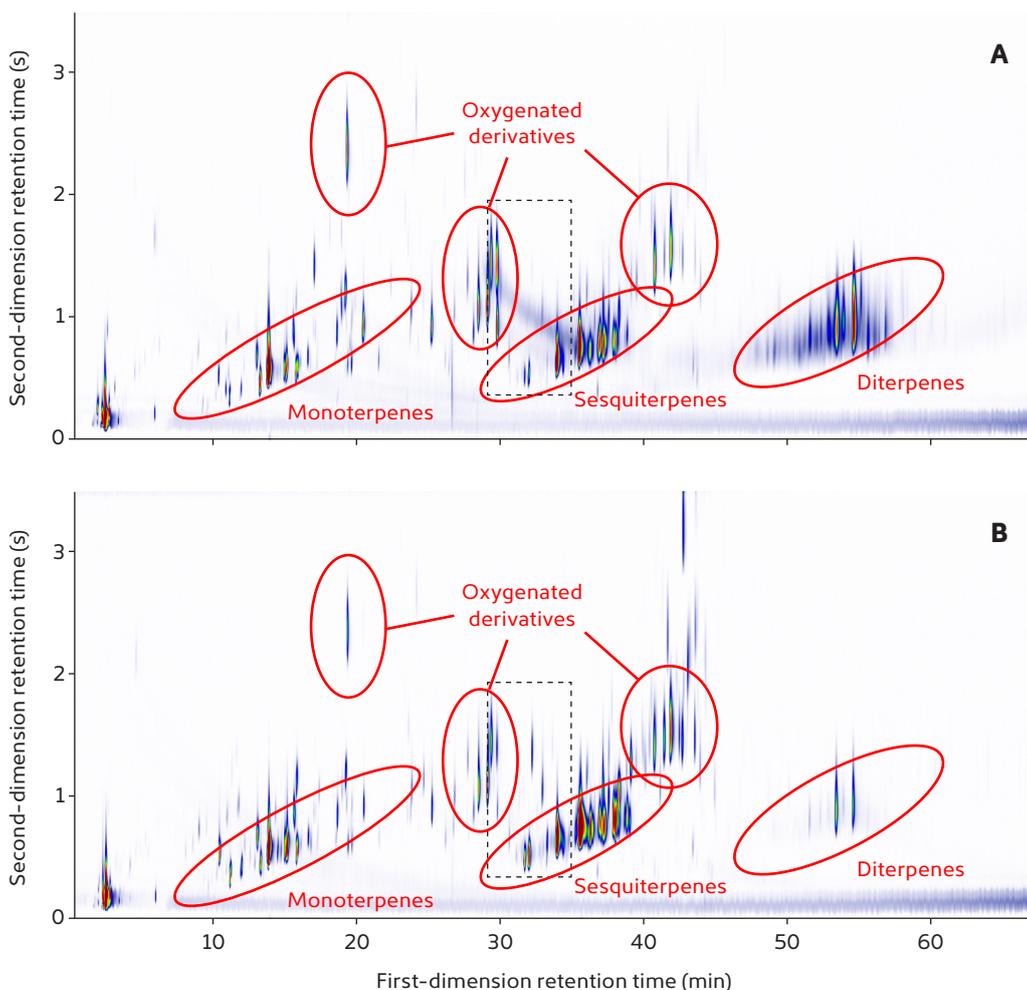
Figure 1

GC×GC–MS colour plots for two hop oils, showing the structured ordering and separation of chemical classes. The dashed boxes indicate the regions expanded in Figure 2.

Compositional differences were easily observed in the GC×GC–MS colour plots, with a number of compounds found to be present in only one sample. For example, Figure 2 shows major differences in the abundance of four compounds between hop oils A and B. The use of flow-modulated GC×GC–MS allows these components to be chromatographically resolved and confidently identified (Figure 3), with minimal running costs compared to thermally modulated systems.

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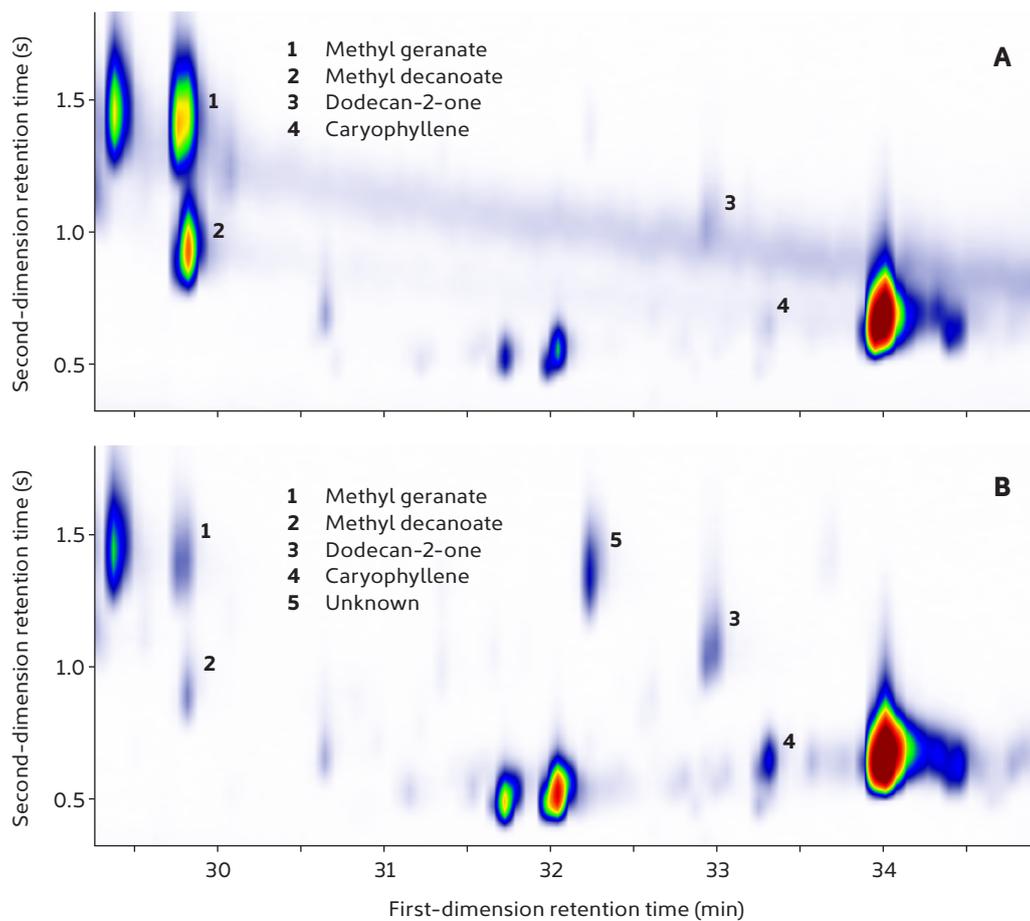
Figure 2

Expanded regions of Figure 1, showing matches against the NIST 14 library for four compounds with markedly different abundances between the two samples. Note that methyl geranate (**1**) and methyl decanoate (**2**) would have co-eluted in a one-dimensional separation.
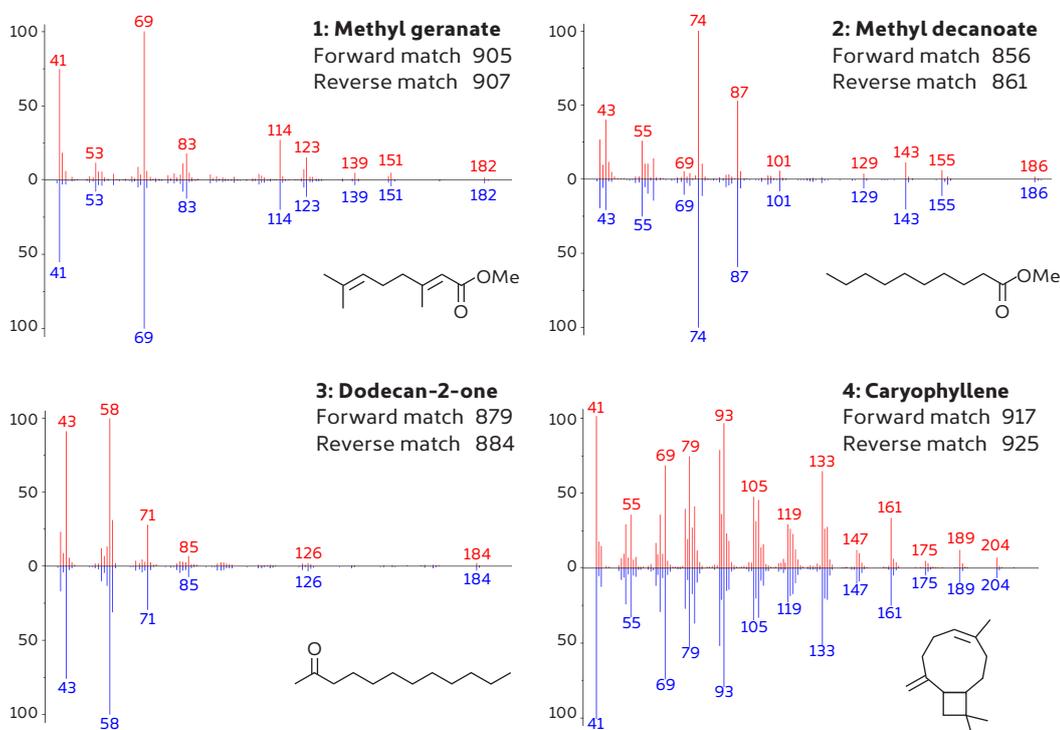
Figure 3

BenchTOF spectra (top, red) and NIST 14 spectra (bottom, blue) for components **1–4** labelled in Figure 2.

An additional peak (**5**) was detected in the same retention time window for hop oil B, but a suitable library match could not be found in the NIST 14 database. However, given the retention indices (in the 1st and 2nd dimensions) of neighbouring compounds, it is likely to be an oxygenated terpenoid, sharing spectral similarities with lavandulyl acetate.
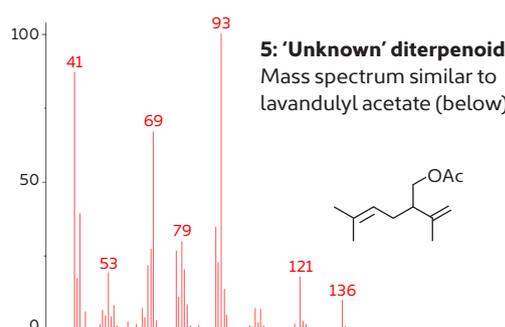
5: 'Unknown' diterpenoid
Mass spectrum similar to lavandulyl acetate (below)

This example illustrates how the ability to provide retention indices in two dimensions allows GC×GC–MS to be used to make tentative assignments for compounds that would otherwise remain unknown. As a result, it is an attractive technique for analysing fragrance and aroma samples containing large numbers of structurally similar compounds such as terpenes.

Other compositional differences were observed between the two hop oils that could markedly affect their aroma. For example, only hop oil B contained sulfur compounds – including *S*-methyl 3-methylbutanethioate and *S*-methyl hexane-thioate, which are thought to give an undesirable 'cooked vegetable' aroma to hops.[2] On the other hand, 3-methylbut-2-enal (delivering an 'almond' aroma[3]) and 2,6-dimethylocta-2,6-diene were only identified in hop oil A.

Even minor differences in such components can have major impacts on the aroma of the final beer, underlining the importance of confident identification of hop-oil components. Such analytical rigour is also important when analysing essential oils used in the perfume industry, where batch-to-batch variations must be minimised by following strict quality control procedures.

Tandem Ionisation at 70 eV and 12 eV

Despite the increased separation by GC×GC, it can often still be challenging to identify individual terpenoids, due to weak molecular ions and/or similar spectra using conventional (70 eV) electron ionisation.

To address this, Tandem Ionisation was used to simultaneously collect spectra at both hard (70 eV) and soft (12 eV) ionisation energies (Figures 5 and 6), providing another level of information from this analytical system. This soft ionisation technology gives increased intensity for the larger diagnostic ions, while still retaining a degree of fragmentation (unlike other soft ionisation techniques). These spectra therefore aid structural elucidation and improve selectivity, as illustrated by the example of peaks **7** and **8**. Both peaks gave strong hits for *m*- and *p*-camphorene at 70 eV, but noticeable differences in the ion ratios at 12 eV (Table 1), giving enhanced confidence in their identity.

Figure 5

Expanded region of Figure 1, showing the location of the peaks in hop oil A giving rise to the spectra in Figure 6.
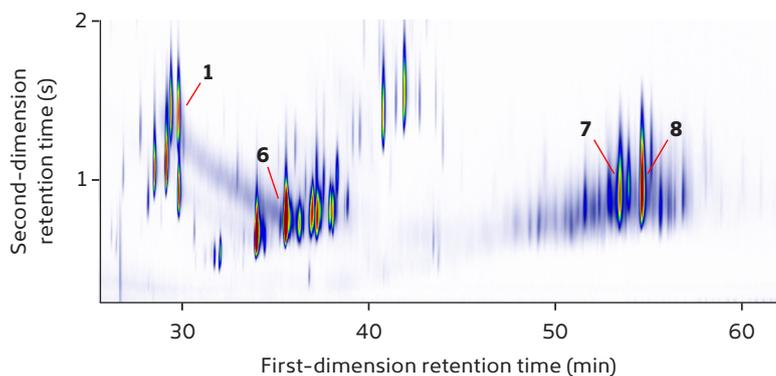
1 Methyl geranate
6 Humulene
7 *m*-Camphorene
8 *p*-Camphorene
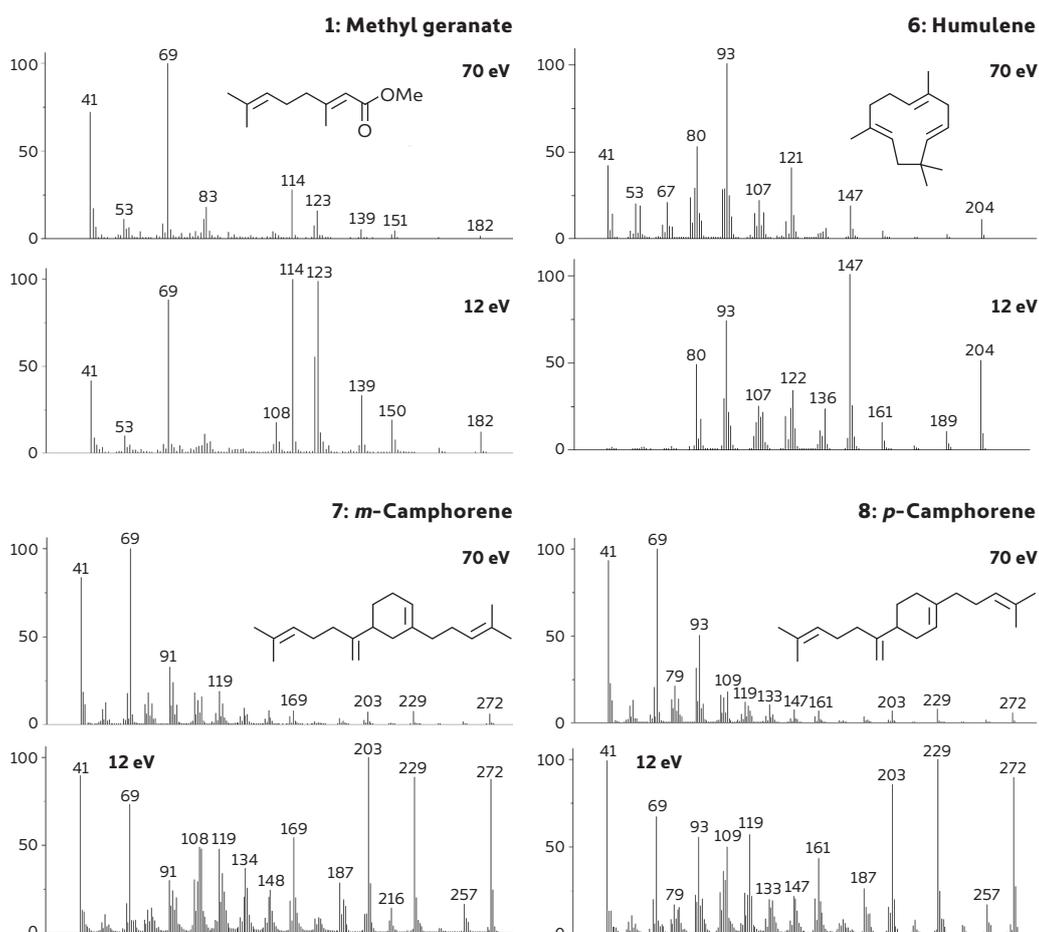
Figure 6

Comparison of 70 and 12 eV spectra for four compounds in hop oil A, obtained by Tandem Ionisation.

	70 eV		12 eV	
Ion ratio	*m*-Camphorene	*p*-Camphorene	*m*-Camphorene	*p*-Camphorene
272 : 203	0.82	0.87	0.87	1.14
203 : 229	1.00	0.91	1.13	0.83
161 : 91	0.24	0.22	1.80	2.04
93 : 69	0.24	0.51	0.33	0.96

Table 1

Ion ratios at 70 eV and 12 eV for *m*- and *p*-camphorene. At 70 eV, the only significant difference is for m/z 93 : 69, but at 12 eV all four ion ratios show differences.

Conclusions

In this study, we have shown that GC×GC–MS with flow modulation can be successfully applied to the analysis of complex hop oils. The enhanced separation offers confident identification of compounds that would ordinarily co-elute on a 1D GC–MS system. Moreover, this type of GC×GC–MS screening enables key compositional differences to be established quickly and routinely, without the need for expensive liquid cryogen.

We have also shown how the Tandem Ionisation capability of BenchTOF instruments can provide additional information that is valuable for analyte speciation, by generating conventional 70 eV and soft-EI spectra simultaneously across every peak.

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

References and notes

[1]   Select-eV® capability, available on the BenchTOF-Select™ mass spectrometer from Markes International, allows soft EI spectra to be collected down to 10 eV, and is fully automated by the instrument's software with no inherent loss in sensitivity or need for manual intervention. Tandem Ionisation® now allows soft and hard ionisation spectra for a single peak to be simultaneously obtained, in both GC and GC×GC analyses, enabling challenging compounds (such as structurally similar isomers) to be discriminated without impacting laboratory workflows. Contact SepSolve for more details.

[2]   T.L. Peppard, Volatile organosulphur compounds in hops and hop oils: A review, *Journal of the Institute of Brewing*, 1981, 87: 376–385, http://doi.org/10.1002/j.2050-0416.1981.tb04054.x.

[3]   G.A. Burdock, *Fenaroli's Handbook of Flavor Ingredients* (5th edition), CRC Press, 2004.