

# Detailed characterisation of essential oils by flow-modulated GC×GC and Tandem Ionisation mass spectrometry

In this study, we describe the use of comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF MS) with Tandem Ionisation to characterise essential oils in fine detail, allowing the in-depth comparison of their compositions that aids rigorous quality control.



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## Introduction

Volatile oils of plants, also known as essential oils, have been used for many years for their flavour, fragrance and medicinal properties. However, their extreme complexity and diversity presents analysts with a significant challenge when it comes to quality control. They are generally composed of monoterpenes and sesquiterpenes, as well as their oxygenated derivatives, such as aliphatic aldehydes, alcohols and esters.<sup>[1]</sup>

In many cases, trace components can impart a distinctive fragrance character to the oil, meaning that rigorous quality control procedures are required to ensure that essential oils do not vary significantly from the initial reference batch, or contain any unwanted adulterants. Furthermore, because essential oils are raw materials for the perfume industry, analysts must now also ensure that they adhere to new legislation on fragrance allergens.

Traditionally, gas chromatography (GC), coupled with mass spectrometry (MS) or flame ionisation detection (FID), have been the standard methods for qualitative and quantitative analysis of such samples, relying on the combination of mass spectra and Kovats retention indices to confirm the identity of individual terpenes.

However, in recent years, comprehensive two-dimensional GC with time-of-flight MS (GC×GC–TOF MS) has become an attractive technique for characterisation of essential oils.<sup>[2]</sup> The enhanced separation capacity offered by the coupling of two columns with different selectivities, combined with highly sensitive mass spectral identification, provides a high-performance solution for

rapid screening of essential oils. Nevertheless, the structurally similar terpenes found in essential oils can be difficult (or impossible) to speciate when using conventional 70 eV electron ionisation, even with the added power of retention indices in two dimensions.

Here we examine the use of the Tandem Ionisation<sup>® [3]</sup> to provide two complementary data sets from a single analysis – one containing 70 eV data, and the other with soft EI for enhancement of molecular ions and improved speciation of isomers. This capability is illustrated by comparing a range of essential oils by flow-modulated GC×GC–TOF MS combined with multivariate statistical analysis for fast evaluation of compositional differences.

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## Experimental

**Sample preparation:** 1% (v/v) dilutions of eight essential oils were prepared in ethyl acetate.

**GC×GC:** Injector: Split/splitless; Liner: Single taper with wool, 4 mm (i.d.); Carrier gas: Helium, constant-flow at 0.6 mL/min; Mode: 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°C/min to 280°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; Modulation period ( $P_M$ ): 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 45–400; Data rate: 100 Hz. Tandem Ionisation: Simultaneous acquisition of 70 eV and 12 eV data.

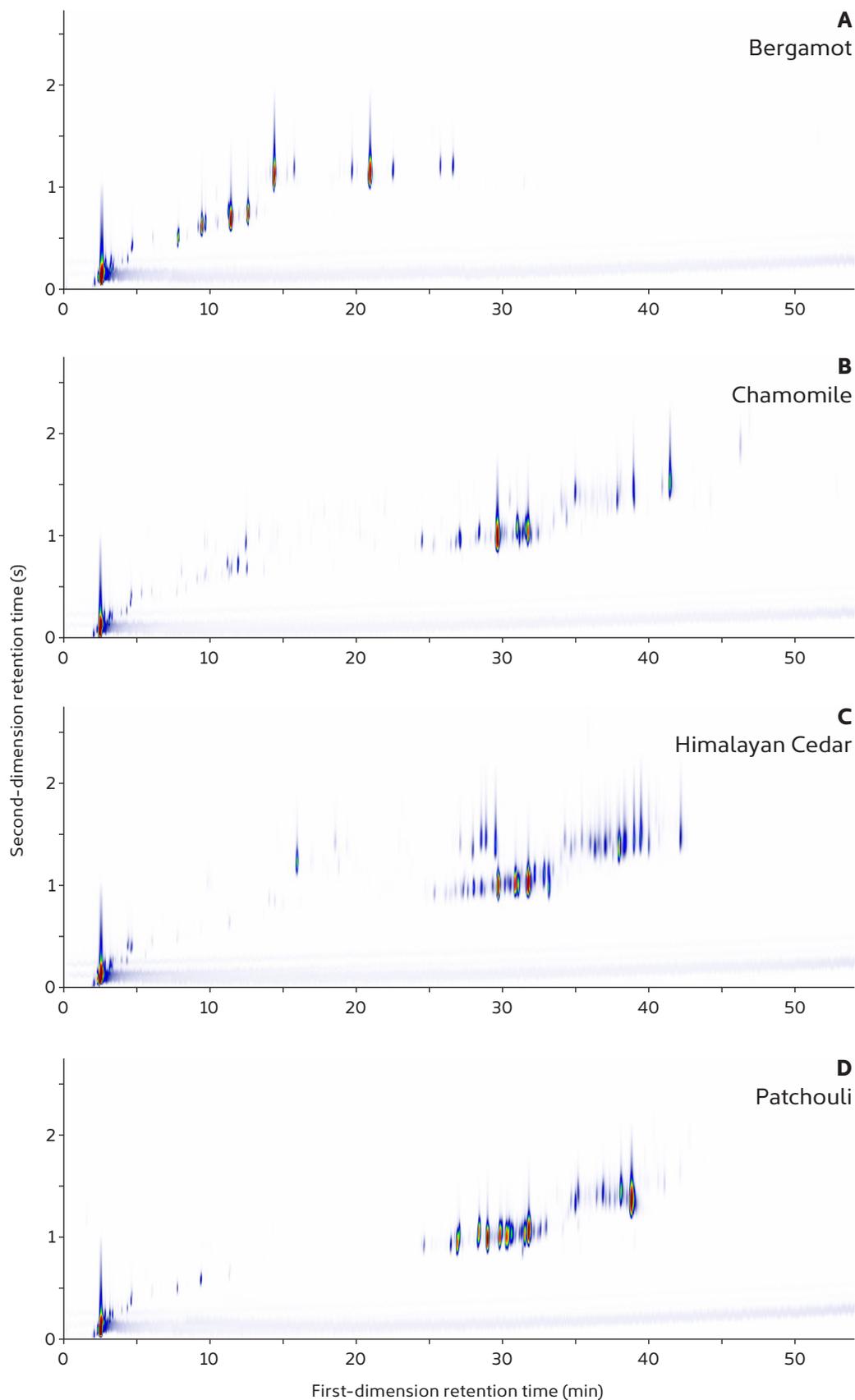
**Software:** Image processing: *GC Image*™ (GC Image, LLC); Statistical analysis: MATLAB® (R2011a, MathWorks).

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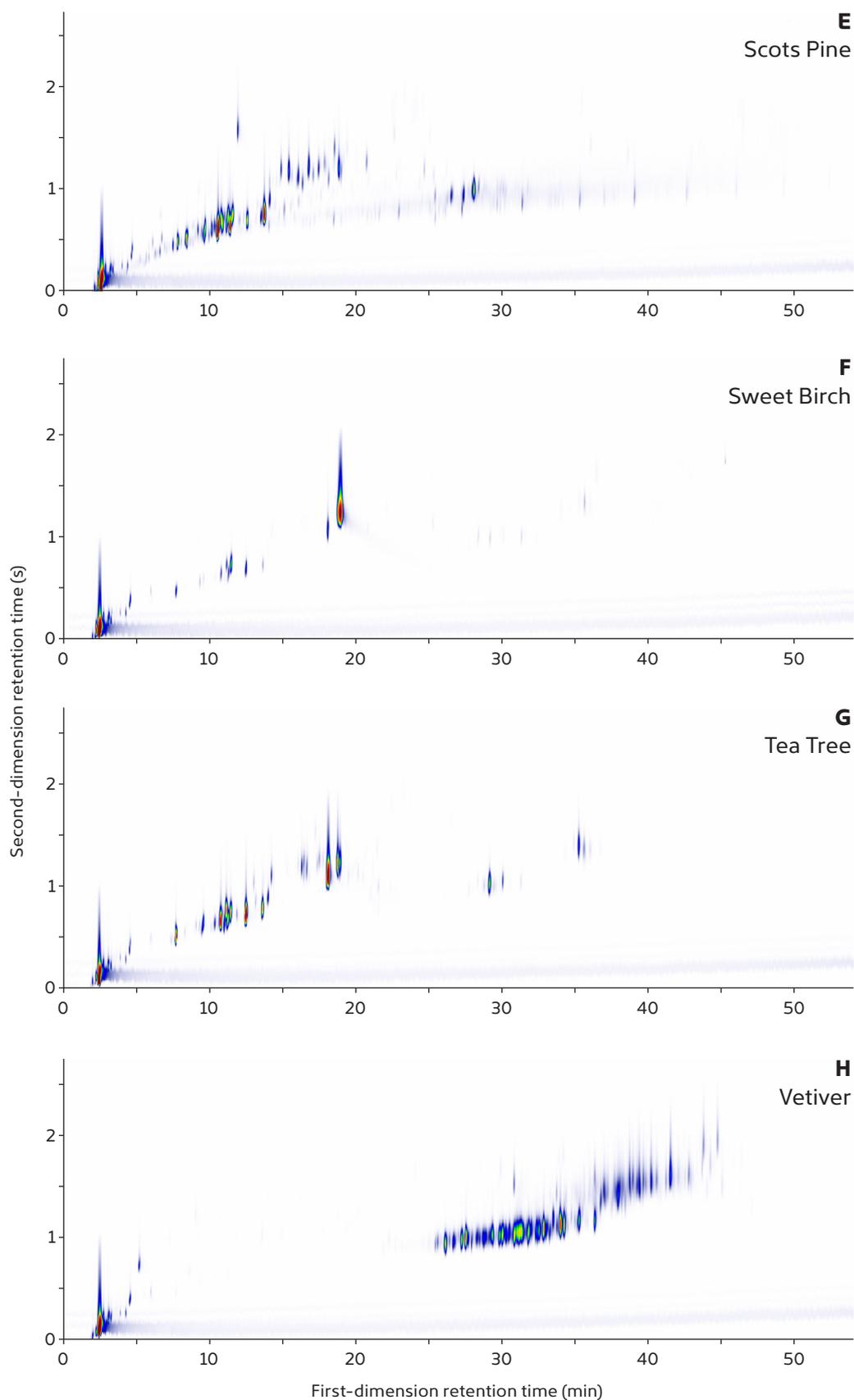
## Results and discussion

### Screening of essential oils

The GC×GC–TOF MS colour plots for eight essential oils (Figure 1) display dramatic differences in monoterpene and sesquiterpene content. Some, such as Sweet Birch (**F**), almost entirely comprise monoterpenes ( $^1t_R$  8–22 min), while others, like Patchouli (**D**), mostly contain sesquiterpenes ( $^1t_R$  >22 min). A summary of the main characteristics of each oil is provided in Table 1.

**Figure 1**

Comparison of GCxGC-TOF MS colour plots for eight essential oils (continued on next page).

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Comparison of GCxGC-TOF MS colour plots for eight essential oils (continued from previous page).

	Essential oil	Characteristics
<b>A</b>	Bergamot	Mainly monoterpenes – including camphene, linalool, limonene, $\gamma$ -terpinene and $\beta$ -pinene.
<b>B</b>	Chamomile	Mainly sesquiterpenes – including farnesenes, bisabolone oxide A and bisabolol oxide A.
<b>C</b>	Himalayan Cedar	Mainly sesquiterpenes – including $\alpha$ - and $\beta$ -himachalene.
<b>D</b>	Patchouli	Mainly sesquiterpenes and oxygenated derivatives, e.g. patchouli alcohol.
<b>E</b>	Scots Pine	Mix of mono- and sesquiterpenes, as well as benzyl alcohol (not observed in significant quantity in any other oil) and several oxygenated terpenes.
<b>F</b>	Sweet Birch	Major peak for methyl salicylate.
<b>G</b>	Tea Tree	Mainly monoterpenes – including $\alpha$ - and $\gamma$ -terpinene and $\alpha$ -pinene.
<b>H</b>	Vetiver	Mainly sesquiterpenes – including $\beta$ - and $\gamma$ -vetivenene.

**Table 1**

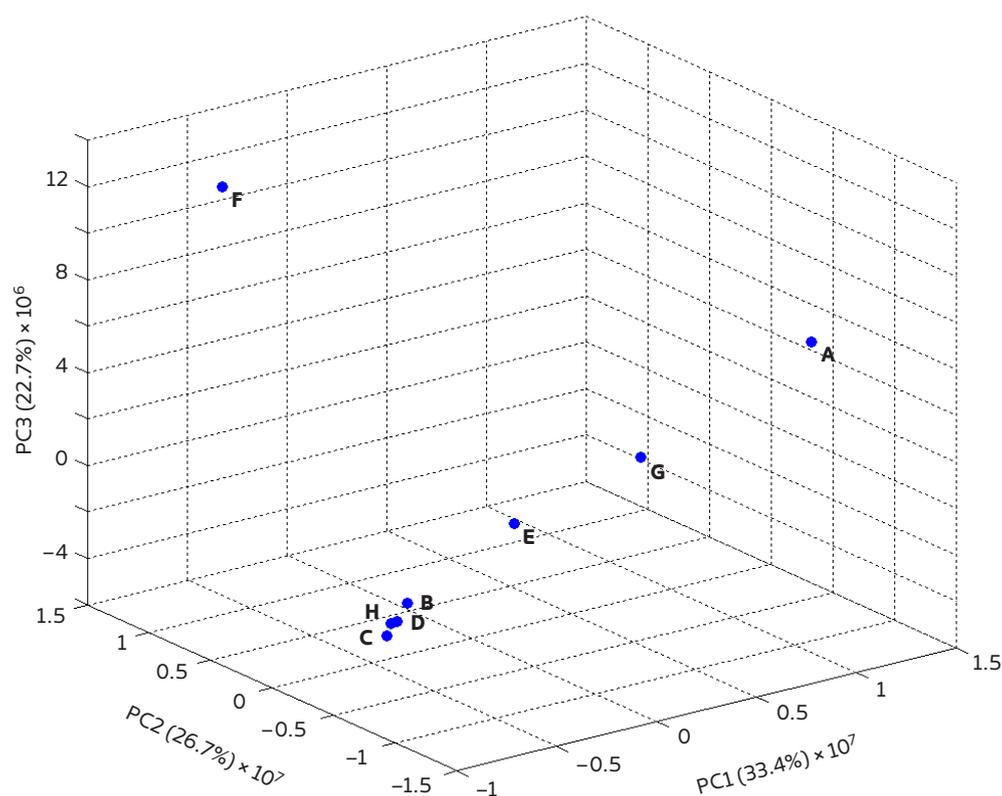
Summary of the major characteristics of essential oils analysed in this study.

The flow-modulated GC $\times$ GC–TOF MS method effectively separated multiple chemical classes that would co-elute in a one-dimensional separation. It is useful to have retention times in both dimensions, as well as mass spectra, for confirming compound identity. This is even more important when investigating final fragrance mixtures or cosmetics, which contain higher matrix levels that can hinder identifications in a 1D separation.

### Fast comparisons of composition

A template of over 100 different target compounds was prepared and applied to each sample, in order to collate the peak area data (using EIC chromatograms for a specified quant ion for each target). Principal components analysis (PCA) was then applied to the peak lists to distil the information into the key differences in sample composition. It is worth pointing out that the high repeatability of  $^1t_R$  and  $^2t_R$  using flow modulation is due to a dedicated EPC (electronic pressure control) module for each column. This makes flow modulation well-suited to large-scale projects and comparisons across multiple data sets, because of the minimal RT differences for any given peak.

The PCA score plot (Figure 2) shows that Patchouli (**D**), Vetiver (**H**), Chamomile (**B**) and Himalayan Cedar (**C**) all cluster closely, while the other oils are spread out across the plot. The first three principal components described 82.8% of the sample variation. The major contributors to PC1 are terpinen-4-ol and  $\alpha$ -terpinene, while for PC2 they are monoterpenes including linalool, limonene, linalyl acetate and  $\beta$ -pinene. Finally, methyl salicylate contributes most to the variation on PC3, explaining why Sweet Birch (**F**, which has a high content of this compound) is separated from the other oils on this axis.



**Figure 2**

PCA score plot comparing the eight essential oils.

This type of GC×GC–TOF MS screening approach, coupled with automated template-based compound identification in *GC Image*, can allow differences in target compounds across multiple essential oil batches to be determined quickly and effectively in a quality control laboratory.

### Tandem Ionisation for confident identification of terpenes

The screening approach described works well in cases where target compounds are known and have distinctive mass spectra (for confident identification using automated templates). However, in many situations, characterisation of the entire sample is required to provide the final fragrance formulation, including ‘unknown’ compounds that may not be present in spectral libraries.

This is a challenging prospect when the dominant constituents are terpenes with very similar EI (70 eV) mass spectra, which are consequently difficult to speciate confidently based solely on 70 eV analyses (note that the TOF MS instrument used here is able to generate ‘reference-quality’ spectra<sup>[4]</sup>).

Tandem Ionisation is of great value in such cases, by allowing fast switching between conventional 70 eV ionisation and Select-eV soft EI. This approach allows two complementary datasets to be acquired simultaneously without impacting laboratory workflow, and with perfectly aligned peaks for easy navigation of the soft EI data.

In fact, laboratory workflows can even be *enhanced* by applying a checklist (Table 2) for identifying constituents of essential oils. Flow-modulated GC×GC

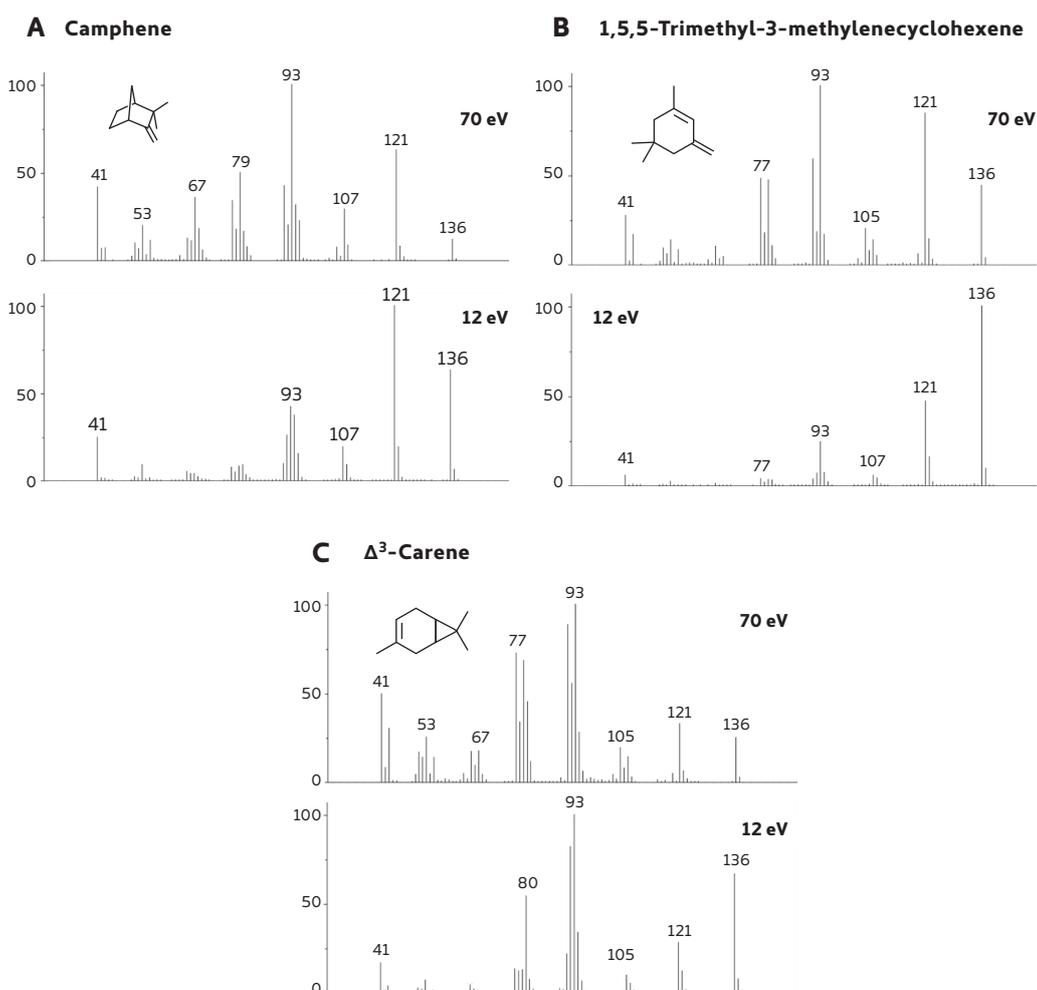
provides repeatable retention times in two dimensions ( $^1t_R$  and  $^2t_R$ ), while Tandem Ionisation delivers confirmatory ion ratios (IRs) from both the 70 eV and soft EI datasets, for robust quality control.

Compound assignment	$^1t_R$	$^2t_R$	Ion ratio (70 eV)	Ion ratio (12 eV)	Ion ratio (70 eV/12 eV)	Identity confirmed?
$\beta$ -Pinene	✓	✗	✓	✓	✓	Y
Linalyl acetate	✓	✗	✓	✗	✗	N
(2E,6E)-Farnesol	✓	✓	✗	✓	✓	Y
(2E,6Z)-Farnesol	✓	✓	✗	✓	✓	Y

**Table 2**

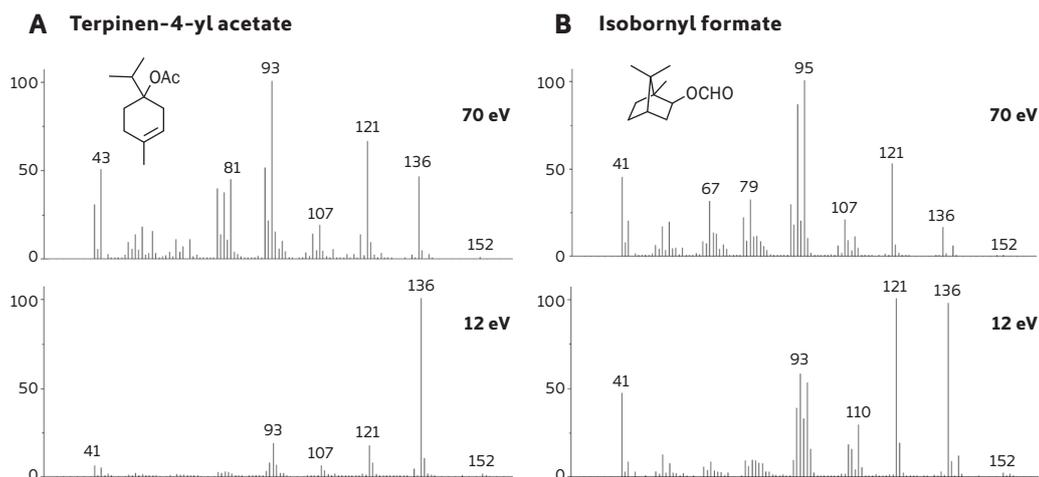
Improved QC checks using GC×GC–TOF MS with Tandem Ionisation.

Figures 3 and 4 show spectral comparisons for three cyclic monoterpenes and two oxygenated terpenes, respectively. The 70 eV spectra within each figure are similar to each other, with identical ions present for each compound and in comparable ratios, making it difficult to identify the individual compounds. The 12 eV spectra, however, have distinct differences, meaning that the use of a library of 12 eV spectra can add confidence to essential oil characterisation.



**Figure 3**

Spectral comparisons at 70 and 12 eV from Tandem Ionisation GC×GC–TOF MS for three cyclic monoterpenes ( $m/z$  136) found in essential oils.

**Figure 4**

Spectral comparisons at 70 and 12 eV from Tandem Ionisation GC×GC–TOF MS for two oxygenated terpenes ( $m/z$  152) found in essential oils.

## Conclusions

In this study, we have shown that GC×GC–TOF MS with flow modulation can be successfully applied for routine and repeatable screening of essential oils. This approach ensures that confident identification is achieved for these complex mixtures – which is particularly important for quality control within the fragrance industry.

The ability of GC×GC–TOF MS to provide structured chromatograms, with chemical classes eluting in bands, can aid data review, enabling swift tentative identifications to be made. The use of Tandem Ionisation then adds an additional level of confidence in situations where 70 eV data alone cannot speciate similar terpenes, but with no inherent loss in sensitivity or inconvenience to the operator.

It is also worth noting that the application of Tandem Ionisation is fully controlled by the instrument's software, with two complementary datasets generated automatically, meaning that there is no inconvenience to the operator, and that no additional analysis time is required. Moreover, the peaks in both hard and soft ionisation data files are perfectly aligned, simplifying data exploration. Tandem Ionisation can also fit seamlessly into laboratory workflows, providing independent ion ratio checks at both 70 eV and low eV for robust QC, alongside  $^1t_R$  and  $^2t_R$ .

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

## References and notes

- [1] B. d'Acampora Zellner, P. Dugo, G. Dugo and L. Mondello, Analysis of essential oils (ch. 7), in *Handbook of Essential Oils: Science, Technology and Applications* (2nd edition), K.H.C. Baser and G. Buchbauer (eds.), CRC Press, 2015, pp. 195–225.
- [2] R.A. Shellie, Volatile components of plants, essential oils and fragrances, in *Comprehensive Two Dimensional Gas Chromatography*, L. Ramos (ed.), Elsevier, 2009, pp. 189–213.
- [3] 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.
- [4] Uniquely among TOF MS instruments, spectra generated by BenchTOF systems are a close match to the quadrupole-acquired spectra in commercial libraries such as NIST or Wiley – even in cases where the molecular ion is weak.

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