White Paper 034
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Comprehensive aroma profiling of cannabis using a discovery workflow

This study describes the use of GC×GC–TOF MS for exploratory profiling of terpenes and other aroma-active compounds in cannabis flowers.

Introduction

The classification of terpenes in cannabis is important due to the distinctive aroma and flavour that they impart, as well as their potential therapeutic effects.[1] Consequently, plant breeders will often attempt to engineer cultivars with specific terpene profiles, in order to encourage particular traits.[2] Robust terpene profiling is therefore necessary for accurate product descriptions and labelling.

Here, we evaluate a discovery-based approach for terpene profiling using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) for enhanced separation and improved sample characterisation. The described system provides another level of confidence in the identification of terpenes, by utilising both hard (70 eV) and soft (12 eV) ionisation. Soft ionisation is shown to aid the identification of terpene isomers that prove too similar when using conventional 70 eV spectra.

However, the acquisition of high-quality data is just the first step – efficient processing workflows are then required to allow meaningful conclusions to be drawn. Here, we evaluate differences in terpene composition for the same cannabis strain grown under different conditions using robust flow-modulated GC×GC–TOF MS and efficient processing workflows in ChromSpace® software.

Experimental

Samples: Two 'Blueberry Kush' strain cannabis samples – Sample A was purchased from a dispensary in Ontario, while sample B was grown outdoors in the summer of 2019 from 'Blueberry Kush' seeds. *Note: phenotyping was not performed.*

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Sample preparation: 0.5 g of cannabis flower was extracted with methanol by vortexing for 20 minutes in a centrifugre tube. The extract was filtered using a 0.2 µm syringe filter, before transfer of 2 mL to a GC vial.

GC×GC: Modulator: INSIGHT® flow modulator (SepSolve Analytical); Modulation period (P_M): 2.6 s.

TOF MS: BenchTOF-Select™; Mass range: m/z 40–350; Acquisition rate: 100 Hz in Tandem Ionisation® mode (with 70 eV and 12 eV data acquired simultaneously).

Software: ChromSpace® GC×GC software for full instrument control and data processing.

Please contact SepSolve for full analytical parameters.

Results and discussion

In this study, two 'Blueberry Kush' cannabis extracts were analysed by GC×GC–TOF MS (Figure 1). The two samples are from the same strain but grown under different conditions – Sample A was purchased from a dispensary in Ontario, while sample B was grown outdoors in the summer of 2019 from seeds.

Figure 1 shows the enhanced separation provided by GC×GC for the hydrocarbon-based terpenes (monoterpenes, sesquiterpenes) and the oxygenated-terpenes (monoterpenoids and sesquiterpenoids) for increased insight into sample composition.

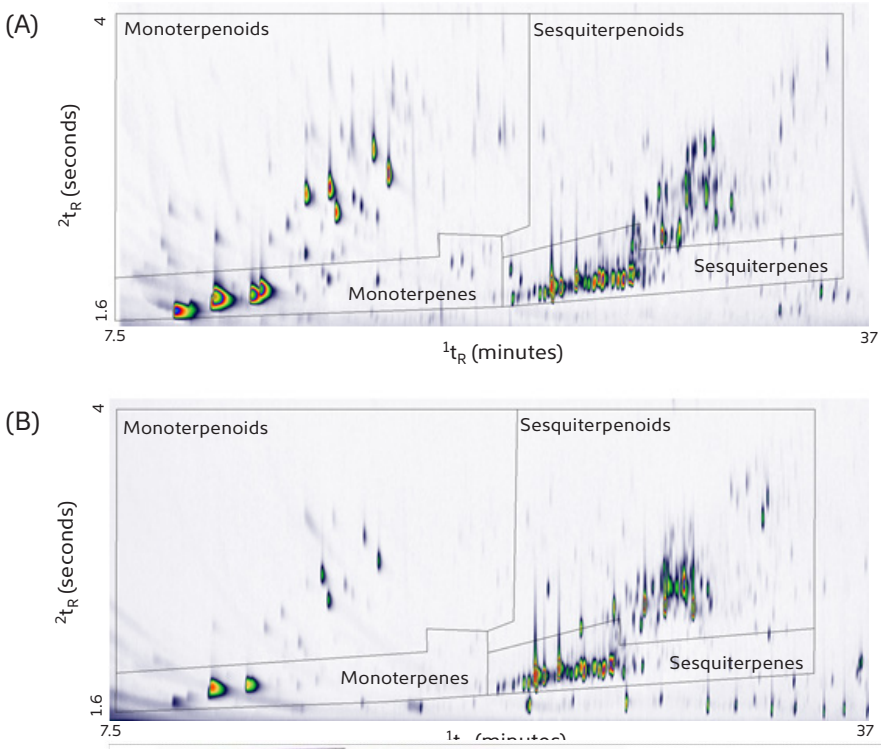

Figure 1
GC×GC–TOF MS chromatograms of (A) dispensary and (B) outdoor grown 'Blueberry Kush' highlighting the excellent terpene class separation.

A discovery-based approach was developed to quickly and efficiently report the terpene content of the sample, based on the following chemical classes – monoterpenes, monoterpenoids, sesquiterpenes and sesquiterpenoids. Using the 'Stencils' function in ChromSpace software, peaks were classified according to the region of the chromatogram they were found.

In order to prevent contributions from other chemical classes that happen to elute in the same regions (e.g. other aroma-active species, such as esters and alcohols), simple scripting expressions were applied to each stencil region to add selectivity. The scripts exploit diagnostic ions from each terpene class in order to correctly classify the terpenes, and exclude interferences, in an automated manner.

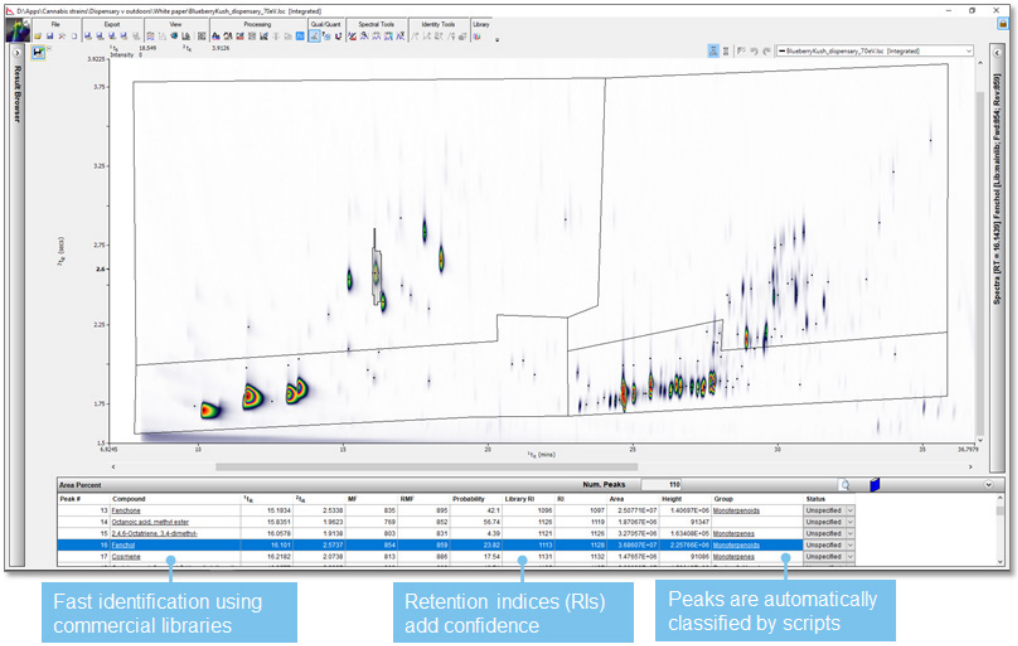

Figure 2

ChromSpace peak table for Sample A showing automated classification of the peaks.

Figure 2 shows an example peak table after applying this automated classification approach, with custom peak apex markers to ensure they are not distracting from the raw data. The 'group' column conveniently reports the chemical class that the peak belongs to, based on the use of simple scripting expressions. This helps to organise the peak table, adds analytical significance to the identified compounds and accelerates the 'discovery' workflow. For example, octanoic acid methyl ester elutes within the 'monoterpenes' region of the chromatogram but is not classified as such because it does not pass the criteria in the scripting expression. Furthermore, ChromSpace enables the use of retention indices (RIs) within the identification process to add another level of confidence to the match.

Terpene class	No. of peaks detected	
	Dispensary	Outdoor
Monoterpenes	31	20
Monoterpenoids	31	12
Sesquiterpenes	57	53
Sesquiterpenoids	94	52
Total terpenes	**213**	**137**

Table 1

Total number of peaks detected for each terpene class in the cannabis extracts.

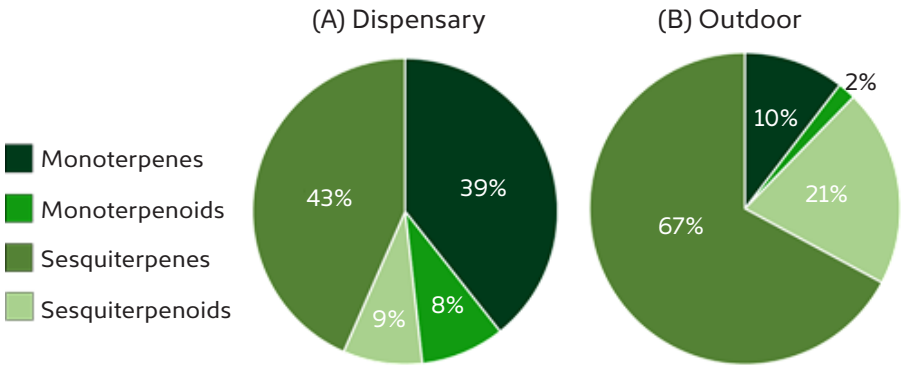

Figure 3

Overview of the area percent contributions for each terpene class (based on summed peak areas from a group-type report).

Table 1 is a compilation of the number of peaks detected per terpene class for both samples using the discovery approach, while Figure 3 summarises the area percent contributions from each terpene class, using a simple group-type report in ChromSpace software.

The dispensary sample was characterised by high relative proportions of monoterpenes and sesquiterpenes, whose combined peak areas account for 82% of the total terpene peak area (see Figure 3). The monoterpenoid and sesquiterpenoid chemical classes represented 8% and 9% of the total terpene peak area, respectively. In contrast, the 'outdoor-grown' sample was depleted of monoterpenes (10%) and monoterpenoids (2%) and possessed relatively higher proportions of sesquiterpenes (67%) and sesquiterpenoids (21%). The results show the importance of growing conditions (e.g. temperature, nutrients, amount of sunlight, etc.) and the impact they can have on the overall terpene profile of the strain.

The dispensary sample was more diverse, containing 213 individual terpenes, and increased contributions from monoterpenes, such as β-myrcene, α-pinene, β-ocimene and limonene. On the other hand, the 'outdoor grown' sample contained an increased abundance of sesquiterpenes and sesquiterpenoids, namely β-caryophyllene, α-humulene, *trans*-nerolidol and α-bisabolol.

The BenchTOF mass spectrometer used in this study provides highly-sensitive detection, which, in conjunction with its reference-quality spectra, allows these terpenes to be identified by screening against commercial libraries, such as NIST and Wiley (Figure 4). Identification of such a diverse range of terpenes is important to allow comprehensive aroma profiling and flavour interpretation, which in turn, enables innovative product labelling strategies to be used. For example, the β-eudesmene peak (Figure 4) was found to be twice as abundant in the dispensary sample and contributes a 'herbal' aroma,[3] while the α-calacorene was only identified in the outdoor-grown sample and contributes a 'woody' aroma.[3]

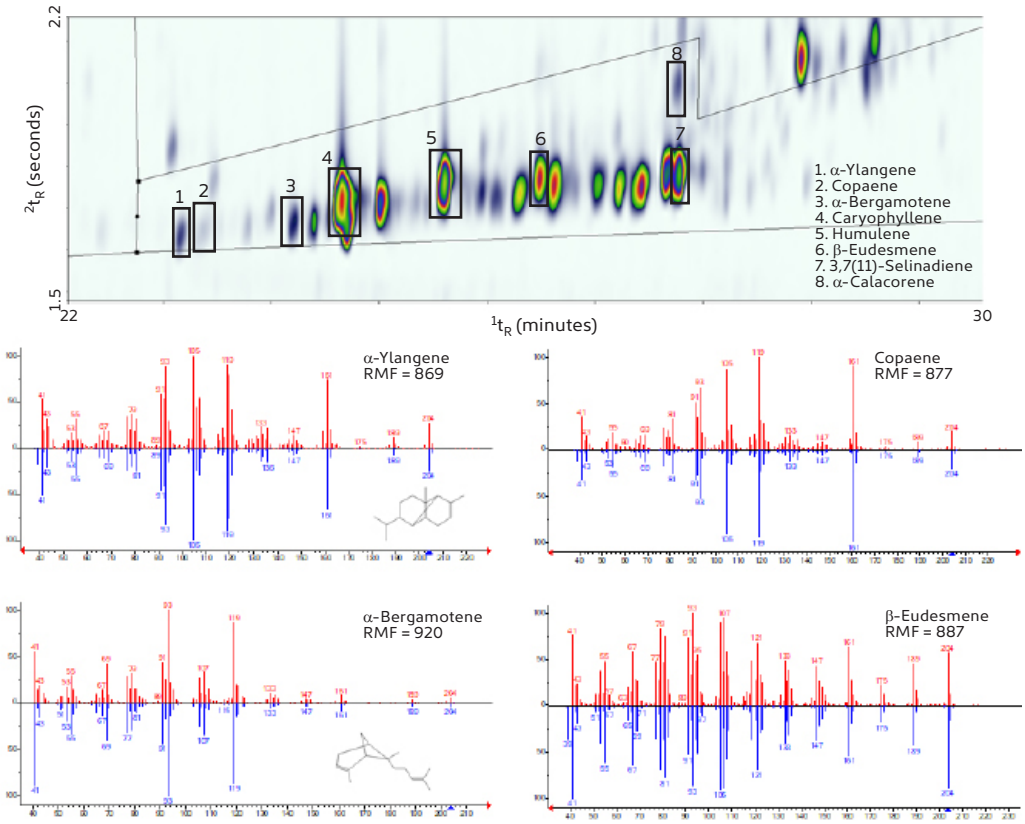

Figure 4

A selection of sesquiterpenes identified in Sample A with BenchTOF spectra (red) compared to NIST17 library (blue).

Although not a prominent terpene class in the cannabis extracts, a few diterpenes, such as *m*-camphorene and phytol, were also identified by spectral matches and expected RIs (Figure 5). Phytol was identified in both extracts, while *m*-camphorene was only detected in the dispensary sample.

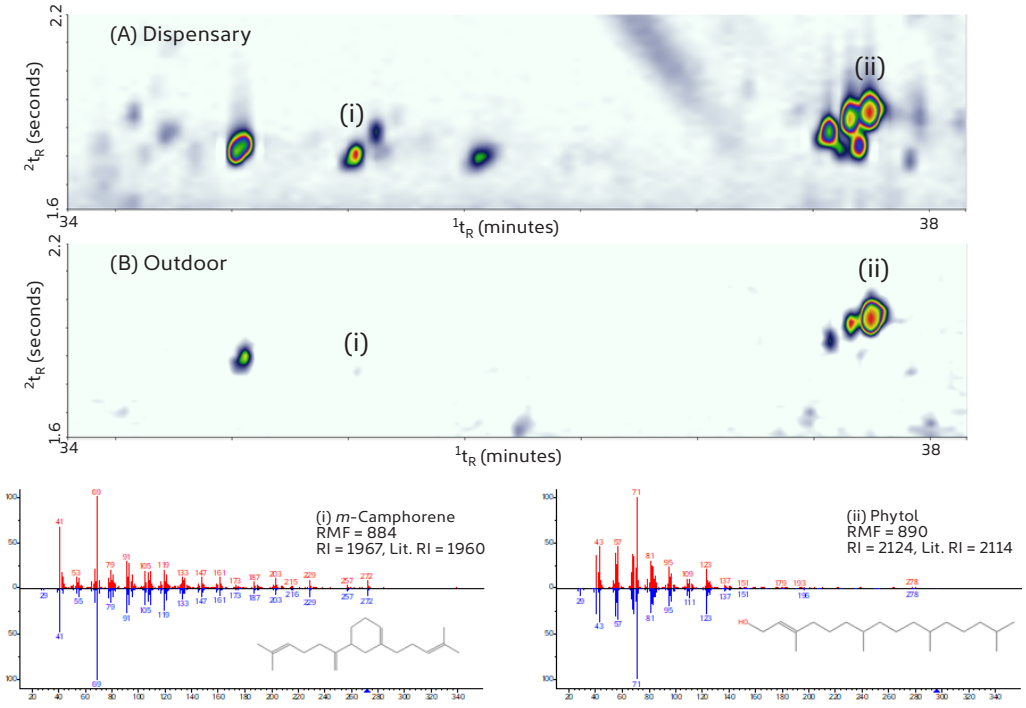

Figure 5

Identification of the diterpenes (i) *m*-camphorene and (ii) phytol in the Blueberry Kush extracts, with BenchTOF spectra (red) compared to NIST17 library (blue).

By utilising a GC×GC–TOF MS discovery workflow, the analysis is not limited to solely terpenes – other aroma-active species can also be identified. Scripting expressions were created for other chemical classes, including n-alkyl methyl esters and n-alkyl ethyl esters. Figure 6 shows the elution pattern of a number of these methyl and ethyl esters as detected in the dispensary sample. Interestingly, the only ester present in the outdoor-grown sample was methyl hexadecanoate – likely resulting in a difference in flavour profile for the two samples.

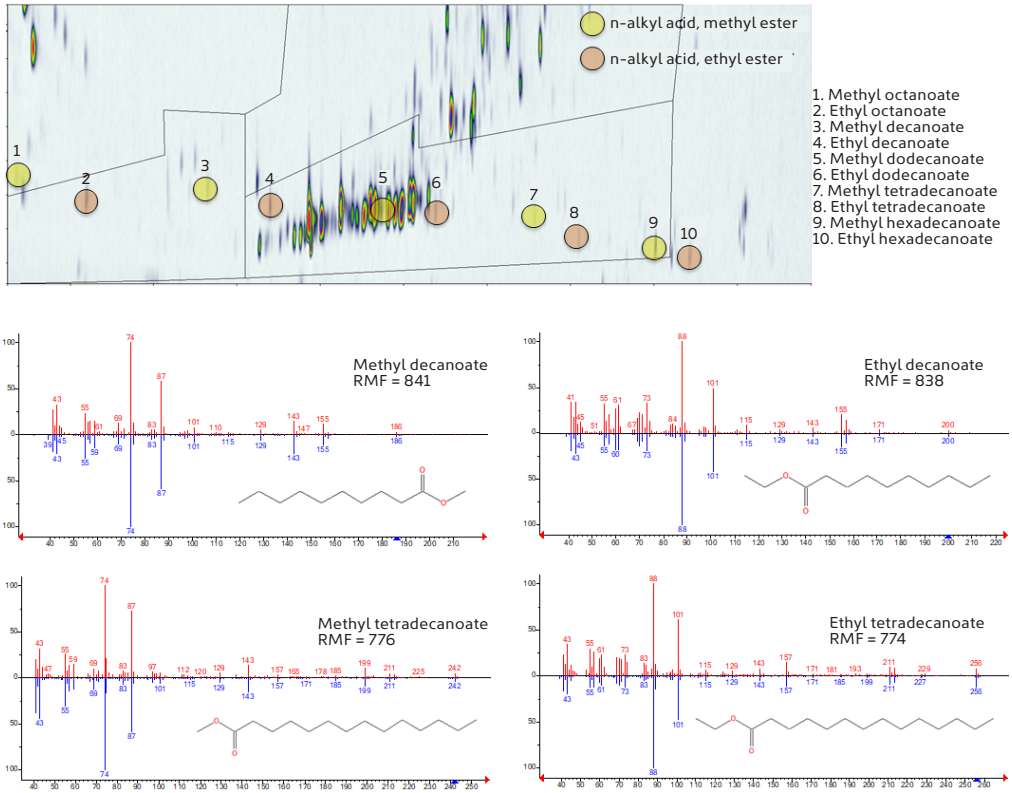

Figure 6

GC×GC–TOF MS chromatogram for sample A, highlighting numerous n-alkyl methyl and ethyl esters, which were flagged by scripting expressions, with BenchTOF spectra (red) compared to NIST17 library (blue) showing identification of a selection of the compounds.

Improving confidence in terpene profiling

Using conventional 70 eV ionisation, many of the terpene isomers share similar spectra, containing the same ions in slightly different ratios. For example, Figure 7 shows the spectra of two isomers of nerolidol, which are close to identical at 70 eV. However, when using soft ionisation, the higher m/z ions are enhanced and differences in ratios emerge for the two compounds.

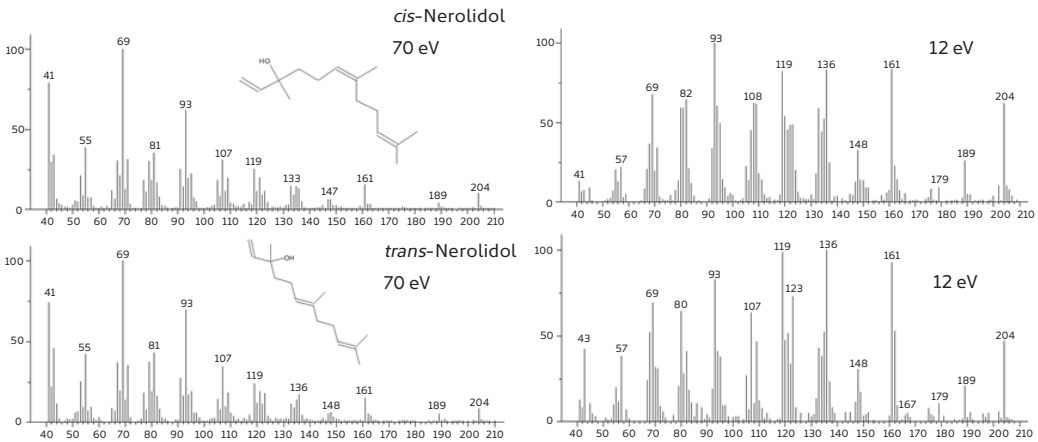

Figure 7

cis-Nerolidol at 70 eV and 12 eV (top) and *trans*-nerolidol at 70 eV and 12 eV (bottom). With hard ionisation (70 eV), the isomers share similar spectra; with soft ionisation (12 eV), it is easier to distinguish between the two by the differences in ratios.

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At 70 eV, this results in strong matches (MF >800) for multiple compounds when comparing to a spectral library (Figure 8, top), while at 12 eV, there is greater distinction between the spectra of similar terpenes, resulting in more confident spectral matching (Figure 8, bottom).

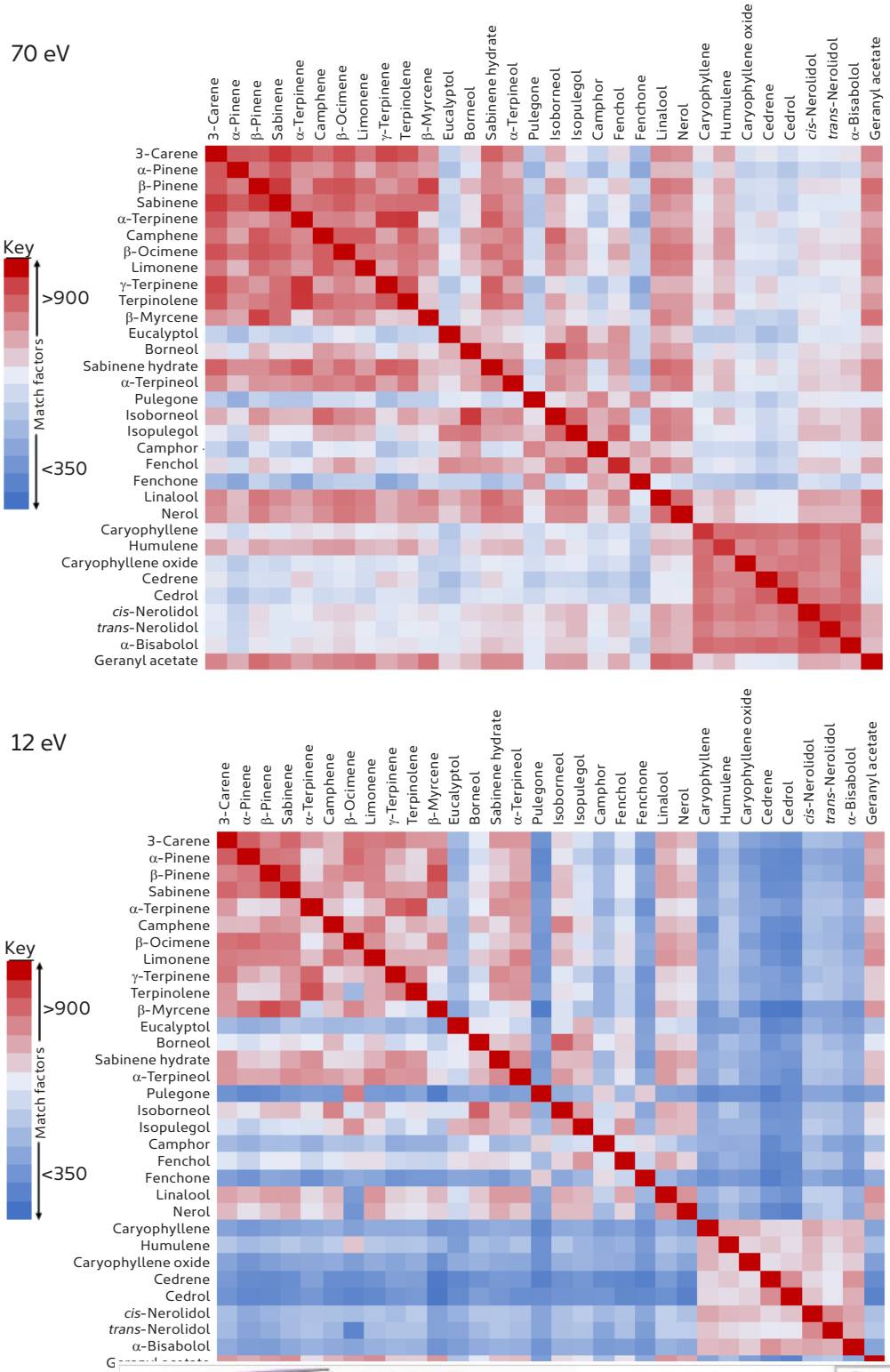

Figure 8

Heatmaps illustrating the improved discrimination in match factors achieved for the key terpenes and terpenoids when using 12 eV (lower) compared to 70 eV ionisation.

Conclusions

The discovery approach described in this study provides:

- The enhanced separation necessary for robust profiling of terpenes and terpenoids, overcoming co-elution issues experienced in 1D GC.
- Identification of the widest possible range of terpenes (and other aroma-active species) through coupling with robust BenchTOF mass spectrometers.
- Increased precision in flavour interpretation, enabling improved product labelling.
- Improved confidence in identification of terpene isomers using unique Tandem Ionisation.
- Streamlined workflow and simplified training requirements, through full instrument control and data processing using ChromSpace GC×GC software.

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

References

[1] R.C. Clarke and D.P. Watson, Chapter 1: Cannabis and Natural Cannabis Medicines, in: *Marijuana and the Cannabinoids*, Mahmoud A. ElSohly (Editor), Springer Science & Business Media, 2007.

[2] C.M. Andre, J.-F. Hausman and G. Guerriero, Cannabis sativa: The plant of the thousand and one molecules, *Front. Plant Sci.*, 2016, http://DOI: 10.3389/fpls.2016.00019.

[3] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 27th April 2020).

Applications were performed under the stated analytical conditions. Operation under different conditions, or with incompatible sample matrices, may impact the performance shown.

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