

# Enhanced characterisation of cannabis extracts using flow-modulated GC×GC–TOF MS/FID with Tandem Ionisation



This study demonstrates the use of flow-modulated GC×GC to analyse complex cannabis extracts, with simultaneous detection by TOF MS for confident identification and FID for robust quantitation. GC×GC provides the enhanced separation necessary to enable a wide range of components, including cannabinoids, terpenoids and pesticides, to be analysed in a single analytical run, while Tandem Ionisation adds an additional level of confidence for compounds exhibiting weak molecular ions and/or similar spectra at 70 eV.

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## Introduction

The rapid growth in medicinal use of cannabis (marijuana), notably in many US states, is driving increased demand for robust testing of cannabis products.

In a medicinal context, the primary compounds of interest are the psychoactive cannabinoids that define potency – namely tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). However, in the light of rising sales of ‘edibles’ containing cannabis oil, there is also a need to analyse the terpenoids responsible for cannabis aroma; in addition, safety screening for potentially harmful residues, such as pesticides, is also required. Detection of these chemicals can be challenging due to sample and/or matrix complexity.

In this study, we use comprehensive two-dimensional gas chromatography (GC×GC), using an INSIGHT™ flow modulator for improved separation capacity in the analysis of these complex plant extracts. Detection is conducted simultaneously on two channels, using flame ionisation detection (FID) for quantitation and time-of-flight mass spectrometry (TOF MS) for analyte speciation.

To aid the characterisation of challenging cannabinoids and terpenoids, an ion source is used that simultaneously generates hard and soft electron ionisation (EI) spectra. This technique, known as Tandem Ionisation®, is shown to provide an extra dimension of information that can aid structural elucidation and enhance confidence in identification of isomers.

## Experimental

**Sample preparation:** Six cannabis extracts (A–F, from plants of different origin) were prepared by QuEChERS extraction, as described by Cochran et al.<sup>[1]</sup>

**GC×GC:** Injector: Split/splitless; Injection volume: 1.0 µL; Split: 5:1; Inlet temperature: 280°C; Flow modulator: INSIGHT™ (SepSolve Analytical);  $P_M$ : 4.0 s. 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™; Mass range  $m/z$  45–550. Tandem ionisation: Simultaneous acquisition of 70 eV and 12 eV data.

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

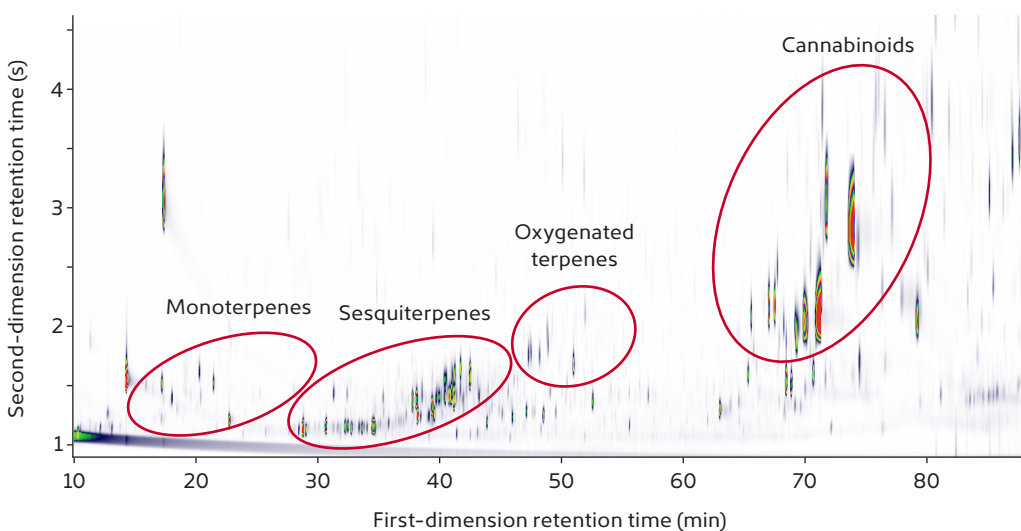
**Software:** ChromSpace® GC×GC software for instrument control and data processing.

Please contact SepSolve for full analytical parameters.

## Results and discussion

### 1. Separation of compound classes

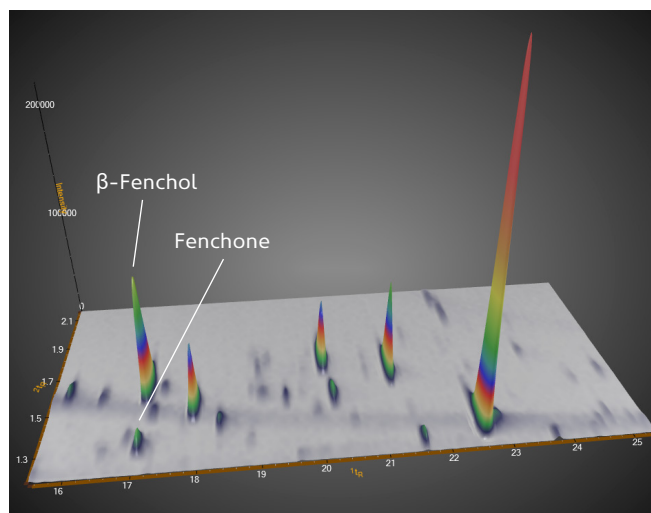
The colour plot resulting from INSIGHT™ flow-modulated GC×GC–TOF MS of a cannabis extract is shown in Figure 1. As is common in GC×GC, the identification process is simplified because chemical classes elute in bands (known as the ‘roof-tiling’ effect), and as demonstrated here this phenomenon assists classification of the diverse terpenes in this sample.



**Figure 1**

GC×GC–TOF MS colour plot of extract A, with key chemical classes annotated.

In contrast, conventional GC-FID or GC-MS may result in the abundance of important terpenes being over-estimated, due to the co-elution of similar compounds or oxygenated derivatives (Figure 2).



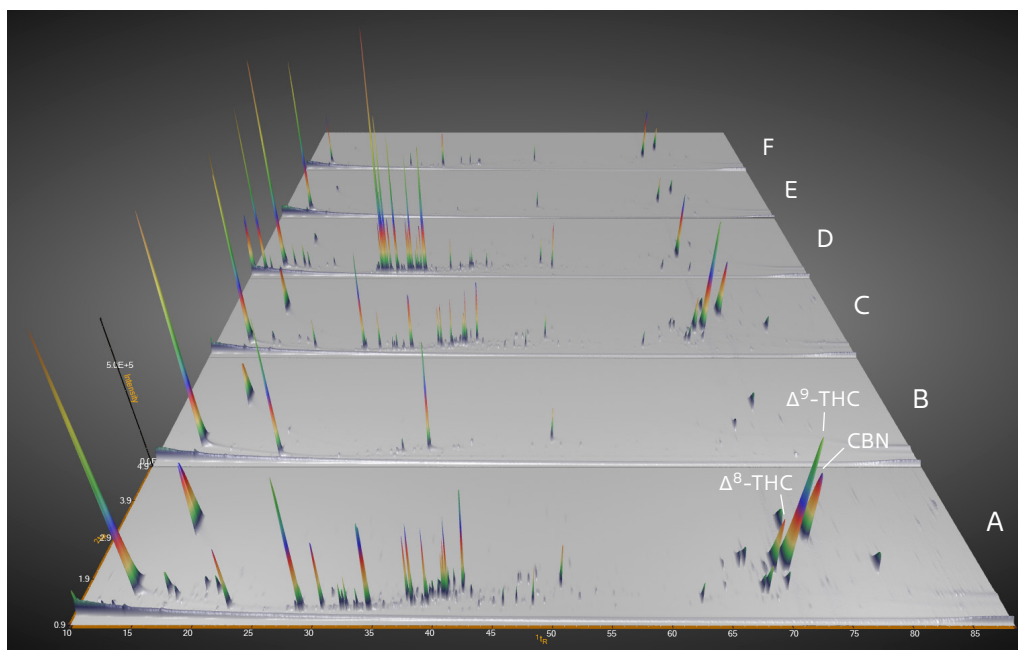
**Figure 2**

Expansion of part of Figure 1, showing how  $\beta$ -fenchol and fenchone would co-elute in a one-dimensional separation.

## 2. Comparison of sample composition

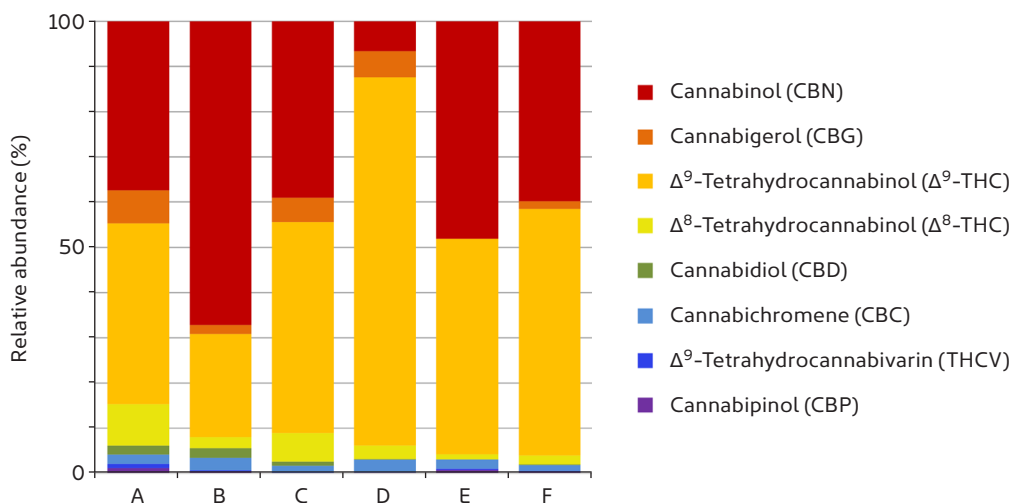
Figure 3 compares the GC $\times$ GC-TOF MS profiles of the six cannabis extracts, and shows that they exhibit significant differences in composition. These differences are also illustrated by the relative abundances of cannabinoids, determined using FID detection (Figure 4).

In the six samples examined, the principal psychoactive ingredient of cannabis,  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), dominates the cannabinoid composition (as is typical of cannabis for recreational use). This is especially true for extract D, which unlike the other samples contains a relatively low proportion of the  $\Delta^9$ -THC degradation product cannabiniol (CBN). The high proportion of CBN in the other extracts makes it highly likely that these samples have been exposed to the air for a considerable period.



**Figure 3**

Comparison of GC $\times$ GC-TOF MS surface charts for the six cannabis extracts, displayed in ChromSpace<sup>®</sup> software.

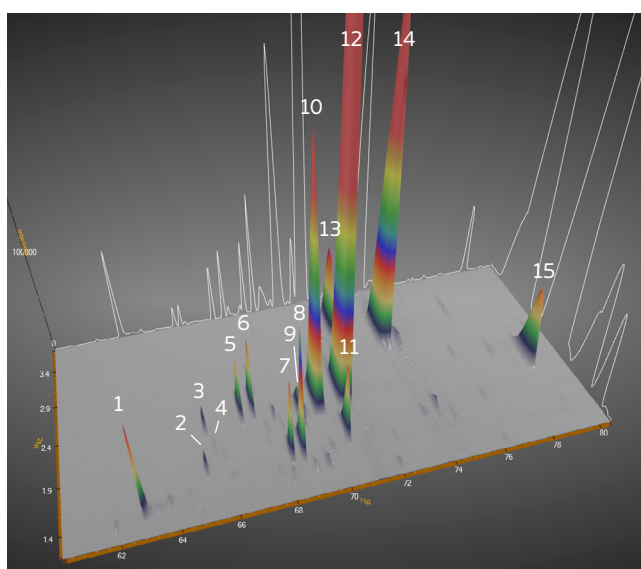


**Figure 4**

Relative abundance of major cannabinoids for extracts A–F using peak areas from GC×GC–FID data files.

### 3. Identification of minor cannabinoids

Although  $\Delta^9$ -THC,  $\Delta^8$ -THC, CBN and CBD are the primary cannabinoids studied, approximately 70 compounds of this class have been identified in cannabis,<sup>[2]</sup> and the enhanced separation of GC×GC combined with TOF MS enables identification of a wide range of these. As an example of this capability, the cannabinoid region of the GC×GC–TOF MS surface plot for extract A is shown in Figure 5, confirming the presence of at least 15 cannabinoids.



- 1 Cannabipinol (CBP)
- 2 Cannabicitran
- 3  $\Delta^9$ -Tetrahydrocannabivarin (THCV)
- 4 Unknown
- 5 Cannabichromene (CBC)
- 6 Cannabidiol (CBD)
- 7 Unknown
- 8 Cannabicyclonone (CBCON)
- 9 *exo*-Tetrahydrocannabinol (*exo*-THC)
- 10  $\Delta^8$ -Tetrahydrocannabinol ( $\Delta^8$ -THC)
- 11 Unknown
- 12  $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC)
- 13 Cannabigerol (CBG)
- 14 Cannabinol (CBN)
- 15 Methoxytetrahydrocannabinol

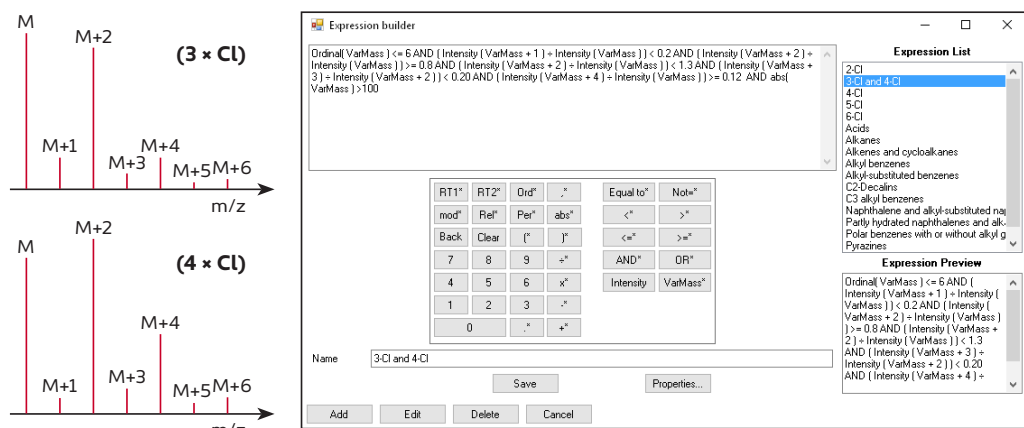
**Figure 5**

Cannabinoids identified in extract A. Note: "Unknown" refers to cannabinoids that were not present in either the NIST 17 database or the Cayman Spectral Library.

#### 4. Use of scripts to identify pesticides

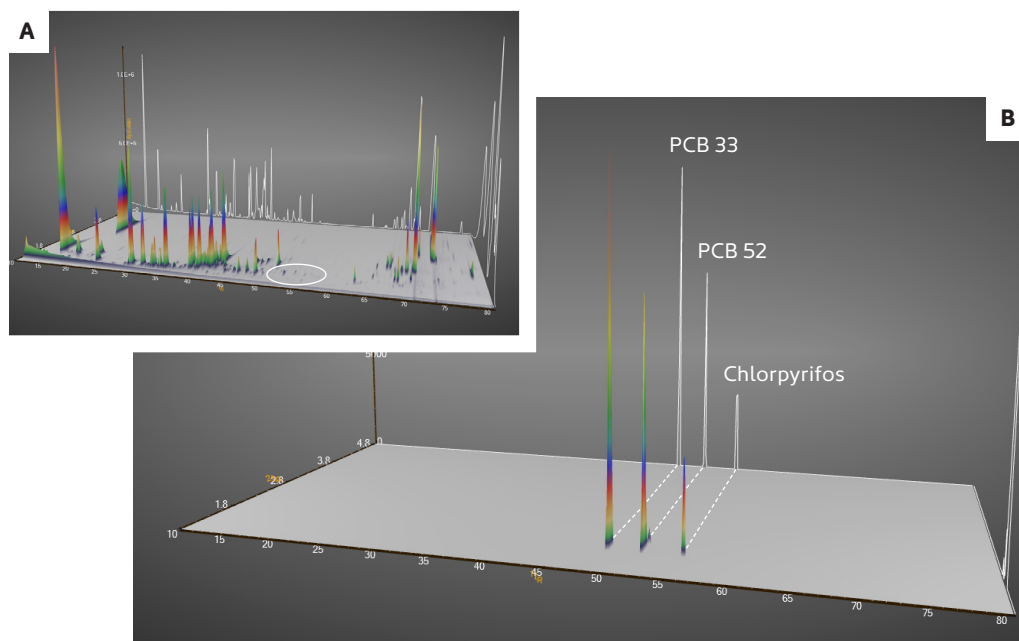
Scripting expressions are extremely useful to filter complex GC-MS or GC×GC-MS data and identify specific compounds and chemical classes. The ChromSpace® software used in this study includes a simple expressions builder, with preconfigured buttons and error flagging to allow novices to effortlessly create scripts.

Figures 6 and 7 show how this scripting function can be applied to the identification of polychlorinated pollutants in cannabis samples, by flagging up compounds containing the distinctive (3 × Cl) or (4 × Cl) isotope patterns in their mass spectra.



**Figure 6**

Scripting expression to identify polychlorinated compounds based on the isotope fingerprints from three or four chlorine atoms.



**Figure 7**

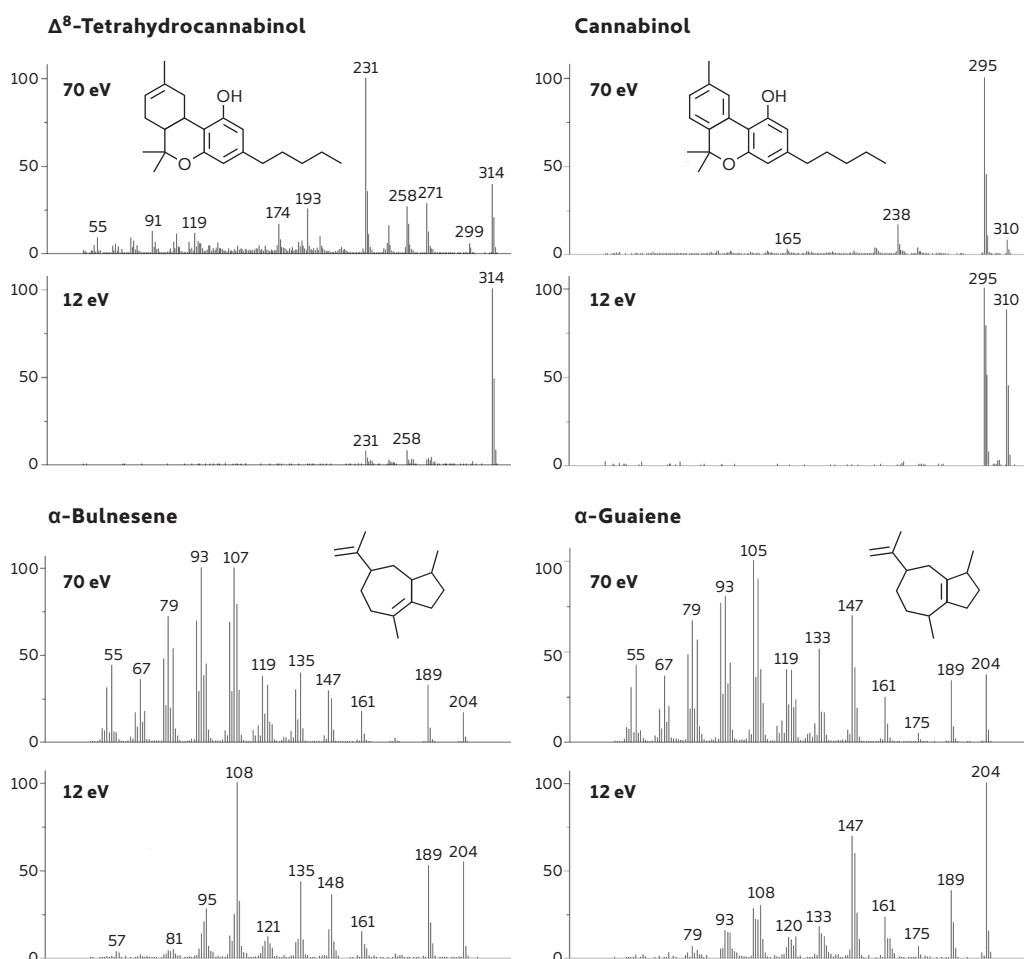
Detection of polychlorinated compounds in extract A (circled) using the scripting expression in Figure 6. (A) TIC surface plot. (B) Filtered surface plot showing only those peaks passing the scripting criteria.

## 5. Tandem Ionisation for confident identification

Confident screening of cannabis composition can be challenging, because many constituents are either not present in commercial spectral libraries, or have very similar 70 eV electron-ionisation (EI) mass spectra. Tandem Ionisation<sup>®</sup> is of great value in such cases, by allowing fast switching between conventional 70 eV EI and low-energy 'soft' EI for improved isomer speciation (without an inherent loss in sensitivity). This approach allows simultaneous acquisition of two datasets without impacting lab workflow, and with perfectly aligned peaks for easy data navigation.

Figure 8 shows spectral comparisons for four compounds identified in the cannabis samples. The spectra of  $\Delta^8$ -THC and CBN clearly demonstrate the reduced fragmentation and enhanced molecular ions in the 12 eV spectra, leading to higher signal-to-noise ratios for target peaks, and improved selectivity due to the lower incidence of commonly-encountered smaller fragment ions.

Another benefit of Tandem Ionisation<sup>®</sup> is illustrated by the isomeric terpenoids  $\alpha$ -bulnesene and  $\alpha$ -guaiene, which have very similar 70 eV spectra that would make it difficult to achieve confident identification. However, the 12 eV spectra have distinct differences in ion ratios that allow easier speciation. Accordingly, a library of such spectra for key cannabis analytes would significantly speed up confident analyte identification.



**Figure 8**

Comparison of spectra at 70 eV (top) and 12 eV (bottom) for two cannabinoids and two terpenoids found in the cannabis extracts.

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## Conclusions

This study has shown that GC×GC–TOF MS/FID using the INSIGHT™ flow modulator provides the sensitivity, spectral quality and chromatographic resolving power necessary for confident analysis of complex cannabis extracts. Parallel detection enables both confident identification by TOF MS and robust quantitation by FID in a single run, while powerful data-mining tools simplify the search for target compounds.

In addition, Tandem Ionisation® adds an additional level of confidence in situations where 70 eV data alone cannot speciate similar compounds, without impacting laboratory workflow.

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

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## Acknowledgements

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## References

- [1] J. Cochran, J. Kowalski, S. Lupo, M. Misselwitz and A. Rigdon, High-quality analysis of pesticides in cannabis using QuEChERS, cartridge SPE cleanup, and GC×GC–TOFMS, *Restek Technical Article FFAR1950-UNV*, 2014, [www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff\\_FFAR1950-UNV](http://www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff_FFAR1950-UNV).
- [2] M.A. El Sohly and D. Slade: Chemical constituents of marijuana: The complex mixture of natural cannabinoids, *Life Science*, 2005, 78: 539–548, <http://dx.doi.org/10.1016/j.lfs.2005.09.011>.

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