White Paper 018
May 2018

Improving confidence in the quantitative analysis of cannabis terpenes using flow-modulated GC×GC–FID

This study demonstrates the use of flow-modulated GC×GC–FID to streamline profiling of cannabis terpenes and terpenoids. A key feature is the enhanced separation provided by GC×GC, which improves confidence in compound identity and data quality.

Introduction

The classification of terpenes and terpenoids is an important aspect of cannabis analysis, due to the distinctive aroma and flavour that they impart, as well as their contributions to physiological effects and psychoactivity. In the case of medical cannabis, specific terpene profiles are engineered by plant breeders in order to give the desired therapeutic effects.[1]

However, the separation and quantitation of these diverse compounds can be challenging. Conventional GC–FID or GC–MS results in the abundance of important terpenes being over-estimated, due to the co-elution of similar compounds or oxygenated derivatives, and poor confidence in data quality.

Comprehensive two-dimensional gas chromatography (GC×GC) offers significant advantages over conventional chromatography for such analyses, with its vastly expanded separation space and the added benefit of highly structured groupings of compounds. This improved separation allows common co-elutions to be resolved without the need for expensive mass spectrometers or complicated deconvolution algorithms that may not correctly apportion the analyte peak area.

Here we demonstrate the fast and efficient profiling of cannabis terpenes and terpenoids, using reverse fill/flush flow modulation for robust, repeatable and affordable GC×GC–FID, combined with simple but effective data-processing workflows.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com
www.sepsolve.com

Experimental

Samples: Two calibration mixtures (Terpene Standards A and B, o2si, Charleston, SC, USA) were prepared at 5–500 ppm in dichloromethane. The resulting calibration curves were used to determine the terpene and terpenoid content of four cannabis oils – 'Pineapple Express', 'Strawberry Banana', 'Candy Kush' and 'CBD Yummy'.

GC×GC: Modulator: INSIGHT™ flow modulator (SepSolve Analytical). 2D column set: 1st dimension: Rtx®-35 MS; 2nd dimension: Rtx®-Wax.

FID: H_2 flow: 30 mL/min; Air flow: 300 mL/min; Temperature: 300°C.

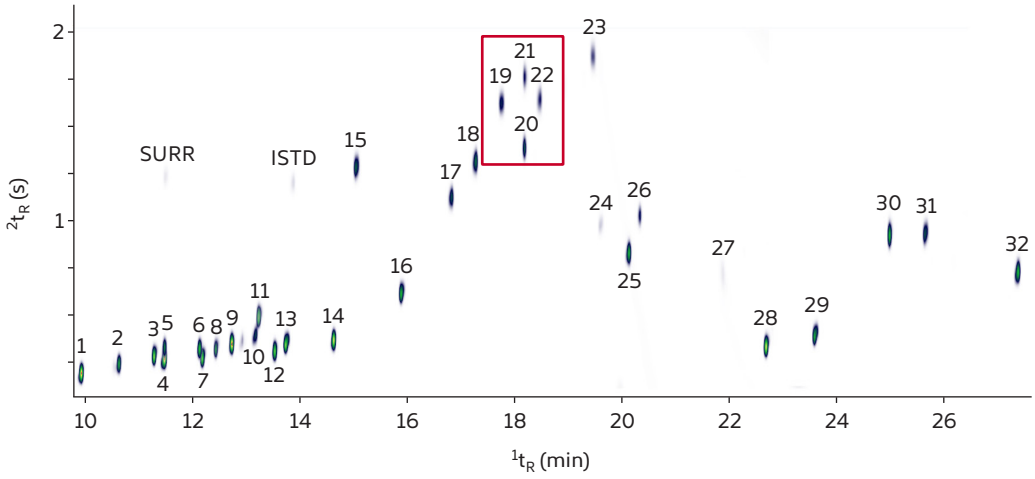
Software: ChromSpace® GC×GC software for full instrument control and data processing.

Please contact SepSolve for full analytical parameters.

Results and discussion

1. Separation of terpenes by GC×GC–FID

The enhanced separation provided by GC×GC enables all terpenes in both standards to be resolved (Figure 1). In contrast, conventional GC–FID or GC–MS can result in the abundance of important compounds being over-estimated, due to the co-elution of similar compounds or oxygenated derivatives. An excellent



1	α-Pinene	12	Cineole	23	Geraniol
2	Camphene	13	γ-Terpinene	24	*cis*-Citral
3	Sabinene	ISTD	1,4-Dichlorobenzene-d_4	25	Pulegone
4	β-Pinene	14	Terpinolene	26	*trans*-Citral
5	Myrcene	15	Linalool	27	δ-Valerolactam
SURR	4-Bromofluorobenzene	16	Fenchone	28	*trans*-Caryophyllene
6	α-Phellandrene	17	Isopulegol	29	α-Humulene
7	Carene	18	Menthol	30	*cis*-Nerolidol
8	α-Terpinene	19	Borneol	31	*trans*-Nerolidol
9	Limonene	20	α-Terpineol	32	Caryophyllene oxide
10	Dimethyloctatriene	21	Dihydrocarveol		
11	Isopropyltoluene	22	Citronellol		

Figure 1

Overlaid GC×GC–FID chromatograms of 32 cannabis terpenes in the two standard mixes. Full details are provided in Table 1, and an expansion of the boxed region is shown in Figure 2.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com

example of this enhanced separation is for α-terpineol and dihydrocarveol, which would have completely co-eluted in a 1D GC analysis (Figure 2).

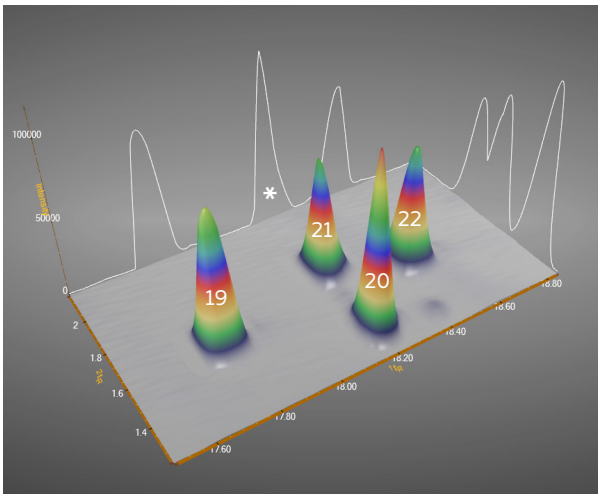


Figure 2

Expansion of part of Figure 1 in ChromSpace, showing the separation of α-terpineol (#20) and dihydrocarveol (#21). The ^1t_R projection indicates that a single peak (*) would have resulted from these two compounds in a 1D GC analysis.

2. Real-time data processing

Figure 3 shows how the ChromSpace instrument control and data-processing software used in this study allows automated classification of terpenes using stencil regions, prior to quantitative analysis. An additional feature of ChromSpace is the ability to save processing parameters in a method alongside the acquisition parameters. This enables data processing to be performed while the sample is acquiring, and saves time by allowing full results to be viewed as soon as the acquisition sequence has completed.

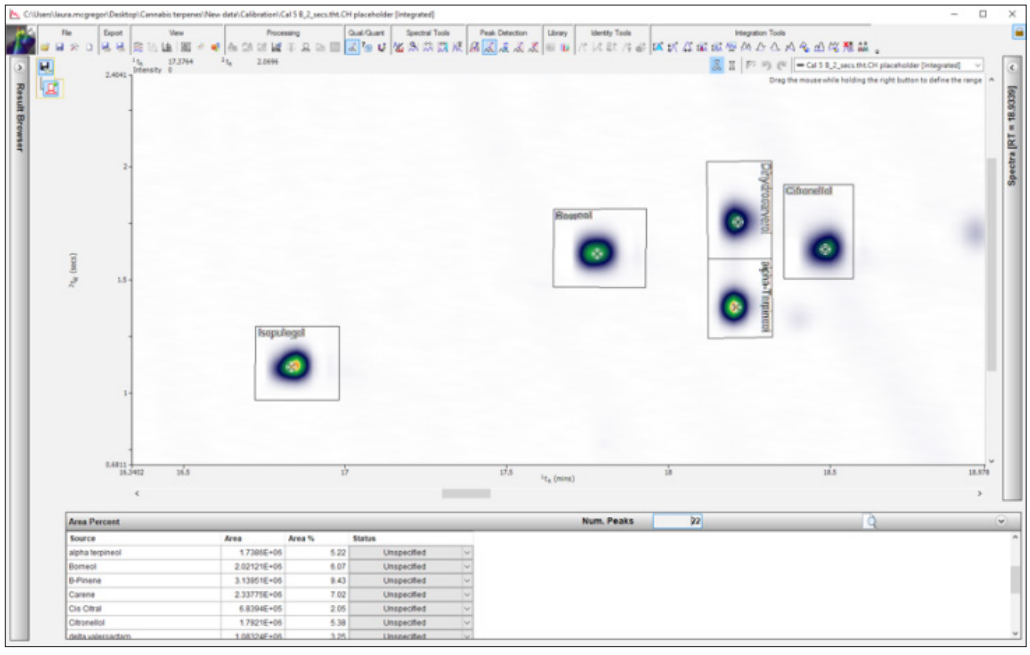


Figure 3

ChromSpace software showing the integration of stencil regions in the 200 ppm calibration standard. The peak table (lower panel) populates while the sample is running.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com

3. Linearity

Calibration curves were prepared for all terpenes from 5–500 ppm, with quantitation fully automated in ChromSpace using integration of the stencil regions. All R^2 values were found to be over 0.997 (see Table 1), indicating excellent linearity, and Figure 4 shows calibration curves for a selection of the cannabis terpenes. Quantitation results are easily exported from ChromSpace, with LIMS compatibility assisting with streamlined lab workflows.

No.	Compound	^1t_R (min)	^2t_R (s)	R^2	Concentration (ppm)			
					'Pineapple Express'	'Strawberry Banana'	'Candy Kush'	'CBD Yummy'
1	α-Pinene	9.9471	0.1857	0.9999	19.18	95.36	11.77	7.61
2	Camphene	10.6494	0.2450	0.9997	8.96	7.20	6.49	—
3	Sabinene	11.3037	0.2785	0.9996	—	—	—	—
4	β-Pinene	11.4936	0.2822	0.9997	135.87	122.62	118.37	111.14
5	Myrcene	11.4980	0.3251	0.9998	312.41	281.55	271.64	254.81
SURR	4-Bromofluoro-benzene	11.5202	1.2333	—	—	—	—	—
6	α-Phellandrene	12.1508	0.3221	0.9999	—	—	—	—
7	Carene	12.2036	0.2822	0.9997	—	—	—	—
8	α-Terpinene	12.4544	0.3194	0.9996	—	—	—	6.35
9	Limonene	12.7484	0.3459	0.9999	89.04	51.58	58.07	51.15
10	Dimethyloctatriene	13.1891	0.3937	0.9990	10.84	9.61	25.37	9.91
11	Isopropyltoluene	13.2527	0.5052	0.9998	—	—	—	—
12	Cineole	13.5537	0.3052	0.9999	4.01	5.60	5.41	5.67
13	γ-Terpinene	13.7558	0.3459	0.9996	3.53	4.23	3.71	3.47
ISTD	1,4-Dichloro-benzene-d_4	13.8946	1.2015	—	—	—	—	—
14	Terpinolene	14.6516	0.3612	0.9998	23.95	21.98	22.88	19.20
15	Linalool	15.0669	1.2883	0.9998	67.81	97.68	51.68	52.10
16	Fenchone	15.9068	0.6135	0.9999	4.33	—	5.28	4.51
17	Isopulegol	16.8468	1.1295	0.9994	—	—	—	—
18	Menthol	17.2952	1.3185	0.9998	—	—	—	—
19	Borneol	17.7720	1.6333	0.9995	11.76	11.27	9.11	9.83
20	α-Terpineol	18.1997	1.3905	0.9994	34.96	25.10	20.37	—
21	Dihydrocarveol	18.2090	1.7730	0.9992	—	—	—	—
22	Citronellol	18.4925	1.6516	0.9991	—	9.01	—	—
23	Geraniol	19.4828	1.8762	0.9992	—	—	—	—
24	*cis*-Citral	19.6222	0.9838	0.9986	—	—	—	—
25	Pulegone	20.1468	0.8316	0.9998	<LOQ	—	—	<LOQ
26	*trans*-Citral	20.3521	1.0385	0.9995	—	—	—	—
27	δ-Valerolactam	21.8909	0.7168	0.9974	—	—	—	—
28	*trans*-Caryophyllene	22.7088	0.3337	0.9997	252.93	345.25	289.56	116.78
29	α-Humulene	23.6032	0.3830	0.9995	91.03	152.07	80.42	34.08
30	*cis*-Nerolidol	24.9991	0.9300	0.9999	—	—	—	—
31	*trans*-Nerolidol	25.6690	0.9410	0.9998	15.63	9.47	3.56	4.16
32	Caryophyllene oxide	27.3981	0.7276	0.9993	8.71	10.97	10.77	1.35

Table 1

Quantitation results for key terpenes in the four cannabis oils.
SURR = Surrogate.
IS = Internal standard.
<LOQ = Value below limit of quantitation.
A dash (—) in the concentration column indicates that the compound was not detected.

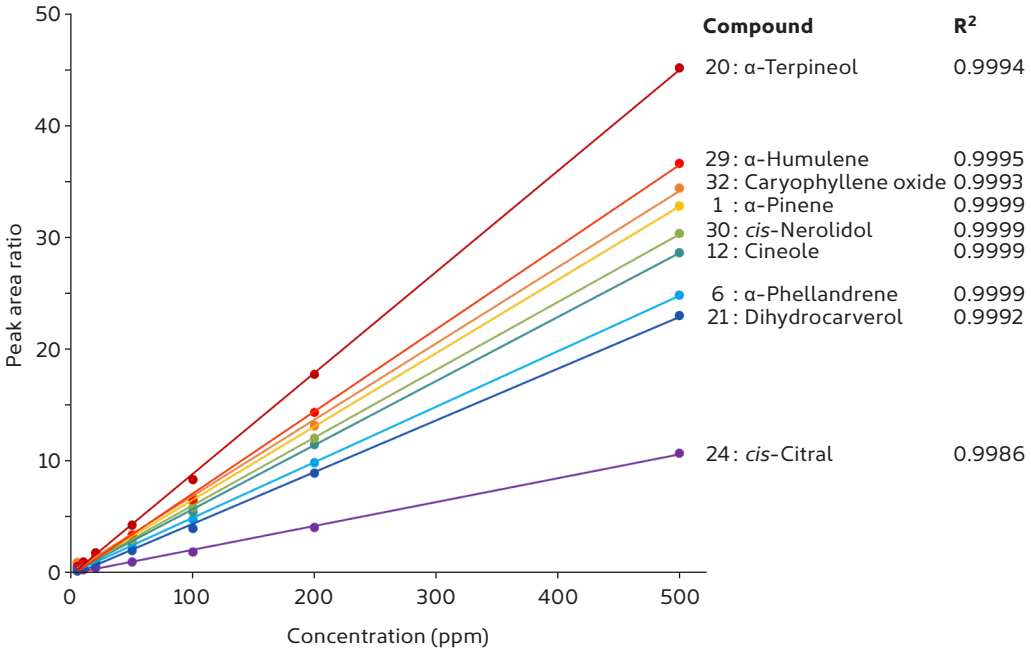


Figure 4

GC×GC–FID calibration curves for a selection of cannabis terpenes in the combined standard mix.

4. Comparison of cannabis aroma profiles

The terpene content of the four cannabis oils was also analysed using the same GC×GC–FID method, and Figure 5 shows the resulting surface charts. It is clear that the enhanced separation of GC×GC resolves co-elutions that would have occurred in conventional 1D GC analyses, with the associated over-estimation of analyte concentrations, and lowered data quality.

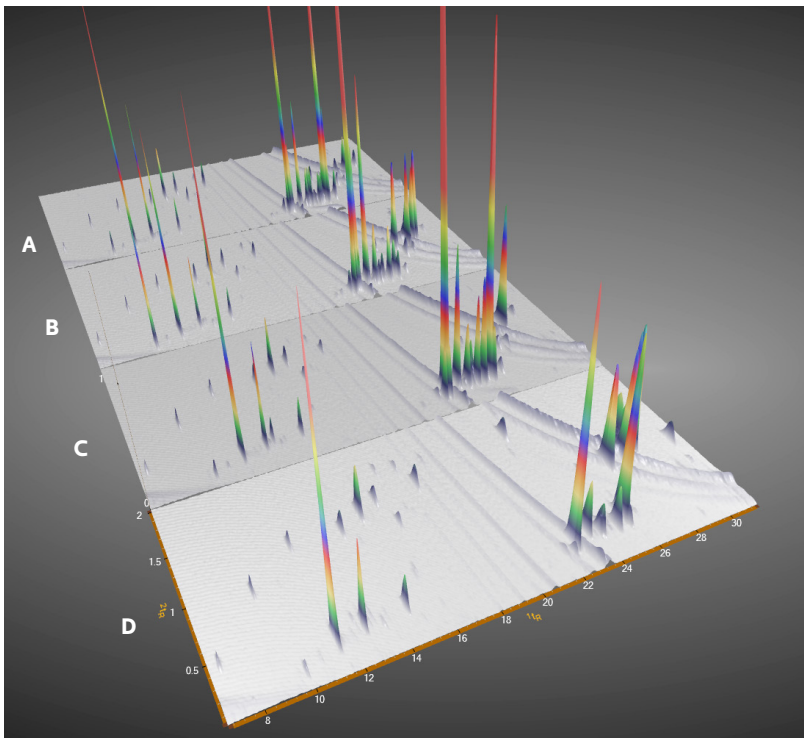


Figure 5

GC×GC–FID surface charts showing the terpene content of the four cannabis oils:
(**A**) 'Pineapple Express',
(**B**) 'Strawberry Banana',
(**C**) 'Candy Kush',
(**D**) 'CBD Yummy'.

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com

The GC×GC–FID calibration curves were used to quantify the terpene composition of the oils – values are listed in Table 1 and summarised using 'aroma wheels' (Figure 6). In general, the aroma profiles of all four samples are fairly similar, but there are a number of differences in terpene composition that are likely to cause differences in the perceived aroma and therapeutic effects:

- 'Strawberry Banana' contained higher levels of α-pinene (#1), known for its 'woody, pine, fresh' aroma[2] and bronchodilation effects.[3]
- 'Strawberry Banana' also contained higher levels of linalool (#15), known to give a 'floral, citrus, sweet' aroma.[2]

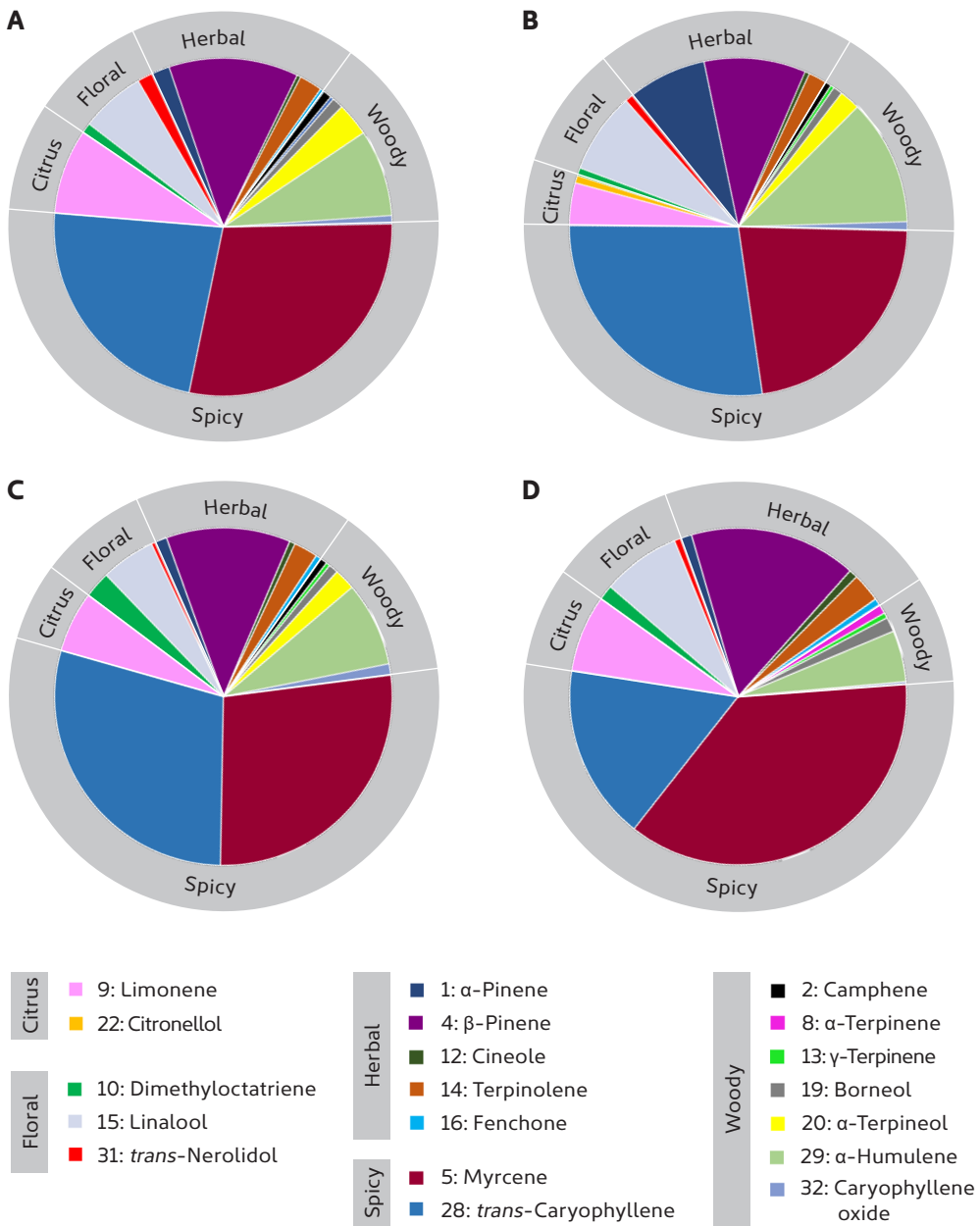


Figure 6

Aroma wheels generated for the four cannabis oils:[2] (**A**) 'Pineapple Express', (**B**) 'Strawberry Banana', (**C**) 'Candy Kush', (**D**) 'CBD Yummy'.

- 'Pineapple Express' contained almost double the concentration of limonene (#9) compared to the other samples. This compound is known to impart a more 'citrus, orange' aroma and a sweet taste.[2]
- 'CBD Yummy' contained lower levels of the sesquiterpenes *trans*-caryophyllene (#28) and α-humulene (#29) compared to the other oils, whereas 'Strawberry Banana' contained the highest levels, likely imparting a 'spicy, woody' aroma.[4] α-Humulene is also thought to suppress appetite and have anti-bacterial and anti-inflammatory effects,[4] while *trans*-caryophyllene is thought to possess anti-inflammatory and analgesic properties.[4]

5. Use of dual-channel GC×GC

An additional feature of this study was the use of a configuration employing two modulators within the same GC oven, enabling the simultaneous analysis of two samples for improved productivity (Figure 7).

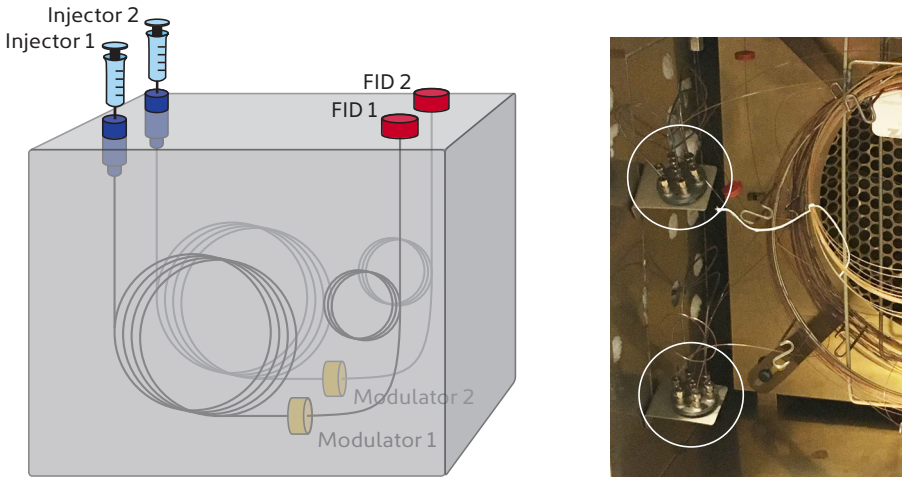


Figure 7

Diagram and photo of a dual injection system with two INSIGHT modulators (circled) configured within the same GC oven.

Conclusions

This study has demonstrated that GC×GC–FID with ChromSpace® data processing can provide:

- The enhanced separation necessary for robust quantitation of terpenes and terpenoids, overcoming co-elution issues experienced in 1D GC.
- Streamlined workflow and simplified training requirements, through full instrument control and real-time data processing using ChromSpace GC×GC software.
- Doubled sample throughput with dual injection, providing a swift return on investment.

- Lower running (and capital) costs through the use of INSIGHT™ flow modulation. The INSIGHT modulator is compatible with most popular gas chromatographs, and is supplied with everything required to start using GC×GC immediately – allowing it to be retrofitted to existing GCs or purchased as part of a new system.
- Simple upgrades to existing GCs by retrofitting INSIGHT.

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

Acknowledgements

SepSolve Analytical gratefully acknowledges David Mathis (Bonafides Laboratory Inc., CO, USA) for analysis of the samples and valuable insights into cannabis testing, and Restek Corporation for supplying the columns.

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] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 15 April 2018).

[3] E.B. Russo and F. Grotenhermen, *The Handbook of Cannabis Therapeutics: From Bench to Bedside*, Routledge, 2006.

[4] Leafly (cannabis information resource), www.leafly.com/news/cannabis-101/humulene-caryophyllene-and-trans-nerolidol-what-are-the-benefits (accessed on 19 April 2018).

D0038_1_020518

SepSolve Analytical Ltd
T: +44 (0)1733 669222 (UK) +1 888-379-383 (US)
E: hello@sepsolve.com

www.sepsolve.com