Quantitation of residual solvents and terpenes in cannabis products – a combined approach

This study describes a combined approach for fast, targeted screening of residual solvents and key terpenes, as well as a discovery route for enhanced separation of terpenes and terpenoids and greater insight into cannabis aroma.

Introduction

Laboratories seeking to maximise productivity in the analysis of cannabis products face numerous challenges due to sample diversity and complexity, as well as the vast array of testing requirements.

One such example is the analysis of residual solvents – chemicals used in the manufacturing process (e.g. of cannabis concentrates) that may remain in the final product. These solvents can be harmful to consumers, making it an important aspect of cannabis testing and the subject of new regulations. In fact, in the state of California, it is now a legal requirement for cannabis products to undergo residual solvent testing,[1] to ensure products do not exceed the defined action levels.

On the other hand, 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.[2] Consequently, plant breeders will often attempt to engineer cultivars with specific terpene profiles, in order to encourage particular traits.[3] Robust terpene profiling is therefore necessary for accurate product descriptions and labelling.

Most laboratories adopt one-dimensional gas chromatography coupled with mass spectrometry (GC–MS) in an attempt to tackle these challenges. However, this may not provide adequate separation of terpenes and terpenoids, resulting in numerous co-elutions that interfere with the compounds of interest and cause difficulties in quantitation. Furthermore, separate methods or even separate systems are typically required for the analysis of specific target classes, e.g. residual solvents or terpenes.

Here, we describe a combined approach for fast, targeted screening of both residual solvents and major terpenes, as well as a discovery route for enhanced separation of terpenes and terpenoids and greater insight into cannabis aroma. The system utilises gas chromatography with parallel detection by flame ionisation and time-of-flight mass spectrometry, as well as an optional secondary separation (using GC×GC) to resolve complex terpene profiles and provide improved flavour interpretation. We will demonstrate how this approach can not only maximise productivity, but also improve data quality.

Experimental

The instrument configuration used in this study is illustrated in Figure 1. The system provides two channels for:

(A)   Fast separation and quantitation by 1D GC–TOF MS:

► Category I and II residual solvents at California action limits (<12 minutes).

► 29 routinely-monitored terpenes from 0.2–10 ppm (<12 minutes).

(B)   GC×GC 'discovery' approach for enhanced separation of terpenes and terpenoids and greater insight into aroma profiles.
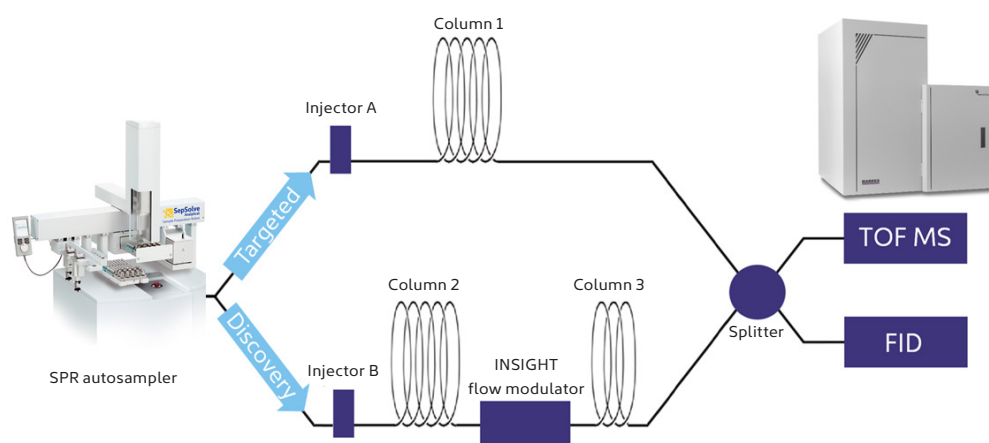

Figure 1
Schematic of the system configured for this study.

Samples: Residual solvents standards were prepared in dimethylacetamide, while a terpenes standard was prepared in methanol.

Sample preparation: Sample preparation robot (SepSolve Analytical). Residual solvents analysis was based on a full evaporation technique headspace method. For the terpenes analysis, liquid injection (1 µL) of extracts was performed.

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

FID: H_2 flow: 30 mL/min; Air flow: 300 mL/min; Temperature: 280°C.

TOF MS: BenchTOF-Select™ (SepSolve Analytical).

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

Please contact SepSolve for full analytical parameters.

Results and discussion

Targeted analysis of residual solvents

A fully automated headspace GC–TOF MS method was developed for the quantitation of Category I and II residual solvents with an injection-to-injection run time of less than 12 minutes (Figure 2). In fact, the target compounds actually elute in <6 min, but the system was ramped to 280°C to remove any late-eluting interferents. Despite co-elutions, all target analytes could be resolved through the use of unique quantitation ions (see Figure 2 inset). Calibrations were performed for all residual solvents, with excellent linearity demonstrated by R^2 > 0.99 for all components, while the repeatability of this fully automated method was also shown to be excellent, with RSD <5% for peak area (Table 1).
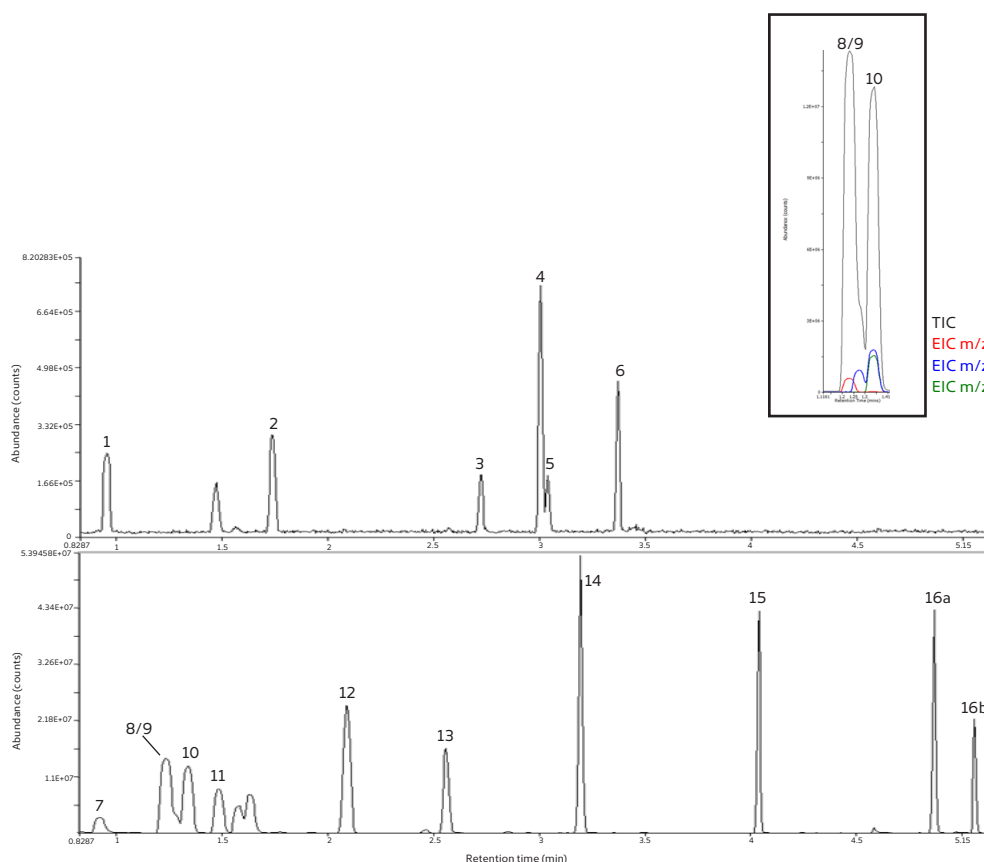

Figure 2

Headspace GC–TOF MS chromatograms for Category I (top) and Category II (bottom) residual solvents. The inset shows an example of how co-eluting compounds can be separated using unique ions. Peak identifications are listed in Table 1.

| Peak no.	Compound name*	Action level** (µg/g)	Calibration range (ppm)	R²	Peak area RSD (%)
1	Ethylene oxide	1.0	0.2–20	0.9977	3.35
2	Dichloromethane	1.0	0.2–20	0.9969	1.53
3	Chloroform	1.0	0.2–20	0.9973	4.07
4	Benzene	1.0	0.2–20	0.9988	4.03
5	1,2-Dichloroethane	1.0	0.2–20	0.9987	4.62
6	Trichloroethylene	1.0	0.2–20	0.9984	4.79
7	Methanol	3000	200–6000	0.9950	4.04
8	Pentane	5000	200–6000	0.9932	3.15
9	Ethanol	5000	200–6000	0.9987	3.56
10	Ethyl ether	5000	200–6000	0.9974	3.59
11	Acetone	5000	200–6000	0.9970	3.93
12	Hexane	290	200–6000	0.9993	3.99
13	Ethyl acetate	5000	200–6000	0.9957	4.34
14	Heptane	5000	200–6000	0.9925	4.94
15	Toluene	890	200–6000	0.9958	4.29
16a	*m/p*-Xylene	2170	300–12000	0.9914	4.12
16b	*o*-Xylene				3.88

Table 1

Validation data for targeted analysis of residual solvents, showing excellent repeatability and linearity. *Note: Butane and propane could also be detected by the method, but due to logistical issues working with the reference standard (low boiling point resulted in loss of reference standard at ambient temperature), calibration data was not acquired. **California action limit as defined in ref. [1].

Targeted analysis of key terpenes

Fast GC–TOF MS for the quantitation of 29 routinely-monitored terpenes was developed with an injection-to-injection run time under 12 minutes (Table 2). Figure 3 shows a GC–TOF MS chromatogram for the terpene standard mix, highlighting the excellent peak shape achieved. A six-point calibration series (from 0.2 to 10 ppm) was also prepared, with all R^2 values >0.99.
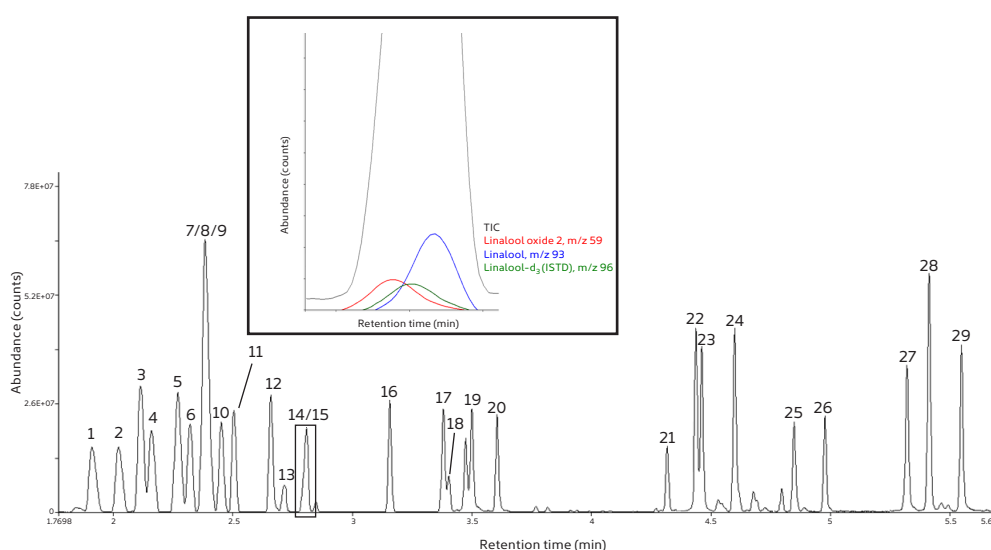
Figure 3

Fast GC–TOF MS for routine monitoring of major cannabis terpenes. Inset shows the separation of target compounds using unique EICs.

Peak no.	Compound name	Quant ion	R^2	Peak area RSD (%)
1	α-Pinene	93	0.9979	2.5
2	Camphene	93	0.9944	2.3
3	β-Myrcene	93	0.9988	4.7
4	β-Pinene	93	0.9989	3.8
5	3-Carene	93	0.9981	4.3
6	α-Terpinene	93	0.9992	4.9
7	Limonene	93	0.9993	2.4
8	p-Cymene	119	0.9981	2.6
9	Ocimene	93	0.9982	5.7
10	Eucalyptol	154	0.9981	4.6
11	γ-Terpinene	93	0.9971	4.4
12	Terpinolene	93	0.9983	4.7
13	Linalool oxide 1	59	0.9990	6.3
14	Linalool oxide 2	59	0.9969	6.3
15	Linalool	93	0.9994	4.8
16	Isopulegol	68	0.9957	4.5
17	α-Terpineol	59	0.9956	5.0
18	Citronellol	69	0.9919	4.1
19	Nerol	69	0.9958	3.9
20	Geraniol	69	0.9992	6.6
21	Damascenone	69	0.9928	4.6
22	trans-Caryophyllene	133	0.9920	4.1
23	β-Damascone	177	0.9939	5.5
24	Humulene	93	0.9918	3.9
25	cis-Nerolidol	69	0.9914	4.4
26	trans-Nerolidol	69	0.9902	4.5
27	Caryophyllene oxide	93	0.9927	4.5
28	Guaiol	119	0.9919	3.4
29	α-Bisabolol	119	0.9927	4.4

Table 2
Validation data for targeted analysis of terpenes showing excellent repeatability and linearity.

'Discovery' approach for greater insight into terpene profiles

A discovery-based GC×GC method was also developed to expand out from the list of 29 routinely-monitored terpenes. The enhanced separation of GC×GC and highly structured groupings give improved confidence in profiling terpenes and terpenoids. Figure 4 shows the comparison of two cannabis strains, highlighting the improved level of detail that can be delivered by this approach.
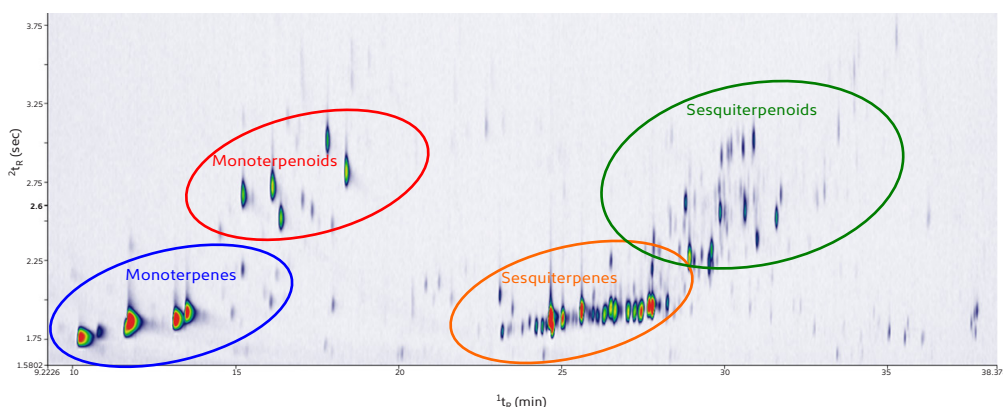

Figure 4

GC×GC–TOF MS colour plot of a Blueberry Kush extract showing the enhanced separation of terpenes and terpenoids by the discovery approach.

The configuration used in this study enables both GC×GC–FID and GC×GC–TOF MS data to be acquired. Flame ionisation detection (FID) is the 'gold standard' for quantitation, while time-of flight mass spectrometry (TOF MS) enables confident identification of both targets and non-targets – meaning that this approach can look beyond the terpenes to investigate other aroma-active species. For example, Figure 5 shows the identification of the ester ethyl caprate (sweet, waxy, fruity[4]) as well as the sesquiterpenoid trans-nerolidol (floral, green[4]), which would have co-eluted with the high-loading sesquiterpenes in a 1D separation, but is well-separated by GC×GC and confidently identified by BenchTOF MS. The same is true for the aromatic sesquiterpene α-curcumene (herbal, turmeric), which is again retained longer on the more polar stationary phase of the second column.

For more detail on the GCxGC–TOF MS 'discovery' approach to terpene profiling, please see our White Paper Comprehensive aroma profiling of cannabis using a discovery workflow.

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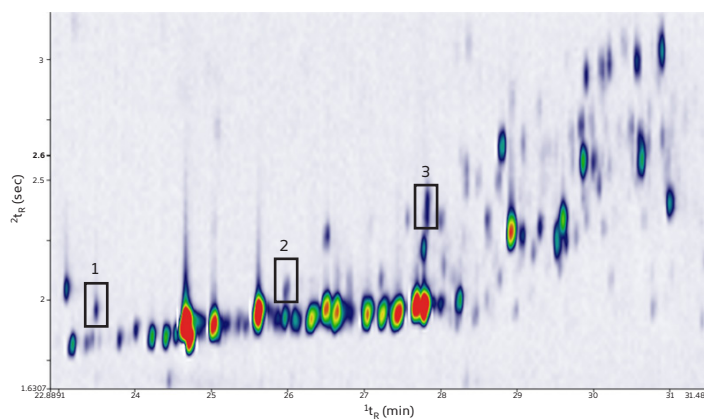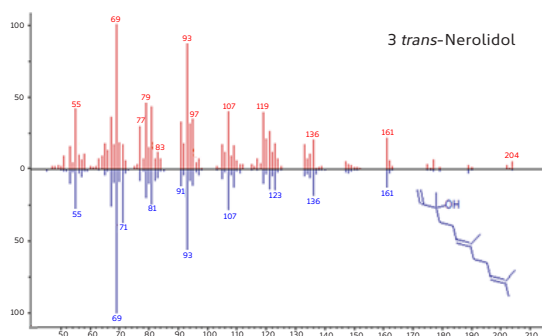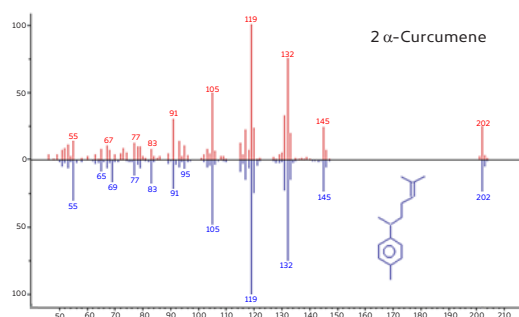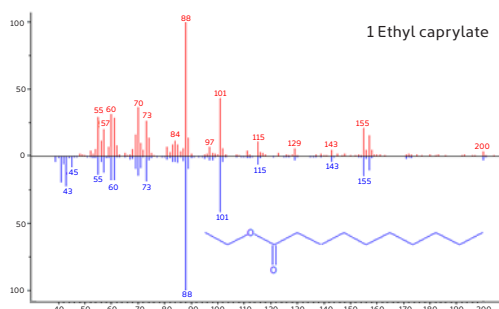A company of the SCHAUENBURG International Group

Figure 5

Expanded region of the GC×GC–TOF MS colour plot of a Blueberry Kush extract showing the separation and identification of aroma-active species using BenchTOF spectra (red) against the NIST17 library (blue).

Conclusions

The combined analytical approach described in this study enables analysts to both discover more and deliver more in the testing of cannabis products. The system offers numerous advantages, including:

► Fully automated sample extraction, standard addition, headspace sampling and injection using robotic tool change.

► Excellent reproducibility and linearity of residual solvents (Category I and II) and terpenes using a fast GC–TOF MS method.

► Enhanced separation by GC×GC for robust profiling of a wider range of terpenes and terpenoids, for increased precision in flavour interpretation and improved product labelling.

► Simple switching between 1D and 2D methods for high productivity and improved data quality in a single platform.

► Streamlined workflows and simplified training requirements, through instrument control and data processing using ChromSpace software for both 1D and GC×GC approaches.

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

References

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[4] The Good Scents Company Information System (search facility), www.thegoodscentscompany.com/search2.html (accessed on 6th April 2020).

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