APPLICATION NOTE AN136

Determination of Terpenes in Cannabis by Headspace GC-SQMS

INTRODUCTION

Hemp and marijuana are becoming more popular because of legalisation in multiple countries. The increase of this market also increases the offer of products and therefore the need to monitor different components. One of these components are cannabinoids or just short potency analysis, which can be found in our portfolio of Cannabis applications (AN091,92,93,130). This application focusses on terpenes analysis using GC-SQMS, for Terpene analysis using GC-FID see application note 133.

Terpenes are organic compounds in marijuana that provide the distinguished aroma and flavour. Every species or strain of marijuana has it's own distinguished profile and therefore it is a perfect method to analyse specific strains. These terpenes do not only provide flavour or aroma but also support other components on producing the desired effects, terpenes can help in producing a calming affect but can also give you more energy (energizing effect).

Figure 1 shows the SCION Instruments 436 GC with single quad (SQ) mass spectrometer together with the Scion HT3 Headspace sampler. On which this method is applicable to.

Figure 1. SCION HT3 Headspace Sampler together with the SCION Instruments 436 GC with SQ-MS.

Determination of Terpenes in Cannabis by GC-FID

EXPERIMENTAL

This analysis can be implemented on the 436-GC and the 456-GC platform. The analysis was performed on the Scion 456-GC analyser equipped with a single quad mass spectrometer and Scion HT3 Headspace autosampler.

There are more than one hundred terpenes known. For this application the focus is on the 21 most important components, which are shown in table 1.

Table 1: Terpenes components

Nr.	Component	Nr.	Component
1	α-Pinene	12	Terpinolene
2	Camphene	13	Linalool
3	β-Mycrene	14	Isopulegol
4	β-Pinene	15	Geraniol
5	Δ-3-Carene	16	β-Caryophllene
6	α-Terpinene	17	α-Humulene
7	d-Limonene	18	Nerolidol-1
8	P-Cymene	19	Guaiol
9	Eucalyptol	20	Caryphyllene oxide
10	Ocimene	21	α-Bisabolol
11	ɣ-Terpinene		

The focus of the calibration lies on these 21 components, the calibration curves for the terpenes standards were prepared between 0.5 and 16 µg/ml.

The Quality Control (QC) sample was made from 4 µg/ml for all components.

To analyse terpenes in marijuana an extraction has to be performed. 0.1g of the sample was added to 30mL of methanol before shaking vigorously for one minute. The extract was left to settle for 30 minutes before being filtered. 2 mL was then transferred into a 20 ml Scion headspace vial, ready for injection.

Please keep in mind that the sample preparation is different than the potency preparation where the sample is heated. Table 2 details the GC, MS and headspace parameters used throughout this method.

Table 2. Instrumentation operating conditions.

Injector	Splitless 50:1, 230 °C
Column	624-MS
Oven Program	50°C (1.0 min), 10°C/min to 250°C (2.0 min)
Carrier	Helium, 1.6 ml/min
Autosampler	Scion HT3
Software	MS work station/ HT3 Teklink
Oven temperature	150°C
Transfer line temperature	150°C
Sample temperature	120°C
Sample equilibrium time	30 min
MS transfer line temperature	250°C
Ion source temperature	200°C
Ionization mode	EI
Scan start	2.00
Scan mode	Full Scan

RESULTS

The precision of the method was obtained by seven consecutive injections containing 4 µg/ml of each component. While reviewing the data it is important to keep in mind that these values are on trace level, and that the headspace injection can cause an extra variance in the data.

It was shown that the RSD for the components lied between 2.0 and 7.0 %., which is really good for this method. The repeatability of each component can be found in table 3.

All the chromatographic peaks were identified with the NIST library and integrated. β-Mycrene and β-Pinene have the same quantification ion and almost have the same retention times, it is advised to check the integration of these components after analysis.

Figure 2 shows examples of these target peaks from a 4 µg/ml standard containing a mix of all the components.

Figure 2. Target compound example peaks.

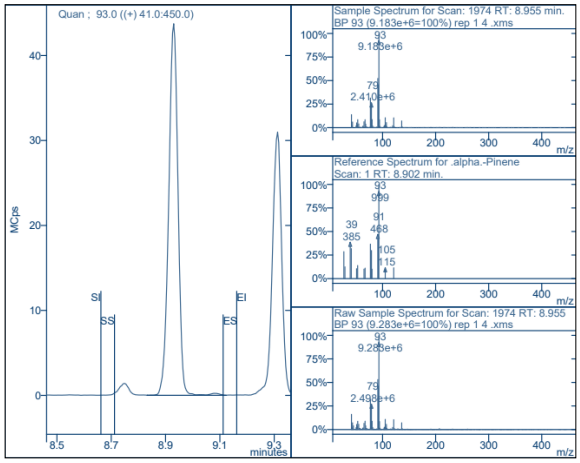
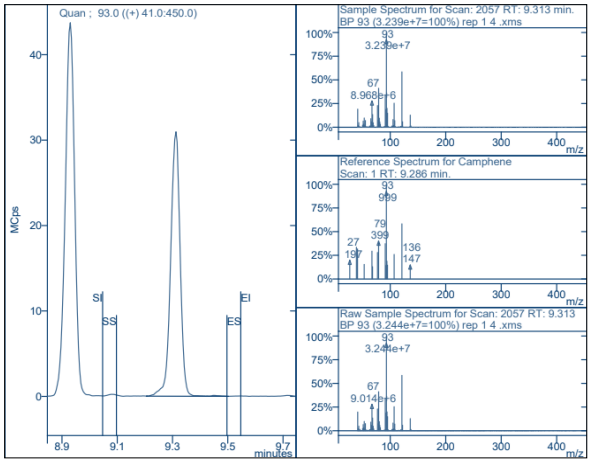
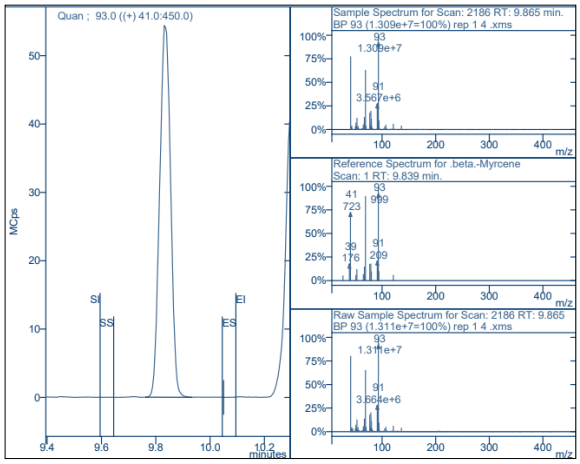
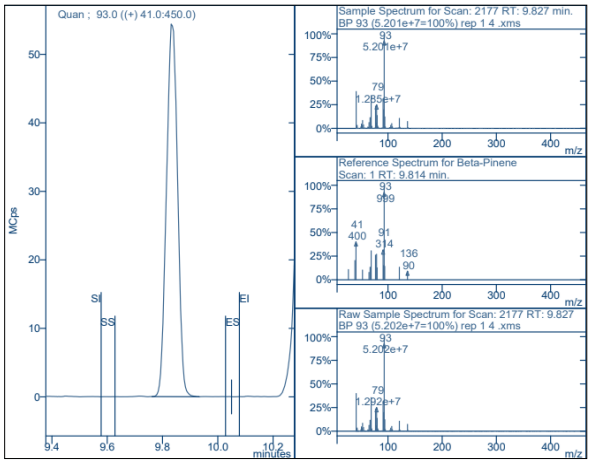
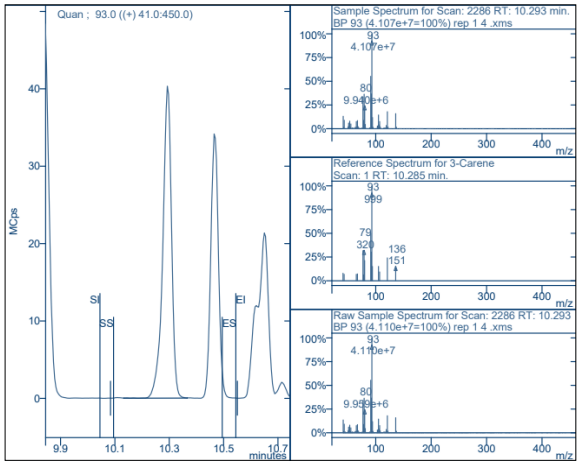
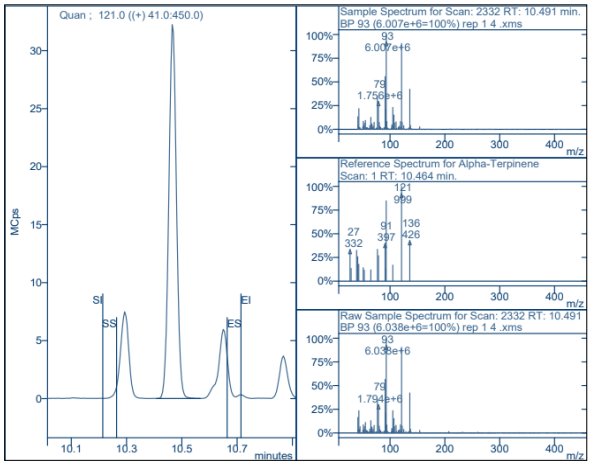
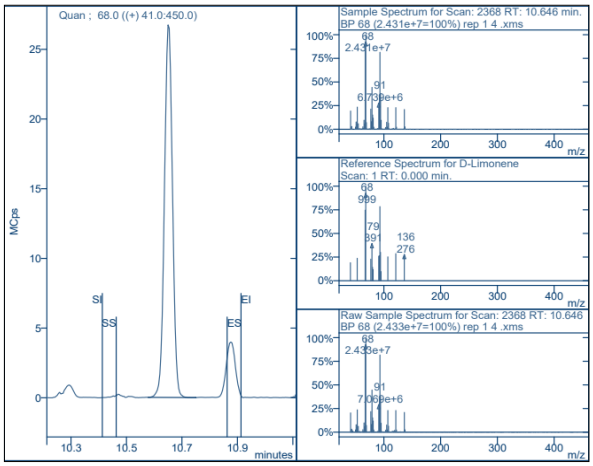
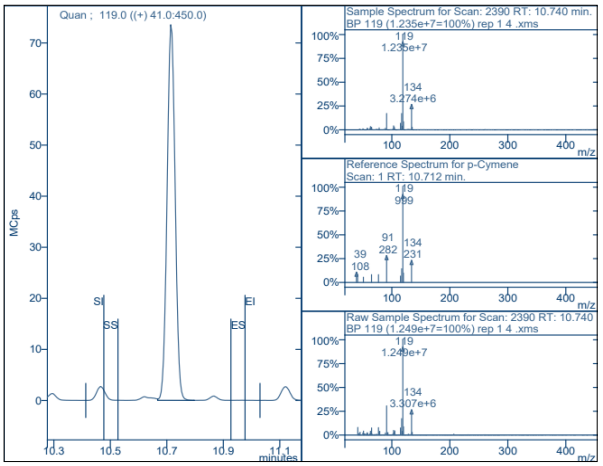
α-Pinene	Camphene
	
β-Mycrene	**β-Pinene**
	
Δ-3-Carene	**α-Terpinene**
	

Table 3. Target compound example peaks part 2.

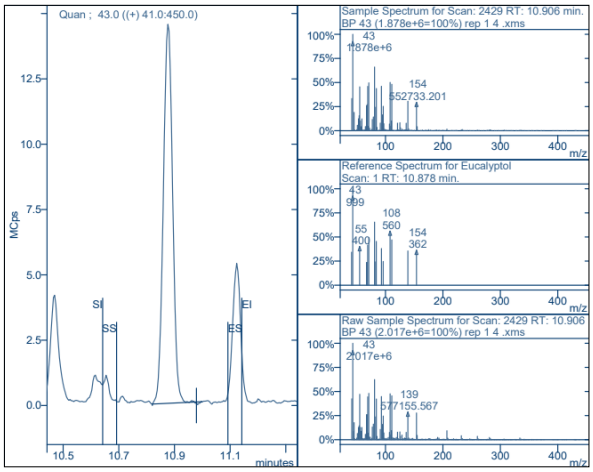
d-Limonene



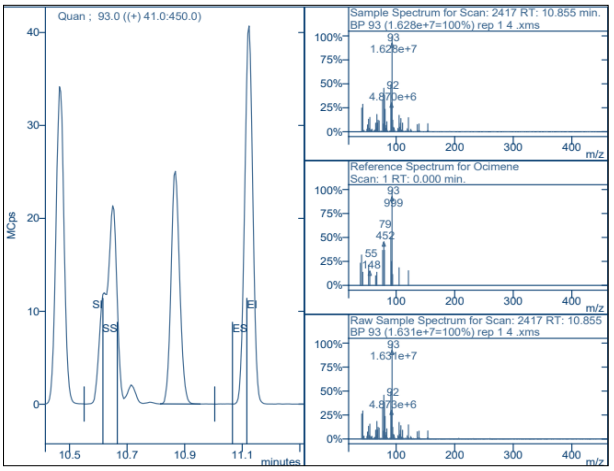
P-Cymene



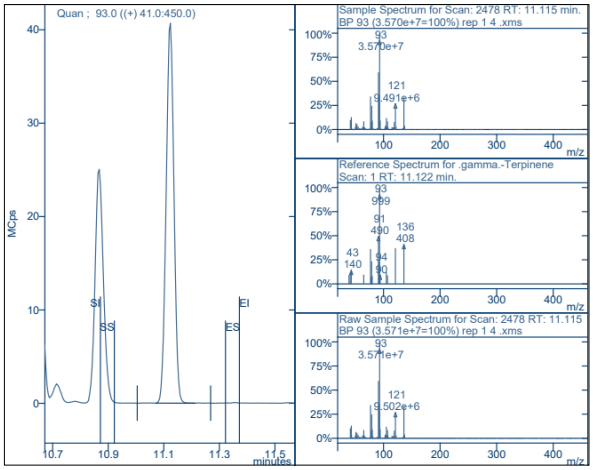
Eucalyptol



Ocimene



ɣ-Terpinene



Terpinolene

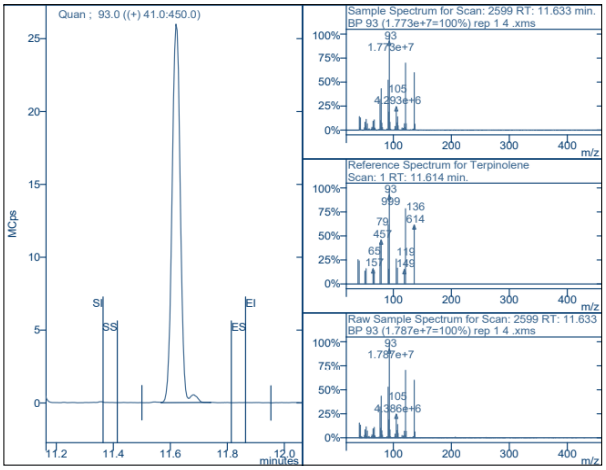


Table 3. Target compound example peaks part 3.

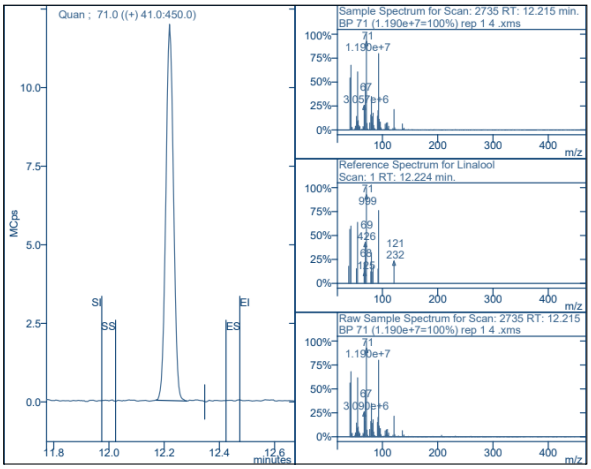
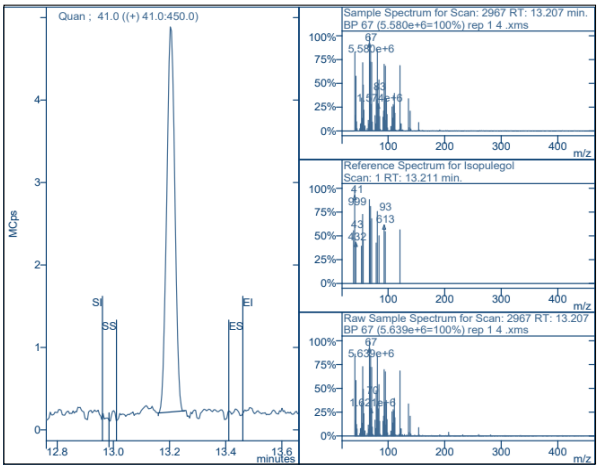
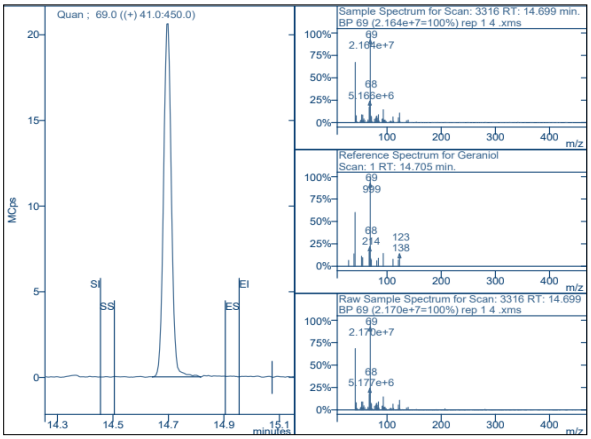
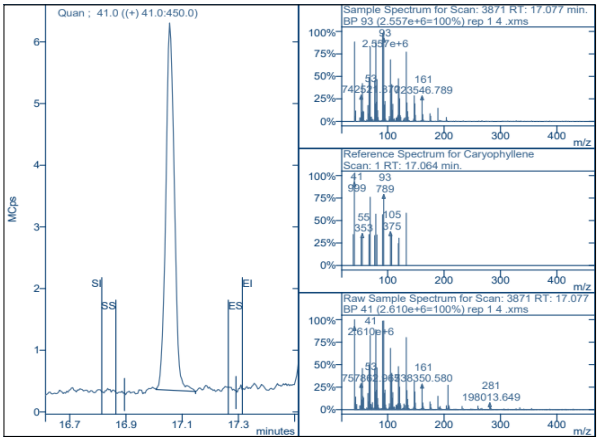
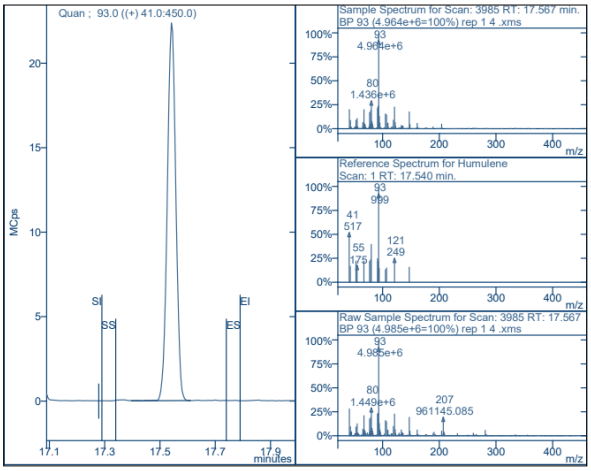
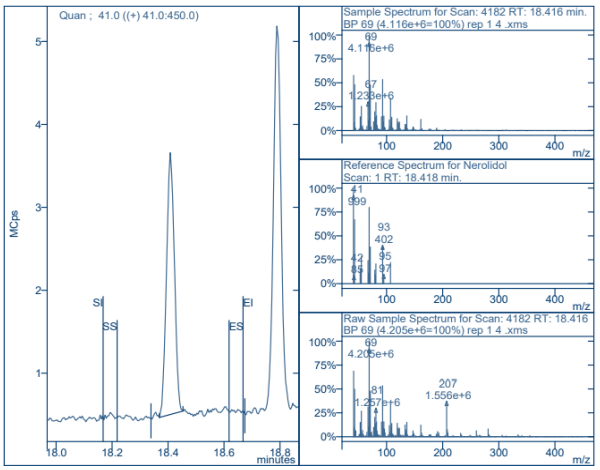
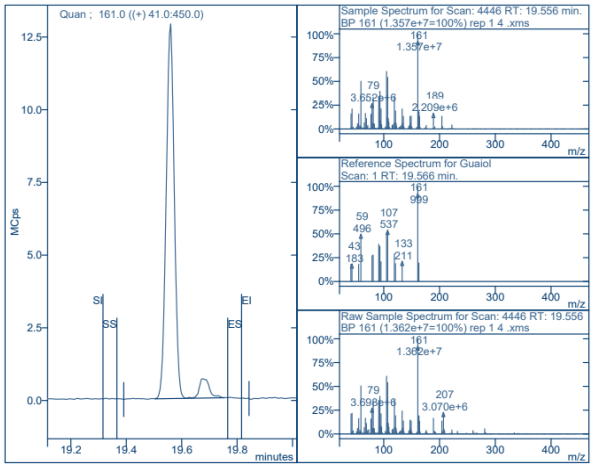
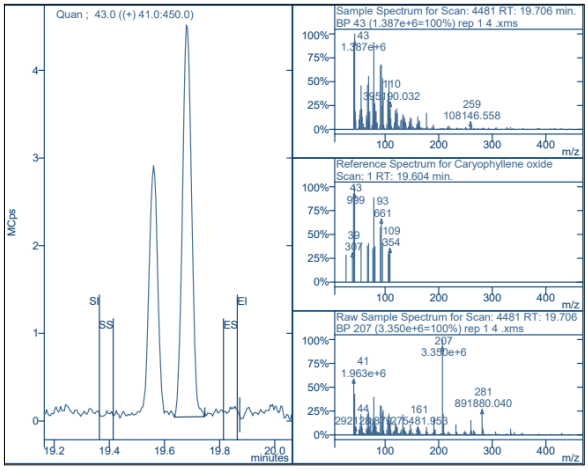
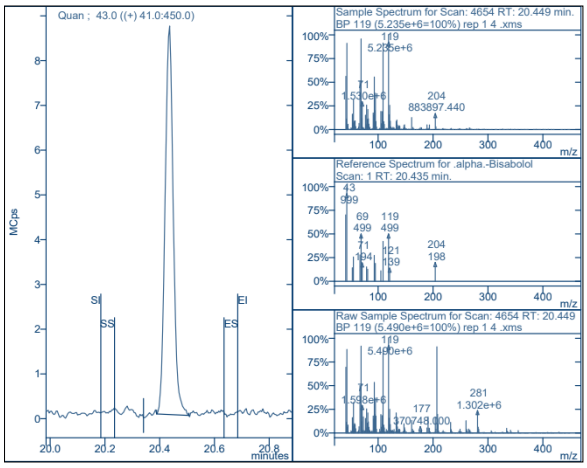
Linalool	Isopulegol
	
Geraniol	**β-Caryophllene**
	
α-Humulene	**Nerolidol-1**
	

Table 3. Target compound example peaks part 4.

Guaiol	Caryphyllene oxide
	
α-Bisabolol	
	

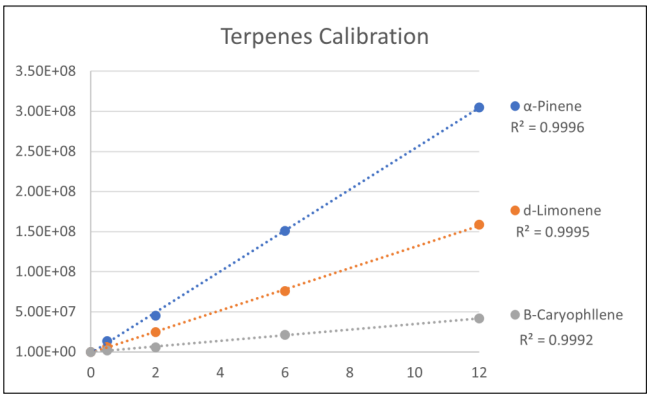


Figure 4: Three example calibration curves of the Terpenes standard.

The calibration curves for the Terpene standards were prepared between 0.5 to 16 µg/ml. Most components had a correlation coefficient (R2) of 0.999 or greater which is perfect for this application. Figure 4 shows an example of the calibration curve for three components.

From the linearity the limit of detection (LOD) and limit of quantitation (LOQ) was calculated.

The QC sample was analysed using seven injections, the average concentrations are shown in table 3 together with the LOD and LOQ.

After analysis of the marijuana extract it showed that the sample contained α-Pinene, Camphene, β-Mycrene, β-Pinene, Linalool, α-Humulene, Guaiol and α-Bisabolol. The concentration of these components are also shown in table 3

In this app note a sample volume of 2 ml is advised, when the components are below the LOD and LOQ it is possible to increase the sample volume as long if the total volume is 10 ml or another option is enrichment of the sample.

Table 3. Results of different Terpenes analysis.

Nr.	Component	R^2	LOD µg/ml	LOQ µg/ml	QC µg/ml	Repeatability %	Sample µg/ml
1	α-Pinene	0.9996	0.24	0.71	4.1	4.	15.1
2	Camphene	0.9999	0.12	0.35	4.3	3.93	0.39
3	β-Mycrene	0.9997	0.21	0.65	4.0	3.13	2.80
4	β-Pinene	0.9997	0.21	0.65	4.0	3.24	2.80
5	Δ-3-Carene	0.9992	0.32	0.98	3.7	3.85	-
6	α-Terpinene	0.9991	0.36	1.08	3.5	3.95	-
7	d-Limonene	0.9995	0.25	0.76	3.9	3.47	-
8	P-Cymene	0.9992	0.33	1.01	3.6	5.71	-
9	Eucalyptol	0.9999	0.11	0.33	3.8	4.54	-
10	Ocimene	0.9998	0.15	0.54	3.5	4.59	-
11	γ-Terpinene	0.9996	0.23	0.71	3.7	6.08	-
12	Terpinolene	0.9990	0.36	1.10	3.5	5.89	-
13	Linalool	0.9980	0.51	1.55	3.7	5.43	6.84
14	Isopulegol	0.9983	0.48	1.46	4.0	5.88	-
15	Geraniol	0.9964	1.21	3.66	4.3	4.67	-
16	β-Caryophllene	0.9992	0.33	1.00	4.0	7.02	-
17	α-Humulene	0.9985	0.46	1.38	3.5	6.38	4.53
18	Nerolidol-1	0.9969	1.31	3.96	3.6	3.13	-
19	Guaiol	0.9982	0.84	2.55	3.3	4.97	8.95
20	Caryphyllene oxide	0.9999	0.28	0.85	3.6	2.09	-
21	α-Bisabolol	0.9935	1.98	5.99	4.4	2.63	38.1

CONCLUSION

The Scion 4X6-GC analyser equipped with a split/spitless injector, Scion Instruments column, SQ-MS and HT3 Headspace Sampler is capable of analysing Terpenes from cannabis products in a qualitative and quantitative way.

The method developed is well suited for Terpenes analysis. It should be taken into account that the concentration of Terpenes in the sample can be very low and that a increase of sample volume or enrichment is necessary.

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