APPLICATION NOTE AN133

Determination of Terpenes in Cannabis by GC-FID

KEY WORDS : Terpenes, Cannabis Analysis, Hemp, Marijuana, Aroma, Flavour

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.

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 4X6 GC's which this method is applicable to.

Figure 1. SCION Instruments 4X6 GC

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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 an FID and 8400 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 200 µg/ml.

The Quality Control (QC) sample was made from 20 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 GC 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 parameters used throughout this method.

Table 2. Analytical conditions

Injector	Splitless 10:1, 250 °C
Column	624-MS
Oven Program	50°C (1.0 min), 10°C/min to 250°C (2.0 min)
Carrier	Helium, 1.5 ml/min
Detector	FID with ceramic jet, 300°C Air: 300 ml/min, Fuel gas (H_2): 30 ml/min, Make up (N_2): 30 ml/min
Inj. Volume	1.0 µl
Autosampler	8400
Software	Compass CDS

RESULTS

The precision of the method was obtained by ten consecutive injections containing 20 µg/ml of each component. Most standardised methods state that the RSD% of an accurate method must be no more than 2%. It was shown that each component except one had an RSD below 1%, which is an excellent result. The repeatability of each component can be found in table 3.

When looking at the chromatogram of figure 2 it is shown that all components are base line separated, except α-Terpinene, d-Limonene, P-Cymene and Eucalyptol. However, these components have a resolution of 1.2 and 1.1 so accurate quantification can be performed.

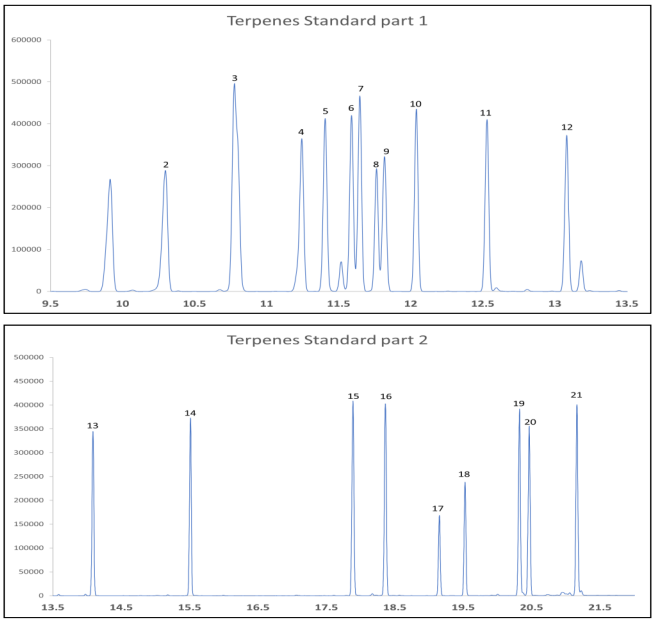

Figure 2: Chromatogram of the Terpenes standard.

Table 3. Results of different Terpenes analysis.

Nr.	Component	R^2	LOD µg/ml	LOQ µg/ml	QC µg/ml	Repeatability %	PTF	Sample µg/ml
1	α-Pinene	0.9999	0.75	2.3	20.5	0.41	0.65	14.1
2	Camphene	0.9999	0.76	2.3	20.6	0.35	0.71	-
3	β-Mycrene	0.9999	0.77	2.3	21.0	0.38	1.73	11.9
4	β-Pinene	0.9999	0.81	2.5	21.0	0.38	0.67	-
5	Δ-3-Carene	0.9999	0.83	2.5	21.1	0.38	0.97	-
6	α-Terpinene	0.9999	1.0	3.1	21.1	0.38	1.02	< 0.5
7	d-Limonene	0.9999	0.74	2.2	21.3	0.46	0.85	-
8	P-Cymene	0.9998	1.2	3.6	21.3	0.38	1.02	-
9	Eucalyptol	0.9998	1.4	4.1	21.7	0.46	0.93	-
10	Ocimene	0.9999	0.91	2.8	21.3	0.33	0.94	-
11	ɣ-Terpinene	0.9999	0.85	2.6	21.4	0.31	0.92	< 0.5
12	Terpinolene	0.9997	1.6	4.8	22.0	0.49	0.91	29.6
13	Linalool	0.9996	1.7	5.2	22.0	0.49	0.92	25.8
14	Isopulegol	0.9996	1.8	5.4	22.2	1.33	0.89	-
15	Geraniol	0.9996	1.7	5.1	21.9	0.39	0.94	< 0.5
16	β-Caryophllene	0.9995	2.0	6.0	22.0	0.44	0.93	-
17	α-Humulene	0.9995	2.0	5.9	21.8	0.68	0.98	< 1.0
18	Nerolidol-1	0.9995	1.9	5.8	21.7	0.69	0.97	< 0.5
19	Guaiol	0.9997	1.5	4.6	22.1	0.68	0.90	< 0.5
20	Caryphyllene oxide	0.9996	1.7	5.0	20.9	0.92	0.90	< 0.5
21	α-Bisabolol	0.9997	1.4	4.2	21.3	0.64	1.02	51.0

In addition to these results, it is shown that the peak tailing factor (PTF) of all components is smaller than two as shown in table 3.

The calibration curves for the Terpene standards were prepared between 0.5 to 200 µg/ml. All components had a correlation coefficient (R2) of 0.9995 or greater which is exceptionally good. Figure 3 shows an example of the calibration curve for four components.

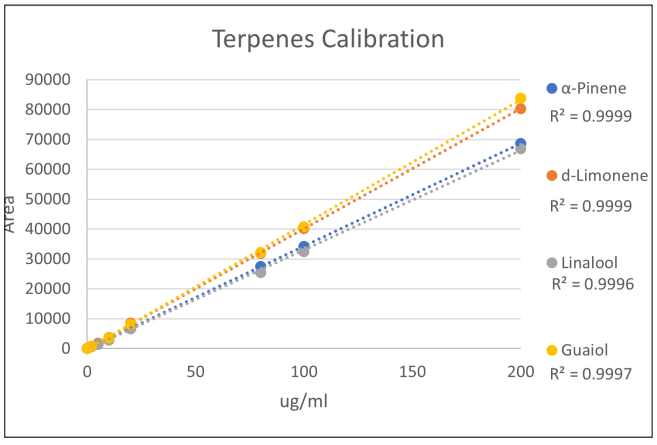

Figure 3: four example calibration curves of the Terpenes standard.

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

The QC sample was analysed using ten 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, β-Mycrene, α-Terpinene, ɣ-Terpinene, Terpinolene, Linalool, Geraniol, α-Humulene, Nerolidol-1, Caryphyllene oxide and α-Bisabolol. The concentration of these components are also shown in table 3.

Some of these components were below the LOD and/or LOQ, if quantification of these components is necessary sample enrichment is needed. Another option is to perform a standard addition method instead of a external calibration to calculate the low concentration of these Terpenes.

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CONCLUSION

The Scion 4X6-GC analyser equipped with a split/spitless injector, Scion Instruments column and FID 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 if quantification is necessary sample enrichment is needed.

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