Technical note #026

LC-UV Detection of Cannabinoids and Terpenes for Potency Testing of Medicinal Cannabis

INTRODUCTION

Cannabis is classed, by US federal law, as a Schedule I drug, which defines it as having no medical value and a high potential for abuse and addiction. However, a large number of US states have now independently legalised the use of cannabis for medicinal purposes. This has led to the emergence of a growing number of analytical testing facilities across the country. These laboratories typically test for potency, in addition to pesticide and mycotoxin residues. Hundreds of cannabinoids and terpenes have been isolated from the *Cannabis Sativa* plant, with each state having its own regulations and testing requirements. Eleven common cannabinoids and six terpenes are often monitored. This technical note details LC methods for the analysis of the cannabinoid and terpene content of cannabis-based therapeutics using Avantor® ACE® HPLC and UHPLC columns.

MEDICINAL CANNABIS

The term cannabis refers to the whole plant, with each strain having a unique chemical make-up, making regulation difficult. The natural plant product can either be used unadulterated, for therapeutic use (most commonly smoked), or specific chemicals can be extracted from the plant to use in other drug formulations.

It is the cannabinoid tetrahydrocannabinol (THC) which elicits the psychoactive effects of cannabis, with cannabidiol (CBD) providing the majority of therapeutic effects, such as pain relief and antiemetic properties. Small amounts of THC are likely to be present in medicinal cannabis products, so it is important to be able to quantify the chemicals present to allow correlation with the label-claimed content. Medicinal cannabis-based products are available in various forms: topical creams, capsules and oils, and can potentially be used for the treatment of several conditions such as multiple sclerosis, arthritis and epilepsy.

FDA APPROVAL

Cannabinoid and terpene reference standards are becoming increasingly more widely available, which

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allows the simplified identification and subsequent quantification of these compounds in real samples. However, due to strict laws in certain states and countries, cannabis-related substances can be difficult to get hold of, with high costs associated. It is for these reasons that cannabis-based therapies are rarely making it to the clinical trial stage. Medical professionals are currently prohibited from prescribing cannabis-based treatments due to the lack of approval from the FDA. Despite these rules and regulations, a number of cannabis-based medicines have been approved by the FDA.

Further research is currently needed into the therapeutic effects of whole-plant cannabis-based drugs. Every

different combination of cannabinoids and terpenes can produce different effects and without further understanding of this, the FDA will be reluctant to approve further drugs.

This technical note presents three methods for the analysis of 10 cannabinoids and 21 terpenes on Avantor® ACE® columns, using mass-spectrometry compatible HPLC conditions. The cannabinoid method (Figure 1) includes the five most commonly analysed compounds: THC, THCA, CBD, CBDA and CBN, and is additionally shown as a fast separation on a fused-core stationary phase (Figure 2).
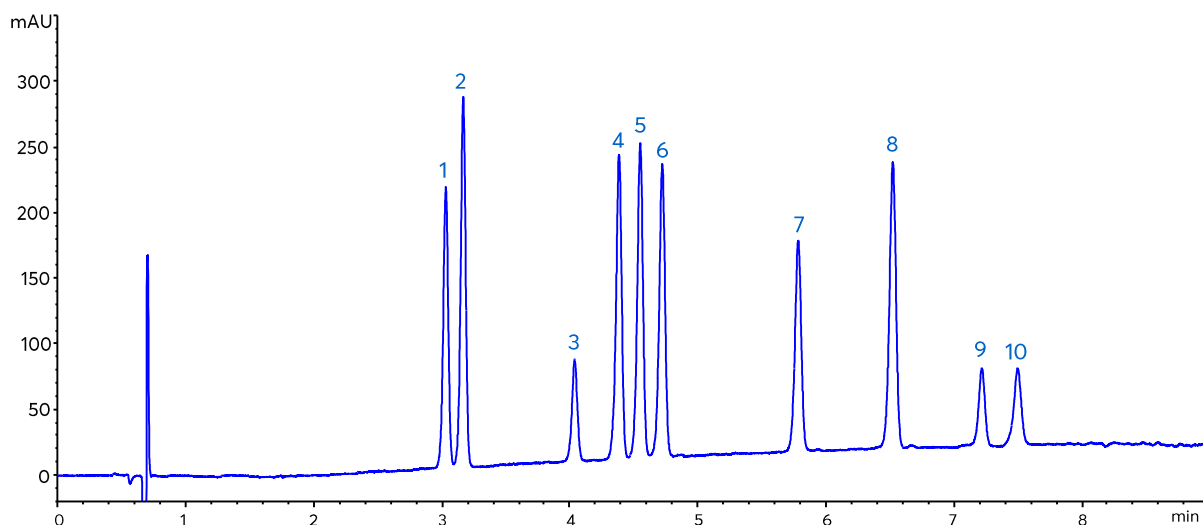

Figure 1: Ten Cannabinoids using LC-UV (Avantor® ACE® Application Note #6850).

Sample: 1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 2. Cannabidivarin (CBDV), 3. Cannabidiolic acid (CBDA), 4. Cannabigerol (CBG), 5. Cannabidiol (CBD), 6. Tetrahydrocannabivarin (THCV), 7. Cannabinol (CBN), 8. (-)-trans-Δ^9-Tetrahydrocannabinol (THC), 9. Cannabichromene (CBC), 10. Δ^9-Tetrahydrocannabinolic acid A (THCA-A).

Table 1: Cannabinoid method details

Column	ACE Excel 2 SuperC18
Dimensions	100 x 3.0 mm
Mobile phases	A: 0.075% formic acid in H_2O B: 0.1% formic acid in MeCN
Gradient	70 – 100% B in 9 mins with 1 min isocratic hold at start
Flow Rate	0.6 mL/min
Injection	5 µL
Temperature	40 °C
Detection	UV, 210 nm

The method in Figure 2 shows the rapid analysis of the cannabinoids listed in Figure 1. A shorter column length was used for this method, packed with Avantor® ACE® UltraCore solid-core particles. The use of a shorter column, combined with the increased efficiency afforded by the solid-core stationary phase, resulted in the separation of all ten peaks in under 3 minutes.
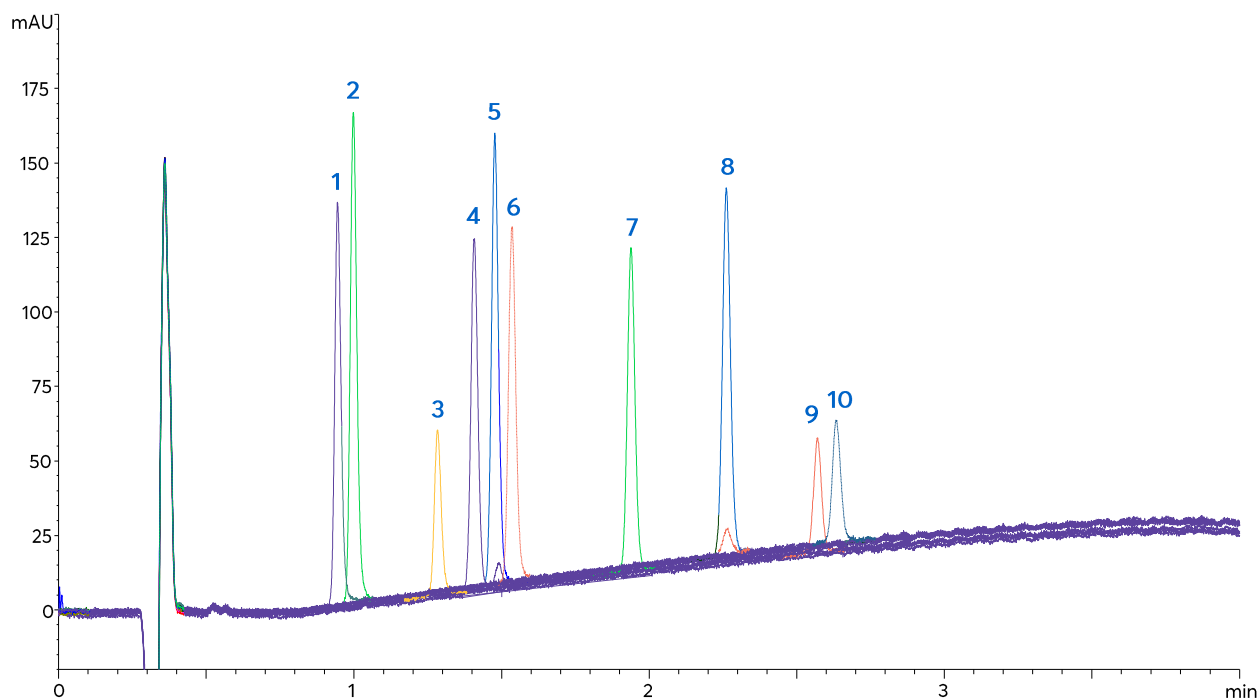

Figure 2: Ten Cannabinoids using rapid LC-UV method (Avantor® ACE® Application Note #6900).

Sample: 1. (-)-11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 2. Cannabidivarin (CBDV), 3. Cannabidiolic acid (CBDA), 4. Cannabigerol (CBG), 5.Cannabidiol (CBD), 6. Tetrahydrocannabivarin (THCV), 7. Cannabinol (CBN), 8. (-)-*trans*-Δ^9-Tetrahydrocannabinol (THC), 9. Cannabichromene (CBC), 10. Δ^9-Tetrahydocannabinolic acid A (THCA-A).

Table 2: Rapid cannabinoid analysis, method details

Column	ACE UltraCore 2.5 SuperC18
Dimensions	50 x 3.0 mm
Mobile phases	A: 0.075% formic acid in H_2O B: 0.1% formic acid in MeCN
Gradient	70 – 100% B in 3.93 mins with 0.25 min isocratic hold at start
Flow Rate	0.6 mL/min
Injection	2 µL
Temperature	40 °C
Detection	UV, 210 nm

Terpenes have historically been analysed using gas chromatography, however, the method below shows an LC-UV method for the analysis of 21 terpenes commonly found in cannabis. The same detection wavelength and mobile phase additive as the cannabinoids method were also used for the terpenes method. The gradient conditions below were developed using ChromSword Auto 5 method development software.
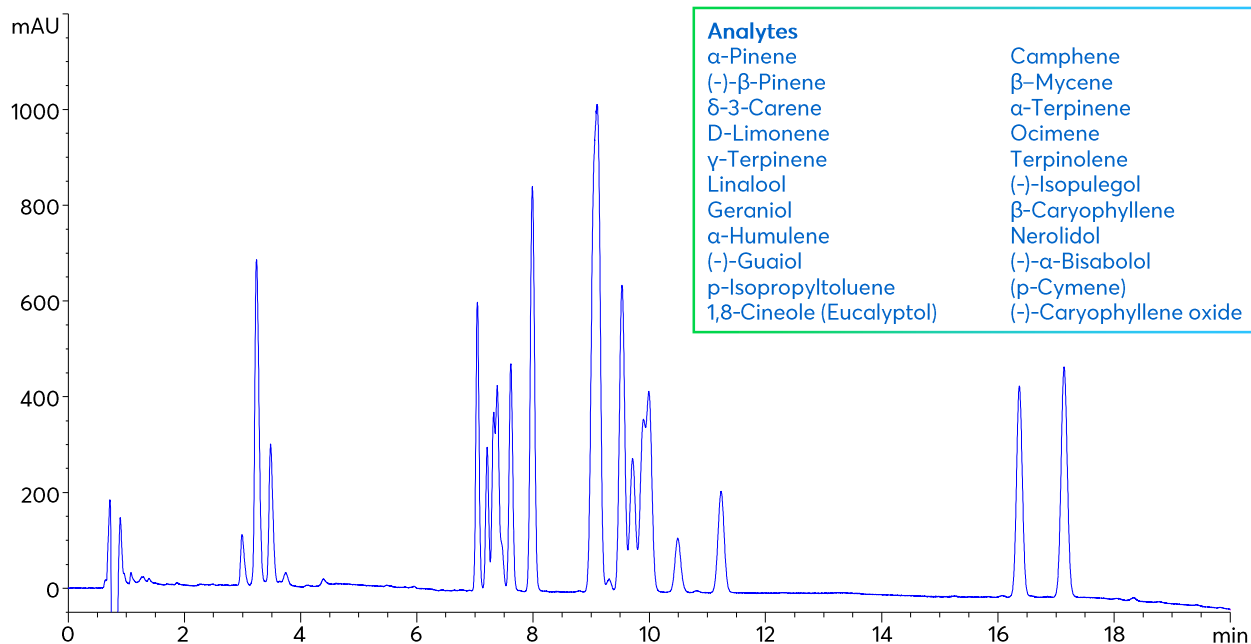

Analytes

α-Pinene	Camphene
(-)-β-Pinene	β–Mycene
δ-3-Carene	α-Terpinene
D-Limonene	Ocimene
γ-Terpinene	Terpinolene
Linalool	(-)-Isopulegol
Geraniol	β-Caryophyllene
α-Humulene	Nerolidol
(-)-Guaiol	(-)-α-Bisabolol
p-Isopropyltoluene	(p-Cymene)
1,8-Cineole (Eucalyptol)	(-)-Caryophyllene oxide

Figure 3: 21 Terpenes using LC-UV method detailed in Table 3 (Avantor® ACE® Application Note #7040).

Table 3: Terpene method details

Column	ACE Excel 2 C18-PFP
Dimensions	100 x 3.0 mm
Mobile phases	A: 0.1% formic acid in H_2O B: 0.1% formic acid in MeCN
Flow Rate	0.6 mL/min
Injection	2 µL
Temperature	30 °C
Detection	UV, 210 nm
Instrument	VWR Hitachi ChromasterUltra Rs

Table 4: Terpene method gradient table

Time (mins)	%B
0	43
3.4	50
5.3	63
9.3	65
12.1	66
13.8	75
16.7	77
19.4	94
28.2	94

ORDERING TABLE

Product	Details	Size	Part number
ACE Excel 2 SuperC18	HPLC/UHPLC column	100 x 3.0 mm	EXL-1011-1003U
ACE UltraCore 2.5 SuperC18	HPLC/UHPLC column	50 x 3.0 mm	CORE-25A-0503U
ACE Excel 2 C18-PFP	HPLC/UHPLC column	100 x 3.0 mm	EXL-1010-1003U

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