Application Note

Cannabis

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Qualitative and quantitative determination of cannabinoid profiles and potency in CBD hemp oil using LC/UV and Mass Selective Detection

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Abstract

With respect to recent legislation in the U.S. legalizing the use of recreational or medicinal marijuana (*Cannabis sativa*), there has been an exponential growth for analytical services that determine the quality and potency of retail products. This Application Note describes using an Agilent 1290 Infinity II UHPLC with an Agilent Mass Selective Detector (MSD) single quadruple system for the analysis of 10 cannabinoids common to *C. sativa* that includes Δ^9-tetrahydrocannabinol and Δ^9-tetrahydrocannabinolic acid (THCA) for total for potency determination and quality profiling.

Introduction

There are no U.S. Federal guidelines for assuring cannabis safety and public health. Regulations are defined on a state-by-state basis where medicinal or recreational cannabis use has been legalized for adult consumption. Most commonly, the states require the quantification and characterization of the cannabinoid profile in botanical *C. sativa* or in products containing cannabinoids extracted from the plant. This testing is necessary to determine potency, and ensure the quality and safety of these commodities.

C. sativa species can contain more than 60 cannabinoids[1] typically with high concentrations of psychoactive Δ^9-tetrahydrocannabinol (THC) content percentage by weight. However, hemp products such as hemp oil extracts contain relatively low levels of THC and higher amounts of other cannabinoids such as cannabinol (CBN) and cannabidiol (CBD). For this study, samples of commercially available hemp oil were purchased and characterized. Samples were prepared using rugged and reliable automated dilution. We characterized and quantified cannabinoids in CBD oils using the Agilent 1290 Infinity II UHPLC system with the Agilent LC/MSD single quadrupole-based system. Mass information was included to provide unambiguous confirmation of the cannabinoids. We further compared the mass spectrometry quantitative results to detection using an electrospray ionization (ESI) mode with a multimode ionization source on the mass spectrometer. To demonstrate statistical reproducibility, the samples were run in replicate (n = 6).

Experimental

The analytical platform was an Agilent 1290 Infinity II UHPLC System, including:

- Quaternary pump
- Multisampler with wash
- Multicolumn thermostat
- DAD

UHPLC Conditions

Parameter	Value
Column	Agilent ZORBAX Poroshell Bonus RP, 3.0 × 50 mm, 2.7 µm
Mobile phase	A) High purity HPLC grade water* B) High purity HPLC grade methanol C) High purity HPLC grade water modified with 1 mL formic acid and 2.2 mL ammonium formate
Flow rate	1.0 mL/min
Run time	6.25 minutes
Post run	2 minutes
Column temperature	50 °C isothermal
Injection volume	0.5 µL
Autosampler temperature	25 °C
Needle wash	3.5 seconds flush port, 25:25:50, H2O:IPA:MeOH
DAD	230 nm

* **Note:** For binary HPLC systems, mobile phase A is modified with formic acid and ammonium formate, as noted above, and a column cleanup time must be defined using 100 % methanol.

LC/MSD Conditions

Parameter	Value
Ionization mode	ESI positive
Nebulizer gas	Nitrogen, 30 psi
Drying gas	Nitrogen, 5 L/min
Drying gas temperature	350 °C
Capillary voltage	70 V
Scan range	150 m/z–700 m/z
SIM ions	315.2 m/z, 287.0 m/z, 311.0 m/z, 359.0 m/z, 317.0 m/z, and, 361.0 m/z
Dwell time	70 ms

Table 1. HPLC mobile phase gradient.

Time (min)	% B	%C
0	60	5
6.25	95	5

The Agilent LC/MSD single quadrupole system was operated with with the multimode ionization source, as shown in Figure 1.

Targeted cannabinoids

Table 2 lists the target cannabinoids purchased as DEA-exempt solutions from Cerilliant (Round Rock, TX).

Results and discussion

UV and DAD

All analytes were chromatographically resolved in less than 5 minutes. For seven levels of calibrator solutions prepared over a range of 0.05 µg/mL to 100 µg/mL, linear regression coefficients (R^2) ≥0.999 were achieved for all targeted cannabinoids (Table 3), and limits of quantitation (LOQ) were determined to be as low as 1.0 ng/mL.

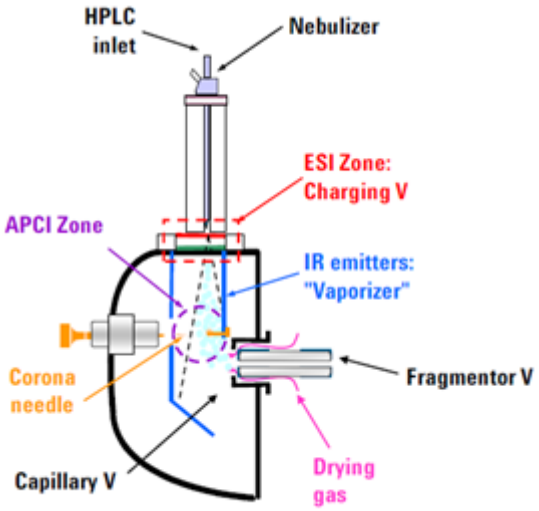

Figure 1. Agilent Multi-mode source performs simultaneous ESI and APCI for the analysis of both polar and nonpolar compounds. See Agilent Technical Overview 5989-2935 for more information.

Table 2. DEA-exempt standards from Cerilliant.

Compound
Cannabidivarin (CBDV)
Tetrahydrocannabivarin (THCV)
(−)-*trans*-Δ⁹-tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabigerol (CBG)
Δ⁹-Tetrahydrocannabinolic acid (THCA)
Cannabidiolic acid (CBDA)
Cannabinol (CBN)
Cannabigerolic acid (CBGA)
Cannabichromene (CBC)

Table 3. Linear regression coefficients.

Acronym	R^2
CBDV	0.9997
THCV	0.9992
CBC	0.9998
CBG	0.9997
CBN	0.9999
CBD	0.9995
CBDA	0.9999
Δ⁹-THC	0.9999
CBGA	0.9998
THCA	0.9994

MSD Standards

Standard calibrator mixtures were analyzed on the LC/MSD using OpenLAB CDS software to establish linearity over the concentration range. Figure 2 shows the extracted ion chromatograms of a 10-compound mix. Single Ion Monitoring (SIM) traces for each compound are illustrated by different colors. Mass selective detection and excellent chromatographic separation of the Bonus RP column ensures unequivocal identification of each analyte.

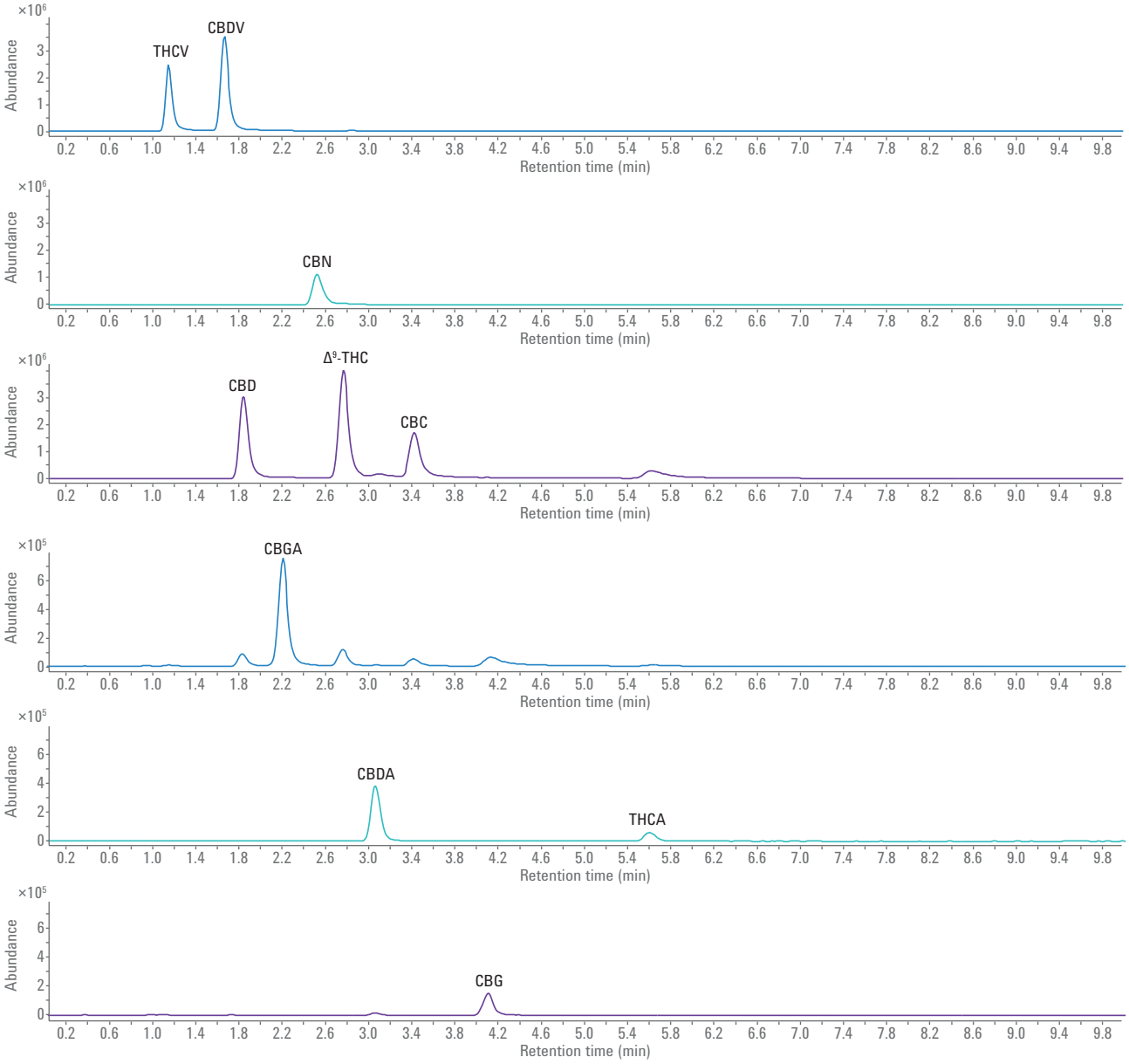

Figure 2. EIC overlay of the SIM ions illustrating compound identification.

In Figure 3, the SIM ion for THC, CBD, and CBC is extracted at 315.2 *m/z* with retention times of 2.779 minutes, 1.843 minutes, and 3.427 minutes, respectively. The 315.2 *m/z* SIM ion for Δ^8-THC can also be seen at 3.099 minutes. We created a standard curve from a known reference solution of Δ^8-THC, and appended that compound to the target list, thereby creating a method for all 10 cannabinoids listed in Table 2. In addition, we added the spectrum and retention time information of Δ^8-THC to a cannabinoid spectral library. The spectral library enables searching and identification of unknown samples.

MSD Investigation of hemp oil extracts

We obtained a sample of natural hemp to serve as a control blank, and six commercially available CBD products derived from hemp oil. Table 4 illustrates the results of these analyses. In all commercial products, CBD was detected at levels consistent with the product labeling, and THC levels were very low, as expected for products derived from hemp oil. CBDA was also detected in samples 1 through 6, in which CBD was detected at moderate levels. CBDA is the naturally occurring carboxylated precursor to CBD that readily decarboxylates upon drying, heating, or other processing conditions. CBG was also detected in samples 1 and 2.

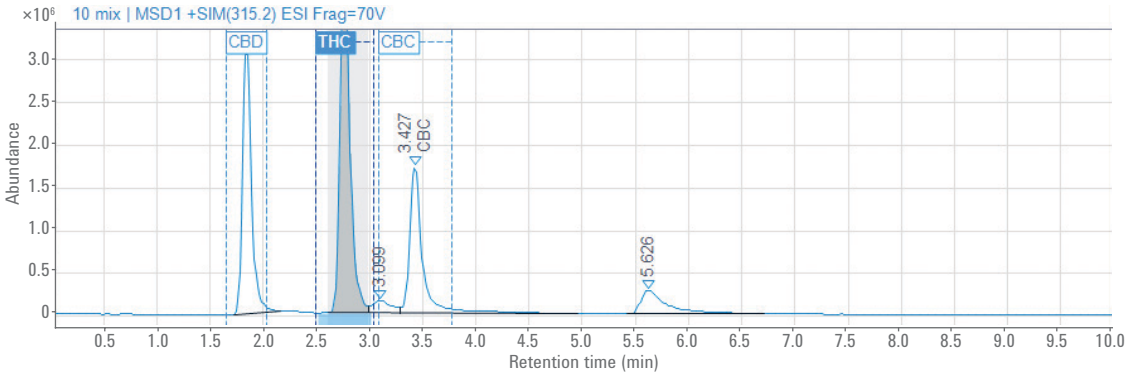

Figure 3. SIM EIC for a standard mixture of CBD, Δ^9-THC, and CBC. Δ^8-THC is shown at 3.099 minutes.

Table 4. Results of hemp oil product testing in mg/mL.

Sample	CBD	CBN	Δ^8-THC	Δ^9-THC	CBC	CBDV	CBDA	THCA	THCV	CBG	CBN
Hemp	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
1	25.0	n. d.	n. d.	0.06	n. d.	n. d.	1.5	n. d.	n. d.	6.0	n. d.
2	24.9	n. d.	n. d.	0.06	n. d.	n. d.	1.5	n. d.	n. d.	5.0	n. d.
3	16.0	n. d.	n. d.	0.12	n. d.	n. d.	3.0	n. d.	n. d.	n. d.	n. d.
4	8.0	n. d.	n. d.	0.04	n. d.	n. d.	4.0	n. d.	n. d.	n. d.	n. d.
5	8.0	n. d.	n. d.	0.04	n. d.	n. d.	4.0	n. d.	n. d.	n. d.	n. d.
6	17.0	n. d.	n. d.	0.07	n. d.	n. d.	2.7	n. d.	n. d.	n. d.	n. d.

Conclusions

This Application Note describes a methodology using Agilent OpenLAB CDS software and the Agilent 1290 Infinity II UHPLC with the Agilent InfinityLab LC/MSD system. This platform offers reliable and robust identification and quantification of the 10 most commonly analyzed cannabinoids found in cannabis and cannabinoid containing products. The addition of mass selective detection using the LC/MSD and the excellent chromatographic reproducibility of the Agilent Bonus RP column provides unequivocal identification of the target cannabinoids and confidence in the analytical results compared to optical detection using LC/UV alone. The analytical method easily characterizes and quantifies cannabinoids in CBD oils available from commercial sources to provide a robust tool for potency, safety, and quality determinations.

Reference

1. Huestis, M. A. Human Cannabinoid Pharmacokinetics. *Chem. Biodivers*. **2007**, *4, 8*, 1770–1804.

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