Cannabinoid Profiling and Quantitation in Hemp Extracts using the Agilent 1290 Infinity II/6230B LC/TOF system

Application Brief

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Abstract

Hemp extracts have recently become an area of scientific interest. Some of these extracts include cannabinoids such as cannabidiol (CBD), cannabidiolic acid (CDBA), cannabinol (CBN), cannabigernol (CBG), and Δ^9-tetrahydrocannabinol (THC). CBD, CBDA, CBN, and CBG occur in relatively high to moderate concentrations in the plant material, and are nonpsychoactive. THC is psychoactive, but occurs in very low concentrations (<0.3 %) in hemp products. However, tetrahydrocannabinolic acid (THCA) is known to convert to THC over time and under heated conditions, so it is often desirable to measure the total THC content as: Total THC = THCA + THC. In this study, we used the Agilent 1290 Infinity II combined with an Agilent 6230B liquid chromatography-time of flight (LC-TOF) mass spectrometry system to determine potency, and quantitatively profile the 11 most commonly targeted cannabinoids. The method achieves excellent chromatographic separation for cannabinoids studied including separation of Δ^8-THC and Δ^9-THC with minimal sample preparation. Detection limits are determined to be as low as 1.0 ng/mL with linear regression coefficients of 0.999 or better over a calibration range of 10 µg/mL to 1,000 µg/mL.

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Introduction

Hemp is a fibrous variety of *C. sativa* with a low psychoactive Δ^9-tetrahydrocannabinol (THC) component, but high quantities of other cannabinoids. Most commonly, cannabinoid profiling and potency quantification is performed through liquid chromatography (LC) with ultra-violet (UV) or diode array detectors (DADs). Although capable to measure the diverse concentration ranges of the compounds in plant material in many cases, even with known references and retention time information, UV detection alone is not sufficient for compound identification. This is because unknown interferences are common in real samples, and can confound compound identification.

An Agilent 1290 Infinity II combined with an Agilent 6230B LC-TOF (Agilent Technologies, Santa Clara, CA USA) system was used to compare range and linearity to data collected on an Agilent 1290 Infinity II ultra-high performance liquid chromatography (UHPLC) system with a DAD. Targeted analytes were THC, tetrahydrocannabivarin (THCV), tetrahydrocannabinolic acid (THCA), cannabinol (CBN), cannabichromene (CBC), cannabidiol (CBD), cannabidivarin (CBDV), cannabidiolic acid (CDBA), cannabigernol (CBG), and cannabigerolic acid (CBGA). Table 1 shows the target compound names, acronyms, exact mass, and empirical formulae. The added information of accurate mass and high mass resolving power offers confidence in compound identification, and greatly improves specificity compared to UV detectors. To assist with compound identification in unknown samples, a personal compound database library (PCDL) containing spectral data, accurate mass, and retention time information was created.

Table 1. Targeted Cannabinoids and Acronyms

Cannabinoids	Acronym	Empirical formula	Exact mass $(M+H)^+$
Cannabidivarin	CBDV	$C_{19}H_{26}O_2$	287.2006 *m/z*
Tetrahydrocannabivarin	THCV	$C_{19}H_{26}O_2$	287.2006 *m/z*
Cannabichromene	CBC	$C_{21}H_{30}O_2$	315.2319 *m/z*
Cannabigerol	CBG	$C_{21}H_{32}O_2$	317.2475 *m/z*
Cannabinol	CBN	$C_{21}H_{26}O_2$	311.2006 *m/z*
Cannabidiol	CBD	$C_{21}H_{30}O_2$	315.2319 *m/z*
Cannabidiolic acid	CBDA	$C_{22}H_{30}O_4$	359.2217 *m/z*
Δ^9-tetrahydrocannabinol	THC	$C_{21}H_{30}O_2$	315.2319 *m/z*
Cannabigerolic acid	CBGA	$C_{22}H_{32}O_4$	361.2373 *m/z*
Tetrahydrocannabinolic acid	THCA	$C_{22}H_{30}O_4$	359.2217 *m/z*

Experimental

HPLC Conditions

Parameter	Value
Agilent 1290 Infinity II UHPLC series Quaternary Pump, Multisampler with wash, Multi Column Thermostat, DAD	
Column	Agilent ZORBAX Bonus RP. 2.1 × 50 mm, 1.8 µm
Column temperature	50 °C
Injection volume	0.25 µL
Autosampler temperature	23 °C
Needle wash	3.5 seconds Flush port (25:25:50) (H_2O:IPA:MeOH)
DAD-UV	230 nm
Mobile phase	A) Water B) Methanol C) 0.1 % CH_2O_2 + 2.2 mL 5 M NH_4HCO_2 in H_2O
Flow rate	0.5 mL/min
Gradient	Time (min) %B %C 0.0 72 5 12.5 95 5
Stop time	12.5 minutes
Post time	1.0 minutes
Overall run time	20.0 minutes (including re-equilibration)

MS Conditions

Parameter	Value
Agilent 6230B Time of Flight Mass Spectrometer	
MS Parameters	
Ionization mode	Dual ESI, Positive Ion
Mass range	100–1,700 *m/z*
Spectral acquisition rate	1.00 Hz
Source parameters	
Drying gas flow	12 L/min
Drying gas temperature	350 °C
Nebulizer gas pressure	40 psi
Capillary voltage	4,000 V
Fragmentor	175 V
Skimmer	65 V
Ref mass enabled	Yes
Ref masses	121.0508 *m/z* 922.0097 *m/z*
Average scan	1
Detection window	100 ppm
Min height	100

Calibrator and sample preparation

HPLC-DAD

Calibrators containing a mixture of the commercial standard solutions were prepared over a range of 50 µg/mL to 1,000 µg/mL.

LC-TOF

Calibrators containing a mixture of the commercial standard solutions were prepared over a range of 100 µg/mL to 5,000 µg/mL for method development, and 10 µg/mL to 1,000 µg/mL for the analysis of the unknown samples. A calibration curve was created to establish THC limits of detection (LOD) over the range of 12.5 ng/mL to 1,000 ng/mL in solvent.

Sample preparation

One hundred-microliter aliquots of each commercially purchased sample were diluted 100-fold with dichloromethane, followed by a 10-fold dilution in methanol. This limited cleanup of the samples requires a longer analysis time to allow for all the noncannabinoid components to elute from the column. Seven samples of commercially available hemp oil products were purchased. Table 2 lists these with product descriptions and masked identifiers.

Table 2. List of Commercially Available Products Purchased for the Analysis

Sample identifier	Product description
SIA-1	Hemp oil*
SIA-2	Hemp oil*
SIB	Cold pressed hemp oil
SIC	Hemp oil†
SID	Hemp oil†
SIE	Unflavored hemp oil‡
SIF	Hemp oil with flavoring‡

* Same manufacturer, product and lot

† Same manufacturer. SIC filtered, sample SID unfiltered

‡ Same manufacturer

Creation of the PCDL

Spectral data were acquired for each standard solution using the LC-TOF method described above. Spectral data were copied into the PCDL. Once the library was built, it was used to identify cannabinoids in unknown samples using library searching workflows in MassHunter Qualitative Analysis.

Results and Discussion

Calibrator data were acquired on both the UHPLC-DAD and LC-TOF systems, and methods and linear curves were constructed for each target analyte. Excellent chromatographic separation was achieved, and allowed resolution of Δ^8-THC and THC (Figure 1). The UHPLC-DAD linear regression coefficients (R^2) were ≥0.999 for all targeted cannabinoids. Similarly, the R^2 coefficients for the LC-TOF data were at least 0.999 for all compounds over both the 100 µg/mL–5,000 µg/mL and the 10 µg/mL–1,000 µg/mL calibrators ranges. The limits of quantitation (LOQ) were similar on both the UHPLC-DAD and LC-TOF systems for all cannabinoids. The LC-TOF data were evaluated down to 12.5 ng/mL for THC LOD and LOQ experiments. An LOD of 1.0 ng/mL could be achieved using a signal-to-noise ratio (S/N) of 3:1 as the criterion, and an LOQ of 3.0 ng/mL (S/N ≥10:1) for THC was determined.

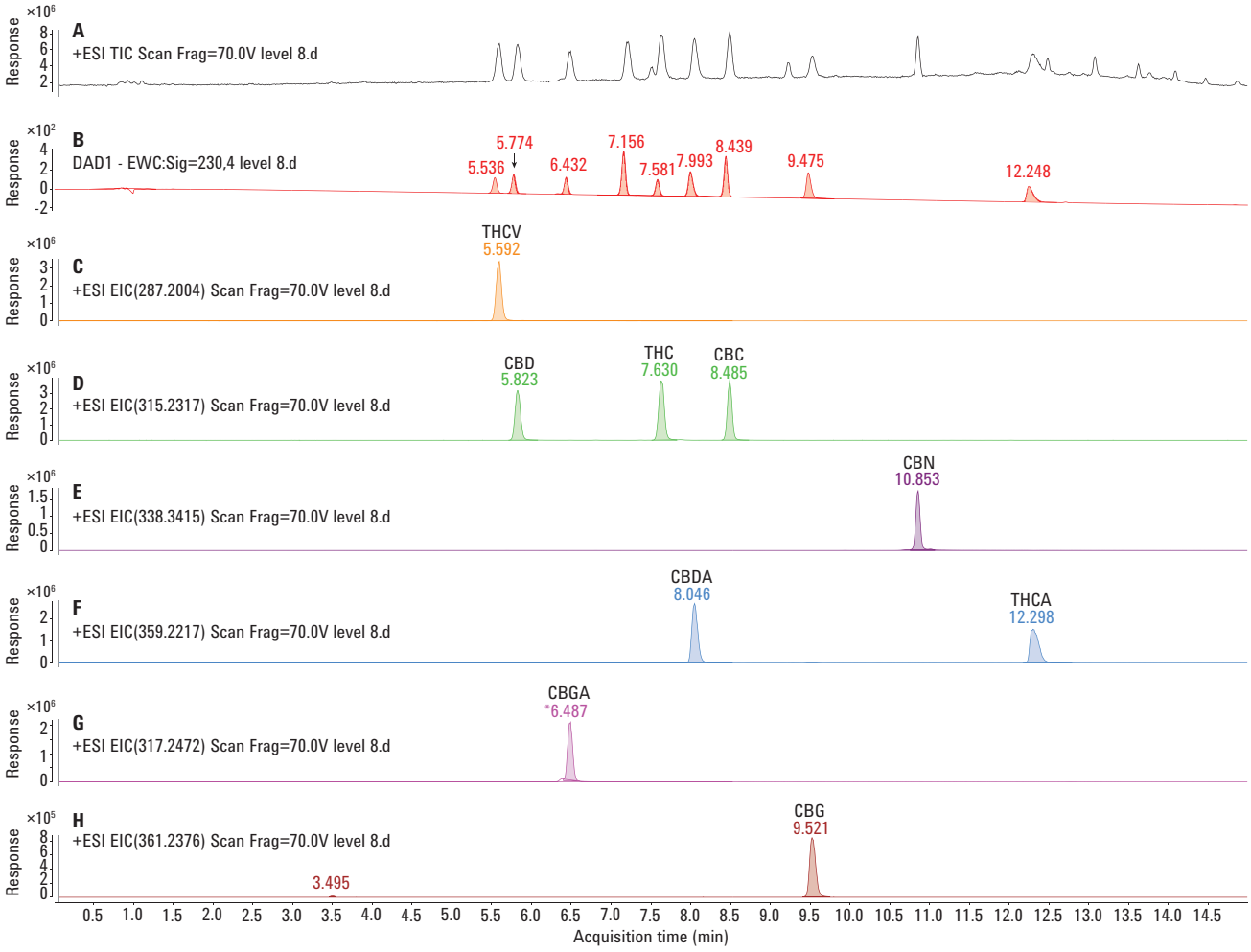

Figure 1. Chromatograms. A) Total ion chromatogram (TIC) from the TOF. B) UV signal at 230 nm. C–H) Extracted ion chromatograms (EICs) for each cannabinoid.

The seven commercial hemp oil samples were prepared with the dilution schema described above and analyzed on the LC-TOF system. Four replicate injections of each sample were acquired. The PCDL was used to accurately identify each targeted cannabinoid. The average concentration, standard deviation, standard error, and confidence interval at the 99% confidence level was determined, and the results given in Table 4.

Conclusion

Hemp extracts contain many cannabinoids. Typically, CBD is found in relatively high concentrations, while THC is found in very low concentrations in hemp oils. Analytical methods using HPLC with UV detection are cost-effective and exhibit good linearity, but lack the dynamic range required to detect both ends of this concentration range. Conversely, LC-TOF offers a wide dynamic range that simultaneously quantitates both high and low concentration cannabinoids in a single run. LC-TOF was used to evaluate the advantages of linear dynamic range, linearity, specificity, and the ability to profile and quantify cannabinoids in unknown hemp oil samples. This method achieves excellent chromatographic separation and LOD for the targeted cannabinoids including separation of Δ^8-THC and THC. The added information of accurate mass and high mass resolving power offers empirical formula generation to assist in compound identification using a custom PCDL, and the ability to extract ions of interest from the background matrix, much improving specificity compared to UV or DAD detection.

Table 3. Linear Regression Coefficients

Acronym	Retention time (min)	R^2 HPLC	R^2 LC-TOF
CBDV	4.360	0.999	0.999
THCV	5.525	0.999	0.999
CBC	5.653	0.999	0.999
CBG	6.421	0.999	0.998
CBN	7.140	0.999	0.999
CBD	7.585	0.999	0.999
CBDA	7.785	0.999	0.999
Δ^9-THC	8.446	0.999	0.999
CBGA	9.046	0.999	0.999
THCA	12.280	0.999	0.999

Table 4. Unknown Analysis Results

Sample identifier	Average concentration (mg/mL)	Standard deviation	Standard error	99 % CI
SIA-1	508.1	9.8	4.9	479.5, 536.7
SIA-2	460.7	14.7	7.4	417.8, 503.6
SIB	Not detected	N/A	N/A	N/A
SIC	2.3	0.17	0.085	1.7, 2.9
SID	9.3	0.86	0.43	6.8, 11.8
SIE	11.6	0.85	0.43	9.1, 14.1
SIF	12.3	0.78	0.39	9.9, 14.5

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