

Quantitation of Phytocannabinoid Oils Using the Agilent Infinity II 1260 Prime/InfinityLab LC/MSD iQ LC/MS System

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Abstract

Determining the concentration of Δ^9 -tetrahydrocannabinol (THC), cannabidiol (CBD), Δ^9 -tetrahydrocannabinolic acid (THCA), and other phytocannabinoids in cannabis or hemp and derived products is critical both from a regulatory testing and forensic perspective. However, the analytical testing is essentially the same regardless of the required outcome and information. In this application note, we employed an Agilent Infinity II 1260 Prime LC System coupled to an Agilent InfinityLab LC/MSD iQ for the analysis of CBD oil products manufactured for the pet industry. Here, we demonstrate excellent chromatographic resolution, and limits of detection (LOD) and quantitation (LOQ).

Introduction

Globally, the hemp market was estimated at \$3.9 billion in 2017, and the hemp seed segment is predicted to grow "at a CAGR of 17.1%"¹ through 2025. Some of the markets affected by hemp production include nutraceuticals, food, textiles, construction materials, and personal care products. It is also anticipated that cannabidiol (a nonpsychoactive cannabinoid) production will grow to support the burgeoning recreational and medicinal cannabis markets in the U.S., Canada, and other countries around the world.

A simple search of the world wide web generates hundreds of hemp and CBD oil products designed for pet consumption. Presently, the USDA only mandates potency testing to ensure that the total THC content of hemp and hemp products is less than 0.3% by weight. In most regulatory laboratories, HPLC with UV detection is used to separate and measure cannabinoids in the various products. However, CBD oils often have coeluting compounds such as

terpenes that may interfere with accurate identification and quantitation of the target cannabinoids when using UV detectors. The addition of the LC/MSD iQ in our work adds the specificity of mass confirmation to mitigate these interferences and greatly improve overall robustness of the method.

As interest in CBD oils and products grows, manufacturers, distributors, and researchers must accurately determine the individual concentrations of both CBD and THC and the total THC and total CBD content as defined by:

$$\text{Total THC} = 0.877 \times [\text{THCA}] + [\text{THC}]$$

Equation 1.

$$\text{Total CBD} = 0.877 \times [\text{CBDA}] + [\text{CBD}]$$

Equation 2.

Where [THCA] is the concentration of the acid analog of THC and [CBDA] is the acid analog of CBD. It is important to note that the *Cannabis sativa L.* genome encodes for the biosynthesis of THCA, CBDA, and other phytocannabinoid acids, and that THC, CBD, etc., are generated upon harvesting, drying, and heating.

Experimental

Hardware and software

An Agilent InfinityLab LC/MSD iQ system was used for all experiments and consisted of the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- 1260 Infinity II Vial Sampler (G7129A with 40 μL metering device and 20 μL loop and G7129C) equipped with an Integrated Column Compartment (3 μL heater; G7130-64430) and Integrated Sample Cooler
- 1260 Infinity II Diode Array Detector WR (G7115A)
- InfinityLab LC/MSD iQ (G6160AA)

Agilent OpenLab CDS Software (version 2.4) was used for data acquisition and data processing.

Data collection

All MSD parameters other than the SIM ions were set by the LC/MSD iQ automatically using Auto Acquire mode. Auto Acquire sets MS parameters based on the HPLC chromatographic settings such as flow rate. The following tables illustrate the LC/MSD iQ analytical parameters.

Table 1. HPLC conditions.

Parameter	Value
Flow	0.5 mL/min
Column	Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 1.9 μm
Stop Time	12.5 minutes
Post Time	3.0 minutes
Injection Volume	0.5 μL
Wavelengths	228 and 270 nm
Sampling Rate	40 Hz

Table 2. HPLC mobile phase gradient.

Time	(A) 100% H ₂ O	(B) 100% ACN	(C) 100% MeOH	(D) 1 mL of Formic Acid in 1 L of H ₂ O
0	25	70	0	5
3.2	25	70	0	5
8.2	5	0	90	5

Table 3. LC/MSD iQ acquisition settings using Auto Acquire.

Acquire Mode	Auto
Tune File	atunes.tune
Ion Source	ESI
Time Filter Enabled	On
Target Points Enabled	On
% SIM	50

Table 4. LC/MSD iQ electrospray source conditions.

Source Parameter	Value
Gas Temperature	325 °C
Gas Flow	13 L/min
Nebulizer	55 psig
Capillary Voltage	3,500

Table 5. iQ SIM/Scan parameters.

Segment	Name	M+H (m/z)	Fragmentor (V)	Polarity
SCAN		200 to 700	100	Positive
SIM	THCA and CBDA	359.2	100	Positive
SIM	CBN	311.2	100	Positive
SIM	CBD, THC, and CBC	315.2	100	Positive
SIM	CBG	317.2	100	Positive
SIM	CBDV and THCV	359.2	110	Positive
SIM	CBGA	361.2	110	Positive

Column and supplies

Table 6. Agilent supplies and orderable part numbers.

Consumables	Part Number
Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 1.9 μm	695675-302
0.45 μm regenerated cellulose (RC) syringe filter	5190-5107
Formic acid	G2453-85060
InfinityLab Ultrapure LCMS Water, 1 L	5191-4498
InfinityLab Ultrapure LC/MS Grade Methanol, 1 L	5191-4497
InfinityLab Acetonitrile, 1 L	5191-4496
Ceramic Homogenizers, 50 mL Tubes, 100/pk	5982-9313
5 mL Disposable Syringe 100/pk	9301-6476

Sample preparation

A 100 µL aliquot of the homogenized sample was pipetted into a tared 10 mL volumetric flask and the weight of the sample aliquot was accurately determined and recorded. Five milliliters of high-purity hexane was added to the flask and after capping it, briefly vortexed to mix. Hexane was added to the 10 mL mark on the flask and the flask was mixed again (100-fold dilution). One hundred microliters of the diluted oils were transferred to new vials, and 900 µL of ethanol was added to each. The vials were capped and briefly mixed before being filtered with 0.45 µm regenerated cellulose (RC) syringe filters and

transferred to 2 mL autosampler vials for analysis. The total dilution factor was 1,000-fold. Note that this dilution factor may be increased or decreased based on the CBD content of the product under analysis.

Serial dilutions of a mixture of 11 cannabinoids shown in Table 7 were performed to create five levels of solvent-based calibrators over the range of 0.1 or 0.25 µg/mL through 50.0 µg/mL. Replicate injections at the lower calibration levels were made for LOD and LOQ determinations.

Conversion from weight/volume was performed using Equation 3.

Results and discussion

The 1260 Infinity II Prime LC quaternary pump with its binary-like gradient profile allows the use of four unique solvents. This saves the analyst from the task of making complicated mobile phases, and allows quick bottle refills. The ionic strength and UV baseline absorption remain constant with the use of the D channel (0.1% formic acid) at 5% of the total mobile phase. The 800-bar limit of the HPLC pump and components allows the 1.9 µm Poroshell column to be used and to obtain the separation benefits of smaller particles. This resulted in excellent chromatographic resolution throughout the analysis. Figure 1 shows the SIM and UV chromatograms. Table 8 shows the calculated LOD and LOQ for each compound. The LOD was determined at three times the signal-to-noise, and the LOQ was determined by 10 times signal-to-noise. Lastly, Table 9 illustrates the quantitative results from six commercially available pet products.

Table 7. Target cannabinoids and retention times in minutes.

Compound	Acronym	Retention Time
Cannabidivarin	CBDV	2.676
Cannabidiolic acid	CBDA	3.693
Cannabigerolic acid	CBGA	3.979
Cannabigerol	CBG	4.267
Cannabidiol	CBD	4.554
Tetrahydrocannabivarin	THCV	4.941
Cannabinol	CBN	7.198
Δ ⁹ -tetrahydrocannabinol	Δ ⁹ -THC	8.290
Δ ⁸ -tetrahydrocannabinol	Δ ⁸ -THC	8.590
Cannabichromene	CBC	9.173
Δ ⁹ -tetrahydrocannabinolic acid	THCA	9.795

$$\text{Cannabinoid Concentration } \left(\% \frac{\text{wt}}{\text{wt}} \right) = \left[\frac{\text{Calculated Concentration} * V * D}{M * 10,000} \right]$$

Equation 3.

Calculated Concentration = concentration of analyte from linear regression analysis (µg/mL)

V = initial volume of sample diluent (mL)

D = dilution factor

M = mass of sample (g)

10,000 = conversion from µg/g to % wt/wt.

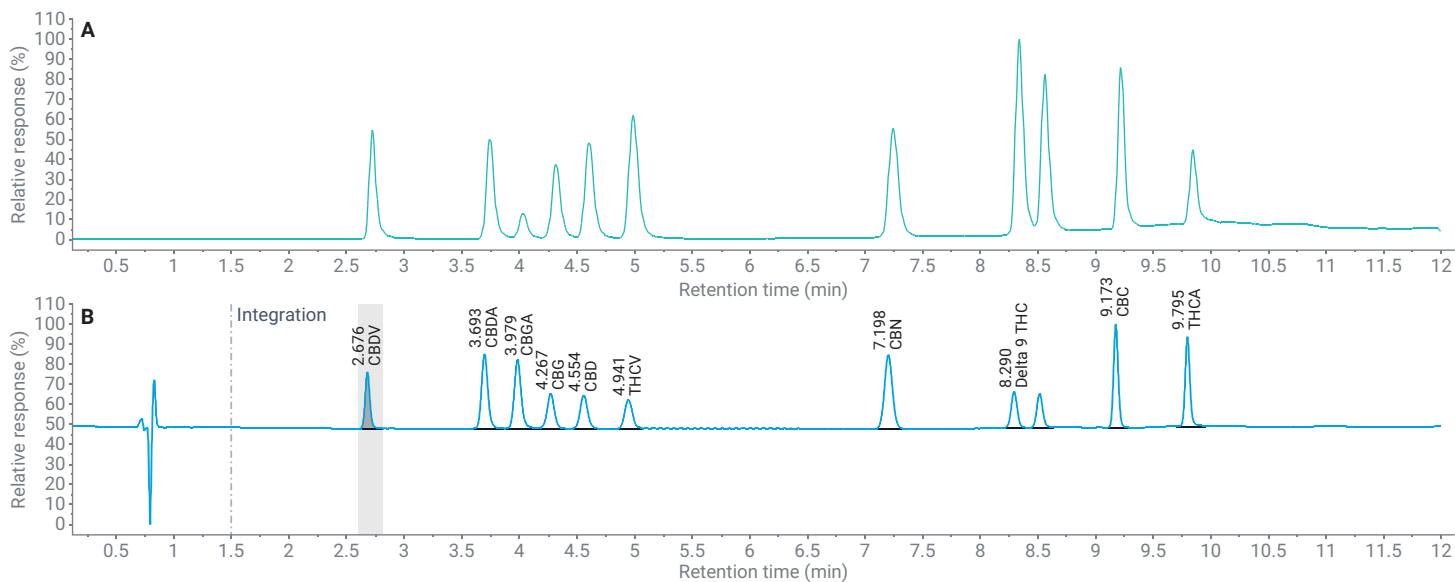


Figure 1. Normalized SIM TIC (A) and DAD signal (B) for the 11-compound mix. The concentration units are $\mu\text{g/mL}$.

Table 8. Correlation coefficient (R^2) for calibration range 0.1 to 50 $\mu\text{g/mL}$, and LOD and LOQ results.

Compound	R^2	LOD ($\mu\text{g/mL}$)	LOQ ($\mu\text{g/mL}$)
CBDV	0.995	0.25	0.50
CBDA	0.996	0.25	0.50
CBGA	0.997	0.25	0.50
CBG	0.997	0.25	0.50
CBD	0.999	0.25	0.50
THCV	0.995	0.25	0.50
CBN	0.997	0.25	0.50
Δ^9 -THC	0.990	0.10	0.50
CBC	0.995	0.25	0.50
THCA	0.995	0.25	0.50
Δ^9 -THC	0.992	0.10	0.25

Table 9. Calculated quantitative results for six commercially available CBD oils for pets in $\mu\text{g/mL}$.

Sample ID	CBD	CBDA	THCA	Δ^9 -THC	Δ^8 -THC	CBG	CBN	CBC	CBDV	THCV
1MS	191.0	2.0	nd	6.9	nd	6.6	nd	9.5	2.0	nd
2MS	67.9	nd	nd	nd	nd	nd	nd	nd	nd	nd
3MS	23.2	2.2	nd	nd	nd	nd	nd	nd	nd	nd
4MS	2.3	0.8	3.1	1.2	nd	nd	nd	nd	nd	nd
5MS	34.7	3.7	nd	0.2	nd	nd	nd	1.7	nd	nd
6MS	17.0	nd	nd	0.8	nd	nd	nd	1.9	nd	nd

Conclusion

In addition to ease-of-use features, the 1260 Infinity II Prime LC System with the LC/MSD IQ system offers increased selectivity compared to UV detection alone for the analysis of cannabinoid products such as CBD oils for pets. The analysis described in this study identified the presence or absence of 11 cannabinoids in the six products, and accurately quantified each when found. This was achieved through excellent chromatographic resolution and mass spectral confirmation of the m/z ions for each of the target compounds. This methodology can be implemented in laboratories chartered to perform regulatory compliance testing where cannabis and cannabinoid products have been legalized for medicinal or adult recreational use, and in forensic and criminalistic laboratories registered with the US DEA required to authentic hemp in compliance with USDA guidelines.

References

1. Grand View Research (June 2018). *Industrial Hemp Market Size, Share & Trends Analysis Report by Product (Seeds, Fiber, Shivs), By Application (Textiles, Personal Care, Animal Care, Construction Materials), By Region, And Segment Forecasts, 2018 – 2025*. Accessed March 20, 2019 from: <https://www.grandviewresearch.com/industry-analysis/industrial-hemp-market>.

Table 10. Total CBD and THC in % wt/wt.

Sample ID	Total CBD (% wt/wt)	Total THC (% wt/wt)
1MS	22.1	2.53
2MS	7.8	nd
3MS	2.9	nd
4MS	0.3	0.04
5MS	4.3	0.50
6MS	1.9	nd

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