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Quantification of Cannabinoids in Industrial Hemp Using the Agilent 1220 Infinity II LC System

Author

Anthony Macherone
Agilent & Johns Hopkins
University School of Medicine

Agilent offers a proven workflow for the quantification of targeted cannabinoids commonly found in hemp.

- Quantify Δ^9-tetrahydocannabinol (THC) in hemp plant to ensure compliance with federal law

- Quantify tetrahydrocannabinolic acid (THCA) to provide a total THC content ($0.877 \times$ [THCA] + [THC]) where required

- Quantify a total of 11 phytocannabinoids in hemp

- Rapid method implementation

- Consulting services to assist and train your staff

Introduction

The U.S. Agriculture Improvement Act, also known as the Farm Bill, was signed into law in December 2018. A major provision in the law legalizes hemp as an industrial commodity. The DEA, USDA, and FDA have published a combined Statement of Principles in the Federal Register (FR 53365). This statement defines industrial hemp as any part or derivative (including seeds) of the plant *Cannabis sativa* L. with a dry weight concentration of tetrahydrocannabinols not greater than 0.3 % (wt/wt).

In the U.S., where recreational or medicinal marijuana programs have been legalized, regulations have been defined to ensure the safety and quality of the products sold to consumers. These regulations include analytical chemistry and biological assays to identify and quantify pesticides, mycotoxins, heavy metals, residual manufacturing solvents, terpenes, and microbial contaminates. In all regions with some form of cannabis legalization, potency testing is also required. At a minimum, potency testing profiles and quantifies THC, THCA, cannabidiol (CBD), cannabidiolic acid (CBDA), and in some states, cannabinol (CBN) or cannabigerol (CBG), but most testing laboratories profile and quantify these and other phytocannabinoids.

The information content of this application note, along with ready to run acquisition, quantitation, etc. methods and extensive support information are available as eMethod G5277#020, Hemp Potency test with the 1220VL LC/VWD system.

Potency testing in hemp plants

Now, only potency testing is required for both hemp and cannabis plants and products, but it is anticipated that more testing will be required for hemp in the coming years. The purpose of potency testing for hemp plants or products such as hemp oils is to ensure that they contain less than 0.3 % (wt/wt) THC to comply with federal law.

Agilent has invested in the development and implementation of the analytical protocol and the services needed to support these assays. Sales and support personnel have been trained to understand the hemp market and its testing needs. Agilent has also invested in the required consumables, reagents, and supplies. Concerning potency testing, Agilent has published, delivered, and implemented high-performance liquid chromatography (HPLC)-based assays to profile and quantify 11 phytocannabinoids including THC, THCA, CBA, CBDA, CBN, and CGB.

Example analysis

The Agilent 1220 Infinity II LC system offers reliable and robust profiling and quantification for 11 of the most commonly targeted cannabinoids found in *Cannabis sativa* L. plants and products (Table 1). The method defines the analytical column, mobile phase modifiers, and full system parameters to separate the targeted cannabinoids in under 10 minutes (Tables 2 to 4, Figure 1). Through our consultative services, Agilent also offers the required consumables for this assay and full training of laboratory personal.

Table 1. Target cannabinoids commonly found in *Cannabis sativa* L. plants and products.

Target Compounds
Cannabidivarin (CBDV)
Tetrahydrocannabivarin (THCV)
(−)-*trans*-Δ⁹-Tetrahydrocannabinol (THC)
Cannabidiol (CBD)
Cannabigerol (CBG)
Δ⁹-Tetrahydrocannabinolic acid (THCA-A)
Cannabidiolic acid (CBDA)
Cannabinol (CBN)
Cannabigerolic acid (CBGA)
Cannabichromene (CBC)
(−)-Δ⁸-THC

Table 2. Hardware and consumables.

Hardware and Software	Part Number
Agilent 1220 Infinity II LC Gradient System VL	G4290C
Agilent OpenLab 2.2 data acquisition software	M8417AA

Table 3. LC conditions.

Parameter	Value
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile Phase	A) 0.1 % (v/v) formic acid, aqueous phase B) 0.05 % (v/v) formic acid, organic phase
Flow Rate	1.0 mL/min
Run Time	9.5 minutes
Post Run	1.5 minutes
Column Temperature	50 °C isothermal
Injection Volume	5.0 µL
Autosampler Temperature	Ambient
Peak Width	>0.0063 minutes (0.13 seconds response time) (80 Hz)
Variable Wavelength Detector	230 nm

Table 4. LC mobile phase gradient.

Time (min)	% B
0.0	60
1.0	60
7.0	77
8.2	95

Sample preparation for plant material

1. Weigh 200 mg of leaf cutting into a 50 mL centrifuge tube. Homogenize using ceramic homogenizers and a commercial grinder.

2. Add 20 mL of methanol. Vortex/shake for 10 minutes. (100 fold dilution)

3. Aliquot 1 mL into a new vial. Centrifuge at 5,000 rpm for five minutes.

4. Transfer 50 µL of supernatant to a new vial. Add 950 µL of methanol. Mix briefly. (20 fold dilution for a total of 2,000 fold)

5. Filter with 4 mm, 0.45 µm regenerated cellulose (RC) syringe filters (p/n 5190-5107).

Sample preparation for oils and concentrates

1. Pipette a 100 µL aliquot of homogenized hemp oil, CBD oil, concentrate, tincture, or resin into a tared 10 mL volumetric flask. Accurately determine and record the weight of the collected product.

2. Add 8 mL of high purity HPLC or LC/MS grade ethanol, cap, and mix well. Bring the volume to 10 mL with ethanol (100 fold dilution).

3. Using a glass syringe fitted with a 0.45 µm regenerated cellulose (RC) syringe filter (5190-5107), filter 2 mL of the solution into a clean glass vessel.

4. Perform an additional 10 fold dilution of the filtered solution by transferring a 100 µL aliquot into an amber glass 2 mL autosampler vial and adding 900 µL of high purity HPLC or LC/MS grade methanol. Cap and vortex briefly to mix (final dilution factor = 1,000).

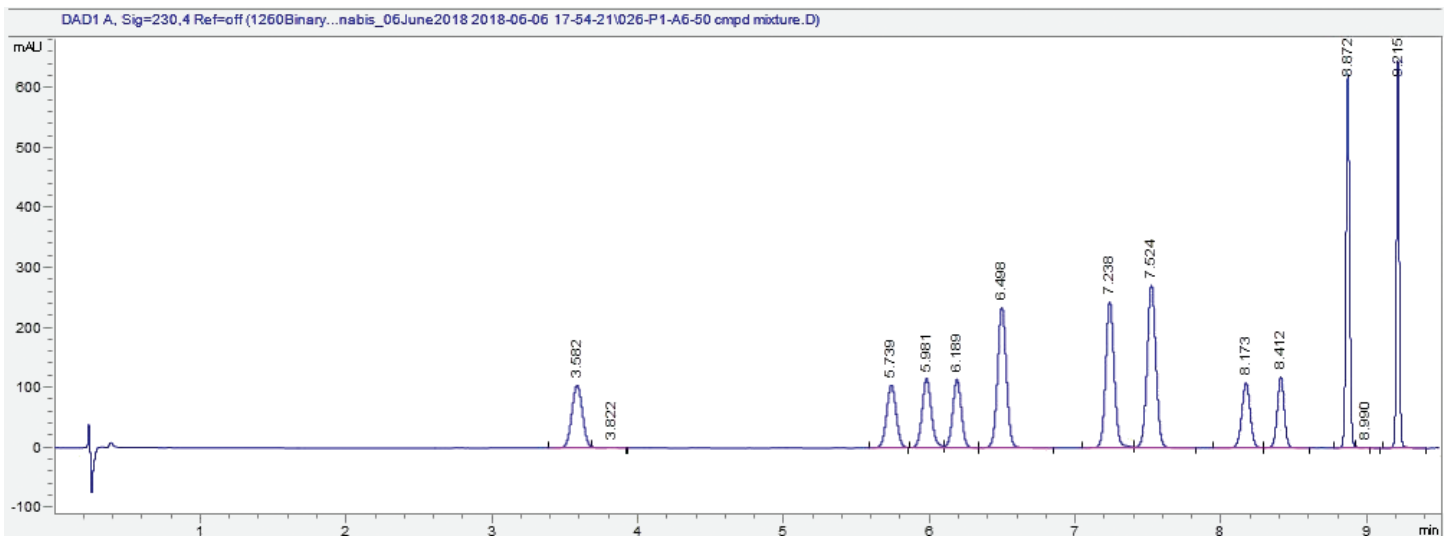**Note:** higher dilution factors may be required depending on the product.

Figure 1. A 50 µg/µL injection of a calibration standard on an Agilent 1220 Infinity II LC system.

Reference

1. Storm, C. *et al.* Dedicated Cannabinoid Potency Testing for Cannabis or Hemp Products Using the Agilent 1220 Infinity II LC System. *Agilent Technologies Application Note*, publication number 5991-9285, **2018**.

Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state/country law.

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