Application Note

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Quantitation of Cannabinoids in Hemp Flower by Derivatization GC/MS

Authors

Jennifer Sanderson and
Jessica Westland
Agilent Technologies, Inc.

Abstract

Total potency and total THC are two important calculations in the distinction of cannabis and hemp. Following U.S. Federal laws, hemp must be less than 0.3% total THC (by dry weight). In this application, offline derivatization of hemp sample extract was performed to determine total THC and quantitate an additional nine commonly analyzed cannabinoids by GC/MS. The derivatization allows for direct analysis and measurement of the thermally labile acids that are naturally occurring in hemp, which simplifies the determination of total THC.

Introduction

Hemp, a multipurpose plant, is a member of the species *Cannabis sativa* L that has long been used for its medicinal properties as well as a source of fiber.[1,2] The United States legal distinction between classifying *Cannabis sativa* L as either hemp or cannabis is defined by the percentage of total THC the plant contains. U.S. Federal law mandates that the total THC percentage of hemp must be less than 0.3% by dry weight.[3] This is a crucial distinction, and laboratories must use the proper sample preparation and analytical methodology to obtain accurate and reliable results.

The challenges of performing this analysis on cannabis flower or hemp is that the plant synthesizes phytocannabinoid acids that decarboxylate under light and heat to their neutral form.[2] Acidic cannabinoids are not GC amendable as they are thermally labile and will decarboxylate as well as degrade into other byproducts in the GC inlet prior to analytical separation. To protect acidic cannabinoids from this process and make them more amenable for GC analysis, derivatization is performed on the extract prior to analysis. This process not only protects the acid from heat degradation, but also makes the analytes more volatile, allowing them to elute faster from the analytical column.

This application presents a workflow to determine total THC by quantitation of (−)-*trans*-Δ9-tetrahydrocannabinol (Δ9-THC) and its acid, (Δ9)-tetrahydrocannabinolic acid (THCA) using sample extraction and offline derivatization. Quantitation of other cannabinoids and the challenges in this matrix are also discussed.

Experimental

Sample preparation

Homogenized ground hemp was obtained from Absolute Standards, Inc. and extracted using the procedure shown in Figure 1.

Approximately 200 mg of homogenized hemp sample was weighed into a 50 mL centrifuge tube (part number 5610-2049). Two ceramic homogenizers (part number 5982-9313) and 20 mL of ethyl acetate were added to the centrifuge tube and mechanically shaken for 10 minutes. The sample was then centrifuged for 5 minutes at 5,000 rpm, filtered using a Captiva regenerated cellulose syringe filter (part number 5190-5109), affixed to a 5 mL Captiva disposable syringe (part number 9301-6476), and diluted for analysis.

Analytical standards preparation

The new suite of cannabinoid reference materials introduced by Agilent offers maximum flexibility for potency analysis by providing 11 individual standards and four unique mixes to meet analyte requirements. The individual standards allow for customization and tailoring the standards to specific regulatory requirements. The pre-made mixes save time and reduce error while allowing for expanded calibration with concentrations at 1 mg/mL for each analyte. The four mixes are listed in Table 1.

To prepare the standards for this workflow, 250 µL of each standard at a concentration of 1.0 mg/mL was dried down under a gentle stream of dry nitrogen and reconstituted in 250 µL of ethyl acetate. Solvent exchange is necessary because these standards come in a methanolic solution, which can interfere with silylation derivatization.[4] A working standard of 100 µg/mL was prepared by adding 100 µL of each standard to 600 µL of ethyl acetate for a final volume of 1 mL. Calibrators were immediately derivatized in order to prevent the acidic cannabinoids from decarboxylating and converting to their respective neutral forms as well as other breakdown products.

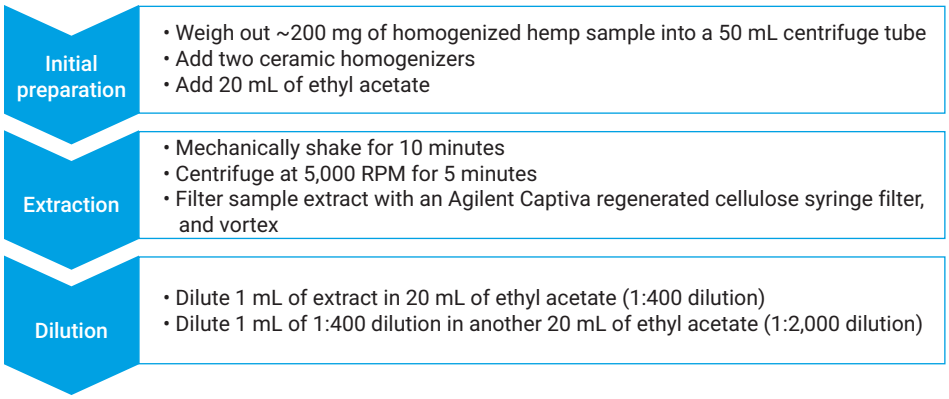

Figure 1. Sample prep method for hemp potency extraction.

Calibration preparation

A total of eight calibrators were prepared by serial dilution of the 100 µg/mL working calibration standard (Table 2). The two lowest level calibrators were prepared from the 10 µg/mL working standard. In this study, all analytes except for CBDA were calibrated from 0.05 to 1 µg/mL. Due to the native concentration of CBDA present in the ground hemp sample, the reporting limit was raised to 0.25 µg/mL and calibrated to 10 µg/mL.

Sample derivatization

Derivatization was carried out with 50 µL of N,O-*bis*(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylsilyl chloride (TCMS) added to 50 µL of calibration standard in 2 mL vials containing 250 µL inserts (part number 5181-1270) to give a 1:1 ratio of sample to derivatizing reagent. Each vial was heated at 70 °C for 60 minutes, a time and temperature that was optimized in concentrated matrix to ensure complete derivatization of all the cannabinoids. The samples were then allowed to come to room temperature prior to analysis by GC/MS.

Instrumentation

An Agilent 8890/5977B gas chromatograph/mass selective detector was employed for this analysis. The 8890 GC was equipped with a multimode inlet and contained a splitless single tapered liner (part number 5190-5112) that contained a sintered frit at the bottom. Separations was carried out on a DB-35MS UI, 30 m × 250 µm, 0.25 µm capillary column (part number 122-3832UI). The mass spectrometer was equipped with an inert ion source with a 9 mm extractor lens (part number G3870-20449). Table 3 provides the GC/MSD parameters used for this workflow.

Data collection and analysis were performed with the Agilent MassHunter Workstation software suite (Acquisition B.10.0.384.1 and Qualitative Analysis for GCMS and LCMS 10.0.707.0).

Table 1. Agilent cannabinoid reference standards.

Cannabinoid Standard	Analyte
Cannabinoid-Mix A: p/n 5190-9430 1.0 mg/mL in methanol	Cannabidiol (CBD) (p/n 5191-3924)
	Cannabinol (CBN) (p/n 5191-3926)
	(–)-*trans*-Δ9-Tetrahydrocannabinol (Δ9-THC) (p/n 5191-3922)
Cannabinoid-Mix B: p/n 5190-9429 1.0 mg/mL in acetonitrile	Cannabigerol (CBG) (p/n 5191-3923)
	(Δ9)-Tetrahydrocannabinolic acid (THCA) (p/n 5191-3925)
	Cannabidiolic acid (CBDA) (p/n 5191-3930)
Cannabinoid-Mix C: p/n 5190-9428 1.0 mg/mL in acetonitrile	Cannabichromene (CBC) (p/n 5191-3928)
	Cannabigerolic acid (CBGA) (p/n 5191-3927)
	Cannabidivarin (CBDV) (p/n 5191-3920)
Cannabinoid-Mix D: p/n 5190-9427 1.0 mg/mL in methanol	Tetrahydrocannabivarin (THCV) (p/n 5191-3921)
	Δ8-Tetrahydrocannabinol (Δ8-THC) (p/n 5191-3922)

Table 2. Calibration level preparation.

Concentration (µg/mL)	W.S. (µg/mL)	Amt W.S. to add (µL)
10	100	100
5	100	50
2.5	100	25
1	100	10
0.5	100	5
0.25	100	2.5
0.1	10	10
0.05	10	5

Table 3. Operating parameters for GC/MS.

Operating Parameters for GC/MS	
Liner	Splitless, UI, fritted, straight (p/n 5190-5112)
Injection Mode	Splitless
Inlet Temperature	280 °C
Oven Program	50 °C (1 min); ramp 20 °C/min to 300 °C (hold for 1.5 min)
Equilibrium Time	0.5 min
Column Flow	Constant, 1.2 mL/min
Column	Agilent DB-35MSUI, 30 m × 0.25 mm, 0.25 µm (p/n 122-3832UI)
Septum Purge Flow Mode	3 mL/min
Purge Flow to Split Vent	15 mL/min after 0.75 min
MS Method-Scan	Scan *m/z* 65 to 600
Solvent Delay	7 min
MS Source	250 °C
MS Quad	150 °C
Tune	atune.u

Results and discussion

Derivatization optimization

Several parameters, such as time, temperature, and ratio of derivatizing agent, all play a role in derivatization. The metric to determine complete derivatization used for this optimization was:

- The absence of CBD-1TMS
- Absolute response of the cannabinolic acids

Cannabidiol, or CBD, has two hydroxyl functional groups to be derivatized, Presence of CBS-1TMS indicates that the second hydroxyl group is not protected and derivatization is not complete.

When CBD-1TMS is present, this is indicative of incomplete derivatization. Varying the ratio of derivatizing agent to spiked matrix was the only variable that proved to affect this metric. The 1:1 ratio was sufficient to convert all of CBD to CBD 2-TMS.

There is an upper limit on temperature that can be used for derivatization with BSTFA. As the temperature exceeds 75 °C, BSTFA will decompose. Therefore, 70 °C was selected and remained constant while varying the time from 0 to 60 minutes in 15-minute increments. The neutral cannabinoids were completely derivatized in 45 minutes in matrix, which is demonstrated by a less than 1% change in response from 45 to 60 minutes. The acidic cannabinoid levels maximized at 60 minutes, so 60 minutes was selected for this study.

Linearity, precision, and accuracy

To meet the maximum concentration for total THC in the hemp flower that is diluted 2,000-fold, an analytical range starting at 50 ppb was analyzed for all of the cannabinoids except CBDA. The resulting linear regression and analytical range for each cannabinoid are listed in Table 4.

Achieving this lower calibration limit is critical for the analysis of Δ9-THC and THCA. The requirement of 0.3% total THC in dry weight of a 200 mg sample equates to a maximum concentration of 0.3 µg/mL. Figure 2 shows the linearity achieved for derivatized Δ9-THC and THCA.

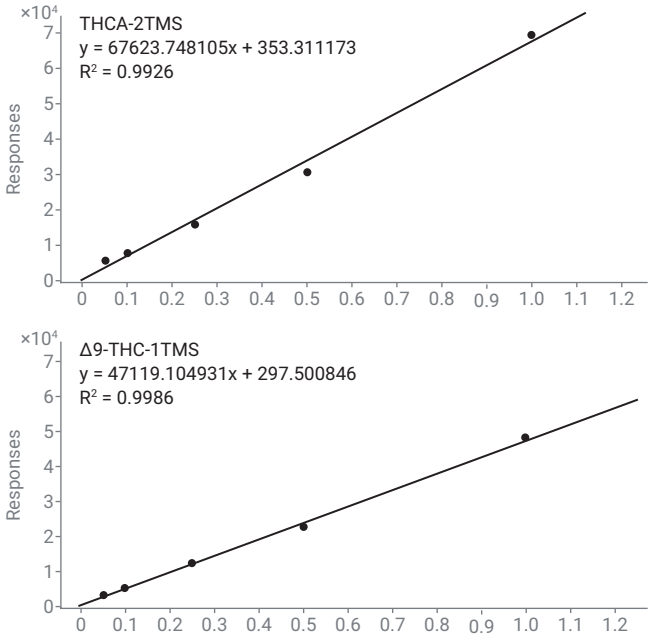

Figure 2. Calibration curves for Δ9-THC and THCA.

Table 4. Calibration, linear regression, and %RSD for 0.05 and 0.10 µg/mL standards.

Analyte	Analytical Range (µg/mL)	Linear Regression (r^2)	%RSD (0.05 µg/mL)	%Recovery (0.05 µg/mL)	%RSD (0.10 µg/mL)	%Recovery (0.10 µg/mL)	Conc. In Hemp (µg/mL)
CBDV-1TMS	0.05 to 1	0.999	5.4	113	4.3	98	N.D.
CBD-2TMS	0.05 to 1	0.994	15.7	71	7.5	79	Below LOQ
THCV-1TMS	0.05 to 1	0.999	8.3	115	3.5	99	N.D.
CBC-1TMS	0.05 to 1	0.997	2.7	148	5.5	108	N.D.
CBG-2TMS	0.05 to 1	0.994	3.1	125	4.6	97	Below LOQ
D8THC-1TMS	0.05 to 1	0.999	6.9	106	8.6	98	N.D.
D9THC-1TMS	0.05 to 1	0.999	12.7	119	3.7	96	Below LOQ
CBDA-3TMS	0.25 to 10	0.998	14.8	696	12.7	392	1,777
CBN-1TMS	0.05 to 1	0.999	3.5	115	4.6	101	Below LOQ
CBGA-1TMS	0.05 to 1	0.989	6.7	193	6.4	137	N.D.
THCA-2TMS	0.05 to 1	0.993	11.2	165	9.5	147	Below LOQ

(N.D. = not detected; LOQ = limit of quantitation)

In addition to linearity, seven replicate injections of hemp matrix spiked at 0.05 and 0.100 µg/mL were performed to determine accuracy and precision. The acidic cannabinoids had high recoveries at both 0.05 and 0.100 µg/mL, all exceeding 150% recovery spiked at 0.05 µg/mL. At 0.100 µg/mL, THCA and CBDA fell below 150% at 137% and 147%, respectively. At both levels, %RSD were well below 15% for all 11 cannabinoids, demonstrating good repeatability. CBDA is present in high quantities in the hemp flower and therefore has a higher reporting limit. The accuracy at the 0.05 and 0.100 µg/mL levels have very high recoveries, at 700% and 400% respectively. CBD recoveries were surprisingly low for both 0.05 and 0.100 µg/mL and had higher injection to injection variability at the 0.05 µg/mL level. The other cannabinoids exhibited good accuracy and precision in the matrix.

Total THC

The derivatized forms of Δ9-THC and THCA will give the total THC by the formula THC_{total} = % THC + (0.877 × %THCA). The factor of 0.877 corrects for decarboxylation of THCA to it's neutral form of THC. In the diluted matrix, levels outside the calibration range (less than 50 ppb) were found for both Δ9-THC and THCA. With the lowest calibrator at 0.05 µg/mL, the total THC value was 0.1 µg/mL, which is well below the maximum allowed legal limit of 0.3 µg/mL. The only cannabinoid found at a quantifiable level in this sample was CBDA, which back-calculates to a concentration of 1,777 µg/mL, or 0.8% by dry weight of the plant sampled.

Other cannabinoids

This hemp flower contains high levels of CBDA, among other phytochemicals. This presents challenges in how samples are prepared, diluted, and analyzed, as the balance between diluting the sample to properly calibrate CBDA could lead to a loss of other cannabinoids of interest. CBN, CBD, and CBG, cannabinoids that can be present in trace levels in hemp plant prior to harvesting, were all found in the sample, but not at a quantifiable level. Spiked recoveries of the cannabinoids suggest that these traces were not created during the sample preparation process.

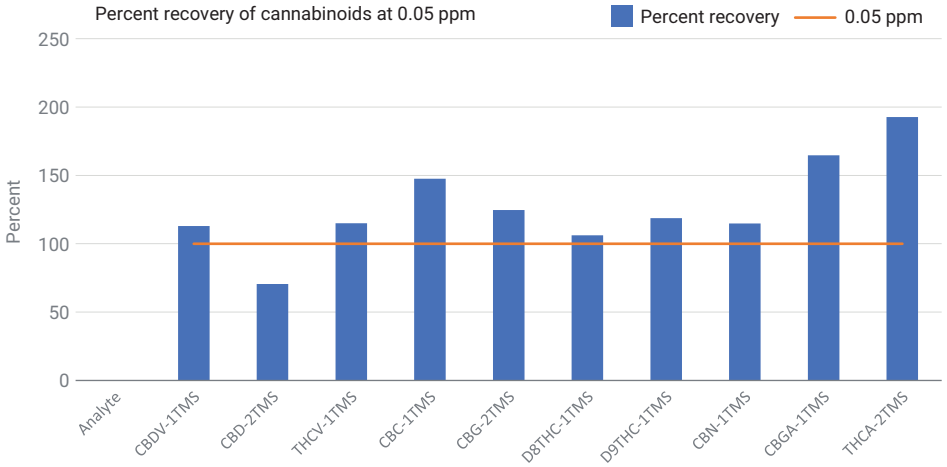

Figure 3. Percent recovery of cannabinoids at 0.05 µg/mL.

Conclusion

The use of offline derivatization using BSFTA with 1% TCMS as a derivatizing agent offers a simple and straightforward method for the analysis of cannabinoids in hemp flower extract. The preservation of the acidic forms of cannabinoids native to the plant allows for direct measurements of THCA corrected for the molecular weight ratio of THC/THCA. This allows for summation of THCA and Δ9-THC to give total THC. It is critical to have fresh calibrators and hemp extract to accurately perform this analysis to prevent any loss of acidic cannabinoids to their neutral form and other byproducts so that accurate THC can be obtained. Despite high levels of CBDA driving the high dilution factor required for analysis, good linearity and spike recoveries were obtained for the cannabinoids, expanding the utility for this method beyond total THC to evaluate other cannabinoids in hemp flower.

Disclaimer

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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