Quantitation of 11 Cannabinoids In Hemp and Cannabis Flower Matrices by GC-FID

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Introduction

Introduction

The legal distinction between classifying *Cannabis Sativa L* as either Hemp or Cannabis is defined by the percentage of total THC the plant contains. Federal law mandates that the percentage must be less than 0.3% by dry weight (1). The THC percentage reported for regulation purposes is a combined value of Δ9-THC and its acid that is native in the plant, THCA. Using liquid chromatography (LC), THCA and Δ9-THC are separated and quantitated, with THCA needing to be corrected for the loss of CO_2 to convert THCA to Δ9-THC by a factor of 0.88. Then the two totals are combined and reported.

Gas Chromatography (GC) has the advantage of decarboxylating THCA in the GC inlet prior to chromatographic separation, resulting in a single peak for Δ9-THC , thus simplifying reporting. Other acidic cannabinoids, such as CBDA and CBGA, are also decarboxylated prior to separation. Presented here is the use of GC with FID to quantitate both neutral and acidic cannabinoids in hemp and cannabis flower.

Figure 1: Agilent 8890 GC/FID.

Experimental

Sample Preparation.

Hemp flower was obtained from Absolute Standards. The flower was homogenized and then was extracted using the procedure below in Figure 1. A sample of cannabis flower from the University of Mississippi was extracted using the same procedure.

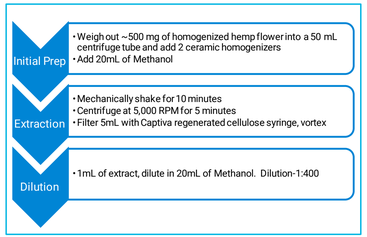


Figure 2: Sample preparation workflow.

Instrumentation

The Agilent 8890 Gas Chromatograph with a flame ionization detector (FID) was employed for this analysis. The 8890 GC was equipped with a MultiMode Inlet and contained a splitless single tapered liner (PN 5190-5112) that contained a sintered frit at the bottom. Separations were carried out on a DB-35MS UI 30 m x 250µm x 0.25 µM capillary column (PN 122-3832UI). Full method parameters can be found in Table 1. Agilent OpenLab v. 3.5 software was used for data acquisition and data analysis.

GC System	8890 GC/FID
Inlet	Multimode Inlet, 300ºC
	5:1 Pulsed Split
Column	DB-35MSUI 30X250µmX0.25µm
Carrier Gas	He, 1.4 mL/min
Oven	90ºC, hold for 0.5 min
	Ramp at 40ºC/min to 300ºC, hold for 4.75 min
FID	Heater 320ºC
	H2 Flow 50mL/min
	Air Flow 400mL.min
	Makeup Flow 50mL/min
Injection	1µL

Table 1: Analytical conditions for analysis of cannabinoids in hemp flower matrix.

Results and Discussion

Separation of the Neutral Cannabinoids

Eight neutral cannabinoids (CBDV, THCV, CBC, CBD, Δ8-THC, Δ9-THC, CBG and CBN) are fully resolved chromatographically as seen in Figure 3 . The acidic cannabinoids THCA, CBGA and CBDA were also analyzed. The acids are decarboxylated in the inlet prior to column injection, combining with their neutral counterparts.

Decarboxylation of Acidic Cannabinoids

The process of decarboxylating acidic cannabinoids is sensitive to both time and temperatures. Some of the neutral cannabinoids, such as Δ9-THC, are known to evaporate at temperatures often employed for rapid decarboxylation. Further, Δ9-THC can degrade at temperatures as low as 85ºC into CBN (2), which is why this analyte is typically monitored during analysis. Quantitation of THCA against a Δ9-THC calibration showed an 85% recovery with this method.

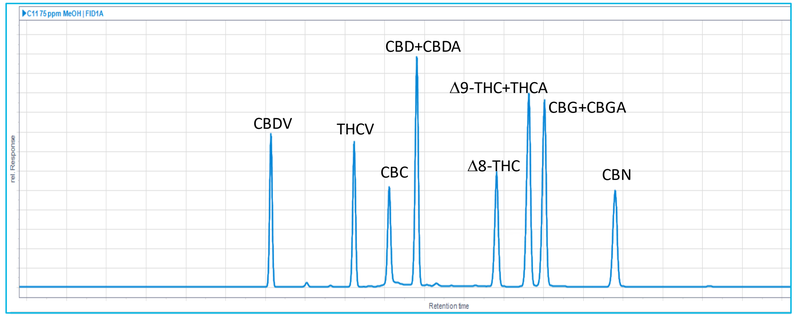
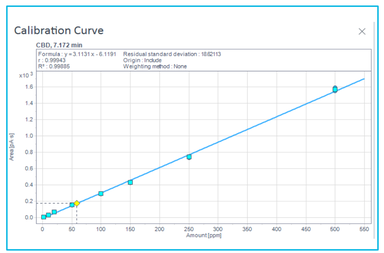


Figure 3: Chromatogram of 11 cannabinoids in methanol solvent. The standards are sourced from Agilent Technologies (**https://www.agilent.com/en/product/chemical-standards/cannabis-testing-standards/cannabinoids**)

Calibration

For each of the analytes, an 8-pt. calibration ranging from 1ppm to 250ppm was analyzed. CBGA, CBDA and THCA were also analyzed combined with their neutral forms, giving a range of 2ppm to 500ppm for the combined neutrals and acids. The calibrations for Δ9-THC and CBD are pictured below in Figures 4 and 4a. All calibration curves were made in methanol solvent and had r^2 values of 0.998 or better.



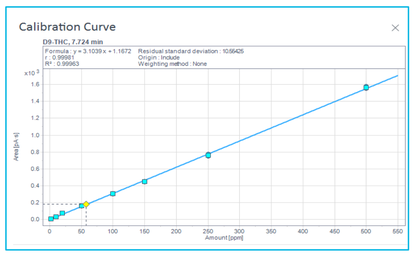


Figure 4 and 4a: Calibration curve for CBD and Δ9-THC in methanol solvent.

Results and Discussion

Cannabinoid Profile of Hemp Flower

Duplicate samples of hemp flower were analyzed for the content of the neutral cannabinoids. Reported values and calculated values are reported in Table 3 . The hemp flower was also spiked at 25ppm to evaluate recovery of cannabinoids in matrix, which is also reported.

Cannabinoid Profile of Cannabis Flower

A partially uncharacterized low-potency cannabis flower was also extracted and analyzed for the 11 cannabinoids as well having matrix spiked to evaluate recovery. The total THC was calculated to be 0.32% w/w, which meets the legal threshold to be classified as cannabis.

	Composition of Hemp Flower			Composition of Cannabis Flower	
Cannabinoid	Reported %	Calculated %	Matrix Spike Recoveries	Calculated %	Matrix Spike Recoveries
CBDV	0.002	N.D.	113	N.D.	113
THCV	0.01	N.D.	109	N.D.	110
CBC	0.03	Below LOQ	129	N.D.	116
CBD + CBDA	3.47	3.15	111	2.41	100
D8THC	0	N.D.	116	N.D.	112
D9THC + THCA	0.17	0.24	121	0.32	110
CBG+ CBGA	0.2	0.39	111	0.41	107
CBN	0	N.D.	113	N.D.	114

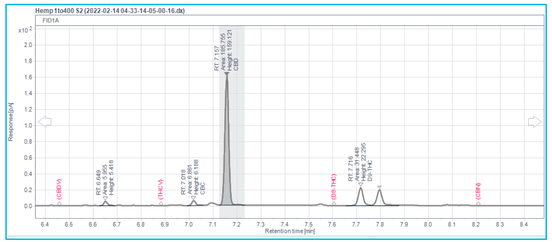


Table 3 (above): Tabulated composition of cannabinoids in hemp and cannabis flower by w/w%. Figure 5 (left): Chromatogram of Hemp flower sample. The cannabinoids in red were not found in the sample.

Conclusions

GC-FID is a quick method to quantitate Total THC as well as other cannabinoids.

- By decarboxylating the acidic cannabinoids into their neutral forms, total THC can be quantitated and reported without further corrections.
- Further work needs to be carried out on ensuring full decarboxylation of the acids while minimizing loss of neutrals through evaporation or breakdown product.
- By using solvent calibrations and matrix spikes, it is possible to quantitate variable cannabis and hemp matrices.

References

[1] H.R.2-Agriculture Improvement Act of 2018.n.b. SEC. 10111.

[2] Repka, M. A., et. Al. (2006). Temperature stability and bioadhesive properties of Δ9-tetrahydrocannabinol incorporated hydroxypropylcellulose polymer matrix systems. Drug development and industrial pharmacy, 32(1), 21-32.

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