Quantitation of Cannabinoids by Derivatization GC/MS

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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). While there is a continued drive towards LC methods for THC and cannabinoid quantitation, GC will continue to have a role and thus presents the need to have reliable methods in this application space.

Figure 1 Agilent 8890/5977B GC/MSD

Presented here is a workflow to determine total THC by quantitation of (−)-trans-Δ9-Tetrahydrocannabinol (Δ9-THC) and its acid, delta(9)-Tetrahydrocannabinolic acid (THCA) using sample extraction and offline derivatization. Quantitation of other cannabinoids and the challenges in this matrix are also discussed here.

Experimental

Standard Preparation

The new suite of cannabinoid reference materials introduced by Agilent offer maximum flexibility for potency analysis by providing 4 unique mixes to meet analyte requirements. This allows for utilizing time-saving prepared mixes at 1 mg/mL per compound to allow for expanded calibration and quantitation for all 11 cannabinoids.

Cannabinoid Standards	Analyte
Cannabinoid-Mix A: P/N 5190-9430 1.0 mg/mL in Methanol	Cannabidiol (CBD)
	Cannabinol (CBN)
	(−)-trans-Δ9-Tetrahydrocannabinol (Δ9-THC)
Cannabinoid-Mix B: P/N 5190-9429 1.0 mg/mL in Acetonitrile	Cannabigerol (CBG)
	delta(9)-Tetrahydrocannabinolic acid (THCA)
	Cannabidiolic acid (CBDA)
Cannabinoid-Mix C: P/N 5190-9428 1.0 mg/mL in Acetonitrile	Cannabichromene (CBC)
	Cannabigerolic acid (CBGA)
	Cannabidivarin (CBDV)
Cannabinoid-Mix D: P/N 5190-9427 1.0 mg/mL in Methanol	Tetrahydrocannabivarin (THCV)
	delta-8-Tetrahydrocannabinol (Δ8-THC)

Table 1 Agilent Cannabinoids Standards

To prepare the standards this workflow, 250 μL of each standard 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 the silylation reaction (2).

Experimental

Calibration Preparation

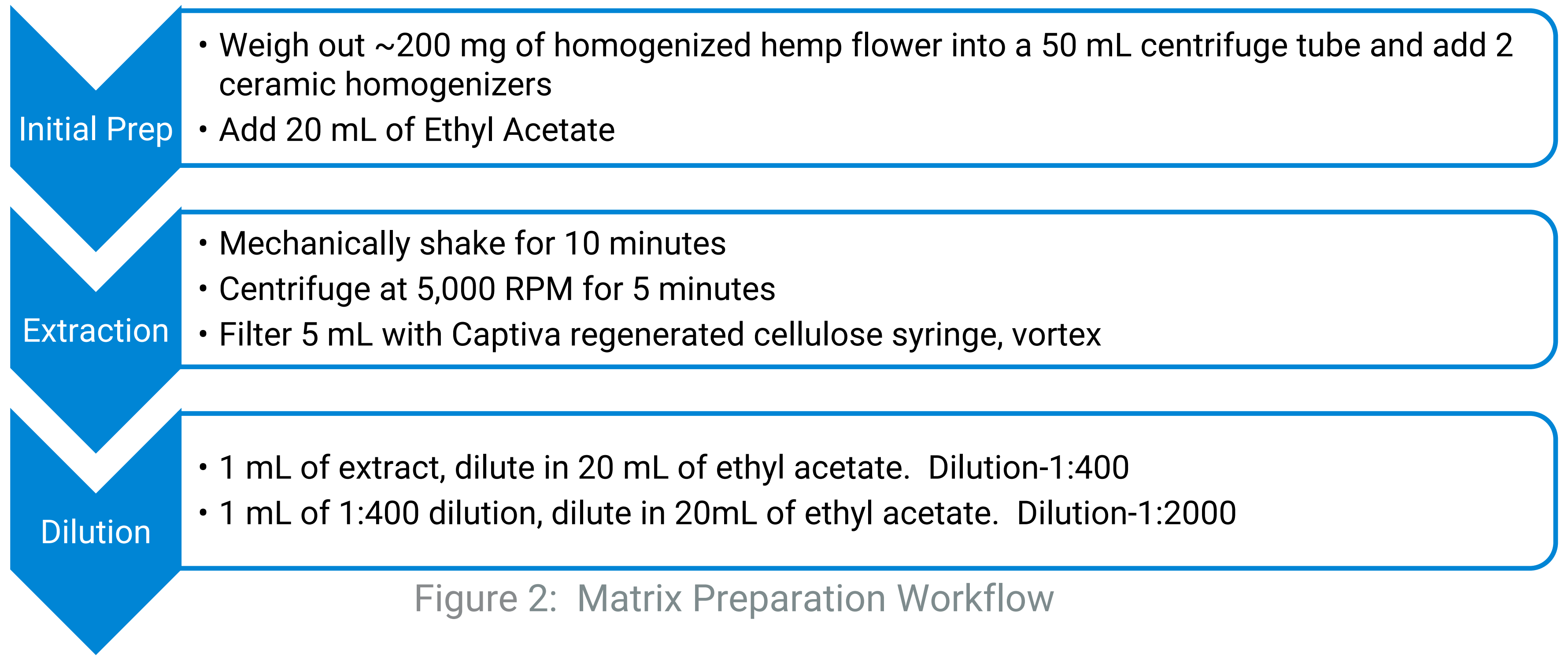
A total of 8 calibrators were prepared in matrix (Figure 2) by serial dilution of the 100 ppm working calibration standard. The two lower level calibrators were made from the 10 ppm working standard (W.S.).

Concentration (ppm)	W.S. (ppm)	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 2 Calibration preparation table

Sample Derivatization

Using the calibration table above in Table 2, 50 μL of N,O-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% Trimethylsilyl chloride (TCMS) is added to each vial containing 50 μL of standard to give a 1:1 ratio of sample to derivatizing reagent. Each vial is then heated to 70 ºC for 60 minutes, a time and temperature that was optimized in concentrated matrix to ensure complete derivatization of all the cannabinoids. The samples are then allowed to come to room temperature and is ready for analysis by GC/MS.



Initial Prep
- Weigh out ~200 mg of homogenized hemp flower into a 50 mL centrifuge tube and add 2 ceramic homogenizers
- Add 20 mL of Ethyl Acetate

Extraction
- Mechanically shake for 10 minutes
- Centrifuge at 5,000 RPM for 5 minutes
- Filter 5 mL with Captiva regenerated cellulose syringe, vortex

Dilution
- 1 mL of extract, dilute in 20 mL of ethyl acetate. Dilution-1:400
- 1 mL of 1:400 dilution, dilute in 20mL of ethyl acetate. Dilution-1:2000

Figure 2: Matrix Preparation Workflow

Results and Discussion

Calibration in Hemp Matrix-Δ9-THC and THCA

Table 3 to the right are the linear regression of the 11 cannabinoids calibrated in this work, with a working lower range of 50-100 ppb. 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 ppm in solution with this dilution of the matrix at 2,000-fold. Figure 3 shows the linearity achieved for derivatized Δ9-THC and THCA.

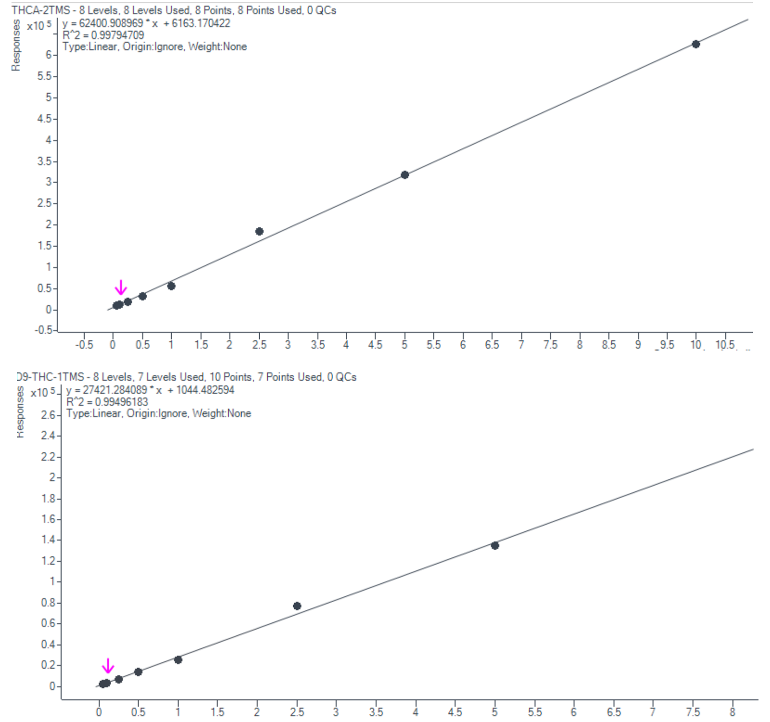


Figure 3 Calibration Curves for Δ9-THC and THCA

In addition to linearity, seven replicate injections of hemp matrix spiked at 100 ppb were performed to determine accuracy and precision. The percent RSD was less than 15 for Δ9-THC and THCA , demonstrating good repeatability and percent recoveries at this level were within 10 % for Δ9-THC and THCA.

Total THC In Hemp Matrix

The derivatized forms of Δ9-THC and THCA allow total THC to be calculated with correction factor 0.877 for THCA . In the diluted matrix, a total of 0.237 ppm was found. This back calculates to approximately 473 μg in 200 mg of hemp flower, or 0.22%, which meets the legal requirement for this plant to be classified as Hemp. Percentage of all cannabinoids by dry weight are tabulated above.

Results and Discussion

Analyte	Analytical Range (ppm)	Linear Regression (r^2)	% RSD (0.100 ppm)	% Recovery	% Cannabinoid in Hemp
CBDV-1TMS	0.1-5	0.994	2.26	140	0
CBD-2TMS	0.1-5	0.993	8.15	169	0.5
THCV-1TMS	0.05-5	0.995	4.80	103	0.2
CBC-1TMS	0.05-5	0.994	2.93	116	0
CBG-2TMS	0.05-1	0.995	6.08	108	0.1
D8THC-1TMS	0.05-5	0.990	6.06	82	0.3
D9THC-1TMS	0.05-5	0.995	13.10	113	0.08
CBDA-3TMS	0.5-10	0.989	25.32	1195	5.3
CBN-1TMS	0.05-5	0.995	2.56	85	0
CBGA-1TMS	0.05-1	0.999	14.90	99	0.2
THCA-2TMS	0.05-10	0.997	7.51	134	0.16

Challenges in Hemp Matrix-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 loss in other cannabinoids of interests. CBDA had variability injection to injection at the 0.1 ppm level due to the already high levels present in the hemp (calculated to be ~5 %).CBD and CBDV had good precision but showed high bias for recoveries. The other cannabinoids exhibited good accuracy and precision in the matrix. Figure 4 graphically shows the percent recoveries for the spiked matrix

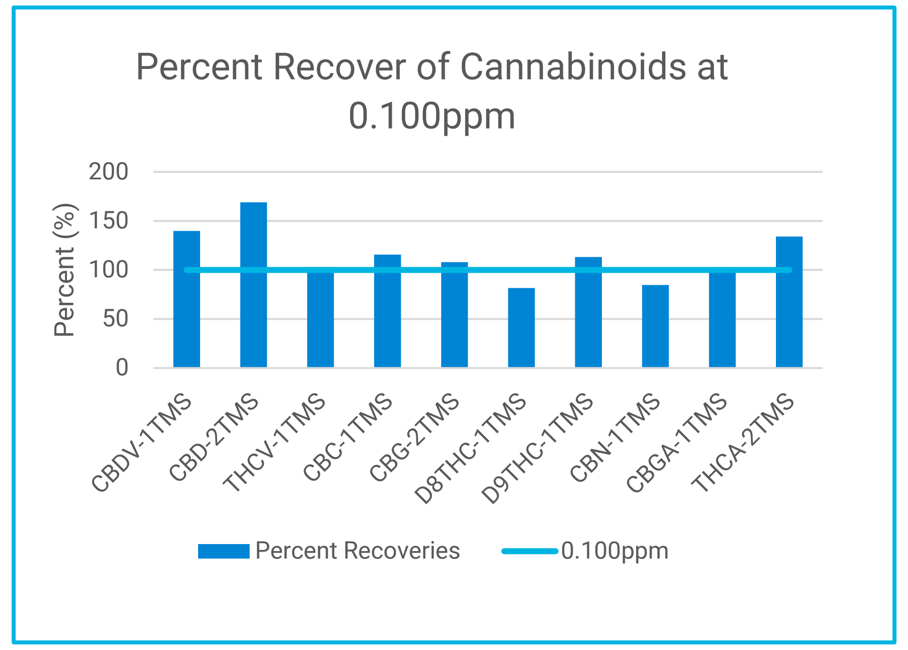


Figure 4 Graph of percent recoveries for the cannabinoids. CBDA is excluded from this graphic.

Conclusions

Agilent Potency Standards Can be used to quantitate cannabinoids

- Derivatization of analytes can be used to determine all cannabinoids, including cannabinolic acids by sample derivatization
- Total THC can be determined in a straightforward calculation by summation of Δ9-THC and THCA, corrected for CO_2 loss by 0.877 .
- Hemp matrix presents challenges as natural percent levels of other cannabinoids interfere with calibration and quantitation.

References

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

[2] Francis Orata, F. (2012) Derivatization Reactions and Reagents for Gas Chromatography Analysis. In: Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications.

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