Instrument: Pegasus® BT

Cannabis Analysis: Hemp Potency Determination

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Introduction

The large increase in hemp production in the United States has mandated standardization for Delta 9-tetrahydrocannabinol (△9-THC) testing across the nation.[1] Hemp or fiber-type cannabis, was legalized as an agricultural commodity by the United States Government in 2018.[2] Hemp contains low concentrations of the psychoactive cannabinoid △9-THC and must be tested to confirm that △9-THC levels are below 0.3%.[3] Currently, there are states using their own extraction and Gas Chromatography-Flame Ionization Detection (GC-FID) methods for hemp potency testing. These methods are in line with extraction protocol and analytical methods for cannabis testing outlined by the United Nations Office on Drugs and Crime.[4] However, for legislative requirements, it is imperative that methods be both selective and sensitive for this type of analysis since cannabis is a complex botanical composed of diverse chemical compounds present in a wide range of concentrations.[5] Gas Chromatography-Mass Spectrometry (GC-MS) is a preferred method for qualitative and quantitative analysis of complex botanicals because of its excellent chromatographic resolution, sensitivity, and increased selectivity. In addition, the use of microwave-assisted extraction (MAE) results in conversion (decarboxylation) of inactive △9-THCA (acid form of THC) to △9-THC before detection, thus facilitating total THC determination in hemp samples by GC-MS. The Time-of-Flight Mass Spectrometry variation of this technology (GC-TOFMS), together with modern processing software with deconvolution capabilities, results in cleaner signals for accurate identification and quantitation regardless of closely eluting compounds and interfering complex sample matrix. In this study, a commercially available hemp Certified Reference Material (CRM) and three other commercially available hemp samples were analyzed to determine their △9-THC levels and thus their legality based on US law. Figure 1 displays the Analytical Ion Chromatogram (AIC) with chromatographic peaks for tribenzylamine (TBA), cannabidiol (CBD), △9-THC, and cannabinol (CBN) for the CRM. Quantitative results for △9-THC are included in the inset table. TBA was used as the internal standard for △9-THC quantitation.

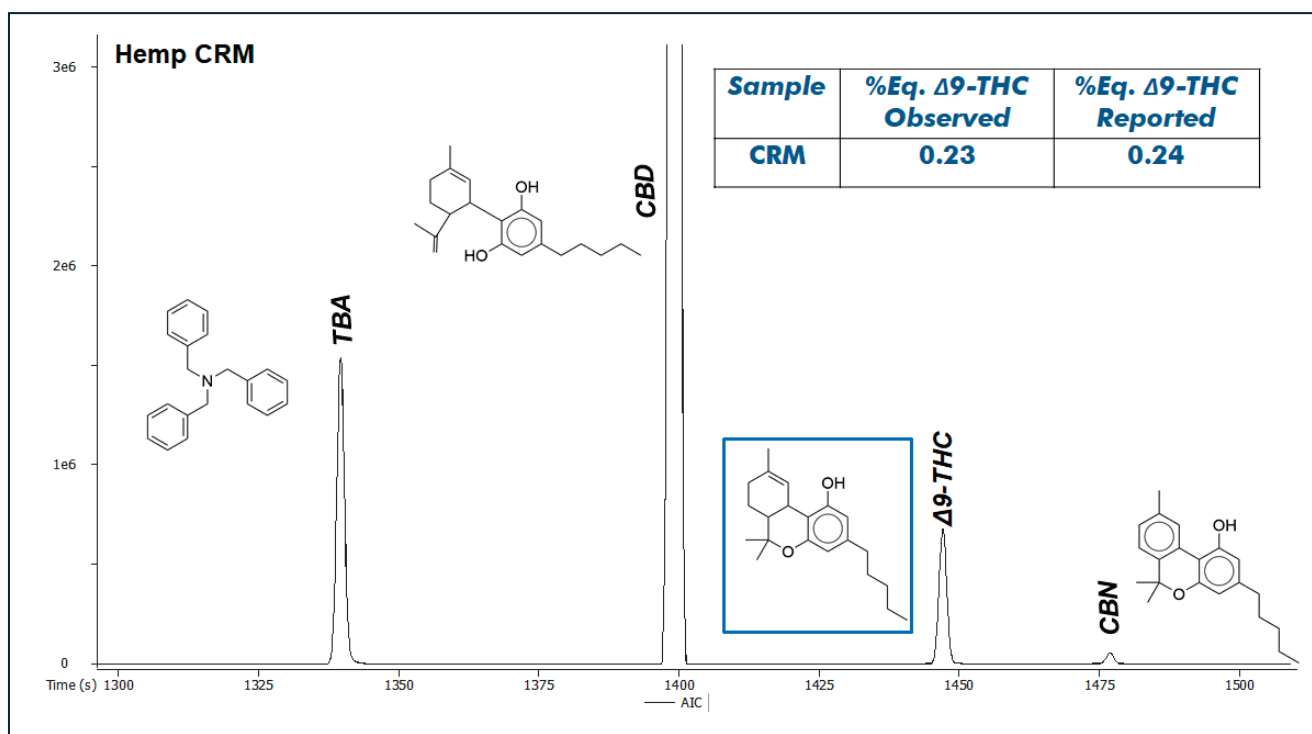| Sample	%Eq. Δ9-THC Observed	%Eq. Δ9-THC Reported
CRM	0.23	0.24

Figure 1. A zoomed-in AIC displaying TBA, △9-THC, CBD, and CBN in a hemp sample. Inset table shows experimentally determined and manufacturer reported percent equivalent (% Eq.) values for △9-THC in the hemp CRM Sample.

Experimental

The TBA internal standard (Cat. No. 90660) and HPLC-grade ethanol (Cat. No. 459836) were purchased from Sigma-Aldrich. The three-component cannabinoid standard (1000 mg/mL of Δ9-THC, CBD, and CBN in methanol) and 50 mL centrifuge tubes were purchased from Restek Corporation, and the cannabis botanicals were obtained from a local vape shop. The hemp CRM was procured from Emerald Scientific (Part No. 54999C).

The internal standard solution was prepared by transferring 50 mg of TBA to a 1 L volumetric flask with 800 mL of ethanol. The flask was shaken and additional ethanol was added to the 1 L mark. A stock solution cannabinoid standard mix (120 ppm) was prepared by mixing 0.5 mL of the Restek cannabinoid standard with 3.67 mL of methanol for a total volume of 4.17 mL. Seven calibration standards (1, 2, 4, 8, 20, 40, and 80 ng/μL) were prepared by mixing the appropriate amount of internal standard solution, cannabinoid stock, and ethanol.

Samples were prepared by weighing 0.05 g of freshly ground hemp sample into a 10 mL reaction tube (Figure 2). An 8 mL aliquot of the internal standard solution was added to the tube, and the heterogeneous mixture was purged with N_2, placed in a microwave reactor, and heated at 180 °C for 30 minutes. During this extraction procedure, the acid forms of the cannabinoids were decarboxylated (activated) to produce the corresponding neutral forms as shown for the acid Δ9-THC-A in Figure 3. The supernatant (500 μL) was then transferred to a GC vial and diluted with 1 mL of ethanol and vortexed for 1 minute. Samples were analyzed with a LECO *Pegasus* BT using the conditions shown in Table 1. Data were processed with ChromaTOF® software.
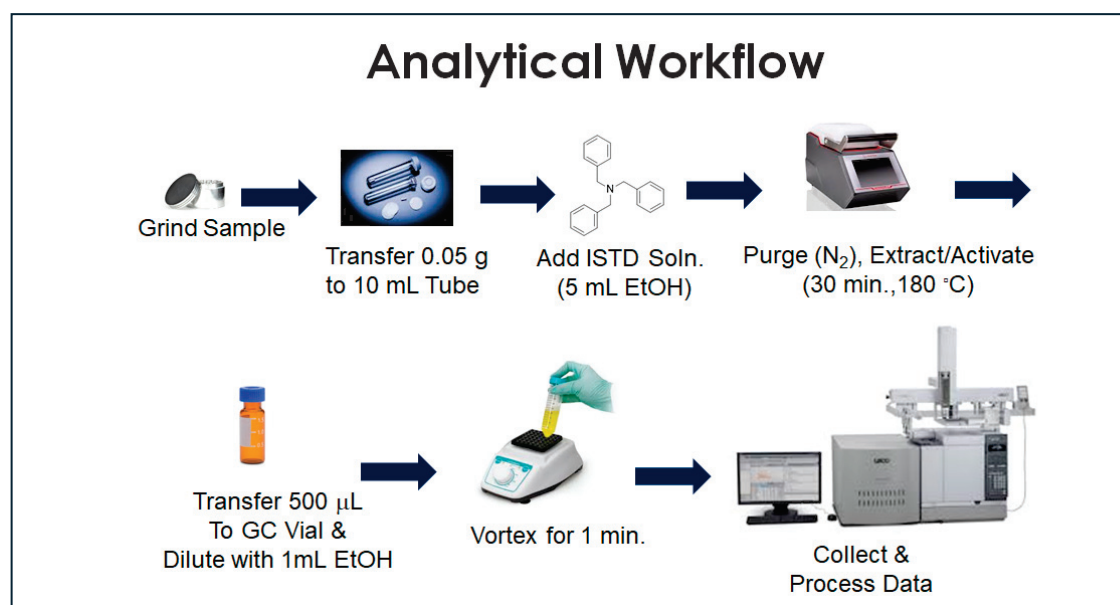

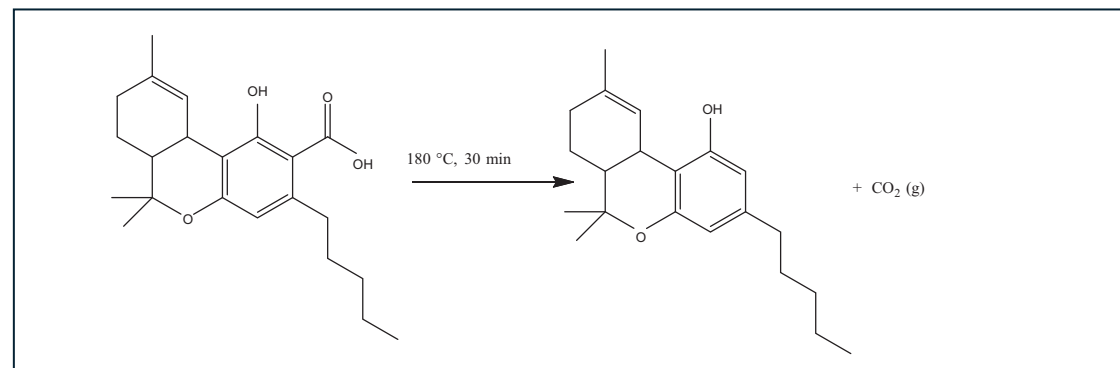*Figure 2. Sample preparation procedure for GC-TOFMS analysis of hemp samples.*

Figure 3. Conversion of THCA to THC.

Table 1. GC-TOFMS (*Pegasus* BT) Instrument Settings

Gas Chromatograph	Agilent 7890 with LECO L-PAL 3 Autosampler
Injection	1 µL, Split 100:1 (250 °C)
Carrier Gas	He @ 1.2 mL/min, Constant Flow
Column	Rxi-5 MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Temperature Program	40 °C (1 min), ramped 10 °C/min to 300 °C (3 min)
Mass Spectrometer	LECO *Pegasus* BT
Ion Source Temperature	250 °C
Ionization Mode	EI
Mass Range (m/z)	45-650
Acquisition Rate	10 spectra/s

Results and Discussion

Comprehensive data was collected for the CRM and three additional hemp samples. The Peak True (deconvoluted) and corresponding library mass spectra for CBD, Δ9-THC and CBN are displayed in Figure 4. The spectral similarity values for the three cannabinoids were 921, 900, and 810 respectively.
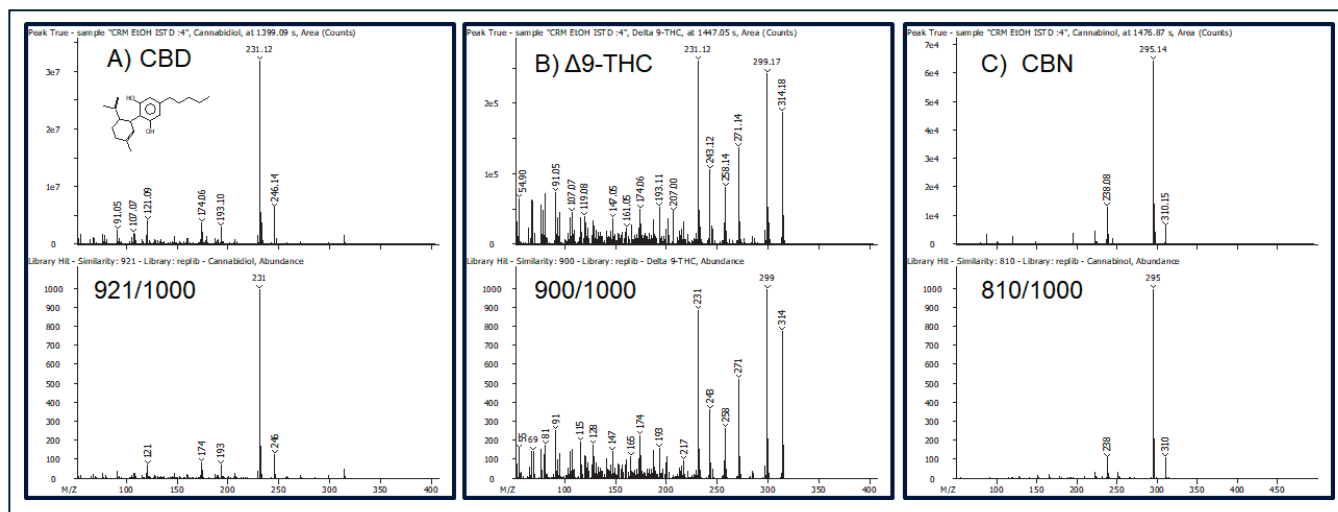

Figure 4. Peak True and Library Mass Spectra for CBD, Δ9-THC, and CBN in a hemp CRM.

The rich botanical data sets were probed for total Δ9-THC content (Δ9-THC tot = Δ9-THC + Δ9-THCA), as well as concentrations for CBD and CBN. Data were processed using the Target Analyte Finding (TAF) feature of LECO's *ChromaTOF* software. The TAF method rapidly processes the full mass range data looking for specific ions with mass tolerances and retention time windows for positive detection. Two ions for each of the cannabinoids were required for positive detection and quantitation in each sample (Figure 5). Calibration curves covering a concentration range from 1-80 ng/µL for Δ9-THC, CBD, and CBN were generated using *ChromaTOF* and used to calculate their concentrations in the hemp samples. Excellent linearity (R^2 > 0.997) was observed for all three cannabinoids (Figure 6).
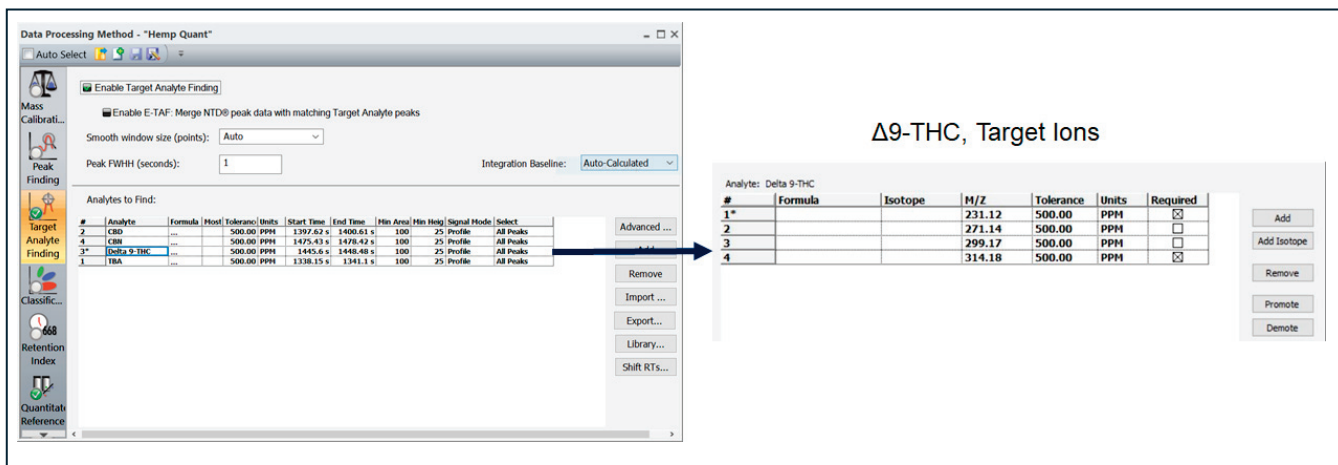

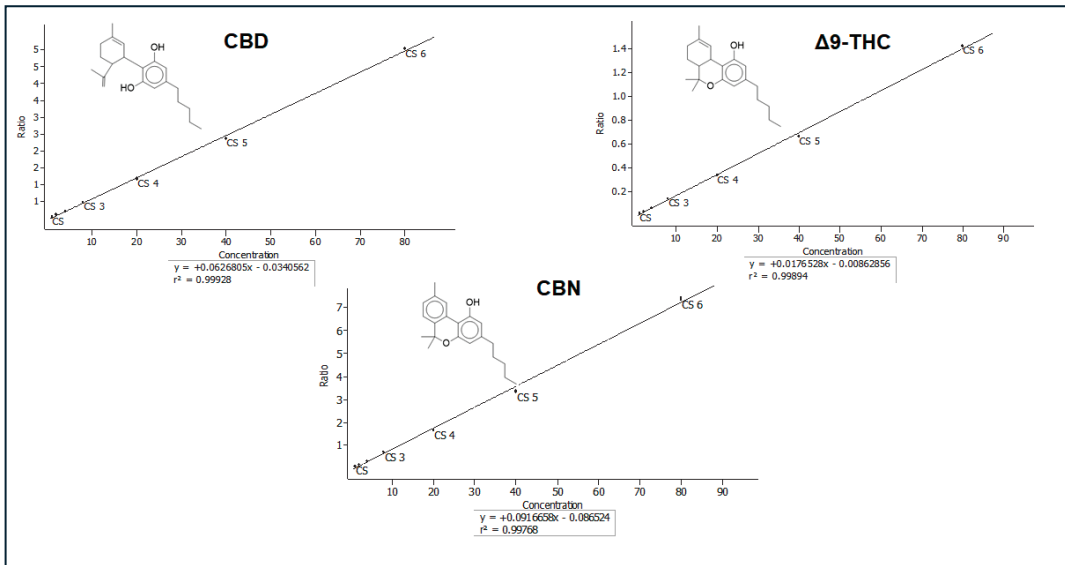*Figure 5. TAF method for quantitative analysis of CBD, Δ9-THC, and CBN in hemp.*

Figure 6. Calibration curves for CBD, Δ9-THC, and CBN (1 to 80 ng/μL).

Hemp samples 1, 2, and 3 were analyzed using GC-TOFMS to quantify their levels of Δ9-THC in order to determine their legality based on US law. Data for these samples were processed using the TAF method described above, and the AICs with peaks for TBA and targeted cannabinoids are displayed below (Figure 7). The cannabinoid three-letter acronyms, calibration concentrations (ng/μL), and % Eq. values are summarized in Figure 8. Concentrations that exceeded the calibration range used in this study were simply reported as greater than 2.4% equivalents (>2.4%). Concentrations that were below the calibration range were reported as less than 0.04% equivalents (<0.04%).
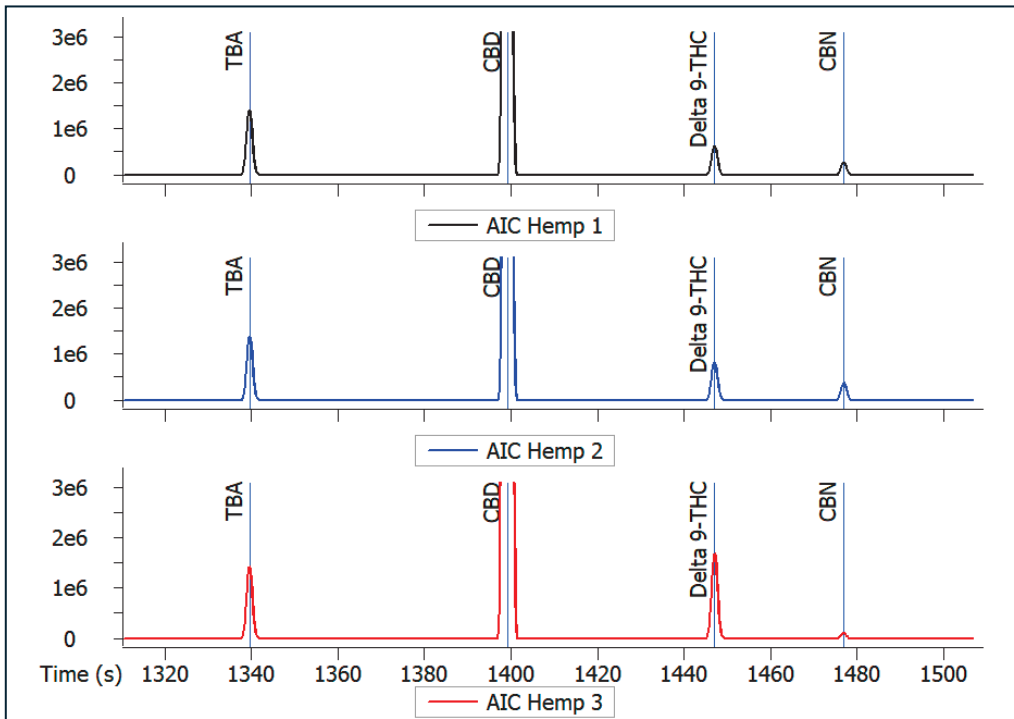

Figure 7. A zoomed-in AIC displaying TBA (internal standard) and targeted CBD, Δ9-THC, and CBN in commercially available hemp samples 1, 2, and 3.
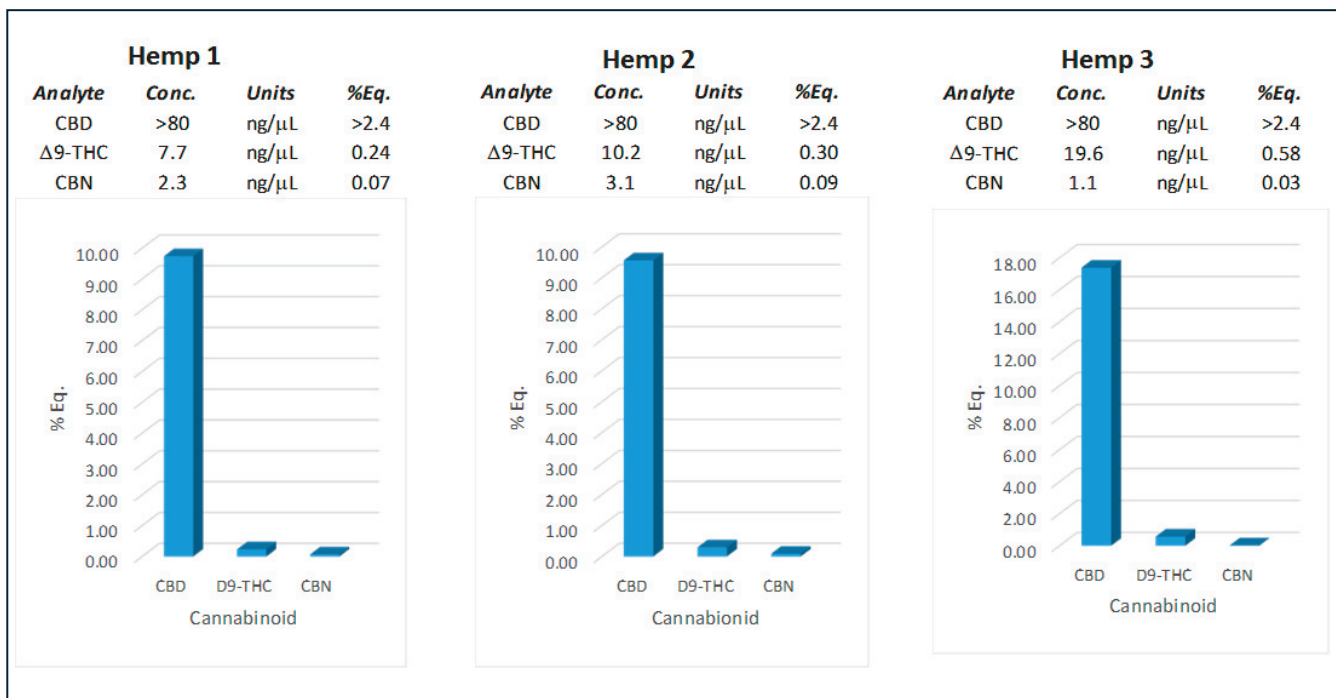
Figure 8. CBD, △9-THC, and CBN concentrations, sample weights and % Eq. in hemp samples 1, 2, and 3.

Each sample was analyzed twice, average △9-THC concentration calculated, and values compared to the distributor's reported values (Table 2). The observed △9-THC for sample 1 was consistent with that reported in its certificate of analysis; however, △9-THC levels for samples 2 and 3 were higher than the ones reported by the distributor. It is interesting to note that the △9-THC value for sample 3 (0.58 % Eq.) was significantly higher than the US legal limit.

Table 2. Reported and Observed (TAF) results for % Eq. △9-THC in hemp samples 1, 2, and 3.

Sample	%Eq. Δ9-THC Observed	%Eq. Δ9-THC Reported
1	0.22	<0.3
2	0.30	ND
3	0.58	<0.3

A major advantage of GC-TOFMS data collection is that it is comprehensive and can be analyzed using untargeted Peak Find processing with deconvolution. This results in a more detailed analysis and improves characterization of the many components in these botanical samples. Untargeted analysis is not restricted by specific analyte target panels and provides additional information regarding the numerous cannabinoids, terpenes, and terpenoids in hemp samples as shown in the filtered chromatogram in Figure 9. The names, formulas, retention times, and spectral similarity values for a small representative set of compounds in hemp sample 3 are listed in Table 3. Spectral similarity values were determined by comparison to well-established databases (e.g., NIST) and averaged 866/1000 for these compounds. Not surprisingly, CBD was the compound present in highest concentration in the hemp samples. Recently, there has been a dramatic increase in the number of CBD-containing products hitting the market. This includes topical creams, oils, edibles, and beverages. There is growing evidence of the benefits of CBD for the treatment of epilepsy, chronic pain, and cancer.[6] Furthermore, CBD modulates some of the adverse effects associated with the use of △9-THC.[7] It is important to shift to a more comprehensive investigation of cannabis to determine appropriate dosing, study long-term risks, and expand on the potential benefits of additional cannabinoids and terpenes in cannabis.[8]
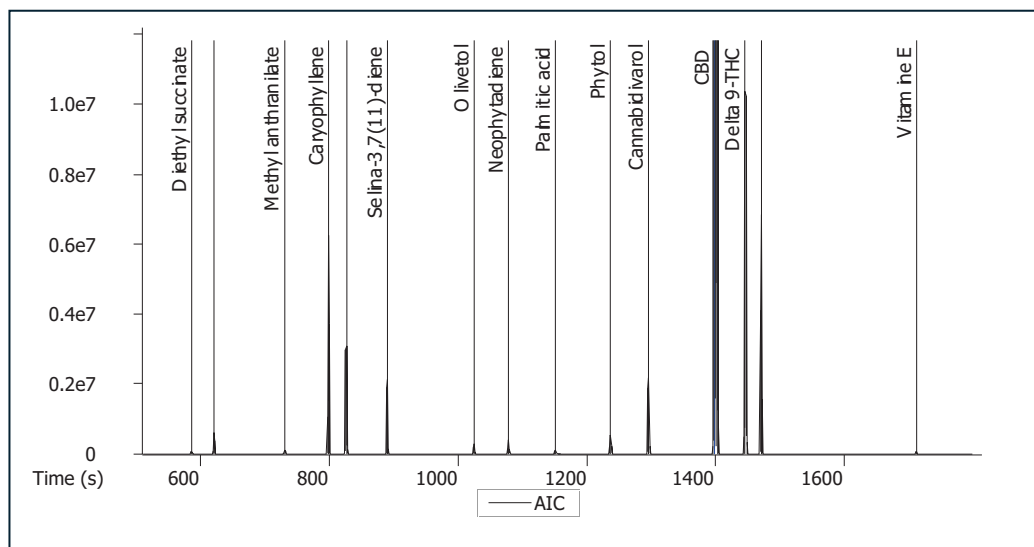
Figure 9: AIC displaying a representative subset of components identified in hemp sample 3.

Table 3. Representative compounds in hemp sample 3.

Name	Formula	R.T. (s)	Similarity
Diethyl succinate	$C_8H_{14}O_4$	586	845
Coumaran	C_8H_8O	621	847
Methyl anthranilate	$C_8H_9NO_2$	731	838
Caryophyllene	$C_{15}H_{24}$	799	888
Humulene	$C_{15}H_{24}$	826	884
Selina-3,7(11)-diene	$C_{15}H_{24}$	890	906
Olivetol	$C_{11}H_{16}O_2$	1025	867
Neophytadiene	$C_{20}H_{38}$	1079	849
Palmitic acid	$C_{16}H_{32}O_2$	1151	777
Phytol	$C_{20}H_{40}O$	1237	875
Cannabidivarol	$C_{19}H_{26}O_2$	1296	880
CBD	$C_{21}H_{30}O_2$	1399	922
Cannabichromene	$C_{21}H_{30}O_2$	1403	845
Δ9-THC	$C_{21}H_{30}O_2$	1447	907
Cannabigerol	$C_{21}H_{32}O_2$	1471	909
Vitamin E	$C_{29}H_{50}O_2$	1713	821

A couple of examples of the spectral quality of cannabis compounds are shown in Figure 10 where the Peak True (deconvoluted) mass spectra for the sesquiterpene caryophyllene and cannabinoid cannabigerol (CBG) are displayed on top, and the corresponding NIST library matches are shown on the bottom of the figure. Spectral similarity values for these compounds were 888 and 909/1000 and the mass delta values ($M\Delta = m/z_{obs} - m/z_{calc}$) were -0.01 and -0.04 respectively.
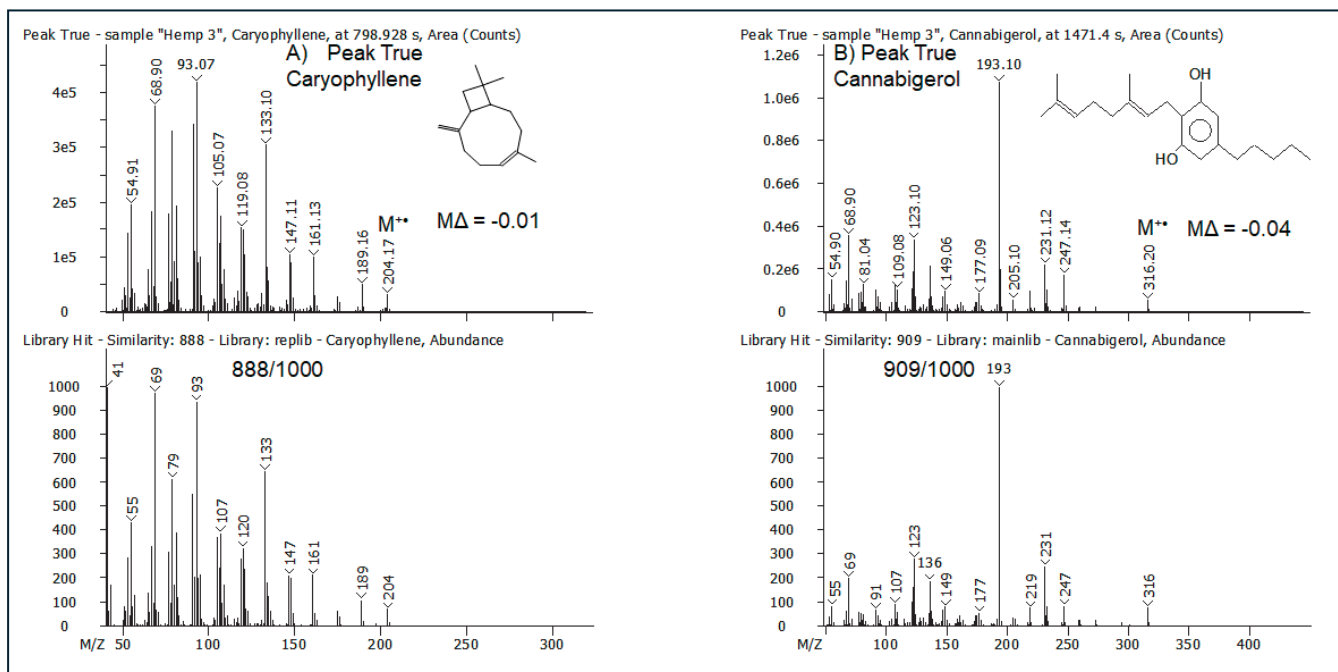
Figure 10. Peak True (Deconvoluted) and Library Mass Spectra for caryophyllene and CBG in hemp Sample 3.

Conclusion

GC-TOFMS is a sensitive and selective tool for quantitative analysis of cannabinoids in hemp samples. Simple extraction procedures and quantitative analysis using GC with high performance TOFMS and TAF provided conclusive potency testing results for Δ9-THC in hemp samples. Leveraging *ChromaTOF's* automated deconvolution, which takes advantage of the comprehensive GC-TOFMS data, also allows for a shift towards a more comprehensive approach of analysis with these samples. This results in an expanded chemical profile of cannabis materials. The ability to achieve the day-to-day routine quantitation workflows while also providing the non-targeted component results in the same analysis will help laboratories streamline their operation with this improved level of efficiency.

References

[1] US Department of Agriculture "Establishment of a Domestic Hemp Production Program" 2019; Doc. No. AMS-SC-19-990-2 IR).

[2] H.R.5485-Hemp Farming Act of 2018.

[3] Congressional Research Service, "Defining Hemp: A Fact Sheet" 2019. https://fas.org/sgp/crs/misc/R44742.pdf, Accessed 11/5/2019.

[4] United Nations Office on Drugs and Crime, "Recommended methods for the identification and analysis of cannabis and cannabis products", 2009.

[5] Hewavitharana A.K., Golding G., Tempany G., King G. and Holling N., Journal of Analytical Toxicology, 2005, 29, 258-261.

[6] Freeman A.M., Petrilli K., Lees R., Hindocha C., Mokrysz C., Curran V. Saunders R. and Freeman T.P., Neuroscience & Biobehavioral Reviews, 2019, 107, 696-712. Lafaye G., Karila L., Blecha L. and Benyamina A., Dialogues in Clinical Neuroscience, 2017, 19, 309-316.

[7] Sledzinski P., Zeyland J., Slomski R. and Nowack A., Cancer Medicine, 2018, 7(3), 765-775. Devinsky O., Cilio M.R., Cross H., Fernandez-Ruiz J., Frech J., Hill C., Katz R., Marzo V.D., Jutras-Aswad D., Notcutt W.G., Martinez-Orgado J., Rboson P.J., Rohrback B.G., Thiele E., Whalley B. and Friedman D., Epilepsia, 2014, 55(6), 791-802.

[8] Hazekamp A., Medical Cannabis and Cannabinoids, 2018, 1, 65-7.