



Complete CANNABIS TESTING SOLUTIONS for the Canadian market

A rapid method for the analysis of hemp potency by GC-FID

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Keywords

Hemp, potency, cannabis, rapid
analysis, gas chromatography,
GC, flame ionization detector, FID,
TRACE 1310, TraceGOLD TG-5MS,
Chromeleon CDS

Goal

The aim of this application note is to demonstrate a quick and simplified GC-FID method to determine the potency of hemp by measuring the concentration of three major cannabinoids: tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN).

Introduction

The plant *Cannabis sativa L.* is grown throughout the world and has been used for centuries in many areas including textiles, manufacturing, building materials, and medicine, in addition to its use as a recreational drug.¹

Marijuana and hemp are both grown from different varieties of the same plant. The difference between them is defined by the level of the main psychoactive ingredient, THC. Today, under Canadian law, the THC content must be no more than 0.3% w/w.²

There are more than 100 cannabinoids present in cannabis, of which the main chemicals of interest are CBD, THC, and CBN. By measuring these components an assessment of the potency of the cannabis can be made and this information can then be used for regulatory and quality control purposes.

For quality control purposes it is also important to determine the levels of terpenes (which contribute to cannabis' flavor profile), potential pesticide residue content, and any residual solvents that may originate from the processing that are present in the final products. Those applications are not the focus of the present work, but they will be covered in the next publications.

Analysis of cannabinoids is usually performed using high performance liquid chromatography (HPLC) and this GC method was designed to be a fast, easy method to complement the full cannabinoid analysis performed on the LC. Cannabinoids can be present in acid form, such as tetrahydrocannabinolic acid (THC-A), and in decarboxylated form, such as THC. Over time or when subjected to heat, for example when smoked, the acid form, THC-A, reacts and the decarboxylated version, THC, is formed. During GC analysis this process occurs in the hot GC inlet and the acid forms are not observed. For GC analyses THC content is defined as total THC (the sum of THC and THC-A). GC offers the advantages of speed and simplicity over other separation techniques and is often the preferred technique for this type of analysis, especially for routine laboratories.

The experiments described here demonstrate a simple and rapid GC-FID method for the analysis of THC, CBD, and CBN in the *Cannabis sativa L.* plant to determine the potency of the material and whether it can be classified as cannabis or hemp for regulatory purposes. This method was designed to co-exist on the same instrument running combined residual solvents and terpenes analysis for even more capability within the same footprint.

Experimental

The Thermo Scientific™ TriPlus RSH™ autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with a Thermo Scientific™ Instant Connect Split/Splitless (SSL) injector and a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID). Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-5MS 20 m × 0.18 mm × 0.18 µm column (P/N 26098-5780) using helium as the carrier gas and a cyclosplitter inlet liner (P/N 453A1365-UI). By selecting this capillary column, high chromatographic resolution and reduced flow rate are achieved. Moreover, additional quality control tests (e.g., pesticide residue analysis) can be

Table 1. Analytical parameters for the GC-FID used for potency determination

TRACE 1310 GC	
Inlet	
Injector and mode:	SSL, Split
Injector temperature:	275 °C
Carrier gas, flow mode:	Helium, constant flow, 0.9 mL/min
Split flow:	90 mL/min
Split ratio:	100:1
Injection volume:	1 µL
Oven	
Temperature:	275 °C, isothermal
Run time:	4 min
FID	
Temperature:	275 °C
Air flow:	350 mL/min
H ₂ flow:	35 mL/min
Makeup flow (N ₂):	40 mL/min
Acquisition rate:	30 Hz

performed without the need of column exchange. Methanol was used as the wash solvent in positions A and B of the autosampler, to limit the amount of carryover.

Standard and sample preparation

Cannabinoid standard mix containing CBD, THC, and CBN in methanol was purchased from Restek (P/N 34014). Linearity was assessed by diluting the cannabinoid standard in extraction solvent (90% acetone, 10% *n*-hexane) to obtain nine calibration points ranging from 1 µg/mL to 500 µg/mL.

Hemp extracts were prepared by adding 0.1 g ± 0.002 g of dried, separated, and finely ground hemp to a tared 15 mL disposable glass vial with PTFE screw cap, then dosing with 10 mL of extraction solvent, vortexing briefly, then sonicating for 60 minutes. The extract was then transferred to a disposable 10 mL syringe and filtered through a 0.2 µm Thermo Scientific™ Titan3™ PTFE syringe filter (P/N 42213-NPL). The liquid was filtered directly into 2 mL autosampler vials for analysis.

Data acquisition, processing, and reporting

The data were acquired, processed and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2. Integrated instrument control ensures full automation from instrument setup to raw data processing, reporting, and storage. Simplified eWorkflow™ procedures deliver effective data management ensuring ease of use, data integrity, and traceability. Chromeleon CDS also offers the option to scale up the entire analytical process in the laboratory from a single workstation to an enterprise environment and works seamlessly with Thermo Scientific™ mass spectrometers used for the pesticides, residual solvents, and terpene analyses that modern testing labs are performing.

Results and discussion

Chromatography

By using the GC conditions described in Table 1, excellent chromatographic separation and peak shape were obtained for standard injections using the low-bleed TraceGOLD TG-5MS column. Chromatographic resolution between peaks was >2.5 and the peak asymmetry factors were ~ 1.1 (both calculated according to the USP formulae³) for all analytes, demonstrating baseline resolution and Gaussian peak shapes (Figure 1). Typically, a resolution of >1.5 would indicate baseline resolution and an asymmetry value of between 0.8 and 1.2 would indicate an acceptable peak shape.

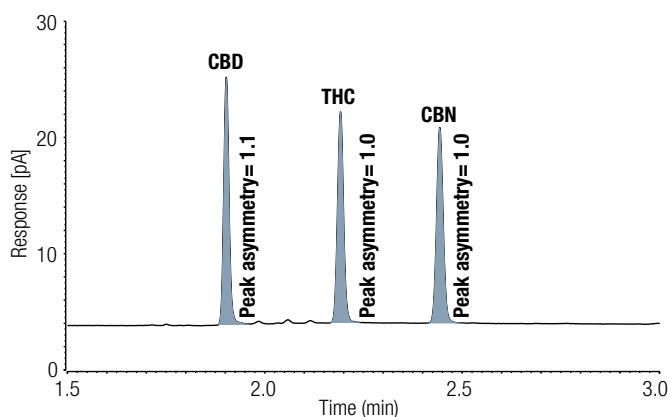


Figure 1. Chromatogram of the 100 µg/mL standard demonstrating peak shape and resolution

Linearity

The linearity of CBD, THC, and CBN tested over a calibration range of 1–500 µg/mL showed coefficient of determination (R^2) values greater than 0.995 (Figure 2).

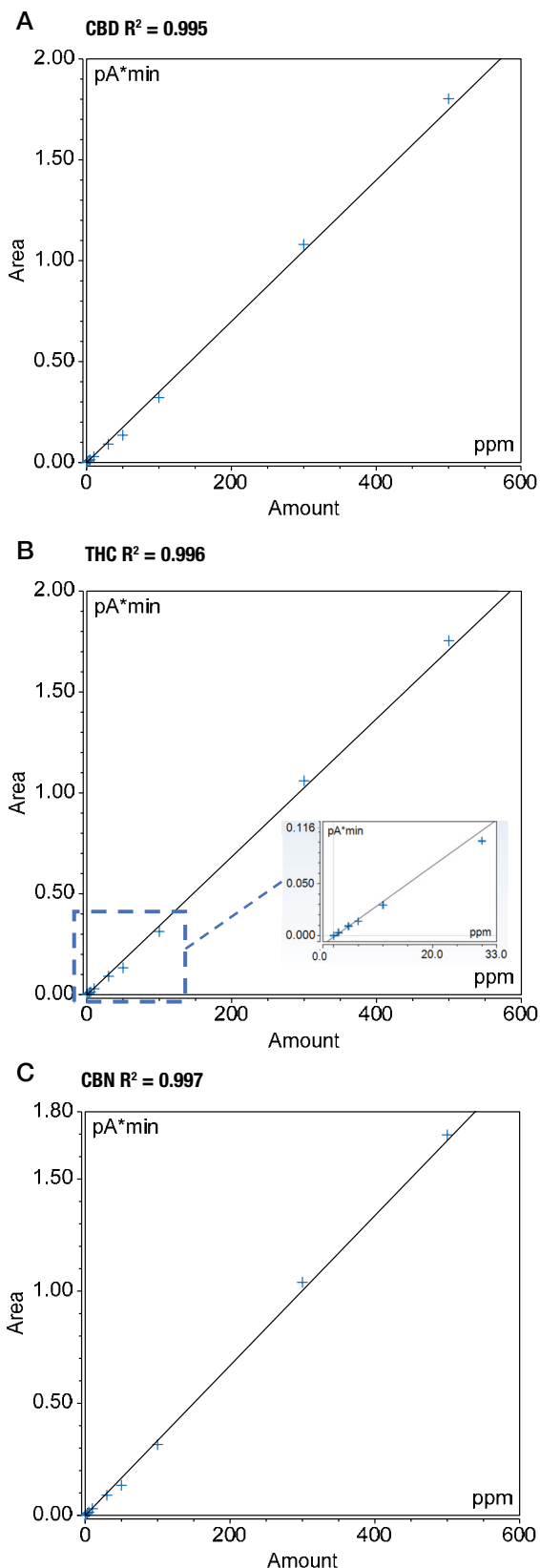


Figure 2. Calibration plots for CBD (A), THC (B), and CBN (C) in the range 1–500 µg/mL (ppm) showing R^2 values of 0.995, 0.996, and 0.997, respectively

Repeatability

Method repeatability was assessed using n=6 consecutive injections of standard solutions, from the same vial, at 3 µg/mL, 30 µg/mL, and 300 µg/mL. Results showed good repeatability for all the target compounds with peak area RSD <6% at all levels (Table 2).

Table 2. %RSD of peak area for n=6 consecutive injections of standard at 3 µg/mL, 30 µg/mL, and 300 µg/mL for CBD, THC, and CBN of less than 6%

Compound	%RSD peak area (n=6)		
	3 µg/mL	30 µg/mL	300 µg/mL
CBD	5.6	5.9	2.1
d9-THC	5.6	5.3	1.9
CBN	3.4	5.1	1.8
Average	4.9	5.4	1.9

Assessment of extracted hemp sample

Testing the method performance with a real hemp sample shows that the peak shape of the target compounds is not compromised with USP peak asymmetry factors remaining at ~1.1. Chromatographic resolution (R_s) remains >1.5 for target analytes despite the presence of additional matrix peaks and significantly more concentrated CBD amounts as compared to THC or CBN (Figure 3). The identity of these matrix peaks could not be confirmed using an FID, but by coupling the GC to a mass spectrometer detector tentative identification of these peaks can be made by searching the measured spectra against the NIST17 spectral library.

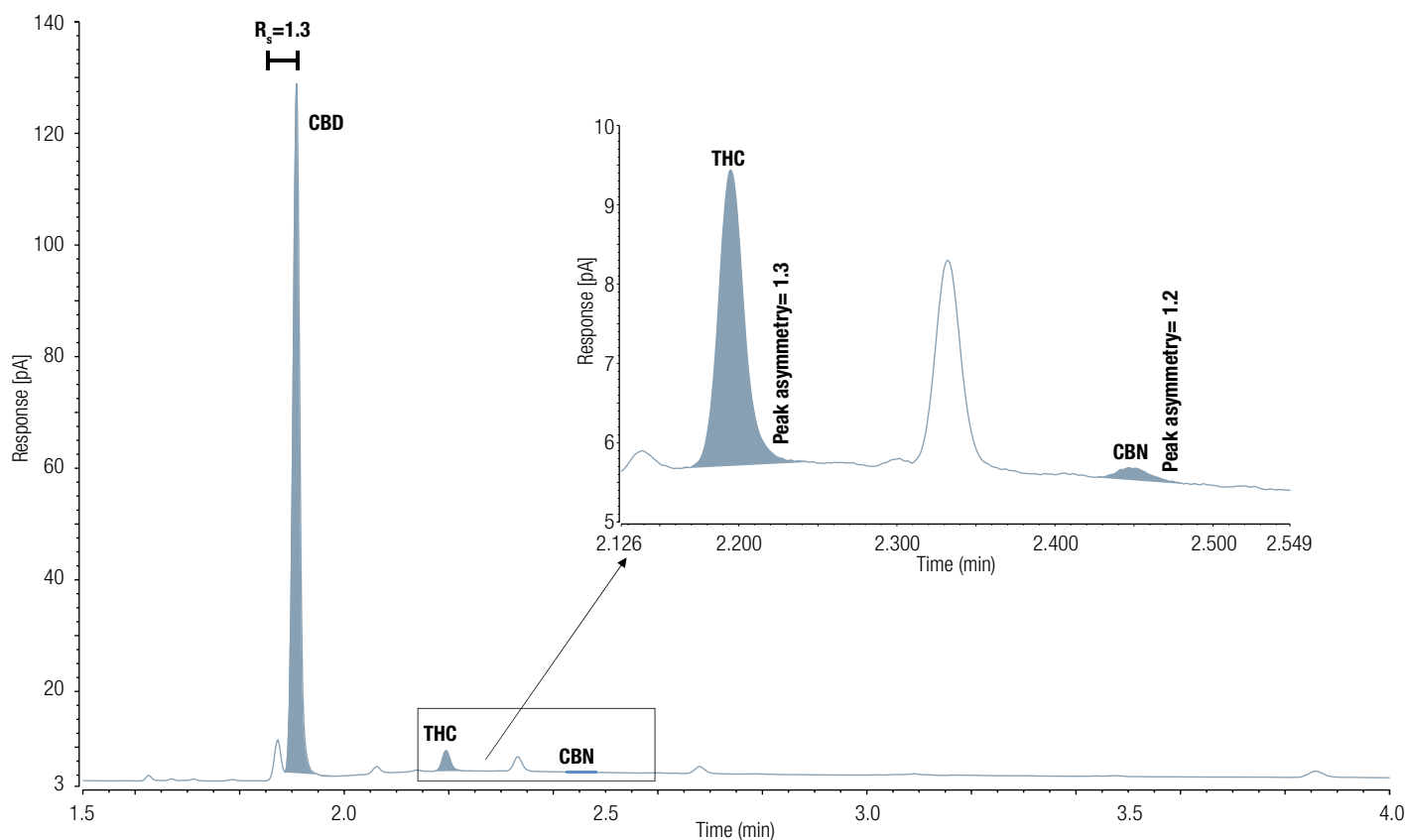


Figure 3. Hemp sample FID chromatogram showing no significant matrix interferences and Gaussian peak shapes for CBD at 1.90 min, THC at 2.19 min, and CBN at 2.44 minutes. A zoomed chromatogram from 2.15 to 2.6 minutes is included to highlight the CBN peak.

Quantitative analysis for CBD, THC, and CBN was easily performed using the dynamic processing features of the CDS. The resulting calculated amounts were 5.5% w/w CBD, 0.21% w/w THC, and 0.01% w/w CBN. Potency results confirm that the samples tested had <0.3% THC

and can be classified as hemp. Cannabis-specific report templates were generated to show the quantitative amounts for each analyte, as well as graphical depictions of the results to better understand the sample composition (Figure 4).

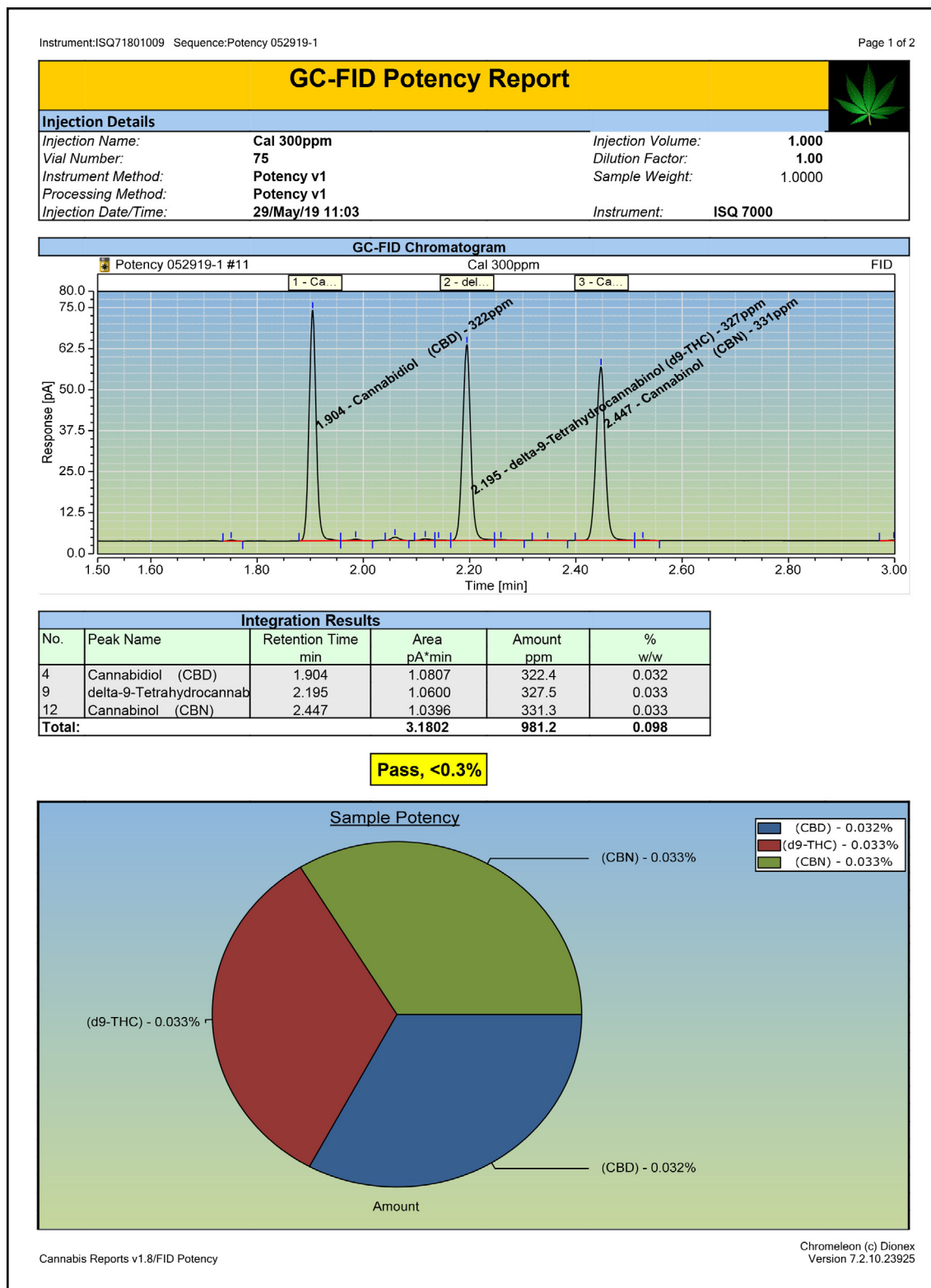


Figure 4. Example of data customized reporting in Chromeleon CDS showing hemp potency with the contribution (in %) of the main constituents

Conclusions

The purpose of these experiments was to assess the performance of the TRACE 1310 GC system and TraceGOLD TG-5MS column for the analysis of cannabinoids in hemp while demonstrating the following:

- The results shown demonstrate a simple and rapid GC-FID method, suitable for potency analysis, allowing for quantitative assessment of CBD, CBN, and THC levels in <4 minutes.
- Quantitative analysis for CBD, THC, and CBN was easily performed using the Interactive Results feature of Chromeleon CDS. The resulting calculated amounts were 5.5% w/w CBD, 0.21% w/w THC, and 0.01% w/w CBN. Potency results confirm that the samples tested had <0.3% THC and can be classified as hemp.
- Excellent chromatographic separation was achieved on the low-bleed Thermo Scientific TraceGOLD TG-5MS column, allowing for fast, high-throughput sample analysis.
- Very good linearity was obtained for each target compound over a concentration range of 1–500 µg/mL with R^2 values >0.995 and calibration factors RSD <30% over the concentration range, for all target analytes.
- The Thermo Scientific TriPlus RSH autosampler delivered very good injection repeatability, as shown by <6% RSD of peak area for n=6 consecutive injections, across three different concentrations, for all target analytes.
- The method presented was shown to be applicable for potency assessment, allowing for the correct classification of the analyzed sample. Quantitative analysis for the target compounds (CBD, THC, and CBN) was easily performed using the Chromeleon CDS. This software ensures data integrity, traceability and effective data management from instrument control to the final report.

References

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