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Application News

Liquid Chromatography Mass Spectrometry

# Quantitative Analysis of Natural Cannabinoids using the Triple Quad LCMS-8050

No. LCMS-050

Liquid Chromatograph Mass Spectrometer
LCMS-8050


## ■ Summary

Quantitative analysis of natural cannabinoids was conducted using the LCMS-8050 triple quadrupole mass spectrometer. A lower limit of quantitation (LLOQ) of 1 – 4 ng/mL was achieved depending on the specific cannabinoid. This method showed certain medicinal oils or tinctures available over the internet contained naturally occurring cannabinoids.


## ■ Background

There are currently three different species of *Cannabis* (*C. sativa, C. indica* and *C. ruderalis*) that contain approximately 85 different types of cannabinoids. The major cannabinoids contained in *Cannabis* are shown in **Figure 1.** Each of these cannabinoid compounds have different physiological interactions with the human body but the Δ9-Tetrahydrocannabinol (Δ9-THC), Cannabigerol (CBG), and Cannabinol (CBN) are considered the main psychoactive cannabinoids with the Δ9-THC being the primary psychoactive component[1,2].

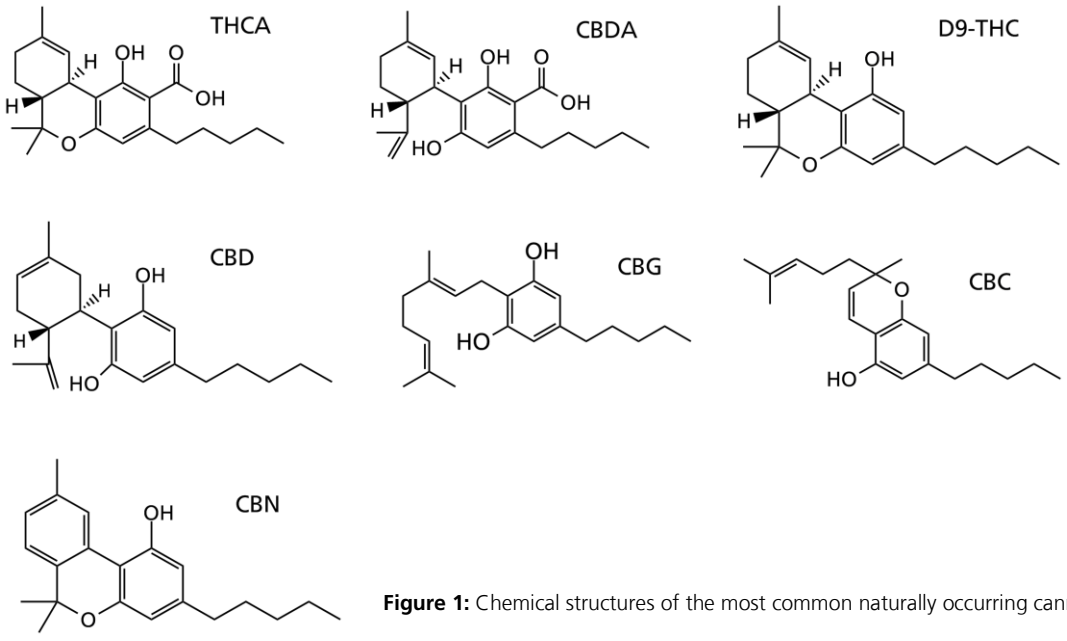


**Figure 1:** Chemical structures of the most common naturally occurring cannabinoids.

Besides having psychoactive constituents, *Cannabis* also has other naturally occurring cannabinoids that may have medicinal value. The analysis of natural cannabinoids is necessary not only because of potential medical uses for these compounds, but also in the regulation and quality control testing of products containing these compounds. Many products are being sold online by large national retailers and locally in states where the legalization of these products has allowed for the distribution of both medical and recreational use of cannabinoids. To ensure the authenticity, quality, and amount of each cannabinoid contained in the product, an LC-MS/MS method was developed using the Shimadzu LCMS-8050 triple quadrupole mass spectrometer.

## ■ Method

After diluting in methanol neat standards of the naturally occurring cannabinoids, flow injection analysis was used to optimize source, CID conditions, and product ion selection. Optimized LC conditions were developed empirically and a 3-minute gradient method was developed using a Restek column. Using solvent standards, calibration curves were created and various medical tinctures were then analyzed.

## ■ Results and Discussion

Cannabinoid optimization identified one quantifier and two qualifier ions for each naturally occurring cannabinoid. The ions were selected using the Shimadzu Optimization for Method Software and were verified with product ion scans. The two ions were selected based on ion intensity and repeatability across multiple collision energies. The precursor ions selected were the $[M+H]^+$ for all of the cannabinoid compounds. Following MRM optimization and development of chromatographic conditions, a standard curve was generated for each cannabinoid with n=6 (**Figure 2**).

The lower limits of quantitation (LLOQ) were established for each cannabinoid at 1 ng/mL except for CBC which was 4 ng/mL (**Table 1**). The minimum signal-to-noise ratio for all of the cannabinoids was determined to be greater than or equal to 20:1. Calibration curve weighting of either 1/Concentration (1/C) or 1/Concentration$^2$ (1/C$^2$) was applied.

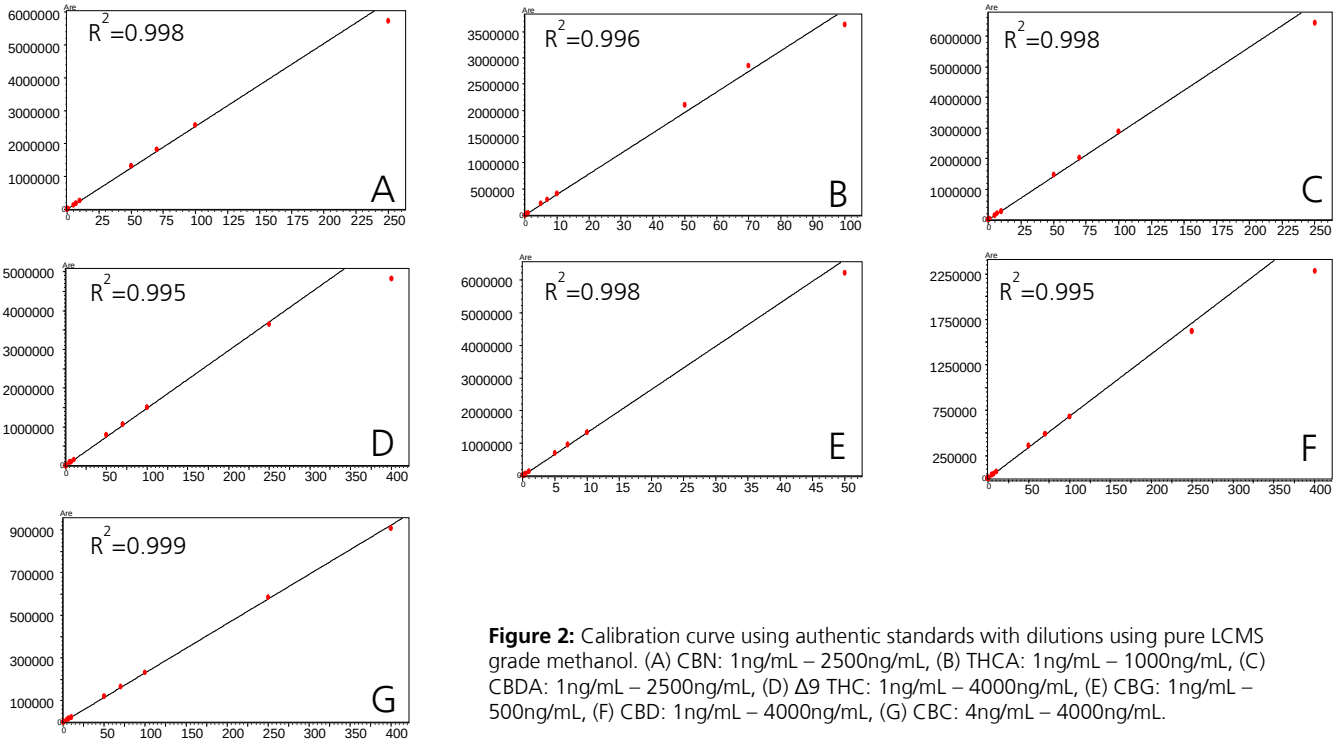


**Figure 2:** Calibration curve using authentic standards with dilutions using pure LCMS grade methanol. (A) CBN: 1ng/mL – 2500ng/mL, (B) THCA: 1ng/mL – 1000ng/mL, (C) CBDA: 1ng/mL – 2500ng/mL, (D) Δ9 THC: 1ng/mL – 4000ng/mL, (E) CBG: 1ng/mL – 500ng/mL, (F) CBD: 1ng/mL – 4000ng/mL, (G) CBC: 4ng/mL – 4000ng/mL.

| Quantitative Results at LLOQ (n=6) | | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | **LOD (ng/mL)** | **%RSD** | **%Accuracy** | **S/N** | **Weighting** | **Commercial Tincture** |
| CBN | 1 | 4.516099 | 99.998± 4.2% | 58.96 | 1/C² | 0.016% ± 0.001% |
| THCA | 1 | 7.023558 | 99.998 ± 9.1% | 21.14 | 1/C | 0.452% ± 0.018% |
| CBDA | 1 | 6.671582 | 100.001 ± 5.7% | 70.42 | 1/C² | 0.019% ± 0.001% |
| Δ9 THC | 1 | 6.414479 | 99.997 ± 6.3% | 85.89 | 1/C² | 0.370% ± 0.021% |
| CBG | 1 | 3.666911 | 100.000 ± 3.7% | 2397.6 | 1/C² | 0.018% ± 0.0004% |
| CBD | 1 | 7.770838 | 100.123 ± 6.8% | 107.4 | 1/C² | 0.006% ± 0.001% |
| CBC | 2.5 | 8.193242 | 100.006± 5.7% | 70.64 | 1/C | 0.029% ± 0.006% |

**Table 1:** Quantitative results for each cannabinoid at the limit of quantitation and the concentration of the commercial tincture.

The chromatographic method that was developed yielded baseline separation of six of the seven cannabinoids with CBG (m/z 317.25) and CBD (m/z 314.95) co-eluting. All seven of the cannabinoids were detected in the commercially available tincture purchased online (**Figure 3**). The concentrations for each cannabinoid are presented in Table 1. There was no measurable carryover in the blank injected immediately after the highest level standard.

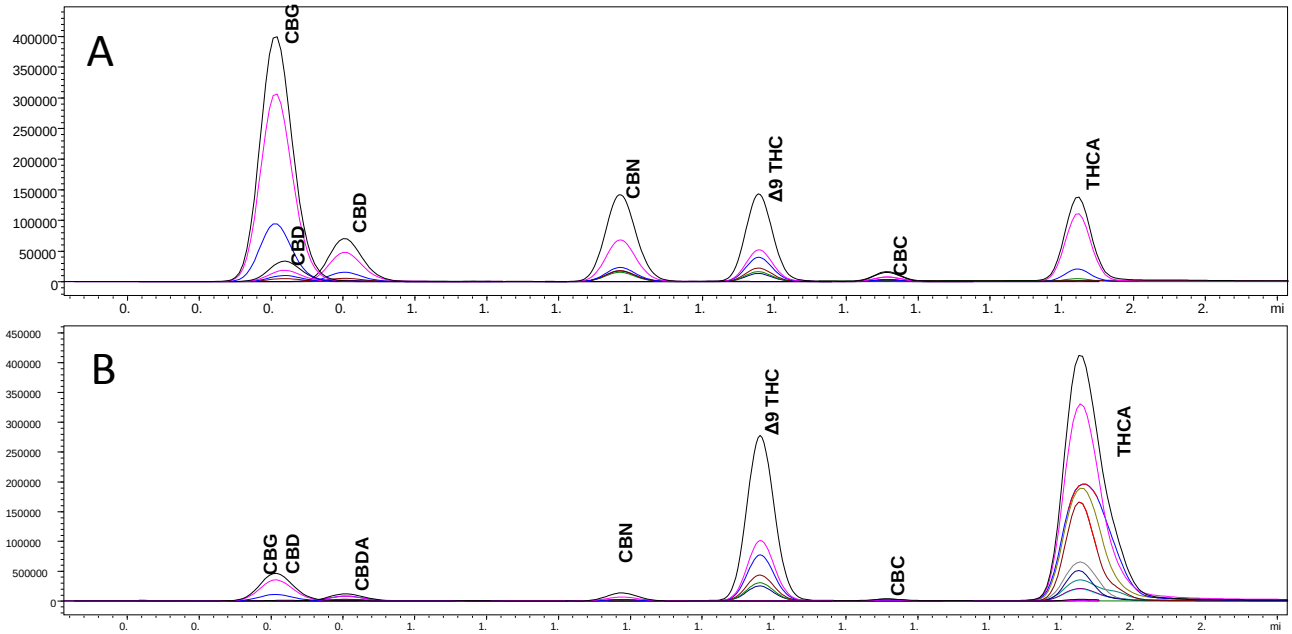


**Figure 3:** (A) Chromatogram of seven cannabinoids at 100 ng/mL in solvent. (B) Chromatogram of a commercially available tincture containing seven cannabinoids.

## ■ Conclusion

This work demonstrates a rapid method for the detection of naturally occurring cannabinoids by using the Shimadzu LCMS-8050. All seven cannabinoids were detected at levels as low as 1 ng/mL (1 pg on column) with a S/N of at least 20:1. This method is useful for quantitating cannabinoids in raw or commercial products.

## ■ References

1. Borgelt LM, Franson KL, Nussbaum AM, Wang GS (February 2013). "The pharmacologic and clinical effects of medical cannabis". *Pharmacotherapy* (Review) **33** (2): 195–209.
2. Gaoni Y, Mechoulam R (1964). "Isolation, structure and partial synthesis of an active constituent of hashish". *Journal of the American Chemical Society* **86** (8): 1646–1647.

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LCMS-8030 LCMS-8040 LCMS-8050 LCMS-2020 LCMS-IT-TOF

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