Quantitative Analysis of Cannabinoids using the LCMS-2020 Single Quad MS

Summary
Evaluation and quantitation of a variety of cannabinoids on an LCMS-2020 single quadrupole mass spectrometer.

Method
Cannabichromene (CBC) and Δ9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), Δ8-THC (d8-THC), Δ9-THC (d9-THC) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for SIM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%) methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The standards (Figure 1) were transferred to autosampler vials and injected into a Nexera-LCMS-2020 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in Figure 2. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45° C and the injection volume was 5 µL.

LCMS-2020 Analysis
Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-2020. A positive and negative scan and selected ion monitoring (SIM) modes were used simultaneously for analysis. Details of the MS parameters are shown in Table 1.

![Chemical structures for cannabinoids](image)

Figure 1. Chemical structures for cannabinoids.
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Results and Discussion
Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.8-5.0 minutes. Linearity was achieved from 0.5-1024ng/mL for CBN and d9-THC, 1-1024ng/mL for d8-THC, 2-128ng/mL for THCA, 2-2048ng/mL for CBD and 4-2048ng/mL for CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r² values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in Figures 3-8.

Table 1. MS Interface parameters.

| Interface DUIS | Nebulizing gas flow  | 2 L/min |
| Drying gas flow | 15 L/min |
| DL Temperature  | 300 ºC |
| Heat Block Temperature | 500 ºC |
| Polarity (+) CBD, CBN, d8-THC, d9-THC, CBC (-) THCA |

Figure 2. Gradient conditions.

Figure 3. Chromatogram and calibration curve for cannabidiol (CBD).

Figure 4. Chromatogram and calibration curve for cannabinol (CBN).
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A representative chromatogram and calibration curve for each analyte are shown in Figures 3-8.

Conclusion

A method for rapid quantitation of various cannabinoids was requested utilizing UHPLC equipment and LCMS to increase the speed and sensitivity of analysis. A 7 minute method was developed, with the main limiting factor being the separation of d8-THC and d9-THC. If d8-THC and d9-THC do not need to be individually quantified then the method could be shortened further.

It is important to note that these results were achieved using standards without matrix, but the ability of the Nexera X2 system and LCMS-2020 mass spectrometer to enable rapid, sensitive quantitative analysis of cannabinoids is highlighted by this method.

Figure 5. Chromatogram and calibration curve for cannabichromene (CBC).

Figure 6. Chromatogram and calibration curve for d9-THC.

Figure 7. Chromatogram and calibration curve for d8-THC.
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Figure 8. Chromatogram and calibration curve for d9-THC Acid A (THCA).
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