The Quantitation and Simultaneous In-Source Fragmentation of 16 Cannabinoids in Hemp Using Single Quadrupole LC-MS

Hui Guo, Craig Young, Rachel Lieberman, Christopher Gilles, Shimadzu Scientific Instruments, Inc.

1. Introduction

With the passing of the Farm Bill in December 2018, which legalized hemp if the psychoactive compound, THC, content is 0.3% or less, and the legalization of cannabis in more than two thirds of US states and Canada, more accurate and sensitive analytical methods are needed for the quantitative determination of cannabinoids besides the widely applied technique of HPLC with UV and photodiode array detectors. The higher sensitivity, specificity, and mass identification provided by LC-MS are increasingly recognized for the quantitative determination of cannabinoids.

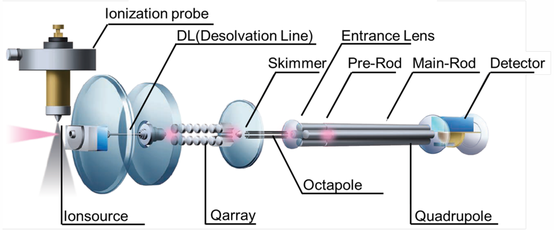
In this study, an LC-MS method of quantitation and simultaneous in-source fragmentation (SID) was developed using the LCMS-2020 single quadrupole MS with an integrated LC front end (2040C 3D) for the quantification of 16 cannabinoids. SID in single-quad MS is performed by manipulating the DC voltage of Q-array. Selected ion monitoring (SIM) was used for quantitation, and simultaneous positive and negative scans were used for identification.

2. Experimental

Experiments were performed using a Shimadzu integrated HPLC (2040 3D) and a single quadrupole mass spectrometer detector (LCMS-2020) with electrospray ionization (ESI) interface.

Table 1 Method Conditions

LC (2040C 3D)	
Column	Shimadzu NexLeaf CBX II, 1.8 µm, 3.0 x 100mm (220-91525-75) Shimadzu NexLeaf CBX II Guard, 1.8 µm (220-91525-76)
Mobile phase A	0.1% formic acid and 5 mM ammonium formate in 100% water
Mobile phase B	0.1% formic acid in 50% methanol/50% acetonitrile
Flow rate	0.5 mL/min
Oven	30 °C
Injection volume	5 µL
Gradient	B conc. 83% (0 min) → 98% (6 min) → 98% (6.5 min) → 83% (6.51-10 min)
MS (LCMS-2020)	
Ionization	ESI
Interface temperature	350 °C
DL temperature	250 °C
Nebulizing gas flow	15 L/min
Heat block	400 °C
Drying gas flow	1.5 L/min
Q-array DC voltage	55 V (Scan)
Q-array DC voltage	0 V (SIM)



3. Results and Discussion

SIM in both positive and negative modes was used for building quantitative standard curves for each compound. In addition, in-source fragmentation (SID) was simultaneously performed in both positive and negative scan mode for target confirmation.

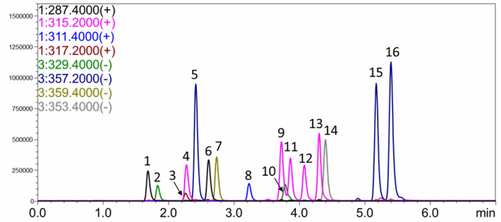


Fig. 1 Chromatogram of 16 cannabinoid standards mixture (1 ppm each). Peaks: 1. CBDV, 2. CBDVA, 3. CBG, 4. CBD, 5. CBDA, 6. THCV, 7. CBGA, 8. CBN, 9. Δ9-THC, 10. THCVA, 11. Δ8-THC, 12. CBC, 13. CBL, 14. CBNA, 15. THCA, 16. CBCA

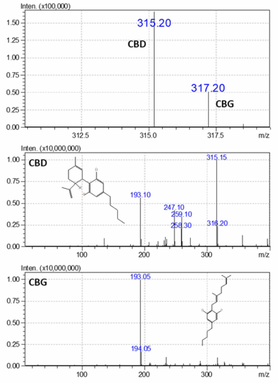


Fig. 2 Electrospray mass spectrum and in-source fragmentation of CBD and CBG.

Although CBD and CBG were co-eluted from the column, identification using SID fragmentation and mass identification can be obtained from mass spectra.

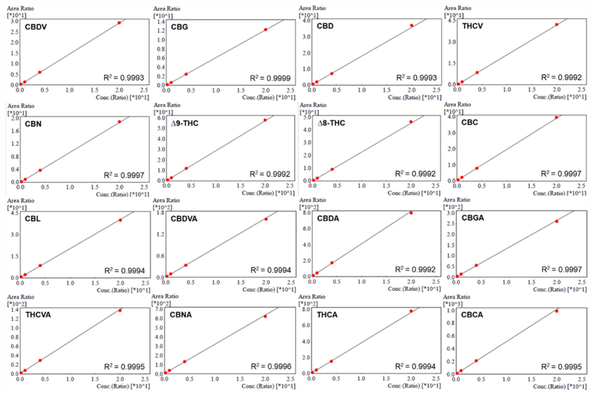


Fig. 3 Standard curves for 16 cannabinoids.

Standard curves were established using the peak area ratio of the cannabinoids to their corresponding internal standard versus the concentration ratio of the cannabinoids and internal standard across the concentration range of 10 to 1000 ppb. A correlation coefficient r2 > 0.999 was obtained for all 16 cannabinoids as shown in Figure 3.

Retention time precision and peak area precision of 16 cannabinoids were determined by making 7 replicate injections of the mixed standard. The method accuracy was investigated by spiking standards with a 100 ppb concentration for each compound into a blank matrix, acceptable recoveries from 85% to 97% were obtained for all 16 cannabinoids. The MDL study shows that a level of less than 0.5 ppb for Δ9-THC, Δ8-THC and THCA can be determined with 99% confidence using this method.

Table 2 Retention time and peak area reproducibility

Cannabinoids	Tr Precision (RSD%)	Area Precision (RSD%)
CBDV	0.024	1.019
CBG	0.038	1.092
CBD	0.041	1.582
THCV	0.031	0.495
CBN	0.033	2.299
Δ9-THC	0.034	0.754
Δ8-THC	0.039	0.699
CBC	0.044	1.399
CBL	0.044	0.693
CBDVA	0.024	3.664
CBDA	0.031	1.376
CBGA	0.024	0.792
THCVA	0.028	3.692
CBNA	0.050	0.997
THCA	0.044	1.034
CBCA	0.044	1.129

Table 3 Method Detection Limits

Cannabinoids	MDL Standard (ppb)	Calculated MDL (ppb)
CBDV	5	0.61
CBG	5	2.76
CBD	5	1.42
THCV	5	0.78
CBN	5	1.87
Δ9-THC	1	0.40
Δ8-THC	1	0.43
CBC	1	0.97
CBL	1	1.77
CBDVA	5	0.81
CBDA	1	1.76
CBGA	1	0.43
THCVA	5	2.05
CBNA	1	0.70
THCA	1	0.45
CBCA	1	0.29

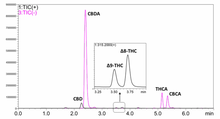


Fig. 4 Hemp flower analysis using LC-ESI-MS

Table 4 Hemp flower analysis

Cannabinoids	Label Claim (%)	Test Result (%)
CBD	1.3	1.8
CBDA	17	16.4
Δ9-THC	0.0	0.1
Δ8-THC	N/A	0.27
THCA	N/A	1.2

Figure 4 shows the chromatogram of a hemp flower sample obtained from an on-line vendor of industrial hemp. Concentrations of CBD and CBDA obtained in this study agree with the concentrations shown in the Certificate of Analysis (CoA) provided by the vendor. However, detectable quantities of Δ8- and Δ9-THC were found, while the vendor's CoA did not mention Δ8-THC and reported that Δ9-THC was not detected. In addition, THC-A was found to be 1.2%. According to the latest regulation (7 CFR Part 990 [Doc. No. AMS-SC-19-0042; SC19-990-2 IR] Establishment of a Domestic Hemp Production Program), this sample would be classified as cannabis and not hemp as its total THC content derived from THC-A and Δ9-THC exceeds the 0.3% guideline. This illustrates the advantage of the LCMS method for its sensitivity and specificity in determining cannabinoids.

4. Conclusion

A quantitative LCMS method for the determination of 16 cannabinoids and their respective acidic forms, was developed using the Shimadzu single quadruple LCMS-2020 with an integrated LC front end (2040C 3D). This method demonstrates the increased sensitivity and specificity of mass spectrometry for the analysis of cannabinoids in industrial hemp.