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Application News

No. HPLC-029

High Performance Liquid Chromatography

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

■ 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 a previous application note (HPLC-028), an in-source fragmentation (SID) method was developed using the Shimadzu single quadrupole MS (LCMS-2020) to characterize 16 cannabinoids.

In this extended study, an LC-MS method of quantitation and simultaneous SID was developed using the LCMS-2020 single quadrupole MS with an integrated LC front end (LC 2040C 3D) for the quantification of 16 cannabinoids, including Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL) and their respective acidic forms, etc. Selected ion monitoring (SIM) was used for quantitation, simultaneous positive and negative scans were used for identification.

■ Experimental

Equipment

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

Materials

A mixture of 16 cannabinoids was prepared using an 11-component cannabinoids mixture (Shimadzu 220-91239-21) with five additional individual cannabinoids obtained from Sigma Aldrich (Cerilliant). LCMS grade solvents, formic acid and ammonium formate were obtained from Honeywell. Degassed deionized water was used (>18.0 MΩ-cm).

(–)-Δ9-THC-D_3 (THC-D_3) and (±)-11-nor-9-Carboxy-Δ9-THC-D_3 (THCCOOH-D_3) purchased from Sigma Aldrich (Cerilliant) were used as internal standards for cannabinoids ionized in positive mode and negative mode, respectively. 50 ppb internal standards (IS) solution consisting both THC-D_3 and THCCOOH-D_3 was prepared with 90% mobile phase B and 10% DI. Standards of different concentrations were prepared by diluting stock standards with IS solution.

Hemp flower sample extraction steps

1. Weigh 200 mg into a 50 mL centrifuge tube.
2. Add two 9.5 mm O.D. steel balls into the tube.
3. Shake at 1000 rpm for 5 min using 2010 Geno/Grinder to grind the sample thoroughly into a fine powder.
4. Add 20 mL of methanol into the tube, vortex 1 min, wait for 15 minutes.
5. Vortex 1 min again, wait for another 15 minutes.
6. Vortex the mixture for 30 seconds, transfer 100 µL supernatant into a 50 mL centrifuge tube, add 9.9 mL methanol and vortex for 30 seconds to make a 100X dilution of the sample.
7. Filter the diluted sample using a 0.45 µm syringe filter.

HPLC-MS conditions

The method conditions for both HPLC and MS are shown in Table 1.

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)

■ Results and Discussion

For this study, 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. Figure 1 shows the separation results of 16 cannabinoids using SIM mode. Under the conditions of the experiment, neutral cannabinoids ionize in positive mode while their respective acidic forms ionize in negative mode. In a previous application note (HPLC-028), CBD and CBG were shown to co-elute from the column; while their molecular weights differ, identification using SID fragmentation and mass identification can be obtained from mass spectra as shown in Figure 2. Target formula weights and adduct ions are shown in Table 2. In order to evaluate the method, linearity, repeatability, method accuracy and method detection limits (MDL) were also determined.

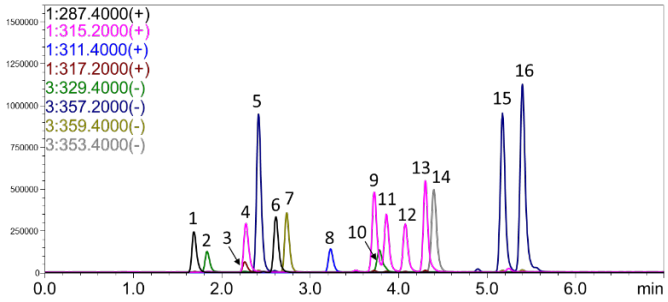

Figure 1: Chromatogram of 16 cannabinoid standards mixture (1 ppm each). Peaks: 1. CBDV, 2. CBDVA, 3. CBG, 4. CBD, 5. CBDA, 6. THCV, 7. CBGA, 8. CBN, 9. Δ9-THC, 10. THCVA, 11. Δ8-THC, 12. CBC, 13. CBL, 14. CBNA, 15. THCA, 16. CBCA

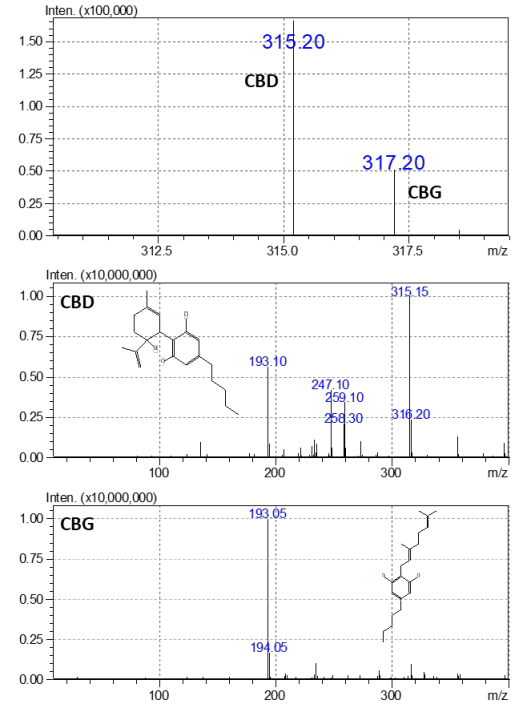

Figure 2: Electrospray mass spectrum and in-source fragmentation of CBD and CBD.

Cannabinoids	Formula Weight	Adduct [+H]
CBDV	286.41	287.4
CBG	316.48	317.2
CBD	314.22	315.2
THCV	286.41	287.4
CBN	310.43	311.4
Δ9-THC	314.22	315.2
Δ8-THC	314.45	315.2
CBC	314.47	315.2
CBL	314.46	315.2
Cannabinoids	**Formula Weight**	**Adduct [-H]**
CBDVA	330.42	329.4
CBDA	358.47	357.2
CBGA	360.49	359.4
THCVA	330.42	329.4
CBNA	354.45	353.4
THCA	358.47	357.2
CBCA	358.47	357.2

Table 2: Target formula weights and adduct ions

Linearity

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 $r^2 > 0.999$ was obtained for all 16 cannabinoids as shown in Figure 3.

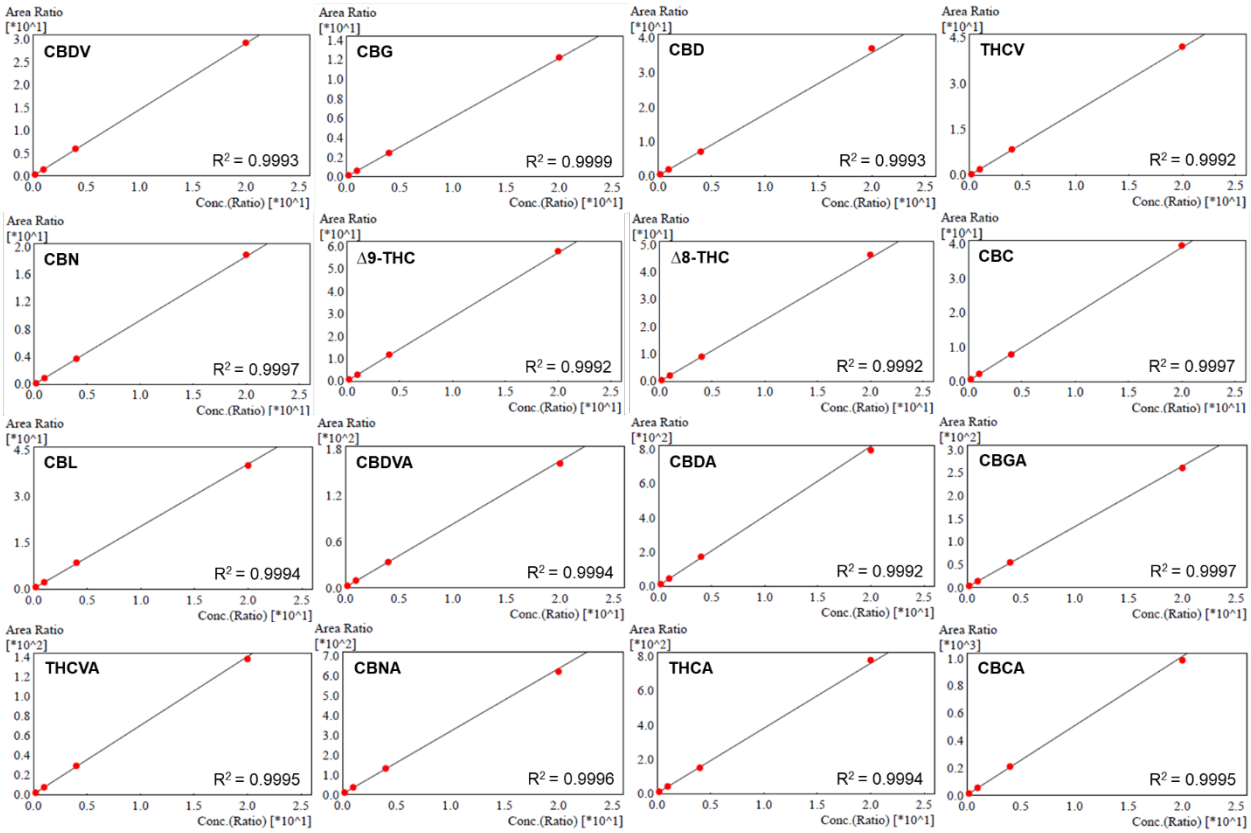

Figure 3: Standard curves for 16 cannabinoids.

Repeatability and Accuracy

Table 3 shows retention time precision and peak area precision of 16 cannabinoids from seven injections of the mixed standard. As shown in the table, excellent method repeatability was obtained with retention time RSDs less than 0.05% and peak area RSDs from 0.5 to 3.6%. 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.

Method Detection Limit

Method Detection Limit (MDL) is defined as the minimum concentration of a compound that can be measured with 99% confidence that its concentration is distinguishable from a blank. The MDL was determined by making seven replicate injections of MDL standards (conc. 3 to 5 times greater than the instrument detection limit). The MDL was calculated as (t) x (S), where t is student's t value for 99% confidence level (t = 3.14 for seven replicates) and S is standard deviation from seven injections. The MDL standard concentration and calculated MDL are shown in Table 4. 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 3: Retention time and peak area repeatability

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 4: MDL standard concentration and MDL

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

Hemp flower analysis

Figure 4 shows the chromatogram of a hemp flower sample obtained from an on-line vendor of industrial hemp. A Certificate of Analysis (CoA) was provided by the vendor. As shown in Table 5, concentrations of CBD and CBDA obtained in this study agree with the concentrations shown in the CoA. 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.

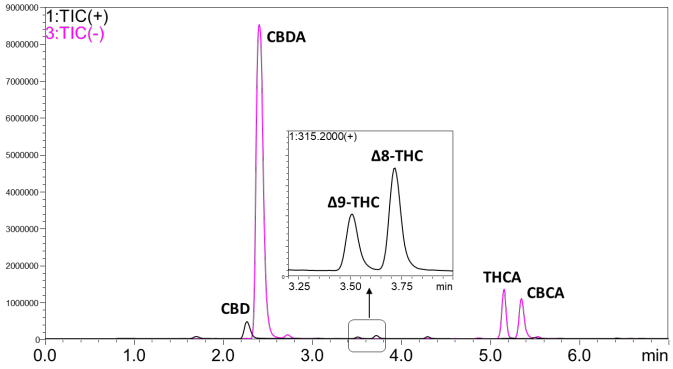

Figure 4: Hemp flower analysis using LC-ESI-MS.

Table 5: 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

■ Conclusion

A quantitative LCMS method for the determination of 16 cannabinoids, including Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-tetrahydrocannabinol (Δ8-THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabicyclol (CBL) and their respective acidic forms, was developed using the Shimadzu single quadruple LCMS-2020 with an integrated LC front end (LC 2040C 3D). This method demonstrates the increased sensitivity and specificity of mass spectrometry for the analysis of cannabinoids in industrial hemp.

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