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Application News

SSI-LCMS-128

Liquid Chromatography Mass Spectrometry

Separation of 4 Tetrahydrocannabinol Isomers: Δ6a/10a, Δ8, Δ9, and Δ10 on a Single Quad LCMS

■ Introduction

The cannabis/hemp market continues to grow each year with more states legalizing recreational marijuana, as well as the 2018 Farm Bill removing hemp from the controlled substance list. This bill also defines that any cannabis sativa L. strain with a total tetrahydrocannabinol (THC) concentration of 0.3% or less can be considered hemp and not cannabis. Due to this definition, there is an even higher demand to differentiate hemp from cannabis by determining the correct concentration of THC.

■ LCMS Instrumentation

A Shimadzu LCMS-2020 single quadrupole mass spectrometer coupled with a modular Nexera 40 series UHPLC system was employed for this evaluation. The Nexera 40 series was equipped with a PDA detector for simultaneous analysis of analyte absorbance and compound ionization.

■ LCMS Method Development

Utilizing a Restek Raptor C18 column (150 mm x 2.1 mm, 2.7 µm) and gradient elution with an overall run time of 8 minutes, four tetrahydrocannabinol isomers, tetrahydrocannabinolic acid, and cannabidiol were chromatographically separated. Mobile phase A consisted of water with 5mM ammonium formate and 0.1% formic acid and Mobile phase B was 50:50 methanol and acetonitrile with 0.1% formic acid. A final flow rate of 0.7mL/min and sample injection volume of 5µL was used. Exact gradient conditions are shown in Figure 1.

Table 1: LCMS-2020 method parameters

Drying Gas	15.0 L/min
Interface Temperature	350 °C
DL Temperature	250 °C
Heat Block Temperature	400 °C
Flow rate	0.7 mL/min
Column Oven Temperature	40 °C
Sample Tray Temperature	15 °C
SIM Channels (*m/z*)	315.15 (+), 357.15 (-)

The MS interface and temperature settings used can be found in Table 1. The final MS method included multiple selected ion monitoring (SIM) events to monitor for each ion of interest as well as scan events for each polarity with a Qarray voltage of 55V. The scan events monitor for in source collision induced disassociation (CID).

Neat calibration curves were run from 0.01ng/µL to 10ng/µL. Two hemp samples were extracted and analyzed against the calibration curve. Sample extraction was completed using 100mg dry flower geno-grinded for 5 minutes at 1000rpm. Ten mL methanol was added and the sample was vortexed for 1 minute before centrifuging. Then, 1 mL of the supernatant was aliquoted into a sample vial for injection. All calibrators and hemp samples were spiked with delta-9 D3-THC for internal standard quantitation.

Time	%B
0	77
3.25	80
3.5	89
5.5	91
6	96
6.5	96
6.51	77
8	77

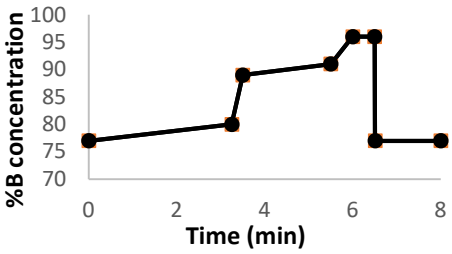

Figure 1: LC gradient parameters

■ Results and Discussion

Separation of Δ6a/10a THC, Δ8 THC, Δ9 THC, and Δ10 THC was achieved in a single chromatographic run. A comparison of the wavelength 220nm PDA chromatogram and the MS TIC can be seen in Figure 2. Representative SIM chromatograms for all four isomers plus CBD and THCA can be seen in Figure 3.

In addition to a SIM method demonstrating chromatographic separation, a scan event was used to monitor in-source fragmentation. A single Qarray voltage of 55V was applied to fragment all analytes of interest. All four THC isomers plus CBD have a nominal mass of 314.5 g/mol and were differentiated using retention time.

Initial retention time references were confirmed using an individual neat standard. The in-source CID allowed for secondary confirmation of different THC isomers by comparing the fragmentation patterns produced. All four THC isomers produced the *m/z* fragments of 193 and 259, but *m/z* 247 is only present with Δ8 and Δ9 THC. Further, the fragment *m/z* 299 is only present for Δ6a/10a THC, and fragment *m/z* 217 is only present for Δ10 THC (Figure 4). Individual confirmation between Δ8 THC and Δ9 THC was completed by the presence of a m/z fragment of 239 (Figure 5).

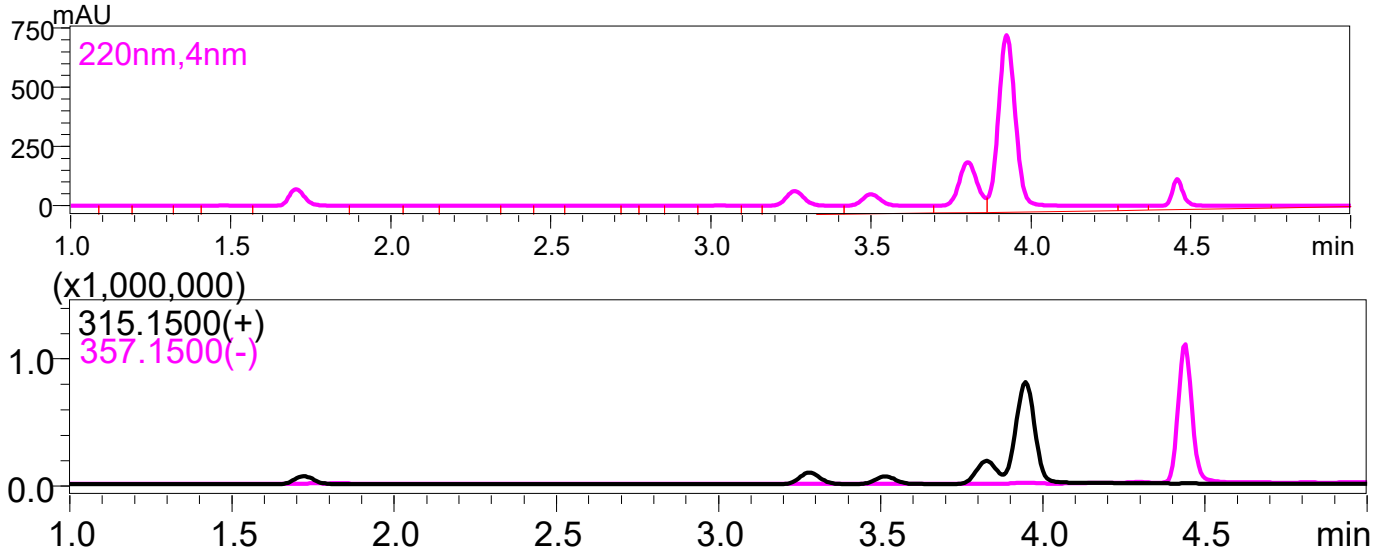

Figure 2: Representative Chromatograms. Top: PDA wavelength 220nm at 10µg/mL Bottom: SIM 315.15 TIC and SIM 357.15 TIC at 0.5µg/mL

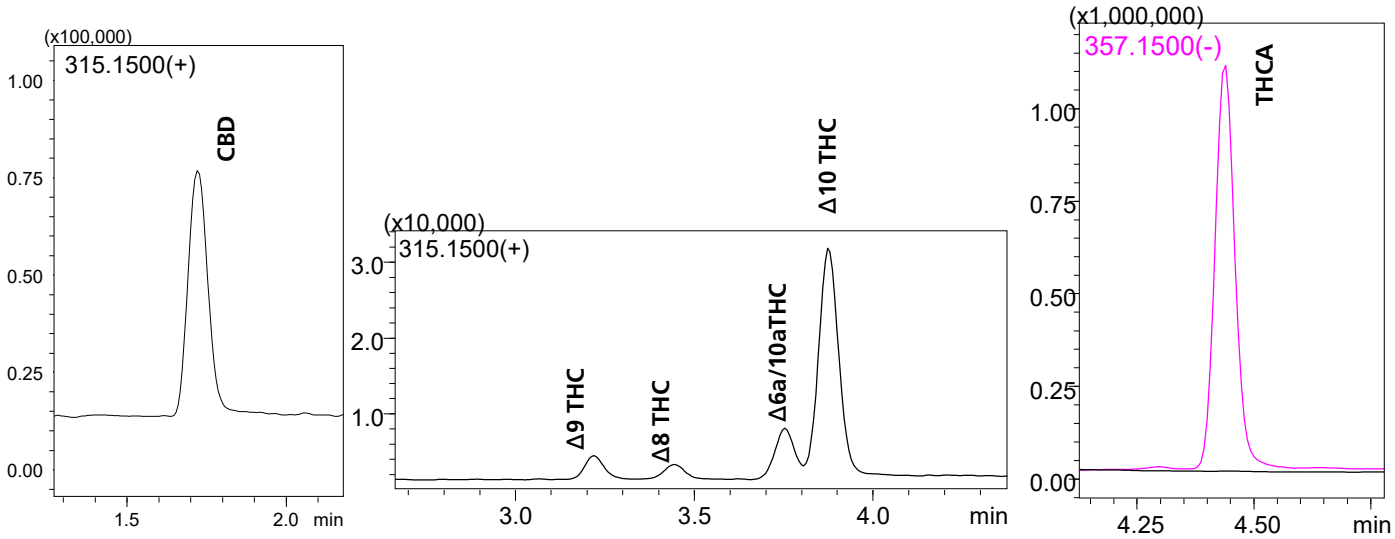

Figure 3: Representative SIM Chromatogram for four THC isomers, CBD, and THCA at 0.5µg/mL

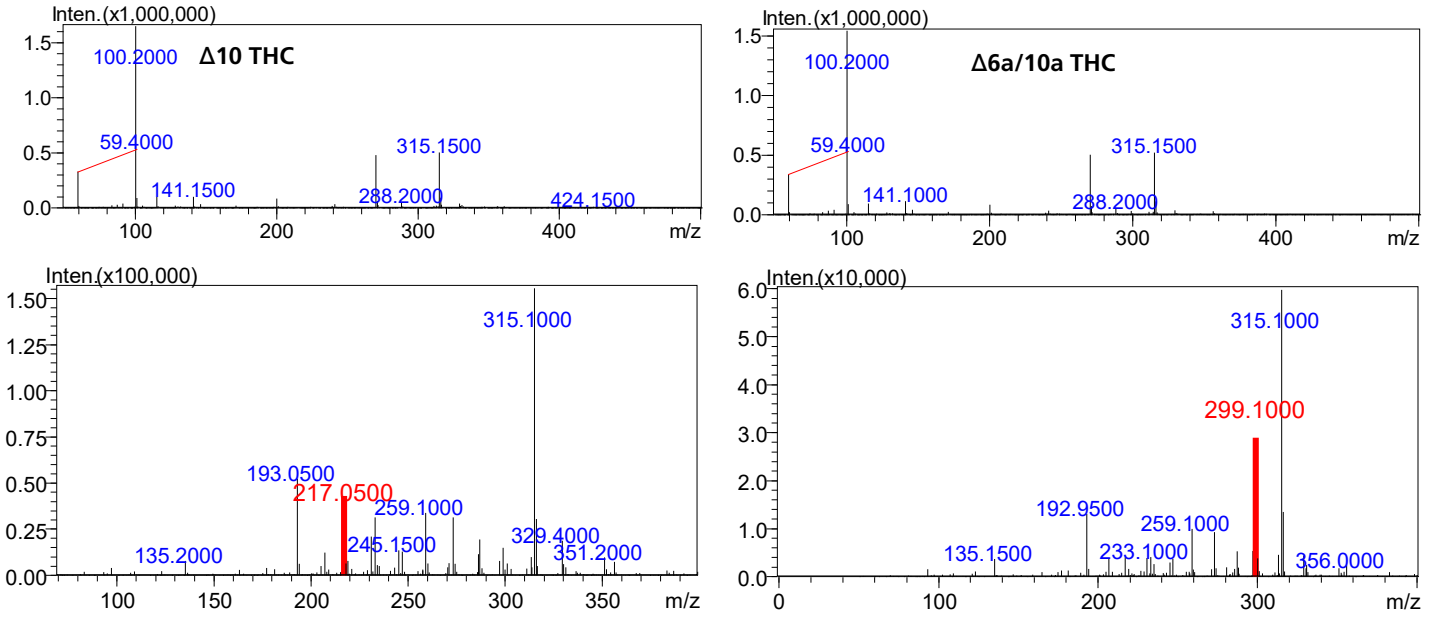

Figure 4: Scan and in-source CID spectra for Δ10 THC and Δ6a/10a THC at 10µg/mL. Top spectrum is a positive scan event with 0V, bottom spectrum is a positive scan event with 55V in-source CID

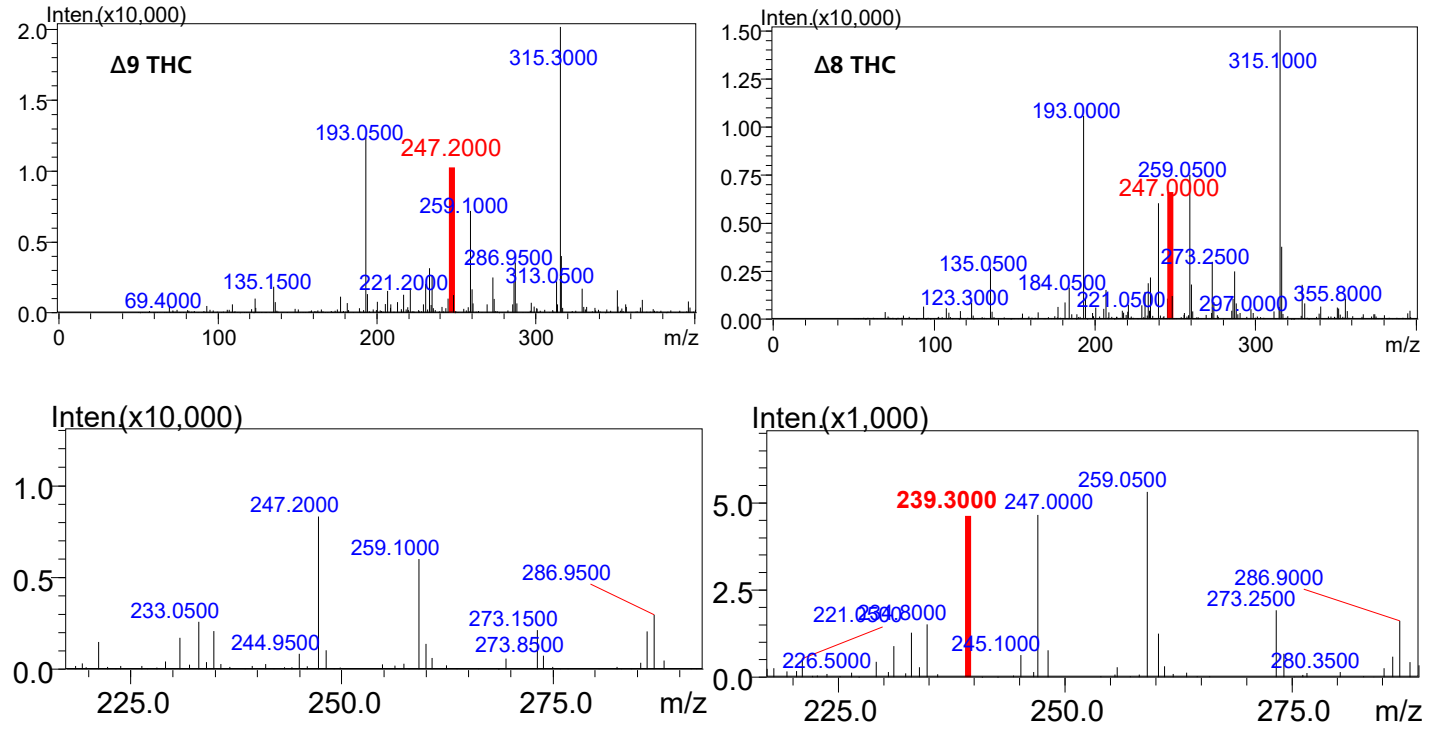

Figure 5: 55V In-source CID spectra for Δ8 THC and Δ9 THC at 10µg/mL

All calibration curves were run in triplicate and had at least 5 points with a correlation coefficient of R^2=0.996 or better. Internal standard quantitation was used with a 1/C weighting for all analytes. Δ9-D3-THC was used as the internal standard and was spiked using 25µL at a 500 ng/µL concentration for all samples.

The accuracy for each calibrator was between 83.7% and 117.3% with a %RSD of 7.85 or better. The average accuracy and %RSD for each calibrator can be found in Table 2.

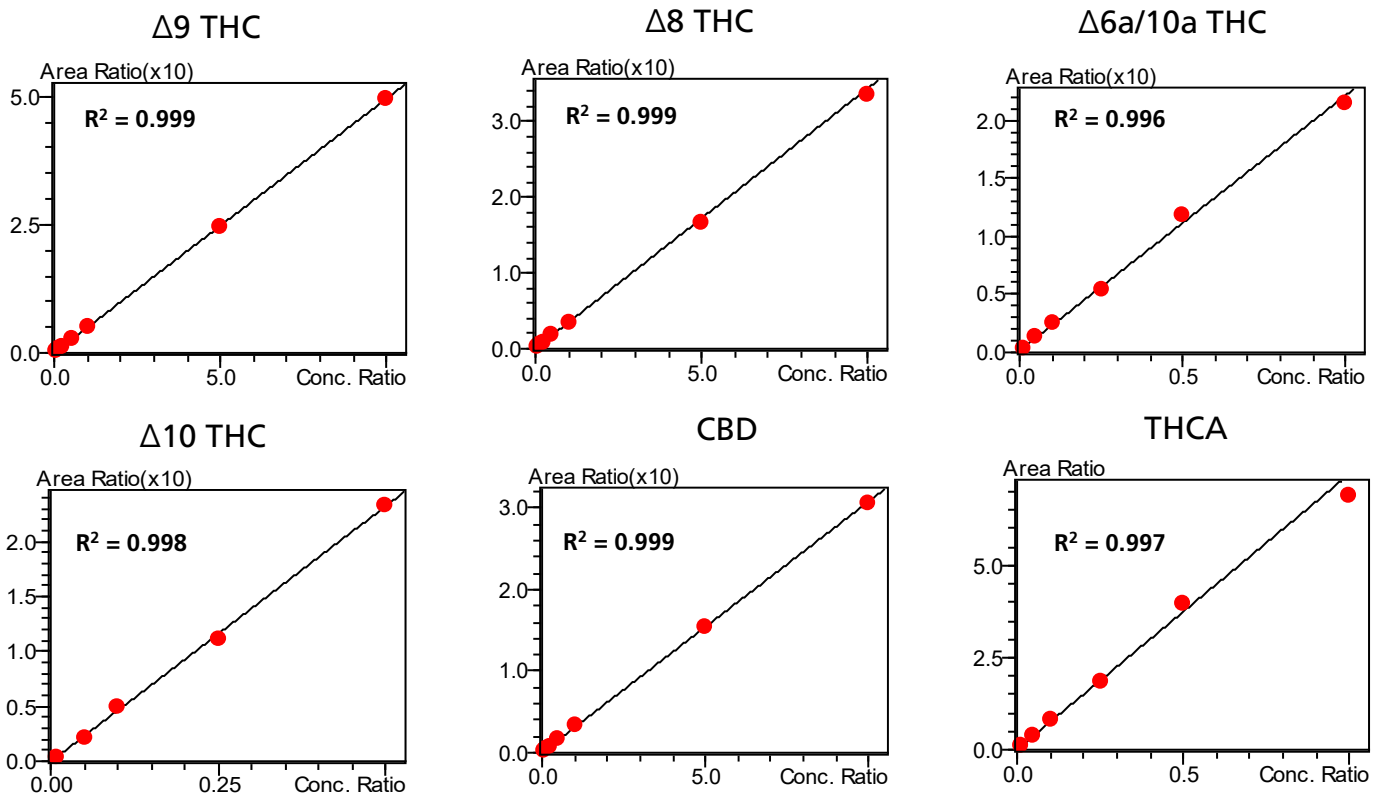

Figure 5: Calibration curves for each compound

Table 2: Average accuracy and %RSD for all calibration levels.
(Level 1 = 0.01 ng/µL, Level 2 = 0.05 ng/µL, Level 3 = 0.1 ng/µL, Level 4 = 0.25 ng/µL, Level 5 = 0.5 ng/µL, Level 6 = 1.0 ng/µL, Level 7 = 5.0 ng/µL)

Analyte	Level 1 Average Accuracy (%RSD)	Level 2 Average Accuracy (%RSD)	Level 3 Average Accuracy (%RSD)	Level 4 Average Accuracy (%RSD)	Level 5 Average Accuracy (%RSD)	Level 6 Average Accuracy (%RSD)	Level 7 Average Accuracy (%RSD)	R^2
CBD	NA	91 (4.67)	101 (4.28)	98 (2.71)	110 (2.30)	103 (2.55)	99 (1.51)	0.999
Δ9-THC	NA	90 (5.3)	101 (1.85)	98 (2.05)	110 (2.79)	102 (2.33)	99 (0.44)	0.999
Δ8-THC	NA	90 (6.95)	108 (5.49)	97 (2.32)	107 (2.68)	100 (2.87)	99 (0.93)	0.999
Δ6a/10a-THC	98 (7.85)	90 (2.00)	108 (1.40)	100 (2.76)	108 (2.41)	96 (3.48)	NA	0.996
Δ10-THC	100 (2.11)	95 (1.87)	108 (2.83)	97 (0.81)	100 (2.80)	NA	NA	0.998
THCA	88 (3.52)	105 (2.58)	108 (0.65)	97 (1.43)	106 (2.15)	97 (0.04)	NA	0.997

Two hemp samples were analyzed against the internal standard calibration curves. Four injections were completed for each hemp sample. One was spiked with Δ8-THC, Δ6a/10a-THC, and Δ10-THC to confirm detection and baseline separation of each isomer in hemp matrix. Representative chromatograms for both hemp samples are shown in Figure 6. The final THC content was calculated two ways. The first is the current protocol using equation 1. The second uses equation 2 and factors in all THC isomers when calculating the final potency of THC. The %THC for each isomer or THCA was calculated using Equation 3.

Final %THC and %RSD for both hemp samples can be found in Table 3 and final THC content can be found in Table 4.

Equation 1: (%THCA x 0.877) + %Δ9-THC = potency of THC
Equation 2: (%THCA x 0.877) + %ΔTHC sum of isomers = potency of THC
Equation 3: [THC] x (VOL/MG) x 100 = %THC

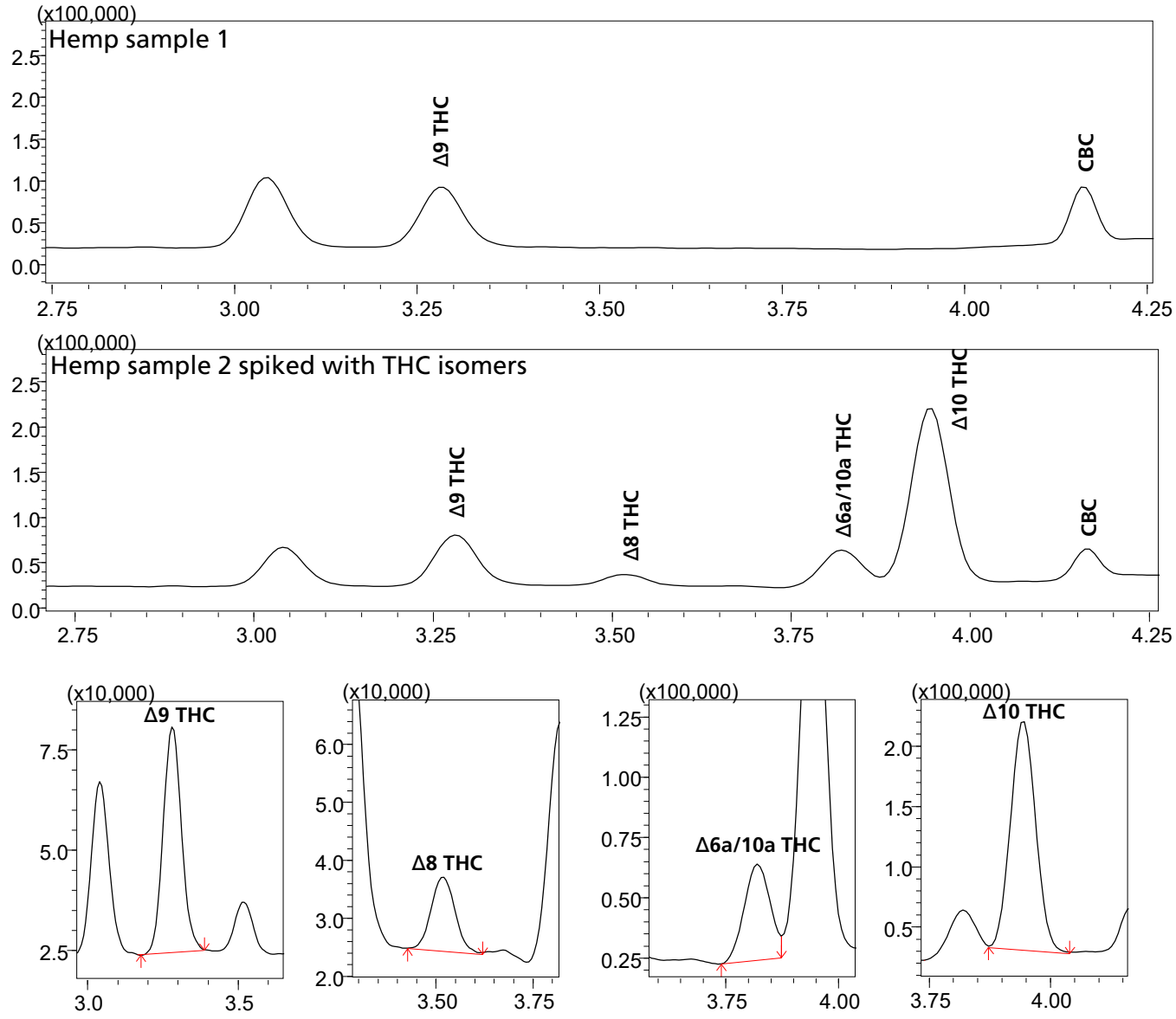

Figure 5: Representative chromatogram for two hemp samples. Hemp sample 2 was spiked with 0.125 ng/µL THC isomers Δ8 THC, Δ6a/10a THC, and Δ10 THC.

Table 3: THC Concentration and Weight % for Hemp Samples (All values based on an average of 4 replicates)

Analyte	Sample 1 Conc. (ng/µL)	Sample 1 Wt %	Sample 1 %RSD	Sample 2 Conc. (ng/µL)	Sample 2 Wt %	Sample 2 %RSD
CBD	5.243	0.0524	1.5	2.811	0.0281	2.7
Δ9-THC	0.550	0.0055	3.3	0.440	0.0044	3.6
Δ8-THC	NA	NA	NA	0.132	0.0013	3.7
Δ61/10a-THC	NA	NA	NA	0.136	0.0014	3.8
Δ10-THC	NA	NA	NA	0.142	0.0014	3.7
THCA	4.22	0.0422	2.8	2.37	0.0237	2.3

Table 4: Hemp Samples

	%THC content	%THC content with isomers
Hemp Sample 1	0.0424	0.0424
Hemp Sample 2	0.0252	0.0293

■ Conclusion

A Shimadzu single quadrupole mass spectrometer, LCMS-2020, with in-line PDA demonstrated its capability for simultaneous detection of four THC isomers with baseline separation. Additional confirmation was completed using in-source fragmentation of four THC isomers. Linear calibration curves were acquired for each analyte with a %RSD of 0.996 or better and a % accuracy between 80 and 120 for all calibrators.

Two hemp samples were analyzed to determine the total THC content to be below 0.3% and confirm that baseline separation of all four isomers can be seen in matrix.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060NX


LCMS-2020


Q-TOF LCMS-9030

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