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Application News

No. LCMS-108

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of 16 Cannabinoids with Complete Baseline Separation of Δ8 and Δ9-THC Utilizing the Triple Quad LCMS-8050

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■ Background

Growing interest in analyzing the inherently complex cannabis- and hemp-based products necessitate the analytical benefits of LC-MSMS such as higher sensitivity, selectivity, and resolution for the determination of cannabinoids. Several methods have been developed for optimal results of resolution, sensitivity, and throughput but this method introduces the list of 16 cannabinoids being analyzed by triple quadruple LCMS. To assist in optimizing for high throughput while maintaining sensitivity and resolution, this application demonstrates a sub-five-minute gradient method utilizing the Nexera UHPLC system and triple quad LCMS-8050.

■ Introduction

The following method application is driven by the potential terpenes interferences with THC quantitation when analyzing fortified Cannabis products on an HPLC system with a UV or PDA detector. Generally, terpene interference is not a problem as naturally occurring terpenoid content does not exceed 0.2% of the total and terpenes are lost in the baseline under the currently run gradient conditions with HPLC-UV or UHPLC-PDA[1]. However, a sample that has been fortified with excess terpene content may face co-elution issues of the terpenes with individual cannabinoids because the terpenes are also quite hydrophobic and exhibit similar retention on the column as the cannabinoids.

This application employs the highly selective capabilities of Shimadzu Scientific Instruments' LCMS-8050 triple quad as well as the efficacy of this instrumentation in achieving chromatographic speed, analyte selectivity and quantitative sensitivity of all cannabinoids.

The method demonstrates ideal chromatographic conditions utilizing the UHPLC in conjunction with the ideal diffusion properties of the column delivering complete baseline separation of all cannabinoids including Delta-8 Tetrahydrocannabinol (Δ8-THC) and Delta-9 Tetrahydrocannabinol (Δ9- THC).

■ Method and Materials

A mixture of 16 cannabinoids was prepared using an 11-component mixture (Shimadzu 220-91239-21) with five additional individual cannabinoids obtained from Sigma Aldrich (Cerilliant). The stock solution included the following Cannabinoids as well as their acidic forms: Cannabidivarinic acid (CBDVA), Cannabidivarin (CBDV), Cannabidiolic acid (CBDA), Cannabigerolic acid (CBGA), Cannabigerol (CBG), Cannabidiol (CBD), Tetrahydrocannabivarin (THCV), Tetrahydrocannabivarinic acid (THCVA), Cannabinol (CBN), Cannabinolic acid (CBNA), Delta 9-Tetrahydrocannabinol (Δ9-THC), Delta 8-Tetrahydrocannabinol (Δ8-THC), Cannabicyclol (CBL), Cannabichromene (CBC), Tetrahydrocannabinolic acid A (THCA-A), and Cannabichromenic acid (CBCA).

The column was obtained from Restek.[2] LCMS grade mobile phase solvent were obtain wed from Honeywell. A stock concentration of 1000 ppb for each standard was used for MRM optimization. The standards were prepared in 25:75 LCMS grade Water: Methanol solvent in varying concentrations ranging from 1 ng/ml – 2000 ng/ml. For quantification, samples were diluted in the ratio of 1:9, 1:99, 1:999 v/v Cannabis extract to ethanol. The samples were loaded onto the Shimadzu Nexera UHPLC with LCMS-8050 for analysis using the analytical conditions listed in **Table 1**.

Table 1: Analytical Conditions

HPLC- Shimadzu Nexera UHPLC	
Column Temperature	40 °C
Injection Volume	5 uL
LCMS-8050 Triple Quad Mass Spectrometer	
Ionization	Heated ESI
Nebulizing Gas	3 L/min
Drying Gas	10 L/min
Heating Gas	10 L/min
DL Temperature	250 °C
Heat Block Temperature	400 °C
Interface Temperature	370 °C

■ Results and Discussion

The chromatographic method that was developed yielded baseline separation of all 16 cannabinoids **(Figure 1)** including the Delta-8 THC and Delta-9 THC **(Figure 2 and 3)**. A twelve-point calibration curve was prepared ranging from 1 to 2000 ng/ml. Due to the wide linear range a best-fit weighting method was used ($1/C^2$). Calibration curves for the all Cannabinoids exhibited R^2 values ≥ 0.99. All the chromatograms show the lowest calibrator of the linear range **(Figure 4)**. All standards were run in series of triplicates as well as calibration checks that were also run in replicates of six injections. The statistical results can be seen in **Table 2**.

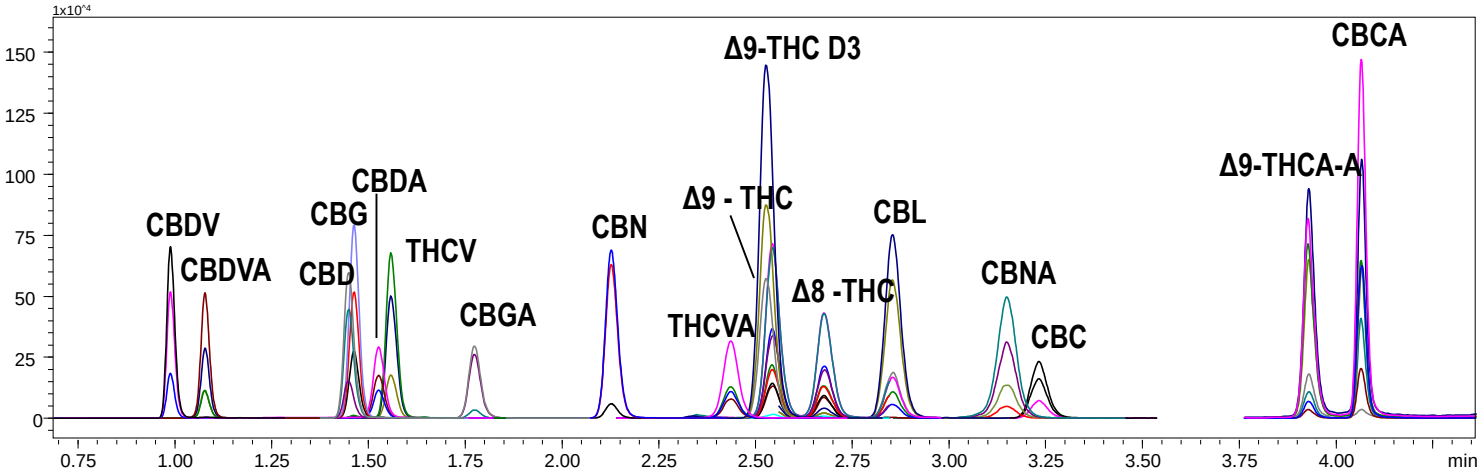

Figure 1: MRM and TIC of simultaneous acquisition of 16 cannabinoids at 100ng/ml.

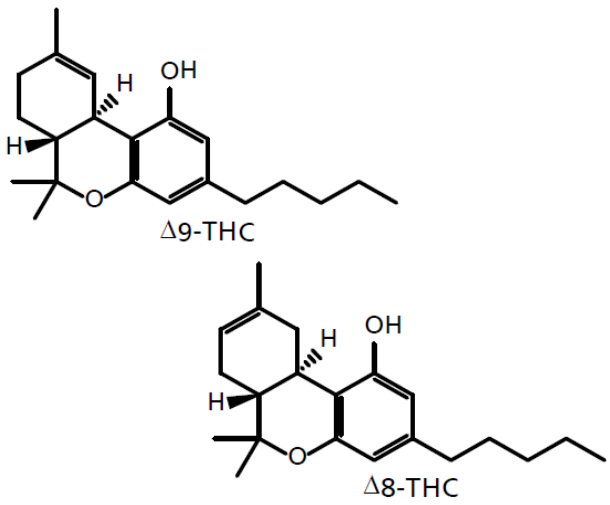

Figure 2: Chemical structure Δ8-THC and Δ9-THC

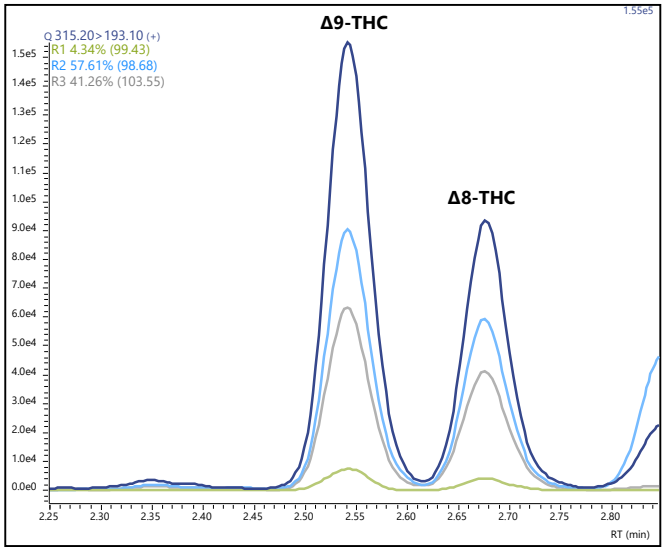

Figure 3: Chemical structure Δ8-THC and Δ9-THC

Table 2: MRM Transitions, calibration range, accuracy and precision results for potency. The table shows the quantitative abilities of this method for each analyte. Values of linearity refer to the linear range specified in the table.

Compound	MRM Transition (quantifier ion)	MRM Transitions (qualifying ion)	Linear Range ng/mL	Linearity r^2	%RSD	% Accuracy
CBDV	287.20>165.00	287.20>123.05	1- 2000	0.997	5.4	101.6
CBG	317.20>192.90	317.20>123.00	1- 2000	0.997	3.2	95.9
CBD	315.20>193.00	315.20>135.05	1- 2000	0.997	0.4	99.0
THCV	287.20>165.20	287.20>231.10	1- 2000	0.997	4.6	96.2
CBN	311.15>223.00	311.15>43.30	1- 2000	0.998	2.0	105.0
CBC	315.20>193.10	315.20>259.05	1- 2000	0.999	9.1	102.0
CBL	315.05>235.10	315.05>81.20	1- 2000	0.999	3.0	103.5
Δ9 - THC	315.20>193.10	315.20>245.00 315.20>123.20 315.20>259.10	1- 2000	0.998	6.7	94.5
Δ8 -THC	315.20>192.95	315.20>135.10 315.20>123.25	1- 2000	0.997	4.2	107.0
CBDA	357.00>244.95	357.00>339.25	1- 2000	0.9929	4.2	106.7
CBGA	359.10>341.20	359.10>315.20	1- 2000	0.996	2.1	101.3
CBDVA	329.15>311.25	329.15>216.95 329.15>107.15	1- 2000	0.9932	2.7	90.9
Δ9-THCA-A	357.00>313.15	357.00>245.20 357.00>191.20	1- 2000	0.9924	0.9	95.6
THCVA	329.10>217.10	329.00>285.10 329.00>163.15	1- 2000	0.996	8.9	105.5
CBNA	353.00>309.15	353.10>279.20 353.00>171.25	1- 2000	0.997	2.8	100.2
CBCA	357.15>191.20	357.15>313.20 357.15>203.10	1- 2000	0.9927	9.9	92.0

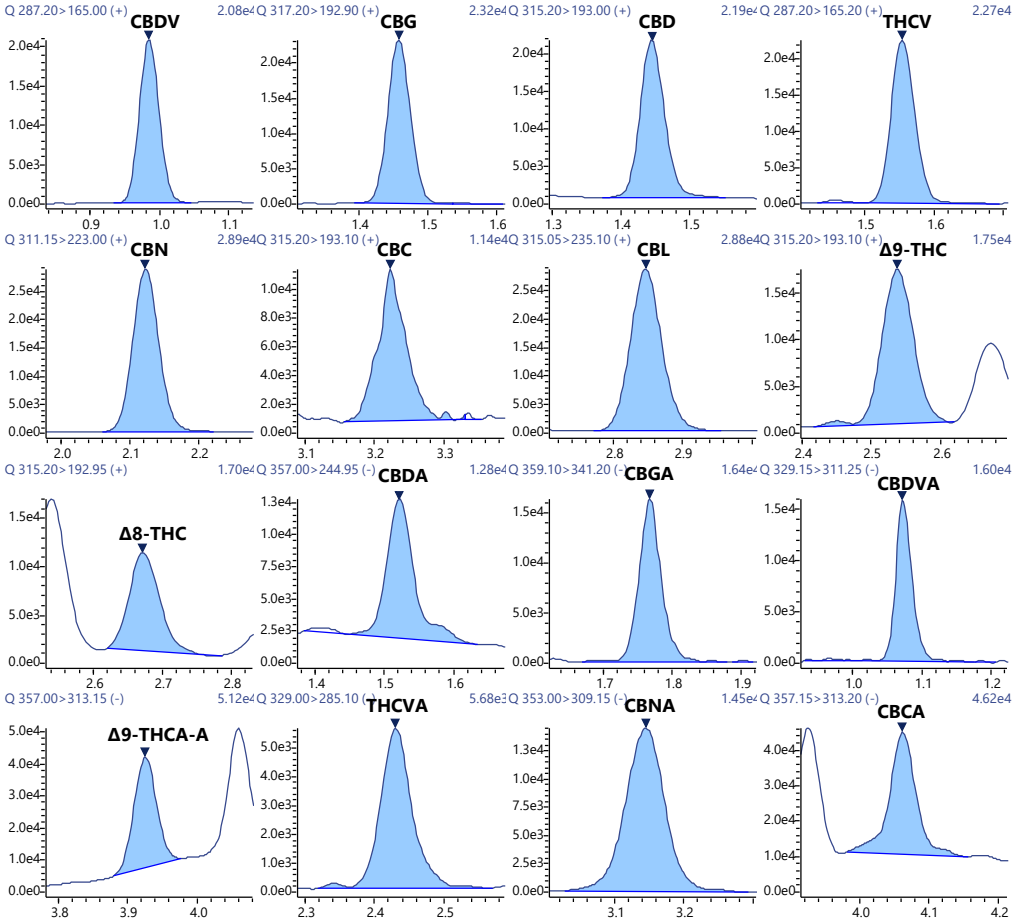

Figure 4: The above chromatograms show the lowest calibrator of the linear range.

■ Conclusion

This application demonstrates a rapid, selective and robust method for the detection of various cannabinoids by using the Shimadzu LCMS-8050. All sixteen cannabinoids were detected at levels as low as 1 ng/mL with a minimum signal-to-noise ratio of 10 with the only limitation in hitting an even lower LOQ being the wide linear range. It is important to note that these results were achieved using standards without matrix. Matrix-matched calibration standards cannot be used because of native amounts of cannabinoids; therefore, utilizing Internal Standards is recommended. This method demonstrates the ability of the Nexera X2 UHPLC and LCMS-8050 mass spectrometer to enable simultaneous, accurate measurement of many of the cannabinoids of interest. This method is suitable for quantitating cannabinoids in raw or commercial products.

■ Acknowledgements

Shimadzu Scientific Instruments would like to thank its collaborators: Cannabusiness (Aquatic Resources Management), who worked to develop the analytical conditions that made this application note possible, and Restek Corporations for providing us with the materials used for developing this method.

■ References

1. Shimadzu Scientific instruments (2016) The Determination of CBD and General Cannabinoid Content in Hemp Oils Using HPLC with UV Detection.
2. Restek. (2017). 16 Cannabinoids on Raptor ARC-18 1.8 µm by LC-UV.

UFMS
ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


Q-TOF LCMS-9030

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