CUSTOMER APPLICATION NOTE 000108

Quantitation of seventeen cannabinoids in dried cannabis, hemp and vape oils by LC-MS/MS

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Goal

Demonstrate accurate and precise quantitation of seventeen cannabinoids extracted from cannabis and hemp dried plant materials, in addition to vape oils with LC-MS/MS detection on a Thermo Scientific™ Vanquish™ Flex UHPLC System coupled to a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer.

Introduction

Regulated cannabis markets have seen sharp increases in production as jurisdictions around the world continue to legalize cannabis for medicinal and adult recreational use. At the same time, the hemp industry produces large amounts of hemp for CBD production, textiles and other uses that must adhere to Δ9-THC regulatory limits. The need for reliable analytical methods able to accurately quantitate cannabinoids in cannabis, hemp, and vape oils has increased in step with the increases in production, quality demands, and the ever-changing regulatory requirements.[1-3] Liquid chromatography with UV detection (LC-UV) has been used predominantly for this application, however it generally lacks performance when analyzing a large suite of cannabinoids or those at very low abundance (e.g., minor neutral and acidic cannabinoids).

By contrast, liquid chromatography—tandem mass spectrometry (LC-MS/MS) is capable of specific and sensitive quantitation of low level analytes in complex matrices, and is well-suited for the analysis of large sets of cannabinoids in cannabis, hemp, and vape oils. While more complex and costly than LC-UV, LC-MS/MS is a viable

alternative when accurate quantification is required for regulatory purposes. The method outlined below is able to quantitate the major cannabinoids, Δ9-THC, Δ9-THCA, CBD and CBDA, and also the lower concentration minor cannabinoids, Δ8-THC CBN, CBNA, CBG, CBGA, CBC, CBCA, THCV, THCVA, CBDV, CBDVA, CBL and CBLA.[4] Detailed method parameters are provided and performance characteristics are described.

Experimental

Calibration standards (STD) and quality control (QC) samples, containing all 17 cannabinoids (Cerilliant®), were prepared in methanol over a range of 10–10,000 ng/mL (Table 1).

Cannabis and hemp sample preparation: dried cannabis and hemp samples were cryo-ground to a homogeneous powder, using a SPEX Freezer/Mill™, and subsampled (100 mg). Sample extraction was performed twice using 5 mL of a mixture of methanol and water (80:20, v/v). The samples were centrifuged after each extraction, with both supernatants retained and combined. The combined supernatants were mixed and an aliquot was diluted appropriately with methanol. For example, a 1/300 dilution of supernatant allows quantitation over a range of 0.3 to 300 mg/g (0.03 to 30%) cannabinoid content.

Vape oil sample preparation: vape oils were removed from the cartridges and sampled (25 mg). Sample extraction was performed twice using 5 mL of a mixture of methanol and chloroform (90:10, v/v) with centrifugation of the samples after each extraction. The supernatants were retained, combined, mixed and an aliquot was diluted appropriately with methanol. For example, a 1/250 dilution of supernatant allows quantitation over a range of 1.0 to 1000 mg/g (0.1 to 100%) cannabinoid content.

After extraction and dilution, aliquots (100 µL) of the diluted supernatants and each STD and QC sample were transferred to HPLC vials containing glass inserts followed by the addition of internal standard (50 µL, 500 ng/mL THC-d3, CBD-d3, CBN-d3 and THCA-d3 in methanol).

Analysis was originally performed on a high-end legacy LC-MS/MS platform and later transferred to the TSQ Altis system with a Vanquish UHPLC system. UPLC gradient separation was performed on a Thermo Scientific™ Accucore™ C18 column, with a guard column of the same phase and mobile phases consisting of 0.1% formic acid in water and acetonitrile at a flow rate of 0.5 mL/min (Tables 2 and 3). The mass spectrometer was operated in positive ion mode and selective reaction monitoring mode (SRM). Two ion transitions were monitored for each cannabinoid for quantitation and confirmation (Table 4 and 5).

Table 1. Calibration standards and QC samples

Standard/QC	Conc. (ng/mL)
STD-7	10,000
STD-6	9,000
STD-5	6,000
STD-4	1,000
STD-3	100
STD-2	20
STD-1	10
STD-0	0
QC-3	8,000
QC-2	1,500
QC-1	30

Table 2a

Vanquish Flex UHPLC Components	Part number	Specifics
Vanquish Autosampler	VF-A10-A	Split Sampler FT
Vanquish Pump	VF-P10-A	Binary Pump F (150 µL mixer)
Vanquish Column Compartment	VH-C10-A	Forced or still air heating/cooling option

Table 2b. Chromatography parameters

Parameter	Setting
Column	Accucore C18, 150 × 2.1 mm, 2.6 µm
Guard column	Accucore C18, 10 × 2.1 mm, 2.6 µm
Mobile phase A	0.1% Formic acid in water
Mobile phase B	0.1% Formic acid in ACN
Flow rate	0.5 mL/min
Run time	18.0 min
Column temperature	40°C
Injection volume	1 µL
Needle wash	ACN:MeOH:Water:FA; 40:40:20:1
Autosampler temperature	4°C

Table 3. UHPLC gradient

Time (min)	% A	% B
0.0	40	60
8.0	32	68
13.5	32	68
13.6	5	95
14.5	5	95
14.6	40	60
18.0	40	60

Table 4. Mass spectrometer parameters

Parameter	Setting
Scan type	SRM
Ion source	HESI
Polarity	Positive
Spray voltage	4000 V
Sheath gas	50
Aux gas	25
Sweep gas	2
CID	1.5 mTorr
Ion transfer tube temperature	325°C
Vaporizer temperature	280°C

Table 5. MS/MS parameters

Name	IS used for quant	Q1 *(m/z)*	Q3 *(m/z)*	RF (V)	CE (eV)
CBDVA	CBN-d3	313.2	191.1	95	26
		313.2	233.1	95	19
CBDV	CBN-d3	287.2	165.1	58	22
		287.2	123.1	58	31
CBDA	THCA-d3	341.2	219.1	105	26
		341.2	261.1	105	19
CBGA	CBN-d3	343.2	219.1	72	22
		343.2	149.1	72	38
CBG	CBN-d3	317.2	193.1	57	16
		317.2	123.1	57	32
THCV	CBN-d3	287.2	165.1	58	22
		287.2	123.1	58	31
CBD	CBD-d3	315.2	193.1	62	22
		315.2	123.1	62	31
THCVA	CBN-d3	313.2	191.1	95	26
		313.2	233.1	95	21
CBN	CBN-d3	311.2	223.1	71	20
		311.2	241.1	71	18
CBNA	THCA-d3	337.2	235.1	156	28
		337.2	253.1	156	23
Δ9-THC	THC-d3	315.2	193.1	62	22
		315.2	123.1	62	31
Δ8-THC	THC-d3	315.2	193.1	62	22
		315.2	123.1	62	31
CBL	CBN-d3	315.2	235.1	55	17
		315.2	81.1	55	30
THCA	THCA-d3	341.2	219.1	125	26
		341.2	261.1	125	21
CBC	CBN-d3	315.2	193.1	62	18
		315.2	259.1	62	14
CBLA	THCA-d3	359.2	261.1	67	25
		359.2	219.1	67	32
CBCA	THCA-d3	341.2	219.1	111	25
		359.21	219.1	60	25
CBD-d3	—	318.2	196.1	62	22
CBN-d3	—	314.2	223.1	71	20
THC-d3	—	318.2	196.1	62	22
THCA-d3	—	344.2	222.1	120	26

quantitation ion
confirmation ion

Results and discussion

This method has been described previously, and is presented here in a modified form. Full validation results are available in the corresponding peer reviewed publication.[4] Calibration standard ion chromatograms (Figure 1) show separation of all cannabinoid critical pairs (within ±2 *m/z*), while cannabis sample chromatograms dominated by THCA (Figure 2) and CBDA (Figure 3) show the ability of the method to efficiently quantitate low levels of minor cannabinoids in the presence of high levels of major cannabinoids. A hemp sample chromatogram (Figure 4) shows chromatographic separation of a sample containing 16 cannabinoids with CBD as a major component and 15 minor cannabinoids, while a vape oil sample chromatogram (Figure 5) shows the presence of neutral cannabinoids only. The acidic cannabinoids form both the molecular ion [M+H]+ and a water loss species [M+H-H_2O]+ in positive ionization mode. Monitoring either or both species can be used to exploit differences in sensitivity to enhance the accuracy of quantitation of closely eluting cannabinoids.

Representative calibration curves (Figures 6 and 7) show linearity, while precision (RSD) and accuracy (Acc.) using QC samples, a cannabis certified reference material (CRM) and hemp CRM, are shown in Table 6.

Table 6. Statistical results for calibration curves, QC samples and matrix CRM samples*

		QC-1 30 ng/mL		QC-2 1500 ng/mL		QC-3 8000 ng/mL		Cannabis CRM		HEMP CRM	
Analyte	**r^2**	**RSD (%)**	**Acc. (%)**	**RSD (%)**	**Acc. (%)**	**RSD (%)**	**Acc. (%)**	**RSD (%)**	**Acc. (%)**	**RSD (%)**	**Acc. (%)**
CBDVA	0.9997	1.2	108.4	1.7	105.5	1.0	99.8	4.1	101.0	1.6	100.1
CBDV	0.9997	1.5	107.3	1.4	103.1	0.5	94.8	3.9	100.4	1.4	94.4
CBDA	0.9996	1.1	102.1	1.6	100.8	1.6	93.4	5.1	101.1	1.5	97.1
CBGA	0.9998	0.8	101.3	2.1	99.7	1.8	91.7	5.1	94.9	1.7	95.7
CBG	0.9954	1.2	112.8	0.9	109.4	0.9	92.0	4.8	100.3	2.0	96.1
THCV	0.9973	2.7	110.6	0.9	105.4	1.2	91.1	4.9	92.6	1.9	104.8
CBD	0.9992	1.5	108.2	1.0	101.2	0.4	94.7	4.7	101.3	1.2	96.2
THCVA	0.9992	1.9	110.5	2.2	109.3	0.8	104.5	3.8	106.5	1.1	97.3
CBN	0.9996	1.9	103.2	0.7	100.7	1.0	96.7	4.3	112.2	1.9	90.4
CBNA	0.9982	2.5	111.6	0.9	113.2	1.1	95.2	4.0	108.1	1.8	107.0
Δ9-THC	0.9990	1.4	105.2	0.9	103.6	1.0	93.9	4.9	96.2	1.6	102.1
Δ8-THC	0.9993	0.7	106.6	0.3	103.7	1.1	94.3	N/AP	N/AP	N/AP	N/AP
CBL	0.9995	0.7	105.4	0.6	101.7	0.9	95.1	N/AP	N/AP	1.4	90.5
Δ9-THCA	0.9995	1.3	106.4	0.7	102.5	1.0	94.2	4.4	95.9	1.1	92.0
CBC	0.9984	2.3	104.0	0.9	101.8	0.8	101.0	3.1	100.0	2.1	90.4
CBLA	0.9992	2.9	103.3	1.8	104.6	1.5	103.9	N/AP	N/AP	1.5	103.7
CBCA	0.9990	2.4	106.2	4.2	104.5	1.9	107.8	5.1	97.3	3.1	98.4

*This data was originally acquired on a high-end legacy LC-MS/MS platform, and was later transferred to the TSQ Altis which provided equal or better method performance.

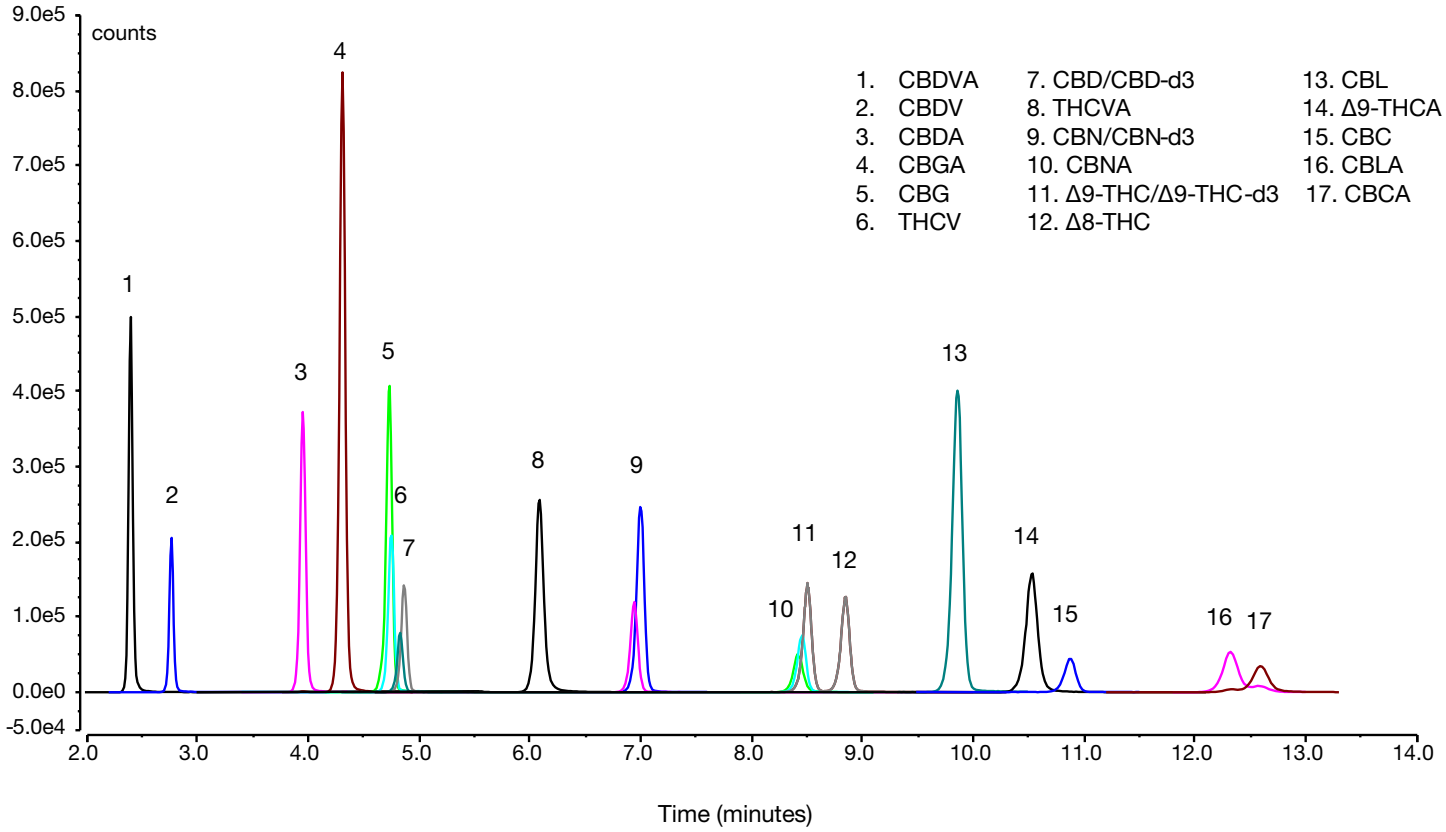

Figure 1. LC-MS/MS ion chromatogram of a calibration standard containing 17 cannabinoids

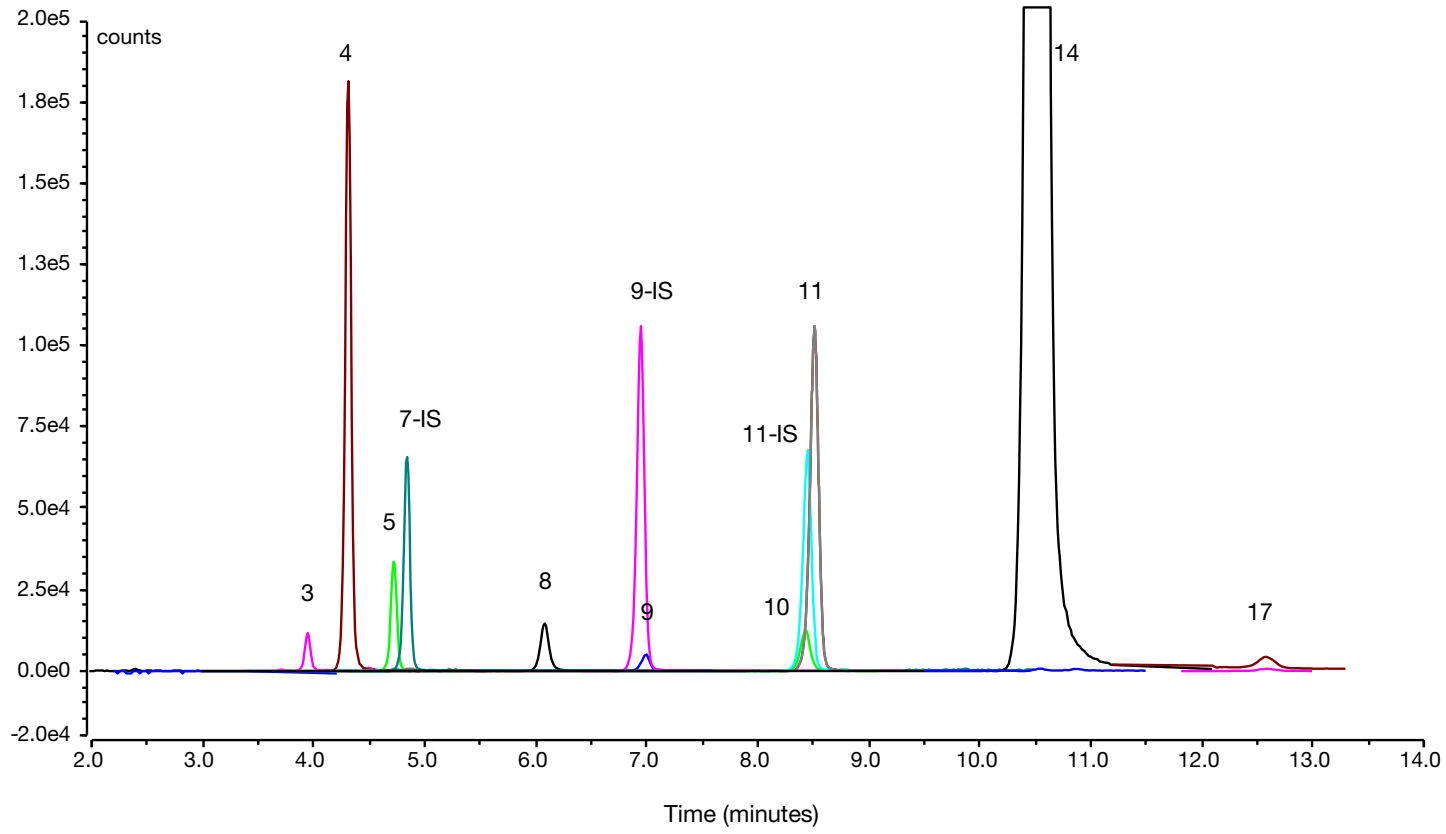

Figure 2. LC-MS/MS chromatogram of a cannabis sample with high THCA content

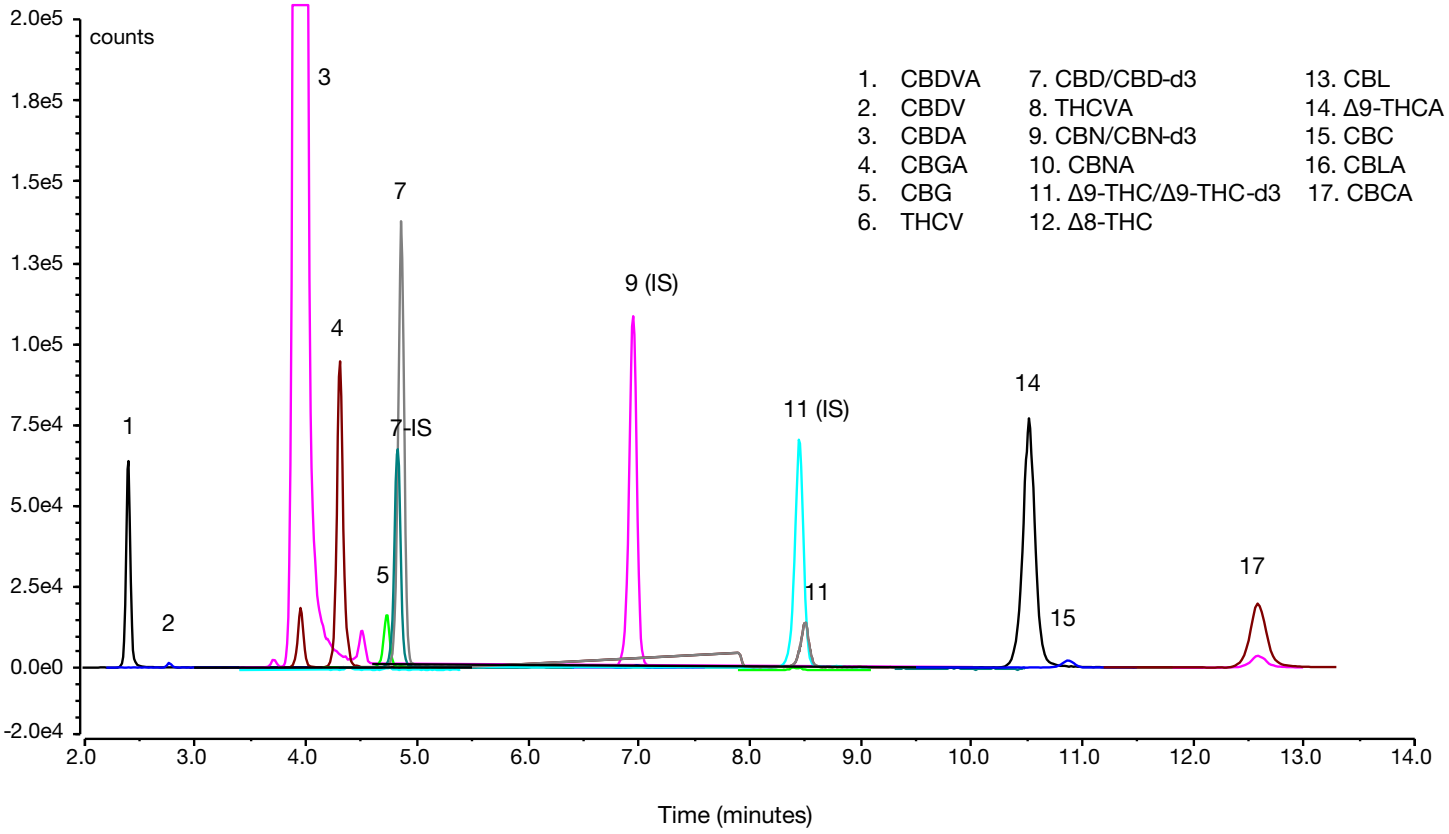

Figure 3. LC-MS/MS chromatogram of a cannabis sample with high CBDA content

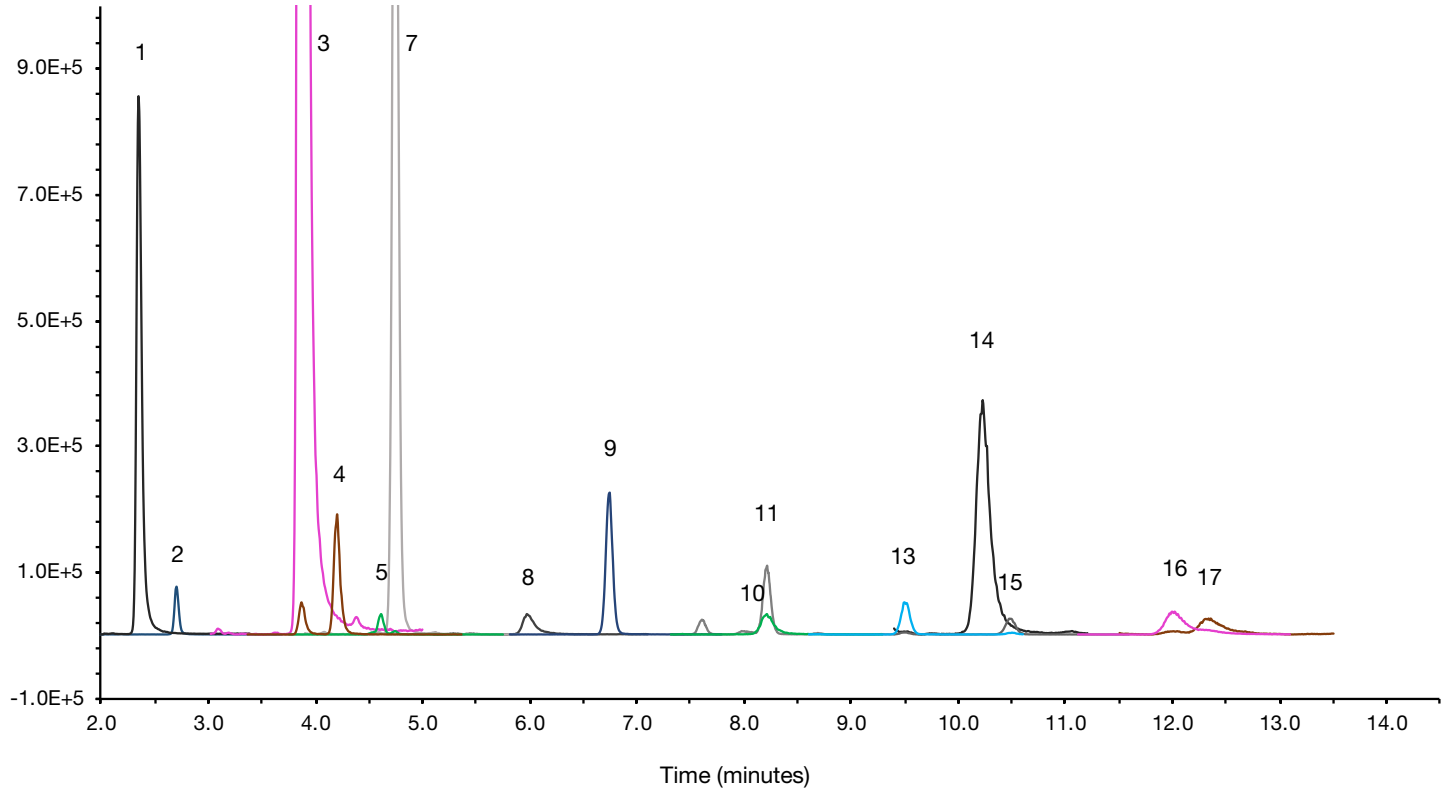

Figure 4. LC-MS/MS chromatogram of a hemp sample with high CBDA content

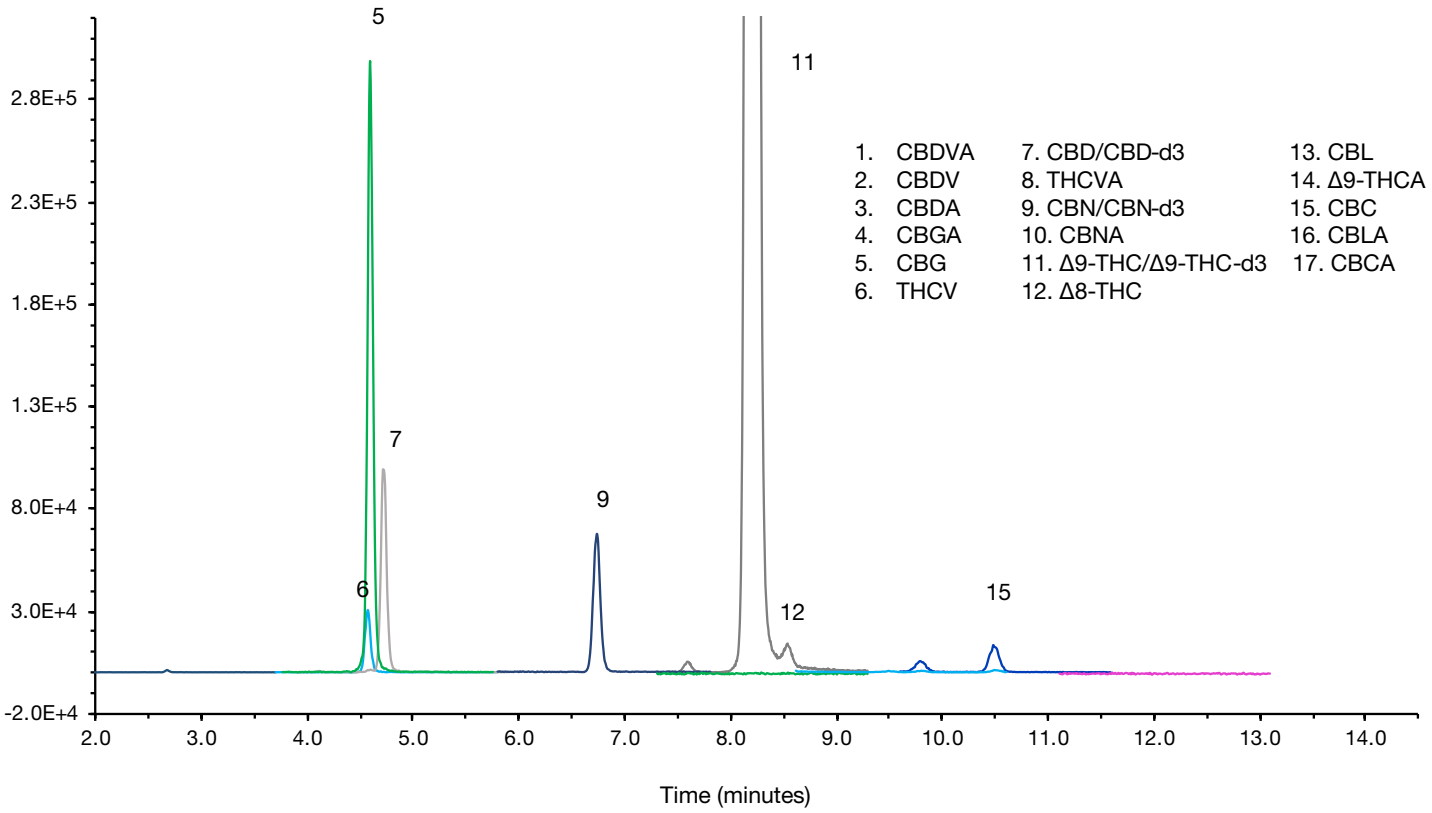

Figure 5. LC-MS/MS chromatogram of a vape oil sample showing neutral cannabinoids without their corresponding acidic components and high THC content

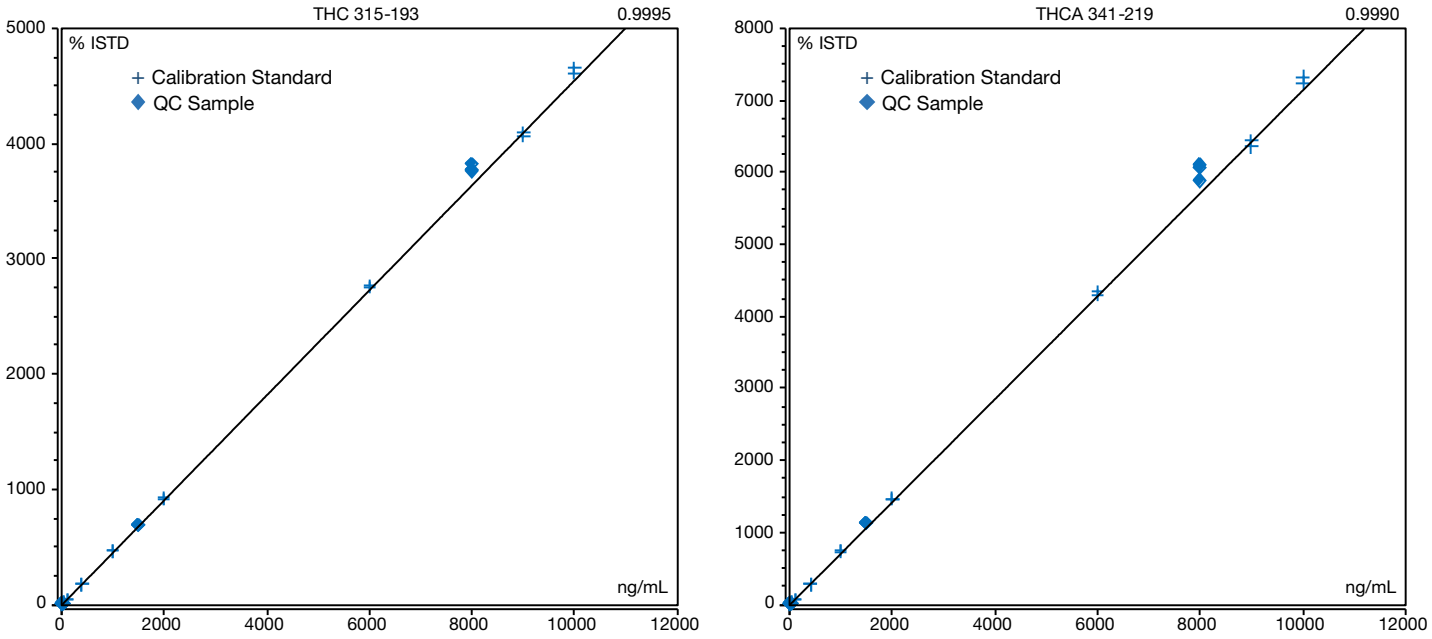

Figure 6. Calibration curve regression for THC and THCA

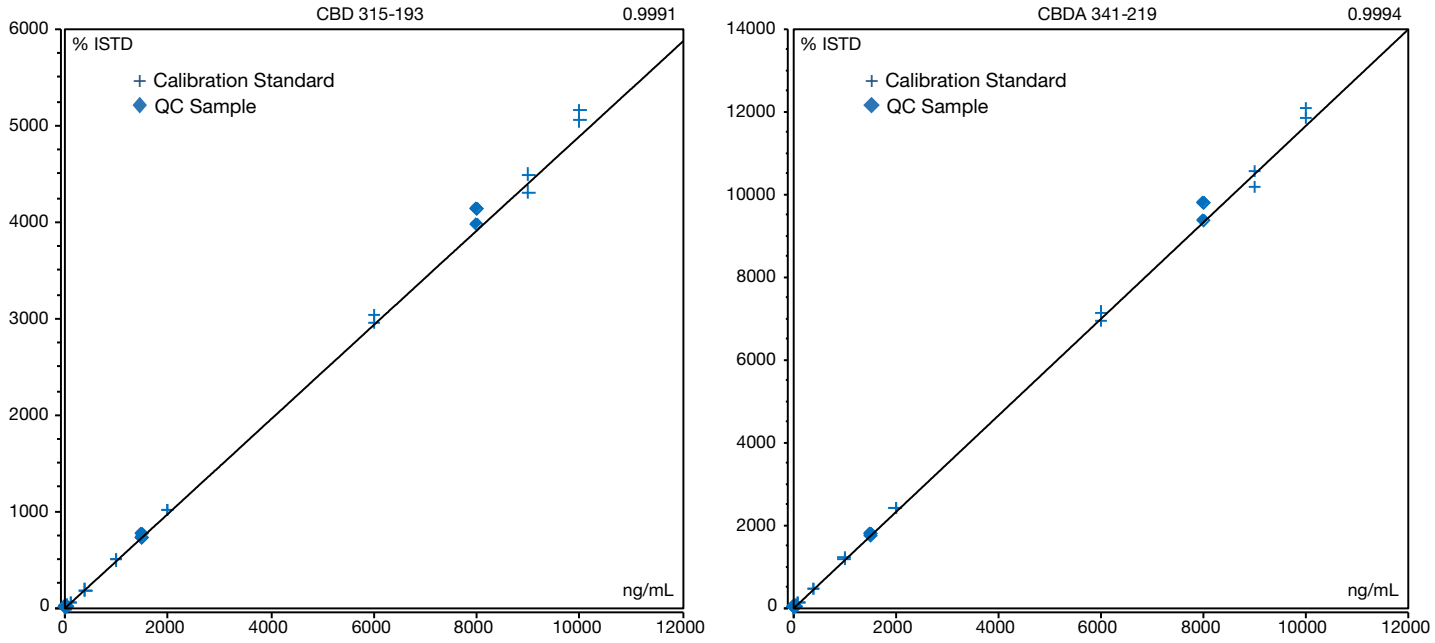

Figure 7. Calibration curve regression for CBD and CBDA

Conclusion

The method described has been shown to provide reproducible and accurate results for cannabinoids in dried cannabis, hemp, and vape oil samples. Separation of the key cannabinoids has been demonstrated in both calibration solutions and extracted matrix samples. While the method has yet to be fully validated for more complex matrices such as edibles and matrices, the high specificity of LC-MS/MS offers significant advantages for these challenging samples types. Therefore, this method can serve as viable starting point for such analyses.

References

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