Determination of Cannabinoids Using LC-MS/MS

Scope and Application

This method is used to quantitate Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), Δ^9-tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), cannabidiolic acid (CBDA), and cannabinol (CBN) in alcoholic beverages (beer, wine, and distilled spirits) using deuterated Δ^9-THC (Δ^9-THC-d3) as the internal standard. The samples are first prepared by diluting (typically 1:100) with an acidic aqueous-organic solvent to an appropriate level and then analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Regulatory Tolerances:

As per Public Law 115-334-Dec. 20, 2018 Sec. 10113 Hemp Production, hemp is defined as "The plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a Δ^9-THC concentration of not more than 0.3 percent on a dry weight basis." Per TTB's Hemp Policy (https://www.ttb.gov/formulation/hemp-policy) alcohol beverages containing hemp or hemp components should have no detectable levels of THC.
Upon dilution of the raw hemp material, or processed extract, into a finished alcoholic beverage, the concentration of Δ^9-THC and other major cannabinoids will be on the order of parts-per-million (ppm). Therefore, the focus of this method is to quantify the major cannabinoids in the low-ppm range and can be extended to higher concentrations by further sample dilution.

Levels and Limitations

Analyte	Method Detection Limit	Method Quantitation Limit	Instrumental Linear Range
Δ^9-THC	0.07 ng/mL	0.21 ng/mL	0.5 – 100 ng/mL
Δ^8-THC	0.06 ng/mL	0.17 ng/mL	0.5 – 100 ng/mL
THCA	0.09 ng/mL	0.27 ng/mL	0.5 – 100 ng/mL
CBD	0.07 ng/mL	0.20 ng/mL	0.5 – 100 ng/mL
CBDA	0.06 ng/mL	0.19 ng/mL	0.5 – 100 ng/mL
CBN	0.06 ng/mL	0.17 ng/mL	0.5 – 100 ng/mL

Note: The densities of the samples of interest are roughly 1.0 g/mL. Therefore, the units of µg/mL (or ng/mL) and ppm (or pbb) may be considered interchangeable.

Equipment

Glassware and Supplies:

Graduated cylinders as needed
Class A volumetric flasks as needed
Positive-displacement auto pipettors capable of delivering 50 µL to 5000 µL
15 mL conical centrifuge tubes with screw cap closure
2 mL microcentrifuge tubes
2 mL autosampler vials with split-top caps
1 L amber solvent bottle (for aqueous mobile phase)

Instrumentation:

Sciex Exion UHPLC (or equivalent)
Sciex 5500+ QTrap triple quadrupole mass spectrometer (or equivalent)

Instrument Parameters (LC):

Column:	Phenomenex Kinetex C18 2.7 µm, 2.1 × 100 mm
Guard:	Phenomenex SecurityGuard ULTRA UHPLC C18, 2.1 mm cartridge
Column temperature:	50 °C
Injection volume:	5 µL
Wash Parameters:	500 µL
Flow rate:	450 µL/min
Mobile phases:	A: 0.1% formic acid in water B: 0.1% formic acid in methanol
Gradient:	0.0 min: 75% B 4.5 min: 75% B 7.5 min: 100% B 8.5 min: 100% B 9.0 min: 75% B 10.0 min: 75% B

Instrument Parameters (Ionization Source):

Ionization mode:	Electrospray (ESI) with pos/neg switching
Ion Spray voltage (IS):	5500 V (-4500 V)
Source temperature (TEM):	550 °C
Curtain gas (CUR):	30
Gas 1 (GS1):	55
Gas 2 (GS2):	55
Collision Gas (CAD):	8 (Medium)

Instrument Parameters (MS/MS):

Analyte	Retention Time (min)	Precursor Ion (m/z)	Product Ions (m/z)	Collision Energy (V)	Declustering Potential (V)
CBD	2.4	315.1	193.1 [a] 259.1 [b]	29 25	114 114
CBDA*	2.9	357.1	339.1 [a] 245.1 [b]	-30 -42	-30 -30
CBN	3.7	311.1	223.1 [a] 195.1 [b]	29 37	126 126
Δ^9-THC-d3 (IS)	4.45	318.1	196.0[a] 123.1[b]	33 45	130 130
Δ^9-THC	4.6	315.1	193.1 [a] 123.1 [b]	31 43	131 131
Δ^8-THC	5.0	315.1	193.1 [a] 123.1 [b]	31 43	131 131
THCA*	6.9	357.1	313.1 [a] 245.1 [b]	-34 -42	-60 -60

*Negative ion mode. [a]Primary transitions. [b]Secondary transitions.

Entrance potential (EP) globally set to 10 V (-10 V). Cell exit potential (CXP) globally set to 12 V (-12 V). Target cycle time set to 1 s for all (+ and -) MRM experiments.

Reagent and Sample Preparation and Handling

(Vendors and product numbers listed are for convenience. Equivalent products may be used.)

Methanol, Optima LC-MS grade, CAS No. 67-56-1
Water, Optima LC-MS grade or 18 Ω, CAS No. 7732-18-5
Formic acid, LC-MS grade, CAS No. 64-18-6

Cerilliant, 2 mL ampoules containing >1 mL @ 1 mg/mL in methanol or acetonitrile:
Δ^9-Tetrahydrocannabinol (Δ^9-THC, 98.1%)
CAS No. 1972-08-3, Cerilliant No. T-005
Δ^9-Tetrahydrocannabinolic acid (THCA, 97.7%)
CAS No. 23978-85-0, Cerilliant No. T-093
Cannabidiol (CBD, 99.5%)
CAS No. 13956-29-1, Cerilliant No. C-045
Cannabidiolic acid (CBDA, 98.3%)
CAS No. 1244-58-2, Cerilliant No. C-144
Cannabinol (CBN, 99.4%)
CAS No. 521-35-7, Cerilliant No. C-046
Δ^8-Tetrahydrocannabinol (Δ^8-THC, 99.2%)
CAS No. 5957-75-5, Cerilliant No. T-032
Deuterated Δ^9-THC (Δ^9-THC-d3, 98.8%)
CAS No. 81586-39-2, Cerilliant No. T-011

Restek, 2 mL ampoules containing >1 mL @ 1 mg/mL in methanol or acetonitrile (2nd source):

Δ^9-Tetrahydrocannabinol (Δ^9-THC, 98%)
CAS No. 1972-08-3, Restek No. 34067

Cannabidiol (CBD, 99%)
CAS No. 13956-29-1, Restek No. 34011

Preparation of Solutions:

1) Mobile phases (stable for 3 months at room temperature)
   a. Mobile phase A: 0.1% formic acid in water (1 L)
      i. To a 1 L graduated cylinder add approximately 250 mL water
      ii. Add 1.0 mL formic acid and gently swirl to mix
      iii. Add water to the 1 L mark
      iv. Decant into a LC solvent bottle and swirl (amber glass recommended)
   b. Mobile phase B: 0.1% formic acid in methanol (1 L)
      i. To a 1 L graduated cylinder add approximately 250 mL methanol
      ii. Add 1.0 mL formic acid and gently swirl to mix
      iii. Add methanol to the 1 L mark
      iv. Decant into a LC solvent bottle and swirl
2) Seal/needle washes (stable for 6 months at room temperature)
   a. Seal/weak needle wash: 20% methanol (1 L)
      i. To a 1 L graduated cylinder add 200 mL methanol
      ii. Add water to the 1 L mark
      iii. Decant into a LC solvent bottle and swirl
   b. Strong needle wash: 100% methanol (1 L)
      i. Add methanol directly into a LC solvent bottle
3) Extraction solvents (stable for 6 months at room temperature)
   a. Extraction solvent 1: 1.0% formic acid in 75% methanol (100 mL)
      i. To a 100 mL graduated cylinder add 25 mL water
      ii. Add 1.0 mL formic acid and gently swirl to mix
      iii. Add methanol to the 100 mL mark
      iv. Decant into a glass solvent bottle and swirl
   b. Extraction solvent 2: 0.1% formic acid in 75% methanol (100 mL)
      i. To a 100 mL graduated cylinder add 25 mL water
      ii. Add 100 µL formic acid and gently swirl to mix
      iii. Add methanol to the 100 mL mark
      iv. Decant into a glass solvent bottle and swirl

Preparation of Standards:

1) Stock standard mixes (stable for 12 months, stored at -20 °C)

   a. Positive mode stock standard mix containing 100 µg/mL each of Δ^9-THC, Δ^8-THC, CBD, and CBN (10 mL) in methanol.
      i. Transfer entire contents from the individual standard ampules (Cerilliant) to 2 mL microcentrifuge tubes using disposable pipettes

ii. Transfer 1 mL of each standard to a 10 mL volumetric flask using an auto pipette
iii. Fill to the line of the flask with methanol
iv. Shake and transfer to a 15 mL centrifuge tube

b. Negative mode stock standard mix containing 100 µg/mL each of THCA and CBDA (10 mL) in acetonitrile.
i. Transfer entire contents from the individual standard ampules (Cerilliant) to 2 mL microcentrifuge tubes using disposable pipettes
ii. Transfer 1 mL of each standard to a 10 mL volumetric flask using an auto pipette
iii. Fill to the line of the flask with acetonitrile
iv. Shake and transfer to a 15 mL centrifuge tube

c. Stock internal standard containing 100 µg/mL of Δ^9-THC-d3 (10 mL) in methanol.
i. Transfer entire contents from the ampule (Cerilliant) to a 2 mL microcentrifuge tube using a disposable pipette
ii. Transfer 1 mL of the standard to a 10 mL volumetric flask using an auto pipette
iii. Fill to the line of the flask with methanol
iv. Shake and transfer to a 15 mL centrifuge tube

2) Intermediate stock standard mixes (stable for 12 months, stored at -20 °C)

a. Positive mode intermediate stock standard mix containing 20 µg/mL of Δ^9-THC, Δ^8-THC, CBD, and CBN (10 mL) in methanol.
i. Transfer 2 mL of positive mode stock standard mix (100 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube

b. Negative mode intermediate stock standard mix containing 20 µg/mL of THCA and CBDA (10 mL) in acetonitrile.
i. Transfer 2 mL of negative mode stock standard mix (100 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with acetonitrile
iii. Shake and transfer to a 15 mL centrifuge tube

c. Positive mode intermediate stock standard mix containing 200 ng/mL of Δ^9-THC, Δ^8-THC, CBD, and CBN (10 mL) in methanol.
i. Transfer 100 µL of the positive mode intermediate stock standard mix (20 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube

d. Negative mode intermediate stock standard mix containing 200 ng/mL of THCA and CBDA (10 mL) in acetonitrile.

i. Transfer 100 µL of the negative mode intermediate stock standard mix (20 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with acetonitrile
iii. Shake and transfer to a 15 mL centrifuge tube

e. Intermediate stock internal standard containing 1 µg/mL of Δ^9-THC-d3 (10 mL) in methanol.
i. Transfer 100 µL of stock internal standard (100 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube

3) Positive mode working standard mixes containing Δ^9-THC, Δ^8-THC , CBD, CBN, and Δ^9-THC-d3 in methanol (stable for 6 months, stored at -20 °C)
a. 10X Calibration level 6 (1 µg/mL, 10 mL)
i. Transfer 500 µL of the positive mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube
b. 10X Calibration level 5 (500 ng/mL, 10 mL)
i. Transfer 250 µL of the positive mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube
c. 10X Calibration level 4 (100 ng/mL, 10 mL)
i. Transfer 50 µL of the positive mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube
d. 10X Calibration level 3 (50 ng/mL, 10 mL)
i. Transfer 2.5 mL of the positive mode intermediate stock standard mix (200 ng/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube
e. 10X Calibration level 2 (10 ng/mL, 10 mL)
i. Transfer 500 µL of the positive mode intermediate stock standard mix (200 ng/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol
iii. Shake and transfer to a 15 mL centrifuge tube
f. 10X Calibration level 1 (5 ng/mL, 10 mL)
i. Transfer 250 µL of the positive mode intermediate stock standard mix (200 ng/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
ii. Fill to the line of the flask with methanol

iii. Shake and transfer to a 15 mL centrifuge tube

4) Negative mode working standard mixes containing THCA, CBDA, and Δ^9-THC-d3 in acetonitrile (stable for 6 months, stored at -20 °C)
   a. 10X Calibration level 6 (1 µg/mL, 10 mL)
      i. Transfer 500 µL of the negative mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube
   b. 10X Calibration level 5 (500 ng/mL, 10 mL)
      i. Transfer 250 µL of the negative mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube
   c. 10X Calibration level 4 (100 ng/mL, 10 mL)
      i. Transfer 50 µL of the negative mode intermediate stock standard mix (20 µg/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube
   d. 10X Calibration level 3 (50 ng/mL, 10 mL)
      i. Transfer 2.5 mL of the negative mode intermediate stock standard mix (200 ng/mL) and 1 mL of the intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube
   e. 10X Calibration level 2 (10 ng/mL, 10 mL)
      i. Transfer 500 µL of the negative mode intermediate stock standard mix (200 ng/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube
   f. 10X Calibration level 1 (5 ng/mL, 10 mL)
      i. Transfer 250 µL of the negative mode intermediate stock standard mix (200 ng/mL) and 1 mL of intermediate stock internal standard (1 µg/mL) to a 10 mL volumetric flask using an auto pipette
      ii. Fill to the line of the flask with acetonitrile
      iii. Shake and transfer to a 15 mL centrifuge tube

5) Positive mode calibration standard mixes containing Δ^9-THC, Δ^8-THC , CBD, CBN, and Δ^9-THC-d3 in mobile phase (stable for 24 hours at ambient temperature)
   a. For 1 mL at each calibration level of the positive mode working standard mixes:
      i. Transfer 100 µL of the working standard mix to an autosampler vial
      ii. Add 900 µL of 0.1% formic acid in 75% methanol
      iii. Cap and shake

6) Negative mode calibration standard mixes containing THCA, CBDA, and Δ^9-THC-d3 in mobile phase (stable for 24 hours at ambient temperature)
   a. For 1 mL at each calibration level of the negative mode working standard mixes:
      i. Transfer 100 µL of the working standard mix to an autosampler vial
      ii. Add 900 µL of 0.1% formic acid in 75% methanol
      iii. Cap and shake

Preparation of Laboratory Control Sample (LCS):

1) Transfer 4.85 mL of distilled spirit LCS (e.g. 40% tequila) to a 15 mL centrifuge tube
2) Add 50 µL of the positive mode stock standard mix containing 100 µg/mL each Δ^9-THC, Δ^8-THC, CBD, and CBN in methanol.
3) Add 50 µL of the negative mode stock standard mix containing 100 µg/mL each THCA and CBDA in acetonitrile.
4) Add 50 µL of stock I.S. solution containing 100 µg/mL Δ^9-THC-d3 in methanol
5) Cap and shake
6) Transfer 100 µL of spiked sample prepared in steps 1-3 to a 2 mL microcentrifuge tube
7) Add 900 µL of 1.0% formic acid in 75% methanol (Extraction Solvent 1)
8) Cap and shake
9) Transfer 100 µL of diluted spiked sample to an autosampler vial
10) Add 900 µL of 0.1% formic acid in 75% methanol (Extraction Solvent 2)
11) Cap and shake
12) Repeat steps 5 – 10 to prepare the 2nd LCS sample

Preparation of 2nd Source Sample (QC):

1) Prepare the QC stock standard mix containing 100 µg/mL each of Δ^9-THC and CBD (Restek) in the same manner as stated above (10 mL).
2) Prepare the QC intermediate stock standard mix containing 20 µg/mL each of Δ^9-THC and CBD in the same manner as stated above (10 mL).
3) Prepare the QC working standard mix containing 100 ng/mL each of Δ^9-THC and CBD in the same manner as stated above (10 mL).
4) Prepare the QC sample by transferring 100 µL of QC working standard mix to an autosampler vial, adding 900 µL of 0.1% formic acid in 75% methanol (Extraction Solvent 2), capping, and shaking.

Preparation of Samples:

1) Determine the density of the sample using a densitometer
2) Transfer 5 mL of sample using to a 15 mL centrifuge tube
3) Add 50 µL of stock I.S. solution (100 µg/mL Δ^9-THC-d3 in methanol)
4) Cap and shake
5) Transfer 100 µL of I.S.-spiked sample prepared in steps 1-3 to a 2 mL microcentrifuge tube

6) Add 900 µL of 1.0% formic acid in 75% methanol (Extraction Solvent 1)
7) Cap and shake
8) Transfer 100 µL of diluted spiked sample to an autosampler vial
9) Add 900 µL of 0.1% formic acid in 75% methanol (Extraction Solvent 2)
10) Cap and shake

Samples should be prepared and analyzed on the same day (within 24 hours).

Procedures

1) Prepare fresh calibration standards from working standards as described in the Preparation of Standards section above.
2) Prepare the samples as described in the Preparation of Samples section above.
3) Prime the LC equilibrate the system to the initial instrument method conditions.
4) Inject blanks, calibrants, samples, and QC using the following recommended sequence template:
   - Solvent blank
   - Positive mode calibration levels 1 – 6
   - Negative mode calibration levels 1 – 6
   - Solvent blank
   - Two LCS
   - Solvent blank
   - QC check (2nd source sample)
   - Solvent blank
   - Sample(s)
   - Solvent blank
   - QC check (2nd source sample)
   - Solvent blank
5) Shut down system and properly store LC column.
6) Process sequence in SciexOS Analytics using processing method.
7) Report results as described in the Reporting Results section below.

Quality Control

1) The correlation coefficient (R^2) for all calibration curves is to be ≥ 0.995. If R^2 is < 0.995, prepare fresh calibration standard mixes and re-run injections.
2) Run 2 LCS samples for accuracy and precision. The values for accuracy and precision are to be within the prescribed limits for both Δ^9-THC and CBD. If the values are outside the prescribed limits, samples are to be prepared fresh and re-run.
3) Run a 2nd source check standard (QC) at least once every 8 samples (including LCS). The QC check is to be 1.0 ± 0.15 ppm for both Δ^9-THC and CBD. If the QC check is not within the prescribed limit, all standards/samples run prior to the QC check are to be re-run.

Sources of Uncertainty

- Pipetting errors
- Impure and/or contaminated standards or reagents
- Matrix interferences (overlapping signal with analyte)

Calculations

$$Actual\ Conc.(ppm) = \frac{Calc.Conc.(ng/mL)}{Sample\ Density\ (g/mL)} \times \frac{Dilution\ Factor}{1000}$$

Reporting Results

Report the results as follows:

Analytes are calculated in ng/mL and reported in ppm (*XX.xx* or *X.xx*).

If calc. conc. is <0.1 ng/mL, result is reported as "Not Detected".
If calc. conc. is ≥0.1 ng/mL and <0.5 ng/mL, result is reported as "Below Quantitation Limit".
If calc. conc. is ≥0.5 ng/mL, result is reported as "*XX.xx* or *X.xx* ppm".
If calc. conc. is >100 ng/mL, analyst must re-analyze with larger dilution factor.

Safety Notes

Anticipated waste volume for each sample preparation and UPLC run is approximately 15 mL consisting primarily of aqueous organic solvent (methanol and/or ethanol with water). Will possibly contain up to percent levels of some cannabinoids.

Required Training, Certification and Re-certification

1) Receive in-house LC-MS/MS training.
2) Initial certification is achieved by running 3 LCS replicates with results of precision and accuracy in agreement with the results of the validation package.
3) Periodically, analysts are retested for competency (e.g. every 5 years) and/or given proficiency test.

Revision History

Rev. 1 –Initial Revision
Rev. 2 – Addition of Δ^8-THC as an analyte and Δ^9-THC-d3 as the internal standard