CHE 10-20 The Quantitation of THC in Plant Material by HPLC-UV-DAD

A. Purpose

1. This procedure specifies the required elements for delta-9-tetrahydrocannibinol ((-)-Delta-9-THC) extraction and concentration determination (0.10-10%) from questioned plant materials after THC identification has been performed per DOM's CHE 10-07 and 10-13.

B. Scope

1. (-)-Delta-9-THC and 12 other cannabinoids are extracted from plant material by solvent extraction. Cannabinoids are detectable at 230 nm on an HPLC-UV-DAD. The ratio of sample peak area to internal standard peak area is compared to a calibration curve to provide the (-)-Delta-9-THC quantitation in the sample.

Cannabidivarin (CBDV)	(-)-Delta-9-tetrahydrocannibinol (THC)
Tetrahydrocannabivarol (THCV)	(-)-Delta-8-tetrahydrocannibinol
Cannabidiol (CBD)	Cannabichromene (CBC)
Cannabigerol (CBG)	Cannabinolic Acid (CBNA)
Cannabidiolic Acid (CBDA)	Delta-9-tetrahydrocannabinoic acid (THCA)
Cannabigerolic (CBGA)	Cannabichromenic Acid (CBCA)
Cannabinol (CBN)	Androstenedione (Internal Standard)

Specimen Criteria

1. Minimum starting weight of evidence for quantitation shall be ¼ ounce (7 g) of plant material. The minimum starting weight may also be evaluated on a case by case basis.

C. Equipment

1. Agilent HPLC-UV-DAD
2. Agilent Open Lab Software
3. Analytical balance
4. Rotator
5. 16 x 125 mm glass screw top round-bottom tubes and caps (or similar)
6. Volumetric flasks (5 mL, 10 mL, 100 mL, 1L)
7. Graduated cylinders
8. Autosampler vials and caps
9. 2-20 µL, 20-200 µL, 100-1000 µL adjustable pipettes
10. Repeater pipette
11. Disposable transfer pipettes
12. 0.45 µm syringe filters

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13. Syringe
14. Oven
15. Thermometer

D. Chemicals, Reagents, Solvents, Standards

1. Internal Standard: 4-Androsten-3,17-dione (Androstenedione)
2. Standards: 13 cannabinoids as stated in scope
   a. (-)-Delta-9-THC
      i. 100 mg/mL for calibrators (Lipomed or similar)
      ii. 50 mg/mL for controls (Lipomed or similar)
3. Control
   a. THC plant analyzed by another Laboratory (Department of Agriculture or similar) as a process control.
4. Methanol (HPLC grade or higher)
5. Ultra-Pure Water
6. Formic acid (HPLC >99.0%)

E. Safety/PPE

1. Gloves
2. Protective eye wear
3. Lab Coat
4. Depending on the chemical being used the chemist may want to perform their procedure in the hood.

F. Procedure

Internal Standard Working Stock Preparation

Drug	External Provider Ampule [ ]	Internal Standard Working Stock 100 µg/mL	Spike into casework
Androstenedione	Powder	500 mg-QS to 100 ml MEOH in 100 ml Volumetric Flask **Final [ ] 5 mg/mL or 5000 µg/mL**	250 µL of IS Working Stock into each sample **Final [ ]** **250 µL of 5000 µg/mL into 5 mL** **250 µg/mL**

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Calibrator Working Stock Preparation

Drug	External Provider Ampule [ ]	Calibrator Working Stock 5000 µg/mL
(-)-Delta-9-THC	100 mg/mL Lipomed or similar	500 µL of 100 mg/mL Working Stock QS to 10 mL MEOH in 10 mL Volumetric Flask Take 250 µL of 100 mg/mL Working Stock QS to 5 mL MEOH in 5 mL Volumetric Flask Combine to have 15 mLs total **Final [ ] 5000 µg/mL**

Control Working Stock Preparation

Drug	External Provider Ampule [ ]	Control Working Stock 2500 µg/mL
(-)-Delta-9-THC	50 mg/mL Lipomed or similar	500 µL of 50 mg/mL Working Stock Mix - QS to 10 mL MEOH in 10 mL Volumetric Flask Take 250 µL of 50 mg/mL Working Stock Mix - QS to 5 mL MEOH in 5 mL Volumetric Flask **Final [ ] 2500 µg/mL**

1. All Working Stocks shall be stored at a freezer temperature.
2. Expiration: 1-year from date of preparation.

13 Cannabinoid Mixture Solution & THC/THCA Mixture Solution

1. 100 µL of each 1 mg/ml solution (13 drug solution) into an autosampler vial, add 250 µL of internal standard solution.
   a. Maintain at freezer temperatures.
   b. Valid for 2 years as qualitative standard or until degradation is seen.

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Calibrators and Controls - (-)-Delta-9-THC
To be created with each analytical batch

Calibrator	µg/mL	mg/ml	250 mg sample % THC	25 mg sample % THC	µL of 5000 µg/mL Solution	Volume of MeOH (µL)
1	50	0.05	0.10%	1.00%	50	4950
2	75	0.075	0.15%	1.50%	75	4925
3	100	0.1	0.20%	2.00%	100	4900
4	200	0.2	0.40%	4.00%	200	4800
5	400	0.4	0.80%	8.00%	400	4600
6	500	0.5	1.00%	10%	500	4500

Control	µg/mL	mg/ml	250 mg sample % THC	25 mg sample % THC	µL of 2500 µg/ml Solution	Volume of MeOH (µL)
1	80	0.080	0.16%	1.60%	160	4840
2	150	0.150	0.30%	3.00%	300	4700
3	325	0.325	0.65%	6.50%	650	4350

Reagent Preparation

1. Aqueous Mobile Phase (0.1% V/V Formic Acid in Water).
   a. 1 mL of Formic Acid in 1 L of Ultra Pure Water.
   b. Store at room temperature.
   c. Expires 2 months after date of preparation.

2. Organic Mobile Phase (0.05% V/V Formic Acid in Methanol).
   a. 500 µL of Formic Acid in 1 L HPLC Grade Methanol.
   b. Store at room temperature.
   c. Expires 6 months after date of preparation.

Casework Documentation

The following information should be documented:

1. Initial weight of the plant material.
2. Weight of the plant material before and after moisture removal procedure.
3. Weights of the ground plant material used in thermo-conversion procedure.
4. Oven temperatures from the moisture removal and thermal conversion procedure.

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Plant Moisture Removal Procedure

1. All plant material (whether assumed to be dry or not) shall be subjected to the plant moisture removal process.
2. Sample approximately 3.5 g of plant material and place on foil and bake at 90°C for 45 minutes. The remaining amount of plant material (non-heated) will be retained for future testing.
3. After 45 minutes, take the plant material out of the oven, manually break up the plant material, and place back into the oven for an additional 45 minutes.
4. Allow the plant material to cool and re-weigh.
   a. If there is more than a 15% change between the first and second weights, the plant material shall be dried at 90°C for an additional hour, then the process of weighting and drying at 90°C for 15 minutes will be repeated until there is less than a 15% change between weights.

Thermal Conversion of THCA to THC Procedure

1. Place the dried plant material in a disposable grinding chamber.
2. Grind the plant material at 25000 rpm until a fine, uniform powder is achieved.
3. Cannabinoid profiling sample: Weigh out either 25 mg or 250 mg (GCMS identification and concentration dependent) of plant material, place in screw cap test tube and proceed to Sample Extraction Procedure #2.
4. THC quantitation samples: Weigh out in duplicate, a target of 25 mg or 250 mg of plant material (GCMS identification and concentration dependent) and place into screw cap test tubes and cap. Record the exact weight from the analytical balance into the worksheet.
5. THC Process Control sample: Weigh out 25 mg or 250 mg (GCMS identification dependent) of ground retained plant material into screw cap test tubes and cap.
6. Heat the test tubes with plant material at 145°C for 30 minutes to force the conversion of THCA to THC.
7. Allow the dried plant material powder to cool.
8. Add 5 mL of Methanol to the test tube and proceed to Sample Extraction Procedure step 2.

Sample Extraction Procedure

1. Accurately prepare aqueous calibrators and controls in screw cap test tubes.
2. With repeater pipette, add 250 µL of internal standard to each test tube including the THC quantitation samples prepared above.
3. Cap all test tubes and rotate at approximately 30 rpm for 15 minutes.
4. Allow them to naturally settle on the countertop for 15 minutes.
5. Transfer approximately 1 mL of sample into an autosampler vial.
   a. For calibrators and positive controls, 1 mL of methanol can be directly transferred into the autosampler vials without the use of the 0.45 µm filter.

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b. For the negative control and all plant samples, use a syringe equipped with a 0.45 µm filter and transfer approximately 1 mL of methanol through the filter directly into an autosampler vial.

6. Load profiling samples onto HPLC-UV-DAD and analyze the samples using **THCQUANT** method for acquisition in the following manner:
   a. Methanol wash(es)
   b. Negative aqueous
   c. 13 component Cannabinoid mixture (non-extracted)
   d. Negative aqueous
   e. Case profile sample
7. Load quantitation samples onto HPLC-UV-DAD and analyze the samples using **THCQUANT** method for acquisition in the following manner:
   a. Methanol wash(es)
   b. Negative aqueous
   c. Calibrators 1, 2, 3, etc
   d. Negative aqueous
   e. 13 component Cannabinoid mixture (non-extracted) (Calibrator 7)
   f. Controls aqueous 1,2,3
   g. Processed control(s)
   h. Negative aqueous
   i. Casework in duplicates (if more than one case is analyzed, must have Negative aqueous between cases)
   j. Negative aqueous
   k. Mid-level calibrator as QC-end
   l. Control 2 as QC-end

Instrumentation:

Agilent 1260 Infinity II LC with Diode Array Detection equipped with 1260 Infinity II Automatic Sampler

Column:

Agilent Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm
Compatible HPLC EC-C18 Guard Column

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Instrument Parameters:

Autosampler Temperature: Ambient

Draw speed: 200 µL/min Eject speed: 400 µL/min

Column Temperature: 50 °C isothermal

Injection Volume: 5.0 µL

Flow Rate: 1 mL/min

Time	% Aqueous	% Organic
0	40	60
1	40	60
7	23	77
8.2	5	95

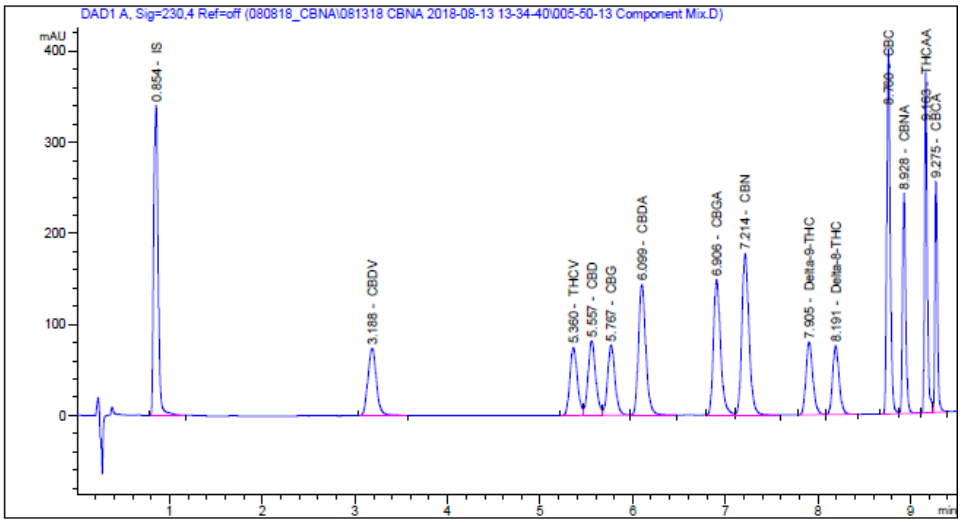
Stop time: 9.50 min

Post time: 1.50 min

Wavelength detector: 230 nm

Peak width > 0.0063 min (0.13 s response time) (40 Hz)

Retention Time order:



Data Analysis

1. Typically, the method shall be calibrated with the calibrator datafiles injected on the instrument from the beginning of the analytical run. However, if necessary, the use of re-injected calibrator datafiles as method calibrators is acceptable.

2. Data Analysis can be conducted with Agilent Chemstation.

Agilent Chemstation

1. Switch to Data Analysis mode. Ensure Recalculate Mode is activated
2. Load your run data files.
3. Select Integration -> Edit Integration Events Table
4. Evaluate the Late Run Baseline Now Time (Approximately 7.6 min) using the 50 µg/mL and 500 µg/mL Calibrators and the 13 component mix.
   a. The time should fall between CBN and delta-9-THC that allows for optimal integration of both the low and high calibrators
5. Save your run as a new master method, ie "101218 THC SMO".
6. Check to make sure the new master method is selected (this should happen automatically).
7. Select the Recalculate with Specific Method icon
   a. Ensure the current method is present in the Method box
   b. Set the Auto step interval to 1 sec
   c. Select As specific in method for the Destination
   d. Click Ok
   e. The software will process the run data
8. When complete, navigate to the Data folder on the C drive, search for .pdf files, and copy and paste pdf's to a folder for analysis.
9. Print your calibration curves (File>Print>Calib table + Curve) to the same folder.
10. Evaluation of data
    a. Ensure that the auto-integration events are correct for the calibrators/controls.
       i. It may be necessary to adjust the integration for case samples.
    b. Evaluate each of the calibrators to ensure that the area counts match the calibration report for both all analytes and the internal standard.
    c. Ensure that the calibration curve for delta-9-THC (r^2) $\geq$0.996
11. At the discretion of the analyst, manual calibration and reprocessing of data is acceptable and may be necessary.
    a. When performing manual calibration, every calibrator must be re-processed.
    b. Double click on the calibrator data file.
    c. Select Calibration -> Recalibrate.
    d. Select the appropriate calibration level.
    e. Choose 'Replace' as the mode.
    f. Repeat the process until all of the calibrator files have been re-processed.
    g. Save the Method as a New Master Method.
    h. Continue from Step 7 above (Recalculate).

12. Any manually integrated files should be identified by the software or analyst.

G. Quality Requirements

1. Calibrators.
   a. A calibration curve shall be derived by comparing the ratios of the calibrator THC peak areas to the internal standard peak area. The ratio of sample peak area to internal standard peak area is compared to the calibration curve to provide a quantitation of THC in the plant sample.
   b. The calibration curve shall be comprised of all six calibrators.
      i. The calibrator regression model shall be linear, non-forced, origin ignored, equal weight and the coefficient of determination (r^2) ≥0.996.
      ii. The calibrator calculated value shall be within ±10% (truncated) of the target value.
      iii. The upper limit of quantitation is the highest calibrator used in the curve.
      iv. All casework greater than this limit should be repeated at a dilution or reported as present greater than the highest calibrator concentration.
      v. A calibration point shall only be removed from the curve for just cause:
         a) Points cannot be removed to improve the accuracy of a control(s).
         b) The lowest and/or middle calibrators shall not be excluded from the calibration curve unless the chromatography is suspect or there is just reason that can be identified in writing.
         c) The highest calibrators may be dropped from the calibration curve if there is an undue influence on the curve causing the low calibrators to be outside the acceptable ±10%.
         d) Chromatography is less than desirable.
         e) Calibrator mis-spike: Determined by the evaluation of the same calibrator from a previous run against this run's curve.
         f) If the removal of a point from the curve changes the high or low reporting limits, the change shall be documented and the range must be adjusted accordingly.
2. Controls.
   a. The retention time of the analytes shall be within ±5% of the retention time of the calibrators.
   b. The positive controls shall fall within ±20% (truncated) of the target value.
   c. The negative control shall be void of all drugs of interest for that method, no greater than 10% of the signal of the lowest calibrator.
   d. The negative control should be analyzed at the beginning of each analytical batch, after the highest calibrator (to provide evidence of no carryover), between case sets of duplicates, and at the completion of the case samples.
3. Case Samples.

a. The retention time of the analytes shall be within ±5% of the retention time of the calibrators.
b. The case duplicate results shall be within 15% of each other, otherwise the samples must be repeated with new re-weighed material and have to begin at the Thermal Conversion Procedure.
   i. Also must include the positive plant control and a negative plant control.
   ii. If the duplicates samples (25 mg sample size) quantitate greater than 500 µg/ml each, results will be reported as >10% THC regardless if duplicate results are within 20%.

H. Interpretations/Opinions/Report Wording Guidelines

1. The case sample duplicate results shall be entered into the controlled %THC Reporting template (excel chart), converted from µg/mL to %THC, averaged, and truncated as shown below:
   a. Results 0.10 to 9.99%, reported to the hundredths place.
   b. Result of 10%, reported as a whole number.
2. The miscellaneous cannabinoids detected in the profile sample will not be reported.
3. All weights and concentrations in this procedure for calibration curves, controls, and samples are targets and will be adjusted for the actual weight or concentration of the prepared calibration curve, control, or sample.
4. Reporting
   a. Measurement uncertainty shall be reported as a relative percentage (rounded), to the same number of decimal places as the result, at a confidence interval of 95%.
      i. Example: UoM for THC quant is 8.31%. THC quantitative result of 0.32%. Report THC 0.32% (±0.03%).
      ii. Example: UoM for THC quant is 8.31%. THC quantitative result of 5.55%. Report THC 5.55 % (±0.46%).
   b. Results are reported as tetrahydrocannibinols per CRS 18-18-102 and CRS 35-61-101.
      i. Tetrahyrocannibinols includes the thermal conversion of delta-9-THC and THCA.
   c. Reporting statements
      i. Analysis identified tetrahydrocannabinols by GC-MS weighing X (±X). See report dated X.
      ii. For results of less than 0.10% THC:
         a) Quantitative analysis of the plant material for Tetrahydrocannabinols by HPLC-UV-DAD was performed but was not detected above the quantitative reporting limit.
      iii. For results of 0.10 – 10% THC:
         a) Quantitative analysis of the plant material by HPLC-UV-DAD determined a concentration of X% (±X%) tetrahydrocannibinols.
      iv. For results greater than 10% THC:

a) Quantitative analysis of the plant material by HPLC-UV-DAD determined a concentration of greater than 10% tetrahydrocannabinols.

v. Quantitative analysis by HPLC-UV-DAD was performed on dried, homogenized plant material. The quantitative reporting limit for this method is 0.10% delta-9-tetrahydrocannabinols. Tetrahydrocannabinols includes the thermal conversion delta-9-tetrahydrocannibinols and delta-9-tetrahydrocannabinolic acid (THCA).

I. Limitations

1. Lower quantitation limit = Lowest Calibrator.
   a. Calibration Curve using either 25 or 250 mg as a sample size: 50 µg/mL = 0.10% THC or 1.00% THC.
2. Upper quantitation limit = Highest Calibrator.
   a. Calibration Curve using either 25 or 250 mg as a sample size: 500 µg/mL = 1.00% THC or 10% THC.

J. References

1. Agilent Dedicated Cannabinoid Potency Testing Using the Agilent 1220 Infinity II LC System, August 2018.
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K. Additional Factors

None