## Abbreviations

| | |
|---|---|
| ACN | Acetonitrile |
| C of A | Certificate of Analysis |
| DAD | Diode Array Detector |
| DI | Deionized, a type of water |
| HPLC | High Performance Liquid Chromatograph |
| IPA | Isopropanol, Isopropyl alcohol, 2-propanol |
| THC/d9-THC | delta-9-tetrahydrocannabinol |
| THCA | delta-9-tetrahydrocannabinolic acid |
| D8-THC | delta-8-tetrahydrocannabinol, an isomer of delta-9-tetrahydrocannabinol |
| S/N | signal-to-noise ratio |
| mAU | milliAbsorbance Units |
| mL | Milliliter |
| MeOH | Methanol |
| PTFE | Polytetrafluoroethylene |
| RC | Regenerated cellulose |
| RPD | Relative Percent Difference: (Difference of 2 results)/Average of 2 results*100 |
| uL | Microliter |
| ug | Microgram |
| UV | Ultraviolet |

## Introduction

The Alaska Statutes list marijuana as a Schedule VI controlled substance. Marijuana is understood to mean *Cannabis sativa L.* have greater than 0.3% THC on a dry weight basis based on the following definition of industrial hemp.

In the Alaska Statutes 2020, Sec. 03.05.100. Definitions. (5) defines industrial hemp as follows:
(5) "industrial hemp" means all parts and varieties of the plant Cannabis sativa L. containing not more than 0.3 percent delta-9-tetrahydrocannabinol.

Additionally, Sec. 03.05.076. Industrial hemp. (g) states:
(g) Industrial hemp products intended for human consumption may not exceed 0.3 percent delta-9-tetrahydrocannabinol.

Finally, Sec. 11.71.900. Definitions. (3) provides the following:
(3) "cannabidiol oil" means the viscous liquid concentrate of cannabidiol extracted from the plant (genus) Cannabis containing not more than 0.3 percent delta-9-tetrahydrocannabinol

To adequately characterize *Cannabis* plant material and cannabinoid concentrates as controlled or non-controlled materials, it is imperative to know the concentration of delta-9-THC in that material. Furthermore, fresh *Cannabis* plant material contains mostly THCA which, when converted to THC via heating or chemical degradation, contributes to the psychoactive effect experienced by the user. For these reasons, this method was implemented to quantitate both THC and THCA in *Cannabis* plant material and cannabinoid concentrates, as well as to determine the Total THC content where applicable.

The method for this testing is high performance liquid chromatography using a diode array detector (HPLC-DAD). The method validation report is located in the laboratory's SharePoint document library.

## Equipment and Instrumentation

### Analytical Balance and Deionizer

A Mettler-Toledo analytical balance (4 decimal places) is available for sampling weighing. A Haug deionizer with U-shaped electrode is provided for static control during weighing operations.

### HPLC-DAD

An Agilent 1260 High Performance Liquid Chromatograph (HPLC) with a quaternary pump, heated column compartment, and Diode Array Detector (DAD) is used for this analysis. The instrument is operated with an attached computer running OpenLab CDS ChemStation Edition to calibrate and analyze unknown case samples using a programmed injection sequence. The current approved HPLC-DAD method as well as any archived methods are stored in the laboratory's SharePoint document library.

Instrument maintenance is recorded in the maintenance log which is also located in SharePoint.

The following preventative maintenance tasks will be performed at the minimum frequencies listed below. Instrument maintenance may be performed via a service contract.

| Service | Minimum Frequency |
|---|---|
| Replace Purge Valve Frit | Every 6 months |
| Replace Guard Column Cartridge (If using**) | Annually (If using**) |
| Replace Purge Valve Seal Cap | Annually |
| Flush All Degasser Lines with 100% IPA | Annually |
| Delete Data > 1 Year Old | Annually |
| Replace Glass Solvent Filters | Annually |
| Replace Piston Seals, Clean Pistons, Seal Wear-In | Every 2 Years |
| Replace Column | As Needed |
| Replace UV Lamp, Wavelength Calibration | As Needed |

**If using a guard column, check that the diameter and particle size match that of the analytical column.

Other maintenance and repair functions may be performed as needed. All maintenance is documented in the maintenance log stored in the laboratory's SharePoint document library.

Electronic copies of the manufacturer's manuals for the HPLC are in the laboratory's SharePoint document library including instructions on instrument maintenance tasks.

### Calibrated Pipettes and Volumetric Glassware

Sample, calibration standard, and control standard dilutions are performed using calibrated microliter pipettes and Class A volumetric glassware including flasks and graduated cylinders.

### Calibrated Pipette Care

Good technique will avoid many potential issues when using calibrated pipettes.

1. After drawing up liquid into a tip, always eject the tip before laying the pipette down.
2. Dial down to the desired volume and lock the volume adjustment before pipetting.
3. Maintain the pipette in a near-vertical position while aspirating.
4. Submerge the pipette tip only as much as needed to avoid bubbles.
5. Draw up solutions slowly to avoid bubbles.
6. If the source container is very narrow, transfer liquid to a secondary container before pipetting.
7. Depress the plunger to the second stop point to ensure complete transfer of the liquid.
8. Store pipettes and tips in a safe location (e.g., drawer) to avoid drops or contamination.
9. If a pipette becomes contaminated, consult the manufacturer for cleaning instructions.

### Pipette Calibration

Calibrated pipettes are recalibrated annually by an external provider. Pipette calibration requirements are communicated to the entity responsible for calibration. Pipette calibration certificates are stored in the laboratory SharePoint document library.

### Volumetric Glassware Cleaning

Cannabinoids are more non-polar than many drugs; however, methanol generally does a sufficient job at removing them from laboratory glassware. Dispose of expired calibrator and control solutions in solvent waste containers.

Rinse volumetric glassware (including caps) thoroughly with methanol (ACS-grade is OK) followed by deionized water followed by HPLC-grade methanol. Place uncapped volumetric glassware in a safe location for drying.

**Note:** Do not use ovens or strong heat sources to dry volumetric glassware. If moisture remains inside before the next use, rinse the glassware with a small amount of the intended solvent (e.g., HPLC-grade methanol) and dispose in the solvent waste. Ensure volumetric glassware is completely dry if used as a weighing container.

## Deionized Water System

A Millipore Direct-Q 3UV-R laboratory water system produces high-purity, Type 1 water for mobile phase preparation and cleaning. Water system maintenance is recorded in the system maintenance log which is located in SharePoint.

## Homogenizer

A SPEX SamplePrep 1600 MiniG homogenizer is available for homogenization of plant material samples. 50 mL conical polypropylene tubes with caps and ceramic milling beads are used for this task. Homogenizer maintenance is recorded in the maintenance log which is located in SharePoint.

## Centrifuge

An Eppendorf 5702 centrifuge (4400 rpm max) is provided for particulate removal from samples and matrix-matched controls.

### Orbital Shaker and Vortexer

An orbital shaker and vial tray are used for sample agitation during solvent extraction. A standard laboratory vortexer is used for mixing, where needed.

### HPLC Instrument Parameters (THCQuant.M Method)

| Parameter Name | Value | | |
|---|---|---|---|
| HPLC Column | Agilent InfinityLab Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm HPLC column | | |
| Mobile Phases | A) 0.1% (v/v) Formic acid in deionized water<br>B) 0.05% (v/v) Formic acid in HPLC-grade methanol | | |
| Flow Rate | 1.0 mL/min | | |
| Maximum Pressure | 600 bar | | |
| Run Time | 9.2 minutes | | |
| Post Run | 1.5 minutes | | |
| Column Temperature | 50°C (Isothermal) | | |
| Injection Volume | 5.0 uL | | |
| Needle Wash | Methanol, Wash vial, 1 cycle | | |
| Autosampler Temperature | Ambient | | |
| Peak Width (method) | >0.0063 minutes (0.13 seconds response time) (40 Hz) | | |
| Diode Array Detector (DAD) | 230 nm (4 nm bandwidth)<br>Reference 360 nm (100 nm bandwidth) | | |
| Mobile Phase Gradient | Time (min) | %A (Aqueous) | %B (Organic) |
| | 0.0 | 40 | 60 |
| | 1.0 | 40 | 60 |
| | 7.0 | 23 | 77 |
| | 8.2 | 5 | 95 |

### HPLC Integration Parameters (Default)

The OpenLab CDS ChemStation software will integrate peaks based on the integration parameters set in the method. The default integration parameters determined during method validation are:

| Parameter Name | Value | Time (min) |
|---|---|---|
| Baseline Correction Type | Classical | Initial |
| Slope Sensitivity | 8.000 | Initial |
| Peak Width | 0.030 | Initial |
| Area Reject | 2.000 | Initial |
| Height Reject | 0.600 | Initial |
| Shoulders | DROP | Initial |
| Area Percent Reject | 0.000 | Initial |
| Integration | OFF | Initial |
| Integration | ON | 1.000 |

Additional manual integration events may be needed on a run-by-run basis to achieve optimal integration. These may include, but are not limited to, Baseline Now, Fixed Peak Width, and Slope Sensitivity.

## Quantitative Standards

### Purchasing

Certified Reference Materials (CRMs) are purchased from reputable manufacturers accredited to ISO 17025 and ISO Guide 34/ISO 17034 to ensure reference material quality. Reference materials used for the preparation of calibration solutions are sourced from a different manufacturer than reference materials used for the preparation of controls. Quantitative cannabinoid standards are treated as Primary Drug Standards according to the Seized Drugs Procedure Manual. The standard Certificate of Analysis (C of A), purchasing information, and verification data will be included with the Drug Standard Control Form in the primary drug standards binders.

### Storage and Expiration

Purchased standards are stored according to the manufacturer storage conditions, where possible. Manufacturer expiration dates supersede laboratory-assigned expiration dates.

Quantitative cannabinoid standards are treated as primary drug standards via the Seized Drugs Procedure Manual. This includes labeling and secure storage until retrieval for use in casework.

### Verification

When a new lot of reference material is purchased, the laboratory will test one vial of the material (duplicate injection) to verify that it contains the expected components at the expected concentrations.

#### Verification of Expected Concentrations

The preferred method of verification of quantitative cannabinoid standards is via HPLC analysis with a routine THC quantitation sequence. This method of verification covers identification of the expected components by retention time comparison to the current lots of standards as well as verification of expected concentration through quantitation like a sample (duplicate injection).

**Note:** Because cannabinoid standards are available at various concentrations, the verifier will determine the appropriate dilution to cause the analytes to be within the calibration range (0.5 to 50.0 ug/mL). This dilution will be used to back-calculate from the average measured concentration to the original solution concentrations. Example:

$$Original\ Concentration\ \left(\frac{ug}{mL}\right) = \ Average\ Measured\ Concentration\ \left(\frac{ug}{mL}\right) * Dilution\ Factor$$

The calculated original solution concentrations for THC and/or THCA must be within +/- 5% of the stated concentration(s) from the C of A. A record of this calculation will be maintained with the verification data.

| [Example] Value from C of A | [Example] Range of acceptable calculated values |
|---|---|
| 1000 ug/mL THC | 950 to 1050 ug/mL THC |
| 1000 ug/mL THCA | 950 to 1050 ug/mL THCA |

If the calculated concentration is not withing +/- 5% of the stated value from the C of A, contact the Technical Lead and/or standard manufacturer.

### Verification of Expected Components

Qualitative verification of the expected components may be achieved by HPLC analysis (Verification of Expected Concentrations) or via GC/MS. Verification of thermally-labile components (e.g., THCA) may not be performed using GC.

### Documentation of Verification

When a cannabinoid standard has been verified, the relevant verification data will be stored with the Drug Standard Control Form. If a dilution was performed, the analyst performing the verification will add a record of the dilution and subsequent calculations to the verification paperwork along with their initials and date. Standard verification must be reviewed by a second analyst competent in THC quantitation.

### New Calibration Solutions

When a new lot of the primary calibration standard is put into use, the HPLC master method must be updated with the new analyte concentrations from the C of A. The prior method will have an end date applied and a copy of the new instrument method will be placed in SharePoint with a start date.

The THC Quantitation Control Worksheet, sheet "1. New Cal or Control" will be updated with the new calibrator THC and THCA concentrations from the certificate of analysis. Also update the manufacturer, lot number, and expiration date.

### Digitally Archiving Verification Records

When a calibration or control standard lot is no longer in use, the printed copy of the Drug Standard Control Form with all attachments will be scanned and placed in the laboratory's SharePoint document library. After ensuring that the scanned record is complete and legible, the printed copy will be destroyed.

## Storage and Use of Purchased Chemicals

### Definition and Quality Requirements

Purchased chemicals are used for calibrator and control preparation, sample dilution, reagent preparation, and cleaning. Purchased chemicals for cleaning will be ACS grade or better. Methanol for calibrator and control preparation, sample dilution, and mobile phase preparation will be HPLC-grade or better (low UV absorbance). Solvents that will pass through the HPLC (e.g., CAN, IPA) will also be HPLC-grade or better.

### Storage Conditions and Expiration Dates

Chemical manufacturers specify the storage conditions of chemicals. Chemicals should be stored according to the manufacturer storage conditions including consideration of any chemical hazards. Refer to the Health and Safety Manual for more information.

**Chemicals stored in the solvent cabinet of the HPLC are at a higher risk of spillage than those stored in cabinets due to elevation above the counter.** Ensure solvent bottles in the HPLC solvent cabinet are tightly capped when possible. Bottles that are not in use should be removed from the solvent cabinet and stored properly.

Chemical manufacturers may print expiration or retest dates on chemical containers. A chemical should not be used for casework beyond the manufacturer expiration date. Additionally, reagents may not have an expiration date later than that of the earliest component expiration date.

Example: If a reagent is prepared that typically has a 1-month expiration using a solvent that expires in 10 days, the prepared reagent must also expire in 10 days.

When a chemical expires or reaches the retest date, the chemical manufacturer may be contacted to determine if the expiration date has been extended. If this is the case, the updated expiration date will be written or printed on the chemical container(s) along with the analyst initials and date. If a chemical manufacturer has not provided a retest or expiration date on the chemical container or associated documentation, an expiration date will be assigned by the laboratory according to the nature of the material. This expiration date will be written or printed onto the chemical container(s) along with the analyst initials and date.

General expiration date guidelines are:
Liquid acids/bases: 2 years from the date received
Organic solvents: 2 years from the date received

## Evidence Handling

Suspected plant material and concentrates containing THC are stored the same as normal laboratory drugs evidence. All unsealed evidence must be secured with access limited to the analyst when the analyst is not present.

When opening evidence for analysis, each sealed layer of packaging must be marked with the laboratory case number, item number, date of opening, and initials of the analyst who opened it.

## Analyst Notes

The analyst's notes for THC quantitation will include at minimum:

- The start and end date of analysis
- The date of sample preparation and analysis
- An item description including the packaging
- The sample type (plant material or concentrate)
- Any unusual aspects of the sample
- Any significant discrepancies or broken seals (refer to Seized Drugs Procedure Manual on evidence discrepancies)
- The overall sample weight
- The weight of the sample used for quantitative analysis
- Identification of the balance used for each weighing operation

- The THC Control Pack identifier for all completed runs that include the request item(s)
- Results for each THC duplicate with RPD, where applicable
- Results for each THCA duplicate with RPD, where applicable
- Calculated THC wt% and THCA wt% (if results are within the calibration range)
- Calculated Total THC wt % (if results are within the calibration range)

## Item Selection and Sampling

### Item Selection

Not all items submitted to the laboratory for analysis will be routinely analyzed. The number of samples analyzed will be kept to a reasonable number and limited to relevant items based on information conveyed to the laboratory by law enforcement and/or a prosecutor. Once probative results are obtained, additional analysis will not be performed. If no information is provided to the laboratory by law enforcement and/or a prosecutor to guide sample selection, the following guidelines are to be utilized.

When multiple containers or packages are submitted for testing, the analyst will choose one for testing. Testing of additional containers may occur in the following circumstances:

- The contents of the first item tested negative
- The combined weight of multiple containers exceeds a legal threshold
    - One ounce of marijuana {Sec 11.71.040 (a) (2)}

### Purpose of Testing and Limitations

The purpose of THC quantitation is to differentiate materials containing greater than 0.3% THC by weight from those with less or no detectable THC. Therefore, this method may not be able to specify the exact concentration of THC or THCA in a material if the concentration is above or below the intended measurement range (e.g., approximately 0.10% to 10% THC in plant material).

The limit of detection (LOD) of this method is 0.5 ug/mL of THC or THCA in solution. This corresponds to 0.10 %w/w THC or THCA in plant material (200.0 mg sample) and 0.25% w/w THC or THCA in a concentrate (20.0 mg sample).

The lower limit of quantitation (LLOQ) is equal to the LOD.

The method quantitation limits are dependent on the amount of sample used according to the following table:

| Sample Type | Measured Weight (g) | Calibrated result (ug/mL) | Sample Concentration (ug/mL) | Wt% Analyte (Min or Max) |
|---|---|---|---|---|
| Plant Material | 0.2000 (200.0 mg) | 0.5 (min) | 500 | 0.10% |
| Plant Material | 0.2000 (200.0 mg) | 50.0 (max) | 500 | 10% |
| Concentrate | 0.0200 (20.0 mg) | 0.5 (min) | 200 | 0.25% |
| Concentrate | 0.0200 (20.0 mg) | 50.0 (max) | 200 | 25% |

### Minimum Sample Quantity

The minimum sample quantities for THC Quantitation are as follows (including remaining material for defense testing):

Plant material (suspected marijuana): 1 gram (1000 mg)

Suspected THC concentrate: 100 mg

If less than the minimum sample quantity (above) but more than the minimum test amount is submitted, the item will be reported as **Insufficient material for quantitation without a letter of consumption from the District Attorney's office.**

Upon receipt of a written permission to consume the item, THC quantitation may still be performed.

If the sample quantity does not meet the minimum test amount, the report will state **Insufficient sample for quantitation.** Qualitative analysis may still be possible for these samples (if not already performed).

### Selective Sampling

Plant material is known to be non-homogenous. AS 11.71.900 Definitions defines marijuana as "the seeds, and leaves, buds, and flowers of the plant (genus) Cannabis, whether growing or not". Furthermore, "it does not include the stalks of the plant, fiber produced from the stalks, oil or cake made from the seeds of the plant".

For the purposes of THC quantitation, the parts of the plant acceptable for testing are the leaves, buds, flowers, and combinations thereof. Cannabis seeds and stalks contain negligible amounts of THC and therefore their inclusion in a test sample will artificially decrease the THC concentration. Analysts should seek to exclude seeds and stalks from the test sample. If an evidence item contains a high percentage of seeds and stalks, quantitation may not be possible event if the sample quantity is greater than the minimum. In this case, the report will state **Sample quality insufficient for quantitation**.

Samples of plant material are homogenized during sample preparation. Cannabinoid concentrates are presumed to be homogenous upon submission and are not homogenized during sample preparation.

## Analysis Procedure – Sequence of Events

1. Mobile phase preparation (30 minutes)
2. Instrument setup and purging (30 minutes - active, 1 hour+ - inactive)
3. Calibrator and control preparation (60 minutes)
4. Sample preparation (depends on sample type and number)
5. Instrument sequence setup (15 minutes)

THC quantitation using HPLC-DAD is performed in batches called "runs". A run consists of negative controls (blanks), calibrator solutions, control solutions (positive controls), matrix-matched control solutions, and samples. The completed sequence of injections constitutes one run. Quality control criteria exist for individual case samples, but also for the run as a whole and are documented in a control pack.

This method is externally-calibrated. Calibration curves are constructed from known calibrator concentrations (ug/mL) and Area Response (area under peak curve) using a linear relationship:

$$y = mx + b$$

Where y = Area Response and x = the known calibrator concentrations in ug/mL. Once the linear curve is constructed during calibration, the slope of the line (m) and the y-intercept (b) are determined mathematically. A separate curve is constructed for each calibrated analyte (delta-9-THC, delta-8-THC, and THCA). See the example calibration table and calibration curves in Appendix A. The curve weighting is linear according to the known analyte concentration of the calibrator (lower calibrators have a greater weighting).

The linear relationship for each analyte is then used to calculate unknown sample concentrations and these concentrations are reported on the instrument report for each injection.

Liquid control solutions are analyzed with every analysis sequence regardless of sample type. Matrix-matched control samples will be run with every analysis sequence; however, a sequence consisting of only one type of sample (e.g., plant material) need only have the matrix-matched of the same type analyzed with it. If plant material and concentrates are analyzed in the same sequence, both matrix-matched controls are required for that run.

The maximum number of samples per run is 10 (20 sample injections accounting for duplicates).

The lot numbers and expiration dates of calibrators and controls are recorded in the THC Quantitation Control Worksheet. Analysts are responsible for ensuring that the lot numbers and expiration dates are reflected correctly in the worksheet.

## Mobile Phase Preparation

NOTE: Mobile phase preparations may be scaled as needed.

### Mobile Phase A (0.1 % (v/v) Formic acid - Aqueous)

#### Equipment Needed

1. 1000 mL Class A volumetric flask with stopper
2. 1000 mL glass solvent bottle with cap
3. Calibrated pipette, 100-1000 uL
4. Pipette tips

#### Chemicals Needed

1. Deionized (DI) water
    a. From DI water system
2. Formic acid, HPLC-grade or better
    a. Stored in the refrigerator

#### Procedure

1. Fill a clean, dry 1000 mL volumetric flask roughly halfway with DI water.
2. Add 1000 uL (100 on display) of formic acid to the flask using a 100-1000 uL calibrated pipette.
3. Gently swirl the flask to mix.
4. Carefully add DI water to bring the flask to volume (bottom of meniscus aligned with the graduated mark).
5. Place the stopper on the flask and mix by inverting several times.
6. Transfer the solution to a clean solvent bottle and cap.
7. Label the solvent bottle with the solution name, hazard information, preparation date, expiration date, and preparer's initials.

#### Expiration and Storage

1 day (end of following day) at Room Temperature (RT)

### Mobile Phase B (0.05 % (v/v) Formic acid - Organic)

#### Equipment Needed

1. 1000 mL Class A volumetric flask with stopper
2. 1000 mL glass solvent bottle with cap
3. Glass funnel
4. Calibrated pipette, 100-1000 uL
5. Pipette tips

#### Chemicals Needed

1. HPLC-grade Methanol
2. Formic acid, HPLC-grade or better
    a. Stored in the refrigerator

### Procedure

1. Using a glass funnel, fill a clean, dry 1000 mL volumetric flask roughly halfway with HPLC-grade methanol.
2. Add 500 uL (050 on display) of formic acid to the flask using a 100-1000 uL calibrated pipette.
3. Gently swirl the flask to mix.
4. Carefully add HPLC-grade methanol to bring the flask to volume (bottom of meniscus aligned with the graduated mark).
5. Place the stopper on the flask and mix by inverting several times.
6. Transfer the solution to a clean solvent bottle and cap.
7. Label the solvent bottle with the solution name, hazard information, preparation date, expiration date, and preparer's initials.

### Expiration and Storage

3 days (end of day) at Room Temperature (RT)

## Instrument Setup

Proper HPLC shutdown and storage conditions are critical to prevent microbial growth. HPLC columns may be removed from the system during storage. Turning off the DAD lamp is critical to preserve UV lamp life.

Preparation of new mobile phases, purging the lines, reinstalling the column, turning on the instrument modules, and equilibrating the system are all necessary before the instrument may be used for casework after a shutdown period.

### Supplies Needed

1. Mobile phase A, check expiration date
2. Mobile phase B, check expiration date
3. Agilent InfinityLab Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm HPLC column

### Turn Instrument On

1. Turn on each of the 3 instrument modules using the power buttons on the front left side. The modules go through an automatic startup sequence.
2. Turn on the attached desktop computer (if not already on).
3. Load the OpenLab CDS ChemStation software by clicking the LC-001 (online) icon on the desktop of the attached computer.
4. When the Loading Method popup appears, select "Download to instrument".
5. Observe the instrument modules listed in the software (Sampler, Quat. Pump, Column Oven, DAD). The module color codes should be green or orange indicating that the software can connect to each module. The DAD lamp icon is purple when the lamp is on. If the DAD lamp is not on, hover over the DAD module and click the green On button that appears. The lamp takes a short time to illuminate.
6. Determine the current solvent bottle for each solvent line by looking where the lines go in the solvent cabinet and observing the white plastic tags attached to each line. Solvent lines A and B are used for the THC Quantitation instrument method.

7. Determine if an HPLC column is currently installed by removing the column oven compartment cover just below the sampler module using the dark gray tab on the front of the compartment.
    a. If no column is installed, locate an appropriate column (Supplies Needed). Refer to Install HPLC Column (if needed) for installation instructions. Replace the column compartment door once a column is installed. The compartment door must be latched closed to allow the column to reach the appropriate temperature.
8. **Ensure the solvent waste bottle located below the HPLC has room for additional solvent. Refer to** Check Mobile Phase Levels **to determine how much waste will be produced. If the solvent waste bottle is 3/4 full or more, replace the bottle with an empty one.**

## Load Mobile Phases

1. Place the solvent bottles containing fresh mobile phase A and mobile phase B (Mobile Phase Preparation) into the solvent cabinet. Place solvent line A into mobile phase A and solvent line B into mobile phase B.
2. **IMPORTANT: In the software, click on the solvent bottle indicators to update the actual level of solvent. This setting prevents the liquid pump from running if the instrument runs out of solvent. Only bottles A and B need to be updated for this method.**

## Purge Mobile Phase Lines

1. In the pump module (bottom module), open the black purge valve one full turn. The real-time pressure display in the Quat. Pump module in the software should drop to near-zero.
   NOTE: Opening the purge valve too far may result in leaking.
2. In the software, ensure that all 4 instrument modules are active by clicking the green On button (hover tooltip says “Turn on device(s)”).
3. From Method, Load Method in the software, load the THCQuant.M method. The pump should begin running at 1.000 mL/min (in the Quat. Pump module). All modules should turn green in the software after a few minutes.
4. Open the method by right clicking on the pump module and selecting Method. With the purge valve open, change the flow rate to 5 mL/min for 5 minutes to flush the old solvent out of solvent lines A and B and prime the new solvent.
5. Observe the solvent frits (glass filters at the end of the solvent lines) and solvent lines themselves to check for bubbles. Lines A and B should be flushed and free of bubbles.
6. Once the lines have been flushed, reduce the flow rate by reopening the method:
    a. If a column has not been installed, change the flow rate to zero mL/min.
    b. If a column is already installed, reduce the flow rate to 0.5 mL/min.
7. Firmly close the black purge valve on the pump head. Do not overtighten. The pressure should rapidly increase under Quat. Pump in the software. **Do not allow the pressure to exceed 600 bar.**

## Install HPLC Column (if needed)

1. With the flow off, open the column oven compartment.
2. Remove the metal union in place of a column by removing the finger-tight plastic fittings.

3. Locate the HPLC column to install. Note the flow direction indicated by the printed arrow on the column label.
4. Remove the plastic end caps from the column.
5. Attach the inlet end of the column using the plastic fitting coming from the guard column (if using).
    a. The flow arrow on the column should point to the right.
    b. Approximately 2 mm of the metal tube from the guard column (if using) should protrude out the tip of the plastic fitting.
        i. If not using a guard column, attach the column directly to the heater outlet line.
    c. **NOTE:** Do not use tools to attach the column. Screw in the fitting as much as possible to prevent leaks.
6. Attach the outlet end of the column to the tubing going to the detector. Again, approximately 2 mm of PEEK tubing should protrude from the tapered fitting when it is screwed into the column. This connection must be firmly tightened to prevent leaks.

### Load Method (Again)

1. Load the THCQuant.M method again to begin flow through the column and load the normal method parameters. **Do not save changes to the method.**
2. Observe the system via the software to watch the system pressure (blue line).
    a. The pressure will rise rapidly, level off, then fall to a stable level.
    b. Ensure that the maximum pressure has been reached before performing other tasks (e.g., calibrator/control preparation) to check for leaks. The most likely place for leaks is at the column fittings.
        i. If a leak does occur, stop the flow and reinstall the leaking connection.
        ii. Once the system pressure has begun to fall, it is safe to perform other tasks while the instrument equilibrates.

## Calibrator Preparation

### Equipment Needed

1. [2] 2 mL Class A volumetric flasks with caps
2. [1] 5 mL Class A volumetric flask with cap
3. [1] 10 mL Class A volumetric flask with cap
4. [1] 20 mL Class A volumetric flask with cap
5. Calibrated pipette, 100 to 1000 uL
6. Calibrated pipette, 20 to 200 uL
7. Calibrated pipette tips
8. Clean glass beaker
9. Disposable transfer pipettes
10. Amber HPLC vials and vial caps

### Chemicals Needed

1. HPLC-grade methanol, ~40 mL

### Primary Standards Needed

1. Phytocannabinoid Mixture 11 certified reference material (CRM), 1000 ug/mL, Cayman Chemical part no. 32841, SCDL Control no. 228 **OR**
2. Phytocannabinoid Mixture 4 certified reference material (CRM), 1000 ug/mL, Cayman Chemical part no. 25106
    a. A comparable Cayman Chemical CRM (1000 ug/mL) may be used that contains, at minimum:
        i. Delta-9-THC
        ii. Delta-8-THC
        iii. Delta-9-THCA

**NOTE: Verify that the calibrator analyte concentrations in the currently approved HPLC method correspond to the exact concentrations for the CRM before starting a sequence.**

## Procedure

### Stock Solution Math (50.0 ug/mL)

$$1000\frac{ug}{mL} * 0.10\ mL\ (100\ uL) = \frac{100\ ug\ of\ analyte}{2\ mL\ final\ volume} = 50\frac{ug}{mL} per\ analyte$$

### Create Stock Solution (50.0 ug/mL)

1. Fill a 2 mL volumetric flask approximately halfway with HPLC-grade methanol.
2. Using a 20-200 uL calibrated pipette, add 100 uL (100 on display) of Phytocannabinoid Mixture 11 to the volumetric flask.
3. Dilute the flask to the mark with HPLC-grade methanol.
4. Cap and invert several times to mix.

### Create Remaining Solutions (10.0, 5.0, 1.0, 0.5 ug/mL)

1. Perform serial dilutions using a 100-1000 uL calibrated pipette to create the following solutions (see table).
2. Begin by filling all remaining volumetric flasks about halfway with HPLC-grade methanol. After pipetting the "Start with…" solution, dilute each flask to volume, cap, and invert several times to mix well ***before*** starting on the next level.

| Start with… | Pipette this much… | Into this flask… | Final Concentration |
|---|---|---|---|
| 50.0 ug/mL | 1000 uL (1 mL)*** | 5 mL | 10.0 ug/mL |
| 10.0 ug/mL | 1000 uL | 2 mL | 5.0 ug/mL |
| 10.0 ug/mL | 1000 uL | 10 mL | 1.0 ug/mL |
| 10.0 ug/mL | 1000 uL | 20 mL | 0.5 ug/mL |

****Transfer just over 1 mL of the 50.0 ug/mL stock to an amber HPLC vial before drawing up with a calibrated pipette. This will prevent vacuum issues when pipetting directly from the 2 mL volumetric flask.*

3. After all calibration solutions have been created, label each of 5 amber HPLC vials with the corresponding concentrations. Place a microliter insert into each vial (to conserve calibrator volume).
4. Transfer calibration solutions to the corresponding vials, cap, and set aside until ready for injection.

#### Expiration and Storage

Calibration solutions may be stored in their volumetric flasks in the freezer. Cannabinoid solutions are sensitive to temperature and light exposure.

Expiration: 15 days in freezer

## Control Preparation

### Liquid Controls (40.0 ug/mL and 1.5 ug/mL)

#### Equipment Needed

1. [1] 2 mL Class A volumetric flask with cap
2. [1] 10 mL Class A volumetric flask with cap
3. Calibrated pipette, 20 to 200 uL
4. Calibrated pipette, 2 to 20 uL
5. Calibrated pipette tips
6. Clean glass beaker
7. Disposable transfer pipettes
8. Amber HPLC vials and vial caps

#### Chemicals Needed

1. HPLC-grade methanol, ~15 mL total

#### Standards Needed

1. Delta-9-THCA CRM, Supelco/Cerilliant, 1000 ug/mL, T-093S-1ML, SCDL Control no. 245
2. Delta-9-THC CRM, Supelco/Cerilliant, 1000 ug/mL, T-005**,** SCDL Control no. 7

#### Procedure (40.0 ug/mL)

1. Fill a 2 mL volumetric flask approximately halfway with HPLC-grade methanol.
2. Using a 20-200 uL calibrated pipette, add 80 uL (080 on the display) each of the THC and THCA standards to the flask.
3. Dilute to the mark with HPLC-grade methanol. Cap and invert several times to mix.
4. Transfer an amount of solution sufficient for injection to a labeled amber HPLC vial and cap.

#### Procedure (1.5 ug/mL)

1. Fill a 10 mL volumetric flask approximately halfway with HPLC-grade methanol.
2. Using a 2-20 uL calibrated pipette, add 15 uL (150 on the display) each of the THC and THCA standards to the flask.
3. Dilute to the mark with HPLC-grade methanol. Cap and invert several times to mix.
4. Transfer an amount of solution sufficient for injection to a labeled amber HPLC vial and cap.

### Expiration and Storage

Control solutions may be stored in their volumetric flasks in the freezer. Cannabinoid solutions are sensitive to temperature and light exposure.

Expiration: 1 day in freezer

### Negative Control (Blank)

Fill an HPLC vial with the same lot of HPLC-grade methanol used to prepare the Liquid Controls (40.0 ug/mL and 1.5 ug/mL) and samples. This vial will serve as a negative control for the HPLC analysis.

### Needle Wash

Fill an HPLC vial with HPLC-grade methanol. This vial will be used to rinse the autosampler needle after a sample is drawn during the HPLC analysis.

### Matrix-Matched Control Samples

Matrix-matched control samples will be Laboratory-Established Reference Materials (LERMs) that have been previously analyzed for THC and THCA content by an external laboratory. Control samples are analyzed to collect data for the ongoing estimation of the uncertainty of measurement. Matrix-matched control sample measurements are compared to a floating average of all measurements for that control over a defined period.

Matrix-matched control samples are prepared in the same manner as the respective case samples using the same preparation methods excluding the final dilution factor. Follow Plant Material (incl. Plant Material Control) and/or Concentrates (incl. Concentrate Control) for the preparation procedures. Matrix-matched control samples are prepared in duplicate in the same manner as the respective samples.

Matrix-matched control sample analyte concentrations (averages of THC and THCA) must fall on the calibration range (0.5 ug/mL to 50.0 ug/mL) to be counted toward the ongoing estimation of uncertainty.

- For the Plant Material Control, an additional 1:2 dilution is needed. Refer to Plant Material (incl. Plant Material Control).
- For the Concentrate Control, an additional 1:4 dilution is needed. Refer to Concentrates (incl. Concentrate Control).

## Sample Preparation

### Plant Material (incl. Plant Material Control)

#### Equipment Needed

1. Calibrated analytical balance
2. Anti-static device
3. Calibrated pipette, 100 to 1000 uL
4. Calibrated pipette, 20 to 200 uL
5. Graduated cylinder for 20 mL
6. Clean tweezers or forceps

7. Clean lab scissors

### Consumables Needed

1. 50 mL polypropylene tubes with caps, one per sample
2. Medium aluminum weigh pan, one per sample
3. HPLC-grade methanol, ~25 mL per sample
4. Disposable test tubes, 4 per sample
5. Calibrated pipette tips
6. Amber HPLC vials and caps, 2 per sample
7. 5 mL plastic Luer lock syringes, 2 per sample
8. Syringe filters, 0.45 um regenerated cellulose, Luer lock, 2 per sample
9. Disposable transfer pipettes
10. Methanol for cleaning utensils

### Plant Material Sample Procedure

1. Ensure the analytical balance performance check has been completed and documented.
2. Label a new 50 mL polypropylene tube with the case number, item number, initials, and date.
3. Pass the capped polypropylene tube through the anti-static device and place on the analytical balance.
4. Tare the balance, then place the tube on the counter. Place a piece of paper towel on the counter.
5. Transfer the plant material from the original or laboratory container to an aluminum weigh pan over the paper towel.
6. Using clean tweezers (or forceps) and lab scissors, cut the plant material into pea-sized pieces over the weigh pan.
    a. The parts of the plant acceptable for testing are the leaves, buds, flowers, and combinations thereof. Analysts should seek to exclude seeds and stalks from the test sample.
7. Transfer 0.2000 g +/- 0.0020 g (200.0 mg +/- 2.0 mg) of cut plant material into the polypropylene tube and cap.
8. Pass the tube through the anti-static device and place on the tared balance. Record the net weight of the plant material to 4 decimal places.
9. Turn off the anti-static device.
10. If preparing more than one sample, repeat steps 2 through 9 for each sample up to a maximum of 6 (the most that can be homogenized at once).
    a. **NOTE:** If using a pre-homogenized plant material control, skip to step 15 for preparation of that control only.
11. Add 2 ceramic homogenizers to each polypropylene tube and recap.
12. Place the polypropylene tubes into the homogenizer tube holder. NOTE: If only one sample is being used, add a second empty tube to the holder to balance the clamp pressure.
13. Place the tube holder with the tubes into the homogenizer following the instructions on the label. Homogenize the tubes at 1400 rpm for 30 seconds (0:30 on the display).

14. Remove the tubes from the homogenizer and place on the counter. **Check the tubes for cracks before adding any liquid to avoid leaking.**
    a. If a tube is cracked, the best method depends on the sample quantity available.
        i. If plenty of sample is available from the original evidence, weigh a new sample of plant material into a new, labeled 50 mL polypropylene tube beginning at step 2. Dispose of the previous sample and tube in hazardous waste.
        ii. If limited sample is available, transfer the plant material to a new, labeled 50 mL polypropylene tube. Pass the cracked tube through the anti-static device to assist in removal of the plant material.
            1. Note: Plant material not transferred to the new tube will result in an artificially low THC/THCA concentration.
15. Add 20 mL of HPLC-grade methanol to each tube using a Class A graduated cylinder.
16. Shake or vortex each sample for 10 minutes.
    a. **NOTE**: Do NOT use the homogenizer to shake tubes containing liquid.
17. Starting with one sample, complete steps 18 through 20 before moving to the next sample.
18. *(duplication point)* Label 2 disposable test tubes per sample with identifying information.
19. Using a transfer pipette, fill to half each of 2 labeled disposable test tubes (i.e., each sample in duplicate) using liquid from the 50 mL tube.
20. Centrifuge the duplicates at 4400 rpm (4.4 rpm on the display) for 5 minutes.
21. Perform a dilution accordingly:
    a. <u>For a case sample:</u> Using a 20 to 200 uL calibrated pipette, transfer 50 uL (050 on the display) of supernatant from one test tube to a clean disposable test tube. Using a 100 to 1000 uL calibrated pipette, add 950 uL (095 on the display) of HPLC-grade methanol to the same test tube. Vortex for 20 seconds to mix.
    b. <u>For Plant Material Control:</u> Using a 20 to 200 uL calibrated pipette, transfer 25 uL (025 on the display) of supernatant from one test tube to a clean disposable test tube. Using a 100 to 1000 uL calibrated pipette, add 975 uL (halfway between 097 and 098 on the display) of HPLC-grade methanol to the same test tube. Vortex for 20 seconds to mix.
22. Repeat step 21 for the 2nd duplicate (if not already completed).
23. Attach a 0.45 um regenerated cellulose syringe filters to each of [2] 5 mL plastic Luer lock syringes. Remove the plunger from each syringe and set aside.
24. Label 2 amber HPLC vials with sample identifying information.
25. Dump the liquid from one test tube into the top of one of the syringes. Insert the plunger and dispense the liquid into one autosampler vial, then cap. Repeat with the 2nd duplicate.
26. *(save sample extracts)* Locate the duplicate test tubes from step 20. Label 2 amber HPLC vials with sample identifying information. Transfer approximately 1 mL of liquid from each test tube to a corresponding vial. Cap the HPLC vials.

    Plant material sample extracts (for case samples only) may be stored in the freezer for up to 6 days after preparation in case of run failure. Warming to room temperature

and re-dilution beginning at Step 21 is required before stored sample extracts may be analyzed.

27. Repeat steps 21 through 26 for each centrifuged sample.
28. If additional samples need to be centrifuged, return to step 18.

Case sample dilution factor: 2000-fold.
Plant Material Control dilution factor: 4000-fold

## Concentrates (incl. Concentrate Control)

### Equipment Needed

1. Calibrated analytical balance
2. Anti-static device
3. Calibrated pipette, 100 to 1000 uL
4. Calibrated pipette, 20 to 200 uL
5. 10 mL volumetric flask with stopper, 1 per sample
6. Narrow laboratory spatula or wooden dowel

### Consumables Needed

1. HPLC-grade methanol, ~12 mL per sample
2. Calibrated pipette tips
3. Amber HPLC vials and caps, 2 per sample
4. 5 mL plastic Luer lock syringes, 1 per sample
5. Syringe filters, 0.45 um regenerated cellulose, Luer lock, 1 per sample
6. Methanol for cleaning utensils

### Concentrate Sample Procedure

1. Ensure the analytical balance performance check has been completed and documented.
2. Locate a clean, dry 10 mL volumetric flask with a stopper. **NOTE: The flask must be completely dry to avoid evaporation issues during weighing.**
3. Label the volumetric flask with the case number, item number, initials, and date.
4. Pass the uncapped volumetric flask through the anti-static device and place on the analytical balance.
5. Tare the balance, then place the uncapped volumetric flask on the counter.
6. Carefully transfer 0.0200 g +/- 0.0010 g (20.0 mg +/- 1.0 mg) of extract or concentrate to the flask using a laboratory spatula or wooden dowel. For liquid samples, use a narrow transfer pipette to place sample into the volumetric flask.
7. Pass the flask through the anti-static device and place on the tared balance. Record the net weight of the extract or concentrate.
8. Turn off the anti-static device.
9. If preparing more than one sample, repeat steps 2 through 8 for each sample.
10. Add approximately 8 mL of HPLC-grade methanol to each volumetric flask. Cap each flask.
11. Vortex or invert each flask until the sample has completely dissolved.
12. Working one extract/concentrate sample at a time, complete steps 13 through 20.

13. Dilute the volumetric flask to the mark with HPLC-grade methanol. Vortex or invert to mix.
14. *(duplication point)* Attach a 0.45 um regenerated cellulose syringe filter to each of two 5 mL plastic Luer lock syringes. Remove the plunger from each syringe and set aside.
15. Place two new amber HPLC vials into a vial tray.
16. Using a disposable transfer pipette, transfer ~1 mL of liquid into the open top of one syringe. Reinsert the syringe plunger.
17. Filter the solution into the first HPLC vial and cap. Repeat steps 16 and 17 with the duplicate and the second HPLC vial.
18. Label 2 additional amber HPLC vials with sample identifying information.
19. Perform a dilution according to the sample type.
    a. <u>For a case sample:</u> Using a 20 to 200 uL calibrated pipette, pipette 100 uL (100 on display) into each labeled HPLC vial. Using a 100 to 1000 uL calibrated pipette, add 900 uL (090 on display) of HPLC-grade methanol to each labeled HPLC vial. Cap and vortex each vial.
    b. <u>For the Concentrate Control:</u> Using a 20 to 200 uL calibrated pipette, pipette 25 uL (025 on display) into each labeled HPLC vial. Using a 100 to 1000 uL calibrated pipette, add 975 uL (halfway between 097 and 098 on the display) of HPLC-grade methanol to each labeled HPLC vial. Cap and vortex each vial.
20. *(save sample extracts)* Locate the remaining duplicate HPLC vials from Step 17. Cap the HPLC vials if not already capped.

    Concentrate sample extracts (case samples only) may be stored in the freezer for up to 6 days after preparation in case of run failure. Warming to room temperature and re-dilution beginning at Step 18 is required before stored sample extracts may be analyzed.

21. Repeat steps 13 through 20 for each extract/concentrate sample.

Case sample dilution factor: 1000-fold
Concentrate Control dilution factor: 4000-fold

## Instrument Sequence Setup/Prepare to Run

### Load Sequence Template

A sequence template is available for THC Quantitation to make setup faster (THCQuant.S). Select Sequence > Load Sequence Template in the software to find a sequence template. Previously saved sequence templates may also be used as a starting point.

Verify the sequence contains the following elements. Refer to the example sequence in Appendix B – Example Sequence Table.

| Injection # | Solution Name | Type | Calibration Level |
|---|---|---|---|
| 1 | Blank (1 or more) | Sample | - |
| 2 | 0.5 ug/mL Calibrator | Calibration | 1 |
| 3 | 1.0 ug/mL Calibrator | Calibration | 2 |
| 4 | 5.0 ug/mL Calibrator | Calibration | 3 |

| Injection # | Solution Name | Type | Calibration Level |
|---|---|---|---|
| 5 | 10.0 ug/mL Calibrator | Calibration | 4 |
| 6 | 50.0 ug/mL Calibrator | Calibration | 5 |
| 7 | Blank (Carryover check) | Sample | - |
| 8 | 40.0 ug/mL Control #1 | Sample | - |
| 9 | 40.0 ug/mL Control #2 | Sample | - |
| 10 | Plant Material Control #1** | Sample | - |
| 11 | Plant Material Control #1** | Sample | - |
| 12 | Concentrate Control #1** | Sample | - |
| 13 | Concentrate Control #1** | Sample | - |
| 14 | 1.5 ug/mL Control #1 | Sample | - |
| 15 | 1.5 ug/mL Control #2 | Sample | - |
| 16 | Sample duplicate #1 | Sample | - |
| 17 | Sample duplicate #2 | Sample | - |
| # | Repeat as needed… | Sample | - |
| # | 40.0 ug/mL Control #1 | Sample | - |
| # | 40.0 ug/mL Control #2 | Sample | - |
| # | Plant Material Control #1** | Sample | - |
| # | Plant Material Control #1** | Sample | - |
| # | Concentrate Control #1** | Sample | - |
| # | Concentrate Control #1** | Sample | - |
| # | 1.5 ug/mL Control #1 | Sample | - |
| # | 1.5 ug/mL Control #2 | Sample | - |
| # | **Save Solvent (LowFlow.M method)** | Sample | - |

**The required matrix-matched control corresponds to the types of samples included in the run. If both plant material and concentrate samples are included in the run, both controls are required.

## Edit Sequence Template

After loading the sequence template, update the Sample Location and Sample Name for each line in the sequence. The Sample Name field must contain:

1. For calibrators: The calibrator concentration and preparation date
2. For liquid controls: The control concentration and preparation date
3. For plant material or concentrate controls: The LERM control number and the preparation date
4. For casework samples: The case number and item number.
5. For blanks: At least the word “blank”

Blank injections may be injected from the same vial position. Duplicates for controls of the same concentration may be injected from the same vial position. Matrix-matched controls must be injected once per duplicate before the samples and once per duplicate after the samples. Sample duplicates must be injected once per duplicate.

The Method Name for all sequence lines (except the Save Solvent) is THCQuant. The Method Name for Save Solvent is THCLowFlow.

Verify that the Update RF field for all calibrators says “Replace”. The Update RT field for the 0.5 ug/mL calibrator must say “Replace”, but the remaining calibrators will say “No update”. Refer to Appendix B – Example Sequence Table.

### Save Sequence Template As

Select “Save Sequence Template As” to save the modified sequence. Sequences are named according to the date and analyst that ran them using the following format: YYYYMMDDInitials (Example: 20220617DJW). Saved sequence templates should be placed in the C:\Users\Public\Documents\ChemStation\1\Sequence\Completed Sequences folder on the computer attached to the instrument.

### Check Mobile Phase Levels

It is critical that both mobile phases are present in sufficient amounts to complete the analysis sequence. The run time/cycle time is ≤11.5 minutes per injection. Use the following table to estimate solvent consumption depending on the number of lines in the sequence. **Note: The waste volume produced is equivalent to sum of the Mobile Phase A and Mobile Phase B volume consumed. Values are approximate.**

| # of Sequence Lines | Run Time | Mobile Phase A Consumed | Mobile Phase B Consumed | Waste Produced |
|---|---|---|---|---|
| 17 (1 sample in duplicate) | 204 mins | 65 mL | 132 mL | 197 mL |
| 23 (4 samples) | 276 mins | 87 mL | 178 mL | 265 mL |
| 31 (8 samples) | 372 mins | 117 mL | 240 mL | 357 mL |
| THCLowFlow Method | 12 hours (overnight) | 15 mL | 22 mL | 37 mL |

### Verify Stable Detector Baseline and Pump Pressure

Observe the real-time output of the pump pressure and the DAD using the graph in the online version of the OpenLab CDS ChemStation software. The arrows at the bottom of the graph may be used to adjust the graph scaling. Look for a stable pump pressure and detector output (baseline) over a 5-minute period. If the baseline and pressure are stable, proceed to Start Run.

### Start Run

Open the sequence table and select Run. The instrument modules will turn purple in the software and injections will begin according to the sequence table.

## Review Default Reports

As injections are completed, PDF reports are produced and added to the Reports folder on the computer desktop. Once the run has completed, review the PDFs to check for the following:

1. A PDF is present for all expected injections from the sequence table (excluding Save Solvent)
2. Blank injections do not contain integrated delta-9-THC, delta-8-THC, or THCA peaks
3. Delta-9-THC, delta-8-THC, and THCA are all integrated in all 5 calibrator injections

4. Delta-9-THC, delta-8-THC, and THCA peaks are integrated and flagged with the corresponding compound name in all applicable sample injections

Retention time windows are used to identify integrated peaks that correspond to the retention times of the analytes in the calibration table (Delta-9-THC, delta-8-THC, and THCA).

If all criteria are met, cut or copy all PDFs to a flash drive. If one or more of these criteria are NOT met, delete all report PDFs for the run and proceed to Check Integration.

NOTE: Cutting/copying/deleting PDFs from the system only impacts the produced PDF reports and does not alter or remove the raw data files from the instrument computer.

### Check Integration

The goals of integration for this method are as follows:

1. Delta-9-THC, delta-8-THC, and THCA are all integrated in all 5 calibrator injections
2. Integration is such that the area of the peak is captured appropriately and compensates for:
    a. Rising or falling baseline
    b. Shoulders
    c. Co-elution
3. Integration is consistent for replicates of the same solutions/samples

If the default integration has not achieved one or more of these goals, the sequence method (the version of the method associated with the specific analysis sequence) may have the integration parameters adjusted until the integration meets the goals listed above. This is performed in the Offline (data processing) version of OpenLab CDS ChemStation. The most adjusted integration parameters are:

1. Slope sensitivity
2. Peak width
3. Area/height reject
4. Manual integration (drawing the integration line using the mouse cursor)

Once the integration achieves the goals listed above, save the sequence method to retain the integration parameters for that sequence.

If it is not possible to achieve the integration goals listed above with reasonable effort, contact the Technical Lead.

### Recalibration and Reprocessing

When integration of a sequence is modified, recalibration is necessary because this may impact the peak areas of the calibration solutions. Recalibration is the process of recreating the analyte calibration curves. Recalibration is performed automatically during sequence reprocessing.

Reprocessing a sequence means the process of integrating, calibrating, calculating the sample concentrations, and producing PDF reports for an entire sequence. The sequence method parameters (such as integration events) are used to perform this action. To begin reprocessing a sequence, click the

green curved arrow above the injection list in the Offline version of the software. Alternatively, the Reprocess button may be selected from the sequence table.

### Copy Reports

Once integration, calibration, and report generation has been completed, cut or copy the corresponding PDFs to a flash drive. The reports are used during data entry into the THC Quantitation Control Worksheet and individual case files.

## Enter Calibrator and Control Data

### Print Control Pack Information

After integration, calibration, and reprocessing are completed, print the calibration table, calibration curves, and sequence table associated with the sequence.

#### Print Calibration Table and Calibration Curves

1. Load the sequence from the Offline version of OpenLab CDS ChemStation.
2. Click File > Print > Calib. Table and Curves.
3. Choose Printer from the print options. This is a digital PDF printer.
4. Select a location to save the PDF and choose a file name.

#### Print Sequence Table

1. Load the sequence from the Offline version of OpenLab CDS ChemStation.
2. Click Sequence > Sequence Table to view the sequence table.
3. Press Print Screen on the keyboard or copy the sequence table using the Snipping Tool.
4. Paste the sequence table into Paint and save it with the PDF instrument reports.

### Enter Data into Spreadsheet

Enter calibrator, control, and uncertainty control sample weight data into the THC Quantitation Control Worksheet in SharePoint.

1. Open the spreadsheet in the Excel desktop app.
2. On the “1. New Cal or Control” sheet, verify:
    a. Calibrator and control manufacturers, lot numbers, and expiration dates
3. On the “2. Enter Calibrators” sheet, enter the following information:
    a. Date and Analyst (initials)
    b. Reported THC and Reported THCA concentrations from the calibrator instrument reports (3 decimal places)
    c. Conditional formatting confirms whether the calibrator accuracy passed or not (PASS/FAIL)
4. If the calibrators all PASS, copy the Date through Reported THCA cells into the first column on the “3. Copy Calibrators” sheet (example below). The corresponding tables in that sheet as well as the graphs in “2. Enter Calibrators” sheet will populate automatically.
    a. If one or more calibrators FAIL, refer to Control Packs and notify the Technical Lead. Enter the calibrator data normally to create a complete control pack. Casework data may not be reported from a run where one or more calibrators fail.

| Date | Analyst | Instrument | Level | Reported THC | Reported THCA |
|---|---|---|---|---|---|
| 4/28/2022 | AAC | LC-UV1 | 0.5 | 0.537 | 0.528 |
| 4/28/2022 | AAC | LC-UV1 | 1.0 | 1.011 | 0.990 |
| 4/28/2022 | AAC | LC-UV1 | 5.0 | 4.768 | 4.632 |
| 4/28/2022 | AAC | LC-UV1 | 10.0 | 9.643 | 9.579 |
| 4/28/2022 | AAC | LC-UV1 | 50.0 | 50.535 | 50.532 |

5. If only plant material samples were run with a plant material control, enter the control results into "4. Enter Ctrls – Plant Material". If only concentrate samples were run with a concentrate control, enter the control results into "5. Enter Ctrls – Conc" sheet. If both types of samples and controls were run, both sheets are used. Liquid control data may be copied from one sheet into the other as it will be the same.
    a. Enter Date and Analyst (initials)
    b. Check that the Control ID is correct for both matrix-matched controls, if applicable
        i. Field is auto-populated from "1. New Cal or Control"
    c. Enter THC and THCA values from the instrument reports (4 values total, 4 decimal places are displayed in the control sheet)
    d. Enter the corresponding matrix-matched control weights in the bottom left
        i. Conditional formatting will cause this field to turn red if an invalid weight is entered.
    e. Conditional formatting confirms whether the control repeatability and accuracy passed or not (PASS/FAIL)
6. If the controls all PASS, copy the Date through THCA Value 2 cells into the first column on the "6. Copy Ctrls" sheet (example below). The corresponding tables in that sheet as well as the graphs in the "4. Enter Ctrls – Plant Material" and "5. Enter Ctrls – Conc" sheets will populate automatically. **Enter the matrix-matched control weight(s) into appropriate columns on the "6. Copy Ctrls" sheet. The wt% values will not be graphed without this/these weight(s).**
    a. If one or more controls FAIL, refer to Control Packs and notify the Technical Lead. Enter the control data normally to create a complete control pack. Casework data may not be reported from a run where any liquid control fails. If one matrix-matched control was run and fails, no casework data may be reported from that run. If both matrix-matched controls are run and one fails, no casework data may be reported for samples with the same type as the control that failed.
        i. Example: If Plant Material and Concentrate matrix-matched controls and samples are analyzed and only the Plant Material control fails, concentrate samples may still be reported from this run but plant material samples will not be reported.

| Date | Analyst | Instrument | Control ID | Before/After Samples | THC Value 1 | THCA Value 1 | THC Value 2 | THCA Value 2 |
|---|---|---|---|---|---|---|---|---|
| 4/28/2022 | AAC | LC-UV1 | 40.0 | Before | 38.1179 | 38.6286 | 38.1789 | 38.6516 |
| 4/28/2022 | AAC | LC-UV1 | 40.0 | After | 38.2177 | 38.6607 | 38.2289 | 38.6196 |
| 4/28/2022 | AAC | LC-UV1 | 248-LERM | Before | 8.0280 | 26.3096 | 8.4336 | 27.4403 |
| 4/28/2022 | AAC | LC-UV1 | 248-LERM | After | 8.0147 | 26.1495 | 8.3351 | 27.8899 |
| 4/28/2022 | AAC | LC-UV1 | 1.5 | Before | 1.4993 | 1.5186 | 1.4742 | 1.4978 |
| 4/28/2022 | AAC | LC-UV1 | 1.5 | After | 1.4824 | 1.5069 | 1.4614 | 1.4984 |

7. Review the "2. Enter Calibrators", "4. Enter Ctrls – Plant Material", and/or "5. Enter Ctrls – Conc" sheets to verify that the following:
    a. All measured values PASS.
    b. Graphs are updated to reflect the new data points and are not distorted.
8. Print a PDF copy of the following sheets: "2. Enter Calibrators", "4. Enter Ctrls – Plant Material", and/or "5. Enter Ctrls – Conc".
    a. Hit File > Print or Ctrl + P to bring up the print window
    b. Select the Adobe PDF printer
    c. Review the print preview to ensure that the required information will be printed on one page (header, tables, and graphs). If not, select Fit Sheet on One Page to correct.

## Check Acceptance Criteria

### Quality Control Acceptance Criteria

The following quality control parameters must be met for a run to be considered valid:

- Negative controls contain no detectable delta-9-THC, delta-8-THC, and THCA
    - "Detectable" means integrated and flagged by the software
- Calibrator values for delta-9-THC and THCA are within +/- 0.10 ug/mL or 10% of the nominal value, whichever is greater
    - Calibrators are analyzed once each at the beginning of the sequence
- Linearity $R^2$ values for delta-9-THC and THCA are each ≥0.999
    - Determined from review of the "Calib Tables + Curves" report printed from the instrument software
- Positive controls (40.0 ug/mL and 1.5 ug/mL) are injected in duplicate immediately before and after the samples
    - RPD is ≤5.0% for liquid controls
    - Liquid control values are within +/- 20% of the nominal value
- Plant Material and Concentrate control duplicates are each injected once before and after the samples
    - RPD is ≤10.0%
    - Plant Material controls are within +/- 30% of the respective running %w/w averages tracked by the THC Quantitation Control Worksheet
    - Concentrate controls are within +/- 30% of the respective running %w/w averages tracked by the THC Quantitation Control Worksheet

### Sample Acceptance Criteria

Plant Material and Concentrate samples must have duplicate results with RPDs of ≤10.0%. If THC and/or THCA RPDs exceed 10.0%, the sample will be re-diluted and reanalyzed. Stored extracts may be used for reanalysis, if available (refer to Plant Material Sample Procedure and/or Concentrate Sample Procedure.

#### *Insufficient Sample Remaining*

If quantitation testing has begun on a sample and more sample is needed (e.g., run failure, sample does not meet quality control requirements) but taking more sample would not leave enough for reanalysis, the report will state **Insufficient sample for identification unless written approval to consume the evidence is provided by the District Attorney's Office.**

## Control Packs

### When to Create a Control Pack

Control packs are generated for each run that is completed, even if the run fails (examples: calibrator or control failure). Control packs are not generated for runs that are not completed (examples: UV lamp burned out, autosampler error, power failure, etc.).

### Control Pack Contents

A control pack is a single PDF document that contains the following:

- Sequence for the run
- Calibration table and calibration curves
- Instrument reports for each non-sample injection (negative controls, calibrators, and positive controls)
- THC Quantitation Control Worksheet printed PDF pages for the following sheets:
    - "2. Enter Calibrators"
    - "4. Enter Ctrls – Plant Material"
      AND/OR (depending on what sample types were tested)
    - "5. Enter Ctrls – Conc"

### Control Pack Identification

Control packs are named with a control pack identifier. The control pack identifier is stamped on each page of the control pack using the format YYYYMMDDInitials (Example: 20220617DJW). Control pack identifiers for failed runs will be in the format YYYYMMDDInitials-F.

### Control Pack Storage

THC quantitation control packs are stored in Seized Drugs THC Quantitation Control Packs in the laboratory SharePoint document library.

## Case Data Entry

### Insert PDFs

PDF reports for each duplicate must be entered into the case file in LIMS. Ensure that the case number, item number, and analyst initials appear on the reports.

### Enter Quantitative Values

In JusticeTrax, Add Result for the item, select the SD THC Quantitation result type and click the 3 dots in the bottom right corner to access the custom form. Enter the analysis date as well as the raw THC and THCA concentrations for each sample. Ensure the Sample Type matches the nature of the item as this impacts further calculations and automated reporting. Enter the control pack identifier into the designated field.

### Check Notes and Report

Preview the JusticeTrax report to observe the results of calculations based on the quantitative values entered into the custom form such as average, RPD, wt% of each analyte, and Total THC wt% (where applicable). Average and RPD values resulting from data where the RPD between the 2 entered results for each analyte is greater than 10.0% will turn red on the report. Additional reporting language may be used if sample quality or quantity is an issue.

## Technical and Administrative Review

THC quantitation requests are reviewed by a second competent analyst. Review includes the control pack and case-specific data.

## Appendix A – Example Calibration Table and Curves

```
==========================================================================

                        Calibration Table

==========================================================================

                      General Calibration Setting
------------------------------------------------------------------------------------------

 Calib. Data Modified  :      4/27/2022
 1:34:19 PM Signals calculated separately :
                              No

 Rel. Reference Window :      5.000  %
 Abs. Reference Window :      0.000 min
 Rel. Non-ref. Window  :      5.000  %
 Abs. Non-ref. Window  :      0.000 min
 Uncalibrated Peaks    :      not reported
 Partial Calibration   :      Yes, identified peaks are recalibrated
 Correct All Ret. Times:      No, only for identified peaks

 Curve Type            :      Linear
 Origin                :      Ignored
 Weight                :      Linear (Amnt)

 Recalibration Settings:
 Average Response      :      Average all
 calibrations Average Retention Time:
                       Floating Average New 75%

 Calibration Report Options :
     Printout of recalibrations within a
         sequence: Calibration Table after
         Recalibration Normal Report after
         Recalibration
     If the sequence is done with bracketing:
         Results of first cycle (ending previous bracket)

------------------------------------------------------------------------------------------
                                  Signal Details
------------------------------------------------------------------------------------------

 Signal 1: DAD1 A, Sig=230,4 Ref=360,100

------------------------------------------------------------------------------------------
```

Overview Table

| RT | Sig | Lvl | Amount [ug/ml] | Area | Rsp.Factor | Ref | ISTD | # Compound |
|---|---|---|---|---|---|---|---|---|
| 8.616 | 1 | 1 | 5.00500e-1 | 3.96241 | 1.26312e-1 | No | No | d9-THC |
| | | 2 | 1.00100 | 7.97581 | 1.25504e-1 | | | |
| | | 3 | 5.00500 | 39.67061 | 1.26164e-1 | | | |
| | | 4 | 10.01000 | 78.47777 | 1.27552e-1 | | | |
| | | 5 | 50.05000 | 403.80093 | 1.23947e-1 | | | |
| 8.795 | 1 | 1 | 5.02000e-1 | 5.59633 | 8.97017e-2 | No | No | d8-THC |
| | | 2 | 1.00400 | 8.73308 | 1.14965e-1 | | | |
| | | 3 | 5.02000 | 43.03818 | 1.16641e-1 | | | |
| | | 4 | 10.04000 | 81.73779 | 1.22832e-1 | | | |
| | | 5 | 50.20000 | 408.12521 | 1.23001e-1 | | | |
| 9.471 | 1 | 1 | 4.99000e-1 | 6.18451 | 8.06855e-2 | No | No | THCA |
| | | 2 | 9.98000e-1 | 12.70661 | 7.85418e-2 | | | |
| | | 3 | 4.99000 | 69.35828 | 7.19453e-2 | | | |
| | | 4 | 9.98000 | 140.70280 | 7.09296e-2 | | | |
| | | 5 | 49.90000 | 747.73163 | 6.67352e-2 | | | |

More compound-specific settings

Compound: d9-THC
Time Window : From 8.399 min To 8.670 min
Compound: d8-THC
Time Window : From 8.689 min To 9.015 min
Compound: THCA
Time Window : From 9.234 min To 9.669 min

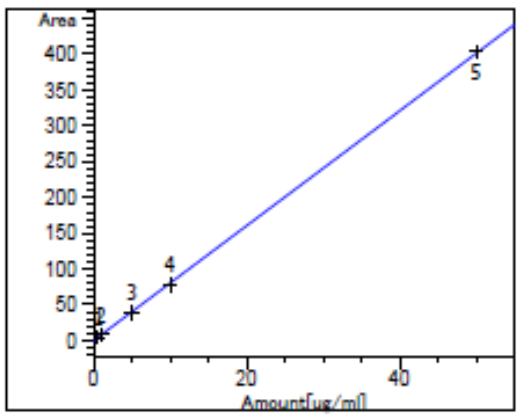
Peak Sum Table

***No Entries in table***

==========

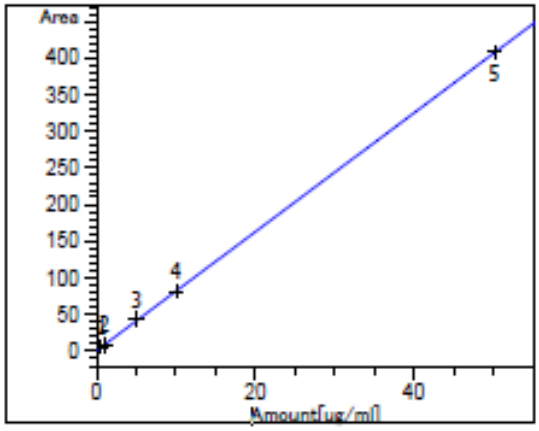
Calibration Curves

d9-THC at exp. RT: 8.616



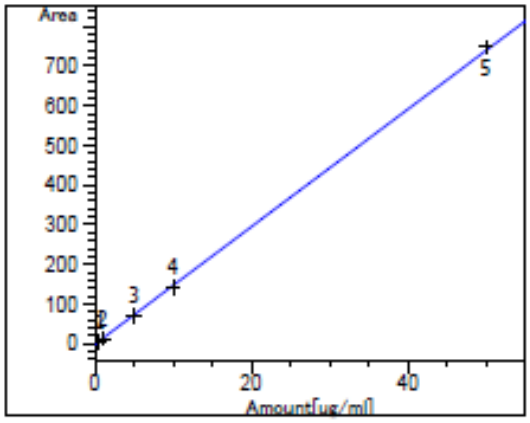

DAD1 A, Sig=230,4 Ref=360,100
Correlation: 0.99994
Residual Std. Dev.: 1.57041
Formula: y = mx + b
m: 8.03014
b: -1.30218e-1
x: Amount (ug/mL
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.5

```
    Level 3     : 0.1
    Level 4     : 0.05
    Level 5     : 0.01
```




d8-THC at exp. RT: 8.795

```
DAD1 A, Sig=230,4 Ref=360,100
Correlation:            0.99988
Residual Std. Dev.:     0.91448
Formula: y = mx + b
      m:       8.10151
      b:       1.26509
      x:
      Amount[ug/ml]
      y: Area
Calibration Level Weights:
    Level 1     : 1
    Level 2     : 0.5
    Level 3     : 0.1
    Level 4     : 0.05
    Level 5     : 0.01
```




```
THCA at exp. RT: 9.471
DAD1 A, Sig=230,4 Ref=360,100
Correlation:            0.99972
Residual Std. Dev.:     6.05037
Formula: y = mx + b
      m:      14.85620
      b:      -1.85549
      x:
      Amount[ug/ml]
      y: Area
Calibration Level Weights:
    Level 1     : 1
    Level 2     : 0.5
    Level 3     : 0.1
    Level 4     : 0.05
    Level 5     : 0.01
```

==========

## Appendix B – Example Sequence Table

| Line | Sample Con... | Sample Location | Sample Name | Method Name | Injection Source | Injection Vol... | Inj/Loc | Sample Type | Cal Level | Update RF | Update RT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Use Current ... | P1-A1 | Method Blank | THCQuant | As Method | | 1 | Sample | | | |
| 2 | Use Current ... | P1-A1 | Method Blank | THCQuant | As Method | | 1 | Sample | | | |
| 3 | Use Current ... | P1-A2 | 0.5 ug/mL Calibrator 6-17-22 | THCQuant | As Method | | 1 | Calibration | 1 | Replace | Replace |
| 4 | Use Current ... | P1-A3 | 1.0 ug/mL Calibrator 6-17-22 | THCQuant | As Method | | 1 | Calibration | 2 | Replace | No update |
| 5 | Use Current ... | P1-A4 | 5.0 ug/mL Calibrator 6-17-22 | THCQuant | As Method | | 1 | Calibration | 3 | Replace | No update |
| 6 | Use Current ... | P1-A5 | 10.0 ug/mL Calibrator 6-17-22 | THCQuant | As Method | | 1 | Calibration | 4 | Replace | No update |
| 7 | Use Current ... | P1-A6 | 50.0 ug/mL Calibrator 6-17-22 | THCQuant | As Method | | 1 | Calibration | 5 | Replace | No update |
| 8 | Use Current ... | P1-A1 | Method Blank | THCQuant | As Method | | 1 | Sample | | | |
| 9 | Use Current ... | P1-B1 | 40.0 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 10 | Use Current ... | P1-B1 | 40.0 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 11 | Use Current ... | P1-B2 | 247-LERM Plant Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 12 | Use Current ... | P1-B3 | 247-LERM Plant Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 13 | Use Current ... | P1-B4 | 248-LERM Concentrate Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 14 | Use Current ... | P1-B5 | 248-LERM Concentrate Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 15 | Use Current ... | P1-B6 | 1.5 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 16 | Use Current ... | P1-B6 | 1.5 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 17 | Use Current ... | P1-C1 | Sample 1 Rep 1 | THCQuant | As Method | | 1 | Sample | | | |
| 18 | Use Current ... | P1-C2 | Sample 1 Rep 2 | THCQuant | As Method | | 1 | Sample | | | |
| 19 | Use Current ... | P1-C3 | Sample 2 Rep 1 | THCQuant | As Method | | 1 | Sample | | | |
| 20 | Use Current ... | P1-C4 | Sample 2 Rep 2 | THCQuant | As Method | | 1 | Sample | | | |
| 21 | Use Current ... | P1-C5 | Sample 3 Rep 1 | THCQuant | As Method | | 1 | Sample | | | |
| 22 | Use Current ... | P1-C6 | Sample 3 Rep 2 | THCQuant | As Method | | 1 | Sample | | | |
| 23 | Use Current ... | P1-B1 | 40.0 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 24 | Use Current ... | P1-B1 | 40.0 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 25 | Use Current ... | P1-B2 | 247-LERM Plant Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 26 | Use Current ... | P1-B3 | 247-LERM Plant Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 27 | Use Current ... | P1-B4 | 248-LERM Concentrate Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 28 | Use Current ... | P1-B5 | 248-LERM Concentrate Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 29 | Use Current ... | P1-B6 | 1.5 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 30 | Use Current ... | P1-B6 | 1.5 ug/mL Control 6-17-22 | THCQuant | As Method | | 1 | Sample | | | |
| 31 | Use Current ... | | Save solvent | THCLowFlow | As Method | | 1 | Sample | | | |

## Revision History

| Location | Revision Made |
|---|---|
| Quality Control Acceptance Criteria | Updated concentrate control accuracy requirement to +/-30% (related to 2024.04.11 Seized Drugs THC Quant Concentrate Control Acceptance Criteria.pdf) |