

Department of Cannabis Control
California Code of Regulations Title 4
Division 19. Department of Cannabis Control

Chapter 6. Testing Laboratories

Article 4. Standard Operating Procedures

§15712.1. Test Method for Cannabinoids.

(a) Notwithstanding section 15712, a licensed laboratory shall utilize the cannabinoids test method required by this section.

(b) The licensed laboratory shall use Determination of Cannabinoids Concentration by HPLC, Standard Operating Procedures (New 05/15/2022), which is incorporated by reference herein, to perform the cannabinoid content analysis required by section 15724.

(c) The cannabinoid test method identified in subsection (b) shall not be altered by the licensed laboratory.

(d) Notwithstanding the requirements of section 15724(a), the licensed laboratory shall analyze the sample size of the representative sample as indicated by the cannabinoid test method identified in subsection (b).

(e) Prior to using the cannabinoid test method identified in subsection (b), the licensed laboratory shall perform verification of the test method, in accordance with section 15712.2, to demonstrate that the laboratory is capable of meeting the test method's performance specifications.

(f) The licensed laboratory is not required to provide the Department with a validation report of the cannabinoid test method pursuant to sections 15702(c)(1) and 15713 for the cannabinoid test method identified in subsection (b).

(g) The licensed laboratory shall provide the Department with its standard operating procedures implementing the cannabinoid test method identified in subsection (b) in accordance with the requirements of section 15702(b) and 15711(a). The SOP shall be submitted with the verification report required by section 15712.2 prior to use of the method for regulatory compliance testing.

(h) The licensed laboratory shall commence utilizing the cannabinoid test method specified in subsection (b) no later than July 1, 2023.

Authority: Sections 26013 and 26100, Business and Professions Code. Reference: Section 26100, Business and Professions Code.

Proposed Text of Regulations

Standard Cannabinoids Test Method and Standardized Operating Procedures
for All Licensed Commercial Cannabis Testing Laboratories

§15712.2. Verification of Test Method for Cannabinoids.

(a) Prior to using a cannabinoid test method identified in section 15712.1(b) for regulatory compliance testing, each licensed laboratory shall perform verification of the test method in their own laboratory to demonstrate that the laboratory is capable of meeting the test method’s performance specifications.

(b) For purposes of this chapter, “Method Verification” means the process of demonstrating that a laboratory is capable of replicating a validated test method with an acceptable level of performance.

(c) To complete the method verification of a cannabinoid test method identified in section 15712.1(b), the laboratory shall address the criteria listed in the following table:

Criteria	Number Required	Notes
Sample matrices	≥1	A single matrix can be selected even if the original method is applicable to multiple matrices
Matrix blanks	≥1	
Method blanks	≥1	
Spike concentration levels	≥2	
Spike replicates	≥3	

(d) The licensed laboratory shall calculate and establish the Limit of Detection (LOD) and Limit of Quantification (LOQ) for all analytes in accordance with section 15731 as part of the method verification.

(e) The licensed laboratory shall evaluate the linear dynamic range for all analytes to ensure they meet the needs for the method as part of the method verification.

(f) The licensed laboratory shall generate a verification report for each cannabinoid test method used. Each verification report shall include the following information:

(1) Instrument calibration data;

(2) Raw data, including instrument raw data;

(3) Cannabis reference materials or certified reference material results;

(4) Data and calculations pertaining to LOD and LOQ determinations; and

(5) Laboratory Quality Control report, as described in 15730 (j).

(g) The supervisory or management laboratory employee shall review, approve, sign, and date the verification report for each cannabinoid test method used.

Proposed Text of Regulations

(h) The licensed laboratory shall provide the Department with the verification report demonstrating verification of the cannabinoid test method prior to use of the method for regulatory compliance testing of cannabis and cannabis products.

Authority: Sections 26013 and 26100, Business and Professions Code. Reference: Section 26100, Business and Professions Code.

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Title: Determination of Cannabinoids Concentration by High Performance Liquid Chromatography (HPLC)

Scope: This Standard Operating Procedure (SOP) describes the standard procedures and instructions for the determination of cannabinoids concentration by High Performance Liquid Chromatography (HPLC).

Application: This method covers the determination of cannabinoid concentration in cannabis in the following forms: smoked (e.g. flowers, hash), inhaled (e.g. vape oil), oral ingestion (e.g. edibles, beverages) and topical (e.g. lotions) products.

Introduction: Samples are extracted with solvent methanol or a mixture of acetonitrile/methanol (80:20). The extracts are diluted with acetonitrile/methanol and then analyzed by High Performance Liquid Chromatography (HPLC) with a C18 column.

Definitions:

1. "Acceptance criteria" means the specified limits placed on the characteristics of an item or method that are used to determine data quality.
2. "Continuing calibration verification" (CCV) means a type of quality control sample that includes each of the target method analytes that is a mid-range calibration standard which checks the continued validity of the initial calibration of the instrument.
3. "Certified reference material" (CRM) means a reference material in cannabis or similar non-cannabis matrix prepared at a known concentration by a certifying body or a party independent of the laboratory with ISO/IEC 17034 accreditation. The laboratory will calculate the percent recovery of the certified reference material based on measured concentration relative to the known concentration.
4. "High Performance Liquid Chromatography" (HPLC) means the technique to separate, identify and quantify analytes in solution placed in a chromatography column at a high pressure, 500 psi or above. An HPLC system includes but not limited to, a solvent delivery module, a sample injection module, a column, and a detector.
5. "Initial Calibration Verification" (ICV) means a solution of each of the target method analytes of known concentration that is obtained from a source external to the laboratory and different from the source of calibration standards.

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6. "Liquid Chromatography" (LC) means the technique to separate, identify and quantify analytes in solution placed in a chromatography column. A LC system includes, but not limited to, a solvent delivery module, a sample injection module, a column, and a detector.
7. "Laboratory Control Sample" (LCS) means a blank matrix to which known concentrations of each of the target method analytes are added. The spiked concentration must be at a mid-range concentration of the calibration curve for the target analytes. The LCS is analyzed in the same manner as the representative sample.
8. "Limit of detection" (LOD) means the lowest quantity of a substance or analyte that can be distinguished from the absence of that substance within a stated confidence limit.
9. "Limit of quantitation" (LOQ) means the minimum concentration of an analyte in a specific matrix that can be reliably quantified while also meeting predefined goals for bias and imprecision.
10. "Method Verification" means the process of demonstrating that a laboratory is capable of replicating a validated test method with an acceptable level of performance.
11. "Moisture content" means the percentage of water in a sample, by weight.
12. "Recovery" means measured concentration relative to the added (spiked) concentration in a reference material or matrix spike sample.
13. "Resolution" means a quantitative measure of how well two elution peaks can be differentiated in a chromatic separation. It is measured by dividing the difference in peak retention times by the average peak width.
14. "Reporting Limit" (RL) means the lowest concentration at which an analyte can be detected in a sample in each analytical batch. RL for each batch of samples are determined by multiplying the lowest concentration of the working calibration standard 0.5 ppm by total dilution factor, depending on the samples.
15. "Relative percent difference" (RPD) means the comparative statistic that is used to calculate precision or random error. RPD is calculated using the following equation:

$$RPD = \frac{|(\text{representative sample measurement} - \text{replicate sample measurement})|}{([\text{representative sample measurement} + \text{replicate sample measurement}] / 2)} \times 100\%$$
16. "Retention Time" (RT) means the time it takes for a solute to pass through a chromatography column. It is used to identify the cannabinoid associated with a specific peak.

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I. Safety

The toxicity and carcinogenicity of each chemical used in this method have not been thoroughly investigated. Therefore, each chemical compound must be treated as a potential health hazard and exposure must be limited to the lowest possible level. See "Laboratory Safety Guidance" by Occupational Safety and Health Administration (OSHA) for detailed requirement.

The general laboratory safety precautions of the laboratory shall be followed at all times. The appropriate personal protective equipment, such as laboratory coats, eye protection, masks, and gloves, fume hoods etc. shall be used at all times. Handling and disposal of any chemicals shall follow the federal, state and local regulations. See "Laboratory Safety Guidance" by Occupational Safety and Health Administration (OSHA) for detailed requirement.

II. Apparatus and Materials

- A. HPLC equipment, consisting of a column module, solvent delivery module, photodiode-array detection module and sampling module that is capable of separating the cannabinoids of interest to achieve a minimum resolution of 1.3.
- B. Computerized chromatography data collection and processing system and printer with data processing software.
- C. Analytical balance capable of weighing to the nearest 0.1 mg
- D. Top Loading balance capable of weighing to the nearest 0.1 g
- E. Disposable glass Pasteur pipette
- F. Pipettes and pipet tips
- G. Conical polypropylene centrifuge tubes (50ml)
- H. Centrifuge that can effectively separate solids or particles from the extraction solvents by centrifugal force
- I. Sonicator
- J. Ice bucket
- K. HPLC vials, amber
- L. HPLC caps
- M. LC Column capable of separating the cannabinoids of interest to achieve a minimum resolution of 1.3
- N. Disposable syringes with Luer-Lok tips, 3 ml
- O. Syringe filter disk, 0.2 μ m Polytetrafluoroethylene (PTFE)
- P. HPLC solvent bottles, 1 L
- Q. Vortex mixer (Analog Vortex Mixer or equivalent)

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- R. Griffin glass beakers
- S. Graduated cylinder
- T. Tissue homogenizer capable of grinding samples to less than 1 mm
- U. Cryogenic grinder capable of grinding samples to less than 1 mm

III. Reagents

- A. Water, HPLC grade or above
- B. Methanol, HPLC grade or above
- C. Acetonitrile, HPLC grade or above
- D. Formic Acid, HPLC grade or above

IV. Calibration Standard

A. Stock standard solution:

1. Cannabidiolic Acid (CBDA), 1.0 mg/ml, (certified reference material (CRM)).
2. Cannabidiol (CBD), 1.0 mg/ml, (CRM).
3. Cannabigerol (CBG), 1.0 mg/ml, (CRM).
4. Cannabinol (CBN), 1.0 mg/ml, (CRM).
5. Delta 9-Tetrahydrocannabinol (Delta 9-THC), 1.0 mg/ml, (CRM).
6. Tetrahydrocannabinolic Acid (THCA), 1.0 mg/ml, (CRM).
7. Tetrahydrocannabivarin (THCV), 1.0 mg/ml, (CRM).
8. Delta 8-Tetrahydrocannabinol (Delta 8-THC), 1.0 mg/ml, (CRM).
9. Cannabichromene (CBC), 1.0 mg/ml, (CRM).

B. Cannabinoids mix working standard solutions:

Prepare 100 ppm and 10 ppm cannabinoids mix working standard solutions as follows:

1. Working Standard (A): Prepare 100 ppm cannabinoids mix working standards (A) using cannabinoids CRMs from a first source: add same amount of each of the nine cannabinoids 1 mg/ml stock standard solution from Section IV.A (1-9), then add acetonitrile/methanol (80:20) as diluent. Vortex to mix well.
2. Working Standard (B): Prepare 10 ppm cannabinoids mix working standards (B) by appropriate dilution of the 100 ppm solution from Section IV.B.1 using acetonitrile/methanol (80:20) as diluent.

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3. Working Standard (C): Prepare 100 ppm cannabinoids mix working standards (C) using cannabinoids CRMs from a second source: add same amount of each of the nine cannabinoids 1 mg/ml stock standard, then add acetonitrile/methanol (80:20) as diluent. Vortex to mix well.
4. Working Standard (D): Prepare 10 ppm cannabinoids mix working standards for Initial Calibration Verification (ICV) (D) by appropriate dilution of the 100 ppm solution from Section IV.B.3 using acetonitrile/methanol (80:20) as diluent.
5. Store all four working standards (A) (B) (C) (D) in Freezer (-20°C).

C. Calibration standard solutions:

Prepare 0.5, 2, 5, 10, 20, 50 and 100 ppm calibration standard solutions as follows:

1. Prepare 20, 50 and 100 ppm calibration standards by appropriate dilution of the 100 ppm cannabinoids mix working standards (A) using acetonitrile/methanol (80:20) as diluent.
2. Prepare 0.5, 2, 5 and 10 ppm calibration standards by appropriate dilution of the 10 ppm cannabinoids mix working standards (B) using acetonitrile/methanol (80:20) as diluent.

V. Procedure

A. The moisture content of dried flower, including pre-rolls, shall be tested and reported as required by California Code of Regulations, title 4, section 15717. The percentage moisture content shall be used to calculate the dry-weight percent cannabinoid by the equation specified in section IX.A.

B. Sample Preparation:

Notes: Group samples by type (e.g., plant material, juice, hemp oil, chocolate, hard candy, gummy and cookie).

1. Homogenize the samples as follows:

For plant material, use a tissue homogenizer or grinding device which can grind the samples to less than 1 mm, following the manufacturer's instructions.

For chocolate, hard candy, gummy and cookie samples, use a cryogenic grinder which can grind the samples to less than 1 mm, following

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manufacturer's instructions.

For juice and oil samples, invert the container 3 or more times to ensure homogeneity of the liquids.

- From the homogenized composite sample, weigh appropriate amount of sample, indicated below, that corresponds to the sample type into a labeled 50 ml centrifuge tube and record the weight.

Plant material/concentrate/vape oil: 200 mg.
 Cannabis infused oil: 0.5 g.
 Chocolate/hard candy/gummy/cookie/other edibles: 2 g.
 Juice/water/beverage: 5 ml.

C. Sample Extraction

- Add 40 ml extraction solvent to the 50 mL centrifuge tube with the sample. For plant material, use acetonitrile/methanol 80:20 as extraction solvent. For everything else, use methanol as extraction solvent.
- Vortex each centrifuge tube for 1 minute to mix the sample and extraction solvent well.
- Extract in a sonicating bath for 30 minutes with ice in the water bath.
- Centrifuge to 3900 rpm for 15 minutes.
- Take approximately 1.5 ml of the supernatant and filter through a 0.2 um PTFE filter into an HPLC vial.
- Dilute the sample extract to obtain expected concentration within the range of calibration curve used for the analysis. The expected concentration can be calculated based on labels of samples or past experience on similar samples.

Typical dilutions are given in the following table:

Sample matrix	Dilution
Flower/plant material	20
Concentrate/vape oil	80
Edibles	10
Beverages	Without dilution

- If the concentration is found to be out of the calibration range, make adjustments of the dilutions to obtain expected concentration within the range

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of calibration curve and re-analyze the sample. This step should be repeated until the concentration is within the range of calibration curve.

D. Instrumental Parameters

Note: Instrumental Parameters are column and system specific and will vary according to the specific HPLC column and system used. Parameters listed below are for a Perkin Elmer Altus A-30 with Restek Raptor ARC-18 2.1 x 150mm, 2.7um which was used in validating the test method.

1. LC Column: Restek Raptor ARC-18 2.1 x 150mm, 2.7um or an equivalent column that can separate the cannabinoids of interest to achieve a minimum resolution of 1.3.

LC Parameters for Perkin Elmer Altus A-30 with Restek Raptor ARC-18 2.1 x 150mm, 2.7um:

Mobile phase A: Water with 0.05% formic acid

Mobile phase B: Acetonitrile with 0.05% formic acid

Gradient Program:

Time (min)	Flow rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0	0.4	25	75
7.00	0.4	25	75
7.01	0.4	0	100
9.00	0.4	0	100
9.01	0.4	25	75
12.00	0.4	25	75

Flow Rate: 0.4 ml/min

Run time: total 12.00 min: 7.00 min + 2 min washing period + 3 min column re-equilibration

Column Temperature: 35°C

Autosampler Temperature: 15°C

Injection Volume: 2 µl

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2. PDA detector

Spectrum data range: 210 - 400 nm

Wavelength for detection:

Compound	Wavelength for detection
CBDA	220 nm
CBG	220 nm
CBD	220 nm
THCV	220 nm
CBN	220 nm
Delta9-THC	220 nm
Delta8-THC	220 nm
CBC	220 nm
THCA	220 nm

E. Instrument Analysis:

1. Equilibrate the HPLC system with the mobile phases for at least 30 minutes.
2. Inject the standards used to generate the seven-point calibration curve and the Initial Calibration Verification (ICV).
3. Inject the samples, including 1 method blanks, 1 laboratory control sample (LCS), sample and sample duplicate, 1 post-dilution spiked sample.
4. After every 10 injections, re-inject a check standard using one of the calibration standards and a blank for quality control purpose.
5. At the end of the run, re-inject a check standard (section VI.3) using one of the calibration standards and a blank for quality control purpose.
6. Store samples and Standards in the HPLC autosampler or a refrigerator in dark at 4°C.

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VI. Method Limit of Quantification (LOQ) and Reporting Limit (RL)

The calibration standard range is from 0.5 to 100 ppm. The LOQ shall be established as part of the method verification required by California Code of Regulations, title 4, section 15712.2 and in accordance with California Code of Regulations, title 4, section 15731.

	CBDA	THCV	CBD	CBG	CBN	Δ9-THC	Δ8-THC	THCA	CBC
LOD in sample (mg/g)	0.011	0.035	0.055	0.037	0.003	0.014	0.036	0.013	0.008
LOQ in sample (mg/g)	0.032	0.105	0.165	0.111	0.009	0.041	0.108	0.039	0.025

The RLs for each batch of samples are determined by multiplying the lowest concentration of the working calibration standard 0.5 ppm by total dilution factor, depending on the individual dilutions needed for the samples to be within the calibration range.

VII. Quality Control

The laboratory quality control samples should be analyzed in accordance with the requirements contained in California Code of Regulations, title 4, section 15730.

- A. A sample batch is defined as a group of 20 samples or less that is processed together. A sample batch must be accompanied by the following:
1. Solvent Blank to determine that the instrument system is clean and free of contamination. The solvent blank is the same as dilution solvent (acetonitrile/methanol (80:20)). This solvent blank should always run pairing with Initial Calibration Verification (ICV) or Continuing Calibration Verification (CCV).
 2. Initial Calibration Verification (ICV) prepared from a set of cannabinoids CRMs from a second source, to check whether the calibration standards are good. ICV should fall within +/- 30% of the expected value of 10 ppm.
 3. Continuing Calibration Verification (CCV) using established calibration from Section IV.D.2. Check the calibration of the instrument at every 10th injection by analyzing one of the calibration standards (e.g. 50 ppm). CCV should fall within +/- 30% of the chosen calibration standards concentration.

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4. Actions to take when quality control failures occur are specified in California Code of Regulations Section, title 4, 15730(f), which include the frequency of calibration when CCVs fail.
- B. Every sequence/sample batch processed should include at least 1 method blank, 1 laboratory control sample (LCS), 1 sample duplicate and 1 matrix post-dilution spike.
1. A Method Blank is used to determine that no contamination resulted from sample extraction procedures. Use Deionized (DI) water as the method blank for juice sample matrices and follow the same extraction procedures. For all other cannabis matrices, use 40ml extraction solvent as the method blank.
 2. A Laboratory Control Sample (LCS) is a blank matrix to which known concentrations of each of the target method analytes is added. The LCS is analyzed in the same manner as the representative sample. Cellulose powder is used as a blank matrix for this method and a mixture of 9 cannabinoids (CRM) at known amount is spiked into the blank matrix. Recovery of the LCS must be 70-130% of the spiked amount.
 3. A Sample Duplicate measures the precision of the analytical process. Duplicate analysis involves a replicate sample, sub-sampled in the laboratory. Method precision is documented and controlled based on the relative percent difference (RPD). The RPD must meet the acceptance criteria of $RPD \leq 30\%$ as required by California Code of Regulations, title 4, section 15730.
 4. A Matrix Post-dilution spike is used to evaluate the effects of sample matrices on the performance of the analytical method. A post-dilution spike is used because, given the limit of concentrated cannabinoids stock standards, matrix spike is not applicable. Prepare the post-dilution spike by spiking known amount of cannabinoids mix standards into the diluted samples. The recovery must be 70-130% of the spiked amount.

C. Integration

All chromatograms should be processed by automatic detection and integration of peaks using instrument software. For manual integration, if done, laboratories shall have an integration policy that outlines the proper way to integrate chromatographic peaks, the preference of automatic integration for consistency, and if occasion for manual integration is needed, justification for the manual integration is documented and supported with documentation including

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chromatograms showing both automatic and manual integration. The policy shall include review of manual integration documentation by management and documentation of management approval or denial. Excessive manual integration indicates there may be a problem with the instrument requiring investigation and preventative maintenance.

D. Retention time (RT) Acceptance Window

Calculate the retention time acceptance window of each cannabinoid using the average retention time of the calibration standards in the same run of the samples. There are 7 calibration standards in the run so 7 retention times of each cannabinoid are collected from the standards. The average retention time of each cannabinoid is calculated by averaging the 7 retention times. The acceptance window is set as the average retention time +/- 2.5% of the average retention time.

E. UV-Visible Spectrum

Whenever identification of a sample analyte peak is in doubt, the UV-Visible spectrum of that peak shall be visually compared to the UV-Visible spectrum of a standard CCV cannabinoid peak or compared using the method from the instrument's software. If the sample peak has a retention time within the acceptance window determined in section VII.D. but the spectra does not match the cannabinoid's UV-Visible spectrum, then the sample peak shall not be reported as the cannabinoid. If there is evidence of a cannabinoid plus an impurity, the laboratory may deconvolve the cannabinoid from the interference following the requirements for manual integration in section VII.C.

VIII. Acceptance Criteria for Quality Control Samples

Calibration curves must have correlation coefficient >0.99. All the calibration check standards the LCS and Matrix Post-dilution spikes must be within 70-130% recovery of spiked amount. If any of the quality control samples did not meet the acceptance criteria, the samples associated with failed QCs need to be re-analyzed in accordance with California Code of Regulations, title 4, section 15730.

IX. Reporting Results

A. Results shall be calculated and reported in accordance with the cannabinoid reporting requirements in California Code of Regulations, title 4, section 15724

(New 05/15/2022)

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and the data package requirements in California Code of Regulations, title 4, section 15732.

- B. Results for all samples shall be reported with 3 significant figures.
- C. Results that are below the reporting limit determined in Section VI are reported as “<RL”.