

**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. Cannabinoid Test Method for Dried Flower, including Non-Infused Pre-Rolls.**

(a) Notwithstanding section 15712, a licensed laboratory shall utilize the cannabinoid test method required by this section and shall not utilize any other cannabinoid test method for the purpose of regulatory compliance testing and reporting results for dried flower, including non-infused pre-rolls. A licensed laboratory is not required to use the cannabinoid test method required by this section for cannabis products, including infused pre-rolls.

(b) The licensed laboratory shall use Standard Operating Procedures: Determination of Cannabinoids Concentration by High Performance Liquid Chromatography (HPLC) for Dried Flower, including Non-Infused Pre-Rolls (New 4/10/2023), which is incorporated by reference herein, to perform the cannabinoid testing 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 specified in 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 a method verification of the cannabinoid test method, in accordance with section 15712.2, to demonstrate that the laboratory is capable of meeting the cannabinoid 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(d) 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 sections 15702(b) and 15711(a). The standard operating procedures shall be submitted with the verification report required by section 15712.2 prior to use of the cannabinoid test method for regulatory compliance testing.

(h) The licensed laboratory shall commence utilizing the cannabinoid test method identified in subsection (b) no later than January 1, 2024.

**Final Text of Regulations (July 2023)**

(i) The licensed laboratory may test for additional cannabinoid analytes beyond those specified in section IV(A) and in additional matrices beyond those covered in the cannabinoid test method identified in subsection (b). The licensed laboratory shall provide a method validation for additional cannabinoid analytes and additional matrices in accordance with section 15713.

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

**§15712.2. Verification of Cannabinoid Test Method for Dried Flower, including Non-Infused Pre-Rolls.**

(a) Prior to using the cannabinoid test method identified in section 15712.1(b) for regulatory compliance testing, each licensed laboratory shall perform a method verification of the cannabinoid test method in their own laboratory to demonstrate that the laboratory is capable of meeting the cannabinoid 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) For purposes of this chapter, “reagent blank” means reagents that are used in the procedure taken through the entire method and which are added in the same volumes as used in the sample preparation. A reagent blank is analyzed in the same manner as the representative sample.

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

<u>Criteria</u>	<u>Number Required</u>
<u>Reagent blanks</u>	<u>≥1</u>
<u>Method blanks</u>	<u>≥1</u>
<u>Spike concentration levels</u>	<u>≥2</u>
<u>Spike replicates</u>	<u>≥3</u>

(e) As part of the method verification:

(1) The licensed laboratory shall calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ) for all analytes in accordance with section 15731; and

(2) The licensed laboratory shall evaluate the linear dynamic range for all analytes to ensure they meet the needs for the cannabinoid test method.

(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 section 15730(j).
- (g) The supervisory or management laboratory employee shall review, approve, sign, and date the verification report for each cannabinoid test method used.
- (h) The licensed laboratory shall provide the Department with the verification report demonstrating verification of the cannabinoid test method prior to use of the cannabinoid test method for regulatory compliance.

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) for Dried Flower, including Non-Infused Pre-Rolls**

**Scope:** This Standard Operating Procedure (SOP) describes the standard procedures and instructions for the determination of cannabinoids concentration for dried flower, including non-infused pre-rolls by High Performance Liquid Chromatography (HPLC).

**Application:** This method covers the determination of cannabinoid concentration in dried flower, including non-infused pre-rolls. This method does not cover the determination of cannabinoid concentration in cannabis products, including infused pre-rolls.

**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. "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 is not limited to, a solvent delivery module, a sample injection module, a column, and a detector.
4. "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.
5. "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.

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6. “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.

7. “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.

8. “Method Blank” (MB) means an analyte free matrix to which all reagents are added in the same volumes or proportions as used in the sample preparation and which is processed in exactly the same manner as the representative sample.

9. “Method Verification” means the process of demonstrating that a laboratory is capable of replicating a validated test method with an acceptable level of performance.

10. “Moisture content” means the percentage of water in a sample, by weight.

11. “Matrix Post-dilution Spike” means spiking a known amount of cannabinoids mix standards into a diluted sample after extraction. A Matrix Post-dilution Spike is used to evaluate the effects of sample matrices on the performance of the analytical method.

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. Resolution is measured by dividing the difference in peak retention times by the average peak width.

14. “Relative percent difference” (RPD) means the comparative statistic that is used to calculate precision or random error. RPD is calculated using the following equation:

$$RPD = \left| \frac{\text{representative sample measurement} - \text{replicate sample measurement}}{(\text{representative sample measurement} + \text{replicate sample measurement}) / 2} \right| \times 100\%$$

15. “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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16. “Solvent Blank” means the same dilution solvent used to create the calibration working standards, acetonitrile/methanol (80:20), and is run in pairing with the ICV and CCV. A Solvent Blank is used to determine that the instrument system is clean and free of contamination.

17. “Standard” means a certified reference standard comprised of one or more of the target analytes prepared at a known concentration by a certifying body or a party independent of the laboratory with ISO/IEC 17034 accreditation.

## **I. Safety**

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.

## **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 separate solids or particles from the extraction solvents by centrifugal force
- I. Sonicator
- J. Ice bucket
- K. HPLC vials, amber
- L. HPLC caps

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- M. HPLC 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 µm Polytetrafluoroethylene (PTFE)
- P. HPLC solvent bottles
- Q. Vortex mixer
- R. Griffin glass beakers
- S. Graduated cylinder
- T. Tissue homogenizer or any size reduction equipment 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 with the following analytes at the listed concentration. Mixtures or combined standard solutions of the listed analytes at their specified concentration or single standard solutions of the analytes at their specified concentrations may be used for the following stock standard solution.
  1. Cannabidiolic Acid (CBDA), CAS number 1244-58-2, 1.0 mg/mL standard
  2. Cannabidiol (CBD), CAS number 13956-29-1, 1.0 mg/mL standard
  3. Cannabigerol (CBG), CAS number 25654-31-3, 1.0 mg/mL standard
  4. Cannabinol (CBN), CAS number 521-35-7, 1.0 mg/mL standard
  5. Delta 9-Tetrahydrocannabinol (Delta 9-THC), CAS number 1972-08-3, 1.0 mg/mL standard
  6. Tetrahydrocannabinolic Acid (THCA), CAS number 23978-85-0, 1.0 mg/mL standard

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7. Tetrahydrocannabivarin (THCV), CAS number 31262-37-0, 1.0 mg/mL standard
8. Delta 8-Tetrahydrocannabinol (Delta 8-THC), CAS number 5957-75-5, 1.0 mg/mL standard
9. Cannabichromene (CBC), CAS number 20675-51-8, 1.0 mg/mL standard

**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 standards from a first source. Using single standard solutions of the target analytes, add the 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 Volume:Volume) as diluent. Vortex to mix well. For mixtures or combined standard solutions, add acetonitrile/methanol (80:20 Volume:Volume) 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 Volume:Volume) as diluent.
3. Working Standard (C): Prepare 100 ppm cannabinoids mix working standards (C) using cannabinoids standards from a different source of the calibration standards and source external to the laboratory. Using single standard solutions of the target analytes, add the same amount of each of the nine cannabinoids 1 mg/mL stock standard, then add acetonitrile/methanol (80:20 Volume:Volume) as diluent. Vortex to mix well. For mixtures or combined standard solutions, add acetonitrile/methanol (80:20 Volume:Volume) 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 Volume:Volume) as diluent.
5. Store all four working standards (A) (B) (C) (D) in the Freezer (-20°C) or per the manufacturer's specifications.



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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 Volume:Volume) 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 Volume:Volume) as diluent.
3. Additional calibration standards may be added to the standards above. The 0.5, 2, 5, 10, 20, 50 and 100 ppm calibration standards are the minimum required.

**V. Procedure**

A. The moisture content of dried flower, including non-infused pre-rolls, shall be tested and reported as required by California Code of Regulations, title 4, section 15717.

1. 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:

1. Homogenize the samples by using a tissue homogenizer or any size reduction equipment which can grind the samples to less than 1 mm, following the manufacturer's instructions. For non-infused pre-rolls, include the rolling paper in the homogenized samples.
2. From the homogenized composite sample, weigh 200 mg of sample into a labeled 50 mL centrifuge tube and record the weight.

C. Sample Extraction

1. Add 40 mL extraction solvent to the 50 mL centrifuge tube with the sample. Use acetonitrile/methanol (80:20 Volume:Volume) as extraction solvent.
2. Vortex each centrifuge tube for at least 1 minute to mix the sample and extraction solvent well.

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3. Extract in a sonicating bath for at least 30 minutes with ice in the water bath.
4. Centrifuge to 3900 rpm for 15 minutes.
5. Take approximately 1.5 mL of the supernatant and filter through a 0.2 µm PTFE filter into an HPLC vial.
6. Dilute the sample extract with acetonitrile/methanol (80:20 Volume:Volume) to obtain an expected concentration within the range of calibration curve used for the analysis.

The typical dilution for dried flower, including non-infused pre-rolls, is 20.

7. If the concentration is found to be out of the calibration range, make adjustments of the dilutions to obtain an expected concentration within the range of calibration curve and re-analyze the sample. This step shall 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, 2.7µm which was used in validating the test method.

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

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

Mobile phase A: Water with 0.05 Volume/Volume% formic acid

Mobile phase B: Acetonitrile with 0.05 Volume/Volume% formic acid

Gradient Program:

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

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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 + 2.00 min washing period + 3.00 min column re-equilibration

Column Temperature: 35°C

Autosampler Temperature: 15°C

Injection Volume: 2 µL

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). If a valid calibration curve and a valid ICV already exist for this method and specific instrument, a CCV may be analyzed in place of a new calibration curve and ICV, so long as

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the CCV meets the requirements in California Code of Regulations, title 4, section 15730.

3. Inject the samples, including 1 Method Blank, 1 Laboratory Control Sample (LCS), sample and sample replicate, 1 Post-dilution Spiked sample.
4. After every 10 samples, re-inject a Continuing Calibration Verification (CCV) using one of the mid-range calibration standards and a Solvent Blank for quality control purposes.
5. At the end of the run, re-inject a mid-range CCV (Section VII.A.3) using one of the calibration standards and a Solvent Blank for quality control purposes.

## VI. Method Limit of Quantification (LOQ)

The minimum 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	$\Delta^9$ -THC	$\Delta^8$ -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

LOQ for analytes tested shall be within the range of the calibration curve.

## VII. Quality Control

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

- A. An analytical sequence is defined as a group of samples that are analyzed sequentially using the same instrument calibration curve. An analytical sequence must be accompanied by the following:

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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 Volume:Volume)). This Solvent Blank should always run pairing with Initial Calibration Verification (ICV) or Continuing Calibration Verification (CCV). The solvent blank should be free of the target analytes such that no target analyte is present over the LOQ to meet acceptance criteria. If target analytes are present over the LOQ, rerun the solvent blank once or until the target analytes are no longer present over the LOQ. If the problem persists, locate the source of contamination and rerun the CCV or ICV.
  2. Initial Calibration Verification (ICV) prepared from a set of cannabinoids standards from a source external to the laboratory and different from the source of the calibration standards, to check whether the calibration curve is valid. ICV shall fall within +/- 30% percent recovery of the expected value of 10 ppm.
  3. Continuing Calibration Verification (CCV) using established calibration from Section IV.C. Check the calibration of the instrument at every 10th sample by analyzing one of the mid-range calibration standards (e.g., 50 ppm). CCV shall fall within +/- 30% percent recovery of the chosen calibration standards concentration.
  4. Actions to take when quality control failures occur are specified in California Code of Regulations, title 4, section 15730(f), which include the frequency of calibration when CCVs fail.
- B. An analytical batch is defined as a group of 20 samples or less that are prepared together. Every analytical batch processed shall include at least 1 Method Blank, 1 laboratory control sample (LCS), 1 laboratory replicate sample (LRS) and 1 Matrix Post-dilution Spike.
1. A Method Blank is an analyte free matrix and is analyzed in the same manner as the representative sample. Cellulose powder is used as the blank matrix for this method. The Method Blank is used to determine that no contamination resulted from the sample extraction procedures.
  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

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(standards) at a known amount is spiked at mid-range into the blank matrix. Recovery of the LCS must be 70-130% of the spiked amount.

3. A Laboratory Replicate Sample (LRS) 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 matrix post-dilution spike is used because, given the limit of concentrated cannabinoids stock standards, matrix spike is not applicable. Prepare the matrix 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 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 curve standards injected during the same analytical sequence of the samples. Calibration curve standards injected during the same analytical sequence include CCVs and the calibration curve standards. If no calibration curve was injected during the same analytical sequence as the samples, use the CCVs injected during the same analytical sequence as the samples. There are 7 calibration curve standards in a

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calibration curve so 7 retention times of each cannabinoid can be collected along with 1 retention time of each cannabinoid from every CCV injected in the analytical sequence. The average retention time of each cannabinoid is calculated by averaging the total 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. If the laboratory is unable to deconvolve the cannabinoid from the interference, the sample shall be re-analyzed in accordance with the requirements of California Code of Regulations, title 4, section 15730.

**VIII. Acceptance Criteria for Quality Control Samples**

Calibration curves must have a coefficient of determination or r<sup>2</sup> value ≥ 0.99. All CCVs, LCS, and Matrix Post-dilution Spikes must be within 70-130% recovery of the spiked amount. The Method Blank must not exceed the LOQ for any analyte. If any of the laboratory quality control samples (LQCs) did not meet the acceptance criteria, the samples associated with failed LQCs shall be re-analyzed in accordance with California Code of Regulations, title 4, section 15730.

**IX. Reporting Results**

Results shall be calculated and reported in accordance with the cannabinoid reporting requirements in California Code of Regulations, title 4, section 15724 and the data package requirements in California Code of Regulations, title 4, section 15732.