



Cannabinoid Concentration Sample Preparation (CCSP)

1.0 Scope and Application

- 1.1 This method was adapted from the New York State Department of Health procedure NYS DOH MML-301.
- 1.2 This method addresses the extraction of cannabis samples for cannabinoid analysis by High Performance Liquid Chromatography (HPLC) with Photodiode Array (PDA) Detection or similar detector. It contains information specifically relevant to the extraction and preparation of cannabis products in Section 8.0. This preparation method is used in conjunction with 976-CLASP Method Cannabinoid Concentration Analysis (CCA). Refer to the analytical procedure (976-CLASP Method CCA) for information on analyte list, calibration, analysis, quality control and data reporting.
- 1.3 This method is restricted to use by or under the supervision of analysts experienced in the preparation of cannabis products. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in Section 9.0.

2.0 Summary of the Method

- 2.1 A portion of cannabis product, typically from 10 to 1200 mg, is weighed into a centrifuge tube. The amount weighed depends upon the specific product produced by a licensed producer or processor and the declared concentrations of cannabinoids in the cannabis product. A surrogate (SUR) and typically 20.0-mL of methanol (MeOH) are added. The solution is mixed well and is either diluted further or used directly for analysis. If necessary, this extract is diluted an additional 2- to 20-fold based on the concentrations of cannabinoids in the cannabis sample as declared by the licensed producer or processor. The internal standard working diluent (IWD) is then added to the extract or dilution thereof, and the potency measurement is made using HPLC-PDA (see 976-CLASP METHOD Cannabinoid Concentration Analysis).

3.0 Definitions

- 3.1 **Analytical Batch:** A group of samples, standards, and blanks which are analyzed together with the same method sequence and same lots of reagents and with the manipulations common to each sample within the same time period usually no more than 24 hours. Batch size is usually limited to instrument loading capacity.
- 3.2 **Brand:** A defined cannabis product that has a homogenous and uniform cannabinoid concentration (total THC and total CBD) and product quality, produced according to an approved and stable processing protocol, and shall have the same inactive ingredients as that defined for that form of the brand.
- 3.3 **Continuing Calibration Verification Standard (CCV):** One of the primary calibration standards used to verify the acceptability of an existing calibration.

- 3.4 Form: A type of a cannabis product approved by the WSLCB that shall refer to the final preparation of an approved cannabis brand; for example, an extract in oil for sublingual administration, an extract for vaporization or an extract in a capsule for ingestion.
- 3.5 Inactive ingredients: Inactive ingredient means any component other than an active ingredient.
- 3.6 Internal Standard (IS): A pure compound that should not be found in any sample. The IS is a compound added to samples, standards, and quality-control samples at a known concentration to provide a basis for peak area ratios used in quantitation. The IS is also used to monitor instrument and extraction performance for each analysis and to correct for solvent evaporation during the analysis.
- 3.7 Internal Standard Working Diluent (IWD) – A solution of IS that is and added to all samples at the same concentration. This working diluent is used to dilute the samples and monitor the integrity of the sample injections.
- 3.8 Laboratory Control Sample (LCS): A portion of appropriate clean matrix that is spiked with known quantities of target analytes and processed as a sample. The LCS measures the accuracy of the methodology. Acronyms include Method Blank Spike (MBS) and Laboratory Fortified Blank (LFB).
- 3.9 Limit of Detection (LOD): The minimum amount or concentration of analyte that can be reliably distinguished from zero. The term is usually restricted to the response of the detection system and is often referred to as the detection limit. Acronym: Method Detection Limit (MDL).
- 3.10 Limit of Quantitation (LOQ): the minimum amount or concentration of analyte in the test sample that can be quantified with acceptable precision and accuracy.
- 3.11 Matrix Spike Duplicate Sample (MSD): A second portion of an actual sample that was used to prepare the MS and is spiked and processed in an identical manner to that used for the MS. The MS and MSD are used together to measure the precision of the method.
- 3.12 Matrix Spike Sample (MS): A portion of sample that is spiked with known quantities of target analytes and processed as if it were a sample. The sample from which the portion to be spiked was taken must be analyzed separately to determine any background analyte concentrations. The MS is corrected for background concentrations and used to determine whether the sample matrix contributes bias to the sample results. The MS is used to evaluate the accuracy of the method in the same way that the MBS is used.
- 3.13 Matrix Blank (MB): An aliquot of appropriate pure matrix void of target analytes that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The matrix blank (MB) is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.
- 3.14 Preparation Batch: Samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch consists of

one to twenty samples (not including matrix blanks, LCS, matrix spikes, matrix duplicates, and CCV) of the same matrix.

- 3.15 Stock Standard: A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards, where available, or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source, where available.
- 3.16 Surrogate Standard (SUR): A pure compound that should not be found in any sample but is similar in nature to the compounds of interest. This compound is added to a sample in a known amount before processing to monitor method performance for each sample. It is quantified in a manner analogous to that used for the analytes. The SUR is useful in ensuring that there were no problems in sample preparation.
- 3.17 Surrogate Stock Diluent (SSD): A concentrated solution of SUR that is prepared in acetonitrile (MeCN). This stock diluent is used to prepare the surrogate working diluent (SWD).
- 3.18 Surrogate Working Diluent (SWD): A solution of SUR that is prepared from the SSD and is added to all samples. This working diluent is used to monitor method performance.
- 3.19 System Blank (SBLK): A portion of appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.

4.0 Health and Safety Warnings

- 4.1 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.
- 4.2 Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling, and disposal of solvents, reagents, and standards. These guidelines must be made available to all personnel involved in the chemical analysis.
- 4.3 Lab coats, safety glasses and gloves must be worn when performing standard or sample preparations, working with instrumentation, disposing of waste, and cleaning glassware.
- 4.4 The fume hood must be used when using or preparing standards, reagents, or samples that require proper ventilation.
- 4.5 The IS norgestrel is a suspected carcinogen and is known to be hazardous during pregnancy. Other internal standards may be used in place of norgestrel.

5.0 Interferences

- 5.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts observed as chromatographic peaks or elevated baselines in the chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running extracted blanks as described in 976-CLASP Method Cannabinoid Concentration Analysis.

- 5.2 All glassware must be washed and, if applicable, verified to be free from background contamination.
 - 5.2.1 All new glassware and processing apparatus must be thoroughly cleaned according to lab SOP.
 - 5.2.2 All routine glassware and processing apparatus must be thoroughly cleaned according to lab SOP. If glassware contamination is suspected, wash per Section 5.2.1.
 - 5.2.3 The use of high-purity reagents and solvents helps to minimize interference problems.
 - 5.2.4 After cleaning, glassware is stored in a clean storage area away from standards and syringes to prevent cross-contamination.
- 5.3 When interferences or contamination are evident in samples, the re-preparation of the original sample is recommended after the source of contamination has been identified.
- 5.4 Interfering contamination known as “carry over” may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Rinsing of the sample syringe and associated equipment between samples with solvent/mobile phase can minimize this sample cross contamination. After analysis of a sample containing high concentrations of analytes, one or more injections of solvent/mobile phase should be made to ensure that accurate values are obtained for the next sample.
- 5.5 Matrix interferences may occur due to inactive ingredients in the sample. Disclosure of inactive ingredients is sometimes held propriety by processors. If an inactive ingredient or other matrix interference is believed to be present, the sample may be spiked with target analytes and analyzed together with the non-spiked sample to verify the results. If these analyses verify the original results, report only the results from the original non-spiked sample. This may not always be possible if a limited amount of sample is received for analysis.
- 5.6 Samples and standards must be prepared in the same final solvent to allow for chromatographic comparability of samples to standards.

6.0 Equipment and Supplies

- 6.1 Sampling Equipment
 - 6.1.1 Pre-cleaned 50-mL plastic bottle fitted with Teflon-lined screw cap.
- 6.2 Equipment
 - 6.2.1 Analytical balance, Mettler-Toledo Model # 205DU or equivalent.
 - 6.2.2 Sonicator, Branson, Model # 2510R-DTH or equivalent.

6.2.3 Vortex, Maxi Mix 11 Model #37615 or equivalent.

6.2.4 Centrifuge, Eppendorf Model # 5415D or equivalent.

6.2.5 Shaker, Labline, Model# 3540 or equivalent.

6.3 Support Equipment

6.3.1 Centrifuge tubes, various sizes.

6.3.2 Stainless steel spatulas.

6.3.3 Class A volumetric flasks, various sizes.

6.3.4 Glass graduated test tubes.

6.3.5 Disposable pipettes.

6.3.6 Macro pipette controller, various sizes.

6.3.7 Pipettes, pipette bulbs.

6.3.8 Aluminum foil squares and plastic weighing dishes for weighing out chemicals.

7.0 Reagents and Standards

7.1 Inorganic Chemicals: Chemicals are obtained from one of the major manufactures such as Sigma-Aldrich, VWR or equivalent. All inorganic chemicals are of reagent grade quality, unless specified in 976-CLASP Method CCA, see Section 7.0.

7.1.1 Stable solid materials are stored in the laboratory on shelves at room temperature. Concentrated acids are also stored at room temperature in an appropriate cabinet.

7.2 Solvents: All solvents used in sample preparation must be HPLC grade or better (976-CLASP Method CCA, see Section 7.0.). Solvents not in use are stored in solvent cabinets.

7.2.1 HPLC grade Acetonitrile (MeCN), Macron or equivalent.

7.2.2 HPLC grade Methanol (MeOH), J.T. Baker or equivalent.

7.3 Matrix Reagents: Cannabis excipients or reagents that may be used as a “representative matrix” for matrix evaluation are listed below.

Matrices	Representative Commodity
Flower	Hemp
Concentrates	Hemp Seed Oil
Edibles	Baked Goods
Other	*

- 7.3.1 New matrices and excipients may also be provided by the licensed producer or processor for evaluation.
- 7.4 Standards: Standards potency analysis are currently purchased from Cerilliant, Cayman, Restek, Sigma-Aldrich or equivalent (976-CLASP Method CCA, see Section 7.2).
 - 7.4.1 Note: Stock standard solutions or neat materials may be purchased from any vendor. When available, standards/stocks materials are purchased from vendors that can provide NIST traceability accompanied by a Certificate of Analysis.
- 7.5 Syringes: Syringes are obtained from one of the major manufactures such as Hamilton, SGE or equivalent. Manual syringes with fixed or removable needles are stored after cleaning. On arrival in the laboratory, new glassware is cleaned as per Section 5.2.
- 7.6 Glassware: Glassware is obtained from one of the major manufactures of laboratory glassware such as Kimble, Ace Glass, Corning or equivalent. On arrival in the laboratory, new glassware is cleaned as per Section 5.2.

8.0 Preparation of Reagents, Solutions, Standards, Matrices and Samples

- 8.1 Standards, SUR and IS are prepared as per 976-CLASP Method Cannabinoid Concentration Analysis (CCA), Section 8.0.
- 8.2 Licensed producer or processor excipient materials and blank matrix.
- 8.3 Sample extract preparation procedure (including MB, MS, MSD, LCS):
 - 8.3.1 A direct dilution method is applied for most of the cannabis and cannabis products. This method can also be used for extraction of solid material. All samples are prepared in this manner unless problems are encountered with a specific sample matrix (i.e. form, brand). Any deviations from this sample preparation method are documented and recorded in the data packages. All recoveries are documented and recorded in the data packages. The documentation must be available for review and approval by the department.
 - 8.3.2 The amount of cannabis product to be extracted is based on the cannabis product type. The weight of matrix and/or cannabis product used is based on the concentration of cannabinoids in each product to ensure the final concentrations are within the analytical curve. The sample matrix and/or cannabis product extract, usually from 10 to 1200 mg, is weighed into a centrifuge tube. Depending on form, alternate preparation steps may be required (See Appendix).
 - 8.3.3 The volume of surrogate, 0.005 to 0.040 mL is spiked into the centrifuge tube. The amount is based on predicted cannabinoid levels in the sample and dilutions needed to ensure the final concentration of the SUR is within the calibration curve.

Typically, a sample that is diluted less than 5-fold will receive 5 µL of SUR standard stock solution at a concentration of 50 mg/mL as the spike into the sample. Based on the final cannabinoid concentrations, if further dilutions are necessary, the SUR is spiked into the sample at a higher concentration to ensure that the measured concentration is within the calibration range of the SUR standard curve.

8.3.4 For extraction, add 20.0 mL of MeOH to the centrifuge tube and mix well for 30 minutes on a shaker to extract the sample. Scale volume as necessary.

8.3.4.1 The following modification for formulations requiring an aqueous extraction step may be made:

8.3.4.1.1 Add 20% water and sonicate for 15 minutes prior to surrogate and methanol addition.

8.3.4.1.2 The substitution or addition of other solvents is acceptable as long as the extraction efficiency is equal to or greater than that of methanol alone.

8.3.4.1.3 Laboratories may adjust solvents and volumes for different cannabis product matrixes to improve extraction efficiencies and minimize interferences.

8.3.4.2 The final concentration of the cannabinoids in cannabis extract must fall within the range of the calibration curve. In some circumstances, an additional methanol dilution of 2 to 20-fold is necessary to analyze the samples. The dilutions are determined based on the concentrations of the cannabinoids in the sample reported by the licensed producer or processor. A larger dilution is needed to bracket high concentration cannabinoids, while a direct injection of the extract or a less diluted sample is required for the analysis of the lower-concentration cannabinoids present in the same sample. Some samples may need to be analyzed twice to measure the primary cannabinoids.

8.3.4.3 Follow 976-CLASP Method CCA as per Section 11.0 for MB, MS, and MSD preparation.

8.3.4.4 Sample extracts (section 8.3.4) are best stored in a freezer at $\leq -20^{\circ}\text{C}$ until analysis is final. (976-CLASP Method CCA see Section 9.5).

8.3.4.5 If necessary, transfer 1 mL of extract into a 2.0 mL centrifuge tube and centrifuge at 12,000 g for 5 min.

8.3.5 Transfer 500 μL IWD preparation @ 10 $\mu\text{g/mL}$ into 2.0 mL HPLC vial (976-CLASP Method CCA see Section 8.0).

8.3.6 Transfer 500 μL of diluted sample supernatant prepared (Section 8.3.4) into the HPLC vial with IWD (Section 8.3.5) and mix well providing a 1:1 ratio.

8.3.7 Follow 976-CLASP Method CCA as per Section 13.0, for sample analysis and data reporting.

9.0 Quality Control/Assurance

9.1 Demonstration of Capability (DOC)

- 9.1.1 All laboratory staff must perform an initial demonstration of capability in using the extraction procedures described in this SOP. The initial DOC must consist of the analysis of four or five extracted spike samples that have been fortified with all analytes of interest to a concentration of one (1) to four (4) times the LOQ. The spiking solution used must be from a source independent from those used to prepare the calibration standards.
 - 9.1.2 The initial DOC is performed under the supervision of a trained analyst. The DOC must meet all acceptance criteria, as described in the analytical procedure 976-CLASP Method CCA, see Section 11.0, before the analyst may perform the procedure without supervision.
 - 9.1.3 Annually, each analyst who will be performing the extraction method must complete a continuing DOC for each target analyte (see 976-CLASP METHOD CCA Table 1). The continuing DOC may be completed by one of the following techniques if available:
 - 9.1.3.1 Acceptable performance of a blind sample, such as an external proficiency test.
 - 9.1.3.2 Acceptable performance of an initial DOC as described above and in 976-CLASP METHOD CCA (see Section 11.0) at any concentration within the calibration range.
 - 9.1.4 If major changes to the method or instrument are made, or the laboratory/analyst has not performed the method in a twelve (12) month period, each analyst must complete an initial DOC as described in 976-CLASP METHOD CCA, Section 11.0. Refer to 976-CLASP METHOD CCA, Section 11.0. for additional information on quality control measures, acceptance criteria and corrective actions for nonconforming data. Minor changes to the method are evaluated using the extracted spike, samples, or the secondary source standard per (976-CLASP METHOD CCA, see Section 11.0).
- 9.2 LOD and LOQ
- 9.2.1 An initial LOD study for each method must be completed and documented for all target analytes in each representative matrix (see Section 7.3), on each instrument used to analyze sample extracts. Since this method is being used for quantitative analysis, the LOD may be set at the same level as the LOQ.
 - 9.2.2 An initial LOQ study for each method must be completed and documented for all target analytes in each representative matrix. The LOQ mean recovery shall be within 70-130% of the spiked value.
 - 9.2.2 On an ongoing basis, the laboratory shall prepare and analyze a minimum of one LOQ verification sample spiked at the same concentration as the initial LOQ verification study on each instrument during each year in which samples are analyzed for each representative matrix, method, and analyte combination. The recovery of the LOQ verification samples shall be within 70-130%.
- 9.3 Extraction (Preparation) Batch-Specific Quality Control

- 9.3.1 The preparation batch size consists of a maximum of 20 cannabis samples (see Section 3.17). The following quality control samples must also be extracted, where applicable, at the prescribed frequency:
 - 9.3.1.1 Matrix Blank, one (1) per preparation batch.
 - 9.3.1.2 Matrix Spike/Matrix Spike Duplicate, one (1) each per preparation batch, if sample is provided.
 - 9.3.1.2.1 A laboratory sample duplicate (duplicating one of the compliance samples in the batch) and a singular matrix spike is acceptable, when a matrix spike duplicate is not appropriate, for each preparation batch.
 - 9.3.1.3 A laboratory may choose to use a Laboratory Control Sample (LCS). The following may also meet the LCS requirement.
 - 9.3.1.3.1 A laboratory control sample (LCS) may be used in place of a continuing calibration verification (CCV) (but not as a replacement for a failing CCV) for methods where the calibration goes through the same process as the LCS. Note that the more stringent acceptance criteria must be met.
 - 9.3.1.3.2 The matrix spike (MS) may be used in place of the LCS as long as the acceptance criteria are as stringent as for the LCS.
- 9.3.2 Refer to the analytical procedure (976-CLASP METHOD CCA) for information on the quality control measures, the applicable acceptance criteria and the corrective actions for nonconforming data.
- 9.4 Analytical Batch-Specific Quality Control
 - 9.4.1 Refer to analytical procedure (976-CLASP METHOD CCA) for information on quality control measures, applicable acceptance criteria and corrective actions for nonconforming data.

10.0 Data Acquisition, Reduction, Analysis and Calculations

- 10.1 See Analytical Batch-Specific Quality Control requirements in 976-CLASP Method CCA.

11.0 Sample Transport, Receipt, Preservation, Handling, and Storage

- 11.1 Cannabis products from licensed producer or processor are received, handled, verified, and documented ensuring method regulatory and accreditation body requirements are met.
- 11.2 Prior to analysis, the extracts are stored in a freezer at $\leq -20^{\circ}\text{C}$ unless otherwise noted (976-976-CLASP METHOD CCA see Section 9.0.).
- 11.3 Cannabinoids are light-sensitive; therefore samples must be protected from the light.

12.0 Waste Management/Pollution Prevention

- 12.1 Minimize solvent, chemical, reagent, and standard use whenever possible to reduce the amount of hazardous waste generated.
- 12.2 Dispose of solvent waste in an appropriate solvent waste container, properly labeled and vented.
 - 12.2.1 All other solvents are separated into two categories, chlorinated and non-chlorinated and are disposed per local, state, and federal regulations.
- 12.3 Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.
- 12.4 Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal.
- 12.5 Consult federal, state, and local regulations for additional information or for information on the disposal of products not described in this method.

13.0 References

- 1. Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations, of the State of New York.
- 2. Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (NYS DOH MML-300).
- 3. Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11 Environmental Protection Agency, 40 CFR (7-1-95 Edition) Part 136, Appendix B.
- 4. *Norgestrel*; MSDS No. N2260 [Online]; Sigma-Aldrich: Saint Louis MO, September 03, 2014 <http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do>.
- 5. 21 CFR 210.3(b)(8): Definitions. Current Good Manufacturing Practice in Manufacturing. Code of Federal Regulations. Title 21, Chapter 1, Subchapter C.
- 6. Alternate Approved Preparation Steps/ Method Validation Summary by Form (MML-301-AppA).
- 7. New York State (NYS) Department of Health (DOH) Environmental Laboratory Approval Program website, <https://www.wadsworth.org/regulatory/elap/medical-marijuana>.

17.0 Acknowledgements

The above method was adapted from the New York State Department of Health procedure NYS DOH MML-301 by the Cannabis Laboratory Analysis Standards Program to meet the recommendations of the Cannabis Science Task Force as a standard extraction method for determining cannabinoid concentrations for certified cannabis laboratories in the state of Washington.

Appendix

1. Product/Form: Chewables

a. Method Development Narrative/Background

- i. Matrices that contain methanol insoluble materials present a challenge for good recovery of cannabinoids during sample preparation in 976-CLASP Method Cannabinoid Concentration Analysis or 977-CLASP Method Cannabinoid Concentration Sample Prep. Products containing excipients which are highly water soluble, but mostly methanol insoluble are common. This alternate procedure was developed to address the sample preparation of such methanol insoluble preparations.
- ii. Roughly 30% cannabinoids by weight 1:1 THC:CBD vape oil preparation of accurately determined concentration by the analytical method 976-CLASP Method Cannabinoid Concentration Analysis was used as the source of cannabinoids for spiking. The roughly 5-g chewable dose forms were cut in half length-wise with a razor blade to afford ~2.5 g samples of matrix. Three sets of five samples each were prepared for this study. The first set were samples containing matrix (~2.5 g). The low spike set of samples contained matrix (~2.5 g) and were spiked with an accurately weighed amount of vape oil around (~7 mg). The high spike set of samples containing matrix (~2.5 g) were spiked with an accurately weighed amount of vape oil (~35 mg). The low spike represents ~2 mg of both THC and CBD, and the highest spike is just above the 10 mg per dose limit of total THC allowed under current regulation.

b. Analysis of Results

- i. Initial data on the extracted samples was collected and processed as described in 976-CLASP Method Cannabinoid Concentration Analysis. The surrogate recovery was 89% and the precision was <2.0% CV at all spike levels. The low-spike level showed nearly quantitative recovery for both total THC and total CBD. The standard deviation was moderate with a %CV of around 7%. The recovery fell slightly for the high-spike samples to 95.8 and 87.9% for total THC and total CBD respectively, while the precision improved to under 2% CV for both.
- ii. The same samples were retested after 24 hours on the autosampler at about 4 °C. The recovery remained constant within experimental variation, while the precision decreased slightly to 8.7% and 7.6% for total THC and total CBD respectively. The surrogate recovery was constant at around 89% in all cases and a precision of <2.8% CV at all spiking levels. This result demonstrates the stability of the sample in the cooled autosampler for 24 hours.
- iii. A fresh dilution of the initial extraction after being stored at 4 °C for 5 days was prepared and analyzed according to 976-CLASP Method Cannabinoid Concentration Analysis. All data for surrogate, total THC, and total CBD recoveries and precision %CV from these samples were unchanged from the initial preparation. This result demonstrates a 5-day storage stability at 4 °C after

initial sample preparation.

- iv. Two preparations of fully formulated chewable products were tested by two separate analysts (one analyst per product) and found to be within $\pm 10\%$ of the manufacturer's laboratory value. On Formulation #1 (1:1 product), the differences between the laboratories for total THC and total CBD were 2.7% and -6.6%, respectively. For Formulation #2, a high-THC low-CBD product, the differences between the laboratories for total THC and total CBD were -2.3% and -2.0% respectively.

c. Procedure

- i. A weighed-portion of homogenous chewable is cut lengthwise with a razor blade and added to a 50-mL centrifuge tube.
- ii. Surrogate, SUR, 0.040 mL is spiked into a clean, 50-mL centrifuge tube. The amount of surrogate is based on cannabinoid levels in the sample reported by the RO and dilutions needed to ensure the final concentration of the SUR is within the calibration curve.
- iii. Add 1.0 mL of Water and 19.0 mL of MeOH to the 50-mL centrifuge tube. Cap the tube tightly and sonicate at 55 °C for 15 minutes. Remove the tube and vortex for 10 seconds. Repeat. Immediately transfer about 2.0 mL of the well-mixed dispersion to a 2.0-mL centrifuge tube and centrifuge at 12,000 rpm for 5 minutes.
- iv. Transfer the supernatant to a new 2.0-mL centrifuge tube and place in -20 °C freezer for >8 hours. Remove from freezer and immediately centrifuge at 12,000 rpm for 5 min. Remove supernatant and dilute with MeOH to the desired ratio based on the expected cannabinoid content as required for 976-CLASP Method CCA.

2. Product/Form: Whole Flower

a. Method Development Narrative/Background

- i. Products which are whole flower provide a challenge for homogeneity. This alternate procedure was developed to address the sample preparation of whole flower.
- ii. See Guidance Document for the Homogenization of Whole Flower for Composite Analysis (most current revision) on the NYS ELAP website at: <https://www.wadsworth.org/sites/default/files/WebDoc/Guidance%20Document%20for%20the%20Homogenization%20of%20Whole%20Flower-10-05-21.pdf> A 3-5 g sample of the homogenized flower is set aside for Cannabis Laboratory potency and contamination testing.

b. Procedure

- i. A statistical number of samples are prepared from the homogenized flower sample as per this method.

- ii. The potency and % CS is reported as an average of the pooled specimen sample. For this test, only one pooled result is reported.