NEW YORK STATE OF OPPORTUNITY. Department of Health

ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Commissioner

SALLY DRESLIN, M.S., R.N.
Executive Deputy Commissioner

New York State Department of Health - Wadsworth Center
Laboratory of Organic Analytical Chemistry–
NYS ELAP Laboratory ID 10763

Division of Environmental Health Sciences
Albany, New York

Medical marijuana sample preparation protocols for potency analysis
NYS DOH MML-301

Table of Contents

1.0. Scope and Application

1.1. This method (NYS ELAP Method ID 9981) addresses only the extraction of medical marijuana samples for cannabinoid analysis using high performance liquid chromatography (HPLC) with photodiode array (PDA) detection. It contains information specifically relevant to the extraction and preparation of medical marijuana products in Section 8.0. Refer to the analytical method (NYS DOH MML-300, NYS ELAP Method ID 9980) for information on the analyte list and for procedures for calibration, analysis, quality control, and data reporting of cannabinoids.

1.2. This method is restricted to use by or under the supervision of analysts experienced in the analysis of medical marijuana products. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in Section 10.0.

2.0. Summary of the Method

2.1. Approximately 10-200 mg of medical marijuana product is weighed into a 50 mL centrifuge tube. A surrogate is added to the tube containing the medical marijuana product, and methanol is added as the extraction solvent. The solution is mixed well, and a portion of the extract is removed for analysis. Internal standard is added, and the extract is diluted up to 100-fold for analysis based on the concentrations of cannabinoids in the medical marijuana samples as declared by the submitting Registered Organization (RO). The potency of the product in terms of cannabinoid content is then determined using HPLC-PDA (See NYS DOH MML-300).

3.0. Definitions

3.1. 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.

3.2. Internal Standard (IS) – A pure compound that should not be found in any sample. This compound is added to a sample in a known amount and is measured in a similar manner to that of the analytes. The purpose of the internal standard is to provide a basis for the peak area ratios used in quantitation and to monitor instrument performance for each sample analysis.

3.3. Internal Standard Stock Diluent (ISD) – A concentrated solution of internal standard that is prepared in a mixture of 3:1, methanol:chloroform. This stock diluent is used to prepare the internal standard working diluent (IWD).

3.4. Internal Standard Working Diluent (IWD) – A solution of internal standard that is prepared from the ISD 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.

3.5. Surrogate Standard (SS) – A pure analyte, which should not be found in any sample, but is similar in nature to the compounds of interest. This compound can be added to a sample in a known amount before processing to monitor method performance for each sample.

3.6. Surrogate Stock Diluent (SSD) – A concentrated solution of surrogate standard that is prepared in acetonitrile. This stock diluent is used to prepare the surrogate working diluent (SWD).

3.7. Surrogate Working Diluent (SWD) – A solution of surrogate that is prepared from the SSD and is added to all samples. This working diluent is used to monitor method performance.

3.8. Sample Batch – A group of sample extracts that are processed together as a unit using the same procedure and materials. A typical batch consists of 20 samples. A batch includes applicable quality controls, such as method blanks, method blank spikes, matrix spikes, duplicates, or quality control samples.

3.9. System Blank (SBLK) – A portion of appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.

3.10. Method Blank (MB) – An aliquot of appropriate pure matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The method blank is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.11. 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). A CCV or CCR may also serve as an LCS for this procedure. A separate LCS does not have to be included in an analytical batch when either of the designated CCV or CCR samples meets the LCS criteria.

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 is used to determine whether the sample matrix contributes bias to the sample results. The MS is also used to evaluate the accuracy of the methodology in the same way that the MBS is used.

3.13. Matrix Spike Duplicate Sample (MSD) – A second portion of actual sample used to prepare the MS that is spiked and processed just like the MS. The MS and MSD are used together to measure the precision of the methodology.

3.14. Limit of Detection (LOD) – The calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero. Acronym: Method Detection Limit (MDL).

3.15. Limit of Quantitation (LOQ) – The minimum concentration that can be quantitatively reported for a target analyte. This limit can be no lower than the lowest calibration standard.

4.0. Health and Safety Warnings

4.1. The toxicity and carcinogenicity of each chemical used in this method have not been defined. All chemicals must therefore be treated as potential health hazards, and exposure must be limited to the lowest level possible.

4.2 Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling, and disposal of solvents, reagents, and standards. SDSs are located within the laboratory. These guidelines must be made available to all personnel involved in the chemical analyses.

4.3 Appropriate lab coat, safety glasses and gloves must be worn when performing standard or sample preparations, working on instrumentation, disposing of waste, and cleaning glassware.

4.4 The fume hood must be used when preparing standards, reagents, or samples that require proper ventilation.

5 Interferences

5.1 Solvents, reagents, glassware, and other sample processing apparati may lead to artifacts or elevated baselines in HPLC chromatograms. The processing of method blanks is used to verify the absence of any interferences.

5.2 All glassware is washed following the procedures described below and, if applicable, verified to be free from background contamination. Store all glassware and processing apparati in a clean environment to minimize possible contaminants.

5.2.1 Glassware used in the laboratory is cleaned following laboratory specific procedures.

5.2.1.1 Rinse with the last solvent used.

5.2.1.2 Wash in hot water with Alconox® (or equivalent) detergent, using scrub brushes as appropriate. *NOTE: PROLONGED SOAKING OF GLASSWARE IN DETERGENT IS NOT RECOMMENDED!*

5.2.1.3 Rinse thoroughly with hot tap water.

5.2.1.4 Rinse with Organic Free Reagent Water.

5.2.1.5 Dry in an oven at 105 °C for a minimum of one (1) hour.

5.2.1.6 **Exception:** Large glassware, such as separatory funnels and their components (stoppers and stopcocks) and 1000-mL graduated cylinders are allowed to air dry, then rinse one time with acetone followed by two rinses with dichloromethane.

5.2.1.7 Upon removal from the oven, allow the glassware to cool to room temperature.

5.2.1.8 Syringes:

5.2.1.8.1 Immediately after use, rinse three times with the last solvent used.

5.2.1.8.2 If syringe will not be cleaned immediately, store in a mason jar containing a small amount of solvent.

5.3 Interferences may occur when a sample containing low concentrations of analytes is analyzed following a sample containing high concentrations of analytes. If this type of interference is believed to have occurred, system blanks must be run through the system until contamination is eliminated and the affected sample must be reanalyzed to verify results.

5.4 Matrix interferences may occur as a result of contaminants present in the sample. If matrix interference is believed to have occurred, it is recommended that a matrix spike be analyzed with the sample to verify the results. This may not always be possible, given the amount of sample that is provided for analysis.

6 Instrumentation, Equipment and Supplies

6.1 Sampling Equipment

6.1.1 Pre-cleaned 50-mL plastic bottles fitted with Teflon-lined screw caps.

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, 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 Reagents and Standards

7.1 Inorganic/Organic Chemicals – Chemicals are obtained from one of the major manufacturer's such as Sigma-Aldrich, VWR or equivalent. All chemicals are of reagent grade quality, unless specified in NYS DOH MML-300. 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.

7.1.1 Formic Acid 99% liquid, Fluka, or equivalent.

7.1.2 Ammonium Formate ≥ 99.0% purity, Fluka, or equivalent.

7.1.3 Chloroform, ACS reagent grade, or equivalent.

7.2 Solvents – All solvents used in sample preparation are HPLC grade. 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, J.T. Baker, or equivalent.

7.2.3 Reagent water from Barnstead Nanopure Diamond water purifier, High Purity HPLC Water or equivalent.

7.3 Matrix Reagents

7.3.1 Gold Hemp Oil Hemp meds or equivalent.

7.3.2 Olive Oil Sigma-Aldrich (Cat # 01514) or equivalent.

7.3.3 Propylene Glycol, J.T. Baker (Cat # U510-07) or equivalent.

7.3.4 Ethanol (Absolute), PHARMCO-AAPER (Cat # 111000200) or equivalent.

7.4 Standards – The standards currently used for potency analysis are purchased from Cerilliant, Cayman, and Sigma-Aldrich.

Stock standard solutions or neat materials may be purchased from any vendor. When available, standards/stocks materials are purchased from vendors which can provide NIST-traceable standards accompanied by a Certificate of Analysis.

7.4.1 The commercially available Cannabinoid standards from Cerilliant and Cayman are listed in Table 1 and Table 2, respectively. Table 3 lists the provider(s) of the IS and SS.

Table 1. – Cerilliant analytical reference standards

Standard	Catalog #	Concentration	Solvent
Cannabinol (CBN)	C-046	1.0 mg/mL	MEOH
Cannabidiol (CBD)	C-045	1.0 mg/mL	MEOH
Delta-9 Tetrahydrocannabinol (THC)	T-005	1.0 mg/mL	MEOH
Cannabigerolic Acid (CBGA)	C-142	1.0 mg/mL	MeCN
Cannabigerol (CBG)	C-141	1.0 mg/mL	MEOH
Cannabidivarin (CBDV)	C-140	1.0 mg/mL	MEOH
Cannabichromene (CBC)	C-143	1.0 mg/mL	MEOH
Cannabidiolic acid (CBDA)	C-144	1.0 mg/mL	MEOH

Table 2. – Cayman Chemical analytical reference standards

Standard	Catalog #	Concentration	Solvent
Cannabichromene (CBC)	16398	1.0 mg/mL	MEOH
Tetrahydrocannabivarin (THCV)	16397	1.0 mg/mL	MEOH
Cannabidiolic Acid (CBDA)	15673	1.0 mg/mL	MEOH
Tetrahydrocannabinolic Acid (THCA)	15679	1.0 mg/mL	MEOH
Cannabigerol (CBG)	15362	1.0 mg/mL	MEOH

Table 3. – Cayman Chemical and Sigma-Aldrich analytical reference standards

Norgestrel (IS)	10006319	n/a	Solid
4-pentylphenyl 4-methybenzoate (SS)	665754	n/a	Solid

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**.** Syringes are compliant with relevant standard requirements.

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. Glassware is compliant with relevant standard requirements.

8 Preparation of Reagents, Solutions, Standards, and Samples

8.1 Standards, Surrogate and Internal Standards are prepared as per NYS DOH MML-300.

8.2 Medical Marijuana products (stored as per instructions provided by the RO.)

8.3 Tincture and Olive Oil Matrix

8.3.1 Tincture matrix with hemp oil spike preparation (stored at -20 °C for up to 6 months)

8.3.1.1 Tincture matrix is prepared using Gold grade RSHO hemp oil. The instructions below are for demonstration, and weights can vary as long as the proportions remain the same. This preparation should yield 10 g of a tincture matrix.

8.3.1.2 Tincture matrix preparation:

8.3.1.2.1 Hemp Oil	~0.4-1.0 g
8.3.1.2.2 Propylene Glycol	~5.1 g
8.3.1.2.3 Ethanol (Absolute)	~4.4 g

8.3.1.3 The tincture matrix preparation is sealed and placed into a water bath for about 1 minute at 50 ^{o}C. Afterward, the tincture preparation is shaken and/or vortexed for several minutes to insure that it is uniform.

8.3.1.4 The resulting tincture matrix has the following approximate composition:

8.3.1.4.1 Hemp Oil	~4-10 % wt/wt
8.3.1.4.2 Propylene Glycol	~50 % wt/wt
8.3.1.4.3 Ethanol (Absolute)	~40 % wt/wt

8.3.2 Olive oil matrix with hemp oil spike preparation (stored at -20 °C for up to 6 months)

8.3.2.1 Olive Oil matrix is prepared using Gold grade RSHO hemp oil. The instructions below are for demonstration, and weights can vary as long as the proportions remain the same. This preparation should yield 10 g of olive oil matrix.

8.3.2.2 Olive Oil Matrix preparation:

8.3.2.2.1 Hemp Oil	2-5 g
8.3.2.2.2 Olive Oil	5-8 g

8.3.2.3 The olive oil matrix preparation is sealed and placed into a water bath for about 1 minute at 50 °C. Afterward, the olive oil matrix is shaken and/or vortexed for several minutes to insure that it is uniform.

8.3.2.4 The resulting olive oil matrix has the following approximate composition:

8.3.2.4.1 Hemp Oil	20-50 % wt/wt

8.4 Sample Preparation Procedure

8.4.1 A direct dilution method is used for sample extracts that are oil-based and contain more than 1% cannabinoids. This direct dilution method will be used for all samples unless problems become apparent with a specific type of sample matrix.

8.4.2 Approximately 10-200 mg of sample matrix and/or medical marijuana product extract (the actual sample amount will be dependent on the cannabinoid concentrations of the various medical marijuana products) is weighed in a tared centrifuge tube.

8.4.3 The surrogate amount of 0.010 to 0.100 mL is spiked into the centrifuge tube. The amount is based on the RO's reported cannabinoid content of the sample. Typically, 5-10 µL of surrogate stock diluent at a concentration of 50 mg/mL is spiked into the sample (see NYS DOH MML-300).

8.4.4 Add 20.00 mL of methanol and mix well for 30 min to achieve extraction of the cannabinoids.

8.4.5 Transfer 1 mL of extract into 1.5-mL centrifuge tube, and centrifuge at 12,000 x g for 5 min.

8.4.6 Dilute the supernatant up to 100-fold with methanol for the potency measurement. The dilution factor must be determined based on the RO's reported cannabinoid

concentrations in the sample. This is necessary to insure that the final analytical values fall within the concentrations specified by the calibration curves. It may be necessary to analyze the samples twice. A larger dilution may be needed to analyze high-concentration cannabinoids, whereas a direct injection or smaller dilution may be needed to measure the cannabinoids present at lower concentrations within the same sample.

8.4.7 Transfer 500 µl of Internal Standard Working Diluent preparation (IWD) @ 10 µg/mL (prepared in section 8.3 of NYS DOH MML-300) into a 1.5-mL HPLC vial.

8.4.8 Transfer 500 µl of diluted sample supernatant into an HPLC vial prepared in Section 8.4.6 and mix well with IWD, providing a 1:1 ratio.

8.4.9 Follow the NYS DOH MML-300 Method for sample analysis and data reporting.

9 Shipping Conditions, Receiving, Preservation, and Storage

9.1 Sample shipping conditions:

9.1.1 The medical marijuana products from the Registered Organization (RO) are shipped as per manufacturer's specifications and must adhere to all regulatory requirements.

9.2 Sample receiving conditions:

9.2.1 Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met.

9.3 Sample and sample extract storage:

9.3.1 All medical marijuana products must be stored under the conditions recommended by the manufacturer prior to extraction. The storage is documented.

9.3.2 Prior to analysis, the extracts are stored in a freezer at -20 °C unless otherwise noted (See section 8.0).

9.3.3 Cannabinoids are light-sensitive; samples must be protected from the light.

9.3.4 The medical marijuana product, product extract location and product extraction date must be traceable in documentation.

10 Quality Control and Assurance

10.1 Demonstration of Capability (DOC)

10.1.1 Routine Samples

10.1.1.1 All laboratory staff must perform an initial demonstration of capability in using the extraction procedures described in this method. The initial DOC must consist of the analysis of four or five extracted spike samples that have been fortified with all analytes of interest at a mid-level concentration. The spiking

solution used must be from a source independent from those used to prepare the calibration standards.

10.1.1.2 The initial DOC may be completed by extracting four to five method blank spike samples prepared specifically for a DOC under the supervision of a trained technician. The DOC must meet all acceptance criteria, as described in the analytical method (NYS DOH MML-300), before the technician may perform the method without supervision.

10.1.2 Annually, each applicable analyst must complete a DOC for each target analyte in each of the matrices (See NYS DOH MML-300).

10.1.2.1 Acceptable performance of an initial demonstration of capability as described in 10.1.1.

10.1.3 If major changes to the method or instrumentation 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 Section 10.1.1. Minor changes to the method are evaluated using the extracted spikes, routine samples or the secondary source standards per (NYS DOH MML-300).

10.2 Method Detection Limits

10.2.1 A method-specific LOD study must be completed for all target analytes in each matrix, as listed in NYS DOH MML-300, Section 1.1 and NYS DOH MML-301, Section 8.0, on each instrument used to analyze sample extracts. An LOD study consists of the analysis of seven to ten matrix blank samples that have been fortified with all target analytes at no more than twice the laboratory's anticipated minimum reporting limit. The fortified samples must be treated as real samples and taken through all applicable method procedures. It is recommended that the fortified matrix blank samples be prepared and analyzed over a period of several days, so that day-to-day variations are reflected in the precision data; however, this is not a requirement.

10.2.2 Calculate the mean recovery and standard deviation for each analyte. Use the following equation to calculate the LOD:

LOD = standard deviation of the n samples (µg/mL) * t^1

t^1 is the value for the 99% confidence level with n-1 degrees of freedom where n is the number of replicates (Table 4).

Table 4.

n	t
7	3.143
8	2.998
9	2.896
10	2.821

10.2.3 The LOD for each cannabinoid is then used to calculate a LOQ for each cannabinoid by multiplying the LOD by 5.

Equation 1

$$LOQ = LOD * 5$$

10.2.4 The LOQ for each cannabinoid in each matrix must then be verified by extracting and analyzing a LCS that has been spiked at no more than twice the LOQ. The measured value should be within 80-120% of the expected value.

10.2.5 Annually, or when minor changes to the method are made, an LOQ verification must be completed as described in NYS DOH MML-300, Section 11.2.4 to demonstrate continued sensitivity at the LOQ.

10.2.6 A new LOD study must be verified annually on each instrument for each method, matrix and analyte. In addition, LODs are also determined each time there is a significant change in the test method or instrument type.

10.3 Batch-Specific Quality Control

10.3.1 The batch size consists of a maximum of 20 samples. The following quality control samples must be extracted, when applicable, with each preparation of a batch of samples at the prescribed frequency:

10.3.1.1 Method Blank, one (1) per extraction batch or every (20) samples.

10.3.1.2 Method Blank Spike, one (1) per batch or every twenty (20) samples.

10.3.1.3 Matrix Spike, one (1) per batch or every twenty (20) samples, if sample is provided.

10.3.1.4 Laboratory Control Sample (LCS) one (1) per batch or every twenty (20) samples.

10.3.2 Refer to the analytical method (NYS DOH MML-300) for information on the quality control measures, the applicable acceptance criteria, and the corrective actions for nonconforming data.

11 Data Acquisition, Reduction, Analysis, and Calculations

11.1 Not applicable; refer to the appropriate analytical method (see NYS DOH MML-300).

12 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.

12.2.1 All other solvents are separated into two categories, chlorinated and non-chlorinated, and are disposed of in red, 5-Gallon solvent cans.

12.3 Dispose of non-hazardous water 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.

13 References

13.1 Measurement of Phytocannabinoids by HPLC-PDA, NYS DOH MML-300

13.2 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.