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**Medical Marijuana Sample Preparation Protocols for Potency Analysis
NYS DOH MML-301**



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Table of Contents

1.0.	Scope and Application	3
2.0.	Summary of the Method.....	3
3.0.	Definitions.....	3
4.0.	Health and Safety Warnings	6
5.0.	Interferences.....	6
6.0.	Equipment and Supplies	7
7.0.	Reagents, Standards and Matrix (Consumables).....	8
8.0.	Preparation of Reagents, Solutions, Standards, Matrices and Samples.	9
9.0.	Quality Control/Assurance	11
10.0.	Data Acquisition, Reduction, Analysis and Calculations	13
12.0.	Waste Management/Pollution Prevention	13
13.0.	References.....	14
14.0.	Appendices	15
15.0.	Addendum	19



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1.0. Scope and Application

- 1.1. This method (NYS ELAP Method ID 9981) addresses the extraction of medical marijuana (MM) samples for cannabinoid analysis by High Performance Liquid Chromatography (HPLC) with Photodiode Array (PDA) Detection. It contains information specifically relevant to the extraction and preparation of MM products in **Section 8.0**. This preparation method (**NYS DOH MML-301**), is used in conjunction with the analytical method, *Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA* (**NYS MML-300**), in support of cannabinoid analyses required per Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations, of the State of New York. Refer to the analytical procedure (**NYS DOH MML-300**) for information on analyte list, calibration, analysis, quality control and data reporting.
- 1.2. This method is restricted to use by or under the supervision of analysts experienced in the preparation of MM 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 MM product, typically from 10 to 1200 mg, is weighed into a 50-mL centrifuge tube. The amount weighed depends upon the specific product produced by a Registered Organization (RO) and the declared concentrations of cannabinoids in the MM 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 MM sample as declared by the RO. 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 **NYS DOH MML-300**).
- 2.2. It's important to note that MM products are distinguished by brand and form (see **Section 3.0 Definitions**). Based on the current regulations, approved medical marijuana products shall be limited to the forms of administration approved by the Department, including but not limited to: metered liquid or oil preparations; solid and semisolid preparations (e.g. capsules, chewable and effervescent tablets, lozenges); metered ground plant preparations; whole flower; topical forms and transdermal patches. Medical marijuana may not be incorporated into food products by the registered organization, unless approved by the commissioner.

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.



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- 3.2. Internal Standard (IS) – A pure compound that should not be found in any sample. The IS is a compound added to both samples and standards at a known concentration to provide a basis for peak area ratios used in quantitation. The IS is also used to monitor instrument performance for each analysis and to correct for solvent evaporation during the analysis.
- 3.3. Internal Standard Stock Diluent (ISD) – A concentrated solution of IS that is prepared in solvent. This stock diluent is used to prepare the internal standard working diluent (IWD).
- 3.4. Internal Standard Working Diluent (IWD) – A solution of IS 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 (SUR) – 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 SUR that is prepared in acetonitrile (MeCN). This stock diluent is used to prepare the surrogate working diluent (SWD).
- 3.7. 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.
- 3.8. System Blank (SBLK) – A portion of appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.
- 3.9. 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.
- 3.10. Continuing Calibration Verification Standard (CCV) – One of the primary calibration standards used to verify the acceptability of an existing calibration.
- 3.11. Cross Check Reference Standard (CCR) – A solution of method standards prepared from a stock standard solution that is obtained from a source that is independent of that used to prepare the calibration standards (i.e. independent vendor, independent lot, or independent preparation). The CCR is used to verify that the original calibration source is acceptable.
- 3.12. 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).



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- 3.13. 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.
- 3.14. Matrix Spike Duplicate Sample (MSD)** – A second portion of the sample that was used to prepare the MS that 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.
- 3.15. Limit of Detection (LOD)** – The statistically 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).
- 3.16. 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.
- 3.17. 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 medical marijuana samples of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.
- 3.18. Analytical Batch** - An analytical batch consists of prepared samples which are analyzed together as a group. An analytical batch can include prepared samples originating from different matrices and can exceed twenty samples.
- 3.19. Brand** - A defined medical marijuana 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.
- 3.20. Form** - A type of a medical marijuana product approved by the commissioner that shall refer to the final preparation of an approved medical marijuana brand; for example, an extract in oil for sublingual administration, an extract for vaporization or an extract in a capsule for ingestion.
- 3.21. Inactive ingredients** - *Inactive ingredient* means any component other than an *active ingredient*.

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. SDSs are located within the laboratory in labeled, yellow binders. 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.

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 **NYS DOH MML-300**.
- 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. Before using new glassware or equipment the first time, wash with hot water and detergent, rinse with tap water and reagent water and final rinsing with methanol.
 - 5.2.2. All routine glassware and processing apparatus must be thoroughly cleaned. After each use, rinse all glassware and processing apparatus three times with the last solvent used and dry in a clean area to prevent cross-contamination. If glassware contamination is suspected wash as 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 are sometimes held propriety by RO’s. 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. If additional sample is not available for reanalysis, the original results must be qualified on the final report.
- 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, 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, Standards and Matrix (Consumables)

7.1. Inorganic Chemicals – Chemicals are obtained from one of the major manufacturers such as Sigma-Aldrich, VWR or equivalent. All inorganic chemicals are of reagent grade quality, unless specified in **NYS DOH MML-300**, 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 (**NYS DOH MML-300**, 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- MM excipients or reagents that may be used as a “representative matrix” for matrix evaluation are listed below.

7.3.1. Medium-chain triglycerides (MCT), Warner Graham (Cat # 812N) or equivalent.

7.3.2. New matrices and excipients may also be provided by the ROs for evaluation.

7.4. Standards – Standards potency analysis are currently purchased from Cerilliant, Cayman, Restek, Sigma-Aldrich or equivalent (**NYS DOH MML-300**, 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 **NYS DOH MML-300**, see **Section 8.0**.

8.2. RO excipient materials and blank matrix (stored as per RO instructions).

8.3. Sample extract preparation procedure (including MB, MS, MSD, LCS):

8.3.1. A direct dilution method is applied for most of the MM 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 MM product to be extracted is based on the RO brand. The weight of matrix and/or medical marijuana 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 medical marijuana product extract, usually from 10 to 1200 mg, is weighed into a 50-mL centrifuge tube. Depending on form, alternate preparation steps may be required (See **MML-301-AppA**).

8.3.3. The volume of surrogate, 0.005 to 0.040 mL is spiked into the 50-mL centrifuge tube. The amount 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.

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 50-mL centrifuge tube and, mix well for 30 minutes on a shaker to extract the sample.
- 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
- 8.3.4.2.** The following modifications may be made to increase the extraction efficiency where analysis requires using high sample volumes of the product due to low cannabinoid concentration (for example Balms containing < 1% cannabinoids):
- 8.3.4.2.1. Once extracted with 20 ml methanol place the samples in -20 °C freezer overnight. Afterwards remove from the freezer and sonicate the samples for 15 minutes.
- 8.3.4.3.** The final concentration of the cannabinoids in medical marijuana 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 RO. 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.
- 8.3.4.4.** Follow **MML-300-SOP** as per **Section 11.0** for MB, MS and MSD preparation.
- 8.3.4.5.** Sample extracts (**section 8.3.4**) are stored in a freezer at $\leq -20^{\circ}\text{C}$ until analysis is final. (**MML-300-SOP** see **Section 9.5**)
- 8.3.4.6.** 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 (**NYS DOH MML-300** see **Section 8.0**).



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- 8.3.6. Transfer 500 μ L 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 **NYS DOH MML-300** 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 **NYS DOH MML-300**, 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 **NYS DOH MML-300 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 **NYS DOH MML-300** (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 **NYS DOH MML-300, Section 11.0**. Refer to **NYS DOH MML-300, 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 (**NYS DOH MML-300, see Section 11.0**).



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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 **MML-301-SOP, see Section 7.3**), on each instrument used to analyze sample extracts. If the laboratory intends to report results below the LOQ, an ongoing LOD verification is also required.
- 9.2.2. Based on the LOD, the laboratory shall select an LOQ that is greater than the LOD (typically 3-5x the LOD) and consistent with the needs of its client. An LOQ is required for each representative matrix, method and analyte combination. For each method, the lowest calibration standard concentration must be at or below the corresponding LOQ.
- 9.2.3. An initial LOQ study for each method must be completed and documented for all target analytes in each representative matrix. The initial LOD samples may be used for this purpose as long as the concentration used is at or below the LOQ. The mean recovery shall be within 70-130% of the spiked value.
- 9.2.4. 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 quarter 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%.
- 9.2.5. The 2017 Method Update Rule finalized in the Environmental Protection Agency's (EPA's) Federal Register on August 28, 2017, prescribes a revised approach to Method Detection Limit (MDL)/LOD data collection and calculation per Part 136 Appendix B. The New York State (NYS) Environmental Laboratory Program (ELAP) requires that the revised procedure detailed within the EPA's document *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016* be implemented for all NYS ELAP accredited methods.

9.3. Extraction (Preparation) Batch-Specific Quality Control

- 9.3.1. The preparation batch size consists of a maximum of 20 medical marijuana samples (see **Section 3.17**). The following quality control samples must also be extracted, where applicable, at the prescribed frequency:
 - 9.3.1.1. Method 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.
 - 9.3.1.3. Laboratory Control Sample (LCS) one (1) per preparation batch. The following may also meet the LCS requirement.



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

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

- 11.1. Medical Marijuana Products from Registered Organizations are received, handled, verified and documented ensuring method regulatory and Accreditation Body requirements are met.
- 11.2. Follow instructions provided by the RO for storage prior to sample extraction.
- 11.3. Prior to analysis, the extracts are stored in a freezer at $\leq -20^{\circ}\text{C}$ unless otherwise noted (**NYS DOH MML-300** see **Section 9.0.**)
- 11.4. 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.
 - 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 aqueous waste in the laboratory sink followed by flushing with tap water.



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- 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

- 13.1. Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations, of the State of New York.
- 13.2. Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (NYS DOH MML-300)
- 13.3. 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.
- 13.4. *Norgestrel*; MSDS No. N2260 [Online]; Sigma-Aldrich: Saint Louis MO, September 03, 2014 <http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do>
- 13.5. 21 CFR 210.3(b)(8) – Definitions. Current Good Manufacturing Practice in Manufacturing. Code of Federal Regulations. Title 21, Chapter 1, Subchapter C.
- 13.6. Alternate Approved Preparation Steps/ Method Validation Summary by Form (MML-301-AppA)
- 13.7. New York State (NYS) Department of Health (DOH) Environmental Laboratory Approval Program website, <https://www.wadsworth.org/regulatory/elap/medical-marijuana>



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14.0. Appendices

Appendix A (MML-301-AppA) – Alternate Approved Preparation Steps/ Method Validation Summary by Brand/Form (see MML-301-SOP, section 8.3)

1. Product/Form – Metered Dose Inhaler

a. Method Development Narrative/Background

- i. The metered dose inhaler (MDI) is a device typically used for inhaled respiratory medications. MDI is one of the approved forms of medical marijuana products in New York State (NYS) for the certified patients. It is a pressurized container with specific formulation in a plastic holder with a mouthpiece. When sprayed, it gives a reliable, consistent dose of medication. Our current sample preparation method is not suitable for preparation of MDI testing samples. Herein we developed a new procedure to address MDI sample preparation.
- ii. The alternate preparation avoids potential contamination during the transfer of the product (i.e. metal particulate contamination) and allows quicker sampling of product by the separate labs (organic chemistry, inorganic chemistry, and microbiology).
- iii. Following the sample preparation steps listed below, five duplicates sample were prepared from Inhaler # 2.
- iv. The Medical Marijuana Laboratory (MML) also prepared two other preparations as benchmarks (the controls) to compare against the alternative procedure proposed below.
- v. Benchmark # 1 involved preparing Inhaler # 1 by the procedure sent from the RO (cooling, puncturing, warming, pouring into vial, prep as a liquid). For this calculation, 17.6 mg is used as the dose ($1.848 \text{ g EtOH mix per can} \div 105 \text{ doses per can}$).
- vi. Benchmark # 2 involved preparing Inhaler # 2 in the finalized alternative procedure outlined below, except the inhaler was sprayed into a small zip-lock style bag (50 x 75 mm). The flexible bag seals around the inhaler mouthpiece and inflates to trap the gas/alcohol/distillate. The bag is then rinsed with 10 mL Methanol (MeOH) twice, then fully submerged in the solvent in the tube. The second benchmark provides a best-case preparation as the entire spray is contained within the bag.

b. Analysis of Results:

i. Table 1 – Summary of Results

Table 1. Prep	1x (mg)	2x (mg)	3x (mg)	4x (mg)	5x (mg)	Average	%RSD	% of Benchmark 1 (RO Prep)
BM 1 (RO)	4.53	--	--	--	--	4.53	--	--
BM 2 (Bag)	4.66	--	--	--	--	4.66	--	102.9%
Inhaler 2 (Tube)	4.08	4.82	4.56	4.58	4.73	4.55	6.3%	100.5%

- ii. Table 1 lists the measured results of the potency (THC, mg/dose). The finalized procedure is within 5% of both the RO preparation and the best-case preparation. There should not be a noticeable difference in preparation between these methods. The laboratory then investigated the reproducibility between different devices and analysts, and since we already have data on the extraction method, the reproducibility was determined only from the mass dispensed. Using two analysts, they each sprayed a single actuation from inhalers 2, 3, 4, and 5, repeating five times per inhaler (20 weights per analyst, 40 overall). Analyst 1 sprayed an average mass of $46.8 \text{ mg} \pm 1.46$ (3.1 %RSD). Analyst 2 sprayed an average mass of $47.1 \text{ mg} \pm 1.19$ (2.5 %RSD). The relative percent difference between the two averages was 0.65%. There is not a noticeable difference between inhalers (%RSD <5%) or between analysts (%RPD <5%).

c. Procedure:

- For Metered Dose Inhalers (MDI) where part of the formulation includes a gas (EX: Freon) a mass of material removed from the inhaler is used, not a measured mass of collected material.
- Shake the inhaler and prime the unit at least twice by spraying into a waste container. If the inhaler has already been used for testing, priming is not necessary.
- Place the inhaler on a balance and tare it. Shake and spray a single actuation into a tube by placing the mouthpiece within the opening of the tube, and keeping the inhaler upright, in an "L" shape. Cap quickly after spraying.
- Weigh the inhaler on the tared balance (the value will be negative). The absolute value of this mass is the value used for calculating potency, not the mass of sample in the tube

2. Product/Form - Chewables

a. Method Development Narrative/Background

- i. Matrices that contain methanol insoluble materials prevent a challenge for good recovery of cannabinoids during sample preparation in the MML-300/301 methods. 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. A roughly 30% cannabinoids by weight 1:1 THC:CBD vape oil preparation of accurately determined concentration by the analytical method (MML-300) 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 method MML-300. 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 MML-300. 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 ±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. This agreement is within the $\pm 10\%$ acceptable range and is acceptable to comply with NYS Medical Marijuana Regulation 1004.11(c)(3).

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 MML-300.

3. 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>
- iii. A 3-5 g sample of the homogenized flower is set aside for Medical Marijuana Laboratory potency and contamination testing.

b. Procedure:

- i. A statistical number of samples are prepared from the homogenized flower sample as per MML-301-SOP.
- ii. The potency and % C_s is reported as an average of the pooled specimen sample. For this test, only one pooled result is reported.



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15.0. Addendum

GUIDANCE REGARDING FLEXIBILITY INHERENT TO MM METHODS AND THE PRECEDENCE OF QUALITY CONTROL CRITERIA

The specific products and instrument settings cited in the DOH methods represent those products and settings used during method development or subsequently evaluated by the Department for use in the method. Glassware, reagents, supplies, equipment and settings other than those listed in this method may be employed, provided that method performance appropriate for the intended application has been documented. Such performance includes consideration of precision, accuracy (or bias), recovery, representativeness, comparability, and sensitivity (quantitation or reporting limits, now referred to as limit of quantitation (LOQ) relative to the reporting or regulatory limits) for the intended use of the analytical results.

In response to this inherent flexibility, if an alternative analytical procedure is employed, then the Department expects the laboratory to demonstrate and document that the procedure is capable of providing appropriate performance for its intended application. This demonstration must not be performed after the fact, but as part of the laboratory's initial demonstration of proficiency (or capability; iDOC) with the method. The documentation should be in writing, maintained in the laboratory, and available for inspection upon request by the Department. The documentation should include the performance data as well as a detailed description of the procedural steps as performed (i.e., a written standard operating procedure).