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Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA
NYS DOH MML-300

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1.0. Scope and Application

1.1. This method (NYS ELAP Method ID 9980) is to be used for the analysis of cannabinoid profiles in medical marijuana (MM) products. The method is for the determination of concentrations of the cannabinoids listed below (**Table 1**) as required by the New York State (NYS) medical marijuana regulations delineated in 10NYCCR § 1004.11(c)(2).

Table 1. Analyte List

Analyte	CAS Number	LOQ[1] MCT Matrix (µg/mL)
Cannabichromene (CBC)	20675-51-8	0.60
Tetrahydrocannabivarin (THCV)	31262-37-0	0.60
*Cannabidiolic Acid (CBDA)	1244-58-2	0.60
*Tetrahydrocannabinolic Acid (THCA)	23978-85-0	1.00
Cannabigerol (CBG)	25654-31-3	0.60
Cannabinol (CBN)	521-35-7	0.60
*Cannabidiol (CBD)	13956-29-1	0.60
*Delta-9-Tetrahydrocannabinol (Delta-9-THC)	1972-08-3	0.60
*Delta-8-Tetrahydrocannabinol (Delta-8-THC)	5957-75-5	0.90
Cannabidivarin (CBDV)	24274-48-4	0.60
4-Pentylphenyl 4-Methylbenzoate (Surrogate)	50649-59-7	0.60
Norgestrel (Internal Standard)	6533-00-2	N/A

[1] The Limit of Quantitation (LOQ) is the lowest concentration that can be accurately quantified for a target analyte (**Section 3.15**). LOQs were determined with medium-chain triglycerides (MCT) as the matrix. LOQs referenced within **Table 1** are subject to change based on LOD/LOQ determinations detailed within **Section 11.2**.
*Major analytes of interest see **Section 11.1.1.1.2**

1.2. This method is restricted to use by or under the supervision of analysts experienced in the use of high-performance liquid chromatography with photodiode array detection (HPLC-PDA) and the interpretation of ultra-violet (UV) spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in **Section 11.1**

1.3. This procedure covers only the analysis of phytocannabinoids by using HPLC-PDA. It does not contain procedures relevant to sample extraction or the purification of sample extracts. Details of sample preparation are contained in **MML-301-SOP**.

2.0. Summary of the Method

2.1. Samples from each lot of MM product are diluted/dissolved with organic solvents (See **MML-301-SOP** for sample preparation details)**.** The diluted samples fortified with internal standard (IS) are injected onto an HPLC. The targeted analytes are separated and subsequently detected online by monitoring UV absorbance using a PDA detector. The separation of nine cannabinoids is achieved on a C18 reversed-phase column 150 mm in length. Based on the summary data provided in **Table 1**, the limit of quantification (LOQ) for most of the cannabinoids is approximately 0.60 µg/mL. This method can be used to quantify the cannabinoid components that are present as low as 0.04% (percent by weight; the actual values for various MM products are dependent on how much material is used for testing) in the MM products.

2.2. 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; and 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.

3.2. Internal Standard (IS) – A pure compound that should not be found in any sample. The 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 also used to monitor instrument performance for each analysis and to correct for solvent evaporation during the analysis.

3.3. Internal Standard Working Diluent (IWD) – A solution of IS that is prepared from the IS that is added to all samples at the same concentration. This working diluent is used to dilute the samples and to monitor the integrity of the sample injections.

3.4. 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 can be 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.

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

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

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

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

3.9. Calibration Standard (CalS) – A solution of method analytes prepared from stock or working standard solutions used to calibrate the instrument response with respect to analyte concentration.

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 pure matrix that is spiked with known quantities of target analytes and processed as if it were a sample. The LCS is used to evaluate the accuracy of the methodology. Acronyms include: Method Blank Spike and Laboratory Fortified Blank.

3.13. Matrix Spike Sample (MS) – An aliquot of sample prepared, taken through all sample preparation and analytical steps of the procedure unless otherwise noted in a referenced method, by adding a known amount of target analyte to a specified amount of sample for which an independent test result of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency. When sample is not suitable, a "representative" matrix may be used instead. Synonym: Laboratory Fortified Sample Matrix.

3.14. 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 analogous manner to the MS. The MS and MSD are used together to determine the precision of the methodology.

3.15. Lower Limit of Quantitation (**LOQ**) – The minimum concentration that can be quantitatively reported for a target analyte. For routine analyses, the lowest calibration

standard must be at or below the **LOQ** for each analyte. **LOQ** is typically 3-5 times the **LOD**. Synonym: Method Reporting Limit (MRL).

3.16. 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.

3.17. Demonstration of Capability (DOC) – a procedure to establish the ability of the analyst to generate acceptable accuracy and precision using the method.

3.18. 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 method blanks, LCS, matrix spikes and matrix duplicates) of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.

3.19. 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.

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 a 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 **Section 11.4**.

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 perform a final rinse 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 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 avoid 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 and eliminated, if possible.

5.4. Interfering contamination known as “carryover” 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 system blank 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 because of excipients present in the sample. If a matrix interference is believed to be present, the sample may be spiked with target analytes and analyzed together with the nonspiked sample to verify the results. If these analyses verify the original results, report only the results from the original nonspiked 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.

5.7. See **Appendix A (MML-300-AppA)** for examples of blanks, compound retention times and elution order.

6.0. Instrumentation, Equipment and Supplies

(Vendors and catalog numbers are included for illustration only. These are examples of the products currently used in the laboratory. This is not a fully inclusive list, and inclusion should not imply product endorsement. Instrumentation, equipment and supply substitutions may be made provided that the substitutions meet the method criteria. Refer to MML-301-SOP for extraction related equipment and supplies.)

6.1. Standard and Sample Preparation Equipment

6.1.1. Syringes, various sizes.

6.1.2. Eppendorf pipets, various sizes.

6.1.3. Disposable Eppendorf tips, various sizes.

6.1.4. Positive pipet, Handy Step S.

6.1.5. Positive pipet tips of various sizes.

6.1.6. Centrifuge tubes, various sizes.

6.1.7. Class "A" volumetric flasks with stoppers, various sizes.

6.1.8. Disposable glass pipettes and bulbs.

6.1.9. 2-mL autosampler vials with Teflon-lined screw caps or vials with crimp-top caps.

6.2. Instrumentation

6.2.1. Analytical balance, Mettler-Toledo Model # 205DU

6.2.2. Sonicator, Branson, Model # 2510R-DTH.

6.2.3. Vortex, Maxi Mix 11 Model #37615.

6.2.4. Centrifuge, Model # 5415D.

6.2.5. Shaker, Labline, Model# 3540.

6.2.6. A complete HPLC system, equipped with a column oven which is suitable for use with a variety of columns, as well as all the required accessories including:

syringes, analytical columns, gases, detectors and a data system for instrument control and data analysis/processing.

6.2.6.1. Components of the Shimadzu HPLC system used:

6.2.6.1.1. Micro vacuum degasser, model # DGU-20A3.

6.2.6.1.2. Solvent selector model# FCV-11A2**.**

6.2.6.1.3. Pumps, model # LC-20ADxR.

6.2.6.1.4. Column oven, model # CTO-20A.

6.2.6.1.5. Autosampler, model # SIL-20ACxR.

6.2.6.1.6. System controller, model # CBM-20A.

6.2.6.1.7. Photodiode array detector, model# SPD-M20A.

6.2.6.1.8. Operating software, Shimadzu LabSolutions.

7.0. Reagents and Standards

7.1. Solvents (HPLC Grade) and reagents – All solvents and reagents must have records that trace their origins and preparations, including Certificates of Analysis, laboratory receipts and preparation records.

7.1.1. Methanol (MeOH) – HPLC grade.

7.1.2. Acetonitrile (MeCN) – HPLC grade.

7.1.3. Water – HPLC grade.

7.1.4. Acetone – HPLC grade.

7.1.5. Ammonium formate – 98+ % purity, Fluka catalog # 3272-02.

7.1.6. Formic acid – MSD grade, Sigma-Aldrich catalog # 39,938-8.

7.1.7. Miglyol – Miglyol 812, Warner Graham, catalog # 140325.

7.2. Stock standards

7.2.1. When available, stock standards are purchased from vendors who can provide NIST- traceable standards accompanied by Certificates of Analysis.

7.2.2. The commercial standards/materials listed in **Sections 7.2.4** (**Table 2**), **7.2.5** (**Table 3**), **7.2.6** (**Table 4**), **7.2.7** (**Table 5**) and **7.2.8** (**Table 6**) are examples of those currently used in the laboratory, alternate vendors may be used. This is not a fully inclusive list, and substitutions may be made if the criteria described above are met.

7.2.3. At a minimum, commercial standards/materials are stored per the manufacturer's recommended storage conditions and expiration dates are as prescribed by the vendor on their Certificate of Analysis.

7.2.4. Cerilliant analytical reference standards

Table 2.

Standard	Catalog #	Concentration	Solvent
CBN	C-046	1.0 mg/mL	MeOH
CBD	C-045	1.0 mg/mL	MeOH
Delta-9-THC	T-005	1.0 mg/mL	MeOH
Delta-8-THC	T-032	1.0 mg/mL	MeOH
CBG	C-141	1.0 mg/mL	MeOH
CBDV	C-140	1.0 mg/mL	MeOH
CBC	C-143	1.0 mg/mL	MeOH
CBDA	C-144	1.0 mg/mL	MeCN
THCV	T-094	1.0 mg/mL	MeOH
THCA	T-093	1.0 mg/mL	MeCN

7.2.5. Cayman Chemical analytical reference standard

Table 3.

Standard	Catalog #	Concentration	Solvent
CBN	ISO60183	1.0 mg/mL	MeOH
CBD	ISO60156	1.0 mg/mL	MeOH
Delta-9-THC	ISO60157	1.0 mg/mL	MeOH
Delta-8-THC	ISO60158	1.0 mg/mL	MeOH
CBG	20164	1.0 mg/mL	MeOH
CBDV	20165	1.0 mg/mL	MeOH
CBC	ISO60163	1.0 mg/mL	MeOH
CBDA	18090	1.0 mg/mL	MeCN
THCV	18091	1.0 mg/mL	MeOH
THCA	ISO60175	1.0 mg/mL	MeCN

7.2.6. Restek analytical reference standards

Table 4.

Standard Name	Catalog #	Concentration	Solvent
CBN	34014	1000 µg/mL	MeOH
CBD	34014	1000 µg/mL	MeOH
Delta-9-THC	34014	1000 µg/mL	MeOH

7.2.7. Sigma-Aldrich analytical reference standards

Table 5.

Standard	Catalog #	Concentration	Solvent
4-pentylphenyl 4-methybenzoate (SUR)	665754	n/a	Solid

7.2.8. Fluka analytical reference standards

Table 6.

Standard	Catalog #	Concentration	Solvent
Norgestrel (IS)	10006319	n/a	Solid

8.0. Preparation of Reagents, Solutions and Standards

8.1. General preparation information

8.1.1. All reagents, solutions and standards must be traceable to stocks and, when available, have NIST-traceable documentation. The preparation method, date of preparation, expiration date and analyst must also be traceable in laboratory documentation.

8.1.2. Standard preparation steps are offered for guidance only. Alternate preparations, concentrations and stock mixtures may be utilized provided that they meet the requirements detailed herein.

8.1.3. A syringe or positive pipet is used to deliver any volume of sample or standard that will be quantified in the analysis. Eppendorf pipets are used for transferring volumes only when quantification is not necessary. Replace pipet tips after each solution change.

8.2. IWD Preparations (from neat)

8.2.1. IWD preparation from neat standard @ 10 µg/mL. The IWD is prepared annually and stored at -20 °C.

8.2.1.1. The IWD is prepared directly from the neat material.

8.2.1.1.1. Weigh 10.0 mg of norgestrel (IS) into a 1-L volumetric flask.

8.2.1.1.2. Dilute to volume with MeOH.

8.2.1.1.3. Sonicate for 1-2 min or until all solids are in solution.

8.2.1.1.4. Invert several times to mix well and transfer to Wheaton bottles for storage.

8.3. SSD @ 50 mg/mL. The SSD is prepared annually and stored at -20 °C.

8.3.1. The SSD is prepared by weighing 500 mg of 4-pentylphenyl 4-methylbenzoate into a weighing vessel and transferring it into a 10-mL volumetric flask containing MeCN. The volumetric is then diluted to volume with MeCN.

8.3.2. The SSD is mixed well and labeled appropriately.

8.4. SWD @ 100 µg/mL. The SWD is prepared monthly and stored at -20 °C.

8.4.1. Transfer 200 µL of SSD prepared in **Section 8.3** into a 100-mL volumetric flask.

8.4.2. Fill to volume with MeOH, mix well and label.

8.5. Primary cannabinoid standard stock solution @ 90 µg/mL. The Primary cannabinoid standard is prepared annually and stored at -80 °C.

8.5.1. Aliquots (0.9 mL) of each standard solution (1000 µg/mL) purchased from vendors are added to a 10-mL volumetric flask.

8.5.2. Fill to volume with MeOH and invert 3 times to mix.

Table 7. (*The primary cannabinoid standard mixture is prepared in a 10-mL volumetric flask as a combined preparation)*

Standard number	Volume Added	Standard name	Catalog #	Diluted Concentration
1	0.9 mL	CBN	C-046	90 µg/mL
2	0.9 mL	CBD	C-045	90 µg/mL
3	0.9 mL	Delta-9-THC	T-005	90 µg/mL

4	0.9 mL	Delta-8-THC	T-032	90 µg/mL
5	0.9 mL	CBC	C-143	90 µg/mL
6	0.9 mL	THCV	T-094	90 µg/mL
7	0.9 mL	CBDA	C-144	90 µg/mL
8	0.9 mL	THCA	T-093	90 µg/mL
9	0.9 mL	CBG	C-141	90 µg/mL
10	0.9 mL	CBDV	C-140	90 µg/mL

8.6. Primary cannabinoid working solution and surrogate @ 45.0 µg/mL. The primary cannabinoid working solution is prepared annually and stored at -80 °C.

8.6.1. Take 1.00 mL of the primary cannabinoid stock solution at 90 µg/mL prepared in **Section 8.5,** place into a 2-mL volumetric flask and add 0.900 mL of SWD prepared in **Section 8.4.**

8.6.2. Fill to volume with MeOH and invert 3 times to mix.

8.7. A cross check reference standard (CCR) stock standard prepared as a secondary and separate cannabinoid stock solution using Cayman standards is prepared at 9.0 µg/mL. The CCR is prepared annually and stored at -80 °C.

Table 8. CCR (*This CCR is prepared in a 25-mL volumetric as a combined preparation)*

Standard number	Volume Added	Standard name	Catalog #	Diluted Concentration
1	0.225 mL	CBN	C-046	9.0 µg/mL
2	0.225 mL	CBD	C-045	9.0 µg/mL
3	0.225 mL	Delta-9-THC	T-005	9.0 µg/mL
4	0.225 mL	Delta-8-THC	T-032	9.0 µg/mL
5	0.225 mL	CBC	C-143	9.0 µg/mL
6	0.225 mL	THCV	T-094	9.0 µg/mL
7	0.225 mL	CBDA	C-144	9.0 µg/mL
8	0.225 mL	THCA	T-093	9.0 µg/mL
9	0.225 mL	CBG	C-141	9.0 µg/mL
10	0.225 mL	CBDV	C-140	9.0 µg/mL

8.7.1. Portions (0.225 mL) of each standard solution as purchased from vendors (1000 µg/mL) are added to a 25-mL volumetric flask.

8.7.2. Fill to volume with MeOH and invert 3 times to mix.

8.8. As needed, an additional CCR standard (Restek) is prepared @ 10 µg/mL to verify the calibration curve. The CCR (Restek) is prepared annually and stored at -80 °C.

The Restek stock standard contains only 3 Cannabinoids at 1000 µg/mL. Since this stock contains only 3 cannabinoids, it is used, when necessary, in addition to the CCR (Cayman) prepared in **Section 8.7**.

Table 9.

Standard Number	Volume used	Standard Name	Catalog #	Diluted Concentration
1	10 µL	CBN	34014	10 µg/mL
2	10 µL	CBD	34014	10 µg/mL
3	10 µL	Delta-9-THC	34014	10 µg/mL

8.8.1. 0.01 mL of standard solution mix as purchased from vendor at 1000 µg/mL is added to a 10-mL volumetric flask.

8.8.2. Fill to volume with MeOH and invert 3 times to mix.

8.9. Mobile Phases. The mobile phases are maintained at room temperature and must be prepared monthly.

8.9.1. HPLC mobile phase A: 25 mM ammonium formate, 0.1% formic acid in HPLC water.

8.9.1.1. 1.575 g of ammonium formate is added to a scintillation vial and dissolved with a small volume of HPLC grade water.

8.9.1.2. Ammonium formate solution prepared in **Section 8.9.1.1** is transferred to a 1-L volumetric flask. The scintillation vial is rinsed well with HPLC grade water and 1.0 mL of formic acid is added to the 1-L volumetric flask. Dilute to volume with HPLC grade water and mix well.

8.9.2. HPLC mobile phase B: MeCN containing 0.1% formic acid.

8.9.2.1. Add 1.0 mL of formic acid into a 1-L volumetric flask. Add MeCN to a total volume of 1-L and mix well.

8.9.3. Matrices – stored as per Manufacturer's recommendations.

Table 10.

Matrices	Manufacturer	Catalog #
Miglyol – Oil based matrix	Warner Graham Co	140325

8.9.3.1. MCT is used as a representative matrix in the preparation of DOC, LOD, LOQ, MS and MSD.

9.0. Shipping Conditions, Receiving, Preparation, Analysis and Storage

9.1. Sample shipping conditions

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

9.2. Sample receiving

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

9.2.2. All MM products must be stored under the conditions based on the manufacturer's recommendation. The storage conditions are documented.

9.2.3. All MM products must be stored under the conditions based on the manufacturer's recommendation. The storage conditions are documented.

9.3. Sample preparation

9.3.1. Follow MM sample preparation as per **MML-301-SOP** and document preparation of all samples.

9.4. Sample analysis:

9.4.1. Samples for analysis are placed into the autosampler, which is maintained at 4-10 °C. Samples are analyzed by HPLC-PDA using a Poroshell C18 column. Ultraviolet (UV) absorption spectra are recorded over the wavelength range of 190 to 800 nm. The absorbance at 227 nm is displayed to provide a chromatogram of the peaks representing the cannabinoid components, which are then integrated for analyte quantitation. Chromatography is achieved using the mobile phases described in **Section 8.9** and the instrumental parameters as outlined in **Tables 11** and **12**.

9.4.2. HPLC analytical parameters

9.4.2.1. Injector

9.4.2.1.1. The Injection volume is 10-µL.

9.4.2.2. Mobile phases

9.4.2.2.1. Mobile phase A: 0.1% formic acid in 25 mM ammonium formate (aqueous).

9.4.2.2.2. Mobile phase B: 0.1% formic acid in MeCN.

9.4.2.3. Column

9.4.2.3.1. Column: Agilent Poroshell 120, EC-C18, 3.0 x 150 mm, 2.7 µm particle size, Cat # 693975-302 or equivalent.

9.4.2.3.2. Column oven temperature: 30 °C.

9.4.2.4. HPLC conditions

9.4.2.4.1. HPLC: Flow rate of 0.625 mL/min with a gradient of mobile phase A/mobile phase B composition as shown in the **Table 11** for the Agilent Poroshell 120 column (the gradient may be modified depending on the column used).

9.4.2.4.2. These parameters serve as a guideline and may be adjusted to optimize separation if the quality performance criteria are met in **Section 11.0.**

Table 11. Mobile phase gradient

Time (min)	%Mobile A	%Mobile B
0.0	27	73
18.00	27	73
19.00	0	100
21.00	0	100
22.10	27	73
25.00	27	73

9.4.2.4.3. Data collection time: 18.0 min.

9.4.2.4.4. Total run time: 25.00 min.

9.4.2.5. PDA Detector

9.4.2.5.1. Wavelength scan range: 190 - 800 nm.

9.4.2.5.2. Wavelength for display and peak integration: 227 nm (If necessary, an alternate wavelength may be used).

9.5. Extract storage

9.5.1. Sample extracts (20 mL) are stored in a freezer at ≤ -20 °C until analysis, which must be completed within 7 days of extraction. After testing is completed, the remaining extracts are stored at ≤ -20 °C for one month for reanalysis if it is necessary.

9.6. Mobile phase storage

9.6.1. When they are maintained at room temperature, it is common practice to dispose of any aqueous mobile phases after one month. This is to prevent any microbial growth and changes in the mobile phases.

10.0. Calibration

10.1. Initial calibration

Examples of currently used instrumental integration parameters are listed below (see **Table 12)**. Results are calculated using peak area. These settings serve as guidelines and may be adjusted for optimization of integration. If interferences preclude use of IS (norgestrel), an external standard calibration may be used to calculate the results (see **Section 13.3 and Section 14.11**) provided that other quality performance criteria are achieved (see **Section 11.0**).

Table 12. Integration parameters for a calibration curve using absorbance at 227 nm wavelength

Description	Units	Value
Channel	nm	227
Width	s	1
Slope	µV/min	15500
Drift	µV/min	0
Time to double peak	min	1000
Minimum Area/Height	counts	1000

10.1.1. The primary standard stock solution prepared in **Section 8.5** is used to prepare calibration standards for the cannabinoids at concentrations appropriate for the range of the instrument and the sample content. A minimum of 5 calibration concentrations must be analyzed for each cannabinoid.

10.1.2. The lowest level calibration standard must be at or below the LOQ values listed in **Section 1.0, Table 1** for each analyte, or the LOQ values must be adjusted accordingly.

10.2. Primary cannabinoid stock standard curve preparation. The calibration curve standards are prepared monthly and stored at -20 °C for up to 2 months.
Concentration range for all compounds including the surrogate is 45.0 µg/mL to 0.46 µg/mL. It is highly recommended that positive pipets or glass syringes are used for calibration curve preparation (See **Table 13** for dilution schedules).

10.2.1. ***CalS-6*** **45.0 µg/mL**

10.2.1.1. Fill one 1.5-mL centrifuge tube with 1.00 mL of primary standard stock solution prepared in **Section 8.5** and surrogate prepared in **Section 8.4**. This solution is labeled as **CalS 6**.

10.2.2. ***CalS-5*** **18.0 µg/mL**

10.2.2.1. Transfer 400 µL **CalS 6** taken from the initial centrifuge tube in (see **Section 10.2.1.1)** into **CalS 5** vial.
10.2.2.2. Add 600 µL MeOH.
10.2.2.3. Mix well.

10.2.3. ***CalS-4*** **7.20 µg/mL**

10.2.3.1. Transfer 400 µL **CalS 5** taken from the HPLC vial (see **Section 10.2.2.3**) into **CalS 4** vial.
10.2.3.2. Add 600 µL MeOH
10.2.3.3. Mix well.

10.2.4. ***CalS-3*** **2.88 µg/mL**

10.2.4.1. Transfer 400 µL **CalS 4** taken from the HPLC vial (see **Section 10.2.3.3**) into **CalS 3** vial.
10.2.4.2. Add 600 µL MeOH
10.2.4.3. Mix well.

10.2.5. ***CalS-2*** **1.15 µg/mL**

10.2.5.1. Transfer 400 µL **CalS 3** taken from the HPLC vial (see **Section 10.2.4.3**) into **CalS 2** vial.
10.2.5.2. Add 600 µL MeOH
10.2.5.3. Mix well.

10.2.6. ***CalS-1*** **0.46 µg/mL**

10.2.6.1. Transfer 400 µL **CalS 2** taken from the HPLC vial (see **Section 10.2.5.3**) into **CalS 1** vial.
10.2.6.2. Add 600 µL MeOH
10.2.6.3. Mix well.

10.2.7. Prepare calibration standard mixtures with IWD prepared in **Section 8.2**

10.2.7.1. Following **Table 13** to prepare the standards in HPLC vials: from **CalS-IWD-1** through **CalS-IWD-6.**

Table 13. Dilution schedule of CalS-1 thru CalS-5
Initial Standard dilution schedule w/out IWD (for final standard prep in MeOH)

Cal std	Std conc ug/mL	Cannabinoid std ref. Section ID	Volume of cannabinoid std	MeOH
CalS-6	45	**Section 8.5**	-	-
CalS-5	18	**10.2.1.1**	400 µL	600 µL
CalS-4	7.2	**10.2.2.3**	400 µL	600 µL
CalS-3	2.88	**10.2.3.3**	400 µL	600 µL
CalS-2	1.15	**10.2.4.3**	400 µL	600 µL
CalS-1	046	**10.2.5.3**	400 µL	600 µL

Table 14. Final std dilution schedule mixed with IWD (for analysis on instrument).

Cal std w/IWD	Cal std (**Table 13**)	Volume of cannabinoid stock std	IWD As prepared in **Section 8.2**
CalS-IWD-6	CalS-6 (**10.2.1.1**)	500 µL	500 µL
CalS-IWD-5	CalS-5 (**10.2.2.3**)	500 µL	500 µL
CalS-IWD-4	CalS-4 (**10.2.3.3**)	500 µL	500 µL
CalS-IWD-3	CalS-3 (**10.2.4.3**)	500 µL	500 µL
CalS-IWD-2	CalS-2 (**10.2.5.3**)	500 µL	500 µL
CalS-IWD-1	CalS-1 (**10.2.6.3**)	500 µL	500 µL

10.2.8. Starting with the lowest standard concentration, analyze each calibration standard and tabulate the responses (analyte peak area/IS peak area). The results are used to prepare a calibration curve for each target analyte (weighted 1/C linear regression).

10.3. Initial calibration criteria

10.3.1. The absolute IS response in each chromatographic run must not deviate by more than 10% from average of its initial calibration values.

10.3.2. The correlation coefficient (r) of the calibration curve for each analyte must be verified to be ≥ 0.995 before any analysis of samples can begin.

10.3.3. Each calibration standard, processed under the new initial calibration, must be within 90-110% of the known value for each analyte for the initial calibration to be considered valid. Exception: standards ≤LOQ must be within 70-130%. Evaluation of each standard also serves as the measure of % Relative Error with the same acceptance criteria.

10.3.4. If these criteria cannot be met, a new calibration must be established.

10.4. Initial verification of calibration

10.4.1. The initial calibration for each cannabinoid must be verified by analyzing a CCR. The CCR is prepared as described below but higher or lower levels or volumes may be prepared. It is recommended that the CCR is prepared at a concentration within the middle of the calibration curve.

10.4.2. Cayman CCR solution (**Section 8.7**) @ 4.5 µg/mL. The CCR is stored for up to 1 year at -80 °C or up to 1 month at -20 °C.

10.4.2.1. In a 20-mL centrifuge tube, add 5 µL of SSD (**Section 8.3**) and add 20 mL of MeOH.

10.4.2.2. Add 250 µL MeOH with surrogate from **Section 10.4.2.1** into an HPLC vial.

10.4.2.3. Add 250 µL CCR standard solution @ 9.0 µg/mL from **Section 8.7** into the same vial.

10.4.2.4. Add 500 µL of IWD prepared in **Section 8.2** into the same vial and vortex well.

10.4.2.5. Label as CCR @4.5 µg/mL.

10.4.2.6. The measured recovery values for the analytes of the CCR must fall within 85-115% of the known value for the cannabinoids.

10.4.3. Restek CCR solution (**Section 8.8**) @ 5.0 µg/mL. The CCR is prepared as needed and store up to 1 year at -80 °C and up to one month at -20 °C.

10.4.3.1. The Restek CCR is prepared as described below but higher or lower levels or volumes may be prepared. It is recommended that the Restek CCR is prepared at a concentration within the middle of the calibration curve. An example of Restek CCR @ 5.0 µg/mL is prepared as follows:

10.4.3.2. Into a 1-mL volumetric add 500µL of MeOH with surrogate solution from **Section 10.4.2.1** and add 500 µL of Restek CCR at 10 µg/mL as prepared in **Section 8.8** and labeled as Restek CCR Stock.

10.4.3.3. Into a 2-mL HPLC vial, add 500µL of Restek CCR as prepared in **Section 10.4.2.10** and 500 µL of IWD in **Section 8.2**. Transfer solution to a label vial and vortex.

10.4.3.4. Label as Restek CCR @ 5.0 µg/mL. An appropriate volume for instrument analysis is transferred to labeled HPLC vials with inserts, capped and stored. Since this stock contains only 3 cannabinoids, it is used, when necessary, in addition to the CCR (Cayman) prepared in **Section 8.7**

10.4.3.5. The measured recovery values for the analytes of the Restek CCR must fall within 85-115% of the known value for CBN, CBD and Delta-9-THC.

10.5. Initial and Ongoing Calibration Verification

10.5.1. The initial calibration curve for each cannabinoid must be verified by the analysis of a mid-level CCR

10.5.1.1. The mid-level CCR must be within 85-115% of the known value for each analyte within the initial calibration.

10.5.2. After verifying the initial calibration, a CCV that is ≤ 1/2 the highest calibration standard must be analyzed with each analytical batch (typically 20 samples). For external calibration, a CCV is required at the beginning and end of each analytical batch. For internal standard calibration, a CCV is only required at the beginning of the analytical batch.

10.5.2.1. Low-level CCVs that are ≤ the LOQ must be within 70-130 % of the known value for each analyte. CCVs > the LOQ must be within 90-110% of the predicted concentration.

10.5.2.2. CCVs may also be interspersed throughout the analytical batch at varying concentrations provided that the CCVs analyzed at the beginning and end (for external calibration) of each analytical batch are equal to or less than half the highest calibration level. Additional CCVs may also be run at higher levels to evaluate the upper end of the calibration curve.

10.5.2.2.1. Examples of CCV levels are as follows: 18.0µg/mL, 7.20 µg/mL, 2.88 µg/mL and 1.15 µg/mL (see **Section 10.2**, **CalS-IWD- 5** through **CalS-IWD-2**).

11.0. Quality Control and Assurance

11.1. DOC

11.1.1. Each analyst must perform a DOC using the procedures described in this SOP for each target analyte listed in **Table 1**.

11.1.1.1. Initial DOC

11.1.1.1.1. Prior to analyzing samples, the analyst must perform an initial DOC consisting of four or five solvent-spike samples that have been fortified with all analytes of interest at a concentration of one (1) to four (4) times the LOQ. If possible, the spiking solution should be from a source independent of those used to prepare the calibration standards.

11.1.1.1.2. For each individual analyte, the recovery value for all replicates must fall within the range of 85 -115 %. The precision of the measurements, calculated as relative standard deviation (RSD), must be < 5% for the major analytes such as Delta-8-THC, Delta-9-THC or CBD and < 10 % for minor analytes. Each analyst must complete a successful initial DOC prior to analyzing samples.

11.1.1.2. Continuing DOC

11.1.1.2.1. Annually, each analyst must complete a continuing DOC for each target analyte. The continuing DOC may be completed by one of the following techniques:

11.1.1.2.1.1. Acceptable performance on the analysis of a blind sample, such as an external proficiency test, when available.

11.1.1.2.1.2. Acceptable performance on an initial DOC as described above in **Section 11.1.1.1** at any concentration within the calibration range.

11.1.2. If major changes to the method or the instrumentation are made, or the laboratory/analyst has not performed the method in a twelve (12) month period,

the analyst must complete an initial DOC as described in (**Section 11.1.1.1**). Minor changes to the method are evaluated using the LCS (**Section 11.5**).

11.1.3. All initial and continuing DOCs must be documented.

11.2. LOD and LOQ

11.2.1. An initial LOD study for each method must be completed and documented for all target analytes in each representative matrix (see **MML-301-SOP, 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.

11.2.2. 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.

11.2.3. 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.

11.2.4. 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 being analyzed for each representative matrix, method, and analyte combination. The recovery of the LOQ verification samples shall be within 70-130%.

11.2.5. 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.

11.3. SBLK

11.3.1. Prior to beginning analysis, the analyst must demonstrate that the instrument is free from background interference by analyzing an SBLK.

11.3.2. Fill an HPLC vial with MeOH and analyze as an SBLK. Background contamination found, which could interfere with the measurement of target analytes, must be < 1/3 LOQ for routine samples.

11.4. MB

11.4.1. Before processing samples, the analyst must demonstrate that all glassware and reagent interferences are under control. For each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample) or each time reagents are changed, an MB must be analyzed. If, within the retention time window of any target analyte, the MB produces a peak that would prevent the determination of the analyte, identify the source of contamination and eliminate the interference before processing the samples. Background contamination observed must be <1/3 the LOQ for each target analyte.

11.4.1.1. Fill an HPLC vial with 500 µL of blank extracted matrix and 500 µL of IWD.

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11.5. LCS

11.5.1. One LCS is required with each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample). The following rules may also be applied to the LCS requirement.

11.5.1.1. 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.

11.5.1.2. 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.

11.5.2. The LCS must be spiked with all target analytes at a mid-level concentration in the curve.

11.5.3. The recovery of the LCS must be within 80-120% of the expected prepared value.

11.5.4. The LCS is stored up to 1 year at -80 °C and up to 1 month at -20 °C.

11.6. SUR

11.6.1. The SUR is spiked into all samples. The measured concentration for the SUR in each sample should be within 80-120% of the expected prepared value.

11.7. IS

11.7.1. The IS is spiked into all samples. The IS peak area in all the analyzed samples must be within 10 % of the mean values of the initial calibration curve.

11.8. MS and MSD

11.8.1. A matrix spike and matrix spike duplicate are required with each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample). If sample is not available, a "representative matrix" is used to prepare the MS/MSD and is spiked at a mid-level (4.5 µg/mL) concentration with the target analytes.

11.8.2. To determine the accuracy, calculate the percent recovery of the concentration for the analyte in the **MS**. Recovery must be within 80 – 120% of the true value.

11.8.3. To determine the precision, calculate the relative percent difference (RPD) between the MS/MSD. The RPD must be <20% (**Section 13.3.3**).

11.8.4. MS and MSD are stored at -20 °C until sample analysis.

11.9. System Performance Mix Requirements

11.9.1. Method specifications

Table 16

Test	Analyte	Concentration	Requirement
Sensitivity	CBN	CalS @ LOQ	Detection of analyte at S/N >3
Chromatographic Performance	Delta-9-THC	CalS @ LOQ	0.80 <PGF[a] < 1.15
Column Performance	Delta-9-THC and Delta-8-THC	CalS @ LOQ	[b]Resolution >1.0

[a]PGF = Peak Gaussian Factor (See Equation 3)
[b]Resolution between the two peaks (See Equation 4)

Equation 3.

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

W = (1/2) is the peak width at half height, and W (1/10) is the peak width at tenth height.

Equation 4.

$$R = t/W$$

t = the difference in elution times between the two peaks
W = the average peak width at the baseline of the two peaks.

11.9.2. Instrument specifications

11.9.2.1. An instrument performance system check is carried out daily using a standard at or below the detection limit. Documentation of this is maintained to ensure instrument hardware is functional (i.e., detectors and pumps).

11.9.2.2. Planned maintenance (PM) must be performed once a year per Manufacturer's specifications. Documentation of this must be maintained in the laboratory. The purpose of PM is to establish the initial installation and performance procedures that are required for evaluating the acceptability of the instrument's performance. PMs address immediate and future service issues on instrumentation to maximize system productivity.

12.0. Extraction Procedure

12.1. See appropriate extraction procedure (see **MML-301-SOP**) for more information

13.0. Data Acquisition, Reduction, Analysis and Calculations

13.1. HPLC

13.1.1. Other HPLC columns and/or chromatographic conditions may be used if the retention time acceptance limits are within 2%. See **Appendix A (see MML-300-AppA)** for relative retention times**.**

13.1.2. Perform an initial calibration (**see Sections 10.1 – 10.3**) or verify the initial calibration on each day of analysis by analyzing a CCV as described in **Section 10.5.2.** For all analyses the standards and sample extracts must be in MeOH.

13.1.3. If the response for a target analyte exceeds the working range of the instrument, dilute the extract in diluent and reanalyze.

13.1.4. If concentrations above the calibration curve are expected, the sample extract may be diluted and analyzed to prevent detector saturation and/or negative impacts on the column and/or injector. Since the IS is added after the dilution of the extract, there is no impact on the IS.

13.1.5. When the software inadequately integrates a peak and manual integration does become necessary, laboratory specific procedures must be used a guidance for any manual integration of peaks.

13.1.6. If interference is suspected due to carryover (**see Section 5.4**) and target cannabinoids are present in an unusually concentrated extracted sample, the analyst must demonstrate that the compounds in the subsequent sample are not due to carryover. After the analysis of a sample containing high concentrations of cannabinoids, SBLK should be analyzed to evaluate cross-contamination (**see Section 14.7**). Alternatively, if the sample immediately following the high concentration sample does not contain the cannabinoids present in the high-level sample, freedom from contamination has been established. It is the responsibility of the analyst to confirm that no peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

13.1.7. A SBLK should be analyzed prior to sample analyses to ensure that the total system (i.e., syringe, lines and HPLC system) is free of contaminants. It is also recommended that new and currently in-use columns be washed with 100% mobile phase B for a period of 2 h prior to startup for the analysis of new batches.

13.2. Identification of analytes

13.2.1. Identify a sample component by comparison of its retention time with the retention time of a reference chromatogram (standard). If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound and IS, then identification is considered positive.

13.2.2. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of an analytical sequence. Three times the standard deviation (SD) of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

13.2.3. Current retention time windows are set to allow less than 2% deviation from the expected retention times for all analytes. The expected retention time might change slightly with extended column usage. The expected retention time for each analyte must be verified as necessary using the calibration standards.

13.2.4. Identification of analytes requires expert judgment when sample components are not resolved chromatographically or if any doubt exists over the identification of a peak on a chromatogram. If necessary, the analyst may need to employ appropriate alternate techniques to help confirm peak identification, such as alternate wavelengths and columns. Alternate methods to verify the identification of unknown peaks are under development.

13.2.5. See MML-300-AppA for example chromatograms and retention times by analyte.

13.3. Calculations

13.3.1. Evaluation of Initial Calibration Standards (external calibration)

13.3.1.1. The software calculates the recoveries for the calibration standards. Low-level CCVs that are ≤ the LOQ must be within 70-130 % of the known value for each analyte. CCVs > the LOQ must be within 90-110% of the predicted concentration.

13.3.1.2. Calculate the retention time of each standard compound in the calibration curve. The retention time of the standard compound must be within 2% of the average retention time of that standard in the curve.

13.3.2. Initial calibrations of IS

13.3.2.1. Use the instrument software and specified parameters to perform peak integration for all identified peaks.

13.3.2.2. The IS is used for response and retention time reference. If there is an interfering component present in the sample that precludes accurate determination of the IS, the SUR (4-pentylphenyl 4-methylbenzoate) should be used as retention time reference.

13.3.2.3. Calculate the average retention time of the IS in the calibration curve and in each sample. The retention time of the IS in each sample must be within 2% of the average retention time of the standards in the curve.

13.3.2.4. Calculate the average response factor of the IS in the calibration curve and in each sample. The response factor for the IS must not vary by more than 10% from this average for each of the standards in the calibration curve.

Equation 5.

$$\text{Relative Retention Time} = \frac{RT_{STD}}{RT_{IS}}$$

Where RT_{STD} = Retention time of standard
RT_{IS} = Retention time of internal standard

13.3.3. QC and unknown samples

13.3.3.1. Apply the linear regression generated from the calibration standards to all QA/QC and unknown samples to calculate the concentration (µg/mL) of each cannabinoid using the instrument software or Excel spreadsheet. CCVs must not be used to calculate the concentration of analytes in samples.

13.3.3.2. **MS** and **MSD**

13.3.3.2.1. Calculate the % recovery of the MS and MSD.

13.3.3.2.2. To determine the precision, calculate the relative percent difference (RPD). The RPD must be <20%.

Equation 6.

$$RPD = \frac{|MS - MSD|}{\left(\frac{|MS + MSD|}{2}\right)} * 100$$

Where RPD is in percent (%).
MS = Matrix Spike concentration in ppb.
MSD = Matrix Spike Duplicate concentration in ppb.

13.3.3.3. Medical marijuana products

13.3.3.4. The final results for the marijuana products are reported as weight percentage (% C_S) using the following equations:

Equation 7.

$$C_s = \frac{C_X\left(\frac{\mu g}{mL}\right) * V_F(mL) * D}{M_I(mg) * 1000)}$$

Equation 8.

$$\% C_S = C_S * 100$$

Where C_S = Concentration of analyte in Sample (mass ratio)
$\% C_S$ = Concentration of analyte in Sample (%)
C_x = Concentration of analyte in Extract (µg/mL)
V_F = Final volume of extract (mL)
M_I = Initial mass of sample (mg)
D = Dilution factor, if applicable

13.4. Reporting of results

13.4.1. Non-detected analytes and analytes with a concentration <LOQ are reported as less than (<) the LOQ as specified in **Table 1.**

13.4.2. Analytes detected at a concentration at or above the LOQ are reported using 3 significant figures.

13.4.3. Total THC and total CBD are reported as mg/dose and calculated as follows:

Equation 9. Total THC = $(C_{delta\text{-}9\text{-}THC} + C_{delta\text{-}8\text{-}THC} + C_{THCA}) * M_{dose}$
Equation 10. Total CBD = $(C_{CBD}+C_{CBDA}) * M_{dose}$

C_{THC} = Concentration of THC calculated using equation 8.
$C_{THC\text{-}delta\ 9}$ = Concentration of THC delta-9 calculated using equation 8.
$C_{THC\text{-}delta\ 8}$ = Concentration of THC delta-8 calculated using equation 8.
C_{CBD} = Concentration of CBD calculated using from equation 8.
C_{CBDA} = Concentration of CBDA calculated using equation 8.
M_{dose} = Total mass of the dose (mg) calculated using equations 9 and 10.

13.4.4. For equations 9 and 10, where a component is less than the LOQ, the value of '0' is substituted in the equation.

13.4.5. All results are reported through CLIMS.

14.0. Data Assessment, Acceptance Criteria and Corrective Actions for Out-of-Control Data

14.1. All analytical batches must meet all quality control criteria as described within this procedure and all QC results must be documented.

14.2. The acceptance criteria for standards and QC samples are defined in **Sections 10.0 and 11.0**. The sections below (**Sections 14.3 through 14.14**) outline the most common corrective action procedures for nonconforming data and inconsistent results. Since re-injection of a standard or sample is a routine corrective action for most nonconformities, it is not included in each individual section below, but may be used whenever applicable.

14.3. Failure to meet QC criteria for a calibration curve correlation coefficient of ≥0.995

14.3.1. Assess the calibration curve to determine if a standard appears to be prepared incorrectly.

14.3.2. If necessary, perform instrument maintenance.

14.3.3. A correlation factor of ≥ 0.995 must be achieved before sample analysis can begin. If samples were analyzed before an acceptable calibration curve was established, all affected samples must be re-analyzed under an acceptable curve or the results will be appropriately qualified.

14.4. Failure to meet required QC criteria for CCR of 85-115% recovery

14.4.1. A new CCR is prepared and re-analyzed. It may necessary to prepare it from a new working or stock solution.

14.4.2. If a newly prepared CCR still doesn't meet the required criteria, a new initial calibration curve is analyzed using existing or new calibration standards. The initial calibration curve is then verified with a CCR.

14.4.3. An acceptable CCR must be achieved prior to sample analysis. All samples associated with an unacceptable CCR must be reanalyzed.

14.5. Failure to meet required QC criteria for CCV of 90% to 110% recovery

14.5.1. A new CCV is prepared and analyzed. If necessary, prepare a new working or stock solution.

14.5.2. If a newly prepared CCV still doesn't meet the required criteria, a new initial calibration curve is analyzed using existing or new calibration standards. The initial calibration curve is then verified with a CCR.

14.5.3. All samples associated with an unacceptable CCV will be reanalyzed. If reanalysis in not possible due to lack of remaining extract or sample, the original sample results must be appropriately qualified.

14.6. Failure to meet required QC criteria for LOQ of 70 – 130% recovery

14.6.1. A new LOQ is prepared and re-analyzed. Prepare from a new working or stock solution, if necessary.

14.6.2. If a newly prepared LOQ still doesn't meet the required criteria, the instrument is recalibrated with new calibration standards, which may be prepared from new or existing working standard solutions or stock standard solutions. A new initial calibration curve is prepared, analyzed and verified with a CCR.

14.6.3. Acceptable LOQ recovery must be achieved before sample analysis can begin. If samples were analyzed before an acceptable LOQ was achieved, all affected samples must be re-analyzed after an acceptable LOQ is achieved.

14.7. Failure to meet required QC criteria for SBLK of <1/3 LOQ for target analyte(s) in routine sample batches

14.7.1. Change the HPLC column.

14.7.2. Inject SBLK and run through the system until background contamination is removed or reduced to an acceptable level.

14.7.3. An acceptable SBLK must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be re-analyzed. If re-analysis of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

Exception - If the samples do not contain target analytes at or above the LOQ, the original results may be reported without re-analysis and qualification is not necessary.

14.8. Failure to meet required QC criteria for MB of <1/3 LOQ for target analyte(s)

14.8.1. The source of contamination shall be investigated, and measures taken to minimize or eliminate the problem. Affected samples will be reprocessed or data shall be appropriately qualified if:

a) the concentration of a targeted analyte in the blank is at or above the reporting limit as established by the method or by regulation, AND is greater than 1/10 of the amount measured in the sample;
b) the blank contamination otherwise affects the sample results as per the method requirements or the individual project data quality objectives; and
c) a blank is determined to be contaminated. The cause shall be investigated, and measures taken to minimize or eliminate the problem. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g., reprocessing or data qualifying codes). In all cases, the corrective action shall be documented.

14.9. LCS with <80% or >120% recovery

14.9.1. See Section 14.10.

14.10. MS and MSD with <80% and >120% recovery or RPD >20%

14.10.1. If the MS and/or MSD fails to meet the acceptance criteria for any target analytes, it is recommended that the MS and/or MSD be re-prepared and analyzed if sufficient sample remains; this may require preparation from a new stock standard.

14.10.2. Re-analysis is not required; however, if the MS and/or MSD fails with high recovery and no target analytes are detected in the batch, as LOQ sensitivity is shown, or if all other quality control measures within the batch are acceptable.

14.10.3. If re-analysis is performed and the new MS and/or MSD meets the acceptance criteria, only report the results from the re-analysis.

14.10.4. The relative percent difference (RPD) for each spiked analyte in the MS and MSD must be <20%.

14.10.5. If the new MS and/or MSD still fails, the original MS and/or MSD and corresponding sample results must be appropriately qualified.

Exception – if the MS and/or MSD fails with high recovery and no target analytes are detected in the batch, qualification is not necessary as LOQ sensitivity is shown.

14.11. IS area in sample deviates by > 10% from area in most recent CCV

14.11.1. Re-inject the sample extract.

14.11.1.1. If the results of the re-injection meet acceptance criteria, only report the results of the re-injection.

14.11.1.2. If re-injection still fails, re-extract and re-analyze the sample.

14.11.1.3. If additional sample is not available, results must be reported with appropriate qualifiers.

14.11.2. In the event of an interference(s) (causing >10% error) with the internal standard.

14.11.2.1. Cannabinoid concentrations are calculated using an external calibration.

14.11.2.2. Cannabinoid concentrations in the QC samples should be calculated the same way as the other unknown samples without using the internal standard correction.

14.11.2.3. When the internal standard correction is not used in determining the cannabinoid concentrations, all sample analyses must be finished within 48 h of sample preparation to avoid significant error caused by evaporation of solvent from the samples.

14.11.2.4. If all quality assurance criteria are met in **Section 11.0**, samples are reported with the appropriate qualifiers.

14.12. SUR with <80% or >120% recovery

14.12.1. Re-prepare the sample and analyze if a duplicate sample remains.

14.12.2. If the re-analysis meets acceptance criteria, only report those results.

14.12.3. If the re-analysis still fails, the original sample results will be appropriately qualified.

Exception – if the SUR fails with high recovery and no target analytes are detected in the sample, qualification is not necessary as LOQ sensitivity is shown.

14.13. Inconsistent baseline

14.13.1. Perform appropriate instrument maintenance, if applicable.

14.13.2. Repeat the sequence using the same standards/samples. If repeat analysis is acceptable, report only the analytical results from the repeated analysis.

14.13.3. If instrument maintenance and repeat analysis fails to produce acceptable data, the sample results will be appropriately qualified.

14.14. All other nonconforming data, not addressed within this procedure, requires the completion of a nonconformance/corrective action report.

15.0. Method Performance

15.1. LOD and DOC study results are maintained by the laboratory.

15.2. Preventative Maintenance is performed on the instrument once per year. This maintenance pertains to the lamp, pumps and data system.

16.0. Waste Management/Pollution Prevention

16.1. It is the responsibility of the laboratory to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

16.2. Minimize solvent, chemical, reagent and standard use whenever possible to reduce the amount of hazardous waste generated.

16.3. Dispose of solvent waste in an appropriate solvent waste container (red, 5-gal solvent can), properly labeled (separate chlorinated and non-chlorinated solvents).

16.4. Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.

16.5. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal

17.0. References

17.1. Definition and Procedure for the Determination of the Method Detection Limit, Environmental Protection Agency, 40 CFR Part 136, Appendix B.

17.2. Shimadzu LabSolutions "LC Getting Started Guide" http://www.shimadzu.com/an/data-net/labsolutions/labsol-2.html

17.3. Public Health Law, section 502 of the Public Health Law (PHL), Title 10 (Health) of The Official Compilation of Codes, Rules and Regulations of the State of New York (NYCRR) subpart 55-2 (Approval of Laboratories Performing Environmental Analysis). http://w3.health.state.ny.us/dbspace/NYCRR10.nsf/56cf2e25d626f9f785256538006c3ed7/c9252587bc832b3485256c390055920a?OpenDocument&Highlight=0,section,55)

17.4. *Norgestrel*; MSDS No. N2260 [Online]; Sigma-Aldrich: Saint Louis MO, September 03, 2014 http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do

18.0. Supporting Documents

18.1. Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (MML-300-AppA).
18.2. Medical marijuana sample preparation protocols for potency analysis (MML-301-SOP)

19.0. Appendices

Appendix A - MML-300-AppA

Figure 1- 5 µg/mL ISTD in blank solvent

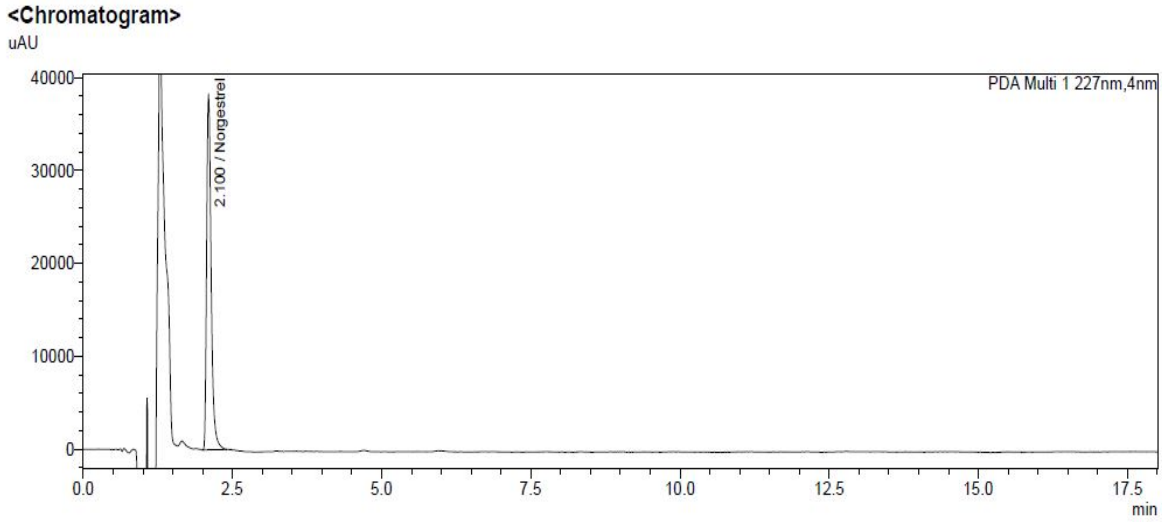

Figure 2- 45.0 µg/ml w/ ISTD and Surrogate

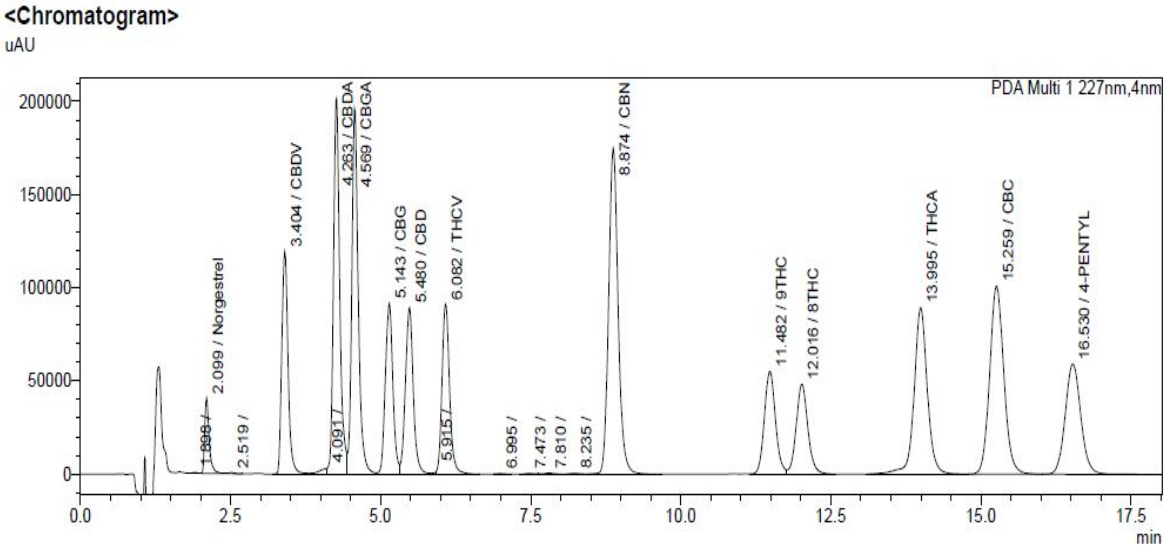

Table 1 – HPLC-PDA

Cannabinoids, Internal Standard and Surrogate with corresponding retention times.

Analyte	Full name	Nickname	Retention time*
1.	Norgestrel	ISTD	2.09
2.	Cannabidivarin	CBDV	3.40
3.	Cannabidiolic Acid	CBDA	4.26
4.	Cannabigerolic Acid	CBGA	4.57
5.	Cannabigerol	CBG	5.14
6.	Cannabidiol	CBD	5.48
7.	Tetrahydrocannabivarin	THCV	6.06
8.	Cannabinol	CBN	8.87
9.	Delta-9 Tetrahydrocannabinol	THC-9	11.48
10.	Delta-8 Tetrahydrocannabinol	THC-8	12.02
11.	Tetrahydrocannabinolic Acid	THCA	14.00
12.	Cannabichromene	CBC	15.26
13.	4- pentylphenyl 4-methylbenzoate	Surrogate	16.53

*Retention Time acceptable within 2%
Retention times are approximate based on current column setup and may vary slightly over different column installations and the lifetime of the columns.
Columns and analytical conditions are described in Section 9.