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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 NY State 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
Cannabigerolic Acid (CBGA)	25555-57-1	0.60
Cannabinol (CBN)	521-35-7	0.60
Cannabidiol (CBD)	13956-29-1	0.60
Delta-9-Tetrahydrocannabinol (THC)	1972-08-3	0.60
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

- 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 **NYS DOH MML-301**.

2.0. Summary of the Method

- 2.1. Samples from each lot of MM product (currently includes but not limited to capsules, tinctures, formulations for vaporization, oral solutions, oral sprays, powders and dry leaf pods) are diluted/dissolved with organic solvents (See **NYS DOH MML-301** 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 ten cannabinoids is achieved on a C18 reversed-phase column 150 mm in length. 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.

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. A CCV or CCR may also serve as an LCS for this procedure. It is not necessary to include a separate LCS in an analytical batch when either of the designated CCV or CCR samples meets the LCS criteria.
- 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. Limit of Quantitation (LOQ) – The minimum concentration that can be quantitatively reported for a target analyte. This LOQ can be no lower than the lowest calibration standard.
- 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. Sample Batch – A group of samples that are processed together as a unit using the same procedure and materials. A typical batch consists of twenty (20) samples. A batch includes the necessary quality controls, including method blanks, method blank spikes, matrix spikes, duplicates and quality control samples.
- 3.19. Analysis batch – A set of samples that are analyzed on the same instrument during a 24-hour period which includes all appropriate blanks and spikes for an extraction batch.

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 **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 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 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** 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 **NYS DOH MML-301** 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. Stock standard solutions or neat materials may be purchased from several vendors. When available, standards/materials 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. 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
THC	T-005	1.0 mg/mL	MeOH
CBGA	C-142	1.0 mg/mL	MeCN
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	MeOH
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
CBC	16398	1.0 mg/mL	MeOH
THCV	16397	1.0 mg/mL	MeOH
CBDA	15673	1.0 mg/mL	MeOH
THCA	15679	1.0 mg/mL	MeOH
CBG	15362	1.0 mg/mL	MeOH

7.2.6. Restek analytical reference standards

Table 4.

Standard Number	Standard Name	Catalog #	Concentration	Solvent
1	CBN	34014	1000 µg/mL	MeOH
2	CBD	34014	1000 µg/mL	MeOH
3	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 for guidance only. These may be interchanged. In addition, different concentrations or alternate stock mixtures may be prepared as necessary.
- 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	THC	T-005	90 µg/mL
4	0.9 mL	CBGA	C-142	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 Cerilliant standards is prepared at 9.0 µg/mL. The CCR is prepared annually and stored at -80 °C. Currently, a separate preparation of the primary stocks is used as a CCR. This stock is prepared at a different concentration and uses the remaining volume in the ampule.

Table 8. CCR (This CCR is prepared in a 10-mL volumetric as a combined preparation)

Standard number	Volume Added	Standard name	Catalog #	Diluted Concentration
1	0.09 mL	CBN	C-046	9.0 µg/mL
2	0.09 mL	CBD	C-045	9.0 µg/mL
3	0.09 mL	THC	T-005	9.0 µg/mL
4	0.09 mL	CBGA	C-142	9.0 µg/mL
5	0.09 mL	CBC	C-143	9.0 µg/mL



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Table with 5 columns: Standard Number, Volume used, Standard Name, Catalog #, Diluted Concentration. Rows 6-10 showing standards for THC, CBDA, THCA, CBG, and CBDV.

8.7.1. Portions (0.09 mL) of each standard solution as purchased from vendors (1000 µg/mL) are added to a 10-mL volumetric flask.

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

8.8. 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 (Cerilliant) prepared in Section 8.7.

Table 9.

Table with 5 columns: Standard Number, Volume used, Standard Name, Catalog #, Diluted Concentration. Rows 1-3 showing standards for CBN, CBD, and THC.

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 Manufacture 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, 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.

9.3. Sample preparation

9.3.1. Follow MM sample preparation as per **NYS DOH MML-301** 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 **Table 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.



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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 13.0 and Section 14.12) 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

Table with 3 columns: Description, Units, Value. Rows include Channel (227 nm), Width (1 s), Slope (15500 uV/min), Drift (0 uV/min), Time to double peak (1000 min), and Minimum Area/Height (1000 counts).

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 is used 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 ug/mL to 0.19 ug/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 ug/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.



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10.2.2. CalS-5 15.0 µg/mL

- 10.2.2.1. Transfer 300 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 5.00 µg/mL

- 10.2.3.1. Transfer 300 µL **CalS 5** taken from the initial 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 1.67 µg/mL

- 10.2.4.1. Transfer 300 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 0.56 µg/mL

- 10.2.5.1. Transfer 300 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 0.19 µg/mL

- 10.2.6.1. Transfer 300 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	10.2.1.1	-	-
CalS-5	15	10.2.1.1	300 µL	600 µL



CalS-4	5	10.2.2.3	300 µL	600 µL
CalS-3	1.67	10.2.3.3	300 µL	600 µL
CalS-2	0.56	10.2.4.3	300 µL	600 µL
CalS-1	0.19	10.2.5.3	300 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. The exception is the lowest calibration point (around the limit of quantitation), which may be within 70-130% of the expected value for each analyte.

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 at a minimum one 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.** CCR solution @ 4.5 µg/mL. The CCR is stored for up to 1 year at -80 °C or up to 1 month at -20 °C.
The CCR solution is prepared using Cerilliant standards (**Section 8.7**).
- 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 with insert.
- 10.4.2.3.** Add 250 µL CCR standard solution @ 9.0 µg/mL from **Section 8.7** into the same vial with insert.
- 10.4.2.4.** Vortex well.
- 10.4.2.5.** Add 500 µL of IWD prepared in **Section 8.2** into an HPLC vial and vortex well.
- 10.4.2.6.** Label as CCR @4.5 µg/mL.
- 10.4.2.7.** The measured recovery values for the analytes of the CCR must fall within 90-110% of the known value for the major cannabinoids.
- 10.4.2.8.** Restek CCR solution @ 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.
- 10.4.2.9.** 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:
- 10.4.2.10.** 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.
- 10.4.2.11.** Into a 2-mL HPLC vial, add 500µL of Restek CCR is prepared in **Section 10.4.2.10** and 500 µL of IWD in **Section 8.2**. Transfer solution to a label vial and vortex.
- 10.4.2.12.** 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. A vial is analyzed with each calibration curve.
- 10.4.2.13.** The measured recovery values for the analytes of the Restek CCR must fall within 90-110% of the known value for CBN, CBD and THC.

10.5. Minimum daily calibration verification and CCVs

- 10.5.1.** The working calibration curve for each cannabinoid must be verified with each analytical batch by the analysis of a mid-level CCR and a CCV at or near the LOQ. The opening



mid-level CCR and CCV at or near the LOQ may be substituted with a full initial calibration and CCR. A bracketing CCV is analyzed at the end of batch/sequence.

- 10.5.1.1. The mid-level CCR, processed under the minimum daily calibration verification, must be within 90-110% of the known value for each analyte for the initial calibration to be considered valid.
 - 10.5.1.2. A low-level standard which is at or near the LOQ processed under the minimum daily calibration verification, must be within 70-130 % of the known value for each analyte for the initial calibration to be considered valid.
 - 10.5.1.3. Bracketing CCVs must be interspersed with samples at regular intervals during the course of the analysis batch and/or analysis sequence at varying concentrations. CCVs are prepared at the following levels: 15 µg/mL, 5.0 µg/mL and 1.67 µg/mL (see **Section 10.2, CalS-IWD- 5 through CalS-IWD-3**). The analytes in the CCVs must be recovered within 90-110% of the predicted concentration
- 10.5.2. For extended periods of analysis, where two or more analysis batches are analyzed simultaneously over a period exceeding 24 h, the minimum daily calibration verification must be followed between the analysis batches. See **Section 10.5.1.**

11.0. Quality Control and Assurance

11.1. DOC

- 11.1.1. Each analyst must perform an initial DOC in using the procedures described in this method for each target analyte as listed in **Table 1**. The initial DOC must consist of the analysis of four or five solvent-spike samples that have been fortified with all analytes of interest at a mid-level concentration (approximately 5-10 µg/mL). If possible, the spiking solution used should be from a source independent of those used to prepare the calibration standards.
 - 11.1.1.1. For each individual analyte, the recovery value for all replicates must fall within the range of 90 -110 % of mean. The precision of the measurements, calculated as relative standard deviation (RSD), must be < 5% for the major analytes such as THC or CBD and < 10 % for minor analytes. For analyses of compounds that fail for these criteria, this procedure must be repeated until satisfactory performance has been demonstrated.
 - 11.1.1.2. Annually, each analyst who will be performing the method 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. Acceptable performance on the analysis of a blind sample, such as an external proficiency test, when available.
 - 11.1.1.2.2. Acceptable performance on an initial DOC as described above in **Section 11.1.1.**



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). 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 LOD study must be completed and documented for all target analytes in each representative matrix (see NYS DOH MML-301), on each instrument used to analyze sample extracts. An LOD study consists of the analysis of seven to ten laboratory control samples in a blank sample matrix that have been fortified with all target analytes at no more than twice the laboratory’s minimum reporting limit. The fortified samples must be treated as real samples and taken through all applicable method procedures.

11.2.2. Calculate the mean recovery and SD for each analyte. Use equation 1 to calculate the LOD:

Equation 1.

$LOD = \text{standard deviation of the } n \text{ samples } (\mu\text{g/mL}) * t^l$

t^l is the value for the 99% confidence level with n-1 degrees of freedom, where n is the number of replicates.

Table 15

n	t
7	3.143
8	2.998
9	2.896
10	2.821

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

Equation 2.

$LOQ = LOD * 5$

11.2.4. The LOQ for each cannabinoid in a representative matrix must then be verified by extracting and analyzing an MS that has been spiked at no more than twice the LOQ. The measured value should be within 70-130% of the expected spike concentration.

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



11.3. SBLK

11.3.1. Before analyzing each batch of samples, the analyst must run an SBLK. Before processing the batch, the analyst must demonstrate that the instrument is free from background interference by analyzing an SBLK.

11.3.1.1. Fill an HPLC vial with MeOH and analyze as an SBLK.

11.3.2. 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. Each time a set of samples is extracted, or 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 μ L of blank extracted matrix and 500 μ L of IWD.

11.5. LCS

11.5.1. An LCS is analyzed every twenty samples or one per sample batch (all samples prepared within a 24-h period), whichever is greater.

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

11.5.3. The value obtained for 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 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 sample must be analyzed every twenty samples or one per sample set (samples prepared with in a 24-h period), whichever is more frequent. A “representative matrix” is used to prepare the MS and is spiked at a mid-level (4.5 $\mu\text{g}/\text{mL}$) concentration with the target analytes.



- 11.8.2. A duplicate matrix spike must be prepared and compared against the original MS sample.
- 11.8.3. 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.
- 11.8.4. To determine the precision, calculate the relative percent difference (RPD). The RPD must be <20% (Section 13.3.4).
- 11.8.5. The MS and MSD are stored up to 1 year at -80 °C or up to 1 month at -20 °C.

11.9. System Performance Mix Requirements

11.9.1. Method specifications

Table 16

Test	Analyte	Concentration	Requirement
Sensitivity	CBN	0.19 mg/mL	Detection of analyte at S/N >3
Chromatographic Performance	THC	0.56 mg/mL	0.80 <PGF ^a < 1.15
Column Performance	CBDA CBGA	0.56 mg/mL	^b Resolution >1.0

Where ^aPGF = Peak Gaussian Factor (See Equation 3.)

Equation 3.

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

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

^bResolution between the two peaks (See Equation 4.)

Equation 4.

$$R = t/W$$

Where t is the difference in elution times between the two peaks and W is 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 instruments 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 **NYS DOH MML-301**) 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** for relative retention times.
- 13.1.2. Calibrate or verify the system calibration on each day of analysis as described in **Section 10.4**. 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. Contamination by "carryover" can occur whenever high-and low-concentration samples are analyzed in sequence. If 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, one or more blanks should be analyzed to check for cross-contamination (see **Section 14.7 for SBLK criteria**). 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 method blank should be analyzed prior to sample analyses to ensure that the total system (i.e., syringe, lines and HPLC system) is free of contaminants. A preventive technique is between-sample rinsing of the sample syringe and filter holder with two portions of diluent. 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 and should be available in the near future.

13.3. Calculations

13.3.1. Initial calibration of Standards

- 13.3.1.1. Calibrate the response factors for the calibration standards used to prepare the calibration curve. These response factors must not vary by more than 10% from the values predicted by the calibration curve.
- 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.

Equation 5.

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

Where RT_{STD} = Retention time of standard

RT_{IS} = Retention time of internal standard



13.3.2. Initial calibrations of IS

- 13.3.2.1.** Use the instrumental 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. 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. 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.
- 13.3.2.5.** Calculate the retention time of the IS in each sample. The retention time of the sample must be within 2% of the average retention time of the standards in the curve.
- 13.3.2.6.** Calculate the IS response factor for each sample in a batch. The IS response factor for each sample in the batch must be within 10% of the average of the IS response factors determined in **Section 13.3.2.4** of the standard curve.

13.3.3. Minimum daily calibration verification for an analytical batch is as follows:

- 13.3.3.1.** The working calibration curve and average response factors must be verified on each working day. Calculate the minimum daily calibration requirements for passing CCR and a standard at or near the LOQ (see **Section 10.4**).
- 13.3.3.2.** Calculate in subsequent analysis batches, bracketing CCVs. It is highly recommended that the CCVs are alternated between medium and high concentration CCV standards within the working calibration curve (see **Section 10.5**).

13.3.4. QC and unknown samples

- 13.3.4.1.** Apply the linear regression calibration curve generated from the calibration standards to all QA/QC and unknown samples to calculate the concentration ($\mu\text{g/mL}$) of each cannabinoid using the instrument quantification software.
- 13.3.4.2. MS and MSD**
 - 13.3.4.2.1.** Calculate the % recovery of the MS and MSD.



13.3.4.2.2. 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.

13.3.4.3. Medical marijuana products

13.3.4.4. 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

13.3.5. Do not use CCVs to calculate the concentration of analytes in samples.

13.4. Reporting of results

13.4.1. Non-detected analytes 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 using Equations 9 and 10.

Equation 9. Total THC = (C_{THC}+C_{THCA}) * M_{dose}



Equation 10. Total CBD = $(C_{\text{CBD}} + C_{\text{CBDA}}) * M_{\text{dose}}$

C_{THC} = Concentration of THC calculated using equation 8.

C_{THCA} = Concentration of THCA 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 equation 9 and 10.

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 whether there is one standard that appears to be prepared incorrectly. If so, re-prepare that standard and analyze. If more than eight (8) h have elapsed since the original failing calibration standard was analyzed, then all calibration standards must be re-analyzed. If the standard is on the high or low end of the curve, it may be removed, so long as the curve has a **minimum of five points in succession**. Additionally, the LOQ must be met for analysis and samples must be analyzed within the concentration range of the curve.
 - 14.3.2. If more than one standard appears to be prepared incorrectly, or the calibration curve is erratic, re-prepare all calibration standards and analyze. This may involve re-preparing the working standard solution or opening new stock standard solutions.
 - 14.3.3. If necessary, perform instrument maintenance.
 - 14.3.4. A correlation coefficient of ≥ 0.995 must be achieved before sample analysis can begin. If samples were analyzed before an acceptable calibration curve/ARF was established, all affected samples must be re-analyzed under an acceptable curve/ARF or the results will be appropriately qualified.
- 14.4. Failure to meet required QC criteria for CCR of 90-110% recovery
 - 14.4.1. A new CCR is prepared and re-analyzed. It may necessary to prepare it from a new working or stock solutions.
 - 14.4.2. If a newly prepared CCR 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 calibration curve must be prepared, run on the instrument and be verified with a CCR using the new curve.



- 14.4.3.** All samples must have an acceptable CCR. Any samples that are analyzed without an acceptable CCR must be reanalyzed when an acceptable CCR is achieved or a new calibration is established.
- 14.5.** Failure to meet required QC criteria for CCV of 90% to 110% recovery
- 14.5.1.** A new CCV is prepared and re-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, the instrument is recalibrated with initial calibration standards, which may be prepared from new or existing working standard solutions or stock standard solutions. A new calibration curve is prepared and analyzed on the instrument and verified with a CCR using the new curve.
- 14.5.3.** All samples must be bracketed by an acceptable CCV.
- 14.5.3.1.** If a CCV is unacceptable, a second uninterrupted CCV is analyzed on the established instrument. If the second CCV is acceptable, the analyst can continue analyzing the batch.
- 14.5.3.2.** If a second uninterrupted bracketing CCV is still unacceptable, the analyst must perform a new initial calibration.
- 14.5.3.3.** All samples analyzed before an acceptable bracketing CCV must be re-analyzed on the instrument. If reanalysis is 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 recovery for routine sample batches
- 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 calibration curve is prepared and analyzed on the instrument and verified with a CCR using the new curve.
- 14.6.3.** The 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 minimum daily calibration verification

- 14.8.1.** A new LOQ and/or CCR are prepared and re-analyzed. Prepare from a new working or stock solution, if necessary.
- 14.8.2.** If a newly prepared LOQ and/or CCR 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.
- 14.8.3.** If one of the bracketing CCVs interspersed through the analysis fails to meet required criteria during the analysis, the failing CCV and/or CCVs are reanalyzed as well as the samples that they bracket.

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

- 14.9.1.** Analyze MB to ensure that the system is free from background contamination. If background contamination is discovered in the system blank, follow the corrective actions described above (**Section 14.7**)
- 14.9.2.** Re-inject the MB once a contaminant-free system is achieved.
- 14.9.3.** If the re-injection still fails, extract and analyze a new MB to ensure that a systemic problem does not exist. If a new MB has already been extracted with a subsequent batch, then the extraction of an additional blank is not required.
- 14.9.4.** An acceptable MB must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be re-analyzed (if system contamination is suspected) or re-extracted and analyzed (if extraction contamination is suspected).

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.

- 14.9.5.** If subsequent MBs continue to show unacceptable levels of background contamination, the extraction of additional samples must be halted until the source of the contamination can be determined and eliminated or reduced to acceptable levels.

14.10. LCS with $<80\%$ or $>120\%$ recovery

- 14.10.1.** As the CCR or CCV may be used to meet the LCS criteria, the corrective actions above (**Section 14.4 and 14.5**) apply.



14.10.2. If an LCS is prepared and analyzed independently of the CCR and CCV and it fails to meet the acceptance criteria, the LCS must be re-prepared and analyzed. If necessary, prepare from a new stock standard.

Exception – If the LCS fails with high recovery and no target analytes are detected in the batch, re-analysis may not be necessary as LOQ sensitivity is shown. Continued failure in subsequent batches, however, will require a corrective action.

14.10.3. If the new LCS meets the acceptance criteria, only report those results.

14.10.4. If the new LCS still fails, the original LCS and corresponding sample results will be appropriately qualified.

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

14.11. MS and MSD with <80% and >120% recovery

14.11.1. 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.

14.11.2. 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.

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

14.11.4. The relative percent difference (RPD) for each spiked analyte in the MS and MSD must be <20%, until in-house precision limits can be established.

14.11.5. 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.

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

14.12.1. Re-inject the sample extract.

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

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

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



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

14.12.2.1. Cannabinoid concentrations are calculated without the use of the internal standard.

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

14.12.2.3. When the internal standard correction is not used in determining the cannabinoid concentrations, all sample analyses must be finished within 24 h of sample preparation to avoid significant error caused by evaporation of solvent from the samples.

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

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

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

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

14.13.3. 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.

14.14. Inconsistent baseline

14.14.1. Perform appropriate instrument maintenance, if applicable.

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

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

14.15. 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. Shimadzu performs a PM 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
- 16.6.** Consult federal, state and local regulations for additional information or for information on the disposal of products not described in this method.



17.0. References

- 17.1. 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.
- 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 (NYS DOH MML-300, Appendix A).
- 18.2. Medical marijuana sample preparation protocols for potency analysis (NYS DOH MML-301)



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19.0. Appendices

Appendix A

Figure 1- 5 µg/mL ISTD in blank solvent

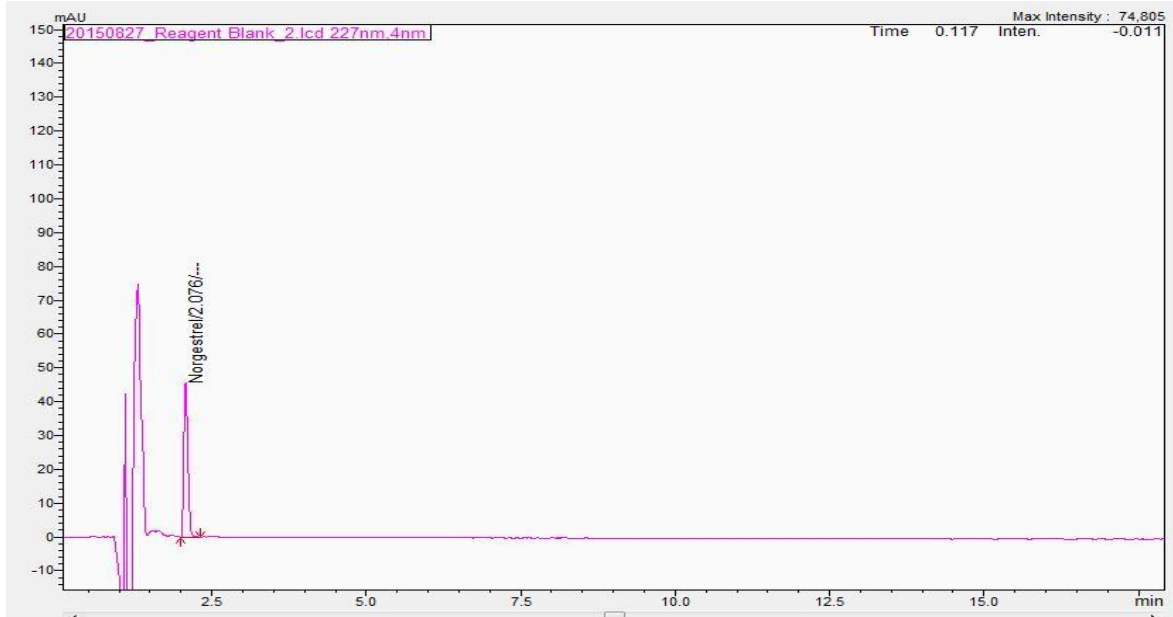
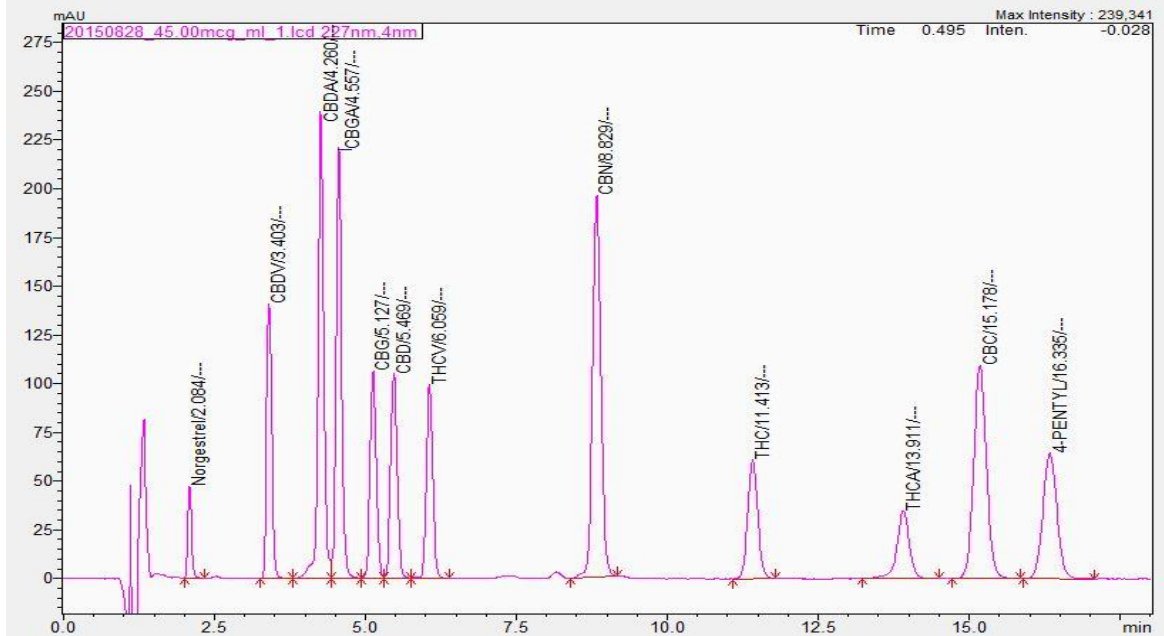


Figure 2- 45.0 µg/ml w/ ISTD and Surrogate





**Table 1 – HPLC-PDA
Cannabinoids, Internal Standard and Surrogate with corresponding retention times.**

Analyte	Full name	Nickname	Retention time*
1.	Norgestrel	ISTD	2.07
2.	Cannabidivarin	CBDV	3.40
3.	Cannabidiolic Acid	CBDA	4.26
4.	Cannabigerolic Acid	CBGA	4.55
5.	Cannabigerol	CBG	5.13
6.	Cannabidiol	CBD	5.45
7.	Tetrahydrocannabivarin	THCV	6.05
8.	Cannabinol	CBN	8.80
9.	Delta-9 Tetrahydrocannabinol	THC	11.40
10.	Tetrahydrocannabinolic Acid	THCA	13.90
11.	Cannabichromene	CBC	15.15
12.	4- pentylphenyl 4-methylbenzoate	Surrogate	16.25

*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.