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Executive Deputy Commissioner

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

# Division of Environmental Health Sciences
# Albany, New York

# Measurement of Phytocannabinoids 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 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 Title 10 (Health), Subpart 55-2.15 and Chapter XIII, Section 1004.14.

**Table 1 –** Analyte List

| Analyte | CAS Number | LOQ[1] (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, IS ) | 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 3.17).

**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 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.

**1.4.** The Norgestrel IS is a suspected carcinogen and is a known to be hazardous during pregnancy (see reference 17.4).

## 2.0. Summary of the Method

**2.1.** Representative samples of the medical marijuana products (in capsules, tinctures, or formulations for vaporization) are diluted/dissolved with organic solvents (See NYS DOH MML-301 for sample preparation details). The diluted samples fortified with internal standard 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 reverse-phase column 150 mm in length. The limit of quantification 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) in the medical marijuana products.

## 3.0. Definitions

**3.1.** <u>Internal Standard</u> (IS) – A pure compound that should not be found in any sample. The IS is a compound added to both samples and standards at a known concentration in order to provide a basis for peak area ratios used in quantitation. The IS is also used to monitor instrument performance for each analysis.

**3.2.** <u>Internal Standard Stock Diluent</u> (ISD) – A concentrated solution of IS that is prepared in a mixture of 1:1 acetonitrile (MeCN): chloroform. This stock diluent is used to prepare the IS working diluent (IWD).

**3.3.** <u>Internal Standard Working Diluent</u> (IWD) – A solution of IS that is prepared from the ISD 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.** <u>Surrogate Standard</u> (SS) – 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.

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

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

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

**3.8.** <u>Method Blank</u> (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 surrogates 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.** <u>Laboratory Control Sample</u> (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

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used to evaluate the accuracy of the methodology.  Acronyms include:  Method Blank Spike (MBS) and Laboratory Fortified Blank (LFB).  A CCV or CCR may also serve as an LCS for this procedure.  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.

**3.10.** Stock Standard – A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards, when available, or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source, when available.

**3.11.** Working Standard Solution (WS) – A solution of method analytes prepared from stock standard solutions and diluted as necessary to prepare calibration standards or other necessary analyte solutions.

**3.12.** 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.

**3.13.** Continuing Calibration Verification Standard (CCV) – One of the primary calibration standards used to verify the acceptability of an existing calibration.

**3.14.** 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.

**3.15.** Matrix Spike Sample (MS) – A portion of an actual sample that is spiked with a known quantity of target analytes and analyzed as if it were a sample.  The sample from which the portion to be spiked was taken must be analyzed separately to determine background analyte concentrations.  The MS is used to correct for background concentrations of the analytes and to determine whether the sample matrix contributes bias to the sample results.

**3.16.** 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.

**3.17.** 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.

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

**3.19.** Sample Batch – A group of samples that are processed together as a unit using the same procedure and materials. A typical batch consists of 20 samples.  A batch includes the necessary quality controls, including method blanks, method blank spikes, matrix spikes, duplicates, and quality control samples (Batch ID#).

**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. 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.

**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.

    **5.1.1.** Glassware used in the laboratory is cleaned following laboratory specific procedures.

        **5.1.1.1.** Rinse with the last solvent used.

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

        **5.1.1.3.** Rinse thoroughly with hot tap water.

        **5.1.1.4.** Rinse with Organic Free Reagent Water.

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

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

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

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**5.1.1.8.**     Syringes:

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

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

**5.1.2.**     The use of high-purity reagents and solvents helps to minimize interference problems.

**5.1.3.**     After cleaning, glassware is stored in a clean storage area away from standards and syringes to prevent any cross-contamination.

**5.2.**     When interferences or contamination are evident in samples, the re-preparation of the original sample is recommended after the source of contamination has been identified.

**5.3.**     Interfering contamination known as "carry over" may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes.  Rinsing of the sample syringe and associated equipment between samples with solvent/mobile phase can minimize this sample cross contamination.  After analysis of a sample containing high concentrations of analytes one or more injections of solvent/mobile phase should be made to ensure that accurate values are obtained for the next sample.

**5.4.**     Matrix interferences may occur as a result of contaminants present in the sample.  If a matrix interference is believed to be present, a matrix-spike experiment must be performed.  The MS must be analyzed together with the unspiked sample to verify the results.  If these analyses verify the original results, report only the results from the original unspiked sample.  This may not always be possible if a limited amount of sample is received for analysis.  In the event that additional sample is not available for reanalysis, the original results must be qualified on the final report.

**5.5.**     Samples and standards must be prepared in the same final solvent to allow for chromatographic comparability of samples to standards.

## 6.0.   Instrumentation, Equipment and Supplies
(All specifications are suggested. Catalog numbers are included for illustration only. 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 or equivalent.

**6.1.5.**     Positive pipet tips of various sizes.

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**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.** Sample Extraction Equipment (See NYS DOH MML-301)

**6.3.** Instrumentation

**6.3.1.** Analytical balance, Mettler-Toledo Model # 205DU or equivalent.

**6.3.2.** Sonicator, Branson, Model # 2510R-DTH or equivalent.

**6.3.3.** Vortex, Maxi Mix 11 Model #37615 or equivalent.

**6.3.4.** Centrifuge, Model # 5415D or equivalent.

**6.3.5.** Shaker, Labline, Model# 3540 or equivalent.

**6.3.6.** An analytical system complete with an HPLC, equipped with a column oven which is suitable for use with a variety of columns, as well as all of the required accessories including: syringes, analytical columns, gases, detectors, and a data system for instrument control and data analysis/processing (e.g., Shimadzu HPLC, or equivalent). See Appendix A for further clarification and description of the instrumentation.

> **6.3.6.1.** Shimadzu HPLC system or equivalent:
>
> > **6.3.6.1.1.** Micro vacuum degasser, model # DGU-20A3 or equivalent.
> >
> > **6.3.6.1.2.** Solvent Selector model# FCV-11A2 or equivalent.
> >
> > **6.3.6.1.3.** Pumps, model # LC-20ADxR, or equivalent.
> >
> > **6.3.6.1.4.** Column Oven, model # CTO-20A or equivalent.
> >
> > **6.3.6.1.5.** Autosampler, model # SIL-20ACxR or equivalent.
> >
> > **6.3.6.1.6.** System Controller model # CBM-20A or equivalent Photo diode Array Detector Model# SPD-M20A or equivalent.
> >
> > **6.3.6.1.7.** Shimadzu Labsolutions software on a compatible computer system for data collection, or equivalent.

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**7.0.  Reagents and Standards**

  **7.1.**  Solvents (HPLC Grade) and reagents – All solvents and reagents must have records to trace their origins and preparations including certificate 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.**  Chloroform, ACS grade.

   **7.1.6.**  Ammonium formate (98+ purity, Fluka catalog # 3272-02 or equivalent).

   **7.1.7.**  Formic acid  (MSD grade, Sigma-Aldrich catalog # 39,938-8 or equivalent).

  **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 a Certificate of Analysis.

   **7.2.2.**  The commercial standards/materials listed in Sections 7.2.4 (Table 2), 7.2.5 (Table 3), and 7.2.6 (Table 4) are examples of those currently used in the laboratory. This is not a fully inclusive list and substitutions may be made as long as 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)

**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 |

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**7.2.5.** Cayman Chemical analytical reference standards (Table 3)

**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 |
| Norgestrel (*IS*) | 10006319 | n/a | Solid |

**7.2.6.** Sigma-Aldrich analytical reference standards (Table 4)

**Table 4**

| 4-Pentylphenyl 4-Methylbenzoate (*Surrogate*) | 665754-5G | 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, if possible, have NIST-traceable documentation. The preparation method, date of preparation, expiration date, and analyst must also be traceable in laboratory documentation.

**8.1.2.** Standards 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 on any volume 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.** ISD @ 10 mg/mL (prepare yearly and store at -20 °C):

**8.2.1.** The ISD is prepared by weighing 10.0 mg of norgestrel (IS) into a 1-mL volumetric.

**8.2.2.** Separately, a solution of 20 mL of MeCN and 20 mL of chloroform is prepared.

**8.2.3.** The 50/50 solution of MeCN/chloroform prepared in 8.2.2 is used to dilute the norgestrel to the 1 mL volume.

**8.2.4.** The ISD is mixed well and labeled appropriately.

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**8.3.** IWD @ 10 µg/mL (prepare monthly):

    **8.3.1.** Transfer 100 µL of ISD prepared in Section 8.2 to a 100 mL volumetric flask.

    **8.3.2.** Fill with MeOH to volume, mix well and label.

**8.4.** SSD @ 50 mg/mL (prepare yearly and store at -20 °C):

    **8.4.1.** 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 with MeCN. The volumetric is then filled to volume with MeCN.

    **8.4.2.** The SSD is mixed well and labeled appropriately.

**8.5.** SWD @ 100 µg/mL (prepare monthly):

    **8.5.1.** Transfer 200 µL of SSD prepared in Section 8.4 into a 100 mL volumetric flask.

    **8.5.2.** Fill to volume with MeOH, mix well and label.

**8.6.** Primary cannabinoid standard stock solution @ 90 µg/mL (prepare yearly and store at -20 °C).

    **8.6.1.** 0.9 mL of each standard solution (1000 µg/mL) purchased from vendors are added to a 10 mL volumetric flask.

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

**Table 5 –** *(The primary cannabinoid standard mixture is prepared in a 10 mL volumetric 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 | 16398 | 90 µg/mL |
| 6 | 0.9 mL | THCV | 16397 | 90 µg/mL |
| 7 | 0.9 mL | CBDA | 15673 | 90 µg/mL |
| 8 | 0.9 mL | THCA | 15679 | 90 µg/mL |
| 9 | 0.9 mL | CBG | 15362 | 90 µg/mL |
| 10 | 0.9 mL | CBDV | C-140 | 90 µg/mL |

**8.7.** Primary cannabinoid working solution and surrogate @ 45.0 µg/mL (prepare yearly and store at -20 °C).

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**8.7.1.** Take 1.00 mL of the primary cannabinoid stock solution at 90 µg/mL as prepared in section 8.6 and place into a 2 mL volumetric flask, add 0.900 mL of SWD as prepared in Section 8.5.

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

**8.8.** Secondary cannabinoid stock solution 90 µg/mL (stable for 1 year at -20 °C). Currently, a separate preparation of the primary stocks is used as a secondary source. This stock is prepared at the same concentration but uses the remaining volume in the ampule. The remaining 90 µL of stock is diluted into a 1-mL volumetric flask (See Table 6).

**8.8.1.** 0.09 mL of each standard solution as purchased from vendors (1000 µg/mL) is added to a 1.0 mL volumetric flask.

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

**Table 6 -** Secondary cannabinoid stock standard (*The secondary standard mixture is prepared in a 1 mL volumetric as a combined preparation.)*

| Standard number | Volume Added | Standard name | Catalog # | Diluted Concentration |
|---|---|---|---|---|
| 1 | 0.09 mL | CBN | C-046 | 90 µg/mL |
| 2 | 0.09 mL | CBD | C-045 | 90 µg/mL |
| 3 | 0.09 mL | THC | T-005 | 90 µg/mL |
| 4 | 0.09 mL | CBGA | C-142 | 90 µg/mL |
| 5 | 0.09 mL | CBC | 16398 | 90 µg/mL |
| 6 | 0.09 mL | THCV | 16397 | 90 µg/mL |
| 7 | 0.09 mL | CBDA | 15673 | 90 µg/mL |
| 8 | 0.09 mL | THCA | 15679 | 90 µg/mL |
| 9 | 0.09 mL | CBG | 15362 | 90 µg/mL |
| 10 | 0.09 mL | CBDV | C-140 | 90 µg/mL |

**8.9.** Cross check reference standard solution with surrogate @ 45.0 µg/mL (prepare yearly and store at -20 °C). The CCR is prepared as described below but higher or lower levels or volumes may be prepared. The CCR should be prepared at a concentration within the middle of the calibration curve.

**8.9.1.** Take 0.5 mL of a secondary cannabinoid stock standard at 90 µg/mL as prepared in section 8.8 and place into a 1-mL volumetric flask and add 0.45 mL of SWD as prepared in section 8.5.

**8.9.2.** Fill to volume with MeOH and vortex to mix. This solution is used to prepare a cross check reference working solution (see Section 10.4).

**8.10.** Mobile Phases

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

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**8.10.1.1.**   1.575 g of ammonium formate is added to a scintillation vial and dissolved with a small volume of HPLC grade water.

**8.10.1.2.**   Ammonium formate solution prepared in step 8.10.1.1 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.

**8.10.1.3.**   This solution must be prepared monthly.

**8.10.2.**   HPLC mobile phase B:  100% MeCN with 0.1% Formic Acid.

**8.10.2.1.**   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.

**8.10.2.2.**   This solution must be prepared monthly.

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

**9.1.**   Sample shipping conditions:

**9.1.1.**   The medical marijuana products from Registered Organizations (RO) are shipped as 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 medical marijuana products must be stored under the conditions recommended by the manufacturer. The storage is documented.

**9.3.**   Sample preparation:

**9.3.1.**   Follow medical marijuana sample preparation as per NYS DOH MML-301, and document the preparation of all samples.

**9.3.1.1.**   Approximately 20 to 30 mg of sample matrix and/or medical marijuana product extract is weighed into a 1.5-mL centrifuge tube.

**9.3.1.2.**   The volume of surrogate, 0.010 to 0.100 mL, is spiked into the centrifuge tube. The amount is based on cannabinoid levels in the sample reported by the RO.

**9.3.1.3.**   Add 20.00 mL of MeOH and mix well for 30 minutes on a shaker to extract the sample.

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**9.3.1.4.** Transfer 1 mL of each extract into 1.5-mL centrifuge tubes and centrifuge at 12,000 g for 5 min.

**9.3.1.5.** Dilute the supernatant 1:100 with MeOH for determination of the cannabinoid profile. The dilution factor should be determined based on the concentrations of the cannabinoids in the sample reported by the RO. This is to insure that the final concentration will fall within the range of the calibration curve. Samples may need to be analyzed twice. A larger dilution may be needed to bracket high concentration cannabinoids, while a direct injection of the extract or a less diluted sample may be required for analysis in order to measure the lower-concentration cannabinoids present in the same sample.

**9.3.1.6.** Transfer 500 µL of IWD @ 10 µg/mL, prepared in Section 8.3, into 1.5 mL HPLC vial.

**9.3.1.7.** Transfer 500 µL of sample extract supernatant (Section 9.3.1.5) into the HPLC vial prepared in 9.3.1.6. The supernatant is mixed well with IWD providing an equal volume of extract to IWD.

**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, 3.0 x 150 mm; 2.7 µm partial size (Cat # 693975-302 or equivalent) 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 cannabinoid peaks in the samples, which are then integrated for analyte quantitation. Chromatography is achieved using the mobile phases described in Section 8.10, and the instrumental parameters outlined in Tables 7 and 8 are employed.

**9.4.2.** HPLC analytical parameters:

**9.4.2.1.** Injector:

**9.4.2.1.1.** Injector: The injection volume is 10µL

**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.

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**9.4.2.3.2.** 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 mobile phase A/mobile phase B gradient shown in the Table 7 for the Agilent Poroshell 120 column (the gradient may vary depending on the column used).

**Table 7** – Gradient

| Time (min) | %Mobile A | %Mobile B |
|:-:|:-:|:-:|
| 0.0 | 27 | 73 |
| 18.00 | 27 | 73 |
| 19.00 | 27 | 100 |
| 21.00 | 5 | 100 |
| 22.10 | 27 | 73 |
| 25.00 | 27 | 73 |

**9.4.2.4.2.** Data collection time: 18.0 min.

**9.4.2.4.3.** Total run time: 25.00 min.

**9.4.2.5.** Photodiode Array 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 (An alternate wavelength could be used, if necessary).

**9.5.** Extract storage:

**9.5.1.** Sample extracts at a volume of 20 mL are stored in a freezer at ≤ -20 °C until analysis. Analysis must be completed within 7 days of extraction. After testing is completed, the remaining extract is stored at ≤ -20 °C for one month for reanalysis, if needed. The medical marijuana product extract location and extraction are traceable in documentation.

**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 changes in mobile phases and microbial growth.

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**10.0. Calibration**

**10.1.** Initial calibration

**10.1.1.** Integration parameters are listed below in Table 8. Results are calculated using peak area. The integration parameters are examples from the current instrument. These settings serve as a guideline, and may be adjusted for optimization of integration.

**Table 8** – Integration parameters for a calibration curve using absorbance at 227 nm

| 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 |

**10.1.2.** The primary stock solution prepared in Section 8.6 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.

**10.1.3.** The lowest level calibration standard must be at or below the LOQ values listed in Section 1.1 for each analyte, or the LOQ values must be adjusted accordingly.

**10.2.** Primary cannabinoid stock standard curve preparation (Concentration range for all compounds including surrogate is 45.0 µg/mL to 0.19 µg/mL.)

**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 cannabinoid working solution prepared in Section 8.7 and SWD prepared in Section 8.5. This solution is labeled as CalS 6.

**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 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.** Pipet 300 µL CalS 5 taken from the initial HPLC vial (of Section 10.2.2.3) into CalS 4 vial.
**10.2.3.2.** Add 600 µL MeOH.
**10.2.3.3.** Mix well.

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**10.2.4.** _CalS 3_ **1.67 µg/mL**

**10.2.4.1.** Pipet 300 µL CalS 4 taken from the HPLC vial (of 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.** Pipet 300 µL CalS 3 taken from the HPLC vial (of 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.** Pipet 300 µL CalS 2 taken from the HPLC vial (of 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 8.3

**10.2.7.1.** Following Table 10 to prepare the standards in HPLC vials: from CalS-IWD-1 through CalS-IWD-6.

**Table 9 –** Dilution schedule of CalS 1 thru 6, Initial Standard dilution schedule w/out IWD (for final standard prep in MeOH)

| Cal std | Final Std conc µg/mL | Standard used to make the dilution, ref section ID | Volume of cannabinoid std | Volume of MeOH |
|---|---|---|---|---|
| CalS6 | 45 | 10.2.1.1 | - | - |
| CalS5 | 15 | 10.2..1.1 | 300 µL | 600 µL |
| CalS4 | 5 | 10.2.2.3 | 300 µL | 600 µL |
| CalS3 | 1.67 | 10.2.3.3 | 300 µL | 600 µL |
| CalS2 | 0.56 | 10.2.4.3 | 300 µL | 600 µL |
| CalS1 | 0.19 | 10.2.5.3 | 300 µL | 600 µL |

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**Table 10 –** Final standard dilution schedule mixed with IWD (for analysis on instrument)

| Cal std w/IWD | Cal std (Table 9) | Volume of cannabinoid stock std | IWD As prepared in Section 8.3 |
|---|---|---|---|
| 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 its initial calibration.

**10.3.2.** The correlation coefficient (r) of the calibration curve for each analyte must 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 detection), 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 a CCR.

**10.4.2.** **CCR@** 4.50 µg/mL

**10.4.2.1.** Add 900 µL MeOH into an HPLC vial.

**10.4.2.2.** Add 100 µL cross check reference standard solution with surrogate @ 45.0 µg/mL from 8.9 into vial.

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**10.4.2.3.**   Mix well and label as CCR without IS.

**10.4.2.4.**   Add 500 µL of IWD prepared in Section 8.3 into an HPLC vial.

**10.4.2.5.**   Add 500 µL from 10.4.2.3 and mix well.

**10.4.2.6.**   Label as CCR-IWD.

**10.4.3.**   The measured recovery values for the analytes of the CCR must fall within 90-110% of the known value for the major cannabinoids.

**10.5.**   CCV

**10.5.1.**   The working calibration curve for each cannabinoid must be verified on each working day by the analysis of a CCR, a low-level standard and a minimum of two mid-level CCVs, one at the beginning and one at the end of the analytical sequence. The opening CCV and low-level standard may be substituted with a full initial calibration and CCR.

**10.5.2.**   For extended periods of analysis, CCVs must also be interspersed throughout the analytical sequence at regular intervals (every 20 samples, including quality control samples), with the CCVs at varying concentration levels. A sequence is always bracketed with a final CCV at the end of batch/sequence.

**10.5.3.**   The analytes in the CCVs must be recovered within 90-110% of the predicted concentration.

**10.5.3.1.**   CCVs are prepared at the following levels: 15 µg/mL, 5.0 µg/mL and 1.67 µg/mL (same as CalS-IWD- 3 through CalS-IWD-5 in Section 10.2).

**11.0.   Quality Control and Assurance**

**11.1.**   Demonstration of Capability (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 ± 10% of mean. The precision of the measurements, calculated as relative standard deviation (RSD), must be ± 5% or less for the major analytes such as THC or CBD. 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 if available:

**11.1.1.2.1.** Acceptable performance of a blind sample, such as an external proficiency test.

**11.1.1.2.2.** Acceptable performance of an initial DOC as described above in 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 ongoing DOCs must be documented.

**11.2.** LOD

**11.2.1.** An LOD study must be completed and documented for all target analytes in each matrix (See section 1.1 and 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. It is recommended that the laboratory control samples be prepared and analyzed over a period of several days, so that day-to-day variations are reflected in the precision of the data, however, this is not a requirement.

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

$$LOD = \text{standard deviation of the n samples } (\mu g/mL) * t^1$$

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

**Table 11**

| n | t |
|---|---|
| 7 | 3.143 |
| 8 | 2.998 |
| 9 | 2.896 |
| 10 | 2.821 |

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**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 1.**

$$LOQ = LOD * 5$$

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

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

**11.3.** SBLK

**11.3.1.** Before processing samples, the analyst must demonstrate that the instrument is free from background interference by analyzing a SBLK.

**11.3.1.1.** Fill HPLC vial full with MeOH.

**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, determine 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.** Prepare a MB sample as per NYS DOH MML-301. Fill HPLC vial with 500 µL of blank extracted matrix and 500 µL 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.

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**11.6.** Surrogate spike

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

**11.7.** IS

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

**11.8.** MS and MSD

**11.8.1.** After samples are diluted/extracted in MeOH, a selected MS and MSD supernatant extract is spiked with 5.0 µg/mL of cannabinoids standard mixtures as the matrix spiked sample. A MS and MSD are analyzed after every twenty samples or one per sample batch (all samples prepared within a 24-h period), whichever is greater.

**11.8.2.** When a matrix spike is not available, the extraction solvent is fortified with each target analyte at a mid-level concentration (5.0 µg/mL is recommended). Duplicates of extraction solvent spiked sample must be analyzed.

**11.8.3.** The accuracy is calculated as percent recovery. The recovery for the individual cannabinoid analytes must fall within the range of 80-120% of the spiked concentration. The precision between duplicates is calculated using relative percent difference (RPD). The RPD must be <10.

**11.9.** System Performance Mix Requirements

**11.9.1.** Method specifications

**Table 12 –** Method specifications

| 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:
[a]PGF = Peak Gaussian Factor, calculated using:

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

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

[b]Resolution between the two peaks as defined by the equation:

$$R = t/W$$

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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.** Planned Maintenance (PM) must be performed twice a year per Manufacturer's specifications. This documentation 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. PM addresses immediate and future service issues on instrumentation to maximize system productivity.

**11.9.2.2.** Daily, an instrument perform system check is performed and this documentation is maintained to make sure instrument hardware is functional. (i.e., detectors and pumps).

## 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, chromatographic conditions, or detectors may be used if the requirements of Appendix A are met.

**13.1.2.** Calibrate or verify the system calibration on each day of analysis as described in Section 10.4. For all of the 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 "carry-over" can occur whenever high-concentration 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 carry-over. After the analysis of a sample containing high concentrations of cannabinoids, one or more blanks should be analyzed to check for cross-

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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 in order to ensure that the total system (syringe, lines and LC 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 to wash new columns and currently in use columns prior to startup of a new batches with 100% mobile phase B for a period of 2 hours.

**13.2.** Identification of analytes

**13.2.1.** Identify a sample component by comparison of its retention time to 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.

**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.** Calculate 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

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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 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.

**13.3.2.3.** Calculate the average retention time of the IS in the calibration curve. The retention time of each standard IS 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 of 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 13.3.2.4 from the analysis of the standard curve.

**13.3.3.** QC and unknown samples

**13.3.3.1.** Apply the linear regression calibration curve generated from the calibration standards to all QA/QC and unknown samples to calculate the concentration (μg/mL) of each cannabinoids using the instrument quantification software.

**13.3.4.** Medical marijuana products

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

**Equation 2.**

$$C_S = \frac{C_x\,(\mu g/mL) * V_F\,(mL) * D}{M_I\,(mg) * 1000}$$

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**Equation 3.**

$$\% \, C_S = \, C_S \,*100$$

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 daily calibration verification standards 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, unless the low-level standard in the calibration was not at or below the LOQ, in which case the LOQ must be adjusted accordingly.

**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 4 and 5.

**Equation 4**   Total THC = $(C_{THC}+C_{THCA})*M_{dose}$

**Equation 5**   Total CBD = $(C_{CBD}+C_{CBDA})*M_{dose}$

$C_{THC}$ = Concentration of THC calculated using equation 2.
$C_{THCA}$ = Concentration of THCA calculated using equation 2.
$C_{CBD}$ = Concentration of CBD calculated using from equation 2.
$C_{CBDA}$ = Concentration of CBDA calculated using equation 2.
$M_{dose}$ = Total mass per dose (mg)

## 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 14.10) 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.

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**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 there is one particular standard that appears to be prepared incorrectly. If so, re-prepare that standard and analyze. If more than eight (8) h has 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 limit of quantitation 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 cross check reference standard (CCR) of 90-110% recovery.

    **14.4.1.** A new CCR is prepared and re-analyzed. It may be 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, 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 run on the instrument, and it is 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 90-110% recovery.

    **14.5.1.** A new CCV is prepared and re-analyzed. It may be necessary to prepare it from 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 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.

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**14.5.3.** All samples must be bracketed by an acceptable CCV. If samples were analyzed before an acceptable CCV was achieved, all affected samples must be re-analyzed once an acceptable CCV is achieved or a new calibration curve is established on the instrument. 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 low level standard (CalS-IWD-1) of 70-130% recovery for routine sample batches.

**14.6.1.** A new CalS-IS-1 is prepared and re-analyzed; may need to prepare from new working or stock solutions.

**14.6.2.** If a newly prepared CalS-IS-1 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.** A CalS-IS-1 within 70-130% 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.** 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).

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**14.8.2.** Re-inject the MB once a contaminant-free system is achieved.

**14.8.3.** If the re-injection still fails, request that a new MB be extracted and analyze 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.

**14.8.4.** 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.

**14.8.5.** 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.

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

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

**14.9.2.** 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; this may require preparation 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.

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

**14.9.4.** 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.

**14.10.** MS and MSD with < 80% and > 120% recovery.

**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.

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**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 those results.

**14.10.4.** The relative percent difference (RPD) for each spiked analyte in the MS and MSD must be < 15%, until in-house precision limits can be established.

**14.10.5.** If the new MS and/or MSD still fails, the original MS and/or MSD and corresponding sample results will 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 re-injection meets 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 will be reported with appropriate qualifiers.

**14.12.** Surrogates 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 surrogate 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.

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**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 laboratory.

**15.2.** The instrumentation's PM is performed twice a year by Shimadzu. This maintenance pertains to the lamp, pumps and computers.

## 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-Gallon solvent can), properly labeled (separate chlorinated and non-chlorinated solvents).

**16.4.** Dispose of water 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

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**18.0.  Supporting Documents**

> **18.1.**   See Appendix A.
>
> **18.2.**   NYS DOH MML-301

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### APPENDIX A

**Figure 1- 5µg/mL ISTD in blank solvent**



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

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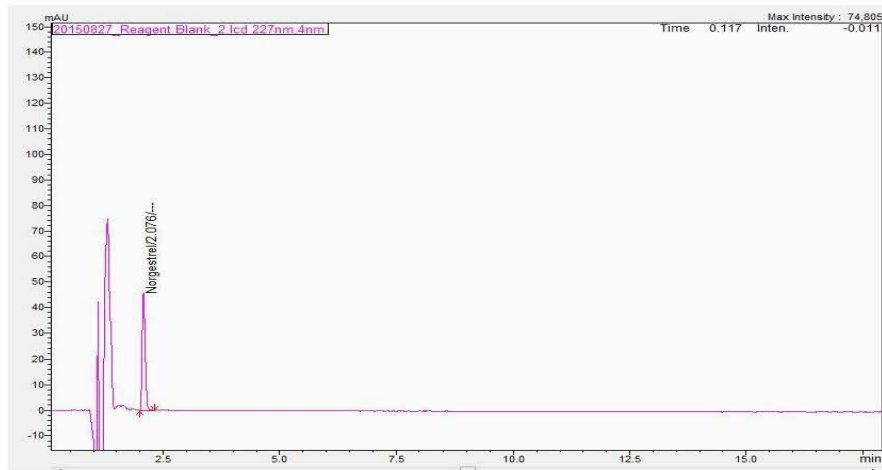**SALLY DRESLIN, M.S., R.N.**
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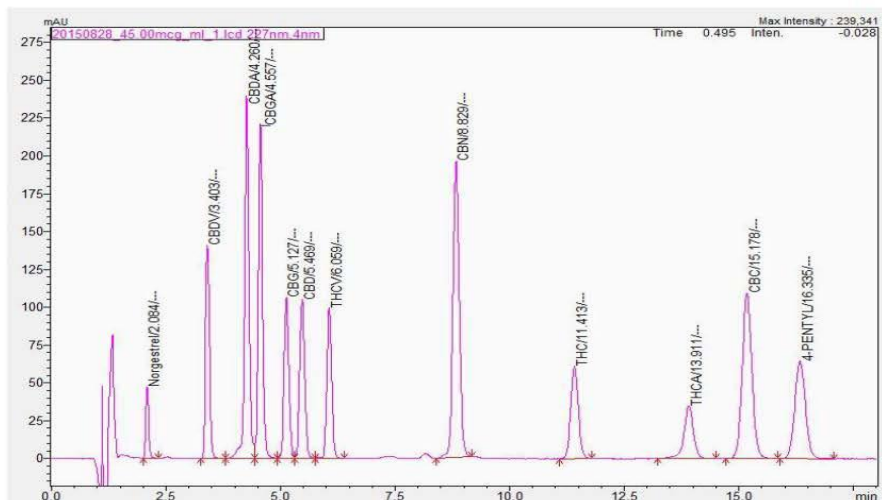
### Table 1 – HPLC-PDA
**Cannabinoids, Internal Standard and Surrogate with corresponding retention times.**

| 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%