Method:	ECLS-CT-MM-1
Method Issued:	03/11/2013
Revision (#):	04
Revised:	06/07/2019

Standard Operation Procedure for Qualitative and Quantitative Determination of Major Cannabinoids in Cannabis Plant Material

Method ECLS-CT-MM-1

New Jersey Department of Health

Public Health and Environmental Laboratories

Environmental and Chemical Laboratory Services

3 Schwarzkopf Drive

West Trenton, NJ 08628

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Qualitative and Quantitative Analysis of Cannabinoids in Cannabis Plant Material by Liquid Chromatography–Diode Array Detector/Mass Spectrometry Detector (LC-DAD/MSD) Trap

1. Identification of the Test Method

This procedure illustrates a method for determination of **Cannabinoid Profile in Marijuana Plant Samples.**

2. Applicable Matrices

Cannabis Sativa and *Indica* Plant Materials

3. Detection Limits

3.1 The upper reporting limit is the concentration of the highest calibration standard.

3.2 The method detection limits for this method are recorded in **Appendix L** and vary by cannabinoid.

3.3 Method detection limit (MDL) is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero. The MDL is compound dependent and is particularly dependent on extraction efficiency and sample matrix.

3.3.1 A Method Detection Limit for each listed analyte was obtained by analyzing 7 replicates of the lowest calibration standard used in this method and is recorded in Appendix L. The raw data calibration reports used to calculate the MDL are located in **Appendix L**.

3.3.2 The method detection limits (MDL) were calculated using the formula:

$$\mathbf{MDL = S\ t_{(n-1,\ 1\text{-}alpha=0.99)}}$$

where:
t (n-1, 1-alpha = 0.99) = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses
Student t-values are listed in the Environmental Protection Agency – Code of Federal Regulations Part 136 (40CFR136) which can be found online. Student t-value for 7 replicates = 3.143.

Analyte Name	CAS #	Molecular Weight (g/mol)	Lower Reporting Limit (%)
Delta-9-Tetrahydrocannabinol (Δ^9-THC)	1972-08-3	314.46	0.012
Cannabidiol (CBD)	13956-29-1	314.47	0.012
Delta-9-Tetrahydrocannabinoic Acid (THCA)	37347-91-4	358.48	0.012
Cannabigerol (CBG)	25654-31-3	316.48	0.012
Cannabigerolic Acid (CBGA)	25555-57-1	360.49	0.012
Cannabidiolic Acid (CBDA)	1244-58-2	358.48	0.012
Cannabinol (CBN)	5211-35-7	310.43	0.012
Delta-8-Tetrahydrocannabinol (Δ^8-THC)	5957-75-5	314.47	0.012

4. Scope and Application

This method is a qualitative and quantitative procedure for the measurement of the cannabinoids in a plant material by liquid solid extraction and a High-Performance Liquid Chromatography (HPLC)–Ultraviolet Diode Array Detector (UVDAD) and Mass Spectrometry Detector (MSD) Trap. It also has the potential to be used for the analysis of other Cannabis matrices after validating the method for a particular product.

5. Summary of Method

Ground cannabis plant material is extracted with a methanol: chloroform (9:1) mixture twice. The combined extract is filtered through a 0.2 µm Nylon membrane. The combined extract is diluted and 200 µL of diluted extract is then concentrated to dryness under a nitrogen stream at room temperature using the Turbo-Vap unit. The residue is reconstituted in 200 µL of methanol: water (65:35) and then is analyzed by HPLC-UVDAD. Quantitation is performed at the 235 nm UVDAD signal with the Agilent "ChemStation" software using a linear regression. The MSD Trap is used for an additional qualitative identification. Cannabinoid compounds are reported as percent by weight of medicinal marijuana plant material.

This method is optimized from the following publication.

De Backer, B., ***et.al. Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material*****. 2009.** ***Journal of Chromatography B,*** **877, 4115-4124.**

6. Definitions

6.1 ATC (Alternative Treatment Center) – Centers that grow and distribute the medicinal marijuana tested in this method.

6.2 Calibrator – A solution containing the calibration standard(s) at a known concentration.

6.3 CB (Calibration Blank) – The reagent blank at 0 ppm analyzed at the beginning of each analytical batch to demonstrate that none of the target analytes or any interference is observed at or above the limit of detection.

6.4 COC – Chain of Custody

6.4.1 External COC– will be maintained on the official NJDOH Sample Submittal Form (**CTL-1**). It shall include: the mode of collection, the collector's name, the date and time of collection, preservation method, requested analyses, storage, and all movement of the sample from collection to receipt.

6.5 CRL (Control) – a quality control sample used to assure analyses are properly performed and the results produced are reliable.

6.6 CV – Calibration Verification

6.6.1 CCV (Continuous Calibration Verification) – a solution comprised of primary source standards that is used as a verification of the primary source standards used in the initial calibration.

6.6.2 HCV (High Calibration Verification) – a solution comprised of secondary source standards at a high concentration that is processed and analyzed as a demonstration of higher calibration range recoveries.

6.6.3 LCV (Low Calibration Verification) – a solution comprised of secondary source standards at a low concentration that is processed and analyzed as a demonstration of lower calibration range recoveries.

6.7 ECLS - Environmental & Chemical Laboratory Services, at 3 Schwarzkopf Drive, West Trenton, NJ 08628.

6.8 ECLS/CT/MML – Environmental & Chemical Laboratory Services/Chemical Terrorism/Medicinal Marijuana laboratory.

6.9 IS (Internal Standard) – A non-cannabinoid added to a sample, extract, or standard solution in a known amount. The responses of method analytes and surrogates relative to internal standard are used in all concentration calculations.

6.10 MC (Method Control Sample) – A composite sample comprised of previously-analyzed medicinal marijuana samples that is extracted and analyzed parallel to

any samples received by the NJDOH to ensure that the method is consistent for analysis of the appropriate matrix.

6.11 Method Detection Limit (MDL) – the lowest concentration or amount of the target analyte that can be determined to be different from zero by a single measurement at a stated level of probability. Detection limits are analyte-specific.

6.12 MS (Matrix Spike)/MSD (Matrix Spike Duplicate) – A sample spiked with a concentrated solution of one or more of the target analytes. The percentages of analytes recovered from the spiked sample are compared to the percentages in the unspiked sample and the recovery is calculated as a percentage to assess the ability of the method to extract the cannabinoids of interest.

6.13 ND – Not detected.

6.14 NJDOH – New Jersey Department of Health.

6.15 NJMMP – New Jersey Medicinal Marijuana Program.

6.16 OP (Organically Pure) – Free of organic solids that could potentially interfere with an HPLC analysis. Organic purity is measured in units of total organic carbon (TOC).

6.17 PPE (Personal Protective Equipment) – Equipment worn by analysts in order to protect them from occupational and laboratory hazards. PPE always includes a laboratory coat and safety glasses and may also include latex or rubber gloves, a face shield or surgical mask, or any other items required to ensure that the analyst is safe during his or her laboratory activities.

6.18 PT (Proficiency Testing) – Analysis of samples with unknown levels of cannabinoids. The University of Kentucky offers a proficiency testing program for the determination of cannabinoids (Δ^9-THC, Δ^9-THCA, CBD, CBDA, CBN, total Δ^9-THC, Total CBD) in hemp.

6.19 QA (Quality Assurance) – An integrated system of activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence.

6.20 QAO (Quality Assurance Officer) – The officer responsible for the implementation and management of all quality systems and procedures outlined in the QM (Quality Manual).

6.21 **QC (Quality Control)** – The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users. The term "QC Samples" refers to the CRL, CCV, HCV, and LCV that are analyzed alongside the plant samples and must meet acceptance criteria outlined in **Section 12.5** for the results of the analysis to be deemed acceptable.

6.22 **QM (Quality Manual)** – A document stating the management policies, objectives, principles, organizational structure and authority, responsibilities, accountability, and implementation of an agency, organization, or laboratory to ensure the quality of its product and the utility of its product to the users.

6.23 **RC (Regulatory Compliance Sample)** – A sample of medicinal marijuana analyzed to ensure that the ATC is adhering to the regulations set forth by the NJMMP.

6.24 **RL (Reporting Limit)** – The lowest detection value that can be reliably achieved.

7. Interferences

7.1 Unknown compounds from the plant matrix can interfere with the peaks of analytes of interest.

7.2 CBD and CBG elute adjacently and should be monitored to ensure peaks are correctly integrated.

Note: Resolution needs to be monitored and adjustment is to be made if necessary. See **Section 9.4.1** and **Appendix H**.

8. Safety

8.1 Lab coats and safety glasses are mandatory for all personnel in the laboratory.

8.2 The health impacts of chemicals used in this method have not been fully investigated. Each chemical should be treated as a potential health hazard. Exposure to these chemicals must be reduced to the lowest possible level by the chemical fume hood, protective lab coat, and face shield/safety glasses. For acetonitrile use butyl or nitrile gloves. For methanol use neoprene or rubber glove. For ammonium acetate use nitrile gloves.

8.3 Safety Data Sheets (SDS) containing the potential hazards and other health and safety information associated with specific chemicals or reagents are available in binders in **Room L-425B**. Please refer to them for usage and safety information.

8.3.1 Acetic Acid, Glacial [64-19-7] – Flammable Liquid

Health Effects:
Skin Corrosion, Serious Eye Damage
Recommended Gloves – butyl-rubber, natural latex/chloroprene

8.3.2 Ammonium Acetate [631-61-8]
Health Effects: nonhazardous

8.3.3 Chloroform [67-66-3]
Health Effects:
Carcinogen, Acute toxicity, Harmful by Ingestion, Eye & skin Irritant
Target Organs – Eyes, skin, liver, kidneys, central nervous system
Recommended Gloves – fluorinated rubber

8.3.4 Ethyl Alcohol (Ethanol) [64-17-5] – Flammable Liquid
Health Effects:
Eye Irritant
Recommended Gloves – butyl-rubber or nitrile rubber

8.3.5 Hydrogen Peroxide [7722-84-1]
Health Effects:
Acute toxicity, skin corrosion, eye damage
Target Organ – respiratory system
Recommended Gloves – nitrile rubber

8.3.6 Isopropyl Alcohol [67-63-0] – Flammable Liquid
Health Effects:
Eye & skin Irritant,
Target Organ: Central Nervous System
Recommended gloves – nitrile rubber

8.3.7 Methanol [67-56-1] – Flammable Liquid
Health Effects:
Acute toxicity – Oral, Inhalation, & Dermal,
Recommended Gloves – butyl-rubber or nitrile rubber

8.3.8 Nitric Acid [7697-37-2] – Oxidizing Liquid, Corrosive to Metals
Health Effects:
Skin corrosion, serious eye damage
Recommended Gloves – fluorinated rubber, natural latex/chloroprene

8.4 Mechanical Hazards: Laboratorians should read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the LC and mass spectrometer unless all power to the instrument is off. Generally, mechanical and electronic maintenance and repair should be performed only by qualified technicians. The LC and the mass spectrometer contain several areas which are hot enough to cause burns. Precautions should be used when working in these areas.

8.5 General Guidelines for Working with Chemical Substances:

8.5.1 Use safety carriers when transporting 2-liter or larger bottles. Handle in the chemical fume hood.

8.5.2 Use recommended gloves. Change gloves to avoid cross-contamination of samples, reagents and glassware.

8.5.3 Wear safety glasses when pouring from one container into another.

8.5.4 Use nostril / nose masks, gloves and goggles when grinding medicinal marijuana plant material.

8.5.5 When diluting acids, **always add the acid to water**.

8.5.6 Keep chemicals in tightly closed containers and protect from physical damage.

8.5.7 Store in cool, dry, ventilated areas away from sources of heat, and incompatibles.

9. Equipment, Supplies, and Maintenance

9.1 Sample Extraction and Preparation

9.1.1 Mettler Toledo XPE205DR Analytical Balance (Material No. 3008770) or equivalent

9.1.2 Sartorius Cubis MSA225S-100DI Analytical Balance (Cat. No. MSA225S100DI) or equivalent

9.1.3 VWR Disposable, Square, 85 x 85 x 24 mm, 100-mL Weighing Boats (Cat. No. 10803-150) or equivalent

9.1.4 Sentry Safes (4, assorted sizes) with Combination Locks (available at several retail locations) or equivalent

9.1.5 Assorted Glass Beakers

9.1.6 Assorted Graduated Cylinders – Glass – 100 mL, 250 mL, 500 mL, 1000 mL

9.1.7 VWR 1 L Glass Media Storage Bottles with Cap (Cat. No. 10754-820) or equivalent

9.1.8 Millipore Milli-Q Gradient A10 Water Purification System (Cat. No. QGARD00D2) or equivalent

9.1.9 Mettler Toledo FE20 FiveEasy Benchtop pH Meter (Cat. No. 01-915-661) or equivalent

9.1.10 Mettler Toledo LE409 pH Electrode (Cat. No. 01-915-676) or equivalent

9.1.11 VWR Spin Bar Magnetic Stir Bar 9.5 x 25 mm (Cat. No. 58948-983) or equivalent

9.1.12 VWR Standard Hot Plate Stirrer (Cat. No. 97042-638) or equivalent

9.1.13 Thermosafe Dry Ice Machine (Model No. 460) or equivalent

9.1.14 Airgas Bone Dry 3.0 Grade 300 L Carbon Dioxide Tank with Siphon (Part No. CD BD300S) or equivalent

9.1.15 Robot Coupe Blixer 2 Single Speed Blender/Mixer or equivalent

9.1.16 Parafilm 2 in. x 250 ft. Plastic Paraffin Film (Sigma Aldrich Product No. P7543) or equivalent

9.1.17 Rubbermaid Icing Blade Scraper (Item No. 1913) or equivalent

9.1.18 Retsch Mixer Mill MM400 (Cat. No. 207450001) or equivalent

9.1.19 Retsch Falcon Tube Adapter for MM400 (Cat. No. 220010015) or equivalent

9.1.20 Retsch 10 mm stainless steel grinding ball (Cat. No. 053680063) or equivalent

9.1.21 VWR TraceClean 120 mL Straight Sided, Wide Mouth Amber Glass Jars (Cat. No. 89094-030) or equivalent

9.1.22 Pipettes and Tips

- **9.1.22.1** 10 – 100 µL Eppendorf "Reference" Pipette (Eppendorf Cat. No. 4920000059) or equivalent
- **9.1.22.2** 2 – 200 µL Eppendorf epT.I.P.S. (Eppendorf Cat. No. 022491539) or equivalent
- **9.1.22.3** 20 – 200 µL Rainin "Pipet-Lite" Pipette (Rainin Cat. No. 17008652
- **9.1.22.4** 20 – 250 µL Rainin pipette tips (Rainin Cat. No. 17001116)
- **9.1.22.5** 100 – 1000 µL Eppendorf "Reference" Pipette (Eppendorf Cat. No. 4920000083) or equivalent
- **9.1.22.6** 50 – 1000 µL Eppendorf epT.I.P.S (Eppendorf Cat. No. 022491555) or equivalent
- **9.1.22.7** 0.5 – 10 mL Eppendorf "Repeater Plus" Pipette (Eppendorf Cat. No. 4982000322) or equivalent
- **9.1.22.8** 50 mL Eppendorf "Combitips Plus" (Eppendorf Cat. No. 0030089480) or equivalent
- **9.1.22.9** VWR Disposable 5.75" Borosilicate Glass Pasteur Pipettes (Cat. No. 14673-010) or equivalent
- **9.1.22.10** VWR 1 mL Latex Pipette Bulbs (Cat. No. 82024-550) or equivalent

9.1.23 VWR Disposable, Graduated, Conical, Sterile 50 mL Centrifuge Tubes with Screw Caps (Cat. No. 21008-178) or equivalent

9.1.24 Traceable 99M/59S Timer (Model No. 23609-196) or equivalent

9.1.25 Thermo Scientific MaxiMix I Vortex Mixer, 120 V (Model No. M16715Q) or equivalent

9.1.26 Burrell Scientific Variable Speed Wrist Action Shaker Model 95-FF 115 V (Part No. 0757952419) or equivalent

9.1.27 Burrell Scientific Single Speed Wrist Action Shaker Model 75-CC 115V (Part No. 0757751219) or equivalent

9.1.28 Eppendorf Centrifuge 5910R refrigerated, with Rotor S-4 x Universal, 120 V (Part. No. 5942000342) or equivalent

9.1.29 BD 5 mL Single-Use, Sterile Syringe with Luer-Lok Tip (Cat. No. 309646) or equivalent

9.1.30 Pall Life Sciences Acrodisc 13 mm Syringe Filter with 0.2 µm Nylon Membrane (Part No. 4540) or equivalent

9.1.31 VWR Disposable 12 x 75 mm Borosilicate Glass Culture Tubes (Cat. No. 47729-570) or equivalent

9.1.32 VWR Disposable 16 x 125 mm Borosilicate Glass Culture Tubes (Cat. No. 47729-578) or equivalent

9.1.33 Evaporator and Accessories

9.1.33.1 Biotage TurboVap LV 110 V Concentration Evaporator Workstation (Part No. C103198) or equivalent

9.1.33.2 Biotage TurboVap Tube Rack for 10 x 75 mm Tubes (Part No. C48950) or equivalent

9.1.33.3 Airgas Industrial Grade 300 L Nitrogen Tank (Part No. NI 300) or equivalent

9.1.34 Vials and Caps

9.1.34.1 AQ Brand 300-µL Screw Top Polypropylene Autosampler Vials (Cat. No. 9532S-MS) or equivalent

9.1.34.2 AQ Brand Screw-Top Autosampler Caps (Cat. No. 9502S-10M-B) or equivalent

9.1.34.3 Agilent 2 mL Screw Top Amber Glass Vials (Part No. 5182-0716) or equivalent

9.1.34.4 Agilent 9 mm Screw Caps (Part No. 5185-5864) or equivalent

9.1.35 SCP Science DigiBLOC 3000 Graphite Digestion Block (Cat. No. 010-500-205) or equivalent

9.2 Sample Analysis

9.2.1 LC Supplies –See Agilent Manual

9.2.2 LC Column – Agilent Poroshell 120 SB-C18, 3.0 x 75mm 2.7-micron Agilent Cat PN # 687975-302

9.2.3 MS Supplies - Refer to list found in Agilent 1100 LC-MSD Trap (SL)Maintenance Manual located on the system desktop.

9.3 Instrumentation/Software

9.3.1 The analysis is performed on Agilent 1100 LC-UVDADMSD (Trap

SL) with a 5983B liquid handling autosampler. Agilent "ChemStation" software data system is used to control the instruments. Data analysis is performed using ChemStation in the "Enhanced Data Analysis" mode and MSD Trap Data Analysis Module.

Agilent 1100 Series LC system with following modules:

9.3.1.1 G1379A Degasser
9.3.1.2 G1312A Binary Pump
9.3.1.3 G1313A Autosampler
9.3.1.4 G1316A Column Compartment
9.3.1.5 G1315B UV Diode Array Detector (DAD)
9.3.1.6 G2445D Agilent 1100 Series LC/MSD trap (SL)

9.3.2 The "Chem Station" software can acquire, store, reduce, and output DAD data. It identifies each analyte within a specified retention time window for each LC peak. Integration is then performed on these quantitation and confirmation ions Quantitation is performed using linear regression.

9.4 Maintenance

9.4.1 **Column –** When chromatographic peak shape deteriorates, the column can be cleaned by passing a higher polarity solvent (95% Methanol: 5% 25 mM Ammonium Acetate in water at 0.5 mL per minute setting column temperature at 40 ℃ for few hours. Re-equilibrate the column using initial method mobile phase conditions for an hour before and analytical peak shapes should improve. See **Appendix H.**

9.4.2 **LC/MS** – ECLS has a service contract with Agilent for a preventative maintenance schedule. This contract includes LC inlet maintenance, mass spectrometer source cleaning, and rough pump maintenance. If necessary, this maintenance can be performed between service calls. Service maintenance videos are available on CDs located at the work station.

10. Reagents and Standards

10.1 **Acetic Acid** (CAS # 64-19-7), "Baker analyzed" ACS reagent, J.T. Baker, Cat # 9508-03 or equivalent.
10.2 **Ammonium Acetate** (CAS # 631-61-8) Reagent Grade, >98.0% Sigma Aldrich, Cat. # A7262 or equivalent.
10.3 **Chloroform** (CAS # 67-66-3), "Baker analyzed" ACS reagent, J.T. Baker, Cat # 9180-01 or equivalent.

10.4 **Ethyl Alcohol, 200 Proof [64-17-5],** Millipore Sigma ACS Grade (Millipore Sigma Cat. No. 57188) or equivalent.

10.5 **Ibuprofen** (CAS # 15687-27-1) ≥98% (GC) Sigma Aldrich, Cat. # I4883 or equivalent.

10.6 **Isopropyl Alcohol** (2-Propanol) (CAS # 67-63-0) Fisher Scientific, Cat # A998-4 or equivalent.

10.7 **Methanol** (CAS # 67-56-1) HPLC Grade J.T. Baker, Cat # BJAH230-4 or equivalent.

10.8 **Nitric Acid** (CAS # 7697-37-2), 69-70 %, Baker Analyzed, Cat # 9601-04 Fisher Scientific, Cat # A450-4 or equivalent.

10.9 **Water** Type 1 – Organic Pure (OP) Water - (18MΩ) (< TOC 5ppm).

10.10 Mobile Phases

10.10.1 Mobile Phase A: 25 mM Ammonium Acetate in 1L OP water.

Weigh 1.925g of Ammonium Acetate in a weighing boat and dissolve in 1000 mL of organic pure (OP) water. Using a calibrated pH meter for pH buffers 7.0 & 4.0, adjust the pH to 4.75 (± 0.01) by adding acetic acid drop wise and with continuous stirring.

10.10.2 Mobile Phase B: Methanol, HPLC grade

10.11 **MeOH: Water (65:35) 10mL –** Combine 6.5 mL of methanol in 3.5 mL of organic pure water (measure separately and combine). Prepare fresh daily as methanol will evaporate

10.12 Calibration Standards

10.12.1 Primary Standards:

10.12.1.1 **Cannabinoids standard,** 1000 µg/mL in Methanol; 3-Components: **Delta-9-Tetrahydrocannabinol (Δ^9-THC), Cannabidiol (CBD),** and **Cannabinol (CBN)** Restek Cat No. 34014 or equivalent

10.12.1.2 **Delta-8-Tetrahydrocannabinol (Δ^8-THC) Standard,** 1000 µg/mL in Methanol, Restek Cat. No. 34090 or equivalent

10.12.1.3 **Delta-9-Tetrahydrocannabinoic Acid (THCA),** 1000 µg/mL in Methanol, Restek Cat. No. 34093 or equivalent

10.12.1.4 Cannabigerol (CBG), 1000 µg/mL in Methanol, Restek Cat. No. 34091 or equivalent

10.12.1.5 Cannabigerolic Acid (CBGA), 1000 µg/mL in methanol (Cayman Chemical Cat. No. 9001572, solid) or equivalent

Note: CBGA solid (1 mg) is dissolved in 1 mL of methanol prior to the creation of the standards.

10.12.1.6 Cannabidiolic Acid (CBDA), 1000 µg/mL in acetonitrile (Cayman Chemical Cat. No. 18090) or equivalent

10.12.2 Quality Control Samples (LCV, HCV) The low and high calibration verification samples contain the same compounds as **10.12.1.1 – 10.12.1.6** but from a separate vender. If a separate vendor cannot be obtained, a separate lot from the same vendor is acceptable.

10.12.3 Secondary Standards:

10.12.3.1 Delta-9-Tetrahydrocannabinol (Δ^9-THC), 1000 µg/mL in Methanol, Cerilliant Cat. No. T-005 or equivalent

10.12.3.2 Cannabidiol (CBD), 1000 µg/mL in Methanol, Cerilliant Cat. No. C-045 or equivalent

10.12.3.3 Cannabinol (CBN), 1000 µg/mL in Methanol, Cerilliant Cat. No. C-046 or equivalent

10.12.3.4 Delta-8-Tetrahydrocannabinol (Δ^8-THC) Standard, 1000 µg/mL in Methanol, Cerilliant Cat. No. T-032 or equivalent

10.12.3.5 Delta-9-Tetrahydrocannabinoic Acid (THCA), 1000 µg/mL in acetonitrile, Cerilliant Cat. No. T-093 or equivalent

10.12.3.6 Cannabigerol (CBG), 1000 µg/mL in Methanol, Cerilliant Cat. No. C-141 or equivalent

10.12.3.7 Cannabigerolic Acid (CBGA), 1000 µg/mL in acetonitrile, Cerilliant Cat. No. C-142 or equivalent

10.12.3.8 Cannabidiolic Acid (CBDA), 1000 µg/mL in acetonitrile, Cerilliant Cat. No. C-144 or equivalent

10.12.4 Delta-9-Tetrahydrocannabinol (Δ^9-THC), 50 mg/mL in ethanol (Lipomed Cat. No. THC-139-50LE) or equivalent

10.12.5 Method Control (MC) Sample

The method control sample is a composite of ground plant material prepared from previously-analyzed samples. This sample is extracted and analyzed parallel to any samples received by the NJDOH and the results are compared to previous analyses of the MC to assure that the method is consistent for analysis of the appropriate matrix. [An example of a method control sample would be ID#12-073-59-01 which is a composite composed of previously-analyzed samples.]

11. Sample Collection, Preservation, Shipment and Storage

11.1 Medicinal marijuana plant samples are collected by NJDOH Medicinal Marijuana Program Enforcement officials and delivered to the ECLS Chemical Terrorism/Medicinal Marijuana laboratory. The sampling agency is responsible for initiating the COC for each sample and other appropriate sample documentation. An external chain-of–custody or sample submittal form must accompany each sample, and must contain all relevant information regarding the sample, including but not limited to:

Sample ID Number
Client, Address, Phone #, Client ID#
Grower's Name, Sampling Site, Address
Name of Sample Collector, Time and Date of Sampling.
Type of Sample-Flowers, Shakes, etc. and Sample Weight
Analysis Requests –Regulatory Compliance, Cannabinoid Profile, Pesticide Residue, Heavy Metals, Mycotoxins, etc.
NJMMP personnel delivering the material

11.2 Chain of Custody Information should also include: Relinquished by, Received by, Sample weight upon the receipt, Date and Time of receipt and Reason for transfer for each sample.

11.3 Cultivar Samples: A cannabinoid profile for each new cultivar (strain) of medicinal marijuana must be assessed to establish a baseline for future quality check analyses. A total of 7.5 g (5 individual samples of 1.5 g) are required for a cultivar analysis.

11.4 Regulatory Compliance (RC) Samples: Once a cannabinoid profile of a particular cultivar has been established, the product must be re-checked intermittently to ensure that the plant material is free of pesticides, heavy metals, and mycotoxins

and that the cannabinoid profile has not deviated significantly from the original cultivar sample. A total of 7 g is required for an RC analysis.

11.5 The criteria for unacceptable specimens are an insufficient amount of sample submitted, tampered or damaged sample container. A description of reasons for each rejected sample should be recorded on the sample submittal form.

11.6 Sample Storage and Preservation – ECLS/CT/MML will login marijuana samples immediately upon receipt. All samples are then stored in the designated safes at ambient room temperature.

11.6.1 Medicinal marijuana samples are stored at ambient room temperature in 4 safes. The 4 safes are in room L425.

11.7 Once the samples have been analyzed at the ECLS/CT/MML and results are released, the samples are transferred to a different safe for a long-term storage. These samples are controlled dangerous substances and as such, their possession must be traceable from the time the samples are collected until they are destroyed.

11.8 All QC, calibration and reference testing materials must be stored in a freezer at -20˚C.

12. Quality Control

12.1 The ECLS Chemical Terrorism/Medicinal Marijuana laboratory follows the quality control program required by ECLS. The requirements for this program consist of participation in the validation study to demonstrate the laboratory's capability of generating acceptable accuracy and precision. Quality control samples are analyzed with each analytical run for accuracy assessment and to confirm that measurements were performed in a control mode of operation. Quality control samples are analyzed after the calibration standards have been run and after the unknowns have been run. Up to 20 unknown samples may be included in a run. For a run of more than 10 unknowns, include the CCV and CB after every ten samples. The HCV and LCV are run at the beginning and end of every run. The LCV and HCV are from a secondary source to further ensure proper results are being collected. The laboratory maintains records to document the quality of the data that is generated by using ongoing data quality checks that are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

12.2 The analyst participates in the initial validation study to demonstrate the capability to generate acceptable accuracy and precision for this method.

12.3 Before processing any samples, a calibration blank is analyzed to demonstrate that interferences from the analytical system and glassware are under control.

12.4 The laboratory demonstrates for each run that the operation of the LC-UVDAD system is in control through the analysis of performance check samples.

12.5 The QC results are compared with the established QC acceptance criteria. If the concentrations fall outside the designated range (70-130%) for the LCV/HCV and (85-130%) for the CCV, the laboratory performance for this analysis is judged to be out of control and the problem is identified and corrected. There is also a low QC standard CRL which is run before samples. It is the same concentration as the second lowest standard at 0.5 ppm and has an acceptance range of (50-150%). See **Appendixes C and D.**

12.6 The MS and MSD samples must have recoveries of the spiking analytes between 70 and 130% of the theoretical value (the percentages of the analytes detected in the unspiked sample + the percentage of analyte added). See **Appendix E.**

12.7 The laboratory maintains performance records to document the quality of data that is generated.

12.8 For the results of an inter-laboratory testing study, see **Appendix F**.

13. Calibration and Standardization

13.1 Preparation of Calibration Standards

13.1.1 **Preparation of Level 7 (50 ppm/100 ppm) Intermediate Calibration Working Standard:** Add 200 µL of THCA and 100 µL of each of the remaining standards, **10.12.1.1 – 10.12.1.6**. This creates a 700 µL mixture. To this, add 600 µL of methanol and 700 µL of OP water. Vortex for 60 seconds. This produces 2 mL of the 50 ppm (100 ppm THCA) Level 7 Standard.

Note: *The concentration of each standard solution refers to the concentration of all standards except THCA with the understanding that the concentration of THCA will be equal to twice times that concentration.*

13.1.2 The Preparation of Levels 1 – 6 can be found in the **Preparation of Cannabinoids Working Calibration Standards Table** below. Each Calibration Standard is prepared by combining the appropriate amount of standard solution and solvent in a 2-mL brown vial and vortexing to mix.

Preparation of Cannabinoids Working Calibration Standards Table					
Level #	Desired Concentration ppm	Volume of 50 ppm Intermediate	Volume of Level 4 Working Calibration Standard at 5 ppm	Amount of 65:35 (Methanol: OP Water)	Final Volume
6	20 ppm	400 µL		600 µL	1000 µL
5	10 ppm	200 µL		800 µL	1000 µL
4	5 ppm	200 µL		1800 µL	2000 µL
3	1 ppm		200 µL	800 µL	1000 µL
2	0.50 ppm		100 µL	900 µL	1000 µL
1	0.25 ppm		50 µL	950 µL	1000 µL

13.2 Preparation of Primary Source QC Solutions

13.2.1 Preparation of CCV – Two CCVs are prepared identically to calibration standard level 5

13.2.2 Preparation of CRL – One CRL is prepared identically to calibration standard level 2

13.3 Preparation of 50 ppm Intermediate Quality Control Standards – Each of the 8 cannabinoids are purchased as their own solution from a separate vendor as that which was used for the calibration standards. To create the 50 ppm intermediate solution, 200 µL of THCA and 100 µL of every other cannabinoid (**10.12.3.1 – 10.12.3.8**) is added to an autosampler vial. This produces a 900 µL mixture. To this, add 400 µL of methanol and 700 µL of OP water. Vortex for 60 seconds. This produces 2 mL of the 50 ppm (100 ppm THCA) Intermediate QC Standard.

13.4 Preparation of 30 ppm HCV and 5 ppm LCV – The HCV is prepared by adding 480 µL of 65:35 (Methanol: OP water) to 720 µL of the 50 ppm Intermediate QC Standard. The LCV is prepared by adding 900 µL of 65:35 (Methanol: OP water) to 100 µL of the 50 ppm Intermediate QC Standard.

13.5 Internal standard calibration procedure is used in this method.

13.6 Preparation of Ibuprofen Internal Standard

13.6.1 Preparation of 10,000 ppm Ibuprofen Internal Standard (IS) – Weigh out 100 mg of Ibuprofen and dissolve in 10 mL of 65:35 (Methanol: OP water). Vortex for 1 minute.

13.6.2 Preparation of 200 ppm Ibuprofen Internal Spiking Standard – Add 9.8 mL of 65:35 (Methanol: OP water) to 200 µL of the 10,000 ppm IS. Vortex for 1 minute.

13.7 **Preparation of Spiking Solution:** Combine 200 µL of 50 mg/mL Δ^9-THC solution (**Section 10.12.4**) with 800 µL of ethyl alcohol in a 2-mL amber glass vial. Vortex to mix. Label the vial and store at -20.0 ^{0}C.

13.8 The slope, intercept and R^2- value for a 7-point calibration curve is generated using a linear regression. These parameters are determined by linear least squares fit using the "ChemStation" software.

13.9 A working calibration curve is generated each day samples are analyzed. If the R^2 – value (coefficient of determination) for the calibration curve is less than 0.995, a new calibration curve is prepared.

13.10 Linearity of the standard curve should extend over the entire range**.**

13.11 The calibration curve is forced through the y-intercept (0,0).

13.12 A CB is prepared and analyzed in each analytical run. It is used to verify that the reagents and materials used in the method are free from contamination and that the instrument is free from interferences.

14. Analytical Procedure

Note: *Any incident which results in the loss of medicinal marijuana plant material during any parts of the below procedure must be brought to the attention of the Program Manager and documented in the Medical Marijuana Sample Prep Logbook (CHEM-16) or black lab notebook "Sample Inventory".*

14.1 Grinding of Medicinal Marijuana Plant Material Using the Retsch Mixer Mill MM400

14.1.1 Label a clean, amber jar with the sample ID# of the plant material that is about to be ground. Weigh the empty amber jar and cap and record its weight in the Medical Marijuana Sample Prep Logbook (CHEM-16).

14.1.2 Label 2 clean, 50-mL centrifuge tube with the sample ID # of the plant material that is about to be ground. Label the cap of the tube with a sharpie with the samples ID #.

Note: The analyst may weigh the centrifuge tubes and cap and record its weight in the Medical Marijuana Sample Prep Logbook (CHEM-16) or the black lab notebook "Sample Analysis".

14.1.3 Transfer four – 10 mm stainless-steel balls into each of the pre-weighed 50-mL centrifuge tubes.

Note: The analyst may weigh the centrifuge tubes, cap, and 4- 10 mm stainless steel balls and record its weight in the Medical Marijuana Sample Prep Logbook (CHEM-16) or the black lab notebook "Sample Analysis".

14.1.4 Remove the sample from the container provided by the ATC and weigh the marijuana plant material in a large, tared polystyrene weighing dish. Record the weight in the Medical Marijuana Sample Prep Logbook (CHEM-16).

14.1.5 Divide the plant material and distribute to the 2 prelabeled 50-mL centrifuge tubes which contains the 10 mm stainless-steel balls for grinding.

Note: *The analyst may choose to weigh the centrifuge tubes, cap, 4-10 mm stainless steel balls, and plant material and record its weight in the Medical Marijuana Sample Prep Logbook (CHEM-16) or the black lab notebook "Sample Analysis".*

14.1.6 Loosely cap the centrifuge tubes. Place the centrifuge tube into a -70˚C freezer for at least 30 minutes.

14.1.7 Remove the centrifuge tubes from the -70˚C freezer and remove the cap for 5 seconds. Recap the centrifuge tubes and wipe off any excess ice formed on the outside of the tube with a paper towel.

14.1.8 Place the centrifuge tubes in the arms of the Falcon tube adapters on the MM400 and hand tighten the arms.

Note: *The left and right arm of the Falcon Tube Adapter for the MM400 must be balanced, 2 samples need to be mixed at the same time on opposing arms.*

14.1.8.1 To loosen the arm of the adapter: lift the stainless-steel nobs on the top of the adapter and turn the stainless-steel nob so the grooves are not locked into place. Turn the black knob on the side of the adapter to loosen.

14.1.8.2 To tighten the arms of the adapter: lift the stainless-steel nobs on the top of the adapter and turn the stainless-steel nob so the grooves are locked into place. Turn the black knob on the side of the adapter to tighten.

14.1.9 Set the MM400 to a frequency of 25 Hz and the timer to 5 minutes.

14.1.10 Press the start button to begin the grinding of the plant material at 25 Hz for 5 minutes.

14.1.11 Remove the tube from the arm of the adapter (refer to **Section 14.1.8.1** to loosen the arm of the adapter).

14.1.12 Uncap the centrifuge tube and inspect the plant material. Scrap the sides of the centrifuge tube to ensure all plant material is adequately ground.

14.1.12.1 If the plant material is not adequately ground enough, recap the centrifuge tube and grind again (refer to **Sections 14.1.6 – 14.1.8**).

14.1.12.1.1 Set the MM400 for a frequency of 20-25 Hz for 5-10 minutes **(depending on the ground material).** Press the start button to begin the grinding of the tube at 20-25 Hz for 5-10 minutes.

14.1.12.1.2 Remove the tube from the arm of the adapter and uncap the tube. Inspect the ground plant material.

14.1.13 If plant material is finely ground, scrap the cap and the sides of the centrifuge tube with a spatula to loosen the plant material. Remove the stainless-steel balls with tweezers. If any excess plant material is stuck to the balls, scrap each ball with a spatula over a weighing boat.

14.1.14 Combine the plant material from both centrifuge tubes, caps, and stainless-steel balls in the previously-weighed amber jar **(Section 14.1.1)**.

14.1.15 Weigh the ground sample, amber jar, and cap together and record the weight in the Medical Marijuana Sample Prep Logbook (CHEM-16).

14.1.16 Place the labeled, closed amber jar in a safe and secure the safe.

14.1.17 Cleaning the Retsch Mixer Mill 400 Accessories

14.1.17.1 Rinse the stainless-steel balls with warm sink water.

14.1.17.2 Clean the balls with a soap solution.

14.1.17.3 Rinse the balls with sink water, DI water, OP water, isopropyl alcohol and methanol.

14.1.17.4 Dry the stainless-steel balls at room temperature for a few hours before reuse.

14.2 Grinding of Medicinal Marijuana Plant Material using Robot Coupe Blixer 2

Note: *The Robot Coupe Blixer 2 is a secondary sample preparation device which can be used in the event that the Mixer Mill MM400 is unavailable.*

Note*: Plant material should always be ground in a fume hood. The analyst must wear a lab coat, safety goggles, gloves, and a surgical mask to minimize bodily contact with the finely-ground plant material*.

14.2.1 Remove the sample from the container provided by the ATC and weigh the marijuana plant material in a large, tared polystyrene weighing dish. Record the weight in the Medical Marijuana Sample Prep Logbook (CHEM-16). Transfer the plant material to a clean, dry, stainless steel Robot Coupe Bowl.

14.2.2 Label a clean, amber jar with the sample ID # of the plant material that is about to be ground. Weigh the jar and record its weight in the Medical Marijuana Sample Prep Logbook (CHEM-16).

14.2.3 Prepare a block of dry ice from liquid carbon dioxide using a ThermoSafe Dry Ice Machine and a tank with a siphon tube.

Note*: Formation of a block of dry ice takes approximately 1 minute. If the block has not formed after 2 minutes, the CO_2 tank needs to be changed. Changing the tank should follow safety procedures outlined by the laboratory safety officer*.

14.2.4 Break the dry ice block. Weigh small pieces of dry ice roughly 2-3 times the weight of the marijuana sample and add them to the Robot Coupe bowl.

14.2.5 Place the lid on the bowl and tighten it. Cover the opening at the top of the lid with paraffin film to prevent moisture from entering into the bowl and to reduce the physical loss of the plant material during grinding.

14.2.6 Grind the plant material in the "pulse" mode of the Robot Coupe for 30 seconds, then wait for the blades to stop spinning and the dust to settle in the bowl. Remove the lid and scrape the ground plant material off the lid and sides into the bottom of the bowl.

14.2.7 Replace and tighten the lid. Repeat step **14.2.6**. Carefully transfer the ground plant material in the previously-weighed amber jar (**Section 14.2.2**).

14.2.8 Leave the amber jar uncovered in a separate fume hood for 30 min for any moisture or remaining dry ice to evaporate. After 30 min, put the lid on the jar. Weigh the ground sample and jar together and record the weight in the Medical Marijuana Sample Prep Logbook (CHEM-16).

14.2.9 Place the labeled, closed jar in a safe and secure the safe.

14.2.10 Cleaning the Robot Coupe Blixer 2 Single Speed Blender/Mixer

14.2.10.1 Rinse the bowl, blades, and lid with warm sink water to remove any remaining ground plant material.

14.2.10.2 Clean all parts with a soap solution using a soft brush.

14.2.10.3 Rinse all parts first with laboratory deionized water, then with isopropyl alcohol, and finally with OP water.

14.2.10.4 Dry all parts at room temperature overnight or for at least a few hours before reuse.

14.3 Sample Preparation

14.3.1 Extraction

14.3.1.1 Tare a pre-labeled 50-mL propylene centrifuge tube without a cap using a 100-mL beaker.

14.3.1.2 Weigh 200 mg of ground medicinal marijuana plant material into the pre-labeled tube and record in the black lab notebook “Sample Analysis”.

14.3.1.3 Pipet 100 µL of the Δ^9-THC spiking solution (**Section 13.7**) into the ground plant material of the MS and MSD samples. Allow the ethanol from the solution to evaporate before proceeding to the next extraction step.

14.3.1.4 Pipet 20.0 mL of Methanol: Chloroform (9:1) into the centrifuge tube and cap.

14.3.1.5 Hand shake for 30 seconds and vortex for 30 seconds.

14.3.1.6 Shake on a shaker for 30 minutes.

14.3.1.7 Centrifuge the tube for 10 minutes.

14.3.1.8 Decant the liquid extract into a clean, labeled second 50-mL centrifuge tube and cap.

14.3.1.9 Pipet another 20.0 mL of Methanol: Chloroform (9:1) aliquot into the first centrifuge tube containing the sample and cap.

14.3.1.10 Repeat steps **14.4.1.5 – 14.4.1.7.**

14.3.1.11 Decant and combine this second liquid extract into the second centrifuge tube with the first extract. Cap and mix by inverting a few times.

14.3.1.12 Filter about 2 mL of extract through a 0.2 µm Nylon membrane filter into clean, labeled amber glass 2-mL vial. Cap it and store at -20 ℃ along with unfiltered extract.

14.3.2 Sample Dilutions and Reconstitution

14.3.2.1 Samples are prepared with a 1 to 20 dilution.

14.3.2.1.1 1 to 20 Dilution: Pipet 50 µL of extract into a culture tube and then dilute with 950 µL of methanol. Vortex to mix.

14.3.2.2 Transfer 200 µL of the diluted extract into a 12 x 75 mm culture tube.

14.3.2.3 Evaporate off methanol to dryness using nitrogen at room temperature using TurboVap.

14.3.2.3.1 Turn TurboVap evaporator unit on, open nitrogen valve, and place the correctly-sized test tube rack in the water bath. Set the temperature to 25 ^{0}C and the timer to 10 minutes.

14.3.2.3.2 Place tubes into TurboVap and ensure that no caps remain on the nitrogen spouts above the tubes. Close the lid and select the rows containing tubes by pressing the corresponding buttons to the left of

the window. Press "Start" and quickly set nitrogen flow to 10 psi.

14.3.2.3.3 Once the 10 minutes are up, check each tube individually to ensure the solvent has evaporated by lifting the tube, wiping off the excess water with a paper towel, and visually assessing the tube's contents.

14.3.2.3.4 If moisture remains inside the tube, the tube can be dried down again in 2-minute intervals until the solvent has completely evaporated. Dry residue can be reconstituted following the steps outlined below.

14.3.2.4 **Internal Standard Method** Add 200 µL of Methanol: OP water (65:35) to the dry residue, and vortex for 30 seconds to dissolve completely. Then pipette 50 µL of 200 ppm Internal Standard (**Section 13.6.2**) into the culture tube with the sample and vortex for another 30 seconds. Transfer this solution into an appropriately labeled micro auto-sampler vial using 200 µL pipette. Tap the vial gently to release the trapped bubbles.

14.4 HPLC Instrumentation Analysis

14.4.1 System Preparation

14.4.1.1 The valve should be opened, and the conditions monitored on the "ChemStation" software to ensure that the pressure drops to atmospheric conditions.

14.4.1.2 Connect the mobile phases by switching the caps on the glass media bottles with the caps containing the appropriate leads attached to the instrument.

14.4.1.3 The column should first be flushed with 100% Mobile Phase B (**Section 10.10.2**) at a high flow rate (approximately 5 mL/min) for 5 minutes. The leads should be inspected visually in this time to ensure that any air bubbles pass through the system.

14.4.1.4 The column should be flushed secondly with 100% Mobile Phase A (**Section 10.10.1)** at a high flow rate (approximately 5 mL/min) for 5 minutes. The leads should be inspected

visually in this time to ensure that any air bubbles pass through the system.

14.4.1.5 The flow rate should be decreased to 0.7 mL/min and changed to 32% Mobile Phase A (**Section 10.10.1**) and 68% Mobile Phase B (**Section 10.10.2**). The valve should be closed, and the conditions monitored on the "ChemStation" software to ensure that the pressure begins to rise again.

14.4.1.6 Leave the system under these conditions for at least one hour prior to analysis to equilibrate the system and establish a stable baseline.

14.4.2 Agilent HPLC System

14.4.2.1 1100 Degasser
14.4.2.2 1100 Binary Pump
14.4.2.3 1100 Auto-sampler
14.4.2.4 1100 Thermo column compartment
14.4.2.5 1100 Ultra Violet Diode Array Detector
14.4.2.6 Agilent MSD Trap(SL) Detector

14.4.3 HPLC Conditions

14.4.3.1 Column: LC Column – Agilent Poroshell 120 SB-C18, 3.0 x 75mm 2.7-micron, Agilent Cat PN # 687975-302

14.4.3.2 Column temperature: 30˚C

14.4.3.3 Mobile phases: Methanol: 25 mM Ammonium Acetate in organic pure water, **Gradient Program**

14.4.3.4 Pump Flow Parameters:

14.4.3.4.1 Flow Rate: 0.7 mL/min
14.4.3.4.2 Stop Time: 10.0 min.
14.4.3.4.3 Post Time: 4.0 min

Time	% B (Methanol)	% A (25 mM Ammonium Acetate)	Flow (mL/min)
0.00	68.0	32.0	0.7
8.25	85.0	15.0	0.7
9.0	95.0	5.0	0.7
10.0	68.0	32.0	0.7

14.4.3.5 Detection: UVDADMSD (SL Trap)

14.4.3.6 Lamp: UV

14.4.3.7 Spectrum Scan:

14.4.3.7.1 Store: All in peak
14.4.3.7.2 Range: 200 to 400
14.4.3.7.3 Step: 2.0 nm
14.4.3.7.4 Threshold: 1.0 mAU
14.4.3.7.5 Autobalance: Prerun
14.4.3.7.6 Slit: 4 nm
14.4.3.7.7 Peak width (Response time): >0.1 min (2s)

14.4.3.8 Quantitation:

14.4.3.8.1 UV-DAD signals data at 230, 235, 240, 260 and 290 nm are collected with bandwidth = 4 using at 360 nm as a reference with a bandwidth = 10 nm.

14.4.3.8.2 UV signal at 235 nm only is used for quantitation.

14.4.3.9 Autosampler:

14.4.3.9.1 Injection Volume: 5 µL
14.4.3.9.2 Injection with a Needle Wash: Vial # 91
14.4.3.9.3 Optimization: None
14.4.3.9.4 Draw speed: 100 µL/min
14.4.3.9.5 Eject Speed: 200 µL/min
14.4.3.9.6 Draw Position: 0.0

14.4.3.10 Run time: 10 minutes

14.4.3.11 Post run time: 4 minutes

14.4.3.12 Analyte and IS Retention Times listed below are approximate and for information only.

Analyte	Run Time (min)
Ibuprofen	1.990
CBDA	3.448
CBGA	4.150
CBD	5.591
CBG	5.888
THCA	6.453
CBN	7.584
Δ^9-THC	8.381
Δ^8-THC	8.670

14.4.3.13 Mass Spectrometer Detector (MSD Trap SL) –Used for Qualitative confirmation of analytes when the identification of the analyte is in question.

15. Data Analysis and Reduction

15.1 Raw data files are quantitated using the Data Analysis Menu of the "ChemStation" software. The peaks are automatically integrated using the "ChemStation" integrator, and the integration of each peak is reviewed and manually corrected as appropriate in the data analysis mode.

15.2 A linear calibration curve is generated, and the QCs are quantitated and evaluated against established acceptance criteria. If acceptable, each sample is then quantitated against the calibration curve.

15.3 The results from reviewed data files are electronically uploaded into the previously created batch in the Element database.

15.4 After uploaded, the supervisor must review the data and sign off before it can be reported.

16. Method Performance

16.1 To establish the ability to generate acceptable accuracy and precision, the analyst must participate in the initial validation study as required by ECLS.

16.2 For each analytical run after the initial validation, the analyst must demonstrate method validity by successfully perform the following:

16.2.1 Prepare a quantitation curve utilizing external standard spiked with calibrating standards.

16.2.2 The calibration curve must be linear with an R^2 value > 0.995.

16.3 A CB is run with every set and should not show any interference at the peaks of interest.

16.4 The CCV sample is analyzed right after calibration with a batch of samples and then after every ten samples. The QC results must meet the established acceptance criteria. See **Section 12.5** and **Appendix C** for acceptance limits.

16.5 The HCV and LCV samples are analyzed before and after all samples are run. See **Section 12.5** and **Appendix D** for acceptance limits.

16.6 The CRL sample is run after the initial HCV and LCV samples but before the actual samples to be analyzed. See **Section 12.5** for acceptance limits.

16.7 Analyze one matrix spiked sample and one matrix spiked duplicate per analysis. See **Section 12.6** and **Appendix E** for acceptance limits.

17. Pollution Prevention

17.1 This method utilizes an extraction procedure that requires the use of methanol and chloroform. All extracts are discarded into the extraction laboratory's organic waste beakers or containers containing concentrated nitric acid after the results of the analyses are given to data management and the supervisor has reviewed and approved the results of the analyses.

17.2 The range and quantity of hazardous substances used in this laboratory require preplanning to respond safely to chemical spills. For this laboratory, spill kits with instructions, absorbents, reactants, and protective equipment are available to clean up minor spills. A minor chemical spill is one that the laboratory staff is capable of handling safely without the assistance of safety and emergency personnel. All other chemical spills are considered major and should only be done by knowledgeable and experienced personnel.

17.3 The ECLS Chemical Terrorism/Medicinal Marijuana laboratory is equipped with a spill-kit (**SpillSolv**) and all accidental spills are cleaned up using these spill kits.

17.4 Each spill kit contains an absorbent that can be used to clean up acids, bases, or solvents depending on the type of spill.

17.5 If a minor chemical spill occurs in the laboratory, use the following procedure:

17.5.1 Alert the lab personnel in the immediate area of spill, including the supervisor.

17.5.2 Wear protective equipment, including safety goggles, gloves, and a long – sleeved laboratory coat.

17.5.3 Avoid breathing vapors from the spill.

17.5.4 Use appropriate spill kit to absorb spill.

17.5.5 Collect residue; place in container and dispose as chemical waste.

17.5.6 Clean spill area with water.

17.5.7 After the instrument has finished the analytical run, and the performance of the instrument is judged acceptable, the analytical vials located on the autosampler tray are uncapped and any remaining content is discarded into the waste containers containing nitric acid.

17.5.8 If a spill occurs with the medicinal marijuana samples, the laboratory supervisor is made aware.

18. Data Assessment and Acceptance Criteria for Quality Control Measurements

18.1 Acceptance limits are adopted from the initial demonstration of the laboratory capability as referenced in Method Performance (**Section 16**).

18.2 The calibration curve must be linear, with an R^2 value of at least 0.995.

18.3 The linearity of the standard curve should extend over the entire range. The y intercept is fixed at (0.0).

18.4 The calibration blank must show that there is no interference at the masses of interest.

18.5 The results for QC-control must meet validation and QC acceptance criteria found in **Appendixes C and D**.

19. Contingencies for Unacceptable QC or Calibration Data

19.1 Process for determining the acceptability of the entire analytical run: All quality controls must meet the method requirements as referenced in **Section 16** of this SOP.

19.2 Process for determining the acceptability of data for part of an analytical run.

19.2.1 If an error or problem with the analytical run is determined, the problem is corrected and the entire analytical run is repeated.

19.2.2 If the analytical problem persists, assistance is requested to help trouble shoot the method to resolve any out of control QC.

19.2.3 After the analytical problem has been corrected, a set of QC samples are run. If the QC results are satisfactory, analysis of the samples is continued.

19.3 Notification to QAO of any persistent instrument failure must be submitted in writing with two days

19.4 If the R^2 value for the calibration curve is < 0.995, run the standards again. If the curve fails again, prepare new standards and re-run another calibration curve.

20. Waste Management

20.1 Each laboratory is responsible for safely disposing materials and for maintaining awareness of OSHA regulations regarding safe handling of the chemicals used in this method. Periodically extraction solvents are transported from the extraction laboratory to the disposal area located at the warehouse dock in the presence of the safety officer who is responsible for disposing of all solvents produced by the laboratory.

20.2 Marijuana Waste Management: Disposal of Medicinal Marijuana Plant Material and Analysis Extracts.

20.2.1 Extracted Plant Material

Note: *The digestion procedure should take place in the fume hood to minimize contact with corrosive chemicals.*

Note: *This method can be modified for the destruction of medicinal marijuana samples that have been analyzed and the results have been reported. The modification of the procedure only applies to the amount of plant material to be destroyed, the amount of nitric acid added to the sample, and the amount of hydrogen peroxide added to the sample.*

20.2.1.1 Turn on DigiBLOC 3000 digestion system and allow to warm.

20.2.1.2 Uncap the 50-mL plastic centrifuge tubes containing the 200 mg of plant material and place the tubes in the DigiBLOC.

If free of plant material, the caps may be thrown in the garbage.

20.2.1.3 Once the DigiBLOC and centrifuge tubes have heated to approximately 90 °C, pipet 5 mL of concentrated nitric acid into each tube.

***Note**: Nitric acid is highly corrosive in both its liquid and vapor states. Exercise caution and wear the appropriate PPE.*

20.2.1.4 Leave the tubes to digest until the liquid inside is pale yellow and no bubbles or orange-brown fumes are being produced.

20.2.1.5 Place the tubes in a rack and turn the DigiBLOC off.

20.2.1.6 Pipet 2.5 mL of 30% hydrogen peroxide into each tube.

***Note**: Hydrogen peroxide can cause severe skin and eye damage. Exercise caution and wear the appropriate PPE.*

20.2.1.7 Once cool and transparent, dump the liquid contents of each tube down the drain with running water. The empty tubes may be thrown in the garbage.

20.2.1.8 For previously analyzed samples, the date, time and material disposed of will be kept in a black laboratory notebook marked "Medical Marijuana Sample Disposal." The destruction of the extracted plant material is not required to be recorded in the "Medical Marijuana Sample Disposal" black laboratory notebook.

20.2.1 **Methanol: Chloroform (9:1) Extracts**: After the results of the analyses have been given to data management and the supervisor has reviewed and approved the results of the analyses, all methanol and chloroform extracts are to be discarded into an organic waste bottle containing concentrated nitric acid.

20.2.2 **Diluted Extracts from Autosampler Vials**: Use a vacuum to remove extract from an autosampler vial. Combine all the extracts into a beaker. Let solvents evaporate off. To the dry residue add concentrated nitric acid and heat using a hot plate or DIGI block to digest. **The plant material oxidizes readily as indicated by the color change from green to a pale yellow.** Then the residuals can be discarded as

general waste.

20.3 Reagent Disposal

20.3.1 Never pour corrosive materials or flammable liquid compounds that give off toxic vapors down the drain.

20.3.2 Segregate chlorinated and non-chlorinated wastes into glass containers.

20.3.3 Label each container with type of waste, initial and final date of collection.

20.3.4 When bottles are full, contact the Laboratory Safety Officer to arrange for their disposal.

20.3.5 Transfer the bottles to the PHEAL loading dock and discard the waste into the appropriate 55 Gal waste drum for later pickup by the contracted waste disposal agency. The amount of disposed waste and the date/time of the discard into the waste drum are documented by the Laboratory Safety Officer.

20.4 Spill Cleanup

20.4.1 Use appropriate kit to neutralize and absorb inorganic acids and bases. Collect residue, place in container and dispose as chemical waste.

20.4.2 For other chemicals, use appropriate kit or absorb spill with vermiculite, dry sand or diatomaceous earth. Collect residue, place in container and dispose as chemical waste.

20.4.3 Clean spill area with water.

21. References

De Backer, B., et.al. Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. 2009. Journal of Chromatography B, 877,4115-4124.

22. Appendix A: Initial Demonstration of Capability (DOC)

23. Appendix B: Sequence Template

24. Appendix C: Continuous Calibration Verification Data and Acceptance Criteria