

	<p>HEALTH & ENVIRONMENTAL TESTING LABORATORY</p> <p>Forensic Toxicology</p> <p>221 State Street</p> <p>Augusta, ME 04333</p>
---	--

**DETERMINATION OF CANNABINOIDS IN BLOOD
BY LIQUID-LIQUID EXTRACTION AND LC-MS/MS ANALYSIS**

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Contents

Principle and Scope:	3
Equipment and Supplies:	3
Specimen Requirements:	4
Reagents and Reference Materials:	4
Safety Precautions:.....	1
Reagent Preparation:.....	1
Calibration and Quality Control Stock Preparation:	1
Quality Control Stock Preparation:.....	2
Extraction Procedure:.....	3
Instrument Maintenance Procedure:	4
Instrumentation and Data Acquisition Parameters:.....	4
Identification & Quantitation	5
Quality Control Requirements:	5
Uncertainty of Measurement:.....	5
Documentation:.....	6
Waste Management:.....	6
References:.....	6
Revision:	7

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Maine HETL- Forensic Toxicology**DETERMINATION OF CANNABINOIDS IN BLOOD
BY LIQUID-LIQUID EXTRACTION AND LC-MS/MS ANALYSIS****Principle and Scope:**

Δ^9 THC is the primary psychoactive cannabinoid found in cannabis. This compound has been documented to produce a sense of euphoria, relaxation, and various sensory alterations. Δ^9 THC is metabolized into the active metabolite OH-THC which is commonly recognized to indicate recent use. The inactive metabolite THC-COOH is commonly recognized to have no psychoactive effect but is an indicator of prior use.

This method describes the procedures for the quantitative determination of Δ^9 THC, OH-THC and THC-COOH in whole blood. Deuterated internal standards for the compounds of interest are added to the whole blood samples, the compounds of interest and corresponding deuterated internal standards are then efficiently partitioned from the blood sample via a liquid/liquid extraction with organic solvent and separated on an HPLC column. The samples are then analyzed using a tandem mass spectrometer utilizing positive-ion electrospray.

Equipment and Supplies:

Volumetric Flasks various sizes

Volumetric cylinders various sizes

Disposable Glass vials 5-15 mL

Teflon lined caps

Autosampler vials with silianized inserts

Autosampler caps

Vortex mixer

Disposable transfer pipettes

Pipettes- various

Evaporator

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Specimen Requirements:

- Only whole blood samples shall be analyzed using this method.
- Whole blood samples are collected in tubes provided by HETL or by a qualified medical professional and upon receipt, stored under refrigeration at HETL (<10°C).
- No dilutions shall be performed for cannabinoids testing. If a case sample is found to be above the upper limit of quantitation, then the case sample shall be result out as “Compound detected: (Compound) > (Upper Limit of Quantitation) ng/mL”. If a case sample is found to have insufficient quantity to extract and analyze as a neat sample for cannabinoids testing shall not be performed.
- If a blood sample is received by the laboratory with volume not sufficient to perform standard OUI blood drug panel and only a portion of panel can be screened and confirmed for then the following comment shall be included in the COA: “Unable to perform standard OUI blood drug panel due to low sample volume.” In addition, prior to starting testing the analyst shall reach out to the investigating officer notifying them that the sample is QNS to perform the standard OUI blood drug panel and discuss course of testing.
- Blood samples that were collected using non-DHHS certified blood collection tubes shall have the following comment included in the COA: “(Color) topped tube used for analysis.”

Reagents and Reference Materials:

- Hexane
- Ethyl acetate
- Methanol - High purity LC-MS quality
- Water - High purity LC-MS quality
- Formic Acid
- Water with 0.1% Formic Acid- High purity LC-MS quality, Fisher brand or equivalent
- 1N HCl
- **Stock Standard Solutions** – Individual solutions of target compounds and internal standards are purchased from traceable, approved vendors such as Cerilliant or Lipomed. All standards shall be stored at conditions recommended by the manufacturer.
 - **Δ 9 THC 1mg/mL**
 - **OH-THC 100 µg/mL**
 - **THC-COOH 100 µg/mL**
 - **Δ 9 THC-d3 100 µg/mL**
 - **OH-THC-d3 100 µg/mL**
 - **THC-COOH-d3 100 µg/mL**

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

- **Blank Blood matrix**- approved vendor supplied and checked for quality control prior to purchase. Store at <10°C.

Safety Precautions:

The solvents used are considered toxic. Repeated or prolonged exposure can produce targeted organ damage. Proper PPE shall be used when handling solvents.

Blood from unknown case samples shall be handled following Universal Precaution guidelines. Face shield in addition to PPE shall be utilized while pipetting blood.

Reagent Preparation:

All reagent preparations are to be recorded in the LCMS Reagent Preparation Log.

- **Mobile Phase A:** Water with 0.1% Formic Acid. This reagent is good for 12 months from the date of opening. This can be purchased as a prepared reagent or prepared in the lab (1 mL formic acid QS to 1 L with LC-MS grade water, good for 1 year from prep date or until the earliest expiration date of any components used in the making.)
- **Mobile Phase B:** Methanol. This reagent is good for 12 months from the date of opening or until the earliest expiration date of any components used in the making.
- **80:20 Hexane: Ethyl Acetate-** To a graduated cylinder or 100 mL volumetric flask, add 80 mL Hexane and QS to 100 mL with ethyl acetate. Different volumes may be prepared as long as proportions are kept the same. This reagent is good for one month or until the earliest expiration date of any components used in the making.
- **50:50 LCMS grade Methanol: LCMS grade Water with 0.1% Formic Acid-**To a graduated cylinder or a 100mL volumetric flask add 50mL of LCMS grade Methanol and QS to 100mL with LCMS grade Water with 0.1% Formic Acid. Different volumes may be prepared as long as the proportions are kept the same. This reagent is good for one month or until the earliest expiration date of any components used in the making.

Calibration and Quality Control Stock Preparation:

All Cannabinoids working stock preparations shall be recorded in the LCMS Reagent Preparation Log.

- **Cannabinoids Stock A 1000/5000 ng/mL:** Add a portion of methanol to a 10 mL volumetric flask. Using only Cerilliant stock or equivalent add 10µL of Δ 9 THC 1mg/mL Stock, 100µL of OH-THC 100 µg/mL Stock, and 500µL of THC-COOH 100 µg/mL Stock or equivalents to the volumetric flask to obtain a final

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

concentration of 1000/5000ng/mL. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.

- **Cannabinoids Stock B 100/500 ng/mL:** Add a portion of methanol to a 10 mL volumetric flask then add 1mL of Cannabinoids Working Stock 1000/5000 ng/mL to the volumetric flask. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.
- **Cannabinoids Stock C 10/50 ng/mL:** Add a portion of methanol to a 10 mL volumetric flask then add 1mL of Cannabinoids Working Stock 100/500 ng/mL to the volumetric flask. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.
- **Cannabinoids Internal Standard 1000 ng/mL:** Add a portion of methanol to a 10 mL volumetric flask then add 100 uL of each Δ 9 THC-d3 (100 ug/mL), OH-THC-d3 (100 ug/mL), THC-COOH-d3 (100 ug/mL) or equivalents to the volumetric flask to obtain a final concentration of 1000ng/mL. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.

Quality Control Stock Preparation:

Blank matrix samples will be fortified respectfully at low, medium, and high levels using working stock created from stock standards from a separate vendor than the calibration source or a different lot number of the same vendor. A negative control will be created using an unfortified blank matrix. Internal standard shall be added and quality control samples will then be extracted and analyzed with each batch immediately following calibration. A full set of controls shall be run for each extraction batch of twenty samples. All quality control working stock preparations are recorded in the LCMS Reagent Preparation Log.

- **Cannabinoids QC Stock A 1000/3000 ng/mL:** Add a portion of methanol to a 10 mL volumetric flask. Using only Lipomed stock or equivalent add 10 μ L of Δ 9 THC 1mg/mL Stock, 100 μ L of OH-THC 100 μ g/mL Stock, and 300 μ L of THC-COOH 100 μ g/mL Stock or equivalents to the volumetric flask to obtain a final concentration of 1000/3000ng/mL. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.
- **Cannabinoids QC Stock B 100/300ng/mL:** Add a portion of methanol to a 10 mL volumetric flask then add 1mL of the Quality Control Working Stock 1000/3000 ng/mL to the volumetric flask. QS to 10mL with LCMS grade Methanol. This working stock is good for 12 months or until the earliest expiration date of any components used in the making.

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Table 1: Calibration Levels						
Calibration Level	Target Concentration THC, OH-THC	Target Concentration THC-COOH	Vol 1000/5000 ng/mL Stock	Vol 100/500 ng/mL Stock	Vol 10/50 ng/mL Stock	Blank Whole Blood
	ng/mL	ng/mL	(μ L)	(μ L)	(μ L)	(μ L)
1	0.5	2.5			25	500
2	1	5			50	500
3	5	25		25		500
4	20	100		100		500
5	50	250	25			500
6	80	400	40			500
7	100	500	50			500

Table 2: Quality Control Levels					
Quality Control Level	Target Concentration THC, OH-THC	Target Concentration THC-COOH	Vol 1000 ng/mL Stock	Vol 100 ng/mL Stock	Blank Whole Blood
	ng/mL	ng/mL	(μ L)	(μ L)	(μ L)
Low (QCL)	5	15		25	500
Medium (QCM)	20	60		100	500
High (QCH)	80	240	40		500
Negative (Neg)	--	--			500

Extraction Procedure:

1. Case samples shall be removed from refrigeration storage, allowed to warm to room temperature and placed on a rocker for a minimum of ten minutes.
2. Calibrators are prepared as per Table 1: Calibration Levels in glass tubes.
3. Quality Controls are prepared as per Table 2: Quality Control Levels in glass tubes.
4. Transfer 500 μ L of each case sample into a glass tube.
5. Pulse vortex
6. Add 25 μ L Internal Standard to each tube (Note: the same lot of internal standard shall be used for all samples in an analytical batch.)
7. Pulse vortex
8. Add 500 μ L HPLC Grade Water to each tube
9. Pulse vortex
10. Add 100 μ L 1N HCl to each tube
11. Pulse vortex
12. In a fume hood add 2.5 mL 80:20 Hexane: Ethyl acetate to each tube and cap.
13. Pulse Vortex 1 minute
14. Centrifuge at high speed for ten minutes
15. In a fume hood remove the supernatant top layer and transfer into new labeled glass tubes

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

16. Dry down at room temp w/ Nitrogen
17. Reconstitute in 100 µL 50:50 mobile phase Water w/0.1% Formic Acid: Methanol
18. Pulse vortex
19. Transfer to an autosampler vial with silanized insert for analysis

Instrument Maintenance Procedure:

Refer to the HETL LC-MS/MS Instrument Maintenance Procedure a copy of which is located in the immediate area of the instrument.

Instrumentation and Data Acquisition Parameters:

The instrument method parameters will be printed out and placed in a method binder close to the instrument.

- Shimadzu 8030 Tandem Mass Spectrometer and LC system, Agilent 6470A Tandem Mass Spectrometer and LC system (or equivalent)
- LC Guard Cartridge – Raptor Biphenyl 2.7µm 5x3.0mm
- LC Column- Raptor Biphenyl 2.7µm 100x2.1mm
- Flow rate 0.5 mL/Min
- Mobile phase A: Water with 0.1% Formic Acid
- Mobile Phase B: Methanol
- Injection volume 20 µL
- LC oven 40°C

Table 3: LC Pump Gradient

Time	% A: Water with 0.1% Formic Acid	% B: Methanol
0	50	50
0.5	50	50
1.0	To MS	To MS
8.5	0	100
8.9	0	100
9.0	50	50
10.0	To Waste	To Waste
10.0	Stop	Stop

Table 4: Instrument Parameters for Target Analytes

Target Analyte	Precursor Ion (m/z)	Product Ion	
Δ9-THC	315	Quant	193
		Qualifier	123
11-OH-THC	331	Quant	193
		Qualifier	313
THC-COOH	345	Quant	299
		Qualifier	193

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Table 5: Instrument Parameters for Internal Standards

Internal Standard	Precursor Ion (<i>m/z</i>)	Product Ion	
		Quant	196
Δ9-THC-d3	318	Qualifier	123
		Quant	316
11-OH-THC-d3	334	Qualifier	196
		Quant	302
THC-COOH-d3	348	Qualifier	330

Identification & Quantitation

- A calibration curve shall be run for each new sequence. An acceptable calibration curve shall consist of a minimum of 4 calibration points. For all other identification and quantitation criteria and guidelines please Refer to: LCMSMS Analysis, Acceptance, & Reporting Criteria Standard Operating Procedure.
- **Limit of Detection, Limit of Quantification & Reporting Levels:** the limits of detection and quantification have been determined as illustrated in Table 6: Limits of Detection and Quantification and are linear within the range indicated. If the lowest or highest calibrator has been dropped, then the quantification window shall be adjusted to reflect the change as needed.

Table 6: Reporting Limit/Lower Limits of Detection/Lower Limit of Quantitation		
Compound	RL/LLOD/LLOQ	Upper LOQ
THC	1ng/mL	100ng/mL
OH-THC	1ng/mL	100ng/mL
THC-COOH	5ng/mL	500ng/mL

Quality Control Requirements:

- For quality control requirements, acceptance criteria, and guidelines please Refer to: LCMSMS Analysis, Acceptance, & Reporting Criteria Standard Operating Procedure.

Uncertainty of Measurement:

- For estimation of uncertainty of measurement please Refer to: LCMSMS Analysis, Acceptance, & Reporting Criteria Standard Operating Procedure.

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Documentation:

Each batch (example: THC082819EAF) folder shall contain:

- Raw/Summary data from the instrument for all calibrators and quality controls
- Calibration Report from the instrument
- LC-MSMS Calibration & QC Review Form
- Internal Standard Area Report
- Qualifier Ion Ratio Report (when applicable)
- Instrument Sequence Table
- Cannabinoids in Blood Bench Sheet Form

Each Sample folder shall contain:

- Laboratory Blood Analysis Request Form
- Receipt/Contract for Examination Form/Chain of Custody
- Blood Kit Inventory Worksheet
- Blood Drug Screen Worksheet
- Raw/Summary data from the instrument including reinjections if applicable
- LC-MSMS Sample Review Form
- Blood Drug Results Worksheet
- Case Review Form

Waste Management:

Residual organic solvent, standards and instrument waste must be disposed in accordance with Federal and Maine law.

Any lab ware exposed to blood shall be disposed of in hazardous waste containers for proper disposal.

References:

Determination of Δ^9 -THC in Whole Blood using Gas Chromatography-Mass Spectrometry. Chu and Drummer. JAT 26 November/December 2002.

Development and validation of an automated liquid-liquid extraction GC/MS method for the determination of THC, 11-OH-THC, and free THC-carboxylic acid (THC-COOH) from blood serum. Purschke, Heini, Lerch, Erdmann, and Veit. Analytical and Bioanalytical Chemistry 408: 4379-4388 April 2016.

An Efficient, Robust Method for the Determination of Cannabinoids in Whole Blood by LC-MS-MS. Tiscione, Miller, Shan, Sprague, and Yeatman. JAT 40:639-648 July 2016.

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version

Revision:

REVISED BY	REV#	DATE	Revisions
LN	1	11/04/2019	All references to silanized tubes were changed to glass tubes. Table 6 LLOQ was increase from 0.5ng/mL to 1ng/mL , and 2.5ng/mL to 5ng/mL.
EAF	2	01/13/20	Removed references to c-18 HPLC column and replaced with raptor biphenyl. Removed dilution information and added that no dilutions shall be perform for cannabinoids testing. Updated instrument method per December 2019 addendum study. Added qualifier ion ratio report to batch documentation list.
EAF	3	10/09/2020	Annual Review: Table 1 calibration levels changed calibrator 4 from 10uL of 1000/5000ng/mL stock to 100uL of 100/500ng/mL stock and moved position of table within SOP. Quality Control Preparation: added “or a different lot number of the same vendor”. Extraction section removed “no brake”. Table 6 removed LLOD column, combined RL/LLOD/LLOQ. Instrument and data parameters: Removed Nebulizing gas flow, DL temperature, Heat block, Drying gas flow, CID gas, all CE, and all Dwell times. All of these parameters are instrument specific and cannot be used for method replications. All of these parameters are still located in the printed instrument data acquisition methods located by the instrument. Documentation: qualifier ion ratio report added “when applicable” and removed STARLIMs batch sequences.
EAF	4	03/03/2021	Added Agilent 6470A information/reworded to include Agilent 6470A. Reworded reporting limit wording regarding removing calibration points. Added QNS sample and non-DHHS information to Specimen Requirement section.

DETERMINATION OF CANNABINOIDS IN BLOOD SOP: Doc # = 019

Approved by: Forensic Lab Director – Lauren Niskach

Originally issued: 10-16-2019

Date Revised: 03-15-2021

Electronic Copy is Controlled

Printed Copy is Convenience

Refer to SharePoint for the most current version