CANNABINOID SCREENING BY LIQUID CHROMATOGRAPHY – TANDEM MASS SPECTROMETRY

42.1 METHOD

This test method may be used to identify Δ^9-THC (THC) and its metabolite, 11-nor-9-carboxy-Δ^9-THC (THCCOOH), in biological specimens and other submitted evidence. The targeted compounds and internal standards are isolated from biological specimens or evidence by the use of liquid-liquid extraction (LLE). The extracts are injected into a high performance liquid chromatograph (HPLC) coupled to a tandem mass spectrometer (MS-MS) detector equipped with an atmospheric pressure electrospray ionization source.

42.2 SPECIMENS

The specimen volume is 0.25 mL. Specimens include, but are not limited to, whole blood, serum, plasma, tissue homogenate and non-biological aqueous solutions. Dilutions of specimens may be analyzed at the Forensic Scientist's discretion.

NOTE: Matrix-matching of all calibrators and the negative and positive control is required for analysis of liver (tissue) homogenate or serum/plasma specimens (see 42.4.2 and 42.4.3).

When analyzing non-biological evidence (e.g., infused beverages), care should be taken to prevent carryover on the instrument (e.g., analyze at dilution, include solvent blanks following injection, run at the end of the batch). Analysis of urine specimens is performed using the cannabinoids confirmation test method, TCc12727.

42.3 REAGENTS, MATERIALS AND EQUIPMENT

42.3.1 REAGENTS

NOTE: Unless use of LC-MS grade (or equivalent from a high-purity filtration system) deionized water (DI H_2O) is specified, laboratory general-use DI H_2O is used in reagent preparation. Organic solvents are reagent grade unless otherwise specified.

- 10% Acetic acid

  Add 10 mL of concentrated acetic acid to approximately 50 mL DI H_2O in a 100 mL flask. Dilute to 100 mL with DI H_2O and mix. The solution is stored in a glass bottle at room temperature and expires one year from the date of preparation.

- Acetic acid (glacial)
- Acetonitrile (ACN), LC-MS grade
- Certified blank blood (specified for THC) and/or other biological matrices
- DI H_2O, laboratory general-use and LC-MS grade (or equivalent from a high-purity filtration system)
- Ethyl acetate (EtAC)

- Extraction solvent; hexanes:ethyl acetate 9:1

  Add 90 mL hexanes to a glass flask. Add 10 mL ethyl acetate and mix. Store the solvent in a glass flask/bottle at room temperature. Use on date of preparation only.

- Formic acid, concentrated
- 0.1% Formic acid

  Add 1 mL of concentrated formic acid to 800 mL LC-MS grade DI H_2O in a 1 L flask. Dilute to 1 L with LC-MS grade DI H_2O and mix. Filter this solution prior to use on the HPLC. The solution is stored in an amber glass bottle at room temperature and expires one year from the date of preparation.

- Hexanes
- Methanol (MeOH)
- Reconstitution solution, 50:50 LC-MS grade ACN:LC-MS grade DI H_2O

  Add 2 mL of LC-MS grade acetonitrile to 2 mL of LC-MS grade DI H_2O in a glass tube, cap and mix. Use on date of preparation only.

- Sodium hydroxide (NaOH), concentrated, 10N

NOTE: Adjustments to final volumes of prepared reagents are permitted as long as the proportions are maintained.

42.3.2 MATERIALS

- Disposable extraction tubes (16 x 100 mm recommended) and screw-cap or centrifuge tubes with closures
- Disposable glass transfer pipettes
- Glass autosampler vials with integrated conical inserts and caps
- HPLC column (Agilent Poroshell 120 EC-C18, 2.1x75 mm, 2.7 µM particle size, or equivalent)
- Laboratory glassware (graduated cylinders, flasks)
- Solvent filters (0.45 µm pore size; reduced cellulose, other)

42.3.3 EQUIPMENT

- Shimadzu HPLC, or equivalent
- Sciex API 3200 MS-MS, or equivalent
- Calibrated, adjustable piston pipettes and verified, adjustable repeater-pipette with disposable pipette tips
- General-use equipment (centrifuge, evaporator, rotary mixer, solvent filtration apparatus, vortex mixer)

42.4 STANDARDS, CALIBRATORS AND CONTROLS

42.4.1 STANDARDS

- Working standard (WS): 0.1 ng/µL THC/5 ng/µL THCCOOH
- Working control standard (QC): 0.1 ng/µL THC/0.5 ng/µL THCCOOH
- Stock internal standard: 1 ng/µL THC-d_3/5 ng/µL THCCOOH-d_3
- Working internal standard: 0.1 ng/µL THC-d_3/0.5 ng/µL THCCOOH-d_3

42.4.2 SEMI-QUANTITATIVE (SEMI-QUANT) CALIBRATORS

Semi-quant calibrators are prepared in certified blank blood at the time of analysis, as detailed in 42.5 SAMPLE PREPARATION.

Analysis of liver (tissue) homogenate or serum/plasma specimens requires that all semi-quant calibrators be prepared in blank alternate matrix. If testing only an alternate matrix, a whole blood semi-quant curve is not required.

42.4.3 CONTROLS

42.4.3.1 At least one negative whole blood control and one positive whole blood control are tested with each batch. For analysis of liver (tissue) homogenate or serum/plasma specimens only, whole blood controls are not required.

42.4.3.2 Samples with known target concentrations (semi-quant calibrators and positive/negative controls) must make up at least 10% of the extracted batch (based on number of case specimen samples), with case specimens bracketed by positive known samples (semi-quant calibrators or positive control).

42.4.3.3 For analysis of liver (tissue) homogenate or serum/ plasma specimens, matrix-matching of all semi-quant calibrators and the negative and positive controls (must meet 10% for that matrix) is required.

42.5 SAMPLE PREPARATION

NOTE: Laboratory general-use DI H_2O is used in sample preparation. LC-MS grade DI H_2O (or equivalent) and LC-MS grade ACN are used in reconstitution 42.5.15). Organic solvents used in sample preparation are reagent grade.

42.5.1 Label a clean extraction tube for each member of the test batch (i.e., semi-quant calibrator, control, case sample).

42.5.2 Add 0.5 mL DI H_2O to each tube.

42.5.3 Using a calibrated pipette, add 0.25 mL of certified blank whole blood into each of the semi-quant calibrator tubes, and negative and positive control tube(s).

42.5.4 Prepare a 1:10 dilution of the working standard. (0.1, 0.5 ng/µL)

a. Using a calibrated pipette, combine 0.1 mL of the working standard with 0.9 mL of ACN or MeOH in a labeled tube.

b. Cap and vortex mix. This dilution shall be disposed of after semi-quant calibrator preparation.

42.5.5 Using a calibrated pipette, spike the semi-quant calibrators according to the following table, using the prepared dilutions.

Calibrator Description	Volume (µL) Added	Standard Concentration	Dilution of WS (or WS)
Calibrator 1 – 1/5 ng/mL	25	0.01/0.05 ng/µL	1:10
Calibrator 2 – 2/10 ng/mL	50	0.01/0.05 ng/µL	1:10
Calibrator 3 – 10/50 ng/mL	25	0.1/0.5 ng/µL	WS

42.5.6 Prepare a 1:10 dilution of the control working standard. (0.01, 0.05 ng/µL)
a. Using a calibrated pipette, combine 0.1 mL of the control working standard with 0.9 mL of ACN or MeOH in a labeled tube.
b. Cap and vortex mix. This dilution shall be disposed of after control preparation.

42.5.7 Using a calibrated pipette, add 75 µL of the 1:10 dilution of the working control standard to the positive control tube. The target concentration for the control is 3.0 ng/mL THC and 15 ng/mL THCCOOH.

42.5.8 Using a calibrated pipette, sample 0.25 mL of each case specimen into its respective tube.

42.5.9 Using a calibrated pipette or verified repeater-pipette, add 25 µL of the working internal standard solution to each tube. Final concentration of the internal standard is 10 ng/mL THC-d_3 and 50 ng/mL THCCOOH-d_3.

42.5.10 Add 200 µL of 10% acetic acid and vortex-mix.

42.5.11 Add 2 mL extraction solvent (hexanes:ethyl acetate, 9:1) to each tube.

42.5.12 Cap the tubes and place on a rotary mixer for 30 minutes.

42.5.13 Centrifuge the tubes for 10 minutes at 3500 rpm (recommended for 16 x 100 mm tubes) to achieve separation.

42.5.14 Transfer the organic layer to a clean, labeled centrifuge or screw-cap tube.

42.5.15 Transfer the tubes to the evaporator and evaporate the extracts to dryness at 40°C.

42.5.16 Reconstitute samples with 50 µL of reconstitution solvent (50:50 LC-MS grade ACN:LC-MS grade DI H_2O) and briefly vortex-mix. If necessary, centrifuge the tubes for 2 minutes at 2000 rpm to collect the extracts at the bottom of the tubes.

42.5.17 Transfer the extracts to labeled glass autosampler vials with integrated inserts and cap.

42.6 INSTRUMENTAL PARAMETERS/DATA ANALYSIS

- Acquisition method – CANNSCREEN (instrumental parameters in Appendix A)
- Calibration curve – linear, $1/a^2$ weighting factor
- Updating calibrator (retention times ±2%, transition ratios ±20%) – Cal 3
- Result comparisons – all units in ng/mL

  Cal 1: truncated to two decimal places (acceptable range 0.75 – 1.25 ng/mL for THC; 3.75 – 6.25 ng/mL for THCCOOH).

  Cals 2-3, Pos Ctl: truncated to one decimal place for target concentrations ≤10 ng/mL; truncated, whole integer values for target concentrations >10 ng/mL.

  NOTE: The positive control must meet acceptability criteria for chromatography, retention time and ratios. Where control values for THC or THCCOOH are not within ±20% of target, qualitative results are still reportable from this screen; however, this should be considered when comparing screening values to those from confirmation/quantitative testing (TCc12727).

42.7 REPORTING

Results at or above the cutoff concentrations (Cal 1) are reported as positive for all matrices, provided that the specimen results meet all criteria for acceptance (e.g., retention time, chromatography, transition ratios), including results >Cal 3 concentrations (10 ng/mL THC, 50 ng/mL THCCOOH).

42.8 METHOD PERFORMANCE

- Limit of detection (for qualitative reporting): 1.0 ng/mL THC
  5.0 ng/mL THCCOOH
- Dynamic range: 1.0 – 10 ng/mL THC, 5.0 – 50 ng/mL THCCOOH

APPENDIX A
INSTRUMENTAL PARAMETERS

Shimadzu/Sciex LC-MSMS System

SHIMADZU LIQUID CHROMATOGRAPH

Gradient Elution	
Flow rate	0.5 mL/min
Solvent A	0.1% Formic acid
Solvent B	ACN (LC-MS grade)
Initial composition	60% A, 40% B
0 – 1.0 min	40% B
1.0 – 7.0 min	95% B
7.0 – 10.0 min	95% B
10.1 – 12.5 min	40% B
Post time	2.5 min
Column temp	50°C
Autosampler	
Injection volume	20 µL
Rinsing volume	1000 µL
Rinsing solvent	75:25 MeOH:DI H_2O
Cooler temperature	25ºC

SCIEX MASS SPECTROMETER

Scan type	(+) MRM	Curtain/collision gas	Nitrogen
Ion mode	ESI	Curtain gas flow	40 L/min
Resolution (Q1)	Unit	Collision gas flow	4 L/min
Resolution (Q3)	Unit	Gas 1 temp	40ºC
Valve position A	To waste	Gas 2 temp	80ºC
Valve position B (all transitions)	To MS	Ion voltage	5.5 kV
Valve position A	To waste	Interface temp	650ºC

Compound	MRM Transitions	Dwell Time
THCCOOH-d_3	348.3→330.0, 302.0	50 msec
THCCOOH	345.4→299.2, 193.3	50 msec
THC-d_3	318.3→196.3, 123.1	100 msec
THC	315.2→193.3, 123.2	100 msec

LIST OF CHANGES

Revision Date	Description	Page Number
6/19/19	Method approved by Washington State Toxicologist. See DRA dated 5/17/19. Method released for use in evidentiary testing on 6/19/19.	All