1 PRINCIPLE/SCOPE

This procedure is mainly used for detection of various cannabinoids and their respective metabolites within biological specimens (e.g., blood/serum and urine) within a variety of forensic-related requests (e.g., driving under the influence, drug-facilitated assault). Deuterated internal standards are added to specimens for both qualitative measures (extraction monitoring) and quantitation purposes. In this procedure, blood specimens undergo a protein precipitation process to clean up any matrix before instrumental analysis. Urine samples undergo hydrolysis prior to being diluted using acetonitrile before instrumental analysis. After sample preparation, samples are analyzed by LC/MS/MS.

2 SPECIMENS

This procedure uses biological fluids (e.g., blood, serum, plasma, or urine). Typically, 0.5 mL of sample is used during the analysis, but differing volumes may be necessary depending on the matrix. Dilution of samples due to limited specimen, due to suspicion of high drug or metabolite concentrations (as possibly indicated by presumptive results), due to possible matrix-effects, or similar reasons is acceptable. All dilutions and other significant changes should be recorded and communicated appropriately.

3 REAGENTS

3.1 In the event that a part number or item number listed is not available, an equivalent may be purchased. Note: When possible, calibrators and controls are prepared with reference materials from different manufacturers. In the event that different manufacturers are not available, a calibrator and control may be made using the same manufacturer provided a different analyst prepares each solution.

3.2 If the equivalent reference material purchased has a different concentration, an appropriate volume (µL) shall be used.

3.3 All reagents must be ACS grade or better.

3.3.1	Formic Acid	Fisher 27048
3.3.2	Water	Millipore, Deionized (DIW)
3.3.3	Acetonitrile	Fisher A955

3.3.4	Abalonase Ultra	UASBETA-GLUC
3.3.5	Drug Free Blood	UTAK 44600-WB(F)
3.3.6	Drug Free Human Urine	UTAK 88121-CDF(F)
3.3.7	Delta-9 THC (1.0 mg/mL)	Cerilliant T-005
3.3.8	Delta-9 THC (1.0 mg/mL)	Cayman ISO60157
3.3.9	Delta-9 THC-d_3 (0.1 mg/mL)	Cerilliant T-003
3.3.10	Delta-9 THC-COOH (0.1 mg/mL)	Cerilliant T-018
3.3.11	Delta-9 THC-COOH (1.0 mg/mL)	Lipomed THC-726-1LM
3.3.12	Delta-9 THC-COOH-d_3 (0.1 mg/mL)	Cerilliant T-004
3.3.13	11-hydroxy-delta-9 THC (0.1 mg/mL)	Cerilliant H-026
3.3.14	11-hydroxy-delta-9 THC (1.0 mg/mL)	Lipomed THC-318-1M
3.3.15	11-hydroxy-delta-9 THC- d_3 (0.1 mg/mL)	Cerilliant H-041
3.3.16	(6aR,9S)-delta-10-THC (1.0 mg/mL)	Cerilliant T-159
3.3.17	(6aR,9S)-delta-10-THC (1.0 mg/mL)	Cayman 33012
3.3.18	Delta-8 THC (1.0 mg/ml)	Cerilliant T-032
3.3.19	Delta-8 THC (1.0 mg/ml)	Cayman ISO60158
3.3.20	Delta-8 THC-d_3 (0.1 mg/ml)	Cerilliant T-153
3.3.21	Delta-8 THC-COOH (1 mg/mL)	Cerilliant T-170
3.3.22	Delta-8 THC-COOH (1 mg/mL)	Cayman 34024
3.3.23	Delta-8 THC-COOH-d_3 (0.1 mg/mL)	Cerilliant T-158

3.3.24 Delta-9 THC-COOH Glucuronide (0.1 mg/mL) Cerilliant T-038

4 EQUIPMENT

4.1 LC-MS/MS

4.1.1 Agilent 6475AA QQQ with a 1260 Infinity II LC stack or equivalent

4.2 LC Column

4.2.1 50 x 3.0mm, 2.7 µm particle size, Poroshell 120 EC-C18, Agilent, or equivalent

4.3 Guard Cartridge

4.3.1 Poroshell 120 UHPLC Guard Column, EC-C18 3.0mm, Agilent, or equivalent

4.4 Centrifuge capable of at least 3000 rpm

4.5 Vortexer

4.6 Disposable borosilicate test tubes (e.g. 16x100 mm, round bottom, borosilicate glass with applicable caps)

4.7 Autosampler vials with inserts (1.8 mL or equivalent)

4.8 Heating element

5 SOLUTION PREPARATION

Different volumes may be prepared as long as the concentration/ratio is consistent.

5.1 Mobile phase A (5mM Ammonium Formate/0.1% Formic Acid in water)

5.1.1 Combine 0.315 g Ammonium Formate, 1.0 mL of formic acid to a final volume of 1000 mL in a glass bottle.

5.1.2 Make Fresh

5.2 Mobile phase B (Methanol)

5.2.1 Store in glass at room temperature.

5.3 0.1% Formic Acid in Water (Used to make reconstitution mixture)

5.3.1 Combine 1.0 mL of formic acid to a final volume of 1000 mL of water in a glass bottle.

5.3.2 Make Fresh

5.4 0.1% Formic Acid in ACN (Used to make reconstitution mixture)

5.4.1 Combine 1.0 mL of formic acid to a final volume of 1000 mL of ACN in a glass bottle.

5.4.2 Make Fresh

5.5 Reconstitution Mixture (Dilution Solution/Solvent Blank)

5.5.1 Combine 95 mL of 0.1% Formic Acid in Water with 5.0 mL of 0.1% Formic Acid in Acetonitrile and mix.

5.5.2 Stable for 6 months when stored at room temperature.

5.6 Stock Calibration Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 1.0/1.0/5.0 µg/mL)

5.7 Add the following to a 10 mL volumetric flask:

Analyte Name	Standard Concentration (Cerilliant)	Amount to Pipette (uL)
Delta-9 THC	1.0 mg/mL	10
Delta-8 THC	1.0 mg/mL	10
Delta-10 THC	1.0 mg/mL	10
11-Hydroxy-Delta-9 THC	0.1 mg/mL	100
Delta-9 Carboxy THC	0.1 mg/mL	500
Delta-8 Carboxy THC	1.0 mg/mL	50

5.7.1 Dilute to the line using ACN. Mix.

5.7.2 Stable for 3 months when stored in the freezer.

5.8 Dilute **Blood** Calibration Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 0.1/0.1/0.5 µg/mL)

5.8.1 To a 10 mL volumetric flask, add 1000 µL of Stock Calibration Solution (1.0/1.0/5.0 µg/mL).

5.8.2 Dilute to the line using ACN. Mix.

5.8.3 Stable for 3 months when stored in the freezer.

5.9 Dilute **Urine** Calibration Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 50/50/250 ng/mL)

5.9.1 To a 10 mL volumetric flask, add 500 µL of Stock Calibration Solution (1.0/1.0/5.0 µg/mL).

5.9.2 Dilute to the line using ACN. Mix.

5.9.3 Stable for 3 months when stored in the freezer.

5.10 Working IS Solution (THC-d3 (delta-8 and delta-9)/11-Hydroxy-delta-9-THC-d3/THC-COOH-d3 (delta-8 and delta-9)– 1.0/1.0/5.0 µg/mL)

5.10.1 Add the following to a 10 mL volumetric flask:

Analyte Name	Standard Concentration (Cerilliant))	Amount to Pipette (uL)
Delta-9 THC-d3	0.1 mg/mL	100
Delta-8 THC-d3	0.1 mg/mL	100
11-Hydroxy-Delta-9 THC-d3	0.1 mg/mL	100
Delta-9 Carboxy THC-d3	0.1 mg/mL	500
Delta-8 Carboxy THC-d3	0.1 mg/mL	500

5.10.2 Dilute to the line using ACN. Mix.

5.10.3 Stable for 6 months when stored in the freezer.

5.11 Stock Control Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 1.0/1.0/5.0 µg/mL)

5.12 Add the following to a 10 mL volumetric flask:

Analyte Name	Standard Concentration (Lipomed or Cayman)	Amount to Pipette(uL)
Delta-9 THC	1.0 mg/mL	10
Delta-8 THC	1.0 mg/mL	10
Delta-10 THC	1.0 mg/mL	10
11-Hydroxy-Delta-9 THC	1.0 mg/mL	10
Delta-9 Carboxy THC	1.0 mg/mL	50
Delta-8 Carboxy THC	1.0 mg/mL	50

5.12.1 Dilute to the line using ACN. Mix.

5.12.2 Stable for 3 months when stored in the freezer.

5.13 Dilute **Blood** Control Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 0.1/0.1/0.5 µg/mL)

5.13.1 To a 10 mL volumetric flask, add 1000 uL of Stock Control Solution (1.0/1.0/5.0 µg/mL).

5.13.2 Dilute to the line using ACN. Mix.

5.13.3 Stable for 3 months when stored in the freezer.

5.14 Dilute **Urine** Control Solution (THC (delta-8, delta-9 and delta-10)/11-Hydroxy-delta-9 THC/THC-COOH (delta-8 and delta-9) – 50/50/250 ng/mL)

5.14.1 To a 10 mL volumetric flask, add 500 µL of Stock Calibration Solution (1.0/1.0/5.0 µg/mL).

5.14.2 Dilute to the line using ACN. Mix.

5.14.3 Stable for 3 months when stored in the freezer.

5.15 Hydrolysis Control Solution (3.3 µg/mL)

5.15.1 The purchased 0.1 mg/mL THC-COOH glucuronide contains 0.066 mg/mL of THC-COOH

5.15.2 To a 10 mL volumetric flask, add 500 µL of THC-COOH glucuronide (0.1 mg/mL).

5.15.3 Dilute to the line using ACN. Mix.

5.15.4 Stable for 2 months when stored in the freezer

6 PROCEDURE

6.1 Sample Preparation

6.1.1 Label clean test tubes accordingly: Calibrators, controls and case specimen identifier.

6.1.2 Add 10 µL of Working IS Solution to all tubes.

6.1.3 **URINE SAMPLES:** Turn on heating block and allow to come to temperature for at least 30 minutes prior to heating samples.

6.1.4 **URINE SAMPLES:** Add 10 µL of Abalonase Ultra to all tubes

6.1.5 Pipette 0.5 mL of case specimen to the appropriately labeled tube unless directed to run the sample diluted.

6.1.5.1 If a dilution is needed, make up remaining blood or urine volume using blank blood or human urine to ensure total volume used is 0.5mL (i.e., x2 dilution would require 0.25mL blank blood in addition to 0.25mL case sample). NOTE: Serum/Plasma case specimen will not receive any additional blank matrix, resulting in total volume less than 0.5mL.

6.1.6 To prepare the **blood** calibration curve, pipette the following volumes of the Designated Calibration Solution into 0.5 mL of drug-free blood:

Calibrator	Dilute Cal Solution (µL)	Stock Cal Solution (µL)	Final Concentration of Target Analytes (ng/mL)THC/THC-OH/THC-COOH
Level 1	5	N/A	1.0/5.0
Level 2	10	N/A	2.0/10
Level 3	25	N/A	5.0/25
Level 4	N/A	5	10/50
Level 5	N/A	10	20/100
Level 6	N/A	25	50/250

6.1.7 To prepare the **blood** in-house controls, pipette the following volumes of the Designated Control Solution into 0.5 mL of drug-free blood.

Control	Dilute Control Solution (µL)	Stock Control Solution (µL)	Final Concentration of Target Analytes (ng/mL)THC/THC-OH/THC-COOH
Negative	0	0	0
Low	15	N/A	3.0/15
Mid	N/A	7.5	15/75
High	N/A	15	30/150

6.1.8 To prepare the **Urine** calibration curve, pipette the following volumes of the Designated Calibration Solution into 0.5 mL of drug-free human urine:

Calibrator	Dilute Cal Solution (µL)	Stock Cal Solution (µL)	Final Concentration of Target Analytes (ng/mL)THC/THC-OH/THC-COOH
Level 1	10	N/A	1.0/5.0
Level 2	100	N/A	10/50
Level 3	N/A	20	40/200
Level 4	N/A	50	100/500
Level 5	N/A	100	200/1000

6.1.9 To prepare the **Urine** in-house controls, pipette the following volumes of the Designated Control Solution into 0.5 mL of drug-free human urine.

Control	Dilute Control Solution (µL)	Stock Control Solution (µL)	Hydrolysis Control Solution (µL)	Final Concentration of Target Analytes (ng/mL)THC/THC-OH/THC-COOH
Negative	0	0	N/A	0
Low	50	N/A	N/A	5.0/25
Mid	N/A	35	N/A	70/350
Hydrolysis	N/A	N/A	20	132* (THC-COOH only)

*The target value of THC-COOH is 132 ng/mL, however, concentrations ≥100 ng/mL will be acceptable. If the concentration is <100 ng/mL, consult a FSE2 or higher.

6.1.10 **Urine Samples**:

6.1.10.1 Cap tubes, vortex and heat at 55°C for at least 30 min. Allow to cool completely.

6.1.10.2 Add 1.5 mL of cold ACN (stored in Freezer) to the sample while vortexing

6.1.11 **Blood Samples**:

6.1.11.1 Add 0.75 mL cold ACN (stored in Freezer) dropwise to the sample while vortexing.

6.1.12 Centrifuge the tubes for 10 minutes at a minimum of 3000 rpm.

6.1.13 Add 50 µL 95:5 reconstitution mixture to each LC-MS/MS vial.

6.1.14 Transfer 150 µL ACN supernatant to appropriate LC-MS/MS vial.

6.1.15 Cap the LC-MS/MS vials and mix.

6.1.16 Ensure the appropriate instrumental quality assurance/quality control (QA/QC) procedures were performed. The instrument must have passing QA/QC results prior to preparing and loading of samples.

6.1.17 Prepare the sequence and enter the samples in appropriate order. Negative controls will be analyzed prior to evidentiary samples. Blank samples (i.e., those containing just reconstitution mixture) may be analyzed in between evidentiary samples to avoid carry-over.

6.1.18 Verify the sequence:

6.1.18.1 Print the sequence list

6.1.18.2 Check that the physical placement of the autosampler vials and the vial positions within the instrument's sequence list match. Indicate completion of this check using sequence checked, sequence verified or similar on the sequence page along with analysts initials and date.

6.1.19 Print the instrument method and include both the method and sequence printouts with the batch documents.

7 INSTRUMENT PARAMETERS

For complete method parameters see TX 41.2. Documentation of changes must be included with batch data so that any instrumental parameter change can be associated with data and casework until this procedure has been updated.

8 DATA PROCESSING

8.1 Refer to TX 43 TOX Quality Control Manual for specific batch processing criteria.

8.2 Ion ratios should compare favorably to ion ratios of an extracted calibrator or positive control at a comparable concentration. Generally, ion ratios are within the limits specific within the section procedure related to mass spectral comparisons.

NOTE: With the exception of the internal standard, it is recognized that some ion ratios are concentration dependent; thus, concentrations at the ends of the calibrations curve may not be within the updated ratios and may be acceptable.

8.3 For Urine Specimens: If a case specimen exceeds the upper limit of quantitation and a solvent blank was not run immediately after it, repeat or reinject (with a solvent blank prior) the next case specimen if that specimen is within 20% of the LOQ for that analyte.

9 STABILITY POST-EXTRACTION

9.1.1 Calibrators, controls or case specimens may be re-injected within 72 hours of being injected with a solvent blank prior. The chromatograms for both injections are saved with the data packet. A positive control must be re-injected with case specimens to verify that the curve is still acceptable if the sequence was completed.

9.1.2 If a case specimen(s) is re-injected after 72 hours, the calibrators and controls shall be re-injected along with the case specimen(s). NOTE: This would be considered a new analytical run and prepared separately from the original runs.

9.1.3 If a case specimen(s) was inadvertently not injected and it is more than 72 hours since the first calibrator was injected, you shall re-inject the calibrators and controls with the case specimen(s). NOTE: This would be considered a new analytical run and prepared separately from the original runs.

10 ASSAY CHARACTERISTICS

10.1 The limit of detection for each analyte is listed below:

Analyte	LOD-Blood	LOD-Urine
Delta-9 THC	1.0 ng/mL	1.0 ng/mL
Delta-8 THC	1.0 ng/mL	1.0 ng/mL
Delta-10 THC	1.0 ng/mL	1.0 ng/mL
Delta-9 THC-COOH	5.0 ng/mL	5.0 ng/mL
Delta-8 THC-COOH	5.0 ng/mL	5.0 ng/mL
Delta-9 THC-OH	1.0 ng/mL	1.0 ng/mL

10.1.1 Results with values less than 10 ng/mL will be truncated and reported to one decimal point (i.e 0-9.9).

10.1.2 Results with values greater than or equal to 10 ng/mL will be reported as a whole number.

10.1.3 All analytes demonstrate a linear regression model using 1/x weighting.

10.1.4 LOQ is equal to the lowest cal

10.1.5 ULOQ is equal to the highest cal

10.1.6 Dilutions of up to x10 (1:9) may be performed.

10.1.7 Delta-10 THC will be reported as qualitative only using the level 1 calibrator as the cut-off limit.

10.1.8 Exo-THC is observed in the delta-9 THC window however differs by retention time (exo-THC elutes prior to delta-9 THC) but may produce a spilt peak if both are present.

10.1.9 9s-delta6a, 10a-THC is observed in the delta-10 THC window however differs by retention time (9s-delta6a, 10a THC elutes prior to delta-10 THC) but may produce a spilt peak if both are present.

10.1.10 Delta-8 THC OH coelutes with delta-9 THC OH, therefore if a peak for delta-8 THC is present and a case is positive for delta-9 THC-OH, the analyte will be reported as Delta-9/8 THC-OH.

10.1.11 Delta-8-ISO THC co-elutes with delta-8 THC, therefore if a peak for delta-8 THC is present and a case is positive delta-8 Carboxy THC, the analyte will be reported as delta-8 THC. If the case is not positive for delta-8 Carboxy THC, the analyte will be reported as delta-8/delta-8 ISO THC.

10.1.12 Delta-9 THCP and Tetrahydrocannabivarin produce no peaks in any of the analyte windows.

11 SAFETY PRECAUTIONS

Refer to the DSS GL 2 Safety Manual for precautions.