Title: Analysis of Biological Specimens for Cannabinoids by Liquid Chromatography/Mass Spectrometry (LC/MS)

1. Introduction/Scope

Marijuana, obtained from the Cannabis sativa plant, is a commonly abused drug. It is typically dried and smoked but there are a variety of alternative ways for ingestion. The chemical Δ^9-tetrahydrocannabinol (THC) is the primary psychoactive component of marijuana. Another chemical, 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) is a major active metabolite of THC and 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH) is a major inactive metabolite of THC.

2. Principle

Biological specimens are assayed for the presence of THC, THC-OH, and THC-COOH. A deuterated internal standard solution is added to specimens which contain deuterated analogs to the analytes of interest. Extraction of analytes occurs through the use of polymeric solid phase extraction (SPE) technology. Eluted solutions are analyzed using high pressure liquid chromatography/mass spectrometry (HPLC/MS or LC/MS) including multiple reaction mode (MRM) detection.

3. Specimens

This procedure involves the analysis of a biological fluid (e.g., blood, serum/plasma, urine, vitreous). Blood and serum will be treated both qualitatively and quantitatively the same way. Typically 0.5 mL of sample is used during the analysis but differing volumes may be necessary. Dilution of samples due to limited volume of specimen, due to suspicion of high drug or metabolite concentrations (as possibly indicated by presumptive results), due to possible matrix-effects, or similar reasons are acceptable. All dilutions and other significant changes will be recorded and communicated appropriately within casework documentation.

4. Equipment/Materials/Reagent

4.1 Disposable borosilicate test tubes (e.g., 16 x 100 mm, round bottom, borosilicate glass with Teflon caps)

4.2 Vortex mixer

4.3 Automatic pipettes (with disposable tips)

4.4 Positive pressure solid phase extraction device (e.g., Cerex, or equivalent)

4.5 Sample concentrator with nitrogen (e.g., Cerex 48 heated, or equivalent)

4.6 Centrifuge

4.7 Liquid Chromatograph/Mass Spectrometer (Shimadzu LCMS-8030, or equivalent)

4.8 HPLC column (Kinetex 2.6µ, C18, 100Å, 50 mm x 2.1 mm (Phenomenex), or equivalent)

4.9 Pre-Column – SecurityGuard ULTRA Cartridge UHPLC Kinetex for 2.1mm ID Columns (Phenomenex or equivalent)

4.10 Autosampler vials with inserts (LC/MS, 1.8mL or equivalent)

4.11 Acetic acid, glacial (Reagent grade or equivalent)

4.12 Acetonitrile (CH_3CN or ACN, Optima grade or equivalent)

4.13 Ammonium acetate (NH_4OAc ; Reagent Grade or equivalent)

4.14 Ammonium hydroxide (NH_4OH, Reagent grade or equivalent)

4.15 Ethyl acetate (HPLC grade or equivalent)

4.16 Hexane (HPLC grade or equivalent)

4.17 Hydrochloric acid – concentrated (HCl ; Reagent grade or equivalent)

4.18 Methanol (MeOH, Reagent grade or equivalent)

4.19 Water (H_2O, Millipore, Deionized (DIW) or equivalent)

4.20 $NH_4OAc_{(aq)}$ (5M or 38.5% (w/v): Can be prepared by dissolving 3.85 g of NH_4OAc in 10mL of water. Stable for at two (2) months in refrigerator.

4.21 SPE Conditioning-Solution – $HCl_{(aq)}$ (0.1M or 0.83% (v/v)) – Can be prepared by slowly adding 8.3 mL of $HCl_{(conc)}$ to water within a 1 L volumetric flask. Dilute to volume with water. Stable for one (1) year at room temperature. Purchased solutions may be used. This solution can be stored in a re-pipetter bottle.

4.22 SPE Sample Wash Mixture – $H_2O:CH_3CN:NH_4OH$ (85:15:1): Can be prepared by combining 85 mL of deionized H_2O, 15 mL of CH_3CN, 1 mL NH_4OH and mix well. Store in glass at room temperature. Should be prepared fresh.

4.23 Elution Solvent (THC, THC-OH) – Ethyl acetate

4.24 Elution Mixture (THC-COOH) – Hexane:Ethyl Acetate:Acetic Acid (80:18:2): Can be prepared by combining 80 mL of hexane, 18 mL of ethyl acetate and 2 mL of acetic acid and mixing well. Store in glass at room temperature. Stable for one (1) month.

4.25 Mobile Phase A – $NH_4OAc_{(aq)}$ (5mM or 0.04% (w/v)): Can be prepared by diluting 1mL of the 5 mM (38.5% (w/v)) $NH_4OAc_{(aq)}$ in 1 L of water (1:1000 dilution) and mixing well. Store in glass at room temperature. Stable for one (1) week.

4.26 Mobile Phase B – (ACN or CH_3CN). Store in glass at room temperature

4.27 Δ^9-tetrahydrocannabinol (THC) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

4.28 Δ^9-tetrahydrocannabinol-D_3 (THC-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

4.29 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

4.30 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-D_3 (THC-COOH-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

4.31 11-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH) Reference Standard (1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

4.32 11-hydroxy-Δ^9-tetrahydrocannabinol-D_3 (THC-OH-D_3) Reference Standard (0.1 mg/mL or equivalent). Storage conditions per manufacturer's instructions.

5. Standards and Controls

5.1 Negative and Positive Controls: Can be purchased (e.g., UTAK Labs) or generated in-house (e.g., spiked blank blood, spiked blank urine, spiked vitreous). If prepared in-house, reference standards from different manufacturers or from different lot numbers should be used when making controls and calibrator solutions.
Store frozen, refrigerated, or obtain fresh. If purchased, the stability should be determined by the manufacturer. If frozen, samples should be brought to room temperature prior to use within the procedure. Appropriate positive/negative controls and calibrators (for quantitations) will be extracted and analyzed with each assay or batch.

5.2 Stock Deuterated Internal Standard (IS) Mix Solution –
((THC-D_3 + THC-OH-D_3 + THC-COOH-D_3 (1 µg/mL each))):
Can be prepared by individually combining 100 µL each of 0.1 mg/mL solutions of THC-D_3, THC-OH-D_3, and THC-COOH-D_3 into a 10 mL volumetric flask and diluting to volume (10 mL) with MeOH. Stable for 6 months from date of preparation.
Alternatively can be prepared, if using 1 mg/mL solutions of the reference standards, only add 10 µL each of the reference standards to a 10 mL volumetric flask and diluting to volume with MeOH.

5.3 Working Deuterated Internal Standard (IS) Mix Solution –
((THC-D_3 + THC-OH-D_3 + THC-COOH-D_3 (0.1 µg/mL each))):
Can be prepared by diluting the Stock Deuterated Internal Standard (IS) Mix Solution 1:10 with MeOH (e.g., 1 mL of Stock IS Mix Solution in a total volume of 10 mL of MeOH). Stable for 6 months from date of preparation.

5.4 Working Standard Solution for Controls (High) –
((THC + THC-OH + THC-COOH (1 µg/mL each))):
Can be prepared by individually combining 25 µL each of the 1 mg/mL purchased reference standard solutions of THC, THC-OH, and THC-COOH into a 25 mL volumetric flask and diluting to volume (25 mL) with MeOH. Stable for 3 months from date of preparation.

5.5 Working Standard Solution for Controls (Low) –
((THC + THC-OH + THC-COOH (0.1 µg/mL each))):
Dilute the Working Standard Solution for Controls (High) 1:10 with MeOH. This can be done by transferring 2.5 mL of the Working Standard Solution for Controls (High) into a 25 mL volumetric flask and diluting to volume with MeOH. Stable for 3 months from date of preparation.

5.6 Working Standard Solution for Calibrators (High) –
((THC + THC-OH + THC-COOH (1 µg/mL each))):
Can be prepared by individually combining 25 µL each of the 1 mg/mL purchased reference standard solutions of THC, THC-OH, and THC-COOH into a 25 mL volumetric flask and diluting to volume with MeOH. The reference standard solutions should be from a different lot number, manufacturer, or should be prepared by a different analyst compared to the Working Standard Solution for Controls (High). Stable for 3 months from date of preparation.

5.7 Working Standard Solution for Calibrators (Low) –
((THC + THC-OH + THC-COOH (0.1 µg/mL each))):
Can be prepared by diluting the Working Standard Solution (High) 1:10 with MeOH. This can be done by transferring 2.5 mL of the Working Standard Solution (High) into 25 mL volumetric flask and diluting to volume (25 mL) with MeOH. Stable for 3 months from date of preparation.

5.8 High Positive Control (Hi-Ctrl) Blood/Serum
(THC + THC-OH + THC-COOH (50 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution (High ; 1 µg/mL)) to a screw-capped test tube containing 0.5 mL of blank blood. Prepare fresh and see table below.

5.9 Low Positive Control (Lo-Ctrl) Blood/Serum
(THC + THC-OH + THC-COOH (5 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution (Low ; 0.1 µg/mL)) to a screw-capped test tube containing 0.5 mL of blank blood. Prepare fresh and see table below.

5.10 Positive Control Cut-Off Solution (non-Blood)
(THC + THC-OH + THC-COOH (5 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution for Calibrators (Low ;

0.1 µg/mL) to a screw-capped test tube containing 0.5 mL of blank matrix (e.g., urine, vitreous fluid). Store refrigerated in glass. Prepare fresh.

Note: If qualitative analysis is only needed for a blood sample, then that matrix can follow the same process as is done for the qualitative analysis of non-blood samples with the exception that the Level 1 calibrator solution will be used as the Positive Control Cut-Off Solution for blood.

5.11 Positive Control Solution– Non-Blood (e.g., Urine, Vitreous)
(THC + THC-OH + THC-COOH (5 ng/mL)):
Can be prepared by adding 25 µL of the Working Standard Solution for Controls (Low ; 0.1 µg/mL) to a screw-capped test tube containing 0.5 mL of blank matrix (e.g., Urine, Vitreous). Store refrigerated in glass. Prepare fresh.

5.12 Calibrator Solutions (THC + THC-OH + THC-COOH (2, 5, 20, 50, 100 ng/mL))
Can be prepared by using either the Working Standard Solution for Calibrators (Low) or Working Standard Solution for Calibrators (High) according to Table 2. Prepare fresh.

5.13 Negative Control Solution – Non-Blood (e.g., Urine, Vitreous):
Purchased or in-house negative control (e.g., urine). Store refrigerated or obtain fresh. If purchased then the stability should be determined by the manufacturer. A negative control should be extracted and analyzed with each appropriate assay or batch. Water can be used as a negative control for vitreous fluid.

5.14 Negative Control Solution – Blood:
Purchased (e.g., Diagnostics Products Corporation, UTAK Labs), donated (e.g., American Red Cross), or in-house negative control blood (e.g., from a proven blank specimen). Store frozen, refrigerated, or obtain fresh. If purchased or donated, stability should be determined by manufacturer. A negative control blood should be extracted and analyzed with each blood assay or batch.

Table 1: Control Solutions Used for Quantitation (Blood/Serum)

Level	THC THC-OH THC-COOH (ng/mL)	Volume of Working Std. Solution for Controls (Low) (0.1 µg/mL) (µL)	Volume of Working Std. Solution for Controls (High) (1 µg/mL) (µL)	Volume of Neg. Ctrl. Blood [drug-free] (mL)
Negative Ctrl.	–	–	–	0.5
Low Positive Control	5	25	–	0.5
High Positive Control	50	–	25	0.5

6. Quantitative Analysis

This procedure may be used quantitatively through the construction of a multipoint calibration graph for the analyte(s) of interest. Use the table below and analyze the extract solutions from all of the calibrator level solutions.

Table 2: Calibrator Solutions

Level	THC THC-OH THC-COOH (ng/mL)	Volume of Working Std. Solution for Calibrator (Low) (0.1 µg/mL) (µL)	Volume of Working Std. Solution for Calibrator (High) (1 µg/mL) (µL)	Volume of Neg. Ctrl. Blood [drug-free] (mL)
1	2	10	–	0.5
2	5	25	–	0.5
3	20	100	–	0.5
4	50	–	25	0.5
5	100	–	50	0.5

7. Qualitative Analysis

This procedure may be used qualitatively through the construction of a single-point calibration graph for the analyte(s) of interest. Use the table below and analyze the extract solutions from the negative, cut-off, and positive control solutions along with samples.

Table 3: Control Solutions Used for Qualitative (non-Blood)

Level	THC THC-OH THC-COOH (ng/mL)	Volume of Working Std. Solution for Calibrator (Low) (0.1 µg/mL) (µL)	Volume of Working Std. Solution for Control (Low) (0.1 µg/mL) (µL)	Volume of Neg. Ctrl. Urine (non-Blood) [drug-free] (mL)
Negative Ctrl.	–	–		0.5
Positive Control Cut-Off	5	25	-	0.5
Positive Control	15	-	75	0.5

8. Sampling

Not applicable.

9. Procedure

9.1 Where applicable, prepare calibrator and control solutions according to tables above and label appropriately. These should contain 0.5 mL of sample each. Cap when not in use.

9.2 Add 0.5 mL of unknown specimen(s) to properly labeled test tubes and cap when not in use.

9.3 Add 100 µL of the Working Deuterated Internal Standard (IS) Mix Solution [10 ng of each component] to each test tube, cap, and vortex-mix.

9.4 Slowly add cold ACN to each blood/serum sample while vortex-mixing for at least 30 seconds. Protein precipitation should occur. This step can be skipped for urine (or other applicable non-blood) specimens.

9.5 Cap and centrifuge each tube (e.g., ~6 minutes at ~5200 RPM).

9.6 Add 2 mL of 0.1M $HCl_{(aq)}$ to each SPE column to be used in the extraction process.

9.7 Individually transfer the supernatant from each centrifuged sample into properly labeled SPE columns. Be careful not to transfer protein sediments.

9.8 Allow solutions to migrate through SPE columns via a small amount of pressure in order to push solutions through the columns.

9.9 Wash SPE columns with 1 mL of the SPE Sample Wash Mixture (H_2O:ACN:NH_4OH (85:15:1)) via a small amount of pressure in order to push solutions through the columns.

9.10 Dry the SPE columns for ~5-8 minutes using high pressure (60-80 psi). Do not dry for longer than 8 minutes.

9.11 Elute SPE columns with 2 mL of ethyl acetate (Elution Solvent for THC and THC-OH) at the rate of ~0.5 mL/min. into properly labeled test tubes. Increase the pressure briefly and expel remaining elution solvent from the SPE sorbent beds and from the Luer tips.

9.12 Dry the SPE columns for 2 minutes using high pressure (60-80 psi).

9.13 Elute SPE columns with 2 mL of Elution Mixture for THC-COOH (Hexane:Ethyl Acetate:Acetic Acid (80:18:2)) at the rate of ~0.5 mL/min. into <u>the same</u> test tubes as were used for the THC and THC-OH extracts. Increase the pressure briefly and expel remaining elution solvent from the SPE sorbent beds and from the Luer tips.

9.14 Evaporate the combined sample extracts to dryness (e.g., $N_{2(g)}$ at <40°C).

9.15 Reconstitute the dried residue in 50 µL ACN and vortex-mix the solutions to solubilize the residues from the surfaces of the collection tubes.

9.16 Add 50 µL of water to each test tube and vortex-mix. Total volume of each solution should be ~100 µL.

9.17 Individually transfer solutions to properly labeled autosampler vials containing volume inserts. No solid particulates or colloidal solutions should be visible within autosampler vials. Contact the Lead Examiner (or higher) for assistance if this situation arises.

9.18 Analyze solutions by LC/MS instrumentation.

10. Instrumental Conditions

The following are the typical operating parameters for the instrument used in this procedure. The instrument conditions may be modified to adjust or improve the procedure. The appendix contains an abbreviated version of the procedure and this checklist can be used by analysts.

<u>HPLC Parameters</u>:

HPLC Column: Phenomenex, Kinetex, 2.6µ, C18, 100 Å, 50 mm x 2.1 mm
Mobile Phase A: $NH_4OAc_{(aq)}$ (5mM or 0.04% (w/v))
Mobile Phase B: Acetonitrile (neat)
Column Temperature: 40 °C
Constant Flow: 0.45 mL/min.
Autosampler Temperature: 15 °C

Note: While not mandatory, checking pH of mobile phase and recording background ions of mobile phase prior to starting instrumental sequence will help troubleshoot if any problems arise within batch data acquisition.

Gradient Program: Initial 20% B Flow 0.45 mL/min

Time (min.)	**Mobile Phase A** **$NH_4OAc_{(aq)}$ – (5mM ; 0.04% (w/v))** **(%)**	**Mobile Phase B** **Acetonitrile (100%)** **(%)**
Initial	80	20
0.25	80	20
1.75	10	90
2.40	10	90
2.41	80	20
4.50	80	20

Mass Spectrometer Parameters:

Ionization Source	Electrospray	Polarity	Negative Ion
Ion Spray Potential	-3.5 kV	Heat Block	500 °C
Nebulizer Gas	Nitrogen	Nebulizer	2.0 L/min
Drying Gas	Nitrogen	Drying Gas	15 L/min.
Scan Type	MS/MS (&/or Full Scan)	Resolution	Unit
Desolvation Line	250 °C		

Transition Ions:

Compound	Precursor (m/z)	Product (m/z)
THC	313	245
THC	313	191
THC	313	203
THC-OH	329	311
THC-OH	329	268
THC-OH	329	173
THC-COOH	343	245
THC-COOH	343	191
THC-COOH	343	299
THC-D3	316	194
THC-D3	316	248
THC-OH-D3	332	271
THC-OH-D3	332	314
THC-OH-D3	332	173
THC-COOH-D3	346	302
THC-COOH-D3	346	248
THC-COOH-D3	346	233

Note: For specific instrument parameters refer to the appendix.

11. Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. Retention time (chromatographic characteristic), fragmentation pattern and qualified ion ratios (mass spectrometric characteristics), and other characteristics are used as the basis for detection and identification. In most cases all of the criteria below should be met in order to identify the appropriate drugs within biological specimens.

11.1 Chromatography

All chromatographic peaks for the analytes of interest should show good chromatographic characteristics, with reasonable peak shape, width, and resolution. For low concentrations of an analyte (e.g., ≤5 ng/mL), there may be transitions that are not optimal. In order to be determined as acceptable, a chromatographic peak in a sample should compare favorably to the same analyte's chromatographic peak in a known sample which has been analyzed on the same system and in the same, or subsequent, analytical timeframe.

Retention Time (RT):
The retention time of a peak of interest should be within 0.1 minute of the retention time of a reference standard (i.e., calibrator or positive control).

11.2 Mass Spectrometry

Ion ratios should compare favorably to ion ratios of an extracted calibrator or positive control at a comparable concentration (e.g., positive control). Generally, ion ratios are within the limits as specified within the Section procedure related to mass spectral comparisons.

11.3 Batch Acceptance

In order for a batch to be acceptable:

11.3.1 No analytes of interest will be detected in the Negative Control.

11.3.2 Significant carry-over will be brought to the attention of the lead Examiner to determine if evidentiary samples have been negatively impacted. If so, re-analyses will occur and sample re-extraction may be necessary. Appropriate case documentation will accompany these instances within affected case files to record events.

11.3.3 With Unit Lead (or higher approval) quantitative values greater than the upper limit of quantitation can be reported as "greater than" and no uncertainty value will be associated with that result. Alternatively, in order for a more accurate quantity to be reported, dilution and re-injection can occur (no approval necessary).

11.3.4 The chromatographic area ratio of analyte(s) from the Positive Control Solution will be equal to or greater than those from the Positive Control Cut-Off Solution in order for the batch to be acceptable qualitatively.

Note: An analyte is considered applicable if it is important to the batch.
Analytes of interest are considered those compounds that are being reported.

11.3.5 All applicable analytes of interest within Positive Controls, as well as internal standards, will be identified.

11.3.6 Quantitative results of positive controls must fall within ±20% of the analyte's target concentration. If some drugs within the positive control solutions do not demonstrate acceptable quantitative results, then other drugs that do have acceptable quantitative results may be reported within samples within the batch.

11.3.7 Statistical data of positive controls should be recorded and evaluated within appropriate charts

11.4 Reporting Qualitative Results

11.4.1 A one-point calibration line is generated by plotting data from the chromatographic area ratio of the positive control cut-off solution versus concentration.

11.4.2 Force the one-point calibration graph through the origin. This can be done by using the force-through origin setting within software.

11.4.3 The chromatographic area ratio of analyte(s) from samples will be equal to or greater than those from the Positive Control Cut-Off Solution in order for the analyte(s) to be reported.

11.5 Reporting Quantitative Results

11.5.1 Calculations are performed by the applicable instrument software.

11.5.2 Calibration graphs and quantitative results are obtained by comparing chromatographic peak responses [or peak areas] to corresponding peak responses [areas] from internal standards. Calibration graphs are created and equations are obtained by plotting peak area ratios against corresponding calibrant concentrations for each analyte of interest. Based on resulting calibration equations concentrations of select analytes within evidentiary samples are obtained based on corresponding ratios of analyte peak areas to internal standard areas. The calibration line shall not be forced through the origin.

11.5.3 A coefficient of determination (R^2) should be ≥ 0.990 when using deuterated internal standards. If the coefficient of determination is lower than 0.990 then approval from the appropriate Lead Examiner (or higher) must occur and, if deemed acceptable, justification must accompany the applicable data. Calibrators shall not be removed from calibration graphs without approval from the Assistant Director (or higher). Such removal of calibration points will be appropriately documented within batches and within each case file (e.g., case notes, summary sheets). Calibration data are used for the analysis of each batch and should not be used with other batches without the approval of the Assistant Director (or higher).

11.5.4 The chromatographic area ratio of analyte(s) from samples will be equal to or greater than those from the Level 1 Calibrator Solution in order for the analyte(s) to be reported.

11.5.5 Samples resulting in analyte concentrations higher than the highest calibrator and needing a specific concentration to be reported should be diluted (the option to not dilute and report the analyte as ‘greater than’ are allowed, but approval must be obtained by the appropriate Unit Lead or higher ; this approval may be in the form of a Batch/Technical review approval).

11.5.6 The lower limit of quantitation (LLOQ) is the concentration of the lowest calibrator. The upper limit of quantitation (ULOQ) is the concentration of the highest calibrator. These values may be administratively set (meaning that the procedure may be able to quantitate beyond those values but going beyond such concentrations is not necessary for this procedure).

12. Limitations

Limits of Detection (LOD), Limit of Quantitation (LOQ), Upper Limit of Quantitation (ULOQ)

Cannabinoids	**LOD – **Urine (ng/mL)**	**LOD – Blood (ng/mL)**	**LLOQ – Blood (ng/mL)**	**ULOQ – Blood (ng/mL)**
THC	5*	2*	2*	100
THC-COOH	5*	2*	2*	100
THC-OH	5*	2*	2*	100

*Administratively set – values may actually be lower
**Unconjugated – analytes simply spiked in urine

The THC analytes within this procedure relate to the delta-9 form and are not related to delta-8-THC nor any other cannabinoid analog. Additionally, this procedure does not involve the analysis of THCA within specimens.

13. Safety

This procedure is carried out in a laboratory environment and standard safety procedures appropriate for such an environment should be utilized, including gloves, safety glasses, and protective clothing (e.g., lab coat). Biological specimens will be handled using universal precautions and will be treated as biohazardous. Potentially contaminated items and surfaces will be cleaned prior to use. When casework samples are being processed/analyzed brown paper (or other similar barrier) should be placed in between the surface and the specimens.

14. References

FBI Laboratory, Analysis of Cannabinoids from Biological Specimens by LC/MS/MS

D.M. Schwope, K.B. Scheidweiler, M.A. Huestis. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. Analytical Bioanalytical Chemistry. 401(4):1273-1283 (2011).

Monroe County MEO, 11-Nor-9-Carboxy-Δ^9-Tetrahydrocannabinol,11-Hydroxy Tetrahydrocannabinol and Δ^9-Tetrahydrocannabinol Quantitation In Blood.

SPEware Corporation, Extraction of Δ^9-Tetrahydrocannabinol (THC) and its Metabolites from Whole Blood for LC-MS Confirmations using Cerex® THC Extraction Columns, 3mL, 35mg

Appendix: Instrument Parameters (Shimadzu LCMS-01)

==== Shimadzu LabSolutions Method Report ====
THC LC_negative_acquistion_2022_2.lcm

<<System Controller>>
Model : CBM-20A
Power On : On
Event1 : Off
Event2 : Off
Event3 : Off
Event4 : Off
Sample Load Timing : Off
Sample Loading Overlap Time : 0.00 min

<<Data Acquisition>>
LC Stop Time : 4.50 min

<<Pump>>
Mode : Binary gradient
Pump A : LC-20AD
Pump B : LC-20AD
Total Flow : 0.4500 mL/min
B Conc. : 20.0 %
B Curve : 0
PressMax : 6000 psi
PressMin : 0 psi
Pump A Valve Model : FCV-11AL
Pump A Valve : B-B-A

<<Autosampler>>
Autosampler Model : SIL-20AC
Enable Autosampler : Use
Sample Rack : Rack 1.5mL 70 vials
Rinsing Volume : 500 uL
Needle Stroke : 52 mm
Control Vial Needle Stroke : 52 mm
Rinsing Speed : 35 uL/sec
Sampling Speed : 15 uL/sec
Purge Time : 25.0 min

Rinse Mode : Before/After
Rinse Dip Time : 0 sec
Cooler Temperature : 15 C

<<Oven>>
Oven Model : CTO-20A
Enable Oven : Use
Oven Temperature : 40 C
Maximum Temperature : 90 C
Valve 2/R : FCV-12AH
Valve 2/R Position : 0
Ready Check : On

<<LC Time Program>>

Time	Module	Command	Value	Comment
0.25	Pumps	B.Conc	20	
1.75	Pumps	B.Conc	90	
1.75	Column Oven	CTO.RVR	1	
2.40	Pumps	B.Conc	90	
2.41	Pumps	B.Conc	20	
3.25	Column Oven	CTO.RVR	0	
4.50	Controller Stop			

<<MS Parameter>>
CID Gas: Use the Data in the Tuning File
Conversion Dynode: Use the Data in the Tuning File
Initial Valve Position: -

--Segment 1 Event 1--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min
End Time: 3.250 min
Compound Name: THC-COOH-D3
Ch1 (Precursor m/z) 346.10 (Product m/z) 302.25 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 23.0 (CE) 20.0 (Q3 Pre Bias) 30.0
Ch2 (Precursor m/z) 346.10 (Product m/z) 233.35 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 17.0 (CE) 30.0 (Q3 Pre Bias) 24.0
Ch3 (Precursor m/z) 346.10 (Product m/z) 248.15 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 16.0 (CE) 29.0 (Q3 Pre Bias) 26.0
Event Time: 0.054 sec

Q1 Resolution: Unit
Q3 Resolution: Unit
Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File
Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data

--Segment 1 Event 2--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min
End Time: 3.250 min
Compound Name: THC-COOH
Ch1 (Precursor m/z) 343.00 (Product m/z) 299.30 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 23.0 (CE) 20.0 (Q3 Pre Bias) 30.0
Ch2 (Precursor m/z) 343.00 (Product m/z) 245.30 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 16.0 (CE) 29.0 (Q3 Pre Bias) 16.0
Ch3 (Precursor m/z) 343.00 (Product m/z) 191.05 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 26.0 (CE) 31.0 (Q3 Pre Bias) 20.0
Event Time: 0.054 sec
Q1 Resolution: Unit
Q3 Resolution: Unit
Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File
Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data

--Segment 1 Event 3--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min

End Time: 3.250 min
Compound Name: 11-OH-THC-D3
Ch1 (Precursor m/z) 332.15 (Product m/z) 314.30 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 16.0 (CE) 19.0 (Q3 Pre Bias) 15.0
Ch2 (Precursor m/z) 332.15 (Product m/z) 271.20 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 16.0 (CE) 30.0 (Q3 Pre Bias) 13.0
Ch3 (Precursor m/z) 332.15 (Product m/z) 173.20 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 16.0 (CE) 34.0 (Q3 Pre Bias) 19.0
Event Time: 0.054 sec
Q1 Resolution: Unit
Q3 Resolution: Unit
Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File
Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data

--Segment 1 Event 4--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min
End Time: 3.250 min
Compound Name: 11-OH-THC
Ch1 (Precursor m/z) 329.10 (Product m/z) 311.30 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 23.0 (CE) 19.0 (Q3 Pre Bias) 21.0
Ch2 (Precursor m/z) 329.10 (Product m/z) 173.15 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 15.0 (CE) 28.0 (Q3 Pre Bias) 18.0
Ch3 (Precursor m/z) 329.10 (Product m/z) 268.20 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 23.0 (CE) 26.0 (Q3 Pre Bias) 18.0
Event Time: 0.054 sec
Q1 Resolution: Unit
Q3 Resolution: Unit
Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File

Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data

--Segment 1 Event 5--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min
End Time: 3.250 min
Compound Name :THC
Ch1 (Precursor m/z) 313.10 (Product m/z) 245.15 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 22.0 (CE) 29.0 (Q3 Pre Bias) 25.0
Ch2 (Precursor m/z) 313.10 (Product m/z) 191.20 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 15.0 (CE) 30.0 (Q3 Pre Bias) 20.0
Ch3 (Precursor m/z) 313.10 (Product m/z) 203.20 (Pause Time) 3.0 (Dwell Time) 15.0 (Q1 Pre Bias) 13.0 (CE) 34.0 (Q3 Pre Bias) 19.0
Event Time: 0.054 sec
Q1 Resolution: Unit
Q3 Resolution: Unit
Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File
Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data

--Segment 1 Event 6--
Acquisition Mode: MRM
Polarity: Negative
Start Time: 1.750 min
End Time: 3.250 min
Compound Name: THC-D3
Ch1 (Precursor m/z) 316.10 (Product m/z) 248.30 (Pause Time) 2.0 (Dwell Time) 15.0 (Q1 Pre Bias) 22.0 (CE) 28.0 (Q3 Pre Bias) 25.0
Ch2 (Precursor m/z) 316.10 (Product m/z) 194.10 (Pause Time) 2.0 (Dwell Time) 15.0 (Q1 Pre Bias) 11.0 (CE) 29.0 (Q3 Pre Bias) 20.0
Event Time: 0.034 sec
Q1 Resolution: Unit
Q3 Resolution: Unit

Micro Scan Width: 0.00 u
Interface Volt.: Use the Data in the Tuning File
DUIS Corona Needle Volt.: Use the Data in the Tuning File
Pause Time / Dwell Time: Set Value of Each Channel (Pause Time & Dwell Time)
DL Bias: Use the Data in the Tuning File
Qarray Bias: Use the Data in the Tuning File
Qarray RF: Use the Data in the Tuning File
Q1 Prerod Bias: Set Data
Q3 Prerod Bias: Set Data
<<MS Program>>
<<Analog Table>>
<<Interface>>
Interface: DUIS - DUIS
Interface Temperature: 350 C
DL Temperature: 250 C
Nebulizing Gas Flow: 2.00 L/min
Heat Block: 500 C
Drying Gas: On
Drying Gas Flow: 15.00 L/min

Rev. #	History
1	New procedure for blood or other appropriate matrices.
2	General formatting updates throughout document. Sections 1, 2, 3 updated. Updated description of controls within Section 5. Positive Control Cut-Off Solution added within Section 5. Replaced 'Other Matrix' with 'non-Blood' throughout document. Removed 1 ng/mL Calibrator Solution – the lowest calibrator is 2 ng/mL in blood. Separated tables for qualitative (non-blood) and quantitative (blood) preparations. Added a note-statement regarding qualitative-only blood assays. Renamed the Quantitative section and added a Qualitative section. Removed option to use gravity during SPE process. Added requirement to properly label SPE columns. Added statement regarding clarity of reconstituted extract solutions and when to inject onto the instrument. Flow rate change within instrumental section. Added statement regarding checking mobile phase pH and obtaining mobile phase background spectra prior to start of instrumental sequences. Removed mass spectral comparison specifics from document and referred to Section-specific procedure. Revised the Decision Criteria section which included batch acceptance information. Changed retention time acceptability from percent to a fixed range. Updated criteria for qualitative and quantitative reporting of results. Updated the Limitations Section - changed the administrative limit of detection (LOD) and administrative limit of quantitation (LOQ) in blood for all cannabinoid analytes to 2 ng/mL. Added comment about delta-8-THC and THCA. Added an appendix for instrumental parameters that came from the actual instrument.