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1.0 Principle

1.1 Delta-9-tetrahydrocannabinol, or THC, is a lipophilic molecule that is introduced into the body through the use of the plant *Cannabis sativa* or cannabis products. THC is a psychoactive compound that acts as an agonist for the CB1 receptors in the central nervous system. Its primary metabolites, 11-hydroxy-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) are also detectable in blood following the use of cannabis products.

1.2 THC and its metabolites can be extracted from whole blood using a process of cell lysis, protein precipitation, and solid phase extraction. Due to the lipophilic and non-polar nature of the analytes of interest, a Hydrophilic-Lipophilic Balance (HLB) solid phase sorbent is used to preferentially extract THC and its metabolites from the biological matrix. Based on differential affinity for the organic solvents passed through the sorbent bed, other potentially interfering compounds such as phospholipids are largely removed from the resulting eluent.

1.3 The eluent is analyzed on the Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) system. Chromatographic separation between the analytes of interest using a C18 Bridged-Ethylene Hybrid (BEH) column. Each analyte has a different parent mass and will fragment in a unique and predictable pattern as its ions pass through the tandem mass spectrometer, allowing for confirmatory identification and quantitation.

2.0 Equipment

2.1 Pipettes

2.2 Class A volumetric glassware

2.3 Graduated cylinders

2.4 HPLC grade glass bottles

2.5 Glass culture tubes

2.6 Analytical balance

2.7 Vortex mixer

2.8 Centrifuge

2.9 Positive pressure manifold (PPM)

2.10 96-well Oasis PRiME HLB µElution plates

2.11 96-well collection plates and sealing mats

2.12 Waters Acquity H-Class UPLC / Xevo TQ-S micro MS system.

3.0 Solvents & Reagents

3.1 Materials

3.1.1 Zinc sulfate heptahydrate

3.1.2 Ammonium acetate

3.1.3 Formic acid (HPLC grade)

3.1.4 Acetonitrile (HPLC grade)

3.1.5 Methanol (HPLC grade)

3.1.6 Isopropanol (HPLC grade)

3.1.7 Deionized water

3.2 Zinc sulfate/ammonium acetate 0.1 M solution

3.2.1 Add 28.76 g zinc sulfate heptahydrate and 7.71 g ammonium acetate to 500 mL diH_2O.

3.2.2 Fill bottle to 1L.

3.2.3 Assigned lot number is ZA-MMDDYYYY.

3.3 Wash solvent – 25% methanol in water

3.3.1 Add 25 mL methanol to 75 mL diH_2O.

3.3.2 Assigned lot number is MH-MMDDYYYY.

3.4 Elution solvent – 15% isopropanol in acetonitrile

3.4.1 Add 15 mL isopropanol to 85 mL acetonitrile.

3.4.2 Assigned lot number is AI-MMDDYYYY.

3.5 Solvents and reagents do not require a performance check prior to use.

3.6 All solvents and reagents are stored at room temperature and expire one year from date of preparation unless otherwise noted.

3.7 Reagent preparation will be recorded in the Reagent Preparation Log and containers labeled with lot number, preparation date, expiration date, and preparer initials, unless otherwise noted.

3.8 Volumes specified in each preparation can be changed, provided the final concentration or ratio of components remains consistent.

4.0 Standards & Controls

4.1 Materials

4.1.1 Negative Control Stock (NEG) see TOX_P700

4.1.2 Methanol (HPLC grade)

4.1.3 Δ^9-THC standard

4.1.4 Δ^9-THC-D_3 standard

4.1.5 11-hydroxy-Δ^9-THC standard

4.1.6 11-hydroxy-Δ^9-THC-D_3 standard

4.1.7 11-nor-9-carboxy-Δ^9-THC standard

4.1.8 11-nor-9-carboxy-Δ^9-THC-D_3 standard

4.2 Internal standard preparation

4.2.1 Add 5 mL methanol to a 10 mL volumetric flask.

4.2.2 Pipette the following volumes of each standard:

Standard	Concentration	Volume Added	Final Concentration
Δ^9-THC-D_3	100 µg/mL	50 µL	500 ng/mL
11-hydroxy-Δ^9-THC-D_3	100 µg/mL	50 µL	500 ng/mL
11-nor-9-carboxy-Δ^9-THC-D_3	1 mg/mL	50 µL	5 µg/mL

4.2.3 Fill flask to volume with methanol.

4.2.4 Assigned lot number is THC-IS-MMDDYYYY.

4.2.5 Prior to being placed in service, solutions must be performance checked.

4.2.5.1 Prepare one vial by adding 25 µL of currently in-service internal standard solution to 475 µL diH_2O and vortex to mix.

4.2.5.2 Prepare a second vial as above, using the newly prepared lot number of internal standard solution.

4.2.5.3 Analyze these vials on the UPLC system using the currently validated analytical method.

4.2.5.4 Retention times should be consistent between the currently in-service lot number and the newly prepared lot number.

4.2.5.5 The monitored transitions for each analyte of interest should exhibit no measurable interference from the deuterated internal standard compounds.

4.2.5.6 Peak area counts for each compound should match between the currently in-service lot number and the newly prepared lot number to within ± 30%.

4.3 Calibrator working stock preparation

4.3.1 Add 5 mL methanol to a 10 mL class A volumetric flask.

4.3.2 Pipette the following volumes of each standard:

Standard	Concentration*	Volume Added	Final Concentration
Δ^9-THC	100 µg/mL	100 µL	1 µg/mL
11-hydroxy-Δ^9-THC	100 µg/mL	100 µL	1 µg/mL
11-nor-9-carboxy-Δ^9-THC	1 mg/mL	100 µL	10 µg/mL

*Parent stocks may require dilution to achieve this starting concentration

4.3.3 Fill flask to volume with methanol.

4.3.4 Assigned lot number is THC-CAL-MMDDYYYY.

4.3.5 Prior to being placed in service, solutions must be performance checked.

4.3.5.1 In triplicate, prepare samples at the highest calibrator concentration, as specified in section 5.1, using the newly prepared calibrator working stock.

4.3.5.2 Extract these samples and analyze them against a valid calibration curve prepared using the currently in-service calibrator working stock.

4.3.5.3 Each analyte should fall within ± 20% of the target value at each concentration.

4.3.5.4 Retention times should be consistent between the currently in-service lot number and the newly prepared lot number.

4.3.5.5 Qualifier ion ratios for the newly prepared lot number should fall within ± 20% of the currently in-service lot number average ion ratio.

4.4 Quality control working stock preparation

4.4.1 Add 5 mL methanol to a 10 mL volumetric flask.

4.4.2 Pipette the following volumes of each standard:

Standard	Concentration*	Volume Added	Final Concentration
Δ^9-THC	100 µg/mL	100 µL	1 µg/mL
11-hydroxy-Δ^9-THC	100 µg/mL	100 µL	1 µg/mL
11-nor-9-carboxy-Δ^9-THC	1 mg/mL	100 µL	10 µg/mL

*****Parent stocks may require dilution to achieve this starting concentration

4.4.3 Fill flask to volume with methanol.

4.4.4 Assigned lot number is THC-QC-MMDDYYYY.

4.4.5 Prior to being placed in service, solutions must be performance checked.

4.4.5.1 In triplicate, prepare samples at the high-range quality control concentration, as specified in section 5.2, using the newly prepared quality control working stock.

4.4.5.2 Extract these samples and analyze them against a valid calibration curve prepared using the currently in-service calibrator working stock.

4.4.5.3 Each analyte should fall within ± 20% of the target value at each concentration.

4.4.5.4 Retention times for each analyte should be consistent with the calibration standards.

4.4.5.5 Qualifier ion ratios for the newly prepared lot number should fall within ± 20% of the calibration average ion ratio.

4.5 Reagent preparation will be recorded in the Reagent Preparation Log and containers labeled with lot number, preparation date, expiration date, and preparer initials, unless otherwise noted.

4.6 All working stocks are stored in the freezer and expire one year from date of preparation.

4.7 Chromatograms from the analysis will be reviewed and documentation of passing QC recorded in the Reagent Preparation Log. Analytical results will be kept on file with the Toxicology Section.

4.8 Volumes specified in each preparation can be changed, provided the final concentration or ratio of analytes and components remains consistent.

4.9 Concentrations of purchased reference materials may vary depending on availability from the manufacturer. Alternative ratios or volumes may be used in

reagent preparation, provided the final concentration of analytes remains consistent.

4.10 If the currently in-service lot number is not adequate for comparison purposes (e.g. insufficient volume, expired, failing acceptability criteria), reagent performance can be based on the general acceptability criteria for the analytical method.

5.0 Procedure

5.1 Prepare calibration samples

5.1.1 Serially dilute calibration working stock material in methanol to each calibration concentration.

5.1.1.1 For Δ^9-THC and 11-hydroxy-Δ^9-THC, calibrators will be prepared at 0.50, 1.0, 2.5, 5.0, 25, and 50 ng/mL.

5.1.1.2 For 11-nor-9-carboxy-Δ^9-THC, calibrators will be prepared at 5.0, 10, 25, 50, 250, and 500 ng/mL.

5.1.1.3 Refer to THC sample preparation sheet for suggested volumes and concentrations to use in serial dilution.

5.1.2 Aliquot 450 µL NEG into clean glass culture tubes and label one for each concentration.

5.1.3 Add 25 µL of the appropriate calibrator concentration to each tube.

5.1.4 Add 25 µL of internal standard to each tube.

5.1.5 Vortex to mix.

5.2 Prepare quality control samples

5.2.1 Dilute quality control working stock material in methanol to each of the required quality control concentrations.

5.2.1.1 For Δ^9-THC and 11-hydroxy-Δ^9-THC, QC samples will be prepared at low (1.5 ng/mL), mid (25 ng/mL), and high (40 ng/mL) concentrations.

5.2.1.2 For 11-nor-9-carboxy-Δ^9-THC, QC samples will be prepared at low (15 ng/mL), mid (250 ng/mL), and high (400 ng/mL) concentrations.

5.2.1.3 Refer to THC sample preparation sheet for suggested volumes and concentrations to use in serial dilution.

5.2.2 Aliquot 450 µL NEG into clean, labeled glass culture tubes. The number of tubes at each concentration will depend upon the QC requirements of the analytical batch.

5.2.3 Add 25 µL of the appropriate QC dilution to each tube.

5.2.4 Add 25 µL of internal standard to each tube.

5.2.5 Vortex to mix.

5.3 Prepare negative control samples

5.3.1 Aliquot 450 µL NEG into clean, labeled glass culture tubes.

5.3.2 Add 25 µL of methanol to each tube.

5.3.3 Add 25 µL of internal standard to each tube.

5.3.4 Vortex to mix.

5.4 Prepare casework samples

5.4.1 Select one item from each evidential blood kit for analysis.

5.4.2 Thoroughly vortex each tube to ensure homogeneity of the sample.

5.4.3 Pipette 450 µL of blood into a clean, labeled glass culture tube.

5.4.4 Add 25 µL of methanol to each tube.

5.4.5 Add 25 µL of internal standard to each tube.

5.4.6 Vortex to mix.

5.5 Add 50 µL 0.1 M zinc sulfate/ammonium acetate solution to clean, labeled glass culture tubes.

5.6 Pipette 100 µL of prepared samples into each zinc sulfate/ammonium acetate tube; vortex to mix.

5.7 Add 400 µL ice cold mobile phase B; vortex to mix.

5.8 Centrifuge samples at 3030 rcf for 10 min.

5.9 Transfer supernatant into clean, labeled glass culture tubes containing 1 mL diH_2O.

5.10 Transfer samples into µElution plate wells in two aliquots of ~750 µL; apply each at 1-2 mL/min using PPM.

5.10.1 Ensure each sample position is documented on THC sample preparation sheet.

5.11 Wash twice with 250 µL of wash solvent.

5.12 Add 50 µL diH_2O to each sample well in 96-well collection plate.

5.13 Elute into collection plate wells using two aliquots of 25 µL 15% isopropanol in acetonitrile.

5.14 Cap collection plate with pre-slit sealing mat.

5.15 Gently vortex plate before loading onto instrument.

6.0 Instrumental Analysis

6.1 Complete all required maintenance procedures as outlined in the Toxicology Confirmation Manual (TOX_P700) and document in the Instrument Maintenance Log.

6.2 Ensure the "THC & Metabolites" inlet method and tune files are loaded and active.

6.3 Turn on gas flows, source electronics, and mobile phase pumps; allow all metrics to stabilize before running samples.

6.4 Place the collection plate in the autosampler.

6.5 In MassLynx, generate a sequence list for the analytical batch.

6.5.1 Inlet method, tune file, and MRM method columns should all be set to "THC & Metabolites".

6.5.2 All calibration and control standards should be set to "standard" and "QC" sample types, respectively, and include concentration levels in the appropriate columns.

6.5.3 All calibration standards should include a "1" in the quantitative reference column.

6.6 Save sequence list and begin sample acquisition.

6.7 After analysis, sample plates or vials are kept in either the autosampler set to 4°C or in the refrigerator if reinjection is required.

6.7.1 Plates must be covered with a sealing mat or parafilm to prevent sample evaporation.

6.7.2 Calibrators may be reinjected up to 72 hours after preparation.

6.7.3 Casework samples and controls may be reinjected up to 48 hours after preparation.

7.0 Data

7.1 Upon completion of the run, process all acquired samples in TargetLynx using the "THC and Metabolites" method or appropriate submethod for reanalysis.

7.2 Ensure all samples meet the quality criteria outlined in section 7.0 of the Toxicology Confirmation Manual (TOX_P700).

7.3 Generate a data packet and perform analyst review as outlined in section 9.0 of the Toxicology Confirmation Manual (TOX_P700).

8.0 References

8.1 Toxicology Screening Manual (TOX_P600)

8.2 Toxicology Confirmation Manual (TOX_P700)

8.3 Reagent Preparation Log

8.4 Instrument Maintenance Log

8.5 THC Control Chart

DOCUMENT HISTORY			
DATE	**VERSION**	**APPROVED BY**	**ACTIVITY OR REVISION**
2/11/2019	1	Lab Director	First edition
11/9/2020	2	Lab Director	Quality assurance and data interpretation practices improved; general edits for clarity
6/14/2021	3	Lab Director	Minor formatting changes throughout