4.3.4 Quantitative Confirmation for Marihuana Metabolite in Blood

4.3.4 Quantitative Confirmation for Marihuana Metabolite in Blood

4.3.4.1 Analytes

11-nor-9-carboxy-9-tetrahydrocannabinol (THC metabolite) and 9-tetrahydrocannabinol (parent THC)

4.3.4.2 General Description of Method

An internal standard GC/MS identification and optional quantitation of derivatized THC and the THC-COOH metabolite using pentafluoropropionic anhydride (PFPA) and hexafluoro-isopropanol (HFIP). Extraction of the THC parent and metabolite from the blood matrix is accomplished by using a solid phase extraction (SPE) method that has been adapted from a United Chemical Technologies (UCT) method, formerly Worldwide Monitoring Corp.

4.3.4.3 Equipment and Reagents

• GC/MS equipped with a suitable column for separating THC compounds from other drugs and coextractives (i.e. 15 meter DB5 capillary column).
• UCT standard SPE vacuum tank, manifold, vacuum source, and reagents as specified in the UCT procedure manual code TCU200THCZ050191.
• UCT SPE Columns intended for THC extraction, such as Clean Screen THC or Styre Screen columns, Phenomenex Strata columns or equivalent.
• Internal standard: 11-nor-9-carboxy-9 tetrahydrocannabinol-D9, 10 ng/ul THC and 11-COOH-THC standards for controls and calibrators (see below).
• The usual assortment of laboratory glassware, reaction vessels, pipettes, reagent grade chemicals, vortexers and shakers.
• Derivatizing reagents: pentafluoropropionic anhydride, Aldrich 25,238-7 (or equivalent), 1,1,1,3,3,3-hexafluoro-2-propanol, Aldrich 32,524-4 (or equivalent)

4.3.4.4 Details: UCT Clean Screen or Styre Screen Columns

A homogeneous blood sample is assured by gently rocking the specimen on the Labquake Shaker for at least 5 minutes. If the specimen is clotted, homogenizing glassware can be used to obtain a liquid sample. All sample handling will be performed in the biological safety cabinet using the universal biohazard handling techniques

4.3.4.4.1 Sample Preparation

• Prepare a standard curve of 1-200 ng/ml THC and 11-COOH-THC from 2 mls blank blood and 20 ul of the appropriate standard:

*Std	Analyte	Concentration	Final Concentration*
1	THC-COOH	0.5 ug/ml	5 ng/ml
	THC	0.1 ug/ml	1 ngml
2	THC-COOH	2.5 ug/ml	25 ngml
	THC	1 ug/ml	10 ng/ml
3	THC-COOH	10 ug/ml	100 ng/ml
	THC	2.5 ug/ml	25 ng/ml
4	THC-COOH	20 ug/ml	200 ng/ml
	THC	10 ug/ml	100 ngml

• Prepare a negative control using 2 mls of blank blood.

• Prepare two positive controls using 2 ml blank blood and 20 ul each of the appropriate standard:

* Std	Analyte	Concentration	Final Concentration*
Low	THC-COOH	1 ug/ml	10 ng/ml
	THC	0.5 ug/ml	5 ng/ml
High	THC-COOH	5 ug/ml	50 ng/ml

THC	2 ug/ml	20 ng/ml

- Pipette 2 mls sample blood (less is allowable if 2 mls is not available) into a clean test tube.

- To each unknown, standard and control add 200 ng (20 ul) 11-nor-9-carboxy-9-tetrahydrocannabinol-D9 as the internal standard (MW = 353).

- Add 200 µl methanol to each unknown, standard and control. Vortex briefly.

- Add dropwise 2 mls cold acetonitrile while vortexing sample at moderate speed. Continue vortexing at least 60 seconds.

- Centrifuge for 10 minutes at 3500 rpm. Decant supernatant into 5 ml test tube, discard pellet.

Dry under nitrogen at 35 °C until volume is 100 - 200 µl (~ 30 minutes).

- Add 3 ml dH_2O and vortex. Sample will be cloudy and may contain particulate matter.

4.3.4.4.2 Condition Clean Screen Extraction Column

Note: Styre Screen Columns do not require conditioning.

Pass the following reagents through all Clean Screen columns in the run:

- 3 ml anhydrous methyl alcohol (CH_3OH), aspirate.
- 3 ml DI water, aspirate.
- 1 ml 100 mM hydrochloric acid (HCl), aspirate.

NOTE: Aspirate at 3 inches Hg to prevent sorbent drying.

4.3.4.4.3 Apply Sample

- Carefully decant the sample mixture into its respective extraction column.

- Allow the sample to saturate the sorbent, and then draw sample through the column at 1 - 2 ml/minute. This may be accomplished by using the pump with the valve open (no vacuum).

4.3.4.4.4 Wash Column

- 1 ml DI water, aspirate.
- 1 ml water/acetonitrile/NH_4OH (89/10/1); make daily; aspirate.
- DRY COLUMN 10 INCHES Hg FOR 20 - 30 MIN

4.3. 4.4.5 Elute Cannabinoids

2 ml of acetonitrile/ glacial acetic acid (98/2). Use gravity flow or minimal vacuum (5 in Hg).

4.3. 4.4.6. Evaporate Eluates

Evaporate to 1 ml 40 °C Transfer to autosampler vial and continue evaporating to dryness.

4.3. 4.4.7 Derivatize

To each vial add:

- 5 µl ethyl acetate
- 25 ul hexafluoroisopropanol (HFIP). Each molecule of HFIP adds 150 amu to the derivatizable analyte.
- 50 ul perfluoropropionic anhydride (PFPA). Each molecule of PFP adds 146 amu to the derivatizable analyte.
- Mix / vortex.
- Incubate 45 min at 60-70 °C.

4.3.4.4.8 Evaporate Derivatives

- Evaporate the solvent in each vial just to dryness (40 °C), until the pungent smell of the anhydride is absent.
- Add 30 ul ethyl acetate (EtOAc). Mix / . vortex. Run on GC/MS.

4.3.4.4.9 Data Interpretation

4.3.4.4.9.1 A standard curve should be generated with r2 0.95 for each analyte. If the r2 value is not within this range for 11-COOH-THC, the analytical run shall be repeated. If the r2 value is within this range for 11-COOH-THC but not for THC, the supervisor shall determine how the data shall be interpreted. If some of the samples in the run cannot be repeated (e.g. insufficient volume remaining), the supervisor shall determine how the existing data shall be interpreted.

4.3.4.4.9.2 Control values should be returned which are within 20% of the target value. If they are not within this range, discuss with supervisor. A determination will be made whether the cause for the variability lies with that particular sample or with the entire analytical run. If it is the latter, the run will be repeated.

4.3.4.4.9.3 The LOD for both THC and 11-COOH-THC has been determined to be approximately 0.5 ng/ml in Full Scan mode, 0.1 ng/ml in SIM mode. The LOQ for both compounds has been designated to be 2 ng/ml in Full Scan mode, 1 ng/ml in SIM mode.

4.3.4.4.9.4 Quantitative values generated for THC and 11-COOH-THC will be reported with the following conditions:

• THC and 11-COOH-THC will not be reported if they are present below the LOQ.
• The analyst has the final determination as to whether the spectrum selected by the computer is valid. If the analyst feels that the spectrum is invalid or spurious, s/he may refrain from reporting the compound as present, regardless of the quantitation returned.
• Ion ratios should be comparable to those of the controls, allowing for concentration variances (see www.soft-tox.org, laboratory guidelines). If the analyst feels that the spectrum is valid, but the quantitation is inaccurate due to interfering peaks, s/he should see the supervisor. The data may be re-processed or calculated manually on a case-by-case basis. The analyst may reprint a spectrum for the sake of clarity if desired. This will not affect the validity of the data processing.
• If the initial sample volume is less than 2 mls, a volume correction should be made on the data sheet, and the corrected value reported.

• No decimal places shall be reported.

4.3.4.5 Alternative Method Using Strata XC or XL-C SPE Columns

4.3.4.5.1 Sample Preparation

• Prepare a standard curve of 1-200 ng/ml THC and 11-COOH-THC from 2 mls blank blood and 20 ul of the appropriate standard:

*Std	Analyte	Concentration	Final Concentration*
1	THC-COOH	0.5 ug/ml	5 ng/ml
	THC	0.1 ug/ml	1 ngml
2	THC-COOH	2.5 ug/ml	25 ngml
	THC	1 ug/ml	10 ng/ml
3	THC-COOH	10 ug/ml	100 ng/ml
	THC	2.5 ug/ml	25 ng/ml
4	THC-COOH	20 ug/ml	200 ng/ml
	THC	10 ug/ml	100 ngml

• Prepare a negative control using 2 mls of blank blood.

• Prepare two positive controls using 2 ml blank blood and 20 ul each of the appropriate standard:

* Std	Analyte	Concentration	Final Concentration*
Low	THC-COOH	1 ug/ml	10 ng/ml
	THC	0.5 ug/ml	5 ng/ml
High	THC-COOH	5 ug/ml	50 ng/ml
	THC	2 ug/ml	20 ng/ml

• Pipette 2 mls sample blood (less is allowable if 2 mls is not available) into a clean test tube.

• To each unknown, standard and control add 200 ng (20 ul) 11-nor-9-carboxy-delta 9-tetrahydrocannabinol-D9 as the internal standard (MW = 353).

• Add 200 µl methanol to each unknown, standard and control. Vortex briefly.

• Add dropwise 2 mls cold acetonitrile while vortexing sample at moderate speed. Continue vortexing at least 60 seconds.

• Centrifuge for 10 minutes at 3500 rpm. Decant supernatant into 5 ml test tube, discard pellet.

Dry under nitrogen at 35 °C until volume is 100 - 200 µl (~ 30 minutes).

• Add 2.5 ml of 100x diluted glacial acetic acid and vortex. pH should be 3.4 - 4.0.

4.3.4.5.2 Condition Strata Extraction Columns

Pass the following reagents through all columns in the run:

- 2 ml anhydrous methyl alcohol (CH_3OH), aspirate.
- 2 ml 100 mM hydrochloric acid (HCl), aspirate.

NOTE: Aspirate at 5 inches Hg to prevent sorbent drying.

4.3.4.5.3 Apply Sample

- Carefully decant the sample mixture into its respective extraction column.

- Allow the sample to saturate the sorbent, and then draw sample through the column at 1 - 2 ml/minute. This may be accomplished by using the pump with the valve open (no vacuum).

4.3.4.5.4 Wash Column

- 2 ml 100 mM HCl, aspirate.
- 2 ml 100 mM HCl/acetonitrile (70:30); aspirate.
- DRY COLUMN 10 INCHES Hg FOR 5 MIN
- Add 200 µl glacial acetic acid, dry a further 2 minutes

4.3. 4.5.5 Elute Cannabinoids

2 ml of acetonitrile/ glacial acetic acid (98/2). Use gravity flow or minimal vacuum (5 in Hg).

4.3. 4.5.6. Evaporate Eluates

Evaporate to 1 ml < 40 deg C Transfer to autosampler vial and continue evaporating to dryness.

4.3. 4.5.7 Derivatize

To each vial add:

- 5 µl ethyl acetate
- 25 ul hexafluoroisopropanol (HFIP). Each molecule of HFIP adds 150 amu to the derivatizable analyte.
- 50 ul perfluoropropionic anhydride (PFPA). Each molecule of PFP adds 146 amu to the derivatizable analyte.
- Mix / vortex.
- Incubate 45 min at 60-70 °C.

4.3.4.5.8 Evaporate Derivatives

- Evaporate the solvent in each vial just to dryness (40 °C), until the pungent smell of the anhydride is absent.
- Add 30 ul ethyl acetate (EtOAc). Mix / . vortex. Run on GC/MS.

4.3.4.5.9 Data Interpretation

4.3.4.5.9.1 A standard curve should be generated with r2 0.95 for each analyte. If the r2 value is not within this range for 11-COOH-THC, the analytical run shall be repeated. If the r2 value is within this range for 11-COOH-THC but not for THC, the supervisor shall determine how the data shall be interpreted. If some of the samples in the run cannot be repeated (e.g. insufficient volume remaining), the supervisor shall determine how the existing data shall be interpreted.

4.3.4.5.9.2 Control values should be returned which are within 20% of the target value. If they are not within this range, discuss with supervisor. A determination will be made whether the cause for the variability lies with that particular sample or with the entire analytical run. If it is the latter, the run will be repeated.

4.3.4.5.9.3 The LOD for both THC and 11-COOH-THC has been determined to be approximately 0.5 ng/ml in Full Scan mode, 0.1 ng/ml in SIM mode. The LOQ for both compounds has been designated to be 2 ng/ml in Full Scan mode, 1 ng/ml in SIM mode.

4.3.4.5.9.4 Quantitative values generated for THC and 11-COOH-THC will be reported with the following conditions:

- THC and 11-COOH-THC will not be reported if they are present below the LOQ.
- The analyst has the final determination as to whether the spectrum selected by the computer is valid. If the analyst feels that the spectrum is invalid or spurious, s/he may refrain from reporting the compound as present, regardless of the quantitation returned.
- Ion ratios should be comparable to those of the controls, allowing for concentration variances (see www.soft-tox.org, laboratory guidelines). If the analyst feels that the spectrum is valid, but the quantitation is inaccurate due to interfering peaks, s/he should see the supervisor. The data may be re-processed or calculated manually on a case-by-case basis. The analyst may reprint a spectrum for the sake of clarity if desired. This will not affect the validity of the data processing.
- If the initial sample volume is less than 2 mls, a volume correction should be made on the data sheet, and the corrected value reported.

- No decimal places shall be reported.