Section Three
Blood Toxicology

3.10 SPE Methods for GC/MSD Confirmation

3.10.1 Extraction of 11-nor-Δ^9-THC-9-COOH (Carboxy-THC) from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® THC Column

3.10.1.1 BACKGROUND

Δ^9-THC (Figure 1) is the chief psychoactive cannabinoid resulting from exposure to marijuana. Δ^9-THC has a peak blood concentration within 5 to 15 minutes following smoking of a marijuana cigarette.[3,4,5] This blood concentration drops rapidly after cessation of smoking.[3,4] The level may fall to less than 5µL within 30 to 60 minutes although longer detection times have been reported.[3,4] Detection of low dose (1.75%) post smoking Δ^9-THC has been reported to vary from 3 to 12 hours.[4] This detection window was based on a limit of quantitation of 0.5ng/mL. The number, duration, and spacing of puffs, hold time, and inhalation volume all impact the degree of drug exposure and thus bioavailability.[4] Longer detection times have been observed for frequent users. The Δ^9-THC metabolite, 11-nor-Δ^9-THC-9-COOH (Carboxy-THC), concentration gradually increases and may plateau for several hours.[4] There is poor correlation between blood Δ^9-THC and psychoactive affects since the Δ^9-THC concentrations begin to decline prior to the time of peak effects.[3,4,5] Work continues on models using the relative amounts of Δ^9-THC and Carboxy-THC to assist with establishing recent drug use.

Negative behavioral effects reported from exposure to marijuana include altered time perception, lack of concentration, impaired learning and memory which can lead to impairment of cognitive and performance tasks.[4] Establishing impairment in an individual is based on evaluation of all available information in conjunction with the quantitative blood levels.

For additional background refer to analytical method 2.4.4 and provided references.

Figure 1.

3.10.1.2 PRINCIPLE

This procedure outlines the use of the 200mg United Chemical Technologies (UCT) CLEAN SCREEN® THC Column for the extraction of

the Cannabinoid, Carboxy-THC, from blood. The CLEAN SCREEN® THC column utilizes a copolymeric sorbent which combines a cationic exchanger and a hydrophobic functionality (reverse phase) to interact effectively, physically and chemically, with analytes of interest while interacting minimally with interfering substances in the blood sample. The cation exchanger utilizes an anionic sorbent to bind to cations. Additional retention mechanisms include hydrophobic interactions and polar adsorption.

For the extraction of Carboxy-THC, a deuterated internal standard is added to the sample. Blood proteins are precipitated with 10% Methanol-Acetonitrile solvent mix and are removed via centrifugation. The supernatant is transferred to a clean tube where the volume is reduced by one-half. The sample pH is then adjusted with an Acetate buffer (pH 4.5) and loaded onto a pre-conditioned SPE column. The conditioning of the SPE column creates an environment which allows for optimal interaction between the sorbent and the analyte of interest. The column is subsequently washed to selectively remove matrix components and interfering substances from the column. Next, the column is dried to remove traces of aqueous and organic solvents. After drying, the analyte of interest is eluted from the SPE column with an organic solvent mixture. Following elution and evaporation of the solvent, the extract is derivatized for compound confirmation using a gas chromatograph equipped with a mass selective detector (GC-MSD).

3.10.1.3 EQUIPMENT AND SUPPLIES

3.10.1.3.1 200mg CLEAN SCREEN® THC Extraction Column
3.10.1.3.2 Disposable inserts for SPE manifold ports.
3.10.1.3.3 Drybath or laboratory oven capable of 70°C
3.10.1.3.4 Evaporative concentrator equipped with nitrogen tank.
3.10.1.3.5 Tube rocker
3.10.1.3.6 Vortex mixer
3.10.1.3.7 Laboratory centrifuge capable of 3400- 3500rpm
3.10.1.3.8 Vacuum Manifold/ Vacuum pump
3.10.1.3.9 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.10.1.3.10 16 x 100mm glass tubes (silanized recommended)
3.10.1.3.11 Screw Cap for 16mm O.D. tubes
3.10.1.3.12 GC/MS Automated Liquid Sample (ALS) vials
3.10.1.3.13 Silanized GC/MS Vial Microinsert
3.10.1.3.14 Gas Chromatograph equipped with a quadruple mass selective detector and a nonpolar capillary column with a phase composition comparable to 100%-dimethylpolysiloxane or 95%-dimethylpolysiloxane with 5%-diphenyl.

3.10.1.4 REAGENTS

Refer to manual section 5.12 for solution preparation instructions.

3.10.1.4.1 Deionized/distilled (DI) water
3.10.1.4.2 Methanol (Certified ACS Grade)
3.10.1.4.3 Hexane (Certified ACS Grade)
3.10.1.4.4 Ethyl Acetate (Certified ACS Grade)
3.10.1.4.5 Acetonitrile (Certified ACS Grade)
3.10.1.4.6 10% Methanol in Acetonitrile
3.10.1.4.7 100mM Acetate Buffer (pH 4.5)
3.10.1.4.8 100mM HCl
3.10.1.4.9 70:30 Hexane:Ethyl Acetate
3.10.1.4.10 70:30 100mM HCl:Acetonitrile
3.10.1.4.11 BSTFA + 1% TMCS

3.10.1.5 QUALITY ASSURANCE MATERIAL

3.10.1.5.1 Calibrator and Control Solutions

Corresponding calibrator and control reference material must be obtained from different vendors, or be from different lot numbers if suitable second vendors are not available.

3.10.1.5.1.1 **Reference Material Stock Solutions**

Concentration: 100µg/mL or 1mg /mL
Carboxy-THC

Store remaining stock solution as recommended by vendor.

3.10.1.5.1.2 **Reference Material Working Solutions**

Working solutions are stable for 6 months when stored under refrigeration.

Concentration: 1.0ng/µL

As appropriate, add 100µL of 100µg/mL or 10µL of 1mg/mL Stock Solution to approximately 9mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

Concentration: 0.1ng/µL

Add 1000µL of 1ng/µL working calibration solution to approximately 8mL Methanol in 10mL volumetric class A flask. QS to 10mL.

3.10.1.5.2 Internal Standard Solutions

3.10.1.5.2.1 **Stock Solution**

Concentration: 100µg/mL or 1mg /mL Carboxy-THC-D_9

Store remaining stock solution as recommended by vendor.

3.10.1.5.2.2 **Working Internal Standard Solution**

Working internal standard solution is stable for 6 months when stored under refrigeration.

Concentration: 1.0ng/µL

Add 100µL of 100µg/mL or 10µL of 1mg /mL stock solution to approximately 9mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

3.10.1.5.3 Whole Blood Negative Control

Negative Whole Blood

3.10.1.6 PROCEDURE

3.10.1.6.1 Initial set-up

Label extraction tubes, 200mg CLEAN SCREEN® extraction columns, and GC/MSD vials with microinserts for calibrators, controls and case samples.

3.10.1.6.2 Calibrator Preparation

Use the same lot of negative blood used to prepare the negative control to prepare calibrators.

3.10.1.6.2.1 Add 1mL of negative whole blood to six extraction tubes.

3.10.1.6.2.2 Add the volume of 0.1ng/µL Carboxy-THC calibrator working solution as indicated in the following table.

Level	Desired ng/mL	µL Working RM
1	2.5	25
2	5	50
3	10	100

3.10.1.6.2.3 Add the volume of 1.0ng/µL Carboxy-THC calibrator working solution as indicated in the following table.

Level	Desired ng/mL	µL Working Reference Material
4	25	25
5	50	50
6	100	100

3.10.1.6.3 Positive Control Sample Preparation

Use the same lot of negative blood used to prepare the negative control for positive control preparation.

3.10.1.6.3.1 Add 1mL of negative whole blood to two extraction tubes.

3.10.1.6.3.2 Add indicated amount of 0.1ng/µL working control solution.

Desired ng/mL	µL Working Control
6.0	60.0

3.10.1.6.3.3 Add indicated amount of 1.0ng/µL working control solution.

Desired ng/mL	µL Working Control
60.0	60.0

3.10.1.6.4 Negative Control Sample Preparation

Add 1mL of negative whole blood into an extraction tube.

3.10.1.6.5 Case Sample Preparation

Place sample container on tube rocker for a minimum of five minutes. If sample is clotted, homogenize as necessary. Transfer 1mL of blood into a labeled extraction tube.

3.10.1.6.6 Internal Standard Addition

3.10.1.6.6.1 To calibrators, controls and case samples, add 25µL of internal standard.

3.10.1.6.6.2 Vortex tube briefly and allow to stand 15 to 30 minutes for sample equilibration.

3.10.1.6.7 Protein Precipitation

3.10.1.6.7.1 While vortexing, add 2mL 10% methanol in acetonitrile dropwise to case, calibrator and control samples.

3.10.1.6.7.2 Cap tube and continue vortexing tube for approximately 30 seconds.

3.10.1.6.7.3 Allow tube to stand for five minutes

3.10.1.6.7.4 Centrifuge at approximately 3500 rpm for10 minutes.

3.10.1.6.7.5 Decant organic supernatant into second labeled glass tube.

3.10.1.6.7.6 Transfer tube to Evaporative Concentrator and evaporate under nitrogen at ≤40°C to approximately 1mL. ***Do not allow extract to go to dryness.***

3.10.1.6.7.7 To the evaporated extract add 2mL 100mM acetate buffer (pH 4.5). Vortex briefly to mix.

3.10.1.6.7.8 If necessary, centrifuge buffered solution for an additional 5 minutes at approximately 3500 rpm to remove blood fragments or foam.

3.10.1.6.8 SPE Column Preparation

3.10.1.6.8.1 Insert labeled 200mg CLEAN SCREEN® THC extraction column into appropriate location on vacuum manifold.

3.10.1.6.8.2 To each SPE column, add 3mL 70:30 **Hexane:Ethyl acetate**. Aspirate at ≤ 3 in. Hg to prevent sorbent drying.

3.10.1.6.8.3 To each SPE column, add 3mL **methanol** to the column. Aspirate at ≤ 3 in. Hg.

3.10.1.6.8.4 To each SPE column, add 3mL **deionized water** to the column. Aspirate at ≤ 3 in. Hg.

3.10.1.6.8.5 To each SPE column, add 1mL **100mM HCl** and aspirate at ≤ 3 in. Hg.

3.10.1.6.9 Blood Extract Loading
Decant buffered blood extract onto the SPE column and allow to gravity flow or apply minimal vacuum.

3.10.1.6.10 Column Wash

3.10.1.6.10.1 To each SPE column, add 2mL of **deionized water**. Aspirate at ≤ 3 in. Hg.

3.10.1.6.10.2 To each SPE column, add 2mL 70:30 100mM **HCl:Acetonitrile**. Aspirate at ≤ 3 in. Hg.

3.10.1.6.11 Dry Disc
Increase vacuum to ≥10 in. Hg (≥34 kPa) for ≥ 5 minutes.

3.10.1.6.12 Compound Elution

3.10.1.6.12.1 Open vacuum manifold, wipe collection tips, and insert the collection rack containing the labeled glass tubes.

3.10.1.6.12.2 To each SPE column, add 200uL **hexane** (important for elution solvent reception).
Gravity flow only. Do not allow column to dry.

3.10.1.6.12.3 To each SPE column, add 3mL 70:30 **Hexane:Ethyl Acetate** elution solvent. *Collect eluate with gravity flow or apply minimal vacuum.*

3.10.1.6.13 Eluate Evaporation
Transfer centrifuge tube to Evaporative Concentrator. Evaporate eluates to dryness, under a gentle stream of nitrogen at ≤ 40°C.

3.10.1.6.14 Derivatization

3.10.1.6.14.1 In fume hood, add 40µL each ethyl acetate and BSTFA (1% TMCS) to each extract.

3.10.1.6.14.2 Cap tubes and vortex briefly.

3.10.1.6.14.3 Place tubes in dry bath or oven set at 70°C for 15 minutes.

3.10.1.6.14.4 Remove tubes from oven and allow to cool to room temperature.

3.10.1.6.14.5 Transfer derivative to labeled GC/MSD ALS vial with microinsert.

3.10.1.6.15 Preparation for GC-MS Run

3.10.1.6.15.1 Perform an AUTOTUNE and TUNE EVALUATION. When tune values are acceptable, samples may be run on the instrument.

3.10.1.6.15.2 Program SEQUENCE TABLE with sample, calibrator and control information.

3.10.1.6.15.3 Load ALS vials into quadrant racks as indicated in the SEQUENCE TABLE.

3.10.1.6.16 GC-MS Calibration Curve

3.10.1.6.16.1 The calibration curve must be established with a minimum of four data points.

3.10.1.6.16.2 Calibrators should be analyzed in order of increasing concentration.

3.10.1.6.16.3 The least squares line resulting from the analysis of the calibrators must have a coefficient of correlation of ≥0.98.

3.10.1.6.16.4 If calibrators are run in duplicate, it is not required that duplicate calibration points be included as long as the linearity requirement is met.

3.10.1.7 GC and MSD ACQUISITION PARAMETERS

Critical parameters are specified below. Parameters not specified are at the discretion of the analyst and should be optimized for the particular GC-MSD instrument. Each laboratory should maintain a centrally stored printed or electronic copy of current and past GC-MSD methods. The data supporting the GC-MSD method should be stored centrally.

3.10.1.7.1 GC Temperature Parameter

Injection Port: 250°C or 260°C

3.10.1.7.2 MSD Instrument Parameters

Detector/Transfer Line: 280°C

3.10.1.7.3 ALS Parameters

Injection Volume: 1µL (1 stop)
Viscosity Delay: A minimum of 1 second
Solvent Washes (A & B): A minimum of 3 pre- and post-wash rinses.

3.10.1.7.4 MS SIM Parameters

Analyte	Target Ion	Qualifier Ion 1	Qualifier Ion 2
Carboxy-THC	371	473	488
Carboxy-THC-D9	380	482	497

3.10.1.8 REPORTING CRITERIA

3.10.1.8.1 Qualitative Chromatographic Criteria

Acceptable retention time window established by calibrators is ± 0.2 minutes.

3.10.1.8.2 Qualitative Mass Spectral SIM Criteria

Ion ratios for the analyte and its corresponding internal standard, established by calibrators for target and qualifier ions, must not differ by more than ±20% (relative). Cut-off for Carboxy-THC is the lowest calibrator (2.5ng/mL), or the lowest calibrator that meets quality assurance requirements. Any analyte with a quantitative value below this cut-off will be reported as "none detected." If the concentration exceeds the calibration range, the sample can either be appropriately diluted with negative whole blood for reanalysis or qualitatively confirmed in full scan mode (refer to sections 3.10.1.8.1 and 3.10.1.8.3 for confirmation criteria).

3.10.1.8.3 Qualitative Mass Spectral Full Scan Criteria

Analytes may be confirmed from full scan data if there are no significant differences in the mass spectral data as compared to the appropriate reference material.

3.10.1.9 REPORTING OF RESULTS

3.10.1.9.1 Qualitative Confirmation

If Carboxy-THC meets confirmation criteria, it may be reported. The administrative cut-off of 2.5ng/mL, or the lowest calibrator meeting quality assurance requirements, will be used to determine if the analyte is "none detected."

3.10.1.10 QUALITY ASSURANCE REQUIREMENTS

3.10.1.10.1 General

3.10.1.10.1.1 Blood samples are to be stored under refrigeration after aliquots are removed for analysis.

3.10.1.10.1.2 Refer to toxicology manual section 5.8 for additional GC-MSD quality assurance requirements.

3.10.1.10.1.3 Refer to toxicology manual section 5.10 for reference material authentication requirements.

3.10.1.10.2 Per Analysis Run Control Requirements

3.10.1.10.2.1 A solvent blank must follow the highest calibrator, as well as precede each case sample.

3.10.1.10.2.2 A minimum of the spiked blood controls described in section 3.10.1.6.3 must be run per batch of samples.

3.10.2.10.2.3 If the number of case samples exceeds 10, in addition to the two spiked described in 3.10.1.6.3, one blood control must be run for each additional 10 case samples,. Additional concentrations may be used.

3.10.2.10.2.4 Analysts may combine their samples into a single run to conserve supplies. However, each analyst with samples in the run must independently comply with the control requirements in sections 3.10.1.6.3 and 3.10.2.10.2.3.

3.10.1.11 ANALYSIS DOCUMENTATION

3.10.1.11.1 A packet containing original data for controls and standards will be prepared for each analysis run and stored centrally in the laboratory where the analysis was performed until archiving.

3.10.1.11.2 A copy of controls and calibrators need not be included in individual case files. When necessary, a copy of the control and calibrator printouts can be prepared from the centrally stored document.

3.10.1.12 REFERENCES AND RECOMMENDED READING

3.10.1.12.1 UCT CLEAN SCREEN® Extraction Columns Application Manual.

3.10.1.12.2 Standard Operating Procedure for Blood SPE Cannabinoids, Montana Department of Justice Forensic Sciences Division.

3.10.1.12.3 Standard Operating Procedure for Blood SPE THC and Carboxy-THC GC/MSD Assay, Edmonton, Canada Office of the Chief Medical Examiners, 2003.

3.10.1.12.4 Huestis, M.A., *Cannabis (Marijuana) - Effects on Human Behavior and Performance*, Forensic Science Rev. 14(1/2): 16-60, 2002.

3.10.1.12.5 Drummer, O.H., *Cannabis*, pp. 178-212. *in:* The Forensic Pharmacology of Drugs of Abuse, Arnold: London, 2001.

3.10.1.12.6 Bock, Peter, *Getting it right - R&D methods for science and engineering*, Academic Press, San Diego, 2001.

Revision History

Section Three

Blood Toxicology

3.10 Manual Solid Phase Extraction (SPE) Methods

3.10.1 Extraction of Carboxy-THC from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® THC Extraction Column

Revision No.	Issue Date	History/Comments
0	11-22-2006	Original Issue Method is approved for qualitative purposes only. Upon review of uncertainty determinations for quantitative analysis this method will be applied for intended use.
1	07-28-2008	Clarified that negative blood used to prepare calibrators and positive controls is the same lot as used for negative control.
2	03-07-2011	Replaced 4°C storage with "under refrigeration", emphasized need for sample homogeneity. Reformatted for clarity.
3	05-17-2013	Removed Δ^9-THC from method. Clarified that stock solutions are to be stored per vendor recommendations. Added provision for qualitative confirmation of analytes from full scan data. Removed quantitative references – method not for quantitation. Clarified that analytes will be deemed "none detected" if values achieved for that analyte is below the lowest calibrator meeting quality assurance requirements. Added provision for shared runs by multiple analysts, and clarified per-analyst control requirements. Made silanized extraction tubes a recommendation, due to supply availability limitations. Formatting for continuity.