Idaho State Police
Forensic Services

Approval for Quality System Controlled Documents

Discipline/Name of Document: Toxicology
3.10.1 – Extraction and Quantitation of Δ^9–Tetrahydrocannabinol (Δ^9-THC) and 11-nor-Δ^9-THC-9-COOH (Carboxy–THC) from Blood Employing the United Chemical Technologies (UCT) 200 mg CLEAN SCREEN® THC Extraction Column **(FOR QUALITATIVE USE ONLY)**

Revision Number: 0

Issue Date: 11/22/2006

APPROVED BY: Corinna C Owsley — Quality Manager

6/26/07 — Date Signed

Original Certificate did not document that the approval was only for reporting qualitative results.

Idaho State Police
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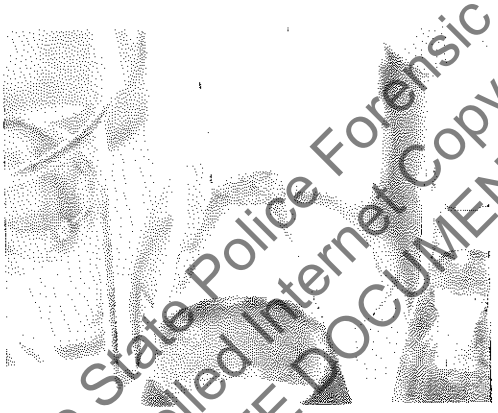

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Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.10 SPE Methods for Quantitative GC/MSD Confirmation

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

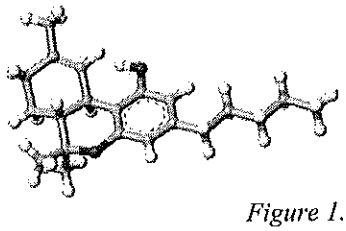

Figure 1.

3.10.1.2 PRINCIPLE

This procedure outlines the use of the 200mg United Chemical Technologies (UCT) CLEAN SCREEN® THC Column for the extraction from blood of the cannabinoids Δ^9-THC and Carboxy-THC. 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 and 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 and quantitation of THC and Carboxy-THC, deuterated internal standard is added, blood proteins are precipitated with Acetonitrile-10% Methanol and removed via centrifugation, the supernatant is adjusted to pH 4.5 with an acetate buffer, and loaded onto a pre-conditioned SPE column. The conditioning creates an environment, which allows for optimal interaction between the sorbent and the analytes 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. When the column is dry, the analytes of interest are recovered from the column with a solvent mixture. Following the elution from the SPE column the extract is derivatized for confirmation on 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 Drybath or laboratory oven
3.10.1.3.3 Evaporative concentrator equipped with nitrogen tank.
3.10.1.3.4 Vortex mixer
3.10.1.3.5 Laboratory centrifuge capable of ≥ 3200rpm
3.10.1.3.6 Vacuum Manifold/ Vacuum pump
3.10.1.3.7 Fixed and adjustable volume single channel air displacement pipetters, and appropriate tips, capable of accurate and precise dispensing of volumes indicated.
3.10.1.3.8 16 x 100mm silanized glass tubes
3.10.1.3.9 Screw Cap for 16mm O.D. tubes
3.10.1.3.10 GC/MS Automated Liquid Sample (ALS) vials
3.10.1.3.11 Silanized GC/MS Vial Microinsert
3.10.1.3.12 Gas Chromatograph equipped with a quadrapole mass selective detector and a nonpolar capillary column with a phase composition comparable to 100%-dimethylpolysiloxane or 95%-dimethyl-polysiloxane 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

3.10.1.5.1.1 **Stock Solutions**

The source of a corresponding calibrator and control must be obtained from a different vendor.

Carboxy-THC or Δ^9-THC
Concentration: 100µg/mL or 1mg/mL

3.10.1.5.1.2 **Working Solutions**

1ng/µL

As appropriate, add 100.0µL 100µg/mL or 10.0µL 1mg/mL Stock Solution to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL.

0.1ng/µL

Add 1.0mL 1ng/µL Carboxy-THC and Δ^9-THC working calibration solution to ≅8mL Methanol in 10mL volumetric class A flask. QS to 10mL. Store remaining stock solution in ALS vial in freezer. Working solutions are stable for 6 months when stored at 4°C.

3.10.1.5.2 Internal Standard Solutions

3.10.1.5.2.1 **Stock Solutions**

Δ^9-THC-D_3 or Carboxy-THC-D_9
Concentration: 100µg/mL or 1mg/mL

3.10.1.5.2.2 **Working Internal Standard Solution [1ng/µL]**
Add 100µL 100µg/mL or 10µL 1mg/mL stock solutions to ≅9mL Methanol in a 10mL volumetric class A flask. QS to 10mL. Solution is stable for six months when stored at 4°C.

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 Calibration Standard Preparation

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 Δ^9-THC and Carboxy-THC mixed calibrator working solution as indicated in the following table.

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

3.10.1.6.2.3 Add the volume of 1ng/µL Δ^9-THC and Carboxy-THC mixed calibrator working solution as indicated in the following table.

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

3.10.1.6.3 Positive Control Sample 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 mixed control solution.

Desired ng/mL	µL Working Control
6	60

3.10.1.6.3.3 Add indicated amount of 1ng/µL working mixed control solution.

Desired ng/mL	µL Working Control
60	60

3.10.1.6.4 Negative Control Sample Preparation
Add 1mL of negative whole blood to an extraction tube.

3.10.1.6.5 Case Sample Preparation
Add 1mL of blood to 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 mix.

3.10.1.6.6.2 Cap tube and vortex tube briefly.

3.10.1.6.6.3 Allow tubes 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 to case, calibrator and control samples.

3.10.1.6.7.2 Cap tube and continue vortexing tube for 30 seconds.

3.10.1.6.7.3 Centrifuge @ 3200-3400 rpm for 10 minutes.

3.10.1.6.7.4 Decant organic supernatant into second labeled tapered bottom centrifuge tube.

3.10.1.6.7.5 Transfer tube to TurboVap and evaporate under nitrogen @ ≅ ≤40°C to approximately 1mL. *Do not allow extract*

to go to dryness.

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

3.10.1.6.7.7 If necessary, centrifuge an additional 5 minutes @ 3200-3400 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 in the vacuum manifold.

3.10.1.6.8.2 Add 3mL 70:30 Hexane:Ethyl acetate. Aspirate at ≤ 3 in. Hg to prevent sorbent drying.

3.10.1.6.8.3 Add 3mL of methanol to the column. Aspirate at ≤ 3 in. Hg.

3.10.1.6.8.4 Add 3mL of deionized water to the column Aspirate at ≤ 3 in. Hg.

3.10.1.6.8.5 Add 1mL of 100mM HCl and aspirate at ≤ 3 in. Hg.

3.10.1.6.9 Blood Extract Loading

Decant buffered blood extract onto column and allow to gravity flow or apply minimal vacuum.

3.10.1.6.10 Column Wash

3.10.1.6.10.1 Add 2mL of deionized water. Aspirate at ≤ 3 in. Hg.

3.10.1.6.10.2 Add 2mL 100mM 70:30 HCl:Acetonitrile.

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 tapered tip centrifuge tubes.

3.10.1.6.12.2 Add 200uL hexane.
Gravity flow only. Do not allow disc to dry.

3.10.1.6.12.3 Add 3mL 70:30 Hexane:Ethyl Acetate elution solvent to the column. *Collect eluate with gravity flow or apply minimal vacuum.*

3.10.1.6.13 Eluate Evaporation
Transfer centrifuge tube to TurboVap. Take solvent 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).

3.10.1.6.14.2 Cap tubes and vortex briefly.

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

3.10.1.6.14.4 Remove tubes from oven and allow to cool.

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.

3.10.1.6.15.2 When tune values are acceptable, 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 All reported results must be bracketed by calibrators.

3.10.1.6.16.3 Calibrators should be analyzed in order of increasing concentration.

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

3.10.1.6.16.5 If calibration standards are run in duplicate, it is not required that duplicate calibration points are 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
Δ9-THC	386	371	315
Δ9-THC-D3	374	306	389
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 minute.

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%.

3.10.1.8.3 Quantitative Mass Spectral and Control Criteria

3.10.1.8.3.1 Quantitative results can be accepted if the calculated concentration of all calibration standards and control samples are within ±20% of their respective concentrations and the coefficient of variation (CV%) for replicates of control samples is ≤ 15%.

3.10.1.8.3.2 Quantitation is achieved through the plotting of the target ion response ratio versus the concentration for each calibrator.

3.10.1.8.3.3 Quantitative values for case samples, calibrators and controls will be truncated for reporting purposes.

3.10.1.8.3.4 Cut-off for Δ^9-THC and Carboxy-THC is 2.5ng/mL, the lowest calibrator.

3.10.1.8.3.5 If the concentration exceeds the calibration range, the sample can either be appropriately diluted with DI water for reanalysis or reported as greater than 100ng/mL.

3.10.1.9 REPORTING OF RESULTS

3.10.1.9.1 Quantitative Value

Analysis results should be truncated and reported out without decimal places.

3.10.1.9.2 Uncertainty Value

Based on the current uncertainty assessment, the +/– range should be included on the analysis report. Refer to method variation spreadsheet for current uncertainty figure.

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 5.1 for pipette calibration options.

3.10.1.10.1.3 Refer to toxicology manual section 5.2 for balance calibration requirements.

3.10.1.10.1.4 Refer to toxicology manual section 5.8 for reference standard authentication and additional GC-MSD quality assurance requirements.

3.10.1.10.2 Per Analysis Run Control Requirements

3.10.1.10.2.1 Solvent blank should follow the highest calibrator as well as each case sample.

3.10.1.10.2.2 A minimum of two blood controls must be run per batch of samples. A control must be run for each additional 10 case samples.

3.10.1.10.3 Monitoring of Control Values

Upon the completion of analysis, input blood control values on spreadsheet used to assess uncertainty for this method.

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 standards need not be included in individual case files. When necessary, a copy of the control and standard 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.

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Idaho State Police
Forensic Services
Toxicology Discipline

Section Three
Blood Toxicology

3.10 Manual Solid Phase Extraction (SPE) Methods

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

Revision #	Issue Date	History
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.

Approval

Discipline Leader: _____ **Date:** _____
Susan C. Williamson

Issuance

QA Manager: _____ **Date:** _____
Alan C. Spanbauer