1.0 PRINCIPLE

The principle psychoactive ingredient in Marihuana is Delta-9-Tetrahydrocannabinol (THC). This compound is metabolized in the body to form the metabolite, 11-nor-9-carboxy-delta-9-tetrahydocannabinol (THC-COOH), which may be further metabolized by liver glucuronidation. THC-COOH is somewhat water soluble and it, along with the glucuronide conjugate are excreted in the urine. Forensic urine samples are therefore treated with aqueous sodium hydroxide and heated to hydrolyze the glucuronide conjugate. Post-hydrolysis urine samples are acidified with glacial acetic acid, and any free THC, or THC-COOH present in the sample is extracted onto a solid phase extraction column. Co-extracting materials are washed from the column, and THC/THC-COOH is subsequently eluted. For blood samples, protein precipitation with acetonitrile is utilized followed by the addition of acetate buffer, and the any THC and/or THC-COOH that may be present is extracted ont a solid phase extraction column, as noted above. Following evaporation of the elution solvent, the extracted drug is derivatized with BSTFA (N,O, -bis trimethylsilyl trifluoroacetamide) with 1% TCMS (Trimethylchlorosilane). The derivatized sample extracts are analyzed by GC/MS.

Qualitative identification of THC and THC-COOH is based on retention time and ion ratios for 3 ions compared to the corresponding ion ratios from a calibrator extracted and run in the same batch. Concentration of each analyte is determined by single point calibration, using d3-analogue of the analyte as the internal standard. Each GC/MS run is separately evaluated using control and blank samples, and is processed by a custom spreadsheet program, on which calibrator, blank and control results are summarized and tabulated, and batch review process by both the analyst and technical reviewer is documented. Matrix-specific (blood and/or urine) positive and negative controls are extracted and analyzed in each analytical batch. The presence of THC-COOH may be confirmed in urine, blood or other aqueous fluids. The concentration of any THC, or THC-COOH present in the sample is qualitatively evaluated by 3-ion SIM, using retention time and 2 qualitative ions as the basis for identification. Quantitative evaluation is made by single point calibration, using deuterated internal standard, and two control samples to define the quantitative range

2.0 SPECIMEN

Specimens requiring confirmation for THC/THC-COOH are listed by lab case number on the clip board marked “THC-COOH List” which is maintained in the Toxicology Instrument room . Analysts preparing a batch for analysis should generate their batch sample list (see form 23.4, appended to SOP 23) from this document.

2.1 All evidence transfers, either between individuals or between an individual and a storage location must be documented on the Chain of Custody for the case, either in the LIMS, or on hard-copy COC document maintained in the Case Jacket.

2.2 When not in the sampling or aliquot process, samples in the Toxicology section must be stored in the secure and locked Toxicology evidence storage room.

2.3 Samples must be maintained in such a manner so that they are protected from contamination or deleterious change. Depending on the nature of the sample, this may mean refrigeration or freezing when not in the analytical process.

2.4 When analysis of samples in the toxicology section is complete, they must be maintained "Under Proper Seal." This is interpreted to mean that the sample, or a container in which the sample is kept, is sealed with tamper-evident tape, with the initials and date of person placing the seal clearly marked on, or proximate to that seal.

2.5 Samples are maintained in the Toxicology Section for 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. After this period, samples are discarded in the appropriate medical waste disposal container. Sample from fatalities, or cases with requests for retention are maintained by the laboratory.

3.0 EQUIPMENT:

GC/MS and associated data station/computer (HP6890/5973 or equivalent)
General laboratory glassware and equipment
Solid phase extraction manifold and associated vacuum equipment.
Analytical evaporator Zymark Turbovap or equivalent.
UCT; ZSTHC020 "Clean Screen"
pH paper strips, range 0-14

4.0 REAGENTS:

4.1 Reagents available as stock items:

Methanol (HPLC grade or equivalent)
Glacial Acetic Acid (Baker reagent grade or equivalent)
Hexane (HPLC grade or equivalent)

Deionized water (DIW; 2-megohm In-House supply)
Sodium Hydroxide (Baker reagent grade or equivalent)
Ethyl acetate (HPLC grade or equivalent)
Acetonitrile (Baker reagent grade or equivalent)
BSTFA with 1% TMCS
Control Blood (Outdated bank blood from Hospital)
Drug free urine
THC-COOH Stock: 1 mL vial 1.0 mg/mL
THC-COOH deuterated Stock: 1 mL vial 1.0 mg/mL
Sodium Phosphate Dibasic (Baker reagent grade or equivalent)
Sodium Phosphate monobasic (Baker reagent grade or equivalent)

4.2 Reagents prepared in the Toxicology Laboratory:

4.2.1 10 N NaOH; dissolve 200 g NaOH/500 mL DIW
4.2.2 0.1 M HCL; dilute 8.4 mL conc. HCl to 1000 mL DIW
4.2.3. 0.1 M HCL / Acetonitrile (70:30)
4.2.4. Hexane / Ethyl Acetate (50:50)
4.2.5 0.1 M phosphate buffer pH 6.0; combine 48.6 g Na_2HPO_4 and 6.8 g NaH_2PO_4 dilute to 4000 mL using DIW.

Note: Reagent Preparation and Validation is documented in the Toxicology "Reagent Preparation/Validation Logbook" maintained in the Toxicology section. Validation is addressed in section 4.5, below.

4.3 Calibrators and Internal Standard:
Note: Preparation of all calibrator and control solutions is documented in the "Calibrator and Control Preparation Log" (maintained in the Toxicology Wet Laboratory)

THC-COOH internal standard (d3-THC-COOH) 10 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/10 mL MeOH) or equivalent
THC-COOH Calibrator stock solution (THC-COOH) 10 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/10 mL MeOH) or equivalent
Delta-9-THC Calibrator stock solution (THC) 10 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/10 mL MeOH) or equivalent

4.4 Controls:

#1 THC-COOH Control stock solution (THC-COOH) 10 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/10 mL MeOH) or equivalent
#2 THC-COOH Control stock solution (THC-COOH) 1 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/100 mL MeOH) or equivalent
#1 Delta-9-THC Control stock solution (THC) 10 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/10 mL MeOH) or equivalent
#2 Delta-9-THC Control stock solution (THC) 1 ng/ul
(Alltech; 100 ug/mL: 1.0 mL/100 mL MeOH) or equivalent

This procedure utilizes controls prepared (spiked) in blank blood and/or urine (as appropriate to batch makeup) as follows:

Blood and Urine: THC-COOH Low Control: 10 ng/mL
High Control: 200 ng/mL
Calibrator: 100 ng/mL)

Each run will incorporate a high and low control for each analyte. Blood matrix controls may be used to validate urine results, but not the reverse. Controls are prepared by addition of THC and THC-COOH from validated stock solutions to blank sample matrix aliquots, prior to extraction, (details in Procedure below). Acceptable control performance is target value +/- 20%.

4.5 Validation of Reagents

Validated reagents are marked with a green dot, detailing the specific procedure for which the reagent was validated, and the batch on which that process was documented. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as validation of reagents. Reagents so validated are marked with a green sticker as noted above. Preparation of reagents, and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory.

5.0 PROCEDURE

Note: Departure from procedures as specified in this SOP is not anticipated. Should an issue arise that may require such a departure, the issue must be raised with the Section Supervisor, Quality Manager and/or the Director. If the proposed change will not present a change of such a magnitude so as to require validation, the change may be approved, and the Director will modify and re-issue the SOP accordingly.

Any such procedural changes would be subject to the review process afforded by the quality control measures of the analytical scheme described herein. Hence, any modification or change that produces an unexpected deleterious effect on the analytical procedure would be expected to trigger analysis or batch failure in the QC review stages.

5.1 Batch Format: Analytical batches for THC-COOH and THC confirmation should follow the format indicated below: Note that samples are analyzed in duplicate.

Calibrator
Matrix blank
Control High
Control Low
Sample 1 rep 1
Sample 1 rep 2
Samples 2-10 (or fewer) Rep 1 and 2
Control High (Final control, in the absence of additional samples)
Additional Samples (10 or fewer)
Final Control

5.2 Label a 16x100 screw cap culture tube for each sample replicate, blank, calibrator and control, similarly label a SPE tube, place a clean thru tip on each end and load in the manifold.

5.3 Using a validated dispensing pipette, place a 5.0 mL aliquot of blank urine or a 1.0 mL aliquot of blank blood as appropriate, into the properly labeled test tubes for each blank, control and calibrator.

5.4 Using a validated dispensing pipette, place a 5.0 mL aliquot of urine or a 1.0 mL aliquot of blood as appropriate, of each sample replicate into the properly labeled test tubes.

Note: Urine with observable turbidity can be filtered with a syringless filter or centrifuged for 5 minutes @ 2000 rpm.

Note: Samples requiring dilution as a function of concentration greater than the high control, should be diluted with 0.1M pH 6.0 Phosphate buffer as appropriate. The initial quantitative values may be used as a guide for the dilution process. The final dilution volume should be greated than the 5.0 mL (urine) or 1.0 ml (blood) aliquot that will be taken. The dilution process shall be documented on the batch worksheet, including the pipette(s) used in the process:

Example: Blood sample; 1st run result, ~ 25 ng/mL THC, ~ 180 ng/mL THC-COOH. A 1:1 dilution would likely be an adequate dilution for both compounds, (THC final result would be expected to be ~ 12 ng/mL, THC-COOH ~ 90 ng/ml)e 1.0 ug/mL high control). Therefore, 600 uL sample is added to a 13 x 100 test tube using a 0 – 1 mL adjustable volume pipette, along with 600 uL 0.1 M pH 6.0 Phosphate buffer. (Note; because equal volumes of sample were utilized, pipette precision determines the accuracy of the dilution). The solution is mixed, and aliquotted as per step 5.4. The dilution details, including the dilution factor (Volume sample: Volume Diluent + Volume Sample) are documented on the batch worksheet.

5.5 Using a validated dispensing pipette, add 25 ul of deuterated internal standard stock solution to each sample replicate, blank, calibrator and control tube.

5.6. For Urine Samples:

5.6.1 Using a validated dispensing pipette, add 50 ul of THC-COOH calibrator standard stock solution to the tube labeled Calibrator.

5.6.2 Using a validated dispensing pipette, add 100 ul of THC-COOH #1 control standard stocks solution to the tube labeled High Control.

5.6.3 Using a validated dispensing pipette, add 50 ul of THC-COOH #2 control standard stock solution to the tube labeled Low Control.

5.6.3 Hydrolysis of Urine Samples

5.6.3.1 Add 100 ul of 10 M sodium hydroxide to each tube and then cap.

5.6.3.2 Heat tubes for 30 min. in 60°C water bath, allow to cool.

5.6.3.3 Adjust the pH to ~3.5 +/- 0.5 with ~1 mL glacial acetic acid, check with pH test strips. Proceed to step 5.8

5.7 For Blood Samples:

5.7.1 Using a validated dispensing pipette, add 25 ul of THC-COOH and THC calibrator standard stock solutions to the tube labeled Calibrator.

5.7.2 Using a validated dispensing pipette, add 50 ul of THC and THC-COOH #1 control standard stocks solutions to the tube labeled High Control.

5.7.3 Using a validated dispensing pipette, add 10 ul of THC and THC-COOH #2 control standard stock solutions to the tube labeled Low Control.

5.7.4 Add 4 mL of water to each tube, vortex for 30 sec. and let stand for 5 minutes.

5.7.5 Centrifuge @ 2000 rpm for 5 minutes. Transfer the supernatant to a clean, appropriately labeled tube.

5.7.6 Adjust pH to ~3.5 +/- 0.5 with ~ 1 mL 100mM HCl.

5.8 (All Samples) Condition the columns:
1 x 3 mL methanol; drain (vacuum assist)
1 x 3 mL DIW; drain (vacuum assist)
1 x 1 mL 0.1 M HCl; drain (vacuum assist)
DO NOT LET COLUMN GO DRY!

5.8.1 Transfer contents of each tube to the appropriately labeled SPE tube, and allow gentle drop wise flow until the level reaches the top if the column bed.

5.8.2 Wash column:
1 x 2 mL DIW; drain (vacuum assist)

1 x 2 mL 0.1 M HCl/acetonitrile (70:30); drain (vacuum assist)
Dry column (15 minutes at > 10 inches Hg)

5.8.3 Position appropriately labeled 13x100 test tubes under each SPE column, with clean thru tip inside collection test tube.

5.8.4 Elute analyte from SPE columns by adding 3 mL hexane/ethyl acetate (50:50). Collect at 1-2 mL/minute.

5.8.5 Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen (Turbovap).

5.9 Reconstitute residue with 150 ul of ethyl acetate and transfer to gc/ms vial with limited volume insert. Add 25 micro liters BSTFA (with 1% TMCS), cap and reserve for GCMS analysis. NOTE: Do not evaporate BSTFA solution

6.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

6.1 Instrument and Setup:

GCMS/Autosampler: (Hewlett-Packard 6890/5973, or equivalent)
Column: 30M RTX-5MS (0.25 mm ID; 0.25 micron film)
Inj. Temp. 250 ^{o}
Det. Temp. 160 ^{o}
Oven (init.): 160^{o}, 30 ^{o} /min to 300 ^{o}, (1.50 min hold).
9.60 min total run time
1 ul inj

6.2 Injection sequence**;** Samples are injected on the GC/MS in the following sequence:

Calibrator
Matrix blank
Control High
Control Low
Sample 1 rep 1
Sample 1 rep 2
Samples 2-10 Rep 1 and 2
Control High
Additional Samples

Final Control

6.3 DETECTION AND IDENTIFICATION

Determination of the presence of THC-COOH and THC in the sample extract is identified by appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification. For a positive Identification of THC-COOH or THC, the retention time must be within 5% of the corresponding analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator sample. Qualitave identification of each analyte is independent of each other.

6.4 CALIBRATION AND QUANTITATION

6.4.1 Calibration

Calibration for each batch is done independently. Hence, no sample analysis conducted under DPS guidelines drug is quantitated based on an historical calibration curve. Calibration is accomplished by the incorporation into the sample procedures of a blank sample of the matrix being analyzed that has known quantities (200 ng/mL for THC-COOH and 100 ng/mL for THC in Urine, 20 ng/mL and 10 ng/mL for blood, respectively) in addition to the deuterated internal standard. The response of the system to this calibrator, and the assumption of a 0 response to a 0 concentration, defines a run-specific standard curve that is used as the basis for the quantitative calculation in all controls and samples. The system is therefore “single-point calibration, multi-point control”.

6.4.2 Quantitation

Quantitation is accomplished by the comparison of the response ratio of the analyte in a specific sample, to the response ratios of the calibrators as expressed as a standard curve. The concentration of the analyte in the sample is then extrapolated from the standard curve, and corrected for any dilution that may have been performed to facilitate the analysis of relatively concentrated samples.

7.0 QUALITY CONTROL AND RUN EVALUATION

7.1 Verification of Vial Sequence: The vial sequence is checked both prior to and after the injection of samples when the auto injector is used. The check after the injection of samples is documented on the run summary sheet.

7.2 Chromatography - Evaluation and Acceptance Criteria: Chromatographic quality is evaluated for each peak. While general guidelines are that a peak should be symmetrical, and be resolved to baseline on at least one side, with 90% resolution on the other side, significant departures from those guidelines may be experienced with forensic samples. In many cases, chromatographic quality will warrant rejection of the chromatographic run, or specific samples, by the operator. Any such action should be clearly documented on the batch summary sheet. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Director, Section Supervisor or Quality Manager.

7.3 Evaluation of Potential Carryover

Carryover in the chromatographic quality is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% (40 ng/mL) requires batch rejection, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover of less than 2% will require operator consideration with regard to the potential for effects on specific samples, and may require re-extraction of specific samples. Carryover is further evaluated on a per sample basis by the requirement that quantitative results between replicates agree within 20%. Any significant carrover effect should cause the first of the two replicate samples to exceed the second by an amount in excess of the 20% differential. If not, any carryover may be considered inconsequential. In practice, when a question of potential carryover exists, the potentially affected sample replicates may be repeated at the end of the batch.

7.4 Control Results: For the batch to be considered acceptable for any particular analyte, quantitative results for both control for that analyte must be within 20% of the target value.

7.5 Internal Standard: Minimal acceptable internal standard abundance is 500k for both THC and THC-COOH. For any individual injection to be acceptable, the internal standard abundance must be at least 20% of the corresponding abundance in the calibrator, and greater than the minimal abundance noted above.

7.4 LINEARITY: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of quantitative results of control materials.

Note; Blood, or body fluid quantitative analysis with quantitative results of either THC or THC-COOH concentrations greater than the corresponding calibrator will be re-analyzed with an appropriate dilution (see section 5.4, above) such that the target analyte concentration will be above the LOQ, but below the calibrator concentration. Because of the lack of correlation between blood and urine concentrations, urine samples will be subject to consideration for such re-analysis on a case-by case basis, as determined by the analyst.

7.5 SENSITIVITY- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ): For the purposes of this procedure, the LOD and LOQ are defined as equal to the lowest concentration of the lowest control. Qualitative Identification and/or Quantitative analysis below the concentration of the low control may be accepted on a case by case basis with the concurrence of the analyst, technical reviewer, Director and/or Quality Manager.

7.6 ACCURACY AND PRECISION:Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of 10 reps of the specific control, and the target value. Precision is expressed as the CV of that value

7.7 SPECIFICITY: Specificity is a function of both the resolution of target analyte during the analytical process, and the mass spectral fragmentation that analyte molecules undergo during the instrumental analysis. There has been no report of any material other than THC-COOH that elutes within 5% of the retention time of known standard materials, and produce the same fragmentation ions and ratios.

7.8 REPORTING OF RESULTS:THC-COOH and THC runs runs are performed as part of GC/MS batches, containing controls and calibrators. The complete batch packets are in the Toxicology Laboratory. This packet contains all run evaluation

documentation. Specific chromatograms for each case are filed in the appropriate case file. Results are documented on the "Batch Summary" sticker on each case file and include a reference number for the batch as a whole. A Batch summary sheet will be produced with each batch. Data on each batch should include fields such as: Sample name, Batch ID (Date of Batch), analysts who generated data, matrix, analyte found (and concentration if applicable), controls run with the batch and results obtained. If controls do not meet the criteria, the batch can be rejected as a whole or by a case by case basis. The supervisor is notified and proper action is taken to correct any problem. Batches and/or cases shall be repeated as needed.

Procedural Uncertainty is reported with all quantitative results, and is calculated and tabulated annually for each analytical method, (See SOP TX-19 section 6.3).

8.0 QUALITY ASSURANCE: Quality Assurance is provided by the multiple layers of checks that are performed both during and after analysis. Specifically:

8.1 The GC/MS run is thoroughly checked by the operator, including vial position on the autosampler, both prior to and following the injection of samples.

8.2 The GC/MS run is reviewed and signed off by a reviewer distinct from the operator, with this review including an evaluation of qualitative and quantitative (where applicable) results, including:

a. Control Results
b. Chromatographic Characteristics
c. Transcription errors

8.3 All results, as transcribed in the Case Summary Form are checked against the original run summary sheet during the process of report preparation, and during the administrative review of case results.

8.4 The original run is compared to the Final Report during the Final Director's review, prior to case sign-off.

9.0 SOURCES OF ERROR

The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification with essentially no uncertainty. Urine drug analyses are reported only as qualitative results.

10.0 REFERENCES

Clarke's Isolation and Identification of Drugs. 2nd Edition

UCT United Chemical Technologies
Solid phase extraction methods

Appendix II:

GC/MS temperature program specifications

Temperature program			
Parameter			
Initial temp	160° C		
Initial Time	0.00min		
Ramps Rate/final temp/final time	rate	temp	time
	30.0	300	4.0 min
Post temp	300°c		
Post time	1.50 min		
Run Time	8.67 min		
Front inlet			
Mode	Pulsed splitless		
Initial temp	250°c		
Pressure	12.91 psi		
Pulse pressure	25 psi		
Pulse time	0.50 min		
Total flow	43.3 mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -3 Solvent B - 3		
Tune file	STUNE		
Acquisition mode	SIM		
Solvent delay	6.25 minutes		
M/Zs group 1 Start time 0.00	374, 371, 476, 473, 491, 488		
Dwell	20		
MS Quad	150°c max 200		
MS Source	230°c max 250		

Example of controlled THC batch document TX THC-1. Batch documents can vary based on nature of batch.

CT DPS; Toxicology Laboratory Batch ID: thc 9-17-10

THC-COOH GCMS Batch Summary Page 1

Autosampler position	Sample (Case Number)	Specimen (Matrix)	Specimen Volume, (ml)	Cal. Stock Vol (ul)	Con. Stock Vol. (ul)	Int. Std. Violume (ul)	Analyte	Theoretical Concentration (ng/ml)	Observed Concentration (ng/ml)	Percent Recovery (Acceptable: 80 - 120)	I.S. Abund (x 1 E 4)	Acceptable **; Analyst	Technical Review**
3	Calibrator	urine	5.0	100		25	thc-cooh	200	200		398		
4	Negative Control	urine	5.0			25	thc-cooh	0.00	nd		335		
5	Control 1	urine	5.0		100	25	thc-cooh	200	201	100	368		
6	Control 2	urine	5.0		50	25	thc-cooh	10.0	8.56	85.6	284		
7	TX-10-1288	urine	5.0			25	thc-cooh		64.2		98.6		
8	TX-10-1288	urine	5.0			25	thc-cooh		63.5		96.1		
9	TX-10-1289	urine	5.0			25	thc-cooh		110		146		
10	TX-10-1289	urine	5.0			25	thc-cooh		110		134		
11	TX-10-1339	urine	5.0			25	thc-cooh		203		127		
12	TX-10-1339	urine	5.0			25	thc-cooh		177		119		
13	TX-10-1340	urine	5.0			25	thc-cooh		31.3		122		
14	TX-10-1340	urine	5.0			25	thc-cooh		35.3		101		
5	Control 1	urine	5.0		100	25	thc-cooh	200	183		384		

Samples Extracted by: _____ Date: _____ GCMS Run Date: _____

Carryover check: THC-COOH < 5 ng/ml ? ___ Yes ___ No

** Acceptability; Peak Shape, Retention Time, IS Area, Fragment Ratio all must be acceptable.

Analyst Review by: _____ Date: _____ Run Accepted? ___ Yes ___ No

Analyst Comments: ***THC-D3 internal Std used for this extraction.***

Technical Review by: _____ Date: _____ Run Accepted? ___ Yes ___ No

Reviewer Comments: _____

** ND = Not Detected

Vial position verified prior to sample removal:_____ Internal stds 20% of Calibrator:_____