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-tetrahydrocannabinol (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. 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-COOH is based on retention time and ion ratios for 3 tons compared to the corresponding ion ratios from a calibrator extracted and run in the same batch. Concentration of each analyte is determined by a calibration using the peak area ratio between the analyte and the internal standard, using d3-analogue of the analyte as the internal standards. Each GC/MS run is assessed separately; the processed data is evaluated using control and blank samples, and a documented batch review process by both the analyst and technical reviewer. Matrix-specific (urine) positive and negative controls are extracted and analyzed in each analytical batch the concentration of any 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. This procedure may be used quantitatively via construction of a multi-point calibration curve for the analyte(s) of interest.

2.0 SPECIMEN

Urine samples requiring confirmation for THC-COOH are scheduled in JusticeTrax (JT) from which a worklist can be generated.

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 in JusticeTrax.

2.2 When not in the sampling or aliquot process samples will be maintained in locked storage within the Toxicology unit.

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 unit is complete, they must be maintained "Under Proper Seal." Refer to SOP TX-19 for further guidance.

2.5 Samples are maintained in the Toxicology Section for a minimum of 8 weeks after case is completed, in the absence of notification of any legal action, or other reason to maintain the samples. Samples from fatalities and sexual assaults are maintained indefinitely by the DSS. Cases with requests for retention are maintained by the laboratory based on the request. Upon request from the courts or submitting agency the evidence will be returned to the submitting agency.

3.0 EQUIPMENT:

Equivalent equipment may be used.

- GC/MS and associated data station/computer
- General laboratory glassware and equipment
- Solid phase extraction manifold and associated positive pressure or vacuum equipment
- Analytical evaporator
- UCT; ZSTHC020 "Clean Screen"
- pH paper strips, range 0-14

4.0 REAGENTS:

4.1 Reagents available as stock items:

Equivalent or better grade reagents may be used.

- Methanol (HPLC grade or equivalent)
- Glacial Acetic Acid (Baker reagent grade or equivalent)
- Hexane (HPLC grade or equivalent)
- Deionized water (DIW; Millipore 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
- Drug free urine
- THC-COOH Stock: 1 mL vial 1.0 mg/mL (Cerilliant)
- THC-COOH deuterated Stock: 1 mL vial 1.0 mg/mL (Cerilliant)
- 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: (Obtained from an approved vendor)

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

4.3.1 d3-THC-COOH internal standard 10 ng/µL (Cerilliant or equivalent)
Dilute 250 µL of 1.0 mg/mL reference standard into 25 mL methanol

4.3.2. THC-COOH Calibrator stock solution 10 ng/µL (Cerilliant or equivalent)
Dilute 100 µL of 1.0 mg/mL reference standard into 10 mL methanol

Note: Equivalent ratios may be used; this will be documented in the 'Calibrator and Control Preparation Log'.

4.4 Controls:

4.4.1 #1 THC-COOH Control stock solution 10 ng/ µL (Lipomed or equivalent) Dilute 100 µL of 1.0 mg/mL reference standard into 10 mL methanol

4.4.2 #2 THC-COOH Control stock solution (THC-COOH) 1 ng/ µL (Lipomed or equivalent). Dilute 25 µL of 1.0 mg/mL reference standard into 25 mL methanol

4.4.3 This procedure utilizes controls prepared in house spiked into urine

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. Controls are prepared by addition of 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% of the expected value.

4.5 Validation of Reagents

Validated reagents are marked with a green dot (sticker), 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

5.1 Batch Format: Analytical batches for THC-COOH confirmation should follow the format indicated below: Note that samples are analyzed undiluted and diluted by a factor of two. Other dilution factors may be used based on the needs of the case. The immunoassay findings can be used as a guide for choosing a dilution factor.

5.2 Label a 16 x100 screw cap culture tube for each, blank, calibrator, control, and

sample. Similarly label a SPE extraction tube and load in the manifold.

Pipet blank, standards and control according to table into 5 mL of blank urine.

	Working Standard #1 10 ng/mL µL to add	Final conc.
blank	0	
Calibrator	50	100 ng/mL

	Working Control #2 1.0 ng/mL µL to add	Working Control #1 10 ng/mL µL to add	Final conc.
Low QC	50		10 ng/mL
High QC		100	200 ng/mL

5.3 Using a validated dispensing pipette, place 5.0 mL and 2.5 mL aliquots of urine into the properly labeled test tubes.

Note: Urine with observable turbidity can be filtered with a syringeless filter or centrifuged for approximately 3 to 5 minutes @ 5200 rpm.

Note: Samples should be diluted with 0.1M pH 6.0 Phosphate buffer. Samples requiring dilution as a function of concentration greater than the upper limit of quantitation should be diluted as appropriate.

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

5.5 Hydrolysis of Urine Samples

5.5.1 Add 100 µL of 10 M sodium hydroxide to each tube and then cap.

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

5.5.3 Adjust the pH to ~3.5 +/- 0.5 with ~1 mL glacial acetic acid, check with pH test strips. Proceed to step 5.6

5.6 Extraction

Condition the columns:

- 1 x 3 mL methanol; drain to hazardous waste stream (1-2 mL/min)
- 1 x 3 mL DIW; drain to non-hazardous waste stream (1-2 mL/min)
- 1 x 1 mL 0.1 M HCl; drain non-hazardous waste stream (1-2 mL/min)
- DO NOT LET COLUMN GO DRY!

5.6.1 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.

5.6.2 Wash column:

- 1 x 2 mL DIW; drain to nonhazardous waste stream (1-2 mL/min)
- 1 x 2 mL 0.1 M HCl/acetonitrile (70:30); drain to hazardous waste stream (1-2 mL/min)
- Dry column (15 minutes at > 10 inches Hg)

5.6.3 Position appropriately labeled 13 x100 test tubes under each SPE column; check that extraction column tip is positioned inside collection test tube.

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

5.6.5 Remove receiver tubes and evaporate to dryness at < 40º C using a gentle flow of heated nitrogen.

5.7 Reconstitute residue with 150 µL of ethyl acetate and transfer to GC/MS vial with limited volume insert. Add 25 µL BSTFA (with 1% TMCS), cap and reserve for GC/MS analysis. Note**:** Do not evaporate BSTFA solution

6.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

6.1 Instrument and Setup:

GC/MS/Auto sampler: (Hewlett-Packard 6890/5973, or equivalent)
Column: 30M RTX-5MS (0.25 mm ID; 0.25 micron film)
Inj. Temp. 250 ^{o} C
Det. Temp. 280 ^{o} C
Oven (initial): 160^{o} C, 30 ^{o} /min to 300 ^{o} C, (2.50 min hold).
7.67 min total run time
1 µL injection

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

Before running casework check that the instrument is in proper working condition (acceptable for use); refer to SOP TX-29 for further guidance.

- Matrix blank
- Control Low
- Calibrator
- Control High
- Reinject matrix blank
- Samples rep diluted
- Samples rep undiluted
- Inject ethyl acetate solvent blanks between cases
- End run batch with reinjecting the high control
- For longer runs, reinject the low control in the middle of the batch

6.3 DETECTION AND IDENTIFICATION

Determination of the presence of THC-COOH 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, the retention time must be within 5% of the analyte in the calibrator injection, and the ion ratios for both qualitative ions must be within 20% of the corresponding ratio calibrator or control.

6.4 CALIBRATION AND QUANTITATION

6.4.1 Calibration

Calibration for each batch is done independently. Hence, no sample analysis conducted under DESPP guidelines 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 (100 ng/mL for THC-COOH in urine) 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 controls.

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.

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 reviewed and approved by with the Unit Lead and Deputy Director. Depending on the nature of the deviation DSS customers may need to be consulted, see GL-20 for guidance.

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 with the batch. Questionable chromatographic peak shape, resolution, or other problems with chromatography can be discussed with the Deputy Director or Unit Lead.

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 comparing quantitative results between sample replicates. When a question of potential carryover exists, the potentially affected sample replicates may be repeated (reinjected) at the end of the batch.

7.4 Control Results: For the batch to be considered acceptable the negative blank should be blank and a positive control should be positive.

7.5 Internal Standard: The sample preparation extraction efficiency and GC/MS instrument performance can be demonstrated through the internal standard abundances. They should be consistent throughout the batch. The abundance should be about the same as the 100ng/mL calibrator. The batch or samples within the batch should be repeated when the abundance of the internal standard is too low and not acceptable.

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

7.7 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

7.8 ACCURACY AND PRECISION: Accuracy is expressed as a mean (absolute value) percentage difference between mean quantitative value of the specific control, and

the target value. Precision is expressed as the CV of that value.

7.9 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.

7.10 REPORTING OF RESULTS: THC-COOH runs are performed as part of GC/MS batches, containing controls and calibrators. The complete batch packets are in the Toxicology Laboratory. Refer to SOP TX-5 for guidance on batch preparation, case reporting and distribution. Qualitative results are reported for urine samples.

8.0 SOURCES OF ERROR

The utilization of 3-ion SIM methodology, with reference to procedural, controls and calibrators yields qualitative drug identification. Interfering substances, if present, can be detected when retention times shift or ion ratios are outside their limits. If interfering substances are suspected, the urine samples maybe diluted further to minimize their impact. Urine drug analyses are reported only as qualitative results.

9.0 REFERENCES

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

UCT United Chemical Technologies
Solid phase extraction methods

Appendix I:

GC/MS temperature program specifications

Temperature program			
Parameter			
Initial temp	160° C		
Initial Time	0.00 min		
Ramps Rate/final temp/final time	rate	temp	time
	30.0	300	2.5 min
Post temp	300°c		
Post time	0.50 min		
Run Time	7.67 min		
Front inlet			
Mode	Pulsed split less		
Injector temp	250°c		
Pressure	12.9 psi		
Pulse pressure	25 psi		
Pulse time	0.50 min		
Purge to split vent	40.0 mL/min		
Total flow	44 mL/min		
Gas type	Helium		
Injection volume	1 microliter		
Post injection washes Solvent A / Solvent B	Solvent A -2 Solvent B - 3		
Tune file	ATUNE		
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		
MS Source	230°c		