1.0 PRINCIPLE

Opioids are a class of drugs, typified by morphine, whose properties include narcosis, analgesia and sedation. Opioids are of forensic significance, due to their potential for abuse and dependence with continued use, and because of the extensive illicit market in the drugs.

Opioids are extracted from blood and urine samples, using solid phase extraction columns. Opioids that may be present are eluted with Methylene chloride/ Isopropanol/ NH_4OH. After evaporation of the solvents, the extracted drug is derivitized with BSTFA, forming the trimethylsilyl derivative and analyzed by GC/MS. Each opiate analytical batch is run separately processed, calibrated, run and evaluated. Batch results are summarized and tabulated on a spreadsheet, including all calibrator, control, and sample results.

Qualitative identification of Opioids 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. Concentrations of Opioids are determined by single point calibration, using d3-analogue of the opioids as the internal standard. Each GC/MS run is separately evaluated using control and blank samples, and is processed by a custom batch 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 Opioids may be confirmed in urine, blood or other aqueous fluids.

2.0 SAMPLES

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

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, the 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; ZSDAU020 "Clean Screen" extraction columns

4.0 Reagents:

4.1 Reagents available as stock items:
Methanol (Baker HPLC grade or equivalent)
Ethyl acetate (Baker HPLC grade or equivalent)
Glacial Acetic Acid (Baker reagent grade or equivalent)
Ammonium Hydroxide (Baker reagent grade or equivalent)
Blank Blood (Received from Blood bank – Outdated supply)
Drug free urine (or equivalent)
Alltech, Cerilliant or IN-HOUSE drug Stocks: Codeine, Oxycodone, Morphine, Hydrocodone, Hydromorphone (1 mL vial 1.0 mg/mL)
Alltech, Cerilliant or IN-HOUSE deuterated drug Stocks: Codeine-d3, Oxycodone-d3, Morphine-d3, Hydrocodone-d3, Hydromorphone-d3 (1 mL

vial 1.0 mg/mL
Alltech, Cerilliant or IN-HOUSE Morphine glucuronide STD: 1 mL vial 1.0 mg/mL
Deionized water (DIW; Millipore or equivalent In-House supply)
β-Glucuronidase; Sigma or equivalent
Sodium Acetate Trihydrate (Baker reagent grade or equivalent)
Sodium Phosphate Dibasic (Baker reagent grade or equivalent)
Sodium Phosphate Monobasic (Baker reagent grade or equivalent)
BSTFA with 1% TMCS (Trimethylchlorosilane)
Sodium Azide (Baker reagent grade or equivalent)

4.2 Reagents prepared in the Toxicology Laboratory:

4.2.1 pH 6 Phosphate Buffer (0.1 M): 48.6 g Na2HPO4 and 6.8 g NaH2PO4, in 4000 mL DIW,

4.2.2 pH 4.5 Acetate Buffer (0.1 M): Add 5.86 g sodium acetate Trihydrate and 3.24 mL glacial acetic acid to 1000 mL DIW.

4.2.3 1 M Acetate buffer pH 5.0; Dissolve 42.9 g sodium acetate trihydrate in 400mL DIW; add 10.4 mL glacial acetic acid. Dilute to 500 mL with DIW.

4.2.4 100mM Acetate buffer pH 5.0;Dilute 40 mL 1.0 M acetate buffer to 400 mL with DIW.

4.2.5 β-Glucuronidase solution; Dissolve 100,000 Fishman units lyophilized powder with 20 mL 100 mM Acetate buffer pH 5.0.

4.2.6 Methylene chloride/isopropanol/ammonium hydroxide (39/10/1mL; or equivalent ratio) Note: Must be prepared fresh each day of use.

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

4.3 Calibrators and Internal Standard (I.S.):

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 Deuterated I.S. Stock solutions: Combine 1 mL from each Deuterated internal standard stock solution (1mg/mL) and dilute up to 30 mL with Methanol in a screw-capped flask.

4.3.2 Mixed Calibrator Urine stock solution: Add 100 ul of each standard solution and Q.S. to 10 mL in a volumetric flask with methanol (10

ug/mL).

4.3.3 Mixed Calibrator Blood stock solution: Add 100 ul of each standard solution, Q.S to 10 mL in a volumetric flask with methanol. Further dilute a 1.0 mL aliquot to 10 mL with methanol, in a screw-capped test tube (1.0 ug/mL).

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

4.4.1 Mixed Control Urine stock solution: Add 100 ul of each standard solution, Q.S. to 10 mL in a volumetric flask with methanol (10ug/mL).

4.4.2 Mixed Control Blood stock solution: Add 100 ul of each standard solution, Q.S to 10 mL in a volumetric flask with methanol. Further dilute a 1.0 mL aliquot to 10 mL with methanol, in a screw-capped test tube (1.0 ug/mL).

4.5 Validation of Reagents: Acceptable performance of all batch control materials and overall batch acceptability (although individual samples may fail) is considered as 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. 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. See TX SOP # 11

5.0 Proceedure:

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 Section Quality Manager, Supervisor 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 reasonably expected to trigger analysis or batch failure in the QC review stages.

5.1 Batch Format: Analytical batches for Opioids confirmations should follow the format indicated below: Note that samples are analyzed in duplicate.

Calibrator
Matrix blank
Control High
Control Low
Glucuronide Control
Sample 1 rep 1
Sample 1 rep 2
Samples 2-10 Rep 1 and 2
Control High (Final control in the absence of additional samples)
Additional Samples
Final Control (May be high or low)

5.2 Label a 16x100 screw top culture tube for each sample replicate, blank, calibrator and control.

5.3 Add 2 mL of Negative urine to each control, calibrator and blank to be run if sample matrix is urine. Add 2 mL of negative blood to each control, calibrator and blank to be run if sample matrix is blood.

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 1.0 ml 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, ~ 1250 ng/mL morphine. Since a 1:1 dilution would be adequate for quantitation, a 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 5.3. The dilution details, including the

dilution factor (Volume sample: Volume Diluent + Volume Sample) are documented on the batch worksheet.

5.4 Using a validated dispensing pipette, place 2 mLs aliquot of each sample replicate culture tube for Urine and 2 mL aliquot of each sample replicate culture tube for Blood. Note: Cal/CTL stock addition volumes are the same for blood and urine, however, stock solutions as appropriate for the matrix must be used .

5.5 Using a validated dispensing pipette, add 40 ul of deuterated opioid internal standard stock solution to each replicate, blank, calibrator and control.

5.6 Using a validated dispensing pipette, add 400 ul of opioid control standard stock solutions to the tube labeled Calibrator.

5.7 Using a validated dispensing pipette, add 200 ul of opioid calibrator standard stock solution to the tube labeled High Control.

5.8 Using a validated dispensing pipette, add 100 ul of opioid control standard stock solution to the tube labeled Low Control.

5.9 Using a validated dispensing pipette, add 200 ul of Morphine Glucuronide solution to a tube labeled Glucuronide Control.

5.10 To urine samples, blanks, calibrators and controls only, add 0.5 mL of β-Glucuronidase solution. Hydrolyze for 3 hours at 60°C in water bath.

5.11 Add 3 mL of pH 6.0 0.1 M Phosphate buffer to each sample, calibrator, control and blank.

5.12 Label SPE tubes to correspond with each culture tube, place a clean thru tip on each end and load in the manifold.

5.13 Condition the columns:
1 x 3 mL methanol; aspirate
1 x 3 mL DIW; aspirate
1 x 1 mL 0.1 M Phosphate buffer 6.0; aspirate
DO NOT LET COLUMN GO DRY!

5.14 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.

5.15 Wash column:
1 x 2 mL DIW; aspirate
1 x 2 mL 0.1 M acetate buffer pH 4.5; aspirate
1 x 3 mL methanol; aspirate

5.16 Dry column (15 minutes at > 10 inches Hg)

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

5.18 Elute analytes with: 1 x 3 mL methylene chloride/isopropanol/ammonium hydroxide (39/10/1), Collect Eluent at 1 to 2 mL/minute.

5.19 Remove receiver tubes and evaporate to dryness at < 36 C using a gentle flow of Nitrogen (Turbovap).

5.20 Reconstitute residue with 150 ul of ethyl acetate and transfer to gc/ms vial with limited volume insert, add 50 uL BSTFA (with 1% TMCS), cap and reserve for GCMS analysis with no further evaporation.

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 °
Det. Temp. 120 °
Oven (init.): 80°, 10 ° /min to 300 °, (1 min hold).
19 min total run time
1 ul inj
Method: 6206OP.M

6.2 Injection sequence:

Calibrator
Matrix blank

Control High
Control Low
Glucuronide control
Sample 1 rep 1
Sample 1 rep 2
Samples 2-10 Rep 1 and 2
High Control (Final control in the absence of additional samples)
Additional Samples
Final Control (May be high or low)

6.3 Detection and Identification:

Determination of the presence of Opioids in the sample extract are 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 Opioids, 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. Qualitative identification of each analyte is independent.

6.4 Calibration and Quantitation:

6.4.1 Calibration:

Calibration for each batch and fraction 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 (1000 ug/L for Opiods in urine and 100 ug/L in blood) in addition to the 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, multipoint 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 samples are injected 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 the Section Quality Manager.

7.3 Evaluation of Potential Carryover: Carryover in the chromatograph~~ic quality~~ is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% (10 ng/mL for urine and 1 ng/mL for blood) requires batch evaluation, and remedial action for the instrument (e.g. replacement of injector insert, new septum and perhaps column trim or even replacement). Demonstrated carryover lf 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, both control results for that analyte must be within 20% of the target value.

7.5 Internal Standard:

Note: For any individual injection to be acceptable, the internal standard abundance must be at least 20% of the corresponding abundance in the calibrator.

7.6 Linearity:

7.6.1 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 cocaine or benzoylecgonine concentrations greater than the corresponding calibrator will be re-analyzed with an appropriate dilution (see section 5.3, 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.

7.7 SENSITIVITY- LIMITS OF DETECTION (LOD) and QUANTITATION (LOQ):

7.7.1 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.

7.8 Accuracy and Precision:

7.8.1 Precision of the procedure is evaluated on a case by case basis or by repeat analyses of control or PT materials. 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

7.9 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 Opioid drugs 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:

Opioid 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;** The program of multiple layers of checks that are performed both during and after analysis provides the basis for the Quality Assurance of the procedure. 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 evaluated by a reviewer distinct from the operator, (Batch Technical Review) including an evaluation of qualitative and quantitative results (as applicable)**:**

a. Control Results
b. Chromatographic characteristics of calibrators, controls and samples.
c. Consistency of data between analytical and summary documents
d. Peak area, shape, internal standard response (including

abundance) retention time,

e. Preparation of calibrators, and controls (correct amount and concentration of fortifications).

f. Completion of Analyst batch review

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

GC/MS temperature program specifications

Temperature program			
Parameter			
Initial temp	120° C		
Initial Time	0.00min		
Ramps Rate/final temp/hold time	rate	Temp	time
	10.0	300	1.0
Post temp	120°c		
Post time	0.00 min		
Run Time	19 min		
Front inlet			
Mode	Pulsed splitless		
Initial temp	250°c		
Pressure	10.75 psi		
Pulse pressure	25 psi		
Pulse time	0.50 min		
Total flow	43.4 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	15.4 minutes		
M/Zs Group 1 Group 2 Group 3 Group 4	 374, 359, 271, 256, 343 302,273,299,270,256 432,417,429,414,401,360,303,357,342,286, 390,375,387,372,229		

Dwell	50

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

CT DPS; Toxicology Laboratory

Batch ID: 9-15-10

Opiate GCMS Batch Summary Page 1

Autosampler vial position	Sample (Case Number)	Specimen (Matrix)	Specimen Volume, (ml)	Cal. Stock Vol. (ul)	Con. Stock Vol. (ul)	Int. Std. Volume (ul)	Analyte	Abundance I.S. (x 1E4)	Theoretical Concentration (mg/L)	Observed Concentration (mg/L)	Percent Recovery (Acceptable: 80 - 120)	Acceptable *** Analyst	Technical Review**
3	Calibrator	Blood	1.0	200		80	Codeine 15.58	399	2.00	2.00			
				200		80	Hydrocodone 15.67	438	2.00	2.00			
				200		80	Morphine 15.99	375	2.00	2.00			
				200		80	Hydromorphone 16.01	97.8	2.00	2.00			
				200		80	Oxycodone 16.55	12.9	2.00	2.00			
4	Blank	Blood	1.0			80	Codeine	468	0.00	ND			
						80	Hydrocodone	479	0.00	ND			
						80	Morphine	396	0.00	ND			
						80	Hydromorphone	92.1	0.00	ND			
						80	Oxycodone	20.7	0.00	ND			
5	High Control	Blood	1.0		100	80	Codeine	515	1.00	0.932	93.2		
					100	80	Hydrocodone	569	1.00	0.932	93.2		
					100	80	Morphine	469	1.00	0.952	95.2		
					100	80	Hydromorphone	121	1.00	1.38	138		
					100	80	Oxycodone	17.7	1.00	0.920	92.0		
7	Low Control	Blood	1.0		20	80	Codeine	514	0.200	0.194	97.0		
					20	80	Hydrocodone	565	0.200	0.195	97.5		
					20	80	Morphine	442	0.200	0.192	96.0		
					20	80	Hydromorphone	74.3	0.200	0.750	375		
					20	80	Oxycodone	22.8	0.200	0.212	106		
8	ID-10-1374	Serum	0.5			80	NDD: Nop drugs detected	467					
9	ID-10-1374	Serum	0.5			80	NDD: Nop drugs detected	424					
5	High Control	Blood	1.0		100	80	Codeine	534	1.00	0.937	93.7		
					100	80	Hydrocodone	569	1.00	0.935	93.5		
					100	80	Morphine	500	1.00	0.950	95.0		
					100	80	Hydromorphone	160	1.00	1.45	145		
					100	80	Oxycodone	23.2	1.00	0.908	90.8		

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

Vial position verified prior to sample removal _____ * Carryover check: Opiates < 5 ng/ml ? ____ Yes ___ No

Elution solvent valid? ____ Yes ____ No Derivitizing agent valid? ____ Yes ____ No

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

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

Acccepted quantitative results have internal standard abundances of at least 20% of internal standard abundance observed in calibrator.

Analyst Comments: ND = Not detected.

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

Reviewer Comments: