1.0 PRINCIPLE

Blood and urine samples, that require examination for "Weak Acid," Neutral and/or Basic Drugs (WAN/BDS) by GC/MS, are extracted using mixed-bed solid phase extraction columns.

Weakly Acidic or Neutral (WAN) drugs are extracted into a hexane/ethyl acetate and basic drugs that may be present are extracted into a methylene chloride/isopropanol/ NH4OH mixture After evaporation of the solvents, the extracted drug(s) are analyzed by GC/MS in Scan mode.

Qualitative identification of the WAN and/or Basic Drugs by GC/MS is based on retention time and ion ratios for 3 ions compared to the corresponding ion ratios from a calibrator run in the same batch, or "full-scan" spectra fragmentation pattern compared to a reference library based fit. Matrix-specific (blood and/or urine) positive and negative controls are extracted and analyzed in each analytical batch. Cyproheptadine and ethinamate are used as internal standards for basic and acid neutral drugs. The presence of WAN and Basic Drugs may be confirmed in urine, blood or other aqueous fluids.

2.0 SPECIMEN

A. The LIMS system can be used to generate a worklist for specimens requiring confirmation for WAN/BDS.

B. 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.

C. 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.

D. 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.

E. When all of the analyses of samples in the toxicology section are 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 the person placing the seal clearly marked on, or

proximate to, that seal.

F. Samples are maintained in the Toxicology Section for 8 weeks after the case is completed. Case completed means the final report was issued. The starting date for the hold period begins on the date the final report is mailed. Samples will be held longer when requested. After this period, in the absence of notification of any legal action or other reason to maintain the samples, samples are discarded in the appropriate medical waste disposal container. Samples from fatalities, or cases with requests for retention are maintained by the laboratory. Sexual assault cases are returned to the submitting agency. Cases classified as "other" types may be returned to the submitting agency. Consult with supervisor and/or case management for any special cases.

3.0 EQUIPMENT:

Note: Comparable or equivalent equipment may be used.

GC/MS and associated data station/computer (HP6890/5973, 5975, or 5977)
General laboratory glassware and equipment
Solid phase extraction manifold (SPEware and associated equipment)
Analytical evaporator (SPEware)
Water Bath
UCT; ZSDAU020 "Clean Screen" solid-phase extraction tubes
Centrifuge (Beckman)
Oven (Blue M)

4.0 REAGENTS:

A. Reagents available as stock items: (Sigma or J.T. Baker Reagent Grade or equivalent unless otherwise specified)

Methanol (CH_3OH)
Ammonium hydroxide (NH_4OH)
Ethyl acetate ($C_4H_8O_{2)}$
Hexane ($CH_3(CH_2)_4CH_3$)
Methylene chloride (CH_2CH_2)
Isopropanol (C_3H_8O)
Glacial acetic acid ($C_2H_4O_2$)
Sodium phosphate dibasic (Na_2HPO_4)
Sodium phosphate monobasic ($NaH_2\ PO_4$)

Sodium acetate trihydrate ($CH_3COONa•_3H_2O$)
Hydrochloric acid (HCl)

Other Reagents

β-Glucuronidase (p. vulgata ; Sigma or equivalent)
Blank Blood (may be acquired from Hartford Hospital)
Drug free urine (donated by laboratory personnel)
Morphine glucuronide 1 mL vial 1.0 mg/mL (Alltech, Sigma or equivalent)
Deionized water (DIW; Millipore or equivalent In-House supply)

B. Reagents prepared in the Toxicology Laboratory:
Volumes may be proportionally changed.

1. 0.1 M phosphate buffer pH 6.0 1Liter

   a. Combine 1.7 g sodium phosphate dibasic Na_2HPO_4 and 12.14 g sodium phosphate monobasic NaH_2PO_4
   b. QS to 1000 mL using DIW
   c. Storage: room temperature in glass or plastic. Stability: 6 months.

2. 1.0 M Acetic Acid 500mL

   a. To approximately 400 mL DIW in a graduated cylinder,
   b. Add 28.6 mL glacial acetic acid
   c. Mix, QS to 500 mL
   d. Storage: room temperature in glass or plastic. Stability: 6 months

3. 0.1 M Acetic Acid 500mL

   a. Dilute 50 mL 1.0M acetic acid to 500 mL with DIW
   b. Mix. Storage: room temperature in glass or plastic. Stability: 6 months.

4. 1.0 M Acetate buffer (pH 5.0) 500mL

   a. To approximately 400 mL DIW in a graduated cylinder,
   b. Dissolve 42.9 g sodium acetate trihydrate in approximately 400 mL DIW
   c. Add 10.4 mL glacial acetic acid $C_2H_4O_2$
   d. Dilute to 500 mL with DIW

d. Mix. Check pH, adjust pH to 5.0 ± 0.1 with 1.0 M acetic acid if needed.
e. Storage: room temperature in glass or plastic. Stability: 6 months
f. Inspect daily for contamination.

5. 0.1 M Acetate Buffer (pH 5.0)

a. Dilute 20 mL 1.0 M acetate buffer to 200 mL with DIW
b. Mix. Store at: room temperature in glass or plastic. Stability: 6 months

6. Elution Solvent: Methylene chloride/Isopropanol/ammonium hydroxide (39/10/1)

Note: Adjust volume as needed for the total number of tubes; 3 mL needed for each tube. Must be prepared fresh each day of use.

Example. To make 150 mL of solution:

a. First combine 30 mL isopropanol and 3 mL ammonium hydroxide into a 200 mL graduated cylinder with stopper cap
b. Then add 117 mL methylene chloride (prevents buildup of pressure)
c. Cap, mix.

Note: Dispose of unused elution solvent mixture in the halogenated waste stream container

7. β -Glucuronidase, (5,000 F units/mL) in 0.1 M Acetate Buffer (pH 5.0)

Prepare daily for use, make slight excess for each batch, each 1 mL sample requires 2500 F units. Add 0.5mL of β -Glucuronidase to each tube.

Example: for 48 total tubes prepare 25 mL

Calculate activity for each lot of β-Glucuronidase as follows:
(Lot specific, value from bottle label)
e.g. 1,439,000 -glucuronidase units/g solid

$$\frac{5{,}000 \text{ Units/mL}}{x \text{ mg}} = \frac{1{,}439{,}000}{1000 \text{ mg}}$$

$$x = 3.47 \text{ mg/mL}$$

To make 25 mL

a. Weigh out 87 mg β –Glucuronidase solid. Add to 25 mL of 0.1 M acetate buffer (pH 5.0)
b. Dissolve before use by swirling gently.
c. Make fresh daily as needed for each batch.

8. 1% methanolic HCl 100 mL

a. Dilute 1 mL Concentrated HCl into 100 mL methanol.
b. Store at room temperature in glass or plastic. Stability: 6 months
c. Inspect daily before use for contamination. If any visible bacterial growth is present, discard and make fresh.

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

C. 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 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. 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 SOP #11.

5.0 CALIBRATION STANDARDS/IN-HOUSE CONTROL STANDARDS/INTERNAL STANDARDS:

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

A. Stock Calibrator and Control solutions: Comprised of various drugs as needed; (Alltech or Cerilliant; 1 mg/mL). Composition of the Calibrator (analyte and concentration) will be dependent on the current patterns of observed drugs, and

normal levels at which those drugs are observed. (E.g., Phenobarbital cal = 5.0 mg/L, Ketamine cal = 0.5 mg/L)

Reference material standards target analyte drug stocks are obtained from Sigma/Aldrich, Cerilliant, Lipomed, Grace or other equivalent manufacturers. 1 mL ampules 1.0 mg/mL or 100ug/ mL

IS drug stocks: Cyproheptadine (Powder, stored in CS safe)
IS drug stocks: Ethinamate (Powder, stored in CS safe)

1. Working Standard Solution 10 ug/mL
   a. Into a 10mL volumetric flask partially filled with methanol
   b. Pipette 100 uL of each reference standard (1 mg/mL)
   c. QS with methanol, mix
   d. Store in freezer (-0^{0}C)
   e. Stable 2 years when stored tightly capped

2. Diluted Working Standard Solution 1.0 ug/mL
   a. Pipette 100 uL of Working Standard Solution (10ug/mL) into a borosilicate culture tube
   b. Add 900 uL of methanol
   c. Cap, Vortex mix
   d. Store in freezer (-0^{0}C)
   e. Stable 1 year when stored tightly capped

   Note: This procedure utilizes controls prepared (spiked) in blank blood and/or urine (as appropriate to batch makeup) as follows: Each quantitative assay must 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 WAN and Basic drugs from validated stock solutions to blank sample matrix aliquots, prior to extraction, (details in Procedure below). Acceptable quantitative control performance is target value +/- 20%.

B. Internal Stock Standards

1. Cyproheptadine 1.0 mg/mL in methanol 25 mL

   a. In a 25 mL volumetric flask, weigh out 32.52 mg of cyproheptadine

hydrochloride sesquihydrate (MW 350.88 FW 287.398)

b. Dissolve in and QS to volume with methanol
c. Store in freezer (-0ºC)
d. Stable 2 years when stored tightly capped.

2. Ethinamate 1.0mg/mL in methanol 25 mL
   a. In a 25 mL volumetric flask, weigh out 25 mg of ethinamate
   b. Dissolve in and QS to volume with methanol
   c. Store in freezer (-0ºC)
   d. Stable 2 years when stored tightly capped.

3. Working Internal Standard Solution (Cyproheptadine 1ug/mL, Ethinamate 2 ug/mL)

   a. To a 100 mL volumetric flask partially filled with methanol
   b. Pipette 100 uL of stock 1 mg/mL cyproheptadine
   c. Pipette 200 uL of stock 1 mg/mL ethinamate
   d. QS to volume with methanol
   e. Store in freezer (-0ºC)
   f. Stable 2 years when stored tightly capped.

The following analytes and supplier are listed in the tables: Other manufactures, Lipomed, Alltech may be used.

Weak Acid Neutral Drugs

S.No.	Standard	Concentration of standard	Manufacturer
1	butalbital	1.00 mg/ml	Cerilliant
2	carisoprodol	1.00 mg/ml	Cerilliant
3	ibuprofen	1.00 mg/ml	Cerilliant
4	lorazepam	1.00 mg/ml	Cerilliant
5	meprobamate	1.00 mg/ml	Cerilliant
6	oxazepam	1.00 mg/ml	Cerilliant
7	pentobarbital	1.00 mg/ml	Cerilliant
8	phenobarbital	1.00 mg/ml	Cerilliant
9	phenytoin	1.00 mg/ml	Cerilliant
10	secobarbital	1.00 mg/ml	Cerilliant
11	talbutal	1.00 mg/ml	Grace
12	temazepam	1.00 mg/ml	Cerilliant

13	topiramate	1.00 mg/ml	Cerilliant

Basic Drugs

	Standard	Concentration of Standard	Manufacturer
1	Amphetamine	1.00 mg/ml	Cerilliant
2	Methamphetamine	1.00 mg/ml	Cerilliant
3	3,4-MDA	1.00 mg/ml	Alltech
4	3,4-MDMA	1.00 mg/ml	Cerilliant
5	alprazolam	1.00 mg/ml	Cerilliant
6	amitriptyline	1.00 mg/ml	Cerilliant
7	bupropion	1.00 mg/ml	Cerilliant
8	chlorpheniramine	1.00 mg/ml	Cerilliant
9	citalopram	1.00 mg/ml	Alltech
10	clonazepam	1.00 mg/ml	Cerilliant
11	cocaine	1.00 mg/ml	Cerilliant
12	codeine	1.00 mg/ml	Cerilliant
13	cyclobenzaprine	1.00 mg/ml	Cerilliant
14	desipramine	1.00 mg/ml	Cerilliant
15	dextromethorphan	1.00 mg/ml	Cerilliant
16	diazepam	1.00 mg/ml	Cerilliant
17	diltiazem	1.00 mg/ml	Cerilliant
18	diphenhydramine	1.00 mg/ml	Cerilliant
19	doxepin	1.00 mg/ml	Cerilliant
20	doxylamine	1.00 mg/ml	Cerilliant
21	fluoxetine	1.00 mg/ml	Cerilliant
22	haloperidol	1.00 mg/ml	Cerilliant
23	hydrocodone	1.00 mg/ml	Cerilliant
24	hydroxyzine	1.00 mg/ml	Alltech
25	ketamine	1.00 mg/ml	Cerilliant
26	meperidine	1.00 mg/ml	Cerilliant
27	methadone	1.00 mg/ml	Cerilliant
28	methaqualone	1.00 mg/ml	Cerilliant
29	methylphenidate	1.00 mg/ml	Alltech
30	nordiazepam	1.00 mg/ml	Cerilliant
31	nortriptyline	1.00 mg/ml	Cerilliant
32	oxycodone	1.00 mg/ml	Cerilliant
33	phencyclidine	1.00 mg/ml	Cerilliant
34	phentermine	1.00 mg/ml	Cerilliant
35	sertraline	1.00 mg/ml	Cerilliant

36	tramadol	1.00 mg/ml	Cerilliant
37	trazodone	1.00 mg/ml	Cerilliant
38	venlafaxine	1.00 mg/ml	Cerilliant
39	zolpidem	1.00 mg/ml	Cerilliant
40	propofol	1.00 mg/ml	Cerilliant
41	hydromorphone	1.00 mg/ml	Cerilliant
42	oxymorphone	1.00 mg/ml	Cerilliant
43	EDDP	1.00 mg/ml	Cerilliant
44	Ranitidine	1.00 mg/ml	Cerilliant
45	BE	1.00 mg/ml	Cerilliant
46	Morphine_3β_D_Glucuronide	1.00 mg/ml	Cerilliant

Note: Other analytes can be added to these mixtures. For specific targeted quantitative analyses, only the analytes of interest need to be added to the calibrator and control solutions.

Urine Qualitative

The target concentration for the positive urine calibrator/standard/control is 500 ng/mL
Pipette 50 uL of the Working Standard Solution (10ug/mL) into 1.0 mL blank matrix
Continue to follow sample preparation procedure .

Blood Quantitative
Refer to table in Procedure section
Continue to follow sample preparation procedure

6.0 QUALITY CONTROLS

Calibrators and controls must be independently prepared from a separate weighing or initial dilution or obtained from other sources. When available commercial reference controls will be purchased from an outside vendor. If commercial controls are not available, In-House controls should be prepared from a different provider. When only one supplier is available, a lot different from the calibrator should be used. At the least, when there is only one source, a separate preparation, different from the calibration standard may be used.

A. Urine Control DAU LC 2, Product # 50703, Utak Laboratories Valencia, CA

91355

1. Remove cap from vial
2. Reconstitute control material by adding exactly 10.0 mL of DIW, using a 10 mL volumetric pipette.
3. Replace cap and let sit 10 – 15 minutes.
4. Swirl gently 3 – 4 minutes to ensure homogeneous mixture.
5. Swirl gently each time an aliquot is removed to ensure a homogeneous mixture.
6. Assay control material in the same manner as case specimens.
7. For quantitative assays, record the results obtained on a quality control chart in Excel, that describes statistcal limits for the test method and the particular lot of the control material.
8. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution.

B. Morphine 3-β-D-glucuronide working solution 100 ug/mL

a. pipette 100 uL of stock 1mg/mL reference morphine 3-β-glucuronide into a 10 mL volumetric flask
b. QS with methanol
c. Stable 1 years when stored tightly capped in freezer (-0°C)

C. Urine Control Morphine 3-β-D-glucuronide. (20 ug/mL glucuronide, 12.4ug/mL free morphine)

For hydrolysis batches

a. pipette 200 uL of 100 ug/mL morphine 3-β-glucuronide solution to the Hydrolysis Control tube.
b. Add 800 uL blank urine

7.0 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 Deputy 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 Deputy 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.

Prior to running case work, the GCMS instrument suitability must be acceptable. The acceptance criteria is that all analytes, suitable by GCMS analysis, in the unextracted mixed standard (Daily Standard or External Standard) are present. The responses of each analyte are at least 10 times the signal to noise ratio and the responses haven't changed significantly from previous runs. The retention times should also be consistent, allowing for slight changes when the column is clipped. The peak shapes of analytes should be symmetrical, no unusual tailing. The MS reference library search match must identify all analytes in the suitability mixture.

A. Sample Preparation

1. Blood
   a. Pipette 1 mL blood into an appropriately labeled 16x 100 borosilicate culture tube
   b. Add 3 mL phosphate buffer (pH= 6.0)
   c. Add 100 ng cyproheptadine, 200 ng ethinamate by pipetting 100 uL of working internal standard solution (Cyproheptadine 1ug/mL, Ethinamate 2 ug/mL)
   d. Mix, Sonicate for 15 minutes
   e. Centrifuge 10 minutes at 2500 rpm
   f. Skip to Extraction Apply Sample step

   Note: Transfer supernatant before pellet dissipates

2. Urine Hydrolysis
   a. Pipette 1.0 mL of each urine sample into an appropriately labeled tube
   b. Add 100 ng cyproheptadine, 200 ng ethinamate by pipetting 100 uL of working internal standard solution (Cyproheptadine 1ug/mL, Ethinamate 2 ug/mL)
   c. Add 0.5 mL of 5,000 F units/ mL β-glucuronidase to each tube
   d. Mix/vortex, Incubate for a minimum of 3 hours or overnight at 60°C in water bath or oven.
   e. Cool to room temperature
   f. Add 3 mL phosphate buffer (pH= 6.0)
   g. Centrifuge for 10 minutes at 2500 rpm
   h. Skip to Extraction Apply Sample step

3. Urine Not Hydrolyzed

a. Pipette 1 mL of each urine sample into an appropriately labeled borosilicate culture tube
b. Add 3 mL phosphate buffer (pH= 6.0)
c. Add 100 ng cyproheptadine, 200 ng ethinamate by pipetting 100 uL of working internal standard solution (Cyproheptadine 1ug/mL, Ethinamate 2 ug/mL)
d. Mix
e. Centrifuge 10 minutes at 2500 rpm
f. Skip to Extraction Apply Sample step

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 dilution process shall be documented in the case jacket.

According to the table below, pipette 1mL of sample, blank, calibrator and control to each appropriately labeled 16x 100 screw top borosilicate culture tube

Calibrator Concentration ng/mL	uL Working Standard (10 ug/mL)	uL diluted Working Standard (1.0 ug/mL)	Blank Blood Pipette uL
0-blank	0	0	1000
50	----	50	950
100	----	100	900
200	20		980
500	50		950
1000	100		900
QC Concentration	uL Stock Control (10 ug/mL)		
200	20		980
1000	100		900
	Utak Control		
Utak Low	500		500
Utak High	1000		0

Final volume of each calibrator, blank and control should be 1 mL.

B. Extraction

1. Condition the Clean Screen® Extraction columns:
   1 x 3 mL methanol; drain to hazardous solvent waste stream
   1 x 3 mL DIW; drain to regulated biohazard waste
   1 x 1 mL 100 mM Phosphate buffer; drain to regulated biohazard waste.

Note: Aspirate or drain at <3 inches Hg to prevent sorbent drying

DO NOT LET COLUMN GO DRY!

Note: Conditioning of SPE columns can be started when samples are being centrifuged.

2. Apply sample

3. Transfer contents, supernatants of prepared blood and /or urine samples, ~~contents of each tube~~ to the appropriately labeled conditioned SPE tube, and allow gentle drop wise flow until the level reaches the top of the column bed. Load at 1 mL/minute

4. Wash column:
   1 x 2 mL Phosphate buffer; drain to regulated biohazard waste
   1 x 2 mL 0.1 M Acetic acid; drain to regulated biohazard waste
   Dry column (5 minutes at > 10 inches Hg)

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

6. Elute Acid analytes:
   1 x 1 mL Hexane
   1 x 3 mL Ethyl acetate
   Collect Eluent at 1 to 2 mL/minute

7. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen.

8. Reconstitute residue with 150 uL of ethyl acetate and transfer to gc/ms vial with limited volume insert, cap and reserve for GCMS analysis.

9. Wash column:
   1 x 3 mL methanol; aspirate drain to hazardous solvent waste stream.

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

    Position another set of clean appropriately labeled 13x100 borosilicate test tubes under each SPE column, with tip inside collection test tube.

11. Elute Basic analytes: Elution Solvent Methylene chloride/IPA/NH_4OH (39:10:1)
    1 x 3 mL Methylene chloride/IPA/NH_4OH (39:10:1)
    Collect Eluent at 1 to 2 mL/minute.

12. Add 1 drop of 1% methanolic HCl to each tube

13. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen

14. Reconstitute residue with 150 uL of ethyl acetate and transfer to gc/ms vial with limited volume insert, cap and reserve for GCMS Analysis. Refrigerate vials if extracts won't be injected on the instrument on the same day. Stable at least one week when tightly capped.

8.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

A. Instrument and Setup:
GCMS/Autosampler: (Hewlett-Packard 6890/5973, or equivalent)
Column: 30M RTX-5MS or 30M RTX-1MS (0.25 mm ID; 0.25 micron film)
Inj. Temp. 250 ^{o} Det. Temp. 280 ^{o}
Oven (init.): 80^{o}, 25 ^{o} /min to 300 ^{o}, (5.4 ~~10~~ min hold).
15.2 min total run time 1 uL inj
Scan range 40 to 470 amu
Method: Drugsmwa.M; Method Details Appended (Appendix II)

B. Batch Format: Analytical batches for WAN and Basic drug confirmations should follow the format indicated below:

1. Inject 1 uL into the GCMS using the following sequence

a. Urine Qualitative

1 . Solvent blank
2. Positive urine calibrator/standard 0.500 ug/mL
3. Urine blank
4. Morphine Glucuronide Control (Hydrolysis Batches Only)
5. Utak control
6. Case samples
7. etc
8. Calibrator or control after every 10 case samples (new or reinjection).
9. End batch with calibrator or control

b. Blood Quantitation multipoint calibrators

Note: Number of calibrators and concentration can change based on analytes of interest and the expected therapeutic and toxic range.

1. Solvent blank
2. Prime Calibrator/
3. Blood blank
4. Calibrator 1 (50 ng/mL)
5. Calibrator 2 (100 ng/mL)
6. Calibrator 3 (200 ng/mL)
7. Calibrator 4 (500 ng/mL)
8. Calibrator 5 (1000 ng/mL)
9. Blood blank
   If available run Utak control(s)
10. Low control (100 ng/mL)
11. High control (1000 ng/mL)
12. Case sample
13. etc
    Run set of Low and High controls midway through the batch or approximately after 10 case samples (new or reinjection)
14. End batch with set of controls

c. File name - Include case number (including year) in each sample data file

d. Verify the sequence; verify the vial positions in the tray match sequence.

9.0 DETECTION AND IDENTIFICATION

A. MS Analysis Criteria for a GCMS single nominal mass analysis and EI

Determination of the presence of WAN and Basic drugs in the sample extract are identified by evaluation of full spectra, and/or 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 drugs, all of. the diagnostic ions with a relative abundance of 10% or greater must match a pre-designated and concurrently analyzed reference. There may be additional ions in the 'unknown' spectrum due to minor interferences that cannot be removed by background subtraction, but all of the diagnostic ions present in the reference spectrum must be present in the 'unknown' unless absent due to low absolute abundance. Relative abundances of the diagnostic ions, as well as relative retention times must always be considered in designating a "positive" match. The retention time must be within 2 ~~5~~% of the corresponding analyte in the calibrator injection. When selected ion monitoring is used for identification and/ or quantitation, it must compare ion ratios and retention times between calibrators, controls and unknowns. Whenever possible, three ions must be monitored for the analyte and two ions for the internal standard. The qualifying ions must be +/- 20% relative to a calibrator included in the same run. It is realized that for some analytes it may be difficult to choose multiple ions. Some spectra may contain only one or two ions that are > 5% relative abundance. Therefore, additional or complimentary methods of identification should be used.

Qualitative identification of each analyte is independent.

Note: Qualitative identification of analytes with abundance < 5% of the I.S. abundance should be considered with great care, and evaluation of significance based on the (presumably) low concentration. Detection of metabolites from parent drugs detected adds confidence in the identification of drugs.

The internal standard recovery in the extracted sample is monitored. If the internal standard recovery is substantially reduced, recoveries less than 50% or greater than 200% relative to the calibrators or controls, the quantitative value must be investigated on a case-by-case basis as to whether reporting a quantitative result is appropriate.

10.0 CALIBRATION AND QUANTITATION

Note: Quantitation is not performed on urine analyses.

A. **Calibration:** Calibration for each batch and fraction is done independently. Hence, no sample analysis conducted under DSS guidelines is quantitated based on an historical calibration curve. Urine ~~Calibration~~ positive control is the 0.5 ug/mL calibrator and internal standard spiked into drug-free urine. The negative control is the internal standard spiked into drug-free urine.

B. **Quantitation:** Quantitation is accomplished by the comparison of the response ratio of the analyte in a 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.

For multi-point calibrations, the criteria for acceptability of the calibration, and for individual calibrators is that when the values are read back against the final calibration curve they should be within +/-20% of their value. A slightly wider acceptance value (e.g., +/-25% or +/-30%) is acceptable for calibrators that approach the LOQ of the assay.

Note: The available quantitative range of this procedure is limited to the calibration range. The lowest standard that meets quantitative acceptability criteria and peak identification criteria is the LOQ. Analytes that only meet peak identification criteria may be reported as detected ≤ LOD. LOD is usually the lowest calibrator. Analytes detected ≥ ULOQ may be reported as greater than the highest calibrator or control.

The criteria for a valid calibration GCMS linear regression "r^2" value for a 3 point curve is ≥ 0.98 using non-deuterated internal standards. A significant change in the slope of the calibration line, monitored between runs, may indicate that corrective action needs to be taken.

When more than 3 calibration points are used, one point may be removed if it failed to fall into the acceptable quantitative range. If two or more points need to be removed consult with the Section Supervisor if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative. (In terms of the amount of drug injected on the instrument [thereby allowing for appropriate calculation of diluted samples] is defined for, and validated on, each batch by the high and low control, and the acceptable performance of each.)

11.0 QUALITY CONTROL AND RUN EVALUATION

A. 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 batch ~~run~~ summary sheet.

B. 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 Section Supervisor, Director or the Quality Manager.

C. Evaluation of Potential Carryover: Carryover in the chromatogram is evaluated by injection of a blank sample immediately following the calibrator. Carryover of greater than 2% 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. In practice, when a question of potential carryover exists coming from the previous injection of case sample containing a high concentration of an analyte, the potentially affected sample may be repeated at the end of the batch.

D. Evaluation of Controls: Positive and negative controls are evaluated to allow for procedural batch acceptability.

   Qualitative Control results are documented and evaluated on the batch summary sheet.

E. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics

   Quantitative assays must have controls to verify the calibration and to monitor its stability. Each batch must have at least 10% controls including a positive and negative. The controls can be re-injected in the middle and end of the batch to demonstrate the stability of the calibration. Acceptable results are the mean or target +/- 20% or +/- 2 standard deviations.

Levy-Jennings charts will be used monitor assay performance precision over time.

F. Internal standard abundance should be similar throughout the batch.

G. Linearity: Linearity of the calibration curve is demonstrable in each batch, for each analyte as a function of linear regression and quantitative results of control materials.

H. 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. LOD should be at least 3 times the signal to noise ratio, LOQ should be at least 10 times the signal to noise ratio.

I. Accuracy and Precision: Precision of the procedure is evaluated on a yearly basis, 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.

J. 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 WAN and Basic drugs that elutes within 5% if the retention time of known standard materials, and produce the same fragmentation ions and ratios.

12.0 REPORTING OF RESULTS:

The analyst processes the data and enters results into Justice Trax (LIMS) along with the method used. The final review of toxicology results prior to issuing the toxicology report includes chain of custody documentation, all qualitative and quantitative data, including relevant quality control, in addition to a clerical check of the final report. All data must undergo at least one additional documented technical review by a qualified person, other than the analyst, before a report can be released The responsibility for part of the review may be delegated to quality assurance or other qualified personnel. Different aspects of the review may be conducted by different people. A “qualified” person is defined as someone with sufficient training and experience to perform the

stated review.

Wherever possible, analytical results must be reviewed with reference to whatever case history or other information is available.

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).

13.0 QUALITY ASSURANCE

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

A. 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.

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

1. Control Results
2. Chromatographic Characteristics
3. Transcription errors

C. WAN/BDS 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, the worklist, instrument batch sequence, calibrators, controls, and blanks and is filed separate from the case jacket. Specific chromatograms for each case are filed in the appropriate case jacket file. Results are documented on the case jacket "Summary" sticker on each case file, which includes a reference number for the batch as a whole. A Batch summary sheet will be produced with each batch. Data on each batch should document fields such as: Test name, Batch ID (Date of Batch), analysts who generated data, matrix, and instrument used, Instrument tune and suitability acceptability, vial position verification, acceptability of controls run with the batch and new reagent validation if applicable. Refer to "Batch Summary" form.

D. The original run is compared to the Final Report during the Final Technical

~~Director's~~ review, prior to case sign-off.

Case data from failed runs will be maintained (paper or electronic) with the batch package and available for review, as it forms part of the record of testing performed on any given specimen/case and may be important in the overall context of case review.

14.0 SOURCES OF ERROR

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

15.0 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

Parameter			
Initial temp	80° C		
Initial Time	~~0~~1.00min		
Ramps Rate/final temp/final time	rate	temp	time
	25.0	300	~~14~~7.00
Post temp	80°c		
Post time	0.00		
Run Time	~~14.0~~ 16.8 min		
Front inlet			
Equilibration time	0.5 min		
Mode	Pulsed splitless		
Initial temp	250°c		
Constant Flow	1.0 mL/ min		
Pressure	8.56 psi varies		
Pulse pressure	~~25~~ 35 psi		
Pulse time	0.50 min		
Purge flow	40.0 mL/ min		
Total flow	43.4 mL/min		
Gas type	~~Helium~~		
Injection volume	1microliter		
Post injection washes Solvent A / Solvent B	Solvent A -3 Solvent B - 3		
Tune file	A ~~S~~TUNE.U		
Acquisition mode	Scan		
Solvent delay	~~4.00~~ 2.5 min~~utes~~		
Low mass	40		
High mass	470		
Threshold	250		
MS Quad	150°c max 200		
MS Source	230°c max 250		

Batch Name & Number:				**Batch summary**
	Analyst	Date	Test	Comments
Setup				
Samples Aliquoted				
Instrument # & Run Date				
Tune acceptable	Yes No			
Suitabilty acceptable				
Daily Standard	Yes No			
External Standard	Yes No			
New Reagents for Validation				
Vial Position Verified by				
QC- Qualitative				
QC negative acceptable	Yes No			
QC positive acceptable	Yes No			
Analyst Comments:				
Batch Reviewer Comments:				
Batch Acceptable	Yes No			
Batch Reviewer Initials/ Date				