

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/ NH_4OH 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 SAFETY

This procedure is carried out in a laboratory environment, and standard safety procedures appropriate for such an environment should be utilized, including (minimally) gloves, safety glasses and protective clothing (lab coat). Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be cleaned and disinfected prior to any further use.

3.0 SPECIMEN

- A. Urine
- B. Blood
- C. Serum

D. Other Matrices e.g. meat, tissue, liquids

1. Work lists are generated through JusticeTrax. Specimens comprising an analytical batch are obtained from LIMS system work list, additional samples can be added to the worklist that were not generated by LIMS
2. All evidence transfers, either between individuals or between an individual and storage location must be documented on the Chain of Custody for the case JusticeTrax.
3. When not in the sampling or aliquot process, samples in the Toxicology unit will be maintained in locked storage within the Toxicology unit.
4. 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.
5. 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.
6. Samples are maintained in the Toxicology Section for a minimum of 8 weeks after the 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 the submitting agency the evidence will be returned to the submitting agency.

4.0 EQUIPMENT:

Note: Comparable or equivalent equipment may be used.

- A. GC/MS and associated data station/computer (Hewlett-Packard 6890/5973, Agilent Technologies 7890A, AT-7890B or equivalent)
- B. General laboratory glassware and equipment
- C. Solid phase extraction manifold
- D. Analytical evaporator

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E. Water Bath

F. UCT; ZSDAU020 "Clean Screen" solid-phase extraction tubes

G. Centrifuge

H. Sonicator

I. Vortex

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

1. Methanol (CH₃OH)
2. Ammonium hydroxide (NH₄OH)
3. Ethyl acetate (C₄H₈O₂)
4. Hexane (CH₃(CH₂)₄CH₃)
5. Methylene chloride (CH₂Cl₂)
6. Isopropanol (C₃H₈O)
7. Sodium phosphate dibasic (Na₂HPO₄)
8. Sodium phosphate monobasic (NaH₂PO₄)
9. Sodium acetate trihydrate (CH₃COONa•3H₂O)
10. Hydrochloric acid (HCl)

B. **OTHER REAGENTS:**

1. β-Glucuronidase (p. vulgata ; Sigma or equivalent)
2. Blank Blood (may be acquired from a blood bank)

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3. Drug free urine (donated in-house)
4. Morphine glucuronide 1.0 mg/mL (Alltech, Sigma or equivalent)
5. Deionized water (DIW; Millipore or equivalent In-House supply)

C. REAGENTS PREPARED IN THE TOXICOLOGY LABORATORY:

Volumes may be proportionally changed.

1. **0.1 M PHOSPHATE BUFFER PH 6.0** 1 LITER
 - 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: 12 months.
2. **1.0 M ACETIC ACID** 500 mL
 - 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: 12 months
3. **0.1 M ACETIC ACID** 500 mL
 - a. Dilute 50 mL 1.0 M acetic acid to 500 mL with DIW
 - b. Mix. Storage: room temperature in glass or plastic. Stability: 12 months.
4. **1.0 M ACETATE BUFFER (PH 5.0)** 500 mL
 - 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 $\text{C}_2\text{H}_4\text{O}_2$

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- d. Dilute to 500 mL with DIW
 - e. Mix. Check pH, adjust pH to 5.0 ± 0.1 with 1.0 M acetic acid if needed.
 - f. Storage: room temperature in glass or plastic. Stability: 12 months
 - g. Inspect daily for contamination or turbidity.
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: 12 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 and mix.
 - d. Note: Dispose of unused elution solvent mixture in the halogenated waste stream container
7. **B -GLUCURONIDASE, (5,000 F UNITS/ML) IN 0.1 M ACETATE BUFFER (PH 5.0)**

Example: 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

$$\frac{5,000 \text{ Units/mL}}{x \text{ mg/mL}} = \frac{1,439,000 \text{ Units}}{1000 \text{ mg}} \quad x = 3.47 \text{ mg/mL}$$

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- a. Prepare daily for use, make slight excess for each batch, each 1 mL sample requires 2500 F units. Add 0.5 mL of β -Glucuronidase to each tube.
- b. Example: for 48 total tubes prepare 25 mL
- c. To make 25 mL:
 - i. Weigh out 87 mg β -Glucuronidase solid. Add to 25 mL of 0.1 M acetate buffer (pH 5.0)
 - ii. Dissolve before use by swirling gently.

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.

D. **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 documented on the method run sheet. Newly prepared reagents may be evaluated for validity on an analytical batch, prior to any consideration of sample results. Preparation of reagents and their validation is documented in the Toxicology Section Reagent Preparation Validation Logbook, maintained in the Toxicology laboratory.

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

- A. Note: Preparation of all calibrator and control solutions is documented in the "Calibrator and Control Preparation Log" (maintained in the Toxicology Wet Laboratory).
- B. Stock Calibrator and Control solutions: Comprised of various drugs as needed; (Alltech, Cerilliant or equivalent; 1 mg/mL). Composition of the Calibrator (analyte and

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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)

1. Reference material standards are obtained from Sigma/Aldrich, Cerilliant, Lipomed, Grace or other equivalent manufacturers. 1 mL ampules at concentrations of 1.0 mg/mL or 100 ug/ mL or as available by the provider.

C. Internal Standards:

1. Cyproheptadine
2. Ethinamate

D. Working Standard Solution 10 ug/mL

1. Into a 10 mL volumetric flask partially filled with methanol
2. Pipette 100 uL of each reference standard (1 mg/mL)
3. QS with methanol, mix
4. Store in freezer/refrigerator
5. Stable 2 years when stored tightly capped

E. Diluted Working Standard Solution 1.0 ug/mL

1. Pipette 100 uL of Working Standard Solution (10 ug/mL) into a borosilicate culture tube
2. Add 900 uL of methanol
3. Cap and Vortex
4. Store in freezer/refrigerator
5. Stable 1 year when stored tightly capped

*Approved by Director: Dr. Guy Vallaro*F. 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/refrigerator
 - 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/refrigerator)
 - 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/refrigerator
 - f. Stable 2 years when stored tightly capped
4. Urine Qualitative
 - a. The target concentration for the positive urine calibrator/standard/control is 500 ng/mL
 - b. Pipette 50 uL of the Working Standard Solution (10ug /mL) into 1.0 mL blank matrix
 - c. Continue to follow sample preparation procedure
5. Blood Quantitative
 - a. Refer to table in Procedure section
 - b. Continue to follow sample preparation procedure
6. Weak Acid Neutral Drugs Reference Standards

S.No.	Standard	Concentration of standard	Manufacturer
1	butalbital	1.00 mg/ml	Cerilliant
2	carisoprodol	1.00 mg/ml	Cerilliant

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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

7. Basic Drugs Reference Standards

	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

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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 3 β -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.

Manufacturers listed are suggested. Equivalent manufacturers can be used as needed.

8. Urine Qualitative Test

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

9. Blood Quantitative Test

- Refer to table in Procedure section.
- Continue to follow sample preparation procedure.

7.0 QUALITY CONTROLS

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- A. Calibrators and controls must be independently prepared from a separate 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 (prepared by a 2nd analyst) should be used.
1. Urine Control DAU LC 2, Product # 50703, Utak Laboratories Valencia, CA 91355
 - a. Remove cap from vial
 - b. Reconstitute control material by adding exactly 10.0 mL of DIW, using a 10 mL volumetric pipette
 - c. Replace cap and let sit 10 – 15 minutes
 - d. Swirl gently 3 – 4 minutes to ensure homogeneous mixture
 - e. Swirl gently each time an aliquot is removed to ensure a homogeneous mixture
 - f. Handle and test the control material in the same manner as case specimens
 - g. For quantitative assays, record the results of the controls obtained on the Excel Quality Control Chart on the DSS S drive, that describes statistical limits for the test method and the particular lot of the control material
 - h. Store reconstituted control material refrigerated at 2-8°C, stable for 25 days after reconstitution
 2. Morphine 3-β-D-glucuronide working solution 10 ug/mL
 - a. Pipette 100 uL of stock 1 mg/mL reference morphine 3-β-glucuronide into a 10 mL volumetric flask
 - b. QS with methanol
 - c. Stable 1 year when stored tightly capped in freezer (-0°C)
 3. Urine Control Morphine 3-β-D-glucuronide. (2.0 ug/mL glucuronide, 1.24ug/mL free morphine)

For hydrolysis batches

 - a. Pipette 200 uL of 10 ug/mL morphine 3-β-glucuronide solution to the Hydrolysis Control tube
 - b. Add 800 uL blank urine

8.0 PROCEDURE

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A. Sample Preparation

1. Blood

- a. Pipette 1 mL blood into an appropriately labeled 16 x 100 borosilicate culture tube
- b. Add 3 mL phosphate buffer (pH= 6.0)
- c. Add 2 mL DIW Water
- d. Add 100 ng cyproheptadine, 200 ng ethinamate by pipetting 100 uL of working internal standard solution (Cyproheptadine 1 ug/mL, Ethinamate 2 ug/mL)
- e. Mix, Sonicate for 15 minutes
- f. Centrifuge ~8 minutes at ~ 5000 rpm
- g. Go to 'Extraction' step below
- i. Note: Transfer supernatant before pellet dissipates

2. Urine Hydrolysis (optional)

- 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 1 ug/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
- e. Cool to room temperature
- f. Add 3 mL phosphate buffer (pH= 6.0)
- g. Centrifuge for ~8 minutes at ~5000 rpm (if required due to the nature of the sample)
- h. Go to Extraction step below

3. Urine Not Hydrolyzed

- a. Pipette 1 mL of each urine sample into an appropriately labeled borosilicate culture tube. Turbid samples may be filtered prior to this step.
- 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 1 ug/mL, Ethinamate 2 ug/mL)
- d. Mix
- e. Centrifuge ~8 minutes at ~5000 rpm (if required due to the nature of the sample).
- f. Go to Extraction step below.

Note: Cases requiring reanalysis due to high concentrations (i.e. initial analysis is greater than the high control), should be diluted with 0.1 M

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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. Note: this is for quantitative casework only.

4. Quantitative Test: Example of preparation of calibrators and controls.

- a. According to the table below, pipette 1 mL of sample, blank, calibrator and control to each appropriately labeled 16 x 100 screw top culture tube. (Calibrators may not be extracted if using a historical calibration curve).
- b. The final volume for each calibrator, control and blank should be 1mL.

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

B. Extraction

1. Condition the Clean Screen® Extraction columns:

- a. 1 x 3 mL methanol; drain to hazardous solvent waste stream
- b. 1 x 3 mL DIW; drain to regulated biohazard waste

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- c. 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
 - d. DO NOT LET COLUMN GO DRY!
2. Apply sample:
 - a. Transfer prepared sample (supernatants of prepared blood and /or urine samples), to the appropriately labeled, conditioned SPE tube, and allow gentle drop wise flow until the level reaches the top of the column bed. Elute at 1 mL/minute.
 3. Wash column:
 - a. 1 x 2 mL Phosphate buffer; drain to regulated biohazard waste
 - b. 1 x 2 mL 0.1 M Acetic acid; drain to regulated biohazard waste
 - c. Dry column (5 minutes at > 10 inches Hg)
 4. Position appropriately labeled clean 13x100 borosilicate collection test tubes under each SPE column, with tip inside collection test tube.
 5. Elute Acid analytes:
 - a. 1 x 1 mL Hexane
 - b. 1 x 3 mL Ethyl acetate
 - c. Collect Eluent at 1 to 2 mL/minute
 6. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of nitrogen.
 7. Reconstitute residue with 150 uL of ethyl acetate and transfer to GC/MS vial with limited volume insert, cap and reserve for GC/MS 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. Wash column:
 - a. 1 x 3 mL methanol; aspirate drain to hazardous solvent waste stream.
 9. Dry column (15 minutes at > 10 inches Hg)
 - a. Position another set of clean appropriately labeled 13x100 borosilicate test tubes under each SPE column, with tip inside collection test tube.

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10. Elute Basic analytes: Elution Solvent Methylene chloride/IPA/NH₄OH (39:10:1)
 - a. 1 x 3 mL Methylene chloride/IPA/NH₄OH (39:10:1)
 - b. Collect Eluent at 1 to 2 mL/minute.
11. Add 1 drop of 1% methanolic HCl to each tube
12. Remove receiver tubes and evaporate to dryness at < 40 C using a gentle flow of Nitrogen
13. Reconstitute residue with 150 uL of ethyl acetate and transfer to GC/MS vial with limited volume insert, cap and reserve for GC/MS Analysis. Refrigerate vials if extracts won't be injected on the instrument on the same day. Stable at least one week when tightly capped.
 - a. 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.

9.0 CHROMATOGRAPHY AND MASS SPECTROSCOPY

A. Instrument and Setup:

1. GC/MS/Auto sampler:(Hewlett-Packard 6890/5973, Agilent Technologies 7890A/5975C, 7890B/5977A)
2. Column: 30M RTX-5MS or 30M RTX-1MS (0.25 mm ID; 0.25 micron film)
3. Injection Temperature 250° Detector Temperature 280°
4. Oven (initial): 80°, 25° /min to 300°, (7.0 min hold).
5. 16.8 min total run time, 1 uL injection
6. Scan range 40 to 470 amu
7. Method: Drugsmwa.M; Method Details Appended (Appendix II)
8. Prior to running case work, the GC/MS instrument suitability must be acceptable (see SOP TX-29 for guidance).

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- B. **Batch Format:** Qualitative batches require a positive and negative control. Quantitative batches require known calibrators and controls, case samples should be bracketed by acceptable controls. Analytical batches for WAN and Basic drug confirmations should generally follow the format indicated below. Inject 1 uL into the GC/MS using the following sequence.

1. Urine Qualitative

- a. Solvent blank
- b. Positive urine calibrator/standard 0.500 ug/mL (i.e. In-house control)
- c. Urine blank
- d. Morphine Glucuronide Control (Hydrolysis Batches Only)
- e. Utak control
- f. Solvent blank
- g. Case samples
- h. End batch with calibrator or control

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

- a. Solvent blank
- b. Priming Calibrator
- c. Blood blank
- d. Calibrator 1 (50 ng/mL)
- e. Calibrator 2 (100 ng/mL)
- f. Calibrator 3 (200 ng/mL)
- g. Calibrator 4 (500 ng/mL)
- h. Calibrator 5 (1000 ng/mL)
- i. Blood blank
- j. If available run Utak control(s)
- k. Low control (100 ng/mL)
- l. High control (1000 ng/mL)

- m. Solvent blank
- n. Case sample
- o. Run a control midway through the batch for longer runs
- p. End batch with a control

- 3. File name - Include case number (including year) in each sample data file
- 4. Verify the sequence; verify the vial positions in the tray match sequence.

10.0 DETECTION AND IDENTIFICATION

- A. MS Analysis Criteria for EI (Electron Ionization) ion source fragmentation and detection of nominal mass spectral measurements.

Determination of the presence of WAN and Basic drugs in the sample extract are identified by evaluation of full spectra, and appearance and ratio of the 3 ions characteristic of each species at the appropriate retention time, hence both retention time or relative retention time (a GC characteristic) and fragmentation pattern and ratio (MS characteristics) are used as the basis of qualitative identification.

- 1. For a positive identification of drugs:
 - a. All of the diagnostic ions present in the reference standard must be present in the unknown.
 - b. 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.
 - c. Relative abundances of the diagnostic ions, as well as retention times or relative retention times must always be considered in designating a "positive" match. The retention time or relative retention time must be within 2% of the corresponding analyte, run on the same instrument.
- 2. 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 may be used.

3. Qualitative identification of each analyte is independent.
 - a. 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.
4. 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.

11.0 CALIBRATION AND QUANTITATION

Note: Quantitation is not performed on urine analyses.

- A. Calibration: Calibration for each batch and fraction (WAN/BDS) may be performed independently or historical curves may be used. When historical curves are used the analyst will assure that there have been no major changes to the instrument since the calibration curve was analyzed. Typically curves will be updated monthly (as needed). Controls extracted with the sample batch will be used to assess the appropriateness of the historical calibration curve.

Urine 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.
 1. 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 generally should be within +/-20% of their value. A slightly wider acceptance value (e.g., +/-25% or +/-30%) is acceptable for calibrators that approach the Limit of Quantitation (LOQ) of the assay.

2. 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 \leq LOD. LOD is usually the lowest calibrator. Analytes detected that are above the highest calibrator may be reported as greater than the highest calibrator or control. If a quantitative level is needed, the sample will be reanalyzed at a diluted concentration so that it falls within the calibration curve limits.
3. The criteria for a valid calibration GC/MS 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.
4. 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 Unit Lead if any results can be accepted. On a case-by-case basis, results may be reported qualitatively or semi-quantitative.

12.0 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 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 Unit Lead or Deputy Director.
- 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.
- E. Qualitative Results: Controls must demonstrate the target analyte with acceptable chromatography and spectral characteristics.
- F. Quantitative 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 $\pm 20\%$ or ± 2 standard deviations.
1. Levy-Jennings charts will be used monitor assay performance precision over time.
- G. Internal standard abundance should be similar throughout the batch.
- H. 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.
- I. 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 calibrator or control. LOD should be at least 3 times the signal to noise ratio, LOQ should be at least 10 times the signal to noise ratio.
- J. 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.

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- K. 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% of the retention time of known standard materials, and produce the same fragmentation ions and ratios.

13.0 REPORTING OF RESULTS:

Once the batch is completed and the data is compiled, the batch undergoes a batch review (refer to SOP TX-5 for guidance). Once the batch is accepted the results are entered into JusticeTrax.

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).

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

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Appendix II:

GC/MS temperature program specifications

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

TX 28 WAN BDS Confirmation

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Batch Name & Number:			Batch summary	
	Analyst	Date	Test	Comments
Setup				
Samples Aliquoted				
Instrument #		& Run Date		
Tune acceptable	Yes No			
Suitability 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				
Batch Reviewer Initials/ Date				
State of Connecticut Department of Emergency Services and Public Protection				
Division of Scientific Services				
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