I. INTRODUCTION

Opioids include both natural opiate alkaloids (*eg.* morphine, codeine) as well as their synthetic derivatives (*eg.* hydromorphone, oxycodone, fentanyl). These drugs exert their effects through binding at the mu, kappa, and delta opioid receptors producing sedation and analgesia. Opioids are widely prescribed for pain management, but there is a high potential for abuse and dependence with continued use. In addition, their increasing presence in illicit drug markets makes these drugs of important forensic significance.

Samples that require identification/confirmation by LC-MS/MS are extracted from a buffered, diluted sample aliquot by adsorption onto a solid phase extraction column. Drugs that may be present are then eluted from the SPE column, dried, and reconstituted before injection onto the LC-MS/MS system.

The detection of each specific analyte is determined by single point calibration cut off for urines. Bloods are quantitated with a multipoint calibration using deuterated opioid internal standard(s).

Matrix-specific (blood and/or urine as needed) positive and negative controls are extracted and analyzed in each analytical batch. The presence of opioids or other pain management drugs may be confirmed in urine, blood or other tissues or fluids.

A. Method Targets

1. Codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, 6-monoacetyl morphine (6-MAM), buprenorphine, norbuprenorphine, naloxone, fentanyl, and norfentanyl

Compounds may be added or eliminated from this list upon toxicologist review.

B. Safety

This procedure is carried out in a laboratory environment and standard safety procedures should be utilized, including (minimally) safety glasses and lab coat when deemed necessary. Biological specimens subject to the analytical procedure should be handled using universal precautions. Potentially contaminated items and surfaces should be disinfected prior to and after use.

D. Specimen Requirements

1. 0.5 mL blood or urine

II. MATERIALS

A. Equipment

1. General laboratory glassware
2. Vortex, Thermolyne Maxi Mix 1 or equivalent
3. Sonicator, Fisher-Scientific or equivalent (for blood samples)
4. Centrifuge, Beckman TJ-6 or equivalent
5. Trace B Extraction Columns - SPEWARE (Baldwin Park, CA)
6. SPEWARE CEREX System-48 Solid phase extraction manifold
7. SPEWARE CEREX System-48 Sample Concentrator
8. Shimadzu LC/MS/MS System consisting of:
    a. Degasser: Shimadzu DGU-20A
    b. Pumps: 2 Shimadzu LC-20AD Prominence
    c. Autosampler: Shimadzu SIL 20AC Prominence
    d. Column Oven: CTO-20A
    e. Pre-Column: SecurityGuard ULTRA Cartridge UHPLC Phenyl for 4.6mmID Columns (Phenomenex)
    f. Column: Kinetex Phenyl Hexyl (Phenomenex)
    g. Detector: Shimadzu LCMS-8030 Mass Spectrometer
    h. Controller: Shimadzu CBM-20A
    i. Data Station: Shimadzu LabSolutions software

B. Reagents available as stock items:

Sigma or J.T. Baker reagent grade or equivalent unless specified

1. Methanol (CH3OH): Fisher Optimum LCMS Grade or Burdick Jackson pesticide grade
2. Deionized water (DIW): Milli-Q or LCMS grade
3. Drug-free urine or blood (Drug free blood obtained from Hartford Hospital blood bank)
4. Formic Acid (HCOOH)

5. Ammonium formate (NH4COOH)
6. Glacial acetic acid (CH3COOH)
7. Sodium acetate trihydrate (NaCH3COO·H2O)
8. Sodium carbonate (Na2CO3)
9. Sodium bicarbonate (NaHCO3)
10. UTAK LC-2 Control – UTAK Laboratories (Valencia, CA)
11. UTAK Whole Blood Pain Management 100 Control – UTAK Laboratories (Valencia, CA)
12. β-Glucuronidase (p. vulgata ; Sigma or equivalent)

C. Drug Standard Solutions – Cerilliant Corportation (Austin, TX)

Target Analytes	**Concentration**
Codeine	1.0 mg/mL
Morphine	1.0 mg/mL
Hydrocodone	1.0 mg/mL
Hydromorphone	1.0 mg/mL
Oxycodone	1.0 mg/mL
Oxymorphone	1.0 mg/mL
6-Monoacetylmorphine (6MAM)	1.0 mg/mL
Buprenorphine	1.0 mg/mL
Norbuprenorphine	1.0 mg/mL
Naloxone	1.0 mg/mL
Fentanyl	1.0 mg/mL
Norfentanyl	1.0 mg/mL

D. Deuterated Drug Standard Solution – Cerilliant Corporation (Austin, TX)

Codeine-D3	1.0 mg/mL
Morphine-D3	1.0 mg/mL
Hydrocodone-D3	1.0 mg/mL
Hydromorphone-D3	1.0 mg/mL
Oxycodone-D3	1.0 mg/mL

Oxymorphone-D3	1.0 mg/mL
Buprenorphine-D4	0.1 mg/mL

E. Drug Standard Solutions – Lipomed Inc. (Cambridge, MA)

Target Analytes	**Concentration**
Codeine	1.0 mg/mL
Morphine	1.0 mg/mL
Hydrocodone	1.0 mg/mL
Hydromorphone	1.0 mg/mL
Oxycodone	1.0 mg/mL
Oxymorphone	1.0 mg/mL
6-Monoacetylmorphine (6MAM)	1.0 mg/mL
Buprenorphine	1.0 mg/mL
Norbuprenorphine	1.0 mg/mL
Naloxone	1.0 mg/mL
Fentanyl	1.0 mg/mL
Norfentanyl	1.0 mg/mL

F. Reagents prepared in the Toxicology Laboratory

1. **5M Ammonium formate**:
   a. Dissolve 3.15 g of ammonium formate in 10 mL volumetric flask.
   b. Q.S. to 10 mL with high purity water. Stable for one year.
2. **Mobile Phase A (H_20 with 0.01% formic acid)**:
   a. Add 0.5 mL 5M Ammonium formate and 0.05 mL formic acid to a 500 mL volumetric flask.
   b. Q.S. to 500 mL with high purity water.
   c. Transfer solution to a glass bottle reserved for LC/MS use only. Stable for one week.
3. **0.1M Sodium Acetate buffer (pH 4.5):**
   a. Combine 5.86 g of sodium acetate trihydrate and 3.24 mL glacial acetic acid in a 1000 mL stoppered graduated cylinder.
   b. Q.S. to 1000 mL with deionized water.

c. Store in glass container at room temperature (25°C)

d. Stable for one year

e. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

4. <u>1.0 M Acetate buffer (pH 5.0)</u>

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 C2H4O2

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: 6 months

g. Inspect daily for contamination.

5. <u>0.1 M Acetate Buffer (pH 5.0)</u>

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

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 0.5 mL sample requires 1250 F units. Add 250 uL of β -Glucuronidase to each tube.

Example: for 40 total tubes prepare 10 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
5,000 Units/mL = 1,439,000
x mg 1000 mg
1. x = 3.47 mg/mL

5,000 Units/mL = 1,439,000
x mg 1000 mg
x = 3.47 mg/mL

To make 10 mL

Weigh out 34.7 mg β –Glucuronidase solid. Add to 10 mL of 0.1 M acetate buffer (pH 5.0) Dissolve before use by swirling gently.
Make fresh daily as needed for each batch

6. **0.1M Sodium Carbonate (pH 8.0):**
   a. Add 5.3 g of sodium carbonate to a 500 mL volumetric flask.
   b. Q.S. to 500 mL with deionized water.
   c. Using a validated pH meter, check pH; should be 8.0 ±0.2.
   d. Store in glass container at room temperature (25^{o}C)
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.
7. **0.1M Sodium Bicarbonate (pH 11.0)**:
   a. Add 4.2 g of sodium bicarbonate to a **separate** 500 volumetric flask.
   b. Q.S. to 500 mL with deionized water.
   c. Using a validated pH meter, check pH; should be around 11.0 ±0.2.
   d. Store in glass container at room temperature (25^{o}C)
   e. Stable for one year
   f. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

8. **Bicarbonate buffer pH 9.0: Mixture of 0.1 M Sodium Carbonate and 0.1 M Bicarbonate Solutions**

a. Into a beaker containing the 0.1M Sodium Carbonate solution (lower pH solution)
b. Using a validated pH meter, check pH, adjust with the 0.1M Sodium Bicarbonate solution (higher pH solution) until a pH of 9.0 ± 0.2 is reached.

c. Store in glass container at room temperature (25°C)

d. Stable for one year

e. Inspect for contamination before use. If bacterial contamination is visible, prepare fresh before use.

9. Reconstitution Mixture: 20% Methanol in Deionized Water

a. Into a 100 mL graduated cylinder with a cap, add 80 mL of deionized water.

b. Add 20 mL methanol.

c. Cap and shake. Store at room temperature; stable for 6 months.

d. Before use check for clarity; if cloudy, discard and prepare fresh.

III. PREPARATION OF STANDARDS

A. Opiate 1 Stock Standard Cerilliant Mix 100 µg/mL of hydrocodone, hydromorphone, oxycodone, oxymorphone; 50ug/mL of buprenorphine, norbuprenorphine, fentanyl, norfentanyl

1. Into a borosilicate glass screw-top culture tube, add 100 µL of the following 1mg/mL Cerilliant reference standards:

Hydrocodone	Hydromorphone	Oxycodone	Oxymorphone	6-MAM	100 uL

2. Into the same culture tube, add 50 µL of the following 1mg/mL Cerilliant reference standards:

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl	50 uL

3. Add 300 µL methanol

4. Store in freezer (≤ -10°C)

5. Stable for 6 months when tightly capped.

B. 2000 ng/mL Cerilliant Opiate Working Standard: codeine, morphine, 400 ng/mL hydrocodone, hydromorphone, oxycodone, oxymorphone, 200 ng/mL buprenorphine, norbuprenorphine, fentanyl, norfentanyl

1. Into a 10 mL volumetric flask, add 20 µL of 1 mg/mL codeine, 20 µL of 1 mg/mL morphine reference standard.
2. Add 40 uL of mixed Cerilliant Opiate 1 stock standard
3. Q.S. to 10 mL with DI water. Protect from light, make fresh with each run.

C. <u>Working Internal Standard: 2.0 µg/mL codeine D3, morphine D3, oxycodone D3, oxymorphone D3, hydrocodone D3, hydromorphone D3 ; 500 ng/mL buprenorphine D4</u>

1. To a 50 mL volumetric flask, add 100 µL of each 1 mg/mL Cerilliant reference deuterated opiates .
2. Add 250 µL of 100 µg/mL buprenorphine D4
3. Q.S. to 50 mL with methanol.
4. Store in freezer (≤ -10^{o}C)
5. Stable for 6 months when tightly capped.

IV. PREPARATION OF CONTROLS

A. Mixed Lipomed and/or Grace Stock Control In-House Control Mix 100 µg/mL of hydrocodone, hydromorphone, oxycodone, oxymorphone; 50ug/mL of buprenorphine, norbuprenorphine, fentanyl, norfentanyl

1. Into a screw-top glass culture tube, add 100 µL of the following Lipomed and/or Grace 1 mg/mL stock standards:

<u>100 µg/mL of hydrocodone, hydromorphone, oxycodone, oxymorphone; 50ug/mL of buprenorphine, norbuprenorphine, fentanyl, norfentanyl Mixed Lipomed and or Grace Stock Control</u>

2. Into a borosilicate glass screw-top culture tube, add 100 µL of the following 1mg/mL Cerilliant reference standards:

Hydrocodone	Hydromorphone	Oxycodone	Oxymorphone	6-MAM

3. Into the same culture tube, add 50 µL of the following 1mg/mL Cerilliant reference standards:

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl

4. Add 300 µL methanol
5. Store in freezer (≤ -10^{o}C)
6. Stable for 6 months when tightly capped.

B. 2000 ng/mL Cerilliant Opiate Working Standard: codeine, morphine, 400 ng/mL hydrocodone, hydromorphone, oxycodone, oxymorphone, 200 ng/mL buprenorphine, norbuprenorphine, fentanyl, norfentanyl

1. Into a 10 mL volumetric flask, add 20 µL of 1 mg/mL codeine, 20 µL of 1 mg/mL morphine reference standard.
2. Add 40 uL of mixed Cerilliant Opiate 1 stock standard
3. Q.S. to 10 mL with DI water. Make fresh with each run.
4. Store in freezer (≤ -10^{o}C)
5. Stable for 6 months when tightly capped.

C. External Controls

1. External controls will come from UTAK or equivalent commercially prepared samples. Reconstitute Utak dried whole blood controls with 5 mL DI water using a volumetric pipette or equivalent.

    a. Cap and let sit 10-15 minutes

    b. Gently swirl 3-4 minutes or mix on rotator until all particles are dissolved into a homogeneous mixture, swirl gently each time an aliquot is removed to ensure a homogeneous mixture.

    c. For later use and to extend control stability, pipette the reconstituted control into 700 uL aliquots in borosilicate glass culture tubes. Cap and store frozen. As needed, thaw stored frozen aliquot, swirl gently to ensure a homogeneous mixture.

D. Negative Controls

1. Negative control: Drug-free human urine or drug-free human blood.

V. PROCEDURE

A. Label clean screw cap tubes appropriately with blank, calibrator, control and case number designations.

B. Prepare calibrator and control samples, according to tables below:

a. Blood Calibrators

Standard Concentration			Working Standard Volume to Add:	Blank Blood Volume to Add:
Cerilliant Working Standard				
			Volume to Add uL:	
Blank			**0 µL**	**500 µL**
200 ng/mL	**400 ng/mL**	**2000 ng/mL**		
1	**2**	**10 ng/mL**	**2.5**	**497**
2	**4**	**20 ng/mL**	**5**	**495**
5	**10**	**50 ng/mL**	**12.5**	**487**
10	**20**	**100 ng/mL**	**25**	**475**
20	**40**	**200 ng/mL**	**50**	**450**
50	**100**	**500 ng/mL**	**125**	**375**
100	**200**	**1000 ng/mL**	**250**	**250**
200	**400**	**2000 ng/mL**	**500**	**500**

b. In-House Blood Controls

Control Concentration			Working Control Volume to Add:	Blank Blood Volume to Add:
Working Control				
200 ng/mL	**400 ng/mL**	**2000 ng/mL**		
5	**10**	**50 ng/mL**	**12.5**	**487**
20	**40**	**200 ng/mL**	**50**	**450**
100	**200**	**1000 ng/mL**	**250**	**250**

c. In House Urine Control

Control Concentration			Working Control Volume To Add:	Blank Urine Volume to Add:
Working Control (2000 ng/mL)				
5	**10**	**50 ng/mL**	**12.5**	**487**
200	**400**	**2000 ng/mL**	**500**	**500**

d. Commercial Controls

Control Concentration	Pipette Volume To Add:	Blank Matrix Volume to Add:
UTAK PMWB dilution x4	125 µL	250 µL
UTAK PMWB	500 µL	0 µL

C. **Blood and Urine sample preparations**

1. Add 0.5 mL case specimen, blood or urine, to appropriate labeled tubes.
2. Add 100 µL of deuterated IS mix to each tube.
   a. **Urine Total conjugated and unconjugated**

      Note: All urines do not need hydrolysis, on a case by case basis the analyst may decide which samples to test for total and free analytes.

      Add 250 µL of β –Glucuronidase in 0.1M acetate buffer, pH 5.0 to hydrolyze urine

      Heat for 3 hours at 60^{o}C in water bath. Cool tubes to room temperature.
3. Add 1 mL of 0.10 M sodium acetate buffer (pH 4.5) to each tube.
   a. **Blood sample preparations:** Add 500 µL DI water to each blood tube.

      Note: Adding 500uL DI water to urine samples is not detrimental.

4. Cap tubes, then vortex each tube for 10 seconds.

    a. **Blood sample preparations:** Sonicate blood samples for 15 minutes.

    Note: Sonicating urine samples is not detrimental.

5. Centrifuge **all** tubes for 8 min at about 5200 rpm.

6. Label SPE columns to correspond with each screw-top culture tube.

7. Place labeled Trace B extraction columns in the SPE column rack in the appropriate order. Position plastic waste tray labeled "Methanol" underneath SPE column rack.

8. Condition each column sequentially with:

    a. 1 mL methanol; drain (≈3 psi) to Solvent "Hazardous Waste" stream

9. Remove plastic waste tray labeled "Methanol" and replace with plastic tray labeled "Biohazardous/Buffers"

    a. 1 mL DI water; drain (≈ 3 psi) to "Non-Hazardous" regulated waste stream

10. Carefully transfer the sample to the center of the SPE column. Avoid splashing and/or transferring any sediment found at the bottom of the tube.

11. Wash each SPE column sequentially with:

    a. 1 mL bicarbonate buffer (pH 9.0); drain (≈ 3 psi) to non-hazardous regulated waste stream
    b. 1 mL DI water; drain (≈ 3 psi) to non-hazardous regulated waste stream

12. Dry the columns for 10 minutes using maximum pressure, between 60-80 psi.

13. During this 10 minute window, label autosampler LC vials and place in appropriate order in the SPE collection rack; prepare the elution solvent.

14. The elution solvent ratio is 80: 18: 2 Dichloromethane: IPA: NH4OH. (Adjust volume prepared for the number of tubes, minimize excessive hazardous waste, 2mL needed for each tube)

    Add the IPA and NH4OH together first before adding the dichloromethane portion (following this order prevents unsafe buildup of gases).

15. After 10 minutes, replace plastic waste tray with SPE collection rack containing labeled autosampler vials in order corresponding to SPE columns.

16. Elute column with two 1.0mL aliquots of 80:18:2 Dichloromethane:IPA:NH4OH into the appropriate autosampler vial. Flow at 2-4 mL/min to optimize recovery.

17. Remove top SPE column rack and transfer collection rack from SPE manifold to sample concentrator.

18. Evaporate all vials to dryness at <40^{o} C.

19. Reconstitute each slotted screw cap vial with 520 µL of starting mobile phase of 20% methanol in DI water.

20. Inject 10 µL of each sample for LC/MS/MS analysis.

VI. INSTRUMENT PARAMETERS

A. LC Parameters

1. Shimadzu Prominence LC-20 System

a. Flow	0.6 mL/min
b. Autosampler Temperature	15°C
c. Injection Volume	10 µL
d. Needle Wash	500 µL; before and after aspiration
e. Column	Kinetex 2.6um Phenyl-Hexyl 100A 50 x 4.6 cm
	Manufactured by Phenomenex (Torrance, CA)
f. Oven Temperature	40°C
g. Gradient	

Time (min)	Mobile Phase B (Methanol)	Mobile Phase A (0.01% Formic Acid in Water)
Initial	5%	95%
2.50	45%	55%
4.50	45%	55%
5.50	95%	5%
7.50	95%	5%
7.51	5%	95%
9.00	STOP	STOP

B. M/S Acquisition Parameters: The following conditions can be adjusted if needed based on availability of gases.

Interface	DUIS (APCI and ESI)
DL Temperature	250°C
Nebulizing Gas	2 L/min
Drying Gas	15 L/min

C. Transition Ions Monitored and Retention Times (Times are approximate and may vary)

Analytes may be added or deleted as needed

Using the LabSolutions optimization software**,** the following transitions were identified:

Drug/Metabolite	Precursor Ion	Quantification Ion	Reference Ion(s)	Retention Time Min
Codeine	299.7	58.1	165.1	3.05
Codeine D3	303.0	61.0	128.0	3.22
Morphine	286.1	165.1	181.1	2.30
Morphine D3	288.7	201.1	165.0	2.40
6 MAM	328.0	164.95	58.1	3.31
Oxycodone	315.9	298.15	241.1	3.28
Oxycodone D3	319.0	301.15	129.1	3.41
Oxymorphone	301.9	284.15	227.1	2.43
Oxymorphone D3	305.0	287.1	230.1	2.53
Hydrocodone	299.8	199.1	171.0	3.40
Hydrocodone D3	302.75	199.05	170.9	3.50
Hydromorphone	285.9	185.0	157.0	2.47
Hydromorphone D3	289.0	185.05	157.0	2.68
Buprenorphine	468.2	55.2	84.5	6.55
Buprenorphine D4	472.0	59.1	88.15	6.62
Norbuprenorphine	414.1	57.1	101.1	5.30
Fentanyl	336.75	188.15	105.1	6.28
Norfentanyl	233.1	84.1	55.0	3.93

D. Detection and Identification: The certifying toxicologist will review all chromatography, peak integrations, and transition ion ratios used for identifications.

Determination of the presence of target analytes in the sample extract are identified by appearance and ratio of product ions that are characteristic of each drug at the appropriate retention time. In this manner, both retention time (an LC characteristic) and fragmentation pattern and ratio (an MS characteristic) are used as the basis for qualitative identification. For

the identification of an analyte to be made, the retention time of the chromatographic peak must be within 0.1 minute of the corresponding analyte in the calibrator sample as well as having ion ratios that are within the following limits. The 100 ng/mL Cerilliant Standard sample will be utilized to set the expected ion ratios.

Expected (Set) Ion Ratio	Allowance
> 50%	20%
20-50%	25%
< 20%	30%

E. Calibration: A calibration curve for each drug and metabolite is analyzed with each batch. The calibration correlation coefficient must be ≥ 0.990 when using deuterated internal standards, ≥ 0.98 is acceptable using non deuterated internal standards, or approved by the toxicology Director or Supervisor. A standard may be removed to attain a correlation coefficient of ≥ 0.990. Following linear regression, reprocessed calibrators must be within 20% of their target value to be included in the final calibration curve used to calculate case samples. One calibrator may be dropped from the curve. If the highest calibrator is omitted, samples higher than the next highest calibrator must be diluted or approved by the toxicology Director or Supervisor to be reported. If the lowest calibrator is omitted, the lower limit of quantitation (LOQ) is the next lowest calibrator value. Calibration for each analysis batch is done independently. The urine control will be quantitated based on the concurrently analyzed standards used to create the blood calibration curve. Calibration is accomplished by the addition of a known amount of analytes in addition to the internal standard into a blank sample of the matrix tested. The response of the system to this calibration defines a run-specific standard curve that is used as the basis for the quantitative calculation in all controls and samples. The system for blood samples is "multi-point calibration, multi-point control."

F. Quantitation: Quantitation is accomplished by the comparison of the response ratio of the analyte and the internal standard in a specific sample relative to the response ratios of the calibration curve. The concentration of the analyte in the sample is then extrapolated from the standard curve.

VII. RESULTS INTERPRETATION

A. Positive results will be reported only when:

1. Analyte identification is based on at least two transitions with relative abundances within +/- 20 % of the target, relative to a calibrator.
2. Retention times are within 0.1 min, or +/- 3% relative to a calibrator analyzed in same batch.
3. Qualitative results have at least a 3x signal to noise (S/N) ratio.
4. The integration of the analyte peak has acceptable symmetrical shape and chromatography.
5. All MRMs show peaks at the appropriate retention times.
6. Quality control sample results are acceptable.

VIII. QUALITY CONTROL

A. Criteria for Quantitative Results

1. Statistics will be maintained on all controls.
2. Results must fall within ±20% of the analytes' target concentration.
3. All results are recorded.

B. Verification of Vial Sequence: The vial sequence is checked prior to and following injection of samples. These checks will be documented.

C. Evaluation of Potential Carryover: Potential Carryover will be determined by a blank sample after the highest calibrator. In addition, a solvent blank will be run after each sample to ensure there is no carryover between samples.

D. Linearity: Linearity of the calibration curve is demonstrable in each batch for each analyte as a function of r^2 correlation coefficient and quantitative results of control materials.

E. Sensitivity (LOD, LOQ): For the purposes of this procedure, the limits of detection (LOD) and the limits of quantitation (LOQ) are defined as equal to the lowest concentration of the lowest calibrator. Qualitative Identification must have a signal of at least 3 times the signal to noise ratio and have acceptable ion ratios criteria met.

F. 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.

IX. QUALITY ASSURANCE

A. Quality Assurance is provided by the following multi-layer program:

1. The LC/MS analysis is thoroughly checked by the instrument operator, including vial position on the auto sampler, prior to and following the injection of samples.
2. The LC/MS data is reviewed and signed off by a reviewer distinct from the operator; this review includes an evaluation of qualitative and quantitative (where applicable) results containing:
    a. Control Results
    b. Chromatographic Characteristics
    c. Transcription Errors

1. The reported results are checked against the findings of the run data and during the technical review of case results.
2. The original run is compared to the Final Report during the final technical review, prior to case sign-off.

X. SOURCES OF ERROR

It has been established that no known interferences are present in the calibrators/controls. Ion suppression or enhancement and potential interferences from other analytes have not been found for the common drugs and metabolites typically seen in casework.

XI. References

A. A Comparison of the Validity of Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry Analysis of Urine Samples for Morphine, Codeine,

6-Acetylmorphine, and Benzoylecgonine; Peter R. Stout, Nichole D. Bynum, John M. Mitchell, Michael R. Baylor and Jeri D. Ropero-Miller; J.Anal Toxicol (2009) 33 (8): 398-408

B. Opiates. Connecticut Division of Scientific Services: Toxicology Laboratory.

C. Solid-Phase Extraction Method for Trace B Columns. SPEWARE: Baldwin Park, CA.

Opiate – Abbreviated Method / Procedure for Bench Use: SOP# TX-32 **Batch #:**

ANALYST: **BATCH DATE:** **PREP DATE:** **EXTRACTION DATE:**

	Procedure	Prepared / Lot #	Expiration Date	Being Validated	Pipet Used
1	Properly label screw cap test tubes: (for extraction and a set for collection of eluent				

Standard Concentration			Working Standard Volume to Add:	Blank Blood Volume to Add:
Cerilliant Working Standard				
			Volume to Add uL:	
Blank			**0 µL**	**500 µL**
200 ng/mL	**400 ng/mL**	**2000 ng/mL**		
1	**2**	**10 ng/mL**	**2.5**	**497**
2	**4**	**20 ng/mL**	**5**	**495**
5	**10**	**50 ng/mL**	**12.5**	**487**
10	**20**	**100 ng/mL**	**25**	**475**
20	**40**	**200 ng/mL**	**50**	**450**
50	**100**	**500 ng/mL**	**125**	**375**
100	**200**	**1000 ng/mL**	**250**	**250**
200	**400**	**2000 ng/mL**	**500**	**500**

Control Concentration			Working Control Volume To Add:	Blank Urine Volume to Add:
Working Control (2000 ng/mL)				
5	**10**	**50 ng/mL**	**12.5**	**487**
200	**400**	**2000 ng/mL**	**500**	**500**

Control Concentration	Pipette Volume To Add:	Blank Matrix Volume to Add:
UTAK PMWB dilution x4	125 µL	250 µL
UTAK PMWB	500 µL	0 µL

Control Concentration			Working Control Volume to Add:	Blank Blood Volume to Add:
Working Control				
200 ng/mL	**400 ng/mL**	**2000 ng/mL**		
5	**10**	**50 ng/mL**	**12.5**	**487**
20	**40**	**200 ng/mL**	**50**	**450**
100	**200**	**1000 ng/mL**	**250**	**250**

2	Into the appropriately labeled test tubes: Follow tables for blanks, standards QC Pipette **0.5mL *Case Specimen***				
3	Pipette ***100 µL Internal Standard*** into the tubes				
4	Bloods: Add ***100 µL DI Water,*** *cap, mix,* ***Sonicate 15 min, Centrifuge 8 min at 5200 rpm***				
5	Urine: Hydrolyze B-gluc, 2500 Units/0.5mL, heat 3 hrs. at 60° C Cool to room temperature for 5 minutes				

6	Properly label and set up extraction columns on manifold (3 mL 35mg) (SPEware Trace B *Part# 711-335C)*				
7	Condition the column at 1-2mL/ minute Add ***1 mL*** *of* ***Methanol*** *(collect in Hazardous Organic Waste Tray)* Add ***1 mL*** *of* ***Deionized water*** *(collect in Non-Hazardous Waste Tray)*				
8	Load samples into the center of correlating properly labeled columns at 1-2mL/min				
9	Wash the column 1-2mL/ minute Add **1 *mL bicarbonate buffer pH 9.0*** Add **1 *mL Deionized Water***				
10	Dry the Columns 10 minutes (60-80 psi)				
11	Elute into the correspondently labeled vials at 1-2mL/ minute Add **Two *1mL*** of elution solvent: ***80/18/2 Methylene Chloride /Isopropanol/ NH_4OH*** *Prepare fresh, Isopropanol* *Ammonium Hydroxide* *Methylene Chloride*				
12	Transfer elution vials to the evaporator and dry with Nitrogen at <40°C. Do not over dry				
13	Reconstitute dried extracts by pipetting ***520 µL*** of **20/80 methanol/ DI Water** into each vial. Cap, vortex				
14	Inject 10 uL onto LCMSMS				