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 fluids.

A. Method Targets

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

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 (CH_3OH): 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 or equivalent)

4. Formic Acid (HCOOH)
5. Ammonium formate (NH_4HCO_2)
6. Glacial acetic acid (CH_3COOH)
7. Sodium acetate trihydrate ($NaCH_3COO{\cdot}3H_2O$)
8. Sodium carbonate (Na_2CO_3)
9. Sodium bicarbonate ($NaHCO_3$)
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 (6-MAM)	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
Tramadol	1.0 mg/mL
N-desmethyltramadol	1.0 mg/mL
O-desmethyltramadol	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
Tramadol-C^{13}-D3	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
Tramadol	1.0 mg/mL
N-desmethyltramadol	1.0 mg/mL
O-desmethyltramadol	1.0 mg/mL

F. Reagents prepared in the Toxicology Laboratory

1. <u>5M Ammonium formate</u>:

a. Dissolve 3.15 g of ammonium formate in 10 mL volumetric flask.

b. Q.S. to 10 mL with DIW. Stable for one year.

2. **Mobile Phase A (H_20 with 5mM ammonium formate and 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.
   d. The above instructions make 500 mL of mobile phase; adjust volumes of reagents accordingly if requiring a different final

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. **1.0 M Acetate buffer (pH 5.0)**
   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
   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. **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. **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

7. **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.

8. **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.

9. 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^{o}C)

d. Stable for one year

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

10. 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. <u>Cerilliant Opiate Stock Standard Mix</u>

Note: Equivalent reference standards may substituted if needed. Prepared calibrators and controls should be made from standards from different manufacturers.

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

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

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl

3. Add 300 µL methanol
4. Store in freezer (≤ -10°C)
5. Stable for 6 months when tightly capped.
7. Final concentrations:

100 µg/mL- Hydrocodone, Hydromorphone Oxycodone, Oxymorphone, 6-MAM

50 µg/mL - Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

B. <u>Cerilliant Opiate Working Standard</u>

1. Into a 10 mL volumetric flask, **add 20 µL** of the following 1 mg/mL Cerilliant reference standards:

Codeine	Morphine	Tramadol	N-desmethyl-tramadol	O-desmethyl-tramadol

2. Add 40 uL of mixed <u>Cerilliant Opiate Stock Standard</u>.
3. Q.S. to 10 mL with DI water. Protect from light, make fresh with each run.
4. *Final concentrations:*

2000 ng/mL – Codeine, Morphine, Tramadol, N-desmethyltramadol, O-desmethyltramadol

400 ng/mL – Oxycodone, Oxymorphone, Hydrocodone, Hydromorphone

200 ng/mL – Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

C. <u>Working Internal Standard Mix</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 each 100 µg/mL deuterated standard.
3. 3. Q.S. to 50 mL with methanol.
4. Store in freezer (≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

2.0 µg/mL- Codeine-D3, Morphine D3, Oxycodone-D3, Oxymorphone-D3, Hydrocodone-D3, Hydromorphone-D3

500 ng/mL- Buprenorphine-D4, Tramadol-C^{13}-D3

IV. PREPARATION OF CONTROLS

Note: Alternative controls, both commercial and in-house, to those listed below may be employed at the analyst's discretion.

A. **<u>In-House Stock Control (Lipomed and/or Grace)</u>**

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

Hydrocodone	Hydromorphone	Oxycodone	Oxymorphone	6-MAM

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

Buprenorphine	Norbuprenorphine	Fentanyl	Norfentanyl

3. Add 300 µL methanol
4. Store in freezer (≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

100 ug/mL - Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone, 6-MAM

50 µg/mL- Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

B. **<u>Opiate Working Control</u>**

1. Into a 10 mL volumetric flask, **add 20 µL** of the following 1mg/mL Lipomed/Grace reference standards:

Codeine	Morphine	Tramadol	N-desmethyl-tramadol	O-desmethyl-tramadol

2. Add 40 uL of mixed <u>In-House Stock Control (Lipomed/Grace)</u>.
3. Q.S. to 10 mL with DI water. Make fresh with each run.
4. Store in freezer (≤ -10°C)
5. Stable for 6 months when tightly capped.
6. Final concentrations:

2000 ng/mL- Codeine, Morphine, Tramadol, N-desmethyltramadol O-desmethyltramadol

400 ng/mL- Hydrocodone, Hydromorphone, Oxycodone, Oxymorphone, 6-MAM

200 ng/mL- Buprenorphine, Norbuprenorphine, Fentanyl, Norfentanyl

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.

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 Concentrations (ng/mL)			Opiate Working Standard Volume to Add:	Blank Blood Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
Blank			0 µL	500 µL
1	2	10	2.5 µL	500 µL
2	4	20	5 µL	500 µL
5	10	50	12.5 µL	500 µL
10	20	100	25 µL	500 µL
20	40	200	50 µL	500 µL
50	100	500	125 µL	500 µL
100	200	1000	250 µL	500 µL
200	400	2000	500 µL	0 µL

b. In-House Blood Controls

Control Concentrations (ng/mL)			Opiate Working Control Volume to Add:	Blank Blood Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
5	10	50	12.5 µL	500 µL
20	40	200	50 µL	500 µL
100	200	1000	250 µL	500 µL

c. In-House Urine Control

Control Concentrations (ng/mL)			Opiate Working Control Volume To Add:	Blank Urine Volume to Add:
Buprenorphine Norbuprenorphine Fentanyl Norfentanyl	Hydrocodone Hydromorphone Oxycodone Oxymorphone 6-MAM	Codeine Morphine Tramadol N-desmethyltramadol O-desmethyltramadol		
5	10	50	12.5 µL	500 µL

d. Commercial Controls (optional)

Control Concentration	Pipette Volume To Add:	Blank Matrix Volume to Add:
UTAK PMWB dilution x4	125 µL	375 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 (or earlier), label autosampler LC vials and place in the appropriate position in the SPE collection rack underneath the corresponding SPE column; prepare the elution solvent.

14. The elution solvent ratio is 80: 18: 2 Dichloromethane: IPA: NH_4OH. (Adjust volume prepared for the number of samples, minimize excessive hazardous waste, 2mL needed for each tube)

    Add the IPA and NH_4OH 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:NH_4OH 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
Tramadol	264.0	58.15	30.20, 246.20	4.07
N-desmethyltramadol	250.15	44.00	232.30	4.23
O-desmethyltramadol	250.15	58.10	30.15	3.29
Tramadol-C^{13}-D3	268.20	58.10		4.05

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. Initially the 100 ng/mL Cerilliant Standard sample will be utilized to set the expected ion ratios, however it is recognized that some ion ratios are concentration dependent. As such ratios may be set on a case by case basis using a standard with a concentration close to the concentration of the analyte of interest in the case.

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 generally should be within 20% of their target value to be included in the final calibration curve used to calculate case samples. Higher percent differences are permitted for calibrators at the limit of quantitation. 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 limit of quantitation (LOQ) is defined as equal to the lowest concentration of the lowest calibrator. The Limit of Detection (LOD) must have a response of at least 3 times the signal to noise ratio and have acceptable ion ratios.

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.