Solid Phase Extraction of Opioids from Biologicals with Analysis by LC-HRMS/MS

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1 INTRODUCTION

Opioids are a class of substances that include natural, semi-synthetic and synthetic alkaloidal agents derived from opium or substances that have morphine-like activity.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☒ Quantitation
Matrices	Blood, serum, plasma, urine, bile, gastric contents, vitreous humor, or a previously prepared tissue homogenate
Analytes	Morphine, codeine, hydromorphone, hydrocodone, oxymorphone, oxycodone, 6-acetylmorphine (quantitative) Normorphine, norcodeine, noroxycodone, dihydromorphine, and dihydrocodeine (qualitative)
Personnel	This document applies authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Biological specimens are qualitatively screened and/or quantitated for opioids by this method. Since most opioids are biotransformed to form a glucuronide conjugate during metabolism, these conjugates need to be hydrolyzed to obtain "total" opioid concentrations. The hydrolysis occurs by cleaving the drug-conjugate with the enzyme β-glucuronidase. Analysis without hydrolysis yields "free" opioid concentrations. Analysis with hydrolysis yields "total" opioid concentrations. Specimens are mixed with internal standards, adjusted to a slightly acidic pH, and extracted using mixed mode hydrophobic/cation exchange solid phase extraction cartridges. Target drugs are eluted using a mixed solvent system of methylene chloride, isopropanol, and ammonium hydroxide. The eluent is taken to dryness and reconstituted prior to analysis by liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS).

4 SPECIMEN CRITERIA

This procedure can be used for assaying biological specimens such as blood, serum, plasma, urine, bile, gastric contents, vitreous humor, or a previously prepared tissue homogenate. When available, 1 mL of biofluid or 2 g of a prepared tissue homogenate (1:1) is used in the assay. Blood, bile, gastric content, and tissue homogenate samples are centrifuged prior to analysis. Urine, vitreous humor, plasma, or serum specimens can be directly extracted. Total opiate analysis requires that specimens such as blood, urine, and bile be enzymatically hydrolyzed prior to analysis.

5 EQUIPMENT

5.1 Equipment

A. Centrifuge

B. Evaporator with nitrogen

C. Heating block

D. Homogenizer (for tissue or similar specimens)

E. Solid phase extraction manifold (vacuum or positive pressure)

F. Volumetric flasks

G. Vortex mixer

5.1.1 Columns

A. Xterra Phenyl LC column: 150 x 2.1 mm. 5 µm dp

B. 2 µm prefilter

5.2 Consumables

A. Autosampler vials

B. Clean Screen DAU solid phase extraction (SPE) cartridges (200 mg x 10 mL)

C. Pipette tips

D. Test tubes (16 x 125 mm screw-top, 16 x 100 mm and 12 x 75 mm culture, or comparable)

5.3 Instruments

A. Thermo LTQ Orbitrap XL Hybrid Ion Trap/Fourier Transform Mass Spectrometer
Shimadzu HPLC

5.4 Software

5.4.1 Thermo LTQ Orbitrap XL

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1 / XP Professional
Mass Spectrometer	Foundation	1.0.2 or higher
	Xcalibur	2.1.0 SP1 / 2.0.7
	LTQ Tune Plus	2.5.5
	Shimadzu LC Controller	5.4 / 6.5

5.5 Chemicals/Reagents

5.5.1 Purchased

Acetic acid, glacial	17 M, ≥ACS grade)
Acetonitrile	≥Optima Grade
Ammonium formate	≥LC/MS grade
Ammonium hydroxide, concentrated	≥Reagent Grade
Formic acid	≥Reagent Grade
Hydrochloric acid, concentrated	12M, ≥ACS Grade
Isopropanol	≥HPLC Grade
Methanol	≥Optima Grade
Methylene chloride	≥HPLC grade
Sodium acetate trihydrate	≥Reagent Grade
Water	≥Optima Grade, deionized
β-Glucuronidase	Type H-2 from Helix Pomatia; 100,000+ units/mL
Sodium phosphate monobasic monohydrate	Reagent grade
Sodium phosphate dibasic heptahydrate	Reagent grade

5.5.2 Prepared

A. 1 N Hydrochloric Acid

To a 100-mL graduated cylinder, add 80 mL deionized water. Add 8 mL concentrated hydrochloric acid and mix well. Bring to 96 mL with deionized water. Store in glass at room temperature. Stable 6 months.

B. Sodium acetate buffer (1.1 M)

To a 100-mL volumetric flask, add 14.95 g sodium acetate trihydrate, 60 mL deionized water, and 2.2 mL glacial acetic acid. Mix well to dissolve and bring to volume with deionized water. Verify 5<pH<6. Store refrigerated in glass. Stable 2 months.

C. 0.1 M, pH 6 Phosphate buffer

To a 500-mL volumetric flask, add 400 mL deionized water, 6.1 g sodium phosphate monobasic monohydrate, and 1.6 g sodium phosphate dibasic heptahydrate. Mix well to dissolve. Verify 5.8<pH<6.1. Bring to volume with deionized water. Store refrigerated in glass. Stable 2 months.

D. 1:1 Methanol:Water

Combine 50 mL methanol with 50 mL water (both Optima grade) and mix well. Store in glass at room temperature. Stable 12 months.

E. 0.1 M Acetic acid

To a 100-mL graduated cylinder, add 80 mL deionized water and 0.5 mL glacial acetic acid. Mix well and bring to 85 mL with deionized water. Store in glass at room temperature. Stable 6 months.

F. SPE elution solvent (78:20:2 methylene chloride:isopropanol:ammonia)

Combine 20 mL HPLC grade isopropanol with 2 mL concentrated ammonium hydroxide and mix well. Add 78 mL HPLC grade methylene chloride and mix well. Store in glass at room temperature. To be prepared fresh.

G. Reconstitution solvent (95:5 water:methanol)

Combine 5 mL methanol with 95 mL water (both Optima grade) and mix well. Store in glass at room temperature. Stable for 6 months.

H. LC mobile phase 1 (95:5:0.05 10 mM ammonium formate : acetonitrile : formic acid)

Dissolve 630 mg of ammonium formate in 1 L of Optima grade water. Remove 50 mL of this solution, save for LC Mobile Phase #2, and add 50 mL of acetonitrile. Mix well. Add 500 µL formic acid and mix well. Store in glass at room temperature. Stable for 2 months.

I. LC mobile phase 2 (5:95:0.05 10 mM ammonium formate : acetonitrile : formic acid)

Add 25 mL of the aqueous formate solution from the preparation of LC mobile phase #1 to 475 mL of acetonitrile. Mix well. Add 250 µL formic acid and mix well. Store in glass at room temperature. Stable for 2 months.

5.6 Standards/Controls

5.6.1 Purchased

A. Internal Standard Stock Solutions (0.1 mg/mL)
   1. 6-AM-d3
   2. Codeine-d6
   3. Hydrocodone-d3
   4. Hydromorphone-d3
   5. Morphine-d3
   6. Oxycodone-d6
   7. Oxymorphone-d3

Purchased from Cerilliant or another approved supplier. Stability and storage conditions are determined by the manufacturer.

B. Standard Stock Solutions (1 mg/mL)
   1. 6-AM
   2. Codeine

3. Dihydrocodeine
4. Dihydromorphine
5. Hydrocodone
6. Hydromorphone
7. Morphine
8. Mophine-3-β-glucuronide or Morphine-6-β-glucuronide (0.1 mg/mL)
9. Norcodeine
10. Normorphine
11. Noroxycodone
12. Oxycodone
13. Oxymorphone

Purchased from Cerilliant (typically used for calibrators) and from Lipomed (typically used for controls) or another approved supplier. Stability and storage conditions are determined by the manufacturer.

5.6.2 Prepared

A. Internal Standards Working Solution (4 µg/mL or 1 µg/mL, depending on analyte)

Mix 1 mL each of the d_3-morphine and the d_6-codeine Stock Solutions with 250 µL each of the d_3-hydromorphone, d_3-hydrocodone, d_3-oxymorphone, and d_6-oxycodone Stock Solutions. Dilute with 1:1 methanol:water to a final volume of 25 mL. Store at <0ºC in glass. Stable for at least 1 year.

B. d_3-6-AM Working Solution (2 µg/mL)

Dilute 500 µL of the d_3-6-AM stock solution to 25 mL in acetonitrile. Store in glass at <0ºC. Stable for 6 months.

C. Column Performance Evaluation Mix (0.5 µg/mL each component)

Mix 50 µL each of the morphine, hydromorphone, oxycodone, dihydrocodeine, and norcodeine stock standards. Dilute to 100 mL with Reconstitution Solvent (5:95 methanol:water) and mix well. Store refrigerated in glass. Stable for at least 1 year. A 5 µL portion of this solution is analyzed before each day's samples, in order to confirm acceptable instrument performance.

D. Control Working Solution #1 (3.5 or 0.7 µg/mL, depending on component)

Mix 175 µL each of the morphine and codeine Stock Solutions with 35 µL each of the hydromorphone, hydrocodone, oxymorphone, and oxycodone stock solutions. Dilute with 1:1 methanol:water to a final volume of 50 mL. Store in glass at <0ºC. Stable for at least 1 year.

E. Control Working Solution #2 (1 µg/mL)

Dilute 50 µL of the 6-AM Stock Solution with acetonitrile to a final volume of 50 mL. Store in glass at <0ºC. Stable for 6 months.

F. Control Working Solution #3 (1 µg/mL each component)

Dilute 50 µL each of the normorphine, norcodeine, noroxycodone, dihydromorphine, and dihydrocodeine Stock Solutions with 1:1 methanol:water to a final volume of 50 mL. Store in glass at <0ºC. Stable for at least 1 year.

G. Control Working Solution #4 (2.5 µg/mL)

Dilute 250 µL of the morphine glucuronide Stock Solution with 1:1 methanol:water to a final volume of 10 mL. Store in glass at <0ºC. Stable for 6 months.

H. Calibration Working Solution #1 (20 or 4 µg/mL, depending on component)

Mix 1.0 mL each of the morphine and codeine Stock Solutions with 200 µL each of the hydromorphone, hydrocodone, oxymorphone, and oxycodone Stock Solutions and dilute with 1:1 methanol:water to a final volume of 50 mL. Store in glass at <0ºC. Stable for at least 1 year.

I. Calibration Working Solution #2 (1 or 0.2 µg/mL, depending on component)

Dilute 2.5 mL of the Calibration Working Solution #1 to 50 mL with 1:1 methanol:water. Store in glass at <0ºC. Stable for at least 1 year.

J. Calibration Working Solution #3 (2.5 µg/mL)

Dilute 125 µL of the 6-AM Stock Solution with acetonitrile to a final volume of 50 mL. Store in glass at <0ºC. Stable for 6 months.

K. Calibration Working Solution #4 (0.5 µg/mL)

Dilute 10 mL of the Calibration Working Solution #3 with acetonitrile to a final volume of 50 mL. Store in glass at <0ºC. Stable for 6 months.

Table 1: Blood Calibrator Preparation

Volume of Matrix (mL)	Volume of Cal Solution #1 (µL)	Volume of Cal Solution #2 (µL)	Volume of Cal Solution #3 (µL)*	Volume of Cal Solution #4 (µL)*
Level 1 – 25 ng/mL morphine and codeine, 5 ng/mL for all others				
0.95	0	25**	0	10
Level 2 – 50 ng/mL morphine and codeine, 10 ng/mL for all others				
0.95	0	50	0	20
Level 3 – 100 ng/mL morphine and codeine, 20 ng/mL for all others				
0.85	0	100	0	40
Level 4 – 300 ng/mL morphine and codeine, 40 ng/mL 6-AM, 60 ng/mL for all others				
0.95	15	0	16	0
Level 5 – 500 ng/mL morphine and codeine, 60 ng/mL 6-AM, 100 ng/mL for all others				
0.95	25	0	24	0
Level 6 – 700 ng/mL morphine and codeine, 80 ng/mL 6-AM, 140 ng/mL for all others				
0.95	35	0	32	0
Level 7 – 1000 ng/mL morphine and codeine, 100 ng/mL 6-AM, 200 ng/mL for all others				
0.90	50	0	40	0

* - Calibration solutions #3 and #4 should not be added to samples that will be subjected to hydrolysis.

** - This calibrator will be outside the linear range for hydromorphone.

L. Negative Control

Purchased from Diagnostics Products Corporation, UTAK Laboratories, Inc., Cliniqa, or prepared in-house from an appropriate blank specimen. Store refrigerated or obtain fresh. Stability determined by manufacturer. A Negative Control will be extracted and

analyzed with every assay. When possible, the negative control will be matrix matched.

M. Positive Control

These are normally prepared in-house as per TOX-101 but may be purchased from an appropriate vendor as circumstances dictate. Storage and stability determined by manufacturer. Normally prepared by adding the amounts of Control Working Solution to 1 mL matrix as directed in Table 1 below. Quantitative controls are typically prepared in duplicate. When possible, the Positive Control will be matrix matched. Additionally, deuterated analog internal standards serve as a qualitative positive control for each individual specimen.

Table 2: Opiate Control Preparation

Volume of Control Solution #1 (µL)	Volume of Control Solution #2 (µL)*	Volume of Control Solution #3 (µL)*	Volume of Control Solution #4 (µL)
Qualitative Blood or Urine Control (245 ng/mL morphine and codeine, 49 or 50 ng/mL for all other target analytes)			
70	50	50	0
Low Quantitative Blood Control (70 ng/mL morphine and codeine, 15 ng/mL for 6-AM, and 14 ng/mL all other quantitated analytes)			
20	15	0	0
High Quantitative Blood Control (770 ng/mL morphine and codeine, 80 ng/mL 6-AM, 154 ng/mL for all other quantitated analytes)			
220	80	0	0
Hydrolysis Control (250 ng/mL morphine-glucuronide = 154 ng/mL morphine)**			
0	0	0	100

* - Control solution #2 should not be added to samples that will be subjected to hydrolysis.

** - The Hydrolysis Control is analyzed whenever hydrolysis is performed on case specimens to ensure that the enzyme is working properly

6 PROCEDURE

	Step	Note	Reference/Lot
	A. Samples		
☐	1. To labeled screw top 16 x 100 mm tubes add:		
	☐ i. 1 mL of biological fluid		
	☐ ii. 2 g of a prepared tissue homogenate (1:1) For quantitation, case samples are typically prepared in duplicate		
	B. Controls		
☐	1. Prepare Negative Control(s)		
☐	2. Prepare Positive Control(s) i. See Table 2; for quantitation, controls are prepared in duplicate		
	C. Calibrators		
☐	1. Prepare Calibrators (for quantitation) i. See Table 1		
	D. Internal Standard(s)		
☐	1. Add 50 µL of TOX-418 Internal Standard Working Solution		
	☐ i. For "free" opiate assays: add 25 µL of d3-6-AM Working Solution and 1 mL of deionized water and vortex.		
	E. Hydrolysis (Total Opioid Assays only)		
☐	1. Add 1 mL of 1.1 M sodium acetate buffer (~pH 5.2)		
☐	2. Add 30 µL of β-glucuronidase		
☐	3. Vortex		
☐	4. Incubate overnight at approximately 37°C		
	F. Buffer		
☐	1. Add 4 mL of 100mM phosphate buffer		
☐	2. Vortex		
☐	3. Check pH: 6 ± 0.5		

☐	**G. Centrifuge at ~3000 rpm for 15 minutes**		
☐	**H. Transfer supernatant to 16 x 100 mm tube**		
	I. Extract (SPE, sorbent should not be dried until step 4)		
☐	1. Condition cartridges (1 mL/min)		
	☐ i. Add 3 mL methanol		
	☐ ii. Add 3 mL deionized water		
	☐ iii. Add 1 mL 100mM phosphate buffer		
☐	2. Load samples (1 mL/min)		
☐	3. Wash cartridges (1 mL/min)		
	☐ i. Add 3 mL of deionized water		
	☐ ii. Add 1 mL 100mM acetic acid		
	☐ iii. Add 3 mL of methanol		
☐	4. Dry cartridge under full vacuum for 3 minutes		
☐	5. Elute (1 mL/min)		
	☐ i. Add 3 mL Elution Solvent		
	☐ ii. Collect eluent in 12 x 75 mm tubes		
☐	6. Evaporate to dryness under nitrogen at 40°C.		
	J. Reconstitute		
☐	1. Add 100 µL of reconstitution solvent to 12 x 75 mm tubes		
☐	2. Vortex and transfer to ALS vial.		
	K. Instrumental Analysis		
☐	1. LC/MS: analyze 5 µL i. Analyze LC/MS Performance Standard prior to batch analysis		
	ii. Mobile Phase 1 (aqueous)		
	iii. Mobile Phase 2 (organic)		
	iv. LC Column		
☐	2. Note: analyze an injection of a solvent blank under the column wash conditions specified in Section 7.1.2 of this procedure at least every 15 analytical injections.		

7 ANALYTICAL PARAMETERS

7.1 Shimadzu HPLC

7.1.1 Gradient/Conditions

Time (min)	Mobile Phase %		Flow Rate		
	1-Aqueous	2-Organic	(mL/min)	Column Heater (°C)	30
0	100	0	0.25	Autosampler (°C)	15
2	100	0	0.25	Run Time (min)	25
6	80	20	0.25		
10	80	20	0.25		
11	40	60	0.25		
16	40	60	0.25		
17	100	0	0.25		
25	100	0	0.25		

7.1.2 Column Washing

Time (min)	Mobile Phase %		Flow Rate		
	1-Aqueous	2-Organic	(mL/min)	Column Heater (°C)	30
0	100	0	0.25	Autosampler (°C)	15
1	100	0	0.25	Run Time (min)	25
4	10	90	0.25		
14	10	90	0.25		
17	100	0	0.25		
25	100	0	0.25		

7.2 Thermo LTQ-XL Orbitrap

Source Parameters		
Mode: Electrospray	Spray Voltage: +5 kV	Capillary Temperature: 225ºC
Sheath Gas: 25 (arb units)	Aux Gas: 12 (arb units)	Sweep Gas: 0 (arb units)
All other source parameters are set through the tuning process. See the appropriate IOSS standard operating procedure for details.		

Segment #1 (0-2 minutes) (1 scan event)(also for Column Wash)		
Event #1	full scan m/z 200 – 370, 7500 resolution (minimum)	
Segment #2 (2-6.5 minutes) (3 scan events)		
Event #1	full scan m/z 200 - 370, 7500 resolution (minimum)	
Event #2	MS/MS data dependant scan (unit resolution)	collision energy: 30% (rel)
	precursor: most intense of m/z 272.13, 284.13, 286.14, 288.16	
	isolation width: 2.0 AMU	scan range: software control
Event #3	MS^3 product scan (unit resolution)	collision energy: see below
	precursor: m/z 302.2 (CE = 30%) > m/z 284.2 (CE = 30%)	
	isolation width: 2.0 AMU	scan range: m/z 75-320
Segment #3 (6.5-15 minutes) (4 scan events)		
Event #1	full scan m/z 200 - 370, 7500 resolution (minimum)	
Event #2	MS/MS data dependant scan (unit resolution)	collision energy: 25% (rel)
	precursor: most intense of m/z 284.13, 286.14, 298.14, 300.16, 302.18, 328.15	
	isolation width: 2.0 AMU	scan range: software control
Event #3	MS^3 product scan (unit resolution)	collision energy: see below
	precursor: m/z 316.2 (CE = 25%) > m/z 298.2 (CE = 35%)	
	isolation width: 2.0 AMU	scan range: m/z 80-330
Event #4	MS^3 product scan (unit resolution)	collision energy: see below
	precursor: m/z 302.2 (CE = 30%) > m/z 284.2 (CE = 30%)	
	isolation width: 2.0 AMU	scan range: m/z 75-320
Segment #4 (15-25 minutes) (1 scan event)		
Event #1	full scan m/z 200 – 370, 7500 resolution (minimum)	

8 DATA ANALYSIS

8.1 Decision Criteria

8.1.1 Batch Acceptance Criteria

No analytes of interest should be detected in the Negative Control. For this purpose, analytes of interest are defined as those analytes that will be reported for this batch.

All intended analytes should be present in the Positive Control. Each Quantitative Positive Control shall quantitate within ±20% of the target value. See TOX-101 for more information.

8.1.2 Sample Acceptance Criteria

8.1.2.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

8.1.2.1.1 Retention Time

The retention time of the peak should be within ± 2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, calibrator, or Positive Control.

Table 3: Retention Time Data

Compound Name	RRT (to d_3-morphine)
d_3-Morphine	RT ~ 4 min
Morphine	1.0
Codeine	2.0
6-AM	2.1
Oxycodone	2.1
Oxymorphone	1.1
Hydrocodone	2.2
Hydromorphone	1.4
Normorphine	0.8
Norcodeine	2.0
Noroxycodone	2.1
Dihydromorphine	0.9
Dihydrocodeine	2.0

Note: Norhydrocodone (M+H 286.144) elutes with a RRT of 2.3.

8.1.2.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than

that for any observed peak at similar retention time in a Negative Control or blank injected just prior to the sample.

8.1.2.2 Mass Spectrometry

The mass spectrum of the analyte of interest should match that of a reference standard or an extracted Positive Control within a reasonable degree of scientific certainty. See TOX-104 for further guidance.

Mass spectral fragments of commonly encountered opioids are listed in Table 4. Under the listing of preferred tandem MS product ions, the normal base peak is listed in bold text. Other significant ions may be substituted for preferred ions if uncorrectable interference exists for that ion.

Table 4: Mass Spectrometry Data:

Compound Name	Quantitation Ion(s) from Full Scan MS	Precursor Ion for MS^2 or Precursor Chain for MS^3	Preferred Tandem MS Product Ions
Morphine	286.144	286.14	183, **201**, 211
d_3-Morphine	289.163	NA	NA
Codeine	300.159	300.16	**215**, 225, 282
d_6-Codeine	306.197	NA	NA
6-AM	328.154	328.15	193, **211**, 268
d_3-6-AM	331.173	NA	NA
Oxycodone	316.154, 298.144*	316.2 >298.2*	187, 241, **256**
d_6-Oxycodone	322.192, 304.151*	NA	NA
Oxymorphone	302.139	302.2 > 284.2	199, 227, **242**
d_3-Oxymorphone	305.158	NA	NA
Hydrocodone	300.159	300.16	**199**, 241, 257
d_3-Hydrocodone	303.178	NA	NA
Hydromorphone	286.144	286.14	**185**, 227, 243
d_3-Hydromorphone	289.163	NA	NA
Normorphine	NA	272.13	201, 229, **254**
Norcodeine	NA	286.14	215, 225, **268**
Noroxycodone	NA	302.2 > 284.2*	187, **229** **
Dihydromorphine	NA	288.16	**187**, 213, 231
Dihydrocodeine	NA	302.18	**201**, 227, **245** ***

* Oxycodone and noroxycodone both show large (M-18) fragments in their full scan mass spectra, with significant variation in the ion ratio dependent upon concentration. The instrument method is set to acquire MS/MS spectra of these fragments in addition to the MS^3 spectra of the pseudomolecular ion in case the pseudomolecular precursor is too weak to provide good spectral fidelity. The MS/MS and MS^3 spectra are qualitatively similar, but show different ion ratios.

** Noroxycodone normally yields only two fragment ions of reasonable intensity in MS^3 analysis. A criterion of no other ions present at >15% of the base peak may be used as additional criteria for the presence of this compound.
*** Either m/z 201 or m/z 245 may be the base peak for MS/MS of dihydrocodeine, depending upon the specific sample.

8.2 Calculations

8.2.1 Calibration

Model	Linear
Weighting	1/x or equal (Codeine: quadratic-log-log)

Performed using a ±20 mmu extracted ion mass window in the full scan high resolution data. Refer to TOX-101 for further guidance.

9 REPORTING

9.1 Measurement Uncertainty

Refer to CHEM-100 and TOX-101.

10 CORRECTIVE MEASURES

Refer to TOX-101 for guidance on action steps in the event of a quality control failure.

11 PERFORMANCE CHARACTERISTICS

11.1 LOD, LOQ, Linearity, Bias, Precision

Compound	LOD in Blood (ng/mL)	LOD in Urine (ng/mL)	LLOQ (ng/mL)	Linear Range (ng/mL)	Accuracy (% bias)	Precision (% intermed)
Morphine	10	25	25	25-1000	-0.8	3.5 to 8.6
Codeine	5	10	25	25-1000	+16.5	4.5 to 10.6
Hydromorphone	5	10	10	10-200	-2.8	5.0 to 9.0
Hydrocodone	2	5	5	5-200	+1.6	4.8 to 7.2
Oxymorphone	2	5	5	5-200	-2.0	9.7 to 12.7
Oxycodone	1	2	5	5-200	-1.1	4.9 to 10.2
6-AM	2	10	5	5-100	-13.5	4.3 to 6.8
Normorphine	5	10	*Not evaluated.*			
Norcodeine	10	10				
Noroxycodone	5	10				
Dihydromorphine	10	10				
Dihydrocodeine	5	5				

11.2 Carryover

During validation there was no significant carryover following the analysis of a 1000 ng/mL level sample

12 LIMITATIONS

12.1 General

The enzymatic hydrolysis procedure will convert a large fraction of any 6-AM in a sample to free morphine. Appropriate care should be taken in interpreting total morphine concentration in any sample for which 6-AM was detected in the free opioid analysis.

12.2 Interferences

Grossly decomposed or putrefied samples may affect both detection and quantitation limits. Very high levels of codeine (>1 µg/mL) may interfere with accurate quantitation of oxycodone, and very high levels of naltrexone may interfere with accurate quantitation of oxycodone. In none of these cases will qualitative identification be compromised. High levels of naloxone may interfere with detection and quantitation of hydromorphone but would not yield false positive results. A compound that is present in many blank blood samples has shown to interfere with the quantitation of oxycodone and oxymorphone at unit mass resolution, but this compound can be resolved using high resolution.

12.3 Processed Sample Stability

Not evaluated; all batches contain appropriate internal standards and controls.

13 SAFETY

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

14 REVISION HISTORY

Revision	Issued	Changes
06	08/01/2022	Complete document reformat.