Analysis of Opioids in Hair by UPLC/HRMS

Table of Contents

Analysis of Opioids in Hair by UPLC/HRMS

1 INTRODUCTION

Opioids are a class of substances that include natural, semi-synthetic, and synthetic alkaloidal agents derived from opium or substances which have morphine-like activity. Naturally occurring opioids such as morphine and codeine are typically referred to as opiates. Heroin (diacetylmorphine) is a semi-synthetic opioid that is synthesized by the acetylation of morphine. In humans, heroin is rapidly metabolized to 6-monoacetylmorphine (6-AM) and morphine. Other common opioids include hydromorphone, hydrocodone, oxymorphone, oxycodone, methadone, meperidine, and tramadol. Buprenorphine and fentanyl are two potent synthetic opioids which are usually given at a much lower dose than other opioids. These compounds and their metabolites may be found in the hair of individuals who have been exposed to the drugs.

2 SCOPE

Analyses	☒ Screening ☒ Confirmation ☐ Quantitation
Matrices	Hair
Analytes	Morphine Codeine Hydromorphone Hydrocodone Oxycodone 6-monoacetylmorphine (6-AM) Methadone Meperidine Tramadol Methadone Fentanyl Buprenorphine Norburprenorphine
Personnel	This document applies to authorized personnel who perform the described tasks, singly or in combination.

3 PRINCIPLE

Hair samples are decontaminated with methylene chloride, water, and methanol washes before cryogrinding. The resulting hair powder is digested using a proteinase based solution. The resulting digest solution is purified using solid phase extraction (SPE). Final extracts are analyzed by ultra performance liquid chromatography-high resolution mass spectrometry (UPLC/HRMS).

4 SPECIMEN CRITERIA

20 milligrams of hair is preferred for each analysis. Lower amounts of hair may be used with an increase in limit of detection.

5 EQUIPMENT

Specified items may be substituted with an equivalent material/product if necessary.

5.1 Equipment

Material / Equipment	Grade/Type	Supplier	Product/Part No.
Centrifuge	micro-vial size, 10,000 rpm capable	varies	n/a
Centrifuge	12x75 mm size, 1500 rpm capable	varies	n/a
Concentrator	multiple sample, capable of 40°C	SPEWare	279-0050
Cryogrinder	programmable	Retsch	MM200
Heating block	n/a	Varies	n/a
Laboratory balance	0.1 mg resolution	Varies	n/a
LC Column	BEH UPLC C18: 2.1x100 mm, 1.7u	Waters	186002352
Pipettes	20µL to 1 mL range	varies	n/a
Positive pressure manifold	multi-sample, capable of 25 psi	SPEWare	289-0004
Thermomixer	programmable, capable of 55°C and 1000 rpm	Eppendorf	Model 5355, R

5.2 Consumables

Material / Equipment	Grade/Type	Supplier	Product/Part No.
0.2µ centrifugal filtration vial, Spin-X	0.2µ, nylon	Fisher	CLS8169
3.0 mm grinding balls	stainless steel	Retsch	22.445.0011
Aluminum weighing dish	fluted sides, tab	Fisher-Scientific	08-732-100
Autosampler caps	9 mm, Blue S/T	Wheaton	09-0034B
Autosampler vials with inserts	9 mm with insert	Wheaton	09-1200-101
Eppendorf vials, 2.0 mL, snap-cap	Polypropylene, curved/conical bottom	Fisher Scientific	05-408-138
Falcon 15mL conical tube	n/a	Fisher-Scientific	14-959-49D
Glass tubes, 12 x 75 mm	n/a	Fisher Scientific	14-961-26

SPE Column, Polychrom Clin II	SCX strong cation exchange, 35 mg, 3 mL capacity	SPEWare/Cerex	650-353

5.3 Instruments

Material / Equipment	Grade/Type	Supplier	Product/Part No.
UPLC Binary Pump System	Acquity-I class	Waters	
Mass Spectrometer	Hybrid quadrupole-Orbitrap	Thermo Scientific	Q-Exactive

5.4 Software

Component	Software	Version
Operating System	Microsoft Windows	7 Pro SP 1
Mass Spectrometer	Foundation	3.1
	Xcalibur	3.1
	Q-Exactive Orbitrap MS	2.8 SP1
	Waters Acquity	3.0.0
Chromatography	Acquity Instrument Driver	1.51.3347
	Binary Solvent Manager	1.50.1521
	Column Manager	1.50.1678
	Sample Manager	1.50.2736
Data Analysis	Tracefinder Forensic	5.1

5.5 Chemicals/Reagents

5.5.1 Purchased

Material / Equipment	Grade/Type	Supplier	Product/Part No.
Acetonitrile	HPLC/Optima	Fisher	A996-4
Ammonium hydroxide	ACS	Fisher	A6695-500
Calcium chloride, 1.0 M	Fluka/volumetric	Sigma-Aldrich	21114-1L
Dithiothreitol (DTT)	≥ 98% (TLC)	Sigma-Aldrich	D0632
Ethyl acetate	ACS	Fisher	E195-1
Formic Acid	reagent	Sigma/Fluka	94318

Hydrochloric acid	ACS	Fisher	A144-500
Methanol	HPLC/Optima	Varies	n/a
Methylene chloride	HPLC/Optima	Varies	n/a
Potassium bicarbonate	ACS	Fisher	P9144-500
Potassium carbonate	ACS	Fisher	P208-500
potassium phosphate, dibasic	ACS	Fisher	P-288-500
potassium phosphate, monobasic	ACS	Fisher	P-286-1
Proteinase-K enzyme	Tritirachium album, ≥ 30 units/mg protein	Sigma-Aldrich	P-6556-100MG
Urea	Electrophoresis grade	Sigma-Aldrich	U-6504
Water	Millipore/18mΩ	In-house	n/a
Water	HPLC/Optima	Fisher	W7-4

5.5.2 Prepared

Material / Equipment	Steps
0.1 M hydrochloric acid	Aliquot 800 µL of hydrochloric acid and dilute to 96 mL with deionized water. Stable for at least 3 months. Store in glass at room temperature.
0.1 M potassium phosphate buffer, pH 8	Combine 94 mL of the 1.0 M potassium phosphate, dibasic solution and 6 mL of the 1.0 M potassium phosphate, monobasic solution. Dilute to 1 L with deionized water. Mix well and store refrigerated in glass. Stable for 3 months.
1.0 M potassium phosphate, dibasic	Weigh 87 g of potassium phosphate, dibasic to a 500 mL volumetric flask. Dilute to the mark with deionized water. Mix well and store refrigerated in glass. Stable for 3 months.
1.0 M potassium phosphate, monobasic	Weigh 68 g of potassium phosphate, monobasic to a 500 mL volumetric flask. Dilute to the mark with deionized water. Mix well and store refrigerated in glass. Stable for 3 months.
5% potassium phosphate, monobasic	Weigh 25 g potassium phosphate (monobasic) and dilute to 500 mL with deionized water. Mix well. Store refrigerated, stable for 3 months.
Digestion Solution	To a 15 mL conical tube, add 308 mg of DTT, 900 mg of urea and 0.050 mL of 1.0M calcium chloride. Dilute to 10 mL with 0.1M potassium phosphate buffer, pH 8. Vortex and store in heating block at 55°C until use. Stable for one day.

	Makes 10 mL of solution, which is sufficient for 20 digests (0.5 mL per digest).
Elution Solvent (98% ethyl acetate with 2% ammonia)	To 40 mL of ethyl acetate, add 0.8 mL of ammonium hydroxide. Mix well. Prepare fresh, just prior to elution.
Mobile Phase 1, Aqueous / Weak Wash	In a 500 mL graduated cylinder, add 250 mL of Optima grade water. Add 0.5 mL of formic acid. Dilute to 500 mL and mix well. Stable for 5 days. To prevent microbial growth discard after 5 days.
Mobile Phase 2, Organic / Strong Wash	In a 500 mL volumetric cylinder, add 250 mL of Optima grade acetonitrile. Add 0.5 mL of formic acid. Dilute to 500 mL and mix well. Stable for at least 3 months.
Potassium carbonate buffer, pH 9	Dissolve 20 g of potassium bicarbonate and 10 g of potassium carbonate in 500 mL of deionized water. Adjust the pH to 9 and then dilute to 1 L. Store at ambient temperature. Stable for at least 3 months.
Proteinase-K enzyme working solution, 40 mg/mL	Weigh 40 mg of proteinase-K into a tared Eppendorf snap-cap vial. Add 1mL of 0.1M potassium phosphate buffer, pH 8. Vortex and store refrigerated. Stable for 3 days. 1 mL of working solution is sufficient for 20 digests (0.050 mL per digest).
Reconstitution solvent 95:5 water:acetonitrile with 0.1% formic acid	Combine 5 mL acetonitrile with 95 mL water (both Optima grade) and 0.1 mL formic acid and mix well. Store in glass at room temperature. Stable for 3 months.
Seal Wash, Solvent A2	Methanol:Water 1:1. Combine equal volumes of each solvent. Store at ambient temperature. Stable for at least 6 months.
Solvent B2	Acetonitrile

5.6 Standards/Controls

5.6.1 Purchased

5.6.1.1 Internal Standards

Analyte	Concentration (mg/mL)	Solvent	Product No.	Aliquot for Stock (mL)
d_3-Morphine	0.1	methanol	M-003	0.250
d_6-Codeine	0.1	methanol	C-040	0.250

d_3-Oxymorphone	0.1	methanol	O-003	0.250
d_6-Oxycodone	0.1	methanol	O-005	0.250
d_3-Hydromorphone	0.1	methanol	H-006	0.250
d_3-Hydrocodone	0.1	methanol	H-005	0.250
d_3-Tramadol-13C	0.1	methanol	T-029	0.250
d_3-Methadone	0.1	methanol	M-008	0.250
d_4-Meperidine	0.1	methanol	M-036	0.250
d_4-Buprenorphine	0.1	methanol	B-901	0.025
d_3-Norbuprenorphine	0.1	methanol	N-920	0.025
d_5-Fentanyl	0.1	methanol	F-001	0.025
d_3-6-AM	0.1	acetonitrile	A-010	0.250

Cerilliant or another approved vendor.

5.6.1.2 Controls

Analyte	Concentration (mg/mL)	Solvent	Product No.	Aliquot for Stock (mL)
Morphine	1	methanol	M-030	0.250
Codeine	1	methanol	C-006	0.250
Oxymorphone	1	methanol	O-004	0.250
Oxycodone	1	methanol	O-002	0.250
Hydromorphone	1	methanol	H-004	0.250
Hydrocodone	1	methanol	H-003	0.250
Tramadol	1	methanol	T-027	0.250
Methadone	1	methanol	M-019	0.250
Meperidine	1	methanol	M-035	0.250
Buprenorphine	1	methanol	B-902	0.025
Norbuprenorphine	1	methanol	N-912	0.025
Fentanyl	1	methanol	F-002	0.025
6-AM	1	acetonitrile	A-009	0.250

Cerilliant, Lipomed or another approved vendor.

5.6.2 *Prepared*

5.6.2.1 *Internal Standards*

Stock Internal Standard Solution (methanol, 0.005/0.0005 mg/mL)
Aliquot the standards as indicated in section 5.6.1.1 (except d_3-6-AM) into a 5 mL volumetric flask. Dilute to the mark with Optima grade methanol. Store below 0°C in glass. Stable for at least one year.
Stock Internal Standard Solution, d_3-6-AM (acetonitrile, 0.005 mg/mL)
Aliquot the d_3-6-AM standard as indicated in section 5.6.1.1 into a 5 mL volumetric flask. Dilute to the mark with Optima Grade acetonitrile. Store below 0°C in glass. Stable for at least one year.
Working Internal Standard Solution (aqueous, 0.05/0.005 µg/mL)
Aliquot 0.050 mL of both of the stock internal standard solutions to a partially filled 5 mL volumetric flask. Bring to the mark with HPLC grade water. Store refrigerated in glass. Stable for approximately one week.

5.6.2.2 *Controls*

Stock Control Solution (methanol, 0.01/0.001 mg/mL)
Aliquot the standards as indicated in section 5.6.1.2 (except 6-AM) into a 25 mL volumetric flask. Dilute to the mark with Optima grade methanol. Store below 0°C in glass. Stable for at least one year.
Stock Control Solution, 6-AM (acetonitrile, 0.01 mg/mL)
Aliquot the 6-AM standard as indicated in section 5.6.1.2 into a 5 mL volumetric flask. Dilute to the mark with Optima grade acetonitrile. Store below 0°C in glass. Stable for at least one year.
Working Control Solution (high, aqueous, 80/8 ng/mL)
Aliquot 0.080 mL of both of the stock standard solutions to a partially filled 10 mL volumetric flask. Dilute to the mark with HPLC grade water. Store refrigerated in glass. Stable for approximately one week.
Working Control Solution (low, aqueous, 4/0.4 ng/mL)
Aliquot 0.50 mL of the Working Standard Solution (High) to a partially filled 10 mL volumetric flask. Bring to the mark with HPLC grade water. Store refrigerated in glass. Stable for approximately one week.

5.6.2.3 *Matrix*

Negative Control Hair
Obtained from drug-free donors. Stored in paper or plastic at room temperature. Negative Control Hair does not expire.

Negative Control Hair Powder
Obtained by washing Negative Control hair with methylene chloride, water, and methanol; drying the hair, and pulverizing in a cryogrinder. Negative Control Hair Powder is stored in plastic at room temperature and does not expire.

5.6.2.4 Control Scheme

5.6.2.4.1 Low Positive Control

Analyte	Matrix (mg)	Solution	Spike (mL)	Final Conc. (pg/mg)
Morphine	20	Working Standard Control Solution- Low	0.025	5.0
Codeine				5.0
Oxymorphone				5.0
Oxycodone				5.0
Hydromorphone				5.0
Hydrocodone				5.0
Tramadol				5.0
Methadone				5.0
Meperidine				5.0
Buprenorphine				0.5
Norbuprenorphine				0.5
Fentanyl				0.5
6-AM				5.0

5.6.2.4.2 High Positive Control

Analyte	Matrix (mg)	Solution	Spike (mL)	Final Conc. (pg/mg)
Morphine	20	Working Standard Control Solution- High	0.025	100
Codeine				100
Oxymorphone				100
Oxycodone				100
Hydromorphone				100
Hydrocodone				100
Tramadol				100
Methadone				100
Meperidine				100

Buprenorphine				10
Norbuprenorphine				10
Fentanyl				10
6-AM				100

5.6.2.5 LC/MS Performance Standard

Column Performance Evaluation Mix (2/0.2 ng/mL)
Aliquot 0.05 mL of the Working Standard Solution (low, aqueous) to an autosampler vial. Aliquot 0.05 mL of reconstitution solvent and mix. Stable for approximately one week.

6 PROCEDURE

6.1 Sample Analysis

	Step	Note	Reference/Lot
	A. Sample Handling		
☐	1. Visually inspect hair and record observations		
☐	2. If segmental analysis is required, cut a portion of the hair sample into 2-cm segments.		
☐	3. Weigh approximately 20 mg of each hair sample (or segment) into a properly labeled 2.0 mL Eppendorf tube. Record weight to the nearest 0.1 mg.		
	B. Decontamination		
	1. To each sample tube:		
	i. Organic Wash 1		
☐	a. Add 1.0 mL methylene chloride		
☐	b. Vortex for ~ 1 minute		
☐	c. Discard Wash		
	ii. Aqueous Wash		
☐	a. Add 1.0 mL deionized water		
☐	b. Vortex for ~ 1 minute		
☐	c. Discard Wash		
	iii. Organic Wash 2		
☐	a. Add 1.0 mL methanol		
☐	b. Vortex for ~ 1 minute		
☐	c. This wash may be capped and stored refrigerated for later analysis, if necessary.		
	iv. Drying		
☐	a. Dry hair samples in a heating block or sample concentrator at approximately 40°C to evaporate any remaining solvent. Dry for 30 minutes or until samples are dry.		
	C. Cryogrinding		
☐	1. Add 3mm x 3 grinding balls (new) to each tube and cap		
☐	2. Place tubes into the cryogrinder's 6-position tube holder and screw the retaining ring into place		

☐	3. Cryogrind dry hair samples using the settings in Section 7.2.4. Use all appropriate safety gear for cryogrinder operation.		
☐	4. Upon removing the vials from the cryogrinder, allow the temperature of the vials to rise past the frost point before removing the retaining ring		
☐	5. Tap the tubes on the bench to loosen any cryoground hair from the cap before opening the tubes and proceeding.		
	D. Controls (20 mg each of negative control matrix)		
☐	1. Prepare Negative Control(s)		
☐	2. Prepare Positive Control(s) i. Low Control		
☐	ii. High Control		
	iii. Control Scheme		
	E. Digestion		
	1. Open tubes and add:		
☐	i. Digestion Solution: 0.5 mL		
☐	ii. Proteinase-K Working Solution: 0.050 mL		
☐	iii. Internal Standard Working Solution: 0.020 mL		
☐	2. Recap tubes and vortex briefly		
☐	3. Thermomix for 60 minutes at 55ºC and 750 rpm (may be extended if necessary).		
☐	4. Add 1 mL of 5% potassium phosphate monobasic		
☐	5. Continue thermomixing at 55ºC and 750 rpm for five minutes.		
☐	6. Centrifuge samples at ~10,000 rpm for 5 minutes		
☐	7. Decant supernatant to 12 x 75 mm glass tube.		
☐	8. Add an additional 1 mL of 5% potassium phosphate, monobasic.		
	F. **Extract** (SPE)		
	1. Condition cartridges (1 mL/min)		
☐	i. Add 3 mL methanol		
☐	ii. Add 1 mL deionized water		
☐	2. Load samples (1 mL/min).		
	3. Wash cartridges (1 mL/min)		

☐	i. Add 1 mL of potassium carbonate buffer		
☐	ii. Add 1 mL of deionized water		
☐	iii. Add 1 mL 100mM HCl		
☐	iv. Add 1 mL of methanol		
☐	4. Dry cartridge under full vacuum/25 psi for 5 minutes		
	5. Elute (1 mL/min)		
☐	i. Add 2 mL Elution Solvent		
☐	ii. Collecte eluent in 12 x 75 mm tubes		
	G. Concentrate		
☐	1. Evaporate to dryness under nitrogen at 40°C		
	H. Reconstitute		
☐	1. Add 75 µL of Reconstitution Solvent 2. Vortex thoroughly 3. Centrifuge the 12 x 75 mm tubes for 1 min at 1500 rpm to consolidate the solvent. 4. Transfer the solvent to a 0.2µ filtration vial. 5. Centrifuge at 10,000 rpm for 5 minutes. 6. Transfer the eluent to ALS vial with insert and cap		
☐	**I. Instrumental Analysis** 1. LC/MS: analyze 10 µL i. Analyze LC/MS Performance Standard prior to batch analysis		
	ii. Mobile Phase 1 (aqueous)/Weak Wash		
	iii. Mobile Phase 2 (organic)/Strong Wash		
	iv. Seal Wash/Solvent A2		
	v. Solvent B2		
	vi. LC Column		

6.2 Wash Analysis (Optional)

1. Add 20 µL Internal Standard Solution to each wash.		
2. Evaporate to dryness under a gentle stream of nitrogen at approximately 40ºC.		
3. Reconstitute each sample in 0.5 mL deionized water by vortexing for at least 10 seconds.		
4. Extract (6.1) and analyze (7)		

7 ANALYTICAL PARAMETERS

7.1 Waters Acquity i-Class UPLC Gradient/Conditions

Time (min)	1-Aqueous %	2-Organic %	(mL/min)	Column Heater (°C)	50
0.00	98	2	0.40	Autosampler (°C)	10
0.50	98	2	0.40	Run Time (min)	9.75
5.00	50	50	0.40	**Autosampler Volumes**	**µL**
5.50	10	90	0.40	Weak Wash	600
7.50	10	90	0.40	Strong Wash	200
7.75	98	2	0.40	Sample Loop	20
9.75	98	2	0.40	Needle Overfill Flush	5
				Event Table	6.25 min
		Seal Wash	5 min	Cycle inject valve	

7.2 Thermo Q-Exactive Conditions

Depending upon the case scenario, full scan and either SIM or MS/MS can be used.

SIM provides a slight increase in sensitivity whereas MS/MS provides more information.

7.2.1 Full Scan and SIM

Parameter	Full Scan	tSIM	Parameter	Value
Runtime	0 to 9.75 min	0 to 9.75 min	Mode	ESI
Polarity	positive	positive	Spray Voltage	+3.5 kV
In-Source CID	0.0 eV	0.0 eV	Capillary Temperature	375ºC
Inclusion	-	on	Heater Temperature	350ºC
Microscans	1	1	Sheath Gas	35
Resolution	35,000	35,000	Aux Gas	1
AGC Target	1e6	2e4	Sweep Gas	0
Maximum IT	50 ms	100 ms	S- Lens RF Level	70
MSX Count	-	1	For Inclusion List Values see Section 7.2.3	
Isolation Window	-	1.5 m/z		

Scan Ranges	1	-	
Scan Range	100-650 m/z	100-650 m/z	

7.2.2 *Full Scan and MS/MS*

Parameter	Full Scan	tMS2	Parameter	Value
Runtime	0 to 9.75 min	0 to 9.75 min	Mode	ESI
Polarity	positive	positive	Spray Voltage	+3.5 kV
In-Source CID	0.0 eV	0.0 eV	Capillary Temperature	375ºC
Inclusion	-	on	Heater Temperature	350ºC
Microscans	1	1	Sheath Gas	35
Resolution	17,500	17,500	Aux Gas	1
AGC Target	1e6	2e4	Sweep Gas	0
Maximum IT	50 ms	100 ms	S- Lens RF Level	70
MSX Count	-	1	For Inclusion List Values see Section 7.2.3	
Isolation Window	-	1.5 m/z		
Scan Ranges	1	-		
Scan Range	100-650 m/z	100-650 m/z		

7.2.3 *Inclusion List*

Mass (m/z)	Polarity	Start (min)	End (min)	NCE (%)	CS (z)	Name
286.14445	Positive	1.40	1.62	55	1	morphine
302.13935	Positive	1.60	1.74	50	1	oxymorphone
286.14445	Positive	1.70	1.88	55	1	hydromorphone
300.16007	Positive	2.11	2.28	55	1	codeine
316.15500	Positive	2.28	2.45	42	1	oxycodone
328.15500	Positive	2.30	2.50	55	1	6-AM
300.16007	Positive	2.40	2.59	55	1	hydrocodone
264.19648	Positive	3.05	3.24	10	1	tramadol
248.16517	Positive	3.30	3.45	45	1	meperidine
414.26456	Positive	3.50	3.65	62	1	norbuprenorphine
337.22809	Positive	3.88	4.02	35	1	fentanyl
256.17025	Positive	4.04	4.12	70	1	diphenhydramine*
468.31150	Positive	4.16	4.30	62	1	buprenorphine
310.21720	Positive	4.68	4.78	30	1	methadone

(inclusion optional)

7.2.4 Retsch Cryomill

Cycles	1
Precool	Auto
Run time	6.5 min
Rate	25 hz

8 DATA ANALYSIS

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard, calibrator, or extracted Positive Control.

8.1 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.

8.2 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 or Positive Control.

8.3 Signal-to-Noise

To justify the existence of a peak, its baseline signal to noise ratio should exceed 3. Further, the 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.

8.4 Mass Spectrometry

The mass spectrum of the analyte of interest should match that of a reference standard, extracted calibrator, or an extracted Positive Control. See TOX-104 for further guidance.

8.5 Calculations

Refer to TOX-101 for further guidance.

8.5.1 Software

Qualitative calculations may be performed by one or more of the following software packages:

A. Thermo Xcalibur
    1. QualBrowser
    2. Tracefinder
B. Microsoft
    1. Excel

9 REPORTING

Refer to TOX-100 and TOX-101 for guidance.

In order to report the LOD expressed in Section 11.1, the positive control for that analyte must have been detected in the batch. Values estimating as lower than the LOD may be reported so long as the conditions in Section 8 are met.

9.1 Diphenhydramine

Diphenhydramine and other common over-the-counter/prescription drugs may be detected by this method. Diphenhydramine was frequently identified during matrix evaluations. In order to report such a drug as detected in a case sample, that drug must not be detected in the Negative Control Hair used for the batch.

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

Administratively set, expressed as minimum established during validation.

Analyte	LOD (pg/mg)
morphine	10
oxymorphone	10
hydromorphone	10
codeine	10
oxycodone	10
6-AM	10
hydrocodone	10
tramadol	10
meperidine	10
norbuprenorphine	1
fentanyl	1
diphenhydramine	10
buprenorphine	1
methadone	10

11.2 Carryover

None detected at tested concentrations during validation.

12 LIMITATIONS

12.1 Processed Sample Stability

All analytes are stable in processed sample extracts for at least four days except for buprenorphine and norbuprenorphine. After three days norbuprenorphine partially converts to buprenorphine.

12.2 Selectivity

A low level interferent for tramadol is observed in some samples which coelutes and has a similar parent mass (264.195). In order to establish tramadol as detected in a sample, there must also be:

a) tandem mass spectrometry that passes TOX-104 criteria

b) have a detectable isotope trace (265.199) with a 17 ± 2 % ratio, and

c) have an estimated concentration of greater than 1 pg/mg.

No other interferences were observed.

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
01	02/11/2022	Changed title. Document reformat. Minor updates to wording/phrases. 7.2 -Clarified use of SIM vs MS/MS 9 – Clarified reporting criteria