

	TX-PM 4.1.3 Confirmation of Opiates	
	Document #: 4992	Page 1 of 6
	Revision #: 1	Issued Date: 02/06/2018
	Document Manager: Nicholas Fillingier	Approved By: Jeffrey Nye

4.1.3 Confirmation of Opiates

4.1.3.1 Analyte(s)

Codeine, morphine, hydromorphone, oxycodone and other opiates.

4.1.3.2 Specimen Requirements

Two mL blood, serum, plasma, urine, or other biological fluid (i.e. vitreous fluid, spinal fluid).

4.1.3.3 General Description of Method

An internal standard GC/MS identification of the derivatized opiate. Extraction of the analytes of interest from the biological matrix is accomplished by using a solid phase extraction (SPE) method that has been adapted from a United Chemical Technologies (UCT) method, formerly Worldwide Monitoring Corp.

4.1.3.4 Equipment and Reagents

- GC/MS equipped with a suitable column for separating the analytes of interest from other drugs and coextractives (i.e. 15 meter DB5 capillary column).
- UCT standard SPE vacuum tank, manifold, vacuum source, and reagents as specified in the UCT procedure manual code CBB200DAUZ050191.
- Internal standard(s): deuterated analogs of codeine, morphine or other appropriate compound.
- Authentic drug standards for positive control (see below).
- The usual assortment of laboratory glassware, reaction vessels, pipettes, reagent grade chemicals, vortexers, and shakers.
- Derivatizing reagents: N,O-bis (trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (Pierce prod. # 38831 or equivalent).

4.1.3.5 Sample Preparation for Confirmation of Opiates

4.1.3.5.1 Controls

- Positive and Negative Controls must be prepared in each matrix in which unknown samples are being analyzed.
- Prepare a Negative Control by pipetting 2 mL blank blood and / or 2 mL blank urine into a clean, labeled 16 x 125 mm culture tube.
- Prepare a Positive Control by pipetting 2 mL blank blood and / or 2 mL blank urine and 20 µL of the Opiate Standard into a clean labeled 16 x 125 mm culture tube. Add other analytes as appropriate.
- Prepare a Hydrolysis Control by pipetting 2 mL blank blood and / or urine and 20 µL of the Hydrolysis Control into a clean labeled 16 x 125 mm culture tube.

	TX-PM 4.1.3 Confirmation of Opiates	
	Document #: 4992	Page 2 of 6
	Revision #: 1	Issued Date: 02/06/2018
	Document Manager: Nicholas Fillinger	Approved By: Jeffrey Nye

4.1.3.5.2 Unknowns

- Pipet 2 mL blood or urine into a clean, labeled 16 x 125 mm culture tube.

4.1.3.5.3 Additional Preparation of All Controls and Unknowns

- Add 20 µL Opiate Internal Standard (100 ng/µL each: D-3 Codeine and D-3 Morphine)
- Q.S. to 5 mL with deionized water.
- Mix / vortex, and let stand for 5 minutes.
- Add 40 µL B-glucuronidase
- Mix / vortex.
- Hydrolyze at 60° C for 1 hour. Allow sample to cool to room temperature.
- Centrifuge for 10 minutes at ≥3000 rpm.
- Decant samples into clean, labelled 16 x 125 mm culture tubes.
- Discard tubes containing pellets.
- Add 4 mL acetate buffer (pH 4.5).
- Mix / vortex. pH should be 4.5 ± 0.5.

4.1.3.6 Sample Extraction

4.1.3.6.1 Condition Clean Screen Extraction Column

To appropriately labeled extraction columns placed on a manifold pass the following reagents individually through all columns under gravity or low vacuum (<3 in Hg):

- 3 mL anhydrous methanol, aspirate.
- 3 mL DI water, aspirate.
- 1 mL acetate buffer (pH 4.5), aspirate.

Note: Aspirate at ≤ 3 inches Hg to prevent sorbent drying.

4.1.3.6.2 Apply Sample

- Carefully decant the sample mixture into its respective extraction column.
- Allow gravity to pull the sample through the column. (If vacuum assisted aspiration is used, use a flow rate of no more than 2 mL/min.)

4.1.3.6.3 Wash Column

- 2 mL acetate buffer, pH 4.5, aspirate.
- 2 mL DI water, aspirate.
- 1 mL anhydrous methanol, aspirate.
- DRY COLUMN ≥ 10 INCHES Hg FOR 30 MINUTES

	TX-PM 4.1.3 Confirmation of Opiates	
	Document #: 4992	Page 3 of 6
	Revision #: 1	Issued Date: 02/06/2018
	Document Manager: Nicholas Fillingier	Approved By: Jeffrey Nye

4.1.3.6.4 Elute Analytes

Place clean, labeled 12 x 75 mm culture tubes into rack, place the rack inside the manifold tank assuring each column will elute into its respective tube.

- 3 mL methylene chloride/ isopropanol/ ammonium hydroxide in the ratio of 78:20:2.
- NOTE: **Prepare elution solvent daily**. Add ammonium hydroxide to isopropanol, then add the methylene chloride (pH > 10).
- Collect eluate under gravity. Use vacuum pump to remove the last of the eluate from the column.

4.1.3.6.5 Evaporate Eluates

- Evaporate to dryness $\leq 40^{\circ}$ C in TurboVap
- Reconstitute sample with 200 - 300 μ L ethyl acetate
- Mix / vortex.
- Transfer to autosampler vial and evaporate to dryness $\leq 40^{\circ}$ C in Turbo Vap.

4.1.3.9.6 Unextracted Control

- An unextracted control is prepared by pipetting 5 μ L Opiate Standard into a clean, labeled autosampler vial.
- Evaporate to dryness at $\leq 40^{\circ}$ C in turboVap.
- Unextracted control is prepared in the same manner as controls and unknowns from this point forward.

4.1.3.6.7 Derivatize

To each control and unknown add:

- 40 μ L BSTFA/TMCS
- 40 μ L acetonitrile
- Cap with red rubber septum and metal crimp cap.
- Mix / vortex
- Incubate at 95° C for 30 minutes.
- Do NOT evaporate. Cool to room temperature.
- Run on appropriate GC/MS.

4.1.3.7 GC/MS Instrument Setup

- See the [Toxicology ANB-Dedicated GC-MS \(DSQ\) Schedule](#) for details regarding maintenance prior to batch analysis.

	TX-PM 4.1.3 Confirmation of Opiates	
	Document #: 4992	Page 4 of 6
	Revision #: 1	Issued Date: 02/06/2018
	Document Manager: Nicholas Fillinger	Approved By: Jeffrey Nye

4.1.3.8 GC/MS Data Interpretation

Analyte	Base Ion (m/z)	Molecular Ion (m/z)	Other ions (m/z)
D3-morphine	73	432	149, 239, 199, 223
D3-codeine	73	374	181, 149, 199, 237
morphine	73	429	236, 146, 196, 234
codeine	73	371	178, 196, 234, 42
hydromorphone	357	357	300, 73, 301, 59, 299
oxycodone	387	387	179, 73, 229, 230, 70

4.1.3.8.1 Chromatographic and Mass Spectral Quality Control

- Chromatographic Quality
 - Chromatographic quality for Toxicology SIM data is defined as a reasonably symmetrical shaped peak consistent with those observed in calibrators and positive controls and is able to be differentiated from a negative control.
 - Chromatographic quality for Toxicology Full Scan data is defined as a resolved peak, not always symmetrical in nature, for three m/zs consistent with the analyte of interest and the TIC. The observable peak should be able to be differentiated from a negative control.
 - Flexibility is given to the experienced analyst to prevent misidentification and under-identification.
- Retention Time
 - Whenever possible, the retention time of positive analytes shall match a known reference standard run with each batch of unknowns. If a known reference standard is unavailable, a relative retention time based upon deuterated internal standards should be used. It is recognized that retention times may "shift", slightly, from that of the known reference standard. Flexibility is given to the experienced analyst to prevent misidentification and under-identification.
- Mass spectrum and ion ratios
 - Whenever possible, the mass spectrum and ion ratios of an identified analyte shall match a known reference standard. If a known reference standard is unavailable, a library match using an approved library is acceptable. It is recognized that ion ratios may change, slightly, from that of the known reference standard based upon factors such as analyte concentration, co-eluting substances and background noise. Flexibility is given to the experienced analyst to prevent misidentification and under-identification.
- Library Matches
 - If a known reference standard is unavailable, a library match from an accepted library may be used to aid in identification of an analyte. Approved libraries are:
 - DD2010
 - SWGDRG
 - nistdemo
 - mainlib

	TX-PM 4.1.3 Confirmation of Opiates	
	Document #: 4992	Page 5 of 6
	Revision #: 1	Issued Date: 02/06/2018
	Document Manager: Nicholas Fillingier	Approved By: Jeffrey Nye

- caymanspectrallibrary
- Any MSP in-house library in which the name of the analyte, lot number and manufacturer have been recorded

4.1.3.9 Reporting Results

- Qualitative positive results will be reported as "Detected (Not Quantified)".
- See also [4.6 Drug Reporting Guidelines for Forensic Advantage](#).

4.1.3.10 Preparation of Standards and Controls

Controls and internal standards are to be made in glass screw top vials capable of containing at least 10 mL, or equivalent.

Prepared controls and internal standards are to be stored refrigerated.

Expiration is one year from date made, or the date of earliest component expiration, whichever is earlier.

4.1.3.10.1 Opiate Mix

The Opiate Mix is made using a 1 mg/mL stock standard of all analytes.

Analyte	Volume (mL)	Final Concentration (ng/μL)
codeine	1	100
hydromorphone	1	100
morphine	1	100
oxycodone	1	100
methanol	6	

4.1.3.10.2 Internal Standards

The Opiate Assay Internal Standard is made using a 1 mg/mL stock standard of all analytes.

Analyte	Volume (mL)	Final Concentration (ng/mL)
D3-codeine	1	100
D3-morphine	1	100

	TX-PM 4.1.3 Confirmation of Opiates	
	<i>Document #: 4992</i>	<i>Page 6 of 6</i>
	<i>Revision #: 1</i>	<i>Issued Date: 02/06/2018</i>
	<i>Document Manager: Nicholas Fillinger</i>	<i>Approved By: Jeffrey Nye</i>

methanol	8	
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4.1.3.10.3 Hydrolysis Control

The Opiate Assay Hydrolysis Control is made using a 1 mg/mL stock standard of all analytes.

Analyte	Volume (mL)	Final Concentration
morphine-3-β-D-glucuronide	1	100
methanol	9	