

	<b><i>TX-PM 4.1.4 Qualitative Confirmation of Acidic, Neutral and Basic Drugs in Other Biological Matrices</i></b>	
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	Document Manager: Nicholas Fillinger	Approved By: Jeffrey Nye

## **4.1.4 Qualitative Confirmation of Acidic, Neutral and Basic Drugs in Other Biological Matrices**

### **4.1.4.1 Analyte(s)**

See toxicology scope of analysis in [Toxicology Procedure Manual 1.14](#)

### **4.1.4.2 Specimen requirements**

2 mL vitreous humor, serum, plasma, other biological fluid or tissue suspension.

<2 mL of sample may be used if sample quantity is limited.

### **4.1.4.3 General Description of Method**

- An internal standard GC/MS identification of acidic, neutral and basic drugs.
- An internal standard GC/MS confirmation of the derivatized cocaine metabolite (benzoylecgonine) or opiate(s) using pentafluoro-propionic anhydride (PFPA) and hexafluoro-isopropanol (HFIP).
- 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.

### **4.1.4.4 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, reagents, and SPE columns as specified in the UCT procedure manual code EMB200DAUZ050191.
- Internal standard(s): deuterated analogs of phencyclidine, diazepam, nordiazepam, morphine, benzoylecgonine, and butalbital.
- 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: pentafluoropropionic anhydride, Aldrich 25,238-7 (or equivalent) 1,1,1,3,3,3-hexafluoro-2-propanol, Aldrich 32,524-4 (or equivalent)

### **4.1.4.5 Sample Preparation and Extraction**

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A homogeneous sample is ensured by gently rocking the specimen on the Labquake Shaker for at least 5 minutes. All sample handling will be performed in the biological safety cabinet using the universal biohazard handling techniques.

#### 4.1.4.5.1 Controls

- Prepare a negative control by pipetting 2 mL DI water into a clean, labeled 16 x 125 mm culture tube.
- Prepare one or more positive controls by pipetting 2 mL DI water and 20 µL of the Low, Medium or High Positive General Control and 20 µL Low, Medium or High Positive Cocaine Control into a clean, labeled 16 x 125 mm culture tube.
- Prepare a Tier I and Tier II standard by pipetting 20 µL of the appropriate standard into a clean, labeled autosampler vial.

#### 4.1.4.5.2 Unknowns

- Pipet 2 mL sample into a clean, labeled 16 x 125 mm culture tube. Less is allowable if sample quantity is limited. For low volume samples, add DI water to bring total sample volume to 2 mL.

#### 4.1.4.5.3 Additional Sample Preparation for all Controls and Unknowns

- Add 1 mL of internal standard solution.
- QS to 5 mL with DI water. Mix/Vortex.
- Centrifuge for 10 minutes at  $\geq 3000$  rpm.
- Transfer samples to clean, labeled 16 x 125 mm culture tubes.
- Discard tubes containing pellets.
- Adjust the pH to  $6.0 \pm 0.5$  with 4 mL 100 mM phosphate buffer.

#### 4.1.4.5.4 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 methyl alcohol (CH<sub>3</sub>OH), aspirate.
- 3 mL DI water, aspirate.
- 1 mL 100 mM phosphate buffer (pH 6.0), aspirate.

*NOTE: Aspirate at  $\leq 3$  inches Hg to prevent sorbent drying.*

#### 4.1.4.5.5 Apply Sample

- Carefully, decant the sample mixture into its respective extraction column.

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- 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.)

#### 4.1.4.5.6 Wash Columns

- 3 mL DI water, aspirate.
- 1 mL 100 mM acetic acid, aspirate.
- DRY COLUMN ( $\geq$  10 INCHES Hg FOR 30 MIN)
- 500  $\mu$ L hexane; aspirate.

#### 4.1.4.5.7 Elute Acidic and Neutral Drugs

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.

- 2 mL hexane/ethyl acetate (30/70); collect eluate using gravity or less than 2 mL/min vacuum.
- Use vacuum pump to remove the last of the eluate from the columns.
- Evaporate eluate to dryness ( $\leq$  40° C) in TurboVap.
- Reconstitute in an appropriate volume of ethyl acetate or a 50:50 mixture of methyl alcohol and ethyl acetate.
- Transfer to a dry, clean conical autosampler vial.
- Cap with red rubber septum and metal crimp cap.
- Run on an appropriate GC/MS.

*NOTE: Acidic and Neutral eluate may not be collected and analyzed in every case.*

#### 4.1.4.5.8 Wash Columns

- 3 mL methanol, aspirate
- DRY COLUMN ( $\geq$ 10 INCHES Hg FOR 5 MIN)

#### 4.1.4.5.9 Elute Basic Drugs

- 2 mL methylene chloride / isopropanol / ammonium hydroxide in the following appropriate ratios: (78/20/2), (39/10/1), (23.4/6/0.6), (19.5/5/0.5).
- NOTE: Prepare elution solvent daily. Add ammonium hydroxide to isopropanol then add the methylene chloride (pH  $\geq$ 10).
- Collect eluate under gravity.
- Use vacuum pump to remove the last of the eluate from columns.

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#### **4.1.4.5.10 Evaporate Eluates**

- Evaporate just to dryness ( $\leq 40^{\circ}$  C) in TurboVap;
- Reconstitute in an appropriate volume of methyl alcohol or a 50:50 mixture of methyl alcohol and ethyl acetate.
- Transfer to a dry, clean conical autosampler vial.
- Cap appropriately with metal crimp cap.
- Run on an appropriate GC/MS.

#### **4.1.4.5.11 Derivatize Extracts**

Derivatize the basic extract following the GC/MS run when ruling out benzoylecgonine, opiates, amphetamines or other derivatizable analytes.

##### **4.1.4.5.11.1 Unextracted Derivative Control**

An unextracted Derivative Control is prepared by pipetting 5  $\mu$ L Derivative Standard Mix into a clean, labeled autosampler vial.

Unextracted Derivative Control is prepared in the same manner as all standards, controls and unknowns from this point forward.

##### **4.1.4.5.11.2 Unextracted Opiate Standard**

An Unextracted Opiate Control is required when an unknown sample is analyzed for the presence of hydromorphone and the incubation is performed at room temperature.

An Unextracted Opiate Control is prepared by pipetting 5  $\mu$ L Opiate Standard into a clean, labeled autosampler vial.

Unextracted Opiate Standard is prepared in the same manner as all standards, controls and unknowns with any exceptions detailed below.

##### **4.1.4.5.11.3 Derivatize**

- Evaporate all standards, controls and unknown samples to dryness at  $\leq 40^{\circ}$  C in TurboVap.

***CAUTION: Overdrying may cause loss of volatile analytes.***

To each standard, control or unknown add:

- 50  $\mu$ L ethyl acetate.
- 10  $\mu$ L hexafluoroisopropanol (HFIP). Each molecule of HFIP adds 150 amu to the analyte.
- 15  $\mu$ L perfluoropropionic anhydride (PFPA). Each molecule of PFP adds 146 amu to the analyte.

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- Mix / vortex.
- The Unextracted Opiate Control and unknowns with a request for hydromorphone should be incubated at **room temperature** for 30 – 40 minutes.
- If a case is suspected of having derivatizable analytes in addition to hydromorphone present, the case should be reviewed by a unit supervisor to determine the appropriate incubation temperature.
- All other samples incubate for 30-40 minutes at 60 to 80° C.

**Note #1: Samples with large quantities of derivatizable drugs (e.g., ibuprofen or pseudoephedrine) may preclude the confirmation of the intended analyte(s). If suspected, rederivatize the sample using a larger volume of the above reagents.**

#### 4.1.4.5.11.4 Evaporate Derivatives

- Evaporate the solvent in each vial just to dryness ( $\leq 40^{\circ}$  C) until the pungent smell of the anhydride is absent.
- Reconstitute in an appropriate volume of ethyl acetate; use screening values as a guide. Ex: 40  $\mu$ L

#### 4.1.4.6 GC/MS Instrument Setup

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

#### 4.1.4.7 Data Analysis

##### 4.1.4.7.1 Controls and Standards

- Each full batch analysis shall contain a low, medium, high and negative control. Partial batches require a minimum of one positive control and a negative control.
- The following standards shall be analyzed with each batch, full or partial.
  - Tier I
  - Tier II
- The positive control and standards should contain all analytes of interest. Verification of control lot number and expiration date shall occur at the time calibrator/control packs are reviewed.
- The negative control should not contain enough of the analyte of interest to be confirmed.

##### 4.1.4.7.2 Unknowns

- Casework samples shall be analyzed for a minimum of Tier I substances. Analytes detected during Tier I analysis, outside the scope of Tier I, may be reported by the casework scientist. The requirements in 4.1.4.8 apply.

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- Casework samples shall be analyzed for Tier II substances when requested. Additionally, the casework scientist may initiate a full or partial Tier II analysis based upon observations within the data.
- The casework scientist may report analytes of interest that are outside the scope of Tier I and Tier II. The requirements in 4.1.4.8 apply.

#### **4.1.4.7.3 Blanks**

- Each casework pair of samples, acidic/neutral and basic, should have a minimum of one solvent blank run between them. Additional blanks may be run if necessary.
- The blank should not contain enough analyte of interest to be confirmed. If it does, this indicates carryover and the subsequent casework sample will need to be reinjected.

#### **4.1.4.8 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 the three most abundant m/zs and TIC of any particular analyte. The observable peak should be able to be differentiated from a negative control. It is recognized that in certain instances, such as co-eluting substances, the three most abundant m/zs may not be the most appropriate indicator of chromatographic quality. 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

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

#### **4.1.4.9 Reporting results**

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