# Technical Procedure for the Solid Phase Extraction of Acidic, Neutral and Basic Drugs for GC-MS Analysis

**1.0 Purpose** - This procedure specifies the required elements for the solid phase extraction of acidic, neutral and basic drugs (other than cannabinoids and *gamma*-hydroxybutyric acid) from blood, serum, and urine.

**2.0 Scope** – This procedure applies to the Toxicology Units of the State Crime Laboratory.

## 3.0 Definitions

- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.

## 4.0 Equipment, Materials and Reagents

### 4.1 Equipment

- Centrifuge
- pH meter
- Mechanical pipettes
- Class A volumetric flasks
- Pressure manifold or other solid phase extraction device equipped with nitrogen
- Zymark TurboVap LV or other evaporator equipped with nitrogen

### 4.2 Materials

- Test tubes (16 x 125, 13 x 100, 12 x 75)
- Test tube caps or stoppers
- Vortexer
- Pipet tips

### 4.3 Reagents

- Deionized water

### 4.4 Commercial Reagents

- Methylene chloride, ACS grade
- Acetic acid, ACS grade
- Anhydrous dibasic sodium phosphate ($Na_2HPO_4$), ACS grade
- Anhydrous Monobasic sodium phosphate ($NaH_2PO_4$), ACS grade
- Methanol, ACS grade
- β-glucuronidase, 1,000,000 – 3,000,000 units / gram solid
- Sodium hydroxide pellets, ACS grade
- Hydrochloric acid, concentrated, ACS grade
- Hexane, ACS grade
- Ethyl acetate, ACS grade
- Ammonium hydroxide, concentrated, ACS grade
- Phosphoric acid, 85 %, ACS grade
- Nitrogen, grade 5.0

- UCT Clean Screen® DAU Solid Phase Extraction Columns

**4.5 Primary Reference Materials**

- Prazepam
- Nalorphine
- Hexobarbital
- Methohexital

**4.6 Critical Reagents**

- Negative Blood
- Negative Urine
- BSTFA with 1 % TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide with 1 % trimethylchlorosilane)

**4.7 Prepared Reagents -** Prepared reagents may be prepared in any amount provided that the component ratios are kept constant.

**4.7.1 1.0 M Acetic Acid**

**4.7.1.1** Add 28.6 mL glacial acetic acid to 400 mL deionized water in a 500 mL volumetric flask.

**4.7.1.2** Mix and dilute to 500 mL with deionized water.

**4.7.1.3** Lot Number: Eight digit format year/month/day

**4.7.1.3.1** Example: 20101231

**4.7.1.4** Expirati**o**n: Three years.

**4.7.1.5** Store at room temperature.

**4.7.1.6** QC Check: Tests acidic with pH or litmus paper.

**4.7.2 0.1 M Monobasic sodium phosphate (NaH2PO4)**

**4.7.2.1** Dissolve 1.20 grams monobasic sodium phosphate, anhydrous, in deionized water in a 100 mL volumetric flask.

**4.7.2.2** Mix and dilute to 100 mL with deionized water.

**4.7.2.3** Lot number: Eight digit format year/month/day

**4.7.2.3.1** Example: 20101231

**4.7.2.4** Expiration: Three years.

**4.7.2.5** Refrigerate

**4.7.2.6** QC Check: Tests acidic with pH or litmus paper.

**4.7.3 0.1 M Dibasic sodium phosphate (Na2HPO4)**

**4.7.3.1** Dissolve 1.42 grams dibasic sodium phosphate, anhydrous, in 80 mL deionized water in a 100 mL volumetric flask.

**4.7.3.2** Mix and dilute to 100 mL with deionized water.

**4.7.3.3** Lot number: Eight digit format year/month/day

**4.7.3.3.1** Example: 20101231

**4.7.3.4** Expiration: Three years.

**4.7.3.5** Refrigerate.

**4.7.3.6** QC check: Tests basic with pH paper.

**4.7.4 0.1 M Phosphate Buffer**

**4.7.4.1** Dissolve 1.70 g anhydrous dibasic sodium phosphate ($Na_2HPO_4$) and 12.14 g monobasic sodium phosphate monohydrate ($NaH_2PO_4$-$H_2O$) in 800 mL deionized water.

**4.7.4.2** Dilute to 1 L with deionized water and mix.

**4.7.4.3** Using a pH meter, adjust pH to 6.0 +/- 0.1 with 0.1 M monobasic sodium phosphate (lowers pH) or 0.1 M dibasic sodium phosphate (raises pH).

**4.7.4.4** Lot number: Eight digit format year/month/day

**4.7.4.4.1** Example: 20101231

**4.7.4.5** Expiration: One month

**4.7.4.6** Refrigerate.

**4.7.4.7** QC Check: Record final pH.

**4.7.5 Basic Drug Internal Standard**

**4.7.5.1** Prepare a 2000 ng/mL solution of prazepam reference standard and a 1000 ng/mL solution of nalorphine reference standard in methanol.

**4.7.5.1.1** Example – dilute 1.0 mL of a 1.0 mg/mL solution of prazepam and 0.5 mL of a 1.0 mg/mL solution of nalorphine to 500 mL with methanol.

**4.7.5.2** Lot number: Eight digit format year/month/day

**4.7.5.2.1** Example: 20101231

**4.7.5.3** Expiration: One year.

**4.7.5.4** Refrigerate.

**4.7.5.5** QC check: Successful negative control extraction.

**4.7.6 Acidic/Neutral Drug Internal Standard – Hexobarbital 20 µg/mL**

**4.7.6.1** Prepare a 20 µg/mL solution of hexobarbital reference standard in methanol.

**4.7.6.1.1** Example – dilute 1.0 mL of a 1.0 mg/mL solution of hexobarbital to 50 mL with methanol.

**4.7.6.2** Lot number: Eight digit format year/month/day

**4.7.6.2.1** Example: 20101231

**4.7.6.3** Expiration: One year.

**4.7.6.4** Refrigerate.

**4.7.6.5** QC check: Successful negative control extraction.

**4.7.7 Acidic/Neutral Drug Internal Standard – Methohexital 10 µg/mL**

**4.7.7.1** Prepare a 10 µg/mL solution of methohexital reference standard in methanol.

**4.7.7.1.1** Example – dilute 0.5 mL of a 1.0 mg/mL solution of Methohexital to 50 mL with methanol.

**4.7.7.2** Lot number: Eight digit format year/month/day

**4.7.7.2.1** Example: 20101231

**4.7.7.3** Expiration: One year.

**4.7.7.4** Refrigerate.

**4.7.7.5** QC check: Successful negative control extraction.

**4.7.8 0.1 N Sodium hydroxide (NaOH)**

**4.7.8.1** Dissolve 4.00 grams of sodium hydroxide in 80 mL of deionized water in a 100 mL volumetric flask.

**4.7.8.2** Mix and dilute to volume with deionized water.

**4.7.8.3** Lot number: Eight digit format year/month/day

**4.7.8.3.1** Example: 20101231

**4.7.8.4** Expiration: Three years.

**4.7.8.5** Store at room temperature.

**4.7.8.6** QC check: Tests basic to pH or litmus paper.

**4.7.9 0.5 M Phosphoric acid**

**4.7.9.1** Add 3.4 mL concentrated phosphoric acid to 80 mL deionized water in a 100 mL volumetric flask.

**4.7.9.2** Mix and dilute to volume with deionized water.

**4.7.9.3** Lot number: Eight digit format year/month/day

**4.7.9.3.1** Example: 20101231

**4.7.9.4** Expiration: Three years.

**4.7.9.5** Store at room temperature.

**4.7.9.6** QC check: Tests acidic to pH or litmus paper.

## 5.0 Procedure

### 5.1 Standards and Controls

#### 5.1.1 Positive control

**5.1.1.1** For each basic extraction, the mass spectrum of the basic internal standard (Nalorphine-diTMS if derivatized) must meet the identification criteria in the Toxicology Gas Chromatography/Mass Spectrometry (GC-MS) procedure and the signal-to-noise ratio for the basic internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard/the response at the baseline or valley immediately before the internal standard signal.

**5.1.1.2** For each acidic/neutral extraction, the mass spectrum of the acidic/neutral internal standard must meet the identification criteria in the Toxicology Gas Chromatography/Mass Spectrometry (GC-MS) procedure and the signal-to-noise ratio for the acidic/neutral internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard/the response at the baseline or valley immediately before the internal standard signal.

#### 5.1.2 Negative Control

**5.1.2.1** For each extraction batch of blood/serum samples prepare a negative control as directed in **5.5** with 2.0 mL of negative blood.

**5.1.2.1.1** For each GC-MS analysis, the mass spectrum of the appropriate internal standard must meet the identification criteria in the Toxicology Gas Chromatography/Mass Spectrometry (GC-MS) procedure and the signal-to-noise ratio for the internal standard gas chromatographic peak must be 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard/the response at the baseline or valley immediately before the internal standard signal.

**5.1.2.1.2** The negative control shall be subjected to the same post extraction techniques as any case samples in the batch.

**5.1.2.2** For each extraction batch of urine samples prepare a negative control as directed in **5.6** with 5.0 mL of negative urine.

**5.1.2.2.1** For each GC-MS analysis, the mass spectrum of the appropriate internal standard must meet the identification criteria in the Toxicology Gas Chromatography/Mass Spectrometry (GC-MS) procedure and the signal-to-noise ratio for the internal standard gas chromatographic peak is 5:1 or greater. The signal-to-noise ratio is defined as the response at the peak height of the internal standard/the response at the baseline or valley immediately before the internal standard signal.

**5.1.2.2.2** The negative control shall be subjected to the same post extraction techniques as any case samples in the batch.

**5.2 Calibrations –** N/A

**5.3 Maintenance**

**5.3.1** Ensure that the pressure manifold is clean prior to use and clean after use.

**5.3.2** Add water to the TurboVap if needed.

**5.4 Sampling**

**5.4.1** Allow all solutions and samples to equilibrate to room temperature.

**5.4.2** Ensure that all body fluids are homogenous by shaking and/or vortexing.

**5.4.2.1** If a homogenous sample cannot be obtained, make a notation in the worksheet detailing the condition of the sample and its handling.

**5.5 Blood/Serum sample preparation**

**5.5.1** Add 4.5 mL of deionized $H_2O$ to 2.0 mL of blood.

**5.5.1.1** If an acidic/neutral fraction is to be collected, add 100 µL of an acidic/neutral drug internal standard solution. Use methohexital for samples

where carisoprodol is indicated. Use hexobarbital or methohexital for all other samples.

**5.5.1.2** If a basic fraction is to be collected, add 100 µL of the prazepam internal standard solution. In addition, add 100 µL of nalorphine if derivatization is indicated.

**5.5.2** Mix/Vortex and allow to stand for 5 minutes.

**5.5.3** Mix/Vortex sample.

**5.5.4** Centrifuge for 10 minutes.

**5.5.5** Decant liquid portion of the sample into 2 mL of 0.1 M phosphate buffer and mix.

**5.5.6** If needed, adjust pH to 6.0 ± 0.5 with 0.1 M monobasic sodium phosphate (lowers pH) or 0.1 M dibasic sodium phosphate (raises pH).

## 5.6 Urine sample preparation

**5.6.1** If an acidic/neutral fraction is to be collected, add 250 µL of an acidic/neutral drug internal standard solution to 5.0 mL of urine.

**5.6.2** If a basic fraction is to be collected, add 250 µL of prazepam internal standard solution to 5.0 mL of urine. In addition, add 250 µL of nalorphine if derivatization is indicated.

**5.6.3** If needed, adjust pH to 6.0 ± 0.5 with 0.1 M monobasic sodium phosphate (lowers pH) or 0.1 M dibasic sodium phosphate (raises pH).

**5.6.4** If a majority of the substance(s) of interest is suspected to have formed a glucuronide complex (e.g., a positive or elevated opiate immunoassay and no corresponding substance detected in a non-hydrolyzed extraction), hydrolyze the sample using either enzyme or acid hydrolysis prior to extraction.

**5.6.4.1 Enzyme hydrolysis**

**5.6.4.1.1** Add 2 mL β-glucuronidase and mix.

**5.6.4.1.2** Securely cap and heat for 3 hours at 65 °C.

**5.6.4.1.3** Allow to cool to room temperature.

**5.6.4.1.4** Adjust pH to 6.0 +/- 0.5 with approximately 0.7 mL of 1.0 N NaOH.

**5.6.4.2 Acid Hydrolysis**

**5.6.4.2.1** Add 0.5 mL concentrated HCl and mix.

**5.6.4.2.2** Securely cap and heat for 30 minutes at 120 °C.

**5.6.4.2.3** Allow to cool to room temperature.

**5.6.4.2.4** Mix 1 mL deionized water with 1 mL concentrated ammonium hydroxide. Add 1.0 mL of this mixture to the sample and mix.

**5.6.4.2.5** Adjust pH to 6.0 +/- 0.5 with 1 to 3 mL 0.5 M phosphoric acid.

## 5.7 Procedure to collect an acidic/neutral and/or a basic fraction

**5.7.1** The flow rate for the sample and elution solvent is less than 5 mL per minute. The flow rate for all other additions is 1 to 15 mL per minute. Allow each addition to elute completely prior to adding the next addition.

**5.7.2** Add 3 mL methanol to a UCT Clean Screen® DAU Solid Phase Extraction Column**.**

**5.7.3** Add 3 mL of water to the column.

**5.7.4** Add 1 mL of 0.1 M phosphate buffer to the column.

**5.7.5** Add the blood or urine to be extracted to the column.

**5.7.6** Add 3 mL of water to the column.

**5.7.7** Add 1 mL of 1.0 M acetic acid to the column.

**5.7.8** Dry the column with a nitrogen flow for 5 minutes

**5.7.9** Add 2 mL of hexane to the column.

**5.7.9.1** If only a basic fraction is being collected, proceed to **5.7.11**.

**5.7.10** Elute and collect the acidic/neutral fraction with 6 mL of methylene chloride.

**5.7.10.1** If only an acid/neutral fraction is being collected, proceed to **5.8**.

**5.7.11** Add 3 mL of methanol to the column.

**5.7.12** Dry the column with a nitrogen flow for 2 minutes.

**5.7.13** Mix 20 mL isopropanol and 2 mL ammonium hydroxide. Add 78 mL methylene chloride. (The amount of this mixture may be altered if the component ratios are kept constant.) Elute and collect the basic fraction with 3 mL of the mixture. Dispose of any unused portion according to the State Crime Laboratory Safety Manual.

**5.7.14** Proceed to **5.8**.

## 5.8 Post extraction procedure

**5.8.1** Evaporate to dryness using a TurboVap.

**5.8.2** If a derivatization is not desired, reconstitute the sample in 50 µL of ethyl acetate for basic fractions or 100 µL of ethyl acetate for acidic/neutral fractions. The solvent and/or volume of solvent may be changed based upon analytical needs, but shall be

documented in the case record. Mix and transfer to an insert in auto-sampler vial and cap.

**5.8.3** Derivatization may be performed to improve detection or separation. The derivatization may be performed on either the dried collected sample from **5.8.1,** or to a sample dried after GC-MS analysis following the drying instructions in **5.8.1**.

**5.8.3.1** Add 50 µL of BSTFA with 1 % TMCS and cap securely.

**5.8.3.2** Mix and heat at 80 °C for 30 minutes.

**5.8.3.3** Cool to room temperature.

**5.8.3.4** If needed, transfer to an insert in an auto-sampler vial and cap securely.

**5.8.4** Chromatograph using an appropriate method outlined in the Toxicology Gas Chromatography/Mass Spectrometry (GC-MS) procedure.

**5.9** Create a Quality Control data packet to be reviewed by a Forensic Scientist qualified to perform Solid Phase Extraction of Acidic, Neutral and Basic Drugs for GC-MS Analysis and, if acceptable, approved in the Toxicology Unit section object repository of FA with a file name beginning with the type of extraction performed (Acid or Base) followed by the eight digit year/month/day format ending with the instrument name. A suffix may be added to differentiate between multiple runs.

**5.9.1** Example: Acid20121016T1-XXX or Base20121016T1-XXX

**5.10** The quality control data packet shall contain the following:

- Lot number(s) of internal standard(s), negative blood and/or negative urine
- Extraction date
- The lot number of UCT Clean Screen® DAU Solid Phase Extraction Column used
- If applicable, the lot number of BSTFA with 1 % TMCS
- Completed extraction worksheet
- GC-MS sequence list
- GC-MS tune
- GC-MS method
- Total Ion Chromatogram of the negative control and corresponding blank
- Mass spectra of internal standard(s) and peaks of interest
- Negative Control shall show an indication that the analyst has examined it thoroughly and found no reported substances

**5.11** The case record shall contain the following:

- Total Ion Chromatogram of the sample and corresponding blank
- Mass spectra of internal standard(s) and peaks of interest
- Expanded mass spectra of phenethylamines
- Approved Quality Control data packet for the extraction

**5.12 Calculations –** N/A

**5.13 Uncertainty of Measurement –** N/A

## 6.0 Limitations

**6.1** Refer to the references and other published chemical information as needed to determine the fraction in which a target analyte is expected to elute. Typically, barbiturates, carisoprodol, meprobamate and some benzodiazepines elute in the acidic/neutral fraction. Typically, alkaloids, amphetamines, opiates, zolpidem, tramadol and most benzodiazepines elute in the basic fraction.

**6.2** Refer to the references and other published chemical information as needed to determine the need for derivatization. Typically, morphine and benzoylecgonine need to be derivatized for detection by GC-MS. Some benzodiazepines and other substances may need to be derivatized for detection by GC-MS (e.g., a positive or elevated opiate or benzodiazepine immunoassay and no corresponding substance detected in a non-derivatized sample).

**6.3** Refer to the references and other published chemical information as needed to determine the need for urine hydrolysis.

**6.4** The solid phase extraction columns shall not be allowed to dry during the extraction other than at steps indicated.

**6.5** Store solid phase extraction columns in a closed container.

## 7.0 Safety

**7.1** Refer to Laboratory Safety Manual

## 8.0 References

*UCT Solid Phase Extraction Manual*. United Chemical Technologies Inc. Bristol, PA., (2010) 9 –11, 56 – 58.

Kitchen, Chester J., Michael Telepchak and Thomas F. August. *An Automated Solid PhaseExtraction Method for Thebaine, 6-Acetylmorphine and Other Opiates in Urine*. United ChemicalTechnologies.

Combie, J. Blake et al. "Morphine Glucuronide Hydrolysis: Superiority of β-Glucuronidase from Patella vulgate." *Clinical Chemistry*. 28/1 (1992): 83-6.

*BSTFA with 1 % TMCS Product Specification*, Sigma-Aldrich Co, (1997).

Clean Screen® Extraction Columns have been used in the Toxicology Unit to extract neutral, acidic and basic drugs and the metabolites of these drugs from whole blood and urine since 1995. Use of the Clean Screen® Extraction Columns to extract neutral, acidic and basic drugs and the metabolites of these drugs has been validated through proficiency testing provided by College of American Pathologists.

## 9.0 Records

- Case Record

**10.0 Attachments –** N/A

| **Revision History** | | |
|---|---|---|
| Effective Date | Version Number | Reason |
| 09/17/2012 | 1 | J-05 and J-12 Combination and Conversion to ISO format |
| 10/26/2012 | 2 | 1.0 - removed reference to other matrices; 4.5 - removed Phenobarbital-D5; 4.7.8.1.1 - corrected drug reference in example; Removed 4.7.9 referring to Phenobarbital-D5; 5.1.2.1 - inserted serum, removed "reference standard"; 5.1.2.2 - removed "reference standard"; Removed 5.1.2.3 - unknown or other matrices; 5.5 - inserted Serum; 5.5.1.1 - changed phenobarbital-d5 to Methohexital, removed phenobarbital-d5; 5.5.4 - removed speed requirement; Removed section 5.7 - Other matrix sample preparation; 5.8-5.10 - consolidated into one extraction procedure (now 5.7); Removed conditioning of the columns with elution solvent in consolidated extraction; moved instructions for preparation of elution solvent; Post Extraction Procedure (now 5.8) corrected references within procedure; changed wording; reworded 5.8.1; Inserted 5.8.4 - reference to GC-MS procedure; Inserted new 5.9-5.10 - quality control data packet and criteria; Amended case record requirements (now 5.11) to reflect addition of quality control data packet; grammar |
| 02/15/2013 | 3 | 2.0 - modified for procedure merge<br>5.10 - inserted GC-MS method |
| 05/10/2013 | 4 | 4.7.1.3, 4.7.2.3, 4.7.3.3, 4.7.4.4, 4.7.5.2, 4.7.6.2, 4.7.7.2, 4.7.8.3, and 4.7.9.3 - simplified lot number format and reflected change in example<br>Old 4.7.6 consolidated into 4.7.5 to create one basic internal standard solution<br>5.1.1.1, 5.1.1.2, 5.1.2.1.1 and 5.1.2.2.1 - inserted reference to mass spectral identification criteria and changed signal to noise ratio requirement<br>5.10 - inserted completed extraction worksheet |
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