
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 phenethylamines) 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

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- UCT Clean Screen[®] DAU Solid Phase Extraction Columns

4.5 Primary Reference Materials

- Prazepam
- Nalorphine
- Methohexital
- Alprazolam
- Amitriptyline
- Cocaine
- Codeine
- Diphenhydramine
- Lidocaine
- Meprobamate
- Methadone
- Oxycodone
- Trazodone

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 Expiration: 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 Phosphate Buffer

- 4.7.2.1 Dissolve 1.70 g anhydrous dibasic sodium phosphate (Na_2HPO_4) and 12.14 g monobasic sodium phosphate monohydrate ($\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$) in 800 mL deionized water.

- 4.7.2.2 Dilute to 1 L with deionized water and mix.
- 4.7.2.3 Using a pH meter, adjust pH to 6.0 +/- 0.1 with monobasic sodium phosphate (lowers pH) or dibasic sodium phosphate (raises pH).
- 4.7.2.4 Lot number: Eight digit format year/month/day
 - 4.7.2.4.1 Example: 20101231
- 4.7.2.5 Expiration: One month
- 4.7.2.6 Refrigerate.
- 4.7.2.7 QC Check: Record final pH.

4.7.3 Acid/Base Internal Standard

- 4.7.3.1 Prepare a 2000 ng/mL solution of prazepam reference standard, a 1000 ng/mL solution of nalorphine reference standard, and a 10 µg/mL solution of methohexital reference standard in methanol.
 - 4.7.3.1.1 Example – dilute 400 µL of a 1.0 mg/mL solution of prazepam and 200 uL of a 1.0 mg/mL solution of nalorphine and 2 mL of a 1.0 mg/mL solution of methohexital to 200 mL with methanol.
- 4.7.3.2 Lot number: Eight digit format year/month/day
 - 4.7.3.2.1 Example: 20101231
- 4.7.3.3 Expiration: One year.
- 4.7.3.4 Refrigerate.
- 4.7.3.5 QC check: Successful negative control extraction.

4.7.4 Positive Control Standard

- 4.7.4.1 Prepare a positive control standard containing the following primary reference standards in methanol.
 - Alprazolam at 1400 ng/mL (final blood/urine concentration 70 ng/mL)
 - Amitriptyline at 4000 ng/mL (final blood/urine concentration 200 ng/mL)
 - Cocaine at 2000 ng/mL (final blood/urine concentration 100 ng/mL)
 - Codeine at 4000 ng/mL (final blood/urine concentration 200 ng/mL)

- Diphenhydramine at 2000 ng/mL (final blood/urine concentration 100 ng/mL)
- Lidocaine at 2000 ng/mL (final blood/urine concentration 100 ng/mL)
- Meprobamate at 160 µg/mL (final blood/urine concentration 8.0 µg/mL)
- Methadone at 1000 ng/mL (final blood/urine concentration 50 ng/mL)
- Oxycodone at 1200 ng/mL (final blood/urine concentration 60 ng/mL)
- Trazodone at 10,000 ng/mL (final blood/urine concentration 500 ng/mL)

4.7.4.1.1 Example: add the following volumes of each 1 mg/mL standard to a 10 mL flask and dilute to volume in methanol.

- 14 µL of Alprazolam
- 40 µL of Amitriptyline
- 20 µL of Cocaine
- 40 µL of Codeine
- 20 µL of Diphenhydramine
- 20 µL of Lidocaine
- 1.6 mL of Meprobamate
- 10 µL of Methadone
- 12 µL of Oxycodone
- 100 µL of Trazodone

4.7.4.2 Lot Number: eight digit format year/month/day

4.7.4.2.1 Example: 20101231

4.7.4.3 Expiration: One year

4.7.4.4 Refrigerate

4.7.4.5 QC check: successful positive control extraction.

4.7.5 0.1 N Sodium hydroxide (NaOH)

4.7.5.1 Dissolve 4.00 g of sodium hydroxide in 80 mL of deionized water in a 100 mL volumetric flask.

4.7.5.2 Mix and dilute to volume with deionized water.

4.7.5.3 Lot number: Eight digit format year/month/day

4.7.5.3.1 Example: 20101231

4.7.5.4 Expiration: Three years.

4.7.5.5 Store at room temperature.

4.7.5.6 QC check: Tests basic to pH or litmus paper.

4.7.6 0.5 M Phosphoric acid

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

4.7.6.2 Mix and dilute to volume with deionized water.

4.7.6.3 Lot number: Eight digit format year/month/day

4.7.6.3.1 Example: 20101231

4.7.6.4 Expiration: Three years.

4.7.6.5 Store at room temperature.

4.7.6.6 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 acid/neutral and basic extraction batch of blood/serum samples prepare a positive control by adding 100 µL of the positive control standard to 2.0 mL of negative blood and prepare as directed in **5.5**. If urine samples are extracted in the batch then prepare a urine positive control by adding 250 µL of the positive control standard to 5.0 mL of negative urine and prepare as directed in **5.6**.

5.1.1.1.1 For each extraction, the mass spectra of the internal standards (Nalorphine-di-TMS if derivatized) and all positive control analytes must meet the identification criteria in the procedure for [Toxicology Gas Chromatography/Mass Spectrometry](#).

5.1.1.1.2 All positive controls shall be subjected to the same post extraction techniques as any corresponding case samples in the batch.

5.1.1.1.3 If a drug intended to be in a positive control is not identified in the positive control, all cases affected by the control failure will be re-extracted.

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. If urine samples are

extracted in the batch then prepare a negative control as directed in **5.6** with 5.0 mL of negative urine.

5.1.2.1.1 For each GC-MS analysis, the mass spectrum of the internal standard must meet the identification criteria in the [Toxicology Gas Chromatography-Mass Spectrometry \(GC-MS\) procedure](#).

5.1.2.1.2 The negative controls 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 H₂O to 2.0 mL of blood.

5.5.2 Add 100 µL of the acid/base internal standard solution.

5.5.3 Mix/Vortex and allow to stand for 5 minutes.

5.5.4 Mix/Vortex sample.

5.5.5 Centrifuge for 10 minutes.

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

5.5.7 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 Add 250 µL of acid/base internal standard solution to 5.0 mL of urine.

5.6.2 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).

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

5.6.3.1 Enzyme hydrolysis

5.6.3.1.1 Add 2 mL β -glucuronidase and mix.

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

5.6.3.1.3 Allow to cool to room temperature.

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

5.6.3.2 Acid Hydrolysis

5.6.3.2.1 Add 0.5 mL concentrated HCl and mix.

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

5.6.3.2.3 Allow to cool to room temperature.

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

5.6.3.2.5 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 shall be performed on the dried collected sample from **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 the method that was used to evaluate the certified reference standards.

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:

- Summary page with FA workstation reference
- Completed extraction worksheet
- GC-MS sequence list
- GC-MS tune
- GC-MS method
- Negative and Positive Control data generated in accordance with **5.11** (re-injected negative and positive control data only requires generation of **5.11.1** and **5.11.4**).

5.11 The case record shall contain the following:

5.11.1 Labeled Total Ion Chromatogram (TIC) of the sample and corresponding blank

5.11.2 Total Ion Chromatogram clearly showing the peak of interest with an un-subtracted mass spectrum

5.11.3 Mass spectra of internal standard(s) and peaks of interest with library match

5.11.4 RRTs of the peak of interest and corresponding reference standard

5.11.5 Extracted Ion Chromatogram for an analyte being reported negative

5.11.6 Approved Quality Control data packet for the extraction (N/A for quality controls)

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, 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 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 Phase Extraction Method for Thebaine, 6-Acetylmorphine and Other Opiates in Urine*. United Chemical Technologies.

Combie, J. Blake et al. “Morphine Glucuronide Hydrolysis: Superiority of β -Glucuronidase from *Patella vulgata*.” *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 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 Old 4.7.6 consolidated into 4.7.5 to create one basic internal standard solution 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 5.10 - inserted completed extraction worksheet
11/15/2013	5	Added issuing authority to header
05-09-2014	6	1.0 – Removed reference to gamma-hydroxybutyric acid and added reference to phenethylamines. 4.5 and 4.7.5 - Added positive control. 4.7.2, 4.7.3, and 4.7.6 – Removed 4.7.4.3- Modified due to removal of 4.7.2 and 4.7.3 5.1.1 - Added requirement to prepare positive control for each extraction batch and criteria. Internal standard acceptance criteria moved to GC-MS technical procedure 5.1.2.1 - Added instructions for urine negative control 5.1.2.1.1 - Internal standard acceptance criteria moved to GC-MS technical procedure 5.1.2.2 – Removed due to changes in 5.1.2.1 5.5.1.1, 5.5.1.2 – Removed reference to specific internal standards 5.6.2 – Removed references to Nalorphine Internal Standard since only

		one basic internal standard used. 5.8.4 – Updated requirement. 5.10 - Added requirement for Positive Control to be in QC data packet. Modified criteria to be included in data packet 5.11 - Removed requirement for expanded mass spectrum for phenethylamines. Added additional criteria.
08-29-2014	7	4.7.3-Consolidated 4.7.4 with 4.7.3 5.1.1.1 – modified to be consistent with 5.1.2 5.5.1.1 and 5.5.1.2 – Consolidated to new 5.5.2 5.6.1 and 5.6.2 – Consolidated 5.6.3 and 6.2 - Removed reference to elevated Opiates 5.8.3 – Removed after analysis option 5.10 – Added criteria for re-injected controls 5.11 – Changed from bullets to numbers 5.11.1- added “Labeled” 5.11.4 - changed “sample” to “peak of interest” 6.1 – Removed reference to amphetamines