Technical Procedure for Phenethylamine Liquid-Liquid Extraction, (PHEALLE) for Analysis by GC-MS Analysis

1.0 Purpose - This procedure specifies the required elements for the liquid-liquid extraction of phenethylamine drugs 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
- Vortexer
- Test tube rocker
- Zymark TurboVap LV or other evaporator equipped with nitrogen

4.2 Materials

- Large screw-cap test tubes (16 x 150 mm)
- Pipet tips
- GC-MS vials with caps

4.3 Prepared Reagents

- 2 % HCL in methanol
- Ammonium Chloride buffer pH 9.0
- 0.5 N H_2SO_4

4.4 Commercial Reagents

- Ammonium chloride, ACS grade
- N-butyl chloride, ACS grade
- Methanol, ACS grade
- Sodium chloride, ACS grade
- Hydrochloric acid, concentrated, ACS grade
- Sulfuric acid, concentrated, ACS grade
- Hexanes, ACS grade
- Acetic anhydride derivatizing reagent, ACS grade
- Ethyl acetate, ACS grade
- Ammonium hydroxide, concentrated, ACS grade

- Nitrogen

4.5 Primary Reference Materials

- Amphetamine
- Methamphetamine
- 3,4-Methylenedioxyamphetamine (MDA)
- 3,4-Methylenedioxymethamphetamine (MDMA)
- d-11 Amphetamine
- d-11 Methamphetamine

4.6 Critical Reagents

- Negative Blood
- Negative Urine

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

4.7.1 2 % HCL in Methanol

4.7.1.1 Mix 10 mL of methanol and 200 µL of concentrated hydrochloric acid.

4.7.1.2 This reagent is to be prepared daily prior to use.

4.7.1.3 QC Check: Tests acidic with pH or litmus paper.

4.7.1.4 Dispose of any unused portion as provided in the State Crime Laboratory Safety Manual.

4.7.2 Ammonium Chloride Buffer pH 9.0

4.7.2.1 Dissolve 35.7 grams of ammonium chloride in 100 mL deionized water in a breaker. Wait until fully dissolved, which may take an hour or more.

4.7.2.2 Add 7 mL of concentrated ammonium hydroxide.

4.7.2.3 Slowly add additional concentrated ammonium hydroxide until the pH reaches 9.0.

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

4.7.2.4.1 Example: 20140509

4.7.2.5 Expiration: One year

4.7.2.6 Refrigerate.

4.7.2.7 QC Check: Tests basic with pH or litmus paper.

4.7.3 0.5 N Sulfuric Acid

4.7.3.1 Add 500 mL of deionized water to a glass bottle.

4.7.3.2 Slowly add 7.0 mL of concentrated sulfuric acid.

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

4.7.3.3.1 Example: 20140509

4.7.3.4 Expiration: One year.

4.7.3.5 Store at room temperature.

4.7.3.6 QC check: Tests acidic with pH paper.

4.7.4 Phenethylamine Internal Standard

4.7.4.1 Prepare a 20 µg/mL solution of d-11 amphetamine and d-11 methamphetamine reference internal standard in methanol.

4.7.4.1.1 Example – In a 50 mL volumetric flask, dilute 1.0 mL of a 1.0 mg/mL solution of d-11 amphetamine and 1.0 mL of a 1.0 mg/mL solution of d-11 methamphetamine and fill to the mark (QS) with methanol.

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

4.7.4.2.1 Example: 20140509

4.7.4.3 Expiration: One year.

4.7.4.4 Store in freezer.

4.7.4.5 QC check: Successful negative control extraction.

4.7.5 Positive Control – 10 µg/mL

4.7.5.1 Prepare a 10 µg/mL solution of the following reference standards in methanol:

- Amphetamine 1mg/mL standard
- Methamphetamine 1mg/mL standard
- 3,4-Methylenedioxyamphetamine (MDA) 1mg/mL standard
- 3,4-Methylenediozymethamphetamine (MDMA) 1mg/mL standard

4.7.5.1.1 Example – In a 50 mL volumetric flask, dilute 0.5 mL of each 1.0 mg/mL standards listed above and QS with methanol.

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

4.7.5.2.1 Example: 20140509

4.7.5.3 Expiration: One year.

4.7.5.4 Store in freezer.

4.7.5.5 QC check: Successful positive control extraction.

5.0 Procedure

5.1 Allow all solutions and samples to be analyzed to equilibrate to room temperature.

5.2 Control Sample Preparation

5.2.1 Positive Control Preparation

5.2.1.1 Add 4.975 mL of negative blood to a test tube.

5.2.1.2 Add 25µL of Positive Control solution.

5.2.1.3 Cap and vortex the test tube.

5.2.1.4 For each extraction batch of blood /serum samples, prepare as directed in **5.6** using 2.0 mL of this positive control.

5.2.1.5 Dispose of any unused portion as provided in the State Crime Laboratory Safety Manual.

5.2.2 Negative Control

5.2.2.1 For each extraction batch of blood/serum samples prepare a negative control as directed in **5.6** using 2.0 mL of negative blood.

5.3 Calibrations – N/A

5.4 Maintenance

5.4.1 Add water to the TurboVap if needed.

5.5 Sampling

5.5.1 Allow all solutions and samples to equilibrate to room temperature.

5.5.2 Ensure the blood samples are homogenous by shaking and/or vortexing.

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

5.5.3 Pipet 2 mL of each control and case samples into clean and labeled screw-cap test tubes.

5.6 Extraction Procedure

5.6.1 Add 50 µL of the Internal Standard solution to all test tubes.

5.6.2 Add approximately 1 g of NaCl to each test tube.

5.6.3 Add 1 mL of pH 9.0 ammonium chloride buffer.

5.6.4 Add 100 µL of concentrated ammonium hydroxide.

5.6.5 Vortex each tube to mix.

5.6.6 Add 10 mL of n-butyl chloride to each test tube, cap with screw cap.

5.6.7 Place on rocker for 30 minutes.

5.6.8 Centrifuge the test tubes for 10 minutes.

5.6.9 Transfer the organic (upper, n-butyl chloride) layer into labeled test tubes.

5.6.10 Add 200 µL of 2 % solution of HCl in methanol.

5.6.11 Vortex test tubes to mix thoroughly.

5.6.12 Evaporate to dryness using the TurboVap starting with the temperature set at 50 ºC. Once all of the tubes have been placed in the TurboVap, increase the temperature to 60 ºC.

5.6.13 Add 3 mL of 0.5 N H_2SO_4 and vortex.

5.6.14 Add 3 mL of hexanes and vortex for 30 seconds.

5.6.15 Centrifuge for 5 minutes.

5.6.16 Aspirate the top layer (hexanes) to waste.

5.6.17 Repeat 5.6.14 thru 5.6.16.

5.6.18 Add 3 mL of n-butyl chloride to each test tube.

5.6.19 Add 500 µL of concentrated ammonium hydroxide to each test tube.

5.6.20 Vortex each test tube for 1 minute.

5.6.21 Centrifuge for 5 minutes.

5.6.22 Transfer the n-butyl chloride layer to labeled test tubes.

5.6.23 Inspect the bottom of the tubes to ensure no transfer of an aqueous layer (water).

5.6.24 Add 50 µL of acetic anhydride to each test tube.

5.6.25 Evaporate for 10 minutes just to dryness at 60 ºC.

5.6.26 Inspect the tubes to ensure no moisture remains.

5.6.27 If moisture is present, repeat steps **5.6.18, 5.6.20, 5.6.22**, and **5.6.25**.

5.6.28 Reconstitute by adding 75 µL of ethyl acetate to each test tube.

5.6.29 Transfer the reconstituted specimens to labeled GC-MS micro-vials and cap.

5.7 Post extraction procedure

5.7.1 Analyze samples on a GC-MS as specified in the Toxicology Gas Chromatography-Mass Spectrometry (GC-MS) procedure.

5.7.1.1 For each phenethylamine liquid-liquid extraction, the mass spectrum of the internal standards (d-11 amphetamine and d-11 methamphetamine) must meet the identification criteria in the Toxicology Gas Chromatography-Mass Spectrometry (GC-MS) procedure.

5.7.1.2 For each phenethylamine liquid-liquid extraction, the relative retention time comparison will be based on using the retention time of the respective internal standard as listed below and must meet the identification criteria in the Toxicology Gas Chromatography-Mass Spectrometry (GC-MS) procedure.

5.7.1.2.1 For amphetamine use d-11 amphetamine.

5.7.1.2.2 For all other analytes, use d-11 methamphetamine.

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

5.8 Quality Control Data Packet

5.8.1 Create a Quality control data packet to be reviewed by a Forensic Scientist qualified to perform PHEALLE and placed in the Toxicology Unit Section Object repository of FA with a file name beginning with the type of extraction performed followed by the eight digit year /month/day format ending with the instrument name. A suffix may be added to differentiate between multiple runs.

5.8.2 Example: PHEA20140509T3-XXX

5.8.3 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 Controls evaluated in accordance with **5.9.1**

5.9 Case Record

5.9.1 The case record shall contain the following:

- Total Ion Chromatogram of the sample and corresponding blank
- Total Ion Chromatogram clearly showing the peak of interest with an unsubtracted mass spectrum
- RRTs of the sample and corresponding reference standard
- Mass spectra of internal standard(s) and peaks of interest with library match
- Extracted Ion Chromatogram for an analyte being reported negative
- Approved Quality Control data packet for the extraction

5.10 Calculations – N/A

5.11 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 This extraction procedure is capable of identifying other structurally related analytes.

7.0 Safety

7.1 Refer to Laboratory Safety Manual.

8.0 References

Procedure for *Analysis of Amphetamines in Blood (AMPHE)* Georgia Bureau of Investigation-Division of Forensic Sciences, Revision 10, November 21, 2012.

Disposition of Toxic Drugs and Chemicals in Man, Baselt and Cravey, Eight Edition, 2008, BioMedical Publications.

9.0 Records

- QC Data packet
- Case Record

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
05/09/2014	1	New procedure