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

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 Toxicology in the Raleigh, Triad, and Western locations 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)
- Test tubes (16 x 125)
- Pipet tips
- GC-MS vials with caps

4.3 Commercial Reagents

- N-butyl chloride, ACS grade
- Sodium chloride, ACS grade
- Hexanes, ACS grade
- Acetic anhydride derivatizing reagent, ACS grade
- Ethyl acetate, ACS grade
- Ammonium hydroxide, concentrated, ACS grade
- Nitrogen

4.4 Primary Reference Materials

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

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- d-11 Amphetamine
- d-11 Methamphetamine
- Mepivacaine

4.5 Critical Reagents

- Negative Blood/Urine

4.6 Prepared Reagents - Refer to Toxicology Solution Prep Guidelines for instructions on how to prepare the reagents required by this procedure.

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

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

4.7.1 Phenethylamine Internal Standard

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

4.7.1.1.1 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.

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

4.7.1.2.1 Example: 20140509

4.7.1.3 Expiration: One year.

4.7.1.4 Store in freezer.

4.7.1.5 QC check: Successful negative control extraction.

4.7.2 Mepivacaine Internal Standard

4.7.2.1 Prepare a solution containing 20 µg/mL of mepivacaine reference internal standard in methanol.

4.7.2.1.1 In a 50 mL volumetric flask, dilute 1.0 mL of a 1.0 mg/mL solution of mepivacaine and fill to the mark (QS) with methanol.

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

4.7.2.2.1 Example: 20140509

4.7.2.3 Expiration: One year.

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4.7.2.4 Store in freezer.

4.7.2.5 QC check: Successful negative control extraction.

4.7.3 Positive Control – 10 µg/mL

4.7.3.1 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-Methylenedioxymethamphetamine (MDMA) 1mg/mL standard

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

4.7.3.1.2 Amounts may be increased or decreased proportionally to produce a different final solution volume.

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

4.7.3.2.1 Example: 20140509

4.7.3.3 Expiration: One year.

4.7.3.4 Store in freezer.

4.7.3.5 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/urine 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 /urine samples, prepare as directed in **5.6** using 2.0 mL of this positive control.

5.2.1.4.1 The final concentration of positive control is 50 ng/mL Amphetamine, 50 ng/mL Methamphetamine, 50 ng/mL 3,4-Methylenedioxyamphetamine (MDA), and 50 ng/mL 3,4-Methylenedioxymethamphetamine (MDMA).

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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/urine samples prepare a negative control as directed in **5.6** using 2.0 mL of negative blood/urine.

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/urine 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.

5.5.3.1 Smaller volumes/dilutions of case samples may be used based upon analytical needs, but shall be documented in the case record.

5.6 Extraction Procedure

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

5.6.2 Add 40 µL of the Mepivacaine Internal Standard solution to all test tubes

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

5.6.4 Add 1 mL of pH 9.0 ammonium chloride buffer.

5.6.5 Add 100 µL of concentrated ammonium hydroxide.

5.6.6 Vortex each tube to mix.

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

5.6.8 Place on rocker for 30 minutes.

5.6.9 Centrifuge the test tubes for 10 minutes.

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

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

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5.6.12 Vortex test tubes to mix thoroughly.

5.6.13 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.

5.6.14 Add 3 mL of 0.5 N H_2SO_4 and vortex.

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

5.6.16 Centrifuge for 5 minutes.

5.6.17 Aspirate the top layer (hexanes) to waste.

5.6.18 Repeat **5.6.15** thru **5.6.17**.

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

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

5.6.21 Vortex each test tube for 1 minute.

5.6.22 Centrifuge for 5 minutes.

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

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

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

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

5.6.27 Inspect the tubes to ensure no moisture remains.

5.6.28 If moisture is present, repeat steps **5.6.19, 5.6.21, 5.6.23**, and **5.6.26**.

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

5.6.30 Transfer the reconstituted specimens to labeled GC-MS autosampler vials and cap.

5.6.31 Analyze samples on a GC-MS as specified in the Toxicology Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography–Mass Spectrometry (GC-MS) Data Processing procedures.

5.7 Calculations – N/A

5.8 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 This extraction procedure is capable of identifying other structurally related analytes as well as other basic and neutral analytes.

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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
03/20/2015	2	4.5 – Added Mepivacaine Inserted New 4.7.5 –Instructions for Mepivacaine Internal Standard stock solution 5.6.2 – Inserted the addition of Mepivacaine Internal Standard New 5.6.18 and 5.6.28 – Adjusted line references 5.7.1.1 – Added Mepivacaine as internal standard 5.7.1.2.2 – Modified to "other derivatized analytes" 5.7.1.2.3 – Added the comparison of non-derivatized analytes to Mepivacaine 5.8.2 – Added criteria for re-injected controls 5.9.1 – Changed from bullets to numbers 6.1 – Added limitation of identifying basic and neutral analytes
02/12/2016	3	2.0 – Modified scope to include regional laboratories 4.2 – Added test tubes to Materials 4.3 – Moved to New 4.6 New 4.3 – Removed some items associated with New 4.6 New 4.5 – Reformatted New 4.7 – Now Prepared Standards: Reagent instructions moved to Toxicology Solution Preparation Guidelines 4.7.4.1.2, 4.7.5.1.2 – Removed New 4.7.2 – Modified Mepivacaine Internal Standard concentration, Modified example volume of volumetric flask 5.2.1, 5.2.2 – Modified to include urine Old 5.7.1.1, 5.7.1.2, 5.8, 5.9 - Removed New 5.5.2.1 – Added specification for low sample volume/dilution 5.7.1 – Moved to New 5.6.31 and added GC-MS Data Processing procedure reference
07/07/2017	4	4.7.2.1 – modified to say "solution containing"
02/22/2019	5	4.7.3.1 corrected spelling 3,4-Methylenedioxymethamphetamine 5.2.1.4.1 add final blood concentrations 5.5.2 add Urine 5.6.30 – Replace "micro-seal" with "autosampler"

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