Acidic/Neutral Drug Solid Phase Extraction (ANSPE)

1.0 Purpose - This procedure specifies the required elements for the solid phase extraction of acidic and neutral drugs from blood, serum, and urine for analysis by Gas Chromatography-Mass Spectrometry.

2.0 Scope – This procedure applies to the Toxicology sections in the Raleigh, Triad, and Western locations of the State Crime Laboratory.

3.0 Definitions – see Toxicology Definitions list

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)
- Test tube caps or stoppers
- Vortexer
- Pipet tips

4.3 Reagents

- Deionized water
- 0.1 M phosphate buffer
- 0.1 M monobasic sodium phosphate
- 0.1 M dibasic sodium phosphate
- 0.1 M acetic acid
- Acid SPE Elution Solvent

4.4 Commercial Reagents

- Methanol, ACS grade or higher
- Hexane, ACS grade or higher
- Ethyl acetate, ACS grade or higher
- Nitrogen – Ultra high purity grade
- UCT Clean Screen® DAU Solid Phase Extraction Columns

4.5 Primary Reference Materials

- Methohexital
- Butalbital
- Meprobamate

4.6 Critical Reagents

- Negative Blood/Urine

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

4.8 Prepared Standards – Prepared standards may be made up in any amount provided that the component ratios are kept constant.

4.8.1 Acid/Neutral Internal Standard

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

4.8.1.1.1 Example – dilute 2 mL of a 1.0 mg/mL solution of methohexital to 200 mL with methanol.

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

4.8.1.2.1 Example: 20101231

4.8.1.3 Expiration: One year.

4.8.1.4 Store in freezer.

4.8.1.5 QC check: Successful negative control extraction.

4.8.2 Acid/Neutral Positive Control Standard

4.8.2.1 Prepare a positive control standard containing the following primary reference standards in methanol.

- Butalbital at 10,000 ng/mL
- Meprobamate at 160 µg/mL

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

- 100 µL of Butalbital
- 1.6 mL of Meprobamate

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

4.8.2.2.1 Example: 20101231

4.8.2.3 Expiration: One year

4.8.2.4 Store in freezer.

4.8.2.5 QC check: successful positive control extraction.

5.0 Procedure

5.1 Control Sample Preparation – One negative and positive control will be extracted for every 20 case samples.

5.1.1 Positive controls

5.1.1.1 Blood/Serum Positive Controls

5.1.1.1.1 For each extraction batch of blood/serum samples, prepare a positive control by adding 50 µL of the positive control standard to 1.0 mL of negative blood and prepare as directed in **5.4.3**.

5.1.1.1.2 The final concentration of the positive control is 500 ng/mL Butalbital, and 8.0 µg/mL Meprobamate.

5.1.1.2 Urine Positive Controls

5.1.1.2.1 For each extraction batch of urine samples, 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 **5.4.4**.

5.1.1.2.2 The final concentration of the positive control is 500 ng/mL Butalbital, and 8.0 µg/mL Meprobamate.

5.1.2 Negative Controls

5.1.2.1 Blood/Serum Negative Controls

5.1.2.1.1 For each extraction batch of blood/serum samples, prepare a negative control as directed in **5.4.3** with 1.0 mL of negative blood.

5.1.2.2 Urine Negative Controls

5.1.2.2.1 For each extraction batch of urine samples, prepare a urine negative control as directed in **5.4.4** with 5.0 mL of negative urine.

5.2 Calibrations – N/A

5.3 Maintenance

5.3.1 Manifold

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

5.3.1.2 Ensure manifold strips are not worn. Replace as needed.

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.

5.4.3 **Blood/Serum sample preparation –** Smaller volumes/dilutions of blood/serum may be used based upon analytical needs, but shall be documented in the case record.

5.4.3.1 Add 1 mL of 0.1 M phosphate buffer to 1.0 mL of blood.

5.4.3.2 Add 50 µL of the acid/neutral internal standard solution.

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

5.4.3.4 Add 2 mL of 0.1 M phosphate buffer.

5.4.3.5 Mix/Vortex sample.

5.4.3.6 Centrifuge for 10 minutes.

5.4.4 **Urine sample preparation -** Smaller volumes/dilutions of urine may be used based upon analytical needs, but shall be documented in the case record.

5.4.4.1 Add 250 µL of acid/neutral internal standard solution to 5.0 mL of urine.

5.4.4.2 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).

5.5 Solid Phase Extraction Procedure

5.5.1 The flow rate for sample loading and elution is less than 2 mL per minute. The flow rate for all other additions is 1 mL to 15 mL per minute. Allow each addition to elute completely prior to adding the next addition.

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

5.5.3 Add 3 mL of water to the column.

5.5.4 Add 3 mL of 0.1 M phosphate buffer to the column.

5.5.5 Add the blood or urine to be extracted to the column.

5.5.6 Add 3 mL of water to the column.

5.5.7 Add 1 mL of 0.1 M acetic acid to the column.

5.5.8 Dry the column with a nitrogen flow for 10 minutes.

5.5.9 Add 2 mL of hexane to the column.

5.5.10 Elute and collect the acidic/neutral fraction with 3 mL of Acid SPE Elution Solvent.

5.5.11 Evaporate to dryness using a TurboVap at 40 ºC.

5.5.12 Reconstitute the sample in 150 µL of ethyl acetate.

5.5.12.1 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.

5.5.13 Analyze using a Gas Chromatograph-Mass Spectrometer.

5.6 Calculations – N/A

5.7 Uncertainty of Measurement – N/A

6.0 Limitations

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

6.2 Store solid phase extraction columns in a closed container.

7.0 Safety

7.1 It should be assumed that all body fluids contain bloodborne pathogens and should therefore be handled accordingly.

7.2 If the examination involves a biohazard, the Forensic Scientist shall use proper PPE, such as eye protection, a lab coat, and/or gloves.

8.0 References

UCT Solid Phase Extraction Manual. United Chemical Technologies Inc. Bristol, PA., (2014) 25 – 27.

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
- Toxicology Extraction Worksheet

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
12/01/2023	5	4.4 – added “or higher” and “Ultra high purity grade”