Basic Drug Solid Phase Extraction (BSPE)

1.0 Purpose - This procedure specifies the required elements for the solid phase extraction of basic drugs (other than phenethylamines) 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 – 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
- Base 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
- BSTFA with 1 % TMCS (N,O-bistrimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane)

4.5 Primary Reference Materials

- Mepivacaine
- Nalorphine

- Alprazolam
- Amitriptyline
- Benzoylecgonine
- Cocaine
- Codeine
- Diphenhydramine
- Lidocaine
- Methadone
- Morphine
- Oxycodone
- Trazodone

4.6 Critical Reagents

- Negative Blood/Urine

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

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

4.8.1 Base Internal Standard

4.8.1.1 Prepare a solution containing 20 µg/mL of mepivacaine reference standard and 2.5 µg/ml of nalorphine reference standard in methanol

4.8.1.1.1 Example – In a 50 mL volumetric flask, dilute 1.0 mL of a 1.0 mg/mL solution of mepivacaine and 125 µL of nalorphine and fill to the mark (QS) 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 Positive Control Standard

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

- Alprazolam at 3000 ng/mL
- Amitriptyline at 4000 ng/mL
- Cocaine at 2000 ng/mL
- Codeine at 4000 ng/mL
- Diphenhydramine at 2000 ng/mL

- Lidocaine at 2000 ng/mL
- Methadone at 1000 ng/mL
- Oxycodone at 1200 ng/mL
- Trazodone at 10,000 ng/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.

- 30 µL of Alprazolam
- 40 µL of Amitriptyline
- 20 µL of Cocaine
- 40 µL of Codeine
- 20 µL of Diphenhydramine
- 20 µL of Lidocaine
- 10 µL of Methadone
- 12 µL of Oxycodone
- 100 µL of Trazodone

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.

4.8.3 Derivatized Positive Control Standard

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

- Benzoylecgonine at 10,000 ng/mL
- Diphenhydramine at 2000 ng/mL
- Morphine at 2000 ng/mL
- Trazodone at 10,000 ng/mL

4.8.3.1.1 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 Benzoylecgonine
- 20 µL of Diphenhydramine
- 20 µL of Morphine
- 100 µL of Trazodone

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

4.8.3.2.1 Example: 20101231

4.8.3.3 Expiration: One year.

4.8.3.4 Store in freezer.

4.8.3.5 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 blood/serum base extraction batch, 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 **5.4.3**.

5.1.1.1.1.1 The final concentration of positive control is 150 ng/mL Alprazolam, 200 ng/mL Amitriptyline, 100 ng/mL Cocaine, 200 ng/mL Codeine, 100 ng/mL Diphenhydramine, 100 ng/mL Lidocaine, 50 ng/mL Methadone, 60 ng/mL Oxycodone, and 500 ng/mL Trazodone.

5.1.1.1.2 For each blood/serum derivatized base extraction, prepare a derivatized positive control by adding 100 µL of the derivatized positive control standard to 2.0 mL of negative blood and prepare as directed in **5.4.3**.

5.1.1.1.2.1 The final concentration of positive control is 500 ng/mL Benzoylecgonine, 100 ng/mL Diphenhydramine, 100 ng/mL Morphine, and 500 ng/mL Trazodone.

5.1.1.2 Urine Positive Controls

5.1.1.2.1 For each urine base extraction, 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 For each urine derivatized base extraction, prepare a derivatized positive control by adding 250 µL of the derivatized positive control standard to 5.0 mL of negative urine and prepare as directed in **5.4.4**.

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 2.0 mL of negative blood.

5.1.2.2 Urine Negative Controls

5.1.2.2.1 For each base 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 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 2.0 mL of blood.

5.4.3.2 Add 40 µL of the base 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 100 µL of base 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 the sample and elution solvent 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.

Note: Base samples should be eluted using a gravity fed flow rate.

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 Add 3 mL of methanol to the column.

5.5.11 Dry the column with a nitrogen flow for 5 minutes.

5.5.12 Elute and collect the basic fraction with 3 mL of Base SPE Elution Solvent.

5.5.13 Evaporate to dryness using a TurboVap 40 ºC.

5.5.14 Sample Reconstitution

5.5.14.1 Underivatized

5.5.14.1.1 Reconstitute the sample in 50 µL of ethyl acetate.

5.5.14.1.2 Mix and transfer to an insert in auto-sampler vial and cap.

5.5.14.1.3 The solvent and/or volume of solvent may be changed based upon analytical needs, but shall be documented in the case record.

5.5.14.2 Derivatized

5.5.14.2.1 Add 50 µL of BSTFA with 1 % TMCS and 50 µL of ethyl acetate and cap securely.

5.5.14.2.2 Mix and heat at 70 °C for 30 minutes.

5.5.14.2.3 Cool to room temperature.

5.5.14.2.4 Transfer to an insert in an auto-sampler vial and cap securely.

5.5.15 Analyze using a Gas Chromatograph-Mass Spectrometer.

5.6 Calculations – N/A

5.7 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 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).

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

6.3 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.

7.3 Refer to Appendix 1 for chemical hygiene and safety precautions for high risk and particularly hazardous substances.

8.0 References

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

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 ChemicalTechnologies.

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

- Appendix 1 - Chemical Hygiene and Safety Precautions for High Risk and Particularly Hazardous Substances

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

Appendix 1 - Chemical Hygiene and Safety Precautions for High Risk and Particularly Hazardous Substances

Methylene Chloride/Dichloromethane **DANGER: PARTICULARLY HAZARDOUS SUBSTANCE ***	
	**HEALTH 2** **FLAMMABILITY 1** **REACTIVITY 1**
Detection of Release	Clear colorless liquid. Ether like odor
Signs/Symptoms of Exposure	Serious eye irritation; skin irritation; may cause drowsiness or dizziness.
PEL	ACGIH (TLV) – 50 ppm; OSHA Specifically Regulated Chemicals/Carcinogens – (PEL) 25 ppm
Associated Hazards	Serious eye and skin irritation; suspected of causing cancer
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: Fluorinated rubber (break through time = 148 minutes)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep in a tightly closed container. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Dispose of in Hazardous Chemical Waste.
Emergency Procedures (2.2)(4.1)(6)	**Eye Contact:** Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. **Inhalation Exposure:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Skin Contact:** Wash off with soap and plenty of water. Consult a physician. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, collect with absorbent material, and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).