**Solid phase extraction (SPE) of environmental water samples for bioassay analysis**

**Equipment and Supplies**

* Dichloromethane (DCM), GC grade
* Ethyl acetate, Optima grade
* Methanol, HPLC grade
* Water, HPLC grade
* Aluminum Foil
* Filter flask setup
* Glass fiber filter (Grade A/E or GF/F)
* Glass tubes (16 x 100mm)
* Glass vials, screwtop (2 mL)
* LDPE bottle (i.e. Nalgene; 500mL or 1L)
* 1L amber bottles
* Screw caps for 2mL vials
* SPE cartridge positive pressure manifold
* SPE cartridge vacuum manifold (12-, 16-, or 24-port) with attached vacuum flask
* SPE tubing and adapters
* TurboVap LV Evaporation Unit
* Volumetric flask (500 mL)
* Waters Oasis HLB SPE cartridge; 200mg, 5 mL glass cartridge (part # 186000683)
* All glassware must be pre-cleaned by washing with soap and water followed by solvent rinse (acetone, methanol rinse) or baked (450°C, 4h) prior to use. Glass fiber filters must be baked (450°C, 4h) prior to use.
* Upon arrival, water samples will be monitored for temperature and to note any potential compromise of samples. Samples will ideally be between 4-8°C and may be compromised above 10°C. Relevant chain of custody (COC) forms will be completed by the receiving individual. After completing, scan and place into appropriate study folder on the L drive.
* Water samples should be stored at 4±2°C after arrival and before extraction. Water samples have a hold time of up to 4 days prior to extraction.
* Received samples should be logged in the “Sample Shipping Log” at: "L:\Priv\Fathead Team Experiments\-FHM Logs, Protocols\Logs\Shipping\Sample shipping log.xlsx"
* Extracted samples should be recorded in the brown sample logbook and on the L drive at: "L:\Priv\Fathead Team Experiments\-FHM Logs, Protocols\Logs\SPE log\SPE log.xlsx"

**Procedure**

Filter Water Samples

1. Assemble filtration flask apparatus and pre-cleaned filter.
2. Mix water sample in bottle. Filter ~1000mL of sample through glass fiber filter filter (0.7µm) using a clean glass apparatus.
3. If filter becomes clogged and filtration is proceeding slowly, a new filter can be used. Once the filter flask upper reservoir is empty, turn off vacuum, remove old filter and replace with new filter. Continue filtering sample.
4. Verify balance performance and record in LogBook. If no 500g standardized weight is available, use a 500mL volumetric filled with MQ water.
5. Weigh 500g aliquot for SPE extraction using pre-cleaned 1L amber bottle. Record weight.
6. Remaining sample can be stored at -20°C in Nalgene (LDPE) bottle if archived sample is planned for the study.

Bioassay SPE Loading (Automated SPE station)

* If SPE Station has not been cleaned in past week, follow cleaning procedure prior to loading samples.
* Ensure solvents are connected to correct lines and are sufficiently filled:
  + Solvent line 1: Ethyl Acetate (20 mL for 4 samples, 40 mL for 8)
  + Solvent line 3: Methanol (20 mL for 4 samples, 40 mL for 8)
  + Solvent line 4: Water (80 mL for 4 samples, 160 mL for 8)
* Ensure hazardous waste container and water collection container have enough room for collection:
  + Hazardous waste: 120 mL for 4 samples, 240 mL for 8
  + Water collection bottle: 2 L for 4 samples, 4 L for 8
* Attach white SPE adaptors to SPE cartridges.
* Insert SPE cartridges into the SPE station and attach inlet lines to the white SPE adaptors. Ensure a snug fit but be careful not to press too hard while cartridges are in SPE station or glass tips may break off.
* If not exactly 4 or 8 samples are being run, attach plastic cleaning cartridges to empty slots on SPE station and attach inlet lines. Plastic cartridges do not require adaptor and pipette tip setup.
* Place water samples on tilted platforms on appropriate side of the SPE station and insert sample lines into bottles. Only top sample line needs to be placed into each bottle. Double check that sample lines connect the correct sample with the correct SPE cartridge.
* Turn on SPE Station.
* Select “210817-bioassay load” method.
* Select samples 1-4, 5-8 or both depending on how many samples are being processed and where they are located.
* Press start and select “okay” after prompt regarding solvent levels.
* After loading method is complete, dry cartridges on SPE vacuum manifold for ~1hr
* Either proceed with elution or remove sample SPE cartridges and store in freezer in 237.
* Proceed with cleaning procedure.

Automated SPE cleaning procedure

* Ensure solvents are connected to correct lines and are sufficiently filled:
  + Solvent line 1: Ethyl Acetate (80 mL)
  + Solvent line 3: Methanol (80 mL)
  + Solvent line 4: Water (160 mL)
* Ensure hazardous waste container has enough room for collection (320 mL)
* Empty water collection bottle in sink and replace.
* Insert plastic cartridges from box labeled “cleaning cartridges” into SPE station and attach inlet lines to each cartridge.
* Place sample lines (upper lines only) on each side of SPE station into one flask (250 mL or larger) and cover top with foil.
* Select “210817-bioassay clean” method.
* Select both samples 1-4 and 5-8
* Press start and select “okay” after prompt regarding solvent levels.
* After cleaning method is complete, remove plastic cleaning cartridges and store in labeled box.
* Remove sample lines on side of instrument from flasks. Discard solvents in the flasks in hazardous waste.
* Rinse the outside of each sample line with methanol and clip into holder to dry.

Elute Samples

1. If cartridges were stored in the freezer, place cartridges in desiccator box for 30 minutes to allow cartridges to come to room temperature and prevent condensation.
2. Place cartridges into the positive pressure manifold with 16 x 100 glass tube underneath to collect solvents. Elute cartridge with 6 mL (2 x 3mL) of methanol.
3. Allow cartridges to elute by gravity if possible. If cartridges will not flow without pressure or are flowing very slowly (less than 1 drop every 3-5 seconds), apply positive pressure to elute cartridges at a rate no faster than ~6 mL/ min.
4. Into the same glass tube, elute sample with 6 mL (2x3mL) 50:50 methanol:DCM. Allow cartridges to elute by gravity if possible. If cartridges will not flow without pressure or are flowing very slowly (less than 1 drop every 3-5 seconds), apply positive pressure to elute cartridges at a rate no faster than ~6 mL/ min.

Concentrate Samples

1. Check the water level in the TurboVap and add water if needed. Turn system on at least 10 minutes before beginning evaporations to allow it to come to temperature.
2. Transfer glass tubes to TurboVac tube rack and place samples into the TurboVap.
3. Concentrate samples under a gentle stream of nitrogen at 35±3°C.
4. Rinse tubes twice with ~0.5mL methanol as the solvent level drops to wash down the sides of the vial.
5. After sample is dry, remove from evaporator.
6. Add 0.5mL DMSO (or a volume equivalent to 1000x concentration factor is sample volume was not 500mL; i.e. 0.45mL for 450mL sample).
7. Vortex for 25-30s then sonicate for 10min at room temperature.
8. Transfer sample to a labeled amber screwtop vial. Label should include the following:

Date – SPE ID#; Sample Name; Extraction Method; Date Extracted and Initials

1. Cap vials and store at -20°C. Record storage location on the L drive.

Clean up

* Clean all glassware by washing with soap and water and triple rinsing with DI or MilliQ water. Allow to dry fully overnight. After dried, bake glassware in the muffle furnace (450°C for 4h). Alternatively, glassware can be solvent rinsed after washing by sequentially rinsing with methanol and acetone then allowing to dry.
* Clean SPE tubing, adapters, and manifold ports with acetone, methanol, and HPLC grade water. To clean tubing, pull solvents through using vacuum. Rinse vacuum manifold and empty any remaining liquids.

**Approvals**

**Signature Date**