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Revision Author: S. Madhava Reddy and Marek Topolski
SV-004-2.16

Standard Operating Procedure For:

Extraction and Fractionation of Petroleum Range Organics from Waters and Sediments (FL-PRO and MADEP-EPH)



Department of Environmental Protection
Chemistry Section
Tallahassee Florida

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Note: SV-004 (A) & FL-PRO (A) are Imbedded in this SOP Pertaining to Sediments/Waste Sample Preparation by SW-846, Method 3550C, Ultrasonic Extraction and Method 3580A, Waste Dilution

Note: SV-004 (B) & FL-PRO (B) are Imbedded in this SOP Pertaining to Water Sample Preparation by SW-846, Method 3510C, Liquid-Liquid Extraction

Note: SV-004 (C) & FL-PRO (C) are Imbedded in this SOP Pertaining to Water Sample Preparation by SW-846, Method 3520C, One-Step Continuous Liquid-Liquid Extraction

1 SCOPE AND APPLICATION

1.1 This SOP details the extraction of Petroleum Hydrocarbons (i.e., petroleum-range organics) from water and sediment matrices for subsequent analysis by GC/FID. The analytes isolated by this procedure are listed in the analysis SOP. This SOP can be used for all samples with the LIMS test IDs S-FL-PRO, W-FL-PRO, WAS-FL-PRO, S-EPH, and W-EPH.

1.2 This method is based on the Florida Department of Environmental Protection's Method for Determination of Petroleum Range Organics (FL-PRO) and the Massachusetts Department of Environmental Protection's (MADEP) Method for the Determination of Extractable Petroleum Hydrocarbons (EPH).

2 SUMMARY OF THE METHOD

2.1 For water samples, a measured volume of water is serially extracted with methylene chloride using the separatory funnel technique or One-step continuous liquid-liquid extraction. The resulting methylene chloride extract is concentrated and part of the extract is subjected to silica gel cleanup or fractionation, and analyzed by GC/FID.

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2.2 For sediment samples, a measured weight of sediment is serially extracted with methylene chloride using the ultrasonic technique. The resulting methylene chloride extract is concentrated and part of the extract is subjected to silica gel cleanup or fractionation, and analyzed by GC/FID.

2.3 Waste samples are diluted with methylene chloride and a part of the extract is passed through a one-gram silica column and analyzed by GC/FID. Pure petroleum products are treated as 100% neat standards: weigh 0.20 grams in 10 mL and analyze another 10 times diluted sample (100 mL final volume).

3 APPARATUS AND EQUIPMENT

3.1 For water extraction by separatory funnel: 2 Liter separatory funnels with Teflon stopcocks and stoppers, Erlenmeyer flasks- 250 mL with ground glass mouth and stoppers, Glass wool columns, automatic separatory funnel tumbler and Glass wool.

3.2 For soil extraction: Beakers, 400 mL, Vacuum filtration flasks, 500 mL, Porcelain Büchner funnels - 9 cm, Rubber funnel supports, Whatman #41 filter paper - 9 cm, Metal spatulas and Ultrasonic Bath, Fisher Scientific, Model FS220, 320 Watts power,

3.3 For S-Evap®: S-Evap® - Consists of heated water bath and water-cooled condensers, capable of concentrating eight samples extracts simultaneously, K&D 500 mL evaporative flasks; attach to concentrator tubes with clips, K&D concentrator tubes -- 15 mL, Ground glass stoppers to fit concentrator tubes, K&D Snyder columns -- three ball macro or equivalent, Teflon boiling chips.

3.4 N-Evap®: consists of heated water bath and apparatus to further concentrate the extract using nitrogen gas.

3.5 For Accelerated One-step Extractor/Concentrator: Organomation ROT-X-TRACT-LC Liquid-Liquid Extractor/Concentrator - consists of heated water bath and water-chiller (Neslab Recirculation Bath set at 11 °C), capable of extracting eight samples simultaneously with extraction body, and Corning Membrane Assembly for Accelerated One-step Extractor/Concentrator (catalog number 3918-47).

3.6 Glass Pasteur pipets

3.7 Top-Loading balance, accurate to 0.1 g

3.8 Vortex

3.9 Centrifuge to fit 15 mL centrifuge tubes

3.10 Centrifuge tubes, graduated 15 mL with screw caps with Teflon-lined septa, and 25 mL graduated centrifuge tubes for EPH samples for the collection of 20 mL fractions. For more than one to five grams of waste dilution use 40 mL centrifuge tubes. (Calibrate tubes to the volume used prior to use and record in the Extraction Log Book)

3.11 Gas-tight micro-syringes, 250 µL, 500 µL, 1000 µL and 2000 µL (Required from the manufacturer, the certificate stating that the volume is accurate to < 1%, otherwise calibrate and keep the record of these gas-tight micro-syringes or volumetric pipets every quarterly)

3.12 For EPH extractions: 200 mL chromatographic columns with reservoir and Teflon stopcocks.

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4 REAGENTS AND CHEMICALS

4.1 Methylene chloride, Optima or Pesticide grade or equivalent (Fisher Scientific)

4.2 Acetone, Optima or Pesticide grade or equivalent (Fisher Scientific)

4.3 For EPH extractions: Hexane, Optima or Pesticide grade or equivalent (Fisher Scientific)

4.4 For FL-PRO extraction, see DEP SOP SV-009 for details of standards preparation:

4.4.1 FL-PRO Surrogate Mix for Water/Sediments (100 µg/mL OTP, 100 µg/mL C_{39}).

4.4.2 FL-PRO Matrix Spike Mix for Water/Sediments (500 µg/mL)

4.5 For EPH extraction, see DEP SOP SV-009 for details of standards preparation:

4.5.1 EPH Surrogate (40 µg/mL)

4.5.2 EPH Spiking Standard (40 µg/mL)

4.5.3 EPH Fractionating Surrogate (40 µg/mL)

4.5.4 EPH Aliphatic Internal Standard (200 µg/mL)

4.5.5 EPH PAH Internal Standard (200 µg/mL)

4.6 Sulfuric acid (1:1) (prepare in hood, using a stirring plate to mix!): Prepare by slowly adding 200 mL concentrated H_2SO_4 to 200 mL reagent water. Allow to cool and then transfer to glass storage container.

4.7 Sodium sulfate (granular and powder): Bake in a muffle furnace at 460 °C for at least 8 hours. Allow to cool before using. Store sodium sulfate in a large mouth bottle with cap.

4.8 Silica gel (Davisil, grade 644): Rinse with methylene chloride (do this by packing the gel in a column and pushing the methylene chloride through the column with nitrogen pressure) and dry (this will take 3-4 hours). Then, bake in an oven at 130 °C for at least 8 hours. Store silica gel in an oven at 100 °C. Allow to cool before using.

4.9 Glass wool (Silane treated): Bake in a muffle furnace at 460 °C for at least 8 hours. Store in an oven at 100 °C and do not store in plastic.

4.10 Ottawa Sand Standard: Bake in a muffle furnace at 460 °C for at least 8 hours. Allow to cool before using. Store Ottawa sand in a large mouth bottle with cap.

4.11 One gram Accu Bond Silica Gel Cartridges, 6 mL (J&W/Agilent),or equivalent.

4.12 DEP well water: Ground water tested free of interferences and used for method blanks and lab-fortified blanks.

5 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

5.1 All water samples are collected in 1-liter amber glass bottles, preserved with acid, transported to the lab on ice and stored at 4 °C.

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5.2 All sediment/waste samples are collected in glass jars, transported to the lab on ice and stored at 4 °C.

5.3 W-FL-PRO samples must be extracted within 7 days after collection. W-EPH samples must be extracted within 14 days after collection.

5.4 S-FL-PRO/WAS-FL-PRO samples must be extracted within 14 days after collection. S-EPH samples must be extracted within 7 days after collection.

6 SAMPLE EXTRACTION

NOTE: a second person must witness the addition of spike standard to the proper samples, verify the correct volume, spike LIMS number and concentration, and initial in the logbook. Also, record bottle number if more than one bottle available, lot numbers of reagents and solvents used, and bath temperature for extracts concentration.

Thoroughly clean all glassware by rinsing it with hot tap water, deionized water and acetone, then baking it in an oven at 550 °C for 8 hours. Alternatively, glassware may be thoroughly washed with Alconox and hot water, and then rinsed with hot tap water, deionized water, and acetone. Pre-rinse all glassware with methylene chloride prior to use!

If entire sample is not used for extraction, always mix thoroughly or shake before sub-sampling.

6.1 Water Extraction for W-FL-PRO - EPA 3510C, Liquid-Liquid Extraction

6.1.1 Check out the samples and let them warm up to room temperature before proceeding. If more than one bottle for the sample, then record bottle number extracted in the preparation worksheet.

6.1.2 Remove the FL-PRO Spike Mix for Water and Sediments and the FL-PRO Surrogate Mix for Water and Sediments from the refrigerator, let them warm to room temperature, and then sonicate for several minutes. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution.

6.1.3 Assemble one of the following pieces of glassware for each sample:

6.1.3.1 2 L separatory funnel with Teflon stopcock and Teflon stopper

6.1.3.2 250 mL Erlenmeyer flask with ground glass mouth and stopper

6.1.3.3 Glass wool column packed with glass wool -- Cut approximately 2 inches of glass wool, using clean, methylene-chloride rinsed scissors. Using the scissors, push the glass wool all the way to the bottom of the column.

6.1.3.4 500 mL Kuderna-Danish flask, column, and concentrator tube.

6.1.3.5 Centrifuge tube with Teflon-lined screw cap

6.1.4 Rinse the glass wool columns with approximately 10-15 mL of methylene chloride, using a rubber bulb. Discard rinsate into waste beaker.

6.1.5 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. (Do not use a permanent marker! Methylene chloride

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will wash off the sample ID if it contacts the outside of the glassware.) There should be one blank, two laboratory fortified blanks (i.e., reagent water spikes -- use DEP well water) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

6.1.6 Note any unusual observations made during the extraction process, including sample color or special extraction instructions and enter in the preparation worksheet. The preparation worksheet is a very valuable reference. Care should be taken that entries for each sample are complete and accurate.

6.1.7 Measure the pH of each sample using wide-range pH paper. Record the value in the prepararion worksheet.

6.1.8 The pH of all samples already should have been adjusted to less than 2. If the pH of a sample is greater than 2, do not adjust the pH yourself. Do not acidify the blank or LFB's.

6.1.9 Weigh each amber bottle for later determination of volume. Pour the entire sample into the 2-liter separatory funnel. For the laboratory blank and the duplicate laboratory fortified blanks, use 1000 mL of DEP well water. Choose one sample to spike in duplicate and measure 1000 mL into each of two separatory funnels. **NOTE: If there is insufficient sample to extract two 1000 mL sample matrix spikes and still have one liter left over for possible re-extractions (i.e., less than 4 liters of sample), extract one 1000 mL matrix spike and duplicate lab fortified blanks. If only one bottle of sample was received, extract duplicate lab fortified blanks, and save 100 mL of each sample in case of future re-extractions. If less than 2 matrix spikes are extracted, note this in the preparation worksheet.**

6.1.10 Fortify all blanks, samples, and spikes with 2 mL of the FL-PRO Surrogate Mix for Water/Sediments (100 µg/mL OTP, 100 µg/mL C_{39}). Fortify the matrix spikes and laboratory fortified blank with 0.2 mL of the FL-PRO Matrix Spike Mix for Water/Sediments (500 µg/mL C_8-C_{40}). **Note the amount added and the concentration of analytes in the preparation worksheet.**

6.1.11 Add 60 mL methylene chloride to each sample bottle, swirl the bottle to rinse the inside thoroughly with the methylene chloride, then pour the methylene chloride into the separatory funnel. Swirl each separatory funnel gently to allow CH_2Cl_2 to mix into sample and allow the vapors to escape before capping. Stopper each funnel and secure the tumbler lid.

6.1.12 Turn on the tumbler slowly for a half turn, stopping with the funnels upside down. Slowly open the stopcocks to vent the pressure. Close the stopcocks.

6.1.13 Repeat step 6.1.12 by turning one, two and four full turns, before venting, and continue that until the amount of gas escaping is minimal.

6.1.14 Turn on the tumbler again and set the speed to 5 (about 25-30 turns per minute). Tumble for three minutes.

6.1.15 Stop the tumbler with the funnels upright (stopcocks down) and lock it in place.

6.1.16 Remove the stoppers from the separatory funnels and allow the methylene chloride and water layers to separate for at least 10 minutes.

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6.1.17 Drain the bottom layer through the glass wool column into a 250 mL Erlenmeyer flask. Do not allow water to drain through the stopcock. If there is an emulsion, it may be necessary to follow one or all of these techniques to break it up:

- Allow separatory funnel to stand for several minutes
- Gently agitate emulsion with a long, glass stirring rod
- Remove entire bottom layer of sample and centrifuge to separate water and methylene chloride
- Add more methylene chloride
- Drain the emulsion directly through the glass wool column. The glass wool should retain up to 20 mL of water and break up some emulsions.

6.1.18 After the first extraction, rinse the tip of the separatory funnel by adding 2-5 mL of methylene chloride, draining the rinsate through the glass wool column into the flask. Repeat this step a second time.

6.1.19 Extract two more times with 60 mL of methylene chloride following the procedures in steps 6.1.11-6.1.17, collecting and combining each methylene chloride layer in the Erlenmeyer flask. Proceed to section 7 for K&D concentration or store in the refrigerator at 4 °C until you are ready to concentrate.

6.1.20 Determine the original volume of sample extracted by weighing the amber sample bottles. Assume that the sample has a density of 1 g/mL. If in doubt as to the sample density, weigh an exact volume (5-10 mL) to determine density. Record sample volume to the nearest 10 mL.

6.1.21 Carefully push the glass wool out of each column using a screwdriver or Allen key. Do not use a glass rod, as it may break and cut you. Be very careful not to cut your hand on the bottom of the glass wool column during this step. Place all spent methylene chloride saturated-sodium sulfate/wool, solid sample filter cake with the filter and any other filter media in a red metal can with a plastic bag and close the lid tightly. When it's full dispose of as a hazardous chemical waste.

6.2 Water Extraction for W-EPH - EPA 3510C, Liquid-Liquid Extraction

6.2.1 Follow the procedure for W-FL-PRO above (6.1), with the following exceptions:

6.2.2 When spiking, add 1 mL of the EPH surrogate (40 µg/mL) to all the samples blanks and spikes. Add 250 µL of EPH spike (40 µg/mL) to matrix spikes and LFB's.

6.3 Sediment Extraction for S-FL-PRO - EPA3550C, Ultrasonic Extraction

6.3.1 Check out the samples and let them warm up to room temperature before proceeding.

6.3.2 Remove the FL-PRO Spike Mix for Water and Sediments and the FL-PRO Surrogate Mix for Water and Sediments from the refrigerator and let them warm to room temperature, and sonicate for several minutes. Many compounds easily precipitate out of solution when in the refrigerator or freezer and they need to be re-dissolved into solution by being warmed to room temperature. Also shake the bottle to ensure complete dissolution.

6.3.3 Label all glassware with the appropriate sample number, using labeling tape or pre-printed sample bar code labels. (Do not use a permanent marker! Methylene chloride

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will wash off the sample ID if it contacts the outside of the glassware.) There should be one blank (use Ottawa sand), two laboratory fortified blanks (i.e., Ottawa sand spikes) and two matrix spikes (duplicates) for each set of samples extracted, not to exceed 20 samples per set.

6.3.4 Note the appearance of the sample in the preparation worksheet (e.g., presence of foreign materials, variable particle size, etc.). Care should be taken that entries for each sample are complete and accurate. Any unusual observations made during the extraction process, including sample color or special extraction instructions should be entered in the preparation worksheet.

6.3.5 Do not decant standing water, large rocks, and other foreign material from the samples at any point and treat as whole sample. The analyst must consult his/her supervisor for direction if obvious non-representative material is found in the sample. For most samples, you will need to remove the sample from the jar and place it on a sheet of aluminum foil (dull side up, shiny side down) in order to homogenize it fully. Do not use aluminum foil if the sample is to be analyzed for metals. If the samples have not already been homogenized, homogenize each sample by mixing it thoroughly using a metal spatula.

6.3.6 Perform a dry weight determination on all samples, unless this has already been done:

6.3.6.1 Weigh 5 g of each sample into a labeled, pre-weighed aluminum-weighing dish Record the weight of the dish and the exact weight of the wet sediment in the Dry Weights Notebook and dry it overnight at 105 °C. The next day, allow it to cool in a desiccator before weighing it a second time. Record the exact weight of the dry sediment in the dry weights notebook.

6.3.6.2 A sample's percent solids is equal to the dry sediment weight (not including the dish weight) divided by the wet sediment weight (not including the dish weight) and multiplied by 100%. Enter the wet and dry weights into the computer generated extraction log sheet, which will calculate and display the percent solids.

6.3.6.3 Remember to upload the percent solids of all samples into LIMS. Percent solid data is usually determined by the metals group.

6.3.7 Using a metal spatula, weigh approximately 25 g of each sample into properly labeled 400 mL beakers. If sample contains gasoline odor, the weigh 10 g or follow the waste dilution procedure. Record the weight in the extraction worksheet to the nearest 0.1 g. Weigh out three 25 g portions of Ottawa sand into three 400 mL beakers -- these will serve as a laboratory blank and two laboratory fortified blanks. Select one sample and weigh out three 25 g portions instead of one -- two of these will be used as duplicate matrix spikes. There will generally be enough sediment in a sample to prepare duplicate matrix spikes, but if none of the samples have enough, prepare a single matrix spike or no matrix spike along with duplicate LFB's and a blank. Be sure to note this in the preparation worksheet.

6.3.8 Fortify all blanks, samples, and spikes with 2 mL of the FL-PRO Surrogate Mix for Water/Sediments (100 µg/mL OTP, 100 µg/mL C_{39}). Fortify the matrix spikes and laboratory fortified blank with 0.2 mL (if MDL spike is required then add 0.1 mL) of the FL-PRO Matrix Spike Mix for Water/Sediments (500 µg/mL C_8-C_{40}). Note the amount added and the concentration of analytes in the extraction worksheet.

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6.3.9 Add approximately 60 g of sodium sulfate powder to each beaker and mix it into the sediment with a metal spatula until the sediment appears sandy and free flowing. If the sample is very wet, it will be necessary to mix it repeatedly, at intervals, until it becomes free flowing.

6.3.10 Add 100 mL of methylene chloride to each beaker.

6.3.11 Cover the beakers loosely with aluminum foil (dull side facing the sample) and place them in the ultrasonic bath. Sonicate for 5 minutes.

6.3.12 Decant and filter the extracts through Whatman No. 41 filter paper in a Büchner funnel into a clean, labeled 500 mL vacuum filtration flask.

6.3.13 Due to the fact that the extraction fluid contains only methylene chloride and no acetone, FL-PRO sediments is more likely to clog the filter paper than other sediment samples. If the sample extract will not filter, decant the extract back into the beaker. Rinse the Büchner funnel and filter paper with methylene chloride into the vacuum flask, and then remove them. Assemble a glass wool column as in steps 6.1.3.3 and 6.1.4. Filter the sample extract through this column instead of through a Büchner funnel.

6.3.14 Repeat the extraction (steps 6.3.10-6.3.13) two more times with two additional 100 mL portions of methylene chloride.

6.3.15 After the final sonication, pour the entire sample into the funnel. Rinse the beaker three times with methylene chloride, pouring the rinsate into the funnel. Rinse the funnel cake and the sides of the funnel with methylene chloride.

6.3.16 Discard the contents of the funnels as in step 6.1.21. Cover the openings of the flasks with aluminum foil and store in the refrigerator at 4 °C until you are ready to concentrate or proceed to section 7 for K&D concentration.

6.4 Sediment Extraction for S-EPH - EPA3550C, Ultrasonic Extraction

6.4.1 Follow the procedure for S-FL-PRO above (6.3), with the following exceptions:

6.4.2 Extract only 10 g of each sample, instead of 25 g.

6.4.3 When spiking, add 1 mL of the EPH surrogate (40 µg/mL) to all samples blanks and spikes. Add 250 µL of EPH spike (40 µg/mL) to matrix spikes and LFB's.

6.5 Waste Sample Extraction for WAS-FL-PRO - EPA 3580A, Waste Dilution. The waste dilution procedure may also be used for sediment samples exraction.

6.5.1 Do one methylene chloride solvent blank and two laboratory fortified blanks and two waste matrix spikes in the set.

6.5.2 Fortify all blanks, samples, and spikes with 2 mL of the FL-PRO Surrogate Mix for Water/Sediments (100 µg/mL OTP, 100 µg/mL C_{39}). Fortify the matrix spikes and laboratory fortified blank with 0.2 mL of the FL-PRO Matrix Spike Mix for Water/Sediments (500 µg/mL C_8-C_{40}). Note the amount added and the concentration of analytes in the extraction worksheet.

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6.5.3 Extract one to five grams (solid waste samples) and/or even up to 100 grams (aqueous waste water samples) of well-mixed waste sample depending upon its matrix.

6.5.4 Follow either W-FL-PRO (Section 6.1) or S-FL-PRO (Section 6.3) for the preparation steps when using more than five grams of sample.

6.5.5 For more than one gram to five grams of waste sample, dilute sample to more than 10 - 40 mL in methylene chloride.

6.5.6 Dilute to 10 mL with methylene chloride and Sonicate for 5 minutes, vortex to mix and centrifuge.

6.5.7 Do one gram silica gel column on the part of the extract as in Section 8.1.

6.5.8 Pure petroleum products are treated as neat standards. To analyze these waste samples with in the calibration limits on GC/FID, weigh approximately 0.20 to 0.50 grams in 10 mL of methylene chloride. Pass 1 mL out of 10 mL of extract through one-gram silica gel column and make up to 10 mL for analysis (0.20 g to 100 mL).

6.6 Electronic Sample Preparation Log

Each sample preparation must be recorded by the preparation team in the electronic Prep Worksheet Manager module of LIMS. For FL –PRO analyses there are two, ready- made, partially prefilled templates in LIMS. The template names are identical with LIMS prep IDs: FL-PRO (A), (soil and waste) and FL-PRO (B) (water). Many fields in both the header and the grid are prefilled and seldom change. They need, however, to be always checked for correctness.
Header fields in the FL-PRO preparation log:

- **Worksheet ID#:** *this is assigned automatically by LIMS. Example FL-PRO (A)_361*T
- **Prep ID:** *LIMS prep ID defined in LIMS and connected with test ID. Each test ID may only be connected to one Prep ID, but many test IDs may use the same prep method.*
- **Prepared By**:
- **Prep Start Date/Time**:*This will be the prep date in LIMS reports*
- **Spike Witness**;
- **Prep Completion Date/Time**:
- **Date/Time Reviewed**:
- **Reviewed By**:
- **Balance ID**: *pre-entered , usually the same*
- **Spike 1:** *Std tracker SN; this is usually surrogate*
- **Spike 2:** *Std tracker SN; this is usually TRPH spike*
- **Water bath temp:** *in deg C*
- **Final Extract Solvent:** *methylene Chloride_ lot number*
- **Solvent 1** *methylene Chloride_ lot number*
- **Cleanup procedures:** *1 g silica gel*
- **Prep comments:** *enter other chemicals/supplies here with lot numbers; example ottowa sand.*

Grid Fields in the FL-PRO preparation Log

Prep Batch ID: ***consecutive "P" number assigned by LIMS in the form of P123456. The same number has to be used for sample reporting in QC manager.***
Sample ID: *the sample IDs are selected in LIMS when the worksheet is created.; for QC samples those rows are typically pre-filled in the template*
Bottle #: *Water samples only; entered as ¼ - bottle 1 of*

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- **Lab pH**: *The pH of the sample measured with wide range pH ; waters only.test strips.*
- **Sample ID**; *the laboratory sample Id assigned in LIMS*
- **Test ID**: *The analysis requested for the sample.*
- **Matrix:** *matrix assigned in LIMS*
- **Sample Wt (g)/ Volume (mL) extracted**: *For water samples the volume extracted is determined by subtracting the mass of the empty sample bottle from the mass of the sample bottle + sample. For QC samples required volume is measured using a graduated cylinder. For other sample types the mass of the sample itself is measured e.g. 25g wet sample for soil/sediment 1g for waste samples.*

- **Final extract Volume (mL**: The volume of the sample extract after full concentration step; 2 mL for regular water/sediment/waste sample extraction
  - **Clean-up procedure**: *The clean-up procedure used, if applicable. Write down anything done differently than SOP requirements.*
  - **Spike 1:** *volume of surrogate spike added*
  - **Spike 2:** *volume of TRPH spike added*

- **Comments:** *This section should include any observations regarding unusual appearance or odor of the sample, any dilutions made during preparations or any other remarks about the preparation.*

7 SAMPLE EXTRACT CONCENTRATION

7.1 Concentrate sample extracts using the S-Evap® System Kuderna-Danish (K&D) Concentration at 70 °C and record the temperature in the notebook. It is best to K&D samples to a volume of 3-5 mL and do not let them go dry.

7.2 If water or solid particles are present in the sample extract prior to K&D concentration, remove by adding a small amount of granular sodium sulfate to the flask and swirl the flask to allow the sodium sulfate to absorb excess water. If there is a large amount of water in the extract, or both water and solid particles, remove top water layer with a pipet as much as possible, place a small plug of silanized glass wool into the bottom of a clean glass powder funnel and pour 2-3 cm of granular sodium sulfate on top of it. Carefully pour the sample extract from the Erlenmeyer flask through the funnel and into the K&D flask, rinsing the Erlenmeyer flask three times with 5 mL or so of methylene chloride and adding that to the funnel. Rinse the funnel with 5-10 mL of methylene chloride.

7.3 This step is different for FL-PRO samples and EPH samples

7.3.1 For FL-PRO samples, remove the K&D apparatus from the S-Evap® when there is only 3-5 mL left in the concentrator tube. Let the vapors in the condensing column cool and drain into the tube, then remove the Snyder column and rinse the inside of the K&D flask with a few mL of methylene chloride, allowing this to drain into the concentrator tube. If the sample would not concentrate, is very viscous, or is very dark in color, make comments in the logbook and this sample may need to be made up to a higher volume.

7. 3.2 For EPH samples, remove the K&D apparatus from the S-Evap® when there is only 15 mL left in the concentrator tube. Do not let the tube boil

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dry. Let the vapors in the condensing column cool and drain into the tube for at least 10 minutes. Add 50 mL of Hexane to each K&D flask, pouring the hexane through the Snyder column and add one fresh boiling chip. Increase the temperature of the water bath to 90 °C. Concentrate until volume is less than 3 to 5 mL. When the extract volume is less than 5 mL, remove the flask from the bath and rinse with hexane instead of methylene chloride.

7.4 Remove the concentrator tube from the K&D flask. There may be water on the outside of the flask -- do not allow it to drip into the sample (The water can be removed with a Kim wipe.). Rinse the joint of the K&D flask with methylene chloride for FL-PRO samples or hexane for EPH samples into the concentrator tube. Proceed to N-Evap for final extract concentration.

7.5 N-Evap® Concentration of FL-PRO Samples: Transfer the contents of the concentration tube into a 15 mL screw-top centrifuge tube and make up to 10 mL. If the sample volume is more than 10 mL, then N-Evap under gentle stream of nitrogen at 40 °C water bath temperature. If the sample extract is not dark in color or viscous, bring the volume up to 10 mL with methylene chloride. Cap tightly, vortex to mix, sonicate, and centrifuge. If the sample extract will not concentrate below 10 mL, is very viscous, or is very dark in color, bring it to whatever volume is possible. When performing silica gel cleanup, clean up half of the sample extract with loose silica gel, if possible. If the extract volume is very high, cleaning up half of it may be impossible. In this case, only clean up a smaller aliquot. For example, if the extract volume is 20 mL, you may wish to clean up only 1 mL. In any case, note the volume of the extract and the amount taken for cleanup in the extraction logbook.

7.6 N-Evap® Concentration of EPH Samples: Follow the procedure in 7.5 with the following exceptions, temperature of the water bath should be set at 50 °C instead of 40 °C and when transferring the extract into a centrifuge tube, rinse with hexane instead of methylene chloride. If the sample is not dark in color or viscous, make to a final volume of 1 mL in hexane.

8 SILICA GEL CLEANUP PROCEDURE

8.1 1 g silica cleanup for FL-PRO Samples

8.1.1 For samples that are very dark in color, very viscous, or did not concentrate easily, make up to higher volume, and clean up part of the extract with one gram of loose silica gel or using one gram silica gel solid phase extraction cartridge.

8.1.2 Half of the sample extract should be cleaned up. For most samples, this will be 5 mL out of 10 mL. If a sample extract has a higher volume, clean up half the volume of the extract if possible.

8.1.3 Using loose silica gel

8.1.3.1 For each sample extract, prepare a column by placing a plug of pre-cleaned silanized glass wool in a large glass Pasteur pipette. Add 1 g of loose silica gel to the column.

8.1.3.2 Pour roughly half of the loose silica gel into the extract and shake the mixture for 5 minutes.

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8.1.3.3 Using a Pasteur pipet, transfer the extract to the silica gel column, and collect in a centrifuge tube.

8.1.4 Using a silica gel solid-phase extraction cartridge

8.1.4.1 Condition a 1 g silica gel solid-phase extraction cartridge with 5 mL of methylene chloride.

8.1.4.2 Using a Pasteur pipet, transfer the extract to the silica gel cartridge, and collect in a centrifuge tube.

8.1.5 Rinse the centrifuge tube, which held the extract with 2 mL methylene chloride, adding the rinsate to the column or cartridge, and collecting it along with the sample extract. Repeat the rinse at least twice more. Continue collecting until you have collected a total of 12 mL.

8.1.6 The sample extract will need to be concentrated again, using the N-Evap®. It should be brought to a volume of 1 mL. If the extract is difficult to concentrate, you may not be able to concentrate it all the way to 1 mL. If you concentrate to another volume be sure to note that volume in the logbook.

8.2 5 g silica fractionation for EPH Samples

8.2.1 For each extract, prepare one silica gel column from a 200 mL chromatographic column with a reservoir and a stopcock. With the stopcock closed, add a few mL of hexane to the column. Push a plug of silanized glass wool into the bottom of the column (a glass rod can be used for this) and tamp out all of the air bubbles. Pour approximately one-quarter to one-half inch of granular sodium sulfate on top of the glass wool plug. In a beaker, prepare slurry of 5 g of silica gel and a few milliliters of hexane. Using a funnel and a hexane filled wash bottle, transfer the slurry into the column on top of the sodium sulfate. Rinse the beaker and funnel well with hexane to ensure that no silica gel is left behind. The silica gel slurry will stick to the walls of the column. Rinse it down by opening the stopcock and washing the walls of the column with hexane (a Pasteur pipet can be used for this) until all of the slurry is packed into the column. Do not let the column go dry during this step. Pour another half inch of sodium sulfate on top of the silica gel. Before using the column, rinse with 20 mL of hexane, letting the hexane drip through. Again, do not allow the silica gel to go dry. Just before addition of the sample extract and fractionation surrogate, ensure that the hexane meniscus is resting on the sodium sulfate layer by opening the stopcock and adjusting the level accordingly.

8.2.2 Half of the sample extract should be put through the column. For most samples, this will be 500 µL out of 1 mL. Save half of the sample extract.

8.2.3 To each column, add 500 µL of fractionating surrogate (40 ppm).

8.2.4 To each column, add exactly 500 µL of the appropriate extract with a glass volumetric pipet.

8.2.5 Open the stopcock for each column. In a centrifuge tube labeled for fraction 1, collect 1 mL from each column. This step should result in the 1 mL of sample being completely deposited on the head of the silica gel. This step is important since it determines the sample resolution and if done poorly, the compounds will not separate into well-defined fractions.

8.2.6 Add 19 mL of hexane to the top of each column.

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8.2.7 From each column, continue collection of 19 mL in the same centrifuge tube labeled with the sample number and fraction 1. (Do not collect this fraction in the same centrifuge tube you used to collect the discarded hexane.) This is fraction 1, the hexane fraction, which contains the aliphatic compounds. Due to evaporation, the actual collection volume may be less than 20 mL. Therefore, close the stopcock not when 20 mL have been collected, but when the hexane meniscus just reaches the sodium sulfate layer.

8.2.8 Note: The first 5 mL of the aliphatic fraction is not likely to contain any of the analytes. Therefore, it is possible to collect the first 5 mL into the waste tube and discard it, then collect the next 15 mL as fraction 1. However, if this is done, there is a risk that some analytes may be lost, particularly if the analytes are present in the sample in very high concentrations.

8.2.9 To each column, add 20 mL of methylene chloride, being careful not to disturb the silica gel.

8.2.10 Using a separate centrifuge tube from fraction 1 collect 20 mL from each column, as in step 8.2.7. This is fraction 2, the methylene chloride fraction, which contains the aromatic compounds.

8.2.11 Note: The first 4 mL of the aromatic fraction is not likely to contain any of the analytes. Therefore, it is possible to collect the first 3 mL into the waste tube and discard it, then collect the next 17 mL as fraction 2. However, if this is done, there is a risk that some analytes may be lost, particularly if the analytes are present in the sample in very high concentrations.

8.2.12 Concentrate both fractions to less than 1 mL on the N-Evap. To fraction 1, add 100 µL of Aliphatic Internal Standard (200 ppm). To fraction 2, add 100 µL of PAH Internal Standard (200 ppm). Make each fraction to a final volume of 1 mL, fraction 1 in hexane, fraction 2 in methylene chloride.

9 SAMPLE ANALYSIS PROCEDURE

9.1 For sample analysis, reference SOP **SV-007**, "Analysis of Petroleum-Range Organics by GC-FID (FL-PRO Method) and Analysis of Extractable Petroleum Hydrocarbons (EPH) by GC-FID (The Massachusetts Method)."

10 QUALITY CONTROL

10.1 For every set of samples (not to exceed twenty samples), a reagent blank, two lab fortified blanks and duplicate matrix spikes must be prepared. If there is insufficient sample available to prepare duplicate sample matrix spikes, prepare one reagent blank, two laboratory-fortified blanks, and one matrix spike. If there is insufficient sample available to prepare a single sample matrix spike, prepare one reagent blank, two laboratory-fortified blanks, and no matrix spike.

10.2 See BNA SOP SV-010 for detailed information on quality control, definitions and interferences.

11 SAFETY/HAZARDOUS WASTE MANAGEMENT

11.1 See SOP OG-001, Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories) for specific details on safety and hazardous waste management.

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12 REFERENCES

12.1 Florida Department of Environmental Protection's Method for Determination of Petroleum Range Organics (FL-PRO), Rev. 1, 11/1/1995.

12.2 SW-846, Test Methods for Evaluating Solid Wastes, Method 3510C, Separatory Funnel Liquid-Liquid Extraction, Third Edition, Revision 3, 12/1996

12.3 SW-846, Test Methods for Evaluating Solid Wastes, Method 3520C, Continuous Liquid-Liquid Extraction, Third Edition, Revision 3, 12/1996

12.4 SW-846, Test Methods for Evaluating Solid Wastes, Method 3550C, Ultrasonic Extraction, Third Edition, Revision 3, 2/2007

12.5 SW-846, Test Methods for Evaluating Solid Wastes, Method 3580A, Waste Dilution, Third Edition, Revision 1, 07/1992

12.6 Massachusetts Department of Environmental Protection's Method for Determination of Extractable Petroleum Hydrocarbons (EPH), 01/1998.

12.7 DEP SOP SV-009. BNA Standard Preparation.

12.8 DEP SOP SV-007. Analysis of Petroleum-Range Organics by GC-FID (FL-PRO Method) and Analysis of Extractable Petroleum Hydrocarbons (EPH) by GC-FID (The Massachusetts Method).

12.9 DEP SOP SV-010. Quality Assurance/Quality Control in the Semi-Volatile Organics Laboratory (BNA-GC/MS).

12.10 DEP SOP OG-001. Safety and Waste Management Practices in the Organic Analysis Groups (Volatile, Semi-Volatile and Pesticide Laboratories).

APPENDIX OF CHANGES

07/23/2014 Addition of electronic Prep Worksheet Manager.

07/20/2015 References to extraction notebook or logbook were changed to references to preparation worksheet, since a physical notebook no longer exists. A chapter describing continuous liquid-liquid extraction was removed since that procedure is no longer performed.

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APPENDIX: FL-PRO SAMPLE PREPARATION LOGS EXAMPLES FOR WATERS, SOILS and WASTES

Printed: 7/23/2014 9:48:27 **FL-PRO (B)_305** Page 1 of 1

Worksheet ID: FL-PRO (B)_305
Prep ID: FL-PRO (B)
Prepared By: GHAFFARI_R
Prep Start Date/Time: 6/27/2014 13:00
Spike Witness: SIDDELL_F
Prep Completion Date/Time: 6/27/2014 16:52
Date/Time Reviewed: 6/27/2014 15:52
Reviewed By: GHAFFARI_R
Balance ID: Adventurer ProAV4101c SN#8029281092
Spike 1: 54103
Spike 2: 55224
Water Bath Temperature (C): 70 C
Final Extract Solvent: Methylene chloride_140730
Solvent 1: Methylene chloride_140730
Cleanup Procedures: 1 g Silica Gel
Prep Comments: SPE Silica lot#741820-142943

Prep Batch ID	Bottle #	Lab pH	Sample ID	Test ID	Matrix Type	Sample Volume	Final Extract	Sample Cleanup	Spike 1	Spike 2
P270082		7	MBLK	W-FL-PRO	FDEP WEEL	1000	2 mL	5mL/10mL on 1g silica to 1mL		
P270082		7	LFB_1	W-FL-PRO	FDEP WELL	1000	2 mL	5mL/10mL on 1g silica to 1mL	2mL	200uL
P270082		7	LFB_2	W-FL-PRO	FDEP WELL	1000	2 mL	5mL/10mL on 1g silica to 1mL	2mL	200uL
P270082 Dark yellowish	2/4	<3	MS-1614495	W-FL-PRO	WATER	1000	2 mL	5mL/10mL on 1g silica to 1mL	2mL	200uL
P270082 Dark yellowish	3/4	<3	MSD-1614495	W-FL-PRO	WATER	1000	2 mL	5mL/10mL on 1g silica to 1mL	2mL	200uL
P270082 light yellowish	1/2	<3	1614493	W-FL-PRO	WATER	1050	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 light yellowish	1/2	<3	1614494	W-FL-PRO	WATER	980	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 Dark yellowish	1/4	<3	1614495	W-FL-PRO	WATER	1000	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 Dark brownish	1/2	<3	1614496	W-FL-PRO	WATER	986	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 light yellowish	1/2	<3	1614497	W-FL-PRO	WATER	1046	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 light yellowish	1/2	<3	1614498	W-FL-PRO	WATER	1035	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 Dark brownish	1/2	<3	1614499	W-FL-PRO	WATER	1034	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 light yellowish	1/2	<3	1614500	W-FL-PRO	WATER	1043	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 light yellowish	1/2	<3	1614501	W-FL-PRO	WATER	1009	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	
P270082 colorless	1/2	<3	1614502	W-FL-PRO	W-EQPMT-BK	1022	2 mL	5mL/10mL on 1g Silica to 1 mL	2mL	

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FL-PRO (A)_361

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Worksheet ID: FL-PRO (A)_361
Prep ID: FL-PRO (A)
Prepared By: GHAFFARI_R
Prep Start Date/Time: 7/17/2014 09:00
Spike Witness: SIDDELL_F
Prep Completion Date/Time: 7/17/2014 15:00
Date/Time Reviewed: 7/17/2014 16:00
Reviewed By: GHAFFARI_R
Balance ID: Denver Ins, SN#25054395
Spike 1: 56136
Spike 2: 55224
Equipment ID(s):
Water Bath Temperature (C): 70 C
Final Extract Solvent: Methylene chloride_143172
Solvent 1: Methylene chloride_143172
Cleanup Procedures: 1g Silica Gel
Prep Comments: Ottawa sand lot#120571 SPE Silica lot#805270-Q1-08 Sodium Sulfate lot#132282

Prep Batch ID	Sample ID	Test ID	Matrix Type	% Solids	Sample Dry	Sample Wet	Final Extract	Spike 1	Spike 2	Sample Cleanup
P271066	MBLK	S-FL-PRO	OTTAWA	100	25	25	2			5mL/10mL on 1g silica to 1mL
P271066	LFB_1	S-FL-PRO	OTTAWA	100	25	25	2	2mL	200uL	5mL/10mL on 1g silica to 1mL
P271066	LFB_2	S-FL-PRO	OTTAWA	100	25	25	2	2mL	200uL	5mL/10mL on 1g silica to 1mL
P271066	MS-1619343	S-FL-PRO	S-SOIL	80.4	20.1	25	2	2mL	200uL	5mL/10mL on 1g silica to 1mL
P271066	MSD-1619343	S-FL-PRO	S-SOIL	80.4	20.1	25	2	2mL	200uL	5mL/10mL on 1g silica to 1mL
P271066	1619343	S-FL-PRO	S-SOIL	80.4	20.1	25	2			5mL/10mL on 1g silica to 1mL
P271066	1619344	S-FL-PRO	S-SOIL	63.8	15.95	25	2			5mL/10mL on 1g silica to 1mL
P271066	1619345	S-FL-PRO	S-SOIL	78.9	19.725	25	2			5mL/10mL on 1g silica to 1mL
P271066	1619346	S-FL-PRO	S-SOIL	85.1	21.275	25	2			5mL/10mL on 1g silica to 1mL

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Printed: 7/23/2014 9:55:16 **FL-PRO (A)_356** Page 1 of 1

Worksheet ID: FL-PRO (A)_356
Prep ID: FL-PRO (A)
Prepared By: GHAFFARI_R
Prep Start Date/Time: 6/23/2014 11:30
Spike Witness: SIDDELL_F
Prep Completion Date/Time: 6/23/2014 09:31
Date/Time Reviewed: 6/23/2014 12:31
Reviewed By: TOPOLSKI_M
Balance ID: DENVER MXX-123 SN#25155135
Spike 1: 54103
Spike 2: 55224
Equipment ID(s): DENVER MXXX-123 SN#25155135
Water Bath Temperature (C): NA
Final Extract Solvent: Methylene chloride_140730
Solvent 1: Methylene chloride_140730
Cleanup Procedures: 1g Silica Gel#805270-q1-08
Prep Comments:

Prep Batch ID	Sample ID	Test ID	Matrix Type	% Solids	Sample Dry	Sample Wet	Final Extract	Spike 1	Spike 2	Sample Cleanup
P269721	MBLK	WAS-FL-PRO	CH2CL2			1.0	2	2mL		5/10 on 1g silica to1mL
P269721	LFB_1	WAS-FL-PRO	CH2CL2			1.0	2	2mL	200uL	5/10 on 1g silica to1mL
P269721	LFB_2	WAS-FL-PRO	CH2CL2			1.0	2	2mL	200uL	5/10 on 1g silica to1mL
P269721	MS-1612689	WAS-FL-PRO	WAS-LIQUID			1.004	2	2mL	200uL	5/10 on 1g silica to 1mL
P269721	MSD-1612689	WAS-FL-PRO	WAS-LIQUID			1.016	2	2mL	200uL	5/10 on 1g silica to1mL
P269721	1612684	WAS-FL-PRO	WAS-LIQUID			1.001	10	2mL		1/10 on 1g silica to 1mL
P269721	1612685	WAS-FL-PRO	WAS-LIQUID			0.203	2	2mL		5/10 on 1g silica to1mL
P269721	1612686	WAS-FL-PRO	WAS-LIQUID			0.208	2	2mL		5/10 on 1g silica to 1mL
P269721	1612687	WAS-FL-PRO	WAS-LIQUID			0.201	2	2mL		5/10 on 1g silica to1mL
P269721	1612688	WAS-FL-PRO	WAS-LIQUID			0.210	2	2mL		5/10 on 1g silica to 1mL
P269721	1612689	WAS-FL-PRO	WAS-LIQUID			1.022	2	2mL		5/10 on 1g silica to1mL

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