Standard Operating Procedure for

Oil & Grease – Method EPA1664A

This document is intended to enable a technician new to Solid Phase Extraction (SPE) technology to perform SPE on oil and grease samples while obtaining reportable and reliable results for n-Hexane Extractable Material (HEM) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar material). This utilization of SPE in EPA Method 1664*A, Revision A* is a *Freon- Free* (Clean Air Act Amendment of 1990) technique that employs n-hexane extraction gravimetric analysis and provides a QC procedure designed to monitor Precision and Recovery (PAR). Determination of "Oil and Grease" and "Total Petroleum Hydrocarbons" is a performance based method applicable to aqueous matrices. Testing most often occurs on surface and saline waters; industrial and domestic aqueous wastes including non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps and greases. Results can be obtained in the range of 5 to1000 mg/L for HEM and SGT-HEM while providing a method detection limit (MDL) of 1.4 mg/L and a minimum level of quantification (ML) of 5.0 mg/L. This method is not to be used for the determination of materials that are volatile at temperatures below approximately 85°C, such as petroleum fuels from gasoline may be lost in the solvent through evaporation.

This document includes a materials list, a step-by-step guide to the method as well as Xenosep contact information for technical and sales support. More detailed information can be found at www.xenosep.com

Section 1 – Manifold Starter Kit Assembly Instructions

1. While facing the front of the Manifold (white valve handles face you), remove the drain and hose barb plugs from the handles. Attach the **Side Arm** (#26625) to the right handle opening and tighten until the side arm faces up. Connect the **Swivel Clamp** (#26617) to the top of the Side Arm. Attach the **Vacuum Tubing** (#26615) to the hose barb fitting in the left handle and also to your sample waste container/vacuum trap/vacuum source.
2. Insert a glass **Collection Vial** (#26507) into a stainless steel tapered chamber.
3. Place a **Filter Holder** (#26605) on the tapered chamber ensuring outlet enters vial.
4. Insert the **Stainless Steel Support** (#26611) into the recess of the Filter Holder.
5. Place an **SPE Filter** (#24547, diamond-patterned side down) onto each Stainless Steel Support.
6. Fit the black **Teflon O-Rings** (#26608) into the grooves of the **Teflon® Coupler** (#26610) and firmly press Coupler on top of the SPE Filter.
7. Connect the **Filter Funnel** (#26603) over the Coupler and secure with an **Aluminum Clamp** (#26613).
8. Open the Swivel Clamp on the Side Arm and attach the ***Eluter*** (#26516) to the short length of tubing on the Side Arm.
9. Attach a ***Flat Sided Erlenmeyer Flask*** (#26513) to the outlet of the Eluter and secure using *a Green Clamp (#26512).*
10. Insert an **Inline, Sodium Sulfate Column** (#26524) into the Eluter so that the plastic luer tip of the column extends into the glass luer outlet of the Eluter.
11. Repeat steps 2 – 10 as necessary.

Items included in Xenosep Manifold Kit (#26618)

- 24 Xenosep SPE Filters (#26626)
- 3 Collection Vial (#26507)
- 1 Green Clamp (#26512)
- 1 Flat Sided Erlenmeyer Flask (#26513)
- 24 Transfer Pipets (#26627)
- 1 Eluter (#26516)
- 24 Sodium Sulfate Drying Columns (#26628)
- 25 Aluminum Pans (#26629)
- 5 EPA 1664A Standard 1 x 30 mL, 4 mg/mL (#26532)
- 6 Coupler O-rings (#26608)
- 3 Funnels (#26603)
- 3 Holders (#26605)
- 3 Teflon® Couplers (#26610)
- 3 Stainless Steel Supports (#26611)
- 3 Aluminum Clamps (#26613)
- 1 Tygon® Tubing; 3”, 12”, 48” (#26615)
- 1 Swivel Clamp (#26617)
- 1 Side Arm (#26625)

Lab Supplies: Items needed but not included in Kit

- n-Hexane - 85% min. purity; A.C.S. Grade, **Residue < 1 mg/L (<0.0001%)**
- Methanol - A.C.S. Grade, **Residue < 1 mg/L (< 0.0001%)**
- Acetone - A.C.S. Grade, Residue < 1 mg/L (<0.0001%)
- Hydrochloric acid
- Distilled water
- Gloves
- Protective eye glasses

Equipment: Items not included in the Starter Kit

- 1 lab buret support stand
- 1 swivel clamp (#26616)
- Vacuum pump set at > than 25" of vacuum; air flow greater than 3 CFM
- Distillation apparatus (#26511) – Optional
- Solvent Recovery Apparatus (#26635) – Optional
- Hot plate
- Thermometer
- 1, 5 &10 mL glass pipet and rinsing bulb
- Boiling chips; silicon carbide or fluoropolymer
- 4 wash bottles for Hexane, Distilled water, Acetone or Methanol
- 1, 100 mL volumetric flask
- Analytical balance capable of reading 0.1g
- Glass powder transfer funnel
- 1 Desiccator
- Sample Waste Container – 4L minimum
- Vacuum trap

Section 2 – Method Summary and other information

1) Weigh flask and record value
2) Prepare sample by acidifying and adding PAR standard
3) Condition SPE filter
4) Filter Extract sample
5) Elute & Dry sample extract, collect into flask
6) Distill sample Distillation apparatus or equivalent
7) Evaporate remaining hexane
8) Weigh flask and record value

Safety: Conduct filtration and extraction in a hood or in a well ventilated area. Wear gloves and discard the waste solvents appropriate containers. For details as to the toxicity and carcinogenicity of each reagent refer to OSHA or NIOSH approved personal hygiene monitoring methods.

Definitions:

1) PAR - Precision and Recovery
2) SGT-HEM (Silica Gel Treated – Hexane Extractable Material)
3) MDL – Method Detection Limit

4) ML – Minimal Level of Quantification

Validate PAR Standard and Recovery Technique

1) Tare an aluminum pan.
2) Pipet 10 mL (40 mg) of the enclosed EPA Method 1664A standard (4 mg/mL) into the aluminum pan and place pan on 35ºC hot plate (warm to touch but not will not burn hand).
3) Close hood sash and evaporate solvent using low heat and gentle laminar air flow from hood.
4) As soon as bottom of aluminum pan starts to develop dry spots (no solvent areas) carefully remove pan from hot plate and let it continue to gently evaporate to dryness at room temperature in a hood using laminar air flow if possible.
5) When the aluminum pan appears to be completely dry, place pan in desiccator for 30 minutes until a stable weight can be obtained.
6) Record weight of aluminum pan. Subtract the final pan weight from the initial tare weight. This value should be 40±1 mg.

Section 3 - Preparation of Reagent, Standard and Equipment

1) Use enclosed EPA 1664A Standard; or prepare according to EPA Method1664A.
2) *Acidifying Solution:* 6 N HCl. Mix equal volumes of concentrated HCL and reagent water.
3) Prepare 4 separate wash bottles; Distilled water, acetone, hexane and methanol.

Sampling Equipment:

1) Sample collection bottles should be 1 liter, wide-mouth glass bottles with PTFE-lined screw caps.

Prepare Flask:

Note: See both options under Hexane Extractable Material in Section 4 before proceeding with this step.

1) Label flat sided Erlenmeyer Flask
2) Add 3-4 boiling chips
3) Heat flask for one hour at 105°C
4) Cool in desiccator for 30 minutes
5) Record initial stable weight

OR

1) Label aluminum pans
2) Record initial stable weight

PAR Sample Preparation:

1) Bring sample to room temperature.
2) Acidify sample by adding 6-8 mL of 6 N HCL, shake well.
3) Spike 10 mL (40 mg) PAR standard to the sample bottle. Shake well.

Section 4: Assemble Apparatus and Run method

1) Insert collection vial into the tapered chamber.
2) Place filter holder on top of the tapered chamber.
3) Insert the stainless steel support in recess on top of the filter holder.
4) Place Xenosep SPE Filter diamond-patterned side down onto stainless steel support.
5) Firmly press Teflon collar on top of the SPE filter and into the filter holder.
6) Mate funnel/reservoir on top of the filter holder/over the protruding Teflon collar.
7) Secure funnel to filter holder with the aluminum clamp.
8) Ensure white PTFE valve is closed to vacuum/liquid flow.

Condition SPE Filter:

1) With the filter secured in the SPE filter holder (diamond-patterned side down) rinse side walls of the funnel with 10 mL of n-hexane. Make sure SPE filter is completely covered with solvent.
2) Let stand for 5 seconds then open/close stopcock to vacuum and collect hexane into collection vial.
3) Repeat steps 1 and 2.
4) Air dry SPE filter with vacuum for 1 minute.
5) Add 10 mL methanol to SPE filter and make sure to completely cover the filter.
6) Let stand for 5 seconds then open/close stopcock quickly to pull all the excess solvent through the filter into the collection vial.
7) Remove collection vial containing solvent waste and discard appropriately. Reassemble apparatus.
8) Pour 20 mL of distilled water on to the filter, turn stopcock open and allow vacuum to pull water into the manifold. **Note:** Do not let the filter dry out. If this occurs after step 4, repeat the conditioning process beginning at step 5.

Filter- Extract Sample:

1) *Pour sample into funnel and turn on vacuum. Minimize sample contact with the* funnel by keeping the liquid level below the Xenosep logo.
2) Once the sample is completely filtered, rinse side walls with a small amount of distilled water to move any sediment to the surface of the SPE filter. Allow the vacuum pump to air dry the SPE filter for at least 4 min but not longer than 8 minutes as lower recoveries may occur due to loss of the more volatile analytes. Turn vacuum pump off.
3) Remember to periodically check and empty the sample waste container to prevent overflow of the sample waste into the vacuum trap – vacuum source.
4) Discard sample waste as appropriate.

Remove Water from SPE Filter and Trapped Sediment Layer:

1) If there is a trapped sediment layer on the filter surface, add a small amount of magnesium sulfate on top of the SPE filter and carefully mix with spatula until the sediment/magnesium sulfate appears dry, granular and free flowing.

2) After the SPE filter drying step is complete, turn off vacuum and use a glass powder funnel to transfer the solids into the Xenosep inline sodium sulfate column below.
3) Rinse the spatula and/or funnel used for mixing/scooping/transfer with n-hexane and add it to the final extract.

Analyte Recovery and Water Removal from n-Hexane Extract:

1) Insert an inline column containing sodium sulfate into the Eluter.
2) Remove Filter Holder/Funnel assembly from tapered chamber and place on Eluter.
3) Connect a pre-tared Flat Sided Flask to outlet of Eluter. Secure using a green clamp.
4) Dispense 10 mL of n-hexane into sample bottle.
5) Rinse sample bottle and cap in a horizontal circular motion for 10 sec.
6) Slowly vent cap and place it on counter, PTFE-septa up.
7) Tilt sample bottle so n-hexane extract settles in the shoulder of the sample bottle.
8) Aspirate solvent from sample bottle using a transfer pipet and completely rinse the funnel walls at least 3 times with solvent.
9) Repeat Step 4 though 8, 2 times.
10) Open stopcock and pull n-hexane through sodium sulfate drying column into the flat sided flask
11) Dispense 10 mL of n-hexane from wash bottle for final rinse of funnel/eluter and pre-tared flat sided flask. Close stopcock.

Section 5: Determination of HEM

1) Distillation and evaporation to dryness is a very technique dependent step and is a major source of lower analyte recoveries. As such we have included two options for this section. The first option we recommend for practice and/or ongoing HEM analysis without solvent recovery is evaporation using aluminum pans. The pans are lighter, tare faster and are easier to determine when to take them off the heat source. Check with your regulatory authority if evaporation without solvent recovery is an acceptable option for ongoing analysis as this seems to vary by auditor. If not, use the Xenosep Solvent Recovery Kit (#26635) to recover the evaporated solvent from aluminum pans or the distillation option using the flat sided flasks below. The flat sided Erlenmeyer flasks were specifically designed to speed this process and provide high recoveries. Note: aluminum pans may contain trace amounts of oil from the manufacturing process which will not affect HEM results but could affect SGT-HEM results unless they are pre-cleaned. If pans are to be used for SGT-HEM analysis, pre-rinse inside of pans with hexane, dry in oven at 105°C for 1 hour, place in desiccator for 30 minutes and obtain a stable tare weight before use.

Option 1 – No Solvent Recovery

1) Quantitatively transfer extract from flat sided Erlenmeyer flask into the pre-tared aluminum pan. Rinse Erlenmeyer several times with small amounts of hexane and collect in aluminum pan.
2) Place Pre-tared aluminum pan on warm (35ºC) hot plate (warm to the touch but does not burn hand).
3) Close hood sash to create a laminar flow of air over the aluminum pan/hot plate which helps speed the evaporation process.
4) As soon as bottom of the aluminum pan starts to develop dry spots (no solvent areas) carefully remove the pan from the hot plate and let it continue to gently evaporate to dryness at room temperature in a hood using laminar air flow if possible.

5) When the aluminum pan appears completely dry, place in desiccator for minimum 30 minutes until a stable weight can be obtained.
6) Record weight of aluminum pan and subtract that value from the pre-tared value to obtain the amount of HEM present in the sample and is expressed in mg/L.

Option 2 – > 80% Solvent Recovery (> 90% solvent recovery using water jacket)

1) Attach the flat sided Erlenmeyer flask containing extract to the short side of the Crossover Condenser using a green plastic clamp.
2) If using nitrogen to speed the distillation process, remove black cap with PTFE lined septa and attach nitrogen tubing to side arm above flat sided flask. Note: Use of nitrogen will lower solvent recovery.
3) Place a 125 mL round flat bottom round flask on the opposite side of the crossover condenser (side with water jacket).
4) If using the water jacket, attach tubing with water flow to the middle hose barb. Attach another piece of tubing to the upper hose barb exiting into a sink.
5) Submerge the flat sided flask under water deep enough to cover the Xenosep logo. The thermometer in the water bath should read at least 85°C.
6) As soon as the bottom of the flask starts to form dry spots, remove the flask from the water bath and wipe outside of the flask to remove any water or finger prints.
7) Lay the flat side of the flask on the bench in a hood or in a well ventilated area. As the flask cools down, the excess solvent will gently evaporate and a small amount of n-hexane vapors will condense on the counter because the n-hexane vapors are heavier than air.
8) When the flask appears completely dry, remove flask and place in a desiccator for minimum of 30 minutes until a stable weight can be obtained.
9) Record weight of boiling flask and subtract that value from the pre-tared value to obtain the amount of HEM present in the sample and is expressed in mg/L.

Section 5: Determination of SGT-HEM

1) Re-dissolve “dried” HEM in a flat sided flask with 85-90 mL n-hexane.
2) Quantitatively transfer to a 100 mL volumetric flask and bring up to volume with n-hexane.
3) Aliquot an appropriate amount of extract, dilute and return to flask as described in EPA Method 1664A.
4) Add 3 g of anhydrous silica gel for every 100 mg HEM or fraction thereof.
5) Stir for 5 minutes and filter through a 2nd inline sodium sulfate column into a 2nd Pre-tared flat sided flask.
6) Rinse inline silica gel/inline sodium sulfate column with 10 mL n-hexane to complete the transfer.
7) Distill n-hexane and obtain stable weight per HEM section above.
8) Record weight of the boiling flask and compare value to tare weight. The difference is the amount of SGT-HEM present in the sample and is expressed in mg/L.

Section 6: Clean in Place

1) Remove the used inline sodium sulfate column from the Eluter and discard.
2) Rinse the tip of the Eluter with very small amounts of acetone and n-hexane (< 1 mL each).
3) Collect and discard all waste solvents according to appropriate local, state and/or Federal Regulations.

For more information, please contact:

Xenosep Technologies
508 Main Street
Boonton, NJ 07005

T: 973.239.0247
F: 973.239.4133
www.xenosep.com