United States
Environmental Protection
Agency

Office of Water
Engineering and Analysis Division (4303)
Washington, DC 20460

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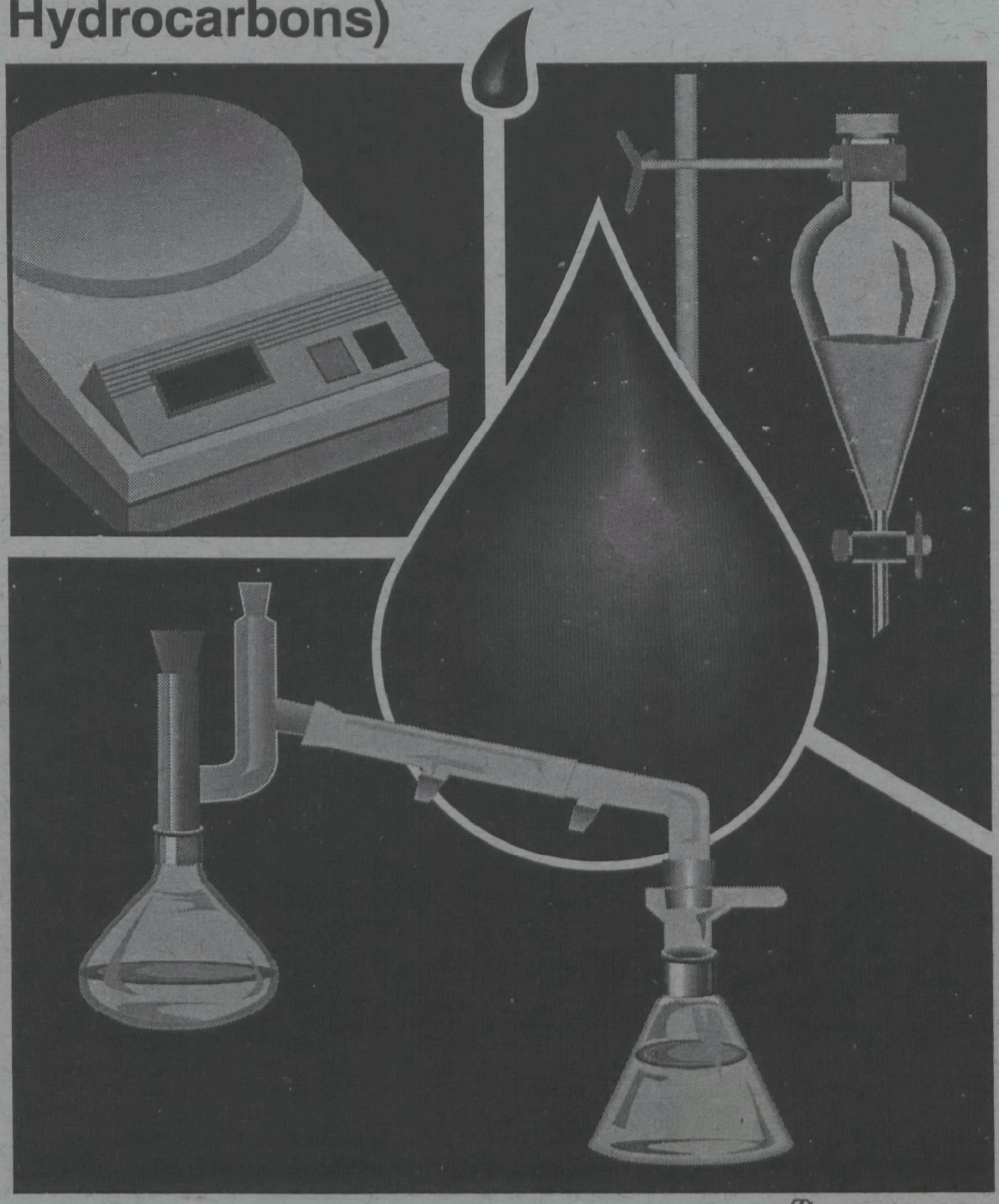
EPA

Method 1664: N-Hexane Extractable Material (HEM) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry (Oil and Grease and Total Petroleum Hydrocarbons)



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Acknowledgments

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Disclaimer

This method has been reviewed by the Engineering and Analysis Division, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

As a party to the Montreal Protocol on Substances that Deplete the Ozone Layer and as required by law under the Clean Air Act Amendments of 1990 (CAAA), the United States is committed to controlling and eventually phasing out the use of chlorofluorocarbons (CFCs). In support of these efforts, Method 1664 was developed by the United States Environmental Protection Agency Office of Science and Technology to replace previously used gravimetric procedures that employed Freon-113, a Class I CFC, as the extraction solvent for the determination of oil and grease and petroleum hydrocarbons.

Method 1664 is a performance based method applicable to aqueous matrices that requires the use of n-hexane as the extraction solvent and gravimetry as the determinative technique. Alternative extraction and concentration techniques are allowed, provided that all performance specifications are met. In addition, QC procedures designed to monitor precision and accuracy have been incorporated into Method 1664.

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Method 1664

N-Hexane Extractable Material (HEM) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM) by Extraction and Gravimetry

(Oil and Grease and Total Petroleum Hydrocarbons)

1.0 Scope and Application

1.1 This method is for the determination of n-hexane extractable material (HEM) and n-hexane extractable material that is not adsorbed by silica gel (SGT-HEM) in surface and saline waters and industrial and domestic aqueous wastes. Extractable materials that may be determined are relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases, and related materials.

1.2 This method is for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Federal Water Pollution Control Act and Amendments. "Oil and grease" is a conventional pollutant defined in the Act and codified at 40 *CFR* 401.16. The term "n-hexane extractable material" reflects that this method can be applied to materials other than oils and greases. Similarly, the term "silica gel treated n-hexane extractable material" reflects that this method can be applied to materials other than aliphatic petroleum hydrocarbons that are not adsorbed by silica gel.

1.3 This method is not applicable to measurement of materials that volatilize at temperatures below approximately 85°C. Petroleum fuels from gasoline through #2 fuel oil may be partially lost in the solvent removal operation.

1.4 Some crude oils and heavy fuel oils contain a significant percentage of materials that are not soluble in n-hexane. Accordingly, recoveries of these materials may be low.

1.5 This method is capable of measuring HEM in the range of 2 to 1000 mg/L and SGT-HEM in the range of 5 to 1000 mg/L, and may be extended to higher levels by analysis of a smaller sample volume collected separately.

1.6 For this method, the Method Detection Limit (MDL; 40 *CFR* 136, Appendix B) has been determined as 0.91 mg/L for HEM and 1.6 mg/L for SGT-HEM (Reference 16.1), and the Minimum Level (ML; Reference 16.3) has been set at 2.0 mg/L for HEM and 5.0 mg/L for SGT-HEM (Reference 16.1).

1.7 This method is "performance-based". The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in Section 9.1.2.

1.8 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 *CFR* 136.4 and 136.5.

1.9 Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

2.0 Summary of Method

2.1 A 1-L sample is acidified to pH <2 and serially extracted three times with n-hexane in a separatory funnel. The extract is dried over sodium sulfate.

2.2 The solvent is evaporated from the extract and the HEM is weighed. If the HEM is to be used for determination of SGT-HEM, the HEM is redissolved in n-hexane.

2.3 For SGT-HEM determination, an amount of silica gel proportionate to the amount of HEM is added to the solution containing the redissolved HEM to remove adsorbable materials. The solution is filtered to remove the silica gel, the solvent is evaporated, and the SGT-HEM is weighed.

2.4 Quality is assured through calibration and testing of the extraction, concentration, and gravimetric systems.

3.0 Definitions

3.1 HEM and SGT-HEM are method-defined analytes; i.e., the definitions of both HEM and SGT-HEM are dependent on the procedure used. The nature of the oils and/or greases, and the presence of extractable non-oily matter in the sample will influence the material measured and interpretation of results.

3.2 Definitions for terms used in this method are given in the glossary at the end of the method.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts that affect results. Specific selection of reagents and purification of solvents may be required.

4.2 All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running laboratory blanks as described in Section 9.4.

4.3 Glassware is cleaned by washing in hot water containing detergent, rinsing with tap and distilled water, and rinsing with solvent or baking. Boiling flasks that will contain the extracted residue are dried in an oven at 105–115°C and stored in a desiccator.

4.4 Sodium sulfate and silica gel fines have the potential to inflate results for HEM and SGT-HEM by passing through the filter paper. If the filter paper specified in this method is inadequate for removal of these fines, use of a 0.45-micron filter is recommended.

4.5 Interferences extracted from samples will vary considerably from source to source, depending upon the diversity of the site being sampled. For those instances in which samples are thought to consist of complex matrices containing substances (such as particulates or detergents) that may interfere with the extraction procedure, a smaller sample may need to be collected for analysis.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely determined; however, each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 n-Hexane has been shown to have increased neurotoxic effects over other hexanes and some other solvents. Inhalation of n-hexane should be minimized by performing all operations with n-hexane in a hood or well-ventilated area.

5.3 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.

5.4 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 16.4–16.6.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.*

6.1 Sampling equipment.

6.1.1 Sample collection bottles: Glass, approximately 1 L, with PTFE-lined screw cap.

Note: *In those instances necessitating collection of a smaller aliquot, a smaller sample container may be used.*

6.1.2 Cleaning.

6.1.2.1 Bottles: Detergent water wash, tap water rinse, cap with aluminum foil, and bake at 200–250°C for 1 h minimum prior to use. Solvent rinse may be used in place of baking.

6.1.2.2 Liners for screw caps: Detergent water wash, tap water and solvent rinse, and bake at 110–200°C for 1 h minimum prior to use.

6.1.3 Bottles and liners must be lot-certified to be free of artifacts by running laboratory blanks according to this method (per Section 9.4). If blanks from bottles and/or liners without cleaning or with fewer cleaning steps than required above show no detectable materials, the bottle and liner cleaning steps that do not eliminate these artifacts may be omitted.

6.2 Equipment for glassware cleaning.

6.2.1 Laboratory sink with overhead fume hood.

6.2.2 Oven: Capable of maintaining a temperature within ± 5°C in the range of 100–250°C.

6.3 Equipment for calibration.

6.3.1 Analytical Balance: Capable of weighing 0.1 mg.

6.3.2 Volumetric flask: 100-mL glass.

6.3.3 Vials: Assorted sizes, with PTFE-lined screw caps.

6.3.4 Volumetric pipette: 5-mL glass.

6.4 Equipment for sample extraction.

6.4.1 Balance: Top loading, capable of weighing 500–2000 g within ± 1%.

6.4.2 Glass stirring rod.

6.4.3 Separatory funnel: 2000-mL, with PTFE stopcock.

6.4.4 Funnel: Large, glass, for pouring sample into separatory funnel.

6.4.5 Centrifuge: Capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm minimum.

6.4.6 Centrifuge tubes: 100-mL glass.

6.5 Equipment for removal of water, sodium sulfate, and silica gel fines.

6.5.1 Funnel: Analytical, glass.

6.5.2 Filter paper: Whatman No. 40 (or equivalent), to fit funnel.

6.6 Equipment for solvent evaporation.

6.6.1 Water bath: Capable of maintaining a temperature of approximately 85°C.

6.6.2 Flask: Boiling, 125-mL (Corning No. 4100 or equivalent).

6.6.3 Distilling head: Claisen (VWR Scientific No. 26339-005, or equivalent), includes Claisen-type connecting tube and condenser.

6.6.4 Distilling adaptor (attached to the distilling head and to the waste collection flask for recovery of solvent).

6.6.5 Waste collection flask (attached to the distilling adaptor for collection of the distilled solvent).

6.6.6 Ice bath (to aid in the condensation and collection of the distilled solvent).

6.6.7 Vacuum, pump or other source of vacuum.

6.6.8 Desiccator: Cabinet- or jar-type, capable of keeping the boiling flask (Section 6.6.2) dry during cooling.

6.7 Equipment for removal of adsorbable materials.

6.7.1 Magnetic stirrer.

6.7.2 PTFE-coated magnetic stirring bars.

6.7.3 Graduated cylinder: 500-mL, capable of measuring ± 5 mL.

6.7.4 Pipettes: Assorted sizes, calibrated to within ± 0.5 percent.

7.0 Reagents and Standards

Note: *Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance meeting requirements of this method is the responsibility of the laboratory.*

7.1 Reagent water: Water in which HEM is not detected at or above the Minimum Level of this method. Bottled distilled water, or water prepared by passage of tap water through activated carbon have been shown to be acceptable sources of reagent water.

7.2 Hydrochloric or sulfuric acid: ACS, 1:1. Mix equal volumes of conc. HCl or H_2SO_4 and reagent water.

7.3 n-Hexane: 85% purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L.

7.4 Acetone: ACS, residue less than 1 mg/L.

7.5 Sodium sulfate: ACS, granular anhydrous.

Note: *Powdered sodium sulfate should not be used because traces of water will cause it to solidify.*

7.6 Boiling chips: Silicon carbide or PTFE.

7.7 Silica gel: Anhydrous, 60 - 200 mesh (Davidson Grade 950 or equivalent). Dry at 200–250°C for 24 h minimum and store in a desiccator or tightly sealed container. Determine the n-hexane soluble material content of the silica gel by extracting 30 g of silica gel with n-hexane and evaporating the n-hexane to dryness. The silica gel must contain less than 5 mg of n-hexane soluble material per 30 g (< 0.17 mg/g).

7.8 Hexadecane: 98% minimum purity.

7.9 Stearic acid: 98% minimum purity.

7.10 Hexadecane/stearic acid (1:1) spiking solution: Prepare in acetone at a concentration of 4 mg/mL each.

7.10.1 Place 400 ± 4 mg stearic acid and 400 ± 4 mg hexadecane in a 100-mL volumetric flask and fill to the mark with acetone.

Note: *The solution may require warming for complete dissolution of stearic acid.*

7.10.2 After the hexadecane and stearic acid have dissolved, transfer the solution to a 100–150 mL vial with PTFE-lined cap. Mark the solution level on the vial and store in the dark at room temperature.

7.10.3 Immediately prior to use, verify the level on the vial and bring to volume with acetone, if required. Warm to redissolve all visible precipitate.

Note: *If there is doubt of the concentration, remove 5.00 ± 0.05 mL with a volumetric pipet, place in a tared weighing pan, and evaporate to dryness in a fume hood. The weight must be 40 ± 1 mg.*

7.11 Precision and recovery (PAR) standard: Spike 5.00 ± 0.05 mL of the hexadecane/stearic acid spiking solution (Section 7.10) into 950–1050 mL of reagent water to produce concentrations of approximately 20 mg/L each of hexadecane and stearic acid. The PAR standard is used for the determination of initial precision and recovery (Section 9.2.2) and ongoing precision and recovery (Section 9.6).

7.12 The spiking solutions should be checked frequently for signs of degradation or evaporation and must be replaced after six months, or sooner if degradation has occurred.

8.0 Sample Collection, Preservation, and Storage

8.1 Collect approximately one liter of representative sample in a glass bottle following conventional sampling practices (Reference 16.7), except that the bottle must not be pre-rinsed with sample before collection.

8.1.1 If analysis is to be delayed for more than a few hours, preserve the sample by adding 5 mL of HCl or H_2SO_4 solution (Section 7.2) at the time of collection, and refrigerate at 0–4°C (40 *CFR* 136, Table II).

8.1.2 If a sample is known or suspected to contain greater than 1000 mg/L of extractable material, collect a proportionately smaller volume of sample (the volume required will depend upon the estimated amount of extractable material) in a glass bottle. Add a proportionately smaller amount of HCl or H_2SO_4 solution to the smaller sample if preservation is necessary.

8.2 Collect an additional two aliquots (1 L, additional smaller volume, or both) of a sample for each set of ten samples or less for the matrix spike and matrix spike duplicate.

Note: *For those circumstances requiring the collection of multiple aliquots of one sample, each aliquot is to be collected in either of the following ways: 1) collect the total volume needed in one container, homogenize by mixing with a stir bar until a vortex forms, and transfer to required aliquots, 2) collect simultaneously in parallel, if possible, or 3) collect as grab samples in rapid succession.*

8.3 The high probability that extractable matter may adhere to sampling equipment and result in measurements that are biased low precludes the collection of composite samples for determina-

tion of oil and grease. Therefore, samples must be collected as grab samples. If a composite measurement is required, individual grab samples collected at prescribed time intervals must be analyzed separately and the concentrations averaged.

8.4 All samples must be refrigerated at 0–4°C from the time of collection until extraction (40 *CFR* 136, Table II).

8.5 All samples must be analyzed within 28 days of the date and time of collection (40 *CFR* 136, Table II).

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 16.8). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analyses of standards and blanks as a test of continued performance, and analyses of matrix spike (MS) and matrix spike duplicate (MSD) samples to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the analyst is permitted certain options to improve separations or lower the costs of measurements, provided that all performance specifications are met. These options include alternate extraction and concentration devices and procedures such as solid-phase extraction and Kuderna-Danish concentration. Alternate determinative techniques, such as infra-red spectroscopy or immuno-assay, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for HEM and/or SGT-HEM in the sample of interest.

9.1.2.1 Each time a modification is made to this method, the analyst is required to repeat the IPR test in Section 9.2.2 to demonstrate that the modification produces results equivalent to or better than results produced by this method. If the detection limit of the method will be affected by the modification, the analyst must demonstrate that the MDL (40 *CFR* 136, Appendix B) is less than or equal to the MDL in this method or one-third the regulatory compliance level, whichever is higher. If the modified method is to be used for compliance monitoring, the discharger must also demonstrate that the modified method recovers at least as much HEM and/or SGT-HEM as this method on each specific discharge. The tests required for this equivalency demonstration are given in Sections 9.1.2.1.1-9.1.2.1.3.

9.1.2.1.1 Collect, extract, concentrate, and weigh the HEM or SGT-HEM in two sets of four aliquots of unspiked wastewater. One set of four wastewater aliquots is analyzed according to the protocol in Section 11 of

this method and the other set of four aliquots is analyzed using the modified method.

9.1.2.1.2 Compute the average percent recovery of HEM and SGT-HEM for the set of results from this method and for the set of results from the modified method. The average percent recovery using the modified method must be equal to or greater than 79 percent of the average percent recovery produced by this method for HEM and 66 percent of the average percent recovery produced by this method for SGT-HEM. If not, the modified method may not be used.

Note: *If the average concentration of the four results produced using this method is below the Minimum Level (Section 1.6), proceed as follows:*

9.1.2.1.3 Extract and concentrate a sufficient amount of wastewater to produce a minimum of 160 mg of HEM or SGT-HEM. Dissolve this material in acetone and spike 20 - 50 mg of the material into each of eight aliquots of wastewater. Use these eight aliquots for the equivalency test (Sections 9.1.2.1.1-9.1.2.1.2).

Note: *If more than 10 liters of wastewater must be extracted to produce 160 mg of HEM or SGT-HEM, and if the equivalency test of the modified method is passed for spikes of reference standards into reagent waster (Section 9.2.2), the modified method is deemed to be equivalent to this method for determining HEM and or SGT-HEM on that specific discharge.*

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A listing of pollutant(s) measured (HEM and/or SGT-HEM).

9.1.2.2.3 A narrative stating reason(s) for the modification.

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration (Section 10).
(b) Calibration verification (Section 9.5).
(c) Initial precision and recovery (Section 9.2.2).
(d) Analysis of blanks (Section 9.4).
(e) Accuracy assessment (Section 9.3).
(f) Ongoing precision and recovery (Section 9.6).

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (weight or other signal) to the final result. These data are to include:

(a) Sample numbers and other identifiers.
(b) Extraction dates.
(c) Analysis dates and times.
(d) Analysis sequence/run chronology.
(e) Sample weight or volume (Section 11.1.4).
(f) Extract volume for SGT-HEM (Section 11.5.2).
(g) Make and model of analytical balance and weights traceable to NIST.
(h) Copies of logbooks, printer tapes, and other recordings of raw data.
(i) Data system outputs, and other data to link the raw data to the results reported.

9.1.3 Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of laboratory blanks are required to demonstrate freedom from contamination. The procedure and criteria for analysis of a blank are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control. These procedures are described in Sections 9.5 and 9.6, respectively.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.7 and 9.6.3.

9.1.7 Accompanying QC for the determination of HEM and/or SGT-HEM is required per analytical batch. An analytical batch is a set of samples extracted at the same time, to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory blank (Section 9.4), an ongoing precision and recovery sample (OPR, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 blank, 1 OPR, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 blank, 1 OPR, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are to be extracted at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial demonstration of laboratory capability.

9.2.1 Method Detection Limit (MDL)—To establish the ability to detect HEM and SGT-HEM, the analyst shall determine the MDL per the procedure in 40 *CFR* 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL in Section 1.6 must be achieved prior to the practice of this method.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Extract and evaporate four samples of the PAR standard (Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X) and the standard deviation of the percent recovery (s) for HEM and for SGT-HEM (if determined). Use the following equation for calculation of the standard deviation of the percent recovery:

Equation 1

$$s = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n - 1}}$$

where:
n = Number of samples
x = Concentration in each sample

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 1. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the precision limit or X falls outside the range for recovery, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3 Matrix spikes—The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site. The two sample aliquots shall be spiked with the hexadecane/stearic acid spiking solution (Section 7.10).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of HEM or SGT-HEM in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of HEM or SGT-HEM in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard (Section 7.11) or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2 Analyze one sample aliquot out of each set of ten samples from each site according to the procedure beginning in Section 11 to determine the background concentration (B) of HEM or SGT-HEM.

9.3.2.1 If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (P) of HEM or SGT-HEM in each aliquot using the following equation:

Equation 2

$$P = \frac{100\ (A - B)}{T}$$

where:
A = *Measured concentration of analyte after spiking*
B = *Measured background concentration of HEM or SGT-HEM*
T = *True concentration of the spike* (40 *mg/L*)

When determining SGT-HEM, the true concentration (T) must be divided by 2 to reflect the concentration of hexadecane that remains after removal of stearic acid (20 mg/L).

9.3.4 Compare the percent recovery of the HEM or SGT-HEM with the corresponding QC acceptance criteria in Table 1.

9.3.4.1 If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria in Table 1, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.4.2 If the results of both the spike and the ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample reanalyzed.

9.3.5 Compute the relative percent difference (RPD) between the two results (not between the two recoveries) using the following equation:

Equation 3

$$RPD = \frac{|\ D_1 - D_2\ |}{(D_1 + D_2)/2} \times 100$$

where:
D_1 = *Concentration of HEM or SGT-HEM in the sample*
D_2 = *Concentration of HEM or SGT-HEM in the second (duplicate) sample*

9.3.6 The relative percent difference for duplicates shall meet the acceptance criteria in Table 1. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected, and the analytical batch reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P_a - 2s_p$ to $P_a + 2s_p$. For example, if $P_a = 90\%$ and $s_p = 10\%$ for five analyses of HEM or SGT-HEM, the accuracy interval is expressed as 70–110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

9.4 Laboratory blanks—Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Extract and concentrate a laboratory reagent water blank initially (i.e. with the tests in Section 9.2) and with each analytical batch. The blank must be subjected to the exact same procedural steps as a sample.

9.4.2 If material is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination.

9.5 Calibration verification—Verify calibration of the balance per Section 10 before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 blank, 1 OPR, 1 MS, and 1 MSD.) If calibration is not verified after the measurements, recalibrate the balance and reweigh the batch.

9.6 Ongoing precision and recovery—To demonstrate that the analysis system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the analyst shall perform the following operations:

9.6.1 Extract and concentrate a precision and recovery standard (Section 7.11) with each analytical batch according to the procedure beginning in Section 11.

9.6.2 Compare the concentration with the limits for ongoing precision and recovery in Table 1. If the concentration is in the range specified, the extraction, evaporation, and weighing processes are in control and analysis of blanks and samples may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. In this event, correct the problem, re-extract the analytical batch, and repeat the ongoing precision and recovery test.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery (s_r). Express the accuracy as a recovery interval

from R – $2s_r$ to R + $2s_r$. For example, if R = 95% and s_r = 5%, the accuracy is 85% to 105%.

9.7 Quality control sample (QCS)—it is suggested that the laboratory obtain a quality control sample from a source different from the source for the hexadecane and stearic acid used routinely in this method (Sections 7.8 and 7.9), and that the QCS be used for verification of the concentrations of HEM and SGT-HEM using the procedure given in the note in Section 7.10.3.

9.8 The specifications contained in this method can be met if the apparatus used is scrupulously cleaned and dedicated for the determination of HEM and SGT-HEM. The standards used for initial precision and recovery (IPR, Section 9.2.2), matrix spikes (MS/MSD, Section 9.3), and ongoing precision and recovery (OPR, Section 9.6) should be identical, so that the most precise results will be obtained.

9.9 Depending upon specific program requirements, field replicates and field spikes into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10.0 Calibration and Standardization

10.1 Calibrate the analytical balance at 10 mg and 1000 mg using class "S" weights.

10.2 Calibration shall be within ± 10% (i.e. 0.5 mg) at 5 mg and ± 0.5% (i.e. 5 mg) at 1000 mg. If values are not within these limits, recalibrate the balance.

11.0 Procedure

This method is entirely empirical. Precise and accurate results can be obtained only by strict adherence to all details.

Note: *The procedure below is based on the preparation, extraction, and analysis of a 1 L sample volume. If a smaller volume of sample is collected for analysis, the laboratory may need to adjust the size of the labware used in order to compensate for the smaller volume being processed. Commensurately smaller volumes of reagents (i.e. HCl or H_2SO_4, n-hexane, and sodium sulfate) may be used.*

11.1 Preparation of the analytical batch.

11.1.1 Bring the analytical batch of samples, including the sample aliquots for the MS and MSD, to room temperature.

11.1.2 Place approximately 1000 mL (950–1050 mL) of reagent water (Section 7.1) in a clean sample bottle to serve as the laboratory blank.

11.1.3 Prepare the OPR (Section 9.6) using the PAR standard (Section 7.11).

11.1.4 Either mark the sample bottles at the water meniscus or weigh the bottles for later determination of sample volume. Weighing will be more accurate. Mark or weigh the MS and MSD.

11.2 pH verification.

11.2.1 Verify that the pH of the sample is <2 using the following procedure:

11.2.1.1 Dip a glass stirring rod into the well mixed sample.

11.2.1.2 Withdraw the stirring rod and allow a drop of the sample to fall on or touch the pH paper.

Note: *Do not dip the pH paper into the bottle or touch it to the sample on the lid.*

11.2.1.3 Rinse the stirring rod with a small portion of n-hexane that will be used for extraction (to ensure that no extractable material is lost on the stirring rod). Collect the rinsate in the separatory funnel to be used for sample extraction.

11.2.2 If the sample is at neutral pH, add 4-6 mL HCl or H_2SO_4 solution (Section 7.2) to the 1 liter sample. If the sample is at high pH, use a proportionately larger amount of HCl or H_2SO_4 solution. If a smaller sample volume was collected, use a proportionately smaller amount of HCl or H_2SO_4 solution.

11.2.3 Replace the cap and shake the bottle to mix thoroughly. Check the pH of the sample using the procedure in Section 11.2.1. If necessary, add more acid to the sample and retest.

11.2.4 Add the appropriate amount of HCl solution to the blank, OPR, MS, and MSD.

11.3 Extraction

11.3.1 Tare a boiling flask containing 3–5 boiling chips as follows:

11.3.1.1 Place the flask containing the chips in an oven at 105–115°C for a minimum of 2 h to dry the flask and chips.

11.3.1.2 Remove from the oven and immediately transfer to cool in a desiccator.

11.3.1.3 When cool, remove from the desiccator and weigh immediately on a calibrated balance (Section 10).

11.3.2 Pour the sample into the separatory funnel.

11.3.3 Add 30 mL n-hexane to the sample bottle and seal the bottle with the original bottle cap. Shake the bottle to rinse all interior surfaces of the bottle, including the lid of the bottle cap. Pour the solvent into the separatory funnel.

11.3.4 Extract the sample by shaking the separatory funnel vigorously for 2 minutes with periodic venting into a hood to release excess pressure.

11.3.5 Allow the organic phase to separate from the aqueous phase for a minimum of 10 minutes. If an emulsion forms between the phases and the emulsion is greater than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, use of solvent phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical

methods. Alternatively, solid-phase or other extraction techniques may be used to prevent emulsion formation, provided that the requirements in Section 9.1.2 are met.

11.3.6 Drain the aqueous layer (lower layer) into the original sample container. Drain a small amount of the organic layer into the sample container to minimize the amount of water remaining in the separatory funnel.

Note: *The amount of water remaining with the n-hexane must be minimized to prevent dissolution or clumping of the sodium sulfate in the solution drying process.*

11.3.7 Place approximately 10 g anhydrous Na_2SO_4 in a filter funnel and rinse with a small portion of n-hexane. Discard the rinsate.

Note: *The specific properties of a sample may necessitate the use of larger amounts of* Na_2SO_4.

11.3.8 Drain the n-hexane layer (upper layer) from the separatory funnel through the Na_2SO_4 into the preweighed boiling flask containing the boiling chips (Section 11.3.1.3).

11.3.9 Repeat the extraction (Sections 11.3.3–11.3.6 and 11.3.8) twice more with fresh 30-mL portions of n-hexane, combining the extracts in the boiling flask.

11.3.10 Rinse the tip of the separatory funnel, the filter paper, and the funnel with 2–3 small (3–5 mL) portions of n-hexane. Collect the rinsings in the flask.

11.3.11 A milky extract indicates the presence of water. If the extract is milky, allow the solution to settle for up to one hour to allow the water to sink to the bottom. Decant the solvent layer (upper layer) through sodium sulfate to remove any excess water as in Sections 11.3.7–11.3.8.

11.3.12 If SGT-HEM only is to be determined, proceed to Section 11.5.

11.4 Solvent evaporation.

11.4.1 Connect the boiling flask to the distilling head apparatus and evaporate the solvent by immersing the lower half of the flask in a water bath or a steam bath. Adjust the water temperature as required to complete the concentration in less than 30 minutes. Collect the solvent for reuse.

11.4.2 When the temperature in the distilling head reaches 70°C or the flask appears almost dry, remove the distilling head. Sweep out the flask for 15 seconds with air to remove solvent vapor by inserting a glass tube connected to a vacuum source. Immediately remove the flask from the heat source and wipe the outside surface dry to remove excess moisture and fingerprints.

Note: *The analyst should carefully monitor the flask during the final evaporation stages to assure that all of the solvent is removed and at the same time to prevent loss of the more volatile sample constituents.*

11.4.3 Inspect the residue in the boiling flask for crystals. Crystal formation is an indication that sodium sulfate may have dissolved and passed into the tared boiling flask. This may happen when the drying capacity of the sodium sulfate is exceeded or if the sample was not adjusted to low pH. If crystals are observed, redissolve the extract in n-hexane, filter into another tared boiling flask, and repeat the evaporation procedure (Sections 11.4.1–11.4.2).

11.4.4 Cool the boiling flask in a desiccator for at least 30 minutes and determine the weight of the material in the flask.

11.4.4.1 If the extract was from the HEM procedure, determine the HEM (W_h) by subtracting the tare weight (Section 11.3.1) from the total weight of the flask.

11.4.4.2 If the extract was from the SGT-HEM procedure (Section 11.5.5), determine the weight of SGT-HEM (W_s) by subtracting the tare weight from the total weight of the flask.

11.4.5 Determine the original sample volume (V_s) in liters by filling the sample bottle to the mark with water and measuring the volume of water in a 1 to 2 L graduated cylinder. If the sample weight was used (Section 11.1.4), weigh the empty bottle and cap and determine V_s by difference, assuming a sample density of 1.00.

11.5 SGT-HEM determination.

11.5.1 Silica gel capacity—To ensure that the capacity of the silica gel will not be exceeded, the amount of HEM must be known.

11.5.1.1 If it is known that the amount of HEM is less than 100 mg, the analyst may proceed with the determination of SGT-HEM per Sections 11.5.3–11.5.5 without determination of HEM.

11.5.1.2 If, however, the amount of HEM is not known, HEM must first be determined using the procedure in Sections 11.3–11.4.

11.5.2 Extractable materials in silica gel—Because the capacity of silica gel is not known for all substances, it is presumed that 3 g will adsorb 100 mg of all adsorbable materials. The amount of silica gel that can be used for adsorption in the SGT-HEM procedure below has been limited to 30 g because of concerns about possible extractable impurities in the silica gel. Therefore, if the extract contains more than 1000 mg of HEM, split the extract per the following procedure:

11.5.2.1 Add 85–90 mL of n-hexane to the boiling flask to redissolve the HEM. If necessary, heat the solution on an explosion-proof hotplate or in a water bath to completely redissolve the HEM.

11.5.2.2 Transfer the extract to a 100-mL volumetric flask. Rinse the boiling flask sequentially with 2–3 small portions of n-hexane and add to the volumetric flask. Dilute to the mark with n-hexane.

11.5.2.3 Calculate the extract volume that contains 1000 mg of extractable material according to the following equation:

Equation 4

$$V_a = \frac{1000\ V_t}{W_h}$$

where:
V_a = *Volume of aliquot to be withdrawn (mL)*
V_t = *Total volume of solvent used in Section* 11.5.2.2 *(mL)*
W_h = *Weight of extractable material HEM measurement (mg)*

11.5.2.4 Using a calibrated pipet, remove the volume to be withdrawn (V_a) and return to the boiling flask. Dilute to approximately 100 mL with n-hexane.

11.5.3 Adsorption with silica gel

11.5.3.1 Add 3.0 ± 0.3 g of anhydrous silica gel (Section 7.7) to the boiling flask for every 100 mg of HEM, or fraction thereof, to a maximum of 30 g of silica gel. For example, if the weight of HEM is 735 mg, add 3 x 8 = 24 g of silica gel.

11.5.3.2 Add a PTFE-coated stirring bar to the flask and stir the solution on a magnetic stirrer for a minimum of 5 minutes.

11.5.4 Filter the solution through n-hexane moistened filter paper into a pre-dried, tared boiling flask containing several boiling chips. Rinse the silica gel and filter paper with several small amounts of n-hexane to complete the transfer.

11.5.5 Evaporate the solution and determine the weight of SGT-HEM per Section 11.4.

12.0 Data Analysis and Calculations

12.1 n-Hexane extractable material—Calculate the concentration of HEM ("oil and grease") in the sample per the following equation:

Equation 5

$$HEM\ (mg/L) = \frac{W_h\ (mg)}{V_s\ (L)}$$

where:
W_h = *Weight of extractable material Section* 11.4.4.1 *(mg)*
V_s = *Sample volume from Section* 11.4.5 *(L)*

12.2 Silica gel treated n-hexane extractable material—Calculate the concentration of SGT-HEM ("petroleum hydrocarbons") in the sample per the equation above, substituting W_s (from Section 11.4.4.2) for W_h. If the extract was split to decrease the total amount of material to 1,000 mg,

determine the corrected total weight of SGT-HEM in the un-split extract (W_c) using the following equation:

Equation 6

$$W_c\ (mg) = \frac{V_t}{V_a} W_d\ (mg)$$

where:
W_d = *Weight in the portion of the extract split for adsorption (Sections* 11.5.2.4 *and* 11.4.4.2)
V_t *and* V_a *are as defined in Equation* 3

Use the corrected total weight of SGT-HEM in the unsplit extract (W_c) to determine the total SGT-HEM in the sample by substituting W_c for W_h in Equation 5.

12.3 Reporting

12.3.1 Samples—Report results to three significant figures for HEM and SGT-HEM found above the Minimum Level (Section 1.6) in all samples. Do not report results below the Minimum Level.

12.3.2 Report results to three significant figures for HEM and SGT-HEM found above the Method Detection Limit (Section 1.6) in all blanks. Do not report results below the MDL.

13.0 Method Performance

This method was validated in 13 laboratories using spiked reagent water samples (References 16.1, 16.2, and 16.9).

14.0 Pollution Prevention

14.1 The solvents used in this method pose little threat to the environment when recycled and managed properly.

14.2 Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

15.0 Waste Management

15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Samples preserved with HCl or H_2SO_4 to pH < 2 are hazardous and must be neutralized before being disposed, or must be handled as hazardous waste.

15.3 For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel", and "Less is Better: Laboratory Chemical Management for Waste Reduction", both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

16.0 References

16.1 "Determination of the Method Detection Limit and Minimum Level for EPA Method 1664 by Global Environmental Services", October 1994. Available from the Sample Control Center (operated by DynCorp Viar), 300 North Lee Street, Alexandria, VA 22314, (703) 519-1140.

16.2 "Summary Results from Phase II of the Freon Replacement Study for work performed by Commonwealth Technology, Inc. under Special Analytical Services Contract 1273", January 1, 1994. Available from the Sample Control Center (operated by DynCorp Viar), 300 N. Lee St., Alexandria, VA 22314, (703) 519-1140.

16.3 40 *CFR* 136, Appendix A, Methods 1624 and 1625.

16.4 "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

16.5 "OSHA Safety and Health Standards, General Industry," (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

16.6 "Safety in Academic Chemistry Laboratories," American Chemical Society, Committee on Chemical Safety, 3rd Edition, 1979.

16.7 "Standard Practices for Sampling Water," ASTM Annual Book of Standards, Part 31, D3370-76, American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

16.8 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-Ci, Cincinnati, OH 45268, EPA-600/4-79-019, March 1979.

16.9 "Results of the Twin City Round Robin Group Interlaboratory Study of Method 1664 HEM analysis", October 1994. Available from the Sample Control Center (operated by DynCorp Viar), 300 N. Lee Street, Alexandria, VA 22314, (703) 519-1140.

17.0 Tables

Table 1. Acceptance Criteria for Performance Tests

Acceptance Criterion	Section	Limit
Initial precision and recovery	9.2.2	
HEM Precision (s)	9.2.2.2	10 percent
HEM Recovery (X)	9.2.2.2	83–101 percent
SGT-HEM Precision (s)	9.2.2.2	13 percent
SGT-HEM Recovery (X)	9.2.2.2	83-116 percent
Matrix spike/matrix spike duplicate	9.3	
HEM Recovery	9.3.4	79–114 percent
HEM RPD	9.3.5	18 percent
SGT-HEM Recovery	9.3.4	66-114 percent
SGT-HEM RPD	9.3.5	24 percent
Ongoing precision and recovery	9.6	
HEM Recovery	9.6	79-114 percent
SGT-HEM Recovery	9.6	66–114 percent

18.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

18.1 Units of weight and measure and their abbreviations

18.1.1 Symbols

°C	degrees Celsius
<	less than
%	percent
±	plus or minus

18.1.2 Alphabetical characters

g	gram
h	hour
L	liter
mg	milligram
mg/g	milligram per gram
mg/L	milligram per liter
mg/mL	milligram per milliliter
mL	milliliter
No.	number
rpm	revolutions per minute

18.2 Definitions, acronyms, and abbreviations

18.2.1 Analyte: The HEM or SGT-HEM tested for by this method.

18.2.2 Analytical batch: The set of samples extracted at the same time, to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory blank (Section 9.4), an ongoing precision and recovery sample (OPR, Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 blank, 1 OPR, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 blank, 1 OPR, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are to be extracted at one time, the samples must be separated into analytical batches of 10 or fewer samples.

18.2.3 Field blank: An aliquot of reagent water that is placed in a sample container in the laboratory or in the field and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

18.2.4 HEM: See n-Hexane extractable material.

18.2.5 n-Hexane extractable material: The material that is extracted from a sample and determined by this method.

18.2.6 IPR: See initial precision and recovery.

18.2.7 Initial precision and recovery (IPR): Four aliquots of the diluted PAR analyzed to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

18.2.8 Laboratory blank (method blank): An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The laboratory blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

18.2.9 Laboratory control sample (LCS): See Ongoing precision and recovery standard (OPR).

18.2.10 Matrix spike (MS) and matrix spike duplicate (MSD): Aliquots of an environmental sample to which known quantities of the analytes are added in the laboratory. The MS and MSD are prepared and/or analyzed exactly like a field sample. Their purpose is to quantify any additional bias and imprecision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations.

18.2.11 May: This action, activity, or procedural step is neither required nor prohibited.

18.2.12 May not: This action, activity, or procedural step is prohibited.

18.2.13 Method Detection Limit: The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

18.2.14 Minimum Level (ML): The lowest level at which the entire analytical system gives a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed.

18.2.15 Must: This action, activity, or procedural step is required.

18.2.16 Ongoing precision and recovery standard (OPR, also called a laboratory control sample): A laboratory blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and accuracy.

18.2.17 OPR: See Ongoing precision and recovery standard.

18.2.18 PAR: See Precision and recovery standard.

18.2.19 Precision and recovery standard: Secondary standard that is diluted and spiked to form the IPR and OPR.

18.2.20 Quality control sample (QCS): A sample containing analytes of interest at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

18.2.21 Reagent water: Water demonstrated to be free from HEM and SGT-HEM and potentially interfering substances at or above the Minimum Level of this method.

18.2.22 SGT-HEM: See Silica gel treated n-hexane extractable material.

18.2.23 Should: This action, activity, or procedural step is suggested but not required.

18.2.24 Silica gel treated n-hexane extractable material: n-Hexane extractable material (HEM) that is not adsorbed by silica gel.

18.2.25 Stock solution: A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.