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Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry

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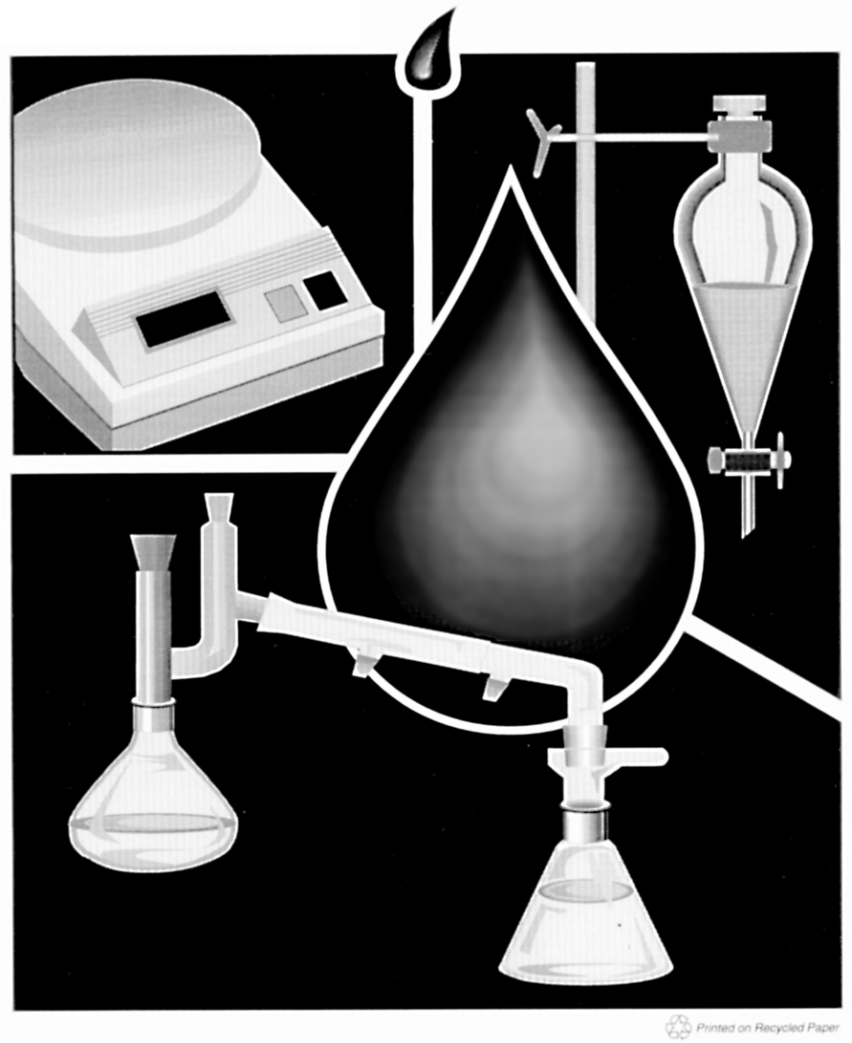

U.S. Environmental Protection Agency
Office of Water (4303T)
1200 Pennsylvania Avenue, NW
Washington, DC 20460

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Disclaimer

This method has been reviewed by the Engineering and Analytical Support Branch of the Engineering and Analysis Division (EAD) in OST and approved for publication. Mention of trade names or commercial products does not constitute and endorsement or recommendation for use.

Contact

Questions concerning this method or its application should be directed to:

CWA Methods Team, or Lemuel Walker
U.S. EPA
Engineering and Analysis Division, MC 4303T
Office of Science and Technology
1200 Pennsylvania Avenue NW
Washington, D.C. 20460
OSTCWAMethods@epa.gov
Walker.Lemuel@epa.gov

Additional copies of this method are available at: http://www.epa.gov/waterscience/methods/

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Introduction

EPA withdrew all oil and grease methods using chlorofluorocarbon-113 (CFC-113; Freon-113) as an extraction solvent in the final rule published March 7, 2007; 72 FR 11199, including approved Method 413.1, and Methods 418.1 and 418.2, which were never approved for use at 40 CFR 136 even though they were listed in the now outdated Methods for Chemical Analysis of Water and Wastes (EPA 600/4-79-020; NTIS PB84-128677). As such, EPA Method 1664, Revision B: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) is the only approved EPA method for the measurement of oil and grease.

The term "n-Hexane Extractable Material (HEM; Oil and Grease)" reflects the material extracted by n-hexane as that which is being measured using Method 1664 and that the common name "oil and grease" is being retained because of its familiarity to the analytical community. The term "Silica-gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material)" designates the substances that remain after n-hexane extractable material is exposed to silica gel. In the final rule published May 14, 1999 (64 FR 26315) the term "Non-polar Material" (NPM) was chosen to avoid confusion in the analytical community with the term "total petroleum hydrocarbons" (TPH), as this term can measure a different property or material, or employ different analytical techniques, including infrared spectroscopy and gas chromatography.

Method 1664B is a performance-based method applicable to the measurement of the method-defined parameters HEM and SGT-HEM from aqueous matrices and requires the use of n-hexane as the extraction solvent, gravimetry as the determinative technique, and includes additional QC procedures designed to monitor precision and accuracy. Although analyst modification of an approved, method-defined analyte method is generally not allowed, some performance-based changes may be acceptable. However, it should be noted that due to the nature of some discharge/waste streams, not all samples may be amenable to analysis with a modified method, (e.g., SPE as the modification) and for these samples, 1664B should be applied as written.

The purpose of this update is to provide additional information to improve method performance and more clearly define by example:

1. Modifications that are allowed for nationwide use without prior EPA review (40 CFR 136.6).
2. Modifications that are not allowed.

The modifications listed below and included in Section 1.7 are not meant to be an exhaustive list of all possible modifications to the method, but rather they represent modifications which have been presented to the EPA by various stakeholders and our determination of their acceptability for use in Method 1664B.

In recognition of advances that are occurring in analytical technology, the laboratory 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 continuous liquid-liquid extraction (CLLE), evaporation, Kuderna-Danish concentration and solid-phase extraction (SPE). Alternate determinative techniques, such as immunoassay and infrared spectroscopy, and changes that degrade method performance or change the chemistry of the method including the use of extraction solvents other than n-hexane (85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L – see Section 7.3) 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. Specificity is defined as producing results equivalent to the results produced by this method for analytical standards (Section 9.2.2) and, where applicable, environmental samples (Section 9.2.3), and that meet all of the QC criteria stated in this method. 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.

Method 1664, Revision B
n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry

1.0 Scope and Application

1.1 This method is for determination of n-hexane extractable material (HEM; oil and grease) and n-hexane extractable material that is not adsorbed by silica gel (SGT-HEM; non-polar material) 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. The method is based on prior Environmental Protection Agency (EPA) methods for determination of "oil and grease" and "total petroleum hydrocarbons" (References 16.1 and 16.2).

1.2 This method is for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Clean Water Act; the Resource Conservation and Recovery Act; the Comprehensive Environmental Response, Compensation, and Liability Act; and other EPA regulatory programs. "Oil and grease" is a conventional pollutant under the Clean Water Act and codified at 40 CFR 401.16. The term "n-hexane extractable material" reflects that this method can be used to determine materials other than oils and greases. Similarly, the term "silica gel treated n-hexane extractable material" reflects that this method can be used to determine material that is not adsorbed by silica gel (non-polar material).

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 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 HEM and SGT-HEM in this method, the method detection limit (MDL) is 1.4 mg/L and the minimum level of quantitation (ML) is 5.0 mg/L (Reference 16.3).

1.7 The laboratory 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 Sections 9.1.2 and 9.2.3.
Some examples of allowed and unacceptable modifications include:

1.7.1 Allowable Modifications

1.7.1.1 Alternate extraction techniques including continuous liquid-liquid extraction, solid-phase extraction, solid-phase-heated solvent extraction, solid-phase-Soxhlet extraction, and others. Acceptable solid-phase extraction (SPE) products include: Xenosep filters for EPA Method 1664, part no. 24547 and 25390H or equivalent; UCT LLC Oil and Grease cartridge part no.

ECUNIOGXF, or equivalent; or other SPE products that meet all method performance criteria such as Horizon part no. 50-021-HT, Snip and Pour QC Standard or equivalent.

1.7.1.2 Alternate concentration techniques including Kuderna-Danish concentration and evaporation using aluminum pans, beakers and flasks as well as user defined temperatures and approximate concentration times.

1.7.1.3 The requirement to dry the boiling flask for 30-45 minutes in an oven maintained at 70 ± 2 °C at Section 11.4.2.2 may be completely omitted or replaced with a user-defined lower temperature and/or shorter drying time.

1.7.1.4 A lower PAR (Precision and Recovery) standard concentration such as 20 mg/L may be used to spike matrix samples provided the concentration of the spike is: (a) greater than the background concentration, (b) less than or equal to the regulatory compliance level and (c) all quality control requirements are achieved.

1.7.1.5 Collection and analysis of a smaller sample volume is permitted provided all the quality control requirements of Section 9 are met. If smaller sample volumes are used, all QC and initial and ongoing demonstration of performance samples must use the same volume as the smaller volume samples. To achieve a proportionately smaller matrix spike at the recommended OPR concentration of 40 mg/L, i.e., spike 0.1 mL of the 4 mg/mL PAR standard for every 10 mL of proportionately smaller sample volume or fraction thereof. To report the most accurate HEM and SGT-HEM values in mg/L, subtract the smaller volume method blank value from the smaller volume sample value provided the recovery value of the smaller volume matrix spike is equal to or greater than the MDL for the modified method. Multiply this proportionately smaller sample value by the appropriate dilution factor and express the final sample result in mg/L. For example, if 4.4 mg/100 mL was the result for the proportionately smaller sample and 0.6 mg/100mL was the result for the method blank, the reported concentration of the sample should be 38.0 mg/L ([4.4 mg/100 mL – 0.6 mg/100 mL] x 10 = 38.0 mg/L). Additionally, all samples must be associated with an uncontaminated, 1-L method blank before the results may be reported for regulatory compliance purposes.

1.7.1.6 Balances should be calibrated with an additional upper class "S" weight in order to bracket the expected final weighing value. In addition, the frequency of balance calibration per Section 9.5 may be modified to before and after each day provided all QC requirements are achieved and the laboratory accepts responsibility for their results.

1.7.1.7 All samples must be acidified and/or verified in the lab to pH<2 immediately prior to analysis. If analysis is to be delayed for more than four hours, adjust the sample in the field to pH<2 with HCl or H_2SO_4 solution (Section 7.2) at the time of collection and refrigerate to 0-6 °C (40 CFR 136, Table II). If the sample is to be shipped by commercial carrier, U.S. Department of Transportation regulations (see 49 CFR Part 172) limit the pH to a minimum

of 1.96 if HCl is used and 1.15 if H_2SO_4 is used. (See 40 CFR Part 136, Table II Footnote 3).

1.7.1.8 The use of optional prefilters, filtration aids, glass wool and/or other means including centrifugation and sedimentation to aid processing of specific samples is allowed per Section 11.3.5. As appropriate, samples acidified to pH<2 which have settled upon refrigerated storage may be analyzed "as is" instead of first mixing and then centrifuging the sample in order to achieve a similar, settled physical state prior to analysis.

1.7.1.9 If SPE filters are used in the analytical batch, they must all be of the same manufacturer brand and diameter. If different manufacturer's brand or different diameter SPE filters are used in different analytical batches or methods, each different SPE method must have its own quality control chart.

1.7.1.10 The laboratory may randomly choose at least one representative discharge/waste stream for the matrix spike for every batch of samples analyzed and must keep a running record of their selection. However, the laboratory may not use the same discharge/waste stream for the matrix spike as was used in the immediately previous batch unless the laboratory only analyzes one discharge/waste stream. If the same discharge/waste stream is selected, the laboratory should randomly select the matrix spike from discharges/waste streams not used for the immediately previous batch.

1.7.1.11 Use of repetitive treatments with silica gel is allowed, as well as using lower amounts, provided the minimum treatment ratio of 3 g silica gel for every 100 mg HEM is maintained, per Section 11.5.

1.7.1.12 The use of solvent phase separation paper or other equivalent means may be used instead of sodium sulfate to remove water from the extract, provided all QC requirements are met, especially Sections 9.3 and 9.4, matrix spike and laboratory blanks respectively.

1.7.1.13 The use of silica gel equivalent to that specified at Section 7.7 may be used, provided all QC performance criteria in this method are met.

1.7.1.14 The use of an optimized extract shaking time (Section 11.3.4) and/or an optimized number of n-hexane extractions (Section 11.3.9) is allowed, provided all QC performance criteria in this method are achieved.

1.7.1.15 The hexadecane and stearic acid reference standards may be stored in the dark in an explosion-proof refrigerator to help prevent acetone evaporation provided: 1) The room temperature volume level is clearly marked on the container and 2) The standard is allowed to re-equilibrate to room temperature before use.

1.7.1.16 Rinsing bottles or apparatus with a polar solvent for glassware cleaning (Section 6.1.2), or other purposes, is allowed, provided the solvent: 1) is immediately sent to waste, 2) is sufficiently removed from contact surfaces so it will not introduce the target analyte into the sample, 3) will not collocate with the extraction solvent n-hexane and 4) will not be collected into the

collection vessel. See Section 1.7.2.1 and the note below that section for additional SPE apparatus requirements.

1.7.1.17 Samples may be analyzed within a user-defined, controlled temperature range to improve extraction efficiency, provided all samples (including QC) are analyzed under similar conditions and all QC performance criteria in this method are achieved.

1.7.2 Unacceptable Modifications

1.7.2.1 The extraction solvent must be n-hexane (85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L – see Section 7.3). Alternate extraction solvents or co-solvents including methanol, acetone and others that react with or introduce the target pollutant into the sample are not allowed.

However, a methanol or other polar solvent rinse after sample filtration may be allowed to remove water residual when using SPE technology in a modified method provided:

1. The methanol rinse is immediately discarded to waste.
2. The SPE filter is sufficiently air dried with vacuum to remove any residual methanol remaining in the SPE filter to trace amounts so as to ensure residual methanol will not introduce the target analyte into the sample and at no time will residual methanol collocate with or be collected with the n-hexane extractions.
3. The laboratory must demonstrate and document the appropriate operating conditions (1 & 2) above to allow this use of methanol.

NOTE: The use of a polar solvent to condition an SPE filter or SPE device in a modified method prior to sample filtration is allowed. The use of methanol or another polar solvent after sample filtration to remove water residual may be allowed provided the polar solvent is immediately discarded to waste and the SPE filter or SPE device is sufficiently air-dried with vacuum to remove any residual polar solvent to trace amounts so at no time will residual polar solvent introduce the target analyte into the sample, collocate with or be collected with the extraction solvent, n-hexane. A simple test to determine sufficient residual polar solvent removal from an SPE filter by vacuum air drying would be to weigh and record the weight of a dry SPE filter to the nearest 0.1 mg. Then analyze a blank using the dry SPE filter, conditioning the SPE filter and filtering the blank sample per the modified SPE method. Immediately after discarding the polar solvent to waste (and prior to addition of the n-hexane extraction solvent), stop the vacuum, remove the SPE filter from the apparatus and weigh the wet SPE filter saturated with residual polar solvent to the nearest 0.1 mg. Record this wet SPE filter weight. Reassemble the wet SPE filter into the apparatus and continue vacuum air drying the SPE filter until the weight of the wet SPE filter is less than 101% of the dry SPE filter weight. For example, if the weight of the dry SPE filter is 1,000.0 mg, continue vacuum air drying the wet SPE filter until the final weight is less than or equal to 1,010.0 mg before contacting the SPE filter with the extraction solvent n-hexane. This simple test could be used by the laboratory to demonstrate and document removal of residual methanol from the SPE filter to trace amounts so as to ensure residual methanol will not introduce the target analyte into the sample and at no time will residual methanol collocate with the n-hexane extraction solvent or be collected in the collection flask.

1.7.2.2 Alternate determinative techniques such as infrared spectroscopy or immunoassay, and changes that degrade method performance are not allowed.

NOTE: EPA Methods 418.1 and 418.2, which employed CFC-113 as the extraction solvent and infrared spectroscopy and gravimetry respectively as the determinative technique, were never approved for use at 40 CFR Part 136, even though they were listed in Methods for Chemical Analysis of Water and Wastes (EPA 600/4-79-020; NTIS PB84-128677). In the final rule published March 12, 2007; 72 FR 11199, EPA withdrew approval of all oil and grease methods that employed chloroflurocarbon-113 (CFC-113, Freon-113) as the extraction solvent and gravimetry or infrared spectroscopy as the determinative technique.

1.7.2.3 Due to the non-homogeneous nature of wastewater samples and the high probability that extractable material may contact and adhere to a wide variety of surfaces (including substances in the sample as well as the sample bottle and cap) and be non-uniformly distributed throughout the sample, sub-sampling or analysis of less than the total collected volume of sample is not allowed.

1.7.2.4 A change in the n-hexadecane and stearic acid standards is not allowed because the performance data for this method were developed using these reference standards.

1.7.2.5 Spiking of the n-hexadecane and stearic acid standards into the extractor (separatory funnel, CLLE body, SPE reservoir, etc.) to potentially improve recovery of the matrix spike is not allowed, as acceptable recovery of the matrix spike from the sample container must be demonstrated per Section 9.3.2.2 (Reference 16.10)

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.

NOTE: Laboratories or dischargers in or regulated by EPA Region 8 may apply to Region 8 for a limited use ATP application to use modified Method 1664 "Cu" to address colloidal sulfur or thiosulfate-inflated results in produced water samples. In order to comply with the new regulation and be able to use modified 1664 "Cu," the laboratory or permittee should, at a minimum:

1. Perform Method 1664B and demonstrate falsely high, out of compliance results
2. Perform modified Method 1664 "Cu" and demonstrate results in compliance with permit levels
3. Document the interference and determine the reason for the out of compliance result is due to the presence of colloidal sulfur or thiosulfate in the specific effluent
4. Apply to Region 8 for approval of a limited use ATP application to use modified Method 1664 "Cu" instead of Method 1664B for the specific effluent.

Use of this modified method in other EPA Regions requires submission to the respective EPA Regional Office or appropriate regulatory authority of a limited-use ATP application for approval (40 CFR 136.4 and 136.5).

1.9 Each laboratory that uses this method, with or without the allowed modifications above, 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 (85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L – see Section 7.3) in a separatory funnel. The extract is dried over sodium sulfate.

2.2 The solvent is distilled from the extract and the HEM is desiccated and 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 polar materials. The solution is filtered to remove the silica gel, the solvent is distilled, and the SGT-HEM is desiccated and weighed.

2.4 Quality is assured through calibration and testing of the extraction, distillation, 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 and as such, the only extraction solvent allowable is n-hexane (85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L – see Section 7.3) with gravimetry as the determinative technique. The use of co-solvents, alternate solvents, or determinative techniques other than gravimetry, including infrared spectroscopy, immunoassay, and gas chromatography, is not allowed because the chemistry of the method is changed. 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 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. It is suggested that the laboratory perform personal hygiene monitoring of each analyst that uses this method. This monitoring should be performed using Occupational Safety and Health Administration (OSHA) or National Institute of Occupational Safety and Health (NIOSH) approved personal hygiene monitoring methods. Results of this monitoring should be made available to the analyst.

5.2 n-Hexane has been shown to have increased neurotoxic effects over other hexanes and some other solvents. OSHA has proposed a time-weighted average (TWA) of 50 parts-per-million (ppm); NIOSH concurs that an 8-hour TWA/permissible exposure limit (PEL) of 50 ppm is appropriate for n-hexane; and the American Conference of Governmental Industrial Hygienists (ACGIH) has published a threshold limit value (TLV) of 50 ppm for n-hexane. Inhalation of n-hexane should be minimized by performing all operations with n-hexane in an explosion-proof hood or well-ventilated area.

5.3 n-Hexane has a flash point of -23 °C (-9 °F), has explosive limits in air in the range of 1-7%, and poses a serious fire risk when heated or exposed to flame. n-Hexane can react vigorously with oxidizing materials. The laboratory should include procedures in its operations that address the safe handling of n-hexane.

5.4 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.

5.5 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 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

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NOTE: In those instances necessitating collection of a sample known or suspected to contain >500 mg/L of HEM (Section 8.1.2), or consisting of a complex matrix containing substances (such as particulates or detergents) that may interfere with the extraction procedure (Section 4.5), 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 ± 2 ºC in the range of 20–250 °C.

6.3 Equipment for calibration

6.3.1 Analytical Balance–Capable of weighing 0.1 mg.

6.3.2 Volumetric flask–Glass, 100-mL.

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

6.3.4 Volumetric pipette–Glass, 10-mL.

6.4 Equipment for sample extraction

6.4.1 Balance (optional)–Top loading, capable of weighing 500–2000 g within ± 1%

6.4.2 Glass stirring rod

6.4.3 Separatory funnel–Glass, 2000-mL, with PTFE stopcock

6.4.4 Funnel–Large, glass, for pouring sample into separatory funnel

6.4.5 Centrifuge (optional)–Explosion proof, capable of spinning at least four 100-mL glass centrifuge tubes at 2400 rpm minimum

6.4.6 Centrifuge tubes (optional)–100-mL glass

6.4.7 Wash bottle (optional)–Fluoropolymer construction for hexane rinses

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 distillation

6.6.1 Water bath or Steam bath–Explosion-proof, capable of maintaining a temperature of at least 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 distillate collection flask for recovery of solvent)

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

6.6.6 Ice bath or recirculating chiller (to aid in the condensation and collection of the distilled solvent)

6.6.7 Vacuum–Vacuum pump or other source of vacuum

6.6.8 Tongs, for handling the boiling flask (Humboldt Manufacturing No. H-23442, or equivalent)

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

6.6.10 Hood-Explosion-proof, capable of accommodating the equipment used for solvent distillation (Section 6.6.1–6.6.5)

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

7.1 Reagent water–Water in which HEM is not detected at or above the minimum level (ML) 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 acid or sulfuric acid–ACS. Mix equal volumes of concentrated HCl and reagent water or 1 part H_2SO_4 and 3 parts reagent water to produce an approximately 6N solution

7.3 n-Hexane–85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L (0.0001% max.)

7.4 Acetone–ACS, residue less than 1 mg/L (0.0001% max)

7.5 Sodium sulfate–ACS, granular anhydrous. Dry at 200-250 °C for 24 h minimum and store in a tightly sealed container until use

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NOTE: Powdered sodium sulfate should not be used because traces of water may cause it to solidify.

7.6 Boiling chips–Silicon carbide or fluoropolymer

7.7 Silica gel–Anhydrous, 75-150 µm, 30 Å pore size (Davisil Grade 923, 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 distilling 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 2 mg/mL each

7.10.1 Place 200 ± 2 mg stearic acid and 200 ± 2 mg hexadecane in a 100-mL volumetric flask and fill to the mark with acetone

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NOTE: The solution may require warming for complete dissolution of stearic acid. If warmed, allow the solution to cool back to the volume mark at room temperature before use as heat generated from vigorous shaking can increase the volume of the solution which can cause low recovery. Best results are obtained if the solution is allowed to cool for at least one hour after warming.

7.10.2 After the hexadecane and stearic acid have dissolved and the solution has cooled back to the volume mark at room temperature, transfer the solution to a 100–150 mL vial with fluoropolymer-lined cap (Kontes No. 482600-0100, or equivalent) and tighten well the 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 if needed but allow the solution to cool back to the volume mark at room temperature before use.

NOTE: If there is doubt of the concentration, remove 10.0 ± 0.1 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. If not, prepare a fresh solution (Section 7.10.1). Best results are obtained using large-diameter smooth-walled aluminum weighing pans (Xenosep # 26506 or equivalent).

7.11 Precision and recovery (PAR) standard - Using a pipet, spike 10.0 ± 0.1 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 (Section 9.2.2) and ongoing (Section 9.6) precision and recovery.

7.12 The spiking solutions should be checked frequently for signs of degradation or evaporation using the test noted in Section 7.10.3, 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. To allow for potential QC failures, it is recommended that additional sample aliquots be collected.

8.1.1 All samples must be acidified and/or verified in the lab to pH<2 immediately prior to analysis. If analysis is to be delayed for more than four hours, adjust the sample pH to less than 2 with HCl or H_2SO_4 solution (Section 7.2) at the time of collection, and refrigerate at 0–6°C (40 CFR 136, Table II). To establish the volume of HCl or H_2SO_4 required, collect a separate aliquot, adjust the pH of this aliquot to less than 2 with acid, and add the volume of acid determined to each sample bottle prior to collection. During the sample collection, do not dip pH paper, a pH electrode, a stirring rod, or other materials into a sample that will be used for HEM or SGT-HEM determination because substances in the sample may adhere to these items.

8.1.2 If a sample is known or suspected to contain greater than 500 mg/L of extractable material or consists of complex matrix containing substances (such as particulates or detergents) that may interfere with the extraction procedure, 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 for preservation as necessary.

8.2 Collect an additional one or two aliquots (1 L, additional smaller volume, or both) of a sample for each set of twenty samples or less for the matrix spike and, if used, the 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 simultaneously in parallel, if possible, or 2) 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 determination 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. Alternatively, samples can be collected in the field and composited in the laboratory. For example, collect four individual 250-mL samples over the course of a day. In the laboratory, pour each 250-mL sample into the separatory funnel, rinse each of the four bottles (and caps) sequentially with 30 mL of n-hexane, and use the 30 mL of n-hexane for the extraction (Section 11.3).

8.4 All samples must be refrigerated at 0–6 °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 analysis of a matrix spike (MS) to assess recovery. 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 laboratory 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 laboratory 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 continuous liquid-liquid extraction, evaporation, Kuderna-Danish concentration, and solid-phase extraction. Alternate determinative techniques, such as immunoassay or infrared spectroscopy, and changes that degrade method performance or change the chemistry of the method including the use of extraction solvents other than n-hexane (85% minimum purity, 99.0% min. saturated C_6 isomers, residue less than 1 mg/L – see Section 7.3) 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. Specificity is defined as producing results equivalent to the results produced by this method for analytical standards (Section 9.2.2) and, where applicable, environmental samples (Section 9.2.3), and that meet all of the QC criteria stated in this method.

9.1.2.1 Each time a modification is made to this method, the laboratory is required to repeat the IPR test in Section 9.2.2 to demonstrate that the modification produces results equivalent to or superior to results produced by this method. If the detection limit of the method will be affected by the modification, the laboratory must demonstrate that the MDL (40 CFR Part 136, Appendix B) is

less than or equal to the MDL in this method or one-third the regulatory compliance limit, whichever is higher. If the modified method is to be used for compliance monitoring, the discharger/generator must also demonstrate that the modified method recovers an amount of HEM and/or SGT-HEM equivalent to the amount recovered by this method on each specific discharge/waste stream (not required for modifications allowed under Section 1.7.1). The tests required for this equivalency demonstration are given in Section 9.2.3.

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 (not required for modifications allowed under Section 1.7.1).

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)
(g) Method detection limit (Section 9.2.1).

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 Analysis of a matrix spike (MS) is required to demonstrate recovery 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 (OPR) 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. For the definition of an analytical batch, see the glossary at the end of this method.

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 laboratory 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 or less than 1/3 the regulatory compliance limit 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 laboratory shall perform the following operations:

9.2.2.1 Determine the concentration of HEM and/or SGT-HEM in 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). 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. Use the following equation for calculation of the standard deviation of the percent recovery:

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

Equation 1

where:
n = Number of samples
x = % recovery 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.

9.2.3 Equivalency demonstration for application of a method modification to compliance monitoring - To establish the ability of a modification of this method to recover an amount of HEM and/or SGT-HEM equivalent to the amount recovered by this method from a specific discharge/waste stream (matrix types), proceed as follows:

NOTE: Equivalency demonstration for specific discharge/waste stream types is not required for the modifications listed as acceptable in Section 1.7.1. For modifications not listed as acceptable at Section 1.7.1, the demonstration is needed only for an example of the specific discharge/ waste stream (matrix type) to be analyzed up to a maximum of the 9 matrix types identified below which would be required to validate the modified method for use with all samples. If validating the modified method for use with all samples, each example waste stream below (except POTW effluent) should be known or suspected to contain an average concentration of 5 to 1,000 mg/L of HEM and/or SGT-HEM to be included in the demonstration:

1. One POTW effluent
2. A second POTW effluent from a different source
3. Saline water
4. Two representative examples of different treated or untreated wastewaters likely to contain petroleum-based substances (hydrocarbons similar in chemical composition to n-hexadecane) at significantly different concentrations
5. Two representative examples of different treated or untreated wastewaters likely to contain animal-based substances (animal fats similar in chemical composition to stearic acid) at significantly different concentrations
6. Two representative examples of different treated or untreated wastewaters likely to contain other hexane extractable materials of various chemical composition including but not limited to, vegetable oils, waxes, soaps, greases and other related materials

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9.2.3.1 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.

9.2.3.2 Calculate the average concentration 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 concentration using the modified method must be 78 to 114 percent of the average concentration produced by this method for HEM and 64 to 132 percent of the average concentration 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 and the average concentration of the four results produced using the modified method are below the minimum level (Section 1.6), and if the equivalency test of the modified method is passed for spikes of reference standards into reagent water (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/waste stream.

9.3 Matrix spikes–The laboratory must spike a minimum of 5 percent of all samples from a given sampling site or, if for compliance monitoring, from a given discharge/waste stream (matrix type). The sample aliquot shall be spiked with the hexadecane/stearic acid spiking solution (Section 7.10). A duplicate matrix spike (MSD) is recommended, but not required.

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, at 1 to 5 times the background concentration of the sample (determined in Section 9.3.2), or at the concentration of the OPR (Section 9.6), whichever concentration is highest.

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 20 samples from each site or discharge/waste stream 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).

NOTE: Samples containing high concentrations (> 100 mg/L) of HEM will require a large volume of spiking solution (Section 7.10) for the MS (and MSD). If the concentration of HEM is expected to exceed 1000 mg/L, smaller sample volumes should be collected for the background measurement and MS (and MSD) so that the amount of HEM plus the amount spiked does not exceed 1000 mg/L.

9.3.2.2 Spike the additional sample aliquot(s) with the spiking solution and analyze the aliquot(s) 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:

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

Equation 2

where:
A = Measured concentration of the analyte after spiking
B = Measured background concentration of HEM or SGT HEM
T = True concentration of the spiked sample

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.

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 or used for regulatory compliance purposes and the laboratory must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge/waste stream should be resampled. If the interference is attributable to a matrix problem, the laboratory must modify the method, repeat the tests required in Section 9.1.2, and repeat the analysis of the sample and the MS (and MSD, if performed). Most matrix interference problems are attributable to the formation of emulsions in the extraction. Section 11.3.5 provides suggestions for overcoming emulsion problems.

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 batch reanalyzed. All samples must be associated with a valid MS (and MSD, if performed).

9.3.5 If an MSD was analyzed, compute the relative percent difference (RPD) between the MS and MSD (not between the two recoveries) using the following equation:

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

Equation 3

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, it is suggested that method precision and accuracy for samples be assessed and records 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 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. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

9.5 Calibration verification–Verify calibration of the balance per Section 10 before and after each analytical batch. If calibration is not verified before and after each day or after measurement of the analytical batch, 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 laboratory 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 recovery with the limits for ongoing precision and recovery in Table 1. If the recovery is in the range specified, the extraction, distillation, and weighing processes are in control and analysis of blanks and samples may proceed. If, however, the recovery 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 the 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 QCS 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. The QCS should be analyzed monthly by laboratories performing routine analyses and less frequently by laboratories performing these analyses intermittently.

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 spike (MS, 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 of the analytes of interest 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 2 mg and 1000 mg, using class "S" or ASTM E 617-1997 Class 1 weights. It is recommended that the balance should also be calibrated with an additional class "S" or ASTM E 617-1997 Class 1 weight that will bracket the final expected weighing value.

10.2 Calibration shall be within ± 10% (i.e. ± 0.2 mg) at 2 mg, ± 0.5% (i.e., ± 5 mg) at 1000 mg, and if applicable, at the appropriate user-specified tolerance for Class 1 weights greater than 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. If a smaller volume is collected for analysis, the laboratory should dilute the sample to 1 L with reagent water so that results across the IPR, blank, OPR, MS, and, if performed, the MSD, are consistent. It is also important that all glassware surfaces be rinsed with n-hexane to effect a quantitative transfer of the constituents in the sample and of the hexadecane/stearic acid in the IPR, OPR, MS, and, if performed, the MSD.

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 bottle at the water meniscus or weigh the bottle 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 less than 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 5-6 mL of HCl or H_2SO_4 solution (Section 7.2) to the 1-L 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 or H_2SO_4 solution to the blank, OPR, MS (and MSD) to adjust the pH of these solutions to <2.

NOTE: The procedure detailed below is for separatory funnel liquid-liquid extraction. Solid-phase extraction (SPE) may be used at the discretion of the discharger/generator and its laboratory. However, if SPE is used, it is the responsibility of the discharger/generator and laboratory to assure that results produced are equivalent to results produced by the procedure below. However, it should be noted that due to the nature of some discharge/waste streams, not all samples may be amenable to analysis with a modified method (e.g., SPE as the modification) and for these samples, 1664B should be applied as written.

11.3 Extraction

11.3.1 Tare a clean 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 a desiccator to cool to room temperature.

11.3.1.3 When cool, remove from the desiccator with tongs 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 of 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 laboratory must employ emulsion-breaking 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 extraction (SPE), continuous liquid-liquid extraction, 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 extract drying process.

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

11.3.7 Place a filter paper (Section 6.5.2) in a filter funnel (Section 6.5.1), add approximately 10 g of anhydrous Na_2SO_4, and rinse with a small portion of n-hexane. Discard the rinsate.

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

NOTE: It is important that water be removed in this step. Water allowed to filter through the Na_2SO_4 will dissolve some of the Na_2SO_4 and carry it into the boiling flask compromising the determination.

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.

NOTE: For samples that are expected to contain a high concentration of salt (e.g., waters from oil production facilities), it may be prudent to collect the extract in a 250-mL separatory funnel and back-extract with reagent water. After back-extraction, the extract should be drained through Na_2SO_4 to remove all traces of water.

11.3.11 A milky extract indicates the presence of water. If the extract is milky, allow the solution to stand for up to one hour to allow the water to settle. Decant the solvent layer (upper layer) through sodium sulfate to remove any excess water as in Sections 11.3.7 and 11.3.8. Rinse the glassware and sodium sulfate with small portions of n-hexane to effect a quantitative transfer.

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

11.4 Solvent distillation

11.4.1 Connect the boiling flask to the distilling head apparatus and distill the solvent by immersing the lower half of the flask in a water bath or a steam bath. Adjust the water temperature as appropriate to complete the concentration. The solvent may be collected for reuse provided the laboratory can demonstrate the solvent meets or exceeds the purity specifications set forth in Section 7.3.

11.4.2 When the temperature in the distilling head reaches approximately 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.

11.4.2.1 Using tongs, immediately remove the flask from the heat source while it still contains approximately 2 mL of residual liquid. Wipe the outside surface dry to remove moisture and fingerprints and place the flask in a hood until visible dryness is achieved while cooling at room temperature.

11.4.2.2 If desired, the flask may be placed in an oven capable of maintaining a user defined temperature within ± 2 °C (up to 70 °C max.) for a user defined period of time (up to 45 minutes max.).

NOTE: The laboratory should carefully monitor the flask during the final stages of distillation to assure that all of the solvent is removed and to prevent loss of the more volatile sample constituents. Best results are achieved when the flask is removed from the heat source while it still contains a few mL of residual liquid and visible dryness is achieved while cooling the flask at room temperature.

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 boiling flask. This may happen if the drying capacity of the sodium sulfate is exceeded or if the sample is not adjusted to low pH. If crystals are observed, redissolve the extract in n-hexane, quantitatively transfer through a filter into another tared boiling flask, and repeat the distillation procedure (Sections 11.4.1–11.4.2).

11.4.4 Continue to dry and cool the flask to room temperature in a desiccator for 30 minutes minimum. Remove with tongs and weigh immediately. While at room temperature and without additional heating, repeat the cycle of desiccating and weighing until the weight loss of the flask and dried residue is less than 4 % of the previous weight or less than 0.5 mg, whichever is less. The final weighing should be used for determining the value for HEM or SGT-HEM as appropriate.

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 less than 100 mg or, if above 100 mg, must be known.

11.5.1.1 If it is known that the amount of HEM is less than 100 mg, the laboratory 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 normally adsorb 100 mg of all adsorbable materials. Therefore, for samples containing 1000 mg HEM, 30 g of silica gel will be needed. 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. If the amount of HEM in the sample is greater than 1000 mg, 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, warm the solution to completely redissolve the HEM.

11.5.2.2 Quantitatively transfer the extract to a 100-mL 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:

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

Equation 4

where:
V_a = *Volume of the 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 from the 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 fluoropolymer-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 Distill 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:

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

Equation 5

where:
W_h = *Weight of extractable material from 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 ("non-polar material") 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 unsplit extract (W_c) using the following equation:

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

Equation 6

where:
W_c = *Corrected total weight of SGT-HEM in the unsplit extract*
W_d = *Weight in the portion of the extract split for adsorption (Sections 11.5.2.4 and 11.4.4.2)*
V_a = *Volume of aliquot withdrawn (mL)*
V_t = *Total volume of solvent used in Section 11.5.2.2 (mL)*

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–Report results to three significant figures for HEM and SGT-HEM found at or above 10 mg/L, and report results to two significant figures for HEM and SGT-HEM found below 10 mg/L.

12.3.1 Samples–Report results for HEM and SGT-HEM found below the ML as < 5.0 mg/L, or as required by the permitting authority or permit.

12.3.2 Blanks–Report results for HEM and SGT-HEM found below the MDL as less than the actual MDL value, i.e., < 1.4 mg/L, or as required by the permitting authority or permit. Do not report results below the MDL unless required by the permitting authority or permit.

12.3.3 Results from tests performed with an analytical system that is not in control (Section 9) must not be reported or otherwise used for permitting or regulatory compliance purposes and do not relieve a discharger or permittee of timely reporting.

13.0 Method Performance

13.1 This method was validated through single-laboratory studies and an inter-laboratory method validation study (Reference 16.9). Combined data from these studies show an average recovery of 93 percent for HEM and 89 percent for SGT-HEM and precision (as relative standard deviation) of 8.7 percent for HEM and 13 percent for SGT-HEM.

13.2 The method detection limit (MDL) and minimum level of quantitation (ML) are based on five studies conducted by EPA and described at proposal of Method 1664 (61 FR 1730) and as verified by data submitted in comments on the proposal of Method 1664 (Reference 16.3).

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 NW, Washington, DC 20036.

16.0 References

16.1 "Methods for Chemical Analysis of Water and Wastes," 3rd Edition, Environmental Protection Agency, Environmental Monitoring Systems Laboratory-Cincinnati (EMSL-Ci), Cincinnati, Ohio 45268, EPA-600/4-79-020, Method 413.1, (1983).

16.2 "Methods for Chemical Analysis of Water and Wastes," 3rd Edition, Environmental Protection Agency, Environmental Monitoring Systems Laboratory-Cincinnati (EMSL-Ci), Cincinnati, Ohio 45268, EPA-600/4-79-020, Method 418.2

16.3 Guidelines Establishing Test Procedures for the Analysis of Oil and Grease and Non-polar Materials; Final Rule; Preamble, Responses to Comments, and Docket, as referenced in the Final Rule, March 7, 2007; 72 FR 11199.

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 "Report on the Method 1664 Validation Studies," April 1995. Available from the CWA Methods Team, OSTCWAMethods@epa.gov.

16.10 **"**Analytical Method Guidance for EPA Method 1664A Implementation and Use (40 CFR part 136)," USEPA Office of Water, EPA/821-R-00-003, February 2000.

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	11
HEM Recovery (X)	9.2.2.2	83 - 101
SGT-HEM Precision (s)	9.2.2.2	28
SGT-HEM Recovery (X)	9.2.2.2	83 - 116
Matrix spike/matrix spike duplicate	9.3	
HEM Recovery	9.3.4	78 - 114
HEM RPD	9.3.5	18
SGT-HEM Recovery	9.3.4	64 - 132
SGT-HEM RPD	9.3.5	34
Ongoing precision and recovery	9.6	
HEM Recovery	9.6	78 - 114
SGT-HEM Recovery	9.6	64 - 132

18.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage to the extent 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 determined by this method.

18.2.2 Analytical batch–The set of samples started through the extraction process in a 12-hour shift, to a maximum of 20 field samples. Each analytical batch of 20 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, (Section 9.3), resulting in a minimum of four analyses (1 sample, 1 blank, 1 OPR, and 1 MS) and a maximum of 23 analyses (20 field samples, 1 blank, 1 OPR, and 1 MS) in the batch. If greater than 20 samples are to be extracted in a 12-hour shift, the samples must be separated into analytical batches of 20 or fewer samples.

18.2.3 Discharge (matrix type)–A sample medium with common characteristics across a given industrial subcategory (40 CFR parts 403-500). For example, C-stage effluents from chlorine bleach mills in the Pulp, Paper, and Paperboard industrial category; effluent from the Continuous Casting subcategory of the Iron and Steel industrial category; publicly owned treatment work (POTW) sludge; and in-process streams in the Atlantic and Gulf Coast Hand-shucked Oyster Processing subcategory are each a matrix type.

18.2.4 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.

18.2.5 HEM–See n-Hexane extractable material.

18.2.6 n-Hexane extractable material–Material that is extracted from a sample using the extraction solvent n-hexane and gravimetrically determined by this method, otherwise known by the common name “oil and grease”. This material includes relatively non-volatile hydrocarbons, vegetable oils, animal fats, waxes, soaps, greases, and related matter.

18.2.7 IPR–See initial precision and recovery.

18.2.8 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 is modified.

18.2.9 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.

18.2.10 Laboratory control sample (LCS)–See Ongoing precision and recovery standard (OPR).

18.2.11 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.

18.2.12 May–This action, activity, or procedural step is neither required nor prohibited.

18.2.13 May not–This action, activity, or procedural step is prohibited.

18.2.14 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.

18.2.15 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.

18.2.16 Must–This action, activity, or procedural step is required.

18.2.17 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.

18.2.18 OPR–See ongoing precision and recovery standard.

18.2.19 PAR–See precision and recovery standard.

18.2.20 Precision and recovery standard–Secondary standard that is diluted and spiked to form the IPR and OPR.

18.2.21 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.

18.2.22 Quantitative transfer–The process of transferring a solution from one container to another using a pipet in which as much solution as possible is transferred, followed by rinsing of the walls of the source container with a small volume of rinsing solution (e.g., n-hexane), followed by transfer of the rinsing solution, followed by a second and third rinse and transfer.

18.2.23 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.

18.2.24 Regulatory Compliance Limit–A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

18.2.25 SGT-HEM–See Silica gel treated n-hexane extractable material.

18.2.26 Should–This action, activity, or procedural step is suggested but not required.

18.2.27 Silica gel treated n-hexane extractable material–Components of n-Hexane extractable material (HEM) that are not adsorbed by silica gel and are gravimetrically determined by this method, otherwise known as non-polar material (NPM).

18.2.28 SPE–Solid-phase extraction is an extraction technique in which the analytes of interest that may be present in a sample are selectively adsorbed onto a disk or cartridge, and subsequently desorbed by an eluting solvent.

18.2.29 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.