Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141

Prepared by

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Washington, DC

July 1996

Disclaimer

This document has been reviewed by the Analytical Methods Staff of the Engineering and Analysis Division in EPA's Office of Science and Technology. This document presents guidance on method format and content as contemplated by EPA as part of its initiative to streamline the water methods approval program. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Introduction

The guidelines and format presented in this document are to be used by EPA and other organizations in preparing new analytical methods for proposal and promulgation under CWA Section 304(h) and SDWA Section 1445(a). These guidelines, in general, follow the "Guidelines and Format for EMSL-Cincinnati Methods" and the American Society for Testing and Materials (ASTM) requirements as stated in "Form and Style for ASTM Standards," November 1994 (13-000001-94). These guidelines and format are a detailed amplification of the Environmental Monitoring Methods Council (EMMC) format. A guideline and format from another organization may be used provided that it is standardized and contains the same elements contained in this document. For example, the method formats from *Standard Methods for Examination of Water and Wastewater*, ASTM, AOAC-International, and the U.S. Geological Survey may be used because these formats are documented and adhered to by these organizations. Methods produced or approved by organizations that allow random formats must be revised into a the format presented in the guidelines and format presented in this document, or into another standardized format, before submission for proposal at 40 CFR part 136 or 141.

The "Guidelines and Format for Methods to be Proposed at 40 CFR part 136 or part 141" (the "Method Guidelines and Format") have been prepared to promote consistency among analytical methods and to streamline the method promulgation process. The elements given in these Method Guidelines and Format are mandatory for all methods proposed at 40 CFR part 136 or 141. This document is presented in four sections that describe standard elements that must be included in the method, required method format, conventions to be used when preparing the method, and required method content.

OW EAD Method 1664, *N-Hexane Extractable Material and Silica Gel Treated N-Hexane Extractable Material by Extraction and Gravimetry (Oil and Grease and Total Petroleum Hydrocarbons),* is given in Appendix B as an example of a method that adheres to these Method Guidelines and Format.

Questions and comments concerning Method Guidelines and Format should be addressed to:

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Requests for additional copies of this publication should be directed to:

Water Resource Center
Mail Code RC-4100
401 M Street, S.W.
Washington, D.C. 20460
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1.0 Elements

1.1 Cover page

For methods submitted to EPA from other organizations or individuals, no cover page is required. Prior to method publication, EPA will prepare the cover page in the standard EPA format, as shown in the sample method in Appendix B. The cover page will use black ink on white or colored paper stock and may include a cover graphic that illustrates the method.

EPA will assign a three- or four-digit method number that correlates with the EPA method series to which the method belongs. The method number is included as the first part of the method title on the cover page.

1.2 Title page

There are two types of title page: a title page prepared by an organization or individual that is submitting a method to EPA, and the final title page that appears in the EPA-published method.

1.2.1 Individuals or organizations submitting methods to EPA should include the following information on the title page of the method:

- Method title
- Date
- Sponsoring organization with address and telephone number

1.2.1.1 When titling the method, use a concise title that cites (in sequence) the particular analyte(s) or property being determined, the type of sample or sample matrix(ces) to which the method is applicable, as appropriate, and the determinative technique or instrumentation. Apply the following guidelines in titling methods:

- If the method applies to numerous matrices (such as water, soil, sediment, sludge, tissue, and others), it may not be practical to include matrices in the title; however, if the method applies to a single matrix or a limited number of matrices, the matrix(ces) should be specified in the title.

- If the method is used to determine a number of analytes or properties, analytes or properties can be named as a group (e.g., trace elements), and the names of specific analytes or properties omitted.

- Avoid the use of the terms "analysis of..." or "determination of..." in method titles, since these terms are understood within the context of the term "method."

- Method titles should use abbreviations or acronyms for familiar parts of the method title, e.g., HRGC/HRMS. The acronym or abbreviation should be defined at first use in the method.

Examples of suitable method titles are:

- Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry
- Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

1.2.1.2 For a methods manual, use a title that identifies the category of methods included in the manual. Examples of suitable methods manual titles are:

- Analytical Methods for Pulp and Paper Industry Wastewater
- Analytical Methods for the Determination of Pollutants in Pharmaceutical Manufacturing Industry Wastewater

1.2.2 Before publishing the method, EPA will generate a title page that mimics the cover page (excluding any cover graphics).

1.3 Acknowledgments

Acknowledgments should identify the author and editor, and provide credit to researchers, peer reviewers, and organizations or individuals that contributed directly and substantively in the development and writing of the method. These acknowledgements are independent of references listed at the end of the method.

1.4 Disclaimer

The disclaimer may appear on the same page with the acknowledgments or may be on the page following the acknowledgments. It may contain one or more disclaimer statements. All disclaimers should include the following statement:

- The mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The disclaimer may not state explicitly or imply that EPA has granted any approval of the method. Once the method has been validated and submitted to EPA for proposal, however, the following statement may be included:

- This method has been submitted to the U.S. Environmental Protection Agency for use in EPA's water programs but has not been approved for use by EPA.

For draft methods, include the following statement:

- This method is in draft form. It has not been released by the U.S. Environmental Protection Agency and should not be construed as an Agency-endorsed method. It is being circulated for comments on its technical merit.

When preparing the method for proposal at 40 CFR part 136 or 141, EPA will edit the disclaimer to cite the Agency review process that the method has undergone.

1.5 Table of contents

A table of contents is required for methods manuals and is recommended for single methods that exceed 25 pages in length. The table of contents should cite the titles and page numbers of all first- and second-order headings (see section 2.9, "Section headings") and all tables and figures.

1.6 Introduction

In the introduction, provide background on the method, describe the purpose of the method, and include a summary-level description of the method. Identify the name, organization, address, and telephone number to contact for questions regarding the method.

When preparing the validated method for submission to EPA for proposal at 40 CFR part 136 or 141, include the following instructions at the end of the introduction:

- Questions concerning this method or its application should be addressed to:

  W. A. Telliard
  USEPA Office of Water
  Analytical Methods Staff
  Mail Code 4303
  401 M Street, S.W.
  Washington, DC 20460
  202/260-7120

- Requests for additional copies of this publication should be directed to:

  Water Resource Center
  Mail Code RC-4100
  401 M Street, S.W.
  Washington, DC 20460
  202/260-7786

1.7 Notice of performance-based method

All methods prepared should be performance-based and should contain the following notice on a separate page directly preceding the body of the method.

- Note: This method is performance-based. The laboratory is permitted to modify or omit any step or procedure, provided that all performance requirements set forth in this method are met. The laboratory may not omit any quality control analyses. The terms "shall" "must," and "may not" indicate steps and procedures required for producing reliable results. The terms "should" and "may" indicate optional steps that may be modified or omitted if the laboratory can demonstrate that the modified method produces results equivalent or superior to results produced by this method.

1.8 Body of method

The body of the method must be presented in the EMMC format. See Section 4.0 for a detailed description of this format.

2.0 Format

2.1 Page numbering

Page numbers should appear in the bottom center of the page. For methods prepared double-sided, page numbers may appear on the outside bottom corner of the page (i.e., on the bottom right for right-hand pages and on the bottom left for left-hand pages).

2.1.1 Numbering front matter -- Number the front matter (i.e., everything preceding the body of the method) consecutively using lower-case Roman numerals. The numerals should appear on the bottom of each page of the front matter, except for the cover and title pages. The cover page is unnumbered. The title page holds the place of page *i* but the numeral is not displayed.

2.1.2 Numbering body of method -- Number the body of the method consecutively with Arabic numerals on the bottom of each page, starting with the number 1.

2.2 Method identification

2.2.1 The method introduction page(s) should contain a header that identifies the method number and revision number or letter. The first page of the body of the method should start with the method number and title in the top center of the page (preceding 1.0 Scope and Application), with no header, as shown in Appendix A. Each pursuant page of the method should contain a header that identifies the method number and revision number or letter. The header also must be separated from the main body of the method by a horizontal line running the width of the page.

2.2.2 If the method was assigned a non-EPA method number during its development and validation, when preparing the method for submission to EPA for proposal at 40 CFR part 136 or 141, edit the header to reflect the method number assigned by EPA (i.e., Method 1664).

2.3 Method date

The date of the method (month and year) should appear on the bottom of each page of the method.

2.4 Font

For text, use an 11-point Times Roman font (typeface). For first-order headings, use a bold, 14-point Univers font. For section numbering, use a bold, 12-point Univers font. For headers and footers, use an italics, 9-point Univers font. Univers or Times Roman fonts may be used in tables as appropriate. If Univers is unavailable, Helvetica may be substituted.

2.5 Margins

Left and right margins should be one inch. The header should be 0.5 inch from the top of the page, with the text starting one inch from the top of the page. The page number should appear 0.5 inch from the bottom of the page, with the text starting one inch from the bottom of the page.

2.6 Justification

Use left justification for text. This results in a ragged-right margin.

2.7 Line spacing

The method should be single-spaced. (If preferred, 1.1 line spacing can be used to enhance readability.) One blank line should appear between each paragraph and section.

2.8 Method sections

Each method must contain the sections given in the EMMC method format. See Section 4.0 for a detailed description of this format. If a section does not apply to a particular method, include the section with a statement that it is not applicable to that method.

2.9 Section headings and numbering

Use the Modified Decimal Numbering (MDN) system to organize material presented in methods and methods manuals. In this system, each method section and subsection is assigned a unique number that shows the relationship of a specific section/subsection to all previous sections/subsections and allows for easy reference. This numbering system is used in this document and in the example method that appears in Appendix A.

The first-order headings are the 17 sections identified in Section 1.7, "Body of Method," starting with 1.0 Scope and Application. First-order headings must appear on a separate line, with a blank line appearing between the heading and the section text. Subsections are numbered and may or may not have a heading preceding the text. Second-order headings or sections are numbered 1.1, 1.2, 1.3, 1.4, etc. Third-order headings or sections are numbered 1.1.1, 1.1.2, 1.1.3, etc. Fourth-order headings or sections are numbered 1.1.1.1, 1.1.1.2, 1.1.1.3, etc.

Do not number beyond the fourth-order heading or section. If additional subdivisions are necessary, use (a), (b), (c), etc. to identify further divisions. Use of subdivisions below the fourth-order heading or section should be avoided where possible by organizing the material differently.

2.10 Indentation

First-order headings should appear flush left. Each subsequent order heading should be block-indented to align with the text of the previous order heading. This indentation method is illustrated in this document and in Method 1664 in Appendix A.

2.11 Electronic submission

Methods and methods manuals must be prepared and submitted to EPA in both hardcopy and electronic formats.

2.11.1 Hardcopy methods should be produced in black type on white or off-white recycled paper and printed or copied double-sided.

2.11.2 Electronic methods must be submitted in machine-readable format, either ASCII or WordPerfect 5.1 or later.

2.11.3 To enable anyone accessing a method electronically to be certain they have retrieved the entire section or method accessed, include a "section end" notice at the end of each first-order section. This is illustrated as follows:

************ End of Section 4.0 ************

2.12 References

Use the following format for order, content, and punctuation when listing references.

2.12.1 Books -- author's name or names (initials last), title of book (underline, period, no quotation marks), name of publisher, address of publisher (city and state), year of publication, and page number, if applicable

2.12.2 Magazines and Journals -- author's name or names (initials last), "title of paper" (quotation marks, comma), volume number, issue number (this may be omitted if the journal page numbers are continuous throughout the volume), date of publications, and page numbers. Example: Jones, J.J., and Smith, R.R., "Correlation of Brinell Hardness and Tensile Strength, *Materials in Design Engineering*. Vol. 10, No. 2, February 1958, pp. 52-67. A list of common journal abbreviations is given in Appendix C.

2.12.3 Proceedings, Transactions, Reports, Bulletins, etc. -- author's name or names (initials last), "title of paper" (in quotation marks), name of publication (underline, no quotation marks, comma), name of publisher, volume number, if any date of publication,and page numbers.

2.12.4 Symposium Volumes or Other Books Comprising Collections of Papers -- Follow style for books, above and add title of paper, in quotes, after author's name.

2.12.5 Patents -- patent number and data.

2.12.6 EPA methods -- Method number and name, EPA report number, U.S. Environmental Protection Agency, laboratory and/or office, location, date.

3.0 Conventions

3.1 Capitalization, italics, underlining, and boldface

3.1.1 Capitalization

3.1.1.1 For first-order headings (numbered 1.0, 2.0, 3.0, etc.), use initial capitalization of major words.

3.1.1.2 For second-, third-, or fourth-order headings, capitalize the first word of the heading only.

3.1.2 Italics--Italicize words or blocks of text for emphasis. Equations and notes interspersed in the text also should be italicized.

3.1.3 Underlining--Underline words that are defined in the Definitions section (or glossary). Use underlining in tables as appropriate for clear presentation of material. Do not use underlining for emphasis; use italics instead but avoid overuse of emphasis.

3.1.4 Boldface--Boldface the following items:
- the method number and title on the cover page, title page, and page 1 of the method
- Acknowledgments, Disclaimer, and Introduction headings
- first-order headings
- section numbering
- equation numbers
- the word "Note:" preceding text notes.

3.2 Punctuation

3.2.1 Always use a comma after the second to last entry in a series.

3.2.2 A dash may be used between a subheading and text that directly follows the subheading. There should be no blank space before or after the dash, e.g., "Matrix Spikes—The laboratory must spike..."

3.2.3 As a general rule, use a hyphen in compound modifiers to avoid ambiguity, e.g., 1-L flask. (In some cases, the hyphen can be left out without ambiguity, e.g., toxic chemical waste.) Do not use a hyphen after an adverb ending in "ly," e.g., commonly accepted practice.

3.2.4 Bullets are not to be used in the body of the method. If used in introductory material, the text following the bullet should start with a capital letter. Short bullets do not require periods at the end; long (multiple-line) bullets do. Semicolons or commas should not be used after bulleted text.

3.3 Footnotes

Use footnotes only in tables. Footnotes should be designated with numbers or lower case letters in superscript, and should appear below the body of the table.

3.4 Text notes

Notes may be used within the text to highlight important information regarding use of the method. Use a margin-to-margin line across the page both preceding and following the note to set it off from the text.

3.5 Equations

Equations should be numbered Equation 1, Equation 2, etc., consecutively as they appear in the text. Use a margin-to-margin line across the page both preceding and following the equation to set it off from the text. Equations should be presented in italics. The equation is followed by "where:" and a list of terms used in the equation (e.g., n = number of samples, x = concentration in each sample).

3.6 Tables and Figures

Tables and figures appear in Section 17.0.

3.6.1 Number tables and figures consecutively with arabic numerals, and give each a title that is complete and descriptive.

3.6.2 In table column headings, specify the quantity being tabulated, followed by the units of measurement shown in parentheses. For example, "Amount spiked (μg/L)".

3.6.3 Place table and figure titles above the information presented.

3.6.4 Figures may be enclosed in a box if desired.

3.7 Trademarks

3.7.1 Avoid the use of trademarks or brand names whenever possible. For examples, use the term "borosilicate glass" rather than the trademarks Pyrex or Kimax; use "fluoropolymer" rather than Teflon. (See Section 4.6.4.)

3.7.2 When a trademark or brand name is used, capitalize it.

3.8 Text references

Text references are references to other locations within the method, not references to any outside source. References to other sources appear in Section 16.0. Do not incorporate essential information into the method by referring to another method.

In the method text, refer to other sections of the method capitalizing the word "Section." Section references should appear in parentheses at the end of the phrase or sentence to which the reference applies, for example, (Section 9.6).

3.9 Units, symbols, abbreviations, and acronyms

3.9.1 Units and symbols from the international metric system (SI, from the French name, Le Systeme International d Unites) are to be used. SI is based on seven basic units that are dimensionally independent. The SI unit of time is the second (symbol = s) which should be used if practical. The SI unit of volume is the cubic meter (symbol = m^3) but the spectral name liter (symbol = L) can be used for liquids and gases. Although the SI unit for mass is kilogram (symbol = kg), the use of gram (g) with or without prefixes is appropriate.

3.9.2 Symbols, not abbreviations, should be used for units. Symbols are not followed by a period except when used at the end of a sentence. Unit symbols are written in lower case except for the symbol for liter (L) or where the unit name was derived from a proper name, such as Pa, from Pascal. When a quantity is expressed as a numerical value and a unit symbol, a space should be left between them, except between the number and symbol for degree Celsius (20°C) and for degree, minute, and second of plane angle.

3.9.3 Use commonly accepted abbreviations in tables as appropriate to conserve space.

3.9.4 Use commonly accepted acronyms and abbreviations in text and tables. An acronym is a word formed from the first or first few letters of other words; everything else is an abbreviation. In many cases, an acronym or abbreviation is more readily identifiable than its narrative counterpart. Always spell out the term the first time it is used and follow it with the acronym or abbreviation shown in parentheses, e.g., material safety data sheet (MSDS), relative percent difference (RPD), or United States Environmental Protection Agency (EPA). Acronyms and nearly all abbreviations have no periods or spaces between letters. As depicted in these examples, although the acronym or abbreviation is capitalized, the narrative version of it is not capitalized unless it is a proper name such as a government agency, society, or association. Once an acronym or abbreviation is introduced in this manner, use only the acronym or abbreviation subsequently.

3.9.5 When a long word or phrase for which there is no standard acronym or abbreviation is used frequently, it may be replaced by an acronym or abbreviation that is explained when it first occurs. For example, relative centrifugal force (RCF).

3.9.6 A list of common symbols, abbreviations, and acronyms is included in Appendix B to this document.

3.10 Numerals

3.10.1 Spell out single-digit numbers (one through nine), with the following exceptions:

3.10.1.1 Use numerals when the quantity is partly fractional, e.g., 1.15, 1- ½.

3.10.1.2 Use numerals when the number is followed by a unit symbol, e.g., 1 m, 9%, 3 ppm. In the method text, units should be spelled out, so the numbers one through nine associated with the units would be spelled out also (e.g., one meter, nine percent, three parts per million).

3.10.1.3 Use numerals to identify equations and tables (e.g., Equation 2, Table 5).

3.10.1.4 In sentences containing multiple numbers, if some numbers must be numerals, use numerals for all (e.g., 2 tests and 16 weighings).

3.10.2 Use numerals for multiple-digit numbers (10 and above), with the following exceptions:

3.10.2.1 Do not begin a sentence with a numeral. When the numeral is spelled out, also spell out the unit following (e.g., One gram is usually sufficient.)

3.10.2.2 Spell out round numbers that are used in an indefinite sense (e.g., a hundred feet or so).

3.10.3 When a number is used as an adjective, insert a hyphen between the number and the unit symbol (e.g., 100-mL volumetric flask, 1-L sample).

3.10.4 When writing decimal numbers of value less than one, place a zero before the decimal point (e.g., 0.45 g).

3.10.5 Do not point-off numbers of four figures (1234) except in tables when they occur in a column containing numbers of more than four figures. Point-off numbers of more than four figures, using commas with no spaces (e.g., 1,325,000).

3.10.6 In expressing ranges and ratios in text, use 1 to 10 or 1:10, not 1-10. A hyphen may be used for ranges in tables.

3.11 Significant digits

Handle numbers with careful regard for correspondence between the data accuracy and the given number of digits. The number of significant digits should neither sacrifice nor exaggerate accuracy.

3.11.1 Any digit that is necessary to define the specific value or quantity is significant. For example, when measured to the nearest 1 m, a distance may be 157 m, which has three significant figures; when measured to the nearest 0.1 m, the distance may be 157.4 m, which has four significant figures.

3.11.2 When adding or subtracting numbers with different degrees of precision, the answer should contain no digits farther to the right than the least precise number. Numbers should first be rounded to one digit farther to the right than that of the least precise number. The answer is then rounded to the same number of significant figures as the least precise number.

3.11.3 For multiplication and division, the product or quotient should contain no more significant figures than are contained in the number with the fewest significant figures.

3.11.4 Examples to distinguish the addition/subtraction and multiplication/division rules are:

Addition:
113.2 + 1.43 = 114.63, rounded to 114.6

Subtraction:
113.1 - 1.43 = 111.77, rounded to 111.8

Multiplication:
113.2 x 1.43 = 161.876, (rounded to 162)

Division:
113.1 ÷ 1.43 = 79.16, rounded to 79.2

Note: The product and quotient above should contain only three significant figures because the number 1.43 contains only three significant figures. The above sum and difference, however, contain four significant figures, because digits that occur to the right of the last significant in the least precise number are rounded.

3.12 Order of magnitude

Zeros may be used to indicate a specific value or to indicate the order of magnitude of a number. For example, in the number 203,185,000, representing population rounded to thousands, the first six digits are significant. The last three digits are zeros that indicate the order of magnitude.

3.13 Rounding

3.13.1 When the first digit discarded is less than five, the last digit retained is not changed.

3.13.2 When the first digit discarded is five or greater, or when five is followed by a digit other than zero, the last digit retained is increased by one.

3.13.3 When the first digit discarded is exactly five followed only by zeros, the last digit retained is rounded upward if it is an odd number and is not adjusted if it is an even number.

4.0 Content

In accordance with EMMC format, each analytical method must contain 17 specific topical sections in a designated order. The required order and content of these sections are listed and described below. All of these sections are mandatory for all methods.

1.0 Scope and Application
2.0 Summary of Method
3.0 Definitions
4.0 Interferences
5.0 Safety
6.0 Equipment and Supplies
7.0 Reagents and Standards
8.0 Sample Collection Preservation and Storage
9.0 Quality Control
10.0 Calibration and Standardization
11.0 Procedure
12.0 Data Analysis and Calculations
13.0 Method Performance
14.0 Pollution Prevention
15.0 Waste Management
16.0 References
17.0 Tables, Diagrams, Flowcharts, and Validation Data

Starting with Section 11.0 Procedure, additional numbered sections may be inserted as required by the particular method; however, the sections listed above must appear in each method in the order listed. See Appendix B for examples of content described in this section.

Note: Subsections within each of the 17 required sections do not need to correlate directly to the subsections included here. In other words, the information mentioned in 4.1.1 below might be covered in two or more subsections in a method.

4.1 Scope and application

This section outlines the purpose, range, limitations, and intended use of the method, and identifies target analytes.

4.1.1 Define the purpose and intended use of the method. State what the method is based upon, noting any relationship of the method to other existing analytical methods. Indicate whether the method is associated with a sampling method. Include the following statement:

- This method is for use in the Environmental Protection Agency's (EPA's) data gathering and monitoring programs under the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act.

4.1.2 List analytes that can be measured by the method, including each analyte's Chemical Abstracts Service Registry Number (CASRN). If regulations cite other than the most commonly used analyte name, refer to the regulation. For pesticides, use "acceptable common names." The use of registered trade names is permitted.

4.1.3 Identify the matrix(ces) for which the method has been found satisfactory.

4.1.4 Indicate the statistically determined method detection limit (MDL) and the analyte concentration range over which the method is applicable. State the matrix(ces) in which MDL was determined. If the MDL is not available, report an instrumental detection limit and define how it was derived. Indicate the minimum level (ML) and water quality criteria if appropriate to the analyte and method.

4.1.5 Describe method limitations, such as "This method is not applicable to saline water," or "This method is not intended for determination of metals at concentrations normally found in treated and untreated discharges from industrial facilities." Indicate any means of recognizing cases where the method may not be applicable to the sample under test.

4.1.6 List any restrictions that may apply, such as "This method is restricted to use by or under the supervision of analysts experienced in ..."

4.1.7 Include the following statement regarding performance-based methods:

- This method is performance-based. The laboratory is permitted to omit any step or modify any procedure (e.g., to overcome interferences, to lower the cost of measurements), provided that all performance requirements in this method are met. Requirements for establishing method equivalency are given in Section 9.1.2.

4.1.8 Include the following statements:

- Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.1.2.

4.2 Summary of Method

This section provides an overview of the method procedure and quality assurance.

4.2.1 Outline, specifying amounts of sample and reagent, the procedure that is followed to determine the presence or absence of the listed analytes. Include any sample pretreatment, such as filtration or digestion. In this description, identify the basic steps involved in performing the method, but omit the details that are a necessary part of the complete statement of procedure.

4.2.1.1 For chemical methods, state the type of procedure (colorimetric, electrometric, volumetric, etc.) and describe the source of color, major chemical reaction, including pertinent chemical equations, etc. For instrumental methods, state the technique.

4.2.1.2 Use the passive voice, e.g., "Instrumental drift is corrected by using internal standardization," rather than "Correct instrumental drift by using internal standardization."

4.2.2 Identify the determinative step in the method.

4.2.3 State in a summary fashion how quality is assured in the method.

4.2.4 List options to the method, if applicable.

4.3 Definitions

This section includes definitions of terms, acronyms, and abbreviations used in the method. If preferred, definitions may be provided in a glossary at the end of the method or manual. In this case, the definitions section must still appear in the method, with a notation that definitions are provided in a glossary at the end of the method. Refer to the specific section number of the glossary.

4.3.1 Include an introductory statement as follows:

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

4.3.2 List units of weight and measure and their abbreviations or acronyms used in the method.

4.3.3 Alphabetically list and define terms, acronyms, and abbreviations used in the method. Where appropriate, include the purpose (e.g., The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample).

4.3.4 Include definitions of the terms may, may not, must, and should, as follows:

- May: This action, activity, or procedural step is neither required nor prohibited.
- May not: This action, activity, or procedural step is prohibited.
- Must: This action, activity, or procedural step is required.
- Shall: This action, activity, or procedural step is suggested but not required.
- Should: This action, activity, or procedural step is suggested but not required.

4.4 Interferences

This section identifies known or potential interferences that may occur during use of the method, and describes ways to reduce or eliminate interferences.

4.4.1 Describe any known or potential problem(s) (e.g., sample or equipment contamination, instrument noise) that may be encountered during the performance of the method and the source of the problem(s). Recommend techniques to avoid or minimize the problem(s) (e.g., ways to reduce sample or equipment contamination, or instrument noise).

4.4.2 Identify any substances, ions, or properties that are known to or likely to cause interference and the amounts that are known to or likely to interfere. Sometimes, this information can be obtained only by observation during the analysis. In such cases, include appropriate notes under "Procedure" or "Data Analysis and Calculations."

4.5 Safety

This section describes special precautions needed to ensure personnel safety during the performance of the method. Procedures described here should be limited to those which are above and beyond good laboratory practices. The section must contain information regarding specific toxicity of analytes or reagents.

4.5.1 Identify and warn analysts of potential hazards associated with using the method (e.g., toxicity or carcinogenicity of analytes or reagents, explosions, fire, radiation). Recommend techniques to minimize hazards where possible (e.g., performing operations in a hood or glove box).

4.5.2 Where the toxicity or carcinogenicity of each compound or reagent has not been precisely determined, include the following statement:

- The toxicity of carcinogenicity of each analyte or reagent 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 using this method and that the results of this monitoring be made available to the analyst.

4.5.3 Indicate the steps in the procedure at which hazards that could damage equipment may occur by use of the word **CAUTION** in boldface type, followed by the details of the precautionary measures that must be taken. If any step in the procedure could result in personal injury or death, include the word **WARNING** in boldface type, followed by the details of the protective measures that must be taken.

4.5.4 Include the following statements:

- 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 ____.

4.6 Equipment and Supplies

This section lists and describes all nonconsumable supplies and equipment needed to perform the method.

4.6.1 Include the following statement as a note preceding the list of equipment and supplies:

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment 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.

4.6.2 Categorize and list required equipment and supplies by the logical order of use; e.g., sampling equipment, equipment for glassware cleaning, equipment for calibration, equipment for sample extraction, etc.. Do not list common laboratory equipment, but do include special or modified forms of unusual sizes or numbers of common equipment that are required or that may require special preparation.

4.6.3 Describe the essential features of each required item. Include schematic drawings as needed to clarify or supplement apparatus descriptions.

4.6.4 Avoid the use of trademarks, brand names, trade names, or suppliers unless a specific manufacturer's product is required for a well-defined reason or the availability of the product is limited (i.e., the apparatus is unique or unusual). For example, when special types of glassware are required, such as heat-resistant, chemical-resistant, etc., state the significant characteristic desired rather than a trademark ("borosilicate glass" rather than Pyrex or Kimax). If only a single source is known, that supplier may be identified.

4.6.5 Whenever a brand name is used, include "or equivalent" following the brand name or part number to demonstrate that use of another product is acceptable.

4.6.6 Include any special glassware cleaning instructions.

4.6.7 List special facilities required, such as a special room for handling hazardous materials.

4.7 Reagents and Standards

This section lists and describes all reagents and standards required to perform the method, and provides preparation instructions and/or suggested suppliers as appropriate.

4.7.1 List the name of the reagent and the necessary purity, followed by any descriptive terms. List reagents in a logical order (e.g., by order of occurrence or use, by group). The method should require that reagents be ACS Reagent Grade unless otherwise specified.

4.7.2 Spell out the full name of inorganic reagents when first used, and include within parentheses the exact chemical formula, showing its water of crystallization, etc. Subsequently, refer to inorganic compounds by formula if they can be specified clearly in this way. As exceptions, always spell out the word "water" and the names of substances in their elemental state (e.g., "lead" not "Pb," "oxygen" not "0_2").

4.7.3 Spell out organic, organometallic, or complex inorganic compounds; chemical formulae are not necessary. Cite the CASRN to avoid ambiguity.

4.7.4 Avoid the use of trademarks and names of patented products. Use chemical names and common names, unless a specific product is required for a well-defined reason. The use of registered trade names is permitted.

4.7.5 Unique and unusual reagents can be named by brand. Whenever a brand name is used, include "or equivalent" following the brand name to demonstrate that another product can be used.

4.7.6 Specify the concentration of inorganic reagents in applicable terms, as follows:

Concentrated acids and bases	density
Dilute acids and bases	volume ratio, x + y (x volume of reagent added to y volume by water)
Nonstandardized solutions	normality, expressed decimally; or the equivalent of 1 mL of solution in terms of grams of a given element expressed as 1 mL = x.xx g of ...

4.7.7 Specify filter paper by describing the significant characteristic such as porosity, rate of filtering ash content, etc., or by reference to ASTM Specification D1100 for *Filter Paper for Use in Chemical Analysis*.

4.8 Sample Collection, Preservation, and Storage

This section provides requirements and instructions for collecting, preserving, and storing samples.

4.8.1 Give detailed directions for collecting, filtering (if applicable), preserving, shipping, and storing samples.

4.8.2 Use preservation procedures and holding times consistent with those specified in current EPA publications or regulations and with other methods for the same analytes.

4.9 Quality Control

This section cites the procedures and analyses required to fully document the quality of data generated by the method. The required components of the laboratory's quality assurance (QA) program and specific quality control (QC) analyses are described in this section. For each QC analysis, the complete analytical procedure, the frequency of required analyses, and interpretation of results are specified.

Note: To ensure data quality, water methods must specify a comprehensive laboratory QA program. The minimum QC requirements that must be included in methods proposed at 40 CFR Part 136 or Part 141 are specified in [use the *Federal Register* citation by which this streamlining initiative is promulgated]. The method must specify QC acceptance criteria in accordance with Chapter 3, “Quality Control Requirements”, of the *Guide to Method Flexibility and Approval of EPA Water Methods* included in the notice referenced above.

4.9.1 Include the following statements in the first subsection (Section 9.1):

- Each laboratory that uses this method is required to operate a formal quality assurance program (Reference ___). 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 [*complete as appropriate to the method* ___________]. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

- 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.

4.9.2 In Section 9.1, cite any options that the analyst is permitted, e.g., alternate extraction, concentration, or cleanup procedures; changes in columns or detectors. Specify that the analyst is required to repeat the required initial demonstration of laboratory capability each time a modification is made to the method. Include the following statements:

- Each time a modification is made to the method, the analyst is required to repeat the procedure in Section 9.2. If the change will affect the detection limit of the method, the laboratory is required to demonstrate that the MDL (40 CFR Part 136, Appendix B) is lower than the MDL for that analyte in this method, or one-third the regulatory compliance level, whichever is higher. If the change will affect calibration, the analyst must recalibrate the instrument according to Section 10.

- 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 the analytes of interest.

- The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:
  - 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.
  - A listing of analytes measured, by name and CASRN.
  - A narrative stating reason(s) for the modification(s).
  - Results from all 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)

  - 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
    - (f) Extract volume
    - (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

4.9.3 In the remainder of Section 9.1, outline the QC requirements that will be described in the section, and the purpose for each type of QC (e.g., blanks, matrix spikes/matrix spike duplicates, calibration verification).

4.9.4 In Section 9.2, describe in detail the initial demonstration of laboratory capability.

4.9.5 Describe the procedure for matrix spikes, calculating percent recoveries, and calculating relative percent difference for duplicates.

4.9.6 Provide instructions for analysis of blanks, e.g., laboratory reagent blanks, method blanks.

4.9.7 Specify requirements for calibration verification.

4.9.8 Provide instructions for analysis of ongoing precision and recovery standards.

4.9.9 Include requirements for analysis of quality control samples (QCS).

4.9.10 Include the following statement at the end of Section 9.0:

- 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.

4.10 Calibration and Standardization

This section describes the method/instrument calibration and standardization process, and required calibration verification. Corrective actions are described for cases when performance specifications are not met.

4.10.1 Specify operating conditions or refer to manufacturer's recommended operating conditions. If appropriate, specify a precalibration routine as needed to document instrument stability.

4.10.2 Give detailed instructions for the use of standards to prepare calibration lines or tables. Include the number of calibration standards, the need for blanks, the frequency of calibration checks, the critical range, etc.

4.10.3 Give detailed instructions for internal standardization, including number and concentration of internal standards.

4.10.4 Include instructions for calibration data storage.

4.11 Procedure

This section describes the sample processing and instrumental analysis steps of the method, and provides detailed instructions to analysts.

4.11.1 For methods used for determination of a method-defined analyte, include the following statement in the introductory portion of Section 11.0 Procedure:

- This method is entirely empirical. Acceptable results can be obtained only by strict adherence to all details.

Do not include this statement in methods for which the analyte is a chemical or physical parameter, the characteristics of which are known (e.g., oil and grease, COD, BOD).

4.11.2 Include in proper sequence detailed directions for performing the analysis.

4.11.2.1 Include steps that are essential to the process and avoid unnecessarily restrictive instructions.

4.11.2.2 Organize the procedure by logical order of activity, e.g., sample preparation, extraction, analysis.

4.11.2.3 Describe the procedure in the imperative mood, present tense, e.g., "Heat the sample aliquot," rather than "The sample aliquot should be heated." Comments and descriptive information that are not in the imperative mood may be included, as appropriate.

4.11.2.4 Write the text so that it is concise and easily understandable.

4.11.2.5 When alternative procedures are given, state which is preferred.

4.11.3 In chemical methods, specify the size of sample aliquot and indicate the required measurement accuracy. (There is no need to weigh a sample to five significant figures in a spectrophotometric method where the final absorbance measurement yields data with only three significant figures).

4.11.4 Include "Notes" throughout the procedure to highlight critical points. Include notes of "WARNING" or "CAUTION" as appropriate to identify known or potential hazards to the analyst or the equipment, respectively.

4.11.5 Indicate steps in which timing is critical, e.g., if a determination may not be interrupted overnight. For a color reaction, indicate how long the color is stable.

4.12 Data Analysis and Calculations

This section provides instructions for analyzing data, and equations and definitions of constants used to calculate final sample analysis results.

4.12.1 Calculations--Provide directions for calculating the results of the analysis, including any equations.

4.12.1.1 Use the imperative mood, e.g., "Report results to three significant figures," rather than "Results should be reported to three significant figures."

4.12.1.2 Where there may be ambiguity of meaning, spell out names in the text (e.g., total Kjeldahl nitrogen) but use the abbreviations (e.g., TKN) in text where the meaning is clear, and in equations.

4.12.1.3 Define the symbols used in the equation immediately under the equation.

4.12.1.4 Use numerical values for any constants. Identify dilution factors, titration factors, etc.

4.12.2 Reporting results

4.12.2.1 Indicate the units in which the results are to be reported (e.g., µg/L, mg/kg).

4.12.2.2 If the sample is a solid material such as a sediment or sludge, indicate whether results are to be reported as wet weight or dry weight.

4.12.2.3 Specify the number of significant figures to be reported.

4.12.2.4 Require that all values obtained by various QC procedures are reported along with the calculated results of the analysis.

4.12.3 Interpretation of results - Use this heading in place of Calculations when the results of the analysis must be expressed in descriptive form, relative terms, or abstract values. List and define the descriptive terms or classifications used.

4.13 Method Performance

This section provides method performance criteria for the method, including precision/bias statements regarding detection limits and source/limitations of data produced using the method. **Note:** Requirements for validating new methods are specified in [cite the volume and page number of the *Federal Register* in which the streamlining is promulgated].

4.13.1 Explain how the method was validated. Provide a detailed description of method performance, including data on precision, bias, detection limits (including the method by which they were determined and matrices to which they apply), and statistical procedures used to develop performance specifications.

> **Note:** This information can be provided through reference to the method validation study.

4.13.2 At a minimum, state single-operator precision and accuracy on reagent water. If other sample types have been investigated, also provide this information for them.

4.13.3 If a collaborative study has been completed, describe the study and report the number of participating operators and laboratories, spike concentrations, level of replication, types of background waters, and any other significant aspects. If the study has been documented, cite the study report and include it in the References section. When citing reference documentation, the details of the study do not have to be included in this section.

4.14 Pollution Prevention

This section describes aspects of the method that minimize or prevent pollution known to be or potentially attributable to the method.

4.14.1 Cite potential sources of pollution attributable to the method.

4.14.2 Recommend ways to minimize pollution.

4.15 Waste Management

This section describes minimization and proper disposal of waste and samples.

4.15.1 Include the following statement as the first subsection:

- 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.

4.15.2 Provide instructions for sample and waste handling and disposal.

4.15.3 Include the following statement as the last subsection:

- 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 DC, 20036.

4.16 References

This section lists references for source documents and publications that contain ancillary information.

Note: Each method should be a free-standing document, providing all information necessary for the method user to perform the method may be found. References within a method should be restricted to associated or source material. Procedural steps or instructions should not be referenced as being found elsewhere, but should be included in total within the method.

4.16.1 Include references for other, related EPA methods; and published studies/articles relating to method performance, techniques, or analytes, and health and safety.

4.16.2 List references in the order cited in the method, and assign each reference an identification number using arabic numerals.

4.16.3 As a rule, do not list documents that are not readily accessible to the reader (e.g., unpublished theses, personal communications, private correspondence). If it is important to list these types of documents, identify where the reader may obtain a copy of the document.

4.17 Tables, Diagrams, Flowcharts, and Validation Data

This section contains all method tables and figures (diagrams and flowcharts), and may contain validation data referenced in the body of the method.

4.17.1 In addition to tables and figures, include additional useful information. Examples of such information include:

4.17.1.1 Notes on significance and interpretation of the method, used to amplify the statement in the text.

4.17.1.2 Development of equations used in the calculations.

4.17.1.3 Charts or supplementary information for computations.

4.18 Glossary

This optional section contains a glossary of terms, acronyms, abbreviations, and symbols used in the method.

Note: This information may appear in the Definitions section of the method (Section 3.0) or may be included in a glossary at the end of the method.

4.18.1 In the first subsection of the glossary, identify units of weight and measure used in the method and their abbreviations.

4.18.2 In the second subsection, define key terms and all acronyms used in the method.

4.18.2.1 List terms, acronyms, and abbreviations alphabetically.

4.18.2.2 Definitions should appear only once. Where an acronym or abbreviation represents a term that is defined under its full name, reference the full name as the definition for acronym or abbreviation.

Appendix A

Standard Acronyms, Abbreviations, and Symbols

ampere	amp
average	avg
calibration standard	CAL
centimeter	cm
cubic centimeter	cm^3
day	(do not abbreviate)
degree Celsius	^oC
diameter	dia
equation	Eq
figure	Fig. (only when followed by a numeral)
foot	ft
gallon	gal
gas chromatograph/chromatography	GC
gel permeation chromatograph/ chromatography	GPC
gram	g
grams per liter	g/L
greater than	>
high performance liquid chromatograph/chromatography	HPLC
high resolution GC	HRGC
high resolution MS	HRMS
hour	h
hydrogen ion concentration, negative logarithm of	pH
inch	in.
initial precision and recovery	IPR
inside diameter	ID
instrument detection limit	IDL
Kuderna-Danish concentrator	K-D
kilogram	kg
laboratory control sample	LCS
laboratory duplicate	LD
laboratory fortified blank	LFB
laboratory fortified sample matrix	LFM
laboratory reagent blank	LRB
less than	<
linear dynamic range	LDR
liter	L
logarithm (common)	log
logarithm (natural)	log e or ln
mass spectrometer/spectrometry	MS
mass to charge ratio	m/z
matrix spike	MS
matrix spike duplicate	MSD
maximum	max
meta	*m*-
method detection limit	MDL
meter	m
microgram	ug
microliter	uL

milliequivalent	meq
milligram	mg
milligram per gram	mg/g
milligram per Liter	mg/L
milligram per milliliter	mg/mL
milliliter	mL
millimeter	mm
millimeter of mercury (pressure)	mm Hg
millisecond	ms
millivolt	mV
minimum	min
minimum level	ML
minute	min
molal	(do not abbreviate)
molar	M
molecular ion	M
mole	mol
month	(do not abbreviate)
most probable number	MPN
nanogram	ng
nanogram per liter	ng/L
nanometer	nm
normal	N
number	No. (only when followed by a numeral)
ongoing precision and recovery	OPR
ortho	*o*-
outside diameter	OD
page	p.
pages	pp.
para	*p*-
part per billion	ppb
part per million	ppm
part per quadrillion	ppq
part per trillion	ppt
per	/ (when used in expressions with unit symbols)
percent	%
pico (prefix)	p
picogram	pg
pound	lb
pounds-per-square inch gauge	psig
precision and recovery	PAR
quality assurance	QA
quality control	QC
quality control sample	QCS
quart	qt
reference	Ref
relative centrifugal force	RCF
relative percent difference	RPD
relative response	RR
relative standard deviation	RSD
response factor	RF

revolutions per minute	rpm
second	s
selected ion current profile	SICP
solid phase extraction	SPE
Soxhlet/Dean-Stark extractor	SDS
specific gravity	sp gr
micrometer	um
volt	V
volume (of a publication)	Vol. (only when followed by a numeral)
volume per unit volume	v/v
watt	W
weight per unit volume	w/v
year	(do not abbreviate)

Appendix B

Example of Method Format

"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)"

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 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. The method is based on prior Environmental Protection Agency (EPA) and association 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. "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 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 1.4 mg/L for HEM and 1.6 mg/L for SGT-HEM (Reference 16.9), and the Minimum Level (ML; Reference 16.3) has been set at 5.0 mg/L for HEM and SGT-HEM (Reference 16.9).

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.

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 using this method and that the results of this monitoring 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. 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 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—Glass, 100-mL.

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

6.3.4 Volumetric pipette—Glass, 5-mL.

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—Glass, 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 or Steam bath—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 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 or recirculating chiller (to aid in the condensation and collection of the distilled solvent).

6.6.7 Vacuum, pump or other source of vacuum.

6.6.8 Tongs, for handling the boiling flask (Baxter Scientific Products No. T5007-2, or equivalent).

6.6.9 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

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 concentrated 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 may cause it to solidify.*

7.6 Boiling chips—Silicon carbide or fluoropolymer.

7.7 Silica gel—Anhydrous, 75 - 150 micrometers, Davisil Grade 923 (Supelco 21447-7A, 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 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 fluoropolymer-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 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 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. 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 If analysis is to be delayed for more than a few 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 0–4°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. 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 materials.

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 siphon into individual bottles using a glass tube, 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 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.

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. 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 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 an amount of HEM and/or SGT-HEM equivalent to the amount recovered by this method on each specific discharge. 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.

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.

9.2.3 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, proceed as follows:

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 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 79 to 114 percent of the average percent recovery produced by this method for HEM and 66 to 114 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 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.*

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 or, if for compliance monitoring, from a given discharge. 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 or discharge 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 and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the tests required in Section 9.1.2, and repeat the analysis of the sample and the MS/MSD.

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 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 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 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 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 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" weights.

10.2 Calibration shall be within ± 10% (i.e. ±0.2 mg) at 2 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 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 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 or H_2SO_4 solution to the blank, OPR, MS, and MSD.

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 cool to room temperature in a desiccator.

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 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, continuous, 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 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.

11.3.12 If only SGT-HEM 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. Using tongs, immediately remove the flask from the heat source and wipe the outside surface dry to remove moisture and fingerprints.

Note: *The analyst should carefully monitor the flask during the final stages of evaporation 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 boiling flask. This may happen if the drying capacity of the sodium sulfate is exceeded or if the sample is not adjusted to a 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 to room temperature in a desiccator and maintain in the desiccator for 30 minutes minimum. Remove with tongs and weigh immediately to 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 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 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 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 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* 4

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 ML (Section 1.6) in all samples. Report results below the ML as < 5 mg/L for HEM and SGT-HEM, or as required by the permitting authority or permit.

12.3.2 Report results to three significant figures for HEM and SGT-HEM found above the MDL (Section 1.6) in all blanks. Do not report results below the MDL unless required by the permitting authority or in the permit.

13.0 Method Performance

This method was validated through single laboratory studies and an inter-laboratory method validation study (Reference 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 "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 "Standard Methods for the Examination of Water and Wastewater", 18th Edition, American Public Health Association, 1015 Fifteenth Street, NW, Washington, D.C. 20005, Method 5520B and Method 5520F, (1992).

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 *Report of the Method 1664 Validation Studies*, April 1995. Available from the Sample Control Center (operated by DynCorp Environmental Programs Division), 300 N. Lee Street, Alexandria, VA 22314, (703) 519-1140.

17.0 Tables

Table 1. Acceptance Criteria for Performance Tests

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

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.