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Method 1654, Revision A: PAH Content of Oil by HPLC/UV

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1. SCOPE AND APPLICATION

1.1 This method is designed to determine the polynuclear aromatic hydrocarbon (PAH) content of oil by high-performance liquid chromatography (HPLC) with an ultra-violet absorption (UV) detector. The PAH content is measured and reported as phenanthrene.

1.2 This method is for use in the Environmental Protection Agency's (EPA's) survey and monitoring programs under the Federal Water Pollution Control Act.

1.3 For oil in drilling muds, this method is designed to be used in conjunction with the extraction procedure in EPA Method 1662.

1.4 The level of PAH in Table 1 typifies the minimum level that can be detected in oil with this method.

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

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC systems and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. SUMMARY OF METHOD

2.1 An oil sample is diluted in acetonitrile and a 20-µL aliquot is injected into the HPLC. The PAHs are partially separated by HPLC and detected with the UV detector.

2.2 Identification of PAH (qualitative analysis) is performed by comparing the response of the UV detector to the response during the retention-time range characteristic of the PAH in diesel oil. PAH is present when a response occurs during this retention-time range.

2.3 Quantitative analysis is performed by calibrating the HPLC with phenanthrene using an external standard technique, and using the calibration factor to determine the concentration of PAH in the sample.

2.4 Quality is assured through reproducible calibration and testing of the extraction and HPLC systems.

3. INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may lead to discrete artifacts and/or elevated baselines causing misinterpretation of chromatograms.

3.1.1 All materials used in the analysis shall be demonstrated to be free from interferences by running method blanks initially and with each sample batch (samples started through the extraction process at the same time, to a maximum of ten). Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.1.2 Glassware and, where possible, reagents are cleaned by solvent rinse and/or baking at 450°C for a minimum of 1 hour.

3.2 When used in conjunction with Method 1662, blanks extracted in that method are treated as an integral part of this method.

3.3 Interferences co-extracted from samples may vary from source to source, depending on the diversity of the site being sampled.

4. SAFETY

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

4.2 The laboratory is responsible for maintaining 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 also be made available to all personnel involved in the chemical analysis. Additional information on laboratory safety can be found in References 1 through 3.

4.3 Methylene chloride has been classified as a known health hazard. All steps in this method which involve exposure to this compound shall be per-formed in an OSHA-approved fume hood.

5. APPARATUS AND MATERIALS

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

5.1 Equipment for glassware cleaning.

5.1.1 Laboratory sink with overhead fume hood.

5.1.2 Kiln: Capable of reaching 450°C within 2 hours and holding 450°C within ± 10°C, with temperature controller and safety switch (Cress Manufacturing Co, Sante Fe Springs, CA, B31H or X31TS, or equivalent).

5.2 Equipment for sample preparation.

5.2.1 Laboratory fume hood.

5.2.2 Analytical balance: Capable of weighing 0.1 mg.

5.2.3 Glassware.

5.2.3.1 Disposable pipettes: Pasteur, 150 mm long by 5 mm i.d. (Fisher Scientific 13-678-6A, or equivalent).

5.2.3.2 Glass pipettes: 1.0- and 10-mL, accurate to 1 % or better.

5.2.3.3 Volumetric flasks: Glass, 10- and 100-mL.

5.2.4 Sample vials: Amber glass, 2- to 5-mL with PTFE-lined screw-cap, to fit HPLC autosampler.

5.3 High-performance liquid chromatograph (HPLC): An analytical system complete with pumps, sample injector, column oven, and ultra-violet (UV) detector.

5.3.1 Pumping system: Capable of isocratic operation and producing a linear gradient from 50% water/50% acetonitrile to 100% acetonitrile in 10 minutes (Waters 600E, or equivalent).

5.3.2 Sample injector: Capable of automated injection of up to 30 samples (Waters 700, or equivalent).

5.3.3 Column oven: Capable of operation at room ambient to 50°C (Waters TCM, or equivalent).

5.3.4 Column: Two C_{18} columns, 150 mm long by 4.6 mm i.d., 300 angstroms (Vydac 201 TP5415, or equivalent) connected in series, preceded by one C_{18} guard column, 30 mm long by 4.6 mm i.d., 300 angstroms (Vydac 201 GCC54T, or equivalent), operated at the conditions shown in Table 1.

5.3.5 Detector: UV operated at 254 nm (Waters 490E, or equivalent).

5.4 Data system.

5.4.1 Data acquisition: The data system shall collect and record LC peak areas and retention times on magnetic media.

5.4.2 Calibration: The data system shall be used to calculate and maintain lists of calibration factors (response divided by concentration) and multi-point calibration curves. Computations of relative standard deviation (coefficient of variation) are used to test calibration linearity.

5.4.3 Data processing: The data system shall be used to search, locate, identify, and quantify the compounds of interest in each analysis. Displays of chromatograms are required to verify results.

5.4.4 Statistics on initial (Section 8.2) and ongoing (Section 12.6) performance shall be computed and maintained.

6. REAGENTS

6.1 Solvents.

6.1.1 Sample preparation: Methylene chloride, distilled in glass (Burdick and Jackson, or equivalent).

6.1.2 HPLC: Methanol, acetonitrile, and water, HPLC quality.

6.2 Standards: Purchased as solutions or mixtures with certification to their purity, concentration, and authenticity, or prepared from materials of known purity and composition. If compound purity is 96% or greater, the weight may be used without correction to compute the concentration of the standard. If PAH in oil from drilling mud is to be tested, the diesel oil standard used in this method should be from the oil used on the drilling rig from which the mud sample is taken. If this oil is not available, No. 2 diesel oil from a local source may be substituted.

6.2.1 Stock solutions: Prepare in methylene chloride or methanol and dilute In acetonitrile for injection into the HPLC. Observe the safety precautions in Section 4.

6.2.1.1 Diesel oil solutions

6.2.1.1.1 Stock solution in methylene chloride (62.5 mg/mL): If QC extracts from Method 1662 are to be tested, use the oil that was spiked in that method. Weigh 6.25 g of diesel oil into a 100-mL ground-glass-stoppered volumetric flask and fill to the mark with methylene chloride.

6.2.1.1.2 Diesel oil calibration solution (1.25 mg/mL): After the oil in the stock solution (Section 6.2.1.1.1) is completely dissolved, remove 1.00 mL and place in a 50-mL volumetric flask. Dilute to the mark with acetonitrile. Mix thoroughly and transfer to a clean 150mL bottle with PTFE-lined cap.

6.2.1.2 Polynuclear aromatic hydrocarbons-naphthalene, phenanthrene, and indeno[1,2,3-*cd*]pyrene: Dissolve an appropriate amount of reference material in a suitable solvent. For example, weigh 10.0 mg of naphthalene in a 10-mL volumetric flask and fill to the mark with methanol. After the naphthalene is completely dissolved, transfer the solution to a 15-mL vial with PTFE-lined cap.

6.2.1.3 Stock solutions should be checked for signs of degradation prior to the preparation of calibration or performance test standards.

6.2.2 PAH calibration standards (CAL): Dilute and mix the stock solutions (Section 6.2.1.2) in acetonitrile to produce the calibration standards shown in Table 2. The three solutions permit the response of phenanthrene to be measured as a function of concentration, and naphthalene and indeno[1,2,3-*cd*]pyrene permit the retention time window for PAH to be defined. The medium-level solution is used for calibration verification (Section 12.2).

6.2.3 Precision and recovery standard: The diesel oil calibration solution (Section 6.2.1.1.2) is used for initial precision and recovery (IPR; Section 8.2) and ongoing precision and recovery (OPR, Section 12.6).

6.2.4 Stability of solutions.

6.2.4.1 When not being used, standards are stored in the dark at -20 to -10°C in screw-capped vials with PTFE-lined lids. A mark is placed on the vial at the level of the solution so that solvent loss by evaporation can be detected. The vial is brought to room temperature prior to use. Any precipitate is redissolved and solvent is added if solvent loss has occurred.

6.2.4.2 Standard solutions used for quantitative purposes (Sections 6.2.1 through 6.2.3) shall be analyzed within 48 hours of preparation and on a monthly basis thereafter for signs of degradation. Standards will remain acceptable if the peak area remains within ± 15% of the area obtained in the initial analysis of the standard.

7. CALIBRATION

7.1 Assemble the HPLC and establish the operating conditions in Table 2.

7.2 Retention time adjustment.

7.2.1 Inject 20 µL- of the medium level calibration standard (Table 2).

7.2.2 Locate the three peaks in this standard.

7.2.3 Adjust the initial solvent mixture, the isocratic hold, the gradient, and the final isocratic hold until the retention times are within $\pm$ 1 minute of the retention times given in Table 2.

7.3 Minimum level: Analyze 20 µL of the low-level calibration standard (Table 2) and verify that the HPLC instrument meets the minimum level for phenanthrene in Table 1.

7.4 External standard calibration.

7.4.1 Analyze 20 µL of each calibration standard (Table 2) beginning with the lowest concentration and proceeding to the highest using to the procedure in Section 11.

7.4.2 Record the areas for the phenanthrene peak and the height of the phenanthrene peak in the high-level standard.

7.4.3 Compute the ratio of response to amount injected (calibration factor) at each concentration by dividing the area of the peak by the concentration of the standard injected. Calculate the mean of the three values to produce an average calibration factor.

7.4.4 Linearity: If the calibration factor is constant over the three point calibration range (< 15% relative standard deviation), linearity through the origin can be assumed; if not, the system shall be recalibrated.

7.5 The average calibration factor is verified on each working 8-hour shift by the measurement of the medium-level calibration standard (Section 12.5).

7.6 Single-point calibration for diesel oil: Inject the precision and recovery standard (Section 6.2.3) to produce a single calibration point for diesel oil.

7.6.1 Integrate the area from the retention time of naphthalene (including the leading edge of the naphthalene peak) through the end of the indeno[1,2,3-*cd*]pyrene peak or until the detector signal returns to a stable baseline, whichever comes later, as shown in Figure 1.

7.6.2 Determine the calibration factor for diesel oil by dividing the integrated area (Section 7.6.1) by the diesel oil concentration (Section 6.2.1.1.2).

8. QUALITY ASSURANCE/QUALITY CONTROL

8.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 4). The minimum requirements of this program consist of an initial demonstration of laboratory capability, an ongoing analysis of standards and blanks as a test of continued performance, analyses of spiked samples to assess accuracy, and analysis of duplicates to assess precision. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method. If the determination of PAH is to be made on extracts from Method 1662, the quality control samples for initial precision and recovery (IPR), spiked samples, duplicate samples, and ongoing precision and recovery (OPR) samples from Method 1662 shall be substituted for those in the QC tests below, and the specifications in Table 1 for extracts from Method 1662 shall be met.

8.1.1 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 8.2.

8.1.2 The analyst is permitted to modify this method to improve separations or lower the costs of measurements, provided all performance requirements are met. Each time a modification is made to the method, the analyst is required to achieve the minimum level (Section 7.3) and to repeat the procedure in Section 8.2 to demonstrate method performance.

8.1.3 Analyses of spiked samples are required to demonstrate method accuracy when extracts from Method 1662 are analyzed. The procedure and QC criteria for spiking are described in Section 8.3.

8.1.4 Analyses of duplicate samples are required to demonstrate method precision when extracts from Method 1662 are analyzed. The procedure and QC criteria for duplicates are described in Section 8.4.

8.1.5 Analyses of blanks are required to demonstrate freedom from contamination. The procedures and criteria for analysis of a blank are described in Section 8.5.

8.1.6 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the precision and recovery standard that the analysis system is in control. These procedures are described in Section 12.5 and 12.6.

8.1.7 The laboratory shall maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 8.3.2 and 12.6.4.

8.2 Initial precision and recovery (IPR): The initial precision and recovery test is performed using the precision and recovery standard. If extracts from Method 1662 are to be analyzed, the extracts from the initial precision and recovery tests in that method shall be used; otherwise, the laboratory shall generate acceptable precision and recovery by performing the following operations.

8.2.1 Using diesel oil, prepare four separate aliquots of the precision and recovery standard (Section 6.2.3). If extracts from Method 1662 are analyzed, the extracts from the initial precision and recovery test in that method shall be used. Analyze these aliquots using the procedure in Section 11.

8.2.2 Using results of the set of four analyses, compute the average recovery (X) of PAH in mg/mL and the standard deviation of the recovery (s) in mg/mL for each aliquot by the external standard method (Sections 7.4 and 14.4).

8.2.3 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 oil samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. In this event, review this method, correct the problem, and repeat the test.

8.3 Method accuracy: If extracts from Method 1662 are to be analyzed, the extract from the accuracy test in that method shall be used; otherwise, an accuracy test is unnecessary. The

procedure for determining method accuracy is given in Section 8.3 of Method 1662, and the specification for accuracy is given in Table 1 of this method.

8.3.1 Compare the percent recovery of PAH with the corresponding QC acceptance criteria in Table 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 12.6.3) is within the acceptance criteria in Table 1, an interference may be present. In this case, the result may not be reported for regulatory compliance purposes. If, however, the results of both the spike and the ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control and the problem shall be identified and corrected, and the sample batch reanalyzed.

8.3.2 As part of the QA program for the laboratory, method accuracy for samples shall be assessed and records shall be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 8.3, compute the average percent recovery (P) and the standard deviation of the percent recovery (P). Express the accuracy assessment as a percent recovery interval from P - $2s_p$ to P + $2s_p$. For example, if P = 90% and s_p = 10% for five analyses of PAH in diesel oil, the accuracy interval is expressed as 70 to 110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

8.4 Duplicates: If extracts from Method 1662 are to be analyzed, the extracts from the duplicates test in that method shall be used. The procedure for preparing duplicates is given in Section 8.4 of Method 1662, and the specification for RPD is given in Table I of this method. If extracts from Method 1662 are not to be analyzed, duplicates of the precision and recovery standard (Section 6.2.3) are analyzed, and the specification for RPD is given for PAH in diesel oil in Table I of this method.

8.4.1 Analyze each of the duplicates per the procedure in Section 11 and compute the results per Section 14.

8.4.2 Calculate the relative percent difference (RPD) between the two results per the following equation:

Equation 1

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

where:
D_1 = *Concentration of diesel oil in the sample*
D_2 = *Concentration of diesel oil in the second (duplicate) sample*

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

8.5 Blanks: If extracts from Method 1662 are to be analyzed, the extracts from blanks in that method shall be analyzed in addition to the blanks in this method.

8.5.1 Rinse the glassware used in preparation of the extracts in this method with acetonitrile and analyze a 20-µL aliquot of the rinsate using the procedure in Section 11 and compute the results per Section 14.

8.5.2 If PAH is detected in a blank at greater than the method detection limit (MDL) in Table 1, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination.

8.6 The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for initial precision and recovery (IPR, Section 8.2) and ongoing precision and recovery (OPR, Section 12.6) should be identical, so that the most precise results will be obtained. The HPLC instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses given in this method.

8.7 Depending on specific program requirements, field replicates and field spikes of diesel oil into samples may be required when Method 1662 and this method are used to assess the precision and accuracy of the sampling and sample transportation techniques.

9. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

9.1 Oil samples are collected in 20- to 40-mL vials with PTFE- or aluminum-foil-lined caps and stored in the dark at -20 to -10°C.

9.2 If extracts from Method 1662 are to be analyzed, the laboratory should be aware that sample and extract holding times for this method have not yet been established. However, based on tests of wastewater for the analytes determined in this method, samples shall be extracted within 7 days of collection and extracts shall be analyzed within 40 days of extraction.

9.3 As a precaution against analyte and solvent loss or degradation, sample extracts are stored in glass bottles with PTFE-lined caps, in the dark, at -20 to -10°C.

10. DILUTION OF OIL AND EXTRACTS

10.1 Neat oil samples: If oil is received in neat form, it should be diluted to bring the concentration within the range of the instrument. If the oil is No. 2 diesel oil, the appropriate concentration will be approximately 2000 µg/mL. Mineral oils and other oils containing a lesser PAH content will require less dilution.

10.2 Extracts from Method 1662: If extracts of samples from Method 1662 are to be analyzed, these extracts (from Section 10.4.2 of that method) are analyzed undiluted unless diesel oil is known or suspected to be present. Extracts of QC samples (IPR, OPR, matrix spikes, and duplicates) from Method 1662 are diluted by a factor of 10 to bring them within the range of the HPLC.

10.3 Dilution of neat oil expected to be diesel oil.

10.3.1 Weigh 100 mg into a 10-mL volumetric flask and dilute to the mark with methylene chloride to produce a concentration of 10 mg/mL. Stopper and mix thoroughly.

10.3.2 Using a calibrated 1.0-mL volumetric pipette, withdraw 1.0 mL of the solution and place in a 10-mL volumetric flask. Then withdraw an additional 0.25 mL of the solution and place in the 10-mL volumetric flask (for a total of 1.25 mL). Fill to the mark with acetonitrile to produce a concentration of 1.25 mg/mL (1250 ug/mL). This solution will be near, but not above, the limit of the calibration range and will match the concentration of the QC samples from Method 1662 (assuming 100% recovery).

11. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

11.1 Table 2 summarizes the recommended operating conditions for the HPLC. Included in this table and in Table 1 are retention times and the minimum level that can be achieved under these conditions. An example of the separation achieved for diesel oil by the multiple HPLC column system is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements for the minimum level (Sections 7.3) and initial precision and recovery (Section 8.2) are met.

11.2 Calibrate the system as described in Section 7 or verify calibration as described in Section 12.

11.3 Analysis of extracts.

11.3.1 Inject 20 µL of the sample extract, Method 1662 extract, or diluted QC extract into the HPLC using a high-pressure syringe or a constant-volume sample-injection loop. Record the volume injected to the nearest 0.1 µL.

11.3.2 Upon injection, begin the solvent program used in calibrating the column (Section 7.2.3). Record the signal from the time of injection until the detector returns to a stable baseline. Return the solvent to the initial conditions.

11.3.3 Using the retention-time data determined during calibration, integrate the area from the retention time of naphthalene (including the leading edge of the naphthalene peak) through the end of the indeno[1,2,3-*cd*]pyrene peak or until the detector signal returns to a stable baseline, whichever comes later.

11.4 If the height of the response during the period recorded (Section 11.3.2) exceeds the height of the response for phenanthrene during calibration (Section 7.4.2), dilute the extract by successive factors of 10 with acetonitrile and reanalyze until the response is within the calibration range.

12. HPLC SYSTEM AND LABORATORY PERFORMANCE

12.1 At the beginning of each 8-hour shift during which analyses are performed, HPLC calibration and system performance are verified. For these tests, analysis of the medium-level calibration standard (Table 2) and of the diluted extract of the precision and recovery standard (Section 6.2.3) shall be used to verify all performance criteria. Adjustment and/or recalibration (per Section 7) shall be performed until all performance criteria are met. Only after all performance criteria are met may samples and blanks be analyzed.

12.2 Inject 20 µL of the medium-level calibration standard (Table 2) into the HPLC instrument according to the procedure in Section 11.

12.3 Retention time: The absolute retention times of the naphthalene, phenanthrene, and indeno[1,2,3-*cd*]pyrene peaks shall be within ±30 seconds of the respective retention times in the initial calibration (Section 7.2.3).

12.4 HPLC resolution: Resolution is acceptable if the peak width at half-height of the phenanthrene peak is less than 30 seconds.

12.5 Calibration verification: Compute the concentration of phenanthrene based on the average calibration factor (Section 7.4.4). The concentration shall be within the limits in Table 1. If calibration is verified, system performance is acceptable and analysis of blanks and QC samples may begin. If, however, the concentration falls outside of the calibration verification range, system performance is unacceptable. In this case, correct the problem and repeat the test, or recalibrate (Section 7.4).

12.6 Ongoing precision and recovery (OPR): If the extract is from Method 1662, the OPR standard from that method shall be used and the specification for the OPR from Method 1662 in Table 1 shall be met; if not, a sample of diesel oil shall be diluted per the procedure in Section 10 and shall be used for the OPR test.

12.6.1 Analyze the appropriate OPR standard.

12.6.2 Compute the concentration of PAH in this standard per Section 14.

12.6.3 Compare the concentration with the limits for ongoing precision and recovery in Table 1. If the concentration is in the range specified, the analytical processes are in control and analysis of blanks and samples may proceed. If, however, the concentration is not in the specified range, these processes are not in control. In this event, correct the problem, re-extract the sample batch if the OPR is from Method 1662, or redilute the oil sample (per Section 10.3). and repeat the ongoing precision and recovery test.

12.6.4 Add results which pass the specification in Section 12.6.3 to initial and previous ongoing data. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of percent recovery (Sr). 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%.

13. QUALITATIVE IDENTIFICATION

13.1 Qualitative determination is accomplished by comparison of data from analysis of a sample or blank with data from analysis of the calibration verification standard (Section 12.5).

13.2 PAH is identified in the sample by the presence of peaks and/or an elevated baseline (hump) between the retention times of the naphthalene and indeno[1,2,3-*cd*]pyrene peaks (Section 11.3.3), as shown in Figure 1. The experience of the analyst shall weigh heavily in interpretation of the chromatogram.

14. QUANTITATIVE DETERMINATION

14.1 Using the data system, compute the concentration of the PAH detected in the solution injected into the HPLC (in µg/mL) using the calibration factor (Section 7.4).

14.2 Concentration of PAH in oil: If neat oil was analyzed, the concentration of PAH in the oil is determined using the following equation:

Equation 2

$$C_o\,(\text{mg/g}) = \frac{C_p\,(\mu\text{g/mL})}{C_i\,(\text{mg/mL})}$$

where:
C_o *= Concentration of PAH in the oil sample*
C_p *= Concentration of PAH measured (from Sections 11.4 and 14.1)*
C_i *= Concentration of oil in the solution injected into the HPLC (from Sections 10.3.2, 11.4, and 14. 1)*

14.3 Concentration of diesel oil in QC extracts from Method 1662: Calculate the concentration of diesel oil in QC extracts from Method 1662 by integrating the area per Section 7.6.1 and using the calibration from Section 7.6.2 of this method, taking into account the dilution of these extracts (Section 10.2).

14.4 Concentration of PAH in oil from Method 1662: The PAH content of oil is complicated by the splitting and possible dilution of these extracts.

14.4.1 Concentration in undiluted extracts: This concentration is determined by Equation 3:

Equation 3

$$CO = \frac{V_e \ x \ C_p}{1/5 x W_T} = \frac{5 \ x \ I \ C_p}{W_T}$$

where:
C_o *= Concentration of PAH in the oil sample*
V_e *= Amount of extract split for HPLC analysis, in mL (1.0 mL)*
C_p *= Concentration of PAH measured*
W_r *= Weight of oil in the concentration tube in Method 1662 (Section 11.5.5 of Method 1662)*
1/5 = Fraction of this weight used for the PAH determination

14.4.2 Concentration in diluted extracts: If the extract was diluted by a factor of 10 (Section 10.3 or 11.4), the concentration determined in Section 14.4.1 is multiplied by 10.

14.5 If the concentration is to be expressed as weight percent, C_o is multiplied by 0.1.

14.6 Report results to three significant figures without correction for recovery.

15. METHOD PERFORMANCE

This method was validated in a single laboratory (Reference 6) using samples of hot-rolled drilling mud (Reference 7).

References

1. "Carcinogens-Working With Carcinogens." Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control [available through National Technical Information System, 5285 Port Royal Road, Springfield, VA 22161, document no. PB2772561: August 1977.

2. "OSHA Safety and Health Standards, General Industry [29 *CFR* 1910], Revised." Occupational Safety and Health Administration, OSHA 2206. Washington, DC: January 1976.

3. "Safety in Academic Chemistry Laboratories (3rd Edition)." American Chemical Society Publication, Committee on Chemical Safety. Washington, DC: 1979.

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

5. "Standard Practice for Sampling Water," *ASTM Annual Book of Standards*, Part 31, D337076, ASTM. Philadelphia, PA: 1980.

6. "Determination of Polynuclear Aromatic Hydrocarbons and Diesel by Modified EPA Method 9310." Prepared for the American Petroleum Institute c/o Shell Development Co, Westhollow Research Center, 3333 Highway 6 South, Houston, TX 77082 by Analytical Technologies Inc., 225 Commerce Drive, Fort Collins, CO 80524: March 29 1991, April 12, 1991, and August 18, 1992.

7. "Results of the API Study of Extraction and Analysis Procedures for the Determination of Diesel Oil in Drilling Muds (Final Report)." American Petroleum Institute, Offshore Effluent Guidelines Steering Committee, Technology Work Group, Prepared by I.C. Raia, Shell Development Co. Houston, TX: April 18, 1991.

Table 1. Performance Data and Method Acceptance Criteria for PAH

Criterion	*Units*	*PAH in Diesel Oil* [1]	*Diesel Oil in Mud Extract* [2]	*Phenanthrene*
Minimum level [3]	ug/mL	100	--	0.1
Method Detection Limit [4]	ug/mg	7.6	--	--
Initial prec and recov				
Precision (std dev)				
PAH in diesel oil [5]	mg/mL	120	--	–
Diesel in mud extract [6]	mg/mL	--	0.55	--
Recovery				
PAH in diesel oil [5]	mg/mL	1090 - 1340	–	–
Diesel in mud extract [6]	mg/mL	--	0.84 - 1.95	–
Calibration verification [7]	ug/mL	--	--	0.39 - 0.61
Ongoing prec and recov				
PAH in diesel oil [5]	mg/mL	1010 - 1450	–	–
Diesel in mud extract	mg/mL	--	0.76 - 2.15	--
Matrix spike recovery [6]	pct	--	0.43 - 2.39	--
Duplicates	RPD	9.5	44	--

Notes:
1 CAS Registry number 68534-30-5; No. 2 diesel oil used for these tests
2 From Method 1662
3 This is a minimum level at which the analytical system shall give recognizable signals and acceptable calibration points.
4 40 *CFR* Part 136, Appendix B; MDL is measured as PAH in oil
5 Test concentration of diesel oil = 1250 ug/mL
6 Test concentration in diluted extract = 1.25 mg/mL
7 Test concentration = 0.50 ug/mL

Table 2. HPLC Calibration Data

		Calibration solution concentration (µg/mL)		
Analyte	*Retention time* (minutes)*	*Low*	*Medium*	*High*
Naphthalene	7.6	-	5	-
Phenanthrene	10.3	0. 1	0.5	2.0
Indeno[l23-*cd*]pyrene	18.9	-	0.5	-
Diesel oil**	7.4-20.0	100	400	2000

* Column system: Two C_{18} columns (150 mm long by 4.6 mm i.d., 300 angstroms) connected in series, preceded by one C_{18} guard column (30 mm long by 4.6 mm i.d., 300 angstroms). Column temperature 30°C; solvent flow rate 1.5 mL/min; linear gradient from 50% water/50% acetonitrile at injection to 100% acetonitrile in 10 minutes, hold at 100% acetonitrile for 15 minutes.

** Diesel oil is calibrated separately using a single point calibration (Section 7.6).

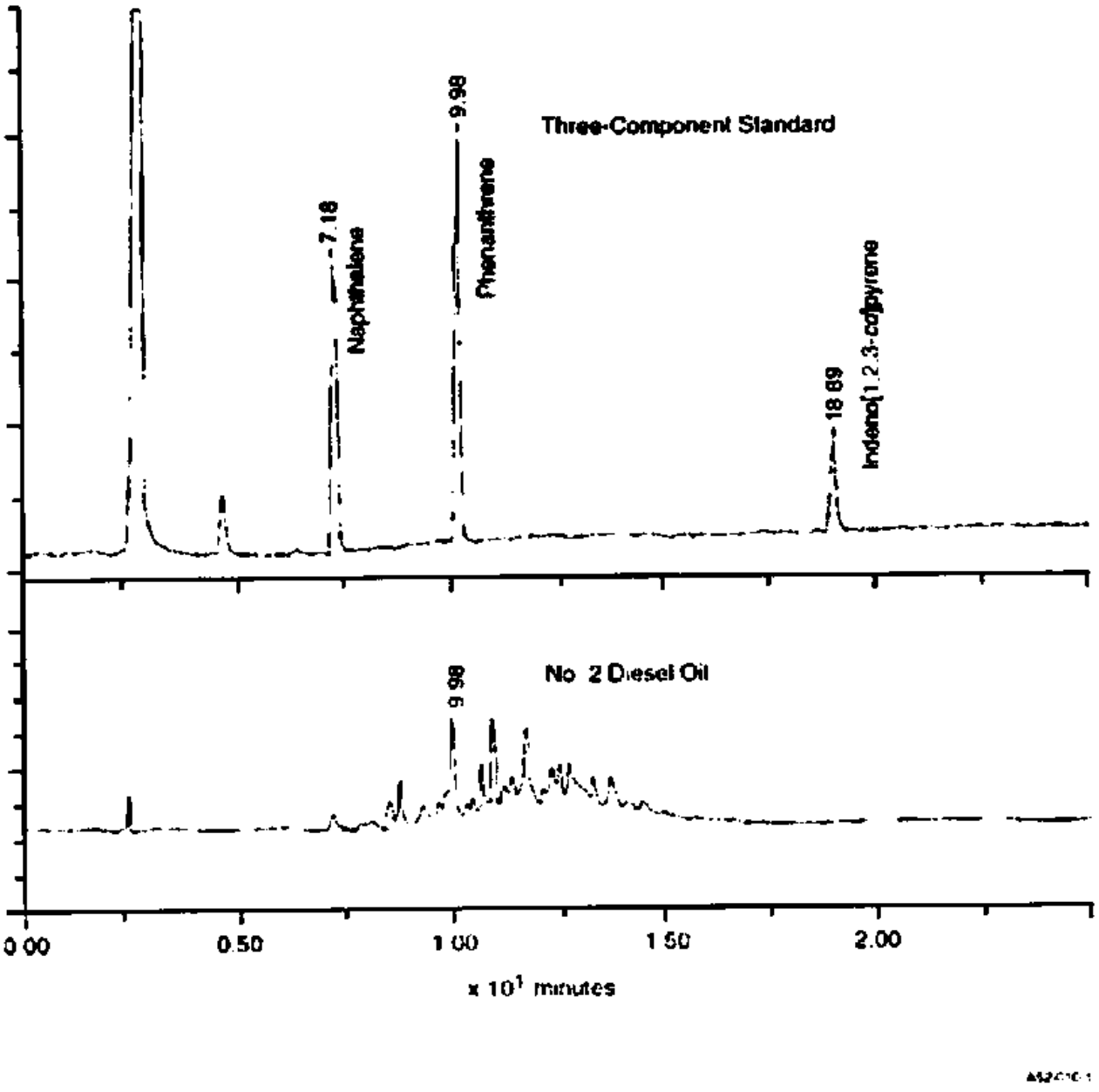

Figure 1. Liquid Chromatography of the Three-Component Standard and of No. 2 Diesel Oil