Designation: D5412 – 93 (Reapproved 2011)$^{\varepsilon 1}$

Standard Test Method for Quantification of Complex Polycyclic Aromatic Hydrocarbon Mixtures or Petroleum Oils in Water[1]

This standard is issued under the fixed designation D5412; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Editorial corrections were made throughout in March 2014.

1. Scope

1.1 This test method covers a means for quantifying or characterizing total polycyclic aromatic hydrocarbons (PAHs) by fluorescence spectroscopy (Fl) for waterborne samples. The characterization step is for the purpose of finding an appropriate calibration standard with similiar emission and synchronous fluorescence spectra.

1.2 This test method is applicable to PAHs resulting from petroleum oils, fuel oils, creosotes, or industrial organic mixtures. Samples can be weathered or unweathered, but either the same material or appropriately characterized site-specific PAH or petroleum oil calibration standards with similar fluorescence spectra should be chosen. The degree of spectral similarity needed will depend on the desired level of quantification and on the required data quality objectives.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water
D3325 Practice for Preservation of Waterborne Oil Samples
D3326 Practice for Preparation of Samples for Identification of Waterborne Oils
D3415 Practice for Identification of Waterborne Oils
D3650 Test Method for Comparison of Waterborne Petroleum Oils By Fluorescence Analysis
D4489 Practices for Sampling of Waterborne Oils
D4657 Test Method for Polynuclear Aromatic Hydrocarbons in Water (Withdrawn 2005)[3]
E131 Terminology Relating to Molecular Spectroscopy
E169 Practices for General Techniques of Ultraviolet-Visible Quantitative Analysis
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers
E388 Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers
E578 Test Method for Linearity of Fluorescence Measuring Systems
E579 Test Method for Limit of Detection of Fluorescence of Quinine Sulfate in Solution

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D1129, Terminology E131, and Practice D3415.

4. Summary of Test Method

4.1 This test method consists of fluorescence analysis of dilute solutions of PAHs or petroleum oils in appropriate solvents (spectroquality solvents such as cyclohexane or other appropriate solvents, for example, ethanol, depending on polarity considerations of the sample). The test method requires an initial qualitative characterization step involving both fluorescence emission and synchronous spectroscopy in order to select appropriate calibration standards with similar fluorescence spectra as compared to the samples (see Annex A1 for the definition of spectral similarity). Intensities of peak

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

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maxima of suitable emission spectra are then used to develop calibration curves for quantification.

NOTE 1—Although some sections of the characterization part of this test method are similar to Test Method D3650, there are also significant differences (see Annex A1). Since the purpose and intent of the two test methods are different, one should not be substituted for the other.

5. Significance and Use

5.1 This test method is useful for characterization and rapid quantification of PAH mixtures including petroleum oils, fuels, creosotes, and industrial organic mixtures, either waterborne or obtained from tanks.

5.2 The unknown PAH mixture is first characterized by its fluorescence emission and synchronous scanning spectra. Then a suitable site-specific calibration standard with similar spectral characteristics is selected as described in Annex A1. This calibration standard may also be well-characterized by other independent methods such as gas chromatography (GC), GC-mass spectrometry (GC-MS), or high performance liquid chromatography (HPLC). Some suggested independent analytical methods are included in References (**1-7**)[4] and Test Method D4657. Other analytical methods can be substituted by an experienced analyst depending on the intended data quality objectives. Peak maxima intensities of appropriate fluorescence emission spectra are then used to set up suitable calibration curves as a function of concentration. Further discussion of fluorescence techniques as applied to the characterization and quantification of PAHs and petroleum oils can be found in References (**8-18**).

5.3 For the purpose of the present test method polynuclear aromatic hydrocarbons are defined to include substituted polycyclic aromatic hydrocarbons with functional groups such as carboxyl acid, hydroxy, carbonyl and amino groups, and heterocycles giving similar fluorescence responses to PAHs of similar molecular weight ranges. If PAHs in the more classic definition, that is, unsubstituted PAHs, are desired, chemical reactions, extractions, or chromatographic procedures may be required to eliminate these other components. Fortunately, for the most commonly expected PAH mixtures, such substituted PAHs and heterocycles are not major components of the mixtures and do not cause serious errors.

6. Interferences

6.1 The fluorescence spectra may be distorted or quantification may be affected if the sample is contaminated with an appreciable amount of other fluorescent chemicals that are excited and which fluoresce in the same spectral regions with relatively high fluorescence yields. Usually the fluorescence spectra would be distorted at levels greater than 1 to 2 % of such impurities before the quantification would be seriously affected.

NOTE 2—**Caution:** Storage of samples in improper containers (for example, plastics other than TFE-fluorocarbon) may result in contamination.

NOTE 3—*Spectroquality* solvents may not have low enough fluorescence background to be used as solvent blanks. Solvent lots vary in the content of fluorescent impurities that may increase with storage time even for unopened bottles.

NOTE 4—This test method is normally used without a matrix spike due to possible fluorescence interference by the spike. If a spike is to be used, it must fluoresce in a spectral region where it will not interfere with the quantification process. Compounds that could be used are dyes that fluoresce at longer wavelengths than the emission of the PAH mixture.

6.2 If the PAH mixture to be analyzed is a complex mixture such as an oil or creosote, it is assumed that a well-characterized sample of the same or similar material is available as a calibration standard so the fluorescent fraction of the mixture can be ratioed against the total mixture. Otherwise, since the samples and standards are weighed, the nonfluorescent portion of the mixture would bias the quantification although the characterization portion of the test method for PAHs given in Annex A1 would be unaffected.

7. Apparatus

7.1 *Fluorescence Spectrometer*—An instrument recording in the spectral range of 250 nm to at least 600 nm for both excitation and emission responses and capable of scanning both monochromators simultaneously at a constant speed with a constant wavelength offset between them for synchronous scanning. The instrument should meet the specifications in Table 1. (Also known as spectrofluorometer or fluorescence spectrophotometer.) Consult manufacturer's instrument manuals for specific operating instructions.

NOTE 5—Although the characterization section of this test method (given in Annex A1) is similar to Test Method D3650 in many respects, there are differences in the purpose and intents of the two test methods. The purpose of the characterization step of this test method is to find an oil with similar fluorescence properties as the sample in order to serve as an appropriate calibration standard for quantification. Other differences between the test methods are instrumentation requirements and the use of synchronous spectra as well as emission spectra for this test method.

7.2 *Excitation Source*—A high-pressure xenon lamp (a 150-W continuous xenon lamp or a 10-W pulsed xenon lamp has been proven acceptable). Other continuum sources (either continuous or pulsed) having sufficient intensity throughout the ultraviolet and visible regions may also be used.

7.3 *Fluorescence Cells*—Standard cells made from fluorescence-free fused silica with a path length of 10 mm and a height of at least 45 mm. Stoppered cells may be preferred to prevent sample evaporation and contamination.

TABLE 1 Specifications for Fluorescence Spectrometers

Wavelength Reproducibility	
Excitation monochromator	±2 nm or better
Emission monochromator	±2 nm or better
Gratings (Typical Values)	
Excitation monochromator	minimum of 600 lines/mm blazed at 300 nm
Emission monochromator	minimum of 600 lines/mm blazed at 300 nm or 500 nm
Photomultiplier Tube	
S-20 or S-5 response or equivalent	
Spectral Resolutions	
Excitation monochromator	spectral bandpass of 2.5 nm or less
Emission monochromator	spectral bandpass 2.5 nm or less
Maximum bandpasses for both monochromators at least 10 nm	

[4] The boldface numbers in parentheses refer to a list of references at the end of this standard.

7.4 *Data Recording System*—Preferably the instrument should be interfaced to a suitable computer system compatible with the instrument and with suitable software for spectral data manipulation. Use of a strip chart or X-Y recorder with a response time of less than 1 s for full-scale deflection is acceptable.

7.5 *Micropipet,* glass, 10 to 50-μL capacity.

7.6 *Weighing Pans,* 5 to 7-mm diameter, 18-mm thick, made of aluminum or equivalent. Check pans for contamination.

8. Reagents and Materials

8.1 *Purity of Reagents*—Use spectroquality grade reagents in all instances unless otherwise stated. Since the goal is to have as low a fluorescence blank as possible, and since different brands and lots of spectroquality solvent may vary, check reagents frequently.

8.2 *Purity of Water*—References to water mean Type IV water conforming to Specification D1193. Since fluorescent organic impurities in the water may introduce an interference, check the purity of the water by analyzing a water blank using the same instrumental conditions as for the solvent blank.

8.3 *Acetone,* spectroquality, (CH_3COCH_3).

8.4 *Cyclohexane,* spectroquality or HPLC grade. The fluorescence solvent blank must be as low as possible and less than 5 % of the intensity of the maximum emission peak for the lowest concentration of PAHs analyzed. Dispense cyclohexane during the procedure from either a TFE-fluorocarbon or glass wash bottle, but, for prolonged storage, store cyclohexane only in glass.

8.5 *Nitric Acid* (1 + 1)—Carefully add one volume of concentrated HNO_3 (sp gr 1.42) to one volume of water..

8.6 *TFE-Fluorocarbon Strips,* 25 mm by 75 mm, 0.25-mm thickness. Use TFE strips when sampling neat PAH films on water as described in Practices D4489.

9. Sampling and Sample Preparation

9.1 Collect a representative sample (see Practices D4489 for water samples).

9.2 Preserve samples in containers as specified in Practice D3325. Do not cool samples below 5°C to avoid dewaxing of oil or creosote samples.

9.3 Neat PAH samples (including surface films or layers on water) require only dilution in spectroquality cyclohexane. Prepare initial concentration for the unknown at 100 μg/mL for a check of the fluorescence signal. Further dilutions down to 1 μ/mL may be needed to bring the fluorescence signal into the linear range and to avoid self-absorption effects in the solution. Most PAH mixtures and oils have been found to be soluble in cyclohexane at the concentrations listed. Alternative solvents can be substituted with appropriate tests.

9.4 If any unknown PAH mixture is dissolved in water, test the mixture with appropriate dilutions or preconcentrations as required. The assumption is that no naturally-occurring fluorescent materials such as humic or fulvic acids are present at levels interfering with the determination (refer to Fig. A2.5 and Fig. A2.6 to show that humic acid does not interfere with the test method even at high (μg/L) levels). This usually becomes a problem only at PAH levels in the low μg/L range. Extraction methods (or separation by column chromatography) are listed in Practice D3326.

9.4.1 An extraction method that proved satisfactory for the collaborative test is as follows:

9.4.1.1 Pour 50.0 mL of the sample into a separatory funnel, add 5.0 mL of cyclohexane and shake for 2 min. Vent the separatory funnel occasionally. Withdraw the aqueous layer (keep this for a second extraction). Collect the cyclohexane extract in a 10-mL volumetric flask. Add 5.0 mL of cyclohexane to the aqueous layer and perform a second extraction. Combine the two extracts and dilute to 10.0 mL with cyclohexane.

9.4.1.2 For field use, it has proven satisfactory to use a reagent bottle instead of a separatory funnel. Pour 50.0 mL of the sample in the bottle and add 5.0 mL of cyclohexane, shake for 2 min and collect most of the top layer with a Pasteur pipet. It is important to collect most of the top layer to maximize percent recovery (tilt the flask to see the separation between the two layers more easily). Add 5.0 mL of cyclohexane to the aqueous layer and perform a second extraction. Combine the two cyclohexane extracts and dilute to 10.0 mL with cyclohexane.

9.4.1.3 See 12.6 to check extraction recoveries. Other extraction methods can be used at the discretion of the analyst, by adding an appropriate solvent exchange step to cyclohexane and by checking for recoveries and interferences. As is always the case, the analyst shall demonstrate method performance when changing the method. At the mg/L level or above, the PAH mixture might not be totally in solution. If the PAH mixture is emulsified in water, is sparingly soluble in water, or if the concentration of the unknown must be known more accurately, it may be necessary to evaporate the solution to dryness or to extract the PAH mixture into a suitable solvent, followed by evaporation, weighing, and redissolving in cyclohexane.

9.4.1.4 At the mg/L level or above, the PAH mixture in water might not be totally in solution.

9.5 Sample bottles must be made of glass, precleaned with dilute nitric acid (1 + 1) and sealed with plastic screw caps having TFE-fluorocarbon liners. Solutions must be prepared in precleaned volumetric flasks. Because many aromatics are subject to photodegradation, flasks must be low-actinic (amber) or covered with aluminum foil. Volumetric flasks and fluorescence cells must be cleaned with dilute nitric acid followed by rinsing with water and then air-drying them. To remove the water more quickly, use a triple rinse with spectroquality acetone. As a final step, triple rinse glassware and cells with the solvent used for analysis, usually cyclohexane.

10. Preparation of Apparatus

10.1 Set up and calibrate the fluorescence spectrometer according to the manufacturer's instructions and Practices E169 and E275 and Test Methods E388, E578, and E579. Include in the calibration procedures a check of wavelength