Designation: D3650 – 93 (Reapproved 2011)

Standard Test Method for Comparison of Waterborne Petroleum Oils By Fluorescence Analysis[1]

This standard is issued under the fixed designation D3650; 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.

1. Scope*

1.1 This test method covers the comparison of waterborne petroleum oils with oils from possible sources by means of fluorescence spectroscopy **(1).**[2] Useful references for this test method include: **(2)** and **(3)** for fluorescence analysis in general and **(4), (5),** and **(6)** for oil spill identification including fluorescence.

1.2 This test method is applicable to crude or refined petroleum products, for any sample of neat oil, waterborne oil, or sample of oil-soaked material. Unless the samples are collected soon after the spill occurs, it is not recommended that volatile fuels such as gasoline, kerosine, and No. 1 fuel oils be analyzed by this test method, because their fluorescence signatures change rapidly with weathering. Some No. 2 fuel oils and light crude oils may only be identifiable up to 2 days weathering, or less, depending on the severity of weathering. In general, samples weathered up to 1 week may be identified, although longer periods of weathering may be tolerated for heavy residual oils, oil weathered under Arctic conditions, or oil that has been protected from weathering by collecting in a thick layer.

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:*[3]

D1129 Terminology Relating to Water
D1193 Specification for Reagent 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
D4489 Practices for Sampling of Waterborne Oils
E131 Terminology Relating to Molecular Spectroscopy
E275 Practice for Describing and Measuring Performance of Ultraviolet and Visible Spectrophotometers
E520 Practice for Describing Photomultiplier Detectors in Emission and Absorption Spectrometry

3. Terminology

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

4. Summary of Test Method

4.1 This test method consists of fluorescence analyses of dilute solutions of oil in spectroquality cyclohexane. In most cases the emission spectra, with excitation at 254 nm, over the spectral range from 280 to 500 nm, are adequate for matching.

4.2 Identification of the sample is made by direct visual comparison of the sample's spectrum with the spectra from possible source samples.

NOTE 1—When weathering has occurred, it may be necessary to consider known weathering trends when matching spectra (Fig. 1 and Fig. 2).

5. Significance and Use

5.1 This test method is useful for rapid identification of waterborne petroleum oil samples as well as oil samples

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] The boldface numbers in parentheses refer to the references at the end of this test method.

[3] 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.

***A Summary of Changes section appears at the end of this standard**

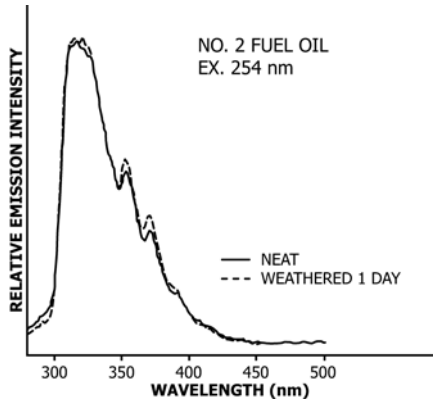

FIG. 1 Fluorescence Spectra for a Typical No. 2 Fuel Oil (Unweathered and Weathered One Day)

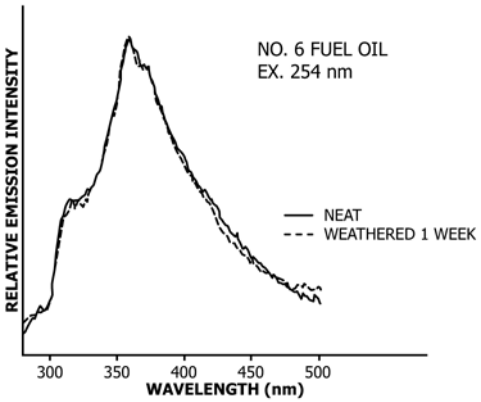

FIG. 2 Fluorescence Spectra for a Typical No. 6 Fuel Oil (Unweathered and Weathered One Day)

obtained from fuel or storage tanks, or from sand, vegetation, or other substrates. This test method is applicable to weathered and unweathered neat oil samples.

5.2 The unknown oil is identified through the comparison of the fluorescence spectrum of the oil with the spectra (obtained at similar instrumental settings on the same instrument) of possible source samples. A match of the entire spectrum between the unknown and possible source sample indicates a common source.

6. Interferences

6.1 The fluorescence spectrum will be distorted if an oil sample has been contaminated by an appreciable amount, for example, 1 % of common chemical impurities such as other oils that are fluorescent on excitation at 254 nm.

NOTE 2—Storage of samples in improper containers (for example, plastics) may result in contamination. This interference can be eliminated by observing proper procedures for collection and preservation of samples. Refer to Practice D3325.

NOTE 3—"Spectroquality" cyclohexane may not have a low enough fluorescence solvent blank. Lots vary in the content of fluorescent impurities, which may increase with storage time even if the bottle is unopened.

6.2 Oil residues may build up in fluorescence cells particularly after prolonged usage with heavy oils. In such a case, follow the procedure using nitric acid for cleaning glassware (10.1.3).

6.3 Possible interferences from Raman or RayleighTyndall scattering are not observed in the emission scan ranges selected.

7. Apparatus

7.1 *Fluorescence Spectrophotometer (or Spectrofluorometer)*—An instrument recording in the spectral range of 220 nm to at least 600 nm for both excitation and emission responses and capable of meeting the specifications stated in Table 1.

7.2 *Excitation Source*—A high-pressure xenon lamp (a 150-W xenon lamp has proven acceptable). Other continuum sources, such as deuterium or high-pressure xenon-mercury, which have sufficient intensity in the ultraviolet region, could be used as excitation sources.

NOTE 4—Line sources such as a low-pressure mercury lamp may also be used for excitation at 254 nm, if the flexibility of using arbitrary excitation wavelengths or excitation spectra is not desired and if source intensity is adequate.

7.3 *Fluorescence Cells*—Standard cells, made from fluorescence-free fused silica with a pathlength of 10 mm and a height of 45 mm.

7.4 *Recorder or Computer*—Strip chart or *X-Y* recorder, with a response time less than 1 s for full-scale deflection, or a computer capable of digitizing the data at a rate of 1 data point per nanometre.

7.5 *Cell-Filling Device*—Disposable Pasteur capillary pipet.

7.6 *Volumetric Flasks*—Low-actinic glass, ground-glass stoppered volumetric flasks (100-mL).

7.7 *Micropipet,* 10 to 50-µL capacity.

7.8 *Analytical Balance,* with a precision of at least ±0.1 mg.

7.9 *Weighing Pans,* 5 to 7-mm diameter, 18 mm deep, made of aluminum or equivalent.

TABLE 1 Specifications for Fluorescence Spectrophotometers

Wavelength Reproducibility	
Excitation monochromator	better than± 2 nm
Emission monochromator	better than ±2 nm
Gratings (Typical Values)	
Excitation monochromator	minimum of 600 lines/mm blazed at 300 nm [A]
Emission monochromator	minimum of 600 lines/mm blazed at 300 nm or 500 nm [A]
Photomultiplier Tube [B]	
Either S-20 [C] or S-5 [D] Response [E]	
Resolution	
Excitation monochromator	better than 2 nm
Emission monchromator	better than 2 nm
Time Constant	
not to exceed one second	

[A] Or designed to have a good efficiency in this spectral region.
[B] See Practice E520.
[C] Photomultiplier tubes such as Hamamatsu R-446-UR.
[D] Photomultiplier tubes such as RCA 1P28 or Hamamatsu R-106.
[E] Or equivalent having a good spectral response in the spectral region from 280 to 600 nm.

7.10 *Test Tubes*, disposable 15-mL glass test tubes.

7.11 *Micropipet*, or microsyringe, 9-µL capacity; with an accuracy of 1 % and reproducibility of 0.1 % of pipet capacity.

7.12 *Micropipet*, 200-µL capacity with disposable tips; with an accuracy of 1 % and reproducibility of 0.1 % of pipettor capacity.

7.13 *Solvent Dispenser*, adjustable to deliver 10 mL.

7.14 *Vortex Mixer*.

8. Reagents and Materials

8.1 *Purity of Reagents*—Spectroquality grade reagents should be used in all instances unless otherwise stated. It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4]

8.2 *Purity of Water*— References to water shall be understood to mean Type IV reagent water conforming to Specification D1193. However, since fluorescent organic impurities in the water may constitute an interference, the purity of the water should be checked by running a water blank using the same instrument conditions as for the solvent blank.

8.3 *Acetone* (CH_3COCH_3).

8.4 *Nitric Acid* (sp gr 1.42)—Concentrated nitric acid (HNO_3).

8.5 *Cyclohexane,* spectroquality grade, with a fluorescence solvent blank less than 2 % of the intensity of the major peak of the sample fluorescence generated with the same instrumental settings over the emission range used. Cyclohexane is dispensed throughout the procedure from a 500-mL TFE-fluorocarbon wash bottle. For prolonged storage, cyclohexane should be stored only in glass. Check the suitability of the solvent by running a solvent blank. The solvent blank can also be used to check for scatter.

NOTE 5—Cyclohexane can be reused, if necessary, after one or more distillations in an all-glass still. The distilled cyclohexane must have no detectable fluorescence (<2 %) in the 280 to 500-nm region of the spectrum when excited at 254 nm.

NOTE 6—Methylcyclohexane can also be used as a solvent, instead of cyclohexane. This is useful, particularly if the solution is needed for low-temperature luminescence measurements as well.

8.6 *Aluminum Foil.*

9. Sampling and Sample Preparation

9.1 Collect a representative sample as directed in Practice D4489.

9.2 Preserve samples in containers as specified in Practice D3325. However, to avoid dewaxing, do not cool samples below 5°C.

9.3 *Preparation of Oil Samples,* as described in Practices D3326. Avoid the use of deasphalting procedures, if possible. Spectroquality cyclohexane is the preferred solvent for sample preparation for fluorescence.

9.4 *Preparation of Solutions for Fluorescence Analysis*—Either of the following techniques for diluting the prepared oil sample with cyclohexane may be used:

9.4.1 *Weighing Technique*—To prepare oil solutions at a concentration of approximately 20 µg/mL, weigh out 0.0016 ± 0.0001 g of oil (equivalent weight for each sample) onto a clean aluminum weighing pan using a micropipet. Transfer weighed oil sample into a clean 100 mL, low-actinic glass volumetric flask by creasing the aluminum pan and washing the oil directly into the volumetric flask using spectroquality cyclohexane dispensed from a TFE-fluorocarbon wash bottle. Dilute the solution up to volume (100 mL) and shake vigorously several times and allow the prepared solution to stand for 30 min and shake again prior to performing the analysis to ensure that all oil dissolves. Occasionally, depending on fluorescence yield of the oil tested and instrumentation used, it may be necessary to use 100 ppm concentration to get adequate fluorescence intensity. In these cases, weigh out 0.0078 ± 0.0001 g of oil and proceed as above.

NOTE 7—It is preferable that the prepared solution be used the same day. Do not use solutions that have been standing for periods in excess of 6 h unless they have been refrigerated. In no case use solutions more than 2 days old.

9.4.2 *Volume Technique*—Allow the prepared oil sample to come to room temperature and shake until they are homogeneous. Transfer 9 µL of the oil to a 15-mL disposable glass test tube with a micropipet or microsyringe and add 10 mL of spectroquality cyclohexane with a solvent dispenser. Place a cap of aluminum foil over the top of the test tube and vortex for approximately 30 s. With a micropipet, transfer 200 µL of this solution to a second 15-mL test tube and then add 10 mL of cyclohexane. Place a cap of aluminum foil over the top of the second test tube and vortex for approximately 30 s. Prepare all samples in this manner.

NOTE 8—If a micropipet with disposable plunger and tips is used, potential cross contamination is avoided. Otherwise, careful cleaning following the procedures specified in 10.1 is required.

10. Preparation of Apparatus

10.1 *Cleaning Glassware:*

10.1.1 Clean all glassware used in this procedure in the following manner: first rinse volumetric flasks and cells three times with spectroquality cyclohexane. Prior to the use of glassware and cells throughout this procedure, rinse again with spectroquality cyclohexane.

10.1.2 If there is water present, rinse the glassware three times with spectroquality acetone, and then three times with cyclohexane as in 10.1.1. Use detergents only if they have been checked for low fluorescence. If laboratory detergent solutions are used, repeated rinsing with Type IV reagent (see 8.3) water will be required.

10.1.3 When working with heavy oils, a cleaning procedure using organic solvents may not be sufficient. Heavy oils build up a residue on cells that solvent cleaning will not remove. If the solvent blank shows significant impurities, a residual film on the cell, rather than an impure solvent, may be the cause.

[4] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Analar Standards for Laboratory Chemicals," BDH Ltd., Poole, Dorset, U.K., and the "United States Pharmacopeia."

Soak the cells in undiluted nitric acid for 1 h. Observe proper safety precautions by using adequate eye and hand protection. Rinse the cells repeatedly with Type IV reagent water, and then proceed as in 10.1.2.

10.2 *Calibration of Spectrophotometer :*

10.2.1 Adjust and calibrate the spectrophotometer (that is, the emission and excitation monochromators) using a low-pressure mercury lamp (or similar line source). Refer to Practice E275 for the approved calibration method.

11. Procedure for Recording Fluorescence Emission Spectrum

11.1 Fill a clean fluorescence cell with oil solution using a Pasteur pipet or similar techniques, taking care not to contaminate the outside of the cell. Gently wipe the outside of the cell with lens paper (nonsilicone-treated) and place the cell in the sample compartment.

11.2 Set the excitation monochromator at 254 nm.

11.3 Set the slit width of the excitation monochromator between 5 and 35 nm depending on the intensity required. E131-nm slit width is recommended. Set the emission slit width to a maximum of 2.5 nm and a minimum of 1 nm.

NOTE 9—Commercial instruments have a wide variation in available slit settings, some being fixed and others adjustable. In the recording of all spectra for a given set of samples, it is important that the excitation and emission slit widths be kept constant. This will eliminate the probability of spectral changes for a given oil that would result from slit-width variations.

11.4 If using a recorder, scan the fluorescence range from 280 to 500 nm to determine the wavelength of the major fluorescence response. Adjust the signal to 95 ± 2 % of full scale. If a computer is used, plot the data at 100 % of full scale at the wavelength of the maximum fluorescence response.

NOTE 10—For comparison of samples, it is important that signals be normalized to the same point. The normalization of 95 ± 2 % of full scale was chosen for recorders to allow slight variations in intensity that might result in an off-scale response.

11.5 Remove the sample cell, discard the solution, and place a fresh (nonirradiated) solution of the sample in the cell (for each spectral scan). Replace the cell in the sample compartment.

NOTE 11—The solution is replaced with a fresh solution to prevent the possibility of errors in the recorded spectrum of the oil through photodecomposition of the sample by prolonged exposure of the sample to high intensity ultraviolet light.

11.6 Scan the emission spectrum from 280 to 500 nm at an emission monochromator scan speed not less than 25 nm/min or greater than 60 nm/min. For chart recorders, a chart speed not to exceed 60 nm/min is recommended.

11.7 If the results (see 12.3) suggest that additional information is needed, excitation at wavelengths other than 254 nm may be used. Follow the procedure for 11.1 to 11.6, except in 11.2 set the excitation monochromator at either 270 nm for light oils or 290 nm for medium and heavy oils. The emission scan should begin at 300 nm or 325 nm, respectively, and should cover a range of at least 220 nm.

12. Interpretation of Spectra

12.1 Overlay the spectrum of the unknown sample with the spectra of the suspect samples. Note five features when comparing the oil spectra: (*1*) general shape, (*2*) number of peaks, (*3*) wavelengths corresponding to the peaks, (*4*) ratios of the major peak intensities, and (*5*) contours of the spectra. Two spectra match if (*1*), (*2*), and (*3*) are the same and (*4*) and (*5*) change ± 4 % relative to the major peak or less. Any change in (*4*) or (*5*) should conform to expected weathering trends.

12.2 Certain spectral changes can be expected with weathering. The degree of weathering depends upon environmental conditions and oil type. For most light oils, the intensity and spectral structure on the long-wavelength side of the major peak increase. (Refer to Fig. 1 for a typical example.) For heavy fuel oils, the long-wavelength side decreases in intensity and structure. (Refer to Fig. 2 for a typical example.) See Refs **(1)** and **(6)** for a discussion of weathering.

12.3 If the spectra are not close enough to be called a match under the criteria in 12.1, but the patterns conform to the weathering changes in 12.2, additional fluorescence spectra exciting at other wavelengths may be taken. This may be useful for additional evidence of matches, or may discriminate between oils if there appears to be more than one match. Also, additional spectra may be useful if contamination is suspected. Wavelengths that may be chosen for additional spectra are 270 nm for light oils and 290 nm for medium and heavy oils. If additional excitation wavelengths are used, the oil spectra so generated must match for every excitation wavelength used for the oils to be considered a match. Otherwise, the oils are a nonmatch or at least one of the samples is contaminated.

NOTE 12—Commercial instrumentation is not uniform in design. The difference in available slits, gratings, and photomultiplier tube selections will produce variations in the recorded fluorescence spectra. Therefore, the comparison of spectra can only be made for spectra recorded on a particular instrument and cannot be compared from instrument to instrument with the possible exception of spectrally corrected spectrofluorometers.

12.4 Based upon the comparison of the spectra according to the criteria in 12.1 and 12.2, classify the comparison of each spill sample with every other spill sample and with suspected source samples as belonging to one of the categories below:

12.4.1 *Match* (M)—The spectra must virtually overlay (less than 1 % deviation relative to the major peak at every point in a point by point comparison) and those minor differences must be attributable to weathering trends discussed in 12.1 and 12.2.

12.4.2 *Probable Match* (PM)—Similar data showing moderate differences (within 4 % deviation relative to the major peak at every point in a point by point comparison) and those minor differences must be attributable to weathering trends discussed in 12.1 and 12.2.

12.4.3 *Indeterminate* (I)—The data appear somewhat similar, but the differences exceed those described in 12.1 or are not consistent with the weathering trends described in 12.2. Contamination by a fluorescent impurity may also result in an indeterminate comparison.

12.4.4 *Nonmatch* (NM)—The data appear different with respect to one or more of the criteria noted in 12.1.

13. Quality Assurance

13.1 If the analysis of the quality control sample described in 5.5 of Practice D3415 does not meet the criteria for a match, the results of all the comparisons are invalid.

14. Reporting Results

14.1 Additional data from other independent analytical methods may be helpful and are desirable in confirming this conclusion. Refer to Practice D3415 for a discussion of other ASTM methods for oil identification.

15. Precision and Bias

15.1 No statement is made about either the precision or bias of this test method since the result merely states whether there is conformance to the criteria for success specified in the procedure.

16. Keywords

16.1 fluorescence spectral emission; fluorescence spectroscopy; oil identification; UV-VIS fluorescence; waterborne petroleum oils

REFERENCES

(1) This test method is based on a modification of a published report, "Oil Spill Identification System," Chemistry Branch, U. S. Coast Guard Research and Development Center, Report No. CG-D-41-75, October 1974. (Available to the public through the National Technical Information Service, Springfield, VA 22161. No. *ADA 003803*).
The following are useful references for fluorescence analysis in general: **(2)** and **(3),** and oil spill identification including fluorescence: **(4), (5), (6), (7),** and **(8).**

(2) Parker, C. A., *Photoluminescence of Solutions,* Elsevier, NY, 1968.

(3) Becker, R. S., *Theory and Interpretation of Fluorescence and Phosphorescence,* John Wiley & Sons, New York, NY, 1969.

(4) Adlard, E. R., *Journal of the Institute of Petroleum*, Vol 58, 1972, p. 63.

(5) Bentz, A. P., "Oil Spill Identification," *Analytical Chemistry*, Vol 48, 1976, pp. 454–472. A general review which lists recent references on fluorescence of oil samples from oil spills.

(6) Fortier, S. H., and Eastwood, D., *Analytical Chemistry*, Vol 50, 1978, p. 334.

(7) Eastwood, D., "Use of Luminescence Spectroscopy in Oil Identification," *Modern Fluorescence Spectroscopy*, D. Eastwood, Ed., *ASTM STP* 822, ASTM, 1983.

(8) Eastwood, D., and Lidberg, R. L., "Application of Fluorescence and FTIR Techniques to Screening and Classifying Hazardous Waste Sample," Proceedings of 7th National Conference on Management of Uncontrolled Hazardous Waste Sites, Washington, D.C., 1986, pp. 370–379.

SUMMARY OF CHANGES

This section identifies the location of selection changes to these test methods that have been incorporated since the 1982 issue. For the convenience of the user, Committee D-19 has highlighted those changes that may impact the use of these test methods. This section may also include descriptions of the changes or reasons for the changes, or both.

(*1*) Paragraph 9.4.2 suggests a simplified solution preparation technique that avoids the variability in weighing out microgram quantities of oil and reduces the volume of solvent used for the Method.

(*2*) Sections referring to sample preparation have been removed. They can be found in the revised version of Practices D3326.

(*3*) References **(7)** and **(8)** have been added.