Designation: D3326 – 07 (Reapproved 2011)

Standard Practice for
Preparation of Samples for Identification of Waterborne Oils[1]

This standard is issued under the fixed designation D3326; 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 practice covers the preparation for analysis of waterborne oils recovered from water. The identification is based upon the comparison of physical and chemical characteristics of the waterborne oils with oils from suspect sources. These oils may be of petroleum or vegetable/animal origin, or both. Seven procedures are given as follows:

	Sections
Procedure A (for samples of more than 50-mL volume containing significant quantities of hydrocarbons with boiling points above 280°C)	8 to 12
Procedure B (for samples containing significant quantities of hydrocarbons with boiling points above 280°C)	13 to 17
Procedure C (for waterborne oils containing significant amounts of components boiling below 280°C and to mixtures of these and higher boiling components)	18 to 22
Procedure D (for samples containing both petroleum and vegetable/animal derived oils)	23 to 27
Procedure E (for samples of light crudes and medium distillate fuels)	28 to 34
Procedure F (for thin films of oil-on-water)	35 to 39
Procedure G (for oil-soaked samples)	40 to 44

1.2 Procedures for the analytical examination of the waterborne oil samples are described in Practice D3415, D3328, D3414, and D3650. Refer to the individual oil identification test methods for the sample preparation method of choice. The deasphalting effects of the sample preparation method should be considered in selecting the best methods.

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.* Specific caution statements are given in Sections 6 and 32.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D95 Test Method for Water in Petroleum Products and Bituminous Materials by Distillation
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D3325 Practice for Preservation of Waterborne Oil Samples
D3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography
D3414 Test Method for Comparison of Waterborne Petroleum Oils by Infrared Spectroscopy
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
E1 Specification for ASTM Liquid-in-Glass Thermometers
E133 Specification for Distillation Equipment

3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *animal/vegetable-derived oils*—a mixture made of mono-, di-, and triglyceride esters of fatty acids and other substances of animal or vegetable origin, or both.

3.2.2 *Simulated weathering of waterborne oils by distillation* considers only the effect of evaporation, which likely is the most significant short-term weathering effect in the environment.

3.2.3 *Simulated weathering of waterborne oils by evaporation* under ultraviolet light simulates the loss of light components on weathering, as well as some oxidative weathering.

4. Significance and Use

4.1 Identification of a recovered oil is determined by comparison with known oils selected because of their possible relationship to the particular recovered oil, for example,

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[1] This practice 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] 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.

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suspected or questioned sources. Thus, samples of such known oils must be collected and submitted along with the unknown for analysis. It is unlikely that identification of the sources of an unknown oil by itself can be made without direct matching, that is, solely with a library of analyses.

5. Reagents and Materials

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society.[3] Special ancillary procedures such as fluorescence may require higher purity grades of solvents. Other grades may be used provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water that meets the purity specifications of Type I or Type II water, as specified in Specification D1193.

6. Caution

6.1 Solvents used in this practice are volatile, flammable, or may cause the harm to the health of the user. Specifically, benzene is a known carcinogen, while chloroform and carbon tetrachloride are suspected carcinogens. Consequently, it is important that extractions and separations utilizing these substances must be carried out in a laboratory hood with a minimum linear face velocity of 38 to 45 m/min (125 to 150 ft/min) located in a regulated area posted with signs bearing the legends: NO SMOKING or (if appropriate) DANGER-CHEMICAL CARCINOGEN-AUTHORIZED PERSONNEL ONLY, or both.

7. Sampling

7.1 Collect representative samples in accordance with Practices D4489.

7.2 Preserve the waterborne oil samples in accordance with Practice D3325.

7.3 The portion of the sample used must be representative of the total sample. If the material is liquid, thoroughly stir the sample as received, warming if necessary to ensure uniformity.

PROCEDURE A—LARGE SAMPLES

8. Scope

8.1 This procedure covers the preparation for analysis of samples in which the volumes of waterborne oil in the environmental and suspect source samples equal or exceed 50 mL and in which the oil portion contains significant amounts of hydrocarbons with boiling points above 280°C.

NOTE 1—The boiling point may be ascertained by injecting the neat samples into the gas chromatograph and checking the elution times above that of pentadecane on a nonpolar column.

8.2 The preparation of samples containing mostly hydrocarbons of boiling points below 280°C, such as petroleum distillate fuels, is beyond the scope of this procedure (see Procedure C or E).

9. Summary of Procedure

9.1 A neat portion of the waterborne oil is retained. If not possible to obtain a neat portion, then retain a portion of the waterborne oil as received. This is to be used in those analyses performed on samples containing significant quantities of hydrocarbons with boiling points below 280°C. Preparation of these samples is beyond the scope of this procedure, but are covered in Procedure C.

NOTE 2—Waterborne oil samples containing significant quantities of hydrocarbons with boiling points below 280°C (see Note 1), such as gasoline and kerosene, can usually be obtained as neat samples without any sample preparation.

9.2 The waterborne oil sample is dissolved in an equal volume of chloroform or dichloromethane and centrifuged to remove the free water, solids, and debris. The water layer, if present, is separated from the organic layer. Other debris, if present, is removed by filtration through glass wool.

NOTE 3—The use of spectrograde cyclohexane is required for the extraction of samples to be analyzed by fluorescence spectrometry by Test Method D3650. Separation of water may be accomplished by centrifugation or dying, or both, with anhydrous sodium sulfate.

9.3 When centrifugation will not separate the water from the chloroform solution of the sample, it is refluxed with an aromatic or petroleum distillate solvent in accordance with Test Method D95.

NOTE 4—Pressure filtration has also been found useful for breaking emulsions.

9.4 A portion of the solvent/sample solution is retained. The solvent may be removed by evaporation. This portion of the sample may be used in the preliminary gas chromatographic analysis, Test Methods D3328 (Test Method A), and other analyses in which the results are unaffected by weathering.

9.5 The remainder of the solvent/sample solution is distilled using nitrogen purge to a liquid temperature of 280°C to remove the solvent and simulate weathering conditions as nearly as possible. The distillate may be discarded or saved for characterization by gas chromatography (Test Methods D3328). This simulated weathering treatment is necessary to bring the unweathered suspect samples and the waterborne oil sample to as nearly comparable physical condition for subsequent analysis as possible. Analyses requiring the use of this treated residue include elemental analysis; gas chromatographic analysis (Test Methods D3328, Test Methods A and B); an infrared procedure (Test Method D3414); a fluorescence test method (Test Method D3650); and any applicable test method or practice described in Practice D3415.

NOTE 5—The distillate might yield useful information but is discarded in this practice.

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[3] *Reagent Chemicals, American Chemical Society Specifications* , American Chemical Society, 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 and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

10. Apparatus

10.1 *Centrifuge,* capable of whirling two or more filled 100-mL centrifuge tubes at a speed that is controlled to give a relative centrifugal force (rcf) between 500 and 800 at the tip of the tubes.

10.2 *Centrifuge Tubes,* cone shaped, 100 mL.

10.3 *Distillation Apparatus for Water Determination,* as specified in Test Method D95.

10.4 *Distillation Apparatus for Simulated Weathering,* as described in Specification E133 except fitted with nitrogen-stripping tubulation as illustrated in Fig. 1.

10.5 *Distillation Flask,* 200 mL, as described in Specification E133.

10.6 *Thermometer,* ASTM high distillation, having a range from − 2 to + 400°C and conforming to the requirements for thermometer 8C as prescribed in Specification E1.

10.7 *Flowmeter,* to regulate flow of nitrogen to distillation flask. It should be calibrated and graduated for the range 10 to 15 mL/min.

11. Reagents and Materials

11.1 *Filter Paper,* medium retention, medium fast speed, prewashed with solvent used.

11.2 *Glass Wool,* prewashed with solvent used.

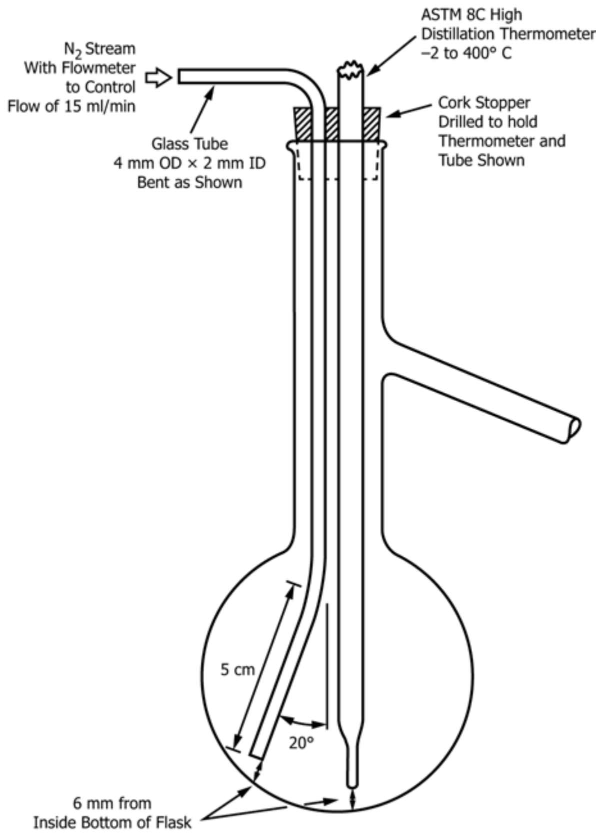

FIG. 1 Adaptation of ASTM Distillation Flask for Topping Chloroform Solutions of Oil to Simulate Weathering

11.3 *Solvent*—Chloroform (stabilized with ethanol) or dichloromethane is used for dissolution of the waterborne oil samples. If water is to be removed by distillation, an aromatic, petroleum distillate, or volatile spirits solvent is required as specified in Test Method D95. The safety precautions associated with the use of the solvent selected should be considered before it is used (see Note 3).

12. Procedure

12.1 *Retention of Neat Samples:*

12.1.1 Decant or siphon off a portion of the neat waterborne oil if possible.

12.1.2 If not possible to obtain a neat sample, retain a portion of the original oil.

12.2 *Removal of Water, Sediment, and Debris:*

12.2.1 Transfer about 50 mL of original waterborne oil to a 100-mL centrifuge tube. Add about 50 mL of chloroform or dichloromethane to the tube and mix thoroughly. For waxy samples, use chloroform. Warm solutions to 50°C to prevent precipitation (see Note 3).

12.2.1.1 Centrifuge the mixture at 500 to 800 rcf (relative centrifugal force) for 10 min to separate free water and solids. For waxy samples, use chloroform. Warm solutions to 50°C to prevent precipitation (see Note 3).

12.2.1.2 Withdraw the water layer if present. Decant the chloroform or dichloromethane solution to a sample bottle. Filter through a glass wool plug, if necessary, to afford a clean separation.

12.2.2 Process those samples from which water cannot be separated by centrifugation by Test Method D95 distillation procedure. Filter the dry solution through medium retention filter paper. Rinse filter paper with solvent to remove oil. For waxy samples, use chloroform and keep filter funnel and contents at 50°C during filtration (see Note 3).

12.2.3 Starting at 12.1, treat all reference or suspect samples in an identical fashion. If it is apparent that the reference or suspect samples contain less than 1 % water and sediment, centrifugation may be eliminated and the reference or suspect samples should be diluted with an equal volume of chloroform or dichloromethane before proceeding.

12.3 *Removal of Solvent and Simulated Weathering:*

12.3.1 Transfer approximately 100 mL of the solution to a chemically clean 200-mL flask. Assemble apparatus so the ASTM high distillation thermometer (8C) and nitrogen stripping tubulation are about 6 mm from the bottom of the flask. Direct flow away from thermometer bulb to prevent local cooling of thermometer (see Fig. 1).

12.3.2 Perform distillation using a nitrogen flow of 10 to 15 mL/min. Terminate distillation at a liquid temperature of 280°C. Shut off the nitrogen flow when the temperature of the liquid in the distillation flask cools below 175°C. Pour the hot residue into a suitable container.

12.3.3 Treat all reference and suspect oils in the same manner as the waterborne oil samples. Repeat 12.2.1 – 12.3.2.

PROCEDURE B—LIMITED SAMPLE VOLUMES OF HEAVY OILS

13. Scope

13.1 This procedure covers the preparation for analysis of waterborne oil samples of petroleum derived origin in which the volumes equal or are less than 1 mL. An aliquot of larger oil samples may also be used.

13.2 The procedure is applicable to oils containing significant amounts of hydrocarbons boiling above 280°C.

13.3 The preparation of samples containing lower boiling hydrocarbon is beyond the scope of this procedure, but is covered by Procedures C and E.

14. Summary of Procedure

14.1 The sample is dissolved in pentane or hexane, and the water and insolubles are removed by centrifugation. The organic solvent phase is dried with anhydrous magnesium sulfate, filtered, and the volatile components and solvents are removed by evaporation under a nitrogen stream (see Note 3).

15. Apparatus

15.1 *Centrifuge,* see 10.1.

15.2 *Centrifuge Tubes,* see 10.2.

15.3 *Flow Control on Nitrogen Cylinder,* to control nitrogen flow over sample surface.

15.4 *Steam Bath,* or commercial temperature controlled solvent evaporator, maintained between 40 and 50°C.

16. Reagents and Materials

16.1 *Magnesium Sulfate,* anhydrous.

16.2 *Nitrogen,* a high purity grade.

16.3 *Pentane or Hexane,* chromatographic grade.

17. Procedure

17.1 Remove approximately 1 mL of the oil phase from the water-oil sample if possible and place it in a 100-mL centrifuge tube.

17.1.1 Add 40 mL of pentane or hexane and 1 g of anhydrous magnesium sulfate. Mix to remove water. If the sample tube is warm, additional magnesium sulfate may be required. Add magnesium sulfate in 1-g aliquots, mixing after each addition until no temperature change is detectable to the touch.

17.2 Alternatively, estimate the volume of oil in the sample and add approximately 40 vol of pentane per 1 vol of oil.

17.2.1 Shake or rapidly mix the oil and solvent.

17.2.2 Allow phases to separate, withdraw the solvent phase with a pipet, and place it in a 100-mL centrifuge tube.

17.3 Centrifuge as described in 12.2.1.1 for 5 min.

17.4 Decant supernatant liquid into a 250-mL beaker and evaporate the solvent and volatiles initially at 25 to 35°C and then at 40 to 50°C for 2 h in the presence of a stream of nitrogen. Transfer the sample to a sample vial when there is approximately 4 mL sample remaining and continue the solvent removal. The samples can then be used for analysis in accordance with Practice D3415.

NOTE 6—This treatment with 70 mg of oil, evaporated at 40°C for 15 min in the presence of an airstream, yielded gas chromatograms resembling those of the distillation test method in 12.3.[4]

PROCEDURE C—OILS BOILING BELOW 280°C

18. Scope

18.1 This procedure covers the preparation for analysis of waterborne oil samples containing significant amounts of components boiling below 280°C.

18.2 The procedure is applicable to samples of distillate fuel oils, light and heavy naphthas, and other petroleum solvents.

19. Summary of Procedure

19.1 The oil and water phases are separated by centrifugation, and the oil phase is dried with anhydrous magnesium sulfate.

20. Apparatus

20.1 *Centrifuge,* see 10.1.

20.2 *Centrifuge Tubes,* see 10.2.

20.3 *Separatory Funnel,* glove or pearshaped, 100 mL, with TFE-fluorocarbon stopcock.

20.4 *Pipets*, disposable glass.

21. Reagents and Materials

21.1 *Magnesium Sulfate,* anhydrous.

22. Procedure

22.1 Transfer up to 10 mL of sample into a 100-mL separatory funnel. If phases separate, withdraw and discard aqueous (lower) phase. Transfer the organic phase into a 12.5-mL centrifuge tube. Alternatively, if there is enough oil on the water, the oil may be transferred directly with a pipet. Proceed to 22.4.

22.2 Prepare emulsified samples in the following manner: Transfer 10 mL of the sample to a centrifuge tube and centrifuge for 30 min at 1000 rcf (relative centrifugal force). If an oil layer appears, remove and proceed as directed in 22.4.

22.3 If a distinct oil layer does not appear, add to the test tube a maximum of 1 g of sodium chloride, mix, and centrifuge as in 22.2. If separation does not occur after centrifugation, add pentane, hexane, or cyclohexane, up to one quarter the sample volume, mix thoroughly, and proceed as in Procedure B.

22.4 Add 1 g of anhydrous magnesium sulfate, and mix for 1 min. If the sample tube is warm, additional magnesium sulfate may be required. Add magnesium sulfate in 1-g aliquots, mixing after each addition until no temperature change is detectable to the touch.

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[4] Gruenfeld, M., and Frederick, R., "The Ultrasonic Dispersion, Source Identification, and Quantitative Analysis of Petroleum Oils in Water," Rapp. P-V, Reun. Cons. int. Explor. Mer. 171:33, 1977.

22.5 Centrifuge as described in 12.2.1.1 for at least 10 min. If magnesium sulfate is not completely removed from the oil, it may interfere with analysis by infrared spectroscopy, Test Method D3414.

22.6 Decant the supernatant, leaving some oil to avoid disturbing the solids, and use for analysis by procedures given in Practice D3415.

PROCEDURE D—SAMPLES COMPOSED OF MIXTURES OF PETROLEUM-BASED AND ANIMAL-/VEGETABLE-DERIVED OILS

23. Scope

23.1 This procedure covers the preparation for analysis of waterborne oil samples composed of mixtures containing significant amounts of petroleum-based and animal-/vegetable-derived oils.

23.2 The procedure incorporates a column chromatographic procedure to separate the animal-/vegetable-derived oil fraction from the mixture.

24. Summary of Procedure

24.1 The waterborne oil phase is separated from the sample and dried with anhydrous magnesium sulfate. The petroleum hydrocarbon phase is separated from the animal vegetable oil phase by dissolving in carbon tetrachloride, followed by column chromatography using a silica gel-alumina column. The animal/vegetable oil fraction may be recovered from the column by elution with methanol.[5]

25. Apparatus

25.1 *Centrifuge,* see 10.1.

25.2 *Centrifuge Tubes,* see 10.2.

25.3 *Separatory Funnel,* glove- or pear-shaped, 100 mL, with TFE-fluorocarbon stopcock.

25.4 *Chromatographic Column,* 10 mm diameter by 250 mm high, loaded as shown in Fig. 2. The column is preconditioned by eluting with 100 mL of carbon tetrachloride and is kept saturated with solvent.

25.5 *Evaporating Dish,* porcelain or borosilicate glass, 100 mL.

26. Reagents and Materials

26.1 *Alumina,* neutral, Brockman activity 1, activated, 80 to 200 mesh, deactivated to 3 % water (wt/wt) vol per weight of water.

26.2 *Carbon Tetrachloride,* spectral or chromatographic grade.

26.3 *Magnesium Sulfate,* anhydrous, prewashed with carbon tetrachloride and dried at 103°C for 1 h.

26.4 *Methanol*, spectral or chromatographic grade.

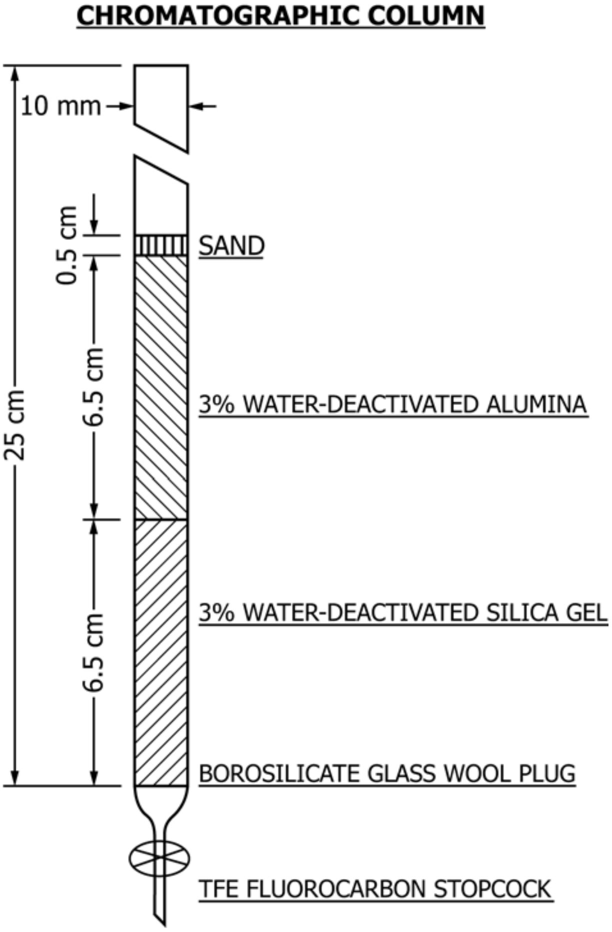

FIG. 2 Chromatographic Column

26.5 *Silica Gel,* activated, 100 to 200 mesh, deactivated to 3 % water (wt/wt).

26.6 *Sand,* sea, washed and ignited, 20 to 30 mesh.

27. Procedure

27.1 Transfer the sample into a 1-L separatory funnel.

27.2 Allow phases to separate.

27.3 Withdraw and discard aqueous (lower) phase.

27.4 Transfer 1 to 10 mL of the organic phase into a 100-mL centrifuge tube.

27.5 Dilute with carbon tetrachloride using a 10:1 volume per volume carbon tetrachloride to sample ratio (see 5.1 regarding purity of solvent).

27.6 Add 5 g of anhydrous magnesium sulfate and shake for 1 min.

27.7 Centrifuge as indicated in 12.2.1.1 for 10 min.

NOTE 7—Centrifugation may not be necessary if a clean supernatant is obtained upon addition of the magnesium sulfate.

27.8 Place lower phase into a 100-mL evaporating dish and evaporate to 2 mL or the original sample volume, whichever is larger.

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[5] Kahn, L., Dudenbostel, B., Speis, D. N., and Karras, G.,“ Determination of Mineral Oils and Animal/Vegetable Oils in the Presence of Each Other,” *American Laboratory*, Vol 9, No. 3, 1977, pp. 61 to 66.

27.9 Place 2 mL of the residue from 27.8 into the chromatographic column (see 25.4 and Fig. 2).

27.10 Elute with carbon tetrachloride at a rate of 3.0 mL/min and collect 30 mL of eluate.

NOTE 8—This eluate contains the hydrocarbons fraction and includes all the petroleum-based oils as well as a small fraction of the hydrocarbons in the liquids from animal/vegetable wastes and oils. This fraction may be analyzed after solvent evaporation by procedures given in Practice D3415.

27.11 Elute the residual material in the column with 100 mL of methanol into a separate container.

NOTE 9—This eluate contains the mono-, di-, and triglycerides, other fatty esters, and polar components of animal-/vegetable-derived oils.

27.12 Evaporate the solvent from 27.10 and 27.11, respectively, at 40 to 50°C by gently blowing nitrogen over the surface of the liquid.

NOTE 10—This fraction may be analyzed by a variety of adjunct procedures such as infrared analysis (Test Method D3414), iodine number, gas chromatography, etc.

PROCEDURE E—LIMITED SAMPLE VOLUMES OF LIGHT OILS

28. Scope

28.1 This procedure covers the preparation for analysis of weathered light crudes and weathered oils of petroleum derived origin having significant amounts of hydrocarbons with boiling points below 280°C.

28.2 The procedure provides a means to simulate the effects of environmental weathering on petroleum oils, thus simplifying comparison of spilled oils to suspected sources.

28.3 The procedure is applicable to simulation of light to moderate weathering, equivalent to one to three days exposure to the marine environment.

29. Summary of Procedure

29.1 Neat samples of unweathered oils (suspected sources), intended to be compared with untreated samples of the weathered waterborne oil, are irradiated as thin films with long wavelength UV light while exposed to an accelerated air flow.

29.2 Exposure of the oil for 3 to 6 h in this manner simulates the effects of one to three days of weathering of light fuel oils in the marine environment.

30. Apparatus

30.1 *Cooling Block,* capable of maintaining a surface temperature of 20°C at room temperature.

30.2 *Fan,* to provide air current on the order of 2.6 m/s.

30.3 *Ultraviolet Lamp,* long wave high intensity, using a high pressure 100 W, sealed beam bulb.

30.4 *Thermometer* (0 to 50°C), or thermocouple.

31. Reagents and Materials

31.1 *Glass Petri Dish,* 7.5 cm, for weathering up to 0.75 mL of oil.

31.2 *Infrared Salt Plate,* (preferably KBr) can be used as the support for the thin film of oil. This is used for infrared analysis of small samples as little as 30 mg.

32. Caution

32.1 Apparatus should be used in a well ventilated area due to hazardous vapors created.

33. Preparation Of Apparatus

33.1 Assemble apparatus as shown in Fig. 3.

33.2 Set fan speed and distance to provide moderate air flow on the order of 2.6 m/s at cooling block.

33.3 Place UV lamp 10 cm from the cooling block (this provides 15.6 mm/cm^2 for the lamp specified).

33.4 With the lamp on and air flow across the cooling block, adjust the cooling block surface temperature to approximately 22°C (20 to 25°C).

34. Procedure

34.1 Transfer 0.25 to 0.75 mL of oil sample to be weathered onto the glass petri dish placed on apparatus thermally equilibrated as described in 33.4. (For infrared analysis, if only small amounts of sample are available, 30 to 100 mg will uniformly coat one side of a 19 by 34 mm KBr salt window.)

34.2 Place the sample on the cooling block. (If salt window is used place a nonabrasive, noncontaminating paper between the window and the cooling block to avoid scratching the salt window).

34.3 Remove the sample after 3 to 6 h of continuous exposure in this apparatus. The sample is now ready for analysis by procedures given in Practice D3415.

PROCEDURE F—THIN FILMS OF OIL-ON-WATER

35. Scope

35.1 This procedure covers the preparation for analysis of waterborne oil samples having an insufficient sample volume for preparation by Procedures A through E.

35.2 The procedure is applicable to both petroleum and nonpetroleum derived oils. It is also applicable to water samples that may or may not contain spilled oil. These samples may be encountered in cases where water is sampled to assess the extent to which oil has dispersed.

35.3 Traces of oil recovered by the techniques in this procedure have usually been subjected to environmental weathering; analysis of such samples may be inconclusive.

36. Summary of Procedure

36.1 Thin films of oil-on-water are removed with a TFE-fluorocarbon strip.

36.2 Water samples are extracted with cyclohexane and either evaporated to a neat oil or used directly for analysis by fluorescence spectroscopy (Test Method D3650) or gas chromatography (Test Methods D3328), or both.

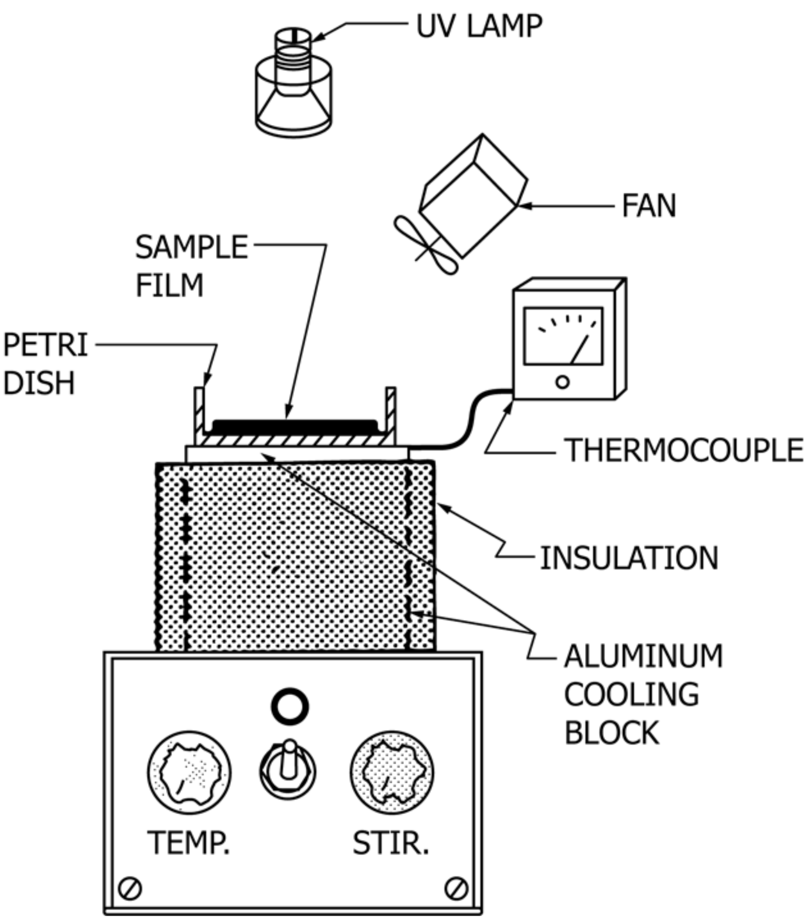

FIG. 3 Weathering Apparatus

37. Apparatus

37.1 *Centrifuge*, see 10.1.

37.2 *Centrifuge Tubes*, see 10.2.

37.3 *Flow Control on Nitrogen Cylinder*, see 15.3.

37.4 *Steam Bath, Hot Plate*, or commercial temperature controlled solvent evaporator, see 15.4.

37.5 *Test Tubes*, disposable, 16 by 125 mm, 15 mL, borosilicate glass culture tubes.

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

38. Reagents and Materials

38.1 *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.

38.2 *TFE-Fluorocarbon Strips*, 25 by 75 mm, 0.25 mm thickness.

38.3 *Magnesium Sulfate*, anhydrous.

38.4 *Nitrogen*, compressed gas cylinder.

39. Procedure

39.1 Samples may be concentrated in the following manner: Dip or pass a TFE-fluorocarbon strip through the oil layer, then allow the oil to drip into a clean aluminum weighing pan. Continue until enough oil has been recovered to use a micropipet. Continue with analysis by fluorescence spectroscopy (Test Method D3650) or gas chromatography (Test Methods D3328), or both.

39.2 To recover oil remaining after the procedure in 39.1 is used, or if insufficient oil is recovered by the TFE-fluorocarbon strip procedure, add 10 mL of cyclohexane to the sample container. (Appropriate sample containers with TFE-fluorocarbon-lined lids are described in Practices D4489.)

39.3 Replace the lid, then gently shake or swirl for approximately 1 min. Allow the sample container to stand undisturbed until the phases have separated.

39.4 Transfer the cyclohexane (upper) phase to a disposable glass test tube or a centrifuge tube. Centrifuge as in 12.2.1.1.

39.5 Transfer the organic phase to a new test tube, add 1 g of magnesium sulfate, and centrifuge as in 22.2.

39.6 Remove solvent under a stream of nitrogen until either all the solvent has evaporated or only a few drops of liquid remain. If the remaining solution appears to contain no visible oil, add 2 mL of cyclohexane. This solution may be used directly for analysis by fluorescence spectroscopy (Test Method D3650) or gas chromatography (Test Methods D3328), or both.

PROCEDURE G—OIL-SOAKED SAMPLES

40. Scope

40.1 This procedure covers the preparation for analysis of oil-soaked samples, such as sand, debris, sorbent pads, or any other substrate having a limited quantity of free flowing oil.

40.2 Oil recovered by the techniques in this procedure is subject to potential interferences from environmental weathering, as well as contamination by organic substances derived from the substrate material.

41. Summary of Procedure

41.1 The oil is separated from the substrate by squeezing or centrifugation.

42. Apparatus

42.1 *Centrifuge*, see 10.1.

42.2 *Centrifuge Tubes*, see 10.2.

42.3 *Test Tubes*, disposable, 16 by 125 mm, 15 mL, borosilicate glass culture tubes.

42.4 *Weighing Pans*, 5 to 7 cm diameter, 18 mm deep, made of aluminum or equivalent. (Double-weight aluminum foil may be substituted.)

43. Reagents and Materials

43.1 *Purity of Water*—References to water in this procedure shall be understood to mean Type IV reagent water conforming to Specification D1193. However, because fluorescent organic impurities in the water may constitute an interference, the purity of the water should be checked by running a water blank.

44. Procedure

44.1 For light oil collected on soft absorbent material, place a portion of the oil-soaked material in the center of a clean aluminum weighing pan or piece of aluminum foil. Fold the sides of the aluminum over the material and squeeze out the oil into a clean aluminum pan. If sufficient oil is recovered, Procedure C may then be followed for sample preparation. Otherwise, microlitre amounts of the oil may be removed with a micropipet and analyzed directly by fluorescence spectroscopy (Test Method D3650) or gas chromatography (Test Methods D3328), or both.

44.2 For oil collected on substrates heavier than the oil, fill a centrifuge tube one-fourth to one-third full with the oil soaked material, plus an equal amount of water. Centrifuge as stated in 12.2.1.1 to free the oil from the material. After this is done, prepare the sample as in Procedure B or C.

44.3 For medium to heavy weight oil collected on light substrates, (grass, feathers, sticks), the oil may be physically removed by transfer with a clean inert material, such as a clean TFE-fluorocarbon strip or spatula, and prepared as in Procedure B or C.

44.4 If none of the above techniques are successful in removing sufficient oil for analysis, a solvent extraction should be used as described in Procedure B.

45. Keywords

45.1 identification; oil spill; preparation; sample; waterborne oil

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