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AMERICAN SOCIETY FOR TESTING AND MATERIALS
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Standard Test Method for Comparison of Waterborne Petroleum Oils by High Performance Liquid Chromatography[1]

This standard is issued under the fixed designation D 5037; 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—Keywords were added editorially in December 1996.

1. Scope

1.1 This test method covers the qualitative comparison of petroleum oils recovered from water or beaches with those collected from suspected sources by the means of high performance liquid chromatography.

1.2 This test method is applicable to weathered or unweathered samples as well as to samples subjected to simulated weathering.

1.3 *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 precautionary statements are given in Section 9.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

D 1193 Specification for Reagent Water[2]

D 3325 Practice for Preservation of Waterborne Oil Samples[3]

D 3326 Practice for Preparation of Samples for Identification of Waterborne Oils[3]

D 3415 Practice for Identification of Waterborne Oils[3]

D 4489 Practices for Sampling of Waterborne Oils[3]

E 131 Terminology Relating to Molecular Spectroscopy[4]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminologies E 131 and D 1129 and Practice D 3415.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *waterborne oil,, n*—an oil, derived from petroleum, carried by a water system (ocean, bay, lake, river, etc.) usually at the surface but occasionally emulsified or dissolved in water. The waterborne oil may also be found deposited on beaches or banks edging the water body.

3.2.2 *weathering of waterborne oil,, adj*—including such effects as evaporation, dissolution, emulsification, oxidation, and biological decomposition.

4. Summary of Test Method

4.1 This test method consists of the chromatographic analysis of a dilute solution of a petroleum oil in acetonitrile injected onto a reverse phase liquid chromatographic column. Isocratic development with acetonitrile as mobile phase is used to separate components. Dual photometric flow cells (ultraviolet and fluorescence) are used to monitor column effluent.

4.2 Identification of the sample is made by visual comparison of the sample's chromatogram with the chromatograms of possible source samples. When weathering has occurred, it is necessary to consider known weathering trends when matching the chromatograms. See 14.4 on weathering effects.

5. Significance and Use

5.1 Samples of oil recovered from a spill are compared with samples of known oils selected because of their possible relationship to the particular recovered oil. The known oils are collected from suspected sources. Samples of such known oils must be collected and submitted along with the unknown for analysis.

5.2 This test method is useful for the identification of the source for petroleum oil spills. Using a fluorescence detector in addition to the ultraviolet detector provides a second, independent profile of the same oil. Significantly more information is available from a single analysis with dual detection.

5.3 The unknown oil is identified by the comparison of its chromatogram to the individual chromatograms (obtained at similar instrumental settings on the same apparatus) of the possible source samples. A match of the entire chromatogram between the unknown and possible source sample indicates a common source. Additional comparisons (refer to Practice D 3415) are needed to strengthen conclusions.

6. Interferences

6.1 Compounds that have the same retention times as the

[1] This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.31 on Identification of Waterborne Oils.

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[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.02.

[4] *Annual Book of ASTM Standards*, Vol 03.06.

sample can interfere in the comparison of the unknown spill to that of the known suspect oils. This is particularly true if animal fat or vegetable oil, naturally occurring hydrocarbons, or spill-treatment chemicals are present in relatively large amounts.

6.2 The spill chromatogram can also be altered significantly if it has been contaminated by an appreciable amount of another oil.

6.3 Storage of samples in improper containers (plastic) may result in contamination. This interference can be eliminated by observing proper procedure for collection and preservation of samples. Refer to Practice D 3325.

7. Apparatus

7.1 *Liquid Chromatograph*—A commercial or custom designed liquid chromatograph that has a pressure range from 0 to 6000 psig, equipped with the following components:

7.1.1 *Solvent Delivery System,*

7.1.2 *Injection System,*

7.1.3 *Ultraviolet Absorption Detector,*

7.1.4 *Fluorescence Detector,* and

7.1.5 *Recorder Device,* (stripchart or chromatographic data system).

7.2 *Chromatographic Column*:

7.2.1 *Analytical Columns,* (4.6 mm inside diameter by 25 cm, with a theoretical plate count greater than 15 000).

7.2.2 *Guard Column or Pre-Column,* packed with the same stationary phase as the analytical column may be installed upstream of the analytical column.

7.3 *Vortex Mixer*.

7.4 *Micropipet,* 10 to 50 µL capacity.

7.5 *SPE (Solid-Phase Extraction) System* with disposable columns.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests 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. [5]

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type IV.

8.3 *Acetonitrile*—Spectroquality solvent for sample treatment and cleanup. Solvent used as the mobile phase in the chromatograph should also be degassed. Dichloromethane, spectroquality.

9. Precautions

9.1 The primary precaution that must be taken to provide the best possible results is that all samples analyzed should be treated in an identical fashion, on the same instrument, on the same column, and preferably on the same day by the same operator. If all samples pertaining to a particular spill case set cannot be run on the same day, one of the first-run samples must be subsequently repeated to ensure chromatograms are essentially unchanged.

9.2 Observe customary safe handling procedures for the organic solvents.

10. Sampling

10.1 Collect a representative sample in accordance with Practices D 4489.

10.2 If the sample is not to be analyzed within one week, it should be preserved in accordance with Practice D 3325 because of the possibility of biological decomposition.

10.3 The sample should be prepared for analysis in accordance with Practice D 3326 because of the great variety of material and circumstances associated with collecting petroleum oils from the environment.

11. Sample Preparation

11.1 Transfer 10 µL of oil sample to a clean test tube using a micropipetor.

11.1.1 The oil is diluted with 50 µL of dichloromethane and swirled until dissolved. This solution is further diluted with 4 mL of acetonitrile and well mixed (using a vortex mixer).

11.1.2 Each oil solution is poured into an individual prerinsed (acetonitrile) disposable extraction column and aspirated through the column via a vacuum manifold. The oil solutions are collected in separate vials in the manifold below each disposable column. If the solutions are not clear, repeat the extractions.

11.2 The prepared samples are either injected manually by syringe or automatically by an auto injector.

12. Preparation of Chromatograph

12.1 If previously used columns are to be employed, proceed to 12.2.4.

12.2 *Column Conditioning for New Analytical or Guard Columns*:

12.2.1 Install the column in the chromatograph but do not connect the column at the detector (outflow) end to avoid deposition of loose packing on the detector cell(s) during conditioning.

12.2.2 Adjust the acetonitrile solvent flow to 1.0 mL/min and purge the column until the solvent becomes clear or for at least 20 min after the solvent exits from the column end.

12.2.3 After conditioning, connect the column to the detector(s).

12.2.4 Adjust the solvent flow to operating parameters (1 mL/min) and monitor the effluent. If there are no peaks in the chromatogram and there is minimal baseline shift, the column is ready for use. Otherwise, continue to equilibrate the column by pumping acetonitrile until these conditions are met.

12.2.5 If the column is to be moved or stored, disconnect and seal the ends of the column to prevent solvent evaporation. When the column is installed, it is always necessary to recondition it.

12.3 *Optimization of Detector(s)*—Adjust the detector's gain to give both maximum response and minimum background noise. Use 254 nm wavelength both for absorption and

[5] *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.

excitation. Set base line at 15 % full scale. Fluorescence emission can be monitored on filter fluorometers using a broad band-pass filter with a cut-off at approximately 375 nm; polychomator instruments should be as similarly operated as practicable.

13. Analytical Procedure

13.1 Operate the instrument and detectors in accordance with the manufacturer's instruction manuals.

13.2 Weekly analysis of a standard reference oil sample prepared in accordance with 11.1-11.1.2 ensures proper column performance and instrumental operation.

13.3 Make appropriate documentation for the chromatograms (date, sample identification, injection volume, column dimensions and packing material, and chromatograph operating parameters).

13.4 Inject a sample and, if necessary, adjust the attenuation(s) so the dominant peak is retained on scale. Repeat the analysis, if necessary, to achieve nearly full scale deflection with the highest peak at a single attenuation setting for the entire chromatogram. (Repeat injections are unnecessary for chromatographs equipped with data systems that store the chromatograms electronically. The detector signal may be replotted from disk storage at an attenuation that meets this requirement).

14. Interpretation of Results

14.1 *Basis of Matching*—The matching of oil samples is essentially a profiling technique based on the premise that identical oils give identical chromatograms. Normally, the matching of a spilled oil to a suspect oil can be accomplished by comparison of the chromatograms for each of the suspect oils to that of the spill.

14.2 It is unnecessary to chemically identify individual components of the chromatographic peaks when comparing chromatograms of a spill with its source; it is sufficient to note their degree of similarity. Proper comparisons of corresponding peaks are readily achieved by using retention times. (These are usually annotated above the peak to the nearest 0.001 min for those chromatographs equipped with data systems.)

14.3 To determine the retention times for those chromatographs not equipped with data systems, mark the strip chart manually at injection (if not done automatically). Using a ruler, take measurements from the injection mark to the peak maxima and multiply these distances by strip chart speed to obtain retention times to the nearest 0.01 min. (A fairly fast chart recorder speed is helpful to provide sizeable measurements to peak maxima for more accurate retention time determinations.)

14.4 *Comparison of Chromatograms*—Normally direct comparison of the chromatograms will suffice for establishing identity or nonidentity between samples. The comparison involves simple peak-for-peak matching, noting the differences and similarities in the relative peak sizes and peak shapes. If the chromatograms are the same on the basis of peak-for-peak matching, there is a high degree of probability that the samples are from the same source. A mismatch is obtained when the chromatographic profiles are different. However, some differences may be due to the presence of one or more extra chromatographic peaks in the spill sample relative to its source. The spill samples may contain contaminants such as bilge cleaning detergents, plasticizers, paint vehicles, etc. Therefore, the presence in a spill sample of one or more extra peaks that are absent from the suspect, is not intrinsically indicative of nonidentity.

14.5 *Weathering Effects*:

14.5.1 When an oil is spilled on water, the oil sample is chemically altered by several processes: evaporation, dissolution, photochemical, and microbial degradation.

14.5.2 Weathering can progress rapidly, even under non open-water conditions (such as in a bilge tank where a relatively small amount of oil is dispersed in a large area). A thin slick on open water may lose significant amounts of its lighter components within 2 h of being spilled. It is important to be cognizant of the effects of weathering when analyzing spill samples. Fig. 1 shows the effect of continued weathering on the chromatograms of a spill oil. Note the progressive loss of the early eluting peaks, while the late eluting profile remains constant.

14.5.3 Light distillate fuels cannot survive heavy weathering, therefore, comparison of heavily weathered residues of

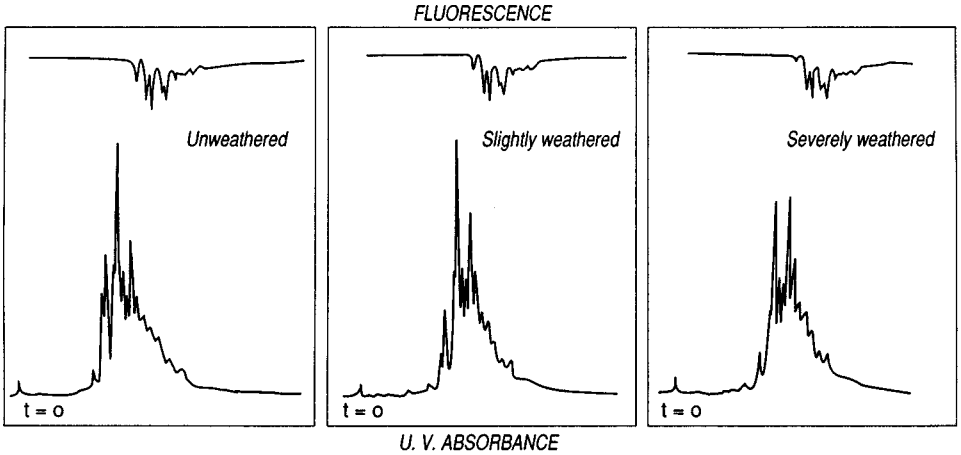

FIG. 1 Liquid Chromatograms of the Same Oil Subjected to Increased Weathering (A → B → C)

these oils is of limited utility.

15. Report

15.1 Based upon the visual comparison of chromatograms, and after considering 10.2 and 14.1-14.5, report the sample of unknown origin as belonging to one of the following categories:

15.1.1 *Match (M)*—Like one or more of the samples submitted for comparison.

15.1.2 *Probable Match (PM)*—Like one or more of the samples submitted for comparison, except for changes that could be attributed to weathering or differences attributable to specific contamination.

15.1.3 *Indeterminate (I)*—Like one or more of the samples submitted for comparison, except for certain differences as in 15.1.2, of such magnitude that it is not possible to ascertain whether the unknown is the same oil heavily weathered or a totally different oil.

15.1.4 *Nonmatch (NM)*—Unlike the samples submitted for comparison.

15.2 If the chromatograms are not similar enough to be called a match under the criteria stated above but the patterns conform to the weathering changes as enumerated, additional chromatographic separations using gradient elution can be employed. This may be useful for additional evidence of matches or may discriminate between similar oils if there appears to be more than one match. Also, additional chromatograms may be useful if contamination is suspected. If the gradient chromatograms do not match, then either the oils are not from a common source or one of the samples is contaminated.

NOTE 1—Operation of the chromatograph for gradient elution is identical to the previously enumerated methodology using isocratic development, except for the mobile phase; this changes linearly from 50/50 acetonitrile/water to 100 % acetonitrile in 30 min. One hundred percent acetonitrile should be pumped until a steady baseline condition is reached. The chromatograph can then be returned to initial conditions and injection of the next sample may proceed when steady baseline is once again achieved. Since trace enrichment of solvent/water impurities on the chromatographic columns can occur during a changing mobile phase (that desorb during the gradient elution), column regeneration to initial conditions and the duration of pumping the initial mobile phase must be reproduced from one injection to the next.

15.3 Additional data from other independent analytical methods may be helpful and are desirable in confirming this conclusion. Refer to Practice D 3415 for a discussion of other ASTM test methods for oil identification.

16. Quality Assurance

16.1 If the analysis of the quality control sample described in 5.5 of Practice D 3415 does not meet the criteria for a match, the results of all the comparisons are invalid.

17. Precision and Bias

17.1 No statement is made about either the precision or bias of this test method for measuring waterborne oils, since the result merely states whether there is conformance to the criteria for comparison specified in the procedure.

18. Keywords

18.1 high performance liquid chromatography; identification; oil spill; waterborne oil; weathering