



Designation: D 5037 – 90 (Reapproved 1996)^{ε1}

Standard Test Method for Comparison of Waterborne Petroleum Oils by High Performance Liquid Chromatography¹

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.

^{ε1} 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 Water²

D 1193 Specification for Reagent Water²

D 3325 Practice for Preservation of Waterborne Oil Samples³

D 3326 Practice for Preparation of Samples for Identification of Waterborne Oils³

D 3415 Practice for Identification of Waterborne Oils³

D 4489 Practices for Sampling of Waterborne Oils³

E 131 Terminology Relating to Molecular Spectroscopy⁴

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.

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 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.

6. Interferences

6.1 Compounds that have the same retention times as the 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 micropipet.

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 pre-rinsed (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 detector gain

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.