Standard Test Method for
Predicting Biodegradability of Lubricants Using a Bio-kinetic Model[1]

1. Scope*

1.1 This test method covers a procedure for predicting biodegradability of lubricants using a bio-kinetic model.

1.2 The values stated in SI units are to be regarded as standard. The values given in parentheses are for information only.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D2549 Test Method for Separation of Representative Aromatics and Nonaromatics Fractions of High-Boiling Oils by Elution Chromatography

D5864 Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components

D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products and Lubricants

D6731 Test Method for Determining the Aerobic, Aquatic Biodegradability of Lubricants or Lubricant Components in a Closed Respirometer

3. Terminology

3.1 *Definitions:*

3.1.1 *aromatics fraction, n*—portion of the sample desorbed with the polar eluants. The aromatics fraction is divided into nonpolar and polar based. They may contain aromatics, condensed naphthenic-aromatics, aromatic olefins, and compounds containing sulfur, nitrogen, and oxygen atoms.

3.1.2 *bio-kinetic model, n*—model that can predict the biodegradability of a lubricant.

3.1.3 *biodegradability, n*—ability of a substance to be broken down into simpler substances by bacteria.

3.1.4 *effective composition to biodegradation (ECB), n*—component of material that can be biodegradable by bacteria.

3.1.4.1 *Discussion*—The term ECB is a part of bio-kinetic model is sum of non-aromatic components in a lubricant.

3.1.5 *nonaromatics fraction, n*—portion of the sample eluted with *n*-pentane. The nonaromatics fraction is a mixture of paraffinic and naphthenic hydrocarbons if sample is a straight-run material. If the sample is a cracked stock, the nonaromatics fraction will also contain aliphatic and cyclic olefins.

4. Summary of Test Method

4.1 A weighed amount of sample is charged to the top of a glass chromatographic column packed with activated bauxite and silica gel. To elute the nonaromatics, *n*-pentane is added to the column. When all of the nonaromatics are eluted, non-polar aromatics fraction is eluted by additions of an equal mixture of toluene and *n*-pentane. The ester fraction is eluted by additions of diethyl ether. Then, the polar-aromatics is eluted by chloroform and ethyl alcohol.

4.2 The solvents are completely removed by evaporation, and the residues are weighed and calculated as the nonaromatics, nonpolar aromatics, ester fractions, and polar aromatics of the sample.

4.3 ECB is calculated based on the amount of nonaromatics and ester fractions with their material ECB coefficients. Then, the biodegradability of a lubricant is calculated using the bio-kinetic model.

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[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.12 on Environmental Standards for Lubricants.

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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.

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

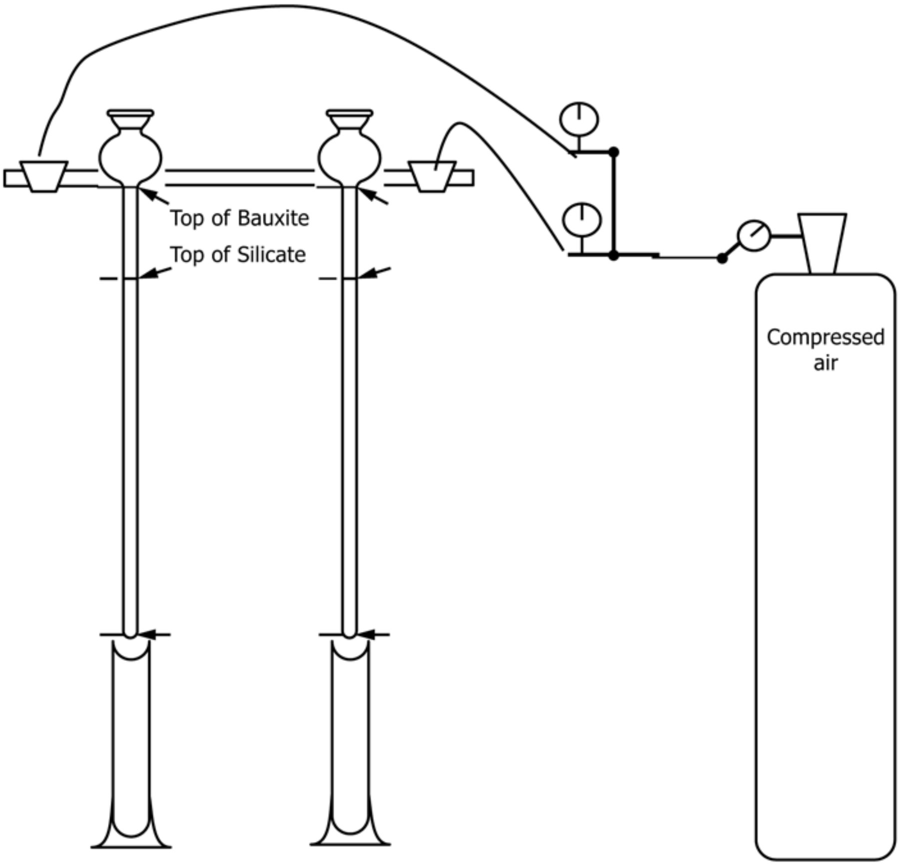

FIG. 1 Test Apparatus

5. Significance and Use

5.1 This procedure is able to predict the biodegradability of lubricants within a day without dealing with microorganisms.[3] Excellent correlation is established between the test results and the conventional biodegradation tests (see Test Method D5864 and Test Method D6731).

6. Apparatus

6.1 *Chromatographic Columns*—as shown in Fig. 1 (see Test Method D2549).

6.2 *Beakers (100, 250, and 600-mL)*—inverted-rim type.

6.3 *Steam Bath.*

6.4 *Electric Vibrator*—for packing column.

6.5 *Weighing Bottles or Erlenmeyer Flasks*—25 and 50 mL.

6.6 *Graduated Cylinders*—50 mL, 100 mL, and 250 mL.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in this test. 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, where such specifications are available. 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.

7.2 *Bauxite, 20- to 60-mesh*—Before use, activate the bauxite by heating at 538°C (1000°F) for 16 h. Transfer the activated material to an airtight container while still hot and protect thereafter from atmospheric moisture.

7.3 *Chloroform*—(**Warning—**Toxic. May be fatal if swallowed.)

7.4 *Cleaning Solution*—Chromic-sulfuric acid. (**Warning—**Causes severe burns. A recognized carcinogen, strong oxidizer, contact with organic material may cause fire.)

7.5 *Diethyl Ether*—Anhydrous. (**Warning—**Extremely flammable.) The ethyl ether used in this test method should be free of peroxides as determined by the procedure in *Reagent Chemicals, American Chemical Society Specifications*.[4]

7.6 *Ethyl Alcohol, Denatured*—Conforming to Formula 2B of the U.S. Bureau of Internal Revenue. (**Warning—**Flammable .)

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[3] Rhee, In-Sik, " Development of Bio-kinetic Model for Lubricants," *NLGI Spokesman*, Volume 69, 2005, pp. 22-29.

[4] *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 *Annual 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.

7.7 *Pressuring Gas*—Dry air or nitrogen, delivered to the top of the column at a regulated gage pressure of 0 to 2 psi (13.8 kPa). (**Warning—**Compressed gas.)

7.8 *n-pentane*—Commercial grade, aromatic–free. Some samples of waxy stocks may not dissolve completely in *n*-pentane, in which case cyclohexane, commercial grade, aromatic-free, may be substituted for *n*-pentane. (**Warning—**Extremely flammable liquid.)

7.9 *Silica Gel*—100- to 200-mesh.

7.10 *Toluene*—Reagent grade minimum purity. (**Warning—**Toluene is flammable. Vapor harmful.)

8. Procedure for Composition Analysis

8.1 Clean the column with chromic-sulfuric acid (7.4), followed by distilled or demineralized water, acetone, and dry air or nitrogen.

8.2 Introduce a small plug of glass wool into column, pressing it firmly into the lower end to prevent the flow of silica gel from the column.

8.3 Clamp the column in a vertical position. Add small increments of silica gel, while vibrating the column along its length, until the tightly packed silica gel extends to the lower mark on the chromatographic column.

8.4 Continue to vibrate the column and add bauxite until the bauxite layer extends to the upper mark on the chromatographic column. Vibrate the column for an additional 3 min after filling is completed

8.5 If the sample is viscous, warm it with intermittent mixing or shaking until it is completely fluid.

8.6 Transfer a representative sample (approximately 2 g) to a 25-mL weighing bottle or flask. Determine the weight of the sample to the nearest 1 mg by weighing the flask before and after sample transfer.

8.7 Add 10 mL of *n*-pentane (7.8) to the flask and dissolve the sample.

8.7.1 If the sample does not dissolve completely in cold *n*-pentane, warm it in warm water or over a steam bath.

8.7.2 If the sample does not dissolve in warm *n*-pentane, take a fresh sample and substitute cyclohexane for the *n*-pentane.

NOTE 1—This procedure is only applicable to liquid-based lubricants. Solid or semisolid lubricants can not be analyzed due to their formations. Therefore, the lubricating grease cannot be analyzed, but its extracted oil can be analyzed using this procedure.

8.8 Add 10 mL of *n*-pentane to the top of the column to prewet the adsorbent.

8.9 When the liquid level reaches the top of the bauxite bed, transfer the sample solution from the weighing flask to the top of the column.

8.10 Rinse the flask with three successive 3-mL washes of *n*-pentane. Add each wash to the top of the column.

8.11 Then rinse the walls of the column bulb with two 3-mL portions of *n*-pentane, allowing the liquid level to reach the top of the bauxite bed before adding the next portion.

8.12 Finally add 35-mL of *n*-pentane to the column bulb.

8.13 Place a 50-mL graduate beneath the column to collect the eluate. The elution rate should be approximately 1 mL/min.

8.14 When the *n*-pentane level reaches the top of the bauxite bed, add 80 mL of an equal mixture of *n*-pentane and toluene (50:50 vol %). Connect the pressuring gas to the top of the column and adjust the pressure to maintain an elution rate of 1 to 2 mL/min. Collect 50 mL of *n*-pentane eluate in the graduate. Rinse the tip of the column with 1 to 2 mL of *n*-pentane, adding this to the 50 mL in the graduate. Label the 50 mL graduate as *n*-pentane eluate.

8.15 When the 50:50 vol % mixture of toluene and pentane level reaches the top of the bauxite bed, release the gas pressure and add 80 mL of diethyl ether (7.5) to the top of the column. Reconnect the gas pressuring system and continue the elution. When 80 mL of elute haven collected in the graduate, rinse the column tip with 1 mL of the 50:50 vol % mixture of toluene and pentane and add the rinse to the 100 mL graduate. Change the receiver to a 100 mL graduate. Label the 100 mL graduate as nonpolar aromatic fraction.

8.16 When the ether level reaches the top of the bauxite bed, release the gas pressure and add 100 mL of chloroform (7.3) to the top of the column. Reconnect the gas pressuring system and continue the elution. When 80 mL of eluate have been collected in the graduate, rinse the column tip with 1 mL of ether and add the rinse to the 100 mL graduate. Change the receiver to a 250 mL graduate. Label the 100 mL graduate as ether-eluted fraction.

8.17 When the chloroform level reaches the top of the bauxite bed, release the gas pressure and add 75 mL of ethyl alcohol (7.6). Reconnect the gas pressuring system and continue the elution until the alcohol level reaches the top of the bauxite bed. Release the gas pressure. Rinse the column tip with 1 mL of chloroform adding this to the graduate. Label the 250 mL graduate as chloroform-alcohol-eluted fraction.

8.18 Weigh a 100 mL inverted-rim beaker to the nearest 1 mg. Quantitatively transfers the *n*-pentane eluate to this beaker and allows the *n*-pentane to evaporate at room temperature. Cyclohexane, if used as the elution solvent, is evaporated on a steam bath. Evaporation is accelerated in both cases by directing a controlled steam of dry nitrogen downward onto the surface of the liquid.

8.19 When all the solvent appears to be evaporated, stop the nitrogen flow, allow the beaker to come to room temperature, and dry the outside of the beaker to remove any condensed moisture. Reweigh the beaker to the nearest 1 mg.

8.20 Repeat the evaporation step for 5-min periods until the weight loss between successive evaporations is less than 20 mg. Heat from a stream bath is generally required during the final evaporation steps to remove completely the elution solvent. The weight of the residue in the beaker is the quantity of the nonaromatics friction.

8.21 Weigh a 250 mL inverted-rim beaker to the nearest 1 mg. Quantitatively transfer the chloroform-alcohol-eluted fraction to this beaker and evaporate on a stream bath with a controlled stream of dry nitrogen directed downward onto the surface of the liquid. When the solvent is evaporated, remove the beaker from the steam bath, cool to room temperature, and add quantitatively the ether-eluted fraction. Evaporate the ether at room temperature as described in 8.18 – 8.20. Determine the weight of the residue (ester fraction) to the nearest 1 mg.

8.22 The weight of aromatics (polar and nonpolar), ester fraction plus the nonaromatics fraction recovered must equal at least 95% of the sample charged. If 95% recovery is not obtained, repeat the test. Recoveries greater than 100% indicate incomplete removal of solvent or the condensation of moisture in the beakers.

8.23 Transfer all sample fractions into suitable size of sample vials for storage pending further analysis.

9. Calculation

9.1 Calculate the percentage of the nonpolar aromatics and polar aromatics fraction, nonaromatics and ester fraction as follows:

$$Nonaromatics,\ wt\ \% = \left[a/\left(total\ recovered\right)\right] \times 100 \quad (1)$$

$$Nonpolar\ aromatic,\ wt\ \% = \left[b/\left(total\ \ recovered\right)\right] \times 100 \quad (2)$$

$$Ester\ fraction,\ wt\ \% = \left[c/\left(total\ \ recovered\right)\right] \times 100 \quad (3)$$

$$Polar\ aromatics,\ wt\ \% = \left[d/\left(total\ \ recovered\right)\right] \times 100 \quad (4)$$

where:

a = weight of nonaromatics recovered by *n*-pentane,
b = weight of nonpolar aromatics recovered by 50:50 mixture of toluene and *n*-pentane,
c = weight of ester fraction recovered by diethyl ether,
d = weight of polar aromatics recovered by chloroform and ethyl alcohol, and
Total recovered = $a + b + c + d$.

9.2 Calculate ECB value of sample as follows:

$$ECB = \sum_{a}^{c} (\eta_a \quad C_a + \eta_c \quad C_c \quad (5)$$

where:

η = ECB coefficient listed in Table 1,
C_a = Fraction of nonaromatics (*n*-pentane), and
C_c = Fraction of ester (diethyl ether).

9.3 Calculate the biodegradability of a lubricant using bio-kinetic model as follows:

TABLE 1 ECB Coefficients for Oils

Lubricant	ECB Coefficient (η)
Mineral oil	0.3
PAO 2[A]	0.8
PAO 4[A]	0.6
PAO 6 or above[A]	0.4
Natural esters	1
Renewable based diester and polyol ester	0.8
Petroleum based ester types	0.01

[A] Viscosity grades for polyalpaolefin (PAO).

$$B(t) = B(1) + \frac{0.49}{\ln\left(6.8 \times ECB^{-2.38}\right)} \ln t \quad (6)$$

where:

t = time, days (i.e., 28),
$B(t)$ = Biodegradability of a lubricant with time, and
$B(1)$ = 0.01.

10. Report

10.1 The value calculated in 9.3 is reported as the biodegradability of a lubricant.

11. Precision and Bias

11.1 *Precision*—The precision of this test method as determined by statistical examination of interlaboratory results in accordance with Practice D6300 is as follows:

11.1.1 *Repeatability*—Two test results obtained within one laboratory obtained by the same operator using the same equipment on the same day in the same laboratory shall be judged not equivalent if they differ by more than the "r" value for that material; "r" is the interval representing the critical difference between two test results for the same material.

Repeatability (r) = 1 %

11.1.2 *Reproducibility*—Two test results obtained by different operators using different equipment in different laboratories shall be judged not equivalent if they differ by more than the "R" value for that material; "R" is the interval representing the critical difference between two test results for the same material.

Reproducibility (R) = 3.8 %

11.2 *Bias*—Bias cannot be determined because there are no reference materials suitable for determining the bias in this test.

12. Keywords

12.1 bio-kinetic model; biodegradability of lubricant; ECB; lubricant

APPENDIX

(Nonmandatory Information)

X1. ROUND ROBIN DATA

X1.1 See Tables X1.1 and X1.2.

TABLE X1.1 Round Robin Samples

Code	Type of Base Oil	Identification
S-1	Bio-based oil	Tractor hydraulic fluid
S-2	Mineral oil	Hydraulic fluid
S-3	PAO 2 cSt	Base oil
S-4	Bio-based oil	Hydraulic fluid
S-5	Water insoluble Polypropylene glycol	...
S-6	Mineral oil	Gear oil
S-7	Mineral oil	Tractor hydraulic fluid
S-8	Bio-based oil	Hydraulic fluid
S-9	Blended bio-based oil	Hydraulic fluid
S-10	PAO 4 cSt	Base oil
S-11	Hydrocarbon soluble polybutylene glycol	...

TABLE X1.2 D7373 Biodegradability of Round Robin Samples (%), 6/20/2011

Cooperator	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
1	65.7	35.3	66.7	78.4	26.7	33.8	35.2	63.3	49.7	52.9	46.9
	65.6	35.3	66.8	78.3	26.3	33.7	34.3	64.9	48.3	57.7	43.3
2	66.1	35.4	67.4	76.2	13.6	34.3	35.4	81.2	52.1	52.6	57.0
	66.4	35.2	67.4	77.4	13.6	34.3	35.2	81.0	51.7	52.7	57.9
3	69.0	34.7	67.7	75.7	11.7	35.5	34.9	81.4	53.2	52.7	66.7
	69.4	34.7	67.7	76.1	11.0	35.2	34.7	82.6	52.5	53.1	64.6
4	69.3	35.2	67.1	80.2	13.6	32.8	35.1	83.1	54.1	52.8	63.0
	69.4	35.2	67.4	80.6	13.6	32.9	35.1	83.1	54.1	52.9	63.9

SUMMARY OF CHANGES

Subcommittee D02.12 has identified the location of selected changes to this standard since the last issue (D7373–07$^{\varepsilon 1}$) that may impact the use of this standard.

(1) Updated Section 11.
(2) Added Appendix X1.
(3) Added Practice D6300 to Referenced Documents and standard text.

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