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AMERICAN SOCIETY FOR TESTING AND MATERIALS
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# Standard Test Method for Semiquantitative Determination of Fish Oil in Drying Oils and Drying Oil Fatty Acids by Gas-Liquid Chromatography[1,2]

This standard is issued under the fixed designation D 3725; 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.

*This standard has been approved for use by agencies of the Department of Defense.*

## 1. Scope

1.1 This test method covers the determination by gas-liquid chromatography of herring and menhaden fish oil when present in small quantities in other drying oils and fatty acids. It describes specific conditions required for use with Test Method D 1983 to identify these oils.

NOTE 1—For general information that contributes to the knowledge of gas chromatography of fats and oils, see Guide D 555, Test Method D 2245 and Practice E 260.

1.2 *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:*

D 555 Guide for Testing Drying Oils[3]

D 1983 Test Method for Fatty Acid Composition by Gas-Liquid Chromatography of Methyl Esters[3]

D 2245 Test Method for Identification of Oils and Oil Acids in Solvent-Reducible Paints[4]

D 2800 Test Method for Preparation of Methyl Esters from Oils for Determination of Fatty Acid Composition by Gas Chromatography[3]

D 3457 Test Method for Preparation of Methyl Esters from Fatty Acids for Determination of Fatty Acid Composition by Gas-Liquid Chromatography[3]

E 260 Practice for Packed Column Gas Chromatography[5]

## 3. Summary of Test Method

3.1 The oil or fatty acids are converted to methyl esters which are then subjected to gas-liquid chromatography. The chromatogram is examined for small amounts of acids common to fish oils but not common to other drying oils or fatty acids used in paints and related products. These specific acids are referred to as *tracer acids*.

## 4. Apparatus

4.1 *Gas Chromatographic Instrument* having the minimal following characteristics:

4.1.1 *Column Oven* operated at a constant temperature between 190 and 210°C.

4.1.2 *Sample Inlet Port*, with the heater characteristics necessary for operation at 300°C.

4.1.3 *Detector*, of the thermal-conductivity or the flameionization type.

4.1.4 *Column*—A 12 ft (3.7 m) long by ⅛ in. (3.2 mm) in diameter aluminum column filled with 10 % (DEGS) (diethylene glycol succinate) on 80 to 100-mesh acid washed, calcined diatomaceous earth is recommended. However, any combination of column and packing, capable of separating these higher acids with good definition and repeatability may be utilized.

## 5. Reagents and Materials

5.1 *Fish Oil or Reference Standards* containing marker acids[6] (known acids added for identification purposes).

5.2 *Standard Reference Drying Oils* (for example, linseed, soya, etc.)

## 6. Standardization and Calibration

6.1 Refer to Test Method D 1983.

## 7. Procedure

7.1 *Preparation of Methyl Esters*:

7.1.1 If sample is an oil, prepare the methyl esters in

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[1] This test method is under the jurisdiction of ASTM Committee D-1 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.32 on Drying Oils.

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[2] This method is equivalent to or better than U.S. Federal Test Method Standard 141, Method 5011.1, Qualitative Test for Fish Oil available from Standardization Documents Order Desk, Bldg. 4 Section D, 700 Robbins Ave., Philadelphia, PA 19111-5094.

[3] *Annual Book of ASTM Standards*, Vol 06.03.

[4] *Annual Book of ASTM Standards*, Vol 06.01.

[5] *Annual Book of ASTM Standards*, Vol 14.02.

[6] The sole source of supply of marker acids (20:5, 22:1, and 22:6), known to the committee at this time is Nu-Check Prep Inc., P.O. Box 172, Elysian, MN 56028. If you are aware of alternative suppliers, please proved this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

accordance with Test Method D 2800.

7.1.2 If sample is a fatty acid, prepare the methyl esters in accordance with Test Method D 3457.

7.2 *Gas Chromatographic Separation*:

7.2.1 Determine optimum conditions required for good separation of methyl esters on the chromatograph and column being utilized. A 300°C injection port temperature is recommended for good reproducible identification of acids beyond C18. It is recommended that the range of the instrument be expanded to produce 10× magnification of the peak areas of fatty acids beyond C18 occurring as contaminants in drying oils.

7.2.2 Subject the prepared methyl esters to gas-liquid chromatography in accordance with Test Method D 1983, but using the above conditions.

## 8. Identification (Refer to Test Method D 1983)

8.1 Using reference drying oils and fish oils or marker acids, identify the fatty acid peaks by relative position on the chart.

8.2 The tracer fatty acids used to identify fish oil in drying oils are:

8.2.1 C20:5 and C22:6 occur in large amounts in fish oil but not in vegetable oils.

8.2.2 C22:1 is useful in differentiating between herring and menhaden since large amounts occur in herring and only small amounts in menhaden.

8.2.3 C14:0 and C16:1 are useful as corroborative acids since small varying amounts do occur in vegetable oils.

NOTE 2—C refers to carbon atoms and : refers to double bonds. For example, C20:5 refers to a fatty acid containing 20 carbon atoms and 5 double bonds.

## 9. Calculation

9.1 This test method is primarily a qualitative test for purity of the drying oil. However, the analysis can be made semiquantitative by comparing the tracer acids in the test oil with the reference oils as follows:

$$A = [(C - O)/F] \times 100 \quad (1)$$

where:

$A$ = fish oil, %,
$C$ = tracer acid found in the test specimen, %,
$O$ = tracer acid found in the reference vegetable oil, %, and
$F$ = tracer acid found in the reference fish oil, %.

9.2 Since a sample of the fish oil actually used in the material under test is not usually available, the calculation in 9.1 may be made using the tracer acid contents in fish oils given in Table 1.

## 10. Precision

10.1 Precision has not been determined. The limit of detectability is about 1 % menhaden or herring oil in linseed oil, but with practice even smaller amounts can be detected.

## 11. Keywords

11.1 chromatography; drying oils; fatty acids; fish oil; gas-liquid chromatography

**TABLE 1 Typical Fatty Acid Composition of Fish Oils**

| | Menhaden | Herring |
|---|---|---|
| 14:0 | 7.5 | 6.5 |
| 16:0 | 18.5 | 10.5 |
| 16:1 | 9.0 | 6.0 |
| 18:0 | 4.5 | 1.0 |
| 18:1 | 17.0 | 9.0 |
| [A] | 3.5 | 16.0 |
| 20:5 | 16.0 | 7.0 |
| 22:1 | 2.0 | 34.0 |
| 22:6 | 13.0 | 6.0 |
| All others | 9.0 | 4.0 |

[A] Acid not positively identified.