W = weight of fish oil containing omega-3 acids taken to prepare the *Sample solution* (g)

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF MERCURY:** Proceed as directed for *Mercury* ⟨261⟩, *Method IIa*, except to use a *Standard Mercury Solution* having the equivalent of 0.1 µg/mL of mercury.

Sample solution: Prepare as directed for the *Sample solution* in the test for *Limit of Arsenic* combining the two duplicate cooled digests into 1.0 mL of *Potassium Permanganate Solution.*

Acceptance criteria: NMT 0.1 µg/g

- **LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS**

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2.0 pg/g of WHO toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls, non-ortho IUPAC congeners PCB-77, PCB-81, PCB-126, and PCB-169, and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) is NMT 10.0 pg/g of WHO toxic equivalents.

SPECIFIC TESTS

- **FATS AND FIXED OILS,** *Acid Value* ⟨**401**⟩**:** NMT 3
- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26, calculated:

$$(2 \times PV) + AV$$

PV = peroxide value
AV = anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 1.5%
- **STEARIN:** 10 mL remains clear after cooling at 0° for 3 h
- **ABSORBANCE**

Sample solution: 0.24 mg/mL in isooctane

Acceptance criteria: The absorbance is NMT 0.70, determined at 233 nm.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature. Protect from light.
- **LABELING:** The label states the amount of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in mg/Capsule.
- **USP REFERENCE STANDARDS** ⟨**11**⟩

USP Docosahexaenoic Acid Ethyl Ester RS
all *cis*-4,7,10,13,16,19-Docosahexaenoic ethyl ester.
$C_{24}H_{36}O_2$ 356.55

USP Eicosapentaenoic Acid Ethyl Ester RS
all *cis*-5,8,11,14,17-Eicosapentaenoic ethyl ester.
$C_{22}H_{34}O_2$ 330.51

USP Fish Oil RS

USP Methyl Tricosanoate RS
Tricosanoic acid methyl ester.
$C_{24}H_{48}O_2$ 368.64

Add the following:

▲Fish Oil Containing Omega-3 Acids Delayed-Release Capsules

DEFINITION

Fish Oil Containing Omega-3 Acids Delayed-Release Capsules are enteric-coated Capsules that contain NLT 95.0% and NMT 105.0% of the labeled amount of Fish Oil Containing Omega-3 Acids where Fish Oil Containing Omega-3 Acids is the purified, winterized, and deodorized fatty oil obtained from fish of the families Engraulidae, Carangidae, Clupeidae, Osmeridae, Scombroidae, and Ammodytidae. The omega-3 acids are defined as the following: alpha-linolenic acid (C18:3 n–3), moroctic acid (C18:4 n–3), eicosatetraenoic acid (C20:4 n–3), eicosapentaenoic acid (EPA) (C20:5 n–3), heneicosapentaenoic acid (C21:5 n–3), docosapentaenoic acid (C22:5 n–3), and docosahexaenoic acid (DHA) (C22:6 n–3). It contains NLT 28.0% (w/w) of total omega-3 acids, expressed as free acids, consisting of NLT 13.0% of EPA and NLT 9.0% of DHA. Suitable antioxidants in appropriate concentrations may be added.

IDENTIFICATION

- **A.** The oil contained in the Capsules meets the requirements for the following test: The retention times of the peaks for eicosapentaenoic acid methyl ester and docosahexaenoic acid methyl ester obtained in the chromatogram of *Test Solution 2* in the test for *Content of EPA and DHA* correspond to those for the respective compounds in the chromatogram of *Standard Solution 1*. The sum of the area for EPA and DHA methyl esters is NLT 22% of the total detected area for the methyl esters, and no other peak has an area higher than 20% of the total detected area for the methyl esters. The chromatogram of *Test Solution 2* exhibits at least 15 additional peaks at the retention times of the methyl esters of unsaturated fatty acids exhibited in *Standard Solution 2*.

STRENGTH

- **CONTENT OF FISH OIL:** Weigh NLT 10 Capsules in a tared weighing bottle, carefully open the Capsules, without loss of shell material, and transfer the combined Capsule contents to a 100-mL beaker. Remove any adhering substance from the emptied Capsules by washing with several small portions of 2,2,4-trimethylpentane. Discard the washings, and allow the empty Capsules to dry in a current of dry air until the 2,2,4-trimethylpentane is completely evaporated. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average net weight per Capsule.

Acceptance criteria: 95.0%–105.0% of the labeled amount

- **CONTENT OF EPA AND DHA**

(See *Fats and Fixed Oils* ⟨401⟩*, Omega-3 Fatty Acids Determination and Profile*.)

Analysis

Samples: *Standard Solution 1*, *Standard Solution 2*, *Test Solution 1*, and *Test Solution 2*

Identify the retention times of the relevant fatty acid methyl esters peaks by comparing the chromatogram of *Standard Solution 2* with the reference chromatogram supplied with the USP Fish Oil RS. Identify the retention time for the internal standard peak from *Test Solution 2* by comparing with that of *Test Solution 1*.

Calculate the percentage of EPA or DHA in the portion of fish oil containing omega-3 acids taken from the Capsules:

$$\text{Result} = (R_U/R_S) \times (W_S/W_U) \times F \times 100$$

R_S = ratio of peak responses of either EPA or DHA relative to the internal standard from *Standard Solution 2*

W_S = weight of either USP Docosahexaenoic Acid Ethyl Ester RS or USP Eicosapentaenoic Acid Ethyl Ester RS used to prepare *Standard Solution 1* (mg)

W_U = weight of the fish oil containing omega-3 acids taken to prepare *Test Solution 2* (mg)

F = factor to express the content of DHA (0.921) and EPA (0.915) as free fatty acids

R_U = ratio of the peak response of either EPA or DHA to the corrected peak response of the internal standard from *Test Solution 2*, calculated as follows:

$$R_U = 1/[(r_{U2}/r_{T2}) - (r_{U1}/r_{T1})]$$

r_{U2} = response of the peak at the locus of the internal standard from *Test Solution 2*

r_{T2} = peak response of EPA or DHA from *Test Solution 2*

r_{U1} = response of any peak at the locus of the internal standard from *Test Solution 1*

r_{T1} = peak response of EPA or DHA from *Test Solution 1*. [NOTE—If no peak is found at the locus of the internal standard, $R_U = r_{T2}/r_{U2}$.]

Acceptance criteria: NLT 13.0% (w/w) of EPA and NLT 9.0% (w/w) of DHA

- **CONTENT OF TOTAL OMEGA-3 ACIDS**

(See *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.)

Analysis: Proceed as directed in the test for *Content of EPA and DHA*.

Calculate the percentage of the total omega-3 acids in the portion of fish oil containing omega-3 acids taken from the Capsules:

$$\text{Result} = EPA + DHA + [A_{n-3}(EPA + DHA)]/(A_{EPA} + A_{DHA})$$

EPA = content of EPA from the test for *Content of EPA and DHA* [% (w/w)]

DHA = content of DHA from the test for *Content of EPA and DHA* [% (w/w)]

A_{n-3} = sum of the areas of the peaks corresponding to C18:3 n–3, C18:4 n–3, C20:4 n–3, C21:5 n–3, and C22:5 n–3 methyl esters from *Test Solution 2*

A_{EPA} = area of the peak corresponding to the EPA methyl ester from *Test Solution 2*

A_{DHA} = area of the peak corresponding to the DHA methyl ester from *Test Solution 2*

Acceptance criteria: NLT 28.0% (w/w) of total omega-3 acids, expressed as free acids

PERFORMANCE TESTS

- **WEIGHT VARIATION ⟨2091⟩:** Meet the requirements
- **DISINTEGRATION AND DISSOLUTION ⟨2040⟩:** Meet the requirements for *Disintegration, Delayed-Release (Enteric-Coated) Soft Shell Capsules*

CONTAMINANTS

- **FATS AND FIXED OILS,** *Trace Metals* ⟨**401**⟩**:** NMT 0.1 ppm each of Pb, Cd, As, and Hg
- **LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS**

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by method No. 1613 revision B of the Environmental Protection Agency. Determine the content of polychlorinated biphenyls (PCBs) by method No. 1668 revision A of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2.0 pg/g of WHO toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls, non-ortho IUPAC congeners PCB-77, PCB-81, PCB-126, and PCB-169, and mono-ortho IUPAC congeners PCB-105, PCB-114, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) is NMT 10.0 pg/g of WHO toxic equivalents.

SPECIFIC TESTS

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- **FATS AND FIXED OILS,** *Anisidine Value* ⟨**401**⟩**:** NMT 20.0
- **FATS AND FIXED OILS,** *Peroxide Value* ⟨**401**⟩**:** NMT 5.0
- **FATS AND FIXED OILS,** *Total Oxidation Value (TOTOX)* ⟨**401**⟩**:** NMT 26, calculated:

$$\text{Result} = (2 \times PV) + AV$$

PV = peroxide value
AV = anisidine value

- **FATS AND FIXED OILS,** *Unsaponifiable Matter* ⟨**401**⟩**:** NMT 1.5%
- **STEARIN:** 10 mL remains clear after cooling at 0° for 3 h.
- **ABSORBANCE**

Sample solution: 0.24 mg/mL in isooctane

Acceptance criteria: NMT 0.70, determined at 233 nm

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at room temperature, protected from light.
- **LABELING:** The label states the amount of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in mg/Capsule.
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$C_{24}H_{36}O_2$ 356.55

USP Eicosapentaenoic Acid Ethyl Ester RS
all *cis*-5,8,11,14,17-Eicosapentaenoic ethyl ester.
$C_{22}H_{34}O_2$ 330.51

USP Fish Oil RS

USP Methyl Tricosanoate RS▲*USP35*

Folic Acid—*see Folic Acid General Monographs*

Folic Acid Tablets—*see Folic Acid Tablets General Monographs*

Forskohlii

DEFINITION

Forskohlii consists of the dried roots of *Plectranthus barbatus* Andrews, also known as *Coleus barbatus* (Andrews) Benth. and *Coleus forskohlii* Briq. (Fam. Lamiaceae). It contains NLT 0.4% of forskolin, calculated on the dried basis.

IDENTIFICATION

- **A. THIN-LAYER CHROMATOGRAPHIC IDENTIFICATION TEST** ⟨**201**⟩

Standard solution A: 50 µg/mL of USP Forskolin RS in acetonitrile. Sonicate for about 10 min.

Standard solution B: 5 mg/mL of USP Powdered Forskohlii Extract RS in acetonitrile. Sonicate for about 15 min, centrifuge, and use the supernatant.