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Fish Oil Omega-3 Acid Ethyl Esters Concentrate

DEFINITION

Fish Oil Omega-3 Acid Ethyl Esters Concentrate is a mixture of omega-3 acids concentrated from the body oil of fish in the form of ethyl esters, mainly eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE) and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). It may also contain ethyl esters of alpha-linolenic acid (C18:3 n-3, EE), moroctic acid (C18:4 n-3, EE), eicosatetraenoic acid (C20:4 n-3, EE), heneicosapentaenoic acid (C21:5 n-3, EE), and docosapentaenoic acid (C22:5 n-3, EE). It contains NLT 60% of total omega-3-acid ethyl esters and NLT 50% of combined omega-3-acid EPA and DHA ethyl esters. Suitable antioxidants in appropriate concentrations may be added.

[NOTE—This material is susceptible to oxidation. Samples should be protected from air exposure and tested promptly.]

IDENTIFICATION

- **A.** The retention times of the principal peaks of *Test solution 4* correspond to those of EPAee and DHAee of *Standard solution 1b* and *Standard solution 1a*, as obtained in the Assay.
- **B.** It meets the *Acceptance criteria* in the Assay.

ASSAY

• CONTENT OF EPAEE, DHAEE, AND TOTAL OMEGA-3-ACID ETHYL ESTERS

(See [Fats and Fixed Oils \(401\)](#), [Procedures, Omega-3 Fatty Acids Determination and Profile](#).)

Test solution 3, Test solution 4, Standard solution 1a, Standard solution 1b, System suitability solution 1, Chromatographic system, and System suitability: Proceed as directed in the chapter.

Analysis

Samples: *Test solution 3, Test solution 4, Standard solution 1a, and Standard solution 1b*

Calculate separately the content of EPAee and DHAee in the portion of Fish Oil Omega-3 Acid Ethyl Esters Concentrate taken:

$$\text{Result} = (R_U/R_S) \times (C_S/C_U)$$

R_U = peak area ratio of the EPAee or DHAee peak to the internal standard peak from *Test solution 3*

R_S = peak area ratio of the EPAee peak to the internal standard peak from *Standard solution 1b* or the DHAee peak to the internal standard peak from *Standard solution 1a*

C_S = concentration of [USP Eicosapentaenoic Acid Ethyl Ester RS](#) in *Standard solution 1b* or [USP Docosahexaenoic Acid Ethyl Ester RS](#) in *Standard solution 1a* (mg/mL)

C_U = concentration of Fish Oil Omega-3 Acid Ethyl Esters Concentrate in *Test solution 3* (mg/mL)

Calculate the content of total omega-3-acid ethyl esters in the portion of Fish Oil Omega-3 Acid Ethyl Esters Concentrate taken:

$$\text{Result} = r_{\text{FAn-3ee}} [(EPAee + DHAee)/(r_{\text{EPAee}} + r_{\text{DHAee}})] + EPAee + DHAee$$

$r_{\text{FAn-3ee}}$ = sum of the peak areas of alpha-linolenic acid ethyl ester (C18:3 n-3, EE), moroctic acid ethyl ester (C18:4 n-3, EE), eicosatetraenoic acid ethyl ester (C20:4 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) from *Test solution 4*

EPA_e = content of EPAee (mg/g)

DHA_{ee} = content of DHAee (mg/g)

r_{EPAee} = peak area of EPAee from *Test solution 4*

r_{DHAe} = peak area of DHAee from *Test solution 4*

Acceptance criteria: See [Table 1](#).

Table 1

Total Omega-3-Acid Ethyl Esters, NLT (%)	Sum of EPAee and DHAee, NLT (%)	EPAee, NLT (%)	DHAee, NLT (%)
60	50	25	20
60	50	—	40
60	50	40	—

IMPURITIES

• OLIGOMERS AND PARTIAL GLYCERIDES

Mobile phase: [Tetrahydrofuran](#)

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin¹ in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively

Sample solution: 1.00 mg/mL of Fish Oil Omega-3 Acid Ethyl Esters Concentrate in *Mobile phase*

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: Differential refractometer

Columns: Three in series, 7.8-mm × 30-cm; 5-μm packing [L21](#), with the following pore sizes.

Column 1: 50 nm

Column 2: 10 nm

Column 3: 5 nm

Connection sequence: Injector–Column 1–Column 2–Column 3–Detector

Flow rate: 0.8 mL/min

Injection volume: 40 μL

System suitability

Sample: *System suitability solution*

[NOTE—The elution order is tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin.]

Suitability requirements

Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin

Analysis

Sample: *Sample solution*

Measure the areas of the major peaks.

Calculate the percentage of oligomers in the portion of Fish Oil Omega-3 Acid Ethyl Esters Concentrate taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak area of oligomers and partial glycerides (sum of the peak areas with retention times less than those of ethyl esters) from the *Sample solution*

r_T = sum of all the peak areas from the *Sample solution*

Acceptance criteria: The sum of oligomers and partial glycerides is NMT 7%.

Change to read:

• LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS (PCBs)

Analysis: Determine the content of polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) ▲ using the current revision of ▲ (USP 1-Dec-2022) Method 1613 ▲ (USP 1-Dec-2022) of the Environmental Protection Agency. Determine the content of PCBs ▲ using the current revision of ▲ (USP 1-Dec-2022) Method 1668 ▲ (USP 1-Dec-2022) of the Environmental Protection Agency.

Acceptance criteria: The sum of PCDDs and PCDFs is NMT 2 pg/g of WHO toxic equivalents. The sum of PCDDs, PCDFs, and dioxin-like PCBs (polychlorinated biphenyls, nonortho 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 \(401\)](#), [Procedures, Acid Value](#): NMT 2.0
- [FATS AND FIXED OILS \(401\)](#), [Procedures, Anisidine Value](#): NMT 20.0
- [FATS AND FIXED OILS \(401\)](#), [Procedures, Peroxide Value](#): NMT 10.0
- **ABSORBANCE**

Sample solution: 0.24 mg/mL of Fish Oil Omega-3 Acid Ethyl Esters Concentrate in [isooctane](#)

Acceptance criteria: NMT 0.6, determined at 233 nm, with [isooctane](#) being used as the blank