Omega-3-Acid Ethyl Esters

DEFINITION

Change to read:

•Omega-3-Acid Ethyl Esters is a mixture of ethyl esters, principally the ethyl esters of eicosapentaenoic acid (EPAee) (C20:5 n–3, EE) and docosahexaenoic acid (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). Tocopherol may be added as an antioxidant.● (IRA 1-Jan-2016)

IDENTIFICATION

Change to read:

- **A.** The retention times of the •principal peaks● (IRA 1-Jan-2016) in *Test solution* •*4*● (IRA 1-Jan-2016) correspond to those • of eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester● (IRA 1-Jan-2016) in *Standard solution* •*1b* and *Standard solution 1a,*● (IRA 1-Jan-2016) as obtained in the *Assay*.

Add the following:

•• **B.** It meets the acceptance criteria in *Table 1* of the *Assay*.● (IRA 1-Jan-2016)

ASSAY

Change to read:

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

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

  •**Standard solution 1a, Standard solution 1b, Test solution 3, Test solution 4, System suitability solution 1, Chromatographic system,** and **System suitability:** Proceed as directed in *Fats and Fixed Oils* ⟨401⟩, *Omega-3 Fatty Acids Determination and Profile*.● (IRA 1-Jan-2016)

  Analysis

  Samples: •*Standard solution 1a*, *Standard solution 1b*, *Test solution 3*, and *Test solution 4*● (IRA 1-Jan-2016)

  Calculate the content of EPAee and DHAee in the portion of Omega-3-Acid Ethyl Esters 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*● (IRA 1-Jan-2016) or DHAee peak to the internal standard peak from •*Standard solution 1a*● (IRA 1-Jan-2016)

C_S = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS •in *Standard solution 1b*● (IRA 1-Jan-2016) or USP Docosahexaenoic Acid Ethyl Ester RS in •*Standard solution 1a*● (IRA 1-Jan-2016) (mg/mL)

C_U = concentration of Omega-3-Acid Ethyl Esters in *Test solution 3* (g/mL)

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

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

$r_{FAn-3ee}$ = 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) in *Test solution 4*

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

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

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

Acceptance criteria: It conforms to the acceptance criteria in *Table 1*. •Articles labeled as Omega-3-Acid Ethyl Esters type A meet *Acceptance Criteria II*.

Table 1

		Acceptance Criteria I		Acceptance Criteria II (For articles labeled as Omega-3-Acid Ethyl Esters type A)	
Name	Relative Retention Time	NLT	NMT	NLT	NMT
C18:3 n–3, EE[a]	0.585	—	—	—	—
C18:4 n–3, EE[b]	0.608	—	—	—	—
C20:4 n–3, EE[c]	0.777	—	—	—	—
C20:5 n–3, EE (EPAee)[d]	0.796	430 mg/g	495 mg/g	365 mg/g	435 mg/g
C21:5 n–3, EE[e]	0.889	—	—	—	—
C22:5 n–3, EE[f]	0.977	—	—	—	—
C22:6 n–3, EE (DHAee)[g]	1.000	347 mg/g	403 mg/g	290 mg/g	360 mg/g
EPAee + DHAee	—	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters	—	90% (w/w)	—	78% (w/w)	—

[a] Alpha-linolenic acid ethyl ester.
[b] Moroctic acid ethyl ester.
[c] Eicosatetraenoic acid ethyl ester.
[d] Eicosapentaenoic acid ethyl ester.
[e] Heneicosapentaenoic acid ethyl ester.
[f] Docosapentaenoic acid ethyl ester (clupanodonic acid ethyl ester).
[g] Docosahexaenoic acid ethyl ester.

● (IRA 1-Jan-2016)

IMPURITIES

- **FATS AND FIXED OILS ⟨401⟩:** NMT 0.1 ppm each of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)

Change to read:

- **CHOLESTEROL**

Internal standard stock solution: 3 mg/mL of 5α-cholestane in *n*-heptane. [NOTE—Prepare fresh before use.]

Internal standard solution: 0.3 mg/mL of 5α-cholestane in *n*-heptane. [NOTE—Prepare fresh before use.]

Standard stock solution: 3.0 mg/mL of cholesterol in *n*-heptane. [NOTE—This solution is stable for 6 months stored in a freezer.] Transfer 1.0 mL of this solution to a 10.0-mL volumetric flask. Dilute with *n*-heptane to volume. [NOTE—Prepare this solution fresh daily.]

Standard solution: Transfer 1.0 mL each of the *Standard stock solution* and the *Internal standard solution* to a 15-mL centrifuge tube. Prepare as directed in the *Sample solution* beginning with "Evaporate to dryness".

Alpha tocopherol stock solution: 1.5–2.0 mg/mL of USP Alpha Tocopherol RS in *n*-heptane. [NOTE—This solution is stable for 12 months stored in a freezer.]

System suitability solution: Mix 1.0 mL of the *Standard stock solution*, 1.0 mL of the *Internal standard stock solution*, and 2.0 mL of the *Alpha tocopherol stock solution* in a 50-mL volumetric flask. Evaporate to dryness with the aid of heat, and dilute with ethyl acetate to volume. Dilute 1.0 mL of this solution with ethyl acetate to 10.0 mL. [NOTE—This solution is stable for 6 months stored in a freezer.]

Sample solution: Transfer 100 mg of Omega-3-Acid Ethyl Esters to a 15-mL centrifuge tube. Add 1.0 mL of the *Internal standard solution*. Evaporate to dryness at about 50° with a gentle stream of nitrogen. Add 0.5 mL of 50% potassium hydroxide and 3 mL of alcohol, fill the tube with nitrogen, and cap. Heat the sample at 100° for 60 min, using a heating block. Cool for about 10 min. Add 6 mL of water to the tube, and shake for 1 min. Extract the solution four times with 2.5-mL portions of ethyl ether, using a vortex mixer or suitable shaker for 1 min for each extraction. Transfer and combine the extracts into a large centrifuge tube, and wash with 5 mL of water, mixing completely with gentle inversion. Remove the water phase, and add 5 mL of 0.5 M potassium hydroxide to the ether phase, mixing carefully to avoid an emulsion. Remove the potassium hydroxide, and add another 5 mL of water, mixing carefully. Transfer the ether phase to a small centrifuge tube. [NOTE—If an emulsion has occurred, a small amount of sodium chloride may be added to obtain a separation of the phases.] Evaporate the ether phase to dryness under a stream of nitrogen with careful heating. Dissolve the sample in 600 µL of ethyl acetate, and mix well. Transfer 200 µL of this solution to a sample vial, and dilute with ethyl acetate to about 2 mL.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary; coated with a G27 phase of 0.25-µm thickness

Temperatures

Injection port: 320°

Detector: 300°

Column: See ● *Table 2*.

Table 2● (IRA 1-Jan-2016)

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	1
170	4	320	1.5

Carrier gas: Helium

Flow rate: 1.3 mL/min

Injection volume: 1 µL

Injection type: Splitless injection system

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 1.2 between alpha tocopherol and cholesterol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the content of total cholesterol in the portion of Omega-3-Acid Ethyl Esters taken:

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

R_U = peak area ratio of the cholesterol peak to the internal standard from the *Sample solution*

R_S = peak area ratio of the cholesterol peak to the internal standard from the *Standard solution*

W_S = weight of cholesterol in the *Standard solution* (mg)

W_U = weight of Omega-3-Acid Ethyl Esters in the *Sample solution* (g)

Acceptance criteria: NMT 3.0 mg/g

- **OLIGOMERS**

Mobile phase: Tetrahydrofuran

System suitability solution: Monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin in *Mobile phase*, with concentrations of about 0.5, 0.3, and 0.2 mg/mL, respectively. [NOTE—Suitable grades of monodocosahexaenoin, didocosahexaenoin, and tridocosahexaenoin may be obtained from Nu-Chek Prep.]

Sample solution 1: 5.0 mg/mL of Omega-3-Acid Ethyl Esters in tetrahydrofuran

Sample solution 2: [NOTE—Use *Sample solution 2* where the results of this test using *Sample solution 1* exceed the *Acceptance criteria* due to the presence of monoglycerides.] Weigh 50 mg of Omega-3-Acid Ethyl Esters into a quartz tube, add 1.5 mL of a 20-g/L solution of sodium hydroxide in methanol, cover with nitrogen, cap tightly with a polytef-lined cap, mix, and heat on a water bath for 7 min. Allow to cool. Add 2.0 mL of boron trichloride–methanol solution, cover with nitrogen, cap tightly, mix, and heat on a water bath for 30 min. Cool to 40°–50°, add 1 mL of isooctane, cap, and shake vigorously for NLT 30 s. Immediately add 5 mL of saturated sodium chloride solution, cover with nitrogen, cap, and shake thoroughly for NLT 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer with 1 mL of isooctane. Wash the combined isooctane extracts with two quantities, each of 1 mL of water. Carefully evaporate the solvent under a stream of nitrogen, then add 10.0 mL of tetrahydrofuran to the residue. Add a small amount of anhydrous sodium sulfate, and filter.

Chromatographic system

(See *Chromatography* ⟨621⟩, *System Suitability*.)

Mode: LC
Detector: Differential refractometer
Columns: Three concatenated, 7.8-mm × 30-cm; 7-µm packing L21, with pore sizes in the range of 5–50 nm, arranged with decreasing pore size from the injector to the detector to fulfill the system suitability requirements
Flow rate: 0.8 mL/min
Injection volume: 40 µL
System suitability
Sample: *System suitability solution*
Suitability requirements
Elution order: Tridocosahexaenoin, didocosahexaenoin, and monodocosahexaenoin
Resolution: NLT 2.0 between monodocosahexaenoin and didocosahexaenoin; NLT 1.0 between didocosahexaenoin and tridocosahexaenoin
Analysis
Samples: *Sample solution 1* and *Sample solution 2*
Measure the areas of the major peaks.
Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 1*:

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

r_I = sum of the areas of the peaks with a retention time less than that of the ethyl esters peaks
r_T = sum of the areas of all peaks
Calculate the percentage of oligomers in the portion of Omega-3-Acid Ethyl Esters taken to prepare *Sample solution 2*:

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

r_I = sum of the areas of all peaks with a retention time less than that of the methyl esters peaks
r_T = sum of the areas of all peaks
Acceptance criteria: NMT 1.0% of oligomers

- **LIMIT OF DIOXINS, FURANS, AND POLYCHLORINATED BIPHENYLS (PCBS):** 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 1 pg/g of WHO toxic equivalents. The sum of PCBs (polychlorinated biphenyls, IUPAC congeners PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180) is NMT 0.5 ppm.

Change to read:

- **LIMIT OF TOTAL UNIDENTIFIED FATTY ACID ETHYL ESTERS**
•[NOTE—This test is not required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]• (IRA 1-Jan-2016)
From the chromatogram obtained with *Test solution 4* in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, determine the peak area of the largest single unidentified peak with a relative retention time different from those in •*Table 3*.

Table 3• (IRA 1-Jan-2016)

Identified Ethyl Ester	Relative Retention Time
Phytanic acid	0.416
C16:3 n–4	0.431
C16:4 n–1	0.468
C18:3 n–6	0.557

Table 3• (IRA 1-Jan-2016) *(Continued)*

Identified Ethyl Ester	Relative Retention Time
C18:3 n–4	0.574
C18:3 n–3	0.585
C18:4 n–3	0.608
C18:4 n–1	0.618
Furan acid 5	0.691
C19:5	0.710
C20:3 n–6	0.720
C20:4 n–6	0.736
Furan acid 7	0.744
C20:4 n–3	0.777
Furan acid 8	0.783
EPA	0.796
Furan acid 9	0.867
C21:5 n–3	0.889
C22:4	0.917
Furan acid 10	0.922
C22:5 n–6	0.939
Furan acid 11	0.963
C22:5 n–3	0.977
DHA	1.000

Calculate the content of unidentified fatty acid ethyl esters in area percentage:

$$\text{Result} = 100 - (100 \times \Sigma\, Aiee/r_T)$$

Aiee = peak area of each identified ethyl ester in •*Table 3*• (IRA 1-Jan-2016)
r_T = sum of the areas of all peaks except solvents and BHT
Acceptance criteria: The area of the largest single unidentified peak is NMT 0.5% of the total area. The total area of unidentified peaks as calculated above is NMT 2%.

Add the following:

•• **LIMIT OF NON-OMEGA-3-ACID ETHYL ESTERS**
[NOTE—This test is only required for the articles labeled as Omega-3-Acid Ethyl Esters type A.]
From the chromatogram obtained with *Test solution 4* in the *Assay* for *Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters*, calculate the amounts of C18:1 n–9 ethyl ester and C20:4 n–6 ethyl ester in the portion of Omega-3-Acid Ethyl Esters taken:

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

Aiee = peak area of C18:1 n–9 ethyl ester or C20:4 n–6 ethyl ester
r_T = sum of the areas of all peaks except solvents and BHT
Acceptance criteria
C18:1 n–9 ethyl ester: NMT 6.0%
C20:4 n–6 ethyl ester: NMT 4.0%• (IRA 1-Jan-2016)

SPECIFIC TESTS

- **FATS AND FIXED OILS** ⟨**401**⟩, *Acid Value*: NMT 2.0
- **FATS AND FIXED OILS** ⟨**401**⟩, *Anisidine Value*: NMT 15
- **FATS AND FIXED OILS** ⟨**401**⟩, *Peroxide Value*: NMT 10.0
- **ABSORBANCE**
Sample solution: Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isooctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isooctane to volume.

Acceptance criteria: NMT 0.55, determined at 233 nm, with isooctane being used as the blank

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers under a nitrogen atmosphere. Store at controlled room temperature.

Change to read:

- **LABELING:** The label states the content of DHA ethyl ester and EPA ethyl ester in mg/g, the sum of the EPA and DHA ethyl esters contents in mg/g, and the content of the total omega-3-acid ethyl esters in weight percentage (w/w). It also states the name of any added antioxidant. ● Articles intended to meet *Acceptance Criteria II* of the *Assay* and the *Limit of Non-Omega-3-Acid Ethyl Esters* are labeled as Omega-3-Acid Ethyl Esters type A.● (IRA 1-Jan-2016)

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