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 (C21:5 n-3, EE), eicosatetraenoic acid (C20:4 n-3, EE), and docosapentaenoic acid (C22:5 n-3, EE). Tocopherol may be added as an antioxidant.*

**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 (IRA 1-Jan-2016) 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:

\[
\text{Result} = \left( \frac{R_0}{R_3} \right) \times \left( \frac{C_i}{C_o} \right)
\]

\[
R_0 = \text{peak area ratio of the EPAee or DHAee peak to the internal standard peak from Test solution 3}
\]

\[
R_3 = \text{peak area ratio of the EPAee or DHAee peak to the internal standard peak from Standard solution 1a (IRA 1-Jan-2016) or DHAee peak to the internal standard peak from Standard solution 1b (IRA 1-Jan-2016)}
\]

\[
C_i = \text{concentration of USP Eicosapentaenoic Acid Ethyl Ester RS in Standard solution 1b (IRA 1-Jan-2016)} \text{ or USP Docosahexaenoic Acid Ethyl Ester RS in Standard solution 1a (IRA 1-Jan-2016)}
\]

\[
C_o = \text{concentration of Omega-3-Acid Ethyl Esters in Test solution 3 (g/mL)}
\]

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

\[
\text{Result} = \frac{\text{EPAee} + \text{DHAee}}{\text{EPAee} + \text{DHAee}} + \text{EPAee} + \text{DHAee}
\]

\[
\text{EPAee} = \text{content of EPAee (mg/g)}
\]

\[
\text{DHAee} = \text{content of DHAee (mg/g)}
\]

\[
\text{rEPAee} = \text{peak area of EPAee in Test solution 4}
\]

\[
\text{rDHAee} = \text{peak area of DHAee in Test solution 4}
\]

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>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Acceptance Criteria I</th>
<th>Acceptance Criteria II (For articles labeled as Omega-3-Acid Ethyl Esters type A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NLT</td>
<td>NMT</td>
</tr>
<tr>
<td>C18:3 n-3, EE</td>
<td>0.585</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C18:4 n-3, EE</td>
<td>0.608</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C20:4 n-3, EE</td>
<td>0.777</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C20:5 n-3, EE (EPAee)</td>
<td>0.796</td>
<td>430 mg/g</td>
<td>495 mg/g</td>
</tr>
<tr>
<td>C22:5 n-3, EE</td>
<td>0.889</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C22:6 n-3, EE (DHAee)</td>
<td>0.977</td>
<td>347 mg/g</td>
<td>403 mg/g</td>
</tr>
<tr>
<td>EPAee + DHAee</td>
<td>—</td>
<td>800 mg/g</td>
<td>880 mg/g</td>
</tr>
<tr>
<td>Total omega-3-acid ethyl esters</td>
<td>—</td>
<td>90% (w/w)</td>
<td>78% (w/w)</td>
</tr>
</tbody>
</table>

*Alpha-linolenic acid ethyl ester; +Moronic acid ethyl ester; +Eicosapentaenoic acid ethyl ester; +Docosapentaenoic acid ethyl ester; +Heneicosapentaenoic acid ethyl ester; +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)

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Change to read:

- **CHOLESTEROL**
  Internal standard stock solution: 3 mg/mL of 5α-cholestanol in n-heptane. [NOTE—Prepare fresh before use.]
  Internal standard solution: 0.3 mg/mL of 5α-cholestanol 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 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 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°C 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°C 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°C
  - **Detector:** 300°C
- **Column:** See Table 2.

### Table 2a (IRA 1-Jan-2016)

<table>
<thead>
<tr>
<th>Initial Temperature (°C)</th>
<th>Temperature Ramp (°C/min)</th>
<th>Final Temperature (°C)</th>
<th>Hold Time at Final Temperature (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>170</td>
<td>0</td>
<td>170</td>
<td>1</td>
</tr>
<tr>
<td>170</td>
<td>4</td>
<td>320</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**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:
  
  \[ R_0 = \frac{W_0}{W} \]
  \[ R_1 = \frac{W_1}{W} \]
  \[ W_0 = \text{weight of Omega-3-Acid Ethyl Esters in the Sample solution (g)} \]
  \[ W_1 = \text{weight of cholesterol in the Standard solution (mg)} \]

**Acceptance criteria:** NMT 3.0 mg/g

- **OILIGOMERS**
  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 polytetra-lined cap, 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°C–50°C, 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:

\[
\text{Result} = \left( \frac{r_i}{r_T} \right) \times 100
\]

\( r_i \) = 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:

\[
\text{Result} = \left( \frac{r_i}{r_T} \right) \times 100
\]

\( r_i \) = 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

- **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 the area of the largest single unidentified peak with a relative retention time different from those in the chromatogram obtained with

\[
\text{Acceptance criteria:} \quad \text{The sum of PCDDs and PCDFs is NMT 2%}
\]

- **LIMIT OF NON-OMEGA-3-ACID ETHYL ESTERS**

[NOTE—This test is only required for the articles labeled No. 1668 revision A of the Environmental Protection Agency. 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.]

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:

\[
\text{Result} = (\text{Aiee}/r_i) \times 100
\]

\( Aiee \) = peak area of each identified ethyl ester in

\( r_i \) = 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 TOTAL UNIDENTIFIED FATTY ACID ETHYL ESTERS**

[NOTE—This test is not 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 percentage of unidentified fatty acid ethyl esters in area percentage:

\[
\text{Result} = 100 - (100 \times \sum \text{Aiee}/r_i)
\]

\( Aiee \) = peak area of each unidentified ethyl ester

\( r_i \) = 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%.

**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.

<table>
<thead>
<tr>
<th>Table 3 (Continued)</th>
<th>Identified Ethyl Ester</th>
<th>Relative Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18:3 n-4</td>
<td>0.574</td>
<td></td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>0.585</td>
<td></td>
</tr>
<tr>
<td>C18:4 n-3</td>
<td>0.608</td>
<td></td>
</tr>
<tr>
<td>C18:4 n-1</td>
<td>0.618</td>
<td></td>
</tr>
<tr>
<td>Furan acid 5</td>
<td>0.691</td>
<td></td>
</tr>
<tr>
<td>C19:5</td>
<td>0.710</td>
<td></td>
</tr>
<tr>
<td>C20:3 n-6</td>
<td>0.720</td>
<td></td>
</tr>
<tr>
<td>C20:4 n-6</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td>Furan acid 7</td>
<td>0.744</td>
<td></td>
</tr>
<tr>
<td>C20:4 n-3</td>
<td>0.777</td>
<td></td>
</tr>
<tr>
<td>Furan acid 8</td>
<td>0.783</td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.796</td>
<td></td>
</tr>
<tr>
<td>Furan acid 9</td>
<td>0.867</td>
<td></td>
</tr>
<tr>
<td>C21:5 n-3</td>
<td>0.889</td>
<td></td>
</tr>
<tr>
<td>C22:4</td>
<td>0.917</td>
<td></td>
</tr>
<tr>
<td>Furan acid 10</td>
<td>0.922</td>
<td></td>
</tr>
<tr>
<td>C22:5 n-6</td>
<td>0.939</td>
<td></td>
</tr>
<tr>
<td>Furan acid 11</td>
<td>0.963</td>
<td></td>
</tr>
<tr>
<td>C22:5 n-3</td>
<td>0.977</td>
<td></td>
</tr>
<tr>
<td>DHA</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

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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*