Omega-3-Acid Ethyl Esters Capsules

DEFINITION

Change to read:

Omegas-3-Acid Ethyl Esters Capsules contain Omega-3-Acid Ethyl Esters, with NLT 95.0% and NMT 105.0% of the labeled sum of eicosapentaenoic acid ethyl ester (EPAee) and docosahexaenoic acid ethyl ester (DHAee) and NLT 95% of the labeled amount of total omega-3-acid ethyl esters, as the sum 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), eicosapentaenoic acid ethyl ester (EPAee) (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), docosapentaenoic acid ethyl ester (C22:5 n-3, EE), and docosahexaenoic acid ethyl ester (DHAee) (C22:6 n-3, EE). Tocopherol may be added as an antioxidant.

IDENTIFICATION

Change to read:

• A. The retention times of the peaks for eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester of the Sample solution correspond to those of the Standard solution, as obtained in the Assay for Content of EPAee, DHAee, and Total Omega-3-Acid Ethyl Esters.

Add the following:

• B. It complies with the Acceptance criteria in the test for Concentration of Omega-3-Acid Ethyl Esters in Specific Tests.

ASSAY

Change to read:

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

[NOTE—Carry out the procedure as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (i.e., copper and iron), and air.]

Antioxidant solution: 50 mg/L of butylated hydroxytoluene in isooctane.

Retention time identification solution: Prepare a mixture containing suitable concentrations 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), eicosapentaenoic acid ethyl ester (C20:5 n-3, EE), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in Antioxidant solution.

Internal standard solution: 7.0 mg/mL of USP Methyl Tricosanoate RS in Antioxidant solution.

System suitability solution: 5.5 mg/mL of docosahexaenoic acid methyl ester and 0.5 mg/mL of tetracos-15-enolic acid methyl ester in Antioxidant solution.

Standard solution: Dissolve 60.0 mg of USP Docosahexaenoic Acid Ethyl Ester RS and 90.0 mg of USP Eicosapentaenoic Acid Ethyl Ester RS in 10.0 mL of Internal standard solution.

Sample solution: Weigh NLT 10 Capsules in a tared weighing bottle. With a sharp blade, 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 diethyl ether. Discard the washings, and allow the empty Capsules to air-dry over a period of NMT 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the empty Capsules in the original tared weighing bottle, and calculate the average fill weight per Capsule (W0). Transfer an amount of the combined Capsule contents equivalent to 225 mg of the labeled amount of total omega-3-acid ethyl esters to a suitable flask, and dissolve with 10.0 mL of Internal standard solution.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm x 25–50-m fused silica capillary; coated with a 0.25-µm film of G16

Temperatures

Injection port: 250°C

Detector: 270°C

Column: See Table 1.

<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>2</td>
</tr>
<tr>
<td>170</td>
<td>3.5</td>
<td>255</td>
<td>9</td>
</tr>
</tbody>
</table>

Carrier gas: Hydrogen or helium

Linear velocity: Adjust to obtain a retention time for docosahexaenoic acid ethyl ester of 26 ± 3 min.

Injection volume: 1 µL

Injection type: Split; split ratio, 1:220

System suitability

Samples: System suitability solution and Standard solution.

Suitability requirements

Resolution: NLT 1.2 between docosahexaenoic acid methyl ester and tetracos-15-enolic acid methyl ester peaks, System suitability solution.

Relative standard deviation: NMT 2.0% for the ratios of the peak responses of DHAee and EPAee relative to the internal standard, Standard solution.

Analysis

Samples: Retention time identification solution.

Identify the retention times of the relevant fatty acid ethyl esters by comparing the peaks from the Sample solution with those from the Retention time identification solution.

Calculate the content, in mg/g, of EPAee and DHAee in the portion of Capsules taken:

\[ \text{Result} = \left( \frac{R_o}{R_i} \right) \times \left( \frac{C_u}{C_0} \right) \]

R0 = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the Sample solution.

Ri = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the Standard solution.
\[ C_i = \text{concentration of USP Eicosapentaenoic Acid Ethyl Esters RS or USP Docosahexaenoic Acid Ethyl Esters RS in the Standard solution (mg/mL)} \]

\[ C_i = \frac{W_{hr}}{L} \times (100/L) \]

Calculate the percentage of the labeled sum of EPAee and DHAee in the portion of Capsules taken:

\[ \text{Result} = \left( \frac{W_{hr}}{L} \times 100 \right) \]

\[ EPAee = \text{content of EPAee in the portion of Capsules taken (mg/g)} \]

\[ DHAee = \text{content of DHAee in the portion of Capsules taken (mg/g)} \]

\[ W_{hr} = \text{average fill weight of the Capsules taken (g)} \]

\[ L = \text{sum of the labeled content of EPAee and DHAee (mg/Capsule)} \]

Calculate the percentage of the labeled amount of total omega-3-acid ethyl esters in the portion of Capsules taken:

\[ \text{Result} = \left( \frac{\text{peak area of EPAee from the Sample solution}}{\text{average fill weight of the Capsules taken (g)}} \right) \times \left( \frac{100}{L} \right) \]

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

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

\[ \text{peak area of EPAee from the Sample solution} \]

\[ \text{peak area of DHAee from the Sample solution} \]

\[ \text{average fill weight of the Capsules taken (g)} \]

\[ L = \text{label claim of total omega-3-acids ethyl esters of 1 mL of water. Carefully evaporate the solvent} \]

\[ \text{add 1.5 mL of a 20-g/L solution of USP Eicosapentaenoic Acid Ethyl Ester RS in the mobile phase} \]

\[ \text{Sample solution 2:} \]

\[ \text{Sample solution 1:} \]

**Chromatographic system**

*See Chromatography (621), System Suitability.*

**Mode:** LC

**Detector:** Differential refractometer

**Columns:** Three concatenated, 7.8-mm \( \times \) 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:** Tridocosahexaenoic acid, didocosahexaenoic, and monodocosahexaenoic

**Resolution:** NLT 2.0 between monodocosahexaenoic and didocosahexaenoic; NLT 1.0 between didocosahexaenoic and tridocosahexaenoic

**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:

\[ \text{Result} = \left( \frac{\text{area of peak} - \text{area of baseline}}{\text{area of peak} + \text{area of baseline}} \right) \times 100 \]

\[ r_i = \text{sum of the areas with retention times less than that of the methyl esters peak} \]

\[ r_f = \text{sum of the areas of all peaks} \]

**Acceptance criteria:** NMT 2% of oligomers
SPECIFIC TESTS

Add the following:

- **CONCENTRATION OF OMEGA-3-ACID ETHYL ESTERS**

  Antioxidant solution, Retention time identification solution, Internal standard solution, System suitability solution, Standard solution, Sample solution, Chromatographic system, System suitability, and Analysis: Proceed as directed in the Assay for Content of EPAee, DHAAe, and Total Omega-3-Acid Ethyl Esters. Calculate the concentration, in mg/g, of EPAee and DHAAe in the portion of Capsules taken:

  Result = \((R_u/R_t) \times (C_u/C_t)\)

  \(R_u\) = peak area ratio of the EPAee or DHAAe peak to the internal standard peak from the Sample solution

  \(R_t\) = peak area ratio of the EPAee or DHAAe peak to the internal standard peak from the Standard solution

  \(C_u\) = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the Standard solution (mg/mL)

  \(C_t\) = nominal concentration of the total omega-3-acid esters in the Sample solution (g/mL)

  Calculate the concentration, in mg/g, of total omega-3-acids ethyl esters in the portion of Capsules taken:

  Result = \((f_{\text{alpha-3ee}} \times [(\text{EPAee} + \text{DHAAe})/(\text{EPAee} + \text{DHAAe})] + \text{EPAee} + \text{DHAAe})\)

  \(f_{\text{alpha-3ee}}\) = 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 the Sample solution

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

  \(\text{DHAAe}\) = content of DHAAe (mg/g)

  \(f_{\text{ox}}\) = peak area of EPAee from the Sample solution

  \(f_{\text{ox}}\) = peak area of DHAAe from the Sample solution

  **Acceptance criteria:** It meets the requirements in Table 2. Capsules labeled as containing Omega-3-Acid Ethyl Esters type A meet Acceptance Criteria II.

<table>
<thead>
<tr>
<th>Name</th>
<th>Acceptance Criteria I</th>
<th>Acceptance Criteria II (For capsules labeled as containing omega-3-acid ethyl esters type A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPAee</td>
<td>NLT 430 mg/g</td>
<td>NMT 495 mg/g</td>
</tr>
<tr>
<td></td>
<td>NLT 365 mg/g</td>
<td>NMT 435 mg/g</td>
</tr>
<tr>
<td>DHAAe</td>
<td>NLT 347 mg/g</td>
<td>NMT 403 mg/g</td>
</tr>
<tr>
<td></td>
<td>NLT 290 mg/g</td>
<td>NMT 360 mg/g</td>
</tr>
</tbody>
</table>

  + Sum 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), and docosahexaenoic acid ethyl ester (DHAAe) (C22:6 n-3, EE).

  - **FATS AND FIXED OILS (401), Acid Value**

    Sample solution: Dissolve about 5.0 g of the oil, accurately weighed, in 100 mL of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 M potassium hydroxide) contained in a flask.

    Acceptance criteria: NMT 2.0

  - **FATS AND FIXED OILS (401), Anisidine Value**

    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 0.60, determined at 233 nm in a 1-cm cell, with isooctane being used as the blank.

  - **MICROBIAL ENUMERATION TESTS (61):** NMT 10^3 cfu/g for the total aerobic microbial count, and NMT 10^5 cfu/g for the total combined yeasts and molds count.

  - **TESTS FOR SPECIFIED MICROORGANISMS (62):** Meet the requirements for absence of Escherichia coli in 1 g and for absence of Salmonella species in 10 g

**ADDITIONAL REQUIREMENTS**

- **PACKAGING AND STORAGE:** Preserve in tight containers, and store at controlled room temperature. Do not freeze. Protect from light.
Change to read:

- **LABELING:** The label states the amount of docosahexaenoic acid (DHA) ethyl ester and eicosapentaenoic acid (EPA) ethyl ester, and the minimum amount of total content of omega-3-acid ethyl esters in mg/Capsule. Capsules intended to meet Acceptance Criteria II of the test for Concentration of Omega-3-Acid Ethyl Esters are labeled as containing Omega-3-Acid Ethyl Esters type A. It also states the name and content of any added antioxidant.

- **USP REFERENCE STANDARDS** (11)
  USP Docosahexaenoic Acid Ethyl Ester RS
  All cis-4,7,10,13,16,19-docosahexaenoic ethyl ester.
  
  USP Eicosapentaenoic Acid Ethyl Ester RS
  All cis-5,8,11,14,17-eicosapentaenoic ethyl ester.
  
  USP Methyl Tricosanoate RS
  Tricosanoic acid methyl ester.
  
  C₂₄H₄₈O₂  368.64