

## Omega-3-Acid Ethyl Esters Capsules

### DEFINITION

#### Change to read:

- Omega-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).<sup>1</sup> (IRA 1-Jan-2016) 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*.<sup>1</sup> (IRA 1-Jan-2016)

#### Add the following:

- **B.** It complies with the *Acceptance criteria* in the test for *Concentration of Omega-3-Acid Ethyl Esters in Specific Tests*.<sup>1</sup> (IRA 1-Jan-2016)

### ASSAY

#### Change to read:

- **CONTENT OF EPAEE, DHAEE, AND TOTAL OMEGA-3-ACID ETHYL ESTERS**.<sup>1</sup> (IRA 1-Jan-2016)

[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), heneicosapentaenoic acid ethyl ester (C21:5 n-3, EE), and docosapentaenoic acid ethyl ester (C22:5 n-3, EE) in *Antioxidant solution*.<sup>1</sup> (IRA 1-Jan-2016)

**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-enoic 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*.

<sup>1</sup> The relevant fatty acid ethyl esters are available from Nu-Chek Prep, Inc. (www.nu-chekprep.com); Cayman Chemical (www.caymanchem.com); and Carbosynth (www.carbosynth.com).

**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 ( $W_{AF}$ ). Transfer an amount of the combined Capsule contents equivalent to 225 mg of the labeled amount of total omega-3-acid ethyl esters.<sup>1</sup> (IRA 1-Jan-2016) 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 × 25–50-m fused silica capillary; coated with a 0.25-μm film of G16

**Temperatures**

**Injection port:** 250°

**Detector:** 270°

**Column:** See *Table 1*.

Table 1

Initial Temperature (°)	Temperature Ramp (°/min)	Final Temperature (°)	Hold Time at Final Temperature (min)
170	0	170	2
170	3.5	255	9

**Carrier gas:** Hydrogen or helium

**Linear velocity:** Adjust to obtain a retention time for docosahexaenoic acid ethyl ester of  $26 \pm 3$  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-enoic 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*,<sup>1</sup> (IRA 1-Jan-2016) *Standard solution*, and *Sample 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*.<sup>1</sup> (IRA 1-Jan-2016)

Calculate the content, in mg/g, of EPAee and DHAee in the portion of Capsules 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 the *Sample solution*

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

## 2 Omega-3-Acid

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

$C_U$  = nominal (IRA 1-Jan-2016) concentration of the total omega-3-acid ethyl esters in the *Sample solution* (g/mL)

(IRA 1-Jan-2016)

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

$$\text{Result} = (EPAee + DHAee) \times W_{AF} \times (100/L)$$

$EPAee$  = content of EPAee in the portion of Capsules taken (mg/g)

$DHAee$  = content of DHAee in the portion of Capsules taken (mg/g)

$W_{AF}$  = average fill weight of the Capsules taken (g)

$L$  = 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:

$$\text{Result} = \{r_{FAn-3ee} \times [(EPAee + DHAee)/(r_{EPAee} + r_{DHAee})] + EPAee + DHAee\} \times W_{AF} \times (100/L)$$

$r_{FAn-3ee}$  = 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) from the *Sample solution*

$EPAee$  = content of EPAee (mg/g)

$DHAee$  = content of DHAee (mg/g)

$r_{EPAee}$  = peak area of EPAee from the *Sample solution*

$r_{DHAee}$  = peak area of DHAee from the *Sample solution*

$W_{AF}$  = average fill weight of the Capsules taken (g)

$L$  = label claim of total omega-3-acids ethyl esters (g/Capsule)

**Acceptance criteria:** 95.0%–105.0% of the labeled sum of EPAee and DHAee and NLT 95.0% of the labeled amount of total omega-3-acid ethyl esters per Capsule. (IRA 1-Jan-2016)

### PERFORMANCE TESTS

• **UNIFORMITY OF DOSAGE UNITS (905), Weight Variation:**

Meet the requirements

• **DISINTEGRATION (701)**

**Medium, tier 1:** Water

**Medium, tier 2:** Simulated gastric fluid TS

**Time:** 30 min

**Analysis:** Perform the test with water as *Medium, tier 1*.

Repeat the test with simulated gastric fluid TS as *Medium, tier 2*, if the disintegration time is more than 30 min in *Medium, tier 1*.

**Acceptance criteria:** Meet the requirements

### IMPURITIES

• **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 the Capsule contents 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 the Capsule contents 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 isoctane, 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 isoctane. Wash the combined isoctane extracts with 2 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 peak areas with retention times less than that of the ethyl esters peak

$r_T$  = sum of the areas of all peaks

Calculate the percentage of oligomers in the portion of the Capsules contents taken to prepare *Sample solution 2*:

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

$r_i$  = sum of the peak areas with retention times less than that of the methyl esters peak

$r_T$  = 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, DHAee, and Total Omega-3-Acid Ethyl Esters. Calculate the concentration, in mg/g, of EPAee and DHAee in the portion of Capsules 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 the Sample solution
- $R_S$  = peak area ratio of the EPAee or DHAee peak to the internal standard peak from the Standard solution
- $C_S$  = concentration of USP Eicosapentaenoic Acid Ethyl Ester RS or USP Docosahexaenoic Acid Ethyl Ester RS in the Standard solution (mg/mL)
- $C_U$  = nominal concentration of the total omega-3-acid ethyl 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:

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

$r_{FA_{n-3ee}}$  = 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

EPAee = content of EPAee (mg/g)

DHAee = content of DHAee (mg/g)

$r_{EPAee}$  = peak area of EPAee from the Sample solution

$r_{DHAee}$  = peak area of DHAee 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 2**

Name	Acceptance Criteria I		Acceptance Criteria II (For capsules labeled as containing omega-3-acid ethyl esters type A)	
	NLT	NMT	NLT	NMT
EPAee	430 mg/g	495 mg/g	365 mg/g	435 mg/g
DHAee	347 mg/g	403 mg/g	290 mg/g	360 mg/g

<sup>a</sup>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).

**Table 2 (Continued)**

Name	Acceptance Criteria I		Acceptance Criteria II (For capsules labeled as containing omega-3-acid ethyl esters type A)	
	NLT	NMT	NLT	NMT
EPAee + DHAee	800 mg/g	880 mg/g	700 mg/g	749 mg/g
Total omega-3-acid ethyl esters <sup>a</sup>	90% (w/w)	—	78% (w/w)	—

<sup>a</sup>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).

- (IRA 1-Jan-2016)
  - **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:** NMT 25
  - **FATS AND FIXED OILS (401), Peroxide Value:** NMT 10 mEq/kg
  - **ABSORBANCE**  
**Sample solution:** Transfer 300 mg, accurately weighed, to a 50-mL volumetric flask. Dissolve in and dilute immediately with isoctane to volume. Pipet 2.0 mL into a 50-mL volumetric flask, and dilute with isoctane to volume.  
**Acceptance criteria:** NMT 0.60, determined at 233 nm in a 1-cm cell, with isoctane being used as the blank
  - **MICROBIAL ENUMERATION TESTS (61):** NMT 10<sup>3</sup> cfu/g for the total aerobic microbial count, and NMT 10<sup>2</sup> 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.

#### 4 Omega-3-Acid

**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.
  - (IRA 1-Jan-2016) 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.

$C_{24}H_{36}O_2$  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