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Omega-3-Acid Ethyl Esters Capsules

DEFINITION

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). Tocopherol may be added as an antioxidant.

IDENTIFICATION

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

ASSAY

• 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 (e.g., 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*.¹

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*.

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 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 phase 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 ± 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 the 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*, *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*.

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*

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 = Capsule fill content of the *Sample solution* (g/mL)

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*

EPA_{ee} = content of EPAee (mg/g)

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