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Omega-3-Acid Ethyl Esters

DEFINITION

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.

IDENTIFICATION

- **A.** The retention times of the principal peaks in *Test solution 4* correspond to those of eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in *Standard solution 1b* and *Standard solution 1a*, as obtained in the Assay.
- **B.** It meets the acceptance criteria in [Table 1](#) of the Assay.

ASSAY

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

(See [Fats and Fixed Oils \(401\), Procedures, Omega-3 Fatty Acids Determination and Profile.](#))

Test solution 3, Test solution 4, Standard solution 1a, Standard solution 1b, System suitability solution 1, Chromatographic system, and

System suitability: Proceed as directed in the chapter.

Analysis

Samples: *Test solution 3, Test solution 4, Standard solution 1a, and Standard solution 1b*

Calculate the content of EPAee and DHAee 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 EPAee or DHAee to the internal standard from *Test solution 3*

R_S = peak area ratio of the EPAee to the internal standard from *Standard solution 1b* or DHAee to the internal standard from *Standard solution 1a*

W_S = weight of DHAee taken for preparing *Standard solution 1a* or weight of EPAee taken for preparing *Standard solution 1b* (mg)

W_U = weight of sample taken for preparing *Test solution 3* (g)

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

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

$r_{\text{FAn-3ee}}$ = 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*

EPAe_e = content of EPAee (mg/g)

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

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

r_{DHAe_e} = 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.

IMPURITIES

• **FATS AND FIXED OILS (401), Procedures, Trace Metals:** NMT 0.1 ppm each of lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)

• CHOLESTEROL

Internal standard stock solution: 3 mg/mL of [5 \$\alpha\$ -cholestane](#) in [n-heptane](#). [NOTE—Prepare fresh before use.]

Internal standard solution: 0.3 mg/mL of [5 \$\alpha\$ -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.

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.

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*.

Prepare as directed in the *Standard solution* beginning with “Evaporate to dryness”.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: GC

Detector: Flame ionization

Column: 0.25-mm × 30-m capillary; coated with a 0.25-µm film of [G27](#) phase

Temperatures

Injection port: 320°

Detector: 300°

Column: See [Table 2](#).

Table 2

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)