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Edition: BP 2025 (Ph. Eur. 11.6 update)

Omega-3-Acid Ethyl Esters 90

General Notices

(Ph. Eur. monograph 1250)

Action and use

Lipid-regulating drug.

Ph Eur

DEFINITION

Ethyl esters of *alpha*-linolenic acid (C18:3 n-3), moroctic (stearidonic) acid (C18:4 n-3), eicosatetraenoic acid (C20:4 n-3), timnodonic (eicosapentaenoic) acid (C20:5 n-3; EPA), heneicosapentaenoic acid (C21:5 n-3), clupanodonic (omega-3 docosapentaenoic) acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA). Omega-3-acid ethyl esters 90 are obtained by transesterification of the body oil obtained from fish of families such as *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Salmonidae* and *Scombridae* or from animals of the class *Cephalopoda* and subsequent physico-chemical purification processes, including urea fractionation followed by molecular distillation.

Content

— *EPA and DHA ethyl esters*: minimum 80.0 per cent and maximum 88.2 per cent, with minimum 43.0 per cent and maximum 49.5 per cent of EPA ethyl esters and minimum 34.7 per cent and maximum 40.3 per cent of DHA ethyl esters;

— *total omega-3-acid ethyl esters (corresponding to the sum of the ethyl esters listed under Definition)*: minimum 90 per cent.

A suitable antioxidant may be added.

PRODUCTION

The content of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), dioxin-like polychlorinated biphenyls (DLPCBs), non-dioxin-like polychlorinated biphenyls (NDLPCBs; 7PCB) and polybrominated diphenyl ethers (PBDEs) is controlled using suitable and validated methods, for example as outlined in Commission Regulation (EU) No 589/2014, Annex III, and in scientific publications of the European Food Safety Authority [EFSA, 2011].

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Persistent organic pollutants	Maximum content
*Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF)**	1 pg/g (WHO-TEQ***)
*Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF) and dioxin-like polychlorinated biphenyls (DLPCBs)**	5 pg/g (WHO-TEQ)
*Non-dioxin-like polychlorinated biphenyls (NDLPCBs; 7PCB) (sum of congeners 28, 52, 101, 118, 138, 153 and 180)**	60 ng/g
*Polybrominated diphenyl ethers (PBDEs) (sum of congeners 28, 47, 49, 99, 100, 153 and 154)***	3 ng/g

*Commission Regulation (EU) No 589/2014, Annex III.
***EFSA Journal* 2011;9(5):2156, [274 pp.].
***WHO-TEQ = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) toxic equivalent (TEQ) as agreed by WHO.

Calculate the upper-bound contents on the assumption that all values of the different congeners below the limit of quantification are equal to the limit of quantification.

CHARACTERS

Appearance

Light yellow liquid.

Solubility

Practically insoluble in water, very soluble in acetone, in ethanol (96 per cent), in heptane and in methanol.

IDENTIFICATION

A. Examine the chromatograms obtained in the assay for EPA and DHA ethyl esters.

Results The peaks due to eicosapentaenoic acid ethyl ester and docosahexaenoic acid ethyl ester in the chromatogram obtained with test solution (b) are similar in retention time to the corresponding peaks in the chromatograms obtained with reference solutions (a_1) and (a_2).

B. It complies with the limits of the assay for total omega-3-acid ethyl esters.

TESTS

Absorbance (*2.2.25*)

Maximum 0.55 at 233 nm.

Dilute 0.300 g to 50.0 mL with *trimethylpentane R*. Dilute 2.0 mL of the solution to 50.0 mL with *trimethylpentane R*.

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Acid value (*2.5.1*)

Maximum 2.0, determined on 10 g in 50 mL of the prescribed mixture of solvents.

Anisidine value (*2.5.36*)

Maximum 20.0.

Peroxide value (*2.5.5, Method B*)

Maximum 10.0.

Unidentified fatty acid ethyl esters (*2.4.29*)

Identify the peaks in the chromatogram obtained with test solution (b) in the assay, using Figure 1250.-2, integrating up to 1.3 times the retention time of DHA ethyl ester. Disregard any peak with an area less than 0.05 per cent of the total area.

Calculate the percentage content of the fatty acid ethyl ester corresponding to the largest single unidentified peak, using the following expression:

$$\frac{100 \times B}{A}$$

A	=	sum of the areas of all peaks, excluding those due to solvents, butylhydroxytoluene and the internal standard;
B	=	area of the largest single unidentified peak, excluding those due to solvents, butylhydroxytoluene and the internal standard.

Calculate the percentage content of total unidentified fatty acid ethyl esters, using the following expression:

$$100 - \frac{100 \times C}{A}$$

C	=	sum of the areas of the peaks due to ethyl esters identified in Figure 1250.-2.

Limits:

— *fatty acid ethyl ester corresponding to the largest single unidentified peak*: maximum 0.5 per cent;

— *total unidentified fatty acid ethyl esters*: maximum 2 per cent.

Cholesterol (*2.4.32*)

Maximum 3.0 mg/g.

Oligomers

Size-exclusion chromatography (*2.2.30*).

Test solution Dilute 50.0 mg of the substance to be examined to 10.0 mL with *tetrahydrofuran R*.

Reference solution Dissolve 50 mg of *monodocosahexaenoin R*, 30 mg of *didocosahexaenoin R* and 20 mg of *tridocosahexaenoin R* in *tetrahydrofuran R* and dilute to 100.0 mL with the same solvent.

Column 3 columns to be connected in series:

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— *size*: *l* = 0.3 m, Ø = 7.8 mm;

— *stationary phase*: *styrene-divinylbenzene copolymer R* (5 µm) with the following pore sizes:

— *column 1*: 50 nm;

— *column 2*: 10 nm;

— *column 3*: 5 nm;

— *connection sequence*: injector – column 1 – column 2 – column 3 – detector.

Mobile phase *tetrahydrofuran R*.

Flow rate 0.8 mL/min.

Run time 38 min.

Detection Differential refractometer.

Injection 40 µL.

System suitability Reference solution:

— *elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin;

— *resolution*: minimum 2.0 between the peaks due to didocosahexaenoin and monodocosahexaenoin; minimum 1.0 between the peaks due to tridocosahexaenoin and didocosahexaenoin.

Calculate the percentage content of oligomers using the following expression:

$$\frac{B}{A} \times 100$$

A	=	sum of the areas of all the peaks in the chromatogram;
B	=	sum of the areas of the peaks with a retention time less than the retention time of the peaks due to ethyl esters.

The ethyl ester peaks, which may be present in the form of an unresolved double peak, are identified as the major peaks in the chromatogram (see Figure 1250.-1).

Where the result obtained exceeds the limit due to the presence of monoglycerides, the following procedure is carried out.

Test solution Weigh 50.0 mg of the substance to be examined into a quartz tube. Add 1.5 mL of a 20 g/L solution of *sodium hydroxide R* in *methanol R*, cover with *nitrogen R*, cap tightly with a polytetrafluoroethylene-lined cap, mix and heat on a water-bath for 7 min. Allow to cool. Add 2 mL of *boron trichloride-methanol solution R*, cover with *nitrogen R*, cap tightly, mix and heat on a water-bath for 30 min. Cool to 40-50 °C, add 1 mL of *trimethylpentane R*, cap and shake vigorously for at least 30 s. Immediately add 5 mL of *saturated sodium chloride solution R*, cover with *nitrogen R*, cap and shake thoroughly for at least 15 s. Transfer the upper layer to a separate tube. Shake the methanol layer once more with 1 mL of *trimethylpentane R*. Wash the combined trimethylpentane extracts with 2 quantities, each of 1 mL, of *water R*. Carefully evaporate the solvent under a current of *nitrogen R* then add 10.0 mL of *tetrahydrofuran R* to the residue. Add a small amount of *anhydrous sodium sulfate R* and filter.

Calculate the percentage content of oligomers using the following expression:

$$\frac{B'}{A} \times 100$$

A	=	sum of the areas of all the peaks in the chromatogram;

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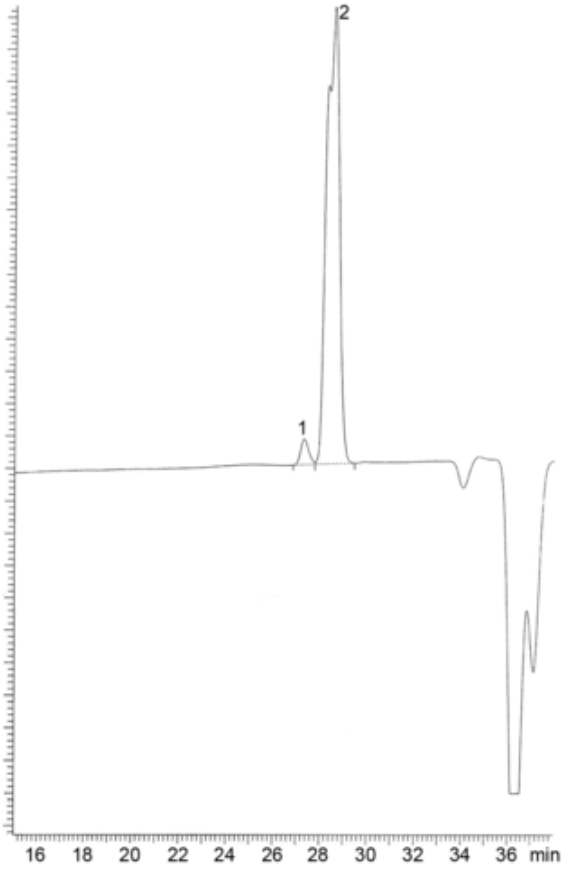
B' = sum of the areas of the peaks with a retention time less than the retention time of the peaks due to methyl esters.

Limit:

— *oligomers*: maximum 1.0 per cent.



1. oligomers 2. ethyl esters

Figure 1250.-1. – *Chromatogram for the test for oligomers in omega-3-acid ethyl esters 90: spiked sample*

ASSAY

EPA and DHA ethyl esters (2.4.29)

For identification of the peaks, see Figure 1250.-2.

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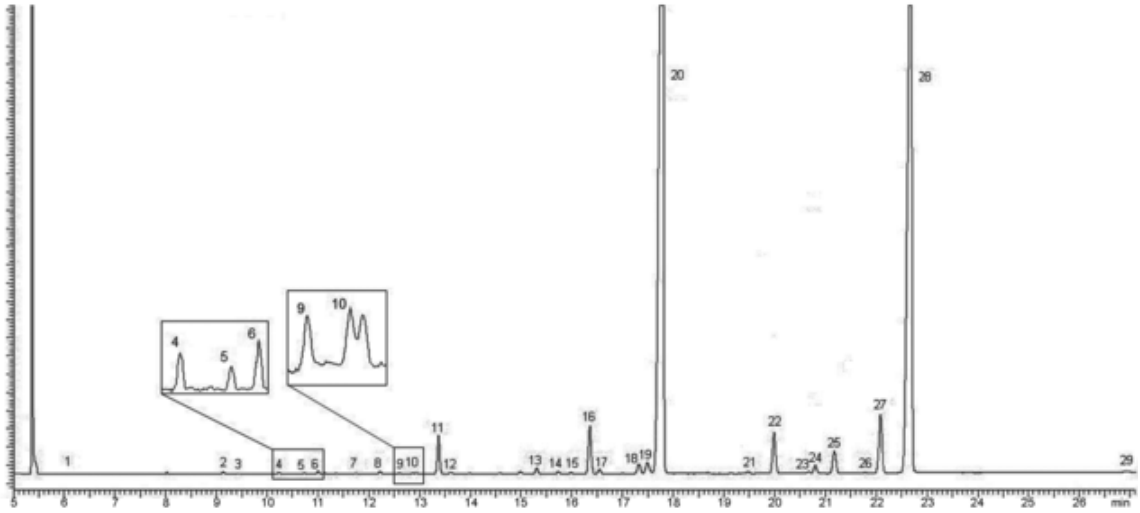

Fatty acid	*r*	Fatty acid	*r*	Fatty acid	*r*	Fatty acid	*r*
1. C14:0	0.278	8. C18:3 *n*-6	0.557	15. C20:3 *n*-6	0.720	22. C21:5 *n*-3	0.889
2. Phytanic acid	0.416	9. C18:3 *n*-4	0.574	16. C20:4 *n*-6	0.736	23. C22:4 *n*-6	0.917
3. C16:3 *n*-4	0.431	10. C18:3 *n*-3	0.585	17. Furan acid 7	0.744	24. Furan acid 10	0.922
4. C16:4 *n*-1	0.468	11. C18:4 *n*-3	0.608	18. C20:4 *n*-3	0.777	25. C22:5 *n*-6	0.939
5. C18:0	0.488	12. C18:4 *n*-1	0.618	19. Furan acid 8	0.783	26. Furan acid 11	0.963
6. C18:1 *n*-9	0.501	13. Furan acid 5	0.691	20. EPA	0.796	27. C22:5 *n*-3	0.977
7. C18:2 *n*-6	0.535	14. C19:5	0.710	21. Furan acid 9	0.867	28. DHA	1.000
						29. C24:6	1.183

r: relative retention with reference to DHA ethyl ester (retention time = about 23 min).

Figure 1250.-2. – *Chromatogram for the assay of omega-3-acid ethyl esters 90 and the test for unidentified fatty acid ethyl esters*

Total omega-3-acid ethyl esters (*2.4.29*)

See Figure 1250.-2.

STORAGE

Under an inert gas, in an airtight container, protected from light.

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