Omega-3-Marine Triglycerides

General Notices
(Omega-3-Acid Triglycerides, Ph Eur monograph 1352)

Action and use
Used in treatment of hypertriglyceridaemia.

Ph Eur

DEFINITION
Mixture of mono-, di- and triesters of omega-3 acids with glycerol containing mainly triesters and obtained either by esterification of concentrated and purified omega-3 acids with glycerol or by transesterification of the omega-3 acid ethyl esters with glycerol. The origin of the omega-3 acids is the body oil from fatty fish species coming from families like *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Salmonidae* and *Scombridae*. The omega-3 acids are identified as the following acids: alpha-linolenic acid (C18:3 n-3), moroctic 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 acid (C22:5 n-3) and cervonic (docosahexaenoic) acid (C22:6 n-3; DHA).

Content
—sum of the contents of the omega-3 acids EPA and DHA, expressed as triglycerides: minimum 45.0 per cent,
—total omega-3 acids, expressed as triglycerides: minimum 60.0 per cent.
Tocopherol may be added as an antioxidant.

CHARACTERS
Appearance
Pale yellow liquid.

Solubility
Practically insoluble in water, very soluble in acetone and in heptane, slightly soluble in ethanol.

IDENTIFICATION
Examine the chromatograms obtained in the assay for EPA and DHA.

Results The peaks due to eicosapentaenoic acid methyl ester and to docosahexaenoic acid methyl ester in the chromatogram obtained with test solution (b) are similar in retention time and size to the corresponding peaks in the chromatogram obtained with reference solution (a).

TESTS
Absorbance *(2.2.25)*
Maximum 0.73 at 233 nm.

Dilute 0.300 g of the substance to be examined to 50.0 ml with *trimethylpentane R*. Dilute 2.0 ml of this solution to 50.0 ml with *trimethylpentane R*.

Acid value *(2.5.1)*

Maximum 3.0, determined on 10.0 g in 50 ml of the prescribed mixture of solvents.

Anisidine value

Maximum 30.0.

The anisidine value is defined as 100 times the absorbance measured in a 1 cm cell filled with a solution containing 1 g of the substance to be examined in 100 ml of a mixture of solvents and reagents according to the method described below.

Carry out the operations as rapidly as possible, avoiding exposure to actinic light.

Test solution (a) Dilute 0.500 g of the substance to be examined to 25.0 ml with *trimethylpentane R*.

Test solution (b) To 5.0 ml of test solution (a) add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R*, shake and store protected from light.

Reference solution To 5.0 ml of *trimethylpentane R* add 1.0 ml of a 2.5 g/l solution of *p-anisidine R* in *glacial acetic acid R*, shake and store protected from light.

Measure the absorbance *(2.2.25)* of test solution (a) at 350 nm using *trimethylpentane R* as the compensation liquid. Measure the absorbance of test solution (b) at 350 nm exactly 10 min after its preparation, using the reference solution as the compensation liquid.

Calculate the anisidine value from the expression:

$$\frac{25 \times (1.2A_s - A_b)}{m}$$

A_s = absorbance of test solution (b),
A_b = absorbance of test solution (a),
m = mass of the substance to be examined in test solution (a), in grams.

Peroxide value *(2.5.5, Method A)*

Maximum 10.0.

Oligomers and partial glycerides

Size-exclusion chromatography *(2.2.30)*.

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

Reference solution In a 100 ml volumetric flask 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 1:
—*dimensions*: *l* = 0.3 m, Ø = 7.8 mm,
—*stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 10 nm.
Columns 2 and 3 placed closest to the injector:
—*dimensions*: *l* = 0.3 m, Ø = 7.8 mm,
—*stationary phase*: *styrene-divinylbenzene copolymer R* (7 µm) with a pore size of 50 nm.

Mobile phase *tetrahydrofuran R.*

Flow rate 0.8 ml/min.

Detection Differential refractometer.

Injection 40 µl.

System suitability Reference solution:

—*elution order*: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin,
— *resolution*: minimum of 2.0 between the peaks due to monodocosahexaenoin and to didocosahexaenoin and minimum of 1.0 between the peaks due to didocosahexaenoin and to tridocosahexaenoin.

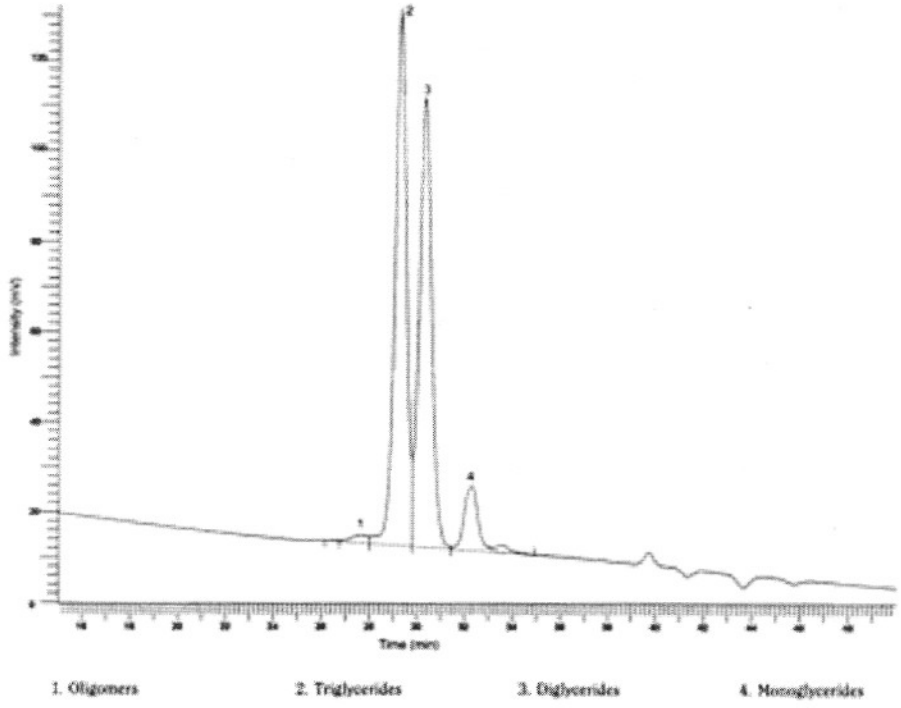

Figure 1352.-1. – *Chromatogram of the test for oligomers and partial glycerides in omega-3-acid triglycerides*

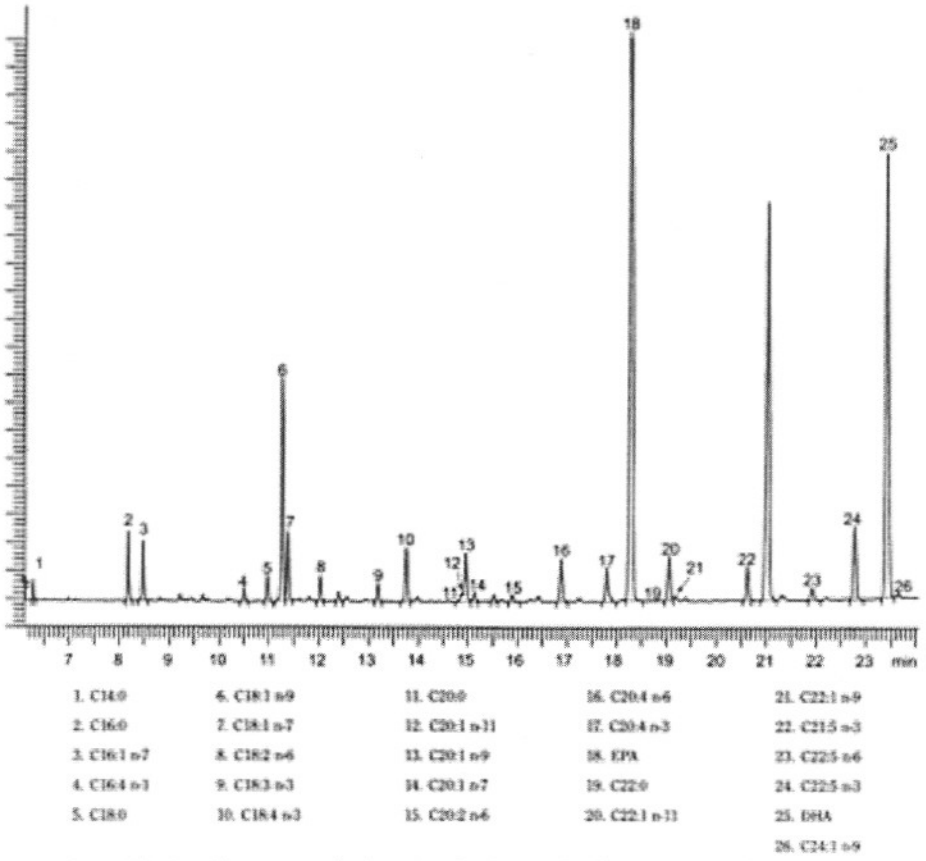

Figure 1352.2. - *Chromatogram for the assay of total omega-3 acids in omega-3-acid triglycerides*

Identify the peaks from the chromatogram (Figure 1352.-1). 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 = area of the peak with a retention time smaller than the retention time of the triglyceride peak.

Calculate the percentage content of partial glycerides using the following expression:

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

C = (sum of the) area(s) of the peak(s) due to the mono- and diglycerides.

Limits:

—*oligomers*: maximum 3.0 per cent,
—*partial glycerides*: maximum 50.0 per cent.

ASSAY

EPA and DHA *(2.4.29)*

See Figure 1352.-2.

Total omega-3-acids *(2.4.29)*

See Figure 1352.-2.

STORAGE

In an airtight, well-filled container, protected from light, under an inert gas.

LABELLING

The label states the concentration of any added tocopherol.

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