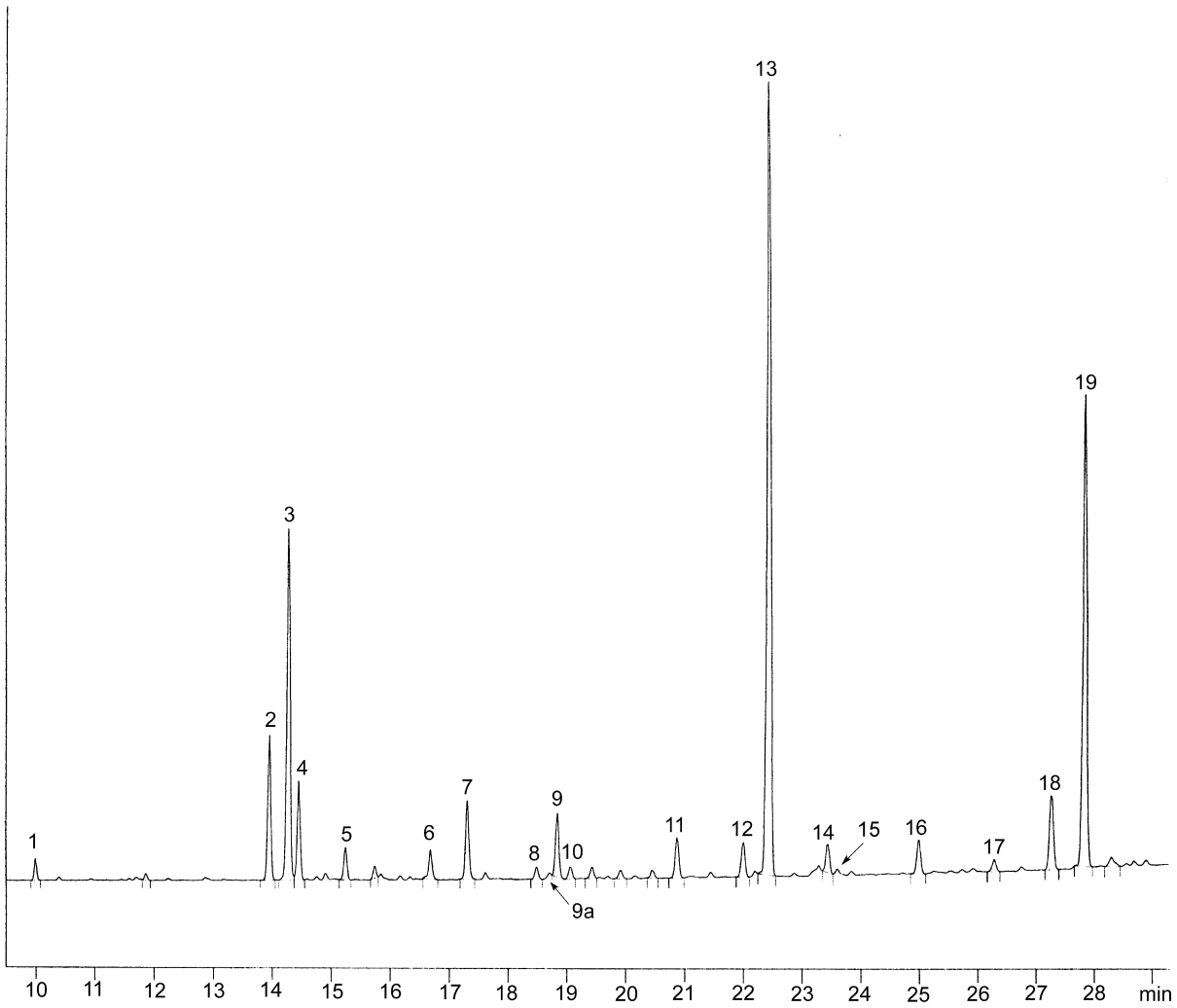


1. C16:0	5. C18:2 n-6	9. C20:1 n-9	12. C20:4 n-3	16. C21:5 n-3
2. C18:0	6. C18:3 n-3	9a. C20:1 n-11	13. EPA	17. C22:5 n-6
3. C18:1 n-9	7. C18:4 n-3	10. C20:1 n-7	14. C22:1 n-11	18. C22:5 n-3
4. C18:1 n-7	8. C20:0	11. C20:4 n-6	15. C22:1 n-9	19. DHA

Figure 2063.-2. – *Chromatogram for the assay of total omega-3 acid ethyl esters in omega-3 acid ethyl esters 60*

01/2008:1250

OMEGA-3-ACID ETHYL ESTERS 90

Omega-3 acidorum esteri ethylici 90

DEFINITION

Ethyl esters of 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). Omega-3-acid ethyl esters are obtained by transesterification of the body oil of fat fish species coming from families such as *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Salmonidae* and *Scombridae* and subsequent physico-chemical purification processes, including urea fractionation followed by molecular distillation.

Content:

– *EPA and DHA ethyl esters*: minimum 80 per cent, with minimum 40 per cent of EPA ethyl esters and minimum 34 per cent of DHA ethyl esters,

– *total omega-3-acid ethyl esters*: minimum 90 per cent.

Tocopherol may be added as an antioxidant.

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

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

Results: the peaks due to eicosapentaenoic acid ethyl ester and to docosahexaenoic acid ethyl ester in the chromatogram obtained with the test solution are similar in retention time and size to the corresponding peaks in the chromatogram obtained with the reference solution.

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 this solution to 50.0 ml with *trimethylpentane R*.

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 A*): maximum 10.0.

Oligomers. 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:

– *size*: *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:

– *size*: *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:

– *elution order* in the chromatogram obtained with the reference solution: tridocosahexaenoin, didocosahexaenoin, monodocosahexaenoin,

– *resolution*: minimum 2.0 between the peaks due to monodocosahexaenoin and didocosahexaenoin and minimum 1.0 between the peaks due to didocosahexaenoin and tridocosahexaenoin in the chromatogram obtained with the reference solution.

Calculate the percentage content of oligomers using the following expression:

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

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

B = sum of the areas of the peaks with a retention time smaller 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 (Figure 1250.-1).

When the result obtained exceeds the limit due to the presence of monoglycerides, the following procedure is carried out.
Test solution. Weigh 10.0 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 a *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*. 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 sulphate R* and filter.

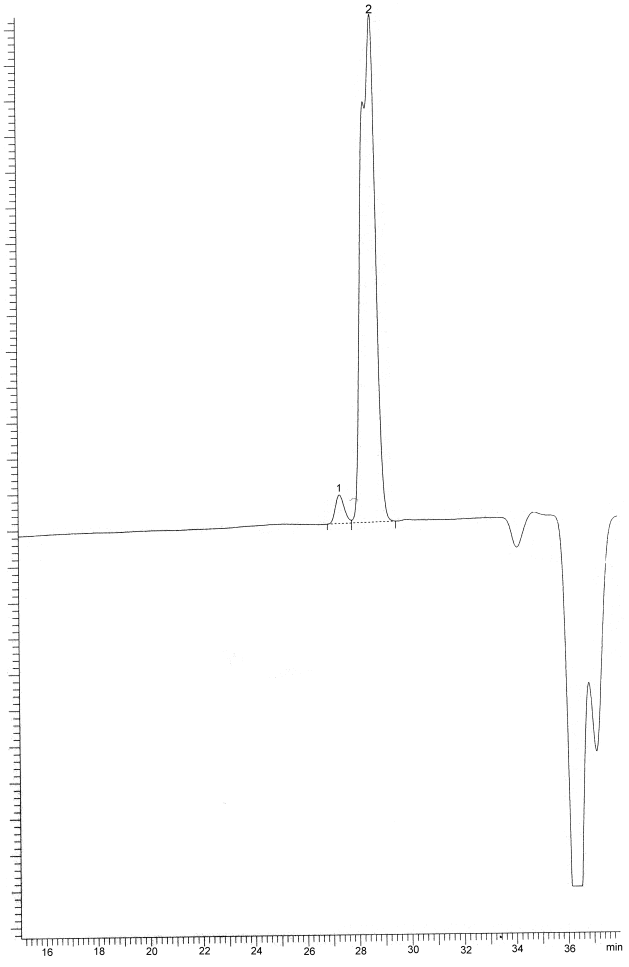
Calculate the percentage content of oligomers using the following expression:

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

B' = sum of the areas of the peaks with a retention time smaller 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 of the test for oligomers in omega-3-acid ethyl esters 90: spiked sample*

ASSAY

EPA and DHA ethyl esters (*2.4.29*). See Figure 1250.-2.

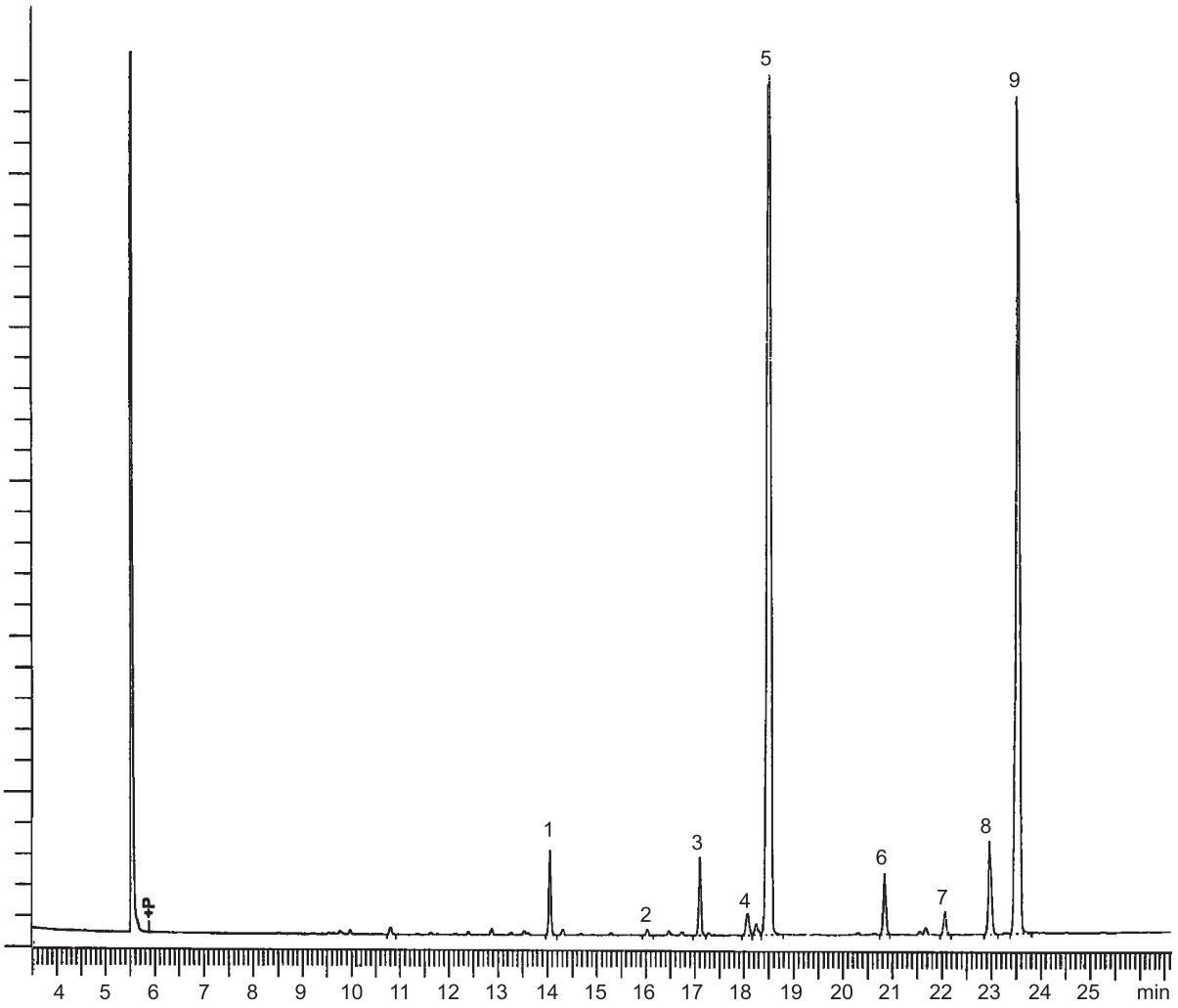
Total omega-3-acid ethyl esters (*2.4.29*). See Figure 1250.-2.

STORAGE

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

LABELLING

The label states the concentration of any added tocopherol.



1. C18:4 n-3
2. C20:2 n-6
3. C20:4 n-6
4. C20:4 n-3
5. EPA
6. C21:5 n-3
7. C22:5 n-6
8. C22:5 n-3
9. DHA

Figure 1250.-2. - *Chromatogram for the assays*

01/2008:1352

OMEGA-3 ACID TRIGLYCERIDES

Omega-3 acidorum triglycerida

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 anhydrous 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*.