01/2008:1912

FISH OIL, RICH IN OMEGA-3 ACIDS

Piscis oleum omega-3 acidis abundans

DEFINITION

Purified, winterised and deodorised fatty oil obtained from fish of the families *Engraulidae*, *Carangidae*, *Clupeidae*, *Osmeridae*, *Scombridae* and *Ammodytidae*. The omega-3 acids are defined 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:

– *EPA, expressed as triglycerides*: minimum 13.0 per cent,
– *DHA, expressed as triglycerides*: minimum 9.0 per cent,
– *total omega-3 acids, expressed as triglycerides*: minimum 28.0 per cent.

Authorised antioxidants in concentrations not exceeding the levels specified by the competent authorities may be added.

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 to the corresponding peaks in the chromatogram obtained with reference solution (a).

TESTS

Appearance. The substance to be examined is not more intensely coloured than a reference solution prepared as follows: to 3.0 ml of red primary solution add 25.0 ml of yellow primary solution and dilute to 50.0 ml with a 10 g/l solution of *hydrochloric acid R* (*2.2.2, Method II*).

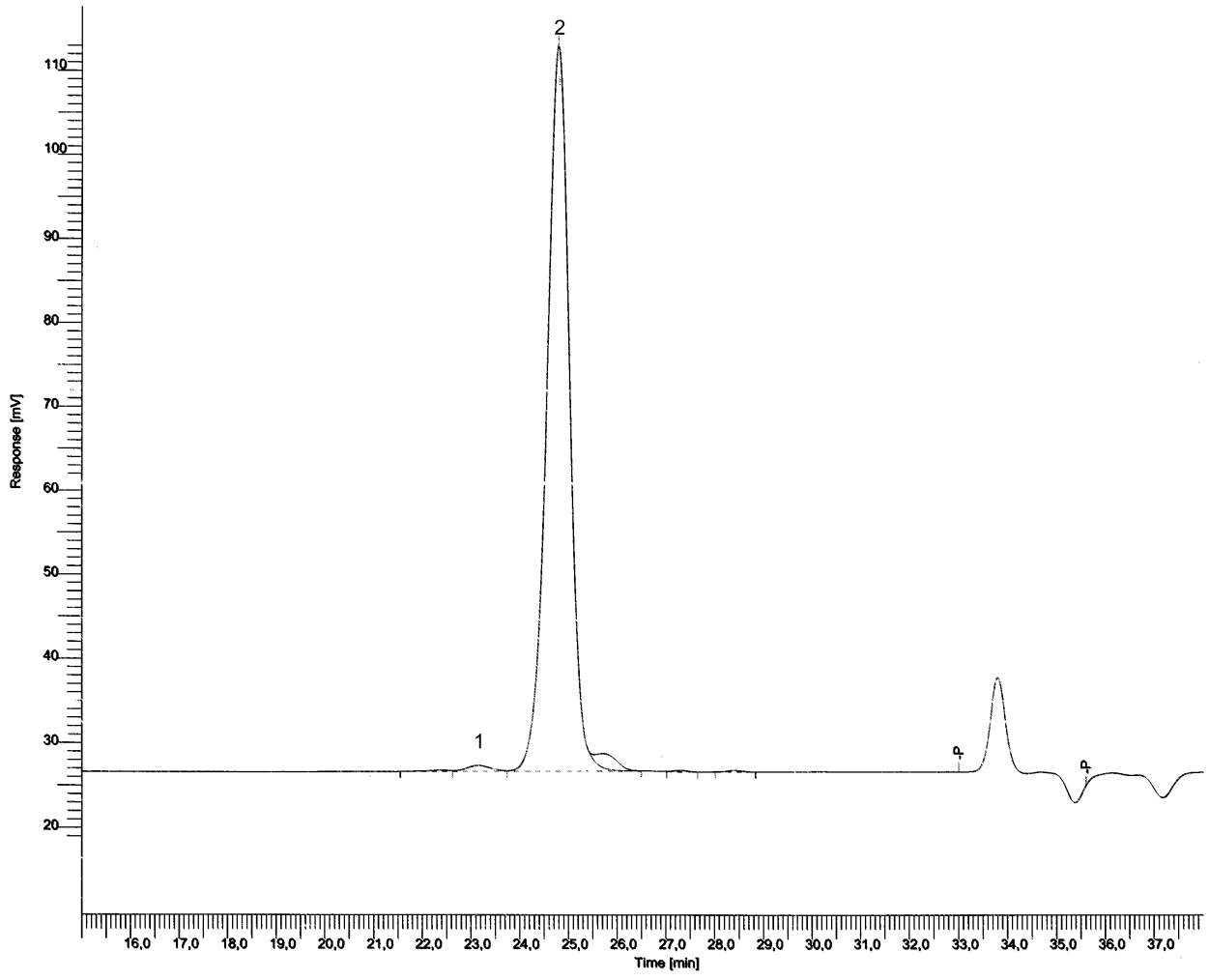
Absorbance (*2.2.25*): maximum 0.70 at 233 nm.

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 0.5, determined on 20.0 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



1. oligomers 2. triglycerides

Figure 1912.-1. – *Chromatogram of the test for oligomers in fish oil rich in omega-3 acids*

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:

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

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.

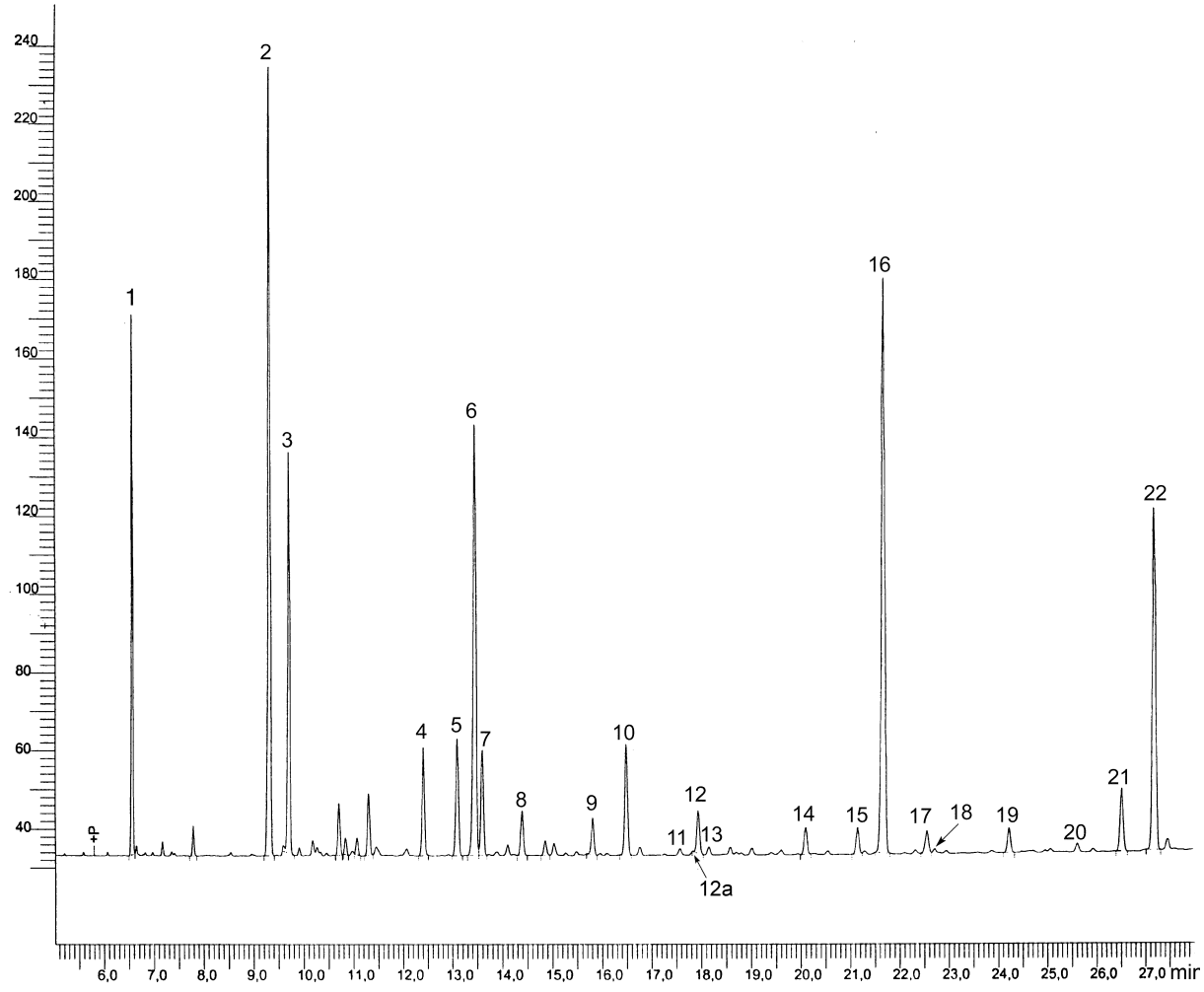
Unsaponifiable matter (*2.5.7*): maximum 1.5 per cent, determined on 5.0 g.

Stearin. 10 ml remains clear after cooling at 0 °C for 3 h.

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.



1. C14:0	6. C18:1 n-9	11. C20:0	15. C20:4 n-3	20. C22:5 n-6
2. C16:0	7. C18:1 n-7	12. C20:1 n-9	16. C20:5 n-3	21. C22:5 n-3
3. C16:1 n-7	8. C18:2 n-6	12a. C20:1 n-11	17. C22:1 n-11	22. C22:6 n-3
4. C16:4 n-1	9. C18:3 n-3	13. C20:1 n-7	18. C22:1 n-9	
5. C18:0	10. C18:4 n-3	14. C20:4 n-6	19. C21:5 n-3	

Figure 1912.-2. – *Chromatogram for the assay of total omega-3 acids in fish oil rich in omega-3 acids*

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: 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.

Identify the peaks from the chromatogram (Figure 1912.-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 less than the retention time of the triglyceride peak.

Limit:
– *oligomers*: maximum 1.5 per cent.

ASSAY

EPA and DHA (*2.4.29*). See Figure 1912.-2.

Total omega-3 acids (*2.4.29*). See Figure 1912.-2.

STORAGE

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

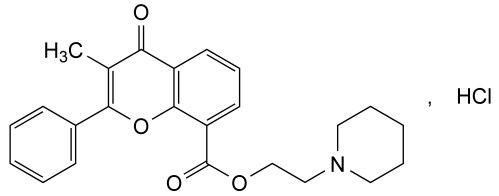
LABELLING

The label states the concentration of EPA, DHA and total omega-3 acids, expressed as triglycerides.

01/2008:1692

FLAVOXATE HYDROCHLORIDE

Flavoxati hydrochloridum



$C_{24}H_{26}ClNO_4$ M_r 427.9
[3717-88-2]

DEFINITION

2-(Piperidin-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4*H*-1-benzopyran-8-carboxylate hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: slightly soluble in water, sparingly soluble in methylene chloride, slightly soluble in ethanol (96 per cent).

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).
Comparison: *flavoxate hydrochloride CRS*.

B. It gives reaction (a) of chlorides (*2.3.1*).

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Use freshly prepared solutions.*

Solvent mixture. Mix 20 volumes of a 0.4 g/l solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 with *phosphoric acid R* and 80 volumes of *acetonitrile R*.

Test solution. Dissolve 10.0 mg of the substance to be examined in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution (a). Dilute 1.0 ml of the test solution to 100.0 ml with the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 6.0 mg of *flavoxate impurity A CRS* and 3.0 mg of *flavoxate impurity B CRS* in the solvent mixture, add 2.0 ml of the test solution and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of this solution to 20.0 ml with the solvent mixture.

Column:
– *size*: *l* = 0.25 m, Ø = 4.6 mm;
– *stationary phase*: spherical *end-capped octadecylsilyl silica gel for chromatography R* (5 µm).

Mobile phase:
– *mobile phase A*: 0.435 g/l solution of *dipotassium hydrogen phosphate R* adjusted to pH 7.5 with *phosphoric acid R*;
– *mobile phase B*: *acetonitrile R*;

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 10	20	80
10 - 20	20 → 10	80 → 90
20 - 25	10	90

Flow rate: 0.8 ml/min.

Detection: spectrophotometer at 254 nm.

Injection: 10 µl.

Relative retention with reference to flavoxate (retention time = about 10 min): impurity A = about 0.2; impurity B = about 0.8.

System suitability: reference solution (c):
– *resolution*: minimum 4.0 between the peaks due to impurity B and flavoxate.

Limits:
– *impurity A*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.3 per cent);
– *impurity B*: not more than the area of the corresponding peak in the chromatogram obtained with reference solution (c) (0.15 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);