

The logo for GOED features the letters 'GOED' in a bold, sans-serif font. The 'GO' is rendered in a light grey color, while 'ED' is in white. A white swoosh underline starts under the 'G' and extends under the 'E', ending with a small upward-pointing arrowhead.

GUIDANCE DOCUMENTS

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GUIDANCE ON METHODS

The PURPOSE of the following Guidance on Methods section is to provide guidance to GOED members on suitable methods for recognized long-chain omega-3 PUFA-containing oils with respect to measurements of fatty acid, oxidation status, and contaminants, as well as other relevant physical and chemical parameters. The overall OBJECTIVE is to provide information that can contribute to the establishment of QUALITY of EPA- and/or DHA-containing oils. Some of the methods specified below also are methods that should be followed as indicated by the GOED Voluntary Monograph (see the GOED Voluntary Monograph).

The first section, “Guidance On Methods Specified By The GOED Monograph” describes the methods that should be followed as indicated by the GOED Voluntary Monograph (see the GOED Voluntary Monograph).

GUIDANCE ON METHODS FOR PARAMETERS SPECIFIED BY THE GOED MONOGRAPH

Description of Tests

A. Fatty Acid Analysis Methods

The following assays for EPA and DHA are recommended to be used:

- ▶ **A-1.** The GOED Analytical Method “Assay for EPA and DHA”.

A full method description can be found below. This method is based on Ph.Eur. General Chapter 2.4.29 “Composition of fatty acids in oils rich in omega-3 acids,” and USP Chapter <401> “Omega-3 fatty acids determination and profile.”

- ▶ **A-2.** USP: 401 “Fats and Fixed Oils”
- ▶ **A-3.** Ph.Eur. 2.4.29 “Composition of Fatty Acids in Oils rich in Omega-3-Acids”
- ▶ **A-4.** AOCS Official Method # Ce 1i-07 “Determination of Saturated, cis-Monounsaturated, and cis- Polyunsaturated Fatty Acids in Marine and Other Oils Containing Long Chain Polyunsaturated Fatty Acids (PUFAs) by Capillary GLC.”

This method permits quantitative determination of EPA and DHA present as triglycerides or fatty acid-ethyl esters in marine oils as fatty acid methyl esters after methylation (“robust, accurate and precise,” depending on individual lab proficiency and improvable using empirical response factors).

(End of section)

GOED Analytical Method “Assay for EPA and DHA” or “GOED Fatty Acid Method”

The assay may be used for the quantitative determination of EPA, DHA and total omega-3 content in omega-3 products, and is applicable to triglyceride and ethyl ester product forms. Results can be expressed as mg DHA/g and mg EPA/g as triglycerides or ethyl esters, respectively, and as free fatty acid equivalents.

EPA and DHA:

Carry out the operations as rapidly as possible, avoiding exposure to actinic light, oxidizing agents, oxidation catalysts (for example, copper and iron) and air. *1

Gas chromatography — The assay is carried out on the methyl esters (after derivatisation of triglycerides - see also step B below), or ethyl esters of (all-Z)-eicosa-5,8,11,14,17-pentaenoic acid (EPA; 20:5 n-3) and (all-Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA; 22:6 n-3) in the sample to be examined. Internal standard: methyl tricosanoate R.

Test solutions: Prepare all test solutions in duplicate.

Step A:

Test solution (a). Dissolve the sample to be examined, according to Table 1, and 70.0 mg of the internal standard in a 50 mg/L solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Gentle heating (up to 60°C) may be applied to dissolve the internal standard. Ethyl esters are now ready for analysis. For triglycerides continue as described in step B.

Table 1.

Approx. sum EPA + DHA	Amount sample to be weighed
30 – 50 %	0.4 – 0.5 g
50 – 70 %	0.3 g
70 – 80 %	0.25 g

Step B:

Introduce 2.0 ml of the solution obtained from Step A into a quartz or borosilicate tube and evaporate the solvent at 40-50°C with a gentle current of nitrogen R.*2 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 to 40-50°C.

Different other heating media may be used as long as the temperature is 100°C. for the heating step.

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. Wash the combined trimethylpentane extracts with 2 quantities, each of 1 ml, of water R and dry over anhydrous sodium sulphate R.

Test solution (b). (to be prepared at the same time as test solution (a))

Dissolve 0.300 g of the sample to be examined in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Proceed as described for test solution (a).

Reference solutions: Prepare reference solutions (a1) and (a2) in duplicate (to be prepared at the same time as test solution (a)).*3 Reference solution (c) only has to be prepared for triglycerides, and only if tetracos-15-enoic acid methyl ester is not clearly observed in the chromatogram with test solution (a).

Note - optional approach: When using EPA and DHA standards of high purity (>99%), reference solution (a1) and (a2) can be combined into one reference solution (a), which is then prepared as follows: Dissolve 60.0 mg of docosahexaenoic acid ethyl ester CRS, about 70.0 mg of the internal standard and 90.0 mg of eicosapentaenoic acid ethyl ester CRS in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Gentle heating (up to 60°C) may be applied to dissolve the internal standard. For ethyl ester samples reference solution (a) is now ready for analysis. For analysis of triglycerides continue as described in step B for preparation of test solution (a) and test solution (b).

Reference solution (a1) Dissolve 70.0 mg of the internal standard and 90.0 mg of eicosapentaenoic acid ethyl ester CRS in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Gentle heating (up to 60°C) may be applied to dissolve the internal standard.

Reference solution (a2) Dissolve 60.0 mg of docosahexaenoic acid ethyl ester CRS, and 70.0 mg of the internal standard in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution. Gentle heating (up to 60°C) may be applied to dissolve the internal standard.

For ethyl esters samples both reference solution (a1) and reference solution(a2) are now ready for analysis. For analysis of triglycerides continue as described in step B for preparation of test solution (a) and test solution (b).

Reference solution (b)

(For system suitability of recovery vs. the theoretical response of the Flame Ionisation Detector (FID))

Introduce 0.300 g of methyl palmitate R, 0.300 g of methyl stearate R, 0.300 g of methyl arachidate R and 0.300 g of methyl behenate R into a 10 ml volumetric flask, dissolve in a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 ml with the same solution.

Reference solution (c)

(For system suitability of chromatographic resolution)

In a 10 mL volumetric flask dissolve a mixture containing 55.0 mg docosahexaenoic acid methyl ester R and 5.0 mg of tetracos-15-enoic acid methyl ester R in a 50 mg/L solution of butylhydroxytoluene R in trimethylpentane R and dilute to 10.0 mL with the same solution.

Gas chromatography:

Column*4:

Material: fused silica

Dimensions: l = at least 25m, Ø = 0.25mm,

Stationary phase: bonded polyethylene glycol polymer / bonded macrogol 20 000 R (film thickness 0.2 µm).

Carrier gas: hydrogen for chromatography R or helium for chromatography.

Split: 1:200, alternatively splitless with temperature control (samples need to be diluted 1:200 with a 0.05 g/l solution of butylhydroxytoluene R in trimethylpentane R before injection)

If necessary, adapt the split ratio and/or sample dilution to obtain a symmetry factor of 0.8-1.5 for the methyl or ethyl esters of eicosapentaenoic acid and docosahexaenoic acid, at the same time observing that for test solution (b) any peaks due to the corresponding esters of linolenic acid, stearidonic acid, eicosatetraenoic acid, heneicosapentaenoic acid and docosapentaenoic acid are clearly detectable.

If necessary, adapt the split ratio and/or sample dilution to obtain a symmetry factor of 0.8-1.5 for the components of reference solution (b).

Table 2: GC Program / Temperatures

	Split Injection		Splitless Injection	
	Time (Min)	Time (°C)	Time (Min)	Time (°C)
Column	0-2	170	0-2	90
	2-25.7	170-240	2-4.7	90-170
	25.7-28	240	4.7-28	170-240
			28-30	240
Injection Port		250		90-250 (90 °C for on-column injection)
Detector		270		270

Detection: flame ionisation.

Injection*5: 1 µl of each solution.

System suitability:

— In the chromatogram obtained with reference solution (b), multiply the area of the peaks due to methyl palmitate, methyl stearate, methyl arachidate, and methyl behenate, by the corresponding factors in Table 3.

Table 3

Fatty acid methyl ester	Theoretical response factor
Methyl palmitate	1.049
Methyl stearate	1.029
Methyl arachidate	1.013
Methyl behenate	1.000

Normalize the corrected areas of the peaks of the fatty acid methyl esters to a sum of 100 percent. The normalized area percentage of each fatty acid methyl ester is to be within +/- 1.0 percent of the corresponding weight percentage.

— Resolution:

Ethyl esters: minimum 1.2 between the peaks due to methyl tricosanoate and heneicosapentaenoic acid ethyl ester in the chromatogram obtained with test solution (a):

Triglycerides: minimum 1.2 between the peaks due to docosahexaenoic acid methyl ester and tetracos-15-enoic acid methyl ester in the chromatogram obtained with test solution (a) or with reference solution (c).^{*6}

In the chromatogram obtained with test solution (a), the peaks due to methyl tricosanoate and any heneicosapentaenoic acid methyl ester present when compared with the chromatogram obtained with test solution (b) are clearly separated.

Calculation of fatty acid content:

Calculate the content of EPA and DHA as mg fatty acid/g oil using the following expression, and taking into account the certified value of the reference substances. The results should be rounded to the nearest 10 mg/g based on the method's precision.

$$\frac{A_x \times m_1}{A_1 \times m_2} \times Rf \times C \times 1,000$$

Rf = response factor for EPA and DHA as given by the expression:

$$\frac{A_{x,3} \times m_{x,r}}{A_{x,r} \times m_{x,3}}$$

m_1 = mass of the internal standard in test solution (a), in milligrams

m_2 = mass of the sample in test solution (a), in milligrams

$m_{x,3}$ = mass of the internal standard in reference solution (a1) (EPA determination), or in reference solution (a2) (DHA determination), in milligrams

$m_{x,r}$ = mass of eicosapentaenoic acid ethyl ester CRS (a1) or docosahexaenoic acid ethyl ester CRS in reference solution (a2), in milligrams

A_x = area of the peak corresponding to eicosapentaenoic acid ester or docosahexaenoic acid ester in the chromatogram obtained with test solution (a)

$A_{x,r}$ = area of the peak corresponding to eicosapentaenoic acid ester in the chromatogram obtained with reference solution (a1), or docosahexaenoic acid ester in the chromatogram obtained with reference solution (a2)

A_1 = area of the peak corresponding to the internal standard in the chromatogram obtained with test solution (a)

$A_{x,3}$ = area of the peak corresponding to the internal standard in the chromatogram obtained with reference solution (a1) (EPA determination), or with reference solution (a2) (DHA determination)

C = conversion factor between ethyl esters and triglycerides:

Ethyl esters: $C = 1.0$

Triglycerides: $C = 0.954$ for EPA
 $C = 0.957$ for DHA

To calculate sample content of EPA and DHA as free fatty acid equivalents, please use the conversion factors provided in the Guidance Documents.

Total Omega-3 Acids

From the assay for EPA and DHA, calculate the percentage content of the total omega-3-acids using the following expression and identifying the peaks from the chromatograms:

$$EPA + DHA + \left(A_{n-3} \times \frac{(EPA + DHA)}{(A_{EPA} + A_{DHA})} \right)$$

EPA = content of EPA obtained from the assay for EPA and DHA

DHA = content of DHA obtained from the assay for EPA and DHA

A_{n-3} = sum of the areas of the peaks corresponding to C18:3 n-3, C18:4 n-3, C20:4 n-3, C21:5 n-3 and C22:5 n-3 esters in the chromatogram obtained with test solution (b)

A_{EPA} = area of the peak corresponding to EPA ester in the chromatogram obtained with test solution (b)

A_{DHA} = area of the peak corresponding to DHA ester in the chromatogram obtained with test solution (b)

Notes

***1** - Nitrogen blanketing over the test samples is recommended and can help protect samples from oxidation. For samples with $\leq 35\%$ EPA/DHA this may not be necessary (verified by one GOED member).

***2** - The solvent can be evaporated with a gentle current of nitrogen and no heating, but heating will speed up the evaporation. Heating the samples on a water-bath in open tubes could introduce moisture that may affect the esterification, and heating done in a heating block can eliminate this risk.

***3** - Because EPA and DHA standards from different pharmacopeial sources are frequently not of high enough purity (i.e. below $< 99\%$), or DHA standards containing significant levels of EPA, the use of two reference solutions ((a1) and (a2)) is preferred over former approaches that combined DHA and EPA standards into one reference solution (a).

***4** - CP-Wax 52CB, 25 m x 0.25 mm I.D. 0.2. μm film thickness, Agilent cat. no. 7713 (formerly Chrompack), or equivalent, will be suitable.

***5** - The use of a focus liner (SGE Analytical Sciences; part number 092002) is recommended in combination with the use of Agilent GC 6890 and FID detection. The focus liner may offer less variability in analytical results, of particular interest when analysing higher purity EPA and DHA grades.

(<http://www.sge.com/uploads/77/29/7729b2910c-346c4004cod1169349bc57/TA-0043-A.pdf>)

***6** - If one knows the samples well and knows that tetracos-15-enoic acid (nervonic acid; 24:1, n-9) should be present, then there is no need to prepare and run reference solution (c), and one can calculate the resolution from test solution (a). But if one knows that tetracos-15-enoic acid should be there but does not show up on the chromatogram for test solution (a), one should rerun the sample and run reference solution (c) in the same sequence. If one is not sure if tetracos-15-enoic acid is present in the sample, then one should prepare and run reference solution (c) with the sample. Loss of resolution between tetracos-15-enoic acid and DHA may indicate that the column has lost its separation abilities.

(End of section)

Total omega-3 content should be expressed as mg per g as the following 7 fatty acid species:

Alpha-linolenic acid (ALA; C18:3 n-3)

Stearidonic acid / Moroctic acid (SDA; C18:4 n-3)

Eicosatetraenoic acid omega-3 (ETA; C20:4 n-3)

Eicosapentaenoic acid (EPA; C20:5 n-3)

Heneicosapentaenoic acid (C21:5 n-3)

Docosapentaenoic acid omega-3 (DPA; C22:5 n-3)

Docosahexaenoic acid (DHA; C22:6 n-3)

To convert between the weight percentages of different chemical forms of long-chain omega-3 fatty acids (Fatty Acid (FA), Triglyceride (TG), Ethyl Ester (EE) or Methyl Ester (ME)), the following conversion factors can be used to express your results as the Fatty Acid (FA).

$$\text{EPA (FA)} = \text{EPA (EE)} * 0.9151$$

$$\text{EPA (FA)} = \text{EPA (TG)} * 0.9598$$

$$\text{EPA (FA)} = \text{EPA (ME)} * 0.9557$$

$$\text{DHA(FA)} = \text{DHA(EE)} * 0.9213$$

$$\text{DHA(FA)} = \text{DHA(TG)} * 0.9629$$

$$\text{DHA(FA)} = \text{DHA(ME)} * 0.9590$$

$$\text{C18:3 n-3(FA)} = \text{C18:3 n-3(EE)} * 0.9085$$

$$\text{C18:3 n-3(FA)} = \text{C18:3 n-3(TG)} * 0.9565$$

$$\text{C18:3 n-3(FA)} = \text{C18:3 n-3(ME)} * 0.9520$$

$$\text{C18:4 n-3(FA)} = \text{C18:4 n-3(EE)} * 0.9078$$

$$\text{C18:4 n-3(FA)} = \text{C18:4 n-3(TG)} * 0.9562$$

$$\text{C18:4 n-3(FA)} = \text{C18:4 n-3(ME)} * 0.9517$$

$$\text{C20:4 n-3(FA)} = \text{C20:4 n-3(EE)} * 0.9156$$

$$\text{C20:4 n-3(FA)} = \text{C20:4 n-3(TG)} * 0.9601$$

$$\text{C20:4 n-3(FA)} = \text{C20:4 n-3(ME)} * 0.9559$$

$$\text{C21:5 n-3(FA)} = \text{C21:5 n-3(EE)} * 0.9186$$

$$\text{C21:5 n-3(FA)} = \text{C21:5 n-3(TG)} * 0.9615$$

$$\text{C21:5 n-3(FA)} = \text{C21:5 n-3(ME)} * 0.9576$$

$$\text{C22:5 n-3(FA)} = \text{C22:5 n-3(EE)} * 0.9217$$

$$\text{C22:5 n-3(FA)} = \text{C22:5 n-3(TG)} * 0.9631$$

$$\text{C22:5 n-3(FA)} = \text{C22:5 n-3(ME)} * 0.9593$$

Primary Reference Standards

It is recommended to use pharmacopeial reference standards. Reference standards from other suppliers are acceptable providing the purity is certified and verified.

Normalizations to 100% purity should be made for the results obtained. Note that high purity

primary reference standards are extremely sensitive to oxidation and should be handled with care. Use of nitrogen cover is recommended.

Densities of oils

Triglyceride oils have a density distinct from that of ethyl esters. The following values may be useful for calculations of liquid formulations:

The density for triglyceride oils is typically 0.93-0.96 g/ml.

The density for ethyl ester oils is typically 0.91 g/ml.

Volumes should be expressed in milliliter (ml). However, volumes used for nutrition labeling purposes are frequently expressed according to spoon size. The definition of spoon size can vary. The following guidance may be useful to understand this:

One tablespoon is 5 ml (Europe) or 15 ml (US). A teaspoon is typically 2.5 ml (Europe) or 5 ml (US)

Gas quality and filters for fatty acid analysis

Grade of carrier gas used: The highest grade of carrier gas is recommended. Hydrogen grade 5.0 (purity $\geq 99.999\%$) or hydrogen from a hydrogen generator can be used. If $<99.999\%$ pure hydrogen is used, then the minimum acceptable grade is 99.95%. To assure the best quality for fatty acid profile (%) and omega content (mg/g) analysis, the GOED and European Pharmacopeia monographs indicate that the carrier gas should be hydrogen for chromatography, minimum 99.95%. A moisture/oxygen/hydrocarbon filter should be used.

Use of gas filters: Oxygen, moisture, and hydrocarbon filters/traps on the hydrogen carrier gas line are recommended.

Note by individual members: If using a hydrogen generator, or if grade 5.0 helium is used as carrier gas, the use of trap filters has been found to not be needed.

Recovery

Recovery of long-chain unsaturated compounds is the most challenging a GC can do. One recommendation to attain optimal analytical recovery is to reduce losses of EPA, DHA and other polyunsaturated fatty acids during the GC-FID analysis to a minimum, in particular by using the

best injector system that avoids thermal cracking and selective evaporation (discrimination). Achieving near 100% recovery has also been demonstrated to contribute to minimize inter-laboratory variability. Near 100% recovery has been achieved by using the following GC-FID instrument, with a specific parts list:

- GC-FID by Scion Instruments (Varian). 436-GC Gas Chromatograph/ Programmed Temperature Vaporizer injector (PTV) type 21 EFC SST Air / FID with electrometer DEFC type 11.

The following column is suitable for analysis in conjunction with the above-mentioned system SCION-WAX, 25m x 0.25mm x 0.20 μ m.

Furthermore, adapting the polyunsaturated fatty acids analyses to splitless injections maintains high recoveries for a longer time as much less non-volatile residues can collect in colder parts of the injector. This recommendation does not preclude the use of other GC-FID systems, but some well-known GC-FID instruments are not able to avoid cracking and discrimination of EPA and DHA specifically.

(End of section)

B. Environmental Contaminants

PCBs, Dioxins, Furans, and Dioxin-like PCBs

There are:

- 12 Dioxin-like (dl)-PCBs (12 PCBs with “dioxin-like” toxic properties)
- 210 PCDDs (“Dioxins”; 75 congeners) and PCDFs (“Furans”; 135 congeners).
- Of the 75 PCDDs, 7 congeners have recognized toxic properties and a TEF value.
- Of the 135 PCDFs, 10 congeners have recognized toxic properties and a TEF value.
- 17 TCDDs (dioxins with a tetrachloro-dibenzo-p-dioxin structure).
- 38 TCDFs (furans with a tetrachloro-dibenzo-furan structure)

The number of dl-PCBs and PCDDs/PCDFs that are quantified using TEF values (sum 29 species) does not add up to the total number of Dioxins, Furans and dl-PCBs isomers (sum 222 species).

Also, the number of isomers of dl-PCBs and tetrachlorinated TCDDs/TCDFs (sum 67 species) does not add up to the total number of Dioxins, Furans and dl-PCBs (sum 222 species).

EC Regulation No. 644/2017 provides basic indications for methods and some requirements, respectively, for the sampling and analysis of dioxins, dioxin-like PCBs and non-dioxin-like PCBs. It is not a precise method description:

- EU Regulation (EU) No 644/2017 (5 April 2017) “Laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014”.

Sample preparation and appropriate methods of analysis for PCDDs, PCDFs, and PCBs are described in EC Regulation (EU) No 644/2017 (stated above). Annex II of 644/2017 describes methods of sampling; Requirements for analytical methods are given in Annex III and IV. The Upper Bound method is required for expressing PCDD, PCDF and PCB content, along with a specific list of PCDD, PCDF and PCB congeners to be included in the calculation. The Limit of Quantitation is

determined as described in European Commission Regulation No 644/2017, Annex 1 section 1.7. Gas chromatography coupled with high-resolution mass spectrometry (GC/HRMS) has proved to provide required sensitivity and specificity. GC/MS-MS is also allowed for confirmatory purposes (since EU 589/2014).

Recommended Methods:

PCBs

- **B-1.1.1.** EPA Method 1668

Only EPA Method 1668 covers all 209 PCB isomers. GMP laboratories apply the method according to USP, and the original EPA method is adapted and validated for fish oils and related products, using HRGC/HRMS systems and matrices not disclosed in the original US EPA method. Note: USP refers to revision A. Revision C is the latest one.

PCDDs and PCDFs

- **B-1.2.1.** EPA Method 1613 revision B: “Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS” (Identification and quantitation of PCDD and PCDF congeners)

GMP laboratories apply the method according to USP, and the original EPA method is adapted and validated for fish oils and related products using HRGC/HRMS systems and matrices not disclosed in the original US EPA method)

- **B-1.2.2.** GLS DF-100 : „Bestimmung von Polychlorierten Dibenzodioxinen (PCDD), polychlorierten Dibenzofuranen (PCDF) und polychlorierten Biphenylen (PCB) inkl. aller 209 PCB Kongenere in Lebens- und Futtermittel sowie anderen Proben mittels Gaschromatographie mit massenspektrometrischer Detektion“.

Method used by an ISO-laboratory, corresponding to an in-house method based both upon European legislation and US EPA methods. The ISO method is accredited by the German Accreditation Body (DAkkS) according to DIN EN ISO/IEC 17025:2005

Dioxin-like PCBs

- ▶ **B-1.3.1.** EPA Method 1668

EPA Method 1668 can also be used only for dioxin-like PCBs. The USP refers to EPA 1668A.

- ▶ **B-1.3.2.** GLS DF-100 : "Bestimmung von Polychlorierten Dibenzodioxinen (PCDD), polychlorierten Dibenzofuranen (PCDF) und polychlorierten Biphenylen (PCB) inkl. aller 209 PCB Kongenere in Lebens- und Futtermittel sowie anderen Proben mittels Gaschromatographie mit massenspektrometrischer Detektion."

Method used by an ISO-laboratory, corresponding to an in-house method based both upon European legislation and US EPA methods. The ISO method is accredited by the German Accreditation Body (DAkkS) according to DIN EN ISO/IEC 17025:2005

Total Dioxins, Furans and Dioxin-like PCBs

- ▶ **B-1.4.1.** EPA Method 1613B for the dioxins, and EPA Method 1668 for the PCBs as required by USP.

- ▶ **B-1.4.2.** GLS DF-100 : "Bestimmung von Polychlorierten Dibenzodioxinen (PCDD), polychlorierten Dibenzofuranen (PCDF) und polychlorierten Biphenylen (PCB) inkl. aller 209 PCB Kongenere in Lebens- und Futtermittel sowie anderen Proben mittels Gaschromatographie mit massenspektrometrischer Detektion."

Method used by an ISO-laboratory, corresponding to an in-house method based both upon European legislation and US EPA methods. The ISO method is accredited by the German Accreditation Body (DAkkS) according to DIN EN ISO/IEC 17025:2005

Heavy Metals

Suitable methods that can be used for the quantitation of heavy metals include the following:

Lead (Pb):

- ▶ **B-2.1.1.** USP Method 233
- ▶ **B-2.1.2.** §64 LFGB L00.00-19/3 (by AAS)
- ▶ **B-2.1.3.** EN 15763:2009 (by ICP-MS)

Cadmium (Cd):

- ▶ **B-2.2.1.** USP Method 233
- ▶ **B-2.2.2.** §64 LFGB L00.00-19/3 (by AAS)
- ▶ **B-2.2.3.** EN 15763:2009 (by ICP-MS)

Mercury (Hg):

- ▶ **B-2.3.1.** USP Method 233
- ▶ **B-2.3.2.** §64 LFGB L00.00-19/4 (by cold vapor

AAS)

- ▶ **B-2.3.3.** EN 15763:2009 (by ICP-MS)

Arsenic (As):

Total arsenic:

- ▶ **B-2.4.1.** §64 LFGB L00.00-19/3 (by AAS)
- ▶ **B-2.4.2.** EN 15763:2009 (by ICP-MS)
- ▶ **B-2.4.3.** DIN EN ISO 11885 (by ICP-OES)

Inorganic arsenic:

- ▶ **B-2.5.1.** USP Method 233
- ▶ **B-2.5.2.** §64 LFGB 25.06 (by Hg-AAS) (note: only for fish oil)
- ▶ **B-2.6.1.** EN 15763. "Foodstuffs - Determination of trace elements - Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion"
- ▶ **B-2.6.2.** AOCS Official Method Ca 18-79. "Analysis for Chromium, Copper, Iron, Nickel, and Manganese in Triglyceride Oils by Atomic Absorption Spectrophotometry Using a Graphite Furnace"
- ▶ **B-2.6.3.** AOCS Ca 15-75. "Analyses for Chromium, Copper, Iron and Nickel in Vegetable Oils by Atomic Absorption Spectrophotometry"

For the reporting of heavy metal concentrations, it is recommended to add information on the detection limit:

The Detection Limit is equal to three times the standard deviation of the mean of blank determinations.

Results below the Detection Limit are reported as <DL. Results between the Detection Limit and the Quantitation Limit are reported as actual values.

(End of section)

C. Oxidation

Peroxide Value (P.V. or PV) and Anisidine Value (or para-Anisidine Value; p-AV): to establish primary and secondary oxidation product levels. These compounds, with others resulting from further decomposition, are responsible for the rancid flavours that develop. Of the two values the p-AV is the more indicative of quality state. Ultra Violet (U.V.) Extinction Values at 233 nm and 269 nm: these figures quantify the conjugated dienes and trienes, respectively, and are related to oxidation levels. Increases in these values are also obtained when an oil is overheated, resulting in colour fixation.

Peroxide Value

- › **C-1.1** Ph. Eur. Method 2.5.5
- › **C-1.2** "Peroxide Value" AOCS Official Method Cd 8b-90

Note: In the context of determination of Peroxide Value (PV) by iodometric titration, the following terms and definitions apply. PV is the quantity of those substances in the sample, expressed in terms of active oxygen, that oxidize potassium iodide under the conditions specified in the official method used. PV is usually expressed in milliequivalents (meq) of active oxygen per kilogram (kg) of oil. It can also be expressed (in SI units) as millimoles (mmol) of active oxygen per kg of oil. The value expressed in mmol of active oxygen per kg is half that expressed in meq of active oxygen per kg. Multiplication of the PV expressed as meq active oxygen per kg of oil by the equivalent mass of oxygen (equaling 8) gives the milligrams of active oxygen per kg of oil.

Note: In Dec. 2015 it was decided by EDQM that for the Ph.Eur. Monograph "Omega-3 Ethyl Esters 90" with regard to Method 2.5.5. "Peroxide Value", Method A (using chloroform as solvent) will no longer be supported, and only the use of Method B will be maintained. The USP monograph is still based on the chloroform solvent. The new situation will entail that for every product destined for both European and US markets two different approaches to measuring PV have to be carried out. New in-house validations may have to be carried out using Method B.

Secondary Oxidation

Para-Anisidine Value (pAV)

- › **C-2.1.1** AOCS Official Method Cd 18-90
- › **C-2.1.2** Ph.Eur. Method 2.5.36 "Anisidine Value"
- › **C-2.1.3** ISO 6885 "Animal and vegetable fats and oils - Determination of Anisidine Value"

This method is almost identical to AOCS Cd 18-90, but can be considered a bit more rigorous: i) The p-anisidine solution preparation is described more precisely,

ii) sample preparation is thoroughly described, iii) spectrophotometric measurement is quantitatively defined, iv) the definition of anisidine value is well-defined in terms of increase of absorbance reacted with anisidine vs not reacted, rather than absorbance itself. A significant difference is that sample blank reading is done with the addition of the glacial acetic acid. This allows for better accounting for any absorbance or matrix interactions of the glacial acetic acid. This removes the 1.2 factor from the normal equation which is present to account for the difference in the volumes of the reacted solution, and the sample blank solution.

The precise measurement of secondary oxidation in oils containing substances that interfere with the colorimetric para-Anisidine Value (p-AV) test requires the employment of novel and validated methods that can specifically measure PUFA-derived secondary oxidation products (such as aldehydes) dissolved in PUFA-rich oils.

(End of section)

USEFUL TECHNICAL INFORMATION ON EPA/DHA-CONTAINING OILS AND PRODUCTS

Information on Additional Testing Methodologies (Not specified by or following the GOED Monograph)

Fatty Acid Analysis

▸ **A-1-1-1** Iodine Value

The Iodine Value (IV) is the mass of halogen, expressed as iodine, absorbed by the fat or oil component in a sample expressed as the mass fraction in grams per 100 g of fat. It is considered a measure of the relative degree of unsaturation of the fat or oil component where a high iodine value is indicative of greater unsaturation.

Distinct marine oils and concentrates have typical IV ranges, and IV used to be extensively used to verify an oil's identity, although much less today.

Most fish oils are in the IV range of 120-200.

Methods:

IV methods suitable for fish oil deal with oils that are close to 150 IV:

- AOCS Cd 1b-87 1993
- AOCS Cd 1d-92 1993
- ISO 3961:1996 E
- USP <401> "Fats and fixed oils", Method I (Hanus method) and Method II.

A variation of 2-3% on IV results can be intrinsically related to the analysis.

The sample amount needs to be adjusted so that the IV falls in the appropriate detection range of the method used. This is especially true for concentrates and shark liver oils with very high concentrations of unsaturated substances. EPA and DHA have IV of 419.56 and 463.57. Shark liver oil has a high content of squalene, rich in double bonds. Squalene (substance) has an IV range of 360-380, and squalene oil (99%) from shark liver has an IV range of 360-395.

The theoretical Iodine Value can be calculated using the IV of the individual unsaturated fatty acids (and other double-bond containing lipids) and their relative concentrations present in an oil.

This table provides indications to the amount of

sample weight to be used for method AOCS Cd 1b-87 1993 and AOCS Cd 1d-92 1993:

IV	Sample Weight		Weighing Accuracy g±
	150% excess g	100% excess g	
< 3	10	10	0.001
3	8.4613	10.5760	0.005
5	5.0770	6.3460	0.0005
10	2.5384	3.1730	0.0002
20	0.8461	1.5865	0.0002
40	0.6346	0.7935	0.0002
80	0.3173	0.3966	0.0002
100	0.2538	0.3173	0.0001
120	0.2115	0.2644	0.0001
140	0.1813	0.2266	0.0001
160	0.1587	0.1938	0.0001
180	0.1410	0.1762	0.0001
200	0.1269	0.1586	0.0001

Oxidation

▸ **C-2.2.1** TAV Test

In order to measure secondary oxidation in EPA/DHA oils where interference due to added flavours precludes the use of the p-AV test, it is possible to use a different approach called the True Anisidine Value (TAV) Test. For any specific omega-3 product the test needs to be validated for the specific oil type, the employed additive (such as flavours), and the specific concentration of the employed additives. Further information can be found here: <http://blog.nutrasource.ca/blog/bid/71638/New-testing-method-the-True-Anisidine-Value-TAV-test-solves-omega-3-industry-problem>

Conjugated Dienes:

- **C-3.1.1** Ph.Eur. Method 2.2.25 "Absorption spectrophotometry, ultraviolet and visible".
This measurement is of relevance in the context of a Eur. Pharmacopeia requirement only.
- **C-3.1.2** USP Monograph "Omega-3 Acid Triglycerides. Specific Tests / Absorbance". (eg USP 38 NF-33)
- **C-3.1.3** AOCS Official Method Ti 1a-64 "Spectrophotometric Determination of

Conjugated Dienoic Acid”.

- **C-3.1.4** AOCS Official Method Th 1a-64 “Diene Value”

- Ph. Eur.: Omega-3 Acid triglycerides
- Ph. Eur.: Omega-3 Acid ethyl esters 60
- Ph. Eur.: Fish oil rich in omega-3

Physico-chemical Parameters

Acid Value

- **D-1.1.1.** AOCS Official Method Cd 3d-63 “Acid Value”

Applicable to crude and refined marine oils. This method permits converting acid value directly to percent fatty acids provided that there are no other acids present in the oil. The method can be modified to measure the acid value of highly colored oils.

- **D-1.1.2.** AOCS Official Method Ca 5a-40 “Free Fatty Acids”

Applicable to all crude and refined, bleached and deodorized marine oils. This method permits measurement of free fatty acid content.

- **D-1.1.3.** USP/NF 401

- D-1.2.1 AOCS Official Method Cd 11-57 “Alpha-monoglycerides”

This method permits the measurement of alpha-monoglycerides in oils.

- D-1.2.2 AOCS Official Method Cd 11b-91 “Determination of Mono- and Diglycerides by Capillary Gas Chromatography”

Applicable to mono- and diglyceride concentrates and mono- and diglycerides in fats and oils.

- D-1.2.3 AOCS Official Method Cd 11d-96 “Mono- and Diglycerides Determination by HPLC-ELSD”

Applicable to oils and emulsifiers.

- D-1.2.4 ISO 18395:2005 “Animal and vegetable fats and oils. Determination of monoacylglycerols, diacylglycerols, triacylglycerols and glycerol by high-performance size-exclusion chromatography”

- D-1.2.5 AOCS Official Method Cd 22-91 “Determination of Polymerized Triglycerides by Gel-Permeation HPLC”

This method measures polymerized (dimeric and higher oligomeric) triglycerides (oligomers) in animal oils.

- D-1.2.6 Ph.Eur. 1352 “Oligomers and partial glycerides” (for re-esterified triglyceride oils)

D-1.2.7 Ph.Eur. 2063 “Oligomers and partial glycerides” (for ethyl ester oils)

Oligomers and Partial Glycerides

Note: The category “Oligomers and partial glycerides” does not have a very precise naming. The current naming can encompass oligomerized triglycerides (“oligomers” formed by oxidative cross-linking), mono- and di-glycerides (“partial glycerides,” which are naturally present in many marine oils), and even lysophospholipids, which are derived from glycerophospholipids. Each of these chemical families require specific methodological approaches for their separation and detection. It is useful that the precise compounds are being referred to when measured and reported. The methods are sensitive to co-elution with other substances and false-positives need to be ruled out.

- The European Pharmacopeia has not made a separate method for mono-glyceride, di-glyceride, tri-glyceride, ethyl ester/FFA and oligomers. Note that Ph. Eur. 2.2.30 (“Size exclusion Chromatography”) is only a general monograph about size exclusion chromatography.

The methods for oligomers and partial glycerides are explained in detail inside each of these four Ph. Eur. Monographs:

- Ph. Eur.: Omega-3 Acid ethyl esters 90

Unsaponifiable Matter

- **D-1.3.1** Ph. Eur. Assay 2.5.7 “Unsaponifiable Matter”

- **D-1.3.2** AOCS Official Method Ca6b-53 “Unsaponifiable Matter”

This method is especially suited for marine oils.

- **D-1.3.3** USP/NF 401 “Unsaponifiable Matter”

Cold Test

GOED recommends the use of the following method to measure the resistance of a test sample to crystallization (often used as an index of

winterization and stearin removal processes), and the confirmation of the removal of solid substances by cooling and filtering.

- › **D-1.4.1** AOCS Official Method: Cc 11-53. "Cold Test"

Note: this method provides a good description how to execute a cold test (3 hours at 0°C.).

- › **D-1.4.2** Ph.Eur. monographs on Cod-Liver Oil (A, B, and farmed), Method "Stearin"

Density

- › **D-1.5.1** AOCS Official Method: Cc 10c-95. "Determination of Mass per Unit Volume ("Liter Weight") in Air of Oils and Fats."
- › **D-1.5.2** AOCS Official Method: To 1a-64. "Specific Gravity 25°C."

Specific Components

Protein

- › **D-2.1.1** GOED provides a summary on methods for the measurement of total protein in oils (e.g. of relevance to allergen content exemptions) in the technical report "Summary on residual protein measurement in EPA/DHA omega-3 oils and allergen labeling." [\[Link\]](#)

Cholesterol

- › **D-2.2.1** AOAC Official Method 994.10 "Cholesterol in Foods"
- › **D-2.2.2** Ph. Eur. Method 2.4.32 "Total cholesterol in oils rich in omega-3 acids"
This method may be used for the quantitative determination of the sum of free and esterified cholesterol in products of fish oils rich in omega-3 acids (as ethyl esters or triglycerides). A new improved method version (ref. 07/2016:2043, EP 8.8) was published on January 1st 2016 with implementation July 1st 2016. It replaces the former Ph.Eur. method 2.4.32 (ref. 07/2010:20432). The improvements relate to the use of solid-phase extraction, a much faster gas chromatographic method, and accuracy down to 0.2 mg/g for total cholesterol.

Specific Contaminants

Poly-Aromatic Hydrocarbons (PAHs)

Benzo(a)pyrene

- › **D-3.1.1** AOCS Official Method Cd 21-91 "Determination of Benzo(a)pyrene in Edible Oils

and Fats"

This method is applicable to crude and refined edible oils and fats.

Sum of 4 poly-aromatic hydrocarbons:

Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (PAH4):

- › **D-3.1.2** PAH4 Assay – Eurofins: in-house method validated with fish oil, for routine analysis of 20-60 samples a day. Caffeine complexation sample clean-up, online-SPE-GC-MS.

Note: EU 836/2011 provides some method requirements for PAH methods.

Phthalates

- › **D-3.2.1** Eurofins: in-house method. Liquid solvent extraction, LC-MS/MS.

Brominated Flame Retardants

The following brominated flame retardants (BFRs) are of relevance:

- › PBDE (Poly Brominated Diphenyl Ethers). If BFRs are monitored these should be measured. The sum of seven PBDEs is often required by FDA for APIs.
- › PBB (Poly Brominated Biphenyls). It is no longer necessary to measure these. They were forbidden 20 years ago and are not found very often in fish oils.
- › HBCD (HexaBromoCycloDodecane) will be found in fish oil, so it makes sense to monitor.
- › TBBPA (TetraBromoBisPhenol A) is the most common BFR but it is chemically bound and the bioavailability is not as high as for other BFRs. It is recommended to monitor.
Note: There are more BFRs, but there is no need to monitor them for the moment.

- › **D-3.3.1** EPA Method 1614

The method is adapted by laboratories in-house for the specific oil matrix that is analyzed.

Pesticides

There are no common global regulations concerning pesticides in fish or fish oil products. Eurofins: routine analysis for 5 DDTs, HCB and lindane (GMP laboratory), and 200 pesticides (ISO

laboratory).

Species Identification / Authentication for Named Oils

D-4.1.1 The authenticity of a lipid sample can be determined by comparing data from nuclear magnetic resonance (NMR) spectroscopy with a database of known reference samples. Depending on the reference data available, the method can provide information about both species and geographic origin. Such a reference database, containing the most commercially relevant named marine oils, has been developed by ORIVO AS (www.orivo.no). The Norwegian company is providing authenticity testing services to companies throughout the value chain on a global basis.

Note: We welcome further indications to methods suitable for species identification and authentication of EPA/DHA-containing oils.

Proficiency Testing

It is recommended that GOED members that have their own analytical capacity for testing fatty acid content, oxidation, and contaminants, participate periodically in the Laboratory Proficiency Program testing organized by AOCS (“Global Organization for EPA and DHA Omega-3 (GOED) Nutraceutical Oils”).

Weblinks:

<https://aocs.personifycloud.com/PersonifyEBusiness/Default.aspx?TabID=251&productId=118332>

[https://www.aocs.org/attain-lab-services/laboratory-proficiency-program-\(lpp\)/laboratory-proficiency-program-series#goed-nutraceutical-oils](https://www.aocs.org/attain-lab-services/laboratory-proficiency-program-(lpp)/laboratory-proficiency-program-series#goed-nutraceutical-oils)

(End of section)

Microbiological Quality

This section provides guidance on the volumes of oil to be tested for microbiological contamination, and maximum limits for microbiological contaminants in omega-3 oils under relevant regulations, pharmacopeial monographs, standard setting organizations, and an example of company standards.

NSF International (Standards developer, formerly called National Sanitation Foundation)

▸ NSF/ANSI 173-2013

Table 2A - Acceptable limits for microbiological contaminants in raw materials

Ingredient	Aerobic	Yeast/Mold	Enterobacteriaceae
Vitamin and/or mineral ingredient	1×10^3 CFU/g	1×10^2 CFU/g	1×10^2 CFU/g
Botanical ingredient — non-extract	1×10^7 CFU/g	1×10^5 CFU/g	1×10^4 CFU/g
Botanical ingredient — extract / other dietary supplemental ingredient	1×10^4 CFU/g	1×10^3 CFU/g	1×10^2 CFU/g

Table 2B - Acceptable limits for pathogenic microbiological contaminants in raw materials

Ingredient	<i>Salmonella spp.</i>	<i>E. coli</i> (1)	<i>S. aureus</i>
Vitamin and/or mineral ingredient	ND ⁽²⁾	ND ⁽²⁾	ND ⁽²⁾
Botanical ingredient — non-extract ⁽¹⁾	ND ⁽²⁾	1×10^2 CFU/g	ND ⁽²⁾
Botanical ingredient — extract / other dietary supplemental ingredient	ND ⁽²⁾	ND ⁽²⁾	ND ⁽²⁾

⁽¹⁾ Upon the presence of *E. coli*, 73.6.2 is to be followed to determine whether the colonies are enterovirulent. There is a zero tolerance for the presence of enterovirulent *E. coli*.

⁽²⁾ ND = Not Detected. Not Detected requires that no colonies shall be present in 10g of sample when tested under the conditions of the USP method cited in 7.3. The detection level for this testing is 10 CFU/g for the period of time tested.

Table 3A - Acceptable limits for microbiological contaminants in finished products(1)

Finished Products	Aerobic	Yeast/Mold	Enterobacteriaceae
Category 1 Vitamin and/or mineral ingredient	1×10^3 CFU/g	1×10^2 CFU/g	1×10^2 CFU/g
Category 2 Botanical ingredient — non-extract	1×10^4 CFU/g	1×10^3 CFU/g	1×10^2 CFU/g
Category 3 Botanical ingredient — extract / other dietary supplemental ingredient	1×10^7 CFU/g	1×10^5 CFU/g	1×10^4 CFU/g

(1) The category designation shall be based on ingredients present at 1% or more by weight in the formula as provided in the full product formulation. For a product containing ingredients from more than one category, the finished product category will be assigned based on the ingredient with the highest category number.

Table 3B - Acceptable limits for microbiological contaminants in finished products⁽¹⁾

Finished Products	<i>Salmonella spp.</i>	<i>E. coli</i> (1)	<i>S. aureus</i>
Category 1 Vitamin and/or mineral ingredient	ND ⁽³⁾	ND ⁽³⁾	ND ⁽³⁾
Category 2 Botanical ingredient — non-extract	ND ⁽³⁾	ND ⁽³⁾	ND ⁽³⁾
Category 3 Botanical ingredient — extract / other dietary supplemental ingredient	ND ⁽³⁾	1×10^2 CFU/g	ND ⁽³⁾

⁽¹⁾ The category designation shall be based on ingredients present at 1% or more by weight in the formula as provided in the full product formulation. For a product containing ingredients from more than one category, the finished product category will be assigned based on the ingredient with the highest category number.

Examples:

- A product containing only Vitamin C and Zinc shall be in category 1.
- A product containing Vitamin C, Zinc, and Green Tea Extract shall be in category 2.
- A product containing Vitamin C, Zinc, and Echinacea shall be in category 3.

⁽²⁾ Upon the presence of *E. Coli*, 73.7 is to be followed to determine whether the colonies are enterovirulent. There is a zero tolerance for the presence of enterovirulent *E. Coli*.

⁽³⁾ ND = Not Detected. Not Detected requires that no colonies shall be present in 10g of sample when tested under the conditions of the USP method cited in 7.3. The detection level for this testing is 10 CFU/g for the period of time tested.

Chinese State Administration for Market Regulation (former CFDA)

- From the SC/T standard, SC/T 3503-2000 (Polyunsaturated fish oil products)

菌落总数 Aerobic plate count	cfu/g (mL)	1000
大肠菌群 Coliforms	MPN/100g (mL)	40
霉菌 Molds	cfu/g (mL)	25
沙门氏菌 <i>Salmonella</i>		None
志贺氏菌 <i>Shigella</i>		None
金黄色葡萄球菌 <i>Staphylococcus aureus</i>		None
溶血性链球菌 <i>Streptococcus hemolyticus</i>		None

NS Method No.	Test items	Standard Method
NS090241	菌落总数 Total plate count	GB 4789.2-2016
NS090242	大肠菌群 Coliforms	GB 4789.3-2016
NS090243	霉菌和酵母菌 Mold and yeast	GB 4789.15-2016
NS090244	沙门氏菌 <i>Salmonella</i>	GB 4789.4-2016
NS090245	志贺氏菌 <i>Shigella</i>	GB 4789.5-2012
NS090246	金黄色葡萄球菌 <i>Staphylococcus aureus</i>	GB 4789.10-2016
NS090247	溶血性链球菌 <i>Streptococcus hemolyticus</i>	GB 4789.11-2014

European Pharmacopeia

- From EP 7.0 - 5.1.4 “Microbiological quality of non-sterile products for pharmaceutical use”

This monograph may be of interest as many fish oil products are used as active ingredients for pharmaceutical forms, and many more EPA/DHA products that are not drugs are produced in accordance to the Pharmacopoeia.

This monograph provides Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use:

- Total aerobic microbial count (TAMC) — 10^3 CFU/g or CFU/ml
- Total combined yeasts/moulds count (TYMC) — 10^2 CFU/g or CFU/ml

The monograph furthermore provides acceptance criteria for microbial quality of non-sterile dosage forms, in particular a special provision for oral dosage forms containing raw materials of natural (animal, vegetal or microbial) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU/g or CFU/ml:

- TAMC — 10^4 CFU/g or CFU/ml
- TYMC — 10^2 CFU/g or CFU/ml
- Specified micro-organisms
 - Not more than 10^2 CFU of bile-tolerant gram-negative bacteria (1 g or 1 ml)
 - Absence of *Salmonella* (10 g or 10 ml)
 - Absence of *Escherichia coli* (1 g or 1 ml)
 - Absence of *Staphylococcus aureus* (1 g or 1 ml)

USP

- General Chapter <2023> Microbial Attributes of Nonsterile Nutritional and Dietary Supplements

The limits are:

- Total aerobic microbial count not more than (NMT) 1000 cfu/g
- Total, combined yeast and mold count NMT 100 cfu/g
- Absence of *Salmonella* species and *E. coli* in 10 g

CODEX WHO/FAO

▸ Microbiological Criteria

The products should comply with any microbiological criteria established in accordance with the Principles for the Establishment and Application of Microbiological Criteria for Foods (CAC/GL 21-1997).

No specific limits on microbial contamination are provided.

Company Standard (Example)

Unless otherwise specified, ingredients used for the manufacture of our products and the products themselves intended for use in human nutrition and health, excluding premixes, are in compliance with the standard set below if tested according to the Methods of Analysis 2.6.12 and 2.6.13 of Ph. Eur. or 61 and 62 of USP.

Microbiological Purity:

Total viable counts:

Total aerobic microbial count	Max. 10 ³ CFU/g or CFU/ml
Total combined yeast/moulds count	Max. 10 ² CFU/g or CFU/ml
<i>Enterobacteria</i>	< 10 CFU/g or CFU/ml
<i>Escherichia coli</i>	Negative in 10 g or 10 ml
<i>Salmonella spp.</i>	Negative in 25 g or 25 ml
<i>Staphylococcus aureus</i>	Negative in 10 g or 10 ml
<i>Pseudomonas aeruginosa</i>	Negative in 10 g or 10 ml

(End of section)

TECHNICAL GUIDANCE DOCUMENTS

The PURPOSE of the following Technical Guidance Documents is to provide information and guidance to GOED members on recognized long-chain omega-3 PUFA-containing oils with respect to suitable testing methods for fatty acids, oxidation status, contaminants, and other relevant physico-chemical parameters, and acceptable levels and ranges of these. The overall OBJECTIVE is the establishment of QUALITY of EPA- and/or DHA-containing oils and product types that fall under the scope of the GOED Voluntary Monograph. The content of the documents is for information and guidance of GOED members to produce quality oils while taking into account the idiosyncrasies of different EPA and DHA chemical forms, formats and species origins of different omega-3 product classes.

Technical Guidance Documents for the following EPA- and DHA-containing product classes are provided in the following pages:

EPA- and/or DHA-containing refined, processed, and concentrated oils (not based on species origin)

- Refined EPA and/or DHA Omega-3 Oil Triglycerides
- EPA and/or DHA Omega-3 Oil Ethyl Ester Concentrates
- EPA and/or DHA Omega-3 Oil Triglyceride Concentrates

Species of Origin EPA- and DHA-containing oils

- Cod Liver Oil
- Tuna oil
- Green-Lipped Mussel Oil
- Salmon Oil
- Anchovy Oils
- Skate Liver Oil

Other types of EPA- and/or DHA-containing oils

- Flavored Oils

(End of section)

EPA- and/or DHA-containing refined, processed, and concentrated oils (not based on species origin).

TECHNICAL GUIDANCE DOCUMENT FOR:

<p>Type of EPA/DHA product class: Refined EPA and/or DHA Omega-3 Oil Triglycerides</p>
<p>Brief description of product class:</p> <p>Refined EPA and/or DHA Omega-3 Oil Triglycerides are purified and/or deodorized oils containing EPA and DHA from fish (including fish of families such as <i>Engraulidae</i>, <i>Carangidae</i>, <i>Clupeidae</i>, <i>Osmeridae</i>, <i>Salmonidae</i>, <i>Scombridae</i>, and <i>Gadidae</i>), cephalopod (including calamari), plant or microbial sources. Refined EPA and/or DHA Omega-3 Oil Triglycerides may also be winterized.</p>
<p>Reason for classification as a separate product class :</p>
<p>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</p> <ul style="list-style-type: none"> A. Oxidation: as specified in GOED Voluntary Monograph B. Environmental contaminants: as specified in GOED Voluntary Monograph C. Fatty acids (EPA and DHA): as specified in GOED Voluntary Monograph
<p>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on Methods):</p> <p>Acid value. Maximum 0.5 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur. Assay 2.5.1 . (In the context of CODEX, for food applications a maximum of 3.0 mg KOH/g would be permitted in specific geographies)</p> <p>Absorbance at 233 nm. Maximum Abs₂₃₃ 0.7; Ph. Eur. Assay 2.2.25</p> <p><i>Note: This test is of relevance in the context of a Eur. Pharmacopeia requirement only.</i></p> <p>Unsaponifiable Matter. Maximum 1.5%; Ph. Eur. Assay 2.5.7 / AOCS Official Method Ca6b-53</p> <p>Stearin. In Ph.Eur. 10th Edition, the instruction to cool the sample to room temperature (15-25°C.) before placing it in the iced water has been introduced to improve repeatability. The sample quantity has been changed to "At least 10 mL." to harmonize with Ph.Eur. Cod-liver oil monographs and USP.</p>
<p>Species identification : Not relevant</p>
<p>Specific comments : GOED does not suggest limits on the content of partial glycerides (MAGs and DAGs) for this product class.</p>

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: EPA and/or DHA Omega-3 Ethyl Ester Concentrates
<p>Brief description of product class:</p> <p>EPA and/or DHA Omega-3 Ethyl Ester Concentrates are ethyl esters of EPA and/or DHA obtained by transesterification of oils from fish (including fish of families such as <i>Engraulidae</i>, <i>Carangidae</i>, <i>Clupeidae</i>, <i>Osmeridae</i>, <i>Salmonidae</i>, <i>Scombridae</i>, and <i>Gadidae</i>), cephalopod (including calamari), plant or microbial sources and subsequent physico-chemical purification processes. An EPA and/or DHA Omega-3 Ethyl Ester Concentrate should have at least 93% fatty acid-ethyl esters.</p>
Reason for classification as a separate product class:
<p>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</p> <ul style="list-style-type: none"> A. Oxidation: as specified in GOED Voluntary Monograph B. Environmental contaminants: as specified in GOED Voluntary Monograph C. Fatty acids (EPA and DHA): as specified in GOED Voluntary Monograph
<p>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):</p> <p>Acid value. Maximum 2.0 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur. 2.5.1</p> <p>Absorbance at 233 nm. Maximum Abs₂₃₃ 0.60; Ph. Eur. Assay 2.2.25</p> <p>Note: This test is of relevance in the context of a Eur. Pharmacopeia requirement only.</p> <p>Oligomers and Glycerides.</p> <p>Ph.Eur. Monograph "Omega-3 Ethyl Esters 60": Maximum 7.0 area% oligomers and glycerides. Ph. Eur. Assay 2.2.30</p> <p>Ph.Eur. Monograph "Omega-3 Ethyl Esters 90": Maximum 1.0 area % oligomers. Ph. Eur. Assay 2.2.30</p> <p>Note: The maximum limits for oligomers and glycerides in ethyl esters oils according to the European Pharmacopeia are indicated here.</p> <p>A number of additional assays are provided in the Guidance on Methods section that can be useful to determine the individual components that constitute oligomers, individual glycerides, and other potentially co-eluting substances.</p> <p>Unsaponifiable Matter. Not applicable.</p> <p>Note: Ethyl ester concentrates are low/devoid of unsaponifiable matter. Ph. Eur. Assay 2.5.7 / AOCS Official Method Ca6b-53 / Ph. Eur. Assay 2.2.30</p>
Species identification: Not relevant

TECHNICAL GUIDANCE DOCUMENT FOR:

<p>Type of EPA/DHA product class: EPA and/or DHA Omega-3 Triglyceride Concentrates</p>
<p>Brief description of product class:</p> <p>EPA and/or DHA Omega-3 Triglyceride Concentrates are mixtures of mono-, di-, and tri-esters of EPA and/or DHA with glycerol containing at least 50% triesters and obtained either by esterification of concentrated and purified EPA and/or DHA, by transesterification of EPA and/or DHA ethyl esters with glycerol, or by enzymatic concentration of omega-3 glycerides. The origin of EPA and DHA is from fish (including fish of families such as <i>Engraulidae</i>, <i>Carangidae</i>, <i>Clupeidae</i>, <i>Osmeridae</i>, <i>Salmonidae</i>, <i>Scombridae</i>, and <i>Gadidae</i>), cephalopod (including calamari), plant or microbial sources.</p>
<p>Reason for classification as a separate product class:</p>
<p><i>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</i></p> <ul style="list-style-type: none"> A. Oxidation: <i>as specified in GOED Voluntary Monograph</i> B. Environmental contaminants: <i>as specified in GOED Voluntary Monograph</i> C. Fatty acids (EPA and DHA): <i>as specified in GOED Voluntary Monograph</i>
<p><i>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):</i></p> <p>Acid value. Maximum 3.0 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur. 2.5.1</p> <p>Absorbance at 233 nm. Maximum Abs₂₃₃ 0.73 ; Ph. Eur. Assay 2.2.25</p> <p><i>Note: this test is of relevance in the context of a Eur. Pharmacopeia requirement only.</i></p> <p>Oligomers and Partial Glycerides.</p> <p>Ph.Eur. monograph 1352 "Omega-3 Acid Triglycerides": Maximum 3.0% oligomers. Ph. Eur. Assay 2.2.30.</p>
<p>Species identification : Not relevant</p>
<p>Specific comments : Specific comments :</p> <p>Triglyceride concentrates are devoid of unsaponifiable matter or have very low levels (e.g. concentrates with high DHA may contain some cholesterol and unsaponifiable matter). Members who do want to test for unsaponifiable matter can use the following methods: Ph. Eur. Assay 2.5.7 / AOCS Official Method Ca6b-53 / Ph. Eur. Assay 2.2.30</p>

Species of origin EPA- and DHA-containing oils

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: **Cod Liver Oil**

Brief description of product class:

Cod Liver Oil is purified fatty oil obtained from the livers of cod, *Gadus morhua* and other *Gadidae* species, solid substances removed by cooling and filtering. Cod Liver Oil may be deodorized and molecularly distilled, and vitamin A and D3 may be added. Cod Liver Oil may be obtained from both wild cod and farmed cod.

Composition of fatty acids:

Trivial name of fatty acid	Nomenclature	Lower limit area (per cent)	Upper limit area (per cent)
<i>Saturated fatty acids:</i>			
Myristic acid	14:0	2.0	6.0
Palmitic acid	16:0	7.0	14.0
Stearic acid	18:0	1.0	4.0
<i>Mono-unsaturated fatty acids:</i>			
Palmitoleic acid	16:1 n-7	4.5	11.5
<i>cis</i> -Vaccenic acid	18:1 n-7	2.0	7.0
Oleic acid	18:1 n-9	12.0	21.0
Gadoleic acid	20:1 n-11	1.0	5.5
Gondoic acid	20:1 n-9	5.0	17.0
Erucic acid	22:1 n-9	0	1.5
Cetoleic acid	22:1 n-11+13	5.0	12.0
<i>Poly-unsaturated fatty acids:</i>			
Linoleic acid	18:2 n-6	0.5	3.0
Alpha-linolenic acid	18:3 n-3	0	2.0
Moroctic acid	18:4 n-3	0.5	4.5
EPA	20:5 n-3	7.0	16.0
DHA	22:6 n-3	6.0	18.0

Reason for classification as a separate product class: Species of Origin EPA/DHA-containing Oil

GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:

- A. Oxidation: as specified in GOED Voluntary Monograph
- B. Environmental contaminants: as specified in GOED Voluntary Monograph
- C. Fatty acids (EPA and DHA): as specified in GOED Voluntary Monograph

The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):

Acid value. Maximum 2.0 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur. 2.5.1.

Note: In Ph.Eur. 10th Edition, for monographs 1192 (Cod liver oil, type A) and 1193 (Cod liver oil, type B), the quantity of substance to be examined has been increased to 20.00 g to improve the accuracy of the method.

Absorbance at 233 nm. Not applicable

Oligomers and Partial Glycerides. Not applicable

Unsaponifiable Matter. Ph.Eur. Monographs on Cod-Liver Oil (A, B, Farmed): Maximum 1.5 %. Methods: Ph. Eur. Assay 2.5.7 / AOCS Official Method Ca6b-53.

Note: In Ph.Eur. 10th Edition, for monographs 1192 (Cod liver oil, type A) and 1193 (Cod liver oil, type B), the quantity of substance to be examined has been increased from 2.0 to 5.0 g to improve the accuracy of the method.

Solid Substances

Cold Test can be used to confirm the removal of solid substances by cooling and filtering.

Ph.Eur. monographs on “Cod-Liver Oil”, Method “Stearin” / AOCS Official Method: Cc 11-53. “Cold Test”.

Fatty acid profile

In order to measure the fatty acid composition by area percentages that is descriptive of cod liver oil the following methods can be used: Ph.Eur. monograph “Cod Liver Oil” Method “Composition of Fatty Acids” / USP monograph “Cod Liver Oil” method “Fatty Acid Profile”.

Iodine Value

Iodine Value can be used to assist in the identification of cod liver oils, as described in the Ph.Eur. Monographs for Cod-liver oil Type A, B and farmed. As the oil has a high iodine value, the quantity of substance to be examined has been decreased to 0.14 – 0.16 g, in order to have an excess of 50-60 per cent, as described in general chapter 2.5.4.

Stearin

Note: In Ph.Eur. 10th Edition, the instruction to cool the sample to room temperature (15-25°C.) before placing it in the iced water has been introduced to improve repeatability.

Species identification : For cod-liver oil of farmed cod, Ph.Eur. 6.3 01/2009:2398 provides a method for identification.

Specific comments :

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: Tuna Oil
<p><i>Brief description of product class :</i></p> <p>Purified, (non-)winterized and deodorized fatty oil from fish of genera <i>Katsuwonus</i> and <i>Thunnus</i> and within the family <i>Scombridae</i>. Skipjack tuna (<i>Katsuwonus pelamis</i>) and Yellowfin tuna (<i>Thunnus albacares</i>) are used as the main raw material. The content of EPA ranges from 4 to 12 percent, DHA is minimally 20%, and total omega-3 is minimally 28% (all expressed as triglycerides). A suitable antioxidant may be added.</p>
Reason for classification as a separate product class : Species of Origin EPA/DHA-containing Oil
<p><i>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</i></p> <ul style="list-style-type: none"> A. Oxidation: <i>as specified in GOED Voluntary Monograph</i> B. Environmental contaminants: <i>as specified in GOED Voluntary Monograph</i> C. Fatty acids (EPA and DHA): <i>as specified in GOED Voluntary Monograph</i>
<p><i>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):</i></p> <p>Acid value. Maximum 0.5 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur. 2.5.1. (In the context of CODEX, for food applications a maximum of 3.0 mg KOH/g would be permitted for some geographies)</p> <p>Absorbance at 233 nm. Maximum Abs₂₃₃ 0.5; Ph. Eur. Assay 2.2.25</p> <p><i>Note: this test is of relevance in the context of a Eur. Pharmacopeia requirement only.</i></p> <p>Oligomers and Partial Glycerides. Ph.Eur. Monograph "Fish Oil, Rich in Omega-3 Acids": Maximum 1.5% oligomers. Ph. Eur. Assay 2.2.30.</p> <p>Unsaponifiable Matter. Maximum 1.5%; Ph. Eur. Assay 2.5.7 / AOCS Official Method Ca6b-53 (For some specific geographies a maximum of 2% would be permitted)</p>
<i>Species identification :</i>
<i>Specific comments :</i>

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: Green-lipped Mussel Oil
<p><i>Brief description of product class:</i></p> <p>Green-lipped Mussel Oil provides a rich source of essential fatty acids. The content of EPA and DHA in Green-lipped Mussel Oil is in the range of 20 - 24 g/100g and 10 - 15 g/100g, respectively. Green-lipped Mussel Oil is produced by supercritical fluid extraction. EPA and DHA are present in Green-lipped Mussel Oil in the form of triglyceride (40-80%), phospholipid (20-60%) and free fatty acid (5-10%). Green-lipped Mussel oil has an orange amber color. During SFE extraction GLM oil is a free flowing oil, but when cooled sets.</p>
Reason for classification as a separate product class: Species of Origin EPA/DHA-containing Oil
<p><i>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</i></p> <ul style="list-style-type: none"> A. Oxidation: as described in the GOED Monograph B. Environmental contaminants: as described in the GOED Monograph C. Fatty acids (EPA and DHA): <i>as specified in GOED Voluntary Monograph</i> AOAC 18th Edn 963.22 if validated
<p><i>Species identification:</i> The only true way to confirm that Green-lipped mussel oil has originated from NZ is through health certificates issued by the NZ food authorities.</p>
<p><i>Specific comments:</i> The exact composition of the oil is dependent on the season, location and reproductive conditions at the time of harvest.</p> <p><i>Note: Specific indications on methods and descriptions for GLMO have been provided by former GOED member Aroma NZ, and text of this Guidance Document here was agreed upon on Sept 22, 2014.</i></p>

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: Salmon Oil
<p>Brief description of product class:</p> <p>Salmon Oil is oil exclusively derived from one or more of the following species: Atlantic salmon (<i>Salmo salar</i>), Chinook salmon (<i>Oncorhynchus tshawytscha</i>), Chum Salmon (<i>Oncorhynchus keta</i>), Coho salmon (<i>Oncorhynchus kisutch</i>), Pink salmon (<i>Oncorhynchus gorbuscha</i>), Sockeye salmon (<i>Oncorhynchus nerka</i>), Masu salmon (<i>Oncorhynchus masou masou</i>). As EPA and DHA level are very variable depending on source and feeding, it is not possible to define what is a salmon oil based on its fatty acid composition. Traceability measurements such as identity preservation (IP) systems and/or analytical instruments such as nuclear magnetic resonance (NMR) testing should therefore be in place to ensure the oil's authenticity. Products labeled with "farmed salmon oil" shall be originating exclusively from farmed fish. Those labeled with "wild (caught/harvest) salmon oil" shall exclusively originate from fish captured in the wild by commercial fisheries.</p>
Reason for classification as a separate product class: Species of Origin EPA/DHA-containing Oil
<p><i>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</i></p> <ul style="list-style-type: none"> A. Oxidation: <i>as specified in GOED Voluntary Monograph</i> B. Environmental contaminants: <i>as specified in GOED Voluntary Monograph</i> C. Fatty acids: <i>as specified in GOED Voluntary Monograph</i>
<p><i>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):</i></p> <p style="padding-left: 40px;">Acid value. Maximum 2.0 mg KOH/g; AOCS Official Method Cd 3d-63 / AOCS Official Method Ca 5a-40 / Ph.Eur 2.5.1</p>
<p><i>Species identification</i> : It is desirable to further develop methodologies to detect and avoid fraudulent mixing of oils.</p>
<p><i>Specific comments</i> :</p>

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: **Anchovy Oils**

Brief description of product class:

Anchovy Oils are purified and/or deodorized oils obtained from fish of the *Engraulidae* family, mainly from *Engraulis ringens* species. Refined Anchovy Oil may be winterized and molecular distilled. A suitable antioxidant may be added.

EPA + DHA content is 27 – 34% (Area). Total Omega 3 ranges from 33 - 40%.

Trivial name of fatty acid	Nomenclature	Lower limit area (per cent)	Upper limit area (per cent)
<i>Saturated fatty acids:</i>			
Myristic	C14:0	5.0	11.5
Pentadecanoic	C15:0	ND	1.5
Palmitic	C16:0	13.0	22.0
Heptadecanoic	C17:0	ND	2.0
Stearic	C18:0	1.0	7.0
Arachidic	C20:0	NA	NA
<i>Mono-unsaturated fatty acids:</i>			
Palmitoleic	C16:1n7	5.0	12.0
Oleic	C18:1n9	5.0	17.0
Vaccenic	C18:1n7	NA	v
Eicosenoic	C20:1n9	ND	4.0
Eicosenoic	C20:1n11	ND	4.0
Cetoleic	C22:1n11	ND	5.0
Erucic	C22:1n9	ND	5.0
<i>Poly-unsaturated fatty acids:</i>			
Linoleic	C18:2n6	ND	3.5
Linolenic	C18:3n3	ND	7.0
Alpha Linolenic	C18:3n6	ND	5.0
Moroctic or Stearidonic (SDA)	C18:4n3	ND	5.0
Arachidonic	C20:4n6	ND	2.0
Eicosatetraenoic (ETA)	C20:4n3	ND	2.0
Eicosapentaenoic (EPA)*	C20:5n3	5.0	26.0
Heneicosapentaenoic (HPA)	C21:5n3	ND	4.0
Docosapentaenoic (DPA) or Clupanodonic	C22:5n3	ND	4.0
Docosahexaenoic (DHA)*	C22:6n3	4.0	24.0

ND: Not detected, defined as $\leq 0.05\%$ | NA: Not applicable | *EPA + DHA range is between 27 – 34%

Reason for classification as a separate product class: Species of Origin EPA/DHA-containing Oil. Anchovy (Peruvian Anchoveta) is the main sea wild species for the production of fish oil

GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:

- A. Oxidation: as specified in GOED Voluntary Monograph*
- B. Environmental contaminants: as specified in GOED Voluntary Monograph*
- C. Fatty acids (EPA and DHA): as specified in GOED Voluntary Monograph*

Specific comments: The Anchovy oil has not undergone any concentration process.

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: Skate Liver Oil
<p>Brief description of product class:</p> <p>Skate liver oil is the refined and ultra-deodorized triglyceride oil obtained from the liver of skate (species <i>Dipturus chilensis</i> and <i>Atlanta raja castelnaui</i> from the Atlantic Ocean). It is a yellow to light brownish colored oil (Gardner color, max. 8). Skate liver oil has a density of 0.920 – 0.930 g/l and a refractive index of 1.481 at 20°C. Suitable antioxidants may be added (mixed tocopherols, alpha-tocopherol, ascorbyl palmitate, citric acid, etc).</p>
Reason for classification as a separate product class: Species of Origin EPA/DHA-containing Oil
<p>GOED members that want to produce and/or sell oils under the scope of the GOED Voluntary Monograph are obliged to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed in the Guidance on Methods:</p> <p>A. Oxidation: as specified in GOED Voluntary Monograph</p> <p>B. Environmental contaminants: as specified in GOED Voluntary Monograph</p> <p>C. Fatty acids: as specified in GOED Voluntary Monograph</p>
<p>The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Technical Guidance Documents):</p> <ul style="list-style-type: none"> • Solid substances: Cold Test can be used to confirm the removal of solid substances by cooling and filtering. Ph.Eur. monographs on "Cod-Liver Oil", Method "Stearin" / AOCs Official Method: Cc 11-53, "Cold Test". • Acid Value: Max. 1.0 mg KOH/g oil. Ph. Eur. Method 2.5.2 • Pesticide residues. Ph. Eur. 2.8.13. • Density: 0.920 – 0.930 g/l • Refractive Index ~1.481 at 20 °C. • Color Gardner - Max. 8
Species identification : <i>Dipturus chilensis</i> and <i>Atlanta raja castelnaui</i> from the Atlantic Ocean.
Specific comments : Added antioxidants can be chosen by the customer.

Other EPA- and/or DHA-containing oils

TECHNICAL GUIDANCE DOCUMENT FOR:

Type of EPA/DHA product class: **Flavored Oils**

Brief description of product class:

Flavored oils are any of the other EPA- and/or DHA-containing oils falling under the scope of the GOED Voluntary Monograph, to which a flavor has been added.

Reason for classification as a separate product class:

Flavored oils are a class of EPA- and/or DHA-containing oils with growing importance for the GOED membership. However, flavored oils cannot always be reliably tested for para-anisidine reactivity by secondary oxidation products using the AOCS Official Method Cd 18-90, since some flavor substances, which are often aldehydes, interfere with the formation of the chromophore measured in the colorimetric assay. Skewing of pAV values is both flavor type- and concentration-dependent, and therefore this Guidance Document is of use in the case that flavoring interferes with pAV value measurements.

GOED members that want to produce and/or sell flavored oils under the scope of the GOED Voluntary Monograph are suggested to measure the following parameters using assays specified in the GOED Voluntary Monograph and further detailed here:

A. Oxidation:

PV: as specified in GOED Voluntary Monograph

pAV: Some flavours added to oils cause interference with the measurement of pAV. The precise measurement of the para-Anisidine Value (pAV) in flavored oilsoils where flavor interference occurs requires the development of validated methods that specifically measure PUFA-derived secondary oxidation products dissolved in the oil (aldehydes). Until the implementation of suitable methods, the following methodological approach will be adopted by GOED:

When oils are flavored with flavors that interfere with the measurement of the pAV value:

1. Measure the baseline pAV of the oil without flavor added, using the method specified in the GOED Voluntary Monograph
2. Determine the difference between the measured pAV value and the maximum pAV value permitted under the GOED Voluntary Monograph (*i.e.* 20). This value of pAV is the allowable oxidation over time (Δ pAV).
3. Admix the flavor into the oil, and measure the batch-specific pAV value of the flavored oil (pAV* value).
4. Calculate the maximum pAV* value that the flavored oil batch may achieve to remain compliant with the GOED Voluntary Monograph, by summing (Δ pAV + pAV* = maximum pAV*). The maximum pAV* value constitutes a useful reference value for shelf-life testing of flavoured oils.

pAV: Some flavours added to oils cause interference with the measurement of pAV. The precise measurement of the para-Anisidine Value (pAV) in flavored oils where flavor interference occurs requires the development of validated methods that specifically measure PUFA-derived secondary oxidation products dissolved in the oil (aldehydes). Until the implementation of suitable methods, the following methodological approach will be adopted by GOED:

TOTOX value: As specified in the GOED Voluntary Monograph

Until replacement by suitable methods for pAV Value determination of flavored oils, the TOTOX value of a flavored oil is calculated as follows:

Maximum 26. Result of the following calculation;

1. *TOTOX at initial time point (or time of manufacture) = 2PV + pAV, where pAV is the anisidine value of the unflavoured oil*
 2. *TOTOX during shelf-life testing = 2PV + (pAV_t - (pAV* - pAV)), where pAV_t is the pAV at a given time, and (pAV* - pAV) are the initial measured values for flavoured and unflavoured oils.*
- ▶ For flavored oils in which the added flavor does not interfere with pAV measurement, pAV can be measured according to the Technical Guidance for the respective oil.
 - ▶ Note that the contribution of added flavors can be variable over time as the flavoring compounds themselves can also be sensitive to oxidation (see Ye et al, JAOCS, 97, 12,1135-1341, 2020: [link](#)). Appropriate additional controls may need to be taken to take this behavior into account.
 - ▶ For flavoured oils in which the added flavor does not cause interference during formulation, but leads to the formation of interfering derivatives during the shelf-life of the flavoured oil, no suitable ways exist to measure secondary oxidation using para-anisidine reactivity.

B. Environmental contaminants: as specified in GOED Voluntary Monograph

C. Fatty acids (EPA and DHA): as specified in GOED Voluntary Monograph. The most suitable method for fatty acid analysis is dictated by the oil type that was flavored, and the applicable Guidance Document for that oil class should be consulted.

The following suggestions for assay methods to be used and limits to take into account for a number of additional parameters, can be of guidance to GOED members on a voluntary basis to produce and sell quality oils (further detail can be found in the Guidance on methods):

Acid value. As specified in the Guidance Document for the corresponding unflavored oil type.

Absorbance at 233 nm. As specified in the Guidance Document for the corresponding unflavored

Oligomers and Partial Glycerides. As specified in the Guidance Document for the corresponding unflavored oil type.

Unsaponifiable Matter. As specified in the Guidance Document for the corresponding unflavored oil type.

Species identification: As specified in the Guidance Document for the corresponding unflavored oil type.

Specific comments: