GOED INDUSTRY ADVISORY:
CORRECT EXPRESSION OF EPA, DHA AND TOTAL OMEGA-3 FATTY ACID CONTENT IN OILS

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GOED

Correct expression of EPA, DHA, and Total Omega-3 Fatty Acid Content in Oils

This industry advisory explains how the content of EPA, DHA and total omega-3 fatty acids in EPA/DHA-containing oils should be correctly expressed in accordance with the GOED Voluntary Monograph. Correct expression of content applies to ingredient oils, as well as finished products. Among other issues, incorrect content expression can lead to non-compliance with label declarations.

The content of EPA, DHA and Total Omega-3 fatty acids should always be expressed by weight (mg/g), and never by area percent (area %). The expression by weight of fatty acids should be as free fatty acid equivalents in milligram per gram (mg/g) of the oil. Furthermore, the weight of each fatty acid should be expressed as mg/g of the specific chemical form of the oil (i.e. Triglyceride (TG) form oils should always be expressed as mg/g of TG, and Ethyl Ester (EE) form oils as mg/g of EE, etc.). The content of EPA and DHA and other omega-3 long-chain polyunsaturated fatty acids (omega-3 LCPUFA) expressed as free fatty acid can be converted to the weight of the fatty acid in the specific chemical form of the oil, using conversion factors provided in the GOED Guidance Documents.

The importance and advantages of expressing the content of EPA/DHA of an oil by weight (mg/g)

- Expression by weight is of critical importance to make label declarations or claims for the content of EPA and/or DHA in encapsulated products, as mg per capsule, or liquid products, as mg per ml serving size. For completeness, it should be noted that manufacturers of consumer-facing products should ideally express EPA and DHA content based on the mass of the serving, rather than the volume, as it cannot be assumed that 1 ml of oil weighs 1 g.

- The amount of EPA and DHA in weight as free fatty acids defines the exact amount of the fatty acids present in a product. Expression as free fatty acids facilitates the comparison of EPA and DHA content across omega-3 oils and between products of differing chemical forms. However, for nutrition statements, the amount is expressed as TG form or EE form respectively. This is necessary to fairly align content expression with the chemical form of other lipids present in blends or real food products. Information on the chemical form should also be present on the label of any food or dietary supplements in order to give an accurate description of the expression of content.

- Expression by weight, but not area %, takes into consideration other constituents in the oil.

- Expression by weight is much more consistent and comparable results are obtained as it is not as sensitive to sample concentration and number of peaks.

The mistake in using area percent (area %) for expressing the content of EPA/DHA of an oil

Fatty acids are most often measured by a combination of gas chromatography, to separate the various fatty acids, and a detection technique, such as flame ionization detection or mass spectrometry. The detector measures each fatty acid as a specific signal peak, where the size of the peak is corresponding to its concentration. By summing the total area of the peaks and determining the area corresponding to a peak of any specific fatty acid, one can calculate an area percent (percent of the total area) of the fatty acid in question. This percentage does not equal the content of that individual fatty acid, nor does it equal a weight percentage. There are additional factors to take into account to determine the content of any specific fatty acid in an oil. These factors are:

1. Depending on the oil type and its processing, oils contain, apart from triglycerides, di- and monoglycerides or ethyl esters that contain esterified fatty acids, other lipids and substances. These can be of many types, including neutral lipids that are not sensitive to hydrolysis (so-called non-saponifiable lipids, such as cholesterol and ether-lipids), cross-linked lipids in the case of a deteriorated oil, as well as substances like glycerol and moisture (water). If one does not correct for the weight of these other substances, the use of an area percent to determine the content of a specific fatty acid will lead to an error in the expression of fatty acid content of the oil. This error is greater if a higher proportion of lipids and other substances are present that do not contribute to the fatty acid profile measured by fatty acid analysis. An area percent value is therefore not the absolute percentage of the fatty acid in the ingredient as a whole.

   This is also of concern in finished products that contain added flavors, antioxidants and other materials (e.g. fat-soluble vitamins) that may not be saponifiable.

   A simple example to illustrate why area % is really not scientifically correct is the following: If you add, for example, water to an omega-3 product, the area % will not change, whereas the expression of content by weight would of course test for much lower levels when the product is diluted in this way.

2. Not all fatty acids generate the same exact peak response in the detector used for analysis. It is incorrect to assume that a certain area percent of one fatty acid corresponds to the same amount of another fatty acid with the same area percent.

Furthermore, the fatty acid-specific peak response factors depend on how the fatty acids are derivatized for analysis, the method of separation and the detector used. Because distinct peaks contribute differentially to the calculated total area % value, the area % determined for any specific fatty acid cannot be assumed to accurately reflect its concentration in the oil.

3. Not all peaks detected in the chromatogram will be fatty acids. Another reason why using area percent is fraught with problems for the quantitation of a specific fatty acid is because often not all peaks in a chromatogram acquired by gas chromatography are fatty acids; some peaks can be naturally-occurring branched chain fatty acids and furan fatty acids, as well as plasticizers and added antioxidants. Summing these peaks with the fatty acid peaks distorts the total peak area belonging to fatty acids. The presence of peaks that are not fatty acids is very different for various oil types.

4. When EPA and DHA content are expressed specifically as triglyceride or ethyl ester, additional errors can occur when a portion of the oil is in the form of partial glycerides. Mono- and diglycerides do contribute with detectable fatty acids but do not correspond exactly to the chemical form of content expression. Hence, it should be known what portion of the oil does not correspond to the chemical form of interest that is labeled so that content can be accurately expressed.

In summary, results generated using area percent methodology should never be used to make correct statements about the content of any particular fatty acid. Using area percent can lead to incorrect overestimations, up to 10% or more, of the content of EPA and DHA in omega-3 oils.

The area percent as such is only used to assess the relative proportions of select fatty acids according to specific methods developed for determining an oil's fatty acid composition (but not the accurate content of specific fatty acids).

Consequences of incorrect content expression

Using area percent will artificially inflate the perceived amount of oil present in a product, because it does not represent the actual concentration. As a consequence, it may then give the impression that less oil is needed to formulate a specific product. Using less oil in formulation will subsequently provide an incorrect and smaller dose than intended for consumers.

Put simply, incorrect content expression can lead to non-compliance with label declarations. If a nutrient must be present at 100% of the declared value, compliance cannot consistently be achieved using area %, and deviations will vary from one oil type to another.

Suitable methods

There are only a limited number of methods that are suitable for the quantitative determination of omega-3 LCPUFA in fish oils, concentrates and other oils rich in LCPUFA. The use of an adequate method allows for appropriate peak separation, the minimization of errors that can be made in assigning fatty acid identity to the correct peaks, and the use of internal standards for accurate quantitation. Suitable quantitative methods are provided in the GOED Technical Guidance Documents.

Regulations on content expression

- European Union:

  In the EU, the provision of food information to consumers (FIC) (Reg. 1169/2011) Annex XV, provides indications that mass, with units of measurement in grams (g), milligrams (mg) or micrograms (µg)), should be used for the expression of LCPUFA content.

  In EU food legislation the nutrition values are usually reported as triglycerides, not as free fatty acids. In practice, finished product manufacturers have successfully achieved novel food applications using expression of fatty acid by weight.

- U.S.A.:

  In the US, the FDA "Dietary Supplement Labeling Guide: Chapter IV. Nutrition Labeling" provides indications to the correct expression of content:

  The constituents of a dietary supplement (such as EPA and DHA in a fish oil) may be listed on a product label in the supplement facts panel along with their quantitative amounts by weight per serving. If EPA/DHA are the dietary ingredients, they should be listed by weight. Depending upon the classification of the nutrient as either Class I or II, dietary supplements found to contain less than the required amount of a nutrient will be deemed to be misbranded and in violation of the law.

  In terms of listing constituents as dietary ingredients, the Guidance states (Item 33) that one may list constituents of a dietary ingredient intended under the dietary ingredient and followed by their quantitative amounts by weight per serving. One may declare the constituents in a column or in a linear display (see: 21 CFR 101.36(b)(3)(iii)).

- Australia:

  The compositional guidelines for Natural Fish Oil by the Therapeutic Goods Administration (TGA, Australian Government, Department of Health) provide indications on content measurement and expression here, see Table 1 - Ingredient Specific Requirements). For the measurement of the sum of EPA and DHA, and of Total Omega-3 acids, the use of the European Pharmacopeia method 2.4.29 or the British Pharmacopeia method are stipulated. Content should be expressed as triglycerides, and relative peak area calculations should not be used.

  Therapeutic Goods Order number 92 on Standard for labels of non-prescription medicines provides legislative information for labelling of complementary medicines, and includes the following:

  "...the information on the main label of the medicine must include:

  .... (b) the name(s) of all active ingredients in the medicine; and

  (c) the quantity or proportion of all active ingredients in the medicine"

  "Section 11 - How information is to be expressed

  (1) Use of appropriate metric units

  (a) For active ingredient(s), where a particular is a statement of mass for which there is a metric unit of measurement, the metric units must be expressed as follows:

  ... (iii) a statement of quantity for more than 1 milligram up to 999 milligrams inclusive must be expressed in terms of milligrams; "

  (2) Expression of quantity or proportion of active ingredients

  Subject to paragraph 11(2)(i) and subsection 11(3) below, the quantity or proportion of an active ingredient to be included on a label must be expressed:

  (a) for a discrete dosage unit - as the quantity of the active ingredient in the dosage unit;

  (b) for a solid for ingestion, where there is no discrete dosage unit - as the quantity of the active ingredient contained in the stated weight of a suitable dose of the solid;

  (c) ...., if the medicine is a liquid for ingestion:

  (i) as the quantity of the active ingredient contained in the stated volume of a suitable dose of the liquid;

  .... (j) for any other medicines:

  (i) where the medicine is a liquid and includes an active ingredient which is a liquid - as the appropriate amount of the active ingredient, either by weight or volume, in a stated volume of the medicine "

  In no instance is the use of area % stated to be used for expression of content.

Conclusions

Content of EPA, DHA and Total Omega-3s of ingredients and products should be expressed by weight, as mg per g of oil. Information on the chemical form (e.g. as TG or EE) should also be present on the label of any food or dietary supplements in order to give an accurate description of the expression of content to consumers. The use of area percent as a means of content expression is incorrect and introduces errors in manufacturing, marketing, label claims and dosing.

The GOED Technical Guidance Documents provide additional information on suitable methods using internal standards for quantitative determination of EPA, DHA and total omega-3s in EPA/DHA omega-3-rich oils.

Examples

The real examples on this and the following page are provided to show the difference between expression of content of EPA and DHA by weight (appropriate) and area % (inappropriate).

Example 1

	EPA area %	mg of EPA calculated from area %	EPA analyzed using GOED method (mg/g TG)	DHA area %	mg of DHA calculated from area %	DHA analyzed using GOED method (mg/g TG)
Sample 1	23.24	232	216	37.19	372	330
Sample 2	20.85	209	193	38.14	381	350
Sample 3	20.74	207	196	38.68	387	346
Sample 4	20.92	209	196	38.3	383	341
Sample 5	26.05	261	246	36.54	365	328

Example 2

Let's consider a typical 3322EE fish oil type. In the specs you will find the following data to express EPA and DHA content:

Analytical specifications Parameter	Limit	Unit	Method
EPA	Min 33		
DHA	Min 22	A%	GC
Total Omega-3 Fatty acids	Min 65		
EPA (EE)	Min 300	mg/g	
DHA (EE)	Min 200	mg/g	GC
Total Omega-3 Fatty acids (EE)	Min 570	mg/g	
EPA (FFA)	Min 270	mg/g	
DHA (FFA)	Min 180	mg/g	GC
Total Omega-3 Fatty acids (FFA)	Min 510	mg/g	

Let us imagine a finished product manufacturer wants to claim 250 mg of EPA+DHA per single capsule (for example, for an EFSA heart health claim).

Considering the minimum value:

a. Starting from A% data you will need to use ≈ 454,5 mg of this fish oil[1]

b. Starting from mg/g of EE you will need to use ≈ 500 mg of the same oil[2]

c. Starting from mg/g of FFA you will need to use ≈ 555,5 mg of the same oil[3]

Clearly the highest difference is between case a) and c). That means using around 100 mg more of oil, with a clear impact on cost (and also on shape, especially in combo products with reduced space in the capsule due to the presence of other ingredients).

Between b) and c) the issue is less relevant, as long as on the label it is mentioned that the calculation has been made on EE basis.

Using an equal mode of expressing content on the label makes competition fair.

Note on calculations:

1. If (inappropriately) using area % to make quantitative statements, then (22% EPA + 33% DHA =) 55% of the area percent of the oil corresponds to EPA+DHA. For 250 mg EPA+DHA, one would need 250/0.55 = 454 mg oil (or 0.454 ml if one would not take the oil density into account).

2. Based on EE, this oil contains 300 + 200 = 500 mg EPA-EE + DHA-EE per g oil, which equals 0.5 g oil for 250 mg EPA+DHA (as ethyl ester).

3. When the content of EPA and DHA is expressed as free fatty acid equivalents, then 1 gram of this oil contains 270 + 180 = 450 mg EPA + DHA, and hence one would need to use 250/0.45 = 555 mg oil.