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# Comprehensive study of the composition of fish fat extracts and quantitative criteria for distinguishing standardized omega-3 polyunsaturated fatty acids extracts

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| *Background* | Effects of drugs and biologically active supplements based on omega-3 polyunsaturated fatty acids (ω3 PUFA) considerably depend on the standardized content of eicosatetraenoic acid (EPA), docosahexaenoic acid (DHA), and other fatty acids in the extracts. |
| *Material and methods* | In this study, we comprehensively examined the composition of 10 ω3 PUFA samples with chromatographic measurement of more than 40 metabolites of fatty acids and other compounds. The data on extract composition were analyzed with current methods of intelligent data analysis (metric condensation method; multidimensional scaling; principal component analysis with axis identification; topology-metrical approach to recognition). |
| *Results* | Quantitative markers were obtained, which allowed separating the standardized ω3 PUFA-based samples (Omacor, Solgar omega-3 700, Femibion Natalker-2, Omega-3 concentrate, Omegamama) from less standardized ones (Fish oil-Teva, Omegatrin, Omeganol, etc.) based on results of a chromatographic analysis of fatty acid composition in the studied samples (EPA+DHA marker, ω6+ω11 marker, and standardization coefficient showing conformity of measured ω3 PUFA levels with the content stated by the manufacturer). |
| *Conclusions* | Among the studied samples, the pharmaceutical product Omacor showed the best values of standardization indexes. |

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| *Keywords* | Omega-3 PUFA standardization; nutritional support; metric analysis of data |
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Fundamentals of the pharmacological action of omega-3 polyunsaturated fatty acids (ω3 PUFAs) have been well studied and described in numerous original studies and review articles [1]. In brief, such ω3 PUFAs as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) form the essential mediators of inflammation – eicosanoids and docosanoids, including neuroprotectins, resolvins, and maresins. Eicosanoids and docosanoids, synthesized from the EPA and DHA in the arachidonic acid cascade, are critical to the physiological resolution of inflammation. This is why ω3 PUFAs prepared from fish oil extracts are effective for the treatment and prevention of various pathologies, including the prevention and treatment of coronary artery disease, stroke, and nutritional support [2]. Taking into account an extremely low level of consumption of ω3 PUFAs by the Russian population, ω3 PUFA medications and dietary supplements are required to compensate for nutritional deficiency. The efficacy of different ω3 PUFA products depends significantly on the standardization of the extract content by EPA, DHA, and other FAs. Unfortunately, there is still a terminological confusion among physicians who refer to all ω3 PUFAs using the term used 300–500 years ago – fish oil. This is a very conditional term that is not suited to today's pharmacology of ω3 PUFAs.

Firstly, medications and dietary supplements containing ω3 PUFAs can be manufactured without using fish oil extracts, rather using marine mammal oil extracts, algae extracts, synthetic ω3 PUFAs, etc. [1].

Secondly, the very use of the term «oil» is not completely biochemically sound, since fats are glycerin esters of fatty acids. In real-life, the prepa-

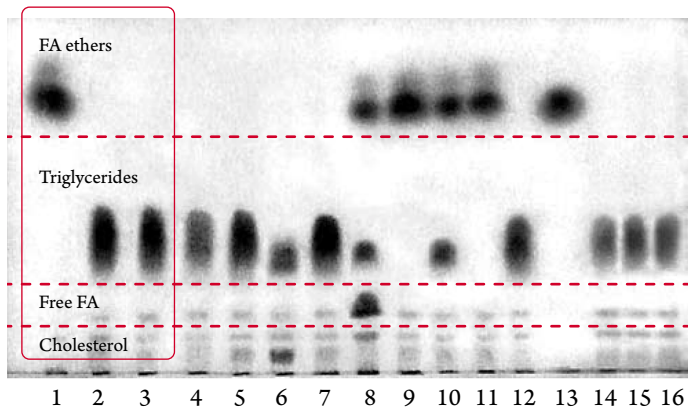
ration of ω3 PUFAs medications and dietary supplements from fish oil extracts, partial saponification of the original extract fats is often carried out with the removal of glycerin and subsequent etherification of the resulting mixture with methyl, ethyl and other short-chain monoatomic alcohols. The resulting mixture cannot be called «oil» in the scientific sense of this word.

Thirdly and most importantly, ω3 PUFAs medications and dietary supplements made from any natural substance can be substantially standardized in terms of the composition. For example, FA esters in Omacor® (Abbott Laboratories LLC) contain at least 90% of ω3 PUFAs (more than 97% in this study). Such a highly standardized extract containing the declared amounts of EPA and DHA is obviously a high purity pharmaceutical product. Thus, the use of the term «fish oil» is not correct.

All this leads to the need to study the chemical composition of various ω3 PUFAs extracted from fish oil [3, 4] and others, in order to establish the real values of the quantitative and qualitative composition of ω3 PUFA medications and dietary supplements used in real-life medical practice. For example, a qualitative study was carried out of the composition of ω3 PUFA products marketed in Russia [5]. Using thin-layer chromatography most of the products studied were classed in two groups: FA esters are the main components of Samples 1, 9, 11 and 13 and triglycerides are the main components in Samples 2–4, 12, 14 (fish oil), 15 and 16 (Figure 1).

In this paper, we present the results of the quantitative chromatographic analysis of 10 ω3 PUFAs samples extracted from fish oil. The findings on the composition of extracts were analyzed using modern methods of data mining (topological and metric recognition, metric thickening, multidimensional scaling, and principal component analysis with axis decoding [6]).

**Figure 1.** Chromatographic analysis of the ω3 PUFA samples, adapted from [5]




*1 – Ateroblock; 2 – Doppelherz Aktiv omega-3; 3 – NutraSource omega 3; 4 – Omeganol; 5 – Omega 3 Fortex; 6 – Omega 3 Forte Plus; 7 – Tandemax; 8 – inessential substances (from bottom to top: cholesterol, free FA, triglycerides, FA ethers); 9 – Viking Omega 3 Forte; 10 – OmegaTrin; 11 – VitrumCardio omega-3; 12 – Oceanol; 13 – Omacor; 14 – fish oil; 15 – Unik omega-3; 16 – Amber drop.*

## Material and Methods

The ω3 PUFAs samples examined in this paper are listed in Table 1. It should be noted that all samples examined were made of the liver oil of various types of fish.

### *Chromatographic determination of the fatty acid composition*

The samples were dissolved in hexane and subjected to hydrochloric hydrolysis in the presence of methanol (Methanol-HCl (3N) Supelco) in tightly sealed vials at 90°C for 1 hour. The resulting FA methyl esters were analyzed in a Shimadzu GCMS-QP2010 Ultra chromatograph under the following conditions: helium carrier gas, linear velocity 35.6 cm/sec (0.9 mL/min), flow division 4:1; capillary column MDN-5 (Supelco), length 30 meters, internal diameter 0.25 mm. Chromatograph parameters: temperature gradient mode, de-

**Table 1.** Summary of the ω3 PUFA samples examined

| Medication / Dietary supplement | Capsule, mg | Omega-3, mg | EPA, mg | DHA, mg |
|---|---|---|---|---|
| Omega-3 Doppelherz Aktiv | 1186 | 300 | 144 | 96 |
| Fish oil-Teva | 500 | 165 | na | na |
| Omegatrin | 780 | 397.8 | na | na |
| Omega-3 concentrate | 1000 | 600 | 330 | 220 |
| Omegamama | 700 | 150 | 23 | 105 |
| Femibion Natalcare II | 500 | 200 | 0 | 200 |
| Omeganol | 500 | 32 | na | na |
| Omacor | 1000 | 900 | 460 | 380 |
| Dear-Natura DHA | 1200 | 500 | 60 | 400 |
| Solgar Omega-3 700 | 1730 | 700 | 360 | 240 |

na, not available; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.


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**Table 2.** Compounds found in the samples examined using chromatographic analysis

| Compound | PUFA | MW |
|---|---|---|
| 11 eicosene acid, methyl ether | ω9 | 325 |
| 11 eicosene acid, propyl ether | ω9 | 353 |
| 13 docosene acid, methyl ether | ω9 | 353 |
| 13 docosene acid, propyl ether | ω9 | 381 |
| 13 methyltetradecanoic acid, methyl ether | sat. | 256 |
| 4, 7, 10, 13, 16, 19 docosahexaenoic acid, butyl ether | DHA | 384 |
| 4, 7, 10, 13, 16, 19 docosahexaenoic acid, methyl ether | DHA | 342 |
| 5, 11, 14, 17 eicosatetraenoic acid, methyl ether | ETA | 318 |
| 5, 8, 11, 14, 17 eicosapentaenoic acid, ethyl ether | EPA | 330 |
| 5, 8, 11, 14, 17 eicosapentaenoic acid, methyl ether | EPA | 316 |
| 5, 8, 11, 14, 17 eicosapentaenoic acid, propyl ether | EPA | 344 |
| 6, 9, 12, 15 hexadecatetraenoic acid, methyl ether | ω3 | 262 |
| 6, 9, 12, 15 octadecatetraenoic acid, butyl ether | ω3 | 332 |
| 6, 9, 12, 15 octadecatetraenoic acid, ethyl ether | ω3 | 304 |
| 6, 9, 12, 15 octadecatetraenoic acid, methyl ether | ω3 | 290 |
| 6, 9, 12 hexadecatrienoic acid, methyl ether | ω3 | 264 |
| 6 octadecenoic acid, ethyl ether | ω11 | 310 |
| 6 octadecenoic acid, methyl ether | ω11 | 296 |
| 7, 10, 13, 16, 19 docosahexaenoic acid, butyl ether | DHA | 384 |
| 7, 10, 13, 16, 19 docosapentaenoic acid, methyl ether | DPA | 342 |
| 7 methyl-6 hexadecenoic acid | ω9 | 282 |
| Linoleic acid, ethyl ether | ω6 | 308 |
| Linoleic acid, methyl ether | ω6 | 294 |
| Palmitoleic acid, ethyl ether | ω7 | 282 |
| Palmitoleic acid, methyl ether | ω7 | 268 |
| Oleic acid, ethyl ether | ω9 | 310 |
| Oleic acid, methyl ether | ω9 | 296 |
| Decanoic acid, methyl ether | sat. | 186 |
| Dodecanoic acid, methyl ether | sat. | 214 |
| Eicosanoic acid, methyl ether | sat. | 327 |
| Eicosanoic acid, propyl ether | sat. | 355 |
| Margaric acid, methyl ether | sat. | 284 |
| Heptadecanoic acid, ethyl ether | sat. | 298 |
| Palmitic acid, methyl ether | sat. | 270 |
| Methylnicotinate | – | 137 |
| Stearic acid, ethyl ether | sat. | 312 |
| Stearic acid, methyl ether | sat. | 298 |
| Caprylic acid, methyl ether | sat. | 158 |
| Pantolactone | – | 130 |
| Pentadecanoic acid, methyl ether | sat. | 256 |
| Myristic acid, ethyl ether | sat. | 257 |
| Myristic acid, methyl ether | sat. | 243 |
| Myristic acid, methyl ether | sat. | 243 |
| 12 methyltridecanoic acid, methyl ether | sat. | 242 |
| 12 methyltridecanoic acid, methyl ether | sat. | 242 |
| Tridecanoic acid, methyl ether | sat. | 228 |

MW, molecular weight, g/mol; sat., saturated fatty acid.

tector 200°C, interface 205°C, measurement mode 45–450 m/z. The qualitative composition of the resulting mixtures was determined using the NIST11 mass spectral library.

After the series of experiments, each sample was described by using a 44-position vector; each vector representing the peak area corresponding to a particular compound. The compounds identified in this study are listed in Table 2.

For the standard processing of the study results, mathematical statistics methods were used. These included calculation of numerical characteristics of random values, verification of statistical hypotheses using parametric and non-parametric tests, correlation analysis, and analysis of variance. The predicted and observed incidence rates of the parameters studied were compared using the $\chi^2$ test, Wilcoxon test, Mann – Whitney test, and Student's t-test in Microsoft Excel spreadsheets.

In addition to the standard statistical methods, the screening data was analyzed using new mathematical approaches to data mining based on the metric thickening method. An approach based on the fundamental concept of metrics was used (in mathematics, a metric is a function of measuring a distance between points that satisfies the triangle axiom). In this case, the points are the patient parameters being studied. Metric configuration is a set of points with the given metrics. The pairwise measurement of the paired distances between these points makes it possible to define metric thickening (clusters of close points) and build metric maps (plane projections of metric configurations), which are visual diagrams reflecting the entire array of investigated correlations of the parameters studied [6].

## Results

The experiments produced chromatograms for each of the ten samples examined; these are listed in Table 1 (Table 3). The NIST11 mass spectral library was used to identify each peak on the chromatogram. The peak area displays the percentage of a relevant compound in the sample examined.

### *Preliminary analysis of the resulting chromatograms*

Even a simple visual analysis of the resulting chromatograms shows that there is a group of samples with a large number of peaks (Fish oil-Teva, Omegatrin, Omega-3 concentrate) corresponding to a lower-quality standardization of fatty acid composition. The samples with a small number of peaks (Omacor, Solgar, etc.) correspond to a higher-quality standardization of the composition.

**Table 3.** Chromatograms of the compounds examined.
The figures above the peaks indicate the respective compounds

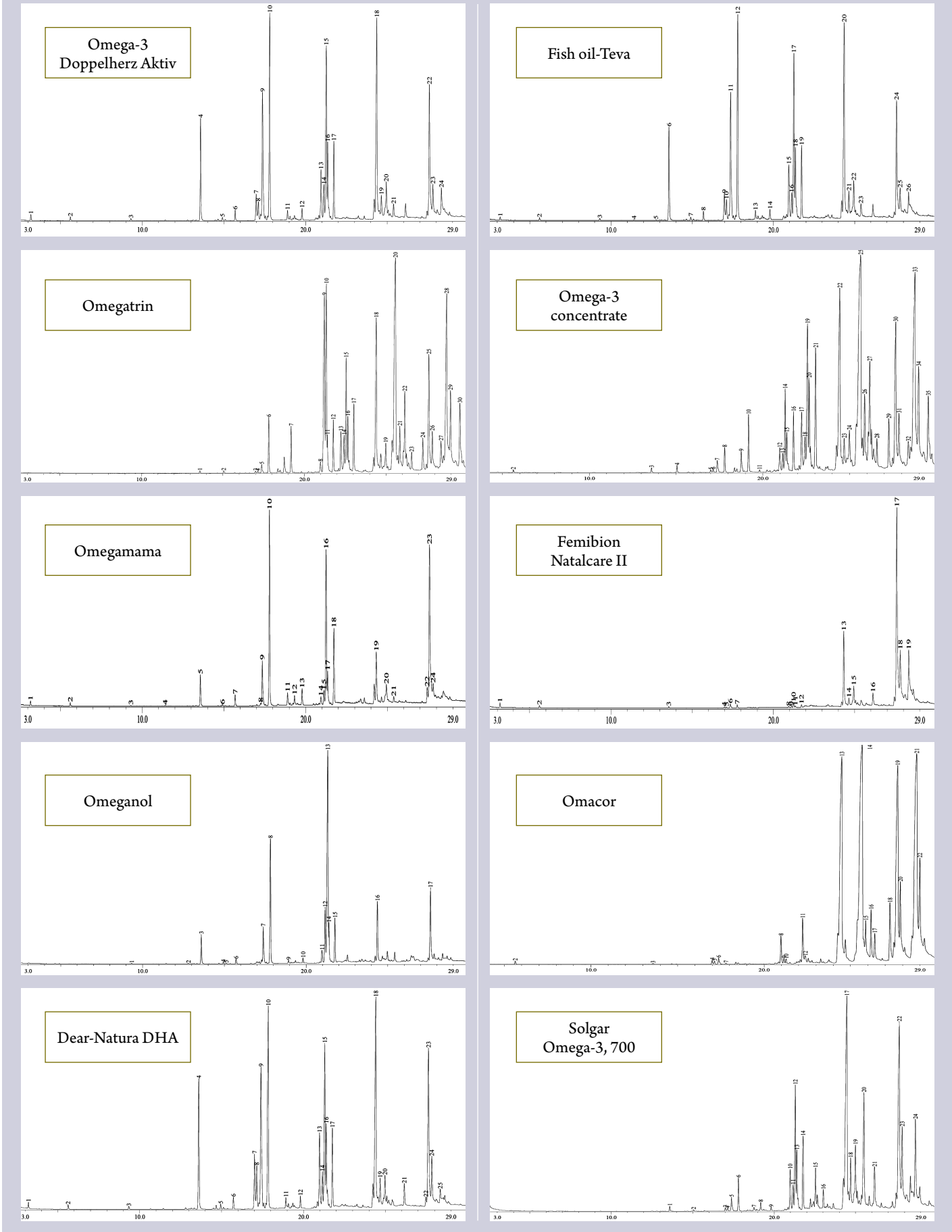



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**Figure 2.** Interpretation of the ω3 PUFA sample chromatogram on the example of Sample #8 (Omacor)

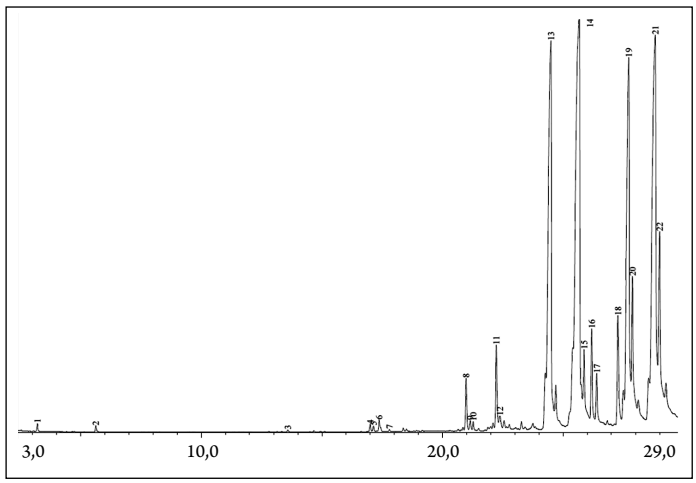



**Table 4.** Informative predictors. The concentrations are used to predict the concentrations of all other components

| Rating | Predictor |
|---|---|
| 100% | Eicosanoic acid, methyl ether, sat |
| 99% | 4, 7, 10, 13, 16, 19 docosahexaenoic acid, butyl ether, DHA |
| 80% | 13 docosene acid, methyl ether, ω9 |
| 70% | 6, 9, 12, 15 hexadecatetraenoic acid, methyl ether, ω3 |
| 70% | 5, 11, 14, 17 eicosatetraenoic acid, methyl ether, ETA |
| 53% | 7, 10, 13, 16, 19 docosapentaenoic acid, methyl ether, DPA |
| 50% | Linoleic acid, ethyl ether, ω6 |
| 50% | 11 eicosene acid, propyl ether, ω9 |
| 47% | 5, 8, 11, 14, 17 eicosapentaenoic acid, methyl ether, EPA |
| 43% | 6, 9, 12 hexadecatrienoic acid, methyl ether, ω3 |
| 43% | 7 methyl-6 hexadecenoic acid, ω9 |
| 40% | 11 eicosene acid, methyl ether ω9 |
| 23% | 4, 7, 10, 13, 16, 19 docosahexaenoic acid, methyl ether, DHA |

The predictor rating corresponds to the percentage of molecular components of extracts. The predictor is used to predict the concentrations of these components.

Samples can be conditionally divided into two types taking into account the qualitative composition [the presence of ethyl, propyl, and butyl FA esters (Table 2)]: (1) «natural» fats similar to fish oil by their fatty acid composition (e.g., see the chromatogram of sample No. 2 – Fish oil-Teva) and (2) «synthetic» fats containing large amounts of non-lipids but FA esters – ethyl, propyl and butyl esters of FA obviously have an artificial origin (e.g., see chromatogram of sample No. 8 Omacor, Figure 2).

The total content of ω3 PUFAs was 97%. The Peaks: 8–6, 9, 12, 15-octadecatetraenoic acid, methyl ether (ω3), 11–6, 9, 12, 15-octadecatetraenoic acid, ethyl ether (ω3), 12 – Linoleic acid, ethyl ether (ω6), 13–5, 8, 11, 14, 17-eicosapentaenoic acid, methyl ether (ω3-EPA), 14–5,8, 11, 14, 17-eicosapentaenoic acid, ethyl ether (ω3-EPA), 15–6, 9, 12, 15-octadecatetraenoic acid, butyl ether (ω3), 16–11-eicosene acid, propyl ether (ω9), 17 – Eicosanic acid, propyl ether (sat.), 18–5, 8, 11, 14, 17-eicosapentaenoic acid, propyl ether (ω3-EPA), 19–4, 7, 10, 13, 16, 19-docosahexaenoic acid, methyl ester (ω3-DHA), 20–7, 10, 13, 16, 19-docosapentaenoic acid methyl ester (ω3-DPA), 21–4, 7, 10, 13, 16, 19-docosahexaenoic acid that, butyl ether (ω3-DHA), 22–7, 10, 13, 16, 19-docosahexaenoic acid, butyl ether (ω3-DHQ).

### *Chromatogram data mining*

In order to establish more explicit quantitative criteria for the qualitative differentiation of ω3 PUFAs extracts, we used data mining for the chromatographic findings on sample composition. Each sample was represented by the concentrations of 44 substances (Table 2).

It should be noted that the concentrations of each molecular component listed in Table 2 are correlated to each other in the ω3 PUFA samples under examination. The correlation is significant, and the concentrations of any molecule from Table 2 can be predicted using topological and metric recognition provided that the concentrations of specific predictor components are known (Table 4). Thus, the prediction had quite a high level of accuracy: the mean correlation coefficient between the predicted and measured concentrations was r=0.77±0.22. It should be noted that the predictors include concentrations of various forms of DHA, EPA, ω6 linoleic acid, and ω9 FAs. Some of the predictors listed were important in order to obtain the informative markers for the standardization of ω3 PUFA extracts (see below).

Using the Kolmogorov–Smirnov metric together with the 44-dimensional vectors, we calculated the «distances» between the samples, investigated the presence of clusters, and plotted the results in a metric map (Figure 3).

The analysis of metric thickening allowed us to identify a cluster of samples with relatively low-quality standardization of composition (Figure 3). Indeed, the manufacturers of three of the five samples included in the cluster (Fish oil-Teva, Omegatrin, Omeganol) did not even mention the approximate contents of EPA and DHA. The lack of such information is an indirect

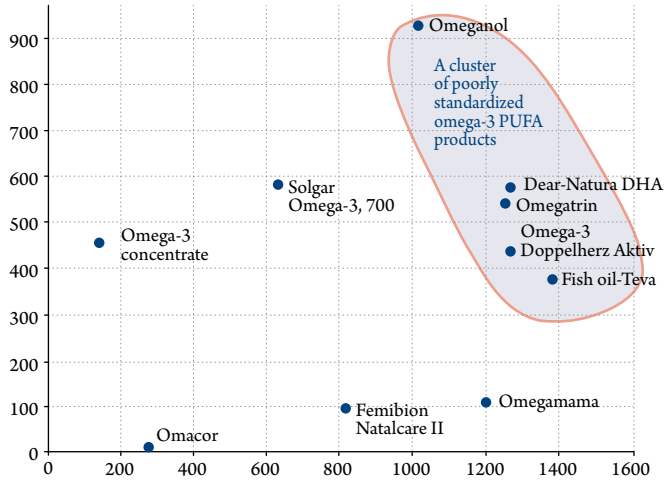
indication of the absence of proper standardization of the composition during the production of samples (Table 1). The composition of different ω3 PUFAs is standardized differently. For this reason the points corresponding to other samples are located in different directions from the cluster of poorly standardized samples.

### *Interpretation of the axes of the metric map and informative markers of the fatty acid composition of the ω3 PUFA extracts*

An examination of the presence of specific components of the fatty acid composition allows for samples in the cluster of lower-degree standardization of composition to be differentiated from those with a higher-degree of standardization. The «conditional axes» X and Y of the metric map should correspond to the content of more specific substances or groups of substances. We compared the content of all 44 substances in the cluster of poorly standardized samples, contained in all other samples, to establish specific indicators of the biochemical quality of standardization. The differences are summarized in Table 5.

The analysis of data and the interpretation of the axes of the metric map produced two of the most informative markers of the fatty acid composition. These allow for the most effective differentiation of the more and less well-standardized ω3 PUFA samples.

**Figure 3.** Metric map of the fatty acid composition of the ω3 PUFA samples examined




*The X and Y axes show (CU) some of the main components interpreted below. A metric map is the planar projection of a matrix of pairwise distances between all samples. The closer points on the map correspond to the samples with similar fatty acid composition. The metric thickening method allowed us to establish the presence of a single cluster corresponding to the samples with poor standardization of composition.*

The «EPA+DHA» marker is summarized at peak areas of the three specific substances: 5, 8, 11, 14, 17 EPA (propyl ether), 4, 7, 10, 13, 16, 19 DHA (butyl

**Table 5.** The differences in the fatty acid composition of the ω3 PUFA samples (chromatographic peak areas, %) and markers used to distinguish more standardized samples from less standardized samples (cluster), ω

| Compound | FA | Standardized | Less well-standardized | p |
|---|---|---|---|---|
| 6-octadecenoic acid, methyl ether | ω11 | 1.26±1.53 | 3.25±1.67 | 0.009 |
| 13-methyltetradecanoic acid, methyl ether | sat. | 0.02±0.04 | 0.11±0.10 | 0.013 |
| Myristic acid, methyl ether | sat. | 0.62±1.18 | 3.03±2.78 | 0.016 |
| Myristic acid, methyl ether | sat. | 0.62±1.18 | 3.03±2.78 | 0.016 |
| Dodecanoic acid, methyl ether | sat. | 0.01±0.02 | 0.05±0.06 | 0.018 |
| Palmitoleic acid, methyl ether | ω7 | 1.28±2.00 | 5.09±4.81 | 0.019 |
| 6,9,12,15-octadecatetraenoic acid, methyl ether | ω3 | 0.93±0.89 | 2.18±1.88 | 0.029 |
| Linoleic acid, methyl ether | ω6 | 0.72±0.67 | 6.35±3.90 | 0.035 |
| 5,8,11,14,17-eicosapentaenoic acid, propyl ether | EPA | 1.44±1.16 | 0.43±0.74 | 0.041 |
| Palmitic acid, methyl ether | sat. | 3.76±7.80 | 10.6±7.74 | 0.042 |
| 6, 9, 12, 15-hexadecatetraenoic acid, methyl ether | ω3 | 0.08±0.09 | 0.68±1.12 | 0.047 |
| 6, 9, 12-hexadecatrienoic acid, methyl ether | ω3 | 0.11±0.10 | 0.55±0.90 | 0.053 |
| 4, 7, 10, 13, 16, 19-docosahexaenoic acid, butyl ether | DHA | 12.5±12.4 | 3.95±6.84 | 0.055 |
| Oleic acid, methyl ether | ω9 | 4.43±6.12 | 18.8±15.6 | 0.057 |
| 4, 7, 10, 13, 16, 19-docosahexaenoic acid, methyl ether | DHA | 19.3±14.6 | 8.97±2.67 | 0.059 |
| Standardization markers | | | | |
| EPA+DHA marker | – | 33.3±10.3 | 13.3±4.92 | 0.0016 |
| ω6+ω11 marker | – | 2.06±2.20 | 10.2±1.89 | 0.0004 |

The "EPA+DHA" marker is summarized at peak areas of 5, 8, 11, 14, 17 EPA (propyl ether), 4, 7, 10, 13, 16, 19 DHA (butyl ether), 4, 7, 10, 13, 16, 19 DHA (methyl ether). The "ω6+ω11" marker is summarized at peak areas of the following components: 6 octadecenoic acid, methyl ether, 6, 9, 12, 15 octadecatrienoic acid, methyl ether, linoleic acid, methyl ether.


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**Figure 4.** The diagram of the composition of the samples examined with axes corresponding to the markers of fatty acid composition "ω6+ω11" and "EPA+DHA"

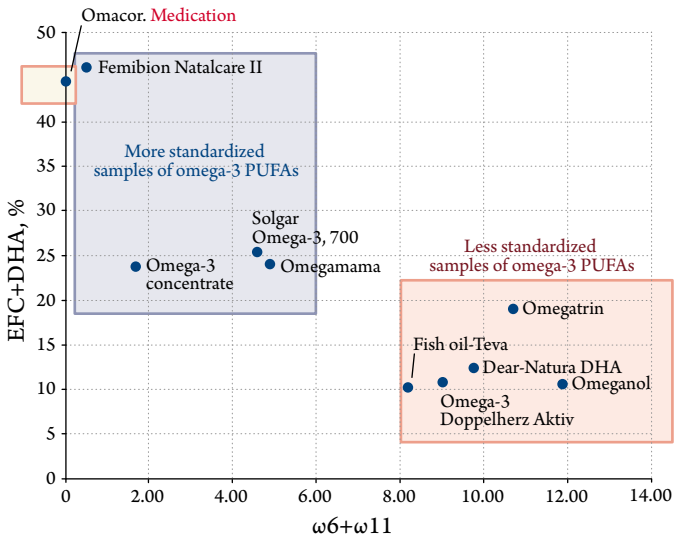
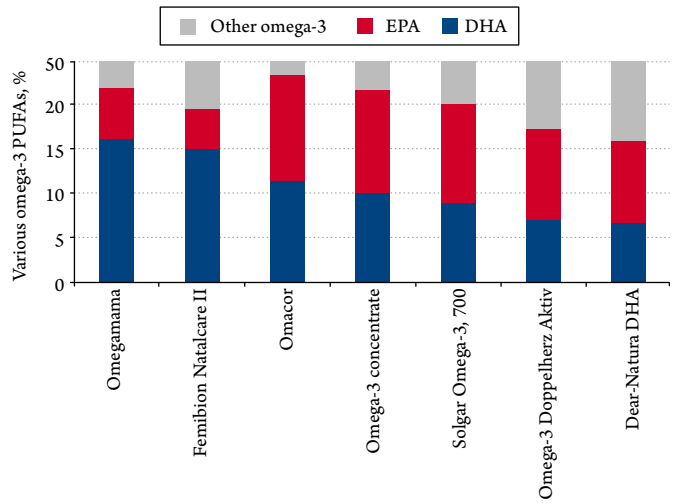



**Figure 5.** The relative content of DHA and EPA in the samples examined with higher quality standardization




*Samples are ordered in descending order of relative DHA content.*

ether), 4, 7, 10, 13, 16, 19 DHA (methyl ether). The values of the «EPA+DHA» marker were statistically significantly 2.5 times higher in the group of more well-standardized samples, than in the group of less well-standardized samples (p=0.0016).

The «ω6+ω11» marker is summarized at peak areas of the following components: 6 octadecenoic acid, methyl ether, 6, 9, 12, 15 octadecatrienoic acid, methyl ether, linoleic acid, methyl ether. The values of the «ω6+ω11» marker were statistically significantly five times lower in the group of more well-standardized samples (%), than in the group of less well-standardized samples (p=0.0004).

Figure 4 shows the diagram relating to the composition of the samples examined with the «ω6+ω11» – «EPA+DHA» axes. The data on the relative content of DHA and EPA in the samples examined is presented in Figure 5. More well-standardized samples obviously lie in the area corresponding to the values of «EPA+DHA»>23%, and «ω6+ω11» <5% (Figure 4). Less well-standardized samples lie in the area corresponding to the values of «EPA+DHA» <20% and «ω6+ω11»>8%.

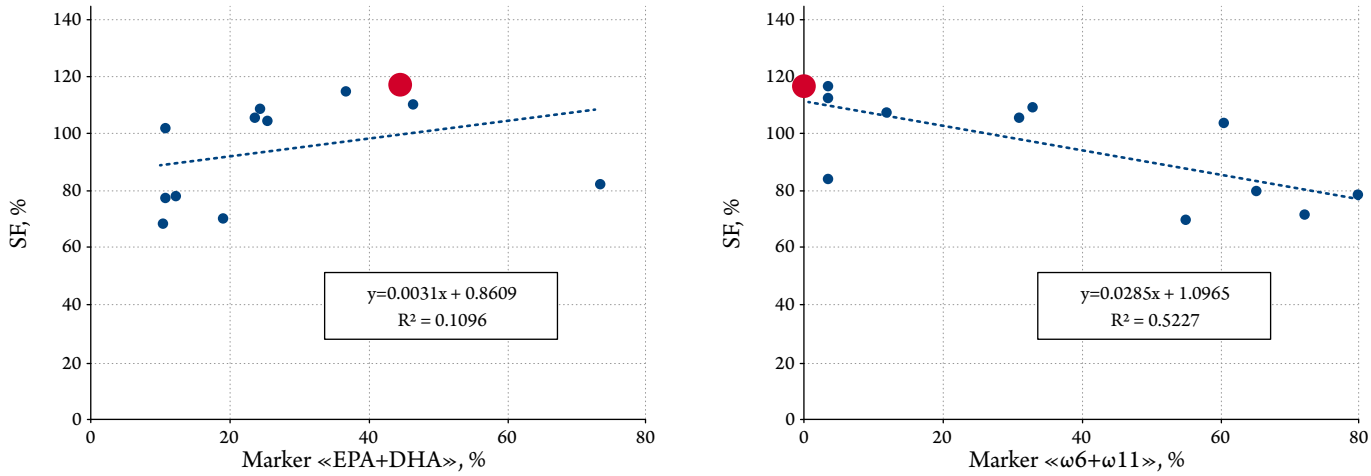
Thus, dietary supplements differ significantly by the values of «ω6+ω11» and «EPA+DHA». This corresponds to a significant unconformity in the content of the active substance. Sample #8 (Omacor) has a unique position on the diagram (Figure 4), actually corresponding to a zero value of the «ω6+ω11» marker. The present study also showed other features of the sample's composition which distinguish it from the samples of the dietary supplements studied. Firstly, the pharmaceutical product consists of 97% ω3 PUFAs (Table 6), and the dietary supplements of not more than 70%. Secondly, 91.5% of the fatty acid composition of the drug is the ω3 PUFAs EPA and DHA with high pharmacological significance for the prevention of cardiovascular mortality [7, 8], improvement of lipid profile, reduction of inflammation [9], etc. (hundreds of the relevant sources are cited in the monograph [7]). Thirdly, the drug does not contain ω11 PUFAs and contains very little ω6 PUFAs (0.55%), and little ω9 PUFAs (1.6%). For comparison: Femibione dietary supplement contains 20.9% ω9 PUFAs.

### *Markers of fatty acid composition and ω3 PUFA extract standardization factor*

In general, the markers obtained allow for a certain «screening» study of the quality of the standardization of substances for the production of ω3 PUFA medications and dietary supplements to be carried out. Let us assume that chromatographic analysis of the sample gives the following marker values: «EPA+DHA»=13%, «ω6+ω11»=9%. Obviously, these values correspond to low standardization of the sample by ω3 PUFAs and the high level of impurities characteristic of so-called «fish oil». If the sample is standardized by ω3 PUFAs, then the value of the «EPA+DHA» marker cannot be less than 23%, and the value of the «ω6+ω11» marker should not be more than 5%.

Thus, the more standardized ω3 PUFA samples are characterized by higher levels of ω3 PUFAs (EPA and DHA) and lower levels of ω6 and ω11. This result is of essential practical significance, since ω6 acids, including arachidonic acid, have hypercoagulation, proinflam-

**Figure 6.** Correlations between the standardization factor SF (%) and the values of the standardization quality markers ("EPA+DHA", "ω6+ω11")




*The red dot shows the composition of Omacor with the highest SF (%) = 114.2%.*

matory, vasoconstriction effects, and cause fluid retention. There is a strong bias in the ω6: ω3 ratio in the typical diet of a modern individual towards ω6, varying from 10:1 to 20:1. Therefore, the maximum reduction of ω6 in the ω3 PUFA samples is not only a marker of the standardization of fatty acid composition but also is in line with the increased pharmacological efficacy of ω3 PUFAs [7].

In addition to the «EPA+DHA» and «ω6+ω11» markers which allow the study samples of ω3 PUFAs to be classified as more or less well-standardized, a standardization factor (SF) is also offered. This factor assesses whether the actual levels of ω3 PUFAs conform to the ω3 PUFA content declared by the manufacturer. The SF is calculated as a composition of four components:

$$KSF\ (\%)=1\ \omega3_{conform} - EPA_{conform} - DHA_{conform} - Other\ \omega3_{conform},$$

where $\omega3_{conform}=\omega3_{declared}$ (%) – $\omega3_{measured}$ (%) is the equivalence of the declared total content of ω3 PUFAs to the measured levels. If a sample contains more ω3 than declared, this increases the value of $\omega3_{conform}$;

$EPA_{conform}=EPA_{declared}$ (%) – $EPA_{measured}$ (%), if the contents of EPA is declared. If not, a «penalty percentage» is assigned (we used a value of 15% that reflects the mean EPA content in poorly standardized samples).

$DHA_{conform}=DHA_{declared}$ (%) – $DHA_{measured}$ (%) if the DHA content is declared, otherwise «penalty interest» is applied (10%).

Other $\omega3_{conform}=|Other\ \omega3_{declared} - Other\ \omega3_{measured}|$ – an absolute value of the difference between the declared and measured content of other ω3 PUFAs. Other ω3 is defined as the difference between the total content of ω3 and the total content of EPA+DHA.

**Table 6.** Detailed fatty acid composition of Sample #8 (Omacor)

| Fatty acid components | Peak, % | Type of FA |
|---|---|---|
| 5, 8, 11, 14, 17-eicosapentaenoic acid, ethyl ether | 25.47 | EPA |
| 4, 7, 10, 13, 16, 19-docosahexaenoic acid, butyl ether | 24.95 | DHA |
| 5, 8, 11, 14, 17-eicosapentaenoic acid, methyl ether | 18.59 | EPA |
| 4, 7, 10, 13, 16, 19-docosahexaenoic acid, methyl ether | 17.12 | DHA |
| 7, 10, 13, 16, 19-docosahexaenoic acid, butyl ether | 2.69 | DHA |
| 5, 8, 11, 14, 17-eicosapentaenoic acid, propyl ether | 2.43 | EPA |
| 6, 9, 12, 15-octadecatetraenoic acid, ethyl ether | 1.99 | ω3 |
| 7, 10, 13, 16, 19-docosapentaenoic acid, methyl ether | 1.87 | ω3 |
| 11-eicosene acid, propyl ether | 1.61 | ω9 |
| 6, 9, 12, 15-octadecatetraenoic acid, methyl ether | 1.06 | ω3 |
| 6, 9, 12, 15-octadecatetraenoic acid, butyl ether | 0.84 | ω3 |
| Eicosanoic acid, propyl ether | 0.83 | sat. |
| Linoleic acid, ethyl ether | 0.55 | ω6 |

The components are sorted by descending order of the peak values.

Examples of the calculation of SF (%) are given in Table 7. Omacor had the highest value of SF among the samples examined. The results of the assessment of the «quality» of standardization using SF (%) correspond to those of the evaluation using the «EPA+DHA» and «ω6+ω11» markers, since there are statistically significant correlations between the standardization factor and the values of the «EPA+DHA» and


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**Table 7.** Standardization factor for the ω3 PUFA samples

| Medication/ dietary supplement | Declared, % | | | Measured, % | | | SF (%) |
|---|---|---|---|---|---|---|---|
| | EPA | DHA | Other ω3 | EPA | DHA | Other ω3 | |
| Omacor | 46.0 | 38.0 | 6.0 | 46.5 | 44.8 | 5.8 | 114.2% |
| Femibion Natalcare II | 0 | 40.0 | 0.0 | 13.4 | 46.0 | 16.0 | 110.5% |
| Omegamama | 4.6 | 21.0 | 4.4 | 7.8 | 22.3 | 4.0 | 108.2% |
| Omega-3 concentrate | 33.0 | 22.0 | 5.0 | 32.1 | 26.2 | 8.4 | 106.6% |
| Solgar Omega-3, 700 | 32.7 | 21.8 | 9.1 | 31.2 | 25.4 | 12.9 | 104.1% |
| Omega-3 Doppelherz Aktiv | 15.2 | 10.1 | 6.3 | 15.5 | 10.8 | 11.5 | 102.1% |
| Dear-Natura DHA | 5.0 | 33.3 | 3.3 | 16.4 | 11.8 | 15.5 | 79.8% |
| Omeganol | – | – | – | 0.0 | 10.7 | 1.9 | 77.7% |
| Omegatrin | – | – | – | 24.7 | 21.7 | 5.7 | 70.4% |
| Fish oil-Teva | – | – | – | 16.2 | 10.3 | 13.0 | 68.5% |

The samples examined are sorted by descending order of SF (%) estimating the correspondence of the fatty acid composition measured in comparison with the declared composition. "Others ω3" is the difference between the total content of ω3 and the sum of EPA and DHA.

«ω6+ω11» markers (Figure 6). However, SF (%) provides additional information on the quality of standardization. For example, the value of SF (%) for Omega-3 Doppelherz Aktiv was quite high (102,1%), since the actual measured content of various PUFAs in the corresponding sample almost completely conformed to the content declared by the manufacturer (Table 7).

## Conclusion

Patients learn through the mass media and the Internet that the consumption of ω3 PUFAs should be corrected. Much less frequently they are advised by physicians who recommend a particular ω3 PUFA medication or dietary supplement. Thus, the final choice of the ω3 PUFA medication / dietary supplement is made by the patient (often on the pharmacist's advice). The efficacy of ω3 PUFA medications and dietary supplements depends significantly on the standardization of the extract content by EPA, DHA, and other FAs. We performed a comprehensive study of 10 samples of ω3 PUFAs and chromatographic definitions of more than 40 metabolites of FAs and other compounds.

We obtained the quantitative markers to differentiate some samples (Omacor and several dietary supplements, including Femibion natalcare II, Omega-3 concentrate, etc.) from less well-standardized samples (Fish oil-Teva, Omegatrin, Omeganol, etc.) based on the standardized ω3 PUFAs. For the standardized samples, the values of the «EPA+DHA» marker is 2,5 times higher (p=0.0016), and the «ω6+ω11» marker is five times lower (p=0.0004). The standardization factor for assessing the conformity of the levels of omega-3 PUFAs measured with the content declared by the manufacturer, was calculated in order to show that Omacor had the best standardization. In should be noted that Omacor (Lovaza in the United States) is the only prescription ω3 PUFA drug approved by the FDA. There is a vast amount of evidence for the use of standardized ω3 PUFA medications for secondary prevention of myocardial infarction and reduction of cardiovascular mortality [7, 8].

In conclusion, it should be noted that the quality of the pharmaceutical standardization of the ω3 PUFA samples should be evaluated not only in terms of fatty acid but also in terms of micronutrient composition. The standard requirement for the inclusion of a dietary supplement in the Unified Register of Marketing Authorization is the low content of certain toxic elements (lead <1.0 mg/kg, cadmium <0.2 mg/kg, mercury <0.3 mg/kg). However, due to the fact that there are more than ten toxic elements, the micronutrient profiles in various drugs, including from 18 to 68 micronutrients, are informative of the degree of purification and standardization [10, 11]. They are also extremely useful in the comparative analysis of the pharmaceutical quality of medications and dietary supplements [12].

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