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# GC Determination of Docosahexaenoıc Acid, Eıcosapentaenoic Acid and Other Fatty Acids in food Supplements by Percentage Method

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**Original Research Article**

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**Abstract:** The aim of current study was the application of GC method for separation of Docosahexaenoic acid and Eicosahexaenoic acid from other fatty acids in food supplements and their further determination by percentage method. This method for quantitative analysis is based on the measuring of the area of all peaks in the chromatogram of the hydrolyzed and methylated samples and calculation of their sum, whereby the area of each peak is calculated as a percentage of the total area of the chromatographic peaks. After hydrolysis of fatty acid esters, the free forms were preesterificated with methanol solution of 14 % boron trifluoride. For separation of Docosahexaenoic acid and Eicosahexaenoic acid from other fatty acids was applied GC method: flow rate of carrier gas: hydrogen: 45 ml/min., inlet pressure: 15 Psi, temperature programm: 140 °C for 5 min, increasing the temperature to 240 °C at a rate of 4 °C/min, 240 °C for 20 min, increasing the temperature to 280 °C at a rate of 6 °C/min and 280 °C for 10 min. The suitability of the system was confirmed by the lack of a statistically significant difference between the values of the chromatographic parameter retention time in the analysis of Methylmyristate (SD = 0.185, RSD = 1.22 %), Methylpentadecanoate (SD = 0.39, RSD = 2.18 %), Methylheptadecanoate (SD = 0.31, RSD = 1.34 %), Methyldocosahexanoate (SD = 0.017, RSD = 0.05 %), Methyleicosapentaenoate (SD = 0.013, RSD = 0.04 %), Methylbehenate (SD = 0.15, RSD = 0.43 %), Methylerucate (SD = 0.13, RSD = 0.37 %. Maximum concentration was found for Methyleicosapentaenoate (20.58 %) and minimun concentration was observed for Methylnonadecanoate (0.30 %). The described GC persentage method can be applied for rutine analysis of Docosahexaenoic acid and Eicosahexaenoic acid in combination with other fatty acids in food additives.

**Keywords:** Docosahexaenoic acid, Eicosahexaenoic acid, GC, persentage method, food supplements.

## INTRODUCTION

Omega-3 fatty acids are now generally recognized as potential nutrients for the prevention of the pathological conditions associated to the aging process, cognition and bone health [1]. In humans ω-3 fatty acids decrease the inflammation [2, 3], are important for the neurodevelopment [4, 5] and the prevention of cognitive decline [6] and reduce the abnormal heart rhythm [7], attacks and stroke in people with heart disease [8]. Omega-3 fatty acids possess hypotriglyceridemic effect in type 2 diabetes [9] and have beneficial properties in Crohn disease [10] and chronic obstructive pulmonary disease [11]. By clinical Diet and Re-infarction Trial (DART) is proved their antiarrhythmic effects and role in decreasing of total mortality and sudden death in patients with myocardial infarction [12]. Docosahexaenoic acid (DHA) is important for function of nervous system [13, 14], improves cognitive decline in elderly people with mild Alzheimer's disease [15], shows beneficial effects during pregnancy and lactation [16] and is important for neurodevelopment in infants [17].

For the determination of free fatty acids fourier-transform infrared spectroscopy is presented [18]. Fatty acids of plasma lipids are separated by thin-layer chromatographic method by developing of plates with methanol, followed by chloroform: methanol = 10: 10 v/v and then in hexane: diethyl ether: acetic acid = 80: 20 : 1 v/v/v [19]. Capillary zone electrophoresis is appropriate for monitoring of total trans fatty acids in cheese [20] and in hydrogenated oils [21]. For analysis of fatty acids are applied non-aqueous capillary electrophoresis by indirect UV-detection [22], micellar electrokinetic chromatography under UV-detection [23], capillary zone electrophoresis, microemulsion electrokinetic chromatography employing different detection systems, such as ultraviolet-visible, capacitively coupled contactless conductivity, laser-induced fluorescence and mass spectrometry [24].

GC method is applied for the identification of components of different plant extract: from roots of *Rubia cardifolia* [25] and leaves of *Cassia angustifolia* Vahl [26], *Catharanthus roseus* [27], *Rhododendron arboreum* [28] and *Teucrium capitatum L.* [29].

For the identification of components of extract of roots of *Rubia cardifolia* is applied GC with capillary column ZB-5 (30 m × 0.25 mm × 0.25 μm); oven temperature: initially maintained at 50 °C for 5 min and then programmed to 250 °C at 10 °C/min; carrier gas: helium at a flow rate of 1 ml/min; electron-impact ionization of the MS [25].

The GC-MS analysis of the extract of the leaves of *Cassia angustifolia Vahl* is performed at capillary column (phenyl methyl siloxane: 25 m × 0.25 mm); oven temperature: 80 °C/2 min to 280 °C at 40 °C/min, detector temperature: 280 °C, carrier gas: helium with a flow rate : 0.9 ml/min [26].

For the analysis of extract of leaves of *Rhododendron arboreum* is proposed GC method with column (30 mm × 0.25 mm x 0.25 μm); column initial temperature: 80 °C/ 3 min, further programmed to increase to 280 °C with rate of 10°C/min; temperature of the injector: 250 °C; carrier gas: helium [28].

The chemical composition of the essential oil extracted from the leaves of *Theuctium capitatum L.* is obtained by GC with capillary column HP - 5 (30 m × 0.25 mm x 0.25 μm); temperature of the column: from 20 °C to 230 °C at 20°C/min; carrier gas: helium with a flow rate: 1.5 ml/min [29].

Gas chromatography (GC) methods with flame ionization detector and mass (MS) detector with electron impact ionization are the most widely used techniques for the determination of the fatty acids [30]. GC/MS is applied for analysis of Trans fatty acid [30], fatty acids in food fats [31] and in biological samples [32].

In biological samples is applied GC with BPX-70 fused silica capillary column (30 m × 0.25 mm × 0.2 μm); temperature programme: initial column temperature 100 °C, programmed to increase at a rate of 10 °C/min up to 160 °C and then at 3 °C/min up to 220 °C, injector temperature: 260 °C; detector temperature: 280 °C; carrier gas: helium at a flow rate of 1 min [32].

The aim of current study was the application of GC method for separation of Docosahexaenoic acid and Eicosahexaenoic (EPA) acid from other fatty acids in food supplements and their further determination by percentage method. After hydrolysis of fatty acid esters, the free forms were preesterificated with methanol solution of 14 % boron trifluoride.

## MATERIALS

### Reference substances

Docosahexaenoic acid (≥ 98% for GC) (Sigma Aldrich, N:D2534), Methyldocosahexanoate (Sigma Aldrich, N:D5518), Methyleicosapentaenoate (Sigma Aldrich, N:17266), Nonadecanoic acid (Serva, N:72332), Methyl Nonadecanoate (Serva, N:74208), Methylmyristate (Sigma Aldrich, N:M3378), Methylpentadecanoate (Sigma Aldrich, N:P6250), Methylheptadecanoate (Sigma Aldrich, N:H 4515), Methylheneicosanoate (Sigma Aldrich, N: 51535), Methyl Behenate (Serva, N:855278), Methyl Erucate (Merck N:E 3385), Methyl Lignocerate (Sigma Aldrich, N:L 6766).

### Reagents with analytical grade quality

Derivatizing reagent: boron trifluoride (99.5%) (Sigma Aldrich, N:339963); n-hexane (Valerus, N:UN 1208); isooctane (99.7%) (Sigma Aldrich, N:59030); methanol (99.9%) (Sigma Aldrich, N: SZBD 063AV UN 1230); nitrogen (Messer Grisheim, N: 00474); potassium hydroxide (Fluka, N:757 551).

### Food supplements (Table 1)

**Table-1: Food supplements containing fatty acids**

| N: | Food supplement | Produser | Content |
|---|---|---|---|
| 1. | Doppelherz Омега-3 aktiv + Vit. E caps. | Doppelherz | 120 mg DHA (12%)/180 mg EPA (18%) |
| 2. | Omega-3, 6, 9 caps. | Ramkopharm | 51 mg Omega-3; 105 mg Omega-6<br>111 mg Omega-9<br>100 mg Oil Semeni *Linum usitatissimum*<br>100 mg Oil Semeni *Carthamus tinctorius*<br>100 mg Oil Semeni *Camellia oleifera* |
| 3. | Omega-3 + Vit. E Solution | Jamieson | 15 ml:<br>300 mg EPA, 400 mg DHA<br>600 mg Vitamin E, 25 μg Vitamin $D_3$ |
| 4. | Norwegian Fish Oil 12/18 1000 mg caps. | ABO Pharma | 120 mg DHA (12%)/180 mg EPA (18%) |
| 5. | Omega 3 Forte 1000 mg caps. | Adipharm | 1000 mg Omega-3 (Salmon oil)<br>120 mg DHA (12%)/180 mg EPA (18%)<br>10 mg Alfa-tocopherol acetate |

## METHODS Gas chromatography

### Equipment

Gas chromatograph" Autosystem" (Perkin Elmer, USA), equipped with a split-splitter injector, flame ionization detector, capillary column ZB-1701 (cyanopropyl-methylsilylsiloxane) (30 m x× 0.25 mm x 0.25 μm) (Phenomenex inc.); hydrogen generator (HGH-300E, Beijing uiland, China); compressor for compressed air with a system of filters; analytical balance; air thermostat.

### Gas chromatographic conditions

Carrier gas hydrogen with flow rate: 45 ml/min., inlet pressure of carrier gas 15 Psi and temperature programm: maintaining at 140 °C for 5 min, increasing the temperature to 240 °C at a rate of 4 ˚C/min., holding on at 240 °C for 20 min, increasing the temperature to 280 °C at a rate of 6 °C/min. maintaining at at 280 °C for 10 min.

### Preparation of sample for determination of DHA, EPA and other fatty acid content

After hydrolysis with solution of potassium hydroxide of esters of fatty acids in fish oil food supplements, their free forms were preesterificated with methanol solution of 14 % boron trifluoride by the following analytical procedure: in a glass reaction vessel of 6 ml were introduced 200 μl of in isooctane solution of Nonadecanoic acid (C19/0) used as an internal standard. The solvent was removed by gently flushing with nitrogen at room temperature and then into the vessel were added 2 ml of precooled reagent: 14 % boron trifluoride in methanol. The reaction vessels were immediately sealed with teflon laminated septum and purged with nitrogen by two needles (inlet and outlet). Nitrogen used as protection gas ensures less oxidation and higher stability of the constituent contents. Purging should be short in order to avoid reducing the concentration of boron trifluoride in methanol. Into the vessel were added 5 μl of sample of fish oil. Sample was incubated for 12 min. at 65 °C and after cooling was added 1 ml distilled water and 1 ml n-hexane. The samples were shaken for 1-2 min for an extraction of the methylated acids, transfered rapidly to 10 ml tubes and after separation of the phases, 200 μl of n-hexane extract was introduced in an autosampler container. Aliquots of the methylated acid extract (1 μl) are introduced into the gas chromatograph for analysis.

## RESULTS AND DISCUSSION

In the natural products fatty acids are present in free form and in connected forms, such as triglycerides, phospholipids, and others. The determination of the total content of Omega-3 fatty acids requires hydrolysis of connected forms and methylation of the free fatty acids. This is necessary for providing of conditions for possible complete hydrolysis and methylation as well as for the protection of polyunsaturated fatty acids from oxidation, to which they are particularly sensitive.

For qualitative and quantitative analysis of fish oil is reported GC/MS method at DB-5 capillary column (30 m × 0.25 mm × 0.25 μm); carrier gas helium with flow rate of 0.8 ml/min; injection volume: 1 μl; injector mode: split with a split ratio: 10 : 1; temperature programme: maintaining the oven 1 min at 80 °C, and increasing at a rate of 10 °C/min to 250 °C, reaching 280 °C at a rate of 8 °C/min and maintaining 5 min at 280 °C; mass spectrometer in electron-impact mode (200 °C) with electron energy: 70 eV. In this methods fish oil is hydrolized in 0.5 M potassium hydroxide methanol solution at 60 °C for 20 min and by the esterification boron trifluoride methanol solution at 60 °C for 5 min, are obtained a volatile methyl esters [33].

For analysis of Docosahexaenoic acid from fish oil is described other GC method by using silica capillary column (30 m × 0.25 mm × 0.25 μm), carrier gas nitrogen, with flow rate 2 ml/min., injection volume: 1 μl, injector mode: split, injector temperature 70 °C, flame ionization detector, oven temperature 110 °C [34].

In previous our investigation after application of GC method with inlet pressure of carrier gas hydrogen: 10 Psi and temperature gradient: from 60 °C to 135 °C for mixture of Methyl Laurate, Methyl Palmitate, Methyl Heptadecanoate, Methyl Stearate, Methyl Nonadecanoate, Methyl Behenate, Methyl Lignocerate, Methyl Hexacosanoate, Methyl Palmitoleate, Methyl Oleate, Methyl Linolenate, was obtained good separation for all methyl esters, excluding of critical couple of methyl esters of Stearic acid and Linoleic acid. For the optimization of their separation were found the more suitable conditions: inlet pressure of carrier gas was changed from 10 Psi to 15 Psi and temperature gradient was modified from 60 °C to 160 °C. By this method methyl esters of Stearic acid and Linolenic acids were separated completely, but the separation of the esters of Oleic acid and Linoleic acid was not satisfactory. In previous our wotk it is proved that GC Method 3 with inlet pressure of carrier gas 15 Psi and temperature program from 140 °C to 280 °C, provide completely separation of all methyl esters of fatty acids, inluding methyl esters of Oleic acid and Linoleic acid and can be applied for rutine analysis of fatty acids in food additives, after derivatization to methyl esters. In the difference with the described literature GC methods in our investigation after is applied hydrolysis and preesterification of free fatty acids is applied GC method with split-splitter injector, capillary column ZB-1701 (cyanopropylmethylsilylsiloxane) (30 m × 0.25 mm × 0.25 μm); carrier gas: hydrogen: 45 ml/min (inlet

pressure 15 Psi), flame ionization detector and the temperature program of from 140 °C to 280 °C [35].

**Selectivity**

A blank solution without the active substance DHA and internal standard Nonadecanoic acid was prepared in the same manner like sample solutions. The selectivity of the applied GC method [36] was proved by the fact that on the chromatograms with blank solution there was no peak with retention time, corresponding to the retention times of fatty acids. The obtained chromatograms are presented in Fig. 1-5.

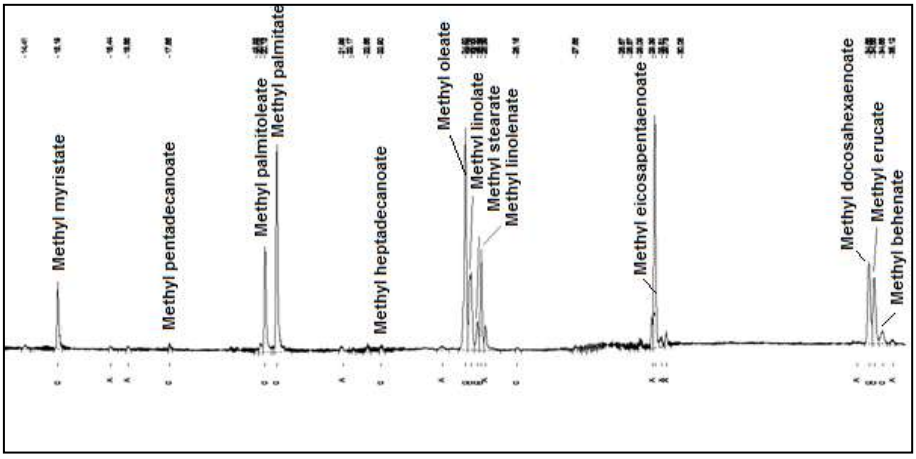


**Fig-1: GC chromatogram of food supplement Dopppelherz Omega-3 Aktiv +Vit. E caps**

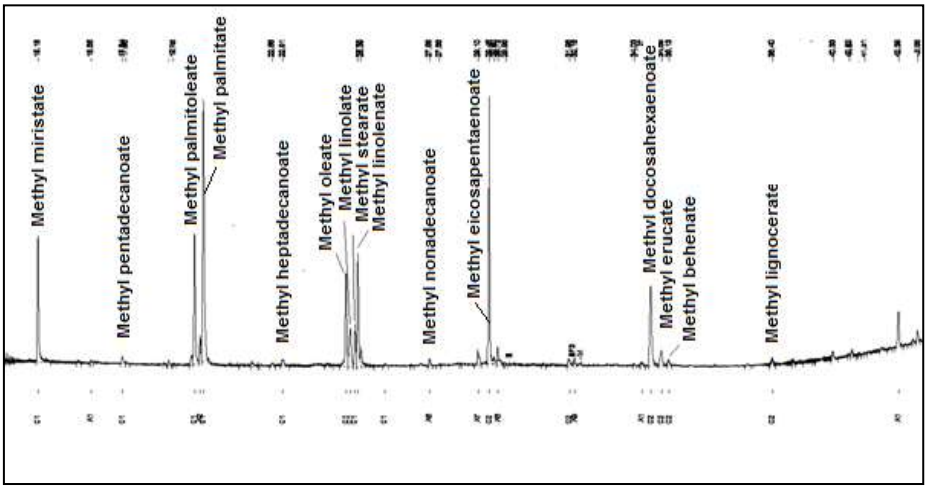


**Fig-2: GC chromatogram of food supplements Omega-3, 6, 9 caps.**

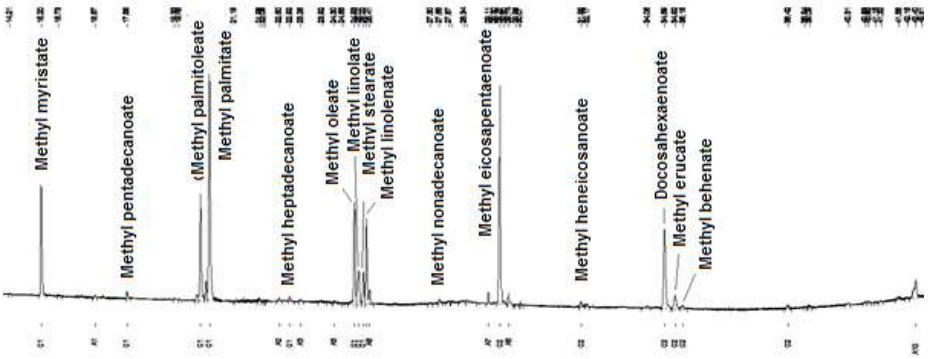


**Fig-3: GC chromatogram of food supplements Omega-3 + Vit. E Solution**

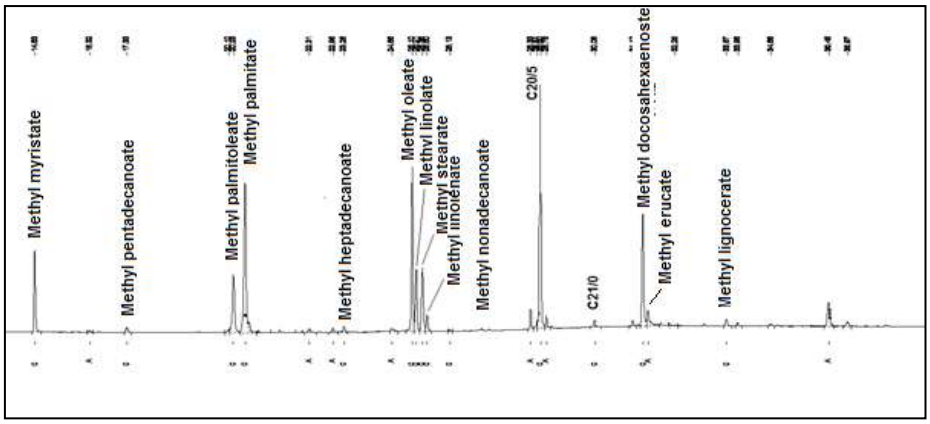


**Fig-4: GC chromatogram of food supplement Norwegian fish oil 12/18 1000 mg caps**

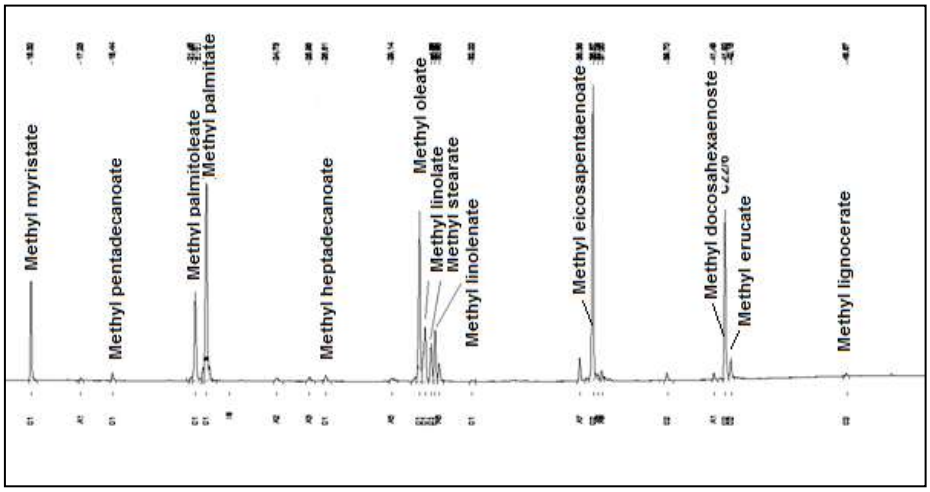


**Fig-5: GC chromatogram of food supplement Omega 3 Forte 1000 mg caps**

**Qualitative analysis**

The available options allow the identification of the peaks of the methylated fatty acids by comparing the retention times with the available comparative standarts of methyl esters: Methyldocosahexanoate, Methyleicosapentaenoate, Methyl Nonadecanoate, Methylmyristate, Methylpentadecanoate, Methylheptadecanoate, Methylheneicosanoate, Methyl Behenate, Methyl Erucate, and Methyl Lignocerate.

**Test for system suitability**

The results for retention times ($t_R$) and Chauvenet's criterion for $t_R$ are summarized in Table 2. for Methylmyristate (MM), Methylpentadecanoate (MPDA) and Methylheptadecanoate (MHDA) and in Table 3. for Methyldocosahexanoate (MDHA), Methyleicosapentaenoate (MEPA). Methylbehenate (MB) and Methylerucate (ME).

For all of the samples the calculated Chauvenet's criterion for $t_R$ are lower than the maximum value (Umax = 1.68, n = 3).

**Table-2: Retention times for Methylmyristate (MM), Methylpentadecanoate (MPDA) and Methylheptadecanoate (MHDA)**

| N: | $t_{R\ MM}$ [min.] | $U_{MM}$ | $t_{R\ MPDA}$ [min.] | $U_{MPDA}$ | $t_{R\ MHDA}$ [min.] | $U_{MHDA}$ |
|---|---|---|---|---|---|---|
| Doppelherz Омега-3 aktiv + Vit. E caps. | 15.188 | 0.24 | 17.851 | 0.04 | 22.900 | 0.70 |
| Omega-3, 6, 9 caps. | 15.179 | 0.19 | 17.844 | 0.05 | 22.909 | 0.67 |
| Omega-3 + Vit. E solution | 15.198 | 0.30 | 17.856 | 0.02 | 22.920 | 0.63 |
| Norwegian Fish Oil 12/18 1000 mg caps. | 14.828 | 1.70 | 17.332 | 1.37 | 23.246 | 0.42 |
| Omega 3 Forte 1000 mg caps. | 15.320 | 0.96 | 18.441 | 1.48 | 23.604 | 1.57 |
| $\bar{x}$ | 15.143 | | 17.865 | | 23.116 | |
| SD | 0.185 | | 0.39 | | 0.31 | |
| RSD [%] | 1.22 | | 2.18 | | 1.34 | |

**Table-3: Retention times for Methyldocosahexanoate (MDHA), Methyleicosa- Pentaenoate (MEPA), Methylbehenate (MB) and Methylerucate (ME)**

| N: | $t_{R\ MDHA}$ [min.] | $U_{MDHA}$ | $t_{R\ MEPA}$ [min.] | $U_{MEPA}$ | $t_{R\ MB}$ $t_R$ [min.] | $U_{MB}$ | $t_{R\ ME}$ $t_R$ [min.] | $U_{ME}$ |
|---|---|---|---|---|---|---|---|---|
| Doppelherz Омега-3 aktiv + Vit. E caps. | 34.562 | 0.65 | 29.450 | 0.54 | 34.884 | 1.15 | 34.683 | 1.13 |
| Omega-3, 6, 9 caps. | 34.564 | 0.53 | 29.448 | 0.69 | 35.131 | 0.49 | 34.888 | 0.45 |
| Omega-3 Select Softgel | 34.592 | 1.12 | 29.472 | 1.15 | 35.155 | 0.65 | 34.919 | 0.68 |
| $\bar{X}$ | 34.573 | | 29.457 | | 35.057 | | 34.830 | |
| SD | 0.017 | | 0.013 | | 0.15 | | 0.13 | |
| RSD [%] | 0.05 | | 0.04 | | 0.43 | | 0.37 | |

The suitability of the system was confirmed by the lack of a statistically significant difference between the values of the chromatographic parameter retention time in the analysis of Methylmyristate (SD = 0.185, RSD = 1.22 %), Methylpentadecanoate (SD = 0.39, RSD = 2.18 %), Methylheptadecanoate (SD = 0.31, RSD = 1.34 %) (Table 2.); Methyldocosahexanoate (SD = 0.017, RSD = 0.05 %); Methyleicosapentaenoate (SD = 0.013, RSD = 0.04 %; Methylbehenate (SD = 0.15, RSD = 0.43 % Methylerucate (SD = 0.13, RSD = 0.37 % (Table 3.).

**Quantitative analysis**

The quantitative analysis can be accomplished as well by comparison of the chromatographic peak areas of the components of the test sample with comparative reference standard, having a known quantity, by using of methods of the external standard or the internal standard, and by the application of the method of the assessment of the relative values of the chromatographic peak areas of analytes to the sum of the areas of all of the components of the analyzed sanple, when they all are exhibited in the chromatogram and their analytical signals are comparable in intensity.

In the case of analysis of the investigated in the present work fatty acids, the application of GC method with flame ionization detector, provide practically the same respons factors of the signal and for the determination of the content the methyl esters is suitable and appropriate for administration a percentage method.

This method for quantitative analysis is based on the measuring of the area of all peaks in the chromatogram of the hydrolyzed and methylated samples and calculation of the sum, whereby the area of each peak is calculated as a percentage of the total area of the chromatographic peaks.

For analytical samples with entirely volatile components and in case of using of a flame ionization detector, relative peak areas correspond to their percentage content in the analyzed object (Table 4– Table 8)

**Table-4: Retention times, peak areas and content of fatty acids methyl esters in food Supplement Dopppelherz Omega-3 Aktiv + Vit. E caps**

| N: | Methyl ester | $t_R$ [min.] | A | A [%] | C [%] |
|---|---|---|---|---|---|
| 1. | Methylmyristate | 15.188 | 7308.37 | 4.15 | 4.18 |
| 2. | Methylpentadecanoate | 17.851 | 793.91 | 0.45 | 0.45 |
| 3. | Methylheptadecanoate | 22.900 | 1244.24 | 0.71 | 0.00 |
| 4. | Methylnonadecanoate | 26.148 | 476.46 | 0.27 | 0.27 |
| 5. | Methyleicosapentaenoate | 29.450 | 26348.09 | 14.95 | 15.06 |
| 6. | Methyldocosahexanoate | 34.562 | 13821.02 | 7.84 | 7.90 |
| 7. | Methylerucate | 34.683 | 11048.26 | 6.27 | 6.31 |
| 8. | Methylbehenate | 34.884 | 2860.23 | 1.62 | 1.63 |
| 9. | Methyllignocerate | 38.404 | 655.68 | 0.37 | 0.37 |

**Table-5: Retention times, peak areas and content of fatty acids methyl esters in food Supplement Omega-3, 6, 9 caps**

| N: | Methyl ester | $t_R$ [min.] | A | A [%] | C [%] |
|---|---|---|---|---|---|
| 1. | Methylmyristate | 15.179 | 15762.49 | 8.47 | 8.55 |
| 2. | Methylpentadecanoate | 17.844 | 1273.16 | 0.68 | 0.69 |
| 3. | Methylheptadecanoate | 22.909 | 1724.91 | 0.93 | 0.00 |
| 4. | Methylnonadecanoate | 26.138 | 549.48 | 0.30 | 0.30 |
| 5. | Methyleicosapentaenoate | 29.448 | 32866.03 | 17.67 | 17.83 |
| 6. | Methylheneicosanoate | 31.975 | 1135.18 | 0.61 | 0.62 |
| 7. | Methyldocosahexanoate | 34.564 | 14756.01 | 7.93 | 8.01 |
| 8. | Methylerucate | 34.888 | 3410.13 | 1.83 | 1.85 |
| 9. | Methylbehenate | 35.131 | 1176.27 | 0.63 | 0.64 |
| 10. | Methyllignocerate | 38.404 | 1946.56 | 1.05 | 1.06 |

**Table-6: Retention times, peak areas and content of fatty acids methyl esters in food supplement Omega-3 + Vit. E Solution**

| N: | Methyl ester | $t_R$ [min.] | A | A [%] | C [%] |
|---|---|---|---|---|---|
| 1. | Methylmyristate | 15.198 | 20507.55 | 8.33 | 8.46 |
| 2. | Methylpentadecanoate | 17.856 | 1477.74 | 0.60 | 0.61 |
| 3. | Methylheptadecanoate | 22.920 | 1197.53 | 0.49 | 0.00 |
| 4. | Methyleicosapentaenoate | 29.472 | 43292.09 | 17.59 | 17.86 |
| 5. | Methylheneicosanoate | 31.992 | 1524.21 | 0.62 | 0.63 |
| 6. | Methyldocosahexanoate | 34.592 | 24660.61 | 10.02 | 10.17 |
| 7. | Methylerucate | 34.919 | 4737.36 | 1.93 | 1.95 |
| 8. | Methylbehenate | 35.155 | 1478.43 | 0.60 | 0.61 |
| 9. | Methyllignocerate | 38.417 | 1497.00 | 0.61 | 0.62 |

**Table-7: Retention times, peak areas and content of fatty acids methyl esters in food Supplement Norwegian fish oil 12/18 1000 mg caps**

| N: | Methyl ester | $t_R$ [min.] | A | A [%] | C [%] |
|---|---|---|---|---|---|
| 1. | Methylmyristate | 14.828 | 3337.23 | 6.31 | 6.42 |
| 2. | Methylpentadecanoate | 17.332 | 275.82 | 0.52 | 0.53 |
| 3. | Methylheptadecanoate | 23.246 | 341.18 | 0.65 | 0.66 |
| 4. | Methylnonadecanoate | 26.128 | 65.73 | 0.12 | 0.13 |
| 5. | Methyleicosapentaenoate | 28.598 | 10191.58 | 19.28 | 19.60 |
| 6. | Methyldocosahexanoate | 31.399 | 5542.15 | 10.48 | 10.66 |
| 7. | Methylerucate | 31.538 | 1055.07 | 2.00 | 2.03 |
| 8. | Methyllignocerate | 33.675 | 377.95 | 0.71 | 0.73 |

**Table-8: Retention times, peak areas and content of fatty acids methyl esters in food supplement Omrga-3 Forte 1000 mg caps**

| N: | Methyl ester | $t_R$ [min.] | A | A [%] | C [%] |
|---|---|---|---|---|---|
| 1. | Methylmyristate | 15.320 | 4745.45 | 6.19 | 6.30 |
| 2. | Methylpentadecanoate | 18.441 | 399.03 | 0.52 | 0.53 |
| 3. | Methylheptadecanoate | 26.604 | 390.83 | 0.51 | 0.52 |
| 4. | Methylnonadecanoate | 32.215 | 97.74 | 0.13 | 0.13 |
| 5. | Methyleicosapentaenoate | 36.865 | 15507.24 | 20.23 | 20.58 |
| 6. | Methyldocosahexanoate | 41.924 | 8978.30 | 11.71 | 11.92 |
| 7. | Methylerucate | 42.151 | 992.31 | 1.29 | 1.31 |

On Table 9 are summarized the experimental results for the obtained by the applied GC method minimum and maximum quantity of methyl esters of fatty acids in food supplements.

**Table-9: Minimum and maximfum content of fatty acids methyl esters in food Supplements**

| N: | Methyl ester | C Min [%] | C Max [%] |
|---|---|---|---|
| 1. | Methylmyristate | 4.18 | 8.55 |
| 2. | Methylpentadecanoate | 0.45 | 0.69 |
| 3. | Methylheptadecanoate | 0.0 | 0.66 |
| 4. | Methylnonadecanoate | 0.13 | 0.30 |
| 5. | Methyleicosapentaenoate | 15.06 | 20.58 |
| 6. | Methyldocosahexanoate | 7.90 | 11.92 |
| 7. | Methylerucate | 1.31 | 6.31 |

## CONCLUSION

In the analysed food supplement the maximum concentration was found for Methyleicosapentaenoate (20.58 %) and Methyldocosahexanoate (11.92 %) and the minimun concentration was observed for: Methylheptadecanoate (0%), Methylnonadecanoate (0.13%); Methylpentadecanoate (0.45 %).

The method is useful for identification and determination of the content of Omega-3 fatty acids in certain food additives.

## REFERENCES


1. Ubeda N, Achon M, Varela-Moreiras G. Omega 3 fatty acids in the elderly. Br J Nutr, 2012; 107(Suppl 2):137-151.
2. De Caterina R, Basta G. n-3 fatty acids and the inflammatory response – biological background. Eur Heart J Suppl, 2001;3(Suppl. D):2-9.
3. Mori TA, Beilin LJ. Omega-3 fatty acids and inflammation. Curr Atheroscler Rep, 2004;6(6):461-467.
4. Campoy C, Escolano-Margarit MV, Anjos T, Szajewska H, Uauy R. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. Br J Nutr. 2012;107(Suppl. 2):85-106.
5. Innis SM. Dietary (n-3) fatty acids and brain development. J Nutr. 2007;137(4):855-859.
6. Cederholm T, Salem N, Palmblad J. ω-3 fatty acids in the prevention of cognitive decline in humans. J Adv Nutr. 2013;*4(6):672-676*.
7. Breslow J. n-3 fatty acids and cardiovascular disease. Am J Clin Nutr. 2006;83(6 Suppl):1477-1482.
8. Delgado-Lista J, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. Br J Nutr, 2012;107 (Suppl 2):201-213.
9. Connor WE, Prince MJ, Ullmann D, Riddle M, Hatcher L, Smith FE, Wilson D. The Hypotriglyceridemic Effect of Fish Oil in Adult-Onset Diabetes without Adverse Glucose Control a. Annals of the New York Academy of Sciences. 1993 Jun;683(1):337-40.
10. Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli *M*. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med, 1996;334(24):1557-*1560*.
11. Shahar E, Folsom AR, Melnick SL, Tockman MS, Comstock GW, Gennaro V, Higgins MW, Sorlie PD, Ko WJ, Szklo M. Dietary n-3 polyunsaturated fatty acids and smoking-related chronic obstructive pulmonary disease. New England Journal of Medicine. 1994 Jul 28;331(4):228-33.
12. Burr ML, Gilbert JF, Holliday RA, Elwood PC, Fehily AM, Rogers S, Sweetnam PM, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). The Lancet. 1989 Sep 30;334(8666):757-61.
13. Guesnet P, Alessandri JM. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)–implications for dietary recommendations. Biochimie. 2011 Jan 1;93(1):7-12.
14. McCann JC, Ames BN. Is docosahexaenoic acid, an n− 3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals–. The American journal of clinical nutrition. 2005 Aug 1;82(2):281-95.
15. Quinn JF, Raman R, Thomas RG, Yurko-Mauro K, Nelson EB, Van Dyck C, Galvin JE, Emond J, Jack CR, Weiner M, Shinto L. Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. Jama. 2010 Nov 3;304(17):1903-11.
16. Carlson SE. Docosahexaenoic acid supplementation in pregnancy and lactation–. The American journal of clinical nutrition. 2008 Dec 30;89(2):678S-84S.
17. Uauy R, Hoffman DR, Mena P, Llanos A, Birch EE. Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. J Pediatr. 2003;143(Suppl. 4):17-25.
18. Al-Alawi. A, Van de Voort FR, Sedman J. New FTIR method for the determination of FFA in oils. J Amer Oil Chemist Soc. 2004;81(5):441-446.
19. Ohta A, Mayo MC, Kramer N, Lands WEM. Rapid analysis of fatty acids in plasma lipids. Lipids, 1990;25(11):742-747.
20. De Castro Barra PM, Barra MM, Azevedo MS, Fett R, Micke GA, Costa AC, de Oliveira MA. A rapid method for monitoring total trans fatty acids (TTFA) during industrial manufacturing of Brazilian spreadable processed cheese by capillary

zone electrophoresis. Food Control. 2012 Feb 1;23(2):456-61.
21. Oliveira MAL, Solis VES, Gioielli LA, Polakiewicz B, Tavares MFM. Method development for the analysis of trans-fatty acids in hydrogenated oils by capillary electrophoresis. Electrophoresis. 2003;24(10):1641-1647.
22. Vergara-Barberan M, Escrig-Domenech A, Lerma-Garcia MJ, Simo-Alfonso EF, Herrero-Martinez JM. Capillary electrophoresis of free fatty acids by indirect ultraviolet detection: application to the classification of vegetable oils according to their botanical origin. J Agric Food Chem, 2011;59(20):10775-10780.
23. Collet J, Gareil P. Micellar electrokinetic chromatography of long chain saturated and unsaturated free fatty acids with neutral micelles-Considerations regarding selectivity and resolution optimization. J Chromatogr A. 1997;792(1-2):165-177.
24. De Oliveira MA, Porto BL, Faria ID, de Oliveira PL, de Castro Barra PM, Castro RD, Sato RT. 20 years of fatty acid analysis by capillary electrophoresis. Molecules. 2014 Sep 9;19(9):14094-113.
25. Gazi S, Ali S, Luqman S, Rizwan SH. Gas chromatography mass spectrometry analysis of ethanolic extract of roots of *Rubia cardifolia* (Manjistha). Asian J Pharm Clin Res, 2016;9(1):91-93.
26. Parveen S, Shahzad A, Upadhyay A, Yadav V. Gas chromatography-mass spectrometry analysis of methanolic leaf extract of *Cassia angustifolia Vahl*. Asian J Pharm Clin Res, 2016;9(3):111-116.
27. Doshi GM, Matthews BD, Chaska PK. Gas chromatography-mass spectrometry studies on ethanolic extract of dried leaves of *Catharanthus roseus*. Asian J Pharm Clin Res, 2018;11(6):336-340.
28. Painuli S, Rai N, Kumar N. Gas chromatography and mass spectrometry analysis of methanolic extract of leaves of *Rhododendron arboreum*. Asian J Pharm Clin Res, 2016;9(1):101-104.
29. El-Amri J, El-Badaoul K, Haloui Z. The chemical composition and the antimicrobial properties of the essential oil extracted from the leaves of *Theuctium capitatum L*. Asian J Pharm Clin Res. 2017;10(2):112-115.
30. Huang Z, Wang B, Crenshaw AA. A simple method for the analysis of trans fatty acid with GC-MS and AT™-Silar-90 capillary column. Food Chem. 2006;98(4):593-598.
31. Salimon J, Omar TA, Salih N. An accurate and reliable method for identification and quantification of fatty acids and trans fatty acids in food fats samples using gas chromatography. Arabian J Chem. 2017;10(2):1875-1882.
32. Quehenberger O, Armando AM, Dennis EA. High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography-mass spectrometry. Biochim Biophys Acta. 2011; 1811(11):648-656.
33. Yi T, Li SM, Fan JY, Fan LL, Zhang ZF, Luo P, Zhang XJ, Wang JG, Zhu L, Zhao ZZ, Chen HB. Comparative analysis of EPA and DHA in fish oil nutritional capsules by GC-MS. Lipids in health and disease. 2014 Dec;13(1):190.
34. Nikhade RR, Deshpande SA, Kosalge SB. Column chromatographic isolation of Docosahexaenoic acid from fish oil and its assessment by analytical techniques. Int J Pharm Pharm Res Human, 2017;9(1):49-58.
35. Hadjieva BR, Tsvetkova DD, Obreshkova DP. Development and optimization of chromatographic systems for separation of fatty acids by gas chtomatographic method. World J Pharm Pharm Sci. 2017;6(4):12-23.
36. Madhav NVS, Ojha A, Singh S. Validation: a significant tool for enhancing qualities of pharmaceutical products. Sch Acad J Pharm. 2017;6(6):288-299.