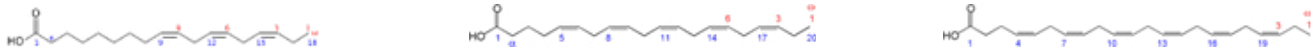
Analysis of omega 3 supplements by GC-FID

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are lipidic compounds that present more than two double bonds distributed along the backbone. Based on the position of the first double bond, starting from the methyl end, PUFAs are mainly classified as omega 3 and omega 6 fatty acids. The most important fatty acids from the omega 3 group are short chain PUFA alpha-linolenic acid (ALA, 18:3n-3), and long chain PUFA eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (structures shown in Figure 1).



ALA EPA DHA

Figure 1. Structures of the three most relevant omega 3 fatty acids.

ALA is an essential fatty acid but its deficiency is rare. EPA and DHA can be produced from ALA, but this bioconversion is not very efficient, and in rich omega-6 diets it is even less promoted. Dietary sources of EPA and DHA are fatty fish, such salmon, herrings, sardines, mackerel, and anchovies, or increasingly common via nutritional supplementation derived from fish, algae or krill oils. Supplementation with EPA and DHA have been reported to have positive effects on human health, such as on heart disease in specific genetic groups [1], on insulin resistance in diabetes [2], on decreasing the ratio of preterm birth [3], on gut microbiome acting as a prebiotic [4], or on chronic diseases that could be linked to a high ratio omega-6/omega-3 [5, 6].

Analytical determination of fatty acids in fish oil through the American Oil Chemists' Society AOAC Ce 1i-07 and 991.39 official methods use gas chromatography coupled to flame ionization detector (GC-FID) employing a polyethylene glycol (PEG) capillary column. PEG columns are an excellent choice for determining the composition of fatty acid in samples were trans isomers are not expected to be present.

Naturally occurring EPA and DHA are all-cis isomers. However, trans isomers can be formed in highly enriched oils¬ during deodorization and enrichment processes through thermal isomerization. This could be explained by the high temperatures (up to 200ºC) that can be reached during the deodorization step [7], and for the repeated distillation processes for EPA and DHA purification (up to 160 ºC under high vacuum) [8].

The presence of trans isomers not only can led to overestimate the amount of EPA and DHA, but also are potentially harmful to human health [9]. Thus, it is pertinent to monitor trans isomers in omega 3 supplements.

Trans isomers cannot be analyzed in PEG columns due to the lack of selectivity towards these compounds, but it is feasible with a long and efficient cyanopropyl stationary phase column. Here, we used a 100% polyethylene glycol (PEG) and a 100% biscyanopropyl column for the GC-FID analysis of three commercial EPA and DHA supplements, targeting FAME compounds with the former, and the presence of *cis/trans* isomers with the latter for a more comprehensive analysis.

MATERIAL, METHODS AND RESULTS

Fish oil analytical standard from marine source (PUFA nº1), 12% BF3 methanolic solution, sodium chloride, and sodium hydroxide were purchased from Merck (Madrid, Spain). Romil solvents were obtained from Teknokroma (Sant Cugat del Vallès, Spain).

Omega-3 supplements were purchased online or in local stores. All three supplements were soft gel capsules containing different amounts of DHA and EPA (see Table 1).

Table 1. Declared information on sample product label

Sample	Source	Processing	DHA (mg/capsule)	EPA (mg/capsule)
Sample A	Anchovies	Molecular distillation	800	300
Sample B	Cold waters wild fish	Molecular distillation	240	360
Sample C	Anchovies	Filtration	250	350

Sample methylation was done according to procedure described in AOAC official method 991.39 but adjusted to 100mg of oil. A blank of the methylation process was prepared as a reference. The addition of internal standard was not considered due to the qualitative nature of the study. As a standard for FAME identification, we used a marine source fish oil, PUFA nº 1 (Sample D in Figures 2 and 3).

FAME samples were manually injected into a gas chromatograph Agilent 7890B GC-FID equipped with a split/splitless injector. Columns and chromatographic conditions used for each type of analysis are detailed in Tables 2 and 3.

Table 2. Column and chromatographic conditions for FAME analysis

Column	**Sapiens-FAMEWAX**, 20m x 0.18mm x 0.18µm, **TR-900984** (Teknokroma Analítica, Sant Cugat del Vallès, Barcelona, Spain)
Carrier gas	H2, ct. flow 2ml/min
Injector	275ºC Split 200:1 Single Taper with wool 0.3µl FAME sample
Oven program	Ramp 1: 140ºC @ 20ºC/min to 190ºC (3min) Ramp 2: @ 5ºC/min to 220ºC (5min)
Detector	FID, 280ºC H2, 30ml/min Air, 400ml/min Make-up gas N2, 25ml/min

Table 3. Column and chromatographic conditions for *cis/trans* FAME analysis

Column	**TR-CN100**, 75m x 0.18mm x 0.14µm, **TR-881674** (Teknokroma Analítica, Sant Cugat del Vallès, Barcelona, Spain)
Carrier gas	H2, ct. flow 1.5ml/min
Injector	260ºC Split 100:1 Single Taper with wool 0.3µl FAME sample
Oven program	Ramp 1: 60ºC (6min) @ 25ºC/min to 180ºC Ramp 2: @ 1ºC/min to 220ºC (5min)
Detector	FID, 260ºC H2, 30ml/min Air, 400ml/min Make-up gas N2, 25ml/min

FAME and *cis/trans* FAME composition of omega 3 supplements were analyzed using two 0.18mm narrow bore capillary columns. Column dimensions were specifically selected to reduce analysis time and increase separation efficiency, even though the separation conditions were not fully optimized. The Sapiens-FAMEWAX is a polyethylene glycol stationary phase column specially designed for the optimal separation of FAME in food samples. The TR-CN100 column is coated with a very polar polymer (biscyanopropylsilicone) that establishes different degree of interaction with *cis* and *trans* isomers and therefore can be identified separately. The chromatographic separation of FAMES present in the three omega 3 supplements, and studied using both columns, are shown in Figure 2 and 3 respectively.

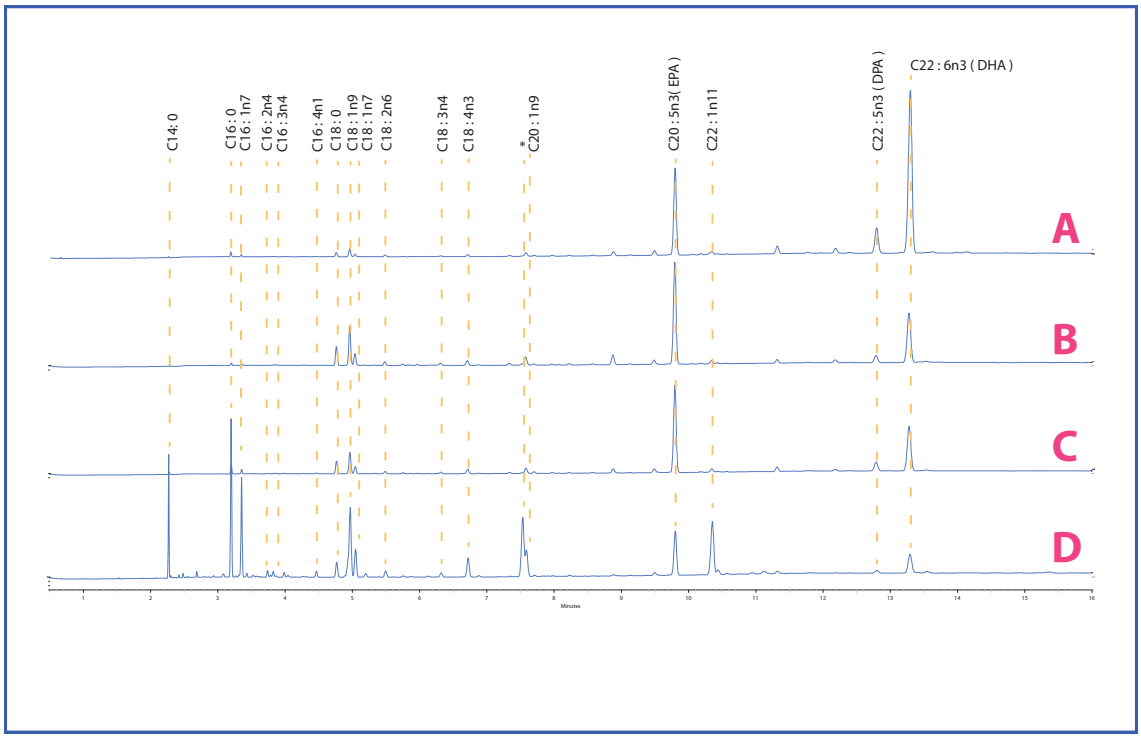


Figure 2. Chromatographic separation of FAME compounds found present in the three omega 3 supplements and the standard fish oil obtained with the **Sapiens-FAMEWAX 20m x 0.18mm x 0.18µm** capillary column. Trace A, B, C correspond to Samples A, B, C respectively and trace D corresponds to PUFA nº 1 standard.

Qualitatively, trace in Figure 2 corresponding to sample A confirms the higher concentration of DHA and the higher purity regarding C18:0 and C18:1 isomers in the region around minute 5 compared to samples B and C while EPA concentration is similar in all three samples. Since molecular distillation separates by boiling point, another DHA closely related omega 3 fatty acid is also concentrated during the process, the docosapentaenoic acid (n-3 DPA, C22:5n3, minute 20 to 21). Traces B and C have a very similar chromatographic profile, suggesting that fish type employed to produce Sample B is also anchovy, one of the most used fishes for producing omega 3 supplements due to the naturally low concentration of heavy metals, PDB and other contaminants. As can be seen by comparison with the reference fish oil at minute 2 to 4.5, distillation mostly clears out all the C14:0 and C16 FAME.

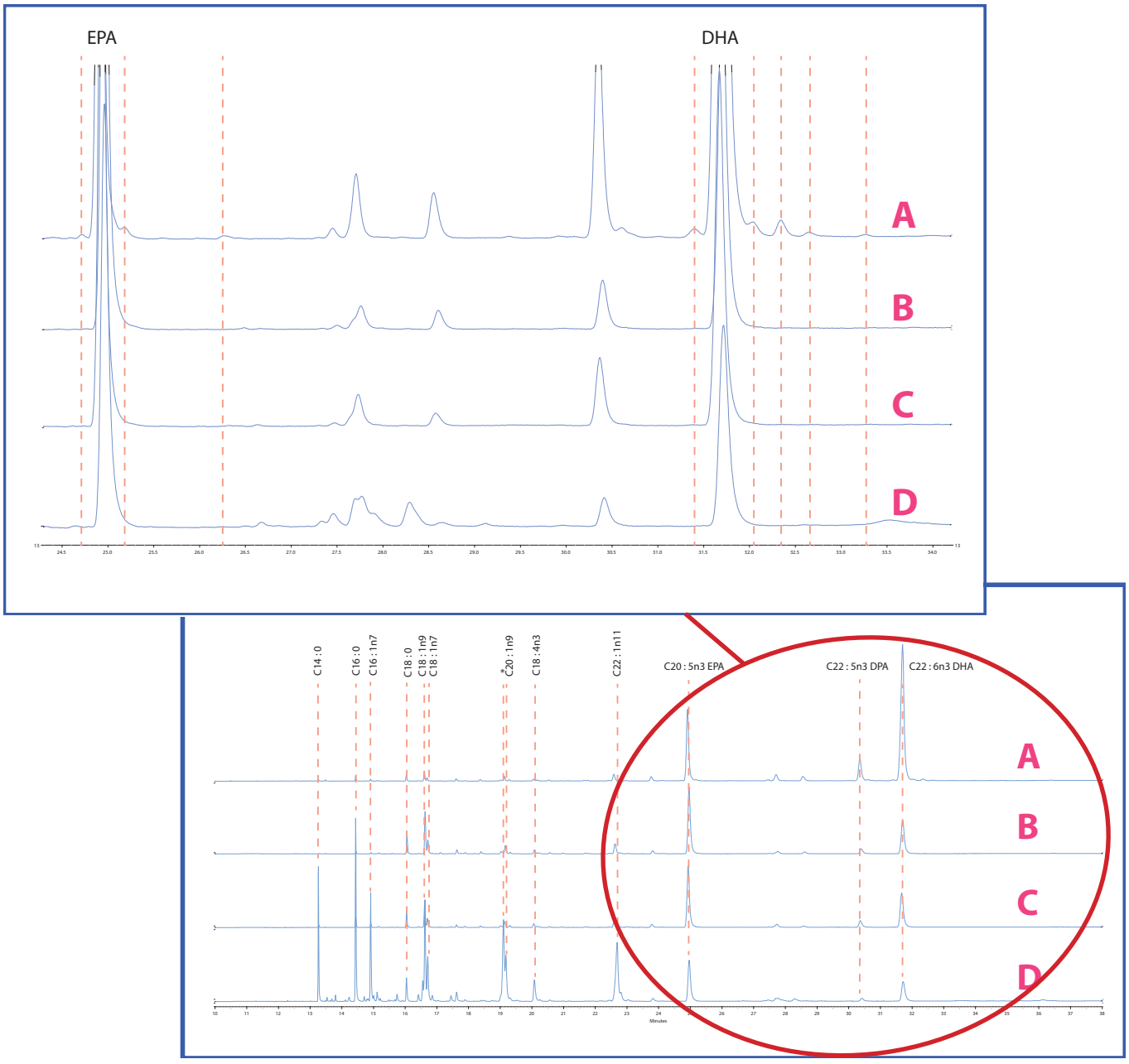


Figure 3. Gas chromatograms of FAME composition of the omega 3 samples and the standard analyzed with the **TR-CN100 75m x 0.18mm x 0.14µm.** Trace A, B, C correspond to Samples A, B, C respectively and trace D corresponds to PUFA nº 1 standard. Zoom of minute 24 to 34, reveals scattered small peaks surrounding EPA and DHA in trace A. These peaks allegedly correspond to *trans*-isomers formed during the processing of fish oil.

In Figure 3, the most substantial difference at macro level compared to the separation with the PEG column is the inversion in the elution order of peaks at minute 22 to 26 (cetoleic acid and EPA). A closer look to trace A also reveals that the surroundings of EPA and DHA peaks are populated with several small peaks. Based on the literature [10], these peaks spread ahead and after all-cis EPA and DHA correspond to trans isomers of these omega 3 fatty acids. Those peaks are not observed in any of the other two supplements nor on the fish oil standard. Differences between sample A and samples B and C could suggest that none of the latest have been subjected to deodorization, or that sample A has gone through multiple distillation steps, ending in the partial thermal isomerization of EPA and DHA. Further experiments will be needed for fine tuning the chromatographic method for an optimized separation of the isomers and for accurate peak identification.

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