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EPA and DHA Quantity in Fish Oil Capsules

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Abstract

Omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenic acid (DHA) are essential to the human diet, and have been linked to many health benefits.[6] Fish oil supplements containing EPA and DHA are produced at varying cost to consumers; however, studies have found no correlation between the price of a fish oil supplement and the quantity of EPA and DHA[4]. This research will allow consumers who are selecting a fish oil based on the quantity of EPA and DHA in their fish oil for health reasons to choose the product with the best quantity and price for the quantity of EPA and DHA that the consumer desires. Gas Chromatography (GC) was used to identify and quantify EPA and DHA in three brands of fish oil supplements: generic Walgreens brand, Dollar Store brand, Nature's Measure and Whole Foods brand, Barleans.

Keywords: EPA, DHA, Fish Oil

1. Introduction

Increasing consumption of omega-3 fatty acids such as EPA and DHA has shown to reduce the risk of Alzheimer's disease, depression, high blood pressure, myocardial infarction, and cerebrovascular accident (stroke)[1,4]. EPA and DHA are essential fatty acids, meaning they are not naturally produced in the body, therefore, they must be consumed in the diet. A rich dietary source of EPA and DHA is marine fish.

The American Heart Association (AHA) recommends that a person eat two servings of fish a week[1], however, many consumers prefer to obtain DHA and EPA in the form of supplements. Fish oil supplements are not regulated by any federal agency so there is the possibility of adulteration. It has been found that there is no correlation between the price of fish oil and the quality[4]. Our goal was to compare the amount of EPA and DHA in three different brands of commercially available fish oil. palmitate, a 16-carbon saturated fatty acid, was also analyzed because it is the third major component of fish oil. Gas chromatography was used to quantify these three fatty acids in each fish oil.

EPA and DHA are unsaturated omega-3 fatty acids with five and six double bonds, respectively. In fats and oils, fatty acids occur in the form of a triglyceride ester which must be hydrolized and transesterified to facilitate GC analysis. A methyl ester of the fatty acid was chosen because the lower boiling point reduces the time needed for chromatographic analysis. A two-step transesterification reaction was performed based on work by Wang et. al.[2]. This process involves hydrolyzing the triglyceride in methanolic KOH, then completing the reaction with the addition of sulfuric acid. The initial KOH step hydrolyzes the triglyceride and forms the methyl ester, but is complicated by co-formation of some carboxylate (because of moisture present in the reaction), so the subsequent acid step is thought to increase the yield of the methyl ester by protonating and methylating this carboxylate.

Separating the methyl esters of EPA and DHA using gas chromatography is difficult because the multiple sites of unsaturation make isomerization under these acidic transesterification conditions likely. This causes multiple unresolved peaks with closely spaced retention times. For this experiment, we integrated three areas of interest in the chromatogram corresponding to the methyl palmitate (which was well resolved because it is a saturated fatty acid

not prone to isomerization upon transesterification), methyl EPA and its isomerization products, and methyl DHA and its isomerization products. Under the conditions of our experiment, the MXT-Wax column separates methyl esters based on boiling point, so the first eluting component was the methyl palmitate, and the methyl ester of the 20 carbon EPA fatty acid eluted next, followed by the methyl ester of the 22 carbon DHA fatty acid. Structures of the fatty acids are palmitic acid $CH_3(CH_2)_{14}COOH$; EPA $CH_3(CH_2CHCH)_5(CH_2)_3COOH$; and DHA $CH_3(CH_2CHCH)_6(CH_2)_2COOH$.

2. Method

2.1 Transesterification

The fish oil was heated with 53 mL of methanol and 7 mL of 10M methanolic KOH in a 55ºC water bath for 1.5 hours. Then 3 mL of concentrated H_2SO_4 was slowly added, and the mixture was heated for an additional 1.5 hours. Next, the mixture was cooled and added to 30 mL of hexane in a separatory funnel, and the fatty acid methyl esters were extracted in the hexane solution. Excess hexane was evaporated, and the methyl esters were analyzed by gas chromatography. Each of the three fish oils was transesterified in triplicate, and the gas chromatography was performed once for each transesterification reaction. There were three main areas of fatty acid methyl esters apparent on the chromatograph, assigned to methyl palmitate, methyl EPA and methyl DHA. These areas were integrated, and percentages calculated for the three components. The percent of each component (palmitate, EPA and DHA) was averaged for each fish oil supplement. We are assuming that each fatty acid is methylated to the same extent, so that the relative amounts of each in the triglyceride is still correctly described by the gas chromatography even if the transesterification is not 100%.

2.2 Gas Chromatography

A SRI 310 Gas Chromatograph was used with a 30m MXT-Wax capillary column. Several different heating protocols were examined, and the protocol that gave the best separation was as follows: initial temperature 80 ºC, hold 3.0 min, ramp 20 ºC/min to 160 ºC, then ramp 0.6 ºC/min to 176 ºC, then ramp 5 ºC/min to 233 degrees Celsius. Injection volume was 1 μL, and the sample was diluted with hexane in order to get the best resolution possible.

The Gas Chromatography column separates based on the boiling point of the components in the fish oil. As palmitate has the lowest boiling point then EPA or DHA, it was the first fatty acid methyl ester to come off the column, followed by EPA, then, DHA which has the most carbons of the three, indicating that it would be the last component off the column.

3. Results

For each fish oil, the transesterification reaction was carried out in triplicate, and the resulting methyl ester mixture was analyzed by gas chromatography. The mean and standard deviation for each of the three fatty acid methyl esters was calculated from percent composition deduced for each chromatogram.

Table 1. Chromatographic data

		percent palmitate	percent EPA	percent DHA
Walgreens 1		16.2	60.2	23.6
Walgreens 2		15.5	57.5	27.0
Walgreens 3		15.1	65.8	19.1
		Mean = 15.6, S.D. = 0.6	Mean = 61.2, S.D. = 4.3	Mean = 23.2, S.D. = 4.0
Nature measure 1		24.9	44.5	32.1
Nature measure 2		17.1	54.4	28.6
Nature measure 3		16.7	35.4	48.0
		Mean = 19.6, S.D. = 4.6	Mean = 44.7, S.D. = 9.5	Mean = 36.2, S.D. = 10.3
Barleans 1		13.5	38.0	48.5
Barleans 2		10.1	30.9	59.0
Barleans 3		16.7	38.0	12.7
		Mean = 13.5, S.D. = 3.3	Mean = 35.6, S.D. = 4.1	Mean = 40.1, S.D. = 24.3
P-value		0.1524	0.0079	0.4257

Our work did not show any significant difference in the palmitate or DHA levels in the fish oils tested. For EPA, there was a p-value of .0079 to indicate that there is a significant difference in EPA values.

3.1 Example Chromatographs:

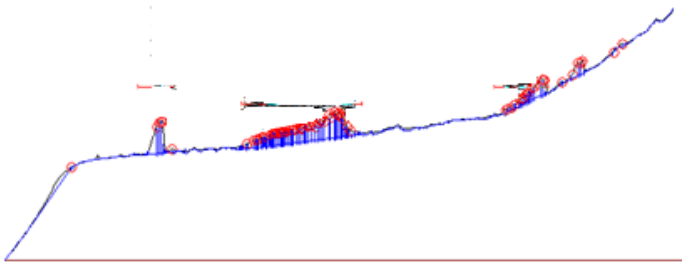

Figure 2. Walgreens

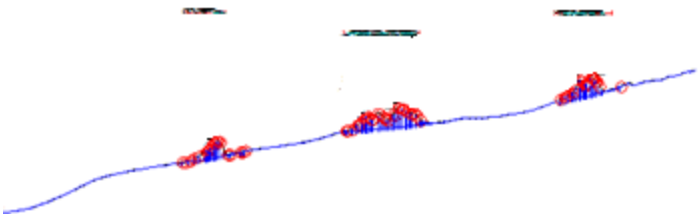

Figure 3. Nature's Measure

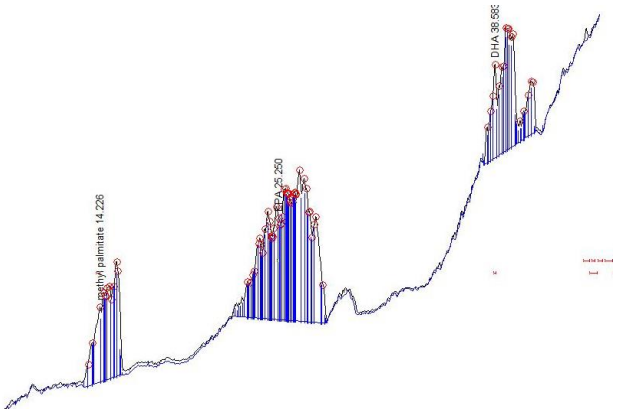

Figure 4. Barleans'

4. Conclusion

The three brands of fish oil did not show a significant difference in palmitate levels, but the content level of EPA in the three brands did show a significant difference. The Family Dollar store brand, Nature's Measure, showed almost twice as much EPA as the more expensive Wholefoods brand, Barleans and significantly more than the Walgreens brand. As the brands of fish oil are sold at different prices, the consumer would obtain more EPA from the Family Dollar brand at a more reasonable price. It can be concluded that consumers interested in obtaining more EPA in their diet would benefit from obtaining the inexpensive Nature's Measure brand from Family Dollar as it contained the most EPA out of the three brands.

5. Acknowledgement

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6. References

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