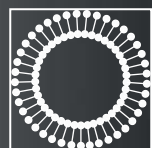


Fatty Acid Analysis

GC/MS

Analytical Services



Avanti[®]
POLAR LIPIDS, INC.

Quantitative Fatty Acid Analysis in Commercial Fish Oils by Gas Chromatography with Mass Spectrometric Detection

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Introduction:

Cold water fish oils contain high quantities of eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) acids. These fatty acids have multiple general health benefits when consumed in the daily diet or as a nutritional supplement. The measurement of these and other essential omega-3 and omega-6 fatty acids have historically been performed by gas chromatographic methods utilizing flame ionization detection. This method is most commonly performed to provide a fatty acid profile based on total normalized peak intensity; however, this technique is not suitable for content or content uniformity assay of the desired fatty acids on a weight / sample basis necessary to fulfill the requirements of current good manufacturing practices (cGMP's) enforced for pharmaceutical and nutraceutical industries. Avanti's Analytical Services utilizes gas chromatography / mass spectrometry (GC/MS) to measure the content of fatty acid in lipid samples. We demonstrate this methodology in the measurement of 40 potential fatty acids in commercially available fish oil samples.

Principle:

GC/MS has long been used for the selective analysis of non-polar compounds. The carboxylic fatty acids require prior hydrolysis from their glycerolipid sources and derivatization to a respective ester form for separation on capillary chromatographic columns. The detection of structural molecular ions generated from the MS source provides more sensitive and selective assay of varied arrays of fatty acids present in lipid samples. Fast quadrupole technology present in most modern mass spectrometers facilitate for selective ion monitoring with simultaneous full scan capabilities. This allows selective quantitation of molecular specific ion peaks while retaining a full mass spectrum of each peak for interpretation, confirmation or library searching.

The quantitative analysis of fatty acids using GC/MS requires several pre-requisites. For each compound to be accurately measured, a standard for each fatty acid of known concentration and purity must be utilized. Fatty acids of differing carbon lengths and degree of unsaturation produce varied concentration versus response curves when ionized at a common electronic potential of -70 eV. The analytical range, calibration curve equation and limits of accurate quantitation must be known prior to reporting results. This is also a reason why the GC/MS should not be utilized for normalized area % profiling as performed by flame ionization detectors. Secondly, an internal standard should be utilized in the extraction / hydrolysis / derivatization of the sample. This internal standard normalizes or corrects for sample-to-sample preparation variance and standard to sample matrix differences. Of course, the internal standard(s) should be chosen based on the diversity, complexity and abundance

of fatty acids and should not be naturally present in the sample matrix.

The data below reports the GC/MS analysis of fatty acids present in commercially available fish oils. Figures 1 and 2 show the total ion chromatograms of a standard mixture and a oil sample, with the respective mass spectra generated from the DHA peak of each. Table 1 reports the assay of 5 separate lots of fish oil performed by our GC/MS method.

Data:

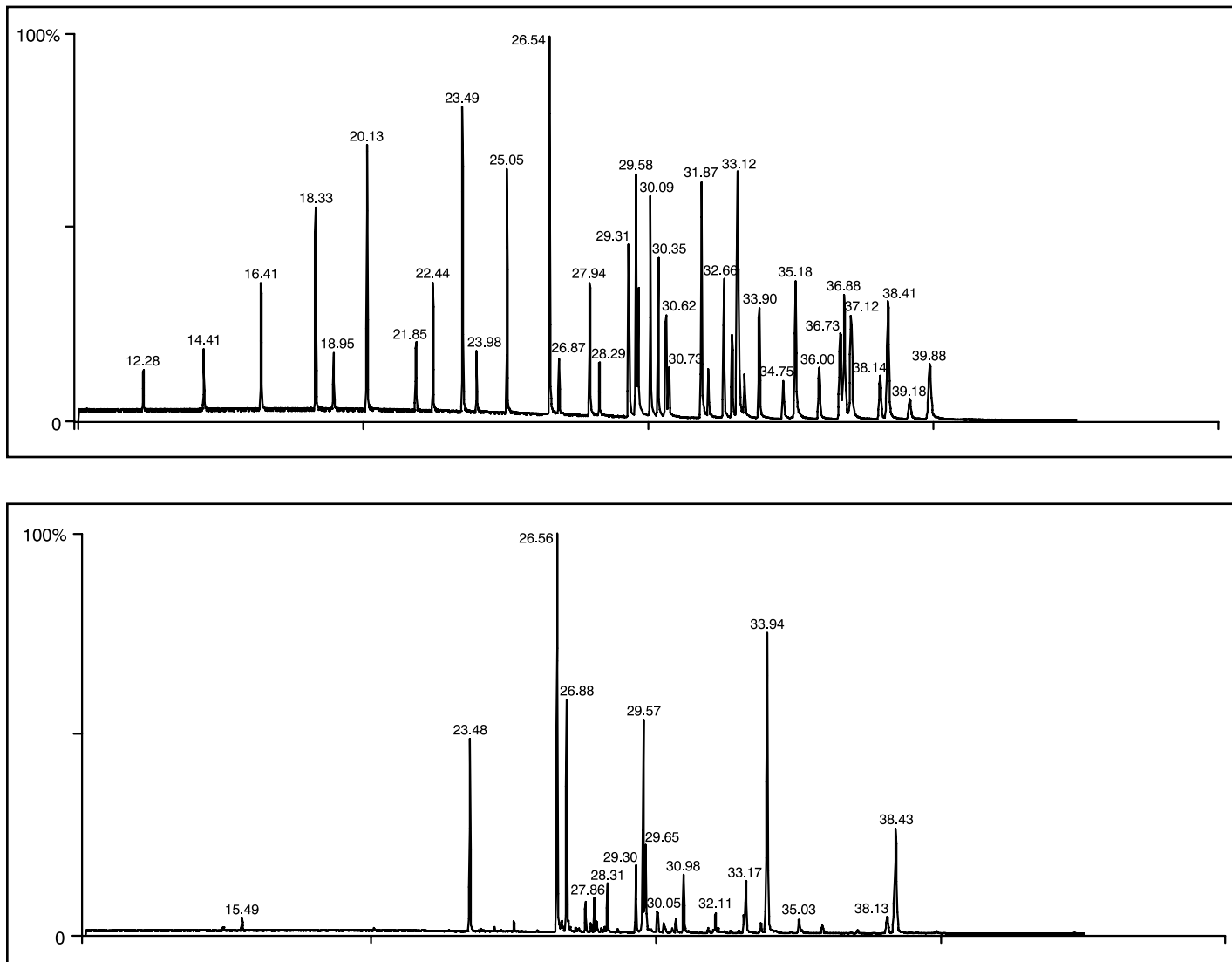


Figure 1: Total ion chromatogram of fatty acid standard (Top panel) and fish oil sample (Bottom panel). Peak at 33.2 min is 21:0 internal standard.

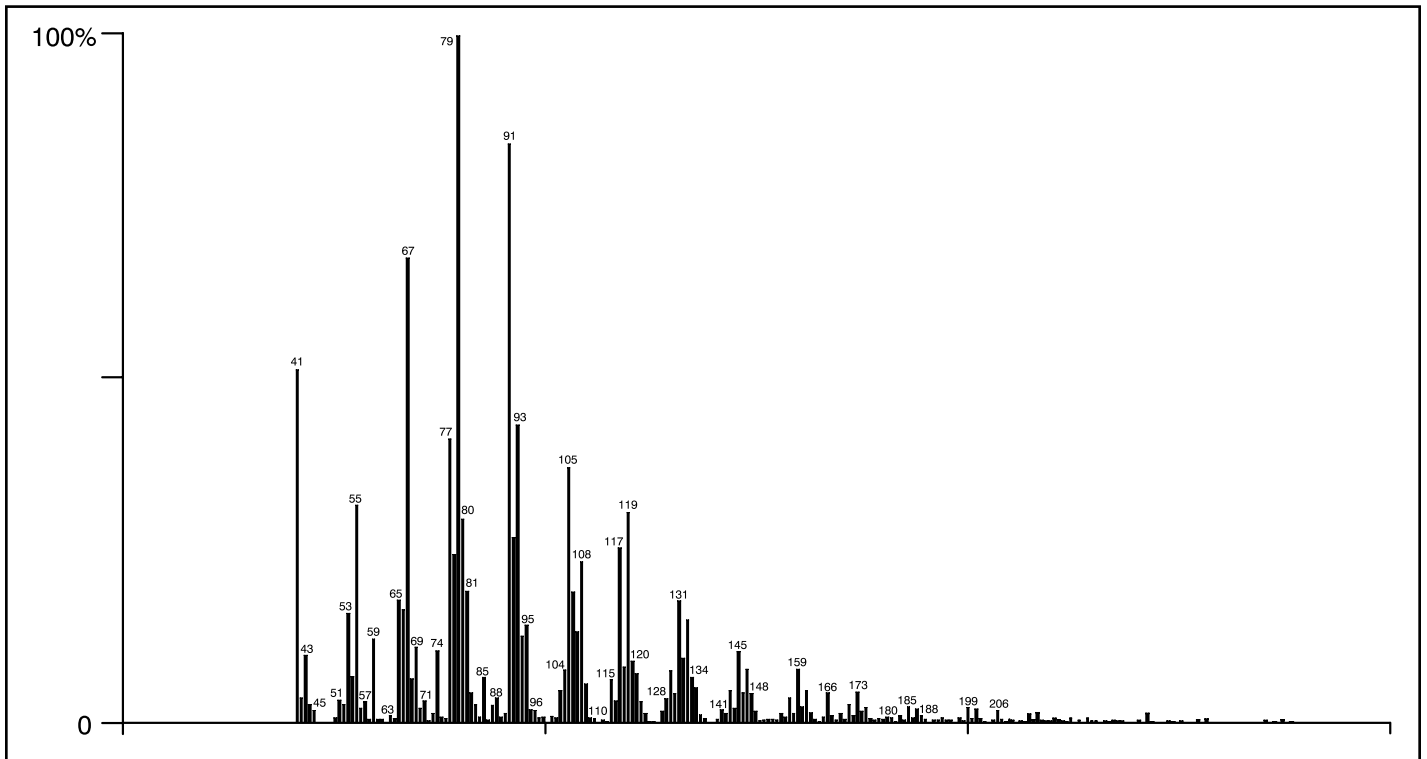
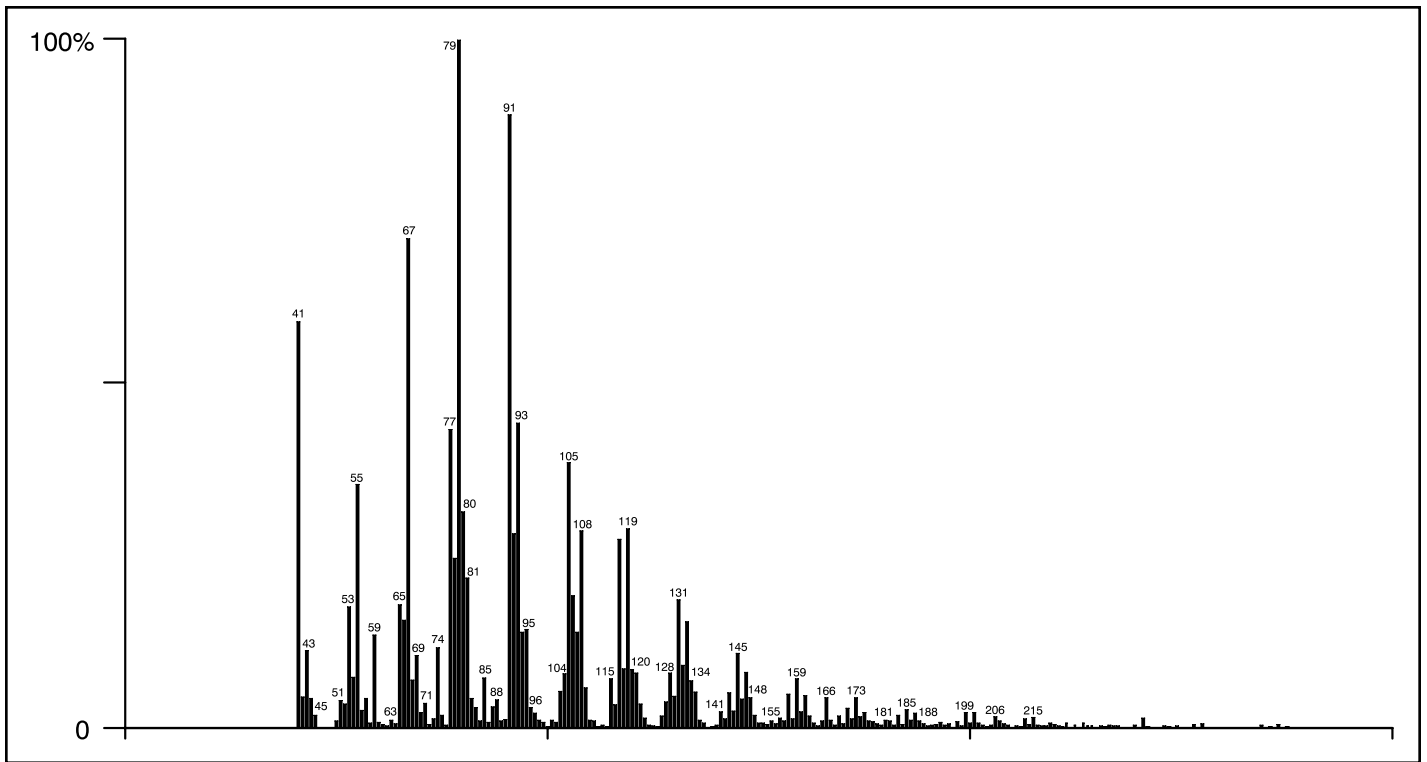


Figure 2: Mass spectrum of DHA in standard and fish oil sample.
 Standard @ 38.41 min. (Top panel)
 Fish oil @ 38.43 min. (Bottom panel)
 Background subtracted.

Table 1: Fatty acid quantitative data for 5 lots of fish oil.
Results expressed in weight % of fatty acid in sample.

Rt (min.)	Fatty Acid	M.W.	#1	#2	#3	#4	#5	mean	s.d.
12.28	8:0	144.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14.10	9:0	158.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.41	10:0	172.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18.31	11:0	186.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19.95	11:1	184.30	0.00	0.00	0.00	0.04	0.00	0.01	0.02
20.13	12:0	200.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21.83	13:0	214.35	0.02	0.02	0.03	0.04	0.02	0.03	0.01
22.44	13:1	212.43	0.00	0.00	0.00	0.10	0.00	0.02	0.04
23.47	14:0	228.38	7.43	6.97	6.80	6.85	7.22	7.05	0.26
23.97	14:1	226.38	0.07	0.06	0.08	0.09	0.06	0.07	0.01
25.03	15:0	242.41	0.36	0.35	0.36	0.37	0.36	0.36	0.01
26.52	16:0	256.43	15.93	15.41	14.89	15.81	15.12	15.43	0.44
26.85	16:1	254.43	6.40	6.32	6.10	6.21	6.27	6.26	0.11
27.94	17:0	270.48	0.46	0.46	0.45	0.48	0.44	0.46	0.01
28.27	17:1	268.48	0.27	0.03	0.04	0.04	0.02	0.08	0.11
29.29	18:0	284.48	3.17	3.14	2.98	3.19	3.13	3.12	0.08
29.56	18:1 cis (n-9)	282.48	8.83	9.41	10.14	10.07	10.29	9.75	0.61
29.64	18:1 cis (n-6)	282.48	2.80	2.61	2.79	2.59	2.63	2.68	0.10
30.06	18:2 (n-6)	280.48	1.65	1.71	1.70	1.75	1.65	1.69	0.04
30.35	18:3 (n-6)	278.48	0.26	0.25	0.33	0.31	0.24	0.28	0.04
30.59	19:0	298.51	0.16	0.16	0.17	0.19	0.16	0.17	0.01
30.72	18:3 (n-3)	278.48	0.85	0.89	0.92	0.95	0.85	0.89	0.04
31.85	20:0	312.54	0.32	0.32	0.33	0.51	0.33	0.36	0.08
32.11	20:1(n-9)	310.54	1.39	1.41	1.38	1.61	1.37	1.43	0.10
32.66	20:2 (n-6)	308.53	0.28	0.29	0.36	0.40	0.28	0.32	0.05
32.94	20:3 (n-6)	306.53	0.18	0.19	0.23	0.24	0.18	0.21	0.03
33.12	20:4 (n-6)	304.52	0.98	0.98	1.26	1.41	0.90	1.11	0.22
33.18	20:3 (n-3)	306.53	0.00	0.00	0.13	0.18	0.00	0.06	0.09

Table 1: (cont.) Fatty acid quantitative data for 5 lots of fish oil.
Results expressed in weight % of fatty acid in sample.

Rt (min.)	Fatty Acid	M.W.	#1	#2	#3	#4	#5	mean	s.d.
37.73	22:0	340.59	0.11	0.12	0.14	0.16	0.12	0.13	0.02
35.15	22:1 (n-9)	338.59	0.28	0.43	0.47	0.52	0.40	0.42	0.09
33.90	20:5 (n-3)	302.52	15.03	15.02	15.13	14.74	14.85	14.96	0.16
35.99	22:2 (n-6)	336.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37.13	22:3 (n-3)	334.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00
37.86	22:4 (n-6)	332.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
38.12	22:5 (n-3)	330.57	2.19	2.22	2.21	2.05	2.12	2.16	0.07
38.39	22:6 (n-3)	328.57	10.60	10.64	11.45	12.07	10.22	11.00	0.75
36.70	23:0	354.61	0.00	0.00	0.12	0.13	0.00	0.05	0.07
39.16	24:0	368.64	0.09	0.00	0.11	0.13	0.00	0.06	0.06
39.86	24:1	366.63	0.43	0.44	0.49	0.55	0.43	0.47	0.05
	TOTAL		80.57	79.86	78.80	83.78	77.02	80.01	2.50
	Sat.		28.07	26.96	26.37	27.86	26.90	27.23	0.71
	Unsat.		52.50	52.90	55.22	55.93	52.76	53.86	1.59
	S/U		1.87	1.96	2.09	2.01	1.96	1.98	0.08
	n-3		28.67	28.77	29.84	29.98	28.04	29.06	0.83
	n-6		6.16	6.02	6.67	6.71	5.88	6.29	0.38
	n-3 / n-6		4.65	4.78	4.48	4.47	4.77	4.63	0.15

Discussion:

The GC/MS analysis of fish oil reports quantities of each detected fatty acid present in the sample as weight % of total sample. For dose calculation, a percent of weight is equivalent to 10 milligrams / gram of oil. The amount of fatty acid administered is calculated as the dose in grams multiplied by mg / gram content. The total fatty acid administered in a 100 mg dose would be ~ 80 mg. The dose of EPA and DHA for the same dose would be 15 and 11 mg respectively. The variance in analysis for the major components is quite small at NMT 5% with variance of trace components as large as 25%. The sensitivity of the GC/MS is approximately 0.05% of each fatty acid by weight of sample.

