Analysis of Fatty Acid Methyl Esters

An Easy, Quick and Cost Effective Solution from GS-Tek

Introduction

Around 30% of fish oil is made up of omega-3. Omega-3s found in fish oil have greater health benefits than the omega-3s found in some plant sources. The main omega-3s in fish oil are EPA and DHA, while the omega-3 in plant sources is mainly ALA. Along with omega-3 fatty acids, omega-6s play a crucial role in supporting brain function and regulating metabolism. LA and ARA are the main compounds of Omega-6s in fish oil. They are the important compounds in the separation and qualification of fish oil.

GsBP-FAMEWax is an application-specific crosslinked PEG-type GC column stationary phase. GsBP-FAMEWax columns are used to generate reproducible separation of fatty acid methyl esters (FAMEs), fatty acid ethyl esters (FAEEs), and fatty acids within 20-30min. Typical applications are fish oils, omega-3/-6 in nutrition. GsBP-FAMEWax columns are more robust and cheaper prices comparing to conventional 100m length FAME columns like HP-FAME/HP-88 or SP2560. These columns are specially tested with FAME mix to ensure the critical separations. The following are GsBP-FAMEWax column offers:

cat no	Description	Temperature Limit, ℃	Similar to JW-FATWAX
6818-2001	GsBP-FAMEWax, 20m x 0.18mm x 0.18um	40 to 260/280	G3909-63002
6825-3002	GsBP-FAMEWax, 30m x 0.25mm x 0.25um	40 to 260/280	G3903-63008
6825-6002	GsBP-FAMEWax, 60m x 0.25mm x 0.25um	40 to 260/280	
6832-3002	GsBP-FAMEWax, 30m x 0.32mm x 0.25um	40 to 260/280	G3903-63009
6832-6002	GsBP-FAMEWax, 60m x 0.32mm x 0.25um	40 to 260/280	

Fish Oil FAME standard

Samples and Instrument Conditions

The Fish oil FAME standard mix (20 components) was analyzed. Table 2 listed the concentration of each component in the FAME standard. The analyses were performed using an Agilent 7890A GC equipped with a flame ionization detector (FID) on a FAMEwax 250°C capillary column 6825-3002 as described. The instrumental configuration and analytical conditions of fish oil standard analysis were summarized in Table 1 and the compounds were identified in table 2. The chromatogram of fish oil standard analysis was recorded in figure 1.

Table 1. Instrumental conditions for fish oil standard analysis

Column	GsBP-FAMEWax, 30m x 0.25mm x 0.25um, cat no 6825-3002
Carrier gas	Hydrogen, 1ml/min
Inlet	S/SL, 250°C, split ratio 50:1
Detector	FID, 280°C
Oven temp	160 °C 4°C/min to 250°C (5min)
Sample	fish oil FAME standard, 1ul

Figure1. Chromatogram of Fish oil standard analysis

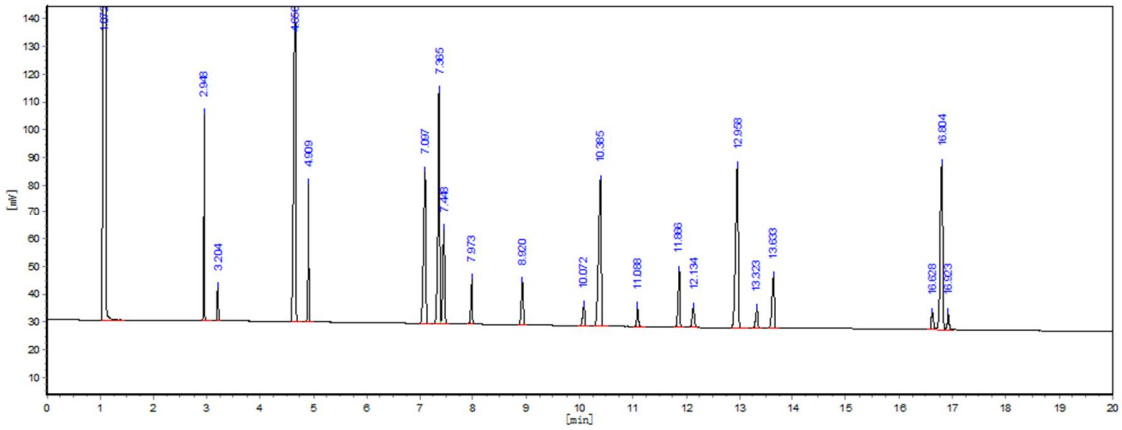

Table 2. Peak Identifications for fish oil standard compound

Compound(methyl ester)	RT (min)	Compound(methyl ester)	RT (min)
Myristic acid C14:0	2.948	cis-11-Eicosenoic acid C20:1n9	10.385
Tetradecenoic acid (cis-9) C14:1	3.204	Eicosadienoic acid C20:2	11.088
Palmitic acid C16:0	4.656	Arachidonic acid C20:4(ARA) (Ω-6)	11.866
Palmitoleic acid C16:1	4.909	cis-8,11,14-Eicosatrienoic acid C20:3	12.134
Stearic acid C18:0	7.097	cis-5,8,11,14,17-Eicosapentaenoic acid C20:5(EPA)(Ω-3)	12.958
Oleic acid C18:1n9	7.365	Behenic acid C22:0	13.323
Vaccenic acid C18:1n7	7.448	Erucic acid C22:1	13.633
Linoleic acid C18:2n6(LA)(Ω-6)	7.973	Lignoceric acid C24:0	16.628
gamma-Linolenic acid C18:3n6(GLA) (Ω-6)	8.92	Docosahexaenoic Acid C22:6n3(DHA)(Ω-3)	16.804
Eicosanoic acid C20:0	10.072	Nervonic acid C24:1	16.923

Conclusion

According to the above results, the FAMEs eluted in order of increasing carbon number, however, if the carbon numbers were the same, the unsaturated esters exhibited longer retention time compared to the same carbon number saturated esters, for example, Tetradecenoic acid (cis-9) C14:1 eluted later than Myristic acid C14:0. The more double bonds, the later compounds eluted. With the same carbon number and the same double bond number, the greater polarity compounds eluted later.

In this case, the baseline separation of main fish oil FAMEs could be achieved. We could adjust the instrumentation condition to improve the peak resolution and adjust the elution time. The lower oven temperature resulted in the higher resolution and the longer analysis during time. We need to choose a suitable temperature program to get the acceptable resolution and analysis during time.

Commercial Fish Oil and Prescription Fish Oil

Samples and Instrument Conditions

To improve the peak shape, the fish oil was usually methylated to FAMEs with KOH as catalyst and analyzed by GC-FID. But in this experiment, we directly

injected fish oil sample (diluted by Hexane) to simplify production processes and reduce the sample loss. According to the previous FAME separation rules, we could figure out the fish oil compound elution order.

Samples of a commercially purchased 1000mg+ fish oil capsule (Vitamin E is included) and prescription fish oil were analyzed. The instrumental conditions were the same with that recorded in Table 1 and the compounds were identified in table 3. The chromatograms were shown in Figure 3 and Figure 4.

Figure3. Chromatogram of 1000mg+ commercial fish oil Analysis

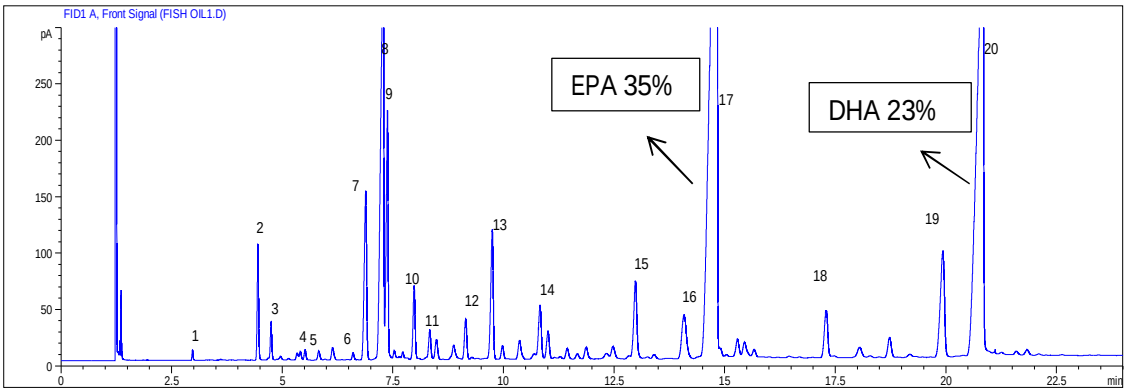

Figure4. Chromatogram of 1000mg+prescription fish oil Analysis

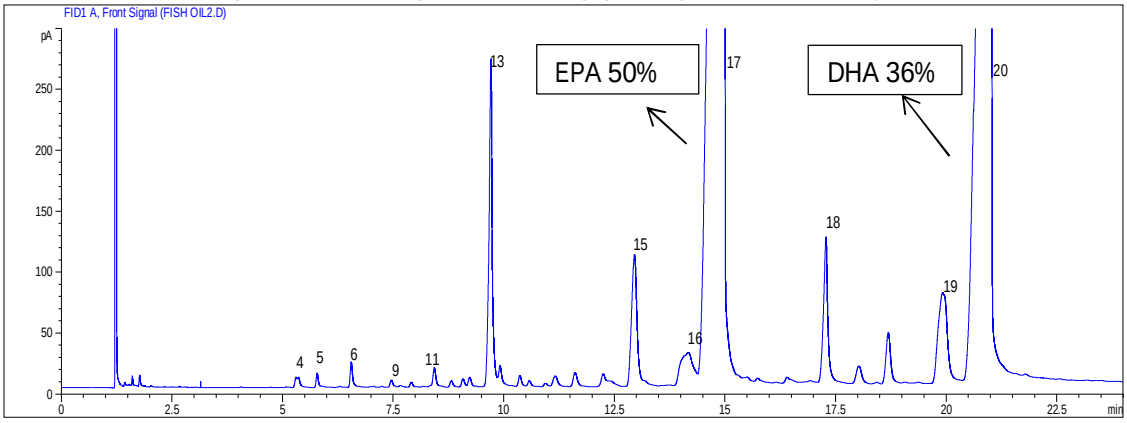

Table 3. Peak Identifications for fish oil compounds

Peak#	Compound(methyl ester)	Peak#	Compound(methyl ester)
1	Myristic acid C14:0	11	gamma-Linolenic acid C18:3n6(GLA)(Ω-6)
2	Palmitic acid C16:0	12	8,11,14-octadecatrienoic acid C18:3n4
3	Palmitoleic acid C16:1n7	13	α-linolenic acid C18:3n3(ALA)(Ω-3)
4	9,12-Hexadecadienoic acid C16:2n4	14	cis-11-Eicosenoic acid C20:1n9
5	6,9,12-Hexadecatrienoic acid C16:3n4	15	Arachidonic acid C20:4n6(ARA) (Ω-6)
6	6,9,12,15-Hexadecatetraenoic acid 16:4n1	16	Eicosatetraenoic acid C20:4n3
7	Stearic acid C18:0	17	cis-5,8,11,14,17-Eicosapentaenoic acid C20:5n3(EPA)(Ω-3)
8	Oleic acid C18:1n9	18	Adrenic acid C22:4n6
9	Vaccenic acid C18:1n7	19	Docosapentaenoic acid C22:5n3
10	Linoleic acid C18:2n6(LA) (Ω-6)	20	Docosahexaenoic Acid C22:6n3(DHA)(Ω-3)

Conclusion

Recent studies attested to the wonders that fish oil can do to overall health. The Effective compounds are Omega-3 including Docosahexaenoic Acid (DHA)/ Eicosapentaenoic Acid (EPA) fatty acids. The above results showed that there was a significant difference in nutrient composition between the commercial fish oil and prescription fish oil. The commercial fish oil had a complex composition, which means there might be some compounds which do not have any health benefit. The main effective compounds EPA and DHA had a lower concentration, 35% and 23%, respectively. In Figure 4, the prescription fish oil had a simple composition and higher concentration of effective compounds, especially EPA (50%) and DHA (36%).

FAMEs Separations

Samples and Instrument Conditions

In this part, we used the FAMEs standard mix (37 compounds from Restek) and demonstrated that main FAMEs could be separated using our GsBP-FAMEwax column. Table 4 listed the instrumental configuration and analytical conditions for the 37 FAMEs mix analysis. The chromatogram of 37 FAME mix analysis was recorded in figure 4 and the compounds were identified in table 5.

Table 4. Instrumental conditions for 37 FAME mix Analysis

Column	GsBP-FAMEWax, 30m x 0.32mm x 0.25um, cat no 6832-3002
Carrier gas	Hydrogen, 2ml/min
Inlet	S/SL, 250C, split ratio 50:1
Detector	FID, 280C
Oven temp	50 °C (1min) 10°C/min to 200C (1min) 2C/min to 260C hold
Sample	37 FAME mix

Figure4. Chromatogram of 37 FAME mix Analysis

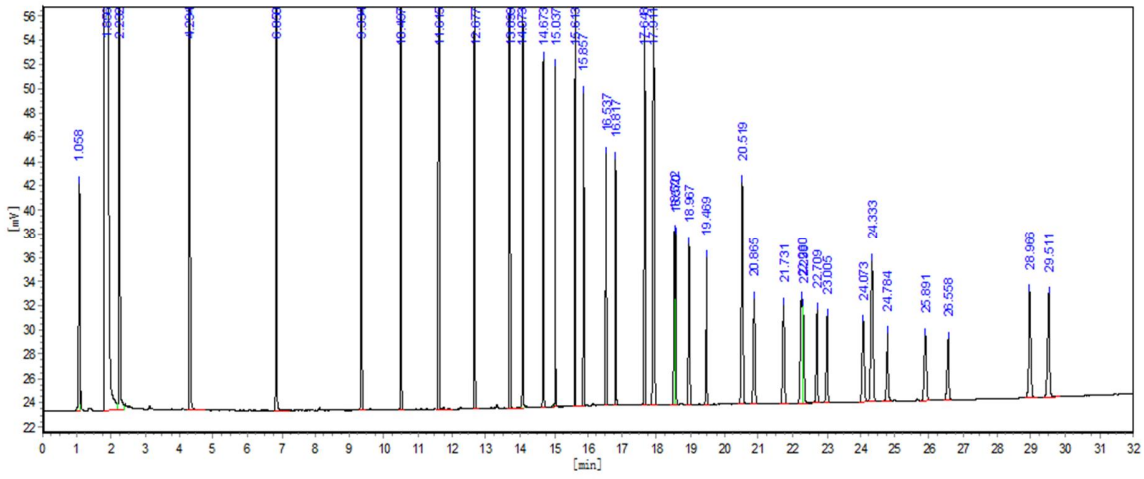

Table 5. Peak Identifications for 37 FAME mix compounds

Compound	RT(min)	Compound	RT(min)
Butyric acid C4:0	2.232	Octadecadienoic acid C18:2(all-cis-9,12)	18.57
Hexanoic acid C6:0	4.294	Gamma-linolenic acid C18:3(all-cis-6,9,12) (GLA)(Ω-6)	18.967
Caprylic acid C8:0	6.85	Alpha-linolenic acid C18:3(all-cis-9,12,15) (ALA)(Ω-3)	19.469
Decanoic acid C10:0	9.334	Eicosanoic acid C20:0	20.519
Undecanoic acid C11:0	10.497	cis-11-Eicosenoic acid C20:1(cis-11)	20.865
Lauric acid C12:0	11.615	Eicosadienoic acid C20:2(all-cis-11,14,)	21.731
Tridecanoic acid C13:0	12.677	all-cis-11,14,17-Eicosatrienoic acid C20:3(all-cis-11,14,17)	22.26
Myristic acid C14:0	13.699	Henicosanoic acid C21:0	22.299
Tetradecenoic acid (cis-9) C14:1(cis-9)	14.073	cis-8,11,14-Eicosatrienoic acid C20:3(all-cis-8,11,14)	22.709
Pentadecanoic acid C15:0	14.673	Arachidonic acid C20:4(all-cis-5,8,11,14) (ARA) (Ω-6)	23.005
Pentadecenoic acid C15:1(cis-10)	15.037	cis-5,8,11,14,17-Eicosapentaenoic acid C20:5(all-cis-5,8,11,14,17) (EPA)(Ω-3)	24.073
Palmitic acid C16:0	15.613	Behenic acid C22:0	24.333
palmitoleic (C16:1 cis-9) acid C16:1(cis-9)	15.857	Erucic acid C22:1(cis-13)	24.784
Heptadecanoic acid C17:0	16.537	cis-13,16-Docosadienoate C22:2(all-cis-13,16)	25.891
cis-10-Heptadecenoic acid C17:1(cis-10)	16.817	Tricosanoic acid C23:0	26.558
Stearic acid C18:0	17.648	Lignoceric acid C24:0	28.966
elaidic acid. C18:1(trans-9)	17.911	Docosahexaenoic acid C22:6 (all-cis-4,7,10,13,16,19) (DHA)(Ω-3)	29.511
oleic acid C18:1(cis-9)	17.911	Nervonic acid C24:1(cis-15)	29.511
Linolelaidic Acid C18:2(all-trans-9,12) (LA) (Ω-6)	18.522		

Conclusion

This Application Note highlighted the benefits of the GsBP-FAMEwax GC column for the analysis of FAMEs commonly found in fish oil. Comparing with the traditional FAMEs separation column, our new column has a longer life time, lower price and shorter analysis duration time (within 30min). The baseline separations of main FAME compounds are achieved. But two critical pairs of 37 FAMEs C22:6 (all-cis-4,7,10,13,16,19)/C24:1(cis-15) and C18:1 (trans-9)/C18:1(cis-9) are not separated completely.

Order Contact:

Email: orders@gs-tek.com

GS-Tek
A USA GC and HPLC column manufacturer
625 Dawson Drive
Newark, DE 19713
USA
Phone: +1-302-533-5646
Web: www.gs-tek.com