

Demonstration of Robustness and Selectivity for the Analysis of FAMES in Salmon Oil and Pet Food Using a Zebron™ ZB-FAME Column

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GC-FID FAME analysis is widely employed to characterize edible fats and oils in food for human and animal food consumption. Zebron ZB-FAME GC columns provide superior selectivity for a mixture of 49 commonly analyzed FAMES in a salmon oil and a pet food sample and a high level of chromatographic reproducibility for real matrix analysis of FAMES.

Overview

The analysis of FAMES in edible fats and oils is one of the most widely employed assays in food quality testing laboratories worldwide. This includes identifying adulteration in expensive oils like olive oil, classifying and quantifying omega 3, 6 and 9, ascertaining percentage (%) of trans fats in food and many other analyses. Owing to the complexity of fatty acids, a viable analytical approach must demonstrate a high degree of selectivity to adequately separate the large mixture of closely related compounds and their cis/trans isomers. In addition, high temperature resistance and high cross-linking in the stationary phase are necessary to accommodate the demanding matrix components. At the same time, such analyses must be performed rapidly with a high degree of robustness and minimal chromatographic alteration over many samples.

In recent years, gas chromatography columns designed specifically for FAME analysis demonstrated significant improvement in selectivity, reproducibility, crosslinking, temperature limits and robustness. Zebron ZB-FAME is the most recent contribution to this evolutionary trend. In the current work, commercial food testing samples were analyzed to demonstrate the suitability and robustness of Zebron ZB-FAME for the routine analysis of FAMES.

Materials and Methods:

The GC-FID analytical conditions are presented below. Standard test mix along with real sample matrix like fish oil, salmon oil, pet food samples were analyzed on a 100 meter ZB-FAME GC column. Analyte retention time and resolution are presented along with chromatograms in subsequent pages

GC-FID Conditions

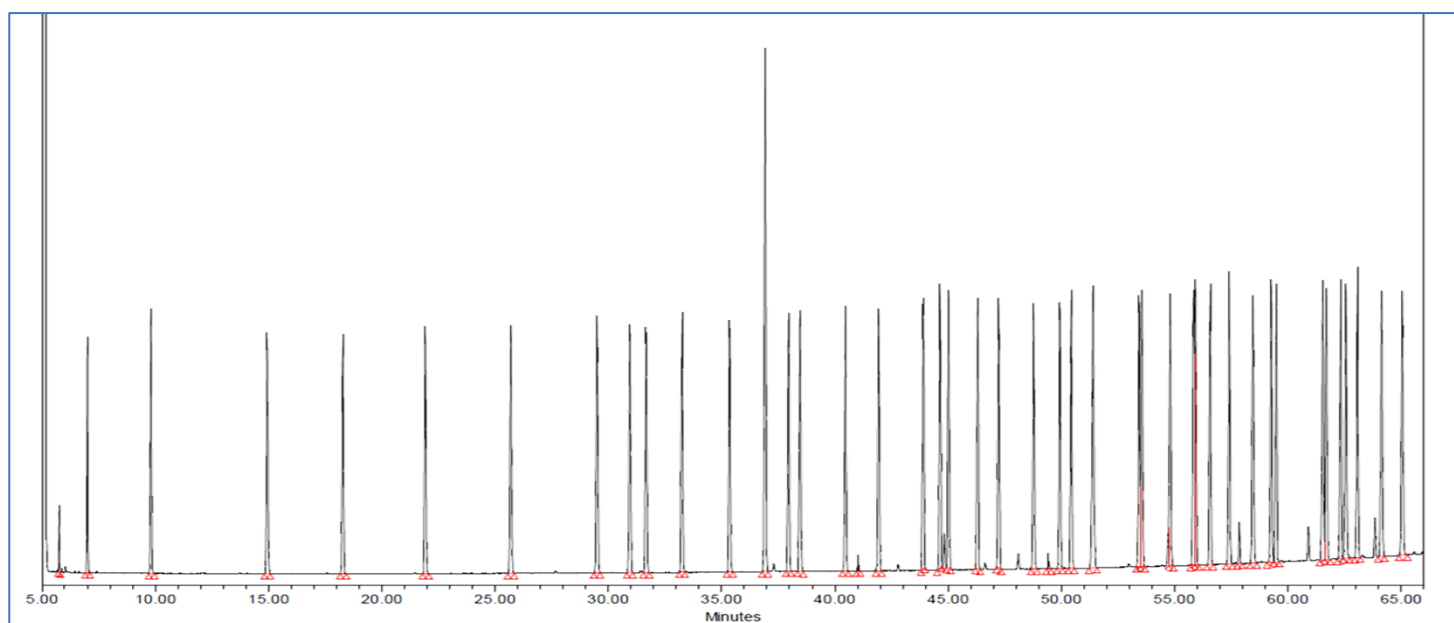
Column:	Zebron ZB-FAME
Dimensions:	100 meter x 0.25mm x 0.20 µm
Part No.:	7MG-G033-10
Recommended Z-Guard™:	7AG-G000-00-GZO
Injection:	Split (100:1) @ 250 °C, 1µL
Recommended Liner:	Zebron PLUS Z-Liner™ (Compatible with Agilent® & Thermo® GC instrument)
Liner Part No.:	AG2-0A03-05
Carrier Gas:	Hydrogen (constant flow)
Injection Temperature:	250 °C
Injection Volume:	1 µL
Flow Rate:	1.65 mL/min
Oven Program:	100 °C for 5 min to 240 °C @2 °C/min for 10 min
Detector:	GC-FID
Detector Temperature:	285 °C



Table 1: Retention and Resolution of 43 FAMES on a 100 Meter Zebron™ ZB-FAME GC Column

#	Name	RT	USP Resolution	#	Name	RT	USP Resolution	#	Name	RT	USP Resolution
1	01-C4:0	5.754	-	20	21-C18:1n-9	45.02	3.1	39	56-C24:0	62.337	5.2
2	02-C6:0	6.999	23.8	21	C18:2 trans n-6	46.306	10.7	40	53-C22:5n-6	62.559	1.8
3	03-C8:0	9.802	37.5	22	24-C18:2n-6	47.232	7.5	41	57-C24:1n-9	63.079	4.2
4	04-C10:0	14.937	51.9	23	28-C18:3n-6	48.779	12.3	42	52-C22:5n-3	64.142	8.7
5	05-C11:0	18.28	29.7	24	27-C18:3n-3	49.932	4.1	43	55-C22:6n-3	65.05	7.2
6	06-C12:0	21.92	31.2	25	31-C20:0	50.443	4.2				
7	C13:0	25.701	31.8	26	32-C20:1n-9	51.401	7.7				
8	07-C14:0	29.503	31.9	27	C21:0	53.436	15.9				
9	C14:1 trans n-5	30.95	12	28	35-C20:2n-6	53.555	1				
10	08-C14:1n-5	31.668	5.8	29	37-C20:3n-6	54.808	10.2				
11	09-C15:0	33.254	13.1	30	41-C20:4n-6	55.839	6.2				
12	10-C15:1n-5	35.352	17.3	31	36-C20:3n-3	55.916	0.4				
13	11-C16:0	36.923	13	32	45-C22:0	56.572	4.5				
14	C16:1 trans n-7	37.955	8.6	33	46-C22:1n-9	57.412	7.1				
15	12-C16:1n-7	38.464	4.2	34	43-C20:5n-3	58.457	5.3				
16	17-C17:0	40.466	16.9	35	C23:0	59.265	6.6				
17	18-C17:1	41.932	8.1	36	49-C22:2n-6	59.495	2				
18	19-C18:0	43.905	16.5	37	51-C22:4n-6	61.544	17.5				
19	C18:1 trans n-9	44.644	6.2	38	50-C22:3n-3	61.703	1.3				

Figure 1. FAME Standard Mix Chromatogram



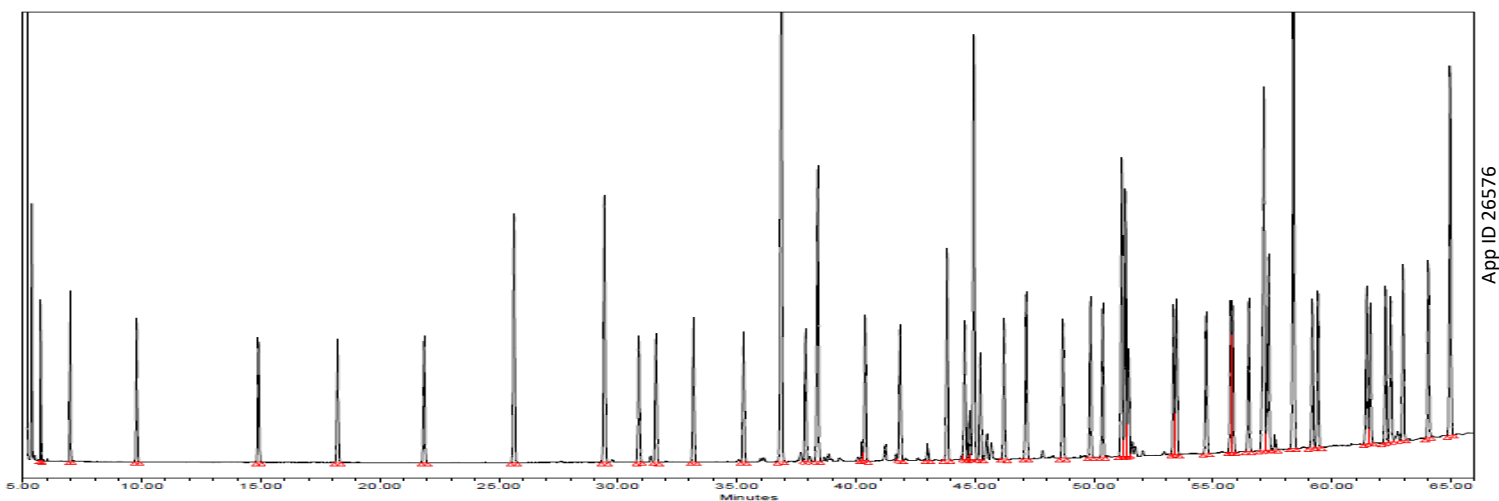
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Table 2: Retention and Resolution of 49 FAMES in Salmon Oil on a 100 Meter ZB-FAME GC Column

#	Name	RT	USP Resolution	#	Name	RT	USP Resolution	#	Name	RT	USP Resolution
1	01-C4:0	5.753	-	23	21-C18:1n-9	44.948	1.5	45	56-C24:0	62.2	5.245
2	02-C6:0	6.991	23.2	24	20-C18:1n-7	45.215	2.1	46	53-C22:5n-6	62.5	1.7
3	03-C8:0	9.78	36.3	25	C18:2 trans n-6	46.22	7.9	47	57-C24:1n-9	63	4.174
4	04-C10:0	14.896	50.1	26	24-C18:2n-6	47.145	7.3	48	52-C22:5n-3	64	8.404
5	05-C11:0	18.227	28.5	27	28-C18:3n-6	48.691	12.1	49	55-C22:6n-3	65	7.048
6	06-C12:0	21.859	29.9	28	27-C18:3n-3	49.842	9				
7	C13:0	25.636	30.7	29	31-C20:0	50.363	4.2				
8	07-C14:0	29.435	30.8	30	33-C20:1n-11	51.161	6.5				
9	C14:1 trans n-5	30.878	11.7	31	32-C20:1n-9	51.322	1.3				
10	08-C14:1n-5	31.594	5.7	32	30-C18:4n-3	51.451	1				
11	09-C15:0	33.176	12.8	33	C21:0	53.343	14.8				
12	10-C15:1n-5	35.273	16.8	34	35-C20:2n-6	53.464	1				
13	11-C16:0	36.858	12.5	35	37-C20:3n-6	54.712	10.1				
14	C16:1 trans n-7	37.876	8.1	36	41-C20:4n-6	55.743	6.1				
15	12-C16:1n-7	38.39	4.1	37	45-C22:0	56.498	4.4				
16	14-C16:2	40.256	14.1	38	47-C22:1n-11	57.14	5.1				
17	17-C17:0	40.384	1	39	40-C20:4n-3	57.331	1.5				
18	18-C17:1	41.849	12.1	40	43-C20:5n-3	58.372	5.9				
19	15-C16:3n-4	43.012	9.3	41	C23:0	59.168	6.3				
20	19-C18:0	43.827	6.6	42	49-C22:2n-6	59.405	2				
21	C18:1 trans n-9	44.564	5.9	43	44-C21:5n-3	61.446	16.7				
				44	51-C22:4n-6	61.601	1.2				

Figure 2: Separation of 49 FAMES in Salmon Oil on a 100 Meter ZB-FAME GC Column



Salmon Oil Sample Analysis

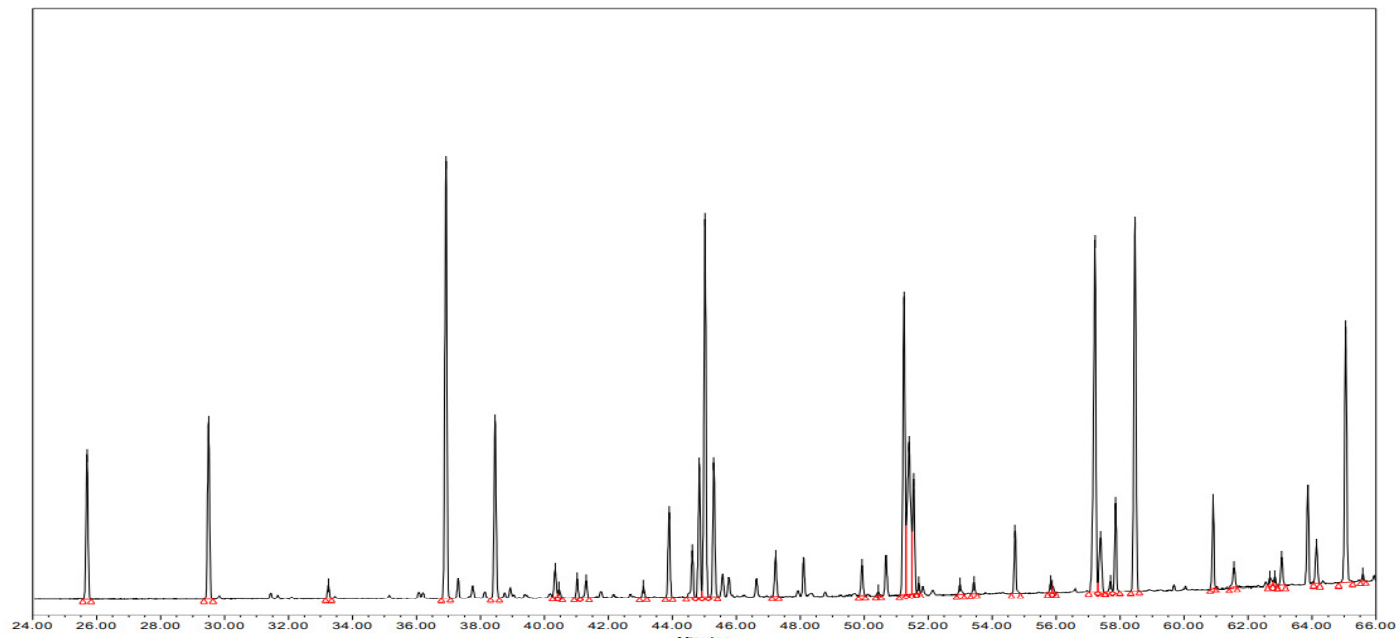
A salmon oil supplement was procured from a retail source. A sample was prepared according to AOCS Official Method Ce 1b-89 “Fatty Acid Composition of Marine Oils by GLC”, spiked with a C13:0 internal standard and analyzed by GC-FID under the conditions mentioned in page 1. The retention time of individual FAME components along with resolution are presented in **Table 2**.



Table 3: Retention and Resolution of 31 FAMES in Fish Oil on a 100 Meter ZB-FAME GC Column

#	Name	RT	USP Resolution
1	C13:0	25.698	-
2	07-C14:0	29.499	31.9
3	09-C15:0	33.247	31.8
4	11-C16:0	36.919	31.1
5	12-C16:1n-7	38.46	12.7
6	14-C16:2	40.334	15.3
7	17-C17:0	40.455	
8	15-C16:3n-4	43.095	14.5
9	19-C18:0	43.896	6.7
10	C18:1 trans n-9	44.623	6.5
11	22-C18:1n-11	44.841	1.9
12	21-C18:1n-9	45.02	1.5
13	20-C18:1n-7	45.297	2.3
14	24-C18:2n-6	47.226	15.9
15	27-C18:3n-3	49.927	21.9
16	31-C20:0	50.431	4.2
17	33-C20:1n-11	51.239	6.8
18	32-C20:1n-9	51.396	1
19	30-C18:4n-3	51.537	0.8
20	C21:0	53.424	3.5
21	37-C20:3n-6	54.712	11.7
22	41-C20:4n-6	55.83	-
23	36-C20:3n-3	55.877	-
24	47-C22:1n-11	57.21	-
25	46-C22:1n-9	57.384	1.2
26	43-C20:5n-3	58.461	5.2
27	44-C21:5n-3	60.906	21.4
28	51-C22:4n-6	61.559	5.7
29	57-C24:1n-9	63.049	1.7
30	52-C22:5n-3	64.134	8.8
31	55-C22:6n-3	65.05	7.2

Figure 3: Separation of 31 FAMES in Fish Oil on a 100 Meter ZB-FAME GC Column



App ID 26575

Fish Oil Sample Analysis

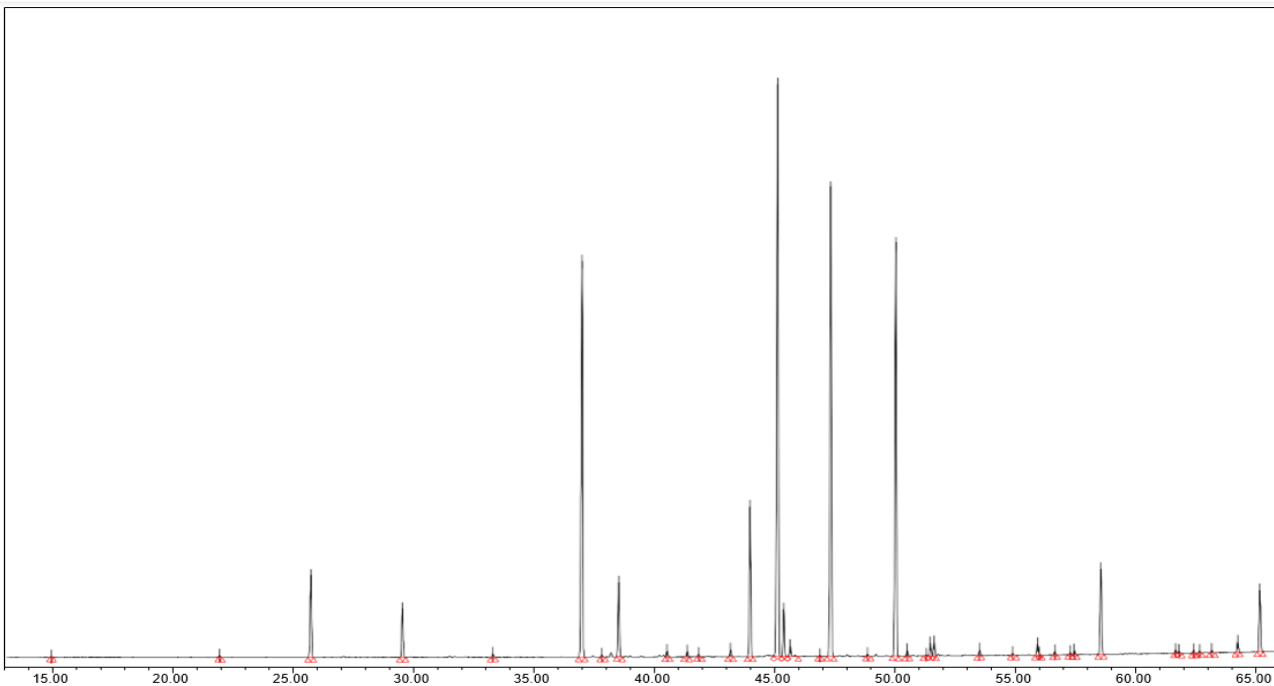
A fish oil supplement was procured from a retail source. A sample was prepared according to AOCS Official Method Ce 1b-89 “Fatty Acid Composition of Marine Oils by GLC”, spiked with a C13:0 internal standard and analyzed by GC-FID under the conditions mentioned in page 1. The results are presented in **Table 3** and the associated chromatogram is presented in **Figure 3**.



Table 4: Retention and Resolution of 36 FAMES in Pet Food on a 100 Meter ZB-FAME GC Column

#	Name	RT	USP Resolution	#	Name	RT	USP Resolution	#	Name	RT	USP Resolution
1	04-C10:0	14.946	-	17	28-C18:3n-6	48.855	13.1	33	53-C22:5n-6	62.644	2.3
2	06-C12:0	21.944	71.6	18	27-C18:3n-3	50.027	10.3	34	57-C24:1n-9	63.143	4.9
3	C13:0	25.736	36.9	19	31-C20:0	50.506	4.4	35	52-C22:5n-3	64.225	9.5
4	07-C14:0	29.545	35.6	20	32-C20:1n-9	51.468	1.4	36	55-C22:6n-3	65.142	8
5	09-C15:0	33.3	35.2	21	30-C18:4n-3	51.619	1.4				
6	11-C16:0	36.996	33.6	22	35-C20:2n-6	53.51	17.3				
7	C16:1 trans n-7	37.818	7.4	23	37-C20:3n-6	54.882	12.6				
8	12-C16:1n-7	38.523	6.9	24	41-C20:4n-6	55.915	9				
9	17-C17:0	40.524	18.2	25	36-C20:3n-3	55.99	-				
10	18-C17:1	41.843	4.3	26	45-C22:0	56.631	-				
11	15-C16:3n-4	43.166	12.2	27	40-C20:4n-3	57.276	6.2				
12	19-C18:0	43.983	7.3	28	46-C22:1n-9	57.446	1.5				
13	21-C18:1n-9	45.122	9.8	29	43-C20:5n-3	58.543	9.4				
14	20-C18:1n-7	45.374	2.2	30	51-C22:4n-6	61.651	29				
15	25-C18:2n-9	46.877	11	31	50-C22:3n-3	61.783	1.3				
16	24-C18:2n-6	47.33	4	32	56-C24:0	62.393	5.9				

Figure 4 Separation of 36 FAMES in Pet Food on a 100 Meter ZB-FAME GC Column



App ID 26574

Pet Food Sample Analysis

A high Omega 3 pet food was procured from a retail source. A sample was prepared according to AOAC Official Method 996.06 “Fat (Total Saturated and Unsaturated) in Foods”, spiked with C13:0 internal standard and analyzed under the conditions presented in page1. The chromatographic results along with retention of individual FAME and resolution values are presented in **Figure 4** and **Table 4**.



Discussion and Conclusions

Zebtron™ ZB-FAME GC columns are designed for robust analysis of FAME components. The stationary phase in ZB-FAME unlike traditional cyano phase is highly bonded and cross-linked through a proprietary process called Engineered Self Crosslinking™ (ESC™). Demonstrated in this study is the separation of such FAME components that are commonly tested in food matrices like fish oil, salmon oil and pet food. In addition to the initial separation, robustness of the column was evaluated after multiple real food matrix samples. The results proved intact stationary phase in ZB-FAME that provided reproducible retention profile for FAMEs analysis. In addition to providing efficient separation for food FAMEs, ZB-FAME GC column offers a higher temperature limit of 280 °C to bake out high boiling contaminants.

Acknowledgement

We thank Eurofins Nutrition Analysis Center, Des Moines, IA and Mr. Keith Persons in performing the analytical contribution. Phenomenex is not affiliated with Eurofins.



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