



TN-2069

Determination of 37 FAMES Including Omega-3 and -6 Fatty Acids by GC-FID in Under 12 Minutes

Bryan Tackett, PhD

Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Introduction

Since unsaturated fatty acids contain a double bond, the bond orientation can be in either a cis or trans configurations. Natural fats favor the cis configuration. Due to the placement of the cis double bond, they tend to have a lower melting temperature, making them liquid at room temperature. Another popular type of unsaturated fats are polyunsaturated fatty acids (PUFA), which have multiple double bonds. An example of these are Omega-3 and Omega-6, which have been popular in recent years due to their potential health benefits and have the double bond at the third or sixth carbon from the tail of the carbon chain, respectively (for the Omega position we count backwards from the end of the carbon chain). Two common essential Omega-3 fatty acids are Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). A common Omega-6 essential fatty acid is Linoleic acid (LA).

The general method of analysis is GC-FID. Mass spectrometry offers minimal value as these are isobaric compounds and the resulting fragments are the same regardless of cis or trans configuration or the number of carbons in the FAME compounds. The difficulty arises from many of these compounds eluting close to each other and many scientists find that they need greater resolution between various peaks for their projects.

Table 1. 37 FAME Standard.

Peak No.	Compound	ID
1	Butanoic Acid Methyl Ester	C4:0
2	Hexanoic Acid Methyl Ester	C6:0
3	Octanoic Acid Methyl Ester	C8:0
4	Decanoic Acid Methyl Ester	C10:0
5	Undecanoic Acid Methyl Ester	C11:0
6	Dodecanoic Acid Methyl Ester	C12:0
7	Tridecanoic Acid Methyl Ester	C13:0
8	Myristic Acid Methyl Ester	C14:0
9	Myristoleic Acid Methyl Ester	C14:1 cis 9
10	Pentadecanoic Acid Methyl Ester	C15:0
11	cis-10-Pentadecenoic Acid Methyl Ester	C15:1 cis 10
12	Hexadecanoic Acid Methyl Ester	C16:0
13	Palmitoleic Acid Methyl Ester	C16:1 cis 9
14	Heptadecanoic Acid Methyl Ester	C17:0
15	cis-10-Heptadecenoic Acid Methyl Ester	C17:1 cis 10
16	Stearic Acid Methyl Ester	C18:0
17	Elaidic Acid Methyl Ester	C18:1 trans 9
18	Oleic Acid Methyl Ester	C18:1 cis 9
19	Linolelaidic Acid Methyl Ester	C18:2 trans 9,12
20	Linoleic Acid Methyl Ester	C18:2 cis 9,12
21	γ-Linolenic Acid Methyl Ester	C18:3 cis 6,9,12
22	α-Linolenic Acid Methyl Ester	C18:3 cis 9,12,15
23	Arachidic Acid Methyl Ester	C20:0
24	cis-11-Eicosenoic Acid Methyl Ester	C20:1 cis 11
25	cis-11,14-Eicosadienoic Acid Methyl Ester	C20:2 cis 11,14
26	Heneicosanoic Acid Methyl Ester	C21:0
27	cis-8,11,14-Eicosatrienoic Acid Methyl Ester	C20:3 cis 8,11,14
28	Arachidonic Acid Methyl Ester	C20:4 cis 5,8,11,14
29	cis-11,14,17-Eicosatrienoic Acid Methyl Ester	C20:3 cis 11,14,17
30	Behenic Acid Methyl Ester	C22:0
31	Erucic Acid Methyl Ester	C22:1 cis 13
32	cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	C20:5 cis 5,8,11,14,17
33	cis-13,16-Docosadienoic Acid Methyl Ester	C22:2 cis 13,16
34	Tricosanoic Acid Methyl Ester	C23:0
35	Lignoceric Acid Methyl Ester	C24:0
36	Nervonic Acid Methyl Ester	C24:1 cis 15
37	cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	C22:6 cis 4,7,10,13,16,19

GC-MS Conditions – Figure 1

Column: Zebron™ ZB-FAME
Dimensions: 30-meter x 0.25 mm x 0.20 μm
Part No.: [7HG-G033-10](#)
Injection: Split 50:1 @ 240 °C, 1 μL
Recommended Liner: Zebron PLUS Single Taper with Wool
Liner Part No.: [AG2-0A11-05](#) (for Agilent® systems)
Carrier Gas: Helium @ 1.2 mL/min (constant flow)
Oven Program: 100 °C for 2 min to 140 °C @ 10 °C/min to 190 °C @ 3 °C/min to 260 °C @ 30 °C/min for 2 min
Detector: FID @ 260 °C
Sample: 37 FAME standard (Table 1)

GC-MS Conditions – Figure 2

Column: Zebron ZB-FAME
Dimensions: 20-meter x 0.18 mm x 0.15 μm
Part No.: [7FD-G033-05](#)
Injection: Split 100:1 @ 250 °C, 1 μL
Recommended Liner: Zebron PLUS Single Taper Z-Liner™
Liner Part No.: [AG2-0A13-05](#) (for Agilent® systems)
Carrier Gas: Helium @ 1.0 mL/min (constant flow)
Oven Program: 80 °C for 1.5 min to 160 °C @ 40 °C/min to 185 °C @ 5 °C/min to 260 °C @ 30 °C/min
Detector: FID @ 260 °C
Sample: 37 FAME standard (Table 1)



Results and Discussion

A commonly used standard 37 FAME (fatty acid methyl ester) mix, often requires a long GC capillary column to obtain baseline resolution. The Zebron™ ZB-FAME column has similar, but modified phase chemistry compared to the traditional GC columns used for the 37 FAME mixture analysis. These 37 FAMES are baseline resolved on a 30-meter ZB-FAME (**Figure 1**) in contrast to the traditional longer columns of 60 meter or more. Additionally, runtime can be further reduced by selecting a 20-meter ZB-FAME column (**Figure 2**) resulting in an analysis under 12 minutes. **Table 2a** and **2b** show the peak order for Omega-3 and Omega-6 EFA.

Figure 1. 37 FAME Mix Using a ZB-FAME 30-Meter Column.

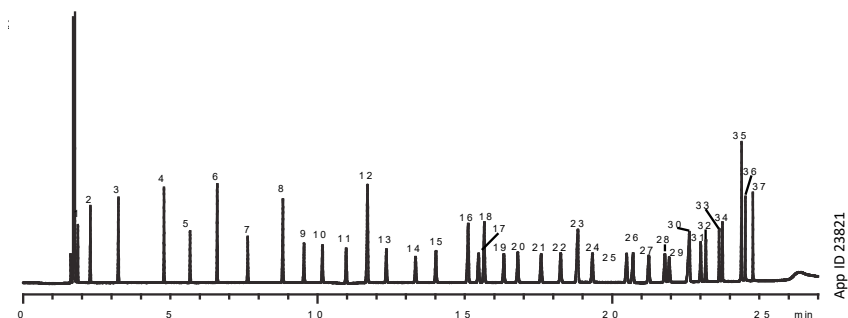


Figure 2. 37 FAME Mix Using a ZB-FAME 20-Meter Column.

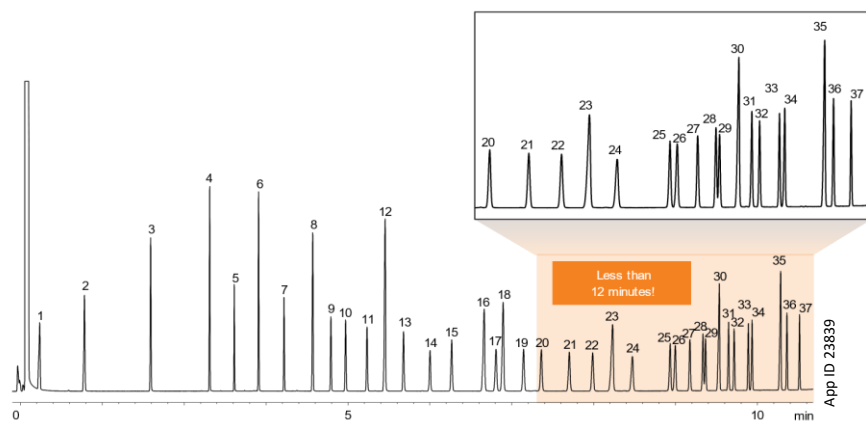


Table 2a. Omega-3 Fatty Acid Methyl Ester.

α -Linolenic Acid (ALA)	18:3 (n-3)	All-cis-9,12,15-octadecatrienoic Acid	Peak 22
Eicosatrienoic Acid (ETE)	20:3 (n-3)	All-cis-11,14,17-eicosatrienoic Acid	Peak 29
Eicosapentaenoic Acid (EPA)	20:5 (n-3)	All-cis-5,8,11,14,17-eicosapentaenoic Acid	Peak 32
Docosahexaenoic Acid (DHA)	22:6 (n-3)	All-cis-4,7,10,13,16,19-docosahexaenoic Acid	Peak 37

Table 2b. Omega-6 Fatty Acid Methyl Ester.

Linolenic Acid (LA)	18:2 (n-6)	All-cis-9,12,15-octadecatrienoic Acid	Peak 20
γ -Linolenic Acid (GLA)	18:3 (n-6)	All-cis-6,9,12-octadecatrienoic Acid	Peak 21
Eicosadienoic Acid	20:2 (n-6)	All-cis-11,14-eicosadienoic Acid	Peak 25
Dihomo- γ -Linolenic Acid (DGLA)	20:3 (n-6)	All-cis-8,11,14-eicosatrienoic Acid	Peak 27
Arachidonic Acid (AA)	20:4 (n-6)	All-cis-5,8,11,14-eicosatetraenoic Acid	Peak 28
Docosadienoic Acid	22:2 (n-6)	All-cis-13,16-docosadienoic Acid	Peak 33



Conclusions

Fatty acids are commonly analyzed components in the food testing industry, as well as clinical and biofuels. Global regulatory changes and research have resulted in an increased interest in the analysis of FAMES to determine the cis/trans structures of fatty acids. A reliable FAME GC column, such as the Zebron™ ZB-FAME column, can be used to achieve chromatography with separation of common Omega-3s and Omega-6s which are highlighted in this technical note.

A high cyano selectivity to analyze Omega-3 and Omega-6 fatty acid methyl esters provides significant resolution improvement using ZB-FAME. Optimized column dimension can lead a to run time as short as 11 minutes which increases throughput.

Ordering Information

Zebron ZB-FAME GC Columns

ID (mm)	df (μm)	Temp. Limits °C	Part No.
20-Meter			
0.18	0.15	-20 to 280	7FD-G033-05
30-Meter			
0.25	0.20	-20 to 280	7HG-G033-10
30-Meter with 5-Meter Guardian™ Integrated Guard			
0.25	0.20	-20 to 280	7HG-G033-10-GGA
60-Meter			
0.25	0.20	-20 to 280	7KG-G033-10
100-Meter			
0.25	0.20	-20 to 280	7MG-G033-10

Zebron PLUS Liners Compatible with Agilent® GC Systems

Description	Application	Inlet Style	Dimensions ID x L (mm)	Deactivation	Part No.	Unit
For 5890, 6890, and 7890 Models						
Direct Connect 	Trace analysis, Splitless injections	S/SL	4 x 78.5	PLUS Inert	AG2-0A50-01 AG2-0A50-05 AG2-0A50-25	Ea 5/pk 25/pk
Single Taper 	Pesticides	S/SL	4 x 78.5	PLUS Inert	AG2-0A10-01 AG2-0A10-05 AG2-0A10-25	Ea 5/pk 25/pk
Single Taper Z-Liner™ 	Semi-volatiles, Dirty samples	S/SL	4 x 78.5	PLUS Inert	AG2-0A13-01 AG2-0A13-05 AG2-0A13-25	Ea 5/pk 25/pk
Single Taper with Wool 	Semi-volatiles	S/SL	4 x 78.5	PLUS Inert	AG2-0A11-01 AG2-0A11-05 AG2-0A11-25	Ea 5/pk 25/pk
Straight 	Volatiles	S/SL	4 x 78.5	PLUS Inert	AG2-0A00-01 AG2-0A00-05 AG2-0A00-25	Ea 5/pk 25/pk
Straight Z-Liner 	Dirty samples, Volatiles, High initial oven temperatures	S/SL	4 x 78.5	PLUS Inert	AG2-0A03-01 AG20A03-05 AG2-0A03-25	Ea 5/pk 25/pk
Straight Single Baffle 	Semi-volatiles, Pesticides	S/SL	4 x 78.5	PLUS Inert	AG2-1F06-01 AG2-1F06-05 AG2-1F06-25	Ea 5/pk 25/pk



Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia

t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
cz-info@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong

t: +852 6012 8162
hkinfo@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia

t: +62 21 3952 5747
indoinfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Japan

t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Poland

t: +48 22 51 02 180
pl-info@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: 800-852-3944
sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
sk-info@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
info@phenomenex.com

🌐 **All other countries/regions**
Corporate Office USA

t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com

BE-HAPPY™
GUARANTEE

Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.

www.phenomenex.com/behappy

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/phx-terms-and-conditions-of-sale.

Trademarks

Zebron, Guardian, Z-Liner, and BE-HAPPY are trademarks of Phenomenex. Agilent is a registered trademark of Agilent Technologies, Inc..

Disclaimer

Phenomenex is in no way affiliated with Agilent Technologies, Inc.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2024 Phenomenex, Inc. All rights reserved.