phenomenex
...breaking with tradition℠

APPLICATIONS

Timothy Anderson
GC Product Manager
Tim was raised in Texas where it was completely too hot, then moved to Pennsylvania and Ohio where it was entirely too cold. He finally settled on California where the weather is just right.

37 FAMEs including Omega-3 and -6 Fatty Acids, by GC-FID in Under 12 Minutes

Timothy Anderson and Ramkumar Dhandapani
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Fatty acids are important for all systems of the body to function normally, including your skin, respiratory system, circulatory system, brain, and organs.

The two essential fatty acids (EFA) that the human body cannot produce are omega-3 fatty acid and omega-6 fatty acid, which are important for brain development, immune system function, and blood pressure regulation.

Accurate determination and quantitation of these EFAs, especially the separation of LNA and GLA, can be performed by capillary gas chromatography (GC). The Zebron™ ZB-FAME GC column is ideal to provide the composition of the EFAs found in flax seed oil, black currant seed oil, borage oil, and olive oil.

Introduction
Highlights on Omega-3s and -6s

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).

Best Analysis of Omega-3s and -6s

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.

A commonly used standard 37 FAME (fatty acid methyl ester) mix, often requires a long GC capillary column to obtain baseline resolution. The Phenomenex ZB-FAME column has similar, but modified phase chemistry compared to the traditional GC columnns 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.

Materials and Methods

Figure 1 conditions:
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 as shown below

Peak	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

Figure 1.
37 FAME mix using a ZB-FAME 30 meter column

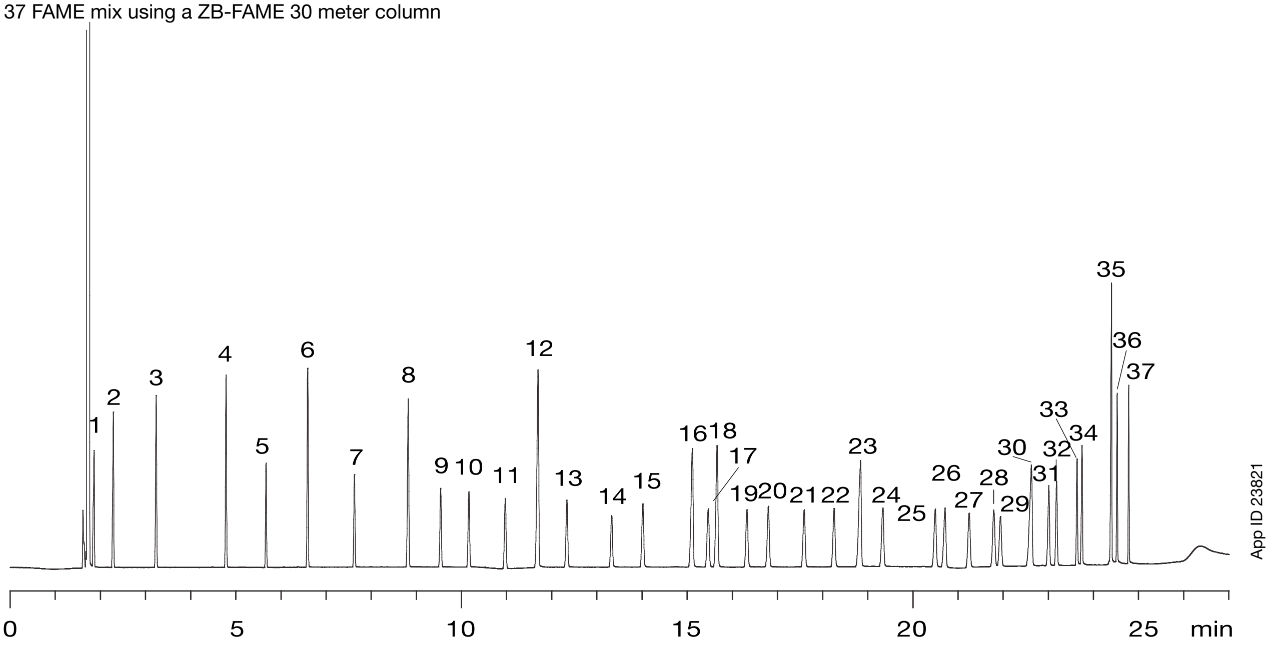


Figure 2.
37 FAME mix using a ZB-FAME 20 meter column

Figure 2 conditions:
Column: Zebron ZB-FAME
Dimensions: 20 meter x 0.18 mm x 0.15 µm
Part No.: 7FD-G0033-05
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 as shown on page 1

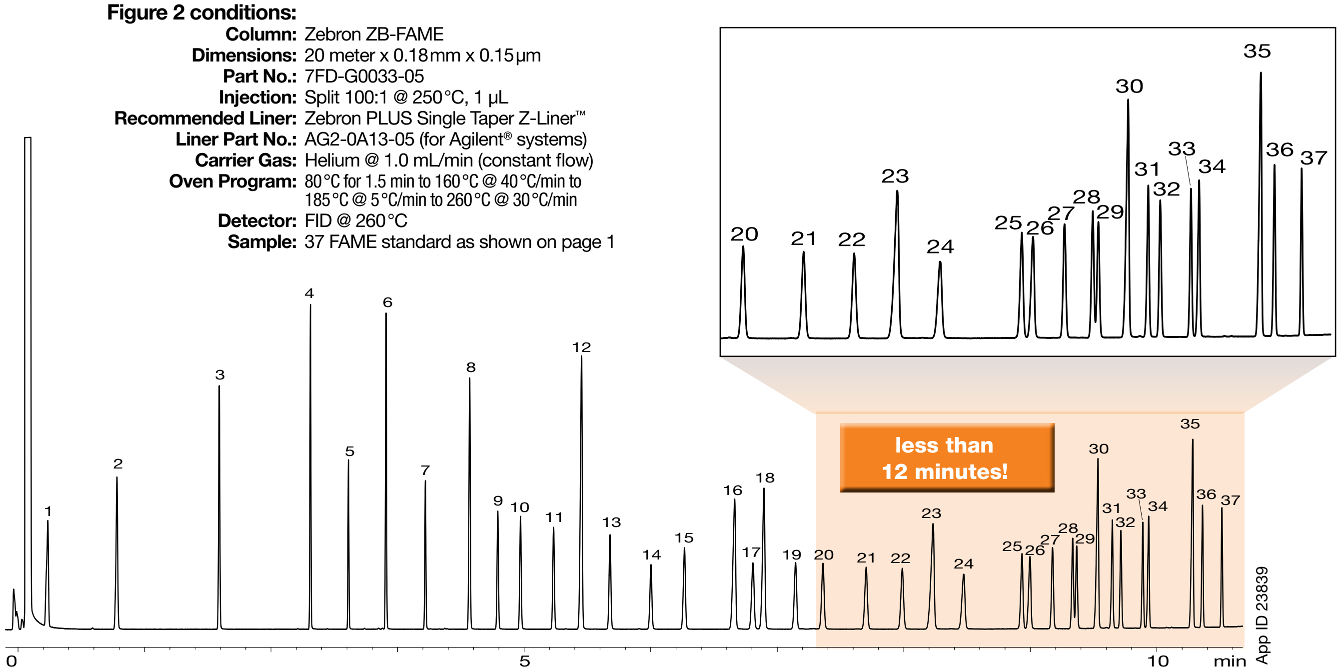


Table 2a.

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-gamma-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

Conclusion

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.

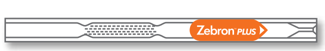
Optimized column dimension can lead a to run time as short as 11 minutes which increases throughput.

Ordering Information

Zebron ZB-FAME GC Columns

Length (m)	ID (mm)	Film (µm)	Temp Limits (°C)	Part No.	5m Guardian™ Part No.
20	0.18	0.15	-20 to 280	7FD-G033-05	-
30	0.25	0.20	-20 to 280	7HG-G033-10	7HG-G033-10-GGA
60	0.25	0.20	-20 to 280	7KG-G033-10	-

Zebron PLUS GC Inlet Liners

	Description	Application	Dimensions ID x L (mm)	Unit	Part No.
For Agilent® or Thermo Scientific® GC Systems					
	Single Taper Z-Liner™	Semi-volatiles, dirty samples	4 x 78.5	5/pk 25/pk	AG2-0A13-05 AG2-0A13-25
	Single Taper with Wool	Semi-volatiles	4 x 78.5	5/pk 25/pk	AG2-0A11-05 AG2-0A11-25

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

phenomenex ...breaking with tradition℠

APPLICATIONS

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 (0)22 2532-1032
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

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

Terms and Conditions
Subject to Phenomenex Standard Terms and Conditions which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks
Zebron, Guardian, and Z-Liner are trademarks of Phenomenex.
Agilent is a registered trademark of Agilent Technologies, Inc.
Thermo Scientific is a registered trademark of Thermo Fisher Scientific Inc.

Disclaimer
Phenomenex is in no way affiliated with Agilent or Thermo.

TN51870417_W