Determination of Omega-3 (n-3) and Omega-6 (n-6) Fatty Acid Composition in Evening Primrose Oil, Flax Seed Oil, Black Currant Oil, and Borage Oil

Essential fatty acids (EFAs) are polyunsaturated fatty acids (PUFAs) that the human body requires, yet cannot produce, and therefore must be obtained through dietary sources or nutritional supplements. α-Linolenic acid (LNA) and γ-Linolenic acid (GLA) are important Omega-3 (n-3) and Omega-6 (n-6) fatty acids. Accurate determination and quantitation of these EFAs, especially the separation of LNA and GLA, can be performed by capillary gas chromatography (GC). The FAMEWAX™ column is ideal to provide the composition of the EFAs found in evening primrose oil, flax seed oil, black currant seed oil, and borage oil.

Why are these fatty acids essential?

The two families of EFAs are the Omega-3 (n-3) series and the Omega-6 (n-6) series. The Omega-3 (n-3) series includes LNA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The Omega-6 (n-6) series includes linoleic acid (LA), GLA, dihommogamma-linolenic acid (DGLA), and arachidonic acid (AA).

These EFAs are nutrients that perform key functions in our bodies. For example, they determine membrane fluidity and reactivity, oxidation rate, metabolic rate, and energy production. In addition, they are a factor in maintaining body temperature, insulating nerves, and cushioning body tissue. EFAs also are precursors to prostaglandins, hormone-like substances that are critical to the body's overall health maintenance. Prostaglandins regulate blood pressure, blood clotting, stimulation of the immune system, and general regulation of heart, kidneys, liver, lungs, and brain. They are short-lived molecules and constantly need to be replenished. Without EFAs this can be impossible.

Background

EFAs are similar to vitamins in their importance to one's overall health. However, vitamins are required in small dietary quantities (µg/day), whereas EFAs are a macronutrient (i.e., necessary in g/day). A joint study released by the Food and Agriculture Organization and the World Health Organization recommends that at least 3% of our daily calorie intake be in the form of EFA.[1]

Polyunsaturated oils, such as safflower, sunflower, and corn oil are good sources of LA. Once ingested, LA can be converted to the other Omega-6 acids: GLA, DGLA, and AA.

Green, leafy vegetables and flax oil are good sources of LNA. From the LNA provided in our diets, our bodies can produce the other Omega-3 (n-3) acids: EPA and DHA.

Unfortunately, one's diet may not be well-fortified with these food sources. Also, physiological conditions can inhibit the conversion process of LA and LNA to the other essential Omega-3 and Omega-6 fatty acids.[1] Therefore, nutritional supplements can be used to help people attain the suggested daily intake. Evening primrose oil, flax seed oil, black currant seed oil, and borage oil are rich sources of these EFAs and are available in capsules as nutritional supplements.

A number of clinical conditions have been treated with oils rich in GLA. Oral dosages of evening primrose oil have been used to treat premenstrual tension, rheumatoid arthritis, breast disorders, and atopic eczema.[2]

Analysis

The oils were obtained from soft-gel capsules of evening primrose oil, flax seed oil, black currant oil, and borage oil. The fats were initially in the form of triglycerides. They were saponified into their free acids and esterified for better volatility and inertness by GC. To do this, 5mL of hexane and 250µL of 2N KOH were added to 0.24g oil. The mixture was shaken for 2 minutes in a closed 20mL vial. After settling, the supernatant was injected.

In the 1980s, packed and capillary GC, as well as liquid chromatography (LC), were evaluated for the analysis of EFAs in evening primrose oil and soybean oil.[2] According to one reference, "gas chromatography using a capillary column (25m, Carbowax® 20 M) was the best tool for the separation of GLA (C18:3n6) and LNA (C18:3n3)."[3]

Based on this finding, we used a Restek column—the 30m, 0.25mm ID, 0.25 µm FAMEWAX™ column (cat.# 12497)—to analyze these oils. The FAMEWAX™ column contains a polyethylene glycol stationary phase, which is slightly more polar than the Stabilwax® column. The FAMEWAX™ column offers excellent selectivity and efficiency, not only to separate saturated (C16:0 and C18:0) and monounsaturated (C18:1n9) fatty acids from the Omega-3 and -6 fatty acids of interest, but also to resolve the isomers of linolenic acid (C18:3n3 and C18:3n6).

We used an HP 5890 GC with a flame ionization detector (FID) and a split/splitless injection port, used in the split mode, with a split vent flow of 40mL/min. The inlet liner was a deactivated 4mm ID split sleeve (cat.# 20781). The injector and detector ports were set at 225°C and 230°C, respectively. The oven temperature program was initially set at 165°C for the first 30 minutes, and then increased at a rate of 1.5°C/min to 220°C, where it remained for the last 15 minutes. The carrier gas was helium and the linear velocity of 40cm/sec. was measured at the initial temperature.

Results

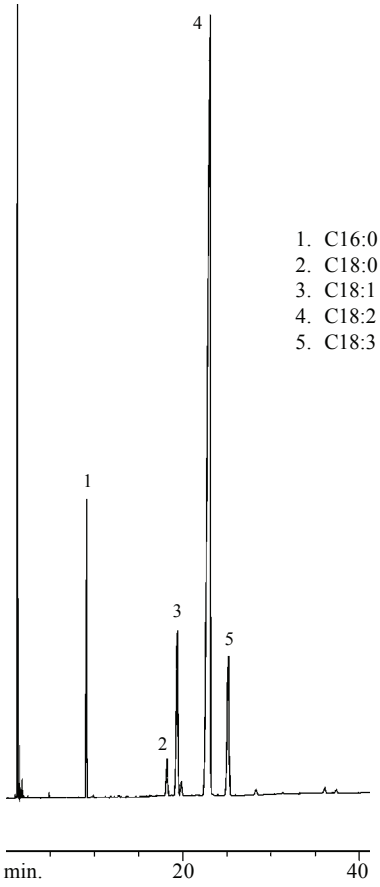
Regarding the content of the Omega-3 and Omega-6 essential fatty acids in the four oils studied, all contain LA (18:2n6) (***Figures 1-4 and Table 1***). Evening primrose oil (***Figure 1***) contains the largest amount of LA and would be the best source. Flax seed oil (***Figure 2***) reveals a significant amount of LNA (C18:3n3), and would be the best source of this EFA. Only black currant seed oil (***Figure 3)*** contains an approximately equal amount of both isomeric linolenic acids. All chromatograms, except that of flax seed oil, illustrate the presence of GLA (C18:3n6). In fact, borage oil (***Figure 4***) and black currant seed oil are significant sources of GLA.

Conclusion

Omega-3 (n-3) and Omega-6 (n-6) EFAs perform key functions in our bodies. Quantifying these compounds in nutritional supplements such as evening primrose oil, flax seed oil, black currant seed oil, and borage oil is successfully achieved using the FAMEWAX™ column. This column offers excellent efficiency and selectivity towards these polyunsaturated methyl esters, providing an accurate determination of the fatty acid profiles. Thus the FAMEWAX™ column is an excellent column choice for this and similar applications.

Figure 1

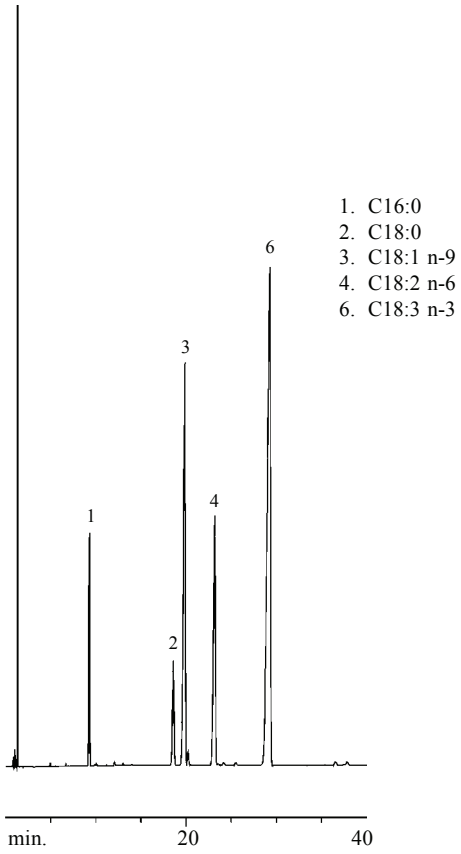
Evening primrose oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Figure 2

Flax seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

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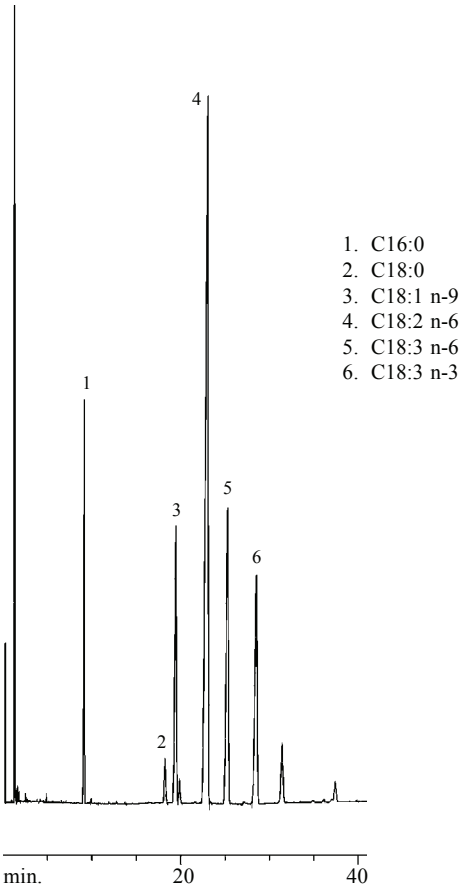
Table I

Composition of Omega-3 and Omega-6 EFAs in the four oils (% area).

	C16:0	*C18:0*	*C18:1n9*	*C18:2n6*	*C18:3n6*	*C18:3n3*
Evening Primrose Oil	6.5	1.8	8.6	**73.5**	8.7	n/a
Flax Seed Oil	4.9	5.2	23.7	15.2	n/a	**50.1**
Black Currant Seed Oil	6.7	1.6	11.3	**47.1**	15.3	13.1
Borage Oil	11.5	4.9	19.5	**40.3**	22.1	n/a

Figure 3

Black currant seed oil



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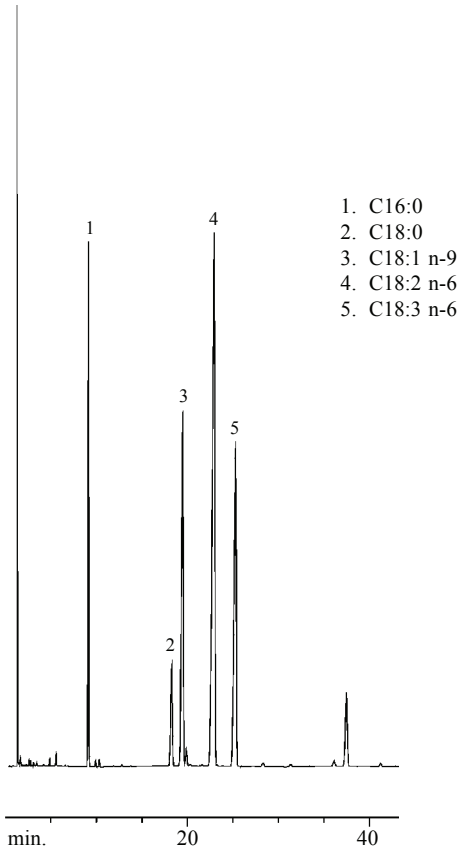
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

Figure 4

Borage seed oil



30m, 0.25mm ID, 0.25µm FAMEWAX™ (cat.# 12497).
Oven temp.: 165°C (hold 30 min.) to 220°C @ 1.5°C/min. (hold 15 min.); **Inj. temp.:** 225°C; **Det. temp.:** 230°C;
Carrier gas: Helium @ 40cm/sec.

References

1. Health and Healing News, "Evening Primrose Oil—Superfood for the '90s." http://www.hhnews.com/epo.htm
2. Robert A. Gibson, David R. Lines and Mark Neumann, "Gamma Linolenic Acid (GLA) Content of Encapsulated Evening Primrose Oil Products," *Lipids*, Vol. 27, no. 1 (1992).
3. M.S. Manku, "A Comparison of GLC and HPLC Methods for Determining Fatty Acid Composition of Evening Primrose and Soybean Oil," Journal of Chromatographic Science, Vol. 21, August (1983).

Product Listing

FAMEWAX™ Columns

- Ideal for FAME analysis.
- Similar to Omegawax™ columns.

ID	df (µm)	Stable to	30m
0.25mm	0.25	250°C	12497
0.32mm	0.25	250°C	12498
0.53mm	0.50	250°C	12499

Thermolite® Septa (green)

- Lowest bleed on FIDs, ECDs, and MSDs.
- Excellent puncturability.
- Preconditioned/ready to use.
- Does not adhere to hot metal surfaces.
- Usable to 340°C inlet temperatures.
- Packaged in non-contaminating tins.

Septum Diameter	25-pk.	50-pk.	100-pk.
9.5mm (3/8")	20359	20360	20361
10mm	20378	20379	20380
11mm (7/16")	20363	20364	20365
Shimadzu Plug	20372	20373	20374

High-Capacity Split Vent Trap

- Reduces the release of hazardous materials into the lab when using a split injection mode.
- Lasts one month or 1,500 injections.
- Connecting lines and mounting kit included.

Each	5-pk.
20698	20699

Fatty Acid Methyl Ester Mixtures

Neat fatty acid methyl esters can be used to prepare specific mixtures not commercially available. These products are of the highest purity available. Each compound is packaged under a nitrogen blanket to ensure product stability. A Certificate of Analysis is provided with each ampul. *Packaged 100mg/ampul.*

Carbon No.	Compound	CAS#	cat.#
C14:0	methyl myristate	124-10-7	35045
C14:1Δ9 cis	methyl myristoleate	56219-06-8	35046
C15:0	methyl pentadecanoate	7162-64-1	35047
C16:0	methyl palmitate	112-39-0	35048
C16:1Δ9 cis	methyl palmitoleate	1120-25-8	35049
C17:0	methyl heptadecanoate	1731-92-6	35050
C18:0	methyl stearate	112-61-8	35051
C18:1Δ9 cis	methyl oleate	112-62-9	35052
C18:2Δ9, 12 cis	methyl linoleate	112-63-0	35053
C18:3Δ9,12,15 cis	methyl linolenate	301-00-8	35054
C19:0	methyl nonadecanoate	1731-94-8	35055
C20:0	methyl arachidate	1120-28-1	35056
C20:1Δ11 cis	methyl eicosenoate	2390-09-2	35057
C20:2Δ11,14 cis	methyl eicosadienoate	2463-02-7	35058
C20:3Δ11,14,17 cis	methyl eicosatrienoate	55682-88-7	35059
C20:4Δ5,8,11,14 cis	methyl arachidonate	2566-89-4	35060

Autosampler Syringe 6-Packs for HP 7673 GCs

- Hamilton and SGE syringes are designed and tested to meet critical autosampler specifications.
- Needle point styles are developed to withstand multiple, fast septum injections.

Volume (µL)	Needle Term.	Gauge	Hamilton Restek cat.#	SGE Restek cat.#
5	ASN/F	23s	20170	24783
5	ASN/F	26s	21230	24782
5	ASN/F	23s-26s	24594	21214
10	ASN/F	23s	20169	24787
10	ASN/F	26s	24599	24786
10	ASN/F	23s-26s	24600	21215

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