NEW! 200 m GC Columns for Detailed Analysis of *cis/trans* FAME Isomers

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Over the last half of the previous century, the use of partially hydrogenated vegetable oil (PHVO) replaced the use of animal fats for baking purposes in most western countries. Initially developed for supply/demand and economic reasons, it was discovered that the use of PHVO could increase a food's shelf-life and/or increase its taste. It was also suggested that the unsaturated fatty acids in PHVO were healthier than the saturated fatty acids in animal fat.

In nature, the overwhelming majority of unsaturated fatty acids occur in the *cis* orientation. As such, humans evolved metabolic pathways to break down *cis* fatty acids. However, the process to make PHVO converts *cis* fatty acids into *trans* fatty acids. Scientific research over the last decade has shown that this situation (the increased intake of *trans* fatty acids coupled with our inability to properly metabolize them) can increase the risk of coronary disease. This is most evident by the proliferation of this disease in countries where the use of PHVO has replaced the use of animal fats. To help combat this trend, in June 2015 the US FDA mandated that food manufacturers must eliminate the use of all artificial *trans* fats (i.e. they can no longer use PHVO) within three years.[1]

The qualitative and quantitative testing of *cis/trans* fatty acids is best accomplished using gas chromatography (GC) after conversion of the fatty acids to fatty acid methyl esters (FAMEs). To assist with this testing, Supelco® recently developed two new capillary GC columns. These 200 m versions of SP™-2560 and SLB®-IL111 are specifically designed for and specially tested for the detailed analysis of *cis/trans* FAME isomers. Specifications for both columns are shown in **Table 1**. This article will show the suitability of these columns for analysis of *cis/trans* FAME isomers as well as other FAME isomer applications.

C18 FAME Isomer Mix

Some of the most studied fatty acids are the C18 family. A custom mixture was made by combining a C18:0 FAME standard, a custom C18:1 PHVO sample (containing multiple C18:1 FAME isomers), a 4-component C18:2 FAME isomer standard, and an 8-component C18:3 FAME isomer standard. This mixture was injected on each column, and run conditions were adjusted to achieve maximize resolution. The optimized chromatograms are shown in **Figure 1**. Peak identification was assigned based on previous work.

While neither column can separate every isomer, both columns provide a high degree of separation of *trans* FAME isomers from *cis* FAME isomers. Of interest is that with SLB-IL111, no *trans* C18:1 FAME isomer co-elutes with C18:1Δ9c, one of the most abundant naturally occurring unsaturated fatty acids. It often results in a very large peak area when analyzing food extracts. This is significant because this entire peak area must be considered as being contributed by the *trans* FAME if there is a co-elution, resulting in *trans* fat values that are biased high.

Table 1. Column Specifications

SP-2560

- **Application:** This highly polar biscyanopropyl column was specifically designed for detailed separation of geometric-positional (*cis/trans*) isomers of fatty acid methyl esters (FAMEs). It is extremely effective for FAME isomer applications.
- **USP Code:** This column meets USP G5 requirements.
- **Phase:** Non-bonded; poly(biscyanopropyl siloxane)
- **Temp. Limits:** Subambient to 250 °C (isothermal or programmed)

SLB-IL111

- **Application:** World's first commercial column to rate over 100 on our GC column polarity scale. Selectivity most orthogonal to non-polar and intermediate polar phases, resulting in very unique elution patterns. Maximum temperature of 270 °C is very impressive for such an extremely polar column. Great choice for separation of polarizable analytes (contain double and/or triple C-C bonds) from neutral analytes. Also a good GCxGC column choice. Launched in 2010.
- **USP Code:** None
- **Phase:** Non-bonded; 1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide
- **Temp. Limits:** 50 °C to 270 °C (isothermal or programmed)

CLA FAME Isomer Mix

The stomachs of several mamalian species have four compartments. These mammals are known as ruminants, and include cows, sheep, goats, and deer. Ruminant fat contains conjugated linoleic acid (CLA) isomers, which are C18:2 fatty acids in which a single carbon-carbon bond separates the two double bonds. A custom mixture containing four CLA FAME isomers was prepared and injected on each column. Run conditions were adjusted to achieve maximize resolution. The optimized chromatograms are shown in **Figure 2**. Peak identification was assigned by injecting each isomer individually. Both columns were able to provide resolution, although with slightly different elution patterns.

Rapeseed Oil FAMEs with CLA FAME Isomers

Rapeseed oil is a simple vegetable oil that contains a series of saturated and unsaturated fatty acids ranging from C14 through C24 in carbon number. A custom mixture containing rapeseed oil FAMEs plus four CLA FAME isomers was prepared and injected on each column. Run conditions were identical to those previously used. The resulting chromatograms are shown in **Figure 3**. Peak identification was assigned based on previous work.

Monitoring the elution locations of the polyunsaturated C18 FAME isomers (peaks 5-10) relative to the saturated and monounsaturated FAME isomers is an indication of a column's ability to undergo dipole-induced dipole interactions. Both columns exhibited great relative retention of these isomers. In fact, the SLB-IL111 retained the CLA FAME isomers (C18:2 species) after C22:0. Also of note is that the co-elutions on one column are fully resolved on the other, providing complementary data.

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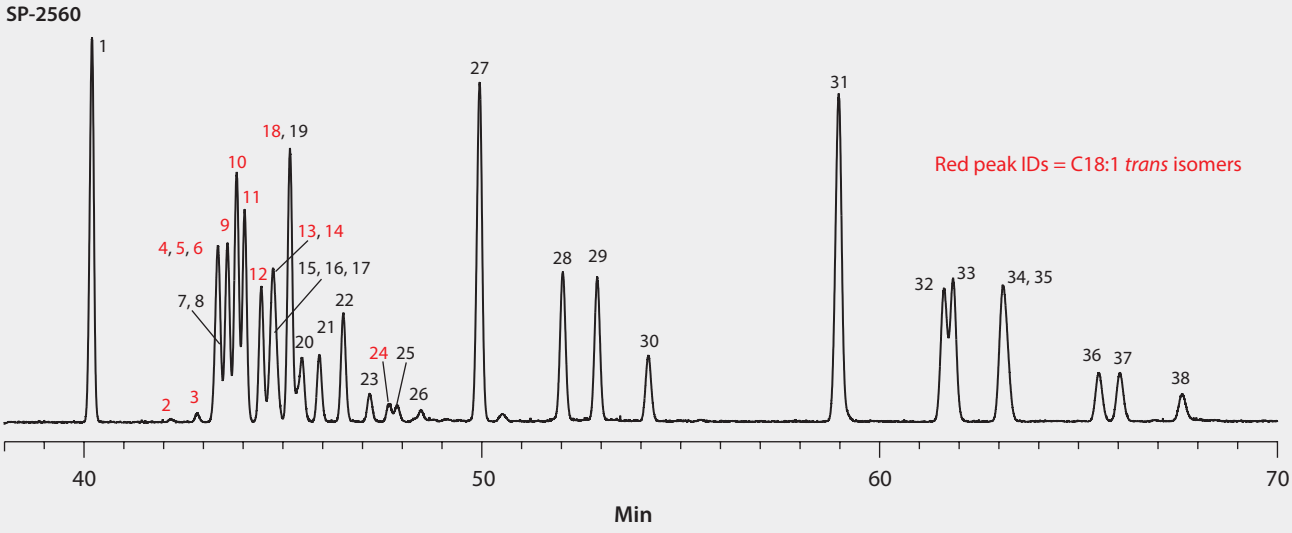
Figure 1. C18:0, C18:1, C18:2, and C18:3 FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Mix of C18:0, C18:1 (from partially hydrogenated vegetable oil [PHVO]), C18:2, and C18:3 FAME isomers

1. C18:0
2. C18:1Δ4t
3. C18:1Δ5t
4. C18:1Δ6t
5. C18:1Δ7t
6. C18:1Δ8t
7. C18:1Δ4c
8. C18:1Δ5c
9. C18:1Δ9t
10. C18:1Δ10t
11. C18:1Δ11t
12. C18:1Δ12t
13. C18:1Δ13t
14. C18:1Δ14t
15. C18:1Δ6c
16. C18:1Δ7c
17. C18:1Δ8c
18. C18:1Δ15t
19. C18:1Δ9c
20. C18:1Δ10c
21. C18:1Δ11c
22. C18:1Δ12c
23. C18:1Δ13c
24. C18:1Δ16t
25. C18:1Δ14c
26. C18:1Δ15c
27. C18:2Δ9t,12t
28. C18:2Δ9c,12t
29. C18:2Δ9t,12c
30. C18:2Δ9c,12c
31. C18:3Δ9t,12t,15t
32. C18:3Δ9t,12t,15c
33. C18:3Δ9t,12c,15t
34. C18:3Δ9c,12c,15t
35. C18:3Δ9c,12t,15t
36. C18:3Δ9c,12t,15c
37. C18:3Δ9t,12c,15c
38. C18:3Δ9c,12c,15c



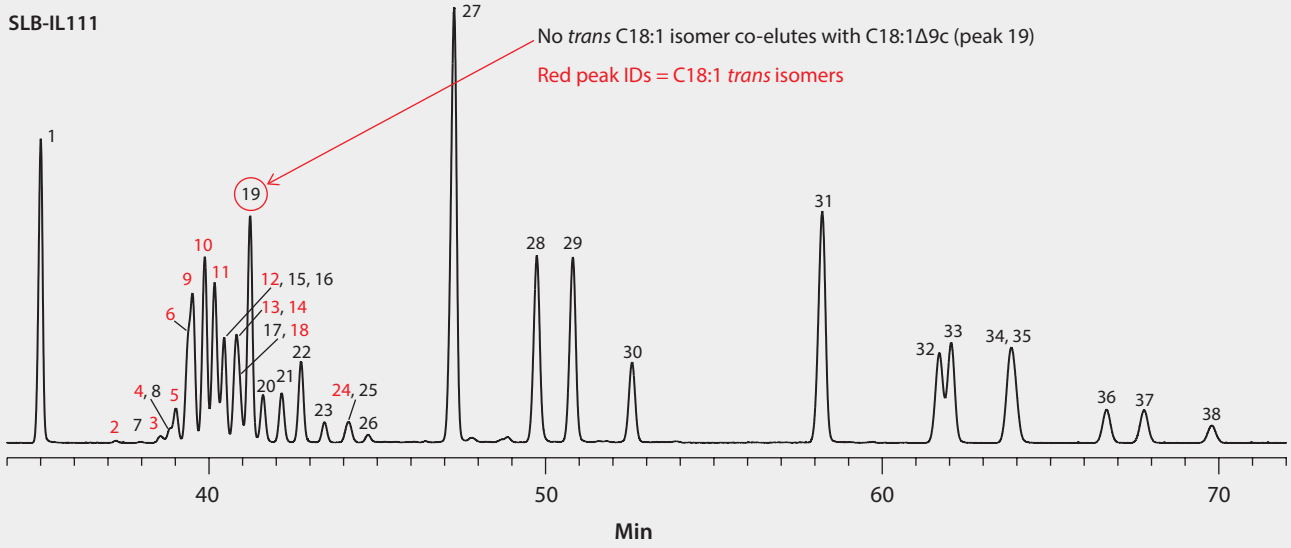
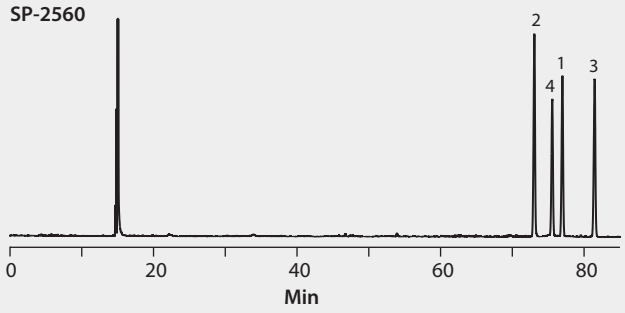
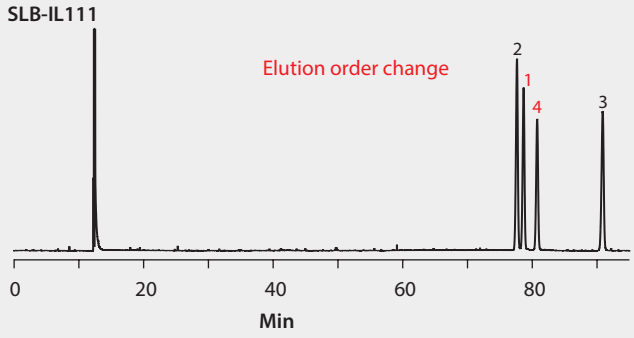


Figure 2. CLA FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D. , 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: 4-component CLA FAME isomer mix, 0.5 mg/mL in methylene chloride

1. C18:2Δ9t,11t (methyl 9-*trans*,11-trans octadecadienoate)
2. C18:2Δ9t,11c (methyl 9-*trans*,11-cis octadecadienoate)
3. C18:2Δ9c,11c (methyl 9-*cis*,11-cis octadecadienoate)
4. C18:2Δ10c,12t (methyl 10-*cis*,12-trans octadecadienoate)





38-Component FAME Isomer Mix

Determining the degree of fatty acid unsaturation of a food product is difficult because foods can contain a complex mixture of saturated, monounsaturated, and polyunsaturated fatty acids with a variety of carbon chain lengths.

The Supelco 37-Component FAME Mix contains methyl esters of fatty acids ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, making this standard very useful to food analysts since it can be used to identify fatty acids in many different types of foods. A custom standard comprised of this mix plus C22:5n3 FAME was prepared, and analyzed on each column under identical conditions. **Figure 4** shows the chromatograms obtained from both columns.

Discussion

The SP-2560/SLB-IL111 pairing allows the most comprehensive fatty acid composition information possible, able to provide accurate results (qualitative and quantitative) for both saturated and *trans* fatty acids. Observations include:

- While not shown, increased resolution was achieved when comparing chromatograms from 200 m versions to chromatograms from 100 m versions.
- Analytes tend to elute from the SLB-IL111 at a lower oven temperature (see **Figures 1-4**)
- SLB-IL111 provides resolution of C18:1Δ9c (one of the most abundant naturally occurring unsaturated fatty acids) from all *trans* FAMEs (see **Figure 1**).
- SP-2560 and SLB-IL111 provide different elution patterns for the CLA FAME isomers analyzed (see **Figures 2** and **3**).
- SLB-IL111 provides increased retention of unsaturated FAME isomers (see **Figures 3** and **4**).
- SP-2560 provides better resolution of saturated FAME isomers from unsaturated FAME isomers (see **Figure 4**).

Conclusion

The SP-2560 chemistry was first introduced in 1983, and the SLB-IL111 chemistry was first introduced in 2010. The new 200 m versions of these chemistries indicate our commitment to remain at the forefront of detailed analysis of *cis/trans* FAME isomers. The SP-2560/SLB-IL111 pairing allows the most comprehensive fatty acid composition information possible.

Reference

1. FDA Cuts *Trans* Fat in Processed Foods. http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm372915.htm (accessed August 18, 2015)

Featured Products

Description	Cat. No.
SP-2560, 200 m × 0.25 mm I.D., 0.20 µm	29688-U
SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm	29689-U
37-Component FAME Mix	CRM47885

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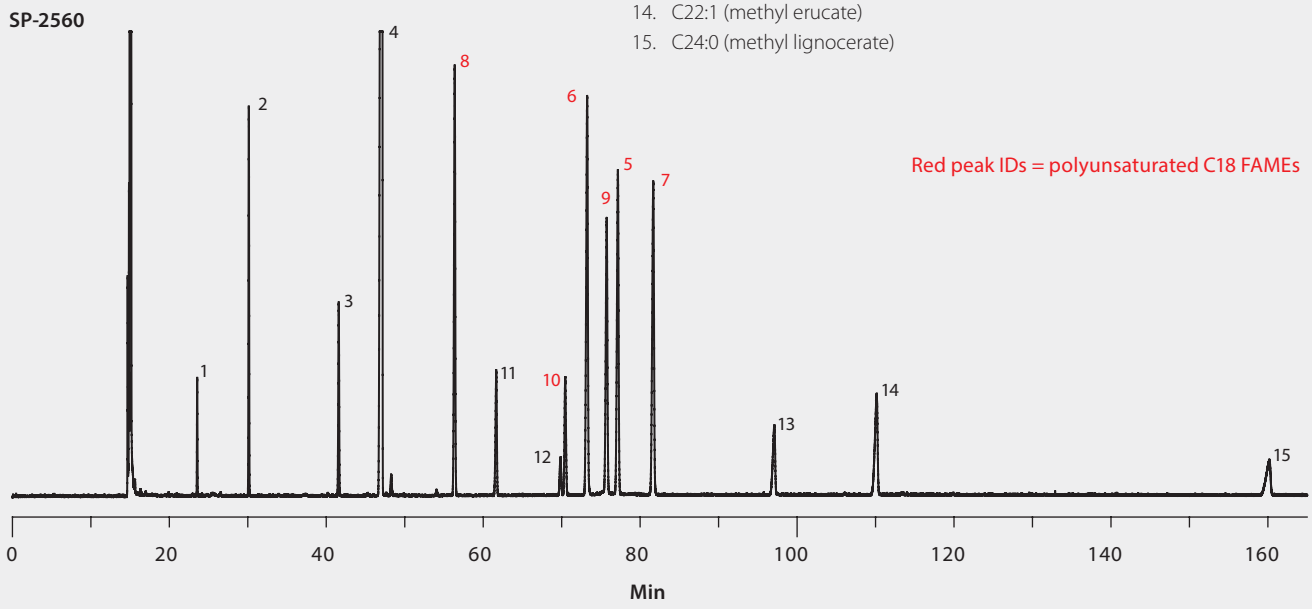
Figure 3. Rapeseed Oil FAMEs with CLA FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 175 °C (SP-2560), 150 °C (SLB-IL111)
inj. temp.: 250 °C
carrier gas: hydrogen
detector: FID, 250 °C
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Mix of rapeseed oil FAME isomers and CLA FAME isomers

1. C14:0 (methyl myristate)
2. C16:0 (methyl palmitate)
3. C18:0 (methyl stearate)
4. C18:1n9c (methyl oleate)
5. C18:2Δ9t,11t (methyl 9-*trans*,11-trans octadecadienoate)
6. C18:2Δ9t,11c (methyl 9-*trans*,11-cis octadecadienoate)
7. C18:2Δ9c,11c (methyl 9-*cis*,11-cis octadecadienoate)
8. C18:2n6c,9c (methyl linoleate)
9. C18:2Δ10c,12t (methyl 10-*cis*,12-trans octadecadienoate)
10. C18:3n3c,6c,9c (methyl linolenate)
11. C20:0 (methyl arachidate)
12. C20:1 (methyl eicosenoate)
13. C22:0 (methyl behenate)
14. C22:1 (methyl erucate)
15. C24:0 (methyl lignocerate)



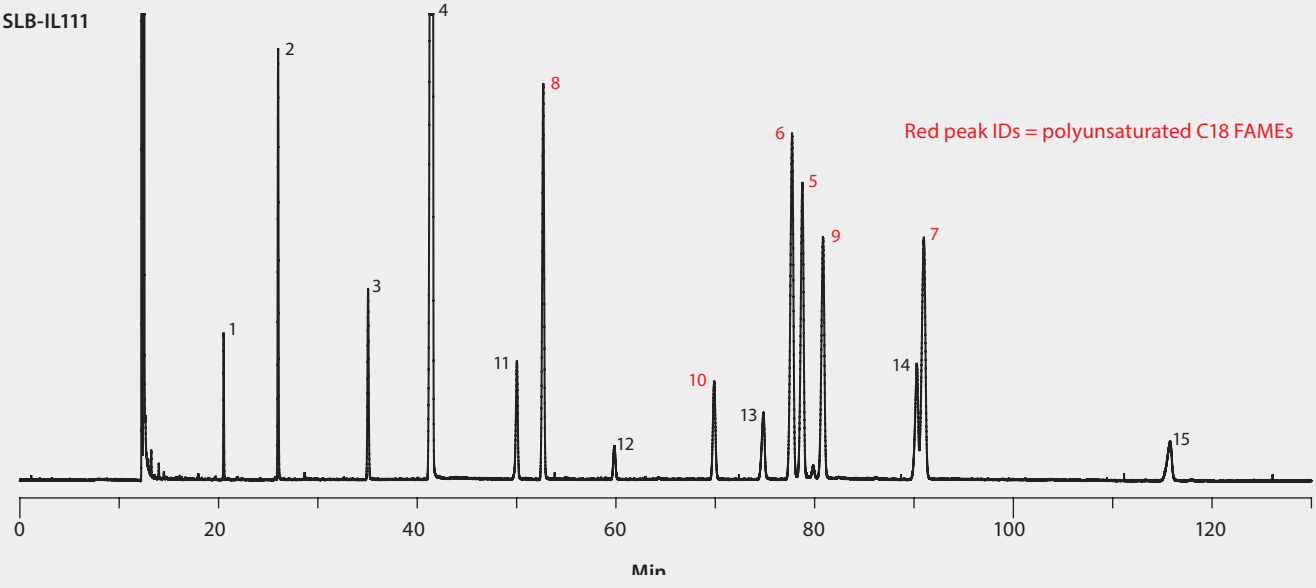


Figure 4. 38-Component FAME Isomers

column: SP-2560, 200 m × 0.25 mm I.D., 0.20 µm (29688-U)
column: SLB-IL111, 200 m × 0.25 mm I.D., 0.20 µm (29689-U)
oven: 100 °C (13 min), 4 °C/min to 240 °C (30 min)
inj. temp.: 250 °C
detector: FID, 250 °C
carrier gas: hydrogen, 27 cm/sec
injection: 1.0 µL, 50:1 split
liner: 4 mm I.D., split type, cup design
sample: Supelco 37-Component FAME Mix (47885-U) + C22:5n3 FAME

1. Butyric Acid Methyl Ester (C4:0)
2. Caproic Acid Methyl Ester (C6:0)
3. Caprylic Acid Methyl Ester (C8:0)
4. Capric Acid Methyl Ester (C10:0)
5. Undecanoic Acid Methyl Ester (C11:0)
6. Lauric 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)
10. Pentadecanoic Acid Methyl Ester (C15:0)
11. *cis*-10-Pentadecenoic Acid Methyl Ester (C15:1)
12. Palmitic Acid Methyl Ester (C16:0)
13. Palmitoleic Acid Methyl Ester (C16:1)
14. Heptadecanoic Acid Methyl Ester (C17:0)
15. *cis*-10-Heptadecenoic Acid Methyl Ester (C17:1)
16. Stearic Acid Methyl Ester (C18:0)
17. Oleic Acid Methyl Ester (C18:1n9c)
18. Elaidic Acid Methyl Ester (C18:1n9t)
19. Linoleic Acid Methyl Ester (C18:2n6c)
20. Linolelaidic Acid Methyl Ester (C18:2n6t)
21. γ-Linolenic Acid Methyl Ester (C18:3n6)
22. α-Linolenic Acid Methyl Ester (C18:3n3)
23. Arachidic Acid Methyl Ester (C20:0)
24. *cis*-11-Eicosenoic Acid Methyl Ester (C20:1n9)
25. *cis*-11,14-Eicosadienoic Acid Methyl Ester (C20:2)
26. *cis*-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6)
27. *cis*-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3)
28. Arachidonic Acid Methyl Ester (C20:4n6)
29. *cis*-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3)
30. Heneicosanoic Acid Methyl Ester (C21:0)
31. Behenic Acid Methyl Ester (C22:0)
32. Erucic Acid Methyl Ester (C22:1n9)
33. *cis*-13,16-Docosadienoic Acid Methyl Ester (C22:2)
34. *cis*-7,10,13,16,19-Docosapentaenoic Acid Methyl Ester (C22:5n3)
35. *cis*-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (C22:6n3)
36. Tricosanoic Acid Methyl Ester (C23:0)
37. Lignoceric Acid Methyl Ester (C24:0)
38. Nervonic Acid Methyl Ester (C24:1n9)