Highly Detailed Analysis of *cis/trans* FAME Isomers

Using 200 m SP™-2560 and SLB®-IL111 Capillary GC Columns

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. During the process to make PHVO, *cis* fatty acids are converted into *trans* fatty acids, which increases the shelf life of food products. Scientific research over the last decade has shown that the increased intake of *trans* fatty acids coupled with our inability to properly metabolize them can increase the risk of coronary disease. In June 2015, the US FDA mandated that food manufacturers must stop using 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 GC after conversion of the fatty acids to fatty acid methyl esters (FAMEs). The 200 m SP-2560 and SLB-IL111 are specifically designed and tested for the highly detailed analysis of *cis/trans* FAME isomers. Specifications for both columns are shown in **Table 1**.

Table 1. Column Specifications

SP™-2560	
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	
USP Code	None
Phase	Non-bonded; 1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethanesulfonyl)imide
Temp. Limits	50 °C to 270 °C (isothermal or programmed)

C18 FAME Isomer Mix

Some of the most studied fatty acids are the C18 family. A custom mixture was injected in each column, and run conditions were adjusted to maximize resolution. The optimized chromatograms are shown in **Figure 1**. While neither column can separate all isomers, both provide a high degree of separation of *trans* FAME isomers from *cis* FAME isomers. Of interest is that SLB-IL111 provides resolution of C18:1Δ9c (a *cis* FAME) from all *trans* FAMEs. This is significant because C18:1Δ9c often results in a very large peak area while analyzing food extracts. Its entire peak area must be considered as being contributed by a *trans* FAME if there is a co-elution, resulting in *trans* fat values that are biased high.

38-Component FAME Isomer Mix

A custom standard comprised of FAMEs ranging from C4 to C24, including key monounsaturated and polyunsaturated fatty acids, was analyzed on each column under identical conditions. **Figure 2** shows the chromatograms obtained from both columns. Of relevance is that SP-2560 provides better resolution of saturated FAME isomers from unsaturated FAME isomers, and that SLB-IL111 provides increased retention of unsaturated FAME isomers.

Rapeseed Oil FAMEs with CLA FAME Isomers

A custom mixture containing rapeseed oil FAMEs plus four CLA FAME isomers was injected in each column using oven temperature programs which optimized resolution. Rapeseed oil is a simple vegetable oil that contains a series of C14-C24 saturated and unsaturated fatty acids. CLAs are C18:2 fatty acids in which a single carbon-carbon bond separates the two double bonds. The resulting chromatograms are shown in **Figure 3**. Of note is that the co-elutions on one column are fully resolved on the other, providing complementary data.

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:

- Analytes elute from SLB-IL111 at a lower oven temperature (**Figures 1–3**)
- SLB-IL111 provides resolution of C18:1Δ9c from all *trans* FAMEs (**Figure 1**)
- SP-2560 provides better resolution of saturated FAME isomers (**Figure 2**)
- SLB-IL111 provides increased retention of unsaturated FAME isomers (**Figures 2–3**)

Ordering Information

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

Reference

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

Related Information

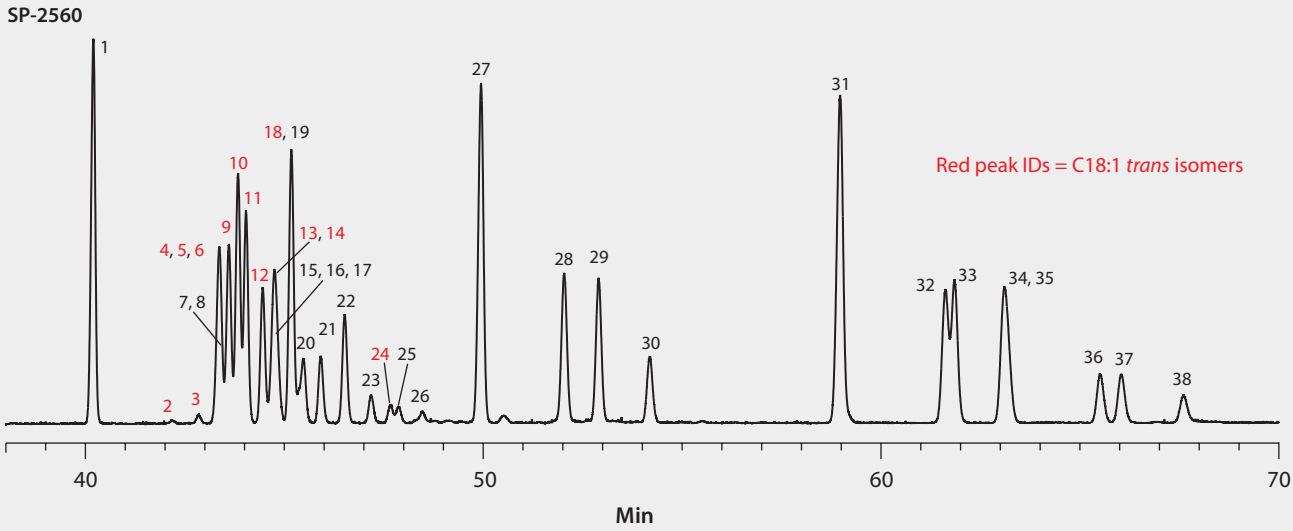
Additional chromatograms, product information, real-time availability, and ordering information is available 24 hours at

sigma-aldrich.com/gc-food

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



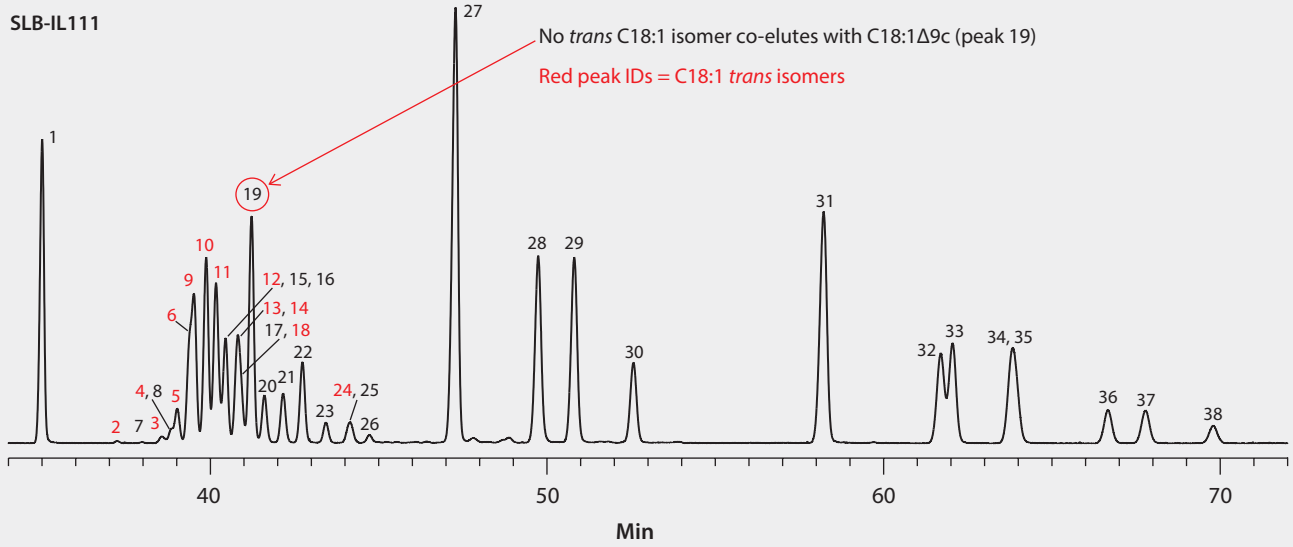
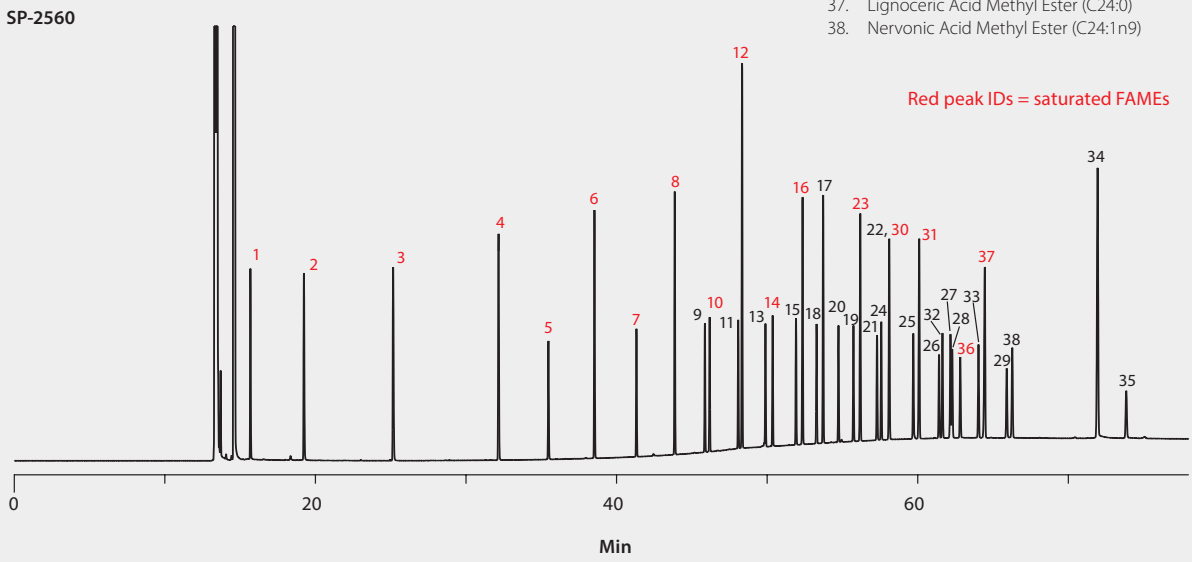


Figure 2. 38-Component FAME Isomers

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)



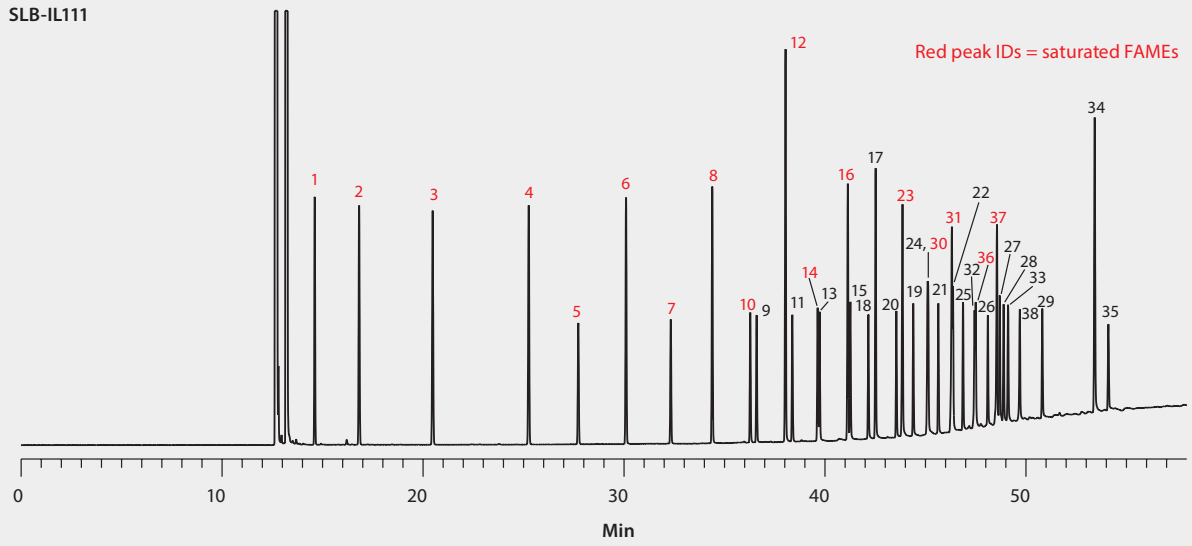
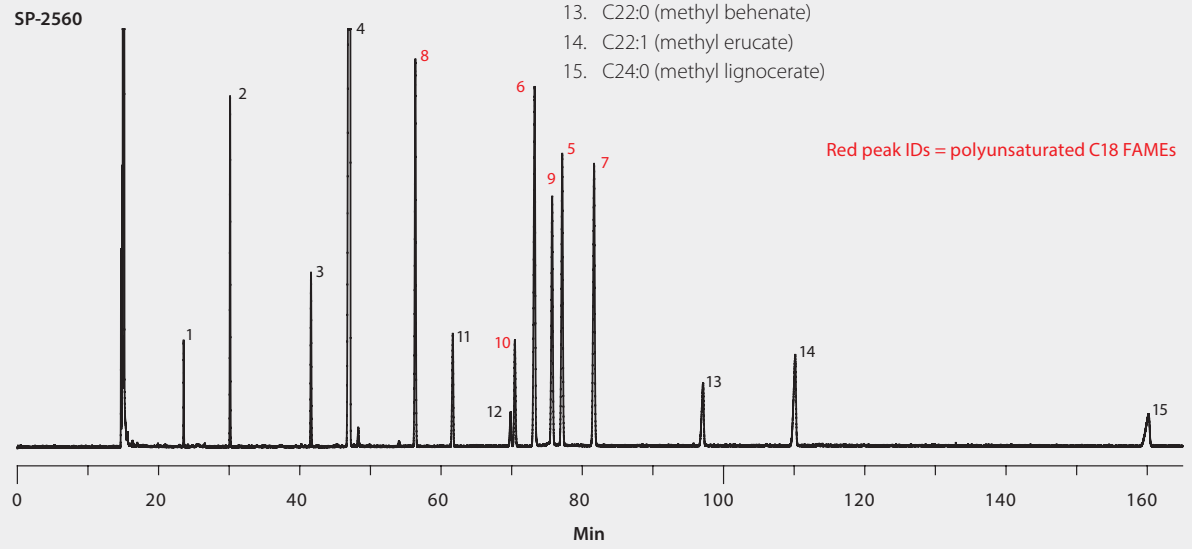
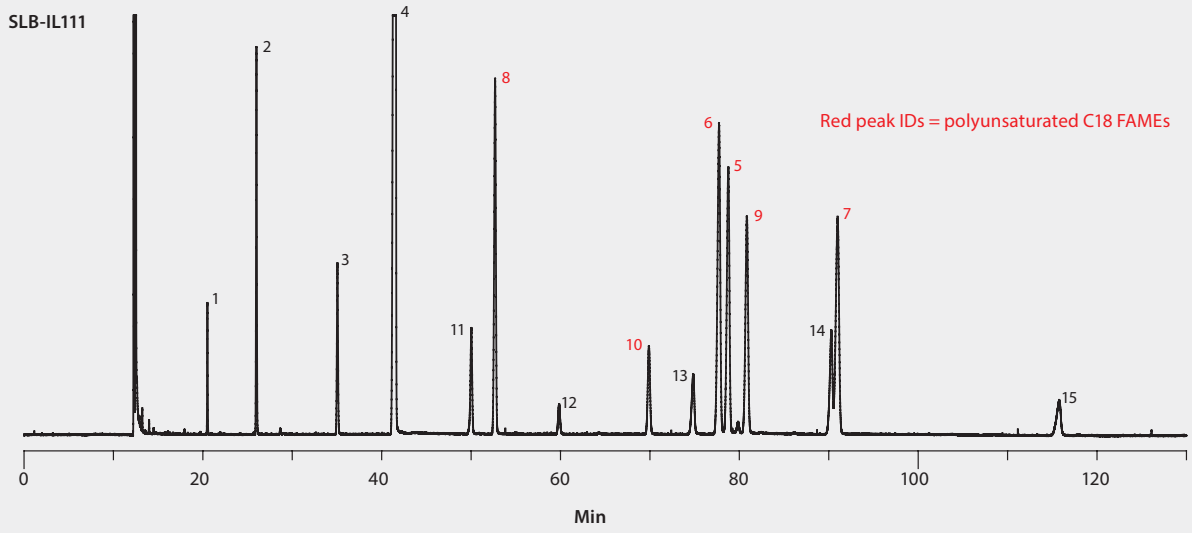


Figure 3. Rapeseed Oil FAMEs with CLA FAME Isomers

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)





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