Technical Report

SLB-IL111 for Fatty Acid Methyl Ester (FAME) Applications

Michael D. Buchanan, Katherine K. Stenerson, and Leonard M. Sidisky

The extremely polar SLB™-IL111 column exhibits the highest polarity of any GC phase, allowing it to resolve key cis/trans fatty acid methyl ester (FAME) isomers that cannot be resolved on other columns. It also provides an alternative selectivity for FAME applications typically performed on highly polar cyanopropyl siloxane columns. Its maximum temperature of 270 °C is very impressive for such an extremely polar column. The chromatograms shown here demonstrate the utility of the SLB-IL111 as a great column choice for the analysis of FAMEs.

Fatty Acid Chemistry

The most commonly analyzed fatty acids consist of C4-C24+ hydrocarbon chains with a carboxyl group (-COOH) at one end. Many types of fatty acids exist, including:

- **Saturated** = all single bonds between carbon atoms.
- **Unsaturated** = at least one double bond.
- **Polyunsaturated** = at least two double bonds.
- **cis** = bonds on either side of the double bond are on the same side.
- **trans** = bonds on either side of the double bond are on opposite sides.
- **Omega 3** = initial double bond located directly after the third carbon atom as measured from the methyl end.
- **Omega 6** = initial double bond located directly after the sixth carbon atom as measured from the methyl end.

It is important for food manufacturers to report the levels of each type of fatty acid, as each has known or suspected health effects. Figure 1 shows the structures of several varieties of fatty acids.

Derivatization of Fatty Acids to Methyl Esters

GC can be used to analyze fatty acids, either as free fatty acids or as fatty acid methyl esters. The primary reasons to analyze fatty acids as fatty acid methyl esters include:

- In their free, undervatized form, fatty acids may be difficult to analyze, because these highly polar compounds tend to form hydrogen bonds, leading to adsorption issues. Reducing their polarity may make them more amenable for analysis.
- To distinguish between the very slight differences exhibited by unsaturated fatty acids, the polar carboxyl functional groups must first be neutralized. This then allows column chemistry to perform separations by degree of unsaturation, position of unsaturation, and even the cis vs. trans configuration of unsaturation.

The esterification of fatty acids to FAMES is performed using an alkylation derivatization reagent. Methyl esters offer excellent stability, and provide quick and quantitative samples for GC analysis. As shown in Table 1, the esterification reaction involves the condensation of the carboxyl group of the fatty acid and the hydroxyl group of an alcohol. Esterification is best done in the presence of an acidic catalyst (such as boron trichloride). The catalyst protonates the oxygen atom of the carboxyl group, making the acid much more reactive. Nucleophilic attack by an alcohol then yields an ester with the loss of water. The catalyst is removed with the water. The alcohol used determines the alkyl chain length of the resulting esters; the use of methanol will result in the formation of methyl esters whereas the use of ethanol will result in ethyl esters. (1,2)

**Table 1. Typical Esterification Procedure**

1. Weigh 1-25 mg of sample into a 5-10 mL Micro Reaction Vessel.
2. Add 2 mL BCl₃-Methanol, 12% w/w. A water scavenger (such as 2,2-dimethoxypropane) can be added at this point.
3. Heat at 60 °C for 5-10 minutes. Derivatization times may vary, depending on the specific compound(s) being derivatized.
4. Cool, then add 1 mL water and 1 mL hexane.
5. Shake the reaction vessel (it is critical to get the esters into the non-polar solvent).
6. After allowing the layers to settle, carefully transfer the upper (organic) layer to a clean vial. Dry the organic layer either by passing through a bed of anhydrous sodium sulfate during the transfer step to the clean vial, or by adding anhydrous sodium sulfate to the clean vial and then shaking.
7. Proceed to the appropriate GC analysis procedure.
GC Column Polarity Scale

A visual depiction of our GC column polarity scale is shown in Figure 2, showing the relationship of several columns to one another. The positions/maximum temperatures of several non-ionic liquid capillary GC columns are shown to the left of the scale. Listed to the right of the scale are the positions/maximum temperatures of Supelco® ionic liquid capillary GC columns. All polarity number values are relative to both squalane (0 on the scale) and SLB-IL100 (100 on the scale). This simple but useful scale allows multiple columns to be quickly compared. Detailed information concerning the scientific basis used to generate this scale can be found at sigma-aldrich.com/il-gc

Figure 2. GC Column Polarity Scale, Positions/Maximum Temperatures of Columns

Standard: 37-Component FAME Mix

To properly identify fatty acid composition, standards of known reference must be used. One such standard is the Supelco 37-Component FAME Mix (47885-U). This standard 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. This mix was analyzed on the SP™-2560, a popular cyanopropyl siloxane column, and the SLB-IL111. Optimized chromatograms are shown in Figure 3. Observations are:

- **Baseline and Peak Shape.** Both columns produced chromatograms with minimal bleed and sharp peaks. Signal-to-noise ratios for the later eluting peaks are slightly better with the SLB-IL111 column.
- **Run Time.** Dispersive interaction is weaker on the SLB-IL111; therefore, overall analysis time is shorter than on the SP-2560 (22 minutes compared to 26 minutes).
- **Selectivity.** Dipole-induced dipole interactions are stronger on the SLB-IL111 than on the SP-2560. These interactions result in increased retention of polarizable analytes (those with double bonds) relative to non-polarizable analytes. In fact, the higher the degree of unsaturation (the greater the number of double bonds), the stronger the retention is. Overall, this results in alternative selectivity to the SP-2560. A comparison of elution orders and elution locations are summarized in Table 2 and Table 3, respectively.

Table 2. Comparison of Elution Orders

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<tr>
<th>Peak ID</th>
<th>Isomer</th>
<th>SP-2560*</th>
<th>SLB-IL111*</th>
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<td>19</td>
<td>C18:2n6c</td>
<td>C18:0</td>
<td>C20:0</td>
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<td>C18:3n6</td>
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<td>33</td>
<td>C22:2</td>
<td>C23:0</td>
<td>C24:0</td>
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* On this column, this isomer elutes after this saturated FAME.

Table 3. Comparison of Elution Locations

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<th>SLB-IL111</th>
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<td>28</td>
<td>C20:4n6</td>
<td>Right after C23:0</td>
<td>Right before C24:0</td>
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<tr>
<td>29</td>
<td>C20:5n3</td>
<td>Right after C24:0</td>
<td>&gt;1 min. after C24:0</td>
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<tr>
<td>32</td>
<td>C22:1n9</td>
<td>Between C22:0 and C23:0</td>
<td>Right before C23:0</td>
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<tr>
<td>34</td>
<td>C22:6n3</td>
<td>~2.5 min. after C24:0</td>
<td>~3.2 min. after C24:0</td>
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Figure 3. 37-Component FAME Mix

SP-2560 Conditions
- column: SP-2560, 100 m x 0.25 mm I.D., 0.20 μm (24056)
- oven: 140 °C (5 min.), 8 °C/min. to 180 °C, 4 °C/min. to 210 °C, 20 °C/min. to 250 °C (7 min.)
- inj.: 250 °C
- det.: FID, 250 °C
- carrier gas: hydrogen, 40 cm/sec
- injection: 1 μL, 100:1 split
- liner: 4 mm I.D., cup design
- sample: Supelco 37-Component FAME Mix (47885-U), analytes at concentrations indicated in methylene chloride

SLB-IL111 Conditions
- column: SLB-IL111, 100 m x 0.25 mm I.D., 0.20 μm (29647-U)
- oven: 140 °C (5 min.), 8 °C/min. to 180 °C, 5 °C/min. to 260 °C
- det.: FID, 260 °C
- All other conditions the same as those used for the SP-2560

1. Butyric Acid Methyl Ester (C4:0) at 4 wt %
2. Caproic Acid Methyl Ester (C6:0) at 4 wt %
3. Caprylic Acid Methyl Ester (C8:0) at 4 wt %
4. Capric Acid Methyl Ester (C10:0) at 4 wt %
5. Undecanoic Acid Methyl Ester (C11:0) at 2 wt %
6. Lauric Acid Methyl Ester (C12:0) at 4 wt %
7. Tridecanoic Acid Methyl Ester (C13:0) at 2 wt %
8. Myristic Acid Methyl Ester (C14:0) at 4 wt %
9. Myristoleic Acid Methyl Ester (C14:1) at 2 wt %
10. Pentadecanoic Acid Methyl Ester (C15:0) at 2 wt %
11. cis-10-Pentadecenoic Acid Methyl Ester (C15:1) at 2 wt %
12. Palmitic Acid Methyl Ester (C16:0) at 6 wt %
13. Palmitoleic Acid Methyl Ester (C16:1) at 2 wt %
14. Heptadecanoic Acid Methyl Ester (C17:0) at 2 wt %
15. cis-10-Heptadecenoic Acid Methyl Ester (C17:1) at 2 wt %
16. Stearic Acid Methyl Ester (C18:0) at 4 wt %
17. Oleic Acid Methyl Ester (C18:1n9c) at 4 wt %
18. Elaidic Acid Methyl Ester (C18:1n9t) at 2 wt %
19. Linoleic Acid Methyl Ester (C18:2n6c) at 2 wt %
20. Linoleadiacid Acid Methyl Ester (C18:2n6d) at 2 wt %
21. α-Linolenic Acid Methyl Ester (C18:3n6) at 2 wt %
22. γ-Linolenic Acid Methyl Ester (C18:3n3) at 2 wt %
23. Arachidic Acid Methyl Ester (C20:0) at 4 wt %
24. cis-11-Eicosanoic Acid Methyl Ester (C20:1n9) at 2 wt %
25. cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2) at 2 wt %
26. cis-8,11,14-Eicosatrienoic Acid Methyl Ester (C20:3n6) at 2 wt %
27. cis-11,14,17-Eicosatrienoic Acid Methyl Ester (C20:3n3) at 2 wt %
28. Arachidonic Acid Methyl Ester (C20:4n6) at 2 wt %
29. cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester (C20:5n3) at 2 wt %
30. Heneicosanoic Acid Methyl Ester (C21:0) at 2 wt %
31. Behenic Acid Methyl Ester (C22:0) at 4 wt %
32. Erucic Acid Methyl Ester (C22:1n9) at 2 wt %
33. cis-13,16-Docosadienoic Acid Methyl Ester (C22:2) at 2 wt %
34. cis-4,7,10,13,16,19- Docosahexaenoic Acid Methyl Ester (C22:6n3) at 2 wt %
35. Tricosanoic Acid Methyl Ester (C23:0) at 2 wt %
36. Lignoceric Acid Methyl Ester (C24:0) at 2 wt %
37. Nervonic Acid Methyl Ester (C24:1n9) at 2 wt %
Application: Trans Fats

Fatty acids in the cis configuration are the dominant form in nature. Correspondingly, enzymes have evolved to efficiently digest and metabolize them with a high degree of specificity. Conversely, trans fatty acids are relatively rare in nature. However, they have become predominant synthetic additives to processed foods, especially fried foods and baked goods, because they can increase the shelf life and flavor stability of foods containing them. It is now known that trans fatty acids, formed by partial hydrogenation of vegetable oil, interfere with natural metabolic processes. Studies have linked their nutritional contribution to be similar to that of saturated fatty acids, possibly playing a role in the heightened risk of coronary artery disease. (3-5)

Because of this, consumer groups have pressured manufacturers and restaurants for the elimination of trans fats. Many regulatory agencies worldwide now require content labeling, to inform buyers of ‘trans fat’ levels of foods and some dietary supplements.

The analysis of nutritionally significant C18 cis and trans fatty acids, as their methyl esters requires the use of a highly polar column to resolve the geometric positional isomers. The 100 m SP-2560 column is extremely useful for this type of analysis. Based on the results seen with analysis of the 37-component FAME mix, the SLB-IL111 was expected to offer selectivity for this application that is complementary to the SP-2560. To compare the two columns, a partially hydrogenated vegetable oil (PHVO) sample containing various C18:1 geometric positional isomers was analyzed on both. The PHVO extract was graciously provided by Dr. Pierluigi Delmonte at the United States Food and Drug Administration (US FDA). A trans fraction extract and a cis fraction extract, both prepared from PHVO using an Ag-ion fractionation procedure, were also supplied by Dr. Delmonte. The PHVO extract contains thirteen C18:1 trans FAME isomers (from C18:1\(\Delta_4\)t to C18:1\(\Delta_{16}\)t), and thirteen C18:1 cis FAME isomers (from C18:1\(\Delta_4\)c to C18:1\(\Delta_{16}\)c). Complete details of the PHVO extract preparation and Ag-ion fractionation can be found in Reference 6.

Once optimal conditions were established, the PHVO, trans fraction, and cis fraction extracts were sequentially analyzed on each column. Figure 4 shows the resulting chromatograms; on the SP-2560 at 180 °C isothermal, the method-specified oven temperature, and the SLB-IL111 at 168 °C isothermal, the experimentally-determined optimal oven temperature. Following the completion of these analyses and a review of Reference 6, several observations were made.

- **Elution Temperature.** Even at a lower oven temperature, analytes eluted faster from the SLB-IL111 than the SP-2560. This is due to the higher polarity of the SLB-IL111 phase.
- **Elution Order.** The SLB-IL111 resulted in a different elution order than that obtained with the SP-2560. This was predicted due to the different selectivities of the columns observed during the analysis of the 37-component FAME mix, and based on the data used to generate our GC column polarity scale.
- **C18:1 Isomers.** The SLB-IL111 was able to provide resolution of C18:1\(\Delta_{15}\)t from C18:1\(\Delta_9\)c, a separation not possible with the SP-2560. Additionally, the SLB-IL111 offered improved resolution of some isomers that cannot be completely resolved with the SP-2560, such as C18:1\(\Delta_{10}\)t from C18:1\(\Delta_{11}\)t, and the pair C18:1\(\Delta_{3}\)t/C18:1\(\Delta_{4}\)t from other isomers.
- **C18:2 Isomers.** Delmonte et al. reported that the SLB-IL111 is able to resolve C18:2\(\Delta_9\)c,11t from C18:2\(\Delta_7\)t,9c. These are the two most abundant conjugated linoleic acid (CLA) isomers found in ruminant fats. These two important isomers cannot be resolved using any other GC column without first performing time-consuming Ag-ion fractionation. (6)

Based on the data presented here and the work reported by Delmonte et al., it appears the SP-2560 and SLB-IL111 can be used in a complementary fashion, to provide more complete and accurate fatty acid identification and composition information than currently possible. It requires less time and labor to inject one extract on two different columns, than to perform Ag-ion fractionation of an extract prior to injecting multiple fractions on a single column.
Figure 4. PHVO Sample and cis/trans Fractions on SP-2560 and SLB-IL111

SP-2560 Conditions
- column: SP-2560, 100 m x 0.25 mm I.D., 0.20 μm (24056)
- oven: 180 °C isothermal
- inj.: 250 °C
- det.: FID, 250 °C
- carrier gas: hydrogen, 1 mL/min.
- injection: 1 μL, 100:1 split
- liner: 4 mm I.D., split liner with cup (2051001)

SLB-IL111 Conditions
- column: SLB-IL111, 100 m x 0.25 mm I.D., 0.20 μm (29647-U)
- oven: 168 °C isothermal
- All other conditions the same as those used for the SP-2560. All peak IDs as labeled
Application: Edible Oils

The edible oil industry boasts revenue measured in the tens of billions of dollars. As such, it can be subject to criminal acts of fraud aimed at increasing profits. A GC fingerprinting technique can be used to monitor product for adulteration (adding a cheaper, inferior oil to boost the volume of a premium, higher priced oil), and also identify the source of oils in unknown samples.

To properly identify an edible oil through pattern recognition, it is necessary to have standards of known reference; such as, our Characterized Reference Oils. These can be used as part of a quality control program, providing an excellent means of standardizing procedures and comparing results between facilities.

The esterification of fatty acids to FAMEs is performed using an alkylation derivatization reagent. Methyl esters offer excellent stability, and provide quick and quantitative samples for GC analysis. The esterification reaction involves the condensation of the carboxyl group of the fatty acid and the hydroxyl group of an alcohol. Esterification is best done in the presence of an acidic catalyst (such as boron trichloride). The catalyst protonates the oxygen atom of the carboxyl group, making the acid much more reactive. Nucleophilic attack by an alcohol then yields an ester with the loss of water. The catalyst is removed with the water. The alcohol used determines the alkyl chain length of the resulting esters; the use of methanol will result in the formation of methyl esters, whereas the use of ethanol will result in estyl esters. (6,7)

Following derivatization, five of our Characterized Reference Oils were analyzed on the SLB-IL111. The resulting chromatograms are shown in Figure 5. A 30 m column was selected because the higher resolution provided by a longer column was not necessary for this application. Additionally, the shorter column resulted in shorter run times. The % compositions, based on peak areas, agree with those listed on the Certificate of Analysis for each mix.

Figure 5. Characterized Reference Oils

column: SLB-IL111, 30 m x 0.25 mm I.D., 0.20 μm (28927-U)
oven: 180 °C
inj.: 250 °C
det.: FID, 260 °C
carrier gas: helium, 25 cm/sec
injection: 1 μL, 50:1 split
liner: 4 mm I.D., split type, cup design
samples: characterized reference oils, methylated using BF3-Methanol prior to analysis

1. Lauric Acid Methyl Ester (C12:0)
2. Myristic Acid Methyl Ester (C14:0)
3. Palmitic Acid Methyl Ester (C16:0)
4. Palmitoleic Acid Methyl Ester (C16:1)
5. Stearic Acid Methyl Ester (C18:0)
6. Oleic Acid Methyl Ester (C18:1n9c)
7. Linoleic Acid Methyl Ester (C18:2n6c)
8. Linolenic Acid Methyl Ester (C18:3n3)
9. Arachidic Acid Methyl Ester (C20:0)
10. cis-11-Eicosenoic Acid Methyl Ester (C20:1)
Conclusion

The extreme polarity of the SLB-IL111 phase, in combination with a 270 °C maximum temperature, makes the SLB-IL111 useful for several FAME applications. Specifically:

- **cis/trans FAME isomers:** when paired with the SP-2560, provides more complete and accurate fatty acid identification/composition information than currently possible
- **FAME profile fingerprinting of edible oils:** provides great resolution and peak shapes in a quick analysis

Additionally, the SLB-IL111 provides alternative selectivity to cyanopropyl siloxane columns for other applications. The extremely polar SLB-IL111 exhibits weaker dispersive interactions than less polar phases; which, in the applications shown here, results in lower elution temperatures and shorter analysis times.

References


SLB-IL111

- **Application:** This extremely polar ionic liquid column was the world’s first commercial column to rate over 100 on our GC column polarity scale. As such, it has the most orthogonal selectivity compared to commonly used non-polar and intermediate polar columns, providing increased selectivity for polar and polarizable analytes. Its temperature limit of 270 °C is very impressive for such an extremely polar column. The 60 m version is excellent at resolving benzene and other aromatics in gasoline samples. The 100 m version is suitable for detailed cis/trans FAME isomer analysis, and is a great complementary column to the SP-2560. Launched in 2010.
- **USP Code:** None
- **Phase:** Non-bonded; proprietary
- **Temperature Limits:** 50 °C to 270 °C (isothermal or programmed)

Ordering Information

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Characterized Reference Oils

- Canola Oil, 1 g
- Coconut Oil, 1 g
- Corn Oil, 1 g
- Cottonseed Oil, 1 g
- Lard Oil, 1 g
- Linseed (Flaxseed) Oil, 1 g
- Menhaden Fish Oil, 1 g
- Olive Oil, 1 g
- Palm Oil, 1 g
- Peanut Oil, 1 g
- Safflower Oil, 1 g
- Soybean Oil, 1 g
- Sunflower Seed Oil, 1 g

Derivatization Reagents

- BCl₃-Methanol, 12% w/w, 20 x 1 mL
- BCl₃-Methanol, 12% w/w, 20 x 2 mL
- BF₃-Methanol, 10% w/w, 20 x 1 mL
- BF₃-Methanol, 10% w/w, 20 x 2 mL
- BF₃-Methanol, 10% w/w, 10 x 1 mL
- BF₃-Methanol, 10% w/w, 400 mL
- Micro Reaction Vessels and Caps
  - 5 mL Clear, with Hole Caps, 12 ea
  - 5 mL Clear, with Solid Caps, 12 ea
  - 5 mL Amber, with Hole Caps, 12 ea
  - 10 mL Clear, with Hole Caps, 12 ea

Water Scavenger

- 2,2-Dimethoxypropane, 98%, 25 mL

Sodium Sulfate, Anhydrous, ≥99.0%

- Granular, 500 g
- Granular, 1 Kg
- Granular, 2.5 Kg

Trademarks

SLB, SP, Supelco – Sigma-Aldrich Co. LLC
# Sigma-Aldrich Worldwide Offices

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