Detailed Analysis of C18:1 cis/trans FAME Isomers Using the New Supelco SLB-IL111

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Introduction

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 process. 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 (1-3).

Because trans fatty acids have adverse health consequences and have no known nutritional benefits over other fats, consumer groups have pressured manufacturers and restaurants for their elimination. Many regulatory agencies worldwide now require content labeling to inform buyers of trans fat’levels of foods and some dietary supplements. Analysis of fatty acids is routinely performed using gas chromatography (GC) following established methodologies that first require derivatization of the fatty acids into fatty acid methyl esters (FAME). Because the differences between cis isomer FAMES and trans isomer FAMEs of the same carbon length and degree of unsaturation are very small, very efficient capillary GC columns with highly polar phases are required to separate them. This article will determine the suitability of our newest GC column to perform this application through direct comparison to a well-established and widely-referenced column.

SP™-2560 Column

Launched in 1983, the highly polar SP-2560 is specifically designed for the detailed analysis of cis/trans FAME isomers, including those found in hydrogenated vegetable oil. The SP-2560, with 100 m x 0.25 mm I.D., 0.20 µm dimensions, is considered by some as the benchmark column for FAME applications. It is referenced in several methods that employ GC analysis for determining fat content in food (4,5).

Complete column specifications are:

- **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
- **Temperature Limits:** Subambient to 250 °C (isothermal or programmed)

![Figure 1. Positions and Maximum Temperature (x °C) of Columns on Our GC Column Polarity Scale](image)

Supelco Ionic Liquid Capillary GC Columns

Ionic liquids are a class of solvents with low melting points that consist of organic cations associated with (inorganic or organic) anions. Several properties have long made them desirable as GC stationary phases. In 2005, Prof. Daniel W. Armstrong (University of Texas at Arlington) showed that dicaticionic and polycationic ionic liquids could successfully be used as viable GC stationary phases. Ionic liquid GC columns have the opportunity to impact current practices along several paths (6).

1. **Identical selectivities as traditional, often flawed, non-ionic liquid columns;** but with higher operating temperatures, lower column bleed, and/or less susceptibility to damage from moisture/oxygen
2. **High thermal stability;** decreasing analysis times, and/or allowing additional higher boiling compounds to be analyzed
3. **Completely unique selectivities compared to any/all traditional non-ionic liquid columns;** producing good peak shape and resolution for compounds of varying functionality, and/or expanding the polarity range up
4. **In multidimensional separations;** due to their engineered orthogonality and high thermal stability

Since the introduction of this innovative column platform, several chemistries have undergone evaluation. In 2008, Supelco launched the world’s first commercially available GC column based on ionic liquid stationary phase technology, the SLB™-IL100. Six unique ionic liquid columns are currently available in our evaluation program. Figure 1 is a visual depiction of our GC (continued on page 4)
column polarity scale, showing the relationship of ionic liquid columns to one another and also to non-ionic liquid 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. The positions/maximum temperatures of several of our 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 our current Supelco ionic liquid capillary GC columns. Detailed information concerning the scientific basis used to generate this scale can be found at sigma-aldrich.com/il-gc

SLB-IL111 Column

Highly polar columns are well suited for the resolution of polarizable analytes, such as aromatics and unsaturated FAMEs. This is due to their ability to undergo various dipole-induced dipole analyte-phase interactions. It was predicted that the SLB-IL111, the only column to rate over 100 on our GC column polarity scale, would show a high degree of selectivity for these types of analytes. Initial evaluations of a 30 m x 0.25 mm I.D., 0.20 µm SLB-IL111 column showed promise. Based on this knowledge, a 100 m version matching the column dimensions typically used for the detailed analysis of cis/trans FAME isomers was developed and tested.

The specifications of the SLB-IL11 are:

- **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. It should also be considered for use in GCxGC applications. Its temperature limit of 270 °C is very impressive for such an extremely polar column. Launched in 2010.
- **USP Code**: None
- **Phase**: Non-bonded; proprietary
- **Temperature Limits**: 50 °C to 270 °C (isothermal or programmed)

Analysis of PHVO and Fractions

A partially hydrogenated vegetable oil (PHVO) extract was provided by Dr. Pierluigi Delmonte from the United States Food and Drug Administration (US FDA) for our work. A cis fraction extract and a trans fraction extract, both prepared from PHVO using an Ag-ion fractionation procedure, were also supplied by Dr. Delmonte. Complete details and references pertaining to the PHVO extract preparation and Ag-ion fractionation procedures can be found in Reference 7.

The established AOCS method for determining fat content requires analysis on the 100 m SP-2560 column operated with a 180 °C isothermal oven temperature (5). To determine the optimal oven temperature for these analytes on the 100 m SLB-IL111, the PHVO extract was analyzed at several different oven temperatures. In concurrence with work published by Delmonte et al. (7), we determine that an isothermal analysis at 168 °C provided the best resolution for these analytes on this column.

Once optimal conditions were established, the PHVO, cis fraction, and trans fraction extracts were sequentially analyzed on each column to determine their ability to separate thirteen C18:1 cis FAME isomers (in blue, from C18:1 4c to C18:1 16c) and thirteen C18:1 trans FAME isomers (in green, from C18:1 4t to C18:1 16t). Figure 2 shows the resulting chromatograms on the SP-2560 at 180 °C isothermal, the method-specified oven temperature. Figure 3 shows the resulting chromatograms on the SLB-IL111 at 168 °C isothermal, the experimentally-determined optimal oven temperature.

Results

Following the completion of this study and a review of Reference 7, several observations were made.

**Elution Temperature**: Due to the higher polarity of the SLB-IL111, the FAME isomers eluted in less time compared to the SP-2560, even though the oven temperature necessary for optimal resolution was lower for the SLB-IL111.

**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, based on the data used to generate our GC column polarity scale.
C18:1 Isomers. The SLB-IL111 was able to provide resolution of C18:1Δ9c from C18:1Δ11t, 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 either, such as C18:1Δ10t from C18:1Δ11t, and the pair C18:1Δ13t/C18:1Δ14t from other isomers.

C18:2 Isomers. Although not shown here, Delmonte et al. reported that the SLB-IL111 is able to resolve C18:2Δ9c,11t from C18:2Δ7t,9c. These are the two most abundant conjugated linoleic acid (CLA) isomers found in ruminant fats. This is encouraging as these two important isomers cannot be resolved using any other GC column and thus requires time-consuming Ag-ion fractionation (7).

Saturated FAMEs. Delmonte et al. also reported that a drawback of the SLB-IL111 was the co-elution of saturated FAMEs with minor trans FAME isomers of one carbon less in chain length. Conversely, the SP-2560 is able to resolve many of the saturated FAMEs from unsaturated FAMES, the exception being C19:0 and C21:0.

Conclusion

The SLB-IL111 column proved to be a highly efficient column for the analysis of cis/trans FAME isomers. Of particular interest is the ability of this column to resolve C18:1Δ9c from C18:1Δ11t, and also C18:2Δ9c,11t from C18:2Δ7t,9c. Neither of these separations can be performed by the SP-2560 column, or any other column made with a cyanopropyl siloxane phase.

Based on the data presented here and the work reported by Delmonte et al., it appears that 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.

References