Reproducible Analyses of Omega-3 and Omega-6 Fatty Acid Methyl Esters by Capillary GC

Omegawax 250 and Omegawax 320 bonded polyethylene glycol phase columns were designed specifically for analyses of omega-3 and omega-6 fatty acid methyl esters. They are well suited for many FAME applications, including analyses of fish tissues and oils, poultry, and samples from land animal sources. Omegawax columns are tested to ensure reproducible column-to-column performance – for 55 Omegawax 320 columns, representing 3 production lots, equivalent chain length (ECL) values for representative FAMEs were highly consistent.

Key Words:
- fatty acids
- fatty acid methyl esters
- omega-3 fatty acids
- omega-6 fatty acids
- fish oils

Analysts evaluating vegetable, animal organ, or marine fish oils must monitor even-numbered, straight chain fatty acid methyl esters (FAMES) containing single and multiple cis double bonds. To prevent errors in identifying and quantifying these esters, the GC column used should elute the compounds primarily by carbon chain length and secondarily by the number of double bonds (1). There should be minimal overlap in elution order among FAMES having different chain lengths (all C18 esters should elute before the C20 esters, etc.). Analysts also have recognized the importance of using equivalent chain length (ECL) values (2, 3) to predict the identity of fatty acids in natural samples. Because the trans isomer content of such samples is negligible, group separations of cis isomers from trans isomers are not important.

Polyethylene glycol (PEG) phase capillary columns resolve these compounds with little or no overlap in the elution order of FAMES of different carbon chain length. However, column to column variability in performance among PEG columns has made it difficult to rely on equivalent chain length values for identifying sample components, particularly when making interlaboratory comparisons. Peak coelutions also are often encountered when using PEG columns for FAMES analyses. Omegawax™ 250 and Omegawax 320 bonded PEG phase capillary columns are tested to ensure consistent equivalent chain length values from column to column, with minimal overlap among carbon chains of differing length. They are specifically prepared and tested for analyses of omega-3 and omega-6 fatty acid methyl esters, as described in AOAC and AOCS methods (4, 5, 6).

Figure A, an analysis of our test mixture for Omegawax columns, is representative of column performance in fish oil FAMEs analyses. There is good separation between C23:0 and C21:5n3 and near baseline resolution between C24:0, C22:6n3 (DHA), and C24:1. Resolution of the other FAMES also is very good. There is only one overlap of even carbon numbered FAMES – C24:0 elutes before C22:6n3.

Table 1 shows the reproducibility of Omegawax 320 column performance. As polarity markers, we monitored the equivalent chain length values for four highly unsaturated FAMES – C18:0/C18:1n9 separation is a measure of column efficiency cited in the AOAC and AOCS methods. The small standard deviations and percent relative standard deviations for ECL values for 55 columns (rep-
Figure B. Omegawax Column Resolves Omega-3 and Omega-6 FAMEs

Column: Omegawax 320, 30m x 0.32mm ID, 0.25µm film
Cat. No.: 24152
Oven: 200°C
Carrier: helium, 25cm/sec, set at 200°C
Det.: FID, 260°C
Inj.: 1µL hexane containing polyunsaturated FAMEs (50mg/mL), marine source (Cat. No. 4-7033) or animal source (Cat. No. 4-7015), split 100:1, 250°C

Omega-3 FAMES: Cod Liver Oil

Omega-6 FAMES: Animal Source

Figure 7. Linoleic acid eluted at 17.6 min, alpha-linolenic acid eluted at 18.8 min.

References

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Omegawax, Supelco = Supelco, Inc. Fused silica columns manufactured under HP US Pat. No. 4,293,415.

Ordering Information:
Omegawax 250 Fused Silica Capillary Column 30m x 0.25mm ID, 0.25µm phase film 24136
Omegawax 320 Fused Silica Capillary Column 30m x 0.32mm ID, 0.25µm phase film 24152
For detailed information on alternative analyses of fatty acids and FAMEs on other Supelco™ capillary GC columns, request free Bulletin 855.

To demonstrate the performance of the Omegawax 250 column, we analyzed samples of catfish filet, brain, and liver. The resolution provided by the Omegawax 250 column revealed a number of tissue to tissue differences in the amounts of specific FAMEs. Filet and liver, for example, contained higher amounts of methyl linoleate (C18:2n6) than did the brain. Also, a larger number of compounds eluted from the brain sample between C16 and C18:2n6. More C22:6n3 was present in the brain and liver than in the filet. Chromatograms and additional details of this comparison are presented in Bulletin 855 (available on request).

Omegawax columns can be used isothermally, to 280°C, to provide excellent, highly reproducible production of nonhydrogenated FAMEs. The thermally stable columns provide a wide temperature range within which a particular analysis can be performed without changes in the sample component elution order.

C22:5n3 and C22:4n6 acids. When naturally occurring fatty acids are synthetically modified to form complex mixtures of cis and trans isomers (e.g., by heating), both a PEG phase column and a polar SP-2340 cyanoalumoxane phase column may be required to obtain the most information (7).