

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:

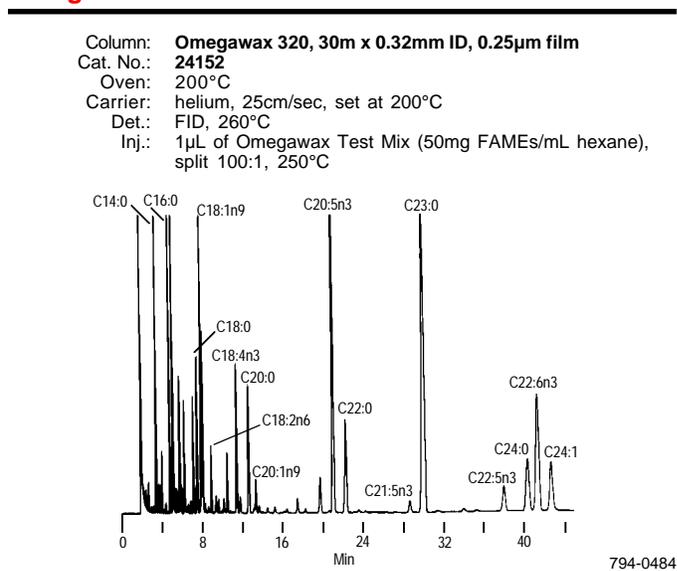
- fatty acids • fatty acid methyl esters
- omega-3 fatty acids • omega-6 fatty acids • fish oils

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

Figure A. Omega-3 FAMEs on an Omegawax Column



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. Even slight column to column differences in polarity will be revealed by these probes. The resolution value for the 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-

Table 1. Omegawax 320 Columns Ensure Consistent Equivalent Chain Length Values*

	FAME / ECL Value				Resolution
	C18:4n3	C20:5n3	C21:5n3	C22:5n3	
Mean	19.64	21.81	22.84	23.80	3.99
Std. Dev.	0.01	0.01	0.01	0.01	0.11
% RSD	0.05	0.06	0.06	0.06	2.85

* Mean, standard deviation, and relative standard deviation for 55 columns from 3 production lots.

resenting three production lots) reveal minimal variability, indicating column polarity is very consistent from column to column. The small standard deviation and percent relative standard deviation for C18:0/C18:1n9 resolution show very consistent column efficiency. Similar results are obtained from Omegawax 250 columns.

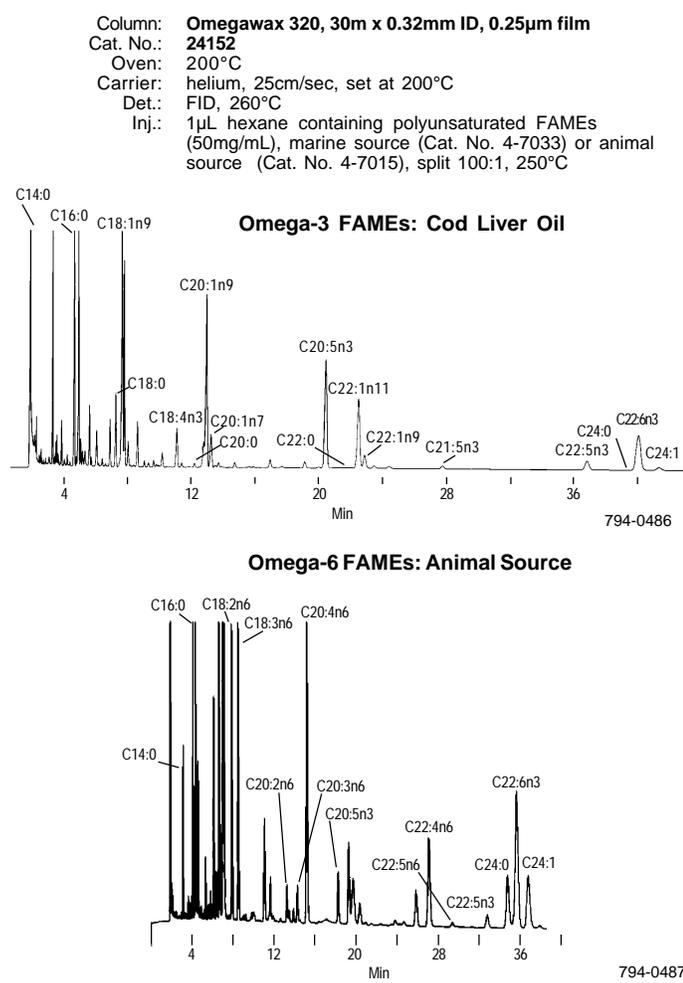
Cod liver oil contains large amounts of omega-3 fatty acids. Figure B1 shows an Omegawax 320 column provides good resolution of these various components and reveals differences in the relative ratios of key components, compared to the menhaden oil-based test mix. Like other marine fish oils, cod liver oil contains large amounts of EPA and DHA. In contrast to marine fish oils, land animal tissue oils contain a high percentage of omega-6 fatty acids. Figure B2 shows an analysis of a land animal organ extract containing significant amounts of C20:4n6

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 cyanosilicone phase column may be required to obtain the most information (7).

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 C:16 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 resolution 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.

Figure B. Omegawax Column Resolves Omega-3 and Omega-6 FAMES



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.

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