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Comparison of SPB-PUFA and Omegawax Capillary Columns for FAMEs Analysis by GC

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Omegawax columns, developed to meet the requirements of AOAC Official Method 991.39 for the analysis of fatty acids, are based on polyethylene glycol phase chemistry. A new capillary column, SPB-PUFA, is coated with a polyalkylene glycol stationary phase, which has a slightly lower polarity than the traditional PEG phase. In comparing these two columns, we found the SPB-PUFA column produces a different elution pattern and, in some analyses, gives unique separations.

The Association of Official Analytical Chemists (AOAC) Method 991.39, *Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters*, recommends analysis using a column with a bonded polyglycol phase, based on Carbowax® 20M (1). The Omegawax™ 320 column was developed to meet these requirements. It applies a polyethylene glycol (PEG) phase chemistry and is the primary column used and recommended for the analysis of highly polyunsaturated FAME samples. The new SPB™-PUFA column is based on a polyalkylene glycol (PAG) stationary phase, providing a lower polarity than the Omegawax column.

This study compares the SPB-PUFA and the Omegawax columns in the analyses of two FAME standards — Supelco™ 37 Component FAME Mix, which mimics the fatty acid composition of a broad range of food samples, and PUFA No. 1 Mix, which is representative of marine fish oil samples. The 37 component mix was analyzed using temperature programmed runs (Figure A). The PUFA mix was analyzed at 200°C on the Omegawax column and at 210°C on the SPB-PUFA column (Figure B). Analyses were performed at other temperatures, but are not shown here.

Overall, the SPB-PUFA and the Omegawax 320 analyses show differences in the elution patterns of key FAMEs, especially with the more highly unsaturated longer chain length FAMEs. Comparing these analyses indicates that the less polar SPB-PUFA column provides a truer carbon chain length separation. All even carbon number FAMEs elute according to carbon number and degree of unsaturation. On the Omegawax column, there is some chain overlap, as the C22:6n3 FAME elutes after the C24:0 FAME.

In order to quantify the difference in polarity of the two columns, we compared the equivalent chain length (ECL) values for a series of unsaturated FAMEs (Table 1). Also listed are the absolute differences in the ECL values (ΔECL), at 200°C and per °C. The ΔECL/°C figures indicate the temperature effect on the selectivity of the columns. The values for the saturated FAMEs are identical on each column, as, by definition, the ECL value for a saturated FAME is always equivalent to its carbon number.

Table 1 shows the Omegawax column is more polar than the SPB-PUFA column, as demonstrated by the larger ECL values for each

Figure A. 37 Component FAME Mix

Column: **SPB-PUFA**, 30m x 0.32mm ID, 0.20µm film
Cat. No.: **24323**
Column: **Omegawax 320**, 30m x 0.32mm ID, 0.25µm film
Cat. No.: **24152**
Oven: 50°C (hold 2 min), 4°C/min to 210°C
Carrier: 25cm/sec, set at isothermal temp.
Det.: (2 x 10^{-11}), 260°C
Sample Conc.: 50mg/mL
Inj.: 1µL of 37 Component FAME Mix, split 100:1, 250°C

Component	Weight (%)	Component	Weight (%)	Component	Weight (%)
1. C4:0	4	13. C16:1n7	2	25. C20:2n6	2
2. C6:0	4	14. C17:0	2	26. C21:0	2
3. C8:0	4	15. C17:1	2	27. C20:3n6	2
4. C10	4	16. C18:0	4	28. C20:3n3	2
5. C11:0	2	17. C18:1n9c	4	29. C20:4n6	2
6. C12:0	4	18. C18:1n9t	2	30. C20:5n3	2
7. C13:0	2	19. C18:2n6c	2	31. C22:0	4
8. C14:0	4	20. C18:2n6t	2	32. C22:1n9	2
9. C14:1n5	2	21. C18:3n6	2	33. C22:2n6	2
10. C15:0	2	22. C18:3n3	2	34. C23:0	2
11. C15:1	2	23. C20:0	4	35. C24:0	4
12. C16:0	6	24. C20:1n9	2	35. C22:6n3	2
				37. C24:1n9	2

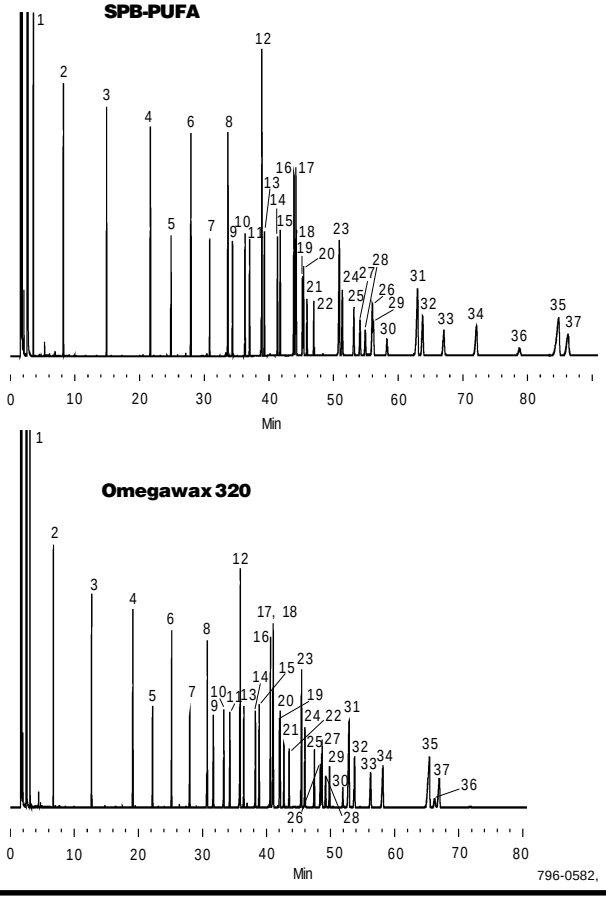

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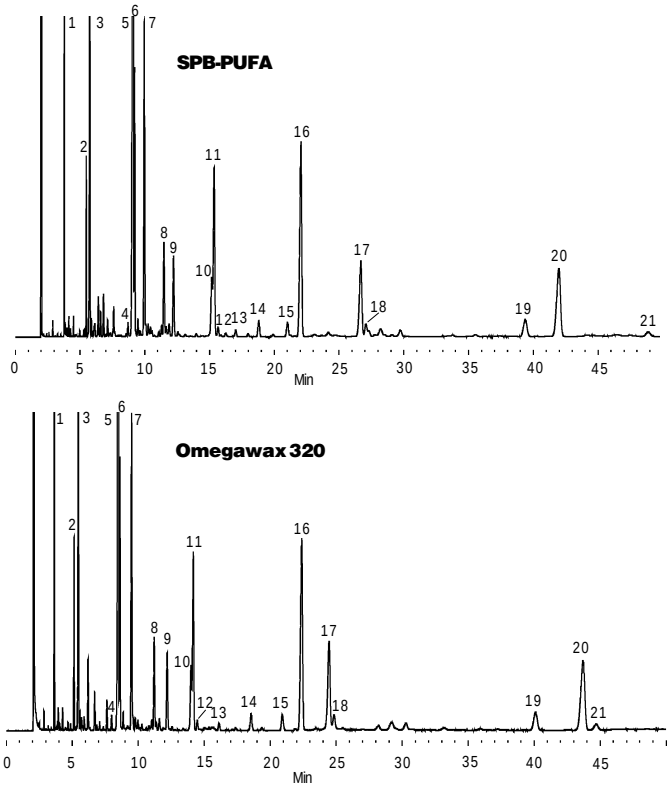
Figure B. PUFA No. 1 Mix

Column: **SPB-PUFA**, 30m x 0.32mm ID, 0.20µm film
Cat. No.: **24323**
Column: **Omegawax 320**, 30m x 0.32mm ID, 0.25µm film
Cat. No.: **24152**
Oven: 210°C Isothermal (SPB-PUFA)
200°C Isothermal (Omegawax 320)
Carrier: 25cm/sec, set at isothermal temp.
Det.: (2 x 10^{-11}), 260°C
Sample Conc.: 50mg/mL
Inj.: 1µL of PUFA No. 1 Mix, split 100:1, 250°C

1. C14:0
2. C16:0
3. C16:1n7
4. C18:0◆
5. C18:1n9
6. C18:1n7
7. C18:2n6
8. C18:3n3◆
9. C18:4n3
10. C20:1n11◆
11. C20:1n9
12. C20:1n7◆
13. C20:2n6
14. C20:4n6◆
15. C20:4n3◆
16. C20:5n3
17. C22:1n11
18. C22:1n9
19. C22:5n3
20. C22:6n3
21. C24:1n9◆



◆Components not always present in this naturally-derived mix. Components not marked are guaranteed to be present in mix.

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of the unsaturated FAMEs. The ECL data also reveal that as the degree of unsaturation increases from monoenoic (Cxx:1) to hexaenoic (Cxx:6) for the same carbon number, the selectivity difference between the columns increases. The ΔECL /°C data show that the more unsaturated the FAME, the more temperature sensitive is its reaction with the column phase. Though not shown, the data generated by evaluating the columns at a series of isothermal temperatures show that the polarity of the columns increases as the temperature increases. This concurs with a previous study showing a temperature-based effect on column selectivity (2).

Table 1. ECL Values for SPB-PUFA and Omegawax 320 at 200°C

FAME	SPB-PUFA ECL	Omegawax ECL	ΔECL	SPB-PUFA ECL/°C	Omegawax ECL/°C
C14:1n5	14.33	14.43	0.10	0.001	0.001
C15:1	15.32	15.42	0.10	0.001	0.001
C16:1n7	16.21	16.30	0.09	0.002	0.002
C17:1	17.20	17.29	0.09	0.002	0.002
C18:1n9c	18.14	18.23	0.10	0.002	0.001
C18:1n9t	18.14	18.23	0.10	0.002	0.001
C18:2n6c	18.50	18.70	0.20	0.005	0.002
C18:2n6t	18.54	18.70	0.17	0.001	0.002
C18:3n6	18.72	19.00	0.28	0.004	0.003
C18:3n3	19.04	19.33	0.30	0.004	0.003
C20:1n9	20.11	20.20	0.09	0.002	0.002
C20:2n6	20.48	20.67	0.19	0.003	0.003
C20:3n6	20.66	20.94	0.28	0.004	0.005
C20:3n3	20.81	21.17	0.36	0.005	0.005
C20:4n6	21.02	21.32	0.30	0.004	0.004
C20:5n3	21.35	21.81	0.46	0.006	0.006
C22:1n9	22.10	22.19	0.09	0.002	0.002
C22:2n6	22.47	22.66	0.19	0.003	0.003
C22:5n3	23.33	23.82	0.49	0.006	0.006
C22:6n3	23.53	24.09	0.55	0.007	0.006
C24:1n9	24.09	24.18	0.09	0.002	0.002

The SPB-PUFA column's FAME elution pattern was found to be different from that of the Omegawax column; therefore, the SPB-PUFA column can be used as a confirmational tool. In some cases the SPB-PUFA column gives unique separations not possible on the Omegawax column, allowing it to be the primary column for some analyses.

Ordering Information:

Description	Cat. No.
SPB-PUFA Column 30m x 0.32mm ID, 0.20µm film	**24323**
Omegawax 320 Column 30m x 0.32mm ID, 0.25µm film	**24152**
Supelco 37 Component FAME Mix 10mg/mL each component in methylene chloride. Components listed in Figure A.	**47885-U**
PUFA No. 1 Mix 100mg. Components listed in Figure B.	**47033**

References

1. Official Methods of Analysis of AOAC International, 16th Edition, 1995; Supplement, March 1996, AOAC International, Gaithersburg, MD, Chapter 41, p.21. *Fatty Acids in Encapsulated Fish Oils and Fish Oil Methyl and Ethyl Esters,*
2. L.M. Sidisky and H.R. Ridley, HRC & CC 3 (1991) 191.

References not available from Supelco.

For more information on the 37 Component Mix, request publication 196907, *Comparison of 37 Component FAME Standard on Four Capillary GC Columns.*

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