Journal of Chromatography, 627 (1992) 87–96
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 569

Determination of fatty acids in fish oil dietary supplements by capillary liquid chromatography with laser-induced fluorescence detection

Jong Shin Yoo and Victoria L. McGuffin

Department of Chemistry, Michigan State University, East Lansing, MI 48824 (USA)

(First received March 5th, 1992; revised manuscript received August 18th, 1992)

ABSTRACT

The 4-bromomethyl-7-methoxycoumarin derivatives of 14 saturated and unsaturated fatty acids, including the $\omega - 3$ fatty acids, were separated by reversed-phase liquid chromatography and detected by laser-induced fluorescence. Baseline resolution was obtained by using a high-efficiency packed capillary column with 240 000 theoretical plates, together with a systematic optimization of the mobile phase composition. The retention indices of the fatty acid derivatives correlated well with a predictive empirical model, showing accuracy better than 0.46% relative error and reproducibility better than $\pm 0.1\%$ relative standard deviation. The physiologically important fatty acids with 12–22 carbon atoms and 0–6 double bonds were determined at the femtomole level in fish oil dietary supplements by using this methodology.

INTRODUCTION

The polyunsaturated $\omega - 3$ fatty acids such as eicosapentaenoic and docosahexaenoic acids are highly enriched in fish oil extracts from cod, mackerel, sardine, and menhaden [1]. These extracts are commonly ingested as dietary supplements because of their putative therapeutic benefits. Several clinical studies have shown that the administration of fish oils to humans and animals can produce antihypertensive, antihyperlipidemic, and antiaggregatory effects [1,2]. However, these fish oil extracts may contain other saturated and unsaturated fatty acids whose physiological effects are less beneficial or are not fully understood. Consequently, the identification and quantitation of all constituents is an important problem for clinical studies as well as for routine evaluation of commercially available dietary supplements.

Correspondence to: V. L. McGuffin, Department of Chemistry, Michigan State University, East Lansing, MI 48824, USA.

Reversed-phase liquid chromatography (LC) is one of the most important techniques for analytical [3–10] and preparative [11,12] scale separations of fatty acids from animal and plant samples. Most naturally occurring fatty acids possess an even number of carbon atoms (N) with a number of double bonds (n) in the *cis* configuration. The fatty acids, which are represented symbolically as $N{:}n$ (for example, eicosapentaenoic acid is 20:5), exhibit a wide retention range depending on the number of carbon atoms and double bonds. It is commonly believed that the retention order of fatty acids can be expressed in terms of the equivalent chain length (ECL $= N - 2n$) [4]. Separation of fatty acids that have the same ECL has been reported under both isocratic [5–8] and gradient (9,10] mobile phase conditions. However, the resolution of polyunsaturated fatty acids containing three or more double bonds (18:3, 20:3, 20:4, 20:5, and 22:6) is usually incomplete due to co-elution with physiologically important saturated and unsaturated fatty acids with similar retention characteristics [4]. Moreover,

0021-9673/92/$05.00

these polyunsaturated fatty acids do not appear to follow the usual ECL retention rule [4].

The use of fluorescence detection to determine fatty acids at high sensitivity has already been reported for LC. The 4-bromomethyl-7-methoxycoumarin [13], anthryldiazomethane [14,15], 9-aminophenanthrene [16], and 9-chloromethylanthracene [17] derivatives of fatty acids can be analyzed at the picomole level and the 4-bromomethyl-7-acetoxycoumarin [18] derivatives at the femtomole level using conventional fluorescence detection. Using laser-induced fluorescence (LIF) detection, an exceptional sensitivity at the attomole level was achieved for the 4-bromomethyl-7-methoxycoumarin derivatives of fatty acids [19] and related substances such as prostaglandins [20].

In this research, an analytical method has been developed to determine the most important saturated and unsaturated fatty acids in fish oil dietary supplements. The fatty acids were derivatized with 4-bromomethyl-7-methoxycoumarin, separated with high efficiency by using packed capillary LC columns, and detected with high sensitivity by using the LIF method.

EXPERIMENTAL

Reagents

The standard saturated fatty acids such as dodecanoic (lauric, 12:0), tridecanoic (13:0), tetradecanoic (myristic, 14:0), pentadecanoic (15:0), and hexadecanoic (palmitic, 16:0) acids were obtained from Sigma (St. Louis, MO, USA). The standard unsaturated fatty acids such as 9-tetradecenoic (myristoleic, 14:1), 9-hexadecenoic (palmitoleic, 16:1), 9-octadecenoic (oleic, 18:1), 9,12-octadecadienoic (linoleic, 18:2), 6,9,12-octadecatrienoic (gammalinolenic, 18:3), 8,11,14-eicosatrienoic (homogammalinolenic, 20:3), 5,8,11,14-eicosatetraenoic (arachidonic, 20:4), 5,8,11,14,17-eicosapentaenoic (20:5), and 4,7,10,13,16,19-docosahexaenoic (22:6) acids were obtained from Cayman Chemical Company (Ann Arbor, MI, USA) and Nu Chek Prep (Elysian, MN, USA). The commercially available fish oil dietary supplements were Promega Pearls from Parke-Davis (Morris Plains, NJ, USA), Omega-3 and Fish-EPA from North Laboratories (Montague, MI, USA). The fluorescence derivatization reagent 4-bromomethyl-7-methoxycoumarin was purchased from Aldrich (Milwaukee, WI, USA) and was stored at 4°C. The dibenzo-18-crown-6 (Aldrich), sodium sulphate (J. T. Baker, Phillipsburg, NJ, USA), and potassium hydrogencarbonate (Matheson Coleman & Bell, Norwood, OH, USA) were reagent-grade chemicals and were used without further purification. All organic solvents were high-purity, distilled-in-glass grade (Baxter Healthcare, Burdick & Jackson Division, Muskegon, MI, USA); water was deionized and doubly distilled (MP-3A, Corning Glass Works, Corning, NY, USA).

Analytical methods

The fluorescence derivatization procedure was similar to that described previously [19]. A 10^{-3} *M* solution of the fatty acid standards was prepared in dry acetone. To a 500-μl aliquot of this solution were added 5 mg of a powdered, anhydrous mixture (1:1) of sodium sulphate and potassium hydrogencarbonate, and 3.6 mg (10 μmol) of dibenzo-18-crown-6 with stirring. The 4-bromomethyl-7-methoxycoumarin (2.7 mg, 10 μmol) reagent was added, and the derivatization reaction was allowed to proceed in the dark at 37°C. After 6 h, the supernatant liquid was removed from the product mixture, evaporated with dry nitrogen, and kept less than 1 day at 4°C.

Prior to derivatization, a 0.8-g sample of each fish oil dietary supplement was dissolved in 50 ml tetrahydrofuran. The triglyceride or esterified form of the fatty acids was saponified with 10 ml of 1 *M* NaOH solution for 1 h at 45°C. The solution was then acidified with 20 ml of 1 *M* HCl solution and dried with 10 g anhydrous sodium sulphate. The free fatty acids were extracted several times with 50 ml chloroform, and the combined extracts were evaporated with dry nitrogen and stored at 4°C. The resulting fatty acids were derivatized with 4-bromomethyl-7-methoxycoumarin according to the procedure described above. The validation of this analytical methodology, including the accuracy and precision of both qualitative and quantitative measurements, has been described previously [19,20].

Apparatus

A single-piston reciprocating pump (Model 114M, Beckman Instruments, San Ramon, CA, USA) was used to deliver the mobile phase under constant-pressure conditions. Samples were introduced by means of a split injection system using a 1-μl

electrically actuated valve (Model ECI4UW1, Valco Instruments, Houston, TX, USA) at a split ratio from 1:100 to 1:200. The column and splitter were maintained at a constant temperature of 30°C in a thermostated bath (Model 182, Precision Scientific, Chicago, IL, USA). Packed capillary columns were prepared from fused-silica tubing (96 to 178 cm length, 200 μm I.D., Hewlett-Packard, Avondale, PA, USA) according to the slurry-packing procedure described previously [21]. The packing material was a 3-μm spherical silica that was chemically bonded with 3.5 μmol/m^2 octadecylsilane and capped with trimethylsilane (Micro-Pak SP-18, Varian, Walnut Creek, CA, USA). The theoretical plate numbers for these packed capillary columns were 158 000 to 275 000, calculated under standard test conditions [21].

The LIF detector has been described previously [19]. A helium–cadmium laser (Model 3112-10S, Omnichrome, Chino, CA, USA) was used as the excitation source at 325 nm wavelength. The fluorescence emission at 420 nm wavelength was isolated by using a combination of two bandpass interference filters (Corion, Holliston, MA, USA) and a liquid filter (1% aqueous $NaNO_2$), and was detected by using a photomultiplier tube (Model Q4249B, Centronic, Bailey Instruments, Saddle Brook, NJ, USA). The resulting photocurrent was amplified with a picoammeter (Model 480, Keithley Instruments, Cleveland, OH, USA) and displayed on a chart recorder (Model 585, Linear Instruments, Reno, NV, USA).

Computer optimization method

To optimize the separation of the fatty acid derivatives, the window-diagram approach developed by Laub [22,23] was employed. The computer program was revised and rewritten in the BASIC programming language to operate on an IBM personal computer system (Model XT, Boca Raton, FL, USA).

First, the capacity factor (k') for each fatty acid was measured experimentally at different compositions of the mobile phase. A semi-empirical equation, originally developed by Hsu *et al.* [24], was used to relate the inverse of the capacity factor and the volume fraction of organic component in the mobile phase. The fitting parameters for this equation were determined by linear regression analysis. From these regression equations, the resolution (R_s) was calculated for each adjacent pair of solutes:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{k'_{i+1} - k'_i}{2 + k'_i + k'_{i+1}}\right) \quad (1)$$

where N is the number of theoretical plates.

A window diagram was then prepared to display the resolution as a function of the mobile phase composition. From this diagram, the optimum mobile phase composition and the least-resolved solute pair were visually determined.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

The retention behavior of physiologically important fatty acids was studied in a reversed-phase LC system using binary aqueous mixtures of methanol and acetonitrile as mobile phases. Four saturated fatty acids (12:0, 13:0, 14:0, and 15:0) and five unsaturated fatty acids (14:1, 18:3, 20:4, 20:5, and 22:6) derivatized with 4-bromomethyl-7-methoxycoumarin were selected as model compounds for initial studies. As shown in Figs. 1 and 2, a non-linear relationship exists between the logarithm of the capacity factor and the mobile phase composi-

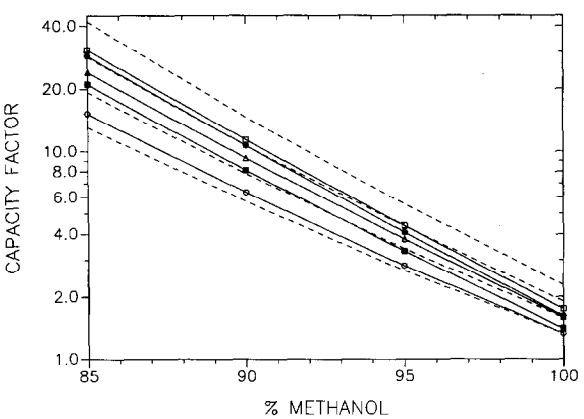

Fig. 1. The relationship between the logarithm of capacity factor (k') for fatty acid derivatives and the mobile phase composition. Column: 96 cm × 200 μm I.D. fused-silica capillary, 3 μm Micro-Pak SP-18 stationary phase. Mobile phase: methanol–water mixtures, 0.75 μl/min, 30°C. Solutes: 14:1 (○), 18:3 (△), 20:4 (□), 20:5 (■) and 22:6 (●); saturated fatty acids from 12:0 (bottom) to 15:0 (top) are represented as dashed lines. Detector: laser-induced fluorescence, 325 nm excitation wavelength, 420 nm emission wavelength.

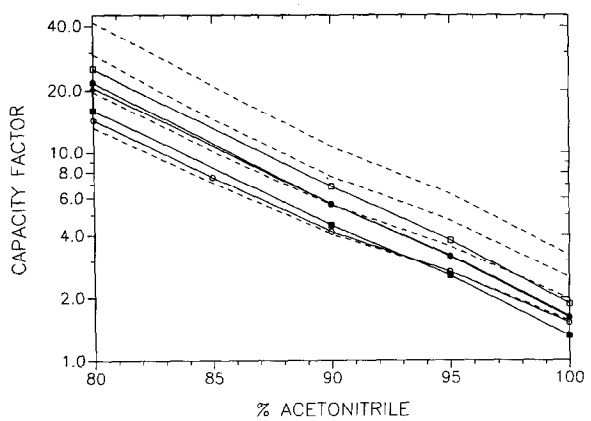

Fig. 2. The relationship between the logarithm of capacity factor (k') for fatty acid derivatives and the mobile phase composition. Mobile phase: acetonitrile–water mixtures, 0.75 μl/min, 30°C. Other experimental conditions and fatty acid symbols as in Fig. 1.

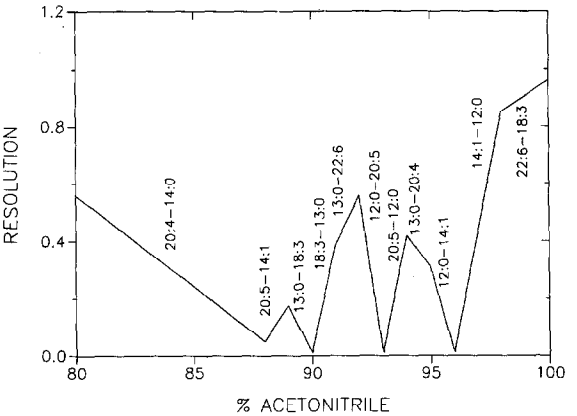

Fig. 4. The relationship between the resolution (R_s) of fatty acid derivatives and the mobile phase composition from Fig. 2.

tion. A significant positive deviation from linearity is observed for all solutes as the fraction of organic component increases from 85 to 100% methanol and from 80 to 100% acetonitrile. It is well known that such non-linear retention behavior can arise from non-ideal solute–solvent and solvent–solvent interactions [25–27].

In general, the logarithm of the capacity factor decreases more rapidly with increasing mobile phase strength for unsaturated than for saturated fatty acid derivatives. In methanol–water mixtures (Fig. 1), the retention order of the unsaturated fatty acids is not changed with mobile phase composition. In acetonitrile–water mixtures (Fig. 2), however, two solute pairs 14:1/20:5 and 18:3/22:6 exhibit an inversion in retention order at 93% and 90% acetonitrile, respectively. For both of these solute pairs, the polyunsaturated fatty acid derivative is less retained at higher acetonitrile composition. It is also apparent, by comparison of the capacity factors for these solute pairs at the same nominal mobile phase composition, that the polyunsaturated fatty acids are less retained in acetonitrile–water than in methanol–water mixtures. These results indicate that acetonitrile has a strong, selective interaction with double bonds, probably arising from dipole induction and orientation forces.

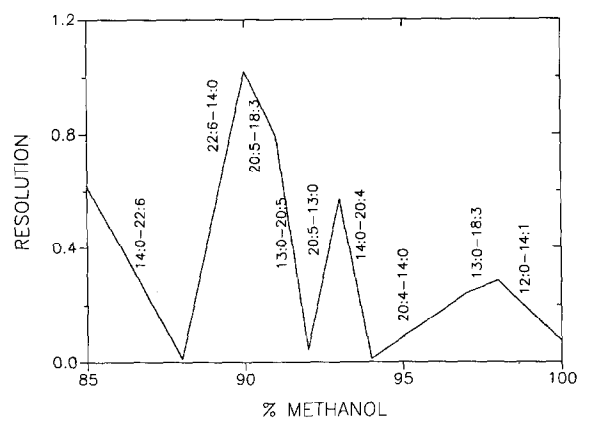

Fig. 3. The relationship between the resolution (R_s) of fatty acid derivatives and the mobile phase composition from Fig. 1.

To optimize the separation of both saturated and unsaturated fatty acid derivatives, a modified window-diagram method was employed [22–24]. The retention data shown in Figs. 1 and 2 were analyzed by linear regression, the resolution between each adjacent pair of solutes was calculated by using eqn. 1, and the resolution of the limiting pair was displayed graphically as a function of the mobile phase composition. Based on the results summarized in Figs. 3 and 4, the optimum composition of the mobile phase was predicted to be 90% methanol and the solute pair 14:0/22:6 was predicted to be the least resolved ($R_s \approx 1.0$). As shown in the experimental study (Fig. 1), however, this solute pair was completely overlapped at the predicted optimum mobile phase composition due to deviation from linear retention behavior. In order to separate 14:0

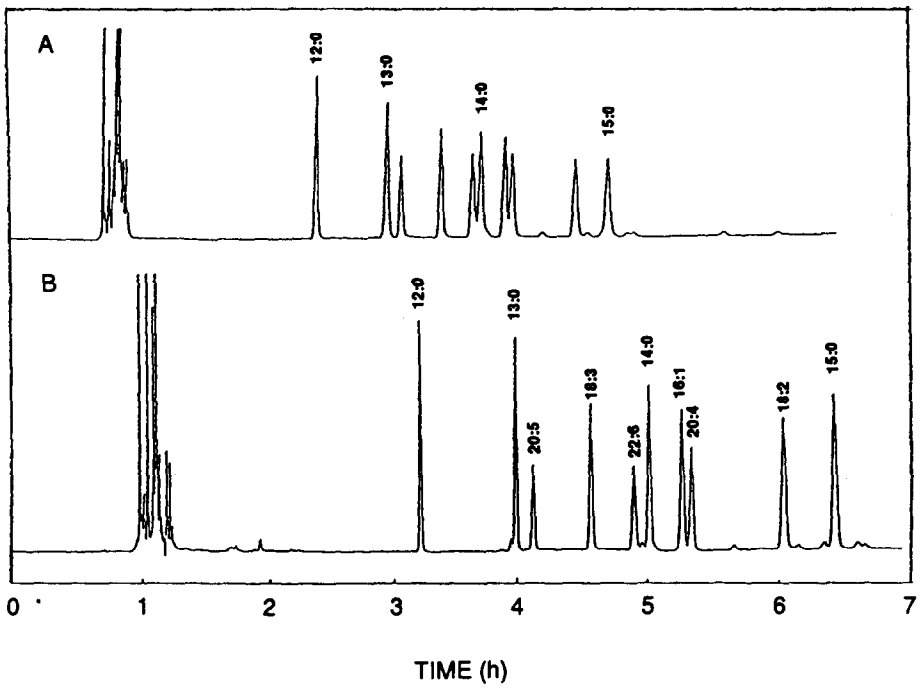

Fig. 5. Separation of standard fatty acid derivatives at the 5–10-femtomole level. Mobile phase: methanol–acetonitrile–water (90:2:8), 0.75 μl/min, 30°C. Column: (A) 96 cm × 200 μm I.D. (158 000 plates), (B) 178 cm × 200 μm I.D. (275 000 plates). Other experimental conditions as in Fig. 1.

and 22:6 without substantially altering the resolution of other fatty acid derivatives, a semi-empirical optimization procedure was employed. Because these solutes differ significantly in the number of double bonds, a small amount of acetonitrile was added to the predicted optimum mobile phase composition in order to reduce the capacity factor of 22:6. Fig. 5A shows the separation obtained for the fatty acid derivatives using a ternary mobile phase mixture of 90% methanol, 2% acetonitrile, and 8% water. Under these conditions, the least-resolved solute pairs were 14:0/22:6 and 16:1/20:4, both of which showed resolution of approximately 1.0. Any further increase in the acetonitrile concentration would be expected to increase the resolution of 14:0/22:6, but would decrease the resolution of 16:1/20:4. Therefore, this mobile phase composition was maintained and a longer chromatographic column was employed to increase the number of theoretical plates. Under these conditions, all fatty acid derivatives showed resolution greater than 1.5 (Fig. 5B).

The optimal separation of 14 fatty acid derivatives using a packed capillary column with 240 000 theoretical plates and the ternary mobile phase mixture of 90% methanol, 2% acetonitrile, and 8% water is represented in Fig. 6. Several additional saturated and unsaturated fatty acids were added to the standard sample for this separation. Nearly baseline resolution was obtained for each component, with no interference from the derivatization reagents or their by-products (Fig. 6, top). It is interesting to note that Halgunset *et al.* [3] also employed a ternary mobile phase mixture of 82% methanol, 9% acetonitrile, and 9% water to separate the *p*-bromophenacyl derivatives of fatty acids. They obtained baseline resolution of 9 fatty acid derivatives, but did not include the polyunsaturated $\omega - 3$ fatty acids.

Determination of fatty acids in fish oil dietary supplements

The fatty acids obtained from fish oil dietary supplements were derivatized with 4-bromomethyl-7-methoxycoumarin and were separated and de-

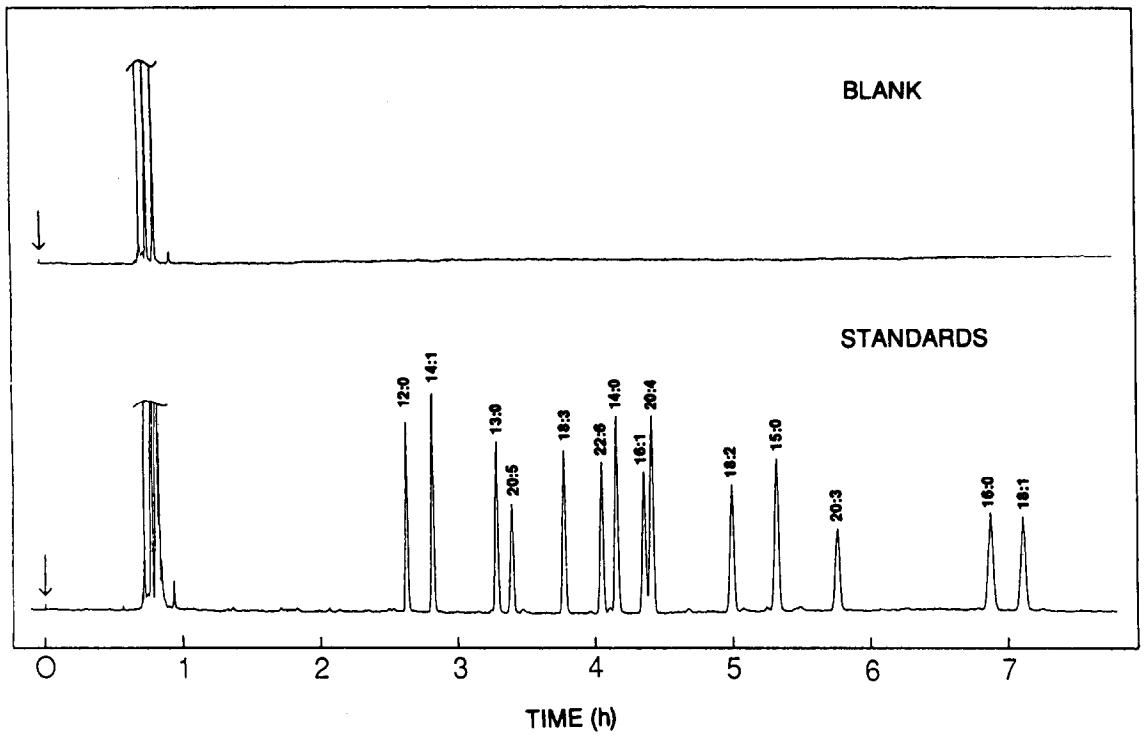

Fig. 6. High-efficiency separation of 14 standard fatty acid derivatives at the 5–10 femtomole level (bottom) and blank (top). Column: 152 cm × 200 μm I.D. (240 000 plates). Other experimental conditions as in Fig. 5.

tected using the analytical methodology described above. The fatty acid composition of Promega Pearls, Omega-3, and Fish-EPA is illustrated in Fig. 7. The major constituents of the fish oil dietary supplements were eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids. Other $\omega - 3$ fatty acids, such as hexadecatetraenoic (16:4) and octadecatetraenoic (18:4) acids, as well as saturated and monounsaturated fatty acids, such as myristic (14:0), palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acids, were the next most abundant components. The minor components were identified as lauric (12:0), hexadecadienoic (16:2), hexadecatrienoic (16:3), linoleic (18:2), linolenic (18:3), arachidonic (20:4), and docosapentaenoic (22:5) acids. The relative amounts of these fatty acids appear to be similar in the Promega Pearls and Omega-3 dietary supplements and are in reasonably good agreement with the manufacturer's specifications (Table I). In contrast, the Fish-EPA supplement contains proportionately greater amounts of saturated and monounsaturated fatty acids, which are of questionable health benefits.

Recently, Beebe and Brown [28] reported a similar analysis of fish oil dietary supplements using reversed-phase LC with a ternary mobile phase of 36% acetonitrile, 20% tetrahydrofuran, and 44% water. They identified the principal components as 20:5, 22:6, 14:0, 16:0, 16:1, 18:1, and 18:2, but were not able to separate and identify the minor components such as 12:0, 16:2, 16:3, 16:4, 18:3, 18:4, 20:4, and 22:5. In addition, the physiologically important $\omega - 3$ fatty acids were not completely resolved from adjacent solutes under their chromatographic conditions. We believe that the results shown in Fig. 7 clearly demonstrate the ability to resolve the physiologically important fatty acids and to detect them at the femtomole level in fish oil dietary supplements. Although the resolution and sensitivity are clearly superior to previous results, these advantages are gained only with a considerable compromise in the analysis time (7.5 h).

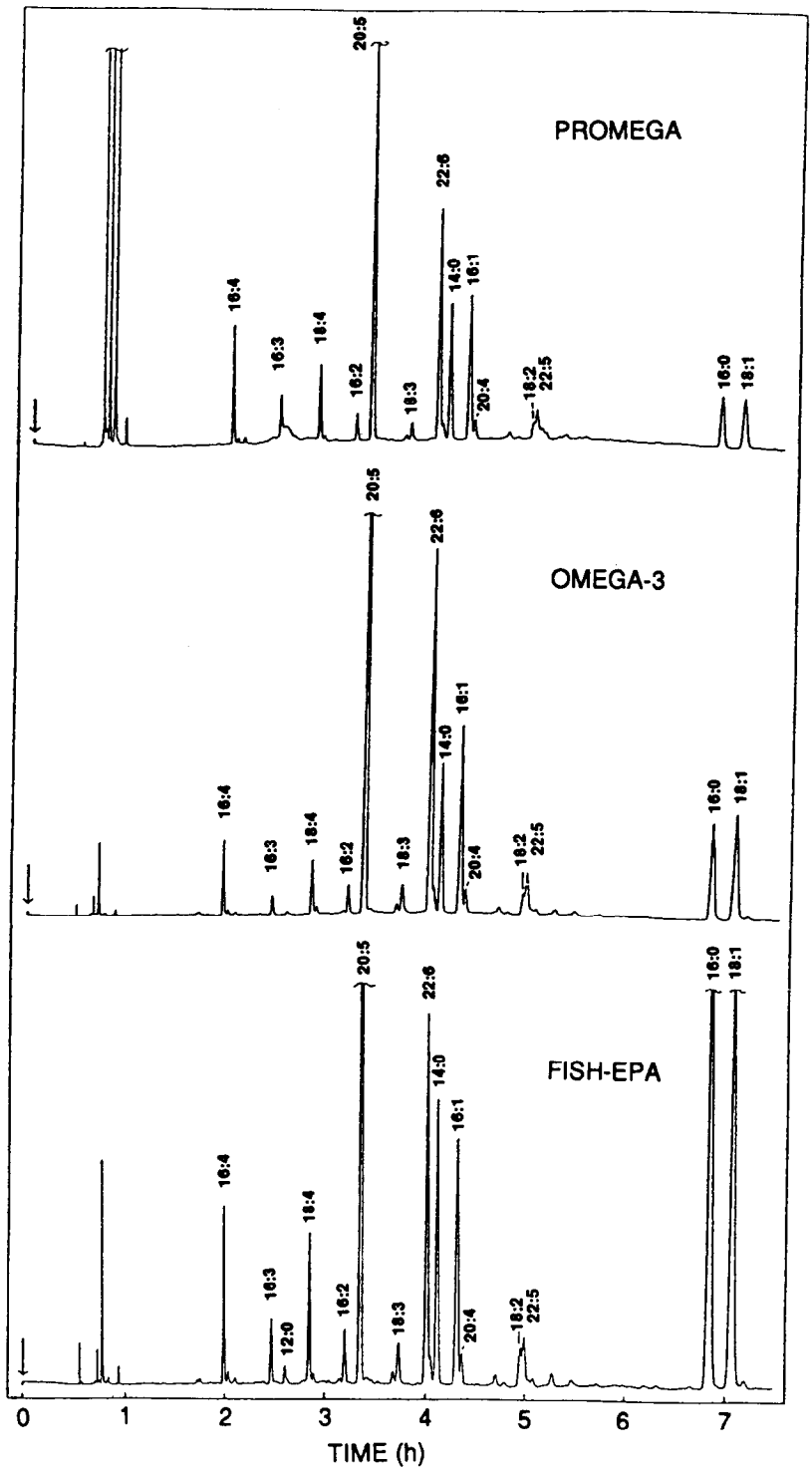

Fig. 7. The chromatograms for the analysis of fatty acid constituents at the femtomole level in fish oil dietary supplements of Promega Pearls, Omega-3, and Fish-EPA. Experimental conditions as in Fig. 6.

TABLE I

COMPOSITION OF POLYUNSATURATED $\omega - 3$ FATTY ACIDS IN COMMERCIALLY AVAILABLE FISH OIL DIETARY SUPPLEMENTS

Data from manufacturer's specifications.

Fatty acid	Promega	Omega-3	Fish-EPA
20:5 (mg/capsule)	168	280	180
22:6 (mg/capsule)	72	120	120
Other $\omega - 3$ (mg/capsule)	60	100	–

Predictive models of fatty acid retention

The retention behavior of the fatty acid derivatives can be described and predicted by means of the retention index system developed by Kovats [29,30]. By using the saturated fatty acids as a standard scale for comparison, the retention index (I) of unsaturated fatty acids can be calculated as follows:

$$I = 100 \cdot \left[N + \frac{\log (k'_i/k'_N)}{\log (k'_{N+1}/k'_N)} \right] \quad (2)$$

where k' is the capacity factor for the unsaturated fatty acid (i) that elutes between the saturated fatty acids with carbon numbers N and $N + 1$. Because the unsaturated fatty acids are expressed in terms of the chain length of an equivalent saturated fatty acid, the Kovats retention index system is conceptually equivalent to the ECL model of Özcimder and Hammers [5]. These models are mathematically related in the following manner:

$$I/100 = \text{ECL} = N - yn \quad (3)$$

where N is the number of carbon atoms, n is the number of double bonds, and y is an empirically derived coefficient. When the retention index values for the fatty acids calculated from the experimental data (Figs. 6 and 7) are substituted in eqn. 3, the coefficient y is determined to be 1.419 ± 0.020 by multiple linear regression analysis. The experimentally measured and theoretically predicted values for the retention index of each unsaturated fatty acid are summarized in Table II. Although the average error incurred by using eqn. 3 appears to be rather small (1.83% relative error), it is significantly greater than

TABLE II

RETENTION INDICES (I) OF UNSATURATED FATTY ACID DERIVATIVES

Unsaturated fatty acids	Retention index (I)				
	Experiment	Theory eqn. 3[a]	Relative error[c] (%)	Theory eqn. 4[b]	Relative error[c] (%)
16:4	1064	1032	−3.10	1059	−0.47
16:3	1171	1174	+0.26	1177	+0.51
14:1	1228	1258	+2.44	1229	+0.08
18:4	1238	1232	−0.49	1243	+0.48
16:2	1292	1316	+1.86	1295	+0.23
20:5	1312	1291	−1.60	1309	−0.23
18:3	1358	1374	+1.18	1361	+0.22
22:6	1388	1349	−2.81	1375	−0.94
16:1	1419	1458	+2.75	1414	−0.35
20:4	1424	1432	+0.56	1427	+0.21
18:2	1475	1516	+2.78	1480	+0.34
22:5	1486	1491	+0.34	1493	+0.47
20:3	1532	1574	+2.74	1546	+0.91
18:1	1613	1658	+2.79	1598	−0.93
Average			1.83		0.46

[a] Calculated from the equation $I/100 = N - 1.419n$.

[b] Calculated from the equation $I/100 = 0.922N - 1.184n + 0.570$.

[c] Calculated from the expression $(I_{\text{Experiment}} - I_{\text{Theory}})/I_{\text{Experiment}}$.

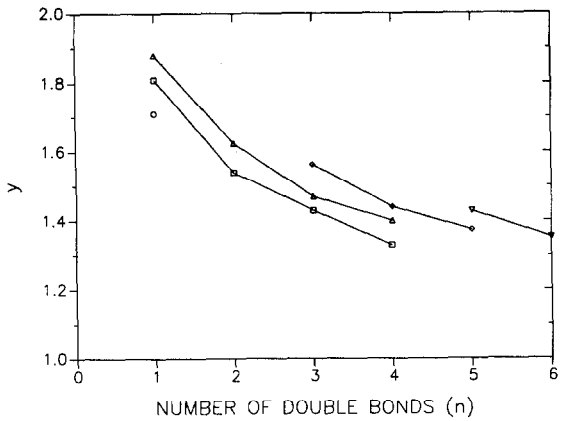

Fig. 8. The relationship between the y coefficient in eqn. 3 and the number of double bonds (n) in fatty acid derivatives with the same number of carbon atoms. Solutes: 14:n (○), 16:n (□), 18:n (△), 20:n (◇) and 22:n (▽). Experimental conditions as in Fig. 6.

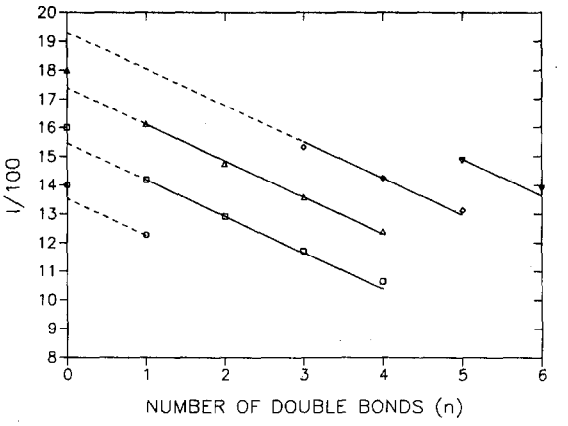

Fig. 10. The relationship between the retention index (I) divided by 100 and the number of double bonds (n) in fatty acid derivatives with the same number of carbon atoms. Solutes: 14:n (○), 16:n (□), 18:n (△), 20:n (◇) and 22:n (▽). Experimental conditions as in Fig. 6.

the precision of replicate measurements (±0.1% relative standard deviation, R.S.D.). More importantly, however, this error results in the incorrect prediction of elution order for all of the polyunsaturated fatty acids with retention characteristics similar to saturated and monounsaturated fatty acids (Table II).

In order to examine this problem in more detail, individual values for the coefficient y were calculated according to eqn. 3 for each unsaturated fatty acid and graphed as a function of the number of double bonds (n). It is apparent from Fig. 8 that the coefficient y decreases as n increases for fatty acids with the same number of carbon atoms, as noted previously by Özcimder and Hammers [5]. In addition, however, the coefficient appears to decrease as N decreases for fatty acids with the same number of double bonds.

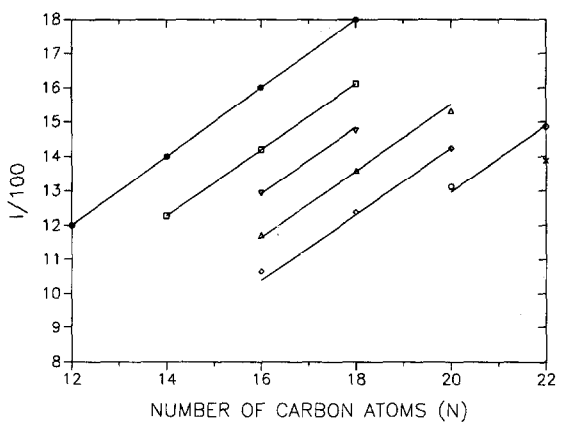

Fig. 9. The relationship between the retention index (I) divided by 100 and the number of carbon atoms (N) in fatty acid derivatives with the same number of double bonds. Solutes: N:0 (●), N:1 (□), N:2 (▽), N:3 (△), N:4 (◇), N:5 (○) and N:6 (×). Experimental conditions as in Fig. 6.

Based on these observations, a more reliable equation to describe and predict the retention behavior of unsaturated fatty acids may be derived. From Fig. 9, a linear relationship is observed between $I/100$ and N for fatty acids with the same number of double bonds. Likewise from Fig. 10, a negative linear relationship is observed between $I/100$ and n for fatty acids with the same number of carbon atoms. Therefore, the retention index can be expressed as a simple linear function of both N and n:

$$I/100 = \text{ECL} = xN - yn + z \quad (4)$$

where x, y and z are empirically derived coefficients. To determine the values of these coefficients, the experimental retention index values for the fatty acids were substituted into eqn. 4 and analyzed by multiple linear regression. The solution of this equation yields $x = 0.922 \pm 0.015$, $y = 1.184 \pm 0.022$, and $z = 0.570 \pm 0.224$ for the unsaturated fatty

acids. As summarized in Table II, the average error in the retention index is 0.46% and all fatty acids are predicted in the correct elution order.

Eqn. 4 represents a simple modification of the classical ECL model for fatty acid retention developed by Özcimder and Hammers [5]. Because this expression is more accurate and precise than eqn. 3, it permits more confident identification of unknown fatty acids in complex mixtures. Although the specific values cited above for the empirical coefficients in eqn. 4 are applicable only under the experimental conditions used in this study, such coefficients can be readily determined under different conditions by using the method described above.

CONCLUSIONS

The retention behavior of 4-bromomethyl-7-methoxycoumarin derivatives of fatty acids with 12–22 carbon atoms and 0–6 double bonds has been studied in reversed-phase LC with aqueous methanol and acetonitrile mobile phases. The baseline resolution for the separation of 14 saturated and unsaturated fatty acids was obtained by using a packed capillary column with 240 000 theoretical plates and a mobile phase composition of 90% methanol, 2% acetonitrile, and 8% water. The fatty acid derivatives were determined at the femtomole level in fish oil dietary supplements with laser-induced fluorescence detection. It was found that the dietary supplements contain predominantly the polyunsaturated eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids as well as lesser amounts of hexadecatetraenoic (16:4) and octadecatetraenoic (18:4) acids. However, they may also contain significant amounts of saturated and monounsaturated fats such as myristic (14:0), palmitic (16:0), palmitoleic (16:1), and oleic (18:1) acids. The retention indices of the unsaturated fatty acid derivatives increased linearly with the number of carbon atoms and decreased linearly with the number of double bonds. The retention indices predicted by using a modified ECL equation showed less than 0.46% relative error and $\pm 0.1\%$ R.S.D.

ACKNOWLEDGEMENTS

This work was supported by the US Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences under Contract No. DE-FG02-89ER14056. The authors wish to thank Dr. Kirk Maxey (Cayman Chemical, Ann Arbor, MI, USA) for providing the fatty acid standards and Dr. Thomas V. Atkinson (Michigan State University) for assisting with the multiple linear regression analysis.

REFERENCES

1 W. E. M. Lands, *Fish and Human Health*, Academic Press, New York, 1986.
2 P. M. Herold and J. E. Kinsella, *Am J. Clin. Nutr.*, 43 (1986) 566.
3 J. Halgunset, F. W. Lund and A. Sunde, *J. Chromatogr.*, 237 (1982) 496.
4 P. R. Brown, J. M. Beebe and J. Turcotte, *Crit. Rev. Anal. Chem.*, 21 (1989) 193.
5 M. Özcimder and W. E. Hammers, *J. Chromatogr.*, 187 (1980) 307.
6 J. P. Roggero and S. V. Coen, *J. Liq. Chromatogr.*, 4 (1981) 1817.
7 A. K. Batta, V. Dayal, R. W. Colman, A. K. Sinha, S. Shefer and G. Salen, *J. Chromatogr.*, 284 (1984) 257.
8 C. Osterroht, *Chromatographia*, 23 (1987) 419.
9 H. C. Jordi, *J. Liq. Chromatogr.*, 1 (1978) 215.
10 R. F. Borch, *Anal. Chem.*, 47 (1975) 2437.
11 H. Traitler, H. J. Wielle and A. Studer, *J. Am. Oil Chem. Soc.*, 65 (1988) 755.
12 T. Rezanka and M. Podojil, *J. Chromatogr.*, 346 (1985) 453.
13 W. Dunges, *Anal. Chem.*, 49 (1977) 442.
14 S. N. Barker, J. A. Monti, S. T. Christian, F. Benington and R. D. Morin, *Anal. Biochem.*, 107 (1980) 116.
15 N. Ichinose, K. Nakamura, C. Shimizu, H. Kurokura and K. Okamato, *J. Chromatogr.*, 295 (1984) 463.
16 M. Ikeda, K. Shimada, T. Sakaguchi and U. Matsumoto, *J. Chromatogr.*, 305 (1984) 261.
17 W. D. Korte, *J. Chromatogr.*, 243 (1982) 153.
18 H. Tsuchiya, T. Hayashi, H. Naruse and N. Takagi, *J. Chromatogr.*, 234 (1982) 121.
19 V. L. McGuffin and R. N. Zare, *Appl. Spectrosc.*, 39 (1985) 847.
20 V. L. McGuffin and R. N. Zare, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 8315.
21 J. C. Gluckman, A. Hirose, V. L. McGuffin and M. Novotny, *Chromatographia*, 17 (1983) 303.
22 R. J. Laub and J. H. Purnell, *J. Chromatogr.*, 112 (1975) 71.
23 R. J. Laub, *J. Liq. Chromatogr.*, 7 (1984) 647.
24 A. J. Hsu, R. J. Laub and S. J. Madden, *J. Liq. Chromatogr.*, 7 (1984) 615.
25 H. B. Patel and T. M. Jefferies, *J. Chromatogr.*, 389 (1987) 21.
26 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
27 E. D. Katz, C. H. Lochmuller and R. P. W. Scott, *Anal. Chem.*, 61 (1989) 349.
28 J. M. Beebe and P. R. Brown, *J. Chromatogr.*, 468 (1989) 225.
29 E. Kovats, *Helv. Chim. Acta*, 41 (1958) 1915.
30 L. S. Ettre, *Chromatographia*, 6 (1987) 489.