Combination of Silver Ion and Reversed-Phase High-Performance Liquid Chromatography in the Fractionation of Herring Oil Triacylglycerols

Päivi Laakso[a] and **William W. Christie**[b,*]

[a]University of Turku, Department of Chemistry and Biochemistry, Laboratory of Food Chemistry, 20500 Turku, Finland and the Foundation for Nutrition Research in Finland, and [b]The Hannah Research Institute, Ayr, Scotland KA6 5HL

Triacylglycerols from North Atlantic herring *(Clupea harengus)* were separated according to the degree of unsaturation by high performance liquid chromatography (HPLC) in the silver ion mode. Each of the eleven fractions collected was then separated by reversed-phase HPLC, which in these circumstances separated the molecules according to the combined chain-lengths of the fatty acyl residues only. One hundred thirty fractions were obtained for fatty acid analysis.

Almost 50% of the triacylglycerol molecules had six or more double bonds in their fatty acyl residues. Saturated-dimonoenes and disaturated-monoenes, 18.9% and 10.4%, respectively, were the most plentiful fractions of the more saturated species. Such a complex mixture of molecules was present that the most abundant subfractions from reversed-phase HPLC represented less than 5% of the total. Indeed, the largest single molecular species [16:0-22:1-22:6(n-3)] represented only 2.8% of the total. These sequential analyses by complementary techniques made it possible to obtain a considerable amount of information on the composition of molecular species, but it was still not possible to identify all components.

KEY WORDS: Fish oil, herring *(Clupea harengus)*, high performance liquid chromatography, reversed-phase chromatography, silver ion chromatography, triacylglycerols.

Natural triacylglycerols, especially those of fish oils, are such complex mixtures of different molecular species that it is impossible to separate all of them by a single chromatographic method. Silver ion chromatography has been used extensively to separate molecules according to degree of unsaturation (1). Bottino (2) and Dolev and Olcott (3) utilized thin-layer (TLC) and column chromatographic methods including silica gel impregnated with silver ions to separate fish oil triacylglycerols. Recently, a cleaner and more efficient method taking the advantage of high-performance liquid chromatography (HPLC) with cation exchange columns loaded with silver ions has been developed (4,5). This technique was applied to fractionate triacylglycerols from North Atlantic herring, sandeel and Baltic herring (6).

Natural triacylglycerols have been analyzed often by reversed-phase HPLC (7). The separation is effected by the combined chain-lengths of the fatty acyl moieties and the total number of double bonds. The dual nature of the separation process can make identification of the fractions difficult. The combination of silver ion TLC and reversed-phase HPLC in sequence is complementary, and it has been used for analyzing triacylglycerols from evening primrose oil (8) and from palm oil and cocoa butter (9), for example. Myher *et al.* (10) and Lund (11) fractionated milk fat triacylglycerols with silver ion TLC before gas-liquid chromatographic (GLC) analyses. There is little detailed information about fish oil triacylglycerols from combined analytical techniques. Wada *et al.* (12) separated Black cod triacylglycerols first according to the partition number by reversed-phase HPLC and then according to the carbon number by GLC, and a similar approach was used with triacylglycerols from the Rock crab (13). Silver ion TLC and reversed-phase HPLC have been utilized in combination also (14). However, the major analytical problems were avoided in this work because of the low polyenoic fatty acid content of the fish oil.

In this paper, a combination of silver ion and reversed-phase HPLC in sequence has been utilized to obtain information on the composition of the molecular species of herring triacylglycerols. It is the first such analysis of a fish oil containing a typical range of polyunsaturated components.

EXPERIMENTAL PROCEDURES

Samples and reagents. Lipids from North Atlantic herring *(Clupea harengus)*, recovered by an industrial pressing process, were donated by J. Pettersen (Norwegian Herring Oil & Meal Industry Research Institute, Bergen, Norway). Pure triacylglycerols were obtained by preparative HPLC on a column of silica gel (Spherisorb™-5 micron; 250 × 5 mm i.d. column) using hexane-tetrahydrofuran (99:1, v/v) as mobile phase at a flow-rate of 1 mL/min. All solvents and reagents were Analar or HPLC grades and were supplied by FSA Scientific (Loughborough, U.K.).

HPLC. The HPLC system consisted of a Spectra-Physics Model 8700 solvent delivery system (Spectra-Physics, St. Albans, U.K.), an ACS Model 750/14 mass detector (Applied Chromatography Systems, Macclesfield, U.K.) and a Spectra-Physics SP 4290 integrator. An adjustable stream-splitter was installed between the column and the detector.

The silver ion column was prepared as described previously (5). The fractionation of fish oil triacylglycerols was carried out as reported earlier (6) using a ternary solvent gradient consisting of (A) 1,2-dichloroethane/dichloromethane (1:1, v/v); (B) acetone; and (C) acetone/acetonitrile (2:1, v/v); as the three components. In brief, linear gradients of 100% A to 50% A-50% B over 10 min, to 70% B-30% C over 30 min, to 50% B-50% C over 20 min, and then to 100% C over a further 20 min, were utilized, and the final solvent mixture was maintained for an additional 5 min. The column was kept at ambient temperature and the flow rate was 1.0 mL/min. Samples were dissolved in 1,2-dichloroethane and aliquots of 1–2 mg triacylglycerol in 10–25 μL of 1,2-dichloroethane were injected on to the column. In order to have sufficient

*To whom correspondence should be addressed.

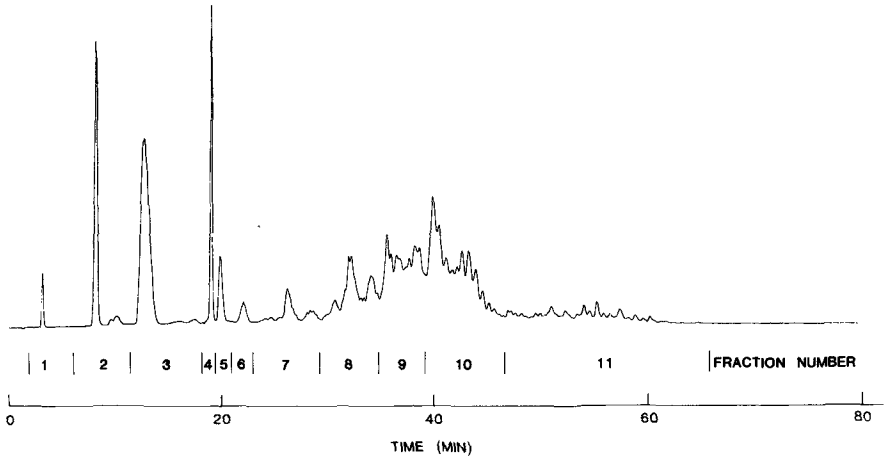

FIG. 1. Fractionation of the triacylglycerols from herring oil by HPLC with a silver ion column and mass detection. The chromatographic conditions are described in the Experimental section. Fraction numbers are the same as in Table 1.

material available, the separation was repeated up to 15 times and corresponding fractions were pooled.

Reversed-phase HPLC analyses were performed using two columns of Spherisorb 5ODS2™ (250 × 4.6 mm i.d., 5 μm) (Hichrom Ltd., Reading, U.K.) in series at ambient temperature. Triacylglycerols were separated with a solvent gradient of (A) 1,2-dichloroethane/dichloromethane (1:4, v/v); and (B) acetonitrile. The gradient was changed over 60 min from 30% A-70% B to 40% A-60% B and then over a further 40 min to 55% A-45% B and held for 5 min; the flow rate was 0.75 mL/min. Samples were dissolved in 1,2-dichloroethane and volumes of 10 μL or less were injected onto the column.

Fatty acid analysis by gas chromatography (GC). Triacylglycerol fractions were collected via the stream-splitter from the HPLC analyses to determine the fatty acid compositions and amounts. Fatty acid methyl esters were prepared by sodium methoxide-catalyzed transesterification (15) in the presence of an internal standard (methyl nonadecanoate). GC analyses were carried out with a Carlo Erba Model 4130 capillary gas chromatograph (Carlo Erba, Milano, Italy) equipped with a split/splitless injector and a fused silica capillary column (25 m × 0.22 mm i.d., film thickness 0.2 μm) coated with Carbowax 20M (Chrompack Ltd, London, U.K.); hydrogen was the carrier gas. The column temperature was programmed with three minutes isothermally at 160°C, then to 195°C at a rate of 4°C/min and was kept at the final temperature for 22 more min. Fatty acids were identified by reference to a standard fish oil (cod liver oil) and were quantified by electronic integration.

RESULTS AND DISCUSSION

In this research, the sequential combination of silver ion and reversed-phase HPLC was investigated for the separation of the triacylglycerols of herring oil, chosen because of its wide fatty acid and complex molecular species compositions as well as its commercial importance.

Silver ion HPLC was used first to fractionate the triacylglycerols according to the degree of unsaturation. They were separated into eleven fractions via the stream-splitter. For analytical purposes, each one was transesterified with sodium methoxide in the presence of internal standard for identification and quantification of the fatty acids by GC. The fractionation was repeated several times to obtain enough material for further analysis with the reversed-phase system. With the latter, the separation was according to the differences in the combined chain-lengths of the fatty acyl constituents only as the influence of the double bonds had, in effect, been neutralized. The peaks obtained by reversed-phase HPLC were collected for analysis from each silver ion fraction. Herring triacylglycerols were divided into 130 fractions in this way.

With silver ion HPLC, the eleven fractions shown in Figure 1 were obtained initially. The more saturated molecules eluted first and the degree of unsaturation increased, the elution order of the main components being SSS, SSM, SMM, MMM, SMD, MMD, SMT, SMTe, SMP and SMH, where S = saturated, M = monoenoic, D = dienoic, T = trienoic, Te = tetraenoic, P = pentaenoic and H = hexaenoic fatty acid moieties. The average numbers of double bonds in the triacylglycerol molecules (double bond indices) were calculated from each silver ion fraction and the results are presented in Table 1. The first six fractions had the compositions expected of simple triacylglycerol mixtures. Fractions 7 to 11 had more complex fatty acid compositions, with one or more moles of polyunsaturated components, and as can be seen from Figure 1 they were composed of several peaks. Thus, the later fractions especially were mixtures of many different kinds of molecules. In a previous paper (6), herring triacylglycerols were divided into 20 fractions and some other fish triacylglycerols into as many as 26, but the polyunsaturated fractions were still mixtures of many molecular species. Only eleven fractions were collected here to limit the amount of practical work.

Some specificity for fatty acids of different chain-lengths, especially the monoenes, in molecular species was evident in that higher proportions of 16:1 were detected in the early fractions than might have been anticipated from the relative amount in the oil as a whole, while

TABLE 1

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions and Double Bond Indices of Triacylglycerol Fractions, Obtained by Silver Ion HPLC, from North Atlantic Herring

Fatty acid	Total	Silver ion HPLC fraction											Recombinant composition
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	
14:0	11.1	39.8	24.6	11.7	1.8	9.2	1.3	5.3	6.5	7.7	6.2	4.9	9.4
15:0	0.8	3.0	1.8	1.0	0.1	0.7		0.7	0.8	0.8	0.5	0.4	0.8
16:0	17.8	51.4	36.4	23.0	4.4	18.4	5.1	17.4	19.7	20.0	12.5	9.3	18.6
16:1	6.6		6.0	10.7	20.6	5.4	6.4	5.1	3.7	4.1	6.0	3.2	6.8
16:2	0.4					5.4	3.8	0.4	0.2	0.2	0.4	0.6	0.4
16:4	0.3							0.2	1.3		0.3	0.8	0.3
17:0	0.3	0.8	0.5					0.3	0.4	0.4	0.2	0.2	0.2
18:0	1.2	3.4	2.2	2.0	0.4	1.0	0.9	1.6	1.7	2.0	1.4	0.9	1.6
18:1	11.1		9.9	20.3	29.4	10.9	19.4	10.4	7.7	7.9	11.9	5.3	12.4
18:2	1.9			0.7	1.5	24.7	21.1	2.0	0.7	0.8	1.5	2.3	1.9
18:3(n-3)	2.1							24.3	1.1	0.3	1.0	4.7	2.1
18:4(n-3)	4.7							0.3	21.7	2.6	1.1	13.2	4.2
20:1	8.2		6.4	12.0	16.2	7.7	14.7	10.0	7.0	7.5	7.7	3.1	8.3
20:4(n-6)	0.3								0.5	0.9		0.6	0.2
20:4(n-3)	0.7								4.0	0.4	0.2	2.7	0.8
20:5(n-3)	6.4								4.8	23.3	3.0	17.5	6.4
22:1	14.5		11.2	18.6	24.4	14.8	25.8	16.5	15.5	14.1	17.8	6.8	15.3
22:5(n-3)	0.5									2.0	0.6	1.3	0.6
22:6(n-3)	8.7									5.2	25.7	19.7	8.3
other	2.5	1.5	1.0		1.2	1.8	1.5	5.1	2.2	1.0	1.7	2.8	1.5
Amount (mole %)		1.3	10.4	18.9	5.6	2.2	1.3	4.7	9.1	14.1	20.4	12.1	
Double bond index	4.9	0.0	1.0	1.9	2.8	3.0	3.5	3.7	5.2	6.2	6.9	9.6	4.8

TABLE 2

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 1

Fatty acid	Total	Reversed-phase HPLC fraction								Recombinant composition
		(1-1)	(1-2)	(1-3)	(1-4)	(1-5)	(1-6)	(1-7)	(1-8)	
14:0	39.8	82.9	57.3	64.7	35.4	31.0	18.8	9.6	11.7	35.3
15:0	3.0		20.7	0.9	13.1	1.2	9.9	0.6	2.4	3.2
16:0	51.4	17.1	12.4	34.4	39.2	65.7	56.6	82.0	60.3	54.5
17:0	0.8				1.7		3.4		3.0	0.7
18:0	3.4				1.2	2.2	3.2	7.9	19.3	4.4
other	1.5		9.5		9.5		8.1		3.3	1.9
Amount (mole %) of the fraction)		3.5	2.4	24.3	7.7	26.5	7.6	16.8	11.1	
Amount (mole %) of the total	1.3	<0.1	<0.1	0.3	0.1	0.3	0.1	0.2	0.1	
Partition number			42		44		46		48	
Approximate carbon number			42.0		44.0		46.0		48.0	

proportionately more of the 20:1 and 22:1 fatty acids were in the more polyunsaturated species.

The reversed-phase HPLC chromatogram of the total herring triacylglycerols is very complex (Fig. 2A). The separation of molecules is based on both the combined chain-lengths of the fatty acyl residues and on the total number of double bonds, so that components elute in ascending order of chain-length but with each of the double bonds reducing the retention time of the molecules by the equivalent of about two methylene groups. In contrast, much simpler chromatograms were obtained when the oil was fractionated according to the degree of unsaturation before analysis by reversed-phase HPLC. Some examples are shown in Figures 2B–2E. The fatty acid compositions and amounts of different molecular types from each silver ion fraction, expressed as mole %, are presented in Tables 2–12. The silver ion fractions, as numbered in the Tables, correspond to those in Figure 1. Fatty acids found at less than 0.05% of the total are included with the "others". Several isomers of monoenoic components were identified but are not differentiated here. To check the recoveries, the fatty acid composition

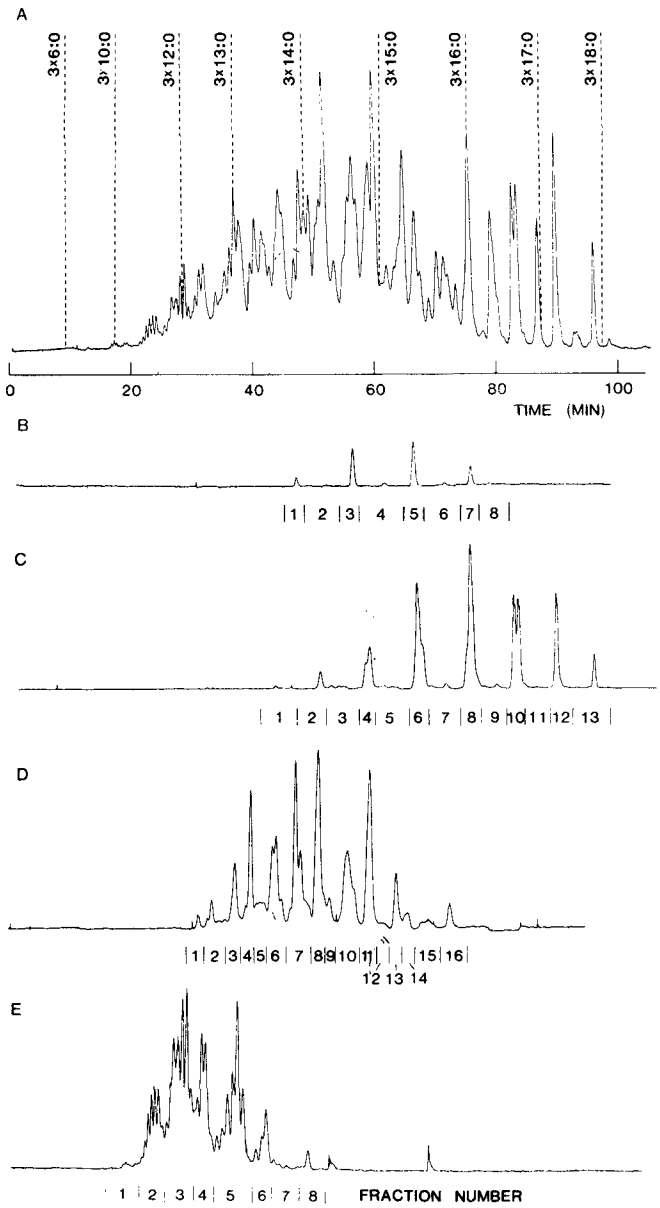

FIG. 2. A. Separation of the total triacylglycerols from herring oil by reversed-phase HPLC with mass detection. Dotted lines show the places where saturated monoacid triacylglycerol standards would elute. As examples, 2B, 2C, 2D and 2E show the reversed-phase HPLC separations of silver ion HPLC fractions 1, 3, 8 and 11, respectively. The chromatograms are placed so that the peaks are approximately in their correct positions when compared to the total chromatogram. Fraction numbers refer to Tables 2, 4, 9 and 12, respectively. The chromatographic conditions are described in the Experimental section.

of each silver ion fraction was reconstituted from the relative proportions in each of the reversed-phase subfractions (Tables 2–12). Recoveries were acceptable in each instance.

Reversed-phase chromatograms of the more saturated fractions from silver ion HPLC were formed of peaks resolved to the baseline. Some peaks were split into two, probably because of differences in the positions of double bonds in fatty acyl constituents. Others were asymmetric and evidently contained more than one molecular species. Most chromatograms had small peaks eluting between the main ones that contained, for example, odd- and branched-chain fatty acids; e.g., silver ion fraction 2 (Table 3) had minor components (sub-fractions 2-2, 2-4, 2-6, 2-8, 2-10 and 2-12) with increased proportions of 15:0 and 17:0 fatty acids. More unsaturated silver ion fractions eluted earlier in the reversed-phase chromatogram than the more saturated ones and were split into several peaks which could no longer be separated to the baseline.

A number of methods have been devised for defining relative retention times of triacylglycerol fractions in a manner that relates to structure and as a guide to the identification of unknowns [reviewed elsewhere (7)], but most of these require isocratic elution conditions rather than gradient elution, as was the case here. By co-injecting the total herring triacylglycerols and a mixture of standards containing saturated monoacid triacylglycerols, "approximate carbon number" (ACN) values for each chromatographic peak in the fish oil were calculated. The places where standards would elute in comparison are shown in Figure 2A. To estimate the relationships between the reversed-phase HPLC peaks from fractions obtained by silver ion HPLC and those of the total herring triacylglycerols, co-injections of each silver ion fraction together with the total triacylglycerols were done when possible. An ACN value was then given to each main peak to give an approximate idea of where peaks in different fractions eluted in comparison to each other, and they are listed in Tables 2 to 12. The error involved is too great to permit certain identification of components with similar values.

By comparing the chromatograms and investigating the fatty acid compositions, the "partition numbers" (PN) of the fractions could also be estimated, i.e., the combined chain-lengths of the fatty acyl groups less two for each double bond in the molecule, and these are also listed in Tables 2 to 12. When the degree of unsaturation increased, the PN values became of less practical value because of molecular shifts caused by positional isomers of double bonds and other overlapping components. The retention times of the peaks *per se* could not be used for identification, because they varied according to the stabilization time before the gradient was started, to minor changes in the gradient or to the ambient temperature. In Figures 2B–2E, the reversed-phase chromatograms of the silver ion fractions are placed so that the chromatographic peaks are as close as possible to their correct positions when compared to those in the chromatogram obtained from total herring triacylglycerols.

The average chain-length of fatty acids of a given degree of unsaturation within one silver ion fraction increased towards the end of the reversed-phase chromatograms as might be anticipated. For example, from the reversed-phase chromatogram of saturated-dimonoene triacylglycerols (Table 4), the amount of 16:1 fatty acid was higher than the amounts of 20:1 and 22:1 up to fraction 3-6, and the proportion of 18:1 was higher than that of 22:1 up to fraction 3-8. The amount of 22:1 exceeded that of 20:1 just after fraction 3-6 and increased up to 62.1% of the total fatty acids in the last fraction.

Silver ion fraction 1, the trisaturated molecules, represented 1.3% only of the total triacylglycerols. This was simplest of all in structure and with reversed-phase HPLC, it was divided into eight sub-fractions from which

TABLE 3

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 2

		Reversed-phase HPLC fraction												Recombinant
Fatty acid	Total	(2-1)	(2-2)	(2-3)	(2-4)	(2-5)	(2-6)	(2-7)	(2-8)	(2-9)	(2-10)	(2-11)	(2-12)	composition
14:0	24.6	53.8	41.1	30.5	10.7	19.6	8.5	21.7	13.6	19.3	8.0	3.2	2.2	20.0
15:0	1.8	0.2	5.0	0.4	6.2	0.4	7.4	0.3	13.1	0.3	8.9	0.3	1.0	1.6
16:0	36.4	9.4	18.5	26.3	32.7	35.8	33.2	38.5	26.7	43.1	31.9	55.7	33.5	36.5
16:1	6.0	27.0	18.0	22.4	12.7	8.7	3.7	2.1	2.2	1.5	0.9	0.3	1.6	6.2
17:0	0.5		3.3		0.8		2.3		3.9		4.7		2.5	0.5
18:0	2.2	0.9	1.0	0.3	1.0	1.0	2.1	2.0	3.3	3.5	3.8	6.5	13.2	2.6
18:1	9.9	6.4	8.5	19.1	21.8	21.5	22.3	13.4	7.7	2.5	7.8	1.1	4.1	12.1
20:1	6.4	0.8	1.7	1.0	5.0	11.1	8.2	9.7	8.4	7.0	6.4	2.8	5.8	7.4
22:1	11.2	1.5	1.4		2.4	1.3	6.1	11.3	18.0	22.1	24.1	30.2	34.3	12.0
other	1.0		1.5		6.7	0.6	6.1	0.9	3.1	0.8	3.6		1.8	1.2
Amount (mole %) of the fraction		3.4	3.2	7.3	3.1	17.8	4.0	27.6	3.2	18.2	2.3	7.1	2.8	
Amount (mole %) of the total	10.4	0.4	0.3	0.8	0.3	1.9	0.4	2.9	0.3	1.9	0.2	0.7	0.3	
Partition number		42		44		46		48		50		52	54	
Approximate carbon number		41.1		43.3		45.0 45.2		47.0 47.2	48.0	49.0	50.0	50.9	52.8	

TABLE 4

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 3

		Reversed-phase HPLC fraction												Recombinant
Fatty acid	Total	(3-1)	(3-2)	(3-3)	(3-4)	(3-5)	(3-6)	(3-7)	(3-8)	(3-9)	(3-10)	(3-11)	(3-12)	(3-13)	composition
14:0	11.7	20.9	16.0	16.5	15.4	9.0	17.1	8.4	13.1	5.0	8.2	5.4	7.3	1.6	12.3
15:0	1.0	1.0	1.1	3.9	0.2	3.9	0.2	5.5		4.5		5.9	0.5		0.9
16:0	23.0	19.1	18.5	27.2	19.2	25.1	19.9	18.0	21.1	15.5	23.9	13.7	21.8	29.2	21.2
16:1	10.7	29.9	32.5	13.1	23.6	11.6	15.8	9.4	10.0	3.2	1.2	1.3	0.5	0.2	11.3
16:2		1.7	0.5	1.6		1.7		2.3							0.3
17:0										1.8		2.7			0.1
18:0	2.0	5.6	1.5	1.6	0.7	1.7	0.8	3.3	1.3	4.7	1.7	5.6	2.8	2.3	1.7
18:1	20.3	11.1	23.6	17.7	32.2	22.2	26.2	20.0	20.7	19.9	18.1	11.7	2.3	1.0	20.2
18:2	0.7	6.5	2.6	9.2	0.3	6.3		1.0							0.8
18:3(n-3)			0.4												0.0
18:4(n-3)		0.8	0.4												0.0
20:1	12.0	1.8	1.5	3.5	7.2	9.2	12.0	11.8	15.5	16.4	20.7	17.6	21.1	3.5	13.3
22:1	18.6	1.6	0.8	1.8	1.1	4.6	7.9	13.9	18.2	24.7	26.1	35.1	43.7	62.1	17.3
other			0.5	3.9		4.7		6.4		4.4		1.0			0.7
Amount (mole %) of the fraction		1.7	4.7	2.7	9.3	4.2	21.8	4.0	20.5	2.9	15.2	1.7	8.1	3.3	
Amount (mole %) of the total	18.9	0.3	0.9	0.5	1.8	0.8	4.1	0.7	3.9	0.5	2.9	0.3	1.5	0.6	
Partition number			44		46		48		50		52		54	56	
Approximate carbon number		41.1	42.6		44.6		46.2 46.4		48.0 50.1		49.9		51.7	53.6	

four peaks dominated (Fig. 2B, Table 2). From the fatty acid analyses, these were mainly 14:0-14:0-14:0 (fraction 1-1), 14:0-14:0-16:0 (fraction 1-3), 14:0-16:0-16:0 (fraction 1-5) and 16:0-16:0-16:0 (fraction 1-7). Fraction 1-7 contained small amounts of 14:0-16:0-18:0 species also. (The fatty acids are listed in a manner that bears no relationship to the positional distribution within the triacylglycerols). The small peaks between the main ones contained odd- and branched-chain fatty acids in addition to the major components.

The reversed-phase chromatogram of the disaturated-monoenes (SSM, silver ion fraction 2, Table 3) was not quite as simple as that of the trisaturated triacylglycerols, and the former were subdivided into twelve fractions when separated according to the combined chain-lengths of the fatty acyl residues. By comparing the reversed-phase chromatogram of this fraction and that of the total triacylglycerols, and from the fatty acid compositions, it was estimated that the ACN values varied between 41.1 and 52.8; the partition numbers of the corresponding

TABLE 5

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 4

		Reversed-phase HPLC fraction													Recombinant composition
Fatty acid	Total	(4-1)	(4-2)	(4-3)	(4-4)	(4-5)	(4-6)	(4-7)	(4-8)	(4-9)	(4-10)	(4-11)	(4-12)	(4-13)	
14:0	1.8	7.0	7.8	1.6	5.5	0.6	7.5	0.7	3.3	0.3	4.2	0.3	0.6	0.9	1.5
15:0	0.1		0.5						0.2		0.6				0.0
16:0	4.4	11.2	7.1	3.8	9.9	1.3	17.9	3.8	12.2	1.5	13.5	1.2	2.0	2.6	3.9
16:1	20.6	65.6	45.4	31.7	14.4	32.1	13.6	22.3	4.6	12.5	2.7	8.3	1.0		20.4
16:2			1.0				2.2								0.1
18:0	0.4	3.9	1.1	0.3	1.7	0.2	2.8	0.2	2.3		7.9	0.4	1.0	2.0	0.7
18:1	29.4	9.4	28.4	44.6	33.5	42.9	24.4	33.5	22.3	31.8	20.1	20.9	16.1	2.5	31.9
18:2	1.5	2.9	5.5	2.1	1.6	0.3	4.9	2.7	6.3	0.3	3.5				1.6
20:1	16.2		1.7	9.9	9.7	10.9	7.3	16.3	10.5	26.1	16.8	27.4	29.2	27.6	17.0
22:1	24.4		1.6	5.3	17.2	11.7	14.3	20.6	29.0	27.3	26.0	41.5	49.8	64.4	22.0
other	1.2			0.7	6.3		5.2		9.2		4.8		0.3		0.7
Amount (mole %) of the fraction		0.9	4.3	10.4	1.9	16.9	3.5	23.2	2.4	17.0	2.4	10.5	4.8	1.7	
Amount (mole %) of the total	5.6	0.1	0.2	0.6	0.1	0.9	0.2	1.3	0.1	1.0	0.1	0.6	0.3	0.1	
Partition number		42	44	46		48		50		52		54	56	58	
Approximate carbon number		40.0	42.0	43.8 44.0		45.5		47.2 47.4		49.0 49.1		50.9 52.8	52.6	>54.0	

TABLE 6

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 5

		Reversed-phase HPLC fraction						Recombinant composition
Fatty acid	Total	(5-1)	(5-2)	(5-3)	(5-4)	(5-5)	(5-6)	
14:0	9.2	24.7	16.0	7.2	7.9	2.7	6.8	9.9
15:0	0.7	1.1	0.6	0.8	0.6	1.4		0.8
16:0	18.4	11.5	17.9	17.1	16.3	26.4	24.0	18.3
16:1	5.4	21.2	12.0	2.2	1.3	1.3	2.1	5.4
16:2	5.4	11.8	7.9	5.8	3.5	0.7		5.1
18:0	1.0	0.8	0.5	1.1	1.4	1.9	6.1	1.3
18:1	10.9	8.0	17.8	17.5	5.2	3.3	9.9	10.7
18:2	24.7	18.7	22.4	24.6	27.2	27.8	12.7	24.6
20:1	7.7	1.2	3.6	14.7	13.3	3.8	8.8	9.0
22:1	14.8	1.0	0.7	7.6	21.5	27.8	23.1	13.4
other	1.8		0.5	1.6	1.8	3.1	6.6	1.7
Amount (mole %) of the fraction		8.7	19.4	24.3	28.3	16.5	2.9	
Amount (mole %) of the total	2.2	0.2	0.4	0.5	0.6	0.4	0.1	
Partition number		42	44	46	48	50		
Approximate carbon number		40.4	42.3	43.8 44.0	45.9	47.6	49.2	

species varied between 42 and 54. As examples, fraction 2-1 was composed mainly of 14:0-14:0-16:1, fraction 2-5 was a mixture of 14:0-16:0-18:1, 14:0-14:0-20:1 and 16:0-16:0-16:1, fraction 2-9 was a mixture of 14:0-16:0-22:1, 14:0-18:0-20:1, 16:0-16:0-20:1, 16:0-18:0-18:1 and 18:0-18:0-16:1 and fraction 12 contained 16:0-18:0-22:1 as the major species. The largest SSM sub-fraction (2-7) had possible fatty acid combinations including 14:0-16:0-20:1, 14:0-14:0-22:1, 16:0-16:0-18:1 and 14:0-18:0-18:1.

With reversed-phase HPLC, the SMM fraction was divided into thirteen sub-fractions (Fig. 2C, Table 4). Small amounts of SSD species were also present, and the latter was expected to elute just after SMM as found previously (4,6), since a molecule with one dienoic fatty acyl residue is more strongly retained by the silver ions than one with two monoenoic fatty acyl residues. Most of the sub-fractions were complex mixtures. For example, peak 3-2 comprised 14:0-16:1-18:1 and 16:0-16:1-16:1,

TABLE 7

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 6

Fatty acid	Total	Reversed-phase HPLC fraction							Recombinant composition
		(6-1)	(6-2)	(6-3)	(6-4)	(6-5)	(6-6)	(6-7)	
14:0	1.3	9.3	3.4	1.2	0.8	1.0	1.1	2.4	1.9
15:0		0.8	0.3	0.2					0.1
16:0	5.1	12.3	9.5	4.1	3.2	2.7	2.8	4.7	4.6
16:1	6.4	31.0	22.7	14.3	12.6	2.5	0.9	1.0	10.9
16:2	3.8	9.3	8.8	8.3	6.4	5.4	4.6	1.2	6.5
18:0	0.9	1.0	0.7	0.6	0.5	0.4	0.4	0.8	0.6
18:1	19.4	16.2	27.2	26.1	18.5	20.2	6.2	3.1	18.6
18:2	21.1	14.4	18.7	19.5	23.4	23.3	22.6	23.7	21.4
20:1	14.7	2.0	6.0	14.9	11.4	16.3	23.7	7.7	13.3
22:1	25.8	1.3	1.1	9.1	22.1	26.0	35.6	52.3	20.3
other	1.5	2.4	1.4	1.7	1.2	2.2	2.0	3.1	1.8
Amount (mole %) of the fraction		6.4	11.9	18.5	23.1	21.0	13.3	5.9	
Amount (mole %) of the total	1.3	0.1	0.2	0.2	0.3	0.3	0.2	0.1	
Partition number		42	44	46	48	50	52	54	
Approximate carbon number									

TABLE 8

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 7

Fatty acid	Total	Reversed-phase HPLC fraction													Recombinant composition
		(7-1)	(7-2)	(7-3)	(7-4)	(7-5)	(7-6)	(7-7)	(7-8)	(7-9)	(7-10)	(7-11)	(7-12)	(7-13)	(7-14)	
14:0	5.3	23.0	30.7	12.5	11.5	11.4	8.0	7.4	1.4	7.0	1.5	2.1	1.7	0.5	1.8	1.6
15:0	0.7	1.0	1.6	1.2	1.3	1.0	1.0	0.5	0.8	0.2	1.2	0.1	0.5	0.3	0.3	0.8
16:0	17.4	11.1	13.0	15.8	18.0	23.8	20.7	21.6	18.6	12.1	12.2	26.0	9.6	12.0	9.8	16.8
16:1	5.1	21.4	11.1	12.2	10.8	5.4	4.6	1.1	7.8	0.8	1.1	0.4	0.6	0.3	0.7	4.9
16:2	0.4	2.0	0.5	1.6	0.7	0.7			0.6							0.4
16:4	0.2	1.7			0.7											0.1
17:0	0.3					0.2			0.4				1.0		0.5	0.1
18:0	1.6	1.0	0.8	0.8	0.6	0.6	1.1	3.1	2.2	1.5	2.5	1.9	2.3	7.0	4.4	1.8
18:1	10.4	7.7	9.0	19.3	17.8	15.5	17.0	4.9	9.4	6.0	13.4	1.4	4.8	1.9	3.9	10.0
18:2	2.0	2.8	3.5	4.0	3.4	2.7	2.5	1.4	2.5	0.5	2.6	0.3	1.2	0.6	0.8	2.0
18:3(n-3)	24.3	14.0	21.9	21.6	23.5	24.8	26.2	10.4	28.4	27.5	22.2	27.4	24.6	10.6	20.4	23.6
18:4(n-3)	0.3			2.3	0.3	1.4	0.2		0.5							0.3
20:1	10.0	2.6	0.8	0.5	4.9	5.1	9.1	14.9	10.7	15.5	10.6	5.5	20.6	2.9	6.6	8.8
20:4(n-6)	0.5			1.6	0.2	0.7	0.3		0.7	0.6	0.3	0.3	1.4	0.5	1.0	0.5
22:1	16.5	5.1	1.0	1.6	1.8	2.5	4.7	24.4	15.1	24.9	27.7	29.8	26.1	46.2	39.9	17.4
other	5.1	6.8	6.1	5.1	4.5	3.9	4.4	10.5	0.8	3.4	4.6	4.7	5.8	17.0	10.1	5.1
Amount (mole %) of the fraction		6.3	3.3	2.4	6.9	9.9	11.9	2.6	9.2	12.4	12.0	10.1	6.0	3.3	3.8	
Amount (mole %) of the total	4.7	0.3	0.2	0.1	0.3	0.5	0.6	0.1	0.4	0.6	0.6	0.5	0.3	0.2	0.2	
Partition number																
Approximate carbon number																

while sub-fraction 3-4 was composed of 14:0-16:1-20:1 and 16:0-16:1-18:1. The most abundant sub-fractions from the saturated-dimonoenes were 3-6, 3-8 and 3-10. Fraction 3-6 might have contained molecules such as 14:0-18:1-20:1, 16:0-18:1-18:1, 16:0-16:1-20:1 and 14:0-16:1-22:1. Fraction 3-8 probably had four major components, 16:0-18:1-20:1, 16:0-16:1-22:1, 14:0-18:1-22:1 and 14:0-20:1-20:1. Fraction 3-10 appeared as a double peak in the chromatogram (Fig. 2C), perhaps because of differences in the positions of double bonds in the molecules. The possible fatty acid

TABLE 9

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 8

Fatty acid	Total	Reversed-phase HPLC fraction																Recombinant composition
		(8-1)	(8-2)	(8-3)	(8-4)	(8-5)	(8-6)	(8-7)	(8-8)	(8-9)	(8-10)	(8-11)	(8-12)	(8-13)	(8-14)	(8-15)	(8-16)	
14:0	6.5	18.5	30.1	13.1	17.0	4.5	7.1	0.9	1.7	1.5	2.5	2.4	2.1	1.2	0.9	1.4	1.0	5.8
15:0	0.8	0.5	1.1	1.9	0.9	0.7	1.5	0.4		0.4	1.1		1.5	0.3	0.3	0.3		0.7
16:0	19.7	6.2	5.8	14.4	27.8	20.7	20.5	25.8	11.5	19.8	10.8	31.0	14.1	3.2	20.2	18.8	8.3	18.1
16:1	3.7	22.4	10.0	12.1	3.3	7.9	2.2	3.0	0.4	1.5	1.0	0.3	0.8		0.2	0.4	0.2	3.0
16:2	0.2	1.3	0.8	0.3	0.3													0.1
16:4	1.3	3.7	1.6	2.6	0.6	2.4	1.9	0.6		3.0	0.8		0.5		2.2			1.0
17:0	0.4				0.1		0.5		0.6	0.5	0.5		0.6	0.9	0.3	0.5	0.4	0.3
18:0	1.7	0.6	0.2	0.2	0.3	0.5	0.7	2.5	1.5	1.5	5.1	1.3	5.2	0.7	2.9	6.6	2.5	2.0
18:1	7.7	7.9	6.2	15.9	6.2	17.3	16.7	8.7	2.6	5.5	13.4	0.6	12.1	1.9	2.2	3.0	1.0	7.9
18:2	0.7	2.3	4.2	1.7	1.1	3.4	0.7	0.5		3.7								0.7
18:3(n-3)	1.1	2.5	5.9	1.7	2.3	3.4	1.1	0.8		4.6	0.3		0.6		0.4	0.4		1.1
18:4(n-3)	21.7	27.2	18.8	26.1	18.0	11.6	26.3	15.7	28.6	10.3	19.4	28.7	8.6	29.0	5.6	10.4	22.5	21.2
20:1	7.0	0.9	1.1	2.5	3.0	5.1	10.6	8.6	15.4	4.4	10.6	2.7	6.9	28.0	6.1	7.0	5.0	8.1
20:4(n-6)	0.9	0.3	0.3	0.7	0.2	1.0	1.4	0.6	1.7	1.0	1.0	1.5	1.1	1.2	1.6	5.1	1.7	1.2
20:4(n-3)	4.0	0.8	1.2	3.6	0.7	10.5	2.9	5.2	1.2	11.4	7.1	0.7	15.4	0.8	18.8	6.4	1.7	4.3
20:5(n-3)	4.8	0.6	8.1	1.4	15.6	5.3	1.5	13.8	0.7	1.3	2.6		1.2		1.5	2.4	1.1	4.2
22:1	15.5	1.4	0.5	0.5	0.5	3.0	3.2	12.9	32.5	25.3	21.6	28.7	21.6	29.2	32.6	25.0	47.2	17.6
22:5(n-3)									0.6	0.6			0.9					0.1
other	2.2	2.8	4.0	1.5	2.1	2.6	1.1		0.9	3.9	2.1	2.1	6.6	3.7	4.4	12.5	7.4	2.6
Amount (mole %) of the fraction		2.1	4.0	6.6	8.0	3.0	10.4	11.2	8.6	2.6	12.0	12.6	2.4	5.4	3.4	3.9	3.8	
Amount (mole %) of the total	9.1	0.2	0.4	0.6	0.7	0.3	0.9	1.0	0.8	0.2	1.1	1.1	0.2	0.5	0.3	0.4	0.3	
Partition number												46		48	48		50	
Approximate carbon number		37.1	38.0	38.9	39.8		41.1	41.7 41.9	42.6		43.8	44.6		45.5	45.9	46.8	47.2-47.4	

combinations were 16:0-20:1-20:1, 14:0-20:1-22:1 and 16:0-18:1-22:1, and the relative amounts were approximately 17, 25 and 54%, respectively. As the areas of the two chromatographic peaks were nearly identical, it is reasonable to assume that 16:0-20:1-20:1 coeluted with 14:0-20:1-22:1 and 16:0-18:1-22:1 was the other peak. Fraction 3-12 was 16:0-20:1-22:1 and 14:0-22:1-22:1, mainly. Fraction 3-13 was the only sub-fraction which nearly represented a single molecular species, i.e., 16:0-22:1-22:1.

Silver ion fractions 4 (MMM), 5 (SMD), 6 (MMD) and 7 (SMT) were all minor components with proportions from 1.3 to 5.6% of the total triacylglycerols (Tables 5–8).

Most of the sub-fractions obtained from silver ion fraction 8 with reversed-phase HPLC (Fig. 2D, Table 9) had the ratio of saturated to monoenoic to tetraenoic fatty acids of approximately 1:1:1, i.e., they were SMTe species. A few peaks contained the pentaenoic acid, 20:5(n-3), and a smaller amount of monoenoic fatty acids than on average, so might have included SSP molecular species (fractions 8-2 and 8-4). Also, some of the fractions had greater monoenoic and lower saturated fatty acid concentrations and might have contained some MMTe species, e.g., fractions 8-8 and 8-13, while fraction 8-7 probably contained some SMP species. Because of the complex fatty acid compositions, it was not always easy to identify or predict particular molecular species in different fractions. The elution pattern did not simply rise to a maximum and then fall off, as in many of the other reversed-phase chromatograms, but exhibited several maxima and minima (this was also true of the next two fractions). This must be because fatty acids exist in the sub-fractions in highly specific combinations. The most abundant sub-fractions from silver ion fraction 8 were 8-7, 8-10 and 8-11, the amounts of which varied between 11.2% and 12.6% of the total. Only fraction 8-1 [mainly 14:0-16:1-18:4(n-3)], fraction 8-11 [16:0-22:1-18:4(n-3)], fraction 8-13 [20:1-22:1-18:4(n-3)] and fraction 8-16 [22:1-22:1-18:4(n-3)] had relatively simple compositions.

Those molecular species eluting in silver ion fraction 9 could be classified as mainly of the SMP type, although other kinds of species were certainly present (Table 10). Sub-fractions 9-1 and 9-2 differed from the others because of their low saturated and high tetraenoic fatty acid contents, and some other fractions had an increased hexaenoic fatty acid content. Fractions of the latter type (9-4 and 9-6) might have been composed of both SMP and SSH species. Fraction 9-9 contained MMP species as did fractions 9-12 and 9-14. Only a few sub-fractions had relatively simple compositions, i.e., fraction 9-5 [mainly 16:0-18:1-20:5(n-3)], fraction 9-7 [14:0-22:1-20:5(n-3) and 16:0-20:1-20:5(n-3)], fraction 9-10 [16:0-22:1-20:5(n-3)], fraction 9-12 [20:1-22:1-20:5(n-3)] and fraction 9-14 [22:1-22:1-20:5(n-3)].

Even though the reversed-phase chromatogram was complicated, most of the peaks of fraction 10 (Table 11) were separated nearly to the base-line. It obviously contained several types of molecular species, and only in a few sub-fractions from reversed-phase HPLC were the ratios of saturated to monoenoic to hexaenoic fatty acids approximately 1:1:1 (fractions 10-7, 10-10, 10-12, 10-14

TABLE 10

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 9

Fatty acid	Total	Reversed-phase HPLC fraction														Recombinant composition
		(9-1)	(9-2)	(9-3)	(9-4)	(9-5)	(9-6)	(9-7)	(9-8)	(9-9)	(9-10)	(9-11)	(9-12)	(9-13)	(9-14)	
14:0	7.7	0.9	2.1	8.4	13.0	6.0	2.3	12.2	8.6	1.4	0.5	3.3	0.7	1.5	1.4	2.7
15:0	0.8	0.2	0.5	1.3	1.0	1.5	0.7	0.3		1.0	0.2	0.4	0.3	0.4	0.2	0.7
16:0	20.0	4.2	6.5	18.3	25.3	23.4	32.4	19.1	17.0	8.2	25.2	21.9	10.2	13.6	11.5	20.2
16:1	4.1	10.3	7.2	12.5	5.8	1.5	5.6	0.8	2.9	0.5	0.2	0.3		0.3	0.2	2.9
16:2	0.2	0.5	0.3		0.2											0.0
16:4					0.1											0.0
17:0	0.4				0.2	0.4	0.4	0.6		0.5			1.3	0.6	0.3	0.4
18:0	2.0	1.2	0.7	0.9	0.3	1.0	1.9	2.0	2.5	6.1	1.7	2.8	1.9	14.4	3.7	2.4
18:1	7.9	4.4	11.9	15.8	11.1	20.2	8.6	2.0	4.1	14.5	1.0	3.6	1.7	1.7	0.8	8.2
18:2	0.8	8.1	4.4	1.8	1.3	0.3	1.2	0.2								0.7
18:3(n-3)	0.3	4.9	3.3		0.3									0.2		0.2
18:4(n-3)	2.6	13.9	13.2	2.5	5.9	0.8	4.6	0.4	1.6	1.1				0.3	0.4	2.3
20:1	7.5	1.9	1.5	0.6	3.2	7.8	5.4	14.0	9.8	10.9	1.4	7.0	20.2	6.1	5.4	7.3
20:4(n-6)		0.2	0.4		0.1									0.2		0.0
20:4(n-3)	0.4	0.9	2.0		0.1	0.3	0.6		3.8			0.9				0.3
20:5(n-3)	23.3	44.3	26.2	35.0	12.8	30.9	13.2	31.8	14.0	23.4	34.5	10.4	24.8	20.2	20.6	25.0
22:1	14.1	2.0	1.2		0.7	0.7	7.0	13.9	16.5	22.9	33.6	29.5	30.3	22.4	44.5	14.7
22:5(n-3)	2.0		2.5	1.0	1.5	1.7	1.9	0.8		4.8	0.6	2.1	4.9	4.0	2.2	1.9
22:6(n-3)	5.2	1.5	15.0	1.4	14.1	2.6	13.9	1.4	2.7	4.4	0.7	2.6	1.5	4.7	1.8	5.3
other	1.0	0.5	1.2	0.6	3.1	1.0	0.4	0.6	16.5	0.3	0.5	15.2	2.3	9.4	6.9	2.0
Amount (mole %) of the fraction		1.4	2.9	5.8	10.9	12.2	13.2	15.0	1.8	9.0	13.0	1.7	6.2	3.6	3.5	
Amount (mole %) of the total	14.1	0.2	0.4	0.8	1.5	1.7	1.9	2.1	0.2	1.3	1.8	0.2	0.9	0.5	0.5	
Partition number						42		44			46		48		50	
Approximate carbon number		37.1	38.0	39.2	40.0	41.1	41.9	42.6		43.8	44.6		45.5		47.4	

and 10-17). Some sub-fractions had a very small amount of saturated and an increased amount of monoenoic fatty acids as compared to the others, and these may contain MMH molecular species (fractions 10-11, 10-13, 10-15 and 10-18). Fractions 10-1, 10-2, 10-3 and 10-6 were exceptional in having lower 22:6(n-3) and higher 20:5(n-3) contents. Sub-fractions 10-11 and 10-12, as well as 10-13 and 10-14, were the largest and corresponded to 2.2 to 2.8% of the total triacylglycerols. Sub-fraction 10-11 and 10-13 were of the MMH type, and the former contained mainly 16:1-22:1-22:6(n-3) and 18:1-20:1-22:6(n-3), while the latter was 18:1-22:1-22:6(n-3) and 20:1-20:1-22:6(n-3). SMH molecular species, 14:0-22:1-22:6(n-3) and 16:0-20:1-22:6(n-3), were the main components of sub-fraction 10-12. In spite of the complicated fatty acid compositions, a few relatively simple molecular species were present: fraction 10-14 [mainly 16:0-22:1-22:6(n-3)], fraction 10-15 [20:1-22:1-22:6(n-3)], fraction 10-17 [18:0-22:1-22:6(n-3)] and fraction 10-18 [22:1-22:1-22:6(n-3)].

The last silver ion fraction (11) had a very complex composition and was not apparently simplified by HPLC in the reversed-phase mode (Table 12, Fig. 2E). No simple means of identifying the components of this fraction was possible. It had on average 10 double bonds per molecule and all the molecular species eluted at the beginning of the reversed-phase chromatogram. Better results might have been obtained if more fractions had been collected in the appropriate region of the silver ion chromatogram for separate analysis by reversed-phase HPLC.

Herring oil triacylglycerols are so complex in their molecular structure that the amounts of the most abundant fractions were less than 5% of the total. All of the sub-fractions in reversed-phase HPLC obtained from the silver ion fractions 1, 5, 6 and 7 corresponded to less than 1% of the total, and even the sub-fractions from the larger silver ion fractions 4 and 8 were less than 2% of the total triacylglycerols. The most abundant molecular species were found in the saturated-dimonoenes, where the proportions of sub-fractions 3-6, 3-8 and 3-10 were 4.1, 3.9 and 2.9%, respectively of the total, although these each contained many different components, as discussed above. The SMH sub-fraction 10-14 was the largest polyunsaturated one (2.8%) that could almost be represented by a single molecule.

It is evident that combining silver ion and reversed-phase HPLC techniques in this way can greatly simplify the analysis of complex natural triacylglycerol samples, such as those of fish oils. Even greater simplification might have been achieved by taking narrower silver ion fractions for further analysis. Many fractions remained rather complex and the detailed compositions of their molecular species have still to be determined. It would also be advantageous if the positional distributions of fatty acids on the glycerol moiety for each of the fractions could be obtained by stereospecific analysis procedures. In biochemical terms, the compositions of the molecular species are determined by the specificities of the acyltransferases responsible for esterifying each position of

TABLE 11

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 10

		Reversed-phase HPLC fraction																		Recombinant
Fatty acid	Total	(10-1)	(10-2)	(10-3)	(10-4)	(10-5)	(10-6)	(10-7)	(10-8)	(10-9)	(10-10)	(10-11)	(10-12)	(10-13)	(10-14)	(10-15)	(10-16)	(10-17)	(10-18)	(10-19)	composition
14:0	6.2	7.8	1.0	6.8	10.1	13.2	3.4	7.4	0.9	2.8	6.2	0.9	10.4	0.8	0.7	1.0	2.9	0.8	0.8	2.5	3.7
15:0	0.5	0.5	0.5	0.2		0.8	0.8	0.4		1.0	0.2	0.7		1.5		0.2				0.4	0.4
16:0	12.5	11.1	6.7	8.6	8.1	4.0	4.4	16.9	5.4	7.7	19.2	4.7	13.3	1.8	31.2	3.5	30.3	4.0	3.0	11.7	11.7
16:1	6.0	8.1	8.9	18.0	19.7	6.7	16.5	10.1	2.4	9.0	1.9	12.5	1.5	0.6	0.3	0.4	0.8	0.2	0.3	1.3	5.0
16:2	0.4	4.4	2.1	1.8	1.6	0.7	0.7	0.2		0.7											0.2
16:4	0.3	1.5		0.3																	0.0
17:0	0.2					0.3				0.3		0.3		0.4		1.2				0.4	0.2
18:0	1.4	1.5	1.0	0.4	0.8	0.7	0.5	0.5	0.7	0.3	1.5	0.5	2.0	0.4	1.6	0.3	4.9	26.3	0.6	5.7	1.4
18:1	11.9	7.2	12.1	4.4	4.5	7.9	22.9	16.7	15.4	28.0	20.9	12.9	2.9	22.9	1.3	2.2	7.4	1.3	1.3	5.7	11.7
18:2	1.5	2.2	11.7	8.5	3.9	7.0	3.3	6.3	3.9	1.4	0.7	2.1	0.3	0.4						1.2	1.6
18:3(n-3)	1.0	12.0	13.1	6.8	3.0	5.7	2.1	1.1	9.9	0.5	1.3	0.2	0.2							0.3	1.1
18:4(n-3)	1.1	15.0	14.0	7.6	1.1	9.6	2.6	0.5	11.6	0.8	0.3	0.5									1.1
20:1	7.7	2.8	1.3	1.2	0.6	5.6	1.6	1.1	5.1	8.7	11.7	13.4	14.2	12.0	2.0	27.8	4.1	6.2	4.5	4.1	9.7
20:4(n-6)			0.5	0.3		0.3	0.2														0.0
20:4(n-3)	0.2	0.7	2.4	0.5	1.2	0.7	0.6	0.4	0.7		0.3										0.2
20:5(n-3)	3.0	9.3	12.1	14.9	4.1	4.4	14.9	3.4	2.8	10.8	0.8	6.3	0.5	1.4	0.1	0.6					3.5
22:1	17.8	4.1	1.8	4.4	2.7	4.2	4.1	1.2	13.7	3.1	1.5	17.5	19.2	26.2	29.1	30.8	7.3	31.0	56.5	27.8	17.7
22:5(n-3)	0.6		0.8	0.3		1.5	0.5		1.9		0.8	0.3	1.1			0.9	1.1	2.2		0.7	0.5
22:6(n-3)	25.7	8.3	10.1	14.1	38.6	25.7	19.1	33.0	24.5	23.7	32.4	26.6	33.6	31.4	32.8	31.1	25.0	27.8	31.0	23.6	29.1
other	1.7	3.4		1.0		1.0	1.8	0.3	1.3	1.2	0.3	0.6	0.8	0.5		1.0	16.3		1.9	14.5	1.0
Amount (mole %) of the fraction		1.4	0.9	2.2	1.3	1.4	4.1	5.5	1.7	7.5	9.4	13.1	12.8	10.7	13.7	7.7	0.7	1.7	3.2	1.2	
Amount (mole %) of the total	20.4	0.3	0.2	0.4	0.3	0.3	0.8	1.1	0.3	1.5	1.9	2.7	2.6	2.2	2.8	1.6	0.1	0.3	0.6	0.2	
Partition number							40			42	42	44	44	46	46	48		48	50		
Approximate carbon number		34.8	36.0	36.8	37.3	38.0	38.5 38.7	39.2	40.0	40.4	41.1	42.1	42.9	44.0 44.2	44.7	45.9	46.4	46.8	47.6	49.1	

TABLE 12

Fatty Acid Compositions (Mole % of the Total Fatty Acids), Proportions (Mole % of the Fraction and of the Total), Partition Numbers and Approximate Carbon Numbers of Triacylglycerol Species Obtained by Reversed-Phase HPLC from Silver Ion HPLC Fraction 11

Fatty acid	Total	Reversed-phase HPLC fraction								Recombinant composition
		(11-1)	(11-2)	(33-3)	(11-4)	(11-5)	(11-6)	(11-7)	(11-8)	
14:0	4.9	5.0	12.8	3.6	1.8	0.6	0.5	0.2	0.2	3.8
15:0	0.4		0.4	0.7	0.2	0.1		0.1		0.4
16:0	9.3	3.2	1.5	15.5	12.4	5.1	2.2	4.6	1.9	8.6
16:1	3.2	4.0	10.6	1.5	1.4	0.6	0.5	0.7	0.4	2.7
16:2	0.6	4.5	0.5	0.3	0.6	0.3	1.0			0.5
16:4	0.8	5.6	1.4	0.4	1.0	0.1				0.7
17:0	0.2				0.5	0.2				0.1
18:0	0.9	0.8			1.0	2.4	0.8	2.0	0.6	0.8
18:1	5.3	0.9	1.3	11.0	4.4	2.4	1.1	4.6	2.2	5.2
18:2	2.3	1.2	3.2	0.7	1.5	2.3	2.9	1.1	19.5	2.2
18:3(n-3)	4.7	13.9	3.7	3.8	5.8	3.5	12.2	4.4	2.3	4.8
18:4(n-3)	13.2	20.8	17.3	12.8	10.5	16.4	8.6	5.9	1.2	13.3
20:1	3.1	0.4		0.6	12.3	4.7	4.0	3.5	2.6	3.7
20:4(n-6)	0.6	1.0	0.3	0.3	1.1	0.6	0.7	3.3	0.5	0.6
20:4(n-3)	2.7	2.5	1.9	1.7	4.1	1.8	6.3	3.5	1.0	2.5
20:5(n-3)	17.5	21.1	23.0	23.4	16.6	16.3	8.0	10.1	1.8	18.6
22:1	6.8				1.0	17.3	25.4	19.1	27.6	6.9
22:5(n-3)	1.3		0.7	0.9	2.0	1.1	2.7	2.7	0.7	1.3
22:6(n-3)	19.7	10.9	18.9	21.3	20.3	21.6	21.5	24.2	32.0	21.0
other	2.8	4.0	2.5	1.6	1.6	2.5	1.6	10.1	5.5	2.3
Amount (mole %) of the fraction		1.8	16.7	31.1	16.8	20.9	7.3	2.9	2.4	
Amount (mole %) of the total	12.1	0.2	2.0	3.8	2.0	2.5	0.9	0.4	0.3	
Partition number										
Approximate carbon number		30.0–32.2	32.2–34.4	34.4–36.5	36.5–37.7	37.7–39.6	39.6–40.7	40.7–41.8	41.8–43.2	

the triacyl-*sn*-glycerol molecules. Such analyses on fish oils are, however, technically daunting and the sheer magnitude of the task with such a large number of subfractions means that it cannot be contemplated at present.

With existing technology, it would appear that HPLC linked to mass spectrometric detection would afford greater opportunities to identify the difficult fractions, especially if combined with the HPLC separation procedures described here. With simpler triacylglycerol samples, the methods used in this research can lead to comprehensive analyses.

REFERENCES

1. Litchfield, C., *Analysis of Triglycerides,* Academic Press, New York, 1972.
2. Bottino, N., *J. Lipid Res. 12*:24 (1971).
3. Dolev, A., and H.S. Olcott, *J. Am. Oil Chem. Soc. 42*:1046 (1965).
4. Christie, W.W., *J. High Res. Chromatogr. Chromatogr. Commun. 10*:148 (1987).
5. Christie, W.W., *J. Chromatogr. 454*:273 (1988).
6. Laakso, P., W.W. Christie and J. Pettersen, *Lipids 25*:284 (1990).
7. Christie, W.W., *High-Performance Liquid Chromatography and Lipids,* Pergamon Press, Oxford, 1987.
8. Ratnayake, W.M.N., D.G. Matthews and R.G. Ackman, *J. Am. Oil Chem. Soc. 66*:966 (1989).
9. Takano, S., and Y. Kondoh, *Ibid. 64*:380 (1987).
10. Myher, J.J., A. Kuksis and L. Marai, *J. Chromatogr. 452*:93 (1988).
11. Lund, P., *Milchwissenschaft 43*:159 (1988).
12. Wada, S., C. Koizumi, A. Takiguchi and J. Nonaka, *Nippon Suisan Gakkaishi 45*:611 (1979).
13. Takeuchi, T., and R.G. Ackman, *Ibid. 53*:2249 (1987).
14. Wada, S., C. Koizumi, A. Takiguchi and J. Nonaka, *Ibid. 45*:615 (1979).
15. Christie, W.W., *J. Lipid Res. 23*:1072 (1982).

[Received August 21, 1990; accepted January 15, 1991]