Capillary Supercritical Fluid Chromatographic Analysis of Shark Liver Oils

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ABSTRACT: The liver oils of six different shark species have been analyzed by supercritical fluid chromatography (SFC). The liver oils were from the species *Pseudotriakis microdon* (False catshark), *Centroscymnus coelepsis* (Portuguese dogfish), *Centrophorus squanosus* (Leafscalp gulper shark), *Deanea calceus* (Birdbeak dogfish), *Etmopterus princips* (Greater lantern shark), and *Centroscymnus crepidater* (Longnose velvet dogfish). The method was capable of direct quantitation of squalene and cholesterol, while quantitation of triacylglycerols, cholesterol esters, and diacylglycerol ethers required thin-layer chromatographic fractionation prior to SFC analysis. The iodine values of the liver oil samples gave a linear correlation when plotted against the squalene content found by SFC. The variation of squalene content within one shark species is large, and there are large differences in squalene content from species to species. The squalene contents varied between 0.22 and 82.54 wt%. The identity of the glycerol ethers was investigated by SFC of the unsaponifiable matter. The major glycerol ethers contained chimyl, batyl, and selachyl alcohol.
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KEY WORDS: Diacylglycerol ether, glycerol ethers, iodine value, marine oil, shark liver oil, squalene, supercritical fluid chromatography, thin-layer chromatography, triacylglycerols, unsaponifiable matter.

Liver oils of deep-sea sharks are of great interest because these marine oils contain squalene and diacylglycerol ethers at high levels. Squalene, or 2,6,10,15,19,23-hexamethyl-tetracosa-hexane, is an important raw material in many industries, including the pharmaceutical, rubber, and surfactants industries (1). Moreover, squalene is easily hydrogenated into squalane, which is used as skin lubricant in the cosmetic industry and as a carrier of lipid-soluble drugs (1). Hydrogenated liver oils of some shark species are sold as technical-grade squalane. Diacylglycerol ethers or 1-alkyl-2,3-diacylglycerols are lipids with an alkyl group linked to the 1-position in glycerol by an ether linkage and two acyl groups linked to the 2- and 3-position by ester linkages. These lipids are believed to have beneficial effects on health because of the similarities to plate-activating factors (2). In nature, the most frequently occurring alkyl groups are monopalmityl, monostearyl, and monooleyl (3). The ratios of liver to body-weight and oil to liver weight are normally high for sharks, which makes them good sources for squalene and diacylglycerol ethers. The weight of a shark liver is, however, not constant, and there are large variations in the squalene content within the same shark species.

The reason why deep-sea sharks have large, oily livers with high levels of squalene and diacylglycerol ethers is still unclear, but it is believed that buoyancy is part of the explanation (4) because the shark family is one of the early animal families and its members do not have swim bladders like members of the later evolved fish families. Squalene has a low density (0.86 g/mL) compared to the density of a normal fish liver oil (typically 0.92 g/mL).

Until now, most characterization of shark liver oils and liver oils from other marine animals with high squalene and diacylglycerol ether levels has been performed by measuring physical parameters, such as density, refractive index, and viscosity (5), or by chemical methods, such as iodine value (IV) and unsaponifiable matter (5). IV and unsaponifiable matter are calculated for the whole liver oil, but these methods are not exact measures of the squalene content because unsaturated fatty acids contribute to the IV, and diacylglycerol ethers contribute to the unsaponifiable matter. Chromatographic techniques have also been applied. The saponifiable part of shark liver oils has been analyzed by gas chromatography (GC) as fatty acid methyl esters (FAME) (6–9). Thin-layer chromatography (TLC) has been used to recover lipid groups for high-temperature GC analysis (6,8). Analysis of the nature of the alkyl groups in these oils has been done by means of trimethylsilyl (TMS) derivatives of glycerol ethers recovered as the unsaponifiable matter of the oils (6,8,10). A review with 152 references on chromatographic methods for the analysis of diacylglycerol ethers has been published (11).

In this work, we have studied the composition of liver oils from six different species, *Pseudotriakis microdon* (False catshark), *Centroscymnus coelepsis* (Portuguese dogfish), *Centrophorus squanosus* (Leafscalp gulper shark), *Deanea calceus* (Birdbeak dogfish), *Etmopterus princips* (Greater lantern shark), and *Centroscymnus crepidater* (Longnose velvet dog-

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fish) by supercritical fluid chromatography (SFC). The intact oils were analyzed to determine the content of squalene, cholesterol, and vitamins and to get a fingerprint of each shark species. To check on the content of squalene, the IV for each shark oil was determined and compared to the SFC results. The SFC method, however, was not capable of separating the triacylglycerols, diacylglycerol ethers, and cholesterol esters. By preparative TLC, we individually recovered these three fractions and, by using an internal standard, it was possible to calculate the percentage of triacylglycerols, diacylglycerol ethers, cholesterol esters, and squalene in each liver oil. To check on the recovery, the squalene content was compared to the content found by analysis of the intact liver oils. To learn about the origin of the glycerol ethers, we carried out SFC on the unsaponifiable matter of the squalene-free oil samples. With this technique, it was not necessary to convert the polar glycerol ethers into TMS derivatives.

EXPERIMENTAL PROCEDURES

Shark oils. The shark liver oils used in this work were obtained from sharks caught during spring 1994 in the Nordic Sea. After landing the sharks, the livers were removed, weighed, and pressed. The shark liver oils were stored at −18°C during the working period. Table 1 shows the characteristics of the six shark species.

Chemicals and gases. Carbon dioxide (99.995%), atmospheric air, hydrogen (99.95%), helium (99.995%), and nitrogen (99.998%) were obtained from Hede Nielsen (Ballerup, Denmark). *n*-Heptane, LichroSolv, chloroform (99.4%), diethylether (99.5%), and acetic acid (100%) were obtained from Merck (Darmstadt, Germany). Petroleum ether was obtained from Mallinckrodt (Paris, KY). The standards of FAME (99.9%), triacylglycerols (99.9%), and cholesterol esters (99.9%) were obtained from Nu-Chek-Prep (Elysian, MN). The standard of squalene (99.0%) was obtained from Sigma Chemical Co. (St. Louis, MO), and glycerol ether standards were obtained from Doosan/Serdary (Enlewood, NJ). All materials were used without further purification.

Apparatus. The SFC used for the analysis of the raw liver oils was a Carlo Erba SFC3000 system (Carlo Erba, Milan, Italy), equipped with a flame-ionization detector (FID). Carbon dioxide was used as the mobile phase. Injections were performed manually by a time split injection method. The injection time was 0.2 s in all experiments. The pressure drop over the system was maintained by integral restrictors nominally rated at 1 mL/min (J&W Scientific, Folsom, CA). The cylinder pump was cooled by circulating ethylene glycol at −5°C. The injection port was thermostatted at 60°C. Control of pump and oven was effected by means of the SFC3000 software (Carlo Erba), and acquisition of data as well as integration of peaks was achieved by means of the software Baseline 810 (Waters, Milford, MA).

The SFC used for analysis of the TLC fractions and of the unsaponifiable matter was an HP-SFC (Hewlett-Packard, Avondale, PA), equipped with FID. The SFC was operated in the upstream mode, meaning that pressure control was performed upstream to the column. Carbon dioxide was used as the mobile phase. Injections were performed by an HP-7673 autosampler, and a flow split method was used. The pressure drop over the system was maintained by frit restrictors (Dionex, Sunnyvale, CA). The flow rate of the expanded carbon dioxide, measured at the end of the column, was 1 mL/min. The split ratio was 1:100.

Peak identification and quantitation. In all SFC methods, peak identification was effected by a comparison of chromatograms with those of relevant standard compounds. In case of doubt, the samples were spiked with standard components and re-analyzed. Identification of bands on the TLC plates was achieved by applying standards that were representative of each lipid group on the plate, together with a shark liver oil sample, and comparing the position of the bands with bands of the sample. Standards of diacylglycerol ethers were unavailable, and only a limited number of glycerol ethers could be purchased.

Determination of IV. IV of the shark liver oils were determined according to the official AOAC method (12).

SFC analysis of intact shark liver oils. The shark liver oils were dissolved in *n*-heptane, Lichrosolv, to a concentration of 75 mg/mL and injected to the Carlo Erba SFC. The oven temperature during the analysis was 170°C. The density was raised from 0.3 g/mL to 0.452 g/mL at a rate of 0.004 g/mL/min, and after a constant density of 0.452 g/mL/min for 14 min, raised to 0.52 g/mL at a rate of 0.001 g/mL/min. The final density of 0.52 g/mL was kept constant for 40 min. Start and end densities correspond to 20.3 and 36.2 MPa, respectively. The total analysis time was 140 min. The column used for these analyses was a DB-5 (J&W Scientific), 5% phenyl

TABLE 1
Characteristics of the Shark Liver Oils[a]

Species	Total weight (kg)	Liver (%) (kg liver/kg shark)	Oil (%) (kg oil/kg liver)	Squalene (%) (kg sq/kg oil)
Pseudotriakis microdon	51.5	23.2	73.5	0.3
Centroscymnus coelepsis	10.2	25.6	77.6	48.7
Centrophorus squasmus	11.7	18.1	77.2	79.6
Deanea calceus	2.3	20.3	84.3	65.2
Etmopterus princeps	2.2	20.5	73.5	36.3
Centroscymnus crepidater	2.0	20.0	82.1	76.3

[a]All liver oils are sample number 1; sq = squalene.

(95% dimethylpolysiloxane) fused-silica capillary column with a length of 20 m, an inner diameter of 100 µm and a film thickness of 0.1 µm.

Preparative TLC and capillary SFC of shark liver oils. Each shark liver oil was dissolved in *n*-heptane to a concentration of 1 g/mL. The samples were applied to Silica 60 TLC plates (Merck). The plates measured 20 cm × 20 cm. Each sample was applied to two plates to ensure enough sample output: 75 µL was added to each plate corresponding to a load of 3.75 mg per centimeter of plate. The elution solvent system was petroleum ether/diethyl ether/acetic acid mixed in the ratio 85:15:1.5 (vol/vol/vol). When the solvent front was 0.5 cm from the top of the plates, they were taken out of the tank and allowed to dry for 5 min. A smaller plate of 5 cm × 20 cm was treated likewise, but after elution, it was sprayed with a 5 vol% sulfuric acid in methanol solution, followed by charring to detect the retention factors of the different bands. The elution order of the shark liver oil components in this developing system was from the bottom: free fatty acids, cholesterol, triacylglycerols, diacylglycerol ethers, and at the top, squalene coeluted with the cholesterol esters. From the large plates, the triacylglycerol, diacylglycerol ether and squalene/cholesterol ester fractions were individually recovered by scraping off the bands. The silica material with the sample was placed in a screw-cap vial, and 4.5 mL of chloroform was added. Addition of chloroform seemed to prevent formation of emulsions. After extraction with chloroform, two times 4.5 mL of hexane was added, and the silica was extracted. After each addition of solvent, the vial was capped well and shaken. The rest of the silica material was transferred to a filter by means of 2 mL hexane. Solvents were completely evaporated from the combined extracts by a stream of nitrogen. The residue was dissolved in 0.5 mL heptane with an internal standard. The standard was wax ester 16:0-16:0 at a concentration of 6.58 mg/mL heptane. The samples were immediately injected on the HP-SFC. Analysis conditions and column were the same as in the analysis of the raw shark liver oils.

Preparation and analysis of glycerol ethers. Preparation and analysis of glycerol ethers could be carried out with the TLC fraction of the diacylglycerol ethers, described above. In this work, however, we wanted to ensure a larger amount of sample for saponification, followed by SFC analysis of the unsaponifiable matter. Therefore, we prepared a new TLC fraction that contained triacylglycerols and diacylglycerol ethers. Separation of these two lipid groups from the squalene has the advantage that interference from squalene and overloading of the SFC column with this component during analysis is avoided. Removal of the squalene/cholesterol ester fraction was performed by a method similar to the previously described TLC method but with a different solvent system. In this method, the system was petroleum ether/diethyl ether/acetic acid mixed in the ratio 90:10:1 (vol/vol/vol). The advantage of this solvent system for this particular case was that triacylglycerols, cholesterol, and diacylglycerol ethers eluted together in one band at the bottom of the plate, while squalene and the cholesterol esters eluted near the top of the plate. In this way, we could run the TLC plates with a higher load. The amount of sample applied to the plates was 7.5 mg per centimeter plate. After the removal of squalene and cholesterol esters, determination of the identity of the glycerol ethers in the shark liver oils was effected by isolating the unsaponifiable matter as described in an AOCS Official Method (13). By this method, triacylglycerols and the two acylglycerol groups in the diacylglycerol ethers are converted to FAME by saponification and transmethylation, while the glycerol ether part of the diacylglycerol ethers is not affected by the treatment. Normally, the next step would be a TMS derivatization of the hydroxy groups on the 2,3-positions, followed by gas-chromatographic analysis. However, polar column SFC provides the possibility to analyze the unsaponifiable extract directly. The dried unsaponifiable extract was dissolved in 0.5 mL hexane and injected on the SFC. The capillary column was a polar 50%-cyanopropylphenyl-50%-methylpolysiloxane, DB-225 (20 m × 100 µm × 0.4 l µm) column (J&W Scientific). The oven temperature was 170°C. The density was raised from 0.3 g/mL to 0.4 g/mL at a rate of 0.002 g/mL/min. Total analysis time was 50 min.

RESULTS AND DISCUSSION

SFC analysis of intact shark liver oils. Table 2 reports compositions of the raw liver oils as found by SFC. Figures 1 and 2 show chromatograms of the liver oils from *Pseudotriakis microdon* and *Etmopterus princeps*. Free fatty acids, vitamin E, squalene, cholesterol, triacylglycerols, cholesterol esters, and diacylglycerol ethers are eluted by this method, making it possible to determine the content of free fatty acids, squalene, vitamin E, and cholesterol. The triacylglycerols, cholesterol esters, and diacylglycerol ethers co-elute by this method. The composition of the oils, shown in Table 2, is the result of duplicate determinations. The fingerprints of the two shark oils show the differences that can occur from species to

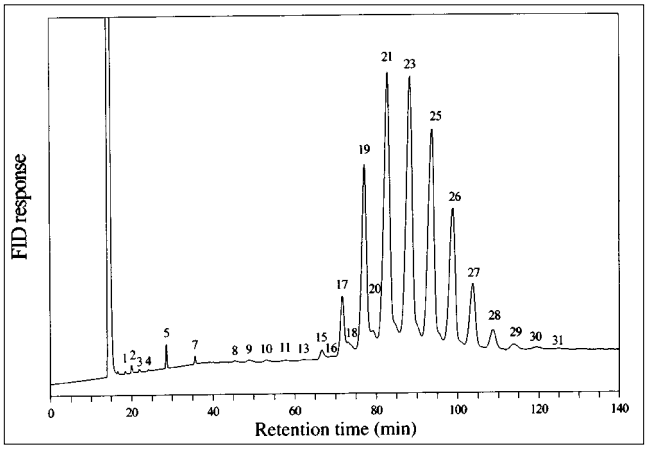

FIG. 1. Capillary supercritical fluid chromatography of the liver oil from *Pseudotriakis microdon*. See text for analysis conditions. Peaks as in Table 2; FID, flame-ionization detector.

TABLE 2
The Composition of the Shark Liver Oils in Weight Percentage as Found by Capillary SFC of the Raw Oil

Peak number	Compound	*Pseudotriakis microdon*	*Centroscymnus coelepsis*	*Centrophorus squasmus*	*Deanea calceus*	*Etmopterus princeps*	*Centroscymnus crepidater*
1	FFA_{14}	0.03	0.00	0.00	0.00	0.00	0.00
2	FFA_{16}	0.05	0.00	0.00	0.00	0.00	0.00
3	FFA_{20}	0.13	0.00	0.00	0.00	0.00	0.00
4	FFA_{22}	0.05	0.00	0.00	0.00	0.00	0.00
5	Squalene	0.26	48.67	79.58	65.16	36.28	76.28
6	Vitamin E	0.00	0.06	0.03	0.03	0.04	0.02
7	Cholesterol	0.10	0.12	0.09	0.09	0.10	0.04
8	DAG_{34}	0.09	0.00	0.00	0.00	0.00	0.00
9	DAG_{36}	0.16	0.00	0.00	0.00	0.00	0.00
10	$TAG_{42} + DAGE_{42}$	0.12	0.00	0.00	0.00	0.00	0.00
11	$TAG_{44} + DAGE_{44}$	0.07	0.00	0.00	0.00	0.00	0.00
12	$TAG_{45} + DAGE_{45}$	0.00	0.03	0.00	0.00	0.00	0.00
13	$TAG_{46} + DAGE_{46}$	0.06	0.07	0.02	0.00	0.10	0.00
14	$TAG_{47} + DAGE_{47}$	0.00	0.05	0.00	0.00	0.00	0.00
15	$TAG_{48} + DAGE_{48}$	0.44	0.77	0.40	0.37	0.62	0.36
16	$CE_{14} + TAG_{49} + DAGE_{49}$	0.16	0.28	0.16	0.14	0.25	0.13
17	$TAG_{50} + DAGE_{50}$	3.08	3.30	1.93	2.50	2.57	1.87
18	$CE_{16} + TAG_{51} + DAGE^{51}$	0.99	1.96	1.40	1.27	1.67	0.32
19	$TAG_{52} + DAGE_{52}$	12.09	9.01	4.11	7.50	6.91	4.75
20	$CE_{18} + TAG_{53} + DAGE_{53}$	1.33	1.40	0.74	0.96	1.90	0.00
21	$TAG_{54} + DAGE_{54}$	22.19	7.98	3.00	5.72	8.33	3.20
22	$CE_{20} + TAG_{55} + DAGE_{55}$	0.00	1.36	0.83	0.80	1.91	0.40
23	$TAG_{56} + DAGE_{56}$	22.16	8.81	2.81	4.91	11.32	4.45
24	$CE_{22} + TAG_{57} + DAGE_{57}$	0.00	0.00	0.00	0.45	0.00	0.37
25	$TAG_{58} + DAGE_{58}$	17.45	7.36	2.14	3.70	10.83	4.41
26	$TAG_{60} + DAGE_{60}$	10.66	4.31	1.21	2.59	7.77	1.82
27	$TAG_{62} + DAGE_{62}$	5.05	2.32	0.84	1.68	5.66	0.90
28	$TAG_{64} + DAGE_{64}$	1.87	1.04	0.38	0.95	2.45	0.38
29	$TAG_{66} + DAGE_{66}$	0.77	0.53	0.22	0.47	0.86	0.19
30	$TAG_{68} + DAGE_{68}$	0.47	0.35	0.11	0.39	0.29	0.08
31	$TAG_{70} + DAGE_{70}$	0.16	0.13	0.00	0.13	0.14	0.04

[a]All liver oils are sample number 1. FFA, free fatty acid; DAG, diacylglycerol; TAG, triacylglycerol; DAGE, diacylglycerol ether. Subscripted numbers refer to the total number of carbon atoms in the acyl groups; SFC, supercritical fluid chromatography.

species. The peaks appearing between the triacylglycerol/diacylglycerol ether peaks in Figure 2 were also observed in the other liver oils.

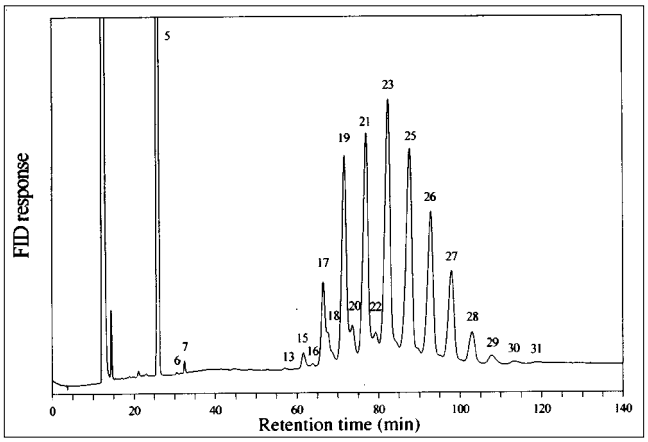

FIG. 2. Capillary supercritical fluid chromatography of the liver oil from *Etmopterus princeps*. See text for analysis conditions. Peaks as in Table 2. See Figure 1 for abbreviation.

As more samples of the same shark species became available to us, we investigated the variation in squalene content for the six different species by SFC analysis of the raw oils. The results are presented in Table 3, which shows that the variation in squalene content within the same species can be large.

Squalene content. Figure 3 shows a plot of weight percentage squalene, found by SFC, vs. IV, which is a measure of the number of double bonds in the sample. The IV of pure squalene is included (4). The graph shows that there is an almost linear correlation between the IV and the squalene content found by SFC. The line does not go through the origin because of the double bonds present in oil components other than squalene. This means that the IV cannot be used to determine the accurate content of squalene in a shark liver oil, but the method is a good indicator of the squalene level in an oil. From the shark liver oil samples analyzed in this work, there is a simple correlation between the IV and the mass fraction of squalene, *W*:

$$IV = 70(1 - W) + 360W) \quad [1]$$

thus the weight percentage of squalene, wt% sq, can be

TABLE 3
The Squalene Content in the Shark Liver Oils as Found by SFC of the Raw Liver Oils[a]

Species	Sample number				
	1	2	3	4	5
Pseudotriakis microdon	0.26 ± 0.1	0.22 ± 0.1			
Centroscymnus coelepsis	48.67 ± 0.1	37.14 ± 0.1	40.57 ± 0.1	52.79 ± 0.1	52.17 ± 0.1
Centrophorus squasmus	79.58 ± 0.2	69.56 + 0.2	78.29 ± 0.2	82.54 ± 0.2	63.74 ± 0.2
Deanea calceus	65.16 ± 0.2	73.87 ± 0.2			
Etmopterus princeps	36.28 ± 0.1	31.77 ± 0.1			
Centroscymnus crepidater	76.28 ± 0.2				

[a]The composition of sample 1 of each species is given in Table 3. See Table 2 for abbreviation.

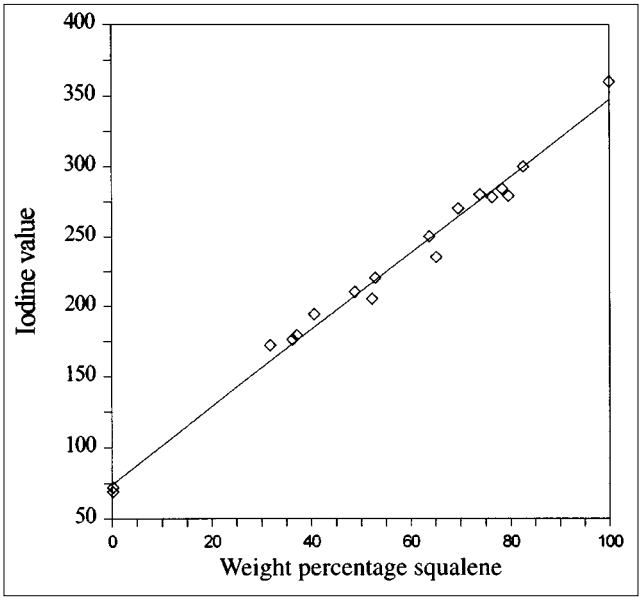

FIG. 3. Weight percentage of squalene found by capillary supercritical fluid chromatography vs. iodine value of the shark liver oils from six different species.

found as:

$$\text{wt\% sq} = [(\text{IV} - 70)/(2.90)] \quad [2]$$

Preparative TLC of shark liver oils. Results of the SFC analysis of the three TLC fractions are shown in Figure 4 for the liver oil of *Etmopterus princeps*. The internal standard is wax ester 16:0-16:0. The retention time scale is the same for all chromatograms, and they can be compared directly. This comparison shows that the triacylglycerols and diacylglycerol ethers have similar retention times, which will result in a larger peak in the chromatogram of the intact oil (Fig. 2). The cholesterol esters have retention times between the triacylglycerols and diacylglycerol ethers, which means that these components cause the shoulders, which were also seen in the chromatogram of the intact oil (Fig. 2). Table 4 gives the content of triacylglycerols, diacylglycerol ethers, cholesterol esters, and squalene in the six shark liver oils, based on the TLC fractions. It is remarkable that much of the cholesterol in the shark liver is present as cholesterol ester. The squalene content found by this method should match the content found by SFC of the intact liver oils. For *Etmopterus princeps,* the deviation between these two determinations is as high as 10%, but generally, the deviation is below 2%, which means that the preparative TLC method is quite quantitative. Table 4 also gives the number of TLC determinations performed. Table 4 shows that there is no relation between the content of squalene and the content of diacylglycerol ethers in the oils.

Analysis of unsaponifiable matter. The chromatogram from the analysis of the unsaponifiable matter of shark liver oil from *Etmopterus princeps* is shown in Figure 5. As only three different standards of glycerol ethers, 1-monostearyl glycerol ether, 1-monopalmitoyl glycerol ether and 1-monooleyl glycerol ether or batyl, selachyl and chimyl alcohol, were available to us, these glycerol ethers are the only ones identified on the chromatogram. However, more than these three typical glycerol ethers are present in the samples but at low concentrations. Cholesterol is also part of the unsaponifiable matter. It should be stressed that the unsaponifiable mat-

TABLE 4
The Content of Squalene, TAG, Diacylglyceryl Ethers (DAGE), and Cholesteryl Esters (CE)[a]

Species	Squalene (TLC–SFC)	TAG (TLC–SFC)	DAGE (TLC–SFC)	CE (TLC–SFC)	Squalene (SFC)	n^b
Pseudotriakis microdon	0.3	33.9	64.2	1.5	0.3	1
Centroscymnus coelepsis	49.7	12.7	32.7	4.8	48.7	2
Centrophorus squasmus	78.6	6.3	12.1	2.9	79.6	1
Deanea calceus	67.9	4.9	25.1	2.2	65.2	2
Etmopterus princeps	40.9	12.5	39.8	6.8	36.3	3
Centroscymnus crepidater	77.6	2.9	19.3	0.2	76.3	2

[a]In the shark liver oils found by preparative thin-layer chromatography (TLC)–SFC. The squalene content found by SFC of the raw liver oil is included for comparison. All liver oils are sample number 1.
[b]*n,* Number of determinations performed. See Table 2 for other abbreviations.

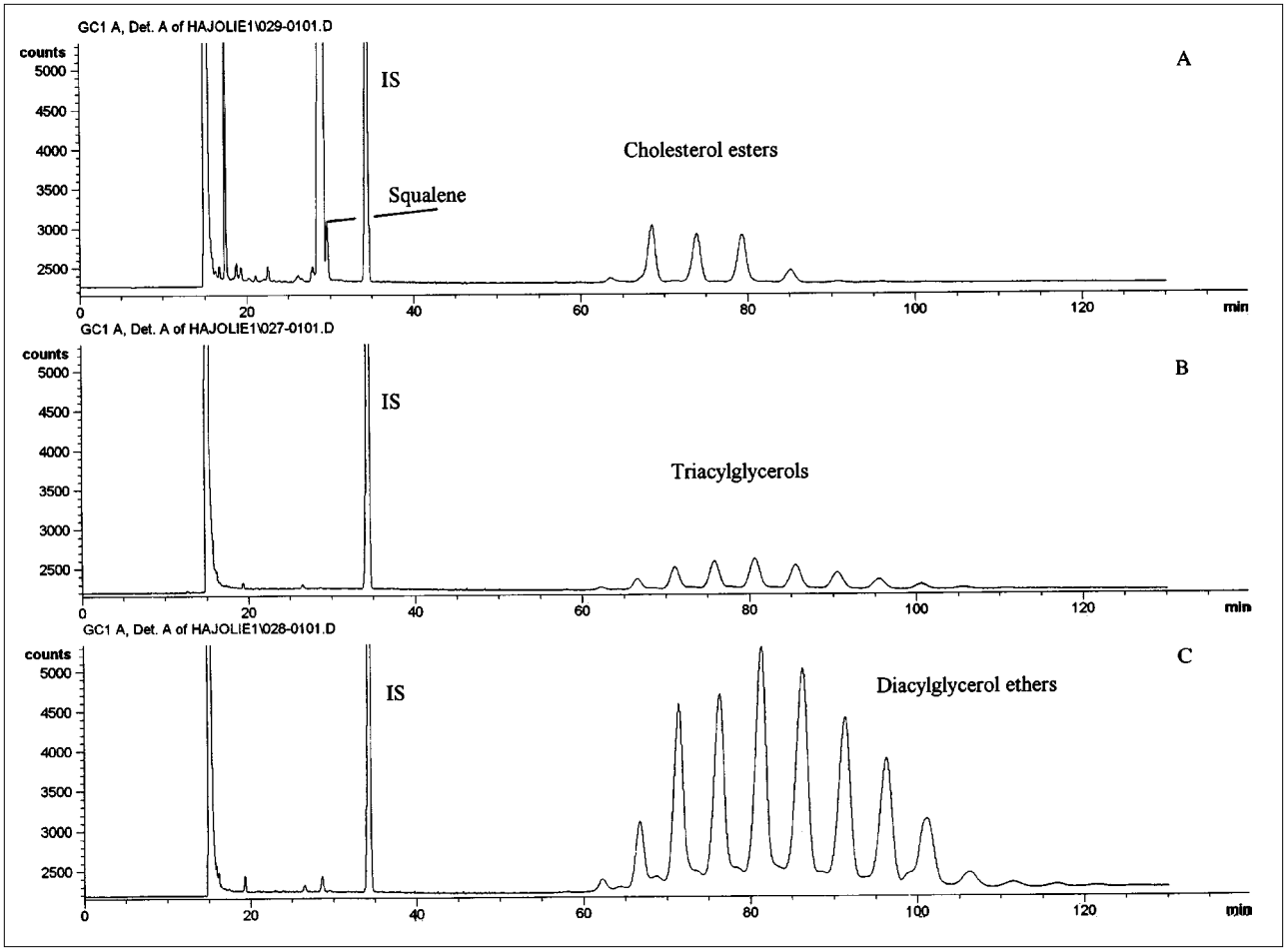

FIG. 4. Capillary supercritical fluid chromatography of the thin-layer chromatography fractions from the liver oil of *Etmopterus princeps.* A, Squalene and cholesterol esters; B, triacylglycerols, and C, diacylglycerol ethers. See text for analysis conditions.

ter is isolated after removing the squalene from the sample by TLC. Otherwise, squalene would have been present in the sample in such high concentrations that the column would have been overloaded.

The results of this work have shown that the industrially important component squalene is present at high levels in some shark species, and that the variations in squalene content within the single species can be large. In the samples analyzed, there is a simple correlation between the IV and the squalene content. The biologically interesting diacylglycerol ethers are found in all shark species analyzed, but their content varies widely. Though the content of free cholesterol in shark liver may seem low, much cholesterol is present in the liver oils as cholesterol esters. The nature of the alkyl groups in the diacylglycerol ethers, studied by analysis of the unsaponifiable matter, is primarily batyl, chimyl and selachyl alcohol, though minor components contain other alcohols. The analysis of glycerol ethers, recovered as unsaponifiable matter, was effected by capillary SFC without converting these polar components into TMS derivatives. This is a great advantage because TMS reagent is expensive and the conversion method can be time-consuming.

In this paper, we have shown that shark liver oils can be analyzed in great detail by combining preparative TLC and capillary SFC. High-temperature GC, instead of SFC after TLC, could be used, but this method requires that the sample be hydrogenated before analysis. This means that the information on fatty acid composition that one could obtain from the following transmethylation would no longer be available. By SFC, analysis of squalene content is straightforward and only requires dissolution of the liver oil sample. Determination of the ratio of diacylglycerol ethers to triacylglycerols is more time-consuming, but the results obtained are accurate.

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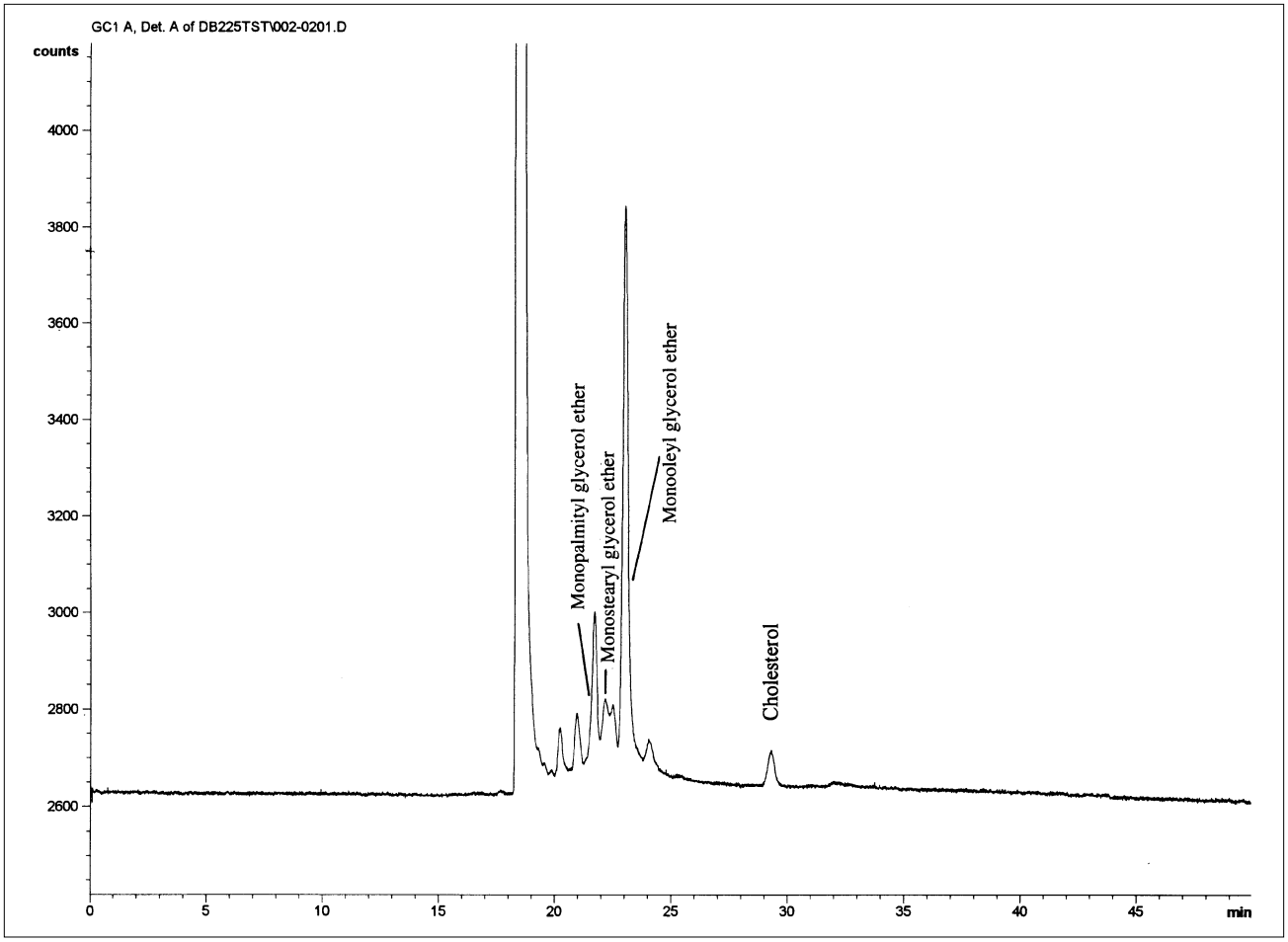

FIG. 5. Capillary supercritical fluid chromatography of the unsaponifiable matter from the liver oil of *Etmopterus princeps*. See text for analysis conditions.

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