Separation of γ- and α-Linolenic Acid Containing Triacylglycerols by Capillary Supercritical Fluid Chromatography

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ABSTRACT: The separation of γ- and α-linolenic acid containing triacylglycerols with an identical acyl carbon number and degree of unsaturation was obtained on capillary supercritical fluid chromatography using a 25% cyanopropyl–75% methylpolysiloxane stationary phase. The resolution of 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol and 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol was 1.35 on a 10 m × 50 μm i.d. column, whereas the resolution was enhanced to 1.66 by combining two 10-meter columns in series. The difference in the position of double bonds in one linolenic acid residue of triacylglycerols resulted in two series of peaks in the separation of alpine currant *(Ribes alpinum)* and black currant *(R. nigrum)* seed oils. The use of the 10-meter column was found to be appropriate for the screening of the triacylglycerol profile in both seed oils studied.
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Edible oils containing γ-linolenic acid, 18:3n-6, are of physiological and nutritional importance. It has been considered that γ-linolenic acid should be regarded as an n-6 essential fatty acid having a notable role in health and disease, as recently reviewed by Horrobin (1). The most important functions of the n-6 essential fatty acids are related to the modulation of membrane structure and the formation of eicosanoids (1). The occurrence, as well as the physical and chemical properties of γ-linolenic acid, are well reviewed by Gunstone (2).

Of the several seed oils containing γ-linolenic acid known today (3), evening primrose (*Oenothera biennis*) oil and borage oil (*Borago officinalis*) are the most well-known. Such oils also have value as commercial products and are consequently under study. Usually the amount of γ-linolenic acid is measured by analyzing the fatty acid composition of the corresponding oil by gas chromatography, as their methyl esters. However, more detailed data of the triacylglycerol composition of edible oils is needed, e.g., to evaluate their nutritional importance and to improve their technological properties.

Using gas chromatography, it has not been possible to separate triacylglycerols with an identical acyl carbon number (ACN) and degree of unsaturation containing either γ- or α-linolenic acid, as reported by Řezanka and Mareš (4). Some articles have been published in which high-performance liquid chromatography (HPLC) has been applied to the separation of triacylglycerols containing γ-linolenic acid residue (5–9). These studies are mostly concerning evening primrose oil and borage oil, in which triacylglycerol compositions were analyzed by silver-ion or reversed-phase HPLC (5,6). However, these oils contain none or very small amounts of α-linolenic acid. Similar separation of triacylglycerols containing γ-linolenic acid also has been reported from the seeds of *Anemone* species by reversed-phase HPLC (7). Perrin *et al.* (8) separated triacylglycerols in black currant seed oil having identical ACNs and degree of unsaturation, containing γ- and α-linolenic acid, with good resolution using two reversed-phase columns in series. Recently, Aitzetmüller and Grönheim (9) produced a similar separation using a 25-cm reversed-phase column for *Primula florindae* and *Ribes nigrum.* Due to the dual nature of the separation mechanism on reversed-phase HPLC, using silver-ion HPLC to separate triacylglycerols according to the degree of unsaturation only has been recommended (5). Furthermore, it may be advantageous to use a combination of silver-ion and reversed-phase HPLC for the analysis of γ-linolenic acid oils.

Supercritical fluid chromatography (SFC) with a packed capillary column impregnated with silver nitrate has been applied to the separation of triacylglycerols in borage oil (10). This technique was able to produce partial separation of γ-linolenoyl-linoleoyl-palmitoyl-glycerol and α-linolenoyl-linoleoyl-palmitoyl-glycerol triacylglycerols. However, no articles of similar separations with open-tubular capillary columns have been published, although capillary SFC has been shown to be able to separate free fatty acids according to the degree of unsaturation (11).

In addition, fatty acids also can be separated as methyl esters by capillary SFC, as shown in this study. Triacylglycerols have been separated according to the ACN and the degree of unsaturation by capillary SFC by using a 25% cyanopropyl–75% methylpolysiloxane stationary phase, and the general elution behavior of triacylglycerols on this particular column has been discussed in more detail in a concurrent pub-

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Abbreviations: ACN, acyl carbon number; c.v., coefficient of variation; HPLC, high-performance liquid chromatography; MS, mass spectrometry; SFC, supercritical fluid chromatography.

lication (Manninen, P., Laakso, P., and Kallio, H., unpublished data). In this study, a column with the same stationary phase was used to separate triacylglycerols containing α- and γ-linolenic acid residues. The separation was studied on both 10- and 20-meter columns. The method was applied for the analysis of alpine currant and black currant seed oils.

EXPERIMENTAL PROCEDURES

Materials. The reference compounds 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol (*sn*-18:1n-9-18:3n-6-18:1n-9) and 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol (*sn*-18:1n-9-18:3n-3-18:1n-9) (custom synthesized by Larodan, Malmö, Sweden) were diluted in *n*-hexane (Rathburn Chemical Ltd., Walkerburn, Scotland) to a concentration of approximately 0.5 mg mL^{-1}, and 1 mg of the mixture of trilinoleoyl-glycerol (18:2n-6-18:2n-6-18:2n-6), dilinoleoyl-γ-linolenoyl-glycerol (18:2n-6-18:2n-6-18:3n-6), and linoleoyl-di-γ-linolenoyl-glycerol (18:2n-6-18:3n-6-18:3n-6) (kindly donated by Dr. David Horrobin of Scotia Pharmaceuticals, Guilford, England) was diluted in 1 mL of dichloromethane (Merck, Darmstadt, Germany) for SFC analysis. Alpine currant (*Ribes alpinum)* seed oil and sea buckthorn (*Hippophaë rhamnoides*) seed oil were extracted with supercritical carbon dioxide at Flavex (Rehlingen, Germany) using production scale plant, and black currant (*R. nigrum)* seed oil was isolated by extraction using methanol/chloroform (1:2, vol/vol). The oils were stored under nitrogen below 0°C, and diluted 1:23 by volume in dichloromethane before chromatography. The fatty acid methyl esters were prepared by sodium methoxide catalyzed transesterification of 40 mg of alpine currant and sea buckthorn seed oils (12). The unpurified fatty acid methyl esters were diluted in 0.7 mL dichloromethane before SFC.

Methods. SFC was obtained on a Lee Scientific Series 600 supercritical fluid chromatograph (Lee Scientific, Dionex, Salt Lake City, UT) equipped with a flame-ionization detector and a pneumatically and electrically-controlled Valco switching valve with an internal loop volume of 1.0 μL. The flame-ionization detector was held at the temperature of 340°C, and nitrogen was used as a make-up gas. Frit restrictors (30 cm × 50 μm i.d.; Lee Scientific, Dionex) were connected at the split outlet and to the end of the analytical column. Sample introduction was performed by a combined timed and dynamic split technique. The columns used were: 10 m × 50 μm i.d. SB-Octyl-50 (50% octyl–50% methylpolysiloxane; Lee Scientific, Dionex) with a film thickness of 0.25 μm, and two 10 m × 50 μm i.d. SB-Cyanopropyl-25 (25% cyanopropyl–75% methylpolysiloxane; Lee Scientific, Dionex) with a film thickness of 0.25 μm. A 1/16" zero dead volume butt connector (SGE, Austin, TX) was used to combine two columns in series. SFC-grade CO_2 (Scott Specialty Gases, Plumsteadville, PA) was used as the carrier fluid. The column flow rate of CO_2 was 0.37 mL min^{-1} with the columns of 10 meters and 0.17 mL min^{-1} with the combination of the two 10-meter columns, as measured with propane at a column temperature of 140°C and CO_2 density of 0.140 g mL^{-1}. Chromatography on the 10-meter columns was performed at the constant temperature of 140°C using a linear density ramp from 0.140 g mL^{-1} at a rate of 0.005 g mL^{-1} min^{-1}, unless otherwise mentioned. With the combination of the two 10-meter columns, the linear density ramp used was from 0.150 g mL^{-1} using a 0.004 g mL^{-1} min^{-1} programming rate. Mass spectrometric analyses were obtained on a Finnigan MAT (San Jose, CA) TSQ-700 triple quadrupole mass spectrometer using a direct exposure probe for sample introduction. Ammonia negative-ion chemical ionization with an ammonia pressure of 8500 mtorr and a temperature of 200°C was used to produce the mass spectra of triacylglycerols (13).

RESULTS AND DISCUSSION

The main components in both alpine currant and black currant seed oils, according to the ACN group separation on a nonpolar stationary phase, were triacylglycerols containing 54 acyl carbons (Fig. 1). In addition, the composition of triacylglycerols obtained on mass spectrometry (MS) analysis was very similar, with only minor differences, in both oils (Table 1). The amount of γ-linolenic acid in alpine currant and black currant seed oils studied were 11.5 and 14.7% in weight, respectively, as measured by methyl esters *via* gas

TABLE 1
The Proportions of Triacylglycerols in Alpine Currant and Black Currant Seed Oil Analyzed Using Ammonia Negative-Ion Chemical Ionization Mass Spectrometry

	Alpine currant seed oil		Black currant seed oil	
Triacylglycerol	Proportion[a,b] (%)	SD	Proportion[a,b] (%)	SD
52:7	0.71	0.03	0.64	0.02
52:6	2.57	0.10	2.89	0.11
52:5	5.19	0.12	7.10	0.17
52:4	5.74	0.30	8.54	0.24
52:3	2.43	0.25	3.10	0.18
52:2	0.89	0.09	0.71	0.04
54:10	1.37	0.06	0.81	0.03
54:9	5.46	0.23	3.97	0.08
54:8	13.33	0.47	11.66	0.18
54:7	19.41	0.67	19.60	0.32
54:6	17.82	0.82	19.18	0.28
54:5	12.21	0.30	10.79	0.19
54:4	6.25	0.24	4.81	0.10
54:3	2.20	0.15	1.36	0.04
54:2	0.39	0.04		
56:7			0.48	0.03
56:6			0.80	0.03
56:5	0.37	0.04	0.81	0.02
56:4	0.58	0.07	0.43	0.02
56:3	0.78	0.12		
56:2	0.71	0.11		
56:1	0.45	0.09		
Trace[c]	1.14	0.15	2.38	0.26

[a]The proportions of triacylglycerols are calculated according to the ^{13}C-corrected intensities of $[M - H]^-$ ions.
[b]Four replicates.
[c]The combined proportion of components corresponding to less than 0.3% of the total.

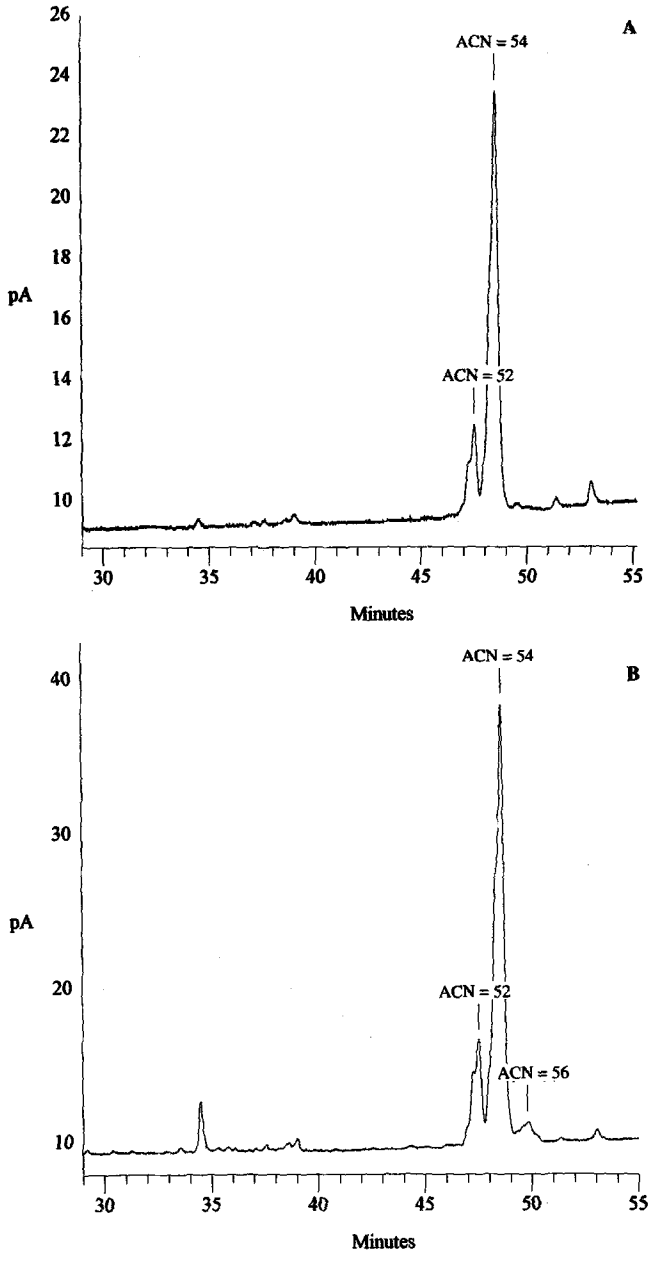

FIG. 1. Separation of alpine currant and black currant seed oils on an SB-Octyl-50 column (Lee Scientific, Dionex, Salt Lake City, UT): (A) alpine currant seed oil, and (B) black currant seed oil. Loop open time 0.7 s. ACN, acyl carbon number.

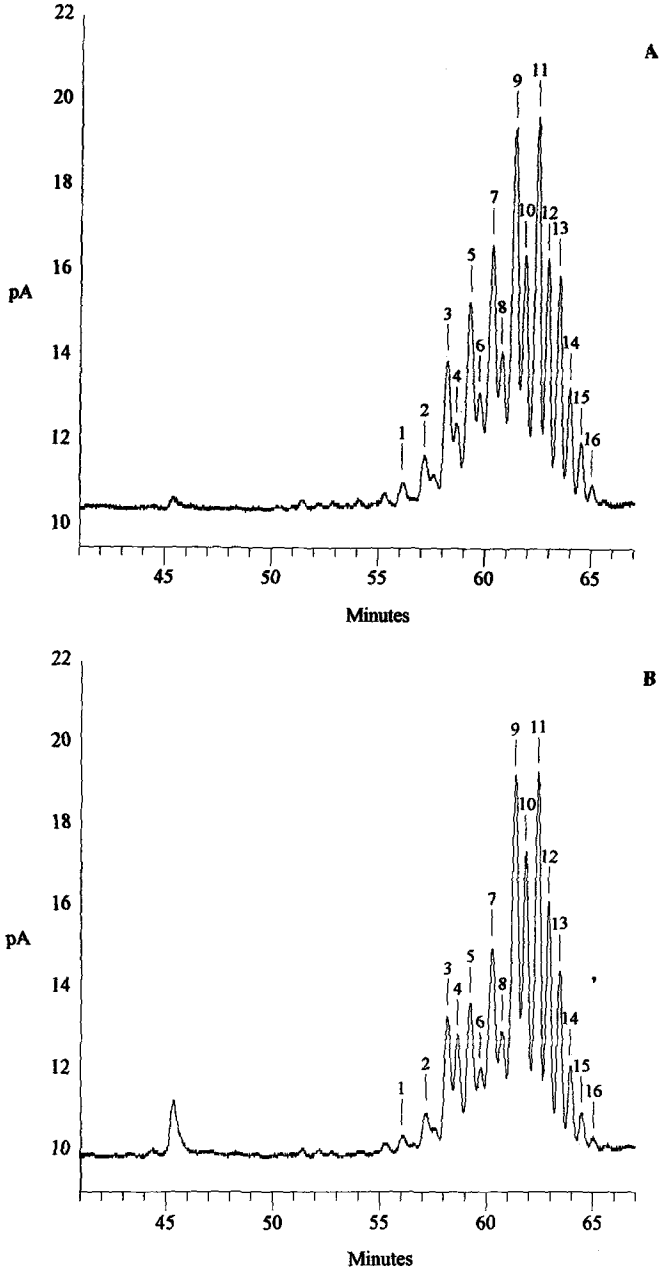

FIG. 2. Separation of triacylglycerols on an SB-Cyanopropyl-25 column (10 m × 50 μm i.d.; Lee Scientific, Dionex): (A) alpine currant seed oil, and (B) black currant seed oil. For peak identification see Table 2. See Figure 1 for company's location.

chromatography. The content of α-linolenic acid was found to be 18.3% by weight in alpine currant seed oil and 14.6% by weight in black currant seed oil fatty acids.

The separation of alpine currant and black currant seed oils on an SB-Cyanopropyl-25 column resulted in two series of prominent peaks (Fig. 2). This chromatographic resolution was not observed in the earlier study of sea buckthorn pulp and seed oil, and cloudberry seed oil (Manninen, P., Laakso, P., and Kallio, H., unpublished data). Such oils do not contain any γ-linolenic acid. The difference in the retention of triacylglycerols containing γ-linolenic acid from those containing α-linolenic acid or no 18:3 fatty acid residues could explain the additional series of triacylglycerol peaks in alpine currant and black currant seed oils. In addition, both alpine currant seed oil and black currant seed oil contained a small amount of stearidonic acid, 18:4n-3, which also could have influenced the resultant separation.

The retention behavior of triacylglycerols containing either γ- or α-linolenic acid could be predicted to some extent by studying the elution pattern of their fatty acid methyl esters. Triacylglycerols do not contain any free carboxylic acid group; therefore, it was more reasonable to study methyl ester derivatives than free fatty acids. The methyl esters of fatty acids of sea buckthorn oil were used to confirm the elution

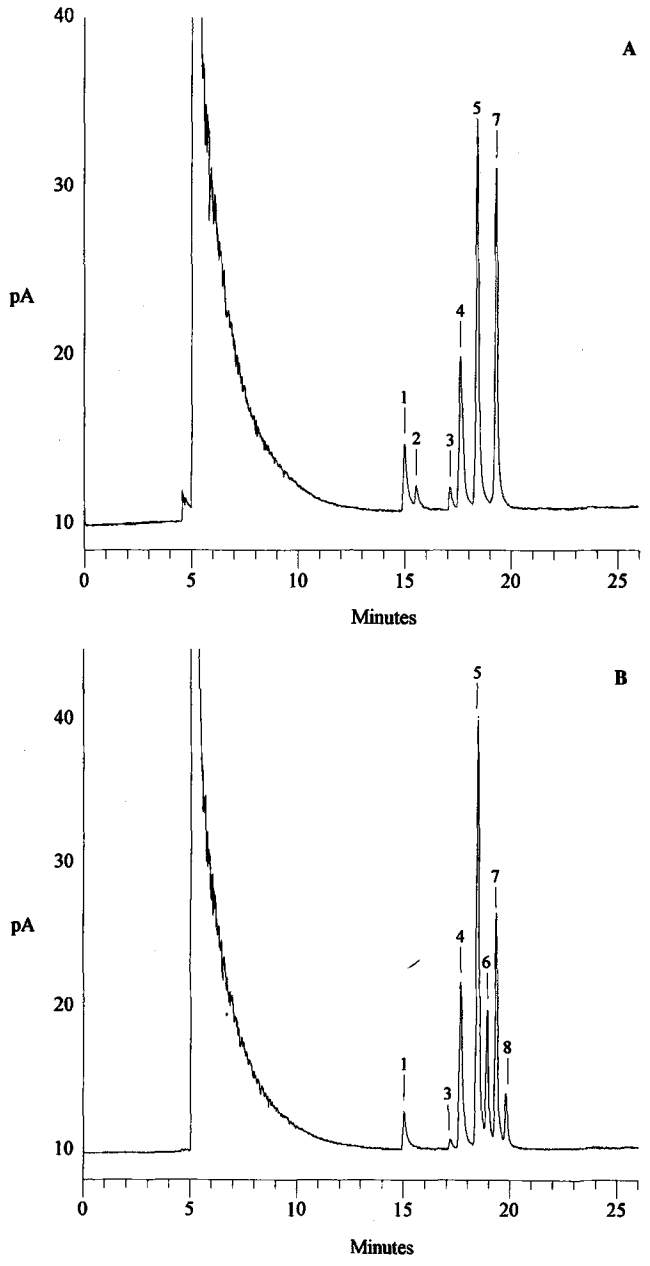

FIG. 3. Separation of fatty acid methyl esters on an SB-Cyanopropyl-25 column (10 m × 50 μm i.d.; Lee Scientific, Dionex): (A) sea buckthorn seed oil and (B) alpine currant seed oil. Chromatographic conditions: linear density ramp from 0.16 g mL^{-1} with 0.010 g $mL^{-1}min^{-1}$. Peak identification: 1 = 16:0, 2 = 16:1n-7, 3 = 18:0, 4 = 18:1n-9, 5 = 18:2n-6, 6 = 18:3n-6, 7 = 18:3n-3, and 8 = 18:4n-3. See Figure 1 for company's location.

order of the fatty acid methyl esters of alpine currant seed oil (Fig. 3). The retention of the fatty acid methyl esters increased with the increasing number of double bonds. In addition, the methyl ester of γ-linolenic acid eluted before that of α-linolenic acid, which indicates that the closer the double bonds are positioned to the ester group, the weaker their interaction between the stationary phase. Therefore, it was reasonable to assume that triacylglycerols with identical ACN and degree of unsaturation, containing either γ- or α-linolenic acid residues, could have slightly different retention.

A two-component mixture containing *sn*-18:1n-9-18:3n-6-18:1n-9 and *sn*-18:1n-9-18:3n-3-18:1n-9 was used to confirm this assumption (Fig. 4A). The component containing the γ-isomer of linolenic acid eluted before that containing the α-isomer, with a resolution of 1.35. The elution behavior was similar to that observed with fatty acid methyl esters. A three-component mixture containing 18:2n-6-18:2n-6-18:2n-6, 18:2n-6-18:2n-6-18:3n-6, and 18:2n-6-18:3n-6-18:3n-6 was separated with the resolutions (R) of $R_{3,4} = 1.12$ and $R_{4,5} = 1.46$ (Fig. 4B). *sn*-18:1n-9-18:3n-6-18:1n-9 and *sn*-18:1n-9-18:3n-3-18:1n-9 were spiked in alpine currant seed oil to confirm the elution order of triacylglycerols with identical ACN and degree of unsaturation having either one γ-linolenic acid or α-linolenic acid moiety. Spiking a real sample with the three-component mixture revealed that triacylglycerols containing two γ-linolenic acid moieties co-eluted with triacylglycerols having identical ACN and one less double bond without any γ-linolenic acid; for example, 54:7 with two γ-linolenic acid moieties co-eluted with 54:6 with only α-isomer of linolenic acid or with no linolenic acid moieties. The difference in the resolution of triacylglycerols containing two α-linolenic acid residues and those with one α- and one γ-linolenic acid was expected to be similar to that of triacylglycerols containing either one α- or one γ-linolenic acid moiety.

The separations were also tested with a longer column (Figs. 4C and 4D). Two 10-meter columns were combined *via* a zero dead volume SGE butt connector, because 20 m × 50 μm i.d. SB-Cyanopropyl-25 columns were, unfortunately, no longer available. The resolution of 1,3-dioleoyl-2-γ-linolenoyl-*sn*-glycerol and 1,3-dioleoyl-2-α-linolenoyl-*sn*-glycerol was enhanced to 1.66 (Fig. 4C). Similarly, the resolution of the three component mixture increased to $R_{3,4} = 1.67$ and $R_{4,5} = 1.46$ (Fig. 4D).

The analysis of alpine currant and black currant seed oils on a 20-meter column improved the separation of the early eluted peaks (Fig. 5). Although the resolution was enhanced and chromatographic conditions further optimized, very similar proportions of the peaks were obtained with both lengths of the column (Table 2). The repeatability of the proportions of triacylglycerols was good in both cases. However, the repeatability in retention times was not satisfactory using the 20-meter column. It would be appropriate to use the 10-meter column for screening the triacylglycerol profile of both oils studied, even though the two peaks could not be effectively integrated using the 10-meter column.

The proportion of the peaks of triacylglycerols containing one γ-linolenic acid was 35.0% in alpine currant seed oil, which was in agreement with the proportion of γ-isomer of linolenic acid (38.6%). In black currant seed oil, the proportion of corresponding peaks was 37.7%, whereas the proportion of γ-isomer of the linolenic acid was 50.1%. Both alpine currant seed oil and black currant seed oil also contained stearidonic acid, 18:4n-3, 4.4 and 3.2% by weight, respectively. The effect of stearidonic acid to the retention of tria-

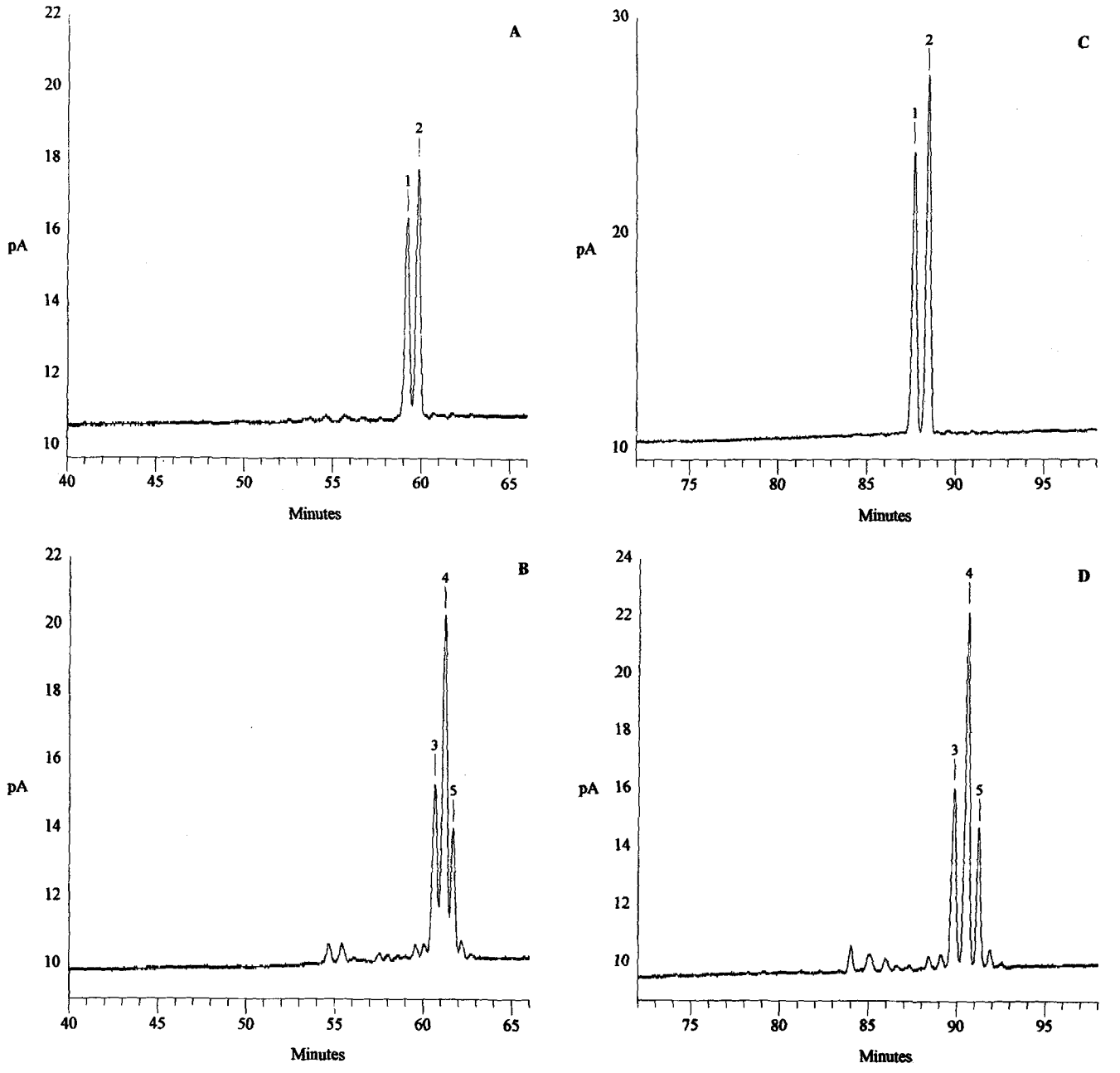

FIG. 4. The separation of reference mixtures of triacylglycerols on an SB-Cyanopropyl-25 column (Lee Scientific, Dionex): (A) a two-component mixture on 10 m x 50 μm i.d. column, (B) a three-component mixture on 10 m x 50 μm i.d. column, (C) a two-component mixture on 20 m x 50 μm i.d. column, and (D) a three-component mixture on 20 m x 50 μm i.d. column. Peak identification: 1-sn-18:1n-9-18:3n-6-18:1n-9, 2 = sn-18:1n-9-18:3n-3-18:1n-9, 3 = 18:2n-6-18:2n-6-18:2n-6, 4 = 18:2n-6-18:2n-6-18:3n-6, and 5 = 18:2n-6-18:3n-6-18:3n-6. See Figure 1 for company's location.

cylglycerols could not be examined during this study because of the lack of reference compounds. However, the difference between the proportion of triacylglycerols containing γ-linolenic acid in black currant seed oil expected and that actually observed (according to the proportion of γ-isomer of the linolenic acid) could not be explained by the amount of stearidonic acid alone, because it was too low. However, the greater difference in proportion indicates that black currant seed oil may contain triacylglycerols having two γ-linolenic acid residues. The presence of such triacylglycerols in black currant seed oil has been reported by Perrin *et al.* (8) and Aitzetmüller and Grönheim (9).

The most abundant triacylglycerols in alpine currant and black currant seed oils based on the MS analysis were 54:5, 54:6, 54:7, and 54:8. The distribution of triacylglycerols containing one γ-linolenic acid residue was quite similar in both oils studied. Among isomeric triacylglycerols (equal number of acyl carbons and double bonds), the proportion of triacylglycerols containing one γ-linolenic acid residue increased with increasing degree of unsaturation. However, one notable

TABLE 2
The Retention Times and the Proportions of Triacylglycerols in Alpine Currant and Black Currant Seed Oils Using 10- and 20-Meter SB-Cyanopropyl-25 Columns[a]

Peak no.	Compound[b] major/minor(s)[c]	Alpine currant seed oil (10 m × 50 μm i.d. column) t_R[d] (min)	SD	Proportion (%)	SD	c.v.[e] (%)	Alpine currant seed oil (20 m × 50 μm i.d. column) t_R[d] (min)	SD	Proportion (%)	SD	c.v.[e] (%)
1	52:2	56.15	0.09	1.03	0.08	8.14	83.38	0.26	0.73	0.07	9.73
2	52:3 (γ)	not integrated					84.00	0.21	0.49	0.03	5.20
3	52:3/54:2,56:1	57.17	0.07	2.11	0.09	4.04	84.66	0.25	1.95	0.06	3.02
4	52:4/54:3 (γ)	not integrated					85.28	0.24	1.33	0.06	4.73
5	52:4/54:3,56:2	58.22	0.07	6.18	0.11	1.85	86.02	0.26	6.08	0.06	0.96
6	54:4/52:5,56:3 (γ)	58.69	0.08	2.98	0.08	2.76	86.70	0.25	2.89	0.10	3.62
7	54:4/52:5,56:3	59.27	0.08	8.68	0.17	2.02	87.36	0.29	8.59	0.10	1.16
8	54:5/52:6,56:4 (γ)	59.76	0.08	4.01	0.22	5.52	88.04	0.31	4.00	0.12	2.92
9	54:5/52:6,56:4	60.30	0.07	11.09	0.11	1.01	88.64	0.36	10.71	0.10	0.90
10	54:6/52:7,56:5 (γ)	60.77	0.06	5.75	0.09	1.51	89.32	0.31	5.60	0.06	1.14
11	54:6/52:7,56:5	61.35	0.08	14.85	0.16	1.06	90.07	0.33	14.59	0.13	0.88
12	54:7 (γ)	61.84	0.07	8.67	0.12	1.37	90.71	0.31	8.61	0.08	0.94
13	54:7	62.41	0.08	13.49	0.09	0.68	91.44	0.33	13.24	0.08	0.64
14	54:8 (γ)	62.92	0.07	7.83	0.06	0.77	92.10	0.32	7.86	0.09	1.11
15	54:8	63.45	0.07	7.29	0.06	0.80	92.76	0.32	7.27	0.15	2.13
16	54:9 (γ)	63.97	0.07	3.56	0.07	2.08	93.43	0.29	3.60	0.07	2.00
17	54:9	64.52	0.06	1.89	0.05	2.58	94.08	0.29	1.90	0.10	5.22
18	54:10 (γ)	65.07	0.07	0.58	0.03	4.30	94.76	0.28	0.62	0.05	8.08

Peak no.	Compound[b] major/minor(s)[c]	Black currant seed oil (10 m × 50 μm i.d. column) t_R[d] (min)	SD	Proportion (%)	SD	c.v.[e] (%)	Black currant seed oil (20 m × 50 μm i.d. column) t_R[d] (min)	SD	Proportion (%)	SD	c.v.[e] (%)
1	52:2	56.02	0.03	0.70	0.11	15.19	83.43	0.48	0.49	0.05	9.25
2	52:3 (γ)	not integrated					83.99	0.46	0.44	0.07	14.92
3	52:3	57.09	0.05	1.79	0.11	6.34	84.72	0.48	1.85	0.28	15.25
4	52:4/54:3 (γ)	not integrated					85.24	0.47	1.22	0.15	12.41
5	52:4/54:3	58.13	0.04	5.83	0.10	1.73	86.08	0.48	5.90	0.10	1.73
6	54:4/52:5 (γ)	58.61	0.05	4.61	0.07	1.57	86.74	0.48	4.28	0.13	3.15
7	54:4/52:5	59.19	0.05	6.91	0.14	1.96	87.42	0.47	7.01	0.12	1.71
8	54:5/52:6,56:4 (γ)	59.70	0.04	3.28	0.05	1.49	88.10	0.47	3.67	0.25	6.83
9	54:5/52:6,56:4	60.20	0.05	9.70	0.11	1.09	88.64	0.51	8.94	0.14	1.54
10	54:6/52:7,56:5 (γ)	60.68	0.06	4.84	0.10	2.06	89.29	0.49	4.89	0.08	1.57
11	54:6/52:7,56:5	61.26	0.07	16.75	0.19	1.11	90.04	0.51	16.62	0.29	1.77
12	54:7/56:6 (γ)	61.77	0.06	11.62	0.14	1.20	90.70	0.50	11.34	0.18	1.58
13	54:7/56:6	62.34	0.06	14.61	0.12	0.85	91.40	0.52	14.01	0.19	1.39
14	54:8/56:7 (γ)	62.84	0.07	8.71	0.10	1.13	92.07	0.52	8.60	0.12	1.41
15	54:8/56:7	63.37	0.05	6.24	0.10	1.56	92.73	0.53	6.24	0.07	1.07
16	54:9 (γ)	63.88	0.04	2.88	0.06	2.21	93.40	0.54	2.84	0.11	3.73
17	54:9	64.43	0.04	1.18	0.09	7.40	94.10	0.56	1.24	0.08	6.27
18	54:10 (γ)	64.97	0.07	0.35	0.11	31.49	94.77	0.62	0.42	0.11	25.47

[a]From Lee Scientific. [b]Identifications are based on the spiked reference compounds and the mass spectrometric analysis. [c](γ) Denotes the triacylglycerols containing one γ-linolenic acid residue. [d]Five replicates. [e]Coefficient of variation.

exception to this trend was found in black currant seed oil, in which the proportion of the peak representing triacylglycerols 54:4 and 52:5 was nearly twice as high as the corresponding peak in the alpine currant seed oil.

The described method gives a new tool for achieving more information of the composition of triacylglycerols in the seed oils containing γ-linolenic acid. However, a method yielding more detailed separation is needed. Fractionation of triacylglycerols according to the number of acyl carbons before SFC on a 25% cyanopropyl–75% methylpolysiloxane stationary phase would be advantageous to enhance chromatographic resolution. The analysis and identification of co-eluting components having different ACNs could also be improved by combining SFC with MS. Nevertheless, chromatographic separation of triacylglycerols containing α- and γ-linolenic acid residues is important, because double-bond positional isomers of linolenic acid cannot be distinguished by MS without fractionation of the sample.

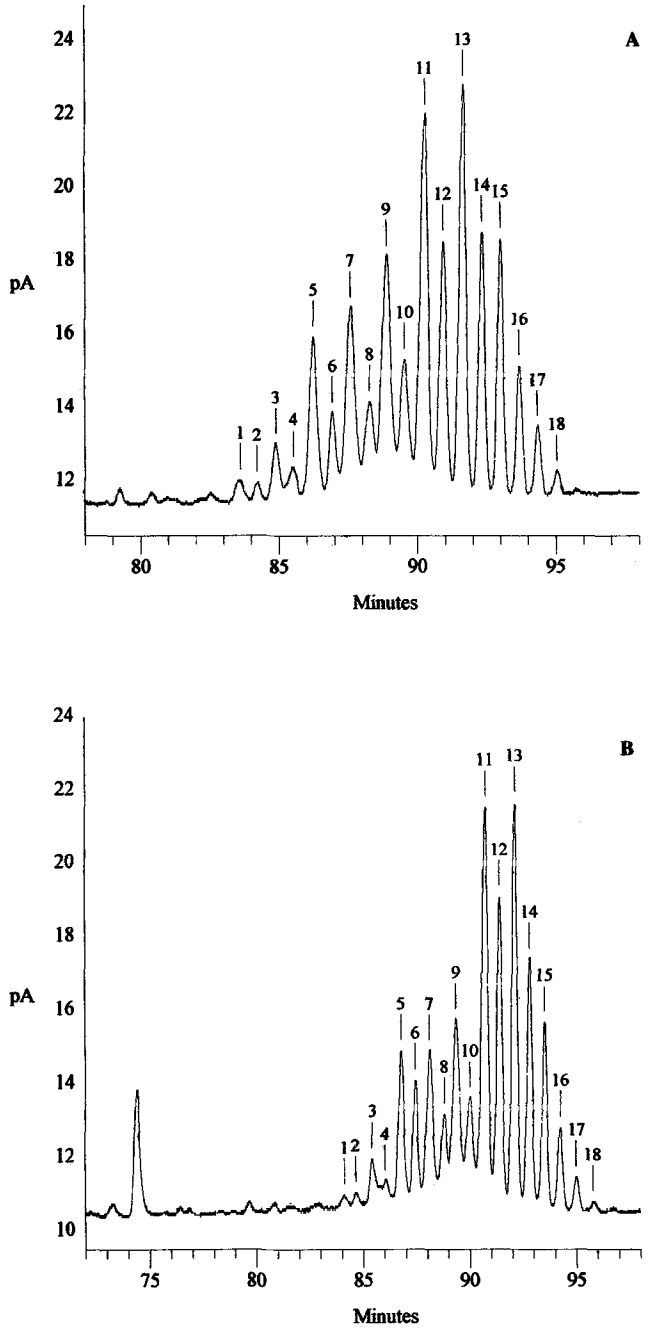

FIG. 5. Separation of triacylglycerols on an SB-Cyanopropyl-25 column (20 m × 50 μm i.d.; Lee Scientific, Dionex): (A) alpine currant seed oil, and (B) black currant seed oil. For peak identification see Table 2. See Figure 1 for company location.

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