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Gas Chromatographic Equivalent Chain Length (ECL) Values of Fatty Acid Methyl Esters on a Highly Polar Ionic Liquid Column, SLB–IL111

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Abstract

This paper reports the retention data of fatty acid methyl esters on a novel ionic liquid column, SLB–IL111, which has higher polarity than commercially available columns for gas chromatography (GC). The fatty acids analyzed using GC include branched-chain saturated fatty acids, C16–C22 monounsaturated fatty acid *cis*/*trans* and positional isomers, and polyunsaturated fatty acids commonly found in natural products. Fatty acids of sardine oil were also analyzed using GC. GC was carried out on a 100–m column under isothermal conditions within the range 120–170°C, using helium as a carrier gas. The retention data of fatty acid methyl esters were represented by equivalent chain length (ECL) values calculated from the retention times of each fatty acid related to those of saturated fatty acids with an even carbon number. This is novel information, obtained on an SLB–IL111 column being operated at various temperatures with a single GC system. The results show that the column temperature greatly affected the ECL values of unsaturated fatty acids. Thus, when using this column for the analysis of fish oil fatty acids, it is necessary to precisely control the column temperature.

Key words : gas chromatography, fatty acid, ionic liquid, SLB–IL111, equivalent chain length, fish oil

Introduction

Gas chromatography (GC) has long been carried out on polymer–type columns. For analysis of fatty acid methyl esters, polar polymer–type stationary phases are preferred. Examples of such stationary phases include polyethylene glycol (e.g., Carbowax, Supelcowax, and Omegawax) and cyanopropyl polysiloxane (e.g., Silar 5CP, SP–2560, and CP–Sil88). These columns can lead to favorable separation of fatty acids with a wide range of carbon number, unsaturation, and olefinic bond position (Christie and Han, 2010). However, the separation is not sufficient to resolve some specific problems. For example, some positional isomers of eicosenoic acid (20 : 1) and docosenoic acid (22 : 1) found in fish oils are not clearly separated from each other even when 50–100 m capillary columns are used (Ota et al., 1995 ; Delmonte and Fardin Kia, 2009 ; Shimizu and Ando, 2012).

In recent years, a new type of GC column, SLB–IL, has become commercially available. The columns of the SLB–IL series use ionic liquid as the stationary phase, and have made it possible to perform high–temperature GC on polar stationary phases, which was impossible using polymer–type columns (Anderson and Armstrong, 2005 ; Ragonese et al., 2009 ; Zeng et al., 2013). A column of SLB–IL100 can be used for the separation of 20 : 1 and 22 : 1 isomers of fish oils (Ando and Sasaki, 2011 ; Shimizu and Ando, 2012). However, the more polar SBL–IL111 column, which became available on the market later, has not yet been detailed on the behavior of fatty acids, except for a few papers that specifically targeted the analysis of partially hydrogenated seed oils (Delmonte et al., 2011) and fatty acid standards (Zeng et al., 2013).

In this study, retention data of various kinds of fatty acid methyl esters were revealed using the SLB–IL111 column. The previous data lacked information on the influence of column temperature on the retention of various kinds of fatty acids. In order to fill this gap in information, the present study has determined the equivalent chain length (ECL) values of fatty acids at different temperatures. Because ECL values vary among different GC systems (Christie, 1988), the same gas chromatograph and column were employed throughout this study. In addition, *cis*/*trans* positional isomers of C16–C22 monounsaturated fatty acids were prepared and subjected to GC using an SLB–IL111 column.

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Materials and Methods

Materials

Methyl esters of normal-chain saturated fatty acids were obtained from GL Science (Tokyo, Japan) and Sigma-Aldrich Japan (Tokyo, Japan). Branched-chain saturated fatty acids were obtained from Applied Science Laboratories (State College, USA). Oleic acid (18 : 1n-9) methyl ester and docosahexaenoic acid (22 : 6n-3) ethyl ester were obtained from Wako Pure Chemical (Osaka, Japan). Palmitoleic acid (16 : 1n-7) methyl ester and eicosapentaenoic acid (20 : 5n-3) ethyl ester were obtained from Doosan Serdary Research Laboratories (Toronto, Canada) and Larodan Fine Chemicals (Malmö, Sweden), respectively. A standard mixture of common fatty acids (Supelco 37 Component FAME Mix) was obtained from Supelco (Bellefonte, USA). Sardine oil was obtained from NOF Corporation (Tokyo, Japan). The oil was converted to fatty acid methyl esters by heating it with 7% BF_3-methanol solution at 100°C for 1 h in the presence of toluene.

Preparation of monounsaturated fatty acid isomers

A series of hexadecenoic acid (16 : 1) and octadecenoic acid (18 : 1) *cis*/*trans* positional isomers were prepared by repeated HBr addition followed by HBr elimination of 16 : 1n-7 and 18 : 1n-9 methyl esters as starting materials, respectively (Delmonte et al., 2008 ; Delmonte and Fardin Kia, 2009). Through one cycle of reaction, *cis*/*trans* isomers with an olefinic bond at positions C_{n-1}, C_n, and C_{n+1} are generated with a ratio of approximately 1 : 2 : 1. The final products obtained in the form of free fatty acids were converted to methyl esters in a manner similar to that described above. The *cis* and *trans* isomers were separated by argentation thin-layer chromatography (Ag-TLC) described below.

The 20 : 1 and 22 : 1 fatty acids were prepared by partially hydrogenating 20 : 5n-3 and 22 : 6n-3, which were released from the ethyl esters by saponification, in 10% hydrazine hydrate-methanol solutions in the presence of air at 50°C for 15 h (Ratnayake et al., 1990). After the *cis*-monounsaturated fatty acids were separated from other products by Ag-TLC, positional isomers were isolated by further Ag-TLC and argentation high-performance liquid chromatography (Ag-HPLC). Each positional isomer was subjected to HBr addition followed by HBr elimination to generate *cis*/*trans* isomers with an olefinic bond at the C_{n-1}, C_n, and C_{n+1} positions.

Ag-TLC and Ag-HPLC separations

Ag-TLC was carried out on 10% $AgNO_3$-impregnated silicic acid plates (20 cm × 20 cm, 0.5 mm thickness) with hexane/acetone (95 : 5, v/v) and toluene for development (Gunstone et al., 1967 ; Wilson et al., 2000). The former solvent was used for isolation of *cis*/*trans* monounsaturated fatty acids and the latter for separation of the positional isomers of the monounsaturated fatty acids.

For the final isolation of *cis*/*trans* positional isomers of monounsaturated fatty acids, Ag-HPLC (Nikolova-Damyanova, 2003) was carried out with a Shimadzu LC-6A pump (Shimadzu, Kyoto, Japan), a Shimadzu CTO-10ASvp column oven, and a Hitachi L-4200 UV-VIS detector (Hitachi, Tokyo Japan). The column used was Silver Column KANTO (25 cm × 4.6 mm i.d., 5 μm particles ; Kanto Chemical, Tokyo, Japan), with hexane/acetonitrile (1,000 : 2, v/v) as the mobile phase at a flow rate of 0.3 mL/min. The column temperature was held at 15°C and detection was carried out at a wavelength of 220 nm. Before sample injection, the injector was washed with hexane/acetonitrile (100 : 2, v/v). The peaks were monitored with a Shimadzu C-R3A integrator.

GC analysis

GC analysis was carried out with a GC-4000 gas chromatograph (GL Sciences) equipped with a capillary column of SLB-IL111 (100 m × 0.25 mm i.d., 0.20 μm film thickness ; Supelco) and a flame ionization detector. The column temperature was isothermal in the range 120-170°C. Injector and detector temperatures were 240°C. Helium was used as carrier gas at a linear velocity of 20 cm/s at 170°C (345 kPa). The split ratio was 25 : 1. The peaks were monitored with a Shimadzu C-R3A integrator.

Results and Discussion

Calculation of ECL values

In this study, each sample was subjected to GC together with normal-chain saturated fatty acids to determine the ECL values from their retention times. The gas chromatogram of methyl esters of sardine oil fatty acids with the saturated fatty acids is shown in Fig. 1. The ECL values were calculated from equation (1). For example, the ECL of 18 : 2n-6 appearing between the peaks of saturated 20 : 0 and 22 : 0 is calculated as follows :

$$ECL_{18:2n-6} = 20.00 + 2\,(\log RRT_{18:2n-6} - \log RRT_{20:0})/(\log RRT_{22:0} - \log RRT_{20:0}) \quad (1)$$

where RRT represents the relative retention times of 18 : 2n-6, 20 : 0, and 22 : 0 compared to the retention time of 18 : 0. Tables 1-5 summarize the ECL values of fatty acid methyl esters on the SLB-IL111 column.

Branched-chain saturated fatty acids

The ECL values of branched-chain saturated fatty acids at 160 and 170°C are shown in Table 1. Monobranched *iso*-fatty acids had almost common decimal fractions of the ECL (0.54-0.56) among the carbon numbers, and *anteiso*-fatty acids 0.77-0.79. These values were not different between

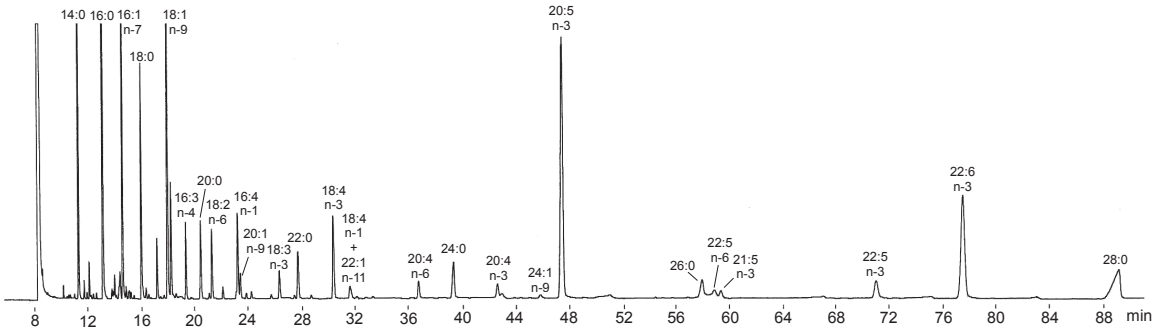

Fig. 1. Gas chromatogram of methyl esters of sardine oil fatty acids with saturated fatty acids on a 100-m SLB-IL111 column at an isothermal temperature of 170°C.

Table 1. ECL values of branched chain saturated fatty acid methyl esters on SLB-IL111

Fatty acid	160°C	170°C	Fatty acid	160°C	170°C
14 : 0	14.00	14.00	*anteiso*-18 : 0	17.78	17.78
TMTD	14.00	14.00	18 : 0	18.00	18.00
iso-15 : 0	14.54	14.54	*iso*-19 : 0	18.55	18.54
anteiso-15 : 0	14.78	14.79	*anteiso*-19 : 0	18.77	18.78
15 : 0	15.00	15.00	19 : 0	18.98	18.99
Pristanic	15.34	15.28	*iso*-20 : 0	19.56	19.56
iso-16 : 0	15.54	15.54	*anteiso*-20 : 0	19.77	19.77
16 : 0	16.00	16.00	20 : 0	20.00	20.00
iso-17 : 0	16.55	–	*iso*-21 : 0	20.55	20.55
Phytanic	16.67	16.62	*anteiso*-21 : 0	20.77	20.78
anteiso-17 : 0	16.78	16.78	21 : 0	20.98	–
17 : 0	16.99	17.00	*iso*-22 : 0	21.56	21.56
iso-18 : 0	17.55	17.54	22 : 0	22.00	22.00

Dash represents that the ECL value could not be detemined because of trace amount.

the analyses at 160°C and 170°C. On the other hand, multi-branched fatty acids (pristanic acid and phytanic acid) showed lower ECL values at the higher temperature. Phytanic acid and *iso*-17 : 0 were well separated at 160°C, whereas their peaks were very close to each other when the analysis was performed at 170°C.

Isomers of monounsaturated fatty acids

The ECL values of *cis*/*trans* and positional isomers of 16 : 1 and 18 : 1 were determined under the isothermal conditions in the range 120-160°C (Table 2). The *trans* isomer eluted faster than the *cis* isomer that had an olefinic bond at the same position. Positional isomers generally eluted in the order of olefinic bond position, with the exception of the terminal olefinic bond (*cis*-15-16 : 1). At all temperatures, some pairs of positional isomers could not be split into two peaks, specifically, pairs of *cis*-6/7-16 : 1, *cis*-6/7-18 : 1, *trans*-6/7-18 : 1, and *trans*-8/9-18 : 1. These fatty acids appeared to be inseparable critical pairs, even on the 100-m column. In contrast, it was found that some pairs separated on changing the column temperature. For example, *trans*-6/7-16 : 1 could be split into two peaks at 140°C. The ECL values of 16 : 1 and 18 : 1 isomers were higher at higher temperatures.

The GC elution time of longer-chain 20 : 1 and 22 : 1 was very long at temperatures below 150°C. Their ECL values were determined at 160 and 170°C using the samples consisting of three positional isomers having an olefinic bond at the C_{n-1}, C_n, and C_{n+1} positions (Table 3). These fatty acids also showed higher ECL values at higher temperature. Pairs of *trans*-5/6-20 : 1, *trans*-8/9-20 : 1, *cis*-9/10-22 : 1, and *trans*-9/10/11-22 : 1 were not clearly resolved at both temperatures. Delmonte et al. (2011) reported GC profile of *cis*/*trans*-4- to 12-20 : 1 on a 100-m column of SLB-IL111 at 168°C. The separations of these isomers observed in the present study were similar to those in their GC profile.

This is the first report providing the ECL values of 20 : 1 and 22 : 1 isomers found in marine fish. These fatty acids are *cis*-5-, 7-, 9-, 11-, 13-, and 15-20 : 1, as well as *cis*-7, 9-, 11-, 13-, and 15-22 : 1 (Ratnayake and Ackman, 1979 ; Ackman et al., 1980 ; Ota et al., 1995). These positional isomers showed ECL values different from each other. The

Table 2. ECL values of C16 and C18 monounsaturated fatty acid methyl esters on SLB-IL111

Olefinic bond position	*cis*-16 : 1					*trans*-16 : 1				
	120°C	130°C	140°C	150°C	160°C	120°C	130°C	140°C	150°C	160°C
5	16.44	16.49	16.54	16.60	16.65	16.46	16.50	16.54	16.58	16.61
6	*16.68*	*16.74*	*16.80*	*16.85*	*16.91*	*16.54*	*16.59*	16.59	*16.67*	*16.71*
7	16.68	16.74	16.80	16.85	16.91	16.54	16.59	16.62	16.67	16.71
8	16.77	16.84	16.89	16.95	17.01	16.60	16.66	16.68	*16.77*	*16.80*
9	16.85	16.91	16.97	17.02	17.08	16.64	16.69	16.72	16.77	16.80
10	16.92	16.98	17.04	17.10	17.16	16.69	16.74	16.77	16.82	16.85
11	17.01	17.07	17.12	17.18	17.24	16.74	16.78	16.81	16.86	16.89
12	17.09	17.15	17.21	17.27	17.33	16.76	*16.78*	*16.81*	*16.86*	*16.89*
13	17.17	17.22	17.28	17.33	17.40	16.81	16.85	16.87	16.91	16.94
14	–	–	17.72	17.79	17.86	–	17.18	17.22	17.27	17.31
15	–	–	–	17.39	–	–	–	–	–	–
	cis-18 : 1					*trans*-18 : 1				
	120°C	130°C	140°C	150°C	160°C	120°C	130°C	140°C	150°C	160°C
4	–	–	–	–	–	–	–	–	18.33	18.36
5	–	–	18.47	18.53	18.58	18.41	18.45	18.48	18.53	–
6	*18.55*	*18.62*	*18.68*	*18.74*	*18.80*	*18.45*	*18.50*	*18.54*	*18.59*	*18.63*
7	18.55	18.62	18.68	18.74	18.80	18.45	18.50	18.54	18.59	18.63
8	18.62	18.69	18.75	18.82	18.88	*18.52*	*18.57*	*18.62*	*18.67*	*18.71*
9	18.68	18.74	18.80	18.87	18.93	18.52	18.57	18.62	18.67	18.71
10	18.72	18.79	18.85	18.92	18.98	18.56	18.62	18.66	18.72	18.76
11	18.79	18.86	18.92	18.98	19.04	18.60	18.65	18.70	18.75	18.79
12	18.87	18.93	18.99	19.05	19.11	18.65	18.69	18.73	18.78	18.82
13	–	–	19.07	19.14	19.20	–	18.74	18.77	18.82	18.86

Italics represent that the peak was not resolved from the neighboring major peak.
Dash represents that the ECL value could not be detemined because of trace amount.

differences between the positional isomers, which differed in the position of their olefinic bond by two carbons, were 0.06-0.16 (20 : 1) and 0.03-0.17 (22 : 1). This result indicates clear separations among the six and five positional isomers of 20 : 1 and 22 : 1, respectively.

The authors previously revealed the separation of these isomers on a 60-m SLB-IL100, which was the first commercially available ionic liquid column (Ando and Sasaki, 2011 ; Shimizu and Ando, 2012). When compared with SLB-IL100, the ECL values observed on SLB-IL111 were higher and almost parallel (Fig. 2). The interaction between the monounsaturated fatty acids and the ionic liquid stationary phase seems stronger on SLB-IL111 because of its higher polarity compared to SLB-IL100. The structures of the ionic liquids are 1,5-di(2,3-dimethylimidazolium)pentane bis(trifluoromethylsulfonyl)imide (SLB-IL111) and 1,9-di(3-vinylimidazolium)nonane bis(trifluoromethylsulfonyl)imide (SLB-IL100).

Polyunsaturated fatty acids

Table 4 shows the ECL values of fatty acids in Supelco 37 Component FAME Mix at 160 and 170°C. Long-chain saturated fatty acids (26 : 0 and 28 : 0) were co-injected onto the GC to calculate the ECL of highly polyunsaturated fatty acids. Commonly found polyunsaturated acids showed increase in the ECL values with increasing unsaturation. In addition, the ECL values were higher at the higher temperature. Three pairs of fatty acids co-eluted at 160°C, specifically, 21 : 0/18 : 3n-6 (ECL 21.00), 24 : 0/22 : 2n-6 (ECL 24.00), and 24 : 1n-9/20 : 5n-3 (ECL 24.75). These pairs

Table 3. ECL values of C20 and C22 monounsaturated fatty acid methyl esters on SLB-IL111

Olefinic bond position	*cis*-20 : 1		*trans*-20 : 1		*cis*-22 : 1		*trans*-22 : 1	
	160°C	170°C	160°C	170°C	160°C	170°C	160°C	170°C
4	20.45	20.49	20.35	20.37	22.39	22.43	22.32	22.34
5	20.54	20.56	20.54	20.56	22.47	22.51	22.47	22.51
6	20.73	20.78	*20.54*	*20.56*	22.68	22.70	*22.54*	*22.54*
7	20.71	20.77	20.57	20.61	22.64	22.67	22.54	22.54
8	20.77	20.83	20.61	20.65	22.68	22.70	*22.54*	*22.54*
9	20.80	*20.83*	*20.61*	*20.65*	*22.71*	*22.76*	*22.58*	*22.61*
10	20.84	20.89	20.65	*20.72*	22.71	22.76	22.58	22.61
11	20.89	20.95	20.68	20.72	22.74	22.79	*22.58*	*22.61*
12	20.94	21.00	20.71	*20.72*	22.77	22.82	22.60	22.64
13	21.01	21.07	20.76	*20.83*	22.83	22.88	22.65	22.68
14	21.09	21.14	20.80	20.83	22.88	22.93	22.68	22.71
15	21.17	21.22	20.84	*20.83*	22.95	23.01	22.72	*22.80*
16	21.24	21.27	20.85	20.88	23.04	23.09	22.77	22.80
17	21.32	21.37	20.89	20.91	23.11	23.16	22.81	22.84
18	21.77	21.82	21.24	21.27	23.19	23.24	22.80	22.83
19					23.25	23.30	22.85	22.87
20					23.68	23.74	23.19	23.22

Italics represent that the peak was not resolved from the neighboring major peak.

Table 4. ECL values of fatty acid methyl esters in Supelco 37 Component FAME Mix on SLB-IL111

Fatty acid	160°C	170°C	Fatty acid	160°C	170°C
10 : 0	10.00	10.00	20 : 1n-9	20.87	20.93
11 : 0	11.00	11.00	21 : 0	21.00	20.99
12 : 0	12.00	12.00	18 : 3n-6	21.00	21.15
13 : 0	13.00	13.00	18 : 3n-3	21.52	21.67
14 : 0	14.00	14.00	22 : 0	22.00	22.00
15 : 0	15.00	15.00	20 : 2n-6	22.09	22.19
14 : 1n-5	15.25	15.31	22 : 1n-9	22.81	22.86
16 : 0	16.00	16.00	23 : 0	22.93	22.99
15 : 1n-5	16.25	16.31	20 : 3n-6	22.99	23.07
17 : 0	17.00	17.00	20 : 3n-3	23.40	23.53
16 : 1n-7	17.07	17.13	20 : 4n-6	23.45	23.61
18 : 0	18.00	18.00	24 : 0	24.00	24.00
17 : 1n-7	18.06	18.12	22 : 2n-6	24.00	24.07
18 : 1n-9*t*	18.69	18.73	24 : 1n-9	24.75	24.80
18 : 1n-9*c*	18.92	18.98	20 : 5n-3	24.75	24.96
18 : 2n-6*t*	19.63	19.70	26 : 0	26.00	26.00
20 : 0	20.00	20.00	22 : 6n-3	27.13	27.36
18 : 2n-6*c*	20.18	20.28	28 : 0	28.00	28.00

Table 5. ECL values of fatty acid methyl esters, prepared from sardine oil, on SLB-IL111

Fatty acid	160°C	Fatty acid	170°C	Fatty acid	160°C	Fatty acid	170°C
14 : 0	14.00	14 : 0	14.00	20 : 1n-11	20.79	20 : 1n-11	–
TMTD	14.00	TMTD	14.00	–	–	16 : 4n-1	20.89
iso-15 : 0	14.54	*iso*-15 : 0	14.53	20 : 1n-9	20.88	20 : 1n-9	–
anteiso-15 : 0	14.78	*anteiso*-15 : 0	14.79	20 : 1n-7	21.02	20 : 1n-7	21.06
15 : 0	15.00	15 : 0	15.00	18 : 3n-6	21.02	18 : 3n-6	21.16
Pristanic	15.34	Pristanic	15.31	18 : 3n-3	21.54	18 : 3n-3	21.68
iso-16 : 0	15.53	*iso*-16 : 0	15.54	22 : 0	22.00	22 : 0	22.00
16 : 0	16.00	16 : 0	16.00	20 : 2n-6	22.11	20 : 2n-6	22.21
iso-17 : 0	16.54	*iso*-17 : 0	16.54	18 : 4n-3	22.37	18 : 4n-3	22.55
Phytanic	16.66	Phytanic	16.70	18 : 4n-1	22.62	18 : 4n-1	22.79
anteiso-17 : 0	16.78	*anteiso*-17 : 0	16.70	22 : 1n-11	22.72	22 : 1n-11	22.79
16 : 1n-9	16.90	16 : 1n-9	17.00	22 : 1n-9	22.82	22 : 1n-9	22.88
17 : 0	16.99	17 : 0	17.00	22 : 1n-7	22.95	22 : 1n-7	23.00
16 : 1n-7	17.07	16 : 1n-7	17.14	20 : 3n-6	22.95	20 : 3n-6	23.09
16 : 1n-5	17.24	16 : 1n-5	17.30	20 : 3n-3	–	20 : 3n-3	–
iso-18 : 0	17.53	*iso*-18 : 0	17.53	20 : 4n-6	23.46	20 : 4n-6	23.64
18 : 0	18.00	18 : 0	18.00	24 : 0	24.00	24 : 0	24.00
16 : 2n-4	18.51	16 : 2n-4	18.62	20 : 4n-3	24.24	20 : 4n-3	24.43
18 : 1n-11	18.80	18 : 1n-11	18.86	24 : 1n-9	24.78	24 : 1n-9	24.81
18 : 1n-9	18.93	18 : 1n-9	18.99	20 : 5n-3	24.78	20 : 5n-3	24.99
18 : 1n-7	19.05	18 : 1n-7	19.11	22 : 5n-6	–	–	–
18 : 1n-5	19.20	18 : 1n-5	19.27	21 : 5n-3	25.90	–	–
16 : 3n-4	19.42	16 : 3n-4	19.57	26 : 0	26.00	26 : 0	26.00
20 : 0	20.00	20 : 0	20.00	–	–	22 : 5n-6	26.07
18 : 2n-6	20.18	18 : 2n-6	20.29	–	–	21 : 5n-3	26.11
18 : 2n-4	20.45	18 : 2n-4	20.56	22 : 5n-3	26.76	22 : 5n-3	26.96
16 : 4n-1	20.69	–	–	22 : 6n-3	27.14	22 : 6n-3	27.36
20 : 1n-13	20.69	20 : 1n-13	–	28 : 0	28.00	28 : 0	28.00

Dash represents that the ECL value could not be detemined because of trace amount.

could be separated at 170°C, because 18 : 3n-6, 22 : 2n-6, and 20 : 5n-3 showed increase in their ECL values to 21.15, 24.07, and 24.96 at 170°C, respectively. This result indicates that the column temperature is an important factor for the separation of fatty acids with a wide range of carbon number and unsaturation. In the present study, all fatty acids of Supelco 37 Component Mix were resolved into single peaks at 170°C. In a recent report of Zeng et al. (2013), the column temperature was programmed to rise from 40°C (held for 4 min) to 260°C, at a rate of 4°C/min. Under their conditions, the last eluent (22 : 6n-3) showed an ECL value of 28.47, which was much higher than that observed in the present study (27.36 at 170°C).

Fatty acids of fish oil

Compared with the above fatty acids, sardine oil contains wider variety of fatty acids, including C16 polyunsaturated fatty acids and positional isomers of polyunsaturated fatty acids. The ECL values obtained at 160 and 170°C are shown in Table 5. Hexadecatrienoic acid (16 : 3n-4) and hexadecatetraenoic acid (16 : 4n-1) are polyunsaturated fatty acids characteristic of Japanese sardine oil (Ando et al., 1988). The ECL values of 16 : 3n-4 and 16 : 4n-1 were 19.42 and 20.69 at 160°C, respectively, and 19.57 and 20.89 at 170°C, respectively. Although 16 : 3n-4 appeared as a single peak at both temperatures, 16 : 4n-1 overlapped with 20 : 1n-13 (160°C) and 20 : 1n-11 (170°C). Thus, when quantifying 16 : 4n-1, it is necessary to analyze it twice, once at 160 and once at 170°C.

Fish oils usually contain 18 : 4n-3, 18 : 4n-1, 20 : 4n-3, 21 : 5n-3, 22 : 5n-6, and 22 : 5n-3 as highly polyunsaturated fatty acids, which were not included in Supelco 37 Mix. These fatty acids appeared as single peaks except for 18 : 4n-1 at 170°C, where 22 : 1n-11 showed the same ECL value. At 160°C, this pair could be separated from each other. At both temperatures, 22 : 6n-3 (DHA) appeared as a single peak and was the last peak of the sardine oil fatty acids.

The ionic liquid column SLB-IL111 had characteristics

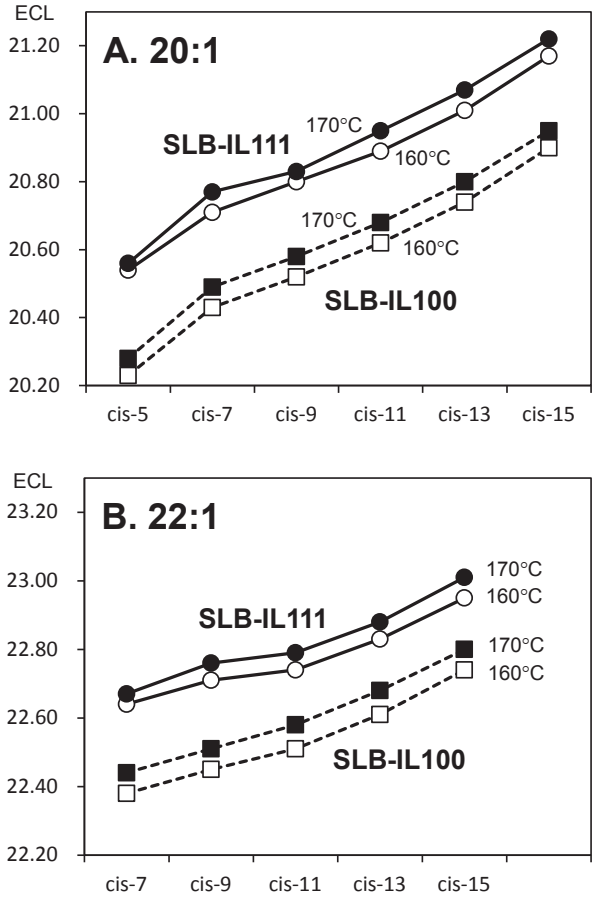

Fig. 2. Equivalent chain length (ECL) values of *cis*-20 : 1 (A) and *cis*-22 : 1 (B) positional isomers on SLB-IL111 and SLB-IL100 columns at isothermal temperature conditions of 160 and 170°C

usually found in polar stationary phases. Retention of unsaturated fatty acids greatly depended on column temperature. At higher column temperature and at higher unsaturation of fatty acids, stronger retention was observed on this column. Although several pairs of fatty acids different in chain length and unsaturation co-eluted from this column, analyses at different temperatures could change the overlap of fatty acid pairs. Therefore, precise control of column temperature is needed when using this column. Alternatively, preliminary fractionation by Ag-TLC and Ag-HPLC seems effective for detailing the fatty acid profile by GC on SLB-IL111. Two-dimensional GC (GC × GC) is also an effective analytical technique for this purpose (Villegas et al., 2010). Even though a relatively complicated technique is required, SLB-IL111 is found to be usable for analysis of fish oil fatty acids, especially monounsaturated fatty acid isomers.

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