



Title	GC Separation of cis-Eicosenoic Acid Positional Isomers on an Ionic Liquid SLB-IL100 Stationary Phase
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Citation	Journal of the American Oil Chemists' Society, 88(6), 743-748 https://doi.org/10.1007/s11746-010-1733-4
Issue Date	2011-06
Doc URL	https://hdl.handle.net/2115/44587
Rights	The original publication is available at springerlink.com .
Type	journal article
File Information	JAOCS-10-0248.pdf



1 GC Separation of *Cis*-Eicosenoic Acid Positional Isomers on an Ionic Liquid SLB-IL100

2 Stationary Phase

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1 **Abstract**

2 Gas chromatography (GC) of *cis*-eicosenoic acid (20:1) positional isomers has been investigated
3 on a capillary column of ionic liquid 1,9-di(3-vinyl-imidazolium)nonane bis(trifluoromethyl)-
4 sulfonylimidate stationary phase (SLB-IL100). A test mixture of isomeric 20:1 methyl esters
5 was prepared from flathead flounder flesh lipids. On a 60-m column operated at 150–180°C, six
6 peaks appeared in the elution order of 20:1n-15 → 20:1n-13 → 20:1n-11 → 20:1n-9 → 20:1n-7
7 → 20:1n-5. These peaks were baseline resolved within 20 min at 180°C. The 20:1n-13 and
8 20:1n-11 isomers, poorly resolved on conventional polar polysiloxane stationary phases, were
9 completely separated from each other with separation factor $\alpha=1.02$ and peak resolution
10 $R_s \geq 1.57$. When equivalent chain length (ECL) values were compared between the SLB-IL100
11 and CP-Sil 88 (biscyanopropyl polysiloxane), those of 20:1n-15 and 20:1n-13 exceptionally
12 tended to be lower on the SLB-IL100. The excellent separation of 20:1 isomers seems due to
13 less retention of 20:1n-15 and 20:1n-13 on SLB-IL100 rather than simply due to its high
14 polarity. Analysis of herring oil 20:1 revealed the occurrence of 20:1n-13 in the Pacific herring
15 but not in the Atlantic herring. The ionic liquid stationary phase, SLB-IL100, is effective to
16 analyze 20:1 isomers occurring in fish and other natural oils.

17

18 **Key words:** GC, Eicosenoic acid, Ionic liquid, SLB-IL100, Fatty acid, Fish oil, Flathead
19 flounder, Herring, Methyl ester.

20

1 **Introduction**

2 Eicosenoic acid (20:1) containing *cis*-olefinic bond exists in many plant and animal lipids [1].
3 Especially in marine fish lipids such as herring, mackerel, capelin, and cod liver oil, 20:1 is one
4 of the major fatty acids accounting for 5–15 % of total fatty acids [2-5].

5 In such fish lipids, there are various isomers of 20:1 different in *cis*-olefinic bond position
6 [2-6]. For example, in the north east Pacific herring, *cis*-11-eicosenoic acid (11*c*-20:1 or 20:1*n*-
7 9) was the most abundant isomer (57.2% of total 20:1) followed by 20:1*n*-11 (36.8%), 20:1*n*-7
8 (3.3%), and 20:1*n*-13 (1.5%) [6,7]. In the Great Lakes alewife, the isomers were 20:1*n*-9 (75%),
9 20:1*n*-7 (21%), and 20:1*n*-11 (4%) [6,7]. Flathead flounder contained wider range of 20:1
10 isomers with *cis*-olefinic bond in the *n*-15, *n*-13, *n*-11, *n*-9, *n*-7, and *n*-5 positions, and 20:1*n*-13
11 and 20:1*n*-11 were the principal isomers [8]. Composition of 20:1 isomers varies among fish
12 species or samples.

13 Although capillary gas chromatography (GC) on polar stationary phase is an effective tool
14 to separate monounsaturated fatty acid isomers, it has not been easy to separate some pairs of
15 positional isomers with central olefinic bond [9]. On columns such as 100-m length SP-2560
16 and CP-Sil 88, it is difficult to separate 18:1*n*-10, 18:1*n*-11 and 18:1*n*-12 as methyl esters [10].
17 A pair of 22:1*n*-11 and 22:1*n*-13 is unresolvable at least on a 50-m column of Silar 5CP [8].
18 Separation of 20:1*n*-11 and 20:1*n*-13 is very poor on the same column [8]. The 20:1 isomers in
19 the above instances were analyzed by indirect methods, i.e., GC of oxidative ozonolysis
20 products [7,8] and gas chromatography-mass spectrometry (GC-MS) of dimethyl disulfide
21 (DMDS) adducts [8].

22 Recently novel stationary phases based on ionic liquids were developed for GC [11]. A
23 commercially available ionic liquid stationary phase, SLB-IL100, has two advantages [11,12].
24 One of them is the polarity much higher than those of polyethylene glycol and biscyanopropyl
25 polysiloxane stationary phases currently used in fatty acid analysis. The other one is the

1 maximum temperature (230°C) significantly higher than that of the corresponding highly polar
2 stationary phase, 1,2,3-tris(2-cyanoethoxypropane) (145°C). The SLB-IL100 stationary phase
3 was applied to the analysis of octadecenoic acid isomers different in olefinic bond positions and
4 *cis/trans* geometries [13,14].

5 In the present study, a column of SLB-IL100 was tested in order to reveal whether this
6 ionic liquid stationary phase is usable for analysis of 20:1 isomers of fish origin. The test sample
7 was prepared from the flathead flounder flesh lipids, including six positional isomers of 20:1.
8 This paper reports the separation, identification, comparison with CP-Sil 88, and application to
9 herring oil 20:1 analysis.

11 **Materials and Methods**

12 **Sample Preparation**

13 Eicosenoic acids of flathead flounder flesh lipids. Fatty acid methyl esters were prepared from
14 the flesh lipids of flathead flounder *Hippoglossoides dubius* [8]. The lipids were saponified in
15 1M KOH (Wako Pure Chemical, Osaka, Japan)-ethanol solution at 90°C for 1 h. The resulting
16 fatty acids were methylated in 7% BF₃-methanol solution (Merck, Darmstadt, Germany) at 70°C
17 for 15 min. *Cis*-monounsaturated fatty acids were concentrated by thin-layer chromatography
18 (TLC) on 10% AgNO₃-impregnated Silica gel 60G plates (20 × 20 cm, 0.5 mm thickness;
19 Merck) with benzene/chloroform (9:1, v/v) for development. The concentrate was fractionated
20 according to their carbon number by reversed-phase TLC on Partisil KC18F plates (20 × 20 cm,
21 0.2 mm thickness; Whatman, Maidstone, England) with acetonitrile for double developments.
22 The 20:1 methyl esters recovered in diethyl ether was purified by TLC on a Silica gel G plate
23 (10 × 10 cm, 0.25 mm thickness, Analteck, Newark, USA) with hexane/diethyl ether (85:15,
24 v/v) for development.

25 Eicosenoic acids of herring flesh lipids. Pacific herring *Clupea pallasii* caught in Ishikari

1 Bay, Hokkaido, Japan and Atlantic herring *C. harengus* landed on Norway were obtained at a
2 food market in Hakodate at May, 2009. Total lipids extracted from the flesh by the method of
3 Bligh and Dyer [15] were converted to fatty acid methyl esters by transesterification in a 7%
4 BF₃-methanol solution at 100°C for 1 h. The 20:1 methyl esters were concentrated by Ag-TLC
5 and reversed-phase TLC in the manners described above.

6 7 GC-FID

8 GC on SLB-IL100. GC was done with a Shimadzu GC-17A gas chromatograph (Shimadzu,
9 Kyoto, Japan) equipped with a flame ionization detector and an open-tubular capillary column
10 of ionic liquid stationary phase, 1,9-di(3-vinyl-imidazolium)nonane bis(trifluoromethyl)-
11 sulfonylimidate, SLB-IL100 (60 m × 0.32 mm i.d., 0.26 μm film thickness; Supelco, Bellefonte,
12 USA). Column temperature was 150, 160, 170, and 180°C. Injector and detector temperatures
13 were 240°C. The carrier gas was helium at a liner velocity of 20 cm/s (117.5 kPa). The split
14 ratio was 25:1. Peaks were monitored with a Shimadzu C-R3A integrator. The 20:1 sample
15 dissolved in hexane was co-injected with the saturated fatty acids, 20:0 and 22:0.

16 GC on CP-Sil 88. GC was carried out with the above system equipped with open-tubular
17 capillary column of biscyanopropyl polysiloxane, CP-Sil 88 (50 m × 0.25 mm i.d., 0.20 μm film
18 thickness; Chrompak, Middelberg, Netherlands). Column temperature was 180°C. Injector and
19 detector temperatures were 240°C. The carrier gas was helium at a liner velocity of 27 cm/s
20 (190 kPa). The split ratio was 33:1.

21 22 Ag-HPLC Fractionation of 20:1 Isomers

23 Silver ion high-performance liquid chromatography (Ag-HPLC) [16-19] was carried out with a
24 Shimadzu LC-6A pump, a Hitachi L-4200 ultraviolet spectrophotometric detector (Hitachi,
25 Tokyo, Japan) and a Shimadzu C-R6A integrator. A column of Silver Column KANTO (25 cm ×

1 4.6 mm i.d., 5 μm particles; Kanto Chemical, Tokyo, Japan) was used with hexane/acetonitrile
2 (1000:2, v/v) as mobile phase at a flow rate of 0.3 mL/min at 15°C. Detection was done at 206
3 nm. The flounder 20:1 dissolved in hexane was injected ten times (each 10 μL of the 20 $\mu\text{g}/\mu\text{L}$
4 solution).

6 GC-MS Analysis of DMDS Adduct

7 Methyl ester of each 20:1 isomer (30-700 μg) was reacted with 1 mL of DMDS (Nakarai Tesque,
8 Kyoto, Japan) in the presence of I_2 (13 mg) as the catalyst for 1 h at 35°C [20-22]. The adduct
9 was purified by TLC on a Silica gel G plate (10 \times 10 cm, 0.25 mm thickness; Analteck) with
10 hexane/diethyl ether/acetic acid (80:20:1, v/v/v) for development.

11 GC-MS was carried on a Zebron ZB-1ms column (30 m \times 0.25 mm i.d., 0.25 μm film
12 thickness; Phenomenex, Torrance, USA) in a HP model 6890 series gas chromatograph
13 (Hewlett-Packard, Palo Alto, USA) linked to a JEOL JMS-700TZ mass spectrometer (JEOL,
14 Tokyo, Japan). Electron impact ionization was used. Column temperature was programmed as
15 follows: isothermal at 40°C for 1 min, increased from 40 to 120°C (40°C/min), increased from
16 120 to 280°C (20°C/min), and held for 20 min. Injector temperature was 280°C. All spectra
17 were obtained at an ionization energy of 70 eV and at a source temperature of 280°C.

19 **Results and Discussion**

20 **Peak Identification**

21 Figure 1 shows the gas chromatograms of the 20:1 isomers on the SLB-IL100 at the column
22 temperatures 150–180°C. Six peaks appeared after the elution of 20:0 methyl ester. Each peak
23 component was isolated by Ag-HPLC fractionation, and then the olefinic bond position was
24 determined by GC-MS of the DMDS adduct. For example, the component of the peak 2 gave
25 strong fragment ions at m/z 229 and 189 due to cleavage between the methylthio-substituted

1 carbons of C7 and C8 and m/z 157 due to loss of methanol (m/z 32) from the ion at m/z 189. The
2 peak 2 was assigned to 20:1n-13 (7c-20:1). On the SLB-IL100, the 20:1 isomers eluted in the
3 order of 20:1n-15 → 20:1n-13 → 20:1n-11 → 20:1n-9 → 20:1n-7 → 20:1n-5.

4 5 Chromatographic Parameters

6 The six isomers were almost or completely baseline resolved at 150–180°C. Table 1 shows the
7 chromatographic parameters characterizing the separations. Separation factors α were 1.02–1.05
8 between the isomers different in olefinic bond position by two carbons. The mean numbers of
9 theoretical plates N of the six peaks were 185,000–213,000. Peak resolutions R_s were 1.57–4.73.
10 The R_s values higher than 1.5 indicate complete separation of the six isomers of 20:1. Under the
11 present conditions, complete separation was achieved within 20 min at 180°C.

12 In the range of 150–180°C, higher R_s values were observed at lower temperatures. The α
13 values were not different at the different temperatures. Column temperature was not found to
14 affect the selectivity to resolve the 20:1 isomers. The N values tended to decrease with
15 decreasing temperature. The higher R_s values are attributable to much increase in retention
16 factors k .

17 Equivalent chain length (ECL) values [9,23] were 20.24–20.99. The ECL values
18 increased with increase in column temperature. At higher temperature, higher selectivity
19 towards unsaturated fatty acids is generally observed on polar stationary phases [9]. This
20 tendency held for the SLB-IL100.

21 22 Separation of the Critical Pair of 20:1 Isomers

23 On the SLB-IL100, the pair of 20:1n-13 and 20:1n-11 was completely separated from each other
24 with the R_s values higher than 1.57. Separation factor α was 1.02. ECL values at 180°C were
25 20.56 and 20.65. The difference ($\Delta\text{ECL}=0.09$) was higher than that can be expected for good

1 separation of peaks on most capillary columns ($\Delta ECL=0.04$) [9].

2 On the 50-m column of CP-Sil 88 (biscyanopropyl polysiloxane), 20:1n-13 and 20:1n-11
3 were poorly but very slightly split at the top of single peak. ECL values at 180°C were 20.43
4 (20:1n-15), 20.56 (20:1n-13), 20.58 (20:1n-11), 20.63 (20:1n-9), 20.73 (20:1n-7) and 20.87
5 (20:1n-5). ΔECL between 20:1n-13 and 20:1n-11 was 0.02.

6 Compared with the CP-Sil 88, higher ECL values were observed for 20:1n-11 through
7 20:1n-5 on the SLB-IL100 (Table 1). This result is consistent with the higher polarity of SLB-
8 IL100 [11-14]. On the other hand, the ECL values of 20:1n-15 and 20:1n-13 tended to be lower
9 on the SLB-IL100 (20.37 vs. 20.43; and 20.56 vs. 20.56). The SLB-IL100 showed less retention
10 of 20:1n-15 and 20:1n-13 inconsistent with the high polarity. As a result, ΔECL of 20:1n-13 and
11 20:1n-11 increased from 0.02 (CP-Sil 88) to 0.09 (SLB-IL100). The excellent separation of this
12 critical pair seems due to the less retention of 20:1n-13 on the SLB-IL100.

13 14 Analysis of Fish Oil 20:1 Isomers

15 Flathead flounder. The 20:1 isomer composition was calculated from the peak area percentages
16 (Table 2). The composition obtained by the GC resembled those previously analyzed by the
17 indirect methods and ozonolysis fission in particular [8]. The major isomer was 20:1n-13
18 (34.5%) and 20:1n-11 (26.2%).

19 Herring. The herring flesh 20:1 isomers were analyzed at 180°C (Fig. 2). The 20:1n-13
20 isomer was not detected in the Atlantic herring, whereas the Pacific herring contained this
21 isomer at the concentration of 0.8% of total 20:1. The 20:1n-11 isomer was the most abundant
22 one in the Pacific herring (76.7%). It is in contrast to the Atlantic herring (9.7%) and other
23 popular fish known to be highest in 20:1n-9 [2]. Similar profile was found in Pacific salmon
24 [24] and saury [25].

1 Fatty Acid Analysis on the SLB-IL100

2 The SLB-IL100 was revealed to be powerful for analysis of 20:1 positional isomers. In this
3 study, the column was frequently used for ten months. At this point, intraday retention time and
4 peak area repeatability ($N=10$) of 20:1 isomers were not over 0.07% and 8.1% in terms of
5 coefficient of variation, respectively (180°C). On the other hand, retention time remarkably
6 decreased during the ten months. The retention times of 20:1n-15 through 20:1n-5 changed from
7 15.3–17.0 min (Fig. 1) to 11.7–12.8 min. ECL values changed as follows: 20.37 → 20.33
8 (20:1n-15), 20.56 → 20.55 (20:1n-13), 20.65 → 20.65 (20:1n-11), 20.73 → 20.74 (20:1n-9),
9 20.84 → 20.86 (20:1n-7), and 20.99 → 21.01 (20:1n-5). Nonetheless, separation of the 20:1
10 isomers remained almost complete (R_s , 1.48–3.33) at 180°C.

11 GC on the SLB-IL100 is a great improvement to analysis of fatty acids including longer-
12 chain monounsaturated fatty acids. However, when total fatty acids of fish origin were subjected,
13 such a highly polar stationary phase gives very complicated chromatogram due to overlapping
14 components of different chain-lengths [9]. On the SLB-IL100, α -linolenic acid (18:3n-3)
15 overlapped the 20:1 isomers. For accurate analysis of 20:1 isomers, preliminary fractionation is
16 necessary.

18 **Acknowledgment**

19 The authors wish to thank Ms. Seiko Oka, Instrumental Analysis Division, Equipment
20 Management Center, Creative Research Institution, Hokkaido University, for the GC-MS
21 analyses.

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- 17

1 **Figure Legends**

2

3 **Fig. 1** GC of *cis*-eicosenoic acid (20:1) methyl esters, prepared from flathead flounder flesh
4 lipids, on SLB-IL100 ionic liquid stationary phase at column temperatures of 180 (a), 170
5 (b), 160 (c) and 150 (d) °C. See the text for the GC conditions. Peak identifications: 1,
6 20:1n-15; 2, 20:1n-13; 3, 20:1n-11; 4, 20:1n-9; 5, 20:1n-7; 6, 20:1n-5; and I.S., 20:0.

7

8 **Fig. 2** GC of *cis*-eicosenoic acid (20:1) methyl esters of the Pacific (a) and Atlantic (b)
9 herring on SLB-IL100 ionic liquid stationary phase at column temperature of 180°C. Peak
10 identifications: 1, 20:1n-13; 2, 20:1n-11; 3, 20:1n-9; 4, 20:1n-7; and 5, 20:1n-5.

Table 1 Chromatographic parameters of 20:1 isomers on the SLB-IL100 ionic liquid stationary phase

Isomer	180°C				170°C				160°C				150°C			
	k^a	α^b	Rs^c	ECL ^d	k	α	Rs	ECL	k	α	Rs	ECL	k	α	Rs	ECL
20:1n-15	2.06			20.37	3.26			20.33	5.11			20.28	8.50			20.24
20:1n-13	2.16	1.05	3.48	20.56	3.42	1.05	4.19	20.51	5.35	1.05	4.35	20.46	8.93	1.05	4.73	20.41
20:1n-11	2.20	1.02	1.57	20.65	3.48	1.02	1.80	20.59	5.47	1.02	2.03	20.54	9.12	1.02	2.06	20.48
20:1n-9	2.25	1.02	1.57	20.73	3.56	1.02	1.98	20.68	5.60	1.02	2.16	20.62	9.35	1.02	2.38	20.57
20:1n-7	2.31	1.03	2.19	20.84	3.66	1.03	2.69	20.79	5.77	1.03	2.81	20.74	9.68	1.04	3.45	20.69
20:1n-5	2.39	1.04	2.67	20.99	3.81	1.04	3.50	20.95	6.02	1.04	3.68	20.90	10.13	1.05	4.47	20.85
$N_{20:1}^e$	201,771				213,959				186,009				185,847			

See the text for the GC conditions. Each parameter was calculated from the chromatograms shown in Fig. 1 ($N=1$).

^a Retention factor.

^b Separation factor (the ratio of the retention factors).

^c Peak resolution. $Rs = 1.18 \times (t_2 - t_1) / (w_1 + w_2)$, where t is retention time and w is the width of the peak at half that height.

^d ECL value calculated on the basis of retention times of eicosanoic acid (ECL 20.00) and docosanoic acid (ECL 22.00) methyl esters.

^e Mean number of theoretical plates of the 20:1 isomer peaks. $N = 5.54 \times (t/w)^2$.

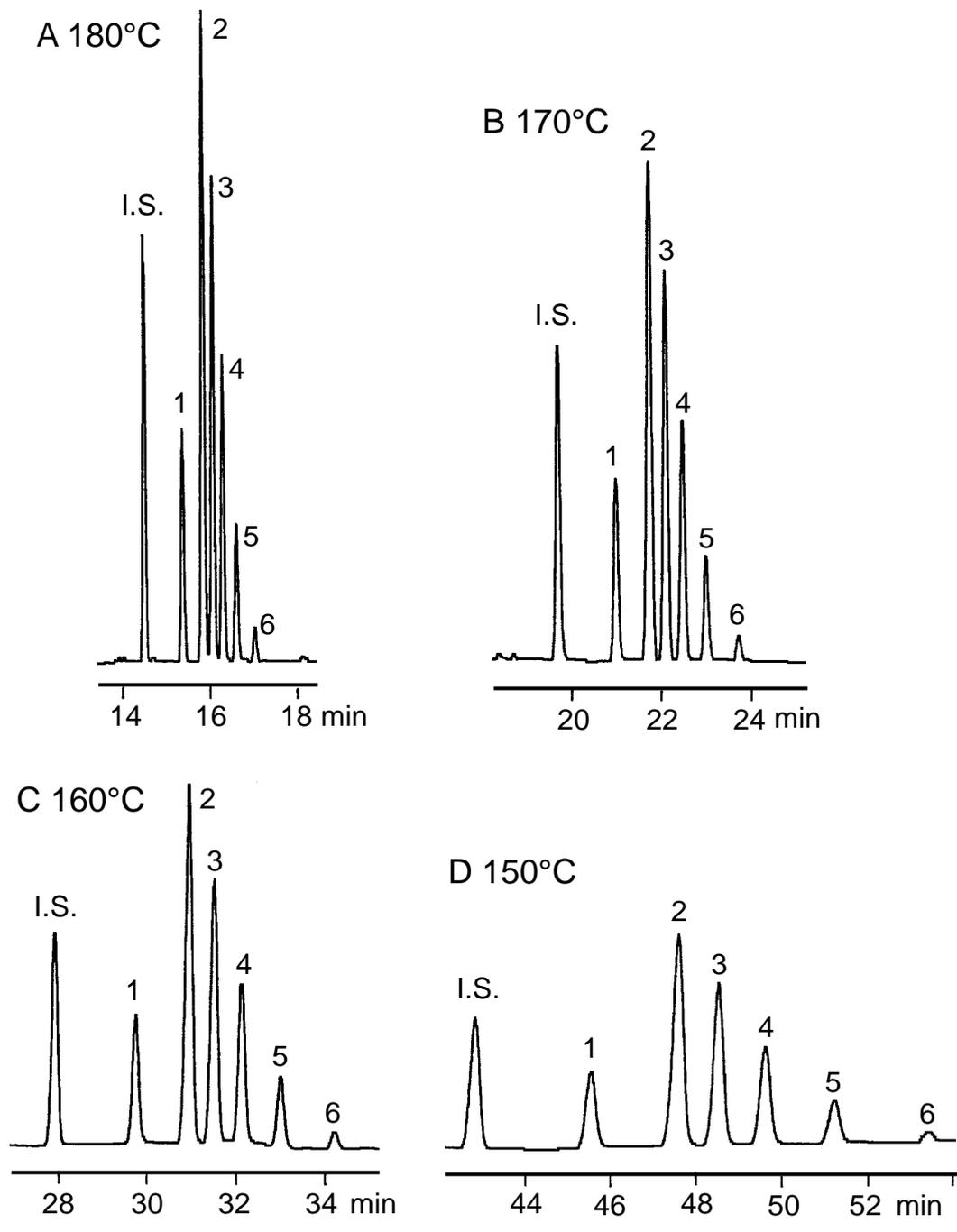
Table 2 Composition of the 20:1 isomers in the flesh lipids of marine fish (wt%)

Isomer	Flathead flounder			Pacific herring	Atlantic herring
	GC ^a <i>N</i> =10	O ₃ ^b <i>N</i> =1	DMDS ^c <i>N</i> =1	GC ^a <i>N</i> =3	GC ^a <i>N</i> =3
20:1n-15	12.3 ± 0.3	8.5	7.9	—	—
20:1n-13	34.5 ± 0.3	36.1	32.8	0.8 ± 0.0	—
20:1n-11	26.2 ± 0.2	28.1	32.7	76.7 ± 0.1	9.7 ± 0.0
20:1n-9	16.7 ± 0.1	18.3	17.5	17.4 ± 0.0	87.5 ± 0.1
20:1n-7	8.1 ± 0.2	7.4	7.4	3.8 ± 0.0	2.2 ± 0.1
20:1n-5	2.3 ± 0.1	1.5	1.7	1.4 ± 0.0	0.6 ± 0.0

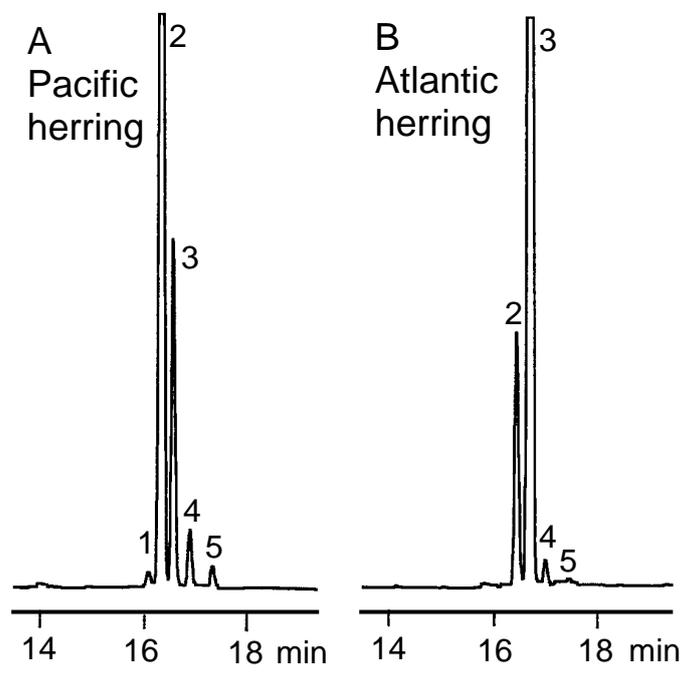
^a Analyzed by GC on SLB-IL100 at 180°C; Mean ± SD of replicate determinations.

^b Previously analyzed by GC of the oxidative ozonolysis products [8].

^c Previously analysed by GC-MS of the dimethyl disulfide adducts [8].



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Figure 1



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Figure 2