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1 **Detection and identification of furan fatty acids from fish lipids by**
2 **high-performance liquid chromatography coupled to electrospray**
3 **ionization quadrupole time-of-flight mass spectrometry**

4
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15 Running title: HPLC/ESI-Q-TOF-MS analysis of furan fatty acids in fish lipids

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25 ABSTRACT

26 Using high-performance liquid chromatography coupled to high-resolution
27 electrospray ionization quadrupole time-of-flight mass spectrometry
28 (HPLC/ESI-Q-TOF-MS), we have developed a new method for detection and
29 identification of furan fatty acids (F-acids), which are widely distributed in living
30 organisms and foods as minor lipid components and are known to have antioxidant
31 and anti-inflammatory effects. For this purpose, total fatty acids prepared from the
32 testis lipids of Japanese chum salmon (*Oncorhynchus keta*) were examined without
33 any concentration or isolation of F-acids. In negative ESI mode, F-acids gave a
34 prominent $[M - H]^-$ ion, by which individual F-acids could be detected and identified.
35 High-resolution extracted ion chromatograms clearly showed the occurrence of five
36 major F-acid homologs as already reported by GC/MS. The method was successfully
37 applied to several fish samples and revealed the occurrence of F-acids for the first time
38 in the two New Zealand fish, hoki (*Macruronus novaezelandiae*) and school shark
39 (*Galeorhinus galeus*).

40

41 *Keywords:*

42 Furan fatty acids

43 High-performance liquid chromatography-electrospray ionization quadrupole

44 time-of-flight mass spectrometry

45 Fish lipids

46 *Oncorhynchus keta*47 *Macruronus novaezelandiae*48 *Galeorhinus galeus*

49

50 1. Introduction

51 Furan fatty acids (F-acids) were first discovered in the 1960–1970s from plant
52 seeds (Morris, Marshall, & Kella, 1966) and fresh-water fish (Glass, Krick, & Eckhardt,
53 1974), and are widely distributed in the lipids of living matter and food, with usually low
54 abundance. F-acids are potent antioxidants and radical scavengers which can protect
55 polyunsaturated fatty acids from spoilage and thus they have been classified as
56 valuable minor compounds in foodstuff (Spiteller, 2005; Vetter & Wendlinger, 2013). A
57 lipid extract of the New Zealand Greenshell™ mussel (*Perna canaliculus*) displays
58 anti-inflammatory effects in animal models and in human controlled studies (Gibson &
59 Gibson, 1998; Tenikoff, Murphy, Le, Howe, & Howarth, 2005), and some F-acids were
60 recently detected in Lyprinol®, a lipid extract of *P. canaliculus*, as active compounds
61 (Wakimoto et al., 2011).

62 Analysis of F-acids is exclusively performed by gas chromatography with flame
63 ionization detection (GC/FID) or coupled to mass spectrometry (GC/MS), which are
64 powerful tools for separation and identification of F-acid homologs in fish lipids. To
65 obtain their clear detection and unambiguous identification, both methods need prior
66 isolation and/or concentration of F-acids, as they usually exist in very low levels in fish
67 lipids (less than 1% of total fatty acids). Multidimensional GC/MS is nowadays applied
68 for identification of F-acids as methyl esters without prior separation from common
69 saturated and unsaturated straight-chain fatty acids from biological samples (Wahl,
70 Chrzanowski, Mfiller, Liebich, & Hoffmann, 1995). The multidimensional GC/MS
71 method is a useful technique for detection of F-acids in large amounts of common fatty
72 acids, but the procedures appear to be complex for screening F-acids in biological
73 materials. In the method, the use of appropriate F-acid standards, which are not easily

74 available, are also essential for careful separations of F-acid-containing fractions on
75 the first GC column.

76 In the present study, using high-resolution quadrupole-time-of-flight mass
77 spectrometry (Q-TOF-MS), we have tried to specifically detect F-acids present in trace
78 to large amounts of common saturated and unsaturated fatty acids by paying attention
79 to a fact that in addition to the two carboxyl oxygen atoms in fatty acid molecules,
80 F-acids have one more oxygen atom in a furan ring. This shows that an F-acid having
81 the same nominal mass as that of a straight-chain fatty acid, such as 10,13-epoxy-11-
82 methyloctadeca-10,12-dienoic acid (F2) and eicosadienoic acid (20:2), can be
83 discriminated by their exact masses (see **Table 1**). This paper describes a new method
84 for direct detection and identification of F-acids in fish oil fatty acids by using
85 reversed-phase HPLC/electrospray ionization (ESI)-Q-TOF-MS. The method is
86 standardized with a synthetic F-acid (9,12-epoxyoctadeca-9,11-dienoic acid) and
87 applied to the Japanese salmon (*Oncorhynchus keta*) and the New Zealand fish (hoki,
88 *Macruronus novaezelandiae* and school shark, *Galeorhinus galeus*) species. The
89 former salmon species is known to contain a series of F-acid homologs in the testis
90 (Ota & Takagi, 1991), whereas no data have been reported for the later fish species.

91

92 **2. Materials and methods**

93

94 *2.1. Fish samples*

95 Lipids from the testis of the mature chum salmon (*O. keta*), the roe and liver of the
96 fin-fish species hoki (*M. novaezelandiae*) and the liver of school shark (*G. galeus*) were
97 extracted by the conventional Bligh-Dyer method (Bligh and Dyer, 1959). Fatty acid

98 methyl esters were prepared as described by Christie and Han (2010, chap. 7) with a
99 slight modification. Briefly, to the fish lipid samples (up to 10 mg) dissolved in toluene
100 (1 mL), a 0.5M sodium methoxide-methanol reagent (Supelco, Bellefonte, PA) was
101 added and the solution was kept at 50°C for 10 min; acetic acid (0.1 mL) was then
102 added, followed by water (3 mL). The reaction products, including fatty acid methyl
103 esters and non-esterified fatty acids (free fatty acids), were extracted with
104 ether-hexane (1:1, v/v; 3 x 3 mL). After evaporation of the solvent, the free fatty acids
105 present were converted into methyl esters using ethereal diazomethane in the
106 presence of a little methanol. A synthetic F-acid methyl ester,
107 methyl-8-(5-hexyl-2-furyl)-octanoate (methyl 9,12-epoxyoctadeca-9,11-dienoate),
108 used as a reference standard, was purchased from Santa Cruz Biotechnology, Inc.
109 (Santa Cruz, CA, USA). Prior to use, this standard F-acid methyl ester was purified on
110 a Sep-Pak Plus Silica cartridge (Waters, Tokyo, Japan) using hexane/ether (95:5, v/v)
111 as the mobile phase after pre-conditioning with hexane. The purity was 75% by
112 GC/FID. For HPLC/MS analysis, methyl esters were saponified to produce free fatty
113 acids by keeping for 30 min at 50°C in a 5% (w/v) potassium hydroxide-ethanol
114 solution.

115

116 2.2. HPLC/ESI-Q-TOF-MS

117 Reversed-phase HPLC/ESI-MS was performed by admitting the entire HPLC
118 column effluents to a Bruker MicrOTOF Q II mass spectrometer (Bruker Daltonics,
119 Yokohama, Japan). HPLC separations were performed using a UltiMate™ 3000 RS
120 HPLC system, consisting of a pump, an autosampler, a photodiode array detector and
121 a column oven (Thermo Scientific, Yokohama, Japan). The analysis was done on a

122 Poroshell 120 EC-18 column (100 x 2.0 mm I.D., 3 μ m particles) at 30°C using an
123 isocratic elution with a mixture of solvent **A** and solvent **B** (5:95, v/v) at the flow-rate of
124 0.3 mL/min, where solvent **A** was water and solvent **B** was acetonitrile-water (95:5, v/v).
125 Both solvents contained 50 mM formic acid and 2 mM ammonium formate. A 5 μ L
126 methanol solution of fish fatty acids (0.5 mg/mL) was injected into the column. MS
127 analysis was performed in ESI negative ion mode. The capillary voltage, nebulizer gas
128 pressure, dry gas temperature and dry gas flow were –3500 V, 1.6 bar, 180°C and 10
129 L/min, respectively. Mass calibration was done using lithium formate cluster ions.
130 Extracted ion chromatograms (EICs) of F-acids were created using a narrow mass
131 extraction width (± 0.01 m/z) of the deprotonated $[M - H]^-$ molecules from total ion
132 chromatograms (TICs). Accurate mass spectra were recorded across the range 0–700
133 m/z .

134

135 2.2. GC and GC/MS

136 GC analysis of fatty acid methyl esters was performed using an Agilent 6890N gas
137 chromatograph (Agilent, Santa Clara, CA) equipped with an Omegawax 250 column
138 (30 m x 0.25 mm i.d. 0.25 μ m film thickness; Supelco, Bellefonte, PA). Helium was
139 used as the carrier gas at a flow rate of 1.0 mL/min. The split ratio was 1:25. The
140 column temperature was elevated from 180 to 240°C at 1°C/min and held for 30 min at
141 240°C. The sample injection port and FID temperatures were maintained at 240°C.
142 GC/MS analysis was performed on an Agilent 6890N gas chromatograph equipped
143 with an Agilent 5973N mass selective detector, under the same conditions as those
144 used in GC/FID. Electron impact mass spectra were recorded at an ionization energy
145 of 70 eV. The ion source temperature was maintained at 240°C.

146

147 3. Results and discussion

148 3.1 Structures of the major furan fatty acids in fish

149 The structures of F-acids commonly found in marine lipids are shown in **Table 1**,
150 including the chum salmon testis lipids examined in this study. Three abbreviations
151 have been used for F-acids (Glass et al., 1974; Rahn, Sand, Krick, Glass, & Schlenk,
152 1981; Vetter et al., 2012). In this study, the original abbreviations described by Glass et
153 al. (1974) are used. F-acids can be subdivided into two groups: one with a pentyl side
154 chain ($m = 4$) and another one with a propyl side chain ($m = 2$). The most abundant
155 F-acid in chum salmon testis lipids is known to be
156 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F6), followed by
157 12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoic acid (F4) and
158 12,15-epoxy-13-methyleicosa-12,14-dienoic acid (F5); the sum of these three F-acids
159 occupies *ca.* 90% of total F-acids (Ota & Takagi, 1991). A similar F-acid composition
160 was also obtained for the salmon testis lipids examined in this study using both
161 HPLC/MS and GC/MS (see **Table 1** and **Fig. S1**).

162

163 3.2 HPLC/MS analysis of furan fatty acids in fish samples

164 The TIC, EIC, and MS spectrum of the unnatural F-acid standard
165 (9,12-epoxyoctadeca-9,11-dienoic acid, $C_{18}H_{29}O_3$, 75% purity) by reversed-phase
166 HPLC/ESI-Q-TOF-MS are shown in **Fig. 1**. A 5 μ L aliquot of methanol solution of
167 F-acid standard (5 μ g/mL) was injected into the column and introduced into the
168 HPLC/MS system. Negative ESI-MS gave a prominent $[M - H]^-$ ion at m/z 293.2128

169 (calculated exact mass: m/z 293.2122), which enabled unambiguous identification of
170 the compound. The detection limit was at or below 2.3 pg when signal-to-noise (S/N)
171 was 3. As with straight-chain fatty acids, the deprotonated molecules were observed
172 for all the F-acids identified in this study, as described below.

173 The TIC of the total fatty acids prepared from the chum salmon testis lipids by
174 HPLC/ESI-Q-TOF-MS and the EICs for each deprotonated molecule of F-acids are
175 shown in **Fig. 2**. The EICs were obtained using narrow ($[M - H]^- \pm 0.01 m/z$, **Fig. 2**)
176 and wider ($[M - H]^- \pm 0.2 m/z$, **Fig. S2**) extraction widths. The overall EICs of F-acids
177 are shown in **Fig. 3**. When the wider extraction width was applied (**Fig. 3**, lower panel
178 and **Fig. S2**), F2 ($C_{19}H_{32}O_3$) with the $[M - H]^-$ ion at m/z 307.2258 (calculated exact
179 mass: m/z 307.2279) was extracted together with the straight-chain 20:2 fatty acid
180 ($C_{20}H_{36}O_2$) with the $[M - H]^-$ ion at m/z 307.2638 (calculated exact mass: m/z
181 307.2642) which co-eluted with F6 under the HPLC conditions employed, while when
182 the narrow extraction width ($[M - H]^- \pm 0.01 m/z$) was applied (**Fig. 3**, upper panel), F2
183 and 20:2 were clearly resolved based on the differences of their exact masses (see **Fig.**
184 **S3**). Similarly, when the narrow extraction width ($[M - H]^- \pm 0.01 m/z$) was applied, F5
185 ($C_{21}H_{36}O_3$) with the $[M - H]^-$ ion at m/z 335.2583 (calculated exact mass: m/z
186 335.2592) was also clearly resolved from a straight-chain 22:2 fatty acid ($C_{22}H_{40}O_2$)
187 with the $[M - H]^-$ ion at m/z 335.2933 (calculated exact mass: m/z 335.2956) which has
188 a close retention time with F8 (**Fig. 3**). These observations demonstrate that high
189 resolution Q-TOF-MS enables direct and accurate identification of F-acids existed at
190 low levels of less than 1% in total fatty acids from biological samples.

191 **Table 1** also lists the F-acids and their interfering straight-chain fatty acids
192 detected in the salmon testis lipids by HPLC/negative ESI-Q-TOF-MS. Five F-acids

193 (F6, F4, F2, F5, and F8) were identified. Although a very low amount of F6' was found
194 by GC/MS (see **Fig. S1**), it was undetectable by HPLC/MS because of the overlapping
195 with the major F6 peak on the reversed-phase column used. Also previously reported
196 minor F1, F3, and F7, which were found in an F-acid concentration from salmon testis
197 lipids by GC/MS (Ota & Takagi, 1991), were not detected in the sample examined in
198 this study by direct analysis of total fatty acids on both HPLC/MS and GC/MS, probably
199 because of their very low amounts being below the detection level. Although
200 quantification was not performed in this study, due to lack of highly pure standards of
201 individual F-acids, peak area percentages obtained from the EICs resembled the
202 GC/MS data, showing that the most abundant F-acid was F6 (63% by HPLC/MS; 69%
203 by GC/FID), followed by F4 (19%, 22%) and F5 (10%, 5%) (**Table 1**). GC/FID analysis
204 of a purified F-acid fraction of salmon testis fatty acids also gave a similar profile,
205 showing F6 (61%), F4 (20%) and F5 (8.4%) as the major ones (Ota & Takagi, 1991).

206 The TIC, EIC and mass spectrum obtained by HPLC/ESI-Q-TOF-MS also
207 suggested the occurrence of two unique F-acids having a hydroxyl or a double bond
208 conjugated to furan ring in the carboxyl side chain (**Fig. 4**), that is,
209 12,15-epoxy-13,14-dimethyleicosa-10,12,14-trienoic acid ($C_{22}H_{36}O_3$, $[M - H]^-$ at m/z
210 347.2568; calculated exact mass: m/z 347.2591) and
211 3-hydroxy-14,17-epoxy-15,16-dimethydocosa-14,16-dienoic acid ($C_{24}H_{42}O_4$, $[M - H]^-$
212 at m/z 393.2992; calculated exact mass: m/z 393.3005), which had not been found
213 previously from salmon lipids. Schodel and Spiteller (1987) reported that
214 transesterification procedures may generate artifacts in the form of unsaturated
215 F-acids with a double bond in the carbon chains. Ishii, Okajima, Koyamatsu, Okada,
216 and Watanabe (1988) reported that unsaturated F-acids are artifacts which were

217 produced during GC analysis. Spiteller (2005) also reported that such unsaturated and
218 hydroxyl F-acids are artifacts detectable after processing biological materials. On the
219 other hand, Boselli, Grob, and Lercker (2000) confirmed that F-acids with unsaturation
220 in the alkyl and carboxyl side chains were naturally occurring in olive oil and were not
221 formed during sample preparation. The occurrence of unsaturated F-acids has also
222 been reported in Adriatic fish (Pacetti, Alberti, Boselli, & Frega, 2010). F-acids with
223 unusual highly unsaturated side chains in alpha-positions were isolated from marine
224 sponges (Ciminiello et al., 1991). Confirmation that the unique F-acids suggested in
225 the chum salmon sample examined in this study are artifacts produced during
226 experiments or naturally occurring compounds is now under investigation.

227 Subsequently, the present method was applied to some New Zealand fish oils, in
228 which F-acids had previously not been known to exist, and we found for the first time
229 several F-acid homologs in the roe and liver oils of two edible fish, hoki and school
230 shark. In the hoki roe oil (**Fig. 5**, upper panel) and school shark liver oil (**Fig. 5**, lower
231 panel), F2, F4, F5, and F6 were the main F-acids; these profiles were similar to those
232 of the salmon F-acids (see **Fig. 3**), while the hoki liver oil contained F2 and F5 as the
233 major ones (Fig. 5, middle panel). The different F-acid compositions in the different
234 tissues of the same species suggest different metabolism and specific functions of
235 F-acids in the tissues. It has been suggested that plants, algae and bacteria via the
236 food chain are the main source of F-acids in animals and fish, especially F-acids
237 having a pentyl side chain (e.g., F2, F5, F6, F8), which the precursor fatty acids are
238 *cis*-vaccenic acid (18:1n-7) or linoleic acid (18:2n-6). In fish, the origin of F-acids
239 having a propyl side chain (e.g., F4) is less certain, but they may be derived from
240 metabolism of 9,12-hexadecadienoic acid (16:2n-4) in algae such as *Phaeodactylum*

241 *tricornutum* (Christie, 2013; Spiteller, 2005), although almost no 16:2n-4 has been
242 detected in the fish samples examined in this study and previous reports (Miller, Perry,
243 Burgess, & Marshall, 2011; Ota & Takagi, 1991; Tenikoff et al., 2005).

244

245 **4 Conclusion**

246 To our knowledge, this is the first report on the detection and identification of
247 F-acids by HPLC/MS. The method is based on direct detection of a trace amount of
248 F-acids in total fatty acids from fish lipids without any concentration or isolation and on
249 their unambiguous identification using their exact masses that differ from those of
250 straight-chain saturated and unsaturated fatty acids. The present study demonstrates
251 that the HPLC/high-resolution MS method is simple and rapid and can be used widely
252 to screen F-acids in biological samples. Complete validation of the method remains a
253 challenging task to quantify exactly small amounts of F-acids in fish and food samples.

254

255 **Declaration of interests**

256 The authors declare no conflict of interests with other people or organizations.

257

258 **Acknowledgements**

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266 **Appendix A. Supplementary data**

267 Supplementary data associated with this article can be found, in the online version,
268 at <http://dx.doi.org/10.1016/j.foodchem.2018.01.044>.

269

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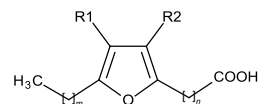
Highlights

- A convenient method to screen bioactive furan fatty acids in fish was developed.
- For this purpose, reversed-phase HPLC/high-resolution ESI-Q-TOF-MS was used.
- Accurate assignments of furan fatty acids were achieved by measuring exact masses.
- Clear extracted ion chromatograms were obtained for all furan fatty acid homologs.
- The method was successfully applied to Japanese and New Zealand fish oil samples.

Table 1 Structures of common furan fatty acids (F-acids) found in fish lipids and the F-acids detected in the chum salmon testis lipids by HPLC/negative ESI-Q-TOF-MS, along with interfering straight-chain fatty acids.

Retention time (min)	Chemical name	Abbreviation ^a	Alkyl substituent				Molecular formula	Selected ion, [M-H] ⁻			Intensity (peak area %)	
			m	n	R ₁	R ₂		Calcd (m/z)	Found (m/z)	Error (10 ⁻³ u)	HPLC/MS	GC/MS
–	10,13-Epoxy-11,12-dimethylhexadeca-10,12-dienoic acid	F1	2	8	CH ₃	CH ₃	C ₁₈ H ₃₀ O ₃	281.2122	nd ^b	–	nd	nd
2.5	10,13-Epoxy-11-methyloctadeca-10,12-dienoic acid	F2	4	8	H	CH ₃	C ₁₉ H ₃₂ O ₃	307.2279	307.2258	2.1	0.9	1.3
–	10,13-Epoxy-11,12-dimethyloctadeca-10,12-dienoic acid	F3	4	8	CH ₃	CH ₃	C ₂₀ H ₃₄ O ₃	321.2436	nd	–	nd	nd
2.9	12,15-Epoxy-13,14-dimethyloctadeca-12,14-dienoic acid	F4	2	10	CH ₃	CH ₃	C ₂₀ H ₃₄ O ₃	321.2436	321.2433	0.3	19.2	22.0
3.7	12,15-Epoxy-13-methyleicosa-12,14-dienoic acid	F5	4	10	H	CH ₃	C ₂₁ H ₃₆ O ₃	335.2592	335.2583	0.9	10.0	4.5
4.3	12,15-Epoxy-13,14-dimethyleicosa-12,14-dienoic acid	F6	4	10	CH ₃	CH ₃	C ₂₂ H ₃₈ O ₃	349.2748	349.2760	-1.2	63.3	68.6
–	14,17-Epoxy-15,16-dimethyleicosa-14,16-dienoic acid	F6'	2	12	CH ₃	CH ₃	C ₂₂ H ₃₈ O ₃	349.2748	nd	–	nd	1.3
–	14,17-Epoxy-15-methyldocosa-14,16-dienoic acid	F7	4	12	H	CH ₃	C ₂₃ H ₄₀ O ₃	363.2905	nd	–	nd	nd
6.9	14,17-Epoxy-15,16-dimethyldocosa-14,16-dienoic acid	F8	4	12	CH ₃	CH ₃	C ₂₄ H ₄₂ O ₃	377.3061	377.3022	3.9	6.6	2.3
3.5	13-Hydroxy-14,17-epoxy-15,16-dimethyldocosa-14,16-dienoic acid ^c	–	4	–	CH ₃	CH ₃	C ₂₄ H ₄₂ O ₄	393.3005	393.2992	1.3	tr ^d	nd
3.5	12,15-Epoxy-13,14-dimethyleicosa-10,12,14-trienoic acid ^c	–	4	–	CH ₃	CH ₃	C ₂₂ H ₃₆ O ₃	347.2591	347.2569	2.2	tr	nd
4.4	Eicosadienoic acid ^e	20:2	–	–	–	–	C ₂₀ H ₃₆ O ₂	307.2642	307.2638	0.4	tr	nd
7.1	Docosadienoic acid ^e	22:2	–	–	–	–	C ₂₂ H ₄₀ O ₂	335.2956	335.2933	2.3	tr	nd

^a For furan fatty acids (F1-F8), see Glass et al. (1974).



^b nd, Not detected.

^c The structures were tentatively identified based on their high-resolution MS data obtained in this study and the literature data (Ishii et al., 1998; Spiteller, 2005; Boselli et al., 2000; Pacetti et al., 2010).

^d tr, Trace amount.

^e The double bond positions were not determined in this study.

1 **Figure legends**

2

3 **Fig. 1** HPLC/negative ESI-Q-TOF-MS profile of the synthetic F-acid,
4 9,12-epoxyoctadeca-9,11-dienoic acid (25 ng). Upper panel, total ion chromatogram
5 (TIC); middle panel, extracted ion chromatogram (EIC) for $[M - H]^- \pm 0.01$ (m/z
6 239.2122 ± 0.01); lower panel, mass spectrum of the peak.

7

8 **Fig. 2** Total ion chromatogram (TIC) of the total fatty acids prepared from the chum
9 salmon testis lipids by HPLC/negative ESI-Q-TOF-MS and the extracted ion
10 chromatograms for each deprotonated molecule ($[M - H]^- \pm 0.01$ m/z) of furan fatty
11 acids (F2–F8).

12

13 **Fig. 3** Comparison of the extracted ion chromatograms for each deprotonated
14 molecule of the furan fatty acids from the chum salmon testis lipids between narrow
15 and wider extraction widths. Upper panel: $[M - H]^- \pm 0.01$ m/z ; lower panel: $[M - H]^- \pm$
16 0.2 m/z .

17

18 **Fig. 4** Extracted ion chromatograms for each deprotonated molecule ($[M - H]^- \pm 0.01$
19 m/z) of the two unique furan fatty acids suggested in the chum salmon testis lipids,
20 along with their mass spectrum. Upper panel:

21 12,15-epoxy-13,14-dimethyleicosa-10,12,14-trienoic acid ($C_{22}H_{36}O_3$); middle panel:
22 13-hydroxy-14,17-epoxy-15,16-dimethydocosa-14,16-dienoic acid ($C_{24}H_{42}O_4$); lower
23 panel: mass spectrum obtained from the trace between 3.4 and 3.5 min of the total ion
24 chromatogram (see **Fig. 2**) and the peak (3.5 min) of the extracted ion chromatograms.

25 The double bond and hydroxyl group positions in the alkyl side chain were tentatively

26 identified based on literature data (Ishii et al., 1998; Spiteller, 2005; Boselli et al., 2000;
27 Pacetti et al., 2010). HPLC and MS conditions are the same as those in **Fig. 2**.

28

29 **Fig. 5** Extracted ion chromatograms for each deprotonated molecule ($[M - H]^- \pm 0.01$
30 m/z) of furan fatty acids from New Zealand materials. Upper panel: hoki roe oil; middle
31 panel: hoki liver oil; lower panel: school shark liver oil. HPLC and MS conditions are the
32 same as those in **Fig. 2**.

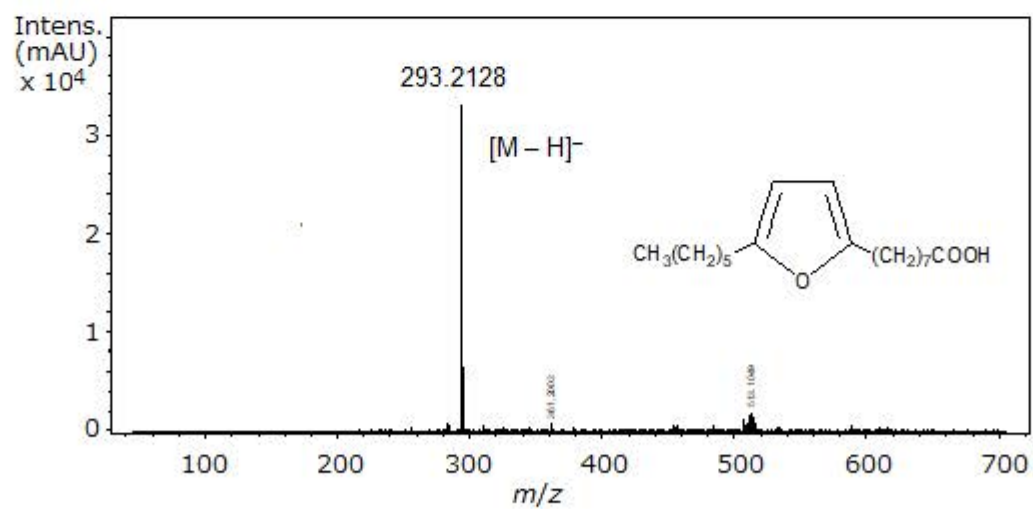
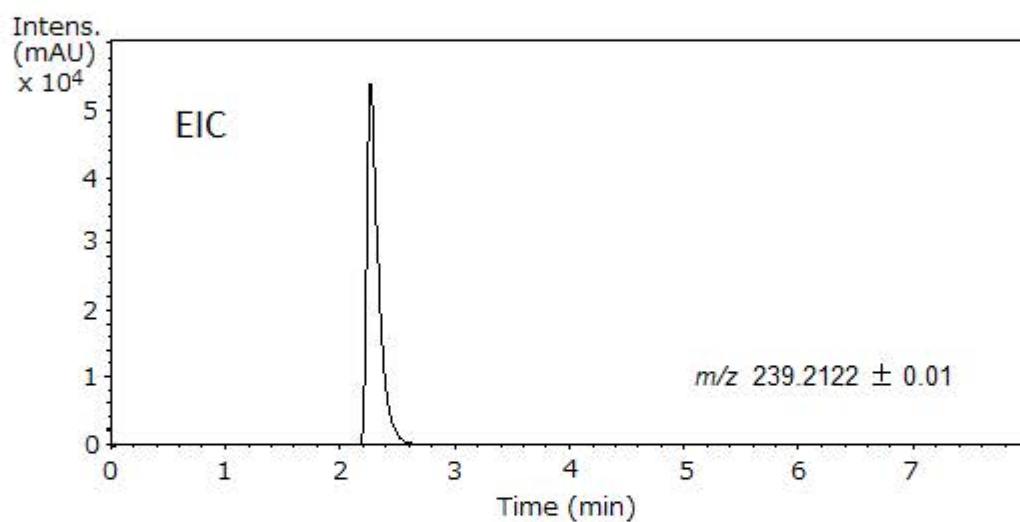
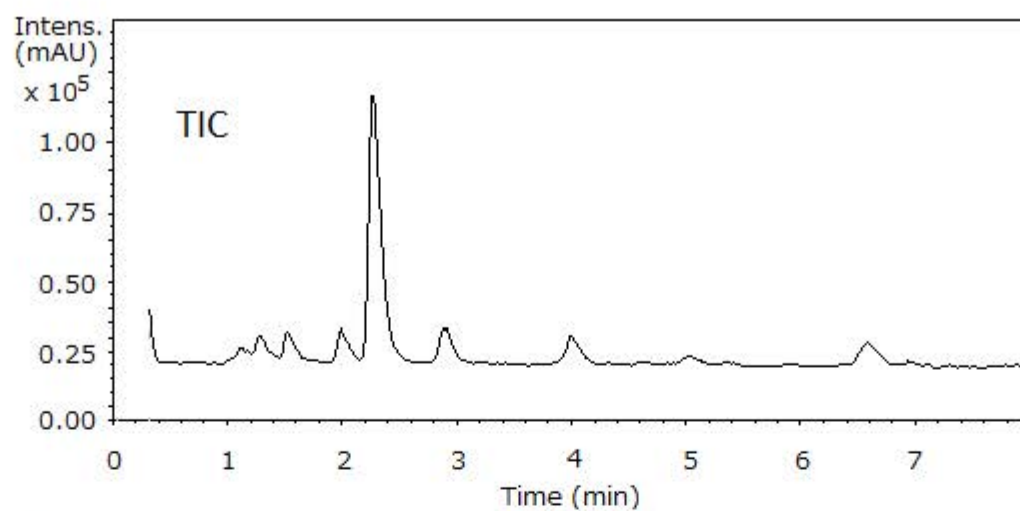


Fig. 1

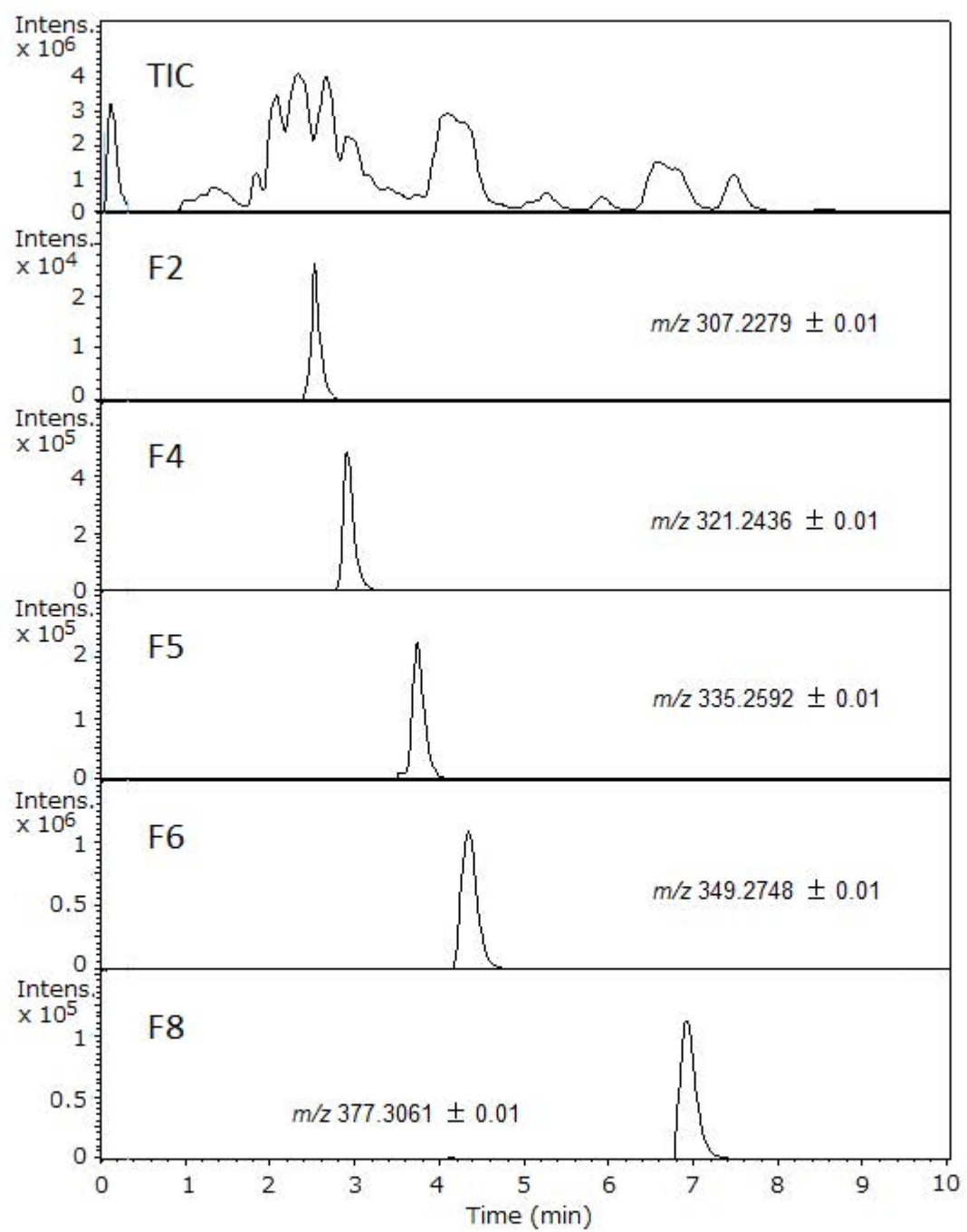


Fig. 2

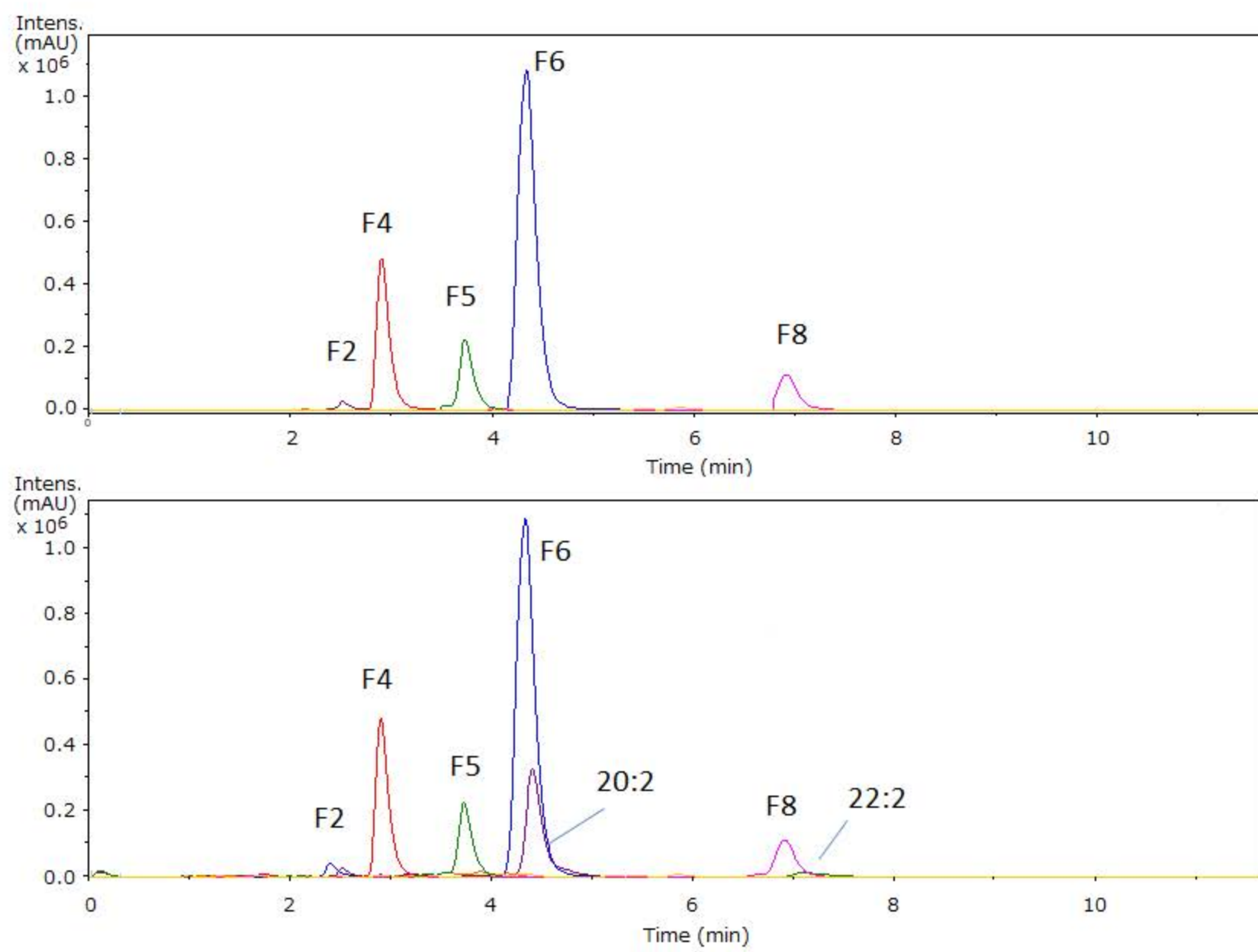


Fig. 3

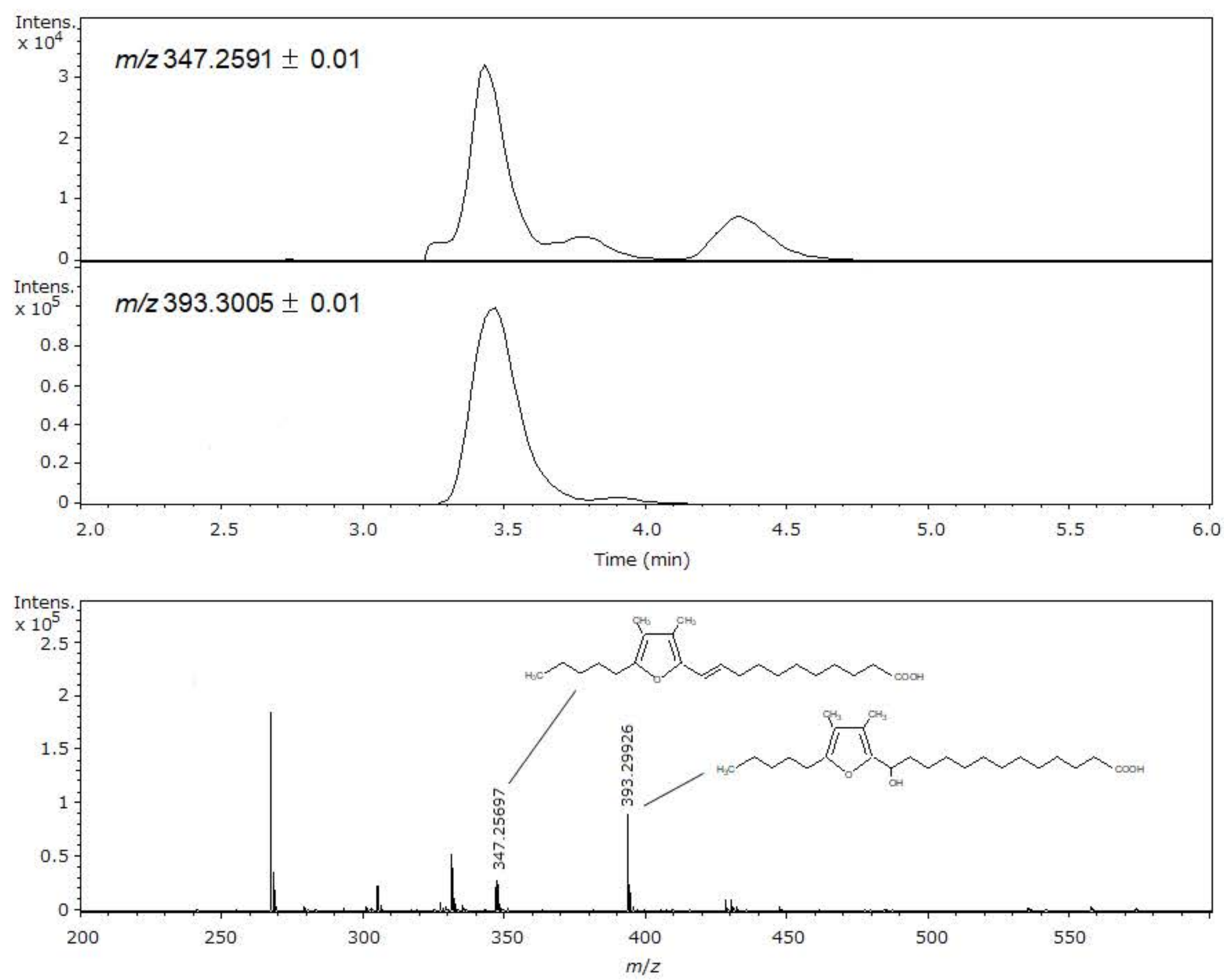


Fig. 4

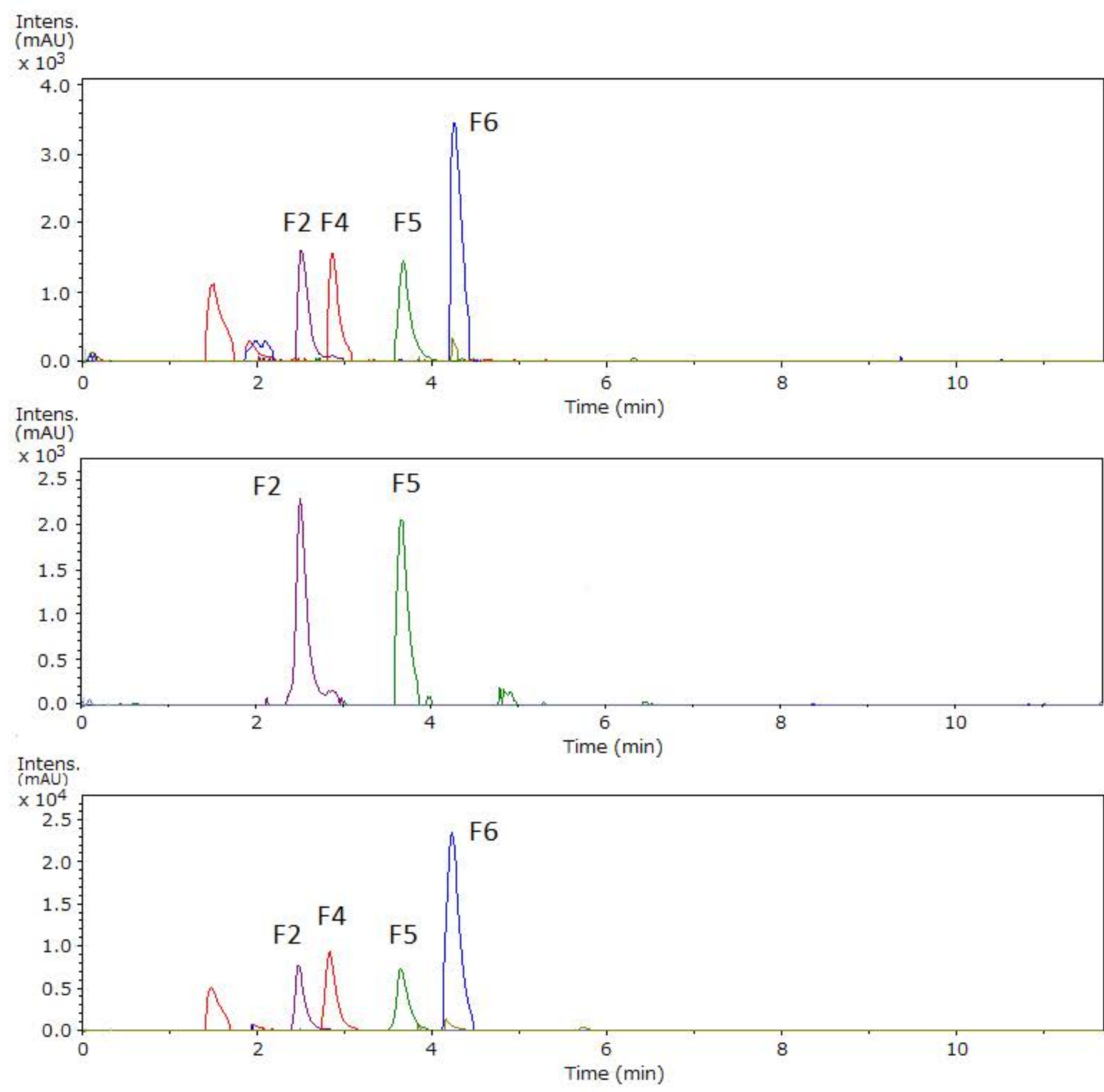


Fig. 5

1 **Supplementary data**

2

3 **Fig. S1** GC/MS identification of furan fatty acids (as methyl esters) from salmon testis
4 lipids. GC and GC/MS conditions are given in text. Molecular ion: m/z 322 (F2), 336
5 (F4), 350 (F5), 364 (F6), 364 (F6'), 392 (F8). M – (alkyl ester): m/z 165 (F2), 151 (F4),
6 165 (F5), 179 (F6), 151 (F6'), 179 (F8). M – (alkyl): m/z 265 (F2), 307 (F4), 293 (F5),
7 307 (F6), 336 (F6'), 335 (F8). For details of the characteristic ions of furan fatty acids,
8 see the reference: Glass, R. L., Krick, T. P., Sand, D., Rahn, C. H., & Schlenk, H.
9 (1975), Furanoid fatty acids from fish lipids. *Lipids*, 10(11), 695–702.

10

11 **Fig. S2** Total ion chromatogram of the total fatty acids prepared from the chum
12 salmon testis lipids by HPLC/negative ESI-Q-TOF-MS and the extracted ion
13 chromatograms for each deprotonated molecule ($[M - H]^- \pm 0.2 m/z$) of furan fatty
14 acids.

15

16 **Fig. S3** Extracted ion chromatograms of the F2 ($C_{19}H_{32}O_3$) and 20:2 ($C_{20}H_{36}O_2$) fatty
17 acids that have the same nominal mass (307). Upper panel: $[M - H]^- \pm 0.01 m/z$; lower
18 panel: $[M - H]^- \pm 0.2 m/z$. Calculated exact mass: m/z 307.2279 for F2; m/z 307.2642
19 for 20:2. Observed mass: m/z 307.2258 for F2; m/z 307.2638 for 20:2.

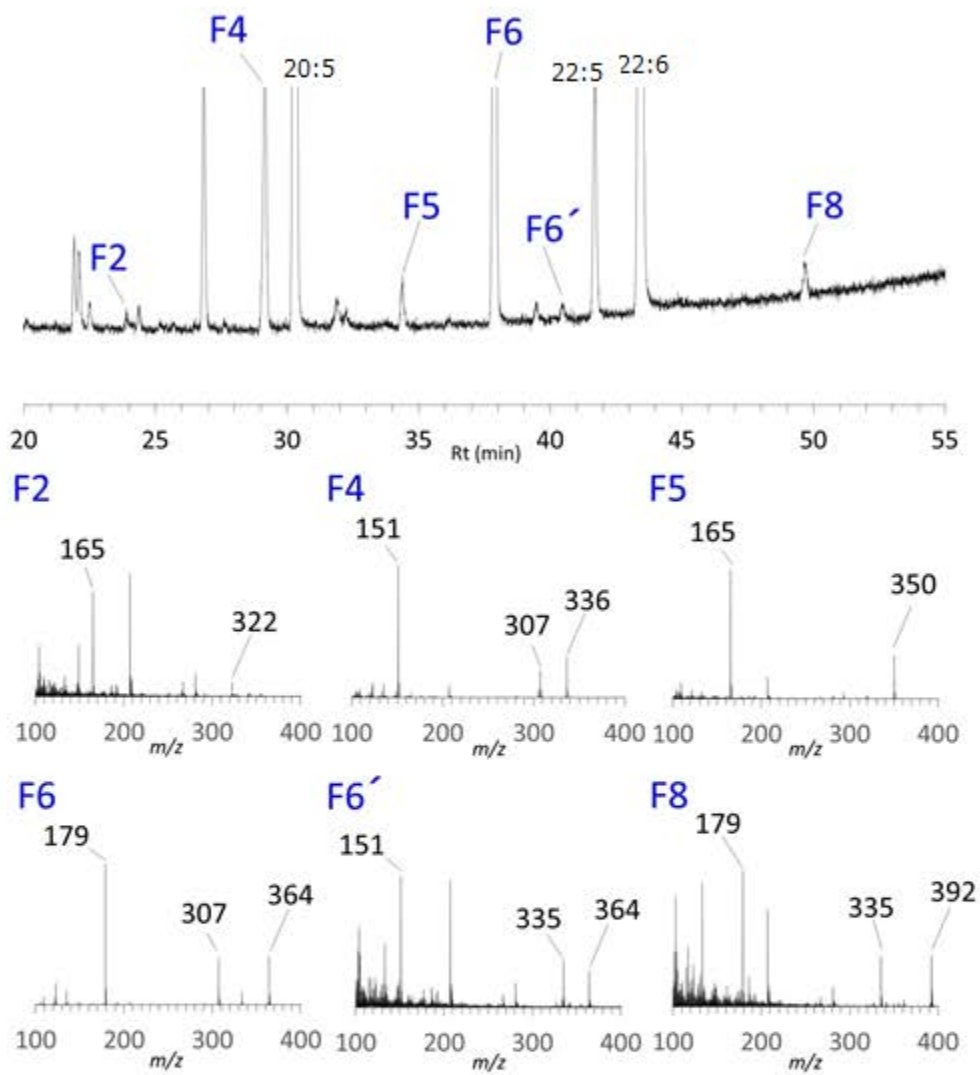


Fig. S1

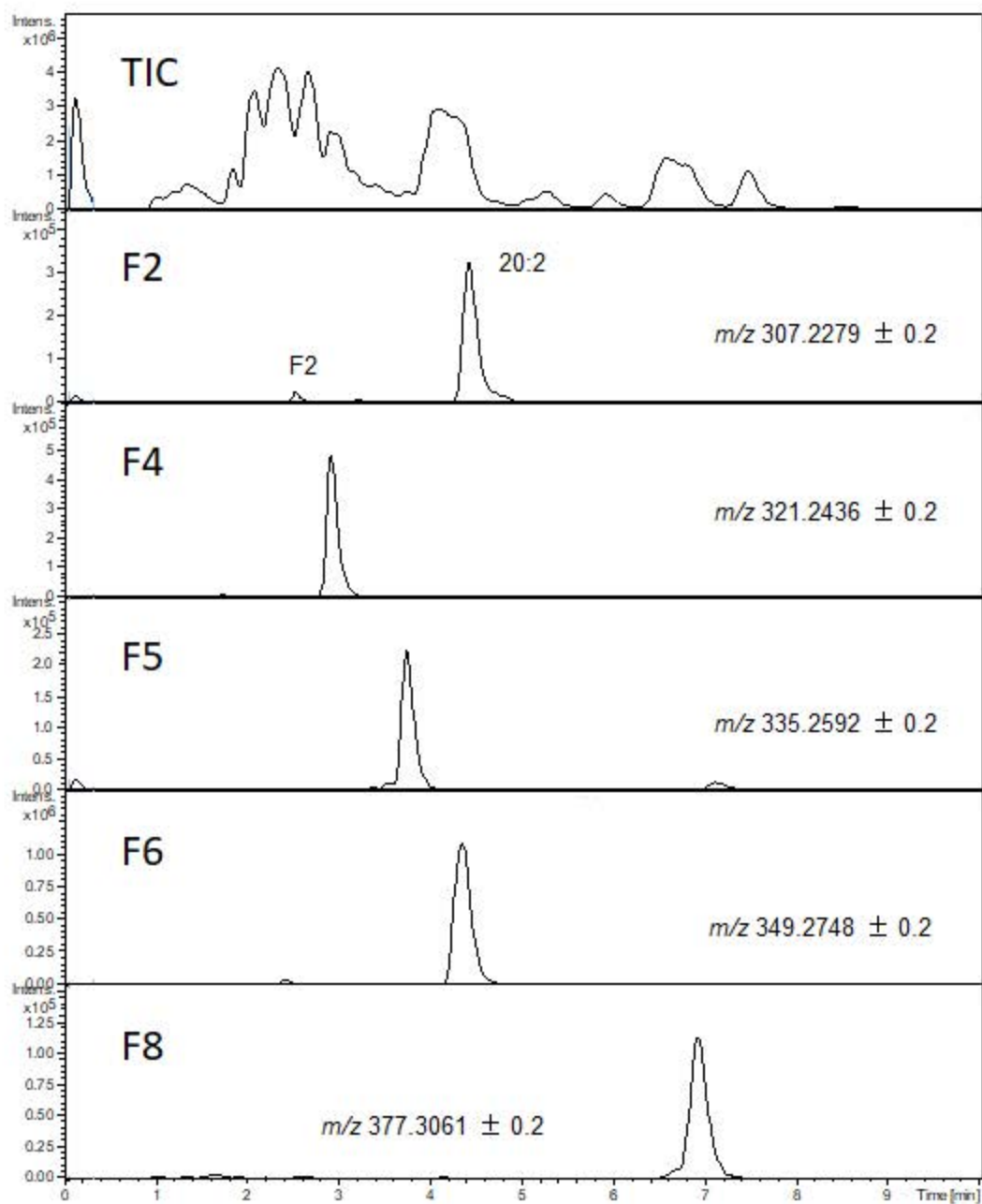


Fig. S2

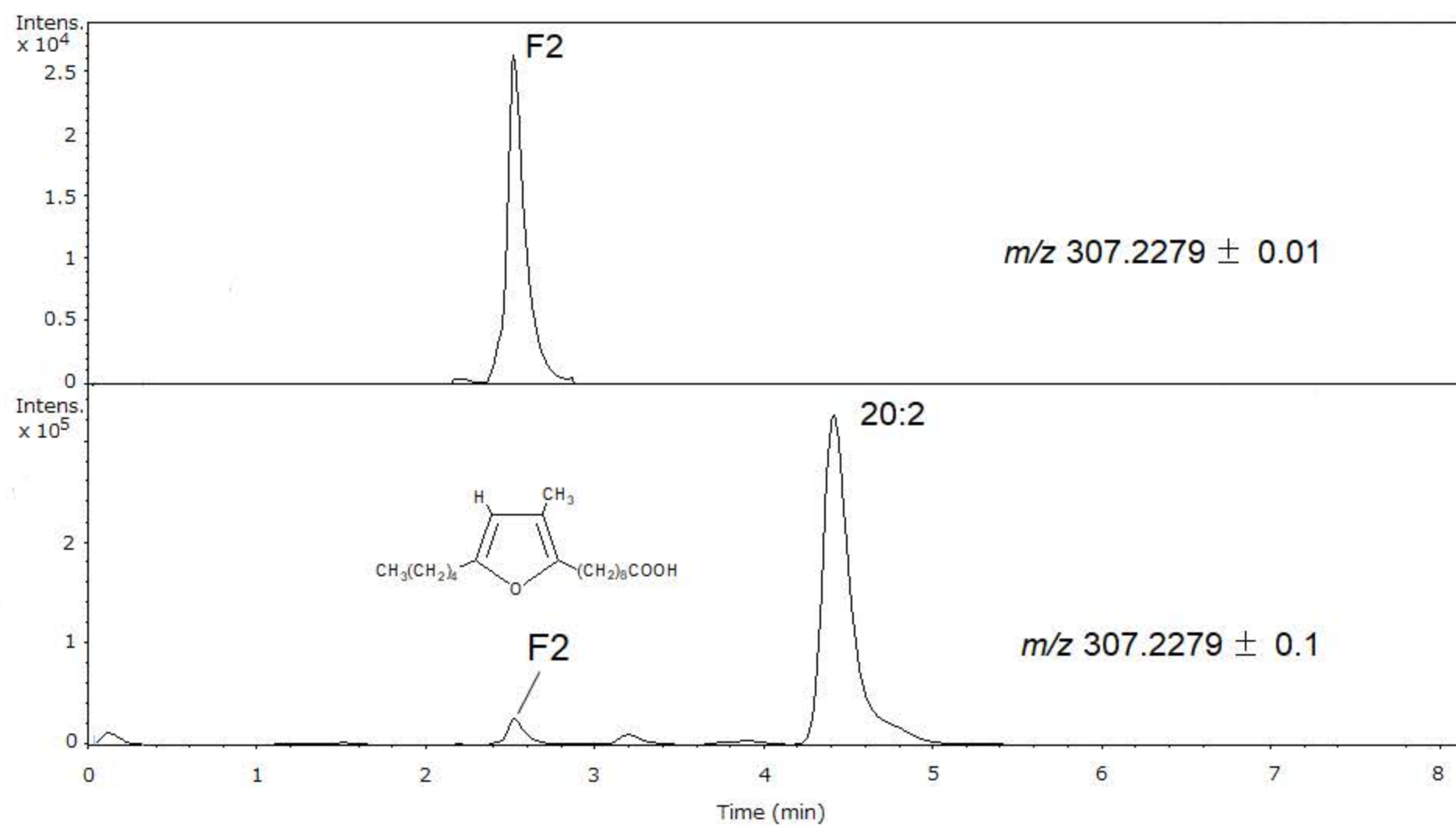


Fig. S3