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

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

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ABSTRACT

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

Keywords:

Furan fatty acids

High-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry

Fish lipids

*Oncorhynchus keta**Macruronus novaezelandiae**Galeorhinus galeus*

1. Introduction

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

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

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

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

2. Materials and methods

2.1. Fish samples

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

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

2.2. HPLC/ESI-Q-TOF-MS

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

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

2.2. GC and GC/MS

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

3. Results and discussion

3.1 Structures of the major furan fatty acids in fish

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

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

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

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

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

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

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

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

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

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

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

4 Conclusion

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

Declaration of interests

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

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Appendix A. Supplementary data

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

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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.

Figure legends

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

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

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

Fig. 4 Extracted ion chromatograms for each deprotonated molecule ($[M - H]^- \pm 0.01$ m/z) of the two unique furan fatty acids suggested in the chum salmon testis lipids, along with their mass spectrum. Upper panel: 12,15-epoxy-13,14-dimethyleicosa-10,12,14-trienoic acid ($C_{22}H_{36}O_3$); middle panel: 13-hydroxy-14,17-epoxy-15,16-dimethydocosa-14,16-dienoic acid ($C_{24}H_{42}O_4$); lower panel: mass spectrum obtained from the trace between 3.4 and 3.5 min of the total ion chromatogram (see **Fig. 2**) and the peak (3.5 min) of the extracted ion chromatograms. The double bond and hydroxyl group positions in the alkyl side chain were tentatively

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

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

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Supplementary data

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

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

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

Fig. S1

Fig. S2

Fig. S3