Identification of new, very long-chain polyunsaturated fatty acids in fish by gas chromatography coupled to quadrupole/time-of-flight mass spectrometry with atmospheric pressure chemical ionization

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Abstract
The characterization of very long-chain (>C24) polyunsaturated fatty acids (VLC-PUFAs), which are essential in the vision, neural function, and reproduction of vertebrates, is challenging because of the lack of reference standards and their very low concentrations in certain lipid classes. In this research, we have developed a new methodology for VLC-PUFA identification based on gas chromatography coupled to quadrupole/time-of-flight mass spectrometry with an atmospheric pressure chemical ionization source (GC-APCI-QTOF MS). The mass accuracy attainable with the innovative QTOF instrument, together with the soft ionization of the APCI source, provides valuable information on the intact molecule, traditionally lost with electron ionization sources due to the extensive fragmentation suffered. We have identified, for the first time, VLC-PUFAs with chains up to 44 carbons in eyes, brain, and gonads of gilthead sea bream, a commercially important fish in the Mediterranean. The added value of ion mobility-mass spectrometry (IMS), recently developed in combination with GC-QTOF MS, and the contribution of the collisional cross section (CCS) parameter in the characterization of novel VLC-PUFAs (for which reference standards are not available) have been also evaluated. The methodology developed has allowed assessing qualitative differences between farmed and wild fish, and opens new perspectives in a still scarcely known field of research.

Keywords Gas chromatography · Quadrupole/time-of-flight mass spectrometer · Very long-chain polyunsaturated fatty acids · Fish

Introduction
The existence of very long-chain polyunsaturated fatty acids (VLC-PUFAs) ranging from 26 to 36 carbons in length in mammal spermatozoa and vertebrate retina was reported in 1986–1987 by Pouls et al. [1, 2] and Aveldaño and Sprecher [3]. Since then, low concentrations of up to C38 VLC-PUFAs have been reported in phosphatidylcholine (PC) of human brain [4]; triacylglycerides (TG) and cholesterol esters (CHE) of mammalian testis [5]; sperm and brain of mammals; PC, sphingomyelin (SM), and cerebrosides (CE) of human retina [6–9]; and PC of fish retina [10, 11]. VLC-PUFAs have been associated with macular dystrophy [6] and Zellweger’s syndrome [4], and are considered essential for the development of the nervous system, retinal function, and reproduction in vertebrates [10, 12–14]. It is widely accepted that, rather than deriving from diet, VLC-PUFAs are produced endogenously (biosynthesis) by successive elongations from C20 to C22 PUFAs, where eicosapentaenoic acid (C20:5n-3) arises as a preferred substrate for elongation to VLC-PUFAs over C20:4n6 (arachidonic acid) and C22:6n3 (docosahexaenoic acid) [7, 15]. The elongation of very long-chain fatty acid protein 4 (Elovl4) plays a major role in VLC-PUFA biosynthesis in vertebrates [6, 7, 10, 16]. Notably, mutations in the murine Elovl4 gene reduce the...
content of VLC-PUFAs in the retina, leading to vision loss [17]. Fish Elovl4 also participate in VLC-PUFA metabolism and are able to elongate C20 and C22 PUFAs to polyenes of up to 36 carbons [16]. Moreover, the pattern of elovl4 expression shows that VLC-PUFA metabolism in fish occurs primarily in the brain, eye, and gonads [18–21], where these compounds may exert pivotal biological functions despite their relative low abundance in both saturated and polyunsaturated chains [22]. Functions of VLC-PUFAs can be associated with their unique physical characteristics, as their long chains allow them to simultaneously have properties characteristic of both saturated and polyunsaturated chains [22].

The very low concentrations of VLC-PUFAs in specific classes of cellular lipids of vertebrates together with the lack of reference standards have made the characterization of these compounds challenging. Gas chromatography coupled to mass spectrometry (GC-MS) with electron ionization (EI) is the most commonly used technique for analyzing VLC-PUFAs [15]. Nevertheless, the extensive fragmentation of these compounds in the EI source leads to the partial or total loss of diagnostic molecular ions (M+) from the spectrum [23]. In addition, the low resolution of simple quadrupole analyzers commonly used until the last decade has made difficult the identification of VLC-PUFAs. Yet, APCI has been revealed in the last years as a deserving rival to the widely accepted EI source, as it promotes the formation of an abundant molecular ion (M+) and/or protonated molecule ([M+H]+). This results in (i) a better sensitivity/specificity in target SRM methodologies and (ii) enhanced identification capabilities, especially when coupled to high-resolution mass spectrometers (HRMS) as hybrid quadrupole time-of-flight (QTOF) [24–26]. The late elution of VLC-PUFAs supposes an additional problem because their chromatographic separation is challenging due to the column degradation after the separation of a high amount of short-chain fatty acids.

In this work, we have developed advanced methodology for the identification of VLC-PUFAs, based on GC-APCI-QTOF MS, in biological samples. Gilthead sea bream was selected because of its relevance in the Mediterranean aquaculture and because it is a valuable model to investigate how the current feed formulations containing high vegetable oil levels can impact the VLC-PUFA metabolism in farmed fish [27, 28]. We herein illustrate that GC-APCI-QTOF MS, together with the use of a chromatographic column suitable for temperatures up to 400 °C, allows the detection and identification of novel VLC-PUFAs, and the assessment of qualitative differences between farmed and wild fish. As a novel application, the contribution of IMS in combination with GC-APCI-QTOF MS, as well as the role of the collisional cross section (CCS), has been evaluated for the characterization of VLC-PUFAs with chains up to 44 carbons, reported for the first time in eyes, brain, and gonads of gilthead sea bream.

**Experimental**

**Samples and samples’ preparation**

As mentioned above, VLC-PUFAs are endogenously produced in vivo by the action of an enzyme called Elovl4 [6, 7, 10, 16]. Thus, we first isolated the gene encoding an Elovl4 from gilthead sea bream (*Sparus aurata*) and expressed its coding region in yeast *Saccharomyces cerevisiae* to generate a set of VLC-PUFA standards. Individual cultures of transgenic yeast containing the sea bream elovl4 were grown in the presence of one of the following polyunsaturated fatty acid (PUFA) substrates: C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-3, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, C22:6n-3, and C24:5n-3. Sea bream Elovl4, like all Elovl enzymes [16], can generate a series of stepwise elongation products varying in 2-carbon units, and therefore, we were able to produce polyenes up to 36 carbons [29] of the following series: 2n-6, 3n-3, 3n-6, 4n-3, 4n-6, 5n-3, and 6n-3. These compounds can be unequivocally identified using MSD EI standard conditions and thus easily located in the chromatogram, since only the transgene (gilthead sea bream elovl4) and not the *S. cerevisiae* endogenous enzymes recognize the exogenously supplemented PUFAs as metabolic substrates.

Adult specimens of gilthead sea bream were dissected and eyes, brains, and gonads were collected and stored at −20 °C until further analysis. Crystalline lenses were removed from eyes. Total lipids were extracted using the method of Folch et al. [30]. Subsequently, an aliquot of total lipids (~200 mg) was further developed by thin-layer chromatography (TLC 20 × 20 silica gel G60, Merck, Darmstadt, Germany) using a polar solvent system (methyl acetate:propan-2-ol:chloroform:methanol: 0.25% (w/v) aqueous KCI (25:25:25:10:9 by vol). The three fractions where VLC-PUFAs were detected in previous analyses, namely SM, PC, and CE, were scrapped off the plate [31], eluted in chloroform:methanol (2:1, v/v) containing BHT (0.01%, w/v), and used to prepare fatty acid methyl esters (FAMEs) [32]. Methyl esters (ME) were stored in hexane/BHT (0.01%) under nitrogen at ~20 °C (for more details, see Garlito et al. [11]). Prior to the analysis by GC-APCI-QTOF MS and GC-APCI-IMS-QTOF MS, samples were dried with a gentle flow of N2 and reconstituted in 50 μL of hexane.

The experimental procedures were performed in accordance with the principles of the European Animal Directive (86/609/EEC) for the protection of experimental animals [33] and were approved by the Ethics Committees of the Spanish National Research Council (CSIC) and the Institute of Aquaculture of Torre la Sal.
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GC-APCI-(Q)TOF MS

As in a previous work [11], an Agilent 7890 N gas chromatography (Palo Alto, CA, USA) equipped with an Agilent 7683 autosampler was coupled to a quadrupole orthogonal acceleration time-of-flight mass spectrometer, Xevo G2 QTOF (Waters Corporation, Manchester, UK), equipped with APGC v2.0 as ionization source, working in positive APCI mode. A fused silica VFT capillary column with a length of 15 m × 0.32 mm i.d. and a film thickness of 0.10 μm (J&W Scientific, Folson, CA, USA) was used for GC separation. The oven temperature was programmed as follows: 130 °C (1 min); 20 °C/min to 340 °C; 2.5 min with a total runtime of 14 min. Pulsed splitless (25 psi) injections of 1 μL were carried out at 280 °C with a splitless time of 1 min. Helium 99.999% (Praxair, Spain) was used as carrier gas at a flow of 4 mL/min.

The interface and ionization source temperatures were set to 340 °C and 150 °C, respectively. N2 was used as auxiliary gas at 200 L/h, as cone gas at 5 L/h and as make-up gas at 300 mL/min. The APCI corona discharge pin was operated at 1.7 μA and the cone voltage was set to 20 V.

The QTOF was operated at 2.5 spectra/s acquiring the mass range m/z 50–1000. The TOF MS resolution was approximately 15,000 (FWHM) at m/z 264. Acquisition was done in MSE mode in which two alternating acquisition functions with different collision energies were generated: the low-energy (LE) function, selecting a collision energy of 4 eV to avoid or minimize fragmentation, and the high-energy (HE) function, with a collision energy ramp ranging from 25 to 40 eV to obtain a greater range of fragment ions.

Heptacose (Sigma-Aldrich, Madrid, Spain) was used for the daily mass calibration. Internal calibration was performed using octafluoronaphthalene (Sigma-Aldrich, Madrid, Spain) as lock mass (monitoring the molecular ion, m/z 271.9872).

In order to work under proton transfer conditions, an uncapped vial containing water was placed in a designed holder into the APCI source door to enhance protonation. MS data were acquired in centroid.

GC-APCI-IMS-(Q)TOF MS

Chromatograph and autosampler described above were interfaced to a VION IMS-QTOF mass spectrometer (Waters Corporation, Manchester, UK), equipped with APGC v2.0 as ionization source, working in positive APCI mode. Chromatographic conditions were kept as described for the previous instrument.

The APCI corona discharge pin was operated at 2.0 μA and the cone voltage was set to 20 V. The interface and ionization source temperatures were set to 350 °C and 150 °C, respectively. N2 was used as auxiliary gas at 300 L/h, as cone gas at 160 L/h and as make-up gas at 275 mL/min.

MS data were acquired using the VION in high-definition (HD) MSE mode, in the range 50–1000 m/z, with N2 as the drift gas, an IMS wave velocity of 250 m/s, and wave height ramp of 20–50 V. Two independent scans with different collision energies were acquired during the run: a collision energy of 6 eV for LE and a ramp of 20–56 eV for HE. HDMSE implies drift time alignment between LE and HE spectra keeping only fragment ions related to parent ions. The LE and HE functions’ setting was in both cases a scan time of 0.25 s. Argon (≥99.999%) was used as collision-induced dissociation (CID) gas. For MS/MS experiments, the QTOF was operated in daughter scan mode at 2.5 spectra/s acquiring the mass range from m/z 50 to the parent ion. Acquisition was done in MS/MS mode defining different windows for each compound, with a collision energy ramp from 20 to 60 eV to obtain sufficient fragment ions. All data were examined using an accurate mass screening workflow within UNIFI informatics platform from Waters Corporation.

The (de)protonated molecule of leucine-enkephalin, at m/z 556.2771, was used for the calibration of the instrument, which must be made in electrospray ionization mode. Internal calibration was performed using two bleeding ions as lock mass (monitoring the molecular ions, m/z 355.06693 and 223.06365 corresponding to decamethylcyclopentasiloxane and hexamethylcyclotrisiloxane, respectively). Prior to the standard injection, the instrument was calibrated for both m/z measurements and CCS calculation following the manufacturer’s instructions. Then, a “system suitability test” (SST) containing 9 compounds was injected ten times (i.e., n = 10) to check the accuracy of the instrument measurements.

Results and discussion

Detection and identification of VLC-PUFAs

From our previous experience [11] and taking into account the literature regarding PUFAs analysis by GC [8, 23, 24, 26], three chromatographic columns (DB-5MS 60 m × 0.25 mm, 0.25 μm; BD-EN14103 30 m × 0.32 mm, 0.25 μm; VF-5HT 15 m × 0.32 mm, 0.10 μm) were tested. The best results were obtained with the VF-5HT column, which was selected to continue the work. The characteristics of this column allowed to work robustly at high flow rates, up to 4 mL/min, resulting in narrower peaks (around 2 s FWHM) and increased sensitivity, compared to the other columns. Under these conditions, we were able to detect chromatographic peaks (that were expected to correspond to novel VLC-PUFAs) that were not observed using longer column with lower maximum oven temperatures [11]. Table S1 (see Electronic Supplementary Material, ESM) summarizes column properties and outputs.

The investigation of novel VLC-PUFAs (>C38) observed in the studied biological matrices (brain, retina, and gonads) was conducted looking for a chromatographic peak for each of the corresponding [M+H]+ ions using 0.02-Da narrow
window eXtracted Ion Chromatograms (nw-XICs). Figure 1 shows VLC-PUFAs (C38:6-ME-C44:6-ME) detected in several matrices.

The MSE spectra of the different VLC-PUFAs peaks were studied for further identification of the compounds. The mass accuracy of [M+H]+ was evaluated in the low-energy (LE) function, and mass errors were below 3.2 ppm in all cases. It is remarkable that the ions observed corresponded to the protonated molecule in all cases, while M+· was not observed, a fact that sometimes occurs in GC-APCI-MS-based methods [24–26]. This can be explained due to the protonable character of these compounds together with the presence of a vial with water in the APCI source, precisely to promote the formation of the protonated molecules.

Unfortunately, the fragmentation observed in HE function was found to be insufficient for identification purposes. This made unfeasible the application of ion ratio rule developed in our previous work for up to C34 VLC-PUFA [11]. Such evaluation of the ion ratios in HE spectra of analogous shorter chain (<C24) PUFAs had allowed us to predict the positions of key double bonds, enabling the tentative classification of these compounds as n-3 or n-6.

Taking advantage of the full capabilities of the hybrid QTOF analyzer, several experiments were performed selecting [M+H]+ as precursor ions, and using a collision energy ramp. The results obtained improved the possibilities for reliable identification because accurate mass fragment ions, compatible with the chemical structure, were obtained in all cases with low mass errors. Figure 2 shows MS/MS spectrum for [M+H]+ of C42:6-ME (C43H75O2, m/z 623.576). Fragmentation routes based on intramolecular nucleophilic substitution suggest that the detected C42:6-ME is n-3 compound, which is consistent with previous findings in which all the >C24 VLC-PUFAs detected in fish were n-3 species [11]. Fig S1 (see ESM) shows the proposed fragmentation routes that would support this hypothesis. This behavior was also observed for the rest of VLC-PUFAs. Similarly, Fig. S2 (see ESM) shows MS/MS spectrum for [M+H]+ of C40:6-ME (C41H71O2, m/z 623.576). Table 1 shows the new VLC-PUFAs found in all matrices, including molecules with chains between 38 and 44 carbons. These are present in different lipids classes of the brain, eye and gonads of gilthead sea bream.

**Contribution of IMS in combination with GC-APCI-QTOF MS to the characterization of VLC-PUFAs**

Ion mobility spectrometry (IMS) is a powerful technique that has been revisited in the last years [34, 35]. IMS separates ions drifting through a tube filled with buffer gas in a weak electric field according to their shape-to-charge ratio. IMS separates ions on the basis of the size-to-charge ratio in the IMS dimension and on the mass-to-charge ratio in the MS dimension. The mobility of an ion is determined by its mass and charge, and by the collisional cross section (CCS), a specific parameter.
parameter valuable for compound identification that represents the rotationally averaged surface area of an ion [36].

The incorporation of IMS in hyphenated chromatography-mass spectrometry methods significantly improves peak capacity, enabling the separation of isomers, isobars and conformers, as well as reducing background noise, especially when using high-resolution mass spectrometry (HRMS). IMS combined to LC-MS has been recently applied in different fields thanks to the development of several commercial systems, with the ion mobility spectrometers based on drift time IMS (DTIMS) or traveling wave IMS (TWIMS) being the most commonly used [17, 35, 37–39]. On the contrary, the coupling of IMS with GC-MS remains practically unexplored [40]. However, GC can be efficiently coupled to IMS-QTOF making use of an APCI source (GC-APCI-IMS-QTOF), a powerful combination that has been little studied until now.

In this work, the use of CCS in a GC-APCI-IMS-QTOF instrument has been of help for VLC-PUFA identification [37, 41]. This is of especial relevance when reference standards are not available for the final identification. CCS data were automatically translated by the software from drift time values prior instrument calibration to construct the translating equation. Experimental CCS (ESM Table S2) were obtained from the injection of elongation products (see Samples and Samples preparation section). This enabled, apart from identification and retention time confirmation of VLC-PUFAs up to C38, the creation of linear regression models correlating the CCS value with the number of carbons for a given number of unsaturation’s and position of the double bounds. It can be seen (ESM Fig. S3) the good correlation between the number of carbons of the VLC-PUFA and the observed CCS, regardless of the unsaturation degree, double bond position and elongation substrate, with correlation factors greater than 0.99 in all cases. Taking into account that CCS value can differ up to a 2% in replicate analyses of the same compound [37, 42], data represented in Fig. S3 (see ESM) suggest that CCS models can clearly distinguish VLC-PUFA with the same number of carbons and with a difference of, at least, 2 unsaturations.

The created CCS model for 6n3 series was finally applied to the detected C40 and C42 species in fish organs in order to test whether the observed CCS values correlated well to the model. As can be appreciated in Fig. 3 observed CCS values for detected C40 and C42 fall just above the predicted curve,

Table 1  List of new VLC-PUFAs identified in the different classes of lipids in the brain, eye, and gonads of wild and cultured gilthead sea bream

| Compound  | Brain SM | Brain PC | Eye SM | Eye PC | Gonads CE SM | Gonads CE PC |
|-----------|----------|----------|--------|--------|--------------|--------------|
| C38:6n-3  | **       | **       | **     | **     | **           | **           |
| C40:6n-3  | **       | **       | **     | **     | **           | **           |
| C42:6n-3  | **       | **       | **     | **     | **           | **           |
| C44:6a    | *        |          |        |        |              |              |

SM, sphingomyelin; PC, phosphatidylcholine; CE, cerebrosides.

**Detected in wild and cultured fish. *Detected only in wild fish.

aC44:6 was characterized by GC-APCI-QTOF, based only on the accurate mass of the protonated molecule. No fragmentation information was obtained due to the low concentration of the analyte. No IMS information was obtained due to sample limitations.

Fig. 2  GC-APCI-QTOF MS/MS product ion scan (CE ramp 20–60 eV) spectra for the ion m/z 623, corresponding to C42:6n-3 identified in PC of the brain of wild gilthead sea bream.

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and below the superior error limit, confirming they belong to the VLC-PUFA family.

These models are also helpful to avoid reporting false positives, such as other isomers that might be present with the same exact mass. This would be the case of three reported isomers of C42:6n3-ME (C43H75O2) in LipidMaps® and Pubchem databases (i.e. cholest-5-en-3β-yl (9Z-hexadecenoate), cholest-5-en-3β-yl (7Z-hexadecenoate) and (5Z, 7E)-(3S)-9,10-seco-5,7,10(19)-cholestatriene-3-yl hexadecanoate (Vitamin 3 Palmitate). Although these isomers, derived from oleic acid, could be discarded by GC retention time, their different spatial conformation would also imply notable differences in the CCS values compared to C42:6n3-ME.

**Biological interpretation**

The analyses carried out in organs and lipid fractions studied from both cultured and wild fish, except 42:6n-3 that was absent in the SM from the gonads and eyes, and present only in the PC from the gonads. Interestingly, the longer chain 44:6n-3 was only found in the PC fraction of the brain of wild specimens (Table 1). These results are particularly important in light of published information [18–21], since it has been suggested that the presence of adequate amounts of these compounds can impact the health and performance of farmed species. Such differences may reflect the influence of environmental conditions, especially diet, on the composition of VLC-PUFAs in brain, eye, and gonads [27, 28, 42] and highlight the potential negative impacts of current diet formulations that lack sufficient essential VLC-PUFAs precursors (i.e., C20–22 PUFA) due to fishmeal and fish oil substitution on neural function and reproduction. The results from the present work open new perspectives in our understanding of VLC-PUFAs and their impact on basic biological functions. In a practical scenario like fish farming deficiency of key VLC-PUFAs can translate into altered visual acuity (critical in visual predators including most cultured fish species), fertility issues of broodstock, and disruptions of brain functioning that can affect growth performance and eventually the economic profits.

**Conclusions**

In this research, we have developed advanced analytical methodology based on GC-APCI-QTOF MS for reliable identification of VLC-PUFAs. The methodology developed has allowed, for the first time, the identification of VLC-PUFAs with chains up to 44 carbons long in the SM and PC of the eyes, brain, and gonads of gilthead sea bream based on their accurate mass and fragmentation pattern.

The quality of the analytical information obtained is the result of the high mass accuracy attainable with QTOF instrument, together with the soft ionization of the APCI source for GC, which provides valuable information from the intact molecule, combined with a thorough sample treatment process and optimized chromatographic separation. The application of ion mobility separation in the GC-HRMS system offers the possibility to use the CCS values to help to confirm that compounds detected did belong to the VLC-PUFA family. Additionally, the full characterization of the abovementioned substrates with very long carbon chains can provide useful information for the scientific community. The application of this method has allowed the assessment of qualitative differences between farmed and wild fish, opening new perspectives in a still incipient field of research involving the role of VLC-PUFAs.

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Data availability Data and material are available.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Code availability Not applicable

References

1. Poulos A, Sharp P, Johnson D, White I, Fellenberg A. The occurrence of polyenic fatty acids with greater than 22 carbon atoms in mammalian spermatozoa. Biochem J. 1986;240:891–5.
2. Poulos A, Johnson DW, Beckman K, White IG, Easton C. Occurrence of unusual molecular species of sphingomyelin containing 28–34 carbon polyenoic fatty acids in ram spermatozoa. Biochem J. 1987;248:961–4.
3. Aveladano MI, Sprecher H. Very long chain (C24 to C36) polyenoic fatty acids of the n-3 and n-6 series in dipolyunsaturated phosphatidylcholines from bovine retina. J Biol Chem. 1987;262:1180–6.
4. Poulos A, Sharp P, Johnson D, Easton C. The occurrence of polyenoic very long chain fatty acids with greater than 32 carbon atoms in molecular species of phosphatidylcholine in normal and peroxisome-deficient (Zellweger’s syndrome) brain. Biochem J. 1988;253:645–50.
5. Furland NE, Maldonado EN, Aveladano MI. Very long chain PUFA in murine testicular triglycerides and cholesterol esters. Lipids. 2003;38:73–80.
6. Agbagha M-P, Brush RS, Mandal MNA, Henry K, Elliot MH, Anderson RE. Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. Proc Natl Acad Sci U S A. 2008;105:12843–8.
7. Agbagha M-P, Mandal MNA, Anderson RE. Retinal very long-chain PUFA: new insights from studies on ELOVL4 protein. J Lipid Res. 2010;51:1624–42.
8. Liu A, Chang J, Lin Y, Shen Z, Bernstein PS. Long chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. J Lipid Res. 2010;51:3217–29.
9. Liu A, Terry R, Lin Y, Nelson K, Bernstein PS. Comprehensive and sensitive quantification of long-chain and very long-chain polyunsaturated fatty acids in small samples of human and mouse retina. J Chromatogr A. 2013;1307:191–200.
10. Deák F, Anderson RE, Fessler JL, Sherry DM. Novel cellular functions of very long chain fatty acids: insight from ELOVL4 mutations. Front Cell Neurosci. 2019;13:428.
11. Garlito B, Portolés T, Niessen WMA, Navarro JC, Hontoria F, Monroig O, et al. Identification of very long-chain (>C24) fatty acid methyl esters using gas chromatography coupled to quadrupole/time of flight mass spectrometry with atmospheric pressure chemical ionization source. Anal Chem Acta. 2019;1051:103–9.
12. Poulos A. Very long chain fatty acids in higher animals—a review. Lipids. 1995;30:1–14.
13. Harkewicz R, Du H, Tong Z, Alkuraya H, Bedell M, Sun W, et al. Essential role of ELOVL4 protein in very long chain fatty acid synthesis and retinal function. J Biol Chem. 2012;287:11469–80.
14. Barabas P, Liu A, Xing W, Chen C-K, Tong Z, Watt CB, et al. Role of ELOVL4 and very long-chain polyunsaturated fatty acids in mouse models of Stargardt type 3 retinal degeneration. Proc Natl Acad Sci U S A. 2013;110:5181–6.
15. Tejada-Casado C, Hernández-Mesa M, Monteau F, Lara FJ, del Olmo-Iruela M, García-Campaña AM, et al. Collision cross section (CCS) as a complementary parameter to characterize human and veterinary drugs. Anal Chem Acta. 2018;1043:52–63.
16. Castro LFC, Tocher DR, Monroig O. Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire. Prog Lipid Res. 2016;62:25–40.
17. Paglia G, Angel P, Williams JP, Richardson K, Olivos HJ, Thompson JW, et al. Ion-mobility-derived collision cross section as an additional measure for lipid fingerprinting and identification. Anal Chem. 2017;89:1137–44.
18. McMahon A, Jackson SN, Woods AS, Kedzierski WA. A Stargardt disease-3 mutation in the mouse Elovl4 gene causes retinal deficiency of C-32-C36 acyl phosphatidylcholines. FEBS Lett. 2007;581:5459–63.
19. Monroig O, Rotllant J, Cerdá-Reverter JM, Dick JR, Figueras A, Tocher DR. Expression and role of Elovl4 elongases in biosynthesis of very long-chain fatty acids during zebrafish Danio rerio early embryonic development. Biochim Biophys Acta Mol Cell Biol Lipids. 1801;2010:1145–54.
20. Monroig O, Webb K, Ibarra-Castro L, Holt GJ, Tocher DR. Biosynthesis of long-chain polyunsaturated fatty acids in marine fish: characterization of an Elovl4-like elongase from cobia Rachycentron canadum and activation of the pathway during early life stages. Aquaculture. 2011;312:145–53.
21. Carmona-Antonanzas G, Monroig O, Dick JR, Davie A, Tocher DR. Biosynthesis of very long-chain fatty acids (>24) in Atlantic salmon: cloning, functional characterization, and tissue distribution of an Elovl4 elongase. Comp Biochem Physiol B. 2011;159:122–9.
22. Monroig O, Wan SQ, Zhang L, You CH, Tocher DR, Li Y. Elongation of long-chain fatty acids in rabbitfish Signatus canaliculatus: cloning, functional characterization and tissue distribution of Elovl5 and Elovl4 like elongases. Aquaculture. 2012;350:36–70.
23. Kloos DP, Gay E, Lingeman H, Bracher F, Müller C, Mayboroda O, et al. Comprehensive gas chromatography-electron ionisation
mass spectrometric analysis of fatty acids and sterols using sequential one-pot silylation: quantification and isotopologue analysis. Rapid Commun Mass Spectrom. 2014;28:1507–14.

24. Portolés T, Ibáñez M, Garlito B, Nácher-Mestre J, Karalazos V, Silva J, et al. Comprehensive strategy for pesticide residue analysis through the production cycle of gilt-head sea bream and Atlantic salmon. Chemosphere. 2017;179:242–53.

25. Hernández F, Ibáñez M, Portolés T, Cervera MI, Sancho JV, Benet FL. Advancing towards universal screening for organic pollutants in waters. J Hazard Mater. 2015;282:86–95.

26. Portolés T, Mol JGJ, Sancho JV, Hernández F. Validation of a qualitative screening method for pesticides in fruits and vegetables by gas chromatography quadrupole time-of-flight mass spectrometry with atmospheric pressure chemical ionization. Anal Chim Acta. 2014;838:76–85.

27. Torres M, Navarro JC, Varó I, Agulleiro MJ, Morais S, Monroig O, et al. Expression of genes related to long-chain (C18-22) and very long-chain (>C24) fatty acid biosynthesis in gilt-head seabream (Sparus aurata) and Senegalese sole (Solea senegalensis) larvae: investigating early ontogeny and nutritional regulation. Aquaculture. 2020;520:734949.

28. Torres M, Navarro JC, Varó I, Agulleiro MJ, Morais S, Monroig O. Nutritional regulation of genes responsible for long-chain (C20-24) and very long-chain (>C24) polyunsaturated fatty acid biosynthesis in post-larvae of gilt-head seabream (Sparus aurata) and Senegalese sole (Solea senegalensis). Aquaculture. 2020;525:735314.

29. Morais S, Torres M, Hontoria F, Monroig Ó, Varó I, Agulleiro MJ, et al. Molecular and functional characterization of elovl4 genes in Sparus aurata and Solea senegalensis pointing to a critical role in very long-chain (>C24) fatty acids synthesis during early neural development of fish. Int J Mol Sci. 2020;21:3514. https://doi.org/10.3390/ijms21103514.

30. Folch J, Lees M, Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. Biol Chem. 1957;226:497–509.

31. Christie WWJ. Esterification of fatty acids in adipose-tissue. J Sci Food Agr. 1982:33:809.

32. Navarro JC, Batty RS, Bell MV, Sargent JR. Effects of two Artemia diets with different contents of polyunsaturated fatty acids on the lipid composition of larvae of Atlantic herring (Clupea harengus). J Fish Biol. 1993;43:503–15.

33. Comission E. COUNCIL DIRECTIVE of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. 1986/669/EEC(L358/1).

34. Armenta S, Alcalá M, Blanco M. A review of recent, unconventional applications of ion mobility spectrometry (IMS). Anal Chim Acta. 2011;703:114–23.

35. Cumeras R, Figueras E, Davis CE, Baumbach JI, Gracia I. Review on ion mobility spectrometry. Part 1: current instrumentation. Analyst. 2015;140:1376–85.

36. Kanu AB, Dwivedi P, Tam M, Matz L, Hill HHJ. Ion mobility–mass spectrometry. J Mass Spectrom. 2008:43:1–22.

37. Bijlsma L, Bade R, Celma A, Mullin L, Cland G, Stead S, et al. Prediction of collision cross-section values for small molecules: application to pesticide residue analysis. Anal Chem. 2017;89:6583–9.

38. Paglia G, Astarita G. Metabolomics and lipidomics using traveling-wave ion mobility mass spectrometry. Nat Protoc. 2017;12:797–813.

39. Levy AJ, Oranzi NR, Ahmadireskety A, Kemperman RHJ, Wei MS, Yost RA, et al. Recent progress in metabolomics using ion mobility-mass spectrometry. TrAC Trends Anal Chem. 2019;116:274–81.

40. Lipok C, Hippler J, Schmitz OJ. A four dimensional separation method based on continuous heart-cutting gas chromatography with ion mobility and high resolution mass spectrometry. J Chromatogr A. 2018;1536:50–7.

41. Tian L, Zeng Y, Zheng X, Chiu Y, Liu T. Detection of peanut oil adulteration mixed with rapeseed oil using gas chromatography and gas chromatography-ion mobility spectrometry. Food Anal Methods. 2019;12:2282–92.

42. Li S, Monroig Ó, Wang T, Yuan Y, Navarro JC, Hontoria F, et al. Functional characterization and differential nutritional regulation of putative Elolv5 and Elolv4 elongases in large yellow croaker (Larimichthys crocea). Sci Rep. 2017;7:1–15.

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