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Detection of perfluoroalkyl acids and sulphonates in Italian eel samples by HPLC-HRMS Orbitrap

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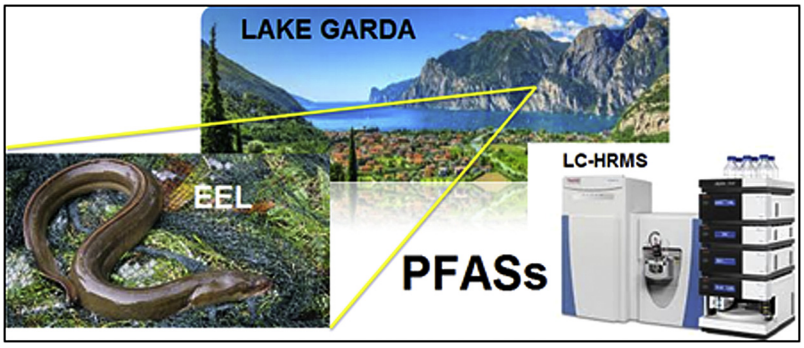
HIGHLIGHTS

- Perfluoroalkyl substances (PFASs) accumulate in environment and in human through diet.
- One sensitive HPLC-HRMS method for PFASs in eels is reported.
- Eels from the Lake Garda in North Italy were analysed.
- Up to eleven PFASs were contemporaneously detected in several eel samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Perfluoroalkyl substances (PFASs) contain one or more carbon-bound hydrogens substituted by fluorine. Since the 1950s, these compounds have been used to manufacture fat- and water-resistant fabrics, paper and food containers, and to produce photographic films, firefighting foams, detergents and insecticides. The widespread use and global distribution of PFASs, have led to their accumulation in the environment. Food, particularly fish and other seafood, is considered the main route of human exposure to PFASs. Consequently, the European Food Safety Authority (EFSA) recommends that more data be collected, to build a database on the contamination levels of the individual PFASs in food, to evaluate a reliable chronic risk to the European consumers. This requires high-sensitivity analytical methods, to increase the number of quantifiable samples and, thereby, improve the credibility of exposure assessments. In this context, the aim of the present research is to develop and validate a sensitive and specific method based on high-performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) analysis, to monitor the presence of 16 PFASs in Italian eels (*Anguilla anguilla*) from the Italian Lake Garda. The detection limits (CCα) and detection capability (CCβ) in the order of pg g^{-1}, the recoveries between 80 and 101% and the other validation parameters fulfilled the requirements of Commission Decision 657/2002/EC. The identification and quantification of PFASs, up to 11 in the same sample, showed a similar distribution among 90 eels. Perfluorooctane sulphonic acid (PFOS) and perfluorobutanoic acid (PFBA) were the analytes more frequently found in the eel samples (94 and 82%, respectively).

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1. Introduction

Perfluoroalkyl substances (PFASs) are molecules in which one or more carbon-hydrogen (C–H) bonds, are replaced by carbon-fluorine (C–F) bonds (Lau et al., 2004). Fluorine is a reactive element in its ionic form and very stable in a bound form. Therefore, perfluorocarbons are stable in the environment, even at > 150 °C, are non-flammable, not subject to photolysis and not readily degraded by alkalis, strong acids or oxidising agents. These stability characteristics make them non-biodegradable and highly persistent in the environment (Lau et al., 2004). Perfluoroalkyl acids have also a unique partitioning behaviour that reveals their hydrophobic and oleophobic nature when they are mixed with water and hydrocarbons, forming three immiscible phases. By attaching a charged moiety, such as carboxylic acid, sulphonic acid, or phosphate, to the perfluorinated chain, the molecule becomes more hydrophilic. All known, biologically produced, fluorinated organics contain only one fluorine atom. However, partially or fully fluorinated organic molecules are synthesised in the laboratory on a large-scale, for their many useful properties (Key et al., 1997).

PFASs are used in a lot of industrial and chemical sectors, as well as for packaging materials, fire-extinguishing fluids, textiles, carpets, paper, furniture, floor polishing agents, cleaning agents, varnish, polish, photograph paper, and insecticides (3M Company, 1999). The global utilisation and distribution of PFASs has caused their accumulation in the environment and human body. Perfluorooctane sulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most common PFASs. Both cause adverse health effects and have shown immunotoxicity, hepatotoxicity, neurobehavioral toxicity, developmental toxicity, reproductive toxicity, lung toxicity, hormonal effects, weak genotoxic and carcinogenic potential (Eriksen et al., 2010; Pinkas et al., 2010).

Food, particularly fish and seafood, is considered the main exposure route to PFASs in the human population. However, scarce information is available in the literature about the detection of PFASs in eels. One research report focused on the detection of PFOS in the liver of three freshwater fish species (gibel carp, carp and eel), in Belgium (Hoff et al., 2005). In another study, PFOS and PFOA residues were investigated in 51 wild eels, among other wild fish, in Germany (Schuetze et al., 2010). Kwadijk et al. (2010) measured the distribution of 15 PFASs among water, sediment and eels, in The Netherlands. Furthermore, PFOA and PFOS in the organs of 35 wild eels, from two Italian locations, were analysed by Giari et al. (2015).

Food exposure can derive by accumulation from the environment or by contact with cookware or packaging materials containing PFASs (Trier et al., 2011). The EFSA Panel on Contaminants in the Food Chain (CONTAM) set a tolerable daily intake (TDI) of 150 ng kg^{-1} body weight (b.w.) per day for PFOS and 1500 ng kg^{-1} b.w. per day for PFOA (EFSA, 2008). However, the scarce data allowed only a limited exposure assessment. Therefore, CONTAM recommended increasing the database, through more studies about PFASs in food, by evaluating the contamination levels, which would improve the accuracy of the chronic dietary exposure risk to the European populations (EFSA, 2012). For this purpose, high-sensitivity analytical methods that increase the proportion of quantified data and accurately monitor PFASs in food, are required, thereby, improving the reliability of the exposure assessments.

Regarding the analytical strategies present in literature, the extraction of perfluorinated compounds from biological samples is usually performed through an alkaline digestion with potassium hydroxide (KOH) (So et al., 2006) or the ion-pair extraction method (Hansen et al., 2001) based on ion pairing of the ionic PFASs with tetra-*n*-butylammonium hydrogensulfate (TBA), followed by a liquid–solid extraction with methyl-*tert*-butylether (MTBE). For purification of the samples the HLB, WAX cartridges or Dispersive Envi-carb are used based on the different matrices (van Leeuwen and de Boer, 2007).

Several methods based on liquid chromatography coupled with triple quadrupole mass spectrometry (MS), have been proposed in the literature for the analysis of PFASs, for several matrices. Also in the few studies on eels reported above, the analyses were performed by LC-MS/MS system. In particular, high-resolution mass spectrometry (HRMS) represents a powerful tool for the determination of trace analysis of various compounds in complex matrices. The advantages of Orbitrap-MS, such as the high MS resolving power and mass accuracy down to 1 ppm, combined with the rapid scan speed, results in high sensitivity, selectivity and specificity, providing new improvements for confirmatory analytical methods, in the challenge against emerging contaminants (Krauss et al., 2010).

In this context, the present research aimed to develop and validate a sensitive and specific method based on high-performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) analysis, to monitor the presence of 16 PFASs in Italian eels (*Anguilla anguilla*) from Lake Garda (Northern Italy). The choice of eel was due to the authors' assumption of potential bioaccumulation of PFASs in this species, facilitated by their length and body composition and, also, because it is an edible matrix, intended for human consumption. Moreover, Lake Garda is a semi-enclosed environment, which has shown an increasing pollution level in recent years, in which the majority of plastic particles have been found (Imhof et al., 2013).

2. Materials and methods

2.1. Chemicals and reagents

All solvents were of HPLC or analytical grade and were purchased from Fluka (Sigma-Aldrich, St. Louis, MO, USA). Water was purified by a Milli-Q system (Millipore, Merck KGaA, Darmstadt, Germany). The extraction cartridges (Oasis HLB WAX 3 mL, 60 mg) were provided by Waters (Milford, MA, USA). Sixteen perfluorinated compounds including both perfluorinated sulphonates and perfluorinated carboxylates, were examined in this study: perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluorobutane sulphonic acid (PFBS), perfluoroheptanoic acid (PFHpA), PFOA, perfluorohexane sulphonate (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), PFOS, perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUnDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), and perfluorooctadecanoic acid (PFODA) (see Table 1 for the formula pertaining to the individual compounds). All these compounds and the two ^{13}C-labeled internal standards (ISs) perfluoro-[1,2,3,4,5–13C5]nonanoic acid (MPFNA) and perfluoro-[1,2,3,4–13C4]octanesulfonic acid (MPFOS) were purchased from Fluka. Ammonium formate, sodium acetate, acetic acid (99.9%) and 25% ammonia solution, were purchased from Fluka.

2.2. Sample collection and preparation

Muscle tissue from ninety farmed eel (average weight 909.2 ± 434.1 g; average length 74.5 ± 10.0 cm; average fat percentage 26.1 ± 5.4%), collected from Lake Garda (Northern Italy), was used. After catching, the eels were immediately taken to the laboratory, and eviscerated. As the high water content of many food samples previously showed to affect the extraction performance of

Table 1
Main information of investigated PFASs (formula, parent, main product, polarity and retention time (RT)).

Compound[a]	Formula	Parent [m/z]	Main product [m/z]	Polarity	RT (min)
PFBA	C4HF7O2	212.97920	168.98836	(−)	9.07
PFPeA	C5HF9O2	262.97601	218.98560	(−)	11.68
PFBS	C4F9HO3S	298.94299	98.95434	(−)	12.02
PFHxA	C6HF11O2	312.97281	268.98288	(−)	13.22
PFHpA	C7HF13O2	362.96962	318.97949	(−)	14.36
PFHxS	C6F13HO3S	398.93660	98.95437	(−)	14.39
PFOA	C8HF15O2	412.96643	368.97681	(−)	15.27
PFNA	C9HF17O2	462.96323	418.97385	(−)	16.03
PFOS	C8F17HO3S	498.93022	79.95598	(−)	16.00
PFDA	C10HF19O2	512.96004	468.97064	(−)	17.96
PFUnDA	C11HF21O2	562.95684	518.96729	(−)	18.48
PFDoA	C12HF23O2	612.95365	568.96387	(−)	18.98
PFTrDA	C13HF25O2	662.95046	618.96057	(−)	19.50
PFTeDA	C14HF27O2	712.94726	668.95823	(−)	20.06
PFHxDA	C16HF31O2	812.94088	768.95184	(−)	20.80
PFODA	C18HF35O2	912.93449	868.94513	(−)	21.81
MPFNA	[13]C5C4HF17O2	467.98001	422.98703	(−)	16.03
MPFOS	[13]C4C4F17HO3S	502.94364	79.95592	(−)	16.00

[a] Refer to text (materials and methods section) for full names of the abbreviated compounds.

PFASs, we lyophilised eel muscle tissues, according to other studies that used freeze-drying prior the sample clean-up (Vestergren et al., 2012). Then the samples were stored at 4 °C, until analysis.

2.3. Standard solutions

Stock solutions (1 mg mL^{-1}) of each standard, were prepared in methanol and kept at −20 °C. Working solutions, containing each of the studied analytes, at 10 and 100 ng mL^{-1}, were prepared daily. Each working solution was maintained at 4 °C, during the method validation procedures.

2.4. Sample extraction

A 2-g aliquot of lyophilised eel sample, was spiked with the two ISs, to obtain a final concentration of 5 ng g^{-1}. Then, 10 mL acetonitrile were added for the protein precipitation and analytes extraction, before the sample was vortexed and sonicated for 15 min. After centrifugation (2500 × g, 4 °C for 10 min), the supernatant was transferred to a glass flask and rotary evaporated to dryness at 35 °C. The extract was suspended in 10 mL water and solid-phase extraction (SPE) performed using Oasis WAX-SPE cartridges under vacuum, for further purification and extraction. The SPE cartridges were preconditioned with 3 mL of 0.5% ammonium hydroxide (NH_4OH) in MeOH, 3 mL MeOH and 3 mL Milli-Q water. The sample was loaded, and, then, the cartridges were washed with 3 mL of 25 mM acetate buffer, pH 4.5, to remove interferences, as well as lipids or proteins and to improve adsorption of target anions to the cartridge, followed by 2 mL MeOH. Finally, the compounds were eluted using 3 mL of 0.5% NH_4OH in MeOH and were collected in a 15-mL polypropylene tube. The eluate was rotary evaporated at 35 °C. The dried extract was reconstituted in 100 μL of 20 mM MeOH:ammonium formiate (10:90 v/v), and, then, transferred to an auto-sampler vial. The injection volume was 10 μL. The method was developed and optimised, taking into consideration the work of Taniyasu et al. (2005), about the different effect of pH of acetate buffer, the percentage of NH_4OH in MeOH, and the influence of elution volume of NH_4OH in MeOH, on recoveries of PFASs. Moreover, considering the ubiquity of PFASs in the environment of analytical laboratories, several precautions were taken, such as washing glassware with MeOH and the execution of at least 10 procedural blanks, on the analysis days, to subtract any background contamination.

2.5. HPLC-HRMS analyses

HPLC analysis was performed by an HPLC system (Thermo Fisher Scientific, San Jose, CA, USA), equipped with a Surveyor MS quaternary pump and degasser, a Surveyor AS autosampler and column oven, and a Rheodyne valve with a 20-μL loop. The analytes were chromatographically separated, using a Synergi Hydro-RP reverse-phase HPLC column (150 × 2.0 mm, i.d. 4 μm), with a C18 guard column (4 × 3.0 mm; Phenomenex, Torrance, CA, USA). Stainless-steel tubes and peeks were used, to minimise background PFAS contamination in the system. Moreover, since PFOA and PFOS were always present in the blank of the chromatographic system, a small Megabond WR C18 column (5 cm × 4.6 mm, i.d. 10 μm) was introduced between the pump and injector, to allow delaying the target analytes by 2 min compared to those already present in the system.

The mobile phase used for the gradient, consisted of a binary mixture of solvents A (20 mM aqueous ammonium formate) and B (MeOH). The elution started with 10% B, which increased to 40% in 4 min. Subsequently, mobile phase B was gradually increased to 95% at the 12th min, which remained constant up to the 18th min. The initial conditions were reached in the 20th min, with an equilibration time of 7 min. The run was performed at 0.3 mL min^{-1}.

The detector, was a Thermo Q-Exactive Plus (Thermo Scientific, San Jose, CA, USA), equipped with a heated electrospray ionisation (HESI) source. Capillary and vaporiser temperatures were set at 330 and 280 °C, respectively, while the electrospray voltage was set at 3.50 kV, operating in negative mode. The sheath and auxiliary gas were set at 35 and 15 arbitrary units (AU). Xcalibur 3.0 software (Thermo Fisher Scientific, San Jose, CA, USA) was used to control the HPLC-HRMS system. The exact mass of the compounds was calculated, using Qual Browser in Xcalibur 3.0 software. Instrument calibration was done every analytical session, using LTQ Velos ESI negative ion calibration solution (Pierce Biotechnology Inc., Rockford, IL, USA).

The full scan (FS) acquisition, was combined with a data-independent acquisition (DIA) strategy, providing the MS^2 spectra for a confirmatory response, based on an inclusion list. The FS resolution was 70,000 FWHM. On the basis of the compound list, a scan range of 200–950 *m/z* was chosen; the automatic gain control (AGC) was set at 1E6, and the maximum injection time was 200 ms. The DIA segment operated in negative mode at 35,000 FWHM. The

AGC target was set to 5E4, with the maximum injection time of 100 ms. The precursor ions are filtered by the quadrupole, which operates at an isolation window of 2 *m*/*z*. Fragmentation of the precursors was optimised with a two-step normalised collision energy (10 and 70 eV). The mass tolerance window was set to 2 ppm. Detection of the analytes was based on the retention time (RT) of the target compounds, and on the calculated exact mass of the deprotonated molecular ions, and at least one specific and typical fragment (Table 1). The formula of the compounds, with the exact theoretical mass of the parents and the diagnostic transition, used to confirm the various PFASs, are reported in Table 1. The extracted parent ion chromatograms, acquired from FS analysis of each analyte in the matrix, are reported in Fig. 1. Acquisition data were recorded and elaborated using Xcalibur™ software (Thermo Fisher).

2.6. Method validation

After the identification of the "blank" eel samples, based on a preliminary screening, the validation was performed according to the criteria of the Commission Decision 657/2002/EC (European Community, 2002). For each compound, the method performance was assessed, through both qualitative and quantitative parameters, providing molecular identification in terms of RT and transition ion ratios; evaluating recovery, linearity, accuracy in terms of trueness, precision as intra- and inter-day repeatability; and through the analytical decision limit (CCα) and detection capability (CCβ), as indicated in SANCO/2004/2726-revision 4 (European Community, 2008).

Twenty blank samples were analysed, to evaluate specificity and selectivity, check for any interference (signals, peaks, ion traces), verify the presence of analytes by a signal-to-noise (S/N) ratio of >3 at the expected RT, and to confirm the ion abundance ratio for the different fragmentations. Validation was performed, by spiking the eel samples at three concentration levels in six replicates, repeated for three independent days, resulting in three analytical series (matrix validation curves). The three concentration levels (C0, 2C0, and 3C0) were previously chosen, according to the minimum concentration detectable with the instrumentation (C0) used, for each analyte (Table 2).

The instrumental linearity was also evaluated, by drawing six-point calibration curves for the solvent containing a fixed amount of the ISs (5 ng mL^{-1}) and the initial analyte concentration, corresponding to C0 up to 100 ng mL^{-1}, for all analytes. The recovery, expressed as a percentage of the measured concentration with respect to the spiked concentration, was evaluated using the data from the validation points of the three analytical series. The precision, in terms of intra- and inter-day repeatability, was evaluated by calculating the relative standard deviation of the results obtained from the six replicates of each analyte, at the three

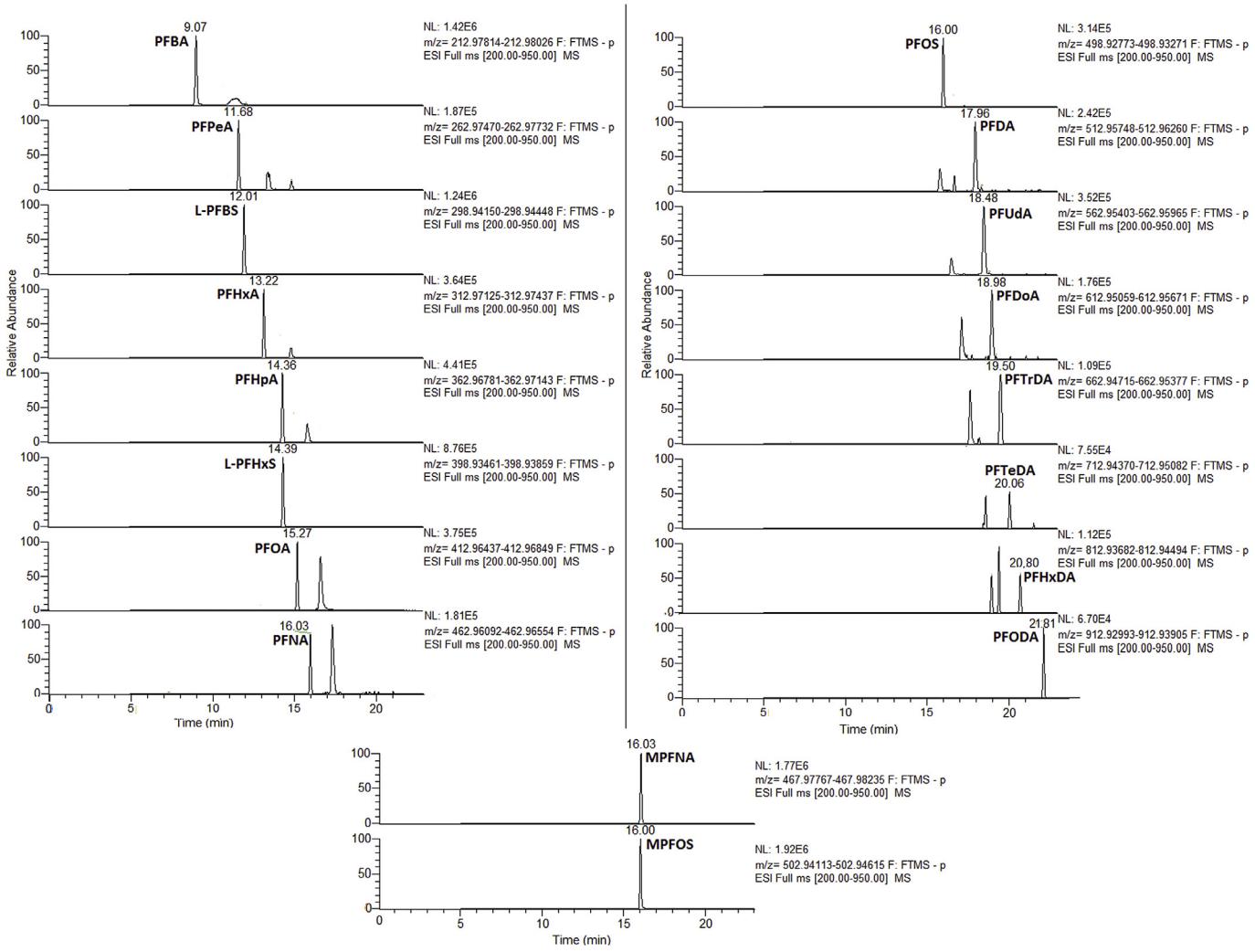

Fig. 1. Extracted parent ion chromatograms from full scan HPLC-HRMS analysis of each PFAS in the eel matrix, at the lowest validation level.

Table 2
Validation parameters[a] of the investigated perfluoroalkyl substances (PFASs).

PFAS[b]	C0, 2C0, 3C0 (pg g^{-1})[c]	CCα (pg g^{-1})	CCβ (pg g^{-1})	Recovery %	CV% Intra-day	CV% Inter-day
PFBA	5, 10, 15	10	12	80	5	18
PFPeA	10, 20, 30	12	15	117	12	12
PFBS	10, 20, 30	12	15	105	10	14
PFHxA	20, 40, 60	30	35	113	4	10
PFHpA	5, 10, 15	10	12	115	4	8
PFHxS	15, 30, 35	20	25	105	7	9
PFOA	5, 10, 15	8	10	116	3	7
PFNA	5, 10, 15	10	12	93	11	18
PFOS	5, 10, 15	5	8	80	14	20
PFDA	20, 40, 60	25	30	80	14	20
PFUnDA	20, 40, 60	30	35	82	6	16
PFDoA	20, 40, 60	35	39	88	7	12
PFTrDA	15, 30, 35	20	25	89	6	10
PFTeDA	5, 10, 15	10	13	93	17	20
PFHxDA	5, 10, 15	8	10	87	19	20
PFODA	5, 10, 15	10	12	85	19	21

CCα: decision limit; CCβ: detection capability; CV: coefficient of variation.
[a] According to Commission Decision 657/2002/CE (European Community, 2002).
[b] Refer to text (materials and methods section) for full names of the abbreviated compounds.
[c] Validation was performed, by spiking the eel samples at three concentration levels (C0, 2C0, 3C0), in six replicates, repeated for three independent days, resulting in three analytical series (matrix validation curves).

concentration levels during the three analytical series. Robustness was evaluated, using the approach of Youden (European Community, 2002). The seven factors selected for the robustness study were: the volume of acetonitrile used for extraction and protein precipitation, the sonication time, the centrifugation time, the centrifugation temperature, the percentage and the volume of ammonium hydroxide (NH4OH) in MeOH using during the SPE purification and the temperature of the rotary evaporator. The matrix effect was assessed based on Matuszewski et al. (2003), by calculating the percentage ratio between the corresponding peak areas of the standards spiked after extraction and the peak areas of the neat standard solution.

3. Results and discussion

3.1. Development and optimisation of sample preparation

The method was developed and optimised, taking into consideration the work of Taniyasu et al. (2005), that reported useful comparisons about the different effect of pH of acetate buffer, the percentage of NH4OH in MeOH, and the influence of elution volume of NH4OH in MeOH on extraction and consequently on recoveries of PFASs. In particular, the samples considered in the work were water and biota pre-treated through an alkaline digestion before the WAX SPE. We chose acetonitrile for the pretreatment step because was useful not only for the extraction but also for protein precipitation to avoid interferences during analysis. As regard WAX purification, we decreased the volumes of the solutions and solvents used during the SPE, we used a higher percentage (0.5% instead of 0.1%) of NH4OH in MeOH and we analysed only the final eluate because it was purified by any interference and contained all the analytes we were interested in. The choices and modifications made to the sample clean-up protocol have been fundamental to obtain satisfactory validation parameters, reported and discussed in the next paragraph.

3.2. Validation performance

The method showed high specificity, without interference signals close to the RT of the analytes. Consequently, a high S/N ratio in the presence of analytes, even at concentrations in the order of pg g^{-1}, was demonstrated. Selectivity demonstrated a good compliance with the relative RTs for each analyte, which, in this instance, was within 2.5% tolerance, with an S/N ratio >3, when compared with the standard solution mix, both in FS and MS^2 chromatograms. Moreover, diagnostic fragments showed an ion ratio within the recommended tolerances (European Community, 2002). The mean recoveries for all analytes ranged between 80 and 117%, indicating the efficiency of the extraction protocol.

The matrix validation curves were linear over the working range, demonstrating a good fit for all analytes with an $R^2 > 0.99$. Precision, in terms of intra- and inter-day repeatability (Thompson, 2000), were calculated using one-way analysis of variance (ANOVA), expressed as coefficients of variation (CVs), and were below 19 and 21%, respectively. The detection limits (CCα) ranged from 5 to 35 pg g^{-1} and detection capability (CCβ) from 8 to 39 pg g^{-1} (Table 2). These limits indicate the potentiality of the method to detect these emergent analytes that currently, do not have established maximum residue limits in edible matrices. Our detection limits resulted lower than the ones in the few literature studies regarding the detection of PFASs in eels. In the work of Hoff et al. (2005) about PFOS and other organohalogen pollutants in liver of three freshwater fish species of Belgium, the detection limits ranged from 0.1 to 1 ng g^{-1} wet weight; in the study of Schuetze et al. (2010) LODs were 0.019 and 0.27 μg kg^{-1} fresh weight for PFOS and PFOA, respectively; in the study of Kwadijk et al. (2010) about distribution of perfluorinated compounds in aquatic systems in The Netherlands, no information regarding the detection limits is reported.

The Youden approach showed a good robustness. There was a modest matrix effect, with values ranging from 84 to 109%, for the studied compounds.

3.3. Application in eel samples

The optimised and validated method was then applied to the analysis of 90 lyophilised eel samples, farmed and collected from Lake Garda. The results showed the presence of several PFASs, up to 11 in the same eel. The average concentrations, standard deviations, percentages of positivity, medians, minimum and maximum concentrations detected and expressed as concentrations in muscle wet weight, are reported in Table 3. The distribution of the various contaminants, in the order of ng g^{-1}, was mostly similar in each sample, representing the low contamination level of the lake, without any relation to the weight, length or the percentage of animal fat.

Usually organic molecules tend to transfer from abiotic to biotic compartments, with persistent lipophilic compounds concentrating in the adipose tissue, but this partitioning approach cannot be applied to the bioaccumulation of perfluorinated compounds (Houde et al., 2006), for their proteinophilic nature (Jones et al., 2003).

PFOS was the more frequently found analyte but the average concentrations did not appear concerning, although it was slightly higher than the average muscle concentrations (0.89 ± 0.58 ng g^{-1} wet weight) present in the eels from north Italian waters (Giari et al., 2015) but considerably lower than those reported in eel liver (17–9031 ng g^{-1} wet weight) in Belgium (Hoff et al., 2005) and in eel muscle tissue (37–83 ng g^{-1} fresh weight) in Germany (Schuetze et al., 2010).

PFOS was found to be the predominant compound in all eel samples of the Netherlands (Kwadijk et al., 2010), with concentrations ranging from 7 to 58 ng g^{-1} wet weight. In the same work PFHxS and PFDoA, were the PFASs detected at the next highest level, approximately 10 times lower than that of PFOS. These three

Table 3
Distribution of perfluoroalkyl substances in Italian eel muscle samples from Garda Lake (ng g^{-1} wet weight).

Compounds[a]	Average (ng g^{-1})	SD (ng g^{-1})	Min (ng g^{-1})	Max (ng g^{-1})	Median	% Positives
PFBA	8.96	11.50	0.00	61.48	3.66	82
PFPeA	0.04	0.02	0.00	0.06	0.00	7
PFHpA	0.10	0.01	0.00	0.12	0.00	6
PFOA	0.22	0.08	0.00	0.54	0.18	77
PFNA	0.39	0.25	0.00	1.51	0.29	74
PFOS	2.18	1.70	0.00	7.81	1.67	94
PFDA	0.88	0.70	0.00	4.42	0.58	82
PFUnDA	0.62	0.57	0.00	1.84	0.00	11
PFDoA	0.93	1.12	0.00	5.35	0.02	51
PFTrDA	0.40	0.39	0.00	1.45	0.00	42
PFTeDA	1.73	2.75	0.00	10.13	0.00	23

[a] Refer to text (materials and methods section) for full names of the abbreviated compounds.

PFASs were also the only compounds ~~to be~~ detected in all the samples.

The PFOA concentrations were also remarkably lower than the previous above-mentioned studies. In the current study, the highest concentrations found in the eel samples were associated with PFBA, with a wide standard deviation, which was observed for the sulphonate form (PFBS) in the sediment and water samples of the Netherlands that was attributed to various sources, given the presence of industries along the Rhine (Kwadijk et al., 2010). In this last work, although at some locations PFBS was not detected in the water samples, low levels of PFBS were detected in eel (0.1–2.3 ng g^{-1} of wet weight), despite the fact that PFBS is considered nonbioaccumulative (Conder et al., 2008): the absence of PFBS in our eel samples agrees with the statement of no bioaccumulation.

Based on the literature and the findings of the Water Research Foundation project #4322 (Fulmer, 2016), conventional treatment at wastewater treatment plants and most drinking water treatment plants, is ineffective at removing short-chain PFASs, as well as PFBA from water. This could explain the higher level of PFBA in respect to other PFASs in our samples. In a study on distribution and sources of polyfluoroalkyl substances in the River Rhine watershed, the dominant concentration of PFBA likely originated from industrial point sources (Möller et al., 2010).

In another work about the sources of polyfluoroalkyl compounds in the North Sea, Baltic Sea and Norwegian Sea, the Authors hypothesised that an additional water contamination source can be the contaminated sewage sludge applied to neighboring agricultural fields (Arehns et al., 2010). It should be emphasised that PFBA was always present, even in the background contamination of our extractive procedure. The average value of 4 ng g^{-1} was detected in the analysis of a batch of 10 procedural blanks and subtracted to each sample analysed.

4. Conclusions

The HPLC-HRMS Orbitrap represents a powerful technical approach, for the analysis of emerging contaminants, due to its resolving power and scanning speeds that contribute to the high selectivity, specificity and sensitivity of the instrumentation. Moreover, the effectiveness of the extraction method, facilitated the instrumental analysis, by the lack of particular interferences, considering the complexity of the studied matrix: *A. anguilla*. Application of the validated method, to the analysis of 90 farmed eel samples collected from Lake Garda, showed a homogeneous situation of modest PFASs contamination compared to eels from other European countries, despite simultaneous detection of up to 11 compounds, in each sample.

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