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Quantitative profiling of perfluoroalkyl substances by ultrahigh-performance liquid chromatography and hybrid quadrupole time-of-flight mass spectrometry

Yolanda Picó · Marinella Farré · Damià Barceló

Abstract The accurate determination of perfluoroalkyl substances (PFSAs) in water, sediment, fish, meat, and human milk was achieved by ultrahigh-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC–QqTOF-MS) with an ABSciex Triple TOF®. A group of 21 PFSAs was selected as target to evaluate the quantitative possibilities. Full scan MS acquisition data allows quantification at relevant low levels (0.1–50 ng L^{-1} in water, 0.05–2 ng g^{-1} in sediments, 0.01–5 ng g^{-1} in fish and meat, and 0.005–2 ng g^{-1} in human milk depending on the compound). Automatic information dependent acquisition product ion mass spectrometry (IDA-MS/MS) confirms the identity even for those compounds that presented only one product ion. The preparation of a homemade database using the extracted ion chromatogram (XIC) Manager of the software based upon retention time, accurate mass, isotopic pattern, and MS/MS library searching achieves not only the successful identification of PFSAs but also of some pharmaceuticals, such as acetaminophen, ibuprofen, salicylic acid, and gemfibrozid. Mean recoveries and relative standard deviation (RSD) were 67–99 % (9–16 % RSD) for water, 62–103 % (8–18 % RSD) for sediment, 60–95 % (8–17 % RSD) for fish, 64–95 % (8–15 % RSD) for meat, and 63–95 % (8–16 %) for human milk. The quantitative data obtained for 60 samples by UHPLC–QqTOF-MS agree with those obtained by LC–MS/MS with a triple quadrupole (QqQ).

Y. Picó (✉)
Food and Environmental Safety Research Group (SAMA-UV),
Facultat de Farmàcia, Universitat de València, Av, Vicent Andrés s/n,
46100 Burjassot, València, Spain
e-mail: yolanda.pico@uv.es

M. Farré · D. Barceló
Department of Environmental Chemistry, IDAEA-CSIC, C/Jordi
Girona 18-26, 08034 Barcelona, Spain

D. Barceló
Catalan Institute for Water Research- ICRA, C/Emili Grahit, 101,
Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona,
17003 Girona, Spain

Introduction

Perfluoroalkyl substances (PFASs) are long, fully fluorinated carbon chains with different functional head groups. These compounds are used in products to resist grease, oil, stains, and water [1, 2] and also in fire-fighting foam [3, 4]. The European Food Safety Authority (EFSA) [5] fixed provisional tolerable daily intakes (TDIs) for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). PFOS and its salts were added to Annex B of the Stockholm Convention as new persistent organic pollutants [6] and the European Commission also prohibited their general use [7] and proposed maximum allowable concentrations in inland surfaces [8]. Concern about PFASs in the environment has increased since PFOS and related compounds were detected in blood plasma of non-occupationally exposed humans and in animal tissues [9–15]. Recurring news appears periodically highlighting high levels of these compounds in water from impacted areas [16–20]. Although the first PFASs were based on PFOS and PFOA, as a result of environmental reports warning about

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the bioaccumulation of these compounds, new and more readily biodegradable substances have been introduced [4, 21, 22].

The analytical methods to determine PFASs are based on liquid chromatography–mass spectrometry (LC–MS) [1, 22, 23]. Triple-quadrupole tandem mass spectrometry (QqQ-MS/MS) in multiple selected reaction monitoring (SRM) mode is predominant owing to its selectivity and sensitivity [14, 19, 20]. However, high resolution mass spectrometers, such as time-of-flight (TOF), Fourier transform ion cyclotron resonance (FT-ICR), or Orbitrap can also be used and have the ability to allow post-target and non-target screening [24, 25]. These techniques were reported for the structural identification of perfluoroalkyl and polyfluoroalkyl substances in end-user products and their biodegradation products formed using activated sludge [2]. However, the analyte concentration must be at least of several hundreds of nanograms per milliliter. For environmental matrices where PFASs are found at trace levels (a few nanograms per liter or per gram), LC–MS/MS is the preferred technique owing to is high sensitivity in comparison with accurate mass techniques [14, 17, 18, 21]. The problem of target screening is that a number of related compounds remain undetected in the sample just because they were not selected a priori as the analyte [24]. The ability to obtain mass spectra with a very high degree of mass accuracy at sufficient mass resolutions and high sensitivity opens the possibility for combining both non-target and target quantification [26–28].

The acquisition of experimental data of sufficient mass resolving power and accuracy has, until a few years ago, only been possible with severe limitations with respect to sensitivity [2]. The new generation of instruments available nowadays is more sensitive and rapid. Consequently, the routine application of high resolution instruments in high-throughput determination of contaminants at trace levels is very recent [29, 30]. High resolution quadrupole time-of-flight mass spectrometry (QqTOF-MS) is particularly interesting for hyphenated LC–MS applications using ultrahigh-performance liquid chromatography (UHPLC), where sub-2-µm-particle columns generate chromatography peaks with peak widths of only a few seconds [30].

The objective of this study was to evaluate a latest-generation QqTOF (ABSciex TripleTOF™) in combination with UHPLC to develop a sensitive and accurate non-target screening method focusing on PFASs. The comparison of two quantitative methods based (i) on UHPLC–QqQ-MS/MS operating in the SRM mode and (ii) on high resolution UHPLC–QqTOF-MS/MS for the quantification of PFASs in water, fish, meat, and human milk is presented herein. Their applicability and reliability are demonstrated by identifying and quantifying several target compounds at low levels (from nanograms per liter to nanograms per gram) in the selected matrices. To the best of the authors' knowledge, the analytical method described in this study is the first to use UHPLC–QqTOF-

MS to simultaneously screen PFASs in environmental and food samples.

Experimental

Chemicals

Sodium perfluorodecanesulfonate (L-PFDS, >98 %), perfluorohexanoic acid (PFHxA, >98 %), pefluoroheptanoic acid (PFHpA, >98 %), perfluorononanoic acid (PFNA, 97 %), perfluoro-7-methyloctanoic acid (i,p-PFNA), perfluorohexanesulfonate (PFHxS), perfluoroheptanesulfonate (PFHpS), sodium perfluoro-7-methyloctanesulfonate (i,p-PFNS), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA), perfluorohexadecanoic acid (PFHxDA), and perfluorooctadecanoic acid (PFODA) were purchased from Wellington Laboratories (Guelph, Ontario, Canada) as 50 µg mL^{-1} methanolic solutions (1.2 mL). Sodium perfluorooctanosulfonate (PFOS, 98 %), perfluorobutanoic acid (PFBA, 98 %), perfluoropentanoic acid (PFPA, 97 %), perfluorooctanoic acid (PFOA, 96 %), and perfluorodecanoic acid (PFDA, 97 %) were obtained from Aldrich (Steinheim, Germany). The internal standards perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid (MPFBA), perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid (MPFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid (MPFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid (MPFNA), perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate potassium salt (MPFOS), perfluoro-n-[$^{18}O_2$]hexasulfonate (MPFHxS), perfluoro-n-[1,2-$^{13}C_2$]decanoic acid (MPFDA), and perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid (MPFUdA) were also purchased from Wellington Laboratories as 50 µg mL^{-1} methanolic solutions (1.2 mL).

All stock standards and solutions were prepared in methanol and stored in polypropylene tubes or vials at 4 °C. A working mixture of all PFASs excepting the internal standard at 500 ng mL^{-1} was prepared in methanol and was used to spike the samples and to prepare diluted solutions at the appropriate concentration. A mixture of the isotopically labeled internal standards (IS) at 100 ng mL^{-1} each was used to spike samples and standards at the appropriate concentration that was 25 ng mL^{-1} in the final extract (62.5 µL).

LC-grade 'suprasolv' methanol and ammonium hydroxide (25 % in water) were purchased from Merck (Darmstadt, Germany). Deionized water (<18 MΩ cm resistivity) was from a Milli-Q SP reagent water system (Millipore, Bedford, MA, USA). Ammonium formate (p.a., for HPLC, ≥99 %) and acetic acid (analytical grade, ≥98 %) were from Sigma–Aldrich. STRATA-X 33 µm polymeric reversed-phase cartridges

(200 mg sorbent/6 mL) of 800 m^2/g surface area were from Phenomenex (Torrance, CA, USA).

Liquid chromatography–tandem mass spectrometry

The chromatography was performed on an Agilent 1260 Infinity (Agilent, Waldbronn, Germany) using a Poroshell 12 D EC-C18 column of 50×30 mm internal diameter, 2.7 μm (Agilent). The flow rate was 0.4 mL/min and the injection volume was 5 μL. Mobile phases consisted of 10 mM ammonium formate in Milli-Q water (A) and 10 mM ammonium formate in methanol (B). Separation was carried out in 20 min under the following conditions: 0 min, 30 % B; 10 min, 85 % B; 15 min, 98 % B. The column was equilibrated for 10 min prior to each analysis. The analyses were performed using the Turbo V source in electrospray (ESI) negative ion (NI) mode at an ionspray voltage of −4,500 V on an AB SCIEX TripleTOF™ 5600 system. The curtain gas was set at 25 psi, nebulizer gas (gas 1) at 60 psi, drying gas (gas 2) at 50 psi, CAD gas at medium, and the temperature at 400 °C. The MS was acquired using an automatic information dependent acquisition (IDA) acquisition method with two experiments: the survey scan type (TOF-MS) and the dependent scan type (product ion) using 45 V of collision energy with a spread of

Table 1 Accurate mass measurement, retention time, intensity, and instrumental LOQ of selected PFASs as determined by UPLC/ESI-QqTOF in MS

| Name	Formula	Mass (Da)	Extraction mass	Width (Da)	Found at mass (Da)	Error (ppm)	Expected RT (min)	Intensity	Instrumental LOQ (ng/mL)
Perfluorinated carboxylic acids (PFCAs)									
PFBA	$C_4F_7O_2H$	213.98648	212.9792	0.02	212.97873	−2.2	1.69	2,560	25
PFPA	$C_5F_9O_2H$	263.98328	262.97601	0.02	262.97527	−2.8	2.00	2,110	25
PFHxA	$C_6F_{11}O_2H$	313.98009	312.97281	0.02	312.97352	−2.27	7.84	2,356	2.5
PFHpA	$C_7F_{13}O_2H$	363.9769	362.96962	0.02	362.96873	−2.5	9.38	10,667	0.5
PFOA	$C_8F_{15}O_2H$	413.9737	412.96643	0.02	412.96476	−4	10.41	2,360	2.5
i,p-PFNA	$C_9F_{17}O_2H$	463.97051	462.96323	0.02	462.96238	−1.8	10.94	2,985	1
PFNA	$C_9F_{17}O_2H$	463.97051	462.96323	0.02	462.96238	−1.8	11.14	3,298	1
PFDA	$C_{10}F_{19}O_2H$	513.96732	512.96004	0.02	512.95976	0.55	11.84	6,224	1
PFUnDA	$C_{11}F_{21}O_2H$	563.96412	562.95685	0.02	562.95789	−1.85	12.42	4,746	1
FFDOA	$C_{12}F_{23}O_2H$	613.96093	612.95365	0.02	612.95373	0.1	12.92	5,790	1
PFTrA	$C_{13}F_{25}O_2H$	663.95774	662.95046	0.02	662.94985	−0.9	13.35	7,220	0.7
PFTeA	$C_{14}F_{27}O_2H$	713.95454	712.94727	0.02	712.94861	1.9	13.73	6,031	1
PFHxDA	$C_{16}HF_{31}O_2$	813.94816	812.94088	0.02	812.94323	2.9	14.35	12,741	0.3
PFODA	$C_{18}F_{35}O_2H$	913.94177	912.93449	0.02	912.93759	3.4	14.89	10,336	0.3
Perfluorinated sulfonates (PFSs)									
PFBS	$C_4F_9SO_3H$	299.95027	298.94299	0.02	298.94301	−0.1	5.68	42,987	0.08
PFHxS	$C_6F_{13}SO_3H$	399.94388	398.93661	0.02	398.93657	−0.1	9.43	54,425	0.08
PFHpS	$C_7F_{15}SO_3H$	449.94069	448.93341	0.02	448.93339	0	10.42	46,088	0.08
PFOS	$C_8F_{17}SO_3H$	499.9375	498.93022	0.02	498.93032	0.2	11.2	71,672	0.05
i,p-PFNS	$C_9F_{19}O_3SH$	549.9343	548.92703	0.02	548.92681	−0.4	12.03	61,845	0.08
PFDS	$C_{10}F_{21}SO_3H$	599.93111	598.92383	0.02	598.92456	1.2	12.44	48,987	0.08
Perfluorinated sulfonamides (PFSAs)									
PFOSA	$C_8H_2F_{17}NO_2S$	498.95348	497.9462	0.02	497.94706	1.7	12.23	25,302	
Isotopically labeled internal standards									
MPFBA	$^{13}C_4F_7O_2H$	217.9999	216.99262	0.02	216.99289	−1.2	1.69	2,690	25
MPHxA	$^{13}C_2C_4F_{11}O_2H$	315.9868	314.97952	0.02	314.98027	2.4	7.58	2,286	2.5
MPFOA	$^{13}C_4C_4F_{15}O_2H$	417.98712	416.97985	0.02	416.97998	0.3	10.41	3,228	1
MPFNA	$^{13}C_5C_4F_{17}O_2H$	468.98728	467.98001	0.02	467.98085	1.8	11.14	3,930	1
MPFDA	$^{13}C_2C_8F_{19}O_2H$	515.97403	514.96675	0.02	514.96646	−0.6	11.84	7,233	0.5
MPFUnDA	$^{13}C_2C_9F_{21}O_2H$	565.97083	564.96356	0.02	564.96481	2.2	12.42	2,246	1
MPFDOA	$^{13}C_2C_{10}F_{23}O_2H$	616.08672	615.07878	0.02	615.07674	3.3	12.92	6,025	0.5
MPHxS	$^{18}O_2C_6F_{13}SOH$	403.95237	402.9451	0.02	402.94542	0.8	9.45	38,788	0.08
MPFOS	$^{13}C_4C_4F_{17}SO_3H$	503.95092	502.94364	0.02	502.94387	0.5	11.22	88,774	0.05
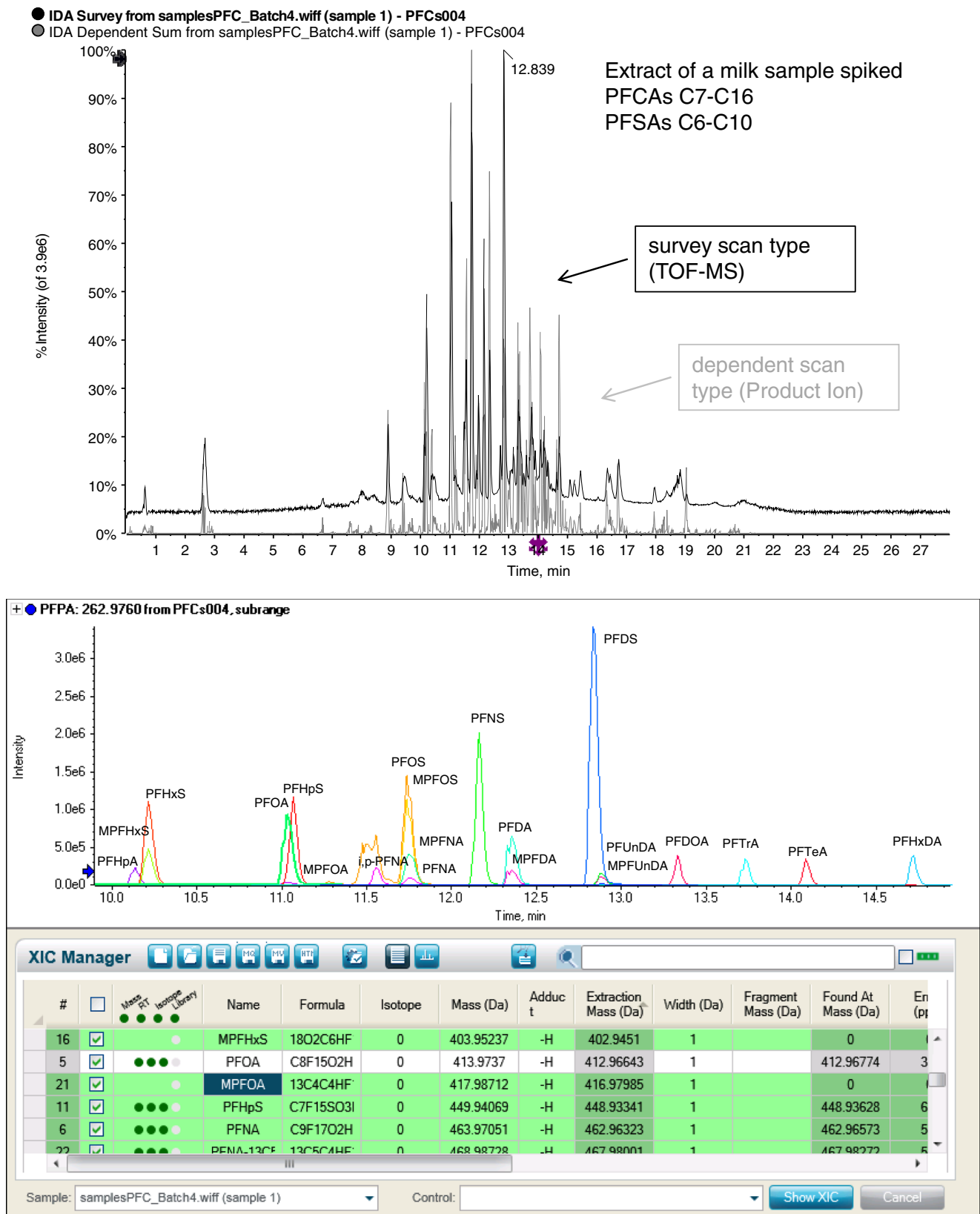
Fig. 1 UHPLC–QqTOF-MS/IDA-MS chromatograms obtained from a spiked human sample extract: *top* total ion chromatogram (TIC) and *middle* extracted ion chromatogram (XIC) of the standards (PFCAs C7– C18 and PFSAs C6–C10) and *bottom* the XIC Manager table with data on the 21 PFASs selected

35 V to gain the maximum information from each product ion scan. This allowed not only quantitation but also the identification of PFASs in the same single analysis.

Data acquisition and processing was carried out using the software Analyst, Peak View 1.0 with the application XIC Manager and Formula Finder; and MultiQuant 2.0. Peak View was used for targeted and non-targeted data processing launching the XIC Manager tool. The XIC Manager automatically generates extracted ion chromatograms (XICs), performs compound identification, and displays results within an XIC table that was self-edited for the selected PFASs. These tables include name, formula, adduct/modification, retention time, and width and are also editable. The results displayed include found mass, mass error (ppm), found at retention time and library (MS and MS/MS) search results.

For comparative purposes, PFASs were also determined using an Agilent 6410 QQQ equipped with an automatic injector, a desgasser, a binary pump, and a column oven (conditions for LC–QqQ-MSMS can be found in the Electronic Supplementary Material (ESM) in Tables S1 and S2).

Extraction procedure

Water, sediment, and fish samples were taken from the Jucar, Ebro, Llobregat, and Guadalquivir as a part of an extensive monitoring study within the SCARCE project. Meat samples (lean beef parts) were purchased from local retail markets. Individual breast milk samples from 10 women from the province of Valencia (Spain) were collected. Informed consent of all volunteer mothers was obtained according to the rules of the local ethics committee. The samples were taken according to the appropriate protocols in order to get a representative sample and to avoid contamination [3, 31]. Samples of river water ($n=15$), sediment ($n=15$), fish ($n=10$), meat ($n=10$), and human milk ($n=10$) were extracted using reported procedures [15, 31, 32]. Samples that did not contain PFASs were spiked with the 21 target analytes to validate the method.

ISs (62.5 µL, 100 ng mL^{-1}) were added to water samples (250 mL) to a concentration of 25 ng L^{-1}. Then, the sample was passed under the application of vacuum through STRA TA-X cartridges, previously preconditioned with 4 mL 0.1 % of NH$_4$OH in methanol, 4 mL of methanol, and 4 mL of H$_2$O. Once the sample passed, the cartridges were air-dried and analytes were then eluted with 4 mL of 0.10 % of NH$_4$OH
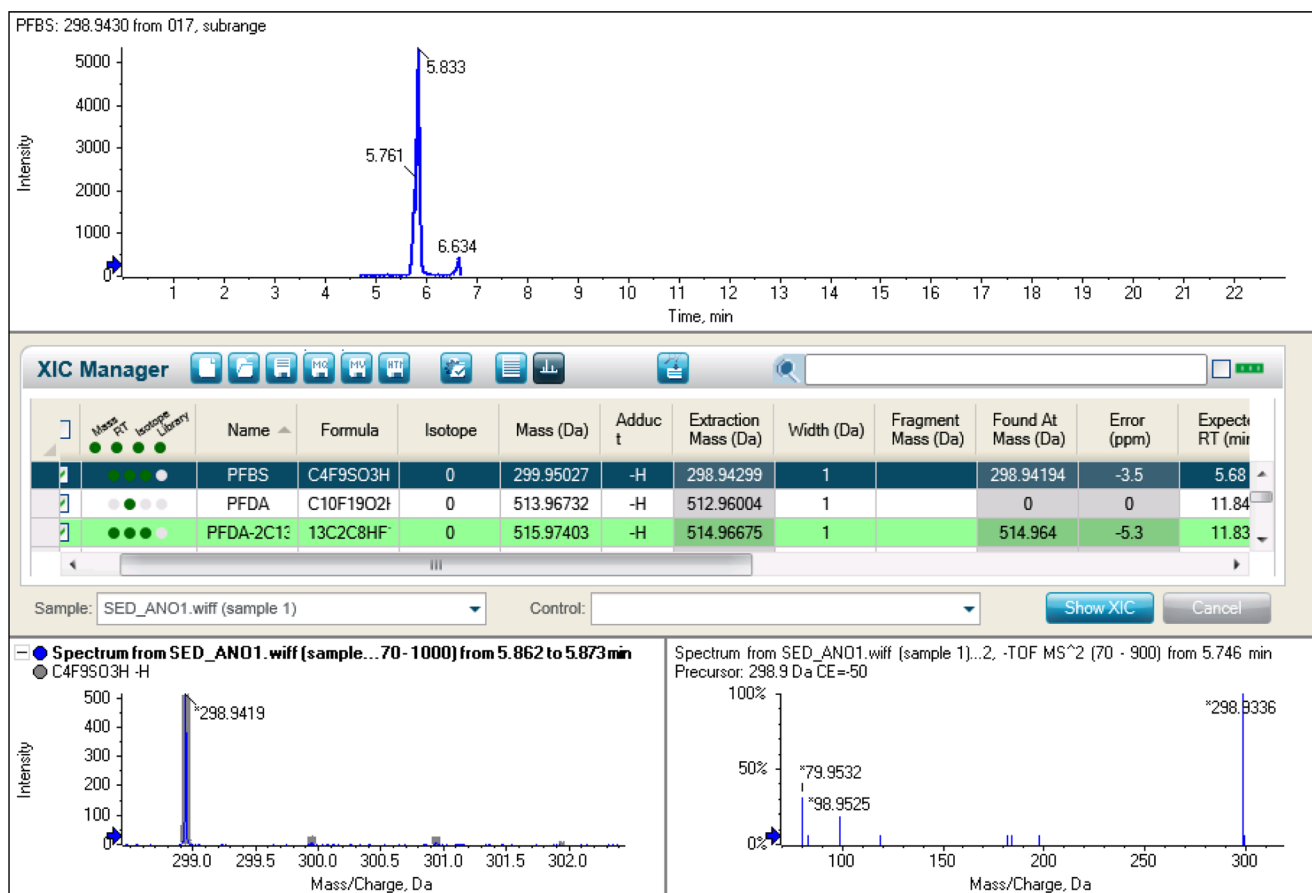

Fig. 2 Sediment sample: PFBS was identified using the XIC Manager by accurate mass and isotopic profiling. The MS/MS spectrum also confirms the identity of the compound

in methanol drop by drop. Extracts were evaporated to dryness and reconstituted with 250 μL of methanol acidified with 1 % acetic acid.

Sludge sample (5 g) was weighed into a 50-mL polypropylene tube and the ISs (62.5 μL, 100 ng mL^{-1}) were added to a concentration of 1.25 ng g^{-1}. This sample was homogenized with 10 mL of 1 % acetic acid in water (vortexed for 1 min, ultrasonicated for 15 min at 40 °C, and centrifuged at 3, 000 rpm for 2 min). The supernatant was transferred into a second polypropylene tube. The extraction step was repeated twice: first with 2.50 mL of methanol/acetic acid 1 % (90:10 v/ v) and second with 10 mL of 1 % acetic acid in water. The three supernatants were combined and the volume was adjusted to 250 mL with water for further SPE clean-up (as for water samples).

About 2 g of fish or meat muscle or 10 g of human milk, exactly weighted (fresh weight), was placed in a 15-mL polypropylene tube and spiked with the ISs (62.5 μL, 100 ng mL^{-1}) to a concentration of 3.125 and 0.625 ng g^{-1}, respectively. Each sample was homogenized for 2 min with 5 mL of water using an Ultraturrax T-25 digital homogenizer (Stauffen, Germany). Then, the sample was added to 8 mL of 10 mM sodium hydroxide in methanol and digested by shaking on an orbital shaker table at room temperature for 16 h. After digestion, samples were centrifuged at 2,000×g for 5 min, and 3 mL of the supernatant was diluted with 27 mL of deionized water prior to SPE as for water.

Background contamination issues

Background interferences from the standard laboratory and solvents as well as contamination can easily occur at all stages of collection and analysis [33–35]. Since PFAS are widely used as plasticizers, in the UHPLC system, trace amounts of PFASs (mostly PFOS and PFOA) leached from polytetrafluoroethylene-based (PTFE) tubing and fingertight fittings as also reported by other authors [34]; therefore, all PTFE tubing was removed from the system and replaced with stainless steel tubing. Furthermore, all glassware and plastic material used in this study for sampling and extraction were first thoroughly washed with soap and water and then cleaned using solvents in sequence, such as water, methanol, dichloromethane, and then again methanol. The Strata X cartridges used were preconditioned first with the solutions used to elute the analytes in order to eliminate any endogenous contamination.

Moreover, all solvents and reagents utilized were carefully checked to be analyte-free, by performing blank procedure analysis. Solvent or ammonium formate contaminations were not observed, even though they were reported by several authors [9, 34, 35]. To ensure the reliability of the process and the absence of background contamination, matrix blank and procedural blanks were carried out with each batch of samples. Furthermore, travel blanks were performed.

Fig. 3 Fish sample: PFOS was identified using the XIC Manager by accurate mass and isotopic profiling. The MS/MS spectrum also confirms the identity of the compound

Validation

The method was validated by calculating linearity, matrix effect, limits of quantification (LOQs), precision as repeatability and within-lab reproducibility, and recovery as widely recommended [1, 22, 26]. The LOQ was estimated by injecting decreasing concentrations of standards and measuring the response to find the concentrations that would give a UHPLC peak height value 1.0×10^4 as recommended elsewhere [24, 30]. The instrumental LOQs were verified for each matrix and established in a reliable way through the recovery studies.

Quantification was achieved using internal calibration with isotopically labeled standards added as surrogates in the beginning of the sample treatment, thus correcting quantification errors due to matrix interferences and extraction and/or MS acquisition discrepancies. The ratio of the most intense transition peak area to the corresponding surrogate peak area was plotted versus concentration.

Linearity was evaluated by preparing calibration curves from LOQs to 100 ng mL^{-1} for each compound. The calibration curves were prepared in methanol and in extracts of water, fish, meat, and human milk (after ensuring that the samples were free of PFASs). The matrix effects were studied by comparing the slope of the calibration curves in methanol and in the matrix extracts.

Recoveries and relative standard deviations (RSDs) of PFASs were calculated by spiking samples in which the target PFASs were not found at three different concentrations (low level, LOQ; medium level, 10×LOQ; and high level, 100× LOQ) and then analyzing them in quintuplicate. The precision of the method was determined by the repeatability and reproducibility studies, and expressed as the RSD (%). The intraday precision was measured by comparing the standard deviation of the recovery percentages of the spiked samples carried out during the same day. The interday precision was determined by analyzing the spiked samples on five distinct days.

Results and discussion

Qualitative screening

Table 1 outlines the mass error obtained as well as the retention time and the instrumental LOQ obtained in nanograms per milliliter of the extract. The mass error ranged from −4 to 3.4 which demonstrated good mass accuracy. Using the
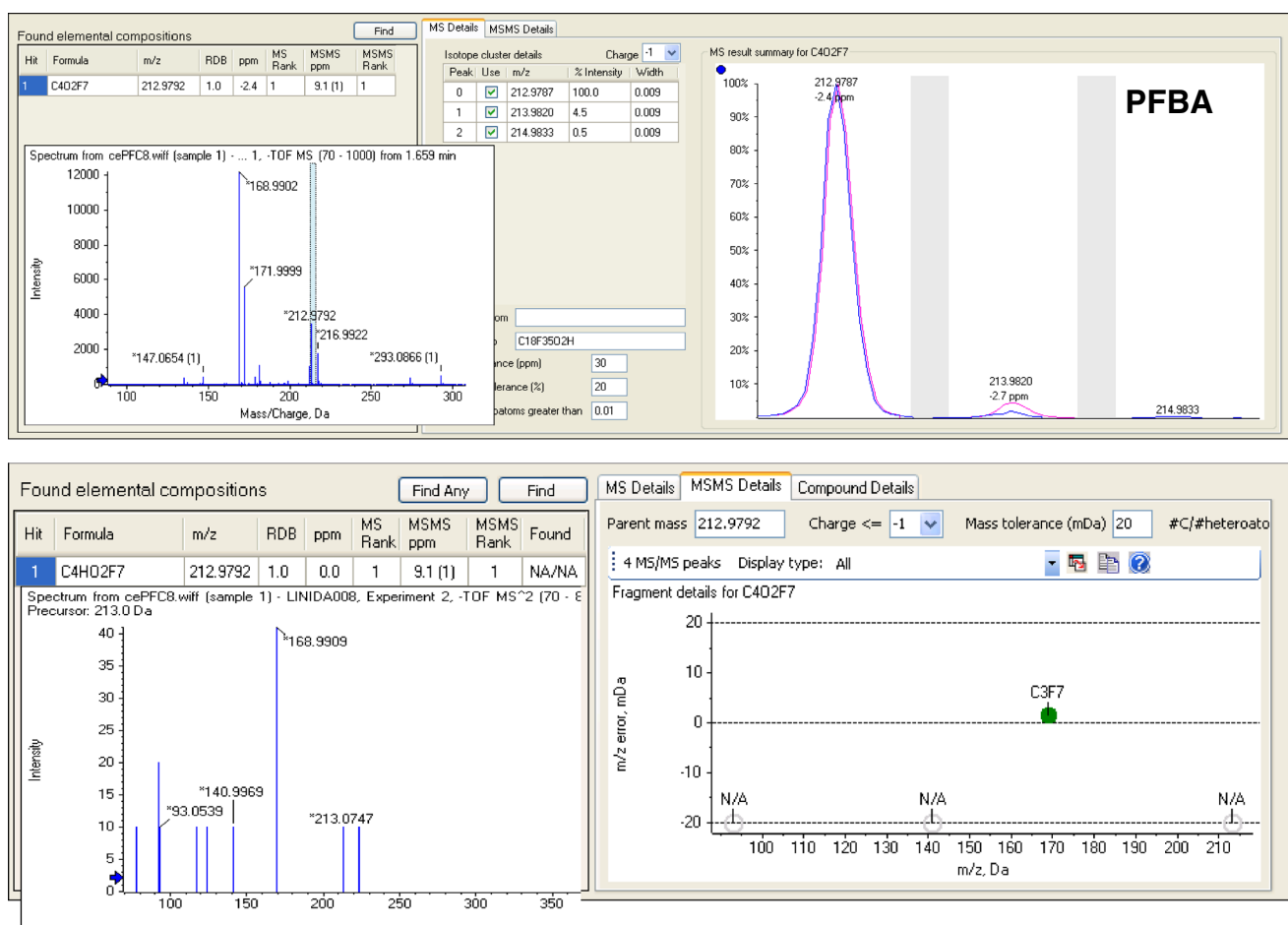

Fig. 4 Water sample: PFBA was identified using Formula Finder and combining TOF-MS and TOF-MS/MS information

proposed gradient all PFASs were eluted in less than 14 min. However, the gradient was enlarged by an additional 10 min in order to ensure the cleaning of the system when dirty samples are injected. The method, as can also be observed in Table 1, was much more sensitive for perfluoroalkyl sulfonates than for the perfluoroalkyl carboxylates. In fact, it is quite difficult to develop a method for the simultaneous determination of both types of compounds using IDA because it is impossible to optimize the collision cell parameters for each different precursor selected to obtain the MS/MS. The use of the same collision energy (CE) might preclude the chance of obtaining appropriate product-ion mass spectra because perfluoroalkyl carboxylates are fragmented at low collision energies, whereas sulfonates require high collision energies to fragment. This parameter was then carefully optimized including the use of energy spread that was needed to attain appropriate sensitivity to quantify carboxylates in environmental and food samples (from 0.5 to 25 ng mL^{-1} in the extract) as well as very good instrumental LOQs for sulfonates (from 0.05 to 0.08 ng mL^{-1}).

Figure 1 shows the total ion chromatogram (TIC) of both the survey scan and the data dependent acquisition, obtained for a blank human milk sample spiked at 2 ng g^{-1} with the PFASs as well as the extracted chromatogram together with an XIC Manager table developed for the 21 selected PFAs using the program template. This table was created by introducing the name, empirical formula, and retention time of each compound, ionization mode, and adduct formation (the program calculates the accurate mass of the molecule and extracted mass as well as the isotope ratio). Then, when a sample is injected and the exact mass extracted, the XIC Manager is able to calculate the mass error (in ppm), isotope ratio (as a percentage difference), and purity score (in percent). Furthermore a MS/MS library was created using Analyst® and Microsoft Access software in order to better identify the compounds. As could be observed in the chromatogram, most of the visible identified peaks correspond to the target analytes, which demonstrated the appropriate selectivity of the method as well as the lack of false positives and negatives. The qualitative identification of target compounds is also noticeable. Figure 2 shows the identification of PFBS in a sediment sample from the Guadalquivir River Basin using this XIC Manager (the library match appears in red because it was done without incorporating the library searching) and Fig. 3 shows the identification of PFOS in a fish sample using the XIC Manager (neither library nor retention time was incorporated). However, the MS/MS recorded clearly confirms the identity

Table 2 Linearity at a concentration range from LOQ to 100 ng mL^{-1} and LODs and LOQs obtained for the PFASs using the IS approach by UHPLC–QqTOF-IDA-MS/MS

PFCs	IS	Equation	r	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Perfluorocarboxylic acids					
PFBA	MPFBA	$y=0.065x-0.099$	0.98339	8	25
PFPA	MPFBA	$y=0.182x+0.042$	0.98752	8	25
PFHxA	MPFHxA	$y=0.755x-0.099$	0.99885	0.8	2.5
PFHpA	MPFHxA	$y=2.354x-0.204$	0.99099	0.2	0.5
PFOA	MPFOA	$y=0.036x-0.005$	0.99877	0.8	2.5
PFNA	MPFNA	$y=0.111x+0.003$	0.99924	0.3	1
i,p-PFNA	MPFNA	$y=6.493x-0.283$	0.99943	0.3	1
PFDA	MPFDA	$y=0.066x+0.005$	0.99719	0.3	1
PFUnDA	MPFUnDA	$y=0.268x-0.016$	0.99612	0.3	1
PFDoA	MPFDoA	$y=0.304x-0.020$	0.99907	0.3	1
PFTrA	MPFDoA	$y=0.462x+0.005$	0.99603	0.2	0.7
PFTeA	MPFDoA	$y=0.755x-0.052$	0.99651	0.3	1
PFHxDA	MPFDoA	$y=1.288x-0.007$	0.99947	0.1	0.3
PFODA	MPFDoA	$y=1.094x-0.020$	0.99907	0.1	0.3
Perfluorosulfonates					
PFBS	MPHxS	$y=0.120x-0.000$	0.99472	0.03	0.08
PFHxS	MPHxS	$y=0.402x-0.025$	0.99879	0.03	0.08
PFHpS	MPHxS	$y=0.666x-0.034$	0.99870	0.03	0.08
PFOS	MPFOS	$y=0.057x-0.004$	0.99956	0.02	0.05
i,p-PFNS	MPFOS	$y=1.230x-0.000$	0.99972	0.03	0.08
PFDS	MPFOS	$y=5.723x-0.049$	0.99788	0.03	0.08
Perfluorosulfonamides					
PFOSA	MPFOS	$y=2.935x+0.010$	0.99406	0.1	0.3

of the compounds. The presence of branched isomers is clearly observed. More examples are displayed in the ESM (Figs. S1–S5).

The compounds can also be identified with other algorithms of the software, such as Formula Finder, which is a non-target algorithm recommended for the identification of unknown compounds. However, it can also be of help to get information on the empirical formula of the product ions of target or suspected compounds as well as on the product ion mass errors. Figure 4 shows the unequivocal identification of PFBA in a water sample and other examples are illustrated in the ESM (Figs. S6 and S7).

The possibility to apply accurate mass to determine PFASs is important because some of the low molecular weight compounds, e.g., PFBA, PFPA, and PFOSA, only give one product ion; in such cases, the only way to achieve enough confidence in the identification is to confirm the identity by accurate mass spectrometry. This method focused on the 21 selected PFASs, but is not a target method and can, therefore, detect any other compound that gave signals in the negative mode and confirm those that gave a product ion mass spectrum under the selected conditions of the collision cell. A search of other possible compounds that ionized in negative mode

was carried out in the analyzed samples. Acetaminophen, ibuprofen, salicylic acid, atorvastatin, and gemfibrozid were frequently detected in water and sediment samples but not always confirmed by MS/MS (acetaminophen mass spectrum is illustrated in ESM Fig. S8). In the samples of milk, meat, and fish, other compounds were not identified, probably because the extraction method is selective, because it requires a digestion with sodium hydroxide to remove the protein binding which probably degrades any other organic pollutant of polar character that could be detected by UHPLC–QqTOF-MS. There are other reasons that justify the lack of other compounds in these samples, e.g., they were simply not present or these compounds cannot be detected by the applied UHPLC method.

The instrumental LOQ was determined empirically, by injecting a series of spiked extract, as the concentration for which the peak height was greater than 1.0×10^4, and it is reported in Table 1. The instrumental LOQ obtained was between 0.05 and 25 ng mL^{-1} in the extract injected in the QqTOF, which will be equivalent to LOQs between 0.05 and 25 ng L^{-1} in water, 0.003 and 1.25 ng g^{-1} in sediment, 0.007 and 3.25 ng g^{-1} in fish and meat, and 0.002 and 0.75 ng g^{-1} in human milk. These estimated LOQs need to
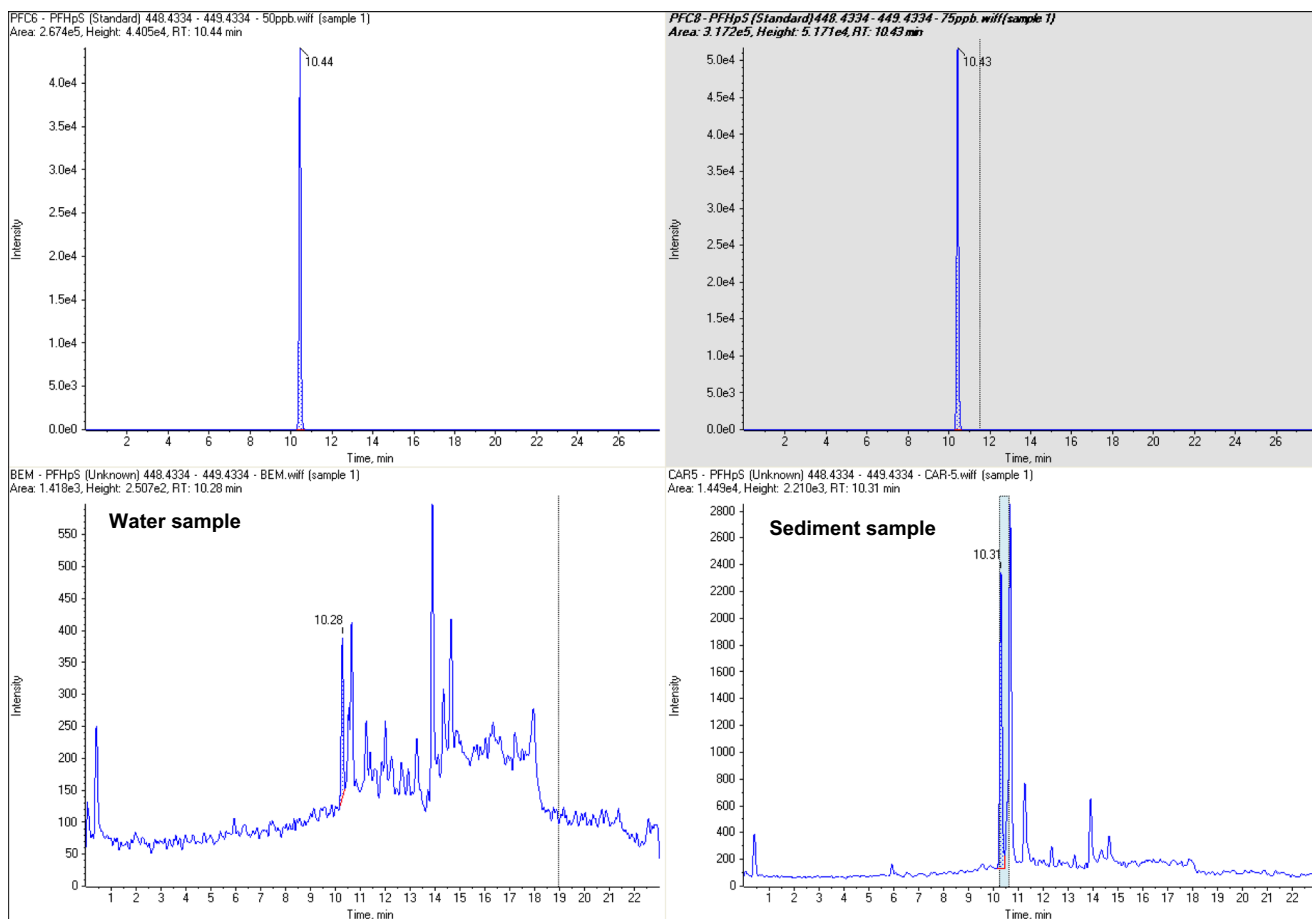

Fig. 5 Results of the quantification in a water and sediment sample

be further confirmed experimentally because low extraction recoveries and high matrix effects can greatly increase them. However, UHPLC–QqTOF-MS (IDA-MS/MS) seems to attain enough sensitivity to determine PFASs at low levels in food and environmental samples.

Quantification of PFASs

The calibration curves were linear with coefficients of determination (R) of at least 0.99 (Table 2). These results correspond to calibration curves prepared in methanol. When the curves were prepared using matrix-matched standards, the matrix effects were present particularly for water and sediment samples. The use of internal standards corrected the matrix interferences for quantifications resulting in accurate quantification and similar slopes between curves obtained in matrix and in methanol. The peak area vs. concentration showed a linear signal over more than three orders of magnitude (ESM Fig. S9 illustrates the regression curve obtained for PFHpS). Thus, QqToF-MS showed good linearity for all compounds ($R \geq 0.99$) except for PFBA and PFPA that gave lower R values. Figure 5 illustrates the quantification of PFHpS in a

water sample and a sediment sample. This PFAS constitutes a minor component.

The most common matrix effect is suppression. In water, the observed matrix effect is always suppression and reaches 25 %; in sediments suppression of the response reaches 40 % and there is also enhancement of the response for some compounds, e.g., up to 20 % for PFHxA and PFOA. Fish, meat, and milk samples also presented a suppression effect for some compounds of up to 30 %. This affects the sensitivity of the method but not the quantification because the matrix effects are corrected for by using the isotopically labeled ISs. The matrix effect depends not only on the compound and matrix but also on the design of the ESI interface. In comparison with the UHPLC–QqQ-MS/MS, the matrix effects observed with this system was always in general significantly lower.

Table 3 outlines the LOQs established by spiking non-contaminated matrices and following the whole extraction process. They are in the range of 0.1–50 ng L^{-1} in water, 0.05–2 ng g^{-1} in sediments, 0.01–5 ng g^{-1} in fish and meat, and 0.005–2 ng g^{-1} in human milk depending on the compound. An increase in the LOQs is observed comparing them with the instrumental ones reported in Table 1. The LOQs obtained using UHPLC–QqTOF-MS are up to 10-fold higher

Table 3 Experimental LOQ, recoveries, and RSDs at this LOQ using UHPLC–QqTOF-MS

	Water			Sediments			Fish			Meat			Human milk		
	LOQ (ng L^{-1})	R (%)	RSD (%)	LOQ ng g^{-1}	R (%)	RSD (%)	LOQ (ng g^{-1})	R (%)	RSD (%)	LOQ (ng g^{-1})	R (%)	RSD (%)	LOQ (ng g^{-1})	R (%)	RSD (%)
PFBA	50	67	16	2.0	62	18	5	65	17	5	68	15	2	70	11
PFPA	50	70	14	2.0	84	16	5	60	15	5	65	14	2	63	14
PFHxA	5	75	13	0.2	100	10	1	82	12	1	76	12	0.1	85	12
PFHpA	1	84	13	0.1	95	11	0.1	85	14	0.1	83	12	0.05	92	12
PFOA	5	83	12	0.2	99	8	1	83	11	1	84	10	0.1	80	10
i.p-PFNA	2	82	10	0.1	103	9	1	83	11	1	86	13	0.1	82	10
PFNA	2	90	10	0.1	95	12	1	86	12	1	89	12	0.1	79	13
PFDA	2	94	11	0.1	100	10	1	82	10	1	92	11	0.1	89	11
PFUnDA	2	92	9	0.1	72	10	1	85	11	1	92	10	0.1	87	11
FFDOA	2	94	9	0.1	90	11	1	87	9	1	90	10	0.1	86	12
PFTrA	1	93	10	0.1	94	12	0.5	90	8	0.5	93	9	0.1	90	10
PFTeA	1	98	10	0.1	88	11	0.5	89	10	0.5	90	9	0.1	92	8
PFHxDA	0.5	91	11	0.1	93	11	0.5	89	9	0.5	91	8	0.05	91	9
PFODA	0.5	90	10	0.1	79	8	0.5	88	9	0.5	90	8	0.05	65	16
PFBS	0.1	89	14	0.1	88	8	0.05	78	15	0.05	82	13	0.01	80	13
PFHxS	0.1	91	12	0.1	89	10	0.05	90	12	0.05	84	10	0.01	85	12
PFHpS	0.1	95	12	0.1	94	12	0.05	93	13	0.05	90	10	0.01	89	8
PFOS	0.1	99	10	0.1	98	9	0.01	94	11	0.01	92	9	0.005	90	8
i.p-PFNS	0.1	94	10	0.1	99	10	0.05	95	11	0.05	93	11	0.01	93	10
PFDS	0.1	93	10	0.1	100	9	0.05	95	10	0.05	95	10	0.01	95	8
PFOSA	0.2	78	10	0.1	70	9	0.1	71	9	0.1	64	14	0.03	69	15

R recovery

Table 4 Comparison of QqQ-MS/MS in SRM and QqToF-MS for the quantification of PFASs in the different matrices

MS analyzer			Water (n=15) ng L⁻¹				Sediments (n=15) ng g⁻¹				Fish (n=10) ng g⁻¹				Meat (n=10) ng g⁻¹				Human milk (n=10) ng g⁻¹			
			Min	Max	Mean	Freq	Min	Max	Mean	Freq	Min	Max	Mean	Freq	Min	Max	Mean	Freq	Min	Max	Mean	Freq
PFBA	QqQ		52.1	644	65.4	5	2.70	10.6	5.83	15	5	264	71.9	6	ND	ND	–	–	ND	ND	–	–
	QqTOF		56.1	700	68.7	5	2.16	9.48	5.80	15	5.3	259	68.8	6	ND	ND	–	–	ND	ND	–	–
PFHxA	QqQ		5.02	18.7	4.24	6	ND	ND	–	–	48.1	592	85.5	3	2.01	20.9	5.55	5	ND	ND	–	–
	QqTOF		5.54	20.2	4.35	6	ND	ND	–	–	51.8	602	87.9	3	1.52	23.2	5.77	5	ND	ND	–	–
PFHpA	QqQ		1.64	20.1	4.52	6	0.39	1.06	0.15	3	38.3	52.9	31.2	7	1.25	35.0	3.85	3	2.25	10.8	2.98	5
	QqTOF		1.58	22.4	4.47	6	0.40	1.26	0.17	3	40.2	54.2	32.5	7	1.32	34.6	3.84	3	2.03	11.2	3.02	5
PFOA	QqQ		0.15	52.2	8.29	8	0.15	6.69	1.69	9	19.6	62.8	8.24	2	0.53	92.3	12.7	7	10.5	162	59.3	8
	QqTOF		0.19	53.9	8.41	8	0.14	7.09	1.86	9	20.1	60.3	8.04	2	0.58	91.8	12.2	7	9.94	159	64.9	8
PFNA	QqQ		0.85	19.8	2.24	4	3.63	3.63	0.48	1	17.3	24.3	6.16	3	1.25	18.6	3.01	3	3.25	15.9	7.32	7
	QqTOF		0.92	20.3	2.35	4	3.23	3.23	0.43	1	17.9	25.2	6.21	3	1.32	19.2	3.19	3	3.33	14.8	7.61	7
PFDA	QqQ		0.98	213	23.6	3	0.37	1.65	0.31	4	19.4	60.2	12.5	3	3.64	56.3	7.82	4	1.09	12.4	1.90	3
	QqTOF		1.03	214	24.5	3	0.35	1.56	0.29	4	19.6	61.9	12.4	3	3.58	55.9	7.90	4	0.98	11.9	1.98	3
PFUnDA	QqQ		0.62	0.62	0.08	1	ND	ND	–	–	17.0	70.8	20.7	5	5.23	32.8	4.82	3	ND	ND	–	–
	QqTOF		0.59	0.59	0.08	1	ND	ND	–	–	16.9	71.4	20.0	5	5.36	32.5	4.91	3	ND	ND	–	–
PFBS	QqQ		ND	ND	–	–	2.17	29.2	4.49	4	7.06	7.92	5.27	7	ND	ND	–	–	ND	ND	–	–
	QqTOF		ND	ND	–	–	2.32	30.1	4.68	4	6.97	8.01	5.18	7	ND	ND	–	–	ND	ND	–	–
PFHxS	QqQ		12.1	36.7	3.25	2	0.52	0.58	0.15	4	0.82	10.4	3.92	9	1.25	15.4	2.20	3	1.09	34.1	6.58	5
	QqTOF		11.9	37.3	3.28	2	0.54	0.51	0.14	4	0.92	11.8	4.20	9	1.32	15.6	2.12	3	1.13	34.8	7.01	5
PFHpS	QqQ		0.25	1.16	0.09	2	0.12	1.45	0.20	4	ND	ND	–	–	ND	ND	–	–	ND	ND	–	–
	QqTOF		0.32	1.12	0.10	2	0.14	1.24	0.22	4	ND	ND	–	–	ND	ND	–	–	ND	ND	–	–
PFOS	QqQ		0.15	128	15.2	6	0.06	9.83	3.50	10	17.9	29.8	18.5	7	2.23	32.9	4.71	5	1.28	189	71.53	10
	QqTOF		0.13	129	15.1	6	0.05	10.0	3.54	10	17.1	30.4	18.4	7	2.74	34.2	5.05	5	1.30	191	70.65	10

Min min value different from zero, *mean* non-detected (ND) was counted as zero

than those obtained by UHPLC–QqQ-MS/MS in SRM for few compounds but almost equal for most of them (see ESM Table S3). However, they are appropriate to determine these PFASs in the selected matrices and of the same order of magnitude as those already reported using other instrumental set-ups [15, 31, 32]. The recoveries and RSDs at these LOQ levels were calculated by extracting and determining five different spiked samples on different days. Average recoveries (and RSD) were 67–99 % (9–16 % RSD) for water, 62–103 % (8–18 % RSD) for sediment, 60–95 % (8–17 % RSD) for fish, 64–95 % (8–15 % RSD) for meat, and 63–95 % (8–16 %) for human milk. Recoveries and RSDs were also calculated at 10 and 100 times the LOQs (data shown in ESM Table S4). These values were also in the range of those published [15, 31, 32]. Recovery and precision obtained using the UHPLC–QqQ-MS/MS were almost equal, as expected because recovery depends mostly on the extraction procedure that was the same for both instruments.

Some samples were analyzed by UHPLC–QqTOF-MS and the results were compared with a previous method developed using UHPLC–QqQ-MS/MS (see conditions in ESM Tables S1 and S2). Table 4 shows that good agreement between both methodologies was obtained, indicating the reliability of the proposed methods.

Conclusions

The screening of PFASs in water, sediments, meat, fish, and human milk by accurate high resolution mass spectrometry using UHPLC–QqTOF-MS is reported for the first time. The operation in IDA without any preselection of the precursor ion by the analyst is an interesting feature because the method is not limited to the analysis of target PFASs as reported here, but is also applicable for the identification of a wide range of other compounds, including pharmaceuticals and pesticides. The results also enhance the reliability of UHPLC–QqTOF-IDA-MS/MS when used to quantify target analytes, by showing appropriate recovery, linearity, precision, and sensitivity. The obtained validation results demonstrated the great confirmatory and quantitative strength of this newly developed UHPLC–QqToF-MS/MS method, which additionally offers great potential for untargeted screening. This system is also confirmed as a time-competitive approach for routine analysis compared to other LC–MS quantitation methods. The methodology is not restricted to this application but can find widespread utilization in the efficient identification of organic contaminants and even other compounds in food and environmental matrices.

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