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A validated analytical method for the determination of perfluorinated compounds in surface-, sea- and sewagewater using liquid chromatography coupled to time-of-flight mass spectrometry

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ABSTRACT

Perfluorinated compounds (PFCs), which are extensively used in a wide variety of applications because of their specific surfactant properties, have recently appeared as an important new class of global environmental pollutants. Quantitative analysis of PFCs in aqueous matrices remains, however, a challenging task. During this study, a new analytical method for the determination of 14 PFCs in surface-, sewage- and seawater was developed and validated. The target analytes were extracted using solid-phase extraction followed by liquid chromatography coupled to a time-of-flight mass spectrometer (LC-ToF-MS). The use of very narrow mass tolerance windows (<10 ppm) resulted in a highly selective MS-technique for the detection of PFCs in complex aqueous matrices. Validation of this analytical method in surface-, sewage- and seawater resulted in limits of quantification (LOQs) varying from 2 to 200 ng L⁻¹, satisfying recoveries (92–134%), and good linearity ($R^2 = 0.99$ for most analytes). Analysis of samples of the North Sea, the Scheldt estuary, and three harbours of the Belgian coastal region led to the detection of four different PFCs. Perfluorooctane sulfonate (PFOS) was found to be the most abundant PFC in levels up to 38.9 ng L⁻¹.

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1. Introduction

Perfluorinated compounds (PFCs) constitute a large group of chemicals characterized by a fully fluorinated hydrophobic carbon chain attached to various hydrophilic heads [1]. The chemical class of PFCs includes the perfluoroalkyl carboxylates, the perfluoroalkyl sulfonates, the perfluoroalkyl sulfonamides, and related products. Their chemical and thermal stability and surface tension lowering properties make them very useful for a wide variety of applications and products: as additives in fire-fighting foam and food packaging, as fat and water repellents for textile, paper and leather treatment, as performance chemicals, and as polymerization aid for the production of fluorinated polymers such as polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) [1,2]. Within the group of PFCs, perfluorooctane sulfonate (PFOS), the final degradation product of the frequently used sulfonated fluorochemicals, has been identified as the most important contaminant [2]. Moreover, PFOS

fulfils the criteria of a persistent organic pollutant (POP) under the Stockholm convention [3]. As a result, EU legislation established the PFOS directive 2006/122/EC [4] which aims at ending the use of PFOS.

In general, PFCs have been reported as extremely persistent environmental contaminants with bioaccumulative and toxic properties [5,6]. Consequently, the concern about the environmental fate and prevalence of PFCs has increased in recent years. Recent monitoring studies have reported the widespread occurrence of PFCs in water [7], air [8], and biological matrices [9]. In surface water [10–12] as well as in wastewater [13,14], PFCs have generally been detected in the ng L⁻¹ up to μg L⁻¹ concentration range. Furthermore, PFCs have been found in seawater and open ocean waters, implying the transport of PFCs from surface water through estuaries to coastal regions and consequently to open oceans [15–17]. According to Van Leeuwen et al. [18], the quality of data obtained from analysis of PFCs in environmental matrices is a major issue of concern. The occurrence of branched isomers, matrix interferences, and cross contamination rendered quantitative analysis of PFCs in aqueous matrices a challenging task. To the best of our knowledge, the use of accurate mass high-resolution mass spectrometric tech-

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Fig. 1. Sampling stations in the North Sea (W01–W06), the Scheldt estuary (S01 and S22) and in the harbour of Nieuwpoort (NP1–NP3), Ostend (OO01–OO04) and Zeebrugge (ZB01–ZB04).

niques for the quantification of PFCs in water samples has not been reported earlier [19].

Therefore, in this study, an analytical methodology was developed using liquid chromatography (LC) coupled to a time-of-flight mass spectrometer (ToF-MS). ToF-MS provides sensitive full scan data and allows the detection of the target PFCs by accurate mass measurements, resulting in a highly selective MS-technique. Fourteen environmentally relevant PFCs were selected, including four perfluoroalkyl sulfonates, nine perfluoroalkyl carboxylic acids and perfluorooctane sulphonamide. A validation study was carried out to demonstrate the applicability of this analytical approach. Finally, the developed method was applied to marine water samples from the North Sea and Scheldt estuary to examine the presence of PFCs in the Belgian marine environment.

2. Materials and methods

2.1. Study area and sampling

The study area is located in the three Belgian coastal harbours (Ostend, Nieuwpoort, and Zeebrugge), the Scheldt estuary, and the offshore coastal area of Belgium. An overview of the study area and sampling stations is depicted in Fig. 1. Ten sampling stations were selected in three coastal harbours; four in the harbour of Zeebrugge (ZB01–ZB04) and three in the harbours of Nieuwpoort (NP01–NP03) and Ostend (OO02–OO04) each. In each harbour, one sampling station was representative for the major freshwater inputs into the harbour, while a second sampling location represented the water at the harbour mouth, and at least one station between these points was sampled as well. An additional station was selected at the Sluice Dock in Ostend (OO01) since at this location aquacultural activities take place. Two stations were sampled in the Scheldt estuary: one station

located at the river mouth near Vlissingen, the second more upstream near Antwerp. Six sampling stations were chosen in the Belgian coastal area: three (W01, W02 and W03) were located close to the harbour mouth of Ostend, Nieuwpoort and Zeebrugge; the remaining three (W04, W05 and W06) were situated more offshore. The sampling campaign was carried out in June 2009.

The 'Zeekat', a rigid inflatable boat, was used for sampling the harbour stations. North Sea and Scheldt estuary stations were sampled with the larger research vessels 'Belgica', 'Zeeleeuw', and 'Scheldewacht'. Water samples were collected at each sampling site using Go-Flo bottles® (General Oceanics Inc., Miami, Florida, USA) at a depth of 4–5 m. Go-Flo bottles® avoid sample contamination at the surface, internal contamination, loss of sample on the deck, and exchange of water from different depths. Samples were stored at 4 °C in the dark before analysis.

2.2. Reagents and chemicals

Fourteen PFCs were examined in this study: four perfluoro-sulfonates (potassium perfluoro-1-butane sulfonate, sodium perfluoro-1-hexane sulfonate, sodium perfluoro-1-octane sulfonate, and sodium perfluoro-1-decane sulfonate), nine perfluorocarboxylates (perfluoro-*n*-pentanoic acid, perfluoro-*n*-hexanoic acid, perfluoro-*n*-heptanoic acid, perfluoro-*n*-octanoic acid, perfluoro-*n*-nonanoic acid, perfluoro-*n*-decanoic acid, perfluoro-*n*-undecanoic acid, perfluoro-*n*-dodecanoic acid, and perfluoro-*n*-tetradecanoic acid) and perfluoro-1-octane sulphonamide. All analytical standards were purchased from Wellington Laboratories (Guelph, Ontario, Canada) with chemical purities of more than 98%. Six ¹³C-labelled internal standards were used as well: sodium perfluoro-1-[1,2,3,4-¹³C₄] octane sulfonate, perfluoro-*n*-[1,2-¹³C₂] hexanoic acid, perfluoro-*n*-[1,2,3,4-¹³C₄] octanoic acid, perfluoro-*n*-[1,2,3,4,5-¹³C₅] nonanoic acid,

perfluoro-*n*-[1,2-¹³C₂] decanoic acid, and perfluoro-*n*-[1,2-¹³C₂] dodecanoic acid.

Methanol was purchased from Rathburn Chemicals (LTD Walkerburn, Scotland), while HPLC-grade water was obtained from Biosolve (Biosolve Chemicals, The Netherlands). Ammonium acetate (2.5 mM) in water was obtained from dilution of LC-MS Chromasolv[®] water containing 0.1% ammonium acetate (Sigma-Aldrich Laborchemikalien GmbH, Seelze). Except for PFOSA, primary stock solutions of all individual analytes were prepared in methanol at a concentration of 50 μg mL⁻¹. PFOSA was purchased in nonane at the same concentration. Working standard mixture solutions were prepared by appropriate dilution of the stock solutions in methanol. All solutions were stored at -20 °C in the dark.

2.3. Extraction and clean-up

The sample preparation protocol was based on the ISO 25101/2006 method [20], which was in its turn derived from the method of Taniyasu et al. [21]. Sewage water samples and water samples, visibly containing particulate matter, were filtered through a glass fibre paper (GF 52 Ø110 mm, Schleicher & Schuell, Dassel, Germany) prior to extraction. Depending on the aqueous matrix, different volumes of water were extracted. In case of surface- and sewage water, 50 mL water was extracted, while 250 mL was used for seawater samples. The ¹³C-labelled internal standards were supplemented to every sample prior to extraction to a final concentration of 100 ng L⁻¹. Solid-phase extraction was carried out using OASIS HLB cartridges (6 cm³, 200 mg, Waters, Milford, MA). The cartridges were pre-conditioned with 2 mL methanol and 2 mL Biosolve water. After loading, the cartridges were rinsed with 2 mL Biosolve water for surface- and sewage water. For seawater, 3 × 2 mL Biosolve water was applied. Subsequently, the cartridges were dried under vacuum for 10 min. Elution was achieved using 2 × 2 mL methanol. Next, extracts were concentrated to 0.5 mL under a gentle stream of nitrogen. Finally, 0.5 mL of 2.5 mM ammonium acetate in water was added before transfer to LC-MS vials. Samples were stored at 4 °C before analysis.

2.4. Chromatographic instrumentation

The LC-apparatus comprised of a 1200 series binary gradient pump and a 1100 series autosampler (Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was achieved using a Luna[®] C18 (2) HPLC column (5 μm particle size, 250 mm × 2.0 mm; Phenomenex Inc., Utrecht). The mobile phase consisted of a mixture of (A) 2.5 mM ammonium acetate in water and (B) methanol. A linear gradient of 0.3 mL min⁻¹ was used starting with a mixture of 50% A and 50% B, increasing to 90% B in 10 min. This ratio was kept for 6 min before reversion to the initial conditions.

Analytes were detected with a time-of-flight mass spectrometer equipped with a dual electrospray ionisation interface (ESI MSD ToF, Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was operated in the negative ion mode. Instrument parameters were: drying gas temperature of 325 °C, drying gas flow of 5 L min⁻¹, nebuliser pressure of 20 psi, capillary voltage of 3500 V, and chamber voltage of 3000 V. Before analyzing a series of samples, the ToF-MS apparatus was tuned and calibrated using the ESI Tuning Mix (Agilent Technologies, Santa Clara, CA, USA). During analysis, a reference solution was pumped into the MS system at a rate of 50 μL min⁻¹ using a separate sprayer connected to a 1100 series pump (Hewlett Packard, Palo Alto, CA, USA). This reference solution consisted of purine with a *m/z* ratio of 119.0363 and HP-0921 (hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine) with a *m/z* ratio of 980.0164 in ACN/H₂O (95/5) (Agilent Technologies,

Santa Clara, CA, USA). Accurate mass measurements could only be achieved if these reference masses were detectable. Chromatograms and spectra were recorded and processed using Agilent MassHunter Quantitative Analysis[®] software (Agilent Technologies, Santa Clara, CA, USA).

2.5. Validation of the method

To demonstrate the applicability of this analytical approach, a validation study was carried out. Besides the validation procedure of the accredited lab of the Flemish Environment Agency (FEA), the SANCO/2007/3131 document [22] was also used as guideline for the validation of this new analytical method. Validation comprised the assessment of specificity/selectivity, linearity, recovery, precision, and the limits of detection (LODs) and quantification (LOQs). Eight-point calibration curves were constructed in surface water (six replicates). To demonstrate the multi-matrix capacity of our analytical procedure, a limited identical validation study was performed using sea- and sewage water as well (four replicates). Representative water samples, being water from the river Kale – which is a small river near Ghent – for surface water, coastal water from the North Sea for seawater, and wastewater from industrial plants for sewage water were used to this purpose. These water samples were spiked with a standard mixture at final concentrations of 5; 7.5; 10; 20; 50; 100 and 250 ng L⁻¹ and analysed as described in Sections 2.3 and 2.4. In addition, blank samples were analysed as well.

2.6. Quality assurance

Before and after analysis of a series of samples, a standard mixture (0.5 ng on column) of the targeted PFCs and the internal standards was injected to check the instrument parameters of the LC-ToF-MS system. Quality control of the method was performed by analysis of a blank sample, together with a linear calibration curve constructed using matrix samples spiked with standard solutions at seven concentration levels in the range of 5 and 250 ng L⁻¹. This was performed for every series of samples. The obtained calibration curves were used for quantification.

3. Results and discussion

3.1. Background contamination

Background contamination in the analytical blanks is a major problem in the analysis of PFCs [21,23,24]. Contamination from laboratory products and instrumentations containing polytetrafluoroethylene and perfluoroalkoxy compounds, is hard to avoid. Therefore, as suggested in literature [23,25], several measures were taken to minimize this kind of contamination. Teflon materials were avoided throughout the extraction procedure. All glass material was cleaned and placed in a drying oven (400 °C) in advance. Furthermore, an additional HPLC column was placed between the pump and the injector. As a result, PFCs originating from tubing or solvents, obtained extra retention and were thus separated from the target PFCs in the sample. Thanks to the elimination of these potential sources of contamination, none of the target compounds were detected in instrumental (direct injection of the mobile phase) and procedural blanks (extracted samples of Biosolve water).

In addition, due to the ubiquitous character of PFOS, analysis of unspiked water samples, i.e. water from the river Kale for surface water, coastal water from the North Sea for seawater, and wastewater from industrial plants for sewage water, frequently resulted in its detection in the low ng L⁻¹ concentration range. For the validation of our new analytical method, the calibration curves were corrected for these concentrations.

Table 1
Validation results: limits of detection (LODs) and quantification (LOQs) in ng L⁻¹, correlation coefficients (R²), recoveries (%), and intra-laboratory reproducibility (RSD%) of the targeted PFCs in surface water, sea- and sewagewater.

Compound	Surface water					Seawater					Sewagewater				
	LOD	LOQ	R ²	Recovery	RSD	LOD	LOQ	R ²	Recovery	RSD	LOD	LOQ	R ²	Recovery	RSD
PFBS	50	100	0.99	95	14	20	40	0.99	100	6	50	100	0.99	102	8
PFHxS	10	20	0.99	92	10	1	2	0.99	108	7	5	10	0.99	99	9
PFOS	7.5	15	0.99	92	14	1	2	0.99	101	7	5	10	0.99	96	16
PFDS	20	40	0.99	108	26	10	20	0.99	96	18	10	20	0.99	113	13
PFPA ^a	–	–	–	–	–	50	100	0.99	97	7	100	200	0.99	100	7
PFHxA	50	100	0.99	102	8	20	40	0.99	103	12	50	100	0.99	98	6
PFHpA	10	20	0.99	99	10	10	20	0.99	101	8	10	20	0.99	100	7
PFOA	7.5	15	0.99	95	13	5	10	0.99	99	10	10	20	0.99	103	11
PFNA	10	20	0.99	95	13	10	20	0.99	102	11	20	40	0.99	100	8
PFDA	20	40	0.99	98	18	10	20	0.99	103	11	20	40	0.99	101	9
PFUnA	50	100	0.98	103	16	10	20	0.99	103	17	50	100	0.99	99	12
PFDoA	20	40	0.98	123	23	80	160	0.98	104	19	50	100	0.99	99	13
PFTeA	100	200	0.95	134	12	100	200	0.96	100	18	100	200	0.99	93	13
PFOSA	10	20	0.99	99	17	5	10	0.99	98	7	20	40	0.99	105	18

^a Could not be quantified in this aqueous matrix.

3.2. Optimization of sample preparation

De Voogt and Sáez [25] suggested to avoid filtration because of the surface-active nature of PFCs. For this reason, filtration was only executed in case of sewage-water samples and water samples, visibly containing particulate matter, to avoid clogging of the cartridge during SPE. Preconcentration was necessary to determine low concentrations of PFCs in the water samples. To this end, solid-phase extraction is certainly the most suitable and commonly used technique [17,21,23,26,27].

Optimization of the SPE-procedure was performed by varying the sample volume, sample pH and the type of SPE cartridge. Dependent on the aqueous matrix, different sample volumes were extracted. For detection in surface- and sewagewater, a sample volume of 50 mL was found to be sufficient to meet satisfying LODs of ≤ 10 ng L⁻¹ for the major contaminants PFOS and PFOA. Compared to limnic systems, concentrations of most organic pollutants in the open sea are low [28]. Therefore, the sample volume was increased to 250 mL for PFC-analysis in seawater samples, resulting in LODs of ≤ 5 ng L⁻¹ for PFOS and PFOA (Table 1).

For solid-phase extraction of PFCs in water samples, the use of Oasis HLB or Oasis WAX cartridges has been reported by several studies [7,21,29–32]. Therefore, these two types of cartridges were examined within this study. The choice of the SPE-sorbent was mainly determined by the obtained recovery rates. Adjusting the sample pH to 3, Oasis WAX provided good results for the majority of the target analytes. However, using the Oasis HLB cartridges at neutral sample pH, higher recoveries for all target PFCs were obtained. Therefore, the Oasis HLB sorbent was selected for further experiments. These cartridges allow extraction of acidic, neutral, and basic analytes at neutral pH due to a combination of hydrophilic and lipophilic characteristics [33]. The Oasis HLB cartridges were rinsed with 2 mL of Biosolve water before elution (3×2 mL for seawater samples). Washing the cartridges has been shown to remove interfering matrix components and remaining sea salt (in the case of seawater samples) from the cartridge [34,35]. Optimal elution of the PFCs was achieved using methanol.

3.3. LC–ToF-MS optimization

A Luna[®] C18 (2) HPLC column was used for chromatographic separation of the analytes. The stationary phase of this LC column consists of ultrapure metal-free silica (99.99% purity) bounded to C18-groups. The performance of the column was evaluated by the separation efficiency for the structurally related PFCs. Good chro-

matographic separation of the compounds under investigation was achieved using the Luna C18 (2) column. In addition, high peak efficiencies, measured as peak width at the baseline, were obtained using this column (Fig. 2).

With respect to PFC-analysis, Berger et al. [36] compared three different MS-techniques coupled to LC: ion-trap MS, triple-quadrupole MS, and ToF-MS. ToF-MS was reported to be the optimal detector for quantification of PFCs, combining high selectivity with high sensitivity. For analysis of PFCs, LC–ToF-MS has been applied as a screening [37] and confirmation [38,39] technique in biological matrices. To our knowledge, the use of ToF-MS for the quantification of PFCs in water samples has not been published earlier. The ToF-MS system used during this study, is capable of producing spectra with a mass resolution of 4000 (at m/z 200) to $>10,000$ (at m/z 2722) (Agilent Technologies, technical overview). According to Van der Heeft et al. [40], the mass resolving power needs to be greater than 10,000 for the entire mass range to qualify for high resolving power MS. However, Kauffmann and Butcher [41] concluded that a mass resolution of 5000–10,000 is sufficient to discriminate analytes from co-eluting sample matrix compounds. The exact masses, the mean measured masses, and the mean mass errors of the detected PFCs at LOD-level were calculated and presented in Table 2. Except for PFPA and PFBS, the mean mass errors were below 5 ppm. Generally, using ToF-MS with a mass resolution of 10,000 FWHM (full width at half maximum; ~ 5000 mass resolution), a deviation of the detected mass of 10 ppm is acceptable [42]. In conclusion, our ToF-MS application enabled the use of a very narrow mass tolerance window of ± 5 ppm, thus providing a high mass accuracy.

The use of isotopically labelled internal standards for PFC-analysis is highly recommended [43]. However, ionization suppression caused by internal standards may occur and result in lower sensitivity [43,44]. Therefore, the internal standard concentration (100 ng L⁻¹) was kept quite low. For six PFCs, the corresponding ¹³C-labelled internal standards were used, while the most appropriate internal standard available was used for the other compounds (Table 2).

Analytes were identified on the basis of their relative retention time, which is the ratio of the retention time of the analyte to that of the internal standard. In addition, the accurate mass of the deprotonated molecular ions ($[M-H]^-$) in the spectrum was taken into account when the chromatographic peak of interest had a signal-to-noise ratio of at least 3:1. As mentioned before, errors in measured masses of known compounds are generally in the range of 5–10 ppm [42,45]. Therefore, within this study,

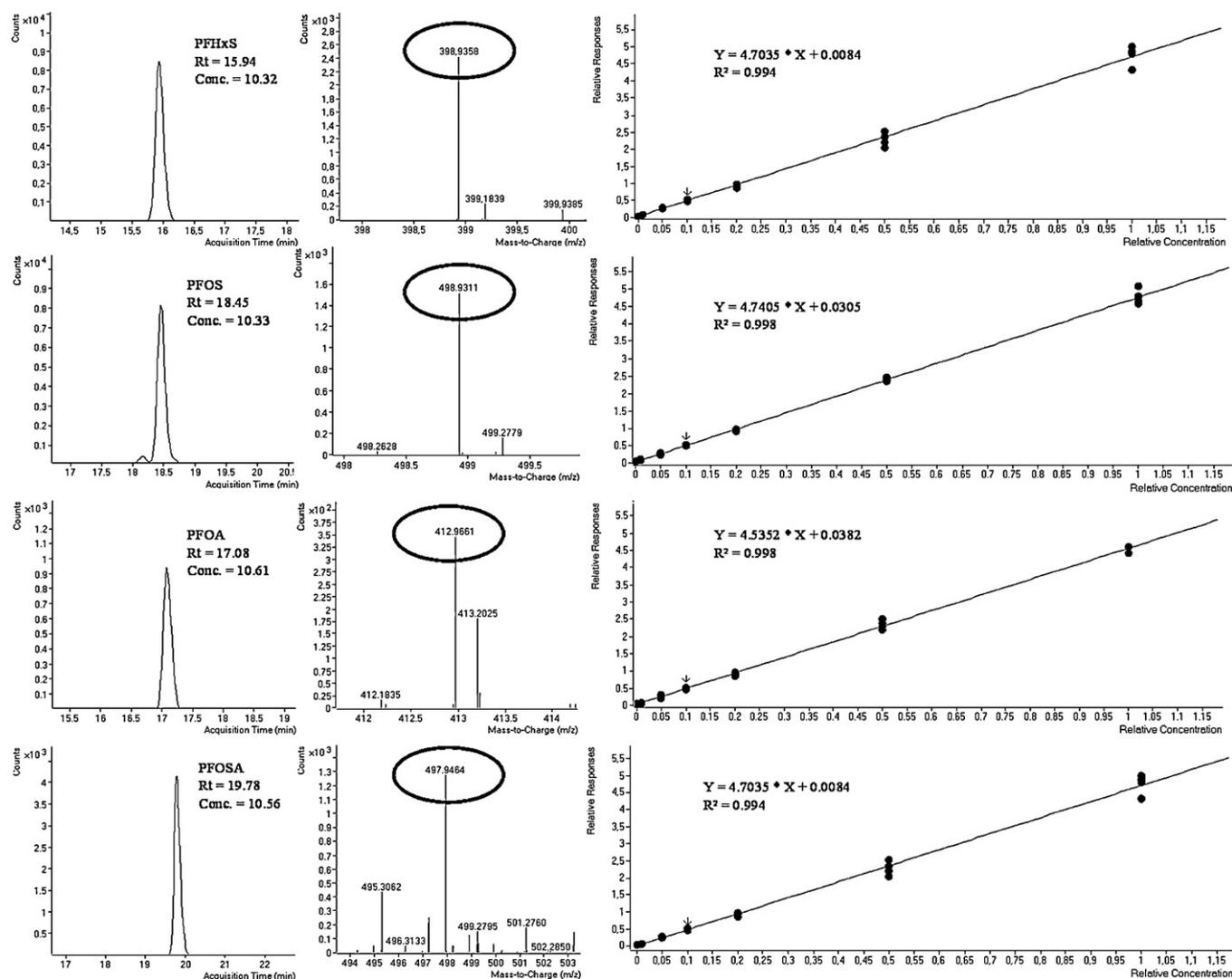


Fig. 2. Chromatograms, spectra and calibration curves of PFHxS, PFOS, PFOA and PFOSA (the four analytes detected in the seawater samples) in seawater spiked at 10 ng L⁻¹.

a maximum mass error of 10 ppm was allowed. Upon identification, area ratios were determined by integration of the area of an analyte under the obtained chromatograms in reference to the integrated area of the internal standard (Fig. 2). The analyte concentrations were calculated by fitting their area ratios in an eight-point calibration curve, established by matrix samples spiked with a standard mixture obtaining concentrations in the range of 0–250 ng L⁻¹.

3.4. Validation study

According to Van Leeuwen et al. [18], the quality of data obtained from analysis of PFCs in environmental matrices is a major issue of concern. Therefore, a validation study was carried out to demonstrate the method's performance. The method was evaluated for specificity/selectivity, linearity, recovery, intra-laboratory reproducibility, and limits of detection (LODs) and quantification (LOQs).

Table 2

Characteristics of the PFC-analysis using a ToF-MS: theoretical masses, mean measured masses, mean mass errors, and internal standards used.

Compound	Theoretical m/z [M–H] ⁻ ion	Mean measured m/z	Mean mass error (ppm)	Internal standard
PFBS	298.9430	298.9459	6.0	¹³ C ₄ PFOS
PFHxS	398.9366	398.9375	2.3	¹³ C ₄ PFOS
PFOS	498.9302	498.9319	3.8	¹³ C ₄ PFOS
PFDS	598.9233	598.9243	2.0	¹³ C ₄ PFOS
PFPA	262.9755	262.9772	5.4	¹³ C ₂ PFHxA
PFHxA	312.9728	312.9734	3.0	¹³ C ₂ PFHxA
PFHpA	362.9696	362.9701	2.7	¹³ C ₄ PFOA
PFOA	412.9664	412.9684	4.8	¹³ C ₄ PFOA
PFNA	462.9632	462.9649	4.0	¹³ C ₅ PFNA
PFDA	512.9600	512.9625	4.8	¹³ C ₂ PFDA
PFUnA	562.9563	562.9584	3.8	¹³ C ₂ PFDA
PFDoA	612.9531	612.9554	3.7	¹³ C ₂ PFDoA
PFTeA	712.9467	712.9479	3.4	¹³ C ₂ PFDoA
PFOSA	497.9457	497.9467	2.1	¹³ C ₄ PFOS

Matrix-matched calibration curves, in which the analytes were spiked into the representative aqueous matrix, were used for quantification. As such, the study of matrix-induced suppression or enhancement effects could be neglected.

3.4.1. Specificity/selectivity

The specificity of our method was evaluated through the analysis of water samples spiked with each compound separately, and of water samples spiked with a mixture of all compounds at a concentration of 100 ng L⁻¹. The specificity of the analytical approach was confirmed since no interferences were demonstrated by using LC–ToF–MS as described above. No other significant peaks with a signal-to-noise ratio of 3 or more were observed at the specific retention times of the targeted PFCs, suggesting a high specificity of the analytical method. This was in accordance with previous reports, since excellent specificity for unequivocal compound identification is guaranteed when using a ToF–MS system [37].

3.4.2. Linearity

Linearity was evaluated in the 0–250 ng L⁻¹ concentration range. Eight-point calibration curves were constructed in surface water (six replicates), sea- and sewagewater (both four replicates). The water samples were spiked with a standard mixture obtaining concentrations of 5; 7.5; 10; 20; 50; 100 and 250 ng L⁻¹. In addition, unspiked matrix samples were analysed as well, to check the occurrence of PFCs in blank samples. The mean regression coefficients (R^2) of the calibration curves were calculated by plotting area ratio versus concentration. For most target compounds, regression coefficients of 0.99 or higher were found, suggesting a good linear correlation (Fig. 2 and Table 1). Regression coefficients below 0.99 were found for the compounds with a longer carbon chain: R^2 was 0.98 for PFUnA in surface water and for PFDoA in both surface and seawater, while R^2 was 0.95 and 0.96 for PFTeA in surface and seawater, respectively. In addition, using the regression equation, the residuals were calculated as the difference of the obtained concentrations and the expected concentrations. Next, the obtained residuals were plotted versus the concentrations (data not shown). For all compounds, the residuals were randomly distributed, thus indicating a linear correlation in the 0–250 ng L⁻¹ concentration range.

3.4.3. Limits of detection (LODs) and quantification (LOQs)

Limits of detection (LODs) and quantification (LOQs) were determined based on the outcome of the eight-point calibration curves of Section 3.4.2. The concentrations of the analytes were calculated using the overall equation of the calibration curves. The LOD was defined as the higher value of the following two alternatives: (1) the lowest detectable concentration of the calibration curve with a signal-to-noise ratio of at least 3:1; (2) three times the standard deviation of the analytes concentration at the lowest detectable concentration level. The LOQs were defined as the final LOD multiplied by 2. This procedure was executed for the different aqueous matrices. The obtained LOQs of the targeted PFCs varied from 15 to 200 ng L⁻¹ in surface water, from 2 to 200 ng L⁻¹ in seawater, and from 10 to 200 ng L⁻¹ in sewagewater (Table 1). Thanks to the higher sample volume, the LOQs were lower in seawater samples. Since the method was in particular optimized for the detection of PFOS and PFOA in surface water, the two major contaminants within the group of PFCs, their LOQs were the lowest: i.e. 15 ng L⁻¹. Generally, higher LOQs were found for the analytes with both the shortest (PFBS, PFPA) and longest carbon chain length (PFUnA, PFDoA, and PFTeA).

In general, these quantitation limits are considered acceptable and are comparable to previous reported LOQs for the same analytes. For example, Taniyasu et al. [46] determined LODs between 4 and 60 ng L⁻¹ for PFOS, PFHxS and PFBS. More sensitive methods are

reported in literature as well, thanks to higher sample volumes or to the use of more sensitive mass spectrometers (triple–quadrupole mass spectrometers). The analysis of ppq (pg/L) concentrations of PFCs was reported by Yamashita et al. [15,23], Taniyasu et al. [21], and Ahrens et al. [17]. For example, the latter study reported method quantification limits of 0.004–0.367 ng L⁻¹ for the same analytes. However, to obtain these LOQs, 5 L water samples were extracted, while the sample volume in this study varied between 50 and 250 mL. Once more, we would like to underline that none of the mentioned studies from the literature used accurate mass, high-resolution time-of-flight mass spectrometry to detect PFCs in aqueous matrices.

3.4.4. Recovery and precision

Since no certified reference material was available, trueness of the measurements and intra-laboratory reproducibility (samples were measured on different days and by different analysts) were assessed using blank matrix samples spiked at both the LOQ level and two times the LOQ level. This was performed in six replicates. The intra-laboratory reproducibility of the method was determined by calculating the relative standard deviation (% RSD). Table 1 summarizes the obtained results for the different matrices. According to SANCO/2007/3131 [22], typically a recovery within the range of 70–120% and a reproducibility RSD ≤20% are required. As can be deduced from Table 1, except for PFDoA and PFTeA in surface water, all obtained recoveries were satisfactory. The obtained RSD values indicated satisfying precision for most analytes in the different matrices. Except for PFDS and PFDoA in surface water, the analytical method was sufficiently precise for quantitative analysis of the selected PFCs in all three matrices.

3.5. Application to North Sea samples

The developed method was applied to water samples collected during the INRAM project (see Section 2.1). Six offshore samples, 11 harbour samples and 2 samples of the Scheldt estuary were collected in June 2009. As can be seen from Table 3, four different PFCs were detected in all waters samples. The other PFCs were not detected at any of the sampling stations. PFOS was detected in every sample in levels up to 38.9 ng L⁻¹, while PFOSA was found once at a concentration of 26.4 ng L⁻¹ at sampling location S22 in Antwerp. PFHxS and PFOA were frequently detected up to concentrations of 13.1 and 23.5 ng L⁻¹, respectively, both at sampling location S22 (Antwerp).

Despite the limited monitoring study, certain differences could be observed between the sampling stations in the study area. As can be seen from Table 3, the harbour of Ostend and in particular the Scheldt estuary were most contaminated with PFCs. Analysis of the S22-sample resulted in the detection of four different PFCs, up to 38.9 ng L⁻¹. Since S22 is located in the industrial zone of Antwerp, large inputs of PFCs could be expected. Samples of the North Sea (W01–W06) were the least contaminated with PFCs. At these locations, only PFOS could be quantified in concentrations below 5 ng L⁻¹. These concentrations of PFOS were in the same range as those reported in previous studies of the German Bight, which is the south-eastern bight of the North Sea [17,47,48]. In accordance with Ahrens et al. [17], a decreasing contamination of PFCs with increasing distance from the coast, could be observed.

Compared to the derived PNEC values of 25 μg L⁻¹ for PFOS [49] and 250 μg L⁻¹ or 1.25 mg L⁻¹ for PFOA [49,50], adverse risks to aquatic organisms are not anticipated from these measured concentrations. However, the possible combined effects that the abundance of several PFCs may cause, possibly even with other micropollutants, cannot be excluded. Next to these toxicity thresholds, two studies determined a health-based guidance for PFOA in drinking water as well. According to the US Safe Drinking Water

Table 3

Detected concentrations (ng L⁻¹) of the PFCs of interest in water sampled at the different sampling locations in the Belgian coastal zone: harbour of Nieuwpoort (NP1–3), harbour of Ostend (OO1–4), harbour of Zeebrugge (ZB1–4), the North Sea (W01–W06), and the Scheldt estuary (S01: near Vlissingen, S22: near Antwerp) (n.d. = not detected).

	Sampling stations																		
	W01	W02	W03	W04	W05	W06	S01	S22	NP1	NP2	NP3	OO1	OO2	OO3	OO4	ZB1	ZB2	ZB3	ZB4
PFHxS	<LOQ	n.d.	n.d.	<LOQ	n.d.	n.d.	3.7	13.1	n.d.	n.d.	n.d.	<LOQ	6.7	2.9	2.4	<LOQ	<LOQ	<LOQ	<LOQ
PFOS	4.2	2.0	<LOQ	2.9	<LOQ	<LOQ	5.2	38.9	3.6	2.9	2.3	6.5	12.8	5.9	4.3	4.8	4.7	3.3	4.4
PFOA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	23.5	n.d.	n.d.	n.d.	<LOQ	n.d.						
PFOSA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	26.4	n.d.										

Act [51], these studies reported drinking water equivalent levels (DWEL: the lifetime exposure level of a contaminant at which adverse health effects are not anticipated to occur, assuming 100% exposure from drinking water). Tardiff et al. [52] found DWELs for PFOA ranging from 0.88 to 2.4 µg L⁻¹, while a guidance value of 0.040 µg L⁻¹ was recommended by Post [53]. The detected PFOA concentrations did also not exceed these drinking water levels.

4. Conclusion

A validated analytical method for the determination of 14 PFCs in surface, sewage and seawater is presented. The analytical procedure consisted of SPE applied to the water samples followed by LC–ToF–MS. The use of very narrow mass tolerance windows (<10 ppm) resulted in a highly selective MS-technique for the detection of PFCs in complex aqueous matrices. The LOQs varied between 2 and 200 ng L⁻¹ and recoveries obtained in surface water (92–134%) were satisfactory. Application of the method to North Sea and Scheldt estuary samples confirmed the occurrence of several PFCs in the marine environment in levels up to 38.9 ng L⁻¹.

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