

# Solid-phase extraction of PFOA and PFOS from surface waters on functionalized multiwalled carbon nanotubes followed by UPLC–ESI-MS

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**Abstract** This is the first report on the analytical application of multiwalled carbon nanotubes (MWCNTs) as solid-phase extraction (SPE) sorbents for determination in surface waters, at the nanograms per litre level, of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), the two predominant contaminants among the perfluorinated compounds detected. After the preconcentration step, the quantification was achieved by ultraperformance liquid chromatography–electrospray ionization mass spectrometry. To increase the extraction efficiency towards these amphiphilic compounds, MWCNTs were derivatized with amino-terminated alkyl chains, thus producing a mixed-mode material (MWCNT-R-NH<sub>2</sub>) combining hydrophobic affinity and anion-exchange properties. Experiments with distilled, tap and river water (pH 3) spiked at different concentrations (10, 15, 30, 100, 200 and 500 ng L<sup>-1</sup>) provided absolute recoveries in the range 71–102 % ( $n=3$ , relative standard deviations less than 10 %). Analytes were eluted in a single fraction with 6 mL methanol ( $3 \times 10^{-4}$  M NaOH). The within-laboratory reproducibility of the MWCNT-R-NH<sub>2</sub> SPE sorbent was evaluated with raw river water, and relative standard deviations less than 15 % were obtained ( $n=4$ ). Preconcentration factors up to 125 (500-mL sample) made it possible to quantify PFOA and PFOS at low nanograms per litre levels in naturally contaminated river water. The method quantification limits of 10 ng L<sup>-1</sup> for PFOA and 15 ng L<sup>-1</sup> for PFOS were well below the advisory levels for drinking and surface waters.

Comparison with non-derivatized MWCNTs highlighted the role of functionalization in improving the adsorption affinity towards these contaminants. MWCNT-R-NH<sub>2</sub> maintained their extraction capability for at least eight repeated adsorption/desorption cycles.

**Keywords** Carbon nanotubes · Emerging pollutants · Perfluorinated compounds · Solid-phase extraction · Surface waters

## Abbreviations

CNT	Carbon nanotube
EQS	Environmental quality standard
ESI	Electrospray ionization
GC	Gas chromatography
HLB	Hydrophilic–lipophilic balanced
LC	Liquid chromatography
MDL	Method detection limit
MQL	Method quantification limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MWCNT	Multiwalled carbon nanotube
PFC	Perfluorinated compound
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
MWCNT-R-NH <sub>2</sub>	Amino-terminated alkyl-functionalized multi-walled carbon nanotubes
RSD	Relative standard deviation
SIM	Selected ion monitoring
SPE	Solid-phase extraction
SRM	Selected reaction monitoring
UPLC	Ultraperformance liquid chromatography
WAX	Weak anion exchange

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## Introduction

The potential of carbon nanotubes (CNTs) as adsorbent solid phases for analytical applications is well evidenced in a recent review focusing on the use of these nanomaterials in separation science [1]. The good sorption properties of CNTs have been exploited to design a number of chromatographic stationary phases for gas chromatography (GC) [2–5] and liquid chromatography (LC) [5–7]. However, the use of CNTs in solid-phase extraction (SPE) is one of the most important applications of these materials in analytical chemistry [1]; their excellent performance for preconcentration of organic and inorganic species is mainly due to their larger surface area, especially on the outside and interstitial spaces within nanotube bundles, their chemical/mechanical stability and their capability to exert different interactions ( $\pi$ – $\pi$  stacking, van der Waals, electrostatic forces) with a number of solutes [1]. Despite this, chemical derivatization of pristine CNTs greatly contributed to improving their capability of extracting organophosphorus pesticides [8] and polyhalogenated organic contaminants [9] from aqueous matrices.

At present, there is no evidence in the literature of the application of CNTs as SPE sorbents for the preconcentration of perfluorinated compounds (PFCs) from water samples. PFCs embody a large group of highly stable man-made compounds which are amphiphilic and consist of a perfluorinated hydrophobic, linear carbon chain attached to one or more hydrophilic heads [10]. They are used to prepare water- and stain-resistant coatings for clothing, leather, upholstery, and carpets; oil-resistant coatings for food contact paper; aviation hydraulic fluids; fire-fighting foams; paints, adhesives, waxes, and polishes; surfactants, emulsifiers, wetting agents, additives, and coatings [11].

Among PFCs, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are global environmental pollutants. As for most “emerging” contaminants, conventional water treatments such as sand filtration and chlorination are not able to quantitatively reduce the levels of these compounds in urban and industrial effluents; consequently, their release into water basins is widespread [12]. Owing to resistance to hydrolysis, photolysis, metabolism and biodegradation, PFCs can be regarded as persistent contaminants. PFCs exhibit great chemical stability and are very resistant to typical environmental degradation processes owing to the carbon–fluorine bond, one of the strongest found in organic chemistry [11]. PFOA and PFOS were proved to possess oestrogenic activities and could be a source of xeno-oestrogens to humans and wildlife in the environment [13].

PFCs are found worldwide in water bodies, including river and lake water [12, 14, 15], wastewater [16], seawater [17] and even rainwater [18] at concentrations in the range from parts per trillion (nanograms per litre) to parts per

billion (micrograms per litre). Aquatic systems are the major sink for these contaminants, and a 2009 EU-wide survey of polar organic persistent pollutants proved the presence of these compounds in European rivers [19], at concentrations even higher than the environmental quality standard (EQS), fixed at  $30 \text{ ng L}^{-1}$  for surface waters [20, 21]. PFOA and PFOS have also been found in drinking water at levels up to some tens of nanograms per litre [22], and the US Environmental Protection Agency established provisional health advisory levels for PFOS and PFOA of  $200 \text{ ng L}^{-1}$  and  $400 \text{ ng L}^{-1}$ , respectively, in drinking water [23].

Different analytical methods have been developed [12, 14, 18, 24–26] or applied [10, 27] for their determination in natural waters. Owing to the low concentrations in actual samples, a preconcentration step is required; among the sorbents tested, reversed phases [14, 22, 26], hydrophilic–lipophilic-balanced (HLB) polymers [12, 15, 25] and weak anion exchange (WAX) phases [10, 18, 24, 26, 27] have been used. Usually, LC coupled with mass spectrometry (MS) or tandem MS (MS/MS) has been adopted for the identification/quantification after SPE [10, 12, 18, 22, 24, 25, 27, 28]; also, fluorescence detection after derivatization with (3-bromoacetyl)coumarin was recently proposed for perfluorinated carboxylic acids [26]. GC–MS determination after derivatization with isobutyl chloroformate has also been proposed for  $C_5$ – $C_{12}$  perfluoroalkyl carboxylic acids [15].

As mentioned above, the strong adsorption affinity of CNTs for organic contaminants has been widely reported [1]. A recent article investigated the environmental implication of the adsorption of toxic compounds on multiwalled CNTs (MWCNTs) released into the environment [29]; besides 4-nonylphenol and 2,4-dichlorophenoxyacetic acid, the affinity of some PFCs for (oxidized) MWCNTs has also been investigated.

On the basis of the above observations, in this article the application of CNTs as a SPE sorbent for PFOA and PFOS is explored for the first time. Non-modified MWCNTs and amino-terminated alkyl-functionalized MWCNTs (MWCNT-R-NH<sub>2</sub>) were tested and compared as SPE sorbents for their preconcentration from natural waters, and ultraperformance LC (UPLC)–electrospray ionization (ESI) MS was chosen for their quantification to shorten the analysis time and improve the sensitivity [10, 28]. MWCNT-R-NH<sub>2</sub> proved to be most suitable for the purpose and could be reused for consecutive extractions; their extraction efficiency was tested on different aqueous matrices spiked at environmentally significant concentrations. The main figures of merit of the SPE–UPLC–MS analytical procedure were evaluated. The MWCNT-R-NH<sub>2</sub> sorbent was then used in the analysis of real samples collected from Italian rivers.

## Experimental

### Chemicals and materials

Pristine MWCNTs [outer diameter 20–50 nm, inner diameter 5–10 nm, length 10–20  $\mu\text{m}$ , specific surface area 60  $\text{m}^2 \text{g}^{-1}$ , electrical conductivity greater than 100  $\text{S cm}^{-1}$ , bulk density 0.28  $\text{g cm}^{-3}$ , true density approximately 2.1  $\text{g cm}^{-3}$ , purity greater than 95 % (w/w), ash content less than 1.5 % (w/w)] were obtained from Cheap Tubes (Brattleboro, USA). Ultrapure  $\text{HNO}_3$  (65 %, w/w),  $\text{H}_2\text{SO}_4$  (98 %, w/w) and  $\text{HCl}$  (37 %, w/w) from Merck Chemicals (Milan, Italy) were used. Methanol, tetrahydrofuran, thionyl chloride and 2,2'-(ethylenedioxy)diethylamine were used for derivatization of the MWCNTs and were supplied by Sigma-Aldrich. Pellets of anhydrous  $\text{NaOH}$  (97 %) were obtained from Carlo Erba (Milan, Italy). Polytetrafluoroethylene filters (0.45- $\mu\text{m}$ ; Sigma-Aldrich) and paper filters (Schleicher and Schuell) were used. Ultrapure water (resistivity 18.2  $\text{M}\Omega \text{cm}^{-1}$  at 25  $^\circ\text{C}$ , total organic carbon less than 5  $\mu\text{g L}^{-1}$ ) was produced by a Milli-Q system (Millipore, Milan, Italy). An Orion 420A pH meter (Thermo Electron, Rodano, Italy) was used. PFOA (96 %) and PFOS (40 % aqueous solution) and ultrapure acetic acid (purity 99 % or greater) were purchased from Sigma-Aldrich. LC-MS grade methanol and water were from Fisher Scientific (Loughborough, UK).

### Purification and derivatization of MWCNTs

Pristine MWCNTs were purified free of metal traces by  $\text{HCl}$  washings, as reported elsewhere [30, 31], and were derivatized following a consolidated procedure involving nucleophilic substitution on acyl chloride derivatized MWCNTs, to give MWCNT-R-NH<sub>2</sub> [30]. The complete characterization of the final material was reported in a previous article [32].

### Water samples

Distilled water prepared in the laboratory and tap water from the Pavia municipal waterworks (pH 7.8, conductivity 271  $\mu\text{S cm}^{-1}$ , dissolved organic carbon 0.43  $\text{mg L}^{-1}$ ,  $\text{Ca}^{2+}$  35  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  10  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  4.8  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  0.6  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  4.4  $\text{mg L}^{-1}$ ) were used for the preliminary recovery tests. Thermal spring water (pH 6.4, conductivity 23,000  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  560  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  1,100  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  8,100  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  1,200  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  4  $\text{mg L}^{-1}$ ) collected in Montecatini Terme (Pistoia, Italy) was used to investigate the salinity effect. Surface water samples were collected in June 2013 from the Staffora River (pH 7.5, conductivity 353  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  59  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  11  $\text{mg L}^{-1}$ ,  $\text{Na}^+$  6  $\text{mg L}^{-1}$ ,  $\text{K}^+$  1.6  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  12.6  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  2.5  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  17.7  $\text{mg L}^{-1}$ ), the Bòrmida River (pH 7.0, conductivity 602  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  78.5  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  21.2  $\text{mg L}^{-1}$ ,  $\text{Na}^+$

27.6  $\text{mg L}^{-1}$ ,  $\text{K}^+$  3.7  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  60  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  13.7  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  58  $\text{mg L}^{-1}$ ), the Tànarò River (pH 6.6, conductivity 412  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  60  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  11.2  $\text{mg L}^{-1}$ ,  $\text{Na}^+$  13.5  $\text{mg L}^{-1}$ ,  $\text{K}^+$  1.8  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  18  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  6  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  33  $\text{mg L}^{-1}$ ), the Ticino River (pH 8.0, conductivity 380  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  37  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  8  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  10.5  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  8.5  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  33.2  $\text{mg L}^{-1}$ ) and the Po River (pH 7.8, conductivity 327  $\mu\text{S cm}^{-1}$ ,  $\text{Ca}^{2+}$  31  $\text{mg L}^{-1}$ ,  $\text{Mg}^{2+}$  6  $\text{mg L}^{-1}$ ,  $\text{Cl}^-$  9.7  $\text{mg L}^{-1}$ ,  $\text{NO}_3^-$  8.1  $\text{mg L}^{-1}$ ,  $\text{SO}_4^{2-}$  26.4  $\text{mg L}^{-1}$ ) by means of subsurface grab-sampling at a depth of approximately 20 cm. Polypropylene bottles were preferred to glass containers, to avoid binding of the target analytes to the surface [14, 28]; before collection, each bottle was rinsed with the surface water, and then the samples were shipped to the laboratory and stored in the dark (4  $^\circ\text{C}$ ). Before analysis, samples were not filtered but were simply decanted [25]. Samples from the Staffora River were collected in the mountain region of the province of Pavia (Varzi) and were used as the blank matrix for spiking with PFCs.

### SPE procedure

The cartridges (6-mL polypropylene tubes) were prepared by placing 200 mg MWCNT-R-NH<sub>2</sub> between two polyethylene frits, washed before use, under a vacuum, with 50 mL methanol followed by 50 mL ultrapure water to remove impurities and minimize void/channelling effects, and dried for 5 min under a strong vacuum.

In the procedure, the cartridge was conditioned with 5 mL methanol, 5 mL ultrapure water and 5 mL ultrapure water acidified with  $\text{HCl}$  (pH 3); 500-mL water samples (pH 3) were fed to the column at a flow rate of about 10  $\text{mL min}^{-1}$ , and then the cartridge was completely dried under vacuum for 10 min. The analytes were eluted with 6 mL methanol ( $3 \times 10^{-4}$  M  $\text{NaOH}$ ) at a flow rate of 1  $\text{mL min}^{-1}$ . The extract, acidified with 0.07 M  $\text{HCl}$ , was injected into the UPLC system.

### UPLC-ESI-MS analysis

The chromatographic analysis was performed with a JASCO (Lecco, Italy) X-LC system interfaced with a Thermo Scientific (Milan, Italy) LTQ XL ESI-MS/MS system. An Agilent EC-C18 Poroshell column (2.1  $\text{mm} \times 50 \text{mm}$ , 2.7  $\mu\text{m}$ ) was used with methanol/water–0.1 % acetic acid (85:15) as the mobile phase, at a flow rate of 0.5  $\text{mL min}^{-1}$ . The injection volume was 18  $\mu\text{L}$ . The column temperature was maintained at 40  $^\circ\text{C}$ , and the total run time was 6 min. In accordance with the literature [33], a single peak was obtained for PFOA, whereas for PFOS the two overlapped peaks observed resulted from the branched/linear isomers present in the commercial standard.

MS detection was performed in negative ion electrospray mode. Selected reaction monitoring (SRM) was adopted to

quantify PFOA by checking a single ion product transition (413→369); the  $m/z=369$  ion corresponds to the  $F_{16}C_7^-$  fragment. PFOS was quantified by selected ion monitoring (SIM) by detection of the  $m/z=499.01$  ion. The ESI source values were as follows: spray voltage, 5.00 kV; source heater temperature 152 °C; capillary temperature, 275 °C; desolvation and cone gas, nitrogen of 99.99 % purity. The analyzer setting were as follows: normalized collision energy, 12 %; collision gas, helium (99.9995 %).

#### Quality control

To avoid contamination throughout the sample preparation and instrumental analysis, all the polytetrafluoroethylene materials and polyether ether ketone tubes were replaced by stainless-steel pieces. Before use, the glassware was rinsed with methanol and ultrapure water and heated to a high temperature for 3 h [34].

Identification of PFOA by MS/MS (SRM) was based on (1) the deprotonated molecule, (2) one selective fragment ion and its relative intensity and (3) the retention time; the fragment was selected for quantification. Identification and quantification of PFOS by MS (SIM) were based on the analyte retention time and on the signal intensity of the deprotonated molecule, respectively.

Procedural blanks were prepared every ten samples to check for any contamination potentially occurring during sample extraction, and methanol blanks were run after every five samples to verify the instrumental background and potential memory effects. Since external calibration provides good accuracy for determination of PFCs [10, 26, 27], and in view of the good accuracy (recovery and precision) and negligible matrix effect observed (see “Results and discussion”), the use of labelled internal standards could be avoided. Five-point calibration curves in methanol were generated for each analyte in the range 0.5–10  $\mu\text{g L}^{-1}$ . Duplicate spikes were performed on SPE extracts from water samples, and the results from external quantification were compared with those obtained by the standard addition method.

Recovery was evaluated on blank distilled, tap and river waters fortified with different amounts of PFCs, in the range 10–500  $\text{ng L}^{-1}$  ( $n=3$ , for each sample). The intraday precision (repeatability) was evaluated on independently fortified samples (10–500  $\text{ng L}^{-1}$ ,  $n=3$ ). The interday precision (within-laboratory reproducibility) was assessed on blank river water spiked with the PFCs at 30  $\text{ng L}^{-1}$  ( $n=4$ ).

## Results and discussion

Prior to determination of the PFCs in unknown environmental samples, a series of experiments were performed to evaluate the extraction efficiency of MWCNTs and MWCNT-R-NH<sub>2</sub>

in the preconcentration of PFOA and PFOS from natural waters, and to characterize the main figures of merit of the analytical method, namely, linearity, method detection limits (MDLs), method quantification limits (MQLs), selectivity and accuracy (recovery and precision).

#### Solid-phase extraction

MWCNT-R-NH<sub>2</sub> can behave as a mixed-mode sorbent, similarly to the new stationary phases proposed for LC [35] and GC [4]. Since adsorption of PFCs is largely governed by hydrophobic interactions [29], the CNT structure is expected to result in an aspecific interaction with the hydrophobic chains of the analytes, mainly through van der Waals forces; at the same time, an anion-exchange mechanism can be established between the anionic PFC head and the protonated amino group of the MWCNT-R-NH<sub>2</sub>, resulting in a specific interaction. Conversely, on MWCNTs, the anion-exchange mechanism is absent. Comparison of the two sorbents highlighted the role of the derivatization.

To begin with, the amount of the CNTs (200 mg) was chosen on the basis of the most used SPE sorbents commercially available for enrichment of trace contaminants from surface waters [36], and considering the extraction procedures specifically designed for PFCs, based on HLB [12, 15, 25] and WAX [10, 18, 27] polymeric materials. Larger amounts were not considered, taking into account the high aspect ratio of the nanomaterials resulting in a large surface area and, consequently, in a greater number of potential adsorption sites compared with commercial phases [1].

Recovery was first tested with distilled water to exclusively evaluate the adsorption capability of the derivatized nanotubes towards PFOA and PFOS, in the absence of any other inorganic/organic species. Samples were spiked with the PFCs at 100–500  $\text{ng L}^{-1}$  and were acidified to pH 3 to promote the protonation of the amino groups of the MWCNTs. Adsorption of PFCs on CNTs is favoured at pH 3, with the pH effect becoming less pronounced on increasing the density of the polar groups on the CNT skeleton [29]. Sample volumes from 100 to 750 mL provided mean recovery as high as 80 % [ $n=3$ , relative standard deviations (RSD) below 8 %] when methanol ( $3 \times 10^{-4}$  M NaOH) was used as the eluting solvent ( $2 \times 4$  mL). Recovery lower than 50 % ( $n=3$ , RSD < 10 %) was obtained by using methanol not containing NaOH (100-mL sample). The poor recovery was due to the poor efficiency of neat methanol to desorb the analytes from the MWCNT-R-NH<sub>2</sub>, thus substantiating the parallel anion-exchange mechanism taking place on this mixed-mode material.

The NaOH concentration proved to be determinant in the elution step, as the recovery decreased to around 60 % ( $n=3$ , RSD < 10 %) with  $3 \times 10^{-6}$  M OH<sup>-</sup>. This is in agreement with the analytical procedures for determination of PFCs based on anion-exchange phases such as WAX materials, requiring



bases to be added to the eluting solvent [10, 26, 27]. Further, recovery of PFOA and PFOS in ultrapure water not greater than 60 % was obtained when hydrophilic–lipophilic materials such as HLB polymers were eluted with methanol [25] or mixtures of organic solvents, i.e. methanol, acetone and ethyl acetate [12]. On the basis of the preliminary results, and considering the EQS of 30 ng L<sup>-1</sup> for PFOA and PFOS [20, 21], additional recovery tests were performed at lower concentrations, and recovery greater than 72 % was obtained for 15–30 ng L<sup>-1</sup> spikes (*n*=3). The recoveries obtained in tap water and in river water enriched at environmentally significant concentrations, together with their standard deviations, are reported in Table 1.

Generally, the target analytes were quantitatively eluted into 2×4 mL extracting solution, but also a single extraction with 6 mL allowed us to achieve optimal sensitivity and accuracy. Therefore, under the optimized conditions, 500 mL river water spiked with the PFCs at 10–15 ng L<sup>-1</sup> followed by elution with 6 mL methanol (3×10<sup>-4</sup> M NaOH) gave recovery as high as 72–81 %, with good intraday precision (RSD<10 %). The largest sample volume that could be preconcentrated at these PFC concentrations was 750 mL (from the breakthrough curve, data not shown).

The importance of derivatization could be appreciated by preconcentrating 100 mL distilled water spiked with the PFCs at 500 ng L<sup>-1</sup> (*n*=3) on non-derivatized MWCNTs purified from the metallic fraction. In this case, the PFCs were not quantitatively retained on the cartridge, with more than 40 % of the initial amount found in the percolated liquid, and complete recovery of the sorbed amount (57 % of the initial amount) required 2×4 mL neat methanol fractions. From these findings, it is evident that a mixed-mode sorbent such

as MWCNT-R-NH<sub>2</sub>, endowed with apolar affinity and anion-exchange properties, is particularly suitable for adsorption/desorption of amphiphilic compounds such as PFCs. Since, generally, the commercial SPE materials do not guarantee optimal performance for a second run [37], we investigated the possibility of reusing the MWCNT-R-NH<sub>2</sub> phase. The same cartridge was found to be reusable with no significant loss of adsorption capability (recovery greater than 70 %) for at least eight consecutive extractions, as verified by preconcentration of tap water samples spiked with both analytes at 30–100 ng L<sup>-1</sup>.

The batch-to-batch reproducibility was verified on a second sample of MWCNTs, independently derivatized following the same procedure, and recoveries higher than 90 % were obtained (30 ng L<sup>-1</sup> spike, *n*=3, RSD<9 %).

Because the two PFCs are always present in their ionic form in environmental waters (p*K*<sub>a</sub> -0.5 and -3.27 for PFOA and PFOS, respectively [29]), inorganic ionic species present in real matrices are expected to compete with the analytes for adsorption on the ion-exchanger sites of the sorbent. We, therefore, further investigated the influence of water salinity on the recovery rate. For this purpose, thermal waters characterized by high conductivity (2,300–23,000 μS cm<sup>-1</sup>) enriched with the PFCs at 100 ng L<sup>-1</sup> were preconcentrated on the MWCNT-R-NH<sub>2</sub> (500-mL sample, preconcentration factors 50–125). Strong interference owing to the competition between the analytes and the inorganic anions for the SPE adsorption sites was observed; specifically, the mean recovery was lowered to 35(5)% (*n*=4), further evidencing the anion-exchange role of the mixed-mode behaviour of MWCNT-R-NH<sub>2</sub>.

## Analytical evaluation of the SPE–UPLC–MS method

### Selectivity

MS detection does not require derivatization and ensures certain identification of the target analytes, PFCs included [28]; for this it provides higher selectivity with respect to other detection systems, for instance, UV and fluorescence detections, which, however, are still widely used for quantitative analysis of water pollutants [37].

PFOA and PFOS were simultaneously and selectively determined by MS in SRM and SIM modes, respectively, after a quick UPLC elution. SIM was used for PFOS identification/quantification as the instrumental configuration did not allow us to detect its low molecular weight fragmentation ions. The selectivity of the method was evaluated from the chromatograms of blank water extracts where no peaks were evidenced at the retention times of the PFCs. This excluded the interference of matrix substances accountable for false-positive signals.

**Table 1** Recovery rate obtained in different aqueous matrices by solid-phase extraction (SPE) on amino-terminated alkyl-functionalized multiwalled carbon nanotubes

Spike (ng L <sup>-1</sup> )	Distilled water		Tap water		River water	
	Recovery (%)		Recovery (%)		Recovery (%)	
	PFOA	PFOS	PFOA	PFOS	PFOA	PFOS
500	93(5)	92(5)	90(4)	90(5)	90(6)	88(5)
200	89(4)	88(6)	91(6)	-	-	-
100	102(7)	89(6)	71(5)	89(6)	-	-
30 <sup>a</sup>	99(7)	77(6)	99(7)	79(6)	92(6)	72(6)
15	93(6)	79(7)	80(5)	75(6)	81(6)	72(7)
10	82(5)	<70	77(5)	<70	72(5)	<70

The standard deviation related to the intraday precision (repeatability) is reported *parentheses* (*n*=3)

PFOA perfluorooctanoic acid, PFOS perfluorooctane sulfonate

<sup>a</sup> Environmental quality standard for PFOA and PFOS

### Linearity and matrix effects

To assess the ion suppression/enhancement effect of the matrix in the ion source, three independent calibration curves were prepared both in clean methanol and in the methanol SPE extracts obtained from preconcentration of 500 mL blank river water (matrix-matched calibration). Good linearity, determined by ordinary linear least-squares regression, was observed in the range 0.5–10  $\mu\text{g L}^{-1}$  for both analytes ( $R^2 > 0.999$ ), with slopes not significantly different (difference less than 4 %) in the two matrices (see Table 2). This means that the matrix effect was lower than 4 %, and thereby it was included in the range of the method precision [10, 38]. The matrix effect, in terms of signal suppression/enhancement, which is known to be often generated by co-extraction of natural organic matter present in environmental water samples [36, 37], and/or by excessive salinity of the extract [39], can be therefore considered negligible. Optimal linear regression coefficients ( $R^2 > 0.999$ ) were obtained by duplicate spiking (1 and 2  $\mu\text{g L}^{-1}$ ) of SPE extracts from preconcentrated samples, and excellent accordance was observed between the concentrations found by external quantification and those obtained by the standard addition method (difference less than 6 %). In agreement with recent literature [10, 27], external calibration proved to be a convenient choice, affording accurate quantification.

### Method detection and quantification limits

Preconcentration factors up to 125 provided MDLs and MQLs suitable for analyses of PFOA and PFOS in contaminated surface waters. In this sense, concentrations in the range 0.8–1.25  $\mu\text{g L}^{-1}$ , which are realistic values expected to be found in the SPE extracts of contaminated environmental

waters, can be accurately measured. Realistic MQLs that provided acceptable recovery (more than 70 %) and precision (RSDs below 20 %) were 10 and 15  $\text{ng L}^{-1}$  for PFOA and PFOS, respectively. These levels are achieved also considering the recovery rate [38, 40] with a maximum sample volume of 500 mL and a single SPE eluted fraction (6 mL). Both MQLs obtained with the proposed preconcentration are well below the drinking water advisory levels [23] and the EQS for surface waters [20, 21]; owing to the use of UPLC coupled with MS detection [28] the MDLs are lower than those achievable by high-performance LC with fluorescence detection [26].

### Accuracy: recovery and precision

SPE recovery was evaluated with distilled, tap and blank river water spiked at different levels (10–500  $\text{ng L}^{-1}$ ) according to the concentrations expected in surface waters [12], and resulted in the range 71–102 % ( $n=3$ , intraday RSD < 10 %), as reported in Table 1. The within-laboratory interday precision, evaluated with blank river water samples ( $n=4$ ) independently fortified (30  $\text{ng L}^{-1}$ , EQS) and analyzed on different days, showed RSDs lower than 15 % for both compounds (see Table 2).

The results obtained proved that the MWCNT-R-NH<sub>2</sub> sorbent is a valid alternative to the polymeric phases tested in work reported in the literature. With regard to the commercial packages, the HLB cartridge resulted in quantitative recovery for PFOA from water samples spiked at 0.14–1.4  $\mu\text{g L}^{-1}$ , but only on the addition of tetrabutylammonium bromide (50  $\text{mg L}^{-1}$ ), as an ion-pairing agent, to the sample [15]. On the other hand, recovery not greater than 60 % was observed for the preconcentration of ultrapure water spiked at 10–100  $\text{ng L}^{-1}$  [12, 25]; higher values (85 % and 65 %, for

**Table 2** Analytical parameters obtained for PFOA and PFOS by the SPE–ultraperformance liquid chromatography–mass spectrometry (UPLC–MS) procedure

	PFOA	PFOS
Calibration curve equation in methanol <sup>a</sup>	$y=5299(219)x+6246(1656)$	$y=7051(393)x - 419(2257)$
Calibration curve equation in matrix-matched solution <sup>a</sup>	$y=5263(231)x+6193(1748)$	$y=7351(83)x - 1172(624)$
Linearity ( $R^2$ )	0.999	0.999
MDL ( $\text{ng L}^{-1}$ ) <sup>b</sup>	3	6
MQL ( $\text{ng L}^{-1}$ ) <sup>b</sup>	10	15
SPE–UPLC–MS intraday precision (RSD, %) <sup>c</sup>	6	8
SPE–UPLC–MS interday precision (RSD, %) <sup>d</sup>	13	14

MDL method detection limit, MQL method quantification limit, RSD relative standard deviation

<sup>a</sup> Calculated as peak areas versus concentration. The slope and intercept errors obtained by ordinary linear least-squares regression are given in parentheses

<sup>b</sup> Preconcentration factor 83

<sup>c</sup> River water spiked at 30  $\text{ng L}^{-1}$  ( $n=3$ )

<sup>d</sup> River water spiked at 30  $\text{ng L}^{-1}$  ( $n=4$ )

**Table 3** Concentrations of perfluorinated compounds determined in naturally contaminated river waters.

River	Concentration (ng L <sup>-1</sup> )	
	PFOA	PFOS
Bòrmida	780(48)	<MQL
Po	30(3)	<MQL
Tànaro	212(13)	<MQL
Ticino	<MQL	15(2)
Stàffora	15(2)	<MQL

The standard deviation is reported in *parentheses* ( $n=3$ )

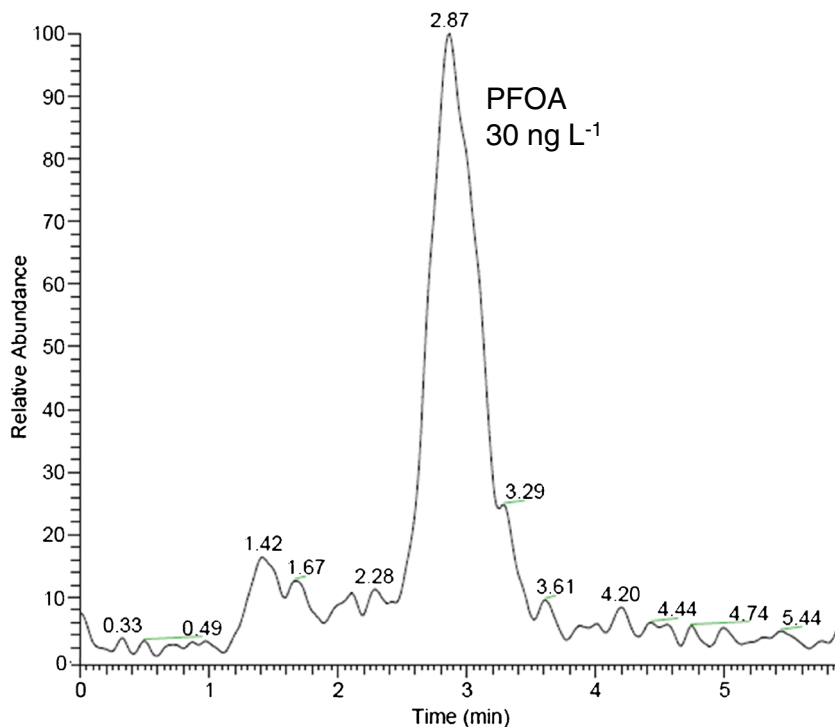
PFOA and PFOS, respectively) were obtained in ultrapure water with a slight modification of the elution step [19].

The WAX phase performed well for determination of the two PFCs in rainwater, providing recovery in the range 96–108 % both in ultrapure water and in rainwater enriched with the PFCs at 10 ng L<sup>-1</sup> [18]. Determination of PFOA and PFOS in river water by UPLC–MS after WAX preconcentration [10] showed recovery of around 60 % and 80 % for PFOA and PFOS, respectively (6–60 ng L<sup>-1</sup> spikes), with RSDs in the range 25–30 %.

#### Analysis of contaminated waters

The optimized method was applied to the determination of PFOA and PFOS in surface waters. Samples were collected from rivers expected to be contaminated with these PFCs, being the final sink of urban and industrial effluents. As reported in Table 3, PFOA at a concentration of 30 ng L<sup>-1</sup> was found in the Po River (a typical chromatogram is shown

**Fig. 1** Typical ultraperformance liquid chromatography–mass spectrometry chromatogram of a solid-phase extraction extract obtained by preconcentration of a 500-mL Po River sample on amino-terminated alkyl-functionalized multiwalled carbon nanotubes. PFOA perfluorooctanoic acid



in Fig. 1), whereas the Bòrmida and Tànaro rivers, sampled near the industrial zone of the city of Alessandria, exhibited PFOA concentrations of several hundred nanograms per litre; PFOA was quantified at a concentration as low as 15 ng L<sup>-1</sup> in the Stàffora River, sampled in the hill region of Pavia, away from factories. The PFOS concentrations were below the MQL in all the samples analyzed, with the exception of samples from the Ticino River, collected in Pavia. The RSDs for these analyses were lower than 11 % ( $n=3$ ).

From a comparison of the levels of PFOA in the Bòrmida and Tànaro rivers with those in the Ticino River, it appears that manufacturing is the major source of contamination. These results are consistent with those previously found by the Joint Research Centre in the 2008 Po watershed monitoring campaign [25] that showed a PFOA concentration of 1,270 ng L<sup>-1</sup> in the Tànaro River, and that PFOS was present at a few nanograms per litre in the rivers monitored.

#### Conclusions

For the first time, CNTs were tested and evaluated as a SPE sorbent for the determination of PFOA and PFOS in natural waters, and the chemical modification of the carbon skeleton with amino-terminated alkyl chains was proved to be successful and determinant. MWCNT-R-NH<sub>2</sub> behaved as a mixed-mode sorbent with high adsorption capability towards these amphiphilic analytes, allowing quantitative recovery, also in raw river water. The MQLs obtained (10–15 ng L<sup>-1</sup>) are well below the advisory concentrations for surface and drinking

water. The MWCNT-R-NH<sub>2</sub> cartridge preserved the adsorption capability for consecutive extractions. The application of the method to real samples proved that PFOA is present in Italian rivers at concentrations higher than the EQS. Summing up, the goal of this work was combining a novel analytical application of functionalized CNTs with a straightforward procedure for screening analyses of PFOA and PFOS in natural waters.

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