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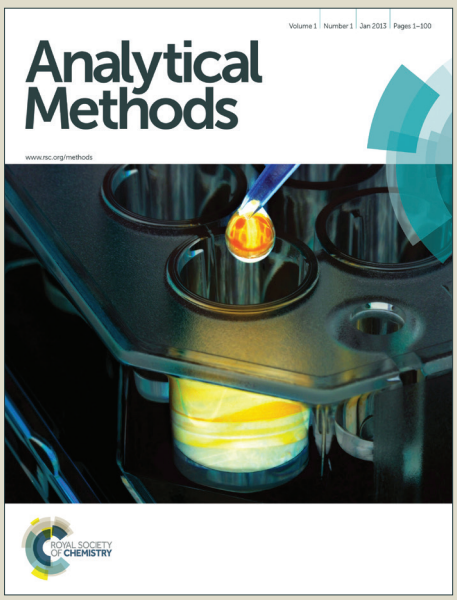

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Simultaneous determination of eighteen perfluorinated compounds in dissolved and particulate phases of wastewater, and in sewage sludge by liquid chromatography - tandem mass spectrometry

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ABSTRACT

Perfluorinated compounds (PFCs) are known chemicals that are used in a wide variety of industrial and consumer products, and have been reported to occur in the environment as contaminants. In this study, a liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) method was developed for simultaneous determination of 10 perfluoroalkyl carboxylate acids (PFCAs), 5 perfluoroalkyl sulfonates (PFASs), and 3 perfluoroalkyl sulfonamides (PFSAs) (18 PFCs in total), in dissolved and particulate phases of wastewater (raw and treated), and in dewatered sewage sludge. The target PFCAs were perfluoropentanoic acid (PFPeA; C5), perfluorohexanoic acid (PFHxA; C6), perfluoroheptanoic acid (PFHpA; C7), perfluorooctanoic acid (PFOA; C8), perfluorononanoic acid (PFNA; C9), perfluorodecanoic acid (PFDA; C10), perfluoroundecanoic acid (PFUdA; C11), perfluorododecanoic acid (PFDoA; C12), perfluorotridecanoic acid (PFTrDA; C13), and perfluorotetradecanoic acid (PFTeDA; C14). The target PFASs were potassium perfluorobutanesulfonate (PFBS; C4), sodium perfluorohexanesulfonate (PFHxS; C6), sodium perfluoroheptanesulfonate (PFHpS; C7), sodium perfluorooctanesulfonate (PFOS; C8), and sodium perfluorodecanesulfonate (PFDS; C10), and the target PFSAs were perfluorooctane sulfonamide (PFOSA), *N*-methylperfluorooctane sulfonamide (*N*-MeFOSA), and *N*-ethylperfluorooctane sulfonamide (*N*-EtFOSA). Wastewater samples were filtered after collection and extracted/purified/pre-concentrated by a solid-phase extraction (SPE) procedure. Particulate matter and sludge samples were extracted by a liquid-solid extraction (LSE) and ultra-sonication procedure, and thereafter purified /preconcentrated by the same SPE procedure that was followed for the dissolved phase of wastewater. The internal standards, perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid ($^{13}C_4$-PFOA), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate ($^{13}C_4$-PFOS), and *N*-methyl-d_3-perfluoro-1-octanesulfonamide ($^2D_3$-*N*-MeFOSA) provided adequate compensation for variations in the extraction percentages and instrumental response. The limits of quantification (LOQs) ranged from 0.29 (PFHpS) to 3.0 ng L^{-1} (PFDoA) for dissolved phase samples, and from 0.15 (PFHpS) to 1.5 ng g^{-1} dry weight (dw) (PFDoA) for particulate matter and sludge samples. The developed methods were applied successfully to wastewater and sludge samples originated from Athens WWTP. PFCs concentrations up to 113 ng L^{-1} (PFUdA), 33 ng L^{-1}(PFOA) and 1042 ng g^{-1}(PFUdA) were determined in influent wastewater, treated wastewater and dewatered sludge, respectively. Analysis of PFCs in particulate matter of wastewater is needed to avoid underestimation of their concentrations.

Introduction

Perfluorinated compounds (PFCs) are persistent organic pollutants (POPs)[1] that consist of a fully fluorinated hydrophobic alkyl chain attached to a hydrophilic end group.[2] PFCs include perfluoroalkyl carboxylate acids (PFCAs), perfluoroalkyl sulfonates (PFASs), and perfluoroalkyl sulfonamides (PFSAs).[1] For over 50 years, they are widely used in several industrial and household applications due to their unique physicochemical properties, i.e. thermal and chemical stability.[1-5] They are extensively used in surfactants, fire-fighting foams and food packing paper.[1-5] Additionally, the applications of PFCs in textile, carpet and leather treatment are well-documented.[1-5]

PFCs are regarded as persistent, bio-accumulative and potentially hazardous to humans and wildlife.[1,6] Wastewater treatment plants (WWTPs) are considered as one of the major transfer routes of these compounds to the aquatic environment.[7,8] Therefore, the development and application of adequate analytical methodologies for the determination of different classes of PFCs in wastewater and sewage sludge is of high importance. Up-to-date, several analytical methods are available for the determination of PFCs in a variety of matrices. Solid phase extraction (SPE) and liquid-liquid extraction (LLE) protocols have been predominately used for extraction, purification and pre-concentration purposes in environmental media due to their ease of applicability.[9-12] Other less common protocols have also been applied such as solid phase micro-extraction (SPME), and SPE based on mixed hemimicelles and magnetic separation.[13,14] The most common analytical technique for PFCs analysis is liquid chromatography (LC) coupled with mass (MS) or tandem mass spectrometric (MS/MS) detection; for some PFCs very low limits of detection can be achieved, reaching even the picogram range.[9,11-12,14-29]

To the best of our knowledge, despite the high number of available analytical methodologies for the determination of PFCs in the environment, few analytical methodologies report the simultaneous determination of multi-class PFCs in both dissolved and particulate phase of wastewater. Moreover, even though long-chain PFCs tend to accumulate on the particulate phase of wastewater due to their hydrophobicity, most available analytical papers aim to their determination only in the dissolved phase of wastewater; however, this practice may underestimate PFCs levels. On this aspect, a liquid chromatography-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) methodology was developed, validated and applied for simultaneous determination of 10 PFCAs, 5 PFASs, and 3 PFSAs (18 PFCs in total; Table 1), in dissolved and particulate phases of wastewater

(raw and treated), and in dewatered sewage sludge. A SPE protocol was developed and optimized for sample preparation, while sources of PFCs contamination during analysis were identified and effectively controlled. The internal standards (ISs) provided adequate compensation for variations in the extraction percentages and instrumental response. The developed methods were applied successfully in wastewater and sludge samples taken from Athens WWTP (Greece).

Experimental

Chemicals and materials

Target compounds and ISs (Table 1) were purchased from Wellington Laboratories (Guelph, Ontario, Canada) (≥ 98%). The target PFCAs were perfluoropentanoic acid (PFPeA; C5), perfluorohexanoic acid (PFHxA; C6), perfluoroheptanoic acid (PFHpA; C7), perfluorooctanoic acid (PFOA; C8), perfluorononanoic acid (PFNA; C9), perfluorodecanoic acid (PFDA; C10), perfluoroundecanoic acid (PFUdA; C11), perfluorododecanoic acid (PFDoA; C12), perfluorotridecanoic acid (PFTrDA; C13), and perfluorotetradecanoic acid (PFTeDA; C14). The target PFASs were potassium perfluorobutanesulfonate (PFBS; C4), sodium perfluorohexanesulfonate (PFHxS; C6), sodium perfluoroheptanesulfonate (PFHpS; C7), sodium perfluorooctanesulfonate (PFOS; C8), and sodium perfluorodecanesulfonate (PFDS; C10), and the target PFSAs were perfluorooctane sulfonamide (PFOSA), *N*-methylperfluorooctane sulfonamide (*N*-MeFOSA), and *N*-ethylperfluorooctane sulfonamide (*N*-EtFOSA). Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid ($^{13}C_4$-PFOA), sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate ($^{13}C_4$-PFOS), and *N*-methyl-d_3-perfluoro-1-octanesulfonamide ($^2D_3$-*N*-MeFOSA) were used as internal standards. Formic acid (98%), acetic acid (98%), ammonium acetate and ammonium formate were supplied by Fluka (Buchs, Switzerland). LC-MS grade methanol (MeOH) and acetonitrile (ACN) were obtained from Merck (Frankfurt, Germany). Milli-Q grade water was purified by an ultrapure water system (Millipore Direct-Q UV, Bedford, MA, USA). Polytyrosine-*1,3,6* standard solution for MS/MS mass axis calibration was purchased from Thermo Electron Corporation (San Jose, CA, USA). Oasis HLB 6 cm^3/200 mg (Waters, Milford, MA) solid-phase extraction (SPE) cartridges with 30 μm average particle diameter, 82 Å average pore diameter, and 823 m^2 g^{-1} specific surface area were used during sample preparation.

All standard stock solutions were prepared in MeOH and stored in the dark at 4 ˚C. Mixtures of target analytes standard solutions were prepared in MeOH at concentrations of

10, 100 and 2500 ng mL^{-1}. Glass fiber pre-filters (0.45 μm; Millipore, Bedford, MA, US) were used to filter wastewater samples and to collect particulate matter from the samples. Mini-UniPrep® syringeless RC filter membranes (0.2 μm; Whatman, Middlesex, UK) were used for the filtration of extracts prior to instrumental analysis. Eppendorf tubes (Sarstedt, Nümbrecht, Germany) were used during sample preparation.

Sample collection

Wastewater and sludge samples were collected from Athens WWTP (Greece). Information concerning Athens WWTP has been reported in our previous study[30]. Twenty four-hour flow-proportional composite samples of sewage influents and secondary effluents were obtained during two consecutive days in 2012, as well as grab samples of primary, secondary and dewatered sludge. All wastewater and sludge samples were collected and stored in high-density polyethylene bottles and bags, respectively. Wastewater samples were immediately filtered after collection, and stored in the dark at 4 ˚C until extraction. The particulate matter derived from samples' filtration and the dewatered sludge samples were stored at -20 °C until analysis. For the development and validation of analytical methods, dissolved phase of wastewater and dewatered sewage sludge were used.

Sample preparation for the dissolved phase of wastewater

An aliquot of 50 mL of filtered wastewater (applies to all liquid samples) was transferred into a 50 mL Eppendorf tube and adjusted to pH = 4.0 ± 0.1 with acetic acid solution 1 M prior to the loading step of the SPE. All blanks and samples were spiked with a known amount of ISs (1.25 ng for each IS) before extraction. Matrix spikes were fortified with the same amount of ISs and an appropriate amount of target analytes prior to extraction (referred to as pre-extraction matrix spikes). All samples prior to SPE were vortex mixed for 1 min. Extraction and isolation of target analytes from the samples were performed by Oasis HLB cartridges. The cartridges were conditioned by passage of 6 mL of MeOH and equilibrated by 10 mL of Milli-Q grade water. Then, the samples were passed through the cartridges. In order to remove any matrix interferences, the cartridges were washed with 2 mL of MeOH/Milli-Q water (40:60, % v/v) and then dried under vacuum. The compounds were eluted with 4 mL MeOH and collected in a 15 mL Eppendorf tube. The eluents were evaporated to near-dryness, under a gentle stream of nitrogen gas (N_2). Then, the eluents were diluted to 500 μL with MeOH/5mM ammonium formate (50:50, % v/v), filtered and transferred for analysis.

For the calculation of recoveries and matrix effects, post-extraction matrix spikes were prepared by spiking ISs and target analytes into final extracts prior to instrumental analysis.

Sample preparation for the particulate matter of wastewater and sludge

An aliquot of 100 mg (±10 mg) dewatered sludge or a filter containing the particulate matter (typical masses on the filters were: 10–20 mg for influent samples, 0.2–0.5 mg for effluent samples) was transferred into a 50 mL Eppendorf tube. Samples were spiked prior to extraction with the ISs (1.25 ng for each IS), and when required (i.e. preparation of quality control samples), they were also spiked with the target analytes. The spiked samples were left over-night in a fume hood in order to evaporate solvent spike. Then, 7.5 mL of 1% v/v acetic acid and 1.5 mL of MeOH were added, liquid-solid extraction (LSE) was performed by vortex-mixing for 1 min, and the mixture was ultra-sonicated for 15 min. The supernatant was collected after centrifugation (×1) at 3500 rpm for 15 min. The LSE procedure was performed three successive times for each sample (3×7.5 mL), and all three supernatants were transferred into a 50 mL Eppendorf tube. Dilution was performed to 50 mL with Milli-Q grade water, and thereafter, pH adjustment of extracts was realized to 4.0 ± 0.1 with acetic acid solution 1 M. Then, SPE extraction followed using the procedure as aforementioned for the dissolved phase samples of wastewater. Post-extraction matrix spikes were prepared as described in the above section.

Instrumental analysis

The measurements were carried out using a UHPLC Thermo Accela pump incorporating a column thermostat, a degasser, and an autosampler (San Jose, CA, U.S.). The mass spectrometric system was a Thermo TSQ Quantum Access triple quadrupole mass analyzer. Chromatographic separation was performed by XTerra MS C18 (100 mm×2.1 mm, 3.5 μm) column from Waters and the column temperature was set at 25 °C; Phenomenex C18 guard columns (4.0 mm×2.0 mm, 5 μm) were used at all times. The operating parameters of ESI, sheath gas, auxiliary gas, capillary temperature, and spray voltage were optimized. Tandem MS parameters for PFCs analysis are presented in Table 1.

(Insert Table 1)

Chromatographic analyses were carried out using a gradient elution program with 5 mM ammonium formate aqueous solution (solvent A) and MeOH (solvent B) as binary mobile phase mixture at a flow rate of 100 μL min^{-1}. The gradient elution started with 30% (v/v) MeOH and increased linearly to 75% MeOH in 1.5 min, and then to 100% MeOH in

12.0 min which was held for 5.0 min (until 17.0 min), reverted to 30% MeOH and re-equilibrated for 13.0 min (from 17.0 to 30.0 min) at 30% MeOH for a total run time of 30.0 min. Divert valve configuration was used in order to divert unwanted flow away from the ion source and increase the ruggedness of the detector; the flow was passed to the mass spectrometer only from the 5.0 to 16.0 min of the run. The electrospray ionization voltage was applied at -2.5 kV. The sheath gas (N_2) flow rate was set at 60 A.U. (Arbitrary Units), the auxiliary gas (N_2) flow rate was set at 20 A.U., the ion transfer capillary temperature was set at 270 °C, and the collision pressure was set at 1.5 mTorr. Multiple Reaction Monitoring (MRM) was applied for all PFCs, except for PFASs and PFSAs where pseudo-MRM was applied. Pseudo-MRM is the technique where the two quadrupoles monitor the same m/z and no fragmentation occurs (Table 1). The final in-vial composition of all samples and standard solutions were in MeOH/5mM ammonium formate (50:50, % v/v), and were injected on column with full-loop injection (10 µL). Data were acquired with the Xcalibur 2.0.6 software package (Thermo Scientific).

Results and discussion

ESI parameters and properties of PFCs

PFCs demonstrate a typical ESI fragmentation pattern that has previously been reported.[25,31-32] The tandem MS fragmentation patterns of PFASs exhibit an array of common product ions such as those observed at 80 and 99 m/z that correspond to $[SO_3]^-$ and $[FSO_3]^-$, respectively. For PFSAs, with the exception of PFOSA, the product ions observed at 269 and 169 m/z corresponded to $[C_4F_9]^-$ and $[C_3F_7]^-$, respectively. For PFOSA, in particular, the predominant product ions were observed at 78 and 169 m/z, corresponding to $[SNO_2]^-$ and $[C_3F_7]^-$, respectively. For PFCAs, the deprotonated molecular ions $[M-H]^-$ induced decarboxylation and formation of various perfluoroalkyl anions. The precursor and product ions, the collision energies and the tube lens offsets of all target analytes and ISs were determined by infusing standard solutions (1.0 µg mL^{-1}) of every compound directly into the ion source (Table 1). From all PFCAs, PFPeA was the only compound demonstrating poor fragmentation, since only one MRM transition could be monitored (263>219 m/z).

When applying the MRM technique, lower sensitivity was obtained for PFASs and PFSAs compared to that of PFCAs. Thus, we applied pseudo-MRM for the analysis of PFASs and PFSAs, since this technique, as suggested by Haug et al. (2009), offers increased sensitivity compared to that of MRM (Fig.1).[16] On this aspect, we assessed the pseudo-MRM

technique under the application of two collision energy (CE) values, 5 and 10 eV, of all target PFASs and PFSAs and that of $^{2}D_{3}$-*N*-MeFOSA (IS) (Fig.1).

(Insert Fig. 1)

Our results supported the findings of Haug et al. (2009),[16] and the optimal CE proved to be at 10 eV for PFBS, PFHxS, PFHpS, PFOS, and PFOSA, whereas for the remaining compounds only slight differences were observed between the two CE values.

LC mobile phase

For the development of the LC-MS/MS chromatographic system, four mobile phase mixtures were examined under isocratic elution conditions, MeOH/5mM ammonium acetate (70:30, % v/v), ACN/5mM ammonium acetate (70:30, % v/v), MeOH/5mM ammonium formate (70:30, % v/v), and ACN/5mM ammonium formate (70:30, % v/v); a high organic fraction (70 % v/v) of mobile phase was assessed in order to achieve faster elution with the C18 column. Full loop injections (10 μL) of the mixed target analyte solution (100 ng mL^{-1}) were made into each binary mobile phase mixture, prior to entering the ion source at a flow rate of 200 μL min^{-1}. For each mobile phase combination, six loop injections (N=6) were performed, and the average peak area of all target analytes was calculated (Fig. 2). Loop injection experiments are of great importance since ionization is simulated in almost actual conditions of LC-MS/MS analysis. It should be stated that loop injection experiments were performed with a FIA (flow injection analysis) system coupled to a loop, providing a continuous supply of mobile phase into the ion source.

(Insert Fig. 2)

For most target PFCs, optimal sensitivity was achieved by the two mobile phase combinations of MeOH, and consequently, based on these combinations, six chromatographic systems (four gradient and two isocratic; Table S1) were evaluated in terms of sensitivity by performing on-column injections of the mix target analyte solution (100 ng mL^{-1}) (Figs. S1 and S2). Overall, best performance was achieved by a binary gradient elution program consisted of MeOH/5mM ammonium formate (chromatographic system B; Table S1). The flow rate was found optimal at 100 μL min^{-1} with respect to the obtained chromatographic separation of target analytes.

Extraction and purification by SPE

Oasis HLB cartridge is suitable for PFCs since it ensured low background levels and acceptable recoveries for most of the target chemicals.[9,33] Thus, Oasis HLB sorbent was

evaluated under three different sample (Milli-Q grade water) pH values, 3.0 ± 0.1, 4.0 ± 0.1, and 6.0 ± 0.1 for effective extraction and isolation of target analytes. The pH values were adjusted with acetic acid solution 1 M. All target PFCs were fortified in 50 mL Milli-Q grade water at the level of 10 ng, and the recovery of every target analyte was calculated based on Eq. 1.

[(Peak area of pre-extraction spiked Milli-Q water) - (Peak area of reagent blank)] / [(Peak area of standard solvent solution) - (Peak area of instrumental blank)] ×100 (1)

Higher recoveries were obtained for PFPeA, PFHxA, and PFSAs when adjusting to pH 4 (Fig.3). For the rest target PFCs, recoveries did not vary considerably between pH 3 and 4 (Fig.3).

(Insert Fig. 3)

Method performance and validation

Calibration curves based on the internal standard method and with a matrix-matched calibration standard (pre-extraction matrix spikes) were prepared for quantification of all PFCs, except for PFOSA. When using the internal standard method, a calibration curve is constructed for every target analyte from the ratio of the analyte response to the internal standard response in every measured standard solution (solvent or matrix matched), plotted against the concentration (amount) of the spiked analyte. Each IS was fortified at an amount of 1.25 ng in all standard (solvent and matrix) solutions. Quantification of PFOSA based on the IS of $^{2}D_{3}$-*N*-MeFOSA was not performed since unacceptable method linearity was obtained, $r < 0.99$; and consequently, the calibration curves were constructed from the peak area of the analyte plotted against the concentration (amount) of the fortified analyte. The instrumental linear range of all target analytes was verified by injecting standard solvent solutions at seven fortification levels (0.05, 0.125, 0.25, 0.5, 1.25, 2.5, and 5.0 ng) and showed an excellent linearity ($r > 0.997$) (Table 2). At all times, it was acknowledged that the constant coefficient (or intercept) of the matrix and solvent calibration curves was not statistically different from zero (t-exp<t-theor; F-test). The limits of detection (LODs) and quantification (LOQs) were calculated for each target analyte as 3 and 9.9 times the signal from the baseline noise (S/N ratio), respectively. For the dissolved phase, the LODs and LOQs were in the ranges of 0.09 (PFHpS) - 0.92 ng L^{-1} (PFDoA), and 0.29 (PFHpS) - 3.0 ng L^{-1} (PFDoA), respectively. For the particulate matter, the LODs and LOQs were in the ranges of 0.04 (PFHpS) - 0.46 ng g^{-1} (PFDoA), and 0.15 (PFHpS) - 1.5 ng g^{-1} (PFDoA),

respectively. Our LODs and LOQs for both matrices were similar to those reported by previous studies.[12,14,19,24]

(Insert Table 2)

The accuracy (trueness) of the methods was evaluated through absolute recovery experiments in six (N=2) replicate analyses at the fortification level of 10 ng. The results are demonstrated in Table 2. Replicate analyses are defined as the measurement of two or more standard solutions (or samples) which are independently carried through all steps of sample preparation and instrumental analysis in an identical manner. The absolute recovery for each target analyte at a specific fortification level was calculated based on Eq. 2.

[(Peak area of pre-extraction spiked matrix) - (Peak area of reagent blank)] / [(Peak area of post-extraction spiked matrix) - (Peak area of reagent blank)] ×100 (2)

For the dissolved phase samples, all target analytes demonstrated absolute recoveries in the ranges of 80.5 -114%. For the particulate matter samples, all target analytes, except for the long-chain PFCs (PFDoA, PFTrDA, PFTeDA and PFDS), demonstrated absolute recoveries in the ranges of 71.0-115%. The long chain PFCs (PFUdA, PFDoA, PFTrDA, PFTeDA and PFDS) demonstrated low absolute recoveries in the ranges of 26.4-38.7% denoting their high affinity to organic matter.[34-36] The results of analytical precision of the methods were demonstrated through repeatability (Intra-day precision, RSDr %; N=9, k=1 day) and reproducibility (Inter-day precision, RSD_R %; N=3, k=3 days) (Table S2). For repeatability and reproducibility experiments, samples from both matrices were fortified at the level of 10 ng, and nine replicate analyses (N=9) were performed within the same day (k=1) and in-between three different days (k=3), respectively. The results showed satisfactory precision for both dissolved phase and particulate matter media, with the majority of target analytes presenting RSD <15%. The suitability of the internal standards, $^{13}C_4$-PFOA, $^{13}C_4$-PFOS, and $^{2}D_3$-N-MeFOSA were assessed in terms of compensation for variations in chromatographic retention for PFCAs, PFASs, and PFSAs, respectively. An aqueous standard solution was prepared containing each target analyte at 0.25 ng and each internal standard at 1.25 ng, and the intra-day precision (N=6 replicate injections, RSD %) of analyte retention time, and analyte relative retention time (RRT; analyte retention time /internal standard retention time) were demonstrated (Table S3). Overall, the results denoted that the use of $^{13}C_4$-PFOA, $^{13}C_4$-PFOS, and $^{2}D_3$-N-MeFOSA as ISs offered excellent chromatographic retention precision of chemicals since most RT and RRT values demonstrated RSDs <0.8%.

Matrix effects (ME %) were present for all chemicals (Table S4), and were quantified in both matrices for three samples taken in different days at the fortification level of 10 ng (Eq. 3).

{[(Peak area of post-extraction spiked matrix) - (Peak area of each sample)] / [(Peak area of standard solvent solution) - (Peak area of reagent blank)] -1} ×100 (3)

Ionization suppression occurs when ME % < 0, while ionization enhancement occurs when ME % > 0. Thus, according to Table S4, ionization enhancement was demonstrated for all chemicals, rendering quantification of PFCs based on the internal standard method and with a pre-extraction matrix matched calibration standard mandatory for the accomplishment of accurate measurements.

The main drawback of PFCs analysis is background contamination.[9,12,31,33] To minimize this effect, previous experiments have been performed to investigate the sources of procedural and instrumental contamination using different types of cartridges, syringe filters, and pure water or/and replacing HPLC tubing, solvent inlet filters and autosampler vials septum.[9] In this study, a number of actions were taken in order to control and minimize sources of contamination. All disposable materials used herein from sample preparation to instrumental analysis were from polypropylene (PP). Lids and other materials containing polytetrafluoroethylene (PTFE) were avoided, due to possible leaching of fluorinated materials. The use of Oasis HLB cartridges did not pose an important contamination source; nonetheless, two reagent blanks (plain Milli-Q water) were carried out through all steps of sample preparation and instrumental analysis at all times for every measured sample batch. If instrument background levels of PFCs were found, they were eliminated before analyses by injecting sufficient blanks to cleanse the system (8 to 10 blanks were required). Additionally, to minimize build-up of PFCs during mobile phase equilibration and to keep background levels constant, the time the system was kept under initial conditions was as short as possible. Prior to daily use, we flushed the LC column with elution solvents [MeOH/5mM ammonium formate (70:30, % v/v)] before initiating a sequence.

The ion ratio % of all PFCAs, except for PFPeA (that demonstrates one MRM transition), were shown in both matrices at the fortification level of 10 ng (pre-extraction spiked samples) in Table S5 and were considered acceptable according to EE guideline 2002/657/EE[37]. The ion ratio % was calculated from the ratio of two MRM transitions that were monitored for each chemical, and compared with that calculated for solvent standards to

confirm the identity. A chromatogram of dewatered sludge fortified with the target analytes prior to extraction (pre-extraction matrix spikes) and passed through the entire analytical procedure is presented in Fig. 4, demonstrating adequate chromatographic separation.

(Insert Fig. 4)

Application of the methods

The developed methods were successfully applied to wastewater and sewage sludge samples obtained from Athens WWTP, in order to monitor the levels of the target PFCs. According to the results (Table S6), 11 out of 18 target PFCs were detected either in wastewater (dissolved/particulate phase) or in sludge samples. The highest total concentrations (sum of dissolved and particulate concentration) in influent and effluent wastewater were determined for PFUdA (113 ng L^{-1}) and PFOA (33 ng L^{-1}), respectively. For the sludge samples, the highest levels of concentration (as ng g^{-1} dw) were determined for PFDoA (447 ng g^{-1} for primary sludge and 224 ng g^{-1} for secondary sludge) and PFUdA (1042 ng g^{-1} for dewatered sludge). PFCs concentrations detected in the current study were similar or lower than those reported in previous studies for WWTP samples.[30,34,35,38-46] The results of this study showed that a significant part of target compounds is detected in the particulate phase of wastewater, ranging from 8 to 100% (influent wastewater) and 9 to 100% in treated wastewater (Table S6). Based on these observations, particulate matter of wastewater should always be analyzed in order to avoid underestimation of PFCs concentrations.

Conclusions

Integrated methods were developed for the determination of PFCs in wastewater (both dissolved and particulate phase) and sludge samples. The proposed methods were proved adequate for environmental monitoring, taking into account the complexity of the matrices and the small amounts of the extracted samples. This approach was proven to be highly selective and sensitive. The performance of the method was demonstrated successfully by its application, and the presence of PFCs chemicals in wastewater and sewage sludge in Greece was presented.

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395 (http://www.env.aegean.gr/WaterMicropol/).

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Figure Captions

Fig. 1. MRM versus pseudo-MRM (at collision energy of 5 and 10 eV) for PFASs and PFSAs.

Fig. 2. Ionization efficacy of PFCs under four different binary mobile phases.

Fig. 3. Oasis HLB sorbent evaluated under three different sample (Milli-Q grade water) pH values, 3.0 ± 0.1, 4.0 ± 0.1, and 6.0 ± 0.1.

Fig. 4. TIC and MRM chromatograms of fortified sludge sample (m/z transitions depicted).

481 **Table 1.** Tandem MS parameters for the analysis of PFCs.

Chemicals	Abbreviation	Precursor ion (*m/z*)	Quantification Product ion (*m/z*)	Collision energy (eV)	Tube lens (V)
Perfluoropentanoic acid	PFPeA	263	219	11	30
Perfluorohexanoic acid	PFHxA	313	269(119 [b])	9	50
Perfluoroheptanoic acid	PFHpA	363	319 (169 [b])	11	50
Perfluorooctanoic acid	PFOA	413	369 (169 [b])	11	37
Perfluorononanoic acid	PFNA	463	419 (169 [b])	11	50
Perfluorodecanoic acid	PFDA	513	469 (169 [b])	13	50
Perfluoroundecanoic acid	PFUdA	563	519 (169 [b])	11	50
Perfluorododecanoic acid	PFDoA	613	569 (169 [b])	13	50
Perfluorotridecanoic acid	PFTrDA	663	619(169 [b])	13	60
Perfluorotetradecanoic acid	PFTeDA	713	669 (419 [b])	13	70
Potassium perfluorobutanesulfonate[a]	PFBS	299	299	10	50
Sodium perfluorohexanesulfonate[a]	PFHxS	399	399	10	50
Sodium perfluoroheptanesulfonate[a]	PFHpS	449	449	10	50
Sodium perfluorooctanesulfonate[a]	PFOS	499	499	10	104
Sodium perfluorodecanesulfonate[a]	PFDS	599	599	10	50
Perfluorooctane sulfonamide[a]	PFOSA	498	498	10	50
N-Methylperfluorooctane sulfonamide[a]	N-MeFOSA	512	512	10	112
N-Ethylperfluorooctane sulfonamide[a]	N-EtFOSA	526	526	10	103
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA (MPFOA)	417	372 (172 [b])	11	38
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate[a]	$^{13}C_4$-PFOS (MPFOS)	503	503	10	92
N-Methyl-d^3-perfluoro-1-octanesulfonamide[a]	$^2D_3$-N-MeFOSA	515	515	10	89

[a] Pseudo-MRM approach; [b] Confirmation ion

482 **Table 2.** Analytical parameters of the developed methodology.

Chemicals	Instrumental linear range (ng)	Instrumental correlation coefficient (r^2)	Dissolved phase of wastewater		Sewage Sludge		Average Recovery % (N=6)	
			LOD (ng L^{-1})	LOQ (ng L^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})	Dissolved phase of wastewater	Sewage sludge
PFPeA	0.05-5	0.9992	0.52	1.7	0.26	0.86	80.5	25.1
PFHxA	0.125-5	0.9998	0.44	1.5	0.22	0.73	94.2	78.2
PFHpA	0.05-5	0.9993	0.60	2.0	0.30	1.0	96.8	85.2
PFOA	0.05-5	0.9995	0.72	2.4	0.36	1.2	91.2	111
PFNA	0.05-10	0.9998	0.76	2.5	0.38	1.3	88.7	82.9
PFDA	0.05-5	0.9998	0.52	1.7	0.26	0.86	89.0	59.8
PFUdA	0.05-5	0.9994	0.11	0.36	0.05	0.18	95.0	34.7
PFDoA	0.05-5	0.998	0.92	3.0	0.46	1.5	93.0	26.4
PFTrDA	0.05-5	0.998	0.68	2.2	0.34	1.1	97.0	30.6
PFTeDA	0.05-5	0.9996	0.37	1.2	0.18	0.61	85.6	32.3

PFBS	0.05-5	0.9995	0.11	0.37	0.06	0.18	112	112
PFHxS	0.05-5	0.9998	0.12	0.40	0.06	0.20	111	113
PFHpS	0.05-5	0.9998	0.09	0.29	0.04	0.15	114	115
PFOS	0.05-5	0.9998	0.18	0.58	0.09	0.29	92.5	95.9
PFDS	0.05-5	0.997	0.48	1.6	0.24	0.79	85.0	38.7
PFOSA[a]	0.25-5	0.9994	0.16	0.54	0.08	0.27	87.0	71.0
N-MeFOSA	0.25-5	0.9991	0.29	1.0	0.14	0.48	91.5	87.2
N-EtFOSA	0.25-5	0.999	0.52	1.7	0.26	0.86	81.6	81.2

[a] Calibration curve was constructed from the peak area of the analyte plotted against the concentration of the fortified analyte.

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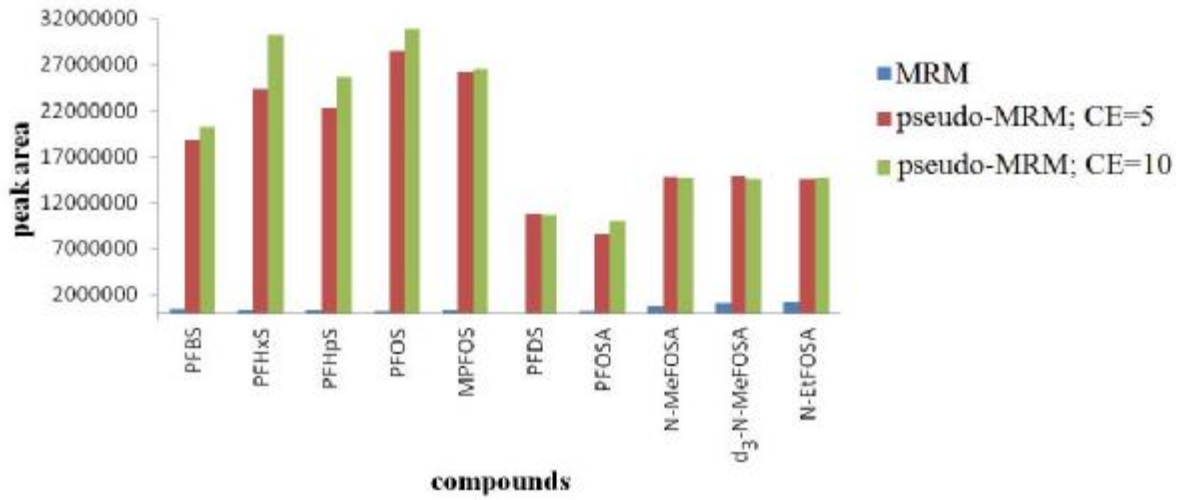

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494 **Fig.1**

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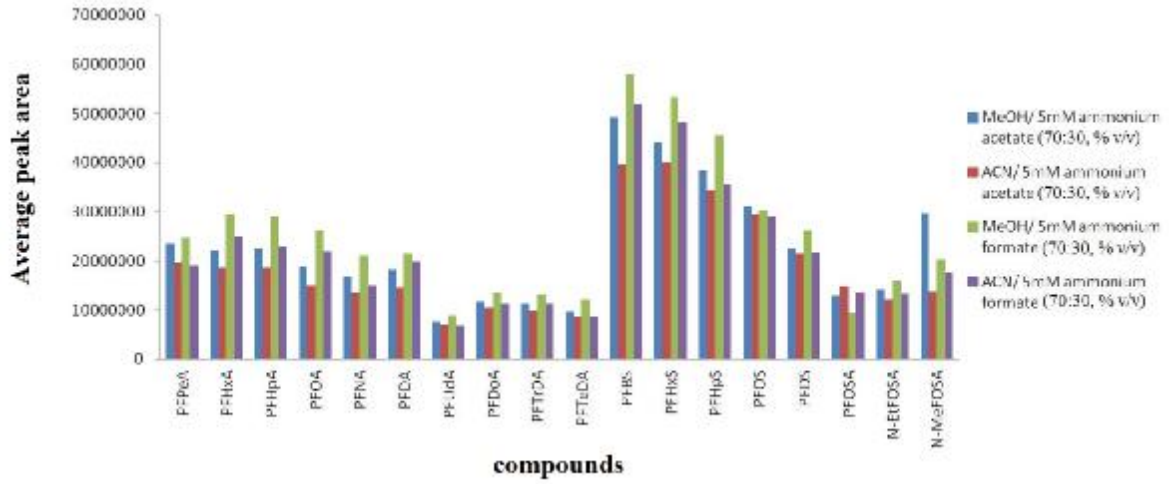

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498 **Fig.2**

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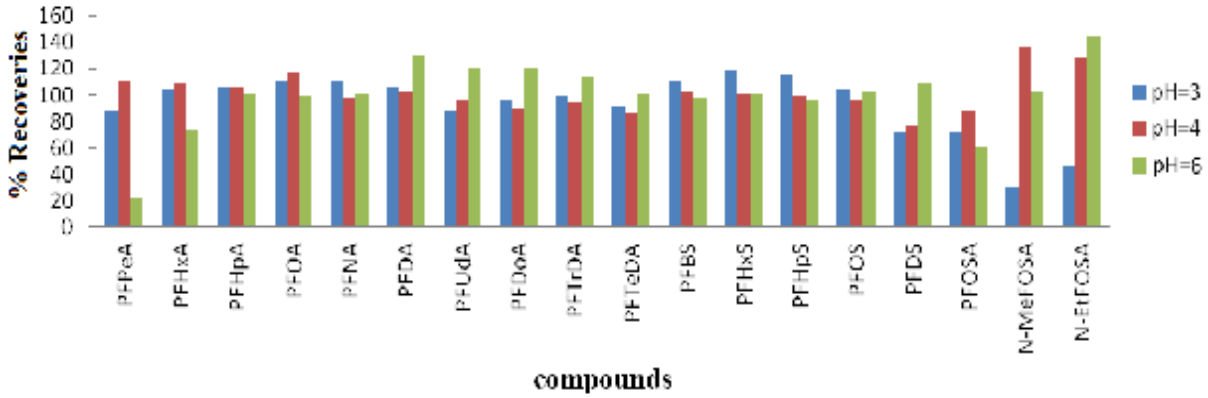

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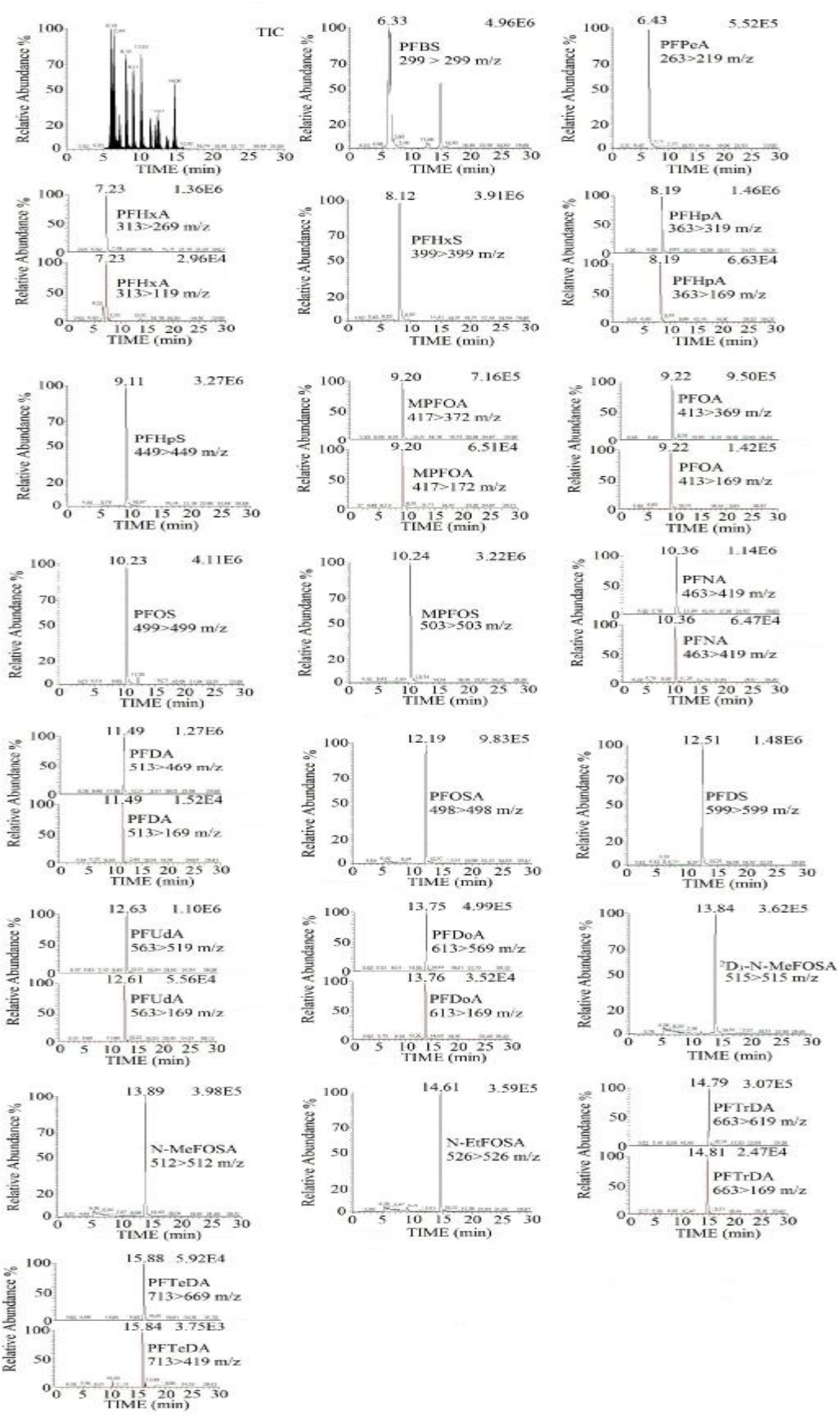

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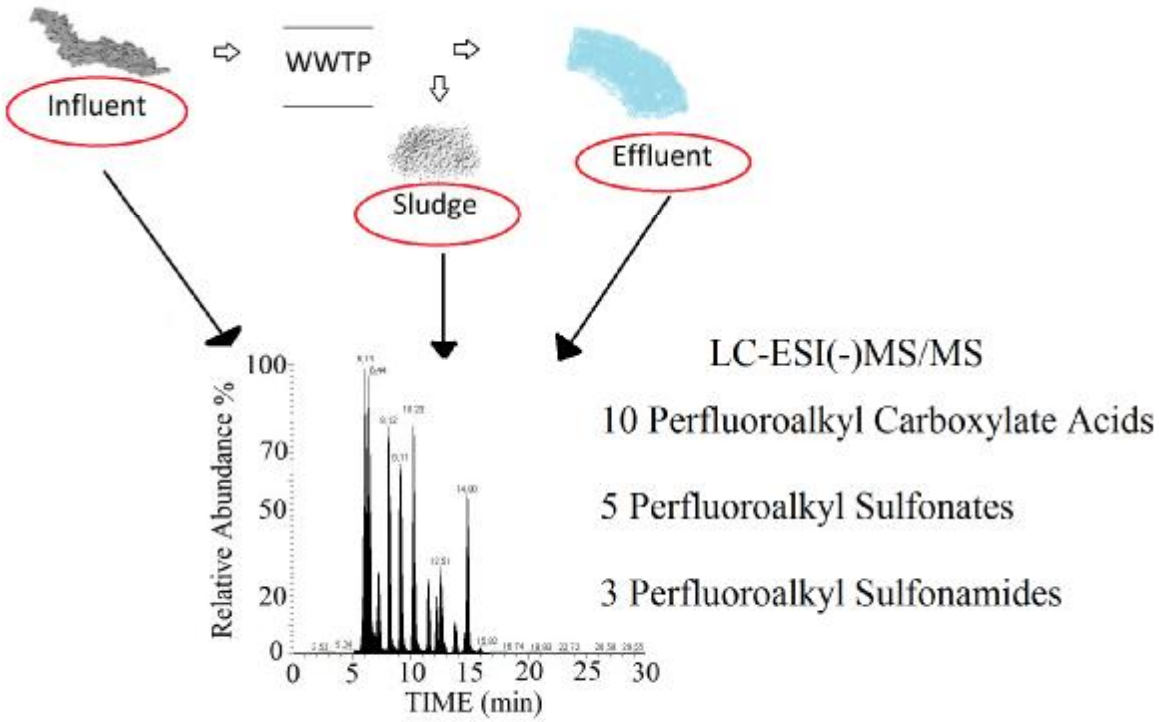
505 **Fig. 4**

506 **TOC GRAPHIC ABSTRACT**



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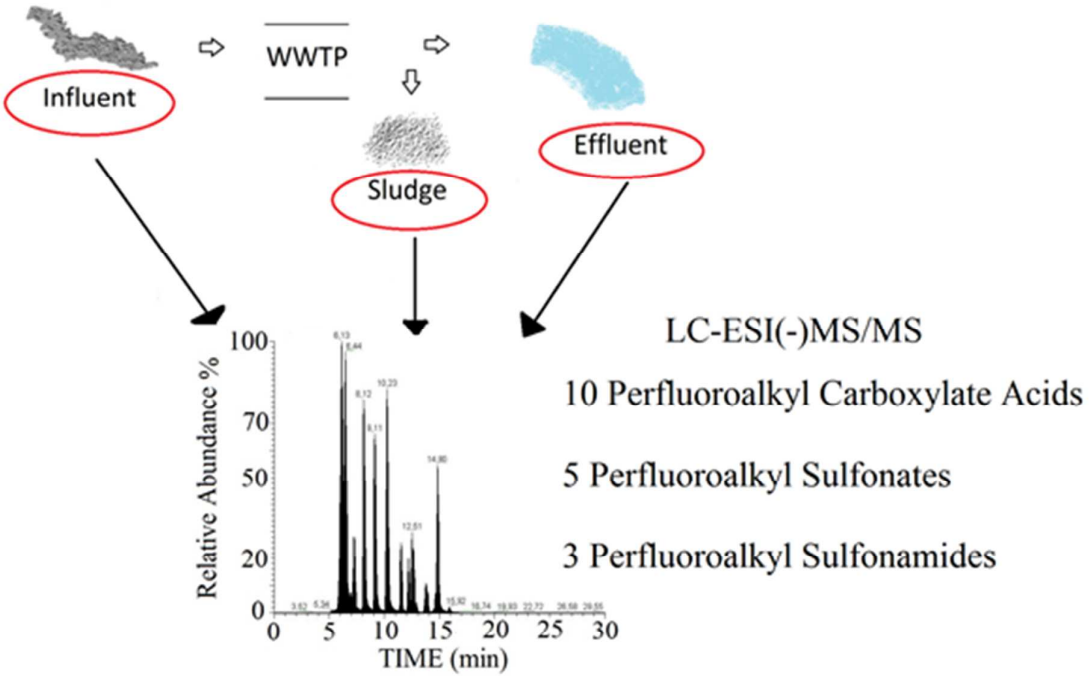

An integrated method for the determination of 18 perfluorinated compounds in dissolved and particulate phase of wastewater and in sludge