Talanta 176 (Jan) 2018 ; 412-421

DOI: 10.1016/j.talanta.2017.08.052

Applicability of the direct injection liquid chromatographic tandem mass spectrometric analytical approach to the sub-ng L^{-1} determination of perfluoro-alkyl acids in waste, surface, ground and drinking water samples

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Abstract

The applicability of a direct injection UHPLC-MS/MS method for the analysis of several perfluoroalkyl acids (PFAAs) in a wide range of water matrices was investigated. The method is based on the direct injection of 100 µL of centrifuged water sample, without any other sample treatment. Very good method detection limits (0.014-0.44 ng L^{-1}) and excellent intra and inter-day precision (RSD% values in the range 1.8-4.4% and 2.7-5.7%, respectively) were achieved, with a total analysis time of 20 minutes per sample. A high number of samples – i.e. 8 drinking waters (DW), 12 ground waters (GW), 13 surface waters (SW), 8 influents and 11 effluents of wastewater treatment plants ($WWTP_{IN}$ and $WWTP_{OUT}$) were processed and the extent of matrix effect (ME) was calculated, highlighting the strong prevalence of |ME|<20%. The occurrence of |ME|>50% was occasionally observed only for perfluorooctanesulphonic and perfluorodecanoic acids. Linear discriminant analysis highlighted the great contribution of the sample origin (i.e. DW, GW, SW, $WWTP_{IN}$ and $WWTP_{OUT}$) to the ME. Partial least square regression (PLS) and leave-one-out cross-validation were performed in order to interpret and predict the signal suppression or enhancement phenomena as a function of physicochemical parameters of water samples (i.e. conductivity, hardness and chemical oxygen demand) and background chromatographic area. The PLS approach resulted only in an approximate screening, due to the low prediction power of the PLS models. However, for most analytes in most samples, the fitted and cross-validated values were such as to correctly distinguish between | ME | higher than 20% or below this limit. PFAAs in the aforementioned water samples were quantified by means of the standard addition method, highlighting their occurrence mainly in WWTP influents and effluents, at concentrations as high as one hundred of µg L^{-1}.

Keywords - Direct injection UHPLC-MS/MS; Perfluoroalkyl acids; Drinking water; Environmental water; Wastewater; Matrix effect chemometrics.

1 Introduction

Perfluoro-alkyl acids (PFAAs) are a class of compounds having a CF_3-$(CF_2)_n$-R structure, where R is a carboxylic or a sulfonic or a phosphonic group, and "n" ranges mostly between 2 and 10. PFAAs are characterized by high resistance to physical, chemical and biological degradation and have been widely employed since the 1950s in a wide range of industrial and commercial applications, as well as in fluoropolymer production, giving rise to a widespread contamination of environmental matrices. More in detail, PFAAs have been determined in wastewater [1-3], surface water [4-6] and drinking water [7]. Moreover, PFAAs have been detected in remote areas like open oceans [8] and Arctic [9, 10].

Among PFAAs, perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been the most industrially employed until 2006, when some regulatory restrictions have been promulgated both in Europe and United States [11, 12]. Furthermore, in 2013 PFOS has been included in the list of priority hazardous substances, within the Directive 2013/39/EU [13], whereas PFOA has been included in the candidate list of Substances of Very High Concern because of its carcinogenic, mutagenic or toxic for reproduction effects as well as persistent, bioaccumulative and toxic properties [14].

For PFOS an annual average environmental quality standard (EQS) and a maximum allowable concentration (MAC) of 0.65 ng L^{-1} and 36 µg L^{-1} have been respectively established for inland waters by the European Community (EC) [13]. As regards drinking water, Provisional Health Advisories of 0.4 µg L^{-1} and 0.2 µg L^{-1} have been proposed by the Environmental Protection Agency of Unites States (USEPA) for PFOA and PFOS, respectively [15]. For these compounds concentration limits in drinking water of 0.5 µg L^{-1} (PFOA) and 0.03 µg L^{-1} (PFOS) have been recommended by the Italian Health Institute, on the basis of maximum tolerable daily intake (TDI) data reported by the European Food Safety Authority [16]. Conversely, to the best of our knowledge, no limits have been established for the presence of PFAAs in groundwater.

Several analytical methods for the determination of PFAAs in water media at trace level have been published, mostly employing solid-phase extraction (SPE) and liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) [1, 17-19].

However, physicochemical properties (i.e. solubility/lipophilicity and acidity) of these molecules greatly vary depending on the chain length and the acidic group present in the molecule, thus making challenging the recovery of all analytes during extraction and clean-up processes. Furthermore, special care should be taken during sample manipulation, treatment and analysis, since, as demonstrated by various inter-laboratory studies, there is an actual risk of contamination

during the whole analytical process, owing to the presence of fluorinated polymers in commonly used laboratory materials and equipment [20].

In order to minimize sample manipulation and treatment, as well as to increase the analytical throughput, several on-line SPE-LC-MS/MS methods have been developed for PFAAs determination in water samples [21-24].

The direct injection (DI) approach is the best choice to overcome any contamination of the sample due to its manipulation and treatment, as well as to ensure a high analytical throughput. However, this approach may suffer a lower sensitivity, compared to SPE-based methods. Moreover, when the DI approach is used, matrix effect (ME) may significantly affect the precision and/or the sensitivity of the method, owing to the absence of extraction and clean-up steps. Several applications of the DI technique have been reported in literature for the determination of different classes of organic micropollutants in water samples [3, 20, 25-28]. Furthermore, the DI approach is also included in official methods for the analysis of selected organic contaminants in drinking water [29, 30], where ME is usually less important than in freshwater or wastewater [31-34]. Nevertheless, to the best of our knowledge, only two papers have been published to date concerning the application of DI-LC-MS/MS to PFAA analysis [3, 20]. Furdui and co-workers evaluated ME for DI-LC-MS/MS analysis of C7-C12 perfluoro-carboxylic acids (PFCAs), C6 and C8 perfluoro-sulfonic acids (PFSAs) and C8 perfluoro-sulfonamide in lake water and effluent wastewater, highlighting in all real samples a suppressive ME [3]. Conversely, on a larger group of PFAAs, including also C4-C6 PFACs and C4 PFSA, Wolf and Reagen evidenced the absence of ME in various synthetic and real drinking water samples, as well as in groundwater, cooling-water and effluent wastewater samples [20]. It should be however noted that in both studies ME was not systematically investigated, and the applicability of the DI-LC-MS/MS approach to a wide range of aqueous matrices, including those characterized by a strong matrix component (e.g. wastewaters and environmental waters), still remains worth to be further investigated. In this regard, it should be remarked that the analysis of PFAAs in aqueous samples provides a realistic picture of their presence in the whole environmental compartment (i.e. water with sediments and particulate matter), since these analytes are almost completely partitioned in the dissolved phase [8].

According to the considerations reported above, the aim of this work was to evaluate the feasibility of using the DI-LC-MS/MS analytical approach for the determination of PFCAs and PFSAs in a very wide range of water samples (i.e. drinking water, groundwater, river water, lake water and wastewater). For each sample ME was investigated by the standard addition method and tentatively interpreted as a function of a set of physicochemical parameters of water samples (i.e. conductivity, hardness, and organic carbon content), as well as chromatographic outputs.

Moreover, since water samples herein analysed were collected in zones never investigated before for PFAA occurrence (i.e. various rural, urban and industrial districts of Tuscany, Italy), this study provides for the first time information regarding the contamination by PFAAs of these areas.

2 Material and methods

2.1 Standards and reagents

Perfluorobutanesulphonic acid (PFBuS, CAS number: 375-73-5), perfluoropentanoic acid (PFPeA, CAS number: 2706-90-3), perfluorohexanoic acid (PFHxA, CAS number: 307-24-4), perfluorohexanesulphonic acid (PFHxS, CAS number: 355-46-4), perfluoroheptanoic acid (PFHpA, CAS number: 375-85-9), perfluorooctanoic acid (PFOA, CAS number: 335-67-1), perfluoro-n-(1,2,3,4 $^{13}C_4$)octanoic acid (MPFOA), perfluoroactanesulphonic acid (PFOS, CAS number: 1763-23-1), and perfluoro-1-(1,2,3,4 $^{13}C_4$)octanesulphonate (MPFOS), perfluorononanoic acid (PFNA, CAS number: 375-95-1), perfluorodecanoic acid (PFDA, CAS number: 335-76-2), methanol stock solutions (50 µg mL^{-1}) were purchased by Wellinghton Laboratories Inc. (Guelph, ON, Canada).
Water and methanol (LC-MS grade) were obtained from Carlo Erba (Milan, Italy).
Ammonium acetate (Sigma-Aldrich, St. Louis, MO, USA) 1 M solution was freshly prepared in water LC-MS grade.

2.2 UHPLC-MS/MS analysis

Ultra-high performance liquid chromatographic (UHPLC) analyses were performed on a Shimadzu (Kyoto, Japan) chromatographic system consisting of a low pressure gradient quaternary pump Nexera X2 LC-30AD, a DGU-20A 5R degassing unit, a SIL-30AC autosampler equipped with a 100 µL loop, a CTO/20AC thermostatted column compartment and a CBM-20A module controller. A delay column (C_{18}, 100 x 4.6 mm) was installed between the mixer and the sample injector, in order to separate the impurity PFAAs originating from the LC system from the analyte PFAAs of the sample.
Chromatographic separation was obtained with a Waters ACQUITY UPLC BEH C_{18} column (100 x 2.1 mm, particle size 1.7 µm) equipped with a guard column (Waters, Milford, MA, USA), thermostatted at 50° C, employing a mixture of 95% water 5% methanol solution of 2 mM ammonium acetate (solvent A) and a methanol solution of 2 mM ammonium acetate (solvent B) at a flow rate of 0.5 mL min^{-1}. All glassware was thoroughly rinsed with methanol before use. The eluents were freshly prepared by adding a suitable aliquot of 1 M ammonium acetate solution filtered on 0.2 µm polyethersulfone filters. The chromatographic gradient was the following: 25% B for 2 min, from 25% to 90% in 6 min, 90% for 5 min, from 100% to 25% in 0.5 min and final hold

for 6.5 min for system re-equilibration. Total analysis time was 20 mins. The injection volume used was set to 100 µL. **Fig. S1** of the Supplementary material section illustrates a reconstructed DI-LC-MS/MS chromatogram of the quantifier and qualifier transitions of target PFAAs obtained by injecting 100 µL of a standard mixture in Milli-Q water at the concentration of 5 ng L^{-1} each.
In order to minimize MS source contamination, the first 2 min. and the last 8 min. of the chromatographic run have been diverted to waste by means of a two-position six-port valve installed before the mass spectrometer.

2.3 Mass spectrometric detection

The Shimadzu LC system was coupled to a 5500 QTrap mass spectrometer (Sciex, Framingham, MA, USA), equipped with a Turbo V® interface by an ESI probe. MS/MS analysis was carried out using the Multiple Reaction Monitoring (MRM) in negative ionization mode. The MS source parameters were optimized in flow injection analysis (FIA) at optimal LC flow and mobile phase composition and were as follows: Curtain Gas (CUR) 50, CAD Gas (CAD) Medium, Temperature (TEM) 650°C, Gas 1 (GS1) 50, Gas 2 (GS 2) 50, and IonSpray Voltage (IS) -4500 V in scheduled MRM(−).
MRM mass transitions used for quantification and identity confirmation, together with compound-dependent parameters are listed in **Table S1** of the Supplementary material section.
Confirmation criteria proposed by the Commission Decision 2002/657/EC [35] concerning retention time and quantifier-to-qualifier ion intensity ratio were adopted for compound identification.

2.4 Method performance evaluation

Standard solutions containing 1, 5, 10, 30, 50, 100 and 250 ng L^{-1} of each analyte were employed for calibration curves. For each investigated compound, method detection limits (MDLs) and method quantification limits (MQLs) were evaluated by replicated (n=5) analysis of procedural blanks, according to the following equation:

$$\text{MDL (MQL)} = \frac{k \cdot \sigma_b}{m}$$

where k is the critical value given by $2t_{1-\alpha}$ with n-1=4 degree of freedom and α=0.05 (k=4.264 and k=10, for MDLs and MQLs, respectively); σ_b is the standard deviation of the blank and m is the slope of the calibration curve.
The method of standard additions was used for the evaluation of ME, as well as for analyte quantification in real samples. Each sample was split into four aliquots, three of which were spiked with increasing concentration of standard solutions of target PFAAs. The spike levels were selected

so that the final concentrations of the compounds fell into the linear range. Spike levels of 10, 50 and 100 ng L^{-1} were employed. The extent of ME was evaluated according to the following equation:

$$ME\% = \frac{m_{sample}}{m_{standard}} \cdot 100 - 100$$

where m_{sample} is the slope of the calibration curve obtained in matrix, and $m_{standard}$ is the slope of the calibration curve in solvent; accordingly, ME values <0 indicate ion suppression, whereas ME values > 0 indicate ion enhancement.

2.5 Sample collection and handling before analysis

A very wide range of wastewater and environmental water samples, comprising eight drinking waters (DW), twelve ground waters (GW), thirteen surface waters (SW), as well as eight influents and eleven effluents of wastewater treatment plants ($WWTP_{IN}$ and $WWTP_{OUT}$), were included in this study (see **Table S2** of the Supplementary material section).

Glass bottles equipped with screw top and aluminium under-cap were employed for collecting the samples. All the samples were obtained through 24-h composite collections using Endress + Hauser Liquiport 2010 CSP44 autosamplers (Reinach, Switzerland). After collection, all samples were stored at +4 °C until analysis, which was performed in triplicate within 48 h after sampling.

Ultracentrifugation of samples at 30,000 x g was performed in polypropylene Eppendorf vials for particulate matter removal before UHPLC-LC-MS/MS analysis. Afterwards, the samples were fortified with labelled MPFOA and MPFOs, as internal standards, in order to highlight any possible leakage during sample injection in the UHPLC system.

2.6 Statistical analysis

Linear discriminant analysis (LDA) and partial least square (PLS) regression with leave-one-out cross validation were performed using the Minitab software packages version 17.0.1 (Minitab Inc., State College, PA, USA).

3 Results and discussion

3.1 Figures of merit of the analytical method

Method performance were evaluated by estimating method detection limits (MDLs), method quantification limits (MQLs), linearity, intra-day and inter-day precision via the replicated injection in the DI-LC-MS/MS system of 100 µL standard solutions in Milli-Q water. **Table 1** summarizes the results obtained for these performance parameters. More in detail, MDLs and MQLs were

evaluated by replicated (n=5) analysis of procedural blanks, according to the equation reported in Section 2.4.

Very good sensitivities were achieved for all investigated compounds, being the MDLs in all cases well-below 1 ng L^{-1}. Furthermore, the MQL achieved for PFOS (0.17 ng L^{-1}) was fully compatible with its quantification at the concentration level of the European EQS for inland waters (0.65 ng L^{-1}), which is commonly considered a challenging limit for every LC-MS analytical method [21].

For all compounds, linearity range covered at least two magnitude orders, ranging from MQLs to 100 ng L^{-1}, with determination coefficient of regression ≥0.9981. Intra-day and inter-day precisions were evaluated for all compounds at a concentration equal to 5 ng L^{-1}, achieving $RSD\%_{intra}$ and $RSD\%_{inter}$ values comprised in the range 1.8-4.4% and 2.7-5.7%, respectively.

3.2 Matrix Effect evaluation and interpretation

Table S3 of the Supplementary material section summarizes the values of ME determined for each compound and sample investigated. Very different extents of signal suppression or enhancement were observed, depending on the analyte and the sample considered, thus giving rise to a very complex dataset.

In order to make easier the discussion of data shown in **Table S3**, a graphical summary of the ME distribution determined for each investigated PFAA in the 52 water samples, is reported in **Fig. 1**. More in detail, **Fig. 1A** illustrates the frequency of occurrence of the absolute values of ME within the following groups: (i) |ME|<20%, (ii) 20≤|ME|≤50% and (iii) |ME|>50%. For most investigated analytes ME was found always lower than 50%. Furthermore, |ME|<20%, which is considered to have a negligible influence on the performance of an analytical method [36-38], showed the highest frequency of occurrence. The occurrence of |ME|>50%, from -51.2% to -77.5%, was observed only for PFOS and PFDA; these extremely high signal suppressions interested mainly GW-6, GW-7 and GW-8 samples, which showed a quite strong suppression also for most of the other investigated compounds (**Table S3**). As illustrated in **Fig. 1B**, PFOS and PFDA were the only analytes showing the mean and median outside the ±20% range; the ME distribution of these two compounds was characterized by a more pronounced suppressive effect and was statistically different from those observed for PFBuS and PFHxS (P≤0.01). In fact, PFOS and PFDA were the most affected by ME (mean values equal to -23.7% and -21.1%, respectively), whereas PFBuS and PFHxS the less influenced by the matrix (mean values equal to -6.1% and -5.5%, respectively). It should also be noted that the ME distribution of PFOS was significantly different (P=0.018) from that of PFOA, as well.

LDA has been carried out on the whole ME dataset in order to verify if suppressive or enhancement effects due to the matrix is a parameter actually suitable for correctly categorizing a certain sample, according to the classification given in **Table S2** (i.e. DW, GW, SW, $WWTP_{IN}$ and $WWTP_{OUT}$). The squared distance between groups and the summary of the results obtained in fitted and cross-validated (leave-one-out method) classification are illustrated in **Table 2** and **Table 3**, respectively.

As shown in **Table 2**, the highest between-group distance was observed by comparing the SW and the WWTP groups (23.4 and 21.7 for $WWTP_{IN}$ and $WWTP_{OUT}$, respectively). SW group was also quite well-separated from DW (distance of 13.0) and GW (distance of 17.6) ones. Accordingly, SW samples were correctly classified in 11 out of 13 cases, both in fitting and in cross-validation (**Table 3**), with probability levels that, in the worst case, were 98% and 95%, respectively, thus evidencing the robustness of this classification. It should also be remarked that the two misclassified samples (i.e. SW-3 and SW-13) were however attributed to GW and DW, without any misleading attribution to the wastewater category.

DW and GW groups were characterized by very low between-group squared distance; a low distance was also observed by comparing these groups with the $WWTP_{OUT}$ one (**Table 2**). Accordingly, some DW and GW samples were mutually misclassified, as well as erroneously attributed to the $WWTP_{OUT}$ group, especially in cross-validation (**Table 3**). However, it is remarkable that no erroneous attribution to the $WWTP_{IN}$ group was generated by the LDA model.

Interesting results were found for the $WWTP_{IN}$ and $WWTP_{OUT}$ groups, which were very low-separated (**Table 2**). The samples of these groups were correctly classified in approximately 70% and 50% of the cases, for fitting and cross-validation, respectively (**Table 3**). It should be however underlined, that most of misclassified samples, with the only exceptions of $WWTP_{OUT}$-7 and $WWTP_{OUT}$-9, were identified as WWTP samples. In almost all these cases, fitting and cross-validation probabilities of attribution $\geq$ 80% were obtained. $WWTP_{OUT}$-7 was wrongly attributed to the GW group, probably as the result of the high dilution rate due to a strong run-off rain event, which occurred during few days before the collection of this sample. As regards $WWTP_{OUT}$-9, it should be noted that this facility was the only one treating wastewater almost exclusively deriving from domestic activities, differently from all the other treatment plants, which receive a significant contribution of industrial origin.

The LDA results highlighted the contribution of the sample origin to the ME observed during PFAA analysis by LC-MS/MS. Accordingly, PLS regression with leave-one-out cross-validation, was attempted on the whole sample dataset, adopting ME as response variable. Conductivity, hardness and COD measured in each water sample were used as sample dependent predictors of the PLS regression (3-variable models), due to the fact that these parameters are routinely determined

in laboratories of potabilization facilities and WWTPs and could be usefully adopted for avoiding an over-application of time-consuming and expensive approaches such as the standard addition method and the isotope dilution technique.

PLS failed to correctly fit and cross-validate the experimental ME values, when the whole sample dataset was used for the regression. The inclusion in the model of a further variable representing the signal area at the retention time of target analytes under negative ionization (4-variable models) did not improve significantly the model.

Better results were achieved when PLS regression was separately applied to the WWTP, DW and especially GW sample categories. As reported in **Table S4** of the Supplementary material section, which illustrates the parameters of the 3-variable and 4-variable fitting and cross-validation models, for these sample categories statistically significant ($P \leq 0.1$) fitting models were obtained for most analytes. Moreover, in some cases the cross-validation models were found to be significant, even though they provided low prediction powers. The 4-variable models were associated to better fittings and predictions, compared to those based on 3 variables, evidencing the role of the signal area at the retention time of target analytes for explaining ME data. Even though the PLS approach resulted only in an approximate screening, due to the low prediction powers of the models, it is interesting to note that for most analytes in most samples, the fitted and cross-validated values were such as to correctly distinguish between |ME| higher than 20% or below this limit. As an example, the experimental, fitted and cross-validated ME values found for PFOA and PFOS in DW, GW and WW samples are plotted in **Fig. 2**. As illustrated in **Table S4**, for PFOA the prediction model was found to be statistically not significant, mainly due to the results obtained for the sample DW-8, while for the other samples a quite good agreement was observed between experimental and fitted/cross-validated data (**Fig. 2A**). In the same DW category, the agreement was more significant for PFOS, allowing to correctly identify the samples characterized by absolute values of ME much higher than 20%, with the main exception of sample DW-1 (**Fig. 2B**). In the GW category the percentage of correct attribution of absolute ME was even greater. In particular, only in the samples GW-4 and GW-6 a strong underestimation of ME was provided by the model for PFOA (**Fig. 2C**), whereas for PFOS all samples had a good estimation (**Fig. 2D**). Quite good agreements between experimental and fitted/cross-validated ME data were also observed in WW samples for PFOA (**Fig. 2E**) and PFOS (**Fig. 2F**), the latter even characterized by a statistically not significant prediction model. The main errors were indeed observed for PFOS in WW-6 and WW-8 samples, for which a strong suppression of the signal should be observed according to the prediction model, while the experimental ME was around 20%.

3.3 Comparison with previously published high-throughput methods

Analytical methods must be characterized by high precision, selectivity and sensitivity, in order to achieve reliable results at ultra-trace concentration levels, requiring at the same time the minimum sample preparation and analysis time (high-throughput methods). The achievement of all these requirements is necessary for the development and optimization of analytical methods suitable to be applied to the PFAA monitoring within various environmental contexts, such as the evaluation of their removal efficiency in WWTPs and the entity of their release into the environment, as well as the assessment of their actual concentrations in drinking water, thus obtaining information on the human exposure towards these micropollutants.

Table 4 illustrates the main characteristics of the analytical method herein proposed in comparison with those provided by elsewhere published high-throughput methods using the same approach (i.e. DI-LC-MS/MS) or other sample preparation techniques (i.e. online SPE-LC-MS/MS and in-tube SPME-LC-MS/MS) for the analysis of PFAAs in water matrices.

Our method represented a general improvement in terms of sensitivity in comparison with previously published online SPE-LC-MS/MS and in-tube SPME LC-MS/MS methods. The difference in sensitivity was particularly remarkable when the method proposed herein was compared with that of Saito and co-workers [39], which was limited to PFOA and PFOS, and above all, of Gosetti et al. [24], which however had the advantages to be very rapid (about 7 min. per sample) and to provide negligible matrix effects, at least in the three river samples investigated. Moreover, the online SPE-LC-MS/MS approach for the analysis of perfluorinated compounds was frequently associated with very high intra-day and above all inter-day variations, in most cases much higher than 10%. For instance, for PFOS, Llorca et al. [23] reported inter-day RSD equal to 26%, whereas Castiglioni and colleagues [40] found intra-day and inter-day RSD as high as 49% and 48%, respectively. It should also be noted that in this latter study, which involved the analysis of WWTP influents and effluents, river and groundwater samples, as well as drinking waters, a matrix effect extent similar to that observed in our study was reported, notwithstanding the presence of the washing step of SPE sorbent before LC-MS/MS determination of PFAAs [40].

Furdui et al. [3] proposed a very fast DI-LC-MS/MS method (4 min. per sample) for the determination in lake waters of nine perfluorinated compounds, including a group of six analytes (i.e. PFHxS, PFHpA, PFOA, PFOS, PFNA and PFDA) in common with target compounds of this study. MQLs reported by Furdui and co-workers were much higher to those achieved herein (**Table 4**). The very short analysis time was mainly due to the use of an isocratic elution with buffered CH_3OH/H_2O 80/20 (v/v), thus avoiding the time-consuming column re-equilibration before a new injection. However, the isocratic elution with high organic solvent percentages is not compatible

with the determination of short-chain PFAAs (i.e. PFBuS, PFPeA and PFHxA), which are eluted with the dead volume. Furthermore, the high eluting strength of the mobile phase results in low retention factors ($k \leq 2.1$ for PFAAs with carbon chain lower than 10 carbon atoms) and may translate in strong ME, especially when no sample preparation is performed [41]. Actually, a strong suppressive ME was reported by Furdui and colleagues for PFOS (mean value and standard deviation of the ME in various lake water samples: -70±15%). It should also be noted that the elution window of PFOS (the fourth eluting compound) was adjacent to the chromatographic band of polar unretained matrix constituents (i.e. salts and highly polar compounds), which are addressed to act as ‘‘endogenous suppressors’’ [42]. It should also be underlined that the isocratic elution does not provide an effective cleaning of the chromatographic column from the more retained matrix components, thus shortening the life of the column itself.

The DI-LC-MS/MS approach was also employed by Schultz et al. [43] for the analysis of perfluoroalkyl sulfonates, fluorotelomer sulfonates, perfluorocarboxylates, and selected fluorinated alkyl sulfonamides in wastewaters. This method was characterized by analysis time and sensitivity comparable to the ones achieved in our method. However, as reported by Schultz and co-workers, background contamination developed periodically within the instrument as detected in solvent blanks, probably due to the very high sample volume injected in the analytical column. In this regard, it is very surprising that no ME was found during the processing of effluent and even influent wastewater samples. In fact, the ESI-LC-MS/MS analysis of PFAAs in water samples usually involves the occurrence of high signal suppression/enhancement phenomena [3, 40]

3.4 Method application to real samples

Real samples were analysed in accordance with the identification criteria reported in the paragraph 2.3. As an example, **Fig. 3** illustrates the overlapped quantifier and qualifier MRM transitions of PFOA in the $WWTP_{IN}$-1 (**Fig. 3B**), $WWTP_{OUT}$-1 (**Fig. 3C**) and $WWTP_{OUT}$-10 (**Fig. 3D**) samples, together with that of PFOS in $WWTP_{OUT}$-1 (**Fig. 3F**) sample, in comparison with the corresponding standard solutions in Milli-Q water (**Figs. 3A** and **3E**).

Table 5 summarizes the concentrations found for target PFAAs in all the DW, GW, SW, $WWTP_{IN}$ and $WWTP_{OUT}$ samples previously investigated for ME (**Table S3**). Target compounds detected in between MDL and MQL or not detected in real samples were reported as below MQL_{sample} or below MDL_{sample}, respectively. MQL_{sample} and MDL_{sample} were calculated using the equations reported below:

$$\mathrm{MDL_{sample}} = \frac{\mathrm{MDL} \cdot 100}{100 + \mathrm{ME\%}} \qquad \mathrm{MQL_{sample}} = \frac{\mathrm{MQL} \cdot 100}{100 + \mathrm{ME\%}}$$

where ME% are the values of matrix effect determined in each sample and reported in **Table S3**.

This study was not designed for the investigation of occurrence and fate of perfluorinated compounds in the different water matrices investigated. However, many kind of water samples were analysed in this study and therefore it is interesting to evaluate the occurrence of target analytes in these samples, as well as to compare the data obtained herein with those found elsewhere. As a general finding, concentrations of carboxylates were higher than those of sulphonates. More in detail, PFPeA and above all PFOA were by far the most abundant perfluorinated compounds in water samples. The prevalence of PFOA is a result generally reported in literature. Furthermore, various recent European studies reported high PFAA concentrations, mainly with short carbon chain [44]. As expected, PFAAs were much less abundant in environmental waters than in wastewaters. In these latter samples concentrations as high as tens of ng L^{-1} of PFBuS, PFPeA, PFHxA, PFHpA and PFOA were observed in some cases. The comparative evaluation of PFAA concentrations in correlated WWTP influent and effluent 24-h composite samples (e.g. $WWTP_{IN}$-1 vs. $WWTP_{OUT}$-1 or $WWTP_{IN}$-2 vs. $WWTP_{OUT}$-5, see **Table S2**) clearly evidenced the recalcitrant behaviour to biological treatment of these compounds. PFAAs showed even an increasing trend during the treatment, in agreement with findings elsewhere observed by different authors [44]. Conversely, as shown in **Table 5**, the use of O_3-based advanced oxidation and activated carbon treatments were effective for the reduction of PFAA concentrations (see $WWTP_{OUT}$-1 vs. $WWTP_{OUT}$-2 vs. $WWTP_{OUT}$-3). The effectiveness of PFAA adsorption on activated carbon was also clearly highlighted by the comparison of PFAA occurrence in groundwater before and after the potabilization treatment. In fact, DW-6, which underwent an activated carbon treatment, did not show any significant residual PFAA contamination in comparison with GW-7, from which it derived. On the contrary, the counter-current air pumping together with the sand filtration treatments were not compatible with PFAA removal, as clearly shown by the comparison of GW-9 and DW-2 samples.

A higher number of PFAA positive samples was found in groundwater than in surface water, probably due to the much lower turn-over rate of the former compared to the latter, together with the high environmental persistence of these compounds. In this regard, the high PFOS concentration found in GW-8 (i.e. 2.5 ng L^{-1}, see **Table 5**), which exceeded the European EQS (0.65 ng L^{-1}) for about four times, should be underlined. It is remarkable that in the samples collected along the Arno river (the most important river of Tuscany and one of the biggest of Italy) the PFAA contamination was very low. More in detail, only in SW-7 two perfluorinated compounds were determined at concentrations higher than MQLs, probably due to the contribution of the WWTP effluent from an important leather industrial district, which enters the Arno river just before this sampling station.

PFOA and PFOS concentrations in DW samples were found to be much lower than the limits recommended by the Italian Health Institute (500 ng L^{-1} and 30 ng L^{-1} for PFOA and PFOS, respectively) and USEPA (400 ng L^{-1} and 200 ng L^{-1}).

4 Conclusions

This study demonstrates the applicability of the high-throughput DI–LC–MS/MS analytical approach for the identification and quantitative determination of PFAAs in a wide range of water matrices, with sensitivities in the sub-ng L^{-1} range, intra-day and inter-day RSD lower than 4.5% and 6.0%, respectively. These performances were generally higher than those recently achieved with on-line SPE–LC–MS/MS, in-tube SPME–LC–MS/MS and DI–LC–MS/MS methods.

Absolute values of ME herein observed were frequently lower than 20% and however below 50% with very few exceptions. The application of LDA on the whole ME dataset identified according to the classification given in **Table S2** (i.e. DW, GW, SW, $WWTP_{IN}$ and $WWTP_{OUT}$) highlighted the significant contribution of the sample origin to the ME. Hence, PLS and leave-one-out cross-validation were performed in order to interpret and predict the signal suppression or enhancement phenomena as a function of conductivity, hardness and chemical oxygen demand of water samples as well as background chromatographic area. Even though the PLS approach gave rise to models characterized by low prediction powers, for most analytes in most samples, the fitted and cross-validated values were such as to correctly distinguish between |ME| higher than 20% or below this limit. Accordingly, the possible use of this chemometric tool for avoiding the over-application of time-consuming and expensive approaches, such as the standard addition method and the isotope dilution technique, should be further investigated.

The application of this DI–LC–MS/MS to the quantification of target PFAAs in real samples provided for the first time data regarding the occurrence of perfluorinated compounds in Tuscany water samples of various origin. Although our study was not designed as an environmental investigation, many kind of 24-hour composite water samples were analysed herein, thus providing interesting information. Among them, PFOA and PFOS concentrations in DW samples were found in all cases much lower than the limits recommended by the Italian Health Institute. Furthermore, the ineffectiveness of activated sludge treatments for the removal of PFAAs was observed, thus highlighting the possible role of biological WWTPs in the punctual release of perfluorinated contaminants in the receiving water bodies, when tertiary treatment stages based on chemical oxidation or activated carbon are not implemented.

5 Acknowledgement

The present work was funded by the ERANETMED network funded by the European Commission's 7th Framework Programme, within the project "Assessing the chemical/microbiological contamination and productivity in the agricultural production chain of model fruit species grown under irrigation with different kinds of reclaimed wastewater" (project ERANETMED_WATER-13-069).

6 References

[1] B. Liu, H. Zhang, L. Xie, J. Li, X. Wang, L. Zhao, Y. Wang, B. Yang, Spatial distribution and partition of perfluoroalkyl acids (PFAAs) in rivers of the Pearl River Delta, southern China, Science of The Total Environment 524 (2015) 1-7.
[2] S. Valsecchi, M. Rusconi, M. Mazzoni, G. Viviano, R. Pagnotta, C. Zaghi, G. Serrini, S. Polesello, Occurrence and sources of perfluoroalkyl acids in Italian river basins, Chemosphere 129 (2015) 126-134.
[3] V.I. Furdui, P.W. Crozier, E.J. Reiner, S.A. Mabury, Trace level determination of perfluorinated compounds in water by direct injection, Chemosphere 73(1, Supplement) (2008) S24-S30.
[4] Y.-C. Lin, W.W.-P. Lai, H.-h. Tung, A.Y.-C. Lin, Occurrence of pharmaceuticals, hormones, and perfluorinated compounds in groundwater in Taiwan, Environ. Monit. Assess. 187(5) (2015) 256 (19 pages).
[5] C. Eschauzier, K.J. Raat, P.J. Stuyfzand, P. De Voogt, Perfluorinated alkylated acids in groundwater and drinking water: Identification, origin and mobility, Science of The Total Environment 458-460 (2013) 477-485.
[6] K. Kuroda, M. Murakami, K. Oguma, H. Takada, S. Takizawa, Investigating sources and pathways of perfluoroalkyl acids (PFAAs) in aquifers in Tokyo using multiple tracers, Science of the Total Environment 488-489 (2014) 51-60.
[7] G.B. Post, P.D. Cohn, K.R. Cooper, Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature, Environmental Research 116 (2012) 93-117.
[8] L. Ahrens, Z. Xie, R. Ebinghaus, Distribution of perfluoroalkyl compounds in seawater from Northern Europe, Atlantic Ocean, and Southern Ocean, Chemosphere 78(8) (2010) 1011-1016.
[9] J.M. Armitage, M. MacLeod, I.T. Cousins, Comparative Assessment of the Global Fate and Transport Pathways of Long-Chain Perfluorocarboxylic Acids (PFCAs) and Perfluorocarboxylates (PFCs) Emitted from Direct Sources, Environ. Sci. Technol. 43(15) (2009) 5830-5836.
[10] J.P. Benskin, D.C.G. Muir, B.F. Scott, C. Spencer, A.O. De Silva, H. Kylin, J.W. Martin, A. Morris, R. Lohmann, G. Tomy, B. Rosenberg, S. Taniyasu, N. Yamashita, Perfluoroalkyl Acids in the Atlantic and Canadian Arctic Oceans, Environ. Sci. Technol. 46(11) (2012) 5815-5823.
[11] European Commission, Directive 2006/122/EC of the European Parliament and of the Council of 12 December 2006 amending for the 30th time Council directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations (perfluorooctane sulfonates), Official Journal of the European Union, 27.12, L 372/32 (2006).
[12] US Environmental Protection Agency, PFOA Stewardship Program, (2006).
[13] European Parliament and Commission, Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy, Official Journal of the European Union (2013).
[14] European Chemicals Agency (ECHA), Inclusion of Substances of Very High Concern in the Candidate List, (2013).

[15] US Environmental Protection Agency, Provisional Health Advisories for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS), (2009).
[16] Istituto Superiore di Sanità, Prot 16/01/2014-0001584, (2014).
[17] X. Esparza, E. Moyano, J. de Boer, M.T. Galceran, S.P.J. van Leeuwen, Analysis of perfluorinated phosphonic acids and perfluorooctane sulfonic acid in water, sludge and sediment by LC–MS/MS, Talanta 86 (2011) 329-336.
[18] A. Jahnke, U. Berger, Trace analysis of per- and polyfluorinated alkyl substances in various matrices-How do current methods perform?, J. Chromatogr. A 1216(3) (2009) 410-421.
[19] R. Loos, G. Locoro, S. Contini, Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS2 analysis, Water Research 44(7) (2010) 2325-2335.
[20] S.T. Wolf, W.K. Reagen, Method and validation for the analysis of perfluorinated compounds in water by pre-sampling isotope dilution-direct injection-LC/MS/MS, Anal. Methods 5(10) (2013) 2444-2454.
[21] M. Mazzoni, M. Rusconi, S. Valsecchi, C.P. Martins, S. Polesello, An on-line solid phase extraction-liquid chromatography-tandem mass spectrometry method for the determination of perfluoroalkyl acids in drinking and surface waters, Journal of analytical methods in chemistry Volume 2015, Article ID 942016 (2015) (13 pages). doi: 10.1155/2015/942016.
[22] M. Takino, S. Daishima, T. Nakahara, Determination of perfluorooctane sulfonate in river water by liquid chromatography/atmospheric pressure photoionization mass spectrometry by automated on-line extraction using turbulent flow chromatography, Rapid Communications in Mass Spectrometry 17(5) (2003) 383-390.
[23] M. Llorca, M. Farré, Y. Picó, J. Müller, T.P. Knepper, D. Barceló, Analysis of perfluoroalkyl substances in waters from Germany and Spain, Sci. Total Environ. 431 (2012) 139-150.
[24] F. Gosetti, U. Chiuminatto, D. Zampieri, E. Mazzucco, E. Robotti, G. Calabrese, M.C. Gennaro, E. Marengo, Determination of perfluorochemicals in biological, environmental and food samples by an automated on-line solid phase extraction ultra high performance liquid chromatography tandem mass spectrometry method, Journal of Chromatography A 1217(50) (2010) 7864-7872.
[25] S. Bayen, X. Yi, E. Segovia, Z. Zhou, B.C. Kelly, Analysis of selected antibiotics in surface freshwater and seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry, Journal of Chromatography A 1338 (2014) 38-43.
[26] J.-D. Berset, R. Brenneisen, C. Mathieu, Analysis of llicit and illicit drugs in waste, surface and lake water samples using large volume direct injection high performance liquid chromatography – Electrospray tandem mass spectrometry (HPLC–MS/MS), Chemosphere 81(7) (2010) 859-866.
[27] T.S. Oliveira, M. Murphy, N. Mendola, V. Wong, D. Carlson, L. Waring, Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS, Sci. Total Environ. 518-519 (2015) 459-478.
[28] D. Shang, M. Kim, M. Haberl, A. Legzdins, Development of a rapid liquid chromatography tandem mass spectrometry method for screening of trace naphthenic acids in aqueous environments, Journal of Chromatography A 1278 (2013) 98-107.
[29] U.S. EPA, Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS), Revision 1.0, U.S. Environmental Protection Agency, Washington DC, EPA/600/R-09/149 (2009).
[30] J.A. Shoemaker, Development and multi-laboratory verification of US EPA method 538 for the analysis of drinking water contaminants by direct aqueous injection-LC/MS/MS, Analytical Methods 3(7) (2011) 1628-1636.
[31] L. Ciofi, C. Ancillotti, U. Chiuminatto, D. Fibbi, L. Checchini, S. Orlandini, M. Del Bubba, Liquid chromatographic–tandem mass spectrometric method for the simultaneous determination of

alkylphenols polyethoxylates, alkylphenoxy carboxylates and alkylphenols in wastewater and surface-water, Journal of Chromatography A 1362 (2014) 75-88.
[32] L. Ciofi, D. Fibbi, U. Chiuminatto, E. Coppini, L. Checchini, M. Del Bubba, Fully-automated on-line solid phase extraction coupled to high-performance liquid chromatography–tandem mass spectrometric analysis at sub-ng/L levels of selected estrogens in surface water and wastewater, Journal of Chromatography A 1283 (2013) 53-61.
[33] C. vom Eyser, K. Palmu, R. Otterpohl, T.C. Schmidt, J. Tuerk, Determination of pharmaceuticals in sewage sludge and biochar from hydrothermal carbonization using different quantification approaches and matrix effect studies, Analytical and Bioanalytical Chemistry 407(3) (2015) 821-830.
[34] Z. Sosa-Ferrera, C. Mahugo-Santana, J.J. Santana-Rodríguez, New developments in liquid chromatography mass spectrometry for the determination of micropollutants, Chromatography Research International 2012 (2012). Article ID 748989, 18 pages. doi:10.1155/2012/748989.
[35] European Commision, COMMISSION DECISION of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities (2002).
[36] V.C. Fernandes, V.F. Domingues, N. Mateus, C. Delerue-Matos, Multiresidue pesticides analysis in soils using modified QuEChERS with disposable pipette extraction and dispersive solid-phase extraction, Journal of Separation Science 36(2) (2013) 376-382.
[37] A.G. Frenich, R. Romero-González, M.L. Gómez-Pérez, J.L.M. Vidal, Multi-mycotoxin analysis in eggs using a QuEChERS-based extraction procedure and ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry, Journal of Chromatography A 1218(28) (2011) 4349-4356.
[38] M. Mei, Z.-X. Du, Y. Cen, QuEChERS-Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry for Determination of Five Currently Used Herbicides, Chinese Journal of Analytical Chemistry 39(11) (2011) 1659-1664.
[39] K. Saito, E. Uemura, A. Ishizaki, H. Kataoka, Determination of perfluorooctanoic acid and perfluorooctane sulfonate by automated in-tube solid-phase microextraction coupled with liquid chromatography–mass spectrometry, Analytica chimica acta 658(2) (2010) 141-146.
[40] S. Castiglioni, S. Valsecchi, S. Polesello, M. Rusconi, M. Melis, M. Palmiotto, A. Manenti, E. Davoli, E. Zuccato, Sources and fate of perfluorinated compounds in the aqueous environment and in drinking water of a highly urbanized and industrialized area in Italy, Journal of Hazardous Materials 282 (2015) 51-60.
[41] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Matrix Effect in Quantitative LC/MS/MS Analyses of Biological Fluids: A Method for Determination of Finasteride in Human Plasma at Picogram Per Milliliter Concentrations, Analytical Chemistry 70(5) (1998) 882-889.
[42] H. Trufelli, P. Palma, G. Famiglini, A. Cappiello, An overview of matrix effects in liquid chromatography–mass spectrometry, Mass spectrometry reviews 30(3) (2011) 491-509.
[43] M.M. Schultz, D.F. Barofsky, J.A. Field, Quantitative determination of fluorinated alkyl substances by large-volume-injection liquid chromatography tandem mass spectrometry characterization of municipal wastewaters, Environmental science & technology 40(1) (2006) 289-295.
[44] O.S. Arvaniti, A.S. Stasinakis, Review on the occurrence, fate and removal of perfluorinated compounds during wastewater treatment, Science of The Total Environment 524–525 (2015) 81-92.

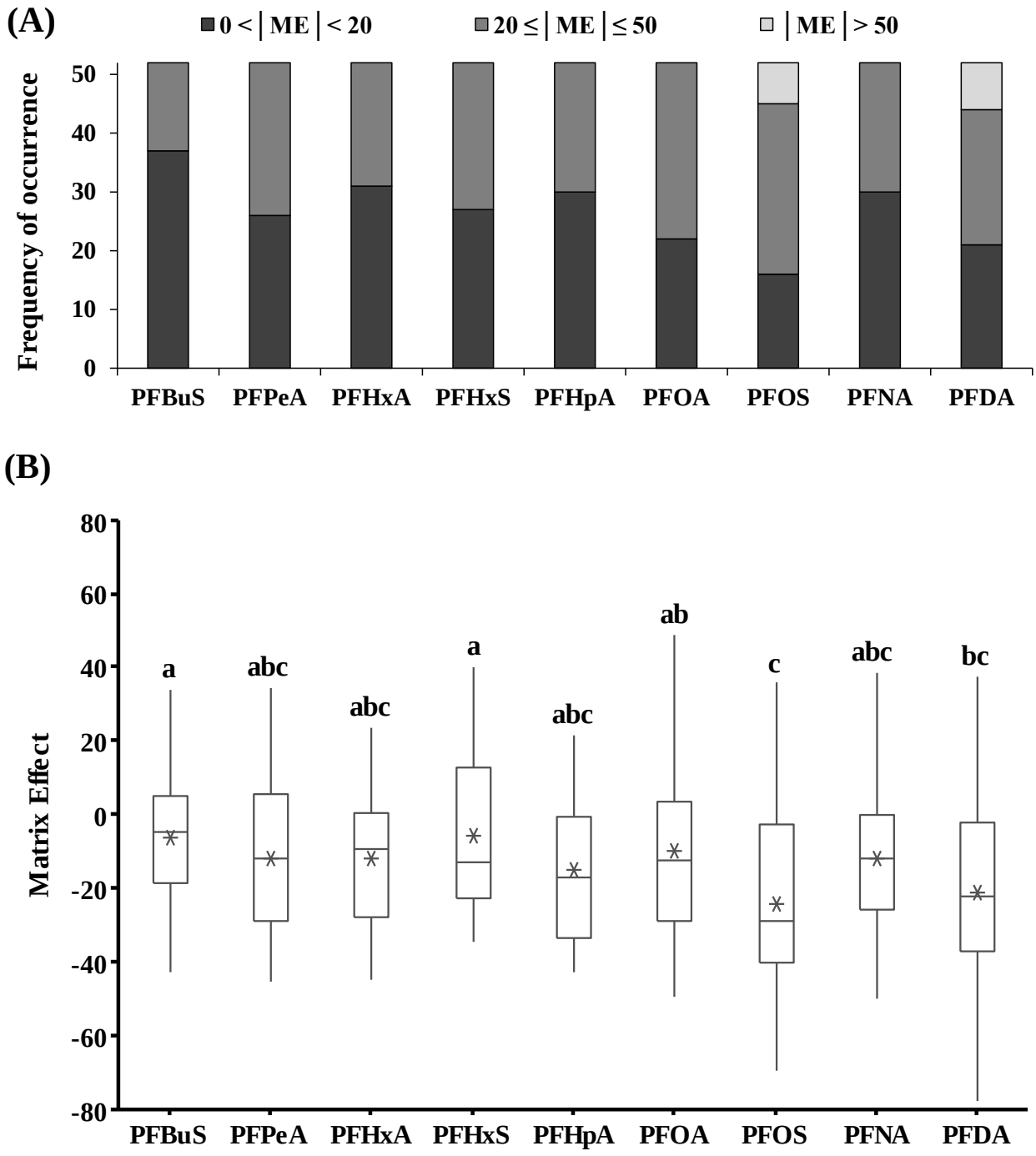

Figure 1 – Graphical summary of the matrix effect dataset. (A) Histogram reporting the frequency of occurrence of matrix effect (ME) within the ranges $|ME|<20$, $20\leq|ME|\leq50$, $|ME|>50$. (B) Boxplot of the distribution of ME dataset determined for each investigated PFAA: each boxplot represents the interquartile range (75% of the $|ME|$ data are less than or equal to the top value of the box and 25% of the $|ME|$ data are less than or equal to the bottom value of the box); upper and lower whiskers refer to the maximum and minimum data point, respectively; the line within the box represents the median of the data; the asterisk represents the mean value of the $|ME|$. The mean values with at least one letter in common are not statistically different according to the Tukey test ($P<0.05$). See paragraph 2.1 for the meaning of the compound acronyms.

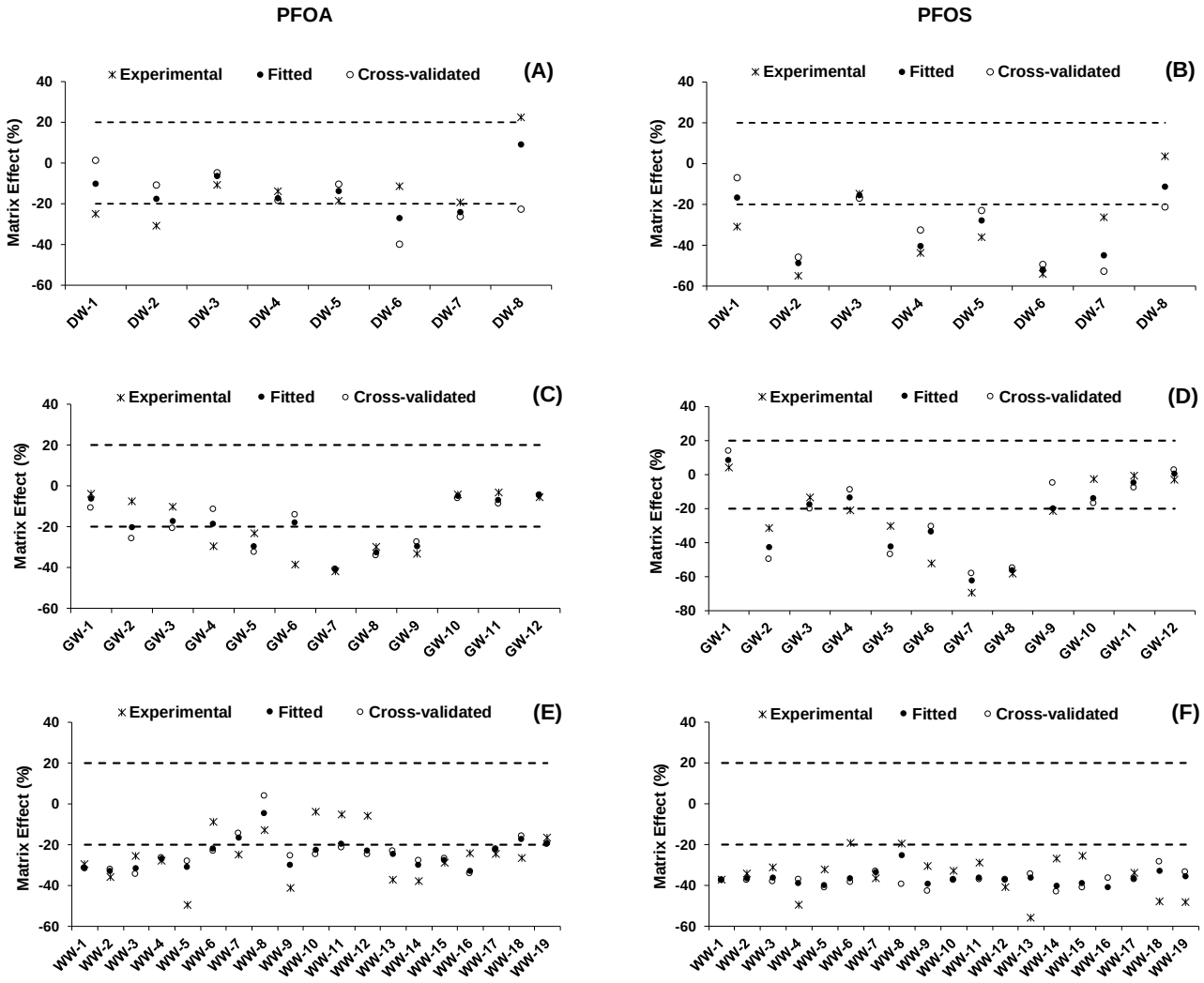

Figure 2 – Fitted and cross-validated matrix effect values determined for PFOA and PFOS in drinking water (A-B), groundwater (C-D) and wastewater (E-F) sample categories using PLS regression, in comparison with experimental data.

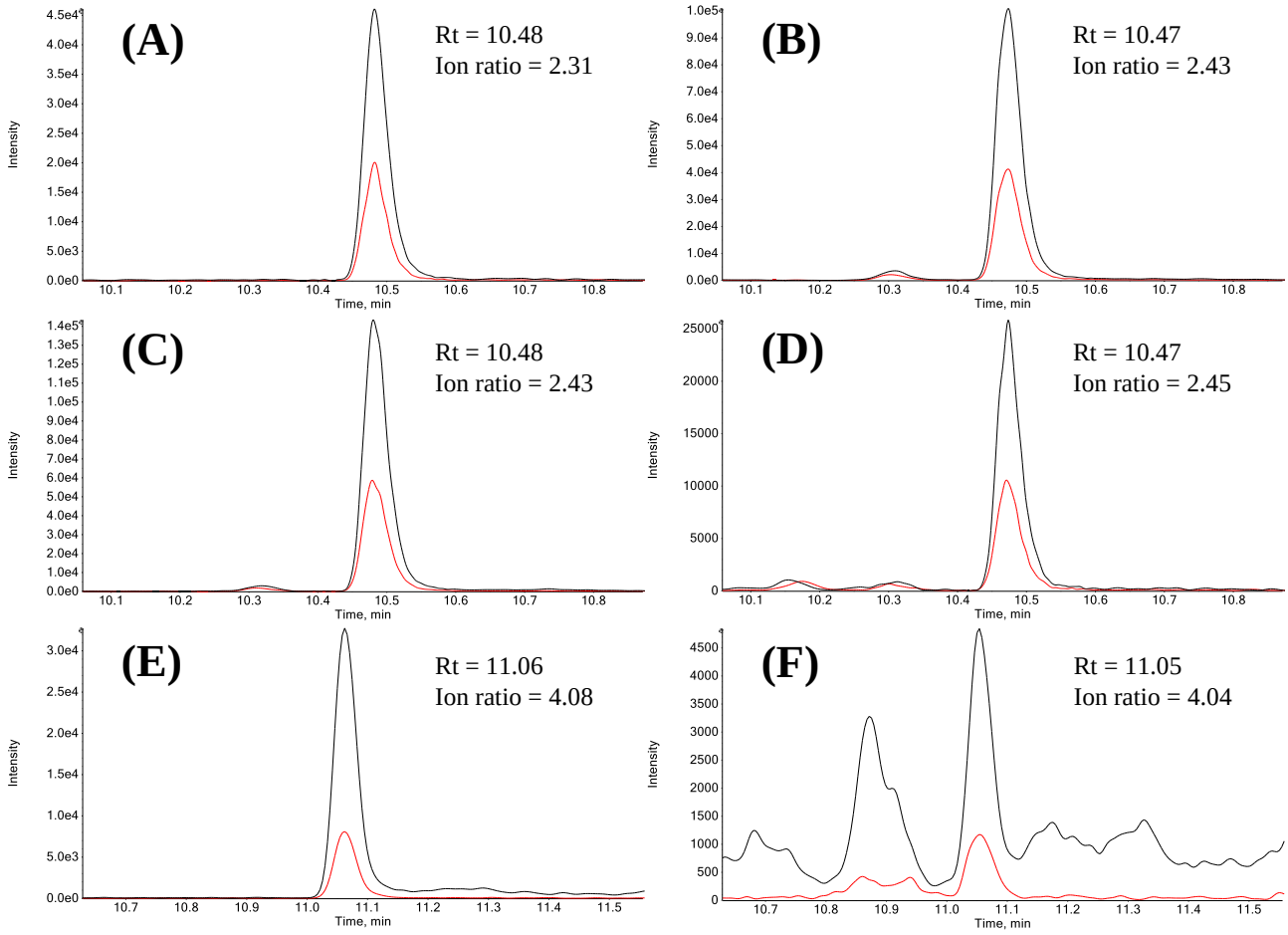

Figure 3 – Overlapped MRM quantifier (*black line*) and qualifier (*red line*) transitions, retention time (Rt) and ion ratio of: (A) PFOA, Milli-Q water (5 ng L^{-1}); (B) PFOA, $WWTP_{IN}$-1 (30.4 ng L^{-1}); (C) PFOA, $WWTP_{OUT}$-1 (66.4 ng L^{-1}); (D) PFOA, $WWTP_{OUT}$-10 (4.9 ng L^{-1}): (E) PFOS, Milli-Q water (5 ng L^{-1}); (F) PFOS, $WWTP_{IN}$-1 (2.0 ng L^{-1}). See paragraph 2.1 for acronyms meaning.

Table 2 – Between-group squared distance, calculated by Linear Discriminant Analysis on the basis of values of matrix effects determined for the analysis of PFAAs on the sample reported in Table 1. Groups: drinking water (DW), ground water (GW), surface water (SW), wastewater treatment plant influent ($WWTP_{IN}$) and wastewater treatment plant effluent ($WWTP_{OUT}$).

	DW	GW	SW	$WWTP_{IN}$	$WWTP_{OUT}$
DW	0.0	2.9	13.0	11.4	7.1
GW	-	0.0	17.6	9.7	7.0
SW	-	-	0.0	23.4	21.7
$WWTP_{IN}$	-	-	-	0.0	2.0
$WWTP_{OUT}$	-	-	-	-	0.0

Table 3 – Fitting (FIT) and cross-validation (CV) results of the Linear Discriminant Analysis based on the values of matrix effects determined for the analysis of PFAAs on the samples reported in Table 1. Groups: drinking water (DW), ground water (GW), surface water (SW), wastewater treatment plant influent ($WWTP_{IN}$) and wastewater treatment plant effluent ($WWTP_{OUT}$).

Put into Group		True Group				
		DW	GW	SW	$WWTP_{IN}$	$WWTP_{OUT}$
DW	FIT	4	1	1	0	1
	CV	3	5	1	0	1
GW	FIT	2	9	1	0	1
	CV	2	5	1	0	1
SW	FIT	1	0	11	0	0
	CV	1	0	11	0	0
$WWTP_{IN}$	FIT	0	0	0	6	2
	CV	0	0	0	4	4
$WWTP_{OUT}$	FIT	1	2	0	2	7
	CV	2	2	0	4	5
Total Number of Samples		8	12	13	8	11
Correct Attributions	FIT	4	9	11	6	7
	CV	3	5	11	4	5
Percentage of Correct Attribution	FIT	50%	75%	85%	75%	64%
	CV	38%	42%	85%	50%	45%

Table 4 – Main characteristics of the analytical method herein proposed in comparison with those provided by elsewhere published high-throughput methods using the direct injection approach (i.e. DI-LC-MS/MS) or other sample preparation techniques (i.e. online SPE-LC-MS/MS and in-tube SPME-LC-MS/MS) for the analysis of PFAAs in water matrixes. See paragraph 2.1 for the meaning of compound acronyms.

Technique	Sample volume (mL)	Total analysis time (min)	MQL (ng L^{-1})									[Reference]
			PFBuS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA	
DI-LC-MS/MS	0.100	20.0	0.033	0.38	1.0	0.030	0.42	0.30	0.17	0.61	0.13	This study
DI-LC-MS/MS	0.100	4.0	n.i.[a]	n.i.[a]	n.i.[a]	1.4	1.6	1.3	0.50	1.2	0.80	[3]
DI-LC-MS/MS	0.500	20.0	0.70	n.i.[a]	0.50	0.80	0.50	0.50	0.50	0.50	0.50	[43]
Online SPE-LC-MS/MS	5.0	15.5	10	4.0	1.0	20	5.0	3.0	10	1.0	1.0	[40]
Online SPE-LC-MS/MS	5.0	16.25	8.2	38	50	0.90	17	2.8	1.3	6.3	8.0	[23]
Online SPE-LC-MS/MS	0.35	7.1	25	50	n.i.[a]	25	10	10	50	n.i.[a]	n.i.[a]	[24]
in-tube SPME LC-MS/MS	0.040	25.0	n.i.[a]	n.i.[a]	n.i.[a]	n.i.[a]	n.i.[a]	5.0	10.6	n.i.[a]	n.i.[a]	[39]

[a] n.i. = not investigated

Table 5 – Concentration values (ng L^{-1}) determined for each investigated compound in all the samples analysed in this study. Values higher than MQL_{sample} are reported in bold. See paragraph 2.1 for the meaning of compound acronyms.

Sample	PFBuS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA
DW-1	<0.021[a]	<0.17[a]	**3.3**	<0.016[a]	<0.22[a]	**4.4**	<0.10[a]	<0.35[a]	<0.065[a]
DW-2	<0.015[a]	<0.17[a]	**3.5**	<0.015[a]	**4.5**	**12.8**	<0.16[a]	<0.93[b]	<0.10[a]
DW-3	<0.015[a]	<0.17[a]	<0.46[a]	<0.015[a]	<0.21[a]	<0.15[a]	<0.084[a]	<0.33[a]	<0.058[a]
DW-4	<0.014[a]	<0.15[a]	<1.0[b]	<0.015[a]	**2.5**	**7.2**	<0.13[a]	**3.2**	<0.070[a]
DW-5	<0.016[a]	<0.24[a]	<1.2[b]	<0.016[a]	<0.24[a]	<0.16[a]	<0.11[a]	<0.31[a]	<0.078[a]
DW-6	<0.016[a]	<0.21[a]	<1.2[b]	<0.015[a]	<0.22[a]	<0.15[a]	<0.16[a]	<0.42[a]	<0.11[a]
DW-7	<0.015[a]	<0.20[a]	<1.3[b]	<0.036[b]	<0.58[b]	<0.52[b]	<0.10[a]	<0.34[a]	<0.077[a]
DW-8	<0.015[a]	<0.15[a]	<0.47[a]	<0.011[a]	<0.16[a]	<0.11[a]	<0.069[a]	<0.23[a]	<0.053[a]
GW-1	<0.014[a]	<0.17[a]	<1.1[b]	<0.014[a]	<0.19[a]	<0.44[b]	<0.068[a]	<0.28[a]	<0.058[a]
GW-2	<0.034[b]	<0.18[a]	<1.2[b]	<0.015[a]	<0.22[a]	**4.1**	<0.10[a]	<0.28[a]	<0.065[a]
GW-3	<0.015[a]	<0.19[a]	<1.3[b]	<0.016[a]	<0.21[a]	<0.47[b]	<0.082[a]	<0.28[a]	<0.062[a]
GW-4	<0.016[a]	<0.21[a]	<0.61[a]	<0.018[a]	<0.21[a]	<0.60[b]	<0.090[a]	<0.29[a]	<0.11[a]
GW-5	<0.016[a]	<0.21[a]	<1.2[b]	<0.015[a]	<0.22[a]	<0.55[b]	<0.10[a]	<0.33[a]	<0.064[a]
GW-6	<0.020[a]	<0.26[a]	**3.9**	**1.8**	<0.28[a]	**4.5**	<0.15[a]	<0.46[a]	<0.12[a]
GW-7	<0.019[a]	<0.24[a]	**7.0**	**1.0**	<0.70[b]	**20.8**	<0.55[b]	**3.2**	<0.58[b]
GW-8	<0.040[b]	<0.64[b]	<1.4[b]	<0.016[a]	**4.2**	<0.60[b]	**2.5**	**1.3**	**2.3**
GW-9	<0.012[a]	<0.15[a]	**3.2**	**1.8**	**3.4**	**14.4**	<0.090[a]	**4.8**	<0.064[a]
GW-10	<0.034[b]	<0.17[a]	<0.47[a]	<0.014[a]	<0.18[a]	<0.44[b]	<0.073[a]	<0.27[a]	<0.055[a]
GW-11	<0.033[b]	<0.16[a]	<0.45[a]	<0.013[a]	<0.18[a]	<0.14[a]	<0.072[a]	<0.26[a]	<0.056[a]
GW-12	<0.034[b]	<0.16[a]	<0.47[a]	**0.8**	<0.18[a]	<0.45[b]	<0.073[a]	<0.27[a]	<0.051[a]
SW-1	<0.012[a]	<0.13[a]	<0.41[a]	<0.010[a]	<0.15[a]	<0.10[a]	<0.068[a]	<0.23[a]	<0.054[a]
SW-2	<0.012[a]	<0.13[a]	<0.40[a]	<0.010[a]	<0.14[a]	<0.10[a]	<0.059[a]	<0.21[a]	<0.048[a]
SW-3	<0.013[a]	<0.14[a]	<0.40[a]	<0.011[a]	<0.17[a]	<0.40[b]	<0.10[a]	<0.25[a]	<0.061[a]
SW-4	<0.013[a]	<0.13[a]	<0.40[a]	<0.010[a]	<0.15[a]	<0.10[a]	<0.068[a]	<0.21[a]	<0.052[a]
SW-5	<0.013[a]	<0.13[a]	<0.9[b]	<0.010[a]	<0.16[a]	<0.32[b]	<0.061[a]	<0.22[a]	<0.042[a]
SW-6	<0.013[a]	<0.15[a]	<1.0[b]	<0.026[b]	<0.16[a]	<0.34[b]	<0.058[a]	<0.22[a]	<0.043[a]
SW-7	**2.8**	<0.13[a]	<0.8[b]	<0.010[a]	<0.15[a]	<0.31[b]	<0.052[a]	<0.20[a]	**2.3**
SW-8	**4.7**	<0.15[a]	**2.7**	<0.010[a]	<0.40[b]	**3.6**	<0.21[b]	<0.25[a]	<0.086[a]
SW-9	<0.014[a]	<0.37[b]	**3.2**	<0.010[a]	<0.39[b]	**4.0**	<0.24[b]	**2.7**	<0.11[a]
SW-10	<0.013[a]	<0.15[a]	<0.45[a]	<0.010[a]	<0.16[a]	<0.11[a]	<0.063[a]	<0.23[a]	<0.049[a]
SW-11	<0.010[a]	<0.12[a]	<0.36[a]	<0.010[a]	<0.17[a]	<0.089[a]	<0.064[a]	<0.19[a]	<0.045[a]
SW-12	<0.013[a]	<0.14[a]	<0.44[a]	<0.026[b]	<0.40[b]	<0.34[b]	<0.31[b]	<0.70[b]	<0.11[a]
SW-13	<0.033[b]	<0.16[a]	<0.48[a]	<0.013[a]	<0.18[a]	<0.42[b]	<0.072[a]	<0.27[a]	<0.055[a]
$WWTP_{IN}$-1	<0.047[b]	**24.4**	**22.0**	**9.1**	**13.9**	**30.4**	**2.0**	**6.2**	**10.6**
$WWTP_{IN}$-2	**1.6**	**29.5**	**24.4**	<0.018[a]	**22.7**	**43.1**	<0.26[b]	**7.8**	**4.8**
$WWTP_{IN}$-3	<0.019[a]	<0.22[a]	**9.6**	**4.1**	**7.8**	**24.4**	<0.25[b]	<0.26[a]	**8.6**
$WWTP_{IN}$-4	<0.019[a]	<0.24[a]	<0.80[a]	<0.018[a]	<0.24[a]	<0.18[a]	<0.14[a]	<0.34[a]	<0.083[a]
$WWTP_{IN}$-5	<0.017[a]	**17.5**	**22.0**	<0.017[a]	**8.6**	**18.1**	<0.11[a]	<0.31[a]	**4.8**
$WWTP_{IN}$-6	<0.013[a]	<0.43[b]	**3.9**	<0.012[a]	<0.49[b]	**6.2**	<0.088[a]	<0.27[a]	<0.066[a]
$WWTP_{IN}$-7	<0.016[a]	<0.62[b]	<0.57[a]	<0.035[b]	<0.62[b]	<0.56[b]	<0.27[b]	<0.85[b]	<0.097[a]
$WWTP_{IN}$-8	**3.3**	<0.48[b]	**6.3**	<0.013[a]	**5.5**	**16.9**	**2.3**	**3.2**	**4.9**
$WWTP_{OUT}$-1	<0.042[b]	**39.1**	**45.1**	<0.043[b]	**22.0**	**66.4**	**4.3**	**12.5**	**17.4**
$WWTP_{OUT}$-2	<0.013[a]	**27.5**	**11.9**	<0.012[a]	**9.2**	**21.7**	<0.11[a]	**1.4**	**1.5**
$WWTP_{OUT}$-3	<0.013[a]	**26.7**	**15.8**	<0.012[a]	**8.3**	**23.9**	<0.11[a]	**3.3**	<0.053[a]
$WWTP_{OUT}$-4	<0.013[a]	**18.7**	**6.0**	<0.012[a]	**4.7**	**11.8**	<0.12[a]	<0.63[b]	<0.086[a]
$WWTP_{OUT}$-5	**62.3**	**132.5**	**95.2**	**1.3**	**61.4**	**103.9**	<0.38[b]	**19.2**	**4.2**
$WWTP_{OUT}$-6	**4.4**	**20.3**	**14.9**	**1.0**	**11.6**	**37.5**	<0.23[b]	**3.8**	**1.5**
$WWTP_{OUT}$-7	<0.020[a]	<0.23[a]	<0.73[a]	<0.019[a]	<0.27[a]	<0.19[a]	<0.10[a]	<0.34[a]	<0.077[a]
$WWTP_{OUT}$-8	<0.021[a]	**98.2**	**60.4**	<0.019[a]	**28.2**	**61.9**	<0.21[a]	**23.0**	**6.4**
$WWTP_{OUT}$-9	**3.0**	<0.40[b]	**8.3**	**2.1**	**4.6**	**16.2**	<0.11[a]	<0.75[b]	<0.067[a]
$WWTP_{OUT}$-10	**0.073**	**85.8**	**3.5**	**1.3**	**1.2**	**4.9**	**4.2**	**1.4**	**1.8**
$WWTP_{OUT}$-11	**2.0**	<0.44[b]	**7.0**	<0.012[a]	**2.7**	**14.8**	<0.14[a]	<0.69[b]	<0.092[a]

[a] Method Detection Limit in real samples (MDL_{sample})

[b] Method Quantification Limit in real samples (MQL_{sample})

Table S2 – List of samples included in this study. DMS: degrees, minutes and seconds; $WWTP_{IN}$: influent of wastewater treatment plant; $WWTP_{OUT}$: effluent of wastewater treatment plant; SW: surface water; GW: groundwater; DW: drinking water; AS: activated sludge; MBR: membrane biological reactor.

Sample	DMS Coordinates	Notes
DW-1	43°45'44''N 11°17'32''E	Clariflocculation + sand filtration + activated carbon + disinfection
DW-2	43°43'40''N 10°57'14''E	Counter-current air pumping + sand filtration + disinfection
DW-3	43°45'46"N 11°17'46"E	As DW-1 + activated carbon + 0.2 µm filtration
DW-4	43°43'40''N 10°57'14''E	As DW-2 + activated carbon + 0.2 µm filtration
DW-5	43°51'12"N 11°03'13'E	Disinfection
DW-6	43°50'52"N 11°05'02"E	Activated carbon + sand/activated carbon mixed filtration + disinfection
DW-7	43°49'12''N 11°02'44''E	As DW-1 + activated carbon + 0.2 µm filtration
DW-8	43°23''45''N 11°36'10''E	Disinfection
GW-1	43°27'35''N 11°09'42''E	Phreatic layer, Bernino, Poggibonsi (Siena), mean depth 10 m
GW-2	43°43'41''N 10°55'43''E	Phreatic layer, Vinci (Florence), mean depth 15 m
GW-3	43°42'26''N 10°54'42''E	Phreatic layer, Empoli (Florence), mean depth 15 m
GW-4	43°43'00''N 10°54'04''E	Phreatic layer, Empoli (Florence), mean depth 15 m
GW-5	43°53'12"N 11°02'54"E	Phreatic layer, Lastruccia, Prato, mean depth 75 m
GW-6	43°51'12"N 11°03'13'E	Phreatic layer, Falda 1, Prato, mean depth 71 m
GW-7	43°50'52"N 11°05'02"E	Phreatic layer, Falda 2, Prato, mean depth 70 m
GW-8	43°51'25"N 11°05'17"E	Phreatic layer, Cafaggio (Prato), mean depth 61 m
GW-9	43°43'40''N 10°57'14''E	Phreatic layer, Arno Vecchio, Empoli (Florence) mean depth 17 m
GW-10	43°32'41''N 11°11'59''E	Phreatic layer, Certaldo (Florence), mean depth 10 m
GW-11	43°32'46''N 11°02'02''E	Phreatic layer, Certaldo (Florence), mean depth 10 m
GW-12	43°32'42''N 11°02'02''E	Phreatic layer, Certaldo (Florence), mean depth 10 m
SW-1	43°30'18''N 11°47'59''E	Arno river before the "Canale Maestro della Chiana" (Arezzo), receiving agricultural runoff and untreated urban WW
SW-2	43°30'16''N 11°47'17''E	Arno river after the "Canale Maestro della Chiana" (Arezzo)
SW-3	43°45'53''N 11°17'52''E	Arno river before entering in Florence
SW-4	43°46'43''N 11°06'37''E	Arno river after the discharge of the Florence WWTP
SW-5	43°46'23''N 11°05'35''E	Arno river after the confluence of the Bisenzio river
SW-6	43°43'18''N 10°52'60''E	Arno river after the city of Empoli (Florence)
SW-7	43°40'40''N 11°38'27''E	Arno river after receiving the WWTP effluent from the leather industrial district of Santa Croce (Pisa)
SW-8	43°42'29''N 10°22'30''E	Arno river in the proximity of the mouth (Pisa)
SW-9	43°46'58''N 11°06'03''E	Bisenzio river before the confluence with Arno river (Florence)
SW-10	43°45'51''N 10°20'37''E	Serchio river in the proximity of the mouth (Lucca)
SW-11	43°50'09''N 10°20'59''E	East area of the coastal lake "Massaciuccoli" (Lucca)
SW-12	43°50'07''N 10°18'32''E	West area of the coastal lake "Massaciuccoli" (Lucca)
SW-13	43°58'29''N 11°16'12''E	Central area of the artificial lake "Bilancino" (Florence)
$WWTP_{IN}$-1	43°50'48''N 11°04'47''E	Mixed industrial (mainly textile)-domestic WW
$WWTP_{IN}$-2	43°53'02''N 11°01'08''E	Landfill leachate, septic tank sewage, mixed industrial-domestic WW
$WWTP_{IN}$-3	43°57'04''N 11°07'45''E	Mixed industrial (mainly textile)-domestic WW
$WWTP_{IN}$-4	44°01'26''N 11°08'35''E	Mixed industrial (mainly textile)-domestic WW
$WWTP_{IN}$-5	43°58'37''N 11°07'35''E	Mixed industrial (mainly textile)-domestic WW
$WWTP_{IN}$-6	43°46'28''N 11°08'15''E	Domestic WW
$WWTP_{IN}$-7	43°37'60''N 11°27'50''E	Mixed industrial (pharmaceutical and engineering)-domestic WW
$WWTP_{IN}$-8	43°50'08''N 11°02'02''E	Mixed industrial (mainly textile)-domestic WW
$WWTP_{OUT}$-1	43°50'48''N 11°04'47''E	AS
$WWTP_{OUT}$-2	43°50'48''N 11°04'47''E	AS + O_3
$WWTP_{OUT}$-3	43°50'48''N 11°04'47''E	AS + O_3 + sand filters + activated carbon
$WWTP_{OUT}$-4	43°50'48''N 11°04'47''E	AS + O_3 + sand filters + dilution 1:2 (v/v) with river water
$WWTP_{OUT}$-5	43°53'02''N 11°01'08''E	Treatment of landfill leachate and septic tank sewage by MBR; treatment of the MBR effluent and the mixed industrial-domestic WW by AS + O_3
$WWTP_{OUT}$-6	43°57'04''N 11°07'45''E	AS
$WWTP_{OUT}$-7	44°01'26''N 11°08'35''E	AS
$WWTP_{OUT}$-8	43°58'37''N 11°07'35''E	AS
$WWTP_{OUT}$-9	43°46'28''N 11°08'15''E	AS
$WWTP_{OUT}$-10	43°37'60''N 11°27'50''E	AS
$WWTP_{OUT}$-11	43°50'08''N 11°02'02''E	AS

Table S3 – Values of matrix effect determined for each investigated compound in each sample analysed (see Table 1 for full details). The meaning of the compound acronyms is reported in the paragraph 2.1.

Sample	PFBuS	PFPeA	PFHxA	PFHxS	PFHpA	PFOA	PFOS	PFNA	PFDA
DW-1	-34.5	-7.7	-20.3	-22.2	-21.9	-25.0	-30.9	-25.9	-16.6
DW-2	-8.0	-9.1	-12.6	-12.1	-22.9	-30.8	-55.0	-34.4	-43.4
DW-3	-4.8	-8.5	-3.7	-15.2	-17.5	-10.8	-14.8	-22.9	-5.9
DW-4	0.5	7.7	-3.7	-13.1	-8.6	-13.9	-43.8	-30.8	-23.0
DW-5	-10.5	-34.6	-17.3	-19.2	-25.8	-18.6	-36.1	-17.5	-30.3
DW-6	-15.2	-25.1	-17.7	-16.0	-22.1	-11.4	-54.1	-39.3	-52.3
DW-7	-5.3	-22.5	-20.8	-15.8	-27.5	-19.3	-26.3	-25.2	-29.3
DW-8	-4.9	2.4	-5.0	19.9	11.5	22.4	3.6	10.5	2.6
GW-1	1.7	-9.4	-10.9	-5.5	-8.9	-4.0	4.6	-8.0	-7.0
GW-2	-2.5	-11.7	-17.4	-15.6	-20.3	-7.4	-31.3	-9.1	-16.9
GW-3	-8.9	-17.4	-23.0	-21.4	-17.5	-10.4	-13.2	-7.3	-12.8
GW-4	-14.3	-25.4	-27.6	-28.2	-17.1	-29.5	-20.6	-12.3	-51.4
GW-5	-11.4	-25.6	-18.2	-14.6	-18.8	-23.3	-30.1	-22.0	-16.0
GW-6	-29.7	-39.5	-35.7	-33.1	-38.2	-38.5	-52.0	-43.7	-53.6
GW-7	-24.4	-35.5	-32.2	-27.4	-39.6	-42.1	-69.3	-49.7	-77.5
GW-8	-18.3	-40.5	-26.1	-22.3	-33.9	-29.9	-58.1	-46.9	-65.6
GW-9	20.9	8.5	17.3	-7.6	-7.1	-33.3	-21.0	-43.8	-14.8
GW-10	-2.2	-4.4	-5.9	-10.8	-4.9	-4.1	-2.2	-3.6	-1.4
GW-11	-0.4	-3.5	-1.3	-2.0	-4.4	-3.2	-0.3	-2.9	-3.2
GW-12	-3.5	-1.2	-5.1	-5.7	-1.0	-5.7	-2.6	-4.3	6.9
SW-1	19.0	21.9	7.1	28.0	14.0	31.1	4.6	11.9	-0.5
SW-2	17.2	19.9	10.0	27.7	21.8	31.7	20.6	19.6	11.6
SW-3	10.4	9.0	11.7	14.2	0.3	5.6	-25.4	4.1	-10.8
SW-4	10.2	24.2	11.8	27.3	13.3	33.2	4.7	19.7	3.2
SW-5	8.2	19.5	7.1	27.5	9.8	29.4	17.2	18.0	28.1
SW-6	6.1	6.9	-1.8	13.8	7.7	23.8	22.2	19.1	27.2
SW-7	28.4	24.9	22.6	40.6	16.4	37.3	36.1	31.7	37.8
SW-8	1.6	7.5	-4.9	21.8	5.9	14.4	-18.2	3.7	-37.1
SW-9	3.0	3.4	-2.2	23.6	7.8	14.3	-28.5	-7.5	-49.9
SW-10	4.5	7.0	-1.0	22.0	11.5	16.9	13.0	9.6	11.1
SW-11	34.2	34.8	24.0	33.0	5.8	49.0	12.4	38.9	21.1
SW-12	8.2	13.3	0.7	17.3	5.5	22.3	-44.6	-13.1	-51.2
SW-13	-1.3	-1.3	-7.6	-3.1	-2.3	-0.7	-0.9	-3.6	-1.5
$WWTP_{IN}$-1	-29.6	0.5	-32.6	-24.6	-35.3	-29.5	-37.1	-17.5	-32.4
$WWTP_{IN}$-2	-22.8	-28.5	-34.1	-29.0	-38.4	-35.8	-34.0	-23.5	-28.0
$WWTP_{IN}$-3	-26.6	-28.0	-26.8	-22.6	-33.2	-25.3	-30.9	0.6	-35.3
$WWTP_{IN}$-4	-27.1	-34.7	-44.5	-27.3	-28.5	-27.8	-49.3	-24.9	-35.1
$WWTP_{IN}$-5	-17.2	-29.5	-31.3	-26.5	-39.0	-49.2	-32.1	-17.4	-24.2
$WWTP_{IN}$-6	6.5	-11.3	8.8	10.1	-14.1	-8.5	-19.1	-4.3	-17.7
$WWTP_{IN}$-7	-13.8	-38.8	-22.2	-14.6	-32.4	-24.6	-36.4	-28.4	-44.4
$WWTP_{IN}$-8	-3.7	-20.4	0.6	0.6	-16.2	-12.8	-19.3	-0.7	-18.0
$WWTP_{OUT}$-1	-21.6	-23.6	-34.2	-30.7	-38.5	-41.0	-30.5	-30.1	-26.0
$WWTP_{OUT}$-2	11.7	-22.4	5.4	9.5	-10.9	-3.8	-32.6	-1.6	-24.8
$WWTP_{OUT}$-3	5.6	-16.9	1.8	10.0	-11.8	-4.9	-28.8	9.9	1.5
$WWTP_{OUT}$-4	4.6	-26.2	8.1	8.9	-6.3	-5.6	-40.8	-2.4	-36.9
$WWTP_{OUT}$-5	-36.2	-41.0	-41.4	-28.3	-42.5	-37.0	-55.8	-36.3	-53.6
$WWTP_{OUT}$-6	-42.2	-45.0	-42.2	-33.2	-40.6	-37.6	-26.8	-28.4	-20.4
$WWTP_{OUT}$-7	-28.9	-30.7	-39.4	-32.0	-35.4	-28.8	-25.2	-24.5	-29.4
$WWTP_{OUT}$-8	-34.3	-44.3	-34.1	-34.2	-42.3	-24.0	-65.9	-22.7	-27.8
$WWTP_{OUT}$-9	-7.4	-5.0	-10.1	-14.0	-17.2	-24.2	-33.6	-19.1	-19.5
$WWTP_{OUT}$-10	-14.2	-36.9	-27.6	-15.3	-34.8	-26.5	-47.8	-29.2	-60.2
$WWTP_{OUT}$-11	4.8	-13.3	-1.7	3.6	-8.9	-16.4	-48.0	-11.3	-41.3

Table S4 – Results of the PLS regression and cross-validation (leave-one-out method) analyses of the matrix effect in the drinking water (DW), groundwater (GW), surface water (SW) and wastewater (WW) sample categories, using as predicting variables COD, conductivity and hardness (3-Variable Models) or also the signal area at the retention time of target analytes under negative ionization (4-Variable Models). P_{FIT} = probability level for the fitting model; EV = percentage of explained variance in fitting; MEC = mean error in fitting; SDEC = standard deviation error in fitting; P_{CV} = probability level for the cross-validated model; CVEV = cross-validated percentage of explained variance; MECV = mean error in cross-validation; SDECV = standard deviation error in cross-validation. Note that for $P_{FIT} > 0.100$ the fitting model has been considered not significant and no calculation of the cross-validated model has been considered. The acronym N.S. means not significant and refers to cases for which the cross-validated error sum of squares was found to be higher than the experimental total sum of squares.

	3-Variable Models								4-Variable Models							
	P_{FIT}	EV	MEC	SDEC	P_{CV}	CVEV	MECV	SDECV	P_{FIT}	EV	MEC	SDEC	P_{CV}	CVEV	MECV	SDECV
DW (n=8)																
PFBuS	0.334	-	-	-	-	-	-	-	0.175	-	-	-	-	-	-	-
PFPeA	0.103	-	-	-	-	-	-	-	0.112	-	-	-	-	-	-	-
PFHxA	0.224	-	-	-	-	-	-	-	0.247	-	-	-	-	-	-	-
PFHxS	0.179	-	-	-	-	-	-	-	0.153	-	-	-	-	-	-	-
PFHpA	0.087	77.6	5.0	5.7	N.S.	-	-	-	0.090	77.2	4.8	5.7	0.997	6.8	9.0	11.6
PFOA	0.151	-	-	-	-	-	-	-	0.049	50.2	9.3	10.6	N.S.	-	-	-
PFOS	0.028	76.0	7.6	9.1	N.S.	-	-	-	0.012	67.6	8.6	10.6	0.311	17.0	14.5	17.0
PFNA	0.008	85.3	4.2	5.5	0.283	46.5	7.7	10.4	0.008	85.5	4.4	5.4	0.308	37.6	6.1	11.3
PFDA	0.028	77.2	6.7	8.2	0.309	44.3	10.9	12.7	0.014	82.0	6.5	7.2	0.269	41.0	11.9	13.1
GW (n=12)																
PFBuS	0.069	29.4	8.1	10.8	N.S.	-	-	-	0.031	38.5	7.8	10.0	N.S.	-	-	-
PFPeA	0.046	34.2	10.5	12.6	N.S.	-	-	-	0.008	52.7	9.2	10.6	0.451	13.7	12.3	14.4
PFHxA	0.207	-	-	-	-	-	-	-	0.028	54.7	7.6	9.7	N.S.	-	-	-
PFHxS	0.160	-	-	-	-	-	-	-	0.100	39.1	6.1	7.6	N.S.	-	-	-
PFHpA	0.017	45.1	8.5	9.5	0.364	17.5	10.4	11.7	0.007	52.8	8.0	8.8	0.208	29.6	9.7	10.8
PFOA	0.100	52.2	7.8	9.9	0.909	12.8	11.6	13.3	0.028	65.8	6.1	8.3	0.391	35.0	9.0	11.5
PFOS	0.002	82.8	8.3	9.7	0.094	60.3	12.7	14.7	0.001	85.9	7.2	8.8	0.050	68.8	11.9	13.0
PFNA	0.009	65.1	8.8	10.8	0.208	39.1	11.3	14.3	0.002	63.7	10.2	11.0	0.123	43.0	12.6	13.8
PFDA	0.040	35.9	18.1	21.6	N.S.	-	-	-	0.038	36.5	18.1	21.4	N.S.	-	-	-
SW (n=13)																
PFBuS	0.457	-	-	-	-	-	-	-	0.441	-	-	-	-	-	-	-
PFPeA	0.343	-	-	-	-	-	-	-	0.147	-	-	-	-	-	-	-
PFHxA	0.696	-	-	-	-	-	-	-	0.489	-	-	-	-	-	-	-
PFHxS	0.666	-	-	-	-	-	-	-	0.517	-	-	-	-	-	-	-
PFHpA	0.467	-	-	-	-	-	-	-	0.212	-	-	-	-	-	-	-
PFOA	0.288	-	-	-	-	-	-	-	0.188	-	-	-	-	-	-	-
PFOS	0.390	-	-	-	-	-	-	-	0.219	-	-	-	-	-	-	-
PFNA	0.673	-	-	-	-	-	-	-	0.235	-	-	-	-	-	-	-
PFDA	0.264	-	-	-	-	-	-	-	0.426	-	-	-	-	-	-	-
WW (n=19)																
PFBuS	0.048	31.6	11.3	13.3	N.S.	-	-	-	0.002	42.7	10.5	12.2	N.S.	-	-	-
PFPeA	0.018	28.7	8.3	10.6	N.S.	-	-	-	0.020	28.0	8.4	10.6	N.S.	-	-	-
PFHxA	0.055	30.5	12.2	15.4	0.978	3.0	14.9	18.2	0.065	29.0	12.3	15.6	N.S.	-	-	-
PFHxS	0.028	35.9	10.3	13.0	0.866	6.0	13.3	15.9	0.001	48.8	9.8	11.7	0.219	23.5	12.1	14.7
PFHpA	0.028	36.0	8.3	10.0	0.772	7.9	10.3	12.0	0.007	35.6	8.5	10.0	0.801	3.3	10.7	12.3
PFOA	0.035	23.6	9.0	11.0	N.S.	-	-	-	0.013	31.2	8.8	10.5	0.991	1.0	10.7	12.6
PFOS	0.763	-	-	-	-	-	-	-	0.243	-	-	-	-	-	-	-
PFNA	0.220	-	-	-	-	-	-	-	0.100	15.1	9.2	11.8	N.S.			
PFDA	0.363	-	-	-	-	-	-	-	0.295	-	-	-	-	-	-	-