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Applicability of the direct injection liquid chromatographic tandem mass spectrometric analytical approach to the sub-ng L⁻¹ determination of perfluoroalkyl 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

1 Perfluoro-alkyl acids (PFAAs) are a class of compounds having a $\text{CF}_3\text{-(CF}_2\text{)}_n\text{-R}$ structure, where R
2 is a carboxylic or a sulfonic or a phosphonic group, and “n” ranges mostly between 2 and 10.
3
4 PFAAs are characterized by high resistance to physical, chemical and biological degradation and
5
6 have been widely employed since the 1950s in a wide range of industrial and commercial
7
8 applications, as well as in fluoropolymer production, giving rise to a widespread contamination of
9
10 environmental matrices. More in detail, PFAAs have been determined in wastewater [1-3], surface
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12 water [4-6] and drinking water [7]. Moreover, PFAAs have been detected in remote areas like open
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14 oceans [8] and Arctic [9, 10].
15

16 Among PFAAs, perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA) have
17
18 been the most industrially employed until 2006, when some regulatory restrictions have been
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20 promulgated both in Europe and United States [11, 12]. Furthermore, in 2013 PFOS has been
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22 included in the list of priority hazardous substances, within the Directive 2013/39/EU [13], whereas
23
24 PFOA has been included in the candidate list of Substances of Very High Concern because of its
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26 carcinogenic, mutagenic or toxic for reproduction effects as well as persistent, bioaccumulative and
27
28 toxic properties [14].

29 For PFOS an annual average environmental quality standard (EQS) and a maximum allowable
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31 concentration (MAC) of 0.65 ng L^{-1} and $36 \text{ } \mu\text{g L}^{-1}$ have been respectively established for inland
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33 waters by the European Community (EC) [13]. As regards drinking water, Provisional Health
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35 Advisories of $0.4 \text{ } \mu\text{g L}^{-1}$ and $0.2 \text{ } \mu\text{g L}^{-1}$ have been proposed by the Environmental Protection
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37 Agency of Unites States (USEPA) for PFOA and PFOS, respectively [15]. For these compounds
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39 concentration limits in drinking water of $0.5 \text{ } \mu\text{g L}^{-1}$ (PFOA) and $0.03 \text{ } \mu\text{g L}^{-1}$ (PFOS) have been
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41 recommended by the Italian Health Institute, on the basis of maximum tolerable daily intake (TDI)
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43 data reported by the European Food Safety Authority [16]. Conversely, to the best of our
44
45 knowledge, no limits have been established for the presence of PFAAs in groundwater.

46 Several analytical methods for the determination of PFAAs in water media at trace level have been
47
48 published, mostly employing solid-phase extraction (SPE) and liquid chromatography (LC) coupled
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50 with tandem mass spectrometry (MS/MS) [1, 17-19].

51 However, physicochemical properties (i.e. solubility/lipophilicity and acidity) of these molecules
52
53 greatly vary depending on the chain length and the acidic group present in the molecule, thus
54
55 making challenging the recovery of all analytes during extraction and clean-up processes.
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57 Furthermore, special care should be taken during sample manipulation, treatment and analysis,
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59 since, as demonstrated by various inter-laboratory studies, there is an actual risk of contamination
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1 during the whole analytical process, owing to the presence of fluorinated polymers in commonly
2 used laboratory materials and equipment [20].

3 In order to minimize sample manipulation and treatment, as well as to increase the analytical
4 throughput, several on-line SPE-LC-MS/MS methods have been developed for PFAAs
5 determination in water samples [21-24].
6

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

31 According to the considerations reported above, the aim of this work was to evaluate the feasibility
32 of using the DI-LC-MS/MS analytical approach for the determination of PFCAs and PFSAs in a
33 very wide range of water samples (i.e. drinking water, groundwater, river water, lake water and
34 wastewater). For each sample ME was investigated by the standard addition method and tentatively
35 interpreted as a function of a set of physicochemical parameters of water samples (i.e. conductivity,
36 hardness, and organic carbon content), as well as chromatographic outputs.
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2 Moreover, since water samples herein analysed were collected in zones never investigated before
3 for PFAA occurrence (i.e. various rural, urban and industrial districts of Tuscany, Italy), this study
4 provides for the first time information regarding the contamination by PFAAs of these areas.
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6 **2 Material and methods**

7 **2.1 Standards and reagents**

8
9 Perfluorobutanesulphonic acid (PFBuS, CAS number: 375-73-5), perfluoropentanoic acid (PFPeA,
10 CAS number: 2706-90-3), perfluorohexanoic acid (PFHxA, CAS number: 307-24-4),
11 perfluorohexanesulphonic acid (PFHxS, CAS number: 355-46-4), perfluoroheptanoic acid (PFHpA,
12 CAS number: 375-85-9), perfluorooctanoic acid (PFOA, CAS number: 335-67-1), perfluoro-n-
13 (1,2,3,4 ¹³C₄)octanoic acid (MPFOA), perfluoroactanesulphonic acid (PFOS, CAS number: 1763-
14 23-1), and perfluoro-1-(1,2,3,4 ¹³C₄)octanesulphonate (MPFOS), perfluorononanoic acid (PFNA,
15 CAS number: 375-95-1), perfluorodecanoic acid (PFDA, CAS number: 335-76-2), methanol stock
16 solutions (50 µg mL⁻¹) were purchased by Wellington Laboratories Inc. (Guelph, ON, Canada).
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19 Water and methanol (LC-MS grade) were obtained from Carlo Erba (Milan, Italy).
20

21 Ammonium acetate (Sigma-Aldrich, St. Louis, MO, USA) 1 M solution was freshly prepared in
22 water LC-MS grade.
23

24 **2.2 UHPLC-MS/MS analysis**

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26 Ultra-high performance liquid chromatographic (UHPLC) analyses were performed on a Shimadzu
27 (Kyoto, Japan) chromatographic system consisting of a low pressure gradient quaternary pump
28 Nexera X2 LC-30AD, a DGU-20A 5R degassing unit, a SIL-30AC autosampler equipped with a
29 100 µL loop, a CTO/20AC thermostatted column compartment and a CBM-20A module controller.
30
31 A delay column (C₁₈, 100 x 4.6 mm) was installed between the mixer and the sample injector, in
32 order to separate the impurity PFAAs originating from the LC system from the analyte PFAAs of
33 the sample.
34

35
36 Chromatographic separation was obtained with a Waters ACQUITY UPLC BEH C₁₈ column (100 x
37 2.1 mm, particle size 1.7 µm) equipped with a guard column (Waters, Milford, MA, USA),
38 thermostatted at 50° C, employing a mixture of 95% water 5% methanol solution of 2 mM
39 ammonium acetate (solvent A) and a methanol solution of 2 mM ammonium acetate (solvent B) at a
40 flow rate of 0.5 mL min⁻¹. All glassware was thoroughly rinsed with methanol before use. The
41 eluents were freshly prepared by adding a suitable aliquot of 1 M ammonium acetate solution
42 filtered on 0.2 µm polyethersulfone filters. The chromatographic gradient was the following: 25% B
43 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
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2 for 6.5 min for system re-equilibration. Total analysis time was 20 mins. The injection volume used
3 was set to 100 μL . **Fig. S1** of the Supplementary material section illustrates a reconstructed DI-LC-
4 MS/MS chromatogram of the quantifier and qualifier transitions of target PFAAs obtained by
5 injecting 100 μL of a standard mixture in Milli-Q water at the concentration of 5 ng L^{-1} each.
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7 In order to minimize MS source contamination, the first 2 min. and the last 8 min. of the
8 chromatographic run have been diverted to waste by means of a two-position six-port valve
9 installed before the mass spectrometer.
10

11 12 13 2.3 Mass spectrometric detection

14 The Shimadzu LC system was coupled to a 5500 QTrap mass spectrometer (Sciex, Framingham,
15 MA, USA), equipped with a Turbo V[®] interface by an ESI probe. MS/MS analysis was carried out
16 using the Multiple Reaction Monitoring (MRM) in negative ionization mode. The MS source
17 parameters were optimized in flow injection analysis (FIA) at optimal LC flow and mobile phase
18 composition and were as follows: Curtain Gas (CUR) 50, CAD Gas (CAD) Medium, Temperature
19 (TEM) 650°C, Gas 1 (GS1) 50, Gas 2 (GS 2) 50, and IonSpray Voltage (IS) -4500 V in scheduled
20 MRM(-).
21

22 MRM mass transitions used for quantification and identity confirmation, together with compound-
23 dependent parameters are listed in **Table S1** of the Supplementary material section.
24

25 Confirmation criteria proposed by the Commission Decision 2002/657/EC [35] concerning
26 retention time and quantifier-to-qualifier ion intensity ratio were adopted for compound
27 identification.
28

29 30 31 2.4 Method performance evaluation

32 Standard solutions containing 1, 5, 10, 30, 50, 100 and 250 ng L^{-1} of each analyte were employed
33 for calibration curves. For each investigated compound, method detection limits (MDLs) and
34 method quantification limits (MQLs) were evaluated by replicated (n=5) analysis of procedural
35 blanks, according to the following equation:
36

$$37 \text{MDL (MQL)} = \frac{k \cdot \sigma_b}{m}$$

38 where k is the critical value given by $2t_{1-\alpha}$ with n-1=4 degree of freedom and $\alpha=0.05$ (k=4.264 and
39 k=10, for MDLs and MQLs, respectively); σ_b is the standard deviation of the blank and m is the
40 slope of the calibration curve.
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42 The method of standard additions was used for the evaluation of ME, as well as for analyte
43 quantification in real samples. Each sample was split into four aliquots, three of which were spiked
44 with increasing concentration of standard solutions of target PFAAs. The spike levels were selected
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1 so that the final concentrations of the compounds fell into the linear range. Spike levels of 10, 50
2 and 100 ng L⁻¹ were employed. The extent of ME was evaluated according to the following
3 equation:
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$$5 \quad \text{ME}\% = \frac{m_{\text{sample}}}{m_{\text{standard}}} \cdot 100 - 100$$

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8 where m_{sample} is the slope of the calibration curve obtained in matrix, and m_{standard} is the slope of the
9 calibration curve in solvent; accordingly, ME values <0 indicate ion suppression, whereas ME
10 values > 0 indicate ion enhancement.
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13 14 15 2.5 Sample collection and handling before analysis

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17 A very wide range of wastewater and environmental water samples, comprising eight drinking
18 waters (DW), twelve ground waters (GW), thirteen surface waters (SW), as well as eight influents
19 and eleven effluents of wastewater treatment plants (WWTP_{IN} and WWTP_{OUT}), were included in
20 this study (see **Table S2** of the Supplementary material section).
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24 Glass bottles equipped with screw top and aluminium under-cap were employed for collecting the
25 samples. All the samples were obtained through 24-h composite collections using Endress + Hauser
26 Liquiport 2010 CSP44 autosamplers (Reinach, Switzerland). After collection, all samples were
27 stored at +4 °C until analysis, which was performed in triplicate within 48 h after sampling.
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31 Ultracentrifugation of samples at 30,000 x g was performed in polypropylene Eppendorf vials for
32 particulate matter removal before UHPLC-LC-MS/MS analysis. Afterwards, the samples were
33 fortified with labelled MPFOA and MPFOs, as internal standards, in order to highlight any possible
34 leakage during sample injection in the UHPLC system.
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40 2.6 Statistical analysis

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42 Linear discriminant analysis (LDA) and partial least square (PLS) regression with leave-one-out
43 cross validation were performed using the Minitab software packages version 17.0.1 (Minitab Inc.,
44 State College, PA, USA).
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49 3 Results and discussion

50 51 3.1 Figures of merit of the analytical method

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53 Method performance were evaluated by estimating method detection limits (MDLs), method
54 quantification limits (MQLs), linearity, intra-day and inter-day precision via the replicated injection
55 in the DI-LC-MS/MS system of 100 µL standard solutions in Milli-Q water. **Table 1** summarizes
56 the results obtained for these performance parameters. More in detail, MDLs and MQLs were
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1 evaluated by replicated (n=5) analysis of procedural blanks, according to the equation reported in
2 Section 2.4.

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

8
9 For all compounds, linearity range covered at least two magnitude orders, ranging from MQLs to
10 100 ng L⁻¹, with determination coefficient of regression ≥ 0.9981 . Intra-day and inter-day precisions
11 were evaluated for all compounds at a concentration equal to 5 ng L⁻¹, achieving RSD%_{intra} and
12 RSD%_{inter} values comprised in the range 1.8-4.4% and 2.7-5.7%, respectively.
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19 3.2 Matrix Effect evaluation and interpretation

20
21 **Table S3** of the Supplementary material section summarizes the values of ME determined for each
22 compound and sample investigated. Very different extents of signal suppression or enhancement
23 were observed, depending on the analyte and the sample considered, thus giving rise to a very
24 complex dataset.
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28 In order to make easier the discussion of data shown in **Table S3**, a graphical summary of the ME
29 distribution determined for each investigated PFAA in the 52 water samples, is reported in **Fig. 1**.
30 More in detail, **Fig. 1A** illustrates the frequency of occurrence of the absolute values of ME within
31 the following groups: (i) $|ME| < 20\%$, (ii) $20 \leq |ME| \leq 50\%$ and (iii) $|ME| > 50\%$. For most investigated
32 analytes ME was found always lower than 50%. Furthermore, $|ME| < 20\%$, which is considered to
33 have a negligible influence on the performance of an analytical method [36-38], showed the highest
34 frequency of occurrence. The occurrence of $|ME| > 50\%$, from -51.2% to -77.5%, was observed only
35 for PFOS and PFDA; these extremely high signal suppressions interested mainly GW-6, GW-7 and
36 GW-8 samples, which showed a quite strong suppression also for most of the other investigated
37 compounds (**Table S3**). As illustrated in **Fig. 1B**, PFOS and PFDA were the only analytes showing
38 the mean and median outside the $\pm 20\%$ range; the ME distribution of these two compounds was
39 characterized by a more pronounced suppressive effect and was statistically different from those
40 observed for PFBuS and PFHxS ($P \leq 0.01$). In fact, PFOS and PFDA were the most affected by ME
41 (mean values equal to -23.7% and -21.1%, respectively), whereas PFBuS and PFHxS the less
42 influenced by the matrix (mean values equal to -6.1% and -5.5%, respectively). It should also be
43 noted that the ME distribution of PFOS was significantly different ($P=0.018$) from that of PFOA, as
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1 LDA has been carried out on the whole ME dataset in order to verify if suppressive or enhancement
2 effects due to the matrix is a parameter actually suitable for correctly categorizing a certain sample,
3 according to the classification given in **Table S2** (i.e. DW, GW, SW, WWTP_{IN} and WWTP_{OUT}).
4 The squared distance between groups and the summary of the results obtained in fitted and cross-
5 validated (leave-one-out method) classification are illustrated in **Table 2** and **Table 3**, respectively.
6
7 As shown in **Table 2**, the highest between-group distance was observed by comparing the SW and
8 the WWTP groups (23.4 and 21.7 for WWTP_{IN} and WWTP_{OUT}, respectively). SW group was also
9 quite well-separated from DW (distance of 13.0) and GW (distance of 17.6) ones. Accordingly, SW
10 samples were correctly classified in 11 out of 13 cases, both in fitting and in cross-validation (**Table**
11 **3**), with probability levels that, in the worst case, were 98% and 95%, respectively, thus evidencing
12 the robustness of this classification. It should also be remarked that the two misclassified samples
13 (i.e. SW-3 and SW-13) were however attributed to GW and DW, without any misleading attribution
14 to the wastewater category.
15

16 DW and GW groups were characterized by very low between-group squared distance; a low
17 distance was also observed by comparing these groups with the WWTP_{OUT} one (**Table 2**).
18 Accordingly, some DW and GW samples were mutually misclassified, as well as erroneously
19 attributed to the WWTP_{OUT} group, especially in cross-validation (**Table 3**). However, it is
20 remarkable that no erroneous attribution to the WWTP_{IN} group was generated by the LDA model.
21
22 Interesting results were found for the WWTP_{IN} and WWTP_{OUT} groups, which were very low-
23 separated (**Table 2**). The samples of these groups were correctly classified in approximately 70%
24 and 50% of the cases, for fitting and cross-validation, respectively (**Table 3**). It should be however
25 underlined, that most of misclassified samples, with the only exceptions of WWTP_{OUT}-7 and
26 WWTP_{OUT}-9, were identified as WWTP samples. In almost all these cases, fitting and cross-
27 validation probabilities of attribution $\geq 80\%$ were obtained. WWTP_{OUT}-7 was wrongly attributed to
28 the GW group, probably as the result of the high dilution rate due to a strong run-off rain event,
29 which occurred during few days before the collection of this sample. As regards WWTP_{OUT}-9, it
30 should be noted that this facility was the only one treating wastewater almost exclusively deriving
31 from domestic activities, differently from all the other treatment plants, which receive a significant
32 contribution of industrial origin.
33

34 The LDA results highlighted the contribution of the sample origin to the ME observed during
35 PFAA analysis by LC-MS/MS. Accordingly, PLS regression with leave-one-out cross-validation,
36 was attempted on the whole sample dataset, adopting ME as response variable. Conductivity,
37 hardness and COD measured in each water sample were used as sample dependent predictors of the
38 PLS regression (3-variable models), due to the fact that these parameters are routinely determined
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1 in laboratories of potabilization facilities and WWTPs and could be usefully adopted for avoiding
2 an over-application of time-consuming and expensive approaches such as the standard addition
3 method and the isotope dilution technique.
4

5 PLS failed to correctly fit and cross-validate the experimental ME values, when the whole sample
6 dataset was used for the regression. The inclusion in the model of a further variable representing the
7 signal area at the retention time of target analytes under negative ionization (4-variable models) did
8 not improve significantly the model.
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12 Better results were achieved when PLS regression was separately applied to the WWTP, DW and
13 especially GW sample categories. As reported in **Table S4** of the Supplementary material section,
14 which illustrates the parameters of the 3-variable and 4-variable fitting and cross-validation models,
15 for these sample categories statistically significant ($P \leq 0.1$) fitting models were obtained for most
16 analytes. Moreover, in some cases the cross-validation models were found to be significant, even
17 though they provided low prediction powers. The 4-variable models were associated to better
18 fittings and predictions, compared to those based on 3 variables, evidencing the role of the signal
19 area at the retention time of target analytes for explaining ME data. Even though the PLS approach
20 resulted only in an approximate screening, due to the low prediction powers of the models, it is
21 interesting to note that for most analytes in most samples, the fitted and cross-validated values were
22 such as to correctly distinguish between $|ME|$ higher than 20% or below this limit. As an example,
23 the experimental, fitted and cross-validated ME values found for PFOA and PFOS in DW, GW and
24 WW samples are plotted in **Fig. 2**. As illustrated in **Table S4**, for PFOA the prediction model was
25 found to be statistically not significant, mainly due to the results obtained for the sample DW-8,
26 while for the other samples a quite good agreement was observed between experimental and
27 fitted/cross-validated data (**Fig. 2A**). In the same DW category, the agreement was more significant
28 for PFOS, allowing to correctly identify the samples characterized by absolute values of ME much
29 higher than 20%, with the main exception of sample DW-1 (**Fig. 2B**). In the GW category the
30 percentage of correct attribution of absolute ME was even greater. In particular, only in the samples
31 GW-4 and GW-6 a strong underestimation of ME was provided by the model for PFOA (**Fig. 2C**),
32 whereas for PFOS all samples had a good estimation (**Fig. 2D**). Quite good agreements between
33 experimental and fitted/cross-validated ME data were also observed in WW samples for PFOA
34 (**Fig. 2E**) and PFOS (**Fig. 2F**), the latter even characterized by a statistically not significant
35 prediction model. The main errors were indeed observed for PFOS in WW-6 and WW-8 samples,
36 for which a strong suppression of the signal should be observed according to the prediction model,
37 while the experimental ME was around 20%.
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3.3 Comparison with previously published high-throughput methods

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

10
11 **Table 4** illustrates the main characteristics of the analytical method herein proposed in comparison
12 with those provided by elsewhere published high-throughput methods using the same approach (i.e.
13 DI-LC-MS/MS) or other sample preparation techniques (i.e. online SPE-LC-MS/MS and in-tube
14 SPME-LC-MS/MS) for the analysis of PFAAs in water matrices.

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16 Our method represented a general improvement in terms of sensitivity in comparison with
17 previously published online SPE-LC-MS/MS and in-tube SPME LC-MS/MS methods. The
18 difference in sensitivity was particularly remarkable when the method proposed herein was
19 compared with that of Saito and co-workers [39], which was limited to PFOA and PFOS, and above
20 all, of Gosetti et al. [24], which however had the advantages to be very rapid (about 7 min. per
21 sample) and to provide negligible matrix effects, at least in the three river samples investigated.
22 Moreover, the online SPE-LC-MS/MS approach for the analysis of perfluorinated compounds was
23 frequently associated with very high intra-day and above all inter-day variations, in most cases
24 much higher than 10%. For instance, for PFOS, Llorca et al. [23] reported inter-day RSD equal to
25 26%, whereas Castiglioni and colleagues [40] found intra-day and inter-day RSD as high as 49%
26 and 48%, respectively. It should also be noted that in this latter study, which involved the analysis
27 of WWTP influents and effluents, river and groundwater samples, as well as drinking waters, a
28 matrix effect extent similar to that observed in our study was reported, notwithstanding the presence
29 of the washing step of SPE sorbent before LC-MS/MS determination of PFAAs [40].

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31 Furdui et al. [3] proposed a very fast DI-LC-MS/MS method (4 min. per sample) for the
32 determination in lake waters of nine perfluorinated compounds, including a group of six analytes
33 (i.e. PFHxS, PFHpA, PFOA, PFOS, PFNA and PFDA) in common with target compounds of this
34 study. MQLs reported by Furdui and co-workers were much higher to those achieved herein (**Table**
35 **4**). The very short analysis time was mainly due to the use of an isocratic elution with buffered
36 CH₃OH/H₂O 80/20 (v/v), thus avoiding the time-consuming column re-equilibration before a new
37 injection. However, the isocratic elution with high organic solvent percentages is not compatible
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with the determination of short-chain PFAAs (i.e. PFBS, 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: $-70 \pm 15\%$). 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:

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

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 O₃-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.

1 PFOA and PFOS concentrations in DW samples were found to be much lower than the limits
2 recommended by the Italian Health Institute (500 ng L⁻¹ and 30 ng L⁻¹ for PFOA and PFOS,
3 respectively) and USEPA (400 ng L⁻¹ and 200 ng L⁻¹).
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6 **4 Conclusions**

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8 This study demonstrates the applicability of the high-throughput DI–LC–MS/MS analytical
9 approach for the identification and quantitative determination of PFAAs in a wide range of water
10 matrices, with sensitivities in the sub-ng L⁻¹ range, intra-day and inter-day RSD lower than 4.5%
11 and 6.0%, respectively. These performances were generally higher than those recently achieved
12 with on-line SPE–LC–MS/MS, in-tube SPME–LC–MS/MS and DI–LC–MS/MS methods.
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15 Absolute values of ME herein observed were frequently lower than 20% and however below 50%
16 with very few exceptions. The application of LDA on the whole ME dataset identified according to
17 the classification given in **Table S2** (i.e. DW, GW, SW, WWTP_{IN} and WWTP_{OUT}) highlighted the
18 significant contribution of the sample origin to the ME. Hence, PLS and leave-one-out cross-
19 validation were performed in order to interpret and predict the signal suppression or enhancement
20 phenomena as a function of conductivity, hardness and chemical oxygen demand of water samples
21 as well as background chromatographic area. Even though the PLS approach gave rise to models
22 characterized by low prediction powers, for most analytes in most samples, the fitted and cross-
23 validated values were such as to correctly distinguish between |ME| higher than 20% or below this
24 limit. Accordingly, the possible use of this chemometric tool for avoiding the over-application of
25 time-consuming and expensive approaches, such as the standard addition method and the isotope
26 dilution technique, should be further investigated.
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29 The application of this DI–LC–MS/MS to the quantification of target PFAAs in real samples
30 provided for the first time data regarding the occurrence of perfluorinated compounds in Tuscany
31 water samples of various origin. Although our study was not designed as an environmental
32 investigation, many kind of 24-hour composite water samples were analysed herein, thus providing
33 interesting information. Among them, PFOA and PFOS concentrations in DW samples were found
34 in all cases much lower than the limits recommended by the Italian Health Institute. Furthermore,
35 the ineffectiveness of activated sludge treatments for the removal of PFAAs was observed, thus
36 highlighting the possible role of biological WWTPs in the punctual release of perfluorinated
37 contaminants in the receiving water bodies, when tertiary treatment stages based on chemical
38 oxidation or activated carbon are not implemented.
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Table 21 – Method detection limits (MDLs), linearity ranges, determination coefficients (R^2), intra-day and inter-day precision (expressed as relative standard deviation percentages, RSD%). See paragraph 2.1 for the meaning of compound acronyms.

Compound	MDL (ng L ⁻¹)	Linear Range (ng L ⁻¹) ^a	R ²	RSD% _{intra} ^b	RSD% _{inter} ^b
PFBuS	0.014	0.033-250	0.9981	3.2	4.7
PFPeA	0.16	0.38-250	0.9985	3.7	4.5
PFHxA	0.44	1.0-250	0.9993	2.6	3.6
PFHxS	0.013	0.030-250	0.9999	4.4	5.7
PFHpA	0.18	0.42-250	0.9995	2.6	4.1
PFOA	0.13	0.30-250	0.9995	2.1	3.8
PFOS	0.071	0.17-250	0.9984	1.8	2.7
PFNA	0.26	0.61-250	0.9992	4.4	5.4
PFDA	0.054	0.13-250	0.9982	3.9	4.8

^a The bottom limit of linearity represents the Method Quantification Limit.

^b Evaluated for all compounds at a concentration equal to 5 ng L⁻¹ in Milli-Q water.

Table 4.2 – 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 5-3 – 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 6-4 – 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 7-5 – Concentration values (ng L^{-1}) determined for each investigated compound in all the samples analysed in this study. Values higher than $\text{MQL}_{\text{sample}}$ 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 ($\text{MDL}_{\text{sample}}$)

^b Method Quantification Limit in real samples ($\text{MQL}_{\text{sample}}$)

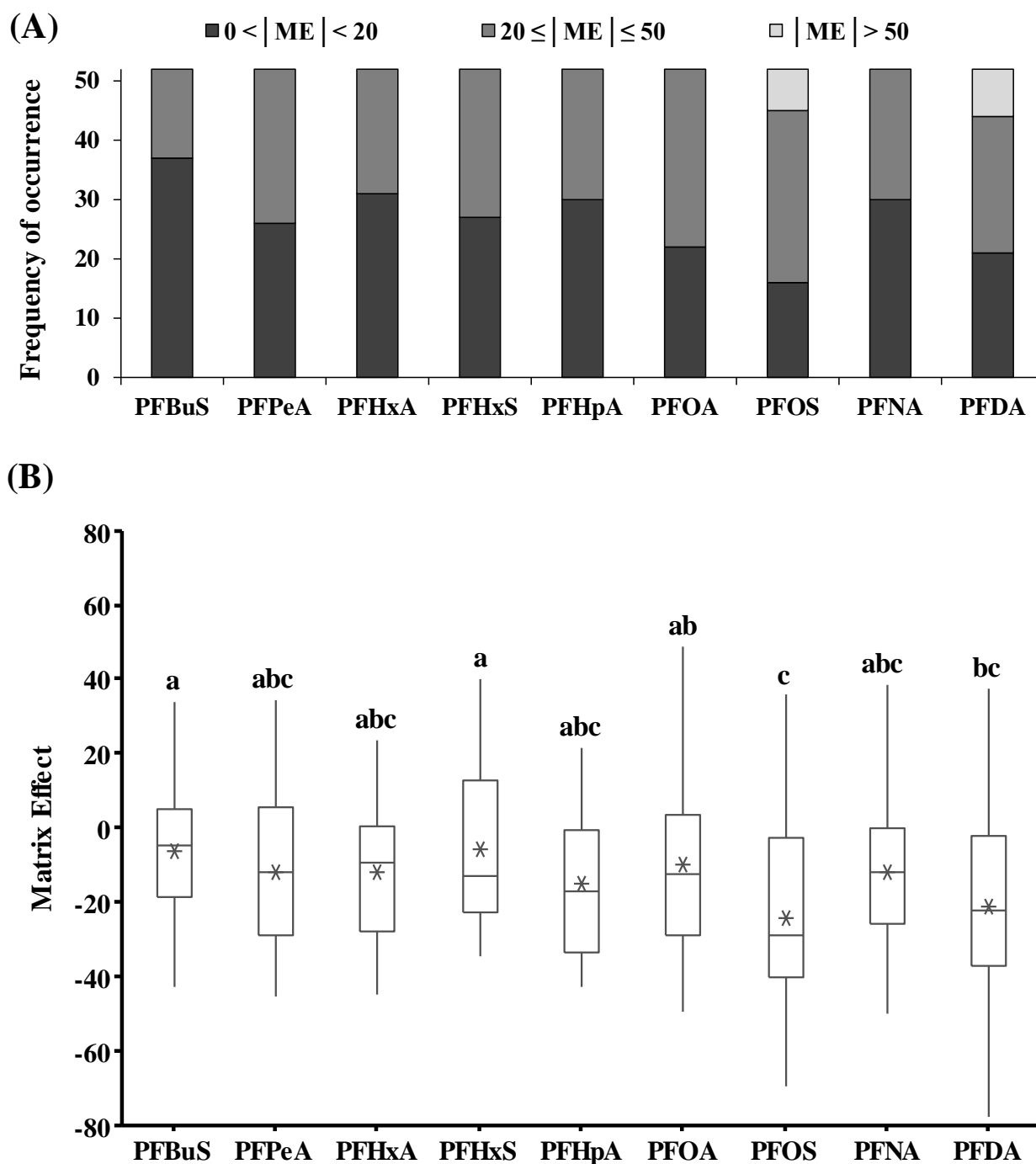


Figure 1 – Graphical summary of the matrix effect dataset. (A) Histogram reporting the frequency of occurrence of matrix effect (ME) within the ranges $|ME| < 20$, $20 \leq |ME| \leq 50$, $|ME| > 50$. (B) Boxplot of the distribution of ME dataset determined for each investigated PFAA: each boxplot represents 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

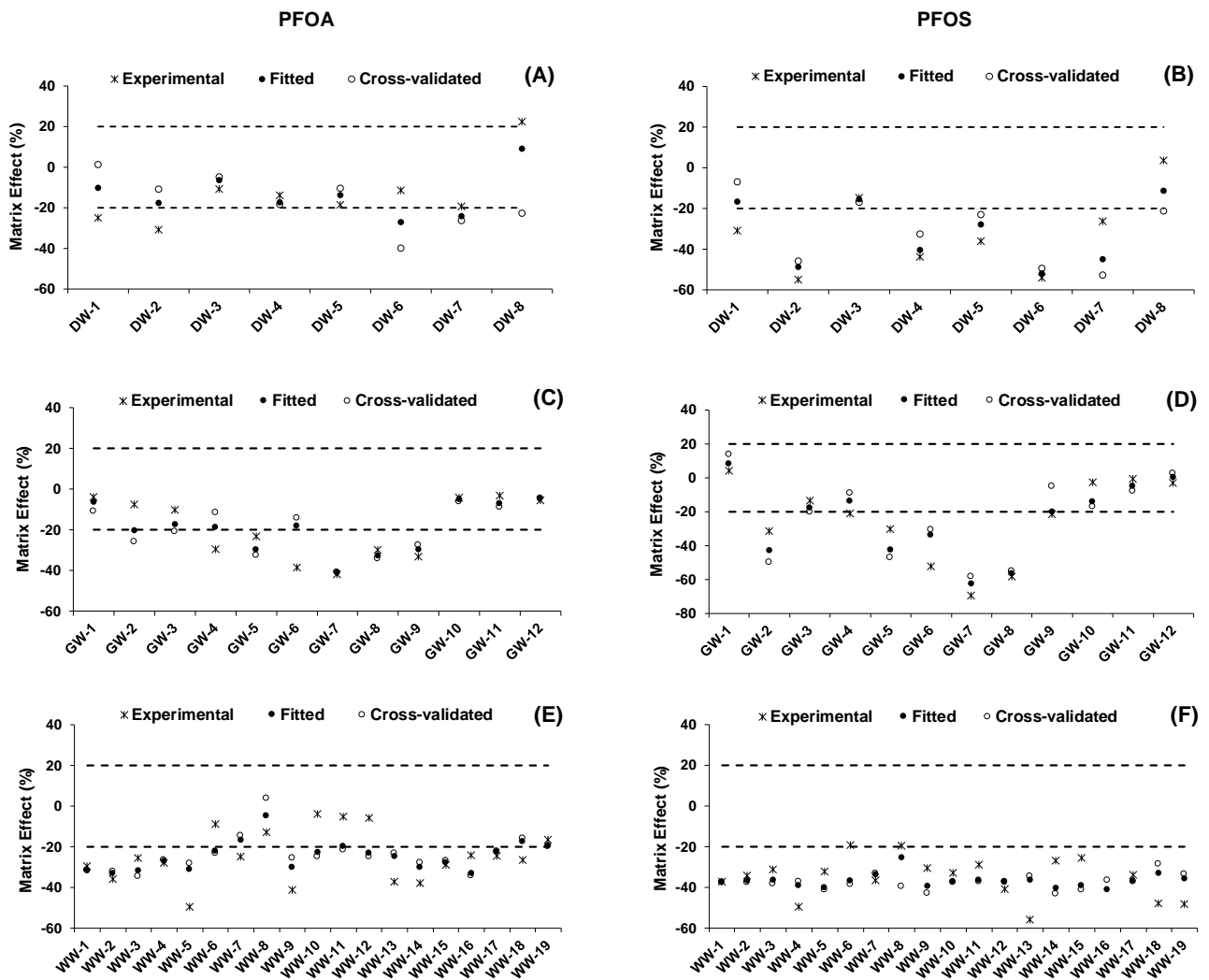


Figure 2 – [Experimental](#), [F](#)-fitted and cross-validated matrix effect values determined for PFOA and PFOS in drinking water ([DWA-B](#)), groundwater ([GWC-D](#)) and wastewater ([WWE-F](#)) sample categories [using PLS regression, in comparison with experimental data.](#)

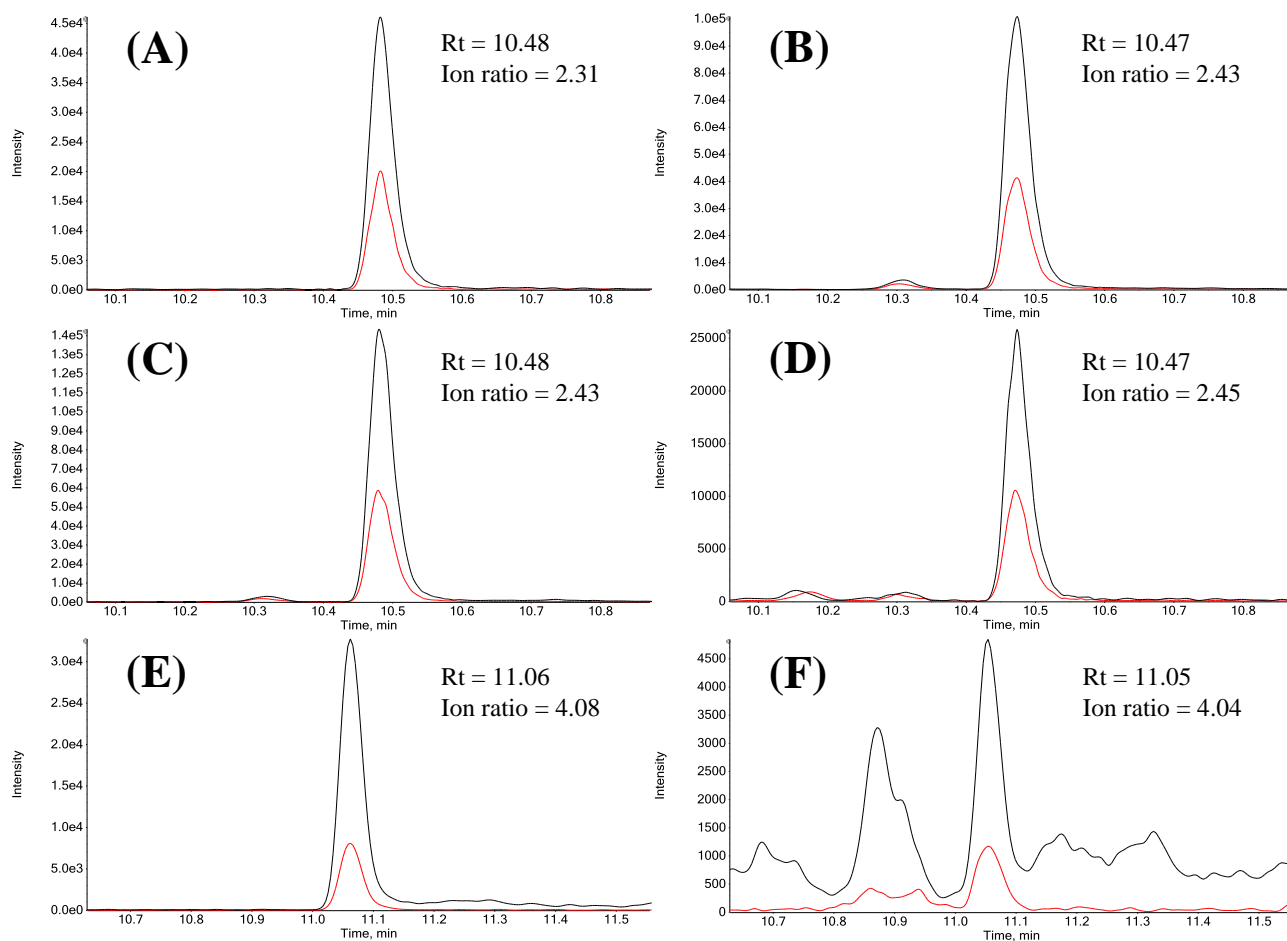
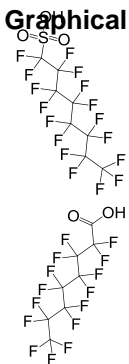


Figure 3 – Overlapped MRM quantifier (*black line*) and qualifier (*red line*) transitions, retention time (R_t) and ion ratio of: (A) PFOA, Milli-Q water (5 ng L^{-1}); (B) PFOA, WWTP_{IN}-1 (30.4 ng L^{-1}); (C) PFOA, WWTP_{OUT}-1 (66.4 ng L^{-1}); (D) PFOA, WWTP_{OUT}-10 (4.9 ng L^{-1}); (E) PFOS,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

Graphical Abstract (for review)

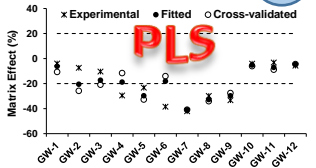
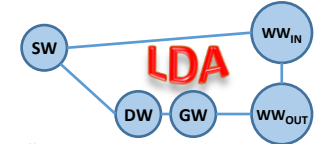


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