

Can qualitative non-target data be indicative of PFAS contamination? First evidence by correlation with EOF in environmental samples

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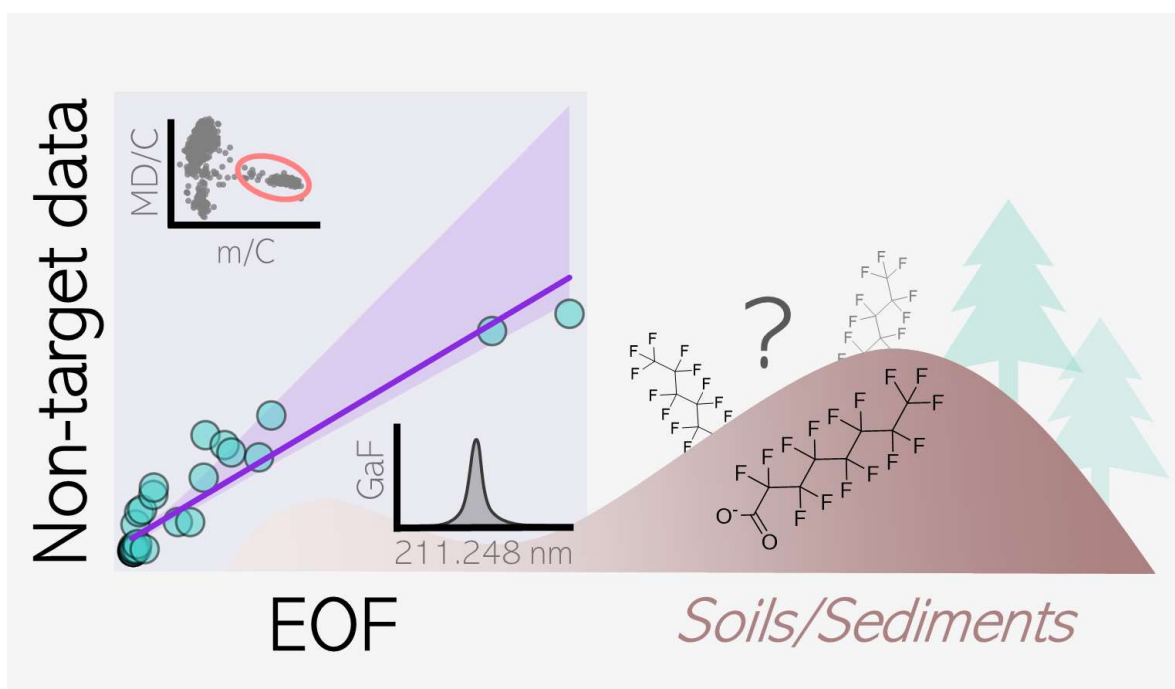
Abstract

We combined and correlated non-target screening (NTS) features and extractable organic fluorine (EOF) data for PFAS analysis in environmental samples. Soils, sediments, and ashes from different locations (Germany, France, Lithuania) (n=34) were extracted and analyzed via liquid chromatography-high-resolution mass spectrometry (LC-HRMS) and high resolution-continuum source-graphite furnace molecular absorption spectrometry (HR-CS-GFMS). NTS feature prioritization based on the mass to carbon (m/C) and mass defect to carbon (MD/C) ratio was applied to discriminate potential PFAS features from common co-extracted organic matrix. Our results show a high correlation between quantitative EOF data with the prioritized feature number ($R^2 = 0.87$, $r_{\text{Pearson}} = 0.93$) as well as with the total peak area of prioritized features ($R^2 = 0.97$, $r_{\text{Pearson}} = 0.98$) while commonly applied MD-ranges for filtering showed much weaker correlation with EOF. The observed highly significant correlations highlight that MD/C-m/C prioritization is associated with a good indication of the overall PFAS burden as evidenced by EOF. Also, semi-quantitative data can be extracted from PFAS-specific NTS as shown for the EOF vs. prioritized peak areas. The correlations indicate that lowly fluorinated compounds were negligible and that the EOF was mostly composed of higher fluorinated substances. We recommend further investigation of the unique complementary EOF vs. NTS combination for PFAS screening.

Keywords: PFAS, extractable organic fluorine (EOF), high-resolution mass spectrometry, non-target-screening (NTS), MD/C-m/C, feature prioritization, HR-CS-GFMAS, fluorine, correlation

Synopsis: We show that PFAS-specific feature prioritization in LC-HRMS data via the m/C and MD/C ratio is associated with a strong correlation of the overall PFAS burden in different environmental samples evidenced by correlation with extractable organic fluorine (EOF) data.

TOC



Introduction

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous contaminants posing adverse effects to humans and the environment.^{1, 2} The analysis of PFAS in environmental samples is very challenging because of their diverse physical/chemical properties and the high number of individual PFAS compounds.³ Target analysis is not feasible for a comprehensive characterization as only a few PFAS can be quantified, thereby often drastically underestimating the total PFAS burden.^{4, 5} Thus, current analytical trends emphasize complementary approaches based on organofluorine sum parameters and non-target screening (NTS) for a higher PFAS coverage.⁶⁻⁸

Organofluorine sum parameters are used to determine the gap between PFAS targets and total organically bound fluorine and thereby indicate the quantity of unidentified extractable organic fluorine (EOF).⁹ EOF sample preparation is combined with fluorine-sensitive and -specific detection systems like high resolution-continuum source-graphite furnace molecular absorption spectrometry (HR-CS-GFMAS).^{10, 11} However, this method is not able to provide information on compound identity and is therefore limited to total fluorine quantification as all species information is lost.

To unravel the unknown gaps in PFAS mass balances while preserving species information, NTS approaches can be utilized.¹² NTS based on high resolution-mass spectrometry (HRMS) is very powerful to identify unknown PFAS in various samples.¹³ Usually after data reduction and prioritization, unknown compounds can be identified by accurate mass, isotope pattern, and characteristic fragmentation.¹⁴ Eventually, the acquired information is usually compared with chemical databases (suspect screening) and molecular structures of the analytes are identified without the need for analytical standards.¹⁵ For PFAS, several intrinsic properties can be used for feature prioritization in NTS.⁶ The chemical mass defect (MD) is often used for prioritization of potentially unknown PFAS by removing features outside a defined MD

range due to the typically high number of F atoms resulting in negative to slightly positive MDs.^{16, 17} However, many other compounds fall in the same MD range as PFAS, hence, further improved prioritization is required to reduce false positives. Therefore, the mass and the MD of every feature can be normalized to the carbon number C ($MD/C-m/C$) which is retrieved from the ratio of the abundance of the monoisotopic mass (I_M) and abundance of the $M+1$ isotopic peak (I_{M+1}) and the natural abundance of ^{13}C according to the following equation: $C \approx I_{M+1}/I_M/0.011145$.¹⁸ Plotting MD/C versus the m/C strongly discriminates PFAS (and other compounds with similar heteroatom fractions) from many common organic matrix compounds (Figure S1). The technique is primarily based on the mass increase when a H is replaced by a F. Even though data filtering in PFAS NTS was much improved, final identification approaches are still time-consuming, especially for novel PFAS poorly represented in databases. Furthermore, most NTS approaches remain solely qualitative and quantitative information is missing.¹⁹

While the EOF provides quantitative information, the identity of individual compounds is not provided. On the other hand, during NTS, usually not all detected features can be identified, but advanced prioritization using the $MD/C-m/C$ dimension is very promising. However, this approach could not be validated with experimental HRMS-data originating from environmental samples which fraction truly originates from PFAS. Hence, the overall aim of our study is to combine EOF analysis with NTS to correlate quantitative information (EOF) with feature prioritization approaches (*e.g.*, $MD/C-m/C$). This approach allows us to draw quantitative conclusions on the comprehensiveness of PFAS-specific NTS by the unique information provided by the EOF. To the best of our knowledge, this study comprises the first approach to correlate EOF and NTS data in various matrices.

Materials and Methods

Soil samples. In total 34 samples from Germany, France, and Lithuania were used for NTS vs. EOF correlation which included 25 soils, 4 compost samples, 2 sediments, 2 sewage sludge ashes and one filter cake after soil washing. The samples included several soils with known PFAS contamination from PFAS containing paper sludge²⁰ and fire-fighting activities near an airport.

Sample extraction. The detailed extraction procedure is provided in the SI.¹¹

EOF analysis by HR-CS-GFMS

EOF analysis using HR-CS-GFMS was based on an optimized method for PFAS analysis by Simon *et al.* A contrAA 800 HR-CS-GFMS system and the software ASpect CS 2.2.2.0 (both Analytik Jena AG) were used for fluorine analysis.²¹ The method is based on the indirect analysis of fluorine using the GaF absorption wavelength at 211.248 nm.

Non-target screening data acquisition. Sample extracts were analyzed with an Agilent 1260 Infinity HPLC (Poroshell 120 EC-C₁₈ column, 2.1 mm × 100 mm, 2.7 µm particles at 40 °C) coupled to an Agilent 6550 quadrupole time-of-flight (QTOF)-mass spectrometer. Details on gradient elution, source- and instrument parameters can be found elsewhere.²² Extracts were measured undiluted and in a 1:10 dilution in negative and positive electrospray ionization (ESI) mode in both full-scan and data-dependent acquisition (ddMS²) (scan range 100 – 1700 *m/z*, 3 spectra/s, details on the ddMS² method in Zweigle *et al.* 2023).²² As quality control, labelled internal standards of several PFAAs (Table S1) were spiked in each sample via the autosampler (20 pg of each labelled PFAA internal standard). At least every 10th sample a MeOH blank and a PFAA standard mix (Table S1) were measured to monitor the instrument response. To avoid cross contamination, a 3-fold needle wash was performed and the batches were measured in order of increasing EOF concentration.

NTS workflow and correlation. Agilent MS data files were converted to mzML using the MSConvert software.²³ To detect features in the HRMS raw data, several modified Python scripts from the PF Δ Screen software tool²² were used (feature detection details in the SI).^{24, 25} To remove background signals from solvent and LC system, a blank correction was performed (details in SI). For a prioritization towards potential PFAS features the MD, m/C and MD/C ratios were calculated. PFAAs were semi-quantified (one-point calibration) via spiked internal standards (Table S1). Several prioritization techniques (*e.g.*, MD/C-m/C) were applied and feature numbers and the sum of the chromatographic peak areas of the prioritized features were correlated with EOF data. To deduce ideal prioritization boundaries (*e.g.*, m/C cutoff), the data were iteratively correlated while removing more features in each step to determine the maximum possible correlation coefficient. Therefore, the boundaries to remove features in the MD/C-m/C dimensions were iteratively changed by moving towards the CF₂-location (MD/C = -0.003, m/C = 50) in 25 steps. For every iteration, the Euclidean distance from the boundary to the CF₂-location was calculated (details in the SI).

Results and Discussion

Correlation of EOF with NTS data. In total, 34 environmental samples originating mainly from different soils were analyzed for quantitative EOF via HR-CS-GFMAS and by NTS via LC-HRMS for potential PFAS. Thereby, during NTS analysis, different PFAS-specific feature prioritization approaches were correlated with quantitative EOF data to gain insights into the advantages and disadvantages of different NTS data treatment approaches for PFAS. To cover a representative range of environmental matrices, the samples investigated included a variety of low contaminated sediments (background levels) and highly PFAS contaminated samples originating from known and unknown pollution sources. For instance, selected soils were from agricultural sites or subject to contamination with PFAS used for paper coatings and fire-fighting activities (aqueous-film forming foams, AFFF) close to an airport. The sample pool includes soil samples from urban and non-urban areas. Besides the “traditional PFAS” with high fluorine content (*e.g.*, PFAAs, diPAPs, different AFFF classes), the agricultural samples might be also influenced by fluorinated pesticides which potentially add to EOF but are difficult to prioritize during NTS.

The determined EOF span a wide range of from $< \text{LOQ}$ up to $15030 \mu\text{g/kg}$ dry weight with a median and mean of 128 and $1445 \mu\text{g/kg}$, respectively (Table S2). After NTS preprocessing, between 202 and 5390 features with a median and mean of 668 and 1189 were detected via LC-HRMS with negative ESI (discussion of ESI^+ later). The applied parameters already included a componentization of related isotopes (total feature number after isotope componentization: 40431, Figure S2). As expected, no correlation between total feature number and EOF was observed ($R^2 = 0$, $r_{\text{Pearson}} = -0.06$; Figure S3), which was also true for $\sum \text{peak areas}$ of all features *vs.* EOF ($R^2 = 0.01$, $r_{\text{Pearson}} = -0.1$). A much better correlation was achieved using the MD/C-m/C dimension as restrictor. Since potential highly fluorinated features (high probability to be PFAS) were shown to be found in the vicinity of $m/C \approx 50$

(*e.g.*, $m/C_{\text{PFOA}} \approx 52$) and $MD/C \approx -0.003$, and only features in a range from m/C of 40 to 60 and MD/C -0.01 to 0.003 were prioritized.¹⁸ This allowed the subdivision of features with high probability to be PFAS although their identity might be unknown at this point. It should be highlighted here, that even a cutoff at $m/C = 40$ resulted in highly efficient prioritization (>98% data reduction) since only 1.6% of the 40431 features in all samples remained (Figure S2). After applying this prioritization strategy, a highly significant positive correlation of the feature number in all samples vs. EOF was observed ($R^2 = 0.87$, $r_{\text{Pearson}} = 0.93$, $p\text{-value} = 7 \times 10^{-16}$; Figure 1a). In this correlation, every feature regardless of its intensity has an identical contribution. This significant correlation allowed several conclusions about the prioritized features without their actual identification: (1) Most features in the prioritized range of the analyzed samples must be PFAS, since a significant number of other features would decrease the correlation coefficient. This is an important finding, since although there is a wide variety of elemental compositions that could locate in the prioritized MD/C - m/C range (*e.g.*, high chlorine, bromine, iodine, or oxygen content), they seem to play only a minor role in the investigated samples. (2) Within the sample pool, although qualitative, the number of prioritized features can be indicative for PFAS content and can therefore be used for sample prioritization. (3) Less fluorinated compounds (*e.g.*, fluorinated pesticides) potentially present in the samples cannot significantly contribute to the EOF since most of them are outside of the used MD/C - m/C range. For example, the 423 fluorinated pesticides from the NORMAN FLUOROPEST suspect list have a mean m/C of 22.6 while more than 95 % have an $m/C < 40$.²⁶ These conclusions are a result of the unique combination of quantitative EOF with NTS

data, since usually not all features can be identified, especially the ones with very low abundance which seem to play a minor role within the EOF as well.

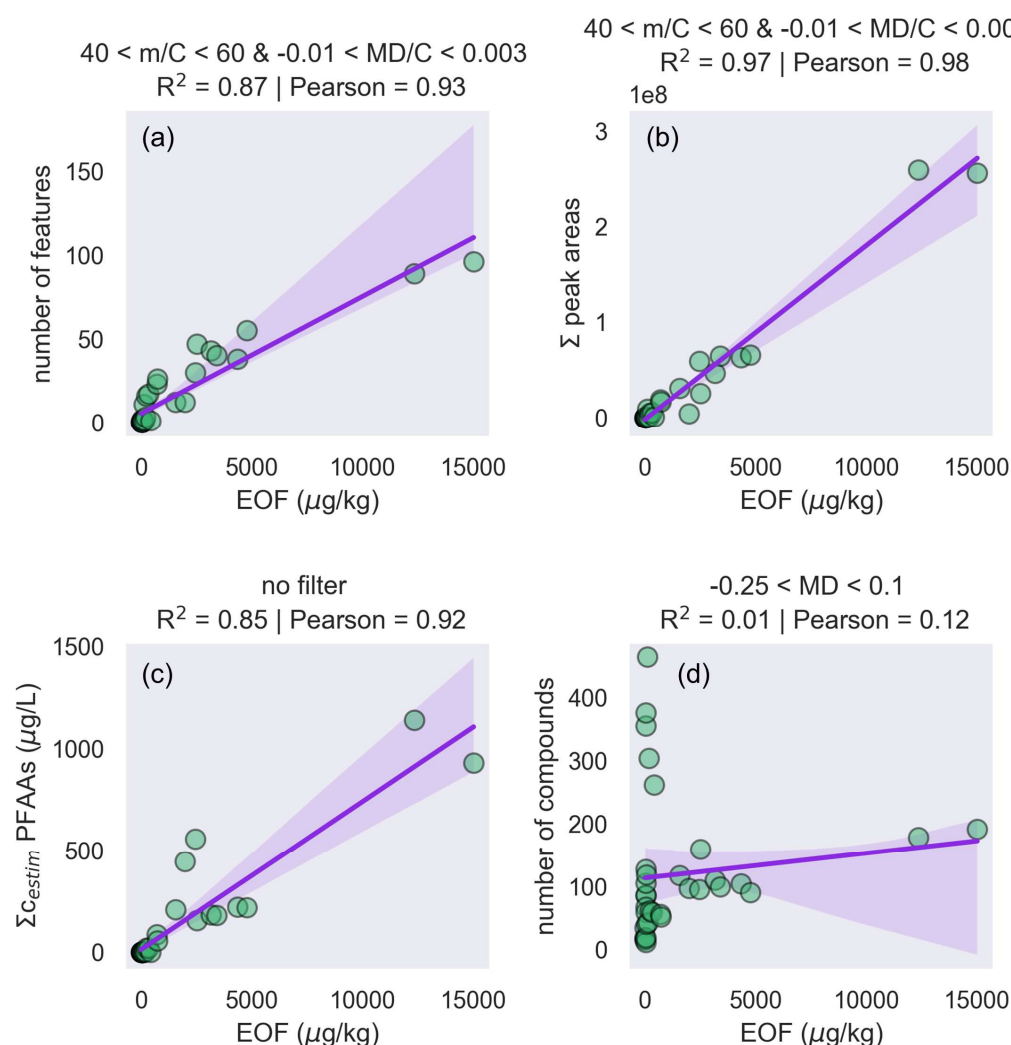


Figure 1: Correlation of different feature prioritization metrics (e.g., MD/C-m/C dimensions, ESI negative) vs. quantitative EOF data for 34 different environmental samples with and without known PFAS contamination. Shown are correlations of EOF vs. (a) number of detected features and (b) sum of peak areas within a range of $40 < m/C < 60$ and $-0.01 < MD/C < 0.003$. In subfigure (c) the correlation of the semi-quantified PFAs (C_4 - C_{18} PFAs and C_4 - C_{12} PFAs) and EOF mass fractions is shown. Further, the EOF was correlated with (d) the number of features after prioritization with a MD-range from -0.25 to $+0.1$ similar as used in other studies (e.g.,^{16, 17, 27, 28}) (sum of peak areas vs. EOF with MD filtering are shown in Figure S4). It becomes obvious that via the MD/C-m/C dimensions prioritized features correlated with EOF mass fractions show a high correlation coefficient indicating no significant fractions of non-PFAS can be present in this feature pool. Violet areas indicate the 95% confidence intervals of the linear regression.

To also correlate more quantitative information present in the HRMS data, the sum of peak areas of prioritized features were correlated with EOF (Figure 1b). Although the ionization response of organic substances in ESI spans several orders of magnitude,²⁹ a highly significant positive correlation was observed with EOF data ($R^2 = 0.97$; $r_{\text{Pearson}} = 0.98$, $p\text{-value} = 1 \times 10^{-25}$).

It should be noted that for this correlation the abundance of only a few features contribute mainly to the sum of the peak areas. For instance, in the three samples with the highest EOF, 50% of the total prioritized peak areas was composed of only 10%, 6% and 7% of the feature numbers in the prioritized space. This shows that peak areas of the most prominent prioritized PFAS features are representative of the majority of determined EOF in the investigated samples. In contrast, the semi-quantification approach with C₄-C₁₈ PFCAs and C₄-C₁₂ PFSAAs showed a slightly lower correlation ($R^2 = 0.85$; $r_{\text{Pearson}} = 0.92$, $p\text{-value} = 1 \times 10^{-14}$; Figure 1c). This result is surprising, as the estimated concentration (one-point calibration) of PFAAs via internal standards is associated with a higher analytical accuracy compared to using the prioritized feature number or Σ peak areas. An explanation could be that several samples are known to be dominated by other PFAS than PFAAs [*e.g.*, diPAPs, fluorotelomer sulfonic acids (FTSAs), N-ethyl perfluorooctane sulfonamide ethanol-based phosphate diester (diSAmPAP), fluorotelomer alkyl phosphate esters (FTMAPs), several substituted PFSAAs (*e.g.*, Cl-PFOS)] that contribute substantially to the determined EOF.²² This highlights that prioritization and semi-quantification via the MD/C-m/C dimension are associated with a good indication of the overall PFAS burden. Considering only PFAAs leads to an underestimation of the PFAS burden with a high uncertainty since variable contributions of unknown PFAS, often overlooked, can be present.³⁰

For the correlation between features prioritized only by typical MD-ranges (*e.g.*, -0.25 to +0.1) as already reported,^{16, 17, 27, 31} a much higher fraction of features that are not PFAS was present (high number of false-positives) (Figure 1d). When applying a MD range from -0.25 to +0.1, the feature number did not show a positive correlation with EOF ($R^2 = 0.01$; $r_{\text{Pearson}} = 0.12$) and also the correlation with Σ peak areas was much lower compared to the filtering via MD/C-m/C ($R^2 = 0.28$; $r_{\text{Pearson}} = 0.53$; Figure S4). This is probably a consequence of high matrix content in samples with high organic matter load. There were many features with masses both below 400

Da and above 800 Da that fall into the used MD range. The high masses often strongly exceeded a positive MD of +0.5 Da, which was erroneously assigned to a negative MD due to mathematical rounding.²² Those features were easily removed by their m/C ratio, however, not by this MD-range (much higher feature number). This is exemplified for the sample with the highest feature number originating from a soil influenced by smoldering fires (Figure S5). It should be noted that if a very narrow MD-range from -0.1 to 0 Da is applied, which somehow fits the prevailing PFAS pattern in the samples, a much higher correlation can also be achieved by MD filtering (Figure S6). However, background information on the kind of PFAS would be required to apply this narrow MD-range, and such narrow filtering increases the risk to exclude true positives which are more easily prioritized by the m/C ratio.

Similar correlations of MD/C-m/C prioritized features with EOF were observed in the samples for positive ESI measurements, however, in general much less features were detected in the prioritized MD/C-m/C range, indicating that most detectable PFAS are anionic (Figure S7).

Overall, the m/C ratio shows a much higher specificity towards PFAS and to the best of our knowledge, this was not yet reported for prioritized (unidentified) features from LC-HRMS data by complementary quantitative EOF data.

Optimization of prioritization within the MD/C-m/C dimensions. To determine ideal prioritization boundaries for the investigated samples, the correlation between EOF and NTS was calculated iteratively while changing the cutoff of m/C, MD/C (and MD) (Figure 2). For the investigated samples, a cutoff at $m/C > 25$ already resulted in a very efficient prioritization (high correlation over a wide prioritization range). This also means that, by applying the m/C dimension, also PFAS with a considerable H-content (m/C between 25 and 30) might be separable from the main organic compounds in those samples. For instance, in our data this would be possible for most PFAS with an $m/C > 25$ (Figure S2). In the case of MD-filtering, the false-positive rate is rather high over a wide MD-range and only a narrow MD-range results

in high correlation. This range, however, strongly depends on the type of PFAS (e.g., increased H-content) and it is more difficult to use the prioritized features as an indicator for the expected PFAS burden since too many compounds fall into a similar MD-range.

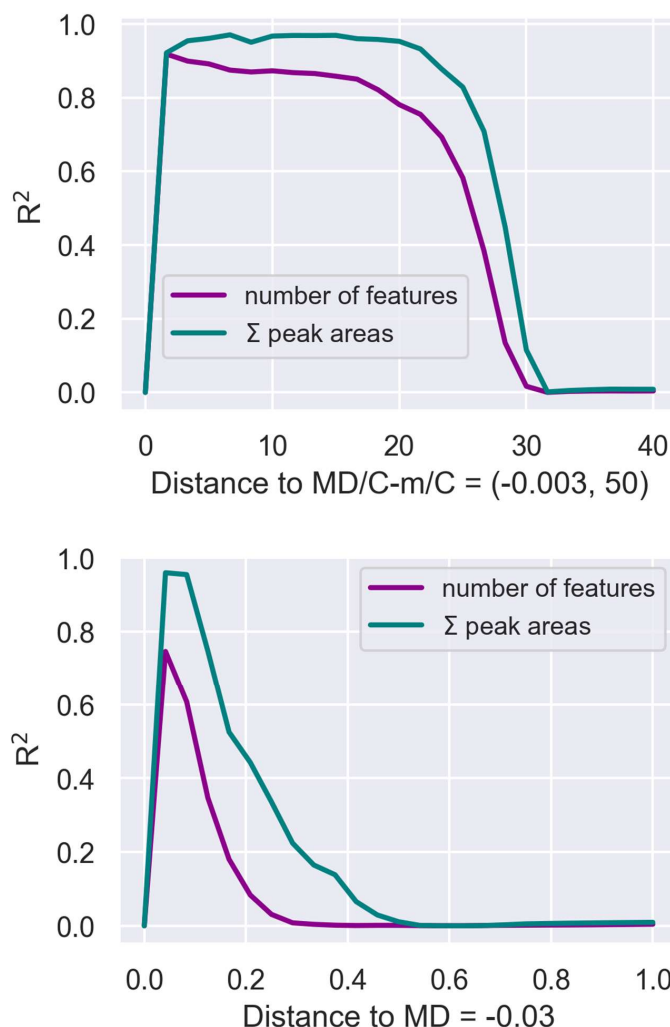


Figure 2: Calculation of optimal restrictions of MD/C-m/C and MD cutoff towards a high correlation of feature number and sum of peak areas with EOF. Top: R^2 vs. Euclidean distance of the prioritization boundaries to the CF₂-location (MD/C-m/C = -0.003, 50) in the MD/C-m/C plot. The range was restricted from m/C = 10 and 110 towards 50 and MD/C = -0.05 and +0.05 towards -0.003 in 25 iterations. Bottom: Similar calculations for MD as prioritization. Here, the MD range was restricted from -0.5 and +0.5 towards -0.03 (typical MD of e.g., PFOA). It should be noted that this only provides an overview of the present samples and is dependent on the present matrix compounds in the HRMS measurement. It becomes obvious that while the MD/C-m/C filtering correlates well over a wide prioritization range, in case of the MD only a very narrow (usually unknown) MD-window results in high correlation. Details on the calculations can be found in the SI.

Environmental implications. The present study highlights the unique complementary combination of PFAS-specific feature prioritization in HRMS data and quantitative EOF analyses. It showed for the first time that most features prioritized by the m/C and MD/C ratio

must originate from highly fluorinated compounds in different environmental samples (n = 34). As a result, we suggest that the EOF was composed of mainly highly fluorinated compounds as well. Thus, using this unique combination of techniques, our approach highlights possibilities for quantification for PFAS in NTS. We, therefore, recommend further investigation of this approach for environmental samples with a wider array of EOF and PFAS profiles, since sample prioritization based on the presented approaches is efficient without comprehensive and time-consuming identification. Future studies could extend correlations to further parameters such as the total oxidizable precursor (TOP) assay (indirect and direct) or absorbable organic fluoride (AOF) as well as complementary separation or ionization types (e.g., gas chromatography and chemical ionization) for HRMS.

Associated Content

Supporting information: SI

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Notes

¶JZ and FS contributed equally to this work and share first authorship.

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