Determination of transformation products of per- and polyfluoroalkyl substances at trace levels in agricultural plants

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Abstract

Per- and polyfluoroalkyl substances (PFASs) are ubiquitous in the environment. However, only a limited number of predominantly persistent perfluoroalkyl acids (PFAAs) have been analyzed in edible plants so far. We present a generic trace analytical method that allows for quantification of 16 intermediate fluorotelomer alcohol (FTOH)- or perfluoroalkane sulfonamidoethanol (FASE)-based transformation products as well as 18 PFAAs in plants. Additionally, 36 suspected intermediate PFAS transformation products were qualitatively analyzed. The ultrasound-assisted solid-liquid extraction of wheat and maize grain, maize leaves, Jerusalem artichoke and ryegrass (1-5 g plant sample intake) was followed by a clean-up with dispersive solid-phase extraction using graphitized carbon adsorbent (5-10 mg per sample) and chemical analysis by reversed phase liquid chromatography-tandem mass spectrometry. The method was based on matrix matched and extracted calibrations (MMEC) and displayed good precision with relative standard deviations in triplicate analyses typically below 15% for all quantified analytes and matrices. An average deviation of 12% between quantified concentrations obtained by MMEC and a method based on isotopically labelled internal standards underlines the good trueness of the method. The method quantification limits for the majority of analytes in all plant samples were in the low ng/kg concentration range. Plant matrices were analyzed from crops grown on agricultural fields that have been contaminated with PFASs. FTOH- and/or FASE-based intermediate transformation products were detected in all samples with *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA) and perfluorooctane sulfonamide (FOSA) as the prevailing compounds in concentrations up to several hundred ng/kg in maize leaves. The 9:3 Acid (a transformation product of 10:2 FTOH) was tentatively identified. In accordance with these findings, the final degradation products perfluorooctane sulfonic acid (PFOS) and perfluorodecanoic acid (PFDA) were frequently detected. For perfluoroalkyl carboxylic acids (PFCAs), according to earlier findings, short chain homologues generally displayed the highest levels (up to 98 µg/kg for perfluorobutanoic acid (PFBA) in maize leaves). However, maize grain was an exception showing the highest concentrations for long chain PFCAs, whereas PFBA was not detected. The uptake of high levels of PFASs into plants is of concern since these may be used as animal feed or represent a direct exposure medium for humans.

Keywords: PFAS transformation products; plant samples; fluorotelomer; perfluoroalkane sulfonamidoethanol; quantification

1 Introduction

Per- and polyfluoroalkyl substances (PFASs) are exclusively man-made compounds that have been produced in large volumes since the 1950s [1, 2]. Their unique ability to lower surface tension is exploited in a wide range of very useful industrial and consumer products including surfactants in fluoropolymer production, metal plating, fire-fighting foams and coatings of textile and paper products [1]. However, increasing evidence shows toxicity of numerous PFASs [3] as well as the ubiquitous presence of PFASs in the environment [2-4]. The final transformation products of many PFASs, the perfluoroalkyl acids (PFAAs), are extremely persistent in the environment. Furthermore, long-chain PFAAs are highly bioaccumulative and thus end up in biota like polar bears [5] and humans [3]. Their short-chain analogues preferably partition to the water cycle [6], from where they find their way into the food chain via uptake into plants [7].

In the federal state of Baden-Württemberg (in the south-west of Germany) a large-scale contamination of agricultural land with PFASs was discovered in 2013 [8]. The source of the contamination is likely to be paper sludge obtained from surface treated paper products brought out on the fields in the early 2000s. At that time, highly fluorinated phosphate esters (PAPs) were frequently applied for surface treatment of water- and grease-repellent products of paper and board [9]. These phosphate esters were typically based on n:2 fluorotelomer alcohols (FTOHs) (n:2 PAPs, also di- and tri-esters) of various fluorinated chain lengths (i. e. various even numbers of n) or on *N*-ethyl perfluorooctane sulfonamidoethanol (EtFOSE) (SAmPAP, usually the di-ester) [9].

Under aerobic conditions n:2 PAPs are hydrolyzed in soil and sludge to form n:2 FTOHs, followed by oxidation to n:2 fluorotelomer carboxylic acids (n:2 FTCAs), n:2 fluorotelomer unsaturated carboxylic acids (n:2 FTUCAs) and finally transformation to highly persistent C_n and C_{n+1} perfluoroalkyl carboxylic acids (PFCAs) [10-13]. In addition, (n-1):3 carboxylic acids ((n-1):3 Acids) and their unsaturated analogues ((n-1):3 UAcids) can also be formed [10, 12-14].

Also EtFOSE-based surfactants are degraded in the environment. The hydrolysis of SAmPAP leads to EtFOSE, which is further microbially transformed to *N*-ethyl perfluorooctane sulfonamidoacetic acid (EtFOSAA), *N*-ethyl perfluorooctane sulfonamide (EtFOSA) and the corresponding *N*-dealkylated substances (FOSE, FOSAA and FOSA). The final persistent transformation products are perfluorooctane sulfonic acid (PFOS) or PFCAs [15-17]. The degradation pathways are exemplarily illustrated in the literature for 6:2 diPAP [10] and SAmPAP diester [15].

Methods for FOSE- or more general perfluoroalkane sulfonamidoethanol (FASE)-based metabolites in plants have not been published so far and the uptake and translocation of these compounds have not been studied. Two published studies analyzed FTOH-based transformation products (FT(U)CAs, (n-1):3 Acids, and PFCAs) in plants. Zhang and co-workers exposed soybean hydroponically to 8:2 FTOH [18]. For sample preparation, the methanol extract of the freeze-dried plant tissues was split. Clean-up of one half was performed by solid-phase extraction (SPE) using a weak anion exchanger (WAX) before screening for acidic transformation products of 8:2 FTOH. The second half was cleaned with graphitized carbon adsorbent before determination of FTOHs. Both, the parent compound, as well as its metabolites were found in all parts of the plant. Bizkarguenaga et al. fortified compost-amended soil with 8:2 diPAP and cultivated carrot and lettuce [19]. Ultrasound-assisted extraction was performed with acetonitrile before clean-up with SPE (WAX). Intermediate transformation products were not detected in the plants, however, a suite of PFCAs were quantified in carrots and perfluorooctanoic acid (PFOA) was found in lettuce. PFAA uptake and translocation has earlier been observed in, e. g., tomato [20] and zucchini [21].

Understanding the potential uptake of FTOH- and FASE-based PFASs and their transformation products into edible plants is a crucial prerequisite for an effective risk assessment in agricultural contamination cases. This requires ultra-trace analytical methods for a suite of (intermediate) PFAS transformation products. The only two published studies to date that are relevant in this respect are described above [18, 19]. However, none of these studies investigated FASE-based substances and both studies used fortification with PFASs to investigate transformation behavior. Hence, no analytical method to measure a broad set of intermediate transformation products of FTOH- and FASE-based compounds in complex matrices is available, yet, or has been applied to study an environmental contamination case. We therefore aimed to develop a quantitative trace analytical method for these compounds (including PFCAs and perfluoroalkane sulfonic acids (PFSAs)) in plant samples. The method was applied to wheat and maize grain, maize leaves, Jerusalem artichoke and ryegrass grown on the contaminated fields in Baden-Württemberg.

2 Materials and methods

2.1 Chemicals and reagents

The target analytes comprised transformation products of fluorotelomer- and perfluoroalkane sulfonamidoethanol-based precursors (see introduction). We focused on compounds with a

chain length between four and sixteen perfluorinated carbon atoms, since these chain lengths have been shown to occur in PAPs used in coatings of paper products [22]. The following substance classes were included: n:2 FTCAs, n:2 FTUCAs, (n-1):3 Acids, (n-1):3 UAcids, (alkyl)FASEs, (alkyl)FASAAs, and (alkyl)FASAs, whereby 'alkyl' could be methyl (Me), ethyl (Et), or missing (i. e. just hydrogen). A total of 16 commercially available authentic reference standards were used in this study together with six stable isotope-labelled compounds used as internal standards (IS). All standard chemicals were purchased from Wellington Laboratories (Guelph, ON, Canada) with a purity of >98% and are listed in Table S1 in the Supplementary Material (SM) together with their acronyms according to Buck et al. [2]. In addition, also the final transformation products, the PFCAs and PFSAs, were analyzed using the same extracts (see section 2.8). Ultrapure water from a Milli-Q system (Merck KGaA, Darmstadt, Germany) was used. LC/MS grade methanol (MeOH), acetonitrile (ACN) and ammonium acetate (NH_4OAc) were obtained from Biosolve (Valkenswaard, the Netherlands).

2.2 Stability of analytes

The stability of standard solutions of the analytes during typical sample processing times (two to four days) and conditions was investigated for at least one compound from each substance class (except (n-1):3 UAcids for which no standard was available) by preparing duplicate individual standard solutions at a concentration of 10 ng/mL in MeOH. One replicate for each compound was stored at daylight and room temperature and the second one in the dark at -18 °C. Signal areas obtained by liquid chromatography-tandem mass spectrometry analysis (LC-MS/MS, see below) were compared after 7, 14 and 21 days.

2.3 Samples

Wheat (*Triticum L.*) grain, maize (*zea mays*) grain and leaves, and Jerusalem artichoke (*Helianthus tuberosus*) were each grown on two different agricultural fields in the area of Rastatt in the Upper Rhine Plain, Baden-Württemberg, Germany. One field was contaminated with PFASs through application of paper fiber biosolids [8], while the other was an uncontaminated control field. Ryegrass (*Lolium perenne*) was cultivated in the lab on contaminated as well as uncontaminated soil taken from the field. An overview of the plant samples used in the present study is given in Table 1. The drying and homogenization procedures for the plant samples are described in detail elsewhere [23]. In brief, after harvest plants were cut in small pieces, dried (by air drying, freeze drying, or in a convection oven at 60 °C), homogenized in a cryogenic grinder, and stored at 6 °C. For method development the

entire uncontaminated Jerusalem artichoke was cut, freeze dried and homogenized while for chemical analysis of contaminated samples the Jerusalem artichoke skin and the peeled Jerusalem artichoke were processed separately.

2.4 Extraction and clean-up

For this study no laboratory tools or vessels containing fluoropolymer parts were used in order to avoid blank contamination. Prior to sample extraction the IS (10 ng of each standard) including IS for PFCAs and PFSAs (see section 2.8 below) were spiked as follows. The homogenized plant samples were placed in a 50 mL polypropylene (PP) centrifugation tube and suspended in ACN. After spiking with 10 ng of each standard in MeOH, the tube was vortex-mixed for 10 s and then left open at room temperature for 72 h for the solvent to evaporate to dryness.

2.4.1 Solid-liquid extraction

Sample extraction was based on solid-liquid extraction with ACN in three cycles. The sample intake and ACN volume for each cycle of extraction was optimized matrix-specifically (Table 1). The sample suspended in ACN was vortex mixed for 10 s, ultra-sonicated for 15 min at room temperature, and placed into a 360 degree rotary mixer for 15 min at 25 rounds per minute (rpm). After centrifugation for 5 min at 5,000 rpm, the supernatant was transferred to a 50 mL PP tube. The extraction was repeated twice as described above but with a reduced duration of 5 min for ultra-sonication. Finally, the combined extracts were concentrated to 1 mL at room temperature under a gentle stream of dry nitrogen. Concentration to dryness was avoided to prevent loss of volatile (neutral) analytes.

2.4.2 Clean-up by dispersive solid phase extraction

The concentrated extract was transferred into a 2.5 mL PP centrifugation tube before adding a matrix-specific amount (Table 1) of granular graphitized carbon (Supelclean ENVI-Carb 120/400, Supelco, Sweden). After vortex mixing for 30 s and microcentrifugation for 2.5 min at 14,000 g, the supernatant was carefully removed and filtered through a syringe filter (0.2 µm membrane, PP housing, Minisart RC4, Sartorius, Germany) into another 2.5 mL cetrifugation tube. The filter was washed twice with 150 µL of ACN. The combined solutions were concentrated to approximately 500 µL under a gentle stream of dry nitrogen at room temperature and the extract was weighed to determine the final volume gravimetrically. Extracts were stored at 8 °C until instrumental analysis. In case precipitation occurred during storage, the extracts were microcentrifuged for another 2.5 min at 14,000 g and only the clear supernatant was transfered to a PP autoinjector vial for LC-MS/MS analysis.

2.5 Instrumental analysis

Chemical analysis of sample extracts was performed by LC-MS/MS employing a UPLC Acquity I-Class system coupled to a tandem mass spectrometer (Xevo TQ-S) equipped with an electrospray ionization (ESI) source (all from Waters). An Acquity UPLC BEH Shield RP18 column (50 x 2.1 mm, 1.7 µm particle size, Waters) was used for chromatographic separation in reversed phase mode at 40 °C at a flow rate of 0.4 mL/min. A 'PFC Isolator^{TM}' column (50 x 2.1 mm, Waters) was placed between the eluent mixer and the injector to trap and delay elution of potential background contamination from the eluents or the UPLC system. The injection volume was 2.5 µL. The applied mobile phase gradient was taken from literature [24] and is given in Table S3 in the SM. MS analysis was carried out in negative ion multiple reaction monitoring (MRM) mode. MRM transitions of compounds with available authentic reference standards were optimized by direct infusion and are displayed in Table S4 in the SM. MRM transitions of target analytes without available reference standard were extrapolated from MRM transitions of structurally similar compounds, usually homologues (Table S5 in the SM).

2.6 Quantification

Signal integration and quantification were carried out using the software MassLynx V4.1 (Waters). Two quantification procedures were applied, i. e. matrix matched and extracted calibration (also known as procedural calibration) and the internal standard (also known as isotope dilution) method. All analytes with reference standards were quantified by matrix matched and extracted calibration using the calibration curves described in section 2.7.2 below. The calibration curve of Jerusalem artichoke was applied for quantification of both Jerusalem artichoke skin and peeled Jerusalem artichoke. Internal standard quantification was additionally performed for FOSA, EtFOSA, MeFOSE, MeFOSAA, 8:2 FTCA and 6:2 FTUCA (as well as for all PFCAs and PFSAs, see section 2.8), which were the analytes with authentic isotope labelled IS in this study. Analytes without reference standards were only analyzed qualitatively (detected with a certain level of confidence (see section 3.4) or non-detected).

2.7 Method validation

A full method validation was made for the FTOH- and FASE-based intermediate transformation products. The following sections 2.7.1 to 2.7.5 thus refer to these analytes. Quantification of PFCAs and PFSAs is described briefly in section 2.8. A full validation was

not performed for the PFAAs since the use of authentic isotopically mass-labeled internal standards for almost all PFAAs assures good quality of the quantified concentrations.

2.7.1 Instrumental blanks, instrumental detection limits and linear range

Instrumental background contamination ("blanks") was evaluated by injection of 2.5 µL pure solvent into the LC-MS/MS system. To obtain instrumental detection limits (IDL) and the linear range of the mass spectrometric detection a dilution series of the standards at 10, 20, 50, 100, 250, 500, 750, 1000, 2000 and 5000 pg/mL in pure ACN (5 µL injection volume) was analyzed. Due to absence of blank contamination for all analytes, the IDL was defined as the lowest injected amount of analyte leading to a signal in the chromatogram with a signal to noise ratio of at least three (S/N ≥3).

2.7.2 Procedural blanks, method detection and quantification limits

Analysis of plant samples grown on uncontaminated soil (see section 2.3) did not show detectable concentrations of any of the analytes. Thus, procedural background contamination did not occur above the method detection limit. Method detection limits (MDLs, in µg/kg sample) were therefore derived from the matrix matched and extracted calibration (5 to 10 calibration points, extracted in duplicates, see Tables S6-S8 in the SM for spike concentration ranges) based on the uncontaminated plant samples. The MDL, derived by rational means, was defined as the lowest spike concentration showing a signal in the chromatogram with S/N ≥3. The method quantification limit (MQL), derived by rational means, was defined as the lowest spike concentration showing a signal in the chromatogram with S/N ≥10 and deviating less than 20% from the matrix matched and extracted linear fitted calibration curve (fit weighting 1/x). To validate the "rational" MDL and MQL values, MDLs and MQLs were additionally determined according to DIN standard 32645 for maize grain and leaves.

2.7.3 Recoveries and matrix effects

Recoveries (covering the whole sample extraction and preparation procedure) and matrix effects were repeatedly evaluated during method development and optimization and in the validation of the final method. In order to consider sample preparation recoveries of the target analytes independently from matrix effects, mean signal areas in the extracts of spiked, uncontaminated plant samples were compared to mean signal areas in spiked extracts of uncontaminated samples. Spiking was performed with 10 ng each of the 16 reference standards (Table S1 in the SM) to the matrix-specifically optimized sample amounts (Table 1), as described above in section 2.4. For the determination of matrix effects on instrumental analysis of the target compounds, mean signal areas in spiked extracts of uncontaminated

samples were compared to mean signal areas of the analytes injected in pure solvent. Matrix effects are expressed as the ratio of the respective signal areas, i. e. a value of 1 means no matrix effect, while values >1 and <1 indicate signal enhancement and suppression, respectively, by the presence of co-extracted sample constituents.

2.7.4 Precision

The precision of the method was evaluated by triplicate analysis (intra-day) of all contaminated plant samples and is expressed as relative standard deviation (RSD) of the quantified concentrations. However, since only relatively few analytes were detectable in the contaminated samples (see section 3.4), repeatability was additionally determined using samples spiked with IS. Contaminated samples (triplicate analysis as described above) and uncontaminated samples (ten repetitions from the matrix matched and extracted calibration curves for all plant types except for Jerusalem artichoke for which 16 repetitions were prepared) were spiked with the six IS before extraction and the RSD of signal areas of the IS in the chromatograms of the final extracts were determined as a quantitative measure of repeatability.

2.7.5 Trueness of quantification

Certified or consensus reference plant materials with the target analytes were not available. The trueness of quantification was therefore evaluated by comparing the quantified concentrations of FOSA, EtFOSA, MeFOSE, MeFOSAA, 8:2 FTCA and 6:2 FTUCA (i. e. the analytes with authentic IS in this study) in contaminated samples obtained by matrix matched and extracted calibration with the results obtained using the independent internal standard method. The latter is generally considered to be the most accurate quantification method using mass spectrometric detection, since it corrects for varying recoveries and matrix effects on a compound- and sample-specific basis.

2.8 Analysis of perfluoroalkyl carboxylic and perfluoroalkane sulfonic acids

A total of 13 PFCAs and four PFSAs were quantified in the contaminated plant samples together with the intermediate transformation products (in the same extracts) using the internal standard method. These two compound classes represent the terminal, stable transformation products from fluorotelomer- and FASE-based precursors, respectively. All reference compounds and isotope labelled IS (Table S9 in the SM) were purchased from Wellington Laboratories (Guelph, ON, Canada) with a purity of >98%. The applied MRM transitions are shown in Table S10 in the SM. MRM transitions of perfluoropentadecanoic acid (PFPeDA, no reference standard available) were extrapolated from MRM transitions of

homologues. The following analytes were quantified using structurally similar IS, since authentic isotope labelled standards were not available: PFTrDA (^{13}C-PFDoDA), PFTeDA (^{13}C-PFDoDA), PFPeDA (^{13}C-PFDoDA, calibration curve for PFTeDA), PFHxDA (^{13}C-PFDoDA), PFODA (^{13}C-PFDoDA), PFBS (^{18}O-PFHxS) and PFDS (^{13}C-PFOS) (for full compound names see Table S9 in the SM).

3 Results and discussion

3.1 Pre-experiments and method development

Fluorotelomer- and FASE-based intermediate transformation products are highly variable in their physico-chemical properties such as charge, pK_A, $logK_{OW}$ or volatility. Thus, the extraction and clean-up procedure needed to be relatively generic and was optimized with regard to a compromise between best possible recoveries, minimizing matrix effects, and achieving low MDLs/MQLs.

For method development the 16 target analytes shown in Table S1 in the SM were employed. The stability of these compounds was experimentally demonstrated (see section 2.2). None of the tested compounds were found to degrade in methanol under the test conditions, thus a potential bias by transformation during sample treatment was not expected. A possible reduction of standard stability in matrix extracts was considered unlikely and not further investigated.

Furthermore, no in-source fragmentation of FTCAs to their unsaturated, co-eluting FTUCA analogues with the same chain length was observed during mass spectrometric detection. Thus, the selectivity of the MS/MS detection was sufficient to distinguish between these structurally very similar compound classes.

Potential losses of the semi-volatile (alkyl)FASAs and (alkyl)FASEs during the different sample drying procedures were not evaluated. The quantified concentrations for these PFASs may thus underestimate the levels originally present in the samples before drying.

Ultrasound-assisted solid-liquid extraction was performed, since this approach has been shown to be highly efficient in numerous studies for a wide variety of different PFASs and sample types [25, 26]. Evaporation to dryness in any sample processing step led to severe recovery losses for (alkyl)FASAs and (alkyl)FASEs and was thus avoided throughout sample preparation. These two compound classes have been reported as volatile in literature [27].

3.2 Selection of the clean-up procedure

A clean-up of all extracts was needed, since complex environmental samples like plant tissue typically display strong matrix effects during analysis by LC-MS with electrospray ionization. The two methods described in literature so far for analysis of FT(U)CAs, (n-1):3 Acids, and PFCAs in plants employed SPE with a weak anion exchange sorbent [18, 19]. We compared different frequently applied approaches for the clean-up procedure, including SPE [11, 28], the QuEChERS (quick easy cheap effective rugged and safe) method [29], a modified version [30] of the ion-pairing method originally developed by Ylinen et al. [31], and dispersive solid-phase extraction using ENVI-Carb graphitized carbon adsorbent as previously applied in various studies [24, 32, 33]. Details of the SPE, the QuEChERS, and ion-pairing method are given in section 1.2 in the SM. Even though we tested SPE with two different mixed mode sorbents (Oasis WAX, Waters and CUNAX22Z, UCT) in conjunction with a large set of solvents of different elution strengths, we observed poor recoveries <10% for both (alkyl)FASAs and (n-1):3 Acids (data not shown) from plant samples. Extracts that were instead cleaned-up with the ion-pairing method still showed massive matrix effects during instrumental analysis for a number of analytes. Due to signal suppression, some analytes were not even detected when spiked into the extracts. SPE and ion-pairing were thus rejected as clean-up methods. In contrast, satisfactory recoveries of all analytes were obtained using ENVI-Carb for clean-up of the extracts. However, strong matrix effects were still observed for many analytes (see below for detailed discussion). Using the QuEChERS method alone resulted in poorer recoveries and even larger matrix effects than for the ENVI-Carb method. An additional clean-up with ENVI-Carb reduced the matrix effects only slightly but also compromised the recoveries. Therefore, we decided to use ENVI-Carb as the only clean-up method for all plant extracts. Figure S1 in the SM compares recoveries and matrix effects for all test compounds extracted from wheat grain and Jerusalem artichoke after extract clean-up with ENVI-Carb alone, QuEChERS alone, and a combination of QuEChERS and ENVI-Carb.

3.3 Optimization and validation of the extraction and clean-up method applying ENVI-Carb

3.3.1 Instrumental blanks, instrumental detection limits and linear range

None of the 16 compounds with available reference standards were detected in the instrumental blanks. Compound specific IDLs are given in Table S11 in the SM. The linear calibration range of the MS/MS instrument covered the entire tested range between the

individual IDLs and 25 pg injected with a Pearson correlation coefficient of the linear regression of at least 0.95 (mean 0.99).

3.3.2 Method detection and quantification limits

As procedural blank contamination did not occur (see section 2.7.2) the MDLs and MQLs were solely a function of sample intake, recovery, matrix effects, instrumental sensitivity and chromatographic noise. Values of MDL and MQL obtained by "rational means" and according to DIN standard 32645 were well comparable in extracts of maize grain and maize leaves and differed from each other on average by less than a factor of three (Tables S12 and S13 in the SM). Rationally derived MDLs and MQLs of MeFOSE and all carboxylic acids excluding 8:2 and 10:2 FTUCA were equal in maize leaves due to our definition of these values in the "rational" approach (see section 2.7.2). Generally, MDLs and MQLs were in the ng/kg range in all tested plant matrices (Tables S12 to S16 in the SM). In literature [18, 19], notably higher MDLs were reported compared to our study. For 6:2 FTUCA 0.3 µg/kg (carrot) and 4 µg/kg (lettuce) vs. 0.004-0.08 µg/kg (our study), for 8:2 FTUCA 0.3 µg/kg (carrot), 3 µg/kg (lettuce), and 0.06 µg/kg (soybean) vs. 0.001-0.5 µg/kg (our study), for 8:2 FTCA 0.3 µg/kg (carrot), 3 µg/kg (lettuce), and 0.22 µg/kg (soybean) vs. 0.03-0.8 µg/kg (our study), and for 7:3 Acid 0.4 µg/kg (carrot), 3 µg/kg (lettuce), and 0.08 µg/kg (soybean) vs. 0.002-0.04 µg/kg (our study).

3.3.3 Recoveries and matrix effects

Sample preparation recoveries and matrix effects on ionization were determined according to section 2.7.3. The spiked amount of 6:2 FTCA did not exceed the MQL in most plant types, due to particularly high MQLs for this analyte (Tables S12 to S16 in the SM). Thus, 6:2 FTCA was not evaluated. However, the FTCAs were represented by 8:2 and 10:2 FTCA. The influence of sample intake and amount of ENVI-Carb used in extract clean-up on recoveries and matrix effects is exemplarily illustrated for maize leaves and Jerusalem artichoke in Figure 1 and for the other tested plant matrices in Figure S2 in the SM. With decreasing sample intake increasing recoveries were achieved for maize leaves (Figure 1a). This also holds true for ryegrass and Jerusalem artichoke (Figure S2 in the SM). Maize leaves and ryegrass are very bulky matrices, thus the increase in recoveries was most likely due to the increase in volume of extraction solvent relatively to the sample amount (and thus less loss of extract in the samples after centrifugation). Consequently, only 1 g of these matrices was extracted in the optimized procedure (Table 1). Jerusalem artichoke aggregated to a solid chunk during evaporation of the ACN (applied to suspend the sample before spiking with IS)

and required mechanical crushing with a specula prior to the extraction procedure. This effect was less pronounced with a lower sample intake. At the same time (as expected) matrix effects decreased with decreasing sample intake; however, even with the final sample intake of 1 g dry weight strong signal suppression (especially for EtFOSE) was observed. Similarly strong matrix suppression for EtFOSE was also observed in extracts of the other tested plants (Figure S2 in the SM). These observations necessitate a quantification approach that takes account of the strong and highly varying matrix effects. The influence of varying amounts of ENVI-Carb in the clean-up of the extracts on recoveries and matrix effects was minor, as representatively shown for Jerusalem artichoke (Figure 1b) and in Figure S2 in the SM. Thus, in the final extraction protocol (Table 1) we consistently applied the lowest tested ENVI-Carb amount in order to assure best possible recoveries during the clean-up procedure. Compound- and matrix-specific recoveries for the finally applied methods are given in Table 2 and matrix effects in Table S17 in the SM.

Zhang and co-workers reported recoveries for isotope-labeled 8:2 FTCA and 8:2 FTUCA from soybean in the range of 68-106% [18]. In the study by Bizkarguenaga et al. only apparent recoveries from carrot and lettuce were presented, hampering a direct comparison with our results [19]. Matrix effects were not presented or discussed in any of these two studies.

3.3.4 Precision

The repeatability of the signal areas of the six IS spiked to uncontaminated plant samples in the preparation of the matrix matched and extracted calibration (n=10 except for Jerusalem artichoke n=16) was used as one measure of precision. Mean RSD for all IS were 9.9% for wheat grain, 5.2% for maize grain, 8.0% for maize leaves, 15% for Jerusalem artichoke, and 9.9% for ryegrass, indicating a high degree of repeatability of the sample preparation and instrumental analytical method. Compound- and matrix-specific results are listed in Table S18 in the SM. The aggregation of Jerusalem artichoke and crushing with a specula prior to the extraction procedure (see section 3.3.3) may be the reason for the slightly elevated RSD in comparison to the other plant samples. Comparable results for repeatability were also obtained considering the signal areas of the IS in the repeated analyses (n=3) of the contaminated samples, with mean RSD of 12% for wheat grain, 4.2% for maize grain, 7.3% for maize leaves, 13% for peeled Jerusalem artichoke, 6.2% for Jerusalem artichoke skin, and 11% for ryegrass (Table S19 in the SM). The high degree of repeatability of IS signal areas is visualized for maize grain as example in Figure 2 and for all other plant matrices in Figure S3 in the SM.

However, analysis of the IS spiked onto the samples 72 h prior to extraction may not fully represent the situation of analytes that were taken up by the plants or formed as transformation products in the plants because the uptake process in the plant is not replicated. Precision of the whole method was thus also evaluated considering quantified concentrations of the analytes in the triplicate analyses of the contaminated samples. The RSD of all quantified values above the respective MQLs are given in Table 3 with a median RSD of 9% and a mean RSD of 17% (mainly driven by one high value of 87% for EtFOSAA in Jerusalem artichoke skin). These values mirror the results obtained for the IS and confirm good precision also for analytes embedded in the matrix at levels down to the MQLs of few ng/kg sample dry weight.

3.3.5 Trueness of quantification

The two independent quantification methods, i. e. matrix matched and extracted external calibration and the internal standard method, yielded well comparable concentrations for the six analytes (with available isotope labelled IS) in the contaminated samples (Table 3). The percentage deviations between the results from the two quantification methods are shown for all detected compounds (including results between the MDL and MQL) and all matrices in Table S20 in the SM. On average the quantified concentrations by the two approaches differed by 12% only, indicating a good trueness of quantification even for detected concentrations below the respective MQLs.

3.4 PFASs in contaminated plant samples

The optimized extraction and clean-up methods were applied to samples of maize grain, maize leaves, peeled Jerusalem artichoke, Jerusalem artichoke skin, wheat grain, and ryegrass, all grown on contaminated fields. All samples were analyzed in triplicates. We quantified the 16 target PFASs with available reference standards (Table 3) and additionally conducted qualitative suspect screening for another 36 representatives of fluorotelomer- and FASE-based transformation products without commercially available reference standards using theoretically derived MRMs for mass spectrometric detection (see Table S5 in the SM). We introduced five levels (a-e) of identification confidence for the analytes lacking reference standards with level “a” indicating highest confidence (Table 4). Results of the suspect screening are given in Table 4 (only detected suspects) and Table S21 in the SM (full list of suspects). Additionally, we also quantified 14 PFCAs and four PFSAs in the same extracts (see section 2.8). Results are summarized in Table S22 in the SM.

Both fluorotelomer- and FOSE-based intermediate transformation products were quantified in different plant samples in relatively low concentrations, all in the ng/kg range (Table 3). However, frequency of detection and concentrations differed amongst the different plant types and parts. Maize leaves were identified as the samples showing the highest contamination concerning both the number and levels of detected analytes. Compounds present in both maize grain and leaves displayed on average a 125-fold higher concentration in the leaves pointing towards uptake with water and accumulation through evaporation of the water from the leaves.

The intermediate FOSE-based transformation products EtFOSAA and FOSA were detected in all plant matrices with EtFOSAA consistently being among the intermediate transformation products with the highest concentration. This indicates that a significant part of the PFAS contamination on the fields is FOSE-based, most likely FOSE-based phosphoric acid esters, which have been used in coatings for paper and board [9]. The relatively high levels of FOSA and especially EtFOSAA are in agreement with literature in which these two compounds were identified as the intermediate transformation products of EtFOSE displaying the longest half-lives during aerobic degradation in activated sludge [17]. Further, this picture is completed by the presence of PFOS in all plant samples that contained concentrations of EtFOSAA >0.01 µg/kg (Table S22 in the SM). PFOS is the final stable biodegradation product of EtFOSE and was recently identified as a metabolite, in this case of FOSA, in a soil/plant environment [34]. In the study of Bizkarguenaga et al., FOSA was only biotransformed to PFOS in the presence of a crop.

The long chain compound 9:3 Acid was tentatively identified (confidence level “a”) in maize leaves, Jerusalem artichoke skin, and wheat grain (Table 4) and thus is one of the fluorotelomer-based intermediates with the highest detection frequency. Confidence level “a” implies that the signal was detected with two MRM transitions and that the retention time was close to the predicted retention time, resulting in a high probability of correct identification. This finding is further corroborated by the detection of PFDA (being a stable transformation product of 9:3 Acid) in all plant samples and with the highest concentrations (reaching µg/kg) among long chain PFCAs (Table S22 in the SM).

The PFCA patterns found in the plant samples, as the terminal degradation products of fluorotelomer-based precursors, were characterized by high concentrations of short chain compounds, with up to 98 µg/kg for PFBA in maize leaves and up to 124 and 94 µg/kg for perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA), respectively, in ryegrass. This is in agreement with literature that identified short chain PFAAs as the

compounds that are predominantly transferred from the roots to the aerial plant parts [19, 35], likely due to their higher water solubility and mobility compared to longer chain analogues [11]. However, in our study maize grain represents an exception. PFBA, PFPeA, and PFHxA were all <MDL in maize grain, even though many long chain homologues were quantified with PFDA as the predominant PFCA at 2.65 µg/kg (Table S22 in the SM). The pattern looks very different in maize leaves with PFBA as the prevalent PFCA at 98 µg/kg. There seem to be plant part-specific uptake barriers that may even discriminate short chain PFCA homologues.

Blaine et al. [20] measured higher concentrations of most investigated PFAAs in shoot or fruit than in root of tomato, radish, celery, and pea. Also in hydroponically grown cabbage, tomato, and zucchini, an uptake of PFAAs by roots and further distribution of predominantly short chain homologues to leaves and fruits was observed [21]. For PFOA and PFOS higher accumulation in the vegetative portion of oat, spring wheat, and maize than in their storage organs was reported [36], while Wen et al. [37] measured higher concentrations of PFAAs in roots than in shoots of wheat. The observed tissue dependent bioaccumulation potential for different classes and homologues of PFASs in our study as well as in literature underlines the need for an independent risk assessment for each plant part.

4 Conclusions

A trace analytical method for the identification and quantification of fluorotelomer- and FASE-based transformation products in plants was developed. It allows quantification of a total of 34 PFASs (16 intermediate transformation products and 18 PFAAs) and qualitative screening of another 36 PFASs in the same extract. To accommodate so many different compounds with a wide variety of physical-chemical properties, the clean-up had to be generic, which partly led to strong matrix effects. The quantification procedure thus has to take matrix effects into account. The developed generic method showed good accuracy (precision and trueness) and can potentially be extended to further groups of PFASs.

Applying the method to plant samples grown on contaminated fields confirmed the uptake of both intermediate transformation products as well as persistent PFAAs. We demonstrated, that FOSE-based precursor PFASs are important constituents of the contamination case in south-west Germany. Accumulation of PFASs in plants grown for human consumption is of concern even if comprehensive toxicity data for many transformation intermediates are lacking. However, FTCAs and FTUCAs have shown acute and chronic toxicity to aquatic invertebrates and green algae [38-40]. Fortunately, in our study edible plant parts like the

grain of maize and wheat were much less contaminated than maize leaves. Other leafy vegetables like lettuce were not investigated.

Perspectively, studies on the uptake mechanisms and possible transformation pathways of PFASs in plants should be undertaken in order to better understand and predict the fate of these anthropogenic chemicals in the environment. Such studies can also help to understand if phytoremediation of contaminated acres could be a viable alternative to excavation.

Acknowledgments

This work was financially supported by the Ministry of the Environment, Climate Protection and the Energy Sector Baden-Württemberg, Germany within the EOFplus project (project no. L75 17016). The authors would like to thank Jörn Breuer, Daniel Beiser and Melanie Zoska for providing the plant samples. Elaine Jennings is thanked for language editing.

Appendix A. Supplementary material

Detailed information on chemicals, MS/MS analysis, matrix matched and extracted calibrations, IDLs, MDLs, MQLs, matrix effects, precision, trueness, as well as results of qualitative and quantitative analysis are given in the Supplementary Material.

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659 **Table 1.** Overview of plant samples as well as matrix-specifically optimized sample intake,
660 solvent volume, and amount of ENVI-Carb used in the extraction and clean-up procedure.

Sample	Extracted sample amount (g)	Volume of ACN in three cycles of extraction (mL)	Amount of ENVI-Carb (mg)
Wheat grain	5	8 / 5 / 5	5
Maize grain	5	6 / 6 / 6	10
Maize leaves	1	10 / 3 / 3	10
Jerusalem artichoke skin	2	7.5 / 5 / 5	10
Jerusalem artichoke peeled	2	7.5 / 5 / 5	10
Jerusalem artichoke (whole)	2	7.5 / 5 / 5	10
Ryegrass	1	10 / 7 / 7	10

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Table 2. Compound- and matrix-specific sample preparation recoveries (in %) of the reference compounds after extraction and clean-up of spiked, uncontaminated plant samples (spiking range 2-10 µg/kg). In cases where the experiment was performed in duplicate both values are given individually.

	Maize grain	Maize leaves	Wheat grain	Jerusalem artichoke	Ryegrass
FOSA	84	71 / 65	79	76 / 71	68 / 57
MeFOSA	63	66 / 59	62	74 / 70	52 / 50
EtFOSA	67	66 / 59	67	65 / 66	52 / 47
MeFOSE	68	72 / 55	69	87 / 83	66 / 53
EtFOSE	80	67 / 68	84	104 / 103	55 / 43
FOSAA	62	47 / 44	72	77 / 75	59 / 53
MeFOSAA	70	58 / 56	78	82 / 81	65 / 55
EtFOSAA	74	61 / 56	83	80 / 83	67 / 59
8:2 FTCA	51	51 / 43	64	76 / 72	43 / 31
10:2 FTCA	59	45 / 43	69	73 / 73	57 / 48
6:2 FTUCA	35	54 / 48	55	71 / 71	45 / 43
8:2 FTUCA	42	54 / 48	65	78 / 75	45 / 36
10:2 FTUCA	53	55 / 50	74	88 / 86	52 / 43
5:3 Acid	64	57 / 51	52	57 / 64	47 / 49
7:3 Acid	65	61 / 53	63	68 / 68	51 / 44

Table 3. Quantified concentrations (on dry weight basis) and RSD (*n*=3) of the target analytes in contaminated plant samples derived by matrix matched and extracted calibration (**bold**) and the internal standard method (*italic*), respectively. All values above MDL were quantified, however, values between the MDL and the MQL are set in parentheses. Empty cells are non-detects (<MDL, see Tables S12-S16 in the SM for MDLs).

	Wheat grain [µg/kg]	RSD [%]	Maize grain [µg/kg]	RSD [%]	Maize leaves [µg/kg]	RSD [%]	Jerusalem artichoke skin [µg/kg]	RSD [%]	Jerusalem artichoke peeled [µg/kg]	Ryegrass [µg/kg]	RSD [%]
FOSA	**0.0022**	**19**	**(0.0019)**		**0.213**	**7**	**(0.0021)**		**(0.0015)**	**(0.0086**)	
	0.0026	*38*	*(0.0016)*		*0.223*	*6*	*(0.0020)*		*(0.0015)*	*(0.0070)*	
MeFOSA	-		-		-		-		-	-	
EtFOSA	-		-		**0.0490**	**9**	-		-	-	
	-		-		*0.0672*	*14*	-		-	-	
MeFOSE	-		-		-		-		-	-	
EtFOSE	-		-		-		-		-	-	
FOSAA	-		**(0.0008)**		**0.152**	**12**	-		**(0.0028)**	**(0.0273**)	
MeFOSAA	-		-		**(0.0017)**		-		-	-	
	-		-		*(0.0017)*		-		-	-	
EtFOSAA	**(0.0059)**		**0.0056**	**21**	**0.405**	**9**	**0.125**	**87**	**(0.0111)**	**(0.0162)**	
6:2 FTCA	-		-		-		-		-	-	
8:2 FTCA	-		-		-		-		-	-	
10:2 FTCA	-		-		-		-		-	-	
6:2 FTUCA	**0.185**	**8**	-		-		-		-	**0.205**	**3**
	0.224	*1*	-		-		-		-	*0.203*	*9*
8:2 FTUCA	-		-		-		-		-	-	
10:2 FTUCA	-		-		-		-		-	-	
5:3 acid	-		-		-		-		-	-	
7:3 acid	-		-		**0.150**	**6**	**(0.0374)**		-	-	

Table 4. Summary of detected analytes in qualitative analysis of target compounds without authentic reference standards in contaminated plant samples and their confidence level of identification (a-e). An explanation of the levels of confidence is also given. Empty cells indicate non-detects.

	Maize leaves	**Maize grain**	**Jerusalem artichoke skin**	**Jerusalem artichoke peeled**	**Ryegrass**	**Wheat grain**
9:3 Acid	**a**	-	**a**	-	-	**a**
11:3 Acid	-	-	-	**a**	-	-
5:3 UAcid	-	-	-	-	c	c
FBSA	-	-	b	-	-	b
MeFBSA	-	**a**	-	-	-	e
MeFBSAA	-	-	e	e	-	e
EtFBSAA	**a**	-	-	e	-	-
MeFHxSAA	-	-	-	-	b	-
EtFBSE	-	b	b	b	c	**a**
FHxSE	-	-	-	-	e	-
EtFHxSE	-	b	-	-	-	-

Level of confidence	**Explanation**
a	Two MRMs were detected. Predicted and experimentally derived retention time differ less than 30 s from each other.
b	One MRM was detected. Predicted and experimentally derived retention time differ less than 30 s from each other.
c	One MRM was detected at low intensity in some but not all triplicates. Predicted and experimentally derived retention time differ less than 30 s from each other.
d	Two MRMs were detected. Predicted and experimentally derived retention time differ more than 30 s from each other.
e	One MRM was detected. Predicted and experimentally derived retention time differ more than 30 s from each other.

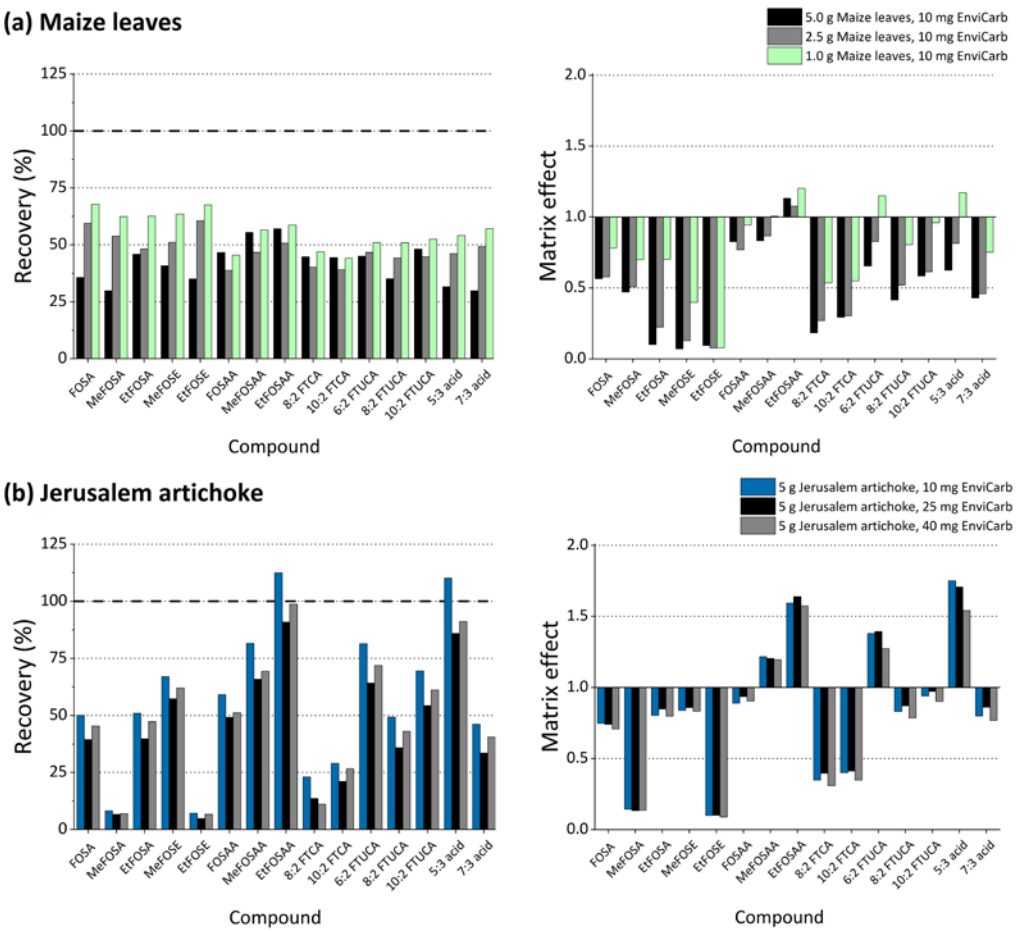
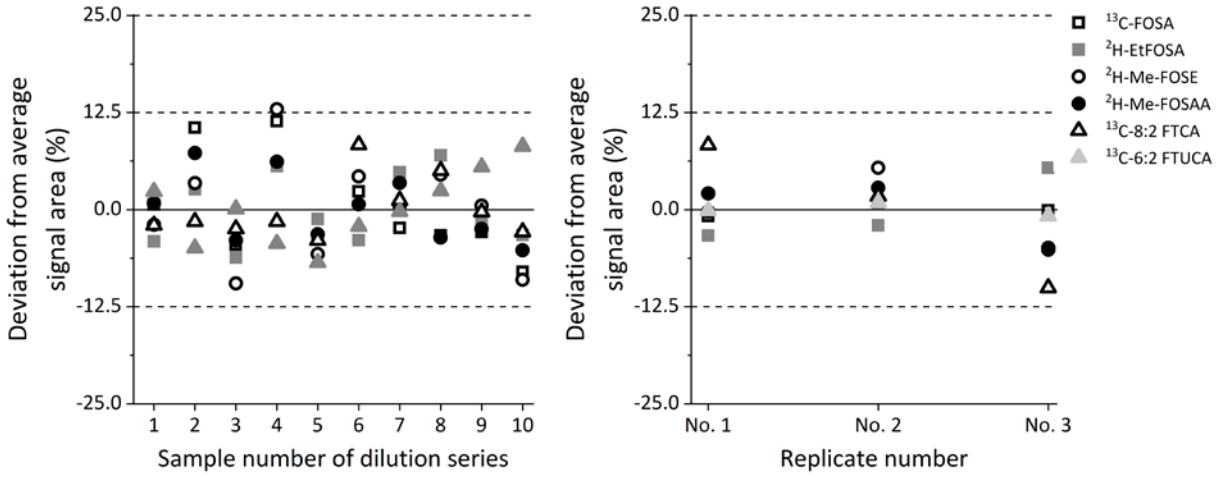


Figure 1. Recoveries and matrix effects of the target analytes after extraction of (a) different amounts of maize leaves (spiked with 2-10 µg/kg) and after application of (b) different amounts of ENVI-Carb during clean-up of Jerusalem artichoke (spiked with 2 µg/kg).



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684 **Figure 2.** Relative variation of individual signal areas of the six internal standards in the
685 extracts of uncontaminated maize grain from the preparation of the matrix matched and
686 extracted calibration (left, mean RSD 5.2%) and in the triplicate analyses of contaminated
687 maize grain (right, mean RSD 4.2%).