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A novel method for extraction, clean-up and analysis of per- and polyfluoroalkyl substances (PFAS) in different plant matrices using LC-MS/MS

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are chemicals of concern due to their persistence, bioaccumulation, and toxic properties. PFAS accumulation in plants poses a risk of human and animal exposure due to consumption of the affected plants, but also allows plants to be used in remediation of PFAS-contaminated soils and groundwater. Therefore, effective extraction, cleanup, and analytical methods for measuring PFAS concentrations in plants are fundamental for research on animal and environmental health. PFAS analysis in plant matrices is complex, due to high matrix interference, and scarcity of methods for analyzing different classes of PFAS. In this study, a simple sample preparation method for PFAS analysis in various plant tissues (leaves, needles, twigs, stems, roots from 10 different species) was developed and validated. Instrumental analysis was performed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The method was optimized considering six different extraction conditions and three different cleanup techniques. Methanol as extraction solvent, combined with 1 g ENVI carb cartridges, showed best performance among all extraction conditions and cleanup techniques tested. Method validation showed good recovery (90–120%), high within-day and between-day precision (<20% relative standard deviation), and low method detection limit (0.04–4.8 ng g^{-1} dry weight (dw)) for different plant matrices. In tests of the method on soil and different plant tissues of silver birch (*Betula pendula*) and Norway spruce (*Picea abies*) at a PFAS-contaminated site, 16 of 24 target PFAS were detected in plants and 17 in soil. ΣPFAS concentration in soil was 43 ng g^{-1} dw. PFAS distribution in silver birch tissues ranged from 7.1 ng g^{-1} dw in roots to 64 ng g^{-1} dw in leaves, and in Norway spruce from 14 ng g^{-1} dw in roots to 16 ng g^{-1} dw in needles. This novel method for PFAS analysis in plants can be valuable in future monitoring, process understanding, remediation, and risk assessments.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic micropollutants that are extremely persistent, bioaccumulative, and potentially toxic to humans and animals [1]. These substances are ubiquitous in humans and the environment [2–4], with previous studies reporting detection of PFAS in e.g., plants and crops at contaminated sites, agricultural fields treated with contaminated biosolids, reclaimed water, and aquatic environments [5–9].

PFAS accumulation in plants is important because plants are a major dietary component for humans and animals, but can also be used in remediation of contaminated sites [10]. Therefore, effective PFAS measurement in plant tissues is crucial for research, monitoring, formulation of remediation strategies, food safety, and regulation. Plants, like other biological matrices, are matrix-rich, which hinders effective extraction and quantification of contaminants [11]. Plants also contain complex elements such as phenolics and photosynthetic pigments, distinguishing them from other environmental and biological matrices [12]. Currently, only limited sample preparation methods for PFAS in plant matrices are available [13–16] and most existing methods have been optimized and validated for only a few compounds. The vast majority of methods used for PFAS analysis in plants to date have applied sample preparation protocols developed for other matrices, such as soil and sediment [17] or biota [18,19].

For extraction, several previous studies have applied solid–liquid extraction, solid-phase extraction (SPE), and the QuEChERS (quick,

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easy, cheap, effective, rugged, and safe) method for PFAS analysis, using polar solvents such as methanol, acetonitrile, methyl ter-butyl ether, or their solutions, with an acid, base, or buffer [5,20,21]. Commonly applied cleanup methods include ion pairing, SPE, activated carbon (as powder or cartridges) or magnesium sulfate, or a combination of SPE and activated carbon [8,22–24]. Several of these approaches have been used without comprehensive method optimization and validation for PFAS analysis in plants, which often poses challenges in obtaining high recovery and low matrix effects for some analysed PFAS [6,20,25]. To our knowledge, comprehensive optimization and validation of methods for PFAS analysis in plants is lacking and, in particular, no studies have investigated the occurrence of PFAS in plants growing at landfill sites.

The aim of this work was to develop and validate a simple extraction and cleanup method for analysis of five PFAS classes in multiple plant tissues, using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Specific objectives were to (i) evaluate the performance of six extraction conditions (acetonitrile, methanol, and their solutions with a weak acid or base), and three cleanup techniques (ENVI-Carb cartridge only, ENVI-Carb cartridge combined with WAX-SPE, and ENVI-Carb powder only); (ii) validate the optimized method; and (iii) apply the method to plant tissues and soil collected from a landfill site with known PFAS contamination as a pilot study of PFAS in landfill plants.

2. Methods

2.1. Chemicals and materials

Target PFAS ($n = 24$) comprised perfluoroalkyl carboxylates (PFCAs) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA), perfluoroalkanesulfonates (PFSAs) (PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS), perfluorooctanesulfonamide (FOSA), methyl- and ethylperfluorooctanesulfonamidoacetic acid FOSAAs (MeFOSAA, EtFOSAA), and 4:2, 6:2, 8:2 fluorotelomer sulfonate (FTSA) (Table S1 in Supporting Information (SI)). Mass-labelled internal standards (ISs) used were: $^{13}C_3$-PFBA, $^{13}C_5$-PFPeA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_8$-PFOA, $^{13}C_9$-PFNA, $^{13}C_6$-PFDA, $^{13}C_7$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_2$-PFTeDA, $^{13}C_3$-PFHxS, $^{13}C_8$-PFOS, $^{13}C_8$-FOSA, d_3-MeFOSAA, d_5-EtFOSAA (Table S1 in SI). Native standards (purity > 98%) and ISs (isotopic purity ≥ 99%) were obtained from Wellington Laboratories (Sweden).

Acetonitrile (ACN), methanol (MeOH), ammonium acetate (NH_4Ac) (≥99%), formic acid (FA) (≥98%), and glacial acetic acid (100%) of high analytical grade were obtained from Merck (LiChrosolv, Merck, Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). MilliQ water was generated by a Milli-Q IQ 7000 Ultrapure Water purification system filtered through a 0.22 μm Millipak Express membrane and an LC-Pak polishing unit (Merck Millipore, Billerica, MA, USA). Oasis solid phase extraction (SPE) WAX cartridges (150 mg, 6 cc, 30 μm) were obtained from Waters (New Bedford, MA, USA). ENVI-Carb cartridges (250 mg, 6 mL & 1 g, 12 mL (120–400 mesh, 100 $m^2\ g^{-1}$)) and ENVI carb powder (120–140 mesh, 100 $m^2\ g^{-1}$) were obtained from Sigma-Aldrich. Plant samples were milled in a blender (230 V; OBH Nordica, Sweden). Homogenization was performed using an overhead shaker (Reax 2, Heidolph, Germany) and an analog vortex mixer (VWR, Leuven, Belgium).

2.2. Sample collection

For method optimization and validation, plant samples were obtained from 10 common plant species at locations without PFAS contamination in the area of Uppsala, Sweden. The plant species were: silver birch (*Betula pendula)*, strawberry (*Fragaria* spp.), Scots pine (*Pinus sylvestris*), mountain ash (*Sorbus aucuparia)*, salix (*Salix* spp.), Norway spruce (*Picea abies*), poplar (*Populus* spp.), bird cherry (*Prunus padus)*, common oak (*Quercus robur)*, and sycamore maple (*Acer pseudoplatanus*).

The suitability of the method developed was tested using plant samples and soil samples collected from a PFAS-contaminated site in Stockholm, Sweden, on 10 October 2019. The site is a landfill, with silver birch and Norway spruce as the main tree vegetation. Tissues (needles, leaves, twigs, bark, roots) were collected separately from different trees ($n = 4$) and combined to produce composite samples for each tissue type. Soil samples were also collected from the same site ($n = 4$) and combined to make 1 composite sample. These composite samples were then analysed in duplicates.

Fresh plant tissue samples were cleaned with tap water (1 time), MilliQ water (2 times), and a mixture of MilliQ and MeOH (50:50; v:v) (2 times). The tissues were freeze-dried for 3 days and then homogenized and milled using a cleaned blender (cleaned 3 times with MilliQ water and 3 times with MeOH between samples).

2.3. Method optimization

The plant tissues collected for each species were sorted into foliage (leaves or needles), twigs, stems, and roots and then cleaned, freeze-dried, and milled using a blender as mentioned above. Thereafter, the leaves and needles (1:1; weight-based) were combined to form one matrix group called 'foliage' (Group I), while the twigs, stems, and roots (1:1:1; weight-based) were combined to form another matrix group called 'woody tissue' (Group II). Samples of these two matrix groups were shaken for 120 h using an overhead shaker, to obtain a respective homogenous mix. The mixes were then stored in the freezer at −20 °C until analysis.

For method optimization, three replicates of 1 g each were weighed into 15 mL PP tubes and spiked with a native PFAS mixture standard to a final concentration of 25 ng g^{-1} dry weight (dw) per compound and IS mixture (5 ng g^{-1} dw).

2.3.1. Extraction conditions

Six different solvents were tested for method validation: i) MeOH, ii) ACN, iii) MeOH:ACN (50:50; v/v), iv) MeOH with 0.1% formic acid (MeOH:FA), v) ACN with 0. 1% formic acid (ACN:FA), and vi) MeOH with 400 mM ammonium acetate (MeOH:NH_4Ac) (Fig. 1). These extraction solvents were applied to the two sample matrices (matrix groups I and II). Each extraction was performed using ultrasonication in three cycles and then the extracts were combined to one extract (for details, see Section 2.5). The combined extract was further cleaned up using an ENVI-carb cartridge prior to concentration and instrumental analysis.

2.3.2. Cleanup technique

Three cleanup methods using MeOH (i.e., best-performing solvent, see Section 3.1.1) as extraction solvent were tested: 1) ENVI-Carb cartridge only (ENVI-Carb cartridge), 2) ENVI-Carb cartridge combined with WAX-SPE (ENVI-Carb cartridge + WAX-SPE), and 3) ENVI-Carb powder only (ENVI-Carb powder) (Fig. 1).

For cleanup 1 (ENVI-Carb cartridge), the extracts (7 mL) were run directly through the ENVI-Carb cartridge (1 g, 12 mL) and collected in 15 mL PP tubes. The cartridges were washed with MeOH (1 mL) after use and then pressed with air using a syringe to collect the wash in the same vial with the extract. No cleaning and conditioning was done prior to using the cartridges.

For cleanup 2 (ENVI-Carb cartridge + WAX-SPE), the extracts were run through the ENVI-Carb cartridge (1 g, 12 mL) as described above, concentrated to 5 mL using nitrogen, and then diluted with 95 mL of Milli-Q water. SPE was performed with Oasis WAX cartridges (150 mg, 6 cc, 30 μm) preconditioned with 0.1% NH_4OH/MeOH (4 mL), MeOH (4 mL), and Milli-Q water (4 mL) sequentially. After sample loading, the cartridges were washed with 25 mM ammonium acetate in Milli-Q water (4 mL) and the WAX cartridges were centrifuged at 3000 rpm for 2 min. Elution was performed with MeOH (4 mL) and 0.1% NH_4OH/MeOH (4

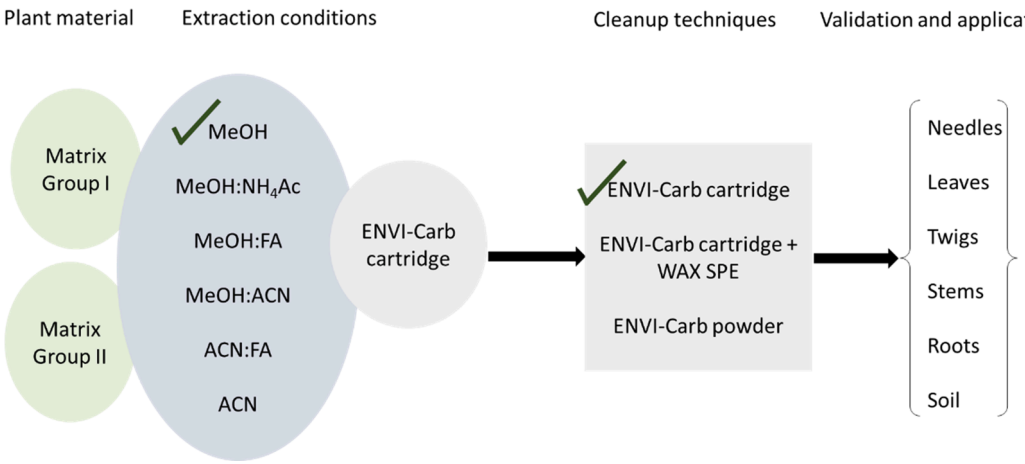

Fig. 1. Workflow used for evaluating the six extraction conditions and three cleanup techniques before validating and applying the method for PFAS analysis in different plant tissues and soil. The green check mark shows the extraction condition and cleanup technique that performed best in PFAS analysis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mL) in 15 mL PP tubes.

All extracts from cleanups 1 and 2 were concentrated to 100 μL using nitrogen and then topped up with methanol to obtain a total volume of 500 μL prior to instrumental analysis.

For cleanup 3 (ENVI-Carb powder), the combined extracts were concentrated to 500 μL and the concentrates were transferred to 2 mL Eppendorf centrifuge tubes containing 25 mg ENVI-Carb powder and 50 μL glacial acetic acid. The tube and its contents were vortexed, followed by 15 min of centrifugation at 4000 rpm. The supernatant was transferred to LC-MS injection vials for instrumental analysis.

2.4. Method validation

Samples (pre-spiked $n = 3$, post-spiked $n = 1$) from each of the matrix groups were used for validation of the optimized method and were analyzed on three different days. Method validation was based on the following parameters: procedural blanks, method detection limits (MDLs), method quantification limits (MQLs), relative recovery, linearity, and within-day and between-day precision (relative standard deviation, RSD(%)) for each of the matrix groups.

In total, six procedural blanks were prepared in the same way as natural samples, but without sample material. MDLs and MQLs were estimated using a signal to noise (S/N) ratio of 3 and 10, respectively, in matrix spiked samples at low concentration levels (c = 5 ng g^{-1} dw). Relative recovery was determined in triplicate for each matrix group.

Linearity was assessed using a nine-point calibration curve (0.01–100 ng mL^{-1}). For within-day precision, RSD (%) and between-day precision, RSD (%) was determined for the mean concentration of triplicate samples run on the same day and on three different days, at a medium concentration level (c = 25 ng g^{-1} dw). The criteria of acceptance for RSD (%) was < 20%.

2.5. Method application

Procedural blanks, fortified samples, and duplicates were used in sample preparation and analysis. Methanol as extraction solvent and ENVI-Carb cartridge alone (cleanup 1) showed the best performance for analysis of PFAS (see Section 3.1.1). In brief, plant tissue (1 g dw) was spiked with 100 μL IS mixture, resulting in 5 ng g^{-1} dw for each IS. The plant tissues were extracted in three cycles using MeOH. During each of these cycles, 3 mL of extraction solvent was added and the samples were vortexed at high speed for 1 min, ultrasonicated for 30 min, and centrifuged at 3000 rpm for 15 min. Combined extracts were run through the ENVI-Carb cartridge (1 g, 12 mL) and collected in 15 mL PP tubes. The cartridges were washed with MeOH (1 mL) after use and then pressed with air using a syringe to collect all solvents trapped within the cartridge. The wash was collected in the same tube as the cleaned extract. The extracts were concentrated to 100 μL using nitrogen and then topped up with methanol to a total volume of 500 μL. The reconstituted extracts were transferred to LC-MS injection vials for instrumental analysis.

For soil samples, sample preparation and extraction was done as described above. Cleanup was performed using 250 mg ENVI-Carb cartridges [26]. The extracts were then concentrated to 500 mL prior to instrumental analysis.

2.6. LC-MS/MS analysis

Instrumental analysis was performed using ultra-high pressure liquid-chromatography (SCIEX ExionLC AC system) coupled to tandem mass spectrometry (SCIEX Triple Quad™ 3500) (UHPLC-MS/MS). The column oven was set to 40 °C, and 20 μL of sample were injected into a Phenomenex Kinetex C18 (30 × 2.1 mm, 1.7 μm) precolumn coupled to a Phenomenex Gemini C18 (50 mm × 2 mm, 3 μm) analytical column for chromatographic separation. The mobile phase consisted of MilliQ water with 10 mM ammonium acetate (A) and MeOH (B). The mobile phase gradient was as follows: 5% B, which was increased to 55% within the first 0.1 min, then further increased to 99% within 4.4 min, kept constant for the next 3.5 min, then decreased to 5% over 0.5 min and kept constant for the next 0.5 min. The flow rate was 0.6 mL min^{-1} and the total run time was 9 min. Information on optimized parameters for the ion source and MS/MS parameters is provided in Tables S2 and S3 in SI. The MS/MS was operated in scheduled multiple reaction monitoring (MRM) mode with negative electrospray ionization (Table S3 in SI). A nine-point calibration curve from 0.01 to 100 ng mL^{-1} was used for quantification. Data evaluation was performed using SciexOS software (2.0).

2.7. Data handling and statistical analyses

For comparison of the different treatments (i.e., extraction conditions and cleanup techniques and their combinations) regarding recovery and matrix effects, descriptive statistics (mean and standard deviation), analysis of variance (ANOVA), and t-tests (significance level, $\alpha = 0.05$) were computed in GraphPad Prism (version 9.2.0 (332)).

3. Results and discussion

3.1. Method optimization

3.1.1. Extraction conditions

Six different extraction conditions (i.e., MeOH or ACN, with or without the additives NH_4OH and 0.1% FA) were assessed for matrix group I (leaves and needles) and matrix group II (twigs, stems. and roots), based on their absolute recovery (Fig. 2). In general, MeOH performed better than ACN, with absolute recovery of 82 ± 12% (41–103%) and 89 ± 24% (64–187%) for matrix groups I and II, respectively. ACN gave generally lower recovery for matrix groups I and II, 61 ± 15% (range 11–79%) and 95 ± 61% (47–291%), respectively. In general, the 24 PFAS investigated showed good absolute recovery except for 6:2 FTSA, 8:2 FTSA, MeFOSAA, PFDA, PFTriDA, and PFTeDA. 6:2 FTSA had the lowest recovery (<50%) under all extraction conditions except MeOH:FA for matrix group I. Low recovery for long-chain PFCAs (PFDA, PFTriDA, PFTeDA) has been reported previously for different matrices, due to strong sorption of these compounds to surfaces, and for PFAS precursors, due to their potential degradation [11]. In matrix group II, PFTriDA showed the highest absolute recovery under all extraction conditions (>150%). High absolute recovery was also observed for PFDA with ACN extraction, MeFOSAA with MeOH:ACN extraction, and 8:2 FTSA with MeOH:ACN, ACN:FA, and ACN extraction.

Additives, especially FA (0.1%), improved the extraction efficiency of both MeOH and ACN for matrix group I, but not group II. For group I, MeOH:FA and ACN:FA generated average recovery of 102 ± 17% (69–139%) and 77 ± 25% (23–158%), respectively, for the targeted PFAS, values which were significantly ($p < 0.0001$) better than those achieved by MeOH and ACN without additives. Similar recovery was reported in a previous study using ACN for extraction with an acidification step (acetic acid) prior to cleanup (ENVICarb cartridges; 500 mg, 6 mL) for PFAS analysis in spinach, tomato, and corn tissue samples [27]. For group II, FA had significantly ($p < 0.0001$) lower extraction efficiency compared with using MeOH and ACN alone, with MeOH:FA giving 73 ± 6.5% recovery (62–91%) and ACN:FA 95 ± 61% (47–291%). MeOH:NH_4OH and MeOH:ACN performed better in extraction of group II than group I samples, but both conditions resulted in greater variation in absolute recovery (see below). For group I, absolute recovery was 100 ± 50 (20–287%) for MeOH:NH_4OH and 78 ± 23% (9.5–133%) for ACN:MeOH for group I. For group II, absolute recovery was slightly higher, 115 ± 67% (51–328%) for MeOH:NH_4OH and 107 ± 47% (41–221%) for ACN:MeOH. Munoz et al. [26] reported good recovery for extraction of soil using MeOH:NH_4OH with a cleanup (ENVICarb cartridges; 250 mg, 6 mL), as found in this study for group II samples.

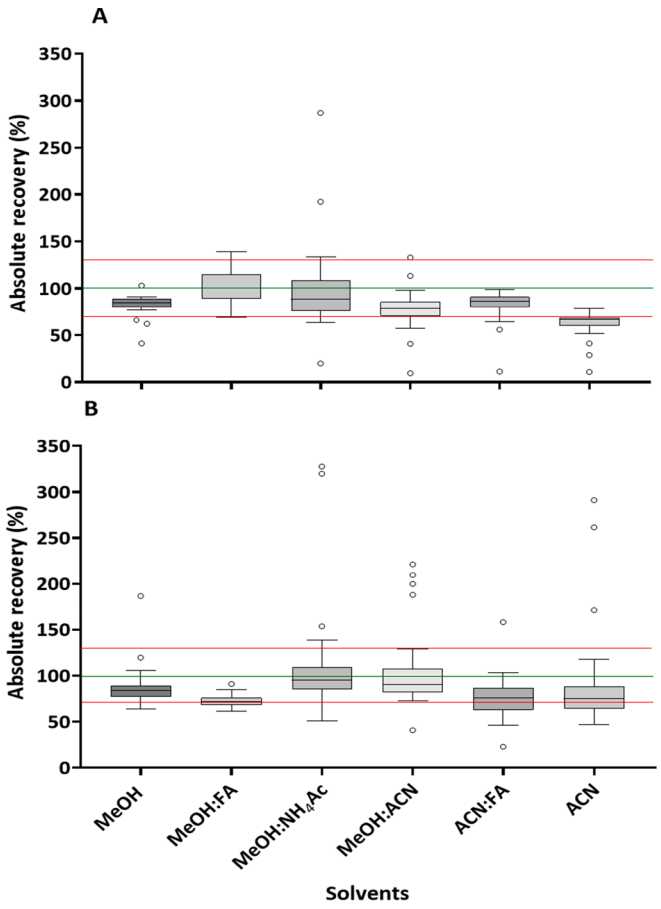

Fig. 2. Absolute recovery of PFAS from A) plant matrix group I (leaves, needles) and B) group II (twigs, stems, roots) under different extraction conditions.

ACN has been widely utilized as an extraction solvent for biological matrices [28] and several applied studies have used this solvent for extraction of PFAS in plants [16,24,27,29]. In a few studies, ACN has been mixed with water during extraction [22,23]. However, this is reported to generate lower recovery compared with using pure acidified organic solvents [28]. In the present study, ACN showed the worst performance of all extraction conditions tested.

Gobelius et al. [6] utilized MeOH and sodium hydroxide (NaOH) for PFAS extraction from plants and obtained absolute recovery of 12 ± 12% to 43 ± 26%. Similarly, Huff et al. [25] observed low absolute recovery (<10%) for several PFAS in plant extraction using MeOH and NaOH. In contrast, good performance was achieved when using MeOH or acidified MeOH for extraction in the present study. Baduel et al. [30] found that addition of acid or buffer improved recovery of acidic polar compounds, which is similar to our findings. Although both MeOH and MeOH:FA performed well for all 24 target PFAS, MeOH was ultimately selected as the extraction solvent in order to have a simple and consistent method for both matrix groups. The extraction method using MeOH was further tested on different cleanup techniques.

3.1.2. Cleanup techniques

For both matrix groups, three cleanup methods were tested: ENVICarb cartridge, ENVICarb cartridge + WAX-SPE, and ENVICarb powder. MeOH was selected as a suitable extraction solvent.

There was a significant difference ($p < 0.0001$) in absolute recovery obtained using the three cleanup methods for both matrix groups. ENVICarb cartridge had the highest absolute recovery, 86 ± 11% (61–119%) and 85 ± 11% (72–115%) for group I and II, respectively (Fig. 3). Use of two cleanup steps, i.e. ENVICarb cartridge + WAX-SPE, slightly improved the matrix effect (from −55 ± 51% to −36 ± 76 % for group I and from −58 ± 44% to −52 ± 53% for group II). However, it significantly reduced the absolute recovery to 60 ± 7.9% (42–72%) and 72 ± 8.4% (40–81%) for group I and II, respectively. Muschket et al. [16] made similar findings when using an additional cleanup step (WAX-SPE and CUNAX22Z-SPE) during sample preparation. ENVICarb powder is the most frequently used cleanup method for PFAS analysis [6,17] but showed the worst performance in this study, with absolute recovery of 53 ± 8.2% (25–60%) for group I and 65 ± 4.7% (59–76%) for group II. This can be explained by lack of proper method optimization (i.e. extraction solvent and ratio of powder to plant material/extract) despite extensive use of the ENVICarb powder to remove pigments from plant tissue in previous studies [6].

Although there were no major differences in matrix effects between the three cleanup methods, ENVICarb powder showed the strongest matrix effects (Fig. S2 in SI). For group I, matrix effects were −55 ± 51%, −36 ± 76%, and −65 ± 40% for ENVICarb cartridge, ENVICarb cartridge + WAX-SPE, and ENVICarb powder, respectively. For group II, the corresponding matrix effects were −58 ± 44%, −52 ± 53%, and −78 ± 24% for ENVICarb cartridge, ENVICarb cartridge + WAX-SPE,

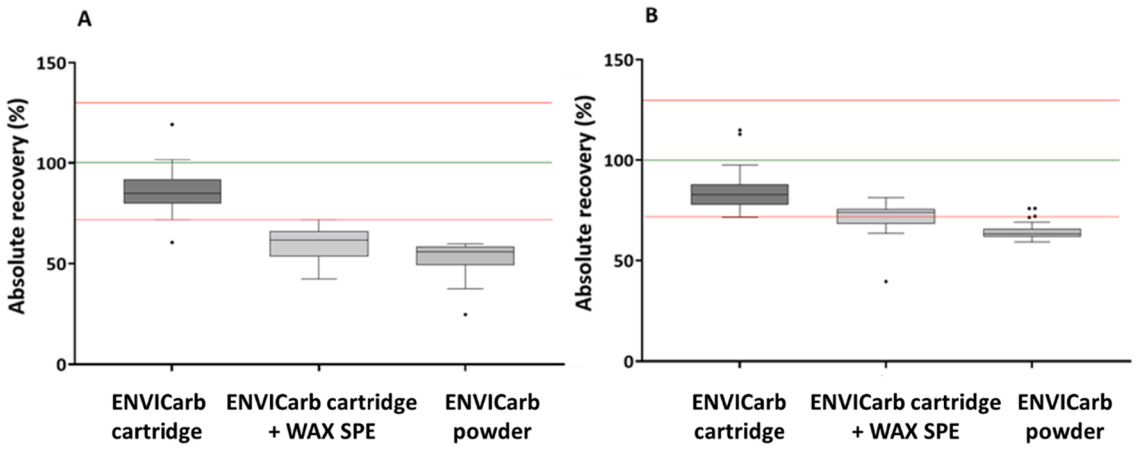

Fig. 3. Absolute recovery of PFAS for (A) matrix group I and (B) matrix group II when using three different cleanup techniques.

and ENVICarb powder, respectively. Based on the recovery and matrix effect results, ENVICarb cartridge was selected as the preferred cleanup method for both matrix groups.

3.2. Method validation

Method validation was performed with the selected extraction solvent (MeOH) and cleanup step (ENVICarb cartridge) (Table 1). None of the PFAS analyzed was detected consistently in the procedural blanks. PFBA (17 ng g^{-1} dw) was found in the matrix blanks (i.e., non-spiked samples) of group I. The MDLs and MQLs were determined from plant matrix samples spiked with low PFAS concentrations. The MDL range was 0.04–2.4 ng g^{-1} dw for group I and 0.1–4.8 ng g^{-1} for group II, while the MQL range was 0.1–8.1 ng g^{-1} dw for group I and 0.3–11 ng g^{-1} dw for group II (Table 1). The plant samples used were composite samples made up of tissues from different plant species on a dry matter basis, and not a single plant species. This represents the worst-case scenario, because the plants represented had different characteristics, with different levels of interferences. Therefore, the values obtained are applicable to other plants.

Relative recovery was within acceptable limits (70–130%) for all compounds except PFBA (140%), in matrix group II (Table 1). Recovery in this study was comparable to, but typically better than, that reported in the literature [13,16,27,29].

Our novel method showed linearity $R^2 \geq 0.99$ for the target compounds over a range of 0.01–100 ng mL^{-1} (Table 1). The precision of the method was < 20% for most target compounds in both matrix groups. For within-day precision, variations slightly above 20% were observed for PFBA and PFTrDA in group I and PFTriDA and 4:2 FTSA in group II, which showed RSD > 20%. For between-day precision, PFBA, PFHpA, PFHpS, PFNS, and PFDS in group 1 and PFBA, PFNS, and PFDS in group II had RSD > 20%.

3.3. Method applications

The novel method was used to investigate PFAS uptake and

Table 1
Validation data for analysis of 24 PFAS in plant matrix group I (leaves, needles) and group II (twigs, stems, roots) using MeOH as extraction solvent and ENVICarb cartridge cleanup.

		Matrix group I					Matrix group II				
Target compound	Linearity R^2	MDL (ng/g dw)	MQL (ng/g dw)	Relative recovery (%)	With-in day precision RSD (%)	Between-day precision RSD (%)	MDL (ng/g dw)	MQL (ng/g dw)	Relative recovery (%)	With-in day precision RSD (%)	Between-day precision RSD (%)
PFBA	0.990	0.05	0.18	115 ± 35	27	25	1.7	5.8	140 ± 25	2.9	31
PFPeA	0.993	2.4	8.1	104 ± 12	6.9	13	1.8	6.1	95 ± 3.3	7.7	13
PFHxA	0.996	0.93	3.1	105 ± 3.6	6.2	3.1	3.4	11	102 ± 5.5	4.7	4.6
PFHpA	0.998	0.27	0.91	108 ± 14	10	33	4.8	16	99 ± 7.5	4.7	32
PFOA	0.998	0.49	1.6	104 ± 4.3	5.1	9.9	0.60	2.0	98 ± 4.0	0.90	6.1
PFNA	0.996	1.3	4.2	102 ± 2.5	5.9	6.1	0.76	2.5	93 ± 12	7.4	7.7
PFDA	0.994	0.41	1.4	98 ± 2.9	7.0	5.6	0.79	2.6	102 ± 3.5	0.55	6.3
PFUnDA	0.990	0.60	2.0	101 ± 12	5.7	14	0.22	0.75	93 ± 9.5	6.5	17
PFDoDA	0.999	0.27	0.91	97 ± 3.5	2.9	1.3	0.20	0.68	94 ± 1.8	3.0	1.2
PFTriDA	0.993	0.21	0.70	95 ± 14	39	0.91	1.4	4.7	105 ± 78	26	14
PFTeDA	0.996	0.34	1.1	102 ± 1.4	2.9	9.2	1.9	6.4	95 ± 1.8	3.3	10
PFBS	0.999	0.22	0.74	96 ± 2.0	5.3	7.2	0.47	1.6	96 ± 6.2	5.0	5.9
PFPeS	0.999	0.19	0.62	101 ± 12	9.9	6.2	0.52	1.7	115 ± 15	12	7.1
PFHxS	0.999	0.43	1.4	97 ± 11	7.9	15	0.95	3.2	96 ± 12	17	5.7
PFHpS	0.997	0.14	0.45	107 ± 24	9.9	35	0.35	1.2	105 ± 15	10	12
PFOS	0.998	0.97	3.2	94 ± 2.2	2.4	14	0.92	3.1	99 ± 13	8.7	5.9
PFNS	0.995	0.18	0.59	109 ± 28	11	28	0.33	1.1	116 ± 32	14	37
PFDS	0.992	0.04	0.13	110 ± 13	15	36	0.20	0.68	110 ± 15	18	36
FOSA	0.999	0.21	0.69	106 ± 5.5	2.0	11	0.17	0.57	101 ± 4.2	3.0	8.0
EtFOSAA	0.999	0.06	0.21	104 ± 6.6	8.6	7.0	0.10	0.32	99 ± 6.3	3.4	5.9
MeFOSAA	0.999	0.17	0.56	107 ± 1.8	9.9	14	1.6	5.4	107 ± 32	18	7.9
4:2 FTSA	0.997	0.18	0.59	89 ± 27	17	40	0.24	0.81	93 ± 29	26	2.4
6:2 FTSA	0.998	0.07	0.24	101 ± 1.4	2.2	5.9	0.13	0.43	96 ± 11	9.1	6.2
8:2 FTSA	0.991	0.20	0.66	100 ± 1.2	5.3	2.1	0.31	1.0	98 ± 8.7	2.7	4.4

distribution in plant and soil samples from a PFAS-contaminated site (landfill) in Sweden. The main plant species at the site were silver birch and Norway spruce. Of the 24 PFAS analyzed, 16 were detected in plant samples (Fig. 4). Previous studies have typically only detected a few PFAS in plants (e.g., PFPeA, PFHxA, PFOA, PFHxS, PFOS, and 6:2 FTSA) [6]. Silver birch had the highest ΣPFAS concentrations, ranging from 7.1 ng g^{-1} dw in roots to 64 ng g^{-1} dw in leaves, while Norway spruce had ΣPFAS concentrations ranging from 14 ng g^{-1} dw in roots to 16 ng g^{-1} dw in needles. Foliage had the highest ΣPFAS concentration in both silver birch and Norway spruce (64 ng g^{-1} in leaves and 16 ng g^{-1} dw needles), followed by twigs (16 ng g^{-1} and 13 ng g^{-1} dw, respectively), bark (11 ng g^{-1}, 10 ng g^{-1} dw, respectively), and roots (7 ng g^{-1} and 14 ng g^{-1} dw, respectively). Particularly dominant PFAS in foliage included PFBA (on average 21% of ∑PFAS), PFHpA (5.8%), PFHxS (4.2%), and PFPeA (3.5%). Roots showed a different composition profile, dominated by the longer-chained PFAS i.e., PFOA (on average 2.1% of ∑PFAS), PFUnDA (2.0%), and PFDA (1.8%). This is in agreement with previous findings of higher concentrations of longer-chained PFAS in roots than in foliage [31]. In general, there are limited PFAS data available on silver birch and Norway spruce at contaminated sites, but our measured concentrations and composition profiles are in general agreement with those in a previous study [6]. Similar PFAS composition profiles, with dominance of short-chain PFAS, have been reported for other plants (vegetables, woody and other herbaceous plants) grown in PFAS-spiked soil [32], spiked water [25,33], and agricultural soils [24].

In soil samples, 17 of the 24 target PFAS were detected and ΣPFAS concentration was 43 ng g^{-1} dw. The PFAS composition profile in the soil differed from that in the two plant species, with PFSAs (PFOS, 17 ng g^{-1} dw, 38% of ∑PFASs) and PFHxS (10 ng g^{-1} dw, 22% of ∑PFAS) being the dominant PFAS in soil. This dominance of PFSAs (i.e., PFHxS and PFOS) in soil was not reflected in the PFAS composition profile of plant tissues, where PFCAs (especially PFBA) were the dominant PFAS. This can be explained by the low mobility of PFHxS and PFOS [31]. A previous study found that PFAS composition profile in plants was different from that in local soil, air, and rainwater [24]. However, Gobelius et al. [6] observed high PFOS concentrations in soil that were reflected in the PFAS distribution profile in plants.

4. Conclusions

A solid–liquid extraction method was developed for extraction of five different classes of PFAS from different plant species and a wide range of plant tissue samples. Methanol outperformed acetonitrile, despite the latter being the most commonly used extraction solvent for biological samples. Combining methanol with ENVICarb cartridges as a cleanup step produced a simple and novel sample preparation method. For most compounds, satisfactory validation parameters were obtained, illustrating good utility of the method for PFAS analysis.

The method was successfully applied to plant tissues of silver birch and Norway spruce from a PFAS-contaminated site, in a pilot investigation of PFAS uptake and distribution in plants at landfill sites. Several PFAS were detected in tissues of both plant species, especially the foliage, at concentration levels similar in magnitude to levels reported in previously [6]. The method presented can be used in future studies on dietary uptake of plant-related PFAS in animals and humans and on plant species for use in phytoremediation.

CRediT authorship contribution statement

Winnie Nassazzi: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Foon Yin Lai:** Conceptualization, Validation, Writing – review & editing, Supervision. **Lutz Ahrens:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision, Project administration.

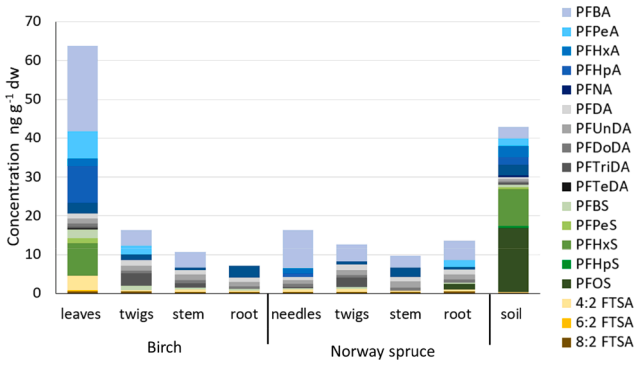

Fig. 4. Composition profiles and concentrations of PFAS in birch tissues, Norway spruce tissues, and soil at a landfill site in Sweden.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2022.123514.

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