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A sensitive method for the detection of legacy and emerging per- and polyfluorinated alkyl substances (PFAS) in dairy milk

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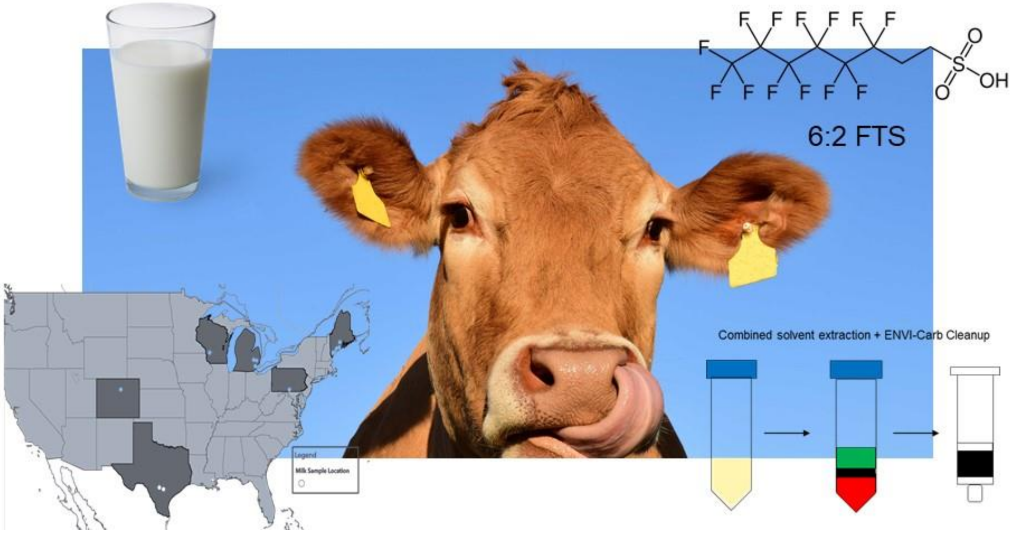
Abstract

There is widespread contamination by per- and polyfluoroalkyl substances (PFAS) across the globe, with adverse effects on human and environmental health. For human exposure, drinking water and dietary exposure have been recognized as important PFAS exposure pathway for the general population. Several documented cases of dairy milk contamination by PFAS have raised concerns over this exposure pathway in general. A sensitive method for determination of 27 PFAS in milk was hence modified and applied on raw and processed milk samples from thirteen farms across the United States (U.S.). A combination of acid and basic extraction method and ENVI-Carb cleanup achieved recoveries of targeted PFAS between 70-141%. The method detection limits (MDL) ranged from 0.8-22 ng/L (for 26 PFAS) and 144 ng/L for perfluorobutanoic acid (PFBA). The uniqueness of this method is considered in the targeted screening of a broad range of legacy PFAS, as well as perfluorinated sulfonamide species and fluorotelomer sulfonates. No legacy PFAS were detected in 13 milk samples from regions of concern given local use of biosolids or proximity to fire training areas. Overall, then, the uptake of perfluoroalkyl acids (PFAA) from dairy milk in the U.S. is considered low.

Graphical abstract



Keywords

AFFF, dairy milk, extraction method, FTS, PFAS

Introduction

Per- and polyfluoroalkyl substances (PFAS) comprise a broad group of anthropogenic chemicals that are widely used in industrial and commercial applications [1]. These chemicals display unique qualities such as lower micellization concentrations, ability to lower surface tension of aqueous phases, hydrophobicity, and are oleophobic [2]. A variety of industries and manufacturers have exploited these physicochemical properties to produce water repellent and stain resistant coatings on textiles, oil-resistant food contact materials, and efficient aqueous film forming foams (AFFF) [3]. As a result of their extensive use and chemical stability, PFAS are ubiquitous in the environment and have been detected in wildlife and humans [4–6].

Extensive PFAS contamination in the environment has been predominantly linked to applications of AFFF near airports, fire training areas, and military bases, as well as agricultural use of biosolids or sludge derived from wastewater treatment plants (WWTP) [5,7–9]. Prolonged applications of AFFF and WWTP biosolids and sludge are attributed to elevated PFAS concentrations in soil and groundwater, as well as surface and well water [10–15]. At numerous sites impacted by AFFF and biosolids, the concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in drinking water dramatically exceeded the U.S. Environmental Protection Agency (EPA) lifetime health advisory level of 70 ng/L for the combined concentration of these two compounds [16–18].

Ingestion of such as contaminated drinking water is a significant human exposure pathway in addition to PFAS ingested through diet [19,20]. In general, human dietary PFAS exposure occurs by two main routes: i) direct exposure to PFAS present in unprocessed, raw products as a result of environmental contamination, and ii) indirect exposure to PFAS present in food contact materials used in manufacturing, packaging, and preparation of processed food [21]. Dietary PFAS exposure pathways and PFAS contribution vary for different populations [21]. For instance, the European Food Safety Authority (EFSA) estimated that fish and seafood are predominant pathways for chronic PFAS exposure in adults to PFOS (up to 86%). The EFSA also projected that milk and dairy products are significant PFAS chronic exposure pathways to vulnerable populations (e.g., toddlers) [20]. The PFAS contamination in milk and dairy products could originate from processing and packaging of the final products, but most likely comes from transfer of PFAS from feed to cows. This was previously demonstrated in both a dosing study [22] and a descriptive model [23] in which dairy milk became a reservoir for PFAS. With a continuous increase in annual milk production in the U.S. over the last decade [24], there is plausible concern for increased risk of dietary exposure to PFAS to the general U.S population.

While a range of retail food studies including raw milk and other dairy products have been conducted in both farm and local market products all over the world [21,25–30] a limited number of reports exists for the U.S. domestic food supply. Previous studies have demonstrated that livestock forage grown on biosolid-amended soils is an important driver of PFAS contamination of the cattle [15,31]. Similarly, the use of organic fertilizers mixed with industrial wastes on cropland has led to contamination of the cattle feedlots and subsequently to elevated PFAS concentrations in animal by-products such as meat [22]. In multiple studies, the cattle exposed to contaminated feed eliminate PFAS via lactational transfer [15,22,32]. Application of contaminated biosolids has also been documented in farms across the U.S. for which PFAS concentrations reached up to thousands of micrograms per kilogram of biosolids [15,31,33]. Therefore, agricultural application of WWTP biosolids or industrial wastes or the proximity of dairy farms to AFFF-impacted areas warrants concern for PFAA contamination in dairy production.

Previous research on PFAS contamination of dairy cow milk has placed a greater emphasis on limited number of legacy perfluorinated alkyl acids (PFAA) with a focus on PFOA and PFOS [21,22,25,28–30,34,35]. Elevated contamination in milk was mostly attributed to the ability of PFAA to bind to β-lactoglobulin proteins in cow milk [26,36] however limited data exists on both milk concentrations and mechanism of binding/releasing of emerging PFAS associated with AFFF and WWTP biosolids applications such as polyfluorinated fluorotelomer sulfonates, perfluoroalkyl sulfonamidoacetic acids, and perfluoroalkyl sulfonamides [17,37].

Previously developed extraction methods for PFAS analysis in milk used a small volume of the samples (1-5 mL) to minimize the lipid and protein content in the final extracts and prevent potential matrix effect during the instrumental analysis [38–40]. Additionally, up-to-date published milk extraction method targeted the legacy PFAA only, without including the novel PFAA alternatives and precursors [21,22,25,26,28–30,35]. The aim of the present study was therefore to i) modify solvent digestion and sample cleanup method for broader group of legacy, emerging and precursor PFAS in raw dairy cow milk and ii) apply the extraction method on raw dairy milk collected from U.S. dairy farms.

Materials and methods

Standards and reagents

The 8-point calibration curve (0.004 – 100 ng/mL), QA/QC instrumental performance check, and surrogate standard were created using analytical PFAS standards purchased from Wellington Laboratories (Ontario, Canada). Individual target PFAS and corresponding isotope labelled analogues are listed in Table SI 1. Formic acid (99+%), ammonium hydroxide (28%-30%), and liquid chromatography-mass spectrometry (LC-MS) grade methanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Oasis WAX solid-phase extraction resin (30 μm) was purchased from Waters (Milford, MA, USA) and ENVI-Carb cartridges (Supelco) were purchased from Sigma-Aldrich.

Extraction Method Evaluation

To evaluate the efficiency of extraction methods, approximately 10 g of local market, whole-milk was weighed into wide-mouth polypropylene jars (*n* = 9), spiked with representative native PFAS standards (10 ng/sample) and placed overnight in a freezer at -15 ^{o}C before freeze drying at -53 ^{o}C for ~60 hours in a LABCONCO FreeZone 2.5L benchtop freeze dry system. Freeze dried milk (~2 g) was transferred to 15mL Corning® Falcon centrifuge tubes and divided into treatment groups (*n* = 3 per treatment) based on extraction solvent and clean-up procedure: i) 0.1% formic acid in methanol followed by clean-up with only ENVI-Carb cartridges, ii) 0.1% formic acid in methanol extraction followed by clean-up with Oasis WAX resin loaded atop of ENVI-Carb cartridges, and iii) 0.1% ammonium hydroxide in methanol followed by clean-up with Oasis WAX powder loaded atop of ENVI-Carb cartridges. To create the paired WAX resin with

ENVI-Carb cartridge, approximately 500 mg of WAX resin suspended in LC-MS-grade methanol was transferred onto the Envi-Carb cartridge (1 g) with a pre-cleaned disposable pipette.

To extract PFAS from the milk matrix, 6 mL of solvent was added to samples according to respective treatment groups. All samples were then vortexed for ~30 s, placed in an ultrasonic bath for 20 min, and centrifuged at 4000 rpm for 10 min. ENVI-Carb cartridges were fixed to a CHROMABOND SPE manifold. All cartridges were pre-cleaned prior to sample loading with ~3 mL each of 0.1% formic acid in methanol, 0.1% ammonium hydroxide in methanol, and finally neutral methanol. The organic layer was then transferred with a disposable transfer pipette and loaded onto the cartridges. 15 mL Falcon centrifuge tubes were placed inside the manifold to capture milk extracts. Samples were allowed to elute under gravity. The pellet formed during centrifugation was rinsed with ~2 mL of neutral methanol and resuspended, vortexed ~30 s, placed in an ultrasonic bath for 20 min, and centrifuged at 4000 rpm for 10 min. The organic layer was then transferred as detailed above onto the respective ENVI-Carb cartridge after the first extract eluted. After gravity elution of the last fraction, a final wash of ~1-2 mL of neutral methanol was used to rinse the inside of the cartridge. Vacuum pressure (<15 psi) was applied to the manifold to remove residual solvent extract bound within the cartridges. Approximately 9-10 mL of extraction eluent was present after SPE clean-up procedures. This eluent was evaporated at 36 ^{o}C to ~0.5 mL under a gentle stream of nitrogen gas and spiked with mass labeled PFAS mix (2 ng/sample). 40 μL of the concentrated extract was diluted with 60 μL of 4 mM ammonium acetate in water prior to LC-MS analysis.

Sample collection and storage

Milk samples (*n* = 13) were collected from 13 individual cattle farms across The United States (Table SI 2, Figure 1). These dairy cattle farms reported use of biosolid amendments on cropland or were located within proximity to AFFF-impacted soils. Samples were shipped on ice in original storage containers. All sample storage containers, extraction vessels, and transfer pipettes were pre-cleaned with ACS-grade methanol, 3% ammonium hydroxide in LC-MS-grade methanol, and LC-MS-grade methanol prior to use. Thawed milk samples were partitioned into pre-cleaned 1 L HDPE bottles for storage at -15 ^{o}C. Locally purchased pasteurized whole milk was used for determinations of dairy matrix interference with instrument detection. Samples were analyzed within their shelf lives. A representative summary of sample collection locations can be found in Table SI 2.

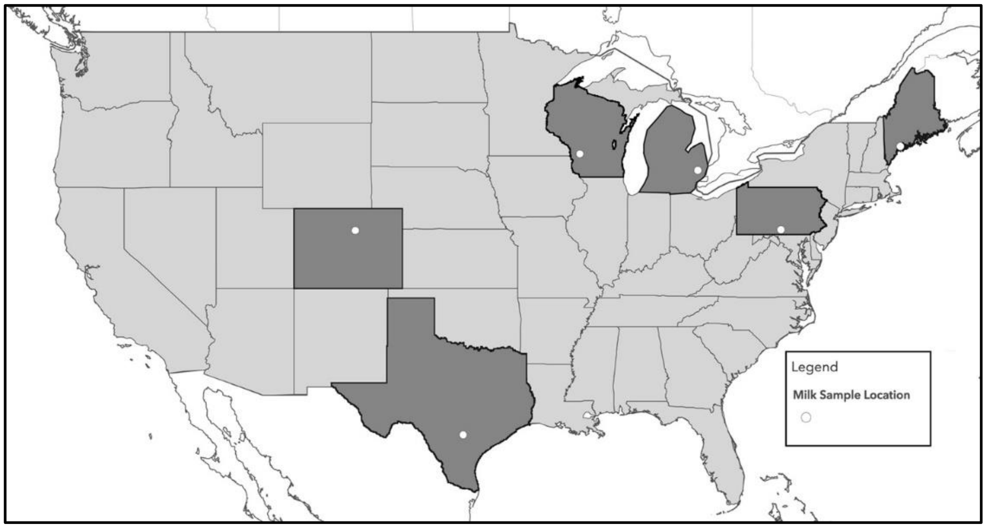


Figure 1: *Map of milk samples location in selected states (Colorado, Maine, Michigan, Pennsylvania, Texas and Wisconsin) in The United States*

Sample preparation

Frozen milk samples were allowed to thaw at room temperature and well mixed before ~25 g of thawed samples were weighed into pre-cleaned 50-mL polypropylene Corning® Falcon centrifuge tubes. All samples, duplicates, matrix spikes and blanks were spiked with mass labeled surrogate PFAS standard mixture (4 ng/sample). Additionally, a native PFAS solution (4 ng/sample) was added to matrix spike milk samples. Sample aliquots were frozen overnight at -15 ^{o}C, followed by -80 ^{o}C for five hours the next day, before freeze-drying in a LABCONCO® FreeZone2.5 for 60 h at -54 ^{o}C. After freeze-drying, sample extraction was conducted using a combined solvent digestion procedure.

Briefly, 12 mL of 0.1% formic acid in LC-MS-grade methanol was added to each freeze-dried milk sample to denature proteins. Samples were then vortexed for ~30 s and placed in an ultra-sonic bath for 25 min at room temperature before centrifugation at 4000 rpm for 10 min. The organic supernatant was then transferred to a 15 mL Corning® Falcon centrifuge tube and concentrated down to ~1 mL under a gentle stream of nitrogen gas to allow room for additional aliquots. Following the initial concentration step, 6 mL of LC-MS-grade methanol was added to the original pellet formed in the first solvent digestion step. The same vortex, sonication, and centrifugation steps were repeated. After centrifugation, the organic supernatant was transferred and combined with the concentrated acidic digestion extract. Lastly, a final solvent digestion was performed with 6 mL of 0.1% ammonium hydroxide in methanol following the same procedures as outlined in the previous solvent digestions. A final sample concentration under a gentle stream of nitrogen gas down to ~4 mL was performed. The final volume extracts were stored overnight at -15 ^{o}C to promote precipitation of residual matrix within extracts.

The sample clean-up procedure was performed with ENVI-carb (1 g, Supelco) cartridges. Cartridges were affixed to a CHROMABOND® SPE manifold and precleaned with 2 mL each of 0.1% formic acid in methanol, 0.1% ammonium hydroxide in methanol, and lastly LC-MS-grade methanol. Prior to loading, samples were taken out of freezer storage, centrifuged to remove residual matrix for 1 min at 4000 rpm. The supernatant was then transferred into ENVI-carb cartridges and allowed to elute under gravity (~1 drop/sec) into fresh 15 mL Corning® Falcon centrifuge tubes. A 1 mL wash with LC-MS-grade methanol was performed on the original storage tube and centrifugation for 1 min at 4000 rpm conducted prior to loading this extract to the cartridge. Additionally, cartridges were rinsed with a final 1 mL LC-MS-grade methanol aliquot. Lastly, vacuum pressure (~10 psi) was applied to elute residual solvent extract bound within the cartridge. Eluents were concentrated down to ~0.5 mL under a gentle stream of nitrogen gas before preparation for HPLC-MS/MS analysis.

Instrumental LC-MS analysis

The LC-MS/MS analysis of targeted PFAS (Table SI 1) was performed using a liquid chromatograph (Shimadzu Prominence UFLC) equipped with a Gemini C18 hybrid column (3 μm, 2.1 mm X 50 mm; Phenomenex) coupled to mass spectrometer (AB Sciex 4500 QTRAP) operating in negative ion mode. To reduce background contamination in the system, a delay column (Luna 5 μm C18(2) 100 Å, LC Column 30 x 2 mm) was installed to the LC system. For analysis, 20 µL of prepared extract was injected on the analytical column and PFAS were separate and determined (all analytical details are listed in SI, Table SI 3, 4 and 5 and in [41]).

QA/QC

The calculations of the PFAS concentration in samples and quality control samples was based on the isotope dilution method of quantitation. To guarantee quality control, three process blanks and two matrix spikes blanks were included within each batch of 14 samples. Blank concentrations were <10% of the measured samples, and due to this low background contamination level, sample concentrations were not blank corrected. The method detection limits (MDL, ng/L) were determined considering the following criteria: in case no analyte signal was detected in the process blanks, instrumental detection limits (IDL) were used as MDL and an appropriate dilution factor was applied. IDL represents the concentration of analyte giving the signal-to-noise ratio of 10 in presence of the matrix. In case the analytes were detected in process blanks, MDL were calculated as average value plus 3 times the standard deviation (SD) of the concentrations in all blanks. MDLs and recoveries for all targeted PFAS are listed in Table 1 (with details in SI). Additionally, recoveries of the surrogate mass labeled PFAS spiked into the real samples, blanks and quality control samples were withing 60-140%.

Results and discussion

Extraction Method Evaluation

The extraction method (solid liquid extraction, SLE) evaluation incorporated nine retail milk replicates for which two different extraction solvents and two different clean-up methods were utilized. When extracting PFAS from milk, it is common to incorporate solvents or salts to denature and precipitate proteins and other biochemical artifacts that may bind PFAS, such a β -lactoglobulin [25,26,28]. For these reasons, a similar approach was applied, relying on either 0.1% formic acid (FA) in methanol (treatment 1 and 2) or 0.1% ammonium hydroxide (AH) in methanol (treatment 3).

A summary of 22 native PFAS recoveries from extraction is provided in Figure 2. Average recoveries for the C4-C10 PFCAs was generally over 60% between treatments (Fig. 2a). Among the C4-C11 PFCAs, incorporation of ~500 mg WAX powder loaded atop of the ENVI-Carb cartridge (1 g) did not result in significantly higher recoveries. Recoveries of the C12-C14 PFCAs were generally over 50% except for PFTeDA (treatment 2). Average recoveries of C4 and C6-C8 PFSAs (Fig. 2b) ranged from 48% to 51% with basic digestion (treatment 3) extraction being most optimal for the recovery of the sulfonates. PFBS had the greatest recovery of the PFSAs at 63%. Recovery of the sulfonamides (Fig. 2c): FBSA, FOSA, *n*-MeFOSA, and *n*-EtFOSA ranged from 23% to 71% across experiments with recovery of FOSA being the highest at 71% in Treatment 1. The recoveries for *n*-MeFOSA, and *n*-EtFOSA were overall low, so these compounds were excluded from further evaluations. Recoveries for the fluorotelomer sulfonates (Fig. 2d) ranged from 42% to 68%, with highest average recovery across treatment groups residing with 6:2 FTS at 68%, and lowest average recovery with 4:2 FTS at 42%. Generally, the target PFAA and polyfluorinated precursors had recoveries of ~60% on average. The WAX powder allowed for greater separation of C4-C12 PFCA, PFBS, PFHxS, PFHpS, PFOS, and other perfluorinated species from the interfering matrix. However, the recoveries for longer chain PFCA which are known for their bioaccumulative properties [42] were higher on average for Treatment 3 which incorporated use of 0.1% ammonium hydroxide in methanol. The recoveries of PFCA were generally higher in Treatment 3 when compared to Treatment 1. A similar pattern can be seen with the PFSA and 6:2 FTS. Slight differences were evident in the recovery for PFOS between Treatment 1 and Treatment 3 (Fig. 2b). These patterns provided justification to utilize a stepwise solvent extraction which incorporates both acidic and basic organic solvents to account for the broad spectrum of predominant PFAS found in WWTP wastewater and biosolids, as well as AFFF [14,37,43–45]. Therefore, the combination of the treatment 1 and 3 was applied on the real milk samples to achieve maximum recoveries for all targeted group of PFAS.

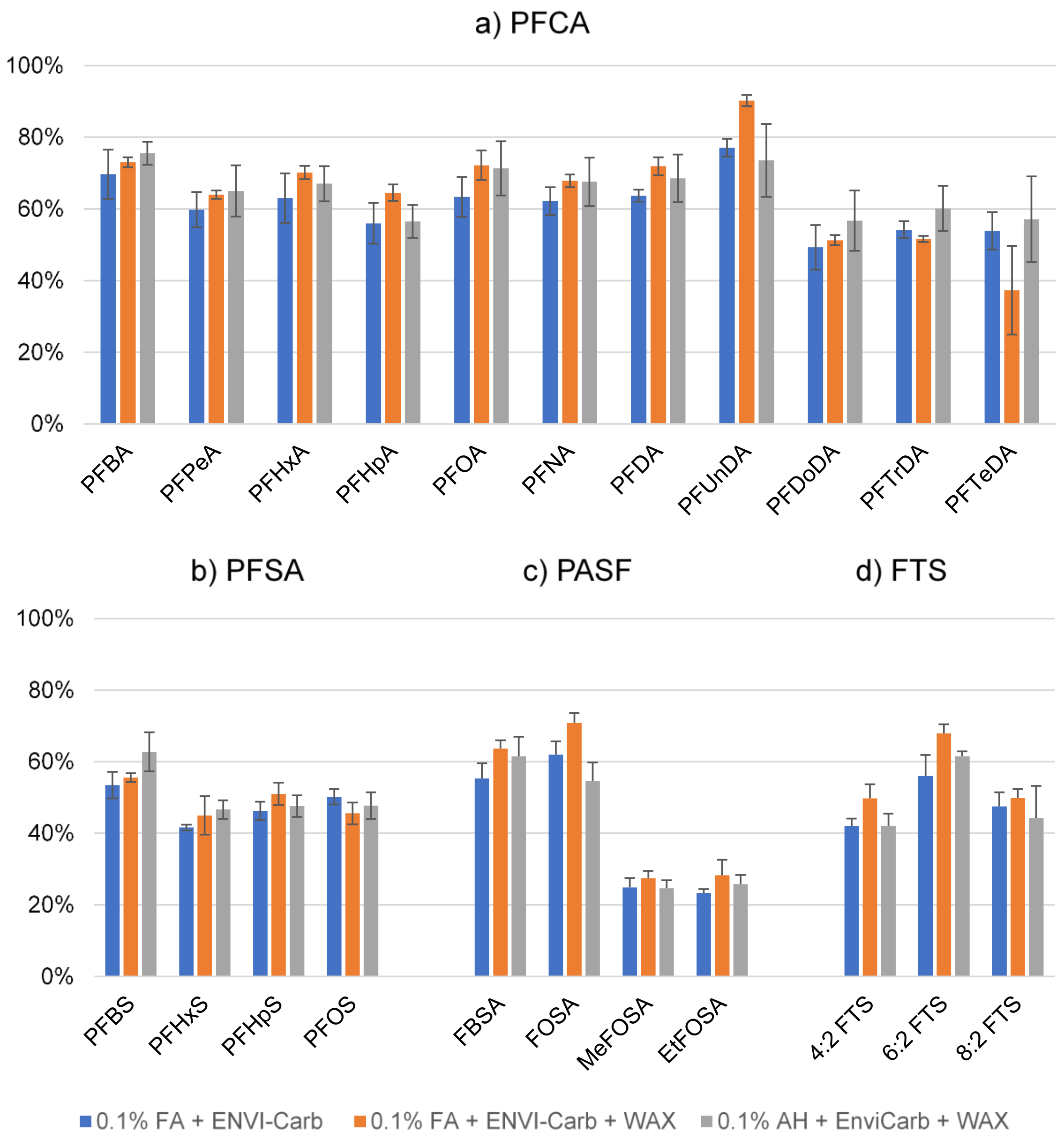


Figure 2: *Recoveries of individual per- (1a, 1b, and 1c) poly- (1c) fluorinated compounds using three different treatment methods. 1a – PFCA (perfluorocarboxylic acids); 1b – PFSA (perfluorosulfonic acids); 1c – PASF based compounds (perfluoro sulfonamides); and 1d – FTS (fluorotelomer sulfonates).Treatments (n=3) : i) 0.1% FA in methanol + clean-up with ENVI-Carb (blue), ii) 0.1% FA in methanol + clean-up with Oasis WAX loaded atop of ENVI-Carb (orange), and iii) 0.1% AH in methanol + clean-up with Oasis WAX loaded atop of ENVI-Carb (grey)*

Analysis of real milk samples

A total of thirteen raw and retail milk samples were collected from U.S. dairy farms that either had confirmed use of biosolids on cropland or were within geographic proximity to military installations with confirmed AFFF use. PFAS present in AFFF utilized at fire training areas and military bases may persist in soils and groundwater leachate [17,46,47]. PFAS recalcitrance in soils due to AFFF leachate irrigation or biosolid amendment application pose reasonable concerns for agriculture [48]. Concentration of PFOA and PFOS in plants grown in biosolid amended soils have previously been found up to 200 ng/g dw and 20 ng/g dw, respectively [49]. PFOS concentrations in biosolids from previous studies found as little as 4.3 to 89 μg/kg dw [50] to as much as 3120 μg/kg in the U.S. [31], reaching elevated concentrations that have ubiquitous concern for biosolid use in agriculture. Where WWTP biosolids have been spread on cropland, PFOS concentrations have been quantified up to 483 μg/kg dw [33,51] took into consideration WWTP biosolid amendments and the likely occurrence of biotransfer from crop to organism by providing toxicokinetic evidence of PFOA uptake and elimination in beef cattle. Following this, Kowalczyk et al. (2013) demonstrated elimination of PFAS from naturally contaminated feed, in part, through lactational transfer [22]. Accumulation of PFAS in animal by-products therefore serves as a possible endpoint for exposure to humans who incorporate dairy milk in their diet. Both the proximity of farms to AFFF-impacted soils and the presence of WWTP biosolids on croplands raises concerns for bioaccumulation in food animals whose feed is obtained from the cropland.

A targeted LC-MS analysis of 27 PFAS (Table SI 1) was conducted on the raw and retail milk samples for which a combined solvent extraction and ENVI-Carb clean-up was performed. The efficiency of the modified solvent digestion extraction and clean-up procedure is summarized in Table 1. The overall recoveries of the 27 PFAS were evaluated by using a real milk samples spiked with native PFAS solution (4ng per sample). The recoveries were calculated using the isotope dilution method. Recoveries for the 27 targeted analytes ranged from 69 ± 9% to 141 ± 5%. The average recovery amongst the PFAA (13 compounds) was 93%, similar to recoveries for PFCA with $CF_2 \leq 10$ previously determined in other studies [21,26,28], which ranged from 70 to 120% . Only Lacina et al. [26] demonstrated similar performance for longer chain PFCA using a multistep ion pair extraction and cleanup method. Within the group of PFAS (8 compounds) the method achieved an average recovery of 113%, ranging from 90 ± 6% to 141 ± 5%, the lowest being PFECHS and PFNS as the highest, respectively. In above mentioned studies the smaller range of PFSA (3 to 5) was dominantly analyzed with recoveries ranging from 70 to 104%. For the 4 sulfonamides and sulfonamide acids, the average recoveries were 97%. Recovery for the only previously determined sulfonamide from this group (FOSA) was 107 ± 1 % which is comparable to previously published recoveries 98% [26]. Lastly, recoveries of the fluorotelomer sulfonates (3 compounds) ranged from 98 ± 3% to 136 ± 7%, with lowest recovery of 6:2 FTS and highest recovery of 8:2 FTS, respectively.

Table 1: *Calculated Recoveries (% ± SD) and Method Detection Limits (MDLs) for analysis of real samples*

Functional group	Fluorination	n (CF_2)	Compound	*Recovery (%) ± SD*	MDL (ng/L)
1 -COOH	Per-	4	PFBA	69 ± 9	144
		5	PFPeA	90 ± 0	7.6
		6	PFHxA	91 ± 3	3.9
		7	PFHpA	120 ± 0	11.0
		8	PFOA	82 ± 0	8.8
		9	PFNA	84 ± 9	2.2
		10	PFDA	86 ± 13	1.6
		11	PFUnDA	118 ± 17	3.6
		12	PFDoDA	110 ± 17	5.7
		13	PFTrDA	91 ± 14	5.3
		14	PFTeDA	88 ± 5	2.8
2 $-SO_3H$	Per-	4	PFBS	117 ± 8	22
		5	PFPeS	94 ± 8	3.6
		6	PFHxS	106 ± 4	11
		7	PFHpS	103 ± 1	11
		8	PFOS	112 ± 6	2.9
		8	PFECHS	90 ± 6	2.3
		9	PFNS	141 ± 5	12.9
		10	PFDS	115 ± 8	2.4
3 $-SO_2N$	Per-	4	FBSA	105 ± 8	1.9
		6	FHxSA	80 ± 12	0.8
		8	FOSA	107 ± 1	5.2
		8	MeFOSAA	112 ± 8	2.1
		8	EtFOSAA	81 ± 1	1.4
4 $-SO_3H$	Poly-	4	4:2 FTS	105 ± 8	1.9
		6	6:2 FTS	98 ± 3	1.6
		8	8:2 FTS	136 ± 7	2.0

We evaluated the method performance on the real samples and calculated the method detection limits (MDLs) for the SLE-HPLC-MS/MS as described above. Generally, MDL ranged from 0.8-22 ng/L for 26 PFAS and 144 ng/L for PFBA, which is known for a strong matrix interference. Achieved MDL are far below the only established action level for PFAS (PFOS; 210 ng/L) in cow's milk developed by Maine Department of Agriculture, Conservation and Forestry (DACF) the Maine Center for Disease Control and Prevention (MECDC) [52].

The uniqueness of this method is considered in the targeted screening of a broad range of legacy PFAS, as well as perfluorinated sulfonamide species and fluorotelomer sulfonates, for which MDL <5.2 ng/L were achieved. To our knowledge, this is one of the first studies to screen such a variety of legacy and emerging PFAS in the U.S. produced milk.

The stepwise solvent digestion method incorporating the use of acidic, and basic methanolic solvents for initial extraction of PFAS in cow milk is the first of its kind in the literature together with a condensed clean-up to a single ENVI-Carb cartridge to help remove milk sample matrix. This improved clean-up and extraction method achieved recoveries that were as good as or better for target PFAS in comparison to other dairy cow milk studies where MDLs varied between hundreds pg/L to tens ng/L for limited number of PFAA [21,22,25,26,28–30,35].

Table 2: *Comparison of concentration (ng/L) of various group of PFAS in dairy milk samples*

Country	**n**	**Concentration range (min-max) ng/L**				**Reference**
		PFCA	**PFSA**	**PASF**	**FTS**	
The Czech Republic	12	<MDL	<MDL	<MDL	NA*	[26]
USA	61	NA	<MDL – 0.16	NA	NA	[21]
Italy	15	<MDL	<MDL	NA	NA	[30]
Germany	14	<MDL – 10.1	<MDL – 8.5	NA	NA	[28]
The Netherlands	17	<MDL	<MDL	NA	NA	[53]
Italy	67	<MDL – 32	<MDL - 97	NA	NA	[29]
China	46	<MDL – 370	<MDL – 120	NA	NA	[34]
China	115	<MDL – 151.8	<MDL – 172.9	NA	NA	[35]
Taiwan	10	30 – 1440	<MDL – 10	NA	NA	[25]
USA	13	< MDL	< MDL	< MDL	<MDL – 6.59	**this study**

** NA – not analyzed in the particular study*

The present study included milk samples that were collected from a variety of rural dairy farms that sell to local markets and larger urban areas (Table SI 1, Figure 1). The only analyte detected in this study was 6:2 FTS at concentration 6.6 ng/L, for which a lack of data is available from previous studies regarding contamination in dairy cow milk (Table 2). To our knowledge, the only other U.S.-based study (Table 2) similarly investigated biosolid-amended croplands and concerns for accumulation of PFAS in dairy milk and quantified only PFOS (0.16 ng/L) above its MDL (0.13 ng/L) [21]. Both 6:2 FTS and PFOS can commonly be found in both AFFF leachate and WWTP biosolids [10,44]. With PFAS concentrations in most milk samples being below their MDLs, and a representative number of dairy farms and locations included in this study, the data suggests that consumption of dairy milk is not a prominent source of dietary PFAS exposure. Similarly, proximity to military zones with historical AFFF use does not seem to be a factor. However, as recent evidence suggests, the presence of biosolids containing PFAS may lead to contamination in soils and plants on cropland [15]. Especially short chain PFAA such as PFBA, PFPeA and PFBS are well known to be accumulated by agriculture plants [54,55] but it is unknown as to whether the cattle on these farms frequently graze on cropland associated with biosolid spreading or if these short chain PFAA which show different elimination kinetics compare to the long chain PFAA due to smaller molecular size have been excreted via urine [22].

Conclusion

The method presented in this study demonstrated enhanced capacity to quantitatively analyzed a broad range of PFAS in dairy milk in sub ng/L using a combined solvent extraction and single step clean-up procedure. Using this method, we screened raw and processed milk samples from dairy cattle farms which reported use of biosolid amendments on cropland or were located within proximity to AFFF-impacted soils. While levels of legacy PFAS formerly known to accumulate in a variety of dairy products were below detection limits, the fluorotelomer sulfonate (6:2 FTS) was detected in one sample. These findings might reflect shifts in the AFFF compositions thus the further exploration of PFAS contamination of dairy products using non-targeted screening or total extractable fluorine approach might be essential.

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Ethics declarations

Conflict of interest

The authors declare no competing interests.

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