JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Determination of Perfluorochemicals in Cow's Milk Using Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT: This study describes a new method developed for detection of 10 different perfluorochemicals (PFCs) in cow's milk, seven perfluorinated carboxylates and three perfluorinated sulfonate salts. After attempting multiple methods employing both acidic and basic extractions, a basic extraction using 10 mM sodium hydroxide in methanol digestion along with weak anion-exchange solid-phase extraction was employed. Vortex mixing and varying sonication times were compared as part of sample processing. Results show that sonication during sample processing yield decreased recovery of longer chain perfluorinated carboxylates. The final method developed was used to determine the concentration of PFCs in 12 raw and 49 retail milk samples from across the United States. With the exception of a single raw milk sample obtained from a dairy farm that had applied PFC containing biosolids to its fields, there were no milk samples containing PFCs.

KEYWORDS: *perfluorochemicals, milk, method development, PFOA, PFOS*

INTRODUCTION

Perfluorinated compounds (PFCs) have been used in a wide variety of manufacturing processes and consumer goods, including cookware, food packaging, stain-resistant coatings, and fire-fighting foams.[1,2] Due to their extensive use and stability, PFCs are now widespread in the environment[3] and have been detected in wildlife,[4] in remote locations far from industrial sites,[5] and in humans from both industrialized and developing countries.[6] These findings have raised concern and prompted international bodies to classify PFCs as persistent organic pollutants (POPs).[7]

Along with quantifying environmental concentrations of PFCs, a number of researchers have identified routes of human exposure. These include but are not limited to inhalation of house dust[8] and air,[9,10] and consumption of water[11] and food.[12] While there is still limited data, a number of researchers have concluded that food is often the primary human exposure route, especially in populations without occupational exposures.[13,14]

When considering human exposure to PFCs from food, the routes of exposure can be divided into two classes. First is the "endogenous" PFC concentration found in raw, unprocessed products due to adsorption from the environment.[15] Second is the migration of PFCs from food contact materials used in manufacturing, packaging, and preparation of food.[16,17] Characterizing and quantifying these pathways is an important first step in assessing human exposures.

The PFC concentrations in a broad range of retail foods have been reported;[12,18,19] however, the number of reports are limited and none addressed the U.S. domestic food supply in detail. The need for a study of the U.S. food supply is apparent considering the concentration of PFCs in human serum. On average, the U.S. human serum concentrations are similar to those of most industrialized countries. Several PFCs (PFOS, PFOA, PFHxS, and PFNA) were regularly detected in the U.S. population that is not occupationally exposed.[20] Determination of PFC concentrations in foods as a result of environmental contributions should focus on foods that are unprocessed or foods that have undergone limited processing and are not exposed to PFC-containing food contact materials. Due to the high consumption of milk in the United States, with per capita usages over 200 pounds per year, as well as its use in other products such as baby formula and prepared foods, milk could be a large contributing factor to the overall dietary human exposure to PFCs.[21,22] The typical (polyethylene food contact) packaging, minimum processing, and large consumption of milk makes it an ideal choice for gauging background concentrations of PFCs in foods.

This study determined PFC concentrations in both raw and retail cow milk samples. There are a variety of methods investigating PFC concentrations in dairy and human milk;[21,23−26] yet detailed characterization and descriptions of method parameters has been limited.[26] This paper presents the development and validation of a new method for determination of 10 PFCs, including seven perfluorinated carboxylates and three perfluorinated sulfonate salts, in cow milk, which was used in a survey of 61 raw and retail milk samples from across the United States (Figure 1).

MATERIALS AND METHODS

Standards, Reagents, and Materials. Perfluoro-*n*-[1,2,3,4-$^{13}C_4$]-octanoic acid ($^{13}C_4$-PFOA), perfluoro-*n*-[1, 2-$^{13}C_2$]undecanoic acid ($^{13}C_2$-PFUnDA), perfluoro-*n*-[1,2-$^{13}C_2$]dodecanoic acid ($^{13}C_2$-PFDoDA), sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate ($^{13}C_3$-PFHxS), and sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]perfluorooctane sulfonate ($^{13}C_4$-PFOS) were used as internal standards (IS) and purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada) along with sodium perfluoro-1-hexane sulfonate (PFHxS) 98%. Perfluoroheptanoic acid (PFHpA) 99%, perfluorooctanoic acid (PFOA) 96%, perfluorononanoic acid (PFNA) 97%, perfluorodecanoic acid

Received: November 8, 2011
Revised: January 9, 2012
Accepted: January 13, 2012
Published: January 13, 2012

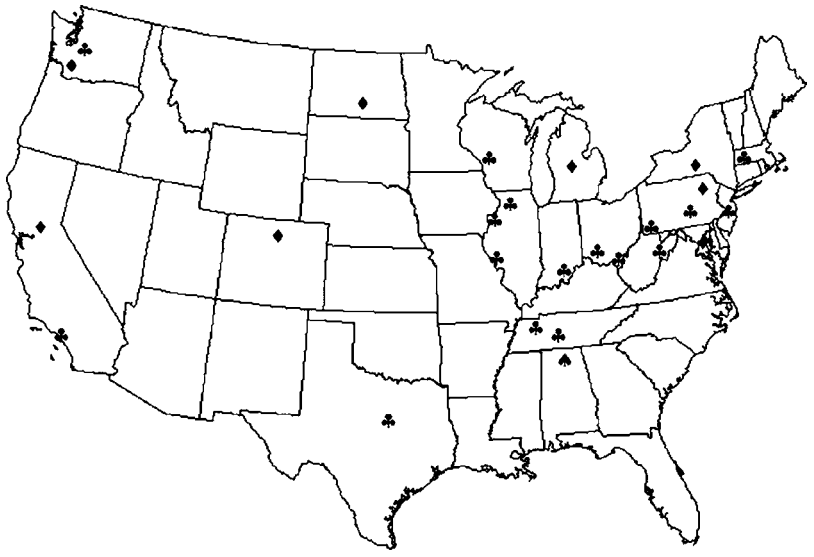

Figure 1. Geographical distribution of milk samples. Multiple sampling occurred in many locations. Key: diamonds, raw milk; spades, raw milk containing PFOS; clubs, retail milk.

Table 1. MS/MS Quantitative Transitions and Collision Energies and Calculated MDLs for PFCs

compound	MDL (n = 9) (ng/g)	percursor ion	product ion quantifier/qualifier	collision energy (eV)	IS used
perfluorobutane sulfonate (PFBS)	0.24	299	80/99	41	$^{13}C_3$-PFHxS
perfluorohexane sulfonate (PFHxS)	0.15	399	80/99	50	$^{13}C_3$-PFHxS
perfluorooctane sulfonate (PFOS)	0.13	499	99/80	60	$^{13}C_4$-PFOS
perfluorohexanoic acid (PFHxA)	0.42	313	269/119	2	$^{13}C_4$-PFOA
perfluoroheptanoic acid (PFHpA)	0.25	363	319/169	3	$^{13}C_4$-PFOA
perfluorooctanoic acid (PFOA)	0.12	413	369/169	4	$^{13}C_4$-PFOA
perfluorononanoic acid (PFNA)	0.28	463	419/219	4	$^{13}C_4$-PFOA
perfluorodecanoic acid (PFDA)	0.43	513	469/219	3	$^{13}C_4$-PFOA
perfluoroundecanoic acid (PFUnDA)	0.11	563	519/269	2	$^{13}C_2$-PFUnDA
perfluorododecanoic acid (PFDoDA)	0.80	613	569/169	8	$^{13}C_2$-PFDoDA
$^{13}C_3$-PFHxS	n/a	402	80/99	50	n/a
$^{13}C_4$-PFOS	n/a	503	99/80	60	n/a
$^{13}C_4$-PFOA	n/a	417	372/172	4	n/a
$^{13}C_2$-PFUnDA	n/a	565	520/320	8	n/a
$^{13}C_2$-PFDoDA	n/a	615	570/319	8	n/a

(PFDA) 98%, perfluoroundecanoic acid (PFUnDA) 98%, perfluorododecanic acid (PFDoDA) 95%, tetrabutylammonium perfluorobutane sulfonate (PFBS) 98%, and ammonium acetate 99.99% were obtained from Aldrich Chemicals (St. Louis, MO, USA). Perfluorooctane sulfonic acid (PFOS) potassium salt 98% standard was obtained from Alfa-Aesar (St. Louis, MO, USA). Perfluorohexanoic acid (PFHxA) was purchased from Oakwood Products Inc. (West Columbia, SC, USA). Two types of formic acid (≥96% and 99+%) were purchased from Aldrich Chemicals (St. Louis, MO, USA). Two additional types of formic acid, J.T. Baker 88% and Acros 88%, were purchased from VWR (Bridgeport, NJ) and Acros (West Chester, PA), respectively. Nitrogen (99.999%) was purchased from Airas (Salem, MS). LC-MS-grade Optima water and methanol, ammonium hydroxide (29%), polypropylene (PP) centrifuge tubes (15 and 50 mL), polystyrene (PS) 5 mL tubes, additive-free low-density polyethylene (LDPE) 500 mL bottles, and colorpHast pH papers were purchased from Fisher Scientific (Pittsburgh, PA, USA). Oasis WAX solid-phase extraction cartridges containing 150 mg (6 cm^3) sorbent with a 30 μm particle size were purchased from Waters (Milford, MA, USA). PP HPLC vials (0.3 mm) with polyethylene (PE) septa were purchased from Agilent Technologies (Palo Alto, CA). Milk samples and extracts were centrifuged using a Fisher 2100 R centrifuge, Model 120 (Needham Heights, MA), and a Herseus Biofuge picocentrifuge with temperature control from Thermo Scientific (Ashville, NC), respectively. Samples in PP centrifuge tubes were sonicated at room temperature using a Branson 2510R-DTH from Branson Ultrasonics Corp. (Danbury, CT). Samples were concentrated using a Techne sample concentrator with a DB-3A dri-block from Techne Inc. (Burlington, NJ).

Sample Collection and Treatment. Raw (n = 12) and retail (n = 49) cow's milk samples were collected from locations across the continental United States. All except for 1 of the 12 raw milk samples were obtained from bulk storage tanks containing milk from multiple cows. The remaining raw milk sample was from an individual cow. This sample and a bulk milk sample were obtained from a dairy farm that had applied PFC-contaminated biosolids to its fields.[27] Raw milk samples were shipped frozen or left liquid and transported on ice. Raw samples were either collected in or transferred into 500 mL LDPE bottles upon receipt and frozen at −20 °C until tested. The retail samples were all pasteurized whole milk, commercially available, and purchased at retail markets. Organic milk samples (n = 9), vitamin D added samples (n = 35), and ultrapasteurized samples (n = 10) were all included in the pasteurized milk collection. After collection all samples were transported on ice. Once received the samples were shaken vigorously and then separated into two separate 50 mL

centrifuge tubes and one 250 mL centrifuge tube and frozen at −20 °C until tested.

Extraction Methods. A 1 g aliquot of milk and 3 ng of internal standard (50 μL of 60 ng/mL each $^{13}C_4$-PFOA, $^{13}C_2$-PFUnDA, $^{13}C_2$-PFDoDA, $^{13}C_3$-PFHxS, $^{13}C_4$-PFOS) were added to a 15 mL centrifuge tube and vortexed. Sodium hydroxide (4 mL of 10 mM) in methanol (0.1% water by volume) was added to the milk, and the samples were vortexed. After mixing, the samples were centrifuged (4000 rcf) at 3 °C for 30 min. Cooling the samples during centrifugation produced a more compact pellet and clearer supernatant. The supernatant was then transferred into a 50 mL centrifuge tube, and the solid pellet was discarded. The supernatant was diluted to 50 mL with LC-MS water, vortexed, and loaded onto a SPE cartridge which had been preconditioned with 5 mL each of 0.1% ammonium hydroxide in methanol, methanol, and water. The sample was eluted to waste, under vacuum, at a rate of about 2 drops/s, and the cartridge was washed with sodium acetate buffer (25 mM, pH = 4, 6 mL), followed by methanol (5 mL). The PFCs were eluted, without vacuum, using 0.1% ammonium hydroxide in methanol (6 mL) and concentrated to 0.3 mL under a steady stream of nitrogen at 60 °C. The concentrates were diluted (1:2) with 2 mM ammonium acetate (0.3 mL) and vortexed. The sample extracts were then transferred to a 1.5 mL PP centrifuge tube and centrifuged at 13 000 rcf for 20 min. A 0.3 mL aliquot was placed in a PP autosampler vial with PE septa and stored at 4 °C until analysis. After analysis samples were recapped with new PE septa and returned to storage at 4 °C.

LC-MS/MS. Samples were analyzed using an Agilent 1100 HPLC interfaced with an Agilent 6410 triple-quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA). A 10 μL aliquot of the extract was injected into a Varian Pursuit XRs C_{18} column (150 × 2.0 mm, 3.0 μm) (Agilent Technologies). PFCs were separated using (A) methanol and (B) water (2 mM ammonium acetate, 5% methanol) with a gradient from 60% to 95% methanol during the first 12 min. Methanol (95%) was maintained for another 1.5 min before re-equilibrating the column for 8 min prior to the next injection. All separations were performed at a flow rate of 0.3 mL/min and a column temperature of 35 °C, resulting in PFCs eluting between 2 and 13 min.

The mass spectrometer was equipped with an electrospray ionization (ESI) source, operating in the negative-ion mode with a capillary voltage of 1000 eV, 20 psi N_2 nebulizer gas at a flow of 6 L/min, N_2 collision gas, and a temperature of 300 °C. Two mass transitions were monitored for each of the 10 analytes (Table 1) using time-segmented multiple reaction monitoring (MRM). Analytes were quantified using the first transition listed in Table 1. Analyte confirmation required the presence of both transitions and that the relative intensity ratios were within ±20% of the calibration standard. Additionally, the second transition must have a S/N peak intensity of ≥3.

Calibration Standards and Quantification. A 1000 ng/mL stock solution of each of the 7 carboxylates and 3 sulfonate salts was prepared in a mixture of 30% water (2 mM ammonium acetate) and 70% methanol by volume. From this stock solution three working stock solutions of 100, 10, and 1 ng/mL were volumetrically prepared in water/methanol (30/70 by volume). Using the 3 working stock solutions, calibration standards were produced by fortifying water (0.1, 0.3, 0.5, 1, 2, 3, 5, 10, 20, and 40 ng/mL), adding internal standard (3 ng), and processing through the sample cleanup and concentration procedure. Matrix-matched calibration standards were not used because it was unknown if a single negative milk could be used as a control for the variety of milk samples analyzed (raw, pasteurized, ultrapasteurized, location). During the study, four different sets of calibration standards were prepared by a single analyst. To address possible contamination issues, negative controls (water blanks) were processed with each sample set. Additionally, positive controls (PFC spiked milk), 1 for every 10 samples, at 2, 10, and 30 ng/g were used to assess the accuracy of the method.

Calibration standards (0.3−40 ng/mL) were analyzed daily prior to and throughout analysis of sample sets. Calibration curves were prepared by plotting the nonweighted simple linear regression of the area ratio (analyte:internal standard) versus analyte concentration (R^2 values of 0.99 for most analytes, 0.97 for PFNA, and 0.96 for PFDA). All of the peak integration and mass spectrometry data processing was performed with Agilent MassHunter Quantitative Analysis (Version B.03.01, Agilent Technologies). PFC concentration in each test portion was calculated from the equation of the line of the standard curves analyzed with the set. Microsoft 2007 Excel (Microsoft Corp., Redmond, WA) was used for all additional data processing. All concentrations reported were determined using the mass of the free acetate and sulfonate ions and not the corresponding salts.

RESULTS AND DISCUSSION

Method Development and Validation. Previous determinations of PFCs in human milk involved the use of a large volume of concentrated formic acid.[24,28] The formic acid was used to precipitate milk proteins, reduce matrix effects, and improve PFC extraction.[29] In this work initial sample extractions, based on the method developed by Tao et al.,[24] utilized concentrated formic acid and produced visibly clean extracts and low background signals. However, these extracts contained a PFOA contamination. After systematic elimination of the other reagents and materials used in the extraction process, the PFOA contamination was determined to originate from the formic acid. The PFOA contamination, calculated by the calibration curve, was large enough (4 ng/mL in extract) to impact the sensitivity and accuracy of the method (Figure 2).

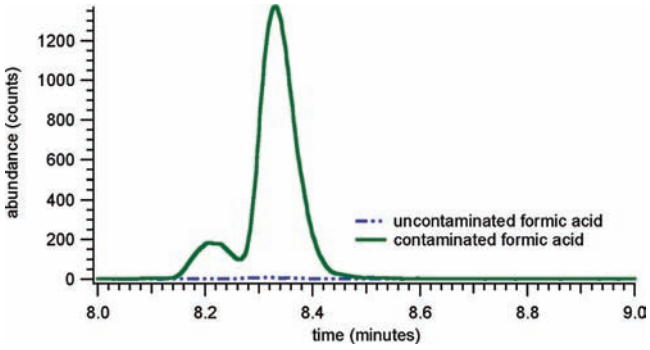

Figure 2. Extracted PFOA ion chromatogram (413 → 369) from samples processed with uncontaminated and contaminated formic acid. Contaminated formic acid shown as having ∼4 ng/g PFOA in blank extract.

To address the PFOA contamination, three formic acids from different vendors (Aldrich, J.T. Baker, and Acros) and different purities were evaluated. All three acids had similar concentrations of PFOA (4 ± 1 ng/mL) in the blank extracts. Therefore, a method to purify the formic acid by pretreatment on an HLB SPE cartridge was evaluated.[29] Pretreatment did not reduce the PFOA concentration. On the basis of these results a different milk extraction method using a smaller volume and lower concentration of formic acid was evaluated.[29] PFOA contamination was reduced but was still at concentrations (∼100 ppt) that would negatively impact the method detection limit.

Due to the background concentration of PFOA attributed to the use of formic acid, additional sample-processing methods were investigated. A number of researchers have reported that the use of alkaline digestion for determination of PFCs in fish,[30,31] meat,[19] and breast milk[21] minimized interferences and matrix effects and improved sensitivity.[32] However, these methods involved sample digestion using an orbital shaker for 3−16 h. A more recent study of PFC concentrations in fish

reported replacing 16 h of orbital shaking with 30 min of sonicaton.[33] Therefore, vortex mixing and/or sonication were evaluated as replacements for the multihour digestion. NaOH (10 mM) was added to milk fortified with PFCs (2 ng/g), and the mixture was briefly (<1 min) vortexed or briefly vortexed and sonicated for 30, 60, or 90 min before centrifuging and SPE cleanup. The absolute recoveries for all of the sulfonates and the shorter chained carboxylates (PFHxA–PFOA) were independent of the sonication time (Figure 3, PFOA data

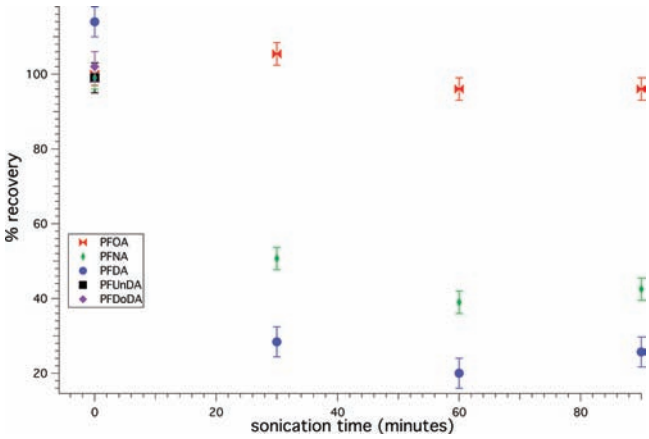

Figure 3. Effect of sonication time on analyte recovery of C_8–C_{12} PFCs. PFUnDA and PFDoDA along with labeled matched IS are not detected at sonication times greater than zero. PFOA data is representative of C_6–C_8 perfluoro sulfonates and perfluoro carboxylates.

shown). However, for the longer chain carboxylates (PFNA–PFDoDA) vortex mixing gave the best recoveries (100 ± 10%), while significant losses were noted for the 30, 60, and 90 min sonication times. PFNA and PFDA show a nearly 50% and 30% recovery, respectively, after 30 min of sonication. Additionally, PFUnDA and PFDoDA were not detected after 30 min of sonication. Although other methods using sonication and/or NaOH have not reported significant analyte losses, those studies used acidic conditions[24] or were extracting from tissue[31] and not the high fat and water conditions found in milk. On the basis of these sonication results all of the samples were processed using vortex mixing after addition of NaOH and prior to centrifugation and SPE cleanup.

In order to reduce potential matrix effects from coextracted contaminants the initial LC gradient was developed to separate PFBS, the first eluting PFC, from unretained or poorly retained compounds and to chromatographically resolve the other PFCs. However, these initial conditions produced poor resolution between the linear and the branched PFOS isomers with the branched isomers eluting as unresolved peaks prior to linear PFOS (Figure 4). The poor resolution led to significant variability in the integrated peak area, particularly at lower concentrations where the branched PFOS peaks were not as prominent.[34,35] Therefore, to get an accurate quantification of PFOS it was necessary to change the chromatographic conditions to resolve the branched and linear isomers. In addition to resolving the isomers, the change also shortened the retention times of the other PFCs.

A potential consequence of the shorter retention times was the interference from coextracted matrix compounds. While a number of researchers used traditional C18 reversed-phase chromatographic columns,[36−38] others reported improved

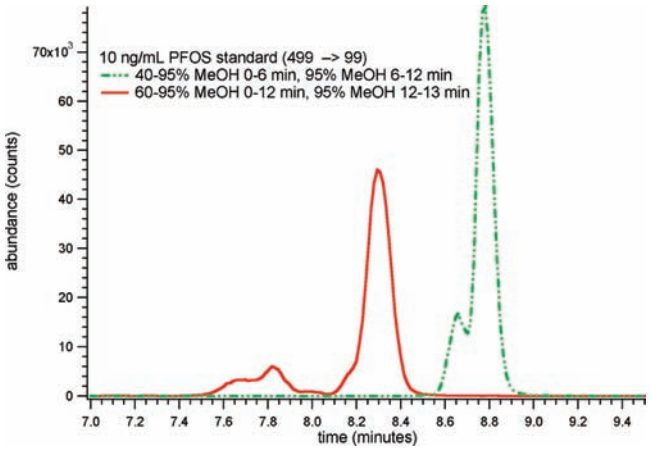

Figure 4. Effect of gradient change on the chromatographic separation of branched and linear PFOS isomers. Initial gradient was 40% methanol, increasing to 95% in 6 min, and held at 95% for 6 min. Improved separation of PFOS was achieved with more a gradual gradient of 60% methanol, increasing to 95% over 12 min, and held at 95% for one minute.

method performance with less traditional phases.[34] To determine if the shorter retention times on the C18 column were affecting our sensitivity, fortified extracts were analyzed using the Fluorosep column and the conditions reported by Lloyd et al.[34] No significant improvement in analyte response or in total run time was noted for the Fluorosep column. On the basis of these results the C18 was used for evaluation of all of the milk samples.

Standard reference materials for determination of PFCs in cow milk were not available; therefore, the accuracy and precision of the method was assessed using raw milk (Norfolk county, MA) fortified at three different concentrations (2, 10, and 30 ng/g). Six aliquots (five for 10 ng/g) from three different stock fortified milk samples were individually processed and analyzed.[39] Average apparent recoveries ranged between 87% and 111% for all 10 PFCs (Table 2). There were no significant differences in the apparent recoveries at the three spike concentrations or between sulfonates and carboxylates, or with increasing CF_2 chain length. Additionally, the relative standard deviation (RSD) values (Table 3) for all but two of the analytes were less than 10%. PFHxA fortified at 2 and 10 ng/g had RSDs of 12.6% and 14%, respectively, and PDoDA (10 ng/g) had a RSD of 10.4%.

Samples which are fortified and immediately processed are not always a good surrogate for incurred residues; therefore, as an additional test of the method performance a retail milk sample was fortified (2 ng/g) and stored for 10 days at 4 °C prior to processing. The average apparent recovery (87–133%) for all but two of the PFCs were consistent with the values determined for other fortified samples (Table 2). PFNA (113 ± 3) and PFDA (133 ± 7) had higher recoveries than reported for the same fortification concentration with the unaged sample. It is possible that the lack of matched isotopically labeled internal standard for PFNA and PFDA contributed to the small change in recovery for the aged sample. In addition to 10 day storage, the effect of a freeze/thaw cycle was also assessed. A retail milk sample, which had not undergone a previous freeze/thaw cycle, was fortified (2 ng/g) and frozen (−20 °C) for 10 days prior to processing. Freeze/thaw test recovery values for the 10 PFCs ranged from 90% to 140% and

dx.doi.org/10.1021/jf204565x | J. Agric. Food Chem. 2012, 60, 1652–1658

Table 2. Average Analyte Spike Recoveries ±95% Confidence Interval

analyte	2 ng (n = 6)	10 ng (n = 5)	30 ng (n = 6)	2 ng aged (n = 3)	2 ng frozen (n = 3)
PFBS	104 ± 18	90 ± 4	97 ± 13	99 ± 4	96 ± 9
PFHxS	99 ± 8	87 ± 5	99 ± 13	101 ± 5	100 ± 9
PFOS	102 ± 13	90 ± 3	97 ± 8	102 ± 4	99 ± 11
PFHxA	111 ± 28	96 ± 16	104 ± 28	87 ± 10	90 ± 2
PFHpA	102 ± 11	95 ± 12	110 ± 15	94 ± 5	99 ± 4
PFOA	100 ± 4	91 ± 3	102 ± 8	100 ± 1	101 ± 5
PFNA	100 ± 7	92 ± 8	108 ± 13	113 ± 3	115 ± 5
PFDA	109 ± 20	90 ± 16	100 ± 13	133 ± 7	140 ± 7
PFUnDA	101 ± 7	93 ± 3	105 ± 16	104 ± 2	105 ± 7
PFDoDA	99 ± 11	88 ± 8	105 ± 22	117 ± 7	113 ± 3

Table 3. Percent RSDs for Different Fortification Concentrations

	% RSD		
analyte	2 ng (n = 6)	10 ng (n = 5)	30 ng (n = 6)
PFBS	9	2	5
PFHxS	4	3	5
PFOS	7	1	5
PFHxA	12	8	7
PFHpA	6	7	6
PFOA	2	2	2
PFNA	4	4	5
PFDA	11	9	4
PFUnDA	4	2	2
PFDoDA	6	5	4

are listed in Table 2. Similar to the aged samples, only PFNA (115 ± 5) and PFDA (140 ± 7) had statistically higher recoveries than the corresponding fortification concentration that did not undergo a freeze/thaw cycle. Although some recovery values were higher, the freeze/thaw cycles did not cause significant analyte loss in milk. Therefore, all results from survey samples which went through freeze/thaw are expected to be accurate.

The method detection limit (MDL) for each PFC was determined using the procedure described in 40.CFR part 136 appendix B.[40] Briefly, 10 g of raw milk was fortified with 10 ng of PFC mixture. Nine, 1 g aliquots of the 1 ng/g fortified milk were processed through the milk extraction method and analyzed to determine PFC concentration in each aliquot. The variance and standard deviation of the nine replicates were then used to determine the MDL. Seven of the PFCs had MDLs under 0.3 ng/g, and the remaining 3 compounds, PFHpA, PFDA, and PFDoDA, had MDLs of 0.42, 0.43, and 0.8 ng/g, respectively (Table 1). The MDLs determined in this study were similar to values previously reported for determination of PFCs in milk using basic digestion[21] and methanol extraction.[34] MDLs for determination of PFCs in human breast milk using formic acid extraction[24,28] were significantly lower than the values reported here (Table 1). While the matrix may contribute to these differences, different procedures for determination of detection limits were used for the two studies. MDL calculations for breast milk analysis used blank water responses and not matrix to determine the detection limit. While it is useful for determining instrument limits of detection the use of blank water responses gives less accurate information about true method performance. The limit of quantitation (LOQ) is estimated as 3.5 times the MDL values.

Sample Analysis. Sixty-one raw and retail milk samples from 17 states were analyzed for PFCs using the method described above (Figure 1). Sixty of the samples had no detectable concentrations of PFCs. Two of the raw milk samples were obtained from a dairy farm that had applied PFC-containing biosolids to its fields.[27] One of the two samples from this dairy farm contained a detectable concentration of PFOS (0.16 ng/g), while the nine other PFC concentrations were below the method detection limits. This sample containing PFOS was from a bulk tank and not an individual cow. The detected PFOS concentration found is below the EPA's Provisional Health Advisory concentration for PFOS in drinking water (0.2 ng/g).[27] These PFC results are consistent with previous reports of PFC concentrations in milk.[18,28,36,37,41] Three of these five previous studies, testing a total of 25 samples, found no detectable concentrations of PFCs.[18,28,37] One of the remaining studies,[36] testing two samples, reported PFOA and PFHpA concentrations (0.05 and 0.015 ng/g, respectively) which were below the method detection limits of the current survey. The other remaining study tested one sample and found less than 10 pg/g of PFOS, PFHxA, PFOA, and PFDA. In addition to milk, a number of researchers have determined PFC concentrations in milk-based products, such as cheese.[18,36,37,41] Out of 22 cheese samples only two had measurable concentrations of PFCs. One sample contained PFOS (0.71 ng/g);[37] another sample contained PFOS (0.012 ng/g), PFHpA (0.0074 ng/g), and PFNA (0.016 ng/g).

In this study milk samples were collected from rural locations and farms close to urban/industrial centers. However, none of the samples, except for a sample from cows grazing in Decatur, AL, had detectable concentrations of PFCs. Studies on surrounding fields found a maximum concentration in soil of 410 ng/g PFOS at the same time that milk sampling occurred.[42] The Decatur milk samples (2) had nondetectable or a low concentration of PFC (0.16 ng/g PFOS). The large number and geographical distribution of the milk samples collected and analyzed in this study suggests that milk is not a significant source of PFC exposure.

The above work has shown that formic acid, a popular reagent used in the cleanup of milk samples, may not be suitable for determination of PFCs in milk due to PFOA contamination. The results also show that long (3−16 h) processing times or sonication are not required when using NaOH digestion for determination of PFCs in milk. Vortex mixing (<1 min) after addition of NaOH gave better overall recoveries than even the shortest sonication time (30 min). Use of the newly developed method for the first large-scale survey of U.S. cow's milk samples supports that milk is not a significant source of PFCs.

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