Development of a U.S. EPA Drinking Water Method for the Analysis of Selected Perfluoroalkyl Acids by Solid-Phase Extraction and LC–MS–MS

Jody A. Shoemaker[1,*] **Brenda Boutin**[2], and **Paul Grimmett**[1]

[1]U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, MS 564, 26 W. Martin Luther King Drive, Cincinnati, OH 45268 and [2]National Council on Aging, c/o U.S. Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, MS 564, 26 W. Martin Luther King Dr, Cincinnati, OH 45268

Abstract

A drinking water method for perfluoroalkyl acids (PFAAs) is presented that addresses the occurrence monitoring needs of the U.S. Environmental Protection Agency (EPA) for a future unregulated contaminant monitoring regulation (UCMR). This paper describes the challenges associated with developing an analytical method for 14 PFAAs that will be used for drinking water occurrence monitoring. The method employs solid-phase extraction with analysis by liquid chromatography–tandem mass spectrometry (LC–MS–MS). The final method preservation scheme requires that samples be stored in polypropylene bottles and that they be buffered and free chlorine removed with Trizma buffer. Mean recoveries of chlorinated surface water samples fortified with the PFAAs at 40–100 ng/L (except for the perfluorooctanesulfonamido–acetic acids at 200 ng/L) are 85–112% with < 5% relative standard deviation. Single laboratory minimum reporting limits of 2.9–14 ng/L are demonstrated with this methodology. The final method meets all of the EPA UCMR survey requirements for sample collection and storage, precision, accuracy, and sensitivity and is expected to be proposed for use under a future UCMR.

Introduction

Perfluoroalkyl acids (PFAAs) have been manufactured for over a half century, and their use has dramatically increased over the years. Due to their unique properties of repelling both water and oil, PFAAs have been used in a wide variety of applications, such as carpets, leather, fabric, upholstery, paper, food containers, fire-fighting foams, and pesticides. These compounds received worldwide attention when the presence of perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonamide (PFOSA) were reported in blood and liver samples of marine mammals, birds, and fish in both urban and remote locations (1). Since this finding, a number of researchers have confirmed the global distribution of PFAAs in wildlife (1–8).

PFAAs have also been detected globally in ground and surface waters that can be potential sources of drinking water. For example, PFOS and PFOA have been found in surface waters in Japan at concentrations typically below 20 ng/L, with the exception of a few locations where the PFOA concentrations were in the range of 100 to 500 ng/L (9–11). Similar PFOS and PFOA concentrations have been found in ocean water and U.S. surface waters (12–16).

The 1996 amendments to the Safe Drinking Water Act required the U.S. Environmental Protection Agency (EPA) to establish a Drinking Water Contaminant Candidate List (CCL) that contains a list of drinking water contaminants that the Agency will consider for future regulation. The first CCL was published in 1998 (17) and is updated every five years. PFOA is listed as a chemical contaminant on the draft CCL3 published in February 2008 (18). One of the key pieces of information that must be available in order to make a regulatory determination on PFAAs is nationwide occurrence data for these contaminants in drinking water. Historically, EPA's Office of Ground Water and Drinking Water (OGWDW) has collected the necessary occurrence data under its Unregulated Contaminant Monitoring Regulations (UCMR). To gather the occurrence data, a rugged analytical method, suitable for determination of PFAAs in drinking water, is needed. The success of this method development task is expected to result in more accurate monitoring for these contaminants in drinking water.

While several methods for PFAAs in water have been reported in the literature, these methods do not adequately address issues specific to analyzing compounds in drinking water for regulatory purposes. Issues such as sample preservation, internal and surrogate standards for QC monitoring, establishing acceptable background levels, and analyte adsorption onto sample bottles have not been addressed. Although a number of health effect studies have been initiated on PFOS and PFOA over the past few years (19–23), drinking water screening levels and health effects for the PFAAs are still being evaluated. A few states have initiated their own guidance levels for PFOA. For example, in early 2007, the New Jersey Department of Environmental Protection set a health-based guidance level for the presence of PFOA in drinking water at 40 ng/L (24). Thus, the goal of this method development effort was to obtain low ng/L detection and quantitation limits using sensitive and selective analytical methodology.

The analyte list contained 14 PFAAs including the C_6

* Author to whom correspondence should be addressed: email shoemaker.jody@epa.gov.

through C_{14} perfluorocarboxylic acids and the C_4, C_6, and C_8 perfluorosulfonic acids, as well as two perfluorooctanesulfonamidoacetic acids. Drinking water samples were concentrated by solid-phase extraction (SPE) and analyzed using liquid chromatography–tandem mass spectrometry (LC–MS–MS). Recovery and precision data for the 14 PFAAs in drinking water are presented as well as a discussion of analytical challenges specific to PFAAs that were overcome during the course of method development.

Experimental

Standards and reagents

Perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), and perfluorotetradecanoic acid (PFTA) were purchased from Aldrich (St. Louis, MO). Perfluorobutanesulfonic acid (PFBS), potassium perfluorohexanesulfonate (PFHxS), and PFOS were purchased from Fluka (Milwaukee, WI). *N*-methylperfluorooctanesulfonamidoacetic acid (NMeFOSAA) and *N*-ethylperfluorooctanesulfonamidoacetic acid (NEtFOSAA) were provided by 3M Company (St. Paul, MN) and perfluorotridecanoic acid (PFTrDA) was purchased from Exfluor (Round Rock, TX). The following internal standards (ISs) and surrogate standards (SURs) were purchased from Wellington Labs (Ontario, Canada): perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonic acid (^{13}C-PFOS, IS#2), d_3-*N*-methylperfluoro-1-octanesulfonamidoacetic acid (d_3-NMeFOSAA, IS#3), perfluoro-*n*-[1,2,-$^{13}C_2$]hexanoic acid (^{13}C-PFHxA, SUR#1), perfluoro-*n*-[1,2,-$^{13}C_2$]decanoic acid (^{13}C-PFDA-SUR#2), and d_5-N-ethylperfluoro-1-octanesulfonamidoacetic acid (d_5-NEtFOSAA, SUR#3). The internal standard, perfluoro-1-[1,2-^{13}C]octanoic acid (^{13}C-PFOA, IS#1), was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Ammonium acetate, diazolidinyl urea (DZU), and Trizma Preset crystals, pH 7.0 (a premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]) were purchased from Sigma-Aldrich (St. Louis, MO). Cupric sulfate ($CuSO_4$), sodium chloride (NaCl), sodium sulfite (Na_2SO_3), and sodium bisulfate ($NaHSO_4$) were purchased from Fisher Scientific (Fairlawn, NJ). Absolv-grade methanol was purchased from Tedia (Fairfield, OH). Deionized water was used from a Gradient A10 Milli-Q water system (Millipore; Bedford, MA).

Standard preparation

With the exception of NMeFOSAA, NEtFOSAA, PFBS, PFHxS, PFOS, PFTrDA, and PFTA, the PFAA individual stock standards (SSs) were prepared by weighing 10–13 mg of neat material and diluting in 2 mL of methanol containing 4% water (5–6.5 mg/mL). PFBS, PFHxS, and PFOS SSs were prepared in pure methanol and NMeFOSAA, NEtFOSAA, PFTrDA, and PFTA were prepared in ethyl acetate. For the purposes of this paper, a primary dilution standard solution (PDS) is defined as a mixture containing the analytes prepared from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions. An intermediate analyte PDS (analyte PDS) at ~2.5–12 ng/µL containing all the target analytes was prepared by adding 5–20 µL of each analyte SS into a 9.88 mL aliquot of methanol containing 4% water for a final volume of 10 mL. A dilute analyte PDS, containing all the target analytes at ~0.5–2.5 ng/µL, was prepared by adding 2 mL of the intermediate analyte PDS to 8 mL of methanol containing 4% water for a final volume of 10 mL. To obtain the fortified analyte concentrations listed in Table II, 20 µL of the ~0.5–2.5 ng/µL analyte PDS was spiked into 250 mL of water.

Calibration

Six calibration standards were prepared in methanol containing 4% water spanning a 40-fold concentration range. For PFOA, this standard curve would represent 2.3–92 ng/L in a 250 mL water sample. The calibration standards were spiked with 10 µL of an IS PDS containing IS#1 (1 ng/µL), IS#2 (3 ng/µL), and IS#3 (4 ng/µL) corresponding to 10, 30 and 40 pg/µL, respectively, in a 1-mL standard. Calibration standards were also spiked with 10 µL of a SUR PDS containing SUR#1 (1 ng/µL), SUR#2 (1 ng/µL), and SUR#3 (4 ng/µL) corresponding to 10, 10, and 40 pg/µL, respectively, in a 1-mL standard. All standards were prepared and stored in polypropylene centrifuge tubes.

During method development, weighted linear curves (^{1}/x) were used for quantitation. Due to the background contamination of the PFAAs observed in every injection, the calibration curve *y*-intercepts of a number of the PFAAs were significantly above zero. This made calculating blank estimates for these PFAAs nearly impossible. Therefore, the calibration curves for all analytes were forced through zero. Forcing zero caused some inaccuracy at low concentrations. This inaccuracy at low concentrations yielded higher lowest concentration minimum detection levels (LCMRLs) (25) because the LCMRL algorithm used is based on both precision and accuracy. However, the need for better estimates of the background levels of the method analytes was deemed more important than achieving lower LCMRLs. To meet our drinking water data quality objectives (DQOs) for a valid calibration curve, each analyte at each calibration point (except the lowest) must calculate to be within 70–130% of its true value when quantitated using the calibration curve. The lowest calibration standard must calculate to be within 50–150% of its true value. These calibration DQOs were met for all the method development research presented here.

Sample preparation

Carbon SPE

Samples were extracted using a J&W Scientific (Folsom, CA) SPE vacuum manifold and Supelco (Bellefonte, PA) ENVI-CARB SPE carbon cartridges (6 mL, 250 mg). The carbon cartridges were conditioned with 20 mL of 20 mM ammonium acetate in methanol (prepared by adding 0.77 g of ammonium acetate to 0.5 L of methanol) followed by 25 mL of deionized water. Water samples (100 mL) in polypropylene bottles (no preservatives), fortified with analytes and surrogates, were passed through the cartridges. Polypropylene tubing was used to transfer the samples from the bottles to the cartridges to prevent any inadvertent

contamination of the extracts from the conventional Teflon transfer tubing. The target analytes were eluted from the carbon cartridges by rinsing the sample bottles with two 8 mL aliquots of 20 mM ammonium acetate prepared in methanol and pulling the solvent through the sample transfer lines and the cartridges in a dropwise fashion (~ 5 mL/min). Extracts were evaporated to dryness with nitrogen in a 65°C water bath, reconstituted with 990 µL of methanol containing 4% deionized water and 10 µL of the IS PDS.

Styrene divinylbenzene SPE

Unless otherwise noted, all 250 mL water samples in polypropylene bottles were preserved with 5 g/L Trizma buffer. Samples were extracted manually using Varian (Palo Alto, CA) styrene divinylbenzene (SDVB) polymer cartridges (6 mL, 500 mg). The cartridges were conditioned with 15 mL of methanol followed by 18 mL of deionized water. Water samples, fortified with method analytes and surrogates, were passed through the cartridges. Polypropylene tubing was used to transfer the samples from the bottles to the cartridges. The cartridges and sample bottles were rinsed with two 4-mL aliquots of deionized water. The target analytes were eluted from the SDVB cartridges by rinsing the sample bottles with two 4-mL aliquots of methanol and pulling the solvent through the sample transfer lines and the cartridges in a dropwise fashion. Extracts were evaporated to dryness with nitrogen in a 65ºC water bath, reconstituted with 990 µL of methanol containing 4% deionized water and 10 µL of the IS PDS.

Autosampler vials

Small aliquots of the extracts were transferred with polyethylene pipettes to SUN-SRi (Rockwood, TN) polypropylene 0.3 mL autosampler vials with molded polypropylene caps. Autosampler vials are a potential source of PFAA contamination. Autosampler vials made of Teflon and Viton have been reported to cause PFAA contamination of blanks (14). Thus, polypropylene autosampler vials and caps were used to prevent any potential contamination of samples from the vials or caps. Unfortunately, these polypropylene autosampler caps do not reseal after injection, causing evaporation losses with time. Therefore, only a small portion of the extracts was placed in the autosampler vial and the remaining extract volume was stored in 15-mL Corning (Corning, NY) polypropylene centrifuge tubes for use in the event that additional analyses were necessary.

LC–MS–MS analysis

Extracts were analyzed on a Waters Micromass (Manchester, U.K.) Premier triple quadrupole MS equipped with an atmospheric pressure ionization source and a Waters (Milford, MA) Acquity LC. The target analytes were ionized by negative ion electrospray. Quantitation was performed using selected reaction monitoring (SRM) MS–MS where the $[M–H]^-$ was selected with the first quadrupole mass analyzer and the third quadrupole mass analyzer scanned the predominant product ion. Ionization and collision cell parameters were optimized for each analyte (Table I). A Waters Atlantis dC18 (2.1 × 150 mm, 5 µm) analytical column was used to separate the target analytes at a flow rate of 0.3 mL/min. The injection volume was 10 µL. The binary mobile phase gradient composition was (A) 20 mM ammonium acetate in deionized water and (B) methanol. The mobile phase was held at initial conditions of 60:40 (A:B) for 1 min, the linear gradient was stepped to 10:90 (A:B) in 24 min and held for 7 min. The post equilibration time was minimized to 5 min.

Table I. Retention Times, MS–MS Parameters, and Internal Standard References

Segment*	Analyte	Retention time (min)	Precursor ion (m/z)	Product ion† (m/z)	Cone voltage (v)	Collision energy‡ (v)	IS # ref.
1	PFBS	8.48	299	80	40	25	2
2	PFHxA	11.38	313	269	15	10	1
3	PFHpA	14.33	363	319	12	10	1
3	PFHxS§	14.48	399	80	40	40	2
4	PFOA	16.73	413	369	15	10	1
4	PFNA	18.76	463	419	12	10	1
4	PFOS§	18.72	499	80	40	40	2
5	PFDA	20.47	513	469	15	10	1
5	NMeFOSAA§	21.28	570	419	25	20	3
5	NEtFOSAA§	22.01	584	419	25	20	3
5	PFUnA	21.95	563	519	15	10	1
5	PFDoA	23.20	613	569	15	10	1
6	PFTrDA	24.32	663	619	15	10	1
6	PFTA	25.27	713	669	15	10	1
2	^{13}C-PFHxA-SUR#1	11.38	315	270	15	10	1
5	^{13}C-PFDA-SUR#2	20.47	515	470	12	12	1
5	d_5NEtFOSAA-SUR#3	21.99	589	419	25	20	3
4	^{13}C-PFOA-IS#1	16.73	415	370	15	10	–
4	^{13}C-PFOS-IS#2	18.71	503	80	40	40	–
5	d_3NMeFOSAA-IS#3	21.24	573	419	25	20	–

* Segments are time durations in which single or multiple scan events occur.
† Ions used for quantitation purposes.
‡ Argon used as collision gas at a flow rate of 0.3 mL/min.
§ Analyte has multiple resolved chromatographic peaks due to linear and branched isomers. All peaks summed for quantitation purposes.

Results

The development of a drinking water method suitable for use in a UCMR involves many steps aimed at providing a sensitive, accurate, precise, and rugged method. The goal is to find an SPE procedure that, combined with LC–MS–MS analysis, produces a method for the analysis of PFAAs that meets our drinking water program's data quality objectives (DQOs) of 70–130% mean recovery (% of true value) and < 30% relative standard deviation (RSD). The processes and decisions involved in the development of this drinking water PFAA method are discussed below.

LC optimization

Literature articles have reported PFAA background contamination arising from LC tubing, valves, and mobile phase solvents

(12,14,26,27). Thus, it was necessary to focus initial studies on evaluating the magnitude of the LC contamination and ways to minimize background levels of the PFAAs. Initially, no modifications were made to the hardware of the Waters Acquity LC used in the development of this method. The PFAA contamination observed in sequential injections, assuming constant post-equilibration times, was reproducible and below the lowest calibration concentration. However, if the LC was idle for more than one day, very high PFAA contamination was observed on the first injection even though the pump was primed (5 min) and the column was flushed (10 min) with methanol prior to injection. It appeared the PFAAs were leaching out of the Teflon solvent transfer lines into the solvent while idle. Typically, 3–5 h were required for this PFAA build up to be flushed out of the LC and column. To prevent long delays in purging high levels of PFAAs from the LC solvent lines, they were replaced with PEEK tubing and the Teflon solvent frits replaced with stainless steel frits.

Methanol is the typical mobile phase solvent used in the analysis of PFAAs. Manufacturers of methanol have indicated that this solvent is filtered through polytetrafluoroethylene (PTFE) frits, contributing to PFAA contamination. Thus, PFAAs in the methanol, LC valves, and tubing accumulate on the analytical column during the post equilibration time. Background area counts were observed for the C_6 through the C_{14} perfluorocarboxylic acids and PFHxS. With the exception of PFTA, all the PFAAs present in the LC background increased with increasing post equilibration time, as expected. This effect can be controlled by keeping the post equilibration time constant. The minimum post equilibration time necessary to minimize the background contamination, yet produce stable retention times, was used. For the hardware and conditions used in this method, a 5 min post equilibration time was chosen.

The most common mobile phase used in the literature for PFAAs is 1–5 mM ammonium acetate–methanol (14,28–34). Initial stages of method development used a 5 mM ammonium acetate–methanol gradient on a C_{18} LC column. Over time, all of the PFAA chromatographic peaks shifted slowly to shorter retention times causing peaks to shift outside their MS–MS time segments. Figure 1A is a graph of retention time shift of PFBS (first eluting peak) and PFTA (last eluting peak) versus days using 5 mM ammonium acetate as the mobile phase modifier. The chromatographic peaks shifted approximately 30 to 60 s over 78 days. This type of retention time shift does not provide sufficient confidence in identification of peaks. LC columns with various types of stationary phases were evaluated, but similar retention time shifts were observed using 5 or 10 mM ammonium acetate in the mobile phase with all columns used (data not shown). Figure 1B shows stable PFBS and PFTA retention times over 88 days using 20 mM ammonium acetate as the mobile phase modifier. These data indicate that the concentration of the modifier used in the LC mobile phase is critical to maintaining constant chromatographic retention times. Thus, all method performance data was collected using 20 mM ammonium acetate in the aqueous mobile phase.

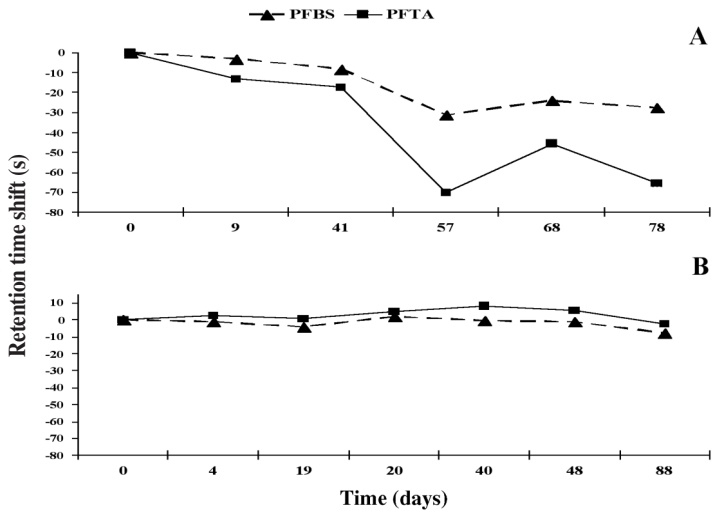

Figure 1. Graph of peak retention time shifts versus time using 5 mM ammonium acetate–methanol (A) and 20 mM ammonium acetate–methanol mobile phase gradients (B). All other LC and MS conditions were identical between experiments.

Preparation of standards

Scientific journal articles reviewed (29,30,35–37) listed 100% methanol as the solvent of choice for making PFAA standards. However, as Figure 2 demonstrates, high SPE recoveries (132–159%) for the C_4 through the C_{14} perfluorocarboxylic acids were obtained when the analyte SSs and the analyte PDS were prepared in 100% methanol and 10 µL of the 2.5 ng/µL analyte PDS was spiked into 100-mL water samples for a final aqueous concentration of 250 ng/L. These 100-mL deionized water extractions were performed using graphitized carbon SPE cartridges. No preservatives were added to the aqueous samples. The same results were obtained regardless of the aqueous sample volume (5 mL to 1000 mL) used. In fact, these same high recoveries were obtained during a study of evaporation recoveries (data not shown). In the evaporation study, the analytes were simply spiked into simulated extracts (10 mL of methanol plus 0.5 mL deionized water), evaporated to dryness and reconstituted (no cartridge extraction performed). The high recoveries obtained in the evaporation study eliminated the deionized water, used for samples and the extraction process, as a source of the high bias. In addition, the PFAA concentrations observed in the laboratory reagent blanks did not account for the high recoveries obtained. It was determined that the individual analyte SSs and the analyte PDS needed to be prepared with a small amount of water. No high bias was observed for the perfluorocarboxylic acids when an analyte PDS made in methanol containing 25% water was used to spike the deionized water samples (Figure 2). It appears the perflourocarboxylic acids were not soluble in 100% methanol, thus insoluble material may have transferred to the aqueous sample during spiking, became solubilized, and resulted in high recoveries when quantitated against standards prepared in pure methanol. Further testing indicated that 4% water in the analyte SSs and analyte PDS was sufficient to solubilize the perfluorocarboxylic acids. The perfluorosulfonamidoacetic acid compounds were insoluble in any amount of water, so those SSs were prepared in pure methanol. Likewise, the longest chain perfluo-

rocarboxylic acids (PFTrDA and PFTA) were not completely soluble in methanol or water, thus these SSs were prepared in ethyl acetate. These data demonstrate the importance of ensuring the PFAAs are completely solubilized in an appropriate solvent during standard preparation to prevent high recoveries from being observed. A recent article (38) on an inter-laboratory study of perfluorinated contaminants in environmental and human samples reported that 72% of the participating laboratories had unsatisfactory PFOA agreement on water sample results. This inter-laboratory study demonstrates that quality quantitation of PFOA and other PFAAs is still a challenge. Although there are many challenges to the analyses of PFAAs which may be contributing to the poor agreement in the interlaboratory study, the standard preparation suggestions presented in this paper may aid in reducing some of the uncertainty in PFAA measurements.

SPE sorbent selection

A number of SPE sorbents have been used to extract PFAAs from water (10,12,14–16,28,29,32,39), such as C_{18}, modified C_{18}, styrene divinylbenzene (SDB), and hydrophilic-lipophilic balance copolymers. When possible, drinking water methods are developed using materials that are commercially available from multiple sources, and C_{18} and SDB meet this criterion. C_{18} (0.5 g, 6 mL) sorbents were evaluated briefly (same extraction procedure and preservation as SDB), but mean (n = 5) recoveries in reagent water were poor for PFBS (5%) and PFHxA (30%). Graphitized carbon SPE sorbents were also evaluated and provided adequate mean recoveries (72–102%) in deionized water. However, low mean recoveries (57–70%) were obtained for six of the PFAAs in chlorinated surface water samples.

SDB sorbents were also evaluated for extraction of the PFAAs from deionized water. In PFAA fortified deionized water without preservatives (pH 4–6), the mean recoveries were low (5–76%) for all analytes except PFHxS and PFOS. These data seemed to indicate a possible pH effect in deionized water (variable measured pH of 4–6) on the recovery of the PFAAs from SDB sorbents. The PFAAs are believed to be more strongly retained on the SDB cartridges under acidic sample conditions and not effectively eluted with methanol. To study the effect of sample pH, PFAA-fortified tap water samples without preservatives (unadjusted pH = 7.5) were extracted and analyzed. Average PFAA recoveries were 98–113% with RSDs of 1–6%. These tap water recoveries indicated that samples need be held at a neutral pH (PFAAs all in ionic form) to obtain recoveries on SDB SPE cartridges that meet DQOs. Trizma buffer can be used to remove free chlorine and buffer the sample to a pH near 7. Mean recoveries on SDB cartridges using Trizma buffer (Table II) in deionized water fortified at low level and high level concentrations met the DQOs for all 14 PFAAs.

Table II. Recovery and Precision of Low- and High-Level PFAA Fortified 250 mL Deionized Water Samples (n = 7) Containing Trizma Buffer

Analyte	Fortified conc. (ng/L)	Mean % recovery	% RSD	Fortified conc. (ng/L)	Mean % recovery	% RSD
PFBS	9.1	93	12	91	92	5.9
PFHxA	5.0	108	9.3	50	103	4.6
PFHpA	8.3	111	6.2	41	106	3.4
PFHxS	11	116	4.9	113	101	2.5
PFOA	9.1	110	6.5	45	104	3.6
PFNA	9.6	121	7.5	48	109	3.5
PFOS	9.6	116	4.1	96	101	3.8
PFDA	7.3	114	4.1	36	106	4.8
NMeFOSAA	20	109	9.3	201	100	3.7
NEtFOSAA	21	113	5.5	214	105	5.3
PFUnA	11	120	5.4	54	107	3.3
PFDoA	7.3	116	4.6	36	107	3.9
PFTrDA	5.5	118	11	54	107	1.9
PFTA	8.7	117	2.4	43	111	2.7
^{13}C-PFHxA	40	89	6.1	40	93	4.4
^{13}C-PFDA	40	102	7.3	40	99	3.5
d_5-NEtFOSAA	160	100	4.9	160	100	4.5

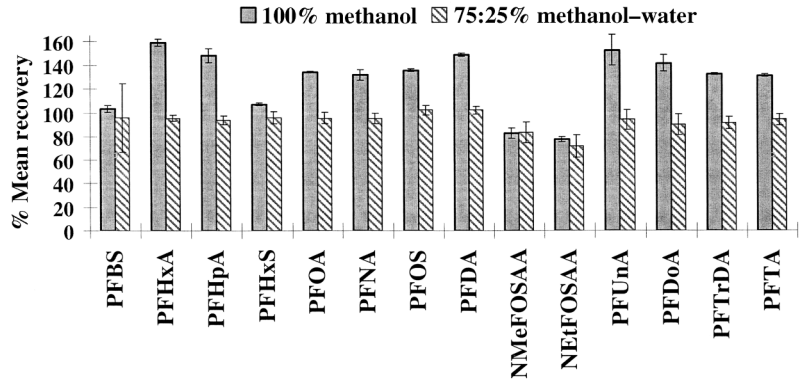

Figure 2. Bar graph of % mean recovery of PFAAs from reagent water samples fortified with standards prepared in either 100% methanol or methanol containing 25% water.

Matrix interferences/effects may be caused by contaminants that are co-extracted from the sample and from drinking water properties, such as hardness. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and/or fulvic material can be co-extracted and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries in SPE (40–43). For the purposes of this method, total organic carbon (TOC) is generally a good indicator of humic/fulvic content of the sample. Chlorinated surface water, measuring 5 mg/L in TOC, was spiked with the PFAAs and extracted with SDB cartridges to evaluate matrix effects on this method. Table III illustrates mean recoveries of 85–109% with 3.1–4.9% RSD in high TOC chlorinated surface water. These results demonstrate that the PFAA extractions are not prone to matrix effects in tap waters containing TOC concentrations up to 5 mg/L. In addition, the effect of hard water was also evaluated. The mean

recoveries of chlorinated ground water (hardness = 342 mg/L) fortified with the PFAAs in Table III were also well within the goals of 70–130% recovery and < 30% RSD.

PFAAs have been reported to irreversibly bind to surfaces, such as glass (14,16,26); therefore, samples and extracts for PFAA analyses are most commonly collected in polypropylene or polyethylene bottles. During development of this drinking water method, adsorption of the PFAAs onto polypropylene sample bottles was investigated. Deionzed water samples, fortified with PFAAs, were extracted without rinsing the sample bottle or sample transfer lines with the elution solvent. Table IV demonstrates that sample bottle elution improves extraction efficiency for PFOS, NMeFOSAA, NEtFOSAA, and the PFAAs with carbon chains ≥ 11. Mean recoveries dropped as low as 75% for these longer chain compounds when rinsing was not performed. Thus, solvent rinsing of the sample bottle and sample transfer lines during elution is necessary for maximum efficiency and aqueous samples cannot be composited.

Table III. Recovery and Precision of PFAA Fortified 250 mL Chlorinated Tap Water Samples (*n* = 7) Obtained From Ground Water and Surface Water Sources*

Analyte	Fortified conc. (ng/L)	Chlorinated ground water†		Chlorinated surface water‡	
		Mean % recovery	% RSD	Mean % recovery	% RSD
PFBS	91	101	3.9	85	4.3
PFHxA	50	112	0.9	101	3.2
PFHpA	41	105	1.8	106	3.7
PFHxS	113	103	2.2	101	4.9
PFOA	46	107	2.2	110	3.1
PFNA	48	103	2.4	112	3.9
PFOS	96	100§	2.2	104	4.0
PFDA	37	101	2.6	109	4.3
NMeFOSAA	202	96	2.6	95	4.0
NEtFOSAA	214	100	2.7	100	3.4
PFUnA	54	99	2.6	106	3.7
PFDoA	37	99	2. 3	104	4.2
PFTrDA	55	96	3.4	102	4.7
PFTA	44	97	4.2	105	3.5
^{13}C-PFHxA	40	98	2.0	85	4.3
^{13}C-PFDA	40	98	2.1	101	3.2
d_5-NEtFOSAA	160	97	5.0	106	3.7

* Trizma buffer was added to tap water samples at 5 g/L.
† TOC = 0.96 mg/L and hardness = 342 mg/L as calcium carbonate.
‡ TOC = 4.95 mg/L and hardness = 137 mg/L as calcium carbonate.
§ Recovery is corrected for a native PFOS concentration of 40 ng/L in the tap water (from a ground water source) used in this study.

Table IV. Recovery and Precision of PFAAs in 250 mL Deionized Water Samples (*n* = 5) Containing Trizma Buffer Without Rinsing the Sample Bottle with the Elution Solvent

	Fortified conc. (ng/L)	No bottle rinse during elution	
		Mean % REC	% RSD
PFBS	91	78	4.1
PFHxA	50	90	5.4
PFHpA	41	102	4.6
PFHxS	113	102	3.3
PFOA	46	105	5.8
PFNA	48	104	5.6
PFOS	96	83	12
PFDA	37	94	6.3
NMeFOSAA	202	82	7.2
NEtFOSAA	214	82	6.9
PFUnA	54	84	8.0
PFDoA	37	76	9.1
PFTrDA	55	75	7.6
PFTA	44	77	7.1

Selection of preservatives

EPA drinking water regulatory methods typically use sample preservatives to prevent microbial degradation (e.g., $CuSO_4$, DZU, $NaHSO_4$) and to dechlorinate (e.g., ascorbic acid, Trizma buffer, Na_2SO_3) at the time of sampling (44). Table V summarizes the recovery and precision results of the PFAAs using various preservative combinations in deionized water without holding the sample (fortify and extract immediately). DZU/ascorbic acid/Trizma buffer yielded low mean recoveries of 5–85%. $NaHSO_4$ (pH = 2)/Na_2SO_3, with NaCl added as a potential salting out agent (aids in reducing the water solubility of the targets), produced poor recoveries (< 42%) for all the perfluorocarboxylic acid compounds, although, the perfluorosulfonates were 100% recovered with this preservative combination. As noted previously, these poor SDB recoveries are probably due to the fact that addition of $NaHSO_4$ to the aqueous samples lowers the pH to 2, causing the perfluorocarboxylic acid compounds to become partially neutral and more strongly retained on the SDB sorbent. Recoveries of 92–108%, with excellent precision, were obtained for all PFAAs with $CuSO_4$/Trizma buffer as the preservatives (Table V).

Further investigation led to the discovery that not all the PFAAs could be held in aqueous samples preserved with $CuSO_4$/Trizma buffer. Chlorinated surface water samples (n = 5), preserved with $CuSO_4$/Trizma buffer, were extracted and analyzed on the day of preparation (day 0). An additional five chlorinated surface water samples, preserved with $CuSO_4$/Trizma buffer were held refrigerated at 4°C for 14 days, then extracted and analyzed. Day 14 mean recoveries for NMeFOSAA, NEtFOSAA, PFUnA, PFDoA, and PFTrDA decreased more than 20% from the Day 0 results. As a result, $CuSO_4$ cannot be used as an antimicrobial due to these adverse holding time effects. Because no suitable antimicrobial could be found that did not adversely affect the recoveries of the analytes, the decision was made to not add an antimicrobial and set the maximum aqueous holding time to 14 days. Tables II and III demonstrate recovery and precision data that meet the DQOs with only Trizma buffer added to the aqueous sample. Using Trizma buffer as the preservative had the added advantages of buffering the aqueous sam-

ples so that pH effects were controlled and removing residual chlorine in the sample.

Aqueous storage and holding time study

An aqueous holding time study was performed to evaluate the chemical stability of the analytes during shipping and during the 14-day holding time. Without an antimicrobial, 14 days is the maximum holding time preferred in current drinking water methods. Replicate samples of a chlorinated ground water were collected, dechlorinated with Trizma buffer, and fortified with analytes. The samples were stored at 10°C for 48 h, before being moved to 4°C storage for the remainder of the storage period. This procedure simulates a typical shipping scenario. A randomly selected set of 5 samples was extracted and analyzed on the day of preparation (day 0) and at 3 additional time points up to and beyond 14 days. The day 0 and 14 aqueous sample mean recoveries and precision are provided in Figure 3. These data support the established 14-day aqueous holding time.

Table V. Recovery and Precision of 250 mL Deionized Water Samples (n = 3) Fortified with PFAAs using Various Preservative Combinations*,†

	Antimicrobial/dechlorination combinations					
	DZU/Ascorbic/Trizma		$NaHSO_4/Na_2SO_4/NaCl$		$CuSO_4$/Trizma	
	Mean % Rec.	%RSD	Mean % Rec.	%RSD	Mean % Rec.	%RSD
PFBS	4.9	90	100	4.6	92	4.4
PFHxA	5.1	82	37	47	99	2.2
PFHpA	9.1	28	31	20	101	3.3
PFHxS	10	23	103	6.4	101	3.4
PFOA	15	15	24	16	101	2.7
PFNA	28	5.4	21	13	97	3.1
PFOS	34	8.5	100	4.3	94	3.3
PFDA	46	14	17	7.1	96	3.9
NMeFOSAA	62	14	27	38	100	3.4
NEtFOSAA	66	17	31	33	108	3.6
PFUnA	72	11	19	26	96	2.1
PFDoA	82	12	22	39	95	4.2
PFTrDA	85	15	41	16	92	4.8
PFTA	82	9.0	42	49	92	2.4
^{13}C-PFHxA	1.8	2.9	37	42	94	3.7
^{13}C-PFDA	43	11	15	11	96	2.9
d_5-NEtFOSAA	60	15	28	30	97	3.1

* All samples were spiked with 37–214 ng/L of the PFAAs (same as Table IV).
† 1 g/L DZU, 0.1 g/L ascorbic acid, 10g/L $NaHSO_4$, 50 mg/L Na_2SO_4, 10 g/L NaCl. 0.5 g/L $CuSO_4$, 5 g/L Trizma buffer.

Extract storage and holding time study

Extracts prepared on day 0 of the aqueous holding time study were stored at room temperature, and analyzed in replicate (n = 5) on day 0, and at 5 additional time points up to and beyond 28 days. The day 28 extract mean recoveries and precision are provided in Figure 3 and support the established 28-day extract holding time.

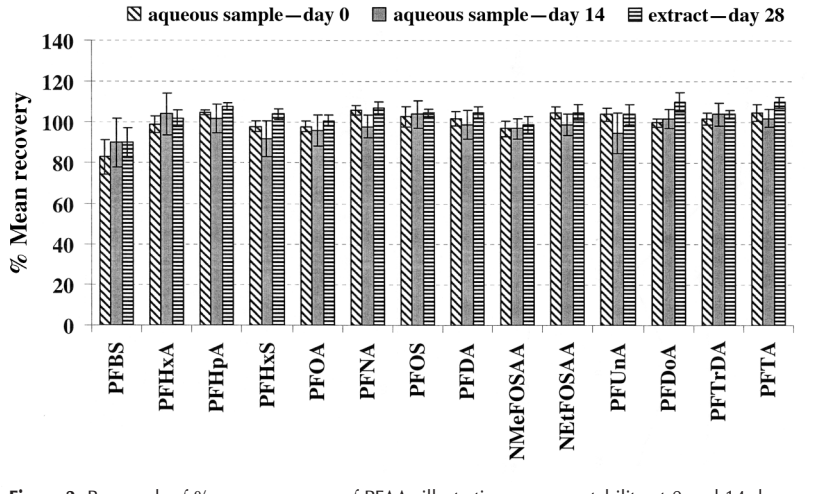

Figure 3. Bar graph of % mean recovery of PFAAs illustrating aqueous stability at 0 and 14 days, and extract stability at 28 days.

LCMRL and DL

Another step in the method development process is the determination of the LCMRLs. The LCMRLs were determined in accordance with OGWDW's new procedure (25). The LCMRL is a single laboratory determination of the lowest true concentration for which a future recovery is expected, with 99% confidence, to be between 50% and 150% recovery. The LCMRL calculation takes into account both precision and accuracy. Therefore, although the calibration curves were forced through zero to obtain better estimates of the blank (see the "Calibration" Section), the accuracy of the calculated LCMRLs, by definition, were between 50% and 150%. Single laboratory LCMRLs for the PFAAs in Table VI ranged from 2.9 to 14 ng/L. The concentrations in Table II, for the low-level fortified deionized water samples, were chosen to be 1 to 2.5 times the calculated LCMRLs (Table VI) to verify method performance at low levels. The resulting recoveries were 89–121% (within our DQOs of 70–130%).

In addition to LCMRLs, detection limits (DLs) were calculated. The DLs were evaluated in accordance with the procedure described by Glaser et al. (45). The DL is the minimum concentration of a substance that can be reported with 99% confidence that the analyte concentration is greater than zero. DLs for the PFAAs in Table VI ranged from 0.5 to 6.5 ng/L.

Conclusions

This paper described the steps involved in the development of an EPA drinking water method for PFAAs. The selection of the SPE sorbent, dechlorination agent, and aqueous and extract holding times were discussed, as well as LC problems encountered during method development. In addition, the need for the

Table VI. Detection Limits and Single Laboratory Minimum Reporting Levels in Deionized Water

Analyte	Fortified conc. (ng/L)*	DL (ng/L)	LCMRL (ng/L)
PFBS	9.1	3.1	3.7
PFHxA	5.0	1.6	2.9
PFHpA	4.1	0.5	3.8
PFHxS	11	2.0	8.0
PFOA	4.6	1.7	5.1
PFNA	4.8	0.7	5.5
PFOS	9.6	1.4	6.5
PFDA	3.7	0.7	3.8
NMeFOSAA	20	6.5	14
NEtFOSAA	21	4.2	14
PFUnA	5.4	2.8	6.9
PFDoA	3.7	1.1	3.5
PFTrDA	5.5	2.2	3.8
PFTA	4.4	1.7	4.7

* Spiking concentration used to determine DL.

PFAAs standards to be prepared in methanol containing a small amount of water to prevent high recoveries was demonstrated. Accuracy and precision under final method conditions meet the DQOs (70–130%) for the analysis of 14 PFAAs in drinking water and the DLs (0.5–6.5 ng/L) and LCMRLs (2.9–14 ng/L) were below any current state action levels for PFAAs. This method is expected to be used by the OGWDW to collect nationwide occurrence data for PFAAs in drinking water in a future UCMR. Better monitoring data will assist OGWDW in making better regulatory decisions.

Disclaimers

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