Method Development for Liquid Chromatography/Triple Quadrupole Mass Spectrometric Analysis of Trace Level Perfluorocarboxylic Acids in Articles of Commerce

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Abstract

An analytical method to identify and quantify trace levels of C5 to C12 perfluorocarboxylic acids (PFCAs) in articles of commerce (AOCs) was developed and rigorously validated. Solid samples were extracted in methanol, and liquid samples were diluted with a solvent consisting of 60:40 (v/v) methanol and 2 mM ammonium acetate (NH_4Ac) aqueous solution. In both cases, the samples were spiked with an isotopically-labeled recovery check standard. The samples were concentrated in a nitrogen atmosphere (solid samples only), filtered, and then analyzed by HPLC coupled with a tandem mass spectrometer. Method evaluation included selection of the extraction solvent and the sample preparation solvent used to facilitate sample injection into the analytical system, method comparison for extraction and sample concentration, determination of extraction efficiency, instrument and method detection limits, and determination of potential sample loss during filtration and sample storage. Results of consecutive extractions demonstrated that a single extraction step accounts for 70% to 100% of the "total" PFCAs in the AOCs with the exception of cookware. The instrument's detection limit was $\leq$ 0.05 ng/mL, and the method detection limit was 1.0 – 3.9 ng/g for solid AOCs and 1.1 – 6.8 ng/g for liquid AOCs. The method has been used to determine the PFCA content in a wide range of AOCs containing or treated with fluoropolymers and fluorotelomers.

Keywords: Method Development, LC/MS/MS Analysis, Perfluorocarboxylic acids (PFCAs), Articles of Commerce (AOCs)

1. Introduction

Perfluorinated compounds (PFCs) such as perfluorocarboxylic acids (PFCAs) have been found in articles of commerce (AOCs). The sources of PFCAs found in AOCs are: (1) fluorotelomers when they exist as unwanted reaction by-products and (2) residual

PFOA and its salts, which are used as a processing aid (surfactant) to make fluoropolymers such as polytetrafluoroethylene (PTFE) polymer. Trace amounts of PFCAs have been regularly detected in humans [1-4], wildlife [5-7], and environmental media [8-11]. They came to the attention of scientists in the U.S. EPA because of their widespread use, developmental toxicity in laboratory animals, and other health effects [12, 13 and references therein]. EPA is investigating the role of AOCs containing or treated with fluoropolymers and fluorotelomers in human exposure in the microenvironments of homes and offices. The purpose of this work is to develop an analytical method to determine PFCA contents of AOCs.

There has been a substantial increase in the number of publications in the literature related to studies of PFCA levels in humans [1-4], biota [5-7], water [6, 8], waste water [14], air [10], and soil [11]. However, data on the PFCA contents of AOCs are limited [15-22]. In addition, most of the reports are limited to a single compound - perfluorooctanoic acid (PFOA). The preferred analytical method for quantitative determination of PFCAs in environmental matrices is LC/MS/MS coupled with solvent extraction [11, 17, 19, and 23-29]. Due to the high contamination of PFCAs in the background introduced through common laboratory facilities and solvents and the low level of PFCAs in most of the AOC samples (non-detectable to µg/g range) , the determination of the PFCA content of AOCs is challenging [24, 30, and 31] and requires sensitive methods with accurate and reproducible data. Larsen et al. [25, 26] compared extraction solvents and measurement methods for PFOA in PTFE polymer. Their results showed that the use of either water, ethanol, or methanol as the solvent for PFOA extraction with both accelerated solvent extractor (ASE) and reflux extraction methods was acceptable. Larsen et al. [26] extended the study to select methanol with ASE as the most efficient extraction method, at an optimized temperature of 150 °C and a solvent residence time of 12 minutes, for quantifying total PFOA in PTFE. They also concluded that thermal treatment greatly increased the quantity of PFOA extracted. Mawn et al. [16] performed single and serial extraction of PFOA using water, methanol, and sweat and saliva simulants for textile and carpet samples. Their results demonstrated that the extraction efficiencies for most samples were lower with water and simulants than with methanol. Twenty-four-hour, wrist-action shaker extraction gave a higher total PFOA result at the specified conditions. Stadalius et al. [19] developed and validated an LC/MS/MS method, which involved extraction using 20 mL of methanol with a wrist-shaker operated at room temperature, for the determination of PFOA in paper and textile products. Risha et al. [29] reported the method and validation for trace level analyses of C8, C9, C10, C11, and C13 PFCAs in water. C18 solid phase extraction was applied, and studies were conducted to assess the stability of samples in mixtures of water and methanol, standards in methanol at room temperature in short-term trials (24 hours), standards at room temperature and refrigerated at 4±2 ^{o}C in long-term trials (14 days), and the stability of stock solutions in methanol. Risha et al.'s experiments suggested that both the solvent used and the chain length of the PFCAs affected the stability of the PFCAs. The issues associated with quantifying PFCAs at low concentrations, including method detection limits, labeled internal standards, recovery, and precision, were discussed by Washington [11] and others [23, 24].

This work reports method development in sample extraction and LC/MS/MS analysis of trace level C5 to C12 PFCAs in AOCs. The PFCAs include perfluoropentanoic acid (PFPeA-C5), perfluorohexanoic acid (PFHxA-C6), perfluoroheptanoic acid (PFHpA-C7), perfluorooctanoic acid (PFOA-C8), perfluorononanoic acid (PFNA-C9), perfluorodecanoic acid (PFDA-C10), perfluoroundecanoic acid (PFUnDA-C11), and perfluorododecanoic acid (PFDoDA-C12). An isotopically-labeled compound, perfluoro-*n*-[1, 2-$^{13}C_2$] decanoic acid (PFDA-$^{13}C_2$) was used as the extraction recovery check standard, and perfluoro-*n*-[1, 2, 3, 4-$^{13}C_4$] octanoic acid (PFOA-$^{13}C_4$) was used as the LC/MS/MS internal standard. In addition to the method development work reported here, the analytical method developed herein was applied to measure PFCAs in various types of AOCs.

2. Experimental

2.1. Standards and Chemicals

One set of PFCA standards was purchased from Oakwood Products, Inc. (West Columbia, SC, USA) and used as calibration standards. They are PFPeA-C5 (97%), PFHxA-C6 (97%), PFHpA-C7 (98%), PFOA-C8 (95%), PFNA-C9 (98%), PFDA-C10 (98%), PFUnDA-C11 (96%), and PFDoDA-C12 (95%). The other set of PFCA standards was purchased from Sigma-Aldrich (Milwaukee, WI, USA). They are PFPeA-C5 (97%), PFHxA-C6 (≥97%, Fluka), PFHpA-C7 (99%), PFOA-C8 (96%), PFNA-C9 (97%), PFDA-C10 (98%), PFUnDA-C11 (95%), and PFDoDA-C12 (95%). They were used as the internal audit program (IAP) standards to evaluate the accuracy and precision of the instrument after calibration. The isotopically-labeled compounds, PFOA-$^{13}C_4$ and PFDA-$^{13}C_2$, which consisted of 50 µg/mL of each in methanol, were purchased from Wellington Laboratories, Inc. (Guelph, Ontario, Canada). Methanol (MeOH), acetonitrile (CH_3CN) and acetic acid ammonium salt (NH_4Ac), all HPLC grade, were purchased from Fisher Scientific. Water (HPLC grade) was purchased from Burdick & Jackson. Ethanol (EtOH, 99.5%, ACS reagent) and methyl tertiary-butyl ether (MTBE, 99.8%, HPLC grade) were purchased from Sigma-Aldrich.

2.2. Standard Preparation

Stock solutions of each individual calibration standard, including a recovery check standard and an internal standard, were prepared in methanol and stored in glass bottles placed in a refrigerator (~ 4 °C). They were discarded two months after the date of preparation due to possible degradation. Prior to instrument calibration, fresh calibration standards were prepared from the stock solutions in 60:40 (v/v) methanol and 2 mM NH_4Ac aqueous solution (referred to as 60:40 solution hereafter) in the range of 0.3 to 100 ng/mL in 10 mL volumetric flasks labeled with a serial number for each concentration level. Eight levels of concentration, 0.3, 0.7, 1.2, 2, 5, 10, 50, and 100 ng/mL, were prepared with 100 µL of 0.5 ng/µL internal standard spiked in each calibration standard. To avoid cross contamination, all glassware and plastic tubes were labeled and designated for a specific usage. Plastic tubes (high-clarity polypropylene

conical centrifuge tubes (BD Falcon™)) and pipettes (Eppendorf Series 2000 Reference ® pipettes and ep TIPS) were disposable. Glassware was rinsed with tap water, de-ionized (DI) water, and HPLC-grade methanol before use and randomly checked for PFCA residuals by LC/MS/MS. The glassware was considered acceptable if all individual PFCAs were below the practical quantification limit.

2.3. Sample Preparation

For solid samples, a few pieces of the selected specimen (0.5 to 3 g) were weighed and placed in a 50-mL high-clarity polypropylene conical centrifuge tubes (BD Falcon™) with 45 mL of MeOH spiked with 100 µL of 2 ng/µL recovery check standard and then extracted with a Nutating Mixer (Model VSN-5, PRO Scientific, Inc., CT, USA) or Dionex ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, USA) for 1 hour (ASE) or 24 hours (VSN-5). When the extraction was done with VSN-5, the sample vial was placed on the rotating table horizontally at a 20-degree angle. The extract aliquots were transferred into a 170-mL borosilicate glass tube and blown down to approximately 1 mL using the RapidVap N_2 Evaporation System (Model 791000, LabConco, Missouri, USA), which was modified at the factory to remove all Teflon® parts and coatings. The 1 mL of the concentrated sample solution was transferred from the 170-mL borosilicate glass tube to a 10-mL volumetric flask using a 60:40 solution rinse. Both the concentrated solution and the rinse were filtered through a 0.1 µm Anotop syringe filter. After adding 100 µL of the 0.5 ng/µL internal standard, the sample was sonicated for 10 minutes before LC/MS/MS analysis. An exception to the solid material extraction procedure was cookware. The fluorinated coatings on the cookware were difficult to remove without incorporating contaminants in the sample. Therefore, the cookware was extracted by covering the entire inner bottom surface with 100 - 150 mL of methanol spiked with 100 µL of recovery standard to a depth of approximately 0.3 mm and then allowed to stand under static conditions at ambient temperature for 24 hours. The extract was collected from the cookware and concentrated to 1 mL in accordance with the procedures of solid sample preparation. To minimize solvent evaporation during extraction, the opening of the cookware was tightly sealed with aluminum foil by compressing the foil to the inside and outside walls of the pan edge to a depth of approximately 0.5 cm.

To prepare the liquid samples, approximately 1.5 mL of liquid sample was weighed, spiked with 100 µL of 2 ng/µL recovery check standard, diluted with 25 mL of the 60:40 solution, sonicated for 10 minutes, and then filtered with a Corning 50-mL, tube-top filter with 0.22 µm pore size (Corning, Inc., NY, USA). Ten mL of the filtrate were transferred into a 10-mL volumetric flask, spiked with 100 µL of the 0.5 ng/µL of the internal standard, and then sonicated for 10 minutes before LC/MS/MS analysis. If the liquid sample contained high levels of PFCAs, a second dilution was conducted before the recovery check standard was added.

The detailed sample acquisition and preparation procedures are described elsewhere.

2.4. Analytical Method

Sample quantification was conducted using an Agilent 1100 HPLC equipped with an Applied Biosystem API 3200 Triple Quadrupole Mass Spectrometer with a Turbo V ion-spray interface. The HPLC column was an Agilent Zorbax Eclipse XDB-C18, 2.1 x 50 mm, 3.5 µm column coupled with an Aglient Eclipse XDB-C18, 2.1 x 15 mm, 3.5 µm guard column. Column temperature was 50 °C and injection volume was 20 µL. The mobile phases included A = 100% 2 mM NH_4Ac in HPLC-grade water and B = 100% HPLC-grade methanol. The flow rate was 0.35 mL/min. The mobile phase was under gradient with the gradient program being 78% A, 22% B for 8 minutes, 50% A, 50% B for 0.5 minutes, 15% A, 85% B for 9.5 minutes, kept 15% A, 85% B for 1.9 minutes, then back to 78% A, and 22% B for 0.1 minutes. The total analysis time, including the washing gradient step, was 20 minutes.

The mass spectrometry was operated in the negative-ion mode, using multiple reaction monitoring (MRM). The MS operating parameters were changed over time to achieve the best sensitivity. Examples of the operating parameters of the MS are: ion source turbo spray, curtain gas – 14 arbitrary unit (setting), collision gas – 7 arbitrary unit (setting), ion spray voltage – -2500 V, temperature – 425 °C, ion source gas 1 – 36 arbitrary unit (setting), ion source gas 2 – 34 arbitrary unit (setting), resolution Q1 unit, and resolution Q2 unit. Table 1 presents the compound-dependent mass spectrometer parameters.

The instrument was calibrated for eight PFCA homologues (C5 to C12) plus the recovery check standard at eight concentration levels in the range of 0.3 to 100 ng/mL with triplicate injections. After calibration, the IAP was conducted to assess the performance of the LC/MS/MS system. The instrument was re-calibrated when QC samples were outside the acceptable range.

Most samples were analyzed shortly after preparation. Otherwise, they were stored in the refrigerator in polypropylene vials at 4 °C and analyzed within two weeks. After refrigerated storage, the samples were equilibrated to room temperature before analysis.

2.5. Method Development

2.5.1. LC/MS/MS Performance

The entire HPLC system was flushed extensively with 100% isopropanol and 100% methanol to eliminate any potential contamination before this work was started. Guard columns were changed routinely when the HPLC pressure was high or peak broadening was observed in the analytical chromatogram.

2.5.2. Dilution Solvent Optimization

To maintain good peak shapes and high sensitivities in the final samples and in the standards prepared for LC/MS/MS analysis, the samples and standards were diluted in a mixture of methanol and 2 mM NH_4Ac in water. The composition of methanol and 2 mM

NH_4Ac in water was optimized from the ratios of 100:0, 90:10, 80:20, 75:25, 70:30, 60:40, 50:50, and 10:90. The peak area responses and peak shapes for the PFCAs were evaluated for each composition.

2.5.3. Extraction

A Nutating Mixer (VSN-5 method) and a Dionex ASE 200 were selected for PFCA extraction based on studies in the literature [16, 24, and 25]. The extraction efficiency of these two instruments was compared. The ASE 200 was operated at the following conditions: preheat – 5 minutes, heat – 5 minutes, static – 3 to 20 minutes, flush% – 30% (volume), purge – 240 seconds, cycles – 3, pressure – 1200 psi, temperature – 60 ºC, and solvent – 100% methanol. It was observed that higher temperatures generated much more suspended particles, making the extracts difficult to filter. The VSN-5 was operated at room temperature and atmospheric pressure. The extraction tubes were placed horizontally at a 20-degree angle on the bed to allow good mixing. Two AOC samples, a non-woven medical garment and a treated mattress protector, in duplicates, along with one field blank, were spiked with the recovery check standard and extracted in methanol with each extraction method for 24 hours. The extracts were collected in a 170-mL borosilicate glass tube and blown down to 1 mL in subsequent preparation for the LC/MS/MS analysis.

Further studies were conducted to evaluate extraction efficiency for various PFCAs with different solvents, including methanol, acetonitrile, MTBE, water, ethanol, and 60:40 (v/v) methanol : water. Duplicate samples of a non-woven medical garment were extracted with each of these solvents following the sample preparation and VSN-5 extraction procedures described above. The extraction efficiencies were compared in terms of individual PFCA concentrations and the recovery of the spiked recovery check standard.

To optimize the number of extraction steps for PFCAs from an AOC sample, six types of AOCs, including mill-treated carpeting, thread sealant tape, non-stick cookware, a treated mattress protector, membrane for apparel (used in breathable, waterproof outerwear), and a treated, non-woven medical garment, were extracted four times with 100% methanol using the VSN-5 method. There was a minimum 24-hour time interval between each sequential extraction. The extraction efficiencies were calculated for the four consecutive extractions.

2.5.4. Blow down

The extracts from most of the AOCs had to be concentrated before the analyses could be conducted. A RapidVap N_2 Evaporation System was used to evaporate extraction solvent. In the solvent comparison tests, the temperatures used to evaporate the solvents were approximately 15 °C below the boiling points of the solvents. For example, methanol extracts were concentrated at 50 °C, whereas the boiling point of methanol is 64.7 °C. Comparison was made between two types of concentration tubes: one with a 1.5-mL end point and the other with a flat bottom. Triplicate 10-mL volumes of methanol

were spiked with 40-ng aliquots of the PFCA standards and blown down to a volume of 1 mL or to dryness. The blown-down standards were prepared for LC/MS/MS analysis following the procedures used for the solid AOC samples. The relative response of a PFCA (analyte peak area response divided by internal standard peak area response) was compared to that of the standard without blow down.

2.5.5. Filtration

Cloudy samples were generated during the extraction, especially with the ASE. Particles from the samples had to be removed prior to LC/MS/MS analysis. The filters evaluated were the 25-mm diameter Whatman Anotop disposable syringe filter with 0.1 µm pore size and the Corning 50-mL, tube-top filter with a pore size of 0.22 µm. The Anotop filter was first tested by standards spiked into 60:40 solution. Then the 0.1-µm filter was evaluated by standards spiked into cloudy samples without detectable PFCAs. The Corning filter was examined by spiking standards into 60:40 solution. Cloudy samples were generated by extracting one apparel sample with methanol and then adding 2 mM NH_4Ac-water to make a 60:40 solution. Standards and cloudy samples were prepared so that they could be split into seven, 10-mL standard solutions without filtration and seven, 10-mL samples with filtration for each type of filter test. The standards were filtered in the same way as the samples. The same LC/MS/MS analysis procedures were followed for all samples and standards.

2.5.6. Stability

The stabilities of C5 to C12 PFCA standards in 60:40 solutions in different storage containers and at different temperatures were investigated. Standards at concentration levels of 1 ng/mL and 10 ng/mL in 60:40 solution, along with field blanks, were prepared and split into 25-mL glass bottles (Pyrex®) and 15-mL, high-clarity polypropylene, conical, centrifuge tubes (BD Falcon™). The bottles and tubes were stored at room temperature (about 23 °C) and in the refrigerator (about 4 °C) for 22 to 35 days. The stabilities of the standards were checked periodically by LC/MS/MS analysis.

2.5.7. Method Detection Limit

The instrument detection limit (IDL) for the LC/MS/MS and the method detection limit (MDL) for PFCA sample analysis were examined. The IDL was determined by evaluating seven injections of the lowest calibration standard (0.3 ng/mL). The MDL was determined by analysis of analytes with the defined analytical method for PFCAs from AOC samples. Standards containing 3 ng of C5 to C12 PFCAs each were spiked in 45-mL methanol in seven replicates for solid MDL determination. Standards containing 75 ng of C5 to C12 PFCAs each were spiked in 25-mL 60:40 solution in seven replicates for liquid MDL determination. And then the same sample preparation procedures that were used in the solid and liquid sample extraction and LC/MS/MS analysis were followed. Method blanks were prepared and analyzed with each set of MDL samples to identify background contamination.

2.5.8. Quality Assurance and Control

A quality assurance project plan (QAPP) was prepared before the project was started. The acceptance criterion for the calibration curve requires a coefficient of determination (r^2) of 0.99 or greater. The internal audit program standards, which contained at least four of the calibrated PFCAs using a different chemical source, were prepared by someone other than the person who prepared the calibration standards and were submitted without concentration information to the analyst who conducted the calibrations. The IAP standards were analyzed after each calibration as a measurement of calibration verification. The criterion for acceptance was that the calculated concentration and the measured IAP standard using the calibration had to be within 15% of each other before and after each batch of samples analyzed. Daily calibration check (DCC) standards, approximately 5 ng/mL for each PFCA, were analyzed to evaluate the LC/MS/MS performance. Analytical results of a sample batch were considered acceptable only when the percent recovery of the DCC was within 100 ± 15% and the percent relative standard deviation (%RSD) of DCCs was within ±15%. All samples and standards were injected in triplicate.

PFCA background levels originating from the methanol solvent, 2 mM NH_4Ac-water, lab coats, gloves, glassware, and HPLC system were routinely evaluated by running solvent blank, system blank, and extracts of samples, e.g., lab coats and gloves. After use, the glassware used for samples was checked for PFCA residuals by measuring the solvent from a heavily-used concentration tube and a 10-mL volumetric flask. A solvent blank was prepared with each set of standards and samples to assess the solvent and HPLC system.

Each AOC sample was extracted in duplicate for LC/MS/MS analysis. Analytical results were considered acceptable when the measured concentrations were in the calibration range, the %RSD of duplicates within ±20%, and the %recovery of the recovery check standard within 100 ± 20%.

3. Results and Discussion

3.1. LC/MS/MS Performance

The identification and quantification of PFCAs were performed by LC/MS/MS. The analytes in LC/MS/MS were confirmed by comparison of retention time of daily calibration check standards and the isotopically-labeled internal standard. The practical quantitation limit (PQL), which is the lowest standard concentration injected, was 0.3 ng/mL. Some C6, C7, and C8 PFCA peaks were detected in the blanks, but they were below the PQL. Linear calibration curves ((1/x)-weighted), with $r^2 \geq 0.99$, were used for quantitation. A representative linear regression equation and the coefficient of determination are given in Table 2. A chromatogram from the analysis of a non-woven medical garment sample is shown in Fig. 1 as an illustration. The sample concentration

was determined from the calibration curve by the relative response, which was the analyte's peak area divided by the internal standard's peak area. The recoveries of DCC ranged from 85% to 115%, and those of the IAP ranged from 85% to 113%.

3.2. Dilution Solvent Optimization

When strong solvents, e.g., MeOH, are used for the extraction of PFCAs from AOC samples, solvent overload effects might result in poor chromatographic performance. The extracts must be diluted with water to weaken the solvent strength prior to LC injection. The methanol and 2 mM NH_4Ac-water in ratios of 100:0, 90:10, 80:20, 75:25, 70:30, 60:40, 50:50, and 10:90 were evaluated. The representative results are shown in Figs. 2a to 2c. The results show that, among the different compositions of methanol and 2 mM NH_4Ac-water tested, methanol and 2 mM NH_4Ac-water in the ratio of 60:40 (v/v) gave the best peak shape and the highest peak area response with the mobile phase gradient used. Thus, this ratio was selected as the optimized injection solvent.

3.3. Extraction

In the extraction comparison between VSN-5 and ASE 200, two AOC samples spiked with the recovery check standard were analyzed. Table 3 summarizes the results of measured PFCAs and the recovery check standard. The results show that these two extraction methods are comparable. However, because ASE was operated under high temperature and high pressure, it often generated a large quantity of suspended particles in the extract, thus causing difficulties in filtration and LC/MS/MS analysis. Some samples, such as thread sealant tape, were completely disintegrated through the ASE and could not be further processed. In addition, random carry-over was detected with the ASE 200 method. For these reasons, the conventional VSN-5 method was selected for use in this research.

The extraction efficiencies of PFCAs with various solvents, including methanol, water, ethanol, acetonitrile, MTBE, and 60:40 (v/v) methanol : water, were evaluated and are summarized in Table 4. A non-woven medical garment sample was extracted with each of these solvents in duplicate by the VSN-5 method. The extraction efficiencies were compared by measuring individual PFCA concentrations and calculating the recovery of the spiked recovery check standard. Water, 60:40 (v/v) methanol : water, and acetonitrile were found to have lower extraction efficiencies than methanol for all C5 to C12 PFCAs. The poor recoveries reflect the combination of ineffective solvent extraction and sample loss due to the difficulty in the blow-down process. Compared to methanol, ethanol had lower extraction efficiency for C5 and C6 and slightly higher efficiency for C7 to C12, but the precision of the results was diminished. MTBE had higher efficiency for C5 and C10 to C12 but lower efficiency for C6 to C9. It was also observed that when MTBE was used as the extraction solvent, it had a solvent compatibility problem with water when other organic compounds were present. These results suggest that methanol is among the best solvents with adequate extraction efficiency, good precision, and best compatibility with the LC mobile phase. Thus, it was chosen as the preferred solvent for this research.

To estimate the efficiency of a single-step extraction, consecutive extractions were conducted for mill-treated carpet, non-stick cookware, thread sealant tape, a treated mattress protector, membrane for apparel, and treated non-woven medical garment samples. They were extracted four consecutive times with methanol using the VSN-5 method. The extraction efficiencies were determined by the amount of each analyte from a single extraction divided by the sum of the four exhaustive extractions. Concentrations below the instrument detection limit were treated as zero. The results shown in Table 5 indicate that the extraction efficiency varies with different AOC matrices and that a single-step extraction can extract 70% to 100% of PFCAs from AOCs, except for cookware. Concentrations of PFCAs measured in cookware were relatively low, resulting in the calculated extraction efficiency being lower than 70%. Only C8 and C7 data were reported for cookware. All our PFCA content results are based on a single-step extraction. The concentrations reported here were not adjusted for recovery.

3.4. Blow down

Comparisons were made between the extracts blown down to dryness and to 1 mL, and the results are summarized in Table 6. The samples blown down to 1 mL have higher recovery and better precision for every PFCA, including the recovery check standard. The lower recoveries observed in blow-down-to-dryness tests most likely were the result of mass losses during solvent evaporation.

3.5. Filtration

Extracts of solid samples and diluted liquids must be filtered prior to LC/MS/MS analysis. Anotop syringe filters were used for solid AOC sample preparation. Corning 50-mL, tube-top filters were used to prepare the liquid AOC samples. Anotop filters have a pigment-free, polypropylene housing and the unique Anopore® membrane made from Gamma-Alumina 6-mm Al_20_3. The Corning 50-mL, tube-top filter has a 50-mm diameter cellulose acetate membrane. The results of both types of filters (Table 7) demonstrate that the filtration process did not add PFCA contamination to the samples.

3.6. Stability

Standards with concentrations of 1 ng/mL and 10 ng/mL for all the analytes, including the recovery check standard, were stored in glass bottles and high-clarity, polypropylene, conical, centrifuge tubes at room temperature (about 23 °C) and in a refrigerator (about 4 °C) for 22 to 35 days. Their concentrations were compared to freshly-prepared standards analyzed on the first day of the test. Field blanks were analyzed for each type of tests. The results are shown in Figs. 3 to 6 using 1-ng/mL standards as an example. The 1-ng/mL standards and 10-ng/mL standards behaved the same way. At the four test conditions, an increasing trend for C5 to C8 concentrations was observed after the standards were stored for 24 to 35 hours whereas C9 to C12 concentrations decreased. For both refrigerator and room temperature storage, the

standards in the polypropylene tubes were more stable than those in the glass bottles. The differences in relative response were within 10% for each PFCA within 35 days. When the standards were stored in glass bottles in the refrigerator, the variation of the relative response of each PFCA was within 20% within 35 days. However, when they were stored in glass bottles at room temperature, after only 13 days, the concentrations of C5 to C7 were gradually increased and doubled the initial concentrations at 23 days, whereas the concentrations of C9 to C12 decreased by a factor of two at 23 days. These results contrast with the findings of Larsen et al. [27], who showed no apparent losses after 3 months at room temperature for samples stored in glass, polyethylene, or polypropylene containers.

3.7. Method Detection Limit

The instrument and method detection limits for PFCA contents in methanol extracts were determined according to the EPA definition and procedure [32]. The limits were calculated using the standard deviation and the correct Student's t-value with 99% confidence level for seven replicates. The results are provided in Table 8. The IDL for each analyte was below the method detection limits for both solid and liquid AOC samples.

3.8. Sample Analysis

The method developed in this study has been applied to analyze 116 articles of commerce (AOCs) treated with fluorinated chemicals. The samples were collected from retail outlets in the United States between March 2007 and May 2008. These AOC samples cover 13 article categories and are divided approximately evenly between domestic and imported products. The samples were analyzed in duplicate. Results from several different types of AOC samples are presented in Table 9. The concentrations reported are not adjusted for recovery. The recoveries listed in Table 9 demonstrate acceptable performance of the method for the analysis of PFCAs in these AOC products. More data from application of the current method to AOC samples will be reported elsewhere.

4. Conclusions

In this study, we presented optimized chromatographic conditions, extraction and sample preparation procedures, analytical recovery, method precision, storage stability, and method detection limits for analysis of PFCAs in both solid and liquid AOCs. The C5 to C12 PFCAs were well separated by Agilent 1100 HPLC equipped with Applied Biosystem API 3200 triple quadrupole mass spectrometer with the gradient mobile phase program. The optimum injection solvent of 60 : 40 methanol : 2mM NH4Ac in water was selected. Though the VSN-5 and the ASE 200 extraction methods are comparable, the VSN-5 method was found to be better suited for PFCAs from solid AOCs than the ASE 200 method. Methanol was identified to be among the best extraction solvents. A single-step extraction can extract 70% to 100% of PFCAs from AOCs, with the exception of cookware. Thus, all of our PFCA content results are based on a single-step extraction

without correction for recovery. The study also demonstrated that using the RapidVap N_2 evaporation system, a 0.1-µm Anotop syringe filter, and a 0.22-µm Corning, tube-top filter for sample preparation did not cause significant interference or sample loss. The PFCA standards stored in the polypropylene tubes were more stable than those stored in the glass bottles both in the refrigerator and at room temperature. Method detection limits for PFCAs in solid AOC samples were less than 3.9 ng/g and those in liquid AOC samples were less than 6.8 ng/g. Overall, the method developed in this study is adequate for detection and quantification of PFCAs at trace levels in general articles of commerce.

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List of Figures

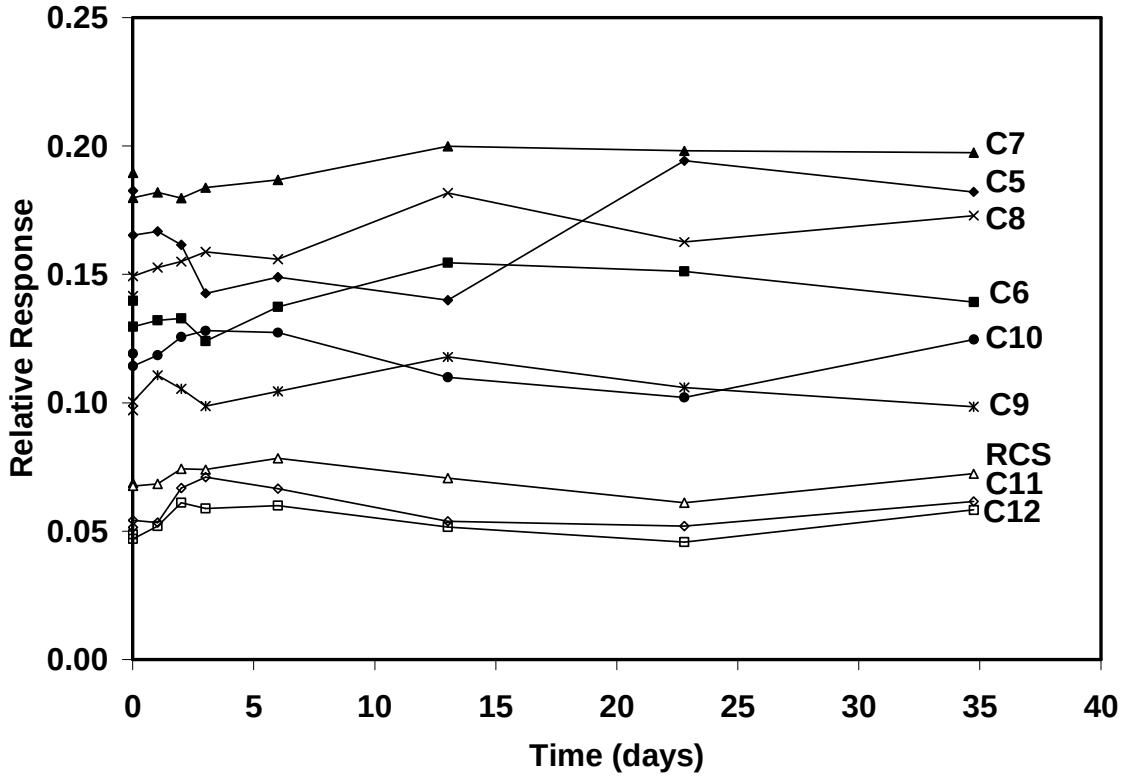

Fig. 6. Time-concentration profile of PFCA standards (1 ng/mL) in polypropylene conical centrifuge tubes at room temperature (about 23 °C; RCS is recovery check standard).

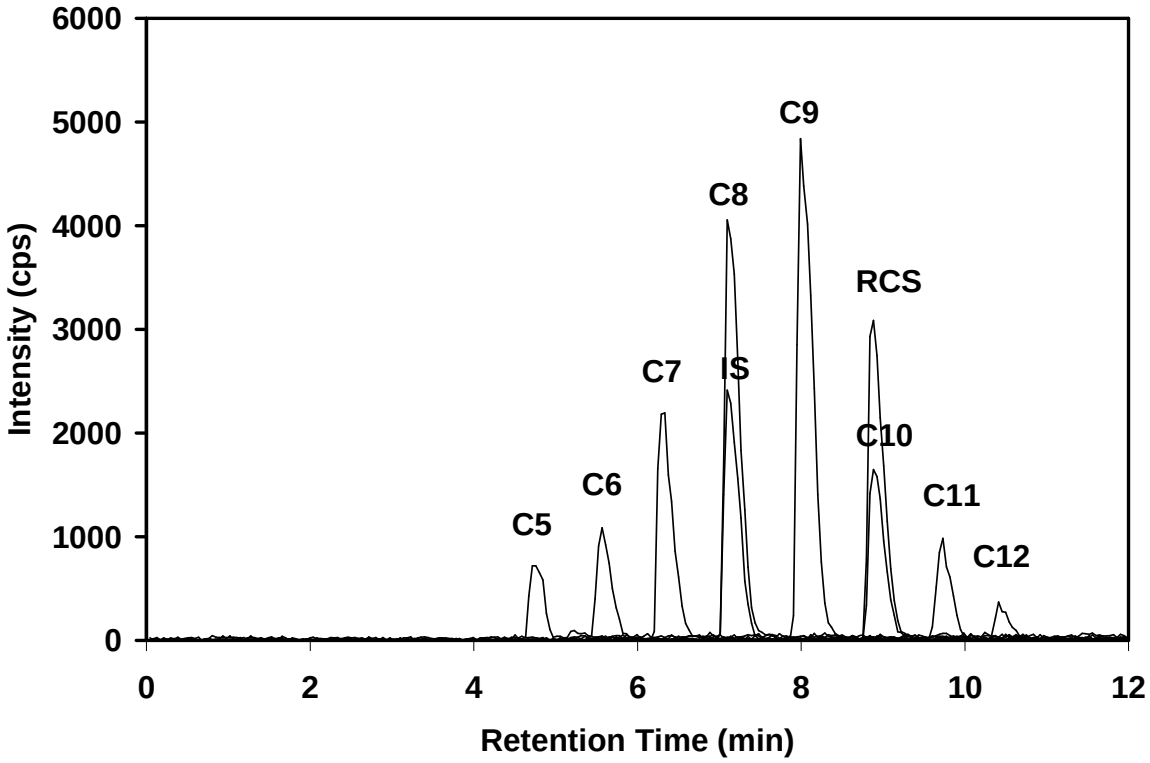

Fig. 1. Chromatogram of a non-woven medical garment sample (IS is internal standard, RCS is recovery check standard).

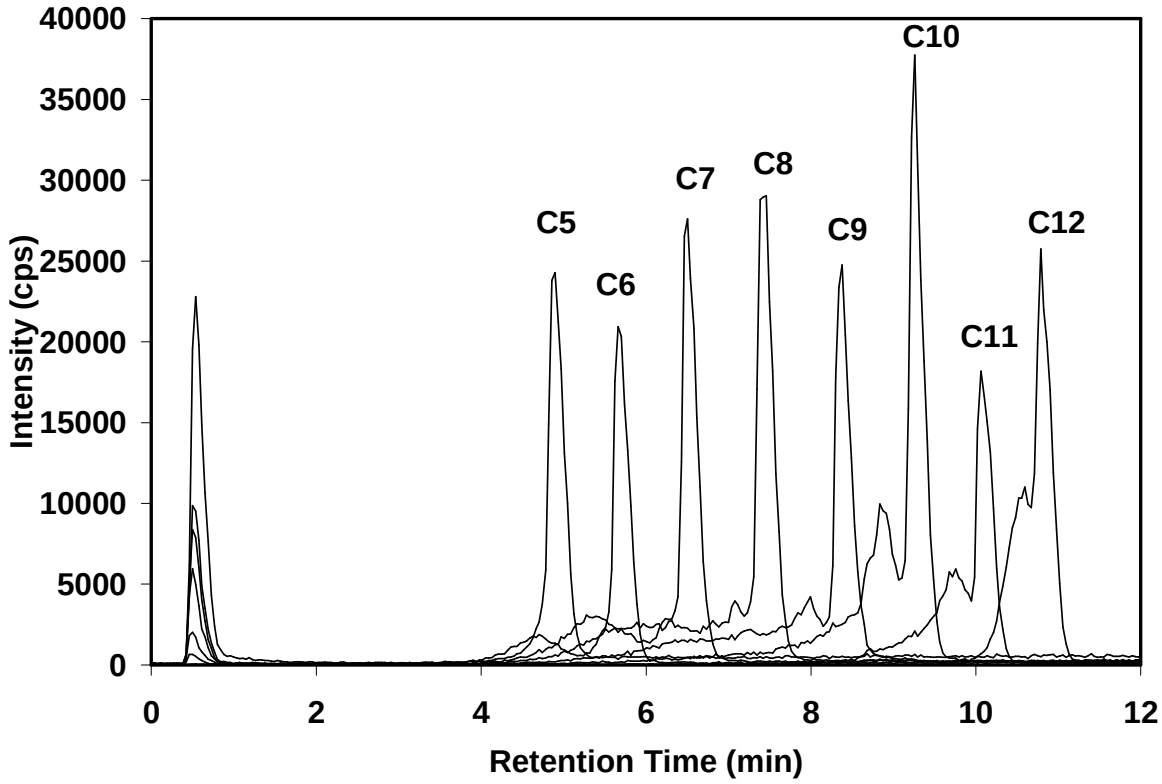

Fig. 2a. The effect of solvent composition for preparing the injection solution on LC/MS/MS performance – Case 1: 100% methanol (showing peak doubling).

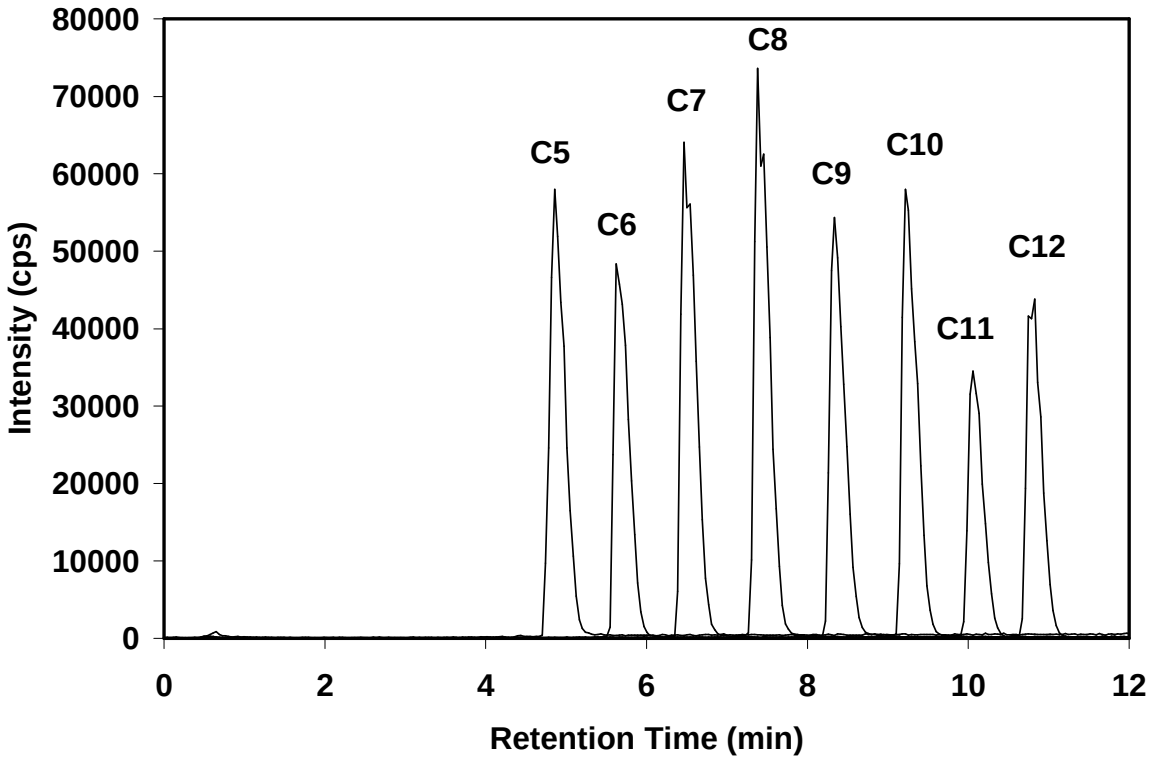

Fig. 2b. The effect of solvent composition for preparing the injection solution on LC/MS/MS performance -- Case 2: 60% (v/v) methanol and 40% 2 mM NH_4Ac aqueous solution (showing good sensitivity and peak resolution).

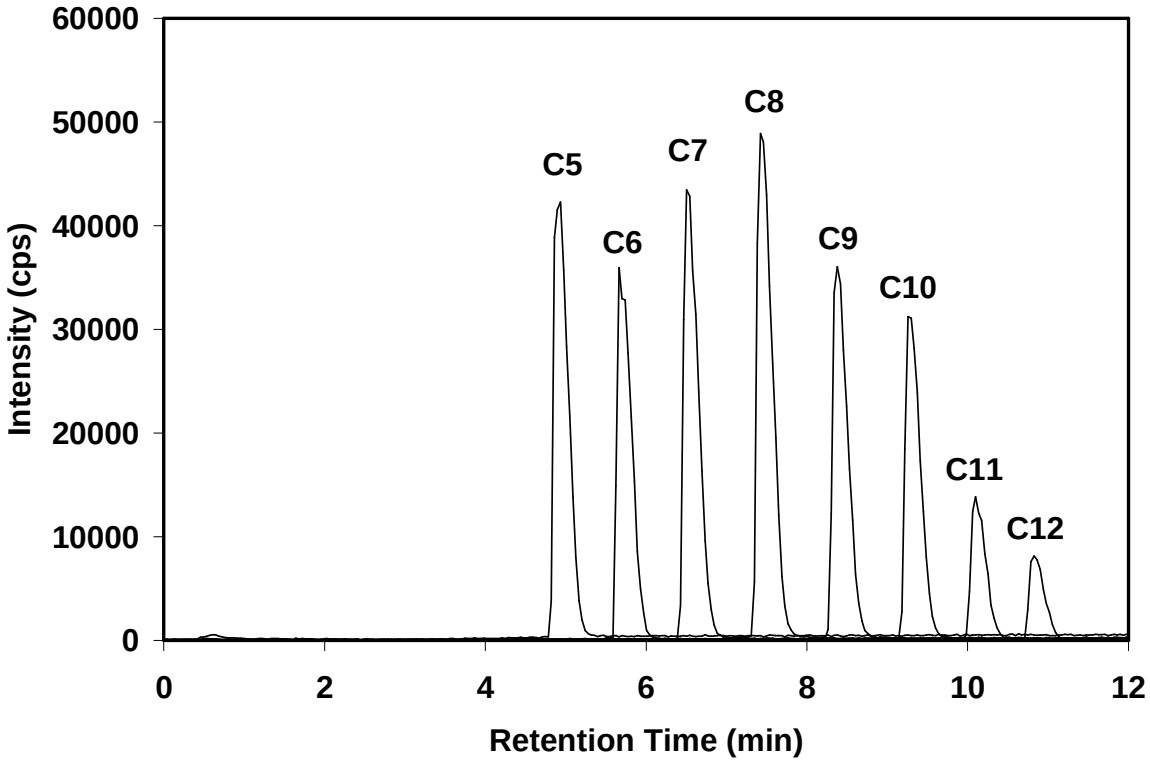

Fig. 2c. The effect of solvent composition for preparing the injection solution on LC/MS/MS performance – Case 3: 10% (v/v) methanol and 90% 2 mM NH_4Ac aqueous solution (showing good peak resolution but low sensitivity).

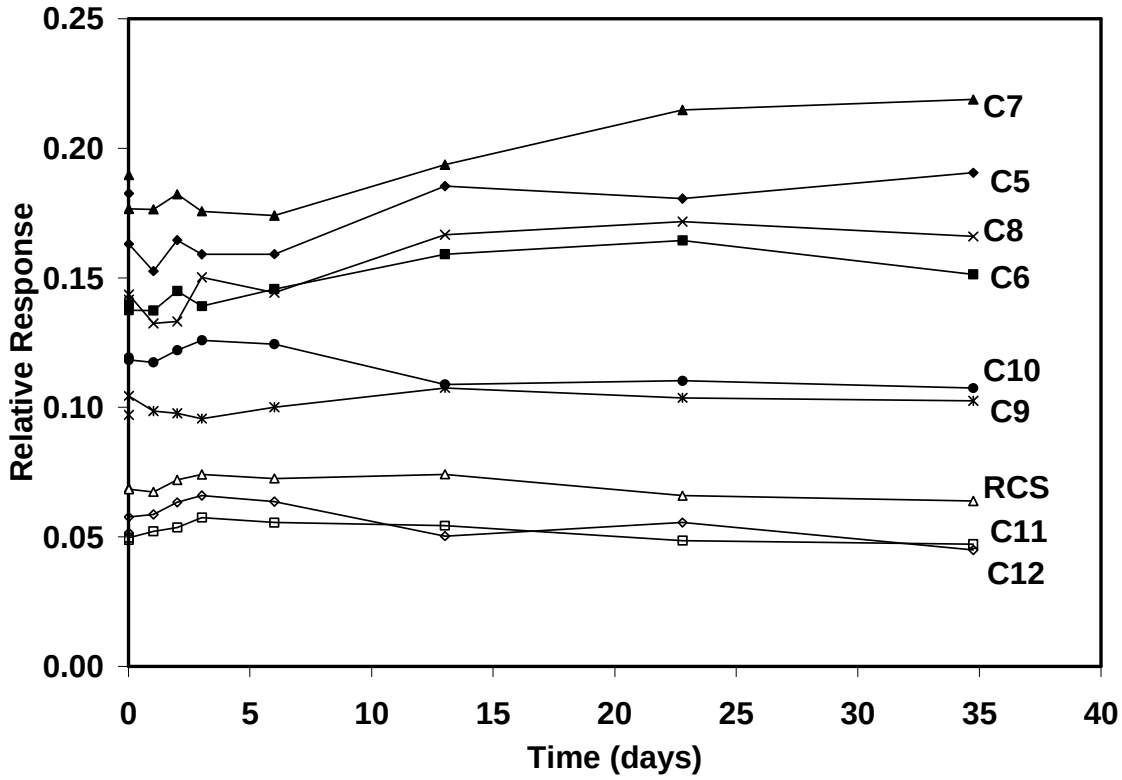

Fig. 3. Time-concentration profile of PFCA standards (1 ng/mL) in glass bottle in refrigerator at about 4 °C (RCS is recovery check standard).

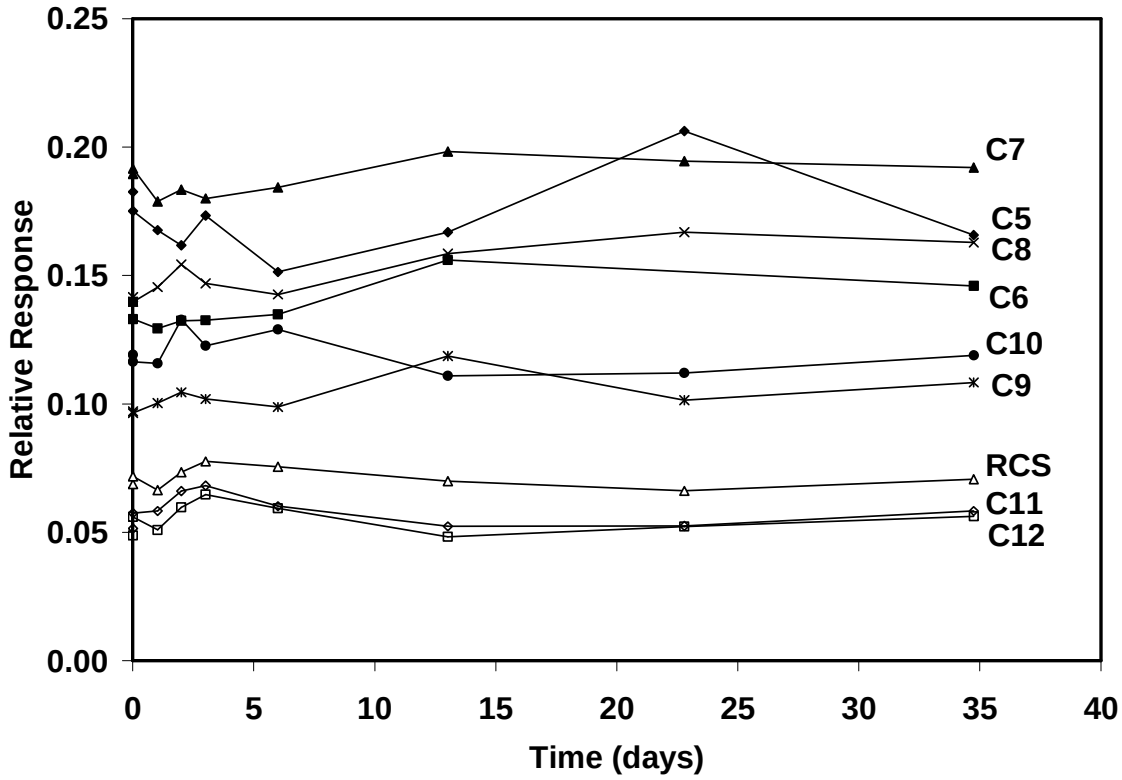

Fig. 4. Time-concentration profile of PFCA standards (1 ng/mL) in polypropylene conical centrifuge tubes in refrigerator at about 4 °C (RCS is recovery check standard).

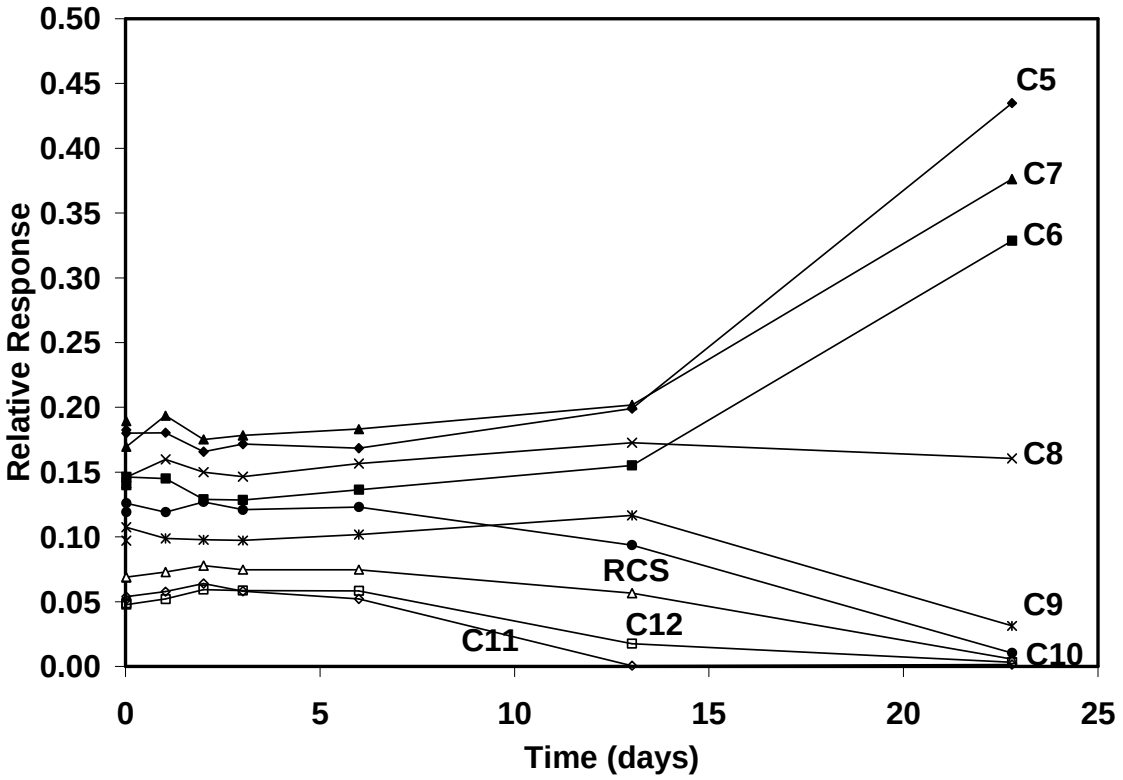

Fig. 5. Time-concentration profile of PFCA standards (1 ng/mL) in glass bottle at room temperature (about 23 °C; RCS is recovery check standard).

Table 1
MS parameters for target analytes

Analytes	Q1 Mass (amu)	Q3 Mass (amu)	Time (msec)	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
PFPeA-C5	263	219	250	-21.1	-2.8	-10	-10
PFHxA-C6	313	269	250	-21.8	-3.2	-14.6	-26.4
PFHpA-C7	363	319	250	-22.8	-3	-13.1	-24.8
PFOA-C8	413	369	250	-23	-2.8	-15	-5.4
PFOA-C8-$^{13}C_4$	417	372	250	23.9	-4.1	-15.4	-24.8
PFNA-C9	463	419	250	-30.6	-3.8	-14.3	-6.8
PFDA-C10	513	469	250	-24.7	-4.6	-15.6	-38
PFDA-C10-$^{13}C_2$	515	470	250	-10	-6	-10	-38
PFUnDA-C11	563	519	250	-24.6	-4.9	-15.2	-38.4
PFDoDA-C12	613	569	250	-26.4	-4.9	-15.2	-38.4

Table 2
Representative results of LC/MS/MS calibrations

	0.3-112 ng/mL			0.3-11.2 ng/mL		
Analytes	**a**	**b**	**r^2 [a]**	**a**	**b**	**r^2**
PFPeA-C5	4.348	-0.046	0.9997	4.376	0.065	0.9977
PFHxA-C6	3.986	-0.072	0.9995	3.949	0.102	0.9982
PFHpA-C7	4.514	-0.012	0.9992	4.503	0.116	0.9965
PFOA-C8	6.410	-0.019	0.9996	6.403	0.117	0.9973
PFNA-C9	8.506	0.103	0.9992	8.539	0.115	0.9969
PFDA-C10	16.219	-0.668	0.9980	14.43	0.059	0.9970
PFDA-C10-$^{13}C_2$	11.379	-0.380	0.9980	10.63	0.037	0.9980
PFUnDA-C11	21.381	0.046	0.9997	22.180	-0.022	0.9988
PFDoDA-C12	35.413	0.114	0.9991	36.017	-0.024	0.9976

[a] r^2 is coefficient of determination.

Table 3
Average concentration (ng/g) ± RSD% of PFCAs from AOCs extracted using VSN-5 vs. ASE extraction method

	Medical Garment (n=4)		Mattress Protector Home Textile (n=4)	
Analytes	**VSN-5**	**ASE**	**VSN-5**	**ASE**
PFPeA-C5	4.9 ±1.4%	4.7 ±2.7%	72.0±3.6%	73.9 ±3.2%
PFHxA-C6	7.5 ±12.8%	7.8 ±8.3%	152.1 ±4.8%	161.3 ±3.6%
PFHpA-C7	11.0 ±8.2%	11.7 ±10.4%	314.5 ±3.6%	326.9 ±2.6%
PFOA-C8	32.0 ±4.4%	34.9 ±4.2%	313.8 ±1.9%	321.1 ±2.2%
PFNA-C9	54.5 ±1.2%	59.7 ±5.1%	291.9 ±1.7%	294.3 ±3.2%
PFDA-C10	18.0 ±4.4%	19.3 ±5.9%	134.4 ±4.7%	134.9 ±4.2%
PFUnDA-C11	18.4 ±7.3%	19.5 ±7.8%	138.5 ±2.7%	143.9 ±3.6%
PFDoDA-C12	7.1 ±8.7%	7.0 ±17.8%	85.9 ±13.2%	86.5 ±4.5%
% RCS Recovery	74.6% ±3.7%	75.5% ±1.5%	94.8% ±2.7%	93.7% ±2.4%

Table 4
Average concentration (ng/g) ± RSD% of PFCAs from non-woven medical garment extracted with different solvents

Analytes	100% MeOH	100% H_2O	40:60 (H2O:MeOH)	100% EtOH	100% ACN	100% MTBE
PFPeA-C5	5.2±7.5%	5.3±5.3%	6.4±2.1%	4.0±3.2%	3.3±10.8%	2.5±67.1%
PFHxA-C6	13.8±5.2%	12.7±4.9%	15.6±2.0%	15.3±4.9%	11.4±7.4%	9.0±16.2%
PFHpA-C7	22.3±4.2%	15.0±1.6%	24.8±2.6%	24.7±7.6%	15.8±7.7%	15.7±14.8%
PFOA-C8	43.0±4.6%	19.8±4.6%	40.6±6.1%	43.0±6.1%	34.5±5.4%	35.6±5.7%
PFNA-C9	80.0±7.9%	15.2±1.8%	57.9±7.8%	81.3±4.1%	59.8±5.4%	75.1±1.7%
PFDA-C10	26.0±5.5%	2.9±22.6%	11.0±9.4%	28.9±0.4%	20.3±4.0%	28.0±0.4%
PFUnDA-C11	25.1±7.6%	1.5±5.2%	3.0±12.3%	30.0±15.7%	21.6±3.4%	29.7±12.5%
PFDoDA-C12	9.7±2.4%	2.4±39.5%	0.8±41.1%	11.5±15.0%	6.6±4.4%	12.6±5.2%
%RCS Recovery	90.8%±1.9%	19.8%±4.0%	46.7%±3.6%	106.3%±1.7%	83.2%±2.7%	81.3%±3.4%

Table 5
PFCAs' average extraction efficiencies from consecutive extractions

AOC	Extraction	C5	C6	C7	C8	C9	C10	C11	C12
Tape [a]	1st	87.4%	87.9%	79.0%	86.7%	91.6%	71.7%	89.8%	95.4%
	2nd	8.5%	8.0%	12.0%	6.4%	4.3%	15.4%	6.6%	4.4%
	3rd	4.1%	2.2%	6.9%	1.4%	1.7%	12.8%	3.6%	0.2%
	4th	BDL [c]	1.9%	2.1%	5.5%	2.4%	BDL	BDL	BDL
Textile Mattress Protector [b]	1st	98.9%	97.1%	99.2%	98.8%	99.7%	98.0%	97.8%	97.2%
	2nd	0.6%	1.8%	0.8%	1.1%	BDL	1.4%	1.2%	2.8%
	3rd	BDL	0.7%	BDL	BDL	0.2%	0.3%	0.5%	BDL
	4th	BDL	0.3%	BDL	BDL	0.1%	0.3%	0.5%	BDL
Garment [b]	1st	100.0%	70.4%	97.0%	95.4%	97.8%	88.5%	83.7%	90.9%
	2nd	BDL	13.2%	3.0%	4.6%	BDL	5.9%	8.7%	9.1%
	3rd	BDL	8.5%	BDL	BDL	1.4%	2.9%	4.0%	BDL
	4th	BDL	7.9%	BDL	BDL	0.8%	2.7%	3.5%	BDL
Carpet [b]	1st	NR [d]	NR	94.7%	96.1%	97.1%	97.0%	89.7%	84.2%
	2nd	NR	NR	4.3%	3.1%	2.9%	3.0%	10.3%	15.8%
	3rd	NR	NR	0.7%	0.4%	BDL	BDL	BDL	BDL
	4th	NR	NR	0.3%	0.3%	BDL	BDL	BDL	BDL
Membranes [b]	1st	NR	NR	NR	95.9%	NR	93.4%	NR	NR
	2nd	NR	NR	NR	1.6%	NR	2.2%	NR	NR
	3rd	NR	NR	NR	1.4%	NR	2.2%	NR	NR
	4th	NR	NR	NR	1.1%	NR	2.2%	NR	NR
Cookware [b]	1st	NR	NR	28.8%	45.7%	NR	NR	NR	NR
	2nd	NR	NR	27.2%	30.0%	NR	NR	NR	NR
	3rd	NR	NR	22.2%	10.0%	NR	NR	NR	NR
	4th	NR	NR	21.7%	11.7%	NR	NR	NR	NR

[a] average of duplicate tests. [b] average of triplicate tests. [c] BDL is below instrument detection limit. [d] NR is not reported due to first extraction below PQL or data not reliable.

Table 6
Average recoveries of PFCAs in blow-down evaluation tests

	Blow Down to Dryness (n=3)			Blow Down to 1 mL (n=3)		
Analytes	**Average**	**STD**	**%RSD**	**Average**	**STD**	**%RSD**
PFPeA-C5	79.1%	0.11	12.5%	90.4%	0.02	1.9%
PFHxA-C6	78.9%	0.12	14.3%	87.9%	0.03	3.3%
PFHpA-C7	79.3%	0.17	19.2%	87.7%	0.03	3.8%
PFOA-C8	71.0%	0.15	18.7%	83.5%	0.03	3.1%
PFNA-C9	77.0%	0.21	22.5%	89.0%	0.01	1.0%
PFDA-C10	78.7%	0.19	20.5%	89.7%	0.02	2.2%
PFDA-C10-$^{13}C_2$	78.6%	0.18	20.1%	90.3%	0.01	1.3%
PFUnDA-C11	78.6%	0.19	20.2%	88.4%	0.02	2.7%
PFDoDA-C12	84.5%	0.13	14.4%	87.8%	0.04	4.5%

Table 7
Average recoveries of PFCA ± RSD% (n = 7) in filter evaluation tests

Analytes	0.22 µm-Corning	0.1 µm- Anotop	0.1 µm-Anotop + SC006 [a]
PFPeA-C5	96.9%±2.4%	100.1%±6.1%	98.7%±3.4%
PFHxA-C6	98.4%±3.0%	100.2%±4.1%	98.5%±2.9%
PFHpA-C7	104.8%±2.9%	100.1%±3.3%	103.7%±2.7%
PFOA-C8	101.9%±1.8%	100.1%±3.8%	98.1%±3.2%
PFNA-C9	102.0%±2.5%	99.9%±2.6%	100.3%±2.9%
PFDA-C10	105.6%±2.9%	100.2%±3.1%	109.7%±1.7%
PFDA-C10-$^{13}C_2$	118.4%±1.8%	96.3%±9.9%	107.2%±3.2%
PFUnDA-C11	100.9%±2.7%	106.1%±3.4%	115%±2.3%
PFDoDA-C12	107.2%±2.2%	98.1%±7.7%	112.8%±2.1%

[a] SC006 is a cloudy sample without detectable PFCAs.

Table 8
Instrument detection limit and method detection limit for target analytes (ng/g) (ng/mL)

Analyte	IDL (ng/mL)	MDL-Solid [a]	MDL-Liquid [b]
PFPeA-C5	0.05	2.380.34	1.800.04
PFHxA-C6	0.05	3.380.39	1.080.15
PFHpA-C7	0.03	3.880.10	3.700.27
PFOA-C8	0.05	0.990.15	6.760.11
PFNA-C9	0.04	1.510.08	2.690.06
PFDA-C10	0.05	0.840.27	1.380.06
PFUnDA-C11	0.05	2.730.09	1.570.07
PFDoDA-C12	0.04	0.850.10	1.650.16

[a.] MDL-Solid is calculated as ng/mL (MDL of injection volume) × 10 (dilution factor) / 1g (AOC mass)

[b.] MDL-Liquid is calculated as ng/mL (MDL of injection volume) × 25 (dilution factor) / 1g (AOC mass)

Table 9
Average concentration (ng/g) ± RSD% of PFCAs from AOCs (n = 2)

	AOC Samples [a]					
Analyte	**A-9**	**B-6**	**C-3**	**D-3**	**E-7**	**F-5**
PFPeA-C5	11.5±6.5%	1939.0±7.4%	140.1±15.1%	16.4±10.7%	21.6±4.8%	6.0±2.7%
PFHxA-C6	19.2±4.3%	5248.0±15.2%	1088.2±0.7%	43.2±14.8%	68.0±1.0%	14.3±2.1%
PFHpA-C7	43.0±2.2%	13319.7±7.4%	2503.1±0.3%	64.9±15.2%	96.6±1.7%	22.4±2.6%
PFOA-C8	19.9±1.4%	5007.8±4.5%	1177.0 ±4.8%	160.5±5.9%	330.0±3.6%	84.2±0.0%
PFNA-C9	20.7±0.4%	8456.9±1.0%	1714.0 ±0.6%	234.6±8.4%	213.5±2.8%	107.7±0.7%
PFDA-C10	18.4±10.3%	2927.2±1.5%	676.0±5.8%	69.2±5.7%	125.0±4.7%	64.2±0.8%
PFUnDA-C11	12.3±17.7%	3050.6±14.0%	800.9±6.3%	61.5±0.3%	45.7±4.1%	41.7±1.1%
PFDoDA-C12	42.0±11.8%	956.9±3.3%	327.7±14.5%	21.2±16.5%	43.0±4.9%	26.9±2.7%
%RCS Recovery	84.7%±0.5%	90.8%±4.3%	102.8%±2.9%	88.9%±15.2%	100.1%±2.0%	100.9%±1.4%

[a.] AOC samples: A-9 is Nylon carpet, B-6 is carpet protector concentrate, C-3 is spot removal kit, D-3 is girl's uniform shirt, E-7 is mattress pad, F-5 is reusable pillow, G-10 is marble & granite sealer, M-1 is tire shine. [b.] The concentration is below practical quantification limit.