A Method for the Low-Level (ng/g) Determination of Perfluorooctanoate in Carpet by LC–MS–MS Using Matrix Extracted Standards

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Abstract

Fluorotelomer-based acrylic polymers are applied to the surface of carpet to impart oil, stain, and water repellence properties. Concerns that fluorotelomer-based polymers are a possible source of "low level" exposure to humans, coupled with their widespread use have prompted the need to develop a method to detect and measure perfluorooctanoate (PFO) in carpet. A liquid chromatography tandem mass spectrometry method for the determination of PFO in carpet using a dual labeled ^{13}C-perfluorooctanoic acid (^{13}C-PFOA) internal standard is successfully developed and validated. Levels of PFO are determined using a gradient, reversed-phase high-performance liquid chromatography (HPLC) method with acetic acid acidified water–methanol, separated on a 50 mm Phenomenex Synergi Polar RP column. Ions monitored are 413 (parent) and 369 (daughter) for PFO and 415 (parent) and 370 (daughter) for dual labeled ^{13}C-PFOA internal standard. Accuracy and precision over three days for 5 to 900 ng/g PFO in carpet ranged from 2.4% to 7.6% and 3.7% to 14.1%, respectively. Overall extraction efficiency for samples (n = 30) fortified with ^{13}C-PFOA at 20 ng/g and perfluorooctanoic acid (PFOA) at 5, 50, and 500 ng/g is 98.9% ± 8.1%. Specificity of the method was evaluated with two different carpet samples.

Introduction

Recent studies indicate potential exposure of the general human population to perfluorooctanoic acid (PFOA) at very low levels (1–7). Other reports showed those low levels of PFOA and other fluorinated compounds could be found in wildlife and in the environment (8,9). Hence, interest in understanding more about the source and mode of exposure of these materials in humans and in the environment has increased.

Fluorotelomer-based acrylic polymers are applied to the surface of carpet fibers to impart oil and water repellency properties (10,11). Small quantities of PFOA may be generated as an unintended by-product in the production of the polymers (12–16). Although fluorotelomer-based polymers are not made using PFOA nor is PFOA added during the manufacture or use of telomer products, questions have arisen as to the possibility of trace level PFOA impurities in telomers and the potential for telomers to transform into PFOA (17). A method to determine extractable perfluorooctanoic acid (PFOA) in water, sweat simulant, saliva simulant, and methanol from textile and carpet samples by liquid chromatograpy tandem mass spectrometry (LC–MS–MS) has been developed (18).

Low level PFOA determinations are particularly challenging as carpet fiber and backing is a complex matrix. Most carpet today is tufted. Tufted carpet consists of face yarn, primary backing fabric, a bonding compound such as latex, polyvinylchloride, or polyurethane, and often a secondary backing fabric. With so many components in carpet samples, the MS–MS signal can either be enhanced or attenuated, depending upon what other compounds might be co-eluting with the analyte. Also background PFOA exceeding the limit of quantitation (LOQ) of 5 ng/g can be observed. Fortification of untreated carpet samples was used to determine if any major problems are evident in recovery of the analyte from the matrix. Ideally an isotopically enriched surrogate might be added during the manufacturing process, but is not practical.

PFOA contamination can originate from solvents, labware, lab contamination, and even from the components of standard HPLC instrumentation. Extraction and injection solvents as well as labware must be evaluated for contamination by routinely running reagent blanks; contamination from work areas must be minimized by washing these areas extensively with methanol, isopropanol, or other appropriate solvent.

Experimental

Reagents

Pure analytical perfluorooctanoic acid (CAS # 335-67-1, Catalog # 001319, chemical purity 97%) was purchased from Oakwood Products, Inc (West Columbia, SC) and perfluoro-

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octanoic acid (1,2-di-^{13}C) PFOA, 100% chemical purity was obtained from DuPont Haskell Laboratory (Newark, DE). Methanol (HPLC grade) and acetic acid were purchased from Fisher Scientific. High purity water was prepared using an NEU-ION system (Baltimore, MD). It is necessary to check the methanol for the presence of contaminants by LC–MS–MS before use since certain lots have been found to be unsuitable for use.

Carpet

Untreated tufted carpet was provided by E.I. du Pont de Nemours and Company.

Equipment

A Shimadzu HPLC equipped with a binary gradient pump and autosampler was used. An Applied Biosystems, Inc. triple-quadrupole mass spectrometer (API 3000) with Sciex Turbo Ion Spray Liquid Introduction Interface (MDS Sciex, Concord, Ontario, Canada) was used as the detector. Negative ions were monitored in MRM mode; 413 (parent) to 369 (daughter) for PFOA and 415 (parent) to 370 (daughter) for dual ^{13}C-PFOA. System control, data acquisition, and processing were done by Analyst software (Applied Biosystems, Inc.). The results were calculated by $1/x^2$ weighted linear regression analysis using Watson LIMS software (v6.4.0.04 InnaPhase Corporation).

The reversed-phase gradient separation was accomplished by injecting 3 µL samples onto a Phenomenex Synergi Polar RP, 2 mm i.d. × 50 mm, 4 µm, column (Torrance, CA) maintained at ambient temperature. The mobile phase comprised of A: water–acetic acid (100:0.1, v:v) and B: methanol–acetic acid (100:0.1, v:v) using the following gradient (Table I). The sample solvent was methanol–water (50:50, v:v).

The MS–MS parameters were optimized to transmit the parent ions, fragment them and monitor the daughter ions. Ions monitored were: 413 (parent) and 369 (daughter) for PFOA; 415 (parent) and 370 (daughter) for dual ^{13}C-PFOA. Negative ions were monitored in MRM mode (Table II).

Table I. HPLC Gradient

Time (min)	% A	% B	Flow (mL/min)
0.0	70	30	0.3
1.0	70	30	0.3
3.0	0	100	0.3
5.0	0	100	0.3
5.1	70	30	0.3

Table II. MS Parameters

Compound	Ionization mode	Declustering potential	Collision energy	Transition
PFO	ESI–	–30	–20	412.7 → 368.8
^{13}C-PF (IS)	ESI–	–30	–20	415.0 → 369.9

Table III. PFO Recovery in Carpet Over Three Days

Sample description	PFO (ng/g)*	Interday average recovery (%)	Interday S.D.	Intraday average recovery (%)	Intraday S.D.
Day 1	5.00	97.8	6.79		
Day 2	5.00	106	3.82	97.8	6.79
Day 3	5.00	120	24.4		
Day 1	15.0	101	11.3		
Day 2	15.0	95.7	1.41	101	11.3
Day 3	15.0	114	0.94		
Day 1	150	104	0.94		
Day 2	150	108	1.89	104	0.94
Day 3	150	99.0	1.41		
Day 1	900	101	3.85		
Day 2	900	104	4.40	101	3.85
Day 3	900	102	5.58		

* n = 2 for each day at each concentration.

Extraction procedure

Samples of approximately 10 grams of carpet with backing (approximately 9 × 9 cm piece) were cut, weighed and placed into glass beakers. The pieces of carpet were spiked (if necessary) with 1.0 mL aliquots of the calibration standard solutions or the fortification solutions. The carpet pieces were also spiked with 1.0 mL of internal standard solution. The spiked carpet pieces were allowed to sit for ~15 min to allow the solvent to dry. The spiked carpet was extracted by adding 200 mL of methanol to the carpet in the beakers. The beakers were covered and allowed to shake on an Eberbach reciprocal shaker for ~15 min; after shaking, the beakers were placed in an ultrasonic water bath for ~30 min. An aliquot of 5.0 mL of the methanol extract from each beaker was transferred into separate glass test tubes. The 5 mL extracts were then evaporated with nitrogen to dryness. The residue was reconstituted with 2.0 mL methanol–water (50:50, v:v). The

Table IV. Interday Accuracy and Precision of PFO in Carpet Over Three Days

	PFOA Fortification Levels in Carpet			
Sample description	5.00 ng/g	15.0 ng/g	150 ng/g	900 ng/g
Day 1	5.13	16.4	156	886
	4.65	14.0	158	935
Day 2	5.14	14.5	165	908
	5.41	14.2	161	964
Day 3	6.85	17.1	150	955
	5.12	17.3	147	884
3 Day Mean	5.38	15.6	156	922
S.D.	0.759	1.52	6.74	34.5
% Coefficient of variance	14.1	9.7	4.3	3.7
% Difference	7.6	4.0	4.0	2.4
n	6	6	6	6

reconstituted samples were centrifuged before transferring to a glass microvial for LC–MS–MS analysis.

Standards and fortification solutions

A stock standard solution of PFOA was prepared at a concentration of 1123.6 µg/mL by dissolving 11.584 mg of the standard (corrected for purity) in methanol. A stock internal standard solution of ^{13}C-PFOA was prepared at a concentration of 1110.6 µg/mL by dissolving 11.106 mg of the standard (corrected for purity) in methanol. Calibration standard solutions from 50 to 10,000 ng/mL were prepared in methanol–water (50:50, v:v). A 200 ng/mL ^{13}C-PFOA internal standard solution was prepared by dilution of the stock solution with methanol–water (50:50, v:v). Fortification solutions of 9,000, 1,500, 150, and 50 ng/mL PFOA were prepared by diluting the stock solution with methanol–water (50:50, v:v).

Standards for analysis were prepared by spiking seven (10 g) untreated carpet pieces on each day of analysis with 1.0 mL of each of the seven calibration standards, respectively. The carpets were also spiked with 1.0 mL of the internal standard solution. The concentration levels of PFOA on the carpet ranged from 5.0 (LOQ) to 1000 ng/g. The concentration level of the internal standard was 20 ng/g. The carpet pieces were processed through the extraction procedure, and the resultant solutions used as calibration standards.

The fortified carpet samples were prepared on each of 3 successive days by spiking duplicate (10 g) untreated carpet samples with the 1.0 mL of each of the fortification solutions to give concentrations on the carpet at the proposed LOQ (5 ng/g), at 3× the proposed LOQ (15 ng/g), at 30× the proposed LOQ (150 ng/g) and at 180× the proposed LOQ (900 ng/g). The carpets were also spiked with 1.0 mL of the internal standard solution. The carpet pieces were processed through the extraction procedure and the resultant solutions were used for determining recovery, precision and accuracy.

Matrix-only samples (methanol extract of carpet samples where the PFOA was added to the methanol after extraction) were prepared for extraction efficiency and matrix effect assessments. Untreated carpet samples (10 g) were extracted with 200 mL of methanol. A 5.0-mL aliquot of supernatant was spiked with 25 µL of standard solutions to give concentrations equivalent to 50, 500, or 5000 ng/mL. A 25-µL aliquot of internal standard solution was added as well. Samples were evaporated with nitrogen to dryness, the dried sample reconstituted with 2.0 mL methanol–water (50:50, v:v) and centrifuged before transfer to a glass microvial for LC–MS–MS analysis.

Neat samples (without carpet matrix) were prepared for extraction efficiency and matrix effect assessment. A 5.0-mL aliquot of methanol was added to each glass test tube. Respective tubes were spiked with 25 µL of solutions at concentration of 50, 500, or 5000 ng/mL, then each tube was spiked with 25 µL of internal standard. Samples were evaporated with nitrogen to dryness, the volume reconstituted with 2.0 mL methanol–water (50:50, v:v) and samples centrifuged before transfer to a glass microvial for LC–MS–MS analysis.

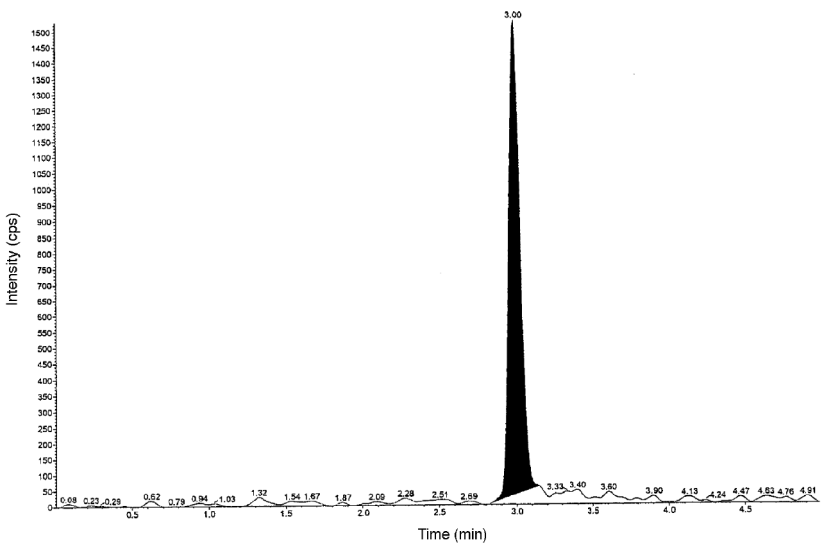

Figure 1. Representative chromatogram of the PFO peak (MRM 415.0 to 368.8) for lowest extracted standard corresponding to 5 ng/mL in carpet (0.625 ng/mL).

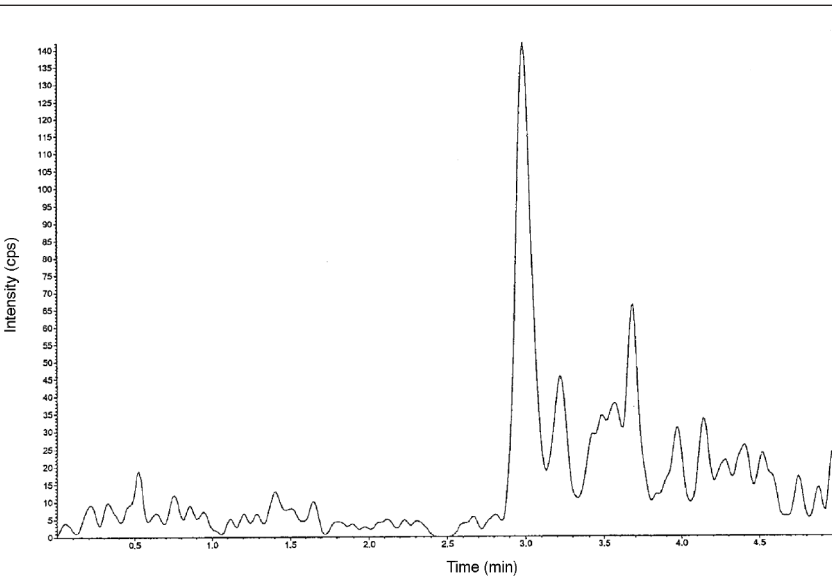

Figure 2. Representative chromatogram of the mobile phase blank (MRM 415.0 to 368.8).

Results and Discussion

Chromatographic performance

Quantitation of PFO, having a retention time of ~3.0 min, was accomplished by tur-

bospray LC–MS–MS analysis. Excellent sensitivity was accomplished at the lowest standard concentration of 5.0 ng/g (which corresponds to a final concentration of 0.625 ng/mL) in carpet extract (Figure 1). To maintain good peak shape and consistent retention, standards and samples were injected in a solvent system with 50% water, and the injection volume did not exceed 3 µL. Total analyses time, including washing, re-equilibration, and injection steps was approximately 10 min.

In this method, carryover and contamination from injection solvents were evaluated by routinely running mobile phase blanks (Figure 2).

Linearity

The linearity of PFO for each analysis set was determined using standard curves in carpet extracts obtained from the peak area ratio between the native analyte and its dual labeled ^{13}C analog (internal standard) at a minimum of seven concentrations, including the LOQ. Untreated carpet was found to contain small amount of PFOA, the source of which is uncertain. In order eliminate the contribution of the background amount the mean response of the three blank plus internal standard samples was subtracted from responses of all samples. Results showed a linear fit from 5.00 to 1,000 ng/g for PFO. Correlation coefficients of $r^2 \geq 0.994$ were readily achievable. Representative linear regression equation with $1/x^2$ weighting (Figure 3)

Matrix effect

The matrix effect study evaluated the suppression or enhancement of the analyte and internal response by the matrix. The matrix effect was determined in carpet fibers with backing at 5, 50, and 500 ng/g PFO and at 20 ng/g internal standard. The neat solutions served as the reference samples. Matrix suppression, determined by percent difference from the solvent peak areas, ranged from 7.6% to 12.0%.

To ensure that samples could be diluted with blank matrix without affecting the calculated concentration, a carpet sample fortified at 200 ng/g was diluted by a factor of ten and five replicates were analyzed. The difference between the mean concentration of the diluted replicates and the nominal concentration was 7.7%.

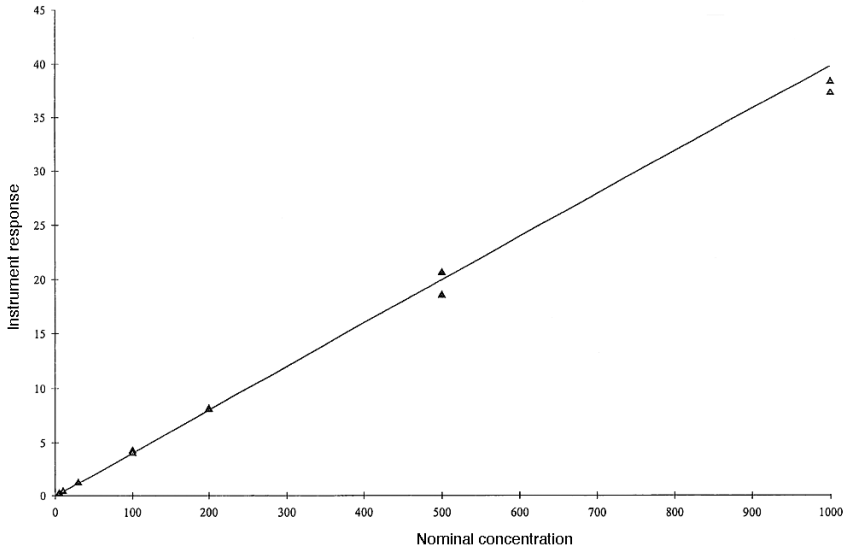

Figure 3. Representative curve for PFO corresponding to concentrations of 5.00 to 1000 ng/g in carpet extract. Linear regression equation with $1/x^2$ weighting: $y = 0.0402x + 0.0124$.

Specificity

Specificity was evaluated by extracting and analyzing two lots of carpet fortified with PFOA and internal standard (10 ng/g). Analyte values (11.477 ± 0.256 ng/g) were within the acceptable range.

Recovery, precision, and accuracy

The method recovery, precision, and accuracy were assessed by measuring the concentrations found in duplicate carpet samples fortified at four levels over three days (Table III). Interday recovery at all concentration levels ranged from 95.7% to 120%.

Interday precision, calculated using the coefficient of variation (CV), ranged from 3.7% to 14.1%. Interday accuracy, determined by the percent difference from the nominal concentration, ranged from 2.4% to 7.6% (Table IV).

Extraction efficiency

The efficiency of the liquid–liquid extraction process was determined comparing carpet extract solutions fortified with 5, 50, and 500 ng/g PFO and at 20 ng/g internal standard to reference samples spiked with an equivalent amount of analyte. The results for recovery of PFO ranged from 96.3% to 110.7% and recovery of internal standard ranged from 96.3% to 98.0%.

Conclusion

Reproducible good peak shape and good gradient retention in carpet extracts are readily obtained with this LC–MS–MS methodology without significant sample clean-up. Excellent sensitivity of this method at 5 ng/g is achieved by the use of a gradient separation and by drying and reconstituting samples in smaller volumes of solvent. Because PFO contamination can originate from work areas, solvents, labware, and HPLC components, low level determinations are particularly challenging as background PFO can exceed the LOQ of 5 ng/g.

The method was validated by extracting duplicate carpet samples fortified at the proposed LOQ (5 ng/g), at 3× the proposed LOQ (15 ng/g), at 10× the proposed LOQ (50 ng/g) and at 180× the proposed LOQ (900 ng/g) on three successive days. The efficiency of the extraction procedure was validated by comparing peak areas of five replicates at the LOQ (5 ng/g), at 10× the proposed LOQ (50 ng/g), and at 100× the proposed LOQ (500 ng/g) with extracts fortified at equivalent concentrations. Based on the data, accuracy, precision, repeata-

bility, recovery and specificity of the method were established. Because PFO was detected in untreated samples, interday precision, ranging from 3.7% to 14.1% and interday accuracy ranging from 2.4% to 7.6% are well within the acceptance criteria for the method. Overall extraction efficiency ranging from 92.4% to 110.7% with no clean-up is quite impressive at these low levels.

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