Method Validation of Two Methods Developed for the Low-Level Determination of Perfluorooctanoic Acid (PFOA) in Paper, Textile and Carpet by LC/MS/MS

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Introduction

Fluorotelomer-based acrylic polymers are applied to the surface of textiles, paper and carpet to impart oil, stain and water repellence properties [1, 2]. 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 methods to detect and measure PFOA in common articles of commerce, namely textile, paper and carpet.

Fluorotelomer-based polymers are not made using PFOA nor is PFOA added during the manufacture or use of telomer products. However, questions have arisen as to the possibility of trace level PFOA impurities in telomers and the potential for telomers to transform to PFOA[3].

The members of the Telomer Research Program (TRP) have committed to evaluating their products to determine whether they contribute to significant human or environmental exposure to PFOA. As part of this effort, the TRP maintained oversight of the development of two analytical methods to determine low levels of PFOA in textile and paper or in carpet. Both methods utilize a methanol extraction and a dual labeled ^{13}C-PFOA internal standard and use electrospray LC/MS/MS for quantitation. The paper and textile method uses standards prepared in equal parts water and methanol and chromatography is performed on a C8 column. The carpet method differs in that the preparation of the calibration standards is accomplished by adding standards directly to untreated carpet samples and extracting, due to the complexity of the carpet matrix. The chromatography is performed on a Synergi™ Polar-RP – (an ether-linked phenyl with polar end-capping) column.

Materials and Methods

Untreated paper was provided by Clariant GmbH. Untreated textile and carpet was provided by E. I. du Pont de Nemours and Company.

Pure analytical perfluorooctanoic acid (CAS # 335-67-1, Catalog # 001319, chemical purity 97%) was purchased from Oakwood Products, Inc (West Columbia, SC, USA) or supplied by Clariant GmbH (Burgkirchen, Germany) and the dual labeled internal standard, perfluorooctanoic acid [1,2-di-^{13}C] PFOA, (100% chemical purity, was obtained from DuPont Haskell Laboratory (Newark, DE, USA). Methanol, HPLC grade, and acetic acid were purchased from Fisher Scientific or EMD (Gibbstown, NJ, USA). High purity water was prepared using an NEU-ION system (Baltimore, MD, USA) or HPLC grade water was purchased from EMD (Gibbstown, NJ, USA). Ammonium acetate, reagent grade, was purchased from JT Baker (Phillipsburg, NJ, USA).

Paper and Textile Method:

Samples of paper or textile (~2 g) were weighed into 50-mL polypropylene centrifuge tubes. The samples were then

fortified, if needed. Six control samples were fortified at the proposed LOQ (5 ng g^{-1}), six at 10x the proposed LOQ (50 ng g^{-1}), and six at 40x the proposed LOQ (200 ng g^{-1}) on three successive days. Twenty mL of methanol was added to each tube, the tube was capped and was allowed to shake on a mechanical, wrist-action shaker for ~30 minutes. The sample was then centrifuged for five minutes at ~3000 rpm. Ten mL of methanol extract was transferred into a centrifuge tube. Fifty µL of a 1.0 mg mL^{-1} ^{13}C-PFOA internal standard solution was added. The samples were then evaporated with nitrogen to < 5.0 mL. The volume was adjusted to 5.0 mL with methanol and then brought to the 10.0 mL mark with water. The samples were then centrifuged at ~5000 rpm for 10 minutes before transfer to an autosampler vial for LC/MS/MS analysis.

Residues of PFOA in paper and textile were determined by injecting aliquots of sample or standard (5.0 µL) onto a 2.1 x 50 mm, 4 mm Genesis C8 column. The mobile phase was comprised of A: 2 mM ammonium acetate in water and B: methanol. The PFOA elutes under the isocratic conditions 40% A and 60% B. After 3.5 minutes the hypercarb cartridges and column are flushed using a gradient that increases the methanol to 100%. The LC/MS/MS was an Agilent 1100 bench top liquid chromatograph coupled to a PE Sciex API 4000 triple quadrupole mass spectrometric detector with a Turbo Ion Spray Liquid Introduction Interface. MS/MS detection was performed in the turbospray negative ionization mode. Ions monitored were 413 (parent) and 369 (daughter) for PFOA and 415 (parent) and 370 (daughter) for dual labeled ^{13}C-PFOA internal standard. Two Thermo Hypersil-Keystone Hypercarb™ cartridges (4 mm) were placed before the HPLC injector to capture any PFOA introduced from the mobile phase and instrument components. Any captured PFOA was removed from pre-columns before the injector by flushing the system with 100% methanol prior to equilibration with the isocratic mobile phase.

Carpet Method:

Approximately ten grams of carpet samples with backing were weighed into glass beakers. Duplicate untreated samples were fortified at the proposed LOQ (5 ng g^{-1}), at 3x the proposed LOQ (15 ng g^{-1}), at 30x the proposed LOQ (150 ng g^{-1}) and at 180x the proposed LOQ (900 ng g^{-1}) on three successive days. One mL of internal standard (20 ng g^{-1}) was added to each sample. The sample was extracted with 200 mL of methanol, the beaker was covered and was allowed to shake on an Eberbach reciprocal shaker for ~15 minutes then ultrasonicated for ~30 minutes. Five mL of the methanol extract were removed and evaporated to dryness under nitrogen The residue was reconstituted with 2.0 mL methanol:water (50:50, v:v). The samples were then centrifuged before LC/MS/MS analysis. Calibration standards ranging from 5.0 to 1,000 ng g^{-1} were prepared by spiking untreated carpet samples (10 g) with standard solutions and extracting as described above.

Levels of PFOA in carpet were determined using a reversed-phase gradient separation. Aliquots of the sample or standard (3-mL) of were injected onto a Phenomenex Synergi Polar RP, 2 mm i.d.´ 50 mm, 4 mm, 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:

Time (min.)	% A	% B	Flow (mL min^{-1})
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

The column was coupled to an Applied Biosystems triple quadrupole mass spectrometric detector with a Turbo Ion Spray Liquid Introduction Interface. MS/MS detection was performed in the turbospray negative ionization mode. Ions monitored in MRM mode included; 413 (parent) to 369 (daughter) for PFOA and 415 (parent) to 370 (daughter) for dual labeled ^{13}C-PFOA internal standard.

Results and Discussion

Chromatographic results:

PFOA evaluated with the method for paper and textile eluted at a retention time of ~2.4 minutes. The lowest standard concentration injected was 0.05 ng mL^{-1}.

PFOA evaluated with the method for carpet eluted at a retention time of ~3.0 minutes. The lowest standard concentration evaluated in carpet extract was 5.00 ng mL^{-1}.

The linearity of PFOA for both methods and for each analysis set was determined using standard curves obtained from the peak area ratio between the native analyte and its dual labeled analog (internal standard) at a minimum of seven standard concentrations. The concentration range of the standards for the paper and textile method was 0.05 to 50 ng mL^{-1} (with a concentration of 5 ng mL^{-1} of the internal standard). The concentration range of the standards for the carpet method was 5.00 to 1000 ng g^{-1} (with concentration of 1 ng g^{-1} of internal standard). Correlation coefficients of r^2[3] 0.992 were readily achievable for both methods.

Validation data:

The recovery data from the fortified paper, textile and carpet samples clearly demonstrate the accuracy and precision of both methods. The overall accuracy and precision was determined by the recovery ± standard deviation. The recovery criteria for validation were defined as recovery of 70 to 120%. The intraday and interday data for paper and textile are presented in Tables 1 and 2. The intraday and interday data for carpet are presented in Table 3.

Table 1 PFOA Recoveries in Textile Over Three Days

Sample Description	Amount Fortified (ng g^{-1})[a]	Average Recovery (%)[a]	Intraday SD [b]	Interday Average Recovery (%)	Interday SD [b]
Day 1	~5	118	2.3		
Day 2	~5	119	0.8	118	2.5
Day 3	~5	118	3.7		
Day 1	~50	114	5.0		
Day 2	~50	115	4.6	114	4.0
Day 3	~50	112	1.5		
Day 1	~200	110	3.3		
Day 2	~200	110	3.2	110	3.9
Day 3	~200	109	5.9		

[a] n = 6 for each day at each fortification level

[b] SD stands for standard deviation

Table 2 PFOA Recoveries in Paper Over Three Days

Sample Description	Amount Fortified (ng g^{-1})[a]	Average Recovery (%)[a]	Intraday SD [b]	Interday Average Recovery (%)	Interday SD [b]
Day 1	~5	122	2.6		
Day 2	~5	120	5.8	119	5.1
Day 3	~5	115	3.3		
Day 1	~50	110	1.4		

Day 2	~50	106	2.1	109	2.8
Day 3	~50	111	2.3		
Day 1	~200	102	1.2		
Day 2	~200	99	3.1	103	3.7
Day 3	~200	107	2.5		

[a]n = 6 for each day at each fortification level; n = 7 on day 3, 200 ng g-1 fortification level.

[b] SD stands for standard deviation

Table 3 PFOA Recoveries in Carpet Over Three Days

Sample Description	Amount Fortified (ng g^{-1})[a]	Average Recovery (%)[a]	Intraday SD [b]	Interday Average Recovery (%)	Interday SD [b]
Day 1	5.00	97.8	0.34		
Day 2	5.00	106	0.19	108	0.76
Day 3	5.00	120	1.2		
Day 1	15.0	101	1.7		
Day 2	15.0	95.7	0.21	104	1.5
Day 3	15.0	115	0.14		
Day 1	150	105	1.4		
Day 2	150	109	2.8	104	6.7
Day 3	150	99.0	2.1		
Day 1	900	101	35		
Day 2	900	104	40	102	34
Day 3	900	102	50		

[a]n = 2 for each day at each fortification level.

[b] SD stands for standard deviation

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