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DATE: 16 January 2003

Analytical Report

TO: George Senkler
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Determination of Perfluorooctanoic Acid from the Surface of Commercial Frying Pans

Abstract

A method was developed to determine if perfluorooctanoic acid (PFOA), a fluoropolymer polymerization aid, might be present in and extracted from the surface of commercial frying pans coated with a fluoropolymer. A modified US FDA method was used for extraction and an LC/MS/MS method used for identification and quantitative analysis. No PFOA was found in the water extract from the fluoropolymer surface to the limit of quantitation in water, 50 ng/L (ppt), from two 500-mL portions of water.

Request

A request was made to determine if any quantifiable PFOA might be found in extract water from a fluoropolymer surface. A US Food and Drug Administration (FDA) method was modified to extract PFOA, if it were present, on the surface of the frying pan.

Fluoropolymer dispersions are used in the commercial processes for coating metal. These products frequently use a fluoropolymer polymerization aid, PFOA, during their manufacture. Recent studies[1] have suggested that some

fluoropolymer polymerization aids might persist in the environment and might exhibit some toxicological effects in animals. Another report[2] indicated that low levels of these materials might be present in human blood serum. Since PFOA is used to suspend and emulsify some polymers during their manufacture, it is necessary to determine if PFOA is still present after the coating and baking processes and thus might be a vehicle for human exposure.

Experimental Section

Ten frying pans, five 8-inch Farberware® Elite Cusine uncoated stainless steel and five 8-inch Anolon® Classic hard-anodizied aluminum coated with a blend of polytetrafluoroethylene (PTFE) and PFA (branded as DuPont Autograph®) were purchased from a local bed and bath specialty store. An FDA procedure[3] was modified as noted. The pans were first washed as recommended by the manufacturer with approximately 50 mL of 0.5 % Palmolive detergent solution and then rinsed with three 50 mL of portions of rinse (deioinzed) water and dried with a Sonora® towel. A custom-made glass lid (9" diameter x 0.125" thickness) with a #19 female ground glass joint attached in the center of the glass (see Figure 1) and a condenser were washed with the detergent solution, rinsed with deionized water and dried. The pan was placed on a Vulcan model 38L gas stove and deionized water was added to approximately 0.635 cm (0.25 inches) from the top. The lid and condenser were placed on the pan, and two 201-g weights were added to the lid to ensure a good seal. The condenser was cooled with wet ice. The frying pan with water and condenser was heated until the water refluxed continuously inside the condenser (Figure 2). The heat was turned down and reflux was allowed to continue for 30 minutes. Then the apparatus was removed from the burner and allowed to cool.

The cooled water was then placed in each of two 500-mL wide-mouth polypropylene bottles. The lid and condenser were rinsed with deionized water, and the pan was rinsed with three 50-mL portions of deionized water. All rinse water was added to each of the two polypropylene bottles.

The procedure was repeated for the remainder of the frying pans. When testing had completed, the polypropylene bottles were placed inside a plastic liner within an insulated cooler. Ice was placed on top of the bottles and the liner folded and taped to prevent leakage. The cooler was then sealed with chain-of-custody tape and Scotch 8981 tape. The cooler was sent to Exygen Research (State College, PA) via Airborne Express for overnight delivery. All samples were received on ice at Exygen Research receiving, examined, logged in, and stored in locked storage until transfer to the Exygen Research analyst.

Samples were treated according to Exygen Research method 01M-008-046 entitled "Method of Analysis for Determination of Ammonium Perfluorooctanoate

(APFO) in Water" issued on January 11, 2002. (See Appendix 1.) Using this method, water samples were treated with 200µL of sodium thiosulfate solution, solid-phase extracted with a C-18 reverse-phase cartridge, eluted with 40% methanol in water, then eluted with 100% methanol, and the latter portion collected for analysis by LC/MS/MS. The treatment results in an eight-fold concentration prior to analysis.

Results and Discussion

All water from the fluoropolymer-coated frying pans showed no quantifiable PFOA present. With a limit of quantitation (LOQ) of 50 ppt and a limit of detection (LOD) of approximately 10 ppt per aliquot, this number corresponds to 100 ng/420.42 cm^2 LOQ or 20 ng/420.42 cm^2 LOD.

One frying pan with no coating (just a stainless steel surface) gave a positive result (Table I) in one instance and a nondetect (with an approximate 10 ppt limit of detection, 1 L water) in another instance. Since PFOA is easily adsorbed onto analytical apparatus, especially fluoropolymeric parts, background levels can be problematic. PFOA can be found in analytical solvents as well as transfer lines in chromatographic systems. The background levels are usually around 10 ppt. The US EPA Guideline[4] recommends that any measurements obtained that are less than five times the blank level should be reported as undetected (U) because the quantity reported cannot reliably be discriminated between the sample and the background. Due to background levels and the possibility for carry over or laboratory cross-contamination, the limit of quantification was set at 50 ppt. We observed that the background level, carry over, or laboratory contamination sometimes exceeds the 50-ppt level.

References

1. Guide to the Safe Handling of Fluoropolymer Dispersions, The Society of the Plastics Industry, Inc., 2001, p. 7.
2. K. J. Hansen, L. A. Clemen, M. E. Ellefson, and H. O. Johnson, *Environmental Science and Technology,* **35**, 766-770 (2001).
3. US FDA Procedure, see reference 21CFR175.300(d) table2 condition of use B.
4. "Data Validation Standard Operating Procedure for Contract Laboratory Program Routine Analytical Services", US EPA Region IV, Athens, Georgia, Revision 2.1, July 1997.

Notebook Numbers

E104373-1 to -15.

Control Terms to be included

PFOA, APFO, frying pans, extraction, LC/MS/MS, US FDA

Table I Analytical Results

Sample ID	Pan ID	PFOA (ng/L)	Peak area original	Peak area lab replicate
376603-1	SS#1, 1st aliquot	nd	792	626
376603-2	SS#1, 2nd aliquot	nd	714	748
376606-1	SS#2, 1st aliquot	36.9	5394	5044
376602-1	SS#2, 2nd aliquot	nd	989	1119
376609-1	SS#3, 1st aliquot	49.0	6802	6858
376609-2	SS#3, 2nd aliquot	nd	600	596
376612-1	SS#4, 1st aliquot	nd	478	435
376612-2	SS#4, 2nd aliquot	nd	513	478
376615-1	SS#5, 1st aliquot	nd	415	364
376615-2	SS#5, 2nd aliquot	nd	439	555
376616	DI water control	nd	233	388
610030	SS #1, 1st aliquot	nd	1300	1379
610035	SS #1, 2nd aliquot	nd	873	1074
610060	Coated #1, 1st aliquot	nd	1284	1353
610065	Coated #1, 2nd aliquot	nd	998	979
610090	Coated #2, 1st aliquot	nd	1104	1336
610095	Coated #2, 2nd aliquot	nd	806	850
610120	Coated #3, 1st aliquot	nd	1039	1036
610125	Coated #3, 2nd aliquot	nd	811	668
610150	SS #2, 1st aliquot	nd	567	544
610156	SS #2, 2nd aliquot	nd	448	702
611030	SS #3, 1st aliquot	223	34861	35556
611035	SS #3, 2nd aliquot	140	22594	20522
611060	Coated #4, 1st aliquot	nd	309	321
615030	SS #4, 1st aliquot	nd	3656	3964
615035	SS #4, 2nd aliquot	nd	2594	2975
615060	Coated #5, 1st aliquot	nd	3966	3524
615065	Coated #5, 2nd aliquot	nd	2538	2998
615090	SS #5, 1st aliquot	nd	2552	2022
615095	SS #5, 2nd aliquot	nd	2610	2360
615120	Coated #6, 1st aliquot	nd	4292	4509
615125	Coated #6, 2nd aliquot	nd	3317	3649
615150	SS #1, 1st aliquot	nd	2559	3148
615155	SS #2, 2nd aliquot	nd	4366	2900
615160	DI water control	nd	2184	2052
611160	DI water control	nd	1858	775
	LOQ = 50 ppt = 50ng/L	nd = not detected		
	LOD = c 10ppt			

Figure 1 Glass Lid for Frying Pan

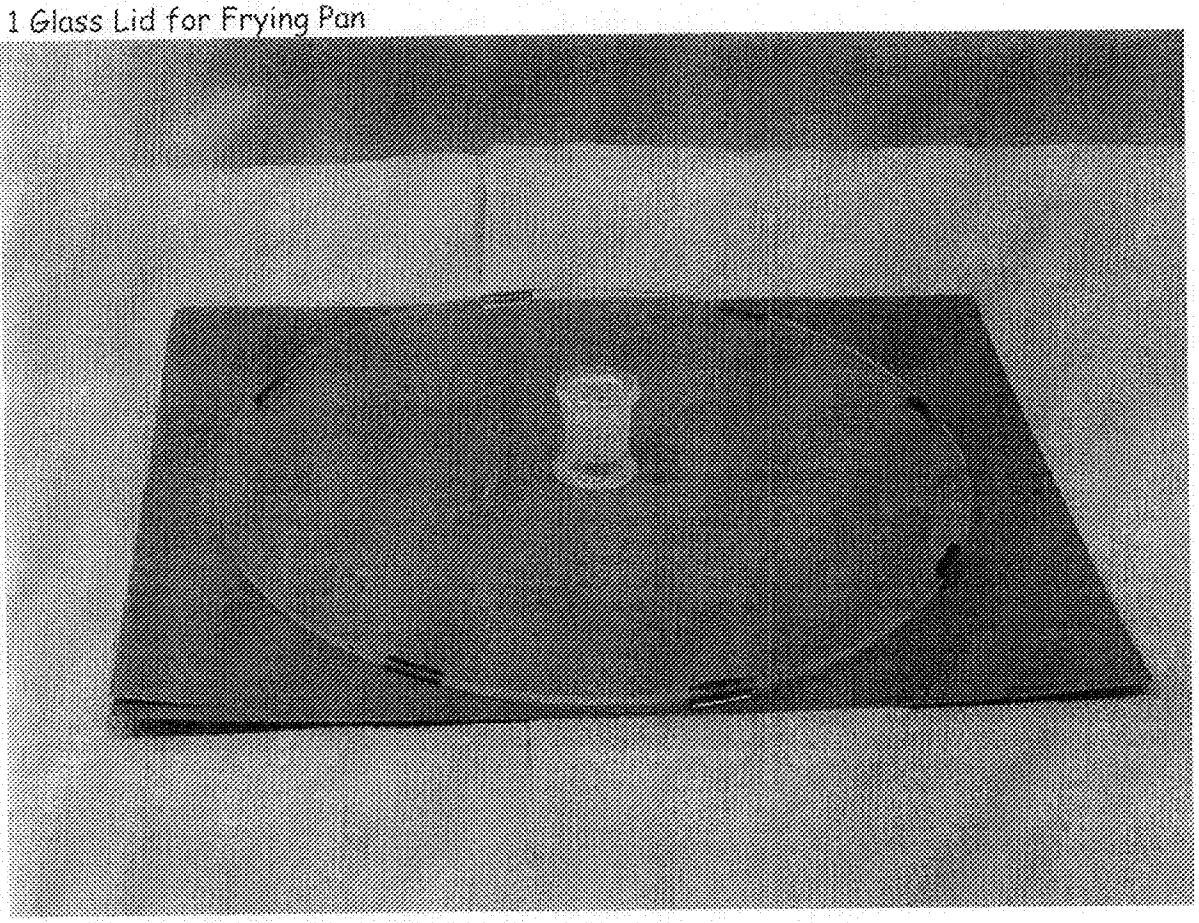
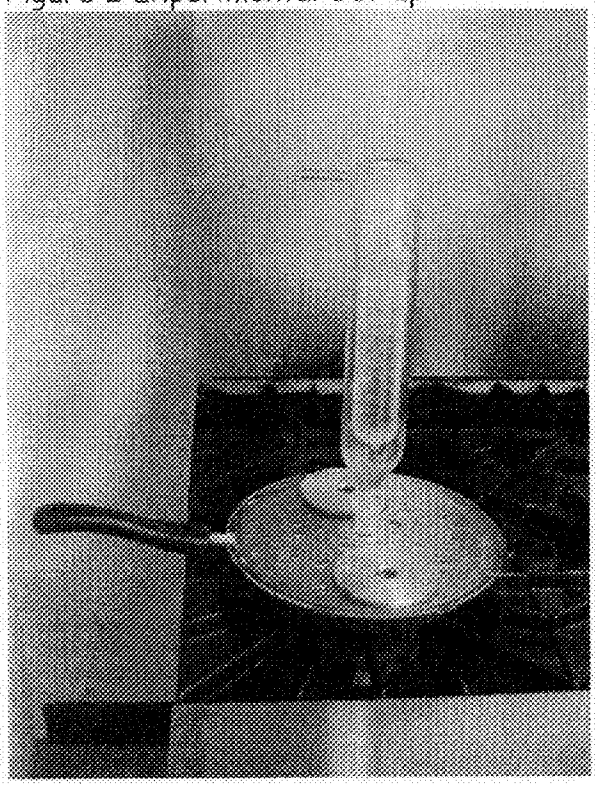


Figure 2 Experimental Set-up



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Appendix 1, Exygen APFO Method

TITLE

Method of Analysis for the Determination of Ammonium Perfluorooctanoate (APFO) in Water

AUTHORS

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DATE ISSUED

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METHOD NUMBER

01M-008-046

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TABLE OF CONTENTS

1. SUMMARY

This report details a method of analysis for residues of ammonium perfluorooctanoate (APFO) in water.

APFO forms perfluorooctanoic acid (PFOA) in solution. PFOA is extracted from water using C_{18} solid phase extraction (SPE) cartridges. Quantification of PFOA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM). Residues of PFOA found are mathematically converted and reported as APFO. The chemical formula of APFO is given in Section 2 of this method.

The proposed limit of quantitation (LOQ; the lowest fortification specified by the method which gives adequate recovery according to EPA guidelines) for this method is 50 ng/L (parts-per-trillion) for APFO.

Quantification is performed using calibration standards prepared in filtered type I water and processed through the extraction procedure, identical to samples. During the extraction procedure, all samples (including standards, controls, fortifications etc.) are concentrated by a factor of eight; therefore, the smallest standard (25 ng/L) injected during the chromatographic run is equivalent to 200 ng/L (concentrated 8x). The method detection limit (MDL) is approximately 6 to 15 ng/L depending on the instrument used for analysis. This value is based on procedures found in 40 CFR Part 136 Appendix B.

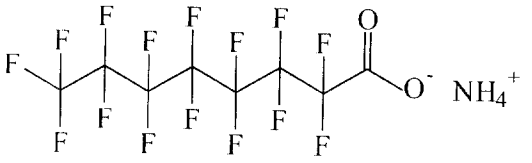
2. Experimental compounds

The structure of ammonium perfluorooctanoate is given below.

$CF_3(CF_2)_6COO^- NH_4^+$

APFO

Chemical Name = Ammonium Perfluorooctanoate
Molecular weight = 431



Note: The PFOA (anion) (formula weight 413) is present in solution and is used for the assay.

3. CHEMICALS AND SUPPLIES

3.1. Chemicals

Chemical	Grade	Source	Catalog No.
Methanol (MeOH)	HPLC	JT Baker	JT9093-2
Ammonium Acetate	Reagent	Sigma-Aldrich	A-7330
Water	Type I	in house	-
Sodium Thiosulfate	Reagent	JT Baker	-

Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25°C, from a Labconco Waterpro™ workstation.

3.2. Standards

Standard	Lot Number	Purity (%)	Source
Pentadecafluorooctanoic Acid	08316DO	96	Aldrich Chem
Perfluorooctanoic Acid	U14D	96	Fluorochem USA

3. Equipment and Supplies

Equipment	Supplier
Balance, analytical (display at least 0.0001 g)	Mettler
Vacuum pump	Buchi
Visiprep vacuum manifold	Supelco
Sep Pak Vac 6 cc (1g) tC18 cartridges (part # WAT 036795)	Waters
50 mL disposable polypropylene centrifuge tubes	VWR
15 mL disposable polypropylene centrifuge tubes	VWR
Disposable glass micropipets (50-100 µL, 100-200 µL)	Drummond (VWR)
25 mm diameter glass fibre acrodisc 0.45 µ (cat	Gelman

#4523)	
Class A pipets and volumetric flasks	various suppliers
Hypercarb drop-in guard column (4 mm) (part # 844017-400)	Keystone
Stand-alone drop-in guard cartridge holder	Keystone
125-mL LDPE narrow-mouth bottles	Nalgene
HPLC pump (LC10AD)	Shimadzu
2 mL clear HPLC vial kit (cat # 5181-3400)	HP
Standard lab equipment (graduated cylinders, disposable tubes etc.)	various suppliers
LC/MS/MS and HPLC systems	As described in section 4.5.

Note: Equivalent materials may be substituted for those specified in this method if they can be shown to produce satisfactory results.

Notes:

1. In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipets, etc.).
2. PTFE or PTFE lined containers or equipment, including PTFE-lined HPLC vials for the HPLC autosampler must not be used.
3. Type I water used during the sample and standard extraction should be filtered through a Hypercarb guard column using a HPLC pump, as described in section 4.2. This water is referred to as "filtered type I water", here after in this report.
4. It is necessary to check the solvents (methanol) for the presence of contaminants by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
5. Use disposable micropipets or pipets to aliquot standard solutions to make calibration standards and sample fortifications.

3.4. Solutions

(1) 50 mM ammonium acetate solution is prepared by weighing 3.86 g of ammonium acetate and dissolving in 1 L of type I water. Dilute the 50 mM solution by a factor of 25 to make the 2 mM ammonium acetate solution used for mobile phase A.

(2) 40% methanol is made by measuring 400 mL methanol and adjusting the volume to 1 L with filtered type I water.

(3) 250 mg/mL sodium thiosulfate solution is made by dissolving 25 g in 100 mL filtered type I water.

Note: The aforementioned examples are provided for guidance, alternative volumes may be prepared as long as the ratios of the solvent to solute ratios are maintained.

3.5. Preparation of Standards and Fortification Solutions

Analytical standards are used for three purposes:

1. Calibration Standards - These standards are prepared in filtered Type I water and are used to calibrate the response of the detector used in the analysis.
2. Laboratory Control Spikes - These fortifications are prepared at concentrations corresponding to the LOQ and 10x LOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in filtered Type I water.
3. Matrix Spikes - These fortifications are prepared by spiking into the field samples at concentrations ranging from 500 ng/L up to 500,000 ng/L. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

3.5.1. Stock solution

Prepare a stock solution of 100 µg/mL of PFOA by weighing out 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 100-mL volumetric flask. The stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 6 months from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a fortification standard of 1.0 µg/mL (1000 ng/mL) of PFOA by adding 1.0 mL of the 100 µg/mL stock solution to a 100-mL volumetric flask and bring up to volume with methanol.
b. Prepare a fortification standard of 0.1 µg/mL (100 ng/mL) by diluting 10.0 mL of the 1.0 µg/mL solution to 100 mL with methanol in a volumetric flask.
c. Prepare a fortification standard of 0.01 µg/mL (10 ng/mL) by diluting 10.0 mL of the 0.1 µg/mL solution to 100 mL with methanol in a volumetric flask.

Example: two hundred microliters of the 10 ng/mL solution spiked into 40 mL of water is equivalent to a 0.05 ppb (50 ng/L) fortification.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of 3 months from the date of preparation, after which time it is necessary to make new standards using the stock solution. Note also that additional concentrations may be prepared if necessary.

3.5.3. Calibration Standards

LC/MS/MS calibration standards are prepared in type I water filtered through a hypercarb drop-in guard cartridge. The calibration standards are processed through the extraction procedure, identical to samples. The extracted standards may be used for a period of two weeks when stored refrigerated (at 2°C to 6°C).

The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ng/L)*	Calibration Standard ID (example)
0	0	40	0	Xcmmddyy-0
10	100	40	25	Xcmmddyy-1
10	200	40	50	Xcmmddyy-2
10	400	40	100	Xcmmddyy-3
100	100	40	250	Xcmmddyy-4
100	200	40	500	Xcmmddyy-5
100	400	40	1000	Xcmmddyy-6

* The extracted concentration of the calibration standard is equal to 8× its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

A zero standard solution (reagent blank) must be prepared with each set of standards extracted. This standard is used only to access the reagents used to prepare the standards and is not included as part of the calibration curve.

Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

3.5.4 Controlling Standard Bias

Prepare a separate stock solution of 100 µg/mL from a different PFOA neat standard from a different source by weighing out 10 mg of analytical standard (corrected for purity only) and dilute to 100 mL with methanol in a 100-mL volumetric flask. The stock solution (in 125-mL LDPE bottles) is to be stored in a refrigerator at 2°C to 6°C and is stable for a maximum period of 6 months from the date of preparation.

Prepare a fortification standard of 1.0 µg/mL (1000 ng/mL) of PFOA by adding 1.0 mL of the 100 µg/mL stock solution to a 100-mL volumetric flask and bring up to volume with methanol. From that solution, prepare a fortification standard of 0.1 µg/mL (100 ng/mL) by diluting 10.0 mL of the 1.0 µg/mL solution to 100 mL with methanol in a volumetric flask.

Prepare a 250 ng/L extracted calibration standard with each set of extracted standards. Add 100 µL of the 100 ng/mL fortification solution to 40 mL of hypercarb filtered water and process through the extraction procedure, identical to samples.

Note: This standard is referred to as the "check standard" from this point forward.

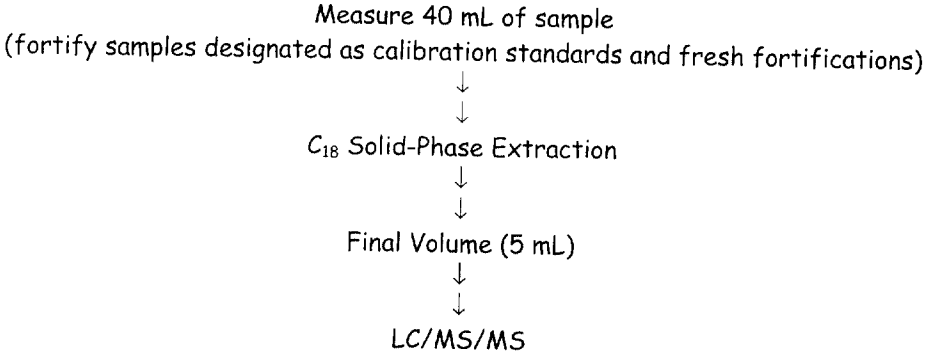
The check standard should be included at least once with each set of extracts analyzed. The check standard should give a response within ± 15% of the average response from the 250 ng/L extracted calibration standards in the set.

4. Method

4.1. Flow Diagram

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram



4.2. Sample Processing

No sample processing is needed for water samples. However, frozen samples must be allowed to completely thaw, un-aided, at room temperature. Samples stored refrigerated should also be allowed to equilibrate to room temperature. All samples must be thoroughly mixed and any visible solids removed by filtering the sample through a 25-mm diameter glass fibre ascrodisc 0.45 μ filter before being sampled for extraction.

Initial studies show that the method is not effected by the pH of the sample. Sample pH is recorded prior to extraction.

Control type I water, used for the preparation of samples and calibration standards as well as water used for equilibrating and washing the SPE cartridges, must be prepared by filtering type I water through a hypercarb guard cartridge using a HPLC pump. Before use, wash the guard cartridge with ~ 25 ml HPLC grade acetonitrile, then ~25 mL of HPLC grade methanol, followed by ~ 25 mL of type I water using the HPLC pump at ~ 2 mL/min flow rate. Then start collecting the filtered type I water eluate from the cartridge. It is recommended that the cartridge be washed, as described above, after filtering ~ 2 liters of type I water. A flow rate of 2 to 3 mL/min is recommended.

4.3. Sample Preparation

a. Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using filtered type I water) and two reagent controls fortified at known concentrations to verify procedural recovery for the batch.

b. Each sample must be extracted in replicate unless the sample is marked as a field blank. Field blanks do not need to be extracted in replicate.

c. Each sample extracted (except for field blanks) must also be separately fortified at a known concentration and carried through the procedure to verify recovery. Matrix spikes should range from 500 ng/L to 500,000 ng/L. If the sample residue is found to be significantly higher than the level at which the sample was initially fortified, the sample should be fortified at a higher concentration (up to 500,000 ng/L) and re-extracted.

4.4. Extraction

1. Measure 40 mL of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
   *Note: For **tap water samples** and any water samples suspected to contain chlorine, add 200 µL of a 250 mg/mL sodium thiosulfate solution to the 40 mL sample and mix well to de-activate chlorine, before fortification.*
2. Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL filtered type I water (~ 2 drop/sec). Do not let column run dry.
   Note: For the following steps, maintain a ~1 drop/ sec flow rate. Do not allow the column to run dry at any time.
3. Load sample on conditioned C_{18} SPE cartridge. Discard eluate.
4. Wash with ~5 mL 40% methanol in water. Discard eluate.
5. Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
6. Analyze samples using electrospray LC/MS/MS.

NOTE: SAMPLES ARE CONCENTRATED BY A FACTOR OF EIGHT DURING THE EXTRACTION; INITIAL VOL = 40 ML → FINAL VOL. = 5 ML.

4.5. Quantitation

4.5.1. LC/MS/MS System and Operating Conditions (Electrospray)

Mass Spec:	Micromass Quattro Ultima (Micromass)
Interface:	Electrospray (Micromass)
	Harvard infusion pump (Harvard Instruments), for tuning
Computer:	COMPAQ Professional Workstation AP200
Software:	Windows NT, MassLynx 3.3
HPLC:	Hewlett Packard (HP) Series 1100
	HP Quat Pump
	HP Vacuum Degasser
	HP Autosampler
	HP Column Oven

Note: A 4 × 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temperature: 35° C

Injection Volume: 15 µL
Mobile Phase (A): 2 mM Ammonium Acetate in Type I water
Mobile Phase (B): Methanol

Time	% A	% B	Flow Rate (mL/min)
0.0	60	40	0.3
0.4	60	40	0.3
1.0	10	90	0.3
7.0	10	90	0.3
7.5	0	100	0.3
9.0	0	100	0.4
9.5	60	40	0.4
13.5	60	40	0.4
14.0	60	40	0.3
15.0	60	40	0.3

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 x 30) and also columns from different manufacturers (Keystone Betasil C_{18} etc.) could be used, provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time
PFOA	Negative	413 → 369	~5 min.

The retention times may vary, on a day to day basis, depending on the batch of mobile phase etc. Drift in retention times (up to ±2 %) is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are included at the beginning and end of the analytical run.

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

4.5.2. Example MS Operating Parameters

The following MS operating values are provided as an example only. Actual values will most likely vary from instrument to instrument. These values may also change over time even when analyzing samples on the same instrument. The values are changed to optimize for greatest sensitivity. For example, hexapole 1 voltage may be set to 2 V; however, as time goes on, hexapole 1 voltage may be increased or decreased. The exact values used each time a set is analyzed will be documented with each data set.

The mass spectrometer is tuned for the analyte by infusing a ~ 0.2 µg/mL standard solution of PFOA (at 10 µL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 40% methanol and 60% 2mM ammonium acetate at 0.2 mL/min flow rate. The analyte is initially tuned for the parent ion and then tuned for the product ion. Once the

instrument is tuned, the optimized parameters are saved as a tune file. This tune file is then used during routine analysis.

Analyte	Dwell (s)	Collision Energy (eV)	Cone (V)
PFOA	0.2	10	10

Source	Set
Capillary	3.0 kV
Hexapole 1	0 V
Aperture 1	0 V
Hexapole 2	0 V
Source Block Temp.	100°C
Desolvation Temp.	300°C

Analyzer	Set
LM Res 1	14.0 V
HM Res 1	14.0 V
IEnergy 1	1 V
Entrance	-2 V
Exit	2 V
LM Res 2	14.0 V
HM Res 2	14.0 V
IEnergy 2	2.0 V
Multiplier	700 V

Gas Flows	Set
Cone Gas	~ 130 L/hr
Desolvation	~ 750 L/hr

Pressures	Read back
Gas Cell	~ 3.0e-3 mbar

4.5.3. Calibration Curve Procedures

a. Inject the same aliquot (between 10 to 25 µL) of each extracted calibration standard processed in matrix (ranging from the lowest level standard to the highest level prepared), into the LC/MS/MS (the final concentrations of these calibration standards will be equivalent to 8× the concentration of the initial standard due to the concentration during SPE).

b. Use linear standard curves for quantitation. The zero calibration standard is not included in the calculation of the calibration curve. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system. Any calibration standard found to be a statistical outlier by using the Huge Error Test,

may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that may be excluded must not exceed 20% of the total number of extracted standards injected.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

4.5. Sample Analysis

a. Inject the same aliquot (between 10 to 25 µL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.
b. Standards corresponding to at least five or more concentration levels (starting with the LOQ level or below) must be included in an analytical set (note that the calibration standards are prepared by spiking filtered type I water and processing similar to actual samples).
c. An entire set of extracted calibration standards should be injected at the beginning of a set followed by extracted calibration standards interspersed approximately every 5-10 samples (to account for a second set of extracted standards). As an alternative, an entire set of extracted calibration standards may be included at the beginning and at the end of a sample set. In either case, extracted calibration standards must be the first and last injection in a sample set.
d. Inject the check standard once with each set of extracts.
e. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. If necessary, dilute the samples to give a response within the standard curve range.
f. Fortification recoveries falling within 70 to 130% are considered acceptable.
g. The total holding time between sample collection and analysis must not exceed 14 days. Extracted samples must be stored refrigerated between 2°C to 6°C until analysis.
h. Field samples in which either no peaks or peaks less than the MDL are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the MDL will be reported as NQ (not quantifiable).

The analysis performed during the method development included fortifications at 100 and 500 ng/L of PFOA in well water, tap water, and stream water (collected locally, State College, PA) and bottled drinking water (from a local grocery store).

4.6. Acceptance Criteria

The following criteria must be met to ensure the presence of PFOA:

1. Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide. This transition would not discriminate between linear and branched forms of PFOA; therefore, both, if present, would be included in the calculation.

2. Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.
3. Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
4. Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.
5. The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
6. The response from the check standard should be within ± 15% of the average response from the 250 ng/L calibration standard concentrations. If the standard response falls outside these limits, appropriate steps must be taken to adjust instrument operation and the set of samples should be reanalyzed. If the standard response is still outside the ± 15% then standard preparations should be reviewed, and if warranted, new stock, fortification and extracted calibration solutions should be prepared.
7. Retention times between standards and samples must not drift more than ± 2 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

4.7. Mass Calibration Criteria

In order to verify mass resolution, a calibration check is performed weekly on the mass spectrometer using a NaI/Rb/Cs tuning solution. The tuning solution is infused onto the mass spec using an infusion pump set to a flow rate of 10 µL/min. An MS scan from 80 to 1380 amu is performed on both MS1 and MS2. The calibration is considered acceptable if the following 11 peaks are found within ± 0.2 amu of their known masses on both MS1 and MS2: 84.9, 132.9, 172.8, 322.7, 472.6, 622.5, 772.4, 922.3, 1072.2, 1222.1 and 1372.0. If any of the masses are outside the ± 0.2 amu, then the instrument will be recalibrated and another calibration check performed.

4.8. Performance Criteria

The following two criteria must be performed as a system suitability test, before the commencement of analysis when using an instrumentation set-up that has not been used for this method.

First Criterion:

Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (50 ng/L) in matrix and obtain a signal to noise ratio of the 413 → 369 transition of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters (or increase the injection volume, if appropriate).

Second Criterion:

Run a set of standards of five or more concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. Generate a calibration curve for PFOA and obtain a linear regression with a coefficient of determination (R^2) of at least 0.985 for PFOA. Once this criterion is met, samples may be analyzed with standards interspersed.

4.9. Method Detection Limit Determination

Before samples can be analyzed on an instrument, a Method Detection Limit (MDL) determination should be performed. The procedure followed can be found in 40 CFR Part 136 Appendix B. The MDL should then be performed on each instrument used for analysis on a quarterly basis.

4.10. Time Required for Analysis

A set of 24 samples (6 standards and 18 samples) can be taken through the extraction procedure in approximately 2-3 hours by one person. The LC/MS/MS analysis (standards and 18 samples) will take approximately 7 hours.

5. Calculations

a. Use Equation 1 to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program.

Equation 1:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

b. For samples fortified with known amounts of PFOA prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

Recovery (%) ={(total analyte found - analyte found in control)/analyte added }x 100

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

Note: Subtract analyte found in control (ng/L) from analyte found (ng/L), if applicable.

b. Use Equation 3 to calculate the amount of APFO found (in ng/L) using the molecular weight ratio of APFO/PFOA:

Equation 3:

$$\text{APFO found (ng/L)} = \text{PFOA Found} \times \frac{\text{MW APFO (431)}}{\text{MW PFOA (414)}}$$

Note: calculations could also be performed in ppb or ng/mL if concentrations were assigned in ng/mL instead of ng/L when performing the analysis.

For reporting purposes, field samples in which either no peaks or peaks less than the MDL are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the MDL will be reported as NQ (not quantifiable).

6. *Safety*

The analyst should read the material safety data sheets for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves.

7. *REFERENCES*

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