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Validation of Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) For Analysis of Perfluorooctanoic Acid (PFOA) In Samples From Chambers Works Wastewater Treatment Facility

Final Report

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DRAFT

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Abstract : A liquid chromatography tandem mass spectrometry analytical technique was used for analysis of perflurooctanoic acid (PFOA) in groundwater and surface waters. This report describes the results of analyses of PFOA in distilled water, salt spiked water and primary clarifier effluent from the Chambers Works (CW) wastewater treatment system.

Keywords high performance liquid chromatography tandem mass spectrometry (LC/MS/MS), perfluorooctanoic acid (PFOA), Chambers Works Secure Environmental Treatment, C8, FC-143, biological treatment, powdered activated carbon treatment (PACT), activated carbon, primary clarification

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Introduction and Objectives

PFOA is a compound that is used for the production of fluoropolymers. PFOA containing wastewaters are discharged to the Chambers Works (CW) site wastewater treatment system, which is operated by Secure Environmental Treatment (SET). A liquid chromatography tandem mass spectrometry (LC/MS/MS) analytical technique was used for analysis of PFOA in groundwater and surface waters. This work evaluated the efficacy of this analytical technique for determining PFOA concentrations in distilled water, a synthetic wastewater matrix and a sample from the CW treatment system. In addition, a preliminarily methanol extraction of PFOA adsorbed to suspended solids was investigated in CW samples.

Summary and Conclusions

The method validation study demonstrates that the LC/MS/MS analytical technique can be used to measure PFOA in the complex wastewater matrix found in the CW SET system. Methanol shows promise as an extractant for PFOA that is adsorbed to suspended solids found in the primary clarification and biological treatment system at Chambers Works. The methanol extraction method will require further development.

Patent Situation

N/A

Program

The program is described elsewhere below.

Publication Status

N/A

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Safety and Waste Disposal

All analytical wastes will be returned to submitter

Environmental Considerations

N/A

Signatures

Signature(s) of the author(s) should appear here.

_____	_____
_____	_____
_____	_____

Experimental

Test Substance

Name:	Ammonium perfluorooctanoate
Synonyms:	Ammonium pentadecafluorooctanoate; Ammonium perfluorocaprylate; DS 101; Fluorad FC 143; Perflurorooctanoic acid ammonium salt; Unidyne DS 101
Active substance:	Octanoic acid, pentadecafluoro-, ammonium salt
CAS Name:	Octanoic acid, pentadecafluoro-, ammonium salt
CAS Number:	3825-26-1
Supplier:	Fluka Chemical Company 1001 West St. Paul Milwaukee, WI 53233 USA
Product Number:	77262
Lot Number:	421207/1 24501
Molecular Formula:	$C_8H_4F_{15}NO_2$
Formula Weight:	431.1 g/mole
Concentration of a.s., nominal:	≥ 98.0%
Certificate of Analysis Date:	24-Jan-01
Solubility:	0.1 g/ml
Appearance:	White Powder

Reagents and Solvents

All chemicals and solvents were of reagent grade or purer and were purchased from the following suppliers: Calcium chloride dihydrate (Mallinckrodt); Sodium sulfate (Baker); Sodium hydroxide (Ricca Chemical); Hydrochloric acid (Baker) Methanol used for extractions (Burdick & Jackson), Methanol HPLC grade (EM Science), and Ammonium Acetate ACS grade (EM Science).

Test Materials

Samples of primary clarifier underflow, primary clarifier effluent, secondary aeration mixed liquor suspended solid (MLSS) and tertiary MLSS samples, and virgin powdered activated carbon (PAC) were used in the study. The primary clarifier underflow sample from primary

clarifier CF-64 and the MLSS samples from Spot 627 (secondary treatment) and Spot 628 (tertiary treatment) were grab samples collected on May 27, 2003 and refrigerated until use. The Primary clarifier effluent (CF-64) was a 24-hr composite sample collected from 529 spot on May 29, 2003 and refrigerated until use. The virgin Powdered Activated Carbon (PAC Calgon WPX-D) was obtained from DuPont Secure Environmental Treatment (SET) and was stored at room temperature. All samples were collected in accordance with the principles explained in Appendix A.

Test solutions were prepared as follows:

- The salt solution was prepared by dissolving calcium chloride (1400mg/L) and sodium sulfate (1250 mg/L) in deionized water. Hydrochloric acid and/or sodium hydroxide were used to adjust the final pH of the solution to 7.0-7.8
- The primary clarifier effluent was centrifuged for 10 minutes at 6000 RPM (Sorvall RC-5 HS-4 Rotor) and supernatants filtered (Pall Gelman 4190) prior to use.
- A mixed MLSS sample was prepared by combining secondary and tertiary MLSS samples at a ratio of 3 parts secondary:1 part tertiary

Analytical

The total solids and total suspended solids of the primary underflow and MLSS samples were determined according to methods 2540B or 2540D, as appropriate and as outlined in Standard Methods for the Examination of Water and Wastewater, 20th Edition (1998), American Public Health Association, Washington, DC. PFOA was analyzed by LC/MS/MS in accordance with the methodology shown in Appendix B.

Experimental Protocol:

Stock solutions were prepared in deionized water and stored at room temperature. Solutions of the test compound were prepared from the stock solution in deionized water, salt solution, and primary clarifier effluent at test concentrations of 0, 10, 100 and 1000 ug/L. The samples were filtered, diluted (if required), and submitted for analysis.

The supernatant fractions of centrifuged primary solids and MLSS samples were filtered and analyzed for the test compound. The solid fraction was extracted by sonicating with methanol for 1 hour. Additional solid fractions were spiked with 100 and 500 ug/L of the test compound and immediately thereafter extracted by sonicating with methanol for 1 hour. Methanol extracts were centrifuged, filtered, diluted (if required) and submitted for analysis.

Discussion of Results

All analytical data are included in Appendix C. Method validation and extraction results are discussed separately below.

A. Method Validation: The candidate aqueous test matrices for method validation included deionized water solution, salt solution and primary clarifier effluent. Table 1 summarizes the results of the sample analyses.

Table I Results of Method Validation

Nominal PFOA (ug/L)	Deionized Water Samples PFOA (ug/L)	Salt Solution PFOA (ug/L)	Filtered Primary Effluent PFOA Measured/Expected (ug/L)
1,000	1,030	1,090	1,500 /1280
100	103	103	150 /198
10	15.2	13.6	88 /90.4
0	<LOQ	<LOQ	78 /0

The test matrices were chosen to evaluate method efficacy in increasingly complex matrices. Deionized water solution was used in order to determine analytical capabilities in a clean background matrix while the salt solution simulates the major ionic components of the CW SET treatment effluent. The primary clarifier effluent represents the most complex wastewater matrix, composed of inorganics and a variety of organic compounds.

The difference between the results for deionized water and salt solution are within the accuracy of the analytical measurement. The expected PFOA concentration in the filtered primary effluent differs from measured due to background concentrations and dilution. The results show that the salt has no impact on the analytical results and that PFOA is present in the primary clarifier system. Increasing the complexity of the test matrix does not appear to interfere with the ability of the analytical method to detect the compound with a reasonable degree of accuracy. For test expediency only single preparations were made for each sample represented in Table 1. Further experiments would define the statistical accuracy in this matrix.

B. Methanol Extraction of PFOA

All analytical data are included in Appendix C. The extraction testing used samples taken from the primary clarifer underflow (primary solids) and the secondary and tertiary mixed liquor suspended solids (MLSS). Secondary and tertiary mixed liquor samples were combined at a ratio of 3:1 (mixed MLSS) to simulate the ratio of tertiary to secondary solids in the CW system. A sample of PAC was also used.

Table II Methanol Extraction from Solids

	Total PFOA micrograms recovered				
Total PFOA micrograms Added	PAC	Primary Solids	Secondary MLSS	Tertiary MLSS	Mixed MLSS
25	0.34	50.6	24.9	25.3	25.0
5	0.04	25.1	8.2	7.4	8.6

0	<LOQ	6.6	2.7	1.9	5.2
Total Suspended Solids gm/L	N/A	140	11.0	8.1	11.9
Solid Test Weight gm	4.3	7.0	0.56	0.4	0.6

The results from the preliminary extraction experiments of the MLSS samples show recoveries of 68% and 110% of the test compound (Table II) with extraction solids weights ranging from 0.4-0.6 grams. The recovery for the primary solids, which were tested at a significantly higher weight (7 grams), ranged from 176% to 369%. Less than 1% was recovered from the PAC samples, which also were tested at high solids weight. These results indicate that extraction with high solid weight samples require additional extraction steps and/or higher extraction volumes. However, optimization of the extraction was beyond the scope of these investigations.

References

N/A

Appendices

Distribution for Company Progress Reports

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APPENDIX A:

SUGGESTED PROTOCOL FOR SAMPLING

The following is a suggested protocol for sample collection. Sample bottles should not be pre-rinsed in the field prior to sample collection. Gloves should be worn during sampling activities and replaced between samples. All samples should be held at a nominal 4°C (not frozen to 6°C) with wet ice from collection to shipping.

Sampling equipment may include stainless steel mixing bowls, trowels, and spoons. All of these items will come in direct contact with the sample and have potential to impact analytical results. Therefore, care should be taken to ensure the cleanliness of all sampling equipment. When possible, pre-cleaned or disposable sampling equipment should be used. Field decontamination may be permitted, provided the following method is applied:

- Wipe off any residual sludge or water with a Chem-wipe™.
- Rinse the equipment with deionized water.
- Rinse the equipment with methanol.
- Place in zip-sealed bag until next use

In addition to the decontamination procedures outlined above, the person collecting the sample should wear clean latex or nitrile disposable lab or exam gloves and should limit his/her contact with the samples. Sample bottles should not be cleaned in the field.

In order to minimize the possibility of introducing PFOA contamination into samples, the following protocol should be followed:

- Avoid polytetrafluoroethylene (PTFE).
- Avoid aluminum foil.
- Do not use self-stick memo notes.
- Avoid blue ice.
- Avoid pre-wrapped foods or snacks.
- Wear clothing that has been washed at least six times.
- Use only containers supplied by contract laboratory.

Samples should be transferred to collection bottles. Samples should be collected and shipped to the designated laboratory following standard chain-of custody procedures.

Appendix B

Liquid Chromatography and Liquid Chromatography Tandem Mass Spectrometry for Perfluorooctanoaic Acid (PFOA) Quantitation

For the rail car grab samples, Perfluorooctanoaic acid (PFOA) was quantitated by liquid chromatography (LC) at Parkersburg lab. PFOA was quantitated in all other samples by the CCAS lab at the Experimental Station using LC combined with tandem mass spectrometry (MS/MS). Both techniques are described below

I. Liquid Chromatography (LC):

Perfluorooctanoaic acid (PFOA) was quantitated by liquid chromatography (LC) at Parkersburg lab in accordance with the following:

Instrument	Agilent 1150
Detector	UV @ 210 nm (Diode Array)
Column	Zorbax XDB-C18 (Reverse Phase)
Mobile Phase	40/60 Acetonitrile/Water + 0.1% Perchloric Acid
Flow Rate	0.4 ml/min
Sample Size	1 - 100 microliters (concentration dependant)
Detection Limit	1 ppm

II. Liquid Chromatography (LC) combined with tandem mass spectrometry (MS/MS):

Perfluorooctanoaic acid (PFOA) was quantitated by liquid chromatography (LC) (Agilent 1100 Liquid Chromatograph) combined with tandem mass spectrometry (MS/MS) (Micromass Quatro Ultima). Negative ion electrospray was used to produce the molecular anion for the perfluorooctanoic acid, which was then subjected to collision-induced dissociation producing the decarboxylated anion. The MS/MS transition was specific to perfluorooctanoic acid and the area of the chromatogram was proportional to the concentration of PFOA.

The standard operating conditions for the method are provided below. Samples were submitted in polystyrene conical bottom tubes which were refrigerated prior to analysis. The samples were diluted (if required) with the appropriate diluent and transferred to polypropylene vials (Agilent 5182-0567) sealed with a natural rubber crimp seal (Agilent 5182-1210).

Appendix B

Samples were analysed by duplicate injections and quantified using a calibration curve generated the day of analysis. Sample concentrations were reported at or above the limit of quantitation (LOQ) for this study.

LC Conditions

Water Samples

The LC column (Waters Symmetry C_{18} 5 µm 2.1x50 mm part# 186000206) is equilibrated with 100% Solvent A (1% Methanol in 2mM Ammonium Acetate) and Solvent B (100% Methanol). The method utilizes a linear gradient with the following conditions:

Time (min)	Solvent B %	Flow (ml/min)
0	0	0.25
5	0	0.25
10	100	0.25
14	100	0.25
15	0	0.25
17	0	0.25

The flow is diverted to waste between 0-5 minutes and directed to the mass spectrometer between 5 to 18.9 minutes. The injection volume is 25 microliters.

Methanol Extracts

The LC column (Agilent Spherisorb ODS 5 µm 2.1x150 part #77916OD-572) is equilibrated with 80% Solvent A (1% Methanol in 2mM Ammonium Acetate) and 20% Solvent B (100% Methanol). The method utilizes a linear gradient with the following conditions:

Time (min)	Solvent B %	Flow (ml/min)
0	20	0.25
17	67	0.25

17.01	20	0.25
20	20	0.25

No flow is diverter is used for this method. The injection volume is 25 microliters.

MS Conditions

The mass spectrometer is optimized for the molecular anion at 413 m/z with the Capillary Voltage set at 2.6 kV, Cone Voltage set at 10V, the source block temperature is set at 125°C and desolvation temperature is set at 250°C. The collision cell is maintained at a pressure of argon 1.0 E-3 and nitrogen flow is maintained at 100 psi. The mass spectrometer monitors the transition 413 > 369.