Determination of Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonic Acid (PFOS) in Water Samples Using On-Line Sample Concentration, Reversed-Phase Liquid Chromatography, and Suppressed Conductivity Detection

INTRODUCTION

Perfluorinated acids (PFAs), including perfluorooctanoic acid (PFOA) and perfluorooctane-sulfonic acid (PFOS), are widely distributed environmental pollutants.[1] PFOS and PFOA are best known for their use in the production of Teflon and other stain-resistant materials.[2] The U.S. Environmental Protection Agency (EPA) as well as international and state public health regulators have recently focused considerable attention on PFOA and PFOS, which are used for a wide variety of industrial, commercial, and consumer applications, because they are persistent, and bio-accumulative depending on the length of the carbon chain. The medium and long chain PCAs are known to be toxic, and some are carcinogenic.

Many HPLC methods have been developed using MS detection for trace analysis of PFOA and PFOS, which offer both good sensitivity and peak identification.[3] However, these methods require larger capital investment, higher operation cost, and sometimes suffer matrix interferences. Conductivity detection detects ionic species, and in suppressed mode provides excellent selectivity and good sensitivity.[4] Moreover, both instrument and operation costs are inexpensive compared to MS detection. Therefore, it offers a reliable and economical approach for trace-level analysis and samples in complex matrices. Because both PFOA and PFOS have low pKa values and are fully charged anions under HPLC conditions, they can both be detected by this suppressed conductivity method.

This technical note describes a methodology to determine FPOA and PFOS in water samples using on-line sample concentration, reversed-phase HPLC separation, and suppressed conductivity detection. Instrument setup, sample concentration, chromatographic method, calibration, and limits of detection are detailed.

EQUIPMENT

Dionex ICS-3000 system equipped with:

DP dual gradient pump, P/N 061713

DC detector/chromatography module, P/N 061767

AMMS® 300 2 mm suppressor, P/N 064559 (recommended) or ASRS® 300 2 mm suppressor, P/N 064555

Conductivity detector, P/N 061830

WPS-3000SL Semipreperative autosampler, P/N 5822.0018 (or 5822.0028) configured with a 1000 µL syringe and sample loop.

MasterFlex C/L peristaltic pump, model 77120-32, with 0.89 mm i.d. tubing

Chromeleon® Chromatography Management software, version 6.7 or higher.

REAGENTS AND STANDARDS

D.I. water, 18.2 MΩ-cm

Acetonitrile, UV grade (B&J, Cat. No. 015-4)

Boric acid, ACS grade (Merck, Cat. No. BX0865-1)

Potassium hydroxide, 40%, electronic grade (J.T. Baker, Cat. No. 3144-03)

Sulfuric acid, semi grade (Jones-Hamilton Co., Cat. No. 85603)

Perfluorooctanoic acid 98% (Aldrich, Cat. No. 171468)

Perfluorooctanesulfonic acid (Accustandard, Cat. No. PFOS-001N)

MOBILE PHASE PREPARATION

Mobile phase A1: Acetonitrile

Mobile phase B1: 100 mM H_3BO_3 and 9 mM KOH, pH 8 (dissolve 6.3 g of H_3BO_3 and 1.05 mL of 40% KOH in 1.0 L of water.)

Mobile phase C1: DI water

Mobile phase A2: Mix 200 g of mobile phase B1 with 800 g of water

Regenerant solution: 10 mN H_2SO_4 (dilute 0.36 mL conc. H_2SO_4 to 1.0 L with DI water.)

SYSTEM SETUP

As shown in Figure 1, the ICS-3000 is configured with a DP dual gradient pump and a DC detector/chromatography module equipped with conductivity detector and 6-port switching valve. In this configuration, the injector valve in the DC module is used as the column-switching valve, while the internal valve in the WPS-3000 is the injection valve. The WPS-3000 autosampler is equipped with 1000 µL syringe and loop. Pump 1 connects to port "P" on the switching valve and provides the elution gradient. Pump 2 connects to the autosampler and loads the sample onto the concentrator and washes away interferences. The autosampler outlet connects to port "S" of the switching valve. The concentrator is connected to ports "L," replacing the sample loop. The analytical column connects to port "C". Port 5 goes to waste through a restrictor capillary (this restrictor reduces the pressure transient on the concentrator column when the valve switches). The peristaltic pump has 0.89 mm i.d. tubing installed, and is set for 0.5 mL/min. Pump 1 of the DP has a GM-4 low-volume static mixer.

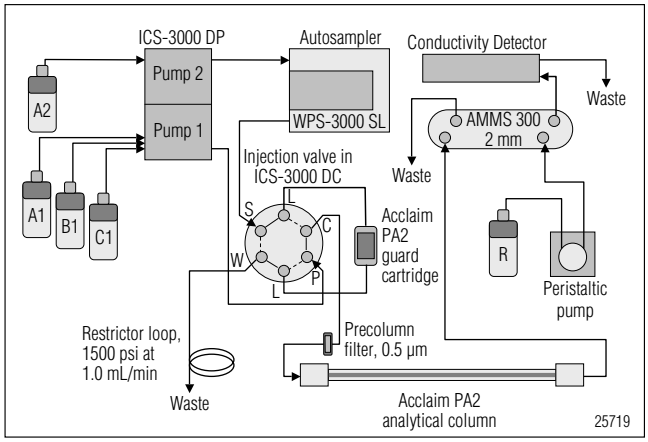


Figure 1. Schematic diagram of the HPLC system configuration.

PREPARATION OF STANDARDS AND SAMPLES

Stock Standard Solutions

Accurately weigh approximately 20 mg of neat standard (PFOS or PFOA) into a 20 mL vial. Assume a density of 0.791 g/mL to calculate the weight of ethanol needed to make a 1000 µg/mL stock standard. Alternatively, pre-made stock standards are commercially available; Wellington Laboratories, Vancouver, BC, Canada is one source.

Working Standards

Pipette 50 µL of each stock solution into a 25.0 mL volumetric flask and dilute to volume with deionized water to make a 20 µg/mL intermediate standard. Serially dilute the intermediate standard with water to 5.0, 1.0, 0.20, 0.050, 0.010, 0.002 and 0.001 µg/mL.

Spiked Tap Water Samples

Dilute the stock standard with degassed, unfiltered drinking water, following the procedure described under Working Standards (above).

CHROMATOGRAPHIC CONDITIONS

Analytical Column:	Acclaim® PA2 3 µm, 2.1 × 150 mm (P/N 063187)
On-Line Concentrator:	Acclaim PA2 5 µm, 4.3 × 10 mm guard cartridge (P/N 063195)
Column Temperature:	30 °C
Detector Cell Temperature:	35 °C
Injection Volume:	1000 µL

Table 1 lists the timed events for the gradient program and valve control.

Table 1. Timed Events for the Gradient Program and Valve Control

Program Time	–7.0	–2.5	0.0	0.1	10.0	15.0	15.1
Flow 1 (mL/min)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
%A1	5	5	5	5	55	55	5
%B1	30	30	30	30	30	30	30
%C1	65	65	65	65	15	15	65
Flow 2 (mL/min)	1.0	1.0	1.0	0.15	0.15	0.15	0.15
%A2	100	100	100	100	100	100	100
valve	load		inject				
autosampler		inject					
data collection			start			end	

RESULTS AND DISCUSSION

Description of the Method

Referring to Figure 1, when the ICS-3000 valve is in the load position, a 1000 µL sample is injected onto the concentrator. The concentrator is washed with buffer by Pump 2 to remove matrix interferences while retaining the hydrophobic analytes of interest, in this case, PFOA and PFOS. Then the concentrator is switched inline with the analytical column. PFOA and PFOS that have been enriched on the concentrator are separated on the reversed-phase analytical column using an acetonitrile gradient from Pump 1. Finally, the anionic PFOA and PFOS are selectively detected by suppressed conductivity detection.

Analytical Column

Silica-based reversed-phase columns are most commonly used in the separation of small molecules because of their ease of use, familiarity, and excellent bed stability and column efficiency compared to their polymer counterparts. The Acclaim PA2 column is chosen for this application because of its excellent hydrolytic stability and high efficiency.[5] In addition, compared to other reversed-phase columns, the Acclaim PA2 exhibits superior peak shape and capacity for this application. Although the separations reported here were performed on an Acclaim PA2 3 µm 2.1 × 150 mm column, other column dimensions can also be used with proper method modifications, depending on specific applications and/or available system setup.

Mobile Phase Selection

Because PFOA and PFOS are anionic, a buffer that can be suppressed for good conductivity detection is needed for stable and reproducible results. Borate buffer was selected for this application because of its low conductance and mildly alkaline buffer range (pH 8 to 9). The organic modifier in the mobile phase is acetonitrile. Methanol is not compatible with borate buffer for this application. Bicarbonate buffer is an alternative and gives similar separations but with somewhat higher background compared to borate buffer.

On-Line Sample Concentration

The detection limit for PFOA and PFOS by suppressed conductivity detection is approximately 1 ng. To achieve the detection limit on the order of 1 µg/L, a minimum of 1 mL of sample needs to be injected. Therefore, an on-line preconcentration technique is used. In this application, an Acclaim PA2 5 µm 4.3 × 10 mm guard cartridge is installed on a 2-position, 6-port valve. A 1 mL sample is injected, the concentrator is washed for 2.5 min with buffer to remove matrix interferences, and the valve is switched into line with the analytical column. The 2500 µL sample loop option and a larger dimension concentrator column may be used to obtain higher sensitivity, but it is necessary that at least twice the volume of the sample loop be passed through the concentrator column during the wash step.

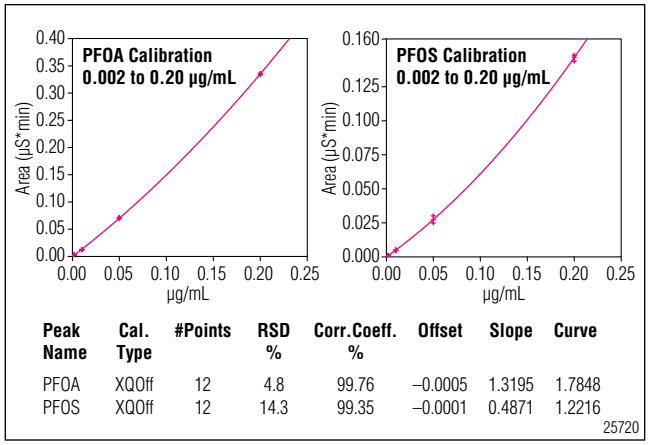
Suppressed Conductivity Detection

A suppressor selectively removes ions bearing the opposite charge of ions of interest in the effluent exiting the analytical column and replaces them with either hydronium ions (anion detection) or hydroxide ions (cation detection). This minimizes the background noise level, resulting in much improved sensitivity. When effluent containing borate buffer and the analytes passes through an anion suppressor, cations are removed with an acidic regenerant solution (10 to 25 mN H_2SO_4 aqueous solution) leaving behind the low conductance boric acid with ionized PFOA and PFOS to be detected by the conductivity detector.

Due to the presence of organic solvent (acetonitrile) in the mobile phase, the suppressor should be used in the chemical suppression mode. The concentration of organic modifier is restrained by the construction of the suppressor and its operating mode. Instructions in the operator's manual[6] must be carefully followed to avoid damage to the suppressor. The recommended suppressor is the AMMS 300 2 mm Anion MicroMembrane™ Suppressor, but others may be used with appropriate caution. The regenerant is delivered to the suppressor with a peristaltic pump at a flow rate of approximately 0.5 to 1.0 mL/min.

Sensitivity, Dynamic Range, and Calibration Curve

The detection limits for PFOA and PFOS are approximately 1 ng per injection. The capacity of the PA2 column (3 µm, 2.1 × 150 mm) is at least 4 µg per injection. Therefore, the method offers a wide dynamic range of 1–40,000 µg/L, based on a 1 mL sample injection. For low-level analysis (1–200 µg/L), quadratic calibration curves are used for both PFOA and PFOS, with R^2 better than 0.99 and 5–15% RSD (Figure 2). For higher-level analysis 200 to 20,000 µg/L, linear calibration curves can be used.



Peak Name	Cal. Type	#Points	RSD %	Corr.Coeff. %	Offset	Slope	Curve
PFOA	XQOff	12	4.8	99.76	–0.0005	1.3195	1.7848
PFOS	XQOff	12	14.3	99.35	–0.0001	0.4871	1.2216

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Figure 2. Calibration at 0.002, 0.010, 0.050, and 0.200 µg/mL.

Precautions for Good Recovery

To obtain reproducible and accurate recovery of PFOA, PFOS, and related compounds, especially at trace levels, care should be taken to eliminate sample adsorption.

Sample Filtration

Several filters have been evaluated, some of which trap PFAs, and some of which are contaminated with PFAs or other interferences. As shown in Table 2, both PTFE and Supor® Polysulfone filters give good recovery for both PFOA and PFOS without contamination. On the other hand, the commonly used nylon membrane filter caused a complete loss of recovery. Elsewhere, it has been reported that glass fiber filters are acceptable.[7]

Table 2. Recovery and Interferences for 200 µg/L PFOA and PFOS for Four Different Filter Media			
Filter Material	**Blank**	**PFOA % Recovery**	**PFOS % Recovery**
PTFE	OK	100	86
Nylon	OK	0	0
Anotop™	Interference	101	99 (interferences)
Supor polysulfone	OK	101	93

Flow Pathway

Fluorocarbon polymers are often used for wetted surfaces in HPLC and IC instruments because they are inert toward many analytes. When the analytes are fluorocarbons, these materials cause carryover and sample adsorption problems. Furthermore, PFAs are used in the manufacturing and processing of fluoropolymers and can interfere with highly sensitive analyses. It is necessary therefore to replace fluorocarbon components with PEEK™ or stainless steel for the surfaces contacted by the sample. For this reason, the UltiMate® WPS-3000SL autosampler is recommended for this application because it provides no noticeable carryover (Note: If the lab has the older Summit® ASI-100, it will also work for this application).

Sample Container

Another issue for a low recovery is adsorption on the walls of the sample container, especially for PFAs longer than C8. Addition of methanol to the sample can prevent adsorption, but this unfavorably reduces retention of PFAs shorter than C8. In this case, different methods may be required for analysis of shorter chain (C < 8) and longer chain (C > 8) PFCAs.

PFOA/PFOS in Tap Water

Figure 3 shows typical chromatography for the analysis of a tap water sample, and Table 3 shows the recovery data. Similar results were obtained for bottled water and synthetic high-ionic-strength water matrices. Ca^{2+} and Mg^{2+} ions affect the retentions of PFOA and PFOS and the efficiency of the suppressor. Therefore the method is designed to flush these interfering ions out of the concentrator column before switching the concentrator inline with the analytical column for separation.

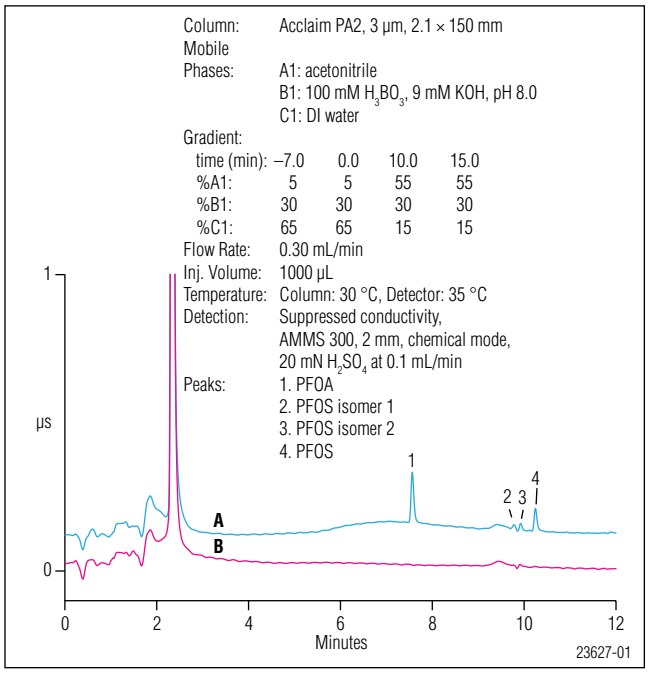

Figure 3. A) Tap water spiked with 10 µg/L each of PFOA and PFOS. B) Tap water blank. Note: The baselines were corrected by blank subtraction.

Table 3. Recovery of PFOA and PFOS from Tap water (n=5)		
Spike level (µg/L)	**PFOA %recovery ±RSD**	**PFOS %recovery ±RSD**
0	N.D.	N.D.
0.5	56 ± 68	106 ± 94
2	98 ± 6	137 ± 26
10	100 ± 4	112 ± 11
50	101 ± 4	102 ± 10
200	100 ± 4	97 ± 6

Other Applications

This method can be used for determination of C6 or larger perfluorocarboxylic acids (PFCAs) without change to the separation conditions. This method detects other anions of intermediate to high hydrophobicity, such as anionic detergents. However, it is unsuitable for analyzing weak acids such as palmitic or stearic acids.

For samples containing higher concentrations of PFCAs (> 200 µg/L), the same method can be applied with direct injection without the on-line preconcentration step. Figure 4 shows the separation of a standard mixture of PFCAs from C4 to C14 with direct injection. Figure 4 also illustrates the use of baseline subtraction in Chromeleon for better peak integration. To minimize the baseline fluctuation due to pump pulsation, the WPS-3000SL is synchronized to Pump 1. As a result, the reagent blank has the same pulsation pattern and baseline slope as the samples, so that baseline subtraction can be properly performed, which greatly improves integration near the detection limit.

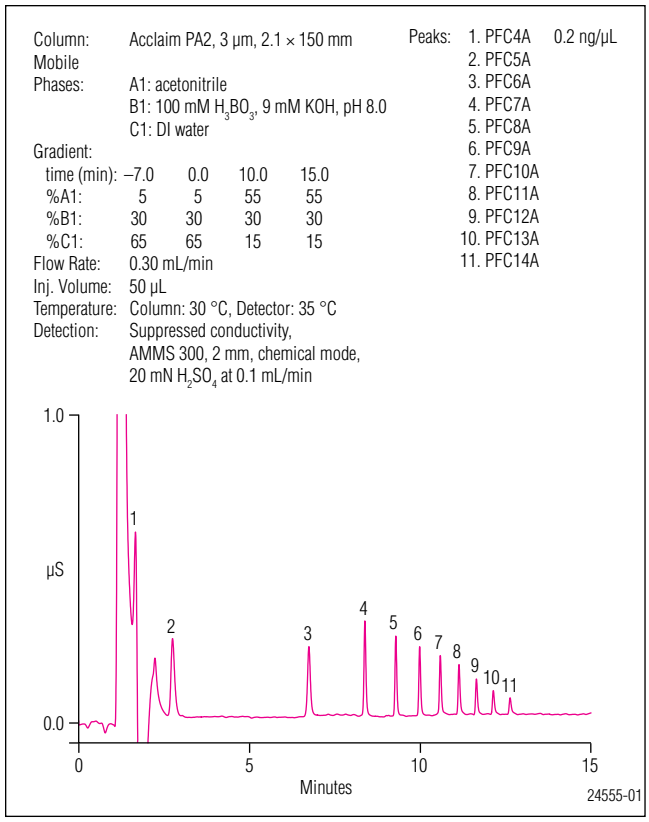

Figure 4. Separation of homologous C4 to C14 Perfluoroalkane-carboxylic acids.

CONCLUSIONS

The reported LC method for analysis of PFOA and PFOS in water samples integrates on-line sample concentration, reversed-phase HPLC, and suppressed conductivity detection. This method uses the Acclaim PA2 column and an ICS-3000 system. This technique can be modified to apply to other fluorinated organic acids (C6–C18 homologs) as well as a variety of anionic surfactants.

REFERENCES

1. Giesy, J.P.; Kannan, K. *Environ. Sci. Technol.* **2001,** *35,* 1339.
2. Kissa, E. *Fluorinated Surfactants and Repellents,* 2nd ed.; Marcel Dekker: New York, 2001.
3. Larson, B.S.; Kaiser, M.A. *Anal. Chem.* **2007,** *79*, 3966.
4. Weiss, J. *Handbook of Ion Chromatography,* 3rd ed.; Wiley-VCH: Weinheim, Germany, 2001.
5. Liu, X.; Bordunov, A.; Pohl, C. *J. Chromatogr., A* **2006,** *1119,* 128.
6. Dionex Corporation. *MicroMembrane Suppressor 300 Manual*; Doc. No. 031727-04: Sunnyvale, CA, 2008 http://www.dionex.com/en-us/webdocs/4366_031727- rev04-Man-MMS%20300.pdf
7. Nakayama, S.F. US EPA, Nat. Exposure Res. Lab., Personal Communication.

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