

Analysis of Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) in Water Samples Using Reversed-Phase Liquid Chromatography (RPLC) with Suppressed Conductivity Detection

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ABSTRACT

This work describes a liquid chromatography method for determination of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in water samples. The method incorporates on-line sample concentration, reversed-phase HPLC using Acclaim® PolarAdvantage II (PA2) columns for both and suppressed conductivity detection. For tap water samples, the LOD and LOQ are expected to be 1 µg/L and 3 µg/L, respectively, for both PFOA and PFOS. The dynamic range is 1 to 40,000 µg/L.

INTRODUCTION

Perfluorinated acids (PFAs), including PFOA and PFOS, are widely distributed pollutants in the environment.¹ PFOS and PFOA are a class of perfluorinated chemicals that are best known for their use in the production of Teflon and other stain-resistant materials.² The U.S. Environmental Protection Agency (EPA), international, and state public health regulators all have recently focused considerable attention on PFOA and PFOS because they are ubiquitous, persistent, and bioaccumulative (depending on the length of the carbon chain). Perfluoro carboxylic acids (PFCAs) are used for a wide variety of industrial, commercial, and consumer applications. The medium and long chain PFCAs 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 matrices 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 all PFAs have low pKa values and are fully charged anions in HPLC conditions, they can be detected by suppressed conductivity method.

We report herein a method to determine PFOA and PFOS in water samples using on-line sample concentration, reversed-phase HPLC and suppressed conductivity detection. We discuss instrument setup, sample concentration, the chromatographic method, calibration, and the limit of detection.

EXPERIMENTAL

Separation Column

Acclaim PA2 analytical column (2.1 × 150 mm, dp = 3 µm, P/N 063187, Dionex, Sunnyvale, CA, USA)
Acclaim PA2 guard cartridge (4.3 × 10 mm, dp = 5 µm, P/N 063195, Dionex)

Instrumentation

Modular ICS-3000 system (Dionex) equipped with a DP dual gradient pump (P/N 061713), DC chromatography module (P/N 061767), conductivity detector (P/N 061830), AMMS-300 2-mm suppressor (P/N 064559), WPS-3000 SL Autosampler (P/N 5822.0018) configured with 1000 µL syringe and sample loop, and MasterFlex C/L peristaltic pump (model 77120-32, with 0.89 mm i.d. tubing). Chromeleon® 6.70 Chromatography Management Software (Dionex) was used for system control and data processing.

System Setup

As shown in Figure 1, the ICS-3000 is configured with DP dual gradient pump; DC detector/chromatography module equipped with conductivity detector and six-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 it 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 W 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.

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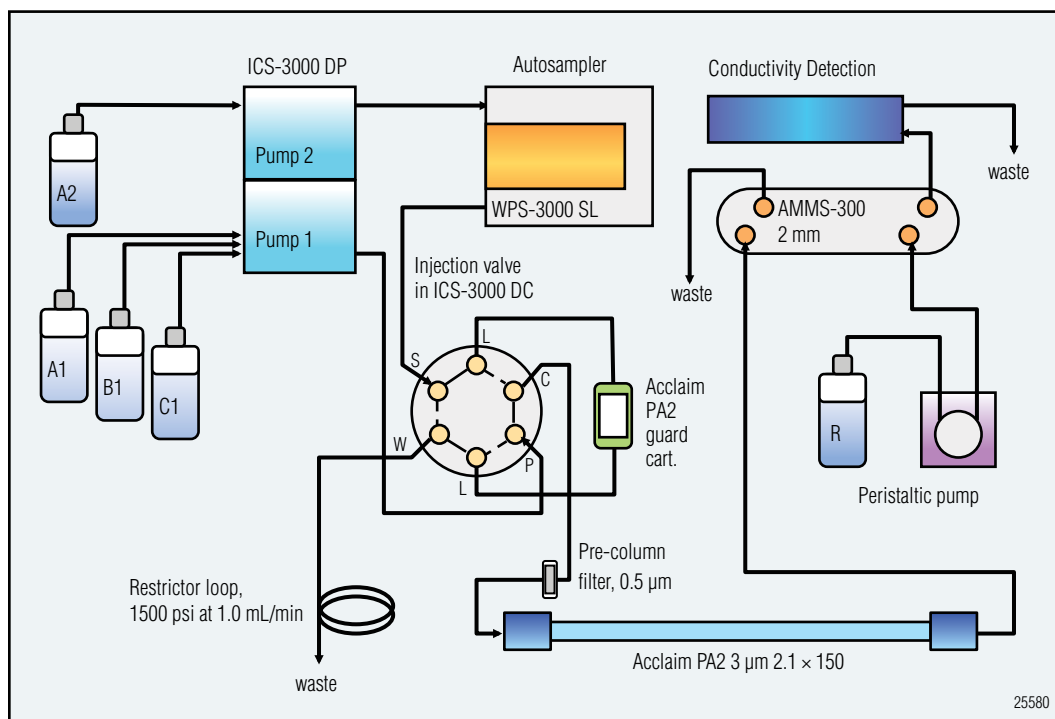


Figure 1. Schematic diagram of the HPLC system configuration.

Reagents and Standards

Acetonitrile:	(B&J, UV grade, Cat. No. 015-4)
Boric acid:	(E. Merck, ACS grade, Cat. No. BX0865-1)
Potassium hydroxide, 40%:	(J.T. Baker, Electronic Grade, Cat. No. 3144-03)
Sulfuric acid:	(Jones-Hamilton Co., Semi Grade, Cat. No. 85603)
Perfluorooctanoic acid (PFOA) 98%:	(Aldrich Cat. No. 171468)
Perfluorooctanesulfonic acid (PFOS):	(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:	D.I. 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 D.I. water)

Chromatographic Conditions

Separation column:	Acclaim PA2 3 μ 2.1 x 150 mm
On-line Concentrator:	Acclaim PA2 5 μ 4.3 x 10 mm guard cartridge
Column temperature:	30 °C
Detector cell temperature:	35 °C
Injection volume:	1000 μ L

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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 (minute):		-7.0	-2.5	0.0	0.1	10.0	15.0	15.1
	Flow 1:	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	10	10	65
	Flow 2:	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

Separation Column

Silica-based reversed-phase columns are most commonly used for separation of small molecules because of the ease of use, familiarity, excellent bed stability and column efficiency. However, they have limited uses in alkaline conditions (pH >7.5). 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 loadability for this application. Depending on specific applications and/or available system set-up, other column dimensions can also be used with proper method modifications.

Mobile Phase Selection

Because PFOA and PFOS are anionic, a buffer that has low conductivity or can be suppressed for good conductivity detection is needed. Borate buffer was selected for this application because of its low conductance and mildly alkaline buffer range (pH 8–9). 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

To achieve the detection limit on the order of 1 µg/L, a minimum of 1 mL sample needs to be injected. If this were injected directly onto the analytical column, matrix effects would disturb the chromatography and detection. Therefore on-line pre-concentration is used. In this application, an Acclaim PA2 guard cartridge (4.3 × 10 mm) is installed on a two-position six-port valve where a 1 mL sample is injected. After washing the concentrator for 2.5 min to remove matrix interferences, it is switched into line with the analytical column. The 2500 µL sample loop option and a larger column dimension for the concentrator could 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 in the wash step.

Suppressed Conductivity Detection

A suppressor selectively removes ions bearing the opposite charge of ions of interest in the effluent exiting the separation column and replaces them with either a hydronium ion (anion detection) or hydroxide ion (cation detection). As the result, the background noise level is greatly minimized resulting in much improved sensitivity. When effluent containing borate buffer and the analytes pass 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 external chemical mode. The concentration of organic modifier is constrained by the construction of the suppressor and its operating mode. Thus, one must carefully follow the operator's manual.⁶ The recommended suppressor is the AMMS[®]-300 2-mm Anion MicroMembrane™ Suppressor. The regenerant is delivered 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 loadability on the PA2 column (3 μm , 2.1 \times 150 mm) is at least 4 μg per injection. Therefore, the method offers a wide dynamic range of 1–40,000 $\mu\text{g/L}$ based on 1 mL sample injection. For low-level analysis (2–200 $\mu\text{g/L}$), 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 $\mu\text{g/L}$, linear calibration curves can be used.

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 Because PTFE contains fluorocarbons, it can potentially affect the recovery of PFAs. Therefore Supor Polysulfone filters is recommended for sample filtration.

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, PFOA is used in the manufacturing and processing of fluoropolymers so that it 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-3000 SL is recommended for this application because it provides no noticeable carryover.

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

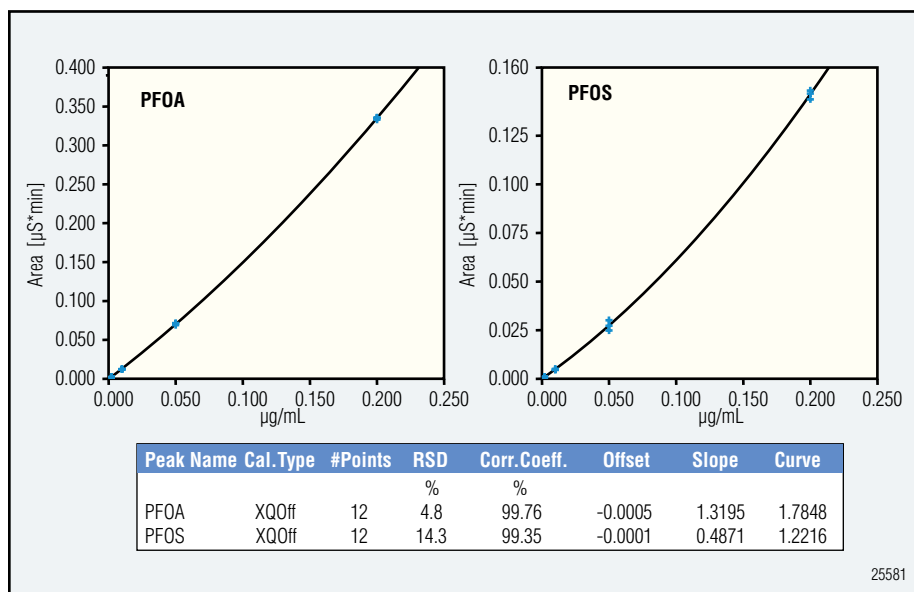


Figure 2. Calibration at 0.002, 0.010, 0.050, and 0.200 µg/mL

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 will result in strong sample diluent, which unfavorably shortens with retention of PFAs shorter than C8. In this case, different methods may be needed for analysis of shorter chains (C < 8) and longer chain (C > 7) 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 a bottled water and a synthetic water matrix. The presence of Ca^{2+} and Mg^{2+} ions affect the retentions of PFOA and PFOS as well as suppression efficiency of the suppressor. Therefore the program is designed to flush these interfering ions out of the concentrator column before switching the concentrator in line with the separation column for analysis. Table 4 shows the profile of gradients A, B, and C.

Table 3. Recovery of PFOA and PFOS from Sunnyvale 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

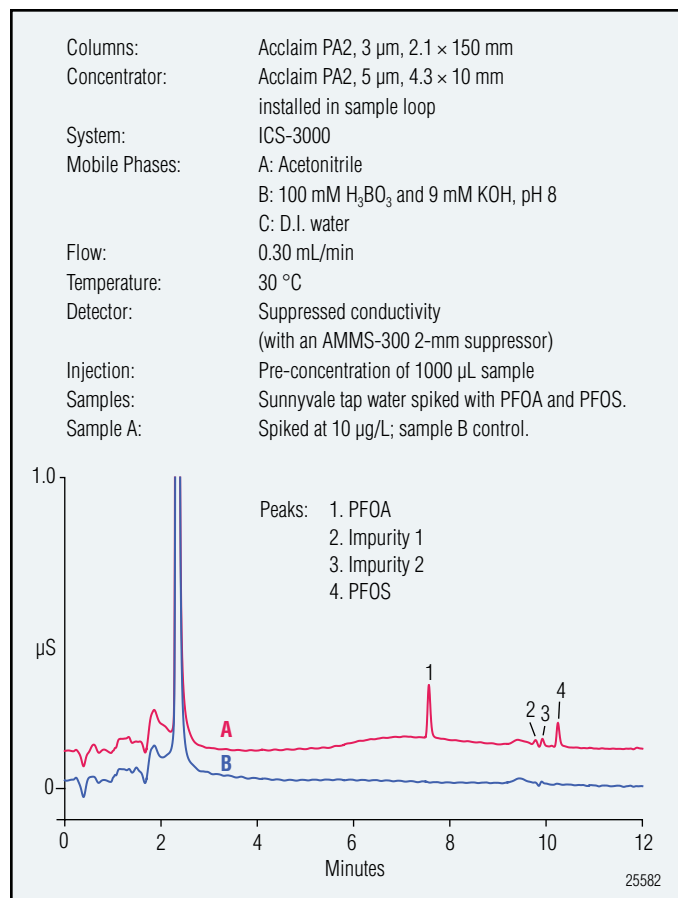


Figure 3. Sunnyvale tap water spiked with PFOA and PFOS.

Table 4. Run Profile Using Three Gradients (A, B, C)							
	-7.0 (min)	-2.5 (min)	0.0 (min)	0.1 (min)	10.0 (min)	15.0 (min)	15.1 (min)
Gradient A	5%	5%	5%	5%	55%	55%	5%
Gradient B	30%	30%	30%	30%	30%	30%	30%
Gradient C	65%	65%	65%	65%	10%	10%	65%

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 does not detect hydrophobic, 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 C5 to C14 with direct injection. Figure 4 also illustrates the use of baseline subtraction in Chromeleon for better peak integration. (Table 4 shows the profile of gradients A, B, and C). To minimize the baseline fluctuation due to pump pulsation, the WPS-3000 SL 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.

CONCLUSIONS

We have developed an LC method for the analysis of PFOA and PFOS in water samples. The method integrates on-line sample concentration, reversed-phase HPLC, and suppressed conductivity detection, using the Acclaim PA2 column and ICS-3000 system to achieve reproducible result with good sensitivity and wide dynamic range. This technique can be modified to apply to other fluorinated organic acids (C6–C18 homologs) as well as a variety of anionic surfactants.

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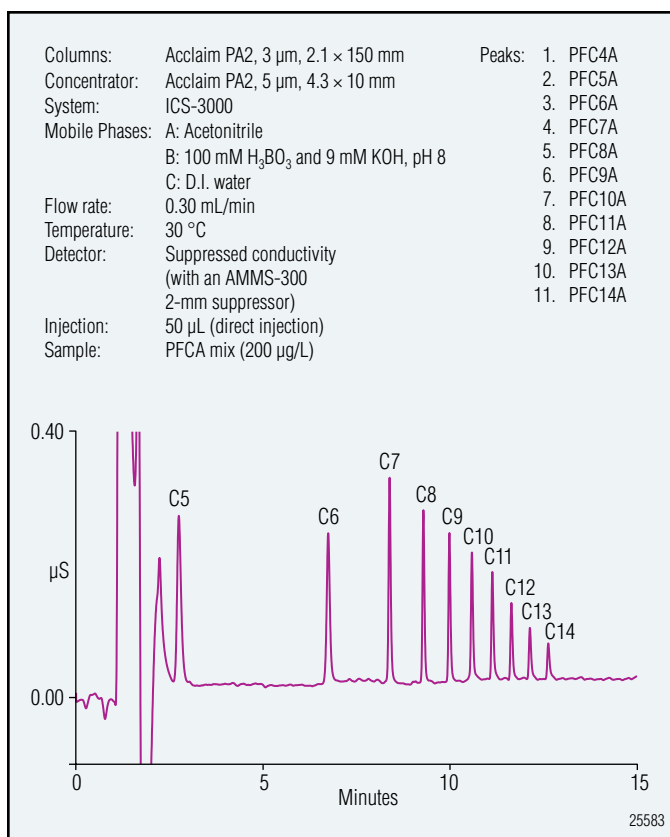


Figure 4. Perfluorocarboxylic acids on the Acclaim PolarAdvantage II column using suppressed conductivity detection.

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