# Optimization of Liquid Chromatography-Tandem Mass Spectrometry for Analysis of C4 to C18 Perfluorinated Acids and Some Fluorotelomers

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## Introduction

Solid phase extraction (SPE), using Sep-Pak C18 or Oasis HLB cartridges, has been followed in the analysis of perfluorinated acids in water samples[1-5]. However, studies have focused on selected anionic perfluorinated compounds. Moreover, these studies have highlighted the need for appropriate sample processing prior to LC-MS analysis to eliminate issues related to ion suppression or enhancement. The above mentioned SPE cartridges are not suitable for the analysis of short chain (C4 to C6) perfluorinated acids and fluorotelomers. In this study, we describe detailed parameters to optimize a new SPE method with HPLC tandem mass spectorometry (HPLC-MS/MS) for the analysis of C4 to C18 perfluorinated acids and some telomer alcohols and telomer acids.

## Materials and Methods

The analytical procedure for the extraction of water samples was similar to that described elsewhere[5]. The major modifications involve the development and optimization of new SPE cartridges (WAX cartridge - weak anion exchange) to enable optimal recoveries for all target chemicals, both ionic and neutral species, in water samples. We have also developed a method for biological samples using KOH digestion followed by WAX extraction; this method provided better results over the ion-pair extraction method, as exemplified by the analysis of human blood and beaver liver samples using these two methods. Analysis of PFCs was performed using an HPLC-MS/MS; an Agilent HP1100 liquid chromatograph interfaced with a Micromass (Beverly, MA, USA) Quattro Ultima Pt mass spectrometer operated in electrospray negative ionization mode was used. A 5- or 10-µL aliquot of the sample extract was injected into a guard column (XDB-C8, 2.1 mm i.d. x 12.5 mm, 5µm; Agilent Technologies, Palo Alto, CA) connected sequentially to a Betasil C18 column (2.1 mm i.d.×50 mm length, 5µm; Thermo Hypersil-Keystone, Bellefonte, PA) with 2 mM ammonium acetate/methanol as mobile phase, starting at 10% methanol. At a flow rate of 300 mL/min, the gradient was increased to 30% methanol at 0.1 min, 75% methanol at 7 min, and 100% methanol at 10 min, and was held there until 12 min before reversion to original conditions, at 20 min.

## Results and Discussion

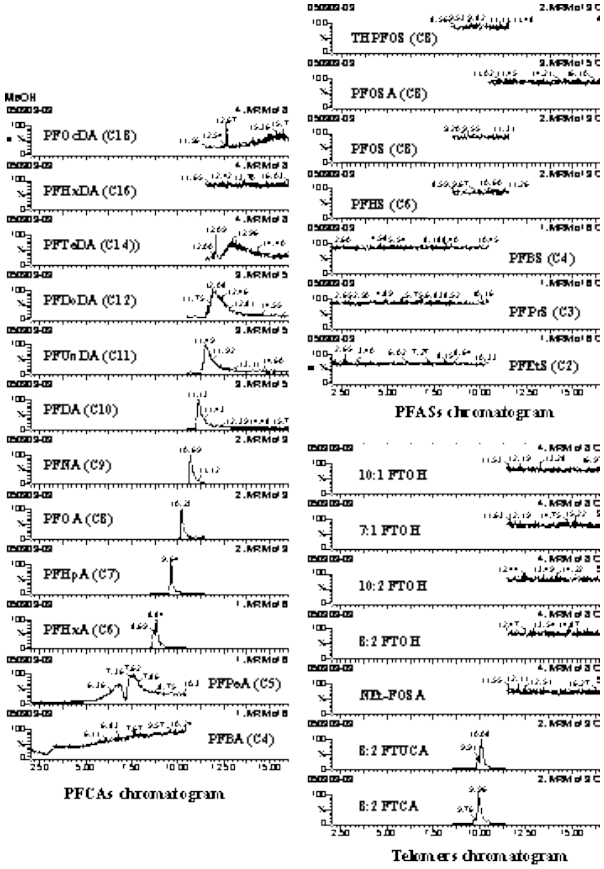


Figure1. Instrumental blank of PFCs

We examined several parameters of the HPLC MS/MS for seven perfluoroalkylsulfonates (PFASs) (C4 to C8) and thirteen perfluoroalkylcarboxylates (PFCAs) (C4 to C18) including THPFOS and 13C-PFOA.

It is evident that background contamination is one of the most difficult aspects of perfluorochemical analysis. The instrumental blank is the first obstruction in the analysis. We successfully decreased blank levels of the HPLC-MS/MS to measure part-per-quadrillion levels of perfluoros in deep sea water. However, we encountered different types of contamination issues when we set up a new HPLC-MS/MS. We found blanks containing C6 to C14 PFCAs and 8:2 FTUCA and 8:2 FTCA (Figure 1).

The source of contamination was found to be fluoropolymer sealing inside of the pump, which is commonly used in normal phase HPLC. Changing several of the plastic materials used in normal phase chromatography to reverse phase was effective in decreasing the contamination in the instrumental blank. After modification of the instrument parts mentioned, all blank peaks disappeared.

**Table 1. Summary of ESI-MS/MS conditions and instrumental quantification limits**

| Analyte | Compound structure | Transition Monitored [m/z] primary>secondary | Cone voltage [V] | Collision energy [eV] | Instrumental quantification limit [pg] | Instrumental detection limit [pg] |
|---|---|---|---|---|---|---|
| **PFASs** | | | | | | |
| PFEtS | $F(CF_2)_2SO_3^-$ | 198.80 > 79.80 | 65 | 20 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| PFPrS | $F(CF_2)_3SO_3^-$ | 248.90 > 79.60 | 70 | 25 | | |
| PFBS | $F(CF_2)_4SO_3^-$ | 298.70 > 79.70 | 35 | 25 | 0.02 | 0.01 |
| PFHxS | $F(CF_2)_6SO_3^-$ | 398.70 > 79.70 | 70 | 30 | 0.02 | 0.01 |
| PFOS | $F(CF_2)_8SO_3^-$ | 498.60 > 79.70 | 90 | 35 | 0.02 | 0.01 |
| PFOSA | $F(CF_2)_8SO_2NH^-$ | 497.70 > 77.70 | 55 | 25 | 0.02 | 0.005 |
| THPFOS | $F(CF_2)_6(CH_2)_2SO_3^-$ | 426.70 > 406.70 | 55 | 20 | 0.02 | 0.005 |
| **PFCAs** | | | | | | |
| PFBA | $F(CF_2)_3COO^-$ | 212.80 > 168.80 | 35 | 7 | 0.10 | 0.07 |
| PFPeA | $F(CF_2)_4COO^-$ | 262.80 > 218.70 | 35 | 7 | 0.02 | 0.01 |
| PFHxA | $F(CF_2)_5COO^-$ | 312.80 > 268.80 | 35 | 7 | 0.02 | 0.005 |
| PFHpA | $F(CF_2)_6COO^-$ | 362.80 > 318.80 | 35 | 8 | 0.02 | 0.01 |
| PFOA | $F(CF_2)_7COO^-$ | 413.00 > 368.70 | 35 | 10 | 0.02 | 0.02 |
| PFNA | $F(CF_2)_8COO^-$ | 462.70 > 418.80 | 35 | 10 | 0.02 | 0.008 |
| PFDA | $F(CF_2)_9COO^-$ | 512.80 > 468.80 | 35 | 10 | 0.02 | 0.004 |
| PFUnDA | $F(CF_2)_{10}COO^-$ | 563.00 > 519.00 | 35 | 10 | 0.02 | 0.007 |
| PFDoDA | $F(CF_2)_{11}COO^-$ | 612.70 > 568.80 | 35 | 10 | 0.02 | 0.007 |
| PFTeDA | $F(CF_2)_{13}COO^-$ | 712.90 > 669.00 | 40 | 12 | 0.02 | 0.01 |
| PFHxDA | $F(CF_2)_{15}COO^-$ | 812.90 > 769.30 | 40 | 15 | 0.02 | 0.01 |
| PFOcDA | $F(CF_2)_{17}COO^-$ | 912.90 > 869.00 | 50 | 15 | 0.02 | 0.07 |
| 13C-PFOA | $F(CF_2)_8{}^{13}CF_2{}^{13}COO^-$ | 414.90 > 369.90 | 35 | 10 | 0.02 | 0.02 |
| **Telomer and Precursor** | | | | | | |
| 8:2FTOH | $F(CF_2)_8(CH_2)_2O^-$ | 463.00 > 354.80 | 35 | 15 | 2 | 1 |
| 10:2FTOH | $F(CF_2)_{10}(CH_2)_2O^-$ | 563.50 > 455.10 | 35 | 15 | 2 | 1 |
| 7:1FTOH | $F(CF_2)_7CH_2O^-$ | 398.90 > 218.90 | 35 | 15 | 0.5 | 0.4 |
| 10:1FTOH | $F(CF_2)_{10}CH_2O^-$ | 549.00 > 369.00 | 35 | 18 | 0.1 | 0.1 |
| 8:2FTCA | $F(CF_2)_8CH_2COO^-$ | 477.00 > 393.00 | 35 | 20 | 0.1 | 0.02 |
| 8:2FTUCA | $F(CF_2)_7CF{=}CHCOO^-$ | 457.00 > 393.00 | 35 | 10 | 0.02 | 0.004 |

| | | | | | | |
|---|---|---|---|---|---|---|
| N-Et FOSAA | $F(CF2)_8\ SO_2\ N(C_2H_5)\ (CH_2COO)^-$ | 583.90 > 418.70 | 60 | 18 | | |
| N-Et FOSA | $F(CF2)_8\ SO_2N\ (C_2H_5)^-$ | 525.90 > 168.90 | 60 | 25 | 0.02 | 0.003 |

We optimized the MS/MS parameters for the above target chemicals including PFASs, PFCAs and fluorotelomers. Transition ions monitored under MS/MS conditions, cone voltage and collision energy are specific to each chemical. Optimized parameters are presented in Table 1. The capillary was held at 1.2 kV. Cone-gas and desolvation-gas flows were held at 60 and 650 L/h, respectively. Source and desolvation temperatures were kept at 120 and 420°C, respectively. MS/MS parameters were optimized so as to transmit the [M-K]- or [M-H]- ions. Instrumental quantification limits calculated from calibration curves prepared for each standard chemical are shown in Table 1. Actual instrumental detection limits are also shown (Table 1). Estimated LOQ, calculated based on spiking known concentrations of standards, and processed through the entire analytical procedure involving the SPE extraction was 1 pg for PFOS, PFHS, PFBS, PFOSA, PFUnDA, PFDA, PFHpA, PFHxA, PFPeA, 13C-PFOA, THPFOS, and 8:2FTUCA, 5 pg for PFOcDA, PFHxDA, PFTeDA, PFDoDA, and PFBA, 8 pg for N-EtFOSA, 25 pg for 8:2FTCA, 40 pg for 10:1FTOH, 800 pg for 7:1FTOH, and 4000 pg for 8:2FTOH and 10:2FTOH in 1L of water sample. The LOQs of target chemicals were evaluated for each sample based on the maximum blank concentration, the concentration factors, the sample volume and a signal-to-noise ratio of 3.

Another source of contamination in blank is standard chemicals themselves. We have quantified impurities in all target perfluorochemical standards commercially available. In most cases, the impurities in standards were negligible. However, 7% of PFBS was found in PFOS standard (3M). Six to 11% of PFBA and 6 to 9% of PFPeA were detected in 13C-PFOA(Perkin Elmer), PFTeDA(SynQuest), PFHxDA(SynQuest), PFOcDA(SynQuest) and 10:1 FTOH (SynQuest) standards. It is clear that inaccurate use of these standards can cause analytical error in the determination of short chain PFCs and telomers. Another important factor in MS/MS determination after alkaline digestion method is ion suppression of specific chemicals. It is necessary to avoid additional inputs of alkali into API and application of switching valve without PTFE contamination are useful. In addition, adsorption of long chain PFCAs on polypropylene tube will be discussed.

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