

# Analysis of Perfluoroalkyl Acids in Wastewater, Sludge, and Liver Extracts Using High-Resolution, Accurate Mass LC-MS

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## Introduction

Perfluoroalkyl acids (PFAAs) are global pollutants and have been shown to bioaccumulate in the food chain. PFAAs have been detected in livers of fish, birds, and marine mammals from Greenland and the Faroe Islands.<sup>1</sup> Biomagnification of perfluorooctane sulfonate (PFOS), the predominant fluorochemical detected, was observed along the marine food chain (Figure 1).

The performance of the Thermo Scientific Exactive mass spectrometer equipped with Orbitrap™ technology has been evaluated for the analysis of ten selected perfluoroalkyl acids in pooled extracts from environmental samples. The following PFAAs were analyzed: perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoate acid (PFTrA), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorooctane sulfonamide (PFOSA) (Figure 2).

The sample extracts were chosen to represent both high and low levels of the analytes in complex matrices. Low levels were expected in liver extracts from Antarctic seals. Medium and high levels were expected in Arctic seals, influent water, and sludge from a wastewater treatment plant. The performance has been evaluated in terms of linearity (range 0.1-50 µg/kg), specificity, and sensitivity.

## Goal

To demonstrate the performance of the Exactive™ high-resolution, accurate mass benchtop liquid chromatography-mass spectrometry (LC-MS) system in the analysis of ten selected perfluoroalkyl acids.

## Experimental Conditions

### Sample Preparation

The sample preparation process is illustrated in Figure 3. Liver samples were extracted by ion pairing with tetrabutylammonium hydrogen sulfate (TBAS) and methyl tertiary butyl ether (MTBE). Sludge samples were extracted by sonication with methanol followed by solid phase extraction (SPE). Effluent water samples were extracted by SPE on C18 columns.

### HPLC

Chromatographic analysis was performed using a Thermo Scientific Accela autosampler and pump. The chromatography conditions were as follows:

|                     |   |
|---------------------|---|
| HPLC column:        | Thermo Scientific Hypersil GOLD, 50 mm x 2.1 mm, 1.9 µm |
| Pre-column:         | Thermo Scientific Hypercarb, 100 mm x 2.1 mm, 5 µm      |
| Column temperature: | 40 °C   |
| Mobile phase C:     | Ammonium acetate (2 mM)                                 |
| Mobile phase D:     | 90% water, 10% ammonium acetate                         |

A trapping column placed in line with the Accela™ pump and autosampler enabled less contamination of perfluorinated compounds (PFC) into the system, thus achieving a lower background.

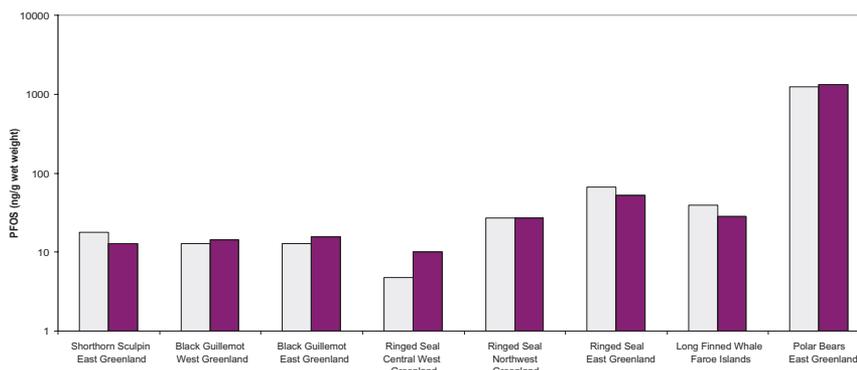


Figure 1. PFOS concentrations (analysis of two samples) in Arctic mammals, birds, and fish [Bossi et al. (2005)]

## Key Words

- Exactive
- Orbitrap technology
- Environmental application
- PFAAs
- PFOS

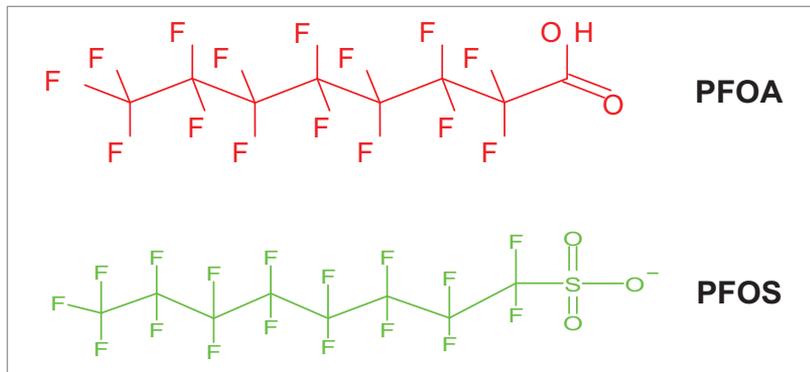


Figure 2. Examples of PFAA target compounds – PFOA and PFOS

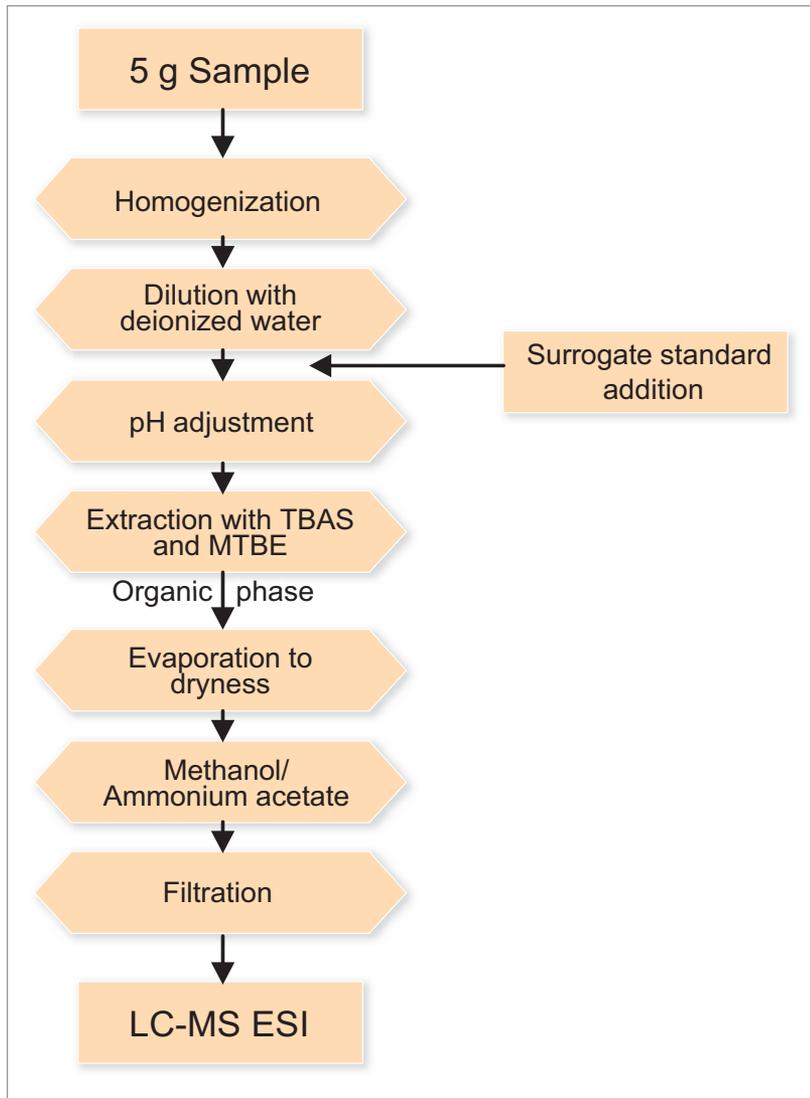


Figure 3. Sample preparation process

## Mass Spectrometry

MS analysis was carried out on an Exactive high performance benchtop LC-MS with an electrospray ionization (ESI) source in negative ion mode. Full scan data with a resolution of 50,000 FWHM at  $m/z$  200-800 was acquired. No lock mass was applied.

## Results and Discussion

The high mass resolution (50,000 FWHM) and mass accuracy (1 ppm) of the Exactive high-resolution, accurate mass system provide efficient peak confirmation and decrease the effects of matrix peaks (Figure 4). The concentration of the target compound in the liver samples was quantified by linear calibration. The use of lock masses could enhance the mass accuracy; however, the Orbitrap instrument was stable for the duration of the sample analysis.

In Figure 5, the extracted ion chromatograms, normalized to the response, are shown. The blank (a) shows very little background and no significant signal at the appropriate retention time. The 0.1  $\mu\text{g}/\text{kg}$  standard (b) has good signal for all compounds, as does the sample of extracted Arctic seal liver (c).

For the calibration curves, three standards per level (0.1, 1, 5, 10, and 50  $\mu\text{g}/\text{kg}$ ) were run. The calibration curve for PFOA is shown in Figure 6.

The results of the analysis are displayed in Table 1. The extracted liver from Antarctic seals showed significantly lower concentrations of PFAAs than the extracted liver from Arctic seals.

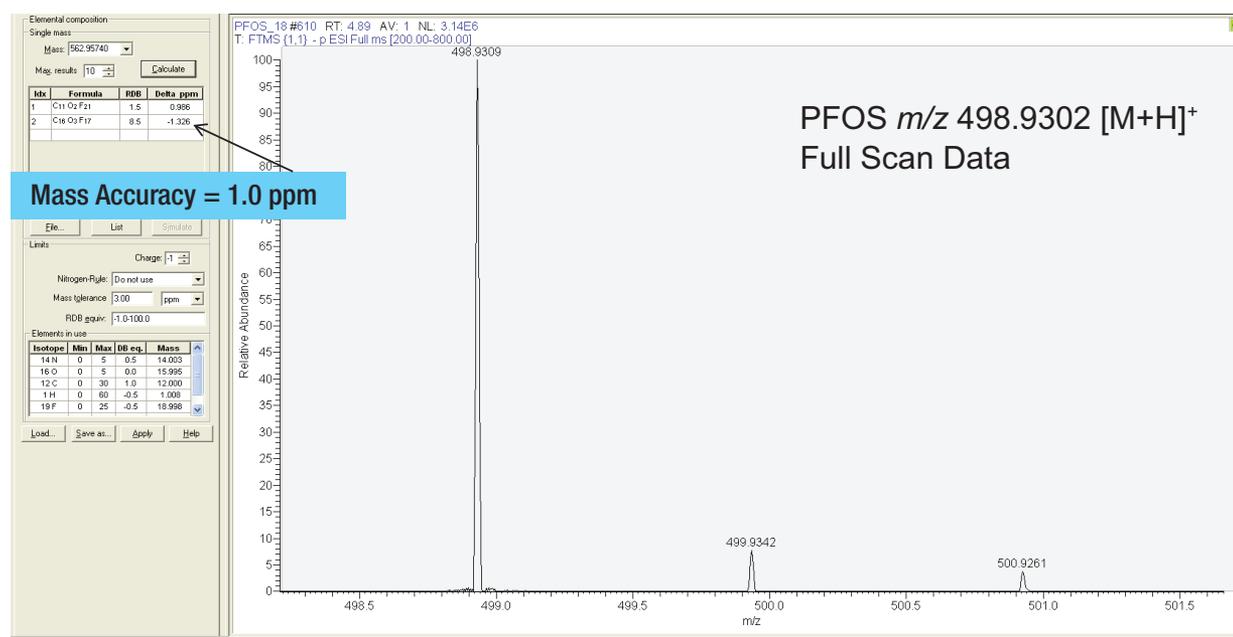
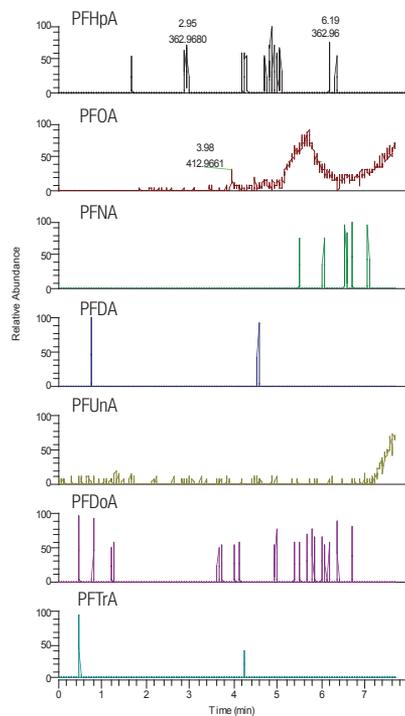


Figure 4. Mass accuracy of PFOS

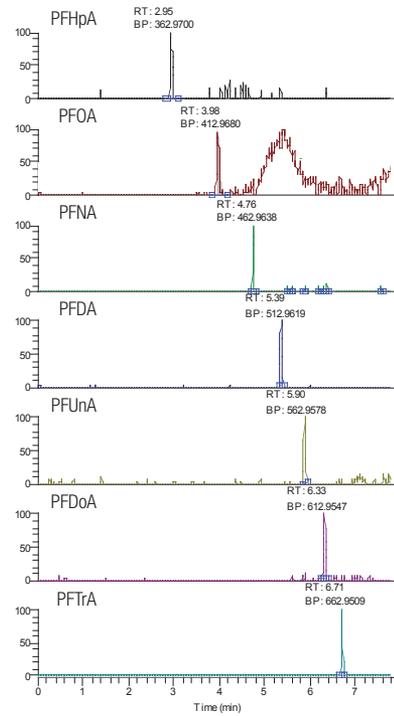
Table 1. PFAAs in Antarctic and Arctic seal liver

|       | Antarctic Seal<br>$\mu\text{g}/\text{kg}$ | Arctic Seal<br>$\mu\text{g}/\text{kg}$ | Arctic Seal<br>$\mu\text{g}/\text{kg}$ | Arctic Seal<br>$\mu\text{g}/\text{kg}$ |
|-------|---|--|--|--|
| PFHpA | –   | –                                      | 0.08                                   | 1.05                                   |
| PFHxS | –   | 0.21                                   | –                                      | 0.21                                   |
| PFOA  | 0.25                                      | 0.35                                   | 2.28                                   | 4.37                                   |
| PFNA  | 0.07                                      | 4.78                                   | 1.72                                   | 1.76                                   |
| PFOS  | –   | 22.95                                  | 17.79                                  | 2.28                                   |
| PFDA  | –   | 2.82                                   | 12.59                                  | 1.09                                   |
| PFOSA | –   | 0.14                                   | –                                      | –                                      |
| PFUnA | –   | 5.45                                   | 0.44                                   | –                                      |
| PFDoA | 0.22                                      | 0.87                                   | –                                      | –                                      |
| PFTrA | –   | 1.97                                   | –                                      | –                                      |

a) Blank



b) Standard



c) Arctic Seal Extract

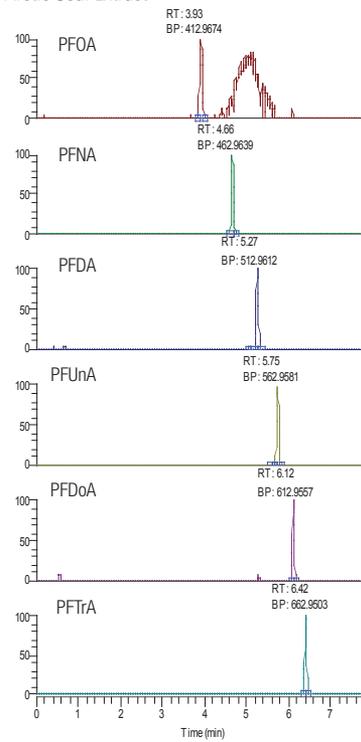


Figure 5. Extracted chromatograms: (a) Blank, (b) Standard, (c) Arctic seal extract

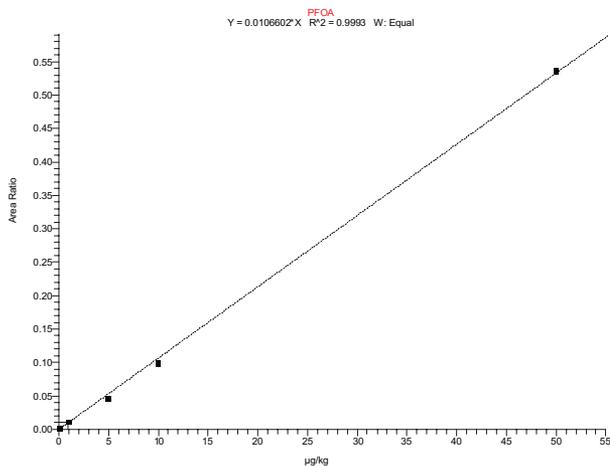


Figure 6. Calibration curve for PFOA

### Conclusion

Full scan in negative mode LC-MS acquisition on the Exactive LC-MS system with Orbitrap technology is suitable for quantification of PFAAs at low concentrations ranging from 0.1 µg/kg to 50 µg/kg in complex matrix extracts. No tuning or fragment determination is required and there is less background with high-resolution, accurate mass acquisition.

### Reference

1. Bossi, R.; Riget, F.F.; Dietz, R.; Sonne, C.; Fauser, P.; Dam, M.; Vorkamp, K. *Environmental Pollution* 2005, 136, 323-329.

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