A METHOD FOR DETERMINATION OF TRACE LEVEL OF TOTAL FLUORINE AND ORGANIC FLUORINE IN BIOTA, WATER, AND SEDIMENT USING COMBUSTION ION CHROMATOGRAPHY FOR FLUORINE

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Abstract
The number of perfluorochemicals (PFCs) that have been found in biological and environmental matrices is increasing as analytical standards and methods evolve. Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) constitute only a fraction of the total of PFCs found in environmental and biological matrices. A robust method and approach is needed to evaluate the mass of fluorinated compounds in environmental and biological matrices. In this study, we describe the analytical issues with the determination of TF and EOF (e.g., separation of inorganic and organic fluorine, co-elution of interferences, etc.), and possible sources of contamination of blank aimed at improving the sensitivity of the method. The method developed in this study is capable of detecting TF and EOF at parts-per-billion (μg as fluorine per litter: μg-F/L) to parts-per-trillion (ng-F/L) levels in biota, water, and sediment samples.

Introduction
A number of poly- and per-fluorinated compounds (PFCs) have been identified in the environment and biological samples. Whereas the number of fluorinated organic chemicals found in biota is increasing, it is not known if there exists any unidentified per-/poly-fluorinated compounds in the environment and biological samples including humans, wildlife tissues, water, and sediment. A robust method and approach is needed to evaluate the mass of fluorinated compounds found in environmental and biological matrices. Total fluorine (TF) and extractable organic fluorine (EOF) analyses are expected to provide useful information on potential discharges of any unknown PFCs into the environment. However, limit of quantitation of TF and EOF using conventional combustion ion chromatography (CIC) has been on the order of sub-parts-per-million (ppm) levels, because of some analytical issues (e.g., co-elution of interferences) and high background levels arising from instrumental blanks. In this study, we identified and removed the analytical issues for the determination of TF and EOF, and possible sources of contamination of blank were identified and eliminated to improve the sensitivity of the method.

Materials and Methods
Extraction. An aliquot of blood, water, and sediment samples was subjected to extraction steps for the analysis of EOF. Briefly, blood samples were extracted by an ion-pair extraction procedure using methyl-tert-butyl ether (MTBE), and the extracts were collected. The residue from this procedure was further extracted with hexane and combined with MTBE extracts for the analysis of EOF. Water samples were extracted by a solid-phase extraction (SPE) procedure using Oasis®WAX cartridges. Sediment samples were extracted by a solid-liquid extraction procedure using methanol and acetonitrile. The extracts from sediment were then subjected to further purification by SPE using Oasis®WAX cartridge described in details elsewhere.[1] TF was determined by taking an aliquot of blood, water, and sediment on a silica boat and placing it directly into the CIC.
CIC analysis. Concentrations of TF and EOF in samples were determined by using CIC. This method involves modifications to traditional combustion ion chromatography (CIC), by the combination of an automated combustion unit (AQF-100 (type AIST; Dia Instruments Co.Ltd. Japan) and an ion chromatography system (ICS-000; type AIST), Dionex Co. Ltd., Japan). The sample was set on a silica boat and placed into a furnace at 900–1000°C. Combustion of the sample in the furnace converted organofluorines and inorganic fluoride into hydrogen fluoride (HF). The HF was absorbed into sodium hydroxide solution (0.2 mmol/L). The concentration of F^- in the solution was analyzed using ion chromatography. The analytical conditions of the ion chromatography have been described in Miyake et al.[2, 3]

Results and Discussion

Separation of inorganic and organic fluorine in extracts. Separation of inorganic fluoride from the organofluorines is crucial for EOF analysis. The separation of inorganic fluorine from organic forms was examined by spiking known concentrations of sodium fluoride (1 mg F/L) into the samples, followed by liquid-liquid extraction for blood and SPE for water and sediment. Concentrations of fluoride in each of the fractions were analyzed by CIC-F. It was found that the organic extracts from the liquid-liquid extraction (i.e., MTBE and hexane extracts) did not contain (<0.006 mg F/L) spiked sodium fluoride and suggested negligible residue of inorganic fluoride in organic solvents. The recoveries of acidic PFCs, including perfluoroalkylsulfonates (PFASs – carbon numbers ranging from C2 to C8), perfluoroalkylcarboxylates (PFCAs – carbon numbers ranging from C5 to C18), and some fluorotelomer carboxylates through ion-pair and hexane extracts were between 71% and 109%. Because of the high water solubility of inorganic F^- (e.g., the water solubility of sodium fluoride is 18,000 mg/L), this anion is expected to partition into the aqueous phase rather than the organic phase.

In the case of SPE, the fluoride ion was found to adsorb strongly onto Oasis®WAX cartridges used for water and sediment samples. Therefore, a modified elution profile was developed in order to completely desorb F- from the cartridge. It was confirmed that the elution profile developed in this study adequately separated inorganic fluorine (i.e., fluoride) from organic fractions (< 0.006 mg F/L) with acceptable recoveries (81%–109%) for a wide range of PFCs including perfluoroalkylsulfonates (PFASs : C4–C8), perfluoroalkylcarboxylates (PFCAs : C4–C18), and some fluorotelomer carboxylates.

Removal of chloride and sulfate in absorbing solution. One of the issues with the determination of TF in samples is the occurrence of large amounts of chloride (e.g., Cl^-) and sulfate (e.g., SO_4^{2-}) that interfere with the analysis of TF in the samples. For example, the respective concentrations of chloride and sulfate ions are generally 20,000 and 2,500 times higher than the fluoride concentration in seawater samples. After combustion of samples, direct IC analysis of absorbing solution offered poor peak shape for the fluoride ion. Furthermore, certain low-molecular-weight organic acids such as formic acid, lactic acid, and acetic acid co-eluted with fluoride ion. The chloride and sulfate ions were removed by passing the solution through OnGuard II Ba, OnGuard II Ag, and OnGuard II H cartridges. The OnGuard II Ba and OnGuard II Ag cartridges respectively removed sulfate and chloride, by precipitation. The OnGuard II H, which has a very high selectivity for multivalent cations, removed sodium, silver, barium, and calcium. The ion chromatograms of samples before and after the passage through the OnGuard cartridges are shown in Figure 1. After the treatment, the fluoride peak and peaks for other organic acids were fully resolved, because the concentrations of chloride and sulfate in the samples had been decreased by <200 µg Cl/L and <500 µg SO_4/L, respectively. The removal rates of chloride and sulfate from water samples were greater than 99.5%, and the recovery of fluoride was 96.5%.

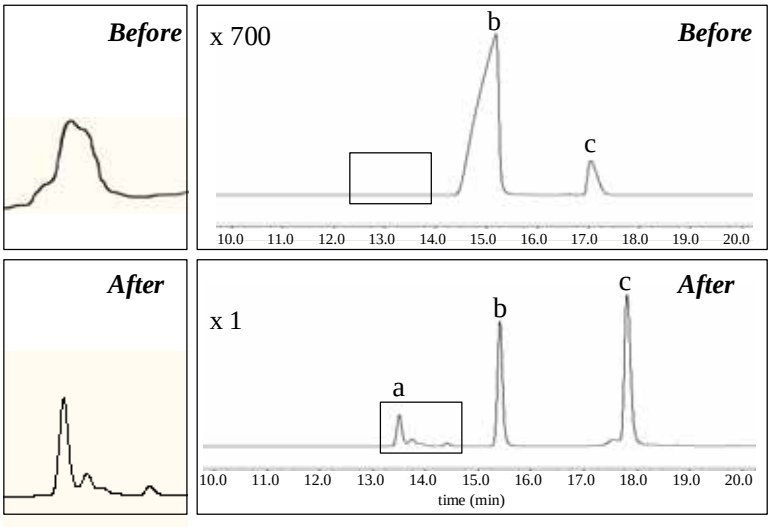

Figure 1. Chromatograms of fluoride, chloride, and sulfate before and after the treatment of samples by OnGuard II Ba/Ag/H cartridges. Peak 'a' corresponds to fluoride ion; Peak 'b' corresponds to chloride ion; Peak 'c' corresponds to sulfate ion.

Co-elution of fluoride and organic acids. One of the issues with the analysis of F^- using CIC is the co-elution of certain low-molecular-weight organic acids (such as formic acid, lactic acid, and acetic acid) with fluoride, resulting in potential interferences in measurements of TF and EOF. The separation of fluoride from the interfering organic acids was examined with two types of chromatographic columns having different ion-exchange properties. We tested an anion exchange column, IonPac AS17 (Dionex Corp., Sunnyvale, CA; 2 mm i.d. x 250 mm length, 10.5 µm), which is routinely used for the determination of inorganic anions. However, this column did not resolve fluoride from the organic acids. In contrast, fluoride and organic acid peaks were fully resolved when IonPac AS20 (77.5 µeq/column; 2 mm i.d. x 250 mm length, 7.5 µm), which has a 10-fold greater ion-exchange capacity than IonPac AS17 (7.5 µeq/column); this column was used under identical operating conditions.

Instrumental and reagent blanks. Several experiments were conducted to check for the sources of contamination of organofluorines and inorganic fluoride in instrumental blanks. Fluoride was detected in the sodium hydroxide solution that is used to absorb HF generated from combustion of samples containing fluorinated compounds. This suggested that the source of contamination is present within the CIC instrument or the gases. Gases were then replaced with high-purity gases (Ar: 99.9999%, O2: 99.9995%). Ion chromatograph tubing, gas lines, valves, and regulator, which contained materials or parts that are made up of polytetrafluoroethylene (PTFE), were replaced with either stainless steel, polyetheretherketone, or polyethylene tubing. Furthermore, a gas purifier containing activated carbon was placed in the gas line, to remove trace levels of fluorine from the gases. Following these modifications, backgrounds levels of fluorine in instrumental and reagent blanks decreased by more than 100-fold, compared to the level found in a traditional CIC (Figure 2).
The limit of detection (LOD) of organofluorine was evaluated for each sample, based on the maximum blank concentration, the concentration factors, and the injection volume of the sample. The LOD for blood, water, and sediment samples were 3 ng F/mL (ppb), 3ng F/L (ppt), and 3 ng F/g (ppb), respectively, when 1 mL of blood, 1L of water, and 1g of sediment were used for analysis, in a final volume of 0.5 mL. Thus, CIC-F with low background contamination and improved sensitivity for fluoride by >2-3 orders of magnitude, compared to that of a conventional CIC, was attained.

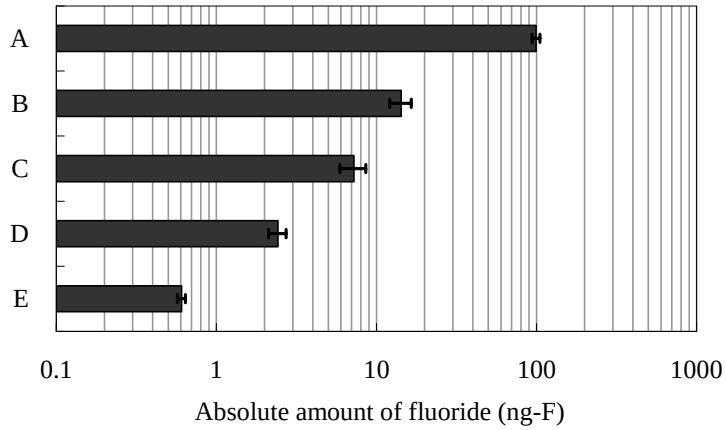

Figure 2. Absolute amount of fluoride in CIC instrumental blanks (n=4). (A) With use of standard gases and equipment; (B) After changing from standard gases to high purity gases; (C) After removal of fluoropolymer parts in ion chromatograph, gas lines, valves, and regulators; (D) After addition of activated carbon to trap impurities from the gases; and (E) After changing from syringe pump made up of fluoropolymer to ceramic syringe pump.

Mass balance analysis of fluorine. Contributions of known PFCs (e.g., PFOA and PFOS) to EOF and TF are shown in Figure 3, as an example of mass balance analysis of fluorine. The contributions of known PFCs to TF in human blood from reference and exposed individuals were 3.6% and 75%, respectively, while in seawater they were <0.0005%. Fluoride ion (F^-) accounted for a major proportion of TF in the seawater (93% and 82.5% for the reference site and the

contaminated site, respectively). This result suggests that the separation of inorganic fluoride from the organofluorines is crucial to provide information on environmental behavior and sources of organofluorines.
In addition, the contributions of known PFCs to EOF in human blood from reference and exposed individuals were 84% and 99%, respectively, while in reference and contaminated seawater they were 0.89% and 34%, respectively. This suggested that the known PFCs contributed little to EOF in seawater. Within the organofluorine fraction, a major portion still remains unidentified in seawater samples, suggesting the occurrence of unknown fluorinated compounds in addition to the known PFCs such as PFOA and PFOS.
Application of the mass balance approach to source materials (e.g., industrial products), and to environmental and biological samples, will provide valuable information on the extent of contamination by other unidentified fluorochemicals in the environment.

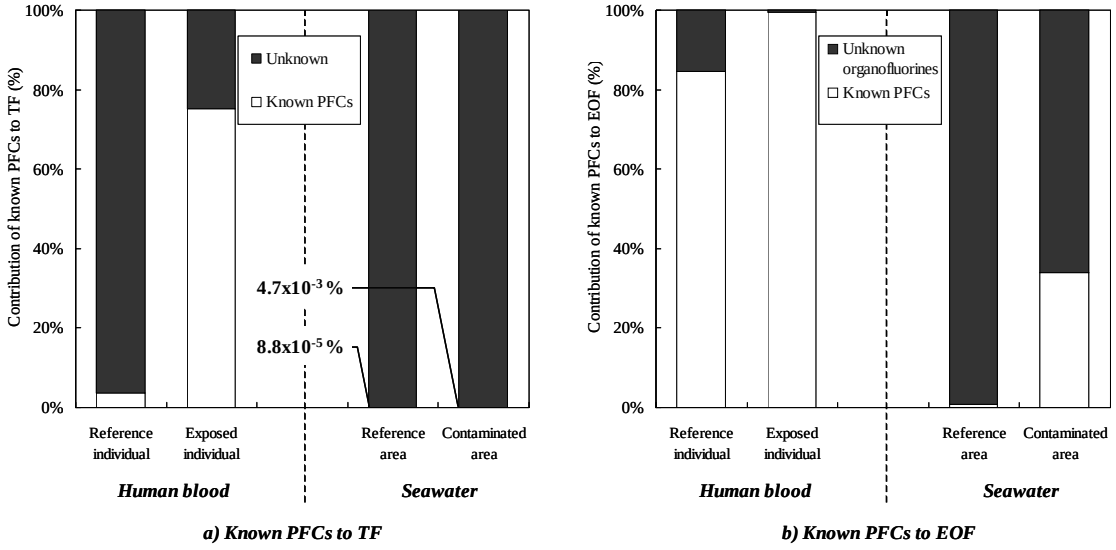

Figure 3. An example of contribution of known PFCs to total fluorine (a) and extractable organic fluorine (b) in human blood and seawater.

References

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3. Miyake, Y., Yamashita, N., So, M.K., Rostkowski, P., Taniyasu, S., Lam, P.K., Kannan, K. *J. Chromatogr. A* (In press).