On-line SPE–Nano-LC–Nanospray-MS for Rapid and Sensitive Determination of Perfluorooctanoic Acid and Perfluorooctane Sulfonate in River Water

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Abstract

An instrumental set up including on-line solid-phase extraction, nano-liquid chromatography, and nanospray mass spectrometry is constructed to improve the sensitivity for quantitation of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in surface water. Sample volumes of 1000 µL are loaded onto a microbore 1.0-mm i.d. × 5 mm, 5 µm Kromasil C_{18} enrichment column by a carrier solution consisting of 10mM ammonium acetate in acetonitrile–water (10:90, v/v) at a flow rate of 250 µL/min, providing on-line analyte enrichment and sample clean-up. Backflushed elution onto a 0.1-mm i.d. × 150 mm, 3.5 µm Kromasil C_{18} analytical column is conducted using an acetonitrile–10mM ammonium acetate solvent gradient from 30% to 70% acetonitrile. Water samples are added with internal standard (perfluoroheptanoic acid) and filtrated prior to injection. The mass limits of detection of PFOA and PFOS are 0.5 and 1 pg, respectively, corresponding to concentration limits of detection of 500 pg/L and 1 ng/L, respectively. The total time spent on sample preparation, chromatography, and detection is approximately 12 min per sample. The method was employed for the determination of PFOS and PFOA in urban river water.

Introduction

Perfluorinated compounds have emerged as an important class of global environmental contaminants. These compounds, which have been used for many industrial applications as well as consumer applications, have been found to be widespread in nature (1,2). Because of concerns about its biopersistance, effects on living organisms, and widespread exposure to workers, human populations, and wildlife, the company 3M announced in 2000 it would discontinue production and distribution of the perfluorinated compound perfluorooctanesulfonate (PFOS). However, these compounds are still produced and processed by others. Thus, monitoring of PFOS and other perfluorinated compounds, such as perfluorooctanoic acid (PFOA), in biological fluids and the outer environment is important to human health and the environment in general.

Lately, liquid chromatography (LC) coupled to mass spectrometric (MS) detection has evolved to be the preferred technique among laboratories monitoring these compounds (1–4). Despite the use of highly selective and sensitive LC–MS methodology, most published methods still often include off-line solid-phased extraction (SPE) or liquid extraction (LE) in their methodology for sample clean-up and analyte enrichment (1–4) because the compounds are often present in the low ppt (ng/L) region. Such SPE procedures can often be time consuming and require manual handling. For instance, the total sample preparation per sample can last as long as 7 h per sample (2). However, the use of analytical instrumentation providing higher sensitivity and potential for automation of the total analytical procedure allows preparation of less amount of sample or improve the total method's limit of detection (LOD), in addition to substantial time and labor savings. Miniaturized LC–MS column switching approaches offer such potential.

It is well documented that the use of columns with smaller inner diameters reduces the radial dilution of the chromatographic band (5). For instance, by injecting the same amount of a compound in a 2.1-mm i.d. column and a 0.1-mm i.d. nano column, the sensitivity can, in theory, be improved by a factor of approximately 400 using the latter (5), using the same MS. Nanobore columns are mostly used at flow rates of 1 µL/min or less, allowing a nano-LC system to be coupled with a nanospray interface. Nanospray interfaces are often more sensitive than regular electrospray interfaces because the initial droplets emitted from the narrow bore fused silica capillary are significantly smaller in size compared with droplets emitted from normal stainless steel capillaries, providing a more efficient ionization (6,7).

LC systems are easily coupled on-line with SPE in a column-switching system (8,9), and this approach has also been used for monitoring PFOS and PFOA in blood and river water (10–12). On-line SPE–LC is easily automated, greatly reducing

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the time used for manual sample preparation. Also, much larger volumes can be quickly injected into an SPE–LC switching system (often 1 mL or more can be injected in a just a few minutes) than with a regular LC system (5–20 µL) without compromising the chromatographic performance. Another possible advantage with a stainless steel housed on-line SPE column is that they are less likely to be a source of contamination than one-time use SPE cartridges with a plastic housing, which have been reported to often be a source of PFOS and other perfluorinated compounds (2).

Methods based on capillary or nano-LC coupled with MS have, to some extent, been used lately for environmental analyses and biomonitoring purposes (13–16). The number of reported nano-LC–MS methods, which provides substantially improved mass sensitivity when compared with capillary LC in addition to the attractive advantage of coupling to the nanospray MS interface, has, for the most part, been reserved for peptide mapping within the field of proteomics. In this paper, the development of a miniaturized on-line SPE–LC–MS method, constructed with the intent of improving the sensitivity for quantitation of PFOA and PFOS in waters, is reported. Justification of using nano-bore columns and nanospray-MS instrumentation was made by sensitivity comparisons with columns of larger diameter and a conventional electrospray interface, but with the same single-MS. The system was optimized with regard to sensitivity, selectivity, and robustness. To the author's knowledge, this is one of the first reported demonstrations of nano-LC coupled with nanospray MS used for water analysis and environmental analysis in general.

Experimental

Materials and reagents

High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) was obtained from Rathburn Chemicals Ltd. (Walkerburn, UK), and HPLC water was obtained from Fluka (Steinheim, Germany). Reagent-grade ammonium acetate (NH_4Ac), 99% perfluoroheptanoic acid (PFHA), and 98% PFOA were provided by Sigma-Aldrich (St. Louis, MO). PFOS (98%) was purchased from Fluka. Fused-silica capillaries were purchased from Polymicro Technologies Inc. (Phoenix, AZ), and nitrogen (99.99%) was purchased from AGA (Oslo, Norway).

Columns and tubing

Analytical columns (0.1 mm i.d. × 150 mm and 0.3 mm i.d. × 150 mm) and HotSep Tracy enrichment columns (1.0 mm i.d. × 5 mm) were purchased from G&T Septech (Kolbotn, Norway). The analytical and enrichment columns were packed with 3.5- and 5-µm 100 Å Kromasil C_{18} particles, respectively.

Fused-silica tubing (30 and 50 µm i.d.) was used for connections with the 0.1-mm column and the 0.3-mm column, respectively.

Chromatographic system

An Agilent Series 1100 capillary gradient pump with an incorporated on-line vacuum degasser was used to deliver the mobile phase, providing back-flushed desorption from the enrichment column and elution on to the analytical column.

Elution of the analytes was conducted using a solvent gradient where solvent A consisted of ACN–H_2O (10:90, v/v) and solvent B consisted of ACN–H_2O (90:10, v/v), both containing NH_4Ac at a concentration of 10mM. The gradient started at 30% B and was increased to 70% B in 5 min, and it was held at 70% B for 2 more min. The mobile phase was delivered at a constant flow rate of 0.7 µL/min. A Hitachi L-7110 isocratic LC pump from Merck (Darmstadt, Germany) was used for sample loading. The solution used for sample loading consisted of ACN–H_2O (10:90, v/v) containing 10mM NH_4Ac, and it was delivered at a flow rate of 250 µL/min. A Valco Cheminert Model C4 injection valve or a Valco Cheminert C2 six-port valve (Cotati, CA) was used for the manual injection of sample volumes of 0.02 to 1000 µL. A Valco Cheminert C2 six-port valve was also used for column-switching.

MS detection

The analytical column was connected to a SiO/Pt coated fused-silica Picotip provided by New Objective (Woburn, MA) with a tip of 50 µm o.d. and of 30 µm i.d. The Picotip was incor-

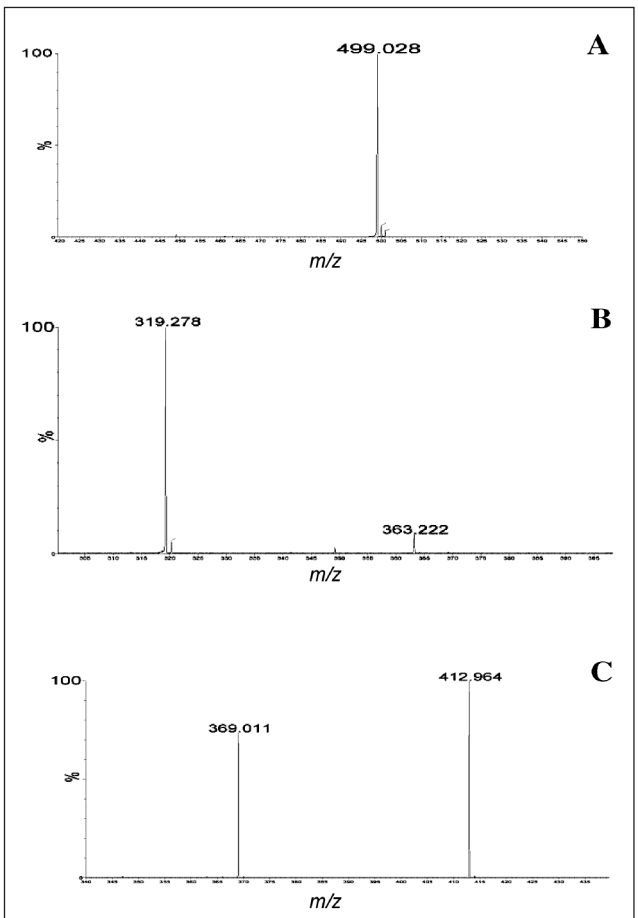

Figure 1. Mass spectra of PFOS (A), PFHA (B), and PFOA (C) (all 1 µg/mL with 1 min acquisition times) using a nanospray interface with a capillary voltage of –1800 V. The mass spectra were obtained by infusing 1 µg/mL solutions of the substances dissolved in ACN–H_2O (50:50, v/v) containing 10mM NH_4Ac.

porated in a t-coupling from Valco modified to provide nanospray action with the option of applying nebulizer gas. The t-coupling was arranged on an *x-y-z* stage for needle position optimization. Mass analysis of the column effluent was provided by a Micromass LCT time-of-flight (TOF)-MS (Micromass, Manchester, UK). Ionization was performed in negative ion mode. PFOS was monitored as $[M-H]^-$ at m/z = 499.0, PFOA was monitored as $[M-H]^-$ and $[M-COOH]^-$ at m/z = 412.9 and 368.9, respectively, and PFHA was monitored as $[M-H]^-$ and $[M-COOH]^-$ at 363.2 and 319.3, respectively (see Figure 1). The following voltages were used: –1.8 kV on the capillary, –30 V on the sample cone, and –3 V on the extraction cone. The TOF-MS instrument was controlled by MassLynx V 4.0 software, and mass spectra were acquired in the m/z range 200–650.

Standard solutions

Stock solutions of 0.1 g/L of PFOA, PFHA, and PFOA were made by dissolving 20 mg in 200 mL ACN–H_2O (50:50, v/v). Calibration solutions (20, 100, and 500 ng/L analyte and 1 µg/L internal standard) were made by diluting stock solution with HPLC-grade water (n = 3). Validation solutions for within- and between-assay studies were of concentrations of 20, 50, 100, 250, and 500 ng/L analyte and 1 µg/L internal standard.

Sample preparation

River water samples from Akerselva River (Oslo, Norway) were collected in polypropylene bottles and stored frozen. Prior to injection, samples were filtered with 0.45-µm Minisart filters (Satorius, Gottingen, Germany), and 9.90-mL samples were added 0.100 mL of 0.1 mg/L PFHA solution.

Quantitation

Calibration curves of PFOA and PFOS used the peak area ratio of the analytes to PFHA (PAR) and the analyte concentration (C) as given in the following equation: (PAR) = slope × (C) + (y-intercept). The slope and y intercept were determined by linear regression analysis.

Precision and LOD and limit of quantitation

Within-assay precision was evaluated by analyzing three levels of controls using four replicates of each, and between-assay precision was evaluated by analysis of one replicate at each level once for 3 days.

The concentration LOD (cLOD) and mass LOD (mLOD) was defined as the concentration or mass that produced peak heights that were three times the noise. The concentration limit of quantitation (cLOQ) was the lowest non-zero concetration level that could be quantitated with RSD (%) ≤ 20%.

Results and Discussion

Analyte enrichment and Chromatography

Mobile phase

Separation of PFOA, PFHA, and PFOS was achieved on a C_{18} column with acetic acid, formic acid, or ammonium acetate as additives using gradient elution. However, electrospray ionization (ESI)-MS direct infusion studies showed that mobile phases containing 10mM ammonium acetate provided the highest signal intensities for both PFOA and PFOS. Thus, 10mM NH_4Ac, which also provides pH control and analyte ion pairing was used as an additive in this study. A 5-min linear mobile phase gradient from 30% to 70% acetonitrile provided separation of the compounds of interest within 6 min.

On-line column switching

Upon transfer of the solutes from the SPE column to the analytical column, refocusing on the analytical column (packed with 3.5 C_{18} µm particles) was supported by using an enrichment column packed with larger and, thus, less retaining 5 µm C_{18} particles.

The use of an enrichment column with a larger inner diameter (1 mm) than the analytical column was used in order to improve the mass and volume capacity of the method. The chromatographic performance on the analytical nano-column was not affected by using an enrichment column with such a large inner diameter, as is often observed for less hydrophobic analytes, as the hydrophobic nature of both PFOA and PFOS provides elution from the enrichment column to the analytical column in a narrow band. The RSD (%) of the retention times for the analytes and the internal standard were ≤ 1% with and without employing the switching system.

Loading solvent

The organic modifier content in the loading solvent in on-line SPE–LC is traditionally adjusted to a low level to ensure solvatization of the alkyl ligands of the stationary phase but without eluting the analytes. However, in order to obtain simultaneous target analyte enrichment and efficient sample clean-up, our goal was to develop a method including as much organic modifier as possible in the loading solvent without analyte break-through. Loading solvents containing 10mM ammonium acetate and various amounts of the organic modifier, ACN, were thus investigated with regard to analyte break-through. Break-through was monitored by connecting the enrichment column directly to the MS and monitoring extracted ion chromatograms (EIC). The loading solvent (250 µL/min) could contain 10% ACN and fully retain the analytes during a test period of 30 min. However, with a loading solvent containing 15% ACN, the analytes eluted from the column after 13 min. Loading solvents with 10% ACN was thus used, still providing on-line sample clean-up for a large portion of other more hydrophilic components of no interest.

Loading flow rates and injection volumes

To obtain low cLODs with an SPE–LC system, as large a sample volume as possible was injected in order to accumulate as much analyte as possible on to the enrichment column. Furthermore, the loading time of the large sample should be short to minimize the total analysis time. HPLC-grade water spiked with PFOS and PFOA was injected at various flow rates and with different injection volumes to establish if elevated loading flow rates or large injection volumes would promote an

undesired compound break-through. The correlation coefficient of peak area as a function of injection volume was 0.99 for both PFOA and PFOS when injecting volumes of 50–1000 µL, indicating that analyte break-through was not a factor when injecting volumes up to 1 mL.

The peak area and peak shape of both PFOA and PFOS were unaltered by loading flow rate, tested from 50 to 250 µL/min, the latter provided a "reasonable" back pressure of 80 bars. With a loading flow rate of 250 µL/min, the loading time of 1 mL was set to 4 min (250 µL × 4 = 1 mL) plus 1 min to ensure complete transfer to the enrichment column, resulting in acceptable total analysis times

Contamination and carry-over

In preliminary experiments, analyte contamination from the HPLC system for both PFOA and PFOS was experienced when injecting blanks. The loading pump was identified as the main source of this problem. However, contamination from the loading pump was unnoticeable after replacing the plastic tubing (possibly treated with fluoropolymers) between the solvent reservoir and the pump with steel tubing. There was no noticeable contamination contributed from the analytical pump, in contrast to another study using an equivalent pump (2). The reason for this may be that the pump flow in our study was 1 µL/min or less, so much less analyte contaminant from tubing etc., would be able to refocus on the analytical column before the onset of a solvent gradient than when operating at a flow rate of 300 µL/min, for example. Thus, miniaturization and low flow rates might have a positive influence by minimizing the carry-over effects originating from the chromatographic system, in addition to the obvious advantages related to improved mass sensitivity. The carry-over effects for the total method was less than 0.5% for PFOA and less than 2.5% for PFOS, tested by injecting blank solutions (i.e., HPLC-grade water) after injections of the standard solution with the highest concentration. Spiked samples containing 10% ACN were injected to examine if the carry-over effect could be reduced, potentially reducing adsorption of the analytes to the valves, which could be the cause of carry-over. However, no difference in carry-over was observed between solutions with or without ACN. The on-line SPE columns had a stainless steel housing (in contrast to most off-line SPE cartridges) and were, therefore, not a source of contamination.

However, as the carry-over of our system cannot be neglected, ACN was injected after analysis of highly concentrated samples. This action eliminated carry-over effects in the method.

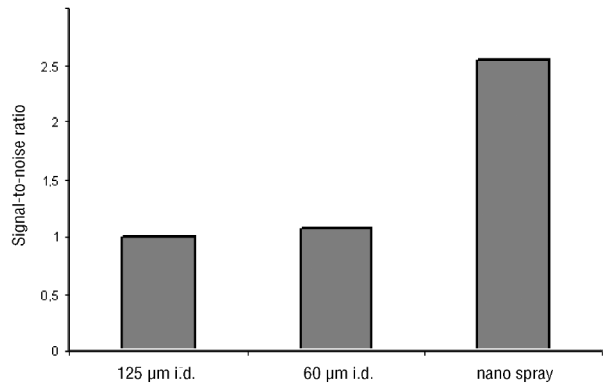

Figure 2. Signal-to-noise ratios of three electrospray interface variations [i.d., 125 µm, 60 µm, and nanospray (30 µm)]. The optimal flow rate for the narrow-bore spray capillary, micro-bore spray capillary, and nanospray capillary was 10, 5, and < 1 µL/mL, respectively.

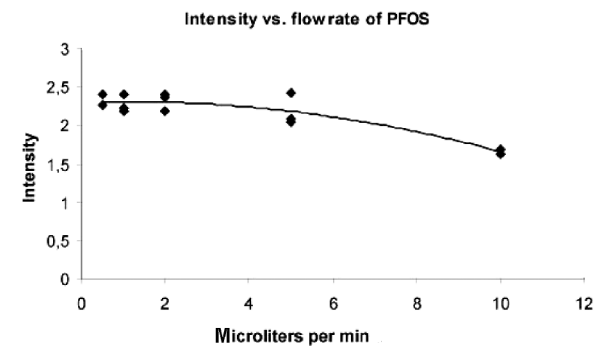

Figure 3. Plot of the PFOS signal intensity as a function of flow rate when employing a nanospray interface.

Column robustness

During preliminary nano-LC experiments without a switching system, the back-pressure on the nano-column often increased after a number of injections. However, when the switching system was included, the pressure was stable throughout the study, as the enrichment column functioned as a solvent and sample filter, providing efficient sample clean-up.

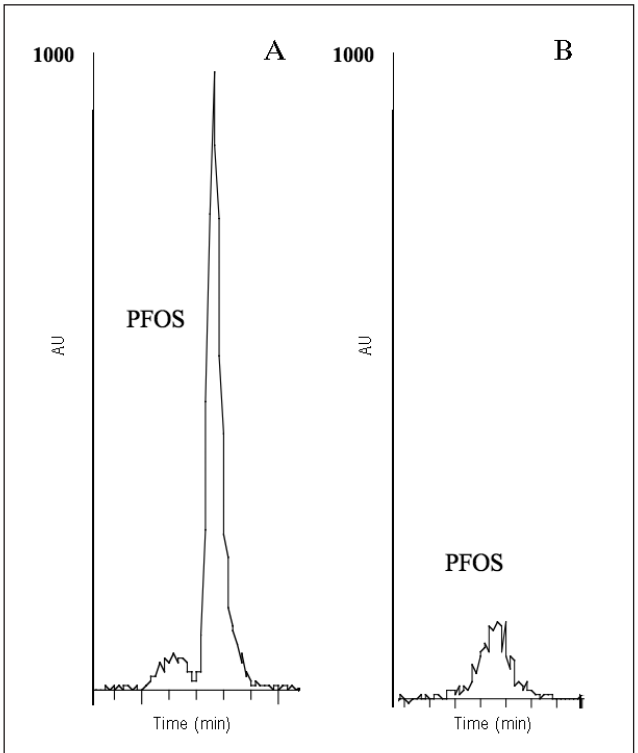

Figure 4. Chromatograms of PFOS (100 pg) injected in to a nano LC-nanospray MS system (A) and a capillary LC-electrospray MS system (B).

Different enrichment columns of the same type produced similar back-pressures of approximately 80 bars at 250 µL/min. Although enrichment column replacement was not necessary during the study, it was confirmed that peak shapes and areas of the solutes of interest were not affected by replacement of a well used enrichment column with a new column.

Negative electrospray MS detection

Interface evaluation

As expected, when coupling a nano-column (flow rates of 1 µL/min or less) with a regular electrospray interface, it was observed that the intensity of the chromatographic peaks was lower than with a 0.32-mm column (flow rate 5 µL/min). This implied that the potential gain in peak height using a nano-column was counterbalanced by the conventional electrospray interface's inability to produce a decent electrospray at nano-LC flow rates. However, direct infusion experiments confirmed that the use of a nanospray interface provided improved sensitivity, illustrated by a signal-to-noise ratio 2.5 times higher than the best S/N values obtainable with the conventional electrospray interface (Figure 2). Furthermore, the sensitivity of the nanospray interface increased when lowering the flow rate and was unaffected by the flow rate from 1 µL/min down to 0.4 µL/min (Figure 3).

Nano-LC coupled with nanospray MS

With the nano column (0.1-mm i.d., pore size 300 Å) connected to the nanospray interface, the PFOS peak intensity increased 7 times compared with that of a capillary column (0.3-mm i.d., 100 Å) coupled to a regular ESI interface (Figure 4), injecting 100 pg in both cases. Theoretically, the peak intensity could increase by a factor of approximately 9 when switching from a 0.3-mm column to a 0.1-mm column if all parameters are equal except for the radius of the column (5). There can be several reasons for this deviation (e.g., the band dilution contribution of the tubing between the column and the spray source was calculated to be 15% with the nano-LC system, in contrast to below 5% with the capillary LC system).

Emitter performance

The applied voltage was found to affect the lifetime of the nanospray emitter. For PFOA and PFOS, optimal conditions were achieved with a relatively low capillary voltage (–1800 V) when employing the SilicaTip emitters. These conditions can be considered to be relatively mild, sustaining the lifetime and performance of the emitter. However, increasing the voltage to –2000 V or more created an electric discharge at the tip, and the emitter quickly suffered performance decay and needed to be replaced within a few hours. It was noticed that the SilicaTips were easily damaged and need replacement after rather mild contact with other objects. However, tip replacement was simple and did not affect the system performance to any noticeable extent. Figure 5 shows that the PFOS extracted ion monitoring signal (intensity vs number of scans) was virtually unaffected by changing from a 5-day old emitter (used approximately 8 h a day) to a new emitter. In the final method, the emitter was replaced routinely every week.

Nanospray performance with solvent gradients

When running solvent gradients with MS detection, electrospray interfaces can lack robustness with regard to producing a stable electrospray if the solvent surface tension differs from the conditions used for optimizing the interface. The extent of this behavior was likely to be dependent on the pH or additive of the mobile phase. For instance, when running gradients with formic acid instead of ammonium acetate, the mass spectra during the start of the gradient were randomly noisy and were vastly dominated by water clusters, followed by a sudden, dramatic decrease of cluster signals and a decrease of the baseline noise when approaching the solvent conditions used for tuning the MS (results not shown). However, when employing ammonium acetate solutions, these effects were not as severe, but the baseline noise varied more during a gradient run than with an electrospray interface with a steel capillary. These conditions did not, however, affect the relative signals of PFHA and the analytes because they all elute at a time where the solvent conditions are approximately equal to the solvent composition used for tuning.

Method evaluation

The carefully optimized method was critically evaluated with respect to performance. For determining the cLOD of the system and to ascertain that no analyte was present before spiking with known amounts, standard solutions of various concentrations in HPLC-grade water were used instead of river water. The cLODs were established to be 0.5 and 1 ng/mL for PFOA and PFOS, respectively, using injection volumes of 1 mL, corresponding to mLODs of 0.5 and 1 pg, respectively. Before and after injecting a spiked sample, a blank was chromatographed to ensure that no unusual carry-over effect would affect the assessment. When the cLOD was established, it was confirmed by three injections with blanks run in between. This is an improvement of a factor of approximately 5 as compared with those obtained by Takino et al., who reported a cLOD of 5.35 ng/L for PFOS using on-line SPE–LC–atmospheric pressure photoionization-MS instrumentation. Using an MS with multiple reaction monitoring (MRM) capabilities in our method might have lowered the LODs even further,

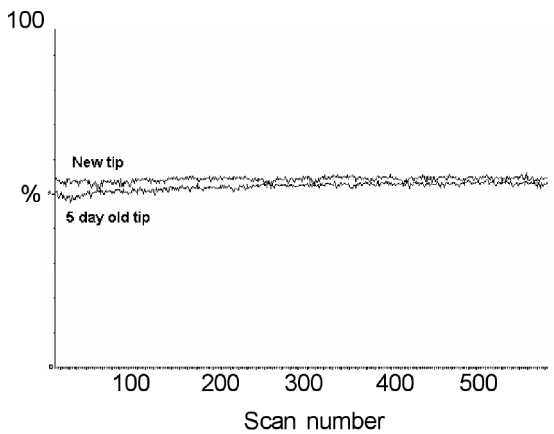

Figure 5. Direct infusion SIM of *m/z* = 499 (PFOS) using a 5-day-old nano emitter and a brand new emitter.

according to our previous experiences with monitoring these compounds in plasma (10). Unfortunately, such instrumentation was not available for this project.

The linearity of the method, expressed as r^2, was 0.99 for both PFOA and PFOS when injecting 1 mL of HPLC-grade solutions with concentrations of the analytes from 20 to 500 ng/L. The accuracy of the method using non-filtered HPLC-grade water calibration solutions for quantitation of real samples was investigated. River water samples were spiked to 5–50 µg/mL of analyte, and only 10 µL was analyzed to eliminate the effect of analyte content present in the sample (low ng/L). The samples were filtered and spiked with internal standard. The response curve of the filtrated solutions was approximately 95% of the slope of the curve for HPLC-grade water solutions spiked at the same concentrations as the samples. All slopes had correlation coefficients of approximately 0.99. The recovery of the filtration step was thus defined as 95%. Hence, the approach of using non-filtered HPLC-grade calibration solutions was considered satisfactory for quantitation. The total method within- and between-day repeatability for PFOA and PFOS are found in Table I, and the RSDs were in the range 4–13 (within-day) and 8–17 (between-day). The method's modest repeatability, which can often be expected when employing a nanospray interface, was expected to be improved by replacing the internal standard PFHA with more expensive labeled standards.

Table I. Method Evaluation

	PFOA	PFOS
Absolute recovery after filtration	95%	95%
Responce factor relative to internal standard	1.13	0.95
Linearity (r^2)	0.99	0.99
Within day repeatabilty (RSD %) n = 4		
Level 1	8.4	3.7
Level 2	13	12
Level 3	8.9	5.7
Between day repeatability (RSD %) *n* = 3		
Level 1	14	17
Level 2	12	12
Level 3	11	8
Limit of detection (1000 µL injected)	500 pg/L	1 ng/mL
Carry over	0.5%	2.5%

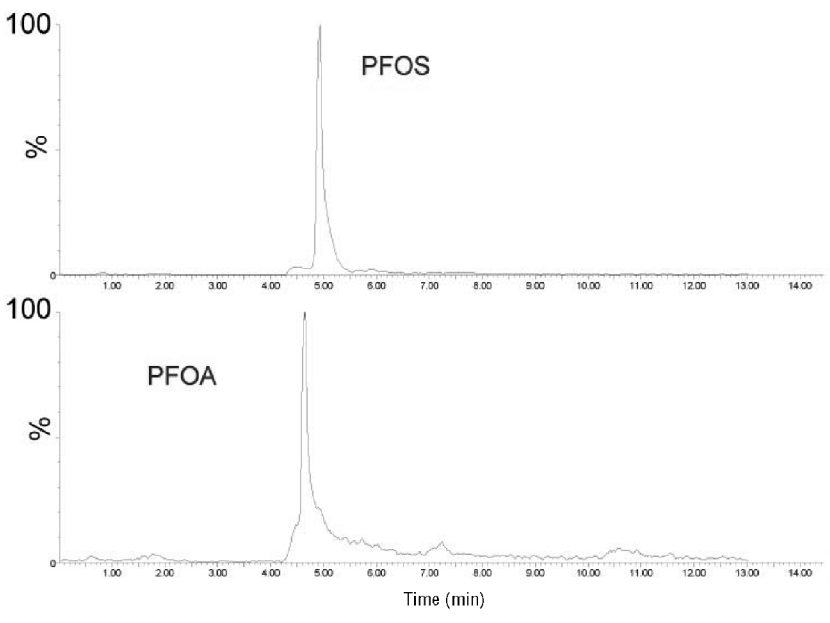

Figure 6. Chromatogram of PFOS and PFOA in a filtered water sample (1 mL injected) collected close to the end of Akerselva River in Oslo. The sample was spiked with PFHA prior to injection.

Determination of PFOA and PFOS in urban river water

The method was applied for determination of PFOS and PFOA of urban river water from Akerselva River (Oslo, Norway). The sample was filtered and spiked with internal standard, and 1 mL was injected in to the system. The time used for filtration, internal standard spiking, enrichment column loading, and chromatographic determination was less than 12 min. Both PFOA and PFOS were found in the sample, and their concentrations were estimated to be 59 and 130 ng/L for PFOA and PFOS, respectively (Figure 6). Subsequent analysis of the same river water sample revealed within assay RSDs in accordance with the performance from the method evaluation studies of spiked HPLC-grade water.

Conclusion

A system based on on-line SPE–nano-LC combined with nanospray-MS is sensitive enough to provide monitoring of PFOA and PFOS present at low ppt range. The method is relatively fast; sample preparation and analysis only takes 12 min per sample. The speed of the method is attributed to the sensitivity of the instrumental set-up, as only 1 mL of sample is necessary for ppt range monitoring. The miniaturized system has proven to be robust, both with respect to quantitation and instrumental aspects. The method is easily automated and is intended to be applied for monitoring of PFOS and PFOA in water samples.

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