

## Fast and Sensitive HILIC-ESI-MS Technique for the Screening of Perfluorooctane Sulphonate in Environmental Water

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**Abstract:** Perfluorooctane sulphonate(PFOS) is in use for several decades as surfactants in a wide variety of industrials and consumer products. Potential health risk of PFOS include developmental toxicity, cancer and bioaccumulation. In the present study a fast and sensitive liquid chromatography mass spectrometry(LCMS) method has been developed using high performance hydrophilic interactive liquid chromatography(HILIC) based stationary phase and electrospray ionization(ESI) mass spectrometry(MS). The limit of detection and quantification of PFOS was 0.1 µg/L and 0.2 µg/L respectively and response was linear up to 1 µg/L with correlation coefficient of 0.9999. Water samples obtained from different environmental sources were spiked with 0.2 µg/L of PFOS and subjected to preconcentration on a solid phase extraction cartridge prior to LCMS analysis showed excellent recoveries ranging within 83.7 to 101.0%.The method was validated with respect to linearity, accuracy and precision using one way analysis of variance(ANOVA) and regression statistics.

**Keywords:** Perfluorooctane sulphonate, Water pollution, HILIC, ESI-MS

### Introduction

In the 21<sup>st</sup> century a continuously growing human population with a limited supply of freshwater based on supply and demand, drinking water resources may be one of the most vital environmental issues. Perfluorooctane sulfonate (PFOS) belongs to the family of perfluorinated chemicals, which have been increasingly used as surfactants in a number of industrial and consumer products which includes paints, adhesives, waxes, polishes, electronics, fire fighting foams, paper as well as grease-proof coatings for food packaging<sup>1</sup>. PFOS is chemically both hydrophobic and lipophobic and contains C-F bond which is one of the strongest known chemical bond. This in fact makes PFOS highly stable in environment and account for its unique profile of distribution in the body<sup>2</sup>. Environmental protection agency of USA (US EPA) set a provisional short-term health advisory level of 0.4 µg/L in drinking water. At the same time, USEPA set a provisional short-term health advisory level of 0.2 µg/L for perfluorooctanesulfonic acid (PFOA)<sup>3</sup>.

Owing to their persistent bioaccumulative and toxic (PBT) characteristics, it has been considered as a major environmental pollutant by several regulatory agencies. A recent study published in the journal of toxicology research indicates the adverse effect of perfluorooctane sulfonate (PFOS) on brain tissue and calcium channel downstream signaling processes<sup>4</sup>.

The erstwhile analytical approaches for the determination of PFOS included combustion methods, infrared spectroscopy(IR), nuclear magnetic resonance(NMR) and x-ray fluorescence<sup>5,6</sup>.

However, these techniques were nonspecific, without the capability of separating different classes of perfluorinated compounds (PFC) and did not have the sensitivity and selectivity required for natural water samples. Another well-utilized methodology for determining environmental contaminants, gas chromatography-mass spectrometry (GC-MS) was also used to detect a wide range of PFCs<sup>5-13</sup>. However, certain classes of fluorinated alkylated substances and perfluorinated surfactants including PFOS, do not form stable volatile derivatives and were not detectable using GC/MS methods.

Recently, high performance liquid chromatography (HPLC) techniques have been developed to be applicable to the analysis of all classes of PFCs even without a derivatization step, with better sensitivity and selectivity than GC methods<sup>5,6</sup>. LC methods reduce sample preparation steps and have flexibility with several conventional detectors applicable for analysis of PFCs. Some of these widely used fluorescence and UV detectors were affordable and applicable with LC for PFC analysis. However, fluorescence and UV detectors were less advantageous than MS because, they often require the addition of chromophores for PFC detection and accurate/precise measurements are often lacking without a secondary detector for confirmation and quantification purposes. Compared to these conventional detectors, LC-electrospray (ESI)-MS and LC-tandem MS were found to be the most suitable and sensitive analytical techniques and therefore used to focus on PFOS and PFOA (Perfluorooctanesulfonic acid) analysis.

The present HILIC-ESI-MS technique is sensitive enough to rapidly determine the amount of PFOS at appreciable lower concentration of 0.1 µg/L. The method has advantages over the previously reported techniques in the literature with respect to shorter analysis time and sensitivity. The method has also been applied for the determination of PFOS in drinking water samples collected in the city of Bhubaneswar, India. To the best of our knowledge studies based on zwitterionic based stationary phase, coupled to ESI-MS for the determination of PFOS in drinking water and environmental water has not been reported so far.

## Experimental

The MS and MS/MS studies were performed on 3200 Q-trap mass spectrometer (AB Sciex, CA, USA) using electrospray ionization source (negative polarity). The instrument was operated in multiple reaction monitoring mode with the following settings: collision energy of -20 V, declustering potential -10 V and the capillary ion spray voltage was -4500 V. Nitrogen was used as curtain gas and CAD gas at a pressure of 15 psi. Zero air at a pressure of 45 psi was used as nebuliser gas and heater gas. MRM transitions were selected from the Q1 MS analysis and collision induced dissociation spectrum of the mixture of PFOS as shown in Figure 1. The instrument response was optimized for PFOS by infusing a constant flow of the standard solutions of PFOS (1000 µg/L).

The HPLC consisted of LC-20AD binary gradient pump, SPD-10AVP UV detector, SIL-10HTC auto sampler and a column oven CTO-10ASVP (Shimadzu Corporation, Kyoto,

Japan). A ZIC HILLIC column (Merck, Darmstadt, Germany, 100 mm x 4.6 mm, 5  $\mu$ m, 200 Å) was used for chromatographic separation. Mobile phase A consisted of 10 mM ammonium acetate, pH adjusted to 5.5 with formic acid and mobile phase B consisted of acetonitrile (20:80v/v) in isocratic mode. The injection volume was 10  $\mu$ L, the flow rate was kept constant at 0.5 mL/min and the temperature of the column was set to 30 °C. The same eluent was also used for preparing the final solution of the standards of the PFOS. An oasis HLB cartridge (Waters Corporation, MA, USA) was used for enrichment of water sample. The total run time of the method was 5 minute including equilibration time.

### *Reagents*

The standard of potassium perfluoro octanesulfonate was obtained from Sigma-Aldrich (Bangalore, India). De-ionized reagent water was prepared using a Milli-Q plus water purification system from Millipore (Bradford, Pennsylvania, USA). LCMS grade methanol and acetonitrile were purchased from Merck India Limited (Mumbai, India). Analytical reagent grade ammonium acetate and formic acid were purchased from Qualigens India Limited (Mumbai, India). sample of surface water was collected from the city of Bhubaneswar, India.

### *General procedure*

Perfluorinated surfactants have been reported to get adsorbed to the glass containers<sup>12</sup>, therefore, all water samples were collected in polypropylene bottles. Water sample collected was divided into two different container of one litre each. One part was spiked with PFOS of 0.2  $\mu$ g/L and homogenized with a stir bar while the other part left untreated to be used as blank. Prior to spiking, the pH of the water sample was measured. The measurement of pH was done to establish that water taken for analysis is not highly alkaline or acidic as extreme pH may affect the efficiency ion exchange cartridge. Both the samples were filtered for solid particles and preconcentrated using an offline approach onto Oasis cartridge. 5 mL of methanol and 5 mL of deionized water passed sequentially to condition the cartridge followed by 1 L of sample water. Finally the compounds trapped on the sorbent were eluted with 5 mL of methanol and 5 mL of deionized water containing 0.1% formic acid. Each of these extracts were reduced to 100  $\mu$ L under nitrogen and brought up to volume of 1 mL with the aforesaid HPLC eluent. The concentrated solution was filtered through 0.22  $\mu$ m GHP filter and analyzed by HILIC-ESI-MS under optimized conditions.

### *Sample preparation*

The drinking water sample collected was filtered through a 0.45  $\mu$ m membrane and stored at 4 °C in previously washed polypropylene bottles. The general procedure described above is then applied without adding any standard of PFOS.

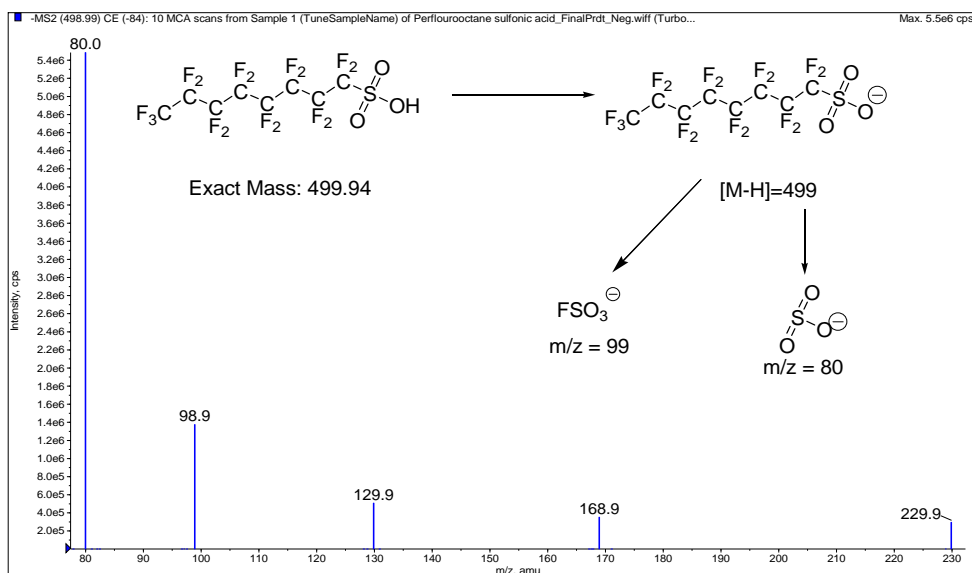
### *Optimization of the extraction step*

The recovery efficiency of the extraction procedure was optimized by adding known amount (0.2 ng/L) of PFOS to water samples which were previously examined for PFOS content. Another set of similar spike and recovery experiment was performed using deionized water that had been previously determined to contain no amount of PFOS. The spiked samples were then loaded to different solid phase extraction cartridges to determine recovery.

The highest recovery was obtained with the ion exchange oasis cartridge and 0.1% formic acid as eluent over classical C-18 cartridges. The matrix effect was also minimized under stated optimized condition.

## Results and Discussion

The retention time of PFOS was 2.14 minute and a run time of 5 minute was sufficient to determine the amount of PFOS content. Wide peaks and tailing were observed in the initial attempts using classical C-18 stationary phase and aqueous rich mobile phase. However use of zwitterionic stationary phase based HILIC column offered superior peak shape in addition to eliminating the need to use high concentration of non volatile buffer or ion pairing modifier. Peak shape also plays a critical role in quantification at very low concentration level and therefore HILIC was considered owing to its unique capability in improving peak shape within a reasonable run time, with MS friendly elutents. Because of an increase in sensitivity in multiple reaction monitoring (MRM) mode over full scan mode, MRM was employed for quantification of PFOS. The molecular ion of PFOS was observed at  $m/z$  499 and produced two key fragment ions at  $m/z$  80 and  $m/z$  99 as depicted in Figure 1. Although the daughter ion of  $m/z$  80 was of highest intensity and has been used for quantification, the other fragment ion at  $m/z$  99 corresponding to  $\text{FSO}_3^-$  was also monitored simultaneously for greater specificity of the fluorinated surfactant.



**Figure 1.** Representative collision induced dissociation spectrum of PFOS indicating product ions

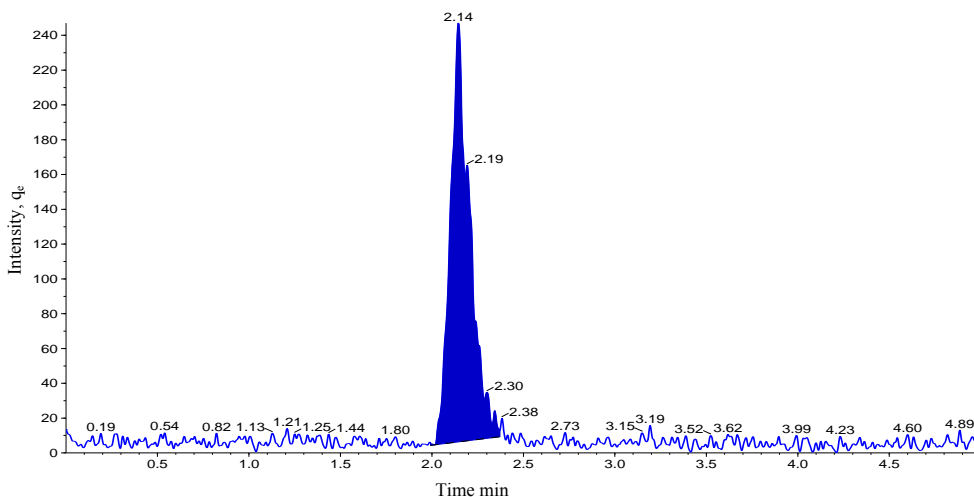
The spike and recovery experiments performed at 4 different concentration level in environmental surface water sample and deionized laboratory water showed almost identical recoveries ranging from 83.7 to 101.0%, with a mean  $95 \pm 3$  in three independent studies.

### *Effect of experimental conditions*

High concentration of organic phase in the eluent also helped to achieve lower DL and QL as they help in droplet formation in electrospray ionization and have an edge over highly aqueous mobile phase<sup>13</sup>. The perfluorinated surfactants are sorptive in nature, therefore methanol was injected periodically after every 10 injection of sample to maintain instrument cleanliness. Attention was also directed towards verification of instrument performance and

calibration curve validity as instrument carryover and contamination related issues can't be ruled out owing to the sorptive nature and ubiquitous presence of PFOS in laboratory environment. A typical chromatogram of PFOS at its quantification limit is shown in Figure 2.

PFOS-0.2 PPb - PFOS (Standard) 499.0/80.0 amu - sample 5 of 17 from POP.wiff  
Area: 1770. counts Height: 241. cps RT: 2.14 min

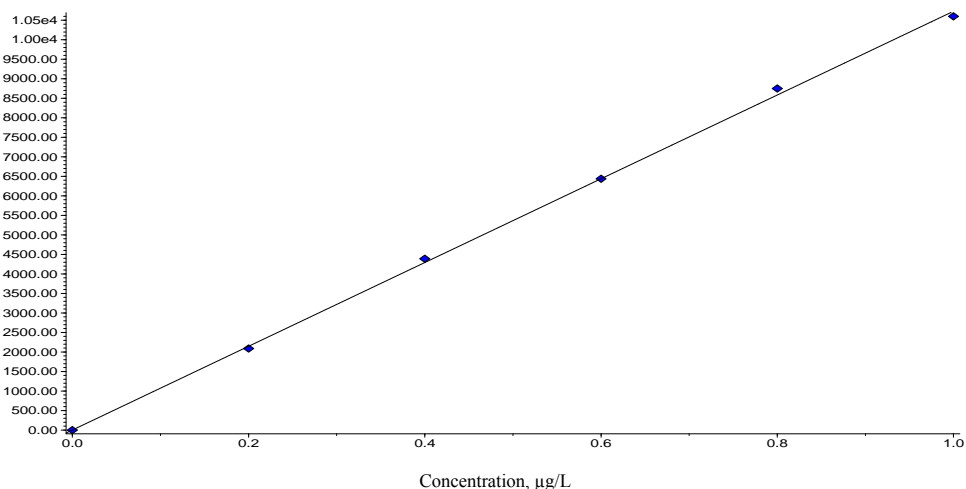


**Figure 2.** Typical chromatogram of PFOS at its quantification limit

#### Validation studies

The developed HILIC-ESI-MS method for the determination of PFOS in water was validated. The linearity was evaluated by preparing and analyzing 5 calibrators in the concentration range 0.2  $\mu\text{g/L}$  to 1  $\mu\text{g/L}$  using the optimized MS condition and MRM transition. The slope, intercept and regression coefficients were determined by the least squares linear regression analysis was achieved  $r^2:0.9999$  as represented in Figure 3.

PFOS.rdb (PFOS): "Linear Through Zero" Regression ("No" weighting):  
 $y = 1.07\text{e}+004 \times$  ( $r = 0.9999$ )



**Figure 3.** Linearity plot of PFOS in the concentration range of 0.2  $\mu\text{g/L}$  to 1.0  $\mu\text{g/L}$

System precision was done by injecting six replicate injection of the standard preparation and its RSD was determined to be 3.62%. Stability of analytical solution was evaluated at 10 °C for 24 hours and the solution was found stable during this period. ANOVA studies carried out at 5% level of significance also indicated significant linearity with F-ANOVA at 1367.64862. The detection limit and quantitation limit of PFOS were calculated on the basis of the lowest concentration of analyte that gives % RSD <3% and %RSD<10% respectively and thus The limit of detection and quantification of PFOS was established to be 0.1 µg/L and 0.2 µg/L respectively.

To confirm accuracy of the procedure PFOS was spiked in surface water sample and recovery was calculated in six independent experiments. The consistent recoveries with mean value of 95±5 indicated that the accuracy of the method is acceptable.

### Application

The proposed method was applied for the determination of PFOS in environmental water samples collected in the city of Bhubaneswar, India. Five samples were analyzed and the concentrations of PFOS were found below detection limit. Recovery test were also performed for two samples and values obtained were 95.3% and 94.9% respectively. The study demonstrates the applicability of this method for the analysis of environmental water samples.

### Conclusion

The HILIC-ESI-MS technique is sensitive enough to rapidly determine the amount of PFOS at appreciable lower concentration of 0.1 µg/L against the advisory level of 0.2 µg/L set by Environmental Protection Agency of USA. The limit of quantification allows determination of PFOS as per international regulations. The simplicity and fastness of the method is also well suited for routine applications in environmental analysis.

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