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Application Note

How to overcome challenges in PFAS analysis ?

Analysis of 11 perfluorinated compounds in waters using AttractSPE® PFAS

Water analysis

Introduction

This application note describes an accurate method for the analysis of 11 per-and polyfluoroalkyl substances (PFAS) in water by LC-MS/MS in accordance with EPA 533. The method relies on AttractSPE® PFAS a specially designed SPE cartridge for the purification and concentration of PFAS, SilactHPLC DELAY - PFAS as delay column, and SilactHPLC LC - PFAS. In addition, numerous precautions are described to avoid contamination and to minimize PFAS presence in blank controls.

Perfluorinated compounds (PFAS) are a large family of molecules consisting of varying lengths of fluorocarbons chains with a functional group such as carboxylic or sulfonic acids attached. They have been widely used for more than 50 years in various products, such as firefighting foams, hydrophobic and nonstick coatings, or surfactants to cite a few examples. Their nature makes them particularly chemically inert and very resistant to degradation in environment. They also tend to accumulate in living organisms. For this reason, some PFAS are classified as persistent organic pollutants (POPs). Finally, PFAS are strongly associated with a variety of human disorders such as neurotoxicity, immune deficiency, and cancer[1].
PFAS have received worldwide attention in recent years. For example, the US EPA have has set an advisory limit of 70 ng/L in drinking water for PFOA and PFOS[2], and the EU Water Framework Directive added PFOA, its salts, and PFOA-related substances to the list of restricted substances in June 2017[3].

To detect trace concentrations of PFAS in water, it is highly recommended to use SPE to concentrate samples prior to analysis. AFFINISEP offers a specific kit containing AttractSPE® PFAS , SilactHPLC DELAY - PFAS, and SilactHPLC LC - PFAS for the analysis of a wide range of emerging and historical PFAS in large water samples in accordance with EPA 533[5].
The presence of PFAS in many common laboratory items (e.g., solvents and some plastics) makes their analysis complex and leads to contamination and high backgrounds in controls. We also describe some precautions to take to avoid this potential problem.

Table 1. List of the tested PFAS.

Compound	Chemical composition	CAS number
Perfluorobutanoic acid (PFBA)	CF3(CF2)2CO2H	375-22-4
Perfluoropentanoic acid (PFPeA)	CF3(CF2)3CO2H	2706-90-3
Perfluorohexanoic acid (PFHxA)	CF3(CF2)4CO2H	307-24-4
Perfluoroheptanoic acid (PFHpA)	CF3(CF2)5CO2H	375-85-9
Perfluorooctanoic acid (PFOA)	CF3(CF2)6CO2H	335-67-1
Perfluorononanoic acid (PFNA)	CF3(CF2)7CO2H	375-95-1
Perfluorodecanoic acid (PFDA)	CF3(CF2)8CO2H	335-76-2
Perfluorotetradecanoic acid (PFTA)	CF3(CF2)12CO2H	376-06-7
Perfluorobutanesulfonic acid (PFBS)	CF3(CF2)3SO3H	375-73-5
Perfluorohexane sulfonic acid (PFHxS)	CF3(CF2)5SO3H	355-46-4
Perfluorooctanesulfonic acid (PFOS)	CF3(CF2)7SO3H	1763-23-1

1 PRECAUTIONS FOR THE ANALYSIS OF PFAS AND RELIABILITY OF THE METHOD

The analysis of PFAS can be challenging because they are persistent and widely used in industry, resulting in trace levels of PFAS appearing almost everywhere including HPLC components and solvents, sampling bottles, and manifolds. This can lead to an increased risk of sample contamination and/or high PFAS backgrounds during analyses. Both problems can hinder PFAS detection at trace levels or lead to false positives, making the elimination of potential PFAS contamination important.

1.1 – PRECAUTION TO USE DURING SAMPLING AND MANIPULATIONS

EPA 537-1[4] and EPA 533[5] describe numerous precautions for sample collection, preservation, storage, analysis, glassware treatment, and discouraged materials that can help avoid accidental contamination, resulting in cleaner analyses and is recommended reading prior to analysis.

Other precautions should be followed. High density polyethylene (HDPE) or polypropylene are recommended for sampling materials. Nitrile gloves should be used and replaced frequently to prevent contamination. The use of glass containers should be avoided because PFAS easily adsorbed to that surface. Also items with nonstick or hydrophobic coatings may contain PFAS and must not be used. If possible, PTFE equipment should be avoided. If PTFE materials are used, the presence of PFAS must be monitored rigorously to avoid unacceptable analyte concentrations. Sampling bottles should be discarded after use to avoid cross contamination. Some plastic materials may contain trace amounts of PFAS (e.g., collection tubes, pipette tips). Finally, all items used (including solvents) should be tested for PFAS to avoid possible false positive results.

1.2 - SPE METHOD

The sample can be contaminated during the sample clean-up step, possibly arising from cartridges, filters, or resins used. For this reason, AFFINISEP has especially designed **AttractSPE® PFAS** for PFAS purification and concentration.

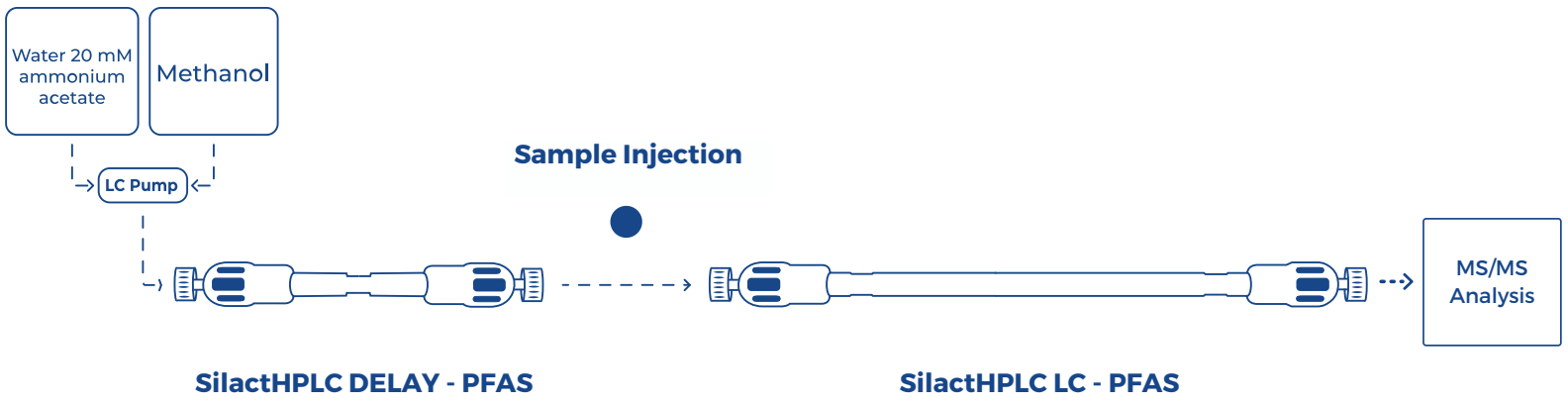
1.3 – HPLC method reliability testing

HPLC devices often contain PTFE parts and tubing. This, coupled with potential traces of PFAS in solvents, can hinder the PFAS analysis at low concentrations. The PFAS released from the HPLC tend to build up at the front of the column, creating interferences. Two solutions are available to minimize these interferences.

The first solution is the replacement of PTFE HPLC parts and perfluorinated tubing and the use of PFAS-free solvents. This solution is expensive to set up, and it is very difficult to obtain solvents totally free of PFAS.

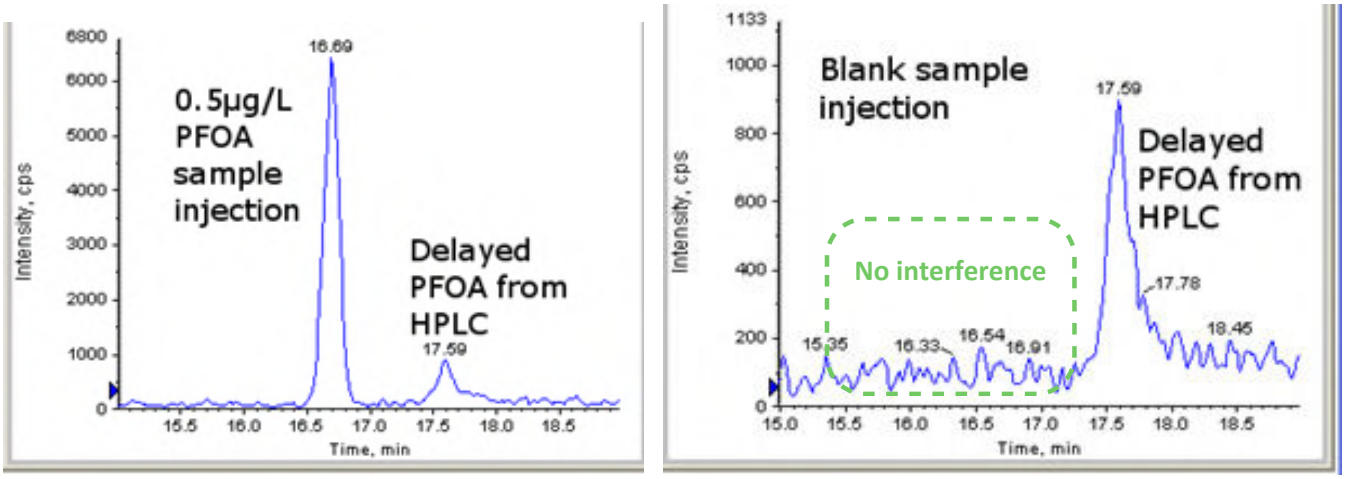
The other solution, which was used in this application note, is the installation of our **HPLC delay column** : SilactHPLC DELAY - PFAS between the LC pumps and the injector. The diagram below (Figure 1) shows the assembly of a delay column in the HPLC system. PFAS from solvents and LC pumps will build up at the front of the delay column, resulting in a shift of the retention time of the interfering PFAS during an analysis. This solution is easy to put in place and is cost effective.

Figure 1. Diagram of delay column installation on HPLC.



To demonstrate the usefulness of the delay column, PFOA was used as an example of an interfering PFAS. Two solutions, a 0.5 µg/L solution of PFOA in methanol and a blank consisting of methanol, were analyzed (Figure 2) to demonstrate the efficiency of the method using SilactHPLC DELAY - PFAS.

Figure 2. Injection of 0.5 µg/L PFOA in methanol (left) and injection of methanol blank (right).



1.4 – RESULTS

The injection of a 0.5 µg/L PFOA solution (Figure 2) shows two peaks. The first one at 16.69 min is PFOA in the injected solution at 0.5 µg/L, while the second one at 17.59 min corresponds to the delayed PFOA interference from HPLC lines and solvents. The injection of a methanol blank showed no interference at the expected retention time. The additional column proved successful at delaying the interfering PFOA at an estimated concentration between 0.05 and 0.1 µg/L.

A calibration curve was also developed in order to check the linearity of the method. Concentrations used were 0.5, 1, 2, 4, and 6 µg/L. The R-square value was found to be greater than 0.998 for the eleven molecules, demonstrating linearity of the method over this concentration range.

2 ANALYSIS OF 11 PFAS IN 500 mL OF TAP WATER

The analysis of tap water was manually carried out using a SPE vacuum manifold with AttractSPE® PFAS 6mL. The tap water hardness was very high (Ca2+ ~300 mg/L, NO3- ~20 mg/L, Cl2 ~0.3mg/L).

The glassware, plastic bottles, and filtration system were thoroughly washed with tap water, rinsed with distilled water, and lastly rinsed with LC-grade methanol. Polypropylene reservoirs (60 mL) with adapters were used to ease the loading.

Four spiked samples were processed to determine reproducibility. An unspiked blank was also processed to determine presence of PFAS in the tap water used. The method is described below.

Step	SPE Protocol
Sample preparation	500 mL of tap water was adjusted to pH ~ 4 with 100 µL of formic acid. The solution was then spiked at 24 ng/L with the 11 PFAS
Conditioning/Equilibration	1. 9 mL 0.1% NH4OH in methanol 2. 9 mL methanol 3. 9 mL ultrapure water (pH = 4)
Loading	500 mL of loading solution (~5 mL/min) pH ~4
Washing	Rinse the walls of sample container with 10 mL of ultrapure water and wash the cartridge with it
Drying	1 min under full vacuum
Elution	1. Rinse the walls of sample container with 4 mL of methanol prior to elution 2. Repeat the step with another 4 mL of methanol 3. Elute with 4 mL 0.1% NH4OH in methanol for a total of 12 mL

The elution was collected in polypropylene vials, homogenized, and analyzed by LC-MS/MS (described in Table 3). To determine the matrix effects, a fraction of the elution of the blank was spiked at 2 µg/L and analyzed.

2.1 – RESULTS

Table 2. Recovery of 11 PFAS in 500 mL of unspiked tap water and spiked tap water (24 ng/L) after purification with AttractSPE® PFAS. The recovery values already take into account the matrix effects. (ND = Not detected)

Compound	Presence in blank	Recovery	RSDr (n=4)	Observed matrix effect
PFBA	ND	106%	3%	-4%
PFPeA	ND	100%	1%	0%
PFHxA	ND	100%	3%	1%
PFHpA	ND	100%	2%	1%
PFOA	ND	98%	1%	-3%
PFNA	ND	100%	6%	-7%
PFDA	ND	93%	2%	-13%
PFTA	ND	93%	3%	-6%
PFBS	ND	97%	2%	5%
PFHxS	ND	104%	2%	0%
PFOS	ND	107%	5%	-5%

The analysis of the blank water sample spiked at 2 µg/L after the elution showed **no significant matrix effects**, with a maximum of 13% signal suppression for PFDA by comparison with the calibration curve. Futhermore, **AttractSPE® PFAS** showed excellent recoveries from **93% to 107%** , and excellent relative standard deviation from **1% to 6%**.

Table 3. LC-MS/MS conditions for the analysis of the 11 PFAS.

LC Conditions

LC Dionex U3000
Column: SilactHPLC LC - PFAS 150 x 2.1 mm, 3 µm and pre-column filter at 30 °C
Delay column: SilactHPLC DELAY - PFAS 50 x 2.1 mm, 5µm

Injection volume: 5 µL
T° sampler: 10°C
Flow rate: 0.25 mL/min

Time (min)	Solvent A	Solvent B
0	60	40
1	60	40
20	10	90
30	10	90
31	60	40
35	60	40

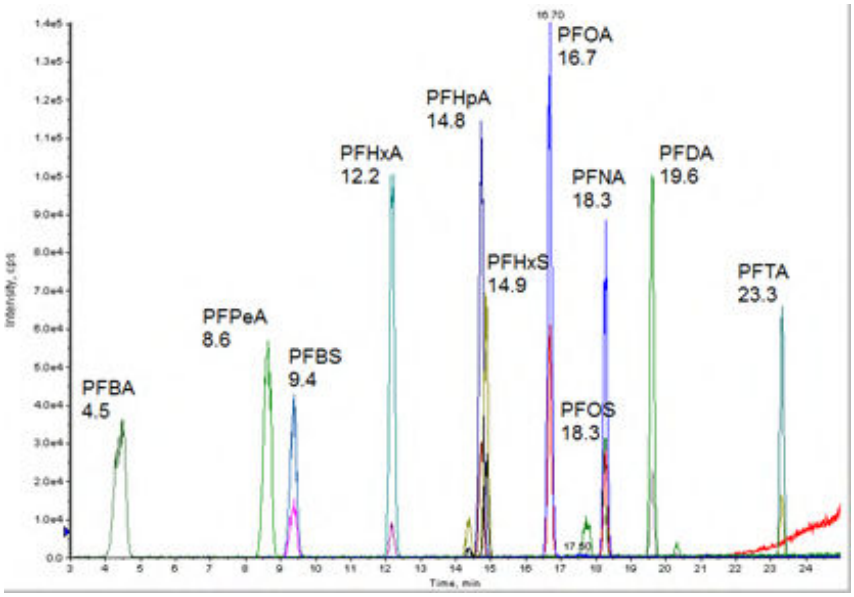
Solvent A: 20 mM ammonium acetate (in water)
Solvent B: methanol

MS Conditions

Qtrap 4000 ESI- MS/MS
Curtain gas: 30
CAD: High
IS: -4500 V
Temperature: 400°C
GS1/GS2: 50/50

Analyte	Retention time (min)	Q1	Q3	CE (V)
PFBA	4.5	213.0	168.8	-14
PFPeA	8.6	263.0	218.8	-12
PFHxA	12.2	313.0	268.9/119	-14/-28
PFHpA	14.8	363.0	318.8/168.8	-16/-26
PFOA	16.7	413.1	368.9/168.8	-14/-26
PFNA	18.3	463.0	418.9/219.0	-16/-24
PFDA	19.6	513.0	469.0/218.8	-13/-11
PFTA	23.3	712.9	668.9/168.8	-18/-42
PFBS	9.4	299.0	79.8/98.9	-52/-44
PFHxS	14.9	399.0	79.9/98.9	-74/-56
PFOS	18.3	499.0	80.1/98.9	-84/-70

Figure 3. LC-MS/MS chromatogram for the 11 PFAS at 5 µg/L.



CONCLUSION

AttractSPE® PFAS was found to be very effective for the analysis of PFAS in water with recoveries higher than **93%** and demonstrated excellent reproducibility with relative standard deviation between **1% and 6%**. Furthermore, the use of SilactHPLC DELAY - PFAS as delay column allows to avoid any PFAS interference during LC-MS/MS analysis.

The method easily concentrated PFAS in 500 mL of water sample more than 40 times without any evaporation step. If needed, the samples can be concentrated more by evaporating the elution.

Particular attention must be paid to verify that the laboratory environment does not contaminate samples and lead to false positives. Some simple precautionary steps are described in the application note (e.g., the use of a delay column). For routine analysis, the use of internal standards to correct the potential matrix effects and adsorption of the largest PFAS is advised.

References :

1. Impact of Perfluorinated Compounds on Human Health, 2014 Academy for Environment and Life Sciences.
2. Drinking water Health Advisory for PFOA and PFOS, EPA
https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos
3. Directive of the European Parliament and of the Council, on the quality of water intended for human consumption, 2018
4. EPA Method 537.1 : Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry(LC/MS/MS).
https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab=NERL
5. EPA Method 533 : Determination of per-and polyfluoroalkyl substances in drinking water by isotope dilution anion exchange solid phase extraction and liquid chromatography/tandem mass spectrometry.
https://www.epa.gov/dwanalyticalmethods/method-533-determination-and-polyfluoroalkyl-substances-drinking-water-isotope

Part number of products used in this application note:

AttractSPE® PFAS 6mL - 150 - 50/pk
- PFAS-50.S.6.150

HPLC column: SilactHPLC LC - PFAS, 150 x 2.1mm, 3 µm 1 unit
- LC-PFAS-150.2.1

HPLC delay column: SilactHPLC DELAY - PFAS, 50 x 2.1mm, 5 µm 1 unit
- DELAY-PFAS-50.2.1

SPE vacuum manifold 1 unit
- ACC-MAN2

Related products :

AttractSPE® PFAS 6mL - 200 - 50/pk
- PFAS-50.S.6.200

AttractSPE® PFAS 6mL - 500 - 50/pk
- PFAS-50.S.6.500

Kit including AttractSPE® PFAS, delay & HPLC columns
- PFAS-50.SDH.6.150