

Automated Solid Phase Extraction (SPE) of 10 Perfluorinated Compounds (PFAs) from Tap Water

APPLICATION NOTE AN1029

APPLICATION BENEFITS

- Ability to extract and concentrate PFAs prior to analysis with an SPE system and consumables that are free of PFAs
- Perfect control through automation of SPE key parameters to achieve good accuracy and reproducibility
- Precautions to improve PFAs HPLC method reliability

ADDRESSED ISSUES

- Compromised sample integrity by PFAs contamination during sample preparation

This collaboration study was performed jointly by Gilson, Inc. and Affinisep.

ABSTRACT

Perfluorinated compounds are a family of molecules consisting of varying lengths of fluorocarbon chains with a functional group such as carboxylic or sulfonic acid attached. Since 2009, PFOs have been classified as POPs (Persistent Organic Pollutants) and the International Agency for Research on Cancer (IARC) classified PFOA as a Group 2B possible carcinogen. The analysis of PFAs can be complex due to their presence in multiple items used in the analytical workflow. This application note describes the automation of the isolation of 10 PFAs from tap water prior to their analysis by LC/MS using a specific SPE polymer AttractSPE® PFAs and the **Gilson ASPEC® 274 Large Volume (LV) System**. The results show good reproducibility of the method without any contamination from the extraction system.

INTRODUCTION

Perfluorinated compounds are a family of molecules consisting of varying lengths of fluorocarbon chains with a functional group such as carboxylic or sulfonic acid attached. To achieve the concentration limit, defined by EU Water Framework Directive and the EPA for perfluorinated compounds in water, it is highly recommended to use solid phase extraction (SPE) to concentrate the sample prior to mass spectrometry analysis.

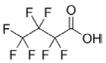
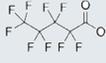
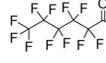
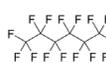
Due to the confusion created by the acronym “PFCs”, and its two different meanings, namely perfluorinated compounds or fluorocarbons, the acronym “PFAs” (per- and polyfluoroalkyl substances), is now preferred to classify this family of compounds.



Figure 1
Gilson ASPEC® 274 Large Volume System

Table 1

List of the tested PFAs sorted from the smallest to the largest.

Compound	Chemical Composition	CAS Number
Perfluorobutanoic acid (PFBA)		375-22-4
Perfluoropentanoic acid (PFPeA)		2706-90-3
Perfluorohexanoic acid (PFHxA)		307-24-4
Perfluoroheptanoic acid (PFHpA)		375-85-9
Perfluorooctanoic acid (PFOA)		335-67-1
Perfluorononanoic acid (PFNA)		375-95-1
Perfluorodecanoic acid (PFDA)		335-76-2
Perfluorobutanesulfonic acid (PFBS)		375-73-5
Perfluorohexane sulfonic acid (PFHxS)		355-46-4
Perfluorooctanesulfonic acid (PFOS)		1763-23-1

This application note describes the analysis of 10 perfluorinated compounds using a specific **AttractSPE® PFAs**. This cartridge chemistry is dedicated specifically to perfluorinated compounds. The cartridges are used in the **ASPEC 274 LV System** (Figure 1) to purify and concentrate the sample prior to analysis by LC-MS/MS.

The analysis of PFAs can be challenging. They are common material components in analytical systems. Their presence in HPLC systems and solvents, sampling bottles, valves, transfer tubing, etc. can lead to significant chance of sample contamination and high PFAs background, masking trace levels of PFAs from detection and/or leading to false positives.

This application ultimately shows the absence of potential PFAs contamination through the **ASPEC 274 LV System** with high recoveries and reproducibility.

PRECAUTIONS FOR THE ANALYSIS OF PFAs AND RELIABILITY OF THE METHOD

PRECAUTIONS FOR SAMPLE HANDLING AND PREPARATION

EPA 537-1^[1] method describes numerous precautions for sample collection, preservation, storage, analysis, and glassware treatment. It also prohibits the use of materials that can cause accidental contamination, ultimately resulting in cleaner analyses.

For this study, Gilson HDPE sample bottles and Gilson PIPETMAN® DIAMOND Tips were used for sample handling and for standard preparation. The **ASPEC 274 LV System** was equipped with PEEK tubing to avoid the use of any fluoropolymer, such as PTFE or FEP, to reduce contamination risk during the sample preparation process.

Cartridges, and the filters and resins contained in them, can also be sources of contamination. For this reason, AFFINISEP has developed a specific **AttractSPE® PFAs** dedicated to the isolation of perfluorinated compounds. AFFINISEP **AttractSPE® PFAs** cartridges will not contaminate any sample with additional PFAs.

To reduce contamination risk, best practice indicates that all sampling containers are discarded after use, nitrile gloves are preferred over other glove materials, the nitrile gloves are often replaced, and all glass or fluoropolymer containers are avoided to prevent surface adsorption of PFAs or contamination from container material.

PRECAUTIONS TO IMPROVE HPLC METHOD RELIABILITY

HPLC devices often contain PTFE parts and tubing. This, coupled with potential traces of PFAs or other fluorine containing material in solvents, can hinder analysis at low concentrations. The fluorine containing materials that are released from the HPLC system tend to build-up at the front of the column creating interference. To minimize this interference, all PTFE or any other fluorinated or perfluorinated polymers are replaced with non-fluorine containing materials. Also, despite the difficulty and added cost, all solvents used must be verifiably PFAs free.

Another approach, which was used in this application note, is the installation of a delay column between the LC pumps and the injector. The diagram below (Figure 2) demonstrates the proper placement of the delay column in the HPLC fluid path. The interfering species from the solvents and LC pumps will concentrate at the front of the delay column resulting in a shift of retention time. This shift in retention time effectively separates interfering species from the analyte PFAs. This is a simple and cost-effective solution.

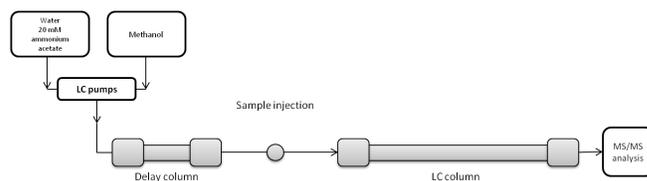
**Figure 2**

Diagram of delay column installation on HPLC.

PFOA is an interfering species when analyzing PFAs. Two solutions, a blank consisting of methanol and a 0.5 µg/L solution of PFOA in methanol were analyzed (Figure 3) to demonstrate the efficiency of the method using a delay column. (Silact™ C18 LC-P 50 x 2.1 cm, 3 µm)

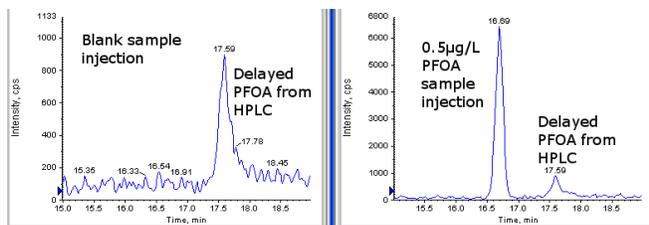


Figure 3
Injection of methanol blank (left) and injection of 0.5µg/L PFOA in methanol (right)

The injection of a 0.5 µg/L PFOA solution (Figure 3) shows two peaks. The first one at 16.69 min is PFOA in the injected solution at 0.5µg/L, while the second one at 17.59 min corresponds to the delayed PFOA interference from HPLC lines and solvents. The injection of a blank methanol showed no interference at the expected retention time. The delay column allows the elimination of interfering PFOA at a concentration estimated between 0.05 and 0.1 µg/L.

ANALYSIS OF 10 PFAS IN 500 mL OF TAP WATER

The SPE protocol was carried out on the **ASPEC 274 LV System** in tap water using **AttractSPE® PFAs** 6 mL cartridges. The polymeric WAX phase contained in these cartridges undergoes a proprietary treatment at Affinispel to ensure it is entirely PFAs free and a specific high loading capacity for PFAs.

The automation of PFAs analysis was carried out using the **ASPEC® 274 Large Volume System** equipped with segregated waste lines and Gilson VALVEMATE® II Valve Actuator. PEEK tubing was used to reduce risk of PFA adsorption and resulting contamination. The solvents and the 500 mL samples of tap water were contained in Gilson HDPE bottles.



SAMPLE PRE-TREATMENT

The tap water hardness was very high (Ca²⁺ ~300 mg/L, NO₃⁻ ~20 mg/L, Cl₂ ~0.3mg/L). For each sample, 475 mL of tap water was mixed with 25 mL of methanol. The pH was adjusted to ~4 with 100 µL formic acid. The solution was then spiked with a mix of 10 PFAs at a concentration of 24 ng/L.

One non-spiked sample blank and four spiked samples were processed and analyzed. The blank was analyzed to verify non-contamination.

The analyses were carried using a manual SPE manifold as a control, and on the automated system (**ASPEC 274 LV System**) to confirm the absence of adsorption and contamination potentially introduced by the automated system.

SPE PROTOCOL

Step	SPE Protocol
Conditioning/ Equilibration	1. 5 mL 0.1% NH ₄ OH in methanol 2. 9 mL methanol 3. 9 mL HPLC grade water with formic acid (pH = 4)
Loading	500 mL of loading solution (475 mL tap water + 25 mL methanol pH = 4)
Drying	20 mL air pushed through the cartridge
Elution	1. 2 mL methanol 2. 4 mL 0.1% NH ₄ OH in methanol

This protocol was easily transferred to **TRILUTION® LH Software** for automation:



Figure 4
Gilson TRILUTION® LH Software SPE method

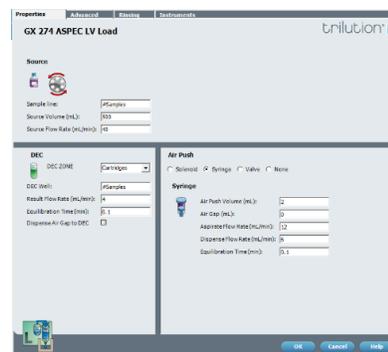


Figure 5
GX 274 ASPEC LV Load task

The elution was collected in polypropylene vials, homogenized, and directly analyzed by LC-MS/MS.

To determine matrix effect, a fraction of the elution of the blank water sample was spiked at 2 µg/L and analyzed.

HPLC/MSMS PROTOCOL

The analytical conditions are presented in the table below:

Table 2

LC-MS/MS conditions for the analysis of the 10 PFAs.

LC Conditions	MS Conditions
LC Dionex U3000	Qtrap 4000 ESI- MS/MS
Column: Silact C18 LC-P 150 x 2.1 cm, 3 µm and pre-column filter at 30°C Delay column: Silact C18 LC-P 50 x 2.1 cm, 3 µm	Curtain gas: 30
	CAD: High
Injection volume: 5 µL	IS : -4500 V
T° sampler: 10°C	Temperature: 400°C
Flow rate: 0.25 mL/min	GS1/GS2: 50/50

Time (min)	Solvent A	Solvent B	Analyte	Retention time (min)	Q1	Q3	CE (V)
0	60	40	PFBA	4.5	213.0	168.8	-14
1	60	40	PFPeA	8.6	263.0	218.8	-12
20	10	90	PFBS	9.4	299.0	79.8/98.9	-52/-44
30	10	90	PFHxA	12.2	313.0	268.9/119	-14/-28
31	60	40	PFHpA	14.8	363.0	318.8/168.8	-16/-26
35	60	40	PFHxS	14.9	399.0	79.9/98.9	-74/-56
Solvent A: 20 mM ammonium acetate (in water) Solvent B: methanol			PFOA	16.7	413.1	368.9/168.8	-14/-26
			PFOS	18.3	499.0	80.1/98.9	-84/-70
			PFNA	18.3	463.0	418.9/219.0	-16/-24
			PFDA	19.6	513.0	469.0/218.8	-13/-11

Table 3

Recovery of 10 PFAs in 500 mL of unspiked tap water and spiked tap water and observed matrix effect after purification with **AttractSPE® PFAs** on **Gilson ASPEC® 274 Large Volume System**.

Compound	Unspiked Tap Water	Spiked Tap Water at 24 ng/L	RSDr (n=4)	Blank water Eluate spiked at 2 µg/L
		Recovery		Observed matrix effect
PFBA	ND*	99%	3%	+16%
PFPeA	ND*	99%	1%	+12%
PFBS	ND*	101%	3%	+10%
PFHxA	ND*	102%	3%	+17%
PFHpA	ND*	100%	4%	+8%
PFHxS	ND*	101%	2%	+1%
PFOA	ND*	102%	2%	-1%
PFOS	ND*	87%	2%	+8%
PFNA	ND*	97%	3%	+8%
PFDA	ND*	83%	2%	-1%

*ND: Not Detected

RESULTS

The method linearity was checked against a calibration curve with PFAs concentrations of 0.5, 1, 2, 4, and 6 µg/L. The R² value was found to be greater than 0.998 for the ten molecules, demonstrating linearity of the method over this concentration range.

As shown in the Table 3, no detectable contamination was found using the **AttractSPE® PFAs** cartridges on the **ASPEC 274 LV System**.

The elution fraction of a blank sample was spiked at 2 µg/L showing **no significant matrix effects**, with a maximum of 16% signal enhancement for PFBA by comparison with the calibration curve. Without an additional concentration step after the SPE protocol, good values of LOQ (6 ng/L) and LOD (2 ng/L) were achieved.

Furthermore, **AttractSPE® PFAs** showed excellent recoveries from **83% to 102%** and the **ASPEC 274 LV System** allowed excellent relative standard deviation from 1% to 4%.

CONCLUSION

The automation of the isolation of 10 PFAs from tap water for analysis by LC-MS/MS, was found to be very effective with good recoveries from **83% to 102%** without any contamination from the extraction system.

The **AttractSPE® PFAs** cartridges have enhanced selectivity and concentrated the sample more than 80 fold, allowing the direct analysis of the 10 PFAs with a LOQ at 6 ng/L without any evaporation step.

The combination of **AttractSPE® PFAs** and the **ASPEC 274 LV System** allowed development of a robust method with excellent repeatability (RSD 1%-4%) that helps eliminate environmental variables and effectively reduces chances of sample contamination.

Furthermore, the automation greatly reduces labor cost, reduces potential exposure to hazardous solvents, and increases overall efficiency for high throughput laboratories.

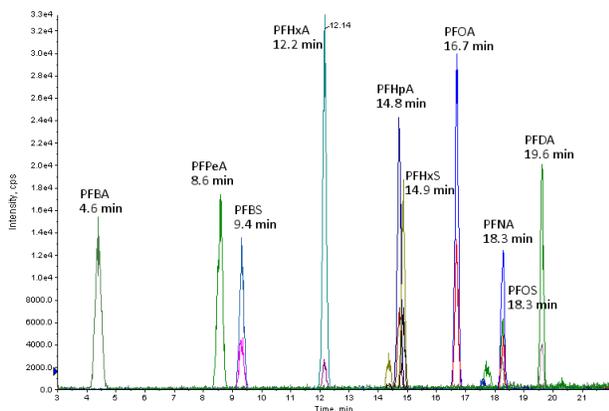


Figure 6

LC-MS/MS chromatogram for the 10 PFAs (see HPLC/MS protocol (Table 2) for retention times)

PART NUMBERS OF PRODUCTS USED IN THIS APPLICATION NOTE

CONSUMABLES		
Part Number	Product Name	Quantity
WAX-PFC-50.S.6	AttractSPE® PFAs 6 mL	50/pk
C18LCP-150.2.1	HPLC column: Silact C18 LC-P 150x2.1cm 3µm	1 unit
C18LCP-50.2.1	HPLC delay column: Silact C18 LC-P 50x2.1cm 3µm	1 unit

GILSON ASPEC 274 LARGE VOLUME SYSTEM		
Part Number	Product Name	Quantity
2614010LV	ASPEC 274 LV System, WITH Z DRIVE	1

Reference

¹Method 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

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